Donald A. Yee *Editor*

Ecology, Systematics, and the Natural History of Predaceous Diving Beetles (Coleoptera: Dytiscidae)

Second Edition

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Editor
Donald A. Yee
School of Biological, Environmental, and
Earth Sciences
University of Southern Mississippi
Hattiesburg, MS, USA

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Cover image: An adult Acilius mediatus from a pond in southcentral Mississippi, U.S.A. (Photo by D.A. Yee).

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To my mother, Patricia, who gave me the tools
and support to pursue my dreams

Preface

There are nearly 200 families of beetle, with many more families in total among the other insect orders, so to devote a single book to this single family of insect should require an answer to the question, Why dytiscids? My answer is simple. They are cool! Within this context, cool can be defined both scientifically (e.g., one of the most diverse beetle families, ubiquitous in freshwater systems, capable of feeding on a multitude of prey including vertebrates) and esthetically (streamlined shaped, wide variety of colors and behaviors, cultural significance). Also, they are cool for another reason. They are understudied as both an aquatic insect group and as an aquatic predator. Thus, they offer even the casual researcher an opportunity to make significant contributions to the knowledge of their biology, ecology, or evolution. Therefore, a book that covers our current understanding of this various aspects of dytiscids, including our gaps in knowledge, seems both timely and warranted.

My hope is that the readers of this second edition will find it a comprehensive overview of the Dytiscidae, a most ubiquitous and amazing family of aquatic predators. It was partly to satisfy what I perceived as a need for such a book that I began developing this book subsequent to a symposium I organized at the 2010 Entomological Society of America meeting held in San Diego, California. Many of the participants in that symposium were kind enough to prepare chapters for this book (i.e., Yves Alaire, Johannes Bergsten, Patrick Crumrine, Lauren Culler, Margherita Gioria, Siegfried Kehl, Kelly Miller, and Shin-ya Ohba). I am grateful to them, and others (i.e., David Bilton, Garth Foster, Mariano Michat, Andrew Austin) whom I met in the meantime and graciously accepted my invitation to contribute. The comprehensive and thoughtful presentations you will find in the following pages are a testament to the authors passion for science in general and dytiscids in particular. My own history with this group is comparably short, spanning from my postdoctoral position at the University of Calgary in 2007 under Steve Vamosi to the present day. However, even at my first sampling foray into a roadside pond, I was struck at the density and variety of adult and larval dytiscids. Once I began to formulate research questions regarding the ecology of these insects, I quickly found that a lack of knowledge, especially ecological, was the rule and not the exception for most species. In fact, in their excellent book, *Predaceous Diving Beetles (Coleoptera: Dytiscidae) of the Nearctic Region*, Larson, Alarie, and Roughley (2000) state

> Very little is known about the habitat, life history, or habits of most North America water beetles.

This statement is apt and can easily apply to dytiscids worldwide. My hope is that this book will help inspire entomologists, ecologists, systematists, and others to make a start to fill in the gaps.

Hattiesburg, MA
October 2021

Donald A. Yee

Introduction to the Second Edition

Since the original volume of this book was produced in 2014, there has been dozens of new publications regrading predaceous diving beetles. To attempt to capture some of this new information, all chapters have been updated with new findings and new citations. Furthermore, a new chapter has been added that details the amazing fauna of the Australian subterranean dytiscids. I thank Dr. Hans Fery for his keen eye and editorial comments on all chapters.

Contents

Chapter 1
An Introduction to the Dytiscidae: Their Diversity, Historical Importance, Cultural Significance, and Other Musings

Donald A. Yee

> *I am surprised what an indelible impression many of the beetles which I caught at Cambridge have left on my mind.*
> *Charles Darwin (1887).*

Abstract Predaceous diving beetles (Family Dytiscidae) are one of the most fearsome predators in freshwater environments, however, most of their biology and ecology remain to be measured. The Dytiscidae exhibit a complex life cycle with both adult and larvae using a variety of aquatic habitats for feeding, reproduction, and intra- and interspecific interactions. Adults are vagile and capable of dispersal across great distances, making them an important component of the terrestrial environment and potentially important for linking various habitats via the movement of energy and materials. Both larvae and adults are predaceous, and the former often possess large curved hollow mandibles that are capable of dispatching large prey, including vertebrates. As predators, they exhibit different hunting behaviors and a full repertoire of chemicals used for defense and communication. Adult dytiscids also display one of the most complex and fascinating examples of sexual selection, with both pre- and post-copulatory mating choice dispersed among different phylogenetic branches of the family. Although the systematics of dytiscids has been of interest for decades, phylogenies are now becoming clearer, allowing us to better understand their dynamic and interesting evolutionary history. These beetles also can instruct us on bigger concepts, like the current focus on conservation both of species and of the habitats that harbor them; to this, dytiscids make a good case study. Although often overlooked in the scientific literature compared to other aquatic insect groups, their importance in human culture, both past and present, is compelling and worthy of note. With all this, perhaps the most intriguing thing about dytiscids is that we know so little about them.

D. A. Yee (✉)
School of Biological, Environmental, and Earth Sciences, University of Southern Mississippi, Hattiesburg, MS, USA
e-mail: donald.yee@usm.edu

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1.1 Dytiscids Past and Present

The pantheon of life is not the sum of mere observations of numbers, like the adding up of so many trading cards or widgets in a collectors case, but instead involves the unique place of each organism on Earth and its rich and often tangled evolutionary past. This book explores the current knowledge of just one family of beetle (among many dozens of families) within one order (among many dozens of orders) within one class (among many dozens of classes) within one phylum (among about 35 phyla of animals) within one of 5 kingdoms nested within one of the three domains of life. The story of the Dytiscidae, both past and current, is interesting and unique, and it is hoped that the reader will gain a better appreciation of their ecology, natural history, and systematics from the chapters that follow.

Of the approximately three dozen families of aquatic or semi-aquatic beetles, predaceous diving beetles (a.k.a. "diving beetles," "water beetles," or "dytiscids") are a common inhabitant of most freshwater lentic and lotic aquatic systems on Earth (Fig. 1.1). The family name, Dytiscidae, derives from the Greek *dytikos*, meaning

Fig. 1.1 An adult and juvenile (water tiger) *Cybister* sp. from a pond in southcentral Mississippi, USA. Note the oar-like rear legs fringed with swimming hairs on the adult. Photo by the author, 2015

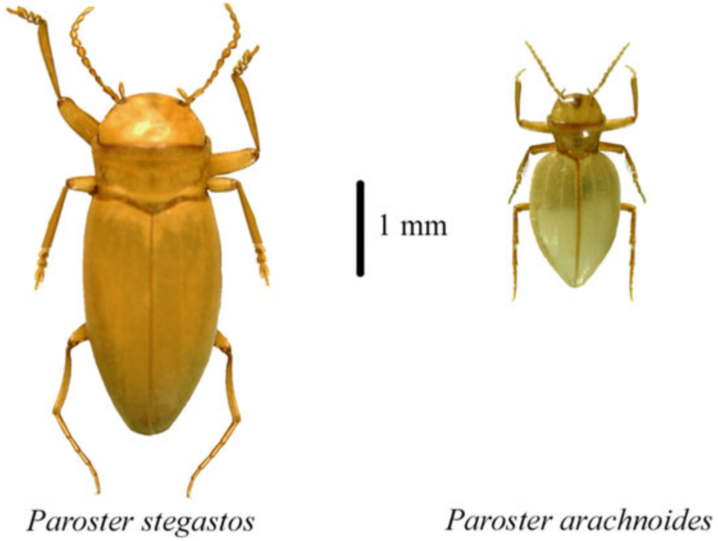

Fig. 1.2 Adult *Paroster stegastos* and *P. arachnoides*, species that are part of the recently discovered Australia subterranean fauna of dytiscids. Note the lack of pigment and eyes, and other morphological adaptations to cave living. Photo courtsey of Chris Watts, 2014

"able to dive," which speaks to their proclivity for submerging in freshwater environments. They occur in almost every type of freshwater (and saline) habitats from large lakes to small plant-held waters (Chap. 10). Many casual and professional scientists have observed adults and larvae when sampling ponds or ditches or other more unlikely places. I myself had the experience of an adult *Cybister* landing in the tailgate of my pickup truck on a summer afternoon, ostensibly thinking that my dark truck bed was a nice refreshing pond. Dytiscids are the most diverse aquatic Coleoptera, with the current number of identified more than 4600 extant species (Nilsson and Hájek 2022). However, like most species of life on Earth (and insects in particular), the actual number of living species will exceed this current total, perhaps by thousands of new species. In fact, the recently discovered Australian subterranean fauna of dytiscids (Fig. 1.2, Chap. 9) has added a whole new world of diversity to this group, and it is possible that other untapped areas (including museum drawers) remain to be explored. The subterranean fauna is unique in many ways, especially in regard to adaptation of beetles, like the loss of pigment, eyes, and some swimming adaptations. Beyond the high diversity of dytiscids within and among habitats, they also exhibit high abundance. In agricultural ponds in Alberta, Canada I routinely collected a dozen or more *Graphoderus occidentalis* adults in a single sweep of my D-net! Other authors note similar experiences with bountiful numbers. Adults are distinguished from members of other aquatic beetle families by their highly specialized aquatic adaptations, including a rounded body shape with dorsal-ventrally flattening, large natatorial oar-like hind legs, and variable respiratory mechanisms (Chap. 5). These adaptations often are useful for distinguishing them from other aquatic coleoptera, including the Hydrophilidae (water scavenger beetles) and Noteridae (burrowing water beetles). Species in the former family are often collected in association with dytiscids but are less streamlined and often swim with alternating strokes of their crawling legs, whereas the latter family was only recently split from the Dytiscidae, and the two families share many conspicuous morphological features.

One of the first references to dytiscids in the literature appears in the *Systema Naturae* (tenth edition, Linnaeus, 1758), although few of the species listed under this family survive under that original set of classifications. Miller and Bergsten in Chap. 3 provide additional history of the scientific literature on dytiscids, although no work of biology can ever be complete without a mention of Charles R. Darwin. As we will see, the eminent Englishman also had a few ties to predaceous diving beetles. His earliest recorded scientific work involves dytiscids, which are part of the insect collections he made near Cambridge in early 1829 at the age of 20 (fully 2 years prior to his voyage on HMS Beagle). At this time, it is thought that he began to cultivate a keen interest in entomology. The collection records from this time appear in several volumes of British insects by James Francis Stephens (1829). Among other insects, Darwin gathered a variety of dytiscids along with notes on their collections, including *Dytiscus conformis* ("Near Cambridge, not rare, in 1829"), *Hydaticus hybneri* ("Near Cambridge in 1829"), *Hygrotus scitulus* ("Near Cambridge"), *Hydroporus areolatus* ("Cambridge"), and several *Colymbetes* including *C. agilis* ("In profusion near Cambridge in 1829") (Fig. 1.3). His early fascination with insects, based especially on collecting beetles, has been noted elsewhere, but one cannot help to think that this early exposure whet his appetite for later beetle collecting (e.g., see the quote that starts this chapter and Chap. 6). Charles Darwin did collect dytiscids (and thousands of other specimens) on the five-year Beagle voyage starting in late December, 1831, including a *Colymbetes signatus* (now in the genus *Rhantus*) that was caught on board the ship, "45 miles from Cape St. Mary" (Monte Video, Uruguay) (Babington 1842). Darwin wonders how much farther it would have flow if stronger winds occurred, and perhaps gives us one of our first observation of a dytiscid dispersal event (Chap. 11). Darwin also discovered several new species of dytiscids on that voyage, which were later compiled and published by C. C. Babington in 1842. After his return, Darwin appeared to lose interest in insects, and focused much effort on various other groups, including earthworms, barnacles, and domesticated animals, as they would provide him with details to help him make his eventual case for natural selection. However, he does return to dytiscids later in life, in a case he published involving dispersal of a freshwater bivalve with the aid of a large dytiscid (Darwin 1882):

> On February 18 of the present year, he [Mr. Walter Drawbridge Crick] caught a female *Dytiscus marginalis*, with a shell of Cyclas cornea clinging to the tarsus of its middle leg. The shell was 0.45 of an inch from end to end, 0.3 in depth, and weighed (as Mr. Crick informs me) 0.39 grams, or 6 grains.

This article was published April 6, 1882, a mere 13 days before his death, and thus represents his last living contribution to science. It is natural for biologists from

Fig. 1.3 Some of the beetles collected by Charles Darwin during his time at Cambridge, England. Note the large dytiscids (perhaps *Cybister* sp.) in the top of the right case. These and other insects collected by Charles Darwin are on display at the University of Cambridge Zoological Museum. Photo courtesy of Richard Carter, 2014

all fields to try and claim Darwin for their own, either because of his effort, if even fleeting, for their study of organism or because of how his work speaks to their current set of questions. Thus, it is of some satisfaction to this author that one could say that his scientific career began and ended with dytiscids.

1.2 Nature Red in Tooth and Claw...and Mandible

When Alfred Tennyson (1809–1892) wrote his famous poem (*Nature, Red in Tooth and Claw*, 1850) he may not have imagined that it would serve as a touchstone to summarize many of the ideas put forth by Charles Darwin in *On the Origin of Species* (1859). However, that phrase, the title to Tennyson's poem about his struggle with his religious faith in the presence of a personal tragedy, has come to encapsulate the very struggle for existence that Darwin outlined in his thesis on natural selection. Dytiscids, as predators, perfectly reflect this struggle (for themselves and their unfortunate prey). Most larvae, including the larger forms (e.g., *Dytiscus*) known as "water tigers," are equipped with large hollow curved mandibles (Fig. 1.4), that easily pierce invertebrate and vertebrate (including human) flesh. They also feed on one another, and therefore aptly conform to the struggle within a species as well. Within the aquatic world of a fishless farm pond or a roadside ditch,

Fig. 1.4 The head of a *Dytiscus* sp. larva, showing the large curved hollow mandibles, stemmata (simple eye spots), and palps. The feeding appendages are powerful enough to restrain and kill tadpoles and fish. Photo courtesy of Neil Phillips 2013

the *Dytiscus* larvae are the great white shark or African lion, seizing upon unexpecting prey in a flash of blood (or hemolymph) and writhing bodies. However, this is not the scale at which we often imagine ourselves, and therefore we may lose sight of the significance of these predators to their prey. Imagine for a minute a larval *Dytiscus* as large as a cheetah. After ignoring the limitations that oxygen consumption demands or the structural qualities that chitin imposes, such a predator would likely surpass the most horrible nightmare that Hollywood could conjure up! Even the chewing and slashing mouthpart of the "Xenomorph" of the *Alien* movie franchise would fail to compare to the piercing and sucking mouthparts of a formidable *Dytiscus* larvae writ large! Such nightmares are all too real for many aquatic prey, including many vertebrates like tadpoles and fish (Chap. 8). However, these predators have received considerably less attention than other predatory taxa in lentic systems, but they are no doubt as important.

I am willing to bet that most researchers feel that their particular study organism is underrepresented in the scientific literature and simultaneously underappreciated by the general public. Although the latter is difficult to quantify, evidence for the former condition is not difficult to find to verify (or refute) that point of view. With this in mind, I gathered evidence to show that, indeed dytiscids are one of the most neglected aquatic insect groups, even among the aquatic Coleoptera and aquatic predators. Using the online search engine Web of Science™ (Thomas Reuters) I gathered citation records for various aquatic insect taxa over the last two decades (1994 to August 2021). I focused on family names for many aquatic groups (e.g., Culicidae, Dytiscidae, Hygrobiidae, Nepidae) but used the ordinal level for others (Plecoptera, Odonata, Trichoptera, Megaloptera) (Fig. 1.5). I used orders for some

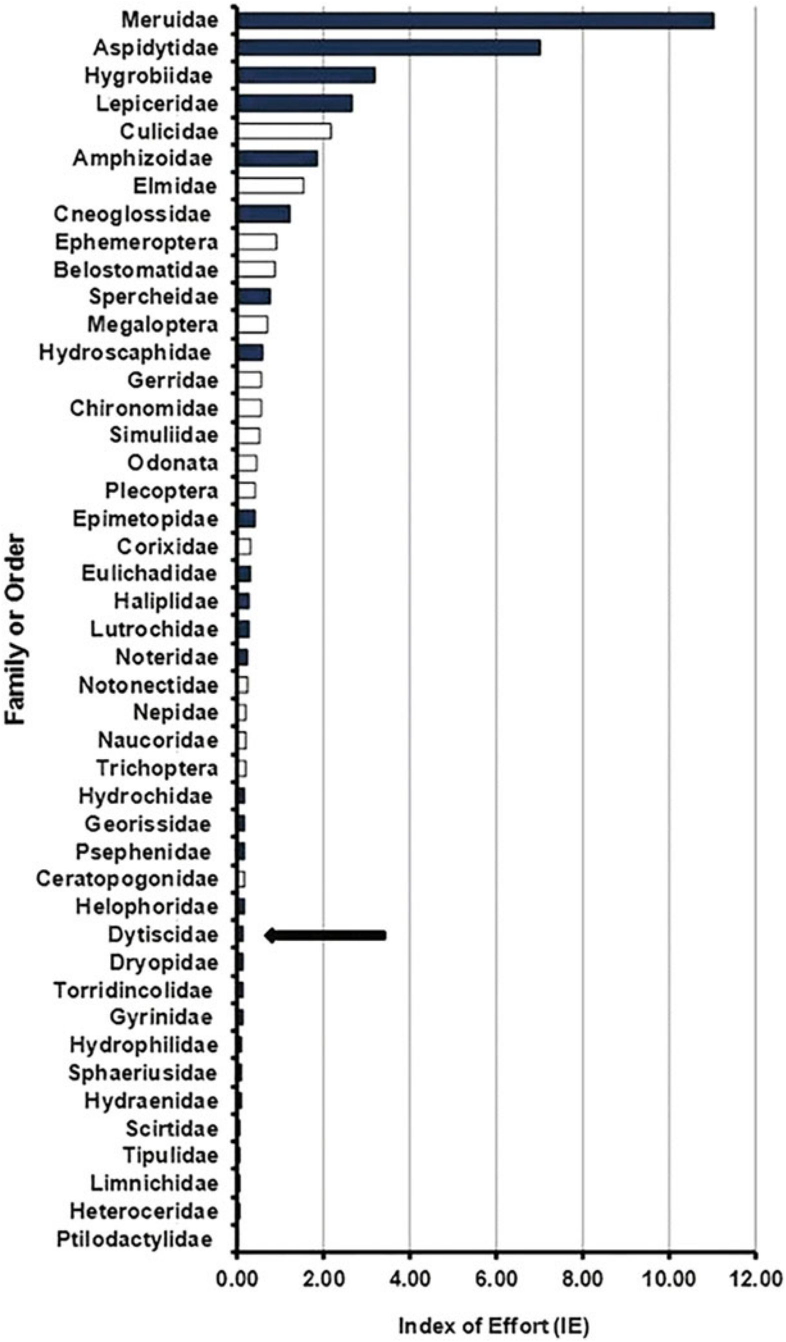

Fig. 1.5 The Index of Effort (IE) for various aquatic insect taxa. IE is defined as the number of scientific publications noted in Web of Science™ from 1994 to 2014 using the family or order names divided by the number of species in each group. Values greater than 1 would indicate that there is more than one publication per species within that taxonomic group. Bars in black are for

groups when they were essentially entirely aquatic. I searched using the "Topic" field, as restricting to publication titles may miss important work where the group was included, but not the major focus (e.g., community ecology studies or broad phylogenies). I also determined the approximate number of species present in each group using various sources. I then divided the total citations over the past 27 years by the number of species in each group to generate an Index of Effort (IE). Values of IE that exceed 1 would indicate more publications than species present (i.e., high effort), whereas values <1 would suggest fewer publications than species (i.e., less effort). There are pros and cons with such an approach, as some groups may receive high effort because of a few important taxa or because some species are of particular interest (e.g., of medical or agricultural importance). Also, this approach is skewed toward more modern interests, and as some groups have ebbed and flowed in scientific focus over the past centuries, this approach may not capture historical interest. It also ignores books and monographs, although they are never as common as journal publications for any group. Finally, this approach will ignore work in journals not covered in Web of Science™, particularly many museum publications or those not written in English. However, I contend that this approach is a good place to start. From the data gathered, I wished to know three things. How much scientific effort have dytiscids received compared to other aquatic taxa? How much effort have dytiscids received compared to other aquatic beetles? How much effort have dytiscids received compared to other aquatic predators?

Dytiscids were found in 926 publications (Fig. 1.5), and with the more than 4200 species had a IE of 0.22 (note that this is about double the number of citations found in 2014 during the first edition of this book!). Larson et al. (2000) list about 500 citations in their work on Nearctic dytiscids, so the publications I found seem a reasonable comparison, as they also include citations back to the eighteenth century and included many published in non-English sources. Of those 926 publications, the majority are related to taxonomy or systematics, which should not be surprising, although it does speak to the general lack of knowledge in other areas (e.g., ecology). Of the 45 aquatic groups considered, their IE value placed them near the bottom (37th. dropping 3 places since 2014), between two less diverse aquatic beetle families (i.e., Georissidae and Gyrinidae) (Fig. 1.5). The only other common aquatic group below them are the Tipulidae (Order Diptera, "crane flies," 4256 species) at IE = 0.08. All the other groups lower in IE than dytiscids are aquatic beetles, many of low diversity (e.g., Ptilodactylidae with 24 species). Some of the higher diversity aquatic insect groups received much more effort. In fact, considering that dytiscids are one of the most speciose families of aquatic insects, they rate only about one publication per every five species, whereas mosquitoes have almost four publications per species (Family Culicidae, IE = 3.69). Other families, like mayflies (Order Ephemeroptera, IE = 1.39), midges (Family Chironomidae,

Fig. 1.5 (continued) aquatic beetles whereas white bars represent other aquatic insect taxa. The Dytiscidae is noted with an arrow

IE = 0.83), and the dragon/damselflies (Order Odonata, IE = 0.82) also have done much better (Fig. 1.5). Compared to other aquatic beetles, here too, Dytiscids had a much lower score, especially when one considers that they are the most diverse aquatic beetle family. Many of the groups with higher IE scores had relatively low number of species, with the highest IE score achieved by Meruidae ("comb-clawed cascade beetles") with only 1 species and 24 publications! Perhaps because of their high diversity other families of beetles did not fare very well overall, so dytiscids were not the exception. For instance, Hydrophilidae (second highest number of species at ~2800) had an IE = 0.19, and both Hydraenidae ("minute moss beetles," IE = 0.15) and Scirtidae ("marsh beetles," IE = 0.11) with over 1000 species each had IE numbers much lower (Fig. 1.5). Of these however only the Hydrophilidae have aquatic larvae and adults, a trait shared with dytiscids, and thus one could argue that they serve as the only true comparison in this regard. Finally, of the aquatic predatory groups, predaceous diving beetles were the lowest ranked, far behind dragon/damselflies, Nepidae (Order Hemiptera, "water scorpions"), Belostomatidae (Order Hemiptera, "giant water bugs"), and the Megaloptera ("dobsonflies" and allies). Thus, based on this examination, it seems clear, even given their high species diversity and prominence as aquatic predators, that dytiscids are neglected in the scientific literature. I attempted to confirm this examination of the scientific literature by searching for the order or family names in the search engine Google™ and recording the number of pages that were returned. This would essentially find how many times the taxa appeared in both academic and non-academic sites and may serve as a loose proxy for public interest. The numbers were very comparable to the academic publication search, with Dytiscidae returning 469,000 pages, compared to more popular taxa including Chironomidae (899,000 pages), Odonata (6,890,000), Culicidae (2,380,000), Ephemeroptera (1,060,000), and Plecoptera (722,000). Some major aquatic groups returned fewer pages than dytiscids (e.g., Ceratopogonidae (467,000)). In general, reference to other aquatic beetle families was found on fewer than 100,000 pages (e.g., Meruidae 27,500 pages). Thus, the lack of interest for dytiscids seems to extend to the general public as well.

1.3 Cultural Notes

There are many wonderful accounts of how insects have permeated into the folklore and mythology of many different societies, both past and present. The scarab beetle in ancient Egypt, the dragonfly in Japan, and the plagues of locust and flies to the Ancient Hebrews are perhaps primary examples of how insects have shaped many cultures worldwide. Oddly enough, there have been reports of plagues of predaceous diving beetle adults in Queensland, Australia (Prain 2011). The presence of dytiscids in myths is rare, although Powell (1900) does document a creation myth among the Cherokee. The mythology centers around the creation of the world, and a water beetle plays a prominent role. Powell writes,

> Beetles are classed together under a name which signifies “insects with shells.” The little water-beetle or mellow-bug (Dineutus discolor) is called Dâyuni’sï, “beaver’s grandmother,” and according to the genesis tradition it brought up the first earth from under the water…They [the animals] wondered what was below the water, and at last Dâyuni’sï…offered to go and see if it could learn. It darted in every direction over the surface of the water, but could find no firm place to rest. Then it dived to the bottom and came up with some soft mud, which began to grow and spread on every side until it became the island which we call the earth.

Many different insects have also worked their way into less dramatic and more common place positions, such as everyday western phrases (“Busy as a bee,” “Nit picking”) or popular culture (e.g., movies like “Them” 1950 and “The Fly,” 1958). Dytiscids have not, as of yet, played a major role in popular culture, but they have nonetheless been part of various cultures and do occasionally make their way into our everyday lives. Although this is not an exhaustive description of their cultural significance, it provides an introduction.

In an interesting (if not bizarre) cultural connection, several different insect types, including dytiscids, are used by east African girls to stimulate breast development (Kutalek and Kassa 2005). This practice has a long history and appears widespread in rural Ethiopia, Tanzania, and Uganda. Specifically, girls collect the beetles, known as *yewha inat* or “mother of water” in Tanzania (e.g., *Rhantus capensis, Hydaticus jeanneli*) from local aquatic habitats during daily chores and are then placed against the girl’s nipple until they bite. Upon biting, they also release defensive compounds from prothoracic and pygidial glands (Chap. 6). After several days the breast is said to be slightly swollen and the year or so after this event the breasts are reported to grow larger. The purpose of this to allow prepubescent girls the chance to feel more adult, however, there is no scientific evidence that this practice delivers the desired results. Oddly enough, young boys in the Njnombe region use the beetles in the exact same way, although they do so to reduce breast growth that may occur during puberty prior to an increase in testosterone levels. In other areas, such as Zimbabwe, boys let the beetles bite their tongues so they may learn to whistle. It appears then that these beetles do not discriminate in helping each sex get an advantage over the other.

Moving away from the warm regions of Africa, dytiscids, like most insects, are not abundant in polar or near-polar regions, however, several species do exist in seasonally high numbers in Greenland. These include *Hydroporus morio* and *Colymbetes dolabratus*. The adults and larvae are active during the brief summer and often feed on chironomids and other small invertebrates. Perhaps because of their prevalence in the relatively barren aquatic systems in Greenland, they do seem to have made their way into local folklore. Böcher (1988), citing older sources, reports that native Greenlanders were afraid of *C. dolabratus* specifically, whom they referred to as either “Pamiortooq”(larvae) and “Minngoq” (adults). Their fear lay in being injured when the beetles would attack and destroy their bowels after accidentally drinking them in water from local sources. To combat this, locals would introduce amphipods (i.e., *Gammarus locusta*) into “infested” waters, where upon a war between these arthropods would result and would end in the eventual destruction

Fig. 1.6 A veritable dytiscid feast. Fried *Cybister japonicus* (now *C. chinensis*), served in a Cantonese restaurant in China, garnished with parsley and orchid flower. Photo courtesy of Manfred Jäch 2003

of both (Böcher 1988). This folklore still remains in Greenland today (L. Culler, personal communication), although it seems to be more about getting bitten when swimming in waters with dytiscids rather than having one's bowels destroyed.

Although Greenlanders are wary of accidentally consuming dytiscids, there are many reports of dytiscids as food for direct human consumption. This entomophagy (dytisciphagy?) is especially prominent in southeast Asia, including China (Jäch and Easton 1998), New Guinea (Gressitt and Hornabrook 1977), and Thailand (Chen et al. 1998). Hoffman reported on dytiscids (*Cybister* sp.) and hydrophilids being sold and consumed in Canton (now Guangzhou), China. He states,

> Beetles of these two families are very commonly eaten in Kwangtung Province and in other places where Cantonese dwell. Although usually kept in separate containers customers very frequently buy some of each family. They care less for the hydrophilids and consequently they are cheaper than the dytiscids...The elytra, legs, and certain other chitinous parts are discarded when eating.

Half a century later, Jäch and Easton (1998) and Jäch (2003) published similar accounts of the practice and Jäch (2003) specifically provides a firsthand account of eating *Cybister japonicus* (now *C. chinensis*) in a local restaurant (Fig. 1.6),

> This species turned out to be rather tasteless, except for the flavor of garlic and other spicy ingredients that had been added. In contrast to *Hydrophilus* [Hydrophilidae], the chitinous structures are not soft, but more or less as prickly as in living specimens, and the abdomen does not contain notable quantities of soft tissue. I tried to eat one specimen, and although I had partly swallowed it, I felt myself forced to spit the majority on the table in front of me (which is not regarded as rude behavior in China, where table manners are quite different from those in the West).

He also comments that eating these aquatic beetles is based on tradition and not economics, as this area of China is quite prosperous, and most of the beetles are reared locally and not wild caught. He ends by noting that the popularity of eating aquatic beetles is waning, perhaps as China continues to modernize. Several sources suggest that the goal of consumption may not be just for nutrition, as the beetle also is seen as having anti-diuretic attributes and thus is perceived to have medicinal value. Other cultures also use dytiscids (e.g., *Cybister tripunctatus*) in traditional medicine (e.g., African cultures, Kutalek and Kassa 2005).

Ingestion of dytiscids by humans is much older, even outside of southeast Asia. Roust (1967) reports the findings of examinations of 186 human fecal droppings (coprolites) from caves in the desert southwest in the USA. The specimens were assumed to be prehistoric based on several lines of evidence, and although there appears to have been no radiocarbon dating conducted on the samples, other artifacts collected in the caves by others seem to confirm the antiquity of this site (e.g., Heizer and Krieger 1956). Besides an abundance of plant material, the remains contained fish bones, mammal teeth, and bird feathers and egg shell, and also included insect parts. Specifically the remains of a large dytiscid. Roust writes,

> . . . undigested remains of the predaceous water beetle Cybister explanatus found in seven (9.46%) of the specimens. Of interest is the fact that no heads of any of these beetles were found, indicating that they were either bitten or torn off prior to ingestion, without chewing, of the whole beetle.

This is not the only account of ingestion of these large aquatic beetles in the Americas. In the past, ancient cultures in area of present day Mexico also consumed aquatic beetles, including *Cybister*, which was termed “Atopinan” and described as, “*a marsh grasshopper of a dark colour and great size, six inches long and two broad (!)*” (Smith 1807). Clearly, the size of this animal is a gross exaggeration, although consumption of these beetles is not! In their review of the caloric content of almost 100 insects consumed in Mexico, Ramos-Elorduy and Pino (1989) cite earlier works regarding the use of *Cybister* (as known as *cucarachas de agua*, “water roaches!”) as food, specifically their consumption by being eaten roasted with salt or as an ingredient in tacos. These authors list that larvae, pupae, and adults are consumed. They further report that *Rantus* (*Rhantus*) sp. adults contain 4015.0 kcal/1000 g, a number comparable to many other beetles examined and much higher than many grains tested (e.g., corn 3640 kcal/1000 g) or other animals (e.g., chicken 1646 kcal/1000 g or cod 3888 kcal/1000 g) (Ramos-Elorduy and Pino 1989). The consumption of predaceous diving beetle adults need not be limited to those areas with a tradition of consuming them, as there are companies that provide them for sale all over the world (Fig. 1.7). The practice of the prehistoric North Americans in removing the heads seems to be another case of ancient wisdom, as even the commercial producers of dytiscids suggest removing the head before consumption.

Although dytiscids are merely viewed as food by some cultures, in other locations, dytiscids are kept as pets. Specifically, the tradition of keeping insects, including dytiscids, exists in Japan (S. Ohba personal communication, Fig. 1.8) and Hong Kong (Jäch and Easton 1998), and based on some accounts were also kept

Fig. 1.7 Canned predaceous diving beetle adults sold for human consumption. Each of these 15 g cans sells for about $6.00 U.S. plus shipping, and as the label indicates, they are cooked and dehydrated and then dusted with barbeque sauce. The instructions indicate to remove the "outer wings" and that everything except the head can be consumed. Photo courtesy of Thailandunique, 2013

in many parts of Europe (i.e., *Cybister*, Wesenberg-Lund 1943 reported in Balke et al. 2004). In Japan, specifically the practice of keeping insects as pets, especially beetles, is long-standing, and various methods exist for purchasing insects, including vending machines (Kawahara and Pyle 2013). The large beetles that are often at the center of this pet trade are held in high esteem, and an entire industry has blossomed around keeping them as pets, including companies that specialize in producing rearing materials, cages, and other accessories for the discerning beetle owner. Related to their use as entertainment, there is an account by Pemberton (1990) who describes the use of large dytiscids in a game of chance. The game requires some people willing to wager a small amount of money, an oval metal tank of water, some prizes, and a live adult *Cybister chinensis*, a species found throughout the region. The game is called *mul bag gae nori*, or the "water beetle game," for reasons that will become obvious. The game is similar to roulette, but instead of a ball that randomly lands within slots along the spinning wheel, here an adult *Cybister chinensis* is allowed to swim and come to rest in one of many vertical flanges that are positioned slightly above the 3–4 cm water level. If the beetle enters or touches a slot, then the player wins the corresponding prize (if any) placed along the outer edges of the tank. The prizes are often of low cost (e.g., small toys, candy) but so is the cost to play. On a related note, Pemberton (1990) also mentions that *mul bang gae* ("water beetle") is also a nickname used for a fat man, likely owing to a similarity to the beetle's round shape.

Fig. 1.8 The interior of a store in Osaka, Japan that specializes in selling insect husbandry supplies and live insects as pets. Such stores are common throughout Japan and often offer a wide range of Coleoptera, including dytiscids for sale. Photo courtesy of Hideyuki Suzuki, 2013

1.4 Final Words

I hope that the readers of this book will find it a comprehensive overview of this ubiquitous and amazing family of aquatic predators. The authors of these chapters have more than a hundred years of combined publishing experience with this family, a fact that hopefully comes out in the comprehensive and thoughtful presentations you will find in the following pages. In each chapter you will find Future Directions that should serve as a starting point for new and less traveled avenues of research. I would add my own suggestion as well, specifically that those who study insects in aquatic systems in particular should take the time to identify and catalog these insects in their community studies. I have met several researchers at scientific meetings who simply ignore them or “lump” species of dytiscids into higher taxonomic groups. Their reasons are varied, but often hinge on a frustration with identification or a general lack of knowledge compared to other aquatic groups (e.g., Odonata). Given the dearth of species-level keys for most dytiscid larvae this is not surprising (Chap. 2), but this should instead be a call to action in producing more keys. In his Forward to this book, Anders Nilsson suggests that the future of taxonomy will likely be focused on molecular approaches, which, if it reaches fruition, should provide a boon to work with larval dytiscids. It is my hope that the book you now have before you will help to mitigate this deficiency and spur interest and new research on this fascinating group of insects.

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Donald A. Yee earned his M.S. in Zoology from Texas Tech University and his Ph.D. in Biological Sciences from Illinois State University. His research mostly focuses on the ecology of container mosquitoes, although for his postdoctoral appointment, he worked on the ecology of predaceous diving beetles in Alberta, Canada. He now works on the ecology of both groups and is particularly interested in how dytiscids affect populations of mosquitoes and other prey in natural wetlands.

Chapter 2

Larval Chaetotaxy of World Dytiscidae (Coleoptera: Adephaga) and Implications for the Study of Hydradephaga

Yves Alarie and Mariano C. Michat

Abstract Although the Dytiscidae (Coleoptera) are among the most common insect inhabitants of freshwaters, knowledge of their larval morphology is scanty throughout the World. The identification of larvae is a continuing problem because the literature available to accomplish this is scattered, limited to certain groups, outdated, difficult to use or non-existent. Recent studies have demonstrated the taxonomic and phylogenetic value of chaetotaxy in studying larval Dytiscidae. The study of body sensilla (setae and pores) was shown to be useful and important both for diagnosis and study of phylogenetic relationships among taxa. The fact that all these studies were conducted separately over a period of more or less 30 years, however, does not facilitate comparison among taxa. This chapter synthesizes these studies into a more comprehensive approach, which should facilitate comparison among the dytiscid subfamilies. Although this framework is useful for the study of larval morphology of the Dytiscidae, it has also recently contributed to the study of larvae of other families of Hydradephaga, namely Aspidytidae, Gyrinidae, Haliplidae, Hygrobiidae, Meruidae and Noteridae. A corollary objective of this chapter therefore is to illustrate the power of larval chaetotaxy for testing hypotheses of phylogenetic relationships of Hydradephaga.

Keywords Larval morphology · Chaetotaxy · Meruidae · Aspidytidae · Gyrinidae · Haliplidae · Hygrobiidae · Noteridae · Dytiscidae

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Y. Alarie (✉)
Department of Biology, Laurentian University, Sudbury, ON, Canada
e-mail: yalarie@laurentian.ca

M. C. Michat
CONICET, Laboratory of Entomology, DBBE, FCEyN, UBA, Buenos Aires, Argentina

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2.1 Introduction

Coleoptera is the largest order of the Kingdom Animalia, comprising a quarter of all known animal species (Gullan and Cranston 2010). The order is represented in almost every non-marine habitat on Earth. It includes many of the most beneficial and destructive insects known, yet an enormous amount of basic taxonomy and biological study is necessary to raise our understanding of this group to the level attained in most other insect orders. The current state of coleopteran taxonomy is uneven in several ways, with many large geographical, developmental and taxonomic gaps (Stehr 1991).

While the state of knowledge of adult beetle taxonomy varies widely across taxa, our knowledge of coleopteran larvae is generally poor. Most beetle larvae are unidentifiable to species, even though the larval stage typically lasts longer than the adult stage and often has the greatest impacts on humans and the environment. As Holometabola, beetle larvae are under differing selection pressures compared to adults and as such show quite different morphological features. As a different expression of the same genotype, each larval instar represents an ontogenetic stage with its own characters, each being important in determining taxa, reconstructing phylogenies, and building classifications.

With over 4600 described species (Nilsson and Hájek 2022), the beetle family Dytiscidae represents one of the largest and most commonly encountered groups of aquatic insects. Up until recently, however, the identification of their larvae was a regular and continuing problem for many because the literature available to accomplish this was widely scattered, limited to certain groups, outdated, difficult to use, or non-existent (Larson et al. 2000). Moreover, larval descriptions were usually lacking or, where present, inadequate because of lack of comparative precision and detail. In part because of this, and also to develop a system useful for phylogenetic analysis, a system of nomenclature of larval chaetotaxy was devised for most Dytiscidae subfamilies but the Hydrodytinae: Agabinae (Alarie 1995, 1998; Alarie et al. 2019; Hájek et al. 2019; Okada et al. 2019; Alarie and Michat 2020), Colymbetinae (Alarie 1995, 1998; Michat 2005; Alarie and Hughes 2006; Alarie et al. 2009), Copelatinae (Michat and Torres 2009), Coptotominae (Michat and Alarie 2013), Dytiscinae (Miller et al. 2007; Alarie et al. 2011a; Michat et al. 2015, 2019), Hydroporinae (Alarie et al. 1990; Alarie and Harper 1990; Alarie 1991; Alarie and Michat 2007a), Laccophilinae (Alarie et al. 2000, 2002b; Michat and Toledo 2015), Lancetinae (Alarie et al. 2002a), and Matinae (Alarie et al. 2001). The fact that all these studies were conducted separately over a period of more or less 30 years does not facilitate comparison among taxa. The main purpose of this chapter therefore is to synthesize these studies into a more comprehensive approach, which should facilitate comparison among the dytiscid subfamilies. Whereas such framework was particularly useful in studies of larval morphology of the Dytiscidae, it has also contributed more recently towards the reconstruction of the larval ground plan of other Hydradephaga families, namely Aspidytidae (Alarie and Bilton 2005), Gyrinidae (Archangelsky and Michat 2007; Michat et al. 2010, 2016, 2017b; Michat and

Gustafson 2016; Colpani et al. 2018, 2020), Haliplidae (Michat et al. 2020), Hygrobiidae (Alarie et al. 2004), Meruidae (Alarie et al. 2011b), and Noteridae (Urcola et al. 2019, 2019a, b, 2020, 2021). A corollary objective of this chapter therefore is to illustrate the power of larval chaetotaxy as a tool for testing hypotheses of phylogenetic relationships of the Hydradephaga families by comparing in particular the generalized leg chaetotaxy pattern derived from that of the Dytiscidae.

2.2 General Morphology of Dytiscidae Larvae

Like other Adephaga, dytiscid larvae are campodeiform with a strongly sclerotized head capsule and prognathous mouthparts. The body is variously shaped, usually elongate and fusiform, generally widest at level of metathorax or middle abdomen (Figs. 2.1a–c, 2.2a–c and 2.3a–l). The dorsal surface of the body is usually distinctly sclerotized, whereas the ventral surface is mostly membranous with sclerotized plates, if present, restricted to the most posterior segments. Sclerites are usually

Fig. 2.1 Dorsal habitus of selected Dytiscidae: (**a**) *Agabus*/*Ilybius* sp.; (**b**) *Cybister fimbriolatus* (Say, 1823); (**c**) *Dytiscus* sp. Courtesy of Dr. Steve Marshall, University of Guelph, ON, Canada

Fig. 2.2 Dorsal habitus of selected Dytiscidae: (**a**) *Hydrovatus pustulatus* (E.F. Melsheimer, 1844); (**b**) *Laccophilus* sp.; (**c**) *Neoporus undulatus* (Say, 1823). Courtesy of Dr. Steve Marshall, University of Guelph, ON, Canada

more pigmented than the rest of the body. Colour patterns occur on the head capsule and terga of most taxa.

The head capsule is strongly sclerotized and variable in shape (triangular, subquadrate, subrectangular, subtrapezoidal, rounded or pyriform (Fig. 2.4a–f). It is divided above by a Y-shaped epicranial suture, which delimits a frontoclypeal region and two lateral epicranial plates (= parietals). An occipital suture may be present, which crosses the back of the head capsule, intersecting the stem of the epicranial suture (Fig. 2.4a). The anterior margin of the frontoclypeus is usually moderately arcuate, but in some groups (e.g., the Hydroporinae) it extends anteriorly, forming a median projecting lobe called the nasale (Fig. 2.4e and f). The first instar of most taxa possesses a pair of spine-like tubercules or egg-bursters (*ruptor ovi* of Bertrand (1972)), usually located on the posterior half of the frontoclypeus (Fig. 2.4a, c and e). Each parietal bears an antennal fossa and six stemmata (absent in subterranean taxa). The antennae are elongated and are comprised of four antennomeres (Fig. 2.5a–d). The antennomere III apically bears a sensory process, which may be short and non-apparent (Fig. 2.5b) or elongate, sometimes as long as the antennomere IV (Fig. 2.5d). The mandibles are well developed, narrow and falcate and in most taxa are grooved mesally as an adaptation for a liquid mode of

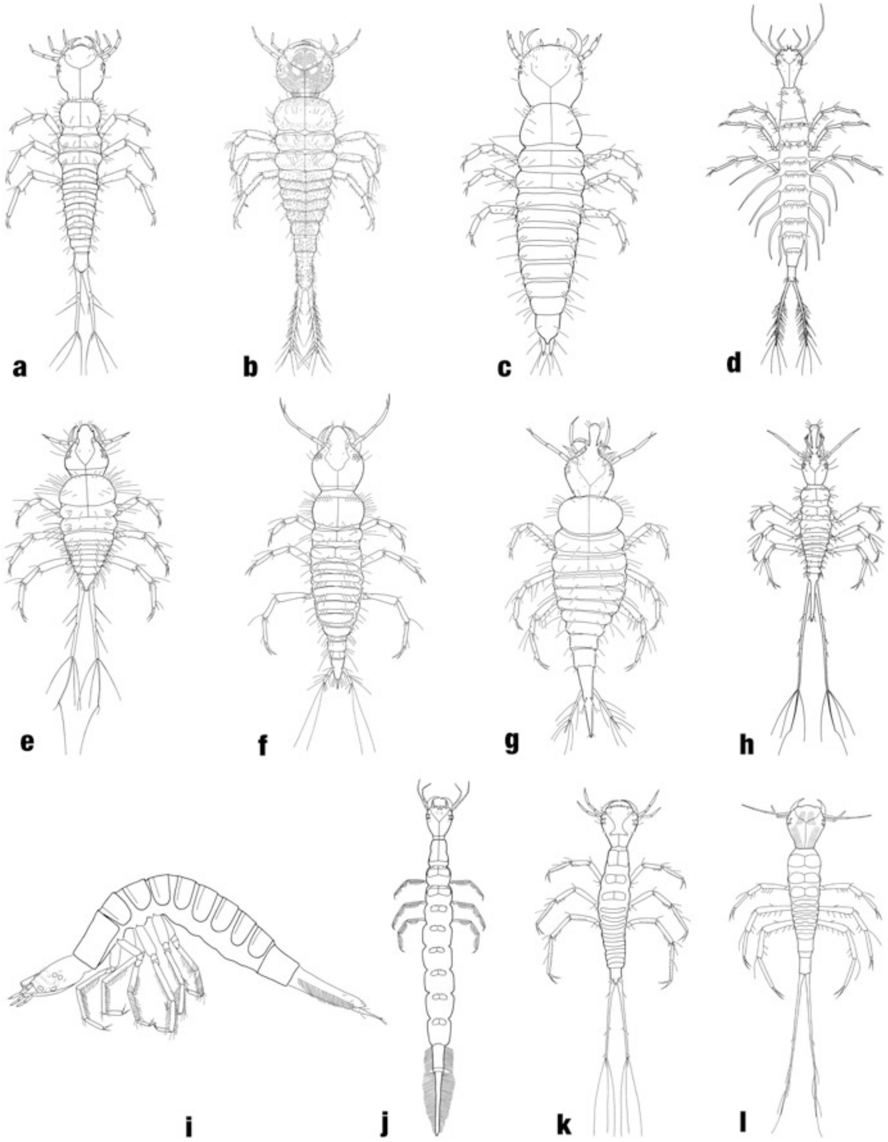

Fig. 2.3 First instars of selected species of Dytiscidae, dorsal view: (**a**) *Platynectes curtulus* (Régimbart, 1899); (**b**) *Bunites distigma* (Brullé, 1838); (**c**) *Copelatus longicornis* Sharp, 1882; (**d**) *Coptotomus longulus lenticus* Hilsenhoff, 1980; (**e**) *Amarodytes duponti* (Aubé, 1838); (**f**) *Celina parallela* (Babington, 1842); (**g**) *Pachydrus obesus* Sharp, 1882; (**h**) *Derovatellus lentus* (Wehncke, 1876); (**i**) *Thermonectus succinctus* (Aubé, 1838); (**j**) *Megadytes glaucus* (Brullé, 1838); (**k**) *Laccophilus obliquatus* Regimbart, 1899; (**l**) *Lancetes marginatus* (Steinheil, 1869)

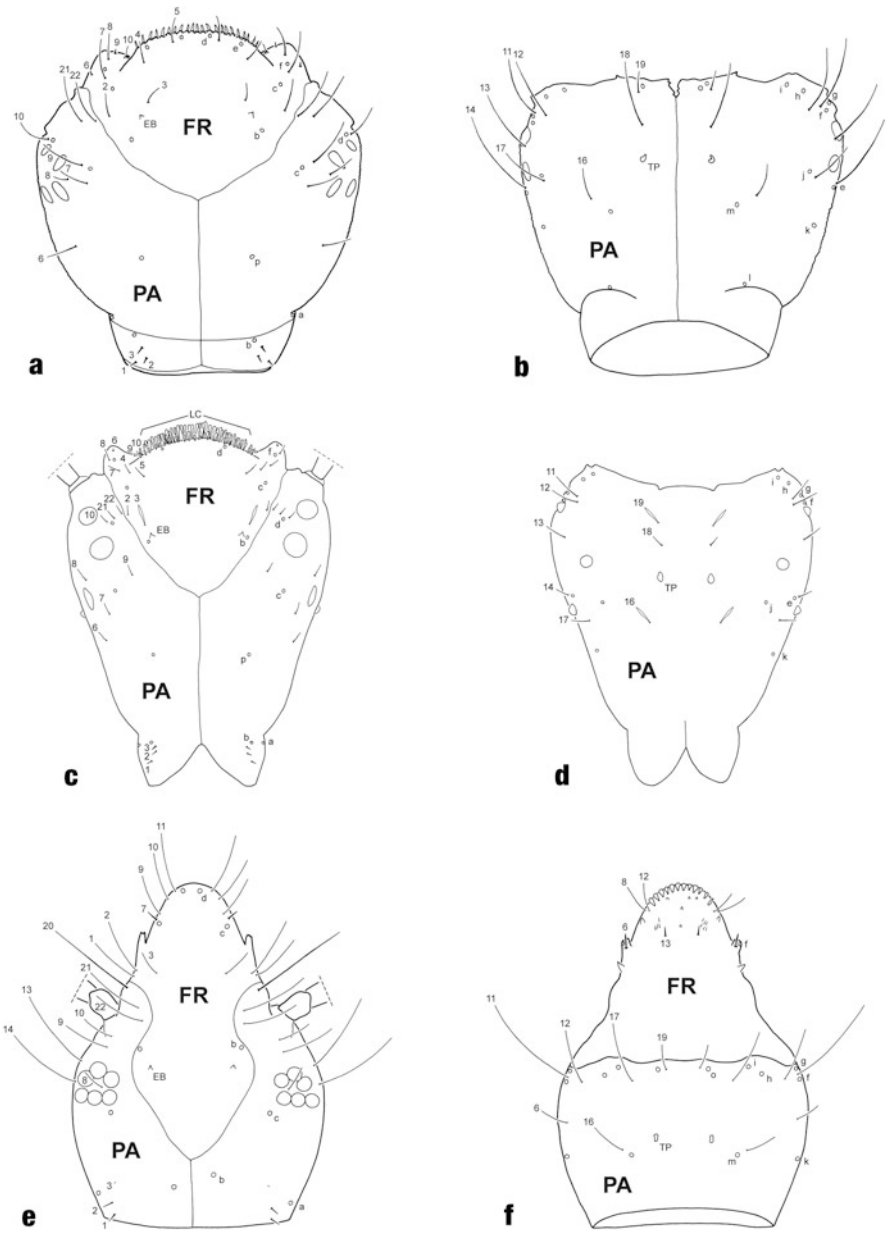

Fig. 2.4 Distribution of ancestral setae and pores on the cephalic capsule of first instars of selected species of Dytiscidae: (**a–b**) *Rhantus calileguai* Trémouilles, 1984, (**a**) dorsal surface, (**b**) ventral surface; (**c–d**) *Acilius semisulcatus* Aubé, 1838, (**c**) dorsal surface, (**d**) ventral surface; (**e–f**) *Anodocheilus maculatus* Babington, 1842, (**e**) dorsal surface, (**f**) ventral surface. *EB* egg burster, *FR* frontoclypeus; *LC* lamellae clypeales, *PA* parietale, *TP* tentorial pit; numbers and lowercase letters refer to primary setae and pores, respectively (see Table 2.1 for list of setae and pores)

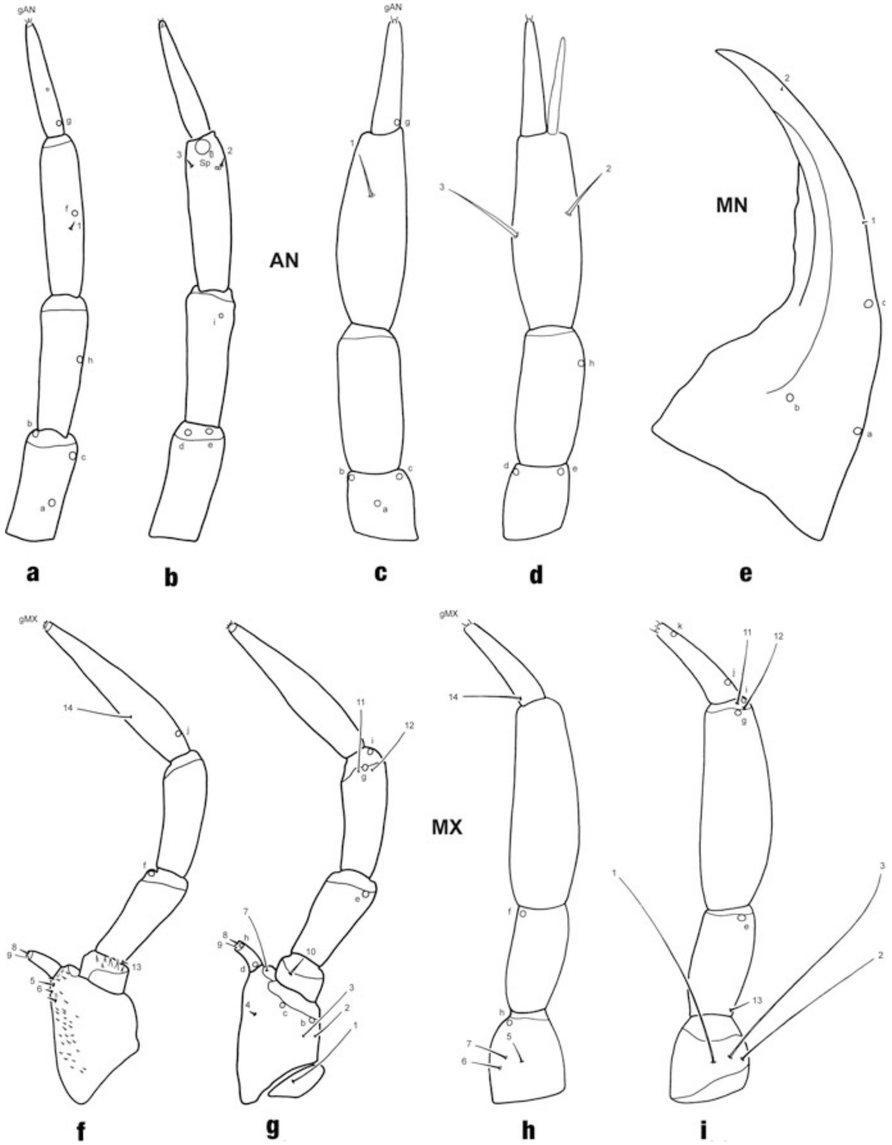

Fig. 2.5 Distribution of ancestral setae and pores on the head appendages of first instars of selected species of Dytiscidae: (**a–b**) *Platynectes curtulus* (Régimbart, 1899), (**a**) right antenna, dorsal surface, (**b**) left antenna, ventral surface; (**c–d**) *Liodessus flavofasciatus* (Steinheil, 1869), (**c**) right antenna, dorsal surface, (**d**) left antenna, ventral surface; (**e**) *Platynectes curtulus*, right mandible, dorsal surface; (**f–g**) *Platynectes curtulus*, (**f**) right maxilla, dorsal surface, (**g**) left maxilla, ventral surface; (**h–i**) *Liodessus flavofasciatus*, (**h**) right maxilla, dorsal surface, (**i**) left maxilla, ventral surface. *AN* antenna, *MN* mandible, *MX* maxilla, *Sp* spinula; numbers and lower-case letters refer to primary setae and pores, respectively (see Table 2.2 for list of setae and pores)

feeding (Fig. 2.5e). The maxilla usually consists of a small basal cardo, a larger stipes, a palp of three palpomeres borne on a palpifer, and a palpiform galea (Figs. 2.5f and g). The galea is reduced or lacking among the Hydroporinae (Fig. 2.5h and i) and Cybistrini. In some hydroporine larvae, the cardo is fused to the stipes (Fig. 2.5i). There has been considerable confusion about the number of maxillary palpomeres, the basic number of segments being three. However, the palpifer may appear to be a basal palpomere, and some Dytiscinae larvae have a secondary segmentation, which increases the apparent number of palpomeres (Fig. 2.6d). Finally, the labium consists of three major parts; the basal postmentum, the apical prementum (sometimes called the mentum), and a pair of labial palps attached to the prementum by a small palpiger (Fig. 2.6e and f). Basically, the labial palp is composed of two palpomeres except for some Hydroporinae (e.g., *Vatellus* Sharp, *Paroster* Sharp), which have one and three, respectively.

The thorax consists of three segments, the pro-, meso- and metathorax, each of which bears a pair of articulated legs (Figs. 2.1a–c, 2.2a–c and 2.3a–l). Each segment has a large tergite and, in most specimens, a pair of smaller laterotergites associated with each leg attachment. Each tergum is usually divided at the midline by a narrow ecdysal suture (e.g., Fig. 2.3a). The ventral region of the thorax is membranous except for a small sclerotized plate or presternum on the prothorax of some groups. One pair of spiracles is usually present on the lateral margin of the mesothorax in the third instar larva. The legs are usually long and slender, the prothoracic legs shortest, the meso- and metathoracic pairs progressively longer and are 6-segmented (sensu Lawrence 1991). The coxa and femur are the longest, and the trochanter is the smallest segment (Fig. 2.7a and b). The tarsal claws are usually unequal in length.

The abdomen is subcylindrical and consists of eight visible segments; segments 1–7 more or less similar in form, segment 8 variously modified for respiration (Figs. 2.1a–c, 2.2a–c and 2.3a–l). Each segment has the dorsum consisting of a large median plate, which extends laterally slightly over the pleura. The tergal plate of segment 8 is usually extending posteriorly well beyond the origin of the urogomphi forming a prolongation of the segment called the siphon (e.g., Fig. 2.3e–h). The ventral surface of the abdominal segments is variously sclerotized. Eight pairs of spiracles are present on the abdomen. The first seven pairs, which are present only in the third instar, are usually located laterodorsally on, or proximad to, the tergal plates. The spiracles on segment 8 are borne dorsally at the apex of the siphon, these being the only spiracles functional throughout the larval stage. The abdominal segment 8 bears a pair of terminal (or subterminal) articulated urogomphi (Figs. 2.1a–c, 2.2a–c and 2.3a–l). These consist of one (e.g., Fig. 2.3a and b) or two (e.g., Fig. 2.3e–h) segments.

2.3 Chaetotaxy Analysis: Methodological Approach

The term ‘chaetotaxy’ is derived from two Greek words: ‘chatite’ = long hairs; and ‘taxis’ = arrangement (Gordh and Headrick 2001) and refers to the arrangement, nomenclature or classification of setae distributed over the insect body (Nichols and Schuh 1989). As pointed out by Solodovnikov (2007), however, in the literature on beetle larvae, which considers chaetotaxy in sufficient detail (e.g., Thomas 1957; Ashe and Watrous 1984; Bousquet and Goulet 1984; Wheeler 1990; Lawrence 1991; Kovarik and Passoa 1993; Makarov 1996; Kilian 1998), the system of characters known as ‘larval chaetotaxy’ is sometimes understood more broadly to include a number of other structures such as cuticular extensions (e.g., microtrichia, setiferous tubercles, scales, spines). As defined in the context of this chapter, however, chaetotaxy is understood more narrowly as a system of setae and pores (sensilla placodea).

The larval chaetotaxy system of the Dytiscidae developed over the past 30 years is a complex of setae and pores demonstrating some patterns in their distribution, similar to the analogous systems of designations originally described for the Carabidae (Bousquet and Goulet 1984). All these systems are based on comparative examination of a certain sample of taxa for evaluating stable versus variable elements of chaetotaxy, finding homologous structures among them, and providing those with a system of designations. Hypotheses of homology were based mainly on the criterion of similarity in position (Wiley 1981) dealing with subsets (i.e., subfamilies). This was based mainly on the assumption that, at lower taxonomic levels, it is possible to determine homology with great precision using stable subpatterns of sensilla distribution.

The value of the nomenclatural system of chaetotaxy that was derived for the Dytiscidae is enhanced because it differentiates the primary setae and pores (found in the first instar) from the secondary ones, which are added in later two instars. There is an overall primary pattern, which is widespread among taxa, though it is modified in a variety of groups. This generalized pattern is consistent enough to be used for phylogenetic analysis and yet sufficiently variable to allow for taxonomic distinction. In addition to this, secondary setae and pores added through the ontogenetic development of the larva often show specific variations in number, position and size that may also serve taxonomic and phylogenetic purposes.

The notation of primary setae and pores of larval Dytiscidae presented in this chapter was based on the study of the first instars of selected taxa belonging to different tribes and genera. Larvae of other adephagan families were also examined for any significant differences in distribution of primary setae and pores within this group of taxa to ensure that the ground plan pattern developed could be extrapolated to related taxa. Descriptions of larval structures were based on specimens cleared either in 10% KOH or lactic acid and mounted on standard glass slides with either Euparal or Hoyer’s medium. Microscopic examination at magnifications of 40–1000X was done using an Olympus BX50 compound microscope equipped with Nomarsky differential interference optics. In these systems, each seta is coded by two capital letters corresponding to the first two letters of the name of the structure on which it is located (e.g., AB, last abdominal segment; AN, antenna; CO, coxa; FR, frontoclypeus; LA, labium) and a number. Pores are coded in a similar manner, except that the number is replaced by a lower case letter.

In the larval chaetotaxy systems proposed for the Dytiscidae, the primary setae and pores were subdivided into two categories: **ancestral**, i.e., those associated with the ancestral pattern (recognized and homologized in most or all of examined taxa), and **additional**, i.e., those evolved secondarily in the first instar (generally restricted to a genus or tribe). Only the setae and pores associated with the ancestral pattern were coded here.

2.4 Ground Plan Pattern of Primary Setae and Pores of the Dytiscidae

Analyses of the primary setae and pores of larval structures such as the head capsule, head appendages, legs, last abdominal segment, and urogomphus have been provided for all dytiscid subfamilies but the Hydrodytinae (c.f., references above). Primary setae and pores are generally easily recognized for most species owing to their similar distribution pattern on the body parts. For some species, however, the homology of some setae and pores may be difficult owing to (1) the presence of additional setae and (or) pores, which could confuse their identification, (2) loss of setae and (or) pores, which disrupts the distribution pattern, and (3) the drastic change of position of setae and (or) pores caused in general by an important modification of the sclerite (e.g., the elongation of the frontoclypeus of the Hydroporinae into a nasale or the variability of the relative elongation of the last abdominal segment into a siphon). The system of primary setae and pores, as defined below for the family Dytiscidae, has a great potential as a source of significant systematic data. The vast number of coded setae (137) and pores (70) and their associated states provide a complex pattern of modification useful at recognizing taxa, at reconstructing phylogeny and at building classification. The characterization of the ground plan pattern of primary setae and pores on selected structures of the Dytiscidae is based on a reconstructed, or generalized, species bearing all primary setae and pores.

2.4.1 Cephalic Capsule

Fifty-two sensilla (32 setae and 20 pores) are coded on the cephalic capsule of the Dytiscidae. These sensilla are illustrated in Fig. 2.4a–f, and they are listed in Table 2.1.

Table 2.1 Ancestral setae and pores on the head capsule of first instars of Dytiscidae subfamilies: *AGA* Agabinae, *COL* Colymbetinae, *CPL* Copelatinae, *COP* Coptotominae, *DYT* Dytiscinae, *HYD* Hydroporinae, *LAC* Laccophilinae, *LAN* Lancetinae, *MAT* Matinae

Setae/pores	AGA	COL	CPL	COP	DYT	HYD	LAC	LAN	MAT
FR1	0	0	0	0	0	1	0	0	0
FR2	1	1	1	1	1	1	1	1	1
FR3	1	1	1	1	1	1	1	1	1
FR4	1	1	1	1	1	0	1	1	1
FR5	1	1	1	1	1	0	1	1	1
FR6	1	1	1	1	1	1	1	1	1
FR7	1	1	1	1	1	1	1	1	1
FR8	1	1	1	1	1	1	1	1	1
FR9	1	1	1	1	1	1	1	1	1
FR10	1	1	1	1	1	1	1	1	1
FR11	0	0	0	0	0	1	0	0	0
FR12	0	0	0	0	0	1	0	0	0
FR13	0	0	0	0	0	0/1	0	0	0
FRb	1	1	1	1	1	0/1	1	1	1
FRc	1	1	1	1	1	1	1	1	1
FRd	1	1	1	1	1	1	1	1	1
FRe	0	1	0	0	0/1	0	0/1	1	0
FRf	1	1	1	1	0/1	1	1	1	1
PA1	1	1	1	1	1	1	1	1	1
PA2	1	1	1	1	1	1	1	1	1
PA3	1	1	1	1	1	1	1	1	1
PA6	1	1	1	1	1	1	1	1	1
PA7	1	1	1	1	1	0	1	1	1
PA8	1	1	1	1	1	1	1	1	1
PA9	1	1	1	1	1	1	1	1	1
PA10	1	1	1	1	1	1	1	1	1
PA11	1	1	1	1	1	1	1	1	1
PA12	1	1	1	1	1	1	1	1	1
PA13	1	1	1	1	1	1	1	1	1
PA14	1	1	1	1	1	1	1	1	1
PA16	1	1	1	1	1	1	1	1	1
PA17	1	1	1	1	1	1	1	1	1
PA18	1	1	1	1	1	0	1	1	1
PA19	1	1	1	1	1	1	1	1	1
PA20	0	0	0	0	0	1	0	0	0
PA21	1	1	1	1	1	1	1	1	1
PA22	1	1	1	1	1	1	1	1	1
PAa	1	1	1	1	1	1	1	1	1
PAb	1	1	1	1	1	1	1	1	1
PAc	1	1	1	1	1	1	0/1	1	1
PAd	1	1	1	1	1	0/1	1	1	1

(continued)

Table 2.1 (continued)

Setae/pores	AGA	COL	CPL	COP	DYT	HYD	LAC	LAN	MAT
PAe	1	1	1	1	1	0/1	1	1	1
PAf	1	1	1	1	1	1	1	1	1
PAg	1	1	1	1	1	1	1	1	1
PAh	1	1	1	1	1	1	1	1	1
PAi	1	1	1	1	1	1	1	1	1
PAj	1	1	1	1	1	0/1	1	1	1
PAk	1	1	1	1	1	1	1	1	1
PAl	1	1	1	1	0/1	0	1	1	1
PAm	1	1	1	1	0/1	1	1	1	1
PAo	1	1	1	1	0/1	1	1	1	1
PAp	1	1	1	1	1	0	1	1	1

FR frontale, *PA* parietale, 0 = absent; 1 = present

Frontoclypeus Thirteen setae (FR1, FR2, FR3, FR4, FR5, FR6, FR7, FR8, FR9, FR10, FR11, FR12, FR13) and five pores (FRb, FRc, FRd, FRe, FRf) compose the basal number of primary sensilla on the frontoclypeus. Except for setae FR1, FR11, FR12 and FR13, which are restricted to the subfamily Hydroporinae (Fig. 2.4e and f), pore FRe, which is only found in the Colymbetinae (Fig. 2.4a), Lancetinae, and some Dytiscinae (*Dytiscus* L., 1758 and *Hyderodes* Hope, 1838) and Laccophilinae (*Neptosternus* Sharp, 1882), and setae FR4 and FR5, which are lacking in the Hydroporinae, all other setae (FR2, FR3, FR4, FR6, FR7, FR8, FR9, FR10) and pores (FRb, FRc, FRd, FRf) are generalized within the Dytiscidae with few exceptions (members of *Notaticus* Zimmermann, 1928 and *Eretes* Laporte, 1833 (Dytiscinae), *Laccornis* Gozis, 1914 (Hydroporinae) and Hyphydrini (Hydroporinae) are the only dytiscids where (1) pore FRf, (2) seta FR13, and (3) pore FRb are lacking, respectively). It is worth noting that the ventroapical margin of the frontoclypeus is also characterized by the presence of a row of typical sensilla [*lamellae clypeales* of Bertrand (1972)] (Fig. 2.4a, c and f). These sensilla have not been included in the ground plan pattern of the frontoclypeus owing to their great variability (both in number and shape).

Parietale 19 setae and 15 pores form the ancestral system of the parietale. The basal half of the sclerite bears five setae (PA1, PA2, PA3, PA6, PA7) and four pores dorsally (PAa, PAb, PAc, PAp), and three setae (PA14, PA16, PA17) and five pores (PAe, PAj, PAk, PAl, PAm) ventrally. The distal portion of the parietale bears six setae (PA8, PA9, PA10, PA20, PA21, PA22) and one pore (PAd) dorsally, and five setae (PA11, PA12, PA13, PA18, PA19) and five pores (PAf, PAg, PAh, PAi, PAo) ventrally. The primary sensilla found on this portion of the head capsule show an extremely consistent pattern within the Dytiscidae except for setae PA6 and PA18, and pores PAl and PAp, which are lacking within the Hydroporinae (Fig. 2.4e and f). Pores PAm, PAo and PAl are also lacking in some genera of the dytiscine tribe

Aciliini (Fig. 2.4f). Hydroporine larvae are also the only dytiscid in which seta PA20 is present, and pores PAd, PAe and PAj are either present or absent (Fig. 2.4e and f).

2.4.2 Head Appendages

Thirty-one setae, 26 pores and three setal groups are coded on the head appendages. The sensilla observed are illustrated in Figs. 2.5a–i and 2.6a–i and their positions are listed in Table 2.2.

Antenna The primary sensilla (three setae, nine pores and a sensillum group) observed on the dytiscid antenna show an extremely consistent pattern among the subfamilies studied (Fig. 2.5a and b). This system is composed of five pores on antennomere I (ANa, ANb, ANc, ANd, ANe), two pores on antennomere II (ANh, ANi), three setae (AN1, AN2, AN3) and one pore (ANf) on antennomere III, and one lateral pore (ANg) and a setal group composed of 2–3 small apical setae and possibly a pore (gAN) on antennomere IV. Antennomere III is also characterized by the presence/absence of a ventroapical spinula (Fig. 2.5b). Hydroporinae is distinctive within the Dytiscidae in that here, the pore ANi is lacking, and pores ANf and ANh are either present or absent (Fig. 2.5c–e). Pores ANe, ANh and ANi are also present or absent within the subfamily Laccophilinae.

Mandible Two setae (MN1, MN2) and three pores (MNa, MNb, MNc) are coded on the mandible of every dytiscid species known as larva (Fig. 2.5e). Seta MN1 is more difficult to homologize in Cybistrini (Dytiscinae) owing to the presence of several additional setae, whereas seta MN2 is minute and pore-like in most Hydroporinae.

Maxilla Fourteen primary setae, ten primary pores and one setal group are coded on the maxilla of the Dytiscidae (Fig. 2.5f and g). One seta (MX1) is either found on the cardo (where present) or the stipes. Six setae (MX2, MX3, MX4, MX5, MX6, MX7) and two pores (MXb, MXc) are the basal number of sensilla on the maxillary stipes. Two setae (MX8, MX9) and two pores (MXd, MXh) appear on the galea (except in Laccophilinae, Hydroporinae and Cybistrini, where some or all of them are either absent (Fig. 2.5h) or located on the stipes (Fig. 2.6d). Five setae, five pores, and a setal group occur on the palpus: one seta (MX10) on palpifer; one seta (MX13) and two pores (MXe, MXf) on palpolmere I; two setae (MX11, MX12) and two pores (MXg, MXi) on palpomere II; one seta (MX14), one pore (MXj) and a setal group (gMX) on palpomere III. This generalized pattern is fairly consistent within the family except for the subfamily Hydroporinae and members of the subfamilies Dytiscinae and Laccophilinae. Indeed the primary pores MXb, MXc, and MXd and to a lesser extent setae MX4 and MX10 are lacking within the Hydroporinae, which is likely correlated with the absence or reduction of the galea, an unsual feature within the Dytiscidae (Alarie and Michat 2007a) (Fig. 2.5h and i). Seta LA9 and pores MXb, MXd, MXf and MXi are either present or absent within

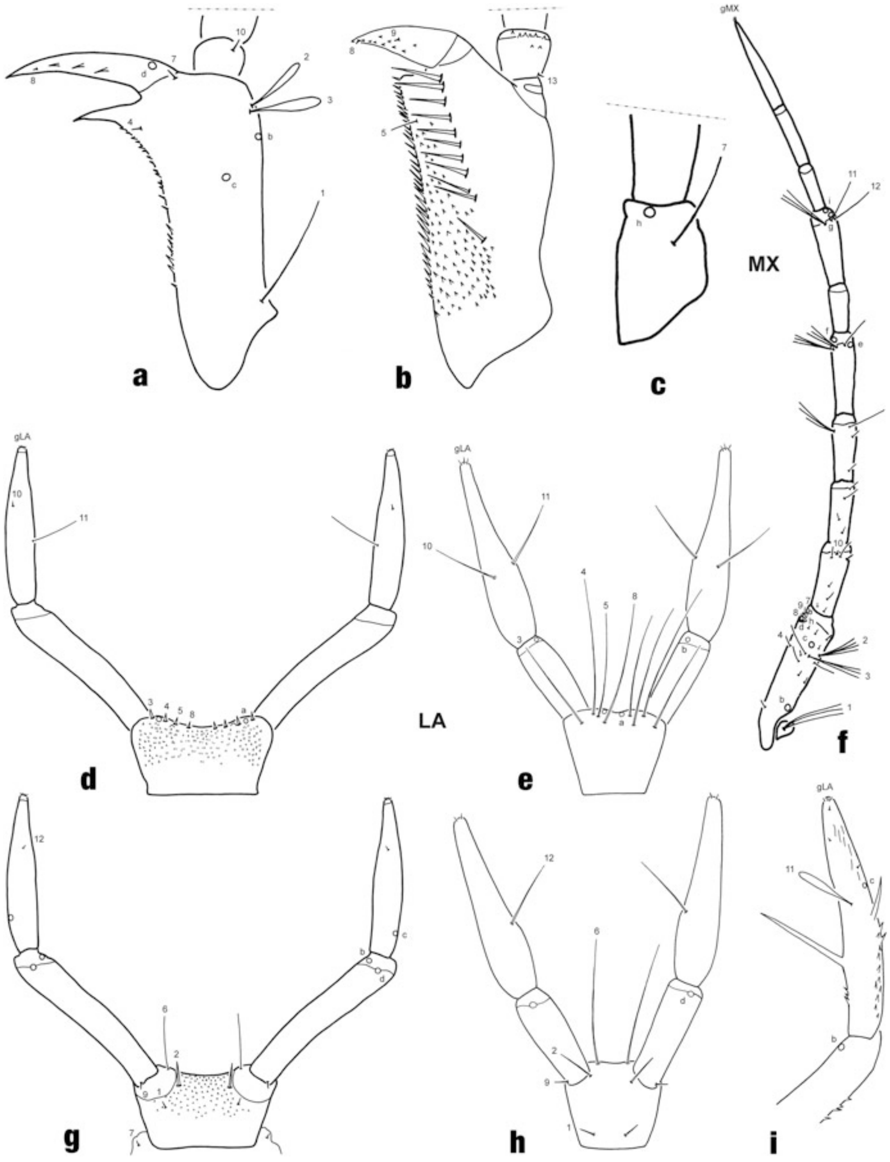

Fig. 2.6 Distribution of ancestral setae and pores on the head appendages of first instars of selected species of Dytiscidae: (**a**) *Eretes australis* (Erichson, 1842), stipes, ventral surface; (**b**) *Acilius semisulcatus* Aubé, 1838, stipes, dorsal surface; (**c**) *Desmopachria concolor* Sharp, 1882, stipes, dorsal surface; (**d**) *Megadytes glaucus* (Brullé, 1837), left maxilla, ventral surface; (**e–f**) *Platynectes curtulus* (Régimbart, 1899), labium, (**e**) dorsal surface, (**f**) ventral surface; (**g–h**) *Liodessus flavofasciatus* (Steinheil, 1869), labium, (**g**) dorsal surface, (**h**) ventral surface; (**i**) *Eretes australis*, labial palpomere 2, dorsal surface. *LA* labium, *MX* maxilla; numbers and lowercase letters refer to primary setae and pores, respectively (see Table 2.2 for list of setae and pores)

Table 2.2 Ancestral setae and pores on the head appendages of first instars of Dytiscidae subfamilies: *AGA* Agabinae, *COL* Colymbetinae, *CPL* Copelatinae, *COP* Coptotominae, *DYT* Dytiscinae, *HYD* Hydroporinae, *LAC* Laccophilinae, *LAN* Lancetinae, *MAT* Matinae

Setae/pores	AGA	COL	CPL	COP	DYT	HYD	LAC	LAN	MAT
AN1	1	1	1	1	1	1	1	1	1
AN2	1	1	1	1	1	1	1	1	1
AN3	1	1	1	1	1	1	1	1	1
ANa	1	1	1	1	1	1	1	1	1
ANb	1	1	1	1	1	1	1	1	1
ANc	1	1	1	1	1	1	1	1	1
ANd	1	1	1	1	1	1	1	1	1
ANe	1	1	1	1	1	1	1	1	1
ANf	1	1	1	1	1	0/1	0/1	1	1
ANg	1	1	1	1	1	1	1	1	1
ANh	1	1	1	1	1	0/1	1	1	1
ANi	1	1	1	1	1	0	0	1	1
MN1	1	1	1	1	1	1	1	1	1
MN2	1	1	1	1	1	1[a]	1	1	1
MNa	1	1	1	1	1	1	1	1	1
MNb	1	1	1	1	1	1	1	1	1
MNc	1	1	1	1	1	1	1	1	1
MX1	1	1	1	1	1	1	1	1	1
MX2	1	1	1	1	1	1	1	1	1
MX3	1	1	1	1	1	1	1	1	1
MX4	1	1	1	1	1	0/1	1	1	1
MX5	1	1	1	1	1	0/1	0/1	1	1
MX6	1	1	0	1	0/1	0/1	0/1	1	1
MX7	1	1	1	1	1	1	1	1	1
MX8	1	1	1	1	1	0/1	1	1	1
MX9	1	1	1	1	1	0/1	1	1	1
MX10	1	1	1	1	1	0/1	1	1	1
MX11	1	1	1	1	1	1	1	1	1
MX12	1	1	1	1	1	1	1	1	1
MX13	1	1	1	1	1	1	1	1	1
MX14	1	1	1	1	1	1	1	1	1
MXb	1	1	1	1	1	0	1	1	1
MXc	1	1	1	1	1	0	1	1	1
MXd	1	1	1	1	1	0	1	1	1
MXe	1	1	1	1	1	1	1	1	1
MXf	1	1	1	1	1	1	1	1	1
MXg	1	1	1	1	1	1	1	1	1
MXh	1	1	1	1	1	1	1	1	1
MXi	1	1	1	1	1	1	1	1	1
MXj	1	1	1	1	1	1	1	1	1
MXk	0	0	0	0	0	1	0	0	0

(continued)

Table 2.2 (continued)

Setae/pores	AGA	COL	CPL	COP	DYT	HYD	LAC	LAN	MAT
LA1	1	1	1	1	1	1	1	1	1
LA2	1	1	1	1	1	1	1	1	1
LA3	1	1	1	1	1	0/1	0/1	1	1
LA4	1	1	1	1	1	1	1	1	1
LA5	1	1	1	1	1	1	1	1	1
LA6	1	1	1	1	1	1	1	1	1
LA7	1	1	1	1	0	1	1	1	1
LA8	1	1	1	1	0/1	1	1	1	1
LA9	1	1	1	1	1	1	1	1	1
LA10	1	1	1	0	1	0/1	0	1	1
LA11	1	1	1	1	1	1	1	1	1
LA12	1	1	1	0	1	0/1	0	1	1
LAa	1	1	1	1	1	1	1	1	1
LAb	1	1	1	1	1	0/1	1	1	1
LAc	1	1	1	1	1	0	1	1	1
LAd	1	1	1	1	1	0/1	1	1	1

[a] Coded as MNd in Alarie (1991)

AN antenna, *LA* labium, *MN* mandible, *MX* maxilla; 0 = absent; 1 = present

the subfamily Laccophilinae. Unique features observed in some Dytiscinae are: (1) the presence of several elongate and spine-like setae along the dorsal margin of the stipes (Aciliini and Eretini) (Fig. 2.6b); (2) the presence of several additional setae on the stipes, palpifer and palpi in the Cybistrini (Fig. 2.6d); (3) setae either multifid (Cybistrini) (Fig. 2.6d) or lanceolate (Eretini) (Fig. 2.6a). It is worth noting that either of setae MX5 and MX6 or both are sometimes lacking (e,g, Dytiscinae (Aciliini and Eretini), Copelatinae, Laccophilinae (*Neptosternus*) and Hyphydrini (Fig. 2.6c)). The primary pore MXk is restricted to the Hydroporinae (Fig. 2.5i).

Labium Twelve primary setae, four primary pores and one setal group are coded on the labium (Fig. 2.6e and f). The prementum is characterized by the presence of seven setae (LA1, LA2, LA3, LA4, LA5, LA6, LA8) and one pore (LAa). Four setae, three pores and a setal group appear on the labial palpus: one small seta (LA9) and two pores (LAb, LAd) on palpomere I; three setae (LA10, LA11, LA12), a setal group (gLA), and one pore (LAc) on palpomere II. Setae LA10 and LA12 are lacking in the Coptotominae, Laccophilinae and Vatellini and are most often minute and very difficult to see in the Agabinae, Colymbetinae, Copelatinae, Dytiscinae and Lancetinae (Fig. 2.6e and f). Pore LAc is consistently lacking within the Hydroporinae (Fig. 2.6g and h) and sometimes within the Laccophilinae. Some laccophilines may also lack pore LAb. Larvae of Eretini and members of the tribe Cybistrini (Dytiscinae) differ from all other Dytiscidae in that here, the seta LA11 is lanceolate (Fig. 2.6i), and the setae LA2, LA6 and LA11 are multifid, respectively. It is worth stressing that the seta LA8 is sometimes absent within some members if the subfamily Dytiscinae (*Notaticus*, *Dytiscus* and *Megadytes carcharias* Griffini, 1895) and that the seta LA3 is absent in some Hydroporinae (Hydrovatini, Methlini) and Laccophilinae (Laccophilini). The pores LAb and LAd are absent in members of the hydroporine tribes Hyphydrini and Vatellini, respectively.

2.4.3 Legs

Sixty-nine sensilla (51 setae and 18 pores) are coded on the leg of the Dytiscidae. These sensilla are illustrated in Fig. 2.7a–j and they are listed in Table 2.3.

Coxa Eighteen setae and two pores compose the basal number of primary sensilla on the coxa (Fig. 2.7a and b). Eleven small setae (CO1, CO2, CO3, CO4, CO5, CO13, CO14, CO15, CO16, CO17, CO18) and one pore (COa) appear on the proximal portion of the segment. Seven setae (CO6, CO7, CO8, CO9, CO10, CO11, CO12) and one pore (COd) appear on the distal portion. This pattern is quite uniform within the taxa studied. The only differences observed are the absence of pore COa in Pachydrini (Hydroporinae), and the relative positions of setae CO6 and CO7 and pore COd.

Trochanter Seven setae and seven pores are coded on the Dytiscidae trochanter (Fig. 2.7a and b). One seta (TR1) and one pore (TRb), and two hair-like setae (TR4, TR7) appear on the dorsal and ventral margin, respectively. The anterior surface is composed of two setae (TR2, TR3) and four pores (TRa, TRc, TRd, TRe) whilst the posterior surface is characterized by the presence of two setae (TR5, TR6) and two pores (TRf, TRg). The seta TR3 is lacking within the Hydroporinae and some Laccophilinae, whilst the seta TR2 is either present or absent amongst the Dytiscinae and the Hydroporinae.

Femur Ten setae and two pores characterize this segment (Fig. 2.7a and b). Seven setae (FE1, FE2, FE3, FE7, FE8, FE9, FE10) and one pore (FEb) appear on the anterior surface of the segment. Three setae (FE4, FE5, FE6) and one pore (FEa) are coded on the posterior surface. Setae FE4 and/or FE5 are lacking in some Dytiscinae (Aciliini, Aubehydrini, Dytiscini and Hydaticini) (Fig. 2.7d), whilst pore FEa is absent in some tribes of Hydroporinae (e.g., Bidessini, Hydrovatini, Hyphydrini, Laccornini and some Hydroporini) (Fig. 2.7f). It is interesting to note that the Dytiscinae larvae are characterized by the presence of a variable number of additional hair-like natatory setae along both the anteroventral and posterodorsal margins of the femur (Fig. 2.7c and d) and that seta FE6 is multifid within the tribe Cybistrini (Fig. 2.7e).

Tibia Seven setae and one pore are coded on the tibia (Fig. 2.7a and b). Three setae (TI2, TI3, TI4) are on the anterior surface and four setae (TI1, TI5, TI6, TI7) and one pore (TIa) are on the posterior surface. Setae TI2 and/or TI6 are absent in some Matinae (Fig. 2.7i and j). The ventral margin of the tibia is characterized by the presence of spinulae, which are generally more strongly developed on the protibia.

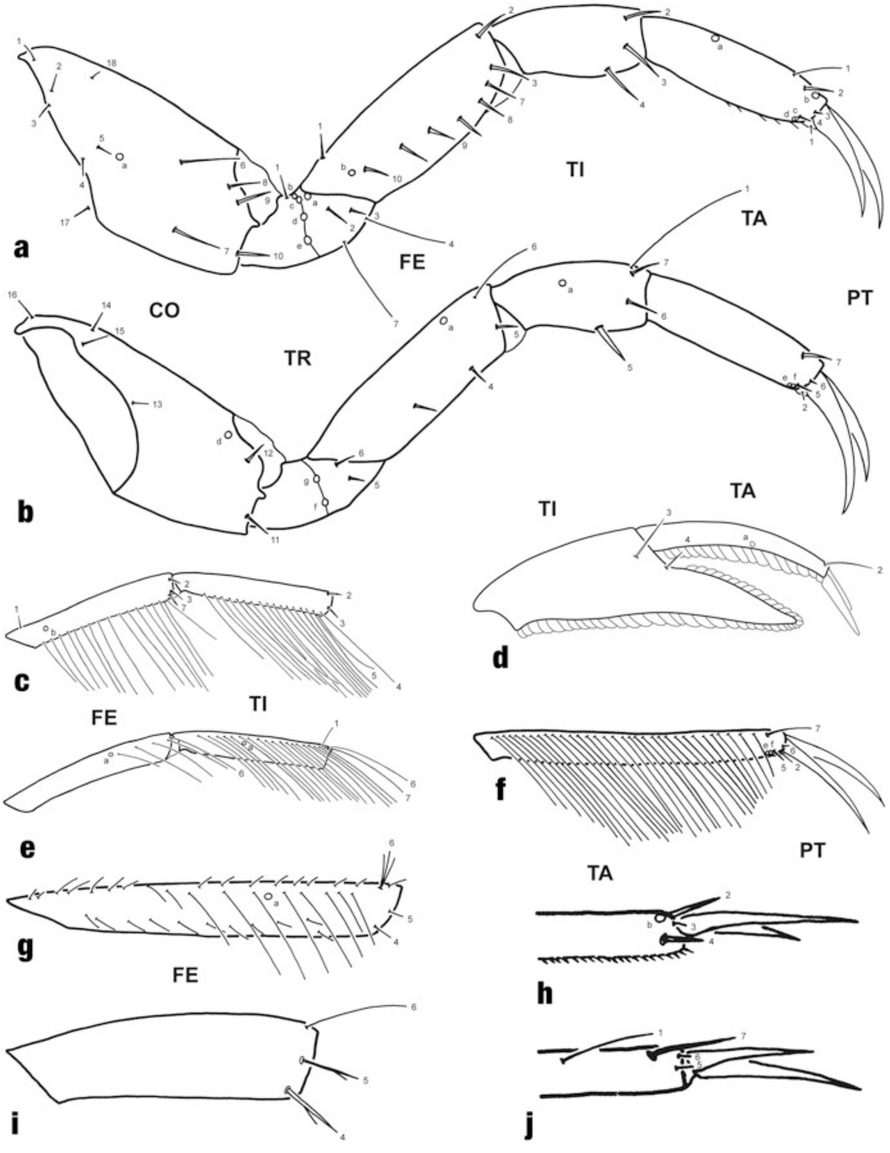

Fig. 2.7 Distribution of ancestral setae and pores on the legs of first instars of selected species of Dytiscidae: (**a–b**) *Copelatus longicornis* Sharp, 1882, metathoracic leg, (**a**) anterior surface, (**b**) posterior surface; (**c–d**) *Hydaticus tuyuensis* Trémouilles, 1996, metafemur and metatibia, (**c**) anterior surface, (**d**) posterior surface; (**e**) *Megadytes carcharias* Griffini, 1895, metafemur, posterior surface; (**f**) *Hydrovatus caraibus* Sharp, 1882, metafemur, posterior surface; (**g**) *Matus bicarinatus* (Say, 1823), protibia and protarsus, anterior surface; (**h**) *Megadytes fallax* (Aubé, 1838), metatarsus, posterior surface; (**i–j**) *Thermonectus succinctus* (Aubé, 1838), apex of metatarsus, (**i**) anterior surface; (**j**) posterior surface. *CO* coxa, *FE* femur, *PT* pretarsus, *TA* tarsus, *TI* tibia,

Larvae of *Matus* Aubé, 1836 (Matinae) are unique in that regard by the presence of characteristic feather-like spinulae on pro- and mesotibiae (Fig. 2.7g). Larvae of the Dytiscinae are characterized by the presence of a row of additional natatory setae on posterodorsal and anteroventral surfaces (Fig. 2.7c and d).

Tarsus Seven setae and six pores are coded on the tarsus (Fig. 2.7a and b). Three setae (TA2, TA3, TA4) and two pores (TAc,TAd) occur on the anterior surface and four setae (TA1, TA5, TA6, TA7) and two pores (TAe, TAf) are found posteriorly. Two other pores (TAa, TAb) are inserted dorsally. The individual pores of the pairs TAc/TAd and TAe/TAf are generally present (except within the tribe Aciliini (Dytiscinae) (Fig. 2.7i and j)) but very difficult to distinguish in some taxa because they are positioned close together and because the ventral margin of the tarsus is generally marked by a pronounced thickening of the marginal spinulae. The pore TAb is also very difficult to locate because of both its apical position and the presence of setae TA2 and TA7. The seta TA1 is generally inserted dorso-apically, and is extremely short and hair-like in some taxa. Members of the tribe Cybistrini (Dytiscinae) are characterized by a row of additional natatory setae on the posterodorsal surface (Fig. 2.7h).

Pretarsus Two short spiniform setae are located basally on the ventral surface of the pretarsus (Fig. 2.7a and b), except within the tribe Aciliini (Dytiscinae) (Fig. 2.7i and j). These may be overlooked easily and incorporated into the row of spinulae of the tarsus.

2.4.4 Last Abdominal Segment

The ground plan pattern of primary setae and pores on the last abdominal segment of the Dytiscidae is illustrated in Fig. 2.8a and b and the sensilla observed are listed in Table 2.4. Fifteen setae and three pores have been coded. Three minute setae (AB1, AB12, AB13) and one pore (ABa) occur on the anterior portion of the segment. The remaining twelve setae and two pores are inserted posteriorly. Setae AB2, AB3, AB4, AB5, AB6 and AB7 along with pores ABb and ABc are dorsal. Their relative distribution varies among taxa more than likely in correlation to the relative elongation of the segment posteriorly (i.e., siphon). Setae AB8, AB9, AB10, AB11, AB14 and AB15 are ventral, although seta AB9 may be more dorsally articulated in some taxa. Because of their small size, marginal position, and spine-like appearance, setae AB7, AB8 and AB14 (= pore ABd within the Hydroporinae) are often extremely difficult to distinguish from the spine-like microsculpture of the siphon. The primary setae AB2, AB6, AB7, AB8, AB13, AB14 and AB15, and the primary

Fig. 2.7 (continued) TR trochanter; numbers and lowercase letters refer to primary setae and pores, respectively (see Table 2.3 for list of setae and pores)

Table 2.3 Ancestral setae and pores on the legs of first instars of Dytiscidae subfamilies: *AGA* Agabinae, *COL* Colymbetinae, *CPL* Copelatinae, *COP* Coptotominae, *DYT* Dytiscinae, *HYD* Hydroporinae, *LAC* Laccophilinae, *LAN* Lancetinae, *MAT* Matinae

Setae/pores	AGA	COL	CPL	COP	DYT	HYD	LAC	LAN	MAT
CO1	1	1	1	1	1	1	1	1	1
CO2	1	1	1	1	1	1	1	1	1
CO3	1	1	1	1	1	1	1	1	1
CO4	1	1	1	1	1	1	1	1	1
CO5	1	1	1	1	1	1	1	1	1
CO6	1	1	1	1	1	1	1	1	1
CO7	1	1	1	1	1	1	1	1	1
CO8	1	1	1	1	1	1	1	1	1
CO9	1	1	1	1	1	1	1	1	1
CO10	1	1	1	1	1	1	1	1	1
CO11	1	1	1	1	1	1	1	1	1
CO12	1	1	1	1	1	1	1	1	1
CO13	1	1	1	1	1	1	1	1	1
CO14	1	1	1	1	1	1	1	1	1
CO15	1	1	1	1	1	1	1	1	1
CO16	1	1	1	1	1	1	1	1	1
CO17	1	1	1	1	1	1	1	1	1
C018	1	1	1	1	1	1	1	1	1
COa	1	1	1	1	1	0/1	1	1	1
COd	1	1	1	1	1	1	1	1	1
TR1	1	1	1	1	1	1	1	1	1
TR2	1	1	1	1	0/1	0/1	1	1	1
TR3	1	1	1	1	1	0	1	1	1
TR4	1	1	1	1	1	1	1	1	1
TR5	1	1	1	1	1	1	1	1	1
TR6	1	1	1	1	1	1	1	1	1
TR7	1	1	1	1	1	1	1	1	1
TRa	1	1	1	1	1	1	1	1	1
TRb	1	1	1	1	1	1	1	1	1
TRc	1	1	1	1	1	1	1	1	1
TRd	1	1	1	1	1	1	1	1	1
TRe	1	1	1	1	1	1	1	1	1
TRf	1	1	1	1	1	1	1	1	1
TRg	1	1	1	1	1	1	1	1	1
FE1	1	1	1	1	1	1	1	1	1
FE2	1	1	1	1	1	1	1	1	1
FE3	1	1	1	1	1	1	1	1	1
FE4	1	1	1	1	0/1	1	1	1	1
FE5	1	1	1	1	0/1	1	1	1	1
FE6	1	1	1	1	1	1	1	1	1
FE7	1	1	1	1	1	1	1	1	1

(continued)

Table 2.3 (continued)

Setae/pores	AGA	COL	CPL	COP	DYT	HYD	LAC	LAN	MAT
FE8	1	1	1	1	1	1	1	1	1
FE9	1	1	1	1	1	1	1	1	1
FE10	1	1	1	1	1	1	1	1	1
FEa	1	1	1	1	1	0/1	1	1	1
FEb	1	1	1	1	1	1	1	1	1
TI1	1	1	1	1	1	1	1	1	1
TI2	1	1	1	1	1	1	1	1	0/1
TI3	1	1	1	1	1	1	1	1	1
TI4	1	1	1	1	1	1	1	1	1
TI5	1	1	1	1	1	1	1	1	1
TI6	1	1	1	1	1	1	1	1	0/1
TI7	1	1	1	1	1	1	1	1	1
TIa	1	1	1	1	1	1	1	1	1
TA1	1	1	1	1	1	1	1	1	1
TA2	1	1	1	1	1	1	1	1	1
TA3	1	1	1	1	1	1	1	1	1
TA4	1	1	1	1	1	1	1	1	1
TA5	1	1	1	1	1	1	1	1	1
TA6	1	1	1	1	1	1	1	1	1
TA7	1	1	1	1	1	1	1	1	1
TAa	1	1	1	1	1	1	1	1	1
TAb	1	1	1	1	1	1	1	1	1
TAc	1	1	1	1	0/1	1	1	1	1
TAd	1	1	1	1	0/1	1	1	1	1
TAe	1	1	1	1	0/1	1	1	1	1
TAf	1	1	1	1	0/1	1	1	1	1
PT1	1	1	1	1	0/1	1	1	1	1
PT2	1	1	1	1	0/1	1	1	1	1

CO coxa, *FE* femur, *PT* pretarsus, *TA* tarsus, *TI* tibia, *TR* trochanter; 0 = absent; 1 = present

pores ABa and ABc are either present or absent amongst the Dytiscinae, Coptotominae, Hydroporinae and Laccophilinae (Fig. 2.8d and e). Larvae of all Dytiscinae are characterized by the presence of several additional elongate hair-like (natatory) setae along the lateral margin (Fig. 2.8d). Larvae of Aciliini and Eretini (Dytiscinae) are unique amongst the Dytiscidae in having the seta AB9 lanceolate (Fig. 2.8d). Larvae of Matinae, Cybistrini and some Colymbetinae (*Bunites* Spangler, 1972, *Meladema* Laporte, 1845, *Neoscutopterus* J. Balfour-Browne, 1943) are characterized by the presence of numerous additional setae (Fig. 2.8c).

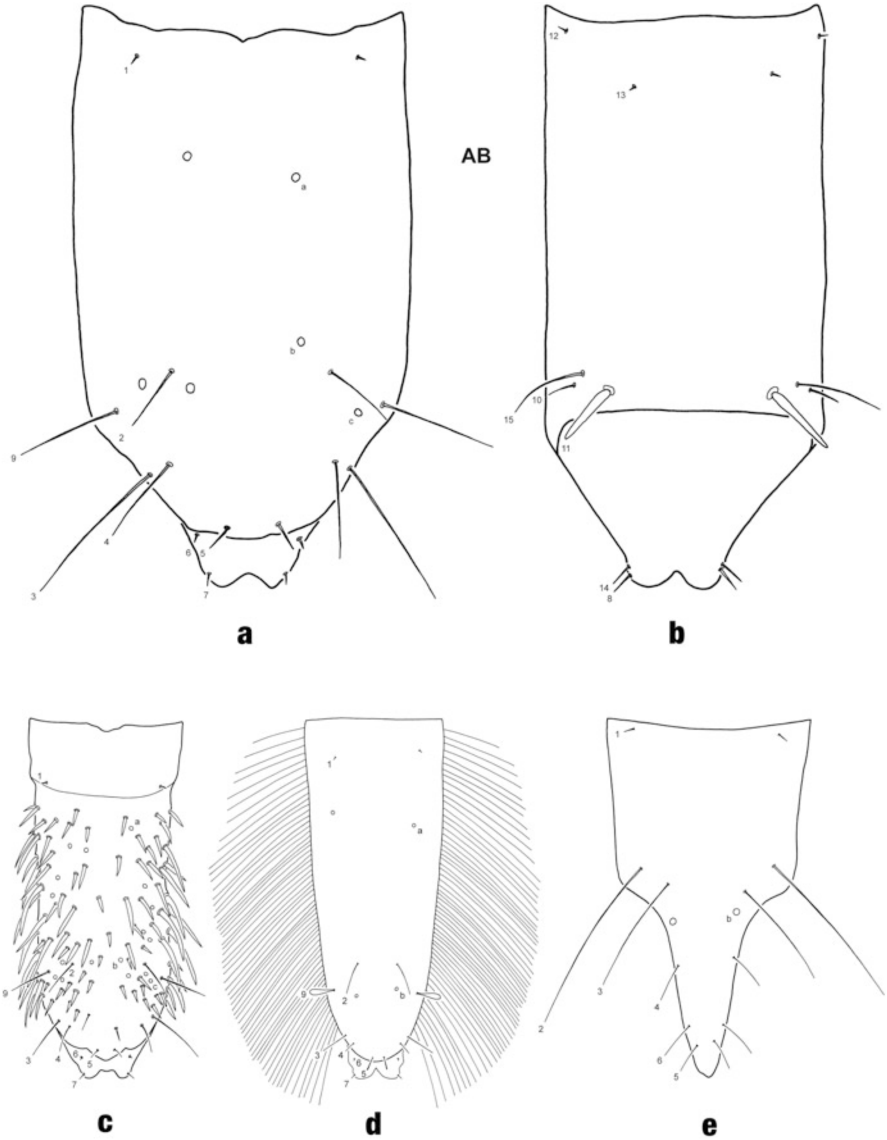

Fig. 2.8 Distribution of ancestral setae and pores on the last abdominal segment of first instars of selected species of Dytiscidae: (**a–b**) *Rhantus calileguai* Trémouilles, 1984, (**a**) dorsal surface, (**b**) ventral surface; (**c**) *Bunites distigma* (Brullé, 1837), dorsal surface; (**d**) *Eretes australis* (Erichson, 1832), dorsal surface; (**e**) *Anodocheilus maculatus* Babington, 1842, dorsal surface. *AB* abdominal segment 8; numbers and lowercase letters refer to primary setae and pores, respectively (see Table 2.4 for list of setae and pores)

Table 2.4 Ancestral setae and pores on the last abdominal segment and the urogomphus of first instars of Dytiscidae subfamilies: *AGA* Agabinae, *COL* Colymbetinae, *CPL* Copelatinae, *COP* Coptotominae, *DYT* Dytiscinae, *HYD* Hydroporinae, *LAC* Laccophilinae, *LAN* Lancetinae, *MAT* Matinae

Setae/pores	AGA	COL	CPL	COP	DYT	HYD	LAC	LAN	MAT
AB1	1	1	1	1	1	1	1	1	1
AB2	1	1	1	1	1	0/1	1	1	1
AB3	1	1	1	1	1	1	1	1	1
AB4	1	1	1	1	1	1	1	1	1
AB5	1	1	1	1	1	1	1	1	1
AB6	1	1	1	1	0/1	1	1	1	1
AB7	1	1	1	1	1	0/1	1	1	1
AB8	1	1	1	1	1	0/1	1	1	1
AB9	1	1	1	1	1	1	1	1	1
AB10	1	1	1	1	1	1	1	1	1
AB11	1	1	1	1	1	1	1	1	1
AB12	1	1	1	1	1	1	1	1	1
AB13	1	1	1	0	0/1	1	1	1	1
AB14	1	1	0	1	1	1[a]	0/1	1	1
AB15	1	1	1	1	1	0/1	0/1	1	1
ABa	1	1	1	1	1	0/1	1	1	1
ABb	1	1	1	1	1	1	1	1	1
ABc	1	1	1	0	0/1	0/1	0/1	1	1
UR1	1	1	1	1	1	1	1	1	1
UR2	1	1	1	1	1	1	1	1	1
UR3	1	1	1	1	1	1	1	1	1
UR4	1	1	1	1	1	1	1	1	1
UR5	1	1	1	1	1	1	1	1	1
UR6	1	1	1	1	1	1	1	1	1
UR7	1	1	1	1	1	1	1	1	1
UR8	1	1	1	1	1	0/1	1	1	1
URa	1	1	1	1	1	1	1	1	1
URb	1	1	1	1	0/1	0/1	1	1	1
URc	1	1	1	1	0/1	1	1	1	1

[a] Pore AB4 in Hydroporinae

AB last abdominal segment, *UR* urogomphus; 0 = absent; 1 = present

2.4.5 *Urogomphus*

The primary sensilla (eight setae and three pores) observed on the urogomphus also show an extremely consistent pattern within the family Dytiscidae. They are represented in Fig. 2.9a–i and listed in Table 2.4. Their relative distribution relies upon the shape of the urogomphus, which is either one- (e.g., Fig. 2.9a, g–i) or two-segmented (e.g., Fig. 2.9b–f). These sensilla are subdivided into three groups. A

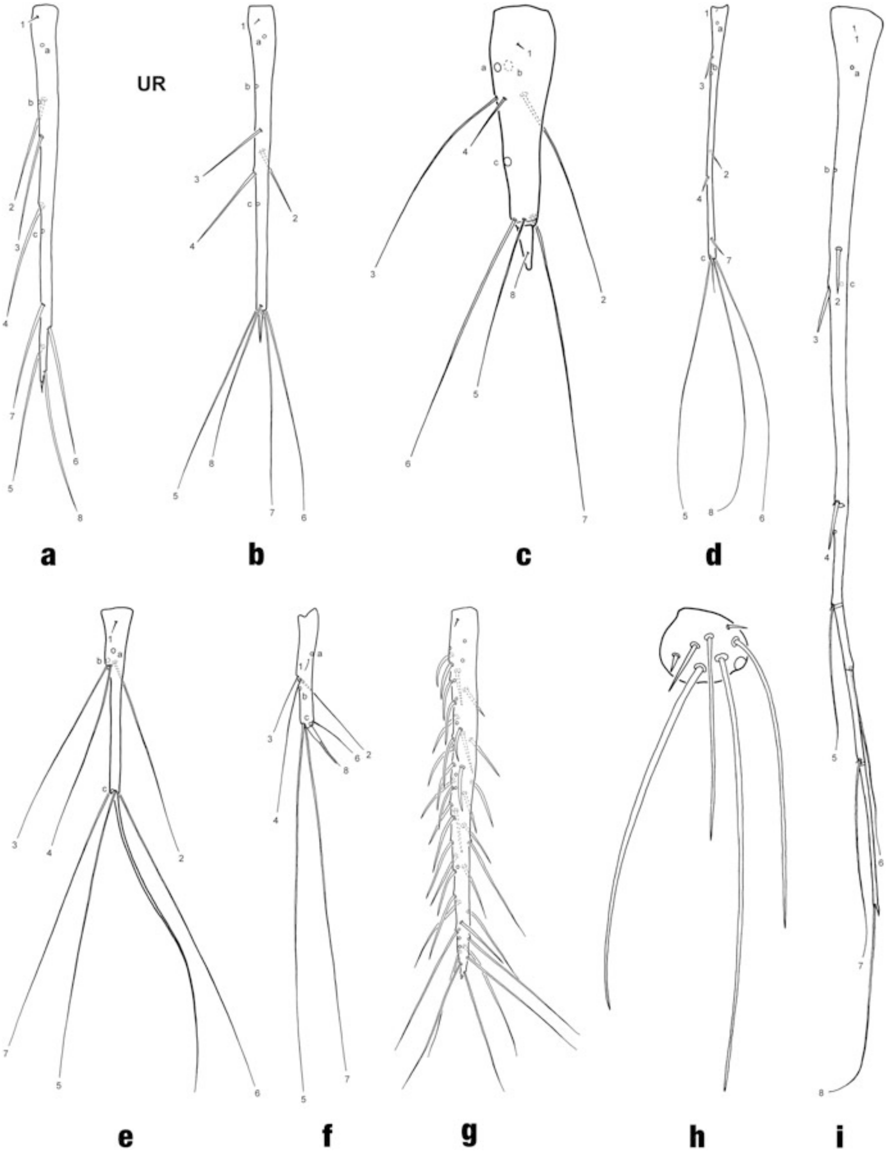

Fig. 2.9 Distribution of ancestral setae and pores on the left urogomphus of first instars of selected species of Dytiscidae: (**a**) *Meridirorhanthus antarcticus nahueli* (Trémouilles, 1984), dorsal surface; (**b**) *Platynectes curtulus* (Régimbart, 1899), dorsal surface; (**c**) *Copelatus longicornis* Sharp, 1882, dorsal surface; (**d**) *Laccophilus obliquatus* Regimbart, 1889, dorsal surface; (**e**) *Laccornellus lugubris* (Aubé, 1838), dorsal surface; (**f**) *Celina parallela* (Babington, 1842), dorsal surface; (**g**) *Bunites distigma* (Brullé, 1837), dorsal surface; (**h**) *Megadytes glaucus* (Brullé, 1837), ventral surface; (**i**) *Lancetes marginatus* (Steinheil, 1869), dorsal surface. *UR* urogomphus; numbers and lowercase letters refer to primary setae and pores, respectively (see Table 2.4 for list of setae and pores)

proximal group is composed of a small spine-like seta (UR1) and a pore (URa) near the base of the urogomphus. Both may be overlooked depending upon the shape of the siphon. The median group is composed of three spine-like setae (UR2, UR3, UR4) and one pore (URb). These setae are variably articulated among taxa. The distal group of primary urogomphal sensilla is composed of four setae (UR5, UR6, UR7, UR8) and one pore (URc). Seta UR8 is inserted on the urogomphomere 2 in Copelatinae (Fig. 2.9c) and Hydroporinae (Fig. 2.9e and f). In some hydroporines (*Canthyporus* Zimmermann, 1919, *Laccornellus* Roughley & Wolfe, 1987, *Hydrovatus* Motschulsky, 1853), it is absent (Fig. 2.9e). Pores URb and/or URc are lacking within the Cybistrini (Fig. 2.9h) and some Hydroporinae (URb in *Desmopachria* Babington, 1841). Larvae of some Dytiscinae (Dytiscini, Hyderodini) differ from other Dytiscidae by the presence of elongate hair-like (natatory) setae along the outer margin. Several Colymbetinae are characterized by the presence of numerous additional spine-like setae (Fig. 2.9g).

2.5 Making the Wealth of the Dytiscidae Chaetotaxy Pattern Available for Study Other Hydradephaga Larvae

The branching pattern of the Hydradephaga families [Aspidytidae, Dytiscidae, Hygrobiidae, Noteridae, Amphizoidae, Meruidae, Gyrinidae, Haliplidae] has received significant attention over the past decade, although no strong consensus on interfamilial relationships has yet emerged. In addition to the paraphyly of Hydradephaga, another long-standing area of phylogenetic uncertainty within Adephaga involves the families of Dytiscoidea: Aspidytidae, Amphizoidae, Hygrobiidae and Dytiscidae (Cai et al. 2020; Gustafson et al. 2021). A way to test these preliminary classifications, however, is to study larval morphology as each larval instar represents an ontogenetic stage with its own characters, each being important in determining taxa, reconstructing phylogenies, and building classifications.

Although little known until very recently the study of larvae of Hydradephaga families other than Dytiscidae has experienced remarkable progress in recent years largely due to the application of the chaetotaxy system developed for the Dytiscidae: Aspidytidae (Alarie and Bilton 2005; Michat et al. 2014b), Gyrinidae (Archangelsky and Michat 2007; Michat et al. 2010, 2016, 2017b; Michat and Gustafson 2016; Colpani et al. 2018, 2020), Haliplidae (Michat et al. 2020), Hygrobiidae (Alarie et al. 2004; Michat et al. 2014a), Meruidae (Alarie et al. 2011b), and Noteridae (Urcola et al. 2019, 2019a, b, 2020, 2021). As demonstrated in these papers, characteristics of setae and pores reveal to be useful and important both for diagnosis and study of the phylogenetic relationships of these taxa and have contributed towards the formulation of several hypotheses of phylogeny.

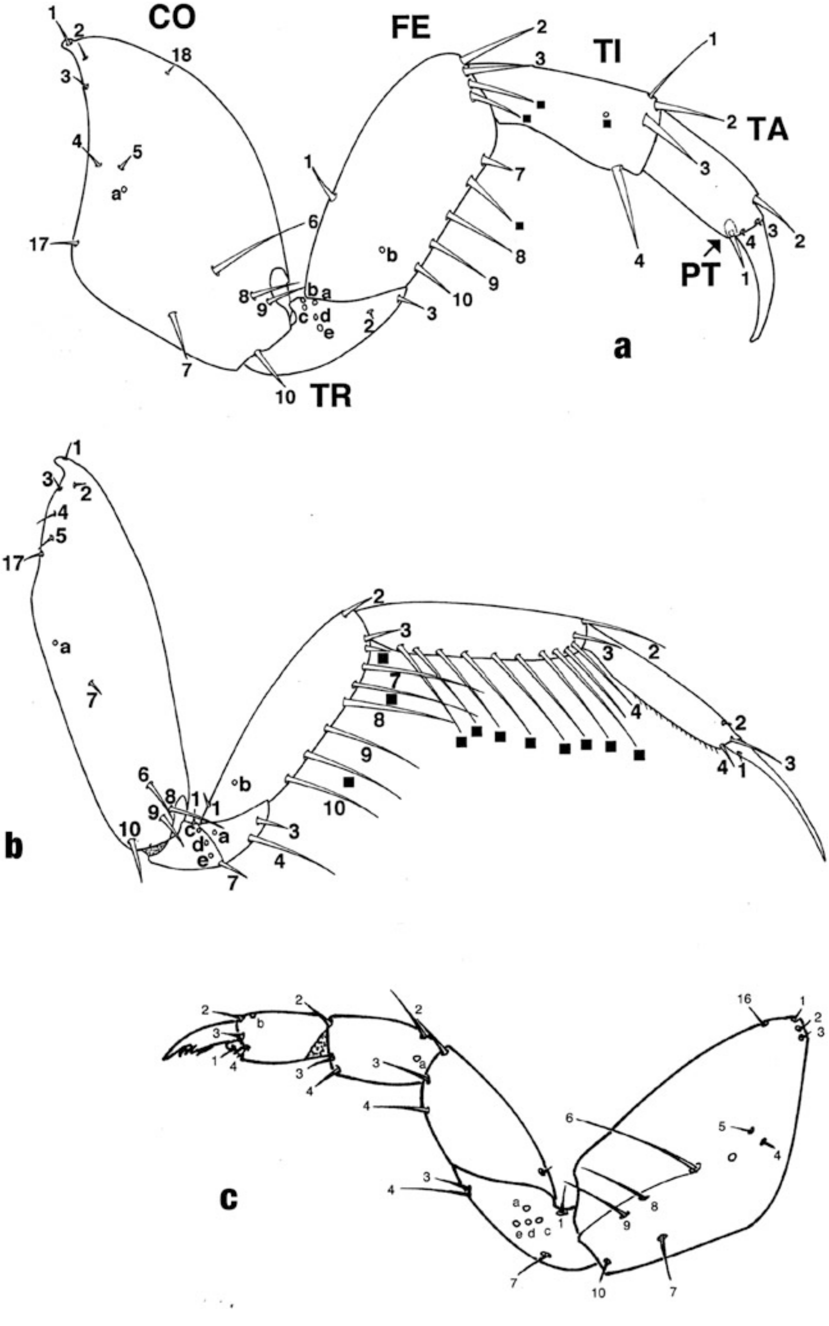

Fig. 2.10 Distribution of ancestral setae and pores on the anterior surface of the metathoracic leg of first instars of selected Hydradephaga families: (**a**) Aspidtytidae: *Aspidytes niobe* Ribera, Beutel, Balke & Vogler, 2002; (**b**) Hygrobiidae: *Hygrobia hermani* (Fabricius, 1775); (**c**) Meruidae: *Meru phyllisae* Spangler & Steiner, 2005. *CO* coxa, *FE* femur, *PT* pretarsus, *TA* tarsus, *TI* tibia, *TR* trochanter; numbers and lowercase letters refer to primary setae and pores, respectively; filled

The study of the pattern of primary setae and pores observed on the leg of the larva of selected species belonging to each of the families of Hydradephaga (with the exception of Amphizoidae, whose larva remains to be studied) allows us to illustrate our point. These sensilla are illustrated in Figs. 2.7a–j, 2.10a–c and 2.11a–c and they are listed in Table 2.5. A quick glance at Table 2.5 shows the great similarity in the number of primary setae and pores observed amongst Hydradephaga larvae, although notable differences can be found there. Among these, we note the presence of the setae FE7-FE10 inserted along the ventral margin of the femur of Aspidytidae, Hygrobiidae and Dytiscidae (Figs. 2.7a and 2.10a and b). These setae are lacking in every other adephagan families (Figs. 2.10c and 2.11a–c), which clearly represent a putative strong synapomorphy supporting the monophyletic origin of the Dytiscoidea (Aspidytidae, Hygrobiidae, Dytiscidae, and Amphizoidae). Some families also have unique characteristics (Table 2.5). The larvae of Haliplidae, for one, share a unique character state in the absence of seta CO6 on the coxa (Fig. 2.11b); similarly, all known Noteridae larvae differ from those of other Hydradephaga by the presence of the primary pore COc located along the posteroventral margin of the coxa; finally the larvae of Meruidae are deemed to miss several primary setae and pores generally observed amongst other Hydradephaga (Fig. 2.11c).

In the past recent years, detailed studies of the primary chaetotaxy of other hydradephagan larval structures (e.g., head capsule, head appendages, last abdominal segment and urogomphi) have developed, in combination with more traditional morphological treatments. As evidenced by the example provided above the utility of exploring the character set provided by chaetotaxy relies not only in presence/absence but also in variations in position, size, and shape of sensilla, which have proven to provide a large number of characters useful to distinguish taxa at different taxonomic levels, and to study the phylogenetic relationships amongst these taxa.

2.6 Larval Chaetotaxy and Ontogeny

The value of the nomenclatural system of chaetotaxy that was derived for the Dytiscidae and other Hydradephaga families over the past 30 years is enhanced because it differentiates the primary setae and pores from the secondary ones that are added through the ontogenetic development of the larva. Secondary setae often show specific variation in number, position and size that may also serve taxonomic and phylogenetic purposes. This is best illustrated by comparing the secondary chaetotaxy of the legs of selected species of the subfamily Hydroporinae.

The Hydroporinae is a large, heterogeneous grouping of minute to small dytiscid species (adult length 1.00–7.10 mm) comprised of ca. 131 genera worldwide

◄

Fig. 2.10 (continued) squares = additional setae or pore, i.e., not included in the ground plan pattern of Hydradephaga (see Table 2.5 for list of setae and pores)

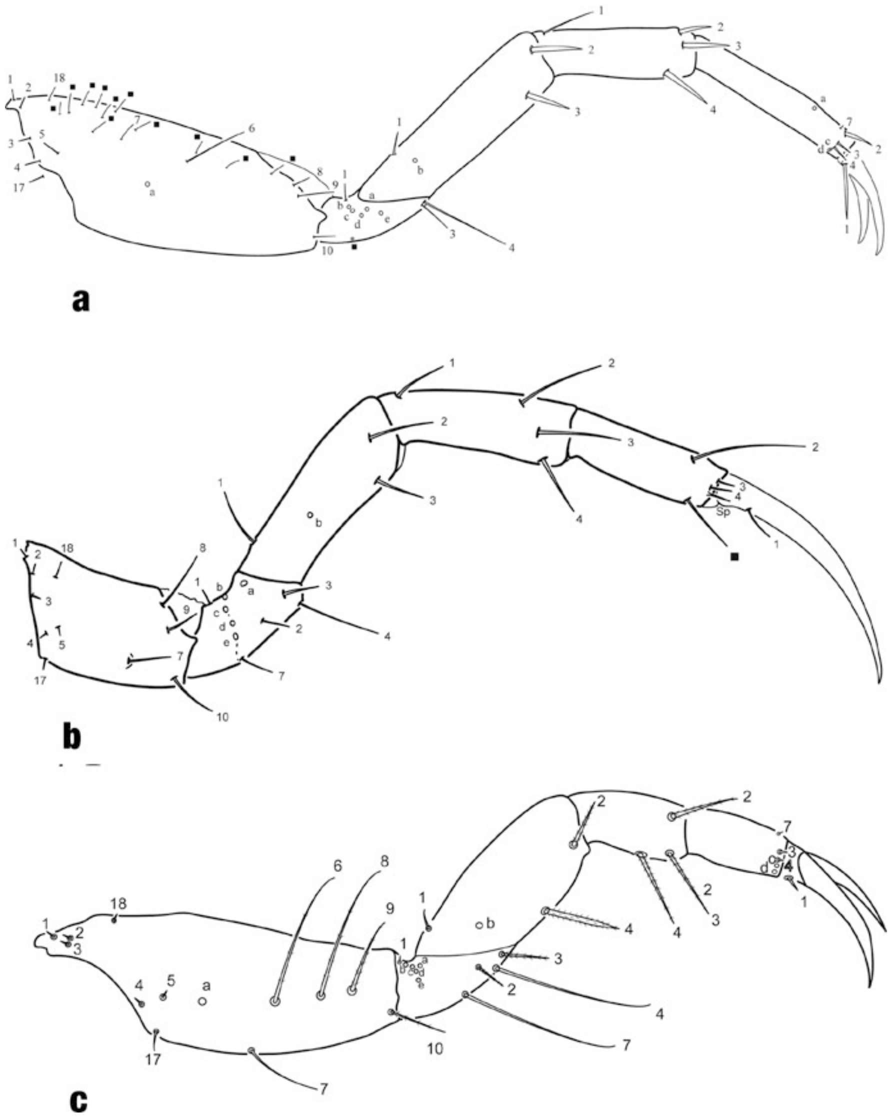

Fig. 2.11 Distribution of ancestral setae and pores on the anterior surface of the metathoracic leg of first instars of selected Hydradephaga families: (**a**) Gyrinidae: *Enhydrus sulcatus* (Wiedemann, 1821); (**b**) Haliplidae: *Haliplus indistinctus* Zimmermann, 1928; (**c**) Noteridae: *Suphisellus nigrinus* (Aubé, 1838). *CO* coxa, *FE* femur, *PT* pretarsus, *TA* tarsus, *TI* tibia, *TR* trochanter; numbers and lowercase letters refer to primary setae and pores, respectively; filled squares = additional setae or pore, i.e., not included in the ground plan pattern of Hydradephaga (see Table 2.5 for list of setae and pores)

Table 2.5 Ancestral setae and pores on the legs of first instars of Hydradephaga families: *ASP* Aspidytidae, *DYT* Dytiscidae, *GYR* Gyrinidae, *HAL* Haliplidae, *HYG* Hygrobiidae, *MER* Meruidae, *NOT* Noteridae

Setae/pores	DYT	ASP	GYR	HAL	HYG	MER	NOT
CO1	1	1	1	1	1	1	1
CO2	1	1	1	1	1	1	1
CO3	1	1	1	1	1	1	1
CO4	1	1	1	1	1	1	1
CO5	1	1	1	1	1	1	1
CO6	1	1	1	0	1	1	1
CO7	1	1	1	1	1	1	1
CO8	1	1	1	1	1	1	1
CO9	1	1	1	1	1	1	1
CO10	1	1	1	1	1	1	1
CO11	1	1	1	1	1	1	1
CO12	1	1	1	1	1	0	1
CO13	1	1	1	1	1	1	1
CO14	1	1	1	1	1	1	1
CO15	1	1	1	1	1	1	1
CO16	1	1	1	1	1	1	1
CO17	1	1	1	1	1	1	1
C018	1	1	1	1	0	1	1
COa	0/1	1	1	0	1	1	1
COc	0	0	0	0	0	0	1
COd	1	1	1	1	1	0	1
TR1	1	1	1	1	1	1	1
TR2	0/1	1	0/1	1	0	1	1
TR3	0/1	1	1	1	1	1	1
TR4	1	1	1	1	1	1	1
TR5	1	1	1	1	1	1	1
TR6	1	1	1	1	1	1	1
TR7	1	1	1	1	1	1	1
TRa	1	1	1	1	1	1	1
TRb	1	1	1	1	1	0	1
TRc	1	1	1	1	1	1	1
TRd	1	1	1	1	1	1	1
TRe	1	1	1	1	1	1	1
TRf	1	1	1	1	1	1	1
TRg	1	1	1	1	1	1	1
FE1	1	1	1	1	1	1	1
FE2	1	1	1	1	1	1	1
FE3	1	1	1	1	1	1	1
FE4	0/1	1	1	1	1	1	1
FE5	0/1	1	1	1	1	1	1
FE6	1	1	1	1	1	1	1

(continued)

Table 2.5 (continued)

Setae/pores	DYT	ASP	GYR	HAL	HYG	MER	NOT
FE7	1	1	0	0	1	0	0
FE8	1	1	0	0	1	0	0
FE9	1	1	0	0	1	0	0
FE10	1	1	0	0	1	0	0
FEa	0/1	1	0	0	1	0	0
FEb	1	1	1	1	1	0	1
TI1	1	1	1	1	1	1	1
TI2	0/1	1	1	1	1	1	1
TI3	1	1	1	1	1	1	1
TI4	1	1	1	1	1	1	1
TI5	1	1	1	1	1	1	1
TI6	0/1	1	1	1	1	1	1
TI7	1	1	1	1	1	1	1
TIa	1	1	1	1	1	1	1
TA1	1	0/1	1	1	1	0	1
TA2	1	1	1	1	1	1	1
TA3	1	1	1	1	1	1	1
TA4	1	1	1	1	1	1	1
TA5	1	1	1	1	1	1	1
TA6	1	1	1	1	1	1	1
TA7	1	1	1	1	1	1	1
TAa	1	1	1	1	1	0	1
TAb	1	1	1	1	1	1	0/1
TAc	0/1	1	1	0	1	0	1
TAd	0/1	1	1	0	1	0	1
TAe	0/1	1	1	0	1	0	1
TAf	0/1	1	1	0	1	0	1
PT1	0/1	1	1	1	1	1	1
PT2	0/1	1	1	1	1	1	1

CO coxa, *FE* femur, *PT* pretarsus, *TA* tarsus, *TI* tibia, *TR* trochanter; 0 = absent; 1 = present

(Nilsson and Hájek 2022). In term of primary setae and pores, the Hydroporinae legs show a pretty consistent pattern, including 50 setae and 18 pores (Table 2.3). Larvae of Hydroporinae, however, are quite variable in regard to both the number and the shape of secondary setae. Indeed, some species (e.g., *Heterosternuta sulphuria* (Matta & Wolfe, 1979) (Alarie and Longing 2010) and *Paroster couragei* Watts, 1978 (Alarie et al. 2009) are characterized by the presence of secondary spine-like setae, which may vary both in position and number (Fig. 2.12a and b). Other species, such as *Antiporus uncifer* Sharp, 1882 (Alarie and Watts 2004), differ from those species in that here a variable number of elongate and hair-like setae (which are deemed to play a role at enhancing the swimming ability and as such are called 'natatory setae') are added in addition to the secondary spine-like setae (Fig. 2.12c).

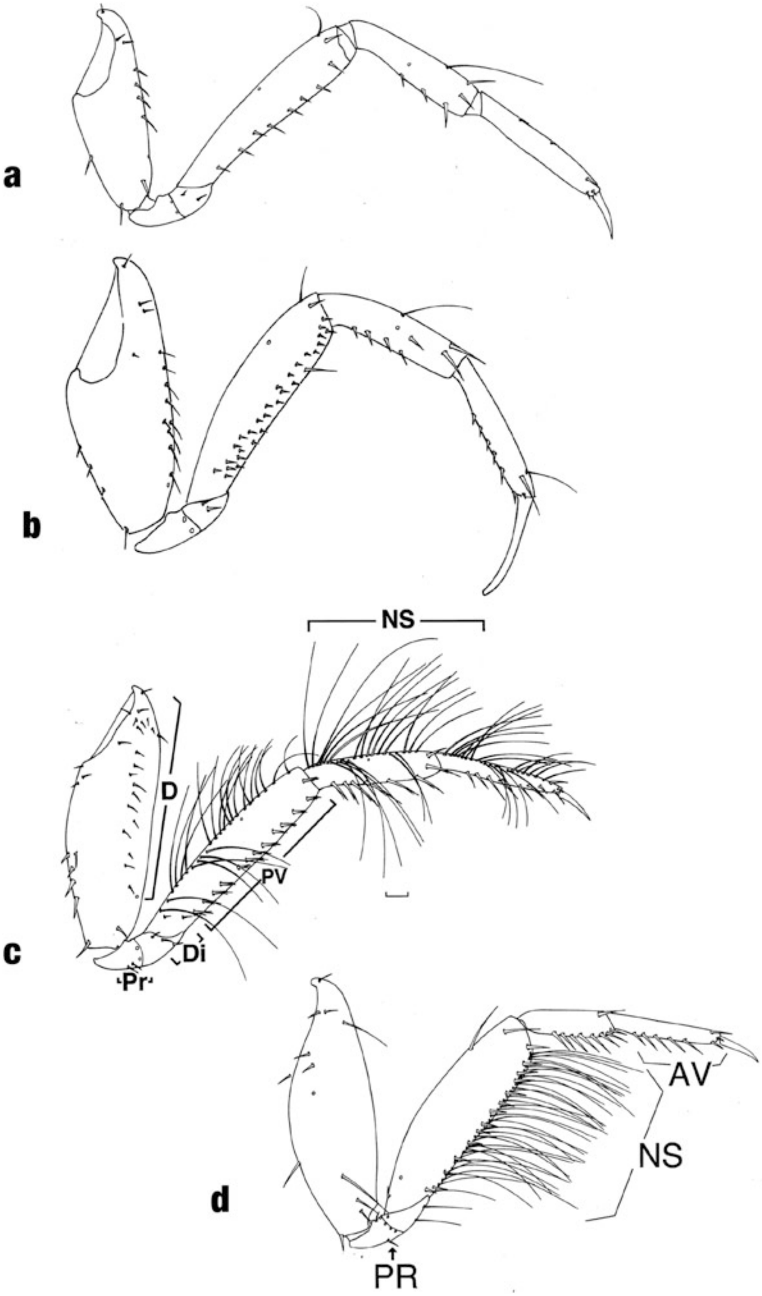

Fig. 2.12 Secondary setae on posterior surface of metathroracic legs of selected species of Hydroporinae: (**a**) *Heterosternuta sulphuria* (Matta & Wolfe, 1979); (**b**) *Paroster couragei* Watts, 1978; (**c**) *Antiporus uncifer* Sharp, 1882; (**d**) *Pachydrus obniger* (Chevrolat, 1863). *D* dorsal, *Di* distal, *NS* natatory setae, *Pr* proximal, *PV* posteroventral, *V* ventral

We stress that these natatory setae may also vary both in number and position, some species being readily distinguished from others in that the natatory setae are restricted to the tibiae and tarsi only compared to the femora, tibiae and tarsi. One of the most intriguing character states in regards to the secondary leg chaetotaxy of the Hydroporinae, however, can be found within the tribe Pachydrini. Indeed, larvae of the genus *Pachydrus* Sharp, 1882 (Alarie and Megna 2006) differ from any other member of the Hydroporinae in that here, the secondary natatory setae are all articulated along the ventral margin of the femora (Fig. 2.12d).

2.7 Summary: Prospective Ideas

The study of the larval morphology of the Dytiscidae over the past 30 years demonstrated a combination of careful attention to detail, thorough consideration of understudied character sets, and appropriate application of phylogenetic theory and methodology can lead to significant advances in our understanding of biodiversity. Such research has demonstrated the power of larval morphology, with its inherent chaetotaxic analysis, as a tool for testing hypotheses of phylogenetic relationships not only of the Dytiscidae but also of other Hydradephaga. Such studies demonstrated that larval structures could be used in phylogenetic reconstruction as a surrogate to adult structures, which have been the traditional cornerstone of systematic biology and subsequent classifications. It is generally held that the more characters support a clade, the more plausible is the hypothesis that the clade represents a natural group (DeSalle and Brower 1997). A more rigorous and stable classification will result from combining different characters from many life stages (Williamson 1992; Wiley 1981). When a phylogenetic hypothesis is supported by several independent lines of evidence, we gain confidence in it as an estimate of phylogenetic history. There is a relative increase in the probability of a tree being true if separate hypotheses of phylogeny from various data sets are congruent with one another. It is an analogue to an increase in statistical power (Lanyon 1993). Thus far, many established views concerning the taxonomic structure of the Dytiscidae have been challenged (e.g., Alarie and Michat 2007b; Michat et al. 2007, 2017a). The continued analyses of larvae of these taxa and those of related groups may possibly lead to a revision of our views on how they are taxonomically organized.

One item of practical significance in studying larval morphology is that association of aquatic beetle larvae with adults has the potential to make the wealth of characters present in the larval stage available for ecological and evolutionary study (e.g., Arnott et al. 2006; Belzile et al. 2006). From an applied viewpoint, the many aquatic ecologists who employ dytiscid beetles in their studies are now in a position to interpret their results from an evolutionary perspective A central tenet emerging from historical analyses of the evolution of morphology is that hypotheses about how these general patterns are generated may only be tested within an explicit phylogenetic framework, which has been the main output of the research conducted on the larval morphology of the beetle family Dytiscidae and other Hydradephaga over the past recent years.

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Yves Alarie obtained his PhD from the Université de Montréal, Québec, Canada in 1990. From 1990 to 1992, he holds a Natural Sciences Engineering Research Council of Canada (NSERC) Postdoctoral Fellowship in the Department of Entomology at the University of Manitoba, Canada; he is now a Full Professor in the Department of Biology, Laurentian University, Sudbury, Ontario, Canada.

Mariano C. Michat was born in Buenos Aires, Argentina. He attended Buenos Aires University, where he received in 2007 the Doctoral degree in Biological Sciences. In 2008 he started his career as a researcher for the Argentinean Research Council (CONICET).

Chapter 3
The Phylogeny and Classification of Predaceous Diving Beetles (Coleoptera: Dytiscidae)

Kelly B. Miller and Johannes Bergsten

Abstract The phylogenetics and higher (family-group) classification of extant members of the beetle family Dytiscidae (Coleoptera), or predaceous diving beetles, is reviewed and reassessed. A phylogenetic analysis of the family is presented based on 168 species of diving beetles and 9 outgroup taxa from Gyrinidae, Noteridae, Amphizoidae, and Paelobiidae. All currently recognized dytiscid subfamilies and tribes are represented, most by multiple genera and species. Data include 104 morphological characters and approximately 6700 aligned bases from 9 DNA sequence fragments from *cytochrome c oxidase I* (COI) and *II* (COII), *histone III* (H3), *16S rRNA* (16S), *12S rRNA* (12S), *arginine kinase* (argkin), *RNA polymerase II* (RNA pol II), *elongation factor 1 alpha* (Ef1α), and *wingless* (wnt). Parsimony and Bayesian analyses were conducted. The topology of the parsimony tree (consensus of 13 equally-parsimonious solutions) exhibits numerous anomalies inconsistent with convincing morphological features and the Bayesian results and has, generally, relatively poor bootstrap support for major clades. The Bayesian topology is more consistent with major morphological features and has strong support for most clades, and conclusions are based primarily on this estimate. Major higher-level phylogenetic relationships with strong support include: (1) monophyly of Dytiscidae Leach,

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[Note that Sects. 3.1–3.4 of this chapter (including tables and figures) represent a semi-reprint of the original study from the first edition of the book in 2014, only typos, misspellings, and formatting errors corrected [in few cases updates added in square brackets]. In contrast, the classification Sects. 3.5–3.7 have been updated in light of later studies. New taxonomic changes in the original study are referenced here (e.g., in the abstract), but are no longer regarded as new, and no new taxonomic changes are introduced in the current version of this chapter.]

K. B. Miller (✉)
Department of Biology and Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM, USA
e-mail: kbmiller@unm.edu

J. Bergsten
Department of Zoology, Swedish Museum of Natural History, Stockholm, Sweden
e-mail: johannes.bergsten@nrm.se

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(2) Matinae Branden sister to the rest of Dytiscidae, (3) Agabinae Thomson + Colymbetinae Erichson, (4) Hydrodytinae Miller + Hydroporinae Aubé, (5) Dytiscinae Leach + Laccophilinae Gistel + Cybistrini Sharp + Copelatinae Branden, (6) monophyly of the subfamilies Matinae, Colymbetinae, Copelatinae, Coptotominae Branden, Lancetinae Branden, Laccophilinae (including *Agabetes* Crotch), Agabinae (support weaker than in other subfamilies) and Hydroporinae (monophyly of Hydrodytinae not tested), (7) paraphyly of Dytiscinae with Cybistrini sister to Laccophilinae (with strong support) and this clade sister to other Dytiscinae, and (8) monophyly of both Agabini (*Agabus*-group of genera) and Hydrotrupini Roughley (*Hydrotrupes* Sharp and the *Platynectes*-group of genera). Major conclusions regarding tribes within Hydroporinae include: (1) monophyly of the tribes Vatellini Sharp, Methlini Branden, Hydrovatini Sharp, Hygrotini Portevin, Hyphydrini Gistel (without *Pachydrus* Sharp) and Bidessini Sharp (including *Peschetius* Guignot, *Hydrodessus* J. Balfour-Browne and *Amarodytes* Régimbart) (monophyly of Laccornini Wolfe and Roughley and Pachydrini Biström, Nilsson and Wewalka not tested), (2) Pachydrini is a problematic, long-branched taxa resolved here as sister to Hydrovatini but with weak support, (3) Hydroporini monophyletic except for *Laccornellus* Roughley and Wolfe and *Canthyporus* Zimmermann, (4) *Laccornellus* and *Canthyporus* together monophyletic and sister to Hydroporinae except Laccornini. Four groups are resolved within Hydroporini exclusive of *Laccornellus* + *Canthyporus* corresponding to the *Deronectes*-, the *Graptodytes*-, the *Necterosoma*- and the *Hydroporus*-groups of genera. The classification of Dytiscidae is revised with the following taxonomic changes [2014]: (1) Hydrotrupini is recognized as a tribe of Agabinae including the genus *Hydrotrupes* and the *Platynectes*-group of genera, (2) the genus *Rugosus* García is moved from Colymbetinae to Copelatinae, (3) Cybistrini is elevated from tribe rank within Dytiscinae to subfamily of Dytiscidae, (4) Hyderodini Miller is placed as a junior synonym of Dytiscini, (5) *Laccornellus* and *Canthyporus* are removed from Hydroporini and placed in their own tribe, Laccornellini, (6) the following family-group names are resurrected from synonymy with Hydroporini and placed as subtribes within Hydroporini, Deronectina Galewski (for the *Deronectes*-group of genera), Siettitiina Smrž (for the *Graptodytes*-group of genera), Sternopriscina Branden (for the *Necterosoma*-group of genera), and Hydroporina (for the *Hydroporus*-group of genera), (7) Carabhydrini Watts is placed as a junior synonym of Sternopriscina, and (8) *Hydrodessus*, formerly *incerta sedis* with respect to tribe, is placed in Bidessini. Each subfamily, tribe and subtribe is diagnosed and its taxonomic history discussed.

Keywords Phylogenetics · Taxonomy · Classification · Water beetles · Evolution

3.1 Introduction

3.1.1 History of Dytiscidae systematics

The 10th edition of the *Systema Naturae* (Linnaeus 1758) included *Dytiscus* Linnaeus among the 25 original genera of Coleoptera with 15 species, though several of these are today not recognized as closely related to Dytiscidae. Continued taxonomic work in the early nineteenth century included descriptions of numerous new taxa by many workers, but especially Aubé (1838), Crotch (1873), Sahlberg (1875) and Régimbart (1879). Numerous more isolated or regional treatments added quite a few new species during this time as well.

Certainly the most significant advance in the history of predaceous diving beetle taxonomy and a very early effort at a phylogenetic classification was by David Sharp, the eminent British coleopterist (Sharp 1882). In this monumental work, he treated the entire family and presented a very early evolutionary understanding of dytiscids. He included about 1140 species, a great many of which are still recognized, and his concepts at or near the genus rank have largely withstood the tests of time, new taxa, changing theories and practice, and additional data. However, his higher taxonomic subdivisions, although deeply influential and persisting well into the twentieth century, have in recent years been shown, with a few exceptions, to not generally reflect the phylogeny. His higher classification divided the family Dytiscidae into series, tribes, and groups. His two series, *Dytisci Fragmentati* and *Dytisci Complicati*, were based on whether the metepisternum is separated from the mesocoxal cavity by the mesepimeron and metepisternum (the former) or reaches the mesocoxal cavity (the latter). His *Dytisci Fragmentati* included beetles in the currently recognized families Paelobiidae Erichson and Noteridae Thomson, but also two diving beetle groups, Vatellini Sharp (all now placed in a tribe of the subfamily Hydroporinae Aubé) and Laccophilini Gistel (all now in a tribe of the subfamily Laccophilinae). All other diving beetles (and the group now recognized as the family Amphizoidae LeConte) were placed in the series *Dytisci Complicati*. *Dytisci Complicati* included the “groups” Cybistrini, Dytiscini, and three tribes: Hydroporides, Colymbetides, and Hydaticides, each with several groups. Sharp’s concepts of higher groups strongly reflected the emphasis at that time on only one or a few characters for hypothesizing relationships as well as on a gradual evolutionary progression towards “perfection,” with cybistrines, in his opinion, near the apex.

The period between Sharp’s *magnum opus* and the development of cladistics (Hennig 1966) was marked by the addition of great numbers of new species and genera, largely within the received higher classification. Standing out as the most influential workers in this period were Maurice Régimbart (1895, 1899) (contemporary with David Sharp), Alois Zimmermann (1919, 1920, 1930, 1931, 1932, 1933, 1934) and his coauthor, Leopold Gschwendtner (Gschwendtner 1935, 1936, 1937, 1938, 1939), and Félix Guignot (1947, 1959a, b, 1961), each of whom also had numerous smaller works. In fact, the combined works by Sharp and Régimbart add up to 71% of the new Dytiscidae names in the period 1870–1909, and the combined productivity of Zimmermann and Guignot include 50% of the new names in the period 1910–1961 (Nilsson 2008). Paelobiidae (Hygrobiidae during this time) and Amphizoidae were separately recognized as their own families during this period, but Noteridae remained treated as a group within predaceous diving beetles.

Post-Hennigian understanding of diving beetle phylogeny and classification began mainly with Burmeister (1976) who was strongly influenced by Hennig. Burmeister focused especially on characters of the female reproductive tract (Burmeister 1976, 1980, 1990), and resulted in further clarification of this system and a few classification changes, including placement of *Agabetes*, previously in Colymbetinae, within Laccophilinae (following Nilsson (1989)) and recognition of Copelatinae as a group separate from Colymbetinae. Other influential morphological cladistic analyses of higher dytiscid taxa in the post-Hennigian period included those by Wolfe (1985, 1988), Beutel (1993, 1994), and Ruhnau (1986); Ruhnau and Brancucci (1984), who refined the classifications of several groups including changes to tribal classification within Hydroporinae, elevation of Lancetinae from Colymbetinae and other results. Beutel and Roughley (1987) presented more definitive evidence that Noteridae is not a close relative of Dytiscidae (with Amphizoidae and Paelobiidae closer to Dytiscidae than Noteridae), and few workers since have continued to recognize noterids as a dytiscid subfamily (but see, for example, Pederzani 1995).

Morphological evidence presented in a cladistic framework continued, and Miller (2000, 2001) summarized many of the known data and conducted some of the first, comprehensive cladistic analyses and revisions to the classification. His work included synonymy of Aubehydrinae with Dytiscinae (Miller 2000) and elevation of Copelatinae, Coptotominae, Matinae, and Agabinae from tribes within a demonstrably non-monophyletic Colymbetinae *sensu auctorum* (Miller 2001). A new subfamily, Hydrodytinae, was also erected (Miller 2001, 2002b). Most recent developments have included comprehensive molecular analyses (Ribera et al. 2002, 2008) or molecular and/or morphological analyses of certain, larger groups (e.g., Balke and Ribera 2004; Balke et al. 2004a, b; Miller 2003; Miller et al. 2007b, 2009a; Ribera et al. 2004).

Other prominent modern developments in dytiscid taxonomy include addition of large numbers of new species with over 4600 valid species now known (Nilsson and Hájek 2022), and probably many more awaiting collection and description from traditional habitats and bioregions. Nilsson-Örtman and Nilsson (2010) predicted a total species richness of around 5400 species, mainly resulting from an increase of species with small body size from the Neotropical, Oriental, and Australian regions. Many large genera (e.g., *Copelatus*, *Laccophilus*) await comprehensive revision that will probably result in description of new species. Recent discovery of new faunas in subterranean, phytotelmatic, hygropetric, and terrestrial habitats will likely result in continued increase in species numbers as these habitats become better collected. Dramatic progress on the larval life stage has been made (especially by Alarie and collaborators, e.g., Alarie 1995, 1998; Alarie et al. 1990, 1997, 1998, 2000, 2001b, 2002a, b, 2011; Alarie and Butera 2003; Alarie and Harper 1990; Alarie et al. 1990;

Alarie and Hughes 2006; Alarie and Michat 2007). Finally, a particularly useful modern world catalog of taxon names (Nilsson 2001, 2003a, b, 2004; Nilsson and Fery 2006) has standardized names and made the nomenclature accessible. The last world catalog was by Zimmermann (1920) and had become seriously out-of-date. Nilsson (2001) not only brought together an updated world catalog for the twenty-first century following the last ICZN code of nomenclature, but also embraced the latest phylogenetic results and scrutinized and consistently treated every original description since Linnaeus. This resulted in, among other things, a substantial number of reinterpreted years of publications. It is today the most highly cited work on Dytiscidae since its publication (Google Scholar). The most recent development is the improved digital dissemination of taxonomic information with all predaceous diving beetle taxon names now in the Integrated Taxonomic Information System based on Nilsson's (2001) work and subsequent updates.

3.1.2 Overview of Current Diving Beetle Classification

Diving beetle reclassification has made progress in recent years, though mainly within subfamilies or tribes. Rather than clarifying relationships among tribes and families, these analyses have tended to illuminate problems with these relationships instead. 10 or 11 dytiscid subfamilies are currently recognized (Larson et al. 2000; Miller 2001; Nilsson 2001). The largest, by far, is Hydroporinae, which includes ten tribes and over half the total species diversity in Dytiscidae (Fig. 3.1). Hydroporinae, as currently defined, is convincingly monophyletic (Miller 2001), but the tribes within it may not be, especially Hydroporini, which is a large, heterogeneous assemblage of genera. Dytiscinae is a subfamily well-supported by morphological characters from both adults and larvae (Alarie et al. 2011; Miller 2000, 2001), but recent molecular analyses have, in some cases, not recovered it as monophyletic with Cybistrini resolved elsewhere (Ribera et al. 2002, 2008). Other tribes within the group are seemingly monophyletic, but there is some ambiguity, especially about their relationships with each other (Alarie et al. 2011; Miller 2000, 2001, 2003; Ribera et al. 2002, 2008). Laccophilinae is a large subfamily, mainly because of the inclusion of two very large genera, *Laccophilus* and *Neptosternus*, with several other smaller genera. Placement of *Agabetes* as sister to all other laccophilines was proposed by Burmeister (1990) based on attributes of the female reproductive tract that was confirmed by Miller (2001) as well as Alarie et al. (2002b) based on larval characters, but disputed by Ribera et al. (2008).

Each of the remaining dytiscid subfamilies comprises taxa formerly placed in Colymbetinae. Lancetinae includes only the monophyletic genus *Lancetes*, with representatives in Australia and temperate or high elevations in South America (Ríha 1961; Ruhnau and Brancucci 1984; Watts 1978; Zimmermann 1924). Coptotominae also includes only a single distinctive genus, *Coptotomus*, with a few species restricted to North America. The three genera in Matinae, *Matus*,

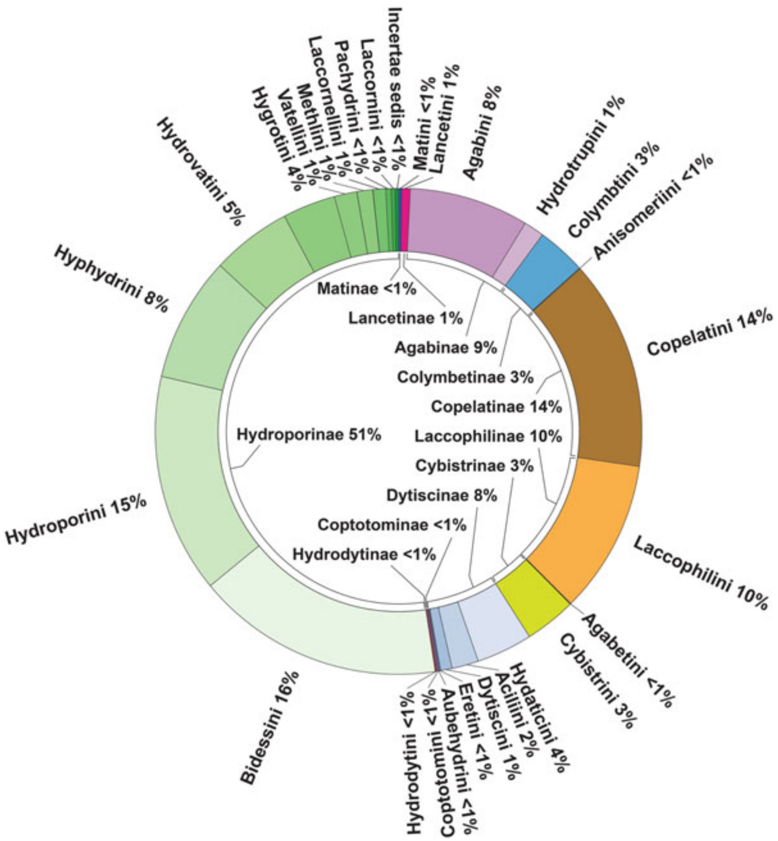

Fig. 3.1 The relative species richness of tribes and subfamilies into which the ~4200 [2022: ~4600] species of Dytiscidae are divided

Batrachomatus, and *Allomatus*, are together apparently monophyletic (Alarie and Butera 2003; Alarie and Watts 2003; Miller 2000), but have an unusual disjunct distribution with *Matus* in eastern North America and the other two genera in Australia. Copelatinae includes an extremely large number of primarily tropical species in several genera, with *Copelatus* and *Exocelina* among the largest dytiscid diversifications (Balke et al. 2007). The most recently described dytiscid subfamily, Hydrodytinae, includes species previously placed in the copelatine genus, *Agaporomorphus*, and is comprised of only two genera and a few, rare species (Miller 2002b). Agabinae, closely associated with Colymbetinae historically, includes a large number of primarily Holarctic genera and species but with several assigned genera found in the Neotropics, southeast Asia and Australia. *Hydrotrupes* was given its own subfamily by Roughley (2000), Hydrotrupinae, based largely on evidence from larva features presented by Beutel (1994). This genus was historically placed in Agabinae and was placed back into that subfamily by Miller (2001). Larval characters have shown some support for this conclusion as well (Alarie et al. 1998).

Hydrotrupes has been placed together with the "austral" agabines in some analyses (Ribera et al. 2004, 2008). Finally, what remains of the subfamily Colymbetinae under its modern definition (Miller 2001) is still quite a large group of several genera, with one, *Rhantus*, very large, heterogeneous, and found worldwide.

Although many of the currently recognized dytiscid subfamilies and tribes appear to be demonstrably monophyletic, relationships among these groups remain ambiguous or poorly supported, with few exceptions. Not only is diving beetle classification in need of a more well-founded phylogenetic hypothesis, but a better understanding of the phylogeny will dramatically enhance work on the evolution of dytiscid diversity, sexual selection, chemical evolution, biogeography, and evolutionary ecology, among other pursuits. The goal of this project is to establish, to the extent possible, a comprehensive phylogeny of extant diving beetles with broad taxon and data sampling and to revise the classification based on it, as needed, with emphasis on the family-group taxa.

3.2 Material and Methods

3.2.1 Taxon Sampling

3.2.1.1 Ingroup

One-hundred and sixty-eight species of diving beetles were included in the analysis (Table 3.1). All currently recognized subfamilies and tribes are represented, most by multiple exemplars. A single exemplar each is included for Hydrodytinae, Agabetini, Aubehydrini, Laccornini, Pachydrini, and Carabhydrini. Ninety-five dytiscid genera (~60% of the total) are represented.

3.2.1.2 Outgroup

Representatives from Amphizoidae, Paelobiidae, Noteridae, and Gyrinidae are included as outgroups (Table 3.1). Trees were rooted using Gyrinidae based on evidence that they may be sister to Hydradephaga (Ribera et al. 2002).

3.2.2 DNA

Whole genomic DNA was extracted using the Qiagen DNEasy kit (Valencia, California, USA) and the animal tissue protocol. Thoracic muscle tissue was taken from large specimens and extracted. Smaller specimens were extracted by removing the abdomen and placing the remaining portion of the specimen in buffer. The portions of the specimens remaining after extraction were retained for vouchering.

Table 3.1 Taxa used in phylogenetic analysis of Dytiscidae, collecting data, KBMC voucher numbers, and GenBank accession numbers for DNA sequences

Family	Subfamily	Tribe	Taxon title	Voucher	Locality	12S	COI	Arg Kin
						16S	COII	RNA Pol II
						H3	Ef1a	Wnt
Gyrinidae			*Spanglerogyrus albiventris*	KBMC Spal472	United States, Alabama, Conecuh Co., 23 km SE Evergreen along CR42, 31°20.059′N 86°47.641′W, 62 m, 12 May 2006, KB Miller, leg., KBM1205063	JX477995	JX478084	KJ548470
						---	JX478172	KJ548857
						JX477914	JX477836	---
Gyrinidae			*Dineutus ciliatus*	KBMC Dici474	United States, Alabama, Conecuh Co., 18.5 km SE Evergreen along CR42, 31°21.38′N 86°50.761′W, 92 m, 12 May 2006, KB Miller, leg., KBM1205062	JX477971	JX478060	---
						---	JX478148	KJ548821
						JX477893	JX477829	---
Gyrinidae			*Gyrinus elevatus*	KBMC Gyel494	Australia, New South Wales, Tuross River, 5 km W Bodalla, 36°8.7′S 149°59.083′E, 39 m, 2 Nov 2000, KB Miller, leg., KBM0211003	JX477977	JX478066	KJ548430
						---	JX478154	---
						JX477898	JX477831	---
Noteridae	*Hydrocanthinae*		*Hydrocanthus*	KBMC Hcsp570	Zambia, Northwestern Province, stream ~4 km NW Ikatu, 11°30.268′S 24°22.747′E, 1384 m, 7 Nov 2007, KB Miller, leg., KBM07110702	KJ548275	---	KJ548490
						KJ548381	KJ548630	KJ548872
						KJ548798	KJ548720	---
Noteridae			*Notomicrus*	KBMC Ncsp642	Venezuela, Amazonas, Communidad Cano Gato, Rio Sipapo, 4°58.838′N 67°44.341′W, 95 m, 16 Jan 2009, KB Miller, leg., VZ09011601	KJ548279	---	KJ548493
						KJ548385	---	---
						KJ548802	KJ548724	---

Noteridae			*Noterus clavicornis*	KBMC Nocl503	Italy, Sardinia, Nuoro Prov., nr Bultei, 40°25.613′N 9°5.149′E, 262 m, 16 Apr 2006, KB Miller, leg., KBM1604064	JX477985	JX478073	KJ548457
						KJ548358	JX478161	---
						JX477904	JX477834	---
Noteridae			*Tonerus wheeleri*	KBMC Tosp639	Venezuela, Amazonas, Tobogan de la Selva, 5°23.207′N 67°36.922′W, 125 m, 14 Jan 2009, KB Miller, leg., VZ09011401	KJ548278	KJ548556	KJ548492
						KJ548384	KJ548633	---
						KJ548801	KJ548723	---
Amphizoidae			*Amphizoa lecontei*	KBMC Amle58	United States, Colorado, Garfield Co., Rifle Falls State Park, East Rife Creek, 10 Aug 2000, KB Miller, leg., KBM100800	KJ548196	KJ548509	KJ548409
						AY071771	---	EU677585
						KJ548745	---	EU677662
Paelobiidae			*Hygrobia hermanni*	KBMC Haho504	Italy, Sardinia, Nuoro Prov., nr Bultei, 40°25.613′N 9°5.149′E, 262 m, 16 Apr 2006, KB Miller, leg., KBM1604064	JX477982	JX478071	KJ548431
						KJ548326	JX478159	KJ548826
						JX477902	---	KJ548151
Dytiscidae	Agabinae	Agabini	*Ilybius*	KBMC Agsp411	United States, Louisiana, Briarwood Preserve, 32°7.9′N 92°59.205′W, 2 Jun 2002, KB Miller, leg., AJA0206021	KJ548194	KJ548507	KJ548407
						KJ548304	KJ548576	KJ548813
						KJ548743	KJ548657	---
Dytiscidae	Agabinae	Agabini	*Agabus didymus*	KBMC Pmsp730	Italy, Sardinia, Nuoro Prov., 4.5 km SE Bottidda, 40°20.332′N 9°1.785′E, 200 m, 16 Apr 2006, KB Miller, leg., KBM1604063	KJ548286	---	---
						KJ548392	---	---
						KJ548809	KJ548727	---

(continued)

Table 3.1 (continued)

						12S	COI	Arg Kin
						16S	COII	RNA Pol II
Family	Subfamily	Tribe	Taxon title	Voucher	Locality	H3	Ef1a	Wnt
Dytiscidae	Agabinae	Agabini	*Agabus disintegratus*	KBMC Agdi412	United States, New Mexico, Hidalgo Co., Coronado Natl. For., Cloverdale Cr., 15 May, MF Whiting, leg.	KJ548192	KJ548505	---
						KJ548302	KJ548574	KJ548811
						KJ548741	KJ548655	---
Dytiscidae	Agabinae	Agabini	*Agabus tristis*	KBMC Agtr60	United States, Colorado, Summit Co., Bogan Flats, 39°26.7′N 106°19.9′W, 2784 m, 6 Aug 2000, KB Miller, leg., KBM0608001	KJ548195	KJ548508	KJ548408
						KJ548305	KJ548577	---
						KJ548744	KJ548658	AF392003
Dytiscidae	Agabinae	Agabini	*Ilybiosoma cordatum*	KBMC Agco57	United States, Colorado, Garfield Co., Rifle Falls State Park, East Rife Creek, 10 Aug 2000, KB Miller, leg., KBM100800	KJ548191	KJ548504	KJ548405
						KJ548301	KJ548573	---
						KJ548740	---	KJ548141
Dytiscidae	Agabinae	Agabini	*Ilybiosoma seriatum*	KBMC Agse413	United States, Utah, Utah Co., Diamond Fork Cyn, river approx 2 mi from turnoff, 29 Apr 2002, DC Ames, leg.	KJ548193	KJ548506	KJ548406
						KJ548303	KJ548575	KJ548812
						KJ548742	KJ548656	KJ548142
Dytiscidae	Agabinae	Agabini	*Ilybius fraterculus*	KBMC Ilfr409	United States, Utah,Utah Co., Goshen Ponds, 14 Jul 2000, JA Robertson, leg.	KJ548236	KJ548533	KJ548440
						KJ548342	KJ548603	---
						KJ548771	KJ548691	---
Dytiscidae	Agabinae	Agabini	*Ilybius gagates*	KBMC Ilga12	United States, New York, Tompkins Co., pond 10 km S Caroline, 10 Sep 1999, KB Miller, leg., KBM1009992	---	KJ548534	---
						---	---	---
						KJ548772	KJ548692	AF392002

Dytiscidae	Agabinae	Agabini	*Ilybius subaeneus*	KBMC Ilsu64	United States, Colorado, Summit Co., Homestake Creek, 39°24.617′N 106°25.35′W, 2798 m, 6 Aug 2000, KB Miller, leg., KBM0608002	KJ548237	KJ548535	KJ548441
						KJ548343	KJ548604	---
						KJ548773	---	AF392029
Dytiscidae	Agabinae	Hydrotrupini	*Platynectes*	KBMC Pysp457	French Guiana, Kaw Mt Res, Amazone Lodge, 4°33′N °12.66′W, 8 Feb 2005, KB Miller, leg., KBM0802051	KJ548256	KJ548543	---
						KJ548365	KJ548618	KJ548852
						KJ548784	KJ548707	---
Dytiscidae	Agabinae	Hydrotrupini	*Agametrus humilis*	KBMC Ansp760	Venezuela, Merida State, Bailadores, 8°14.393′N 71°48.672′W, 1862 m, Cascada Bailadores, stream margins, Short et al, leg., VZ09-0718-02A	---	---	KJ548398
						KJ548294	KJ548568	---
						KJ548733	KJ548647	KJ548138
Dytiscidae	Agabinae	Hydrotrupini	*Agametrus nitens*	KBMC Atsp759	Costa Rica, Cartago Province, Tapanti National Park, Kiri Lodge Trail, small stream in secondary forest, 17 Aug 2010, Short and Gustafson, legs, CR10-0317-SG1	---	---	KJ548397
						KJ548293	KJ548567	---
						KJ548732	KJ548646	KJ548137
Dytiscidae	Agabinae	Hydrotrupini	*Hydrotrupes palpalis*	KBMC Hppa371	United States, Oregon, Lane County, Seal Rock, seeps along cliff at beach, 44°30.217′N 124°4.9′W, 1 Sep 2001, KB Miller, leg., KBM01090101	KJ548223	KJ548526	KJ548435
						KJ548331	KJ548596	---
						KJ548764	KJ548678	KJ548154
Dytiscidae	Agabinae	Hydrotrupini	*Platynectes decemnotatus*	KBMC Plde130	Australia, Victoria, Stoney Creek, 5 km E Meeniyan, 38°35.733′S 146°4.5′W, 25 m, 30 Oct 2000, KB Miller, leg., KBM3010004	JX477993	JX478082	---
						---	JX478170	---
						JX477913	---	---

(continued)

Table 3.1 (continued)

						12S	COI	Arg Kin
						16S	COII	RNA Pol II
Family	Subfamily	Tribe	Taxon title	Voucher	Locality	H3	Ef1a	Wnt
Dytiscidae	Colymbetinae	Colymbetini	*Carabdytes upin*	KBMC Crup251	Papua New Guinea, Eastern Highlands Province, Crater Mtn Research Area, Miamafu Village, 8 Jul 2001, KB Miller, leg., FW0807011	KJ548208	KJ548519	KJ548421
						KJ548317	KJ548588	KJ548820
						KJ548756	KJ548666	KJ548149
Dytiscidae	Colymbetinae	Colymbetini	*Colymbetes fuscus*	KBMC Clsp476	Italy, Sardinia, Nuoro Prov., nr Bultei, 40°25.613′N 9°5.149′E, 262 m, 16 Apr 2006, KB Miller, leg., KBM1604064	KJ548205	KJ548516	KJ548419
						KJ548314	KJ548585	KJ548818
						KJ548753	KJ548664	KJ548147
Dytiscidae	Colymbetinae	Colymbetini	*Colymbetes exaratus*	KBMC Cbin410	United States, Utah, Utah Co., Payson, Payson Lakes, Box Lk., 15 Sep 2004, S Bybee, leg.	KJ548203	KJ548514	KJ548416
						KJ548312	KJ548583	KJ548816
						KJ548751	KJ548663	KJ548146
Dytiscidae	Colymbetinae	Colymbetini	*Colymbetes paykulli*	KBMC Cbpa59	United States, Colorado, Summit Co., Homestake Creek, 39°24.617′N 106°25.35′W, 2798 m, 6 Aug 2000, KB Miller, leg., KBM0608002	KJ548204	KJ548515	---
						KJ548313	KJ548584	---
						KJ548752	---	AF392005
Dytiscidae	Colymbetinae	Colymbetini	*Hoperius planatus*	KBMC Hopl453	United States, Delaware, New Castle Co., Frenchtown Woods Natural Area, 23 May 2004, AEZ Short, leg. AS-04-065	KJ548222	KJ548525	---
						KJ548330	KJ548595	KJ548829
						KJ548763	KJ548677	KJ548153

Dytiscidae	Colymbetinae	Colymbetini	*Meladema coriacea*	KBMC Mdco475	Italy, Sardinia, Nuoro Prov., river upstream from Lake Flumendosa, 39°58.943′N 9°24.722′E, 785 m, 18 Apr 2006, KB Miller, leg., KBM1804063	KJ548246	KJ548539	KJ548452
						KJ548354	KJ548612	KJ548845
						---	---	KJ548166
Dytiscidae	Colymbetinae	Colymbetini	*Neoscutopterus angustus*	KBMC Nean25	United States, New York, Tompkins Co., McLean Bog, 26 May 2000, KB Miller, leg., KBM2605002	KJ548249	KJ548540	---
						KJ548357	KJ548613	---
						---	KJ548703	AF392035
Dytiscidae	Colymbetinae	Colymbetini	*Rhantus atricolor*	KBMC Rhat10	United States, Arizona, Cochise Co., Rucker Canyon, 31°45.067′N 109°22′W, 26 Apr 2000, KB Miller, leg., KBM2604001	KJ548257	KJ548544	---
						KJ548366	KJ548619	KJ548854
						KJ548785	KJ548708	KJ548169
Dytiscidae	Colymbetinae	Colymbetini	*Rhantus binotatus*	KBMC Rhbi3	United States, Arizona, Cochise Co., Rucker Canyon, 31°45.067′N 109°22′W, 26 Apr 2000, KB Miller, leg., KBM2604001	KJ548258	KJ548545	KJ548465
						KJ548367	KJ548620	KJ548855
						KJ548786	KJ548709	AF392040
Dytiscidae	Colymbetinae	Colymbetini	*Meridiorhantus calidus*	KBMC Rhca262	Peru, Madre de Dios, Explorers Inn, 12°50.208′S 69°17.603′W, 10 Dec 2003, KB Miller, leg., KBM1012031	KJ548259	KJ548546	KJ548466
						KJ548368	KJ548621	---
						KJ548787	---	KJ548170
Dytiscidae	Colymbetinae	Colymbetini	*Meridiorhantus sinuatus*	KBMC Rhsi17	United States, New York, St Lawrence Co., Macomb Twp, Fish Cr Marsh, 44°28.333′N 75°33.8′W, 23 May 2000, KB Miller, leg., KBM2305001	KJ548260	KJ548547	KJ548467
						KJ548369	KJ548622	---
						KJ548788	KJ548710	AF392041

(continued)

Table 3.1 (continued)

Family	Subfamily	Tribe	Taxon title	Voucher	Locality	12S	COI	Arg Kin
						16S	COII	RNA Pol II
						H3	Ef1a	Wnt
Dytiscidae	Colymbetinae	Colymbetini	*Meridiorhantus validus*	KBMC Rhsp408	Chile, Prov. Llanquihue, Rio Blanco nr Cascadas, 21 Dec 2001, Whiting, Ogden, Terry, leg.	KJ548261	KJ548548	KJ548468
						KJ548370	KJ548623	KJ548856
						KJ548789	---	KJ548171
Dytiscidae	Copelatinae	Copelatini	*Agaporomorphus silvaticus*	KBMC Apsy268	Peru, Madre de Dios, Posadas Amazonas, 12°48.133′N 69°17.983′W, 13 Dec 2003, KB Miller, leg., KBM13120301	KJ548198	KJ548510	KJ548412
						KJ548307	KJ548579	---
						---	KJ548660	KJ548144
Dytiscidae	Copelatinae	Copelatini	*Copelatus distinctus*	KBMC Cpdi9	United States, Arizona, Santa Cruz Co., Hanshaw Cr., Patagonia, 31°27.25′N 110°43.283′W, 27 Apr 2000, KB Miller, leg., KBM2704001	KJ548207	KJ548518	KJ548420
						KJ548316	KJ548587	KJ548819
						KJ548755	KJ548665	KJ548148
Dytiscidae	Copelatinae	Copelatini	*Exocelina australiae*	KBMC Cpau126	Australia, New South Wales, 6 km SW Braidwood, 35°29.867′S 149°44.15′E, 2 Nov 2000, KB Miller, leg., KBM0211001	KJ548206	KJ548517	---
						KJ548315	KJ548586	---
						KJ548754	---	AF392006
Dytiscidae	Copelatinae	Copelatini	*Lacconectus andrewesi*	KBMC Lsan768	India, Maharashtra, Amboli, 15°55.843′N 73°57.836′E, 3 Oct 2004, KB Miller, leg., KBM0310041	KJ548185	---	KJ548400
						KJ548298	---	---
						KJ548737	---	KJ548139
Dytiscidae	Copelatinae	Copelatini	*Lacconectus regimbarti*	KBMC Lsre769	India, Karnataka, forest stream 30 km E Udupi, 04 Oct 2004, KB Miller, leg.	KJ548186	KJ548501	KJ548401
						KJ548299	KJ548571	---
						KJ548738	KJ548650	KJ548140

Dytiscidae	Copelatinae	Laccornini	*Laccornis difformis*	KBMC Ladi16	United States, New York, St Lawrence Co., Macomb Twp, Fish Cr Marsh, 44°28.333′N 75°33.8′W, 23 May 2000, KB Miller, leg., KBM2305001	KF575591	KF575484	---
						KF575544	KF575441	KJ548835
						KF575363	KF575406	AF392030
Dytiscidae	Coptotominae	Coptotomini	*Coptotomus lenticus*	KBMC Ctle11	United States, New York, Tompkins Co., Ithaca, 42°26.433′N 76°29.8′W, 10 Nov 1999, KB Miller, leg., KBM1009991	KJ548209	KJ548520	---
						KJ548318	KJ548589	---
						KJ548757	KJ548667	AF392007
Dytiscidae	Coptotominae	Coptotomini	*Coptotomus venustus*	KBMC Ctsp530	United States, Alabama, Monroe Co., 3 km E Owassa, 31°29.791′N 86°51.184′W, 99 m, 11 May 2006, KB Miller, leg., KBM1105064	KJ548271	KJ548553	---
						KJ548377	KJ548626	KJ548866
						KJ548794	KJ548717	---
Dytiscidae	Cybistrinae	Cybistrini	*Cybister*	KBMC Cysp757	Venezuela: Amazonas State, 4°58.838′N 67°44.341′W, 95 m, Communidad Cano Gato on Rio Sipapo, 16 Jan 2009, Short, Miller, Camacho, Joly and Garcia, legs. VZ09-0116-01X	KJ548182	KJ548497	KJ548396
						KJ548291	KJ548566	---
						---	---	---
Dytiscidae	Cybistrinae	Cybistrini	*Cybister marginicollis*	KBMC Cyma416	Ghana, Volta Region, road between Nkwanta and Odumase, 8°15.537′N 0°26.562′E, 210 m, 15 Jun 2005, KB Miller, leg., KBM1506051	KJ548210	DQ813682	KJ548422
						KJ548319	DQ813785	---
						DQ813748	KJ548668	DQ813719
Dytiscidae	Cybistrinae	Cybistrini	*Cybister puncticollis*	KBMC Cypu21	Bolivia, Beni, Prov. Cercado, 2.8 km SW Trinidad, 14°50.017′S 64°55.867′W, 17 Jun 1999, KB Miller, leg., KBM1706991	KJ548211	DQ431201	KJ548423
						KJ548320	KJ548590	---
						KJ548758	KJ548669	AF392009

(continued)

Table 3.1 (continued)

Family	Subfamily	Tribe	Taxon title	Voucher	Locality	12S	COI	Arg Kin
						16S	COII	RNA Pol II
						H3	Ef1a	Wnt
Dytiscidae	Cybistrinae	Cybistrini	*Cybister tripunctatus*	KBMC Cytr102	Australia, Victoria, 10 km E Corryong, 36°10.683′S 148°2.867′E, 5 Nov 2000, KB Miller, leg., KBM0511001	KJ548212	DQ813687	KJ548424
						KJ548321	DQ813789	---
						---	---	AF392010
Dytiscidae	Cybistrinae	Cybistrini	*Megadytes fraternus*	KBMC Mefr300	Paraguay, Dpt. Alto Paraguay, Ea. Choroveca, 29 Nov 2002	KJ548247	DQ813698	KJ548453
						KJ548355	DQ813800	KJ548846
						DQ813764	KJ548701	DQ813728
Dytiscidae	Cybistrinae	Cybistrini	*Megadytes robustus*	KBMC Mero254	Peru, Madre de Dios, boat landing nr Infierno, 12°43.493′S 69°13.043′W, 14 Dec 2003, KB Miller, leg., KBM1412031	KJ548248	DQ813702	KJ548454
						KJ548356	DQ813803	KJ548847
						DQ813768	KJ548702	DQ813731
Dytiscidae	Cybistrinae	Cybistrini	*Onychohydrus scutellaris*	KBMC Onsc107	Australia, Victoria, 18.6 km W Casterton, 37°35.95′S 141°9.75′E, 11 Nov 2000, KB Miller, leg., KBM1111001	KJ548253	DQ813704	KJ548461
						KJ548362	DQ813805	---
						DQ813770	KJ548705	DQ813733
Dytiscidae	Cybistrinae	Cybistrini	*Spencerhydrus latecinctus*	KBMC Spla123	Australia, Victoria, 25 km W Casterton, 10 Jun 2000, KB Miller, leg., CHS Watts, leg.	KJ548264	DQ813705	KJ548471
						KJ548372	DQ813806	KJ548858
						DQ813771	---	AF392043
Dytiscidae	Cybistrinae	Cybistrini	*Spencerhydrus pulchellus*	KBMC Sppu108	Australia, Western Australia, Byenup Lagoon, Oct 2000, CHS Watts, leg.	KJ548265	DQ813706	KJ548472
						KJ548373	DQ813807	KJ548859
						DQ813772	KJ548713	DQ813734

Dytiscidae	Cybistrinae	Cybistrini	*Sternhydrus atratus*	KBMC Stat165	Australia, Queensland, Townsville, 27-30 Nov 2001, G. Svenson, leg.	KJ548252	DQ813703	KJ548460
						KJ548361	DQ813804	---
						DQ813769	---	DQ813732
Dytiscidae	Dytiscinae	Aciliini	*Aethionectes*	KBMC Aesp659	Cameroon, Center Region, Mbam Djerem Park, Ganga Camp, 60 km SE Tibati, 5°59.857′N 12°52.668′E, 19 Mar 2009, KB Miller, leg., KBM19030902	KJ548280	KJ548557	KJ548494
						KJ548386	---	KJ548875
						---	KJ548726	---
Dytiscidae	Dytiscinae	Aciliini	*Acilius abbreviatus*	KBMC Acab72	United States, Idaho, Fremont Co., Forest pool on N Big Spring Loop Rd., 44°30.372′N 111°16.56′W, 1957 m, 16 Sep 2000, KB Miller, leg., DLG1609001	KJ548188	KJ548502	KJ548403
						KF979078	KF978924	---
						KF978972	KJ548652	KF979001
Dytiscidae	Dytiscinae	Aciliini	*Acilius semisulcatus*	KBMC Acse7	United States, New York, Tompkins Co., pond 10 km S Caroline, 12 Oct 2000, KB Miller, leg., KBM2310002	KJ548189	DQ275307	---
						KF979085	KF978933	---
						DQ275323	KJ548653	AF391999
Dytiscidae	Dytiscinae	Aciliini	*Aethionectes apicalis*	KBMC Aeop406	South Africa, Eastern Cape Province, Dwesa NP, 24 Jan 2005, J Bergsten, leg.	KJ548190	KJ548503	KJ548404
						KF979088	KF978937	---
						KF978975	KJ548654	---
Dytiscidae	Dytiscinae	Aciliini	*Graphoderus liberus*	KBMC Grli69	United States, New York, Tompkins Co., Ringwood Preserve, 19 Sep 2000, KB Miller, leg., KBM1909001	KJ548218	DQ813693	KJ548429
						KF979094	DQ813795	---
						DQ813759	KJ548674	AF392016
Dytiscidae	Dytiscinae	Aciliini	*Sandracottus bakewellii*	KBMC Saba90	Australia, Northern Territory, Ormiston, CHS Watts, leg.	KJ548262	KJ548549	---
						KF979104	KF978953	---
						KF978986	KJ548711	KF979014
Dytiscidae	Dytiscinae	Aciliini	*Sandracottus dejeanii*	KBMC Sade328	India, Maharashtra, 16°34.992′N 73°35.221′E, 1 Oct 2004, KB Miller, leg., KBM0110041	KJ548263	KJ548550	KJ548469
						KJ548371	KF978954	---
						KJ548790	KJ548712	KJ548172

(continued)

Table 3.1 (continued)

						12S	COI	Arg Kin
						16S	COII	RNA Pol II
Family	Subfamily	Tribe	Taxon title	Voucher	Locality	H3	Ef1a	Wnt
Dytiscidae	Dytiscinae	Aciliini	*Thermonectus basillaris*	KBMC Thba210	United States, New York, Schuyler Co., Texas Hollow, 42°24.73′N 76°47.58′W, 358 m, 12 Sep 2002, KB Miller, leg., KBM120902	KJ548266	DQ431223	KJ548473
						KF979108	KF978958	KJ548860
						KF978991	---	KF979017
Dytiscidae	Dytiscinae	Aciliini	*Thermonectus nigrofasciatus*	KBMC Thni5	United States, Arizona, Cochise Co., Rucker Canyon, 31°45.067′N 109°22′W, 26 Apr 2000, KB Miller, leg., KBM2604001	KJ548267	KJ548551	KJ548474
						---	---	KJ548861
						KF978995	KJ548714	AF392047
Dytiscidae	Dytiscinae	Aubehydrini	*Notaticus fasciatus*	KBMC Nofa52	Bolivia, Beni, Prov. Cercado, 9.5 kmN Trinidad, 14°46.567′S 64°58′W, 17 Jun 1999, KB Miller, leg., KBM1706992	KJ548250	DQ431220	---
						KJ548359	KJ548614	---
						FJ796545	---	AF392036
Dytiscidae	Dytiscinae	Dytiscini	*Dytiscus marginalis*	KBMC Dymg168	Sweden, Västerbotten, Täfteåhalvön, 31 May 2003, J Bergsten, leg.	KJ548214	DQ813691	KJ548425
						KJ548323	DQ813793	KJ548823
						DQ813757	KJ548671	DQ813725
Dytiscidae	Dytiscinae	Dytiscini	*Dytiscus verticalis*	KBMC Dyve24	United States, New York, Tompkins Co., Ithaca, 42°26.433′N 76°29.8′W, 26 May 2000, KB Miller, leg., KBM2605001	KJ548215	DQ813692	KJ548426
						KJ548324	DQ813794	KJ548824
						DQ813758	---	AF392012
Dytiscidae	Dytiscinae	Dytiscini	*Hyderodes crassus*	KBMC Hdcr238	Australia, Western Australia, 6 kmS Pinjarra, 10 Mar 2003, CHS Watts, leg.	KJ548219	KJ548523	KJ548432
						KJ548327	KJ548593	---
						KJ548761	---	KJ548152

Dytiscidae	Dytiscinae	Dytiscini	*Hyderodes shuckardi*	KBMC Hdsh104	Australia, Victoria, 20 km W Cowwarr, 38°0.867′S 146°32.05′E, 7 Nov 2000, KB Miller, leg., KBM07110001	KJ548220	DQ813694	KJ548433
						KJ548328	DQ813796	KJ548827
						DQ813760	KJ548675	AF392018
Dytiscidae	Dytiscinae	Eretini	*Eretes australis*	KBMC Erau103	Australia, South Australia, 15 km N Kingston, 34°5.833′S 140°21′E, 12 Nov 2000, KB Miller, leg., KBM1311001	KJ548216	FJ796579	KJ548427
						KF979073	KF978919	---
						FJ796506	KJ548672	FJ796547
Dytiscidae	Dytiscinae	Eretini	*Eretes griseus*	KBMC Ergr105	Hong Kong, Lantau Ishland, S of Mui Wo, Ngau Kwu Wani, artificial pond, 19 Nov 2000, J. Bergsten, leg.	KJ548217	KJ548522	KJ548428
						KJ548325	KJ548592	KJ548825
						KJ548760	KJ548673	KJ548150
Dytiscidae	Dytiscinae	Hydaticini	*Hydaticus aruspex*	KBMC Hyar68	United States, New York, Schuyler Co., Texas Hollow, 42°24.73′N 76°47.58′W, 358 m, 6 Sep 2000, KB Miller, leg., KBM0609001	KJ548230	FJ796580	KJ548436
						KJ548337	FJ796627	---
						FJ796507	KJ548685	AF392019
Dytiscidae	Dytiscinae	Hydaticini	*Hydaticus cinctipennis*	KBMC Hyci20	United States, New York, St Lawrence Co., Macomb Twp, Fish Cr Marsh, 44°28.333′N 75°33.8′W, 23 May 2000, KB Miller, leg., KBM2305001	KJ548231	FJ796586	---
						KJ548338	FJ796633	---
						FJ796512	KJ548686	AF392021
Dytiscidae	Dytiscinae	Hydaticini	*Hydaticus exclamationis*	KBMC Hyex400	South Africa, Eastern Cape Province, 2 km N Sterkstroom, 31°30.233′S 26°32.160′E, 1414 m, 20 Jan 2005, J Bergsten, leg.	KJ548232	FJ796592	KJ548437
						KJ548339	FJ796639	KJ548832
						FJ796517	KJ548687	FJ796554

(continued)

Table 3.1 (continued)

						12S	COI	Arg Kin
						16S	COII	RNA Pol II
Family	Subfamily	Tribe	Taxon title	Voucher	Locality	H3	Ef1a	Wnt
Dytiscidae	Dytiscinae	Hydaticini	*Hydaticus fabricii*	KBMC Hyfa119	Philippines, Borocay, 11°57′N 121°56′E, 26 Sep 2000, JB2609001	KJ548233	FJ796593	KJ548438
						KJ548340	FJ796640	KJ548833
						FJ796518	KJ548688	AF392022
Dytiscidae	Dytiscinae	Hydaticini	*Hydaticus subfasciatus*	KBMC Hysu23	Bolivia, Dpto. Santa Cruz, Prov. Chiquitos, 2.7 km S San Jose, 17°52.333′S 60°44.467′W, 27 Jun 1999, KB Miller, leg., KBM2706991	KJ548234	FJ796618	---
						---	FJ796662	---
						FJ796539	KJ548689	FJ796574
Dytiscidae	Dytiscinae	Hydaticini	*Hydaticus xanthomelas*	KBMC Hyxa67	Bolivia, Dpto. Santa Cruz, Prov. Velasco, 1.5 km SE San Ignacio, 16°23.4′S 60°57.217′W, 24 Jun 1999, KB Miller, leg., KBM2406991	KJ548235	FJ796624	KJ548439
						KJ548341	FJ796668	KJ548834
						FJ796544	KJ548690	AF392028
Dytiscidae	Hydrodytinae	Hydrodytinin	*Hydrodytes opalinus*	KBMC Hzsp734	Venezuela: Amazonas State, 4°58.838′N 67°44.341′W, 95 m, Communidad Cano Gato on Rio Sipapo, 16 Jan 2009, Short, Miller, Camacho, Joly and Garcia, legs. VZ09-0116-01X	KJ548284	KJ548560	---
						KJ548390	KJ548638	---
						KJ548807	---	KJ548176
Dytiscidae	Hydroporinae	Bidessini	*Amarodytes*	KBMC Arsp282	Peru, Madre de Dios, Explorers Inn, 12°50.208′S 69°17.603′W, 12 Oct 2003, KB Miller, leg., KBM1012031	KF575580	KF575474	---
						KF575533	KF575431	---
						KF575352	---	---

Dytiscidae	Hydroporinae	Bidessini	*Amarodytes*	KBMC Arsp283	Peru, Madre de Dios, Explorers Inn, 12°50.208′S 69°17.603′W, 10 Nov 2003, KB Miller, leg., KBM1012031	KJ548199	KJ548511	KJ548413
						KJ548308	KJ548580	---
						KJ548747	KJ548661	---
Dytiscidae	Hydroporinae	Bidessini	*Anodocheilus*	KBMC Adsp696	Venezuela, Amazonas, ca 15 km S Puerto Ayacucho, 5°30.623′N 67°36.109′W, 110 m, 14 Jan 2009, KB Miller, leg., VZ09011403	KJ548282	KJ548558	---
						KJ548388	KJ548636	---
						KJ548805	---	KJ548175
Dytiscidae	Hydroporinae	Bidessini	*Hypodessus*	KBMC Hosp695	Venezuela, Amazonas, Communidad Cano Gato, Rio Sipapo, 4°58.838′N 67°44.341′W, 95 m, 16 Jan 2009, KB Miller, leg., VZ09011601	KJ548281	---	---
						KJ548387	KJ548635	---
						KJ548804	---	---
Dytiscidae	Hydroporinae	Bidessini	*Liodessus*	KBMC Ldsp755	Zambia, Northwestern Province, stream and meadow ~4 km NW Ikatu, 11°30.268′S 24°22.747′E, 1384 m, 7 Nov 2007, KB Miller, leg., KBM07110702	KJ548181	KJ548496	KJ548395
						KJ548290	---	---
						KJ548730	---	---
Dytiscidae	Hydroporinae	Bidessini	*Neobidessus*	KBMC Nesp698	Venezuela, Apure St., 7°38.660′N 69°18.004W, 90 m, btwn “La Ye” and Bruzual, 18 Jan 2009, Short, Garcia, Camacho, legs., VZ09-0118-03x	KJ548283	KJ548559	---
						KJ548389	KJ548637	---
						KJ548806	---	---
Dytiscidae	Hydroporinae	Bidessini	*Bidessonotus inconspicuus*	KBMC Biin82	United States, New York, Elmira, Aug 2001, KB Miller, leg.	KJ548201	KJ548513	KJ548415
						KJ548310	KJ548582	KJ548815
						KJ548749	---	---

(continued)

Table 3.1 (continued)

						12S	COI	Arg Kin
						16S	COII	RNA Pol II
Family	Subfamily	Tribe	Taxon title	Voucher	Locality	H3	Ef1a	Wnt
Dytiscidae	Hydroporinae	Bidessini	*Hydrodessus*	KBMC Husp735	Venezuela: Amazonas State, 4°58.838′N 67°44.341′W, 95 m, Communidad Cano Gato on Rio Sipapo, 16 Jan 2009, Short, Miller, Camacho, Joly and Garcia, legs. VZ09-0116-01X	KJ548285	KJ548561	---
						KJ548391	KJ548639	---
						KJ548808	---	---
Dytiscidae	Hydroporinae	Bidessini	*Limbodessus compactus*	KBMC Lbco272	Australia, Victoria, 10 km E Lakes Entrance, 34°48.133′S 148°29.617′E, 31 Oct 2000, KB Miller, leg., KBM3110001	KJ548240	KJ548536	KJ548444
						KJ548346	KJ548607	---
						KJ548775	---	KJ548161
Dytiscidae	Hydroporinae	Bidessini	*Liodessus affinis*	KBMC Liaf132	United States, New York, Tompkins Co., Ringwood Preserve, 10 Apr 2001, KB Miller, leg., KBM1005011	KF575592	KF575485	KJ548450
						KF575545	KF575442	---
						KF575364	---	---
Dytiscidae	Hydroporinae	Bidessini	*Peschetius nodieri*	KBMC Peno438	Ghana, Volta Region, Kyabobo National Park border, on road from Koue Village, Togo Border, 8°31.218′N 0°36.153′E, 225 m, 14 Jun 2005, KB Miller, leg., KBM1406051	KJ548255	KJ548542	---
						KJ548364	KJ548617	KJ548850
						KJ548783	---	---
Dytiscidae	Hydroporinae	Bidessini	*Peschetius quadricostatus*	KBMC Pequ345	India, Maharashtra, 16°34.992′N 73°35.221′E, 1 Oct 2004, KB Miller, leg., KBM0110041	KF575599	KF575492	---
						KF575552	KF575449	KJ548851
						---	---	---

Dytiscidae	Hydroporinae	Bidessini	*Uvarus spretus*	KBMC Uvsp289	Costa Rica, Heredia, nr Puerto Viego, La Selva Biological Station, 10°25′N 84°0′W, 23 Feb 2004, KBM2302041	KJ548268	KJ548552	KJ548475
						KJ548374	KJ548624	---
						KJ548791	KJ548715	---
Dytiscidae	Hydroporinae	Hydroporini	*Antiporus blakeii*	KBMC Anbl541	Australia, Victoria, grassy roadside pool nr Carranballac, 03 Oct 2002, 37°42.758′S 142°45.868′E, KB Miller, leg.	KF575614	KF575503	KJ548483
						KF575566	KF575463	---
						KF575385	KF575423	KF575526
Dytiscidae	Hydroporinae	Hydroporini	*Antiporus femoralis*	KBMC Anfe100	Australia, New South Wales, 6 km SW Braidwood, 35°29.867′S 149°44.15′E, 12 Nov 2000, KB Miller, leg., KBM0211001	KF575579	KF575473	KJ548410
						KF575532	KF575430	---
						KF575351	---	KF575512
Dytiscidae	Hydroporinae	Hydroporini	*Barretthydrus geminatus*	KBMC Bage544	Australia, New South Wales, Deva River, 20 km NW Moruya, 35°53.046′S 144°58.756′E, 1 Oct 2002, KB Miller, leg., KBM0110021	KJ548272	FR733550	KJ548485
						KJ548378	KJ548627	KJ548869
						KJ548795	---	---
Dytiscidae	Hydroporinae	Hydroporini	*Carabhydrus niger*	KBMC Cani144	Australia, Tasmania, Dentention River, 0.2 km W Montumana, 27 Nov 2000, CHS Watts, leg.	KJ548202	---	---
						KJ548311	---	---
						KJ548750	---	---
Dytiscidae	Hydroporinae	Hydroporini	*Chostonectes gigas*	KBMC Chgi444	Australia, Victoria, nr Carranballac, 37°42.758′S 142°45.868′E, 3 Oct 2002, KB Miller, leg., KBM0310021	KF575582	KF575476	KJ548418
						KF575535	KF575433	---
						KF575354	KF575399	KF575513

(continued)

Table 3.1 (continued)

						12S	COI	Arg Kin
						16S	COII	RNA Pol II
Family	Subfamily	Tribe	Taxon title	Voucher	Locality	H3	Ef1a	Wnt
Dytiscidae	Hydroporinae	Hydroporini	*Chostonectes nebulosus*	KBMC Chne545	Australia, South Australia, Victoria R. at Williamstown, 12 Oct 2002, 34°40.372′S 138°53.448′E, KB Miller, leg.	KF575616	KF575504	KJ548486
						KF575568	KF575465	---
						KF575387	KF575425	---
Dytiscidae	Hydroporinae	Hydroporini	*Deronectes aubei*	KBMC Deau758	France: Pyr. Or. W Ptrats de Mollo, Torr. Parcigoule, 100m, 13 Aug 2001, H Fery, leg.	---	AF309326	---
						KJ548292	---	---
						KJ548731	KJ548645	---
Dytiscidae	Hydroporinae	Hydroporini	*Deronectes latus*	KBMC Dnla560	Sweden, Västerbotten, Umeå, Bölesholmarna, 6 Aug 2001, J Bergsten, leg.	KJ548274	---	KJ548488
						KJ548380	KJ548629	KJ548870
						KJ548797	KJ548719	---
Dytiscidae	Hydroporinae	Hydroporini	*Deronectes moestus*	KBMC Dcmo514	Italy, Sardinia, Nuoro Prov., river upstream from Lake Flumendosa, 39°58.943′N 9°24.722′E, 785 m, 18 Apr 2006, KB Miller, leg., KBM1804063	KF575609	KF575500	KJ548480
						KF575561	KF575458	KJ548865
						KF575380	KF575419	---
Dytiscidae	Hydroporinae	Hydroporini	*Ereboporus naturaconservatus*	KBMC Ebna538	United States, Texas, Terrell Co., Caroline Sp., 19 May 2007, Denton and Gibson, legs.	KF575613	KF575502	---
						KF575565	KF575462	KJ548868
						KF575384	KF575422	KF575525
Dytiscidae	Hydroporinae	Hydroporini	*Graptodytes ignotus*	KBMC Gpig575	Italy, Sardinia, Sassari Prov., 8 km N Aggius, 40°56.271′N 9°1.113′E, 521 m, 15 Apr 2006, KB Miller, leg., KBM1504064	KF575619	KF575507	KJ548491
						KF575571	KF575467	KJ548873
						KF575390	KF575427	KF575528

Dytiscidae	Hydroporinae	Hydroporini	*Haideoporus texanus*	KBMC Hite536	United States, Texas, Comal Co., Comal Springs Run 3, 8 Nov 2006, Gibson, leg.	KF575612	---	KJ548482
						KF575564	KF575461	---
						KF575383	KF575421	---
Dytiscidae	Hydroporinae	Hydroporini	*Heterosternuta pulchra*	KBMC Hepu136	United States, New York, Tompkins Co., Fall Cr., Ithaca, 15 Sep 1999, KB Miller, leg.	KF575585	KF575478	KJ548434
						KF575538	---	---
						KF575357	KF575401	---
Dytiscidae	Hydroporinae	Hydroporini	*Hydrocolus paugus*	KBMC Hlpa133	United States, New York, Tompkins Co., pond 10 km S Caroline, 16 May 2001, KB Miller, leg., KBM1005012	KF575586	KF575479	---
						KF575539	KF575436	---
						KF575358	KF575402	KF575515
Dytiscidae	Hydroporinae	Hydroporini	*Porhydrus*	KBMC Posp754	Italy, Sardinia, Nuoro Prov., Giara di Gestuari, 39°44.141′N 8°59.924′E, 589 m, 19 Apr 2006, KB Miller, leg., KBM1904061	KJ548180	---	---
						KJ548289	---	---
						---	KJ548644	---
Dytiscidae	Hydroporinae	Hydroporini	*Sanfilippodytes*	KBMC Sasp78	United States, Arizona, Santa Cruz Co., Santa Cruz River nr Beyerville, 31°25.233′N 100°55.25′W, 27 Apr 2000, KB Miller, leg., KBM2704002	KF575601	KF575494	---
						KF575554	KF575451	---
						KF575372	KF575413	KF575520
Dytiscidae	Hydroporinae	Hydroporini	*Hydroporus angustatus*	KBMC Hyan549	Russia, Volgograd Obl., Krasnoslobodsk, pond, 15 May 2001, J Bergsten, leg.	KF575624	KF575511	---
						KF575576	KF575471	---
						KF575395	---	---
Dytiscidae	Hydroporinae	Hydroporini	*Hydroporus obscurus*	KBMC Hyob550	Sweden, Västerbotten, Normjöle Kläppudden, 28 Jul 2001, J Bergsten, leg.	---	KJ548562	---
						KJ548393	KJ548640	---
						KJ548810	KJ548728	---
Dytiscidae	Hydroporinae	Hydroporini	*Hydroporus palustris*	KBMC Hypa548	Sweden, Härjedalen, Rogen, Hundviken, 30 Jun 2001, J Bergsten, leg.	KF575623	KF575510	---
						KF575575	KF575470	---
						KF575394	---	---

(continued)

Table 3.1 (continued)

						12S	COI	Arg Kin
						16S	COII	RNA Pol II
Family	Subfamily	Tribe	Taxon title	Voucher	Locality	H3	Ef1a	Wnt
Dytiscidae	Hydroporinae	Hydroporini	*Hydroporus umbrosus*	KBMC Hyum551	Sweden, Västerbotten, Normjöle Kläppudden, 28 Jul 2001, J Bergsten, leg.	KJ548287	KJ548563	---
						KJ548394	KJ548641	---
						---	---	KJ548177
Dytiscidae	Hydroporinae	Hydroporini	*Megaporus hamatus*	KBMC Mpha96	Australia, New South Wales, 6 km SW Braidwood, 35°29.867′S 149°44.15′E, 2 Nov 2000, KB Miller, leg., KBM0211001	KF575593	KF575486	KJ548455
						KF575546	KF575443	---
						KF575365	KF575407	KF575517
Dytiscidae	Hydroporinae	Hydroporini	*Megaporus howittii*	KBMC Meho547	Australia, Victoria, pond ~25 km S Casterton, 37°39.289′S 141°14.380′E, 3 Oct 2002, KB Miller, leg.	KF575617	KF575505	KJ548487
						KF575569	KF575466	---
						KF575388	---	---
Dytiscidae	Hydroporinae	Hydroporini	*Nebrioporus clarkii*	KBMC Nbcl513	Italy, Sardinia, Nuoro Prov., river upstream from Lake Flumendosa, 39°58.943′N 9°24.722′E, 785 m, 18 Apr 2006, KB Miller, leg., KBM1804063	KF575608	KF575499	KJ548479
						KF575560	---	KJ548864
						KF575379	KF575418	KF575523
Dytiscidae	Hydroporinae	Hydroporini	*Nebrioporus rotundatus*	KBMC Nbro563	United States, New York, Tompkins Co., Ithaca, 21 Jun 2001, KB Miller, leg.	KF575618	KF575506	KJ548489
						KF575570	---	KJ548871
						KF575389	KF575426	---
Dytiscidae	Hydroporinae	Hydroporini	*Necterosoma susanna*	KBMC Ntsu443	Australia, New South Wales, Deva River, 20 km NW Moruya, 35°53.046′S	KF575595	KF575488	KJ548456
						KF575548	KF575445	---

					144°58.756′E, 1 Oct 2002, KB Miller, leg., KBM0110021	KF575367	KF575408	KF575518
Dytiscidae	Hydroporinae	Hydroporini	*Necterosoma undecimlineatum*	KBMC Ntwa95	Australia, New South Wales, nr Brogo Dam, 36°32.067′S 149°46.7′E, 1 Nov 2000, KB Miller, leg., KBM0111001	KF575596	KF575489	KJ548459
						KF575549	KF575446	---
						KF575368	KF575409	---
Dytiscidae	Hydroporinae	Hydroporini	*Neoporus mellitus*	KBMC Npme532	United States, Alabama, Conecuh Co., 23 km SE Evergreen along CR42, 31°20.059′N 86°47.641′W, 62 m, 12 May 2006, KB Miller, leg., KBM1205063	KF575610	KF575501	KJ548481
						KF575562	KF575459	KJ548867
						KF575381	KF575420	KF575524
Dytiscidae	Hydroporinae	Hydroporini	*Nectoporus congruus*	KBMC Orco62	United States, Colorado, Pitkin Co., Crystal River nr Marble, 39°4.25′N 107°12.4′W, 1315 m, 2 Aug 2000, KB Miller, leg., KBM0208001	KF575597	KF575490	KJ548462
						KF575550	KF575447	KJ548849
						KF575369	KF575410	KF575519
Dytiscidae	Hydroporinae	Hydroporini	*Hornectes quadrimaculatus*	KBMC Orqu543	United States, Oregon, Lane Co., Heceta Head, Devil′s Elbow, Cape Cr., 01 Sep 2001, KB Miller, leg.	KF575615	---	KJ548484
						KF575567	KF575464	---
						KF575386	KF575424	KF575527
Dytiscidae	Hydroporinae	Hydroporini	*Oreodytes scitulus*	KBMC Orsc63	United States, Colorado, Pitkin Co., Crystal River nr Marble, 39°4.25′N 107°12.4′W, 1315 m, 2 Aug 2000, KB Miller, leg., KBM0208001	KF575598	KF575491	KJ548463
						KF575551	KF575448	---
						KF575370	KF575411	---
Dytiscidae	Hydroporinae	Hydroporini	*Paroster gibbi*	KBMC Psgi775	NCBI	---	FR732548	---
						AY353842	---	---
						EF670098	---	---

(continued)

Table 3.1 (continued)

						12S	COI	Arg Kin
						16S	COII	RNA Pol II
Family	Subfamily	Tribe	Taxon title	Voucher	Locality	H3	Ef1a	Wnt
Dytiscidae	Hydroporinae	Hydroporini	*Psychopomporus felipi*	KBMC Hysp731	United States, Texas, Val Verde Co., San Felipe Springs, Del Rio, 15 Aug 2008, Foley, leg.	KF575622	---	---
						KF575574	---	---
						KF575393	---	---
Dytiscidae	Hydroporinae	Hydroporini	*Rhithrodytes sexguttatus*	KBMC Rrse508	Italy, Sardinia, Nuoro Prov., 4.5 km SE Bottidda, 40°20.332′N 9°1.785′E, 200 m, 16 Apr 2006, KB Miller, leg., KBM1604063	KF575605	KF575497	KJ548477
						KF575557	KF575455	KJ548862
						KF575376	KF575415	KF575521
Dytiscidae	Hydroporinae	Hydroporini	*Stictonectes optatus*	KBMC Scop512	Italy, Sardinia, Nuoro Prov., river upstream from Lake Flumendosa, 39°58.943′N 9°24.722′E, 785m, 18 Apr 2006, KB Miller, leg., KBM1804063	KF575607	KF575498	KJ548478
						KF575559	KF575457	KJ548863
						KF575378	KF575417	KF575522
Dytiscidae	Hydroporinae	Hydroporini	*Stictonectes rufulus*	KBMC Scru511	Italy, Sardinia, Nuoro Prov., river upstream from Lake Flumendosa, 39°58.943′N 9°24.722′E, 785 m, 18 Apr 2006, KB Miller, leg., KBM1804063	KF575606	---	---
						KF575558	KF575456	---
						KF575377	KF575416	---
Dytiscidae	Hydroporinae	Hydroporini	*Clarkhydrus roffii*	KBMC Stro26	United States, Arizona, Santa Cruz Co., Santa Cruz River nr Beyerville, 31°25.233′N 100°55.25′W, 27 Apr 2000, KB Miller, leg., KBM2704002	KF575603	KF575495	---
						KF575555	KF575453	---
						KF575374	KF575414	---

Dytiscidae	Hydroporinae	Hydroporini	*Hydroporus dorsalis*	KBMC Sudo554	Sweden, Hälsingland, Övre Tälningsån, 2 Jul 2000, J Bergsten, leg.	KF575625	KF575507	---
						KF575577	KF575472	---
						KF575396	---	---
Dytiscidae	Hydroporinae	Hydrovatini	*Hydrovatus parallelipennis*	KBMC Hvsp458	Ghana, Volta Region, road between Nkwanta and Odumase, 8°15.537′N 0°26.562′E, 210 m, 15 Jun 2005, KB Miller, leg., KBM1506051	KJ548229	KJ548532	---
						KJ548336	KJ548602	---
						KJ548770	KJ548684	KJ548159
Dytiscidae	Hydroporinae	Hydrovatini	*Hydrovatus pustulatus*	KBMC Hvpu446	United States, New York, Tompkins Co., Pond 1 km SW Danby, 370 m, 42°20.825′N 26°29.233′W, 23 Sep 2002, KB Miller, leg.	KF575590	KF575483	---
						KF575543	KF575440	---
						KF575362	---	---
Dytiscidae	Hydroporinae	Hydrovatini	*Hydrovatus rufoniger*	KBMC Hvru559	Hong Kong: Lamna Island, Sok Kwu Wan: pond, 18 Sep 2000, J Bergsten, leg.	KJ548273	---	---
						KJ548379	KJ548628	---
						KJ548796	KJ548718	---
Dytiscidae	Hydroporinae	Hydrovatini	*Queda youngi*	KBMC Quyo455	Peru, Madre de Dios, boat landing ~20 km S Infierno, nr Puerto Maldonado, 14 Dec 2003, KB Miller, leg.	KF575600	KF575493	---
						KF575553	KF575450	KJ548853
						KF575371	KF575412	---
Dytiscidae	Hydroporinae	Hygrotini	*Hygrotus compar*	KBMC Htco365	United States, Wyoming, Natrona Co., salty pool, Sand Draw nr Waltman, 43°03.384′N 107°12.106′W, 17 Aug 2002, 1821 m, KB Miller, leg.	KJ548224	KJ548527	---
						KJ548332	KJ548597	---
						KJ548765	KJ548679	KJ548155

(continued)

Table 3.1 (continued)

						12S	COI	Arg Kin
						16S	COII	RNA Pol II
Family	Subfamily	Tribe	Taxon title	Voucher	Locality	H3	Ef1a	Wnt
Dytiscidae	Hydroporinae	Hygrotini	*Hygrotus diversipes*	KBMC Htdi360	United States, Wyoming, Natrona Co., Teapot Cr. ~10 km S Midwest, 43°19.204′N 106°14.311′W, 1534 m, 9 Aug 2002, KB Miller, leg.	KJ548225	KJ548528	---
						KJ548333	KJ548598	---
						KJ548766	KJ548680	KJ548156
Dytiscidae	Hydroporinae	Hygrotini	*Hygrotus impressopunctatus*	KBMC Htim80	United States, Colorado, Mesa Co., Buzzard Cr., pond, 2 May 2000, KB Miller, leg., KBM0205001	KJ548226	KJ548529	---
						KJ548334	KJ548599	---
						KJ548767	KJ548681	---
Dytiscidae	Hydroporinae	Hygrotini	*Hygrotus salinarius*	KBMC Htsa366	United States, Wyoming, Natrona Co., ~12 km S Midwest, salt pool, 43°17.633′N 106°16.375′W, 9 Aug 2002, KB Miller, leg.	KJ548228	KJ548531	---
						KJ548335	KJ548601	---
						KJ548769	KJ548683	KJ548158
Dytiscidae	Hydroporinae	Hygrotini	*Hygrotus inquinatus*	KBMC Hpin578	Namibia, Skeleton Coast NP, Uniab River, pools, 20°13.017′S 13°12.603′E, 14 May 2004, KB Miller, leg., KBM1405041	KF575620	KF575508	---
						KF575572	KF575468	---
						KF575391	KF575428	KF575529
Dytiscidae	Hydroporinae	Hygrotini	*Hygrotus rohani*	KBMC Hpsp574	Zambia, Northwestern Province, stream and meadow ~4 km NW Ikatu, 11°30.268′S 24°22.747′E, 1384 m, 7 Nov 2007, KB Miller, leg., KBM07110702	KJ548276	KJ548554	---
						KJ548382	KJ548631	---
						KJ548799	KJ548721	KJ548173

Dytiscidae	Hydroporinae	Hygrotini	*Herophydrus*	KBMC Hhsp752	Ghana, Volta Region, road between Nkwanta and Odumase, 8°15.537′N 0°26.562′E, 210 m, 15 Jun 2005, KB Miller, leg., KBM1506051	KJ548178	KJ548495	---
						---	KJ548564	---
						---	KJ548642	KJ548136
Dytiscidae	Hydroporinae	Hygrotini	*Cyclopius acaroides*	KBMC Htac370	United States, Alabama, Conecuh Co., 13 km E Evergreen along Hwy 31, Old Town Cr., 31°27.037′N 86°49.81′W, 53 m, 11 May 2006, KB Miller, leg., KBM1105061	KF575589	KF575482	---
						KF575542	KF575439	---
						KF575361	KF575405	KF575516
Dytiscidae	Hydroporinae	Hygrotini	*Clemnius decoratus*	KBMC Htdc582	Sweden, Öland, Borgholm, Runsten, pond, 7 July 2005, 6287995 1550690, J. Geijer leg. BMNH744139.	KJ548277	KJ548555	---
						KJ548383	KJ548632	---
						KJ548800	KJ548722	KJ548174
Dytiscidae	Hydroporinae	Hygrotini	*Hygrotus sayi*	KBMC Htsa34	United States, New York, Tompkins Co., Ithaca, 42°26.433′N 76°29.8′W, 15 Sep 1999, KB Miller, leg., KBM1509991	KJ548227	KJ548530	---
						---	KJ548600	---
						KJ548768	KJ548682	KJ548157
Dytiscidae	Hydroporinae	Hyphydrini	*Desmopachria convexa*	KBMC Dpco81	United States, New York, St Lawrence Co., Macomb Twp, Fish Cr Marsh, 44°28.333′N 75°33.8′W, 23 May 2000, KB Miller, leg., KBM2305001	KF575584	KF575477	---
						KF575537	KF575435	---
						KF575356	---	---
Dytiscidae	Hydroporinae	Hyphydrini	*Desmopachria portmanni*	KBMC Dppo139	United States, Arizona, Cochise Co., Bear Cr. Huachuca Mts, 31°22.796′N 110°21.814′W, 9 May 2003, KB Miller, leg., KBM0905031	KJ548213	KJ548521	---
						KJ548322	KJ548591	KJ548822
						KJ548759	KJ548670	---

(continued)

Table 3.1 (continued)

						12S	COI	Arg Kin
						16S	COII	RNA Pol II
Family	Subfamily	Tribe	Taxon title	Voucher	Locality	H3	Ef1a	Wnt
Dytiscidae	Hydroporinae	Hyphydrini	*Pachydrus*	KBMC Pasp761	Venezuela, Zulia St, 9°51.883′N 72°43.285′W, 96 m, btwn Machiques and Tukuko, 29 Jan 2009, Short, Garcia, Camacho, legs. VZ09-0129-03X	KJ548183	KJ548498	---
						KJ548295	KJ548569	---
						KJ548734	---	---
Dytiscidae	Hydroporinae	Hyphydrini	*Hyphydrus congoanus*	KBMC Hysp753	Zambia, Northwestern Province, Zambeze Source, 11°22.09′S 24°17.917′E, 1501m, 6 Nov 2007, KB Miller, leg., KBM06110701	KJ548179	---	---
						KJ548288	KJ548565	---
						KJ548729	KJ548643	---
Dytiscidae	Hydroporinae	Hyphydrini	*Hyphydrus elegans*	KBMC Hpel440	Australia, Northern Territory, 5 km S Alice Springs, 23°44.74′S 133°52.048′E, 8 Oct 2002, KB Miller, leg., KBM0810022	KF575587	KF575480	---
						KF575540	KF575437	KJ548830
						KF575359	KF575403	---
Dytiscidae	Hydroporinae	Hyphydrini	*Hyphydrus esau*	KBMC Hhsp643	Zambia, Copperbelt Province, nr Chimfunshi Wildlife Orphanage, ~40 km NW Chingola, 12°21.69′S 27°32.52′E, 1271 m, 1 Nov 2007, KB Miller, leg., KBM01110701	---	---	---
						---	KJ548634	KJ548874
						KJ548803	KJ548725	---

Dytiscidae	Hydroporinae	Hyphydrini	*Hyphydrus excoffieri*	KBMC Hpex441	China: Yunnan, 4 kmS Shisong, veg. rich lake, 11 Sep 2000, J Bergsten, leg.	KF575588	KF575481	---
						KF575541	KF575438	KJ548831
						KF575360	KF575404	---
Dytiscidae	Hydroporinae	Hyphydrini	*Hyphydrus signatus*	KBMC Hhsi450	Namibia, Skeleton Coast, Oasis Spring, 14 May 2004, 19°26.746′S 12°49.301′E, KB Miller, leg.	KJ548221	KJ548524	---
						KJ548329	KJ548594	KJ548828
						KJ548762	KJ548676	---
Dytiscidae	Hydroporinae	Hyphydrini	*Microdytes svensoni*	KBMC Mysv765	India, Karnataka, forest stream 30 km E Udupi, 4 Oct 2004, KB Miller, leg.	KF575578	---	---
						KF575531	---	---
						KF575350	KF575397	---
Dytiscidae	Hydroporinae	Laccornellini	*Canthyporus hottentottus*	KBMC Cnho772	NCBI	---	AJ850585	---
						AJ850333	---	---
						EF670118	---	---
Dytiscidae	Hydroporinae	Laccornellini	*Canthyporus nebulosus*	KBMC Cnne773	NCBI	---	AJ850587	---
						AJ850335	---	---
						EF670117	---	---
Dytiscidae	Hydroporinae	Laccornellini	*Canthyporus parvus*	KBMC Cnpa774	NCBI	---	EF056596	---
						EF056668	---	---
						EF056553	---	---
Dytiscidae	Hydroporinae	Laccornellini	*Laccornellus copelatoides*	KBMC Lccp771	NCBI	---	AY334247	---
						AY334131	---	---
						EF056578	---	---
Dytiscidae	Hydroporinae	Laccornellini	*Laccornellus lugubris*	KBMC Lclu770	NCBI	---	AJ850668	---
						AJ850421	---	---
						EF670227	---	---

(continued)

Table 3.1 (continued)

						12S	COI	Arg Kin
						16S	COII	RNA Pol II
Family	Subfamily	Tribe	Taxon title	Voucher	Locality	H3	Ef1a	Wnt
Dytiscidae	Hydroporinae	Methlini	*Celina hubbelli*	KBMC Cehu442	United States, New York, Tompkins Co., Jennings Pond. 1 km SW Danby, 370m, 42°20.825′N 76°29.233′W, 3 Sep 2002, KB Miller, leg.	KF575581	KF575475	KJ548417
						KF575534	KF575432	KJ548817
						KF575353	KF575398	---
Dytiscidae	Hydroporinae	Methlini	*Celina imitatrix*	KBMC Ceim533	United States, Alabama, Monroe Co., 3 km E Owassa, 31°29.791′N 86°51.184′W, 99 m, 11 May 2006, KB Miller, leg., KBM1105064	KF575611	---	---
						KF575563	KF575460	---
						KF575382	---	---
Dytiscidae	Hydroporinae	Methlini	*Methles cribratellus*	KBMC Mtcr459	Ghana, Volta Region, road between Nkwanta and Odumase, 8°15.537′N 0°26.562′E, 210 m, 15 Jun 2005, KB Miller, leg., KBM1506051	KF575594	KF575487	---
						KF575547	KF575444	---
						KF575366	---	---
Dytiscidae	Hydroporinae	Vatellini	*Derovatellus lentus*	KBMC Dele762	Venezuela, Monagas State, S Maturin, morichal margin, 9°36.591′N 63°8.295′W, 45 m, 2 Feb 2010, Short, Garcia, legs. VZ10-0202-01B	KJ548184	KJ548499	---
						KJ548296	KJ548570	---
						KJ548735	KJ548648	---

Dytiscidae	Hydroporinae	Vatellini	*Vatellus bifenestratus*	KBMC Vabi452	Peru, Madre de Dios, Explorers Inn, 12°50.208′S 69°17.603′W, 10 Dec 2003, KB Miller, leg., KBM1012031	KF585004	KF585006	KJ548476
						KF585005	KF585007	---
						KF585003	---	---
Dytiscidae	Hydroporinae	Vatellini	*Vatellus haagi*	KBMC Vaha448	Bolivia, Sta Cruz Prov., San Ignacio, 13 Jan 2004, G Svenson, leg.	KJ548269	---	---
						KJ548375	---	---
						KJ548792	KJ548716	---
Dytiscidae	Hydroporinae	Vatellini	*Vatellus pilacaudus*	KBMC Vapi449	Peru, Madre de Dios, Explorers Inn, 12°50.208′S 69°17.603′W, 10 Dec 2003, KB Miller, leg., KBM1012031	KJ548270	---	---
						KJ548376	KJ548625	---
						KJ548793	---	---
Dytiscidae	Laccophilinae	Agabetini	*Agabetes acuductus*	KBMC Abac15	United States, New York, St Lawrence Co., Macomb Twp, Fish Cr Marsh, 44°28.333′N 75°33.8′W, 23 May 2000, KB Miller, leg., KBM2305001	KJ548187	DQ112634	KJ548402
						KJ548300	KJ548572	---
						KJ548739	KJ548651	AF392048
Dytiscidae	Laccophilinae	Laccophilini	*Australphilus saltus*	KBMC Apsa373	Australia, Victoria, Brodribb River at Sardine Creek, nr Orbost, 37°30.85′S 148°32.617′E, 22 Nov 2000, KB Miller, leg., KBM2211001	KJ548197	DQ112635	KJ548411
						KJ548306	KJ548578	---
						KJ548746	KJ548659	KJ548143
Dytiscidae	Laccophilinae	Laccophilini	*Laccodytes*	KBMC Lmsp764	Venezuela, Monagas State, S Maturin, morichal margin, 9°36.591′N 63°8.295′W, 45 m, 2 Feb 2010, Short, Garcia, legs. VZ10-0202-01B	---	KJ548500	KJ548399
						KJ548297	---	---
						KJ548736	KJ548649	---

(continued)

Table 3.1 (continued)

Family	Subfamily	Tribe	Taxon title	Voucher	Locality	12S	COI	Arg Kin
						16S	COII	RNA Pol II
						H3	Ef1a	Wnt
Dytiscidae	Laccophilinae	Laccophilini	*Laccodytes*	KBMC Lysp376	Peru, Madre de Dios, Explorers Inn, 12°50.208′S 69°17.603′W, 10 Dec 2003, KB Miller, leg., KBM1012031	---	DQ112651	KJ548451
						KJ548353	---	KJ548844
						KJ548780	KJ548700	---
Dytiscidae	Laccophilinae	Laccophilini	*Neptosternus*	KBMC Nrsp379	India, Karnataka, Agumbe Ghats, 13°29.852′N 75°4.221′E, 9 Oct 2004, KB Miller, leg., KBM09100401	KJ548251	DQ112652	KJ548458
						KJ548360	KJ548615	KJ548848
						KJ548781	KJ548704	KJ548167
Dytiscidae	Laccophilinae	Laccophilini	*Laccophilus horni*	KBMC Laho378	United States, Arizona, Cochise Co., Bear Cr. Huachuca Mts, 31°22.796′N 110°21.814′W, 9 May 2003, KB Miller, leg., KBM0905031	KJ548238	DQ112644	KJ548442
						KJ548344	KJ548605	KJ548836
						---	KJ548693	KJ548160
Dytiscidae	Laccophilinae	Laccophilini	*Laccophilus maculosus*	KBMC Lama4	United States, New York, Tompkins Co., Ithaca, 42°26.433′N 76°29.8′W, 23 Oct 2000, KB Miller, leg., KBM2310001	KJ548239	DQ112647	KJ548443
						KJ548345	KJ548606	KJ548837
						KJ548774	KJ548694	AF392031
Dytiscidae	Laccophilinae	Laccophilini	*Philodytes umbrinus*	KBMC Pdum355	Namibia, Skeleton Coast NP, spring at mouth of Khumib River, 18°52.66′S 12°25.539′E, 13 May 2004, KB Miller, leg., KBM1305042	KJ548254	DQ112653	KJ548464
						KJ548363	KJ548616	---
						KJ548782	KJ548706	KJ548168
Dytiscidae	Lancetinae	Lancetini	*Lancetes angusticollis*	KBMC Lcan301	Argentina, Santa Cruz Province, ARG0001011	KJ548241	---	KJ548445
						KJ548347	KJ548608	KJ548838

						KJ548776	KJ548695	KJ548162
Dytiscidae	Lancetinae	Lancetini	*Lancetes lanceolatus*	KBMC Lcla91	Australia, Victoria, Sheepwash Lagoon 10 km E Yea, 37°10.267′S 145°31.733′E, 8 Nov 2000, KB Miller, leg., KBM0811001	JX477983	DQ813695	KJ548446
						KJ548348	DQ813797	KJ548839
						DQ813761	---	AF392032
Dytiscidae	Lancetinae	Lancetini	*Lancetes marginatus*	KBMC Lcma302	Argentina, Buenos Aires Prov., La Escondida, Bragado, Jan 2002	KJ548242	KJ548537	---
						KJ548349	KJ548609	KJ548840
						KJ548777	KJ548696	KJ548163
Dytiscidae	Lancetinae	Lancetini	*Lancetes nigriceps*	KBMC Lcni390	Peru, 9°43S 77°28W, ~11,500′, 29 Mar 2000, MW Hastriter	KJ548243	---	KJ548447
						KJ548350	KJ548610	KJ548841
						KJ548778	KJ548697	KJ548164
Dytiscidae	Lancetinae	Lancetini	*Lancetes varius*	KBMC Lcva196	Chile, Rio Hollenberg, 25 km S Poerto Natales, 14 Nov 2001, L Ström, leg.	KJ548244	DQ813696	KJ548448
						KJ548351	DQ813798	KJ548842
						DQ813762	KJ548698	DQ813726
Dytiscidae	Lancetinae	Lancetini	*Lancetes waterhousei*	KBMC Lcwa303	Argentina, Buenos Aires Prov., La Escondida, Bragado, Jan 2004	KJ548245	KJ548538	KJ548449
						KJ548352	KJ548611	KJ548843
						KJ548779	KJ548699	KJ548165
Dytiscidae	Matinae		*Batrachomatus daemeli*	KBMC Bada129	Australia, New South Wales, Tuross River, 5 km W Bodalla, 36°8.7′S 149°59.0833′E, 39 m, 2 Nov 2000, KB Miller, leg., KBM0211003	KJ548200	KJ548512	KJ548414
						KJ548309	KJ548581	KJ548814
						KJ548748	KJ548662	KJ548145
Dytiscidae	Matinae		*Matus bicarinatus*	KBMC Mabi2	United States, New York, St Lawrence Co., Macomb Twp, Fish Cr Marsh, 44°28.333′N 75°33.8′W, 23 May 2000, KB Miller, leg., KBM2305001	JX477984	JX478072	---
						---	JX478160	---
						JX477903	---	AF392033

“---” are fragments not acquired or analyzed

Vouchers and DNAs are deposited in the Museum of Southwestern Biology Division of Arthropods (MSBA, K.B. Miller). The molecular data include nine genes, *16S rRNA* (16S), *12S rRNA* (12S), *cytochrome c oxidase I* (COI), *cytochrome c oxidase II* (COII), *elongation factor 1α* (Ef1α), *arginine kinase* (AK), *histone III* (H3), *RNA polymerase II* (RNApol), and *wingless* (wnt). Not all fragments were sequenced for all taxa. In particular, the nuclear protein-coding genes did not amplify or sequence for all taxa (except H3, which amplified well for most diving beetles) (Table 3.1). The 3′ end of the fragment of COI and the 5′ and 3′ ends of COII include partial leucine and lycine tRNA coding regions. These were trimmed off because of considerable ambiguity in alignment. DNA fragments were amplified using PCR with TaKaRa Amplitaq (Applied Biosystems, Foster City, CA, USA) on an Eppendorf Mastercycler ep gradient S Thermal Cycler (Eppendorf, Hamburg, Germany). Amplification conditions were similar to those used by Miller et al. (2007b, 2009a) and Miller and Bergsten et al. (2012). Contamination was investigated using negative controls, and PCR products were examined using gel electrophoresis. Products were purified using ExoSAP-IT (USB-Affymetrix, Cleveland, OH, USA) and cycle sequenced using ABI Prism Big Dye (v3.1; Invitrogen, Fairfax, VA, USA) using the same primers used to amplify. Sequencing reaction products were purified using Sephadex G-50 Medium (GE Healthcare, Uppsala, Sweden) and sequenced using an ABI 3130xl Genetic analyzer (Applied Biosystems, Foster City, CA, USA) in the Molecular Biology Facility at the University of New Mexico. Gene regions were sequenced in both directions. Resulting sequence data were examined and edited using the program Sequencher (Genecodes 1999). Five hydroporine taxa amplified a paralogous Ef1α copy (see Miller and Bergsten 2012), and these fragments were discarded. Many of the data were acquired during previous projects (Bergsten and Miller 2007; Miller 2003; Miller and Bergsten 2012; Miller et al. 2007b, 2009a). In a few cases (notably for species in *Laccornellus* and *Canthyporus*), data were acquired from GenBank (Table 3.1). New sequences were deposited in GenBank (Table 3.1).

3.2.3 Morphology

Characters used in this analysis derive from several previous compilations of data (Miller 2000, 2001, 2003, 2005, 2009; Miller et al. 2006, 2007b, 2009a), and those sources should be consulted for more thorough descriptions of the characters and states. Characters and character states are described in the Appendix, and character coding for each species is presented in Table 3.2.

Table 3.2 Morphological[a] data used in combined phylogenetic analysis of Dytiscidae

	00000000000000000000000000000000000000000000 0000000000000000000000000000000000000000000000000000000011111 00000000011111111112222222222333333333344444444444555555555566666666667777777777888888888899999999990000 12345678901234567890123456789012345678901234 567890123456789012345678901234567890123456789012345678901234 + + + +
Spal472 *Spanglerogyrus albiventris*	-00000101101100000001000?0???000000000-0011000000000610000000001010011010001000000--000--000-00000000001
Dici474 *Dineutus ciliatus*	-00000102100100000000000?0???000000000-0000000000000100000000010100120100010000000--000---000-00000000001
Gyel494 *Gyrinus elevatus*	-00000102101100000000000?0???000000000-0000000000000100000000010100120100010000000--000---000-00000000001
Ncsp642 *Notomicrus* sp.	1100001000111000000000?0?1???000000000-0011000000000100100000000100100000111000000011000---000-00000100000
Tosp639 *Tonerus wheeleri*	110000000010100000000000?1???000000000-0011000000000100100000000200100000110000000001000---000-00000100000
Nocl503 *Noterus clavicornis*	110000000010100000000000?1???000000000-0011100000000100100000000200100000110000000001000---000-00000100000
Hcsp570 *Hydrocanthus* sp.	110000000010100000000000?1???000000000-0011100000000100101000000200100000110000000001000---000-00000100000
Haho504 *Hygrobia hermanni*	000000100001100000110000?0???00000000120011000000000100100000000200100000020000000110000001000-00000000000
Amle58 *Amphizoa lecontei*	010000100001000000001000?0???00000000100011000000000100100000000200100000020000000110000001000-00000000000

(continued)

Table 3.2 (continued)

	000000000000000000000000000000 0000000000000000000000000000000000000000000000000000000000000000000011111 00000000011111111112222222222333333333344444444445555555555666666666677777777778888888888999999999900000 1234567890123456789012345678901234 5678901234567890123456789012345678901234567890123456789012345678901234 + + + +
Mabi2 *Matus bicarinatus*	11100001000110001010010001000000000000-001100001000021100000000010100000002000000011000001000-00001000000
Bada129 *Batrachomatus daemeli*	11100001000110001010010001000000000000-001100001000021100000000010100000002000000011000001000-00001000000
Abac15 *Agabetes acuductus*	11000001000110000010010001000000000000-001100000000021100000011020010000003200000011011001000-00000000000
Laho378 *Laccophilus horni*	11000001000000000000110?0???000000000-001100000000021100001000021050000013200000011021000000-01000000000
Lama4 *Laccophilus maculosus*	11000001000000000000110001000000000000-001100000000021100001000021050000013200000011021000000-01000000000
Apsa373 *Australphilus saltus*	11000001000000000000110001000000000000-001100000000021100001000021050000013200000011021000000-01000000000
Pdum355 *Philodytes umbrinus*	11000001000000000000110?0???000000000-001100000000021100001000021050000013200000011021000000-01000000000
Nrsp379 *Neptosternus* sp.	11000001000000000000110?0???000000000-001100000000021100000000021050000013200000011021000000-01000000000
Lmsp764 *Laccodytes* sp.	11000001000000000000110?0???000000000-001100000000021100000000021050000013200000011021000000-01000000000

Lysp376 *Laccodytes* sp.	11000001000000000000110?0???000000000-001100000000021100000000021050000013200000011021000000-01000000000
Ctle11 *Coptotomus lenticus*	1100000100011000001001000100000000000-001100000000021100000001020010000003001000011000000000-00000000000
Ctsp530 *Coptotomus venustus*	11000001000110000010010?0???000000000-001100000000021100000001020010000003001000011000000000-00000000000
Apsy268 *Agaporomorphus silvaticus*	11000001000110000010010?0???000000000-0011000000001200000000010100100001040000000110000---00-01000000000
Cpau126 *Exocelina australiae*	1100000100011000001001000100000000000-001100000001120000000001020010000103000000011000000110-11000000000
Cpdi9 *Copelatus distinctus*	1100000100011000001001000100000000000-001100000001120000000001020010000103000000011000000110-11000000000
Lsan768 *Lacconectus andrewesi*	1100000100011000001001000100000000000-001100000001120000000001020010000103000000011000000110-11000000000
Lsre769 *Lacconectus regimbarti*	1100000100011000001001000100000000000-001100000001120000000001020010000103000000011000000110-11000000000
Hzsp734 *Hydrodytes opalinus*	1100000100011000001001010010000000000-0???000000001200000000010000100????3010000011000000000-00000000000
Ladi16 *Laccornis difformis*	1100000100001010001001010011000000000-101100000000020000000001020010010003010000011000000000-00000000000
Cehu442 *Celina hubbeli*	1100000100011010001001010011000100100-1011000000000200000000010200100100030100000-1000000000-00000000000

(continued)

Table 3.2 (continued)

	0000000000000000000000000000000000 00000000000000000000000000000000000000000000000000000000000000000000000011111 00000000011111111112222222222333333333344444444445555555555666666666677777777778888888888999999999900000 1234567890123456789012345678901234 56789012345678901234567890123456789012345678901234567890123456789012345678901234 + + + +
Ceim533 *Celina* *imitatrix*	1100000100011010001001010011000100100-10110000000002000000000102001001000301000000-1000000000-00000000000
Mtcr459 *Methles* *cribratellus*	11000001000010100010010?0???000100100-10110000000002000000000102001001000301000000-1000000000-00000000000
Lclu770 *Laccornellus* *lugubris*	11000001000010100010010?0???000000000-1011000000000210000000010200100100032100000-0000000001000000000000
Lccp771 *Laccornellus* *copelatoides*	11000001000010100010010?0???000000000-1011000000000210000000010200100100032100000-0000000001000000000000
Cnne773 *Canthyporus* *nebulosus*	11000001000010100010010?0???000000000-1011000000000210000000010200100100030100000-0000000001000000000000
Cnpa774 *Canthyporus* *parvus*	11000001000010100010010?0???000000000-1011000000000210000000010200100100030100000-0000000001000000000000
Cnho772 *Canthyporus* *hottentottus*	11000001000010100010010?0???000000000-10110000000000210000000010200100100030100000-0000000001000000000000
Anbl541 *Antiporus* *blakeii*	1100000100001010001001010011000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Anfe100 *Antiporus* *femoralis*	1100000100001010001001010011000000000-1011000000000110000000010200100100030100000-0000000001000000000000

Bage544 *Barretthydrus geminatus*	11000001000010100010010?0???000000000-10110000000001100000000010200100100030100000-0000000001000000000000
Cani144 *Carabhydrus niger*	11000001000010100010010?0???000000000-10110000000001100000000010200100100030100000-0000000001000000000000
Psgi775 *Paroster gibbi*	11000001000010100010010?0???000000000-10110000000001100000000010200100100030100000-0000000001000000000000
Chgi444 *Chostonectes gigas*	1100000100001010001001010011000000000-10110000000001100000000010200100100030100000-0000000001000000000000
Chne545 *Chostonectes nebulosus*	11000001000010100010010?0???000000000-10110000000001100000000010200100100030100000-0000000001000000000000
Dcmo514 *Deronectes moestus*	11000001000010100010010?0???000000000-10110000000001100000000010200100100030100000-0000000001000000000000
Dnla560 *Deronectes latus*	11000001000010100010010?0???000000000-10110000000001100000000010200100100030100000-0000000001000000000000
Deau758 *Deronectes aubei*	11000001000010100010010?0???000000000-10110000000001100000000010200100100030100000-0000000001000000000000
Ebna538 *Ereboporus naturaconservatus*	11000001000010100010010?0???000000000-10110000000001100000000010200100100030100000-0000000001000000000000
Hysp731 *Psychopomporus felipi*	11000001000010100010010?0???000000000-10110000000001100000000010200100100030100000-0000000001000000000000

(continued)

Table 3.2 (continued)

	0000000000000000000000000000000000000000 00000000000000000000000000000000000000000000000000000000000011111 00000000011111111112222222222333333333344444444445555555555666666666677777777778888888888999999999900000 12345678901234567890123456789012345678901234 567890123456789012345678901234567890123456789012345678901234 + + + +
Posp754 *Porhydrus* sp.	11000001000010100010010?0???000000000-10110000000001100000000102001001000301000000-0000000001000000000000
Gpig575 *Graptodytes ignotus*	11000001000010100010010?0???000000000-10110000000001100000000102001001000301000000-0000000001000000000000
Hepu136 *Heterosternuta pulchra*	1100000100001010001001010011000000000-10110000000001100000000102001001000301000000-0000000001000000000000
Hite536 *Haideoporus texanus*	11000001000010100010010?0???000000000-10110000000001100000000102001001000301000000-0000000001000000000000
Hyan549 *Hydroporus angustatus*	11000001000010100010010?0???000000000-10110000000001100000000102001001000301000000-0000000001000000000000
Hyob550 *Hydroporus obscurus*	11000001000010100010010?0???000000000-10110000000001100000000102001001000301000000-0000000001000000000000
Hypa548 *Hydroporus palustris*	11000001000010100010010?0???000000000-10110000000001100000000102001001000301000000-0000000001000000000000
Hyum551 *Hydroporus umbrosus*	1100000100001010001001010011000000000-10110000000001100000000102001001000301000000-0000000001000000000000
Meho547 *Megaporus howittii*	11000001000010100010010?0???000000000-10110000000001100000000102001001000301000000-0000000001000000000000

Mpha96 *Megaporus hamatus*	11000001000010100010010100110000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Nbro563 *Nebrioporus rotundatus*	11000001000010100010010100110000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Nbcl513 *Nebrioporus clarkii*	11000001000010100010010?0???0000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Npme532 *Neoporus mellitus*	11000001000010100010010?0???0000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Ntsu443 *Necterosoma susanna*	11000001000010100010010?0???0000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Ntwa95 *Necterosoma undecimlineatum*	11000001000010100010010100110000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Orco62 *Nectoporus congruus*	11000001000010100010010100110000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Orqu543 *Hornectes quadrimaculatus*	11000001000010100010010?0???0000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Orsc63 *Oreodytes scitulus*	11000001000010100010010?0???0000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Rrse508 *Rhithrodytes sexguttatus*	11000001000010100010010?0???0000000000-1011000000000110000000010200100100030100000-0000000001000000000000

(continued)

Table 3.2 (continued)

	0000000000000000000000000000000000 000000000000000000000000000000000000000000000000000000000000000011111 00000000011111111112222222222333333333344444444445555555555666666666677777777778888888888999999999900000 1234567890123456789012345678901234 567890123456789012345678901234567890123456789012345678901234567890 + + + +
Scop512 *Stictonectes optatus*	11000001000010100010010?0???000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Scru511 *Stictonectes rufulus*	11000001000010100010010?0???000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Stro26 *Clarkhydrus roffii*	1100000100001010001001010011000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Sudo554 *Hydroporus dorsalis*	11000001000010100010010?0???000000000-1011000000000110000000010200100100030100000-0000000001000000000000
Hlpa133 *Hydrocolus paugus*	11000001000010100010010?0???000000000-1011000000000100000000010200100100030100000-0000000001000000000000
Sasp78 *Sanfilippodytes* sp.	11000001000010100010010?0???000000000-1011000000000100000000010200100100030100000-0000000001000000000000
Vabi452 *Vatellus bifenestratus*	11000001000010110000010?0???010000000-1011000000000110000000010200100100030100000-0000000001000010000000
Vaha448 *Vatellus haagi*	11000001000010110000010?0???010000000-1011000000000110000000010200100100030100000-0000000001000010000000
Vapi449 *Vatellus pilacaudus*	11000001000010110000010?0???010000000-1011000000000110000000010200100100030100000-0000000001000010000000

Dele762 *Derovatellus lentus*	110000010000101100000010?0???010000000-1011000000000110000000010200100100030100000-0000000001000010000000
Husp735 *Hydrodessus* sp.	11000001000010100010011?0???000000000-1011000000000100000000010200100100030100000-0000000001000000000000
Peno438 *Peschetius nodieri*	11000001000010100010011?0???000000000-1011000000000100000000010200101100030100000-0000000001000100000000
Pequ345 *Peschetius quadricostatus*	11000001000010100010011?0???000000000-1011000000000100000000010200101100030100000-0000000001000100000000
Arsp282 *Amarodytes* sp.	11000001000011100010011?0???000000000-1011000000000100000000010200101100030100000-0000000001000100000000
Arsp283 *Amarodytes* sp.	11000001000011100010011?0???000000000-1011000000000100000000010200101100030100000-0000000001000100000000
Biin82 *Bidessonotus inconspicuus*	11010001000011100010011?0???000000000-1011000000000100000000010200101001030100000-0000000001000100000000
Lbco272 *Limbodessus compactus*	11000001000011100010011?0???000010000-1011000000000100000000010200101101030100000-0000000001000100000000
Liaf132 *Liodessus affinis*	11010001000011100010011?0???000000000-1011000000000100000000010200101101030100000-0000000001000100000000
Ldsp755 *Liodessus* sp.	11010001000011100010011?0???000000000-1011000000000100000000010200101101030100000-0000000001000100000000
Adsp696 *Anodocheilus* sp.	11010001000011100010011?0???000000000-1011000000000100000000010200101101030100000-0000000001000100000000
Nesp698 *Neobidessus* sp.	11010001000011100010011?0???000000000-1011000000000100000000010200101001030100000-0000000001000100000000

(continued)

Table 3.2 (continued)

	000000000000000000000000000000000000000 000000000000000000000000000000000000000000000000000000000011111 0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888899999999990000 1234567890123456789012345678901234 5678901234567890123456789012345678901234567890123456789012345678901234 + + + +
Uvsp289 *Uvarus* *spretus*	1100000100001110001001110011000000000-10110000000001000000000102001011010301000000-0000000001000100000000
Hosp695 gen. nr. *Hypodessus* sp.	11000001000011100010011?0???000000000-10110000000001000000000102001011010301000000-0000000001000100000000
Htac370 *Cyclopius* *acaroides*	1100010100001010001001010011000010000-10110000000001000000000102001001000301000000-0000000000-00000001000
Htdc582 *Clemnius* *decoratus*	11000101000010100010010?0???000010000-10110000000001000000000102001001000301000000-0000000000-00000001000
Htsa34 *Hygrotus* *sayi*	1100010100001010001001010011000010000-10110000000001000000000102001001000301000000-0000000000-00000001000
Htco365 *Hygrotus* *compar*	11000001000010100010010?0???000010000-10110000000001000000000102001001000301000000-00000000011000000000000
Htdi360 *Hygrotus* *diversipes*	11000001000010100010010?0???000010000-10110000000001000000000102001001000301000000-00000000011000000000000
Htim80 *Hygrotus* *impressopunctatus*	1100000100001010001001000011000010000-10110000000001000000000102001001000311000000-00000000011000000000000
Htsa366 *Hygrotus* *salinarius*	11000101000010100010010?0???000010000-10110000000001000000000102001001000301000000-00000000011000000000000

Hpsp574 *Hygrotus rohani*	11000101000010100010010?0???000010000-1011000000000100000000010200100100030100000-0000000000-00000001000
Hpin578 *Hygrotus inquinatus*	11000101000010100010010?0???000010000-1011000000000100000000010200100100030100000-0000000000-00000001000
Hhsp752 *Herophydrus* sp.	11000101000010100010010?0???000010000-1011000000000100000000010200100100030100000-0000000000-00000001000
Hvpu446 *Hydrovatus pustulatus*	110001010000101001100100001100001 0100-1011000000000100100000000200100100032000000-0000000000-00000000000
Hvsp458 *Hydrovatus parallelipennis*	11000101000010100110010?0???000010100-1011000000000100100000000200100100032000000-0000000000-00000000000
Hvru559 *Hydrovatus rufoniger*	11000101000010100110010?0???000010100-1011000000000100100000000200100100032000000-0000000000-00000000000
Quyo455 *Queda youngi*	11000101000010100110010?0???000010000-1011000000000100100000000200100100030100000-0000000000-00000000000
Hpel440 *Hyphydrus elegans*	110001010000101000100101001100001 0000-1011000000000000000000010200000100030100000-000000000100000000000
Hpex441 *Hyphydrus excoffieri*	11000101000010100010010?0???000010000-1011000000000000000000010200000100030100000-000000000100000000000
Hhsi450 *Hyphydrus signatus*	11000101000010100010010?0???000010000-1011000000000000000000010200000100030100000-000000000100000000000
Hysp753 *Hyphydrus congoanus*	11000101000010100010010?0???000010000-1011000000000000000000010200000100030100000-000000000100000000000

(continued)

Table 3.2 (continued)

	00000000000000000000000000000000000000000000 0000000000000000000000000000000000000000000000000000000011111 00000000011111111112222222222333333333344444444445555555555666666666677777777778888888888999999999900000 12345678901234567890123456789012345678901234 567890123456789012345678901234567890123456789012345678901234 + + + +
Hhsp643 *Hyphydrus esau*	11000101000010100010011?0???000010000-1011000000000000000000010200000100030100000-0000000001000000000000
Pasp761 *Pachydrus* sp.	1100010100001010011001110011000010000-1011000000000000000000010200000100030100000110000000-00000000000
Dpco81 *Desmopachria convexa*	11000101000010100010011?0???000010000-1011000000000000000000000200000100030100000100000000010000000010000
Dppo139 *Desmopachria portmanni*	11000101000010100010011?0???000010000-1011000000000000000000000200000100030100000100000000010000000010000
Mysv765 *Microdytes svensoni*	11000101000010100010010?0???000010000-1011000000000000000000010200000100030100000100000000010000000000000
Hppa371 *Hydrotrupes palpalis*	11000001000110000010010?0???000000120011000000000211001000000000100000000300000001100000100-00000000000
Plde130 *Platynectes decemaculatus*	11000001000110000010010?0???000000120011000000000211001000100200100000000300000001100000100-00000000000
Pysp457 *Platynectes* sp.	11000001000110000010010?0???000000120011000000000211001000100200100000000300000001100000100-00000000000
Atsp759 *Agametrus nitens*	11000001000100000010010?0???000000120011000000000211001000100200100000000300000001100000100-00000000000

Ansp760 *Agametrus humilis*	110000010001100000010010?0???000000001200110000000002110010001002001000000300000001100000 1000-00000000000
Ilfr409 *Ilybius fraterculus*	110000010001100000100100010000100000110011000000000211001000000100000000030000110110000010 00-00000000000
Ilsu64 *Ilybius subaeneus*	110000010001100000100100010000100000110011000000000211001000000100000000030000110110000010 00-00000000000
Ilga12 *Ilybius gagates*	110000010001100000100100010000000000120011000000000211001000100100100000030000110110000010 00-00000000000
Agsp411 *Ilybius* sp.	110000010001100000010010?0???0000000012001100000000021100100010010010000002000000111000001000-00000000000
Agdi412 *Agabus disintegratus*	110010010001100000010010?0???0000000012001100000000021100100010010010000002000000111000001000-00000000000
Agco57 *Ilybiosoma cordatum*	110000010001100000010010?0???0000000012001100000000021100100010010010000002000000111000011000-00000000000
Agse413 *Ilybiosoma seriatum*	110000010001100000010010?0???0000000012001100000000021100100010010010000002000000111000011000-00000000000
Agtr60 *Agabus tristis*	110010010001100000010010?0???0000000012001100000000021100100010010010000002000000111000001000-00000000000
Pmsp730 *Agabus didymus*	110000010001100000010010?0???0000000012001100000000021100100000010010010002000000011000000000-00000000000
Crup251 *Carabdytes upin*	110000010001100000010010?0???1000000012001100001000021100000000020000000003000000011000001000-00000000000

(continued)

Table 3.2 (continued)

	0000000000000000000000000000000 000000000000000000000000000000000000000000000000000000000000000000000011111 00000000011111111112222222222333333333344444444445555555555666666666677777777778888888888999999999900000 1234567890123456789012345678901234 5678901234567890123456789012345678901234567890123456789012345678901234 + + + +
Rhsi17 *Meridiorhantus sinuatus*	11000001000110000010010?0???1000000010001100001000021100000010010000000002001000011000101000-00000000000
Rhca262 *Meridiorhantus calidus*	11000001000110000010010?0???1000000012001100001000021100000000020000000002001000011000101000-00000000000
Rhat10 *Rhantus atricolor*	110000010001100000100100010010000000100011000010000211000000000100000000020010000110001010 00-00000000000
Rhbi3 *Rhantus binotatus*	1100000100011000001001000100100000001000110000100002110000000001000000000200100001100010100 0-00000000000
Rhsp408 *Meridiorhantus validus*	11000001000110000010010001001000000010001100001000021100000000010000000002001000011000101000-00000000000
Cbin410 *Colymbetes exaratus*	11000001000100000010010?0???1000000010001100001000021100000000010000000002001000011000101000-00000000000
Cbpa59 *Colymbetes paykulli*	11000001000100000010010?0???1000000010001100001000021100000000010000000002001000011000101000-00000000000
Clsp476 *Colymbetes fuscus*	11000001000100000010010?0???1000000010001100001000021100000000010000000002001000011000101000-00000000000
Nean25 *Neoscutopterus angustus*	11000001000100000010010?0???1000000010001100001000021100000010010000000002001000011000101000-00000000000

Mdco475 *Meladema coriacea*	11000001000110000010010?0???1000000010001100001000021100000000010000000002001000011000101000-00000000000
Hopl453 *Hoperius planatus*	110000010001100000100100010010000000100011000010000211000000000100000000020010000110001010 00-00000000000
Lcan301 *Lancetes angusticollis*	11000001000110000010010?0???0000000112001100011000021100000001021000001003100000001100000 1000-00000000000
Lcla91 *Lancetes lanceolatus*	110000010001100000100100010000000001120011000110000211000000010210000010031000000011000001000-00000000000
Lcma302 *Lancetes marginatus*	11000001000110000010010?0???00000001120011000110000211000000010210000010031000000011000001000-00000000000
Lcni390 *Lancetes nigriceps*	11000001000110000010010?0???00000001120011000110000211000000010210000010031000000011000001000-00000000000
Lcva196 *Lancetes varius*	11000001000110000010010?0???00000001120011000110000211000000010210000010031000000011000001000-00000000000
Lcwa303 *Lancetes waterhousei*	11000001000110000010010?0???00000001120011000110000211000000010210000010031000000011000001000-00000000000
Spla123 *Spencerhydrus latecinctus*	01000001000100001010010?0???00000000110011000011000211010101000100000110052000000011000---000-0000000110
Sppu108 *Spencerhydrus pulchellus*	01000001000100001010010?0???00000000110011000011000211010101000100000110052000000011000---000-0000000110

(continued)

Table 3.2 (continued)

	0000000000000000000000000000000000 00000000000000000000000000000000000000000000000000000000000000000011111 000000000111111111122222222223333333333444444444455555555556666666666777777777788888888889999999999900000 1234567890123456789012345678901234 56789012345678901234567890123456789012345678901234567890123456789001234 + + + +
Cyma416 *Cybister marginicollis*	010000010001000000010010?0???0000000011001100111100021101010100010040011005200000011000---000-0000000110
Cypu21 *Cybister puncticollis*	01000001000100000001001000100000000001100110011110002110101010001005001100520000001 1000---000-0000000110
Cytr102 *Cybister tripunctatus*	01000001000100000001001000100000000001100110011110002110101010001005001100520000001 1000---000-0000000110
Cysp757 *Cybister* sp.	01000001000100000001001000100000000001100110011110002110101010001005001100520000001 1000---000-0000000110
Mefr300 *Megadytes fraternus*	010000010001000000010010?0???0000000011001100111100021101010100010020011005200000011100---000-0000000110
Mero254 *Megadytes robustus*	010000010001000000010010?0???0000000011001100111100021101010100010020011005200000011100---000-0000000110
Onsc107 *Onychohydrus scutellaris*	010000010001000000010010?0???0000000011001100011100021100010100010000011005200000011000---000-0000000110
Stat165 *Sternhydrus atratus*	010000010001000000010010?0???0000000011001100011100021100010100010000011005200000011000---000-0000000110
Dymg168 *Dytiscus marginalis*	0100001100010000000100100010010000000100101000110000211100000000100100110052000000011000---000-00000000000

Dyve24 *Dytiscus verticalis*	01000011000100000010010001001000000010010100011000021110000000010010011005200000011000---000-00000000000
Hdcr238 *Hyderodes crassus*	01000001000110000010010?0???100000000-010000011000021110000010010010011005200000011000---000-00000000000
Hdsh104 *Hyderodes shuckardi*	01000001000110000010010?0???100000000-010000011000021110000010010010011005200000011000---000-00000000000
Nofa52 *Notaticus fasciatus*	01000001000000000110010?0???000000000-010000001010021110000000120000011005200000011000---000-00000000000
Hyar68 *Hydaticus aruspex*	01000001000100000010010?0???000000000-010000001010021100000000120000011005200000011000---000-00000000000
Hyci20 *Hydaticus cinctipennis*	01000001000100000010010?0???000000000-010000001010021100000000120000011005200000011000---000-00000000000
Hyex400 *Hydaticus exaratus*	01000001000100000010010?0???000000000-010000001010021100000000120000011005200000011000---000-00000000000
Hyfa119 *Hydaticus fabricii*	01000001000100000010010?0???000000000-010000001010021100000000120000011005200000011000---000-00000000000
Hysu23 *Hydaticus subfasciatus*	01000001000100000010010?0???000000000-010000001010021100000000120000011005200000011000---000-00000000000
Hyxa67 *Hydaticus xanthomelas*	01000001000100000010010?0???000000000-010000001010021100000000120000011005200000011000---000-00000000000

(continued)

Table 3.2 (continued)

	00000000000000000000000000000000000000000000 00000000000000000000000000000000000000000000000000000000000000000011111 00000000011111111112222222222333333333344444444445555555555666666666677777777778888888888999999999900000 12345678901234567890123456789012345678901234 5678901234567890123456789012345678901234567890123456789012345678901234 + + + +
Erau103 *Eretes* *australis*	010000010001100000010010?0???000000000-01000000121002110000000002000001100520000001100---000-00000000000
Ergr105 *Eretes* *griseus*	010000010001100000010010?0???000000000-01000000121002110000000002000001100520000001100---000-00000000000
Grli69 *Graphoderus* *liberus*	010000010001000000010010?0???000000000-01000000121002110000100012000001100520000001100---000-00000000000
Acab72 *Acilius* *abbreviatus*	0100000100010000000100100010000000000-01000100121002110000100012000001100520010001100---000-00000000000
Acse7 *Acilius* *semisulcatus*	0100000100010000000100100010000000000-01000100121002110000100012000001100520010001100---000-00000000000
Thba210 *Thermonectus* *basillaris*	010000010001000000010010?0???000001000-01000100121002110000100012000001100520000001100---000-00000000000
Thni5 *Thermonectus* *nigrofasciatus*	010000010001000000010010?0???000001000-01000100121002110000100012000001100520000001100---000-00000000000
Aeop406 *Aethionectes* *optatus*	010000010001000000010010?0???000000000-01000000121002110000100012000001100520010001100---000-00000000000
Aesp659 *Aethionectes* sp.	010000010001000000010010?0???000000000-01000000121002110000100012000001100520010001100---000-00000000000

Saba90 *Sandracottus bakewellii*	010000010001000000010010?0???000000000-010000001210021100001000120000011005200100011000---000-00000000000
Sade328 *Sandracottus dejeanii*	010000010001000000010010?0???000000000-010000001210021100001000120000011005200100011000---000-00000000000

[a] One character is, in fact, ecological but for simplicity the entire dataset is referred to here and elsewhere as “morphological”

“-” indicates inapplicable data, “?” indicates missing data. Characters are non-additive except those marked with “+”

3.2.4 Analysis

3.2.4.1 Alignment

Several markers are length-invariant among these taxa (H3, COII, COI, EF1α) and alignments of these were unambiguous. Wingless exhibited length variability associated with three-base-pair (or multiple of three-base-pair) indels. Gyrinidae specimens had introns in RNA polymerase II (at positions 269–345), and *Agaporomorphus silvaticus* (Apsy268) had an intron in arginine kinase (at positions 244–298), which were removed. These were aligned using MUSCLE (Edgar 2004) and the default parameters and then adjusted manually as needed to conserve open reading frame. 16S and 12S are each much more length variable, and these were aligned using MUSCLE (Edgar 2004) and the default parameters.

3.2.4.2 Parsimony

Parsimony analysis was done in TNT (Goloboff et al. 2008). The morphological characters 2, 12, 56, 61, 94, 98, 99 and 104 were treated as additive. Tree searches began by generating trees using 30 random addition sequences. These trees were then swapped using tree bisection-reconnection, sectorial search (with the default parameters in TNT), and 30 iterations of tree-drifting (Goloboff 1999). Shortest trees found were then imported into WinClada (Nixon 2002) for examination of topologies, optimization of character states and calculation of the consensus. Bootstrap values were calculated in NONA as implemented in WinClada using 1000 bootstrap iterations and saving the consensus of each iteration.

3.2.4.3 Bayesian

Bayesian analysis was done in MrBayes 3.2 (Ronquist et al. 2012). We used a partitioned model with all parameters of the model, except topology and branch length, unlinked, and estimated separately. Partitioning scheme followed Miller et al. (2009a) with first, second and third codon positions separated into partitions, but the same positions merged across nuclear and mitochondrial protein-coding genes, respectively. Mitochondrial ribosomal 12S and 16S were merged and together given a separate partition. A gamma-distributed rate variation parameter (Γ) and a proportion of invariable sites (I) were allotted the model for each partition. The substitution rate matrix was not selected a priori but estimated using reversible-jump MCMC for each partition across all 203 possible but reversible 4×4 nucleotide models (Huelsenbeck et al. 2004). The morphological data were analyzed under a Markov K model (Lewis 2001) + Γ, with the same characters as in the parsimony analysis treated as ordered and accounting for the bias that only parsimony-informative characters were scored. Three separate MCMC runs, each with one cold and three incrementally heated chains ($T = 0.1$), were distributed across eight cores of two 2.8 GHz Quad-Core Intel Xeon processors (Mac Pro; L2 Cache 12Mb per processor: memory: 4GB 800MHz DDR2 FB-DIMM) and run for 20 million generations. We used a parsimony tree as a starting tree for the chains and sampled the cold chains every 1000th generation. The average deviation of split frequencies, PSRF, ESS, and statistical graphics provided by MrBayes 3.2, and Tracer 1.5 (Rambaut and Drummond 2007), was used to assess mixing and convergence of runs. A burn-in of 25% was discarded before the remaining sampled trees from the three runs were pooled and a majority-rule consensus tree calculated.

3.3 Results

Thirteen equally parsimonious trees were found of length 46,737 (CI = 13, RI = 42) with the consensus of these shown in Fig. 3.2. The consensus is well resolved with few clades collapsed. Support for less-inclusive groupings (genera, tribes) is relatively strong, but support for relationships among the tribes and subfamilies is very low. All of the "backbone," more-inclusive groupings (relationships among tribes and/or subfamilies) are supported by less than 50% bootstrap values (Fig. 3.2).

The three separate runs for the Bayesian analysis converged satisfactory, and the joint tree samples resulted in the close-to fully resolved majority-rule consensus tree in Fig. 3.3. The ingroup, Dytiscidae, was monophyletic (posterior probability, pp = 1.0) and the clade Amphizoidae + Paelobiidae (pp = 1.0) was resolved as its sister group (pp = 1.0). The family Noteridae, containing some of the longest terminal branches in the analysis, was monophyletic (pp = 1.0) and resolved as a sister group to the clade with Amphizoidae, Paelobiidae, and Dytiscidae (pp = 1.0). Within Dytiscidae, the subfamily Matinae was resolved with high support (pp = 0.99) as the sister lineage to remaining Dytiscidae. Matinae apart, the rest of Dytiscidae consist of five well-supported major groupings but where the relative relationship between each other is tentative due to the moderate support. The well-supported higher-level groups are (1) Agabinae + Colymbetinae (pp = 0.96), (2) Hydroporinae + Hydrodytinae (pp = 1.0), (3) Dytiscinae + Laccophilinae + Cybistrini + Copelatinae (pp = 0.96), (4) Coptotominae (pp = 1.0), and (5) Lancetinae (pp = 1.0). The tentative resolution of these five groups places Lancetinae as sister to Agabinae + Colymbetinae (pp = 0.62), Coptotominae as sister to Hydroporinae + Hydrodytinae (pp = 0.63), and Lancetinae + Agabinae + Colymbetinae as sister to the remaining Dytiscidae (pp = 0.62) apart from Matinae. Seven of the ten subfamilies were highly supported as monophyletic (all with pp = 1.0): Matinae, Colymbetinae, Copelatinae, Coptotominae, Lancetinae, Hydroporinae and Laccophilinae. Hydrodytinae had only a single sampled species and hence its monophyly not tested, but it was not nested in any other subfamily. The two exceptions were Agabinae with only moderate support (pp = 0.72) and Dytiscinae recovered as paraphyletic as discussed in detail below.

Fig. 3.2 Strict consensus of 13 most parsimonious cladograms from combined analysis of morphology and DNA sequence data (length of trees = 46,737, CI = 13, Ri = 42). Numbers at branches are bootstrap values. Upper right inset tree is one of 13 parsimony trees with branch lengths proportional to character state changes mapped using "fast" (ACCTRAN) optimization

Fig. 3.2 (continued)

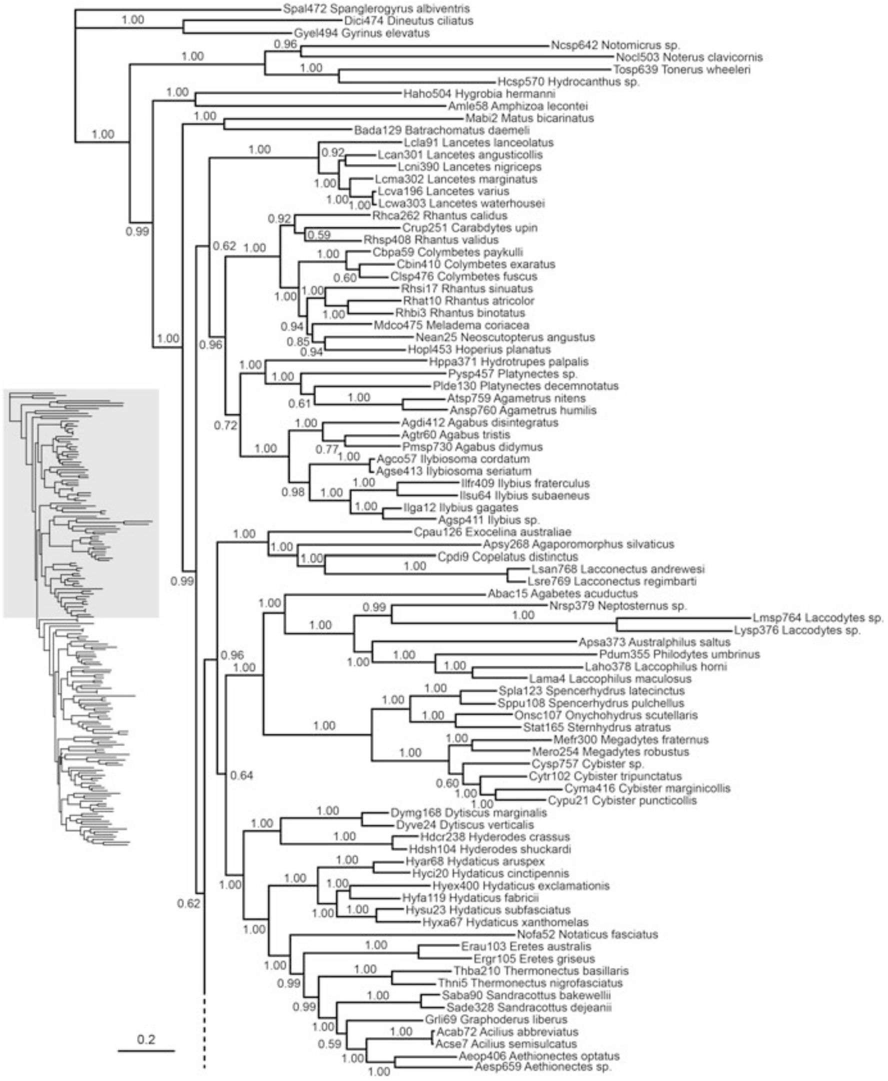

Fig. 3.3 Majority-rule consensus tree derived from the combined Bayesian MCMC analysis with a partitioned model for morphology and DNA sequence data. Numbers at branches are posterior probability clade support values

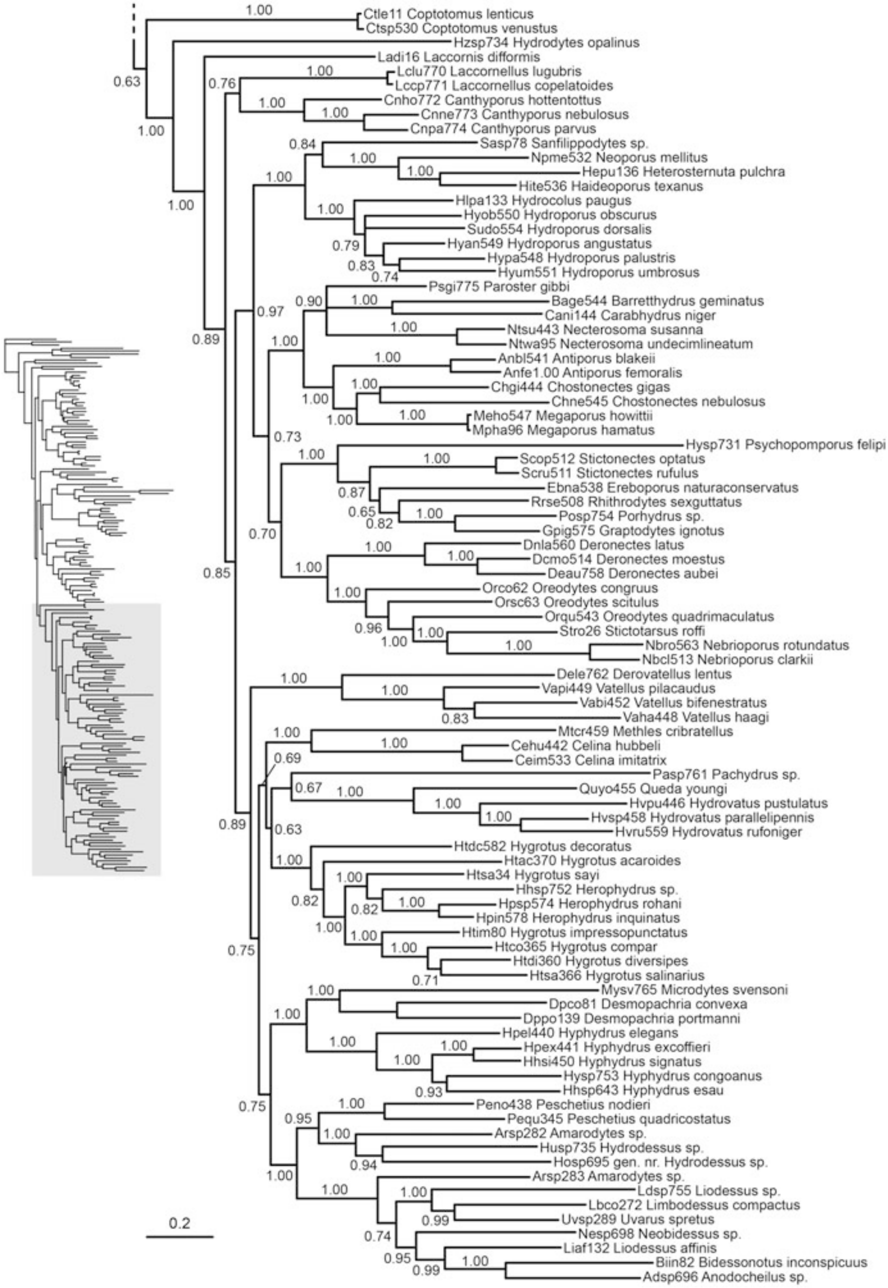

Fig. 3.3 (continued)

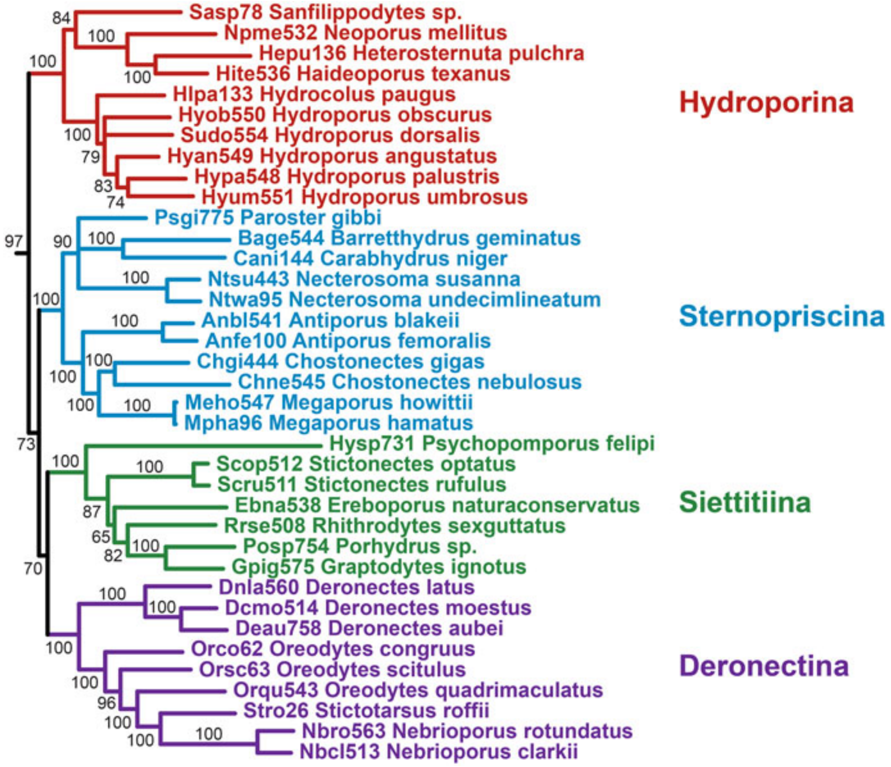

Fig. 3.4 Internal topology of Hydroporini derived from combined (model-partitioned) Bayesian analysis of morphology and DNA sequence data showing relationships among subtribes. Numbers at branches are posterior probability clade support values

The parsimony and Bayes analyses differ considerably in relative support (with much of the parsimony tree poorly supported) and topology. The parsimony tree includes several unexpected results that conflict dramatically with morphology, traditionally recognized groups, and the Bayesian analysis, including placement of *Hydrodytes* (Hydrodytinae) among the outgroups, non-monophyly of Agabinae, the sister group relationship between certain Australian Hydroporini and *Canthyporus*, the nesting of *Laccornellus* among the *Deronectes*-group of genera, and non-monophyly of Methlini with *Methles* nested among certain Australian Hydroporini. Because of this, and because the MrBayes analysis is very well supported in general our preferred conclusions about relationships are based on this estimate of the phylogeny (Figs. 3.3, 3.4 and 3.5). Our discussion centers on this topology and support values for particular conclusions are based on the Bayesian estimate.

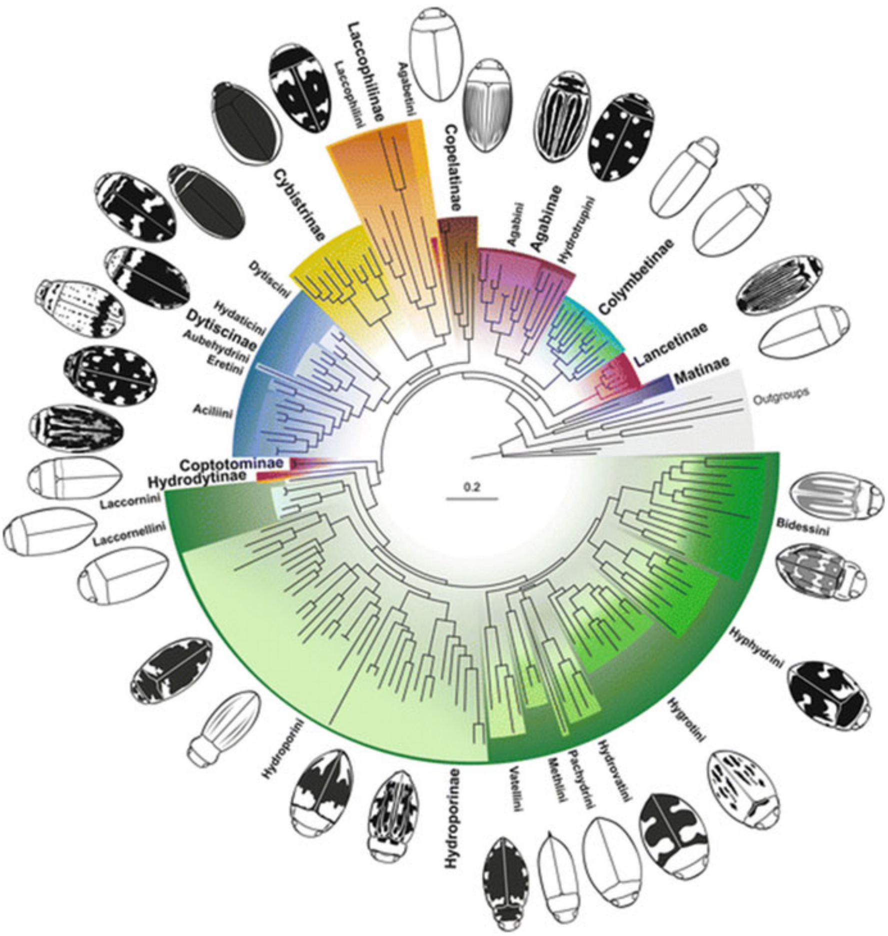

Fig. 3.5 Summary tree derived from combined (model-partitioned) Bayesian analysis of morphology and DNA sequence data for diving beetles (Dytiscidae) showing revised classification of subfamilies and tribes

3.4 Discussion

Diving beetle classification has moved from higher taxa based on authoritative schemes emphasizing few characters (e.g., Régimbart 1879; Sharp 1882), to a post-Hennigian reclassification emphasizing monophyletic groups based in large part on morphology (e.g., Burmeister 1976, 1990; Miller 2001; Wolfe 1985, 1988), to recent sophisticated phylogenetic approaches developing evidence from both morphology and DNA sequence data (Miller 2003; Miller et al. 2007b, 2009a; Ribera et al. 2002, 2008). This history has resulted in considerable phylogenetic progress by developing a much better understanding of diving beetle phylogenetic history, improving the classification, and illuminating areas in need of further study. This analysis similarly develops greater clarity in diving beetle phylogenetic history and updates the classification yet points out areas of weakness in our knowledge. Each of the following treatments discusses the history of phylogenetic ideas about each group as well as conclusions based on this analysis.

3.5 Diving Beetle Phylogeny and Classification

In this second edition, we here include updates from studies published after the first edition in 2014. In general taxonomy, a book was published covering the classification of the family- and genus-groups including fully illustrated diagnoses and keys, treatments of each group including diagnoses, classificiation and distribution of each group, and other aspects of the biology of Dytiscidae, *The Diving Beetles of the World* (Miller, K.B. and Bergsten, J. 2016). That volume was based on the classification presented in the first edition of this chapter. Since then, three more recent studies have been published with taxonomic sampling across the family. Michat et al. (2017) provided a comprehensive phylogenetic parsimony analysis based on 304 larval characters, sampling 113 ingroup taxa representing all current subfamilies and tribes except Hydrodytinae, for which larvae are unknown. Désamoré et al. (2018) analyzed largely the same dataset as here but excluded the morphological characters and a few taxa due to large numbers of missing molecular data. In addition to some changes in taxon relationships, the study provided the first dating of the family based on multiple fossil calibrations. Finally, Gustafson et al. (2020) sequenced ultraconserved elements (UCE) for 55 Adephagan taxa representing all Adephagan families and all Dytiscidae subfamilies but not all tribes. They analyzed a 50% complete matrix with 1076 loci and a 70% complete matrix with 200 loci with multiple methods, which yielded highly interesting phylogenetic results.

After these other analyses, all 11 subfamilies recognized in the first edition remain stable and monophyletic. All tribe and subtribe taxa remain unchanged as well, although a few have been suggested to be paraphyletic such as the current circumscription of Hydrotrupini (Toussaint et al. 2017). Also, a couple of poorly known genera were placed into subtribes within Hydroporini (Kanda et al. 2016; Villastrigo et al. 2021). Continued recovery of the named higher-level clades in Dytiscidae indicates that a set of naturally defined family-group taxa has largely stabilized though relationships among them remain elusive.

A number of additional recent studies have also provided updated classifications, new taxa or phylogenetic insights at the genus level within subfamilies, tribes and subtribes, including in Agabinae (Okada et al. 2019; Alarie and Michat 2020), Colymbetinae (Barman et al. 2014; Morinière et al. 2015, 2016; Balke et al. 2017a), Cybistrinae (Michat et al. 2015a, 2019), Copelatinae (Bilton et al. 2015; Toussaint et al. 2016a), Laccophilinae (Toledo and Michat 2015; Michat and Toledo 2015; Benetti et al. 2019) Sternopriscina (Hendrich et al. 2014, Toussaint et al.

2015b, 2016b; Alarie et al. 2018, 2019b, 2020), Hygrotini (Villastrigo et al. 2017, 2018), Hyphydrini (Alarie et al. 2017), Hydroporini (Villastrigo et al. 2021; Fery and Bouzid 2016; Queney et al. 2020), Deronectina (Fery and Ribera 2018), Siettitiina (Kanda et al. 2016; Ribera and Reboleira 2019) and Bidessini (Miller and Short 2015, Miller and Wheeler 2015, Miller 2016; Balke et al. 2017a, b; Hendrich et al. 2020).

3.5.1 Matinae Branden, 1885

3.5.1.1 Type Genus

Matus Aubé, 1836.

3.5.1.2 Diagnosis

These are Dytiscidae with: (1) the medial portion of the prosternum and prosternal process distinctly longitudinally sulcate, (2) the head with a distinct longitudinal postocular carina, (3) the anterodorsal margins of metatarsomeres I–IV distinctly lobed, and (4) the female genitalia with “amphizoid-type” of configuration (Miller 2001), and with a large accessory gland reservoir attached to the fertilization duct.

3.5.1.3 Discussion

This group has usually been placed as a tribe in Colymbetinae until Miller (2001) elevated it to subfamily rank. Miller (2001) found the group to be sister to all other diving beetles. Ribera et al. (2008) found matines placed near Hydrodytinae, and these two groups, together with Lancetinae and Dytiscini, weakly placed as sister group to the rest of Dytiscidae. Relationships among the genera (based on larvae) were investigated by Alarie et al. (2001b). A recent revision (Hendrich and Balke 2013) synonymized *Allomatus* Mouchamps with *Batrachomatus* Clark and keyed and diagnosed all the Australian species.

In this analysis, Matinae was found to be monophyletic (Fig. 3.3, pp = 1.00) and sister to all other known diving beetles, with good support (Fig. 3.3, pp = 1.00). This corroborates the relationship first proposed by Miller (2001) based on morphology alone. This proposed relationship is interesting in part because of the dramatically disjunct distribution of members of Matinae with *Matus* in eastern North America and *Batrachomatus* in Australia, suggesting a possible ancient vicariance.

The reanalysis by Désamoré et al. (2018) recovered Matinae as sister to all other Dytiscidae but with very poor support (pp = 0.52). The comprehensive phylogeny based on larval characters did not support such a position and instead placed Matinae in a larger clade with all subfamilies except Hydroporinae and Laccophilinae

(Michat et al. 2017). Similarily, the UCE-based analysis found Matinae in a more derived position as sister to Agabinae+Colymbetinae in at least some analyses (Gustafson et al. 2020). The age of Matinae was estimated to 90.6 my (34.6–160.1) for the crown node and 159.2 my (141.5–179.1) for the stem node (Désamoré et al. 2018).

3.5.1.4 Taxon Content

Matinae comprises two genera: *Batrachomatus* Clark, 1863 from Australia and *Matus* Aubé, 1836 from eastern North America.

3.5.2 Lancetinae Branden, 1885

3.5.2.1 Type Genus

Lancetes Sharp, 1882.

3.5.2.2 Diagnosis

Lancetinae are Dytiscidae with: (1) the elytral apices sinuate or subtruncate, (2) the female reproductive tract with two genital openings and a distinctive bursa, and with the spermathecal duct extending from the anterior apex of the bursa, (3) the female gonocoxae weakly, but distinctly fused dorsally, (4) the median lobe asymmetrical with a distinct, elongate ventral sclerite, and (5) the metatarsal claws unequal in length in both sexes.

3.5.2.3 Discussion

Recognized as monophyletic and placed as a tribe in Colymbetinae sensu lato for much of its history, Lancetinae was regarded as potentially closely related to Dytiscinae by Ruhnau and Brancucci (1984) and *Coptotomus* (as a tribe Coptotomini of Colymbetinae) by Brinck (1948). Nilsson (1989) suggested *Lancetes* and Laccophilinae (including *Agabetes*) may be closely related based on larval features. Miller (2001), based on adult characters, and Alarie et al. (2002a), based on larvae, found the group resolved as sister to Dytiscinae. Ribera et al. (2008) found Lancetinae together with Dytiscini, Hydrodytinae and Matinae as sister to the rest of Dytiscidae, though these relationships were not strongly supported in their analysis.

In this analysis, Lancetinae is monophyletic with strong support (Fig. 3.3, pp = 1.00) and is resolved as sister to Colymbetinae + Agabinae, although support for this is not strong (Fig. 3.3, pp = 0.62). The single Australian species is resolved as sister to the South American species in the analysis (Fig. 3.3, pp = 1.00). This is one of the few Australian + South American biogeographic relationships among Dytiscidae.

The reanalysis of Désamoré et al. (2018) increased the support (pp = 0.92) for a monophyletic Lancetinae+Agabinae+Colymbetinae. In contrast, the UCE-based analysis recovered a sistergroup relationship between Lancetinae and Coptotominae with strong support across all analysis (Gustafson et al. 2020), a relationship also relatively strongly supported by larval characters (Michat et al. 2017). The age of Lancetinae was estimated to be 47.2 my (19.4–88.4) for the crown node and 113.0 my (75.5–127.8) for the stem node (Désamoré et al. 2018).

3.5.2.4 Taxon Content

Lancetinae includes a single genus, *Lancetes* Sharp, 1882, with numerous species in temperate and high-elevation areas of South America and one species, *L. lanceolatus* (Clark 1863) in Australia.

3.5.3 Agabinae Thomson, 1867

3.5.3.1 Type Genus

Agabus Leach, 1817.

3.5.3.2 Diagnosis

This fairly homogeneous subfamily is characterized by adults with a series of closely-spaced setae at the anteroventral angle of the metafemur. This is absent in some specimens of *Hydrotrupes*, *Hydronebrius* and some *Platambus*, but secondarily (Nilsson 2000; Ribera et al. 2004).

3.5.3.3 Discussion

This subfamily has usually been recognized as a monophyletic tribe within Colymbetinae until Miller (2001) elevated it to subfamily rank since, in that analysis, it was not found to be related to Colymbetini or other members traditionally placed in that subfamily. This was further confirmed by Ribera et al. (2002) and Ribera et al. (2008), who found Agabinae to be paraphyletic with the *Platynectes*-group of genera not related to the *Agabus*-group. Roughley (2000) placed the anomalous genus, *Hydroptrupes*, in its own subfamily based on larval features presented by Beutel

(1994) that suggested the genus is sister to all Dytiscidae except Copelatinae. This was not supported by Miller's (2001) analysis of adult morphological features or Alarie's (1998) analysis of larval characters, each of which found *Hydrotrupes* related to Agabinae. Ribera et al. (2008) found *Hydrotrupes* resolved together with the *Platynectes*-group of genera. A more focused analysis on the subfamily by Ribera et al. (2004) also supported a distinction between the *Agabus*-group of genera and the *Platynectes*-group. The *Agabus*-group includes a number of primarily Holarctic taxa, whereas the *Platynectes*-group includes several genera from northern and high-elevation South America, Central America, Southeast Asia, and Australia investigated by Toussaint et al. (2017).

This analysis found a monophyletic Agabinae as historically defined, including *Hydrotrupes*, though support for the clade is moderate (Fig. 3.3, pp = 0.72). Agabinae is sister to Colymbetinae with high support (Fig. 3.3, pp = 0.96), and together this clade is sister to Lancetinae, although this last relationship is not strongly supported (Fig. 3.3, pp = 0.62). Within Agabinae, two larger clades are strongly resolved, one including the *Platynectes*-group of genera (including *Hydrotrupes*) (Fig. 3.3, pp = 1.00) and the second including the *Agabus*-group of genera (Fig. 3.3, pp = 1.00). Based on these results, we recognized two separate tribes within Agabinae in the first edition of this chapter, Hydrotrupini Roughley to include *Hydrotrupes* and the *Platynectes*-group of genera and Agabini, to include the remaining, primarily Holarctic genera. Details of their diagnoses and taxon content are described below under each tribe. This definition of Hydrotrupini was found paraphyletic by Toussaint et al. (2017); however, neither Hydrotrupini nor Agabini were monophyletic in the larval analysis by Michat et al. (2017).

The clade of Agabinae+Colymbetinae+Lancetinae was also recovered by Désamoré et al. (2018), but here a deeper rearrangement found a stronger support (pp = 0.95) for a clade that included these with Copelatinae, Laccophilinae, Cybistrinae, and Dytiscinae. Interestingly, the genomic study using UCE loci by Gustafson et al. (2020) further included the last two medium- to large-bodied subfamilies Matinae and Coptotominae in this clade resulting in a basal split within Dytiscidae between this clade and smaller-bodied Hydrodytinae+Hydroporinae. The age of Agabinae was estimated to 83.0 my (53.1–112.3) for the crown node and 97.6 my (65.9–127.8) for the stem node (Désamoré et al. 2018).

3.5.3.4 Taxon Content

Two tribes are currently included in Agabinae, Agabini Thomson, 1867 and Hydrotrupini Roughley, 2000 (see under Hydrotrupini for a discussion of a suggested third tribe by Toussaint et al. 2017).

3.5.4 Agabini Thomson, 1867

3.5.4.1 Type Genus

Agabus Leach, 1817.

3.5.4.2 Diagnosis

These are Agabinae characterized by having: (1) linear, marginal foveae present either at the anterolateral angles of the clypeus or extending entirely across the clypeus, and (2) females without natatory setae along the ventral margins of the metatibia and metafemur (except in the species, *Ilybius discedens*, which is clearly derived within Agabini (Larson 1987; Nilsson 1996, 2000).

3.5.4.3 Discussion

The bulk of the species of Agabinae are in this group, and collectively they have generally been regarded as a natural group and near Colymbetini. One exception to this is *Hydronebrius* Jakovlev which has been placed in its own tribe, Hydronebriini Brinck (and Hydronebriini Guignot), based on the absence of a metafemoral series of setae. Nilsson (2000) found this character unconvincing for tribal status suggesting lack of this series to be simply the result of increased punctation. He synonymized the tribe with Agabini sensu lato. Ribera et al. (2004) investigated relationships among the many species in this group, and Bergsten et al. (2012) explored the degree to which species could be diagnosed based on a mitochondrial marker.

Here Agabini is monophyletic with good support (Fig. 3.3, pp = 1.00) and sister to remaining Agabinae (Fig. 3.3, pp = 0.72). The unusual genus *Hydronebrius* was not included, but based on descriptions of the genus (Brancucci 1980; Nilsson 2000; Toledo 1993) it seems clear the genus is related to the other genera in this group.

Phylogenetic analyses based on larval characters have questioned the monophyly of Agabini and placed *Agabinus*, albeit moderately supported, as sister to all other Agabinae (Michat et al. 2017; Okada et al. 2019).

3.5.4.4 Taxon Content

Based on Nilsson's (2000) work and confirmation of generic limits by Ribera et al. (2004), there are six genera in this tribe. *Platambus* and *Agabinus* were not included, but these are clearly members of this tribe (Nilsson 2000; Ribera et al. 2008; Okada et al. 2019).

Agabinus Crotch, 1873
Agabus Leach, 1817

Hydronebrius Jakovlev, 1897
Ilybiosoma Crotch, 1873
Ilybius Erichson, 1832
Platambus Thomson, 1859

3.5.5 *Hydrotrupini* Roughley, *2000*

3.5.5.1 Type Genus

Hydrotrupes Sharp, 1882.

3.5.5.2 Diagnosis

Members of this group are Agabinae characterized by (1) sublateral elliptical foveae on the clypeus (somewhat ambiguous in *Hydrotrupes*) and (2) females with natatory setae along the ventral margins of the metatibia and metafemur (natatory setae entirely absent in *Hydrotrupes*). Predaceous diving beetle males generally have ventral natatory setae on the metatibia and metafemur, but females of many groups do not. Within Agabinae, only hydrotrupines have ventral setae in both males and females with the exception of the species, *Ilybius discedens* Sharp, which is clearly derived within Agabini (Larson 1987; Nilsson 1996, 2000).

3.5.5.3 Discussion

Hydrotrupini Roughley (at the subfamily rank) was originally erected to include only the genus *Hydrotrupes* but was expanded in the first edition of this chapter to also include the *Platynectes*-group of genera, a unique component of the Agabinae recognized by Brinck (Brinck 1948), Guéorguiev (1971, 1972), Nilsson (2000) and Ribera et al. (2004). *Hydrotrupes* and members of the *Platynectes*-group of genera were found to be monophyletic with strong support (Fig. 3.3, pp = 1.00), with *Hydrotrupes* resolved as sister to the rest of the group, also with high support (Fig. 3.3, pp = 1.00).

The relationships did not change in the reanalysis by Désamoré et al. (2018) but *Hydrotrupes* constitutes an enigmatic taxon that continues to be difficult to place phylogenetically. It represents possibly the longest branch among Agabinae, and the adaptation to the specialized hygropetric habitat may mislead morphological interpretations. Toussaint et al. (2017) investigated the phylogeny of the *Platynectes*-group of genera with a comprehensive species-level sampling and six genes, which led to *Leuronectes* and *Agametrus* included in *Platynectes*. The same study found *Hydrotrupes* more closely related to Agabini than to the *Platynectes*-group in contrast to previous studies, but with moderate support. Although having peculiar lifestyles, including hygropetric habits, which may lead to convergent adaptations, recent analyses with larval characters support a sister group relationship between *Platynectes* and *Hydrotrupes* (Alarie and Michat 2020; Okada et al. 2019). Alarie et al. (2019a) list five shared characters, including a serrate mandible edge. The finding of an extinct representative of the genus *Hydrotrupes* in Baltic amber (Gómez and Damgaard 2014) suggests that the remarkable extant disjunct distribution of the genus, with one species in western North America (Miller and Perkins 2012) and a second in China (Nilsson 2003a, b), may be the relictual remains of a lineage once more widespread (Gómez and Damgaard 2014). Whereas the inclusion of *Hydrotrupes* in Agabinae is no longer doubted (contra Beutel 1994 and Roughley 2000), its relationship to Agabini and the *Platynectes*-group of genera remain unsettled (this analysis, Toussaint et al. 2017, Désamoré et al. 2018; Alarie et al. 2019a, b; Alarie and Michat 2020, Okada et al. 2019).

3.5.5.4 Taxon Content

Hydrotrupini includes the enigmatic genus *Hydrotrupes* Sharp, 1882 and the two genera *Andonectes* Guéorguiev, 1971 and *Platynectes* Régimbart, 1879. The latter has yet to be included in a molecular phylogenetic analysis but based on morphology, it most likely belongs in the tribe. Toussaint et al. (2017) actually proposed the new tribe Platynectini Toussaint and Balke to solve the paraphyly of Hydrotrupini found in their study. They proposed the *Platynectes*-group of genera to be included in Platynectini with Hydrotrupini redefined to only include the genus *Hydrotrupes*. This classification into three tribes is also used in the most recent world catalog (Nilsson and Hájek 2022). However, the proposal to erect the new tribe Platynectini in Toussaint et al. (2017: 504) falls short of making a new family-group name available since no diagnosis for the new name was provided, and the requirement of article 13 (ICZN) not fulfilled. This should be uncontroversial. From a strict interpretation, it would neither fulfill article 16.2 of explicit type genus designation (e.g., see Dubois 2011), but no common agreement exists regarding the interpretation of this article (e.g., see corrigenda Sereno and Larsson 2009 resulting from a discussion on this subject). We refrain from making Platynectini available here and hence maintain the classification of Agabinae into two tribes based on the currently available family-group names. The tribal classification is likely not settled yet for Agabinae and still awaits a stable and well-supported reconstruction between *Hydrotrupes*, the *Platynectes*-group, *Agabinus* and remaining Agabini.

3.5.6 Colymbetinae Erichson, 1837

3.5.6.1 Type Genus

Colymbetes Clairville, 1806

3.5.6.2 Diagnosis

These are Dytiscidae characterized by adults with: (1) the eyes anteriorly emarginate, (2) the male median lobe asymmetrical, but not generally strongly so, (3) the lateral lobes bilaterally symmetrical, (4) the female gonocoxae flattened and apically rounded, (5) the prosternum and prosternal process together in the same plane, and (6) the apices of the elytra evenly rounded, except *Rhantus tristanicola* (Brinck) and *Rhantus selkirki* Jäch, Balke and Michat, (7) abdominal pleurite II with transverse rugae (not visible with elytra closed), and (8) metatarsal claws unequal in length.

3.5.6.3 Discussion

This subfamily included for many years taxa now placed in Lancetinae, Matinae, Agabinae, Coptotominae, Copelatinae, and even Laccophilinae (*Agabetes*) (e.g., Sharp 1882). The taxon content of Colymbetinae changed considerably as these taxa were removed over several years based on recognition of the large-scale paraphyly of the traditional concept (Beutel 1994; Burmeister 1976, 1990; Miller 2001; Ruhnau 1986; Ruhnau and Brancucci 1984). The current definition is considerably restricted (Miller 2001). An unusual species, *Carabdytes upin* Balke, Hendrich and Wewalka, was described from New Guinea and placed in its own tribe, Carabdytini Balke, Hendrich and Wewalka, 1992. Miller (2001) retained the tribal classification, but a molecular analysis by both Ribera et al. (2008) and Balke et al. (2009) resolved *Carabdytes* within Colymbetini, though no formal change was made to the classification until Morinière et al. (2015) synonymized Carabdytini with Colymbetini. Morinière et al. (2015) also tested the position of the two monotypic genera *Senilites* Brinck (from Tristan da Cunha in the South Atlantic) and *Anisomeria* Brinck (from Juan Fernández in the Pacific Ocean) that made up the poorly understood tribe Anisomeriini Brinck 1948. They found both genera nested inside a group of *Rhantus* species and formally synonymized Anisomeriini with Colymbetini. The internal relationships of Colymbetini have been investigated using larvae (Alarie 1995, 1998; Alarie and Balke 1999; Alarie and Larson 1998; Michat 2005; Michat and Archangelsky 2009), but, in general, taxon sampling has not been adequate to determine the relationships among colymbetine taxa given the extreme diversity of the genus *Rhantus* and its evident paraphyly (Ribera et al. 2008). This changed with the extensive sampling in Morinière et al. (2016), a study focused on the latitudinal diversity pattern but highlighting the need for changes to the genus classification, which was implemented in Balke et al. (2017a).

Here, Colymbetinae is monophyletic with high support (Fig. 3.3, pp = 1.00) and is sister to Agabinae with high support (Fig. 3.3, pp = 0.96). *Carabdytes* is nested within Colymbetini (Fig. 3.3), corroborating Ribera et al. (2008), Balke et al. (2009) and Morinière et al. (2016). The sistergroup relationship between Agabinae and Colymbetinae was also supported in the reanalysis by Désamoré et al. (2018) and with strong support in most analyses of UCE data (Gustafson et al. 2020), but not in the larval character phylogenetic analysis (Michat et al. 2017). Within Colymbetinae, there is a sister group relationship between the largely southern hemisphere *Meridioranthus* + *Carabdytes* and remaining Colymbetinae (Morinière et al. 2016; Balke et al. 2017a). Also the large-bodied Holarctic *Colymbetes*, *Hoperius*, *Neoscutopterus* and *Meladema* form a monophyletic group (Morinière et al. 2016; Balke et al. 2017a). The age of Colymbetinae was estimated to be 65.1 my (37.0–95.4) for the crown node and 97.6 my (65.9–127.8) for the stem node (Désamoré et al. 2018).

3.5.6.4 Taxon Content

Following the revised genus-level classification by Balke et al. (2017a), Colymbetinae now includes 11 genera, with the bulk of diversity still in the newly restricted sense of the genus *Rhantus*.

Bunites Spangler, 1972
Caperhantus Balke, Hájek and Hendrich, 2017
Carabdytes Balke, Hendrich and Wewalka, 1992
Colymbetes Clairville, 1806
Hoperius Fall, 1927
Meladema Laporte, 1835
Melanodytes Seidlitz, 1887
Meridiorhantus Balke, Hájek and Hendrich, 2017
Nartus Zaitzev, 1907
Neoscutopterus J. Balfour-Browne, 1943
Rhantus Dejean, 1833

3.5.7 Copelatinae Branden, 1885

3.5.7.1 Type Genus

Copelatus Erichson, 1832.

3.5.7.2 Diagnosis

Copelatinae are Dytiscidae with: (1) the metacoxal lines closely approximated medially (lines absent in *Lacconectus* Motschulsky, *Aglymbus* Sharp and *Madaglymbus* Shaverdo and Balke, but corresponding medial regions of metacoxae narrow), (2) the scutellum externally visible with the elytra closed, and (3) the metatarsal claws subequal in length in both sexes.

3.5.7.3 Discussion

Members of this group have a long history of placement within Colymbetinae. More recently, the group has been recognized as a subfamily sister to the rest of the Dytiscidae based on the presence of a foregut with a crop and serrated mandibles (and presumed ingestion of solid food particles) in larvae of some *Copelatus* (Beutel 1994, 1998; de Marzo 1976; Larson et al. 2000; Ruhnau 1986; Ruhnau and Brancucci 1984), though larvae of most *Copelatus* and several other copelatine genera are unknown and the generality of this feature remains unclear. Recent analyses have contradicted this sister group relationship (Miller 2001; Ribera et al. 2002, 2008), but there has been no consensus regarding copelatine relationships with other dytiscids. In fact, Ribera et al. (2008) found Copelatinae not monophyletic with the Neotropical *Agaporomorphus* related to the Nearctic Coptotominae, instead. Within Copelatinae Balke et al. (2004a) and Shaverdo et al. (2008) used mitochondrial data to test the relationships among the several genera. Bilton et al. (2015) and Toussaint et al. (2016a) added also nuclear genes to datasets, including all currently recognized genera, but with few representatives of each. Basal nodes are not convincingly supported but possibly there is support for a large southern hemisphere clade to the exclusion of *Copelatus* and *Lacconectus* in these analyses. The genera *Copelatus* and *Exocelina* are extremely diverse at the species level, and the Melanesian diversification of the latter has been the focus of several biogeographic analyses (Balke et al. 2007; Toussaint et al. 2014, 2015a) and is estimated to be of Miocene age. The radiation of *Exocelina* also includes specialized stygobiontic and subterranean taxa (e.g., Balke et al. 2004b; Balke and Ribera 2020).

This analysis supports a monophyletic Copelatinae, including *Agaporomorphus*, with good support (Fig. 3.3, pp = 1.00). Also supported strongly is a sister group relationships between Copelatinae and Dytiscinae + (Laccophilinae + Cybistrinae) (Fig. 3.3, pp = 0.96). The reanalysis by Désamoré et al. (2018) yielded a variation to this clade placing Copelatinae as sister to only Laccophilinae+Cybistrinae, whereas Michat et al. (2017) recovered Copelatinae as sister to Agabinae. In most of the UCE-based analyses, Copelatinae was recovered with strong support as sister to the larger clade of cybistrines, dytiscines, matines, colymbetines, and agabines (Gustafson et al. 2020). None of the above relationships are obvious based on any morphological features, but neither is any other relationship of Copelatinae with other predaceous diving beetle groups. Interestingly, Ribera et al. (2002) found copelatines nested within this same clade (with the anomalous addition of Paelobiidae), though a later analysis by Ribera et al. (2008) did not resolve a similar configuration. What is suggested by each of these results, however, including ours, is that the unique larval ingestion of particulate food (Beutel 1994, 1998; de Marzo 1976; Larson et al. 2000; Ruhnau 1986; Ruhnau and Brancucci 1984) is derived in this taxon (at least the known species). The age of Copelatinae was estimated to be 85.9 my (58.0–114.2) for the crown node and 113.9 my (88.8–135.3) for the stem node (Désamoré et al. 2018).

3.5.7.4 Taxon Content

Copelatine currently includes eight genera with no tribal subdivisions. The genus *Rugosus* García was described in Colymbetinae (García 2001), but the holotype specimen of *Rugosus emarginatus* García (in Universidad del Zulia, Maracaibo, Venezuela, J. Camacho, curator; examined by KBM) clearly belongs in Copelatinae based on close approximation of the margins of the medial portion of the metacoxae (metacoxal lines absent), subequal metatarsal claws, and other general features. *Rugosus* García was moved to Copelatinae in the first edition of this chapter and later synonymized with *Aglymbus* by Toussaint et al. (2016a). The very diverse genus *Copelatus* has been divided into multiple genera recently (Balke et al. 2004b) and will likely continue to be subdivided with continued study. The most recent addition was a new species discovered in the South African Western Cape Region, meriting the erection of a new genus, *Capelatus* (Bilton et al. 2015). Members of *Aglymbus*, *Liopterus*, *Madaglymbus*, and *Capelatus* were not included in the analysis, but all have been included in previous (Balke et al. 2004a; Ribera et al. 2008; Shaverdo et al. 2008) and more recent (Bilton et al. 2015; Toussaint et al. 2016a) analyses.

Agaporomorphus Zimmermann, 1921
Aglymbus Sharp, 1880
Capelatus Turner and Bilton, 2015
Copelatus Erichson, 1832
Exocelina Broun, 1886
Lacconectus Motschulsky, 1856
Liopterus Dejean, 1833
Madaglymbus Shaverdo and Balke, 2008

3.5.8 Laccophilinae Gistel, 1848

3.5.8.1 Type Genus

Laccophilus Leach, 1815.

3.5.8.2 Diagnosis

These are Dytiscidae with: (1) the female gonocoxae strongly fused along the dorsal margin with the apex pointed, or bi-pointed with a narrow apical emargination, and the rami fused medially with anteriorly projecting processes and ventrally with distinct teeth, (2) two distinct female genital openings and (3) both males and females with natatory setae along the posteroventral margin of the metatarsomeres but the metatibia without posteroventral natatory setae.

3.5.8.3 Discussion

There are two groups associated with Laccophilinae sensu lato, Agabetini van den Branden, with a single genus *Agabetes* Crotch and two species, and Laccophilini Gistel, which includes the bulk of the diversity in the subfamily. Without the inclusion of *Agabetes*, Laccophilinae has been a consistently recognized group for much of the history of dytiscid classification. Although placed in its own family-group by Branden (1885), *Agabetes* had usually been placed in Colymbetinae until Burmeister (1976) pointed out the unusual female genitalia that linked the genus more closely with Laccophilinae, a result corroborated by Ruhnau and Brancucci (1984). He later (Burmeister 1990) elevated the tribe to subfamily rank within Dytiscidae based on attributes of the female genitalia, though he recognized a close affinity between Agabetinae and Laccophilinae. Many subsequent authors (with some exceptions, see Larson et al. 2000) have instead recognized Laccophilinae with two tribes, Agabetini and Laccophilini, while adding additional evidence from adult and larval morphology (Alarie et al. 2002b; Miller 2001). Ribera et al. (2008) did not, however, find a close association between *Agabetes* and Laccophilinae. There has been no general consensus of relationships of Laccophilinae with other dytiscid groups, though they were historically often placed with Noterinae before that group was removed from Dytiscidae (e.g., Sharp 1882). Larval evidence (Ruhnau and Brancucci 1984) and female reproductive musculature (de Marzo 1997) have suggested some affinities with Hydroporinae, and Nilsson (1989) raised the possibility of close relationship between Laccophilinae and Lancetinae.

In this analysis, *Agabetes* is resolved as the sister to Laccophilini with strong support (Fig. 3.3, pp = 1.00), corroborating Burmeister (1990), Miller (2001), and Alarie et al. (2002b). Here we recognize Laccophilinae with two tribes, Agabetini and Laccophilini. The sister relationship between Laccophilinae and cybistrines and sister group relationship between this clade and Dytiscinae (sensu stricto, i.e., without Cybistrini) is unexpected and perplexing because there is little obviously supporting this from morphology, and there appears to be considerable morphological support for Dytiscinae as historically recognized (i.e., with Cybistrini as a part of it). Dytiscinae sensu lato is supported by several features from adult and larval morphology including: (1) large size in general (compared with small to very small size in Laccophilinae), (2) adults with rounded eyes anteriorly, (3) the median lobe bilaterally symmetrical with a distinct, elongate ventral sclerite, (4) a single genital opening in the female reproductive tract (RT) with the opening for sperm reception into the RT the same opening used for oviposition (laccophilines with two genital openings as with most other Dytiscidae), (5) larval abdominal segments VII-VIII with distinct lateral fringe of setae presumably used during a “shrimping” type of swimming behavior (lateral setae absent in laccophilines), and (6) the larval antennomeres and maxillary and labial palpomeres subdivided into additional sub-segments (not subdivided in laccophilines). Taken together, this has made Dytiscinae among the best-defined predaceous diving beetle groups in analyses based entirely or mainly on morphology (Alarie et al. 2011; Miller 2000, 2001, 2003; Michat et al. 2017). Analyses based entirely or mainly on molecular data, however, have not supported this grouping at all with Cybistrini resolved elsewhere (Fig. 3.3, Ribera et al. 2002) or with Dytiscinae in three separate clades (Ribera et al. 2008). Support for Laccophilinae + Cybistrini is strong in our analysis (Fig. 3.3, pp = 1.00), as is support for Dytiscinae sensu stricto (Fig. 3.3, pp = 1.00), though the clade Dytiscinae sensu stricto + (Laccophilinae + Cybistrini) is not so strongly supported (Fig. 3.3, pp = 0.64). Based on this support and the overall robustness of our results and this analysis in general, it would seem the most prudent thing to do is to change the classification to reflect this best evidence for the phylogeny. Therefore Cybistrini was elevated from tribe to subfamily rank, Cybistrinae, in the first edition of this chapter, and Dytiscinae was restricted to the remaining tribes, Dytiscini (=Hyderodini, see below), Hydaticini, Aubehydrini, Eretini and Aciliini. What these relationships imply is that either the several rather unusually distinctive characteristics shared by Cybistrinae and Dytiscinae are independently derived in those groups or lost (reversed) in Laccophilinae. Possibly, some of these features are closely associated with size that is large in Cybistrinae and most Dytiscinae, but is relatively much smaller in Laccophilinae. The considerable length of many branches within Laccophilinae as over against other nearby taxa suggests this group may have undergone more rapid evolution than have other dytiscid taxa.

Laccophilinae, Cybistrinae, and Dytiscinae do share very similar configurations of the external female genitalia. All have the gonocoxae fused and apically somewhat knifelike and the rami well-developed and fused (modified in Eretini and Aciliini and some Hydatcini to be little or much less knifelike) (Miller 2000, 2001, 2003). In some cases, the rami of Cybistrinae are additionally similar to laccophilines in having the rami ventrally at least somewhat denticulate (Miller 2000, 2001, 2003). Also Cybistrinae and Laccophilinae have asymmetrical male suction cups on protarsus (symmetrical in Dytiscinae sensu stricto), though this is the plesiomorphic condition in Dytiscidae.

Whereas the reanalysis by Désamoré et al. (2018) did not change these results, most of the UCE-based analyses by Gustafson et al. (2020) resulted in, for the first time, a rejoined Dytiscinae and Cybistrinae as sister groups based on molecular data. This strengthens instead the multiple convincing lines of evidence from both adult and larval morphology of their close relationship. Continued recognition as separate subfamilies is fully compatible with these results, however, and is motivated by the fundamental differences in male tarsal suction cups. Laccophilinae was by Gustafson et al. (2020) inferred to be sister to the clade Coptotominae + Lancetinae, but only in some of the UCE-based analyses. The age of Laccophilinae was estimated to be 70.9 my (44.7–98.3) for the crown node and 95.4 my (71.6–120.6) for the stem node (Désamoré et al. 2018).

3.5.8.4 Taxon Content

Two tribes, Agabetini Branden, 1885 with one genus and two species, and Laccophilini Gistel, 1848 with several genera and many species.

3.5.9 Agabetini Branden, 1885

3.5.9.1 Type Genus

Agabetes Crotch, 1873

3.5.9.2 Diagnosis

These are Laccophilinae with: (1) the scutellum visible with the elytra closed, (2) two subequal metatarsal claws, and (3) less strongly lobed metatarsomeres than in Laccophilini. These are medium-sized, darkly colored, oval beetles that are superficially similar to certain agabines and copelatines but lack a series of closely placed setae at the apical angle of the metafemur and have the distinct metacoxal lines broadly separated, among other things. In addition, the dorsal surface is covered with short, fine grooves and males have a distinctive pair of longitudinal grooves on abdominal sternum VI (males of *A. svetlanae* Nilsson not known).

3.5.9.3 Discussion

Agabetini is sister to Laccophilini (all other known Laccophilinae) with good support (Fig. 3.3, pp = 1.00) (see above under Laccophilinae for further discussion).

3.5.9.4 Taxon Content

Agabetini includes one genus, *Agabetes* Crotch, 1873, with two species *A. acuductus* (Harris) in eastern North America and *A. svetlanae* Nilsson, from the Caspian coast of Iran.

3.5.10 Laccophilini Gistel, 1848

3.5.10.1 Type Genus

Laccophilus Leach, 1815.

3.5.10.2 Diagnosis

These are Laccophilinae with: (1) the scutellum not visible with the elytra closed, (2) a single metatarsal claw, and (3) prominent lobes at the anteroapical apices of the metatarsomeres.

3.5.10.3 Discussion

This tribe comprises the bulk of Laccophilinae diversity and is sister to Agabetini with good support (Fig. 3.3, pp = 1.00). Relationships among the numerous genera in the group have not been adequately investigated, though Alarie et al. (2000) and Michat and Toledo (2015) presented some phylogenetic work based on larval features in five genera (see above under Laccophilinae for further discussion). However, Toledo and Michat (2015) delineated the new genus *Laccomimus* based on a cladistic analysis showing it was not very closely related to *Laccodytes*. An additional Neotropical genus was described by Benetti et al. (2019), and intriguing fossil laccophiline taxa has recently been described from Baltic and Saxonian amber (Balke and Hendrich 2019; Balke et al. 2019).

3.5.10.4 Taxon Content

There are 14 genera in Laccophilini with members of several of them very rarely collected and obscure (e.g., *Napodytes*, *Laccosternus*) and others extremely common, abundant, and species-rich (e.g., *Laccophilus*, *Neptosternus*). The newly genus *Laccomimus* from South America was included in the analysis in the first edition of this chapter under the name “*Laccodytes* sp.” (Fig. 3.3, Toledo et al. 2011).

Africophilus Guignot, 1948
Australphilus Watts, 1978
Hamadiana Benetti, Short and Michat, 2019
Japanolaccophilus Satô, 1972
Laccodytes Régimbart, 1895
Laccomimus Toledo and Michat, 2015
Laccophilus Leach, 1815
Laccoporus J. Balfour-Browne, 1938
Laccosternus Brancucci, 1983
Napodytes Steiner, 1981
Neptosternus Sharp, 1882
Philaccolilus Guignot, 1937
Philaccolus Guignot, 1937
Philodytes J. Balfour-Browne, 1939

3.5.11 Cybistrinae Sharp, 1880

3.5.11.1 Type Genus

Cybister Curtis, 1827.

3.5.11.2 Diagnosis

This is one of the most well-defined groups in all of Dytiscidae. Members of the clade exhibit numerous unambiguous adult and larval synapomorphies including adults with: (1) the metafemur and metatibia very broad and short; (2) the metatibial spurs different in size and shape, with the anterior spur acuminate and broader than the posterior one; (3) a posteroapical cluster of bifid setae on the metatibia; (4) a cluster of stiff setae on the apicoventral surface of the elytron; (5) females with two glands near the base of the common oviduct; (6) females with extensive muscles surrounding the vagina; (7) males with the adhesive setae on the mesotarsomeres apically simple (when present); (8) natatory setae present along the dorsal margin of metafemur, and larvae with; (9) the anterior margin of the clypeus prominently dentate; (10) the abdominal tergites reduced; (11) egg bursters absent in instar I; (12) the anterior margin of the prementum with a distinct lobe lacking spinous setae; (13) the antennae, maxillary palpi and labial palpi subdivided in all instars, and (14) the cerci very short or absent (Alarie et al. 2011; Miller et al. 2007b).

3.5.11.3 Discussion

This group has traditionally been recognized as a tribe within Dytiscinae. The group has generally been considered strongly supported as monophyletic (Alarie et al. 2011; Miller 2000, 2001, 2003; Ribera et al. 2002, 2008). The internal phylogeny of Cybistrinae was investigated by Miller et al. (2007b), recovering the Australian genera monophyletic and sister to *Cybister*+*Megadytes*, later confirmed by larval character analyses (Michat et al. 2015a, 2019). Within Dytiscidae, the group has been found to be a member of the Dytiscinae and sister to the rest of the subfamily (Miller 2000, 2001, 2003). Results from other analyses of molecular data, however, suggest the group is not related to other Dytiscinae (Ribera et al. 2002, 2008).

This analysis recovered a monophyletic Cybistrinae (Fig. 3.3, pp = 1.00) sister to Laccophilinae with strong support (Fig. 3.3, pp = 1.00) . See above under Laccophilinae for further discussion of the unexpected relationship of Cybistrinae + Laccophilinae and the later retrieval of Dytiscinae as sister group in Gustafson et al. (2020). The age of Cybistrinae was estimated to be 60.9 my (37.4–81.5) for the crown node and 82.7 my (59.3–110) for the stem node (Désamoré et al. 2018).

3.5.11.4 Taxon Content

This tribe currently includes seven genera with *Cybister* and *Megadytes* each with several subgenera (Miller et al. 2007b).

Austrodytes Watts, 1978
Cybister Curtis, 1827
Megadytes Sharp, 1882
Onychohydrus Schaum and White, 1847
Regimbartina Chatanay, 1911
Spencerhydrus Sharp, 1882
Sternhydrus Brinck, 1945

3.5.12 Dytiscinae Leach, 1815

3.5.12.1 Type Genus

Dytiscus Linnaeus, 1758.

3.5.12.2 Diagnosis

These are Dytiscidae with: (1) the eyes not emarginate along the anterolateral margin, (2) the aedeagus (both the median lobe and lateral lobes) bilaterally symmetrical, (3) a single genital opening in the female, (4) the gonocoxae fused dorsally, (5) the prosternum and prosternal process together in the same plane, (6) the pro- and mesotarsi distinctly pentamerous, (6) males with the protarsal adhesive setae apically with a circular sucker-disc, (7) larval abdominal segments VII-VIII with a distinct lateral fringe of setae, and (8) the larval antennomeres and palpomeres secondarily divided into additional segments. Cybistrinae, until now, has been a part of this subfamily and shares many of the characteristics. Major differences between Cybistrinae and Dytiscinae as here defined include the presence in cybistrines of elongate-oval apices of the male protarsal adhesive setae and the anterior metatibial spur broad and apically acuminate. Dytiscines have the anterior spur slender and similar to the posterior spur.

3.5.12.3 Discussion

This group has maintained its composition of taxa for a long time with a couple of exceptions. One of these is the genus *Notaticus* Zimmermann, with one species, which was originally described in Hydaticini. Guignot (1949), however, subsequently erected the junior synonym *Aubehydrus* Guignot and placed it in its own subfamily Aubehydrinae based on the absence of an externally visible scutellum. Miller (2000) found evidence for placement of the genus within Dytiscinae, a result that was subsequently corroborated using both adult morphology and molecular data (Miller 2001, 2003; Ribera et al. 2002, 2008) and larval features (Miller et al. 2007a). The subfamily (along with Cybistrinae) has been thought to be closely related to Colymbetinae (or the narrower Colymbetini) and, possibly, Lancetinae (Alarie et al. 2002a; Miller 2000, 2001; Ruhnau 1986; Ruhnau and Brancucci 1984) though there has not been a consensus at this point.

In this analysis, Dytiscinae, as traditionally defined, that is, including Cybistrinae, is not monophyletic (Fig. 3.3) (but see discussion above under Laccophilinae). Dytiscinae without Cybistrinae is, though, with the latter group sister to Laccophilinae and that clade sister to the rest of Dytiscinae (Fig. 3.3, see further discussion under Cybistrinae and Laccophilinae above). Dytiscinae as restricted here, is monophyletic with strong support (Fig. 3.3, pp = 1.00). Within Dytiscinae, a clade comprised of Aubehydrini, Hydaticini, Aciliini and Eretini is well-supported, as well (Fig. 3.3, pp = 1.00). This group includes members with series of short, appressed setae along the apical margins of meso- and metatarsomeres I–IV and larvae with characteristic swimming behavior and various morphological features (Alarie et al. 2011; Miller 2000, 2001, 2003; Miller et al. 2007a). Dytiscini + Hyderodini (here regarded as one tribe, Dytiscini, see below) is also well-supported (Fig. 3.3, pp = 1.00). The age of Dytiscinae was estimated to be 112.7 my (84–135.4) for the crown node and 129.1 my (122–141.4) for the stem node (Désamoré et al. 2018).

3.5.12.4 Taxon Content

Dytiscinae currently includes five tribes:

Aciliini Thomson, 1867
Aubehydrini Guignot, 1942
Dytiscini Leach, 1815
Eretini Crotch, 1873
Hydaticini Sharp, 1880

3.5.13 Dytiscini Leach, 1815

3.5.13.1 Type Genus

Dytiscus Linnaeus, 1758.

3.5.13.2 Diagnosis

This group is characterized within Dytiscinae by: (1) absence of short, adpressed setae along the apical margins of the meso- and metatarsomeres and (2) metatarsal claws equal in length.

3.5.13.3 Discussion

Dytiscus and *Hyderodes* were placed in a single tribe by Sharp (1882). Roughley (1990) also regarded them as sister groups. Miller (2000), however, found evidence from morphology that *Hyderodes* is sister to a clade including Aubehydrini, Hydaticini, Eretini and Aciliini and placed the genus in its own tribe, Hyderodini Miller. This was corroborated by subsequent analyses, as well (Miller 2001, 2003). Ribera et al. (2002) found *Hyderodes* nested in a clade of Aubehydrini and Hydaticini, and Ribera et al. (2008) found *Hyderodes* sister to Hydaticini.

In this analysis, the genera *Dytiscus* and *Hyderodes* are together monophyletic with strong support (Fig. 3.3, pp = 1.00), suggesting a more traditional interpretation of the classification of the group. Because of clear support for doing so, we synonymized Hyderodini Miller with Dytiscini Leach in the first edition of this chapter. Dytiscini is sister to the rest of Dytiscinae, as defined here (without Cybistrinae).

3.5.13.4 Taxon Content

Dytiscini includes two genera, the Holarctic *Dytiscus* Linnaeus, 1758 and the Australian *Hyderodes* Hope, 1838.

3.5.14 Hydaticini Sharp, 1880

3.5.14.1 Type Genus

Hydaticus Leach, 1817.

3.5.14.2 Diagnosis

These are Dytiscinae with: (1) the oblique anterolateral margin of the metaventrite (the anterior margin of the metaventral wing) straight or slightly concave, and (2) males with a stridulatory apparatus formed by a reticulate file on the dorsal surface of the male protarsomere II and short spines on the dorsoproximal margin of the protibia (absent in a few taxa) (Larson and Pritchard 1974; Miller 2003).

3.5.14.3 Discussion

Hydaticini has usually been recognized as monophyletic, though one analysis, by Miller (2003), found Aciliini + Eretini nested within Hydaticini, albeit with low support. *Notaticus* (Aubehydrini) was originally included in Hydaticini. However, the distinctively straight anterolateral margin of the metacoxa, the uniquely irregular grooves on the female pronotum and elytron, and the male protarsal / protibial stridulatory device are convincing morphological synapomorphies of the group (Miller et al. 2009a). Historically this group has had two genera, *Prodaticus* and *Hydaticus*, the latter with several subgenera including *Hydaticus* sensu stricto, *H.* (*Guignotites*), *H.* (*Hydaticinus*) and *H.* (*Pleurodytes*). A recent cladistic analysis by Miller et al. (2009a) resulted in a revised classification that recognized the same two genera, but with considerable content rearrangement. *Prodaticus*, which previously included only two species, was synonymized with each of the *Hydaticus* subgenera except *Hydaticus* sensu stricto. Thus, the content of the genus *Hydaticus* was reduced to only seven species, whereas *Prodaticus* included about 130, but with each genus demonstrably monophyletic. Nilsson (2010) preferred to avoid considerable reassignment of species names and placed *Prodaticus sensu* Miller et al. (2009a) as a subgenus of *Hydaticus* sensu lato. Miller (2001) and Miller et al. (2009a) found Hydaticini resolved as sister to Aubehydrini + (Eretini + (Aciliini)) morphologically supported by the presence of short, appressed setae along the apical margins of meso- and metatarsomeres I–IV.

This analysis resulted in a monophyletic Hydaticini with strong support (Fig. 3.3, pp = 1.00). The tribe is resolved as sister to the clade Aubehydrini + (Eretini + Aciliini), also with strong support (Fig. 3.3, pp = 1.00), corroborating Miller (2001).

3.5.14.4 Taxon Content

Hydaticini includes the single genus *Hydaticus* Leach, 1817.

3.5.15 Aubehydrini Guignot, 1942

3.5.15.1 Type Genus

Aubehydrus Guignot, 1942 (=*Notaticus* Zimmermann, 1928).

3.5.15.2 Diagnosis

Within Dytiscinae, members of this tribe are unique in having a concealed scutellum with the elytra closed.

3.5.15.3 Discussion

Notaticus was originally described in Hydaticini by Zimmermann (1928). Guignot (1942) erected a new subfamily, Aubehydrinae, for his new genus, *Aubehydrus* Guignot, which was later synonymized with *Notaticus* by Spangler (1973). *Notaticus* remained in its own subfamily until Miller (2000) placed it back within Dytiscinae based on a phylogenetic analysis of morphology. This was further confirmed by several independent studies (Alarie et al. 2011; Miller 2001, 2003; Miller et al. 2007a; Ribera et al. 2002, 2008). It has been resolved as sister to Hydaticini + Eretini + Aciliini (Miller 2000, 2001), sister to Aciliini (Miller 2003), within Hydaticini (Ribera et al. 2002) or as sister to (Aciliini + Eretini) + (Hyderodini + Hydaticini) (Ribera et al. 2008).

In this analysis, Aubehydrini was resolved as sister to Eretini + Aciliini with strong support (Fig. 3.3, pp = 1.00).

3.5.15.4 Taxon Content

Aubehydrini includes a single genus, *Notaticus* Zimmermann, 1928.

3.5.16 Eretini Crotch, 1873

3.5.16.1 Type Genus

Eretes Laporte, 1833.

3.5.16.2 Diagnosis

Eretini are Dytiscinae with: (1) the prosternal process apically narrow and sharply pointed, (2) the pronotum with a narrow lateral marginal bead, (3) the surfaces of the meso- and metatarsomeres with adpressed, flattened setae, (4) the posterolateral margin of the elytron with a linear series of short, curved, black spines, (5) the elytra very thin and flattened and relatively lightly sclerotized overall; (6) the elytra punctate with each puncture bearing a black spot, and (7) general pale color on all surfaces with small to extensive black markings on the dorsum of the head, pronotum, and elytra.

3.5.16.3 Discussion

Eretes has been recognized in its own tribe for many years, and the species in the group are relatively homogeneous, though they are quite distinctive from other

Dytiscidae. Four species are currently recognized after the revision by Miller (2002a), though there is some disagreement about species limits (Larson et al. 2000). The tribe has long been associated with Aciliini, and this has been confirmed with recent phylogenetic analyses (Alarie et al. 2011; Bukontaite et al. 2014; Miller 2000, 2001, 2003; Ribera et al. 2002) with *Eretes* nested within Aciliini, in some cases (e.g., Ribera et al. 2008).

In this analysis, Eretini is monophyletic (Fig. 3.3, pp = 1.00) and resolved as sister to Aciliini with strong support (Fig. 3.3, pp = 0.99). Eretini and Aciliini are very similar in larval features (Alarie et al. 2011; Miller 2002a), and many adult morphological characters, as well (Miller 2000, 2001, 2002a, 2003).

3.5.16.4 Taxon Content

The tribe has one genus, *Eretes* Laporte, 1833.

3.5.17 Aciliini Thomson, 1867

3.5.17.1 Type Genus

Acilius Leach, 1817.

3.5.17.2 Diagnosis

This tribe includes dytiscines with both metatibial spurs apically bifid.

3.5.17.3 Discussion

Aciliini includes some of the more common large predaceous diving beetles from throughout the world and have attracted considerable attention from biologists. The group has a long history of close association with Hydaticini and Eretini (e.g., Sharp 1882) and its monophyly has not been generally questioned, though the analysis by Ribera et al. (2008) placed *Eretes* within Aciliini. A recent comprehensive analysis of the genera within Aciliini, supported the tribe as monophyletic, Eretini as the sister clade and each of the seven included genera as monophyletic (Bukontaite et al. 2014). Neotropical *Thermonectus* occupied the basalmost position in the tribe, followed by Afrotropical *Aethionectes*+*Tikoloshanes* (Bukontaite et al. 2014).

Here Aciliini is resolved as monophyletic with high support (Fig. 3.3, pp = 0.99). The clade is sister to Eretini, also with high support (Fig. 3.3, pp = 0.99), and these are together in a clade with Hydaticini and Aubehydrini corroborating numerous previous analyses (Miller 2000, 2001, 2003).

3.5.17.4 Taxon Content

There are currently seven genera assigned to Aciliini. *Rhantaticus* and *Tikoloshanes* were not included in this analysis but was included in the analysis by Bukontaite et al. (2014).

Acilius Leach, 1817
Aethionectes Sharp, 1882
Graphoderus Dejean, 1833
Rhantaticus Sharp, 1880
Sandracottus Sharp, 1882
Thermonectus Dejean, 1833
Tikoloshanes Omer-Cooper, 1956

3.5.18 Coptotominae Branden, 1885

3.5.18.1 Type Genus

Coptotomus Say, 1830.

3.5.18.2 Diagnosis

These are Dytiscidae with: (1) a characteristic habitus being medium size (5.5–8.8 mm), elongate and relatively narrow and streamlined, (2) the pronotum with a well-developed lateral bead, (3) the metacoxal lobes large and rounded with the metacoxal lines not closely approximated medially, (4) the prosternum and prosternal process in the same plane, (5) the pro- and mesotarsi distinctly tetramerous, (6) the scutellum externally visible with the elytra closed, (7) the metafemur without an anteroapical series of setae, (8) metatarsomeres I–IV with anteroapical angles lobed, and (9) the metatarsal claws subequal in length in both sexes. In addition, larvae are characterized by having lateral tracheal gills on the abdomen, segment VIII with a lateral fringe of natatory setae (in instars II and III), and the clypeus with a distinct frontal “horn” (Larson et al. 2000).

3.5.18.3 Discussion

Historically, this family-group was recognized mainly at the tribe rank within Colymbetinae, although it has been occasionally recognized as a subfamily (e.g., Bacon et al. 2000). Miller (2001) also placed it at subfamily rank. Recent analyses resolve it in either an isolated position with respect to other subfamilies (Miller 2001) or closely associated with Copelatinae (Ribera et al. 2008). Currently only known from the Nearctic Region, a fossil Palearctic member was described from Baltic amber (Hendrich and Balke 2020).

This analysis resolved a monophyletic Coptotominae (Fig. 3.3, pp = 1.00) as sister to Hydrodytinae + Hydroporinae, though only with modest support (Fig. 3.3, pp = 0.63).

Interestingly, a new hypothesis has emerged following the first edition of this book chapter where Coptotominae now is supported as sister to Lancetinae both based on larval characters (Michat et al. 2017) and based on UCE loci (Gustafson et al. (2020). The age of Coptotominae was estimated to be 6.3 my (1.3–18.7) for the crown node and 138.2 my (118.8–158.7) for the stem node (Désamoré et al. 2018).

3.5.18.4 Taxon Content

Coptotominae includes a single North American genus, *Coptotomus* Say, 1830.

3.5.19 Hydrodytinae Miller, 2001

3.5.19.1 Type Genus

Hydrodytes Miller, 2001.

3.5.19.2 Diagnosis

These are Dytiscidae with: (1) the scutellum visible with the elytra closed, (2) the pro- and mesotarsi distinctly pentamerous in both sexes, (3) the prosternum and prosternal process in the same plane and without a median tubercle, (4) the female gonocoxa with a prolonged anterior apodeme, and (5) the metathoracic wing broad with vein M4 reaching oblongum cell and with distinct subcubital binding patch. The rami of the female genitalia are sinuate, and the male genitalia are bilaterally asymmetrical in the single species known to have males.

3.5.19.3 Discussion

Members of this Neotropical group were placed in the copelatine genus *Agaporomorphus* until that genus was subdivided by Miller (2001), who erected a new genus, *Hydrodytes*, and subfamily for the included species. The entire subfamily was revised by Miller (2002b), including description of a new genus, *Microhydrodytes* Miller. Miller (2001) found Hydrodytinae to be sister to Hydroporinae based on the anterior apodeme of the female gonocoxae and characters of the metafurca. Ribera et al. (2008) found Hydrodytinae resolved as sister to Matinae.

Here, Hydrodytinae is resolved as sister to Hydroporinae, corroborating Miller (2001).

The reanalysis by Désamoré et al. (2018) showed that the same data, excluding the morphological characters, very differently placed Hydrodytinae near Matinae as in Ribera et al. (2008). But UCE-based analyses largely supported the morphology-based sistergroup hypothesis between the species-poor Hydrodytinae and the megadiverse Hydroporinae (Gustafson et al. 2020).

3.5.19.4 Taxon Content

Hydrodytinae includes two Neotropical genera, *Hydrodytes* Miller, 2001 and *Microhydrodytes* Miller, 2002.

3.5.20 Hydroporinae Aubé, 1836

3.5.20.1 Type Genus

Hydroporus Clairville, 1806.

3.5.20.2 Diagnosis

These are Dytiscidae with: (1) the anteromedial portion of the prosternum in a distinctly different plane than the prosternal process (i.e., the prosternal process is declivous with respect to the prosternum, though this is somewhat less dramatic in some taxa such as Methlini), (2) the pro- and mesotarsi pseudotetramerous with tarsomere IV small and hidden within the lobes of tarsomere III (some taxa, such as *Bidessonotus*, *Necterosoma*, and *Sternopriscus* with the pro- and mesotarsi more distinctly pentamerous), and (3) the scutellum concealed with the elytra closed (*Celina* with a distinctively visible scutellum and some *Hydrocolus* with the scutellum partially visible).

3.5.20.3 Discussion

Hydroporinae has been recognized as a natural group for most of the history of dytiscid classification (e.g., Sharp 1882) with a few exceptions. The main one of these is *Celina* (or Methlini inclusive), which has a visible scutellum with the elytra closed and a less strongly declivous prosternal process (though *Methles* has a concealed scutellum). Numerous investigators have recognized this group at the subfamily rank (Bilardo and Rocchi 1990; Franciscolo 1966; Omer-Cooper 1958;

Pederzani 1995; Trémouilles 1995). Other groups of hydroporine have been occasionally elevated to subfamily rank during their history (such as Vatellinae *sensu* Omer-Cooper 1958), but not as commonly. The group has been usually recovered as monophyletic (Burmeister 1976; Miller 2001; Miller et al. 2006; Michat et al. 2017; Gustafson et al. 2020) though Ribera et al. (2002) found Hydroporinae paraphyletic with respect to a large portion of dytiscid diversity and Ribera et al. (2008) found Laccophilini nested within Hydroporinae. Miller (2001) found a sister group relationship between Hydroporinae and Hydrodytinae based on similarities in the female genitalia and metafurca, supported by most UCE-based analyses Gustafson et al. (2020). This relationship was not confirmed in subsequent molecular analyses (Ribera et al. 2002, 2008; Désamoré et al. 2018). The internal tribal phylogeny has been investigated several times within a more modern, cladistic context (Miller et al. 2006; Ribera et al. 2002, 2008; Wolfe 1985, 1988). Because of these efforts, a phylogenetic tribal classification has developed in the past 30 years with clarification of several relationships (see under each tribe below).

In this analysis, Hydroporinae is monophyletic with strong support (Fig. 3.3, pp = 1.00). It is resolved here sister to *Hydrodytes* (Hydrodytinae), as originally suggested by Miller (2001), also with strong support (Fig. 3.3, pp = 1.00). Within the group, most traditionally recognized tribes are monophyletic with a few exceptions (see under each tribe below). However, relationships among the tribes are subtended by relatively shorter branches and lower support values than within the tribes (Fig. 3.3). See below under each tribe treatment for further discussion about relationships among the tribes more specifically. The age of Hydroporinae was estimated to 126.7 my (105.9–148) for the crown node and 138.2 my (118.8–158.7) for the stem node (Désamoré et al. 2018).

3.5.20.4 Taxon Content

There are more genera and species in this group than in any other diving beetle subfamily, about 2300 species or 50% of the total species diversity of predaceous diving beetles (Nilsson 2001). There are currently ten tribes recognized in Hydroporinae. Four genera of hyporheic, subterranean and terrestrial Hydroporinae are currently *incertae sedis* with respect to tribe.

Bidessini Sharp, 1880
Hydroporini Aubé, 1836
Hydrovatini Sharp, 1880
Hygrotini Portevin, 1929
Hyphydrini Gistel, 1848
Laccornini Wolfe and Roughley, 1990
Laccornellini Miller and Bergsten, 2014
Methlini Branden, 1885
Pachydrini Biström, Nilsson and Wewalka, 1997

Vatellini Sharp, 1880
Genera *incertae sedis* with respect to tribe

Kuschelydrus Ordish, 1976
Morimotoa Uéno, 1957
Phreatodessus Ordish, 1976
Typhlodessus Brancucci, 1985

3.5.21 Laccornini Wolfe and Roughley, 1990

3.5.21.1 Type Genus

Laccornis des Gozis, 1914.

3.5.21.2 Diagnosis

Laccornini are Hydroporinae with: (1) the metacoxal lobes large and apically rounded, (2) the metafemur extending to metacoxal lobe along the anterior margin (not separated from it by the metatrochanter), and (3) the female external genitalia with laterotergites.

3.5.21.3 Discussion

Members of this group were included in Hydroporini sensu lato until Wolfe (1985, 1988) investigated more carefully the phylogenetic relationships of *Laccornis* and proposed the genus as sister to the rest of Hydroporinae. *Laccornis* was formally placed in its own tribe by Wolfe and Roughley (1990). Miller (2001) corroborated this relationship, though subsequent molecular analyses have not (Ribera et al. 2008).

In this analysis, *Laccornis* (Laccornini) is resolved as sister to all other Hydroporinae with good support (Fig. 3.3, pp = 0.89), corroborating Miller (2001), Wolfe (1985, 1988), and Wolfe and Roughley (1990). Only a single species of the single genus, *Laccornis*, was included in the analysis, so monophyly of the tribe was not examined, though others have established the probable monophyly of the group (Alarie 1989; Roughley and Wolfe 1987; Wolfe 1985; Wolfe and Roughley 1990; Wolfe and Spangler 1985). Michat et al. (2017) instead found Methlini in the position as sister to remaining Hydroporinae, including *Laccornis*, while in Gustafson et al. (2020) the two tribes formed a monophyletic group in the same position.

3.5.21.4 Taxon Content

Laccornini includes one genus, *Laccornis* des Gozis, 1914.

3.5.22 Laccornellini, Miller and Bergsten, 2014

3.5.22.1 Type Genus

Laccornellus Roughley and Wolfe, 1987.

3.5.22.2 Diagnosis

This taxon includes Hydroporinae species with the following features: (1) the metafemora extend to the metacoxal process, (2) the prosternal pore absent at the anterolateral angle of the prosternum, (3) without an oblique carina across the epipleuron at the humeral angle, (4) abdominal terga VII and VIII apically evenly rounded, and (5) female genitalia without laterotergites. Two other characters were proposed by Roughley and Wolfe (1987): (1) the metacoxal process medially incised and (2) the sublateral row of the mesotibial spines relatively sparse. These are more difficult to adequately homologize across Hydroporinae but help to characterize Laccornellini, as well.

3.5.22.3 Discussion

Members of *Laccornellus* and *Canthyporus* have been historically placed in Hydroporini, but near *Laccornis* (Sharp 1882; Zimmermann 1919, 1920). Wolfe (1985, 1988) and Roughley and Wolfe (1987) suggested that *Laccornellus* and *Canthyporus* may be closely related to each other and together may be phylogenetically near Laccornini, Methlini and Hydrovatini. Ribera's et al. (2008) analysis reinforced the relationship between the two genera as well as their isolated position phylogenetically. The seemingly plesiomorphic character states combined with their unique biogeography as austral disjuncts make *Laccornellus*, from southern South America, and *Canthyporus*, from southern Africa, particularly interesting with respect to the evolutionary history of the subfamily Hydroporinae.

In this analysis, the two genera *Canthyporus* and *Laccornellus* are together monophyletic with modest support (Fig. 3.3, pp = 0.76), corroborating Roughley and Wolfe (1987) and Ribera et al. (2008). This clade is sister to all other Hydroporinae except Laccornini, also with good support (Fig. 3.3, pp = 0.89). Because of the monophyly of the group and its unique phylogenetic position with respect to other members of the subfamily the clade was recognized as a new tribe, Laccornellini in the first edition of this chapter. Although not strongly supported, Michat et al. (2017) found Laccornellini paraphyletic with respect to Hydrovatini.

3.5.22.4 Taxon Content

The tribe includes two genera, *Canthyporus* Zimmermann, 1919 and *Laccornellus* Roughley and Wolfe, 1987.

3.5.23 Hydroporini Aubé, 1836

3.5.23.1 Type Genus

Hydroporus Clairville, 1806.

3.5.23.2 Diagnosis

This tribe includes Hydroporinae with: (1) the metepisternum extending to the metacoxal cavities, (2) the prosternal process extending to the metaventrite between the mesocoxae (except in a few taxa including the North American *Larsonectes minipi* (Larson) and several subterranean taxa which have been variously classified), (3) the metatarsal claws subequal in length, (4) the male lateral lobes of the aedeagus with a single segment, (5) the transverse tooth on the proventriculus without five elongate, finger-like lobes, (6) the medial portion of the metacoxa in a different plane from the base of the abdomen, (7) the metacoxal lobes prominent, (8) the female genitalia with the laterotergites absent, (9) the apex of the elytra and the last abdominal segment not acutely pointed, and (10) the metafemur along the dorsal margin broadly separated from the metacoxal lobes by the metatrochanter.

3.5.23.3 Discussion

Hydroporini historically included a great many Hydroporinae now classified in other tribes, including Laccornini, Hygrotini, and even many Bidessini. Removal of several groups into separate tribes has improved the definition of Hydroporini, but it has seemingly remained a “dumping-ground” for taxa left over after other, more easily diagnosible groups have been recognized, and the character combination above includes no unambiguous synapomorphy for the group. This has been generally recognized, though several apparently monophyletic groups within the tribe have been recognized, including the *Deronectes*-group (Angus and Tatton 2011; Balfour-Browne 1944; Nilsson and Angus 1992), the *Graptodytes*-group (Ribera and Faille 2010; Ribera et al. 2002, 2008; Seidlitz 1887), the *Necterosoma*-group

(Ribera et al. 2002, 2008) and the *Hydroporus*-group (Ribera et al. 2002, 2008). *Laccornellus* and *Canthyporus* have also been historically placed in this group, though they are here removed and placed in their own tribe (see above). Each of these genus-groups was found to be monophyletic by Ribera et al. (2008), but they were not together monophyletic.

In this analysis, Hydroporini, with the exception of *Laccornellus* + *Canthyporus* (Fig. 3.3, see above), is, somewhat surprisingly monophyletic with strong support (Fig. 3.3, pp = 0.97). Within Hydroporini, four well-supported clades are resolved, corresponding to the four genus-groups mentioned above. Given the strength of these clades and the existence of corresponding family-group names, we recognized four subtribes within Hydroporini in the first edition of this chapter. Whereas neither this analysis, nor Désamoré et al. (2018) resolved the relationship between these four subtribes with any significant support, the comprehensive sampling of Hydroporini by Villastrigo et al. (2021) recovered Hydroporina and Sternopriscina as sistergroups and Siettitiina as sister to the other three. All four subtribes were also maximally supported in that study (Villastrigo et al. 2021).

3.5.23.4 Taxon Content

As defined here, Hydroporini includes four subtribes. One subterranean genus *Siamoporus* Spangler, and two recently described genera where authors were unable to place them in subtribes are listed here as subtribe incertae sedis

Deronectina Galewski, 1994
Hydroporina Aubé, 1836
Siettitiina Smrž, 1982
Sternopriscina Branden, 1885
Genera *incertae sedis* with respect to subtribe

  Laodytes Queney, Lemaire and Ferrand, 2020
  Siamoporus Spangler, 1996
  Tassilodytes Fery and Bouzid, 2016

3.5.24 Hydroporina Aubé, 1836

3.5.24.1 Type Genus

Hydroporus Clairville, 1806.

3.5.24.2 Diagnosis

These are Hydroporini with: (1) the elytral epipleuron abruptly narrowed medially and narrow throughout the apical half, (2) the transverse tooth of the proventriculus not apically shallowly multilobed, (3) the rami of the female genitalia variously shaped, but not elongate curved nor apically fused together, (4) the mesosternal fork and the anteromedial process of the metaventrite not connected, (5) male pro- and mesotarsomeres I–III with ventral adhesive discs, and (6) no ring-shaped sclerite (receptacle) on the female bursa.

3.5.24.3 Discussion

This group is monophyletic in the analyses by Ribera et al. (2002, 2008), who recognized the clade as the "*Hydroporus*-group."

In this analysis, the genera included in this group are monophyletic with strong support (Figs. 3.3 and 3.4, pp = 1.00), and these genera are placed in the subtribe Hydroporina. Hydroporina is resolved as the sister to a clade with the remaining Hydroporini, though support for this other clade is not particularly strong (Figs. 3.3 and 3.4, pp = 0.73). The age of Hydroporina was estimated to be 73.6 my (46.6–99.7) for the crown node and 103.5 my (83.1–123) for the stem node (Désamoré et al. 2018).

3.5.24.4 Taxon Content

As defined here, Hydroporina includes six genera after *Suphrodytes* was synonymized with *Hydroporus* by Bergsten et al. (2013). The genera *Lioporeus* and *Stygoporus* were not included in our analysis. *Stygoporus*, a subterranean genus from Oregon, USA, was originally with hesitation placed in this subtribe but found by Kanda et al. (2016) to belong in Siettitiina instead. Similarly, *Lioporeus* was found to belong in Siettitiina and not Hydroporina (Villastrigo et al. 2021). Villastrigo et al. (2021) further found *Hydrocolus* nested within *Hydroporus* and *Heterosternuta* and *Haideoporus* nested within *Neoporus* why some synonymizations or reclassifications may follow.

Haideoporus Young and Longley, 1976
Heterosternuta Strand, 1935
Hydrocolus Roughley and Larson, 2000
Hydroporus Clairville, 1806
Neoporus Guignot, 1931
Sanfilippodytes Franciscolo, 1979

3.5.25 Deronectina Galewski, 1994

3.5.25.1 Type Genus

Deronectes Sharp, 1882.

3.5.25.2 Diagnosis

This group differs from other Hydroporini in at least four diagnostic characteristics: (1) the transverse tooth of the proventriculus is apically shallowly multilobed, (2) the rami of the female genitalia are characteristically shaped, elongate curved, apically fused and together apically rounded (Miller 2001; Miller et al. 2006), (3) the mesosternal fork and anteromedial process of the metaventrite are not connected (Nilsson and Angus 1992), and (4) male pro- and mesotarsomeres I–III lack ventral adhesive discs (though at least some members of Sternopriscina, below, have a similar condition, Nilsson and Angus 1992).

3.5.25.3 Discussion

This group has a history of recognition as a cluster of closely related taxa (Angus and Tatton 2011; Balfour-Browne 1944; Nilsson and Angus 1992). Ribera et al. (2008) found the group to be monophyletic and in a clade together with the *Graptodytes*-group (Siettitiina, see below) and Hygrotini.

In this analysis, the genera of the *Deronectes*-group are together monophyletic with strong support (Figs. 3.3 and 3.4, pp = 1.00). This clade is sister to Siettitiina (the *Graptodytes*-group), though this is not strongly supported (Figs. 3.3 and 3.4, pp = 0.70). Deronectina was resurrected to include members of this clade in the first edition of this chapter (Miller and Bergsten 2014).

A combined morphological and molecular study with an extensive taxon sampling of Deronectina was subsequently published by (Fery and Ribera 2018), which resulted in a major genus-level revision and a more than doubling of the number of genera. Villastrigo et al. (2021) found a sistergroup relationship between Deronectina and Hydroporina+Siettitiina. *Deronectes* is supported as sister to remaining genera within Deronectina (Fery and Ribera 2018; Villastrigo et al. 2021). The age of Deronectina was estimated to be 73.9 my (55.0–95.1) for the crown node and 87.9 my (65.6–106.5) for the stem node (Désamoré et al. 2018).

3.5.25.4 Taxon Content

Following the revision by (Fery and Ribera 2018) this group now includes 20 genera.

Amurodytes Fery and Petrov, 2013
Boreonectes Angus, 2010
Clarkhydrus Fery and Ribera, 2018
Deronectes Sharp, 1882
Deuteronectes Guignot, 1945
Hornectes Fery and Ribera, 2018
Iberonectes Fery and Ribera, 2018
Larsonectes Fery and Ribera, 2018
Leconectes Fery and Ribera, 2018
Mystonectes Fery and Ribera, 2018
Nebrioporus Régimbart, 1906
Nectoboreus Fery and Ribera, 2018
Nectomimus Fery and Ribera, 2018
Nectoporus Guignot, 1950
Neonectes J. Balfour-Browne, 1944
Oreodytes Seidlitz, 1887
Scarodytes des Gozis, 1914
Stictotarsus Zimmermann, 1919
Trichonectes Guignot, 1941
Zaitzevhydrus Fery and Ribera, 2018

3.5.26 Siettitiina Smrž, 1982

3.5.26.1 Type Genus

Siettitia Abeille de Perrin, 1904.

3.5.26.2 Diagnosis

This subtribe has one potential synapomorphy: the female genitalia have a ring-shaped sclerite on the bursa, possibly homologous with the receptacle in other Hydroporinae (Miller 2001; Miller et al. 2006, 2009b).

3.5.26.3 Discussion

This family-group was originally conceived to include multiple unrelated subterranean Hydroporinae (Smrž 1982). Others have noted similarities between certain subterranean Palearctic species and the epigean *Graptodytes* and related genera (Abeille de Perrin 1904; Castro and Delgado 2001). Ribera and Faille (2010) found these genera to be monophyletic and Miller et al. (2013) added the two subterranean Nearctic genera *Psychopomporus* and *Ereboporus* to this otherwise

Mediterranean clade. Later Kanda et al. (2016) showed that also the Nearctic subterranean *Stygoporus* belong to Siettitiina. Ribera and Reboleira (2019) provided a molecular phylogeny of the group, including for the first time the subterranean type genus *Siettitia*. They defined the *Siettitia* group of genera, likely also including the recently described *Etruscodytes*, and reclassified some species of a recovered paraphyletic *Rhithrodytes*. Finally, *Lioporeus*, previously thought to belong to Hydroporina was shown to belong in Siettitiina (Villastrigo et al. 2021). The age of Siettitiina was estimated to 59.5 my (33.8–83.3) for the crown node and 87.9 my (65.6–106.5) for the stem node (Désamoré et al. 2018).

In this analysis, several genera are resolved as monophyletic with strong support (Figs. 3.3 and 3.4, pp = 1.00), corresponding to the *Graptodytes*-group of Ribera and Faille (2010). This includes several Palearctic taxa as well as the Nearctic subterranean taxa *Ereboporus* (Miller et al. 2009b) and *Psychopomporus* (Jean et al. 2012) (Figs. 3.3 and 3.4). This group is sister to Deronectina, though support for this is low (Figs. 3.3 and 3.4, pp = 0.70).

3.5.26.4 Taxon Content

This tribe currently includes 11 genera. The genera *Iberoporus*, *Metaporus*, and *Siettitia,* were not included in the analysis, but are placed in this tribe based on data presented by Ribera and Faille (2010) that they belong to this group.

Ereboporus Miller, Gibson and Alarie, 2009
Etruscodytes Mazza, Cianferoni and Rocchi, 2013
Graptodytes Seidlitz, 1887
Iberoporus Castro and Delgado, 2001
Lioporeus Guignot, 1950
Metaporus Guignot, 1945
Porhydrus Guignot, 1945
Psychopomporus Jean, Telles and Miller, 2012
Rhithrodytes Bameul, 1989
Siettitia Abeille de Perrin, 1904
Stictonectes Brinck, 1943
Stygoporus Larson and Labonte, 1994

3.5.27 Sternopriscina Branden, 1885

3.5.27.1 Type Genus

Sternopriscus Sharp, 1880.

3.5.27.2 Diagnosis

From the other Hydroporini, this subtribe has few discrete distinguishing features though all have the elytral epipleuron relatively broad in the apical half with only gradual narrowing posteriorly. A few other Hydroporini have the elytral epipleuron relatively broad throughout (e.g., *Deronectes*), and members of one genus in this clade, *Paroster*, have the epipleuron narrower apically than others members of the tribe.

3.5.27.3 Discussion

This group of genera has been historically regarded as monophyletic and has been called the *Necterosoma*-group of genera (Balke and Ribera 2004; Ribera et al. 2002, 2008).

In this analysis, the clade that includes the Australian Hydroporini is strongly supported as monophyletic (Figs. 3.3 and 3.4, pp = 1.00), and is here placed in the subtribe Sternopriscina. Sternopriscina is sister to the clade Deronectina + Siettitiina, though support for this is low (Figs. 3.3 and 3.4, pp = 0.73).

The evolution and diversification of Sternopriscina were subsequently investigated by Toussaint et al. (2015b) with an extensive taxon sampling and relationships among the genera are now rather well known (also see Hendrich et al. 2014). The terrestrial Australian genus *Terradessus* with previous uncertain tribal affinity, was found to be nested inside *Paroster* of this subtribe (Toussaint et al. 2016b). Additionally, larvae are becoming increasingly known with descriptions and cladistic analyses for Sternopriscina (Alarie et al. 2018, 2019b, 2020). Villastrigo et al. (2021) found Hydroporina to be the sistergroup of Sternopriscina. The age of Sternopriscina was estimated to 77.4 my (51.9–100.3) for the crown node and 95.8 my (76.1–116.7) for the stem node (Désamoré et al. 2018).

3.5.27.4 Taxon Content

This group comprises 11 genera. *Carabhydrus* was previously placed in its own tribe, Carabhydrini Watts, based in large part on fusion of the metacoxa with visible abdominal sternite I, the weakly deflexed prosternum, and a characteristic habitus (Watts 1978), each potentially derived within other tribes. Although strongly supported as nested well within the *Necterosoma*-group of genera here (Figs. 3.3 and 3.4) and in other analyses (Balke and Ribera 2004; Ribera et al. 2008), it was not synonymized with Hydroporini. Carabhydrini was placed as a junior synonym of Sternopriscina in the first edition of this chapter.

Antiporus Sharp, 1882
Barretthydrus Lea, 1927
Brancuporus Hendrich, Toussaint and Balke, 2014

Carabhydrus Watts, 1978
Chostonectes Sharp, 1880
Megaporus Brinck, 1943
Necterosoma Macleay, 1871
Paroster Sharp, 1882
Sekaliporus Watts, 1997
Sternopriscus Sharp, 1880
Tiporus Watts, 1985

3.5.28 Vatellini Sharp, 1880

3.5.28.1 Type Genus

Vatellus Aubé, 1837

3.5.28.2 Diagnosis

These are Hydroporinae with: (1) the prosternal process not reaching the metaventrite (the mesocoxae are contiguous), (2) abdominal sternite VI with an invaginated, heavily sclerotized gland system ("speleum," Miller 2005), (3) the metepisternum separated from the mesocoxae by the mesepisternum (in extant taxa), and (4) females with an apically expanded and broadly truncate process at the apex of the spermatheca. Members of this tribe are among the most apomorphic and distinctive within the subfamily. They have long legs and an elongate, often somewhat cylindrical body, which is slightly to strongly discontinuous laterally. An extinct member of the group, *Calicovatellus petrodytes* Miller and Lubkin, has the metepisternum extending to the mesocoxal cavities (Miller and Lubkin 2001).

3.5.28.3 Discussion

Relationships of this tribe with other members of Hydroporinae have been among the most unresolved of any in the subfamily (Miller 2001; Ribera et al. 2002, 2008), and there has not been any consensus.

In this analysis, Vatellini is monophyletic (Fig. 3.3, pp = 1.00). It is resolved as sister to a large clade of Hydroporinae with the tribes Methlini, Pachydrini, Hydrovatini, Hygrotini, Hyphydrini and Bidessini, with moderately good support (Fig. 3.3, pp = 0.89).

Michat et al. (2017) recovered Vatellini as sister to Pachydrini and Hyphydrini, albeit with poor support. The age of Vatellini was estimated to be 64.0 my (38.7–90.5) for the crown node and 89.8 my (71.1–109.4) for the stem node (Désamoré et al. 2018).

3.5.28.4 Taxon Content

The group historically included four genera, *Vatellus* Aubé, *Macrovatellus* Sharp, *Derovatellus* Sharp, and *Mesovatellus* Trémouilles. A revision of the classification by Miller (2005) resulted in synonymy of *Macrovatellus* with *Vatellus* and *Mesovatellus* with *Derovatellus*, so that now only two genera are recognized within the tribe today, *Derovatellus* Sharp 1882 and *Vatellus* Aubé, 1837.

3.5.29 Methlini Branden, 1885

3.5.29.1 Type Genus

Methles Sharp, 1882

3.5.29.2 Diagnosis

This tribe includes Hydroporinae characterized by: (1) the metafemur extending to the metacoxal lobe and (2) terga VII and VIII modified, tergum VIII posteriorly acute and with dorsal and ventral lobes, the dorsal lobe posteriorly modified into a trifid structure with a pair of long apodemes extending anteriorly, and tergum VII also with shorter anterior apodemes. In general, the posterior apex of the abdomen and elytra in methlines is acuminate, though more pronounced in *Celina* than *Methles*. Members of the New World *Celina* are characterized additionally by an externally visible and large scutellum (with the elytra closed), which is unique among Hydroporinae genera.

3.5.29.3 Discussion

Sharp (1882) recognized close similarity between the two included genera, *Methles* and *Celina*. Wolfe (1985, 1988) proposed potential synapomorphies for Methlini and suggested that the group exhibited a number of plesiomorphies within Hydroporinae that made them close to *Laccornis*, *Laccornellus* and *Canthyporus*. He also thought Methlini and *Hydrovatus* are sister groups based on a number of similar features associated with the abdominal apex. Ribera et al. (2008) found a monophyletic Methlini sister to *Peschetius*.

In our results, Methlini is strongly supported as monophyletic (Fig. 3.3, pp = 1.00) and is sister to a clade including Pachydrini, Hydrovatini and Hygrotini, though this relationship is only weekly supported (Fig. 3.3, pp = 0.69).

The reanalysis by Désamoré et al. (2018) showed that excluding the morphological characters result in a polyphyletic Methlini although neither *Methles* nor *Celina* had a strongly inferred position in Hydroporinae. Represented only by *Celina* larvae in the larval character analysis of Michat et al. (2017), Methlini was recovered as sister to all remaining Hydroporinae. Although with Hydroporinae very sparsely sampled, also UCE analyses suggested a basal position of *Celina* in a clade together with Laccornini (Gustafson et al. (2020).

3.5.29.4 Taxon Content

Methlini includes two genera, *Celina* Aubé, 1837 and *Methles* Sharp, 1882.

3.5.30 Hydrovatini Sharp, 1880

3.5.30.1 Type Genus

Hydrovatus Motschulsky, 1853.

3.5.30.2 Diagnosis

This tribe is characterized by: (1) the elytral epipleuron with an oblique carina at the humeral angle, (2) the apex of the prosternal process broad and triangular and laterally distinctly margined, (3) the metatarsal claws equal in length, and (4) the metacoxal apices distinctly incised on each side of midline subtending a narrowly or broadly rounded metacoxal lobe. The great majority of the species in this group belong to the genus *Hydrovatus*, which is distinct in having (1) the elytral and abdominal apices acuminate, (2) the metacoxal lobes elongate and slender and marginal incision mediad of lobe deep and narrow, and (3) the female gonocoxae together fused into a knife-like structure with elongate lateral flanges extending from the anterior base. The other species in the group are in the Neotropical genus *Queda* Sharp and are characterized by (1) the elytral and abdominal apices obtusely pointed, (2) shorter and more broadly rounded metacoxal lobes with shorter margin incisions mediad of lobes, and (3) the female gonocoxae not fused, with each gonocoxa apically tri-lobed.

3.5.30.3 Discussion

Sharp (1882) placed *Queda* and *Hydrovatus* together in his tribe Hydrovatini and they were classified this way until Wolfe (1985, 1988) argued that *Hydrovatus*, *Celina* and *Methles* (the last two in the tribe Methlini) share many similarities that he considered plesiomorphic within the Hydroporinae (though without making formal classification changes). Biström (1990, 1996) reviewed the morphological evidence and concluded that *Queda* and *Hydrovatus* do together form a monophyletic group and that Methlini may be sister to Hydrovatini. Monophyly of Hydrovatini was corroborated also by Miller (2001) and Miller et al. (2006), who found the tribe to be phylogenetically near Hygrotini and Hyphydrini. Ribera et al. (2008) did not include *Queda*, but found a monophyletic *Hydrovatus* sister to Vatellini.

In this analysis, *Hydrovatus* and *Queda* are together monophyletic with strong support (Fig. 3.3, pp = 1.00). Hydrovatini is sister to Pachydrini, though this relationship is poorly supported (Fig. 3.3, pp = 0.67). Also in a clade with Pachydrini + Hydrovatini are Hygrotini and Methlini, though branches subtending these relationships are very short and poorly supported (Fig. 3.3). The sister group relationship between Pachydrini + Hydrovatini and Methlini (Fig. 3.3) may support some of the initial observations by Wolfe (1985, 1988) of morphological similarities between *Hydrovatus* and Methlini.

In analysis of larval characters, *Hydrovatini* has affinity with Laccornellini (Michat et al. 2017). The age of Hydrovatini was estimated to be 51.2 my (26.9–76.4) for the crown node and 78.9 my (51.3–104.8) for the stem node (Désamoré et al. 2018).

3.5.30.4 Taxon Content

Hydrovatini includes two genera, *Hydrovatus* Motschulsky, 1853 and *Queda* Sharp, 1882.

3.5.31 Pachydrini Biström, Nilsson and Wewalka, 1997

3.5.31.1 Type Genus

Pachydrus Sharp, 1882.

3.5.31.2 Diagnosis

Pachydrini are Hydroporinae with: (1) the elytral epipleuron with an oblique carina at the humeral angle, (2) the metacoxal lobes absent and the metacoxae medially at the same level as the abdominal sterna, (3) the apex of the prosternal process very broad, laterally unmargined, and broadly in contact with the metaventrite, (4) the metaventral wing broad medially, (5) the anterior metatarsal claw shorter than the posterior, and (6) female genitalia with laterotergites. Members of this group also have the metacoxae fused with the abdomen (shared with Bidessini and *Desmopachria* of the Hyphydrini) and the ventrolateral carina of the elytron thick and undulating, among a few other more obscure characters (see Biström et al. 1997).

3.5.31.3 Discussion

Historically, members of this group were placed in Hyphydrini, but Biström et al. (1997) placed the two included genera in their own tribe, Pachydrini. Pachydrini was synonymized with Hyphydrini by Miller (2001), a result corroborated by Miller et al. (2006) based on morphological data. However, Ribera and Balke et al. (2007) and Ribera et al. (2008) resurrected the tribe based on analysis of molecular data that indicate the genera are not closely related to Hyphydrini. Note that the availability of the family-group name Pachydrini should be attributed to Biström et al. (1997) and not Young (1980) (as cited in Nilsson and Hájek 2022). Young's (1980) sole usage of the name was clearly tentative "They [*Pachydrus* and *Desmopachria*] should probably be placed in a new tribe, the Pachydrini" (Young 1980: 306) and therefore not available following article 15.1 of conditional proposals after 1960 (ICZN). Neither did Young use it as a valid name in the work, and it is therefore also unavailable under article 11.5 (ICZN). The case can be compared with Reithrodontini discussed by Cazzaniga et al. (2019).

Monophyly of the tribe was not tested here because only a single representative of *Pachydrus* was included. It is resolved as sister to Hydrovatini, though this is not well-supported (Fig. 3.3, pp = 0.67). Despite its somewhat uncertain placement, given its phylogenetic position in the subfamily here and other evidence (Biström et al. 1997; Ribera and Balke 2007; Ribera et al. 2008) Pachydrini is here recognized as a tribe.

Among the few represented Hydroporinae taxa in Gustafson et al. (2020), *Pachydrus* is sister to Bidessini.

3.5.31.4 Taxon Content

Pachydrini includes two genera, *Heterhydrus* Fairmaire, 1869 and *Pachydrus* Sharp, 1882.

3.5.32 Hygrotini Portevin, 1929

3.5.32.1 Type Genus

Hygrotus Stephens, 1828.

3.5.32.2 Diagnosis

These are Hydroporinae with the following character combination: (1) the elytral epipleuron with an oblique carina at the humeral angle, (2) the metacoxae with broadly rounded lobes covering the bases of the metatrochanters, (3) the metatarsal claws equal in length (with the exception of males in the *saginatus*-group), and (4) the apices of the abdomen and elytra not acuminate.

3.5.32.3 Discussion

Members of this tribe were placed in Hydroporini by most authors until Nilsson and Holmen (1995) more formally recognized and diagnosed the tribe (following Portevin (1929) and Houlbert (1934)). Until recently, there has been relatively little work done to resolve relationships among the genera within Hygrotini, though Alarie et al. (2001a) presented some evidence for relationships based on the few groups known from larvae. That changed as Villastrigo et al. (2017, 2018) compiled a comprehensive taxon sampling of the tribe, including all five genera recognized at the time, which were reclassified as two genera following the study. The age of Hygrotini was estimated to be 74.4 my (49.8–100.9) for the crown node and 93.9 my (71.3–118) for the stem node (Désamoré et al. 2018).

Hygrotini is monophyletic in our analysis with strong support (Fig. 3.3, pp = 1.00). It is resolved as a sister to Pachydrini + Hydrovatini, though relationships among these groups are not well-supported (Fig. 3.3, pp = 0.69). Previously recognized at the genus rank, *Heroceras* and *Hyphoporus* were not included in this analysis, nor by Ribera et al. (2008), but included in Villastrigo et al. (2017, 2018).

3.5.32.4 Taxon Content

The tribe Hygrotini currently includes two genera, *Clemnius* Villastrigo, Ribera, Manuel, Millán and Fery, 2017 and *Hygrotus* Stephens, 1828.

3.5.33 Hyphydrini Gistel, 1848

3.5.33.1 Type Genus

Hyphydrus Illiger, 1802.

3.5.33.2 Diagnosis

These diving beetles are Hydroporinae with: (1) the elytral epipleuron with an oblique carina at the humeral angle, (2) the metacoxal lobes absent or extremely small and subtriangular and the metacoxae medially at the same level as the abdominal sterna, (3) the apex of the prosternal process narrow and pointed, (4) the metaventral wing narrow medially, and (5) the anterior metatarsal claw shorter than the posterior.

3.5.33.3 Discussion

Some members of Hyphydrini have been variously classified historically, though the numerous genera have been usually grouped together with a few exceptions (see Biström et al. 1997). Prominently, *Pachydrus* and *Heterhydrus* were placed in a separate tribe, Pachydrini, by Biström et al. (1997). This was disputed by Miller (2001) and Miller et al. (2006), who placed these genera back in Hyphydrini based on evidence from morphology. Ribera et al. (2008) argued against this as their analysis placed Pachydrini phylogenetically distant from Hyphydrini and sister to Bidessini, similar to Ribera and Balke et al. (2007), and they resurrected the tribe. Several genera with a restricted or centered distribution in the Cape Regions of South Africa seem to form a monophyletic group together with *Hovahydrus* from Madagascar (Ribera and Balke 2007).

In this analysis, Hyphydrini (excluding Pachydrini) is monophyletic with strong support (Fig. 3.3, pp = 1.00). *Pachydrus* is resolved as sister to Hydrovatini with weak support (pp = 0.67) and is here excluded from Hyphydrini following Biström et al. (1997) and Ribera et al. (2008) (see Pachydrini above). Hyphydrini is here resolved as sister to Bidessini though this is a weekly supported relationship (Fig. 3.3, pp = 0.75).

Michat et al. (2017) found with weak support a sistergroup relationship between Hyphydrini and Pachydrini. A cladistic analysis using larval characters did not support a monophyletic Cape Region clade (Alarie et al. 2017), in contrast to the finding by Ribera and Balke et al. (2007). The age of Hyphydrini was estimated to be 72.5 my (50.4–93.7) for the crown node and 85.1 my (67.5–104.4) for the stem node (Désamoré et al. 2018).

3.5.33.4 Taxon Content

This tribe includes 14 genera. *Pachydrus* Sharp and *Heterhydrus* Sharp are excluded from the tribe and placed back in Pachydrini Biström, Nilsson and Wewalka (see above). Several genera were not included in this analysis, though Biström et al. (1997) argued convincingly for the monophyly of all the genera. Several Afrotropical genera not included in this analysis were included in analyses by Ribera and Balke et al. (2007) and Ribera et al. (2008), finding a monophyletic Hyphydrini as defined here.

Agnoshydrus Biström, Nilsson and Wewalka, 1997
Allopachria Zimmermann, 1924
Andex Sharp, 1882
Anginopachria Wewalka, Balke and Hendrich, 2001
Coelhydrus Sharp, 1882
Darwinhydrus Sharp, 1882
Desmopachria Babington, 1842
Dimitshydrus Uéno, 1996

Hovahydrus Biström, 1982
Hydropeplus Sharp, 1882
Hyphovatus Wewalka and Biström, 1994
Hyphydrus Illiger, 1802
Microdytes J. Balfour-Browne, 1946
Primospes Sharp, 1882

3.5.34 Bidessini Sharp, 1880

3.5.34.1 Type Genus

Bidessus Sharp, 1882.

3.5.34.2 Diagnosis

Current diagnostics of this tribe within Hydroporinae are based on characters that are not readily accessible without dissection of internal tissues. The two primary synapomorphies defining the tribe are: (1) presence of a spermathecal spine and (2) presence of five-lobed teeth on the proventriculus. Additional features include (1) most genera with the metacoxae fused to the first visible abdominal sternum, (2) most genera with two- or three-segmented male lateral lobes (parameres) of the aedeagus, and (3) most genera with metatibia basally slender and apically gradually expanded.

3.5.34.3 Discussion

The classification of this large and important group of dytiscids has been addressed by several influential authors. The historical definition of this group began with Sharp (1882), who placed several taxa, mainly previously placed in *Hydroporus*, in a new tribe based on the fusion of the metacoxae with the first visible abdominal sternum. He believed this to be unique among Coleoptera, and, with this definition, placed in Bidessini the genera *Pachydrus*, *Heterhydrus,* and *Desmopachria*, currently placed in Pachydrini and Hyphydrini. The next main diagnostic effort was by Zimmermann (1919), who defined the group based on the equal-length metatarsal claws and an approximately club-shaped metatibia, which resulted in the removal of *Pachydrus*, *Heterhydrus,* and *Desmopachria* to Hyphydrini. Later influential authors (e.g., Young 1967) used a similar character definition for the group. However, in the most comprehensive modern treatment of the group by Biström (1988), the group was thoroughly reviewed and defined based on the presence of two- or three-segmented male parameres. This resulted in the exclusion of two genera historically placed in the Bidessini, *Amarodytes* Régimbart and *Hydrodessus* J. Balfour-Browne, which, based on his examined specimens, lack segmented lateral lobes. He placed these as Hydroporinae *incertae sedis*. During a phylogenetic analysis of the family by Miller (2001), a new compelling synapomorphy for the tribe was discovered, a heavily sclerotized spine inside the female spermatheca. Members of *Amarodytes* were found to have such a spine, and the genus was placed by Miller (2001) back into Bidessini. It was also discovered that at least some species currently attributed to *Amarodytes*, and specifically *A. duponti* (Aubé), have bisegmented male lateral lobes (Benetti and Régil Cueto 2004), though others do not (suggesting *Amarodytes* itself may not be monophyletic). Most recently, another synapomorphy for Bidessini was discovered by Miller et al. (2006), a five-lobed transverse tooth of the proventriculus. This feature is present in *Amarodytes* and the genus *Peschetius* Guignot, a genus previously placed in the Hydroporini. *Peschetius* also has a distinctive spermathecal spine. *Amarodytes* was, therefore, reconfirmed as a genus of Bidessini and *Peschetius* was formally moved into Bidessini. In contrast to that, Ribera et al. (2008) found *Peschetius* separate from Bidessini and sister to Methlini.

Based on our results, Bidessini is monophyletic, including the genera *Peschetius*, *Amarodytes* and, new to this analysis, *Hydrodessus*, which is placed back into this tribe (Fig. 3.3, pp = 1.00). Numerous *Hydrodessus* specimens were examined as part of an ongoing revision of the genus by the first author, and many of the species have a prominent spermathecal spine, though not all do (Miller, unpublished). Interestingly, the genera historically disputed as bidessines, *Peschetius*, *Amarodytes,* and *Hydrodessus* are all part of one clade except for a species of *Amarodytes* that is sister to the other Bidessini (Fig. 3.3, pp = 0.95). Bidessini is an exceptionally large taxon with many small members in numerous genera. The internal phylogeny of the clade needs considerable phylogenetic revisionary work because of the difficulty of many morphological character combinations defining the various genera and many uncomfortably placed taxa and potentially paraphyletic groups. The phylogenetic affinity of numerous genera remains to be tested, including recently described ones (Balke et al. 2017b; Miller and Wheeler 2015; Miller and Short 2015; Miller 2016). Larvae is known for less than a third of all genera (Michat et al. 2015b). The age of Bidessini was estimated to 76.0 my (57.3–95.8) for the crown node and 85.1 my (67.5–104.4) for the stem node (Désamoré et al. 2018).

3.5.34.4 Taxon Content

This is one of the largest groups in Dytiscidae with about 650 currently recognized species and probably many more unknown species. There are 48 genera with new genera described (and others synonymized) on a regular basis. Many of these are not included here, though sampling included much of the evident phylogenetic diversity of the tribe. Nevertheless, work remains to be done to clarify the placement of the genera and relationships among them.

Africodytes Biström, 1988
Allodessus Guignot, 1953

Amarodytes Régimbart, 1900
Anodocheilus Babington, 1842
Belladessus Miller and Short, 2015
Bidessodes Régimbart, 1900
Bidessonotus Régimbart, 1895
Bidessus Sharp, 1882
Borneodessus Balke, Hendrich, Mazzoldi and Biström, 2002
Brachyvatus Zimmermann, 1919
Clypeodytes Régimbart, 1894
Comaldessus Spangler and Barr, 1995
Crinodessus Miller, 1997
Fontidessus Miller and Spangler, 2008
Geodessus Brancucci, 1979
Gibbidessus Watts, 1978
Glareadessus Wewalka and Biström, 1998
Hemibidessus Zimmermann, 1921
Huxelhydrus Sharp, 1882
Hydrodessus J. Balfour-Browne, 1953
Hydroglyphus Motschulsky, 1853
Hypodessus Guignot, 1939
Incomptodessus Miller and García, 2011
Kakadudessus Hendrich and Balke, 2009
Leiodytes Guignot, 1936
Limbodessus Guignot, 1939
Liodessus Guignot, 1939
Microdessus Young, 1967
Neobidessodes Hendrich and Balke, 2009
Neobidessus Young, 1967
Neoclypeodytes Young, 1967
Novadessus Miller, 2016
Pachynectes Régimbart, 1903
Papuadessus Balke, 2001
Peschetius Guignot, 1942
Petrodessus Miller, 2012
Platydytes Biström, 1988
Pseuduvarus Biström, 1988
Rompindessus Balke, Bergsten and Hendrich, 2017
Sharphydrus Omer-Cooper, 1958
Sinodytes Spangler, 1996
Spanglerodessus Miller and García, 2011
Tepuidessus Spangler, 1981
Trogloguignotus Sanfilippo, 1958
Tyndallhydrus Sharp, 1882
Uvarus Guignot, 1939
Yola des Gozis, 1886

Yolina Guignot, 1936
Zimpherus Miller and Wheeler, 2015

3.6 Family-Group Classification of Dytiscidae Leach, 1815

Subfamily **Agabinae** Thomson, 1867

- Tribe **Agabini** Thomson, 1867
- Tribe **Hydrotrupini** Roughley, 2000

Subfamily **Colymbetinae** Erichson, 1837

- Tribe **Colymbetini** Erichson, 1837

Subfamily **Copelatinae** Branden, 1885

- Tribe **Copelatini** Branden, 1885

Subfamily **Coptotominae** Branden, 1885

- Tribe **Coptotomini** Branden, 1885

Subfamily **Cybistrinae** Sharp, 1880

- Tribe **Cybistrini** Sharp, 1880

Subfamily **Dytiscinae** Leach, 1815

- Tribe **Aubehydrini** Guignot, 1942
- Tribe **Aciliini** Thomson, 1867
- Tribe **Dytiscini** Leach, 1815
- Tribe **Eretini** Crotch, 1873
- Tribe **Hydaticini** Sharp, 1880

Subfamily **Hydrodytinae** Miller, 2001

- Tribe **Hydrodytini** Miller, 2001

Subfamily **Hydroporinae** Aubé, 1836

- Tribe **Bidessini** Sharp, 1880
- Tribe **Hydroporini** Aubé, 1836
    - Subtribe **Hydroporina** Aubé, 1836
    - Subtribe **Sternopriscina** Branden, 1885
    - Subtribe **Deronectina** Galewski, 1994
    - Subtribe **Siettitiina** Smrž, 1982
- Tribe **Hydrovatini** Sharp, 1880
- Tribe **Hygrotini** Portevin, 1929
- Tribe **Hyphydrini** Gistel, 1848

Tribe **Laccornini** Wolfe and Roughley, 1990
Tribe **Laccornellini** Miller and Bergsten, 2014
Tribe **Methlini** Branden, 1885
Tribe **Pachydrini** Biström, Nilsson and Wewalka, 1997
Tribe **Vatellini** Sharp, 1880

Subfamily **Laccophilinae** Gistel, 1848

Tribe **Agabetini** Branden, 1885
Tribe **Laccophilini** Gistel, 1848

Subfamily **Lancetinae** Branden, 1885

Tribe **Lancetini** Branden, 1885

Subfamily **Matinae** Branden, 1885

Tribe **Matini** Branden, 1885

3.7 Future Directions

This dytiscid phylogeny is far from dispositive. It seems, however, that several conclusions are becoming increasingly well supported, including the monophyly of the subfamilies and (most of) tribes as classified here. Within subfamilies, the relationships among the tribes of Dytiscinae are moderately well understood and well supported (Miller 2000, 2001, 2003). Great progress has also been made to elucidate the relationship between and within subtribes of the very diverse Hydroporini (Miller et al. 2013; Toussaint et al. 2015b; Fery and Ribera 2018; Ribera and Reboleira 2019; Villastrigo et al. 2021). What is considerably less clear are the relationships among the subfamilies and relationships among the tribes of Hydroporinae. These relationships are critical for understanding the evolutionary history and current biogeographic patterns of dytiscids and will be major topics for future phylogenetic analyses. Phylogenetic relationships among genera within some of the very large groups, such as Laccophilinae and Bidessini, are also very poorly known, and there is likely to be considerable paraphyly within some of these. As in other groups, genomic scale analyses will be immensely helpful to elucidate the backbone of the diving beetle tree of life. The UCE-based analysis by Gustafson et al. (2020) already gave new perspectives on subfamily relationships, and once these genomic scale datasets are combined with denser taxon sampling, we will likely have solved the most outstanding questions. As of the time of writing there is no reference-quality genome of a dytiscid, but transcriptomes are accumulating (e.g., Vasilikopoulos et al. 2019).

Large numbers of new taxa remain undiscovered, and these may be of considerable help in clarifying the phylogeny of the group. Some of these, such as the great many subterranean taxa remaining undescribed, are difficult to place morphologically, and DNA sequence data, or other data, may be critical for understanding their relationships (Miller et al. 2009b). Certain biogeographic regions are likely to yield large numbers of new species once they have been more thoroughly surveyed, including southeast Asia south through the many islands of the Pacific, much of South America, and central Africa.

Although we are entering a genomic era, there remain numerous morphological systems unexamined that may also prove useful in clarifying relationships. Female reproductive structures were only poorly known until recently, but have proven to have considerable phylogenetic, and evolutionary, significance in the group (Miller 2001, 2003, Chap. 5 in this book). It might be expected that other morphological systems could prove equally rewarding.

Appendix

Morphological and ecological characters and states used in combined phylogenetic analysis of Dytiscidae. Characters used in this analysis are derived from several other recent analyses, which should be consulted for additional description and illustration (Miller 2000, 2001, 2009; Miller and Bergsten 2012; Miller et al. 2006, 2007b, 2009a).

Head

1. Anterolateral margin of eye: (0) Not emarginate, (1) Emarginate.
2. Mandibles: (0) Apically acute, (1) Apically broad.
3. Postocular carina: (0) Absent, (1) Present.
4. Transverse occipital line: (0) Absent, (1) Present.
5. Anterior clypeal groove: (0) Broadly interrupted, (1) Continuous.
6. Anterior clypeal margin: (0) Unmodified, (1) Margin produced or beaded.
7. Fronto clypeal suture: (0) Medially effaced, (1) Entire.
8. Anterior prothoracic glands: (0) Absent, (1) Present.
9. Eyes: (0) Not divided, (1) Divided but contiguous (as in *Spanglerogyrus*), (2) Completely divided (as in most Gyrinidae).
10. Antennae: (0) Simple, (1) With enlarged scape and pedicel and short, compact flagellum (as in Gyrinidae).
11. Scape: (0) Simple, (1) Medially distinctly constricted (as in Noteridae).

Thorax

12. Scutellum: (0) Not externally visible with elytra closed, (1) Externally visible with elytra closed.
13. Lateral pronotal margin: (0) Unbeaded, (1) Beaded.
14. Longitudinal sublateral basal striae on pronotum: (0) Absent, (1) Present.
15. Prosternum, prosternal process: (0) In same plane as prosternum, (1) Declivous, in different plane from prosternum.
16. Prosternal process: (0) Not reaching metaventrite (1) Reaching metaventrite.
17. Prosternal process: (0) Flat to carinate, (1) With distinct, medial, longitudinal sulcus.

18. Prosternal process apex: (0) Pointed or narrowly rounded, (1) Broad, apically truncate.
19. Metepisternum: (0) Not reaching mesocoxal cavities, separated by mesepimeron (1) Reaching mesocoxal cavities.
20. Transverse metaventral suture: (0) Absent, (1) Present.
21. Small lateral lobe on medial portion of metacoxa: (0) Absent, (1) Present.
22. Metacoxa anterior expansion: (0) Not anteriorly expanded, (1) Anteriorly expanded.
23. Metacoxa and abdominal sternite II: (0) Not fused, (1) Fused.
24. Medial cleft of metendosternite: (0) Narrow, (1) Broad and rounded.
25. Noterid platform: (0) Absent, (1) Present.
26. Retractoris mesothoracis muscle insertion on metendosternite: (0) Deeply concave, distinct from margins, (1) Shallow, flattened.
27. Furcodorsalis metathoracis muscle insertions on anterior rami of metendosternite: (0) Medially, (1) Apically.
28. Basal portion of metendosternite: (0) Broad, lateral margin divergent anteriorly, (1) Narrow, lateral margins parallel.

Abdomen

29. Transverse rugae on dorsum of abdominal segment II: (0) Absent, (1) Present.
30. Speleum: (0) Absent, (1) Present (in Vatellini).
31. Apex of female sternum six: (0) Evenly curved, (1) Medially emarginate.
32. Abdominal tergum VIII: (0) Not modified, (1) Apically acute or acuminate with anteriorly directed processes.

Elytron

33. Anterior oblique epipleural carina: (0) Absent, (1) Present.
34. A field of short impressed striae at base of female elytron: (0) Absent, (1) Present.
35. Elytral apices: (0) Not acuminate, (1) Distinctly acuminate.
36. Elytral apices: (0) Not truncate or sinuate, (1) Truncate and slightly sinuate.
37. Apicoventral elytral setae: (0) Absent, (1) Present.
38. Apicoventral elytral setae: (0) A large region of fine setae, (1) A small region of stiff setae, (2) A linear submarginal series.

Legs

39. Pro- and mesotarsi: (0) Clearly pentamerous, (1) Pseudotetramerous.
40. Apices of male protarsal adhesive setae: (0) Oval to elongate, (1) Round, sucker shaped.
41. Anterior protibal spur in male: (0) Absent, (1) Present.
42. Posterior protibial spur in male: (0) Absent, (1) Present.
43. Protibial hooked spur: (0) Absent, (1) Present (as in Noteridae).
44. Ventral series of setae on mesofemur: (0) Shorter than width of mesofemur, (1) Longer than width of mesofemur.
45. Oblique line of setae on mesotarsomeres: (0) Absent, (1) Present.
46. Posterodorsal series of setae on mesotibia: (0) Simple, (1) Bifid.

47. Posterodorsal series of setae on metatibia: (0) Simple, (1) Bifid.
48. Posterodorsal setae on metatibia: (0) A linear series, slightly curved, (1) A large cluster, (2) A strongly oblique series.
49. Posteroapical setae on meso- and metatibia: (0) Simple, (1) Bifid.
50. Appressed striae on metacoxa: (0) Absent, (1) Present.
51. Metacoxal lines: (0) Not closely approximated medially, (1) Closely approximated.
52. Posteromedial metacoxal lobes: (0) Absent, (1) Present small, (2) Present large rounded.
53. Posteromedial metacoxal rim: (0) Discontinuous medially, (1) Continuous medially.
54. Metacoxal cavities: (0) Separated broadly, (1) Contiguous or closely approximated.
55. Metacoxa: (0) Medial portion not concave, (1) Medial portion concave laterally.
56. Deep, oblique groove on metatrochanter: (0) Absent, (1) Present.
57. Linear series of setae in groove near anteroapical angle of metafemur: (0) Absent, (1) Present.
58. Dorsal series of natatory setae on metafemur: (0) Absent, (1) Present.
59. Apices of metatibial spurs: (0) Simple, (1) Bifid.
60. Anterior metatibial spur: (0) Similar to posterior, (1) Acuminate, broad (Cybistrini).
61. Posteroventral series of setae on metatarsomere I: (0) Absent, (1) Present.
62. Posterodorsal series of setae on metatarsomere I: (0) Absent, (1) Present.
63. Adpressed setae along apicodorsal and apicoventral margins of metatarsomeres I–IV: (0) Absent, (1) Present.
64. Natatory setae along posteroventral margin of metatarsi: (0) Absent on males and females, (1) Present on males, absent on females, (2) Present on males and females.
65. Natatory setae along posteroventral margin of metatibia and tarsomeres: (0) Present or absent on both, (1) Present on metatarsomeres but absent on metatibia in both sexes.
66. Anterodorsal margin of metatarsomeres I–IV: (0) Unlobed, (1) Lobed.
67. Metatarsal claws: 0) Male and female unequal, posterior shorter than anterior, (1) Male and female with claws equal, (2) Male equal, female unequal, anterior shorter than posterior, (3) Male and female unequal, anterior shorter than posterior, (4) Male single, female unequal, anterior shorter than posterior, (5) Male and female each with a single claw.
68. Legs: (0) Not expanded, (1) Meso- and metatibia broad, elongate, apically with elongate extension (as in Spanglerogyrus), (2) Meso- and metatibia short and broad, meso- and metataromeres I–IV very broad, subtriangular (as in most Gyrinidae).

Internal

69. Proventriculus: (0) Without five finger-like lobes on crusher teeth, (1) With five finger-like lobes on crusher teeth.

Male Genitalia

70. Median lobe: (0) Asymmetrical, (1) Bilaterally symmetrical.
71. Ventral sclerite on median lobe: (0) Absent, (1) Present.
72. Lateral lobes: (0) With one segment, (1) With two segments, (2) With three segments.
73. Lateral lobes: (0) Symmetrical, (1) Asymmetrical.

Female Genitalia

74. Genital configuration: (0) carabid-type, (1) noterid-type, (2) amphizoid-type, (3) hydroporine-type, (4) *Agaporomorphus*-type, (5) dytiscine-type (Miller 2001).
75. Gonocoxal fusion: (0) Not fused, (1) Weakly fused, (2) Completely fused.
76. Anterior apodeme on gonocoxae: (0) absent, (1) present.
77. Gonocoxal shape: (0) Not short and broad, (1) Short and broad.
78. Gonocoxal shape: (0) Not extremely elongate, (1) Extremely elongate.
79. Dorsolateral carina on gonocoxa: (0) Absent, (1) Present.
80. Dorsolateral carina on gonocoxa: (0) Not dentate, (1) Dentate.
81. Gonocoxae: (0) Rounded or not flattened, (1) Strongly laterally flattened.
82. Articulation of laterotergite and gonocoxa: (0) Strongly angled, laterotergite extending posteriorly, (1) Not angled.
83. Laterotergites: (0) Absent, (1) Present.
84. Series of short spinous setae along medial margin gonocoxosternite: (0) Absent, (1) Present.
85. Rami dentation: (0) Not dentate, (1) Weakly dentate, (2) Strongly dentate.
86. Rami configuration: (0) Not modified, (1) Strongly sclerotized, apically fused, anteriorly divergent.
87. Bursa size: (0) Short, (1) Long, slender, slightly twisted.
88. Bursal shape: (0) Various, (1) Flattened, with thick-walled, parallel margins.
89. Bursal gland reservoir: (0) Absent, (1) Present.
90. Bursal sclerotization: (0) Not heavily sclerotized, (1) Heavily sclerotized.
91. Bursal attachment to gonocoxal apparatus: (0) Anteriorly near bases of gonocoxae, (1) Posteriorly near apices of gonocoxae.
92. Receptacle: (0) Absent, (1) Present.
93. Receptacle shape: (0) Not cone shaped, (1) Cone shaped with basal sclerotized ring.
94. Spermathecal and fertilization ducts: (0) Not coiled, (1) Coiled.
95. Spermatheca: (0) Not reduced, (1) Reduced.
96. Spermathecal spine: (0) Absent, (1) Present.
97. Triangular spermathecal process: (0) Absent, (1) Present.
98. Large accessory gland reservoir near base of fertilization duct: (0) Absent, (1) Present.
99. Large accessory gland reservoir on spermatheca: (0) Absent, (1) Present.
100. Spermathecal disc: (0) Absent, (1) Present.
101. Base of fertilization duct: (0) Without modifications, (1) A heavily sclerotized, irregular ring.

102. Gland reservoirs laterally at base of oviduct: (0) Absent, (1) Present.
103. Thick musculature on vagina: (0) Absent, (1) Present.

Habitat

104. Habitat: (0) Not on water surface, (1) found on water surface.

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Kelly B. Miller is a professor in the Department of Biology and curator of arthropods and assistant director at the Museum of Southwestern Biology at the University of New Mexico. He earned a Ph.D. in Insect Systematics from Cornell University and spent 4 years as a postdoc at Brigham Young University before starting at UNM in 2007. His research is focused on arthropod systematics, especially Hydradephaga, and the evolution of insect sexual strategies.

Johannes Bergsten has been a senior curator since 2009 at the Swedish Museum of Natural History, Stockholm. He holds a Ph. D. in systematics from Umeå University and spent 3 years as a postdoc at Imperial College and the Natural History Museum, London. His research interests involve Hydradephagan systematics, phylogenetic methodology, species delimitation, the water beetle fauna of Madagascar, and sexual conflict in insect mating systems.

Chapter 4
Predaceous Diving Beetle Sexual Systems

Kelly B. Miller and Johannes Bergsten

Abstract Diving beetles have an impressively diverse array of morphological and behavioral attributes associated with sexual systems. These include anatomical dimorphisms with males and females exhibiting many secondary sexual features, behavioral dimorphisms in precopulatory and copulatory activities, extensive variation in male and female genitalia, and sperm complexity that includes sperm conjugation and heteromorphism. Many of these attributes appear to be correlated, suggesting emphasis by certain clades on particular sexual systems. For example, members of Dytiscinae appear to emphasize pre-insemination sexual selection with female resistance behavior possibly associated with oxygen deprivation (hypoxia) of females during copulatory activities, which take place over many hours of mate guarding. In this case, males have large adhesive disks on their protarsi used to better overcome a resistant female, whereas females have modified pronotal and elytral cuticle that interfere with male adhesive disks. This group also has among the simplest male sperm and female reproductive tract morphology, suggesting more limited post-insemination selection, but strong pre-insemination sexual antagonism. In contrast, members of Hydroporinae have no obvious pre-insemination mating behaviors and only short mating durations. This group also has dramatically complex female reproductive tracts and male sperm morphology including conjugation and heteromorphism suggesting intensity in post-insemination sperm choice, sperm cooperation, and sperm competition. Here, dytiscid sexual attributes are reviewed along with discussion of dytiscid sexual system evolution.

Keywords Sexual selection · Evolution · Sexual antagonism · Reproduction · Insemination · Genitalia

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K. B. Miller (✉)
Department of Biology and Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM, USA
e-mail: kbmiller@unm.edu

J. Bergsten
Department of Zoology, Swedish Museum of Natural History, Stockholm, Sweden
e-mail: johannes.bergsten@nrm.se

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4.1 Introduction

Sexual selection is a type of natural selection in which an individual's fitness directly depends on reproductive activities and selective consequences of these activities within a particular species. As such, it involves a range of components from mate finding to copulation to insemination and fertilization to even parental investment in care of offspring, especially when this influences earlier mating decisions. Sexual selection may include competitive interactions between members of the same sex (intrasexual competition) including such things as male–male combat, male dominance hierarchies, resource guarding, mate guarding, parental care, and sperm competition. Other competitive interactions may represent a conflict of interest between males and females over the decision to mate (intersexual competition) because, in general, the sexes have different mating frequency optima because of differential investment in gametes and offspring (Chapman et al. 2003; Arnqvist and Rowe 2005). These interactions include such things as mate choice (generally by females on males, but not always), cryptic female choice (sperm selection), and sexual antagonism (e.g., forced mating). Individuals, populations, species, and even groups of species generally exhibit particular manifestations of sexual selection, with multiple, complex strategies often evident in the same species. These "sexual systems" are expected, like any other phenotypic attributes of organisms, to have a macroevolutionary pattern that may be discovered through phylogenetic investigation. Sexual selection has been invoked to explain many unusual phenotypes including genitalia and "exaggerated" phenotypes such as antlers, bright colors, singing, and courtship behaviors, among many others (reviewed for insects in Shuker and Simmons 2014). In particular, it has been used to explain features that seem to be especially maladaptive when considered from other, more typical natural selection perspectives (Darwin 1871).

Predaceous diving beetles (Coleoptera: Dytiscidae) exhibit some of the richest diversity of sexual systems and related morphology and behavior of any arthropod group. Knowledge of this diversity and its evolutionary patterns and processes is only just beginning, but what is known is suggestive of an excellent system for the study of complex evolution of sexual systems.

All predaceous diving beetles, as far as known, are dioecious, promiscuous, and polygamous. The only known possible exception to this are certain species of *Hydrodytes* Miller (Hydrodytinae), and some species of *Belladessus* Miller & Short (Bidessini) for which only females are known, and which may be parthenogenetic (Young 1989; Miller 2002b; Miller and Short 2015), though this is unconfirmed. As far as known, all other other species have direct sperm transfer and internal fertilization, and, as such, are expected to be subject to the same fitness influences based in sexual selection that are other animals. That is, predaceous diving beetles should be influenced by overall differential interests in mating and mate choice between males and females, associated fitness benefits and costs of mating and mate choice, and the inter- and intrasexual competition that manifests from these effects. Here, emphasis is placed on a review of the diversity of mating-related morphology, behavior, and mating systems in dytiscids.

4.2 Sexual Variation

4.2.1 Secondary Sexual Dimorphisms

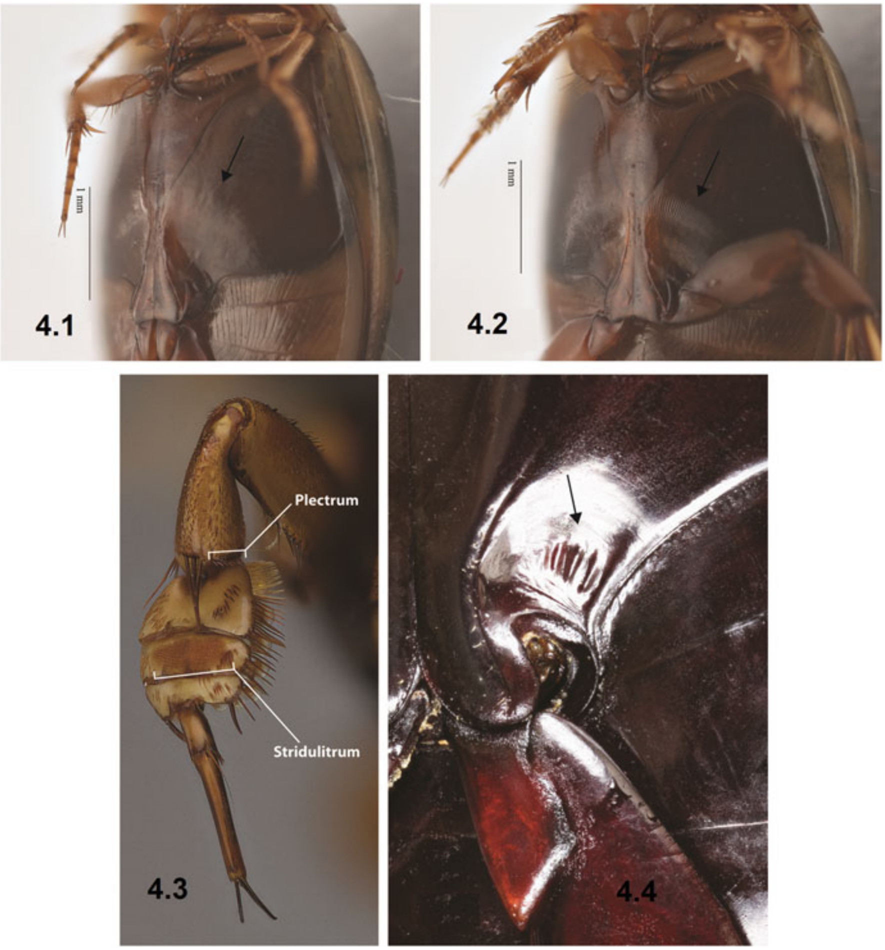
Diving beetles exhibit numerous and diverse secondary sexual phenotypic dimorphisms, with the "unusual" or "exaggerated" state most often, though not always, exhibited by males. Dimorphic features include chemical, behavioral (including sound production), and morphological attributes.

4.2.1.1 Chemical

Among the least known dimorphic systems in diving beetles are pheromones, and the first example of male detection of female release of sexually attractive pheromones (in *Rhantus suturalis* Macleay) was discovered only recently (Herbst et al. 2011). In this case, males were significantly attracted to females using chemical cues (Herbst et al. 2011). Diving beetles are exceptional chemical producers for defense and other purposes (Dettner and Schwinger 1980; Dettner 1985, see Chap. 6 in this book), and it might be expected that they would use this ability in sexual systems, but little is known about the use of chemical sexual signaling in aquatic insects in general, and certainly this is true of Dytiscidae. This may prove to be a fruitful avenue for study in diving beetles. Expanded male antennomeres in numerous groups of diving beetles may be related to this type of signaling (see Sect. 4.2.1.3 below), but this is also unknown.

4.2.1.2 Acoustic

In contrast, at least some acoustic signaling in dytiscids appears to be made by males at least judging from the occurrence of suggested stridulatory devices. Apparent stridulatory devices occur throughout Dytiscidae, but often only on males (Larson and Pritchard 1974) (but present in both males and females in some species such as *Laccophilus hyalinus*). Although discussed extensively in the literature, the documentation of sound production in dytiscids is exceedingly poor, especially underwater (Larson and Pritchard 1974; Aiken 1985). It is clear that many diving beetles are capable of producing sound (Greenhalgh 2018; Desjonquères 2016; Smith 1973; Aiken 1985) but studies of the behavioral and eventual sex-, or mating specific context is rudimentarily known. Some sound production seems to be unrelated to mating and is instead connected to environmental conditions (Smith 1973). Other sound production is initiated during stress and may therefore have a defensive



Figs. 4.1–4.4 Dytiscid stridulatory devices. (4.1, 4.2), *Laccophilus maculosus* (Germar); (1) female; (2) male, arrow indicates stridulitrum. (4.3) *Hydaticus flavolineatus* Boheman, left protibia and tarsus. (4.4) *Cybister fimbriolatus* (Say), left metacoxa

function, or sounds may be related to pre-flight activity and may be accidental without communicative function (Aiken 1985). Possible sound production by males *in copula* is characterized by tapping, rubbing, or stroking movements with the legs (Blunck 1912a; Aiken 1985, 1992). If any sound production by males has a precopulatory function of attracting receptive females, courtship, or discouraging other males, this has not been documented to date. It is also possible that male members of Hydaticini seek instead to attract predators during pre-mating activities (see below). Groups with suspected male stridulatory devices on many or, at least, some species include *Laccophilus* (Figs. 4.1 and 4.2), *Hydrovatus*, Hydaticini

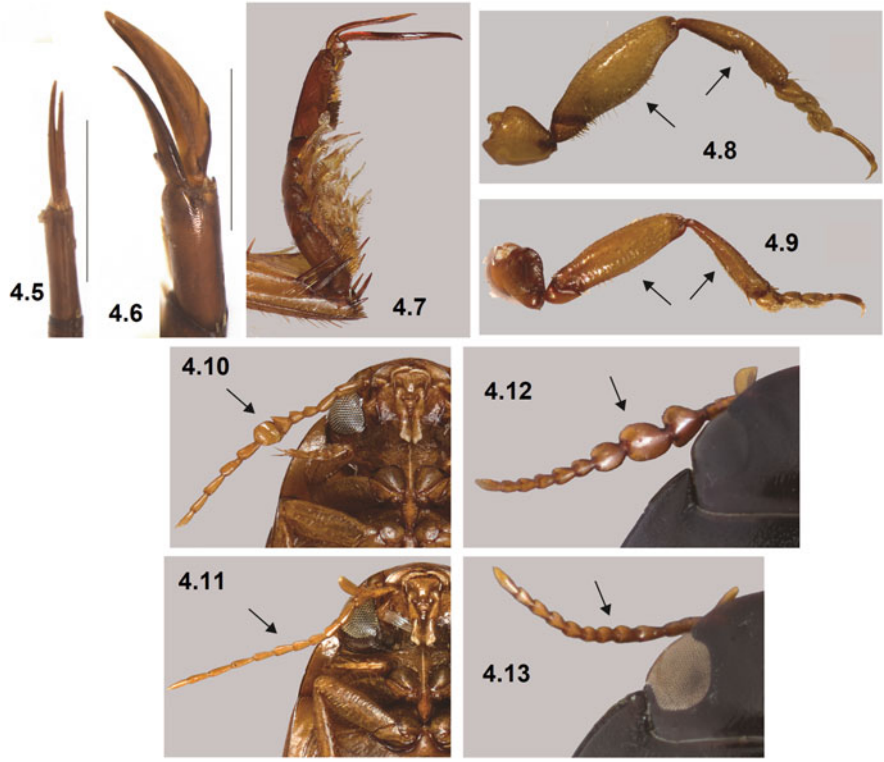
(Fig. 4.3), *Agabus*, *Cybister* (Fig. 4.4)*, Colymbetes* (Larson and Pritchard 1974), and *Agaporomorphus* (Miller 2001b), among many others.

4.2.1.3 Morphology

In addition to stridulatory devices, morphological dimorphisms are common and often obvious in dytiscids, but are certainly better known than chemical or behavioral dimorphisms. For example, sexual size dimorphism is common in predaceous diving beetles. Size may be biased either toward females or males (Zimmerman 1970; Aiken and Wilkinson 1985; Ribera 1994; Schulte-Hostedde and Alarie 2006; Fairn et al. 2007). Selection pressure for or against large relative size in males or females is complex, and in predaceous diving beetles poorly known, as is general knowledge of the degree of size dimorphism in groups across the family (Fairn et al. 2007). Also, body shape can differ between the sexes like in some *Hydroporus* species previously placed in *Suphrodytes* in which females are relatively shorter and broader than males, independent of isometric size differences (Bergsten et al. 2012).

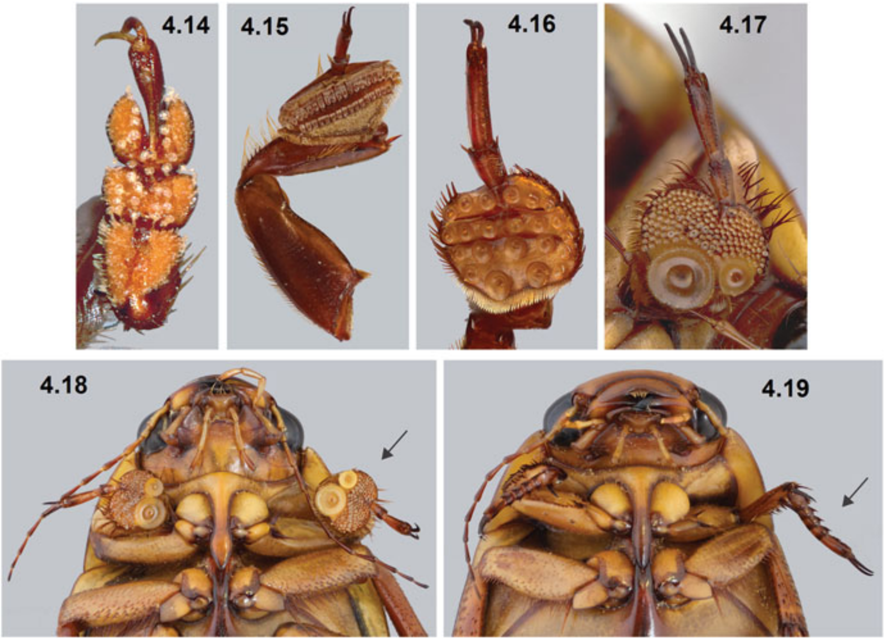
Male diving beetles of many species throughout several families are characterized by secondary morphological features that are often species specific. One common manifestation of this is protarsal or (less commonly) mesotarsal claws that may be asymmetrically more or less strongly curved, hooked, toothed, unequal in length, or otherwise modified (Figs. 4.5, 4.6 and 4.7). These features are commonly used as species diagnostic character states in many groups of diving beetles (e.g., see Nilsson and Holmen 1995; Larson et al. 2000). Within the context of mating systems, presumably these function in these beetles as species-specific grasping devices during mating encounters, but their behavioral correlates remain largely undocumented. Other apparent grasping devices may include conspicuous modifications to protibial shape such as in *Necterosoma* (Figs. 4.8 and 4.9, Watts 1978) or some *Hygrotus* (Leech 1966) and the antennae found in several groups including *Agaporomorphus* (Figs. 4.10 and 4.11, Miller 2001b), *Lioporeus* (Wolfe and Matta 1981), *Allopachria* (Wewalka 2000), *Laccornis* (Wolfe and Roughley 1990), *Queda* (Figs. 4.12 and 4.13, Biström 1990), and others. Some of these may represent expansions of the male antennae for increased chemoreception, though some seem more evidently useful as grasping devices.

One of the most common male sexual dimorphisms across diving beetles is greater lateral expansion of the pro- and/or mesotarsomeres (generally tarsomeres I–III), often with adhesive setae with a greater density, number, type or size of adhesive setae ventrally than (if present) in females (Figs. 4.14, 4.15, 4.16, 4.17, 4.18 and 4.19). These adhesive setae come in a variety of forms, and their presence and variation is not particularly unusual for male beetles in general (Stork 1980). In diving beetles adhesive seta can be radial-symmetrically sucker shaped (Dytiscinae), but also spatulate as in Cybistrinae (Chen et al. 2014) or ellipsoid as in, e.g., studied Hydroporinae (Bilton et al. 2008) and Agabinae (Bilton et al. 2016). These structures are seemingly used for increased adhesion to the female during mating activities, though this is not known for certain for most species. The sucker-shaped adhesive



Figs. 4.5–4.13 Dytiscid sexually dimorphic features. (4.5, 4.6) *Agabus bipustulatus* (Linnaeus), protarsal claws; (4.5) female; (4.6) male. (4.7) *Rhantus frontalis* (Marsham), male protarsus. (4.8, 4.9) *Necterosoma penicillatum* (Clark), left proleg, anterior aspect; (4.8) male; (4.9) female. (4.10, 4.11) *Agaporomorphus knischi* Zimmermann, right side of head, ventral aspect; (4.10) male; (4.11) female. (4.12, 4.13) *Queda youngi* Biström, left side of head, dorsal aspect; (4.12) male; (4.13) female

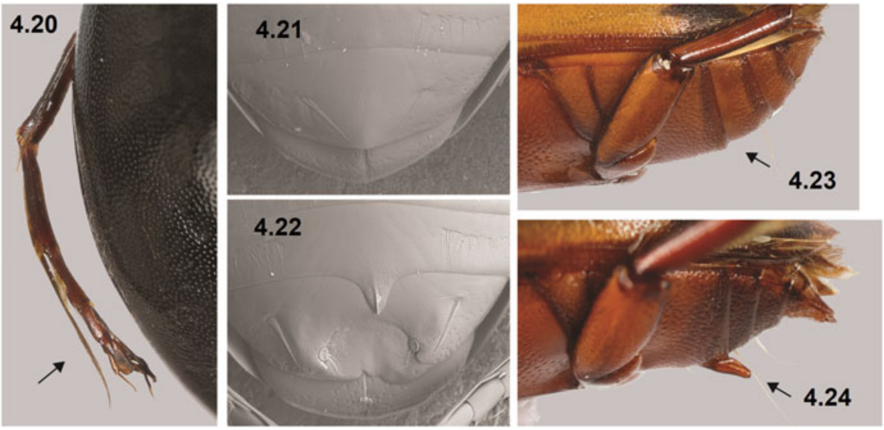
setae on pro- and (sometimes) mesotarsomeres I–III in Dytiscinae (Figs. 4.16, 4.17, 4.18 and 4.19) especially have been interpreted as an improved grasping device for adherence to female surfaces prior to and during the mating event within a sexual conflict context (see below, Bergsten et al. 2001; Miller 2003; Bergsten 2005; Bergsten and Miller 2007). But the ellipsoid-type of adhesive setae as found in Hydroporinae and Agabinae are likely under similar selection as both their sizes and numbers increase in populations with a microstructured matt female elytral morph (Bilton et al. 2008, 2016). Interestingly, the different types of adhesive setae may have different functions and utilize partly different mechanisms. Round sucker-shaped adhesive setae utilize suction force (differential pressure) and have a greater shear resistance (Chen et al. 2014). The spatulate setae of cybistrines have a distally furrowed lip and show a velocity-dependent pull-off response on adhesion implying a viscous force (Chen et al. 2014). Such a response is more dynamic, offering strong resistance to erratic swimming movements but easier detachment and repositioning of the tarsi during mating.



Figs. 4.14–4.19 Dytiscid sexually dimorphic features. (4.14) *Hygrotus impressopunctatus* (Schaller), left protarsus, ventral aspect. (4.15) *Cybister fimbriolatus* (Say), left proleg, ventral aspect. (4.16) *Hydaticus flavolineatus* Boheman, left protarsus, ventral aspect. (4.17) *Dytiscus marginalis* Linnaeus, left protarsus, ventral aspect. (4.18, 4.19) *Dytiscus marginalis*, ventral surface; (4.18) male; (4.19) female

There are many other examples of secondary sexual characters exhibited by dytiscid males. Some males of *Queda* have dramatically modified metatibiae (Fig. 4.20, Biström 1990). Male members of *Graptodytes* (Balfour-Browne 1934; Fery 1995; Ribera and Faille 2010), *Clemnius* (Anderson 1971, as *Hygrotus*), and many Laccophilinae (Zimmerman 1970; Brancucci 1983) have the last visible abdominal sternite variously modified in a species-specific way. Male *Africophilus* have the last two abdominal ventrites modified (Figs. 4.21 and 4.22, Omer-Cooper 1969). Some *Hyphydrus* have males with a large abdominal spine (Figs. 4.23 and 4.24), a modified metatibial spur, or modified protrochanter (Biström 1982). Some *Desmopachria* have males with the prosternal process apically forked with a deep pit between the two branches (Young 1995; Miller 2001a). Some *Hygrotus* have the profemur unusually modified (Leech 1966). Members of *Bidessonotus* have the ventral surface more strongly concave than that of females or most other predaceous diving beetles (Balfour-Browne 1947; Young 1990). Just from these examples, one can appreciate the great number of male-specific modifications affecting many structures across Dytiscidae.

Female-specific modifications are not as common, but there are several conspicuous examples. Many predaceous diving beetle females have the surface of the



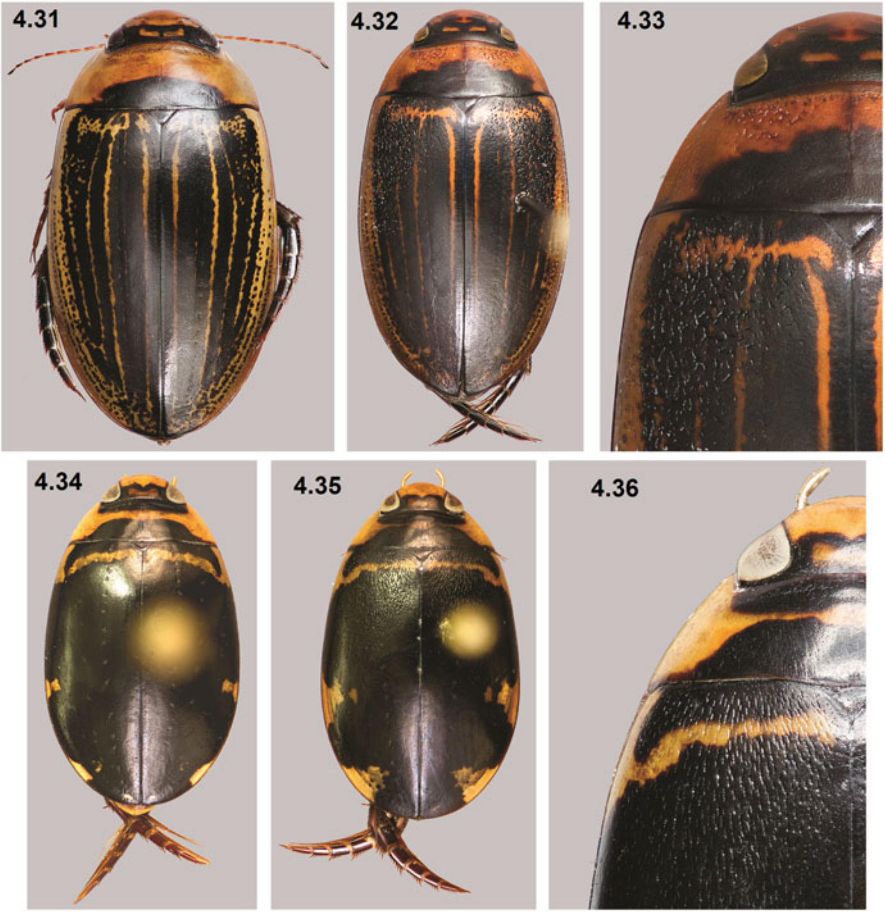
Figs. 4.20–4.24 Dytiscid sexually dimorphic features. (4.20) *Queda youngi* Biström, male left leg, dorsal aspect. (4.21, 4.22) *Africophilus nesiotes* Guignot, apical abdominal sternites, ventral aspect; (4.21) female; (4.22) male. (4.23, 4.24) *Hyphydrus lyratus* Swartz, abdominal sternites, left lateral aspect; (4.23) female; (4.24) male

cuticle, particularly the pronotum and all or portions of the elytron, more heavily microsculptured than in males. In many taxa this is intrasexually dimorphic with some females more extensively microsculptured than others (e.g., Miller 1998; Bilton et al. 2008, 2016; Drotz et al. 2010; Ranarilalatiana et al. 2019). The most dramatic examples of this are in Cybistrinae and Dytiscinae (Figs. 4.25, 4.26, 4.27, 4.28, 4.29, 4.30, 4.31, 4.32, 4.33, 4.34, 4.35, 4.36, 4.37, 4.38, 4.39, 4.40, 4.41 and 4.42, Miller 2003). Many cybistrine females have the elytron with conspicuous striations or "scratches" or reticulate patterns (Brinck 1945; Miller 2003; Miller et al. 2007). Within Dytiscinae, many species of *Dytiscus* have females with deep, elongate grooves in the elytra and densely punctate pronota (Figs. 4.25, 4.26 and 4.27, Roughley 1990; Bergsten et al. 2001; Miller 2003; Härdling and Bergsten 2006). The two species of *Hyderodes* have some females with densely granulose pronotum and elytra (Figs. 4.28, 4.29 and 4.30, Watts 1978; Miller 2003). In *Hyderodes* and *Dytiscus*, many populations have females variable with some individuals modified and others smooth and similar to males (Watts 1978; Roughley 1990; Bergsten et al. 2001; Miller 2003; Härdling and Bergsten 2006). Many females of Hydaticini have deep, irregular grooves laterally on the pronotum and elytron with relatively continuous variation across species from nearly unmodified females to more strongly modified females (Figs. 4.31, 4.32 and 4.33, Roughley and Pengelly 1981; Miller 2003). Females of *Eretes* have an elongate sulcus laterally on the elytron (Miller 2002a). Within Aciliini, *Thermonectus* females have conspicuous scratches on the pronotum and elytron (Figs. 4.34, 4.35 and 4.36), some females of *Graphoderus zonatus* and *G. elatus* have dense granulations (Figs. 4.37, 4.38 and 4.39) and irregular sculpturing on the pronotum and elytron (Bergsten et al. 2001; Härdling and Bergsten 2006; Holmgren et al. 2016), and females of many *Acilius* have broad, deep grooves on the elytron and hairs on the pronotum and in

Figs. 4.25–4.30 Dytiscid sexually dimorphic dorsal surfaces. (4.25–4.27) *Dytiscus dauricus* Gebler, habitus; (4.25) male; (4.26, 4.27) female. (4.28–4.30) *Hyderodes shuckardi* Hope, habitus; (4.28) male; (4.29, 4.30) female

the elytral grooves (Figs. 4.40, 4.41 and 4.42, Bergsten and Miller 2005, 2007; Kiyokawa and Ikeda 2019). Dytiscinae female cuticular modifications in particular have been interpreted within a sexual antagonism scenario (see below for details, Bergsten et al. 2001; Miller 2003; Bergsten and Miller 2007). More conspicuous elytral microsculpture in females is, however, widespread across diving beetles (e.g., Hydroporinae, Cybistrinae, Coptotominae, Lancetinae, Copelatinae, Colymbetinae, Agabinae). In at least some of these species a correlation has been documented between more extensive dorsal microsculpture with male tarsal characteristics (see below; Bilton et al. 2008, 2016) suggesting the features may have evolved under a similar coevolutionary context.

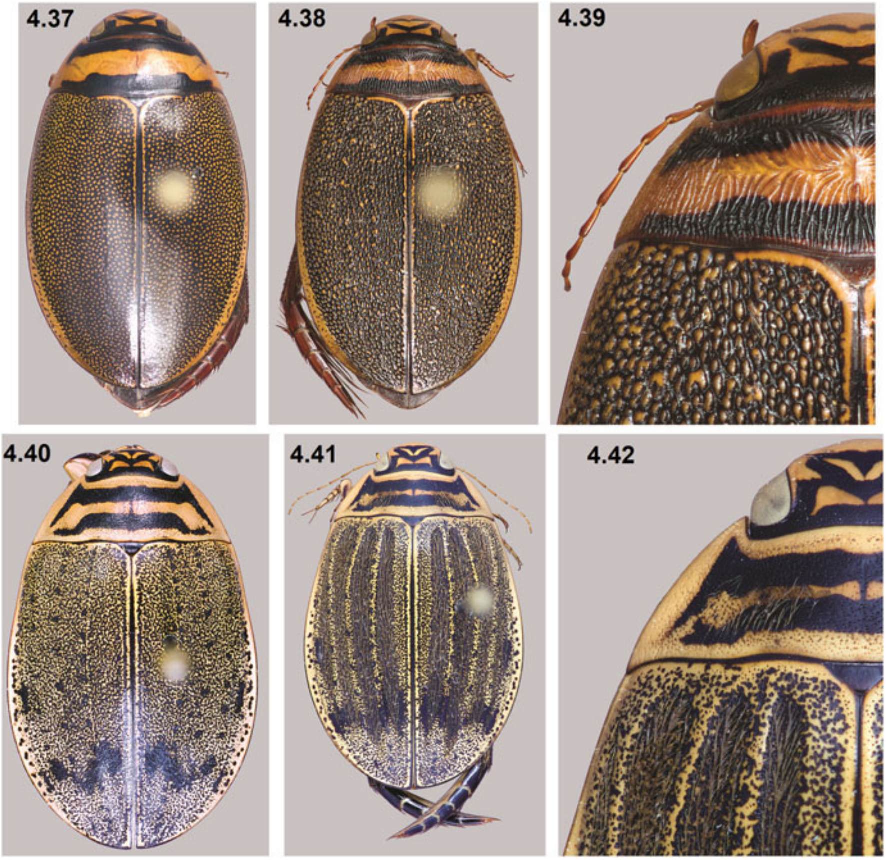
Females of a few dytiscid groups have the elytron with a subapical denticle, including some *Bidessonotus*, *Oreodytes* (Figs. 4.43 and 4.44), *Neobidessodes,* and *Hembidessus* (Balfour-Browne 1947; Young 1990; Nilsson and Holmen 1995;



Figs. 4.31–4.36 Dytiscid sexually dimorphic dorsal surfaces. (4.31–4.33) *Hydaticus continentalis* Balfour-Browne, habitus; (4.31) male; (4.32, 4.33) female. (4.34–4.36) *Thermonectus nobilis* Zimmermann, habitus; (4.34) male; (4.35, 4.36) female

Miller 2001d). The function of this structure is unknown, though it may provide a place for males to grasp using the metatarsal claws. It is present in both males and females of some species in groups like *Nebrioporus* (Toledo 2009) and *Neobidessodes* (Hendrich et al. 2009).

Behavioral dimorphisms (including sound production) are much more poorly known because behavioral observations of predaceous diving beetles are extremely sporadic. It seems clear that there are few or no conspicuous courtship behaviors in many diving beetles (e.g., see video supplement to Bergsten and Miller 2007), though some groups do have characteristic precopulatory or copulatory behaviors that are often species-specific (Aiken 1992; Miller 2003; Cleavall 2009). Many of



Figs. 4.37–4.42 Dytiscid sexually dimorphic dorsal surfaces. (4.37–4.39) *Graphoderus zonatus* (Hoppe), habitus; (4.37) male; (4.38, 4.39) female. (4.40–4.42) *Acilius sulcatus* (Linnaeus), habitus; (4.40) male; (4.41, 4.42) female

the morphological dimorphisms discussed above are likely correlated with behaviors, though most of these are not well known.

4.2.2 Genitalia

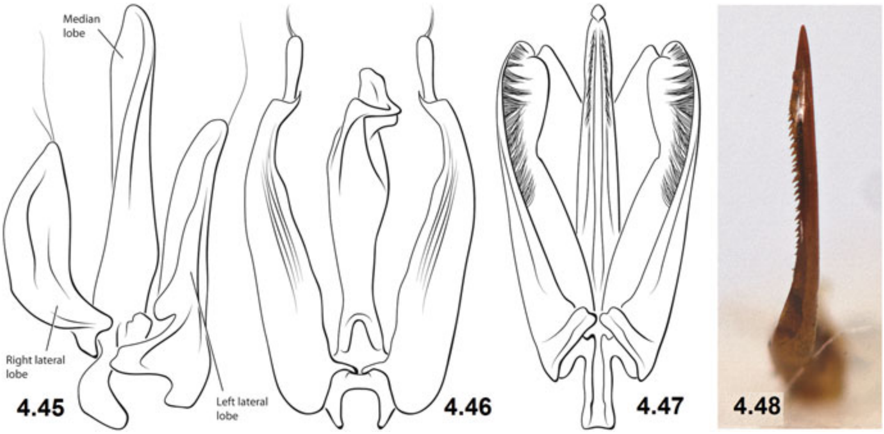
4.2.2.1 Male Genitalia

Male animal genitalia are thought to be under considerable female choice selection pressure and, in many animals, exhibit rapid, divergent evolution (Eberhard 1985). Male external genitalia are often highly characteristic of species of Dytiscidae, and are frequently either the only or the main character system used in species

Figs. 4.43–4.44 *Oreodytes alpinus* (Paykull), habitus; (4.43) male; (4.44) female

diagnostics. The morphology of this system was discussed by Sharp and Muir (1912) and within a taxonomic context by many other authors (detailed in Chap. 5 in this book). The major structures associated with the male external genitalia (aedeagus) are an elongate median lobe that has a variably expanded area at its base where a pair of elongate lateral lobes (parameres) articulate (Figs. 4.45, 4.46 and 4.47). The median lobe has a ventral groove with a weakly developed membranous structure (possibly the remnants of the endophallus) that bears the gonopore through which passes the spermatophore. Other structures appear to be used primarily to facilitate extrusion of the aedeagus. For example, there is a variously sclerotized ring around this tri-lobed structure that may represent components of abdominal sternite VIII and tergite IX, but precise homology of these with other insect abdominal sclerites is difficult. There is also a ventral "strut" comprised typically of an elongate sclerotized structure ventrad to the genital capsule. Finally, abdominal sternite VII is typically longitudinally divided medially into two lateral plates connected anteriorly by a sclerotized ring. There is no large, membranous endophallus or "internal sac." There is exceptional variation in male genitalic shape across diving beetles, and often structures can be somewhat challenging to homologize across many taxa. At least some species exhibit dramatic spines or saw-like structures that may have an antagonistic evolutionary component (see Sect. 4.3.1 below).

A major theme in the morphology of the external male genitalia is "retournement" or rotation of the genitalia at repose (Sharp and Muir 1912; Jeannel and Paulian



Figs. 4.45–4.48 Dytiscidae, male genitalia. (4.45) *Laccophilus maculosus* (Germar), dorsal aspect. (4.46) *Copelatus* sp., dorsal aspect. (4.47) *Dytiscus thianschanicus* (Gschwendtner), dorsal aspect. (4.48) *Copelatus* sp., ventral aspect

1944; Jeannel 1955). Usually, the diving beetle aedeagus (i.e., median lobe and lateral lobes, or parameres) are rotated 90° in repose and subsequently rotated another 90° during copulation (Blunck 1912a; Sharp and Muir 1912; Miller and Nilsson 2003). This configuration may have resulted from conservation of aedeagal position during mating as the "male on top" mating position evolved from an "end to end" position (Jeannel 1955). Additional widespread morphological variation includes degree of symmetry of the median lobe and lateral lobes (Figs. 4.46 and 4.47). The lateral lobes are bilaterally asymmetrical, as is the median lobe, in members of Laccophilini (Laccophilinae, Fig. 4.45) and in a few, isolated groups of Hydroporinae (e.g., some *Bidessonotus* and *Neoporus* within Bidessini (Young 1977, 1981, 1990)). The median lobe is moderately to strongly asymmetrical with symmetrical median lobes in many dytiscids (Fig. 4.46) except the subfamilies Cybistrinae, Dytiscinae, and Hydroporinae, which have distinctly symmetrical genitalia (Fig. 4.47), at least plesiomorphically, with a few other taxa, such as some *Ilybius*, having nearly symmetrical male median lobes. Within Hydroporinae, there are certain groups that have secondarily asymmetrical median lobes including *Graptodytes* (Siettitiina) and several Bidessini genera.

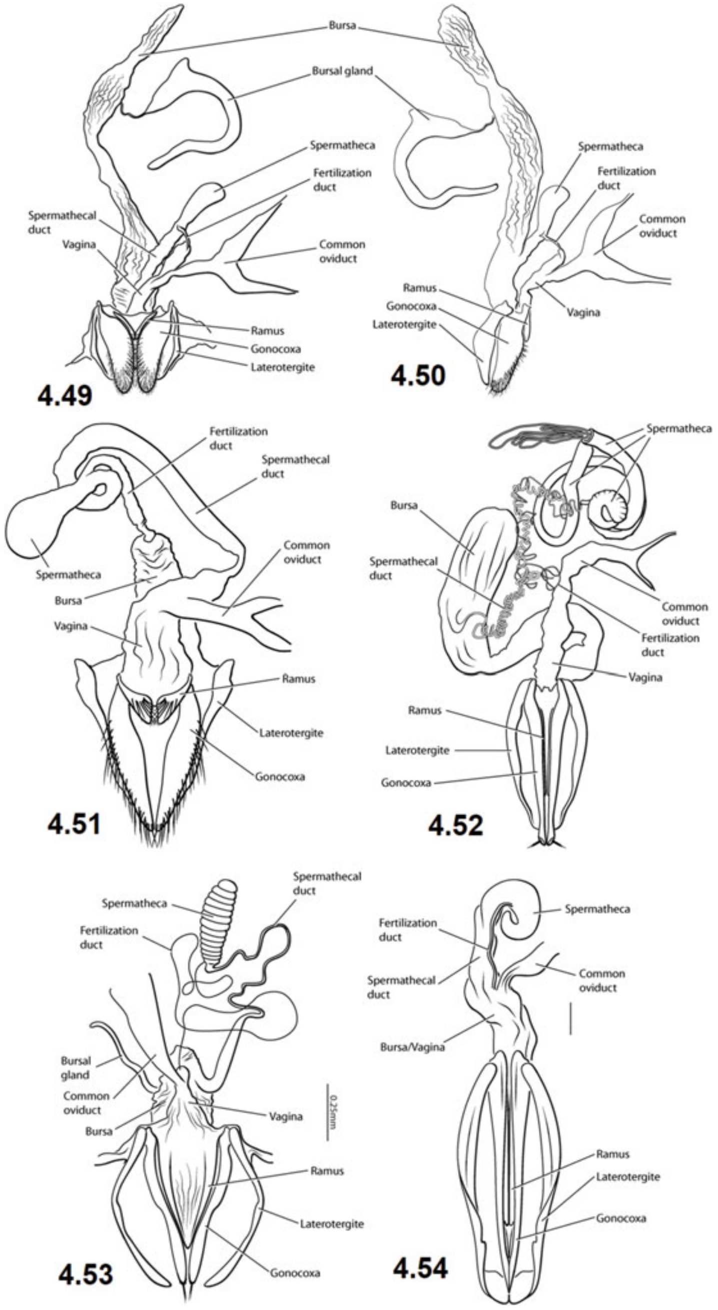
The internal male genitalia has not been carefully investigated in dytiscids, but known species have paired, elongate, tubular testes, and associated glands (Blunck 1912a; Jamieson et al. 1999). It is not known whether male morphology varies significantly with variation in sperm morphology (see below).

4.2.2.2 Female Genitalia

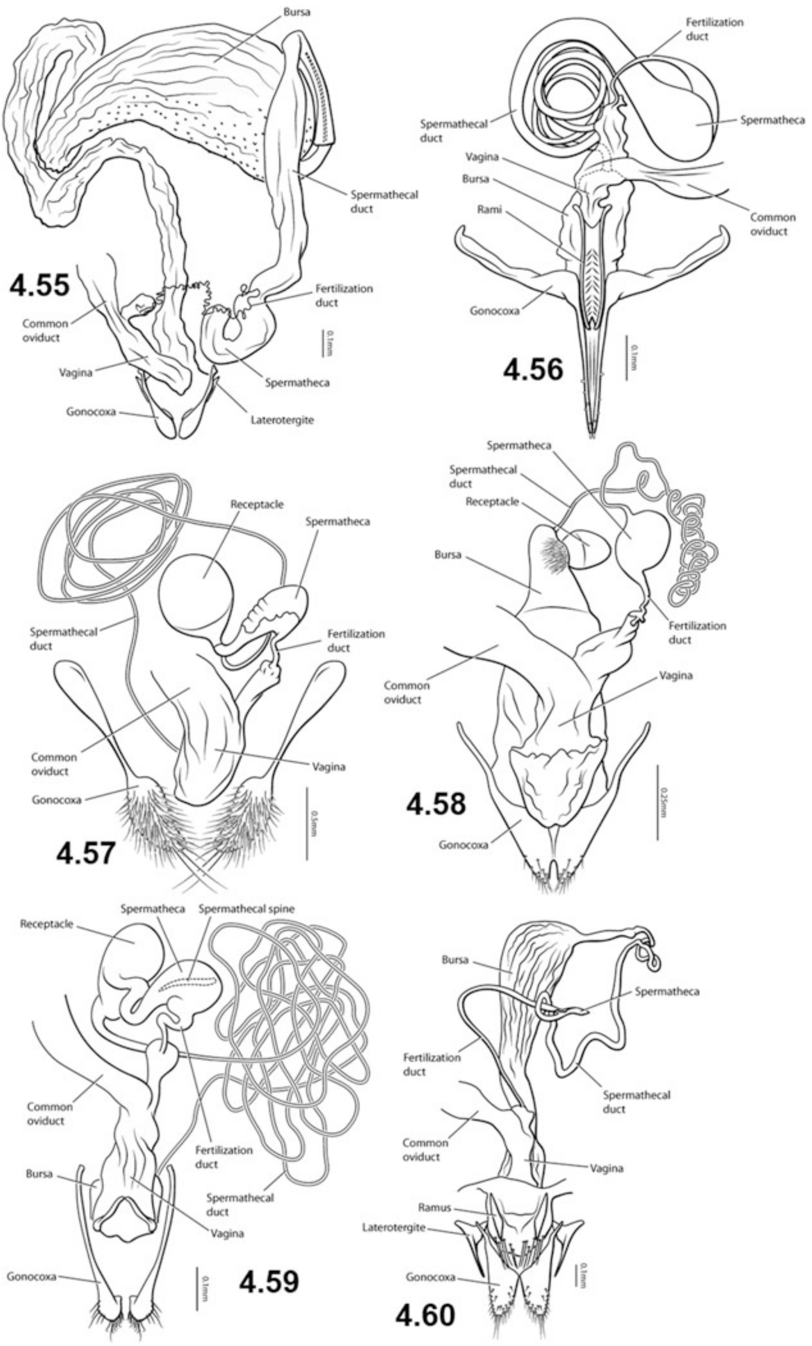
Female genitalia are not expected to be as strongly divergent as male genitalia because they are not under the same types of mate choice selection pressure (Eberhard 1985). Dytiscid female genitalic morphology (external and internal) has been reviewed especially by Deuve (1988, 1993), Burmeister (1976, 1980, 1990b), and Miller (2001c) with less comprehensive work by others (e.g., Böving 1912; Galewski 1974; Angus 1985; de Marzo 1997). Female external genitalia are developed primarily for various activities associated with oviposition. Several groups have specific modifications for endophytic oviposition such as medial fusion of the gonocoxae into a strengthened, knife-like structure, or development of denticles on the rami or gonocoxae. Modifications like these are present in *Hydrovatus* (Hydrovatini), *Ilybius* (Agabini), Laccophilinae, Cybistrinae, and many Dytiscinae (Blunck 1912b; Jackson 1960; Miller 2001c; Inoda 2011). Others have a great many different shapes and configurations, but little is known about most egg laying and how the external genitalia function to do so.

Female internal genitalia in predaceous diving beetles (Figs. 4.49, 4.50, 4.51, 4.52, 4.53, 4.54, 4.55, 4.56, 4.57, 4.58, 4.59 and 4.60) is particularly unusual among arthropods in one important aspect, the organization of the reproductive tract (RT) into a "loop" with two genital openings (e.g., Fig. 4.40, Heberdey 1931; Jackson 1960; Burmeister 1976; Miller 2001c). One opening is to the bursa copulatrix ("bursa") and is for sperm (or spermatophore) reception. The bursa may or may not have an associated gland (or glands and gland reservoir), though the function of the gland is unknown. From the bursa, a variously modified spermathecal duct leads to the spermatheca. From the spermatheca, a fertilization duct leads to the vagina near the base of the common oviduct, and the vagina leads out the apex of the abdomen for oviposition of eggs. Effectively, this decouples the evolution of sperm reception from fertilization and oviposition, thereby releasing constraints on the morphology of the RT that happen when these structures must perform multiple functions. The result of this is dramatic variation in RT morphology across the Dytiscidae, with particular diversity within the Hydroporinae (Figs. 4.55, 4.56, 4.57, 4.58, 4.59 and 4.60), which are characterized by extra chambers, exceptionally long ducts, setae, large spines, sculpturing, and other dramatic modifications (Miller 2001c). In contrast, members of Dytiscinae have the female genitalia reduced, and have, secondarily, a single genital opening (Fig. 4.54; Miller 2001c). These marked differences between Hydroporinae and Dytiscinae may be associated with the evolution of two dramatically different mating systems (see below). Members of Amphizoidae and Paelobiidae also have two genital openings, homologous with the condition in Dytiscidae (Burmeister 1976, 1990a; Miller 2001c). An analogous condition exists in ditrysian Lepidoptera (Scoble 1995).

Miller (2001c) described four basic configurations of internal genitalic morphologies in Dytiscidae. The first is the "Amphizoid-type" with two genital openings, and with the spermathecal duct extending from the posterior base of the bursa (Figs. 4.49 and 4.50). This type characterizes Amphizoidae, Paelobiidae, Matinae, and many Colymbetinae and Agabinae. The second is the "Hydroporine-type" with the spermathecal duct attached at the anterior apex of the bursa (Figs. 4.52, 4.53, 4.55, 4.56, 4.57, 4.58, 4.59 and 4.60). This configuration characterizes Hydroporinae, Lancetinae, Copelatinae, Coptotominae, Laccophilinae, and some Colymbetinae and Agabinae. The third type, the "Dytiscine-type," has a single genital opening with both the fertilization duct and spermathecal duct extending from the vagina/bursa to the spermatheca (Fig. 4.54). This condition is secondarily



Figs. 4.49–4.54 Dytiscidae female reproductive tract, ventral aspect except **b** right lateral aspect. (4.49, 4.50) *Rhantus atricolor* (Aubé). (4.51) *Lancetes lanceolatus* (Clark). (4.52) *Exocelina australis* (Clark). (4.53) *Hydrotrupes palpalis* Sharp. (4.54) *Dytiscus verticalis* Say



Figs. 4.55–4.60 Dytiscidae female reproductive tract, ventral aspect. (4.55) *Pachydrus* sp. (4.56) *Hydrovatus* sp. (4.57) *Megaporus howittii* (Clark). (4.58) *Paroster nigroadumbratus* (Clark). (4.59) *Hemibidessus celinoides* (Zimmermann). (4.60) *Laccornis oblongus* (Stephens)

derived in Cybistrinae and Dytiscinae (Miller 2001c) and represents a reversal to the type of genitalia present in, for example, Noteridae and Gyrinidae. In Cybistrinae and Dytiscinae, the spermatophore is transferred to a separate area ventrad of the main female genitalia (Aiken 1992). The final type is the "*Agaporomorphus*-type" wherein the bursa appears to be completely reduced, which occurs only in the copelatinae genus *Agaporomorphus*.

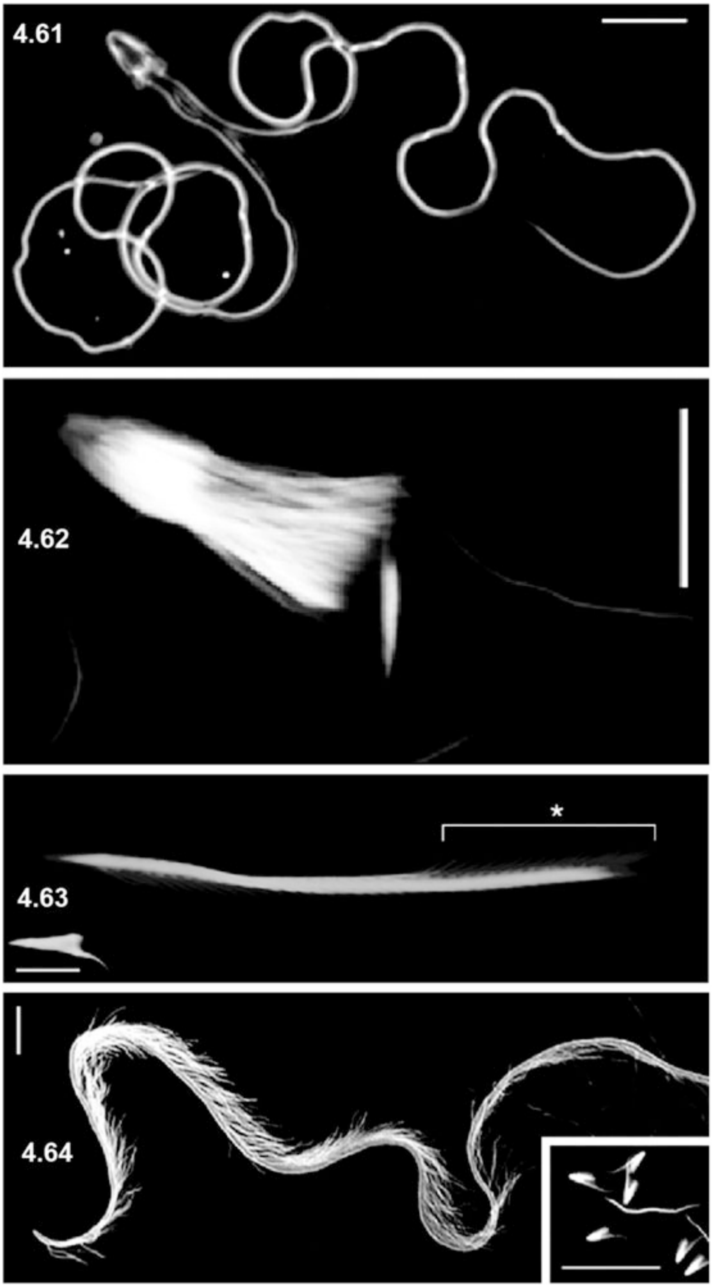
Miller (2001c) investigated the evolution of female genitalia in a phylogenetic context. He found that two genitalic openings is plesiomorphic for Dytiscidae (the condition is present also in Paelobiidae and Amphizoidae) and secondarily reduced to a single opening in Dytiscinae. He also found that adaptations for apparent endophytic oviposition was derived multiple times within Dytiscidae, in Laccophilinae, *Ilybius* (Agabinae), Dytiscinae, and *Hydrovatus* (Hydroporinae). Other larger transitions in dytiscids include loss of the laterotergite in Hydroporinae (except Laccornini) and loss of the bursal gland in numerous lineages, among much other more taxon-specific variation (Miller 2001c).

4.2.3 Sperm

The dramatic variation in female RT morphology in dytiscids suggests that sperm morphology could vary just as dramatically as sperm and RT coevolve. Sperm fitness is heavily influenced both by interactions with the female RT (cryptic female choice) and other male sperm within the RT (sperm competition) (Parker 1970). Sperm fitness may also be influenced by cooperative effects between sperm from the same ejaculate (Higginson and Pitnick 2011). All of these effects appear to be operating on predaceous diving beetle sperm.

Although certain aspects of diving beetle sperm have been known for many years (Auerbach 1893; Ballowitz 1895), this is a relatively poorly studied area of dytiscid sexual biology. Much of the state of knowledge was reviewed by Jamieson et al. (1999). Sperm and sperm selection are clearly major components of dytiscid sexual evolution because in some cases sperm can account for up to 13% of the total male body mass (e.g., *Dytiscus sharpi*, Inoda et al. 2007). Recent studies have begun to shed light on the dramatic and considerable diversity in dytiscid sperm morphology and have attempted to correlate that diversity with female reproductive tract variation within a phylogenetic context (Higginson et al. 2012a, b). Within the wealth of variation in diving beetle sperm, certain patterns can be discerned. Some diving beetles have singleton sperm of a single type, like many animals, and there is much variation in dytiscid sperm length and head shape (Higginson et al. 2012a, b). However, dytiscid sperm is of particular interest because of two notable syndromes: (1) conjugation and (2) heteromorphism, with co-occurrence of each in certain species.

Conjugation refers to a phenotype where two or more sperm that unite at the head to form a cooperative unit (Higginson and Pitnick 2011). In some diving beetles, a simple form of conjugation occurs wherein two sperm heads unite to form a pair (Fig. 4.61). This is found in Cybistrinae, Dytiscinae, and the colymbetinae genus



Figs. 4.61–4.64 Dytiscid sperm. (4.61) Simple conjugate of two sperm, *Graphoderus liberus* (Say). (4.62) Aggregation of multiple sperm, *Ilybius oblitus* Sharp. (4.63) Rouleaux conjugate, *Neoporus undulatus* (Say). (4.64) Rouleaux conjugate, *Hygrotus sayi* Balfour-Browne. Pictures from Higginson et al. (2012b)

Melanodytes (Ballowitz 1895; Mackie and Walker 1974; Werner 1976; Jamieson et al. 1999; Higginson and Pitnick 2011; Higginson et al. 2012a). A second type of conjugation is aggregation of multiple sperm heads together (Fig. 4.62). This is found in many Agabinae, Colymbetinae, *Batrachomatus* (Matinae), some *Pachydrus* (Hydroporinae), *Hygrotus* (Hydroporinae), and possibly *Lioporeus* (Hydroporinae) and *Agabetes* (Laccophilinae) (Ballowitz 1895; Mackie and Walker 1974; Werner 1982; Dallai and Afzelius 1988; Higginson and Pitnick 2011; Higginson et al. 2012a). Finally, a complex type of conjugates in diving beetles are called "rouleaux" (Fawcett and Hollenberg 1963; Shepherd and Martan 1979; Heath et al. 1987) and comprise sperm conjugates with cone-shaped heads that form ordered stacks by nesting together (Figs. 4.63 and 4.64, Higginson and Pitnick 2011; Higginson et al. 2012a). This type of conjugation is found across many Hydroporinae and in *Matus* (Matinae) (Higginson et al. 2012b). Not all diving beetles exhibit conjugation. Singleton sperm are known to be characteristic of Copelatinae, Coptotominae, *Desmopachria*, some *Pachydrus* (Hydroporinae), *Porhydrus* (Hydroporinae), and some *Thermonectus* (Dytiscinae) (Higginson et al. 2012a).

Another unusual phenotype in dytiscid sperm is the presence in certain taxa of heteromorphism, or more than one sperm morphology in the same ejaculate. This occurs in *Agabetes* (Laccophilinae), Coptotominae, *Derovatellus* (Hydroporinae), *Hygrotus* (*Coelambus*) (Hydroporinae), *Ilybius* (Agabinae), *Platambus* (Agabinae) (Higginson et al. 2012a), and, possibly, *Cybister* (Cybistrinae) (Voïnov 1902). Of these, several have both conjugation and heteromorphism including *Agabetes*, *Derovatellus*, *Hygrotus*, *Ilybius,* and *Platambus*. Interestingly, there appears also to be some evidence of eupyrene and apyrene spermatozoa in the same ejaculate in *Cybister tripunctatus* (Mukherjee et al. 1989).

The evolution of sperm in diving beetles was studied by Higginson et al. (2012a, b). Their studies investigated primarily head shape, sperm length, type of conjugation, and heteromorphism in a phylogenetic context. They found aggregation sperm to be the plesiomorphic condition within Dytiscidae with multiple independent transitions to singleton sperm, paired sperm, and rouleaux, and some reversals. Head shape and conjugation were closely correlated, but length and heteromorphism were not. There are within Dytiscidae both long and short conjugated sperm, and heteromorphic sperm in singleton or conjugated systems.

4.3 Dytiscid Sexual Systems

Mating behavior and mating system evolution in dytiscids is just beginning to be investigated, and it is somewhat difficult to generalize, though several lines of evidence are beginning to accumulate based in part on many of the behaviors and morphology described above. A better picture of these systems in Dytiscidae is emerging and revealing a deeply complex range of evolution of syndromes.

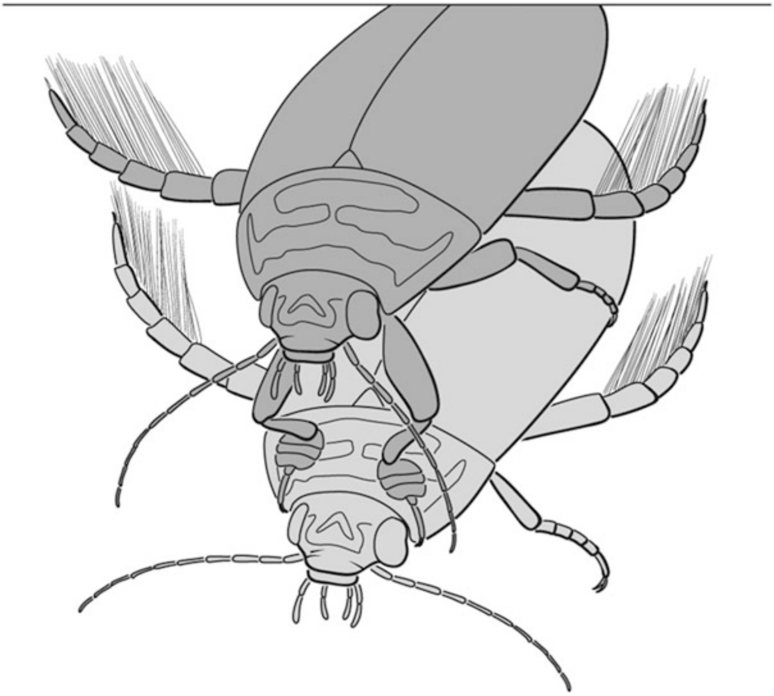


Fig. 4.65 Dytiscidae mating position, *Acilius sulcatus* Linnaeus, male dorsal, female ventral

Presumably both sexes of most predaceous diving beetle species mate multiple times. Males and females of observed species do so (Blunck 1912a; Aiken 1992; Miller 2003; Cleavall 2009). Also, females are often observed with multiple spermatophores in the RT (Miller 2001c). The sexual systems evidently operating in dytiscids based on morphology and behavior (sexually antagonistic selection, sperm selection, and competition, see below) are predicated on multiple matings. Mate finding appears to be either a scramble competition with males actively seeking females, or potentially associated with either male (acoustic) (Larson and Pritchard 1974) or female (chemical) (Herbst et al. 2011) signaling and response. Any signaling would, therefore, represent the traditionally understood combination of competition among males for better signal production or female signal sensing, and female choice on male sound production or ability to sense female signals (Thornhill and Alcock 1983). Other selection effects may be operating to influence signaling as well, such as signal interception by potential predators (Thornhill and Alcock 1983). Other than seemingly limited signal production in predaceous diving beetles, though uninvestigated, there appears to be little or no courtship mating behavioral displays. Copulatory and postcopulatory behaviors, in contrast, are considerably more complex in certain groups of predaceous diving beetles.

Mating takes place in a “male above” position (Fig. 4.65) with the male aedeagus extruded and the median lobe or both the median lobe and lateral lobes placed inside the female RT (Blunck 1912a; Aiken 1992; Cleavall 2009). There is no eversible internal sac, and the mechanism of sperm movement from the male into the female is not known. Most, if not all, predaceous diving beetle males pass a spermatophore to females during copulation. These spermatophores can often be observed within the female bursa upon female RT dissection. Little to nothing is known of spermatophore morphology, production, constituents, or metabolism within the female. Some limited descriptive work has been done with *Dytiscus marginalis* spermatophores (Blunck 1912a).

The many different grasping devices (e.g., modified antennae, claws, legs) in males, and the often dramatically complex and variable male external genitalia probably represent either species-specific variation selected for during female choice of particular stimulations, morphologies, or mechanical fit (Eberhard 1985) or antagonistic selection driven by conflicting interests and reduction of associated costs rather than active choice for better genes (Arnqvist and Rowe 2005). Male predaceous diving beetles seem to routinely attempt mating with other beetles they encounter, even if they are the wrong species or the wrong sex (Cleavall 2009). Signals exchanged during these encounters probably preclude "mating errors," which may help reinforce species isolation as well.

There are no known examples of parental care of fertilized eggs or larvae in predaceous diving beetles beyond female placement of eggs, so contribution of this potential fitness component to sexual evolution in the group is not apparently significant. Given the unusual complexity of female genitalia, it is possible that females differentially invest in eggs internally, but this is completely unknown in predaceous diving beetles.

4.3.1 Pre-insemination Sexual Systems

Among the most dramatic of the sexual systems exhibited by predaceous diving beetles is an apparent sexually antagonistic coevolution scenario exhibited among members of the subfamily Dytiscinae. Sexual conflict involves evolutionary conflicts of interest between males and females that may produce characteristic coevolutionary patterns as each sex seeks greater control over the decision to mate (Parker 2006). Although females, in particular, may derive benefits from mating multiple times (Eberhard and Cordero 1995; Yasui 1998; Arnqvist and Nilsson 2000), there are potentially large costs associated with mating as well (Daly 1978; Wing 1988; Martens and Rehfeldt 1989; Le Boeuf and Mesnick 1991; Magnehagen 1991; Fairbairn 1993; Rowe 1994; Watson and Lighton 1994; Watson et al. 1998). Certain male phenotypes may increase costs to females to the point of intense trauma or even death (e.g., Morrow and Arnqvist 2003; Reinhardt et al. 2003; Rönn et al. 2007). Unlike traditional ideas about sexual selection, wherein choice of a sexual partner increases both male and female average fitness, sexual conflict predicts that males and females may diverge with the development of a strategy that increases fitness in one sex (e.g., manipulative strategies in males) that simultaneously decreases fitness in the other sex (e.g., females) (Pizzari and Snook 2003). The effect of reduced overall lifetime fitness in females can cause them to evolve resistance behaviors and morphologies. Males are then expected to respond by evolving features that are able to overcome the female resistance (e.g., grasping devices), and this adaptation–counteradaptation is expected to develop further into an escalating “arms-race” as each sex seeks to manipulate control of the decision to mate (Parker 1979; Alexander et al. 1997; Pizzari and Snook 2003; Arnqvist and Rowe 2005; Härdling and Smith 2005; Parker 2006). Furthermore, this process is thought to be an important engine of speciation under both allopatric and sympatric conditions (Arnqvist et al. 2000; Gavrilets 2000; Gavrilets and Waxman 2002; Martin and Hosken 2003). It is this type of mating system that appears to be operating in members of Colymbetinae, Dytiscinae, and Cybistrinae, at least, among dytiscids (Bergsten et al. 2001; Miller 2003; Bergsten and Miller 2005, 2007).

Although mating behavior is known for only a few taxa, males of *Dytiscus*, *Thermonectus, Acilius,* and *Rhantus* (Smith 1973; Aiken 1992; Miller 2003; Cleavall 2009) have males that abruptly grasp females in an attempt to mate, whereupon females engage in erratic and swift swimming that sometimes successfully dislodges the male (Aiken 1992; Bergsten and Miller 2007; Cleavall 2009). Aggressive behavior by itself is not sufficient evidence for sexual antagonism over against mutualistic sexual selection since the behavior may simply be seductive or stimulative to females or may represent female assessment without incurring a cost to them (Pizzari and Snook 2003; Parker 2006). However, mate guarding is extremely long in these species, with some mating events lasting hours (Aiken 1992; Miller 2003; Cleavall 2009) as males possibly attempt to ensure paternity through sperm selection or competition. This prolonged mating event duration may explain some of the direct costs inherent to females. Mating pairs may be more susceptible to predation, for example, a situation that occurs in other insects (Magnehagen 1991; Rowe 1994).

A particular cost to mating for female diving beetles may come from the aquatic lifestyle itself combined with prolonged postcopulatory guarding. Although predaceous diving beetles are aquatic, they breathe atmospheric oxygen that they carry with them under their elytra and they have to frequently return to the surface to replenish the oxygen. During mating, males are above females and hold females under water thereby restricting their ability to breathe (Fig. 4.65). During the lengthy period of postcopulatory guarding which, after intromission, can last for several hours, males have been observed tilting females upward so they can access air during the mate guarding phase (Aiken 1992; Cleavall 2009). Whether this should be seen as a coercive strategy by males to subdue females or a female cost simply arising as a byproduct from the selection on males for prolonged mate guarding to secure paternity, remains to be clarified.

Whatever the possible costs to females and coercive abilities of males, compelling evidence that a sexually antagonistic arms race is operating in Dytiscinae also comes from morphology. Males in this subfamily have the protarsi extremely broad with large, sucker-shaped adhesive setae (Figs. 4.16, 4.17 and 4.18, see above). Some groups have sucker setae on the mesotarsomeres, as well. These are used to adhere to the smooth dorsal surface of the female prior to and during mating (Aiken 1992; Bergsten et al. 2001; Miller 2003; Bergsten 2005; Bergsten and Miller 2007; Cleavall 2009). These setae are quite strong, combined on the protarsal palette able to lift 4× the weight of a female (Aiken and Khan 1992), and are presumably an improved grasping device in evolutionary response to the female behavioral resistance (Bergsten et al. 2001; Miller 2003), though it cannot be discounted, based on current evidence, that the expanded protarsi represent a handicap of some kind used by females to determine mate quality.

As further evidence of sexual antagonism, however, females in several groups within Dytiscinae also have unusual morphology with the dorsal surface of the pronotum and elytra irregularly modified with dramatic modifications to the cuticle (Figs. 4.25, 4.26, 4.27, 4.28, 4.29, 4.30, 4.31, 4.32, 4.33, 4.34, 4.35, 4.36, 4.37, 4.38, 4.39, 4.40, 4.41 and 4.42, see above). These modifications interfere with the adhesive ability of the male sucker setae (Bergsten and Miller 2007), and they appear to be an evolutionary response to the male improved grasping device (sucker setae) (Miller 2003). Karlsson Green et al. (2013) measured mechanically the adhesion force of male suction cups on male versus modified female elytra in two species. The adhesion (pull-off force measured in Newtons) of male protarsal setae was two to five times weaker on the modified female elytra compared to the smooth male elytra, confirming the antagonistic effect of the modification. Female morphological "anti-grasping" devices are rare in animals, which has been thought to do considerable violence to the sexual antagonism argument (Eberhard 1985; Arnqvist and Rowe 2002a; Eberhard 2004; Eberhard 2005; Eberhard 2006, but see Rönn et al. 2007). Predaceous diving beetles may, therefore, be relatively unique among animals in exhibiting just such devices.

Thus, it would appear that dytiscines are operating under an escalating sexual antagonism scenario precipitated by (1) male mate guarding, prolonged mating event duration, and coercive male behaviors (such as holding females underwater) that may result in increased costs of matings (and reduced direct fitness) in females, leading to (2) female resistance to male mating attempts, leading to (3) male development of an improved grasping device in the form of circular sucker-shaped setae, and, finally, development of (4) multiple origins of modifications to female dorsal cuticle in response to the male grasping device (Miller 2003). It should be stressed, though, that specific tests of direct and indirect fitness in females of dytiscines, and, therefore, sexual antagonism, have not been done. The behavioral and morphological evidence, while compelling, has not been definitively correlated with differential fitness between males and females, though tests of this may be particularly rewarding in this taxon.

There are several more detailed components to this scenario in predaceous diving beetles that have been investigated. For example, in certain groups, such as *Acilius*, it has been shown that sexual antagonism is driving speciation and the coevolution of changes in male and female secondary sexual characters including curved setae along the margin of the male protarsi, setal tufts on male mesotarsi, setae on the dorsal surface of the female pronotum and elytron, and the presence of longitudinal grooves on the female elytron (Fig. 4.66, Bergsten and Miller 2007). Also,

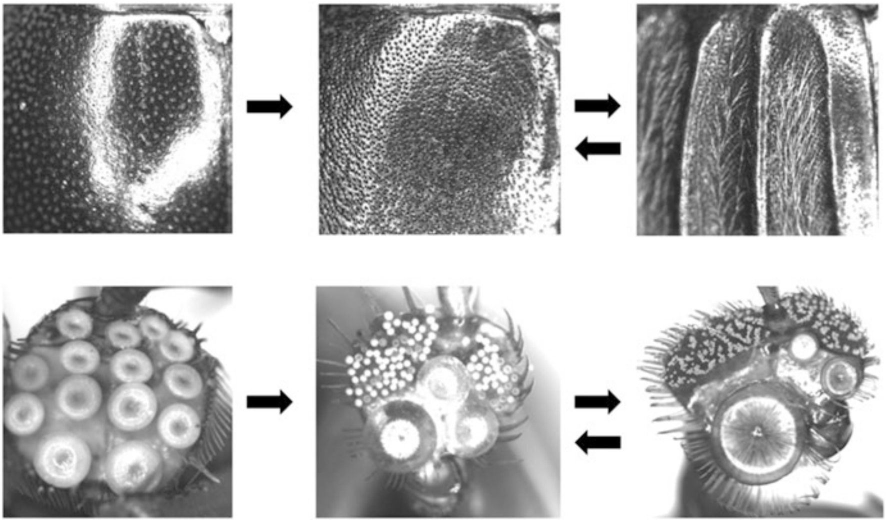


Fig. 4.66 Major coevolutionary transitions in intersexual arms race across phylogeny of *Acilius* species. Top: close-up of female elytra showing transition to dense punctures, to setaceous sulci and back to dense punctures; bottom: male protarsi with adhesive setae showing change in size disparity of sucker setae, to extreme size disparity, and back to a simple size disparity

populations of certain species (e.g. *Dytiscus*, *Hyderodes, Hydroporus*, *Hygrotus, Copelatus*, and *Graphoderus*) have female intrasexual dimorphism with some individuals modified and others smooth, like males (Bergsten et al. 2001; Miller 2003; Härdling and Bergsten 2006; Bilton et al. 2016; Karlsson Green 2010; Karlsson Green et al. 2014; Ranarilalatiana et al. 2019). In *Dytiscus sharpi*, this is controlled genetically with the modified condition dominant (Inoda et al. 2012). There are actually very few examples of the so-called Alternative Mating Phenotypes (AMPs) of females, damselflies and diving beetles being the most convincing examples (Buzatto et al. 2014). The presence of two distinct morphs in a population raises questions about what balancing selection or dynamic enables them to coexist. Theoretical work has indicated that such polymorphism can be maintained through sexual conflict and non-random mating (Härdling and Bergsten 2006; Härdling and Karlsson 2009; Iversen et al. 2019). Non-random mating leads to genetic correlation between male and female morphs. With negative frequency dependent selection the common female morph has a disadvantage, and as the frequency declines the genetic correlation also drags along the associated male morph. Hence, both direct selection on the female morph and indirect selection on the male morph lead to an increase in the more rare morphs until the frequencies and selection forces are reversed. An empirical study on *Graphoderus zonatus* showed both signs of stabilizing selection toward 0.5/0.5 morph frequencies across populations and could confirm assortative (non-random) mating between male and female morphs through wild-caught mating pairs (Iversen et al. 2019). Eventually such a system can maintain equilibrium with polymorphism in both sexes (Härdling and Bergsten 2006; Iversen et al. 2019). In the largely allopatric case of smooth and matt female morphs of *Hydroporus memnonius* with a contact zone across north England and south Scotland, a 30-year time series instead showed expansion of the matt morph at the expense of the shiny morph (Bilton et al. 2016). Whereas dimorphism in dytiscids has mostly been documented in females, the two female morphs of *Hydroporus memnonius* also have two distinct associated male morphs differing in tarsal characteristics (Bilton et al. 2008). The presence or absence of two adhesive setae on the second protarsal segments differs between the two morphs (Bilton et al. 2008). Two male mating clusters or "morphs" with differing adhesive setae constellations, albeit with overlapping variation, exist in *Graphoderus zonatus* (Iversen et al. 2019), as in *Agabus uliginosus* although for the latter in allopatry (Bilton et al. 2016). There is a correlation between the male and female morphs for all three species. In some species the frequency of different morphs show geographic patterns (Bilton et al. 2008; Iversen et al. 2019), which also remain to be fully understood, and it cannot be excluded that environmental factors affecting things like mate finding and mating frequency are involved as well (Karlsson Green et al. 2014; Drotz et al. 2010; Kiyokawa and Ikeda 2019). In a preliminary report of a very interesting study on *Acilius japonicus* by Kiyokawa and Ikeda (2019), a female counteradaptation, fields of setae on the pronotum that interfere with male suction cups, shortened mating duration and was more pronounced in warmer localities (Kiyokawa and Ikeda 2019). This possibly links environmental conditions to the cost of oxygen deprivation during mating and how it affects the antagonistic coevolution of characters. In contrast, the shifting contact zone in *Hydroporus memnonius* could not be explained by environmental conditions as at least differential temperature tolerance between the forms predicted the opposite pattern to that observed for the 30-year period (Bilton et al. 2016). We can clearly expect many idiosyncratic responses, patterns, and environmental correlates as more species and more secondary sexual character systems are investigated.

Male adhesive setal disks have dramatically disparate size in *Dytiscus*, *Eretes*, *Acilius,* and certain other species, such as *Graphoderus zonatus* (Figs. 4.17 and 4.66). These taxa have male protarsi with one or more very large setal disks, and a great many very small ones (Roughley 1990; Bergsten et al. 2001; Bergsten and Miller 2005). Other groups, such as Hydaticini, have these disks more uniform in size within and between species (Fig. 4.16, Bergsten et al. 2001). Variability in size may be correlated with female modifications, as greater variability in setal disk size may increase ability to adhere to irregular surfaces, whereas a smooth surface may instead have an associated optimal size. Indirect evidence of this was presented for *Graphoderus zonatus* in which populations with a high proportion of modified females have males with more divergent setal size, and in populations with a low proportion of modified females (more smooth females) males have more similar-sized setae (Bergsten et al. 2001). A close phylogenetic correlation between inter-specific variability in male adhesive setal size and modification to females was found in *Acilius* (Fig. 4.66, Bergsten and Miller 2007).

Interestingly, once the male has "subdued" the female, many species have species-specific stereotypical male copulatory behaviors, as well. These include rocking or bobbing by the male in *Dytiscus* and *Thermonectus* (Aiken 1992; Cleavall 2009), and "fluttering" or rubbing of the legs during attempted intromission by *Thermonectus* and *Acilius* (Miller 2003; Cleavall 2009). So it would seem that even though females resist mating and males seek to force them to mate, once mating ensues, males still must "entice" a female through copulatory behaviors. In flour beetles it has been shown that the intensity of male rubbing of the female with his legs during copulation is correlated with his fertilization success in multiply mated females, apparently through female choice (Edvardsson and Arnqvist 2000). It is possible that the behavior in dytiscids can have a similar function.

In at least some species of *Dytiscus*, a mating plug is produced by males comprised of an unknown substance smeared around and in the apex of the abdomen of the female (Balduf in Blunck 1912a; Sivinski 1980; Aiken 1992), presumably as an attribute of post-insemination male–male competition for paternity (Alcock 1994). This behavior was observed during matings in the autumn, but spring matings, closer to oviposition time, did not result in a plug (Aiken 1992).

There are a great many unanswered and perplexing questions regarding predaceous diving beetles and this mating system. For example, members of Hydaticini are nested within this group, and females have distinct modifications to the cuticle of the pronotum or pronotum and elytron that would seem to inhibit the sucker setae present in males (Roughley and Pengelly 1981; Miller 2003). It seems reasonable, therefore, to expect this group also exhibits sexual antagonism with females resisting male mating attempts, though mating behavior has never been documented in hydaticines. Unexpectedly, however, males have an apparent stridulatory device on the male protibia and protarsus (Larson and Pritchard 1974; Miller 2003; Miller et al. 2009). If males are signaling to females, and females are responding, then there seems little reason for females to resist male mating attempts because by responding they have already made the decision to mate. If mating was not desired, they could simply not respond to the signal. However, male suction cups are notably undifferentiated in Hydaticini (Fig. 4.16). The protarsal suction cups are few medium-sized and largely same-size in contrast to, e.g., *Dytiscus* (Fig. 4.17), *Acilius*, *Eretes,* and *Graphoderus zonatus* (but similar to in, e.g., *Thermonectus*, *Hyderodes*). It could be that females have actually "won" or lead the antagonistic arms race in hydaticines, or have at least gained a relative advantage in the race to gain control over the mating decision (compare with Arnqvist and Rowe 2002b). Possibly, instead of differentiation of suction cups to better hang-on and overcome female resistance and dorsal modifications, an acoustic signaling device has evolved in male hydaticines to instead attract females, moving the entire mating system from sexual conflict and antagonism to classical sexual selection through female choice (and perhaps same-sex deterrent). This hypothesis predicts a different precopulatory behavior in hydaticines and could be tested in water tank lab experiments monitoring behavior, response, and acoustic recording. Note that underwater sound production by hydaticines is still only presumed based on interpretation of structures on male protibia and tarsus as a stridulation organ but has not to our knowledge been recorded and published. Desjonquères (2016) had a single male *Hydaticus seminiger* in a tank with sound recording equipment for 0.77 days but did not record any sound under these conditions. Other possibilities include that the sucker setae and female cuticular modifications interact in the decision to end the mating encounter, as found by Kiyokawa and Ikeda (2019), or possibly the male sound production is associated with copulatory stimulation. Male sound production may also serve to attract predators to induce females to copulate more quickly (a form of male coercion) as occurs in some water striders (Han and Jablonski 2010).

It seems also possible that the considerable disparity in size between members of Cybistrinae and Dytiscinae and other predaceous diving beetles (some dytiscines and cybistrines reach up to 45–48 mm in length) may reflect this sexual system. As males get larger in order to better “subdue” a reluctant female, females may respond with larger size to better resist their mating attempts. Not all members of this group are unusually large, and variation in size in related taxa within the group may be related to the intensity of evolutionary operation of this system. Considerable variation in size within some groups (e.g., *Thermonectus* species, Miller unpublished) may also be related to intrasexual competition among males interacting with intersexual antagonism. Large size in this group may also reflect a higher demand for atmospheric oxygen allowing for the possibility of male coercion, or a stronger cost of mating to females. Smaller predaceous diving beetles, in at least some cases, are able to breathe directly from the water (Madsen 2012) perhaps removing a large female cost in the equation and disallowing sexual antagonism as a sexual system to evolve.

Finally, another important aspect of this mating system is the observation that Dytiscinae and Cybistrinae have among the simplest configuration of female RT morphology in dytiscids. In these groups there is a secondary reduction to a single genital opening and a simple bursa, fertilization and spermathecal ducts, and spermatheca (Miller 2001c). Remarkably, they also have among the simplest dytiscid sperm morphology, with simple conjugation of sperm pairs (Higginson et al. 2012a). It would seem that among Dytiscinae, and related groups, most of the complexity of sexuality is concentrated in precopulatory and copulatory (pre-insemination) mating behaviors and morphology, with considerably less complexity in the post-insemination environment of the female RT and sperm. The extent to which sperm selection and competition is happening in this group is not known, but observations of their mating behavior and morphology would suggest that they play a much smaller role in these dytiscids than do the pre-inseminatory activities. Even so, in at least some species of *Dytiscus*, sperm production (including associated glands for spermatophore production) can account for up to 13% of total male body mass (Inoda et al. 2007), suggesting there may be more to post-insemination cryptic selection and sperm competition than may be currently evident.

Other predaceous diving beetle groups besides dytiscines and cybistrines may exhibit sexual antagonism as well. For example, several predaceous diving beetle males have unusually modified male intromittent organs with spines (e.g., some *Hyphydrus* (Biström 1982) or slender saw-like or needlelike structures (e.g., some *Copelatus*, Fig. 4.48). In other arthropods (e.g., bruchine seed beetles (Rönn et al. 2007)) spinous median lobes are associated with severe damage to internal female genitalia and sexual antagonism. Needlelike median lobes are associated with "traumatic" or "hypodermic" insemination in other arthropods (e.g., bedbugs (Morrow and Arnqvist 2003)). Mating behavior has not been investigated in *Hyphydrus* or *Copelatus*.

4.3.2 Post-insemination Sexual Systems

In marked contrast, the subfamily Hydroporinae has, overall, the most dramatic diversity of both female RT (Miller 2001c) and sperm morphology (Higginson et al. 2012a, b) within Dytiscidae, and among the most complex in all insects. Hydroporines have female RTs with extra ducts, chambers, internal setae, sculpturing, extremely long and slender spermathecal and fertilization ducts, and other remarkable variation. Complex female reproductive tract morphology is expected to be associated with post-insemination female choice (Hellriegel and Ward 1998; Presgraves et al. 1999). At least some of this complexity may have to do with differential sperm storage strategies and enhanced female control over paternity (Snow and Andrade 2005).

One of the most conspicuous modifications to the female RT in many groups of Hydroporines is the presence of a large, second chamber, often as large as or larger than the spermatheca, called the "receptacle" by Miller (2001c). This structure is either on the bursa, the spermathecal duct, or the spermatheca itself (Miller 2001c). Spermathecal shape is often complex, as well (Miller 2001c), which may also represent differential sperm storage strategies. Secondary chambers and complex sperm storage structures suggest the possibility of extensive female post-insemination mate choice through sperm selection (Hellriegel and Ward 1998; Snow and Andrade 2005), but sperm storage has not been comprehensively investigated in these dytiscids.

Another characteristic of many Hydroporinae (and certain other dytiscids, such as some Agabinae, Coptotominae, and Copelatinae) is long and slender to exceptionally long and slender spermathecal and/or fertilization ducts, or other portions of the female RT (Miller 2001c). Female RT tract length is often closely correlated with sperm selection or other reproductive benefits to females (Birkhead et al. 1993; Miller and Pitnick 2002; Miller and Pitnick 2003), a possibility in predaceous diving beetles.

Finally, other hydroporine RT features include fields of setae, irregular surface structures, or possible glands in different areas of the RT (Miller 2001c). Presumably, these have something to do with sperm selection by the female, but nothing is known about possible correlates with sperm behavior or morphology. One of the most dramatic of these modifications is the large, internal spermathecal spine characteristic of Bidessini (Miller 2001c). Spermathecal spines in other arthropods are known to puncture the spermatophore (Gack and Peschke 1994), but the bidessine spermathecal spine is not apically sharp, and it is not clear what it might be used for.

Consistent with post-insemination sexual selection and sperm competition, Hydroporinae also have dramatically modified sperm with most members of the group having “rouleaux” sperm, or complex conjugations, in some cases with sperm heteromorphism, as well (Higginson et al. 2012a, b). Sperm complexity in the group is certainly interesting and dramatically unusual for animals, but perhaps not entirely unexpected given the phenomenally complex sperm selection environment involved, the hydroporine female RT (see above). Sperm and the female RT are expected to evolve in complex ways because sperm are coevolving with both the female reproductive tract (sperm selection) and other male sperm (sperm competition) (Parker 1970; Birkhead 1996; Presgraves et al. 1999; Miller and Pitnick 2002). Predaceous diving beetles in the Hydroporinae may be particularly suitable for the study of complex post-insemination sexual selection.

Relatively fewer hydroporines have had their mating behavior documented, but the information known suggests that the mating event is short and relatively free of behavior (Miller 2001c). Thus, it would seem that within Hydroporinae, most of the complexity of sexual evolution is post-inseminatory with the pre-insemination sexual activities much simpler and less important in the sexual evolution of the group.

4.4 Summary

Predaceous diving beetles show an extraordinary range of sex-specific internal and external morphological modifications, and recent studies are starting to shed light on their role in pre-, intra-, and postcopulatory phases of the mating system. It appears that within predaceous diving beetles there has been the evolution of two extreme mating system strategies, one (Cybistrinae, Dytiscinae possibly others) that focuses on pre-insemination and copulatory behaviors and morphology, including sexual antagonism, and a second (Hydroporinae) that focuses on post-insemination sperm selection and sperm competition (Fig. 4.67). Other subfamilies are seemingly somewhat intermediate between these extremes or have yet to be studied in detail.

4.5 Future Directions

Although a picture of the evolution of sexual systems is developing in dytiscids, knowledge is extremely fragmentary, often limited to a few species. Nearly every aspect of dytiscid sexual systems requires further investigations. Perhaps the best known components are morphologies of male and female external genitalia and female internal genitalia (e.g. Miller 2001c, 2003). Female internal RT structures are exceptionally diverse, and there is likely to be considerable new information forthcoming as investigators survey more completely the diversity across the group. However, internal male genitalia (testes and associated ducts and glands) are only

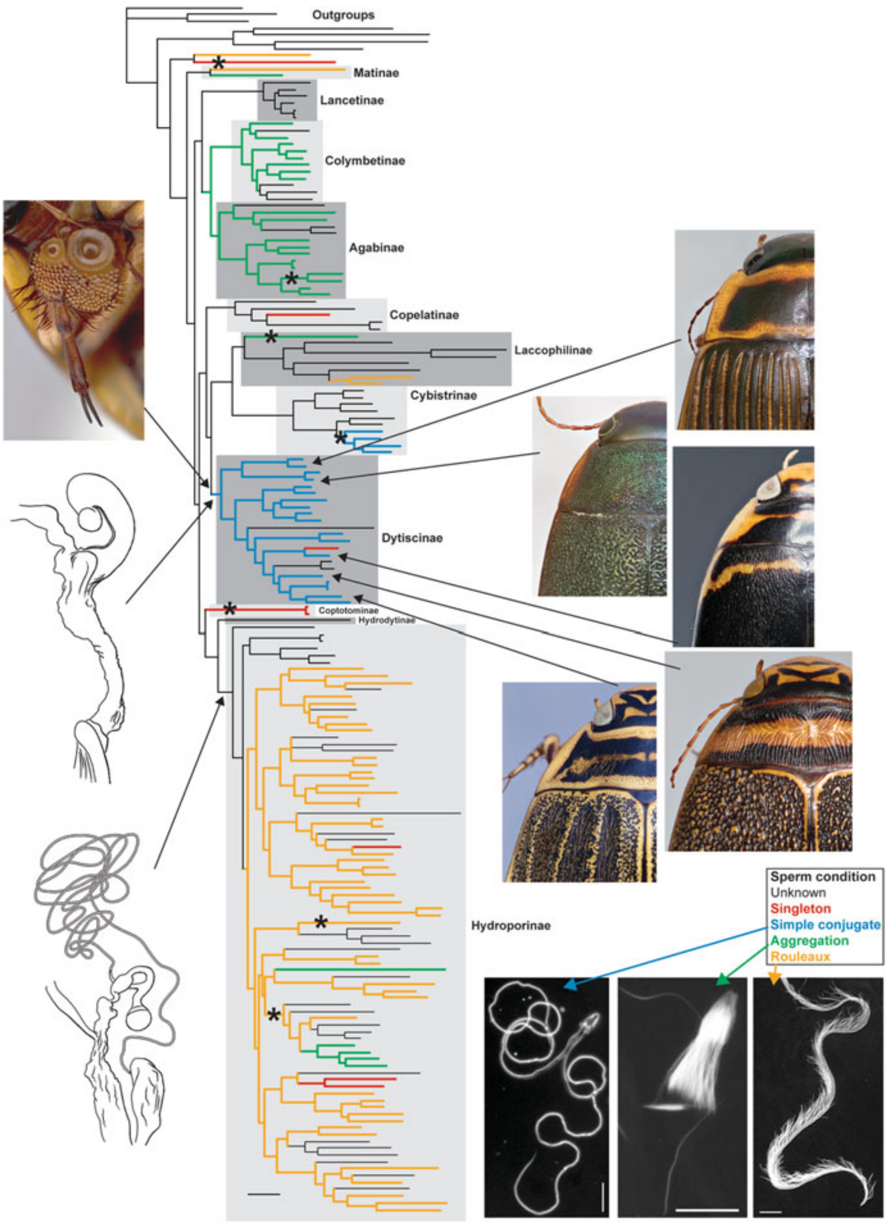


Fig. 4.67 Phylogeny of Dytiscidae from Miller and Bergsten (Chap. 3 in this book) with sexual system characters mapped. Asterisks: sperm heteromorphism

poorly known. Sperm morphology is known for numerous dytiscids, and sperm evolutionary history has been investigated (Higginson et al. 2012a, b), but functional characteristics correlating with sperm morphology as well as sperm activity, location, and storage within the complex female RT is virtually unknown, but likely incredibly interesting for study of sperm cooperation, competition, and selection in animals in general. Mating behavior data is the least known among the various aspects of dytiscid sexual systems. Because of its ephemeral nature and difficulty in acquisition, knowledge of dytiscid mating behavior has lagged along with related aspects such as sexual signaling (visual, chemical, acoustic, etc.). This knowledge will be critical for understanding the evolution of sexual antagonism, especially, but also for other dytiscid groups that could have complex variation in stereotypical behaviors. Finally, beyond the basic characterization of these various components of sexual systems, their environmental and evolutionary interactions, transitions, correlations, and contributions to diversification of dytiscids remain unexplored. Dytiscids have some of the greatest complexity of sexual system evolution in animals and are likely to become a model for the study of such systems in the future.

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Kelly B. Miller is a professor in the Department of Biology and a curator of arthropods and an assistant director at the Museum of Southwestern Biology at the University of New Mexico. He earned a Ph.D. in Insect Systematics from Cornell University and spent 4 years as a postdoc at Brigham Young University before starting at UNM in 2007. His research is focused on arthropod systematics, especially Hydradephaga, and the evolution of insect sexual strategies.

Johannes Bergsten has been a senior curator since 2009 at the Swedish Museum of Natural History, Stockholm. He holds a Ph. D. in Systematics from Umeå University and spent 3 years as a postdoc at Imperial College and the Natural History Museum, London. His research interests involves Hydradephagan systematics, phylogenetic methodology, species delimitation, the water beetle fauna of Madagascar, and sexual conflict in insect mating systems.

Chapter 5
Morphology, Anatomy, and Physiological Aspects of Dytiscids

Siegfried Kehl

> *The Dytiscus and the Hydrophilus...both frequent the water of deep ponds, ditches or pools. With their legs flattened like oars and their very smooth bodies arched on the top, and shaped underneath like the keel of a ship, they are first class swimmers and divers. It is a pleasure to the eye to follow the graceful agility of their oars as they row quietly on the surface, or float under water. Jean-Henri Fabre (2002)*

Abstract Although the morphology of dytiscids is generally distinct from other aquatic insects, there is considerable variation within this highly diverse family. In the first part of this chapter I discuss the external morphology of adult and larval Dytiscidae, as well as highlight the morphological adaptations to the aquatic environment. In the second part of this chapter, the internal anatomy and some physiological aspects, e.g., respiration and digestion, are discussed. The morphology of adult and larval Dytiscidae is very well documented, whereas pupae and the internal anatomy of all stages are neglected. Almost all taxonomic keys (e.g., Epler The water beetles of Florida - an identification manual for the families Chrysomelidae, Curculionidae, Dryopidae, Dytiscidae, Elmidae, Gyrinidae, Haliplidae, Helophoridae, Hydraenidae, Hydrochidae, Hydrophilidae, Noteridae, Psephenidae, Ptilodactylidae and Scirtidae. Florida Department of Environmental Protection, Tallahassee, FL, 2010; Arnett and Thomas, Volume 1. American beetles. Archostemata, Myxophaga, Adephaga, Polyphaga: Staphyliniformia. CRC Press, Boca Raton, FL, 2001; Larson et al., Predaceous diving beetles (Coleoptera: Dytiscidae) of the Nearctic region, with emphasis on the fauna of Canada and Alaska. NRC Research Press, Ottawa, Ontario, Canad, 2000; Nilsson and Holmen, The aquatic Adephaga (Coleoptera) of Fennoscandia and Denmark. II. Dytiscidae. Brill, Leiden, 1995; Franciscolo, Coleoptera-Haliplidae, Hygrobiidae, Gyrinidae, Dytiscidae. Fauna d'Italia, vol XIV. Edizioni Calderini, Bologna, 1979) give descriptions of the morphology of larvae and adults with detailed information

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S. Kehl (✉)
Department of Animal Ecology II, University of Bayreuth, Bayreuth, Germany

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provided in some textbooks (e.g., Balke, 7.6. Dytiscidae leach, 1915. p. 90–116. In: Beutel RG, Leschen RAB (eds) Handbook of zoology. Volume IV. Arthropoda: Insecta. Part 38. Coleoptera, beetles. Volume 1: morphology and systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim.). Walter de Gruyter, Berlin, New York, 2005; Wesenberg-Lund, Biologie der Süsswasserinsekten. Spinger, Berlin, 1943). Internal anatomy and physiological aspects are best documented in larger species (e.g., *Dytiscus marginalis*). Particularly, Korschelt (Bearbeitung einheimischer Tiere. Erste Monographie: Der Gelbrand Dytiscus marginalis L, vol 2. Engelmann, Leipzig, 1923; Bearbeitung einheimischer Tiere. Erste Monographie: Der Gelbrand Dytiscus marginalis L, vol 2. Engelmann, Leipzig, 1924) and his academic staff, as well as Blunck (Z Wiss Zool 100:459–492, 1912a, Z Wiss Zool 102:169–248, 1912b, Z wiss Zool Leipzig 111:76–151, 1914, Z Wiss Zool Leipzig 117(1):1–129, 1917, Z Wiss Zool Leipzig 121(2):172–392, 1923), provide detailed documentation of their observations, experiments, and dissections that occurred almost 100 years ago. These documents still represent some of the best work on these subjects, however, new techniques using advanced microscopic and laboratory methods could provide even great insights into the anatomy and physiology of this group of insects.

Keywords Anatomy · Digestion · Morphology · Physiology · Respiration

5.1 External Morphology

The overall shape and size of adults and larval predaceous diving beetles are highly varied, but there are several features that help to make them distinct from other aquatic beetles. Here, morphology includes aspects of the outward appearance, such as shape, structure, and color, as well as the form and structure of the internal parts (anatomy).

5.1.1 External Morphology of Adults

Adults show considerable range in size (e.g., Fig. 5.1a) and span 1–45 mm in length. The largest dytiscid adults are found in the Dytiscinae (e.g., *Dytiscus latissimus* grows to 45 mm), whereas very small adults are found in Hydroporines (Bidessini) (e.g., *Liodessus flavicollis* 1.5 mm, *Uvarus subtilis* 1.5 mm), and in several stygobiont species that are about 1.0 mm in length. The largest dytiscid in the world is *Megadytes ducalis* (Sharp 1882) and reaches 48 mm in length. The type species found in Brazil in the nineteenth century and with specimens uncovered in Paris (Hendrich et al. 2019) are the only known specimens, hence it is listed in the IUCN red List of threatened species as extinct. The oval outlined and frequently dorsoventrally flattened body of adults give them a streamlined shape, which is, in combination with the natatorial setal fringes on the hind legs of most species, a

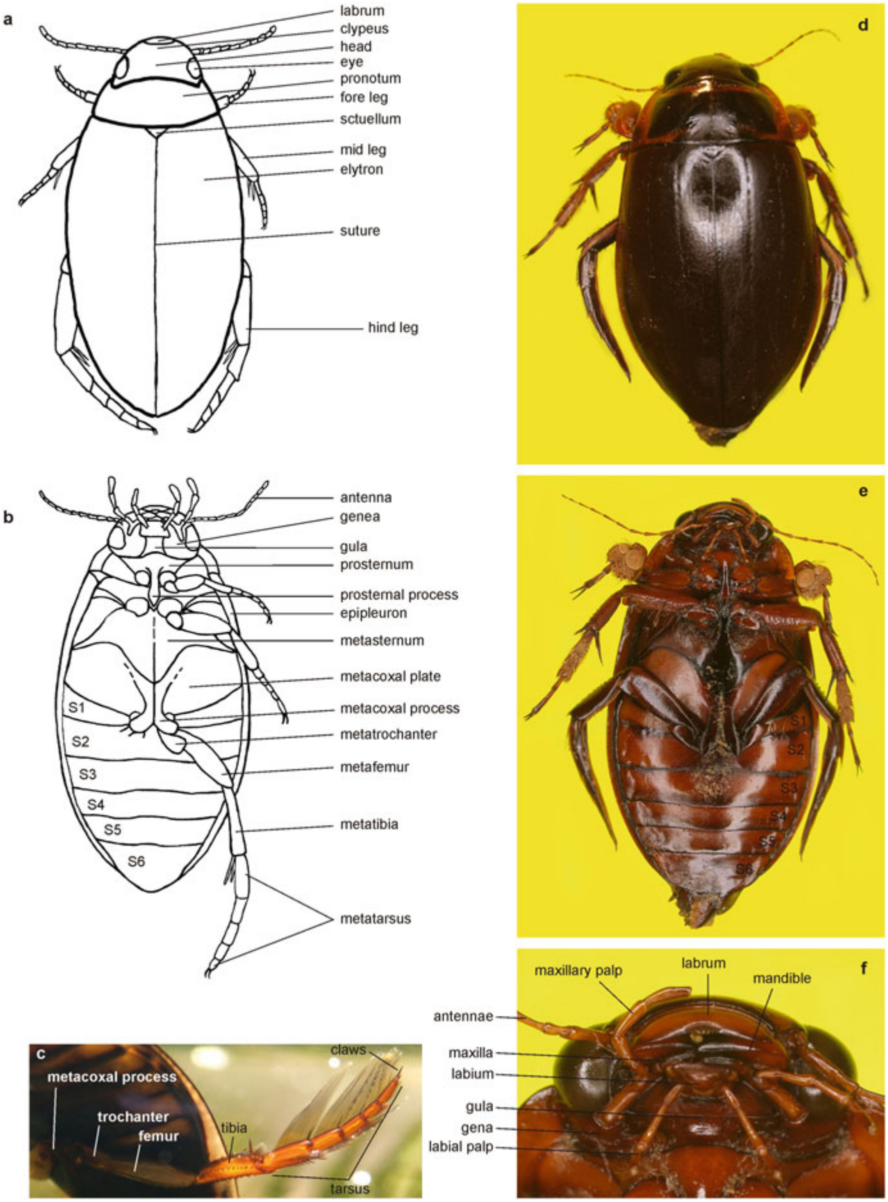

Fig. 5.1 External morphology of adult Dytiscidae. (**a**) schematic dorsal view of Dytiscidae, (**b**) schematic ventral view of Dytiscidae, (**c**) natatorial hind-leg of *Acilius canaliculatus*, (**d**) dorsal view of male *Dytiscus marginalis*, (**e**) *ventral view of male Dytiscus marginalis*, (**f**) head with view on mouthparts of *Dytiscus marginalis*. S1-S6: visible abdominal segments 1–6

Fig. 5.2 External morphology of adult Dytiscidae (**a**) Different size classes; Adult *Liodessus obscurellus* sitting below the head of an adult *Dytiscus* sp. (**b**) *Acilius canaliculatus* in copula. Male (above) attached with the suckers of the forelegs to the pronotum of female. (**c**) Cuticular surface of elytra of *Nectoporus sanmarkii* (SEM picture) with microreticulation and different sensilla. (**d**) Elytral sculpture of *Agabus melanarius* with large polygonal meshes. (**c**) Strongly elongated meshes on the elytra of *Agabus bipustulatus*

perfect adaptation to the aquatic environment (Figs. 5.1a–f, 5.2a, b). Adults are commonly dark in color (i.e., brown, black), sometimes with yellowish margins or spots, but other colors exist, including reddish, testaceous, or pale with a dark patterned dorsal side; subterranean forms are translucent, often appearing testaceous or reddish brown. Coloration patterns can be an effective antipredator defense (Larson 1996; Wohlfahrt and Vamosi 2009), especially in waters with sparse vegetation and sand, silt, or gravel bottoms (Galewski, 1971). Extant species of Laccophilinae often have a greenish tinge from internal organic pigments (i.e., a mixture of carotenoids with the blue bile pigment) (Dettner and Hopstätter 1980 and Chap. 6 in this book). Their elytra cuticle is usually smooth and glabrous or finely setose or strongly punctate (Fig. 5.2c). Many species possess large polygonal impressed meshes (reticulation) that sometimes occur in combination with a smaller, fine reticulation (microreticulation), that are often modified in various ways (Figs. 5.2c–e). Wolfe & Zimmerman (1984) provide detailed Scanning Elector Microscopic examinations of elytral surface of Hydroporinae.

The head of dytiscids is inserted up to the level of the eye into the large pronotum. Ocelli are absent, and compound eyes are generally large, but absent or reduced in stygobiont or subterranean species. Adults usually have filiform antenna with 11 cylindrical segments. The maxillary palpus have 4 segments, whereas the labial palpus have 3. The pro- and mesotarsomeres of the 5 segmented tarsus in males are usually dilated laterally and sometimes are modified into adhesive setae or discs (Figs. 5.1e and 5.3), which enable the males to adhere to the smooth dorsal surface of females (generally on the pronotum, Fig. 5.2b) prior to mating (see also Chap. 4 in this book). The adhesive strength is four times the mass of a female *Dytiscus alaskanus* (Aiken and Khan 1992). In most species, this distinctive character on the front- and mid-legs helps to determine the sexes. Hind legs are often modified for swimming, and are lined with natatorial setae and are sometimes broadened like paddles. Unlike Hydrophilidae and Haliplidae, adult predaceous diving beetles move both hind legs simultaneously for swimming. The midlegs are sometimes additionally used for swimming in small or medium-sized species, but are often restricted to maneuvering (Nachtigall 1977; Ribera and Foster 1997). The abdomen has six visible abdominal segments (ventrites), with the first true segment not visible and the last three segments, which bear the sclerotized genitalia, are invaginated (Larson et al. 2000).

5.1.2 External Morphology of Larvae

Like adults, larvae vary in size among species. Individuals range from 1 mm up to 70 mm long. Larvae are elongated, campodeiform, and more or less parallel-sided, or oblong to ovate, typically broadest near the middle (Fig. 5.3b, d, e). Larvae possess well-developed five segmented legs and a short and inconspicuous pretarsus with claws (Fig. 5.3h). The last abdominal segment has a pair of urogomphi (Fig. 5.3c). Individuals are often heavy sclerotized on the dorsal side (i.e., head, thoraic, and abdominal tergites), whereas the sclerotization on the ventral side varies among species and instars (Balke 2005; Larson et al. 2000). Larvae show color variation, with testaceous, pale yellow to dark grey, or dark brown to black or greenish found on the dorsal side. Moreover, they frequently possess a characteristic color pattern, including stripes. Besides their ferocity, this color pattern helps us to understand the use of "water tiger" as a common term for larvae. The ventral side has unsclerotized parts that are typically yellowish-white or transparent. The integument of most larvae is normally smooth with scattered setae that vary among instars and are useful for taxonomic purposes.

The shape of the head varies strongly from triangular, rectangular, or rounded, with most deviation occurring in Hydroporinae that possess a frontoclypeus with a well-developed frontal projection (nasale or "nose") (Fig. 5.3f). A Y-shaped epicranial suture divides the head dorsally into the frontoclypeal region and two lateral epcranial plates (Fig. 5.3f, Larson et al. 2000). In most species, the first of the three instars possesses egg-bursters on the frontoclypeus (Fig. 5.3f). Most larvae

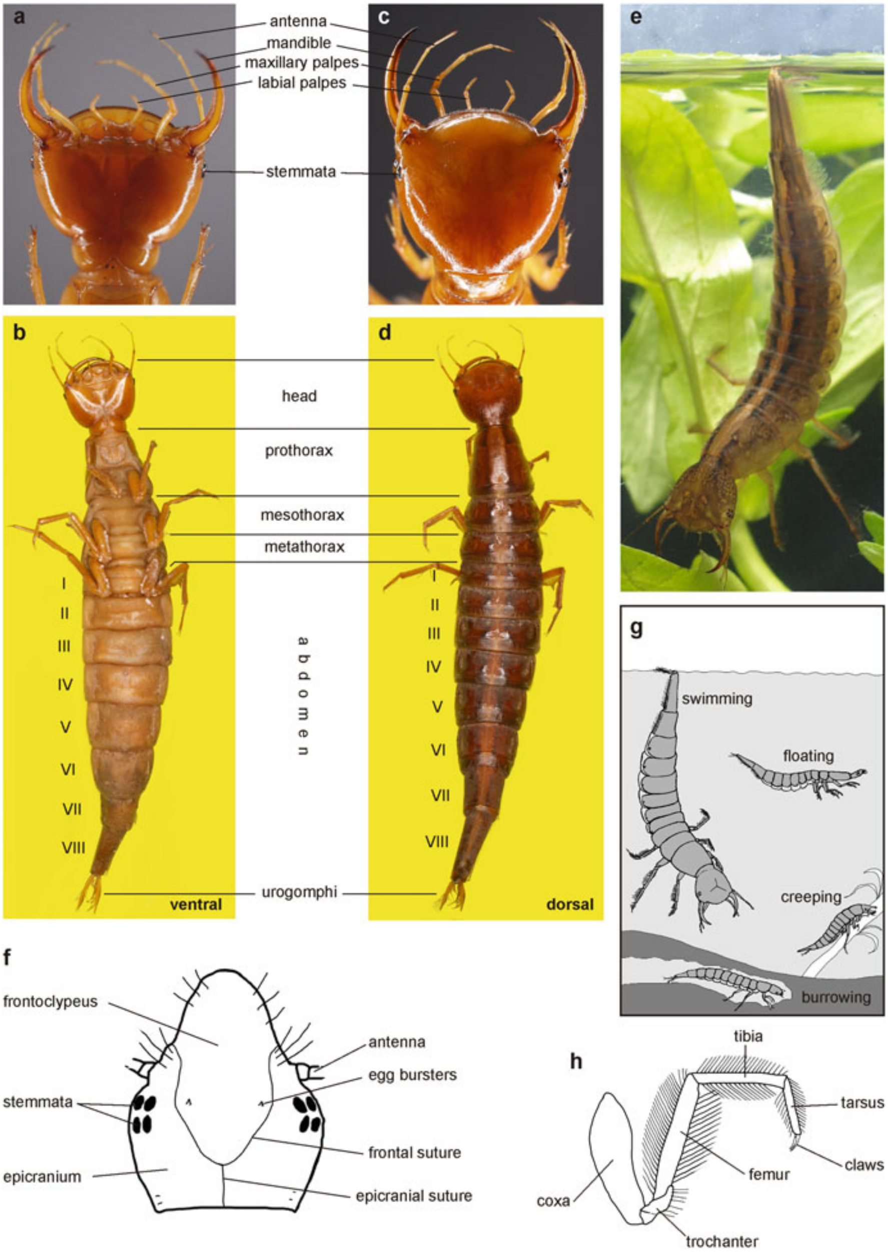

Fig. 5.3 Morphology of predaceous diving beetles larvae. (**a–d**) *Dytiscus marginalis* (**a**) ventral side of head; (**b**) ventral side of larvae (**c**) dorsal side of head (**d**) dorsal side of larvae; (**e**) *Dytiscus marginalis* in typical posture at the water surface for gas exchange. (**f**) head of a hydroporine larvae (**g**) The four main larval life styles of dytiscidae larvae. Actively swimming (e.g., *Dytiscus*), creeping (e.g., *Hyphydrus*), burrowing (e.g., *Matus*) and floating (e.g., *Graphoderus* and *Acilius*). (**h**) typical leg of Dytiscidae larvae (here foreleg of *Dytiscus*)

have a closed mouth opening and prognathous mouthparts with well-developed sucking mandibles (Fig. 5.3b). The mandibles of Hydroporinae are typically curved inwards and upwards.

The slender antenna of larvae are typically 4 segmented, but subdivided in Dytiscinae (Larson et al. 2000). The maxillary palpus typically contain 3 segments, the labial palpus typically has 2 segments, and in some dytiscids the palpes are secondary subdivided. The head has 6 stemmata on each side of the epicranium, which are reduced in stygobiont species. Unlike adults the pronotum is elongated and longer than mesothorax and metathorax (Fig. 5.3c). The abdomen has 8 visible segments, and the apex of the last segment is often elongated into a respiratory siphon.

Within the larvae, different lifestyles, varying in behavior, shape, and morphology, can be distinguished (Fig. 5.3g) (Wesenberg-Lund 1943; Galewski 1971; Wichard et al. 2002). Creeping or crawling larvae move along the bottom of the water close to the substrate or on aquatic plants. These rather bulky small to medium-sized species (many Hydroporines and also *Agabus* and *Ilybius*) have comparatively small eyes and reduced swimming hairs on the legs. Others are able to swim very fast by moving their well-developed legs alternately. These ambush or active predators have moderately large eyes, and most of the larger species (e.g., *Dytiscus*) belongs to this group. The nectonic or pelagic larvae of some species (e.g., *Acilius*, *Graphoderus*) are able to float, move, and skillfully swim in open water. Their legs and last two abdominal segments have well-developed fringes of swimming hairs. Their body is similar to a shrimp with an elongated prothorax, and are specialized for feeding on large zooplankton or small aquatic insect larvae. Larvae are able to bend down their abdomens rapidly when they are attacked or disturbed, so that they propel themselves backwards through the water with great speed. Some species have larvae that are able to burrow in the substrate, most distinctively developed in the larvae of some *Matus*, which have broad pro- and mesotibae (pseudochelate) (Alarie et al. 2001). Finally, many species found in running waters will burrow in the streambed or are found between the roots of aquatic plants.

5.1.3 External Morphology of Pupae

Morphology of predaceous diving beetle pupae is understudied, likely because of difficulties in physically locating them for many species and in difficulties with rearing them under laboratory conditions. Commonly, mature larvae leave the water and pupate in a self-constructed pupal chamber of mud or particles (Fig. 5.4). Within this cell, the pupae lie on its back in an suspended position, held up from the floor only at the anterior and posterior ends (head and urogomphi) by setae. The pupa of exarata type larvae has a 9 segmented abdomen, with the last one rather small and urogomphi present on segment 8. The color of the relatively soft cuticle is whitish with a slight addition of yellow, orange, or brown. Pupation times varied widely from a few weeks to several months, although such information is lacking for the

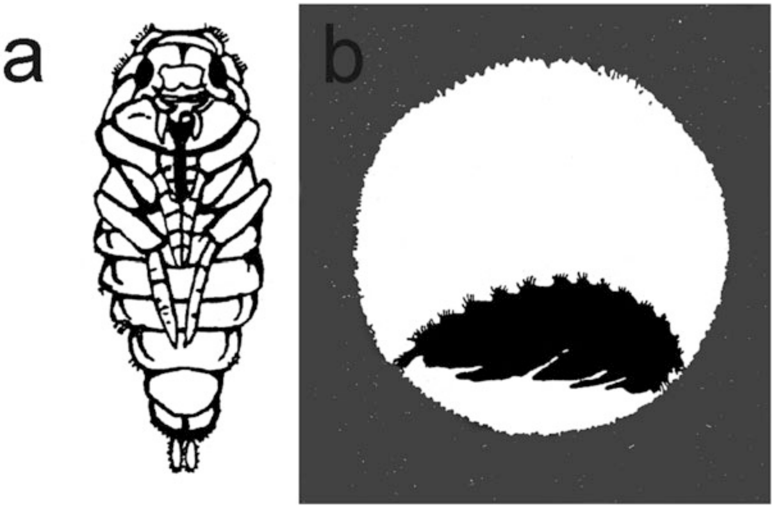

Fig. 5.4 (**a**) Habitus of pupae of *Dytiscus marginalis*. (**b**) Pupae in pupal chamber in its typical position on the back; seen from the side. Redrawn from Naumann (1955)

majority of species. Further information on pupae can be found in Formanowicz and Brodie (1981), Bertrand (1972), Ruhnau (1986), and Korschelt (1924).

5.2 Internal Anatomy and Physiology

Compared to some aspects of their natural history or ecology, the anatomy of Dytiscidae is well studied, which is due to the large size of many species, especially in the Dytiscinae. Nevertheless, knowledge of the physiology and function of their internal structures is still incomplete.

5.2.1 Digestive System and Digestion

Like other insects, the digestive system of Dytiscidae can be divided into three sections: the foregut, midgut, and hindgut. Although the foregut and hindgut are ectodermal invaginations and are lined with cuticle, the midgut is of endodermal origin. Generally, the foregut of adults consists of the oral cavity, the pharynx, esophagus, crop, and proventriculus, whereas the midgut often has diverticles, and the hindgut can be separated into the ileum and rectum with a large rectal ampulla (Fig. 5.5). The crop, as a dilatation of the hind esophagus, functions mainly as food storage area, but it also is the site of the initiation of digestion with digestion fluids from the midgut passing the proventriculus (i.e., filter function). The proventriculus

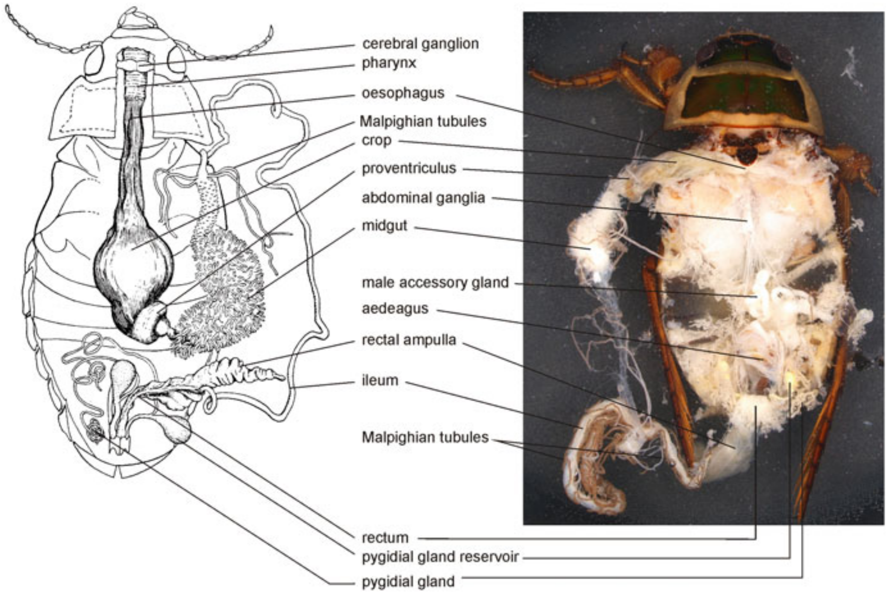

Fig. 5.5 Internal anatomy of *Dytiscus marginalis*. Left side: overview of the digestive system (modified after Rungius 1911). Muscles, fat body, and sexual organs not shown. Right side: Photograph of dissected male for comparison (S. Kehl)

of adult Dytiscidae is armored with four main lobes and four intermediate lobes, and in some species, it is tubular and strongly muscular. The variety of different proventriculi in Dytiscidae is described by Balfour-Browne (1934), who used this part of the anatomy as a taxonomic character. The proventriculus controls the passage of the food to the midgut, but it may also be used to crush larger food items. Indigestible food particles may be arrested by the proventriculus in the crop and expelled by vomiting. The passage time of all food from crop to midgut varies among species and activity. For instance, passage time was measured at approximately 14 hrs in the 4 mm sized *Scarodytes halen*sis (Kehl and Dettner 2003). In general, the foregut can be easily removed from adult beetles and the contents observed under the microscope for diet analysis (Kehl and Dettner 2003; Deding 1988, see also Chap. 8 in this book).

The midgut of predaceous diving beetles consists of a section with many crypts and is the main region of secretion of enzymes and absorption of digestive products. The hindgut is separated by the very long ileum and rectum (Fig. 5.5). Here, absorption of water, salts, and other beneficial substances takes place. Food residues can be stored in the rectal ampulla and if the beetle is disturbed, this strongly smelling material may be released as a form of protection. The rectal ampulla also functions as a hydrostatic organ (Hicks and Larson 1991): the buoyancy of the beetle can be controlled by ingesting and expelling water. Residing between the hind- and midgut is the pyloric valve, which prevents back-flow of material from the hindgut.

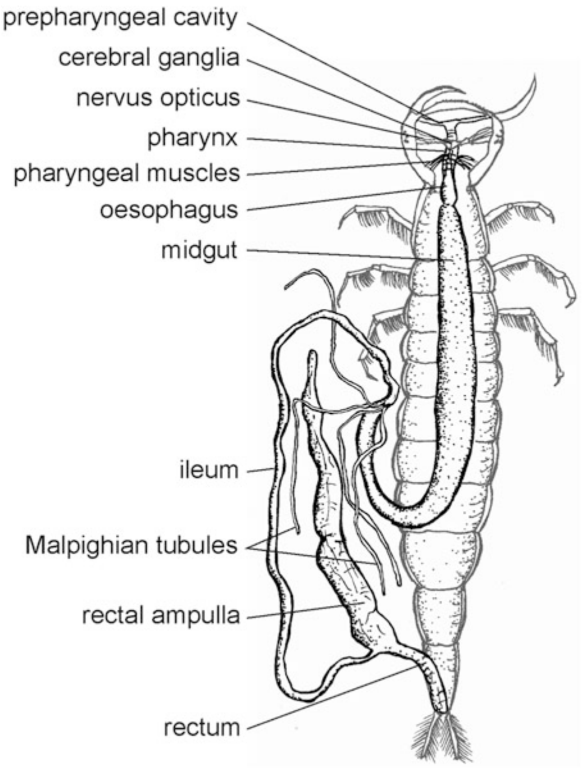

Fig. 5.6 Internal structure of *Dytiscus marginalis* larvae. Muscles, tracheal trunks, fat body removed. After Rungius (1911)

The products of excretion are emptied from the four Malpighian tubules into the alimentary canal at the passage from mid- to hindgut. In terms of osmoregulation, dytiscids cannot achieve sodium balance in fresh water without dietary sodium input, although they are able to regulate sodium loss (Frisbie and Dunson 1988). Dytiscid larvae take up ions into the hemolymph almost exclusively through the intestine. The ileum, which is lined by a highly differentiated transporting epithelium throughout its entire length, is the main site of ion absorption (Schmitz and Komnick 1976).

Larvae have extra-oral digestion and their digestive anatomy differs from that of adults. In larvae, the crop and proventriculus are missing, whereas the midgut is well developed (although a crop is present in Copelatinae, Balke 2005). The rectal ampulla is large and sometimes extends forward into the head (Fig. 5.6). The rectal ampulla in larvae also function as a hydrostatic organ, but there is some evidence that it may also play an important role during molting. For instance, expanding of the rectal ampulla may help to split the outer shell and also may help to form the new cuticle (Naumann 1955). Most remarkably are the modification of the mandibles and internal head structures (Fig. 5.7). Most larvae have a closed mouth opening and use the well-developed, falcate modified suctorial mandibles for piercing the prey, injecting digestive enzymes from the midgut, and ingesting the liquefied food by

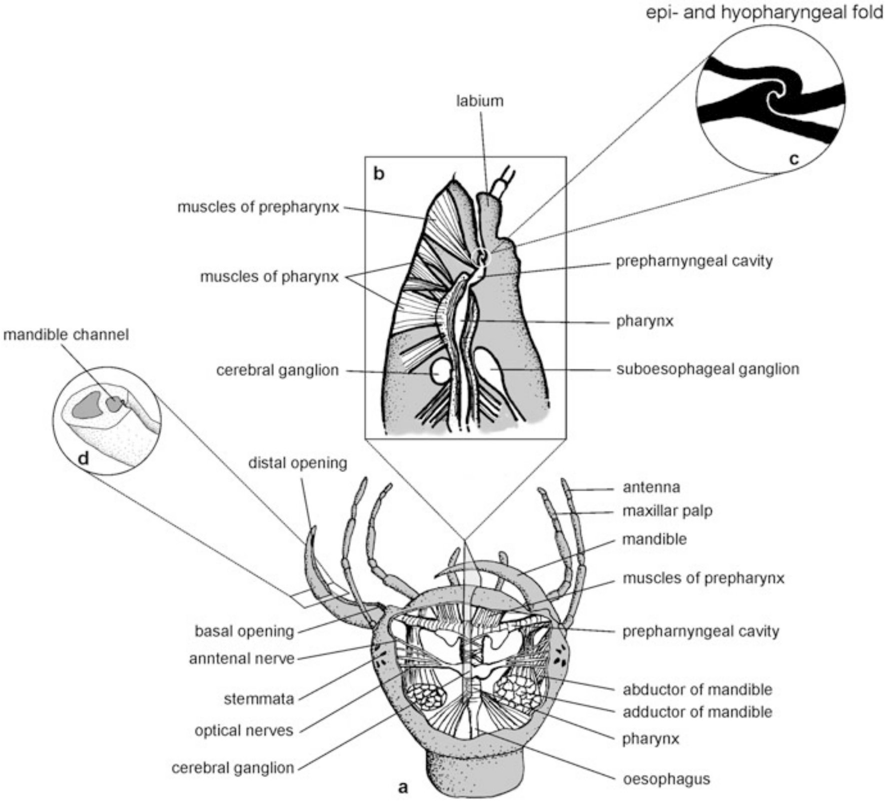

Fig. 5.7 Internal structure of head of *Dytiscus marginalis* larvae. (**a**) opened head capsule with view on the transverse prepharyngeal chamber and muscles of the sucking pump. (**b**) sagittal section of anterior head. (**c**) magnification of the epi- and hypopharyngeal tegumentary folds. (**d**) cross-section of sucking mandible. Modified and combined after Weber (1933), Naumann (1955), Korschelt (1924)

means of a cibarial-pharyngeal sucking pump. The suctorial mandibles have a narrow, almost closed canal or channel extending from near the tip to the base on the inner margin (Fig. 5.7d). In closed position, the mandibles have basal openings that are connected with a transverse prepharyngeal chamber (Fig. 5.7b), which is formed by tightly locked epi- and hypopharyngeal tegumentary folds (Fig. 5.7c) (Wesenberg-Lund 1943; De Marzo 1979; De Marzo and Nilsson 1986; Gorb and Beutel 2000; Korschelt 1924). Thus, most taxa are dependent on liquefied food ingested with their sucking mandibles, but some (e.g., *Graphoderus*, *Acilius*) can still open the mouth and can consume particulate material (Wesenberg-Lund 1943). The sucking channel is absent in Copelatini, Hydrotrupes, and Agabetes (Balke 2005). Mandible geometry has been linked to variation in hunting tactics and prey selectivity behavior (Wall et al. 2006). The mouthparts, especially mandibles and pharyngeal sucking pump are equipped with well-developed muscles (Fig. 5.7a, b).

Salivary glands are missing in adults and larvae. In the past, it was assumed that a paralyzing venom was injected into the prey via the sucking mandibles, but so far no venom glands or toxin has been found. The paralyzing effect that larval feeding appears to have on captured prey is solely caused by the midgut digestion enzymes.

5.2.2 Reproductive System

Due to the phylogenetic importance of the male and female genitalia, the sclerotized structures are well documented in many identification keys (Franciscolo 1979; Larson et al. 2000; Nilsson and Holmen 1995). The internal reproductive organs (ovaries and testis) were often of minor interest, but they can be also helpful, for example, in age structure analysis (Dettner et al. 1986). More recently, the internal genitalia have provided insight into potential post-copulation sexual conflict in many species of dytiscids, especially in the Hydroporinae (see Chap. 4 in this book).

A number of authors have described the sclerotized male genitalia (e.g., Balfour-Browne 1950; Franciscolo 1979; Balke 2005). The male reproductive organs (Fig. 5.8b) consist of paired testes with vas deferentia leading to the seminal vesicles (in Hydroporinae seminal vesicles and testes are closely connected and rolled up). Large paired accessory glands are also present, and in most species, they typically are recurved at approximately half the length in mature adults.

Female reproductive organs (Fig. 5.8a) consist of a pair of ovaries made up of numerous ovarioles, each ovary with a short oviduct, leading to a single common oviduct. A vagina, spermatheca (receptaculum seminis) and in some taxa, a bursa copulatrix is present. Bursa copulatrix and the vagina generally have separate openings. Different configurations of the female genitalia are summarized by Miller (2001) and in Chap. 4 in this book. Several glands and gland reservoirs can be present. The ovaries are, like in all Adephaga, polytrophic-meroistic (nurse cells present, grouped together and alternating with oocytes). The structure of ovipositors can be found elsewhere (Burmeister 1976). Note that the appearance of unsclerotized male and female reproductive organs can vary depending on the age of the beetles. Glands can be of very different sizes (male accessory gland sometimes extending up in the prothorax), and also the ovaries exhibit great variation in size and shape according to the age class (Dettner et al. 1986; Classen and Dettner 1983). The paired or grouped spermatozoa of Dytiscidae have been thoroughly studied by many authors (Dallai and Afzelius 1985, 1987; Werner 1982; Jamieson et al. 1999), and the sperm evolution in diving beetles is discussed by Higginson et al. (2012a, b) as well as in Chap. 4 in this book. Oogenesis in Dytiscidae was studied by Urbani and Russo-Caia (1969, 1972).

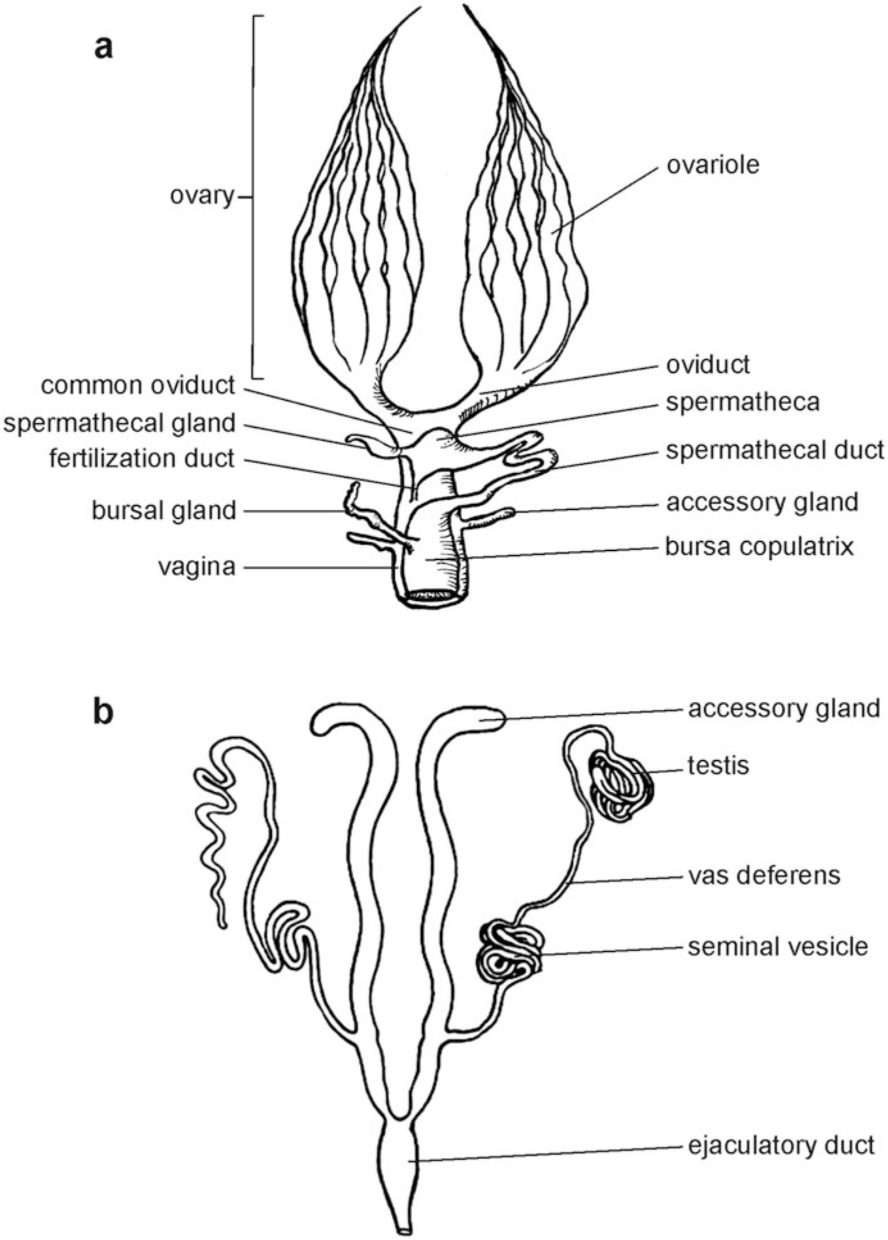

Fig. 5.8 Reproductive systems of Dytiscidae (schematic). Note that, depending on species, glands (gland reservoirs) and ducts could be reduced, shortened or enlarged. Also different parts could be enlarged or reduced depending on age class (see Dettner et al. 1986 and Chap. 4 in this book). (**a**) female reproductive organs; (**b**) male reproductive organs

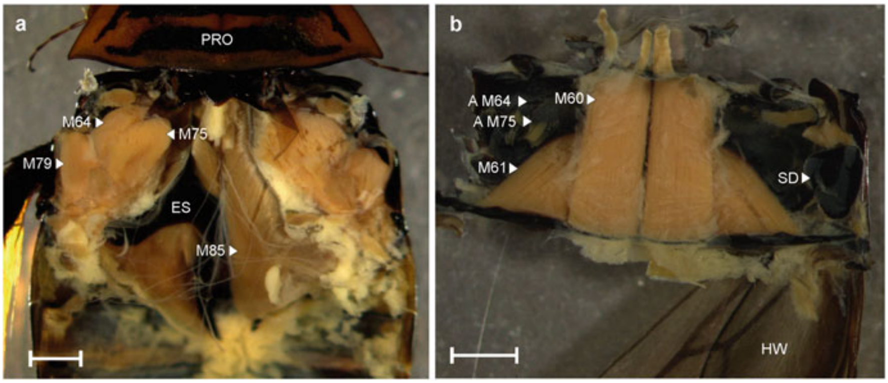

Fig. 5.9 Metathoracic muscles of *Acilius sulcatus*. (**a**) Dorsal view of the opened metathorax, with foregut and left side of M85 removed. (**b**) Dissected metatergum (ventral view) with the dorsal muscles M60 and M61. PRO: pronotum, ES: endosternite, HW: hind wing, SD: subalar plate, M60: *musculus metanoti primus*, M61: *musculus metanoti secundus*, M64: *musculus dorsoventralis primus*, M75: *musculus noto coxalis anterior*, M79: *musculus coxa-subalaris,* M85: *musculus furca-trochanteralis,* A M64: insertion of M64, A M75: insertion of M75. Nomenclature of muscles according to Larsen (1966). Scale bars: 1 mm. (from Kehl and Dettner 2007)

5.2.3 Muscles of Thorax

In most Dytiscidae locomotory musculature (flight and leg musculature) are well developed. A good overview of the locomotory muscles of Dytiscidae is given by Larsen (1966) and Balfour-Browne (1967), with a more physiological approach provided by Kallapur (1970).

Dytiscids adults in general are not considered to be strong flyers, and in several species or specimens the flight muscles are degenerated or reduced, but it remain unclear if the reduction is age dependant ("oogenesis flight syndrome") or if these individuals have lost the ability to fly. Flight capacity and flight muscles analysis can be found in the comprehensive works of Jackson (1952, 1956a, b, 1973). The indirect flight musculature of Dytiscidae (Fig. 5.9) are attached to the thorax and not to the wing base. The longitudinal muscles (e.g., M60) are the depressors, forcing the wings down by arching up the tergite. The dorsoventral muscles (e.g., M64 and M75) are the antagonistic muscles and raise the wings (Jackson 1956a). Several other muscles help to position the wings. For details of the muscles of *Dytiscus marginalis* adults, see Bauer (1910) and Korschelt (1923). More on dispersal and movement via flight in dytiscids can be found in Chap. 11 in this book.

5.2.4 Nervous System

Dytiscidae are similar to other insects in having a relatively simple central nervous system with a dorsal brain linked to a ventral nerve cord. The brain is a complex of

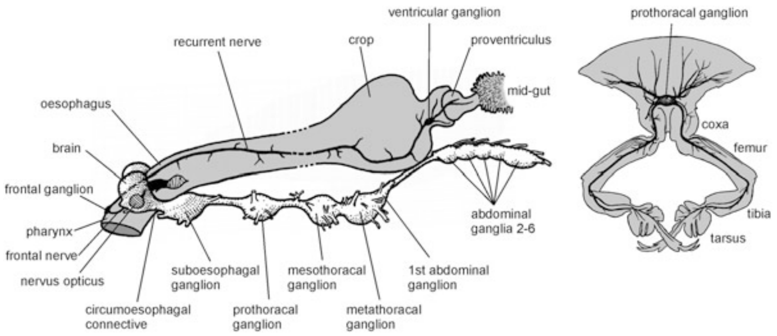

Fig. 5.10 Nervous system and eyes of Dytiscidae. (**a**) Central nervous system (white dotted) and stomatogastric nervous system (black) of adult *Dytiscus marginalis*. (**b**) cross-section prothorax in the region of the prothoracal ganglion of adult *Dytiscus marginalis*. Only central nervous system shown. (**c**) Schematic section through the compound eye of *Dytiscus marginalis*. (**d**) Section through the stemmata of *Dytiscus marginalis*. Figures **a** and **b** modified after Holste (1910) and Wesenberg-Lund (1943). **c** combined from Günther (1912) and Horridge et al. (1970), **d** modified from Günther (1912)

three pairs of ganglia located dorsally within the head capsule above the esophagus. The first pair of the fused ganglia of the brain (protocerebrum) is associated with vision and innervate the compound eyes in adults or the six stemmata on each side in larvae. The second pair (deutocerebrum) processes sensory information from the antennae, and the third pair (tritocerebrum) innervate the labrum and link the brain with the subesophageal ganglion (and the rest of the ventral nerve cord) and with the stomodaeal nervous system via the frontal nerve and the frontal ganglion (Fig. 5.10a). The subesophageal ganglion, located below the brain and esophagus, innervates the mouthparts and is linked to the thoracal ganglia. The three thoracal ganglia are connected with short connections followed by the nearly fused abdominal ganglia. The main function of the thoracal ganglia is to control locomotion by innervating the legs and wings (Fig. 5.10b). The prothoracal ganglia innervates the forelegs and the prothoracal glands, the mesothoracal ganglia the midlegs and the elytra, and the metathoracal ganglia the hind legs and the wings. The first abdominal ganglia is more or less fused with the metathoracal ganglia and also the remaining abdominal ganglia fused into a short column. Holste (1910) supposed that the first abdominal ganglion is a fused ganglion from abdominal segment 1 and 2, and that the last abdominal ganglion (abdominal ganglion 6 in Fig. 5.10) is a fused ganglion of the seventh and last abdominal segment.

The stomodaeal nervous system innervates and controls the internal organs. The paired frontal nerves connect the unpaired frontal ganglion with the tritocerebrum. The frontal ganglion innervates the pharynx and is connected with a long single nerve (recurrent nerve), which runs under the brain on the esophagus to the ventricular ganglion at the end of the crop.

Dytiscids possess a variety of sensilla that allow them to effectively interact with the environment. In addition to several different types of mechanoreceptors (Wolfe and Zimmerman 1984; Hochreuther 1912; Lehr 1914) and chemical receptors (Jensen and Zacharuk 1991, 1992; Baker 2001), Dytiscidae have well-developed visual systems. The compound eyes of adults (Horridge et al. 1970; Meyer-Rochow 1973) (Fig. 5.1f) and the stemmata of larvae (Sbita et al. 2007; Maksimovic et al. 2011; Mandapaka et al. 2006; Buschbeck et al. 2007; Schöne 1951) (Fig. 5.2a) are well studied. The compound eyes (Fig. 5.10c), made up of 9000 single ommatidia in *Dytiscus marginalis* (Günther 1912), are adapted to work in aquatic and terrestrial environments. Unlike air, the refractive index of water is more similar to that of the cornea, so the simple curved corneal lens that is present in many terrestrial insects is unable to focus an image underwater (Lancaster and Downes 2013). The cornea of dytiscids is flat on the external surface and composed of layers of unequal refractive index, with horizontal layers in the distal part and concentrically formed layers around a region of highest refractive index on the axis (Meyer-Rochow 1973). The retina of *Dytiscus* has a tiered structure, with rhabdomeres at three different levels. Crystalline threads stretch from the crystalline cones to the distal layer of rhabdomeres. Between the distal rhabdomere and the proximal rhabdomeres, layers is a wide clear zone (Fig. 5.10c), where light reaching the proximal rhabdomeres of a single ommatidium will have been refracted from several facets of different ommatidia. The proximal rhabdomeres layers therefore seem to be concerned with light perception, while the distal rhabdomere for the perception of form or movement (Horridge et al. 1970). The compound eyes could be light- and dark-adapted, showing a thousand times stronger sensitivity during the night. But the diurnal variations in visual function seem to be independent of retinal pigment migration (Jahn and Wulff 1941, 1943). Several Dytiscidae are known to be sensitive to polarized light, which helps them to find water bodies during flight (Schwind 1995).

Larvae of most dytiscid species have six stemmata located on each side of their head posterior to the origin of each antenna (Fig. 5.1a, c, f). Some species have an additional pair of eyespots. The spatial arrangement of the stemmata on the head capsule varies greatly in dytiscid larvae, which may result from the different hunting strategies. Usually, the stemmata are covered by a corneal (cuticular) lens, and a crystalline body focuses the light on the retina. In *Dytiscus marginalis*, Günther (1912) described two rhabdomere layers (Fig. 5.10d), and in *Thermonectus,* at least two retinas are present (Mandapaka et al. 2006). The proximal retina consists of unusual horizontal rows of long rhabdoms parallel to the light path.

In most insects, stemmata are generally described as simple eyes, with perception involving either bright and dark vision or a rough mosaic vision. This is not so in dytiscid larvae. For example, *Thermonectus marmoratus* is a highly efficient visually-guided predator with highly specialized eye structures and morphologically and functionally different stemmata. The two forward-looking dorsal pairs are tubular and may be primarily used for prey capture, but they have an extremely narrow visual field. The visual field is enlarged through a scanning behavior of the larvae by performing a dorsoventral head and thorax movement, prior to prey capture (Buschbeck et al. 2007). The distal retina is green-sensitive, whereas the proximal retina is UV sensitive (Maksimovic 2011). Furthermore, the proximal retina can support polarization vision, which may allow them to better detect prey.

Two sharp images are focused on the distal and proximal retina by a real bifocal lens, and there is evidence that larvae are able to determine prey distance.

Besides vision, other senses seem to be well developed in the Dytiscidae. These include hearing via chordotonal organs of the antennae (Lehr 1914) or by abdominal mechanoreceptors (Hughes 1952). The olfactory receptors of the antenna appear to work both in water and in the air (Behrend 1971). To detect the presence of predators, adult beetles seem to rely on visual stimuli when visibility is good, while in darkness, they seem to use chemical stimuli (Åbjörnsson et al. 1997). There is also some evidence for the chemical reception of pheromones in adults (Herbst et al. 2011).

5.2.5 Respiration and Tracheal System

The basic physical (Alt 1912; Wesenberg-Lund 1943) and physiological details (Ege 1915; Wolvekamp 1955; Gilbert 1986), of respiration in Dytiscidae are well established (except for stygobiont species), which makes it all the more remarkable that several new findings relating to respiration in Dytiscidae have recently been published (e.g., Calosi et al. 2012; Kehl and Dettner 2009; Madsen 2012).

All aquatic insects, including Dytiscidae, need oxygen for respiration, which can be obtained from the atmosphere (aeropneustic) or directly from the water (dissolved oxygen, hydropneustic). In general, adult dytiscids have an air store under the elytra in the subelytral cavity, where the 8 pairs of abdominal spiracles open (Heberdey 1938; Alt 1912). This air store must be renewed regularly at the water surface, and the beetles accomplish this by breaking the water surface with the tip of the abdomen (Fig. 5.11a). A hydrofuge portion of the apical abdominal tergites guarantees the gas exchange will occur with the subelytral cavity. There are some hints for a controlled air circulation and specialized spiracles for exhalation and inhalation during gas exchange at the water surface (Gilbert 1986). The duration of diving varies depending on species, temperature, and activity (Calosi et al. 2007), but can be prolonged by a small air bubble (Fig 5.11b, c) pressed out from the subelytral cavity and held by the hydrofuge hairs at the tip of the abdomen (Larson et al. 2000). This air bubble acts as a physical gill (compressible gas gill, Rahn and Paganelli 1968; Ege 1915), where dissolved oxygen from the water diffuses in, but at the same time nitrogen diffuses slowly out of the bubble and the size of the bubble shrinks over time. With the decreasing bubble surface, the rate of gas exchange decreases and the beetles must surface again. During inactivity, the physical gill allows the beetle to dive for a long period of time and may be used for survival under the ice during winter. The carbon dioxide from the beetles' metabolism diffuses out in the water due to the high solubility of CO_2. Dive duration varies from a few minutes up to 24 h (Madsen 1967; Calosi et al. 2007; Kehl and Dettner 2009). However, some species remain submerged for very long periods. For example, *Hydroglyphus hamulatus* remained submerged for 10 weeks (Meuche 1937), whereas *Deronectes aubei* can stay submerged for an unlimited time (at 13 °C water temperature) and have

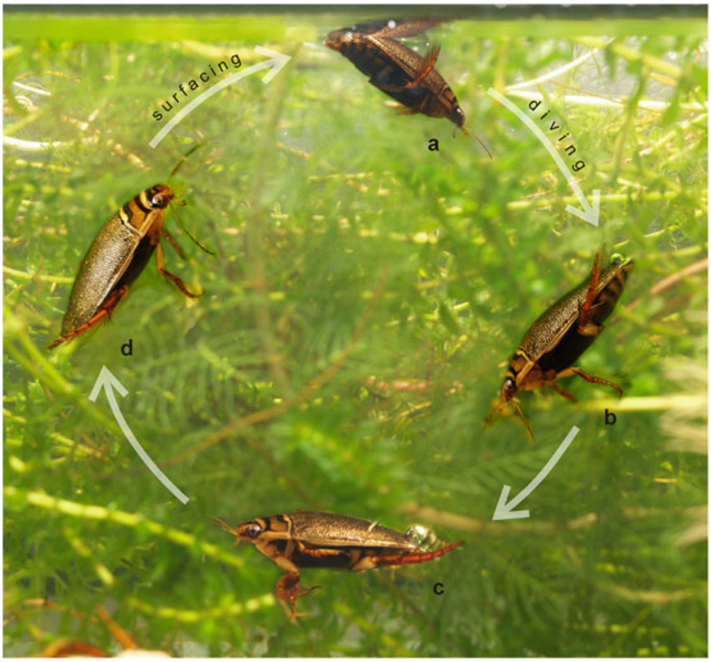

Fig. 5.11 Respiration of adult *Acilius canaliculatus*. (**a**) gas exchange on the water surface. (**b** and **c**) diving, note the air bubble on the tip of the abdomen, acting as physical gill. (**d**) surfacing

specialized setae on the elytra, pronotum, and ventral side that act as tracheal gills (Kehl and Dettner 2009, but see also Madsen 2012) (Fig. 5.12a). These tracheated setae (Figs. 5.13 and 5.2c) also occur in many other small Hydroporinae, enabling them to stay submerged, but they also can use the conventional mode of respiration by surfacing and the subelytral air store. On the basis of the diameters and branching of the intraelytral tracheae (Fig. 5.13a, b) Smrž (1981), it has been assumed that cuticular gas exchange exists via the elytra in stygobiont species. However, the gas exchange of stygobiont species remains unclear, while *Siettitia*, *Phreatodessus*, and *Kuschelydrus* possess these setae for cuticular gas exchange (Kehl and Dettner 2009 and personal observations). It is noteworthy that *Paroster* and other Australian stygobiont species do not have this type of cuticular surface (Bradford 2010, personal observations).

Adults possess two pairs of thoracic spiracles (mesothoracic spiracle situated between the pro- and mesothorax), 8 pairs of abdominal spiracles (Fig. 5.12a, b, c, d) and large thoracic air-sacs (Fig. 5.12e) that can be filled with air in preparation for flight, to reduce weight, and to supply the large muscles with sufficient oxygen during flight. Some time is often required before adults can return to water after flight, as they must deflate these air-sacs before engaging in aquatic respiration. Detailed information of spiracles and the tracheal system of adults can be found in Alt (1912) and Gilbert (1986).

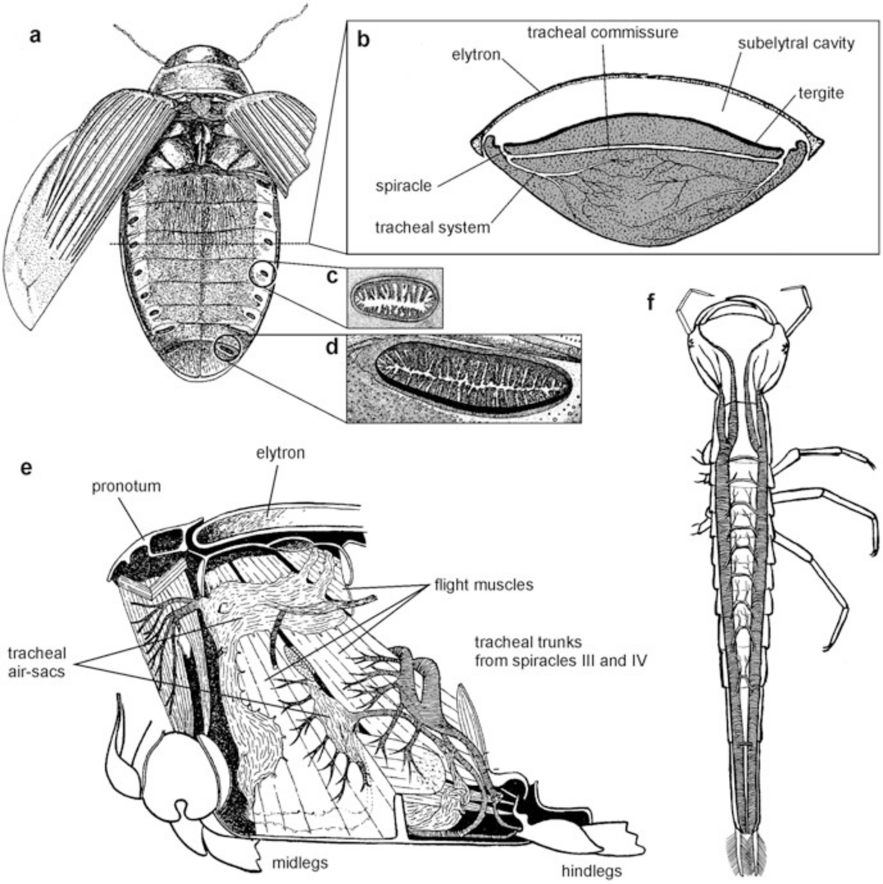

Fig. 5.12 Tracheal system and respiration in Dytiscidae. (**a**) Abdominal spiracles of female *Dytiscus marginalis*. Dorsal view, with elytra folded sideward and hindwings removed. (**b**) Cross-section of *Dytiscus marginalis* in the region of the third spiracle showing the subelytral cavity and tracheal system (hind wings removed). (**c**) abdominal spiracle. (**d**) last abdominal spiracle. (**e**) longitudinal cut of thorax of *Dytiscus marginalis* showing the thoracal air-sacs supplying the strong musculature with enough oxygen. (**f**) tracheal system of *Dytiscus* larvae (first instar). The two strong longitudinal trunks are the only air store of larvae. All figures modified after Alt (1912), except **b** after Naumann (1955)

The tracheal system in larvae (Fig. 5.12f) mainly consists of two strong, longitudinal tracheae, starting at the last abdominal spiracles and proceeding up to the prothorax. In the prothorax, the main tracheae split in an upper and lower tracheae that lead into the head. More detailed information of tracheation of larvae can be found in Alt (1912). The two longitudinal trunks are connected by dorsal commissures in each segment. The two main tracheal trunks can often be seen in live specimens through the cuticle. The taenidium (chitinous fiber forming the spiral thread) is well developed in larvae and often has a dark appearance, so that the

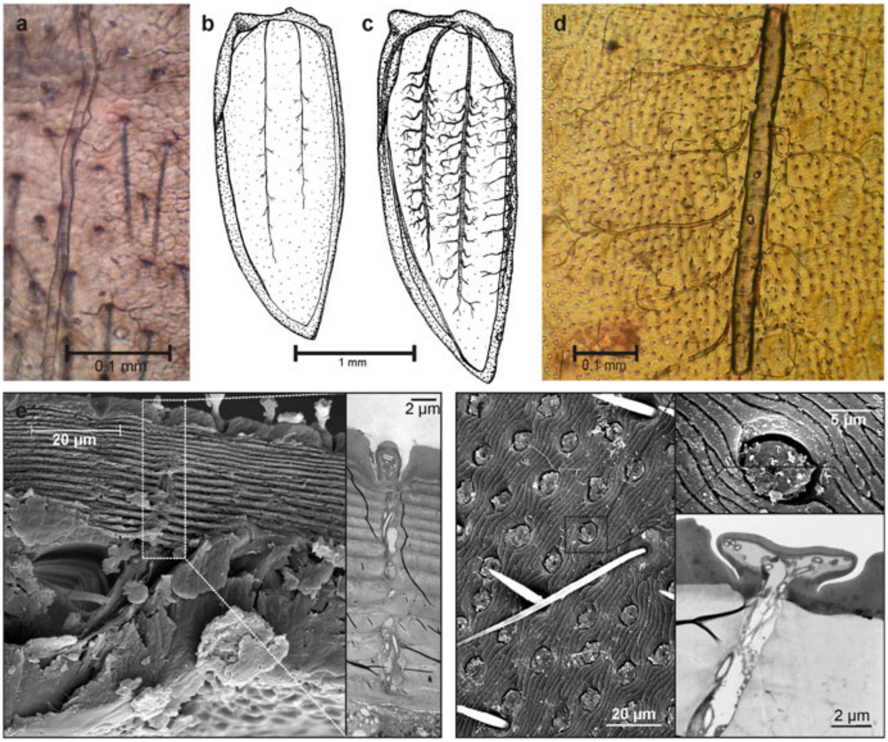

Fig. 5.13 Cuticular gas exchange in adult Dytiscidae with the help of tracheated setae. (**a** and **b**) elytra of *Hydroporus palustris* (no tracheated setae present) with small intraelytral tracheae with only few branchings. (**c** and **d**) elytra of *Deronectes aubei* (with tracheated setae) with a strong, richly branched, longitudinal intraelytral trachea. The diameter of the intraelytral tracheae can give evidence for the capability of cuticular gas exchange via the elytra. (**e**) cross-section of elytra of *Deronectes aubei* with longitudinal trachea cut (left side), from which smaller tracheae and tracheoles go upwards through the "hair channel" into the base of the setae. (**f**) Tracheated setae in *Stictotarsus duodecimpustulatus*. The tiny modified setae (sensilla placoidea type 1 according to Wolfe and Zimmerman 1984) are richly tracheated

tracheal system is not as shiny and silvery as in other insects. The number of functional spiracles in larvae depends on the larval instar. In the first instars, only the last pair of spiracles are present (segment VIII), the lateral abdominal and thoracic spiracles absent, but internal structures are already present. The spiracles on the thorax of the second instars are already visible but closed and not functional (except the last pair). In the last instars, two thoracic and eight abdominal spiracles are present, but remain generally closed by a mechanism except in the last pair (Larson et al. 2000; Blunck 1923; Lawrence 1991). Last instars of *Heterosternuta* and *Neoporus* do not have functional lateral spiracles (Larson et al. 2000; Balke 2005).

Cuticular gas exchange may occur in all larvae, but is only sufficient in smaller species or early instars. Larvae of larger species obtain oxygen at the water surface

Fig. 5.14 Larva of *Coptotomus loticus* with unusual elongated lateral gills on the first six abdominal segments. Photo courtesy of Donald Chandler (2013)

by functional posterior spiracles and store this air in the strong longitudinal tracheal trunks. Unique in Dytiscidae are the larvae of *Coptotomus*, which have elongated lateral gills, a pair on each of the first six abdominal segments (Fig. 5.14). These larvae are able to remain continuously beneath the surface and may go deeper than other dytiscid larvae (Usinger 1956). In *Celina* the apical elytral spines and the spinose ends of the abdomen in adults are supposed to gain oxygen by piercing plants' roots (e.g., *Typha*). It is also possible that the peculiar posteriorly extended lateral tracheal trunks of the abdominal apex in larvae may be used to obtain intracellular air from plants (Hilsenhoff 1993; Spangler 1973).

5.3 Future Directions

There are a number of questions remaining to be answered for dytiscids, especially those that link ecology and physiology. For instance, in relation to their distribution and habitat selection, Why do certain species only occur in certain waters or have a restricted distribution? A step in that direction is given by Calosi et al. (2010), who suggest that the latitudinal range extent and position of *Deronectes* species could be best explained by their absolute thermal tolerance. Specifically, species' northern and southern range limits are related to their tolerance to low and high temperatures, respectively. Further work in this direction should include examinations of larvae, as they are surely more sensitive to environmental conditions than the more mobile adults. Moreover, understanding the larval ecophysiology is a key function to understand habitat requirements. The ecology of dytiscid eggs is also an interesting area in need of more data, considering that for most species egg deposition sites are, and female egg-laying behavior are unknown.

Most morphological and physiological studies are many decades old, and it would be interesting to use new scientific tools (e.g., molecular, electrophysiological, and optical) established and refined over the last few years to understand the fascinating world of predaceous diving beetles. Understanding the microorganism relationships (e.g., gut bacteria and intracellular bacteria, e.g., *Rickettsia* and *Wollbachia*) may give new insights into the biology of the beetles, as well as their physiological functions (see also Chpater 6 in this book). In terms of respiration, there are many unresolved questions, including, How do the tracheated setae function in detail? What is the evolution of the tracheated setae?, and How do subterranean species respire? We are just beginning to understand the visual system in some species (e.g., *Thermonectus*), but the knowledge of other species is poor. The functions and mechanisms of other sensilla and setae on antenna, mouthparts, and body surface are almost entirely unknown. More work on internal structures (e.g., reproductive organs, nervous system including sense organs, gut system, tracheal system) and the comparison in different species may also help support systematics and taxonomic investigations.

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Siegfried Kehl studied Biology at the University of Bayreuth and has been research associate and assistant professor at the department of Animal Ecology II at the University of Bayreuth from 2001 to 2013. His civilian service at the WWF institute for floodplains ecology brought him to the aquatic entomology with particular interests in caddisflies, but his supervisor, Konrad Dettner, kindly convinced him to work on aquatic beetles. He holds a Ph.D. in natural sciences from the University of Bayreuth. His research interests are dytiscid ecology and ecophysiology, including respiration, diet analysis, microorganism interactions, flight capacity, and morphological adaptations to the aquatic environment.

Chapter 6
Chemical Ecology and Biochemistry of Dytiscidae

Konrad Dettner

> *“Wenn man einen solchen Kefer [Cybister lateralimarginalis] fängt, so lässt er insgemein zwischen dem Hals-Schild eine blaulichte Materie hervor fliessen, welche einen widerwärtigen Geruch von sich giebt und vielleicht Ursache ist, dass diese Kefer alle Zeit einen eckelhaften Gestank haben.” [If such a beetle Cybister lateralimarginalis is caught, between the pronotum a bluish fluid appears which is characterized by a disagreeable odor that is probably responsible for the nauseous stench of the whole beetle.], Rösel von Rosenhof (1705–1759)*
>
> *“I must tell you what happened . . . in my early entomological days. Under a piece of bark I found two carabi (I forget which) and caught one in each hand, when . . . I saw a sacred Panagæus crux major. I could not bear to give up either of my Carabi, and to lose Panagæus was out of the question, so that in despair I gently sized one of the carabi between my teeth, when to my unspeakable disgust and pain the little inconsiderate beast squirted his acid down my throat and I lost both Carabi and Panagus!”, Charles Darwin (1809–1882)*

Abstract The chapter deals with chemical mechanisms that help to control intra- und inter-specific interactions with respect to predaceous diving beetles. Apart from chemical receptors and senses within Dytiscidae there are described intraspecific (pheromones) and especially interspecific interactions with respect to this water beetle family. The last group of behavioral modifying compounds includes kairomones and allomones. Allomone constituents from pygidial glands, prothoracic defensive glands, and pupal glands are compiled for a large group of predaceous diving beetles. With respect to the natural compounds, their chemistry, distribution within Hydradephaga, biological activities, and especially their significance for dytiscids are discussed. In addition, further secondary compounds from these beetles

K. Dettner (✉)
Chair of Evolutionary Ecology, University of Bayreuth, Bayreuth, Germany
e-mail: k.dettner@uni-bayreuth.de

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are presented, including epicuticular lipids or pigments that may be responsible for the coloration of the adult beetles and their larvae. Finally, the microorganisms including fungi and their secondary metabolites that are associated with predaceous diving beetles are presented. The described microorganisms range from culturable to non-culturable taxa. The role of hemolymph with respect to internal defense, hemostasis, and regeneration is briefly reviewed.

Keywords Dytiscidae · Chemical ecology · Allomones · Glands · Secondary compounds · Hemolymph

6.1 Chemical Ecology of Freshwater Organisms

Since 1970, after the publication of the book entitled "Chemical Ecology," edited by E. Sondheimer & J. B. Simeone, the field of chemical ecology has been recognized as a distinct interdisciplinary research area. Chemical signals are perhaps the oldest form of communication among organisms, and this discipline investigates how naturally occurring chemicals mediate ecological interactions. In most cases, chemoecological studies focus on ecological mini-systems that include few species or individuals, whereas complex biocenosis are not analyzed. Moreover, chemical ecology often starts with an observation—e.g., chemical defense of a bombardier beetle or attraction of one sex of a moth species to the other sex through sexual pheromones. Chemical ecology is concerned with the identification and synthesis of those substances (semiochemicals = ectohormones) that convey information and interact between different individuals of organisms (allelochemicals as allomones, kairomones, or pheromones). Researchers in chemical ecology also elucidate exocrine gland systems, receptors, and the transduction systems that are recognize and pass on these semiochemicals. In addition, the developmental, behavioral, and ecological consequences of these chemical signals also are investigated. All of these areas rely upon bioassays in the laboratory and in the field. The results of chemoecological studies may be important in plant protection (e.g., Krauss and Nies 2014), in the development of highly selective techniques for pest control, and even in integrated plant protection (e.g., Tabata 2018; Dettner 2019a). Dependent on the research areas of the scientists working on chemical ecology, classification and investigation of these phenomena vary considerably. Natural product chemists and biochemists are interested in biosynthesis and chemical structures of the secondary compounds involved. In contrast, ecologists may favor research that focuses on the interactions among trophic levels. As chemical ecology studies the interactions among different individuals of the same or different species other scientists potentially are interested in knowing the senders and receivers of chemical signals, and in knowing if an ectohormone is of advantage or disadvantage for these individuals. Finally, entomologists interested in chemical ecology may focus on exocrine glands or chemical signals on the body surface or want to learn if the compounds are biosynthesized by the insects, sequestered from plants, or produced by endosymbiontic microorganisms.

As compared with chemoecological studies in terrestrial ecosystems, which has been intensively studied in the 1970s, chemical ecology of aquatic systems was initially neglected, but now there are considerable data available concerning the chemoecology of aquatic systems (e.g., Brönmark and Hansson 2012; Burks and Lodge 2002; Ferrari et al. 2010; Gross 2011; Dettner 2019b). However, marine systems were often studied with the priority in identifying new biologically active natural products. In spite of the fact that freshwater chemical ecology lags behind terrestrial and marine chemical ecology, a constant increase of publications in this interesting field is recognizable (Burks and Lodge 2002). It was found that among allelochemicals kairomones mediate the majority of species interactions in freshwater systems. Fish and predaceous insects act largely as senders, zooplankton, on the contrary, comprise the most studied receivers. Other organisms such as predaceous insects may be both receivers of cues from larger predators and senders of their own cues to lower trophic levels, such as zooplankton (Burks and Lodge 2002). In freshwater systems, chemoecological investigations have especially targeted the study of predator–prey, plant–plant, and plant–herbivore interactions (including microorganisms) and the role of allelochemicals (Ferrari et al. 2010; Gross 2011).

The chapters in recent compilations on chemical ecology in aquatic systems (e.g., Brönmark and Hansson 2012) are of different significance for those who are interested in freshwater systems. Whereas information conveyed by chemical cues (v. Elert 2012) are highly informative, other chapters such as chemical defense (Kicklighter 2012) are only partially valuable, because marine systems are overrepresented and data from freshwater systems are nearly completely lacking. However, taxonomically simple freshwater organisms such as Alveolata, Porifera, Cnidaria, or flatworms (Dettner 2010) are as important as chemically defended Hydrachnidia, water beetles, and water bugs (Coleoptera: e.g., Dytiscidae, Noteridae, Hygrobiidae, Haliplidae; Heteroptera: Corixidae, Notonectidae, Naucoridae, Belostomatidae) or even chemically defended trichopteran larvae.

In this chapter, I focus on all aspects of chemical ecology for adults and to a certain extent pupae of dytiscids. Data on glands or semiochemicals of dytiscid eggs and larvae are, unfortunately, not available, although such information would no doubt be interesting and valuable for our understanding of this family of beetles. For adult dytiscids, there exist only a few data on pheromones (6.3) and kairomones (6.4.1). In contrast, the Dytiscidae possess various complex glands and much is known on allomones (defensive compounds, 6.4.2). Moreover, behavior modifying chemicals may not be volatile or water soluble, but instead may cover the entire body surface as a kind of distinguishing mark, and the nature of such epicuticular lipids is examined here (6.5.2; Dettner and Liepert 1994). Because animal coloration represents secondary compounds, natural pigments of predaceous diving beetles also are reviewed (6.5.3). Finally, various aspects of microorganisms associated with predaceous diving beetles (6.6) and the role of hemolymph with respect to defense, hemostasis, and regeneration is described (Sect. 6.7). Future directions in research are discussed in Sect. 6.8.

6.2 Chemical Senses

Aquatic insects evolved secondarily in aquatic environments and therefore are capable of sensing odors from a diverse range of sources (Crespo 2011). The recent review by Crespo (2011) on chemosensation and related behavior in aquatic insects is mainly focused on hemimetabolous aquatic orders including Ephemeroptera, Odonata, and Plecoptera, and the holometabolous Trichoptera and Diptera. In contrast, aquatic Coleoptera are completely omitted, however specific investigations on dytiscid beetles do exist elsewhere.

Nikolaas Tinbergen (1907–1988), a Dutch born British zoologist (Fig. 6.1a) shared the Nobel prize in 1973 with Karl von Frisch and Konrad Lorenz for research on the social behavior of animals. As early as 1936, he reported on his investigations with adults and larvae of *Dytiscus marginalis*. Although adults possess very large complex eyes (Fig. 6.1b) they do not react to living tadpoles within water filled test tubes. In contrast, adult beetles will quickly move their antennae and swim strongly within an odor plume of meat extract (Fig. 6.1c, d). Tinbergen also discusses the chemosensation of *Dytiscus* larvae. Further results concerning chemical senses of *Dytiscus* larvae are presented by Korschelt (1924).

During the next several decades, the chemical senses of dytiscids were investigated by physiologists and zoologists. Schaller (1926) reported that dytiscids have very good chemical senses (odor, taste) that are especially important for detecting potential food. The receptors for these senses are located on different parts of their body. Dytiscids can taste sweet, sour, salty, and bitter with their taste receptors that are concentrated on their maxillary and labial palpi. Odor receptors (but not taste receptors) are found on the antennal surface. Recently, Song et al. (2016) found two odorant binding proteins in male tarsi of *Cybister japonicus* (now *C. chinensis*). CjapOBP1 represents a classical odorant binding protein, whereas CjapOBP2 belongs to the subclass of C-minus odorant binding proteins. Western blot analysis showed that CjapOBP1 is expressed in male tarsi, antennae, and palpi of both sexes. In contrast, CjapOBP2 is present both in male tarsi and in testis. The authors speculated that *C. japonicus* females could release sex pheromones which are perceived by males when they are fixed with their front tarsi on the backs of females.

Bauer (1938) showed during training experiments (mainly with adult *Dytiscus marginalis*) that beetles can differentiate between a variety of specific chemicals, including saccharose and hydrochloric acid. Furthermore, they can select saccharose when it is offered together with hydrochloric acid, sodium chloride, and the bitter quinine hydrochloride (bitter tasting alkaloid). Finally, they can select hydrochloric acid when it is offered together with glucose, quinine hydrochloride, and sodium chloride. However, beetles cannot differentiate between saccharose and glucose, hydrochloric and tartaric acid, quinine hydrochloride and salicin (bitter tasting alcoholic β-glucoside), or quinine hydrochloride and aloin (anthraquinone glucoside). It was found that these beetles can detect 18 different sugars and may perceive different compounds at different thresholds (e.g., saccharose 0.01 mol; sodium

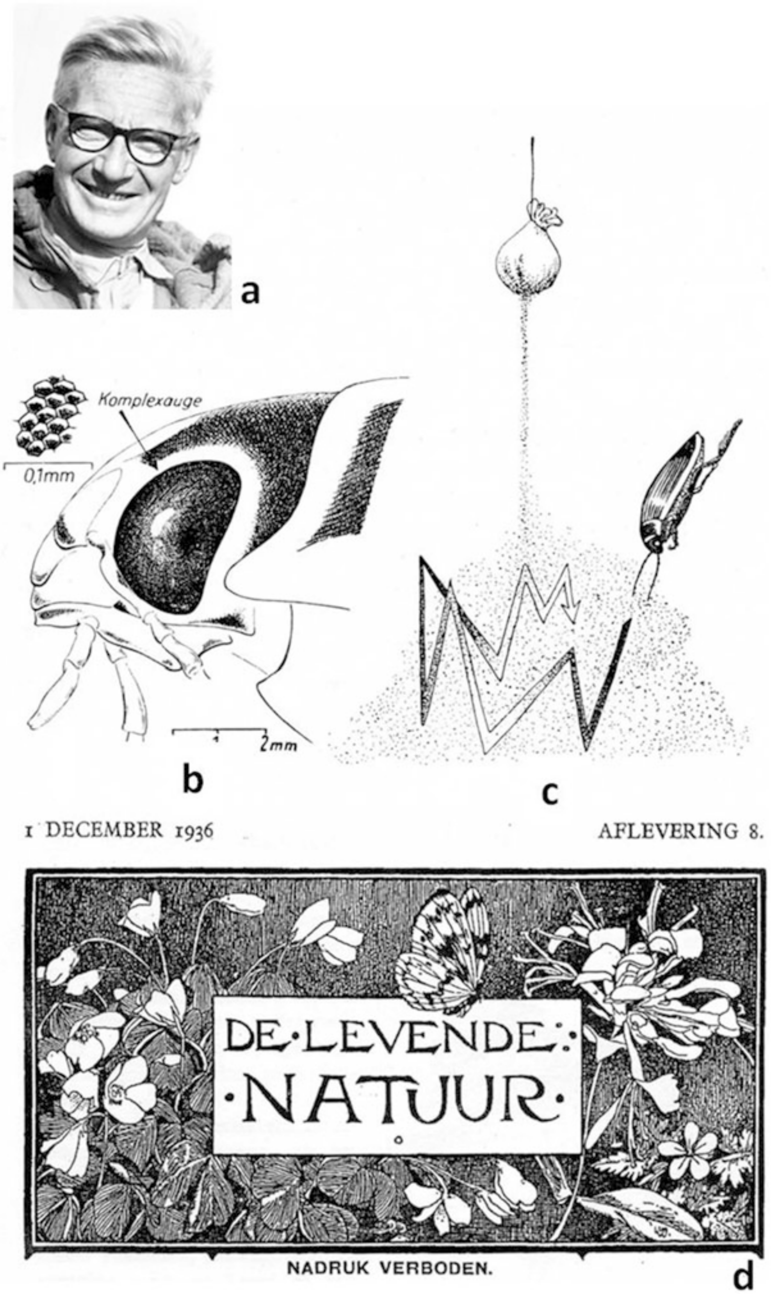


Fig. 6.1 Nobel laureate Nikolaas Tinbergen (1907–1988); (**a**) and his investigations on orientation of *Dytiscus marginalis* (Tinbergen 1936). In spite of the large complex eyes of adults (**b**) during detection of prey the beetle strongly reacts to a meat broth by swimming behavior within an odor plume of a meat extract (**c**; Tinbergen 1951). The title page of the journal "De levende Natuur" from 1936 is figured (**d**)

chloride 0.001 mol, salicin 0.0000625 mol, quinine hydrochloride 0.0000012 mol) (Bauer 1938).

Besides large species such as *Dytiscus,* chemoreception in aqueous and gas phases was studied in the smaller species *Laccophilus maculosus* (Hodgson 1953). In this species the sensilla basiconica are located on the tips of antennae and represent chemoreceptors for gaseous and liquid stimuli. Due to inherent specialization these receptors have the lowest threshold of antennal receptors. Hodgson (1953) also reported that those sensilla basiconica that are located on the tips of the maxillary and labial palpi also represent chemoreceptors, although with higher thresholds. Hydrochloric acid, 1-pentanol, and sodium chloride all stimulated receptor areas on the tips of antennae and palpi. In addition, Hodgson (1951) showed that cations in uniform anion combination stimulated in the following odor of effectiveness according to the order of their ionic motilities: hydronium (= hydroxonium) >> ammonium > potassium > sodium > lithium. Anions in uniform cation combinations stimulated in the following order of effectiveness: hydroxide >> iodine > bromine > sulfate, acetate, chloride > phosphate. In low molecular organic compounds, thresholds to primary to alcohols decreased with increasing in CH_2-groups (e.g., methyl alcohol 3.6 mol, ethyl alcohol 4.3 mol, propyl alcohol 3.2 mol, butyl alcohol 0.046 mol, amyl alcohol 0.0073 mol, hexyl alcohol 0.0011 mol). This trend is apparently directly related to lipid solubility of the alcohols. Behrend (1971) analyzed the responses of single pore plate olfactory cells on odorous compounds in either air or water. The olfactory cells responded either to various organic acids and amino acids (class 1) or to nitrogenic compounds (class 2). Identical stimuli resulted in the same response in air and in water, which does not depend on the physicochemical state of the stimulating molecules within their carriers (air or water).

There exist various light microscopic and electron microscopic studies concerning the sensillae of Dytiscidae. Light microscopic details and a survey were produced by Korschelt (1923). Electron microscopic studies were performed on the fine structure of the sensilla on the distal antennal segment of *Graphoderus occidentalis* (Jensen and Zacharuk 1991), the digitiform from sensilla on the distal segment of maxillar palps of *Agabus bipustulatus* (Guse and Honomichl 1980), and antennal sensillae of *Acilius sulcatus* (Ivanov 1966). Recently, Song et al. (2017) studied the ultrastructure and morphology of antennal sensilla of adult *Cybister japonicus* (now *C. chinensis* Régimbart) beetles. By TEM and SEM they identified five types of sensillae on male and female antennae. Especially Sensilla placodea are abundant and carry multiple pore systems with a typical function of chemoreceptors. Because to the fact that males have longer antennae than females, consequently males have more densilla than females.

6.3 Intraspecific Interactions: Sex Pheromones

Sex pheromones are well known from Lepidoptera and other terrestrial insects, as well as a few examples from marine systems (Wyatt 2003). However, observations on sex pheromones in freshwater systems are very rare in both invertebrates (e.g., *Gammarus*; Borowsky and Borowsky 1987) and vertebrates (Sorensen and Hoye 2010).

As far back as 1912, Blunck (1912b) reported that female *Dytiscus marginalis* produce a certain “Geschlechtsduft” (sexual odor) that leads males to females within an area of 20–30 cm. He also mentioned that males, excited by females, would quickly move their antennae and palpi during an increase in their swimming movements. Blunck (1912b) also found that secretions of female pygidial glands did not arouse males. Smith (1973) reported on sound production in both sexes of different species within genus *Rhantus*, which was observed in a behavioral context of emigration. During his experiments he reported that intra- and inter-specific recognition is achieved through an olfactory clue, and in the laboratory interspecific location even functioned in total darkness.

Recently Herbst et al. (2011) demonstrated the presence of sex pheromones in the predaceous diving beetle *Rhantus suturalis*. Within non-permeable glass flasks, which did not allow the diffusion of chemicals, males and females did not stimulate any reaction by conspecifics of either sex. However, in permeable vessels (e.g., made of finely woven steel) male predaceous diving beetles were significantly attracted to females. In addition, female *R. suturalis* were attracted to other females when they perceived chemical and optical cues simultaneously. Specifically, Fig. 6.2 illustrates the numbers of contacts with (left axis) and the sitting contacts with the vessel in male (a) and female (b) *R. suturalis* to a permeable steel vessel containing one female (F), one male conspecific (M), or an empty control vessel (C). Both with respect to contacts with the vessel and sitting durations on the vessels, males significantly selected females over males of controls. In addition, female *R. suturalis* had significantly more contacts with conspecifics than with males (Fig. 6.2).

With these results in mind, it would be interesting to now elucidate the chemical structure of the substances that modify female behavior in dytiscids. Some aquatic vertebrates (e.g., fishes, amphibians) unlike terrestrial insects use unusual polar compounds that serve as sex pheromones (Sorensen and Hoye 2010) such as L-kynurenine (Masu salmon of genus *Oncorhynchus*), prostaglandin $F_{1\alpha}$, $F_{2\alpha}$ (*Salmo*), a dihydroxypregnan-20-one-3-glucuronide (African catfish *Claria*), dihydroxy-4-pregnen-3-one and prostaglandins (*Carassius auratus*), newts in the genus *Cynops* (decapeptides as sodefrin, silefrin) or the tree frog *Litoria splendida* (25-amino acid peptide splendiferin). Further data which characterize pheromones of aquatic organisms are presented by Breithaupt and Thiel (2011) and Brönmark and Hansson (2012). Remarkably both kynurenine and steroids represent important metabolites of Dytiscidae. An intriguing question for the findings of Blunck (1912b) is if the prothoracic defensive glands are important for sexual pheromone activities.

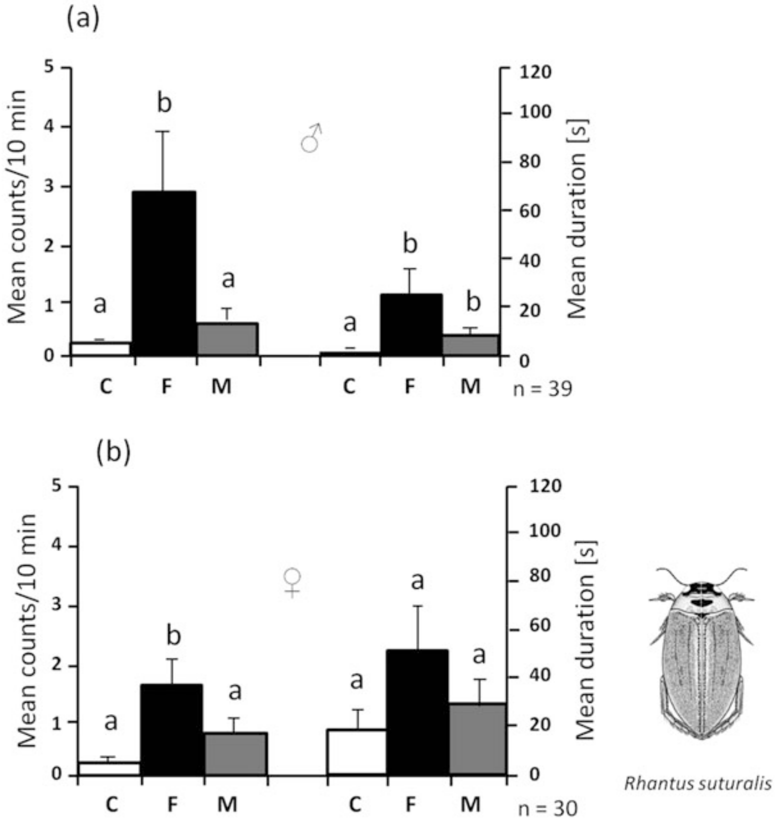


Fig. 6.2 Reaction of male (**a**, above) and female (**b**, below) *Rhantus suturalis* beetles to a vessel made of finely woven steel, containing one female (F) or one male conspecific (M) or to an empty control vessel (C). Left: mean counts of beetle contacts with the vessels. Right: mean sitting duration of beetles on the vessels. Error bars indicate standard errors. Bars with different letters are significantly different at $P \leq 0{,}05$. *n* number of replicates. After Herbst et al. (2011)

6.4 Interspecific Interactions

During evolution, predators, parasitoids, and prey have developed various methods in order to detect, to defend, or generally to interact with each other (see Peckarsky 1984; Williams and Feltmate 1992). Apart from visual communication in aquatic ecosystems with low visibility and effective superposition eyes, predaceous diving beetles seem to especially use non-visual stimuli for their interactions and rely on chemoreception, which is very efficient in both adult and larval dytiscids. Interspecific chemical interactions are generally mediated by allelochemicals, which may be further subdivided depending on whether these chemicals are advantageous for the sending (allomone, 6.4.2) or for the receiving (kairomone, 6.4.1) organisms.

6.4.1 Kairomones and Other Allelochemicals

Kairomones represent interspecific behavioral modifying chemicals that are of advantage for the receiver and in contrast are negative or disadvantageous for the producing organisms. They are important in most predator/prey or host/parasite-systems.

With respect to dytiscids, our knowledge of chemical ecology varies depending on if the dytiscids represent prey (6.4.1.1) or predators (6.4.1.2). In addition, dytiscids may perceive kairomones (6.4.1.1, 6.4.1.2) or may function as kairomone emitters (6.4.1.3). In all cases, there exist many laboratory and field observations, however the mechanisms for these behavior modifying kairomones are unknown.

There is growing evidence both from laboratory and mesocosm studies that insect predators that orientate toward the water surface are often absent in the presence of fishes. However, these insects may have effects on potential prey (e.g., zooplankton) that are analogous to fish predators (Herwig and Schindler 1996). As an example, larval *Acilius semisulcatus* significantly affect the vertical distribution of *Daphnia pulex* prey (especially large specimens; Arts et al. 1981). If dytiscid predators are present, a greater percentage of *Daphnia*-prey was found near the bottom of the experimental cages. Thus, it seems highly probable that chemical signals, such as kairomones produced by dytiscid beetles, are involved in this response.

6.4.1.1 Dytiscid Prey and Fish Predators

The importance of fish predation on aquatic insects, including some species of predaceous diving beetles, was reviewed by Healey (1984) and Sih (1987). Fish can exert strong and negative effects on dytiscid communities (Chap. 7 in this book) and may be important for food web dynamics as dytiscids can be both fish prey and predator (Chap. 8 in this book). In one example (Åbjörnsson et al. 1997), it was determined that *Acilius sulcatus* responded to chemical cues from perch (*Perca fluviatilis*). Whereas odor or visibility alone did not affect the activity of *A. sulcatus*, a significant interaction occurred when the two factors were combined (Fig. 6.3). The lowest activity of the beetles was found when *A. sulcatus* was exposed to water scented by starved perch at night (Fig. 6.3). When the activity was counted as the number of quadrats passed during 10 minutes before and after adding “fish-water,” activity decreased after the addition of odor from starved perch (Åbjörnsson et al. 1997). This finding strongly suggests that beetles may alter their behavior in the presence of fish predators.

6.4.1.2 Dytiscids Predators and Vertebrate Prey

Especially larval dytiscids are often predators of vertebrates (McCormick and Polis 1982) and may use kairomones emitted by their prey. There exist various examples

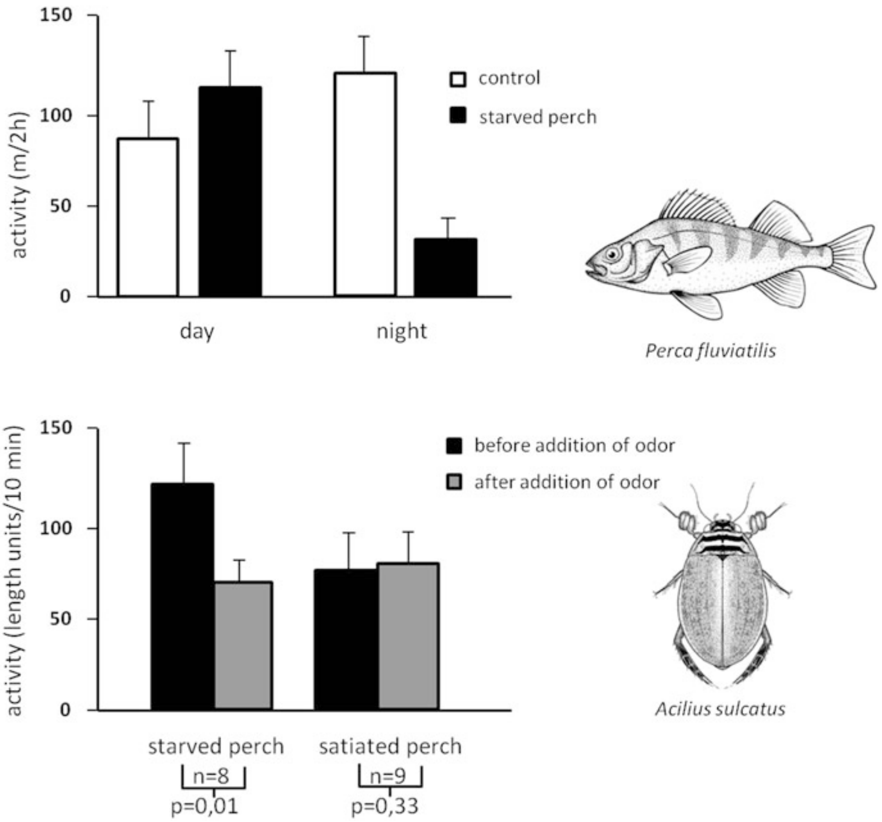


Fig. 6.3 Responses of dytiscid beetles (*Acilius sulcatus*) to chemical cues from perch *Perca fluviatilis*. Above: Activity (m moved within two hours) for *Acilius sulcatus* in the different treatments in the fluviarium experiment (mean + SE). Below: Activity (mean + SE) of *Acilius sulcatus* counted as the number of quadrats past during 10 minutes before and after adding "fish-water" in the aquarium experiment. The *P*-value shows the result of the Wilcoxon-signed-rank test of the difference in activity before and after adding "fish-water." Changed according to Åbjörnsson et al. (1997)

where such interactions are described. In 1995, Mathis et al. reported that alarm pheromones of fathead minnows (*Pimephales promelas*) function as attractants for both predatory fish like pike (Esocidae) and adult predaceous diving beetles. When traps were supplied with skin extracts of alarm substance cells of non-breeding fishes (that had alarm pheromone cells) significantly more beetles were caught in the traps bated with alarm substances as compared with the controls (lacking alarm pheromone cells). These traps recorded seven species including *Acilius semisulcatus, Colymbetes sculptilis, Dytiscus alaskanus, D. circumcinctus, D. cordieri, Graphoderus occidentalis*, and *G. perplexus* Sharp, although only *C. sculptilis* were present in the sufficient numbers for statistical analysis. The evolutionary significance of such alarm signals that attract predators and are useful for alarm signal emitters was summarized by Chivers and Smith (1998) and Chivers et al. (1996).

Recently larvae of *Dytiscus sharpi* Wehncke were recognized as being capable of detect not only prey motion but also prey scent (Inoda 2012). When larvae were exposed only to prey odors in the form of chemical signals from tadpoles they were more likely to be attracted to traps with tadpoles than to empty control traps. In contrast, *D. sharpi* larvae were not attracted to a trap containing conspecific larvae. The author suggested that the larvae are capable of recognizing prey scent (but not prey size), which may increase foraging success but decrease cannibalism.

Manteifel and Reshetnikov (2002) conducted laboratory experiments and allowed different predators to prey on noxious versus non-noxious tadpoles. Whereas predatory fishes and *Aeshna* nymphs actively consumed *Rana* tadpoles, *Bufo* tadpoles were rejected. On the contrary, larvae of *Dytiscus marginalis* attacked both tadpoles. These results are interesting from a chemically perspective, however the degrees of noxiousness of skins and interior bodies of *Bufo* and *Rana* tadpoles were not analyzed in this study. Therefore, interpretation of these results is difficult especially with respect to strategies of nutrition by different predators (i.e., sucking vs. chewing). Hileman et al. (1995) tested the avoidance of unpalatable prey (tails of *Notophthalmus* newts) by *Dytiscus verticalis* larvae. They found that avoidance of unpalatable prey decreased with increased hunger.

6.4.1.3 Dytiscid Predators and Egg-Laying Prey

In temporary pools, larvae of the mosquito *Culiseta longiareolata* are highly vulnerable to the common predatory backswimmer *Notonecta maculata* (Silberbush et al. 2010). It was recently found that adult female mosquitoes use kairomones that are released by these predators to detect the risk of predation. Specifically, oviposition of female mosquito is effectively repelled by n-heneicosane and n-tricosane, two hydrocarbon kairomones produced by *Notonecta* (Silberbush et al. 2010). The same effect was observed recently in females of the wetland mosquito *Culex tritaeniorhynchus* that strongly avoided laying eggs at oviposition sites in the presence of the predaceous diving beetle *Eretes griseus* (Ohba et al. 2012). In contrast, female *Aedes albopictus* mosquitoes laid eggs in both the absence and presence of predator cues, probably because they could not detect the hitherto chemically unknown *Eretes* cues or are not sensitive to them. This was the first report to show that mosquitoes can detect the chemical cues of coleopteran beetles. In addition, Ohba et al. (2012) found that mosquito larvae near the water surface were eaten less frequently by *Eretes griseus* than those at the bottom of the containers. Therefore, filtering at the water surface appears to be an appropriate adaptive response in the presence of this predator.

Beyond the effect of dytiscids on invertebrates, Urban (2008) studied interactions between salamander larvae (*Ambystoma maculatum*) and *Dytiscus* larvae due to kairomones. It was evident that *Dytiscus* kairomones strongly reduced the daytime activity of *A. maculatum* larvae but the presence of beetle larvae did not induce lower larval amphibian body masses, suggesting that perhaps feeding activity was not modified by predator presence.

6.4.2 Allomones

Allomones represent substances that are produced and released by an individual of one species that affects the behavior of an individual of another species. In contrast to kairomones (6.4.1), allomones such as defensive compounds or antibiotics are advantageous for the sender and disadvantageous for the receiver. For both types of interactions there exist many detailed observations and bioassays in the field and the laboratory. However, compared to kairomones, detailed data on the chemical character of these behavior modifying chemicals are completely lacking. In contrast, hydradephagan beetles produce huge amounts of chemically identified natural products in their complex pygidial and prothoracic defensive glands. Therefore, Dytiscidae are well known to harbor elaborate biosynthetic apparatuses for manufacturing either steroids or aromatics (Blum 1981; Morgan 2004; Dettner 2019b). Before reporting on these two complex gland systems where these natural products are produced, it is important to mention other internal structures, the rectal ampullae and probably the venomous gut material of dytiscid larvae.

Both larvae and adult dytiscids possess rectal ampullae. If adults of larger Dytiscidae (Dytiscinae) are handled, they often immediately react by depleting their rectal ampulla. This is evident by an unpleasant odor resembling hydrogen sulfide (H_2S) or ammonia (NH_3). Eisner (1970) named these defensive reflexes enteric discharges and discerns between regurgitation and defecation. These important defensive mechanisms were reviewed recently on the level of insects (Weiss 2006; Rostás and Blassmann 2009). After uptake of water the rectal ampulla may primarily serve as hydrostatic organ to increase the specific weight of the beetle, for example when it lands on a shining water surface (Naumann 1955; Wesenberg-Lund 1943; Hicks and Larson 1991). Moreover, a lot of valuable compounds such as ions and sugars are reabsorbed from the rectal epithelium into the hemolymph (Cochran 1975; Dettner and Peters 2010). In addition, this organ represents the first defecation-defense of adult dytiscids, before prothoracic defensive glands are depleted. Usually the rectal ampulla, which extends through the whole abdomen (Fig. 6.4a), is filled with water and very often with excrements (Wesenberg-Lund 1943). Taxonomically a rectal ampulla is found in representatives of adult Dytiscinae (Fig. 6.4d, e) and Hydroporinae (Fig. 6.4b). Here the hind gut laterally meets the ampulla at its midway point (Fig. 6.4b). The same configuration was observed in *Agabus bipustulatus* L. Within representatives of Colymbetinae the posterior part of the hind gut widens considerably, but otherwise the small hind gut meets the widened hind gut terminally or subapically (Fig. 6.4c). When larger and selected specimens of adult Dytiscinae are molested, odorous irritations are the only threat to humans or other vertebrate predators. It is interesting that large, full-grown larvae, especially of Dytiscinae, possess extremely lengthened rectal papillae

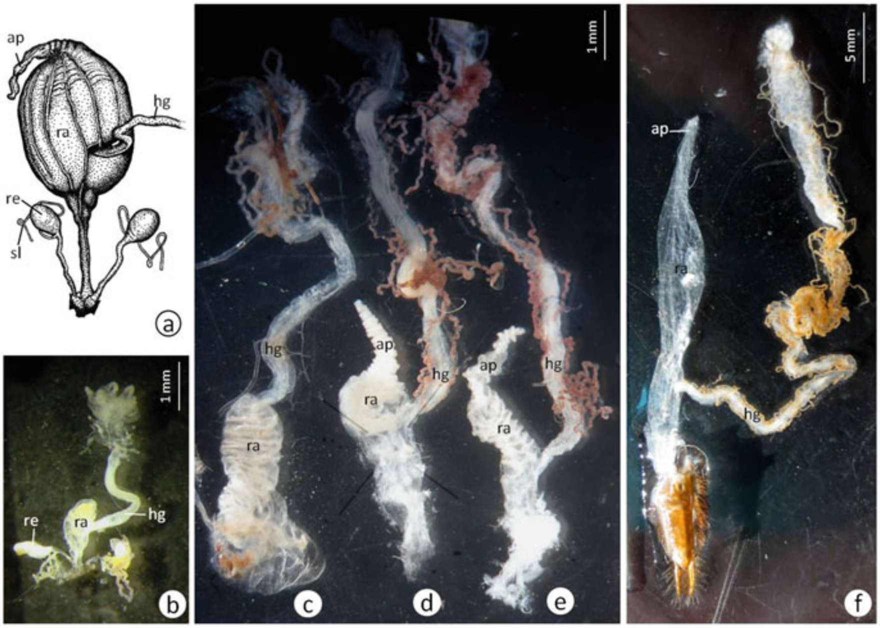


Fig. 6.4 (**a**): Filled rectal ampulla of *Dytiscus marginalis* with appendix, hind gut, and paired pygidial glands. (**b–d**): Mid- and hind gut, rectum and rectal ampulla of *Hyphydrus ovatus* L. (**b**), *Ilybius crassus* C. G. Thomson (**c**), *Acilius sulcatus* (**d**), *Hydaticus seminiger* DeGeer (**e**). Mid- and hind gut, rectum and rectal ampulla together with last abdominal segment and cerci of a *Dytiscus marginalis* larva (L III, **f**). (**a**): According to Naumann (1955). Abbreviations: *re* reservoir of pygidial gland, *sl* secretory lobe, *ra* rectal ampulla, *ap* appendix, *hg* hind gut

(Fig. 6.4f). Sometimes the rectal ampulla, which also serves primarily as hydrostatic organ, is so long and extends into the larval head. It was suggested that this huge larval appendage serves to increase interior pressure in order to burst the last larval skin (Naumann 1955). Korschelt (1924) reports that the rectal ampulla of *Dytiscus* larvae does not represent a defensive mechanism as observed in adults but is filled with water after molting. In contrast to adults, the defensive mechanisms of *Dytiscus* larvae are mechanical and are due to biting movements of sharp mandibles. Bites of full-grown larvae (e.g., “water-tigers”) of large dytiscids are very painful for humans (Dettner 2019b). In addition, for small vertebrates or many water insects larval bites obviously are paralyzing. Since coleopteran larvae possess no salivary glands the origin of these venomous secretions is unknown. Both the presence of esophageal glands and eventual venomous midgut secretions are highly questionable (Korschelt 1924; Walker et al. 2018).

6.4.2.1 Pygidial Glands

According to microtome sections, all hydradephagan families (save one) and neighboring taxa possess pygidial glands and their gland constituents (Dettner and Böhner 2009; Dettner 2019b). Pygidial glands were recorded within the recently identified water beetle family Meruidae (Beutel et al. 2006), however, in Aspidytidae the histological data are absent. There exist various data concerning the anatomy and histology of the pygidial defense glands in Dytiscidae, Noteridae, Haliplidae, Gyrinidae (Forsyth 1968), Amphizoidae, and Hygrobiidae (Forsyth 1970; Figs. 6.5 and 6.6). Paired pygidial defensive glands were described for the first time in more detail in the dytiscid genera *Hyphydrus*, *Stictotarsus*, *Laccophilus,* and *Ilybius* (Forsyth 1968). Later abdominal glands from several other dytiscid species were

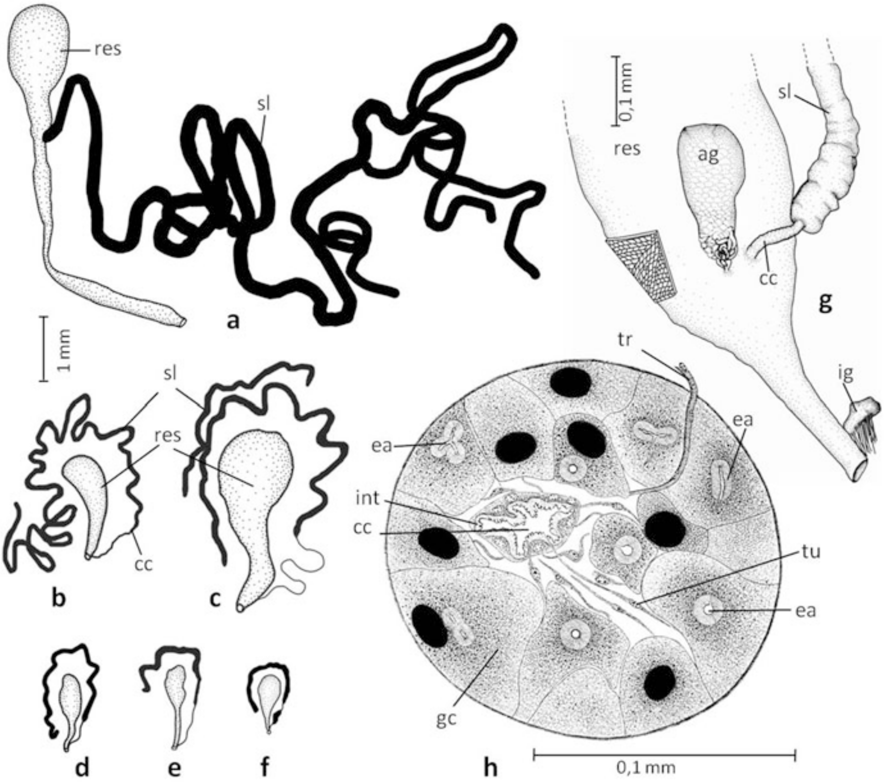


Fig. 6.5 (**a–f**): Structure of one pygidial gland system. (**a**): *Dytiscus marginalis*, (**b**): *Acilius sulcatus*, (**c**): *Colymbetes fuscus* L., (**d**): *Liopterus haemorrhoidalis*, (**e**): *Laccophilus minutus* L., (**f**): *Nebrioporus depressus* Fabricius, (**g**): Enlargement of posterior part of the left pygidial gland system of *Hyphydrus ovatus* (modified after Forsyth 1968). (**h**): Section through secretory lobe of *D. marginalis* (modified after Korschelt 1923). Abbreviations *ag* accessory gland, *cc* collecting canal, *ea.* end apparatus, *gc* gland cell, *ig* integumental gland, *in* intima, *res* gland reservoir, *sl* secretory lobe, *tr* trachea, *tu* tubule. Nuclei are black

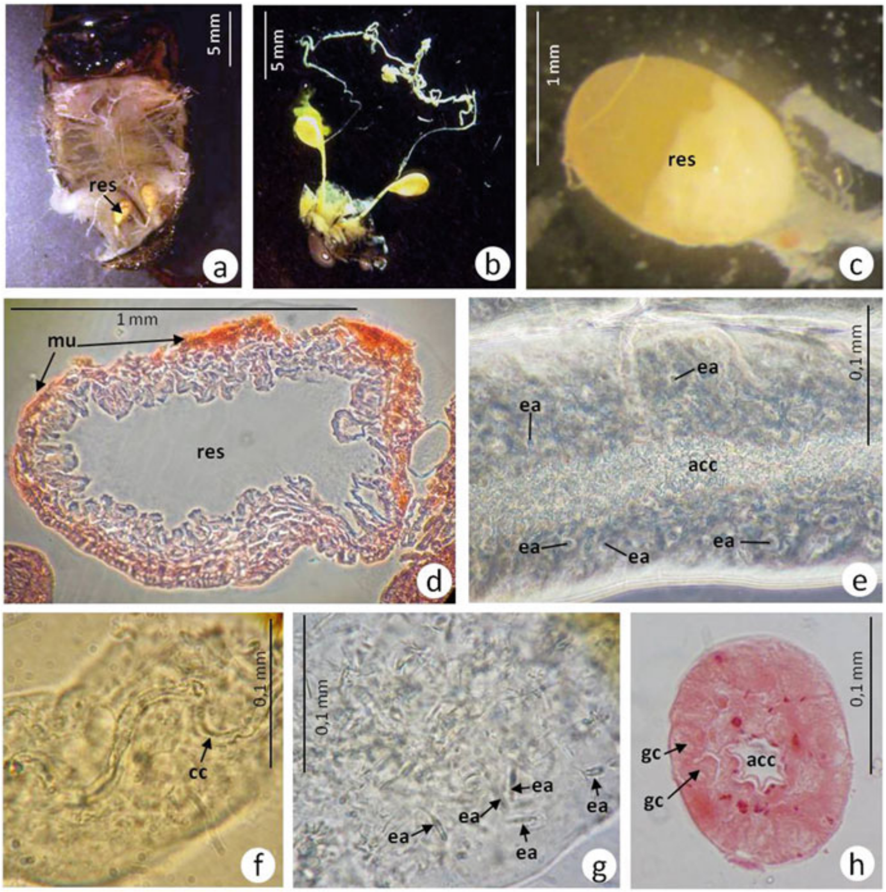


Fig. 6.6 (**a–h**): Structure and histology of pygidial glands of *Dytiscus marginalis*. (**a**): Dissected abdomen with paired pygidial glands. (**b**): Enlargement of two prepared pygidial gland systems. (**c**): Pygidial gland reservoir with 2 organic phases. (**d**): Section through gland reservoir using nuclear fast red-aluminum sulfate solution (**e**): Longitudinal view of squeezed secretory lobe. (**f**): Longitudinal view of squeezed collecting canal. (**g**): Longitudinal view of squeezed secretory lobe with end apparatuses. (**h**): Square section through secretory lobe using nuclear fast read-aluminum sulfate solution

recorded (Dettner 1985). Each gland (Figs. 6.5 and 6.6) comprises an ovoid reservoir that is covered by a muscle coat (Fig. 6.5g inlet figure; 6.6d) and leads into an efferent duct with proximal valve. The lobular secretory tissue or secretory lobe (Figs. 6.5a–g and 6.6b,e,g) is connected to the reservoir by a collecting canal (Figs. 6.5g–h and 6.6f). The openings of the reservoirs are situated on the membranous cuticle behind the eighth abdominal tergite. According to Forsyth (1968, 1970) there exist two types of pygidial gland cells. An organelle of type I is typical for Dytiscidae but absent in Haliplidae, Gyrinidae, and Noteridae. The last three families have organelles of type II, which are also found in Laccophilinae, Hydroporinae, and some Colymbetinae and Dytiscinae. In addition, Laccophilinae and Hydroporinae possess simple unbranched type II organelles, but both simple and branched organelle-forms occur in Dytiscinae and Colymbetinae (Forsyth 1968). Ultrastructural analyses based on pygidial glands of *Dytiscus marginalis* (Kuhn et al. 1972) confirmed that there exist two types of eccrine gland cells. The central cavity of the gland duct is surrounded by microvilli that are stiffened by microfibrils. Kuhn et al. (1972) reported that the Golgi-apparatus is better developed in racemous cells than in the bulbous cells. In addition, the central cavities contain fine-fluffy substances in racemous cells and osmiophilic materials in the bulbous cells. It is interesting that Forsyth (1968) could also describe accessory glands (Fig. 6.5g) in the genera *Hyphydrus* and *Stictotarsus* that open into the reservoir of the pygidial glands close to the opening of the collecting canal. According to Forsyth (1968), these accessory glands are homologous with the basal combustion chamber in bombardier beetles. Vesicle and organelle of accessory glands are similar to the gland cells of thoracic glands and of the type II cells of the pygidial gland. Moreover, an integumental gland (Fig. 6.5g) with about 100 cells opens close to the external reservoir opening in *Hyphydrus* (Forsyth 1968). The secretory lobes are characterized by an axial collecting canal (Fig. 6.5e, h) that is surrounded by gland cells with type I and II organelles (Figs. 6.5h and 6.6e-h).

The secretory lobes may be lengthened (Figs. 6.5a, b and 6.6b) or even branched (Fig. 6.5a, c). The collecting canals may be lengthened as in *Colymbetes, Laccophilus* (Fig. 6.5c, e), or shorter as in *Acilius* (Fig. 6.5b)*, Liopterus* (former *Copelatus*) (Fig. 6.5d)*, Nebrioporus* (Fig. 6.5f)*,* and *Hyphydrus* (Fig. 6.5g)*,* or are even absent as in *Hydaticus* (not figured) and *Dytiscus* (Fig. 6.5a). In most dytiscid species studied the collecting canals unite near the reservoir opening with the efferent duct of reservoirs (Fig. 6.5b–f), however in *Hyphydrus* (Fig. 6.5g) and especially in *Hydaticus* and *Dytiscus* (Figs. 6.5a and 6.6b) the collecting canal unites more anteriorly with the gland reservoir.

According to Korschelt (1923) the pygidial gland system of *Dytiscus marginalis* is innervated by the paired second nervi that originate from the hind border of the last abdominal ganglion (ganglion VI). Obviously this large nerve (called Nervus proctodaeo-genitalis) innervates all organs from the eighth segment onward to the abdominal tip.

6.4.2.1.1 Chemistry of the Pygidial Glands and Distribution of Pygidial Gland Constituents within Dytiscidae and Hydradephaga

Among insects, hydradephagan beetles represent the most prominent taxa producing aromatic exocrines (Dettner and Böhner 2009; Dettner 2019b). Apart from Dytiscidae, aromatic pygidial gland constituents are found in Haliplidae (Dettner and Böhner 2009), Noteridae (Dettner 1997a), Amphizoidae (Dettner and Böhner 2009), and Hygrobiidae (Dettner 1997b), however pygidial gland chemistries of Meruidae, Aspidytidae, and Rhysodidae are unknown. Unusual aromatics that are not present in dytiscid beetles are 3-hydroxyphenylacetic acid and phenyllactic acid

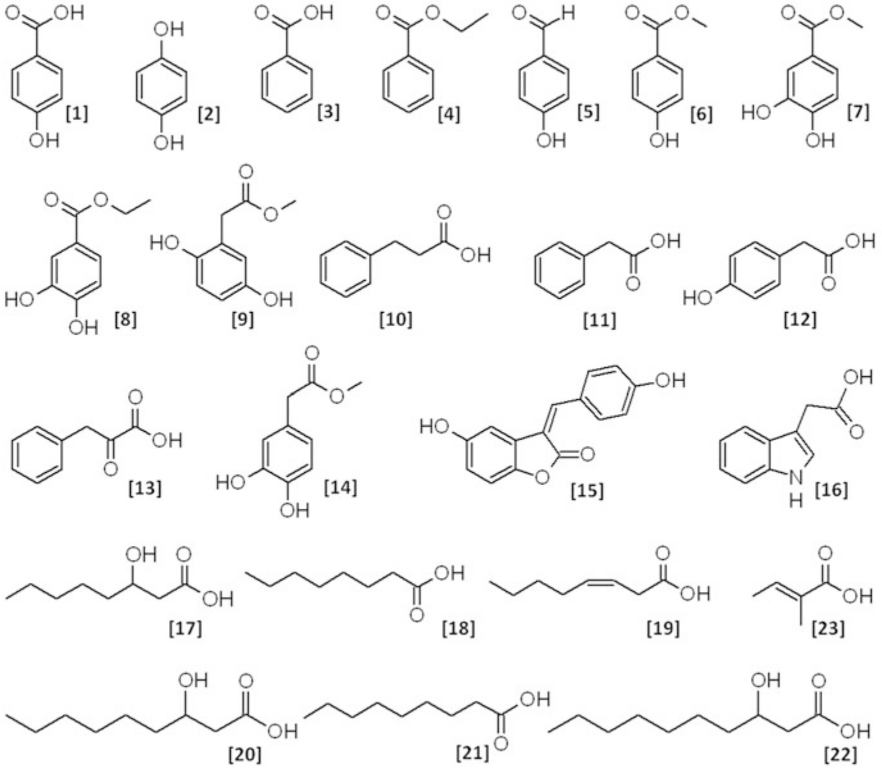


Fig. 6.7 Pygidial gland constituents **1–23** of predaceous diving beetles

in Haliplidae (Dettner and Böhner 2009). In closely related families pygidial glands only contain a few aromatics in usually low amounts. Gyrinidae produce phenylacetaldehyde (Dettner and Böhner 2009), Trachypachidae contain 2-phenylethanole and its esters (Attygalle et al. 2004) and a few carabid and cicindelid taxa contain benzoic acid, phenylacetic acid, and methylsalicylate together with salicylic aldehyde and benzaldehyde (see Francke and Dettner 2005; Dettner and Böhner 2009; Will et al. 2000).

The first results on the chemistry of the pygidial glands of dytiscids were published by Ghidini (1957). He described pygidial gland secretions of Dytiscinae as "disagreeable," whereas representatives of Hydroporinae such as *Hydroporus*, *Potamonectes* (now *Nebrioporus*), *Deronectes*, *Stictotarsus,* and *Coelambus* (now subg. of *Hygrotus*) were characterized as "sweet" and "agreeable" odors. I have supplied the chemical structure of many of the most common pygidial gland products in Fig. 6.7; hereafter I refer to them by number designations (bold).

Subsequently, Schildknecht et al. (1962) reported the presence of benzoic acid (Table. 6.1, Fig. 6.7) and various other aromatics in pygidial glands of different dytiscid species. In the following years, 14 aromatic, 7 aliphatic compounds, a

Table 6.1 Pygidial gland constituents of predaceous diving beetles

DYTISCIDAE, HYDROPORINAE	
Deronectes aubei (Muls.)	(1)#,(3)#,7#,11#,16#
Deronectes latus (Steph.)	(3),(5),11 (Dettner 1985)
Deronectes moestus (Fairm.)	11 (Dettner 1985)
Deronectes platynotus (Germ.)	11 (Dettner 1985)
Geodessus besucheti Branc.	11 (Dettner 1985)
Graptodytes pictus (F.)	(3),11,12,13,16 (Dettner 1979, 1985)
Hydroglyphus geminus (F.)	11,13,16 (Dettner 1979)
Hydroporus angustatus Strm.	11,13,16 (Dettner 1979)
Hydroporus discretus Fairm. & Bris.	11,13 (Dettner 1979)
Hydroporus dorsalis (F.)	(3),11,12,(16) (Dettner 1979, 1985)
Hydroporus ferrugineus Steph.	11,13,16 (Dettner 1979)
Hydroporus incognitus Shp.	11 (Dettner 1985)
Hydroporus lundbladi (Falkenström)	3#,6#,11#
Hydroporus marginatus (Duft.)	11,13,16 (Dettner 1979)
Hydroporus melanarius Strm.	(5),12 (Dettner 1979)
Hydroporus obscurus Strm.	11,12,16 (Dettner 1979)
Hydroporus obsoletus Aubé	11#,19#
Hydroporus palustris (L.)	11,12 (Dettner 1979)
Hydroporus planus (F.)	11,12,13,16 (Dettner 1979)
Hydroporus pubescens (Gyll.)	(2),(3),(6),11 (Dettner 1985)
Hydroporus tristis (Payk.)	11,12,16 (Dettner 1979)
Hydrovatus cuspidatus (Kunze)	(3)#,(11)#,12#,13#,16#,17#,(18)#,21#,22#
Hygrotus inaequalis (F.)	(6)#,7#,11#,12,13,(16),17#,22 (Dettner 1979)
Hygrotus sanfilippoi (Fery)	11,12,13,16 (Dettner 1985)
Hyphydrus aubei Ganglb.	(2),(3),11,12,13,16 (Dettner 1985)
Hyphydrus ovatus (L.)	11, 12, 16 (Dettner 1979)
Nectoporus sanmarkii (C.R. Sahlb.)	(3),(6),11,13 (Dettner 1985)
Nebrioporus canaliculatus (Lac.)	(2),(3),11,13,16 (Dettner 1985)
Nebrioporus depressus (F.)	11,12,(13),16 (Dettner 1979, 1985)
Scarodytes halensis (F.)	(3),(5),11,12,13,16 (Dettner 1979, 1985)
Stictonectes optatus (Seidl.)	(5),(6),11,(16) (Dettner 1985)
Stictotarsus duodecimpustulatus (F.)	11,12,13,16 (Dettner 1979)
DYTISCIDAE: COPELATINAE	
Liopterus atriceps (Sharp)	(1),3,(5),11,13 (Dettner 1985)
Liopterus haemorrhoidalis (F.)	(1),2,3,5,6,11,13 (Dettner 1979, 1985)
DYTISCIDAE: AGABINAE	
Ilybiosoma seriatum (Say)	5,6 (Fescemyer and Mumma 1983)
Platambus obtusatus (Say)	5,6 (Fescemyer and Mumma 1983)

(continued)

Table 6.1 (continued)

Agabus binotatus Aubé	1#,3#,5#,6#,7#
Agabus guttatus (Payk.)	2,3,5,6,7,(11),(13) (Dettner 1979, 1985)
Agabus maderensis Wollaston	2#,3#,4#,5#,6#,7#
Agabus biguttatus (Oliv.)	2#,3#,5#,6#,(11) (Dettner 1979, 1985)
Agabus bipustulatus (L.)	1,2,3,5,6,(11) (Dettner 1979, 1985; Schildknecht 1970)
Agabus wollastoni Sharp	2#,3#,5#,6#,7#
Agabus melanarius Aubé	2,3,4,6,7 (Dettner 1979)
Agabus sturmii (Gyll.)	(1),2,3,5,6,(7),(11) (Dettner 1979, 1985)
Agabus nebulosus (Forst.)	2,3,5,6,7# (Dettner 1979)
Agabus paludosus (F.)	2,3,5,6,7# (Dettner 1979)
Agabus affinis (Payk.)	2,3,5,6,7,(11) (Dettner 1979, 1985)
Agabus congener (Thunb.)	3,5,6 (Dettner 1979)
Agabus didymus (Oliv.)	3,5,6 (Dettner 1979)
Agabus labiatus (Brahm)	(1),2,3,5,6,(7),(11),(13),15 (Dettner 1979, 1985)
Agabus undulatus (Schrank)	(1),2,3,5,6,(7),15 (Dettner 1985)
Agabus serricornis (Payk.)	2,3,5,6,15 (Dettner 1985)
Agabus unguicularis (Thoms.)	2,3,5,6,7,(11) (Dettner 1985)
Agabus brunneus (F.)	2,3,5,6,7 (Dettner 1985)
Platambus maculatus (L.)	(1),2,3,5,6,(7),(11),18,19# (Dettner 1979, 1985)
Ilybius chalconatus (Panz.)	2,3,5,6,7 (Dettner 1985)
Ilybius wasastjernae (C. R. Sahlb.)	5,6 (Dettner 1979)
Ilybius fuliginosus (F.)	2,3,5,6,(7),(11) (Dettner 1979, 1985)
Ilybius fenestratus (F.)	1,2,3,4#,5,6 (Dettner 1985; Schildknecht 1970)
Ilybius hozgargantae (Burm.)	1#,2#,3#,5#,6#,7# (Schaaf 1998)
Ilybius ater (De Geer)	1,2,3,5,6,(7),(11),23 (Dettner 1979, 1985)
Ilybius crassus Thoms.	2#,3#,4#,5#,6#,7, (Dettner 1979)
Ilybius quadriguttatus (Lac.)	3#,6#
Ilybius guttiger (Gyll.)	1,2,3,5,6,7#,(10)#,(23)# (Dettner 1979)
Ilybius aenescens Thoms.	(1),3,5,6,(7),(11) (Dettner 1985)
DYTISCIDAE: COLYMBETINAE	
Colymbetes fuscus (L.)	(1),2,3,5,6,7 (Dettner 1979; Schildknecht 1970)
Colymbetes schildknechti Dett.	2,3,5,6,(11) (Dettner 1985)
Meladema coriacea Laporte	2,3,5,6,(11),(13) (Dettner 1985)
Meladema lanio (F.)	1,2#,3#,4,5#,6#,(7)#
Nartus grapii (Gyll.)	3,5,6 (Dettner 1985)
Rhantus exsoletus (Forst.)	1,2,4,5,6 (Dettner 1985; Schildknecht 1970)
Rhantus suturellus (Harr.)	2,3,5,(6),(7) (Dettner 1985)
Rhantus suturalis (Mcleay)	2,3,5,6,7,(11),(13) (Dettner 1979, 1985)
DYTISCIDAE, DYTISCINAE	
Acilius sulcatus (L.)	2,3,5,6 (Dettner 1979; Schildknecht 1970)
Acilius duvergeri Gob.	1,2,3,5 (Dettner 1985)
Acilius mediatus (Say)	3,5,6 (Newhart and Mumma 1979)
Acilius semisulcatus Aubé	3,5,6 (Newhart and Mumma 1979)

(continued)

Table 6.1 (continued)

Acilius sylvanus Hilsenh.	3,5,6 (Newhart and Mumma 1979)
Dytiscus marginalis L.	3,5,6,7,15 (Dettner 1979; Schildknecht and Weis 1962; Schildknecht et al. 1970)
Dytiscus circumflexus F.	1,2,3,5,6,7,(11) (Dettner 1985)
Dytiscus pisanus Laporte	1,3,5,6,(11),(13) (Dettner 1985)
Dytiscus latissimus L.	3,5,6 (Dettner 1985; Schildknecht 1970)
Eretes sticticus (L.)	(2),3,5,6,(7),(11) (Dettner 1985)
Graphoderus cinereus (L.)	1,(2),3,5,6,7 (Dettner 1979, 1985; Schildknecht 1970)
Graphoderus liberus (Say)	3,5,6 (Miller and Mumma 1973)
Hydaticus seminiger (De Geer)	3,5,6,10,(11)# (Dettner 1979)
Hydaticus leander (Rossi)	2,3,5,(6),10#,(11) (Dettner 1985)
DYTISCIDAE: CYBISTRINAE	
Cybister mesomelas Guignot	3#,6#,10#,11#,
Cybister lateralimarginalis (De Geer)	3,5,6,7,8 (Dettner 1985; Schildknecht 1970)
Cybister tripunctatus (Oliv.)	3,5,6 (Dettner 1985; Schildknecht 1970)
DYTISCIDAE, LACCOPHILINAE	
Laccophilus minutus (L.)	(2),(3),(5),(6),14,17,19,20,22, (Dettner 1985; Schildknecht et al. 1983)
Laccophilus hyalinus (De Geer)	14,17,20,22 (Dettner 1985)

1: 4-hydroxybenzoic acid, **2:** hydroquinone, **3:** benzoic acid, **4:** benzoic acid ethylester, **5:** 4-hydroxybenzaldehyde, **6:** 4-hydroxybenzoic acid methylester, **7:** 3,4-dihydroxybenzoic acid methylester, **8:** 3,4-dihydroxybenzoic acid ethylester, **9:** 2,5-dihydroxyphenylacetic acid methylester, **10:** phenylpropionic acid, **11:** phenylacetic acid, **12:** 4-hydroxyphenylacetic acid, **13:** phenylpyruvic acid, **14:** 3,4-dihydroxyphenylacetic acid methylester, **15:** marginalin (= 4′5-dihydroxy-benzalisocumaranone), **16:** 3-indoleacetic acid, **17:** 3-hydroxyoctanoic acid, **18:** octanoic acid, **19:** *Z*-3-octenoic acid, **20:** 3-hydroxynonanoic acid, **21:** nonanoic acid, **22:** 3-hydroxydecanoic acid, **23:** tiglic acid

(): minor component, without brackets = major component, # new record as compared with Dettner (1985)

tryptophan-metabolite (**16**), and an unusual pigment (**15**) could be identified from this gland system (Table 6.1, Fig. 6.7; e.g., Dettner 1979, 1985; Schildknecht et al. 1983). Since then, several taxa of dytiscid beetles have been checked for their pygidial gland chemistry (Blum 1981; Francke and Dettner 2005) and within insects Dytiscidae represent a valuable source for biosynthesis of various aromatic compounds (Morgan 2004) including 3-indole acetic acid (**16,** Dettner and Schwinger 1977). It is remarkable that a few aromatic main constituents from the pygidial glands (e.g., **5**, **11**) are also present in the thoracic defensive glands of the water bug genera *Ilyocoris* and *Notonecta* or the metapleural glands of various ant genera (see Blum 1981; Staddon and Thorne 1979).

Apart from benzoic acid (**3**) other chemicals (see Fig. 6.7) have been identified including 4-hydroxybenzoic acid (**1**), hydroquinone (**2**), benzoic acid ethylester (**4**), 4-hydroxybenzaldehyde (**5**), 4-hydroxybenzoic acid methylester (**6**), 3,4-dihydroxybenzoic acid methylester (**7**), 3,4-dihydroxybenzoic acid ethylester (**8**), 2,5-dihydroxyphenylacetic acid methylester (**9**), phenylpropionic acid (**10**), phenylacetic acid (**11**), 4-hydroxyphenylacetic acid (**12**), phenylpyruvic acid (**13**), and 3,4-dihydroxyphenylacetic acid methylester (**14**). Remarkably all derivatives of phenylacetic acid (**11**) such as **12** and **13** are typical for the Hydroporinae subfamily (Fig. 6.7) whose representatives share the presence of **11** as a main compound. This strong pleasant odor that is so typical for Hydroporinae is even mentioned in nomenclature. Spangler (1985) described *Hydrodessus fragrans* (now *H. biguttatus* Guignot) due to its strong pleasant fragrance during dissection. This odor is typical for **11** but not for inodorous benzoic acid (**3**). Moreover, gentle molestations of certain living Hydroporinae species, as observed in *Hydroporus lundbladi*, may result in liberation of small amounts of strongly smelling phenylacetic acid from their pygidial gland reservoirs.

Within Colymbetinae and Dytiscinae, phenylacetic acid (**11**) only occurs as a trace constituent (Table 6.1). However, there is one exception, as both species of *Liopterus* (former *Copelatus*) investigated sequester considerable amounts of **11** in their pygidial glands (Figs. 6.7 and 6.8) and are also characterized by the sweetish odor when dissected. In contrast, the ethylester of protocatechuic acid (**8**) was only found in the genus *Cybister* (Table 6.1, Fig. 6.8).

It was suggested that a further aromatic and extremely yellow colored substance from the pygidial glands of *Dytiscus marginalis* (**15,** marginalin, 4′,5-dihydroxybenzalisocumaranone; Schildknecht et al. 1970) was biosynthetically produced from precursors such as 2,5-dihydroxyphenylacetic acid methylester (**9**) and 4-hydroxybenzaldehyde (**5**). Principally both of these aromatics (**5** and **9**) might be produced from a precursor such as 4-hydroxyphenylpyruvic acid. Later on this compound was identified from the pygidial glands of three Agabinae (*Agabus labiatus*, *A. undulatus*, *A. serricornis*; Dettner 1985) that are closely related (Ribera et al. 2004). Moreover, it was shown that the natural marginalin from *Dytiscus* represents an *E*-isomer (Barbier 1987) and may fix solidly on a variety of supports (Barbier 1990). When this compound is distributed on the beetle surface by cleaning behavior, the yellow compound is likely fixed on microorganisms and algae. Marginalin (**15**) is related to aurone, which represents a plant flavonoid that provides yellow coloration to flowers of various ornamental plants. The *Z*-configuration of most aurones represents the more stable configuration.

In addition, phenylpropionic acid (**10**) is typical for the Dytiscinae genera *Hydaticus* and *Cybister* and for one representative of Colymbetinae genus *Ilybius* (Figs. 6.7 and 6.8). In contrast, 3,4-dihydroxyphenylacetic acid methylester (**14**) are restricted to two Laccophilinae species investigated (*Laccophilus minutus, L. hyalinus;* Figs. 6.7 and 6.8). It is astonishing that most Hydroporinae not only produce the sweetish smelling compound **11** but exclusively contain considerable amounts of the tryptophan-derivative 3-indoleacetic acid (**16**), which is also present in Noteridae (Figs. 6.7 and 6.8).

Aliphatic pygidial gland constituents such as 3-hydroxy acids from octanoic (**17**), nonanoic (**20**), and decanoic (**22**) acids are typical for the Laccophilinae and more basally arranged Hydroporinae genera *Hydrovatus* and *Hygrotus* (Table 6.1, Figs. 6.7 and 6.8). These hydroxyacids are also present in pygidial glands from representatives of Haliplidae (Dettner and Böhner 2009) and in metapleural glands

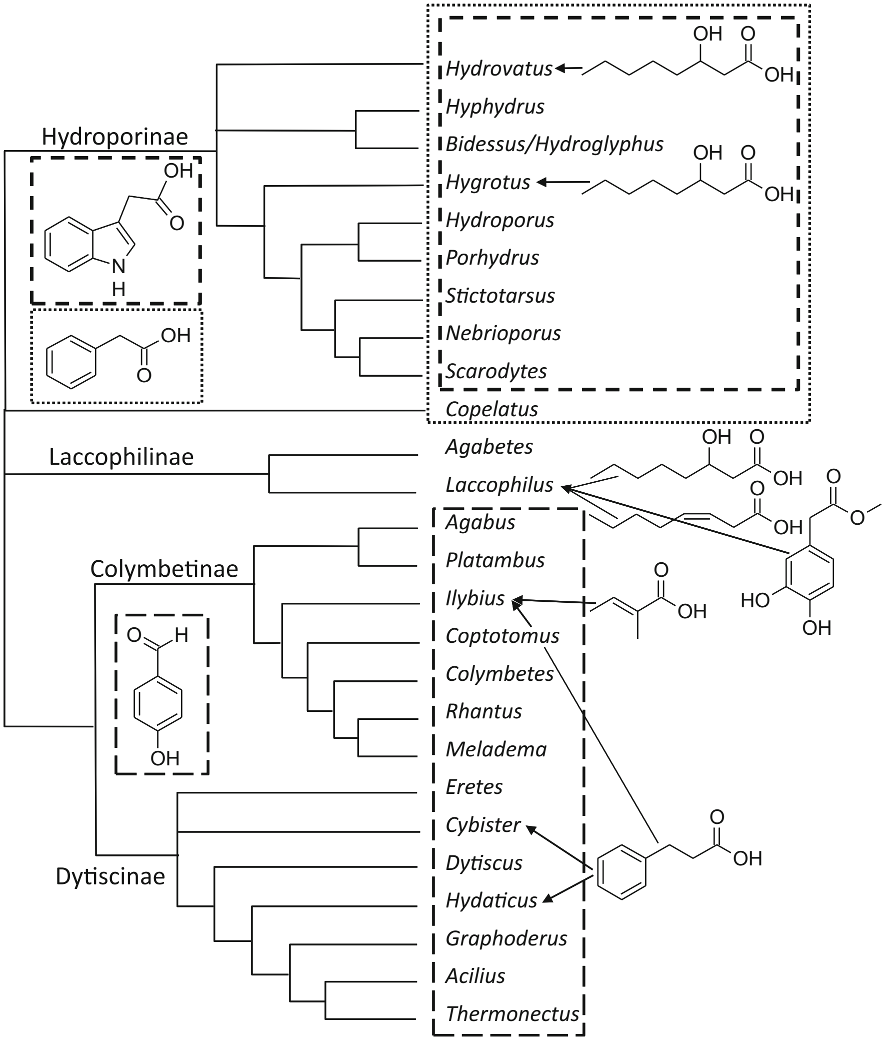


Fig. 6.8 Phylogeny of Dytiscidae genera after Burmeister (1976) and distribution of pygidial gland constituents according to Fig. 6.7. Those compounds which are present in various taxa are figured by boxes. Erratically found chemicals are associated with the genera by arrows. Burmeister's *Potamonectes* was actualized as *Nebrioporus*; *Thermonectes* is now *Thermonectus*

of certain Formicidae (see Blum 1981). Further biosynthetically related acids such as octanic (**18**), 3-octenoic (**19**; Figs. 6.7 and 6.8), and nonanoic (**21**) acids occur in the genera *Hydrovatus*, *Platambus*, and *Laccophilus*. The typical compound of many terrestrial Adephaga (see Blum 1981) that is represented by tiglic acid (**23**) is restricted to two representatives of the genus *Ilybius.*

6.4.2.1.2 Biological Activity of Pygidial Gland Secretions and their Regeneration

The biological significance of the dytiscid pygidial gland secretions is multifunctional. At first, most compounds (apart from marginalin **15** and probably from 3-indoleacetic acid **16**) hitherto identified represent excellent preservatives that are often used in foodstuff industry. This applies especially for both aromatic compounds (**3**, **6,** and **11**) and aliphatic constituents (e.g., **17**, **19**, **20**, **22)** (Dettner 1985; Dettner and Böhner 2009). These compounds are fungicides and bactericides and show an inhibition on germination and growth of plants. Even Z-3-octenoid acid (**19**) chemically resembles the well-known preservative sorbic acid (E,E-2,4-hexadienoic acid). The role of the plant hormone 3-indoleacetic acid (**16**) in hydroporine pygidial glands remains enigmatic. One specimen of *Stictotarsus duodecimpustulatus* sequesters the same amount of compound **16** which can be isolated from 68,000 *Avena* coleoptiles, representing a rich plant source for this compound (Dettner and Schwinger 1977). This plant hormone is found in various gall-forming insects and from the metathoracic glands of few ant species (together with phenylacetic acid **11**). However, there are no gall-forming hydroporine species known. Therefore 3-indoleacetic acid in predaceous diving beetles may represent a soft preservative especially if used together with compound **11.** Finally, derivatives of tryptophan such as 3-indoleacetic acid may represent important excretional products in insects (Cochran 1975).

To distribute their pygidial gland secretions on their body surfaces, dytiscid beetles leave the water. As early as 1967, Maschwitz described this behavior and suggested that these antimicrobial secretions serve to protect the beetles from bacteria and even peritrichic ciliates. This possible protection is illustrated when aqueous dytiscid beetle pygidial gland secretions are tested against the protozoans *Stentor coeruleus* and *Paramecium caudatum* (Fig. 6.9; Cichon, Schneider & Dettner, in preparation). The behavior of both protozoans was recorded under the microscope as activity of cilia at 20 °C. In both species, diluted aqueous solutions (1: 20, v/v) of gland constituents significantly reduced activity of cilia with a stronger effect in *S. coeruleus*, suggesting a negative effect of the beetle secretions on ciliates.

The pygidial gland reservoirs of dytiscids either contain fluids or solid paste-like secretions (Fig. 6.6b). Very often two organic phases, a solid and a fluid, are present within the reservoir (Fig. 6.6c). Depending on their viscosities, the pygidial gland secretions are partly depleted after molestations. Usually only small amounts of the reservoir may be depleted (~13%; Classen and Dettner 1983; Dettner 1985) and therefore the pygidial gland secretions of dytiscids likely do not represent defensive secretions against larger predators.

It is remarkable that the above-mentioned secretion-grooming is also observed under water while the beetles clasp onto water plants or other structure. Kovac and Maschwitz (1990) described this behavior as secretion-grooming, and suggested that the secretion is used to hydrofuge sensitive body parts such as spiraculi and subelytral tergal respiratory structures. However, when contact angles of definite water droplets on elytral surfaces were carefully measured under a contact angle

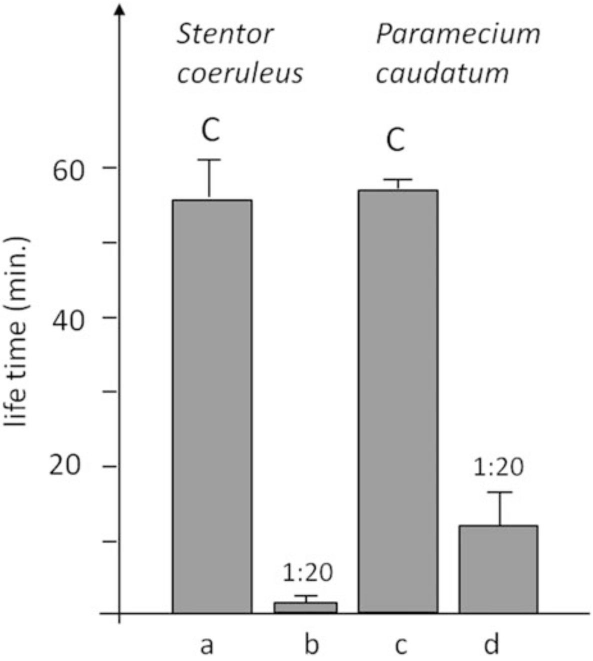


Fig. 6.9 Efficiency of water beetle pygidial gland secretions on protozoans *Stentor coeruleus* (**a–b**) and *Paramecium caudatum* (**c–d**) measured as life time (activity of cilia) at 20 °C. Columns **a**, **c**: water controls; Columns **b**, **d**: secretion of three pygidial gland reservoirs of *Acilius sulcatus* (1:20, v/v)

microscope, all secretions tested from hydradephagan beetles showed a drastic reduction of the contact angle as compared with an untreated elytron of the same beetle specimen when the corresponding second elytron was previously treated with minute amounts of pygidial gland secretion (Dettner 1985; Fig. 6.10). The effect of both pygidial and prothoracic gland secretions on contact angles of water droplets is evident (Fig. 6.10) (Schneider 2008). Male *Acilius sulcatus* possess smooth elytra, whereas females are characterized by grooved and hairy elytra. Therefore, the contact angles of water droplets on female elytral surfaces are distinctly lower than on male elytra. When treated with prothoracic gland secretions both in males and females results in a drastic reduction of contact angles that is more evident in males with their smooth elytra than in females with hairy grooved elytra (Schneider 2008).

Because the contact angle of water on solid surfaces depends both on the surface structure of the elytral epicuticle and from the degree of biofilms on these elytral surfaces, only one freshly collected beetle specimen was used per measurement (Dettner 1985). The wettability after the elytron was treated with gland substance was seen in different species and specimens independently from their pygidial gland chemistries. Even marginalin (**15**), the pigment from the pygidial and preputial glands of *Dytiscus* and few *Agabus* species may significantly lower the contact angle of a water droplet that was placed on a cleaned glass surface (Fig. 6.11). In addition, there was also a significant decrease of the contact angle of 4-hydroxybenzaldehyde (**5**), the main aldehyde of many dytiscid pygidial glands (Fig. 6.11). As many pygidial gland components are amphiphilic (i.e., have a

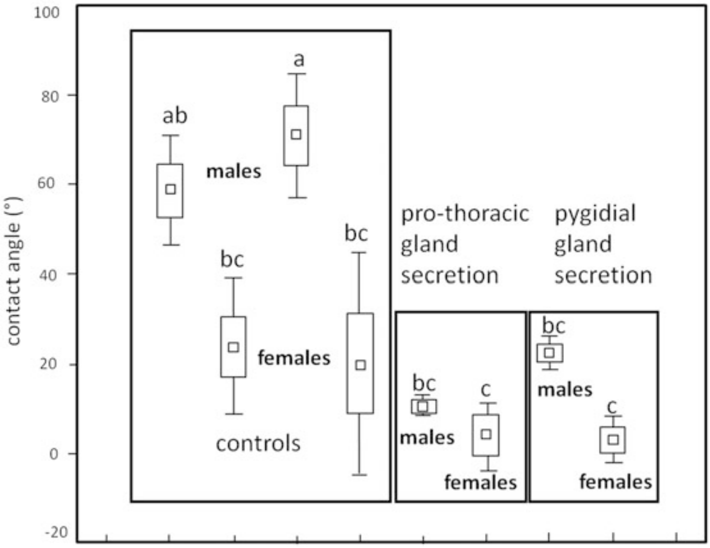


Fig. 6.10 Contact angles of water droplets placed on elytral surfaces of *Acilius sulcatus*. Left box: left elytron (first line) and right elytron (third line) of males; left elytron (second line) and right elytron (fourth line) of females, central box: effect of prothoracic gland secretion on a contact angles of male and female *Acilius* beetles, right box: effect on pygidial gland secretions of male and female (□: standard error and I: standard deviation; Schneider 2008)

lipophilic and hydrophilous part of the molecule) the increase of wettability of a more or less hydrophilous epicuticle after treatment with benzoic (**3**), phenylacetic (**11**), or aliphatic 3-hydroxy acids (**17,20,22**) seems plausible.

In addition to the above-mentioned low molecular compounds, pygidial gland secretions of dytiscids also contain marginalin (**15**) and a glycoproteid consisting of 18 amino acids (Schildknecht and Bühner 1968). The glycoproteid from *Dytiscus marginalis* was shown to contain d-glucose, d-mannose, d-ribose, and the gamma-lactone of glucuronic acid (Schildknecht and Bühner 1969). As described above, marginalin may act as a fixative. In the same way the glycoproteid forms a coherent film (see electron microscopic data in Schildknecht and Bühner 1968), when applied on a glass surface and may fix the low molecular bactericides and fungicides on the beetles surface. In addition, the 3-hydroxy acids **17**, **20**, and **22** may form polyesters that can either fix the metabolites or entangle epizoic microorganisms on the beetles surfaces (Dettner and Böhner 2009).

In general pygidial gland secretion may influence the settlement of external organisms ranging from bacteria to eukaryotic parasites such as Protozoa (Lust 1950; Matthes 1982), fungi (Laboulbeniales, Scheloske 1969), and aquatic mites (Davids et al. 2007). Prothoracic gland secretion was more effective against *Stentor* and *Paramecium* as compared with pygidial gland material (see. 4.2.2.2; Fig. 6.9). Scheloske (1969) found that specimens of Hydroporinae (from 416 specimens 13.0% were parasitized) and Laccophilinae (from 173 specimens 16.8% were

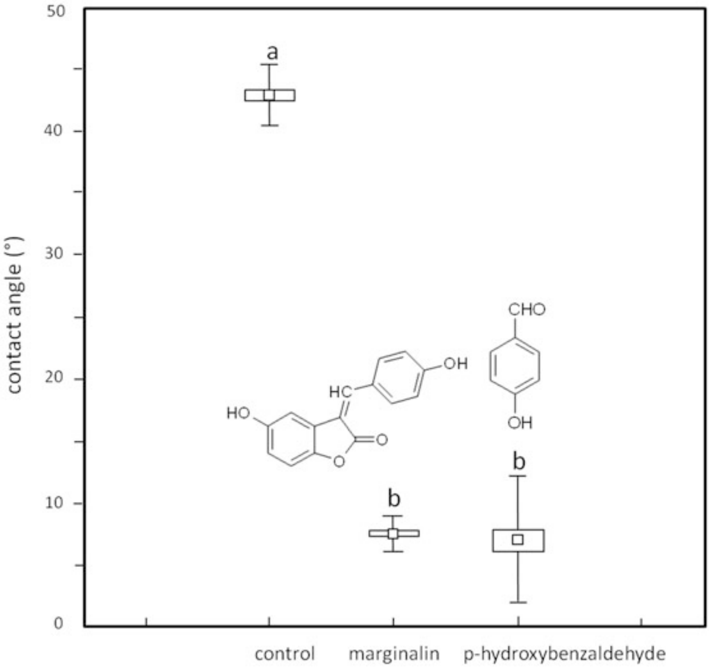


Fig. 6.11 Contact angles of water droplets placed on cleaned glass surfaces (±: standard error and ±: standard deviation; Schneider 2008) which were previously treated with aqueous mixtures of marginalin (**15**) and 4-hydroxybenzaldehyde (**5**). Controls represent untreated glass surfaces

parasitized) showed increased parasitism by Laboulbeniales as compared with Colymbetinae and Dytiscinae (from 815 specimens 10.2% were parasitized). He suggested that the significantly differing pygidial gland compounds, specifically the missing compounds **3**, **5**, and **6** in Hydroporinae may be responsible for this effect (Scheloske 1969). However, he also mentioned that the role of prothoracic gland secretions against Laboulbeniales remains unknown.

In contrast to organisms that settle on the surface of adult dytiscids or their larvae, internal parasites such as hairworms (e.g., *Gordius* and allied genera; Blunck 1922a), trematodes (e.g., Peters 1957; Bray et al. 2012), or gregarines (Geus 1969; Blunck 1923b) are probably not targeted by these glandular secretions. However, it should be investigated if beetles also take up these exocrine secretions orally. In addition, it would be intriguing if maternally derived prothoracic or pygidial gland secretions have any effect on those species of proctrotrupid and chalcid Hymenoptera that parasitize submersed dytiscid eggs.

Seasonal fluctuations of pygidial gland titers were described in the genera *Acilius* (Newhart and Mumma 1979) and *Agabus* (Classen and Dettner 1983). It is unlikely that these fluctuations reflect different degrees of utilization of the gland material, but mainly reflect different age structures of the adult beetles analyzed during a season. It was shown that the secretions of young male and female beetles as determined by analysis of their internal sexual organs quantitatively and qualitatively differ from secretions of older beetle specimens (Classen and Dettner 1983; Dettner 1985), a fact that is probably due to different biosynthetic capacities of beetles of different ages. For example, freshly hatched male and female *Agabus bipustulatus* and *A. paludosus* produce very low amounts of compounds **2**, **3**, **5**, **6**, **7**, whereas older specimens of both species and sexes produce more aromatics per individual with the aldehyde **5** as a main constituent.

Activities of water beetle pygidial gland secretions on other targets are unknown. However, Lousia et al. (2010) reported that pygidial gland secretions resulted in histopathological changes in male accessory glands of *Odontopus varicornis* (Heteroptera, Pyrrhocoridae). These histological changes were described as disintegration of epithelia, disorganized tissues, swollen nuclei, vacuolized cytoplasm, pycnotic and necrotic epithelia, and enlargement of epithelial cells. The effect of these pygidial glands remain one of the largest understudied and potentially most interesting aspect of dytiscid chemical ecology.

6.4.2.2 Prothoracic Defensive Glands

Principally paired endocrine prothoracic glands are present in all insects where they are located within thorax or posterior area of head. These prothoracic glands represent hormone glands and secrete the ecdysteroid ecdysone which is also called molting hormone and elicits the molting process. Insects such as Zygentoma which molt continuously also as adults possess functioning prothoracic glands. Within Pterygota prothoracic glands are reduced during metamorphosis. Within Coleoptera these endocrine glands degenerate during adult or even pupal stage. Therefore, adult beetles have no functioning prothoracic glands which are derived from second maxillary segment and have nerval endings from first thoracic ganglion (Gersch 1964). Apart from Dytiscidae large exocrine and functioning prothoracic glands in adult beetles are only known from Tenebrioninae, Nilioninae, and Alleculinae (Dettner 1987). Exocrine prothoracic glands (Figs. 6.12, 6.13 and 6.14) are only present within a small fraction of hydradephagan beetles (Dettner 1985, 2019b). As reported by Beutel et al. (2006) prothoracic defensive glands are absent in Meruidae, Gyrinidae, and Noteridae, the latter representing the sister group of Dytiscidae. In addition, due to the absence of the prothoracic defensive glands, Aspidytidae (Ribera et al. 2002) are excluded from Dytiscidae and Hygrobiidae, which are both characterized by these peculiar thoracic complex glands (Dettner 1987; Forsyth 1968, 1970). According to the phylogeny of aquatic Adephaga (Beutel et al. 2006), Dytiscidae and Hygrobiidae represent sister groups, Amphizoidae, with no prothoracic defensive glands represent the sister group of Dytiscidae + Hygrobiidae, whereas Aspidytidae form a sister of (Dytiscidae + Hygrobiidae) and Amphizoidae. Forsyth (1970) suggested that the homology of the prothoracic defensive glands between Hygrobiidae and Dytiscidae (Colymbetinae, Hydroporinae, Laccophilinae, Dytiscinae) is uncertain. In Hygrobiidae the prothoracic defensive glands open near

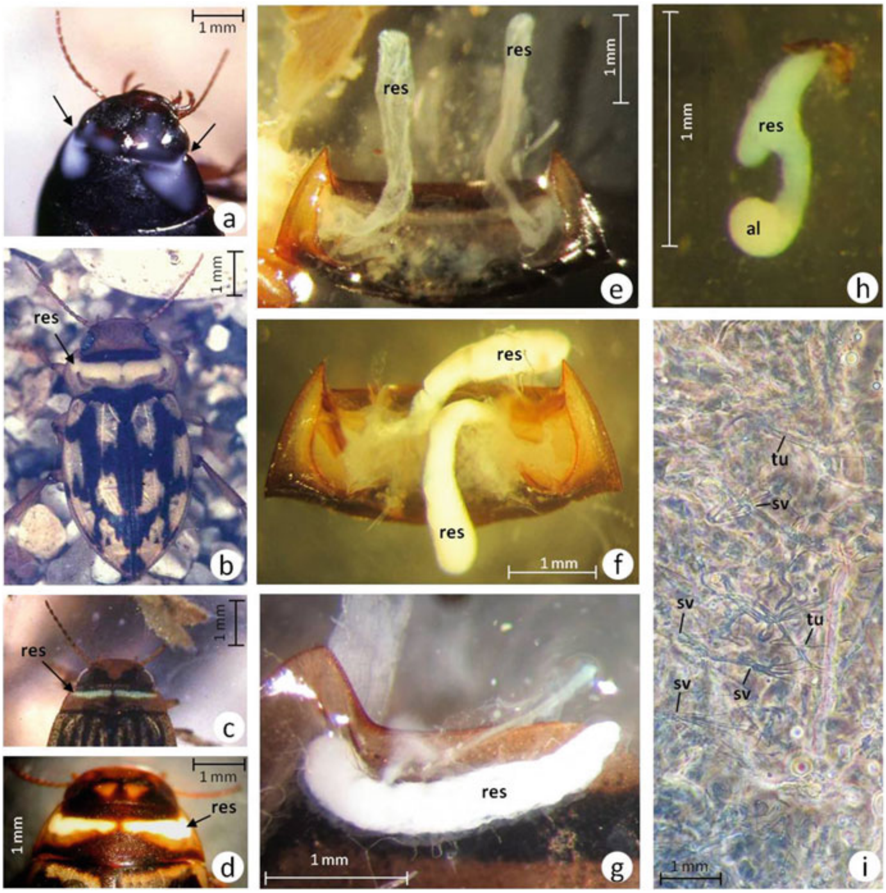


Fig. 6.12 Structure of prothoracic defensive glands of dytiscid beetles. (**a**): Molested specimen of *Ilybius* spec. depleting milky fluid from its paired prothoracic glands. (**b–d**): Prothoracic defensive glands of *Stictotarsus duodecimpustulatus* (**b**), *Hygrotus impressopunctatus* (**c**), *Platambus maculatus* (**d**). Prepared prothoracic defensive glands of *Ilybius fenestratus* (**e**), *Platambus maculatus* (**f**), *Acilius canaliculatus* (**g**), and *Hygrotus inaequalis* (**h**). Abbreviations: *res* reservoir of prothoracic defensive gland, *al* apical limb of reservoir, Squeeze preparation of prothoracic defensive gland tissue of *Hydaticus seminiger* with tubules (tu) and sieve plates (sv) (**i**)

the posterolateral angle of pronotum, in contrast gland reservoirs in Dytiscidae open close to the anterolateral angle of the prothorax (Forsyth 1970).

Both the depletion and chemistry of prothoracic glands of Hygrobiidae are unknown. Therefore, it is important to observe representatives of the above-mentioned Colymbetinae, Hydroporinae, Laccophilinae, and Dytiscinae. When disturbed these dytiscids deplete their milky secretions from their prothoracic defensive glands (see *Ilybius* species Fig. 6.12a). Predaceous diving beetles fixated in ethanol usually show adhering droplets of partly denaturated proteinaceous secretions between the posterior border of head and anterior borders of prothorax. The paired

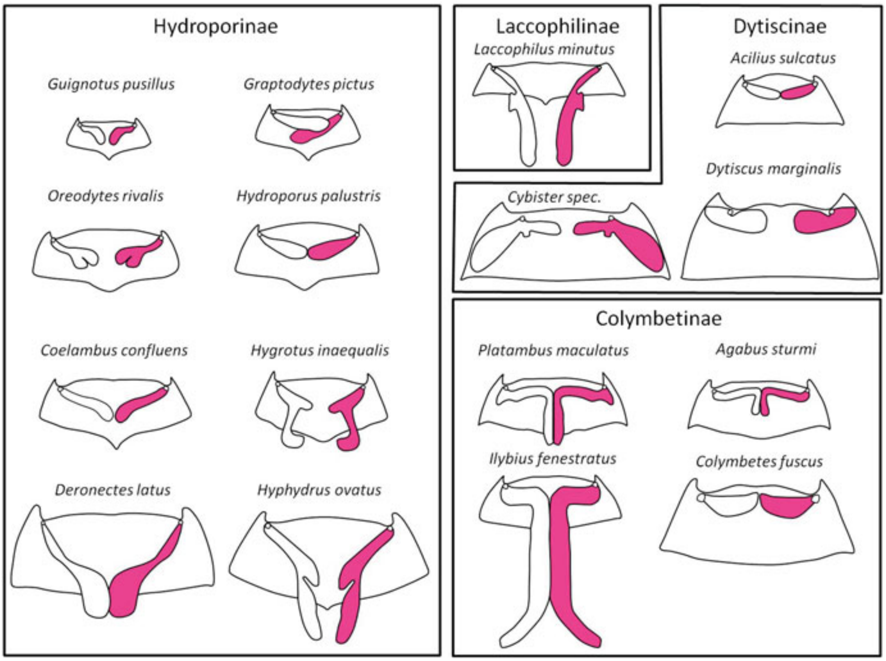


Fig. 6.13 Size and position of prothoracic defensive gland reservoirs in Hydroporinae, Laccophilinae, Dytiscinae, and Colymbetinae

prothoracic defensive glands are sac-like structures (Figs. 6.12, 6.13 and 6.14) and are usually restricted to the anterior border of the prothorax as can be observed in *Stictotarsus* (Fig. 6.12b), *Hygrotus* (*Coelambus*) (Fig. 6.12c), *Platambus* (Fig. 6.12d), or *Acilius* (Fig. 6.12g). Openings of the reservoirs are located dorsolaterally on the cervical membrane of pronotum (Figs. 6.12, 6.13 and 6.14). In several genera such as *Oreodytes* (Fig. 6.13), *Hygrotus* s. str. (Figs. 6.12h and 6.13), *Hyphydrus* (Fig. 6.13), and partly *Laccophilus* (Fig. 6.13) reservoirs are branched. In *Cybister* (Fig. 6.13), *Dytiscus* (Fig. 6.13), and *Hydaticus* (not shown) reservoir openings are shifted more centrally and open near a tooth-like posterior projection of the anterior pronotal border. Prothoracic gland reservoirs are not covered by muscle layers as in pygidial glands (Forsyth 1968), however depletion of reservoirs is achieved by increasing of internal turgor pressure and by contraction of tergo-sternal muscles (Forsyth 1968). Discharge of secretions is finally controlled by a single muscle that has its origin on the cervical membrane.

The gland cells cover the surface of the reservoirs partly or completely depending on species. For example, in *Hygrotus inaequalis* the prothoracic gland reservoir is covered by clusters of gland cells, however an apical limb of reservoir has no glandular cells (Figs. 6.12h, 6.13 and 6.14a). As already described by Forsyth (1968), secretory cells show tubuli that are connected with a typical end apparatus (Figs. 6.12i and 6.14c). Of note is that every gland cell opens individually into the

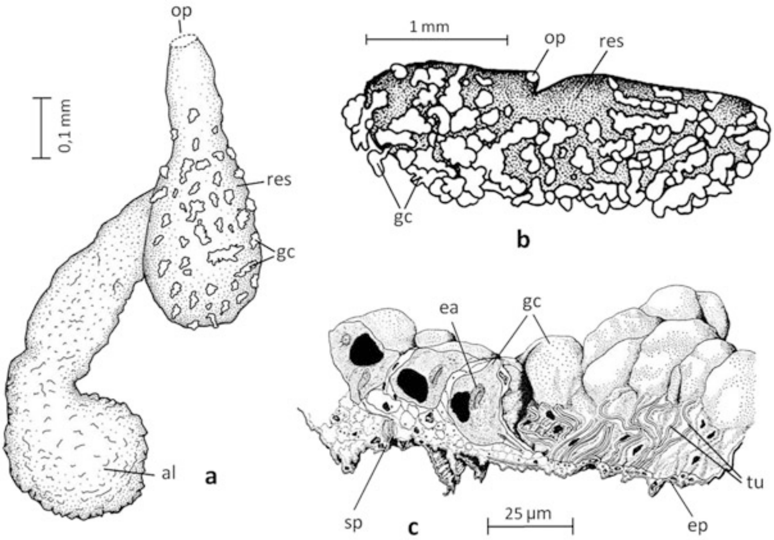


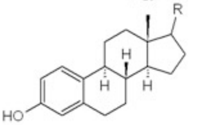
Fig. 6.14 Histology of prothoracic defensive glands. (**a**): *Hygrotus inaequalis*, (**b**): *Dytiscus marginalis* (after Korschelt 1923), (**c**): Section through prothoracic defensive gland of *Hyphydrus ovatus* (modified after Forsyth 1968). Abbreviations *al* apical limb, *ea.* end apparatus, *ep* epidermis, *gc* gland cell, *op* opening of reservoir, *sp.* sieve plate, *tu* tubule. Nuclei are black

prothoracic defensive gland reservoir on circular sieve plates (Figs. 6.12i and 6.14c) covering about 5 to 8 tubules. Sometimes pointed internal projections of the prothoracic defensive gland reservoir are present (e.g., in *Hyphydrus*, Forsyth 1968).

6.4.2.2.1 Chemistry of the Prothoracic Defensive Glands, Emphasizing those Species with Steroidal Vertebrate Hormones

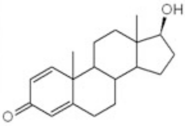
During recent years, few insect taxa were shown to produce steroids that are normally essential for insects (Behmer and Nes 2003; Svoboda 1997; Swevers et al. 1991). These include several chrysomelid (Chrysomelidae, Laurent et al. 2005), carrion (Silphidae, Staphylinidae, Eisner et al. 2005), and lampyrid beetles (Lampyridae, Laurent et al. 2005; Gronquist et al. 2005), as well as giant water bugs (Belostomatidae, Eisner et al. 2005). In some cases, several steroids have been chemically characterized (e.g., toxic steroidal pyrones (lucibufagins) in lampyrid beetles across their developmental stages (Eisner et al. 2005)). As mentioned above, the prothoracic defensive glands of dytiscids produce an impressive array of known vertebrate steroidal hormones together with many novel steroids and these beetles are unique in manufacturing specific steroids including C_{18}, $C_{19,}$ and C_{21} skeletons (Fig. 6.15, Table 6.2). In both predaceous diving beetles and belostomatid bugs some of these molecules are assumed to be synthesized from cholesterol that is acquired from their prey (Eisner et al. 2005).

estratriene (C_{18})

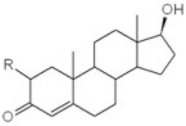


[24]: R: =O: 3-hydroxyestra-1,3,5(10)-trien-17-one; estrone
[25]: R: -OH: 3,17ß-dihydroxyestra-1,3,5(10)-triene; 17ß-estradiol

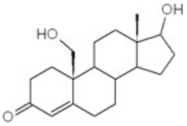
androstene/androstadiene (C_{19})



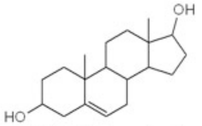
[26]: 17ß-hydroandrost-1,4-dien-3-one; boldenone



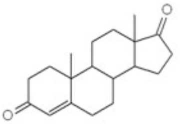
[27]: R: - OH: 2α,17ß-dihydroxyandrost-4-ene-3-one; 2α-hydroxytestosterone
[28]: 2ß,17ß-dihydroxyandrost-4-ene-3-one; 2ß-hydroxytestosterone
[29]: R: -H: 17ß-hydroxyandrost-4-ene-3-one; testosterone



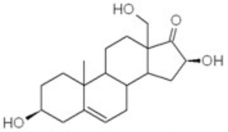
[30]: 17ß,19-dihydroxyandrost-4-ene-3-one; 19-hydroxytestosterone



[31]: 3ß,17ß, dihydroxyandrost-5-ene
[32]: 3α,17ß, dihydroxyandrost-5-ene
[33]: 3ß,17α, dihydroxyandrost-5-ene

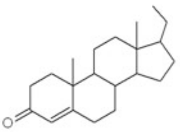


[34]: androst-4-ene-3,17-dione

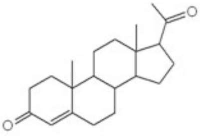


[35]: 3ß,16ß,18-trihydroxyandrost-5-ene-17-one

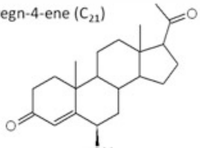
pregn-4-ene (C_{21})



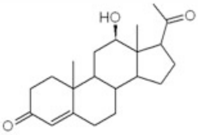
[36]: pregn-4-ene-3-one



[37]: pregn-4-ene-3,20-dione; progesterone

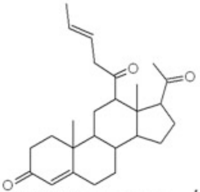


[38]: 6ß-hydroxypregn-4-ene-3,20-dione; 6ß-hydroxyprogesterone

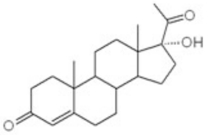


[39]: 12ß-hydroxypregne-4-ene-3,20-dione; 12ß-hydroxyprogesterone

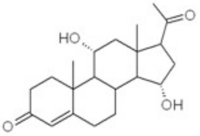
pregn-4-ene (C_{21})



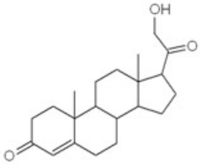
[40]: 12ß-(3'pentenoyl-)pregn-4-ene-3,20-dione



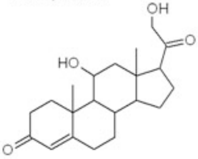
[41]: 17α-hydroxypregn-4-ene-3,20-dione; 17α-hydroxyprogesterone



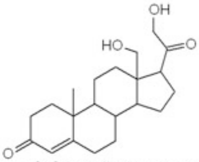
[42]: 11,15-dihydroxypregn-4-ene-3,20-dione; 11,15-dihydroxyprogesterone



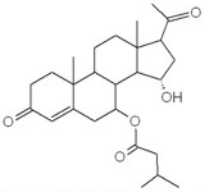
[43]: 21-hydroxypregn-4-ene-3,20-dione;
21-hydroxyprogesterone;
cortexone; deoxycorticosterone



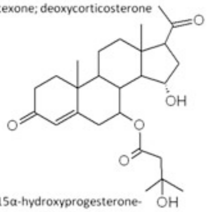
[44]: 11α,21-dihydroxypregn-4-ene-3,20-dione; 11α,21-dihydroxyprogesterone ;
[45]: 11ß,21-dihydroxypregn-4-ene-3,20-dione; 11ß,21-dihydroxyprogesterone; corticosterone



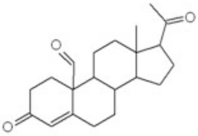
[46]: 18,21-dihydroxypregn-4-ene-3,20-dione;
18,21-dihydroxyprogesterone



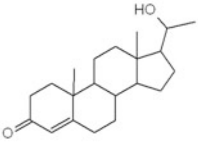
[47]: 15α-hydroxyprogesterone-7α-isobutyrate



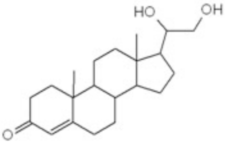
[48]: 15α-hydroxyprogesterone-7α-hydroxyisobutyrate



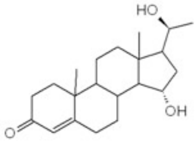
[49]: pregn-4-ene-3,19,20-trione; 19-oxoprogesterone



[50]: 20α-hydroxypregn-4-ene-3-one
[51]: 20ß-hydroxypregn-4-ene-3-one

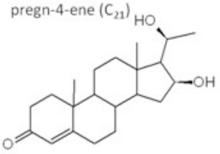


[52]: 20 α,21-dihydroxypregn-4-ene-3-one
[53]: 20ß,21-dihydroxypregn-4-ene-3-one

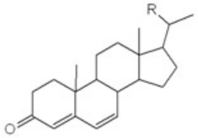


[54]: 15α,20ß-dihydroxypregn-4-ene-3-one

Fig. 6.15 Constituents of prothoracic defensive glands (**24–108**) from dytiscid beetles with continuations pregn-4-ene (C_{21})

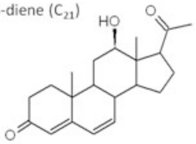


[55]: 16ß,20ß-dihydroxypregn-4-ene-3-one

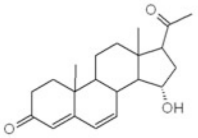


[56]: R = O; pregna-4,6-diene-3,20-dione; 6-dehydroprogesterone
[57]: R = OH; 20α-hydroxypregna-4,6-diene-3-one; cybisterone

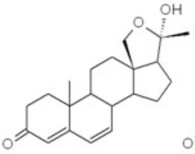
pregna-4,6-diene (C_{21})



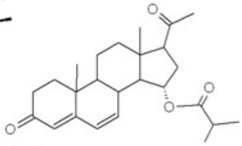
[58]: 12ß-hydroxypregna-4,6-diene-3,20-dione; cybisterol



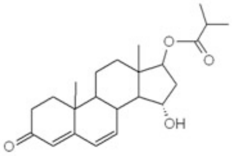
[59]: 15α-hydroxypregna-4,6-diene-3,20-one



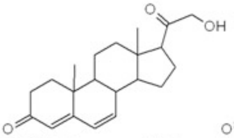
[60]: 18-hydroxypregna-4,6-diene-3-one-18,20-hemiketal; mirasorvone



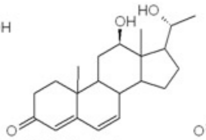
[61]: pregna-4,6-diene-3,20-dione-15α-isobutyrate



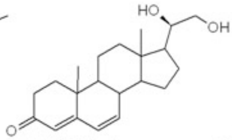
[62]: 15α-hydroxypregna-4,6-diene-3-on-20-isobutyrate



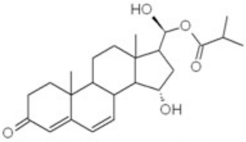
[63]: 21-hydroxypregna-4,6-diene-3,20-dione; 6,7-dehydrocortexone



[64]: 12ß,20α-dihydroxypregna-4,6-diene-3-one

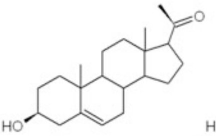


[65]: 20ß,21-dihydroxypregna-4,6-diene-3-one

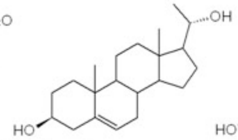


[66]: 15a,20ß-dihydroxypregna-4,6-diene-3-one-20-isobutyrate

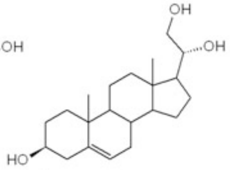
pregn-5-ene (C_{21})



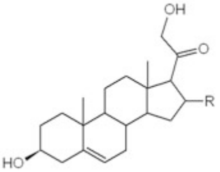
[67]: 3ß-hydroxypregn-5-ene-20-one; pregnenolone



[68]: 3ß,20α-dihydroxypregn-5-ene

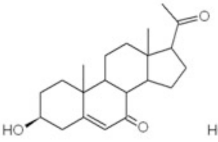


[69]: 3ß,20α, 21-trihydroxypregn-5-ene

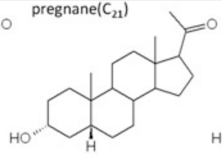


[70]: R = H: 3ß,21-dihydroxypregn-5-ene-20-one; 21-hydroxypregnenolone
[71]: R = OH: 3ß,16α,21-trihydroxypregn-5-ene-20-one; 16α,21-dihydroxypregnenolone

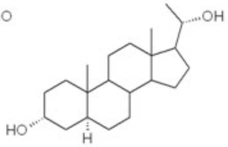
pregnane(C_{21})



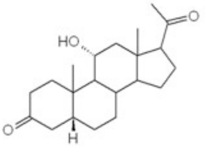
[72]: 3ß-hydroxypregn-5-ene-7,20-dione; 7-oxopregnenolone



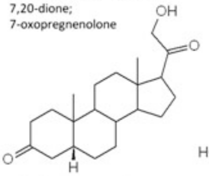
[73]: 3α-hydroxy-5ß-pregnane-20-one



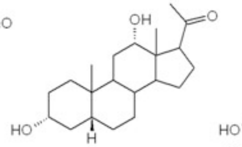
[74]: 3α,20α -dihydroxy-5α-pregnane



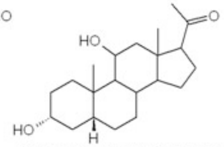
[75]: 11α-hydroxy-5ß-pregnane-3,20-dione



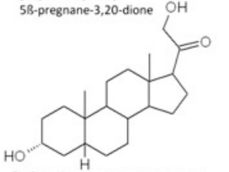
[76]: 21-hydroxy-5ß-pregnane-3,20-dione



[77]: 3α,12 α -dihydroxy-5ß-pregnane-20-one



[78]: 3α,11α-dihydroxy-5ß-pregnane-20-one
[79]: 3α,11ß-dihydroxy-5ß-pregnane-20-one



[80]: 3α,21-dihydroxy-5α-pregnane-20-one
[81]: 3α,21 -dihydroxy-5ß-pregnane -20-one

Fig. 6.15 (continued)

What follows is an examination of the chemistry and biological significance of selected prothoracic defensive gland constituents of predaceous diving beetles that especially act as vertebrate hormones. Specifically, I describe estradienes (**24–25**), androstenes/androstadienes (**26–35**), pregnanes (**72–84**) pregnenes (**36–55, 67–71**), pregnadienes (**56–66**), and other major groups (Fig. 6.15, Table 6.3). In addition, the utilization of predaceous diving beetles as drugs administered to vertebrates is discussed. The significance of these gland constituents for water beetles is reported in Sect. 6.4.2.2.2. Finally, non-steroidal (**87–108**) prothoracic defensive gland constituents are reported.

A considerable fraction of steroids from prothoracic defensive glands in predaceous diving beetles represent well-known sexual (estrogens**:** **24,25**, androgens**:** **26,29,34**), mineralocorticoid (**43**), or glucocorticoid (**45**) hormones in vertebrates. Table 6.3 summarizes those beetle steroids that occur within vertebrates or act as vertebrates hormones. In vertebrate blood androgens or estrogens are bound to globulins which are produced in the liver. Inactivation of steroid hormones in vertebrates takes place in the liver, subsequently there follows excretion via urine or bilefluid (Kleine and Rossmanith 2021). These vertebrate hormones certainly exhibit no hormonal activities in these beetles. In addition, there exist many steroids in predaceous diving beetles whose hormonal or other activities on both vertebrates and invertebrates are unknown (**27, 28, 30–33, 35, 36, 38–40, 42, 44, 46–49, 52–56,**

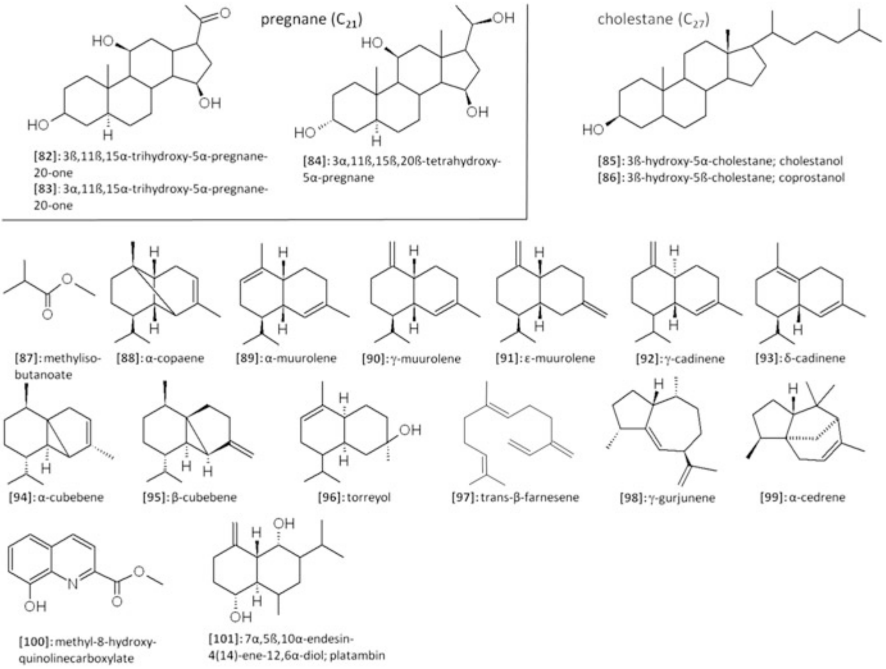


Fig. 6.15 (continued)

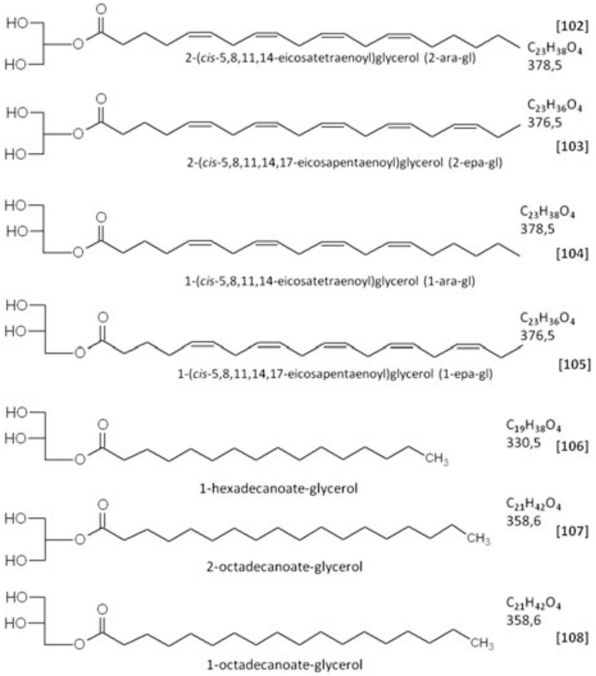


Fig. 6.15 (continued)

59, 60, 62–69, 72–84, 85, 86, 109; see Fig. 6.15). However, there exist interesting reports, where predaceous diving beetles are utilized as hormonal drugs for humans and other mammals. Therefore, these data are critically discussed with respect to the distribution of prothoracic defensive gland constituents in Dytiscidae (Table 6.2).

In a rather interesting (if not perplexing) use, in East Africa predaceous diving beetles (along with whirligig beetles (Gyrinidae) and larvae of ant lions (Myrmeleontidae, Neuroptera)) are preferably collected by young girls who use them to stimulate breast development (Yee 2014). The girls place the insects on their breasts are at first mechanically stimulated by them using the arthropods mouthparts and surfaces and they subsequently apply the secretions from prothoracic and pygidial glands. This procedure is claimed to be an efficient method to stimulate breast growth in these adolescent girls (Kutalek and Kassa 2005), however the results are anecdotal at best. As this activity is widespread in Africa among many ethnic groups it is worthwhile to search for the possible scientific base of this ethnobiologically important behavior, and I explore some of this background in more detail here.

Table 6.2 Constituents of prothoracic defensive glands of predaceous diving beetles

DYTISCIDAE: COLYMBETINAE	
Colymbetes fuscus (L.)	Colymbetin (Schildknecht and Tacheci 1970, 1971)
DYTISCIDAE: AGABINAE	
Agabus affinis (Paykull)	59,102,103,104,105 (Schaaf and Dettner 2000a; Baumgarten et al. 1997; Schaaf 1998)
Agabus bipustulatus (L.)	43,45,50,52,64,80,81 (Schildknecht and Hotz 1970a; Jungnickel 1998; Baumgarten 1995)
Agabus brunneus (F.)	70,73,77,85,86 (Schaaf 1998)
Agabus congener (Thumb)	59,78,104,105 (Schaaf 1998)
Agabus didymus (Ol.)	70,76,81 (Schaaf 1998)
Agabus guttatus (Payk.)	25,29,38,55,68,73,74,80,82,83,84 (Jungnickel and Dettner 1997)
Agabus melanarius Aubé	43, 34 (Jungnickel 1998)
Agabus nebulosus (Forst.)	41,43,44,46,80 (Jungnickel 1998)
Agabus sturmii (Gyll.)	47,48,42,59,61,62, 66 (Baumgarten 1995; Schildknecht and Hotz 1970b)
Agabus undulatus (Schrank)	25,29 (Jungnickel 1998)
Agabus unguicularis (Thoms.)	25,29,43 (Jungnickel 1998)
Ilybiosoma seriatum (Say)	43 (Fescemyer and Mumma 1983; Miller and Mumma 1973,
Ilybius ater (De Geer)	25,29,26,27,28,33 (Jungnickel 1998)
Ilybius crassus Thoms.	25,29,26,27,28,30,35,44,53,57,63,65,72,71 (Jungnickel 1998)
Ilybius fenestratus (F.)	24,25,29,26,27,28,30,31,32,50,53,54,69,71,87,88,89,90,91,92,93,94,95,96,97,98,99,100 (Schildknecht 1977; Schildknecht et al. 1967a, 1971; Schildknecht and Birringer 1969; Jungnickel 1992, 1998)
Ilybius fuliginosus (F.)	29,30,33 (Schildknecht et al. 1967a; Jungnickel 1998)
Ilybius guttiger (Gyll.)	25,29,26,27 (Jungnickel 1998)

(continued)

Table 6.2 (continued)

Ilybius hozgargantae (Burm.)	44,51 (Schaaf 1998)
Platambus maculatus (L.)	54,80,94,101,109 (Schildknecht et al. 1969, 1975; Schaaf 1998)
DYTISCIDAE, DYTISCINAE	
Acilius mediatus (Say)	Unknown steroid (Newhart and Mumma 1979)
Acilius semisulcatus Aubé	43, unknown steroid (Newhart and Mumma 1979; Miller and Mumma 1976a)
Acilius sulcatus (L.)	43,44,49,50,51,56,57,63 (Schildknecht et al. 1967b; Chapman et al. 1977; Jungnickel 1998; Baumgarten 1995)
Acilius sylvanus Hilsenhoff	unknown steroid (Newhart and Mumma 1979)
Dytiscus marginalis L.	43,50,52,53,57,68 (Schildknecht 1966; Schildknecht et al. 1966; Schildknecht and Hotz 1967; Jungnickel 1998; Baumgarten 1995)
Dytiscus pisanus Laporte	36,37,43,56,57 (Schaaf 1998)
Graphoderus cinereus (L.)	67,75,78 (Schaaf et al. 2000)
Graphoderus liberus (Say)	43 (Miller and Mumma 1973; Baumgarten 1995)
Thermonectus marmoratus (Gray)	57, 60 (Meinwald et al. 1998)
DYTISCINAE: CYBISTRINAE	
Cybister confusus Sharp	43 (Chadha et al. 1970; Baumgarten 1995)
Cybister lateralimarginalis (De Geer)	39,40,43,57,58,63,64 (Schildknecht et al. 1967c; Baumgarten 1995)
Cybister limbatus (F.)	39,43,50,57,58,63 (Chadha et al. 1970; Sipahimalani et al. 1970; Baumgarten 1995)
Cybister mesomelas Guignot	Benzoic acid, pentadecanoic acid, octadecanoic acid (Dettner Guignot unpublished)
Cybister tripunctatus (Olivier)	43,51,58,63 (Chadha et al. 1970; Baumgarten 1995)
Cybister spec. Mexico	58,63 (Schildknecht and Körnig 1968)

DYTISCIDAE, LACCOPHILINAE	
Laccophilus minutus (L.)	73,77 (Schaaf et al. 2000; Baumgarten et al. 1997)
DYTISCIDAE, HYDROPORINAE	
Hyphydrus ovatus (L.)	106,107,108, (Baumgarten et al. 1997; Baumgarten 1995)

24: 3-hydroxyestra-1,3,5(10)-trien-17-one; estrone, **25:** 3,17ß-dihydroxyestra-1,3,5(10)-triene; 17ß-estradiol, **26:** 17ß-hydroandrost-1,4-dien-3-one; boldenon, **27:** 2α,17ß-dihydroxy-androst-4-ene-3-one; 2α-hydroxytestosterone, **28:** 2ß,17ß-dihydroxyandrost-4-ene-3-one; 2ß-hydroxytestosterone **29:** 17-ß-hydroxyandrost-4-ene-3-one; testosterone, **30:** 17ß,19-di-hydroxyandrost-4-ene-3-one; 19-hydroxytestosterone, **31:** 3ß,17ß, dihydroxyandrost-5-ene, **32:** 3α,17ß, dihydroxyandrost-5-ene, **33:** 3ß,17α, dihydroxyandrost-5-ene, **34:** androst-4-ene-3,17-dione, **35:** 3ß,16ß,18-trihydroxyandrost-5-ene-17-one, **36:** pregn-4-ene-3-one, **37:** pregn-4-ene-3,20-dione; progesterone, **38:** 6ß-hydroxy-pregn-4-ene-3,20-dione; 6ß-hydroxy-progesterone, **39:** 12ß-hydroxypregne-4-ene-3,20-dione; 12ß-hydroxyprogesterone, **40:** 12ß-(3‘pentenoyl-)pregn-4-ene-3,20-dione, **41:** 17α-hydroxypregn-4-ene-3,20-dione; 17α-hydroxy-progesterone, **42:** 11,15-dihydroxypregn-4-ene-3,20-dione; 11,15-di-hydroxy-progesterone, **43:** 21-hydroxypregn-4-ene-3,20-dione; 21-hydroxyprogesterone; cortexone; deoxycorticosterone, **44:** 11α,21-dihydroxypregn-4-ene-3,20-dione; 11α,21-dihydroxy-progesterone; **45:** 11ß,21-dihydroxypregn-4-ene-3,20-dione; 11ß,21-dihydroxyprogesterone; corticosterone, **46:** 18,21-dihydroxypregn-4-ene-3,20-dione; 18,21-dihydroxyprogesterone**,** **47:** 15α-hydroxy-progesterone-7α-isobutyrate, **48:** 15α-hydroxyprogesterone-7α-hydroxy-isobutyrate, **49:** pregn-4-ene-3,19,20-trione; 19-oxoprogesterone, **50:** 20α-hydroxypregn-4-ene-3-one, **51:** 20ß-hydroxy-pregn-4-ene-3-one, **52:** 20 α,21-dihydroxypregn-4-ene-3-one, **53:** 20ß,21-dihydroxypregn-4-ene-3-one, **54:** 15α,20-ß-dihydroxypregn-4-ene-3-one, **55:** 16ß, 20ß-dihydroxypregn-4-ene-3-one, **56:** pregna-4,6-diene-3,20-dione;6-dehydro-progesterone, **57:** 20-α-hydroxypregna-4,6-diene-3-one; cybisterone, **58:** 12ß-hydroxypregna-4,6-diene-3,20-dione; cybisterol, **59:** 15α-hydroxypregna-4,6-diene-3,20-one, **60:** 18-hydroxypregna-4,6-diene-3-one-18,20-hemiketal; mirasorvone, **61:** pregna-4,6-diene-3,20-dione-15α-isobutyrate, **62:** 15α-hydroxypregna-4,6-diene-3-on-20-isobutyrate, **63:** 21-hydroxypregna-4,6-diene-3,20-dione; 6,7-dehydrocortexone, **64:** 12ß,20α-dihydroxypregna-4,6-diene-3-one, **65:** 20ß,21-dihydroxypregna-4,6-diene-3-one, **66:** 15a,20ß-dihydroxypregna-4,6-diene-3-one-20-isobutyrate, **67:** 3ß-hydroxypregn-5-ene-20-one; pregnenolone, **68:** 3ß,20α-dihydroxypregn-5-ene, **69:** 3ß,20α, 21-trihydroxypregn-5-ene, **70:** 3ß,21-dihydroxypregn-5-ene-20-one; 21-hydroxypregnenolone, **71:** 3ß,16α,21-trihydroxypregn-5-ene-20-one; 16α,21-dihydroxypregnenolone, **72**: 3ß-hydroxypregn-5-ene-7,20-dione; 7-oxopregnenolone, **73:** 3α-hydroxy-5ß-pregnane-20-one, **74:** 3α,20α -dihydroxy-5α-pregnane, **75:** 11α-hydroxy-5ß-pregnane-3,20-dione, **76:**21-hydroxy-5ß-pregnane-3,20-dione, **77:** 3α,12 α-dihydroxy-5ß-pregnane-20-one, **78:** 3α,11α-dihydroxy-5ß-pregnane-20-one, **79:** 3α,11ß-dihydroxy-5ß-pregnane-20-one, **80:** 3α,21-dihydroxy-5α-pregnane-20-one, **81:** 3α,21 -dihydroxy-5ß-pregnane-20-one, **82:** 3ß,11ß,15α-trihydroxy-5α-pregnane-20-one, **83**: 3α,11ß,15α-tri-hydroxy-5α-pregnane-20-one, **84:** 3α,11ß,15ß,20-ß-tetrahydroxy-5α-pregnane, **85:** 3ß-hydroxy-5α-cholestane, **86:** 3ß-hydroxy-5ß-cholestane, **87**: methylisobutanoate, **88:** α-copaene, **89:** α-muurolene, **90:** γ-muurolene, **91:** ε-muurolene, **92:** γ-cadinene, **93:** δ-cadinene, **94:** α-cubebene, **95:** β-cubebene, **96:** torreyol, **97:** trans-β-farnesene, **98:** γ-gurjunene, **99:** α-cedrene, **100:** methyl-8-hydroxy-quinolinecarboxylate, **101:** 7α,5ß,10α-endesin-4(14)-ene-12,6α-diol; platambin, **102:** 2-(*cis*-5,8,11,14-eicosatetraenoyl) glycerol (2-ara-gl), **103:** 2-(*cis*-5,8,11,14,17-eicosapentaenoyl)glycerol (2-epa-gl), **104:** 1-(*cis*-5,8,11,14-eicosatetraenoyl)glycerol (1-ara-gl), **105:** 1-(*cis*-5,8,11,14,17-eicosapentaenoyl)glycerol (1-epa-gl), **106:** 1-hexadecanoate-glycerol, **107:** 2-octadecanoate-glycerol, **108:** 1-octa-decanoate-glycerol, **109:** ledenol

The link between this human behavior and predaceous diving beetles is perhaps based on the biologically active chemicals produced in the prothoracic defensive glands of these insects (Table 6.2). As a girl approaches adolescence, the first outward signs of breast development begin to appear by an increase of blood gonadotropin-titers that are secreted by adenohypophysis (Rosen 2008). Later on the cyclical estrogen and progesterone secretion, and accumulation of fat in the connective tissue result in enlargement of breasts. Later when the duct systems of the milk glands (i.e., branched tubulo-alveolar modified apocrine sweat glands) grow, acquire a thickened epithelium and secretory glands at the end of the milk ducts, normal female breast developmental stages can be observed. Growth hormone and glucocorticoids, insulin and progesterone contribute to the growth and differentiation of these glands. The greatest amount of breast glandular differentiation occurs during puberty, however these processes continue for at least a decade and are enhanced by pregnancy (Rosen 2008).

Based on adult dytiscids, gyrinids, and ant lion larvae, biologically active molecules might be of interest. In Gyrinidae, which have no prothoracic glands (see 6.4.2.1; 6.4.2.2) the pygidial glands are responsible for both defense and surface hygiene. However, the typical gyrinid norsesquiterpenes gyrinidal, isogyrinidal, and gyrinidone, gyrinidione (see Dettner 1985; Meinwald et al. 1972; Schildknecht et al. 1972a) are not known to influence breast development of mammals. The same applies for the antibacterial and smelling low molecular compounds 3-methyl-1-butanol, 3-methyl-1-butanal, 2-methyl-1-propanol, and 6-methyl-5-hepten-2-one from gyrinid pygidial glands (Ivarsson et al. 1996; Schildknecht et al. 1972b). On the other hand, by comparison of prothoracic gland steroids from dytiscid beetles with norsesquiterpenes from gyrinid beetles it is evident that norsesquiterpenes from gyrinids are as effective as certain prothoracic gland steroids from Dytiscidae in their penetrating ability through gill membranes of fishes (Miller and Mumma 1976a, b).

Within predaceous diving beetles there exist a considerable number of species that contain estrone (**24**), 17β-estradiol (**25**), and testosterone (**29**) that can probably influence and stimulate breast growth in females (Tables 6.2 and 6.3). Especially various *Agabus*- and *Ilybius*-species contain these compounds (Table 6.2). In addition, progesterone (**37**), which can also influence breast growth is reported from *Dytiscus pisanus* (Tables 6.2 and 6.3). Another aspect concerns the steroid amounts per beetle. Sequestration of larger amounts of pregnane derivatives was found in *D. marginalis* (deoxycorticosterone **43,** 400 µg/beetle) and *Cybister* spec. (cybisterol **58**, 1000 µg/beetle), however estrone (**24**, 2 µg/beetle *I. fenestratus*) and 17-β-estradiol (**25**, 19 µg/beetle *I. fenestratus*, Miller and Mumma 1976a; see 6.4.2.2) are only found in low quantities. Because there exist natural estrogens,

Table 6.3 Water beetle steroids which act as vertebrate hormones (for reference numbers, see Fig. 6.15)

Type of steroids	Single steroids	Occurrence within vertebrates	Function within vertebrates
C_{18}-steroid estradienes	Estrone (**24**) 17β–estradiol (**25**)	Produced from cholesterol by mammal ovary and placenta but also by the testes in very low amounts, and possibly by the adrenal cortex (increased biological activity of **25** than **24**)**.** Estrone (**24**) in urine of pregnant woman and mares, follicular liquor, bull-urine, palm kernel oil (Budavari et al. 1989)	Both estrogens (**24**, **25** mainly bound to proteins) release estrus and are responsible for development and maintenance of secondary female sexual characters (e.g., breast) and further effects. Especially **24** increases proliferation in mammary epithelial cells and altered cell cycle kinetics (Rosen 2008).
C_{19}-steroids Androstenes Androstadienes	Androstenedione (**34**) Testosterone (**29**) Boldenone (**26**)	Androstenes such as **29** and **34** represent most important androgens (mainly bound to proteins) which are produced from cholesterine in interstitial cells of testes. Apart from gonads androstenedione (**34**) is also produced in adrenal glands and represents intermediate of testosterone (**29**). As compared with **34**, testosterone (**29**) is more effective and has a higher daily production in men. The androstadiene boldenone (**26**) naturally occurs in few other species (Brabander de et al. 2004).	Both compounds (**29**, **34**) responsible for development and maintenance of secondary male sexual characters. Further effects in vertebrates: male behavior, anabolic activities, growth of bones. Boldenone (**26**) from *Ilybius* species (Table 6.2) represents an anabolic steroid in humans but was originally developed for veterinary use (Brabander de et al. 2004).
C_{21}-steroids pregn-4-enes	Progesterone (**37**) 17α-hydroxy-progesterone (**41**) 20α-hydroxy-pregn-4-ene-3-one (**50**) 20β-hydroxy-pregn-4-ene-3-one (**51**) Deoxycorticosterone (**43**) Corticosterone (**45**)	Pregn-4-enes represent active principles of mammal corpus luteum. All human gestagenes such as progesterone (**37,** produced from cholesterol, in corpus luteum during latter half of menstrual cycle), 17α-progesterone (**41**; produced during synthesis of glucocorticoids and sex steroids), and 20α-hydroxy-pregn-4-ene-3-one (**50**; represents an epimer of **51**) are present in predaceous diving beetles. 11-deoxycorticosterone (**43**) is produced by adrenal glands. Corticosterone (**45**) from cortex of adrenal glands is produced in non-human mammals.	Gestagenic steroids are responsible for implantation, development, maintenance of embryos within female uteri. In case of pregnancy: continuous secretion of progesterone (**37**), which results in epithelial expansion in mammary gland and there stimulates growth of alveoli. **37** with antiovulatory effect. 17α-hydroxy-progesterone (**41**) is a natural progestogen and in pregnancy increases in the third trimester primarily due to fetal adrenal production. In ovary, placenta, adrenals **50** and **51** have properties similar to those of progesterone (**37**) and are in equilibrium with **37**. Deoxycorticosterone (**43**) with mineralocorticoid activity (these steroids such as aldosterone maintain normal blood volume, promote sodium and water retention, and increase urinary excretion of potassium

(continued)

Table 6.3 (continued)

Type of steroids	Single steroids	Occurrence within vertebrates	Function within vertebrates
			and hydrogen ions) and represents precursor to aldosterone. Corticosterone (**45**) represents a glucocorticoid (stimulate gluconeogenesis and increase catabolism of proteins and mobilize free fatty acids) just as cortisol.
C_{21}-steroids pregna-4,6-dienes	6-dehydroprogesterone (**57**) Cybisterone (**58**) Mirasorvone (**61**)	**57** represents a synthetic progestin (= progestogen) which prepares the uterus for implantation and pregnancy. Cybisterone (**58**) and mirasorvone (**61**) represent unique 18-oxygenated pregnan structures described for the first time for insects (Meinwald et al. 1998).	Progestins are used for hormonal contraception, prevent endometrial hypoplasia from unopposed estrogen in hormone replacement therapy. A closely related hormone with mineralocorticoid activity: 18-hydroxydeoxy-corticosterone was isolated from rat adrenals (see corticosterone **45,** Fig. 6.15; Meinwald et al. 1998).
C_{21}-steroids pregn-5-ene	21-hydroxypregnenolone (**70**) 16α,21-dihydroxypregnenolone (**71**)	21-hydroxypregnelone (**70**) was isolated from sulfate fraction of neonatal urine, together with **71.** Shackleton et al. (1987) suggest that **70** is produced in the fetal liver.	

synthetic estrogens (e.g., ethinylestradiol, mestranol, turisteron, moxestrol) and non-steroid estrogens (e.g., diethylstilbestrol, dimestrol) it would be interesting to look for any of these compounds in these arthropod groups. Moreover, non-steroid estrogens may be used therapeutically to replace natural estrogenic hormones. It should be also considered that there exist phytoestrogens and mycoestrogens that represent plant- or fungus-derived compounds, which are consumed by animals and might cause estrogenic effects. In some countries, phytoestrogenic plants have been even used in treating menstrual, menopausal, and fertility problems (Müller-Schwarze 2006). Thus, it seems possible that certain arthropod semiochemicals that simultaneously act as vertebrate hormones may bind to estrogen receptors in the mammary glands, or by possibly influencing human hormone regulation or hormone synthesis.

Schildknecht et al. (1967a) report in another paper that water beetles and especially representatives of genus *Gyrinus* were used in European alps as aphrodisiacs against cows and horses (see Ochs 1966). Because *Gyrinus* do not produce steroids, Schildknecht et al. (1967a) suggest that peoples from the alps confused *Gyrinus*-specimens with representatives of *Ilybius*.

The following non-steroid prothoracic gland constituents from adult representatives of Dytiscidae are mainly discussed in Sect. 6.4.2.2.2. Apart from methylisobutanoate (Fig. 6.15, Table 6.2, **87**, Schildknecht 1977) and the preservative benzoic acid (**3**, Fig. 6.7) pentadecanoic and octadecanoic acids have also been identified (Table 6.2). Moreover, several monoglycerides with both saturated (**106–108**, Table 6.2) and unsaturated (**102–105**, Table 6.2, Fig. 6.15) side chains have also been recorded.

Various sesquiterpenes (**88–99**) were identified by Schildknecht (1977) in *Ilybius fenestratus* (Table 6.2, Fig. 6.15). Moreover in *Platambus maculatus*, apart from steroid **55**, an additional sesquiterpene named platambin was recorded **(101**, Table 6.2, Fig. 6.15, Schildknecht 1976, 1977; Weber 1979). Up to now the biological significance of these compounds generally and especially for predacious diving beetles remains obscure.

Even the alkaloid methyl-8-hydroxyquinoline carboxylate (**100**) is abundant in prothoracic defensive gland secretions of *Ilybius fenestratus* (Schildknecht 1976). Due to the yellow color of this compound the *Ilybius* secretion shows a distinct yellow coloration. The free acid could be recently reported from the regurgitate of *Spodoptera* and *Heliothis* larvae (Pesek et al. 2009). The alkaloid derives from the tryptophan metabolism and forms complexes with bivalent metal ions. As an iron-chelator (**100**) it may generally inhibit bacterial infections in the gut. Finally methylesters of leucine and isoleucine were identified from the prothoracic defensive glands of *Ilybius fenestratus*, and in *Dytiscus marginalis*, apart from isoleucine, the valine methylester was also identified (Weber 1979).

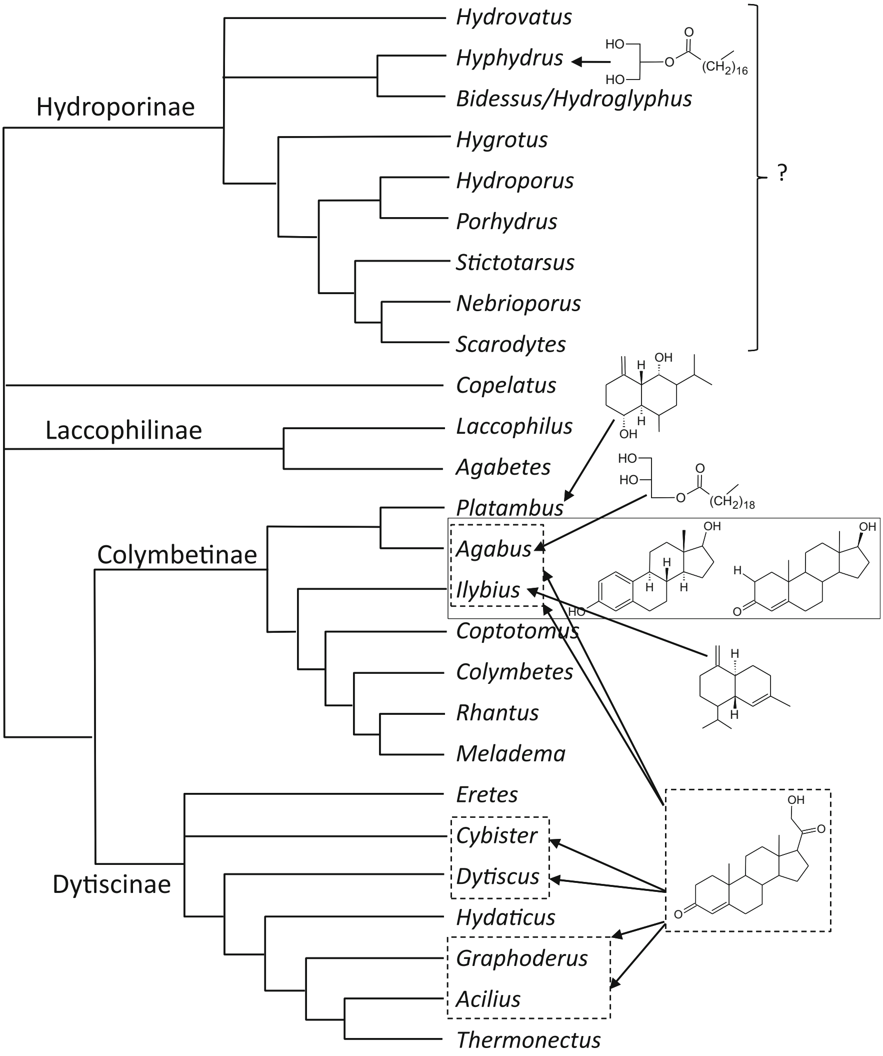


Fig. 6.16 Phylogeny of Dytiscidae genera after Burmeister (1976) and distribution of prothoracic defensive gland constituents according to Fig. 6.15. Those compounds which are present in various taxa are figured by boxes. Erratically found chemicals are associated with the genera by arrows. Burmeister's *Potamonectes* was actualized as *Nebrioporus*; *Thermonectes* as *Thermonectus*

Within the Dytiscidae (Fig. 6.16) the Hydroporinae possess well-developed prothoracic defensive glands, although it remains a mystery that no constituents of the prothoracic defensive glands have been detectable by gas chromatography-mass spectrometry. Only in *Hyphydrus* (with saturated side-chain; **106–108**) and 2 *Agabus*-species monoglycerides (unsaturated side chains) have been recorded (Figs. 6.15 and 6.16, Schaaf and Dettner 2000b). Within *Agabus* and *Ilybius* two estradienes (C_{18}; e.g. 17ß-estradiol **25**) and ten androstenes (C_{19}; e.g. testosterone **29**) have been exclusively recorded. Other representatives from the Dytiscinae and Colymbetinae subfamily may contain up to 21 different pregn-4-enes (C_{21}; e.g. cortexone **43**), 11 pregna-4,6-dienes (C_{21}), 5 pregn-5-enes (C_{21}), 12 pregnanes (C_{21}) and 2 cholestanes (C_{27}). At the moment, biosynthesis of steroids in dytiscids is only partly understood. Therefore, the polarity of the chemical characters (i.e., the differentiation between plesiomorphic and apomorphic characters) is yet to be defined (see Dettner 1987). It is suggested that C_{27}-steroids might represent rather primitive characters, followed by C_{21}-pregn-5-enes and C_{21}-pregne-4-en-3-ones. If the biogenetic pathway is more advanced, C_{21}-steroids with hydroxyl, pregnanes, or other groups are more advanced. Finally, we would assume that C_{18}- and C_{19}-steroids are highly derived.

Volatile sesquiterpenoids such as platambin (**101**) or γ-cadinene (**92**) seem to be present both in Dytiscinae and Colymbetinae, however careful systematic investigations are absent. The nucleoproteid colymbetin is restricted to the genus *Colymbetes*, whereas methylisobutanol (**87**) was found in the secretion of *Ilybius fenestratus* (Table 6.2). Remarkably, *Colymbetes*-species do not produce steroids and instead contain the nucleoproteid colymbetin, which lowers blood pressure.

6.4.2.2.2 Biological Activity and Regeneration of Prothoracic Gland Secretions

In the past, Blunck (1911, 1912a, 1917) performed various experiments to investigate the origin, production, and function of the milky secretion that is sequestered in the prothoracic defensive glands named “Schreckdrüsen.” The author characterized coloration (milky yellowish fluid), odor (very often aromatic odor), and taste (bitter) of these secretions. More recent work has concerned identification of the biological activities (e.g., feeding deterrents, toxicities, anesthetic activities, membrane absorptions) of steroids and especially defensive steroids of predaceous diving beetles and giant water bugs against both fish (Gerhart et al. 1991; Miller and Mumma 1976a, b; Schaaf et al. 2000; Selye and Heard 1943) and mammals (Selye 1941b, 1942). In addition, preliminary results have characterized pygidial and prothoracic gland secretions against epitrichic ciliates (Schneider 2008). Moreover, information on feeding deterrents of polyunsaturated monoglycerides of *Agabus affinis* (Schaaf and Dettner 2000b) and amino acids of *Ilybius fenestratus* (Weber 1979) against fish have been collected. Finally the alkaloid methyl-8-hydroxy-quinolinecarboxylate (**100**) from *Ilybius fenestratus* (Schildknecht 1977) and the nucleoproteid colymbetin from *Colymbetes fuscus* were reported as active against mammal predation (Schildknecht and Tacheci 1971).

Against bluegill sunfishes (*Lepomis macrochirus*) feeding deterrents of three structurally related steroids from prothoracic glands of predaceous diving beetles were determined by using artificial food pellets (Gerhart et al. 1991). It was shown that feeding activities drastically vary depending on specific stereochemistries of the steroids involved. Deoxycorticosterone (= cortexone, **43**) showed the highest activities (94% inhibition), followed by 20α-hydroxypregn-4-ene-3-one (**50**; 58% inhibition), whereas its epimer 20β-hydroxypregn-4-ene-3-one (**51**) did not significantly inhibit feeding. Gerhart et al. (1991) stress that these results are in contradiction with earlier data based on toxicities and anesthetic actions by using fish that were immersed with steroid solutions. Therefore, the authors suggest specific receptor–ligand interactions. Feeding deterrents with fully saturated pregnanes (**72–86**) from *Graphoderus cinereus* and *Laccophilus minutus* against the minnow *Phoxinus phoxinus* also showed that these prothoracic defensive steroids act as strong feeding deterrents against fish (Schaaf et al. 2000).

Other work has been accomplished with the effects of these steroids and mammals. Young et al. (1996) studied the behavioral and pharmacological effects of certain steroids in mice. A neurosedative behavior was found in the progesterone (**37**)-metabolite 3α-hydroxy-5α-pregnane-20-one that is chemically similar to compound **73**. An antiaggressive effect was also observed when the brain titer of the deoxycorticosterone (**43**)-metabolite 3α,21-dihydroxy-5α -pregnane-20-one (**80**) was increased. Compound **73** (3α-Hydroxy-5β-pregnane-20-one, = pregnanolon, eltanolon) was also identified as a quickly acting cardiac active hypnotic (Tassani et al. 1996). The metabolites **73** and **80** obviously interact with the γ-aminobutyric acid$_A$ (GABA$_A$) receptor/chloride canal complex in the central nervous system (Lan and Gee 1994). It is remarkable that the GABA$_A$ receptors are known to contain allosteric modulator sites for therapeutically useful drugs such as benzodiazepines and barbiturates (Lan and Gee 1994).

In detailed investigations, Miller and Mumma (1976a, b) studied toxicities, anesthetic activities. and membrane absorptions of water beetle steroids administered as solutions to immersed minnows (*Pimephales promelas*). Most active steroids in the minnow bioassay were 4-pregnen-3-ones (**36–55**) and related derivatives that are also present in prothoracic defensive glands. The activity of steroids was highly related to the degree of oxygenation. Those steroids oxygenated at the termini of the molecule (C_3 and C_{20} in C_{21}-steroids: **36–55**; C_3 and C_{17} in C_{19}-steroids: **26–35**) were most active; decreased or increased oxygenation of the steroid molecule resulted in a loss of activity. Remarkably, all active steroids were poorly water soluble and 80% of steroid absorption occurred via the gills, which are the primary site of steroid-uptake as compared with the skin (20%).

In comparing bioassays of various structurally different steroids (only a few are also present in dytiscids) against fish (immersed minnows) and mammals (intraperitoneally injected rats), Selye and coworkers showed that those steroids are active in both (Selye 1941a, b, 1942; Selye and Heard 1943) in spite of the fact that both sets of bioassays were completely different. In fishes their activities were even augmented, with lower amounts of tested steroids necessary in fishes (as compared to mammals) to produce deep anesthesia. In mammals, pregnanes with a 3α-OH-5α-H-structure seem to be particularly effective (Purdy et al. 1990), and fast and deep narcosis (intravenous application) in mammals (Gyermek and Soyka 1975) was achieved with 3α-hydroxy-5α-pregnan-20-one and 3α-hydroxy-5β-pregnan-20-one (**73**, *Laccophilus minutus*), with both components being more effective in rats than the barbiturate thiopental (Norberg et al. 1987). Again, stereochemistry plays a central role concerning biological activities of these steroids. The presence of a 3α-OH-group is very important (Phillips 1975; Harrison et al. 1987; Purdy et al. 1990): 3α-OH-5α-H- and 3α-OH-5β-H-Steroids are effective narcotics in mammals, whereas corresponding 3β-OH-steroids are inactive.

It is highly fascinating that four pregnenes (desoxycorticosterone **45**, pregnenolone **67**, progesterone **37**, 3α-hydroxy-pregn-5-ene-20-one) were also recorded from cephalic glands of aquatic belostomatid bugs (Lokensgard et al. 1993). The authors suggest that this remarkable parallel evolution within hemi- and holometabolous fresh water taxa (i.e., belostomatids and dytiscids) may be due to specific predation pressure from fish (Lokensgard et al. 1993).

In a preliminary experiment, epitrichic ciliates in the genus *Opercularia* were isolated from procoxae of *Agabus sturmii* and mixed with droplets of either prothoracic or pygidial gland secretions of the same dytiscid species (Schneider 2008). Under the microscope the movement of the ciliae were registered at the start of the experiment. Cessation of ciliar movement was achieved after 5 minutes when using pygidial gland secretions, however ciliar activity halted after only 2.5 minutes when prothoracic gland secretions were used. This may illustrate that prothoracic gland secretions of dytiscids are also active against protozoans, which settle on the surface of many water insects and may be even more efficient as compared with pygidial gland secretion. In contrast, Lust (1950) treated several species of *Orbopercularia* and *Opercularia* with aqueous prothoracic gland secretion of *Ilybius fuliginosus* and observed that most protozoans recovered few minutes after treatment with the solution. Therefore, it seems necessary to repeat such experiments by using equimolar amounts of various prothoracic and pygidial gland constituents.

The sesquiterpene platambin (**101**) from *Platambus maculatus* (Fig. 6.15) was expected to represent a defensive substance against small mammals (Schildknecht 1977), because poikilothermic vertebrates such as amphibians and fishes should be deterred by the co-occurring steroid. Blum (1981) reports that *Cybister fimbriolatus* exudes a prothoracic defensive secretion enriched with potent odorants as sesquiterpenes. He suggested either intraspecific activities of these terpenes (e.g., alarm pheromone) or activities of these terpenes as chemical alarm signals for those organisms interacting with these toxic beetles. In the laboratory, juvenile eels (*Anguilla anguilla*) are attracted to the sesquiterpene geosmin (Müller-Schwarze 2006).

The yellow colored alkaloid **100** was suggested to deter especially warm-blooded small vertebrates when the sometimes amphibious species *Ilybius fenestratus* stays on land. In contrast, the complex steroid mixture (Table 6.2) of *I. fenestratus* was expected to act against predatory fish (Schildknecht 1977). The corresponding 8-hydroxyquinoline carboxylic acid represents a strong chelator for Mg^{2+}-ions and moreover has antibiotic activities (Pesek et al. 2009). If *I. fenestratus* was fed with radioactive $^{14}COOH$-marked tryptophan significant amounts were incorporated into alkaloid **100** (Schildknecht et al. 1971).

In a feeding bioassay with the two polyunsaturated monoglycerides (1-ara-gl **104**; 1-epa-gl **105**) of *Agabus affinis*, adult minnows (*Phoxinus phoxinus*) were shown to perceive these monoglycerides, and they acted as a deterrent when compared with controls. Moreover, it was shown that this deterrent effect was only achieved by administering higher amounts of both glycerides compared to those occurring in the glands of the *A. affinis* (Schaaf and Dettner 2000b). Because *A. affinis* prothoracic glands contain both four polyunsaturated monoglycerides and the C_{21} steroid 15-α-hydroxy-pregna-4,6-dien-3,20-dione (**59**) it seems probable that the monoglycerides act as emulsifiers for the prothoracic steroid of *A. affinis* that is highly water-insoluble. It is interesting to note that these monoglycerides, such as 2-ara-gl (**102**) have a cannabimimetic potential in mice, which may resemble the anesthetic effects of many steroids in vertebrates.

Amino acids that may be present as free acids or methylesters (Weber 1979) may have various effects on fishes and other predators. Adron and Mackie (1978) found that amino acids such as leucine and isoleucine may represent feeding stimulants for the rainbow trout *Salmo gairdneri*. However, other data indicate that leucine and isoleucine, which are present in the secretion of *Ilybius fuliginosus*, may represent both stimulants and deterrents depending on the fish species were tested (Kasumyan and Døving 2003). A compilation from 2006 (Müller-Schwarze) indicates that various freshwater fish species can recognize various prey or plant food odors by using the chemical cues cysteine (earthworm), L-alanine, L-arginine, L-proline (invertebrates, fish, aquatic plants), tyrosine, phenylalanine, lysine (insects, plankton, crustaceans, fish), free amino acids (injured crustaceans), cysteine, asparagine, glutamic acid, threonine, alanine (plants, small animals), cysteine, and arginine (plants).

According to Hara (2011) cysteine represents the most potent olfactory stimulating amino acid determined electrophysiologically in various fish species. An increased swimming activity is followed by search behaviors depending on fish species. In most species also alanine, lysine as well as proline are active at low concentrations.

The whole water-soluble prothoracic gland secretion of *Colymbetes fuscus*, a certain fraction which was assigned as nucleoproteid colymbetin, lowered blood pressure when injected into the veins of urethane-narcotized rats (Schildknecht and Tacheci 1971). For *C. fuscus* six fractions from the prothoracic glands have been found. The two biologically active fractions had molecular masses of about 700. As compared with the alkaloid methyl-8-hydroxy-quinolinecarboxylate (**100**) from the prothoracic defensive glands of *Ilybius fenestratus* that caused clonic spasms in mice (Schildknecht 1977), the biological significance of the various sesquiterpenes from *I. fenestratus* or of platambin from *Platambus maculatus* has yet to be investigated. Recently Hara (2011) reported that alkaloids stimulate fish gustatory receptors at extremely low concentrations. They induce avoidance behavior and suppress locomotory activities both in salmonids and goldfish. Obviously salmonids are able to avoid noxious substances at a distance whereas goldfish take up the material mixed with gravel, sand, or mud. Because they have a palatal organ they are enabled to manipulate the material mixture in the mouth, separate food (which is ingested) from nonfood and noxious material (which is spitted out).

Seasonal fluctuations of prothoracic defensive gland titers were described in the species *Ilybiosoma seriatum* and *Platambus obtusatus* (Miller and Mumma 1974;

Fescemyer and Mumma 1983). In *I. seriatum* the defensive steroid titer increased from July to September, but low values were obtained during November and December. Further seasonal variations of prothoracic defensive gland constituents were recorded in *Acilius semisulcatus* (Newhart and Mumma 1979), where the steroid titer increased from July to October, in contrast to the pygidial gland constituents that decreased from July to October. Quantization of deoxycorticosterone (**43**) was performed by means of minnow bioassay in aqueous solutions. The survival time of minnows was correlated to known concentrations of steroids (Miller and Mumma 1974). When the prothoracic gland secretions of *Ilybiosoma seriatum* and *Platambus obtusatus* were qualitatively and quantitatively analyzed by HPLC both species regenerated about 80% of their prothoracic gland components within 2 weeks. These defensive gland secretions can be collected simultaneously by electrical shocking with five 20-mA, 90-V DC, 1-sec pulses with 5 minutes within between each pulse (Fescemyer and Mumma 1983).

6.4.3 Other Exocrine Glands

Apart from adults, there are few data concerning exocrine glands from other dytiscid developmental stages. Brancucci and Ruhnau (1985) described parastigmatic glands in dytiscid pupae of the genera *Lancetes*, *Liopterus* (former *Copelatus*), *Agabus*, *Eretes,* and *Dytiscus.* These glands are externally characterized by minute circular openings with a fine peritrema near each spiracle. These unusual pupal glands are described in detail morphologically (as class 3 type according to Quennedey 1998) and chemically in Carabidae (Giglio et al. 2009, 2011). Moreover, when pupal chambers of *Dytiscus* or *Liopterus* (former *Copelatus*) were opened special pupal aromatic odors were identified (Blunck 1923a; Naumann 1955). Blunck (1923a) used litmus paper and was successful in detecting an acid secretion near the spiracles. However, he was in doubt if these pupal secretions might deter shrews, moles, or rats that regularly feed on *Dytiscus* pupae. Casper (1913) suggested that the parastigmatic glands secrete fat-like water repellent agents that cover the pupal cuticle. The 31 low molecular weight volatiles (such as linalool, α-terpinene, β-pinene, 4,8-dimethyl-3,7-nonadien-2-ol) and especially ketones, aldehydes, alcohols, esters, and carboxylic acid from the abdominal glands of carabid pupae were suggested to have a deterrent function against predators and a prophylaxis function against pathogens (Giglio et al. 2009). Unpublished data from our lab (Jakob 2008; Dettner unpublished) showed that pupae of *Dytiscus marginalis* are characterized by a coconut-like odor, and 4-hydroxy-methylbenzoate (**6**) (Fig. 6.7) and δ-decalactone (Fig. 6.22, **109**) could be identified from the seven volatiles collected. Through GC-MS analysis of the peristigmatic glands of the same species we also recorded indole (Fig. 6.22, **110**) and 1,3-dimethoxy-2-hydroxybenzene (Fig. 6.22, **111**).

Finally in various species epidermal adhesive glands are described from suckers of fore and middle tarsi of males (Blunck 1912c; Betz 2010). There were identified glands with gland type class 1. The chemically unknown secretions are excreted outside the actual sucker surface via pore canals at base of the sucker stalk.

6.5 Dermal Glands, Epicuticular Lipids, and Body Coloration by Pigments

6.5.1 Dermal Glands and Epicuticular Lipids

The cuticle of adult predaceous diving beetles is very often covered in oily materials giving the impression that they have been varnished (Fig. 6.17a, b). This appearance is obviously due to the products of dermal glands. According to Korschelt (1923) single-cell dermal glands with tubules and end-apparatuses first originate in the third larval instar, when it has left the water in order to construct a terrestrial pupal chamber; pupae also possess dermal glands. In adult beetles these glandular cells are found on the head and its appendages, the thorax, and the legs. Korschelt (1923) mentions that the density of the dermal glands is significantly larger on the dorsal side of a *Dytiscu*s adult as compared with the ventral side. He mentions about 3000–4000 per square mm and observed dermal cells within the abdominal tergal structures. Many authors suggest that the dermal glands represent varnish-glands, which produce oils that lower the wettability of the epicuticle. In addition, dermal glands in the area of mouth parts and near articulations of legs serve as a kind of lubricating oil (Korschelt 1923).

As far back as 1922b, Blunck states that the wettability of freshly hatched beetles is lower than in older specimens. In addition, the wettability may be significantly modified by hairs (Fig. 6.17i), microsculpture of body surface (Fig. 6.17k), adhering protozoans (Fig. 6.17j), and algae and fungi. However, oily compounds (Fig. 6.17b), which are produced by dermal glands, likely aid in reducing wettability in these beetles.

Various oily materials are known from the surfaces of many dry dytiscid beetles (e.g., *Cybister, Ilybius, Agabus*) and may be recognized when fine surface structures such as microreticulations or colorations are important during determination of the beetles (Roughley 1990). To fully expose morphological features for identification it is often necessary to eliminate these materials by using diethylether, hexane, xylene, ethylacetate, limonene, or 1,1,1-trichloroethane as solvents (Warner 2010; Harrison 2012). In addition, both authors generally remark that greasy beetles especially occur in long-lived species which build up considerable fat reserves that degrade and exude from the pinned specimens as an oily or varnish-like covering. Beament (1976) mentions that oily materials on aquatic insects are used for waterproofing. In addition, he found that representatives of *Agabus* and *Ilybius* are found in warm waters because they have higher transition temperatures of about 32 °C. Beament (1976) suggests that the properties of their oily secretions could limit their distribution and would be correlated with their capacity to osmoregulate. In contrast,

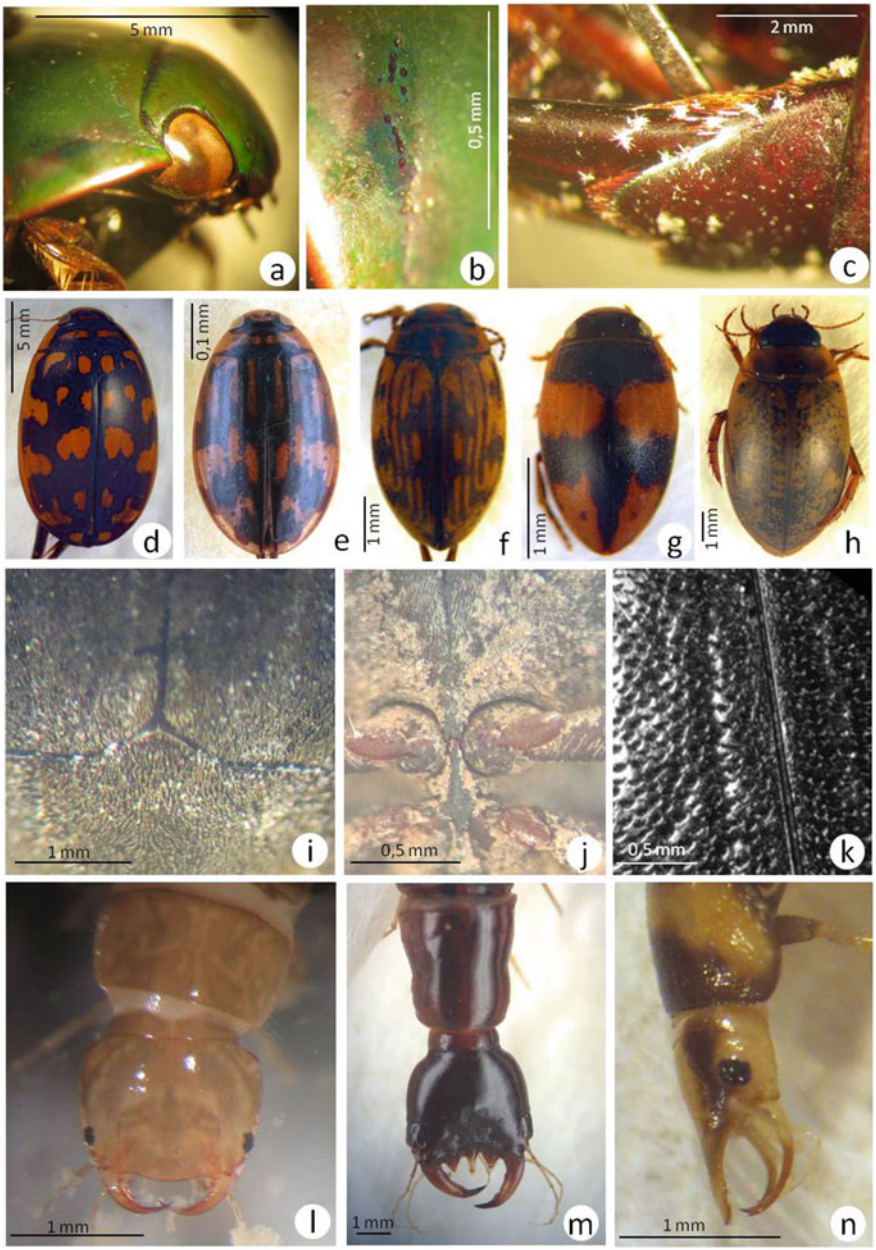


Fig. 6.17 Structural (**a**), secretional (**b**, **c**), and pigmental (**d–h**) coloration in Dytiscidae (adults: **a–k**, larvae: **l–n**). Head and Prothorax of *Cybister vulneratus* (**a**) with structural coloration. Groove on the right pronothal half with fluid epicuticular lipids of *C. vulneratus* (**b**). Tibia and tarsi of *C. vulneratus* with solid crystallized epicuticular lipids (**c**). Black and yellow coloration patterns in Dytiscidae: *Thermonectus* spec. (**d**), *Sandracottus festivus* (**e**), *Scarodytes halensis* (**f**), *Rhithrodytes crux* (**g**), and *Agabus nebulosus* (**h**). Surface structure of *Deronectes moestus* with

transition temperatures in *Dytiscus* are at 24 °C, and representatives of this genus would die at 24 °C and congregate in cold water around an ice cube. Although the chemistry of these solid and oily compounds is unknown in Dytiscidae, there exist data from intersegmental glands in Ponerinae ants (Attygalle et al. 1996). These secretions contain linoleic acid, palmitic acid, methyloleate, and several long-chain hydrocarbons, and have no known behavioral-modifying or antibiotic activities but rather seem to function as lubricants.

6.5.2 Epicuticular Lipids

Within insects, lipids and especially hydrocarbons are widespread and serve primarily as a barrier to water efflux, but also as a waterproofing epicuticular layer and may additionally or exclusively function as signals for chemical communication (Dettner and Peters 2010). According to Blomquist (2010) cuticular hydrocarbons in insects vary from 21 to 60 carbons. As compared with hydrocarbons from plant surfaces, insect hydrocarbons possess various double bonds and methyl branches. It may be that both branching and double bonds may increase informational content of these mixtures in intra- and interspecific chemical interactions, while the waterproofing capabilities remain (Blomquist 2010; Dettner and Liepert 1994).

Concerning freshwater insects and their aquatic developmental stages, there are limited data available with respect to epicuticular hydrocarbons. For several taxa only hydrocarbons from the terrestrial adults are known but aquatic larval stages are unknown (Chrysomelidae: *Donacia*: Jacob and Hanssen 1986; Culicidae: *Anopheles*, *Aedes*, Simuliidae: *Simulium*, Psychodidae: *Phlebotomus*, *Sergentomyia*, *Psychodopygus*, Tabanidae: *Tabanus*, Glossinidae: *Glossina*: Bagnères and Wicker-Thomas 2010). So far, the only work that has identified cuticular hydrocarbons from both aquatic larvae and terrestrial adults is from the stonefly *Pteronarcys californica* (Table 6.4) (Armold et al. 1969). Specifically, adults have more surface lipids and a higher melting surface lipid than larvae, whose surface lipid is an oil at room temperature. Both stages have different surface lipid compositions with adults having a larger percentage of hydrocarbons (adult: 12%; larva: 3%), wax esters (adult: 4%; larva: 1%), free fatty acids (adult: 49%; larva: 12%), and sterols (adult: 18%; larva: 1%), while the surface lipids of larvae contain more triglycerides (adult: 7%; larva: 78%). With respect to hydrocarbons (Table 6.4) n-alkanes dominate in adults, however more alkenes and 3-methylalkanes are present in larvae, whereas internally branches alkanes occur in comparable titers in both stages. Among free

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Fig. 6.17 (continued) hairs and dark body coloration (**i**). Underside of *D. moestus* with secretions which obviously serve as adhesives for detritus particles (**j**). Black elytral surface of *Meladema coriacea* (**k**). Dark and yellow pigments in dytiscid larvae as shown by heads and thoraces of *Liopterus haemorrhoidalis* (**l**), *Cybister* spec. (**m**), and *Hyphydrus ovatus* (**n**)

Table 6.4 Cuticular Hydrocarbons of Stonefly *Pteronarcys* and Dytiscidae (percentual data)

		Alkanes	Alkenes alkadienes	2-Methyl-alkanes	3-Methyl-alkanes	Internally branched monomethyl alkanes	Dimethyl branched alkanes	Unidentified	
Plecoptera/Pteronarcyidae									
Pteronarcys californica	Adult	63.0	Trace	–	24	12	–	–	Armold et al. (1969)
	Larvae	42.0	12	–	31	13	–	–	”
Coleoptera/Dytiscidae									
Dytiscus marginalis	Male	36.0	59.5	1.8	0.9	1.8	–	–	Jacob and Hanssen (1986)
	Male	58.3	25.1	5.4	3.6	4.2	–	3.4	”
	Female	78.5	8.3	1.8	1.6	5.6	–	4.2	”
Agabus bipustulatus		52.7	47.3	–	–	–	–	–	”
Ilybius angustior		43.0	51.6	1.5	–	1.8	–	2.1	”
Agabus anthracinus		46.8	27.8	–	6.1	15.1	2.7	2.0	Alarie et al. (1998)
Nebrioporus baeticus	Female	26.5	8.5	Methylbranched:	59.5	Other branched:	16.5		Botella-Cruz et al. (2017)
	Male	19.3	6.4		41.9		32.3		”
	Larvae	14.9	74.2		2.7		8.2		”

fatty acids, octadecenoic- and octadecatrienoic acids occur in both stages, however hexadecanoic acid dominates in adults, whereas hexadecenoic acid is especially found in females. Because adult *Pteronarcys* specimens do not feed, the differences between adults and larvae cannot be attributed to nutritional effects. Also, if adult stoneflies do not drink, an efficient water conservation mechanism also would be important.

A more recent detailed compilation of epicuticular hydrocarbons from the predaceous diving beetle *Agabus anthracinus* was determined by Alarie et al. (1998). The total ion current chromatogram identified 67 different components, 64 of them could be assigned to n-alkanes (86.4%), alkenes (27.1%), terminally (6.1%) and internally branched monomethylalkanes (15.1%), or dimethylalkanes (2.7%). Other branching points in monomethylalkanes are positions 3, 4, or 5. The main components in *A. anthracinus* were n-nonadecane (6%), n-tricosane (12%), n-pentacosane (6.5%), 11-and 13-methylpentacosane (3.4%), n-heptacosane (7.8%), 3-methylheptacosane (4%), 9-C_{27}: 1 (3.3%), 7-C_{27}: 1 (3.7), 9-C_{29}: 1 (3.7%), and 9-C_{31}: 1 (4%).

Other data with respect to hydrocarbon patterns of Dytiscidae were recorded for *Dytiscus marginalis* (both sexes), *Agabus bipustulatus*, and *Ilybius angustior* (Jacob and Hanssen 1986). It is remarkable that several Carabidae possess internally branched monomethylalkanes between 20–35%, whereas monomethylalkanes in Dytiscidae beetles possess between 3.3 and 21.2%. Also, dimethylbranched alkanes range between 0.4–8.0% in terrestrial Adephaga, whereas they are not present in three Dytiscidae species investigated (apart from *A. anthracinus*: 2.7%). In two samples from males of the same species (*D. marginalis*) a significant variability of cuticular hydrocarbons was evident. As compared with males (alkenes 36.0–58.3%), alkenes in female *D. marginalis* reached 78.5%. Recently Botella-Cruz et al. (2017, 2019) analyzed cuticle hydrocarbons in salinity tolerant water beetles. In *Nebrioporus baeticus* (Table 6.4) they investigated females, males, and larvae (Values in Table 6.4 for larvae cover hydrocarbon chain lengths below C20). They found no specific differences between males and females, where n-alkanes dominated. In contrast and compared with adults the more permeable cuticles of larvae are characterized by a lower diversity of compounds, shorter chain lengths, and a higher proportion of unsaturated hydrocarbons (Botella-Cruz et al. 2019). In addition they found that tolerance to salinity is associated with decrease in cuticular permeability. Moreover, saline species within a short time displayed an extraordinary ability to adjust their hydrocarbon profiles to changing salinity (Botella-Cruz et al. 2019). As a whole these results suggest that osmotic stress of aquatic insects could exert a selection pressure on hydrocarbon profiles similar to aridity in terrestrial species.

6.5.3 Coloration of the Integument

Coloration of the integument is important for all developmental stages of aquatic insects, including dytiscids. As predaceous diving beetle larvae and adults serve as prey for many aquatic and terrestrial predators (see Chap. 8 in this book) body coloration, including crypsis or aposematic coloration plays an important role in the aquatic and terrestrial stages of these beetles (Dettner and Peters 2010; Galewski 1971). There exist three mechanisms of coloration within dytiscids that warrant consideration: structural colors, secretion colors, and pigmentary colors.

Structural colors (Fig. 6.17a, b) result from light scattering, interference, or diffraction (Berthier 2007), and many investigations identified these colors based on beetle elytra (Sun and Bhushan 2012). Structural coloration is seldom found within adephagous water beetles but when these colors survive treatments that remove the outer waxy layer of epicuticle this type of coloration seems to be present. In addition, these colors tend to vary with the direction of the incident light. In certain representatives of *Ilybius*, *Agabus*, *Cybister* (Fig. 6.17a, b), and *Dytiscus*, structural colors (including blue and green as in *Dytiscus*, Blunck 1909b) can be observed. Within hydradephagan beetles diffraction grating has been described in Dytiscidae, Noteridae, and Gyrinidae (Seago et al. 2009; Hinton and Gibbs 1971). Seago et al. (2009) describe diffraction grating as a series of parallel nanoscale ridges that disperses light into ordered spectra.

Secretion colors, which are found in polyphagous water beetles such as within the genus *Helophorus*, are mainly absent in dytiscids. When cuticular surfaces are smooth (Fig. 6.17k) or hairy (Fig. 6.17i) a few species possess epidermal glands that produce a glue that allows for the adhesion of detritus particles on the beetles body surfaces (e.g., *Deronectes moestus*, Fig. 6.17j). These detritus particles may be associated with bacterial biofilms and peritrichic ciliates, which are often associated with aquatic beetles and may aid in crypsis.

The last mechanism for colors in dytiscids are pigmentary colors (Fig. 6.17) that arise from the absorption of light in the visible part of the spectrum by chemical chromophores, also called pigments (Kayser 1985). Adults and most larvae (Fig. 6.17l–n) of dytiscids are commonly dark brown, blackish, or olive in color, and therefore brightly colored (e.g., yellow, red) or marked species are the exception within some genera (Adults: *Thermonectus* Fig. 6.17d, *Sandracottus* Fig. 6.17e, *Scarodytes* Fig. 6.17f, *Rhithrodytes* Fig. 6.17g, *Agabus* Fig. 6.17h; Larvae: *Hyphydrus* Fig. 6.17n). In some cases, pale spots on the elytra are only visible when the elytra are lifted so that light shines through areas of reduced pigments. Vittae (with longitudinal markings) and fasciae may be either pale or dark depending on the background color. In northern latitudes lightly colored or conspicuously striped, spotted, or mottled specimens are usually associated with streams, the margins of lakes (Young 1960a), or sand-pits (Kehl and Dettner 2003, e.g., *Agabus nebulosus*, Fig. 6.17h; *Nebrioporus canaliculatus*; *Scarodytes halensis*, Fig. 6.17f, *Coelambus* (now *Hygrotus*) *confluens*; *Hydroglyphus geminus,* the former *Guignotus pusillus*). Specifically, from the Nearctis Young (1960a) mentions the coloration of *Hydroporus lapponum* (edges of tundra lakes) and *Oreodytes* from streams. In contrast, Young (1960a) mentions species from peat pools or vegetated areas that are uniformly black or brown. In addition, brightly colored species of *Hydroporus* or *Nebrioporus* are found in trout ponds and streams (Galewski 1971). In addition, disruptive color patterns of predaceous diving beetles of genera *Thermonectus* (Fig. 6.17d), *Sandracottus* (Fig. 6.17e), *Hydaticus*, and *Prodaticus* (now subgenus of *Hydaticus*) in Africa, America, and Australia were reported from exposed habitats with clear opened water with mainly mineral substrates (Larson 1996; Miller and Bergsten 2016). Within New Guinea there was found an unusually high portion of dytiscids (e.g., *Laccophilus*) with dark or melanistic forms (Balke et al. 1997). These authors suggested that the shading of the lentic habitats by the luxuriant vegetation may favor the occurrence of dark colored dytiscids.

The aforementioned pigments may be localized in different compartments. Very often all cuticle layers are translucent, and pigments within epidermal cells, within internal organs, compartments, or hemolymph are visible from the outside. In predaceous diving beetles these instances mostly occur in larval and pupal stages. In contrast, adults may possess pigments within different cuticle layers. Larvae of most dytiscid species are rather lightly colored in terms of sclerotized structures and the presence of dark dots is probably due to melanins or represent sclerotin. Some larvae possess dark or black colors (e.g., *Nartus grapii*, several *Hydroporus* species). In other cases, as in larvae of genus *Cybister*, the main total surface of the larvae is membranous and therefore lightly colored.

As in other insects, beetles and especially adephagous predaceous diving beetles may exhibit most chemical classes of biochromes, including carotenoids, chromans, flavonoids, aurones, ternary quinoids, including benzo-, naphtha-, anthra-, and polycyclic quinones, tetrapyrroles, including porphyrins and bilins, indolic melanins, ommochromes, papiliochromes, purines, pterines, and isoalloxazines (Needham 1978). These pigments are either synthesized by the beetles themselves or acquired from their food. In many cases the chemical composition on these zoochromes, their distribution among Dytiscidae, and their biosynthesis are unknown.

Carotenoids represent the only tetraterpenoids found in nature that are built up from eight isoprenoid units. Absorbing visible light across 400–500 nm they display yellow to red colors (Figs. 6.17d–h and 6.18b). These pigments are lipophilic and are therefore especially found in insect eggs, and all droplets of fat in hemolymph or fatbodies are thus yellow. Carotenoids are found in most insects from all insect orders (Coleoptera: e.g., Coccinellidae, Chrysomelidae). Generally they cannot be synthesized de novo by dytiscids who may depend on exogene supply from plants, bacteria, and fungi (Kayser 1985).

Most hydradephagan beetles contain lutein, isozeaxanthin, kryptoxanthin, and β-carotene along with 1–2 unknown carotenoids (Table 6.5, Fig. 6.17; Dettner and Hopstätter 1980; Kayser and Dettner 1984). In addition, in *Gyrinus substriatus* (Gyrinidae) isokryptoxanthin has been found, whereas *Laccophilus minutus* contain astaxanthin. Analysis of carotenoids in *Haliplus ruficollis* (Haliplidae) and *Hydroporus palustris*, as well as in some Dytiscinae (e.g., *Acilius*, *Dytiscus*) has indicated low concentrations of these yellow pigments.

Whereas chromans and flavonoids are absent in Dytiscidae, the heterocyclic aurones that represent a type of flavonoid are present as gland constituents. The yellow colored marginalin (**15**, Fig. 6.7, Table 6.1) was identified in the pygidial and preputial glands of *Dytiscus* and some *Agabus* species (see Sects. 6.4.2 and 6.4.3) (Dettner 1985).

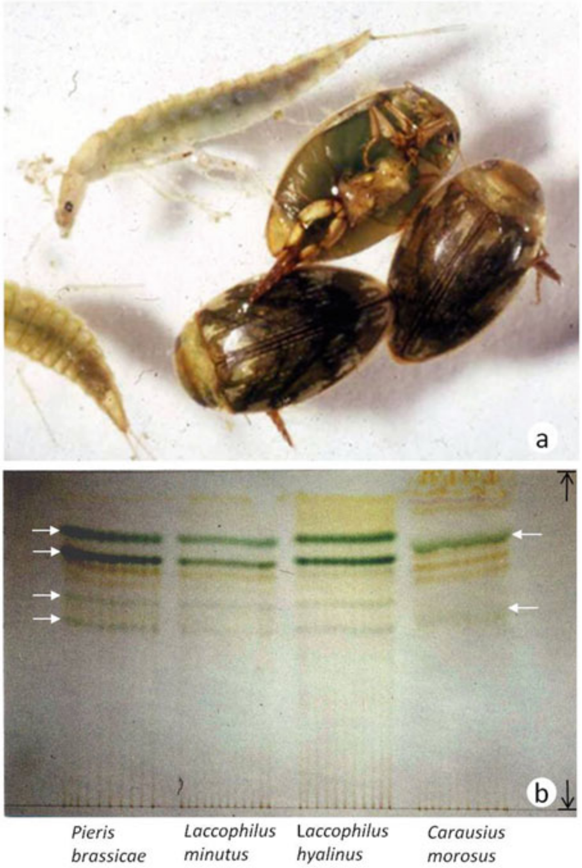


Fig. 6.18 Green coloration of *Laccophilus minutus* beetles and larvae (**a**), and TLC of extracts (**b**) from pierid butterflies *Pieris brassicae*, dytiscid water beetles *Laccophilus minutus*, *L. hyalinus*, and stick insects *Carausius morosus*. In *Pieris* and *Laccophilus* there could be shown 4 pterobilin spots, respectively (white arrows), in *Carausius* biliverdin IXα produces only 2 spots (white arrows). Animals were grinded with sodium sulfate and esterified with 8% HCl/methanol. Chloroform extracts were used for thin-layer chromatography on silica using solvent (benzene/dioxane/glacial acetic acid: 12/2/1; v/v/v). Starting point and solvent front are marked

The green color of certain *Laccophilus* species (*L. minutus*, *L. hyalinus*) is due to the mixture of carotenoids with the blue bile pigment biliverdin IXγ (= pterobiline) (Fig. 6.18). This kind of bile pigment, a tetrapyrrole, was reported for the first time for the order Coleoptera and represents the first identification of biliverdin IXγ outside the lepidopteran order (Kayser and Dettner 1984); biliverdin IXα is present in Odonata, Phasmida (Fig. 6.18b), Orthoptera, Mantodea, Planipennia, and few Lepidoptera (Kayser 1985). The four blue spots in pterobiline and the two spots in biliverdin IXα in Fig. 6.18b probably represent autoxidation products of the pure bile-pigments. Apart from the above-mentioned two species, *Laccophilus complicatus* and *L. maculosus* show a green coloration (Bertrand 1928), and this color is also found in pupae of *Laccophilus maculosus*, *L. proximus*, *L. minutus*, and *L. hyalinus*. Both European *Laccophilus* species are found within dense water plants, hence their green coloration seems to provide an excellent adaptation to this environment. It may be possible that the dominant red or brown colors found in tropical Laccophilinae may be due to a morphological color change.

Table 6.5 Carotenoids in Hydradephaga (see 6.5.3)

	lutein (3,3’-diOH-β,ε-carotene)	isozeaxanthin (4,4’-diOH-β,β-carotene)	kryptoxanthin (3-OH-β,β-carotene)	isokryptoxanthin (4-OH-β,β-carotene)	β-carotene (β,β-carotene)	other carotenoids unidentified	astaxanthin (3,3’-diOH-*β,β*-carotene-4,4′-dione)
Laccophilus hyalinus (De Geer)	+	+	+	–	+	1	?
Laccophilus minutus (L.)	+	+	+	–	+	1	+
Hydroporus palustris (L.)	–	–	–	–	–	–	?
Rhantus suturalis (Mcleay)	+	+	+	–	–	1	?
Agabus bipustulatus (L.)	+	+	+	–	–	–	?
Acilius sulcatus (L.)	+	–	–	–	–	–	?
Dytiscus marginalis L.	+	–	–	–	–	–	?
Noterus clavicornis (De Geer)	+	+	+	–	+	–	?
Gyrinus substriatus Stephens	+	+	+	+	+	2	?
Haliplus ruficollis (De Geer)	+	–	–	–	–	–	?

Ommochromes represent the major part of coloration in insect eyes, but they are also found in the integument of many insect orders and something are responsible for the red color of internal organs (Kayser 1985). They are biosynthetically derived from tryptophan through a degradative pathway via kynurenine and 3-hydroxykynurenine, which is metabolized to xanthurenic acid, 3-hydroxyanthranilic acid, and especially into xanthommatin, acridiommatins, ommins, and ommidins (Kayser 1985). Insects, as well as some fungi and bacteria, can synthesize ommochromes, which are usually bound to protein in intracellular granules. There exist only few records for ommochromes in beetles (Linzen 1974), but dytiscids likely produce these kind of phenoxazine-pigments.

Very often a melanin-type of pigment is used to denote a black pigment without knowledge of its chemical structure. Within dytiscids dark or brown body colorations are likely due to melanins, as melanin-deposition sometimes goes along with the tanning process within the exocuticle and also represents a way of hardening the cuticle. Young (1960b) observed an increase of diffuse melanization in or on the light portions of the color pattern of water beetles, which are likely driven by the environment in humid regions. In contrast, extension of the dark elements of the color pattern may be genetically controlled.

Dark spots are seen in many adult dytiscids (e.g., Fig. 6.17), the dark surfaces of elytra (Fig. 6.17i, k), and the dark colored sclerites (head, pronotum) in larvae (Fig. 6.17l–n). As a whole, melanins are biosynthesized by oxidation of tyrosine and comprise dark, yellow, brown and even red pigments. Their chemical structures are mostly derived from degradation products of the polymers. Melanins are classified into eumelanins, phaeomelanins, and allomelanins (restricted to plants, fungi, and bacteria), which are based on solubilities, color, elementary composition, and type of degradation products (Kayser 1985). Degradation of eumelanins, which may be deposited in the epidermis or other tissues (about 9% nitrogen), yields 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid or pyrrolic acids. In contrast, black allomelanins have lower amounts of nitrogen (1%). Their degradation results in production of catechol, 1,8-dihydroxynaphthalene, and protocatechuic acids.

Sclerotines are generated through sclerotization of insect proteins (arthropodins) by ortho-benzoquinones through the help of phenoloxidase (PO; see Sect. 6.7). They are widespread in insects and especially present in mechanically resistant structures such as the tips of mandibles. It seems highly probable that these pigments also occur in dytiscids, such as in the tips of larval mandibles (e.g., *Liopterus* (Fig. 6.17l), *Cybister* (Fig. 6.17m), and *Hyphydrus* (Fig. 6.17n; Young 1960b).

The white to yellow colored pteridines or pterin pigments are biosynthesized by insects, vertebrates, and bacteria from a purin precursor (guanosine 5′-triphosphate). Lepidoptera and Hemiptera species are rich in pterin pigments (Kayser 1985). In beetles, only xanthopterin, isoxanthopterin, and leucopterin pigments are found (Kayser 1985). The presence of any of these pigments has to be confirmed in dytiscids. Other pigment types, including quinones, papiliochromes, purines, and isoalloxazines are probably absent in dytiscid beetles, however coloration chemistry of light brown or yellow structures (Fig. 6.17c–h) is unknown.

6.6 Bacteria and Fungi from Dytiscids

All developmental stages of Dytiscidae may be associated with other organisms ranging from bacteria and fungi to microsporidia, gregarines, nematodes, mites, and parasitoid insects (Blunck 1923b; Franciscolo 1979; Miller and Bergsten 2016; Poinar and Petersen 1978). In this chapter, there are especially considered microorganisms (bacteria and fungi) which can be localized on the internal or external body surfaces of eggs, larvae, pupae, and adults of Dytiscidae. Internal microorganisms, although present everywhere in the host insect, are often found in mycetocytes or even mycetomes (= bacteriomes), and usually these microbial species either occur intra- or extracellularly (Dettner and Peters 2010). It is possible to isolate and to cultivate microorganisms from compartments within dytiscids, including the gut, rectum, or fat bodies. Due to the fact that certain bacteria are culturable, their biosynthetic capacities can be studied in the laboratory. The number and identity of such culturable (Sects. 6.6.1 and 6.6.2), non-culturable (Sect. 6.6.3) bacteria and fungi (Sect. 6.6.4) from Dytiscidae is described.

6.6.1 Taxonomically Identified Culturable Bacterial Strains from the Dytiscid Beetle Gut, and their Steroid Metabolism under Laboratory Conditions

By using nutrient-rich and nutrient-poor media 30 eutrophic or facultatively oligotrophic bacterial strains were isolated from foregut and other compartments of *Agabus affinis* and *Hydroporus melanarius* (Schaaf and Dettner 1997). Both tyrphophilous species were selected because they are found in waters that are characterized by low pH-values, high titers in humic acids, and low numbers of bacteria. Usually a higher fraction of bacterial species can be isolated and cultivated from the guts of invertebrates (about 5–10%), as compared with other body compartments (König and Varma 2006). The aquatic habitats where both beetle species existed also contained a further 41 strains. All strains from both beetle crops and environments (71 isolates, + 5 reference strains) were compared. Overall the authors found autochthonous bacterial flora in the beetle foreguts and a moderate influence of the aquatic microflora on the bacterial colonization of the beetles (Schaaf and Dettner 1997). How general this pattern is among other species in other habitats is unknown.

Because steroids are essential for insect physiology, it was suggested that the large amounts of dytiscid steroids from prothoracic defensive glands should be biosynthesized from dietary cholesterol with the help of microorganisms. As was evident in the foreguts of the two tyrphophilous dytiscid species (*Agabus affinis*, *Hydroporus melanarius*) that were analyzed microbiologically, several species of microorganisms in large amounts could be isolated and cultivated especially from this body compartment (see Fig. 6.19a). Based on classical methods of identification

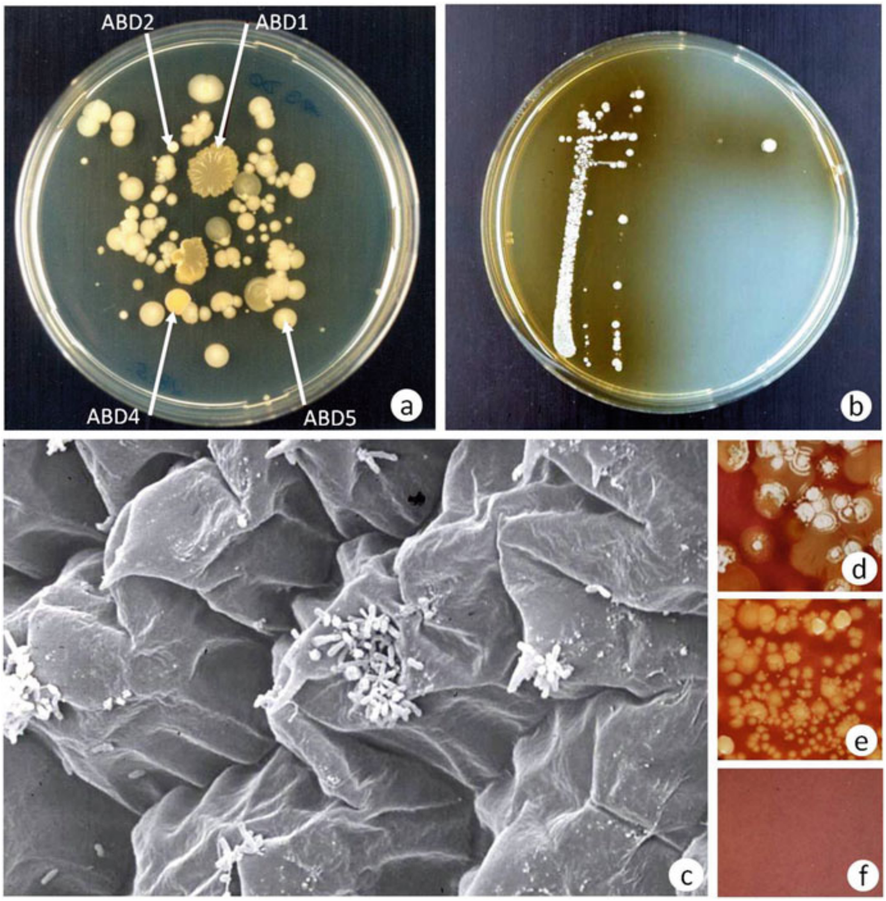


Fig. 6.19 At least four microorganism taxa (ABD1, ABD2, ABD4, ABD5) isolated from gut of *Agabus binotatus* (**a**), colony of *Actinomyces* spec. which was previously isolated from a dytiscid crop producing brown melanin within a Petri dish (**b**). REM of interior crop membrane of dytiscid beetle *Ilybius crassus* with microorganisms between the krypts (**c**). Incubated Petri dishes with isolations from the foregut (**d**), midgut (**e**), and hemolymph/fat body (**f**) of *Agabus melanarius*. There is shown at least one *Actinomyces*-species with its aerial mycelium (**d**). At least two other bacterial species are present in the midgut (**e**), whereas hemolymph/fat body host no cultivable microorganisms at all (**f**)

using shape and coloration of the colonies it was possible to isolate different microorganism strains. As an example, the foregut of *Agabus binotatus* contained at least four colonies (ABD1, ABD2 and ABD4 and ABD5 (Fig. 6.19a)). In addition, high densities of *Actinomyces* were found (Fig. 6.19b). These bacteria are characterized by their air-myceliae (Fig. 6.19b) and their ability to produce melanin as a byproduct when secondary compounds are manufactured. The interior crop membrane of the beetles exhibited a lot of crypts, where rod-shaped or pleomorphic bacterial populations were attached to the gut wall (Fig. 6.19c–d). When these

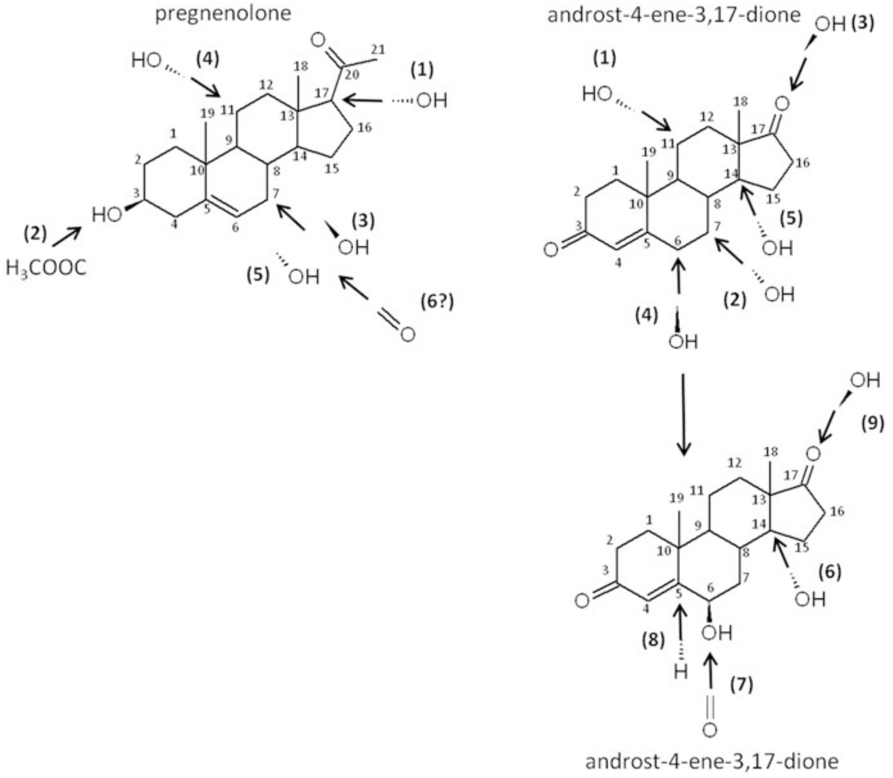


Fig. 6.20 Steroid transformation experiments with *Bacillus*-strains from guts of *Agabus affinis* water beetles. Pregnenolon (left) and androst-4-en-3,17-dione (right) were used as precursors. Arrows indicate those positions within steroid-skeleton where transformations occur. In addition there are indicated functional groups and the number of transformations (brackets)

beetles take up food, crop bacteria subsequently show a drastic increase in number. In addition, after several days/weeks, the colonies become foamy, and aerobic crop-fluid changes from light to dark brown or black, which may indicate a significant increase of microbes and their co-occurring production of colored secondary metabolites.

The foregut microflora of *A. affinis* and *H. melanarius* mainly consists of Pseudomonads, Bacilli, and irregular, gram-positive rods (e.g., *Arthrobacter*, *Corynebacterium*). Of note is that these bacteria groups within the beetle crops are responsible for a multitude of various steroid transformation reactions (Schaaf and Dettner 1998). Generally, microorganisms are well known to modify the steroid skeleton in aqueous solvents through hydroxylations, reduction of carbonyl functions, dehydration, and hydrations, or are important in separating of racemates or asymmetric syntheses.

Two *Bacillus* strains were isolated from foreguts of *Agabus affinis* and were tested for their in vitro steroid transforming ability (Fig. 6.20 right; Schaaf and

Dettner 1998). When incubated with androst-4-en-3,17-dione (Fig. 6.20, right) 13 transformation products were detected. Androst-4-en-3,17-dione was hydroxylated at C_6, C_7, $C_{11,}$ and C_{14} resulting in formation of 6β-,7α-, 11α-, and 14-α-hydroxyandrost-4-en-3,17-diones. One strain also produced minor amounts of 6β,14α-dihydroxyandrost-4-en-3,17-dione from androst-4-en-3,17-dione. Certain amounts of metabolites with a 6β-hydroxy-group were further oxidized to corresponding 6-oxosteroids. Moreover, a specific reduction of the Δ^4-double bond resulted in production of 5α-androstane derivatives. In addition, carbonyl functions at C_3 and C_{17} were reduced leading to the formation of 3ξ-OH or 17β-OH- steroids.

If pregnenolone was used as a precursor (Fig. 6.20, left), dominating reactions were hydroxylations, with 7α-hydroxypregnenolone as major product (Fig. 6.20 left; Schaaf and Dettner 2000a). In addition both strains produced lower yields of 7β- and 15-hydroxypregnenolone. In contrast, 11-, 17-, and 16α-hydroxypregnenolone were only produced by strain HA-V6–3. The second strain HA-V6–11 had the capability to hydroxylate pregnenolone at C11 and C17 as well (see 7, 11α, 7β, 11-α-dihydroxypregnenolone). Both strains oxidized monohydroxylated 7-OH-pregnenolones to 7-oxopregnenolone. One strain (HA-V6–3) also performed 3β-acetylation of pregnenolone in trace amounts. The major difference between the utilization of androst-4-ene-3,17-dione and pregnenolone by these *Agabus* isolates is the shift from C6 to C7, resulting in the formation of 7α-hydroxypregnenolone in contrast to 6β-hydroxy-androst-4-ene-3,17-dione.

If one considers the steroidal prothoracic defensive gland compounds it seems highly probable that they are biosynthesized from cholesterol that is taken up by the beetles with their food. The above-mentioned data illustrate that microorganisms in the crop may produce cholestenone and cholesteryl-3-acetate from cholesterol (Fig. 6.21). To produce defensive steroids a side chain cleavage (Fig. 6.21 **scc**) of cholesterol must be postulated. Functions, localization of these enzymes and their structures in vertebrates are described by Kleine and Rossmanith (2021). For example, in vertebrates **scc** is localized within mitochondria (Kleine and Rossmanith 2021). Pregnenolone (**67**), progesterone (**37**), and pregn-4,16-ene-3,20-dione could be present in the hemolymph (Fig. 6.21). The activity of hydroxysteroid-dehydrogenase-isomerases (Fig. 6.21**, hsd**), dehydrogenases (Fig. 6.21, **dh**), and C_{17}-C_{20}-lyase (Fig. 6.21, **ly**) should be postulated. In vertebrates **hsd** is found within the smooth ER (Kleine and Rossmanith 2021). From **67, 37** and pregn-4,16-ene-3,20-dione the gland cells of the prothoracic defensive glands could produce typical steroidal defensive compounds such as cortexone (**43;** biosynthesized either from **67** or **37**). 21-Hydroxypregna-4,6-diene-3,20-dione (**63**; biosynthesized from **43**), estrone (**24**; biosynthesized from 1,4-androstadiene-3,17-dione), and testosterone (**29**; biosynthesized from 1,4-androstiene-3,17-dione). To produce estrone, an aromatase (Fig. 6.21 **ar**) is necessary. Since cells of breast cancer in vertebrates need estrogens, worldwide there is a search for inhibitors of these aromatases (Kleine and Rossmanith 2021). The presence of enzymes involved in the steroid biosynthesis of vertebrate-type steroids was proven in various insect-tissues, however apart from

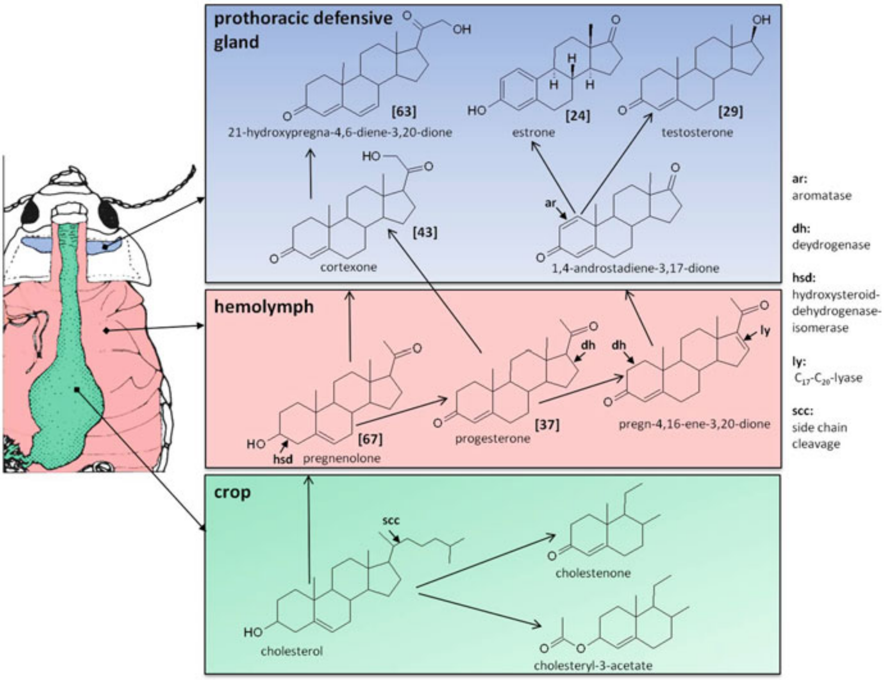


Fig. 6.21 Potential biosynthetic capabilities of microorganisms from the crop (foregut, green) of dytiscid water beetles to metabolize cholesterol. There are indicated further metabolites which should be present in the hemolymph (red) and in the prothoracic defensive glands, respectively, the gland reservoirs (blue). Numbers refer to Fig. 6.15. Important enzymes according to Swevers et al. (1991) are indicated by abbreviations

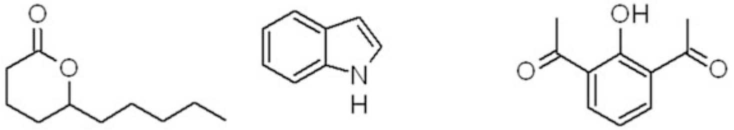
dytiscid beetles such as *Acilius sulcatus*, the steroid concentrations are always very low (Swevers et al. 1991).

At least three investigations concerning biosynthesis of defensive steroids in Dytiscidae have been published. Schildknecht (1970) injected [4-^{14}C]-progesterone, [4-^{14}C]-cholesterol, and [2–^{14}C]-mevalonolactone into *Acilius sulcatus*. In contrast to labeled mevalonolactone, cholesterol and progesterone were incorporated after 6 weeks into 6,7-dehydrocortexone (**63**), cortexone (**43**), cybisterone (**57**), 6,7-dihydrocybisterone (**50, 51**), and 6,7-dehydroprogesterone (**56**). This indicates that these dytiscids absorb cholesterol and other steroids with their food. Biosynthetic experiments with *Agabus seriatus* (now *Ilybiosoma seriatum*) and injected ^{14}C-cholesterol showed that after three weeks 7.5% of incorporation occurred into deoxycorticosterone (**43**) and other prothoracic gland components (Fescemyer and Mumma 1983). In a detailed study, Chapman et al. (1977) found that pregnadiene derivatives (e.g., 6,7-dehydrocortexone (**64**)) were biosynthesized from cholesterol. The introduction of the Δ^4 and Δ^6 bonds was shown to involve the elimination of 4β and 7β hydrogens, respectively (Chapman et al. 1977). Apart from *Acilius sulcatus* and *Ilybiosoma seriatum,* a biosynthesis of vertebrate-type steroids could be only demonstrated in *Manduca sexta* (Swevers et al. 1991).

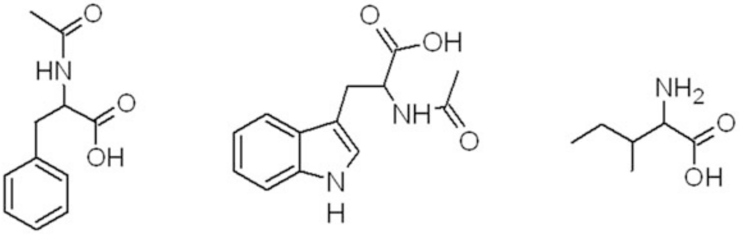
The biotechnological use of microbial steroid transformations has received increasing economical and scientific interest in the recent years. Thus, the isolation and investigation of microorganisms from "exotic" sources associated with steroid-carrying dytiscids deserves further attention.

6.6.2 Taxonomically Identified Culturable Bacterial Strains from the Dytiscid Beetle Gut and their Secondary Metabolites Produced under Laboratory Conditions

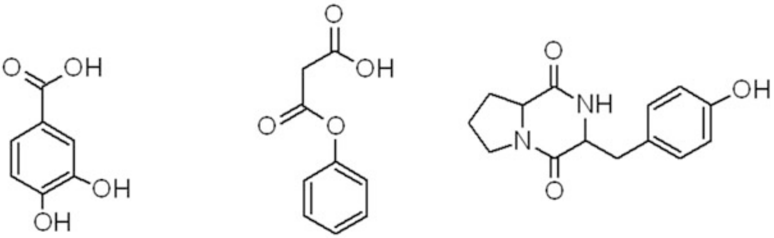
Nearly all insects associate with microorganisms and fungi, and sometimes these interactions are actually symbiotic. To isolate new kind of microorganisms and new natural compounds with biological activity from exotic sources various hydradephagan beetles were externally sterilized and subsequently selected compartments were analyzed for microorganisms (Gebhardt et al. 2002). Among various dytiscid hosts *Laccophilus minutus* was of interest because one bacterial strain, identified as *Bacillus pumilus*, showed remarkable activities in various bioassays. From the *L. minutus* foregut 14 bacterial strains were isolated. The *B. pumilus*-strain exhibited a pronounced herbicidal activity against both duckweed (*Lemna minor*) and a green algae (*Chlorella fusca*) (Gebhardt et al. 2002). After cultivation in a 10 L fermenter, six secondary metabolites were detected from the *B. pumilus* extract (Fig. 6.22): N-acetylphenylalanine (**112**), N-acetyltryptophan (**113**), L-isoleucine (**114**), malonic acid phenylester (**116**), 3,4-dihydroxybenzoid acid (**115**), and cyclo (propyltyrosyl) (**117**). These metabolites show some interesting biological activities. For instance, N-acetylphenylalanine (**112**) is an antidepressant and appears in large amounts in urine of individuals with phenylketonuria. Another acetylated amino acid is represented by N-acetyltryptophan (**113**), which can be used as a stabilizer of some protein solutions. L-isoleucine (**114**) represents an essential proteinogenic amino acid with various biological functions, whereas 3,4-dihydroxybenzoic acid (**115**) is antioxidant and anti-inflammatory and has tumoricidal effects. This latter compound is widely distributed in nature and occurs in various plants (Gebhardt et al. 2002; green tea), in fungi (*Agaricus, Penicillium*, *Phellinus*, Laskin and Lechevalier 1973), in bacteria (*Flavobacterium*, Kieslich 1976), as a tanning agent in the oothecas of blattid insects (Dettner and Peters 2010), and as a constituent of antimicrobial pygidial glands of dytiscids (Dettner 1985). In pygidial glands, 3,4-dihydroxybenzoic acid is found as methyl- (**7**) or ethyl- (**8**) ester. Limited data are available on malonic acid phenylester (**116**). The diketopiperazine cyclo (propyltyrosyl) (**117**) is also known as maculosin I, and was previously isolated from various other microorganisms, including the fungus *Alternaria alternata* and marine sponges (see Dettner 2011). This compound is an extremely host-specific phytotoxin from the *Alternaria*-weed pathogen and causes black leaf blight in



[109]: δ-decalctone **[110]:** 1H-indole **[111]:** 1,3-dimethoxy-2-hydroxybenzene



[112]: N-acetyl-phenyl-alanine **[113]:** N-acetyltryptophan **[114]:** L-isoleucin



[115]: 3,4-dihydroxy-benzoic acid **[116]:** malonic acid phenylester **[117]:** cyclo(prolyltyrosyl)

Fig. 6.22 Constituents of pupal peristigmatic glands of *Dytiscus marginalis* (constituents **109–111**) and secondary compounds (**112–117**) isolated in the laboratory from *Bacillus pumilus* which was isolated from guts of *Laccophilus minutus*

Centaurea maculosa (Strobel et al. 1990). Maculosin represents a prototype of a safe and environmentally friendly antiknapweed herbicide (Bobylev et al. 1996), which binds to cytosolic maculosin-binding proteins (Park and Strobel 1994). In addition, maculosin II (dehydrated maculosin I) and various synthetic analogs inhibit the growth of wheat coleoptiles (Bobylev et al. 2000). More recently, maculosin was found to insert into liquid crystalline phase bilayers of 1,2-palmitoyl-sn-glycero-3-phosphatidyl choline or 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidyl choline. Its orientation within the membranes is modulated by cholesterol (Lopes et al. 2004). Because several dytiscids produce monoglycerides (Fig. 6.15) in their prothoracic glands, maculosin could also interact with these beetle compounds.

These six above-mentioned compounds are produced under laboratory conditions, and thus if they are also biosynthesized under natural conditions in the foregut of *Laccophilus minutus* it would be highly interesting to know their biological significance. When the above-mentioned *Bacillus pumilus*-strain from the collection of microorganisms of BASF was investigated 5 years after the isolation of the microbial material from *Laccophilus* guts the six metabolites were not produced (M. Langer, unpublished data). It seems possible then that this strain was somehow stressed when it produced the six metabolites. In contrast, a different strain (LU 2644) produced small amount of phenylacetic acid, a main pygidial gland constituent (**11**) of Hydroporinae and *Liopterus* species (Fig. 6.7, Table 6.1). In addition, incubation of a *B. pumilus* extract with phenylalanine significantly stimulated the production of phenylacetic acid, which represented the main compound of the bacterial extract. At present it is unknown if microbial metabolites, which were isolated in the lab, are also present within the intact host insect–symbiotic/parasitic bacteria systems.

6.6.3 Non-Culturable Bacteria from Predaceous Diving Beetles

According to König and Varma (2006) only low amounts of gut microorganisms can be cultivated and therefore it is of interest if non-culturable microorganisms can be also quantified. In 2009, it was reported by Küchler et al. that specimens of *Rickettsia* were detected in four species of the genus *Deronectes* (Hydroporinae). The genus *Rickettsia* is represented by gram-negative bacteria that are present in cocci, rods, or thread-like forms. All these bacteria are obligate intracellular parasites and unlike *Chlamydia* or *Mycoplasma* they possess true cell walls. *Rickettsia* species which are usually susceptible to tetracyclines are associated with both human and plant diseases. Human pathogenic species are transmitted by arthropods such as ticks, fleas, or lice and are responsible for typhus, Australian Tick Typhus, Rickettsial pox, or Rocky Mountain Spotted fever.

In *Deronectes platynotus*, 100% of all specimens investigated showed association with *Rickettsia*. In other *Deronectes* species lower numbers of investigated had some associations with *Rickettsia* (e.g., *D. aubei*, *D. delarouzei*: 40%; *D. semirufus*: 33,3%). All individuals of *D. latus*, *D. aubei sanfilippoi*, and *D. moestus inconspectus* were *Rickettsia* negative. Within Hydroporinae *Rickettsia* could also be identified from specimens of *Hydroporus gyllenhalii*, *H. tristis*, *H. umbrosus*, and *H. obscurus*. *Rickettsia*-positive species from Colymbetinae are *Agabus melanarius*, *A. guttatus,* and *Ilybius wasastjernae*. The frequencies of *Rickettsia* infection were maintained across different seasons. *Rickettsia* was also recorded from other coleopteran families including Bruchidae (Fukatsu et al. 2000), Buprestidae (Lawson et al. 2001), Coccinellidae (von der Schulenburg et al. 2001), Curculionidae (Zchori-Fein et al. 2006), and Mordellidae (Duron et al. 2008).

Analysis of 16S rRNA gene sequences revealed a phylogenetic relationship of *Deronectes* rickettsiae with *Rickettsia limoniae*, which also was isolated from the crane fly *Limonia chorea* (Diptera, Limoniidae) and tentatively classified as members of the basal ancestral group. A similarity of *Deronectes* rickettsiae was found to *Rickettsia* of *Cerobasis guestfalica* (Psocoptera, Trogiidae) and *Lutzomyia apache* (Diptera, Psychodinae), whereas *Rickettsia* from *D. semirufus* cluster basally with rickettsiae from leeches. Phylogenetic analysis of *gltA* (citrate synthase) gene sequences showed that *Deronectes* symbionts (from *D. platynotus*, *D. aubei*, *D. semirufus*, *D. delarouzei*) were closely related to rickettsial isolate from the spiders *Pityophantes phrygianus* and *Meta mengei*.

The distribution, transmission, and localization of *Rickettsia* in *D. platynotus* were studied using a diagnostic PCR-assay and FISH. *Rickettsia* could be identified in all compartments of *Deronectes* including the head (ommatidia), soft tissue of elytra, hemolymph, and legs. Those compartments with active metabolism, such as fat body or internal reproductive organs contain numerous Rickettsiae. In the meantime, tissue tropisms and transstadial transmission of *Rickettsia* was also described in *Culicoides impunctatus* (Ceratopogonidae; Pilgrim et al. 2020). In *D. platynotus Rickettsia* is more abundant in females than in males, where the bacteria dominate in accessory glands (and musculature enclosing accessory glands). When eggs of infected females of *D. platynotus* were investigated they were *Rickettsia* positive, which indicates vertical transmission. Due to the predatory lifestyle of *Deronectes*, a horizontal transmission of *Rickettsia* also seems possible, and thus aquatic prey of *Deronectes* should be analyzed in the future. The bacteria could be also found in their oocytes, follicle cells, and second and third larval stages of *Deronectes*, where the bacteria increased from earlier to later stages. In the meantime those *Rickettsia*-isolates belonging to the Torix group are recognized as typical for aquatic invertebrates with predatory larval stages (e.g. midges, predaceous diving beetles, leeches, crane-flies) or alternatively show hematophagy (e.g. biting midges, leeches, sandflies; Pilgrim et al. 2017). Representatives of Torix *Rickettsia* are also found in amoeba, amphipods (Park and Poulin 2020), or Odonata (Thongprem et al. 2020). When *Rickettsia* amplicons are analyzed in the Barcode of Life Data System (184.585 barcode sequences) Pilgrim et al. (2021) showed that *Rickettsia* is observed in about 0.41% of barcode submissions and is more likely to be found than *Wolbachia* (0.17%), another widespread intracellular bacterium of many arthropods (see below). It was shown that Torix *Rickettsia* are overrepresented in aquatic insects (the so-called aquatic hot spot).

The biological role of *Rickettsia* in Coleoptera and especially in aquatic forms is largely unknown. At the moment there are no indications that *Rickettsia* infections have any effects on the fitness of the *Deronectes* host. Neither reduced body weights and fecundities (as in infected aphids) nor remarkable increases in host size as observed in leeches (Kikuchi and Fukatsu 2005) are observed. It is well known that parasitic living bacteria such as *Rickettsia*, *Spiroplasma*, *Cardinium*, and *Wolbachia* can manipulate reproduction of their hosts for their own benefit (including parthenogenesis, cytoplasmatic incompatibility, feminization, and male killing; O’Neill et al. 1997).

Recent data indicate that *Wolbachia* another aforementioned genus of intracellular bacteria (Alphaproteobacteria) is present in predaceous diving beetles. This bacterial genus represents one of the most common microbial parasites ("*Wolbachia* pandemic") and normally infects arthropod species (especially insects) and nematodes. Within Dytiscidae *Wolbachia* was found in *Agabus bipustulatus*, *Liopterus haemorrhoidalis,* and *Hygrotus versicolor* (Sontowski et al. 2015). In contrast following species were negative for this parasite: *Hydroporus dorsalis, H. palustris, H. planus, Hygrotus inaequalis, Hyphydrus ovatus, Laccophilus minutus, L. hyalinus, Hydroglyphus geminus* (formerly *Guignotus pusillus*; Duron et al. 2008), *Platambus maculatus, Rhantus frontalis, R. suturalis, Ilybius quadriguttatus, I. fuliginosus, Agabus bipustulatus, A. sturmii, A. uliginosus, A. undulatus, Colymbetes fuscus* (Sontowski et al. 2015), and genus *Meladema* (Sýkora et al. 2017). The genus *Wolbachia* which occurs in three supergroups (A, B, F) was identified in 204 beetle species and especially in herbivorous species (Kajtoch and Kotásková 2018). Generally *Wolbachia* was recorded from terrestrial species with aquatic life stages (Odonata, Plecoptera, various Diptera). The only fully aquatic hosts among arthropods were a crustacean species and *Hydroglyphus geminus*. Sontowski et al. (2015) suggested that horizontal movements of *Wolbachia* occur less often in aquatic environments than in terrestrial systems. Obviously there exist fewer pathways of such horizontal transmission under water.

6.6.4 Taxonomically Identified and Culturable Fungi from Aquatic Insects and Especially Dytiscid Beetles

There exist manyfold interactions between insects and fungi covering symbiontic interactions but also insect-pathogenic fungi (e.g., Murrin 1996; Spatafora 2004; Vega and Blackwell 2005). Because various insects and also selected Dytiscidae are used as food for animals and humans (see Dettner 2019a, Yee 2014) there were investigated adult specimens of *Dytiscus marginalis* with respect to presence of external (ext.) or internal (int.) Ascomycota species (Ozdal et al. 2012). Several of these compiled and mostly abundant filamentous taxa are able to produce biologically active metabolites which are often targeted against other fungi but also ward off fungivorous insects (Rohlfs et al. 2007). *Acremonium* spec. (ext.) may produce cephalosporines. *Aspergillus niger* (ext, int) and *A. versicolor* (int) produce mycotoxins such as kojiacid, ochratoxins, or sterigmatocystin. *Cladosporium cladosporioides* (ext, int) and *C. herbarum* (ext) belong to the most common fungi outdoors and indoors and were also isolated from other insects. They may produce antifungal metabolites such as cladosporins, 5-hydroxyasperentin, and protein kinase C-inhibitors. *Paecilomyces marquandii* (int) a soilborne filamentous fungus belongs to the taxon Eurotiomycetes. Among Ascomycota there were isolated five *Penicillium* species from *Dytiscus*. *Penicillium brevicompactum* (int) and *P. expansum* (int), which are often found on fruits produce toxins such as mycophenolic acid respectively patulin. *P. frequentans* (int, ext), *P. jensenii* (ext) and *P. notatum* (ext) usually represent abundant saprobionts and produce biologically active metabolites such as frequentin, citrinin, griseofulvin, fumagillin, penicillins, secalonic acid, the sesquiterpenoid PR-toxin or isofumigaclavin. The last taxon isolated from *Dytiscus* surface was *Trichoderma harzianum* (ext). This species produces trichothecene-mycotoxins and can be used as "fungicide" against other fungi such as *Botrytis, Fusarium, and Penicillium.*

Various groups of fungi are entomoparasitic and can infect aquatic larvae especially of Diptera (Boucias and Pendland 1998). Well known is the genus *Lagenidium* (Class Oomycetes now considered to represent Protocists) and *Coelomycetes* (Class Chytridiomycetes now considered to represent Protocista). Two other groups of fungi, the Trichomycetes and the Laboulbeniales represent commensals or even parasites of insects. Trichomycetes are taxonomically isolated Zygomycota which contain polyglucosamine and galactane instead of chitin. The endosymbiontic group is usually found on the linings of hindguts of hosts such as larval stages of Diptera, Ephemeroptera, Plecoptera, and Coleoptera. There they receive minute amounts of nutrients from their hosts and may even produce essential sterols and B-vitamins (Lichtwardt 1996, 2004). Laboulbeniales represent about 2,000 species and are ectosymbionts or parasites of insects (Tavares 1980; Weir 2004). They are treated in Sect. 6.4.2.1.2, moreover literature is compiled in Miller and Bergsten (2016). In an interesting study, Goldmann and Weir (2012) described the position specifity of *Chitonomyces* on *Laccophilus*-beetles and concluded that sexual contacts and transmissions of Laboulbeniales are responsible for position specificities of these ectoparasites. However, they did not discuss how the peculiar secretion-grooming behavior of dytiscid beetles (Kovac and Maschwitz 1990; Dettner 2019b) and the external distribution of fungicides from both prothoracic and pygidial glands, that means host defenses could be also responsible for this enigmatic phenomenon.

6.7 Hemolymph: Aspects Concerning Internal Defense, Hemostasis, and Regeneration Focusing on Dytiscidae

Hemolymph of insects is about 5 to 40% of body weight of insects and their developmental stages. The watery fluid contains small (sugars; amino acids, Schoffeniels 1960; organic acids) and larger organic molecules (proteins, lipids, pigments; for example, see Yadav et al. 1988), ions and blood cells, the so-called hemocytes. As compared with vertebrates insects are generally characterized by very high titers of amino acids, moderate titers of uric acid (often sequestered within fat body), and especially the insect blood sugar trehalose. Concerning ions insect hemolymph generally contains more calcium- and magnesium, or phosphate ions as compared with vertebrates but contains lower concentrations of chloride-ions. This may be illustrated by major inorganic ions within hemolymph of the predatory *Dytiscus marginalis* (concentrations as mequiv/l; Chapman 1998; Na^+: 165, K^+:

6, Ca^{2+}: 22, Mg^{2+}: 37, Cl^-: 44; $H_2PO_4^-$: 3). There exist further ional data with respect to *Colymbetes*, *Cybister* (Crowson 1981), and *Dytiscus verticalis* (Frisbie and Dunson 1988a, b, c). Moreover, various publications cover osmotic concentrations of Dytiscidae hemolymph in order to study osmoregulation and salinity tolerance in larvae or adults of selected species such as Hygrotini or *Hydroporus* (e.g., Pallarés et al. 2015; Villastrigo et al. 2017). The uptake of radioactive sodium chloride via intestine by drinking was studied in larvae of *Acilius sulcatus* and *Dytiscus marginalis* (Schmitz and Komnick 1976).

Metabolic changes between insect tissues/organs and hemolymph are highly important especially with respect to hormones, nutrients, or wastes. In addition, molecules such as glycerin responsible for cold protection that means freezing point depressions are also found in hemolymph (freezing point reduction, e.g. *Acilius* spec.: −0.65 grad C; *Gyrinus* spec.: −0.68 grad C; Frick and Sauer 1973). In most insects there have been described various types of hemocytes such as prohemocytes, plasmatocytes, granulocytes, or oenocytes. These hemocytes may represent about 10% of the hemolymph volume. Concerning Dytiscidae Price and Ratcliffe (1974) could identify six types of hemocytes. Various hemocyte types from Dytiscidae were figured: Coagulocytes by Grégoire (1984), prohemocytes and plasmatocytes by Barrat and Arnold (1910), and oenocytes by Kreuscher (1921). Actual data with respect to blood cells from taxonomically related groups such as Carabidae are from Giglio et al. (2008). Normally hemocytes are fixed on the surface of other tissues, only after injuries, parasitization, or during molting numbers of floating hemocytes within hemolymph are increased.

6.7.1 Internal Defense

Both insect blood cells and insect plasma have various main functions. In case of physical injury of integument due to various kinds of predators there is observed a hemolymph coagulation which results in a wound-healing process, eventually followed by regeneration of body parts. In addition hemocytes may be able to exhibit phagocytosis that means ingestion of small particles such as bacteria or larger metabolites. By the help of hemocytes, even larger parasites such as nematodes or insects eggs of parasitoids may be encapsulated and killed. Finally insect blood represents a storage of nutrients which are additionally distributed with the body. In Dytiscidae a sequestration of distasteful compounds as in Coccinellidae or Meloidae has not been described.

Concerning hemolymph coagulation there is formed a hemolymph clot in order to seal the wound (Fig. 6.23a, b), to reduce hemolymph loss and to inhibit viral or bacterial contaminations. The above-mentioned phagocytosis is known as cellular defense mechanism and is associated with encapsulation and nodule formation of the foreign material. However, not only hemocytes are involved, additionally humoral factors as enzymes or other proteins (Prophenoloxidase, lysozymes, lectins and other proteins) play important roles (Trenczek 1998). As known from various studies

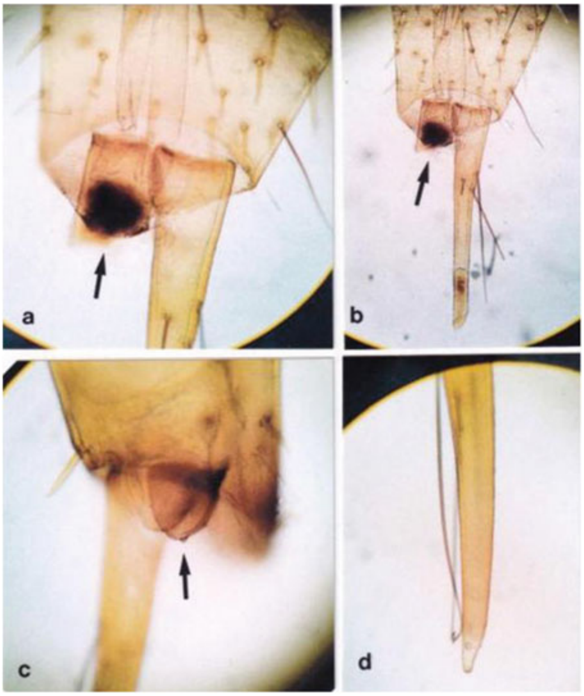


Fig. 6.23 Ventral view of the exuvia of the second-stage larva of *Agabus bipustulatus* with one urogomphus eliminated basally (arrows, **a**, magnification 100X; **b** magnification 36X) and the opposite urogomphus eliminated medially (**b**). Side view of the same specimen in the third larval stage (**c**, magnification 100X) with regenerated closed short (arrow; **c**) and longer (**d**, magnification 100X) urogomphi. Originals

especially with *Drosophila* immunity proteins rapidly are produced in the hemolymph after a primary infection, recognition of the pathogen by hemocytes, and starting of molecular pathways such as Toll and JAK/STAT signaling (Altincicek et al. 2008; Dettner and Peters 2010). These so-called humoral responses (in contrast to cellular responses) may be the very rapid production of antimicrobial peptides (AMPs), lysozyme or phenoloxidase (PO) (see Adamski et al. 2019).

Cioffi et al. (2016) were the first measuring the immune competence in the water beetle genera *Deronectes* and *Hydroporus* (Cioffi 2017) when they studied the physiological niche and the geographical range of these mostly European genera. Although it is very difficult to measure metabolic costs of these different immune responses it is feasible to assess numbers and amounts of antimicrobial hemolymph peptides which are directed against Gram-positive and Gram-negative bacteria, fungi, or viruses. Moreover, the activity of the phenoloxidase (PO) pathway was analyzed in the above-mentioned investigation. According to González-Santoyo and Córdoba-Aguilar (2012), phenoloxidase production and maintenance have fitness costs for the hosts and they also suggested that PO does not seem to be an indicator of resistance but rather of host condition. Apart from melanin production for sclerotization of the insect integument, PO as all-embracing enzyme is also involved in hardening of different structures, in wound healing (Fig. 6.23a, b) and in encapsulation of parasitoid eggs or parasitic nematodes. Whether models assuming that southerly, range-restricted *Deronectes* species need more antimicrobial peptides or

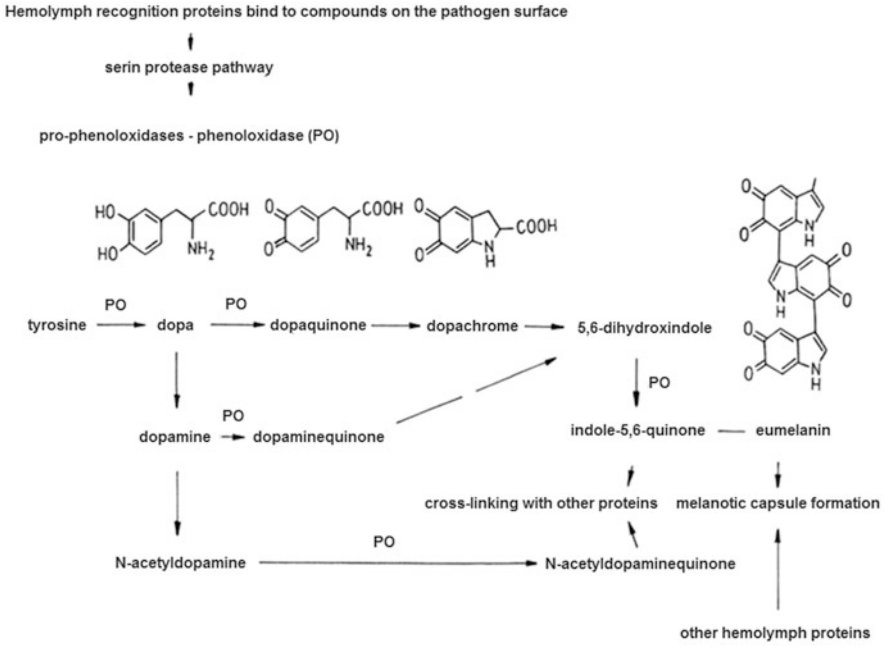


Fig. 6.24 Melanization pathway in insect hemolymph leading to melanotic capsule formation. Modified according to Boucias and Pendland (1998). *PO* Phenoloxidase

high latitude species exhibit seasonal immunocompetence with lower immunity in summer and higher in winter are convincing, this can only be answered when complete aquatic microbiota and other conditions are analyzed simultaneously with insect populations in appropriate biotopes.

6.7.2 Melanization Pathway in Insect Hemolymph and Role of the Key Component Phenoloxidase

A variety of specific and non-specific responses are observed in insects upon foreign particles. Hereby the phenoloxidase system represents the most important defense system in insects leading to melanization of pathogens or damaged tissues (see Fig. 6.23a, b). Various phenoloxidases are found both in the sclerotizing cuticle of insects and the hemolymph. In the cuticle they oxidize o- and p-phenols, within hemolymph they may oxidize mono-phenols and o-diphenols (Urich 2010). Phenoloxidases for activation of process of melanization are activated by prophenoloxidases (Fig. 6.24). The whole system starts when recognition proteins from hemolymph bind to components of the pathogen surface (Götz 1988,) which might represent ß-1,3-glucans, lipopolysaccharides, or peptidoglycans; González-

Santoyo and Córdoba-Aguilar 2012). The activation of an unknown serin protease leads to prophenoloxidase and phenoloxidase activation. Phenoloxidases catalyze oxidations of various phenols or dihydroxyindoles to quinones (see Fig. 6.24), which subsequently are polymerized to form eumelanin one of the most common melanins with dark brown or blackish coloration. Finally melanin is deposited around damaged tissue (Fig. 6.23a, b) or in the neighborhood of pathogens which are subsequently encapsulated. These dark colored capsules in admixture with hemolymph proteins prevent growth and development of pathogens and usually result in its death. In addition these quinoic precursors are crosslinked with proteins from both pathogen and host insect and additionally quinones can generate toxic reactive oxygen species (Boucias and Pendland 1998). It is interesting to note that certain insect-pathogenic bacteria of genus *Photorhabdus* secrete antibiotics which suppress host defenses through phenoloxidase inhibition (Eleftherianos et al. 2007).

6.7.3 Hemostasis and Regeneration of Body Appendages

After strong molestations especially larvae but also adults of Dytiscidae may suffer from predators and if they survive they show various injuries such as scratches or loss of body appendages (Blunck 1923b; Peddle and Larson 1999). In all cases of cuticular damages there is observed a coagulation of hemolymph (hemostasis) along with eumelanin darkening and in larval stages there may be observed regeneration processes which allow to restore lost body parts through regeneration.

If water beetles have been wounded, the wound is sealed through the activity of clotting systems in order to avoid loss of hemolymph and to avoid entrance of pathogenic microorganisms. There were microscopically described several patterns of hemolymph coagulation within various Dytiscidae genera (Grégoire 1984). *Cybister* coagulocytes produce exudations and may show an explosive discharge and gel production (pattern I). Whereas *Hydaticus* coagulocytes did not react, *Dytiscus* coagulocytes produced cytoplasmatic expansions forming meshworks (pattern III; Grégoire 1984; Gupta 2009). Due to their open circulatory system insects extensively use clot formation without potential danger of thromboses. Also insects and especially *Drosophila* show cascades leading to cross-linking through their coagulation system, that means specialized clottable proteins are deposited. In addition there is also observed a phenoloxidase activating cascade, because its function during the wound response has been observed in certain species but not in *Drosophila* (Scherfer et al. 2004; Theopold et al. 2002). If body appendages of dytiscid larvae or adults are eliminated experimentally or under natural conditions, the wound is closed through humoral and cellular hemostasis. In both urogomphi in Fig. 6.23a, b a dark colored clot of coagulocytes and melanin is found sealing the open wound. Also in larvae or adults from the field trunks of body appendages show a black coloration.

During hemostasis hemocytes are degranulated and in addition form microparticles, at the same time certain phospholipids from the coagulocytes are externalized.

At least in the genus *Drosophila* these lipids may be recognized by receptors which are localized on the surface of the hemocytes. These hemocytes also show hemocyte-coagulogens on their surfaces. At the same time and in addition to this cellular activity humoral coagulants the coagulogens are activated and interact with the hemocytes, but also other factors such as lipophorin may be involved.

If insect larvae that means juvenile stages are injured, they can often regenerate body appendages during subsequent moltings. This is especially seen in Blattodea or Hemiptera. That generally means: Without molting no regeneration is possible in adult hemi- or holometabolous insects (Goss 1974; Maruzzo and Bortolin 2013), only closing of wounds through hemostasis and synthesis of cuticle is possible in the aforementioned cases. In many cases regeneration of body appendages is correlated with an atrophy of the appropriate body appendage. Moreover, complete regenerations are often observed, when first stage larvae are concerned. In addition complete regenerations depend on the position of the lesion. If they are situated proximally (e.g., tibia or tarsus), a complete regeneration seems possible. In contrast if these lesion are more basally (e.g., coxa or trochanter) as a rule there are only incomplete regenerations observed (Goss 1974). Finally the time of injury before the next molting is highly important if the degree of regeneration is considered. If there is only few time available, there results either an incomplete regeneration or the developmental time of the larval stage is lengthened.

Concerning Dytiscidae there are several observations by Schaeflein (1989) especially with regard to teratology and regeneration of hydroporine, colymbetine, and dytiscine legs. A more detailed and careful study concerning regenerations in *Dytiscus* larvae was presented more than 110 years ago by Blunck (1909a). He eliminated body appendages in *Dytiscus* larvae or observed larvae with natural damages from the field. In most cases he was highly successful in order to control the living pupal or adult stage. His forgotten data concerning *Dytiscus*-legs and -urogomphi are illustrated in Fig. 6.25. Even earlier Megusar (1907) also investigated regeneration in third-stage larvae of genus *Cybister*. When forelegs of larvae were eliminated, regeneration in the pupal stage was complete, however in few cases the number of tarsal appendages was reduced (see Schaeflein 1989), and foretarsi were narrower in males. When first-stage or second-stage larvae were investigated with respect to legs or urogomphi, Blunck (1909a) always observed a repair in the second or third larval stage. In contrast a complete regeneration was observed in pupae or adults. Blunck also investigated head appendages, however the numbers of experiments were too low. Fig. 6.23 amply illustrates that the short urogomphus with eumelanin in the second larval stage of *Agabus bipustulatus* (Fig. 6.23a) was repaired and closed (with tiny cuticular tip) in the third larval stage (Fig. 6.23c). In the same way the cut larger urogomphus of the second stage larva (Fig. 6.23b) was repaired and also showed a fine tip.

These experiments with respect to developmental biology illustrate, that larvae of larger Dytiscidae are optimal candidates for laboratory experiments (Slack 2013). If it is possible to guarantee a successful pupation, the regeneration capacity of body appendages can be conceived. At the same time even with urogomphi, but also with antennae or legs pattern formations, gradients and polarities can be studied

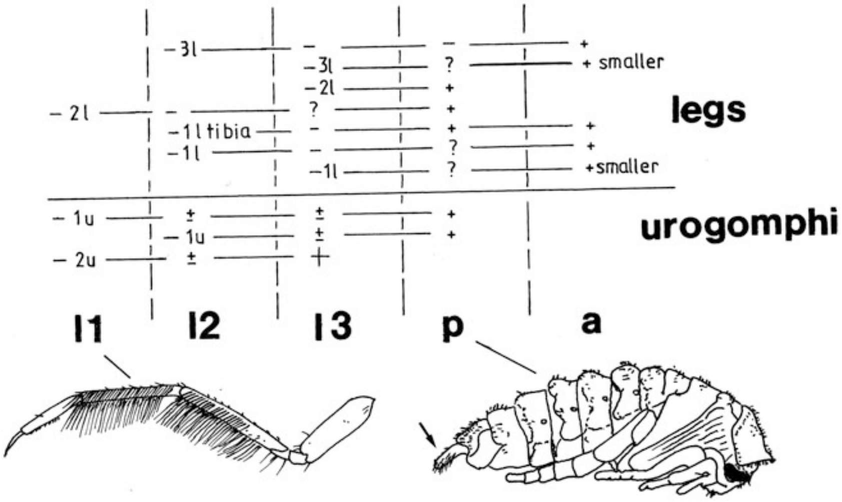


Fig. 6.25 Regeneration experiments by Blunck (1909a) with respect to body legs and urogomphi of *Dytiscus marginalis* larvae. l1, l2, l3: 1. – 3. larval stage; p: pupa; a: adults = beetles, 3 l/1 l: One hind leg (3 l) or one fore leg (1 l) of the appropriate stage was cut. Each horizontal line represents one specimen. -: elimination of appropriate body part; +: complete regeneration; +/−: cut appendage closed, no regeneration

(Lawrence 1993). If genes controlling regeneration are known, it might be also possible to interpret teratological cases which are relatively abundant in all Dytiscidae (Schaeflein 1987).

6.8 Future Directions

It would be interesting if those pheromones and kairomones mentioned in 6.3 and 6.4.1 were characterized chemically in order to perform bioassays with authentic compounds. In addition, further taxa of predaceous diving beetles should be investigated chemically in order to characterize their pygidial and prothoracic defensive gland constituents (6.4.2). Hereby a chemotaxonomic search strategy as practiced with plants of pharmaceutical value and their biologically active natural compounds is recommended. An important question seems to be the chemical characterization of prothoracic gland constituents from Hydroporinae. In addition, both with respect to pygidial and prothoracic defensive glands several taxa of predaceous diving beetles should be investigated, including *Matus* (Matinae), *Agabetes* (Agabetini), representatives of Methlini, Lancetinae, *Carabdytes* (Carabdytini), *Pachydrus* (Hydroporinae), *Paroster* (Hydroporinae), *Necterosoma* (Hydroporinae), or *Laccornellus* (Hydroporinae).

Further field and laboratory bioassays are necessary to detect the effects of gland compounds on beetle relevant pathogenic bacteria, fungi, and ectoparasites. In addition, the biological relevance of the plant hormone indole acetic acid from pygidial glands of Hydroporinae should be investigated. With respect to gland constituents of predaceous diving beetles biosynthetic studies, especially of aromatics and steroids, but also identification and knowledge on localization of appropriate enzymes are urgently required.

Concerning microbiological data it would be worthwhile to isolate culturable microorganisms especially from the guts of other predaceous diving beetle species (see 6.6.2), in order to identify new biological active metabolites. Also, a search for cultivable microorganisms with interesting characteristics will be promising. Of great interest are those beetle species that are found in extreme habitats such as highly polluted waters or hot springs. As in bacteria from guts of larvae of *Heleomyia petrolei* (petroleum fly, Ephydridae) there might be isolated unusual microorganisms that show strong antibiotic resistance or can be grown in organic solvents (Kadavy et al. 2000). Finally larvae of larger Dytiscidae could be well used for laboratory experiments in developmental biology (Slack 2013) in order to investigate the regeneration capacity of body appendages, to know the genes controlling regeneration, to learn more on pattern formation, gradients, polarities, and to interpret teratological cases which are usually found in Dytiscidae.

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Professor Dr. Konrad Dettner studied biology and chemistry at the universities of Stuttgart and Stuttgart-Hohenheim. He was at first interested in population ecology of water beetles, later during preparing his Ph.D., he studied pygidial gland systems and chemical ecology of dytiscid beetles. He received his Ph.D. in 1977 after a postdoctoral time at RWTH Aachen in 1985. He received a habilitation degree and venia legendi, and, in 1986, he was appointed as a full professor of animal ecology at the University of Bayreuth. In 2014, he received the Ernst-Jünger-Prize for Entomology of Baden-Württemberg, 2017 the Karl-Escherich-medal for Applied Entomology of the DGaaE. After his retirement in 2017, he has held the Ernst-Bresslau-Guest-Professorship at University of Cologne. He was the president of the DGaaE and since 2003 he is a curator of an environmental foundation at Black-Forest. His main research areas are the chemical ecology, especially the investigation of defensive compounds, of insects, the role of endosymbiotic bacteria of arthropods, and the biology of freshwater beetles.

Chapter 7
Community Patterns in Dytiscids

Steven M. Vamosi

> *What governs the nature of natural communities? This question has generated much interest among biologists. The major conclusion to come out of the considerable research conducted on the questions seems to be that there is no simple answer. (Larson 1990)*

Abstract Understanding the relative contributions of biotic and abiotic factors to community structure remains a fundamental aim of community ecology. Dytiscid beetles, which occur in a diverse set of aquatic habitats and display considerable variation in their abundance and composition among locales, would appear to be a model system for investigating such questions. Here, I present an overview of investigations into community structure in dytiscids, which reveals that they are understudied relative to their typically high abundance in ditches to bogs to lakes. I discuss emergent trends in the co-occurrence of dytiscids with regard to ecological and phylogenetic similarity, briefly present some investigations into the influence of dispersal on community structure, and discuss some prospects for future progress in this area.

Keywords Competition · Community dynamics · Dispersal · Phylogenetic ecology · Predation

7.1 An Introduction to Natural Communities

If there is no simple explanation of the mechanisms that shape the structure of communities, let us start by defining what natural communities are. In ecology, a community is generally considered to be a group of interacting species coexisting under natural conditions in a defined area. This definition may inspire an examination of the major components of natural dytiscid communities: habitats in which

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S. M. Vamosi (✉)
Department of Biological Sciences, University of Calgary, Calgary, AB, Canada
e-mail: smvamosi@ucalgary.ca

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communities tend to be found, important interactions between dytiscids and the abiotic environment, and species interactions that influence the abundance and distribution of (sets of) dytiscid species.

In general, patterns of species coexistence and diversity in natural communities may be shaped by complex interactions among organisms, and between organisms and the environment (e.g., Vamosi 2005; Östman et al. 2007). Important interactions include competition for limited resources (e.g., Tilman 1982; Schluter and McPhail 1992), predator–prey relationships (e.g., Sih 1987; Vamosi 2005) and their associations (e.g., intraguild predation; Polis et al. 1989, keystone predation; Leibold 1996, Chase 1999), whereas important environmental variables may include the permanence (Wellborn et al. 1996), size (Poethke and Hovestadt 2002), or isolation of a certain habitat (Brown and Kodric-Brown 1977). Freshwater systems are well suited for community studies because it is well established that the composition of freshwater communities can be dramatically influenced by environmental gradients associated with habitat area size, permanence, and with the presence or absence of dominant predators (reviewed by Wellborn et al. 1996).

Public interest in the investigation of wetland insect communities was raised in the 1960s, when researchers examined the role of aquatic insects as food for fish (e.g., Macan 1966a, b) and waterfowl (Murkin and Blatt 1987, reviewed by Batzer and Wissinger 1996). Classic studies often focused on few local water bodies in order to examine the structure of aquatic insect communities (e.g., Macan 1966a, b), whereas more recent studies frequently take into account a larger number of local habitats and/or spatial scales (e.g., Fairchild et al. 2000; Schäfer et al. 2006).

Before I provide an overview of community studies focused on dytiscids, I consider how they tend to be sampled for such studies. Dytiscids are generally more abundant in shallow and densely vegetated microhabitats compared to deeper and more sparsely vegetated parts of water bodies (Larson et al. 2000). Thus, the sweep-net technique is the classic method of dytiscid capture. Following the established method of Larson (1990), dytiscids can be sampled within a defined space among submerged macrophytes along the shoreline with repetitive swipes using a sweep net (e.g., Nilsson and Svensson 1994; Nilsson and Söderberg 1996). To prevent beetles from the surrounding area to be pulled into the sampled space during sweeping, a plastic frame can be placed into the water, with the walls of the frame pressed into the sediment (e.g., Fairchild et al. 2000; Yee et al. 2009). The use of the plastic-frame sweep-netting technique is expected to deliver a more accurate number of specimens per m^2, except for sediment dwelling species and for large active dytiscids (Fairchild et al. 2000). Another method of dytiscid capture is the use of traps consisting of 1.5–2.0 L plastic jars or bottles with inverted funnels, which have been found to be effective at capturing active dytiscids with a sampling range of approximately 10 m (Schäfer et al. 2006, Fig. 7.1). The downside of these "bottle traps" is that not only may dytiscids be caught, but also predators of dytiscids such as large dragonfly larvae, newts, or small fish (personal observation). These predators may eat the trapped dytiscids or prevent dytiscids from entering the traps. As is typically the case, each sampling technique has pros and cons.

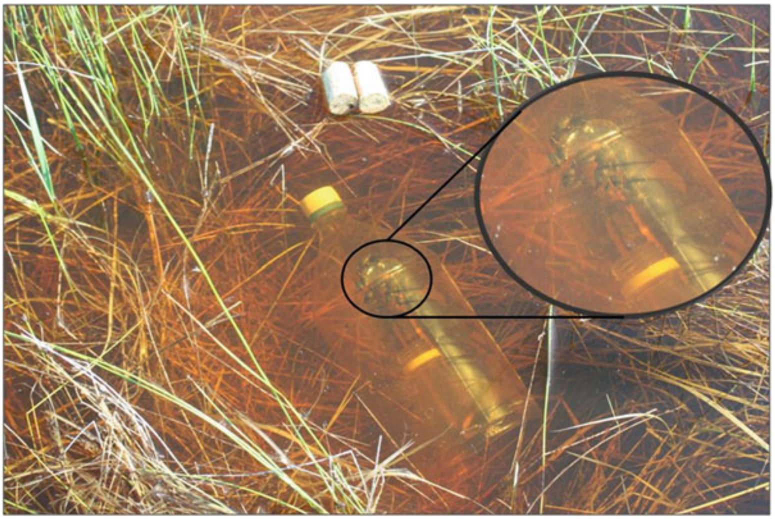

Fig. 7.1 Bottle trap in shallow water, with zoomed inset showing several dytiscid specimens that were successfully captured. Photo courtesy of D.A. Yee

Likely owing to their near-cosmopolitan distribution, high global species richness, and marked among-site variance in species richness at local scales, there is a rich history of studying community patterns in dytiscids. Curiously, I note that there appears to be little attention paid to whether the usual latitudinal biodiversity gradient is observed in dytiscids (but see Nilsson et al. 1994). Anecdotally, it appears it may not exist or, at least, not be very strong, with approximately 500 of the total 4633 species (Nilsson and Hájek 2022) being found in North America, and 276 of the former being present in Canada (Larson et al. 2000). Rather than attempting to present a comprehensive review of all community investigations, I focus on the main findings of a coordinated series of investigations by a few key groups, namely Nilsson and colleagues (Nilsson 1984, 1986; Nilsson and Svensson 1994, 1995; Nilsson et al. 1994; see also Nilsson and Söderberg 1996), Ribera and colleagues (Ribera et al. 2003; see also Baselga et al. 2013), Eyre and colleagues (Eyre et al. 1986, 1992, 1993, 2003; Foster et al. 1990), and Larson and colleagues (Larson 1985, 1990; Larson et al. 2000), primarily in Sweden, western Europe, the United Kingdom, and Canada, respectively. I present a summary of some of the community investigations described in detail, along with that of Wohlfahrt and Vamosi (2012), in Table 7.1. For interested readers, other studies include Lancaster and Scudder (1987), Aiken (1991), Bosi (2001), Arnott et al. (2006), Vamosi et al. (2007), and Vinnersten et al. (2009). In Sect. 7.4, I highlight more recent investigations by Pintar and colleagues (e.g., Pintar and Resetarits Jr 2017a, b, c; Pintar et al. 2018), largely because they focus on species abundances and community patterns in the early stages of the colonization of experimental mesocosms.

Nilsson and colleagues (Nilsson 1984, 1986; Nilsson and Svensson 1994, 1995; Nilsson et al. 1994) documented community structure patterns at a series of sites and,

Table 7.1 Key features of some dytiscid community investigations; see main text for more detail

Locale	Number of sites	Total number of species	Mean species richness	Significant abiotic and biotic associations	Raw data available?	References
Sweden	10	46	~25	Pond successional stage; pond area (but only when fens excluded)	No	Nilsson (1984)
	30	59	10.7	Vegetation structure; prey abundance; relative shore depth	Yes	Nilsson et al. (1994)
	9	69	Not reported	Water permanence; forest cover	No	Schäfer et al. (2006)
England	384	Not reported	7–13[a], depending on habitat type	Water pH; dissolved oxygen levels	No	Eyre et al. (1986)
	157	130[a]	Not reported	Water pH; nitrate levels; water depth; abundance of submerged vegetation	No	Foster et al. (1990)
Canada	312	145	2.4–25.0, depending on cluster	Water salinity; productivity; permanence; temperature; substrate type; flow; vegetation	No	Larson (1985)
	27	35	Not reported	Isolation; surface area; conductivity; water color; shoreline vegetation; complex vegetation; leafy vegetation; top predator	No	Wohlfahrt and Vamosi (2012)

[a] Water beetles, of which dytiscids were one component

in many cases, correlated these with various environmental features. Nilsson (1984), for example, investigated community patterns in 10 kettle-hole ponds located in a very restricted region, which were arrayed along a successional gradient correlated with changes in dominant vegetation. Consistent with other studies, species richness of aquatic beetles was quite high, with 61 species overall (of which 46 were dytiscids), and 14–34 species in the individual ponds. Species richness was negatively associated with successional stage (Fig. 6 in Nilsson 1984), although close inspection reveals that the pattern was largely driven by reduced species richness in the three fen ponds (14, 16, and 24 species), compared to the others (27–34 species). Nilsson et al. (1994) again investigated factors predicting dytiscid abundance and species richness, but on a much larger spatial scale, with 10 lakes each in south, central, and north Sweden. Species richness in these lakes was comparable to that observed in the kettle-hole ponds, ranging from 0 to 32 species, with 17 lakes having 10 or fewer dytiscid species. Partial least square regressions revealed positive associations for abundance and species richness with vegetation, structural complexity, and abundance of two taxa (*Asellus* and immature dipterans) and a negative association with relative shore depth on the first component, as well as weaker positive associations with fish abundance and negative associations with lake area on the second component. I do not review the other three studies here because they either considered a very small number of sites (a single seasonal pond, Nilsson 1986; two boreal snowmelt pools, Nilsson and Svensson 1994) or were comparing clear-cut to natural boreal swamp forest pools in a restricted geographical area when sample size ($N = 40$) was high (Nilsson and Svensson 1995). However, one final remarkable feature about the five studies bears noting here, which is that raw species lists were provided for all water bodies in all cases as well as number of individuals per species in all but Nilsson (1984). The availability of these data, along with key environmental parameters, makes them especially amenable to future phylogeny-informed community ecology analyses (e.g., Vamosi and Vamosi 2007) or meta-analysis approaches.

Although primarily concerned with improving our understanding of speciation in, and relationships among, dytiscids (e.g., Ribera et al. 2004, 2008), other researchers have also delved into related investigations of patterns in species richness. For example, Ribera et al. (2003) investigated large-scale factors associated with species richness in lotic and lentic water beetles (i.e., not just dytiscids) from 15 regions in western Europe, finding an influence of latitude for the former group and influences of geographic connectedness and total area size for the latter group. With reference to incorporating genetic relationships in dytiscid community investigations, I discuss phylogenetic community structure analyses in Sect. 7.5 and the application of DNA barcoding to related questions (Baselga et al. 2013) in Sect. 7.6.

In a series of interrelated studies, Eyre et al. (1986, 1993, 2003, 2006, also Foster et al. 1990) have studied predaceous diving beetles in Scotland and England, with the aim of defining associations between their distributions and environmental conditions to guide environmental monitoring or conservation efforts. Because I am focusing on community patterns here, I will not go into detail on those that used presence/absence from, for example, 10-km national grid squares for distribution data (Eyre et al. 1993, 2003, 2006). Eyre et al. (1986) analyzed the assemblages of dytiscids and other water beetles from 384 sites in northeast England which had been sampled over nearly two decades. Using a largely deprecated clustering algorithm, they produced nine "habitat groups" and the indicator species most representative of each type. Although analyses were not formally presented, they interpreted these habitat groups to suggest that water pH and oxygen levels were dominant influences on water beetle community patterns. In a related analysis, Foster et al. (1990) characterized community patterns for water beetles from 157 sites (primarily ditches in arable land) in England. Using the same clustering techniques, they produced eight habitat groups, which they correlated with nine environmental variables and an index of vegetation management. These analyses revealed that the variables with the greatest influence were water pH, nitrate levels, depth, and abundance of submerged vegetation. Site-specific species lists and environmental conditions were not presented in either paper, but the sample sizes suggest that these would otherwise be excellent candidates for additional analyses.

A trio of researchers have carried out the bulk of the investigations on community patterns in Canada (e.g., Larson 1985; Alarie and Maire 1991; Roughley and Larson 1991; Paquette and Alarie 1999). Building on these works, various aspects of the biology of Nearctic dytiscids were summarized by Larson et al. (2000). Nearctic dytiscids are found to be abundant in a large variety of temporal and permanent freshwater habitats and, correspondingly, display diverse patterns in life history, morphology, and microhabitat use (Larson et al. 2000). One of the main findings of these works relevant to community patterns was documenting significant differences in the distribution of dominant predators such as fish, large active dragonfly larvae (Odonata: Anisoptera), and dytiscid beetles among different lake types: whereas most fish species may only persist in permanent waters, large odonates are more abundant in permanent fishless waters, and dytiscids dominate in temporary ponds (Larson 1990).

Fish are important top predators in many aquatic systems (Wellborn et al. 1996) including important consumers of dytiscids (see Chap. 8). A number of factors lead to the exclusion of many fish species from shallow water bodies, including seasonal increases in water temperature (Magalhães et al. 2002), decay of organic matter, and associated anoxia (Meding and Jackson 2003) and/or oxygen stress due to ice cover (Wellborn et al. 1996). Larger-sized fish are typically able to catch and devour large sized prey. Large predaceous fish thus tend to select for small-bodied prey organisms (McPeek 1990; Wellborn et al. 1996). However, frequently abundant species in shallow ponds may be small-bodied fish species, such as fathead minnows (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*) (Peterka 1989). Although smaller fish may be gape-limited, abundance of fathead minnows has been shown to be associated with decreases in the abundance of aquatic invertebrates and with changes in the pond-community composition (Zimmer et al. 2001).

The aquatic larvae of predaceous dragonflies are less susceptible to oxygen stress than many fish species (Wellborn et al. 1996). However, the larvae of many dragonfly species can be excluded from fish-dominated habitats via predation (Larson 1990; McPeek 1990). In temperate regions, dragonflies may overwinter in the egg stage (i.e., diapause) or as larvae. Large predaceous dragonfly larvae, such as aeshnids (Anisoptera, Aeshnidae), may require one year to several years to complete larval development (Cannings 2002; Askew 2004).

Unlike dragonflies, both the larval and adult stages of dytiscids are aquatic. Dytiscids often inhabit the shallow, vegetated parts of various water bodies, including temporary ponds (Larson et al. 2000). During both life stages, dytiscids need to break the water surface with the tip of their abdomen to take air; thus, most dytiscids in temperate regions leave the water for overwintering in the adult stage (Larson et al. 2000). Dispersing female dytiscids that fly to new habitats may select oviposition sites based on the presence or absence of predators, ovipositing more eggs into fishless habitats (Brodin et al. 2006). Dytiscids in the adult life stage are less susceptible to predation than larvae and possess antipredator defenses such as hardened bodies and cryptic coloration (Larson et al. 2000) or chemicals (Chap. 6). Despite these defenses, small dytiscids may regularly fall prey to large dragonfly larvae (Larson 1990; see also Wohlfahrt and Vamosi 2012).

Correspondingly, Larson (1990) observed increased abundances of large sized dytiscid species that may avoid predation due to their large body size (e.g., species of *Dytiscus*), and of very small-sized dytiscids that may hide from predators among dense patches of vegetation in ponds dominated by large predaceous dragonfly larvae. Thus, in addition to habitats dominated by different predator types, Larson (1990) divided dytiscid communities within dragonfly-dominated ponds into three microhabitat types and prey size morphs: (1) very small dytiscid species, occurring among dense submersed vegetation, (2) very large dytiscid species, and (3) open water species. Overall, although community composition may vary dramatically among water bodies within and among regions, studies to date (Table 7.1) point to recurring influences of key physicochemical features, such as water permanence, vegetation structure, and water chemistry, along with additional effects of species interactions, especially with predators, on shaping community assembly in dytiscids.

7.2 Random vs. Non-random Distributions

As exemplified by the studies reviewed above, the traditional approach to understanding communities has been to group individuals into species, without reference to phylogenetic relatedness or functional trait values, when attempting to understand their presence or abundance in certain locales (e.g., Hutchinson 1959; Hubbell 1979). Niche theory, for example, assumes that multiple species are able to coexist in the same living space, or in their habitat, because different species possess contrasting ecological requirements (Hutchinson 1959). Interspecific competition for limited resources is the classic reason for niche diversification (e.g., Hairston 1949; MacArthur 1958). Thus, it is assumed that over the long term no two species are able to occupy exactly the same niche in the same habitat. Hutchinson (1959) defined the ecological niche as a multidimensional space or hypervolume that is not shaped by competition alone but contains the biological requirements of any species. Within its niche, a species is assumed to experience environmental conditions that allow the species to persist in the habitat. Outside the specific niche, they are expected to experience environmental conditions that prohibit their long-term persistence.

The classic niche theory has not been without its detractors. Hubbell's (1979) main criticism was that high species diversity can be found in many natural habitats, combined with a relatively low number of limiting environmental factors. For example, the high diversity of tree species in tropical forests appears to be at stark odds with the low number of limiting factors such as water, light, and nutrients (Hubbell 1979). In other words, it has been argued that the diversity of species coexisting in a community cannot always be explained by the number of limiting environmental factors. According to the neutral model, species are ecologically identical in the sense that there are no niche differences. Thus, all species are assumed to possess equal ecological requirements and equal per capita fitness (e.g., Hubbell 1979, 2001; Bell 2001; Alonso and Mc Kane 2004). Following the neutral approach, communities are random collections of species, with a composition mainly shaped by metacommunity size, speciation rate, and dispersal among communities (Bell 2001; Hubbell 2001).

More recently, Chase (2005) suggested a synthetic approach to investigating species coexistence within communities. This synthesis would take into account aspects from the niche approach, such as the existence of limiting environmental factors, combined with aspects from the neutral model, such as dispersal effects. Although Chase (2005) has been well received and fruitfully applied to some aquatic systems, literature searches suggest that this integrated approach has not yet been specifically applied to dytiscid communities.

Another approach to investigating community composition that differs from the classic niche perspective is a consideration of metacommunity dynamics. A metacommunity is defined as a set of local communities, which are linked by dispersal and contain groups of interacting species (Wilson 1992; see Levins 1969 for seminal introduction of metapopulations). Theory (Hastings 1980; Amarasekare 2003) predicts that if the species within a community differ in their competitive ability, local coexistence is possible in the presence of limiting factors, which may be abiotic (e.g., wave action) or biotic (e.g., predator presence). However, species may differ in their ability to tolerate environmental factors and, thus, can experience favorable conditions in one habitat type and unfavorable conditions in another habitat type, leading to habitat partitioning (Kneitel and Chase 2004). Thus, spatial heterogeneity among local communities may result in local exclusion and regional coexistence of species within the metacommunity. Habitat partitioning among lake types has been shown in larval dragonflies of the genus *Leucorrhinia*. Shifts from fish lakes to dragonfly lakes have resulted in the loss of abdominal spines, a morphological defense effective in fish presence, but increased the vulnerability of prey in presence of large predaceous dragonflies (Hovmöller and Johansson 2004). Because adaptations exist that increase a species ability to cope with limiting environmental factors, but may have no or opposing effects in different habitats (McPeek 1990; Richardson 2001), species that occur in heterogeneous habitats may be subject to antagonistic selection (Wohlfahrt and Vamosi 2009), which in turn can promote habitat partitioning (Davidowitz et al. 2005).

In contrast, in a spatially homogeneous competitive environment, regional coexistence is expected when a trade-off between competitive ability and dispersal ability exists. In that case, the species that is the weaker competitor must be the better disperser to persist at the regional scale (Hastings 1980). However, local patch densities and habitat fragmentation are predicted to affect the competition–colonization trade-off (Tilman 1994; Yu and Wilson 2001). If local habitat density is reduced, a superior colonizer is more likely to invade the habitat and suppress the stronger competitor. On the other hand, in case the number of isolated habitats declines, it is the better colonizer that is expected to go extinct. Brown and Kodric-Brown (1977) examined the effect of dispersal and immigration on species extinction in patchy habitats. Using island populations as model systems, they found that high immigration rates could reduce extinction rates of conspecifics within habitats. This observation was referred to as the rescue effect. Thus, immigration and recolonization are expected to stabilize the abundance of species, even if these species are not favored by the limiting factors present. This provides researchers with a problem, because locally stable communities may be difficult to tell apart from unstable, dispersal-maintained communities. The question every researcher faces when taking samples from a local habitat patch is to what degree a community was composed as the non-random result of limiting factors and to what degree the community was composed by random dispersal events. Hence, it may be necessary to sample a large number of local habitat patches for patterns in community composition to become evident (Larson 1985). Ultimately, resolving such questions requires the use of manipulative experiments to test the associations observed in the field.

With samples from a series of communities in hand, one can ask several questions, including: (1) are sampled habitat patches occupied by a non-randomly composed community? and (2) does community composition differ among habitat patches? In an extensive study of water bodies in Alberta, Canada, Larson (1985) used cluster analysis to examine the sampled sites for patterns of similarity in dytiscid species distributions. Twelve clusters were identified, which were interpreted as communities with contrasting dytiscid species composition, although there were also unclustered sites. Further analyses revealed that certain environmental factors also varied among the sampled habitat patches that had well-defined community clusters: salinity, productivity, stability, water temperature, substrate type, flow, and vegetation. These differences in the environment may form ecological gradients, which in turn can be associated with differences in dytiscid species distribution. Because communities are typically influenced by a large number of environmental factors, it is often not possible to explain associations between the composition of species and the environment by a single dimension in a statistical analysis (Larson 1985). In more recent studies, the association among multiple gradients in community composition and the environment has been analyzed using ordination analyses, such as redundancy analyses (e.g., Schäfer et al. 2006). Alternatively, canonical correspondence analyses (e.g., Fairchild et al. 2000; Wohlfahrt and Vamosi 2012) can be conducted to identify variance in community data with long gradient lengths (Lepš and Šmilauer 2003).

Recent community analyses have confirmed Larson's (1990) hypothesis that the presence of predaceous fish can be an important biotic factor influencing the composition of dytiscid communities (Wohlfahrt and Vamosi 2012; Liao et al. 2020) and of water beetle communities in general (Fairchild et al. 2000). The negative effects of fish can be mitigated to some extent by the presence of submerged macrophytes, which can serve as refuges from predation (Dionne and Folt 1991) and support high densities of potential prey, such as epiphytic insects (Batzer and Wissinger 1996), for dytiscids (see Chap. 10 for more details on the influence of macrophytes). Other environmental factors such as pond surface area (species richness: Nilsson and Svensson 1994, abundance: Fairchild et al. 2000), elevation

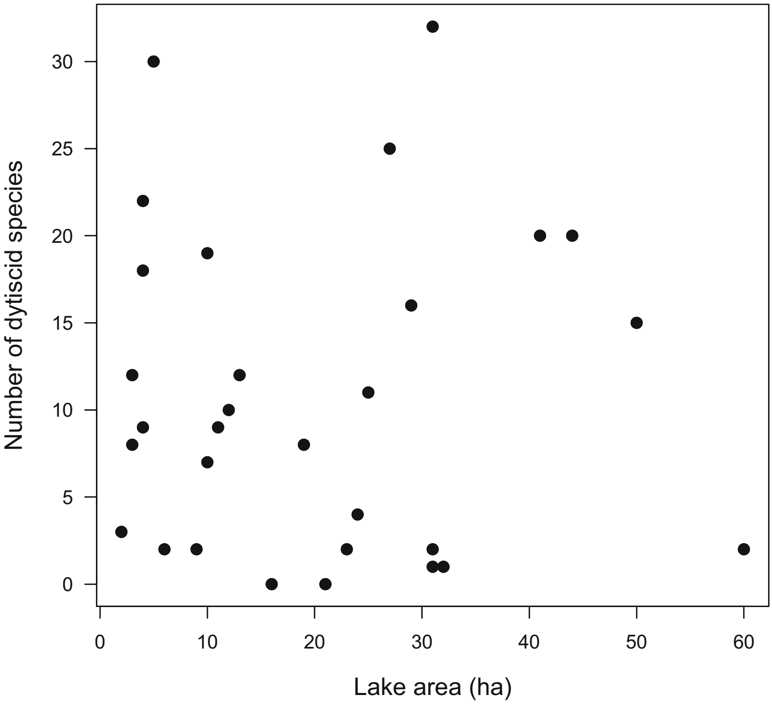

Fig. 7.2 Species richness as a function of lake surface area in 30 Swedish lakes (data from Nilsson et al. 1994)

(Vamosi et al. 2007), pond permanence (species richness: Nilsson and Svensson 1994; Bosi 2001), and habitat isolation (species richness: Suhlman and Chase 2007) may influence dytiscid communities as well.

The number of organisms any habitat can accommodate is typically positively correlated with habitat area size (Poethke and Hovestadt 2002). Dytiscids do not cleanly follow the usual species richness–area relationship, likely owing to the presence of more diverse predator communities in larger water bodies. For example, I present a re-examination of the relationship between lake area and species richness for the 30 lakes considered by Nilsson et al. (1994). There was a negative relationship for the 10 Scania lakes, and a weakly positive but mostly variable relationship for the 10 Södermanland lakes, and no obvious relationship for the 10 Västerbotten lakes. Plotting all the data together reveals considerable variability in species richness for smaller lakes (i.e., those with a surface area <35 ha), and possibly a negative relationship between lake area and species richness for larger lakes (Fig. 7.2). Minimally, the lack of a strong positive relationship deviates from the typical "textbook" pattern (e.g., Smith et al. 2005). I encourage others to carry out more systematic investigations of the relationship between species richness and area for dytiscids, likely in conjunction with additional investigations of latitudinal species richness patterns.

Increasing habitat isolation can result in decreased species richness (Suhlman and Chase 2007) especially if species coexistence is dependent upon immigration from neighboring habitats (reviewed by Taylor 1990), whereas habitat heterogeneity may facilitate species coexistence (e.g., Macan 1966b; Amarasekare 2003) to the point of outweighing the effects of habitat area and isolation on species diversity (e.g., Báldi 2008; Kallimanis et al. 2008; Jonsson et al. 2009; Kruk et al. 2009; Wohlfahrt and Vamosi 2012). In heterogeneous habitats, differences in area, isolation, and dominant predator presence still affect the composition of dytiscid communities and may thus act as environmental gradients (Wohlfahrt and Vamosi 2012). Specifically, the composition of dytiscid communities may differ among habitats depending on both the effect of predator presence or absence, and on the degree of habitat isolation, with different species composition in isolated ponds with the dominant predator present, in isolated ponds with the dominant predator absent, and in less isolated ponds with the dominant predator present or absent. Similarly, the presence of large predaceous dragonfly larvae may affect dytiscid species composition depending on the pond surface area size (Wohlfahrt 2010).

Overall, these results illustrate that differences in the composition of dytiscid communities depend not only upon multiple environmental gradients, but can also be organized along community gradients, from apparently random dytiscid assemblages to patches with significantly contrasting sets of coexisting species. The interpretation of the results from community analyses has evolved from observations of richness and abundance of individual species to investigations of community clusters and community gradients. However, in a world full of scaling issues there remains the question of where a community starts and where it ends. Thus, in a variable environment, a dytiscid "community" may represent no more and no less than a certain point along a dynamic continuum of species coexistence (Larson 1985).

7.3 Ecological Similarity

In the previous section, I explored how dytiscid communities may be influenced by environmental gradients, but what kind of species coexist in communities structured by different biotic interactions and why? Would coexisting species resemble each other or would they differ in their phenotypic traits? A long-standing assumption in community and evolutionary ecology is that organisms with contrasting ecological requirements are better able to coexist in the same habitat (e.g., Hutchinson 1959; Grant 1986; Schluter 2000). Individuals are expected to compete more strongly for limited resources when they share the same ecological niche and, thus, use the same resources in similar ways (Bickel et al. 1995). To predict the outcome of resource competition, Tilman (1982) developed the R* rule for competitive exclusion. According to this rule, the species that suppresses resources to the lowest amount wins in competition, i.e., becomes the dominant competitor. Thus, resource competition may lead to the exclusion or extinction of inferior competitor species and is considered an important factor in structuring communities (Losos 1990; Schluter and McPhail 1992).

Species that occupy similar niches and positions within a community can be grouped into guilds, which have been used in multiple studies to investigate the effects of ecological similarity on patterns of species coexistence (e.g., Williams and Hero 1998; Webb 2000; Gurd 2007). In dytiscids, ecologically similar species can be grouped into guilds by using the criteria of body size and life cycle length (Nilsson 1986; Nilsson and Svensson 1994). Morphological traits are well suited to assess ecological similarity of a species, because morphology is often closely correlated to the species' resource use (e.g., Schluter and McPhail 1992; Gurd 2007). Thus, morphologically similar species are expected to compete more strongly for resources compared to species with contrasting morphology (Juliano and Lawton 1990). Despite this, coexistence of species with similar phenotypic traits has often been observed in natural communities (e.g., Nilsson and Svensson 1994; McPeek and Brown 2000; Hubbell 2001; Scheffer and van Ness 2006).

For dytiscids, competition among coexisting species with similar body size has not been detected (Juliano and Lawton 1990; see also Nilsson 1986). Larson (1985) found generally high species richness in dytiscid communities of the north temperate regions. Combined with high productivity observed in many water bodies, it was concluded that other factors, such as predation, may be more important in shaping dytiscid communities in the temperate regions (Juliano and Lawton 1990; Larson 1990). Body size distributions of dytiscids have also been used to investigate patterns in the composition of communities. For example, Larson (1985) revealed differences in dytiscid size distributions among regions with contrasting climate, such as northern temperate climate in Alberta (Canada) and warm temperate climate in Florida (USA). In general, small-sized (body length: <5 mm) and medium-sized (body length: 5–10 mm) species, including *Hygrotus*, *Hydroporus*, *Laccophilus*, *Rhantus*, and *Agabus*, were found to be more abundant in communities than larger-sized (body length: >10 mm) species. A relatively high number of medium-sized dytiscids coexisted in communities in Alberta compared to dytiscid communities in Florida. It was suggested that the presence of large dragonfly larvae in the more stable habitats in Florida prevented medium-sized dytiscids from coexisting in these communities. However, other studies on dytiscids have found no relationship between body size and frequency of occurrence in dytiscids (e.g., Nilsson et al. 1994; Vamosi and Vamosi 2007).

Although the coexistence of similar species may be limited by the degree of resource competition and may result in competitive exclusion, certain environmental factors may facilitate their coexistence. If phenotypic characters represent adaptations to particular environmental conditions and environmental factors act as filters, the community may be restrained to species with certain sets of phenotypic traits (e.g., Webb 2000; Webb et al. 2002; Vamosi and Vamosi 2007). However, when the environment changes or a species colonizes a new habitat, these traits may no longer be beneficial or may even decrease the species ability to persist (McPeek 1990;

Richardson 2001; Mikolajewski et al. 2006). Thus, species that occur in heterogeneous habitat patches may be subject to antagonistic selection. Antagonistic selection can promote habitat partitioning, and with this, increase the coexistence of ecologically similar species (Davidowitz et al. 2005).

Also within the realm of ecological similarity, predation is an important limiting factor that may instantly reduce the fitness of prey (e.g., Sih 1987, reviewed by Vamosi 2005). Sih (1987) suggested that prey species could coexist with predators by possessing particular antipredator adaptations, such as morphological and/or behavioral traits or, in case heterogeneous local habitats are present, occupy habitats free of predators. For example, habitat heterogeneity can be defined in a region that contains local habitat patches dominated by different top-predator types, such as fish or large active dragonfly larvae (McPeek 1990; Hovmöller and Johansson 2004). Among these heterogeneous habitats, prey body size is likely to be affected by antagonistic selection, because fish and large dragonfly larvae may select for opposite size classes of prey (reviewed by Wellborn et al. 1996). Most larval dragonflies are omnivorous ambush predators, whereas large active dragonfly larvae, such as species of *Anax* and *Aeshna*, often show a more active foraging mode than other odonates (Larson 1990). Because fish are capable of swimming at much higher speeds than most macroinvertebrates, larval dragonflies are less successful in prey capture if the prey performs evasive behaviors (McPeek 1990). Correspondingly, communities with invertebrate top predators, such as large predaceous dragonfly larvae, are associated with the prevalence of larger-sized and more actively foraging prey organisms compared to communities dominated by predaceous fish (Wellborn et al. 1996). Analyses of gut contents confirm that large *Aeshna* dragonfly larvae successfully and regularly prey on small-sized (total body length: <5 mm) dytiscid adults (Larson 1990). In the same study, Larson (1990) demonstrated an interesting negative correlation between larval dragonfly density and dytiscid beetle density along a surface area gradient in bog pools, with dragonfly densities increasing with bog pool size. Bog pools were lumped into only four size classes, making this a tantalizing association that bears further investigation.

Effective antipredator adaptations not only reduce the prey's vulnerability to predation, but they may also involve costs in terms of time or energy expenses (Clark and Harvell 1992). In the absence of predators, prey organisms have to trade-off the risk of predation against the cost of expressing antipredator adaptations. For example, the most common behavioral antipredator adaptation is a change in activity, because reduced activity levels may result in reduced probability of predator encounters (Sih 1987). Because an animal is more likely to encounter food items when it searches actively (Gerritsen and Strickler 1977), reducing activity levels also results in decreased feeding, growth, and development rate (McNamara and Houston 1994; Stoks et al. 2003). Although many studies have investigated activity levels of prey under various combinations of predator presence, ontogenetic stage, and food level (e.g., Wohlfahrt et al. 2007), I am unaware of such studies using larval dytiscids as focal prey.

In the presence of visually hunting predators, differences in the prey species coloration can influence their vulnerability to predation (Brodie 1992) (information

Fig. 7.3 Adult *Hygrotus* (*Leptolambus) marklini* (left) and *H. sellatus* (right) collected from ponds in Alberta, Canada. These species show drastically different marking patterns on the elytra, which may reflect antagonistic selection such as that examined in Wohlfahrt and Vamosi et al. (2009). Photo courtesy of D. A. Yee

on the biological bases of color can be found in Chap. 6). For example, counter shading has been shown to optically flatten the three-dimensional shape of prey animals due to displaying darker dorsal regions contrasting to the ventral region of the body (Ruxton et al. 2004). Prey may also adapt to the background color of their environment using background matching (Endler 1984) or disruptive color patterns (Sherratt et al. 2005). Larson (1990) suggested that longitudinal stripes in larval *Ilybius pleuriticus* LeConte act as an antipredator adaptation against visually hunting dragonfly larvae. Because the success of each type of crypsis depends upon the habitat specific background color (Endler 1984), dytiscid species occurring in similar habitats may tend to possess similar color patterns on their bodies. Larson (1996) suggested that bright color patterns conferred crypsis to dytiscids occurring in habitats with low structure and, therefore, provided protection against visual predators. Conversely, predaceous diving beetles occurring in densely vegetated or shaded habitats with dark substrates are more likely to display dark color patterns (Balke et al. 1997). Species found in multiple habitat types or in different communities across their range may accordingly be expected to experience antagonistic selection on their color patterns.

An experimental test on dytiscids with contrasting body size and color patterns has confirmed antagonistic selection on coloration patterns under conditions of contrasting water clarity (Wohlfahrt and Vamosi 2009) (Fig. 7.3). Small prey species often experience higher vulnerability to predation compared to species with larger body size (e.g., Stein 1977; Richardson and Anholt 1995; Eklöv and Werner 2000). In an environment with clear water, dytiscids with coloration patterns had equally low mortality rates in presence of predaceous aeshnid dragonfly larvae, independent of body size. In contrast, in an environment with dark water conditions, small-sized dytiscids (<5 mm) had higher mortality rates compared to medium-sized dytiscid species (5–10 mm), and larger-sized dytiscids that displayed coloration patterns also experienced increased mortality rates (Wohlfahrt and Vamosi 2009). Thus, whereas dytiscids that possess coloration patterns may compensate for an increased predation risk due to small size under clear water conditions, larger-sized dytiscids may experience antagonistic selection on coloration patterns in habitats with contrasting water clarity. These results illustrate that interactions among predation regime and environment can result in multiple outcomes of predator induced selection and may likely be one factor leading to increased species diversity at the metapopulation and metacommunity scales.

7.4 Dispersal

Thus far, I have largely focused on the influences of "within-site" abiotic and biotic factors on variation in community composition among sites. Various abiotic properties of water bodies and the surrounding shoreline have been shown to be important in influencing the presence or absence and relative abundance of dytiscids. From the studies conducted to date, predation appears to be the dominant species interaction, although additional work with larvae may eventually reveal a role for resource competition. Before moving on to consider the influence of phylogenetic relatedness on community structure, there is one outstanding issue I want to consider: the presence and abundance of species at a site will be influenced by the rate at which individuals disperse from other locations and successfully colonize the focal site, with reliance on dispersal appearing to be negatively correlated with water permanence (Larson et al. 2000) (for more details on dispersal in dytiscids, see Chap. 11). That is, a consideration of the factors structuring local communities is incomplete without a consideration of the role of dispersal. Unfortunately, but unsurprisingly given the relative paucity of community studies in general, the literature on factors affecting dispersal in dytiscids specifically with a view to determining their community consequences is relatively sparse. Larson et al. (2000, p. 9) noted that "*dispersal strategie*s [in dytiscids] *are not well understood*"—my literature surveys suggest that progress in this area has been slow. Indeed, the studies I review here largely provide information on factors affecting dispersal and colonization of sites by dytiscids, rather than their subsequent effects on dytiscid community structure and turnover (see also Yee et al. (2009) for an investigation of factors promoting dispersal in two dytiscid species).

Wilcox (2001) investigated the role of colonization properties on the abundance of predators in seasonal wetlands. In an interesting design, Wilcox (2001) created 27 artificial ponds in a wildlife refuge, resulting in three replicates for each combination of pond size (three levels) and distance from semi-permanent seep (three levels), which served as the source for predators. Unfortunately, because "*identification of dytiscids to genus and species requires examination under a microscope,*" all dytiscids were "*aggregated … for analysis*" (Wilcox 2001, p. 466). Pond size had no effect, whereas there was a significant negative effect of distance from source on dytiscid abundance. Furthermore, there was a significant interaction between pond size and distance from source, with a stronger negative effect of distance on colonization rates in smaller ponds. The effect of distance quickly decreased with time, with all pond sizes having comparable abundances after only 3 weeks. Although these data suggest that dytiscids can quickly colonize newly available habitats, it is worth noting that the farthest block of ponds was only 180 m from the source, which is relatively short compared to the distance individual dytiscids may fly (Lundkvist et al. 2002).

Lundkvist et al. (2002) compared the beetles caught in traps in water and air in an agricultural landscape within two urban landscapes. Although community patterns were not investigated, their flight trapping data revealed that dispersal by dytiscids can be quite significant: two seasons of effort flight trapped 42 species and 1653 individuals. Much of the variation in species distribution among flight traps was explained by three environmental variables: landscape type, distance from water, and vegetation complexity near traps. With regard to possible influences on community structure, their data suggested that flight activity levels are not constant over the season, although the pattern of variation among time periods may vary among years. In the first year of their study, dispersal levels were highest in May and generally decreased with time, although there was a suggestion of a brief increase in August. In the second year of their study, flight activity was low for 4 months (April, May, August, and October), and high in June, July, and September. Because anthropogenic impacts on aquatic communities are only likely to grow with time (e.g., Liao et al. 2020), I hope more studies similar to this one will be carried out in future.

Schäfer et al. (2006) examined the relationship between dytiscid community patterns in nine wetlands and several landscape variables at five spatial scales. Although they did not formally measure flight patterns (all of their traps were located under water), they "*sampled only adult … dytiscids since* [they] *were mainly interested in the dispersing life-stages*" (Schäfer et al. 2006, p. 60). Species richness and diversity of dytiscids were positively associated with water permanence, whereas abundance was negatively correlated with amount of forest cover. A positive association with open areas was interpreted as possibly supporting the notion that wetlands were more visible from the air in open than forested environments. The influence of visibility, however, could not be distinguished from the tendency of ponds in forested areas to have reduced levels of aquatic vegetation, likely due to increased shading compared to those in more open areas.

More recently, in a series of related investigations, Pintar, Resetarits and colleagues (e.g., Pintar and Resetarits Jr 2017a, b, c; Pintar et al. 2018) have experimentally investigated the influence of predation risk and nutrients on aquatic beetle colonization. In both experiments I summarize, mesocosms were small plastic wading pools. Pintar and Resetarits (2017b) examined how variation in zooplankton abundance affected colonization and resulting community patterns of aquatic beetles. Dytiscids overall, and the three most abundant dytiscid species (*Laccophilus fasciatus* Aubé, *Hydroporus rufilabris* Sharp, and *Copelatus glyphicus* (Say)), colonized pools inoculated with zooplankton at a significantly higher rate in the first 2 weeks compared to control pools. Interestingly, colonization by the numerically dominant water scavenger beetles (Hydrophilidae), which are omnivores, did not differ between the treatments. Pintar et al. (2018) assessed the influence of predation risk (0, one, or two golden topminnows *Fundulus chrysotus*) and nutrient abundance (0, 4, or 8 g of rabbit chow) on colonization of by aquatic beetles. During the 14-day duration of their experiment, beetles of 23 species were observed, with only two dytiscid species being abundant enough for analyses (*Copelatus glyphicus* and *Laccophilus fasciatus*). Both species preferentially colonized fishless wading pools, whereas only *Copelatus glyphicus* showed a preference for wading pools with higher nutrient levels. While acknowledging the effort required to set up and properly survey even such small mesocosms, I advocate for more studies in larger replicate experimental ponds with greater variation in predator types/abundances and more realistic nutrient sources. Monitoring such ponds for longer could also provide more insights into (1) responses by less abundant species and (2) changing community patterns over the seasons.

7.5 Phylogenetic Community Composition

In a previous section, I considered how phenotypic traits can be important in structuring natural communities and showed that contrasting combinations of phenotypic traits may lead to differences in prey survival depending on the environment (e.g., Wohlfahrt and Vamosi 2009). The influence of environmental factors on the phenotypes of prey species has been elucidated in several systems, although none perhaps as thoroughly as in the case of the Trinidadian guppy (e.g., Endler 1980, 1995; Reznick 1982; Gordon et al. 2012). Guppy populations have been categorized as belonging to one of two ecotypes: low-predation vs. high-predation populations. Populations of the two ecotypes predictably differ in many traits, with individuals from low-predation populations tending to be more colorful, maturing later, investing more resources into reproduction, and having fewer but larger offspring. These and other differences between the two ecotypes have been shown to have a genetic basis (Reznick 1982; Gordon et al. 2012). An important challenge in community ecology remains the investigation of interactions between species traits and community composition, and the influence of evolutionary processes on the species traits (Cavender-Bares et al. 2004). Phylogenies are increasingly being used in investigations of the influence of evolutionary, ecological, and stochastic processes on community assembly (reviewed by Webb et al. 2002, Cavender-Bares et al. 2009, Vamosi et al. 2009).

The results of an investigation of the interactions among phylogenetic relatedness, habitat-use, and phenotype in anuran species (Richardson 2001) revealed that phenotypic traits could not be predicted by habitat nor taxonomy alone, because

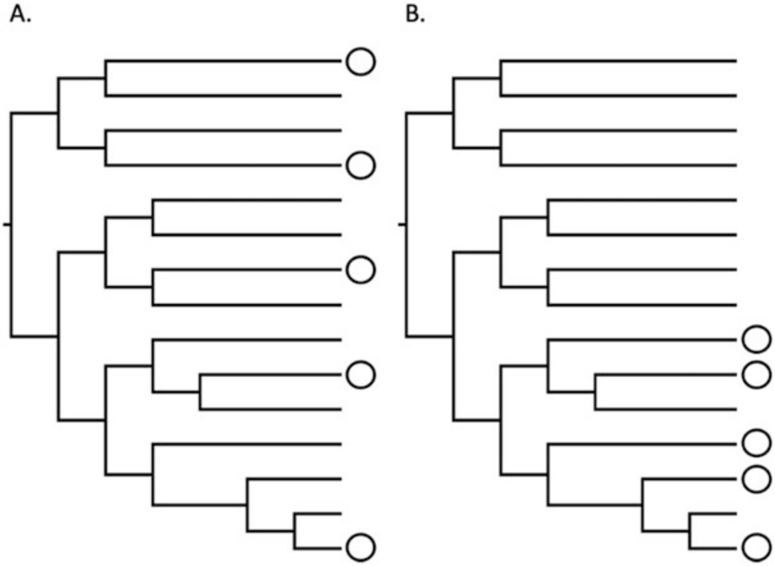

Fig. 7.4 Two hypothetical communities drawn from a regional pool of 15 species. Community A consists of five relatively distantly related species (open circles), which would be considered phylogenetically even, whereas Community B consists of five closely related species, which would be considered phylogenetically clustered. Dytiscid communities tend to more closely resemble the scenario represented in B than that in A, although there is a continuum of patterns observed and relatively few are as clustered as that shown (see Vamosi and Vamosi 2007; Vamosi et al. 2009)

many combinations of traits may result in successful adaptation to a certain habitat. Regardless, phenotypic traits are not taxonomically independent. Closely related species share a common history, and with this, may share many phenotypic characteristics (Stearns and Hoekstra 2001). Thus, closely related species are expected to be ecologically more similar than distantly related species (Webb 2000). Because the development of phenotypic traits may be restricted by the evolutionary history of a lineage, our understanding of the mechanisms that shape communities may be improved by the consideration of phylogenetic relationships among coexisting species (Cavender-Bares et al. 2004; Vamosi et al. 2009; Fig. 7.4). I am aware of concerns raised in the literature about potential flaws in the classic coexistence theory underlying studies of phylogenetic community structure (Mayfield and Levine 2010). In brief, there is growing evidence that interspecific competition is not necessarily strongest between closely related species, even if there is a significant phylogenetic signal to phenotypic traits. Because a full consideration of these issues is beyond the scope of this chapter, I focus on the patterns observed to date in dytiscid communities and largely refrain from definitive statements about possible mechanisms underlying these community patterns.

As mentioned earlier, coexistence of similar species may be facilitated by environmental variables acting as filters, and thereby they may restrict the community to species with certain sets of phenotypic traits (e.g., Webb 2000; Vamosi and Vamosi 2007; Silver et al. 2012). Phenotypic similarity is often a result of trait conservatism in the evolution of species that share a common history (Zimmermann 1931; Ackerly et al. 2006). However, phenotypic similarity can also arise in distantly related species due to convergent evolution, when species evolved under similar environmental conditions (e.g., Webb et al. 2002; Vamosi et al. 2009). Thus, environmental filtering can either increase the relatedness of coexisting species that share conserved phenotypic traits or decrease relatedness due to the retention of species that share convergent traits (Webb et al. 2002).

In general, the phylogenetic composition of communities is expected to depend on the degree of phylogenetic conservatism in traits that are important for the persistence of species in certain habitats. Coexistence of closely related species and, with this, a high degree of phenotypic clustering is expected only if important phenotypic traits are conserved (Webb et al. 2002; Kraft et al. 2007). For example, a study on Caribbean lizards (Losos et al. 2003) showed that a long history affected by competitive interactions resulted in niche divergence and reduced relatedness (i.e., led to phylogenetic evenness) in lizard communities.

A combination of phenotypic traits, as opposed to a single trait, may affect the likelihood of persistence of species in certain environments (Williams and Hero 1998). Multiple phenotypic traits need to be considered in phylogenetic analyses, because natural selection is known to affect whole phenotypes (Endler 1995). Investigations of potential patterns in community composition can thus be complicated by the necessity to simultaneously consider the phylogenetic relatedness of coexisting species, and the degree of conservatism or convergence in multiple phenotypic traits (Kraft et al. 2007). In dytiscids, body size and coloration pattern both are important phenotypic traits influencing survival rates in predator presence depending on the environment (Wohlfahrt and Vamosi 2009). Body size is a phenotypic trait in dytiscids that exhibits strong phylogenetic conservatism (Larson et al. 2000; Vamosi and Vamosi 2007). Closely related dytiscid species are similar enough in body length that they may be grouped into different body size classes (small: <5 mm, medium: 5–10 mm, large: 10–15 mm, very large: >15 mm, Vamosi and Vamosi 2007). In contrast, closely related dytiscid species may vary distinctly in their elytra coloration patterns, especially within the smaller sized species (Larson et al. 2000; Pitcher and Yee 2014, Fig. 7.5). In a study of potential mechanisms promoting the coexistence of congeners, Pitcher and Yee (2014) found few phenotypic differences between two *Laccophilus* species, except for elytra coloration patterns, and also little evidence for strong competitive interactions that would explain their slight habitat differences in the wild.

For statistical analyses of phylogenetic community composition in dytiscids, a dated phylogenetic tree (e.g., Ribera et al. 2004, 2008; see also Vamosi and Vamosi 2007, Pallarés et al. 2018) can be used to investigate whether coexisting dytiscid species are more closely or more distantly related than expected by chance. Null models are used to determine whether the phylogenetic distances of the coexisting species significantly differ from random (Webb 2000). Therefore, phylogenetic distances (i.e., mean phylogenetic distance [MPD] and mean nearest taxon distance

Fig. 7.5 Elytra coloration patterns of coexisting *Laccophilus proximus* (top) and *L. fasciatus rufus* (bottom). Photo courtesy of K.A. Pitcher

[MNTD] values) need to be calculated and compared to the phylogenetic distances of 1000 randomly generated communities, the so-called null communities (Cavender-Bares et al. 2006). These null communities can then be used to compute null distributions of MPD and MNTD values. Finally, null distributions are compared to the observed distributions from natural communities. In apparently the only study of phylogenetic community structure of dytiscids, phylogenetic clustering appeared to be the prevalent pattern (Fig. 7.3), with closely related species with similar body size coexisting in the same habitat more often than expected by chance (Vamosi and Vamosi 2007; see also Larson 1985). These results suggest that phenotypic traits allowing the species to persist in a habitat show strong phylogenetic niche conservatism. Phylogenetic structure was also negatively correlated with mean body size of the community, with a tendency toward weak phylogenetic evenness with increasing mean body size. A possible explanation for this pattern might be that larger-sized dytiscids may compete more strongly with each other for resources than do smaller sized dytiscids, leading to the exclusion of similarly large dytiscid species. Alternatively, large dytiscid species may be most likely to be found in communities with a diversity of smaller species, which would be prey for larger species, leading to evenness at the community level (Vamosi and Vamosi 2007).

The results of a community analysis in dytiscids confirmed that gradients in morphology parameters were associated with environmental filters. An important biotic factor that influenced phenotypic community composition was the presence or absence of the regional top predators, small fish or aeshnid dragonfly larvae (Wohlfahrt 2010). Predation by small-bodied, gape-limited fish may lead to increased body size in coexisting prey organisms (Wellborn et al. 1996). Thus, increased abundance of similar large-sized dytiscid species in fish-dominated habitats may be associated with increased predation risk for small dytiscid species. Correspondingly, abundance of small dytiscid species were increased in habitats with fish absent and in habitats with dense submerged vegetation, which may lead to reduced predation risk (Dionne and Folt 1991). Prevalence of species with plain and blotched elytra patterns coexisting in habitats with increased vegetation density and/or absence of predaceous aeshnids, whereas species with more distinct elytra patterns coexisted more often in habitats with aeshnids present (Wohlfahrt 2010). Therefore, not only conserved phenotypic traits, such as body size, may play a role in shaping dytiscid communities. Traits with weaker phylogenetic signal, such as coloration pattern, may also influence species composition and phylogenetic community structure.

The scale of the community analysis may also influence the results of phylogenetic investigations. For example, investigations of the community composition in oak trees have revealed that on the local scale, important phenotypic traits for the passage through environmental filters may derive from convergent evolution, resulting in phylogenetically even communities (Cavender-Bares et al. 2006). In contrast, on the regional scale, important phenotypic traits may be conserved, resulting in phylogenetic clustering. The phylogenetic composition of communities may depend on the degree of phylogenetic conservatism in traits associated with the persistence of species in certain habitats, with a higher degree of clustering expected when important traits are conserved (Webb et al. 2002; Kraft et al. 2007). Species may coexist in local communities because they possess phenotypic traits that allow them to pass through environmental filters, or because they are abundant in the regional species pool. However, the importance of phenotypic traits, local or regional coexistence cannot be explained without consideration of the species evolutionary history (Webb et al. 2006). In the only analysis of dytiscids that I am aware of, changing the regional scale from one that encompassed only species that were present at the local scale to one that included most species found across the province of Alberta had little qualitative effect on the resulting community patterns (Vamosi et al. 2009, re-analyzing data from Vamosi and Vamosi 2007). Because this was only a pilot investigation of the possible effects of regional pool identity on local patterns, I advocate that more systematic analyses be conducted with other datasets, possibly starting with those readily available in older papers (e.g., Nilsson et al. 1994). With continued interest in phylogenetic community structure, I would like to advocate for more studies applying these methods to dytiscids, to test the generality of the findings (Vamosi and Vamosi 2007) with different regional pools, habitat types, and degrees of connectivity.

7.6 Summary and Future Directions

Community ecology has been a very active sub-discipline of ecology from its inception when Charles Elton documented the feeding relationships among the inhabitants of Bear Island in the Barents Sea (e.g., Summerhayes and Elton 1923). Given the high local species richness and abundance that is often attained by dytiscids in a variety of temperate water bodies, I find it somewhat curious that studying them from a community perspective does not have a longer and richer history. Querying the search phrase "TOPIC: (communit* AND dytiscid*)" in Web of Science returns 167 publications at the time of writing, with the earliest being Larson (1985). For comparison, a similar search (replacing dytiscid* with culicid*) returned ~5× more results (873). Almost one third of the 167 publications are from 2015 and onward, potentially suggesting a recent increase in interest, with the caveat that less than 20% of these recent publications focused on community ecology of aquatic beetles generally, or dytiscids specifically (e.g., Perissinotto et al. 2016; Gomez Lutz and Kehr 2017; Pitcher and Yee 2018; Sheth et al. 2019; Enkhnasan and Boldgiv 2020). I posit that a pair of related impediments continue to underlie this continued paucity of studies: (1) the considerable effort it can take to confidently distinguish members of some of the smaller, locally abundant, and species-rich genera (e.g., *Hydroporus*) and (2) the general inability to identify larvae, which often represent the largest component of samples for much of the field season, below the genus rank with morphological characters alone. Yee (2010), for example, studied predation rates in three dytiscid taxa, which were referred to only as *Graphoderus*, *Rhantus*, and *Dytiscus*; based on relative abundances of adults in the sampled ponds, these were surmised to represent *G. occidentalis* Horn, *R. sericans* Sharp, and *D. alaskanus* Balfour-Browne and/or *D. dauricus* Gebler, respectively. Coupled with a solid grounding in the ecology and natural history of dytiscids, I maintain that a promising way forward for the next generation of community analyses will be the adoption of DNA barcoding techniques for identifying individuals in large samples. There is now a wealth of sequences on the Barcode of Life System (BOLD; Ratnasingham and Hebert 2007), although it is not clear how much of Dytiscidae has been genotyped, with the number of genetic bins (715) being much lower than the number of different species names (2361). Baselga et al. (2013) was an early study demonstrating the potential of this approach, examining beta diversity at three levels of organization (haplotype, nested clade, and species) in 23 local assemblages, using a total of 5066 sequences estimated to represent 274 species of water beetles. That study has now been cited 45 times, although curiously none appear to focus on dytiscid community structure. Regardless, it may be fruitful to investigate whether the patterns described earlier for adults, such as apparent lack of interspecific competition and a general trend for phylogenetic clustering in local communities, will hold for larvae. Such data may also encourage researchers to test more modern views of communities, such as that advanced by Chase (2005), and attempt to better understand the role of dispersal in linking and shaping local assemblages across landscapes.

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Steven M. Vamosi received his Ph.D. from the University of British Columbia, Canada, and is currently a Professor of Population Biology and Scientific Director of the Biogeoscience Institute (Kananaskis Country) at the University of Calgary, Canada (Treaty 7 region of southern Alberta). His main interests are evolutionary and conservation ecology of semi-aquatic and aquatic species, including freshwater fish and amphibians, with a focus on impacts of introduced species, habitat loss, and climate change on native species.

Chapter 8
Predator–Prey Ecology of Dytiscids

Lauren E. Culler, Shin-ya Ohba, and Patrick Crumrine

> *With creamy margined, bronze green wing covers, oarlike hind legs fringed with chestnut-colored hairs, and a pair of formidable, meat-tong mandibles, what a well-fashioned submarine predator the diving beetle is. Wayne H. McAlister (2013)*

Abstract Dytiscids are top invertebrate predators in most freshwater habitats, particularly in lentic systems such as wetlands and ponds. Adult and larval dytiscids are often considered to be generalists, feeding on zooplankton, aquatic macroinvertebrates, larval amphibians, and fish; however, some species selectively feed on certain prey types relative to others and many engage in cannibalism and intraguild predation. These predator–prey interactions cause a variety of consumptive and non-consumptive effects on prey abundance and community composition in freshwater habitats. Dytiscids are also notable predators of mosquito larvae and thus explored as biological agents for mosquito suppression, particularly in areas where mosquitoes are vectors of diseases and in northern areas. Dragonfly nymphs, fish, amphibians, reptiles, birds, and mammals are known predators of dytiscids, although the extent to which these organisms rely on dytiscids for food remains unclear. Given the prominent role of dytiscids in freshwater food webs, future research should be aimed at improving basic knowledge of dytiscid feeding ecology, using dytiscids to test predator–prey and trophic theory, describing the potential for dytiscids in conservation biological control, and examining how environmental change affects the role of dytiscids as predators of vector and nuisance species.

L. E. Culler (✉)
Dartmouth College, Hanover, NH, USA

S.-y. Ohba
Nagasaki University, Nagasaki, Japan
e-mail: ooba@nagasaki-u.ac.jp

P. Crumrine
Rowan University, Glassboro, NJ, USA
e-mail: crumrine@rowan.edu

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8.1 Introduction

Dytiscids are ubiquitous as top invertebrate predators in most freshwater habitats. All dytiscid beetles are carnivorous for at least part of their life cycle. Larvae are exclusively predaceous whereas adults may also feed as scavengers (Johnson and Jakinovich 1970; Larson et al. 2000; Le Sage et al. 2019; Bofill and Yee 2019) and in general they feed on a wide diversity of invertebrate and vertebrate prey. They are also cannibalistic, engage in intraguild predation, and are food for other organisms. These predator–prey interactions are understudied relative to those of fish and odonates (e.g., Batzer et al. 2000; Crumrine et al. 2008) yet are essential for understanding processes that structure freshwater and terrestrial communities (Yee 2010; Klecka and Boukal 2012) as well as cascading predator effects on other ecosystem characteristics (e.g., secondary production, pest abundance).

Dytiscid larvae and adults employ a variety of hunting and feeding strategies depending on species, life stage, and habitat (Michel and Adams 2009). Detection of prey is via visual (Maksimovic et al. 2011; Stowasser and Buschbeck 2014a, b), tactile (Friis et al. 2003), or chemical cues (Formanowicz Jr 1987). Larvae use a variety of hunting modes, including sit-and-wait and active hunting (Yee 2010) and can be broadly classified as swimmers, floaters, and crawlers (Wichard et al. 2002). Larvae of many larger dytiscids, such as those in the genus *Dytiscus*, are swimmers that pursue their prey by ambushing and trapping it against vegetation or the water's surface (Wichard et al. 2002). Floating larvae (e.g., *Graphoderus*, *Acilius*) are more specialized swimmers that move elegantly through open water and are more active during hunting (Wichard et al. 2002). Crawlers, including larvae in the Hydroporinae subgroup, are broad bodied and cling to vegetation and sediment rather than pursuing prey by swimming (Wichard et al. 2002). Once detected and encountered, larval dytiscids grasp their prey with falcate piercing-sucking mandibles. They pre-orally inject digestive protease enzymes that liquefy prey body contents and then proceed to suck the resulting mixture back up through their mandibles for ingestion (Young 1967; Formanowicz Jr 1987). This type of feeding permits tackling prey items that are equal in size or larger (Mathias et al. 2016), including vertebrates like fish (Fig. 8.1) and tadpoles (Fig. 8.2, Smith and Awan 2009). Larvae of one genus of dytiscids (*Copelatus*) lack a mandibular canal and have instead been observed swallowing whole chironomid prey (Watanabe and Hayashi 2019). The terrestrial pupal stage of dytiscids does not feed; however, once they emerge as adults, they have chewing mouthparts like those of other Coleoptera. Adults are less efficacious as predators; they are more gape limited and, relative to their larval counterparts, tend to be clumsy and inept at capturing active prey (Larson et al. 2000).

Fig. 8.1 Larval *Dytiscus* spp. sinking its mandibles into a small fish in a laboratory aquarium (photo credit: Siegfried Kehl)

Fig. 8.2 A larval *Cybister chinensis* Motschulsky grasps and consumes a tadpole in the field (photo credit: Shin-ya Ohba)

8.2 Dytiscid Diets and Selective Predation

Much of what we know about dytiscid diets comes easily from visual observations during field and laboratory studies (Figs. 8.1, 8.2, 8.3, 8.4, 8.5, and 8.6) but other important methods include examination of gut contents, molecular methods, and laboratory experiments. For adults, gut contents can be discerned by dissection of the

Fig. 8.3 A dragonfly nymph succumbs to predation by an adult *Cybister brevis* Aubé (photo credit: Naoto Goto)

Fig. 8.4 Backswimmers (Notonectidae) in a pond becoming food for a *Cybister brevis* larva (photo credit: Shin-ya Ohba)

Fig. 8.5 Larval *Colymbetes dolabratus*, collected from a pond near Kangerlussuaq, Greenland, engaging in cannibalistic interactions (photo credit: Gifford Wong)

Fig. 8.6 A larval *Dytiscus* spp. eats a mosquito larva (photo credit: Ary Farajollahi)

foregut and inspection of the contents using a microscope (see Deding 1988; Bosi 2001; Kehl and Dettner 2003). As with any examination of gut contents, care must be taken in interpreting the results as some of the material could have been ingested via the guts of other prey organisms (Kehl and Dettner 2003) or could have been accidentally ingested. Visual examination of gut contents is not possible for most dytiscid larvae because their prey are liquefied during ingestion. Polyacrylamide gel electrophoresis has been used to assess gut contents of other piercing-sucking predators, such as notonectids (Giller 1984, 1986). From field-collected dytiscids, Bradford et al. (2014) sequenced fragments of the mitochondrial COI gene that are known to species of potential prey. Combined with stable isotope analyses and behavioral observations, they were able to resolve prey preference among three sister species of dytiscids (Bradford et al. 2014). Laboratory feeding experiments and carefully designed preference trials can also help determine what larval and adult dytiscids consume and if they exhibit selective predation. Individuals are offered different types of prey in different proportions. If consumption deviates from the offered proportion, the individual is considered to exhibit selective predation (e.g., Peckarsky 2006). Detailed behavioral observations are required to determine if selective predation is a result of a beetle's ability to detect, encounter, attack, capture, subdue, and digest the various types of prey in its habitat (Culler and Lamp 2009). For example, prey of a given species may be consumed because they are more abundant relative to other species in the habitat and therefore encountered most often. Alternatively, the most abundant prey might be difficult for a dytiscid to successfully capture so alternative prey are pursued.

Many studies and observations suggest that dytiscids are generalists that feed opportunistically on whatever is available (Frelik 2014), including conspecifics and heterospecifics and even decaying animal carcasses (Velasco and Millán 1998; Barrios and Wolff 2011). Occasionally, plant material and algae can also be found in the guts (Deding 1988; Frelik 2014), but plants are considered to be accidentally ingested (Bosi 2001). Invertebrates, specifically zooplankton (Arts et al. 1981; Bradford et al. 2014; Hayashi and Ohba 2018), insects (Figs. 8.3 and 8.4, e.g., Johansson and Nilsson 1992, Hicks 1994, Frelik et al. 2016, Frelik and Pakulnicka 2015), horsehair worms (Watanabe 2019), and amphipods (Bradford et al. 2014) are key parts of the diets of many dytiscid species. In addition, they consume vertebrates including fish (Balfour-Browne 1950; Dillon and Dillon 1961; Le Louarn and Cloarec 1997; Frelik 2014; McDaniel et al. 2019), reptiles (snakes, Drummond and Wolfe 1981), and amphibians (Formanowicz Jr and Brodie Jr 1982; Brodie Jr and Formanowicz Jr 1983; Resetarits 1998; Rubbo et al. 2006; Smith and Awan 2009; Inoda et al. 2009; Inoda and Kamimura 2015; Valdez 2019; Watanabe et al. 2020; Arntzen and Zuiderwijk 2020). Adult *Hydaticus parallelus* Clark have been found to actually oviposit their eggs within frog spawn such that both types of eggs hatch simultaneously, providing beetle larvae access to newly hatched tadpoles (Gould et al. 2019).

Studies have also indicated that larvae and adults of some dytiscid species selectively feed on certain types of prey relative to others (Koegel 1987; Kehl and Dettner 2003; Tate and Hershey 2003; Ohba 2009a, b; Cobbaert et al. 2010; Ohba and Inatani 2012; Ohba and Ogushi 2020), sometimes even preferring dead prey to live prey, as is the case with adults of *Thermonectus marmoratus* (Gray) (Velasco and Millán 1998). Aditya and Saha (2006) showed that adult *Rhantus sikkimensis* Régimbart preferentially fed on chironomids versus culicids. *Dytiscus circumcinctus* Ahrens larvae preferred mayfly nymphs and isopods to caddisfly larvae whereas the co-occurring *D. latissimus* Linnaeus had just the opposite preference (Johansson and Nilsson 1992; Scholten et al. 2018). A study by Yee et al. (2013) showed a preference of larval *Graphoderus* for corixids compared to chironomids or damselflies, but larval *Rhantus* consumed similar proportions of corixids and chironomids. In temporary ponds in North Carolina, *Dytiscus* larvae had a negative effect on the survival of *Pseudacris triseriata* tadpoles relative to *Bufo americanus* tadpoles (Pearman 1995). A few studies have tested the preference of adult and larval dytiscids feeding on dipterans versus microcrustaceans, with preference noted for dipterans, including chironomids by adult *Boreonectes* (Ranta and Espo 1989) and culicids by larval *Agabus* (Culler and Lamp 2009). In freshwater habitats in eastern Poland, Frelik and Pakulnicka (2015) showed that adult dytiscids may actually use only a fraction of the macroinvertebrates available as food; in this case, dytiscids favored Ephemeroptera and Chironomidae over Asellidae, despite that the latter of which reached high numbers in their study sites. Some groups of dytiscids, such as the Hydroporinae, have larvae with elongated nasales that resemble a pig's snout (Friis et al. 2003) and are presumed adaptations for capturing microcrustaceans over other types of prey (Galewski 1971; de Marzo and Nilsson 1986; Hayashi and Ohba 2018). In addition to unique morphological adaptations, beetle size (Bradford et al. 2014), prey behavior (Ohba and Ushio 2015), hunting mode (Yee 2010; Yee et al. 2013), hunger level (Hileman et al. 1995), visual cues (Nilsson 1986), and ontogeny (Friis et al. 2003; Ohba 2009b) are often cited as reasons for greater consumption of certain prey species relative to others.

8.3 Consumptive and Non-consumptive Effects of Dytiscid Predation

Due to high feeding rates, dytiscids are known to decrease total macroinvertebrate abundance or biomass (Arts et al. 1981; Arnott et al. 2006; Magnusson and Williams 2009; Cobbaert et al. 2010), with some macroinvertebrate groups reduced more than others. In fishless ponds in north-central Alberta, adults of *Dytiscus alaskanus* Balfour-Browne, via preferential consumption, lowered biomass of several groups including amphipods, leeches, water bugs, damselflies, dipterans, and snails (Cobbaert et al. 2010). Higher zooplankton biomass was also noted, indicating a possible trophic cascade (Cobbaert et al. 2010). Similarly, Tate and Hershey (2003) used lab experiments and molecular analyses to demonstrate preferential feeding by larval dytiscids (*Agabetes*, *Celina*, *Colymbetes*, *Derovatellus*, *Dytiscus*, and *Rhantus)* on larger prey species, including caddisflies, fairy shrimp, water bugs, Diptera, amphipods, and also young-of-year grayling. Neither of these studies reported changes in taxa richness, but Arnott et al. (2006) found that *Graphoderus liberus* (Say) adults reduced zooplankton biomass by 21% and lowered taxa richness and values of the Shannon–Wiener diversity index for zooplankton. In general, aquatic invertebrate predators have been shown to affect community attributes due to selective predation (e.g., Murdoch et al. 1984; Runck and Blinn 1994), although the studies directed at dytiscids are limited (Arnott et al. 2006).

In addition to consumptive, or lethal, effects on prey communities, dytiscids also trigger changes in prey behavior and physiology, known as non-consumptive effects. For example, removal of aquatic insect predators, including some dytiscids, resulted in altered migration strategies and an increase in body size of daphniids in fishless ponds (Herwig and Schindler 1996). Although specific investigations of non-consumptive effects of dytiscids are uncommon, they do offer insights into how these predators may affect aquatic prey communities. Ohba et al. (2012b) reported that *Culex tritaeniorhynchus* female mosquitoes avoided laying eggs in dytiscid-conditioned water and that smaller mosquitoes emerged from dytiscid-conditioned water as a result of lowered larval activity. Smith and Awan (2009) found that American toad and bullfrog tadpoles altered activity levels and some avoided vegetation when dytiscids were present, presumably to avoid detection and because dytiscids use vegetation as an ambush perch. Similarly, wood frog tadpoles avoided areas containing caged dytiscids in experimental mesocosms (Rubbo et al. 2006). Johnson et al. (2003) found that the presence of dytiscid larvae and other predators of southern leopard frog eggs shortened the time to hatching and decreased hatchling size. In these preceding examples, dytiscid-induced changes in prey behavior and size could be energetically costly and have fitness consequences, but non-consumptive effects can also increase prey fitness. For example, in temporary pools, adult dytiscids facilitated dispersal of their prey (Beladjal and Mertens 2009); consumption, mastication, and the passage of fairy shrimp through the digestive tracts of adult dytiscids (*Ilybius fenestratus* (Fabricius) and *Colymbetes fuscus* (Linnaeus)) led to increased fairy shrimp hatching (Beladjal and Mertens 2009). In another case, by acting as scavengers on tadpole carcasses, dytiscids reduced Ranavirus transmission within a frog population (Le Sage et al. 2019). Non-consumptive predator effects of dytiscids should be further including the possibility of changes in population and community dynamics and ecosystem functions.

8.4 Cannibalism and Intraguild Predation

Intraspecific predation (cannibalism) is quite common among aquatic organisms (Fox 1975) and has been documented among larval dytiscids (Pajunen 1983; Juliano and Lawton 1990; Culler and Lamp 2009; Yee 2010; Carter et al. 2018). There is much less evidence for cannibalism between adults, most likely due to gape limitation (Johnson and Jakinovich 1970). Cannibalism during the larval stage is probably even more prevalent than the literature suggests given the generalist foraging patterns and voracity of many dytiscid species (Fig. 8.5). Cannibalism has the potential to function as a density dependent control on dytiscid populations (Juliano and Lawton 1990) and this effect may be more pronounced when alternative prey is limited in abundance (Culler and Lamp 2009). Under these conditions, cannibalism can be viewed as a lifeboat strategy that allows individuals to persist under sub-optimal ecological conditions and even accelerate development in temporary ponds that are prone to drying (Batzer and Wissinger 1996). In some species, such as *Boreonectes multilineatus* (Falkenström) (formerly *Potamonectes griseostriatus*), conspecifics make up nearly 10% of the diet and are among the more common prey items in the diet of larvae (Pajunen 1983). Cannibalism among dytiscid larvae is also recognized as one factor that must be considered in captive breeding programs for endangered species and can be mitigated by simply raising groups of larvae at high prey density (Inoda and Kitano 2013).

In general, the factors influencing the occurrence and frequency of cannibalism within Dytiscidae are not unlike those across other orders of aquatic insects. In most aquatic insects, population size structure plays a key role in determining the frequency of cannibalism and larger individuals are almost always the cannibal and smaller individuals the victim (Wissinger 1992; Fagan and Odell 1996; Hopper et al. 1996; Wissinger et al. 1996; Yee 2010; Carter et al. 2018). However, the relatively large mandibles possessed by larval dytiscids confer the ability to subdue large prey items including similar-sized conspecifics (Pajunen 1983) and perhaps even larger individuals. Avoidance of cannibalism may be influenced by large differences in size between larvae (Pajunen 1983) and the ability to recognize and avoid conspecifics (Inoda 2012). Given the dearth of studies on cannibalism among dytiscids, these and other aspects of cannibalism deserve further inquiry. This is particularly true for dytiscids because they occupy relatively high trophic positions within fishless systems and recent modeling studies have demonstrated the potential for cannibalism to strongly influence coexistence among predators and structure communities (Rudolf 2007; Ohlberger et al. 2013).

Besides cannibalism, intraguild predation (IGP) is likely to be a common interaction among dytiscids, particularly among larvae for the reasons noted above. IGP is a mixed competition–predation interaction that occurs when species that compete for a common resource also interact as predator and prey (see Figs. 3, 4, and 6 in Polis et al. 1989). Simple mathematical models suggest that IGP should be relatively rare in nature (Holt and Polis 1997), but food web studies indicate that IGP is common across terrestrial, marine, and aquatic systems (Arim and Marquet 2004). More recent theoretical and empirical work indicates that size-structured interactions such as cannibalism may promote the coexistence of predators in IGP systems (Crumrine 2005; Rudolf 2007). There are few studies that specifically examine IGP among larval dytiscids (e.g., Nilsson and Soderstrom 1988; Culler and Lamp 2009; Yee 2010). As is the case with cannibalism, IGP is probably more prevalent than the literature suggests given the generalist foraging patterns of larval dytiscids and high spatial and temporal overlap among species (Yee 2010). Of the studies that have examined IGP among larval dytiscids, not surprisingly, size differences between individuals influence the outcome of predator–prey interactions between intraguild predators. In some cases, larger larvae consume smaller larvae (Nilsson and Soderstrom 1988; Yee 2010), but there are also examples of IGP between individuals similar in size (Culler and Lamp 2009; Yee 2010). In fact, some genera (e.g., *Dytiscus*) do not appear to consume dytiscid prey smaller than themselves and this may promote coexistence between relatively large- and small-bodied dytiscids (Yee 2010). IGP among larval dytiscids can be symmetric; that is, both predators consume each other (Culler and Lamp 2009; Yee 2010). This appears to be most common among congeneric species that are similar in size, although higher levels of aggression may also lead to greater frequency of IGP among some species (Culler and Lamp 2009). Asymmetric IGP appears to be most common when there is a distinct size difference between individuals (Nilsson and Soderstrom 1988; Yee 2010). Large-bodied dytiscids, such as those in the genera *Dytiscus* and *Cybister*, are within the guild of top predators in fishless ponds and likely function as intraguild predators of larval dytiscids as well as other large predatory aquatic insects such as odonate nymphs.

There are surprisingly few studies that have examined IGP within this group of insects; however, Carter et al. (2018) examined IGP and cannibalism within a guild of size-structured predators including *Cybister fimbriolatus* (Say) and the dragonfly *Anax junius* and provide evidence that greater levels of habitat complexity can result in more IGP and lower predation rates on shared prey. This work also highlights how the ecological role of predators can change through development because early instar *C. fimbriolatus* were prey for late instar *A. junius* but late instar *C. fimbriolatus* consumed all size classes of conspecifics and *A. junius* (Carter et al. 2018). This further reinforces the notion that body size rather than species identity may be a better predictor of predator–interactions in guilds of size-selective generalist predators. More complex mesocosm experiments with the same group of predators show that the demographic structure of dytiscid populations can strongly influence community composition and ecosystem processes and is equally, and in some cases more influential than the identity of the predator (Rudolf and Rasmussen 2013). Future studies are warranted because IGP among dytiscids is likely to influence coexistence between competing species and it may help to explain the diversity of species found in some aquatic systems (Yee 2010).

8.5 Dytiscids as Predators of Vector and Nuisance Species

Of coleopteran predators, dytiscids are the most commonly reported predators of vector and nuisance species, specifically of mosquito larvae and pupae (Fig. 8.6, Sailer and Lienk 1954; Roberts et al. 1967; Young 1967; Borland 1971; Notestine 1971; Service 1973; Akmetbekova and Childibaev 1986; Nilsson and Svensson 1994, 1995; Mogi 2007; Quiroz-Martínez and Rodríguez-Castro 2007; Shaalan and Canyon 2009, and references within Table 8.1). Laboratory observations have confirmed that adult and larval dytiscids attack mosquito larvae, but most studies have focused on adults despite that dytiscid larvae are the more voracious predators. Mosquito larvae have been found in the guts of field-collected dytiscids (Deding 1988; Bosi 2001; Vinnersten et al. 2015) and radioisotope studies (James 1965) and precipitin tests (Service 1973) have confirmed a prominent role of dytiscids as mosquito predators. Moreover, the serological method (Service 1977, 1993) and DNA analysis (Ohba et al. 2010; Vinnersten et al. 2015) revealed that some species consumed vector mosquitoes in their natural wetlands. Consumption rates of

Table 8.1 Dytiscids have been documented as predators of *Aedes, Anopheles,* and *Culex* mosquitoes, with most focus on mosquito prey species of medical significance, such as *Ae. albopictus* (yellow fever, dengue fever, Chikungunya, Zika, and others), *An. gambiae* (malaria), and *Cx. tritaeniorhynchus* (Japanese encephalitis)

Mosquito genus	Mosquito species	Dytiscid species	References
Aedes	*Ae. albopictus*	*Agabus disintegratus, A. punctatus, Eretes griseus, Hydaticus vittatus, Platynectes* sp.	Sulaiman and Jeffery (1986), Culler and Lamp (2009), Kumar et al. (2014), Ohba and Ushio (2015), Ohba unpubl. data
	Ae. atropalpus	*Laccophilus maculosus*	James (1964, 1965)
	Ae. communis	*Ilybius erichsoni, I. opacus*	Nilsson and Soderstrom (1988)
	Ae. nigripes	*Colymbetes dolabratus*	Culler et al. (2015), DeSiervo et al. (2020)
	Ae. sticticus	*Agabus biguttulus, A. affinis, Hydaticus aruspex, H. seminiger, Ilybius ater, Nartus grapii, Rhantus exsoletus*	Vinnersten et al. (2015)
	Ae. stimulans and Ae. trichurus	*Ilybius erichsoni, Rhantus frontalis, Hydroporus tenebrosus*	James (1961)
	Ae. vexans	*Laccophilus fasciatus rufus, L. proximus*	Pitcher and Yee (2014)
Anopheles	*An. gambiae*	*Laccophilus simplicistriatus, Copelatus johannis, Hyphydrus impressus, Hydaticus galla, Laccophilus* spp.	Service (1973), Ohba et al. (2010)
Culex	*Cx. annulirostris*	Unknown	Rae (1990)
	Cx. (Culiseta) incidens	*Dytiscus marginicollis*	Lee (1967)
	Cx. mimeticus	*Cybister brevis*	Ohba (2009a)
	Cx. pipiens	*Hydroglyphus geminus, Laccophilus fasciatus, Laccophilus maculosus*	Roberts et al. (1967), Bellini et al. (2000)
	Cx. quinquefasciatus	*Rhantus sikkimensis,* unknown	Aditya et al. (2006), Chandra et al. (2008)
	Cx. tritaeniorhynchus	*Agabus conspicuus, A. japonicus, Cybister brevis, C. chinensis, Eretes griseus, Graphoderus adamsii, Hydaticus bowringii, H. grammicus, H. rhantoides, Hydroglyphus japonicus, Hyphydrus japonicus, Laccophilus difficilis, Rhantus suturalis*	Sugiyama et al. (1996), Ohba and Takagi (2010)
	Cx. spp.	*Colymbetes paykulli, Ilybius fuliginosus, I. ater*	Lundkvist et al. (2003)
Not reported	n/a	*Agabus bipustulatus, Eretes sticticus, Ilybius subaeneus, Rhantus suturalis*	Swamy and Rao (1974), Bosi (2001)

mosquitoes by dytiscids can be as high as 86 mosquito larvae per predator per day (Aditya et al. 2006), thus warranting their consideration as agents for natural mosquito suppression.

Dytiscids are likely significant predators of medically important mosquito species. A large number of studies have documented the presence of dytiscids in various habitats with immature stages of mosquitoes that are vectors of diseases (Mogi and Miyagi 1990; Mogi 1993; Takagi et al. 1996; Mogi et al. 1999; Campos et al. 2004; Carlson et al. 2009; Mwangangi et al. 2008; Hassan et al. 2010; Ohba et al. 2011, 2012a) but fewer studies have examined dytiscid–vector interactions more directly. In Kenyan wetlands, Ohba et al. (2010) used molecular methods to detect that dytiscids consume larvae of the malaria mosquito *Anopheles gambiae.* Based on laboratory studies and field experiments, larval dytiscids were determined to be a potential biocontrol agent against the filarial vector *Culex quinquefasciatus* in India (Chandra et al. 2008). We note that Chandra et al. (2008) reported *Acilius sulcatus* (Linnaeus) as the biocontrol agent, however that species does not occur in India and the larvae examined were likely *Eretes sticticus* (Linnaeus) (M. Jäch, personal communication). Also in India, Kumar et al. (2014) found that dytiscid species in the genus *Platynectes* are a potential biocontrol agent for *Aedes albopictus,* which is a vector of chikungunya and dengue fever. Because dytiscids are a nearly ubiquitous inhabitant of wetlands, rice fields, and rock pools, which are also home to many species of mosquitoes that are vectors of disease, dytiscids likely have a greater role in disease transmission dynamics than we can currently describe.

Northern areas are another location where dytiscids play a role in mosquito population dynamics. Due to short growing seasons, dytiscid life cycles are in synchrony with those of their mosquito prey (e.g., James 1964; Nilsson and Svensson 1994; Culler et al. 2015; DeSiervo et al. 2020). In snowmelt ponds in Greenland, early hatching larvae of *Colymbetes dolabratus* (Paykull) rely almost exclusively on mosquito larvae, one of the only food sources available at that time of year (Culler et al. 2015; DeSiervo et al. 2020). In Canada, *Ilybius erichsoni* (Gemminger and Harold) completes its life cycle in woodland pools, overwintering as both eggs and adults, the appearance of the latter coinciding with the winter hatch of mosquitoes (James 1961, 1967). The impact of these synchronous lifecycles no doubt has an impact on mosquito abundance. In Sweden, Lundkvist et al. (2003) showed that after colonization by large adult dytiscid predators (*Ilybius*, *Rhantus,* and *Agabus* spp.), larval mosquito abundance was significantly reduced. In Canadian rock pools, James (1964) found *Laccophilus maculosus* (Germar) to be the most abundant predator of the mosquito *Aedes atropalpus,* with a significant inverse correlation between densities of *Ae. atropalpus* and larval *L. maculosus*. Mosquitoes in the north are not currently significant vectors of disease; however, they do occur in large numbers, thus dytiscids may serve to reduce the significant nuisance that mosquitoes provide to humans and wildlife (Koltz and Culler 2021)

Although dytiscids seem to have a significant role in suppressing vector and nuisance species, they are difficult to rear, and thus are not likely to work as classical biological control agents. However, conservation biological control (Barbosa 1998) may be a useful technique to employ in management settings (Culler and Lamp

2009). Walton (2012) suggested that the construction of aquatic habitats with a goal of attracting a diverse and abundant predator assemblage may help to reduce pest abundance. According to a study by Schafer et al. (2006), one way to do this is to create permanent wetlands in an open landscape, which they found to favor colonization by diverse dytiscid assemblages and therefore reduce mosquito colonization.

8.6 Dytiscids As Prey

The role of dytiscids in the trophic ecology of freshwater food webs is often investigated from the standpoint of dytiscids as top predators, but dytiscids also make up parts of the diets of many other organisms, both aquatic and terrestrial. Odonates are predators of dytiscids (Fig. 8.7; Larson 1990) and Aykut and Esen (2017) documented that dytiscids, particularly those in the genus *Agabus*, experience parasitism by water mites. *Hydaticus* and *Eretes* also experience parasitism by *Hydrachna* water mites in laboratory conditions (Masuda 1934). These are some of the only published reports of aquatic invertebrates feeding on dytiscids. Dytiscid cuticle has been recovered from dissected fish guts (Laufer et al. 2009) but the extent to which fish rely on dytiscids as a main component of their diet is unknown. Fish and dytiscids do not always co-occur in the same habitat (Schilling et al. 2009; de Mendoza et al. 2012) or dytiscid species richness and abundance tend to be lower in habitats with fish (Liao et al. 2020). In mountain lakes, the distribution of *Agabus bipustulatus* Linnaeus is constrained due to predation by salmonid fish, and thus they are found to only inhabit colder lakes where fish are unlikely to occur (de Mendoza

Fig. 8.7 Dragonfly nymphs and dytiscids frequently co-occur and engage in intraguild predation. Here, a large *Anax* dragonfly nymph (Odonata: Aeshnidae) consumes a *Graphoderus* larvae (photo credit: Donald Yee)

et al. 2012). Gerhart et al. (1991) also showed that dytiscids can secrete defensive hormones that inhibit feeding by fish. Dytiscids are part of the diets of turtles (Chessman 1984; Georges et al. 1986; Demuth and Buhlmann 1997), bullfrogs (Korschgen and Moyle 1955; Bruggers 1973), toads (Whitaker Jr. et al. 1977), salamanders (Whiles et al. 2004; Dasgupta 1996), and snakes (Peddle and Larson 1999). The evidence for snake predation comes from postulation that scratch marks on the beetle's cuticle were caused from predator attacks in areas with known snake populations (Peddle and Larson 1999).

Dytiscids also represent an important linkage between freshwater and terrestrial systems, serving as food for terrestrial predators and sometimes carrying with them pollutants such as microplastics (Kim et al. 2018). Numerous studies have confirmed the role of adult and larval dytiscids in the diets of birds, particularly in birds species that are associated with water (e.g., Schubart et al. 1965; Cramp and Simmons 1977, 1980; Abensperg-Traun and Dickman 1989; Goutner and Furness 1997; Elmberg et al. 2008), but also in hawks (Munro 1929) and finches (Montalti et al. 2005). Pellets collected from colonies of grey herons in northern Poland consisted of 26–51% invertebrate remains, mainly the dytiscid beetle *Dytiscus marginalis* (Linnaeus) (Jakubas and Mioduszewska 2005). Forty-one percent of regurgitate material from Glossy Ibises in Spain were dytiscids, primarily *Cybister* (Macías et al. 2004). In Arkansas, dytiscids make up 19% of the King Rail's diet during the winter months (Meanley 1956). Brooks (1967) presented data on the diets of various species of shorebirds in Illinois, the majority of which contained adults of the dytiscid beetles *Agabus disintegratus* (Crotch) and *Hygrotus*. Raccoons (Capinera 2010) and otters (Brzeziński et al. 1993) are also noted predators of dytiscid beetles. During the warm season, dytiscids are the third most important prey item in terms of biomass for river otters in eastern Poland (Brzeziński et al. 1993). The only other mammals known to ingest dytiscids are humans. Several species in the genus *Cybister* are regularly consumed in parts of China (Jäch 2003), Thailand (Chen et al. 1998), New Guinea (Gressitt and Hornabrook 1977), and Japan (S. Ohba, personal observation).

8.7 Future Research

Dytiscids are ideal study organisms for basic and applied predator–prey research due to their prominent role in freshwater food webs, their ubiquitous distribution, and ease of handling in the laboratory (Fig. 8.8) and field (Fig. 8.9). Two suggested focal areas are (1) improving basic knowledge of dytiscid feeding ecology and (2) learning how dytiscid predator–prey interactions are shaped by the environment in both managed and natural systems. This will help address basic and applied research questions related to feeding strategies and food web structure and composition, consumptive and non-consumptive effects of predation, the utility of dytiscids in natural and managed systems, and how predator–prey dynamics are impacted by climate and land-use change.

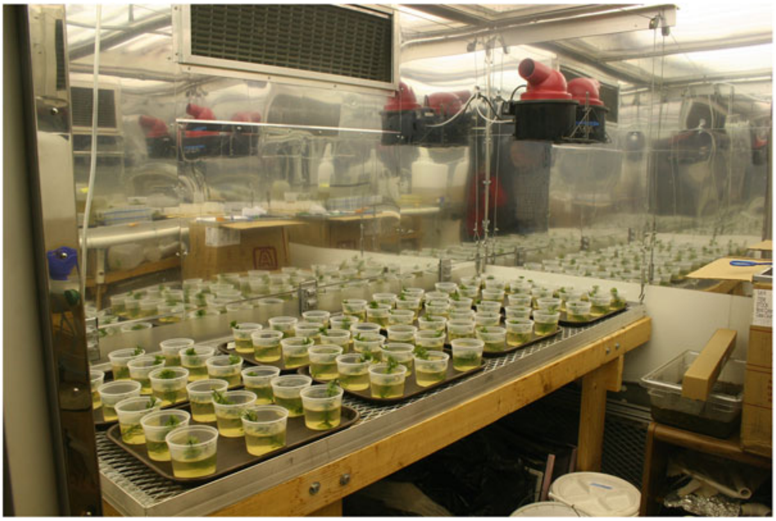

Fig. 8.8 Dytiscids are ideal for use in laboratory experiments where various factors can be manipulated, including habitat structure, temperature, and relative abundance of different types of prey. Here, small plastic cups housing dytiscid larvae are used as microcosms to test the effects of structure and prey density on antagonistic predator–predator interactions (photo credit: Lauren Culler)

Fig. 8.9 Field experiments are useful for measuring effects of dytiscid predation on prey. Here, white pans are set up adjacent to a tundra pond and used to measure consumption rates of mosquito larvae by dytiscid predators (photo credit: Lauren Culler)

Despite a growing number of studies of the feeding preferences of larval and adult dytiscids, more information about basic feeding ecology is needed to resolve the position and relative importance of dytiscids in freshwater and terrestrial food webs. New molecular methods including next-generation sequencing (Bradford et al. 2014) and the use of stable isotopes of carbon and nitrogen are promising techniques to determine the extent that dytiscids are specialists versus generalists and the intensity of intraguild predation and cannibalism. Behavioral studies are needed to elucidate the non-consumptive effects of dytiscid predation on the behavior and life-history traits of prey. The consequences of selective predation, intraguild predation, cannibalism, and non-consumptive effects by dytiscids for populations, communities, and ecosystems remain largely unknown but have the potential to strongly influence population dynamics and species coexistence (Yee 2010; Pitcher and Yee 2014).

Dytiscids also occur in managed systems and thus it is necessary to study how their predator–prey interactions may interfere with or support conservation and management goals. One recent study showed how dytiscids can affect amphibian conservation projects because they consume such large numbers of tadpole prey (Valdez 2019). In such cases it would be desirable to limit dytiscid abundance and the threat of predation. In other cases, dytiscids are favored in freshwater habitats due to their potential as biological control agents for vector and nuisance species. Measuring their effects on nuisance prey populations and testing how habitat and environmental factors influence these effects are essential for projects that aim to construct or restore natural lentic habitats while minimizing increased threats from vectors. Habitat structural complexity has been suggested to enhance predation due to a reduction in negative intraspecific interactions (i.e., cannibalism and intraguild predation, Culler and Lamp 2009, Yee 2010). Wetland construction techniques that include adding coarse woody debris or planting diverse aquatic vegetation could be useful for projects that have goals of encouraging predator colonization to reduce pest abundance (e.g., Walton 2012). This idea largely parallels a practice used in agricultural habitats known as conservation biological control, which is defined as the manipulation of habitats to favor the natural enemies of pests, as to conserve biodiversity and reduce pest problems (Barbosa 1998).

Feeding ecology and dytiscid predator–prey interactions should also be studied in the context of environmental change. Several studies suggest that temperature has a prominent role in the behavior and feeding ecology of dytiscids. Temperature affects predator–prey interactions because of its fundamental effects on the metabolism and physiology of ectothermic organisms. Calosi et al. (2007) showed that temperature can alter the diving behavior of dytiscids, with frequency of diving increasing at higher temperatures, thus decreasing the amount of time available for other activities such as foraging. Nilsson and Svensson (1994) showed that prey mortality from dytiscid predation was higher in warmer pools and Culler et al. (2015) found that predation by *Colymbetes dolabratus* on Arctic mosquitoes increased at warmer temperatures. Understanding temperature effects is a research priority, particularly in regions where there is significant warming occurring (e.g., Arctic and alpine regions) and where dytiscids occur as top predators and have a strong influence on the prey community, particularly mosquito abundance.

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Lauren E. Culler completed her B.S. in Biology in 2005 and M.S. in Entomology in 2008, both from the University of Maryland, College Park, and her Ph.D. in Ecology and Evolutionary Biology from Dartmouth College in 2013. Her interests were shaped by an early fascination with dytiscids and include how environmental factors affect predator-prey interactions in systems that are linked to humans. She works with dytiscids in restored agricultural wetlands on the Eastern Shore of Maryland and tundra ponds in Arctic Greenland.

Shin-ya Ohba has been at Nagasaki University as an associate professor since 2012. He completed his B.S. in Agriculture in 2002 from Tamagawa University, M.S. in Agriculture from Ehime University in 2004, and a Ph. D. in Entomology from Okayama University in 2007. His research interests are basic ecology, the role of predaceous diving beetles as mosquito predators, and also educational effects of dytiscid on the students ("eggs" of teacher) in his laboratory.

Patrick Crumrine earned his B.S. in Biology in 1998 from SUNY Plattsburgh and his Ph.D. in Biology from the University of Kentucky in 2003. The primary focus of his research is to understand how size-structure influences competition and predation among aquatic organisms, particularly odonates and dytiscids. Other research interests include disease ecology of amphibians and population/community structure of aquatic turtles. Most of his work is conducted in small ponds and wetlands in the mid-Atlantic region of the United States.

Chapter 9
The Unique Australian Subterranean Dytiscidae: Diversity, Biology, and Evolution

Andrew Austin, Michelle Guzik, Karl Jones, William Humphreys, Chris Watts, and Steven J. B. Cooper

> *Only recently [. . .] have biologists had access to the vast array of Darwin's "endless forms most beautiful and most wonderful" for in-depth genetic investigations of development, physiology, and evolution. John H. Postlethwait (Postlethwait 2015).*

Abstract The western half of the Australian arid zone harbours the richest diversity of obligate subterranean dytiscids in the world, which are found in isolated calcrete (carbonate) aquifers. Each calcrete usually supports from one to three beetle species that are locally endemic to a specific calcrete, and display the full array of adaptations to living in a permanently dark, aquatic environment. The origin of this dytiscid diversity likely dates back to the late Miocene to Pliocene when central and western Australia was dominated by a more benign, mesic environment. Subsequent aridification led to relictualisation of the fauna to the calcrete aquifers which, because of their physical isolation from each other, have been described as 'islands under the desert'. Here we provide an overview of this remarkable fauna of dytiscids, and outline what is currently known about their diversity, life history, respiratory

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A. Austin (✉) · M. Guzik · K. Jones
Department of Ecology and Evolutionary Biology, School of Biological Sciences, The University of Adelaide, Adelaide, SA, Australia
e-mail: andy.austin@adelaide.edu.au; michelle.guzik@adelaide.edu.au; karl.jones@adelaide.edu.au

W. Humphreys
Department of Terrestrial Zoology, Western Australian Museum, Welshpool DC, WA, Australia

School of Biological Sciences, University of Western Australia, Crawley, WA, Australia
e-mail: bill.humphreys@museum.wa.gov.au

C. Watts · S. J. B. Cooper
Evolutionary Biology Unit, South Australian Museum, Adelaide, SA, Australia
e-mail: chris.watts@samuseum.sa.gov.au; steve.cooper@samuseum.sa.gov.au

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physiology, modes of speciation, population biology, and outline their conservation issues and areas for future research.

Keywords Underground · Cave · Habitat · Conservation · Stygofauna · Aquifer

9.1 Introduction

The arid zone in the south-western half of the Australian continent and Ngalia Basin in the Northern Territory is home to a rich diversity of obligate subterranean groundwater species, (collectively referred to as 'stygofauna'), which are found in isolated calcrete aquifers. Unknown until 25 years ago, each isolated calcrete supports a unique fauna that includes several crustacean groups including bathynellaceans, amphipods, copepods (Bradford et al. 2010; Cho et al. 2005, 2006; Cho and Humphreys 2010; Guzik et al. 2010; Matthews et al. 2020), and oniscidean isopods (Cooper et al. 2008; Guzik et al. 2008, 2019), with the latter group, together with numerous other taxa (spiders, palpigrades, pseudoscorpions, mites, collembolans, myriapods), found in the subterranean terrestrial environment above the water table (referred to as 'troglofauna') (Barranco and Harvey 2008; Guzik et al. 2021; Harrison et al. 2014; Javidkar et al. 2016, 2017, 2018). These calcretes also support the world's greatest diversity of subterranean predaceous diving beetles, which represent a major and the best-studied component of the stygofauna.

The origin of this dytiscid diversity, and that of most other stygofaunal groups, is thought to date back to the mid-late Miocene to Pliocene when central and western Australia were dominated by a more benign, mesic environment (Byrne et al. 2008; Humphreys 2008; Leys et al. 2003). A subsequent period of aridification led to relictualisation of the fauna to habitats that retained water, such as calcrete aquifers (Leys et al. 2003) which, because of their physical isolation from each other (Humphreys 2001), have been aptly described as 'islands under the desert' (Cooper et al. 2002) (Fig. 9.1). The dytiscids and other groups probably entered the subterranean realm by initially colonising the hypogean habitat in river gravels (Leys et al. 2010), with diversification of separate lineages hypothesised to have occurred within the aquifers through sympatric, parapatric and/or microallopatric speciation (Leijs et al. 2012, see below). These processes have resulted in a remarkable fauna of diving beetles that display the full array of adaptations to living in a permanently dark, aquatic environment.

In the following sections, we review the diversity, life history, and respiratory physiology of these beetles, as well as aspects of their evolution including modes of speciation, and conclude with a discussion of their conservation issues and areas for future research.

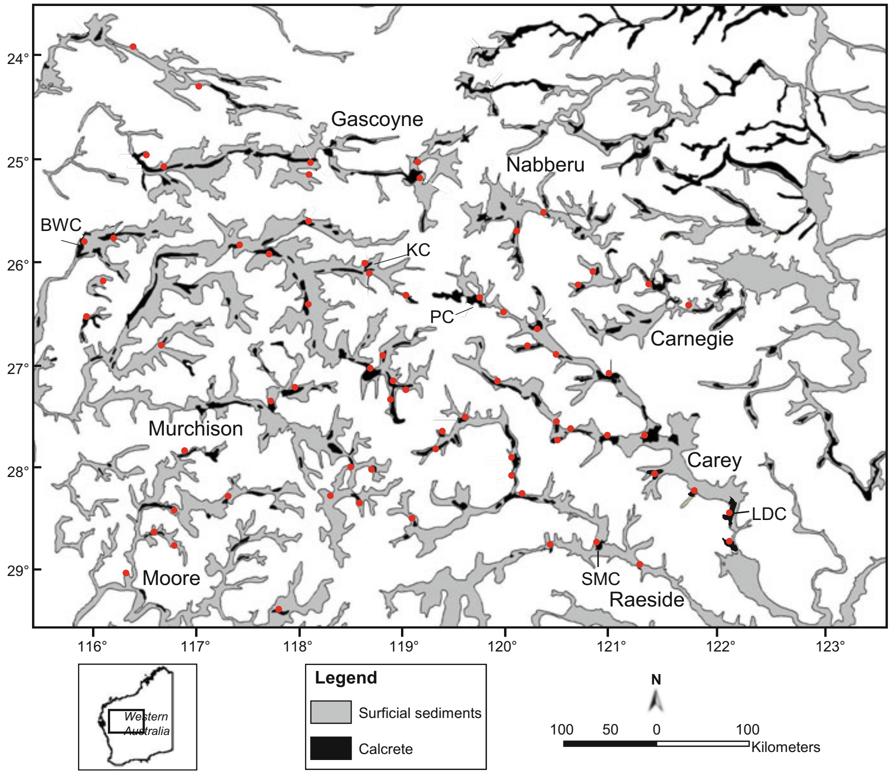

Fig. 9.1 Map of the northern Yilgarn Region of central Western Australia showing the calcrete (black) localities that have been sampled for dytiscids (red dots) and the relative positions of calcretes in the paleodrainages (grey). Carey, Carnegie, Gascoyne, Moore, Murchison, Nabberu and Raeside are the names of the major palaeodrainages. *BWC* Byro West calcrete, *KC* Karalundi calcrete, *LDC* Laverton Downs calcrete, *PC* Paroo calcrete, *SMC* Sturt Meadows calcrete

9.2 Types of Environments

The plateau atop the Yilgarn craton in Western Australia, and the intracratonic Ngalia Basin in the Northern Territory, have been emergent from the sea since the end of the Proterozoic, 543 Mya. The aquifers of each area support a regionally characteristic stygofauna with non-marine affinities, the specific characteristics of which are distinctive between each aquifer (Humphreys 2001).

Such long emergent cratons usually lack major karst limestones within which to develop habitat appropriate for stygofauna, such as an interconnected network of suitably sized voids. However, within the Australian arid zone carbonate deposits are widespread, both as soil and groundwater calcretes (Arakel 1996) because they form in arid climates (Evaporation/Precipitation (E/P) >10; English et al. 2001) which, in the Deserts and Xeric Shrublands Ecoregion of Western Australia, has very high potential evaporation ($E > 3000$ mm per year; Mann and Horwitz 1979) and low annual precipitation ($P < 200$ mm).

These groundwater calcretes have formed in palaeodrainage channels due to solute concentration in the aquifer as a result of evaporation through the sediment surface as the groundwater flow approaches base level, typically a salt lake (playa) (Morgan 1993). As a result, in the Murchison and Gascoyne regions of Western Australia lying to the north of the Mulga-Eucalypt Line, where scrubland dominated by *Eucalyptus* concedes to *Acacia* (Department of the Environment 2014), the region is dotted with more than 210 major bodies of groundwater calcrete varying in extent from about 50–1000 km^2 as well as many smaller ones (Fig. 9.1) (Humphreys 2001).

9.3 Species Diversity and Morphology

More than 100 species of subterranean predaceous diving beetles have now been described from Australia, virtually all coming from the calcretes in the Yilgarn region of Western Australia and the Ngalia Basin of the Northern Territory (Watts and Humphreys 2004, 2006, 2009). In other regions of Australia, which are mostly devoid of calcrete deposits, and other regions globally (i.e., U.S.A., Europe, Africa south-east Asia, Miller and Bergsten 2016), stygobiotic beetles are mostly found in river/stream gravels and cave systems and usually as small numbers of species.

In the Australian calcretes there are two very diverse genera, *Limbodessus* Guignot (Bidessini) and *Paroster* Sharp (Hydroporini) (Fig. 9.2), known from 65 and 34 described species, respectively, and three less speciose genera; *Exocelina* Broun (2 species, Copelatinae), *Carabhydrus* Watts (1 species, Bidessini), and *Neobidessodes* Hendrich and Balke (2 species, Bidessini). However, only about one-third of the known calcretes have been surveyed, and so the true diversity of the fauna is undoubtedly much higher. In large part, the calcretes that have been studied are limited to those that have exploration bore holes drilled by mining companies or wells/bores used by pastoralists. These are the only way to access the calcrete aquifers and sample the fauna using a variety of techniques, such as plankton haul nets or pumps (Allford et al. 2008). Bore holes require specialist drill rigs that are expensive to hire, operate and relocate to remote areas such as the Yilgarn region. Hence, many of the calcretes that do not have existing bore holes will remain unsurveyed for the foreseeable future or until additional bores are drilled by resource companies.

The isolated nature of the calcretes has given rise to a particularly interesting phenomenon; many of the calcretes contain multiple species that are each other's closest relatives, i.e., sister pairs, more rarely as a triplet of species, and in one case, four closely related species. Within a single calcrete the adults of each species are different in size; for example, where there are three species present in an aquifer there is invariably a small, medium and large species (Fig. 9.2).

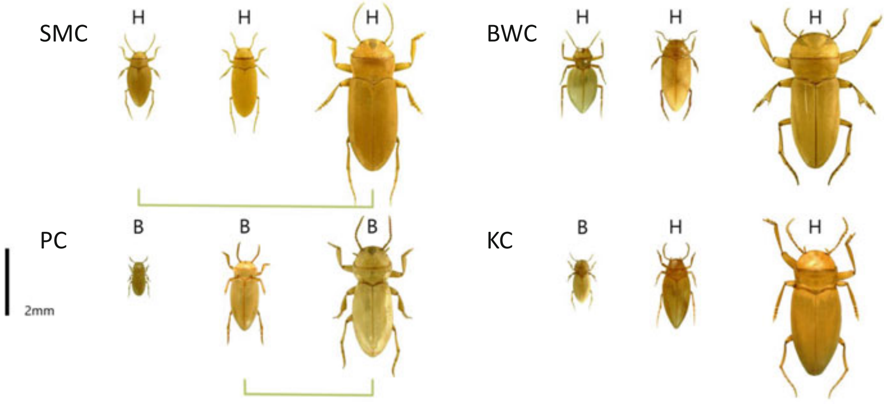

Fig. 9.2 Stygobiotic beetles from the Yilgarn calcretes showing the repeated pattern of size variation within calcretes and morphological variation among species. *SMC* Sturt Meadows calcrete, *Paroster microsturtensis*, *P. mesosturtensis*, *P. macrosturtensis*, *BWC* Byro West calcrete, *P. arachnoides*, *P. dingbatensis*, *P. byroensis*, *KC* Karalundi calcrete, *Limbodessus karalundiensis*, *P. skaphites*, *P. stegastos*, *PC* Paroo calcrete, *L. kurutjutu*, *L. pulpa*, *L. eberhardi*. H and B refer to the tribes Hydroporini and Bidessini respectively. Green lines connect the phylogenetic sister species. Images prepared by Chris Watts, Howard Hamon and Remko Leijs

All species display a reduction in characters typical of permanent inhabitants of a subterranean environment; loss of functional eyes, forewings, colour and thin or soft exoskeleton but, in addition, some display other modifications such as enlarged heads, reduced heads, enlarged prolegs, enclosing elytra (Fig. 9.2) and, in virtually all species, modifications to the shape of the male genitalia. This latter structure, particularly in the morphology of the central lobe in *Limbodessus* species, has evolved into a range of species-specific shapes (Watts and Humphreys 2009). This wealth of different morphologies in these stygobiotic species is in stark contrast to their epigean congeners that differ little among species in their hydrodynamic shape and aedeagal structure. These morphological changes have been observed in most if not all of the discrete calcrete aquifers sampled (Leys et al. 2003). It seems clear that whatever constrained morphological evolution in the surface species was removed, and body shape and appendages were free to evolve unhindered in numerous ways. The enlarged forelegs can be envisaged as an adaptation associated with prey capture, but the advantages of extreme head and aedeagal shapes is less obvious.

Some stygobiotic species from the Yilgarn calcretes show variable degrees of eye reduction, with some 2% of species retaining small, apparently non-functional eye remnants. In one case two sister species from the same calcrete have eyes reduced to about half normal size *Limbodessus microocula* (Watts and Humphreys) and *L. micrommatoion* (Watts and Humphreys), possibly resulting from a more recent entrapment underground (Leijs et al. 2012). The species *L. occidentalis* (Watts and Humphreys), which is found in surface streams as well as calcretes, and *Exocelina saltusholmesensis* Watts et al. from stream gravels near Darwin (Watts et al. 2016) have noticeably smaller than normal but seemingly functional eyes. It seems likely that in these cases the transition from surface to underground is still in progress.

The larval stages for a reasonable number of species are known (14 species of *Paroster*, 25 species of *Limbodessus* and one *Neobidessodes*) and, compared with epigean species, they appear to be less modified compared with adults in response to a stygobiotic lifestyle (Alarie et al. 2009; Michat et al. 2010, 2012). Like the adults, they have lost eyes (none are known to have only partially lost them) and all are depigmented. Otherwise, morphologically they resemble those of their surface congeners with the possible exception of a few species of *Paroster* and *Limbodessus* that have evolved enlarged, spoon-like rostrums which are more extreme than in any epigean species, possibly in response to the presumed preponderance of small copepods in their diet.

9.4 Biology

9.4.1 Life History

Surface dytiscids typically deposit their eggs beneath the water surface, either in or on the vegetation. However, the oviposition sites of subterranean species are so far unknown, although presumably they use firm substrate under the water surface. As for surface species, subterranean taxa have three larval instars (Michat et al. 2012). Epigean species pupate on land near water either under logs, stones or in purpose-built cells in the soil. Pupae of stygobitic species have not been reported but, as occurs in surface species, mature larvae are likely to pupate above the water in cells built in the sediment or naked in crevices in the limestone calcrete.

9.4.2 Feeding

The presence of numerous different-sized pairs and triplets of sympatric sister species (Leys et al. 2003) suggests that they may have evolved by an adaptive shift as a result of ecological-niche differentiation. However, distinct trophic niches have not yet been demonstrated for the sympatric sister beetles tested, although there is evidence their prey comprises both amphipods and copepods (Bradford et al. 2014; Saccò et al. 2019, 2020b).

A common paradigm of subterranean ecology is that subterranean animals (troglobionts) are food limited and have lower metabolic rates than their epigean relatives (Jones et al. 2019; Poulson and Lavoie 2000), although there are exceptions (Bishop et al. 2014; Culver and Poulson 1971). Intense investigation of the trophic dynamics within the calcrete aquifers, using amino acid stable isotope analysis employing $\delta^{13}C$ and $\delta^{15}N$, indicates that stygofauna have a tendency towards opportunistic and omnivorous habits, typical of an ecologically tolerant community (Saccò et al. 2017, 2019, 2020b).

9.4.3 Reproduction

In this arid region, groundwater community trends are closely linked with nutrient fluctuations in the aquifer that can be attributable to the episodic rainfall events typical of the region (Hyde et al. 2018; Sacco et al. 2019). Not unexpectedly, no distinct breeding season has been established for these subterranean beetles. Larvae have been collected in each month samples have been taken (March–November inclusive), although they are over-represented in June and July (winter) as a proportion of all beetles sampled, comprising 15 sampling occasions over 25 years (1992–2016).

9.5 Respiratory Physiology

Most adult epigean dytiscids use an air bubble, called an air store, underneath their elytra to supply O_2 for their dives underwater (Calosi et al. 2007; Ege 1915; Gilbert 1986). Oxygen within the air store can be augmented by O_2 diffusion from the water through a small bubble on the tip of the abdomen which acts as a gas gill (Calosi et al. 2007; Ege 1915; Gilbert 1986; Kehl 2014; Rahn and Paganelli 1968). However, early studies suggested that stygobiotic dytiscids may use cutaneous respiration, where respiratory gasses diffuse through the body surface. These assertions were supported by the lack of a gas gill, a small or non-existent air store, rich tracheation of the elytra, the ability to remain under water for long periods, and the likelihood of having low metabolic rates as in other subterranean species (Ordish 1976; Smrž 1981; Ueno 1957). Additionally, in subterranean species access to air-water interfaces may be limited making replenishment of air stores difficult (Jones et al. 2019).

A recent study shows that three species from the Western Australian calcrete aquifers do use cutaneous respiration (Jones et al. 2019). The study included two sister species from the Sturt Meadows aquifer, *Paroster macrosturtensis* (Watts and Humphreys) and *P. mesosturtensis* (Watts and Humphreys), and *Limbodessus palmulaoides* (Watts and Humphreys) from the separate and isolated Laverton Downs aquifer. Experiments showed that these beetles have an O_2-boundary layer surrounding their bodies and that they consume O_2 directly from the water. The O_2-boundary layer is the fluid layer above a respiratory surface that is deficient in O_2 and indicates O_2 diffusion into that surface. Additionally, these species have small air stores, rendering them slightly negatively buoyant. Unlike most epigean species, they can survive long periods of submergence and have never been observed to use a compressible gas gill (Jones et al. 2019).

The model of O_2-exchange for these species is that O_2 diffuses from the water down its partial pressure gradient through the boundary layer to the surface of the beetle (Jones et al. 2019). O_2 then diffuses through the cuticle where it can enter the tissue directly or the gas within the tracheal system, which in adults includes that under the elytra (Jones et al. 2019). Boundary layer resistance can be reduced by convection of the water above the beetle's surface, either through movement of the beetle or water. This allows for a wider metabolic scope (Jones et al. 2019). The cuticle provides the most significant resistance along the diffusion pathway and resistance is proportional to thickness. In *P. macrosturtensis*, *P. mesosturtensis* and *L. palmulaoides*, cuticle thicknesses are <10 μm, while some slightly larger (~2–3×) epigean species have ~30–40 μm thick cuticles (Jones et al. 2019). Mathematical modelling indicates that the smaller *P. mesosturtensis* (2.0 mm long) has a wider metabolic scope (>10×) than the larger *P. macrosturtensis* (4.0 mm) and *L. palmulaoides* (4.2 mm) where metabolic scope is calculated at 4–5×. Metabolic scope in this case is the factor by which metabolic rate increases above resting metabolism during activity such as swimming or crawling. Therefore, the larger species are more likely to encounter O_2-limitation, particularly if O_2-pressure levels drop within the aquifers. O_2-saturations of <50% have been recorded within the Sturt Meadows aquifer and other aquifers containing dytiscids (Jones et al. 2019; Watts and Humphreys 2006). At an O_2 partial pressure of 10 kPa (~50% saturation) in well-convected water, the metabolic scope of the two largest species declines to ~3× while in *P. mesosturtensis* ~7× (Jones et al. 2019). This highlights the necessity of cutaneously respiring animals to be small due to the unfavourable scaling of surface area for gas exchange and thickness of the cuticle relative to metabolic rate. Cutaneous exchange in dytiscids would be expected to scale with mass (Mb) with the exponent 0.32 ($Mb^{0.66–0.34}$), as cuticle thickness in dytiscids scales to $Mb^{0.34}$ (Jones et al. 2019), while surface area is expected to scale $Mb^{0.66}$. However, metabolic rate in resting insects scales $Mb^{0.82}$ (Chown et al. 2007), creating a large discrepancy between capacity for O_2 gain and demand with increasing size. All of the approximately 100 stygobiotic dytiscids described from Western Australia are <5 mm long (Balke et al. 2004; Watts and Humphreys 2009).

In the three stygobiotic dytiscids where O_2-consumption rate has been measured, metabolic rate is lower than resting insects in general, as well as in plastron breathing aquatic insects (Jones et al. 2019). Plastron breathers use a bubble, which can be maintained indefinitely, on the surfaces of their body to allow O_2 diffusion from the water (Seymour and Matthews 2013). Their low metabolic rates are associated with boundary layer resistance and these species are often in cool fast-flowing water that reduces metabolic demand and thins the boundary layer reducing resistance (Jones et al. 2017; Seymour et al. 2015; Seymour and Matthews 2013; Thorpe and Crisp 1947). The stygobiotic dytiscids have a further reduced metabolic rate associated with resistance of the cuticle (Jones et al. 2019). However, there are other possible explanations for their low metabolic rate. Low metabolic rates are found in stygobiotic isopods and amphipods exposed to low and variable O_2 levels (Hervant et al. 1998; Malard and Hervant 1999), and have been associated with low resource availability in subterranean environments (Hüppop 1985). Additionally, *P. macrosturtensis*, *P. mesosturtensis* and *L. palmulaoides* have reduced wings and cannot fly (Watts and Humphreys 2006), and insects that do not undertake high energy activities like flying have lower resting metabolisms than those that do (Reinhold 1999).

There is likely variation in the mode of respiration undertaken by stygobiotic dytiscids given the diversity of species, where respiration does not simply occur through the unelaborated body surface (Jones et al. 2019). Some epigean *Deronectes* Sharp species have spoon-shaped setae on their body surface, which act like tracheal gills, bringing gas within the tracheal system into close proximity to the water for exchange (Kehl and Dettner 2009). This reduces the diffusion distance through the cuticle to <1 µm (Kehl and Dettner 2009). These respiratory setae appear to occur in the stygobiotic genera *Kuschelydrus* Ordish and *Phreatodessus* Ordish from New Zealand and *Siettitia* Abeille de Perrin from France (Kehl 2014; Kehl and Dettner 2009). Although so far not observed in Australian stygobiotic dytiscids, an interstitial species, *Limbodessus rivulus* (Larson) from northern Australia does have setae which resemble those found in epigean species (Larson 1994). Additionally, there are pore-like structures on submergent tolerant epigean species which may have respiratory function (Madsen 2012). *Limbodessus cueensis* (Watts and Humphreys) and *L. magnificus* (Watts and Humphreys) from the Cue calcrete in Western Australia do have pore-like structures on their body surfaces that may have respiratory function (K. Jones, pers. obs., S. Kehl pers. comm. to WFH). However, further investigation is needed to determine if these structures do have a respiratory purpose.

Respiration in stygobiotic larvae has not been investigated, though they likely use cutaneous respiration given their small size and that smaller larvae and early instars of epigean species use this form of respiration (Miller and Bergsten 2016).

Given the extraordinary diversity of subterranean dytiscids it is possible that variations in respiratory mode do exist. This variation, along with variation in body size, could lead to differences in susceptibility of different species to respiratory limitation in their subterranean environments and is worthy of further investigation.

9.6 Speciation Underground

It is assumed that the majority of subterranean species, particularly within faunal groups that normally live in surface environments (e.g., insects, arachnids and vertebrates), evolved from surface ancestors that were pre-adapted to living underground. Under this scenario, two different modes of speciation have been invoked to explain the evolution of subterranean species: the Climatic Relict Hypothesis (CRH, Barr 1968, Barr and Holsinger 1985, Culver 1982, Sbordoni 1982) and the Adaptive Shift Hypothesis (ASH, Howarth 1986, 1987, Rouch and Danielopol 1987). The CRH is essentially an allopatric mode of speciation, whereby, following an initial colonisation event of the underground environment, speciation is completed following the extinction of colonising surface populations via climatic changes (e.g., by aridification or glaciation events). The ASH represents the case where speciation proceeds, driven by divergent selection (i.e., ecological speciation), and is completed despite the potential for introgression with parapatric surface populations.

These two hypotheses were first explored for the Australian stygobiotic dytiscids using phylogenetic and molecular clock analyses to date the likely time-point that subterranean species evolved (Leys et al. 2003). These analyses revealed that the majority of stygobiotic species evolved independently from surface ancestors, based on the observation that the closest relatives of stygobiotic species within a calcrete were often 100 s of kms away and in different palaeodrainages. Although phylogenetic analyses of these beetles revealed the presence of large monophyletic groups of stygobiotic dytiscids, the lack of a clear geographic pattern to the relationships among many of the species suggested that their ancestors were capable of flight and vision. These features are absent in most stygobiotic dytiscids, but evident in several close microphthalmic relatives of stygobiotic dytiscids (e.g., microphthalmic *L. rivulus* is closely related to stygobiotic *L. cueensis* and *L. magnificus*). Molecular clock analyses further suggested that the emergence of subterranean lineages occurred following the development of aridity on the Australian continent during the Pliocene (Byrne et al. 2008; Leys et al. 2003; Leijs et al. 2012; Sniderman et al. 2016). Under this climatic scenario, many of the surface ancestors went extinct, with the subterranean species surviving within the calcrete aquifers as the water table dropped below ground level. Overall, the analyses by Leys et al. (2003) supported the CRH for the evolution of the majority of stygobiotic dytiscid species from surface ancestors.

As mentioned above, an intriguing pattern that emerged from the phylogenetic analyses was the presence of sympatric pairs and triplets of sister species, with each species of the pair or triplet in distinct size classes (e.g., small species ~1.0 mm, large species ~5.0 mm, see Fig. 9.2), suggesting that they may have evolved from a stygobiotic common ancestor via speciation underground (Cooper et al. 2002; Leijs et al. 2012; Leys et al. 2003). Several additional phylogenetic sister species were found in adjacent/nearby calcretes along the same palaeodrainage system, further suggesting the potential for their evolution from a stygobiotic ancestor. This form of speciation from stygobiotic or troglobiotic ancestors has been referred to recently as "subterranean speciation" (Langille et al. 2021). It appears to be common for several ancient crustacean groups (e.g., Bathynellaceans and Remipedia, Camacho et al. 2020, Hoenemann et al. 2013), but is not thought to be a common form of speciation in insects. An alternative explanation, however, is that the species evolved following repeated colonisation of the calcrete by the same surface ancestor. Mathematical models of the speciation process, incorporating a variety of colonisation parameters and ancestral size pools, were developed by Leijs et al. (2012) who showed that repeated colonisation events from the same ancestral surface species were unlikely to explain the evolution of the 11+ known cases of sympatric pairs and triplets of stygobiotic sister species.

Confirmation of the role of subterranean speciation in the evolution of these sympatric sister species and additional sister species from adjacent calcretes was recently provided using a novel approach based on studying genes involved in vision that were evolving under purifying selection in surface species, but under relaxed selection in the stygobiotic beetles (Langille et al. 2021, 2022). Comparative analyses of the long-wavelength opsin (*lwop*) and arrestin (*arr1* and *arr2*) genes that encode proteins involved in the light detection cascade, revealed shared deleterious mutations (i.e., frameshift mutations or nonsense mutations leading to stop codons in the encoded protein) for a sympatric sister triplet of stygobiotic species and several additional phylogenetic sister species. These shared deleterious mutations suggest their ancestors were most likely stygobionts living underground within the calcretes or interstitial habitat linking the calcrete bodies along palaeodrainages. Overall, the study provided strong evidence for the role of subterranean speciation in the evolution of at least 11 stygobiotic *Paroster* species, out of a total of 28 stygobiotic members of the genus studied to date. Further analyses of *Limbodessus* species are continuing, but it is likely that the role of subterranean speciation in the evolution of the stygobiotic dytiscids has been significantly under-estimated.

In three cases, speciation is likely to have occurred within the confines of a single calcrete body raising the possibility of ecological speciation with gene flow, assuming the distinct size classes of the beetles reflect ecological niche differences. Key mechanisms of sympatric speciation have been outlined in the literature, i.e., trophic niche partitioning (Lu and Bernatchez 1999), prey/host shifting (Rice and Salt 1990), and assortative mating (Erlandsson and Rolán-Alvarez 1998). Interestingly, recent research has identified that stygofaunal niches are closely linked to the hydrodynamic conditions influenced by different rainfall regimes (Saccò et al. 2020b). The ecological variation of food preferences among different species associated with rainfall may be a driver of trophic niche partitioning, especially in larval stages of dytiscids (Saccò et al. 2020c). However, the considerable heterogeneity of calcrete bodies (e.g. variation in thickness and hydrological connectivity), and previous evidence for congruent patterns of genetic sub-structuring in beetles (Guzik et al. 2009, 2011, see below) and other taxa (amphipods, Bradford et al. 2013, isopods, Guzik et al. 2011) makes it difficult to rule out a role of allopatric isolation during their speciation, though periods of restricted gene flow were likely to have been short. Below we further discuss the results of these population genetic and phylogeographic analyses of the stygobiotic beetles within calcrete aquifers and consider the evidence for micro-allopatric speciation.

9.7 Intra-Specific Phylogeography and Population Structure

Prior to 2010, clear evidence of in situ diversification and fragmentation within calcrete aquifer systems was yet detected, but a study by Guzik et al. (2009) investigated population genetic and phylogeographic structure amongst the three sympatric sister species, *P. macrosturtensis*, *P. mesosturtensis* and *P. microsturtensis* (Leys and Watts 2008; Watts and Humphreys 2006) from the

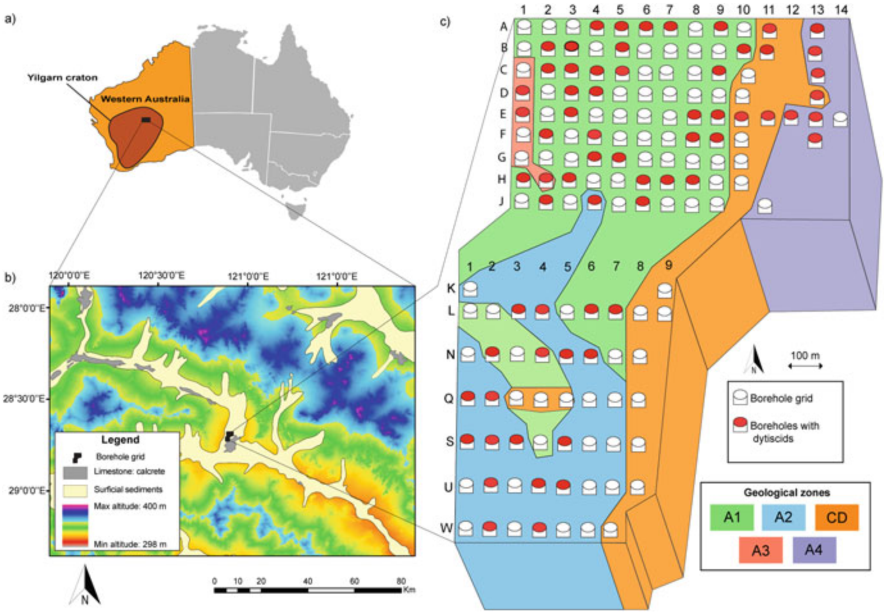

Fig. 9.3 Sturt Meadow calcrete: (**a**) location in the Yilgarn region of Western Australia; (**b**) elevation map showing the surficial sediments and the calcretes nearby the bore hole grid, and (**c**) grid map of the bore holes with those in red indicating locations where dytiscids have been sampled (five geological zones are colour-coded) (after Saccò et al. 2020a)

Sturt Meadows calcrete (SMC). This location provided a unique opportunity for these investigations as it has over 100 mineral exploration bores laid out in a grid pattern across 2.3 km^2 of the calcrete (Fig. 9.3). The aim of Guzik et al. (2009) was to assess the possibility of a shared historical diversifying event by testing for signatures of population fragmentation in the three species using mitochondrial DNA (mtDNA) sequence data. The aquifer was shown to maintain thriving beetle populations with high genetic diversity and potential for intra-calcrete diversification, despite a very small sampling area. There was spatial heterogeneity in the distribution of genetic variation, with some evidence of localised habitation by the beetles, leading to an isolation by distance pattern for *P. mesosturtensis* and *P. macrosturtensis,* but not for the smallest species, *P. microsturtensis*. Similar results were found in three amphipod species from the same calcrete, with some further evidence for phylogeographic structure in one of them (Bradford et al. 2013).

In a later study, Guzik et al. (2011) obtained access to a substantially larger sampling region at the Laverton Downs calcrete (LDC) (13+ km compared to the 3.5 km at SMC) which lies 100 km north-east of SMC within a different palaeodrainage channel. As in the previous SMC study, a comparative approach was used, employing three beetle species, *Limbodessus lapostaae* (Watts and Humphreys), *L. windarraensis* (Watts and Humphreys) and *L. palmulaoides*, which vary in size from largest to smallest (4.2 mm, 2.2 mm and 1.3 mm),

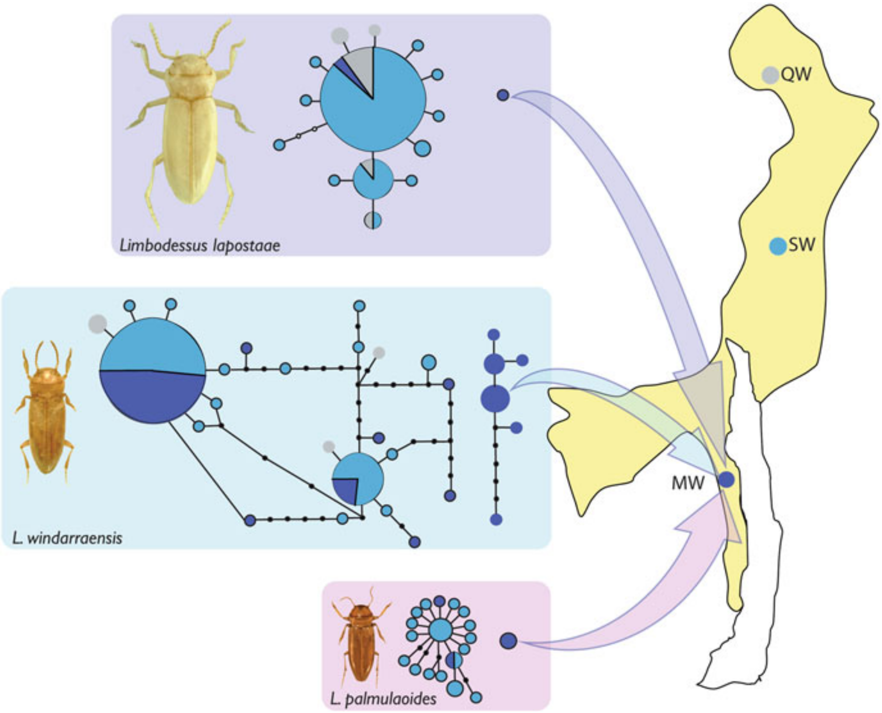

Fig. 9.4 Schematic diagram of Laverton Downs calcrete (~30 km long) (yellow), the adjacent salt lake (white), and relative sampling locations (Mount Windarra (MW)—dark blue dot, Shady Well (SW)—light blue dot, and Quandong Well (QW)—grey dot) for three species of dytiscid diving beetle (*Limbodessus lapostaae*—purple background, *L. windarraensis*—pale blue background, and *L. palmulaoides*—pink background) and their corresponding mitochondrial haplotype networks (inset). Each haplotype is represented by a sphere within each network, with the size of each sphere representative of the relative number of individuals sharing that haplotype. Each haplotype also shows the proportion of individuals sampled at each location. Black lines within networks represent a single base difference between two haplotypes and the smallest black circles represent missing haplotypes. Large shaded arrows emphasise that each of the three species found at Laverton Downs calcrete revealed individuals with divergent but related MW-only haplotypes and were considered to represent micro-allopatric speciation [after Guzik et al. (2011)]. Photos of dytiscid beetles by Chris Watts

respectively, and have probably evolved from different ancestral species (Leijs et al. 2012; Leys et al. 2003; Leys and Watts 2008). At LDC, all three stygobiotic dytiscid species each maintained two genetically distinct clades of haplotypes (mtDNA sequence variants) that showed up to 5% divergence (Fig. 9.4). All the individuals that shared such 'divergent' haplotypes were sampled from the southern-most Mount Windarra (MW) bores (i.e., MW-only haplotypes). In contrast, other individuals sampled from this location shared haplotypes with individuals sampled at the distant northern bores, Shady Well (SW, ~11 km from MW) and Quandong Well

(QW, ~16 km from MW). The processes that led to this pattern were inferred to be two-fold as follows:

1. *Isolation-by-distance within an aquifer*: On first inspection, a model of isolation-by-distance, in which individuals from nearby bores were more closely related to each other than to distant bores, was plausible. Isolation-by-distance was cited as a key mechanism of diversification at SMC (Guzik et al. 2009). A Mantel test showed that while there was evidence of isolation-by-distance between SW and MW, likely caused by the high divergence of MW-only haplotypes, there was no evidence of isolation-by-distance between QW and SW, the latter two sites being 5 km from each other. This finding indicated that such distances were not necessarily a major barrier to dispersal and unlikely to be the cause of the observed divergences. Instead, historical population fragmentation through a vicariant event seemed likely.
2. *Population fragmentation within a calcrete aquifer*: A population genetic signature of fragmentation is supported if there is a shared pattern of phylogeographic structure among taxa, i.e., there are geographically restricted and divergent haplotype lineages or clades with no evidence of shared haplotypes (i.e., reciprocal monophyly). However, in the initial stages of population fragmentation, there may be evidence for paraphyly, where one divergent clade is geographically restricted and a second clade is found in individuals from both populations. Interestingly, it was the latter pattern that was found in all three beetle species at LDC, with the presence of MW-only haplotypes in some individuals of each species and an additional suite of haplotypes shared among other individuals sampled at MW and the northern SW and QW bores (Fig. 9.4). An alternative hypothesis of unidirectional gene flow from north to south, as indicated by a presence of shared haplotypes between SW and MW and an absence of MW-only haplotypes in northern sites, was also considered (Guzik et al. 2011). However, the presence of mechanisms of unidirectional gene flow (e.g., resulting from fast currents), in the strict sense, seemed limited in this system. Instead, the study supported a scenario where a physical barrier may have existed close to the MW region so that individuals from northern sites were physically close to the southern populations but were kept separate. The source of a barrier to dispersal is difficult to identify due to a lack of knowledge of the internal calcrete structure now and in the past. Based on current knowledge of aquifer physicochemistry and structure, it is possible that both water level changes and chemistry associated with a nearby salt lake could be two of many sources of physical isolation to the macro-invertebrate fauna within the aquifer.

Salt lakes are thought to be a source of salinisation for proximate groundwater habitats, resulting in subterranean estuaries (Humphreys et al. 2009). Strong saline stratification and gradients have been recorded vertically in a number of calcrete aquifers (e.g., Watts and Humphreys 2009) and horizontally in a few (Humphreys et al. 2009; Mann and Deutscher 1978). Salinity gradients were certainly a plausible source of isolation at the LDC. Individuals living close to the salt lake that exists alongside the MW site (Fig. 9.4) may have been isolated on either side of a salinity cline, leading to population isolation, and resulting in the fixation of certain haplotypes in each of the geographic regions and subsequent micro-allopatric speciation. The cause of such a change in salinity may have potentially been climate change events resulting from cycles of aridification of the region during the Pleistocene (Byrne et al. 2008).

There are clearly multiple layers to the evolution of stygobiotic diving beetles within calcrete aquifers in the Yilgarn, first at the stage of colonisation of the aquifers and subsequently in situ, where genetic diversity, adaptation and selection pressures have influenced speciation. The inference of micro-allopatric speciation using population genetic and phylogeographic studies by Guzik et al. (2011) has highlighted the possible impact of aquifer hydrogeology and salinity gradients on the isolation of populations, but it also remains plausible for ecological speciation with gene flow to have occurred in this region. The use of genetics as a proxy for elucidating possible origins of speciation in the Yilgarn calcretes has been extremely important. As demonstrated by Langille et al. (2021), in the future, genomics methods will help tie together variation of traits with genetic variation to provide a deeper understanding of speciation.

9.8 Regressive/Adaptive Evolution

The discovery that 50+ individual calcretes contained unique stygobiotic species (Watts and Humphreys 2009 and references therein), most of which had evolved independently from surface species, has offered great potential for the system to be used to explore the regressive (e.g., loss of eyes, wings, and pigment) and adaptive (e.g., respiration, metabolism) changes to the genome that accompany evolution underground (Tierney et al. 2018). In particular, the evolutionary processes that lead to the loss of eyes in subterranean animals have been of considerable debate, with many researchers advocating the role of natural selection (direct selection via energy conservation (e.g., Moran et al. 2015) or indirect selection via antagonistic pleiotropy: selection on constructive traits driving the evolutionary loss of traits; Jeffery 2005) in the loss of eyes in cave animals, while others (e.g., Wilkens 2020) support the role of neutral evolution and genetic drift due to disuse of the character. Although the dytiscid system is limited to date by an inability to conduct breeding experiments, the ancient age of most of the stygobiotic lineages (3–8 Mya) provides sufficient time for mutations to accumulate in genes and become fixed in species, revealing the evolutionary forces that are operating.

One of the first such studies explored the evolution of a gene (*cinnabar*) involved in eye pigmentation in insects (Leys et al. 2005). This study found evidence for loss of function mutations (e.g., insertions/deletions leading to frameshift mutations) and elevated rates of amino acid evolution in the cinnabar gene, suggesting that it was likely to be evolving under neutral processes (i.e., without purifying selection) in the stygobiotic beetles. This research was extended by comparative analyses of the transcriptome of surface and stygobiotic species, focusing on the evolution of opsin genes, a series of phototransduction genes encoding key proteins of the light detection cascade (Tierney et al. 2015). Transcripts were detected for UV (*uvop*), long-wavelength (*lwop*) and ciliary-type (*c-opsin*) opsin genes in two surface beetle species (*Paroster nigroadumbratus* (Clark) and *Allodessus bistrigatus* (Clark)), but the three stygobiotic species showed no evidence of transcription of these genes (Tierney et al. 2015; NB. evidence for transcription of a functional *lwop* protein in the stygobiont *L. palmulaoides* was later shown to be a contaminant; Cooper et al. unpublished analyses). The loss of transcription of the opsin genes provided evidence for neutral evolution of genes that are specific to eye function. Confirmation of this hypothesis was also provided in recent analyses of genomic sequence data from the three opsin genes (and additional photo transduction genes—see above) in stygobiotic dytiscid species (Langille 2019, 2022). These analyses revealed the presence of numerous independent deleterious mutations (frameshifts and stop codons) in *uvop, lwop* and *c-opsin* of subterranean species compared to surface species where each of the genes was intact. These analyses showcase the role of neutral evolutionary processes in the loss of vision by subterranean animals, in further support of the neutral theory of regressive evolution. They also highlight how the stygobiotic dytiscids of the Yilgarn calcretes are in the 'Goldilocks zone' of neutral mutation, where species have evolved in the dark over a sufficient time period to allow mutations to accumulate in neutrally-evolving genes, but not too much time has passed so that the genes have largely disappeared and are no longer detectable in the genomes.

9.9 Conservation Considerations

The restricted distribution of stygobiotic dytiscid species within individual calcrete aquifers, many of which range in size from a few km^2 (e.g. a calcrete harbouring the sister species *Limbodessus melitaensis* Watts and Humphreys and *L. micromelitaensis* Watts and Humphreys of approximately 2.5 km^2) to 100 s of km^2 (Three Rivers calcrete: ~240 km^2) means that many species would be classified as short-range endemics (SREs) under criteria specified by Harvey (2002, distribution $<$10,000 km^2) or Eberhard et al. (2009, distribution $<$1000 km^2). Furthermore, many species could be considered ultra short-range endemics (uSREs; Guzik et al. 2019), with distributions $<$100 km^2, given that the average size of calcretes is only 90.8 km^2 (Harvey et al. 2011). These restricted distributions make the species very vulnerable to habitat disturbances (e.g., removal of calcrete as part of mining operations) or impacts on the volume of groundwater. The region of Western Australia where most stygobiotic dytiscids are located is significant for its mineral resources, including gold, nickel and uranium, and these industries often mine the calcrete to extract resources directly (e.g., uranium), or to neutralise acids that are used during mineral extraction processes, or for road building in the region. Groundwater is also heavily utilised by the resource industry, pastoralists and towns, and while this is often extracted from deep in palaeovalleys, it is, nevertheless, hydrologically connected to groundwater near the surface in shallow calcrete deposits (Arakel et al. 1990), potentially enhancing the rate of drawdown over time. Although Western Australian Government legislation under the *Wildlife Conservation Act 1950* and *Environmental Protection Act 1986* is designed to stop the extinction of stygofaunal species, the state government can overturn recommendations by the Environment Protection Agency (e.g., as in the approval of a uranium mine located in a calcrete at Yeelirrie Station in 2017 that would most likely cause the extinction of stygofaunal species). These decisions can be made despite the listing of 77 calcrete faunas as 'priority communities' by the Western Australian Department of Biodiversity, Conservation and Attractions.

Stygobiotic dytiscids are also likely to be impacted by future climate change despite their long-term survival in calcretes over millennia through multiple ice age cycles since the Pliocene, which cycled the landscape through arid and wet conditions (Byrne et al. 2008). Recent physiological studies have shown that stygobiotic dytiscid species have a lower maximum temperature threshold compared to related surface dytiscids, but are still able to survive temperatures of up to about 35–36 degrees C (Jones et al. unpublished data). Predictions of reduced rainfall events and prolonged droughts in southern Australia (see https://www.csiro.au/en/Research/) may potentially lead to the lowering of the groundwater table below the level of the calcrete, causing extinction of all species within the calcrete. The lowering of the water table will be exacerbated by the removal of significant amounts of groundwater within palaeovalleys or by creating quarries below the level of the water table, where groundwater evaporation is significantly enhanced and causes salination. The stygobiotic dytiscids and associated stygofaunal communities may potentially survive in refugia located in thick deposits of calcrete (>10 m), suggesting that such calcrete habitat should be preserved as a priority.

Overall, there is a need to monitor these subterranean environments on a continuing basis to ensure there is sufficient water and appropriate conditions to maintain groundwater ecosystems. Long-term ecological studies are especially important to document and understand how these ecosystems are being impacted in the future, while baseline information is also needed to understand the natural trends in these ecosystems. With this in mind, significant ecological research has recently been carried out at the Sturt Meadows calcrete that harbours a sister triplet of stygobiotic dytiscids (see above), where the groundwater is accessible via ~100 bore holes arranged in a grid over an area of calcrete spanning ~2.3 km^2 (Fig. 9.3), making it an ideal study system for long-term ecological monitoring in a region that is not yet impacted by mining activities. This research has included studies of the ecosystem dynamics and impact of rainfall events (Hyde et al. 2018; Saccò et al. 2020a, 2021), food webs and trophic positions of the beetles (Bradford et al. 2014; Saccò et al. 2020b), and energy flows (Saccò et al. 2020c), thus enhancing our understanding of how natural climatic changes may impact the dynamics of the groundwater ecosystem.

9.10 Future Work

Given that only about one-third of the more than 210 major calcretes in the Yilgarn have been surveyed to date, there is undoubtedly a considerable diversity of new subterranean dytiscid species yet to be discovered. Unfortunately, access into many of the calcretes is limited by the availability of bore holes or wells (see above), but the expansion of the resources industry into remote locations has the potential to open up new opportunities for access in the future via mineral exploration boreholes. The collection and analysis of additional species would provide an extraordinary opportunity to further explore the biogeographic history of the Australian arid zone and past connections across the landscape. The colonisation history of the beetles and other stygofaunal groups provides a window into past climates, analogous to fossils, but it is only by building up an extensive dataset of the fauna and their phylogenetic relationships that confidence can be gained in their inferred biogeographic history.

The island-like nature of the calcretes and independent evolution of many of the dytiscid species offers an unparalleled opportunity for comparative studies of both the evolution and biology of cave animals (Tierney et al. 2018), allowing investigations of some of the fundamental questions in subterranean biology (Mammola et al. 2020). These include questions associated with the adaptations, origins and evolution of cave animals, and further investigation of the fundamental changes that occur in the genetic architecture of species associated with life in permanent darkness in groundwater habitats. Many aspects of the biology of the beetles is still unknown, such as what are the evolutionary and ecological drivers that led to the size variation of sympatric species and ecological speciation, and what are the potential exaptations that have enabled the surface dytiscids to independently colonise the calcrete aquifers multiple times? The island-like nature of the calcretes and comparative power of the dytiscid system is sure to provide some wonderful research opportunities in the future.

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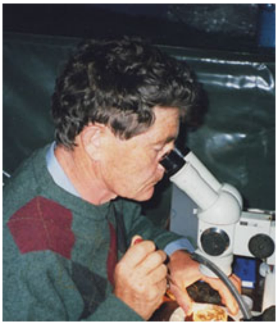
Andrew Austin is Professor Emeritus at the University of Adelaide and has long-standing research interests in the systematics of parasitic wasps and spiders, and the diversity and evolution of subterranean invertebrates.

Michelle Guzik has a Ph.D. in evolutionary biology from James Cook University, Australia. Since then she has applied genetic techniques to understanding the 'dark' biodiversity of subterranean habitats, and the evolution and phylogeography of subterranean dytiscids. Currently she is developing novel genomic approaches for single-species and community detection in groundwater ecosystems.

Karl Jones undertook an honours degree in science at the University of Adelaide and recently completed a Ph.D. investigating gas exchange, respiration, and dive behaviour in aquatic insects. The focus of many of these studies included dytiscids, both surface dwelling and subterranean.

William Humphreys has a B.Sc. Hons in Zoology (University of Wales) and a Ph.D. in ecology (Australian National University). He variously studied the ecology of coral reefs, spiders, and small mammals before specialising in the biology of subterranean animals, both troglofauna and stygofauna at the Western Australian Museum.



Chris Watts is an Honorary Researcher at the South Australian Museum, and published his first paper on Dytiscidae in 1963. He has worked with aquatic beetles since then including describing, mostly with Bill Humphries, over 100 new species of stygobitic Dytiscidae.

Steven J. B. Cooper is a research scientist at the South Australian Museum and an affiliated professor at the University of Adelaide, who uses molecular analyses to explore the diversity and evolution of the Australian fauna. He has a strong focus on subterranean animals, and a long-term interest in the evolution of subterranean dytiscid beetles from the calcrete aquifers of Western Australia.

Chapter 10
Habitats Supporting Dytiscid Life

Margherita Gioria and John Feehan

'No one can hunt long for water-insects without coming across the rapacious Dytiscus'.
Louis Miall 1903

Abstract Predaceous diving beetles (Dytiscidae) are a highly speciose group of insects occurring in a large variety of habitat types, where they often form multispecies assemblages, due to their high diversity and large variation in the degree of habitat specificity. While most species have broad habitat preferences, some are specialized for life under extreme habitat conditions. In this chapter, we provide an overview of the main habitats in which dytiscids occur and summarize some of the habitat variables that contribute most to shaping the distribution of dytiscids across habitats and landscapes. These include a range of abiotic conditions and plant–beetle relationships, which act as major habitat selection factors. We discuss how a variety of habitats in agricultural and urban landscapes can contribute to maintain high dytiscid diversity. We then describe some of the most peculiar habitats where dytiscids occur, including phytotelmata, subterranean and interstitial habitats, rock pools, and terrestrial habitats. Over the past couple of decades, examination of habitats that had been typically underexplored for dytiscids has led to the discovery of new species and even new genera. These studies suggest that further exploration of these habitats and the increasing availability of phylogenetic data will provide important insights into the ecology and evolutionary history of species colonizing extreme habitats. This is in turn critical to improve our understanding of the vulnerability of dytiscids to global environmental changes associated with changes in habitat characteristics and availability.

M. Gioria (✉)
Department of Invasion Ecology, Czech Academy of Sciences, Institute of Botany, Průhonice, Czech Republic
e-mail: margherita.gioria@ibot.cas.cz

J. Feehan
UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland

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10.1 Introduction

Predaceous diving beetles are a highly diverse group of insects, with more than 4600 species being described worldwide (Nilsson and Hájek 2022) from a wide variety of habitats (Ranta 1982; Foster et al. 1992; Larson et al. 2000; Miller and Bergsten 2016). Yet, they vary greatly in the degree of habitat specificity (Fairchild et al. 2000; Valladares et al. 2002). While most species are regarded as habitat generalists and are widely distributed and abundant in commonly distributed habitat types (Foster et al. 1992; Larson et al. 2000), some can be regarded as 'super specialists' (Ribera 2008) and have evolved adaptations to extreme habitat conditions, such as groundwater obligates (Leys et al. 2003; Watts and Humphreys 2006; Leys and Watts 2008), acidophilic (Alarie and Leclair 1988; Hendrich 2001; Shatarnova 2021) or halophilic species (Jäch and Margalit 1987; Bailey et al. 2002), or those adapted to life in phytotelmata (i.e., pools of water within plants, Kitching 2000; Balke et al. 2008; Campos and Fernández 2011). High species diversity and variability in the degree of habitat specificity, combined with the fact that many dytiscids are active dispersers and excellent flyers (Bilton 1994; reviewed in Chap. 11), result in the formation of multispecies assemblages in virtually any habitat type (Larson et al. 2000; reviewed in Chap. 7). These range from running to stagnant waters, freshwater and hypersaline habitats, oligotrophic and eutrophic waters, large lakes and rock pools, rivers and ditches, drinking fountains and stone wells, mires and mosses, bromeliads and tree holes, rain pools and leaf litter in forest floor depressions (Fig. 10.1), subterranean and hygropetric habitats, and a small number of species have even been recorded from terrestrial habitats (e.g., Nilsson 1986; Larson 1997a; Nilsson and Holmen 1995; Foster et al. 1992; Leys et al. 2003; Foster 2010; Post 2010; Fery 2020). In many habitats, especially those characterized by extremely harsh or highly unstable conditions, dytiscids often represent the most diverse or abundant Coleoptera or insect group (e.g., Eyre et al. 1986; Foster et al. 1992; Painter 1999; Fairchild et al. 2003; Gioria et al. 2010a; Pakulnicka et al. 2016a, b; Rolke et al. 2018), including rock pools (Ranta 1985), hot springs (Mason 1939), and bog pools (Downie et al. 1998).

Differences in habitat specificity and in dispersal ability are reflected in large differences in the conservation status of individual species (e.g., Nilsson and Holmen 1995; Larson et al. 2000; Foster 2010; Foster and Bilton 2014). Knowledge of the habitat requirements of individual species and of the frequency, distribution, and conservation value of different habitat types throughout the landscape matrix is key to the conservation of this group. This is especially true for species restricted to uncommon or rare habitats, many of which are threatened by global environmental and socio-economic changes (Foster et al. 1992; Foster 2010; Bilton et al. 2019; reviewed in Chap. 11). In this chapter, we describe some of the major habitat selection factors for dytiscids, including abiotic and physical variables as well as

Fig. 10.1 Wet forest floor lowland secondary forest near Balikpapan (Indonesia, E Kalimantan). Many dytiscid species were recorded among decaying leaves, especially Copelatinae (*Copelatus* and *Lacconectus*) and some *Laccophilus* species (Photo by Jiří Hájek)

biotic interactions that ultimately determine habitat suitability. We describe some of the most peculiar habitats where dytiscids have been collected from, including phytotelmata, subterranean and interstitial habitats, rock pools, and terrestrial and semi-terrestrial habitats. Finally, we discuss some of the major challenges and opportunities in the field. Given that the exploration of traditionally underexplored habitats has allowed identifying new species and even new genera, future explorations and phylogenetic studies will better our understanding of the factors driving both past and current habitat preferences for many species and will likely result in new species being described.

Throughout this chapter, we provide some habitat classifications that have been produced to classify habitats inhabited by water beetles. We refer to ‘habitat’ as that suite of biotic, abiotic, and physical conditions that are suitable for dytiscid life, at least at some stages of their life cycle, using the characterization of ‘habitat within a waterbody’ *versus* that of habitat coinciding with a waterbody (ecosystem). This allows to reconcile the presence of lentic species in lotic ecosystems that provide habitats resembling lentic conditions and vice versa (Larson 1997b), although both characterizations are found in the literature. Large waterbodies such as lakes, ponds, and rivers may in fact support multiple habitat types and often display high habitat heterogeneity (Harper et al. 1997). The distinction between habitat and waterbody or ecosystem is not only important to better classify individual species based on their habitat requirements, but also to ensure the protection of specific habitats that play a key role in supporting rare or uncommon species and maintain habitat connectivity within the landscape matrix. Unless otherwise indicated, we describe the habitats preferences of adults.

10.2 Habitat Requirements and the Importance of Habitat Classifications

The habitat requirements of dytiscids are a function of morphological and physiological traits, swimming and hunting strategies, predation, and food availability (Ribera and Nilsson 1995; Leys et al. 2003; McAbendroth et al. 2005; Yee 2010; Sánchez-Fernández et al. 2012; Pitcher and Yee 2014). The geographical range of individual species and how they use and disperse between ecosystems and habitats within the landscape matrix depend on local environmental conditions as well as on a range of landscape variables such as climate, landform, and landscape history and use (Gray 1981; Williams 1983; Foster et al. 1990; Kholin and Nilsson 1998; Bosi 2001; Leys et al. 2003; Fenoglio et al. 2006; Foster 2010; Gioria et al. 2010a; Pakulnicka et al. 2016a, b; Enkhnasan and Boldgiv 2019). Ultimately, landscape complexity (Newman et al. 2019) is a function of variables such as the type and frequency of habitats present in a landscape, the degree of habitat connectivity (*versus* habitat isolation), and habitat dynamics (e.g., rates of habitat formation and disappearance), determine population dynamics and rates of emigration, immigration, extinction, and speciation (e.g., Hanski 1999; Ribera and Vogler 2000; Ribera et al. 2001, 2003a; Vamosi et al. 2007; Roth et al. 2020). For dytiscids, as for other groups, the importance of landscape variables in shaping species' distributions and community dynamics will depend on their dispersal ability and their preference for lentic *versus* lotic habitats (Ribera et al. 2003a). In regions characterized by high landscape complexity and habitat diversity, including 'extreme habitats', dytiscid diversity is generally high (Picazo et al. 2010; Enkhnasan and Boldgiv 2019).

Knowledge of the past and present distribution of dytiscids across landscapes and habitats is key to identifying the main drivers of habitat preferences and their vulnerability to local and regional environmental changes. Characterizing and classifying the habitats where dytiscids occur and identifying the habitat variables that define the fundamental and realized niche of individual species represents an important step towards acquiring such knowledge. Habitat classifications are useful to identify the habitats that need to be conserved to protect dytiscids and promote the maintenance or enhancement of habitat connectivity. A standard habitat classification applicable to all dytiscids globally would allow making biogeographical comparisons of the importance of specific habitats in supporting dytiscids and how dytiscids respond to different environmental conditions. Yet, predicting the distribution of a species based on broad habitat classifications is hampered by several factors. Any habitat is in fact defined by a variety of unique local and regional conditions that interact with each other in complex ways. The effects of abiotic conditions are in turn confounded by biotic interactions such as predation, inter- and intra-specific competition, the availability and quality of food, and the structure of the vegetation (Wellborn et al. 1996; Lundkvist et al. 2003; Vamosi and Vamosi 2007; Vamosi et al. 2007; Gioria et al. 2010a; Yee 2010, 2014; Liao et al. 2020). Moreover, the distribution of dytiscids has often shown strong nested patterns at the regional level, with small waterbodies often supporting a subset of species that are found in larger ones (Nilsson and Svensson 1995; Kholin and Nilsson 1998; Baber et al. 2004; Florencio et al. 2014). Complex interactions among landscape and habitat variables, resulting in potential nesting effects, coupled with broad habitat preferences for many species (Larson et al. 2000), make it difficult to evaluate the relative importance of individual habitat variables in defining the habitat preferences of different species (e.g., Larson 1997b; Gioria et al. 2010b). This is reflected in the fact that many species have been observed in contrasting habitats (e.g., Nilsson and Holmen 1995; Larson 1997a; Larson et al. 2000; Foster 2010). This is true for eurytopic species, which are tolerant of broad environmental ranges and have a wide distribution (Larson et al. 2000). Moreover, some species occupy different habitats along elevational or latitudinal gradients (e.g., Nilsson and Holmen 1995; Larson et al. 2000; Foster 2010). This is the case of *Cybister lateralimarginalis* (De Geer), a species that in Britain is typically found among the vegetation at the margins of lakes or in calcareous ponds, while it often occurs in acid bog lakes and peat ponds in Scandinavia (Nilsson and Holmen 1995). Or *Hygrotus quinquelineatus* (Zetterstedt), which is mainly associated with temporary, flooded areas in Fennoscandia and Denmark (Nilsson and Holmen 1995) and with turloughs in Ireland (i.e., calcareous temporary wetlands; Foster et al. 1992), where it is considered a moss dweller, however, it is typically associated with reed-beds elsewhere in Europe (Foster et al. 2009). Another example is represented by *Dytiscus lapponicus* Gyllenhal, which is found at high elevations in south and central Scotland, but mostly at sea level in northern Scotland (Balfour-Browne 1962; Downie et al. 1998; Littlewood 2017).

Dispersal affects our understanding of the habitat preferences of active dispersers, especially when it is not possible to study a species' behaviour throughout its life cycle (Bilton 2014; Chap. 11). Adults of many species often migrate to habitats characterized by suboptimal conditions for part of their life cycle, leading to random colonization events (Bilton 2014). This might be driven by seasonal variation in habitat hydrology and permanency, such as floods (Gray 1981) or droughts (Bosi 2001), with species believed to have colonized deep interstitial or subterranean habitats to escape desiccation associated with drought events or increased aridity (Leys et al. 2003; Fenoglio et al. 2006). Colonization of temporary habitats may also be associated with the need to avoid negative biotic interactions, such as fish predation (e.g., Åbjörnsson et al. 1997; De Mendoza et al. 2012; Liao et al. 2020) or intra- and interspecific competition (Balfour-Browne 1962; Wiggins et al. 1980; Ranta 1985; Larson 1997b; Gioria et al. 2010a).

The larval stage plays a critical role in determining dytiscid population dynamics (Bilton et al. 2001). A predominance of information on the distribution and behaviour of adults *versus* that on larvae (Larson 1987, 1997a) complicates our understanding of the habitat requirements and preferences of many species, since these may vary substantially throughout the life cycle (Juliano 1991; Hilsenhoff 1993). The swimming behaviour of adults and larvae also differs, with adults being positively buoyant while larvae generally sink in the water (Miller and Bergsten 2016). Larvae have been increasingly described, and information on their ecology is becoming more widely available, including that of newly discovered species or genera (e.g., Galewski 1973a, b, 1975; Nilsson and Holmen 1995; Alarie and Delgado 1999; Alarie et al. 1998, 1999, 2000; Larson et al. 2000; Alarie and Bilton 2001; Nilsson 2001; Yee et al. 2013; Alarie and Michat 2014; Gustafson et al. 2016; Chap. 2). However, the distribution of dytiscid larvae along many environmental gradients remains largely underexamined (but see Tones 1978; Juliano 1991; Eyre et al. 1992; Fairchild et al. 2003, among others).

Habitat classifications for water beetles are typically based on species distribution (and abundance) data collected from a variety of habitats, broadly characterized, typically over large spatial scales, or species abundance data combined with quantitative data on one or more environmental variables, over small to large spatial scales (e.g., Eyre et al. 1986; Foster et al. 1992; Enkhnasan and Boldgiv 2019). Both approaches are useful to characterize the habitat preferences of many species. While the former is a useful management and conservation tool, the second provides information on the importance of a specific environmental set of environmental conditions in driving the distribution of individual species. The use of large-scale distribution data for water beetles has resulted in robust habitat classifications at the national or regional level (e.g., Eyre et al. 1986; Foster et al. 1992). Eyre et al. (1986) developed a habitat classification for water beetles in north-east England, based on abundance data for 384 sites and identified nine habitat types, including rivers with fast-flowing, shallow, probably highly oxygenated water, characterized by little vegetation, with dytiscids being recorded from gravel; large permanent lakes or ponds, typically characterized by a bare substratum, supporting species with broad habitat preferences; large permanent ponds with a soft substratum, typically found on boulder clay, supporting a vegetation characterized by the presence of plant species such as *Glyceria*, *Carex*, *Juncus*, and *Phragmites* species, which provide a flooded litter zone; ‘transition mires’, with a mixture of *Sphagnum*, *Glyceria*, *Carex*, and *Juncus* species, supporting widely distributed as well as acidophilic species; lowland, typically permanent marshes; lowland, slow-moving sections of streams and seepages, with grassy margins; highly seasonal, lowland temporary pools, supporting active flying dytiscids; upland mires, characterized by the presence of *Sphagnum* and *Carex* species and of considerable amounts of litter, also supporting dytiscids typical of acid conditions; and upland running waters, typically flowing through mosses (*Sphagnum*). Foster et al. (1992) produced a similar habitat classification of water beetles of Ireland, also distinguishing nine main community types defined based on the characteristics of the habitat and the inhabiting species. These include deep rivers supporting dytiscids of deep running water; rivers with riffle sections and beds of unstable shingles, supporting both habitat specialists and generalists, as well as species typically associated with temporary habitats; temporary habitats such as puddles, typically dominated by habitat generalists but also by species associated with seepage; permanent open water bodies characterized by species-rich vegetation, typically enriched or eutrophic waters, as well as canals, supporting habitat generalists other than those occurring in puddles; small lentic water bodies, such as ponds, ditches, and vegetated habitats within enriched lakes, supporting species of permanent habitats; fens supporting small habitat specialist dytiscids; cutover bog and rafts of acid fen vegetation, supporting habitat specialist species and acidophilic species as well as species indicators of eutrophic conditions; montane flushes and ditches in bogs; and turloughs and large, shallow ecosystems on base-rich substrata, supporting species associated with moss and species typically found in newly created habitats where bare substratum is dominant.

In the Ebro delta and other Mediterranean coastal wetlands within the Iberian Peninsula, Ribera et al. (1996) developed a habitat classification for water beetles, with one or more species being used as habitat indicators. Sites were primarily classified depending on sea origin, including non-vegetated dune ponds and lagoons, close to the sea and filled with sea water from storms; sites with water from drainage, rain, or with a mixed origin, sites with fresh water and dense vegetation; small ponds in a clay substratum with marginal vegetation; sites with a mixture of sea water and rainwater from drainage; lagoons with occasional connections with the sea; or lagoons and temporary inundated marshes.

Additional examples of studies that used a similar approach to classify habitats for water beetle assemblages include examinations of species occurrences in fens and drainage ditches among others, including arable fenland and drains in England (Eyre et al. 1990; Foster et al. 1990), and a traditionally managed undrained fen and the ditches of a previously drained cattle-grazed fen meadow (Painter 1999). Each habitat in these classification systems supports characteristic dytiscid communities that depend on the habitat preferences and dispersal ability of individual species. A summary of the environmental variables that are more important in shaping dytiscid communities and distribution is provided below (Sect. 10.3).

10.3 Abiotic Habitat Conditions

Over the past few decades, extensive research efforts have been made to improve our understanding of the relationship between individual abiotic habitat variables and the distribution of dytiscids. Abiotic conditions that have been typically examined include water flow (lentic *versus* lotic), water velocity, permanency, temperature, pH, degree of exposure or shade, salinity, nutrient levels, dissolved oxygen, turbidity, type of substratum, habitat size (surface area and depth), presence of an inflow or outflow for lentic waterbodies, origin (natural *versus* artificial), topography, the type and regime of natural and anthropogenic disturbances, and interactions among these variables (e.g., Nilsson 1984; Larson 1985, 1997b; Ranta 1985; Eyre et al. 1986, 2005; Foster et al. 1990, 1992; Juliano 1991; Fairchild et al. 2003; Schäfer et al. 2006; Gioria et al. 2010a; De Mendoza et al. 2012; Pakulnicka et al. 2016a; Enkhnasan and Boldgiv 2020; Liao et al. 2020). Although the effects of these variables are discussed separately, they strongly interact in determining the habitat preferences of individual species.

10.3.1 Lentic *Versus* Lotic *Habitats*

The distinction between lentic and lotic habitats represents one of the primary criteria of habitat classification for water beetles (e.g., Balfour-Browne 1940, 1962; Foster et al. 1992; Larson 1997a; Larson et al. 2000; Ribera et al. 2001; Ribera 2008). Balfour-Browne (Balfour-Browne 1962) classified habitats available to water beetles into three main categories, recognizing at least two distinct lentic types of habitats: large, open water areas, including waterbodies such as clear lakes with little detritus and vegetation, as well as lakes with much vegetation and detritus; running waters; and stagnant waters, including silt ponds and detritus ponds.

This classification into lentic *versus* lotic waterbodies (rather than habitats) is useful to develop finer classifications based on other habitat features (Balfour-Browne 1962; Williams 1979; Wiggins et al. 1980) but might lead to inconsistencies associated with the low habitat specificity of many species and their dispersal capacity. Moreover, certain waterbodies support a variety of habitat types that may differ in their waterflow (Balfour-Browne 1962; Larson 1997b), with lotic habitats being found in lentic waters and vice versa. For instance, dytiscids often occur within the littoral zone of large lakes, which are exposed to wave action and are characterized by a substrate typically made of gravel, rock, and sand under similar conditions to those found in lotic systems (Williams 1979; Foster et al. 1992; Nilsson and Holmen 1995). Unstable lentic waterbodies support a similar fauna than that of intermittent lotic systems (Larson 1997b). On the other hand, streams support lentic habitats within sluggish areas characterized by abundant mud and vegetation (e.g., Larson 1997a; Foster et al. 1992). These factors often lead to the finding of typically lentic species in lotic waterbodies or vice versa (e.g., Balfour-Browne 1962; Foster et al. 1992; Foster and Eyre 1992; Nilsson and Holmen 1995; Larson et al. 2000). Moreover, the use of certain habitats may vary throughout the year, so that some species are found predominantly in lentic waterbodies at some time of the year, while at other times, they may inhabit lotic ones (Hilsenhoff 1993). Further, the habitat requirements may vary largely throughout the life cycle, so that larvae may be found in lotic habitats while adults prefer lentic habitats, such as the case of *Agabus ambiguus* (Say) (Hilsenhoff 1993).

Dytiscids inhabit a broad variety of habitats within rivers, creeks, and streams, with gravel or muddy substrates (Larson et al. 2000; Enkhnasan and Boldgiv 2020; Shaverdo et al. 2020). These habitats include riffle zones, crevices, floodplains, channels or runs, springs, backwaters, pools of creeks or pools adjacent to streams (Fig. 10.2) or side pools of rivers (Fig. 10.3), waterfalls and wet rock surfaces, some of which support dytiscids that cannot cope with fast current (e.g., Larson et al. 2000; Pederzani et al. 2004; Hendrich et al. 2019; Enkhnasan and Boldgiv 2020; Shaverdo et al. 2020). They also include subterranean (Sect. 10.6.1) and hygropetric habitats (i.e., films of water flowing over rocks; Miller and Perkins 2012; Sect. 10.6.2). Characterizing features of lotic habitats include permanency (Sect. 10.3.3), water velocity (slow, moderate, swift, up to torrential), substrate (rubble, sand, clay, and organic debris), temperature range (minimum winter temperature and maximum

Fig. 10.2 Pools near small stream (diameter ca. 5 cm), inhabited by *Copelatus* and *Exocelina* species (Baliem Valley, Papua, Indonesia; Photo by Jiří Hájek)

Fig. 10.3 Side pools of large river, with gravely bottom, are dominantly inhabited by the enigmatic dytiscid *Huxelhydrus syntheticus* Sharp (Rees River, Otago Lakes, New Zealand; Photo by Jiří Hájek)

Fig. 10.4 Shallow lake near the Canal de Castilla, Canal de Castilla (Palencia Province, Spain) in the northern Iberian Meseta (Valladares et al. 2002; Photo by Luis Felipe Valladares)

summer temperature), and chemical variables (dissolved oxygen, pH, total dissolved organic and inorganic matter, nutrient concentrations) (Pennak 1971; Enkhnasan and Boldgiv 2020). Various classifications of lotic habitats have been proposed. Williams (1979) identified four 'regions' along the length of a river in Canada: eucrenon (the spring region); hypocrenon (the spring brook), rithron (the region extending from the hypocrenon to the point where the mean monthly temperature rises above 20 °C), and the potamon (the region below the rithron extending to the sea or a large lake, where the mean monthly temperature rises to 20 °C) (see also Hynes 1970). Rivers, streams, lakes, and ponds with deep gravel beds can also provide interstitial habitats (the hyporheic zone), where a small number of species has been reported (Sect. 10.6.3). Most dytiscids are found in the potamon and may occupy 'potamon habitats' within the rithron in depositional areas (Williams 1979).

Lentic ecosystems such as lakes, ponds, and mires can support a broad variety of habitats (e.g., Foster et al. 1992; Nilsson and Holmen 1995; Larson et al. 2000). Rock pools, bog pools, rain pools and puddles, tree holes, and phytotelmata, among others, can also be regarded as small lentic habitats as well as discrete ecosystems. Lotic habitats can be found in lentic systems (Foster et al. 1992; Larson et al. 2000). Most dytiscids occur in lentic habitats (Galewski 1971; Williams 1979; Roughley and Larson 1991; Larson 1997a; Larson et al. 2000; Ribera et al. 2001), particularly in smaller and shallow lakes (Fig. 10.4) where wave action is weak, or at the bay of larger lakes (Williams 1979), in shallow ponds or at the margins of running waters, in slow-moving or stagnant habitats within the emergent vegetation along shore

Fig. 10.5 Oxbows and temporary marshes near Baliem River (Papua: Wamena, Indonesia) are unique habitats for numerous (mostly endemic) dytiscid species, such as *Hyphydrus dani* Biström, Balke and Hendrich, *Rhantus dani* Balke, *Hydaticus okalehubyi* Balke and Hendrich, *Limbodessus baliem* Balke and Hendrich, *Hydrovatus enigmaticus* Biström, and *Sternhydrus* Brinck species (Photo by Jiří Hájek)

banks, and within permanent or temporary marshes (Figs. 10.5 and 10.6) or brackish pools (Fig. 10.7) (Balfour-Browne 1940; Jäch and Margalit 1987; Foster et al. 1990, 1992, 2009; Larson et al. 2000; Ribera et al. 2003a; Foster 2010). Among lentic systems, ponds play an especially important role in the conservation of dytiscids (e.g., Foster et al. 1992; Foster and Eyre 1992; Larson et al. 2000; Gioria et al. 2010a) and can be regarded as 'pearls in the landscape' (Probert 1989). In the literature, ponds are often referred to as shallow lakes or pools of still water, albeit with inflow and/or outflow, but the question of what a pond is has been much debated (Probert 1989). Several definitions have been proposed, mostly based on two major variables, i.e., topography and combination of surface area and depth (e.g., Biggs et al. (1998) in the UK, or Oertli et al. (2005) in Switzerland; see Biggs et al. (2005) for a list of definitions of ponds), although some definitions have local significance only. Permanency, pond size, and landscape variables strongly influence the characteristics of the habitats they provide and their importance for dytiscids. Several pond types have been described, with some supporting high dytiscid diversity, such as farmland ponds (Gioria et al. 2010a), urban ponds (Liao et al. 2020), beaver ponds (Larson et al. 2000; Fairchild et al. 2003; Bush and Wissinger 2016), tundra ponds and pools (Nilsson and Holmen 1995; Larson et al. 2000; Lougheed et al. 2011), and kettle ponds and marl holes (Gioria et al. 2010a). As for artificial ponds, quarry ponds (Biström et al. 2015), ponds in golf courses

Fig. 10.6 Shallow marshes in the subalpine zone (ca. 3300 m.a.s.l.) are inhabited with endemic *Limbodessus* Guignot and *Rhantus supranubicus* Balke (Habbema Lake, Papua, Indonesia; Photo by Jiří Hájek)

Fig. 10.7 Densely vegetated brackish pools on sand dunes are inhabited by species such as *Allodessus bistrigatus* (Clark) and *Rhantus suturalis* (Macleay) (Auckland vicinity, New Zealand; Photo by Jiří Hájek)

(Burke 2010), and urban ponds or those created in gardens and demesnes (e.g., Gioria et al. 2010a; Liao et al. 2020) have also been found to support several species.

Fewer species occur in lotic ecosystems or habitats compared to lentic systems, and only a small proportion of those are restricted to running waters, although more habitat specialists and endemic species are found in these than in lentic ecosystems (Ribera et al. 2003a). In a study on the habitat preferences (lentic *versus* lotic) of dytiscids in the Iberian Peninsula, Ribera and Vogler (2000) showed that only three of thirty endemic species were exclusively found in lotic habitats. In Britain, *Deronectes latus* (Stephens) is one of the few species that occurs nearly invariably in lotic habitats, within clear, gravelly rivers and streams (Foster 2010). Most lotic species have been found in lentic waterbodies, particularly at the margins of lakes characterized by some wave action. A strong similarity between the dytiscid fauna of rivers and lakes has thus been reported (Balfour-Browne 1940; Larson et al. 2000), with many widespread eurytopic species being commonly found in both lentic and lotic systems (e.g., Balfour-Browne 1940; Foster et al. 1992; Nilsson and Holmen 1995; Larson 1997a; Larson et al. 2000; Ribera et al. 2001; Foster 2010). In a comprehensive study of the water beetles of springs in Canada, Roughley and Larson (1991) provided quantitative information on the habitat preferences of 260 dytiscid species known from the Nearctic. Of these species, 71% were recorded from lakes, ponds, marshes and lentic saline or forest habitats, while only 29% was found in lotic water bodies, of which 37% were also recorded from springs. In the Nearctic region, only 12 out of 66 species (18%) species unique to Canadian ecozones (Pacific and Atlantic Maritime, Montane Cordillera, Prairies, and Mixedwood Plains) were lotic (Larson et al. 2000). In the Yukon Territory, only 12% of boreal dytiscid species and 15% of arctic species were reported to inhabit lotic habitats (Larson 1997a). Conversely, most species found in the Cordilleran range were lotic (82%, 9 out of 11 species) and represented 45% of the lotic fauna of the Yukon Territory, the westernmost territory of Canada (Larson 1997a).

The relatively low number of lotic species is partly dependent on species-specific dispersal strategies of dytiscids. Differences in the spatial and temporal structure of lentic and lotic habitats within the landscape matrix are in fact generally supposed to select for different dispersal strategies, with active flying dispersal capacity being essential for the long-term persistence of lentic species, while lotic species are assumed to persist without a strong need for long-flying dispersal (Ribera and Vogler 2000; Ribera et al. 2001; Bilton 2014; Chap. 11). Ribera et al. (2003a) evaluated the importance of landscape variables in determining species numbers of water beetles based on available checklists for ten western European countries and the five largest islands. Species numbers were found to differ for beetles of lentic and lotic habitats. The number of lotic species was mainly correlated with latitude of the geographic area, while that of lentic species was correlated with a measure of land connectivity, which reflects geographical constraints to dispersal or the total area under examination. This provides support for the hypothesis that persistence of lentic populations strongly depends on migration and dispersal, while a capacity for long-distance dispersal is less important in lotic species (Ribera and Vogler 2000; Bilton 2014). Yet, broad evidence for this is lacking, and local abiotic and biotic conditions, including a requirement for vegetated habitats for many species (Sect. 10.4.1) or predator–prey interactions (Sect. 10.4.2; Chap. 8), might be more important than the lentic-lotic 'divide' (Ribera 2008) in driving the distribution of many dytiscids (Southwood 1962; Lundkvist et al. 2001).

10.3.2 Springs

Springs originate at the intersection of groundwater, surface water, and terrestrial ecosystems (Scarsbrook et al. 2007). In recognition of their peculiar habitat conditions, the habitat preferences of water beetles have been classified into lentic, lotic, and springs (Roughley and Larson 1991). Dytiscids are frequent inhabitants of springs, with some species being exclusive to springs or spring-fed streams (e.g., Roughley and Larson 1991; Larson et al. 2000; Gioria 2002; Pederzani et al. 2004; Pakulnicka et al. 2016b). However, the dytiscid fauna of springs has received comparatively less attention than that of lentic and lotic ecosystems (Pakulnicka et al. 2016b). Roughley and Larson (1991) showed that 38 species of dytiscids known then from Canada could be categorized as spring-inhabiting (ca. 11%), nine of which (24%) occurred exclusively in springs.

Springs vary greatly in their morphology, chemistry, temperature range (from cold to hot), and permanence, and various classifications have been proposed based on these characteristics (e.g., Danks and Williams 1991; Erman and Erman 1995; White 2005). Williams (1979) recognized three types of springs: rheocrene (springs that flow from a defined opening into a confined channel), limnocrene (springs originating from a large, deep pools of water), and helocrene (springs originating from marshes or bogs). In central Sweden, Hoffsten and Malmqvist (2000) categorized springs depending on their glacial history and hydroperiod into glaciofluvial, moraine, and limestone spring. The thermal regime defines the end of the eucrenal zone (spring) and the beginning of the hypocrenal zone (spring brook) (Smith et al. 2003), with the former being defined as the point where annual variation in water temperature is lower than 2 °C (Erman and Erman 1995). The ecology of spring brooks has sometimes been described as that of springs (see Barquín and Death 2006).

In relation to temperature, dytiscids have been reported from cold, warm, and even hot springs. In a study of the fauna of thermal waters in New Zealand, Winterbourn (1968) recorded larvae of *Antiporus* Sharp species from thermal and warm spring waters of the New Zealand Central Plateau at a temperature of 34 °C. Previous records in the same region include *Rhantus suturalis* (W. S. Macleay) from mineral spring water (Wise 1965) and *Limbodessus plicatus* (Sharp) from warm pools (Ordish 1966). Stark et al. (1976) recorded both adults and larvae of *Limbodessus deflectus* (Ordish) in the outflow of a hot spring in the South Island, New Zealand. Specifically, larvae were common at 28.5 °C, while few larvae were

Fig. 10.8 Karstic region supporting high dytiscid diversity, which are predominantly found in mosses within (**a**) springs and (**b**) slow streams flowing through grassland communities originating from them (County Clare, Ireland; Photos by Margherita Gioria)

found at 35 °C; for adults, few individuals (one to ten per 10 dm^2) occurred at 28.5 and 32.5 °C. Two species, *Hydroporus zackii* Larson and Roughley and *Dytiscus marginicollis* LeConte, have also been recorded from hot springs in North America (Larson et al. 2000). Mason (1939) showed that dytiscids were among the most important group found in Algerian hot springs. Dytiscids recorded from cold springs include species of *Hydrocolus* Roughley and Larson (Larson et al. 2000), whose members occur in springs or seepage (Larson et al. 2000; Ciegler 2001), and *Sanfilippodytes* Franciscolo (Larson 1975; Larson et al. 2000), and several species of *Hydroporus* Clairville (Nilsson and Holmen 1995; Larson et al. 2000; Gioria 2002; Smith et al. 2003) and *Agabus* Leach (Nilsson and Holmen 1995; Larson et al. 2000; Gioria 2002). Dytiscid larvae have also been recorded from cold springs and spring brooks (e.g., Hilsenhoff 1993; Smith et al. 2003). Gioria (2002) recorded larvae of *Dytiscus* L. from an intermittent cold spring in a karst area in western Ireland (Fig. 10.8). Smith et al. (2003) recorded the presence of dytiscid larvae from two intermittent spring brooks in the Peak District National Park, England, from the actual spring or close to the source (2.5 m downstream different points in time), while, in permanent spring brooks, larvae were only recorded 10 m downstream the source.

The duration of the hydroperiod also determines the occurrence of dytiscids in springs. Scarsbrook et al. (2007) collected and compiled data on the ecology of 82 cold springs in New Zealand and showed that permanency plays a major role in determining the distribution of dytiscids. The spring with the lowest permanency supported only one species, *Huxelhydrus syntheticus* Sharp, which had been previously recorded from shallow temporary shingle pools at the margins of larger rivers (Ordish 1966; Winterbourn and Gregson 1981). A species of *Antiporous* Sharp was the only species that was exclusive to temporary spring, while no species were exclusive to permanent springs.

Other factors contributing to shaping the dytiscid fauna of springs include the prevailing substrate, distance from the river, vegetation characteristics, and landscape variables (Pederzani et al. 2004; Pakulnicka et al. 2016b). Pakulnicka et al. (2016b) studied 25 lowland springs along the Krąpiel River, north-western Poland, which were characterized by their substrate (muddy *versus* sandy bottom), overgrown vegetation *versus* bare substratum, and presence or absence of organic matter. *Agabus biguttatus* (Olivier) was the only spring specialist species (crenophile), possibly due to the lowland location of those springs. *Dytiscus dimidiatus* Bergsträsser was one of the most abundant dytiscids, together with *A. bipustulatus* (L.) and *A. paludosus* (Fabricius). Rheophiles, i.e., species preferring fast-flowing waters, included *Ilybius fenestratus* (Fabricius), *I. fuliginosus* (Fabricius), and *Agabus paludosus* (1.9% of specimens). Tyrphophiles and tyrphobionts (i.e., species more or less specific to bogs) included *Ilybius ater* (De Geer), *Acilius canaliculatus* (Nicolai), and several *Hydroporus* species. Only few species were classified as stagnophilic relatively to other water beetles, indicative of the importance of springs for the conservation of dytiscids.

Given the sensitivity of springs to changes in groundwater use and water pollution associated with agricultural and industrial activities, and with urbanization (Dennis and Dennis 2012; Ferguson and Gleeson 2012; Pakulnicka et al. 2016b), knowledge of their specialized fauna can provide important insights into the vulnerability of dytiscids to climatic and other global environmental changes.

10.3.3 Permanency

Permanency, i.e., the duration of the hydroperiod or wet phase, is a major habitat factor affecting the distribution of dytiscids throughout the landscape (Wellborn et al. 1996; Valladares et al. 2002; Gioria et al. 2010a). Many temporary habitats have been named based on local features, such as arroyos, billabongs, caños, cenotes (sinkhole lakes), gnammas, pingos, playas, tinajas, turloughs (disappearing lakes), vleis, or wadis (Fig. 10.9; Curtis 1991; Larson 1996, 1997b; Hall et al. 2004; Foster et al. 1992; Florencio et al. 2014; see Williams et al. 2001). Temporary habitats can be either lentic or lotic. In temporary lotic habitats, Comin and Williams (1994) recognized intermittent (predictable drying cycles) *versus* episodic habitats (low degree of predictability), based on the predictability of the frequency, time of

Fig. 10.9 Rest pools of drying up streams in wadis are the habitat for the majority of dytiscids in arid areas. Wet sand on the border of pools is a typical habitat for Bidessini such as *Glareadessus stocki* Wewalka and Biström (Dhofar, Wadi Shaith, Oman; Photo by Jiří Hájek)

occurrence, and duration of the dry phase. Williams (1996) classified the main types of naturally occurring temporary waterbodies, based on their geographical distribution, into (1) ubiquitous waters (intermittent and episodic ponds, lakes, springs, rivers, and streams, the margin of permanent lakes, ponds, rivers and streams, floodplains, and liquid dung); (2) arid and semi-arid regions (sections of permanent running waters, drypans, billabongs, kopjes, temporary inland saline waters, and desert rain pools); (3) humid tropical regions (e.g., tree holes, coconut shells, and rain pools); (4) temperate regions (e.g., seasonal wetlands, peatland pools, kettle ponds, woodland pools, turloughs, vernal ponds, autumnal ponds; tree holes, and rain pools); (5) maritime regions (e.g., tidal wetlands, supra-littoral tide pools, and saturated moss); and (6) Arctic and Antarctic regions (snowmelt pools, glacial streams, and aestival ponds), which include a variety of wetland types.

An example of early species classification based on tolerance to or avoidance of droughts was provided by Wiggins et al. (1980), who classified the species of temporary vernal and autumnal pools and permanent ponds, based on data from southern Ontario, into four groups: (1) overwintering resident species, which are capable of passive dispersal only, and aestivate and overwinter in the dry basin; (2) overwintering spring recruits, which include species that reproduce in the pool in spring before the beginning of the dry phase, aestivate and overwinter in the dry pool basin. These species are capable of active dispersal, although recruitment and dispersal occur in spring only, with larvae or adults possibly surviving the dry phase; (3) overwintering summer recruits; (4) non-wintering spring migrants, which colonize pools in spring during the wet phase and leave them before the beginning of the dry phase, overwintering in permanent habitats. Williams (1983) argued that this classification was confusing, since 'overwintering residents', 'overwintering spring recruits', and 'overwintering summer recruits' include species that are permanently found in temporary pools and whose active phases in temporary pools often coincide. An alternative classification based on the time of occurrence was thus proposed, distinguishing active 'forms' in a vernal pond in southern Ontario, into five groups: (1) species virtually found over the entire aquatic phase as well as the dry phase, in the pond substratum as semi-torpid adults or immature stages; these species are capable of movement within minutes after being placed in water (two *Hydroporus* species were placed in this group); (2) species found in the pond as active forms within a few days from the beginning of the wet phase in the spring and that completed their life cycles within 4–6 weeks, but disappeared 4–6 weeks before the beginning of the dry phase (*Agabus* and *Neoscutopterus* J. Balfour-Browne); (3) species that colonized the pond 2–5 weeks after the beginning of the wet phase in the spring (adults of *Dytiscus*, *Acilius* Leach, and *Rhantus* Dejean), although they did not breed in the pond and completed their life cycle within a few weeks); (4) species that colonized the pond only 2–3 weeks prior to the beginning of the dry phase, i.e., approximately 10 weeks after the beginning of the wet phase (*Laccophilus* Leach, *Hydaticus* Leach, and *Hydrovatus* Motschulsky); and (5) species that appeared only in the dry phase; no dytiscids species belonged to this group.

Potential inconsistencies between these classifications are associated with differences in the colonization patterns in vernal ponds compared to those in autumnal ponds (e.g., Davy-Bowker 2002). Vernal ponds typically fill up in spring, for instance, from melting snow and rain, dry up in the summer, and remain dry until the following spring (wet phase of approximately 3–4 months). In contrast, autumnal ponds fill in autumn, due to a less permeable substrate or heavy rainfall patterns, and their wet phase of lasts approximately 8–9 months, until summer (Wiggins 1973). In a 3.5 year mark-and-recapture study in seven semi-permanent and temporary ponds in Cheshire, England, characterized by several drying and filling phases, Davy-Bowker (2002) found that the behaviour of *Acilius sulcatus* (L.) and *Dytiscus marginalis* L. was in accordance with that described by Wiggins et al. (1980) and Williams (1983) in Ontario, with these species dispersing from overwintering ponds into temporary ponds in the spring, to then migrate into permanent ponds in the summer, while they generally tend to move among ponds. However, *Agabus bipustulatus* remained in the terrestrial vegetation in damp pond basins for several months after the ponds dried up in the summer and moved back to permanent ponds only when the basin was completely dry. This is consistent with the findings of Eyre et al. (1992), who examined the effects of the duration of the hydroperiod on dytiscid assemblages (both adults and larvae) and observed that the probability of occurrence of *A. bipustulatus* was higher in temporary habitats whose wet phase lasts 3–4 months and decreased at lower and higher durations.

Permanent waterbodies typically support richer and more abundant dytiscid communities compared to temporary ones (e.g., Nilsson and Svensson 1994, 1995; Nilsson and Holmen 1995; Schneider and Frost 1996; Lundkvist et al. 2001; Valladares et al. 2002; Fairchild et al. 2003; Schäfer et al. 2006; Boukal et al. 2007; Gioria et al. 2010a) and a significant turnover in species composition is found along permanency gradients (Wiggins et al. 1980). Dytiscid communities in temporary habitats are often characterized by high similarity in species richness and composition (Kholin and Nilsson 1998; Gioria et al. 2010a) at the genus level, with *Hydroporus*, *Agabus*, and *Ilybius* Erichson usually being the dominant genera, despite differences in other abiotic conditions (e.g., Nilsson 1984; Larson 1985; Foster et al. 1992; Nilsson and Svensson 1995; Lundkvist et al. 2001; Baber et al. 2004; Nicolet et al. 2004; Vinnersten et al. 2009; Gioria et al. 2010a). This is especially true in lentic systems, where dytiscids occur from ephemeral pools to permanent lakes. Baber et al. (2004) recorded *Acilius* Leach, *Dytiscus*, and *Ilybius* species from three categories of wetlands (short, intermediate, or long hydroperiod) but did not find any *Agabus*, *Hydaticus*, *Rhantus*, and *Colymbetes* Clairville species from ‘short hydroperiod’ wetlands, suggesting a preference for more permanent habitats for those species despite being known to temporarily inhabit ephemeral habitats for certain periods of time (e.g., Foster et al. 1992; Larson et al. 2000; Gioria et al. 2010a). Dytiscid communities in temporary habitats are less predictable compared to those occurring in permanent habitats (Nilsson 1986). In a study of aquatic insects in Sycamore Creek, a lowland stream in the Sonoran Desert, Arizona, which is subject to recurrent floods and droughts, Gray (1981) showed that dytiscids avoided droughts principally through habitat selection of oviposition sites, by ovipositing in deep pools that retain water for the duration of larval development or in main channel segments. In contrast, they exhibited a flood avoidance behaviour during floods, by leaving the stream or swimming to habitats where they are protected by the vegetation along the channel edge. Idiosyncratic patterns in the distribution of dytiscids along gradients of permanency might be associated with a preference for temporary habitats that has been observed in many species (e.g., Young 1954; Zimmerman 1959, 1960, 1970; Nilsson and Holmen 1995; Ribera et al. 1995a; Larson et al. 2000; Lundkvist et al. 2001; Valladares et al. 2002; Foster 2010; Pitcher and Yee 2014).

Temporary habitats have been increasingly recognized globally for their important role in the conservation of dytiscids (e.g., Wiggins et al. 1980; Larson 1985; Foster et al. 1992; Nilsson and Svensson 1995; Kholin and Nilsson 1998; Fairchild et al. 2003; Nicolet et al. 2004; Gioria et al. 2010a; Florencio et al. 2014; Bird et al. 2019; Pintar and Resetarits 2020). Differences in the diversity and composition of dytiscid communities in permanent *versus* temporary habitats suggest that their conservation requires the maintenance of habitats of varying degree of permanence across the landscape (Fairchild et al. 2003; Gioria et al. 2010a; Silver and Vamosi 2012). Temporary habitats typically support a mix of specialist and more generalist species, with some occurring temporarily as adults, most of which can fly o more permanent habitats or waterbodies during the dry phase (Nilsson 1986; Bilton et al. 2001; Fairchild et al. 2003; Bird et al. 2019). Breeding species, however, are not found in the most temporary habitats since larvae are not tolerant to desiccation (Bilton et al. 2001).

A generally lower species richness found in temporary compared to more permanent habitats is often associated with lower habitat stability with respect to various biotic and abiotic conditions (Florencio et al. 2014). Variability in the frequency, magnitude, and duration of the wet and dry phases in temporary habitats (Williams 1996) is a key factor determining the response of dytiscids to habitat availability. Some temporary habitats can be characterized by high variability in the duration of the hydroperiod, with periodic, unpredictable drying increasing the risk of desiccation, threatening the persistence of certain species (Wiggins et al. 1980; Ranta 1985; Friday 1987; Valladares et al. 2002). In regions where intra- and inter-annual variation in the duration and frequency of the wet phase is high, the response of dytiscids to habitat availability can be very rapid (Nilsson and Svensson 1995; Larson 1997b; Lundkvist et al. 2001; Fairchild et al. 2003; Vinnersten et al. 2009; Gioria et al. 2010a).

The distribution of dytiscids along gradients of permanency is also associated with variables such as habitat complexity, steepness and depth, temperature, pH, alkalinity, dissolved oxygen, nutrient concentrations, and turbidity, type of substratum, which covary with permanency (e.g., Nilsson et al. 1994; Foster 1995; Nilsson and Svensson 1995; Ribera and Nilsson 1995; Nicolet et al. 2004; Gioria et al. 2010a). Habitat complexity, which is a function of factors such as the structure of the vegetation, the presence of mosses, algal mats, rocks, logs, or stones, the heterogeneity of the substratum (e.g., mud, gravel, rock, detritus, bare substratum, or a combination of those type of substrate; Harper et al. 1997), depth and steepness, wave action, spatial and temporal variations in abiotic conditions (e.g., temperature, dissolved oxygen, or nutrient concentrations), is generally lower in temporary systems and habitats (Fig. 10.10). For instance, gravel is often missing in some temporary habitats, so that species with a preference for a gravel substratum to avoid these habitats even if they are located within a close range to more permanent habitats (Gioria et al. 2010a).

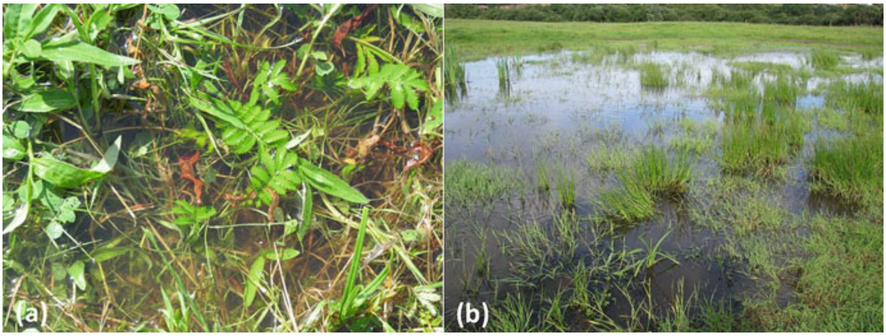

Fig. 10.10 Temporary flooded habitats were the presence of dytiscids has been recorded. (**a**) Temporary rain pool in a grazed grassland community that had been rapidly colonized by two dytiscid species (County Wexford, Ireland). (**b**) Flooded lowland grassland being rapidly colonized by nine dytiscid species (County Westmeath, Ireland; Photo by Margherita Gioria)

The structure and distribution of predators and food resources (Larson 1990), inter- and intra-specific competition (Larson 1990), and vegetation structure (Gioria et al. 2010a, 2011) also vary substantially along gradients of permanency. Temporary habitats may provide a suite of habitat features that are favourable to dytiscid colonization or dispersal, such as reduced competition and risks of predation, despite often lying at the extremes of the physiological requirements of a species. The structure of predators and prey and the number of guilds (e.g., McAbendroth et al. 2005; Tokeshi and Arakaki 2012) tend to be negatively correlated to decreases in the duration of the hydroperiod (Nilsson and Svensson 1995). Temporary habitats typically support fewer predators (fish and Odonata larvae), since these are highly susceptible to habitat drying (e.g., Wiggins et al. 1980; Wellborn et al. 1996; Williams 1996). Thus, species susceptible to fish predation often have a preference for temporary habitats (e.g., Fairchild et al. 2003; van Duinen et al. 2004; Foster 2010; Gioria et al. 2010a). Specialization in temporary habitats as a mechanism to avoid predation is evident in the presence of species that are characteristics of temporary waterbodies but are also found in fishless permanent waterbodies (Sect. 10.4; Chap. 11).

The structure of the vegetation may vary substantially among temporary habitats. Many of those support little or no vegetation, and plant species richness is typically low (Gioria et al. 2010a), with subsequent low dytiscid diversity (Nicolet et al. 2004; Gioria et al. 2010a, 2011). In contrast, some temporary habitats are characterized by dense vegetation associated with high conductivity and nutrient concentrations (Wellborn et al. 1996; Valladares et al. 2002; Nicolet et al. 2004; Gioria et al. 2010a; Silver and Vamosi 2012; Florencio et al. 2014) and provide breeding opportunities (Nilsson et al. 1994; Nilsson and Svensson 1994; Batzer and Wissinger 1996; Fairchild et al. 2003) and abundant food resources, including mosquitoes (Lundkvist et al. 2001, 2003).

Nestedness in temporary habitats has been reported (Nilsson and Svensson 1995; Kholin and Nilsson 1998). Nilsson and Svensson (1995) found strong nestedness in dytiscids recorded from 40 temporary snowmelt pools in Sweden, suggesting that some species may have a minimum habitat size requirement for colonization of these systems. There, the number of guilds and within-guild diversity was positively correlated to increases in the duration of the wet phase for forest pools, although this pattern was not evident for clearing pools.

Habitat connectivity and distance from permanent habitats are also important factors affecting the colonization of temporary habitats. Colonization of temporary habitats is dependent on the dispersal strategies of dytiscids (Bilton 2014). Dytiscids are good colonizers of temporary and newly created habitats, with many species being good, active fliers capable of tracking suitable habitat throughout the year (Larson 1997b). They are often pioneers, being among the first macroinvertebrate predators to arrive in newly formed habitats (e.g., Zimmerman 1960; Yano et al. 1983; Eyre et al. 1986; Foster and Eyre 1992; Fairchild et al. 2003; Lundkvist et al. 2003; Bilton 1994, 2014; Pakulnicka 2008; Gioria et al. 2010a). Dispersal or colonization of newly created habitats may be rapid even in the absence of any emergent or submerged vegetation (e.g., Balfour-Browne 1940; Ranta 1985;

Becerra-Jurado et al. 2009; Gioria et al. 2010a) as well as in anthropogenic habitats, such as clay pits (Pakulnicka 2008; Gioria et al. 2010a), borrow pits (Larson 1997b), gravel pits, quarry pools and highway ditches (Larson et al. 2000), tyre ruts in peaty soils (Larson et al. 2000), newly constructed wetlands (Becerra-Jurado et al. 2009), and cattle troughs (Gioria 2002), among others. Paddy fields are rapidly colonized by dytiscids (e.g., Yano et al. 1983; Larson 1997b; Picazo et al. 2010). In contrast, flightless species are particularly susceptible to low habitat stability with respect to permanency and tend to occupy permanent habitats only. For instance, in northern Queensland, the flightless rheophilic dytiscid *Carabhydrus mubboonus* Larson and Storey was recorded exclusively in forest lotic habitats that provided more stable conditions with respect to the duration and frequency of the wet phase compared to other lentic and lotic habitats (Larson and Storey 1994; Larson 1997b).

Rapid colonization of temporary or newly created habitats may result in a temporary decrease in species richness or abundance of dytiscids in permanent habitats when adjacent temporary habitats experience a wet phase (‘diluting’ effect, see Larson 1997b). Over the duration of the wet phase, it is thus possible that temporary or seasonal habitats might support more species than permanent ones (Nilsson 1984). In 312 sites located in the province of Alberta, Canada, Larson (1985) documented higher dytiscid richness in small seasonal or temporary habitats than in larger permanent water bodies such as lakes.

Biomass of dytiscids per unit area can also be higher in temporary than in permanent habitats. In an investigation on the composition of lentic permanent (small lake shores) and temporary wetlands (depressional wetlands, shallow kettles, and tarns) in the South Island of New Zealand, Wissinger et al. (2009) found that six out of eight species recorded (*Liodessus* Guignot and *Antiporus* Sharp species, *Lancetes lanceolatus* (Clark), and *Rhantus suturalis*) were common in both temporary and permanent habitats but dytiscid biomass was significantly higher in temporary than permanent waterbodies.

Colonization of temporary habitats differs in temperate and tropical zones. In an investigation on habitat and community patterns of tropical Australian beetles, Larson (1997b) found that, in dytiscids, dispersal patterns in *Eucalyptus* woodland regions of Queensland are somewhat different from those in forests in north temperate regions, with tropical species that occur in seasonal habitats responding rapidly to habitat availability and not showing signs of aestivation or hibernation periods during their life cycle. An ability to colonize seasonal habitats is likely an adaptation to highly variable and unpredictable habitat conditions associated with higher inter- and intra-annual variation in precipitation and temperature patterns compared to those characterizing tropical regions (Larson 1997b) (see Chap. 11 for details on dytiscid dispersal in different climatic zones).

Wet meadows and swamps in floodplains represent important temporary habitats for dytiscids (Vinnersten et al. 2009; Pakulnicka and Nowakowski 2012). Peculiar temporary habitats supporting dytiscids include snowmelt pools (Larson et al. 2000), i.e., pools formed by snow melt (Foster 2010), pools of intermittent streams, rain

Fig. 10.11 Turlough (shallow temporary lake fed by a central spring) located in the Burren (County Clare, Ireland; Photo by Margherita Gioria)

pools (Larson et al. 2000), rock pools (Nilsson and Holmen 1995), tree holes (Hendrich and Yang 1997), fen pools (Foster et al. 1990), dry grasslands (Gioria et al. 2010a), forest depressions (Ranarilalatiana and Bergsten (2019) and puddles created by streams (Hájek et al. 2021), and turloughs (Fig. 10.11). Turloughs are karst, seasonal, shallow lakes, some of which with a permanent pond in the centre, which have a highly restricted global distribution, occurring almost exclusively in the west of Ireland (Campbell et al. 1992; Skeffington et al. 2006). These ‘disappearing’ lakes are characterized by a unique hydrology regulated by estavelles (holes and fissures that act both as springs) by which a turlough becomes flooded, as well as swallow holes in the spring, although the water level may raise in response to high precipitation (Skeffington et al. 2006). Turloughs play a central role in the conservation of dytiscids (Bilton 1988; Foster et al. 1992, 2009; Gioria 2002; Skeffington et al. 2006; Reynolds 2014). In the Burren, western Ireland, the majority of turloughs present an upper layer dominated by moss, where many species have been recorded (Foster et al. 1992; Skeffington et al. 2006). In this karstic region, moss dwelling species also inhabit seepages and stone springs (Fig. 10.8; Gioria 2002). Additional examples of unique temporary habitats for dytiscids are described in Sect. 10.6.

10.3.4 Salinity

Dytiscids vary substantially in their tolerance to saline conditions, being recorded from freshwater and highly saline habitats (e.g., Balfour-Browne 1940; Rawson and Moore 1944; Frisbie and Dunson 1988; Timms 1993; Nilsson and Holmen 1995; Ribera et al. 1996; Larson et al. 2000; Chessman 2003). In general, the number of species tends to decrease along gradients of salinity, and only few species can tolerate highly saline conditions (e.g., Jäch and Margalit 1987). Some species have been recorded exclusively from fresh or subsaline habitats (Timms 1993), while others are saline specialists (Tones 1978; Sánchez-Fernández et al. 2010). However, some species are found in both freshwater and saline environments (Galewski 1978; Larson et al. 2000), including hypersaline habitats (Timms 1993). Some species of the tribe Hygrotini (subfamily Hydroporinae) are among the few insects able to tolerate hypersaline concentrations more than twice that of seawater (Villastrigo et al. 2018). Villastrigo et al. (2018) reconstructed the origin and evolution of tolerance to salinity in this lineage and showed that this was gradual, with no direct transitions from freshwater to hypersaline habitats and with some reversals from tolerant to freshwater species. These authors dated the oldest transition to saline tolerance in the late Eocene-early Oligocene, a period of decreasing temperature and precipitation, suggesting a relationship between the development of tolerance to saline conditions and increased aridity (Villastrigo et al. 2018), similarly to what has been hypothesized to drive the colonization of subterranean and interstitial habitats (Leys et al. 2003; Fenoglio et al. 2006).

The osmoregulatory strategies used by dytiscids found in highly saline habitats have been examined for a few species (e.g., *Hygrotus salinarius* (Wallis); Tones 1978; *Dytiscus verticalis* Say; Frisbie and Dunson 1988). In Australia, the Australian Biodiversity Salt Sensitivity Database (Bailey et al. 2002) reported data for 52 dytiscid species, spanning from a tolerance to high salinity levels (up to 93 g l^{-1}), while others have narrow ranges of salinity tolerance. For species recorded in Australian rivers, Chessman (2003) assigned dytiscids a score of 2 (scoring system from 1 to 10, with 1 being high tolerance to a range of environmental conditions, including salinity). In springs of the western Dead Sea area, some species belonging to the genera *Hydroporus*, *Hydroglyphus* Motschulsky, *Hydrovatus* Motschulsky, and *Nebrioporus* Régimbart were recorded from highly saline basin springs (up to 47 g l^{-1}) (Jäch and Margalit 1987). Halophilic species or species associated with distinctly saline waters include several species of *Hygrotus* Stephens (Nilsson and Holmen 1995; Larson et al. 2000), such as *Hygrotus salinarius*, a saline specialist that occurs exclusively in saline water, with its larvae also tolerating a wide range of salinities, with both larvae and adults being recorded in habitats more saline than seawater (Tones 1978; Larson et al. 2000). More recently, the ability to cope with saline conditions was examined in four species belonging to the genus *Nebrioporus* along a fresh-hypersaline gradient in inland waters (Pallarés et al. 2015).

Overall, salinity is considered a good predictor of dytiscid composition and diversity (e.g., Rawson and Moore 1944; Galewski 1971; Larson 1975, 1985; Cuppen 1986). In the Ebro delta and other Mediterranean coastal wetlands in the Iberian Peninsula, Ribera et al. (1996) found that sea water was the main discriminating habitat factor, with *Hydroporus limbatus* Aubé being an indicator species for sea water habitats, while *Rhantus suturalis* was the indicator species for the habitats with water from drainage, rain, or with a mixed origin. In 25 shallow ephemeral lakes of varying salinity, in the semi-desert of north-western New South Wales, Australia, Timms (1993) also recorded *Rhantus suturalis* from fresh- to hyposaline but not in meso- or hypersaline habitats. This is consistent with findings from Williams et al. (1990), who showed that this species recorded from only one lake characterized by low (0.4 g l^{-1}) salinity in the Western District of Victoria, Australia, out of 79 salt lakes.

Among ecologically interesting but overlooked saline habitats for dytiscids are Mediterranean saline streams. Millán et al. (2011) compiled data from saline streams in the Segura and Guadalquivir basins, southeast Iberia, Spain, where only 33% of the streams presented a permanent flow regime, 55% had an intermittent flow, and 12% were ephemeral, occurring only after heavy rainfalls. These authors classified streams into hyposaline, mesosaline, and hypersaline, the former found in larger basins, at higher altitudes, and with lower maximum mean temperatures compared to meso- and hypersaline streams. Eight species were recorded from hyposaline streams, and two of those were also found in mesosaline streams, i.e., *Nebrioporus baeticus* (Schaum) and *N. ceresyi* (Aubé), which are characteristics of inland hypersaline systems (Toledo 2009; Sánchez-Fernández et al. 2010). These species were also recorded from the Rambla Salada, a Mediterranean hypersaline stream in south-eastern Spain (Velasco et al. 2006).

Knowledge of tolerance to salinity for a broad number of species and regions in the future can provide important insights into the potential effects of climate change, agricultural and industrial activities, and changes in land and groundwater use on the distribution of dytiscids (and other taxonomic groups) *via* their effects on salinity (Sánchez-Fernández et al. 2010; Cañedo-Argüelles et al. 2019). While increased salinity in freshwater might negatively affect species with low tolerance to salinity (Cañedo-Argüelles et al. 2019), saline specialists might be threatened by irrigation of intensively farmed areas in inland saline waters. For instance, Sánchez-Fernández et al. (2010) examined the thermal tolerance and acclimatory ability of two hypersaline *Nebrioporus* specialists and found that lowered salinity had negative effects on the tolerance of adults to both high and low temperatures.

10.3.5 Temperature and Temperature-Related Variables, Elevation, and Latitude

Temperature is a major determinant of the distribution of many dytiscids over large spatial scales (e.g., Larson et al. 2000; Eyre et al. 2006), to the extent that some species have been used as palaeoecological indicators to infer past temperatures (Lemdahl 2000). The effects of temperature tend to be especially strong in small, temporary, lentic habitats. For instance, Nilsson and Svensson (1994) found that temperature, pool size, and hydroperiod were among the most important determinant of the composition and distribution of dytiscids in boreal snowmelt pools.

Temperature is typically correlated with elevation and latitude. Vamosi et al. (2007) analyzed species list data collected from over 400 lentic waterbodies in southern Alberta, Canada, and found that species richness did not change with latitude. However, in northern Europe, Heino and Alahuhta (2019) found that latitude was (negatively) correlated with species richness but not mean range size. Calosi et al. (2010) examined relationships between thermal physiology and biogeography of 14 European species belonging to the genus *Deronectes* Sharp and found that absolute thermal tolerance range was the best predictor of both species' latitudinal range extent and position, with species' northern and southern range limits being related to their tolerance of low and high temperatures, while differences in dispersal ability were less important in this group. Proportion of large species increased with latitude but decreased with elevation, suggesting that large species are less prevalent at high elevations. Combining data on thermal physiology with measures of metabolic plasticity and immunocompetence in five closely related European *Deronectes species*, Cioffi et al. (2016) showed that variation in latitudinal range extent and position was explained in part by thermal physiology, but aspects of metabolic plasticity and immunocompetence also contributed to explain such variation. These findings suggest that northerly distributed, wide-ranging species use different energy reserves under thermal stress compared to southern endemic congeners and differ in their antibacterial defences, suggesting a relationship between these processes and distribution range (Cioffi et al. 2016).

In relation to elevation, Vamosi et al. (2007) found a hump-shaped relationship between species richness and elevation, peaking at mid-elevations, while waterbodies at high elevations (>2000 m) had markedly low species richness. Similar findings were reported by Enkhnasan and Boldgiv (2019), who examined community variables of dytiscids (richness, abundance, diversity, and evenness) at three spatial scale (basin, subbasin, and habitat) in two major basins in central and western Mongolia, i.e., the Arctic Ocean Basin and the Central Asian Inland Basin. These authors found that elevation was an important variable structuring dytiscid communities at the basin level, being significantly negatively correlated with dytiscid abundance and diversity. The peak in dytiscid diversity was recorded at mid-elevation (1000–2000 m a.s.l.), possibly due to warmer and better wetland habitat conditions and prey resource availability than at other elevations. Most of the differences among subbasins in terms of dytiscid communities were also associated with elevation, temperature, and dissolved oxygen. High elevation species include *Agabus joachimschmidti* Brancucci and Hendrich, which was found in a temporary brook at 5100 m a.s.l. in south central Tibet and represents the highest elevation record for dytiscids so far (Brancucci and Hendrich 2008), and *Rhantus* species, such as *Rhantus blancasi* Guignot, which was collected in Peru from the margin of permanent lakes with muddy bottom and abundant aquatic vegetation up to an elevation of 4850 m (Balke et al. 2019). Species recorded from broad elevational gradients include *Ilybius hypomelas* (Mannerheim), which was recorded from sea level in Alaska and up to 3000 m in Colorado (Larson et al. 2000).

The effect of temperature on the distribution of dytiscids across habitats is often confounded by its strong correlation with other environmental variables, such as water flow, the degree of exposure to direct sunlight, precipitation, habitat depth, and biotic interactions. Moreover, precipitation is often strongly correlated with elevation (and temperature), so that lentic and lotic habitats are more abundant where precipitation is high (Enkhnasan and Boldgiv 2020). In 82 mountain lakes in the Pyrenees, De Mendoza et al. (2012) found that temperature was the abiotic variable (out of 29 abiotic variables considered) that contributed most to explaining the distribution of species of *Platambus* Thomson along an elevational (thermal) gradient. Species belonging to this genus showed a preference for warmer lakes, consistent with other investigations (e.g., Eyre et al. 1986), while *Agabus* species were confined to colder lakes. The presence of fish (salmonids) and vegetation cover, however, taken individually, explained more variation in species distribution than temperature for most species, indicative of the difficulty in disentangling the interactive effects of multiple biotic and abiotic conditions. There, salmonids had a strong impact on medium size species, such as *Agabus bipustulatus*, despite this being considered a eurythermic species (Ribera et al. 1995a).

Based on distribution data available at the time, Winterbourn (1968) found that the maximum water temperatures at which dytiscids had been recorded ranged between 43 °C and 46 °C (Brues 1927; Mason 1939; Winterbourn and Brown 1967). Sánchez-Fernández et al. (2012) estimated the thermal niche of 12 species of *Deronectes* based on distributional and physiological data and found broad thermal ranges (from −10 °C to 54 °C) for these species in physiological experiments. These ranges were greater than those estimated using distributional data, suggesting that other environmental conditions and dispersal limitations associated with landscape complexity may be more important than thermal physiology in determining the realized niche of these species.

10.3.6 Water pH

Water pH has long been considered a major factor shaping the habitat preferences of dytiscids, with many studies showing a negative correlation between pH and species richness or abundances (e.g., Balfour-Browne 1940; Galewski 1971; Cuppen 1986; Eyre et al. 1986; Friday 1987; Juliano 1991). Dytiscids vary greatly in their tolerance to pH and some species have been recorded along broad pH ranges (e.g., Alarie and Leclair 1988; Juliano 1991; Foster et al. 1990, 1992; Foster 1995, 2010; Arnott et al. 2006; Enkhnasan and Boldgiv 2019), such as *Graphoderus liberus* (Say) (Arnott et al. 2006) or *Graphoderus zonatus* (Hoppe) (Foster 2010; Enkhnasan and Boldgiv 2020). Some species are non-acidic (pH 5.8–7.0; Alarie and Leclair 1988), with some being mainly associated with alkaline habitats. Examples include *Oreodytes* *alpinus* (Paykull), found in large lochs with a pH above 7.2 in Britain (Foster 2010), *Graphoderus elatus* Sharp, recorded in Khatuu River at a pH of 8.06, and *Oreodytes mongolicus* (Brinck), found in waterbodies with a pH ranging from 7.87 to 8.41 in central and western Mongolia (Enkhnasan and Boldgiv 2020).

Some species are strongly associated with acidic habitats (e.g., Hendrich 2001; Eyre et al. 1986; Alarie and Leclair 1988; Foster et al. 1992; Larson et al. 2000; Foster 2010). Acidophilic species include several *Hydroporus* species (Cuppen 1986; Foster et al. 1992; Nilsson and Holmen 1995), which have been found to colonize acidic habitats such as *Sphagnum* pools (pH ~ 4.0–5.0; Galewski 1971; pH ~ 3.9–4.3; Alarie and Leclair 1988). Among those, *Hydroporus rectus* Fall, which is one of the most characteristics species of peatlands within the boreal zone of North America (Larson 1975, 1987). In south-western Australia, several species of *Antiporus* Sharp appear to be restricted to acid peatland swamps and have been classified as acidophilic (Hendrich 2001). In the Arctic Ocean Basin in Mongolia, Enkhnasan and Boldgiv (2020) recorded *Hygrotus impressopunctatus* (Schaller), *Ilybius lateralis* (Gebler), and *Rhantus notaticollis* (Aubé) in an acidic pond with pH 3.85. Shatarnova (2021) examined the diversity and composition of water beetles in a Peat Bog in Belarus and found high dytiscid diversity in lakes but low in hollows. The latter were in fact characterized by the highest acidity, poor plant communities, compared to other systems in that landscape, and supported specialized dytiscid communities.

A tolerance to broad pH ranges observed for many species limits our capacity to predict the role of pH in determining pattern in dytiscid distribution across habitats, such as in boreal lakes (Nilsson and Söderberg 1996) or in ponds (Gioria et al. 2010a, b), and only under extreme conditions (very low pH) have strong correlations been observed (Friday 1987; Alarie and Leclair 1988; Foster et al. 1990; Larson et al. 2000; Arnott et al. 2006). Difficulties in identifying strong relationships between pH and the occurrence of dytiscids are also associated with the fact that, for many species, the effects of pH are mainly indirect, being mediated by biotic interactions such as predation. For instance, Arnott et al. (2006) examined the distribution of water beetles in relation to pH and presence/absence of fish in 29 lakes on the Canadian Shield and found that the occurrence of fish was more important than pH in determining the presence of some species. This was particularly true for *Graphoderus liberus*, which occurred across a wide range of pH in the absence of fish. Similarly, fish predation avoidance was likely the major cause of the presence of *Agabus labiatus* (Brahm) in contrasting habitats with respect to pH in Ireland, with individuals recorded from permanent, dystrophic waters and highly alkaline habitats, such as temporary turloughs and turlough-like pools (Foster et al. 1992).

Besides its indirect effects on predators, water pH can interact with other factors (Foster et al. 1990, 1992). For instance, in an investigation on the effects of calcium carbonate addition on invertebrate assemblages in peat pools, Foster (1995) observed weak effects of pH on the distribution of dytiscids, with many species that had been previously classified as acidophilic breeding successfully in calcium-enriched bog pools, suggesting that the association of certain species with acidic habitats, such as the boreal *Hydroporus morio* Aubé and *Agabus arcticus* (Paykull), is likely due to a broader tolerance to cold temperatures, a poor nutrient status, or a dependence on a soft, organic substratum, rather than a clear preference for acidic habitats. In this study, two main types of community were identified, one found at steep-sided edges of pools, dominated by odonate nymphs and large beetles, and one in shallower pools, which were dominated by *Hydroporus* species.

Mires (bogs, fens, marshes, and swamps) are wetlands that are widely distributed around the globe and can support high dytiscid diversity due to peculiarities in acidity/alkalinity and vegetation. Bogs or peatland obtain most of their water from rainfall (ombrotrophic) and are always acidic and nutrient-poor. Fens are a highly variable habitat that derive most of their water from soil or groundwater (minerotrophic) and may thus be slightly acidic, neutral, or alkaline, and either nutrient-poor or nutrient-rich (Wheeler and Proctor 2003). These systems support a variety of habitats, with *Hydroporus* species often dominating the dytiscid fauna (Shatarnova 2021). Several species of *Acilius*, *Agabus*, *Dytiscus*, *Ilybius*, *Laccophilus*, and *Rhantus* have been recorded in naturally acidic peatland habitats (Larson 1985; Larson et al. 2000; Shatarnova 2021). Fens are an especially important system for dytiscids (Foster 2010). Dytiscids found in fens include *Laccornis oblongus* (Stephens), which is confined to shallow, mossy areas of temporary base-rich fens (Foster 2010), several *Hydroporus* species, *Agabus biguttulus* (Thomson), *Liopterus haemorrhoidalis* (Fabricius) (former *Copelatus haemorrhoidalis*), as well as species that are typically found in peatland, such as *Hydaticus aruspex* Clark, *Hydrocolus rubyi* (Larson) and *Rhantus suturellus* (Harris) (Nilsson and Holmen 1995; Larson et al. 2000).

The effect of pH can differ substantially for adults and larvae. Experimental evidence indicates that larvae of certain species are tolerant to extremely low pH (e.g., *Dytiscus verticalis*, pH = 3.0; Frisbie and Dunson 1988). Juliano (1991) examined patterns of total and relative species abundance for *Hydroporus* species along a pH gradient in a long ditch in North Yorkshire, England, and found a decrease in total abundance of adult *Hydroporus* with decreases in pH, while larvae were most abundant at sites with the lowest pH and were absent where adults were most abundant. While there was no evidence of significant differences in species richness and evenness along the pH gradient, the number of adult *Hydroporus* individuals was higher in less acidic (pH = 5.6–6.2) upstream sites. In contrast, larvae were most abundant along the more acidic (pH = 4.5) downstream sites, suggesting that the absence of predators from these acidic sites was probably more important than the direct effect of pH in determining higher abundance of larvae, being these more susceptible to predation than adults (Juliano 1991).

10.3.7 Habitat Size, Depth, Steepness, and Shading

The effect of size on the distribution of dytiscids can be discussed at two levels, that of the waterbody itself and that of habitat within a waterbody. The size of a waterbody, expressed in terms of surface area or depth, is often strongly correlated with the richness and abundance of dytiscids. A positive relationship between surface area and dytiscid diversity has been reported (Nilsson 1984, 1986; Larson 1985; Fairchild et al. 2003), and strong nestedness patterns have been observed (Sect. 10.2), possibly due to a minimum habitat size requirement for some species (Kholin and Nilsson 1998). However, diversity patterns along gradients of surface area in lentic waterbodies, from pools to lakes, have often been described by a unimodal humpbacked function, with more species and individuals being found in intermediate-size systems, such as ponds, than in large permanent lakes (e.g., Larson 1985; Ranta 1985; Nilsson 1984, 1986; Nilsson and Svensson 1994; Whiteman and Sites 2003; Gioria et al. 2010a). For instance, examination of 12 wetlands in an agricultural landscape in south-eastern Sweden showed that species-area relationships were weak, but species richness was highest in intermediate-size wetlands (Lundkvist et al. 2001). Similarly, in 45 permanent ponds in two agricultural landscapes in Ireland, surface area was only a moderate predictor of species richness and composition of dytiscids, with species richness being higher from intermediate-size ponds (80–120 m^2; 18–22 species) (Gioria et al. 2010a) than in small ponds (<25 m^2; up to 13 species) and larger ponds (120–200 m^2; 10–21 species). Overall, the effect of habitat depth on dytiscid diversity was greater than that of surface area, with shallow habitats being generally richer than deep ones, likely due to a moderate correlation between depth with the structure of predators and with the physical structure provided by the vegetation, which was denser in shallower habitat (Fig. 10.12).

Whether size matters in determining the distribution of dytiscids is not always clear (Oertli et al. 2002). This might be due to a potentially strong correlation between waterbody size and habitat complexity, including the number and types of available habitats, the structure of the vegetation, predators, and food resources. Thus, large waterbodies might support more dytiscid species because they provide more habitat types, although most species prefer shallower and densely vegetated ones (e.g., Fairchild et al. 2000, 2003; Heino 2000; Gioria et al. 2010a; Megna et al. 2019). In small systems supporting one prevailing habitat type, however, size might

Fig. 10.12 Species-rich, dense plant community at the margins of a permanent pond supporting 18 dytiscid species (County Wexford, Ireland; Photo by Margherita Gioria)

be a key determinant of habitat suitability (Gioria et al. 2010a; Roth et al. 2020). Nilsson and Svensson (1994, 1995) showed that higher pool size in snowmelt pools was associated with a higher number of guilds and higher diversity within guilds. In over 100 ombrotrophic bog pools in Newfoundland, Larson and House (1990) found a positive and significant correlation between the size of adult dytiscids and pool size, with small species generally occurring in small pools, except for two *Hydroporus* species that occurred in moss along the water margin.

The presence and abundance of predators strongly affect the relationship between dytiscid diversity and habitat size (Sect. 10.4.1). The presence of fish in larger or deeper habitats may force some species to colonize smaller and shallower habitats to avoid fish predation. For instance, *Dytiscus marginalis* was recorded in small, shallow ponds at high altitudes in the Pyrenees (Ribera et al. 1997), despite a general preference for relatively deep, open waters (Frelik 2014a) as well as an optimum depth of ca. 60 cm in the area (Ribera et al. 1995a). This species was also recorded in a shallow seepage (Gioria 2002; Fig. 10.13), likely to avoid fish predation in adjacent permanent wetlands, as well as in a densely vegetated small pond (Fig. 10.14). Foster (1995) found that *Hydroporus* species were most abundant in shallow bog pools, while larger species, such as *Agabus* and *Ilybius* species, were more abundant in deeper bog pools. Such an effect was likely mediated by biotic interactions, since deeper pools were dominated by odonates, to which smaller species such as *Hydroporus* species are highly susceptible. Similarly, in a long ditch, Juliano (1991) found that larvae and adults of *Hydroporus* species were

Fig. 10.13 Seepage located in a karstic region in western Ireland (Burren, County Clare), where an adult specimen of *Dytiscus marginalis* was recorded

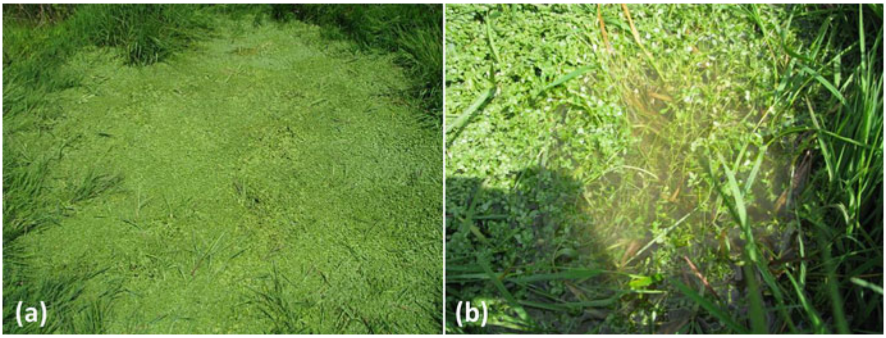

Fig. 10.14 (**a**) Temporary pool with (**b**) details of its dense vegetation (County Kildare, Ireland). A male individual of *Dytiscus marginalis* was recorded from this small pond or pool (diameter ~ 2.5 m, depth ~ 40 cm) together with three *Hydroporus* species and *Hyphydrus ovatus* (Photo by Margherita Gioria)

more abundant in shallow water, possibly to avoid predation by odonates and notonectids and by larger dytiscids (see also Fairchild et al. 2003), although differences in the distribution of prey and a capacity to replace their oxygen storage more easily in shallow waters in small species might have also contributed to these patterns.

The effect of habitat steepness on the distribution and diversity of dytiscids is likely indirect, given its correlation with other habitat variables, such as depth, temperature, light, and nutrient levels. Also, shallow water plants (Fig. 10.12), which are known to support diverse dytiscid communities (e.g., Foster et al. 1992; Fairchild et al. 2003; Gioria et al. 2010a), tend not to grow on the margins of steep-sided habitats (Newbold et al. 1989; Painter 1999). In general, shallow waterbodies with gentle profiles support high dytiscid diversity compared to steep-sided lentic systems (Nilsson et al. 1994; Gioria et al. 2010a). However, in agricultural landscapes, grazing is a major habitat modifier of pond and stream margins, affecting both plant and dytiscid communities (Fig. 10.15; Gioria et al. 2010a). Dispersal limitation in habitats with steep margins might also affect the distribution of dytiscids (Yee et al. 2009). Recently, Liao et al. (2020) examined the dytiscid fauna of 25 ponds with (11 ponds; Fig. 10.16) or without (14 ponds; Fig. 10.17) fish in two urban areas in Finland, and found that steepness of pond margins and the presence (*versus* absence) of predatory fish interacted in determining species richness and abundance, with dytiscids preferring ponds with gently sloping margins and being richer (80%) and more abundant (79%) in fishless ponds than in pond with fish, although medium to large-sized species were more capable of coexisting with fish.

Waterbodies exposed to sunlight are more visible to migrating dytiscids than shaded ones (Nilsson and Svensson 1995; Schäfer et al. 2006), so that, in general, more species have been recorded in open, sun-exposed wetlands (e.g., Nilsson and Svensson 1994; Gee et al. 1997; Fairchild et al. 2003; Schäfer et al. 2006). This is

Fig. 10.15 Permanent pond grazed by cattle, with marginal and emergent vegetation being suppressed and high nutrient levels being recorded. Only five dytiscid species (two *Hydroporus* species, *Agabus nebulosus*, *Hyphydrus ovatus,* and *Rhantus frontalis*) were found in this pond, despite high dytiscid diversity being recorded from temporary and permanent ponds and pools in its proximity (County Wexford, Ireland) (photo by Margherita Gioria)

Fig. 10.16 Urban ponds in the Helsinki Metropolitan Area, Finland, where fish was present. Dytiscids species were found in the marginal vegetation (Liao et al. 2020; Photos by Wenfei Liao)

Fig. 10.17 Urban fishless ponds in the Helsinki Metropolitan Area, Finland. Dytiscids species were found in vegetated and non-vegetated habitats. Poor fliers such as *Graphoderus* species were recorded from the top pond, located in a less urbanized area of Helsinki (Liao et al. 2020; Photos by Wenfei Liao)

reflected in the fact that dispersal occurs more frequently in open than forested landscapes (Nilsson and Svensson 1995; Lundkvist et al. 2002; Schäfer et al. 2006) or urban landscapes (Lundkvist et al. 2002; Liao et al. 2020), although how dispersing dytiscids detect suitable habitats remains largely unknown (Bilton 2014; Chap. 11). Species that prefer shaded habitats to unshaded ones include *Hydroporus striola* (Gyllenhal), which was found to dominate temporary forested wetlands in southern Sweden (Lundkvist et al. 2001) and urban wetlands (Lundkvist et al. 2002), and those inhabiting woodland pools (Nilsson and Holmen 1995; Larson et al. 2000). In addition to affecting dispersal, the degree of shading determines the temperature, vegetation structure, and productivity of a habitat (Lundkvist et al. 2001; Schäfer et al. 2006; Vinnersten et al. 2009), especially where large amounts of debris accumulate on the substrate, often leading to eutrophic and even hypertrophic conditions (Gioria et al. 2010a; Liao et al. 2020; Fig. 10.18). Tree leaf litter inputs to lentic habitats from adjacent plant communities can have a strong effect on dytiscids (Pintar and Resetarits 2017). This is especially true in small, ephemeral, fishless ponds, where dytiscids tend to be the dominant water beetle group (Jeffries 1994; Schneider and Frost 1996; Fairchild et al. 2000, 2003). There, resource quality and abundance can be the most important factor affecting the habitat selection preferences of colonizing dytiscids. Pintar and Resetarits (2017) examined how water beetles respond over time to variation in tree leaf litter composition of pine (slower-decomposing) or hardwood (faster-decomposing) in small fishless ponds. They found that colonization by dytiscids did not differ between pine and hardwood. However, species composition differed and changed through time as habitat conditions varied due to decomposition processes, indicative of the importance of tree leaf decomposition in driving community composition (Pintar and Resetarits 2017).

Fig. 10.18 Eutrophic pond surrounded by dense vegetation causing a major input of leaf litter and subsequent high nutrient levels. This pond supported a relatively species-poor dytiscid community (County Limerick, Ireland; Photo by Margherita Gioria)

10.3.8 Anthropogenic Habitat Degradation, Nutrients, and Pollution

Habitat degradation linked to pollution, eutrophication, and hydrological changes associated with changes in land use, or the intensification of agricultural activities, can have a strong impact on dytiscids (Gioria et al. 2010a; Roth et al. 2020). Roth et al. (2020) examined composition and ecological trends in water beetle communities of Southern Germany, Central Europe, in 33 waterbodies over 28 years, from 1991 to 2018, and found a decrease in the number of species and abundances of many dytiscid species over time, likely due to increased nitrification and/or mineralization, habitat loss and, in general, human-related activities. Nutrient concentrations interact with several habitat variables, such as productivity, vegetation structure, dissolved oxygen, turbidity, and chlorophyll, and are strongly influenced by management practices and land use history (Gioria et al. 2010a). Habitat productivity, in turn, affects the availability and quality of food, the structure of predators, and competitive interactions. Some dytiscids are tolerant of high nutrient concentrations and often represent the dominant water beetle group in farmland ponds (Foster et al. 1992; Painter 1999; Fairchild et al. 2000; Larson et al. 2000; Pakulnicka

Fig. 10.19 Farmland pond supporting diverse dytiscid communities in vegetated habitats with low algal formation (County Limerick, Ireland; Photo by Margherita Gioria)

2008; Gioria et al. 2010a; Verdonschot et al. 2011; Silver and Vamosi 2012; Pakulnicka et al. 2016a; Rolke et al. 2018; Fig. 10.19). Some species have been recorded from pond margins characterized by dense mats of algae (e.g., *Hyphydrus ovatus* (L.); Gioria et al. 2010a; Fig. 10.20) as well as in constructed wetlands created to reducing pollution from nutrients (Fig. 10.21), with *Hydroporus* species dominating these systems (Becerra-Jurado et al. 2009) (see Sect. 10.5.1). While they might differ substantially in their tolerance to nutrient conditions (e.g., Nilsson et al. 1994; Nilsson and Holmen 1995; Foster et al. 1992), dytiscid species recorded in oligotrophic habitats, however, are not generally found in eutrophic habitats (Rolke et al. 2018).

Given their role as predators in the food web, dytiscids are prone to accumulating trace elements and vary substantially in their capacity to cope with and uptake pollution and heavy metals. Burghelea et al. (2011) showed that *Rhantus suturalis* is a suitable bioindicator of trace element pollution in paddy fields, given its high capacity to bioaccumulate Al, Mo and Pb. In contrast, *Laccophilus minutus* (L.) was prone to Se accumulation in reservoirs, while *Hydroglyphus geminus* (Fabricius) exhibited the highest metal uptake in both paddy fields and reservoirs. Aydoğan et al. (2018) examined heavy element accumulation levels by seven *Agabus* species collected from freshwater habitats in four cities in Turkey, in the shallow parts of springs, streams, lakes, ponds, brook, and puddles, and found that *Agabus didymus*

Fig. 10.20 Eutrophic permanent pond dominated by *Cladophora* algae. Despite the high nutrient levels and the presence of a tick mat of algae, seven dytiscid species, belonging to the genera *Agabus*, *Hydroporus*, *Hyphydrus,* and *Ilybius* were recorded from the emergent vegetation (County Wexford, Ireland; Photo by Margherita Gioria)

(Olivier) was the best accumulator of Ca, Ti, V, Cu, As, Se, and Pb, while *Agabus bipustulatus* was the best accumulator of Cr, Mn, Fe, Ni, Zn, and Br. In five cities in Turkey, Erman (2011) found that concentrations of Na, As, Br, and Ba differed significantly between *Dytiscus thianschanicus* (Gschwendtner) and *Dytiscus persicus* Wehncke. For the former, significant differences in Mn and I were also reported between males and females, possibly due to metabolic differences. Differences in concentrations of inorganic elements that were observed among localities suggest that the content of non-essential elements in the body of some dytiscids could be used to evaluate the level of these elements in different waterbodies (Erman 2011). Traces of toxic heavy metals have been reported in *Dytiscus marginalis* by Choudhury et al. (2020) in Assam, India, where this species is commonly consumed by the Bodo tribe. There is evidence that *Dytiscus circumcinctus* Ahrens and *Cybister lateralimarginalis* accumulate mercury, with accumulation probably beginning at the larval stage and mercury not being excreted from the body during the pupal stage (Udodenko et al. 2019).

Fig. 10.21 Pond belonging to a constructed wetland system aimed at reducing pollution from nutrients in a deer farm (County Waterford, Ireland; Photo by Margherita Gioria)

10.4 Biotic Interactions

Physico-chemical properties determine the identity of the species that may permanently or temporarily use certain habitats or waterbodies, depending on their tolerance to one or more abiotic conditions, which define their physiological or fundamental niche. However, individual abiotic habitat conditions have often a low capacity to explain or predict distribution of dytiscids across habitats, especially in the absence of extreme abiotic conditions (e.g., Larson et al. 2000; Arnott et al. 2006; Eyre et al. 2006; Yee et al. 2009; Gioria et al. 2010b). In contrast, biotic interactions play a prominent role in determining the habitat preferences of dytiscids (e.g., Ranta 1985; Larson 1990; Nilsson and Svensson 1994; Nilsson and Söderberg 1996; Bosi 2001; Arnott et al. 2006; Gioria et al. 2010a, 2011; Liao et al. 2020). What follows is a brief discussion of the contribution of plant–dytiscid relationships and predator–prey interactions in determining habitat suitability (the latter are described in detail in Chap. 8).

10.4.1 Vegetation Structure as a Major Habitat Factor

Aquatic and terrestrial plants are a major component of habitat structure and complexity (e.g., Ranta 1985; Friday 1987; Foster et al. 1990, 1992; Nilsson et al. 1994; Gioria et al. 2010a; Wohlfahrt and Vamosi 2012) and play several functional roles for dytiscids in aquatic habitats and at the interface of aquatic-terrestrial habitats (e.g., Nilsson et al. 1994; Fairchild et al. 2000; Gioria et al. 2010a, 2011). Plants determine the physical structure of a habitat, providing oviposition sites as well as refugia against predators, and may mitigate any potential negative effect of inter- and intra-specific competition (e.g., Crowson 1981; Foster et al. 1992; Gee et al. 1997; Painter 1999; McAbendroth et al. 2005; Gioria et al. 2010a; Yee 2010; De Mendoza et al. 2012; Liao et al. 2020). Plants also affect the stability of a habitat's substrate, the cycling of nutrients, and filter the amount of nutrients and pollutants reaching the water (e.g., Becerra-Jurado et al. 2009; see Keddy 2000 for a review). For some species, plants represent a food source (Deding 1988; Yee 2010; Frelik 2014b). Dytiscids are thus more frequently found in vegetated and shallow habitats than deep open waters (Nilsson and Holmen 1995; Heino 2000; Larson et al. 2000; Gioria et al. 2010a; Balke et al. 2019; Megna et al. 2019).

The structure of the vegetation can be defined by various properties, including plant diversity, plant biomass, cover or density, the presence and size of vegetation gaps, plant rigidity, the number and arrangement of stems and leaves, and shoot density, among others (e.g., Nilsson et al. 1994; McAbendroth et al. 2005; Paquette and Alarie 1999; Yee et al. 2009; Gioria et al. 2010a; Tokeshi and Arakaki 2012). The effects of the vegetation on dytiscids depend on species-specific swimming strategies (see Ribera and Nilsson 1995 for a comprehensive study on morphometric patterns of diving beetles). In general, habitats characterized by a complex vegetation structure associated with high plant diversity tend to support species-rich dytiscid assemblages (Nilsson et al. 1994; Downie et al. 1998; Painter 1999; Fairchild et al. 2000; Armitage et al. 2003; Gioria et al. 2010a). In a quantitative evaluation of plant–beetle relationships in 54 farmland ponds in Ireland, Gioria et al. (2010a, 2011) found a statistically moderate (~47%) but biologically high correlation between species richness of vascular plants and that of dytiscids. This correlation was stronger than that observed between plants and water beetles in general, suggesting that the complexity of the vegetation is more important for dytiscids compared to other water beetles. Plant species composition and, to a lesser extent, plant community type composition (*sensu* Rodwell 1995) were also good predictors of species richness and composition. A positive power to predict dytiscid composition observed for plants was likely due to a strong similarity in the response of these groups to abiotic conditions, indicative of a capacity for the vegetation to summarize important information on local habitat conditions. In those ponds, *Juncus* species were characteristic of highly degraded, eutrophic conditions found in ponds grazed by cattle and were good predictors of the presence of species such as *Hyphydrus ovatus, Hydroporus planus* (Fabricius), *H. palustris* (L.), and *Agabus nebulosus* (Forster). In contrast, plant communities dominated by *Typha latifolia* L. were good

Fig. 10.22 Permanent pond characterized by a species-poor plant community dominated by *Typha latifolia*, supporting 22 dytiscid species (County Wexford, Ireland; Photo by Margherita Gioria)

indicators of high dytiscid diversity, often supporting more than 20 species. A good correlation between plant community type and dytiscid species composition and diversity suggests that, even though the majority of dytiscids are not host specific, some plant community types provide conditions that are ideal for the colonization and coexistence of many dytiscids (Gioria et al. 2010a). The beneficial effect of *Typha* on dytiscid species richness is likely associated with the provision of an ideal physical structure for many species since it affords shelter from predators while allowing free movement for active species. These findings are consistent with evidence that cattail ponds dominated by *Typha* species support highly diverse and abundant dytiscid communities (Paquette and Alarie 1999) as well as rare and endangered species (Kolar and Boukal 2020) (Fig. 10.22). Sedge-cattail marshes and swamps also represent a suitable habitat for dytiscids (Hilsenhoff 1993).

Plants may play a critical role as refugia from predators. For instance, an examination of the effects of the vegetation on dytiscids in the presence or absence of predatory fish in urban ponds in Finland showed that, at the pond level, the diversity and abundance of dytiscids were positively associated with increasing plant cover in ponds with fish but not in those without fish (Liao et al., unpublished). For dytiscids, the capacity of the vegetation to provide shelter from predators is dependent on morphometric patterns and swimming behaviour (McAbendroth et al. 2005). In their study on dytiscid communities in mountain lakes in the Pyrenees, De

Mendoza et al. (2012) found that even sparsely vegetated lakes could act as refugia for dytiscids. The refuge effect of the presence of some vegetation in mountain lakes was, however, highly species-specific, being stronger for *Boreonectes ibericus* (Dutton and Angus) but not for *Agabus*, *Platambus* Thomson, and *Hydroporus* species. Some species tolerated dense vegetation, such as *Graphoderus cinereus* (L.), which is usually found in fens or ponds with dense vegetation and, compared to other Dytiscinae Leach, is considered to be adapted to crawl among dense vegetation or detritus (Ribera and Nilsson 1995; De Mendoza et al. 2012). Besides acting as refugia from predators, plants may also offer dytiscids some protection against wave action. For instance, most species recorded in 98 lakes in northern Sweden were found more frequently in samples from protected sites with vegetation than in those from exposed sites without vegetation (Nilsson and Söderberg 1996).

Mosses also play an important role in determining habitat suitability for dytiscids. Many species having been recorded from mosses in lentic and lotic habitats (Figs. 10.8 and 10.23), springs, mossy hollows in bogs or forests, or at the margins of lakes and ponds, and in temporary alkaline fens (e.g., Nilsson and Holmen 1995; Heino 2000; Larson et al. 2000; Foster 2010). Mono-specific habitats composed of *Sphagnum* moss are known to support high densities of *Agabus* and *Hydroporus* species (e.g., Foster et al. 1992; Nilsson and Holmen 1995; Larson et al. 2000; Gioria 2002). In contrast, relatively few species are found in filamentous algal formations. These include *Hydrotrupes palpalis* Sharp, a lotic species also recorded in hygropetric habitats, and *Liodessus flavicollis* (LeConte), which was found in algal mats in relatively deep water, from clear-water pools or ponds with sandy substrates (Larson et al. 2000). In lentic habitats, in fact, the presence of dense algal formations in the water or on its surface can be highly detrimental to dytiscids. For instance, only few specimens of *Hydroporus planus* and *H. palustris* were recorded from ponds characterized by highly dense *Cladophora* formations (Gioria et al. 2010a), despite the proximity of waterbodies supporting diverse dytiscid communities (Fig. 10.20).

10.4.2 Predation, Food Resources, and Competition

Predation represents a major mechanism determining the realized niche of dytiscids. Depending on the characteristics of a specific habitat and on their life stage, dytiscids can be predators and/or prey. Many species are highly susceptible to predation by other aquatic predators, particularly fish and Odonata larvae (e.g., Ranta 1985; Larson 1988, 1990; Bosi 2001; Wohlfahrt and Vamosi 2012; Vamosi and Wohlfahrt 2014; Chap. 8). These predators, like their dytiscid prey, are typically distributed along environmental gradients. The identity and abundance of predators may change within small differences in permanency (Eyre et al. 1992; Jeffries 1994; Larson 1985), with the potential pool of predator species typically decreasing with decreases in habitat permanency and stability (Wellborn et al. 1996). Fish and odonate larvae may have different effects on dytiscid composition and habitat preferences. In

Fig. 10.23 Lotic semi-permanent habitats where several *Hydroporus* species have been recorded. (**a**) Temporary streams where dytiscids were found within marginal mosses. (**b**, **c**) Temporary ditch along a mountain footpath, creating shallow vegetated habitats of slow-moving water (Piedmont, Italy; Photos by Margherita Gioria)

permanent prairie ponds in two Canadian regions, Wohlfahrt and Vamosi (2012) found that the presence of fish alone was a major driver of dytiscid composition. In contrast, the effect of large predaceous odonate larvae interacted with pond surface area, with the latter being the most important variable determining dytiscid composition.

Several studies have shown that dytiscids are found in lower densities in habitats where fish are present, with many species displaying a strong fish predation avoidance behaviour. Species that are highly susceptible to fish predation are known to migrate to temporary habitats during the wet phase or to newly created habitats, even when abiotic conditions are suboptimal, such as more acidic, shallower, or colder habitats compared to those they would occupy based on their physiological requirements (Wiggins et al. 1980; Jeffries and Lawton 1984; Foster et al. 1992; Foster 1995; Larson 1997b; Fairchild et al. 2003; Arnott et al. 2006; De Mendoza et al. 2012). Thus, fishless lakes and ponds generally support higher dytiscid diversity than those where fish is present, with some species being found exclusively in fishless habitats (e.g., Arnott et al. 2006; Schilling et al. 2009; Gioria et al. 2010a; Liao et al. 2020). Some species can sense the presence of fish by using chemical cues, such as *Acilius sulcatus* (Åbjörnsson et al. 1997), which can be found in temporary habitats despite a preference for large, open waters (Silver and Vamosi 2012). Similarly, its presence in a newly created pond and simultaneous absence from permanent, fish-stocked ponds in its proximity is indicative of a strong fish-avoidance behaviour for this species (Gioria et al. 2010a). The effects of fish introduction on dytiscids have also been observed in high elevation lakes, where temperature is the most important abiotic habitat factor (Knapp et al. 2001; Bradford et al. 1998; De Mendoza et al. 2012). In those lakes, predation constrains the distribution of certain species to a lower number of habitats compared to those that could be occupied based on their thermal response.

The susceptibility of dytiscids to fish predation is highly species-specific and is generally assumed to be a function of body size (mean body length), level of activity, and macrophyte cover, among others (Juliano and Lawton 1990; De Mendoza et al. 2012). The presence of fish represents a major constraint, especially for intermediate to large species, which are in contrast successful in fishless habitat (e.g., Wellborn et al. 1996; Knapp et al. 2001; Schilling et al. 2009; Arnott et al. 2006; De Mendoza et al. 2012), while smaller species can cope with fish predation and are frequently recorded in habitats where fish are present. Thus, in the absence of fish, dytiscid biomass is generally high, body size is large, and dytiscid larvae are abundant (e.g., Fairchild et al. 2003; Arnott et al. 2006; De Mendoza et al. 2012). Knapp et al. (2001) examined the fauna of over 500 alpine lakes in the Sierra Nevada of eastern California (never-stocked, stocked-fish-present, and stocked-now-fishless lakes) and found that trout introduction into fishless lakes caused remarkable reduction in dytiscid abundances in the presence of fish. The negative effect of trout introduction was lower on small-bodied species, such as the Hydroporini, which recovered after the disappearance of trout in stocked-now-fishless lakes, while *Agabus* species did not recover. These findings suggest that observed differences in dytiscid

Fig. 10.24 Densely vegetated within permanent ponds where diverse dytiscid communities (21 species) have been recorded despite the presence of fish (County Wexford, Ireland; Photo by Margherita Gioria)

communities between these three lake categories were largely the result of fish stocking history and not due to species-specific habitat requirements (Knapp et al. 2001).

Swimming behaviour also affects the susceptibility of dytiscids to predation and thus contributes to determining their habitat preferences. Species that coexist with fish are typically less active than those facing only predatory invertebrates (mainly odonate) and generally restrict their habitat use to vegetated areas (Fig. 10.24), despite a possible preference for more open or deeper habitats (e.g., Foster 1995; Gioria et al. 2010a). In Pyrenean mountain lakes, a strong negative effect of fish predation observed on *Agabus bipustulatus* but not on *Platambus maculatus* (L.) was likely associated with differences in their swimming behaviour despite both being medium size species (De Mendoza et al. 2012); while the former is a good swimmer and prefers lentic habitats (Ribera and Nilsson 1995), the latter is a poor swimmer, with a preference for lotic ones (Ribera and Nilsson 1995; Ribera et al. 1995b).

Most dytiscid larvae are highly susceptible to predation by odonate larvae (Larson 1985, 1988, 1990; Nilsson 1986; Bosi 2001; Liao et al. 2020), while adults are relatively protected by size, hard cuticle, and, possibly, defensive secretions (Larson 1990). In fishless habitats, smaller species are more susceptible to predation by odonate larvae compared to larger species (e.g., Larson 1990; Wellborn et al. 1996). Along a gradient of permanency and size, predation by odonate larvae may promote the use of larger, less temporary pools by dytiscids. In Newfoundland (Canada), Larson (1990) found that, in certain bog pools, the density of odonate larvae was sufficient to eliminate the presence of vulnerable dytiscids within a matter of days. Predation on larvae was responsible for the differences in the structure of dytiscid communities between two neighbouring marshes in the Eastern plain of the River Po, Italy (Bosi 2001). Larson (1997b) found that in paddy fields and in a borrow pit near a storage reservoir, dytiscids were abundant early in the development of the habitat, bred rapidly, and the larval stages of the smaller species were completed before odonate larvae had become established. Some genera, however, occur regularly with dense populations of these predators. These include large species of *Dytiscus* (Larson 1990; Gioria 2002) and *Cybister* Curtis, *Thermonectus* Dejean species, whose larvae are pelagic and occupy different zones from those occupied by odonate larvae, and *Bidessus* Sharp species, which are very small and generally occur among dense detritus, mosses, or algal mats, or close to the water edge in shallow zones, where populations of odonate larvae tend to be low (Larson 1990).

Food availability and quality is a major habitat variable affecting dytiscids and an important dimension of habitat complexity. Dytiscids are typically considered as generalist predators, and some species have adults that are also scavengers, while larvae are strictly carnivorous (e.g., Nilsson and Svensson 1994, 1995; Larson et al. 2000; Bosi 2001; Nilsson 2001; Pakulnicka 2008; Cobbaert et al. 2010; Yee 2010, 2014; Culler et al. 2014; Frelik 2014b). Yet, for some species, prey identity may be highly important in defining their realized niche (e.g., Deding 1988; Nilsson and Svensson 1994; Lundkvist et al. 2003; Culler and Lamp 2009; Culler et al. 2014). The importance of intra- and interspecific competition for food resource in determining patterns in diversity and community composition is dependent on whether such resources are available in limited supply (Nilsson 1986; Yee 2010). Understanding how food sources and prey consumption affects habitat suitability and the distribution of species across habitats via their effects on intra- and interspecific competition is difficult to assess under natural conditions (Pitcher and Yee 2014). Juliano and Lawton (1990) found that morphological size did not influence competition among dytiscids in a large canal. There, competition for food had density-dependence effects on larvae of *Hydroporus*, which was likely responsible for maintaining the density of adults low, thus minimizing interspecific competition among adults.

10.5 Important Habitats in Anthropogenic Landscapes

10.5.1 Agricultural Habitats

Agricultural landscapes support high dytiscid diversity in several regions (e.g., Foster et al. 1990; Lundkvist et al. 2001, 2002; Gioria et al. 2010a). These landscapes can support a variety of permanent and temporary habitats, and, where habitat connectivity is high, colonization rates are also high (Gioria et al. 2010a). Freshwater systems in agricultural landscapes include temporary and permanent ponds, streams and rivers, drainage and roadside ditches (Foster et al. 1990; Rolke et al. 2018), zacallones (i.e., artificially deepened ponds that supply water for cattle and wild fauna during summer in Spain; Florencio et al. 2014), as well as small habitats such as cattle troughs (e.g., *Agabus nebulosus* and *Hydroporus planus*, M. Gioria, unpublished) and livestock drinking pools (Corsetti and Nardi 2008). Constructed wetlands, including integrated constructed ponds that are used for various water treatments, such as agricultural waste, can improve habitat connectivity in agricultural landscapes and can be colonized by diverse dytiscid communities within few years (Becerra-Jurado et al. 2009). Even highly disturbed systems can play an important conservation role for dytiscids. In Japan, paddy fields have been found to support a 'Near Threatened' species, *Hydaticus bowringii* Clark, which showed a preference to feed on tadpoles rather than insects (Watanabe et al. 2020). Kolar and Boukal (2020) found that extensively managed fishponds provide suitable habitats for the endangered species *Graphoderus bilineatus* (De Geer) in the Czech Republic, a species that is decreasing throughout Europe.

Among lotic habitats, drainage ditches represent an important habitat for dytiscids, with different ditch types being recognized, depending on factors such as pH, nitrate concentrations, permanency, salinity, and vegetation management practices (Foster et al. 1990). Agricultural drainage ditches can support high diversity, with dytiscids dominating the beetle fauna of these systems (Foster et al. 1992; Fairchild et al. 2000; Pakulnicka 2008; Gioria et al. 2010a; Verdonschot et al. 2011; Pakulnicka et al. 2016a), as well as uncommon, threatened, and rare species, such as in traditionally managed and grazing fens in England (Painter 1999) and in drainage ditches in northeast Germany (Rolke et al. 2018).

Despite the potential conservation role of agricultural habitats, livestock grazing can have a negative effect on the diversity and abundance of dytiscid communities (Gioria et al. 2010a; Silver and Vamosi 2012). Grazing can affect dytiscids by increasing nutrient levels through the deposition of cow dung either on the marginal vegetation or directly in the water and by trampling. Trampling by cattle directly affects those species whose larvae occur at the edge of the water in damp soils. Grazing and trampling also suppress the growth of marginal and emergent plants, simplifying habitat structure and increasing turbidity, due to the creation of open zones of bare ground (Gioria et al. 2010a, Fig. 10.15). Grazing was the most important determinant of dytiscid species composition and richness in 54 farmland ponds in Ireland, with grazed ponds supporting significantly less species and individuals than non-grazed ponds. Grazed ponds supported few species, which dominated these systems, such as *Hydroporus planus*, *Agabus nebulosus,* and *Agabus bipustulatus*, which are relatively tolerant of eutrophic conditions (Eyre et al. 1986; Foster et al. 1992; Foster and Eyre 1992; Foster 2010). The negative effect of trampling on dytiscids was evident when a comparison between grazed and fenced ponds was made. The presence of a fence preventing direct access of cattle to the pond margins was, in fact, only beneficial when the distance between the pond margin and the fence allowed the growth of some emergent and marginal vegetation. Evidence of the detrimental effect of grazing on species richness and abundance was also provided by Silver and Vamosi (2012), who examined 13 temporary wetlands subjected to rotational grazing in Alberta, with no dytiscids occurring in early grazed wetlands (wetlands that are grazed during the wet phase). In contrast, *Dytiscus*, *Ilybius*, and *Rhantus* species were indicators of late grazed wetlands, i.e., wetlands that were grazed during the dry phase.

10.5.2 Artificial and Urban Habitats

Evidence collected over the past couple of decades indicates that urban wetlands, created as part of a green infrastructure, can play an important role in the conservation of dytiscids. In their examination in the Helsinki Metropolitan Area, Finland, Liao et al. (2020) showed that urban artificial wetlands can be critical to maintaining dytiscid diversity at the regional level and in urban areas (Figs. 10.16 and 10.17). These authors showed that species richness was related to specific habitat features such as the presence of gently sloping margins *versus* that of steep pond margins, as well as the absence of fish, suggesting that the creation of blue infrastructure must include a diverse range of ponds and wetland habitats if the aim is to promote conservation in urban areas. Their findings support evidence provided by Lundkvist et al. (2002) in two urban landscapes in Sweden, where urban wetlands differing in size, vegetation, and habitat complexity had been recently created. Although diversity in these wetlands was lower than in a close agricultural landscape, they supported dytiscid communities that are infrequent in agricultural landscapes, indicative of their importance in the conservation of dytiscids at the regional level. Additional artificial habitats to those described above and supporting dytiscids include dam and dam lakes (Biström et al. 2015), fish-pond complexes (Buczyńska et al. 2007), fish ponds in gardens and demesnes (M. Gioria, unpublished; Fig. 10.25), ponds created in golf courses (Burke 2010), quarry ponds (Fig. 10.26) and pools (Larson et al. 2000; Biström et al. 2015), roadside ditches (Foster et al. 1990; Shaverdo and Roughley 2011) and other man-made ditch types (Fig. 10.23), drinking fountains, wells and tanks (Corsetti and Nardi 2008), and plastic containers (Fig. 10.27), among others (Larson et al. 2000).

10.6 Peculiar Habitats for Dytiscids

10.6.1 Subterranean Habitats

Subterranean habitats support a high diversity of stygobitic dytiscids, i.e., obligate subterranean species with adaptations for life in wells, boreholes, and caves characterized by complete darkness (Chap. 9, Leys et al. 2003; Balke et al. 2004; Watts et al. 2007; Miller et al. 2009). This is especially true in Western Australia, where an exceptionally diverse subterranean dytiscid fauna has been reported, due to its large network of paleodrainages (e.g., Leys et al. 2003; Humphreys 2008; Watts and Humphreys 2009; Balke et al. 2004; Watts et al. 2007; Watts and Leys 2005; Leys and Watts 2008; Eberhard et al. 2016). There, several species have been recorded from groundwater estuaries of salt lakes and shallow calcretes, i.e., carbonate deposits whose formation is directly associated with groundwater (e.g., Watts and Humphreys 1999, 2000, 2001, 2003, 2004, 2006, 2009; Watts et al. 2007, 2008; Balke et al. 2004; Leys et al. 2010). In the Yilgarn region of Western Australia and

Fig. 10.25 Permanent pond created by the side of Lake Owel (County Westmeath, Ireland) supporting 18 dytiscid species, compared to only three species recorded from the lake margins

the Ngalia basin in central Australia, more than 100 stygobitic dytiscid species have been recorded from calcretes on inland and coastal drainages (Watts and Humphreys 2006, 2009; Watts et al. 2007) and new species are being reported as the search for subterranean diversity continues (Eberhard et al. 2016). While most species have been recorded from northern, western, and central Australia (Watts and Humphreys 2003, 2004, 2006, 2009), truly stygobitic species have also been found in eastern (Watts et al. 2007, 2008) and southern Australia (*Paroster extraordinarius* Leys, Roudnew and Watts; Leys et al. 2010).

Subterranean dytiscids have been recorded in several world regions (e.g., Peschet 1932; Uéno 1957; Sanfilippo 1958; Ordish 1976, 1991; Young and Longley 1976; Franciscolo 1979; Castro and Delgado 2001; Leys et al. 2003; Miller et al. 2009; Spangler 1986, 1996; Larson and LaBonte 1994; Spangler and Barr 1995; Alarie and Wewalka 2001; Balke et al. 2004; Wewalka et al. 2007; Robertson et al. 2008; Deharveng et al. 2009; Jean et al. 2012; Eberhard et al. 2016; Kanda et al. 2016; Ribera and Reboleira 2019; Nilsson and Hájek 2022). Comparatively few species have been found in North America (Young and Longley 1976; Larson and LaBonte 1994; Spangler and Barr 1995; Miller et al. 2009; Jean et al. 2012; Kanda et al. 2016) and in Europe (Castro and Delgado 2001; Mazza et al. 2013; Ribera and Faille 2010) than in Australia.

Fig. 10.26 Quarry pond where dytiscid species were recorded, over the spring time, along the pond margins, despite low emergent and marginal vegetation (County Offaly, Ireland) (photo by Margherita Gioria)

Fig. 10.27 Remnants of human activities found in tropical forest. Artefacts such as this plastic barrel can be suitable substitute habitat for many dytiscids, predominantly Bidessini, *Copelatus*, and *Hydrovatus* species (Tanah Rata, Malaysia; Photo by Jiří Hájek)

Most subterranean species described so far belong to the subfamily Hydroporinae, but exceptions include *Exocelina abdita* Balke, Watts, Cooper, Humphreys and Vogler in Northern Australia (Balke et al. 2004) and *Copelatus cessaima* Caetano, Bená and Vanin in Brazil (Caetano et al. 2013). The subtribe Siettitiina (Dytiscidae, Hydroporinae, Hydroporini) includes the only known European genera of dytiscids having stygobitic members (*Siettitia* Abeille de Perrin, *Iberoporus* Castro and Delgado, and *Etruscodytes* Mazza, Cianferoni and Rocchi) and some North American subterranean species (see Ribera and Reboleira 2019).

Most species colonizing subterranean habitats in Australia are assumed to have evolved from surface ancestors due to aridification, with some species, especially larvae, moving into these habitats to avoid the effects of droughts (Leys et al. 2003; Langille et al. 2020). According to Leys et al. (2010), the evolution of stygobitic dytiscids is an ongoing process. These species have evolved adaptations to darkness, low and heterogeneous food sources, and a relatively constant climate (Balke et al. 2004; Moldovan 2004). These adaptations include reduction or absence of eyes, depigmentation, elongation of the body and antennae, loss of wings and fusion of the elytrae, and anatomical internal alterations (Alarie and Bilton 2001; Balke et al. 2004; Moldovan 2004; Caetano et al. 2013; Ribera and Reboleira 2019). Langille et al. (2020), however, have recently provided evidence of subterranean speciation in at least eight stygobitic species in the genus *Paroster* Sharp that inhabit calcrete aquifers in western Australia, suggesting that these species descend from subterranean ancestors and not from surface ancestors.

Further exploration of subterranean habitats will likely result in the discovery of new species (e.g., Leys et al. 2010; Kanda et al. 2016; Balke and Ribera 2020). Each aquifer can, in fact, be regarded as separate island ecosystem that has been isolated for millions of years from other aquifers (Leys et al. 2003, 2012). As such, each aquifer supports highly unique communities, making the assessment of the fauna of individual aquifers an important conservation goal (Eberhard et al. 2016). The fact that this habitat is underexplored relative to other habitats for dytiscids is evident from the discovery of new species from extensive sampling of aquifers, even in regions where the stygobitic fauna is well known (Eberhard et al. 2016). New species are also increasingly discovered elsewhere (e.g., Miller et al. 2009; Jean et al. 2012; Kanda et al. 2016; Balke and Ribera 2020). First country records of stygobitic species include *Copelatus cessaima*, the first troglomorphic species in Brazil, where it was recorded from caves in water puddles naturally carved on iron rocks (ironstone formation) in the Carajás National Forest (Caetano et al. 2013), and *Iberoporus pluto* Ribera and Reboleira. This is the first stygobitic beetle recorded in Portugal (Ribera and Reboleira 2019) and was described from a single female specimen found at the bottom of a clay pound connected to the margin of the subterranean stream in the well-studied cave Soprador do Carvalho (Coimbra). Mazza et al. (2013) described a new genus and species, *Etruscodytes nethuns* Mazza, Cianferoni and Rocchi, the first subterranean water beetle recorded in Italy, which was collected by pumping water from a well in Tuscany.

10.6.2 Hygropetric Habitats

Hygropetric habitats consist of thin layers of running water flowing over the surface of rocks or through mosses or filamentous algae, and include small waterfalls or margins of larger waterfalls, large boulders in streams, springs, and seepages on vertical cliffs (Larson et al. 2000; Miller and Perkins 2012) (Figs. 10.28 and 10.29). Hygropetric zones have long been overlooked for dytiscids, although in the past couple of decades, several species have been recorded from this habitat, showing that specialized dytiscids are relatively well represented in this habitat globally (e.g., Larson et al. 2000; Ribera et al. 2003b; Pederzani et al. 2004; Miller and Spangler 2008; Fery 2009; Miller 2012; Miller and Perkins 2012; Miller and Montano 2014; Biström et al. 2015; Hájek et al. 2019; Sheth et al. 2021). These include species of *Africophilus* Guignot, *Agabus*, *Fontidessus* K. B. Miller and Spangler, *Hydroporus, Hydrotrupes* Sharp, and *Platynectes* Régimbart. *Africophilus* species have been recorded from hygropetric habitats in Africa, including Madagascar, the Ivory Coast, Tanzania, and Togo (Holmen 1984; Alarie et al. 2000; Bilardo and Rocchi 2014), and, recently, from Gabon, Central Africa (Bilardo et al. 2020). There, a new species, *Africophilus gabonicus* 3 was described, with all specimens being collected from a layer of water on a hygropetric rock cliff next to a roadside fountain, while no other specimens were found in other wet parts of the rock or in a small pool and wet mud and gravel at the bottom of the cliff, suggesting a hygropetric lifestyle for this species. Species formerly in the genus *Hydrotarsus* Falkenström (now in the genus

Fig. 10.28 Wet vertical cliffs were larvae and adults of *Hydrotrupes chinensis* Nilsson have been recorded (Guangdong: Nanling Reserve, China; Photo by Jiří Hájek)

Fig. 10.29 Granite vertical cliff is inhabited by dytiscids such as *Platambus schillhammeri* Wewalka and Brancucci, and *Platynectes dissimilis* (Sharp) (Huashan Mountains, Shaanxi, China; Photo by Jiří Hájek)

Hydroporus) and regarded as highly endemic to Macaronesia (Alarie and Bilton 2001; Ribera et al. 2003b) include hygropetric species, with adults being recorded from damp rocks, especially with bryophytes, around small springs, while larvae were found crawling and burrowing rather than swimming (Alarie and Bilton 2001).

First country or regional records of hygropetric species that have been recently reported suggest that more species will likely be found as this habitat becomes increasingly explored. South America has a relatively rich fauna of hygropetric beetles, including numerous dytiscids, most of which come from the Guiana Shield region of northern South America and Venezuela (Miller and Garcia 2011; Miller and Spangler 2008; Miller and Montano 2014). All known species (seven) of *Fontidessus* are hygropetric (Miller and Montano 2014) and inhabit thin films of water flow over the surfaces of bare rock, where they can be abundant, and some may co-occur at some sites. A new genus and species, *Petrodessus conatus* Miller, was the first hygropetric species recorded from tropical north-eastern Australia (Miller 2012). *Hydrotrupes palpalis* is the only confirmed hygropetric dytiscid in North America, with most specimens being collected from hygropetric habitats but also from less specialized habitats (Miller and Perkins 2012). This species is morphologically similar to the hygropetric *Hydrotrupes chinensis* Nilsson, which has been recently reported from an increasing number of localities in China (Alarie et al. 2019; Fig. 10.28). A new fossil *Hydrotrupes* species, *H. prometheus* Gómez and Damgaard, which was found in Baltic amber, is possibly the first known hygropetric dytiscid fossil, although its morphological similarities with *H. palpalis*, which is also found in other habitats, suggest that this extinct species was not strictly hygropetric (Gómez and Damgaard 2014). A record from Baltic amber and records of extant species found in North America and China suggest that *H. prometheus* might have been widely distributed in northern continents during the Neocene (Gómez and Damgaard 2014).

The genus *Platynectes* includes 71 species occurring in Australian, Neotropical, Oriental, and Palearctic regions, some of which inhabit hygropetric habitats (Nilsson and Hájek 2022). A strictly hygropetric *Platynectes* species was described by Gustafson et al. (2016), *P. agallithoplotes* Gustafson, Short and Miller, from Venezuela, with its name meaning 'joyful-rock-swimmer'. Specimens of this species are associated with seepages on granite outcrops, and the larva has been collected on seeps (Gustafson et al. 2016). Recently, Hájek et al. (2019) described the first hygropetric *Platynectes* species and its larvae from China, *Platynectes davidorum* Hájek, Alarie, Štastný and Vondráček. Specimens of this species were collected at night in water film on rock surface of a small cliff, together with a specimen of *Platynectes dissimilis* (Sharp), a species common in small streams in the area (Fig. 10.29). This habitat is similar to that where *P. agallithoplotes* was found, although morphological adaptations to hygropetric habitats appear enhanced in *P. davidorum* as adults compared to all known *Platynectes* species (Hájek et al. 2019) and are more similar to those of other hygropetric agabine dytiscids (Hájek et al. 2019). These include *Agabus aubei* Perris, which was found (both adults and larvae) in Corsica under mats of the herb *Narthecium reverchonii* Celak. This species grows on steep rocks at the edge of mountain streams, either between the rocky ground and the roots or between the roots (Balke et al. 1997). Recently, Sheth et al. (2021) described the first hygropetric species of *Microdytes* J. Balfour-Browne, i.e., *Microdytes hygropetricus* Sheth, Ghate, Dahanukar and Hájek from the Western Ghats, India, where it appears to inhabit exclusively hygropetric habitats on vertical cliffs.

Some species have been described as semi-hygropetric, such as *Hydroporus sardomontanus* Pederzani, Rocchi and Schizzerotto, which was recorded under stones in wet habitats near springs on Mount Limbara, Sardinia, Italy (Pederzani et al. 2004). This species displays adaptations to life in arid habitats characterized by summer droughts in summer and humid conditions in winter and spring associated with the melting of snow. It was only found under stones, to which it was reported to cling thank to robust front and mid legs, while it avoided pebbles (Pederzani et al. 2004). These features suggest that this species is a poor swimmer but a great climber, whose favourite habitat is in proximity of spring sources (Pederzani et al. 2004). This species belongs to the *H. longulus*-group, whose habitat has been described as not the waterbody itself but the areas close to it, including water seeping through mud or *Sphagnum*, vegetation or decaying leaves, sometimes flowing in thin films of water over the sloping ground or in small puddles (see Fery 2009 for a synopsis of the group).

10.6.3 Interstitial Habitats

Dytiscids are known to have colonized interstitial habitats such as gravel banks along rivers (Fenoglio et al. 2006; Watts et al. 2016). Some species that have been classified as semi-subterranean or interstitial are characterized by peculiar morphological traits such as reduction of eyes, depigmentation, presence of long sensory setae, and the reduction of wings (e.g., Fery et al. 2012; Hernando et al. 2012; Manuel 2013). Watts et al. (2016) described a new interstitial species, *Exocelina saltusholmesensis* Watts, Hendrich, and Balke from a single female collected in a small pool in the bed of a small ephemeral creek through eucalypt woodland near Darwin, tropical northern Australia. The morphological features of this species, including reduced eyes and light pigmentation, and its absence from nearby waterbodies suggest that this is an interstitial (if not subterranean) species and provide a scenario for a transition from epigean to subterranean life (Watts et al. 2016). Adaptations to interstitial habitat have not, however, been reported in some species. Fenoglio et al. (2006) recorded adults and larval stages of *Agabus paludosus* within the interstitial zone of a streambed of the Po River (north-western Italy) at depths comprised between 70 and 90 cm below the surface during a drought when no water was present in the channel. Both adults and larvae likely use this habitat as a refuge under dry conditions, entering and remaining confined to the interstitial zone until water reappeared. Since this species does not show any peculiar adaptation to such an extreme habitat, the use of the interstitial (hyporheic) zone might represent a critical step towards the colonization of aquifers (Fenoglio et al. 2006).

The occurrence of a species in these habitats but not in adjacent waterbodies is strongly indicative of an interstitial lifestyle. For instance, in the Bolu province of north-western Turkey, Hernando et al. (2012) recorded all specimens of *Hydroporus bithynicus* Hernando, Aguilera, Castro, and Ribera from a small pool with upwelling spring water only (ca. one metre in diametre and few centimetres deep) on the side of a stream but not in the stream.

10.6.4 Rock Pools

Rock pools are unique habitats forming in shallow depressions on rocks (Ranta 1985). Characterizing features of this habitat include the unpredictability of the hydroperiod and the small pool volume (Figs. 10.30 and 10.31; Ranta 1982; Jocque et al. 2010). The morphology and hydrology of freshwater rock pools are generally driven by interactions between climate and geology (e.g., limestone, sandstone, granite), with hydroperiods ranging from several days up to the whole year (Jocque et al. 2010). The small size of these pools results in strongly fluctuating environmental conditions, low conductivity, and wide variations in pH (from 4.0 to 11.0) and temperature (from freezing point to 40 °C), often with well-marked daily cycles (Jocque et al. 2010). Survival in these habitats requires stress tolerance to highly

Fig. 10.30 Small pools in the spray zone below the waterfall are often inhabited by minute Hyphydrini: *Microdytes* J. Balfour-Browne species (Tad Yueang waterfall, Champasak, Laos; Photo by Jiří Hájek)

Fig. 10.31 Deep (and stable) rock pools near the river, usually with thick layer of decaying leaves, are inhabited by many dytiscid species, including some larger species as *Rhantus* and *Hydaticus* species, and *Sandracottus maculatus* (Wehncke) (Bolavens Pletaeu, Laos; Photo by Jiří Hájek)

variable environmental conditions as well as a capacity for active emigration followed by recolonization (Jocque et al. 2010). Given the increased interest in this habitat as model system for ecological and evolutionary research (Brendonck et al. 2010), Jocque et al. (2010) reviewed the characteristics and conservation value of freshwater rock pools, showing that dytiscids represent the largest beetle group in this habitat, with 37 species being recorded from various world regions, out of a total of 247 species of active dispersers that had been recorded globally, including North America (Baron et al. 1998; Larson et al. 2000), Botswana (Jocque et al. 2006), Western Australia (Pinder et al. 2000), and Sweden (see Jocque et al. 2010 and references therein). Further, three *Rhanthus* species recorded from rock pools and waterholes in rocks at the hedge of streams in New Caledonia were described by Balke et al. (2010).

In coastal areas, salinity in rock pools may also vary substantially, with water quality ranging from brackish sea water to fresh rainwater (Ranta 1985; Nilsson and Holmen 1995). In an examination of water beetle communities in different habitats in Finland, Ranta (1985) showed that rock pools in the littoral zone of the Baltic Sea and on Baltic islands supported a specialized dytiscid fauna comprising 15 species, representing 58% of the total number of species recorded from these pools and 53% of the total number of individuals, with three species being highly represented in this habitat. A tolerance to salinity might contribute to the habitat specificity of certain species in coastal rock pools, such as *Boreonectes griseostriatus* (De Geer), compared to that of species found in freshwater or brackish rock pools (Nilsson and Holmen 1995).

10.6.5 Terrestrial Habitats

A small number of species in the subfamily Hydroporinae have been collected from terrestrial habitats, although information on the habitats used by immature stages remains largely unknown (Brancucci 1985a; Watts 1982; Balke and Hendrich 1996; Brancucci and Hendrich 2010; Miller and Bergsten 2016; Toussaint et al. 2016). These ‘terrestrial’ species have been collected from damp forest leaf litter in Nepal, northern India (*Geodessus besucheti* Brancucci) and southern India (*Geodessus kejvali* Balke and Hendrich) (Balke and Hendrich 1996; Balke et al. 2008), and from rainforests of north-eastern Australia, i.e., *Paroster caecus* (Watts) and *Paroster anophthalmus* (Brancucci and Monteith) (Brancucci and Monteith 1997), which had been previously placed in the genus *Terradessus* Watts (Toussaint et al. 2016). The genus *Paroster* Sharp is endemic to Australia and is dominated by subterranean species recorded from aquifers and paleodrainages in western Australia, as well as a small number of epigean species (Watts and Humphreys 2004, 2006, 2009; Watts et al. 2008; Leys et al. 2010). Toussaint et al. (2016) examined the evolution of a secondary terrestrial lifestyle in the two terrestrial *Paroster* species in a phylogenetic framework and suggested that colonization of terrestrial habitats was likely linked to the aridification of paleodrainage systems. A fifth terrestrial species, *Typhlodessus monteithi* Brancucci, was described from a single male collected on Mount Panié on the island of Grande Terre in the New Caledonian archipelago (Brancucci 1985b; Brancucci and Hendrich 2010). These species have adapted to a terrestrial life and, in the case of *Geodessus besucheti*, have lost the capacity to swim. Evidence of adaptations to a terrestrial lifestyle is stronger in *Paroster* and *Typhlodessus* Brancucci and Monteith than in *Geodessus* Brancucci. These include small size (around 1.3 mm), depigmentation (*Paroster*) and near-complete lack of eyes (*Typhlodessus*) (Toussaint et al. 2016). More recently, Fery (2020) described *Hydroporus novacula* Fery, which was collected from a steep slope in a beech forest in south-western Georgia (Caucasus), by sifting dry material far from any other water or wet ground. This is possibly the first terrestrial *Hydroporus* species being recorded, given the total absence of any natatorial setae on mid- and hind-legs, which suggest that this species is unable to swim. Yet, Fery (2020) cautioned that more information about the life history of this species is needed to determine whether this could be a terrestrial species.

Ranarilalatiana and Bergsten (2019) highlighted the importance of dry forest floor depression as a potential habitat for dytiscid. These authors described six new species from two genera of Copelatinae known from Madagascar, *Copelatus* Erichson and *Madaglymbus* Shaverdo and Balke. These species were exclusively or almost exclusively found in dry floor depressions with dead leaves in humid forests, but they were not found in nearby streams or other more permanent waterbodies. While the authors suggested that these species should not be regarded as terrestrial or even semi-terrestrial dytiscids, they recognized that they are specialists of very ephemeral aquatic habitats, remaining in dried-up habitats rather than dispersing in other waterbodies (Ranarilalatiana and Bergsten 2019). In Madagascar, this behaviour is likely to be restricted to humid forests where precipitation is high (Ranarilalatiana and Bergsten 2019). Similarly, in their review of the genus *Copelatus* in Madagascar, Ranarilalatiana et al. (2019) described *Copelatus mahajanga* Pederzani and Hájek, which was collected from pitfall traps and from leaf litter, suggesting that the species can survive in dried-up habitats without immediately searching for new ones. Dytiscids from rain pools that may occasionally go dry also include species in the genus *Sanfilippodytes* Franciscolo (Larson et al. 2000). Three *Sanfilippodytes* species have been found from rain pools in the pigmy forest in Mendocino, California, where strongly podsolized soils result in these pools becoming quickly acidified (Post 2010). *Sanfilippodytes setifer* Roughley and Larson appears to have adapted to survive drying periods (Roughley and Larson 2000), although assessments of a potentially terrestrial existence for this species during the dry season is needed (Post 2010).

10.6.6 Phytotelmata

Phytotelmata are habitats consisting of small water reservoirs formed by plant structures capable of retaining water (Varga 1928; Maguire 1971; Kitching 2000;

Richardson and Hull 2000). These habitats are distributed in all continents except Antarctica, but their diversity is larger in the tropics and subtropics (Greeney 2001; Campos and Fernández 2011). Phytotelmata have been classified into various categories, depending on their position on a plant and whether they are formed by rainwater or by a plant-derived fluid, and include bamboo internodes, tree holes or holes in fallen trees, leaves or bracts or open fruit, seed pods (e.g., coconut), and water tanks, such as those found in bromeliads (Kitching 2000; Campos and Fernández 2011; Jalinsky et al. 2014; Campos 2016). These ancient habitats have maintained a specialized dytiscid fauna over evolutionarily extended periods and can affect the spatial distribution, overall abundance, and dispersal of dytiscids (Balke et al. 2008).

The insect fauna of bromeliad (Bromeliaceae) phytotelmata (Frank and Lounibos 2009) and the origin and specificity of bromeliad-associated Copelatinae have been reviewed by Balke et al. (2008). Bromeliad tanks have been colonized by species of the genus *Copelatus*, some of which are strictly specialized to this habitat, despite being highly mobile and phylogenetically related to species occurring in small waterbodies in tropical forests. Species of *Desmopachria* Babington have also been recorded from these habitats. Both adults and larvae have been recorded in bromeliad tanks (Balke et al. 2008; García-Robledo et al. 2005). Bromeliads that support dytiscids include the genera *Aechmea*, *Brocchinia*, *Guzmania*, *Hohenbergia*, *Nidularium*, *Tillandsia*, and *Vriesia* (Balke et al. 2008; Campos and Fernández 2011; Torreias and Ferreira-Keppler 2011). In temperate Argentina, adults of *Copelatus* species have been recorded in *Eryngium cabrerae* Pontiroli (Cyperaceae, Fig. 10.32), while adults of *Liodessus* species have also been found in *Eryngium elegans* Cham. and Schltdl. (Fig. 10.33), and *Aechmea distichantha* Lemaire (Bromelidae) (Fig. 10.34; Campos and Fernández 2011).

Fig. 10.32 *Eryngium cabrerae* (Cyperaceae) in temperate Argentina, where *Copelatus* and *Liodessus* species were recorded (Photo by Raúl Campos)

Fig. 10.33 *Eryngium elegans* (Cyperaceae) in temperate Argentina, where *Liodessus* species were recorded (Photo by Raúl Campos)

Fig. 10.34 *Aechmea distichantha* (Bromeliaceae) in temperate Argentina, where adults of *Liodessus* species were recorded (Photo by Raúl Campos)

Campos (2016) recorded a single adult of a *Liodessus* species from the stump of a bamboo (*Guadua*) species. Water-filled tree holes, consisting in cavities or tree depressions filled with rainwater, represent an important aquatic habitat for some species (Kitching 1971, 2000; Hendrich and Yang 1997), such as *Agabus* (Nishadh and Das 2012) and *Copelatus* species (Yanoviak 2001). Kitching and Orr (1996) investigated the food web of water-filled tree holes in lowland mixed forest in Kuala Belalong, Brunei, and found small dytiscids in tree holes of various origin (root pans, buttress pans, trunk pans, rot-holes, hollow trees, and log holes). Dytiscid larvae have been recorded from older inflorescences of *Xanthosoma* (Araceae) species in Central and South America (García-Robledo et al. 2005).

10.7 Concluding Remarks and Future Directions

Much progress has been made over the past couple of decades in understanding the relationship between habitat characteristics and the distribution of dytiscids. New species and even new genera have been described over the past couple of decades and found in unusual habitats such as hygropetric, subterranean, and even terrestrial or semi-terrestrial habitats. Since many habitats had long been underexplored for dytiscids in many world regions, it is likely that more species will be discovered in the future. Further study of these habitats, coupled with the increasing availability of phylogenetic data and tools, will provide important insights into the ecology and evolution of dytiscids and the potential factors driving species range and habitat shifts (Toussaint et al. 2016; Michat et al. 2017; Villastrigo et al. 2018). This is especially true in the case of species recorded in habitats that may represent an intermediate step towards a truly subterranean or terrestrial lifestyle, such as interstitial habitats or leaf litter. But it is also the case of temporary habitats, whose role in supporting regional dytiscid diversity remains largely unknown in many regions (Bird et al. 2019). Ultimately, knowledge of the habitat requirements and specificity for a broader number of species globally, at different stages of development, is critical to the conservation of this highly diverse taxonomic group (Chap. 12). While larvae of new species and their ecology are increasingly being described, improved knowledge of the distribution and structure of dytiscid larval populations is much needed. This information is key to evaluating the vulnerability of the less abundant species and those inhabiting extreme habitats, especially in the face of global environmental changes (Bilton et al. 2019). Climate change and changes in the frequency and intensity of extreme climatic events will likely have major effects on habitat availability for many species, especially for those already at the edges of their physiological niche or distribution range (Cioffi et al. 2016). This is the case of dytiscids inhabiting temporary habitats in several world regions, especially where droughts are expected to become more frequent and/or of longer duration. Socio-economic changes, including the intensification of agricultural and industrial activities, fish stocking, water use, and urbanization, will likely contribute to further habitat degradation and loss, with implications on habitat availability and connectivity for many species (Arnott et al. 2006; Bilton et al. 2015, 2019). Additional information is needed to predict the potential effects of these changes, which might accelerate extinction rates and lead to a redistribution of species and the formation of novel communities.

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Margherita Gioria is a senior researcher within the Department of Invasion Ecology at the Institute of Botany, Czech Academy of Sciences, where she is the leader of a project aimed at assessing the role of regeneration from seeds in promoting invasions by alien plants. Her work on water beetles and plant–beetle interactions began with her graduate studies on the invertebrate fauna of freshwater springs in the Burren, a unique karstic area in western Ireland. Over the years, she has examined the invertebrate fauna of several freshwater ecosystems and the role of aquatic and marginal plants as predictors of water beetle diversity and composition in agricultural landscapes. At University College Dublin, she led a medium-term project aimed at assessing the impact of agricultural activities on Irish water beetles.

John Feehan is a professor (retired) in the School of Agriculture and Food Science at University College Dublin. His many books include the definitive textbook on Ireland's peatlands, the widely acclaimed *Farming in Ireland: History, Heritage and Environment*, *The Wildflowers of Offaly* (described by Michael Viney as 'a landmark in books about our countryside'), and (with colleagues in UCD) a popular book on Ireland's grasslands: as well as several books on the geology, environmental heritage, and history of Ireland. He has been described as 'one of Ireland's top ecologists and communicators of nature.' Professor Feehan was elected a Member of the Royal Irish Academy in 2021.

Chapter 11
Dispersal in Dytiscidae

David T. Bilton

> *I was reading in bed in Sussex in July, 1937, at 11.15 p.m. when a male Ilybius fuliginosus flew in at the window and settled on my pillow . . . F. Balfour-Browne (1953)*

Abstract Dytiscid beetles live in spatially discrete habitat patches of varying temporal duration and ecological stability. Many species are exemplary active dispersers, moving between suitable localities, sometimes on multiple occasions within an individual's lifetime. Despite this, there is apparently much variation in the ability of individual species to disperse by flight, this having far-reaching consequences for their evolution and persistence. This chapter examines the mechanisms, causes and consequences of dispersal in diving beetles, reviewing work on flight and flightlessness, ultimate and proximate triggers of dispersal, and the biogeographical/macroecological consequences of movement, as well as suggesting areas where further research is required. Most diving beetle species fly, but some do so far more readily and over longer temporal windows than others. The degree to which individual species disperse may be shaped largely by habitat stability and persistence; something which has significant consequences for the composition of regional faunas.

Keywords Flight · Habitat · Pterygote · Wings · Water-borne cues

11.1 Introduction

Like Frank Balfour-Browne's nocturnal *Ilybius*, many dytiscid beetles are active fliers, something which is rarely observed, but nevertheless fundamental to their way of life, allowing them to colonise new areas of habitat (Fernando 1958; Fernando and Galbraith 1973), track the seasonal availability of water (Hilsenhoff 1986; Miguélez

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D. T. Bilton (✉)
Marine Biology and Ecology Research Centre, School of Biological and Marine Sciences, University of Plymouth, Drake Circus, Plymouth, UK
e-mail: dbilton@plymouth.ac.uk

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and Valladares 2008), or move to terrestrial overwintering sites (Galewski 1971). Almost all inland waters on earth contain diving beetles, including those on isolated oceanic islands such as the Azores and Hawaii, and these insects are excellent models for studying a range of questions in ecology and evolution (Bilton et al. 2019). New waterbodies are colonised by a suite of pioneer dytiscids, which often arrive within days of their creation. Even the small pockets of water found in phytotelmata, such as bromeliad tanks harbour specialist diving beetles (e.g. Balke et al. 2008). Like all freshwater organisms, dytiscid beetle populations live and reproduce in discrete localities, surrounded by a relatively inhospitable terrestrial landscape. Whilst the ecophysiology and gas exchange mechanisms of adult diving beetles (Verberk and Bilton 2013) mean that crossing this matrix may be less insurmountable than it is for some freshwater animals, moving between suitable patches nevertheless constitutes a significant challenge.

As with other pterygotes, most dytiscid species possess functional flight wings and utilise these to overcome the challenge of interpatch dispersal. As such, dytiscids can generally be considered active dispersers, powering their own movement between suitable patches of habitat. Such active inter-habitat dispersal involves behaviours that are predominantly initiated, and at least partly controlled, by the individual beetle. Actively dispersing diving beetles may therefore use sensory cues to seek out new areas of habitat or patches occupied by conspecifics. The degree to which dispersal movements can truly be considered active, however, will depend on species, situation and prevailing environmental conditions—for many small beetles, much of their time in the air may be at the mercy of the wind, giving them little control over their direction. As far as is known, however, dytiscids do not utilise animal vectors for movement of adults or other life stages (Bilton et al. 2001; Green and Sánchez 2006).

Following a brief general consideration of the evolution of dispersal, and its consequences, this review will focus on aspects of dispersal biology fundamental for dytiscids, as well as some areas where studies of these beetles have contributed to wider ecological and evolutionary ideas. Specifically, I start by revisiting early studies of flight and flying ability, which directly examine the dispersal apparatus and behaviour of diving beetles. Continuing with a behavioural theme, I review studies of dispersal triggers and timing in diving beetles, considering what cues individuals may use when making the decision to leave an occupied patch, or remain in a new one. Finally, I review the large-scale ecological and evolutionary consequences of dispersal evolution in dytiscids, and show how the origin of both widespread species and narrow-range endemics ultimately depends on the relative strength of selection for dispersal—itself dictated by habitat.

11.2 The Evolution, Maintenance, and Consequences of Dispersal

11.2.1 Why Disperse?

Viewed from the perspective of the individual dytiscid, there are both advantages and disadvantages that may result from dispersing from one site to another (see Bilton et al. 2001; Bonte et al. 2012). Advantages include escape from unfavourable conditions, e.g. limited resources, predators, pathogens and parasites, and inbreeding, and the possibility of locating a new site with low-density occupation and fewer direct competitors. Disadvantages include an inability to locate a suitable new site; a risk of predation en route; an inability to locate a mate; outbreeding depression; and lack of adaptation to the new habitat. The most likely risks to sexually reproducing organisms such as dytiscids in failing to disperse in the short-term are inbreeding, overcrowding and increased competition, predation, and exposure to pathogens and parasites. In the long term, failing to disperse is likely to increase extinction risk at the population and lineage level, if nothing else as a result of stochastic effects.

11.2.2 The Evolution and Maintenance of Dispersal

The evolution of dispersal has received a great deal of theoretical investigation, including the development of numerous mathematical models, and although none of this work has considered dytiscids, the ideas clearly apply when considering these beetles (see e.g. Hamilton and May 1977; Levin et al. 1984; Johnson and Gaines 1990; Cohen and Levin 1991; McPeek and Holt 1992; Dieckmann et al. 1999; Ferriere et al. 2000; Clobert et al. 2001; Ronce 2007). Most models assume that local populations occur in discrete habitats and identify evolutionarily stable strategies based on game theory.

Factors such as habitat stability and permanence are likely to be key in shaping the dispersal strategies of dytiscids over evolutionary timescales, dispersal being more strongly selected for in taxa of relatively unstable habitats, such as the small standing waters which typically hold the bulk of local dytiscid diversity. Such habitats may dry seasonally, forcing the adults of some species to disperse locally to more permanent sites, but are also short-lived on geological timescales (Ribera 2008)—a point I return to below. In addition to the above-mentioned costs and benefits associated with dispersal from the point of view of an individual, the dispersal ability of aquatic invertebrates such as dytiscids is likely to influence the long-term persistence of local populations (e.g. Avise 1992; Hogg et al. 1998), an association that may ultimately influence the success of species. Dispersal ability may be a critical predictor of a species' ability to escape environmental change, such as climate warming, where movement to a more suitable site may be necessary for long-term survival. The climatic changes of the Pleistocene have provided repeated natural experiments which allow us to examine how fauna and flora, including dytiscids, have responded to shifts in temperature, this being particularly well-studied in the northern hemispheres (see Elias 1997 for a review). Whilst there are a number of beetle examples of massive range shifts in response to Pleistocene climatic changes, including the occurrence of the Mongolian and Canadian *Hygrotus unguicularis* (Crotch) on the Isle of Man (between Ireland and Britain) in the Late Glacial period 12,000 years ago (Joachim 1978), almost all of these concern species occurring at high latitudes. Very few examples are known of large-scale range movement in species occupying lower latitudes—most endemic species appear to have evolved in, or close to, the areas where they currently occur, and many may have limited dispersal ability compared to their more widespread relatives, particularly with lotic taxa (Abellán et al. 2011; Sánchez-Fernández et al. 2012a).

11.3 Consequences of Dispersal

Dispersal only makes a difference in an evolutionary sense if it results in successful colonisation of a site—something that involves successful reproduction. Where dispersing individuals enter an existing population, such reproduction results in gene flow, i.e. the transfer of genes from one population to another.

The effects of dispersal and gene flow are varied. Dispersal can result in the expansion of ranges following the colonisation of new sites. An interesting question here is what ultimately sets the limits to dispersal and range expansion in individual species? Whilst there is finally good evidence, including recent meta-analyses (Slatyer et al. 2013) linking geographical range size to niche breadth, a key question is what limits niche breadth evolution itself, particularly at range edges (Kirkpatrick and Barton 1997; Kubisch et al. 2013)? Dispersal can reduce the amount of genetic differentiation amongst populations (Avise 1992; Bohonak 1999), producing panmixia with relatively few successful colonists (Crow and Kimura 1970). On the other hand, in the absence of gene flow, populations are free to evolve along independent trajectories, something which in sexual organisms may lead to the direct or indirect evolution of reproductive isolation and so biological speciation (see Coyne and Orr (2004) for a recent review). Such dispersal limitation processes are believed to have contributed to the diversity of a number of diving beetle radiations, particularly those in running waters (Ribera 2008) and subterranean aquifers (e.g. Vergnon et al. 2013)—indeed the way in which habitat type shapes dispersal evolution, and the way this in turn moulds the evolutionary fate of individual clades is a fundamental feature of freshwater faunas, and something first postulated from studies of water beetles, including dytiscids (see below and Ribera and Vogler 2000; Ribera 2008).

In an ecological sense, dispersal will clearly have important consequences for dytiscid assemblage composition and how species are distributed across patches within a region. Resetarits Jr (2001) points out that random movement among ponds will result in homogenised assemblages, whereas different assemblages will result if adults chose sites in response to their environmental characteristics (see below).

Given the spatial and environmental structure observed in real dytiscid communities (e.g. McAbendroth et al. 2005; Florencio et al. 2011; Picazo et al. 2011), there are no prizes for guessing what most beetles do!

11.4 On Flight and Wings and Flightlessness

Dytiscid hind wings are their primary means of dispersal and have a long history of scientific study; Goodliffe (1939) and Balfour-Browne (1944) outlining key features of venation and discussing its possible taxonomic significance, albeit with differing conclusions. The work of Dorothy Jackson in the 1950s, however, represents the most complete direct investigation of the flight capacity of diving beetles. Whilst this work was almost exclusively concerned with European species, it is worth revisiting here, as no such studies have been undertaken elsewhere, and its findings have much wider relevance. In addition to direct observations of the wings, Jackson also studied the flight musculature and metathoracic exoskeleton associated with flight in diving beetles and subjected living specimens to direct flight tests in the laboratory. As was extensively documented by David Spencer Smith (1964), modifications, especially reduction in size, of certain elements of the metathoracic skeleton such as the pre- and postphragmata on which the flight muscles attach, can indicate flightlessness even if a beetle is fully winged. Jackson published her observations in a series of papers (Jackson 1950, 1952, 1956a, b, c, d, 1958, 1973a, b), categorising the species studied into those which were strongly flying, apparently flightless, and variable.

Jackson's strong fliers are species that readily flew in the laboratory, and in which wings, flight musculature and thoracic skeleton were always well developed. This category includes the majority of larger lentic water dytiscids examined, classic pioneer and generalist species such as *Hygrotus confluens* (Fabricius) and *Hydroporus nigrita* (Fabricius) and *H. tessellatus* Drapiez as well as *Stictonectes lepidus* (Olivier), a species usually associated with lotic habitats. Species Jackson noted as apparently being flightless are a mixed bag of running and standing water species, including the temporary pond agabines *Agabus labiatus* (Brahm), *A. uliginosus* (L.) and *A. undulatus* (Schrank). In only one of these species, *Hydroporus ferrugineus* Stephens (Jackson 1956a), is wing reduction noted (see Fig. 11.1), and even here Jackson reports that whilst there was no trace of flight muscles in the few specimens dissected, and a weakly developed thoracic skeleton, the beetles did show some variation in relative wing size. Whilst such taxa may be relatively poor aerial dispersers, it is difficult to state with certainty that such species *never* disperse by flight. Wing polymorphism is known elsewhere in Dytiscidae, including *Agabus bifarius* (Kirby), in which fully winged and brachypterous individuals have been reported (Leech 1942). Jackson's final category covered variable species in which at least some individuals were known to fly. In some cases, variation was only observed in flight musculature, in others, reductions to the thoracic skeleton were also reported in some individuals (e.g. *Agabus paludosus* (Fabricius)—see Fig. 11.2).

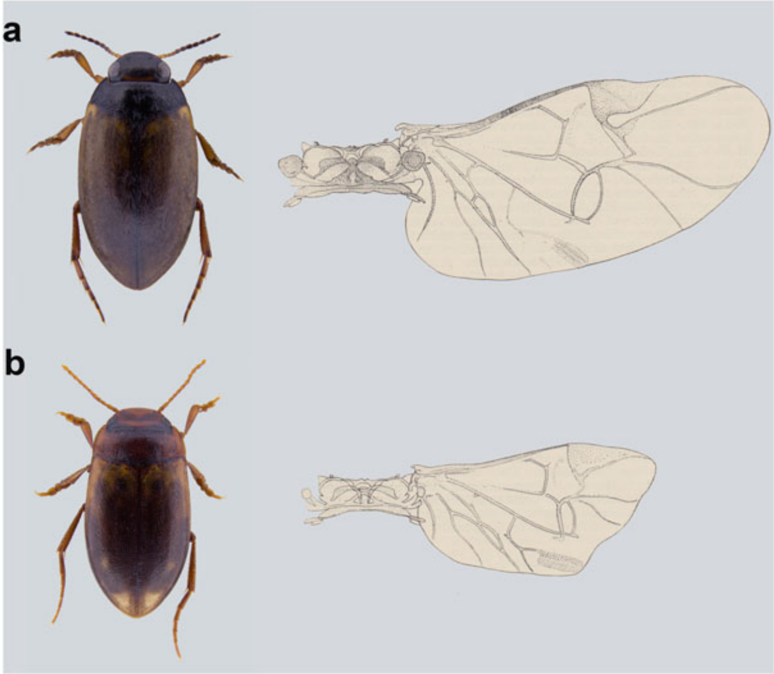

Fig. 11.1 Wings and internal views of metaterga of *Hydroporus* species, with pleural discs attached, drawn to the same scale. (**a**) *Hydroporus planus* (Fabricius)—a strongly flying species, (**b**) *Hydroporus ferrugineus*—a species considered flightless by Jackson. See text for details. After Jackson (1956a)

Whilst there can be no doubt about the strong fliers, it is difficult to be certain that Jackson's flightless species are not simply variable ones with a low proportion of flying individuals, or at least in the populations she examined. There are handful of dytiscids, such as the semi-subterranean *Iberoporus agnus* (Foster) (Bilton and Fery 1996), the island endemic *Agabus maderensis* Wollaston (Balfour-Browne 1950) and some fully subterranean taxa (Spangler 1986), where flight wings are so strongly reduced that flight would be impossible. On the other hand, if species are fully winged, it is hard to discount the possibility that they sometimes use them! As stated above, *A. uliginosus* is a species described as flightless by Jackson (1956b) on the basis of abnormal flight musculature and reduced metaterga and pleural discs, but it is now known that this species does indeed fly on occasion, soon after the emergence of teneral adults (whose morphology was not, unfortunately, examined–Kirby and Foster 1991). In a similar fashion *Agabetes acuductus* (Harris) was considered flightless by Jackson (1956d), but subsequently shown to fly, being captured in UV light traps operated close to occupied woodland pools (Spangler and Gordon 1973). Indeed, it is difficult to see how such species of small isolated lentic waters could adequately disperse in the complete absence of flight, or indeed how flightless species could persist in such habitats given their geological instability (Ribera 2008).

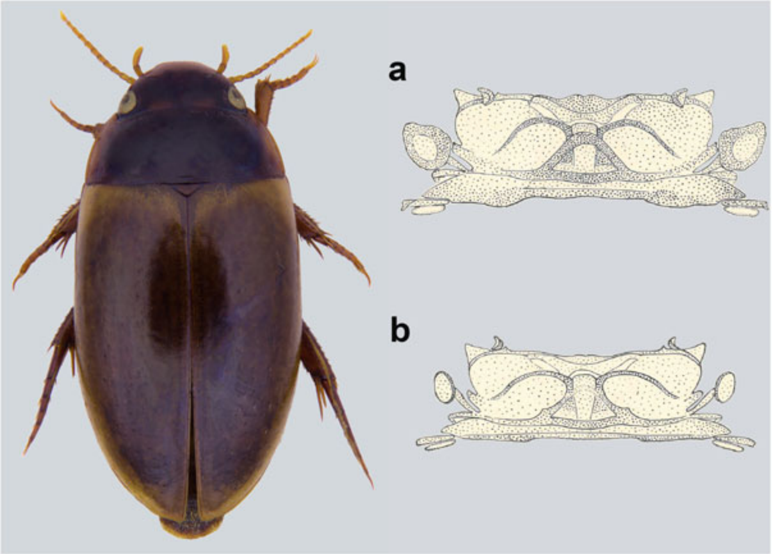

Fig. 11.2 *Agabus paludosus*—one of Jackson's variable species. Internal views of metaterga, with pleural discs attached, drawn to the same scale, (**a**) from a female with normal flight muscles, (**b**) from a male with no flight muscles. After Jackson (1956a)

Indeed when looked at from an ecological and biogeographical perspective, most of Jackson's flightless species probably are capable of flight, or at least some individuals, in some populations are, for part of their adult life. Recent observations on *Hydroporus rufifrons* (Müller) (Fig. 11.3), an inhabitant of seasonally fluctuating pools in northern Eurasia, reveal that despite being fully winged, the species usually has poorly developed flight muscles and cannot be coaxed into flight in the laboratory (D. T. Bilton, *pers. obs.;* G.N. Foster, *pers. comm.*). Despite this, the beetle occurs in isolated ponds in hill country, in both central Europe and the UK (Hess and Heckes 2004; Foster et al. 2008), and it is hard to explain its site occupancy without occasional flight. *H. rufifrons* appears to show a core-satellite metapopulation structure in most regions, being rare in most occupied localities, but superabundant in others, where it can be the dominant dytiscid (D. T. Bilton, pers. obs.; G.N. Foster, pers. comm.). If only a small proportion of beetles in these high-density populations are capable of flight, this would fit with both field and laboratory observations. As discussed in Bilton (1994), it is often assumed that a number of the dytiscids associated with primary fen habitats in western Europe are flightless, following on from the studies of Jackson and the fact that they are typically absent from apparently suitable secondary habitats in many regions, including the UK. Whilst species such *Hydroporus scalesianus* Stephens are indeed restricted to relict patches of primary fen in highly fragmented landscapes such as the UK, the same species are apparently capable of colonising relatively new habitats in other parts of their range, and it is difficult to envisage how this happens without flight. In central Sweden, for example,

Fig. 11.3 *Hydroporus rufifrons*, a northern Palaearctic specialist of temporary and fluctuating waters which has declined significantly in recent decades in much of western Europe in response to agricultural intensification. *H. rufifrons* occupies isolated waterbodies but is apparently an infrequent flier, having never been coaxed into the air in the laboratory. Photo Franz Hebauer

classic relict species such as *Hydroporus glabriusculus* Aubé and *H. scalesianus* occur in small fens formed in the last 200 years as a result of isostatic land uplift around the central Baltic Sea, and in the case of *H. scalesianus*, artificial ponds created *de novo* within the last 50 years (D.T. Bilton, pers. obs). In such regions, the density of suitable habitat remains relatively high, and there is the possibility that elsewhere reduced dispersal ability has evolved recently in response to habitat fragmentation, as reported in *H. glabriusculus* in Britain and Ireland (Bilton 1994). Some species may indeed behave as relicts over part of their range, such populations perhaps representing the 'living dead' in a metapopulation sense (Hanski et al. 1996); Iversen et al. (2013) suggesting that viable population networks of low-dispering species such as *Graphoderus bilineatus* (De Geer) depend on a relatively high density of habitat in the landscape. Roadside pools and elephant footprints are amongst the kind of habitat patches that may serve as 'stepping stones' for dytiscids (Remmers et al. 2017; Pitcher and Yee 2018).

Many of Jackson's findings may be related to the oogenesis-flight syndrome (Johnson 1969), in which individuals disperse early in adult life, often as tenerals, then utilise energy from autolysed flight musculature in reproduction (e.g. Hocking 1952). Although not directly studied to date in dytiscids, such autolysis of flight musculature in fully winged individuals has been observed on commencement of reproductive activity in *Mesovelia* and a range of pond skaters (Galbreath 1975; Vespäläin 1978). In the hydrophiloid beetle *Helophorus brevipalpis* Bedel, fully functional flight musculature is present throughout adult life, as in Jackson's strong fliers. In Spring, flying *H. brevipalpis* females contain mature oocytes, and at this time of year, after snow-melt, dispersal to newly-available temporary water occurs, an individual female which has mated and contains mature eggs representing a very effective coloniser. In the related *Helophorus strigifrons* Thompson flying females are mainly gravid, but with small oocytes, and flight muscle degeneration occurs after dispersal (Landin 1980). This process probably accounts for many of Jackson's observations of flightless and variable species; flight muscle development, and ability to actively disperse, varying over the course of an adult's lifetime. Iversen et al. (2017) found that flight occurred almost exclusively in recently emerged adult *Graphoderus* but throughout the season in *Acilius*, results consistent with oogenesis-flight syndrome in the former but not the latter.

Even in taxa with well-developed wings and flight musculature, species and individuals may differ markedly in their propensity to fly and the distances they are capable of covering, factors which will be important in shaping their ecological and geographical ranges as well as population dynamics. This area is something we know little about in diving beetles, but recent work using flight mills appears very promising in enabling us to address such questions. Matushima and Yokoi (2020) employed such an approach to examine flight behaviour in *Hydaticus bowringii* Clark, *H. grammicus* (Germar) and *Rhantus suturalis* (Macleay) in Ibaraki Prefecture, Japan. Mean flight distances for these taxa were 5.16, 1.97, and 0.58 km, respectively; apparently decreasing with body size. *R. suturalis*, on average, flew the shortest distances, despite being the most widespread predaceous diving beetle species on earth. Some individuals flew extremely long distances: 20.01 km in *H. bowringii* and 12.58 km in *H. grammicus* but only 2.47 km in *R. suturalis*. Interestingly, maximum flight distances were greater in females than males in all three species.

11.5 Proximate Drivers of Dispersal and How to Find Water

11.5.1 Dispersal Triggers at the Individual Level in the Field and the Lab

Factors triggering and regulating dispersal in dytiscids, and indeed freshwater insects in general, have received only limited attention. In many species, dispersal flights are undertaken by teneral individuals (Bilton 1994), this possibly relating to the oogenesis-flight syndrome as discussed above. The proximate cues which may trigger an individual diving beetle to leave a water body are poorly known, although both decreasing water depth and increasing temperature appear to play important roles. In most cases, beetles usually try to leave the water before taking off, but some species, including *Hygrotus salinarius* (Wallis) can fly directly from the water surface itself (Miller 2013). Whilst flight in *Dytiscus marginalis* L. has been seen in the field at temperatures as low as 6.4 °C (Nilsson and Svensson 1992), most observations suggest flight is most common at higher temperatures, even in temperate taxa. In some cases, mass emigrations have been observed in response to changing conditions, such as with *Agabus disintegratus* (Crotch) (Young 1960) and *Eretes sticticus* (L.) (*sensu lato*) (Kingsley 1985). In other instances, dytiscids, including *Hygrotus wardii* (Clark), have been reported as forming a significant portion of diurnal mass swarms of aquatic Heteroptera and Coleoptera, which, when hitting a tine roof, have been likened to hail (Stevens et al. 2007). In one of the few studies of its kind, Velasco and Millán (1998) examined the response of a number of desert stream-dwelling beetles and bugs to simulated drought conditions, by increasing temperature and decreasing water depth in the laboratory. They demonstrated than reduced water depth was the principal trigger of dispersal in the beetles studied, including *Clarkhydrus roffii* (Clark) and *Laccophilus maculosus* (Germar), the threshold depth for dispersal initiation being around 1 cm. More limited dispersal activity was observed in response to warming from 24–40 °C, and here exit responses only occurred at temperatures of 28 °C and above. Pitcher and Yee (2014) showed that dispersal propensity differed markedly between the morphologically similar congenerics *Laccophilus fasciatus rufus* Melsheimer and *L. proximus* Say. *L. proximus* occupies shallower habitats than *L. f. rufus* and was found to have a greater propensity to fly. In a study of the saline water diving beetles *Nebrioporus baeticus* (Schaum) and *N. cereysi* (Aubé) Pallares et al. (2012) found that flight activity in the laboratory increased to a maximum at 40 and 35 °C, respectively, declining significantly thereafter, when high mortality was observed in both species. In the widespread Palaearctic dytiscid *Agabus bipustulatus* (L.), both water depth and individual density influence exit behaviour from aquaria. In response to density, exit rates increased significantly at a density $> 5/l$ (see Fig. 11.4). Decreasing depth also triggered exit behaviour, this increasing significantly below a threshold of around 0.5 cm at 28 °C (see Fig. 11.4). At this depth, beetle elytra were frequently in contact with the surface film during normal activity, and it is thought that this stimulus may have triggered the shift in behaviour observed in the laboratory. It would be interesting to see how such responses are modified by the presence of heterospecifics, and changes in habitat complexity/quality. Using a combination of field and laboratory experiments Yee et al. (2009) showed that higher conspecific density and lower macrophyte density both triggered dispersal in *Rhantus sericans* Sharp and *Graphoderus occidentalis* Horn, and that this effect was modulated by the presence or absence of food, all suggesting that the beetles responded to perceived patch quality.

11.5.2 Weather, Season and the Timing of Field Flights

Some attention has been given to the habitat and climatic conditions which limit flight in water beetles in the field, with temperature and wind speed being important (Landin 1968; Landin and Stark 1973; Zalom et al. 1980; Van der Eijk 1983; Nilsson

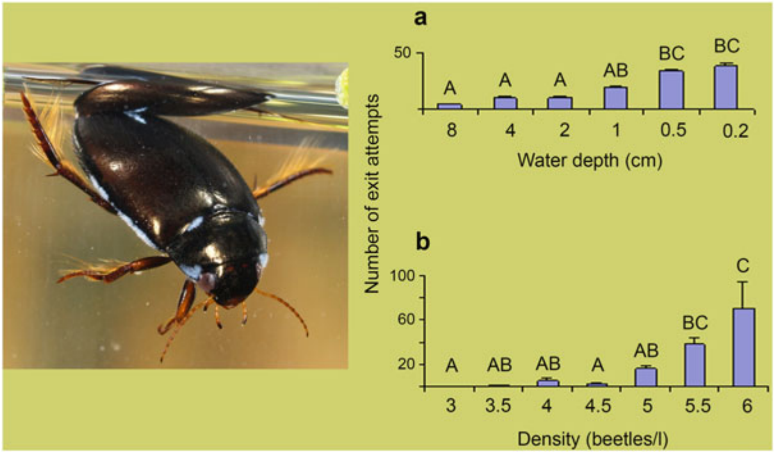

Fig. 11.4 Exit behaviour of *Agabus bipustulatus* in the laboratory. Beetles were placed in 2 l aquaria at 28 °C and after a 5 min acclimation period the number of exit attempts recorded over a 30 min window. A beetle was deemed to have attempted to disperse if it left the water, via the tank margin, or the crossed bamboo sticks supplied. (**a**) The influence of depth on exit behaviour (ANOVA $F = 4.798$; *d.f.* = 5.31; $P = 0.003$)—letters above bars indicate significant differences between means (Fisher's LSD), (**b**) The influence of density on exit behaviour (ANOVA $F = 4.375$; *d.f.* = 6.39; $P = 0.002$)—letters above bars indicate significant differences between means (Fisher's LSD). Data in (**b**) are for total number of exit responses recorded, for convenience, but statistical tests were conducted on data rescaled per individual beetle (Vosper and Bilton, unpublished). Photo, Jonty Denton

and Svensson 1992; Weigelhofer et al. 1992; Williams 2005). In some cases, diurnal flight periodicity has been detected, flight activity peaking in either the mid-morning, around noon, or at nightfall (Nilsson 1997; Csabai et al. 2012). Many species change their diel flight behaviour seasonally, diurnal dispersal being the norm in spring, with evening dispersal becoming more common in summer and autumn. Csabai et al. (2012) suggest that seasonal changes in air temperature may drive such shifts in behaviour, something in keeping with the threshold temperature response observed in many species in the laboratory (see above). As discussed by Csabai et al. (2006), the ability of aquatic insects to detect water polorotactically is at its maximum at high and low angles of solar elevation—i.e. at noon and dawn and dusk. It is thought that this 'polarisation sun-dial' interacts with air temperature to shape the timing of dispersal movements in such animals. Of the dytiscids studied by Csabai et al. (2006) most were evening dispersers, with one, *Rhantus suturalis* dispersing in both morning and evening windows.

The seasonal timing of dytiscid dispersal is poorly understood, with few studies which extend beyond occasional observation, Boda and Csabai (2013) being a notable exception for a regional fauna. Temporary pond breeders appear to disperse mainly in spring, whilst many inhabitants of permanent water predominantly disperse during summer and autumn. Some Nearctic agabines may move between temporary ponds for reproduction in Spring, and more permanent ponds in summer when vernal pools dry (e.g. Hilsenhoff 1986), a phenomenon also seen with some Palaearctic taxa, and in the Western Cape of South Africa, where species such as *Hydropeplus trimaculatus* (Laporte) occupy temporary ponds in spring, and permanent stream pools in summer (D.T. Bilton, pers. obs.). Iversen et al. (2017) contrasted the flight behaviour of *Graphoderus* and *Acilius* species in Estonia, showing that adults of the three *Graphoderus* in the study area (*G. bilineatus*, *G. cinereus* (L.) and *G. zonatus* (Hoppe)) flew almost exclusively soon after emergence in summer, whereas both *Acilius canaliculatus* (Nicolai) *and A. sulcatus* (L.) flew extensively from spring to autumn.

11.5.3 How Do the Beetles Find New Waterbodies and What Persuades Them to Stay?

How dispersing dytiscids detect suitable waterbodies is, again, incompletely understood, although recent work has emphasised the part played by patterns of polarised light. Observations that many water beetles were more strongly attracted to some colour of car than others, particularly red ones (Jäch 1997; Nilsson 1997) were followed up by some elegant experiments by Kriska et al. (2006), who demonstrated that this effect is driven the degree and direction of light polarisation from the surface. Since aquatic insects detect water largely on the basis of the horizontal polarisation of light reflected from water surfaces, they are strongly attracted to red, and other dark, shiny surfaces, such as car bonnets and roofs. Why red in particular should be attractive to some species, such as *Hydroporus incognitus* Sharp, remains unclear. In addition, habitat detection is clearly a process in which different cues may operate at different spatial scales. In addition to the visual, aquatic insects can rely on olfactory cues to detect patches of suitable microhabitat, although such processes remain unstudied to date in diving beetles. Within a waterbody the presence of con- and heterospecifics, vegetation, predators etc. (e.g. Åbjörnsson et al. 1997; Yee et al. 2009), as well as a species niche breadth (see Arribas et al. 2012 for a water beetle example) will clearly be important in determining whether a dispersal event becomes a colonisation. Pintar and Resetarits Jr (2017) manipulated patch 'quality' in a mesocosm experiment designed to mimic seasonal pools, by seeding patches with differing quantities of leaf litter. They found that both numbers of individual beetles and species richness were higher in high-quality patches. Colonisation rates by dytiscids and hydrophilids were higher in fish free patches in the experiments of Resetarits Jr and Binckley (2014). McNamara et al. (2020) show that artificially heated mesocosms were colonised by fewer aquatic insects than unheated ones in Mississippi, USA. Individual dytiscid species differed in their responses: of those abundant enough to analyze, *Copelatus glyphicus* (Say) conformed to this pattern, whereas *Laccophilus fasciatus* Aubé was equally frequent across treatments. Nutrient enrichment of mesocosms had no significant effects, although in a previous study

(Pintar et al. 2018) *C. glyphicus* colonised nutrient enriched and fishless habitats at higher rates.

11.5.4 Splendid Isolation: Predaceous Diving Beetles and Remote Oceanic Islands

The biology of isolated islands has long fascinated naturalists (Wallace 1869; MacArthur and Wilson 1967), particularly the ways in which organisms reach isolated areas of land, and the consequences of colonisation, in terms of community ecology and evolutionary radiation. Diving beetles have reached some of the most isolated islands in the world, including many which are of volcanic origin and so never formerly connected to other land masses (e.g. Balfour-Browne 1945). As with island faunas in most groups, the dytiscid assemblages of oceanic islands are generally disharmonious, being dominated by members of a limited number of genera, which are not always the most speciose in continental areas. In the case of the Pacific Islands, the fauna is mostly made up of species of *Copelatus, Rhantus* and related genera, and a number of Bidessinae, perhaps reflecting the high dispersal propensity of many members of these groups. In most cases, oceanic island dytiscids are endemic to individual islands or archipelagos, reflecting the subsequent absence of gene flow following initial colonisation, although evolutionary radiations *in situ* are typically modest (e.g. Hájek et al. 2021). Even in cases where more extensive intra-archipelago speciation has occurred (e.g. Fijian *Copelatus*—Monaghan et al. 2005), radiations appear to be largely non-adaptive, although this has never been explicitly investigated to date. Some island colonisations have been followed by shifts into new habitats, however, a striking example being the shift into hygropetric habitats on subtropical Macaronesian islands by members of the *Hydroporus fuscipennis* group (Ribera et al. 2003). The detailed biogeography of island colonisation by predaceous diving beetles is poorly known, although recent molecular studies have been illuminating in some cases. Moronière et al. (2015) focused on two morphologically aberrant dytiscids reported from the Tristan archipelago in the South Atlantic and Juan Fernandez Island off the coast of Chile, respectively and formerly classified in their own tribe, the Anisomeriini (Brinck 1948). A molecular phylogeny showed that rather than representing a distinct higher taxon, both these species nested within the genus *Rhantus*, and indeed within the same Neotropical species group, apparent morphological similarities resulting from parallel modifications following island colonisation.

11.6 Dispersal and Biogeography: The Macroecology of Movement in Diving Beetles

11.6.1 Dispersal and Geographical Range Size

It is a longstanding observation that most species on earth are rare, and restricted in their geographical occurrence, but despite numerous reviews (e.g. Gaston 1994, 2003, 2009), our understanding of what drives these patterns remains limited; and not just in the case of dytiscid beetles. A factor that intuitively *should* be involved in many cases, however, is relative dispersal ability, in terms of the ability of a species to establish a new population in a new, discrete, habitat patch (i.e. emigration plus interpatch movement plus immigration, *sensu* Bowler and Benton 2005). A number of recent studies (e.g. Malmqvist 2000; Böhning-Gaese et al. 2006; Rundle et al. 2007) have suggested a relationship between dispersal potential (as assessed by relative wing size) and geographical range, some of these concerning aquatic insects. In the case of dytiscids, Calosi et al. (2010) examined the relative importance of estimated dispersal potential, as assessed from relative wing size, and a number of thermal physiology traits in driving latitudinal range extent in European species of *Deronectes*, a genus of lotic species. They concluded that thermal physiology rather than relative wing size, was the best predictor of geographical range extent, rare species having much narrower thermal limits than common ones, with the highly successful Postglacial colonist *Deronectes latus* (Stephens), distributed from Scandinavia to the Balkans, being by far the most thermally tolerant and plastic species examined. Whether relative dispersal ability plays *any* role in shaping the biogeography of this genus remains unclear, however. Sánchez-Fernández et al. (2012b) examined the fit between actual and potential geographical ranges in the same species, comparing range estimates based on climatic data of occupied areas, with those based on data from physiology experiments. They found that neither approach predicted ranges that closely matched those observed, suggesting a possible role for dispersal limitation. Perhaps when comparing closely related species, measures of wing size tell us little about what species actually do—taxa with similar wings may behave very differently in terms of how frequently they fly, and the thresholds which trigger such movements (see above).

Whatever the role of dispersal in shaping relative range sizes in some individual dytiscid clades, it is difficult to imagine how a widespread diving beetle could colonise large areas without an ability to fly well, particularly given the isolated nature of most inland waters. Perhaps the most widely distributed dytiscid on earth is *Rhantus suturalis*, known almost throughout the old world, from the Azores to New Zealand. Balke et al. (2009) suggest that this species has arisen from within a clade of closely related taxa endemic to the New Guinea highlands between 6 and 2.7 MYA, and has subsequently spread over much of the globe in a rather complex pattern (Fig. 11.5). Data from the British and Irish biological recording schemes for water beetles suggest that *R. suturalis* has also responded rapidly to climatic warming (Fig. 11.6); the species reaching northern Scotland and parts of Ireland

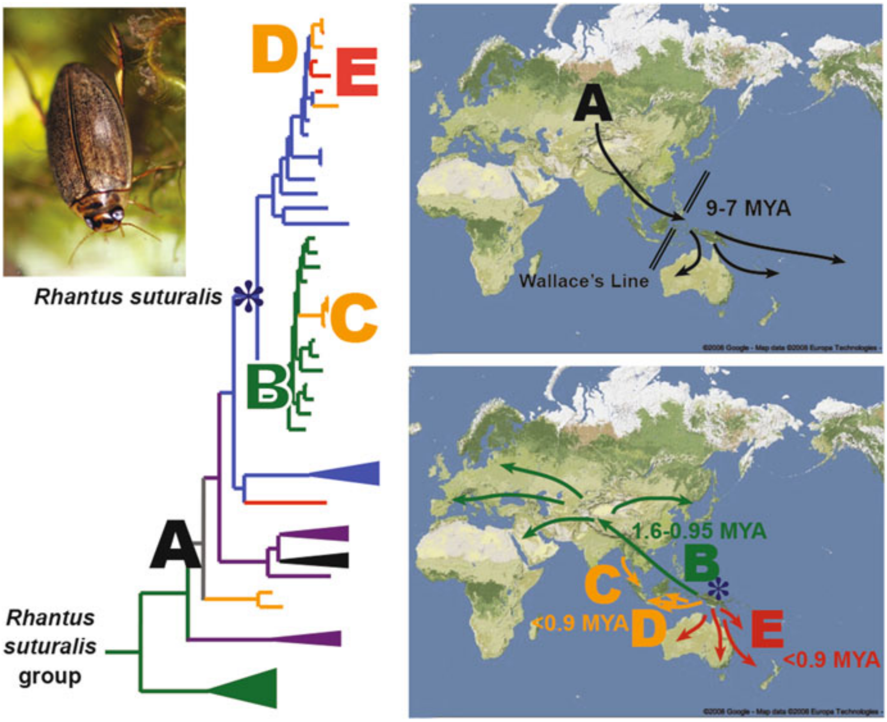

Fig. 11.5 Phylogeography of the supertramp *Rhantus suturalis* and its relatives, showing major colonisation events (A–E). Branch/letter colours: orange, Oriental region; blue, New Guinea; red, Australia, New Zealand and New Caledonia; purple, Melanesia; black, Oceania (Polynesia); green, Holarctic. *R. suturalis* itself originated in the New Guinea highlands (asterisk) and has expanded its range northwards (green arrows—B) and colonised Wallacea, Sumatra and the Australian region (orange and red arrows—C, D, E). Modified after Balke et al. (2009); photo of beetle, Jonty Denton

for the first time in recent decades. This 'supertramp' (*sensu* Diamond 1974) is a very active flier (Jackson 1956b, D. T. Bilton, pers. obs.), and one which has a relatively wide diurnal window for flight activity (Csabai et al. 2006). At the other extreme, dispersal limitation could clearly be an important driver of diversification in dytiscid clades, lineages that are weak dispersers being likely to contain large numbers of narrow-range taxa. Whilst pointing to the importance of long-distance dispersal in island colonisation, Balke et al. (2007) point out that the majority of Exocelina species are endemic to small areas, with over 150 such species likely to present on New Guinea. With one exception so far (Shaverdo et al. 2013), all these New Guinea species occur in running waters. Indeed, as discussed below, habitat type seems to be *the* major ecological/evolutionary driver of range size in dytiscids (and most freshwater organisms for that matter), this effect of habitat being mediated largely through the relative strength of selection for dispersal.

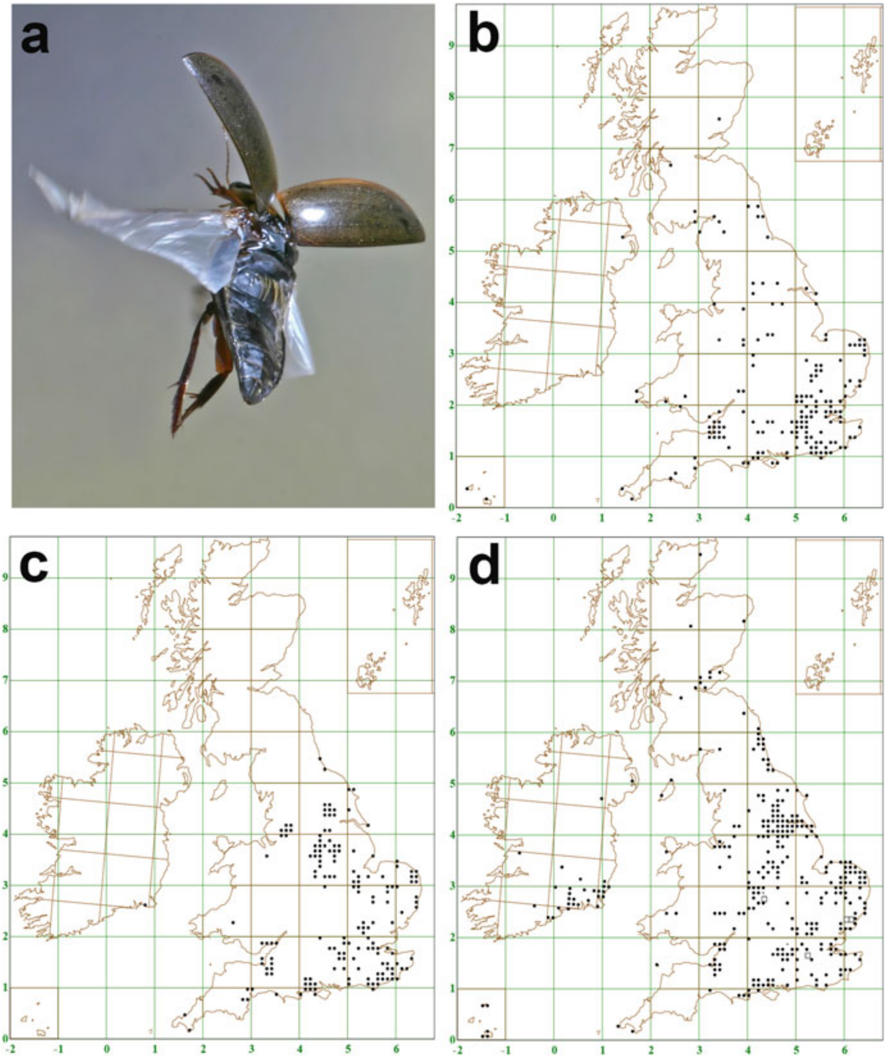

Fig. 11.6 The supertramp *Rhantus suturalis* in Britain and Ireland. (**a**) adult beetle in flight (photo Geoff Nobes), (**b–d**) records from the UK and Irish water beetle recording schemes, by 10 km^2. (**b**) nineteenth century–1979, (**c**) 1980–2000, (**d**) 2000–August 2013. This strong flier has shown apparently rapid shifts in range, at this, the northwestern edge of its global distribution. Whilst apparently present in Scotland and Ireland in the nineteenth century *R. suturalis* was rarely reported in these countries, and relatively southern in distribution in England and Wales between 1980 and 2000. In the current century, it has colonised the northernmost regions of Scotland and expanded greatly in SE Ireland, these expansions likely being a response to recent climate change

11.6.2 Diving Beetles and the Lentic–Lotic Divide

In recent years studies of water beetles, including dytiscids, have been instrumental in the development of a novel macroecological framework for understanding diversity and evolution in freshwater organisms—the lentic–lotic divide (see Ribera 2008 for a review of this topic). First suggested through the study of Iberian aquatic beetles, it is now generally established that in many inland water organisms (e.g. Hoff et al. 2006, 2008), running water species have smaller geographical ranges, and more spatially structured populations, than their standing water relatives. The divide is believed to be driven by differences in the geological persistence of running versus standing waters. Most small isolated standing waterbodies have relatively short lifespans, in geological or evolutionary terms, requiring their occupants to have relatively good powers of dispersal, which result in relatively large geographical ranges. The short lifespan referred to here is not related to short-term seasonal drought, since species have a variety of strategies to cope with these, including, in dytiscids, short larval lifespans (Peters 1972), diapausing eggs/adults (Nilsson and Söderström 1988), or moving to more permanent waterbodies (Hilsenhoff 1986). Instead it refers to the fact that many small standing water basins disappear relatively rapidly through, e.g. successional processes. In contrast, running waters are firstly more connected through a drainage network, and crucially much longer-lived in geological terms, tending to persist as long as there is rainfall and a gradient. In such habitats, there is therefore less need to disperse, and indeed reduced dispersal may in some cases be advantageous due to trade-offs with other life-history traits such as reproduction (e.g. Zera and Denno 1997; Zera and Zhao 2003). The lentic–lotic framework also allows other predictions, including more rapid speciation and greater vulnerability to global change in lotic taxa (Ribera 2008), traits which are a result of their lower ability to disperse.

Since first proposed, these ideas have gained broad acceptance, being supported by a number of empirical studies, some of which have concerned dytiscids. In a study of species of inland saline waters, for example, Abellán et al. (2009) compared phylogeographic structures in two closely related southern European diving beetles *Nebrioporus baeticus* and *N. ceresyi*, which are lotic and lentic, respectively. *N. baeticus* had a higher proportion of its observed nucleotide diversity amongst than within populations, and a faster rate of accumulation of haplotype diversity than its standing water relative, as well as showing higher phylogenetic diversity, despite having a much smaller geographical range. Lam et al. (2018) explored these ideas at the lineage level in the putatively widespread New Guinean diving beetle, *Philaccolilus ameliae* Balke, Hendrich, Larson and Konyorah. *P. ameliae* was revealed to be made up of a complex of genetically distinct lineages, most of which had relatively small geographical ranges, as would be predicted for lotic taxa. Two clades, however, revealed a more complex pattern of low population differentiation, consistent with extensive recent dispersal across rugged mountains and watersheds up to 430 km apart, revealing that not all lineages conform to the pattern, even in closely related taxa. In one of the few papers to question the predictions of the lentic–lotic framework, Short and Caterino (2009) compared phylogeographic structure in the dytiscid *Leconectes striatellus* (LeConte) with that seen in the hydrophilid *Anacaena signaticollis* Fall, and the psenid *Eubrianax edwardsii* (LeConte), in southern Californian running waters. These authors argued that the fact that the species examined spanned observed extremes of genetic and phylogeographical structure argued against the importance of habitat type in structuring aquatic populations. Such a conclusion makes the assumption that dispersal ability/propensity is broadly similar across these taxa, however, something which is unlikely given, for example, their different ecologies and adult lifespans. To effectively test the predictions of the lentic–lotic framework, one needs to compare related taxa which live on either side of the divide. So far, attempts to do this with extensive phylogenetic sampling are very few, with mixed results (Hjalmarsson et al. 2015; Désamoré et al. 2018; Villastrigo et al. 2021).

11.7 Future Directions: Where Do We (and the Beetles) Go from Here?

Whilst our understanding of the causes and consequences of dispersal in dytiscids has improved in a number of ways since Frank Balfour-Browne was disturbed by a nocturnal *Ilybius fuliginosus,* a number of questions remain unanswered, both in terms of the natural history of movement, and the role of dispersal in shaping large-scale patterns. Despite some progress in recent years, there remains a need for empirical studies of individual species and assemblages, particularly those which take a comparative approach. Jackson's work is still, in 2022, the only attempt to examine comparative flight ability in a regional species pool, and similar studies using state-of-the-art methods, such as those which integrate direct observations of anatomy and behaviour with comparative phylogeography remain lacking. In this sense, dytiscids provide excellent models and could be more widely used by researchers; the resulting data allowing better parameterization of dispersal models. Studies such as those of Svensson (1998, 1999) on rockpool whirligig beetles still set the standard for understanding local dispersal movements of individuals in the field, and there is still the need for such work on diving beetles occupying patchy landscapes, particularly if they can be integrated with population genetic approaches. At a larger scale, dytiscids again provide excellent models with which to test biogeographic and macroecological ideas. Diving beetles have featured in a number of recent attempts to understand range size evolution, but there remains a need for further work in this area. Arribas et al. (2012) show that habitat transitions from lotic to lentic waters were accompanied by huge increases in geographical range size, driven by shifts in dispersal potential, in a clade of European *Enochrus* (Hydrophilidae). Dytiscid lineages that show similar habitat transitions could be used to test the generality of such observations. To date, most published work on relative dispersal ability infers this from studies of wing or flight muscle morphology. As stated above, species could also differ in the thresholds which trigger dispersal flights and their timing, factors we are only beginning to understand. Comparative studies of such features, particularly if conducted on a suite of ecologically similar species, may prove highly illuminating.

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David T. Bilton has worked on water beetles one way or another for over 35 years. He has a particular interest in understanding biogeographical patterns and never tires of finding new beetles. After studying zoology at the University of Oxford he obtained his PhD on *Hydroporus* population genetics and phylogeography from the University of London. He is currently Professor of Aquatic Biology at the University of Plymouth in the southwest of England.

Chapter 12

The Conservation of Predaceous Diving Beetles: Knowns, More Unknowns and More Anecdotes

Garth N. Foster and David T. Bilton

> *Long before we have reached even an elementary knowledge of the distinction of the kinds of ecological phenomena, they may have disappeared, owing to the continual erosion of nature that is characteristic of our era.*
> *G. Evelyn Hutchinson (1978), "Father of modern ecology" and in Frank Balfour-Browne's undergraduate class of 1922*

Abstract Aspects of the conservation of Dytiscidae are discussed with particular reference to the benefits, potential and realized, associated with ways of conserving species threatened internationally and nationally. Examples are drawn on a global basis, but inevitably with some bias to the predaceous diving beetles of Western Europe endangered by a history of intensification of agriculture, industrialisation and urban sprawl.

Keywords Biodiversity · Extinction · Pollution · Human impacts · Red list

12.1 Introduction

Few readers of this book will need reminding that the freshwaters of the world are undergoing an unprecedented level of transformation as a result of expanding human populations, and that this impact is intensifying. Freshwaters occupy only a tiny fraction of the global habitat and yet hold a disproportionately large percentage of all known species (Dudgeon et al. 2006; Dudgeon 2020). In the case of

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G. N. Foster (✉)
The Aquatic Coleoptera Conservation Trust, Ayrshire, Scotland, UK
e-mail: latissimus@btinternet.com

D. T. Bilton
Marine Biology and Ecology Research Centre, School of Biological and Marine Sciences, Plymouth University, Plymouth, England, UK

Department of Zoology, University of Johannesburg, Johannesburg, South Africa
e-mail: david.bilton@plymouth.ac.uk

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macroinvertebrates, beetles are second only to flies in terms of the number of species occurring in inland waters, and amongst the beetles, the Dytiscidae represent one of the major aquatic radiations, being found in practically every form of inland waterbody on Earth, where they are often the most ecologically important, or indeed the only, predators.

With over 4600 species worldwide (Jäch and Balke 2008; Miller and Bergsten 2016; Nilsson and Hájek 2022), predaceous diving beetles oblige us to pose the perennial ecological question, "How can so many species occupy the same habitat?" Until we understand the precise ecological requirements of individual taxa, it is risky to provide detailed guidelines for species-level conservation, but at least we have many Red List treatments, mainly national (e.g., Czech Republic by Hejda et al. 2017), occasionally transnational (e.g., Ireland by Foster et al. 2009), and often regional (e.g., Flanders by Scheers 2012 and Schleswig-Holstein by Gürlich et al. 2011). Many case studies and reviews have identified particular species under threat, those cited here being intended to exemplify different aspects of the conservation problem across the world but inevitably drawing heavily on the European experience. Do we have any way of knowing why one species is at risk of extinction and another is doing well? Could the answer be related to why so many species can live together? The only certainty is that we do not know these answers. Investigations of interrelationships between co-existing predaceous diving beetle species are often frustrated by the complexity of responses, e.g., the variation in assemblages of seven *Hydroporus* species co-occurring along a pH gradient could not be explained by pH alone (Juliano 1991).

Conservation effort focused on predaceous diving beetles has been most active in the western Palaearctic, which has borne the brunt of urbanization, intensive agriculture, and industrialization in the past but also, at least in the north, an area with relatively few of the narrowly endemic species, the global loss of which must be an overriding conservation concern. Much has been found out in this European struggle, the degree to which the lessons learnt have wider relevance to conservation globally remaining to be seen. As more and more of the globe shifts from wilderness to development (sensu Sutherland 2004; see also Bradshaw et al. 2021), the European experience may become all too applicable.

12.2 Dytiscidae as a Group Worth Conserving

Predaceous diving beetles are diverse and yet uniform (see Chaps. 3 and 5). Their ground plan is immediately recognizable, whether the largest (the Brazilian *Megadytes ducalis* Sharp—47.5 mm long—Fig. 12.1—Jones 2010; Hendrich et al. 2019) or what could be the smallest (the Australian outback and subterranean *Limbodessus atypicalis* Watts and Humphreys (2006)—0.9 mm long—Fig. 12.2). Nearly all are non-marine aquatic insects with the exceptions a few species living in leaf litter (e.g., Brancucci and Hendrich 2010) and some of Sharp's (1882) Group 1 of *Agabus*, the species with discontinuous outlines, that live in wet areas beside rather than in streams on mountains, extreme forms of which are known as

Fig. 12.1 The first detected specimen of *Megadytes ducalis* Sharp. There is a rumour (Jones 2010) that this specimen was found at the bottom of a dugout canoe by the Amazon. The coin is 22 mm in diameter. Photograph by Garth Foster

"tropicoalpine super specialists" (Nilsson 1992). Predaceous diving beetles vary in distribution from the almost cosmopolitan *Rhantus suturalis* (Macleay), the so-called "supertramp" (Balke et al. 2009), which ranges from New Zealand to Ireland, to many flightless subterranean species confined to single aquifers, as noted in a review by Spangler (1986), with the most numerous examples now known from Australia, e.g., the subterranean *Limbodessus* and former *Nirriperti*—now within *Paroster* (see Miller and Bergsten 2016).

Dytiscidae occur in practically all inland aquatic environments on earth, from wet rock faces on mountains (e.g., *Hydroporus pilosus* group and *Africophilus* species) to fast-flowing streams and rivers (e.g., many of the *Deronectes* group of Hydroporinae), ponds, wetlands, groundwaters and bromeliad tanks (Balke et al. 2008; Miller and Bergsten 2016), and even to tropical forest floors in, for example, India (Brancucci 1979, 1985) and Madagascar (Ranarilalatiana and Bergsten 2019). In many habitats, they are abundant and speciose and may constitute the top predators, having both high ecological importance, and reflecting processes occurring, and assemblage composition, at lower trophic levels. In addition, compared to many other aquatic insects, in particular the Diptera, they are relatively well-known from systematic and biogeographical perspectives (Miller and Bergsten 2016), particularly so in Europe and North America. For these reasons, and others, such as the relative longevity of many species as adults (enabling them to be sampled over an extended season), such beetles provide an excellent surrogate taxon for wider

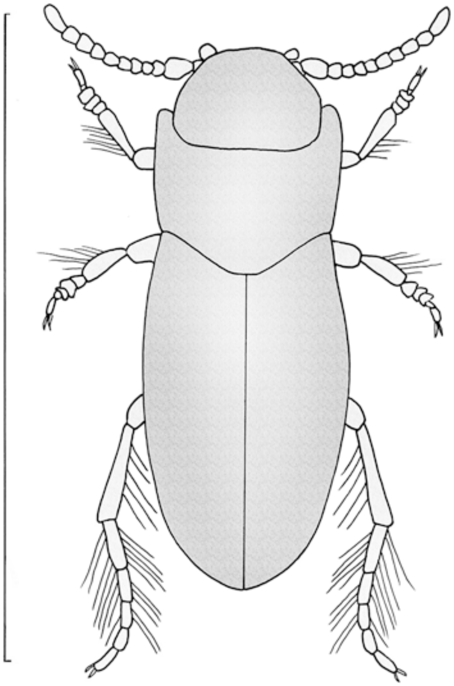

Fig. 12.2 The smallest known dytiscid beetle in the world, *Limbodessus atypicalis* Watts and Humphreys, found in a borehole in the Northern Territory, Australia. The scale is 1 mm. Redrawn from Howard Hamon in Watts and Humphreys (2006)

freshwater biodiversity, an approach that has been applied in a number of different regions, across a wide range of inland water habitats (e.g., Bilton et al. 2006; Picazo et al. 2012). In addition to their application as surrogate taxa, many predaceous diving beetles have narrow ecological niches and so are excellent indicators of ecological quality and the conservation status of sites (e.g., Foster et al. 1990, 1992). Some of the poorly dispersing taxa are claimed as indicators of ecological continuity, such as those associated with pool systems in the remains of ice eruptions formed in periglacial, near-permafrost conditions, now named as lithalsas by Pissart (2003), formerly referred to as pingos (Foster 1993; Bameul 1994), and more generally described as the pools associated with cryogenic mounds (Clay 2015).

12.3 Change

Declines in insect populations have been documented in many parts of the world in recent decades, in both highly impacted and relatively intact landscapes and in both temperate and tropical regions. Whilst the drivers of such changes are complex and incompletely understood, they include both local factors such as urban development and agri-industrialisation, as well as global climate change; the latter often driven by processes occurring many hundreds or thousands of miles away from the study site (see Sánchez-Bayo and Wyckhuys 2019; Wagner et al. 2021 and accompanying papers). A paper extolling an amazing explosion in insect abundance would be very rare indeed, though locust swarms continue to threaten crops from Africa to India (Roussi 2020). In the case of Dytiscidae, and indeed water beetles in general, we have a paucity of data on biomass and abundance, although declines in distribution in recent decades are commonplace, particularly for habitat specialists (e.g., Foster 2010; Foster et al. 2016). Whilst some studies of European freshwater insects have revealed catastrophic declines in abundance since the 1960s (e.g., Baranov et al. 2020), a number of dytiscid studies reveal turnover of taxa, but little change in abundance. A typical example of changes in dytiscid faunas is provided by Roth et al. (2020). They surveyed Hydradephaga in southern Germany in 1991–1995, 2007/8 and 2017/18. Eighty-one species were found, with an annual decline of about 1% in species number and a 2% decline in overall abundance. Community composition also changed over time, partly reflecting natural successional processes. Some species and habitats clearly appear more sensitive than others, although our understanding of why remains largely anecdotal.

Changes in biodiversity may also be intraspecific. For example in those dytiscids with two forms of female, one male-like, the other with dorsal sculpture modified to offer greater resistance to the male, and of course, in the accompanying males engaged in a sexual arms race (Bergsten et al. 2001). The overall tendency is supposedly for the more strongly modified form of female to be more northern, although this has never been formally tested. An exception is *Hydroporus memnonius* Nicolai, in which the matt form of female is absent from Ireland, Anglesey and the Scillies, and is largely southern and eastern in the rest of Britain (Foster et al. 2016). Over a thirty-year period, the matt form (*castaneus* Aubé) and its associated male have expanded significantly at the expense of the male-like form, something which appears to be driven by sexual conflict rather than by climate change (Bilton and Foster 2016). An intraspecific form such as the shining female and its associated males could be considered to have a higher conservation status than the invasive matt form. Loss of population genetic diversity is also clearly a conservation issue, with the effects of bottlenecking being detected in beetle populations in the extremities of their distribution. There is a single population of *Graphoderus bilineatus* (De Geer) in Italy (Nardi et al. 2015; Boscari et al. 2020), posing a dilemma for conservationists. Given localized extinction and a willingness to attempt reintroduction, does one choose individuals genetically close to the bottlenecked population, or does one seek out a range of haplotypes to improve the long-term prospects of the species? Unfortunately, most such studies are still limited to mitochondrial DNA variation, meaning that important local adaptations may be overlooked.

12.4 Apparent Extinction and Discovery as Motivators

There are solid reasons for scientific surveys intended to reveal changes in faunas and the reasons for those changes. But for many of us, it comes down to the thrill of the chase, the chance to find something no-one else has and to put one's mark on it, or, at the other extreme, the chance to prove that others are wrong and that a species is still thriving. Next to finding a species new to science, rediscoveries provide the life blood of enthusiasts, often resulting in a lifetime's devotion. GNF can still recall the pleasure of finding *Agabus striolatus* (Gyllenhal) in the Norfolk Broads in England 122 years after it had last been found there in the mid-nineteenth century (Foster 1982). Another example would be *Ilybius erichsoni* (Gemminger and Harold), found after a gap of 70 years in Brandenburg by the Wendlandts, father and son (Wendlandt et al. 2018). Such a discovery often implies surviving undetected in a relict site rather than a recolonization.

12.5 The Causes of Loss

As with any work on biodiversity loss, the usual litany of human-inspired disasters is difficult to avoid, but it may be better to focus on a few aspects in detail where there is some detailed appraisal or a promise of recovery. With both habitat and species recovery, it is important to consider what one is attempting to recreate. Perceptions of what is 'natural' are clearly prone to generational amnesia, particularly in areas of the globe which have been severely impacted for centuries, and for which historical accounts are scanty (Papworth et al. 2009; Rick and Lockwood 2013).

12.6 Drainage

When Charles Darwin was collecting water beetles in the Fens to the north of Cambridge in the 1820s, he would have had access to Whittlesea Mere, the largest lake in southern England (Fig. 12.3). It and the surrounding fenlands supported aquatic animals now extinct in Britain, including *Rhantus bistriatus* (Bergsträsser) and *Graphoderus bilineatus* (De Geer) (Fig. 12.4). The lake's destruction in the 1850s was the inevitable consequence of drainage begun during the Roman occupation (Rotherham 2013). The lake, becoming ever higher than the shrinking peat of the surrounding land, supposedly posed a tsunami-like threat to the surrounding area, and was easily drained by the newly available steam-driven pumps. Its floor subsequently produced some of the most fertile land in Britain. Some surviving peatland in the area became the first nature reserves in the world. Wicken Fen was set up as a reserve in 1899 by the National Trust, and Woodwalton Fen was acquired as a private reserve in 1910 (Friday 1997; Godwin 1978). These reserves retained

Fig. 12.3 Whittlesea Mere in 1850 immediately prior to its drainage. This was one of the earliest collecting sites for dytiscid beetles in England. As reproduced by Wentworth-Day (1954) from Miller and Skertchly (1878)

Fig. 12.4 *Graphoderus bilineatus* (De Geer)—one of the two dytiscid species listed under the European Union Habitats Directive and the Bern Convention. Photograph courtesy of Claus Wurst

some, but not all, of the predaceous diving beetle rarities. The Norfolk Broads' fenland 50 miles to the east continued to provide mere-like habitat complexes in former mediaeval peat-diggings, with *R. bistriatus* and *G. bilineatus* known until the early twentieth century. These beetles probably disappeared because of a loss of water quality and the resultant change in vegetation. In practice, the networks of drains in the Cambridgeshire Fens continue to support part of the original predaceous diving beetle fauna plus many species of temporary and slow-running fens, the assemblage being artificial and seen at its best in drains fed by clean water upwelling from the gravels beneath the peat (Foster et al. 1990; Foster and Eyre 1992). A very similar story of drainage, loss and faunal change has been played out, or is in active progress, in wetland areas throughout the world.

A counter-intuitive finding coming under the heading of drainage should not go unmentioned. Paddy fields provide important habitats for some of the commoner Dytiscidae in East Asia. It has been found (Watanabe et al. 2013) that the switch from conventional rice production, in which there is midseason drainage of otherwise flooded land, to direct seeding without tillage, in which the land is sown dry and then flooded later until harvest, is beneficial for some Dytiscidae. Populations of *Hydroglyphus japonicus* (Sharp) and *Rhantus suturalis* increased approximately three- to sevenfold, indicating the importance of avoiding disturbance during breeding.

12.7 Pollution

In recent years legislation such as the Clean Water Act in the USA, the Water Framework Directive in Europe and the National Water Act in South Africa has spawned a vast number of papers attempting to bring measurements of performance into line ("intercalibration"), no more so than in Europe (Birk et al. 2012). As Dytiscophiles where are we in all this? You will be generally disappointed if you search for beetles in one of these papers claiming to cover aquatic macroinvertebrates. Even Elmidae get mentioned only rarely, let alone the subdominant dytiscid predators such as *Nebrioporus*, *Oreodytes*, *Nectoporus* and *Platambus*. Perhaps scores based on EPT (Ephemeroptera, Plecoptera and Trichoptera) will suffice, and we should just go on our own way without troubling would-be policymakers? How well beetles follow patterns seen in other taxa is rarely investigated, however, Bilton et al. (2006) providing a rare example, albeit restricted to ponds.

The European Union's Common Agricultural Policy (CAP) was the culmination of post-WWII measures directed at increased food production, so driving agricultural intensification, which has resulted in widespread water contamination with farm fertilizers and pesticides, a multinational approach to pollution that is often suggested as the cause of loss of many predaceous diving beetle species from large areas of Europe. The partial extinctions of the two species listed in the Habitats Directive, *Dytiscus latissimus* L. (Fig. 12.5) and *Graphoderus bilineatus*, are

Fig. 12.5 *Dytiscus latissimus* L.—the largest European dytiscid and the other species listed under the European Union Habitats Directive and the Bern Convention. Photograph courtesy of Lars Hendrich

possible examples, though their decline certainly began before WWII. Land use change must be important, many aspects of this being cited by Hendrich and Balke (2000) and Hendrich (2011) in the case of *D. latissimus*. Cuppen et al. (2006b) note the potential importance of wet and dry acid deposition in moorland pools for damage to populations *G. bilineatus* but identify the importance of moorland systems receiving high-quality seepage water as essential for its survival. In the UK, the extensive and ongoing decline of some taxa appears to follow agricultural intensification. *Hydroporus rufifrons* (Müller) is a species of temporary and fluctuating wetlands, particularly in floodplains, and has been lost from most of the UK in the course of the twentieth century, this decline apparently following the northwestern spread of intensive agriculture from the lowlands of the southeast (Balfour-Browne 1940; Foster et al. 2008). Such data are correlatory, however, and in most cases, we have no clear understanding of how such species are impacted by the intensification process, and whether agricultural chemicals have a direct or indirect effect. Perhaps the best example suggesting that nutrients are a key factor is the recovery or re-colonization of the Naardermeer, Weerribben, Wieden, and Nieuwkoopse Plassen by *G. bilineatus* in the Netherlands following phosphate removal from the Ijmeer water supply (Cuppen et al. 2006b).

12.8 Encroachment

Over and above drainage, habitat loss can come about by many other forms of human ecology—urbanization, industrialization, deforestation and afforestation. It is unfortunate that humans position so many of their greatest trading cities on estuaries and navigable rivers, as huge swathes of wetland habitat have been lost through such encroachments across the world. This was recognized by Adam Smith (1776) . . .

> The inhabitants of a city, it is true, must always ultimately derive their subsistence, and the whole materials and means of their industry, from the country. But those of a city, situated near either the sea coast or the banks of a navigable river, are not necessarily confined to derive them from the country in their neighbourhood. They have a much wider range, and may draw them from the most remote corners of the world, either in exchange for the manufactured produce of their own industry, or by performing the office of carriers between distant countries and exchanging the produce of one for that of another. A city might in this manner grow up to great wealth and splendour, while not only the country in its neighbourhood, but all those to which it traded, were in poverty and wretchedness.

So far as dytiscid beetles are concerned, the wretchedness is more direct. David Sharp (1917) decried the Hammersmith Marshes, in the heart of London, as, “portions and parcels of the dreadful past”, being among, “the very best spots in Britain for Entomology”. Sharp noted that “in London last year [1916] I went to Hammersmith to try and identify the old collecting ground. I quite failed, and what a falling off I found! What people call the advance of civilization produces a very depressing effect on those of us who recollect the beauty of suburban London 60 or 70 years ago.” Now, of course, there is not the remotest portion of this marshland, which Sharp described as running a mile and a half north-west from Holland House to Notting Hill. Sharp might find consolation in the area, however, in that it houses large reservoirs, now abandoned as water supplies, which support a suite of pioneer predaceous diving beetles including *Hygrotus* (*Leptolambus*) *nigrolineatus* (von Steven) not found in Britain until 1983 (Carr 1984) and certainly not a beetle that Sharp could have found in his time.

Urbanization’s impact may be even more marked in areas of high biodiversity. Balke et al. (1997) and Hendrich et al. (2004) highlighted the pressures on predaceous diving beetles and other water beetles in the urban area of Singapore and its surroundings, where a number of species are considered locally extinct or threatened. The informal settlements of the Western Cape of South Africa impinge on the very narrow territory of *Capelatus prykei* Turner and Bilton, as further discussed in Sect. 12.16. Bogotá, the Colombian capital with a population of almost eight million, provides the habitat for the recently described *Rhantus bogotensis* Balke et al. (2019), and other dytiscids confined to the Alto Plano. Blicharska et al. (2017) found that the presence of humans and their dwellings were detrimental to aquatic biodiversity, but could not establish a link to any particular economic status as has been claimed for birds and plants (Iversson and Cook 2000).

12.9 Climate Change

Climate change has rather downplayed the excitement associated with finding a species in a new site, but there is still some pleasure in finding exceptions to the generally accepted rule that biodiversity increases from the Poles to the Equator. Colymbetinae undoubtedly buck this trend; Morinière et al. (2016) explained the inverse latitudinal diversity gradient (iLDG) of this subfamily on the basis of origin in the temperate zone, with niches dictated by fine-tuned responses to seasonal oscillations at relatively low temperatures and phylogenetic niche conservatisms. Climate change may have a greater impact on such species than on the fewer temperate outliers of faunas largely adapted to (sub)tropical climates.

We have plenty of examples of predaceous diving beetles contracting or expanding in distribution in relation to climate changes, but it is difficult to find examples of total losses or extinctions to date. There is, however, a good understanding of changes in the water beetle fauna over the course of the Pleistocene glaciations, with an appreciation of the ability of that fauna to respond to rapid changes in temperature regime, built on the pioneering work of Russell Coope (see Elias 1994, 2010): for example the Mongolian and Canadian *Hygrotus* (*Leptolambus*) *unguicularis* (Crotch) occurred on the Isle of Man (between Ireland and Britain) in the last interglacial period 12,000 years ago (Joachim 1978). In the northern hemisphere at least, shifts in geographical range over the course of the Pleistocene appear to be the norm for many high latitude predaceous diving beetles. Any assumption that these insects are able to cope with current climatic shifts is dangerous on many counts, however. As well as questions regarding the rapidity and direction of current climate change in comparison to that experienced in the Pleistocene, there is the added complication that modern habitats are fragmented such that most species will be unable to track their climate envelopes in the future (Hoegh-Guldberg et al. 2008). In contrast to the story at high latitudes, Abellán et al. (2011) found that for Europe, at least Pleistocene range movements do not appear to have been the norm for narrow-range endemic species distributed around the Mediterranean. Such species may be restricted to individual mountain systems as a consequence of poor heat tolerance (Calosi et al. 2008) and so be in double jeopardy in the face of climatic warming (Bilton et al. 2019), which compromises them physiologically and reduces the extent of their available habitat. Heat tolerance might take two different forms, ability to survive a sudden increase in temperature and ability to acclimate to a change in temperature regime. For *Agabus nevadensis* Lindberg and *Hydroporus sabaudus sierranevadensis* Shaverdo, two taxa endemic to the Sierra Nevada in southern Spain, Pallarés et al. (2019) have established that these beetles are able to withstand higher temperatures than those to which they are currently exposed, whereas neither species showed any ability to acclimate.

The rapidity of climate change in the Anthropocene is generally regarded as being too fast to permit many endangered species to cope in situ, through either evolution or acclimation (Arribas et al. 2012). Some dytiscids do appear to be able to cope with significant change, however. For example, the *Meladema* of the central Sahara, in the Tibesti Mountains of Chad, differs in surface sculpture from typical specimens of *coriacea* Laporte, but are genetically very much part of *coriacea*, differing by only one mutation across 404 base-pairs in mitochondrial COI sequence from specimens from the Moroccan Anti Atlas and Gran Canaria (Ribera et al. 2018). So long as water survives, it seems that there will be a dytiscid to take advantage of it. Diving beetles provide a great test bed for understanding the proteomics of heat tolerance, e.g., work on the *Agabus brunneus* complex (Hidalgo-Galiana et al. 2014a, b, 2015).

In addition to rarity, gas exchange mechanism may shape species' responses to ongoing climate change. As shown by Verberk and Bilton (2013), species with greater ability to control their oxygen uptake are likely to be more able to cope with increasing temperatures and anoxia in freshwater. Being surface exchangers, this means most predaceous diving beetles are likely to be less sensitive than similar taxa which obtain oxygen directly from the water, but it also points to the possible sensitivity of stream and (semi-) subterranean taxa relying on plastrons and/or physical gills (Kehl and Dettner 2009; Madsen 2012; Verberk et al. 2018).

In some eyes, the loss of species following climate change can be offset by the arrival of replacements, although these are usually more widespread and abundant globally speaking than the taxa they replace. An extreme example might be *Eretes* species. *E. griseus* (Fab.) has not been seen in Central Europe for over a hundred years, whereas *E. sticticus* (L.) has been found from 2009 onwards (Hájek et al. 2015). *Cybister lateralimarginalis* (De Geer) appears to be spreading north, as evidenced by finds in Russia (Petrov and Fedorova 2013) and even the first apparent individual in England since the early nineteenth century (Thomas 2009). The success of the *Cybister* in Europe is balanced by its potential competition with *D. latissimus* (Hendrich et al. 2013).

12.10 Globalization and the Fourth Horsemen of the Apocalypse

Invasive species offer a fundamental threat to biodiversity throughout the globe and have a major role in diminishing the diversity of predaceous diving beetles, fish in particular having often displaced them as the top predators. Larson et al. (2000) identified the predator hierarchy in Canadian latitudes whereby fish dominate in the deep, permanent waters that allow survival beneath ice, odonates dominate in shallower water that does not dry up in summer, and beetles dominate in fluctuating or temporary wetland habitats. This model appears to be more widely applicable, but, in terms of conservation, habitat isolation also needs to be taken into account. Upland pools without significant outflows and streams above sills or discharging straight to the sea rather than into rivers provide refuges for predaceous diving beetles unless, as has been so often the case, man intervenes by introducing game fish. Predatory fish such as salmonids, pumpkinseed, *Lepomis gibbosus* (L.), and the western mosquito fish, *Gambusia affinis* (Baird and Girard), are generally regarded as being the most detrimental to insects, but it has been proposed (Kloskowski 2011) that bottom-feeding coarse fish such as the common or European carp, *Cyprinus carpio* L., may do more damage by rendering the habitat permanently turbid. Fish can be lost from unbuffered waters by acid deposition, resulting in increases in some predaceous diving beetle populations (Foster 1991a), but this can hardly be claimed as a victory for conservation!

Invasive wildfowl, turtles, and crayfish are also important. Pederzani and Fabbri (2006) characterized the Louisiana Crayfish, *Procambarus clarkii* (Girard), as the Fourth Horseman of the Apocalypse, fourth that is to "Conquista" (eutrophication associated with agriculture), "Guerra" (chemical warfare) and "Carestia" (famine). They listed eight rare species of predaceous diving beetle under threat from it around Rome. Possibly our most dramatic example of its devastation to date is the Les Marais de la Perge in Médoc, France, where *Graphoderus bilineatus* was discovered in 1990. Bameul (1994) recorded 109 species of water beetle at la Perge but found only two in 2009 (Bameul 2013). It appears that *P. clarkii* colonized large parts of the area following a hurricane in December 1999 that caused extensive flooding in Gironde. Similar instances of the impact of *Procambarus* have been observed in a number of sites in Spain, where endorheic lagoons, with diverse water beetle faunas, have been stocked with crayfish (Andres Millán, pers. comm.). In southern Spanish streams, *Procambarus* appears to affect both the population density and ecology of rheophilic dytiscids; *Agabus brunneus* (F.) and *Deronectes hispanicus* (Rosenhauer), typical river pool species, become restricted to riffle sections in the presence of crayfish, presumably because riffles provide refugia (DTB, personal observations).

Alien plants can also threaten aquatic invertebrate communities though there do not appear to be any examples specific to the Dytiscidae. New Zealand Pigmyweed, *Crassula helmsii* (Kirk), was originally introduced to Britain by aquarists and was first recorded as an escape there in 1956 (Leach and Dawson 1999), subsequently spreading to some of the most remote islands. Although the habitat structure of this plant appears superficially suitable for predaceous diving beetles, *Crassula* beds hold few species or individuals (GNF, personal observations on Alderney, Arran, Guernsey, the Isle of Man and Tiree, and in Belgium; DTB personal observations in Devon and Hampshire). Denton (2001) noted that some predaceous diving beetles survived in the presence of *C. helmsii,* but swards of this plant are known to eliminate native wetland plants. Whether *Crassula*'s apparent impact on predaceous diving beetles is direct or indirect is still unclear.

12.11 Misidentification as a Threat to Understanding

Giving a species the wrong name should not only give the namer a bad name but also undermines attempts at conservation. Establishing the true status of species considered to be extinct is bound to be a rare experience. Misidentification of common species as rarities is more frequent. The publicity associated with the Biodiversity

Action Plan in Great Britain (UK Biodiversity Group 1999) generated false sightings of the Critically Endangered *Laccophilus poecilus* Klug, based on the commonest British *Hydroporus palustris* (L.), as both are about 3 mm long and black with yellow markings although at opposing extremes of the dytiscid body form. Advances since then in the use of photography and online picture galleries have helped to reduce such misidentifications of diving beetles. If only the "Citizen Scientist" would photograph the underside as well as the upper side and appreciate the importance of measuring size, we could improve recording at that level. But photographs cannot be dissected, and we cannot seem to get across the necessity of death in order to keep vouchers. Misidentification is not just a problem for amateur data. Journals covering conservation and ecology are guilty of publishing many papers without any regard for the accuracy of the identifications, and some professional fieldworkers have no more grasp of identification, or the need to maintain voucher material than many amateurs. Limnologists often publish detailed analyses of a site's Physicochemistry and yet reduce the insect assemblage to index scores. They fail to grasp that the presence of just one species will often tell you much more about a site's history than a single pH or conductivity reading or a Simpson's D! Many journals now provide access to raw data as supplementary, but it is surprising how often these supplements are incomplete and worse, how many errors they reveal. In practice, a multivariate analysis is probabilistically just as valid if taxa are *consistently* identified wrongly, but this misses the point. Ellis (1985) appears to have been the first to coin the expression "taxonomic sufficiency" in connection with marine pollution—it is "the concept that in any project organisms must be identified to a level (species, genera, family, etc.) which balances the need to indicate the biology (including for example such matters as diversity) of organisms present with accuracy in making the identification". This concept invites the monitoring of macroinvertebrates as indicators of human impact to be done as a scientific application separate from recording biodiversity. Whilst this means we lose potentially valuable information, if it sees an end to the frustration of dealing with an unlikely record not supported by a voucher, perhaps it would be a good thing!

12.12 Types of Conservation

Conservation efforts in general can be divided into ex situ and in situ (see e.g., Hambler 2004), those concerning predaceous diving beetles to date, with the possible exception of Vahrushev (2011) falling into the former category. For what some might misguidedly regard as an obscure group of insects, it is also important to differentiate between **active** and **passive** conservation. "Active" in the sense that predaceous diving beetles, perhaps just one species, might be the primary focus of the conservation activity—and "passive" in that the survival of the beetles relies on what is being done for another target group or habitat. Whilst some predaceous diving beetles are large enough to have appeal to the general public, and perhaps also to policy-makers (e.g., *Dytiscus latissimus* or *Megadytes ducalis*), sadly most Dytiscidae will always depend on "passive" conservation for survival.

Vahrushev's (2011) work on *Dytiscus* was concerned with laboratory rearing (Fig. 12.7), to which a corollary must be attempted at introduction or, as put IUCN (2013), "assisted colonisation, ... the intentional movement and release of an organism outside its indigenous range to avoid extinction of populations of the focal species". Captive rearing of *D. latissimus* has been extended to Japan, well beyond its known range (Watanabe et al. 2020).

There is very little published experience of translocations of predaceous diving beetles. Balfour-Browne (1962) recounted what was almost certainly the first attempt, in August 1906, when he took *Agabus undulatus* (Schrank) from Yorkshire to the Norfolk Broads: this introduction failed. Recent experience of translocating *Hydroporus rufifrons* within England has been instructive and apparently successful, but the long-term outcome of the project remains to be seen (GNF, personal observations, and see Bray et al. (2012) concerning a trematode parasite detected in the donor population).

Thomas (2011) has noted that the species at greatest risk of extinction caused by man-mediated climate change are often narrow-range endemics, something which has been demonstrated to apply to at least some dytiscids (Calosi et al. 2008). Thomas has argued that it would be better to move such taxa to places with appropriate climate, rather than to try to improve on their current habitats—an approach termed "assisted translocation" by Dawson et al. (2011). In a European context, he noted that Britain is an ideal recipient for translocated species as there are already 2000 introduced species there that are claimed not to have affected indigenous species. A last gasp attempt to build up a population of Iberian Lynx, *Lynx pardinus* (Temminck), in an alien land might find favour conservationists sharing Thomas's view, but could we seriously contemplate harvesting and releasing any of the Iberian or island endemic dytiscids in the same way?

12.13 European Conventions: Including a Case-Study in Conservation Legislation and Its Consequences

International initiatives to protect individual species of predaceous diving beetle began in the 1980s. The Bern Convention (... on the Conservation of European Wildlife and Natural Habitats) was signed in 1979 and has to date been signed up to by 44 countries. Its Appendix II for "Strictly Protected Fauna Species" it lists 710 animal species, including 10 beetles. The two predaceous diving beetles, *Dytiscus latissimus* and *Graphoderus bilineatus*, were chosen from a shortlist of 117 species selected by ten specialists from ten countries (Anonymous 1986; Foster 1991b). Signatory nations were expected to enforce protection of these species by preventing them from being disturbed, captured, killed, or traded. Here we discuss the selection process, and its consequences for dytiscid conservation, based partly on first-hand experience.

The Bern Convention provided the model for the European Union (EU) Habitats Directive (92/43/EEC) of 1992, which required the recognition of “sites of Community importance” and Special Areas of Conservation (SACs), thus setting up an international network of Natura 2000 sites based on scarce or threatened habitat types and species. Its Annex II lists the same two predaceous diving beetles among 38 beetle taxa. Member countries of the EU are required to maintain or, where appropriate, restore sites to favourable conservation status in the natural range of some important habitats listed in Annex I and the species in Annex II. This ‘passive’ approach to the conservation of beetles has produced a good range of dytiscid sites but with some anomalies, for example the absence of inland saline water bodies (Sánchez-Fernández et al. 2008). Gutowski and Przewożny (2013) emphasize the importance of *Dytiscus latissimus* and *Graphoderus bilineatus* as the only beetles to represent aquatic habitats, thus having potential as umbrella species.

What criteria might be applied to select one species over another (Hambler 2004)? Some predaceous diving beetles may qualify as flagship or umbrella species, such as the more conspicuous indicators of high-quality wetlands. In other cases, a species may be recognized as having conservation priority owing to its phylogenetic uniqueness (sensu Vane-Wright et al. 1991). A dytiscid example is *Acilius duvergeri* Gobert, formerly distributed from SW France to Morocco, which has declined severely because of loss of Mediterranean wetlands, and is today recorded certainly only from two sites, one in Sardinia (Dettner 1981; Millán and Castro 2008) and the other in Spain. As well as apparently being the rarest large diving beetle in the western Palaearctic, and one of the most endangered dytiscids on earth, *A. duvergeri* is sister to all other members of the genus (Bergsten and Miller 2006). In biogeographical terms, should one concentrate on a species endemic to a particular mountain range, which may be quite common there (e.g., many southern European *Stictonectes* and *Deronectes*), or to a widely distributed species that is in decline over much of its range, a fate which has beset many northern European fen dwellers? This dichotomy is particularly apparent in western Europe, where intensification of most human activities in the northern lowlands might be contrasted with the climate change associated with some of those activities impacting on the isolated montane and island faunas of the south, which are richer in endemic species. Going back to the original selection process, it is worth noting that we were obliged to select species for the Bern Convention on the basis that their distribution lay mainly within Europe, that the species should be reasonably easy to identify, and that the species should be under serious threat in Europe as a whole, but not necessarily in every place (Foster 1991b). Thus we were guided towards species that might benefit from an international approach. Whether by design or by chance, such criteria may serve to relegate the conservation of a species confined to a particular mountain range or Mediterranean island to being a national issue. All else being equal, such species are generally of greater importance than taxa that are rare in one country but common elsewhere, however, which often form the basis of national Red Lists (see Hunter and Hutchinson 1994).

Settling for widely distributed species under serious threat, the third criterion, “reasonably easy to identify” comes into play. What did this really mean? It seemed to rule out the smaller species, despite the fact that many large Dytiscidae can be just as easily misidentified as smaller ones. Was it just because such large beetles might be more easily detected in illegal transit? Or were they more capable of being viewed as flagship species (Hambler, 2004)? A customs official might have some difficulty deciding the species of *Graphoderus* intercepted. He or she would be in good company as an English specimen of *G. bilineatus* was originally chosen as the neotype for *G. cinereus* (L.) by Jack Balfour-Browne (1960) before it was appreciated (Angus 1976) that *G. bilineatus* had at one time lived in England. That neotype selection was later suppressed (International Commission of Zoological Nomenclature 1989). Little wonder then that a common name proposed for *G. bilineatus* is The Chequered History Beetle! Adding further to the confusion was *G. zonatus* (Hoppe), discovered in England in 1953 but passed over as *G. cinereus* until Angus’s review (1976).

The selection of *Dytiscus latissimus* and *Graphoderus bilineatus* was greeted with indifference by most coleopterists initially. Fears about bureaucratic restrictions on survey work have been little more than restrictions imposed by individual nations before the Bern Convention. Most EU member states enacted legislation to licence the collection, transport, and possession of these beetles as among European Protected Species (EPS). The beetles are not, however, covered by CITES, the Convention on International Trade in Endangered Species of Wild Fauna and Flora. More importantly, the requirement placed upon governments to protect these species by the Habitats Directive has proved to be highly beneficial to our understanding not just of their current distributions, with associated site protection, but also for clarification of their basic biology. Such reviews support the idea that both species have been lost over much of the western lowlands of Europe, but are surviving well in the east and to the north, so much so, for example, that *D. latissimus* was reduced to being of Least Concern in Norway (Kålås et al. 2010). Work in the Netherlands originally was mainly concerned with the analysis of survey data for both species (*D. latissimus*—Cuppen et al. 2006a; *G. bilineatus*—Cuppen et al. 2006b). A model for *G. bilineatus* was particularly useful in relating its distribution to moderate conductivity and to the presence of a rich mixture of emergent and submerged vegetation with some floating-leaved plants such as water-lilies (with duckweed never dominant). Vahruševs and Kalniņš (2013) reviewed data for *D. latissimus* for 26 sites in Estonia, 42 in Latvia, and 37 in Lithuania. The array of ecological data demonstrated a wide tolerance, for example occupied sites ranging in pH from 3.5 to 9.8 and in conductivity from 0.05 to 0.46 mS/cm. Water depth proved of interest, with deeper waters (more than 1 m) being needed in the colder part of the range of *D. latissimus*, presumably so that it can survive in winter, and echoing the model previously used to explain fish dominance in a northern climate. Intensive research on *D. latissimus* and *G. bilineatus* has emphasized their similarities, often being found in the same area, but also fine differences in their habitat requirements, *G. bilineatus* being more or less confined in canals and ditches on peat areas in the Netherlands whilst *D. latissimus* is found in

Fig. 12.6 Large traps have had to be developed to study *Dytiscus latissimus*, which is too big to be caught in the usual kind of bottle trap. In practice, the fisherman's keepnet, suitably baited, has been found most effective. The one illustrated here in fact belongs to a Byelorussian angler—and it was occupied by *latissimus*! The trap illustrated here was found to be baited with beecomb, but many studies (e.g. Volkova et al. 2013) have proved the worth of the use of a bait of red meat or liver. Photograph by Garth Foster

acid boggy ponds (Jan Cuppen, pers. comm.), and that these distributions are narrower than in the past. In Germany Hendrich (2011) has noted that the former association of *D. latissimus* with carp ponds is no longer possible because of their present day management, with liming, removal of vegetation, steep banks and artificial lining, emphasizing a narrowing in habitat requirement in a different way. Recent work in the Netherlands has contrasted the diet of *D. latissimus* larvae with that of *D. lapponicus* Gyllenhal (Scholten et al. 2018). This demonstrated that food availability might be a limiting factor for *D. latissimus* based on early instars being obligate feeders on limnephilid caddis larvae, whereas larvae of *D. lapponicus* feed on a wider range of prey. It was suggested that the promotion of leaf litter on shores using by *D. latissimus* for oviposition might be beneficial in increasing the abundance of shredding caddis larvae.

Non-destructive traps have been developed that have been used for mark-and-recapture, demonstrating, for example that *D. latissimus* can live at least three years in the wild (Schmidt and Hendrich 2013). Traps can, however, prove highly destructive if left untended (Fig. 12.6, and see also Prokin et al. (2018) for the inadvertent effect of ice-hole willow traps, "koshura", in Russia). Attempts to rear both Bern Convention species in captivity have proved difficult, progress being made by Vahrushev (2011) with *D. latissimus* at Latgale Municipal Zoo, Latvia (Figs. 12.7, 12.8, and 12.9), and more recently in Japan (Watanabe et al. 2020).

A major feature of European Union conservation activity is implementation of the Water Framework Directive (WFD or, in full, "Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy"). A principal requirement is to return

Fig. 12.7 A complete breeding system. Vahrushev (2011) gave a complete guide to building equipment suitable for observing the development of *D. latissimus*. "Aqua-terrariums" had a ventilated aerial part suitable for pupation and an aquatic part holding ca 100 US gallons (400 l) and supporting up to 60 individuals. Particular attention was paid to temperature control, water filtration and simulating seasonal variations. Photograph courtesy of Valery Vahrushev

water bodies to "good ecological status" by 2015. Governments of member states have developed catchment management plans. It remains to be seen whether this directive will achieve long-term sustainable water management, and whether "good ecological status" really equates to high water quality, but at least the WFD has obliged government agencies to look beyond the major rivers and lakes of each catchment, and perhaps to devote less attention to fish and more to macroinvertebrates as indicators of ecological status. Predaceous diving beetles have not achieved a high profile in this re-evaluation of aquatic ecology, but their conservation must benefit "passively". In particular, measures to restore near-natural river structure and flow will be of value as well as reductions in diffuse and point source pollution.

12.14 Popularity, Biodiversity and Ecosystem Services

Dytiscid beetles cannot be expected to achieve the popularity among the public or in scientific circles enjoyed by showy insect groups such as butterflies (e.g., Pollard and Yates 1993; Kudrna et al. 2011) and by pollinators (e.g., bumblebees—Goulson 2010). Apart from providing a demonstrably imperfect control of mosquitoes (Larson et al. 2000) and the occasional delicacy in a Cantonese restaurant (Jäch 2003), diving beetles are unlikely to feature highly in delivering the "ecosystem

Fig. 12.8 This is a Japanese representation of the as yet non-Japanese *Dytiscus latissimus* Linnaeus, as illustrated by Agro Bio © The Coleopterological Society of Japan

services" elaborated by the Millennium Ecosystem Assessment (2005). An indication of the importance of predaceous diving beetles in ecosystem function has, however, been demonstrated through the mesocosm studies of Rudolf and Rasmussen (2013). Manipulating the numbers of adults and larvae of *Cybister fimbriolatus* (Say) resulted in significant shifts in animal biomass, phytoplankton, periphyton and decomposition rates, and, as measured via diurnal oxygen cycles, different rates of respiration and net primary productivity. In addition, as discussed above, they can play a role as surrogates of wider aquatic diversity and indicators of ecosystem health. The biodiversity of Dytiscidae results in an array of potentially exploitable corticosteroids in their prothoracic defensive glands (Dettner 1987, 2019) associated with a huge diversity of endosymbiotic bacteria implicated in the transformation of these substances (Gebhardt et al. 2002).

Recognition of the value of predaceous diving beetles is more likely to be associated with their species richness, and therefore their conservation should have benefited most when the paradigm of biodiversity (Convention on Biological Diversity 1992) held sway post-Rio. A possible example is provided by UKBAP, a national Biodiversity Action Plan (UK Biodiversity Group 1999), actually, a non-government initiative (mainly by the Royal Society for the Protection of

Fig. 12.9 Seen here is a larva of *D. latissimus* hatching from its egg in captivity. Unusually amongst dytiscids, the larvae do not appear to be cannibalistic, but separate rearing is still considered necessary to avoid larvae being affected by the external digestion secretions of others (Vahruševs 2009). Photograph courtesy of Valery Vahrushev

Birds) that for a while ran in parallel with government initiatives based largely on UK conservation-based legislation concerned with site protection. The UKBAP listed six dytiscid species for which species action plans drew down funding to research species status, mainly in England. So far as dytiscids are concerned, the UKBAP lived on when these species were placed on the lists in the Natural Environment and Rural Communities Act 2006 (NERC Act) (Sect. 41 in England and Sect. 42 in Wales). The UKBAP has facilitated useful research on the distribution and genetic diversity of dytiscids in Britain (e.g., Foster and Carr 2008; Foster et al. 2008; Lott 2005), but with one of the species (*Laccophilus poecilus*) having last been seen in England in 2002. Any study of species richness must ultimately focus on the individual species involved.

The study of Madagascar’s water beetles (Isambert et al. 2011, drawing on work by Monaghan et al. 2009) takes the study of predaceous diving beetle biodiversity to a new level by comparing species richness, phylogenetic diversity, and endemism in ten national parks. This work demonstrates that our understanding of species-level taxa is high, i.e. molecular and morphological designations were largely (91%) in agreement. The concern is that phylogenetic diversity and endemism are negatively correlated, presumably because many of the endemic species are the result of relatively recent radiations *in situ*. Consequently, ranking sites on the basis of the phylogenetic diversity they support, an approach frequently used in conservation, may mean that globally rare, endemic species are ignored—i.e. just the ones most in need of support! This is surely a powerful conservation message with wide application “serviced” by predaceous diving beetles. The detailed work in Madagascar can also be used in a more orthodox way to identify biodiversity hotspots (see Fig. 12.10 concerning endemic *Rhantus* species—Hjalmarsson et al. 2013).

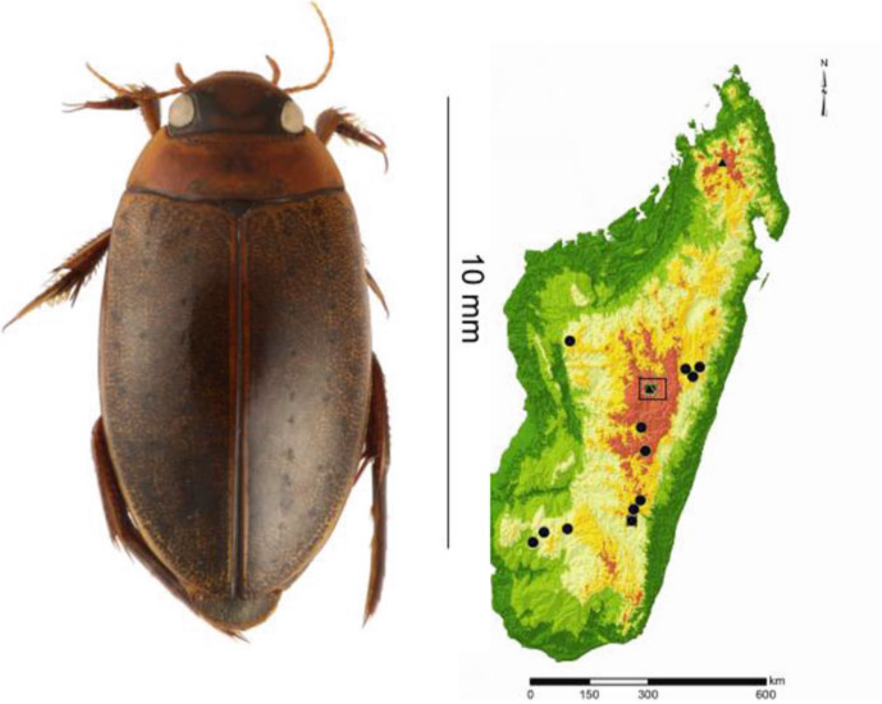

Fig. 12.10 *Rhantus manjakatompo* Pederzani & Rocchi is one of three Madagascan *Rhantus* confined to the high plateaux and all found together only at Manjakatompo (in the rectangle) (Hjalmarsson et al. 2013). Photograph Johannes Bergsten; map adapted from FTM, 1995. *Foiben Taosarintanin'i Madagasikra*. Institut Géographique et Hydrographique National 27/95

12.15 Global Lists

If numbers are important, then world lists ought to provide important tools for conservation of predaceous diving beetles. Unfortunately, the task of listing the entirety, or anything like it, of Dytiscidae fitting the criteria for threat status has proved impossible to date. The first attempt (IUCN 1990) listed fifteen species in the USA, all rated "Indeterminate" apart from one possibly Extinct species, plus the two Bern Convention species, rated as Endangered. Later IUCN Red Lists were based on detailed criteria. The IUCN 1996 List (Baillie and Groombridge 1996), the last to be published as a hard copy, had eight predaceous diving beetles listed as Endangered and four as Vulnerable, the American species having disappeared because no-one could be found to re-evaluate them. Six species were listed as Extinct, and 55 were listed as having been listed in 1994, but "now Not Evaluated". The present IUCN Red List is web-based, version 2020.3, and has 23 dytiscid taxa (Table 12.1).

This list ensures that dytiscids get a mention, but is in serious need of updating, some taxa now known to be not as threatened as their inclusion would suggest. As a

Table 12.1 Dytiscidae in the IUCN Red List 2020.3

Name in IUCN (2020)	Category	Species name	Distribution	Criteria
Acilius duvergeri	VU	*Acilius duvergeri* Gobert, 1874	W Palaearctic	B1 + 2b
Agabus discicollis	EN	*Ilybiosoma discicolle* (Ancey, 1882)	Ethiopia	B1 + 2c
Agabus godmanni	EN	*Agabus godmanni* Crotch, 1867	Azores	B2ab(i, ii, iii,iv,v)
Agabus hozgargantae	EN	*Ilybius hozgargantae* (Burmeister, 1983)	S of Spain	B1 + 2c
Deronectes aljibensis	EN	*Deronectes algibensis* Fery and Fresneda, 1988	S of Spain	B1 + 2c
Deronectes depressicollis		*Deronectes depressicollis* Rosenhauer, 1856	SE Spain	B1 + 2c
Deronectes ferrugineus	VU	*Deronectes ferrugineus* Fery and Brancucci, 1987	NW Iberia	B1 + 2c
Dytiscus latissimus	VU	*Dytiscus latissimus* L., 1758	W Palaearctic	A2c, B1 + 2a
Graphoderus bilineatus	VU	*Graphoderus bilineatus* (De Geer, 1774)	W Palaearctic	B1 + 2 ac
Graptodytes delectus	EN	*Graptodytes delectus* (Wollaston, 1864)	Canaries	B1 + 2c
Hydroporus guernei	EN	*Hydroporus guernei* Régimbart, 1891	Azores	B2ab(i,ii, iii,iv,v)
Hydrotarsus compunctus	CR	*Hydroporus compunctus* Wollaston, 1865	Canaries	B1 + 2c
Hydrotarsus pilosus	EN	*Hydroporus pilosus* (Guignot, 1949)	Canaries	B1 + 2c
Hygrotus artus	EX	*Hygrotus artus* (Fall, 1919)	California	–
Megadytes ducalis	EX	*Megadytes ducalis* Sharp, 1882	Brazil	–
Meladema imbricata	CR	*Meladema imbricata* (Wollston, 1871)	Madeira	A1c
Meladema lanio	VU	*Meladema lanio* (Fab., 1775)	Madeira	B1 + 2b
Rhantus alutaceus	EN	*Carabdytes alutaceus* (Fauvel, 1883)	New Caledonia	A2c
Rhantus orbignyi	EX	*Mediorhantus orbignyi* (Balke, 1992)	Uruguay, Argentina	–
Rhantus novaecaledoniae	EX	*Carabdytes novaecaledoniae* (Balfour-Browne, 1944)	New Caledonia	–
Rhantus papuanus	EX	*Rhantus papuanus* Balfour-Browne, 1939	Papua New Guinea	–
Rhithrodytes agnus	EN	*Iberoporus agnus* (Foster, 1992)	N Portugal	B1 + 2c
Siettitia balsetensis	EX	*Siettitia balsetensis* Abeille de Perrin, 1904	Avignon, France	–

result of trying to put together *A Register of Extinct Beetles* (Anonymous 2020), Anders Nilsson and GNF found that of those six species claimed to be extinct, two (both assigned to new genera since the appearance of the IUCN List) *Carabdytes novaecaledoniae* (Balfour-Browne) and *Meridiorhantus orbignyi* (Balke) were not extinct, and two taxa, the Mono Lake Diving Beetle *Hygrotus artus* Fall and *Rhantus papuanus* Balfour-Browne, were of uncertain status. Challet and Fery (2020) later reported that *H. artus* appears to be more widespread and was probably found in the warm spring above the toxic and alkaline lake itself. This left *Megadytes ducalis* Sharp and *Siettitia balsetensis* Abeille de Perrin. More specimens of the *Megadytes* have been discovered in Paris Museum since it was claimed as extinct (Hendrich et al. 2019), although these are all nineteenth century and originate from the now much transformed Brazilian Cerrado. New candidate species for the *Register* are barely trickling in. One way of reconciling this lack of response with the clamor about mass extinction in the Anthropocene would be that the species that have gone extinct did not get described!

Has appearing on a world list had any benefits? The fact that the list has not been updated recently by water beetle specialists suggests not, but this may be the result of a lack of funding and the fact that IUCN require an evaluation of the *entire* group—a tall order for vertebrates—almost impossible for most insect families. It is also so much easier to prove the existence of a previously unknown species than it is to establish that a known species has really gone extinct. Many of the criteria offered by IUCN to categorize species are difficult to apply to most invertebrates, being based on population size, for example, or are simply unmeasurable with any confidence in taxa such as dytiscids. The often-quoted “extent of occurrence”, defined by a convex polygon encompassing all points of occupation, may also be spurious as a way of defining occupancy, potentially encompassing a lot of empty space between isolated populations and affected by the extent to which an occupied feature such as a river or a coastal strip is linear. However, these problems can be circumvented, and a species consigned to the Red List without the need for large amounts of data.

Appearance on a global list ought to provide leverage for funding to research on individual species. This has occasionally been effective where it is possible to cite treaty or other legal obligations, as discussed above, but is much more limited than for vertebrates.

12.16 Dumbing-Down

Great play is currently being made of the benefits of “Citizen Science”, involving amateurs in research projects. For those of us with a longer view, this supposed recent discovery of the benefits of engaging with the public makes little sense as coleopterists have from the very first come from many walks of life. Professional scientists may well lead in the interpretation of results, but the study of biodiversity was regarded as a respectable hobby for gentlemen and the occasional lady in Victorian times, often associated with professional collectors (Salmon 2000).

Fig. 12.11 Specialist knowledge and lack of sampling technique are not a barrier to generating useful conservation knowledge. One of the rarest of the large diving beetles is *Acilius sinensis* Peschet, originally described from southwest China. No specimens could be found—and not for the want of trying—for 82 years until a taxi driver found two specimens in a water-filled rut by a forest track in 2007 near the Myanmar border (Hendrich 2008). The taxi driver had neither collecting equipment nor, indeed, experience. Photograph courtesy of Lars Hendrich

Certainly, specialist knowledge is not a barrier to finding water beetles (see Fig. 12.10), and harnessing the drive of children to go pond-dipping is probably a prerequisite to a life of hunting for predaceous diving beetles (see Fig. 12.11).

When the first IUCN Red List was put together (IUCN 1990), European coleopterists, if they noticed at all, were intrigued to find that North American candidates had common names; e.g., the Mono Lake Diving Beetle, *Hygrotus artus*. Since then, common names have become more frequent but are still often greeted with hostility. That hostility is justified when the common name is used without the Latin one, or when the name is misleading (in the IUCN Red List "Perrin's Cave Beetle" was coined for *Siettitia balsetensis* Abeille de Perrin, when the species almost certainly lived in gravels under the Var in France). Common names were contrived for all of the species in the Irish list (Foster et al. 2009), though this was resisted for the British list (Foster 2010) save for a few choicer ones. Reaching out to the average conservationist should not really require a dumbed-down common name, but if the expert does not contrive a name then someone less knowledgeable will come up with one instead. A possible advantage of common names is that there is no Law of Priority: the catchiest name will win. Learned societies attempting to control the choice of name (see Ferro 2013) will have no more luck than self-appointed publicists. Scientists might, however, be better employed contriving common names for habitats: for example, stating that a species is "madicolous" means nothing to most people it is misleading anyway as "living on rock" is not the same as "living on wet rock", which it is usually intended to mean. "Hygropetalous" is more accurate, but what is wrong with "living on wet rock" in the first place? One does not have to invoke "film stars" to make the habitat perceivable!

IUCN could learn a similar lesson. IUCN (2020) couples the statement that *Meladema imbricata* (Wollaston) is Critically Endangered on the basis of "A1c" whereas *M. lanio* (F.) is Vulnerable and "B1 + 2b" is not illuminating for most readers. "One-liners"—such as *imbricata* being endemic and confined to four permanent high altitude streams on the three western Canary Islands, whereas *lanio*, although confined to Madeira, is still relatively abundant (Ribera et al. 2003)—make so much more sense. The South African Cape endemic *Capelatus prykei* scores B2ab (i, iii, iv) with an area of occupancy of less than 10 km^2, and was proposed as Critically Endangered by Bilton et al. (2015), but it is also important to recognize its uniqueness as a lineage linked not to other Afrotropical copelatines but to the Palaearctic *Liopterus* and the largely Australasian *Exocelina* and the more down-to-earth problem that its population has been greatly reduced by the development of Cape Town. If the intention was that the criteria might explain the threats affecting unrelated taxa, then they also fall short. For example, the Azorean *Hydroporus guernei* gained its Endangered status by meeting criteria B2b(i–v), whereas Darwin's Frog, *Rhinoderma darwinii* (Duméni and Bibron), achieved the same status through B2ab(iii), an uninformative way of differentiating an island endemic from a species of the South American mainland. The remarkable thing is that such a bewildering muddle of criteria, few of them relevant to macroinvertebrates but too easily misinterpreted when used as such, has produced excellent publicity for endangered species.

12.17 The Way Ahead: "Passive Conservation" and Possible Pitfalls of Connectivity

A recent study by Iversen et al. (2013) has challenged some basic ideas about conserving rare and endangered species. They demonstrate that *Graphoderus bilineatus* could be found in many "unsuitable" habitats in the core area of its distribution, in Estonia, Poland, and Sweden. This is unlike experience in Germany (Hendrich and Balke, 2000) and in the Netherlands (Cuppen et al. 2006b), where the habitat could be more closely defined. Iversen et al. (2013) emphasise the value of a "dynamic landscape", such as is provided by the large floodplains of Poland, the morainic landscape of Estonia, and the myriad of lakes, mires, and bogs on the exposed bedrock of Sweden. They also mention the benefits associated with beaver activity and even the provision of artificial sauna- and fishponds. Such "passive" conservation measures, i.e., those not directed at a particular beetle but perhaps associated with a more populist target (e.g., amphibians in Estonia, Rannap et al. 2009), must provide the principal method of conserving Dytiscidae. Extensification of conservation effort is a natural corollary of metapopulation theory as applied in conservation (Hanski 1998), demanding connectivity of localized populations. In some areas, new wetland landscapes are being created in the hope that they emulate ancient ones; the Great Fen Project in the UK (Bowley 2007) is targeted to reflood 9000 acres of land associated with one of Britain's earliest wetland reserve, Woodwalton Fen (Rothschild and Marren 1997). The isolated fragments of habitat doomed by metapopulation theory may hold the key to survival of endangered species, at least in the short term, and those species may be lost following attempts to reconnect them (see Verberk et al. 2010 for an example in Dutch bog systems). If poor quality water pervades the new system, previously isolated refugia will be lost. Water quality is often a more important issue here than connectivity and should be borne in mind when attempts are made to connect sites or to increase aquatic habitat density. Also, in a time of rapid climate change, it may be alien and invasive species that benefit most from improved connections (Vila and Ibanez 2011). Theory and fashion must not be allowed to triumph over practice.

12.18 Future Directions

Our knowledge of dytiscid systematics and biogeography has improved since the IUCN list was last revised, and the global Red List for Dytiscidae could probably be extended to cover perhaps a quarter of the known species, i.e., about a thousand taxa. In conducting such a revision, at least we might have something authoritative to quote about the extent to which various groups are under threat—tropical rainforest streams, subterranean systems in the Australian outback (Chap. 9), species affected by agricultural intensification in western Europe or by industrialisation and urbanisation in China, mountain endemics affected by climate change, island endemics being lost through tourist development, and so on. Additionally, this would raise the profile of these important and, for us at least, most charismatic insects (e.g., Figs. 12.11 and 12.12).

Given the successes associated with listing two dytiscid species in the Bern Convention and under the European Habitats Directive, it also appears desirable to produce Red Lists at continental scales. If formalized through IUCN, these would fill the gaps for those countries lacking national Red Lists, as well as gaining an overview of the extent of the problems that predaceous diving beetles face at the regional level. Such approaches should, if possible, focus on regions that make sense biogeographically, rather than sticking to political boundaries.

As discussed by Sutherland (2004), the most effective conservation approach at a landscape scale is largely determined by the extent of anthropogenic habitat modification: largely intact ecosystems require less conservation management than highly modified landscapes. Such ideas obviously apply to dytiscids, even if their conservation is largely passive, falling under the umbrella of other taxa or ecosystems. In areas where the extent of wetland habitat has been severely reduced in recent years by human agency, habitat (re)creation may benefit dytiscid assemblages by increasing the density of suitable patches, something which has been demonstrated to be vital for the survival of some threatened taxa at least (e.g., Iversen et al. 2013). Such

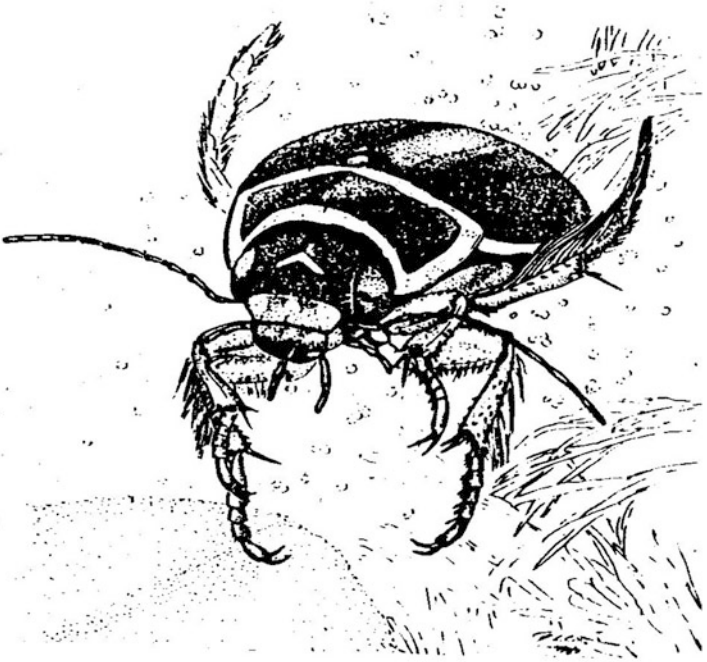

Fig. 12.12 Wirdiena ta' l-Ilma, the Maltese name for *Dytiscus circumflexus* Fabricius, was rated as Vulnerable in the Maltese Red Data Book (Cilia 1989). It was considered "large enough to encourage irresponsible collecting by parties of children on frog-hunts" and was the only beetle illustrated. More recently, it might, as a flagship species, be considered a legitimate target by the next generation of children. Drawing by Jospeh L. Cilia

schemes should always bear water quality in mind, and aim to increase the density of high-quality habitat, rather than just aquatic habitats per se. What works for larger vertebrates may not always work for invertebrates with more exacting requirements at the microhabitat scale.

The Convention on Biological Diversity (CBD) was adopted at the Earth Summit in Rio de Janeiro in 1992. Against expectations, it spawned a potential problem for recognizing biodiversity. This was the *Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity*. This protocol was adopted in 2010, and it has so far been ratified by 127 countries and the European Union. The contracting parties agree to take measures concerning genetic resources, the principal concern having been that natural products emanating from less developed countries could be developed without compensation, particularly by pharmaceutical companies. The developer and this might include those recording new species, needs to obtain consent for the collection of biological material (Watanabe 2015). Many will know the impossibility of trying to obtain permission to collect insect samples, with local, regional and national levels being required, let alone taking obtaining written consent for publishing and storing type material. One upshot is that papers describing new species can now be rather vague about those who collected the material. Even if the Nagoya Protocol has not dampened down enthusiasm for species-chasing, then there is a risk of some economies with the truth surrounding a new find. And the possible antagonism to giant pharmaceutical companies may have lessened a little with the development of some novel vaccines ...

Despite their comparative obscurity, dytiscids have in the past led the way in biological recording. Professor Frank Balfour-Browne set up the first insect recording scheme in 1904 (Foster 2015), and recording effort has continued in Britain and Ireland to the present, with an atlas (Foster et al. 2016) to update the maps produced by Balfour-Browne (1940); similar recording efforts exist in other parts of the world (e.g., for Iberia, Millán et al. 2014). Online recording is now the norm across the developed world, with an unfortunate prolixity of international initiatives, some of which must surely bring biological recording into disrepute because of the mismatch between their claims and their inadequacies. The prospects for recording freshwater life using DNA alone continue to improve, but with some cause for concern.

One of the earliest papers on eDNA (Thomsen et al. 2011) recalled a life-long ambition for some of us, to generate a list of water beetles present in a pond just by putting a “clever stick” into the water, perhaps improved nowadays by the deployment of a drone. The paper described how DNA could be detected for several species of fish and amphibians in a pond water sample, and even the DNA of some animals around the ponds, such as coot, *Fulica atra* L., woodpigeon, *Columba palumbus* L., marsh warbler, *Acrocephalus palustris* (Bechstein), and red deer, *Cervus elaphus* L. Thomsen further noted (pers. comm. to GNF) that they could not detect *Graphoderus bilineatus* that had been seeded into the pond. DNA traces specific to Dytiscidae were too short to pick out particular species, and in any case, dytiscid adult shed much less DNA than many other organisms. Tracking individual water beetles appeared to be a long way off in 2011, but DNA-barcoding of Coleoptera continues to make progress (e.g., Hendrich et al. 2015). In a review (Pawlowski et al. 2018) about the benefits and problems of conventional biological monitoring versus the use of “(e)DNA” found in the water, thirty-three authors from twenty-six institutions recommended a two-step process, use of barcoding in association with existing biological indexes of water quality and then converting to new indices based entirely on metabarcoding. This might obviate the need for any more fieldwork other than a brief dip by a drone. One might never see a beetle again! In contrast (e)DNA-barcoding has been used in Indonesia (de Araujo et al. 2017) to establish the extent of biodiversity of a site, which would presumably encourage more, rather than less, searching for the unnamed species detected.

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Garth N. Foster, in 2004 he retired from the Scottish Agricultural College (now part of SRUC), where he was amongst other things head of the Environment Division. He was at last able to devote himself fulltime to the study of water beetles, having first taken an interest in them in 1961. He is secretary to the Balfour-Browne Club, an international study group for water beetlers started in 1976, and he chairs the Aquatic Coleoptera Conservation Trust, a charity devoted to work on British species under threat. Photograph by Annie Ross.

David T. Bilton has worked on water beetles one way or another for over 35 years. He has a particular interest in understanding biogeographical patterns and never tires of finding new beetles. After studying zoology at the University of Oxford he obtained his PhD on *Hydroporus* population genetics and phylogeography from the University of London. He is currently Professor of Aquatic Biology at the University of Plymouth in the southwest of England.

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