



The Biology of Steninae

11

Oliver Betz, Lars Koerner, and Konrad Dettner

Abstract

Current knowledge of the biology of the megadiverse beetle subfamily Steninae is reviewed here with regard to its systematics, general morphology, life history, behaviour, (chemical) ecology and evolution into various ecomorphs. Comprising >3000 species worldwide, the staphylinid genus *Stenus* is one of the most speciose animal genera on Earth. Steninae are well characterized by a number of adult and larval autapomorphies. Adult *Stenus* beetles are diurnal, optically oriented, epigeic predators of springtails and other small arthropods. The most obvious autapomorphic character defining *Stenus* is its protrusible elongated labium with the paraglossae being modified into adhesive pads. This prey-capture apparatus can be rapidly ejected towards potential prey by increased haemolymph pressure. The paired anal glands of Steninae are described morphologically and with respect to their secretion chemistry. The alkaloid and terpenoid secretions significantly act as defensive compounds against both bacteria and various predators. The unique skimming behaviour of

selected species on water surfaces is described in detail, and the chemotaxonomic value of all gland constituents is discussed.

We describe the developmental stages of these beetles from egg to adult and provide details of the functional morphology of the prey-capture apparatus including its adhesion performance via viscous forces.

Steninae beetles prefer moist habitats and inhabit waterside environments such as reeds or sparsely vegetated sites on river or lake margins or the litter and humus layer in tropical forests. More than 70 distinct behavioural patterns can be assigned to the functional categories of feeding, reproduction, grooming, resting and protection. The ecomorphology of Steninae is reviewed in terms of their compound eyes, the labial prey-capture apparatus including the sticky pads (paraglossae) at its tip, the legs including the tarsi and the abdominal tergites.

11.1 Introduction

The taxon Steninae comprises 3300 species worldwide. Its megadiverse genus *Stenus* (about

O. Betz (✉) • L. Koerner (✉)
Evolutionsbiologie der Invertebraten, Institut für
Evolution und Ökologie, Universität Tübingen, Tübingen,
Germany
e-mail: oliver.betz@uni-tuebingen.de;
Lars.Koerner@springer.com

K. Dettner
Lehrstuhl Tierökologie II, Universität Bayreuth,
Bayreuth, Germany
e-mail: k.dettner@uni-bayreuth.de

3000 species including 24 fossil ones) is one of the most species-rich animal genera on earth. It has a worldwide distribution, with the exception of Antarctica and New Zealand (Puthz 1971, 2010). The body sizes of Steninae span 1.5 mm (*Stenus pernanus*) to 10 mm (*Dianous robustus*) (Fig. 11.1). Adult *Stenus* beetles are diurnal, epigeic and large-eyed, optically oriented predators of springtails and other small arthropods. In adaptation to their predatory behaviour, these beetles possess a fascinating feature (or structure), a rod-like elongated labium that can be rapidly protruded out of the head (Figs. 11.2 and 11.8). It functions in capturing prey by the means of two adhesive pads at its tip. This key innovation may have largely contributed to the evolutionary success of these beetles, since it has opened up a feeding niche to them and makes possible the effective exploitation of elusive prey such as springtails (e.g. Weinreich 1968). Special pygidial glands exhibited by both *Stenus* and “*Dianous*” beetles are used not only for predator defence but, at least in some species, for skimming across the water surface (e.g. Billard and Bruyant 1905).

Research on this intriguing beetle taxon started in 1797 with the description of the genus *Stenus* by Latreille (1797), after Carl von Linné had described the first *Stenus* species as *Staphylinus biguttatus*. Early studies of the unusual mouthparts found in these beetles were conducted by Meinert (1884, 1887) and others. They found their way into early textbooks of entomology (Fig. 11.2).

In the nineteenth century, the genus was subdivided into six subgenera (e.g. Motschulsky 1857, 1860; Rey 1884); they do not represent monophyla and thus have nowadays only some practical relevance for determination purposes (e.g. Lott and Anderson 2011). In the first half of the twentieth century, studies on the taxonomy, morphology and ecology of Steninae are mainly associated with the taxonomist Ludwig Benick (e.g. Benick 1929). His work was continued by the staphylinidologist Volker Puthz, who has described 1722 Steninae species so far and has

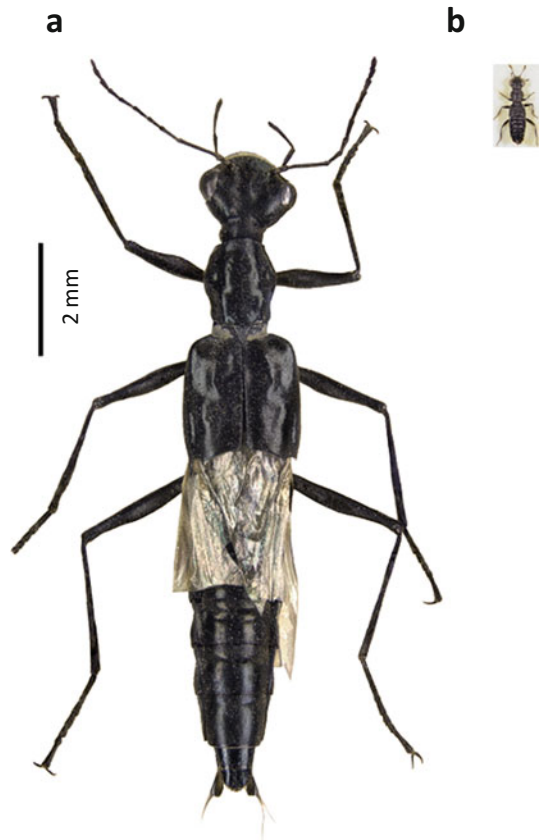


Fig. 11.1 Habitus of the largest and the smallest representatives of Steninae. (a) *Dianous robustus* (body length: 10 mm) and (b) *Stenus pernanus* (body length 1.5 mm). Both the beetles are shown in their natural size relationship (Image of *D. robustus* courtesy of Karin Wolf-Schwenninger)

continuously refined the classification system of this taxon in more than 20 revisions (e.g. Puthz 1971, 1980, 2000a, 2001, 2008, 2013; Naomi et al. 2017). In addition to this continuous taxonomic and systematic work, our biological knowledge of the Steninae has been diversified in many fields such as phylogenetic systematics (e.g. Clarke and Grebennikov 2009; Koerner et al. 2013; Lang et al. 2015; Żyła et al. 2017), population genetics (Serri et al. 2016), autecology (e.g. Renkonen 1934, 1950; Horion 1963), applied ecology (e.g. Schatz et al. 2003; Qui and

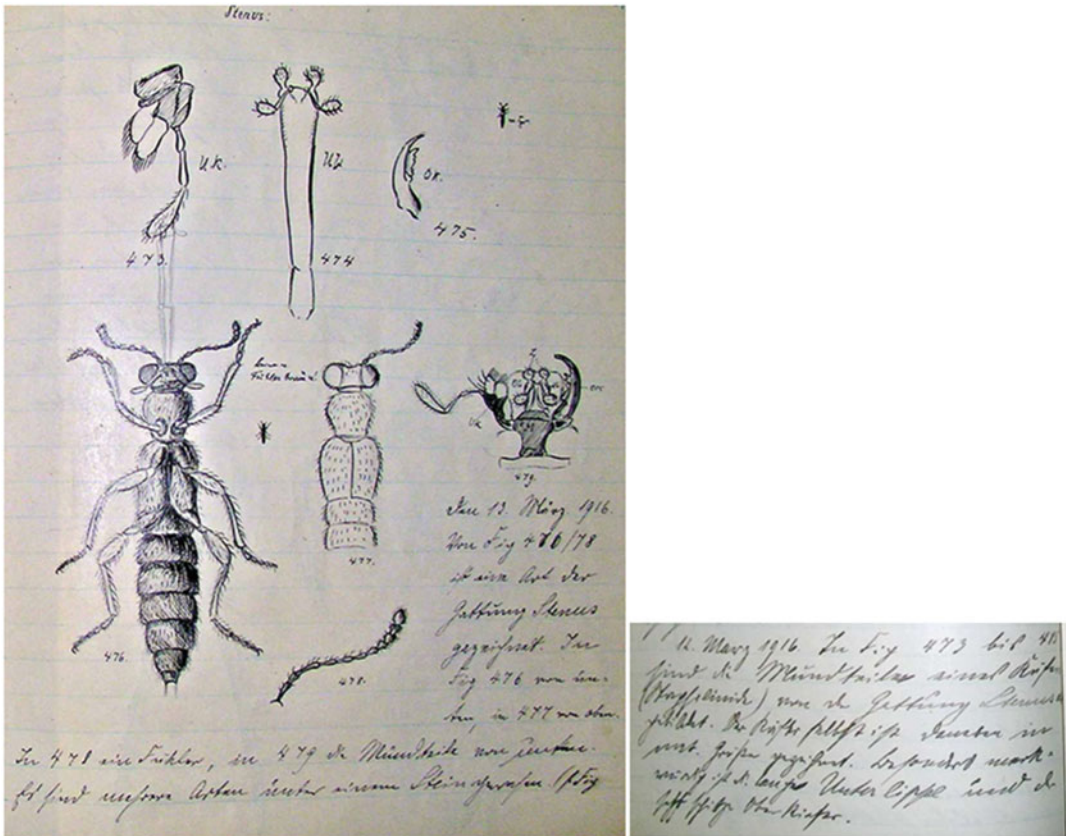


Fig. 11.2 Excerpt of the unpublished “Biological diary” of the German insect morphologist Hermann Weber. His drawings and preliminary notes on the external

morphology of *Stenus* beetles were later introduced into his entomology textbook (Weber 1933)

Zheng 2006), life history (Betz and Fuhrmann 2001), behaviour (e.g. Bauer and Pfeiffer 1991; Betz 1999), ecomorphology (e.g. Betz 2002, 2006), chemical ecology (e.g. Lusebrink et al. 2007, 2008a, b), biophysics (Dietz et al. 2016) and biomimetics (Betz et al. 2009). In particular, the functional morphology and biological role of the unusual labial prey-capture apparatus has attracted much attention (e.g. Schmitz 1943; Weinreich 1968; Bauer and Pfeiffer 1991; Betz 1996; Kölsch and Betz 1998; Koerner et al. 2012a, b, 2017).

In the present contribution, our aim has been to correlate the literature concerning the biology of Steninae and hence to reveal the current interim status of research on these beetles.

11.2 Biodiversity, Phylogenetics and Zoogeography

11.2.1 Current Status of Systematics and Phylogenetic Position

Steninae MacLeay 1825 is a subfamily of the “Staphylinine group” (i.e. subfamily groups of Lawrence and Newton 1982) within the Staphylinidae (rove beetles), containing the extant genera *Stenus* Latreille, 1797 and *Dianous*¹

¹Throughout this text, the genus name *Dianous* is presented in quotation marks according to the presumed paraphyly of *Stenus* with respect to *Dianous* (cf. Koerner et al. 2013; Lang et al. 2015).

Leach, 1819 and additional, probably a new, but as yet undescribed genus from Australia (Clarke and Grebennikov 2009; McKenna et al. 2015; Żyła et al. 2017), and two fossil genera, i.e. †*Eocenostenus* (Cai et al. 2014) and †*Festenus* (Żyła et al. 2017). Steninae currently comprises about 3300 described species (about 3000 *Stenus* spp. and 300 “*Dianous*” spp.).

With regard to their phylogenetic position within the Staphylinine group, the monophyletic Steninae are most closely related to monophyletic Euaesthetinae (Fig. 11.3). Both these groups may represent sister taxa (Clarke and Grebennikov 2009; Grebennikov and Newton 2009; McKenna et al. 2015; Żyła et al. 2017).

Various fossils demonstrate that Steninae and Euaesthetinae, and other subfamilies of the Staphylinine group (e.g. Scydmaeninae, Solieriinae, Megalopsidiinae), were well established as early as the Late Cretaceous (Clarke and Chatzimanolis 2009; Thayer et al. 2012; Jałoszyński et al. 2016; Jałoszyński and Peris 2016; Yamamoto and Solodovnikov 2016; Żyła et al. 2017). Accordingly, all subfamilies of the Staphylinine group are hypothesized to have originated by the Late Jurassic to the Lower Cretaceous and as being well established in the mid-Cretaceous (Clarke and Chatzimanolis 2009; Grebennikov and Newton 2009; Solodovnikov and Schomann 2009; Solodovnikov et al. 2013; Thayer et al. 2012).

Whereas †*Eocenostenus fossilis* has been described from the late Eocene of France (37–34 mya) (Cai et al. 2014), Żyła et al. (2017) have described the extinct genus †*Festenus* from Late Cretaceous Burmese amber (earliest Cenomanian, ca. 99 mya); the latter genus possibly possessed the *Stenus*-like prey-capture apparatus. The phylogenetic analysis of morphological data of extinct and extant taxa of Steninae and allied subfamilies of Staphylinidae has resolved the extinct genus †*Festenus* as a member of Steninae, and has been suggested to form a stem lineage of Steninae. The presence of †*Festenus* in the Later Cretaceous implies an early origin of the

prey-capture apparatus in Steninae, with this apparatus possibly driving evolution towards the crown *Stenus* (Żyła et al. 2017).

Whereas no “*Dianous*” fossils are known so far, the oldest fossil records of *Stenus* beetles date back 70–100 million years to the Cretaceous *Stenus inexpectatus* Schlüter, 1978 from Bezonais, France, and *Stenus imputribilis* Ryvkin, 1988 from Obeshchayushchiy Creek, Russia. Both these fossil taxa are characterized by large eyes, a three-segmented antennal club and antennal insertions positioned on the frons behind the anterior margin of eyes, a character combination similar to those of *Stenus* (Żyła et al. 2017). However, the poor degree of preservation of these fossils makes their more confident identification and characterization impossible (Żyła et al. 2017). Definite *Stenus* with a prey-capture apparatus are known from about 50 mya old Baltic amber (Schlüter 1978; Ryvkin 1988; Puthz 2010; Chatzimanolis 2018). Younger (sub-) fossil material is known from Pleistocene deposits (e.g. Puthz 1971).

The monophyly of Steninae is supported by many larval and adult autapomorphies (Kasule 1966; Puthz 1981; Hansen 1997; Leschen and Newton 2003; Thayer 2005; Clarke and Grebennikov 2009; Grebennikov and Newton 2009) and is also suggested by molecular analyses (Grebennikov and Newton 2009; Koerner et al. 2013; Zhang and Zhou 2013; Lang et al. 2015). According to the phylogenetic analysis of Clarke and Grebennikov (2009) and Żyła et al. (2017), the autapomorphic characters of adult Steninae are (1) the dorsal insertion of the antennae (between the anterior margins of the compound eyes), (2) the presence of apodemes arising from interantennal pits, (3) the absence of the tentorial bridge (Fig. 11.4), (4) the presence of a tentorial loop (Fig. 11.4), (5) the insertion of the labial palps closely together near the anterior margin of the labium, (6) the pronotal marginal carina meeting the pronotosternal suture anterolaterally, (7) the apex of the mesoventral intercoxal process abutting the apex of the metaventral process and (8) the opening of the

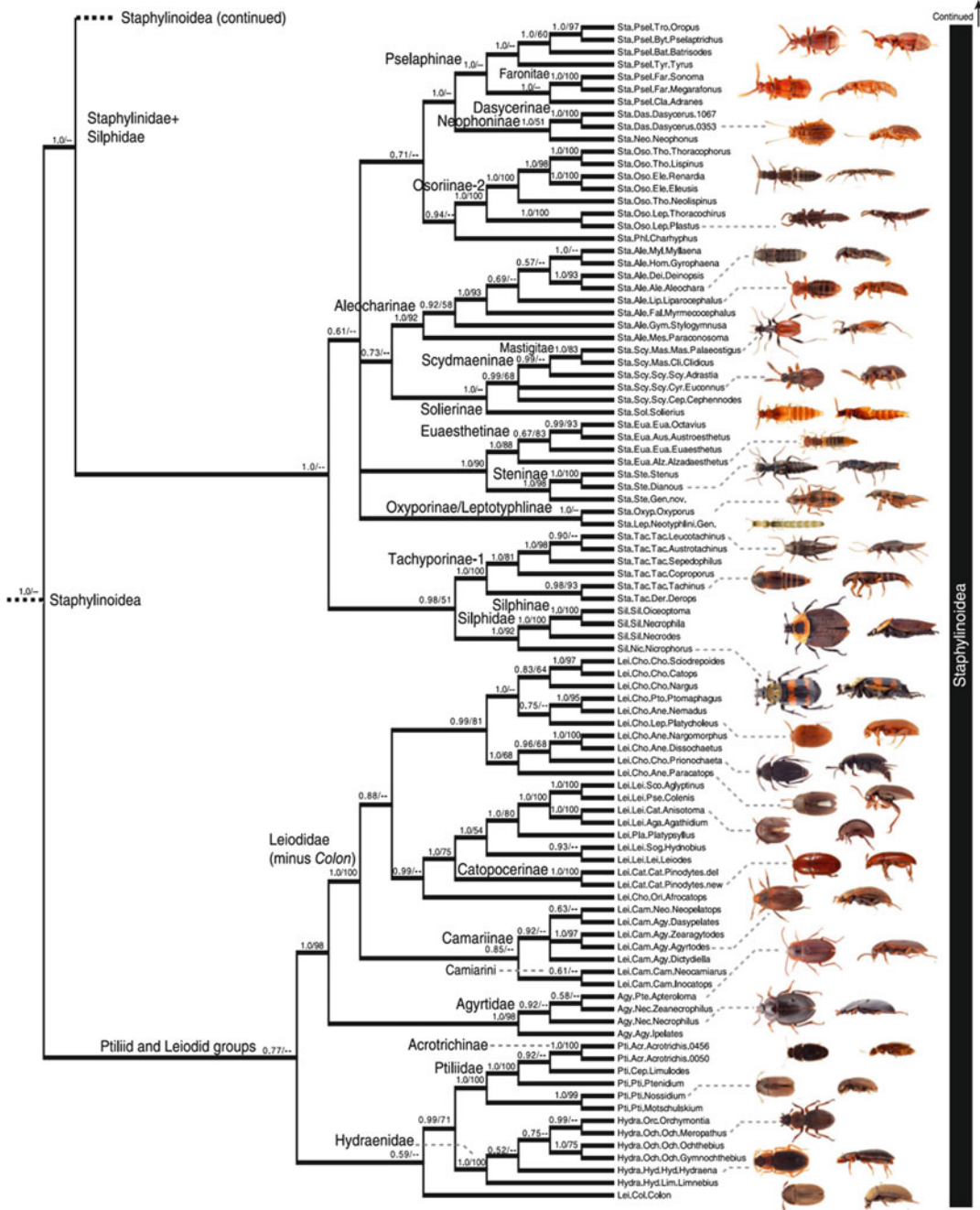


Fig. 11.3 Bayesian 50% MR consensus tree showing the relationships among major lineages of beetles in the infra-ordinal group Staphyliniformia. Bayesian posterior probabilities (first/left number) and partitioned maximum likelihood bootstrap support (second/right number) are

shown above branches. This tree is based on analyses of DNA sequence data from 28S rDNA and the nuclear protein-encoding gene CAD [Excerpt from McKenna et al. (2015). With kind permission from John Wiley and Sons]

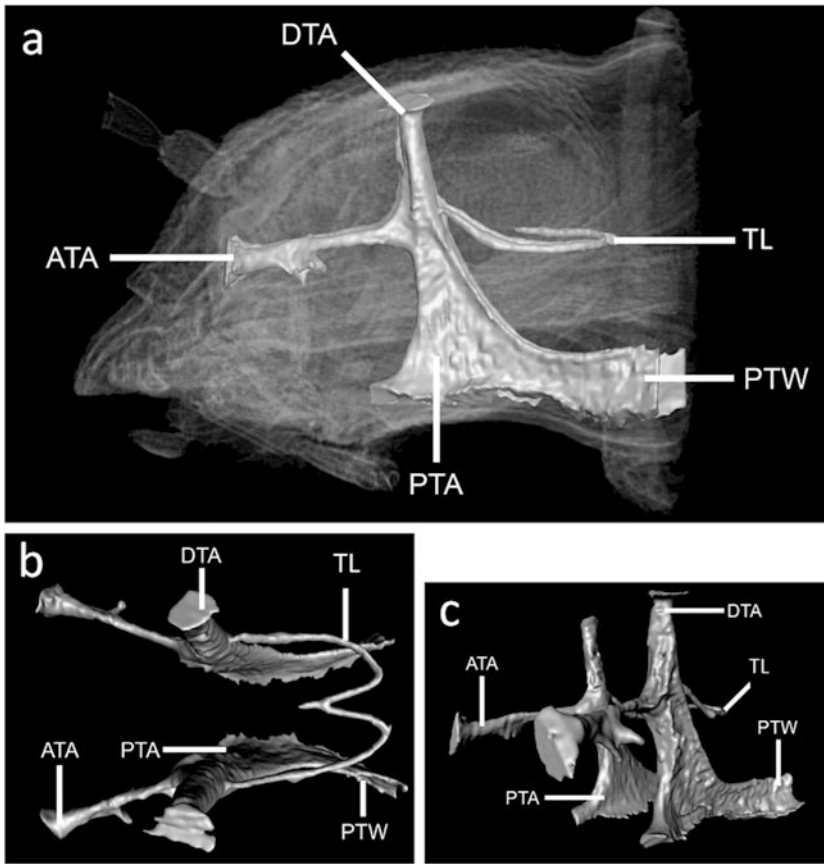


Fig. 11.4 Synchrotron μ CT images of the tentorium of *Stenus cicindeloides*. (a) Lateral, (b) dorsal, (c) fronto-lateral view. Note that a tentorial bridge connecting both the posterior tentorial walls is lacking, whereas a more dorsal tentorial loop that connects both the dorsal tentorial

arms is present. Identical conditions were found in *Dianous coeruleus*. Abbreviations: ATA anterior tentorial arms, DTA dorsal tentorial arms, PTA posterior tentorial arms, PTW posterior tentorial walls, TL tentorial loop

paired pygidial defence glands into the rectum. Several larval characters add to this autapomorphic complex, i.e. (9) the setae on the cranium and the tergites being differentiated in length into long and thick versus short and thin; (10) the first antennomere being constricted, so that the antennae appear four-segmented; (11) the antennomeres 1 and 2 being especially elongate; (12) the second maxillary palpomeres being bent and much longer than wide; (13) the tibia having apical setae being longer than the claws; and (14) the abdominal segment IX showing a latero-ventral projection on each side (Clarke and Grebennikov 2009).

Whereas the genus *Stenus* has been considered monophyletic on the basis of its possession of the labial adhesive prey-capture apparatus and several other adult (mostly related to the prey-capture apparatus) and larval characters, the genus “*Dianous*” is not defined by any autapomorphies (Puthz 1981; Clarke and Grebennikov 2009; Żyła et al. 2017). Molecular analyses based on three genes (COI, 16S rRNA, Histone H3) indicate the evolutionary origin of “*Dianous*” within *Stenus*, suggesting a secondary loss of the specialized prey-capture apparatus and rendering *Stenus* paraphyletic (Koerner et al. 2013; Lang et al. 2015). This view is

consistent with chemotaxonomic analyses (Lusebrink 2007), the morphology of the paired pygidial glands (Jenkins 1957) and the tentorial characters shown in Fig. 11.4 (see also Żyła et al. 2017) as established in both genera. If the hypothesis of paraphyly of *Stenus* with respect to “*Dianous*” gains further support, taxonomists will be forced to abandon the genus “*Dianous*,” which, in this case, would become a subgenus of the genus *Stenus*.

Three as yet undescribed Australian species with protrusible labia bearing adhesive cushions probably form an additional monophyletic genus of Steninae. This hypothesis is confirmed by phylogenetic analyses based on DNA data from 28S rDNA and the nuclear protein-encoding gene CAD (Fig. 11.3), because the undescribed Australian species have a basal position within the Steninae (indicated as “Sta.Ste.Gen.nov.” in Fig. 11.3). Their partial resemblance to the Euaesthetinae (e.g. reduced eye size in at least one species) may have resulted from convergent lifestyles in the litter layer (Clarke and Grebennikov 2009). The basal arrangement of this undescribed genus, which possesses a protrusible prey-capture apparatus similar to that of *Stenus*, further supports the assumption of a secondary loss of the specialized prey-capture apparatus of “*Dianous*.”

With respect to the intrageneric classification, *Stenus* was traditionally subdivided into morphologically defined subgenera (e.g. Schülke and Smetana 2015). The genus *Stenus* was grouped into subgenera based on various morphological features by staphylinid specialists (Rey 1884; Motschulsky in Hermann 2001; Lusebrink 2007; Puthz 2008). Originally, the genus was grouped into six subgenera, i.e. *Stenus*, *Nestus*, *Tesnus*, *Hemistenus*, *Hypostenus* and *Parastenus* (see also the determination key of Lohse 1964, which uses an outdated subgenus concept), mainly based on morphological features. These characteristics, for example, are the appearance of the fourth segment of the metatarsi (simple or bilobed), the relative length of the first and fifth tarsomere of the metatarsi, the relative

length of the metatarsi and the metatibiae and the presence or absence of abdominal paratergites (Cameron 1930; Lohse 1964; Zhao and Zhou 2004; Koerner et al. 2013). Later, subgenera were taxonomically revised resulting in five valid subgenera: *Stenus* s. str., *Hemistenus* Motschulsky 1860, *Hypostenus* Rey 1884, *Metatesnus* Adam 1987 and *Tesnus* Rey 1884 (Puthz 2001, 2008). Here, *Nestus* belongs to *Stenus* s. str., *Hemistenus* is redefined to *Metatesnus* and *Parastenus* is renamed to *Hemistenus*. However, recent findings indicate that this classification is artificial and probably does not reflect authentic phylogenetic relationships. Currently, the genus is grouped into 157 monophyletic species groups that better reflect the phylogenetic relationships within this group (Puthz 2008), a view that is supported by a few available intrageneric molecular studies (Koerner et al. 2013; Lang et al. 2015; Serri et al. 2016).

In “*Dianous*,” according to the morphology of the frons (and some other characters), the species can be classified into species groups I and II (Puthz 1981, 2000b; Shi and Zhou 2011; Tang et al. 2011; Puthz 2016). In contrast to “*Dianous*” group II (about 70% of all “*Dianous*” species), the members of “*Dianous*” group I (about 30% of all “*Dianous*” species) have large *Stenus*-like eyes and were traditionally considered to belong to the genus *Stenus* until the recognition of their lack of the typical prey-capture apparatus of this genus (Puthz 1981). “*Dianous*” group II has been morphologically subdivided into nine “species complexes” that probably form phylogenetic entities (Puthz 2016).

11.2.2 Species Numbers and Zoogeography

The subfamily Steninae has a worldwide distribution, occurring (from the lowlands up to alpine elevations) in the northern temperate, the southern temperate and the tropical zones (Thayer 2005). With respect to geographical regions, Steninae occur in the Nearctic (188/2),

Neotropical (601/0), Palearctic (including China) (1337/151), Afrotropical (358/0), Oriental (including China and Pacific Islands) (629/59) and Australian regions (29/0) [numbers behind each region refer to species numbers (*Stenus*/*Dianous*)] according to Puthz (in litt.). The total number of Holarctic species amounts to 33 (*Stenus*) and 0 (*Dianous*), respectively.

Stenus currently comprises about 3000 species. Its worldwide distribution (with the exception of Antarctica and New Zealand) is indicative of a Gondwanan origin, whereby Puthz (2010) assumes its centre of origin in Asia. The majority of the species have bilobed tarsi in combination with an abdomen that does not show distinct raised margins (Puthz 2017). Most species inhabit the southern hemisphere (Puthz 2017).

Dianous currently comprises >300 species and is distributed in the Oriental, the Palearctic and the Nearctic regions (not in the Ethiopian region) with its main distribution in Asia (India, China and Southeast Asia). The centre of the distribution of the genus *Dianous* is in the mountain areas between the Palearctic and the Oriental regions south of 31° northern latitude (Indochina Peninsula and southern slopes of the Himalaya) (Puthz 1981, 2000b; Shi and Zhou 2011) (Fig. 11.5). From here, it has probably

dispersed in (1) a south-eastern direction, (2) a north-western direction and (3) via the Bering Straits to North America (Puthz 1981).

The overall range of the distribution of *Stenus* species that occur in Central Europe is provided in Horion (1963) and Puthz (2012). The worldwide distribution of the currently known *Dianous* species is provided by Puthz (2000b, 2015, 2016) and Shi and Zhou (2011).

11.3 Morphological Characterization of Eggs, Larvae, Pupae and Adults of Extant Steninae

11.3.1 Eggs

Depending on the species, the eggs of *Stenus* beetles (Fig. 11.11a, b, f–g) come in various colours such as whitish, brownish or yellow–orange (Weinreich 1968). The submillimetre-sized eggs have an ellipsoid shape and may be laid singly or in clusters of up to 20 eggs (Betz 1999), conglutinated to each other and to the substrate by an adhesive that forms a small appendage at one pole of the egg (Weinreich 1968, Fig. 21) (Fig. 11.11f).



Fig. 11.5 The distribution of the genus *Dianous*. Solid line, distribution of *Dianous* species group I; dashed line, distribution of *Dianous* species group II [From Shi and

Zhou (2011, Fig. 25). With kind permission from John Wiley and Sons]

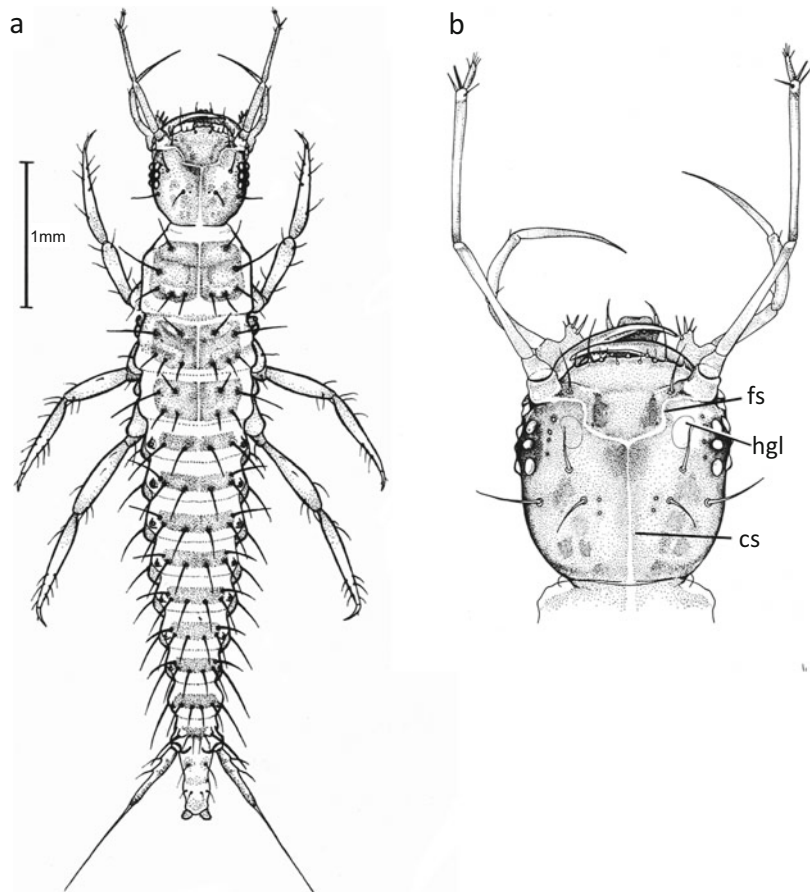
11.3.2 Larvae

Steninae larvae pass through three instars (L_1 – L_3) before they hatch into the pupa. Only a very few larval descriptions are available at the species level (e.g. Welch 1966; Weinreich 1968). The larval morphology of selected *Stenus* species has been described in detail by Weinreich (1968) (Fig. 11.6). Specific characteristics of larval Steninae are also given in Kasule (1966), Newton (1990), Frank (1991), Beutel and Molenda (1997), Leschen and Newton (2003) and Thayer (2005). Their basic colour is whitish, whereby the more strongly sclerotized parts are brown-black. The entire body surface including the setae appears glossy and sticky.

The head capsule (Fig. 11.6b), including the fused and somewhat advanced labrum, is

rounded and bears a characteristic chaetotaxy. It has no neck. Laterally, on each side, the head bears six stemmata that are arranged in an oval shape. The long and slender mandibles are falciform; along the inner side of their apical third, they are denticulate but do not bear any mola or prostheca. In the maxillae, the three-membered palpus rests on a compact palpifer. In the labium, the palpifer bears a two-palpomed palpus. The ligula is broad and bilobed. The antennae are with three antennomeres but appear as with four antennomeres because of a basal constriction of the first antennomere. Their first and second antennomeres are markedly elongated, whereas the third one is very short. The pre-apical antennomere laterally bears a sensory appendage. The frontal suture is Y-shaped. Together with the elongated coronal suture, it divides the

Fig. 11.6 Dorsal habitus of (a) entire larva and (b) larval head of *Stenus comma*. From Weinreich (1968). In (b), the internal position of the head gland according to Beutel and Molenda (1997) is added to the original drawing. Abbreviations: *cs* coronal suture, *fs* frontal suture, *hgl* internal position of head gland



dorsal side of the head capsule into three areas. A pair of glands occurs lateral of the frontal suture. Medially, the ocelli are accompanied by especially arranged pores. Such pores (single or paired) are also found on the vertex.

The dark-coloured thoracic tergites bear a longitudinal median ecdysial line. The anterolateral corners of all three thoracomeres exhibit a pair of gland openings (not shown in Fig. 11.6). Between the pro- and mesocoxae, one pair of spiracles opens at the ends of spiracular tubes. The slender legs bear a uniarticulate tarsus.

The Steninae abdomen is relatively short, making up less than 1.2 times the length of the thorax (Thayer 2005). Abdominal spiracles are equally developed on segments I–VIII. Apart

from their characteristic chaetotaxy, the abdominal tergites bear a pair of anterolateral gland openings. The urogomphi of the ninth abdominal segment are single-membered and originate from a basal dome-like structure.

No “egg bursters” are known from the head or the thoracic tergites. Kasule (1966) provides a key with characters for distinguishing between *Stenus* and “*Dianous*” larvae.

11.3.3 Pupae

The pupal instar of *Stenus* was described by Welch (1966) and Weinreich (1968). These pupae belong to the exarate non-sclerotized type (Fig. 11.7). The head and the mouthparts

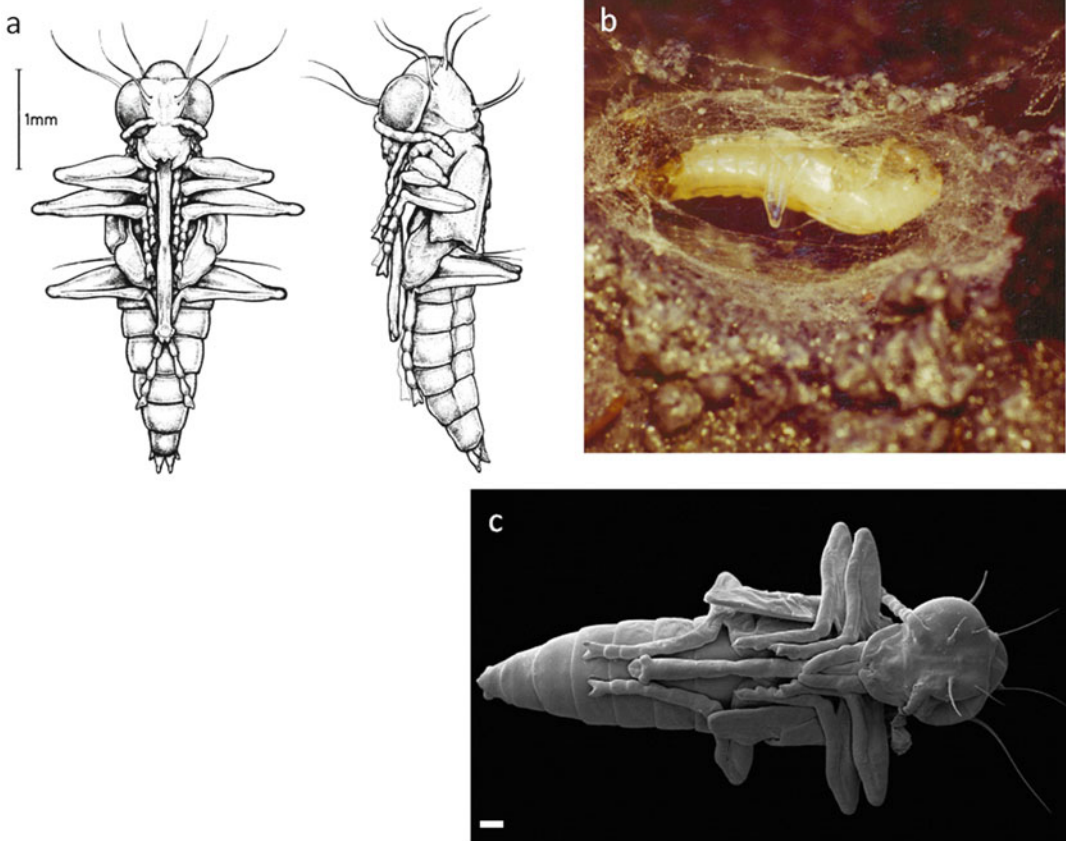


Fig. 11.7 Pupae of *Stenus* species. (a) Ventral and lateral view of *S. comma*. From Weinreich (1968). (b) Lateral view of cocooned pupa of *S. comma* with cocoon

carefully opened. Photo courtesy of Martin Pfeiffer. (c) Scanning electron microscopic (SEM) image of ventral aspect of pupa of *S. guttula*. Scale bar = 200 μm

show a hypognathous orientation. Freshly hatched pupae have a whitish appearance but become darkly pigmented later on. The *Stenus* pupa is instantly identifiable by its laterally protruding eyes and the elongate labium that it bears on its ventral side (the pupa of *Dianous coerulescens* does not show such an elongate labium). On the dorsal head capsule (between the eyes), the pronotum and the first (and potentially the second) abdominal tergite, the pupae possess one to two pairs of elongate spiny projections that darken in 1–2 days after hatching.

11.3.4 Adults

Adult Steninae are characterized by their laterally protruding large eyes, antennae with 11 antennomeres (with two thicker basal antennomeres and a weak club of 3 antennomeres) originating on the vertex between the eyes, a cylindrical pronotum without lateral margins [the pronotum of †*Eocenostenus* is strongly transverse (Cai et al. 2014) and the pronotum of †*Festenus* bears marginal carina reaching the anterolateral prothoracic margin (Żyła et al. 2017)], the small cone-shaped procoxae that are sitting in entirely closed procoxal cavities, cylindrical short elytra not projecting to the metathorax and without an epipleural keel [such epipleural keel is present in †*Festenus* (Żyła et al. 2017)], the markedly separated metacoxae, the pentamerous tarsi, a slender cylindrical abdomen with six visible sterna and paired internal defensive glands that empty into the anus and a usually symmetric copulatory apparatus (i.e. the aedeagus) (e.g. Puthz 1971, 2012; Newton et al. 2001; Lott and Anderson 2011; Thayer 2016). The abdominal musculature of *S. clavicornis* does not show the diagonal crossing type (as characteristic for many other staphylinids with a freely movable abdomen) but the regular longitudinal muscle pattern (Blum 1979). A more comprehensive list of autapomorphic adult characters of Steninae is provided by Clarke and Grebennikov (2009).

The aedeagus is composed of the median lobe (i.e. the penis) and the paired lateral lobes (i.e. the parameres), but, unlike those from other staphylinids, the parameres insert at the dorsal side of the median lobe (Puthz 1971; Naomi 2018). The median lobe consists of a large basal bulb with musculature; a contractor muscle is considered to heighten the pressure of body fluid within the median lobe, by which the internal sac (i.e. the endophallus) is evaginated during copulation. The internal sac is basically furnished with paired expulsion hooks, longitudinal bands, additional teeth, hooks or tufts, or it is modified into a protrusible tube-like flagellum (Puthz 1971; Naomi 2018). Female Steninae possess unsclerotized or well-sclerotized spermatheca (Puthz 1971). Compared with *Stenus*, the aedeagi of “*Dianous*” are more uniform both in outline and in internal structures (Puthz 2000b).

Stenus and “*Dianous*” are mainly distinguished by the presence or absence of the elongated protrusible labium bearing a pair of adhesive cushions at its tip (Figs. 11.2, 11.8, 11.23 and 11.24). In addition, in *Stenus* only, the mentum is divided by a medial longitudinal carina. Other differences such as the relative size of the compound eyes, the presence or absence of long terminal styles at the ninth abdominal segment and the morphology of the genital organs are not consistent throughout the species of the two genera (Puthz 1981) supporting the view that *Stenus* may be paraphyletic with respect to “*Dianous*.”

11.3.5 Functional Morphology of the Labial Prey-Capture Apparatus of *Stenus* Beetles

The labial prey-capture apparatus of adult *Stenus* beetles is composed of a distal elongated rod-like prementum that is connected to the head by an equally elongated membranous connective membrane (Fig. 11.8a). This membrane can be invaginated like the inside-out finger of a glove. At its tip, the prementum bears two adhesive pads (i.e. the modified paraglossae) that attain their sticky function via the viscosity of a

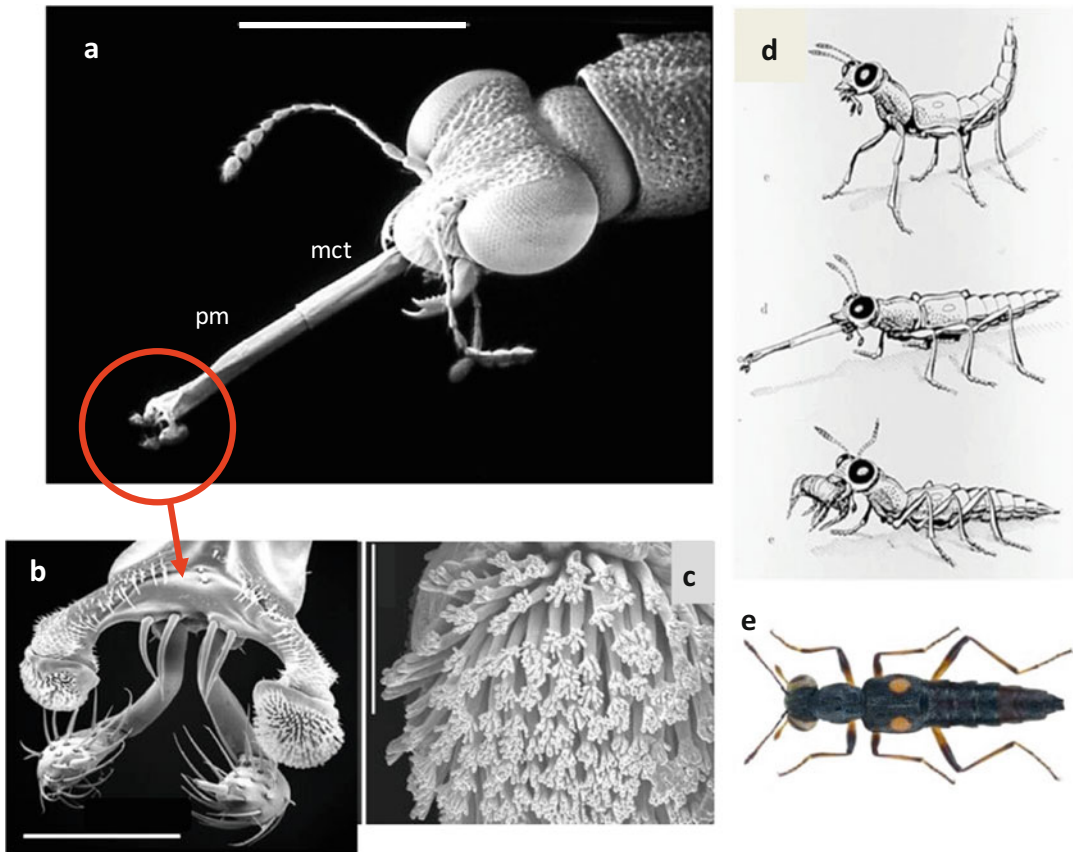


Fig. 11.8 Adhesive prey-capture apparatus of *Stenus* spp. (a–c) SEM images. (a, b) *S. comma*. From Bauer and Pfeiffer (1991); (c) *S. nitidiusculus*. (a) Head with protruded labium. Scale bar = 1 mm. (b) Dorso-frontal view of the apex of the prementum with the paraglossae modified into sticky pads. Scale bar = 100 μ m. (c) Ventrolateral detailed aspect of a sticky pad. Note the terminally branched adhesive trichomes. Scale bar = 20 μ m.

gel-like adhesive secretion that is released through lateral pores and that distributes itself over the whole surface of the paraglossa, which is differentiated into terminally branched trichomes (Fig. 11.8c). The connecting membrane and the prementum contain bundles of ductules that transport the adhesive secretion produced by prominent secretory glands in the head (described in Weinreich 1968; Kölsch 2000; reviewed in Betz 2010) to the sticky cushions. Internally, the sticky cushions are made up of a complex reticulum of endocuticular fibres (Betz 1996; Kölsch and Betz 1998). Together with the mesocuticular nature of their

(d) Prey-capture sequence in *S. comma*. After approaching the prey to a critical distance (top), the prey-capture apparatus is rapidly protruded (middle), and the glued prey is withdrawn to the mandibles (bottom). From Weinreich (1968). (e) Habitus image of *S. guttula*. Length: 4.3 mm. Courtesy of Udo Schmidt (www.kaefer-der-welt.de). Abbreviations: *mct* membranous connecting tube, *pm* prementum

outer wall and their resilin content (Koerner et al. 2012a), this makes these structures highly flexible and elastic, so that they can closely adapt themselves to the shape and surface irregularities of the prey. Moreover, according to their loose arrangement, the sticky cushions can be inflated immediately prior to a strike by increased haemolymph pressure. On their external surface, the sticky cushions are differentiated into a large number of adhesive trichomes (Fig. 11.8c). Each trichome branches out terminally, thereby dramatically increasing the total number of adhesive contacts. The number of both trichomes and terminal branches is species-specific and may

amount to many thousand adhesive contacts per sticky cushion (Bauer and Pfeiffer 1991; Betz 1996; Koerner et al. 2017).

Upon prey-capture, the rod-like labium is hurled out of the body and has to be retracted after prey-capture by dorsal and ventral retractor muscles in order to bring the prey into the range of the mandibles (Fig. 11.8d).

On the basis of several morphological and behavioural studies (e.g. Schmitz 1943; Weinreich 1968; Bauer and Pfeiffer 1991; Betz 1996, 1998a; Kölsch and Betz 1998; Kölsch 2000; Betz and Kölsch 2004; Betz et al. 2009; Koerner et al. 2012a, b, 2017), a functional model can be suggested for the working principles of the prey-capture apparatus (Fig. 11.9) (Kölsch and Betz 1998). When not in use, the labium is withdrawn back into the head, where it is wrapped by the connecting membranous tube. In order to capture prey, the

beetles rapidly protrude their prementum from this resting position towards the prey within only 3–5 ms. The prey adheres to the sticky cushions and is seized by the mandibles after immediate retraction of the prementum. The rapid protrusion of the labium is made possible by a catapult mechanism that involves the antagonistic action of increased haemolymph pressure on the one hand and the contraction of large retractor muscles on the other (Fig. 11.9).

During protrusion, the labium is not simply pressed out of the body by the inflowing haemolymph, because the lumen in the inner tube in the transition zone between the prementum and the membranous connecting tube is filled with tissues (retractor muscles, nerve tissue, gland ductules, tracheae) as indicated by the yellow area in Fig. 11.9c. This largely hinders any influx of haemolymph. However, this changes when the labium is in its

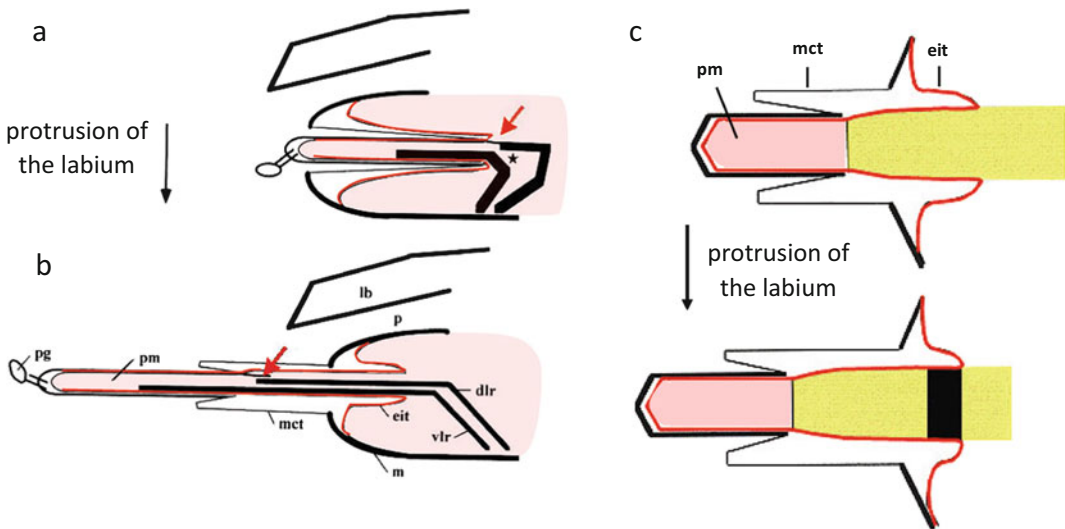


Fig. 11.9 Schematic sagittal section through the labial prey-capture apparatus in *Stenus* spp. Functional model at the resting position (a) and the active capture position (b). The pink area indicates the region filled with haemolymph. The arrow points to the dorsal apodeme of the prementum onto which the dorsal labial retractors are inserted. For further explanations, see text. Details of the way that the internal lumens of the protruding labium is filled are shown in (c). The extra inner tube (red line) turns inside out proximally. The yellow area represents the complex of tissues that fill the inner part of the proximal half in a fully protruded labium. The black

rectangle in the lower scheme shows the position at which the “increasing” volume within the extra inner tube is created during protrusion. Note that, during protrusion, the membranous connecting tube (black line) unfolds distally, whereas the extra inner tube (red line) does so proximally. For further explanations, see text. Abbreviations: *dlr* dorsal labial retractors, *eit* extra inner epidermal tube, which directly wraps the inner complex of tissue, *lb* labrum, *m* mentum, *mct* membranous connecting tube, *pg* paraglossa, *pm* prementum, *vlr* ventral labial retractors. Modified from Kölsch and Betz (1998)

resting position (Fig. 11.9a). In this position, an open cavity forms between the dorsal and the ventral retractor muscles² (asterisk in Fig. 11.9a); through this cavity, the prementum can be filled with haemolymph, while it is still in its resting position. The increase of the haemolymph pressure within the prementum probably also causes the observed erection of the adhesive pads, so that their adhesive surfaces are frontally directed towards the prey (pg in Fig. 11.9b). Despite the increased haemolymph pressure, in this phase, the labium is not yet pressed out of the head, as long as the contracted labial retractor muscles antagonize this pressure. Only once these muscles relax, is the labium (preloaded by haemolymph pressure like a bent bow) catapulted out of the body.

Hence, we are dealing with a catapult mechanism in which the elastic elements of the labium (including the retractor muscles and the resilin-containing membranous connecting tube) are set under an initial stress by an increase of the haemolymph pressure. This haemolymph pressure is probably caused by the contraction of the abdominal body musculature, so that the muscles involved in this antagonism are located at very different parts of the body.

The mechanism of the protrusion of the labium is detailed in Fig. 11.9c. The increasing inner space within the extra inner tube (i.e. the epidermis that has become separate from the cuticular membranous connecting tube) is created at the proximal end of the labium within the head capsule and not in the transition zone between the membranous connecting tube and the prementum. This is a consequence of the extra inner tube (i.e. the epidermis) and the membranous connecting tube (i.e. the cuticle) not lying in parallel (Fig. 11.9). Rather, the complex of the inner tissues and the narrow extra inner tube form an integrated whole, so that the inner

tissue complex does not have to glide within the extra inner tube but moves together with it during both protrusion and retraction.

The trichomes at the surface of the sticky cushions are usually deeply immersed in the adhesive secretion, and hence only the tips of their branches protrude (Fig. 11.10a). The adhesive secretion is released to the exterior via pores that are located in the lateral flank of the sticky cushions (arrow in Fig. 11.10b). From here, it spreads all over their surface. Because of the large amount of the involved secretion, the adhesive working mechanism can be assigned to the viscosity of the adhesive that comes into action during the rapid withdrawal of the labium towards the head after the strike (Fig. 11.10c). Ultrastructural images show that droplets of a lipid-like substance emulsify in a larger aqueous protein fraction (Fig. 11.10b) (Kölsch 2000). Additional histochemical tests have identified water-soluble sugars, proteins and lipids indicating that the secretion is a complex mix of more than one chemical phase (Betz et al. 2009). An emulsion of this kind might be beneficial for the effective distribution of the secretion over various types of surface (hydrophilic *versus* hydrophobic) but might also help precisely to adjust its viscosity. Indeed, the high viscosity of the adhesive secretion has been demonstrated in high-speed video recordings that have shown the sticky pad being retracted from a surface (Koerner et al. 2012a). As is the case with commercially available adhesives, the secretion elongates and forms long parallel fibres, before finally breaking away from the contact area of the substrate. This observation supports the importance of the cohesive forces that give the secretion a high level of internal strength. Additionally, the drag of the fluid is transferred to the pad cuticle whose upper layer is considered to be very soft and compliant, deforming viscoelastically in the direction of the pull (as can be seen in the high-speed video footage of Koerner et al. (2012a)). The elongation of the entire pad upon pull-off is further supported by its resilin content and additional structural peculiarities discussed in Kölsch and Betz (1998).

²The dorsal retractor muscles insert at an apodeme that arises from the dorsal wall of the proximal base of the prementum (see arrow in Fig. 11.9a, b), whereas the ventral muscles insert further distally at the inner side of the ventral wall of the prementum (Fig. 11.9a, b).

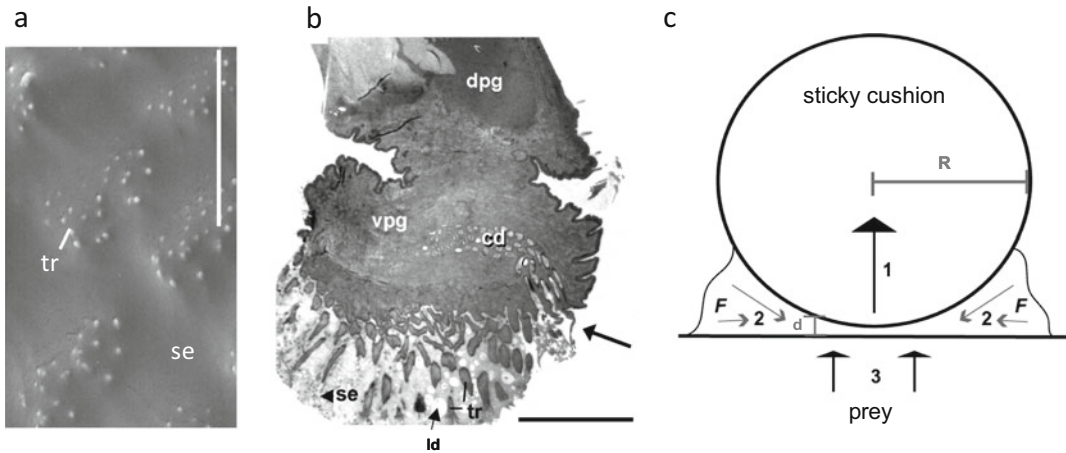


Fig. 11.10 Structure and function of the paraglossae modified into sticky cushions in *Stenus* spp. (a) Cryo-scanning electron microscopic view of a part of the surface of a sticky cushion showing the tips of the adhesive trichomes emerging from the adhesive secretion. Scale bar = 3 μm . (b) Transmission electron microscopic image of a transverse section through a sticky cushion in *S. juno*. The arrow points to the location on the paraglossa at which the secretion is discharged via the lateral pores. Scale bar = 20 μm . For further explanations, see text. (c) Representation of the proposed adhesive mechanism based on the viscosity of the secretion. At withdrawal of the

sticky cushion (arrow 1), the emerging gap between both the sticky cushion and the substrate has to be filled by the secretion (arrow 2) provided that it is sufficiently fluid. If the secretion is too viscous to fill the gap immediately, this gap cannot increase, and the prey is drawn instead towards the adhesive pad (arrow 3). Abbreviations: *cd* chitinous ductules, *dpg* dorsal part of paraglossa, *F* adhesive fluid, *R* radius of the contact area, *d* distance between both the surfaces, *ld* presumed lipid droplet, *se* adhesive secretion, *tr* adhesive trichomes, *vpg* ventral part of paraglossa (from Kölsch 2000; Betz and Kölsch 2004)

11.4 Life History, Development and Larval Biology

11.4.1 Life History and Development

In the temperate zone, adult *Stenus* beetles are long-lived and iteroparous (with overlapping generations), reproducing throughout the season (starting in April) (Larsen 1959, 1963; Weinreich 1968). Betz and Fuhrmann (2001) compared three *Stenus* species with respect to their life history. Their dissections of field-collected females over the entire season revealed that, in Northern Germany, egg production was highest in June and had ceased by the end of summer. The average egg number per female and season/week amounted to 29/1.5 (*S. pubescens*), 63/5 (*S. comma*) and 189/14 (*S. juno*), whereby the egg number was negatively correlated to egg size. Whereas *S. comma* and *S. juno* beetles deposited their clutches on the underside of

moist bark and reed leaves, *S. pubescens* beetles did so into convoluted leaves or hollow stalks (Betz and Fuhrmann 2001). In all these species, one to two egg clusters were laid per week.

Larval development time is short; the whole development from egg to imago can be accomplished within 1 month (Weinreich 1968). Taking the development times (determined at a temperature regime of 18/10 °C) of eggs (8–11 days), larvae (L₁, 4–5 days; L₂, 3–4 days; L₃, 6–7 days) and pupae (7–8 days) and the observed preoviposition time of about 1 month as a basis, we can assume that the generation time is 2 months. This means that an overwintered adult can produce two to three further generations per season (Betz and Fuhrmann 2001). At a constant temperature of 20 °C, the developmental time from egg to imago is accomplished within 3 weeks (Lang 2014). Whereas most *Stenus* species reproduce and develop from spring to summer, a few Central European

species develop in the winter months (e.g. *S. impressus*) (Larsen 1959).

Based on their different locomotory activities, metabolic/consumption rates and egg sizes/numbers, *S. juno* beetles show a reproductive effort that is twice as high as that of *S. comma* and *S. pubescens* (Betz and Fuhrmann 2001). This indicates that different lifestyles are established in *Stenus* entailing different reproductive costs. For inhabitants of open sites, such as *S. comma*, the demand for increased mobility has probably called for spending high maintenance energy at the expense of reproductive effort. For plant-mounting species such as *S. pubescens*, reproductive constraints may arise from an “energy-minimizing” strategy (i.e. low metabolic rates in concurrence with moderate reproductive efforts) that has evolved in adaptation to a diminished food supply.

For “*Dianous*” species, no solid life history data (including egg numbers and developmental times) are available as for *Stenus*.

11.4.2 Larval Biology

Steninae larvae are very rarely found in the field (e.g. Blair 1917), so that our knowledge on their biology is based on laboratory observations. Within an egg cluster, the larvae (as observed in *S. comma*, *S. juno* and *S. pubescens*) mostly hatch synchronously within 1 h. Upon hatching (the entire process takes about 4 min), the larvae penetrate the chorion with their heads first, followed by the extraction of their legs, which are then used to erect the flexed antennae and maxillary palps. Finally, the hind body is drawn out of the chorion (Fig. 11.11c).

Only a few minutes after hatching, the larvae begin to search for prey such as springtails. While searching for prey, the head performs bidirectional pendulum movements to increase its range of perception. While doing so, the antennae and the maxillary palps are directed forward and do not make contact with the ground. Prey detection does not seem to involve visual clues, since a predatory strike is only released upon a direct mechanical contact.

Usually, the first contact is accomplished with the antennae, whereby this contact seems to be below the threshold for the prey. Upon prey contact, the larva rears up its forebody and tries to overwhelm the prey from above. In this phase, the pygopodium attaches to the ground, thus supporting the body. During the strike, the mandibles are first widely opened and subsequently rapidly closed to seize the prey. Larger prey animals are kept away from the ground by the beetle raising its head. Sometimes, the first pair of legs is used to hold the prey. Once the prey is captured, it is held in place by the labium (from below) and by both the maxillae (from the sides). The mandibles penetrate the prey cuticle, so that the prey can be extra-orally digested. In this phase, the prey is further squeezed by the mouthparts and the forelegs and is finally sucked out.

Similar to the imagoes, *Stenus* larvae involve adhesive mechanisms in prey-capture (Larsen 1959, 1963). Springtails often adhere to the mouthparts, the antennae, the legs or other parts of the integument before they are taken off with the legs and/or captured with the mandibles (Betz and Kölsch 2004; Lang 2014). Accordingly, the entire body surface of *Stenus* larvae often has a glossy appearance, and sometimes secretion droplets can be found at the apex of the body setae. Although fast-fleeing prey such as springtails can be captured without any involvement of adhesive mechanisms, adhesion certainly contributes to the high capture success of these larvae towards elusive prey such as springtails. Under laboratory conditions, the capture success of *S. pubescens* and *S. comma* attains 70–90% (Betz and Kölsch 2004). The origin of the adhesive secretion in *Stenus* larvae has not as yet been investigated. Potential candidates are glands associated with the paired openings found dorsally on the head, the three thoracic tergites and the abdominal tergites I–IX. When reared in the laboratory, the larvae of all three stages show an aggressive cannibalistic behaviour.

Before hatching into the next stage, the larvae search a retreat to spin themselves a cocoon. At least 1 day prior to pupation, the L₃ larvae cease to feed and search for an appropriate pupation

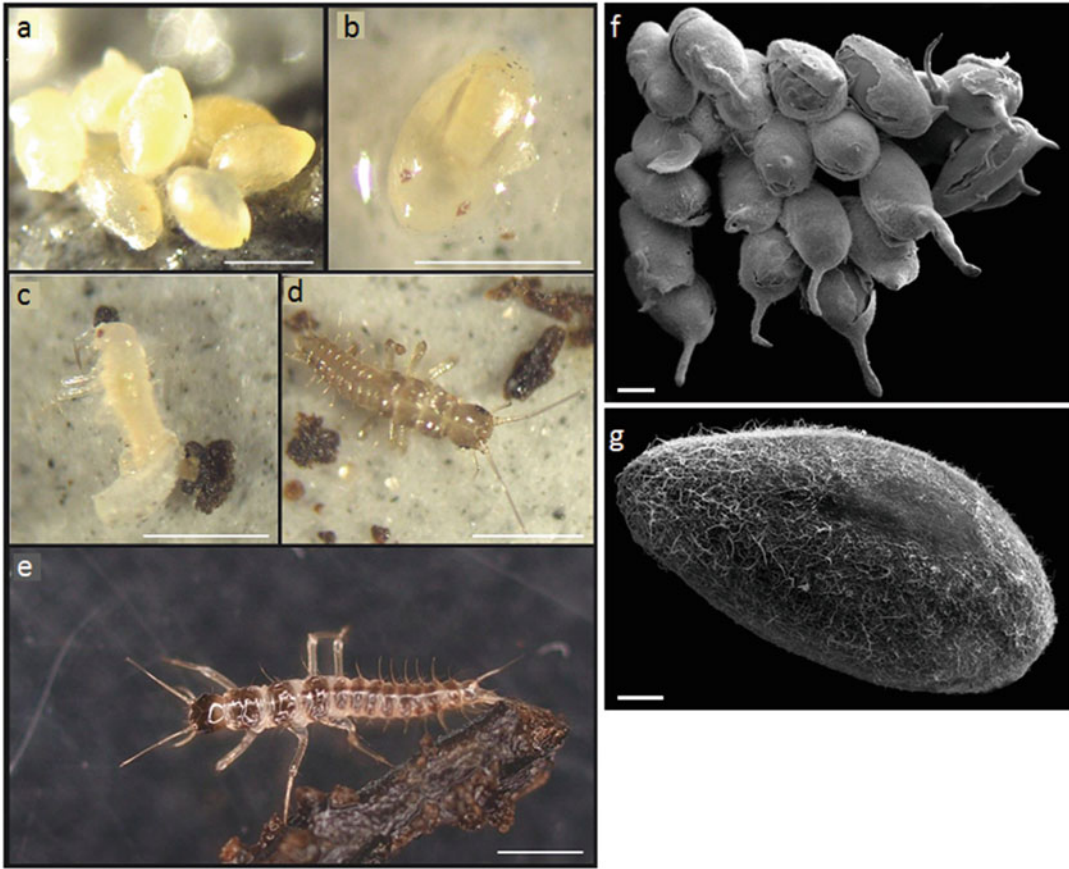


Fig. 11.11 Selected developmental stages of *Stenus junco*. (a) Egg cluster, (b) mature egg with L₁ larva showing ocelli and antennae shining through, (c) hatching L₁ larva disposing of the egg shell, (d) L₂ larva with pigmented and fully sclerotized cuticle, (e) full-grown L3

larva. Note, at the abdominal tip, the pygopodium that is used to attach the larva to the substratum. Scale bars = 1 mm. (f–g) SEM images of *S. bimaculatus* eggs. (f) Egg cluster. Scale bar = 200 μm. (g) Single egg. Scale bar = 100 μm [Images (a–e) from Lang (2014)]

site, where they can spin themselves a cocoon. A *S. comma* pupa sitting in its cocoon is shown in Fig. 11.7b.

During spinning, the larvae perform bidirectional dabbling movements with their pygopodium. The silk is released from the posterior segmental glands of the ninth abdominal segment (Frank and Thomas 1984). In *S. comma*, the silk can be additionally camouflaged by plaiting tiny substrate particles into it. Cocoon building in *Dianous coeruleus* (including the involved gland structures) has thoroughly been described by Jenkins (1958). As also observed in some *Stenus* beetles (e.g. Blair 1917), the cocoon of *D. coeruleus*

is a double structure consisting of an outer and an inner envelope. The silk is produced by paired glands opening in the ninth tergite anterior to the insertion of the urogomphi. Spinning is performed under the aid of the urogomphi and the movable abdomen. While spinning the outer envelope, the larva moves in several directions encircling the chosen pupation site. Once the outer envelope is finished (which takes a couple of hours), the larva fabricates the inner envelope that is situated more closely to its own body. The building of the entire cocoon is finished within 12 h (Jenkins 1958). The larva finally turns into the immobile prepupal stage, with pupal hatching occurring about 1 day after cocoon building.

11.5 Ecology and Behaviour

11.5.1 Ecology

General Lifestyle Adult Steninae beetles are diurnal, free-living and optically oriented predators of springtails and other small invertebrates (mainly soft-bodied arthropods such as aphids and dipteran larvae) (e.g. Betz 1998a; Yang 2003; Ryvkin 2012). As long as the prey species do not exceed a certain size limit, the beetles seem to be rather non-specific, although they reject chemically protected prey immediately after seizure (cf. Larsen 1959; Weinreich 1968; Heethoff et al. 2011). Consumption rates of adult *Stenus* species amount to six medium-sized springtails $\text{d}^{-1} \text{mg}^{-1}$ (Betz and Fuhrmann 2001) and seven aphids d^{-1} and individual *Stenus* (Yang 2003). Because they potentially feed on aphids (in the laboratory, these beetles show a Holling Type II functional response), *Stenus* beetles have been considered as natural enemies for the control of pests in the cultivation of vegetables and rice in China (Li and Zheng 2005).

Parasites In terms of parasites, only infestations by the endoparasitic nematode *Parasitylenchoides* Wachek and members of the fungus genus *Ilyomyces* Picard (Ascomycetes: Laboulbeniaceae) have been reported for Steninae, whereby *Ilyomyces* seems to be host-specific on Steninae (Frank 2018). Investigating two *Stenus* species, Koerner et al. (2016) identified 13 different bacteria species in the adhesive secretion of the labial sticky cushions, although whether these are merely commensals or influence the performance of these pads by other means is unknown.

Habitat Preferences Steninae usually prefer moist habitats and inhabit waterside environments such as reeds or sparsely vegetated sites on river or lake margins or the litter and humus layer in tropical forests. Thayer (2005) specifies the habitat types known to be settled as (1) canopy, (2) foliage of the lower storey, (3) debris (e.g. leaf) layer and grass clumps on ground,

(4) logs, (5) debris (and/or moss) and the ground surface close to the water edges, (6) dung and fermenting plant material and (7) the nests of ants (e.g. the Palearctic species *Stenus aterrimus*), birds and mammals (including middens and bivouacs). Rarely, Steninae have been found nearby fungi (Thayer 2005). Few are adventive, probably being spread by humans (e.g. *S. melanopus*) (Puthz 1971). “*Dianous*” species seem to be strongly hygrobiont (and often bryo- and petrimadiculous including logs and rocks immersed in water) and are particularly associated with the spray zone of running water and cascades (e.g. Champion 1919; Puthz 1971, 1981, 2000b; Rougemont 1983, 1985). In Central Europe, *D. coeruleascens* beetles can also be found associated with the logs of beaver dams. Other (sporadically reported) “*Dianous*” habitats are dead leaves and wet mosses in forests and tussocks, provided that these habitats are sufficiently moist.

In the Palearctic region, most *Stenus* species occur in littoral freshwater environments, where they inhabit waterside environments such as reeds or sparsely vegetated sites on river and lake margins (e.g. Horion 1963). Several *Stenus* species have also been recorded in very dry habitats (e.g. *S. atratulus*) (Puthz 1971). Other surveys provide more detailed information on the habitat choice of mostly European species (Benick 1929; Renkonen 1934, 1950; Horion 1963; Hammond 1975; Anderson 1984; Koch 1989; Betz 1994; Lott and Anderson 2011; Puthz 2012).

11.5.2 Behaviour

By direct observations of the adult focal animals of eight Central European *Stenus* species inhabiting different biotopes in terraria, a genus-specific behavioural inventory was acquired that included more than 70 distinct behavioural patterns (Betz 1999). These could be assigned to the higher functional categories of feeding, reproduction, grooming, resting and protection. Before this general survey, only single observed behaviours in Steninae had been

reported (e.g. Billard and Bruyant 1905; Benick 1922; Delahon 1927; Urban 1928; Renkonen 1934; Voris 1934; Jenkins 1960; Linsenmair 1963; Weinreich 1968; Lecoq 1991). Focal animal sampling revealed that, during the day, the beetles spend their greatest amount of time grooming (mostly 5–30% of the observation time) and searching (for prey) (50–90% of the observation time) (Betz 1999). While searching, the beetles move in a stop and go manner (“saltatory search” according to O’Brien et al. 1989), whereby the movements of potential prey animals are visually detected in the locomotion pauses. Alternatively, these beetles seem to set an ambush, as they remain at a given place and

await prey movements in the surroundings. The predatory behaviour of *Stenus* beetles has been investigated in greater detail by using (high-speed) video techniques (Bauer and Pfeiffer 1991; Betz 1998a; Koerner et al. 2012a). Once the beetles have detected a moving prey, they approach it in a series of runs and stops, orient their longitudinal body axis until it is in alignment with the prey (Fig. 11.22) and, as soon as the critical attack distance is reached, lunge forward to seize the prey. Prey seizure can be performed either by abruptly ejecting the elongated labium or by quickly grasping the prey with the mandibles (Fig. 11.12a) (Bauer and Pfeiffer 1991; Betz 1998a). While hunting in

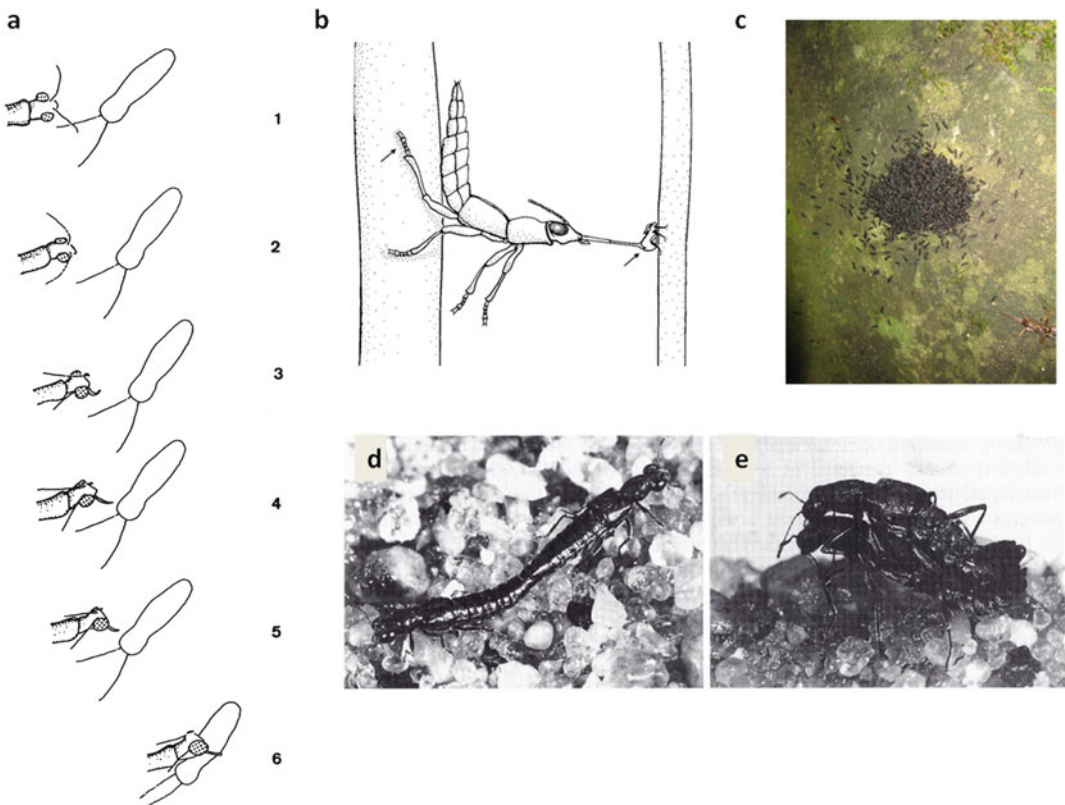


Fig. 11.12 Selected behavioural patterns observed in adult *Stenus* beetles. (a) Schematic drawing of the head of a *S. canaliculatus* beetle grasping a large springtail directly with its mandibles. Sequence drawn from high-speed video footage. (b) *S. cicindeloides*: characteristic prey-capture situation occurring while hunting an aphid in vegetation. The visible length of the labium amounts to 1.3 mm. Arrows indicate structures (tarsi and

paraglossae) in which adhesive forces are required. (c) Gregariousness shown by *Dianous fellowesi* in Guangxi, China (courtesy of Jian-Qing Zhu). (d) *S. bifoveolatus* pair maintaining a copulatory end-to-end position. (e) *S. comma* pair showing a copulatory parallel position. Figures (d)–(e) from Betz (1999). For further explanations see text

the vegetation or in plant debris, the prey-capture instances may be different, forcing the beetles to adapt to the specific situation by, for example, detaching the foretarsi from the substratum and, additionally, bending the thorax against the abdomen in order to attain otherwise unreachable prey (Fig. 11.12b). Attacks with the labium allow larger attack distances than attacks performed with the mandibles (Bauer and Pfeiffer 1991; Betz 1998a). This reduces the chance of premature detection by the prey and makes it possible to catch prey that would otherwise be out of reach (cf. Fig. 11.12b).

Once a prey item is captured, it is kneaded by the mandibles and the maxillae entailing rotatory movements (“rotary-mill method” according to Leschen and Newton 2003). Digestion is extra-oral, followed by the gradually sucking in of the predigested prey material.

In addition to searching (for prey), self-grooming accounts for a substantial part of the time budget of a beetle and consists of quite a variety of different movement patterns (Betz 1999). This behaviour seems to be mainly aimed at spreading a pygidial secretion over the entire body surface. It makes use of the considerable bendability of the abdomen, whose tip may even reach the dorsal surface of the elytra. This will be dealt with in more detail in Sect. 11.6.

With regard to reproductive behaviour, the male attempts to mount the female and tries to copulate. A non-receptive female may forestall this by several antagonistic patterns. Depending on the species, the final copulation position may take the form of an end-to-end position (with the heads facing away from each other) (Fig. 11.12d) or a parallel position (Fig. 11.12e). Copulations in the parallel position do not last longer than several minutes, whereas mating in the end-to-end position may take up to an hour (even with the female continuing its locomotion and dragging the male behind her) (Betz 1999).

One special feature of Steninae beetles is their ability to move on the water surface (cf. Billard and Bruyant 1905; Urban 1928; Jenkins 1960; Linsenmair 1963; Linsenmair and Jander 1963; Betz 1999; Lang et al. 2012; Dietz et al. 2016). This involves three different methods,

i.e. walking (with only the non-wettable tarsi and, additionally, the abdominal tip touching the surface), swimming (performing characteristic swimming movements involving both the legs and the abdomen) and skimming. Skimming involves the release of a spreading-active abdominal secretion (cf. Jenkins 1960) and is dealt with in more detail in Sect. 11.6. We have observed in *Dianous fornicifrons* and *D. srivichaii* that the long terminal styles at the ninth abdominal segment are laterally spread out during skimming, whereas otherwise they are kept parallel.

Another behaviour for both *Stenus* and “*Dianous*” is gregariousness (e.g. Lecoq 1991; Puthz 2000b, Figs. 29–30; Cuccodoro 2017). In this case, numerous individuals flock closely together (touching each other) and may, in this way, form incrustation-like aggregates covering rocks or leaves (Fig. 11.12c). This might be triggered by adverse conditions such as drought or coldness and might represent a kind of protective behaviour. According to Cuccodoro (2017), the aggregations of Steninae (which might be formed of up to more than 100,000 specimens) resemble a composite of several kinds of behavioural patterns, such as hibernation, aestivation, reproductive swarming and possibly hilltopping, with some most likely intermixed.

Recent focal animal observations of nine Thai “*Dianous*” species (including both groups I and II) in terraria revealed large overall correspondences with the behavioural inventory of *Stenus* beetles that was published by Betz (1999).

11.6 Chemical Ecology

Like other members of Staphylinidae, representatives of Steninae are characterized by short elytra resulting in a relatively unprotected and partly flexible abdomen. These characters, which represent true evolutionary success, allow the beetles to colonize habitats with small interstices (Steninae: banks of streams, wet grasslands, swamps, bogs) but make them susceptible to predation and parasitism because of the missing protective elytra. In order to avoid

these hazards, adults of the free-living carnivorous Steninae and many other rove beetle taxa have evolved abdominal defensive glands that synthesize and store a variety of highly effective defensive compounds (Dettner 1987, 1993; Thayer 2016). In addition, several exocrine glands have recently been described in other rove beetle taxa (see Thayer 2016), such as Staphylininae (Quenedey et al. 2002), Paederinae (Schomann and Solodovnikov 2016) or Piestinae (Caron et al. 2008).

11.6.1 Morphology of the Anal Glands

Adults of all at least extant Steninae, including the genera *Stenus* and “*Dianous*” Leach, possess

defensive glands at the tip of their abdomen. The complex glandular system consists of two large translucent sac-like reservoirs (r1) expanding through the last four abdominal segments (Figs. 11.13 and 11.14) and a second smaller reservoir pair (r2), one each at the base of the big reservoirs and the corresponding secretory tissues (g1, g2; Jenkins 1957; Schildknecht 1970; Schierling and Dettner 2013). Basically the reservoirs are characterized by partly eversible membranes, which enable the beetles to deplete volatile secretions, when haemolymph pressure has been previously increased. It is interesting that the large reservoirs contain deterrent and antimicrobial alkaloids (Fig. 11.13), whereas the small reservoirs contain various terpenes (Fig. 11.13).

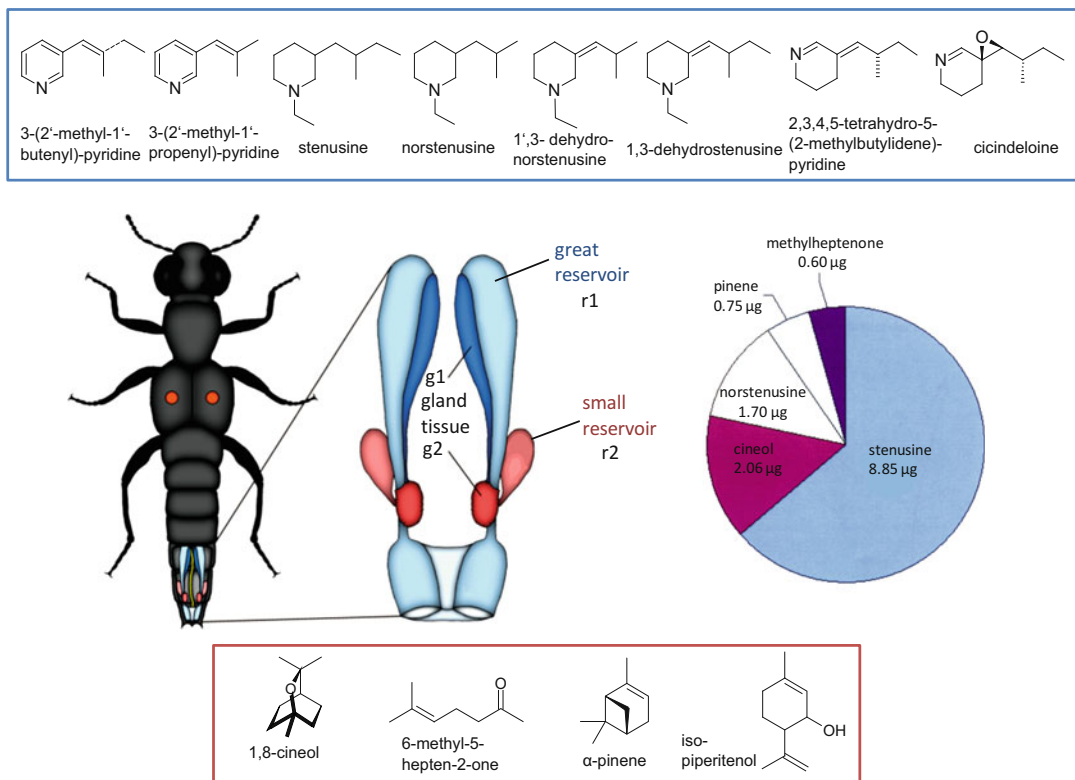


Fig. 11.13 Anal glands and secretions of *Stenus* rove beetles (Schierling 2013). Paired anal glands are characterized by great reservoirs (r1) associated with longitudinal gland tissues (g1, blue). In addition, small paired reservoirs (r2) exist with associated oval gland tissues (g2, centre; red). The identified alkaloids from the large

glands are presented above, depending on species. Typical terpenoid compounds from the small reservoirs are illustrated below. As an example, the circle right symbolizes quantitative amounts of two alkaloids and three terpenoid compounds from the anal gland secretion of *S. comma*

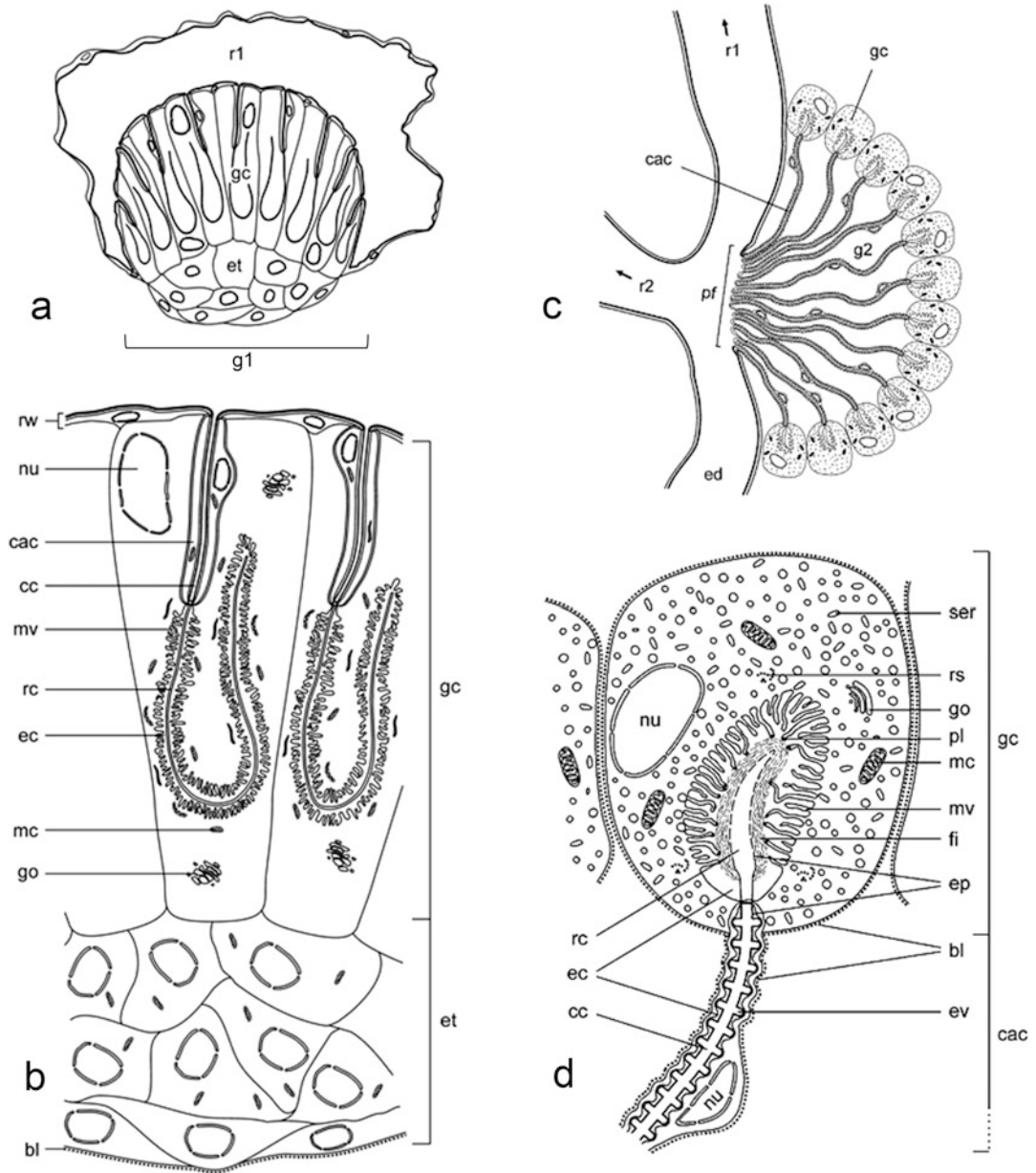


Fig. 11.14 Representation of the large gland system (r1/g1) of *Stenus comma* LeConte and *S. biguttatus* Linnaeus according to Schierling and Dettner (2013). (a) Overview of cross section through r1/g1. The band-shaped gland tissue (gc) and the protective epithelium (et) are located in an invagination of the r1 wall (modified according to Jenkins 1957). (b) Detail of gland cells of the large reservoir and protective epithelium with reservoir wall (rw), nucleus (nu), canal cell (cac), secretion-contacting canal (cc), microvilli of gland cell membrane (mv), secretion-receiving canal (rc), extracellular cavity (ec), mitochondria (mc), Golgi apparatuses (go) and basal intima (bl). (c) Representation of the small gland system

r2/g2 of *S. comma* and *S. biguttatus* with secretion-contacting canals gathering on a pore field on the efferent duct membrane of r1 opposite to r2. Pore field (pf), efferent duct (ed) of r1, canal cells (cac), secretory tissue filling r2 (g2), gland cells (gc). (d) Diagram of the secretion production, receiving and conducting structures of g2. Smooth endoplasmic reticulum (ser), ribosomes (rs), Golgi apparatus (go), plaques at the microvilli apices (pl), mitochondria (mc), microvilli (mv), filament layer (fi), epicuticle (ep), basal lamina (bl), evagination of the conducting canal (ev), receiving canal (rc), extracellular cavity (ec), conducting canal (cc) and nuclei (nu)

The band-shaped glandular tissue (g1) is longitudinally associated with the large reservoir and situated within an invagination of the reservoir membrane (Fig. 11.14a). Each secretory active cell of g1 bears an extracellular cavity, in which the products are secreted by exocytosis and drained by an epicuticular duct. The distal secretion-receiving structure is located in the middle of the extracellular cavity of the gland cell. It is lined with a porous granular epicuticular material of about 50 nm thickness, surrounded by a 120–300-nm-wide filamentous mass that does not resist maceration. In order to achieve a high exocytosis rate, the surface of the gland cell membrane surrounding the extracellular cavity is increased by numerous microvilli (Fig. 11.14b, d). Inside and around the microvilli, the gland cells exhibit large, partially elongated mitochondria with cristae of variable length and frequency. The stability and flexibility of the reservoir wall are attributable to a presumably single layer of epithelium cells containing numerous myofibrils arranged in a right-angled manner. The epithelium cells are covered by a basal lamina. Retraction of the glands is accomplished by retractor muscles (Jenkins 1957; Whitman et al. 1990). Although the large gland system r1/g1 is predominant in every *Stenus* species (Figs. 11.13

and 11.14), the smaller system r2/g2 (Figs. 11.13 and 11.14) is often reduced and difficult to localize (Fig. 11.15). Probably, for this reason, it has been reported only for *S. comma* and *S. biguttatus* (Schildknecht 1970; Schildknecht et al. 1975, 1976; Whitman et al. 1990; Lusebrink 2007), but it is present in all investigated *Stenus* species and might serve as a valuable character for phylogenetic studies of the Steninae.

In contrast to the gland system r1/g1, great interspecific differences are found concerning the morphology and ultrastructure of the smaller gland system r2/g2, which have been especially investigated in *S. comma* (Fig. 11.15a) and *S. biguttatus* (Fig. 11.15b). The small gland system r2/g2 consists of a clear secretion-filled reservoir r2 (up to 330 μm long and 130 μm wide), which opens into the efferent duct of the large reservoir r1. The associated secretory tissue g2 encloses the efferent duct of r1 at the opening of r2, with the main part being located opposite to r2. G2 consists of numerous globular gland cells (16 \times 22 μm in diameter) that make up a “cauliflower-like” structure visible by light microscopy. Each gland cell is equipped with an epicuticular duct that collects the secretion and conducts it into the reservoir through at least one

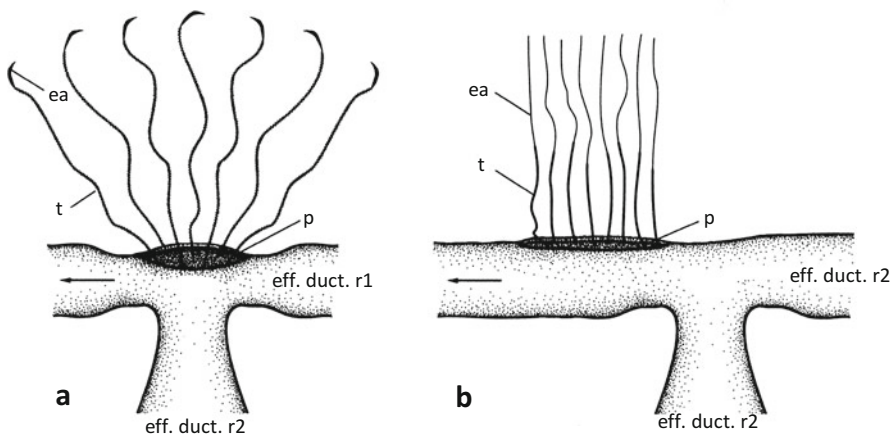


Fig. 11.15 KOH-macerated parts of anal glands of selected representatives of Steninae (**a** *Stenus comma*, **b** *S. biguttatus*) showing the arrangements and reductions of the small reservoirs r2 (eff. duct. r2) and associated tubules (t) and end apparatus (ea). Tubule pores are symbolized by

p and are arranged opposite to the efferent duct of r2 (**a**), respectively, shifted away from efferent duct r2 as in other Steninae (**b**). Both efferent ducts of the large reservoirs (eff. duct. r1) and the direction of secretion emissions (arrows) are indicated (according to Schierling 2013)

canal cell. The canals measure up to 60 μm in total length and can be divided into a distal secretion-receiving, a proximal secretion-conducting and a transition section. The distal secretion-receiving structure of the epicuticular ducts is about 12 μm long and 0.9 μm wide and is situated in an extracellular cavity of the gland cell. As in the secretory cells of g1, the extracellular cavity of the g2 cells is filled with numerous microvilli that are part of the inner gland cell membrane and that are presumably involved in the secretion of the synthesized compounds via exocytosis. The mostly electron-lucent microvilli bear an electron-dense material in their apical region, where they reach the filament layer. Electron-dense material also occurs in the cytoplasm of the whole gland cell. The gland cells are often filled with smooth endoplasmic reticulum. Sparse ovoid mitochondria with short cristae occur near the microvilli. Golgi systems, free ribosomes and rough endoplasmic reticulum are seldom. Each gland cell is coated by an 85-nm-wide basal lamina and is equipped with an ovoid nucleus of 5–6 μm in diameter. The proximal secretion-conducting part of the epicuticular duct transports the secreted compounds into the reservoir. The tubular ducts are up to 50 μm in length and 0.7 μm in diameter and exhibit numerous evaginations all over their surface. The secretion-conducting ducts of g2 are situated in at least one canal cell, which penetrates the gland cell and closely approaches the secretion-receiving part of the canal. As an extracellular structure, the conducting canal is surrounded by an extracellular cavity. In contrast to the secretion-receiving canals, the conducting canals are enclosed by a 60-nm-thick compact non-perforated epicuticle, lined with a cuticulin layer of 5–8 nm in width. The very slim canal cells are poor in internal structures and cell organelles but have an ovoid nucleus. They are surrounded by a basal lamina. Proximally, the canal cells are bundled onto a pore field where the canals open into the efferent duct of r1. The distal secretion-receiving and the proximal secretion-conducting parts of the ducts are linked by a short evagination-free transition section with non-perforated epicuticle. The gland cell and

canal cell are interconnected via septate junctions. The pore field onto which the g2 canals open into the efferent duct of r1 is situated exactly opposite to the reservoir r2. In the living beetles, the r1 efferent duct collapses, and the pore plate is pressed onto the opening of r2. The r2 wall consists of epithelium cells secreting a layer of filaments covered by a puckered epicuticular intima of 8–13 nm in width. The epithelium cells do not contain any muscle fibrils as observed in the r1 wall.

In many species, r2/g2 are massively reduced and functionally altered. The best developed and most complex r2/g2 gland system can be found in *S. comma* and *S. biguttatus* (Fig. 11.15). Other selected species that have been examined show distinct reductions of either g2 or r2 or of both components. Whereas r2 of *S. comma* is up to 330 μm long, in *S. bimaculatus*, a species that is about 1 mm larger than *S. comma*, r2 only reaches a length of approximately 60 μm . Furthermore, in *S. bimaculatus*, the conical outline of r2 is lost, and it becomes a small tubular appendix of the r1 efferent duct. An even more extreme form of reduction of r2 occurs in *S. fulvicornis*, *S. juno* and *S. picipes*. Species such as *D. coerulea*, *S. providus*, *S. solutus*, *S. pubescens* and *S. latifrons* exhibit a small reservoir r2 that keeps its conic form, but its size is dramatically reduced. *S. flavipes* is the only species that possesses a reservoir r2 with a reservoir size/body size ratio comparable with that of *S. comma* and *S. biguttatus*. In all species except *S. comma* and *S. biguttatus*, the epicuticular ducts of g2 do not gather on a pore field but open within a widespread area that does not lie opposite to r2 and from there into the efferent duct of r1. Their comparatively short conducting canals do not exhibit any evaginations, and the distinct secretion-receiving structure observed in *S. comma* and *S. biguttatus* is replaced by a filamentary receiving canal. Overall, their organization is reminiscent of that of the g1 canals. This is also the case for *S. flavipes*.

The eversible membrane parts of the anal glands do not vary in different species (Kanehisa and Tsumuki 1996). They are situated at the base of the efferent ducts of the reservoirs r1 and can

be extruded laterally to the anus between the ninth tergite and sternite (Fig. 11.13). They are each made up of a tubular membrane, which is inverted at the tip and thereby forms a cylindrical double-walled structure bearing a pore at the end for secretion release. Whereas the inner membrane represents an extension of the r1 efferent duct, the outer membrane of the eversible cylinder is continuous with a membrane separating the gut and pygidial gland apparatus from the gonads and other abdominal organs. Jenkins (1957) reports more details concerning the morphology of the eversible membrane parts.

Although the secretion-receiving canals of g1 and g2 gland cells both exhibit a porous granular epicuticle for the reception of the secreted compounds, the conducting canals are lined with a continuous epicuticle and an additional cuticulin layer. This seems important in order to minimize the risk of self-intoxication with cytotoxic defence secretions (Noirot and Quennedy 1974, 1991; Quennedy 1998). As non-proteinous secretions of low molecular weight, the defence compounds of the Steninae are thought to be synthesized in the smooth endoplasmic reticulum and Golgi systems. Whereas the g2 gland cells are completely filled with smooth endoplasmic reticulum, these structures are lacking in g1 gland cells. Thus, the cellular biosynthetic origin of the alkaloids of r1/g1 remains unknown. A sponge-like or filamentous layer surrounding the receiving canal of a gland cell, as is found in both secretory tissues of the Steninae, is usually interpreted as protein or epicuticular filaments (Noirot and Quennedy 1991). In the g2 gland cells, the filament layer surrounding the receiving canal probably does not represent an accumulating secretion. Although the fine filaments do not resist maceration by 10% KOH, they are nevertheless proposed to be of epicuticular origin and are lost following the severe maceration process during preparation for SEM. In contrast to the filamentous layer of the g1 secretion-receiving apparatus, the single filaments of g2 cells are clearly distinguishable as a built-up mesh-like structure. The chemical modification might result in an increasing toxicity during passage through the canal. The conspicuous length of the g2 conducting canals supports

this hypothesis. However, such processes would require enzymes or other compounds located in the canal evaginations, but no structures have been found that suggest secretory activity of the canal cells. Thus, the secretion-modifying compounds or enzymes would also have to be secreted from the g2 gland cells. All the secretion-conducting canals of g2 gather on a moderately sclerotized pore plate on the efferent duct of r1, comparable with those reported for *Eleodes longicollis* (Eisner et al. 1964), but here its position is of fundamental significance for the functionality of r2/g2. The pore plate is situated opposite to the opening of the reservoir r2, and so the secreted compounds have to pass the r1 efferent duct to enter the reservoir r2. The compounds found in r2 have not been detected in r1 (Schierling et al. 2013), and thus a mechanism must exist preventing the g2-synthesized compounds from entering r1. Because of the distinctive position of the pore plate exactly opposite the opening of r2, a transfer of the secretion from g2 to r2 might be facilitated by the collapse of the r1 efferent duct under resting conditions (no secretion release), an event that might result in the pore plate being pressed onto the opening of r2. The wall of reservoirs r1 and r2 is lined by a tight epicuticular intima that prevents the haemolymph and organs from being contaminated with toxic secretion. Furthermore, the reservoir walls are reinforced by myofilaments (r1) or epicuticular filaments (r2). If reservoir r1 is not maximally filled, the myofilaments in the epithelium cells are contracted, and the intima is puckered. A puckered intima has also been observed in the r2 wall, despite the presence of epicuticular filaments instead of muscle fibrils. However, the reservoirs are thus flexible structures that can be adjusted to the actual secretion level. The flat muscular epithelium surrounding r1 is thin and is therefore probably not responsible for the rapid gland eversion and secretion release, which is actually achieved by increasing haemolymph pressure, as proposed by Jenkins (1957). Jenkins (1957) describes the whole pygidial defence gland apparatus of the Steninae as an invagination of the pleural membrane. The duct structures of both secretory tissues g1 and g2 and the corresponding

reservoirs are lined with epicuticular material supporting Jenkins' proposal of the glands' epidermal origin (Noirot and Quennedey 1974; Quennedey 1998).

The appearance and characterization of a second smaller reservoir r2 in Steninae abdominal glands were first described for *S. comma* by Schildknecht (1970). Later, it was reported for *S. biguttatus* and *S. comma* (Lusebrink 2007), but it has never been described for other Steninae. Jenkins (1957) mentioned some structural changes at the basal parts of the gland tissue r1 but was unable to identify it as being independent from g1. We have found r2 and the associated gland tissue g2 in every species examined, including in *D. coerulescens*. However, great species-dependent reductions are apparent in the morphology, ultrastructure and function of this small reservoir r2. As described above, the position of the pore plate exactly opposite to the reservoir r2 seems important for the correct transfer of secretion from g2 to r2 in *S. comma* and *S. biguttatus*. Only by this means can the pore plate be pressed onto the opening of r2 when the r1 efferent duct is collapsed. In the other species examined during this study, the secretion-conducting canals open into the efferent duct of r1 cranial to r2, and so the secretion transfer cannot work. As a consequence, reservoir r2 is reduced, as can be seen in various species. Furthermore, the epicuticular ducts of species with reduced r2/g2 are similar in structure to those of g1, and hence the presence of structurally modified g2 cells or just tightly packed and round g1 cells opposite to the small reservoir r2 is difficult to determine. The small reservoirs r2 contain terpene compounds such as α -pinene, 1,8-cineol (eucalyptol) and 6-methyl-5-hepten-2-one (Schildknecht 1970; Schildknecht et al. 1975, 1976; Lusebrink 2007). Whereas α -pinene and 1,8-cineol have been found in many *Stenus* species, 6-methyl-5-hepten-2-one has only been identified in the r2/g2 secretion of *S. comma* and *S. biguttatus* (Lusebrink 2007; Schierling et al. 2013), which show the best developed r2/g2 gland system within the whole genus. Furthermore, the amount of α -pinene and

1,8-cineol is maximized within these two species. In other Steninae, these terpenes occur, if at all, only in traces (Lusebrink 2007; Schierling et al. 2013). Because *S. comma* and *S. biguttatus* represent the only hitherto investigated species exhibiting distinct evaginations (paratergites) of the secretion-conducting canals, a correlation might be present. *S. comma* and *S. biguttatus* both reveal distinctly raised lateral margins at their abdominal tergites. This character is classified as phylogenetically plesiotypic, and so the two species are arranged at the base of the genus *Stenus* (Puthz 2006, 2010). Because of their better adaption in nearly all areas, the alkaloid compounds of r1 are effective for defence against predators or microorganisms and for locomotion via skimming (Schierling et al. 2013; Lang et al. 2012). Thus, most phylogenetically advanced species have nearly lost or at least dramatically reduced their r2 compounds and the structures responsible for their synthesis and storage. "*Dianous*" with its single Middle European species *D. coerulescens* has hitherto been classified as a phylogenetically basal taxon of the Steninae because of its lack of an adhesive prey-capture apparatus that probably represents an apomorphy in *Stenus* (Puthz 1981; Betz 1996, 1998b, 1999; Leschen and Newton 2003). The molecular and chemotaxonomic analyses of the phylogeny of the Steninae indicate that the genus "*Dianous*" should be integrated into *Stenus* as a species with a secondarily reduced prey-capture apparatus (Koerner et al. 2013; Lang et al. 2015). These analyses are in accordance with the morphology of the small gland system r2/g2 of *D. coerulescens*. It exists only in a strongly reduced form comparable with that of the phylogenetically advanced *Stenus* species.

11.6.2 Secretion Chemistry and Biosynthesis of Secretion

The reservoir r1 of investigated representatives of Steninae is filled with g1-synthesized **pipерidine**- (Fig. 11.13: stenusine; norstenusine; 1',3-dehydrostenusine; 1',3-dehydronorstenusine),

piperidine- (cicindeloin; 2,3,4,5-tetrahydro-5-(2-methylbutylidene)-pyridine) and **pyridine-alkaloids** (Fig. 11.13: (Z)-3-(2'-methyl-1'-butenyl)-pyridine; (E)-3-(2'-methyl-1'-butenyl)-pyridine and 3-(2'-methyl-1'-propenyl)pyridine). The small gland reservoir r2 contains terpenes (e.g. Fig. 11.13; 1,8-cineol and α -pinene and, in few cases, 6-methyl-5-hepten-2-one and isopiperitenol) produced by g2 (Schildknecht 1970; Schildknecht et al. 1975; Kohler 1979; Lusebrink 2007; Lusebrink et al. 2009; Müller et al. 2012; Neumann 1993). In order to verify our trace-analytical investigations, most of the components, some together with their stereoisomers, were synthesized in the laboratory (Müller et al. 2012).

Whereas all terpenoids identified from the small glands of Steninae are widely distributed within plants and arthropods (Blum 1981), all nine alkaloids reported here are uniquely found in Steninae beetles. Nevertheless, other biologically highly active **piperidine-alkaloids** are also found in other animals and plants (Breitmaier 2008). Fire ants of the genus *Solenopsis* contain various toxic and insecticidal 2-alkyl-6-methylpiperidines and even N-alkylpiperidines (Morgan 2010). Moreover, poison dart frogs of the genera *Dendrobates*, *Epipedobates* and *Phylllobates* produce toxic bicyclic piperidine-alkaloids, such as histrionicotoxin A and its derivatives, in their skin. Since these frogs use their skin as a kind of excretion organ, Steninae alkaloids might also be found in frog skin, because these amphibians feed on Steninae beetles (see Dettner 2007). As compiled by Breitmaier (2008), a considerable number of biologically active piperidine-alkaloids are present in various plants such as piperine (*Piper nigrum*, Piperaceae), (–)-coniine (*Conium maculatum*, Apiaceae), (R)-(–)-pelletierine (*Punica granatum*, Punicaceae), (–)-sedamin (*Sedum acre*, Crassulaceae), (–)-lobeline (*Lobelia inflata*, Campanulaceae), pinidin (*Pinus* and *Picea* species) and (+) carpaine (*Carica papaya*, Caricaceae). Bicyclic piperidine-alkaloids also exist such as (+)- α -skytanthin (*Skytanthus acutus*, Apocynaceae), nitramine (*Nitraria*

schoberi, Zygophyllaceae) and (–) sibirin (*Nitraria sibirica*, Zygophyllaceae). A few **piperidine-alkaloids** (see cicindeloin) such as γ -coniceine from the toxic plant *Conium maculatum* (Apiaceae) and toxic 2-methyl-6-alkyl-piperidineines from the ant *Solenopsis geminata* have been reported (Breitmaier 2008; Morgan 2010). Apart from the **pyridine-alkaloids** from Steninae, various biological pyridine-alkaloids are known from plants such as ricinin (*Ricinus communis*, Euphorbiaceae), anibin (*Aniba duckei*, Lauraceae), arecoline (*Areca catechu*, Palmaceae) and nicotine and its derivatives (*Nicotiana tabacum*, Solanaceae). Animals such as various caterpillars of Saturniidae and Lymantriidae produce nicotine and other pyridine-derivatives such as nicotinic acid or nicotinamide in glandular defensive hairs (Deml and Dettner 1995, 1997). Again, a halogenated pyridine-alkaloid from the poison dart frog skin of *Epipedobates tricolor* named (–)-epibatidine acts as a powerful analgesic and is even superior to morphine.

All terpenes are biosynthesized according to the mevalonate pathway (Morgan 2010). In contrast, all *Stenus* alkaloids from the large anal glands are biogenetically derived from amino acids. In particular, piperidine-alkaloids are principally derived either from lysine, acetate or mevalonate. Lusebrink et al. (2008a) have revealed that the N-ethyl-group of stenusine and norstenusine is derived from acetate, a pathway established by using trideuterated sodium acetate (CD₃COONa), whereas L-lysine forms the piperidine ring (stenusine, norstenusine) and the sidechain originates from L-isoleucine. Furthermore, in (Z)-3-(2-methyl-1-butenyl) pyridine, L-lysine yields the pyridine ring, and L-isoleucine yields the 2-methyl-1-butenyl sidechain (Schierling et al. 2011). In the same way, the piperidine ring of cicindeloin is biosynthesized from L-lysine, and, again, the sidechain is biosynthesized from L-isoleucine (Schierling et al. 2012). Wittmann et al. (2015) have additionally revealed that the precursor in the biosynthesis of cicindeloin must be (S, E)-1 [= 2,3,4,5-tetrahydro-5-(2-methylbutylidene)-

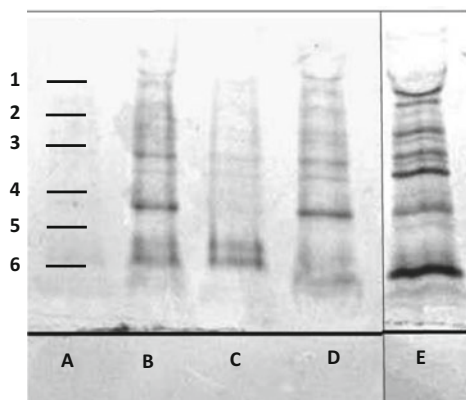


Fig. 11.16 SDS gels (Coomassie staining) of glandular compartments of the two *Stenus* species *S. comma* (A–D) and *S. bimaculatus* (E) (according to Schröder 2013). Vertical numbering of reference compounds (A): 1 phosphorylase b, 2 albumin, 3 ovalbumin, 4 carbonic anhydrase, 5 trypsin

inhibitor, 6 α -lactalbumin. Horizontal lettering: (B) filled whole anal gland system of *Stenus comma*, (C) filled small glandular system of *S. comma*, (D) filled large glandular system of *S. comma*, (E) filled large glandular system of *S. bimaculatus*

pyridine] and not the enantiomer (*S*, *Z*)-1. These results amply indicate that alkaloids not only and exclusively represent so-called secondary plant compounds but are also produced and sequestered by many animals and especially arthropods.

As is well known from the anal defensive glands of carabid beetles (Bombardier beetles; Schildknecht et al. 1968) or recently from chrysomelid larvae (Burse and Boland 2015), biosynthesizing enzymes that are responsible for producing intermediate and end products of the secretions are localized either within glandular cells or within the gland reservoir or are found in both structures. Therefore, the Steninae enzymes for terpenoid biosynthesis via mevalonate should be present in the reservoir or gland cells of the small gland. In contrast, those Steninae enzymes that are responsible for alkaloid biosynthesis should be found in the large gland system. These enzymes have been postulated to process L-lysine and L-isoleucine (decarboxylation of L-lysine; oxidative deamination) and should be responsible for subsequent oxidations, reductions, N-ethylations and epoxidations. When the proteins from the various glandular components of gland 1 and gland 2 of *S. comma* were separated electrophoretically, different protein patterns were evident

(Fig. 11.16). Another species, *S. bimaculatus*, with reduced small glands showed a similar electrophoretic pattern to that of *S. comma*, although typical bands of the reduced small gland were absent. These investigations need to be continued by using other species and by applying enzyme assays in order to differentiate between proteins from alkaloid biosynthesis and structural proteins.

11.6.3 Biology and Role of Behaviour-Modifying Chemicals

Nearly all Steninae secretion compounds show significant antibiotic and deterrent activities (Lusebrink et al. 2009; Schierling et al. 2013) and thus can serve as potent chemical defence compounds. When molested, the beetles bend their abdomen towards the source of irritation, evert their glands and moisten the aggressor with their secretion. In addition, the beetles show typical cleaning movements and use their pygidial gland secretion to coat their body surface, thus avoiding infection by microorganisms (Betz 1999). Moreover, some species of Steninae living on the banks of water use the pygidial defence gland secretion for an exceptional form of locomotion called skimming. This

phenomenon was first described for Steninae by Piffard (1901) and for *Stenus cicindeloides* and *S. tarsalis* by Billard and Bruyant (1905). Supported on the water surface by their hydrophobic tarsi, the beetles touch the surface with the tip of their abdomen and release small amounts of secretion from the everted pygidial glands (see next section).

The secretions of most Staphylinidae, such as Oxytelinae and Aleocharinae, are mainly defensive, but the *Stenus*/*Dianous* pygidial gland secretions appear to be multifunctional. This becomes apparent, when the beetles are observed in nature. When molested, the beetles evert the basal parts of their glands and moisten the aggressor with secretion by bending their flexible abdomen (Jenkins 1957; Betz 1999), a reaction that clearly is defensive. In this regard, the piperidine and pyridine-alkaloids act in particular as feeding deterrents in ant bioassays (Honda 1983; Lusebrink et al. 2009; Schierling et al. 2013). Furthermore, extensive cleaning behaviour is often shown by the Steninae, whereby pygidial gland secretion is dispensed over the entire body surface. Via an analysis of the behavioural inventory of *Stenus*, Betz (1999) observed the beetles spending a large amount of their time budget rubbing their hind legs over the abdominal tip with the everted glands emitting small amounts of secretion. Subsequently, the secretion was transferred to the middle and fore legs and then dispensed over the entire body surface. In view of the wet habitat of most Steninae, stenusine and the pyridine-alkaloid have unsurprisingly been reported as antimicrobial compounds (Schildknecht 1976; Lusebrink et al. 2008b, 2009).

Complete analyses of the pygidial gland secretions have revealed great quantitative and qualitative differences between the analysed species (see Lusebrink et al. 2009; Schierling et al. 2013). Stenusine is usually abundant in most of the species investigated, most often as the main constituent. Analogous to stenusine, the corresponding norstenusine is almost always detected, albeit in lesser amounts. According to Lusebrink et al. (2009), the pyridine- and piperidine-alkaloids are restricted to the

subgenera *Metatesnus* and *Hypostenus*. The piperidine derivative has only been detected in *S. binotatus* (*Metatesnus*), *S. solutus* and *S. cicindeloides* (*Hypostenus*). Although the existence of terpenes is variable in the secretion of the analysed species, some of them seem to be restricted to species of the subgenera *Hemistenus* and *Stenus* s. str. and *Dianous coeruleus*. 6-Methyl-5-hepten-2-one is found only in *S. comma* and *S. biguttatus*, whereas isopiperitenol only occurs in *S. comma*. Except for a few trace terpenes, the results are in agreement with the findings reported by Schildknecht (1976), Lusebrink (2007), Lusebrink et al. (2009) and Müller et al. (2012). The natural isomeric compositions of the alkaloids investigated also seem to be of great interest, as seen in Fig. 11.17 for stenusine.

Ants whose mouthparts come in contact with an alkaloid-containing milk drop (Fig. 11.18) immediately retreat from the drop with panic-like movements, often rubbing their mandibles on the substrate. This action is followed by extensive cleaning activities of the antenna and mouthparts. Among the terpenes, 6–8 (Fig. 11.18a) especially also show significant deterrent activities against ants but do not elicit the drastic reactions evoked by the alkaloids (Fig. 11.18). Additionally, the deterrent responses of the secretion main components have been compared among one another (Fig. 11.18b): the differences in deterrencies between stenusine and the pyridine-alkaloid and the piperidine-alkaloid as compared with the pyridine-alkaloid are extreme.

In order to examine potential synergistic effects of the secretion, we have also tested the three main alkaloids 1, 3 and 5 against naturally occurring secretions (Fig. 11.18c). The secretion of *S. comma* (CO) and that of *S. similis* (Si) is significantly less deterrent than the single main compounds. In addition, the difference in the reaction of *Lasius flavus* to the secretion of *S. solutus* (SO) as compared with the piperidine derivative is remarkable (Fig. 11.18c).

In order to complete the determination of the potential biological activities of Steninae secretions against arthropods, equimolar amounts of Steninae

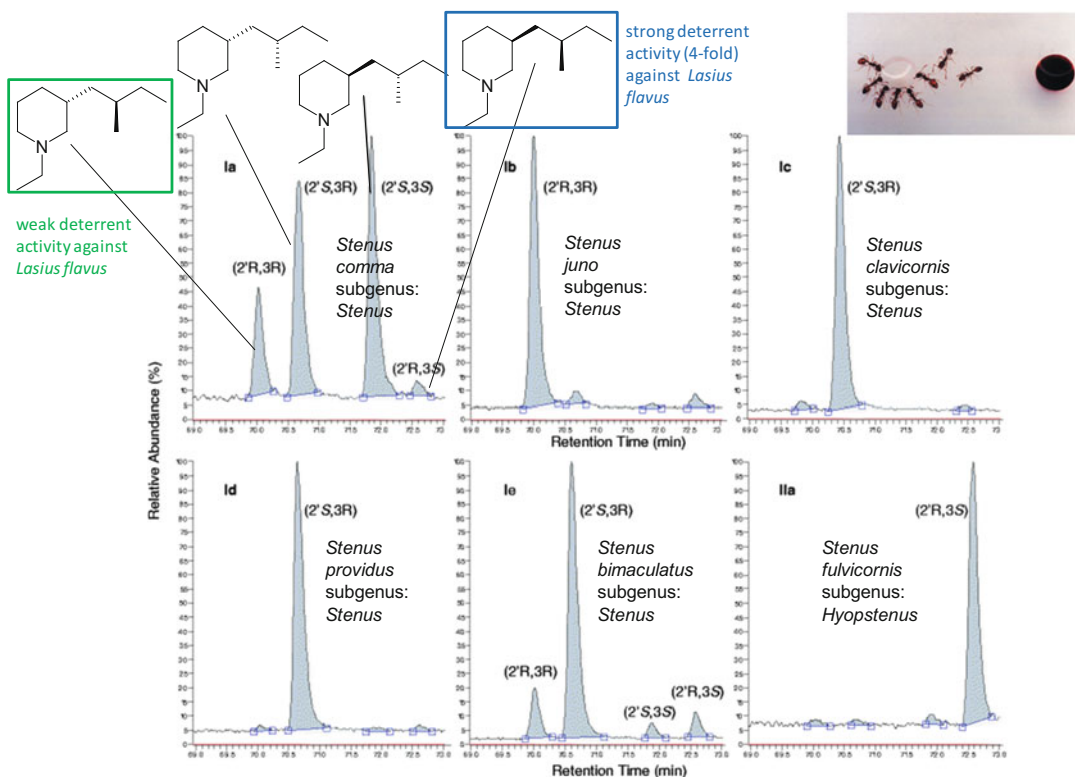


Fig. 11.17 Intrageneric differences of the four stenusine stereoisomers among *Stenus* beetles as shown by enantio-MD/GCMS of selected species of the subgenera *Stenus* and *Hypostenus* (Lusebrink et al. 2007). Concerning the enantiomers (2'R,3R) and (2'R,3S), the

last mentioned exhibits a stronger deterrent activity against *Lasius flavus* ants that were fed with sugar solutions or sugar solutions mixed with an enantiomer (right inlet figure; Schierling et al. 2013)

components were used in a scratch assay involving *Periplaneta americana* cockroaches. An immediate scratching by the hind legs was observed when the terpenes 1,8-cineol and α -pinene were applied (Fig. 11.19). In contrast, stenusine and piperidine-alkaloid were not so effective.

Deterrent activities of Steninae compounds have also been registered against vertebrates such as the fish species *Xiphophorus hellerii* by applying test pellets (Fig. 11.19). In particular, 1,8-cineol, α -pinene, stenusine and the piperidine derivatives show remarkable effects (Rupprecht 2011; Schierling et al. 2013).

Most of the tested Steninae compounds exhibit antimicrobial activities against bacteria (gram positive; gram negative) such as *Escherichia coli*, *Serratia entomophila* and *Bacillus sphaericus*, as determined by the

detection of inhibition zone diameters. As compared with the commercially available Tygacil stenusine, the pyridine derivative and α -pinene are significantly active (Lusebrink et al. 2008b).

Finally, a few Steninae compounds and especially α -pinene are also significantly able to inhibit the growth of *Saccharomyces cerevisiae* (Schierling et al. 2013).

Both bioassays (*S. cerevisiae*, three bacteria species) have revealed that synthetic mixtures of Steninae secretions exhibit similar effects to those of single-tested compounds, indicating that synergistic effects are not as important as compared with the defensive secretions of other staphylinids (Francke and Dettner 2005).

In order to interpret the intrageneric differences in the four stereoisomers of stenusine (Lusebrink et al. 2007), bioassays have been

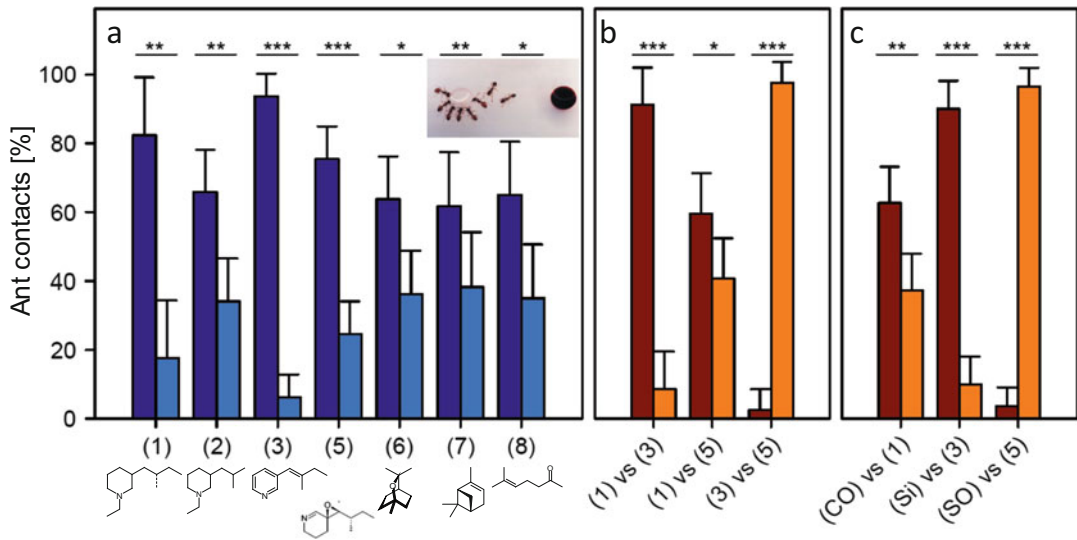


Fig. 11.18 Two-choice bioassays with *Lasius flavus* and synthetic *Stenus* pygidial gland secretion components. The diagrams show the distribution of ant contacts with the control and the test solutions including the standard deviation. (a) Test of the pygidial gland secretion components (light blue) versus controls (dark blue). (b) comparison of the deterencies of three of the main components of the secretion of *S. comma*, *S. similis* and *S. solutus*. (c) Comparison of the deterencies of one of the main components

of the secretion with naturally occurring secretions. **1** Stenusine, **2** norstenusine, **3** 3-(2-methyl-1-butenyl) pyridine, **4** cicindeloine, **5** 1,8-cineole, **6** α -pinene, **7** 6-methyl-5-hepten-2-one. **CO** Secretion of *S. comma*, **Si** secretion of *S. similis*, **SO** secretion of *S. solutus*. Concentration of compounds or secretion in the drops offered: (a) 100 μ g/ml, (b) 30 μ g/ml, (c) 80 μ g/ml (according to Schierling et al. 2013)

performed with two stereoisomers of stenusine. Stereoisomer 2'R,3S exhibits similar inhibition zones against *E. coli*, *S. entomophila* and *B. sphaericus* as stereoisomer 2'R,3R. In contrast, the deterrence of 2'R,3S is stronger than that of isomer 2'R,3R, when *Lasius flavus* ants are used as test organisms (see Fig. 11.17; Schierling et al. 2013).

In nature, Steninae beetles are confronted with several predacious and probably also parasitoid organisms that would not hesitate to attack the small beetles. The list of potential predators includes arthropods such as ants, spiders and carabid beetles and vertebrates such as fish, amphibians, birds and small mammals, all of which have to be deterred by the pygidial gland secretion if the beetles are to survive in their environment. Piperidine- and pyridine-derived alkaloid compounds and terpenes are known from the chemical defence systems of various arthropods (Ekpa et al. 1984; Deml and Dettner 1995; MacConnel et al. 1971; Blum 1981; Huth

and Dettner 1990; Stoeffler et al. 2007). The piperidine-, pyridine- and epoxy-piperidine-alkaloids, however, are restricted to the genera *Stenus* and “*Dianous*.” The distinct deterrent activity of the secretion of living Steninae on attacking ants has previously been demonstrated (Neumann 1993). Furthermore, the pure selected alkaloids and terpenes have been described as ant deterrents by Honda (1983) and Lusebrink et al. (2009). In our experiments, we have been able to confirm the previous observations and to extend these results with the newly available secretion compounds. Ants touching the compound solutions in our tests most often retreat from the milk drops without feeding. Additionally, these ants often exhibit distinct reactions, such as (1) panic movements, (2) mandible rubbing and (3) intensive cleaning that can be classified as typical rejection behaviour (Eisner et al. 1961; Dettner et al. 1996). Since all tested secretion constituents show significant deterrence against *L. flavus* (Fig. 11.18) according to the definition

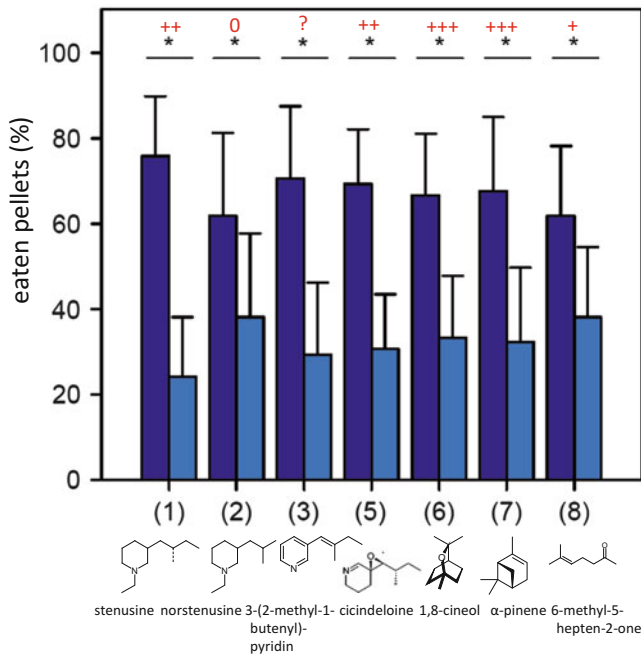


Fig. 11.19 Deterrent activities of synthetic compounds from anal glands of *Stenus* against fish *Xiphophorus hellerii* (Poeciliidae, two-choice-bioassays), as examined by using test pellets. The number of control (dark blue) and test pellets (light blue) are shown (Schierling

et al. 2013; Rupprecht 2011). In addition, note the preliminary results of the scratch assay with *Periplaneta americana* cockroaches (Question mark, not tested; zero, no reaction; plus, weak reaction; double plus, medium reaction; triple plus, strong reaction)

of Schoonhoven et al. (2005), the secretion is with certainty capable of protecting the beetles from attacks by arthropod predators. In our tests, we have applied solutions of maximal 100 µg compound per millilitre milk. The content of stenusine in the glands of a *Stenus comma* (syn. *S. bipunctatus*) beetle (Fig. 11.13) is only about 8,35 µg/beetle (Lusebrink et al. 2008b), but, if a predator attacks a *Stenus*, the beetle emits pure and undiluted secretion. If this secretion is applied to sensitive sensory organs such as mouthparts, the aggressor is deterred immediately. A comparison of the deterrence of naturally occurring secretions of *S. comma* and *S. similis* with the respective main alkaloidal components has revealed that the mixture is less active in both cases. This might be because of the lower proportion of the high-active compounds in the secretion mixture, since the same total amount of compound or mixture is used in both the tests with the pure compounds and those with

the secretion mixture. Hence, the highly active compounds are “diluted” by the less-active ones. The secretion of *S. solutus* turns out to be more active than its main component 4. The better activity of the secretion, however, is more probably attributed to the strong deterrence of 3, which is significantly higher than that of 4. A synergism or quasi-synergism effect of the compounds in nature, as reported for Oxytelinae (Staphylinidae) by Dettner (1987, 1993) and Francke and Dettner (2005), obviously does not exist in Steninae. In the context of the skimming locomotion of some Steninae on the water, a fish deterrence of the secretion seems to be a reasonable goal. Moving objects on the water surface are always attractive to fish, as confirmed by observations of gyridid beetles on fish-inhabited waters (Benfield 1972; Dettner 1985, 2014; Eisner and Aneshansley 2000). Although *X. hellerii* is not present in Central Europe (Frey 1973), *Stenus* species are also expected to

exist in the natural habitat of the tested fish (Hermann 2001; Puthz 1974). Thus, *X. hellerii* can be considered as an appropriate test organism for deterrent experiments with the secretion. Indeed, intact individuals of *S. providedus* offered to *X. hellerii* in early tests were always caught by the fish but were spat out immediately and never swallowed. Thus, unsurprisingly, the pure gland secretion compounds possess significant deterrent activities against the fish *X. hellerii* in experiments. The high concentration of the test compounds necessary for significant avoidance of the test pellets is attributable to the fact that only the amount of the compounds contained in the surface of the pellet can trigger an effect. The main part of the test compounds is embedded in the pellet and is not accessible to the fish. In contrast to the ant bioassays, the alkaloids 1, 3 and 5 do not reveal any different levels of deterrent activity in the fish bioassays. To defeat fish predators, the amount of emitted secretion seems to be more relevant than its composition. Steninae show distinct cleaning behaviour, whereby they take up secretion from their abdominal tip and subsequently dispense it over their entire body surface (Betz 1999). This behaviour, which is called “secretion grooming,” has previously been observed in several Hydradephaga and water bug families (Maschwitz 1967; Kovac and Maschwitz 1989, 1990). The secretion when spread over the body acts as an antimicrobial agent and prevents the beetles from infestation with microorganisms (Schildknecht and Weis 1962; Maschwitz 1967; Schildknecht 1970; Dettner 1985; Kovac and Maschwitz 1990). When kept at very high humidity over several weeks in the laboratory, *S. bimaculatus* beetles partly show a whitish microbial growth on the thorax and elytra, whereas the other parts of the body surface appear clean and glossy. Assuming an antimicrobial activity of the secretion, this observation can be explained on the basis that the thorax and elytra represent those body regions that are most difficult to reach by the legs of the beetle during secretion grooming and therefore are covered by an amount of secretion not sufficient for inhibiting microbial growth (Lusebrink et al.

2008b). Because the pygidial gland secretion of the Steninae has previously been reported as potentially antimicrobial (Dettner 1985, 1993; Betz 1999), Lusebrink et al. (2008b) tested the effects of selected piperidine-alkaloids on several microorganisms. In their bioassays, both tested compounds revealed pronounced antimicrobial activity comparable with that of commercially available antibiotics. Later we attempted to copy exactly the testing conditions we used previously, but we were never able to reproduce these impressive results unless we applied a 200-fold amount of substance, whereas the commercial antibiotic Tygacil (15 Ig) produced comparable inhibition zone diameters in both studies. The tendencies observed in Lusebrink et al. (2008b), however, could be reproduced, whereby stenusine revealed higher antibacterial activity than norstenusine. As with the tests for deterrents, the single secretion compounds showed better antimicrobial activity than the naturally occurring secretions. Thus, a synergistic effect can be excluded concerning the defence against microorganisms. Although the antimicrobial activity of the secretion was not as strong as that reported in the literature, it should have been capable of inhibiting infestation by bacteria and fungi, because it exists pure and undiluted on the body surface of the beetle. Furthermore, the high frequency of cleaning behaviour shown by the beetles indicates that the amount of antimicrobial compounds is sufficient to inhibit microbial growth on the body surface in nature (Betz 1999). The secretion of the Steninae contains various alkaloids that occur as different configurational isomers. The ratio of the four isomers of stenusine (Fig. 11.17) shows great interspecific differences but is always constant within individuals of one single species (Lusebrink 2007; Lusebrink et al. 2007). Accordingly, the ratio is adjusted by the beetles and is not attributable to random synthesis. Concerning the antimicrobial activity, no differences have been observed with the tested stereoisomers, but 2'R,3S was more effective than 2'R,3R in ant bioassays (Fig. 11.17). In *S. comma*, (20R,3S)-1 is only present in minor amounts, whereas it represents the main isomer of 1 in *S. similis*

(Lusebrink 2007; Lusebrink et al. 2007). However, to gain complete insight into the activity of stenusine in natural isomeric composition, further tests should be performed with the remaining isomers. Stereoselective biosynthesis of a compound requires a separate enzyme for each stereoisomer (Morgan 2010). Furthermore, the constancy in the occurrence of the single stereoisomers of stenusine in the secretion demands the exact regulation of every single enzyme involved in the synthesis. This raises the question of selective advantage, because the most active stereoisomer or a random mixture of all stereoisomers should be sufficient for both defence and skimming. Males and females of *S. bimaculatus* are able to perceive the isomeric mixture of alkaloid 1 as determined by electrophysiological tests (Schierling, unpublished results). Thus, a potential pheromone function of some secretion components should be kept in mind because, in this case, the absolute configuration of the compounds is of the highest relevance (Mori 1999; Morgan 2010). However, this possibility is for the moment, speculative, since no confirming experiments have been performed. Corresponding tests are in preparation.

11.6.4 Skimming Behaviour, Evolution of Secretion and Chemotaxonomic Significance

The most unusual function of the pygidial gland secretion in the Steninae is the locomotion on the water surface, called “skimming” (Piffard 1901; Billard and Bruyant 1905; Linsenmair and Jander 1963; Lang et al. 2012), a behaviour typical for many species of the genera *Stenus* and “*Dianous*.” The beetles are equipped with hydrophobic tarsi and are thus able to stand on the water surface. For locomotion, they touch the surface of the water with the tip of their abdomen and release a small amount of secretion by everting the pygidial glands. The secretion spreads immediately on the water surface, forming a monomolecular film and thus

propelling the beetles forward with considerable speed (Schildknecht 1970; Dettner 1991). The alkaloids (Fig. 11.13) presumably represent the main spreading agents for skimming, but some of the terpenes (Fig. 11.13) also show a distinct spreading activity (Schildknecht et al. 1976; Lang et al. 2012). The Steninae alkaloid components occur as various configurational isomers in the secretion. Although great interspecific differences occur in the stereoisomer ratio of stenusine, their composition is constant within different individuals of a single species (Lusebrink et al. 2007). This probably also applies to all other chiral *Stenus* alkaloids. All stereoisomers of stenusine should be equally effective as spreading agents, despite the slight differences in their biological activity (see above) such as deterrence. However, no information is available about a potential pheromone function of any *Stenus* secretion compound.

While hunting for springtails or other arthropods, the hydrophobic beetles might accidentally fall into water. Behaviour of spreading has evolved in these insects as a mechanism to save themselves from drowning and to escape predators such as water striders (Linsenmair 1963; Schildknecht et al. 1975). Piffard (1901) was the first to discover that some species of *Stenus* beetles are able to move over the water surface in a rapid and extraordinary manner. The beetle glides on the water at high velocity without using its legs. A few years later, Billard and Bruyant (1905) observed this locomotion in *Stenus tarsalis* and *S. cincinnatioides*. They report an emission of chemicals that interact with the water surface like a surfactant and propel the beetle rapidly forward comparable with a “soap” boat. This locomotion is driven by Marangoni propulsion (Scriven and Sterling 1960). Marangoni flows are those forced by surface tension gradients. Surfactants such as soap and the emitted substances of the beetles are molecules that find it energetically favourable to reside at the free surface and that act to decrease the local surface tension (Bush and Hu 2006). Jenkins (1960) investigated the spreading behaviour of *Stenus* and “*Dianous*” beetles. He named the spreading action of these two genera

“skimming.” Moreover, he observed the negative phototactic navigation of *Stenus* and “*Dianous*” on the water surface towards the dark bank of a pool. The beetles waste no time in gaining the safe waterside. Schildknecht et al. (1975) found, for the first time, that a secretion of the pygidial glands in the anal region is responsible for the typical skimming action. The gland compounds form a monomolecular film whose front pushes the beetle forward (Dettner 1991). By this kind of locomotion, *Stenus comma*, for example, can achieve a velocity of 0.75 ms^{-1} , and, if the secretion is continuous, a distance up to 15 m can be covered (Linsenmair and Jander 1963). Apart from *Stenus* and “*Dianous*” beetles, such extraordinary movement is only shown by the water cricket *Velia caprai* Tamanini, which uses its rostrum to spread active saliva for skimming (Linsenmair and Jander 1963). No other animal is known to have this unique kind of locomotion. The driving force for skimming is the spreading potential of the gland compounds. Thereby, the gland compounds must be barely soluble in water; otherwise, they could not form a monolayer. Additionally, the spreading agent has to exhibit a lower surface tension than the layer-carrying substance, which is water in case of *Stenus* and “*Dianous*” (Adamson and Gast 1997). The spreading pressures of the compounds can be measured experimentally, for instance, at an interfacial tensiometer (Schildknecht et al. 1976). After the measurement of the surface tension σ of the compounds against air and the interfacial tension γ against water, the spreading pressure P can be calculated according to the following equation defined by Wolf (1957):

$$P = \sigma_{\text{water}} - (\sigma_{\text{substance}} + \gamma_{\text{substance/water}})$$

Spreading action can only be observed if the difference of σ_{water} and $(\sigma_{\text{substance}} + \gamma_{\text{substance/water}})$ is positive, i.e. work is obtained (Wolf 1957). Otherwise, the applied liquid remains as a lens on the water surface. Although almost all Steninae and representatives of “*Dianous*” contain the spreading-active stenusine in their pygidial glands, not every species exhibits skimming behaviour (Jenkins 1960; Linsenmair 1963;

Dettner 1991). The ability to skim is assumed to be linked to the diverse kinds of habitats that are colonized by *Stenus* (Jenkins 1960).

As shown in Fig. 11.20 for ten Steninae species, the skimming behaviour is species-specific and can be characterized by variable features. Whereas some species can skim voluntarily and show a typical linear skimming behaviour with a continuous depletion of secretion (Fig. 11.20), other species skim not always voluntarily and are characterized by a non-linear, partly circular skimming, stepwise probably because of interrupted discontinuous secretion release. Some species exhibit no skimming behaviour at all, whereas other species have to be forced to skim in order to obtain usable values.

Apart from ethological investigations of skimming Steninae beetles, the physicochemical properties regarding the spreading potential of single secretion compounds and mixtures have also been addressed (Lang et al. 2012). An aqueous oversaturated solution was assembled for measurements by a drop volume tensiometer. Differences of spreading pressures between the substances could be revealed, as shown for the pygidial gland secretion of *S. comma* (Fig. 11.20). The monoterpeneoid α -pinene is characterized by the highest spreading pressure followed by 3-(2-methyl-1-butenyl) pyridine (22.22 mN m^{-1} ; not present in *S. comma*), norstenusine, stenusine and 6-methyl-5-hepten-2-one (15.63 mN m^{-1} ; not present in *S. comma*). Cicindeloinine (4.27 mN m^{-1} ; not present in *S. comma*) and the monoterpeneoid 1,8-cineol (Fig. 11.20) are the least surface active. With respect to the first compound, the low value may be attributable both to its extremely low water solubility and its resinous consistency. For the investigation of naturally identical secretion compositions by the tensiometer, four beetle species were chosen based on their gland content (Lang et al. 2012). The species typify representatives of the different *Stenus* groups: *S. clavicornis*, a species that exhibits no skimming behaviour, is characterized by a stenusine-based secretion composition (the so-called piperidine group; see below) and a spreading pressure of 20.67 mN m^{-1} . The second

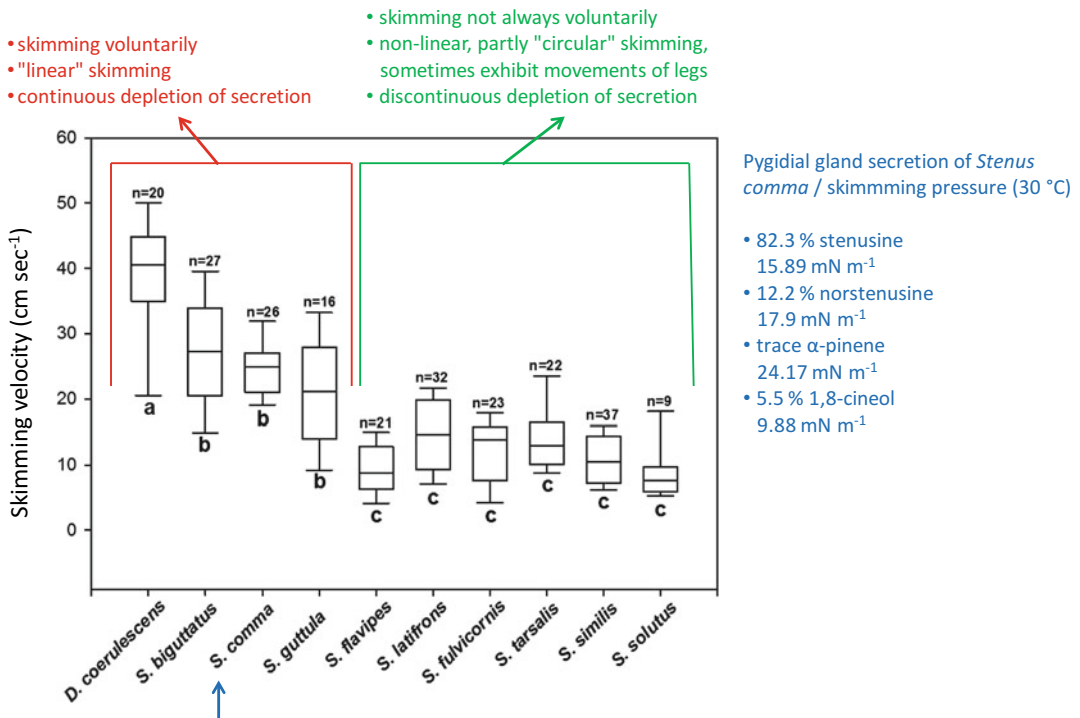


Fig. 11.20 Skimming velocities (cm sec⁻¹) of nine studied *Stenus* species and *Dianous coeruleascens*. The boxplots are shown with the median and corresponding standard deviation bars. *D. coeruleascens* showed the highest spreading rate, followed by *S. biguttatus*, *S. comma* and *S. guttula*. The spreading behaviour of the analysed remaining species (*S. flavipes* to *S. solutus*) cannot be significantly differentiated statistically. The lower-case letters a, b and c indicate significant differences based on calculated *p*-values. Number of specimens

investigated is indicated by *n*. Orange-bracketed species skim voluntarily and show a linear skimming and a continuous depletion of secretions. Green-bracketed species do not always skim voluntarily; show a non-linear, partly circular spreading; and sometimes exhibit movements of their legs. These species are characterized by discontinuous secretion depletions. Right inset: percentual quantitative secretion constituents from the anal glands of *Stenus comma* (arrow) are indicated together with spreading pressures of the single compounds (Lang et al. 2012)

species chosen, *S. comma*, also belongs to the piperidine group and skims voluntarily with high velocities and 19.71 mN m⁻¹. *S. similis* from the so-called pyridine group is characterized by discontinuous secretion release and non-linear skimming (Fig. 11.20) but, nevertheless, has a secretion with a spreading pressure of 20.97 mN m⁻¹. These data illustrate that skimming behaviour is not positively correlated with the spreading pressure of species-specific formulations. In contrast, in *S. solutus*, skimming actions can only rarely be observed, i.e. the beetles skim always involuntarily and very slowly by using their legs (Fig. 11.20). In the same way, the secretion has a low spreading pressure of 11.19 mN m⁻¹.

As a whole, all Steninae species hitherto investigated chemically possess spreading-active substances in their pygidial glands, but not every *Stenus* species actually shows skimming behaviour. As Steninae inhabit interstices (Horion 1963) and banks of open waters (Dettner 1987), the disposition of the beetles in displaying skimming behaviour might be linked to the different habitat claims of the species. *D. coeruleascens*, which is characterized by the significant highest skimming velocity in this study (Fig. 11.20) inhabits the immediate neighbourhood of waterfalls, weirs and fast-flowing mountain streams with declines (Horion 1963). Therefore, for the beetles to survive, they need to be able to skim rapidly in the fast-moving

waters. Consequently, these beetles exhibit distinct skimming behaviour and velocities. Other *Stenus* species, which always display a skimming disposition and high skimming velocities, such as *S. comma* and *S. biguttatus*, can be found in habitats close to the banks of stagnant open waters (Horion 1963). These biotopes also require voluntary and persistent skimming action. Several other *Stenus* species investigated (Fig. 11.20) are characterized by a proportionally slow skimming velocity and a less disposition to skim, such as *S. flavipes* (*Metatesnus*) and representatives of the subgenus *Hypostenus* such as *S. latifrons*. Other species such as *S. fulvicornis*, *S. tarsalis*, *S. similis* and *S. solutus* can be predominantly found in swampy biotopes and wet places within woods, meadows and marshes. If these beetles live in wet places near open waters, they stay in rotten leaves and other detritus material or in places abundantly covered with vegetation (Horion 1963). Therefore, the beetles rarely accidentally fall into open water, and the occasion to skim is uncommon. This might be a reason for the beetles not having developed a distinctive skimming behaviour such as the investigated species *D. coeruleascens*, *S. comma* and *S. biguttatus*. Since many Steninae predominantly prefer moist habitats, the danger of infestation by microorganisms is increased. In order to avoid this infestation, *Stenus* displays secretion grooming (Kovac and Maschwitz 1990; Betz 1999) in which the beetle spreads its antimicrobial secretion of the defensive pygidial glands over the entire body surface. *Stenus* beetles living in interstices in plant debris or detritus might primarily use their pygidial gland secretion for secretion grooming, instead of using it for skimming action. Moreover, another aspect seems to be of importance. In 1960, Jenkins reported, in the context of his ethological studies on "*Dianous*" and *Stenus* species, a negative phototactic skimming behaviour to the dark bank of a pool. Once fallen on the water surface, the beetles at once orient themselves into the "correct" direction and waste no time in gaining the safety of the bank by skimming. These results are supported by the observations of Linsenmair

(1963). As *Stenus* beetles are equipped with extraordinary large eyes in relation to their body size and compared with related staphylinid beetles, the visual sense is evidently essential for their orientation. Furthermore, the beetles not only might orient themselves in the correct direction but also might estimate the distance to the bank. The beetles might be able to balance the distance to the safe bank against the amount of secretion in their glands. Only in exceptional circumstances, do the beetles "decide" to use their precious secretion, e.g. complete physical exhaustion or repeated attacks by predators (Linsenmair 1963). These circumstances might be the reason that skimming velocities of 40–75 cm s⁻¹ and a covered distance of up to 15 m (Linsenmair and Jander 1963 for *S. comma*) have usually not been achieved by the beetles in our studies. In summary, tested *Stenus* species from vegetation-rich habitats show less precise skimming behaviour than Steninae from open habitats possibly because of the lower optical capacity of the former.

The chemical gland content of the different Steninae species and their relationship allows a chemosystematic arrangement to be developed (Francke and Dettner 2005). In the case of Steninae whose gland secretion consists predominantly of stenusine, norstenusine and minor terpenoids, the species can be regarded as phylogenetically basal ("piperidine group" according to Schierling et al. 2012). These species, such as *S. clavicornis* and *S. comma*, use their piperidine-alkaloids mainly for the optionally shown skimming behaviour and as predator avoidance (Connert 1974; Dettner et al. 1996; Hesse 2000). Furthermore, the terpenoids from the small glands are pharmacologically active substances and are used for antimicrobial protection against bacteria and fungi (Schildknecht et al. 1976) and as an insect repellent (Honda 1983; Blum 1981).

The phylogenetically more derived *Stenus* species in this chemotaxonomically based study, as represented by *S. similis* ("pyridine group" according to Schierling et al. 2012), still possess stenusine as the main component in their glands, but the "new" pyridine-alkaloid also comprises a large part of the secretion. Not only

is the spreading pressure of the pyridine higher than the pressure of stenusine, but also the antimicrobial effect is higher against *Bacillus sphaericus*, *Escherichia coli* and *Serratia entomophila* (Lusebrink et al. 2009).

The next phylogenetic level in this chemotaxonomical study is represented by *S. solutus* (“epoxy piperidine group” according to Schierling et al. 2012), which also includes *S. binotatus* and *S. cicindeloides*. These beetles contain remarkable amounts neither of piperidine-alkaloids nor of terpenes but produce the pyridine- and the piperidine-alkaloids. Although the fluid pyridine might be used to gain at least a minor spreading potential together with deterrence against ants together with bactericidal and fungicidal effects, the role of the resinous piperidine cicindeloin cannot be assessed.

In summary, the secretion composition of *Stenus* is subject to evolutionary optimization. From phylogenetically plesiotypic to derived taxa, new alkaloids in the large gland were gradually developed, whereas the small gland containing the terpenes was gradually reduced. The alkaloids were biosynthetically produced from the amino acids L-lysine and L-isoleucine. This observed evolutionary trend in Steninae contrasts with defensive glands of adults of other Staphylinidae such as the Oxytelinae (Francke and Dettner 2005) or Aleocharinae (Steidle and Dettner 1990), where one or a few active principles such as solid quinones are dissolved within different solvents and solvent mixtures. During the evolution of the Oxytelinae or Aleocharinae, these formulations have been optimized in order to increase the insecticidal activities of the glandular mixtures. Another difference exists between the secretions of the Steninae and Oxytelinae/Aleocharinae. In the Steninae, no synergistic or quasi-synergistic effects have ever been observed between the single compounds within the secretion as seen in the Oxytelinae/Aleocharinae (Francke and Dettner 2005). Moreover, all Steninae secretion components represent biologically active natural compounds, whereas secretions from the two other subfamilies contain many inactive solvents. Thus, the *Stenus* secretion meets many different

demands. The spreading potential of the gland compounds is essential not only as a locomotory or escape mechanism but also because of the antimicrobial and protective mixture that can be distributed over the body surface of the beetle while secretion grooming. Whereas the defensive secretions of other insects also serve as surfactants and are able to spread [e.g. the oral secretions regurgitated by *Spodoptera exigua* (Lepidoptera; Rostás and Blassmann 2009) and the secretion of whirligig beetles (Coleoptera, Gyrinidae; Vulinec 1987)], no other insect has developed such striking skimming behaviour. Although extraordinary skimming is a secondary effect of the multifunctional *Stenus* secretion that primarily was evolved for chemical defence, it is essential for the beetles' survival and helps both *Stenus* and “*Dianous*” beetles to be optimally adapted to their various habitats and the challenges of life among staphylinids.

Based on three genes (COI, 16S rRNA and Histone), we have obtained further and comprehensive insights into the molecular phylogeny of the subfamily of Steninae (Fig. 11.21), traditionally comprising the genera *Stenus* and “*Dianous*”. These results indicate that “*Dianous*” belongs to *Stenus* (Koerner et al. 2013; Lang et al. 2015) (see Sect. 11.2). Furthermore, we have shown that our chemotaxonomic approach presented previously by Schierling et al. (2013) is consistent with the obtained molecular data. The piperidine group is the most extensive species group listed. Examples are presented in Fig. 11.21 in red. The next chemotaxonomic level is represented by the pyridine group members *S. similis* and *S. tarsalis* possessing the chemotaxonomic-derived gland compound 3-(2-methyl-1-butenyl) pyridine in their pygidial glands (Schierling et al. 2013; yellow colour). Although these two species cannot be found in the same clade, they are part of a major cluster comprising the species *S. bifoveolatus*, *S. binotatus* and *S. solutus*. Interestingly, the basal piperidine group member, *S. bifoveolatus* (*Metatesnus*), belongs to this cluster, which contains chemotaxonomic-derived species. In this case, the subgenus concept has also not been maintained: *Hypostenus* species (*S. similis*,

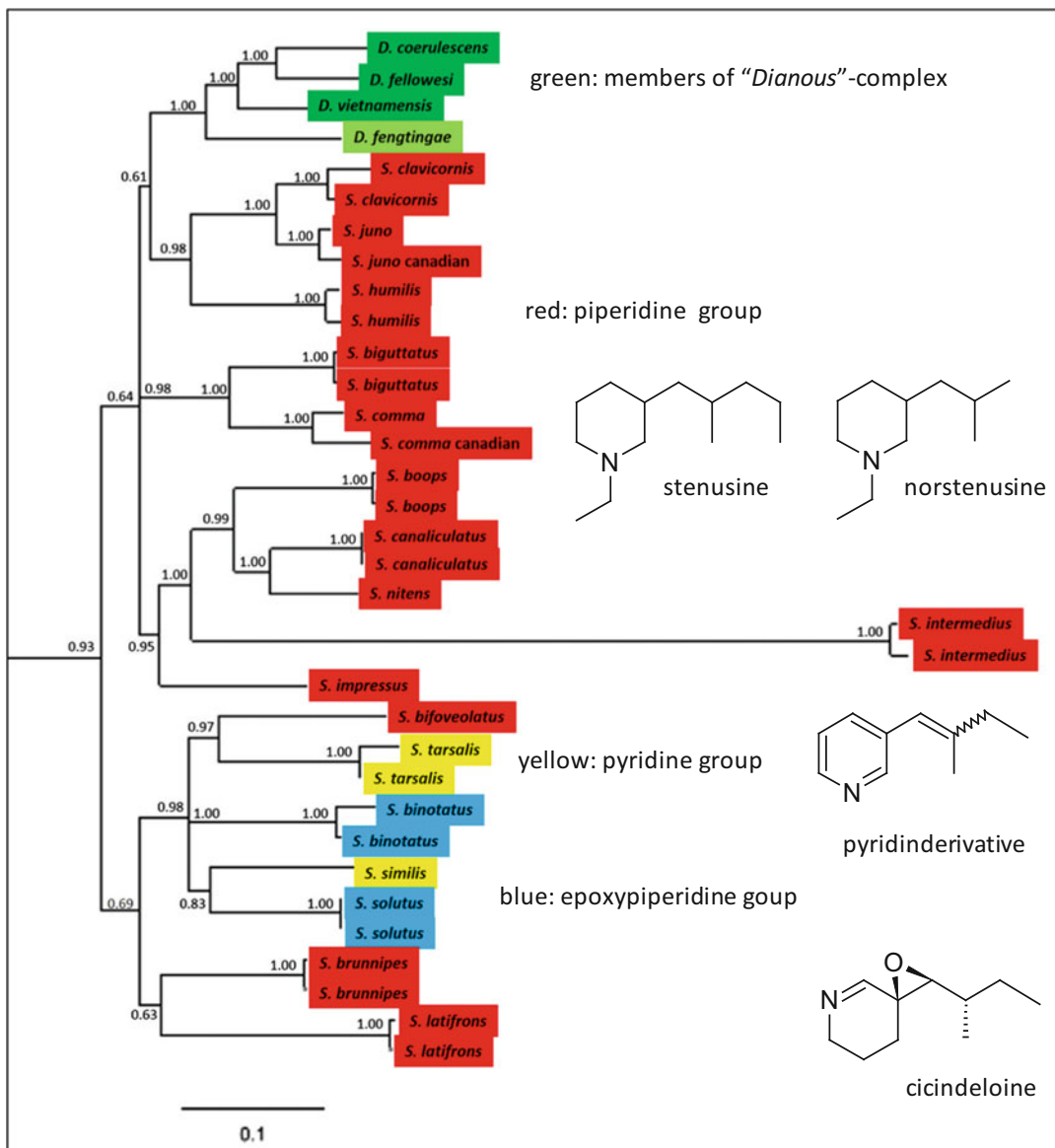


Fig. 11.21 Maximum posterior (MAP) tree for the genera *Stenus* and "*Dianous*" resulting from Bayesian analysis (Ronquist and Huelsenbeck 2003; Lang et al. 2015). The numbers above the branches indicate Bayesian posterior probabilities ≥ 0.50 . The molecular data area is

based on cytochrome oxidase I (COI), 16S rRNA and the histone H3-gene. Members of chemotaxonomic groups according to Schierling et al. (2013), Puthz (1981, 2000b, 2005a), Shi and Zhou (2011) and Tang et al. (2011) (*Stenus*, red, yellow, blue; "*Dianous*," green)

S. tarsalis and *S. solutus*) cluster together with *Metatesnus* species (*S. bifoveolatus* and *S. binotatus*).

The most evolved chemotaxonomic level is represented by the epoxypiperidine group comprising *S. binotatus* and *S. solutus* in our

study. Species of this group are characterized by an extension of the pygidial gland repertoire to the new alkaloid cicindeloin as the main gland constituent (Schierling et al. 2013). Piperidine-alkaloids such as stenusine and norstenusine are completely replaced by

pyridine- and epoxy-piperidine-alkaloids. In the tree constructed (Fig. 11.21), *S. solutus* is positioned in the direct neighbourhood of the pyridine group member *S. similis*, which also possesses the chemotaxonomically derived pyridine-alkaloid but lacks the most evolved piperidine epoxide in its glands. With regard to the species groups of Puthz (2008), the *S. similis* group consisting of *S. similis* and *S. solutus* is supported (BPP = 0.83) in the present chemotaxonomic classification. The other epoxy-piperidine group member *S. binotatus* occupies an isolated position regarding *S. solutus* within a major cluster of both chemotaxonomically derived and most evolved species. However, all *Stenus* species possessing chemotaxonomically evolved gland compounds show a close relationship within the tree constructed.

In Fig. 11.21, the various chemotaxonomic groups are highlighted in colour [red, piperidine group (primitive); yellow, pyridine group (derived); blue, epoxy-piperidine group (most derived)]. However, future investigations of a wider range of *Stenus* and “*Dianous*” species will be necessary to reveal fully the chemotaxonomy and molecular phylogeny of Steninae.

11.7 Ecomorphological Diversity

When morphological features that form direct interfaces to the environment are considered, major interspecific differences occur with respect to the compound eyes, the labial prey-capture apparatus, the legs including the tarsi and the structure of the abdominal tergites. In order to attain a better understanding of the evolution of ecomorphs across the Steninae, we need to understand the functional and ecological consequences of these different morphologies.

11.7.1 Compound Eyes

Steninae beetles have large protruding eyes that are typical for visual hunters among diurnal predatory beetles (e.g. Bauer et al. 1998). Such eyes

favour peripheral vision and may be associated with an array of ommatidia improving the resolution in the frontal field of view (e.g. Burkhardt and de la Motte 1983). Although all Steninae seem to belong to this type, species-specific differences exist that render functional consequences. Although interommatidial angles could not be measured to reconstruct their visual fields, simple comparative measures of the eyes of 18 *Stenus* species correlated well with behavioural features in the study of Betz (1998a). Interspecific differences in approach behaviour and reaction ability in response to unpredictably moving springtails in standardized behavioural experiments were, to some extent, attributable to differences in eye morphology, such as the total number of ommatidia (indicative of the ability to resolve fine details and to detect moving objects at a distance) and the extent of the lateral projection of the eyes (usually corresponding to wide frontal acute zones in predatory insects). Narrow acute zones are unsuited for following fast, unpredictably moving prey, since these are easily lost from the zone (Horridge 1978). Ripicolous species such as *S. comma*, *S. biguttatus*, *S. fossulatus* and *S. guttula* with widely protruding eyes that are composed of many ommatidia are agile and highly responsive (Fig. 11.22a), whereas the representatives of many species mounting the vegetation or inhabiting plant debris are not or behave intermediately (Fig. 11.22b). Their eyes are flatter and consist of fewer ommatidia (Betz 1998a, b).

The “*Dianous*” species also exhibit notable differences in the eye size, all of which have fed into the definition of the species groups (Puthz 2015, 2016).

11.7.2 Labial Prey-Capture Apparatus

The elongate labia of the various *Stenus* beetles differ with respect to their length and the morphology of the sticky cushions (i.e. the paraglossae) at the tip of the prementum. In 220 measured *Stenus* species, the labium length differed between ca. 0.4 mm (*S. canaliculatus*) and 3.4 mm (*S. bilunatus*). Although the absolute

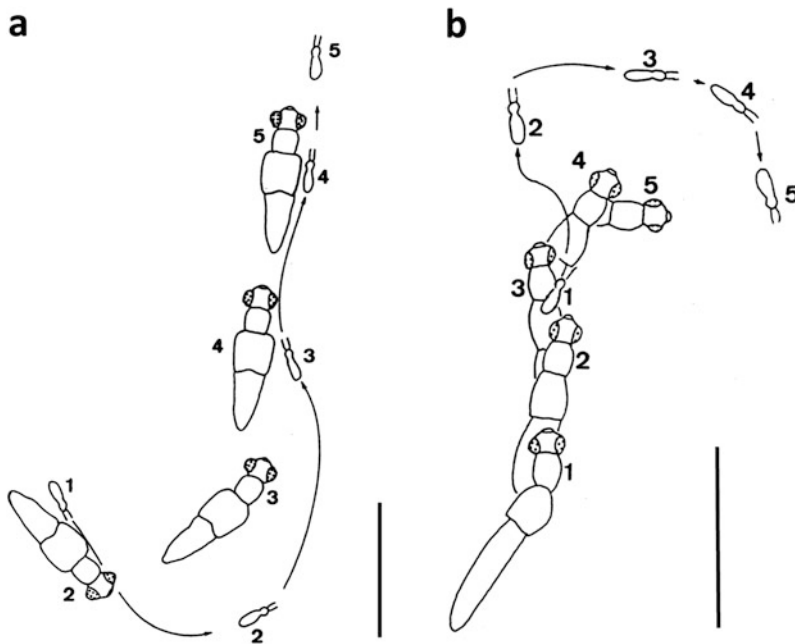


Fig. 11.22 Two opposed forms of optically controlled approaches towards a springtail moving on a plain surface of plaster of Paris. Sequence drawn from video footage. Identical numbers at prey and predator indicate simultaneous constellations. **(a)** *Stenus comma*: time course of the depicted sequence [seconds that lapsed from the start (= picture 1); the first number refers to the prey, the second one to the predator], (1) 0; (2) 1/0; (3) 3/0; (4) 5/5; (5) 8/5. The behaviour of the beetle is characterized

by a high locomotion speed and a barely delayed orientation towards the prey. **(b)** *Stenus pubescens*: time course of the depicted sequence [seconds that lapsed from the start (= picture 1); the first number refers to the prey, the second one to the predator], (1) 0; (2) 1/0; (3) 3/0; (4) 5/8; (5) 9/8. The behaviour of the beetle is characterized by a low locomotion speed and a temporally delayed responsiveness towards the prey. Scale bars = 5 mm

labium length (i.e. the prementum plus the membranous connecting tube) correlates with body length, the comparison of the relative labium lengths (i.e. the labium length divided by the pronotum length) shows that, in some groups of species, the labia have become especially elongate independently of body size, whereas in others, they have become largely shortened (Fig. 11.23). In the members of the *S. canaliculatus* group, the shortened labium length is the result of a secondary reduction of the labium (Betz 1996, 1998b, 2006; Koerner et al. 2013). Large species generally have longer labia and attain higher attack distances than small ones. Since 10–30% of the attack distance is overcome by a forward strike of the entire beetle, the relationship between labium length and attack distance becomes even stronger, when one considers the difference between the

attack distance and the length of the forward strike instead of the pure attack distance (Betz 1996). Variations of the attack distance set by the beetles prior to the predatory strike are probably responsible for interspecific differences in the compressive forces exerted by the labium (Betz 1996, 1998a; Koerner et al. 2012a, b, 2017). Differences in the haemolymph pressure generated to eject the labium for prey-capture might also be responsible for variations in the compressive forces.

An interspecific morphological comparison of the sticky cushions (paraglossae) of the labium of >200 *Stenus* species has established that, in most of the investigated species, they represent a general type (i.e. the ventral surface of the sticky cushions has the shape of an ellipsoid; Fig. 11.24a) that has apparently been modified in different ways during the course of evolution

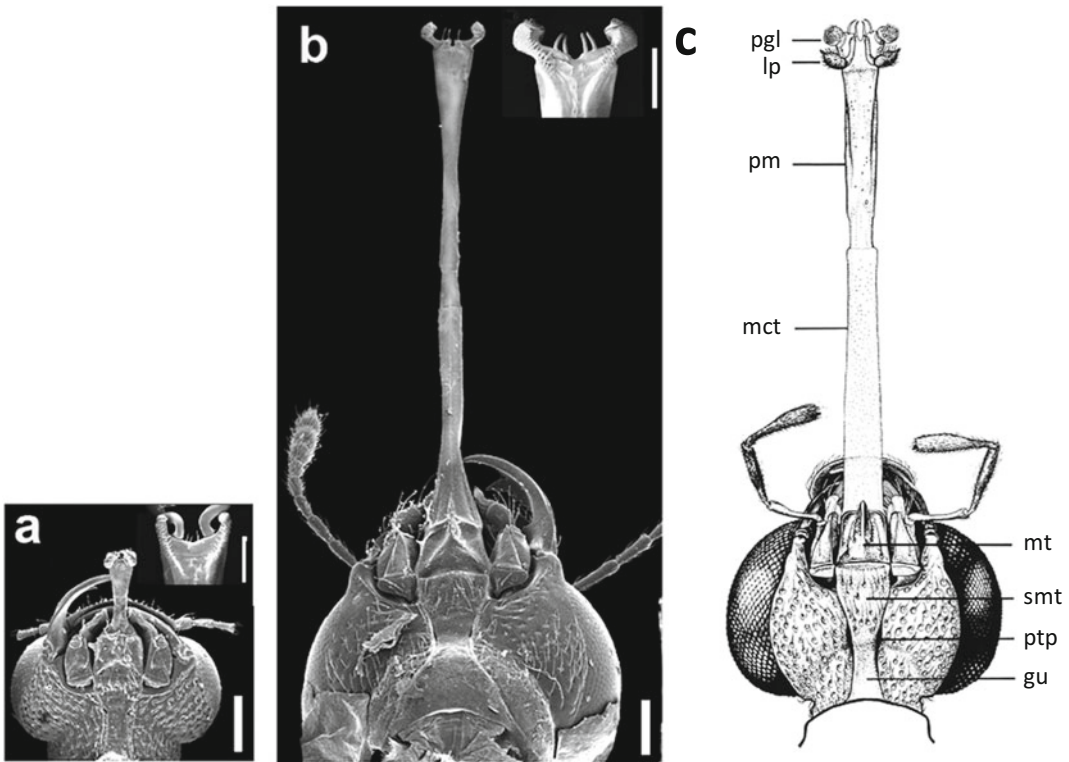


Fig. 11.23 Ventral aspects of the protruded labium in (a) *S. canaliculatus* and (b) *S. comma*. *S. canaliculatus* beetles show a largely shortened labium with vestigialized sticky cushions. Scale bars = 200 μm . Dorsal aspects of the tip of the prementum are shown as insets (scale bar (a), 50 μm ; scale bar (b), 100 μm). In (b),

the labial palps were removed to expose the paraglossae (c) ventral aspect of the head with protruded labium of *S. comma*. From Weinreich (1968). Abbreviations: *gu* gula, *lp* labial palpus, *mct* membranous connecting tube, *mt* mentum, *pgl* paraglossa, *pm* prementum, *ptp* posterior tentorial pit, *smt* submentum

(Betz 1996). These changes primarily concern the number of adhesive trichomes on a cushion, the degree of branching of a single adhesive seta and the manner in which both modes of alteration are combined. Out of the 220 *Stenus* species measured, the area of the sticky pads ranged from 181 μm^2 in *S. canaliculatus* to 10.760 μm^2 in *S. bimaculatus* (L.K. & O.B., unpublished data). As shown experimentally, larger sticky cushions and higher numbers of adhesive trichomes or adhesive contacts improve adhesion and thus lead to enhanced capture success. Hence, the morphology of the sticky cushions is thought to have been exposed to a high selective force that led to an improvement of their selective advantage (Betz 1996). In this regard, the modifications of the sticky cushions in some *Stenus* species (Fig. 11.24) are probably the result of a progressive selection that led to an extension of the

feeding niche by an increase of the maximum prey size or that is connected to a particular suite of potential prey species. For example, the paraglossae of some species within the subgenera *Hypostenus* (*S. latifrons*, *S. fulvicornis*) and *Hemistenus* (*S. persicus*, *S. turk*, *S. parcior*, *S. glacialis*) are considerably elongated (longiform paraglossae, Figs. 11.24b, c), and, hence, a remarkable enlargement of their surface area is attained. Extreme modifications of the paraglossae are also shown by some Asian species with coniform paraglossae [e.g. *S. ananda*, *S. stigmatias* (Fig. 11.24d), *Stenus flammeus* group; Betz 1996; Puthz 1998]; the sticky cushions of these species largely extend in a dorsoventral direction, with continuous narrowing. Again, the surface of the sticky cushions is considerably enlarged. It is also entirely covered by adhesive trichomes (ca 600 per sticky cushion),

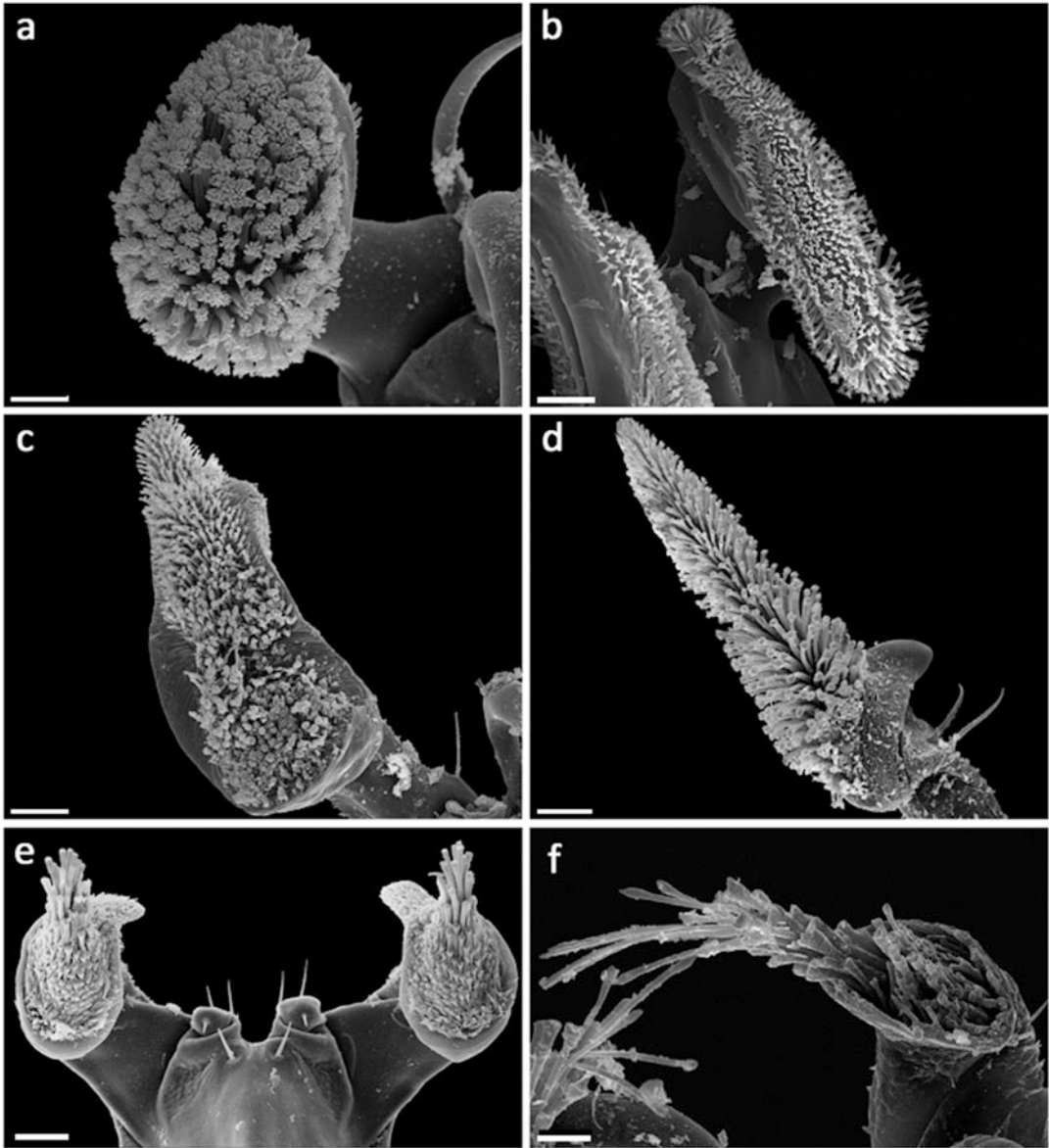


Fig. 11.24 The various paraglossa types as hitherto established in *Stenus* spp. (a) Ellipsoid (*S. biguttatus*). Scale bar = 10 μm . (b, c) Longiform (*S. fulvicornis*, *S. persicus*). Scale bar = 20 μm . (d) Coniform

(*S. stigmaticus*). Scale bar = 20 μm . (e) Actiniform (*S. alpaca*). Scale bar = 20 μm . (f) Sileniform (*S. nepalensis*). Scale bar = 10 μm

some being elongated. Some Middle and South American species [e.g. *S. emily*, *S. electriger*, *S. alpaca* (Fig. 11.24e); Puthz 2005b] have actiniform, and some Asian species have sileniform [e.g. *S. pilicornis*, *S. nepalensis* (Fig. 11.24f); Puthz 2013] paraglossae; both these types are characterized by very long

adhesive trichomes that become longer towards the middle of the sticky pad.

In contrast, some closely related species within the *S. canaliculatus* species group (e.g. *S. canaliculatus*, *S. nitens*, *S. labilis*, *S. caseyi*, *S. dolosus*, *S. vinnulus*) are provided with comparatively simple short labia [i.e. the

labium length ranging from 0.4 mm in *S. canaliculatus* (Fig. 11.23) to 0.9 mm in *S. vinnulus*] that have only very small paraglossae without modified trichomes (i.e. the surface area ranging from $181 \mu\text{m}^2$ in *S. canaliculatus* to $640 \mu\text{m}^2$ in *S. labilis*). Ryvkin (2012) suggested that the reduction of the labium is a characteristic feature of all the members of the *Stenus canaliculatus* group, but this must be verified by further investigations. Molecular, anatomical and ethological findings indicate that these simple labia are vestigial (Betz 1996, 1998b, 2006; Koerner et al. 2013). The development of the presumably vestigial labia of these species might result from a specialization of the beetles involving prey-capture with their mandibles (i.e. these species have comparatively long sabre-like mandibles, which are appropriate for catching large prey).

Species-specific differences have been found in the adhesion of the labia and are thought to result from divergencies in the sticky cushions, especially the surface area of their adhesive surface and the number of adhesive trichomes or adhesive contacts. The influence of the morphology of the sticky cushions on adhesive performance during the predatory strike has been measured in 14 Central European *Stenus* by using micro-force sensors (Koerner et al. 2017). The measurements have revealed strong interspecific differences in the adhesive forces generated during the predatory attack; these differences vary from 0.3 mN in *S. morio* to 1.1 mN in *S. bimaculatus*.

The variations in the adhesive performance are functionally correlated with the morphology of the sticky cushions, i.e. their surface area (Fig. 11.25b) and their number of adhesive

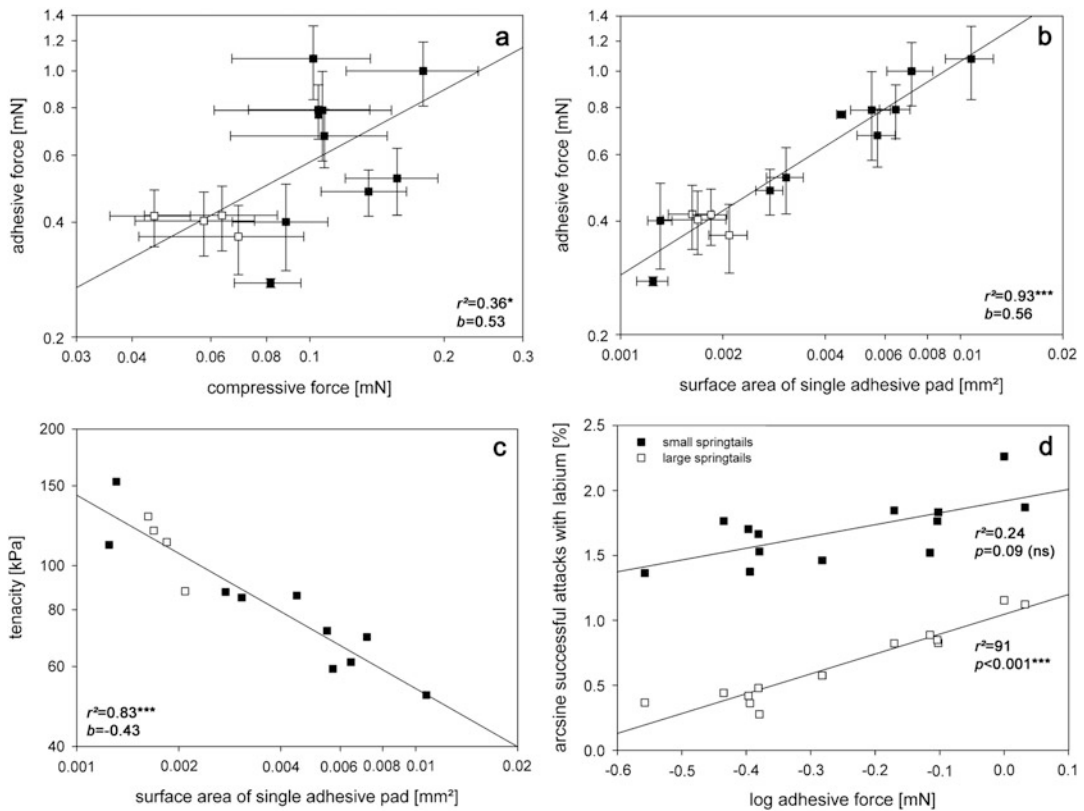


Fig. 11.25 Relationships between morphological and performance parameters of the prey-capture apparatus of 14 *Stenus* species (log-log plots). Trend lines were computed by using phylogenetic regression (PGLS) for gradual model of character evolution (see Koerner et al.

2017). Filled squares in figures (a)–(c) represent inhabitants of moist humus or plant debris near the ground, whereas open squares represent “surface runners” on bare ground [Adapted from Koerner et al. (2017). With kind permission from Oxford University Press]

outgrowths, and divergences in the compressive (impact) forces generated during the predatory strike, ranging from 0.05 mN in *S. biguttatus* to 0.2 mN in *S. juno*. These experiments strongly suggest that the involved adhesive mechanism is pressure-sensitive, i.e. that higher compressive forces result in better adhesive performances (Fig. 11.25a).

The differences in the adhesive forces significantly influence the prey-capture success towards large springtails of *Heteromurus nitidus* (Fig. 11.25d), whereas this relationship is significant only at a 0.1 significance level for small springtails of this species (Fig. 11.25d). Thus, higher adhesive forces lead to higher prey-capture success rates in *Stenus* beetles; this effect is more pronounced towards large-sized springtails (Koerner et al. 2017).

The mean tenacity, as calculated by dividing the mean adhesive force by the mean surface areas of both adhesive pads, amounted to between 51.9 kPa in *S. bimaculatus* and 153.2 kPa in *S. humilis*. Allometric analyses revealed that the tenacity shows negative correlations with the surface area of the adhesive pad and the body size (Fig. 11.25c). Thus, although species with smaller adhesive pads (which usually correspond to smaller body sizes) generated lower absolute values of the adhesive forces, they attained higher tenacities and therefore had more adhesively efficient pads. These negative relationships might mean that the action of the adhesive secretion alone plays a more important role than the size and the morphological complexity of the adhesive pads, i.e. the adhesive strength of the secretion probably overrides the adhesive impact of the pad size and the intercorrelated number of adhesive contacts. This would represent an important functional feature, especially for species with smaller adhesive pads, since it enables them to achieve a relatively high adhesive performance.

11.7.3 Legs Including the Tarsi

Apart from a slight positive allometry of the leg length with respect to the pronotum length, some *Stenus* species (mostly riparian surface runners

on bare ground) show especially elongate slender legs (Betz 1994). Elongate legs usually increase the possibility to effectively and rapidly accelerate/decelerate and improve maneuverability.

The tarsi of all three leg pairs are composed of five tarsomeres. Across the Steninae, a striking diversity of tarsal morphologies exists that manifests itself in (1) the general shape of the single tarsomeres (these are slender non-bilobed, sub-bilobed or wide bilobed³) (Fig. 11.26) and (2) the diversity of morphological types of tenent setae that cover their ventral surface (e.g. Puthz 1971; Betz 1998b, 2000, 2002, 2003, 2006; Puthz 2016).

Wide bilobed tarsi within several species groups are associated with a considerable augmentation of tarsal ventral setae (Betz 2003). About 70% of *Stenus* species and 36% of “*Dianous*” species show bilobed tarsi, whereby these numbers also include species with only weakly bilobed tarsi (cf. Puthz 2016). Contact angle measurements at the underside of the tarsi have revealed that all the investigated species are well supported by the surface of water while walking on it. In this context, the non-wettability of the underside of the tarsi and their total circumference play a major role and not whether they are bilobed or not. At least in *Stenus*, wide bilobed tarsi might have evolved in the context of expanding the adaptive zone towards living plants as hunting sites. Indeed, comparative measurements of the pulling forces attained on vertical substrates have demonstrated that widened bilobed tarsi exhibit a significantly higher adhesive performance on smooth (plant) surfaces than do slender tarsi. This is attributable to the number of tarsal tenent setae and their specific morphology (Betz 2002).

In 18 investigated Central European *Stenus* and one “*Dianous*” species, nine different tarsal seta types could be distinguished; indeed, even within a single species, up to six of these different types might be present. This diversity is partly attributable to some setae representing (mechano) sensilla; others must be considered

³The lobed state is especially established on the antepenultimate and the penultimate tarsomeres.

Fig. 11.26 Ventral aspects of the hind tarsi of various morphological types, i.e. (a) slender (e.g. *Stenus comma*), (b) sub-bilobed (*S. brunnipes*) and (c) wide bilobed (*S. pubescens*). Scale bars = 20 μm . From Betz (2003)



as tenent setae and release an adhesive secretion that is produced by underlying unicellular glands (Betz 2003). In particular, the spatulate type of tenent setae seems to be important for generating a considerable adhesive performance; it has only been found in species with wide bilobed tarsi (Betz 2003).

Most “*Dianous*” species show simple tarsi (all “*Dianous*” group I and most “*Dianous*” group II species), whereby in the “*Dianous*” group II, 16 species with deeply bilobed penultimate tarsi also occur (Puthz 2000b, 2016). One peculiar tarsal character occurring in many “*Dianous*” group II beetles is the “tarsal shoe” that distally projects from the tarsomeres and is formed by a dense array of elongated setae, which may be

modified into tape-like or willow-leaf-like structures (Rougemont 1985; Puthz 2000b; Betz 2003) (Fig. 11.27). These structures might trap air and thus support the beetles on the surface of water (Rougemont 1985). Such a structure might be of special importance, as “*Dianous*” beetles typically live in the immediate vicinity of powerful streams and cascades, where they permanently run the risk of being swept away by the current.

11.7.4 Abdominal Tergites

Stenus species may bear one pair of distinctly raised margins (i.e. the latero- or paratergites) at

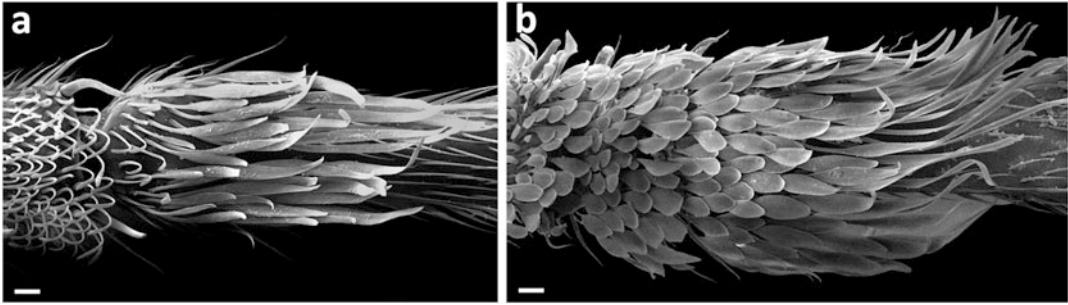


Fig. 11.27 Ventral aspects of the fourth tarsomeres of the hind legs showing distinct “tarsal shoes.” (a) *Dianous obliquenotatus*, (b) *D. fornicifrons*. Scale bars = 20 μm . For further explanations, see text

their tergites IV–VI. However, such margins are absent in about half the *Stenus* species (Puthz 2017), which then have ring-like cylindrical segments. Many other *Stenus* species represent an intermediate situation, since they show only a delicate tergo-sternal suture. Such reductions of the laterotergites might have entailed improved abdominal mobility (Puthz 1971) providing an advantage in many biological contexts in which the abdomen is involved (Betz 1999): terrestrial locomotion, swimming and skimming on the water surface, prey-capture (for building up the haemolymph pressure that is necessary for the ejection of the labium), mating, oviposition, self-grooming, chemical defence and the folding of the hind wings. To assist wing folding, the tergites may bear medial and/or lateral keels (at the first tergites), membranous apical rims (seventh and, occasionally, also eighth tergite), combs (ninth tergite) and spicule patches (tenth tergite and, occasionally, ninth tergite) (Puthz 1971). The folding pattern of the alae is described in Blum (1979).

In “*Dianous*,” the paratergites are usually well established, and only a few species show slight reductions in their widths.

11.7.5 Ecomorphology

For an understanding of the ecomorphological radiation that has occurred in the Steninae, we need to consider the way that morphological, behavioural and ecological traits interact with each other to form certain ecomorphs and the

relationship of this to the phylogeny of the group. Habitat choice experiments on 16 Central European *Stenus* species revealed that these beetles forage in three different zones, i.e. they are (1) inhabitants of moist humus and plant debris near the ground, (2) plant climbers or (3) surface runners on bare ground. A comparison of 18 Central European *Stenus* species has revealed different complexes of adaptations concerning predatory behaviour and linked morphological characters. Surface runners (3) such as *S. comma* have laterally protruding eyes with a large number of ommatidia, long legs and slender tarsi. Standardized prey-capture experiments with springtails have revealed that these predators are highly agile and are capable of pursuing prey that moves quickly and unpredictably. Their searching behaviour is of the ambush-searching type. Moreover, they are less reliant on their specialized labium for prey-capture but have refined the mandible-attack mechanism. The results of Koerner et al. (2017) are indicative of a process of the secondary reduction of their labial prey-capture apparatus, since these species possess, in relation to their body size, only small and simple-structured adhesive pads and generate exceptionally low compressive (impact) forces during their predatory strike (Fig. 11.25a). The improvement of the mandible-attack mechanism in these specialists seems to compensate for the limitation of the adhesive strength of the labium towards larger prey.

However, such ecomorphs are an exception and may have evolved from (1) detriticolous or (2) planticolous predator types that make up the

majority of recent *Stenus* species. These beetles are not particularly agile and stalk in plant debris or in the vegetation in order to hunt stationary or slow moving prey. Consequently, they have flat eyes and wide bilobed tarsi and depend to a higher degree on the labium for prey-capture, since it permits these predators, despite their limited agility, to catch prey in a sudden and surprising manner.

A discriminant function analysis (DCA) considering 91 *Stenus* species has been used here to examine those morphological variables that

determine the three general hunting sites of the species under study (Fig. 11.28). Plant climbers (green triangles) are distinct from soil dwellers (red squares and orange circles) mainly by their widened tarsi, in agreement with experimental results (Betz 2002). This feature might thus represent a key innovation that has made possible the expansion of the adaptive zone to live plants, contributing to the tremendous radiation of this genus. Indeed, approximately 70% of the >3000 *Stenus* species described belong to groups whose representatives have bilobed tarsi. Our DCA

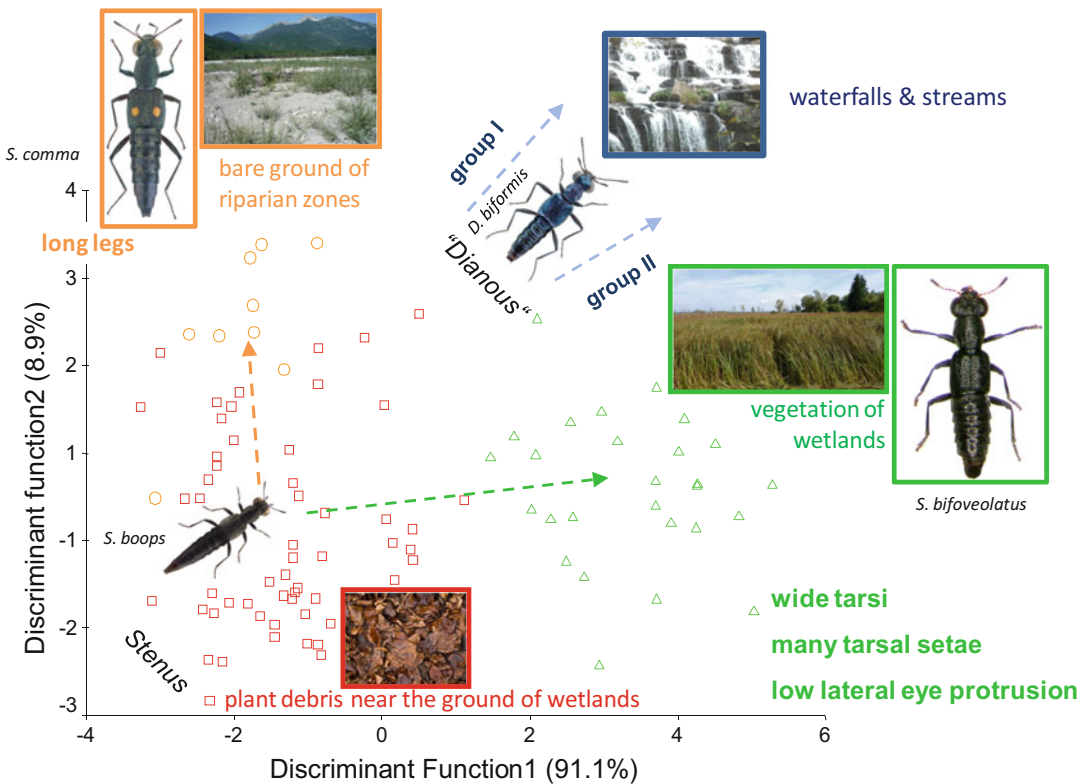


Fig. 11.28 Discriminant analytical separation of the three major hunting sites established in *Stenus* species. Each dot represents a different *Stenus* species. Values were log-transformed and corrected for body size before the analysis. The tarsus width is the most important predictor for the separation of the ground-dwelling versus the vegetation-dwelling species. Proceeding from the debris near the ground (red squares) as the presumed ancestral habitat, the arrows are indicative of the evolutionary shifts of the hunting sites in some phyletic lines towards the vegetation (green triangles) and the bare ground of open riparian habitats (orange circles). Such shifts may have

occurred several times independently. The arrows starting from “*Dianous*” are indicative of its hypothesized (iterative) ecological radiation repeating the ecomorphological diversification found across the *Stenus* subgenera, although this seems to be widely restricted to waterfall environments. For further explanation, see text. Discriminant function 1: relative tarsus width, 0.957*; relative number of tarsal setae, 0.718*; lateral eye protrusion, -0.296*; relative leg length, -0.116. Discriminant function 2: relative tarsus width, -0.109; relative number of tarsal setae, -0.158; lateral eye protrusion, 0.147; relative leg length, 0.952*. Beetle images courtesy of Udo Schmidt (www.kaefer-der-welt.de)

indicates that, among soil dwellers, surface runners on bare ground (orange circles) differ from debris-dwelling species (red squares) by having longer legs (Fig. 11.28) and by adjusting their prey-capture technique to elusive prey, such as springtails, i.e. although they predominantly use their labium for small springtails, they shift to the mandible prey-capture technique for large ones (see above).

If we consider the moist debris on the ground as the ancestral *Stenus* habitat, our analysis suggests that, in some phylogenetic lineages, an ecological radiation has occurred into two different habitat types (arrows in Fig. 11.28). On the one hand, an extension of the hunting habitat has occurred into open, sparsely vegetated sites such as river banks. On the other hand, in connection with widened tarsi, these beetles have effectively explored the vegetation (e.g. reeds) (in the tropics, also the canopy) as a permanent novel adaptive zone. In order to substantiate such an evolutionary scenario further, additional ecomorphological investigations, field studies and molecular phylogenetic analyses need to be conducted taking into consideration the tropical fauna. This also is true for the members of the genus “*Dianous*,” which most probably represents a branch within the genus *Stenus* (Koerner et al. 2013; Lang et al. 2015) and whose biology and evolution have been largely neglected. Although lacking a protrudable elongated labium, “*Dianous*” comprises a diversity of ecomorphs that are similar to those found in *Stenus*, i.e. plant climbers characterized by widened bilobed tarsi, dwellers of bare ground with slender tarsi and largely protruding eyes and inhabitants of moist detritus on the ground (Puthz 2000b). These differences are partly mirrored by the division of this genus into two main species groups (Puthz 1981). Based on the well-supported phylogenetic hypothesis suggesting probable monophyletic “*Dianous*” branches within *Stenus*, this diversity of ecomorphs within “*Dianous*” is indicative of an iterative convergent ecological radiation. Species flocks produced by iterative convergent radiations are produced not by single but by multiple adaptive radiations during their evolutionary history,

where upon subclades radiate across similar ecomorphs related to similar adaptive zones. As a result, within a lower taxonomic unit such as a tribe or a genus, we expect to find ecomorphological diversifications, within certain subclades, similar to those found across the subclades of the superordinate taxon. As possible reasons for such a repeated radiation scenario, Frédéricich et al. (2013) mention constraints caused by developmental processes, pleiotropic effects, morphological integration and competition.

Acknowledgements Our own work that was included in this chapter was supported by the Deutsche Forschungsgemeinschaft to O.B. and K.D. (PAK 478: BE 2233/10-1, BE 2233/11-1, DE 258/12-1, SE 595/14-1) and the Bundesministerium für Bildung und Forschung (Bionics Competition, BNK2-052) to O.B. We thank K. H. Seifert (Bayreuth) for support in identifying and synthesizing *Steninae* alkaloids. We thank Volker Puthz for critical reading of the manuscript and continuous support of our research. The English was corrected by Theresa Jones. Monika Meinert helped with the SEM work.

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