Syst Parasitol (2020) 97:561–567
https://doi.org/10.1007/s11230-020-09939-z

Heterocotyle whittingtoni n. sp. (Monogenea: Monocotylidae) from the gills of the black-spotted whipray, *Maculabatis toshi* (Whitley) (Myliobatiformes: Dasyatidae), collected in coastal waters of Queensland, Australia

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Received: 22 March 2020 / Accepted: 1 September 2020 / Published online: 2 October 2020

Abstract *Heterocotyle whittingtoni* n. sp. (Monogenea: Monocotylidae) is described from the gills of the black-spotted whipray *Maculabatis toshi* (Whitley) (Dasyatidae) collected from Moreton Bay near Dunwich and Peel Island, and from the eastern Gulf of Carpentaria off Weipa, Queensland, Australia. *Heterocotyle whittingtoni* n. sp. has a single sinuous ridge surmounting the haptoral septa and the male copulatory organ lacks an accessory piece. The new species can be distinguished from the two other *Heterocotyle* species that have this combination of characters by the distal portion of the male copulatory organ which is slightly flared with uniquely thickened walls and by the morphology of the testis. The identity of the host of *H. whittingtoni* n. sp. is discussed. We confirm that the host of the monocotylids *Dendromonocotyle lasti* Chisholm & Whittington, 2005 and *Monocotyle caseyae* Chisholm & Whittington, 2005 originally identified as "*Himantura* sp." was *M. toshi*.

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Introduction

The Monocotylidae is a family of monogeneans that parasitise chondrichthyan fishes (sharks, rays and chimaeras). Almost 40% of the described monocotylids have been recorded from Australian waters probably due to research focus in this region and the high diversity of potential chondrichthyan hosts occurring in Australian waters (see Last & Stevens, 2009). Monocotylids infecting rays collected from Moreton Bay, Queensland, Australia, have been examined in previous studies (e.g. Young, 1967; Chisholm & Whittington, 2005; among others). In the present paper we describe a new species of *Heterocotyle* Scott, 1904 from the gills of the black-spotted whipray *Maculabatis toshi* (Whitley) collected in Queensland waters. The identity of the host species is also discussed.

Materials and methods

Five specimens of *Maculabatis toshi* were collected on snorkel by herding them into nets in Moreton Bay near Dunwich, Stradbroke Island (27°15′S, 153°15′E), Queensland, Australia on August 29 and 30, 2002. The rays were transported alive to the laboratory in Dunwich, where they were killed by pithing and assigned the following Stradbroke Island (SI) field codes (SI-06, SI-09, SI-10, SI-11, SI-12) by the first author. A muscle-tissue sample from each ray was fixed in high-grade ethanol and deposited in the Australian Biological Tissue Collection (ABTC), South Australian Museum (SAMA), Adelaide, Australia. Each gill arch was excised and placed in a Petri dish of filtered seawater (FSW) and examined under a stereomicroscope using incident light. The microhabitat of live monogeneans was noted, after which the worms were removed from the gill lamellae and transferred to dishes containing FSW. Three live specimens were examined under a compound microscope equipped with phase contrast optics. The remaining monogeneans were slightly flattened under coverslip pressure and fixed in 10% buffered neutral formalin (BNF) at room temperature.

A single female ray identified as *M. toshi* was collected on May 17, 2004 near Weipa (12°35′11″S, 141°42′34″E) in the eastern Gulf of Carpentaria, off northern Queensland, Australia by J. Caira and K. Jensen. The specimen was assigned autopsy number CM03-81 and further details on the host can be accessed at http://tapewormdb.uconn.edu/index.php/hosts/specimen_search/elasmobranch. The gills were fixed in formalin and later transferred to 70% ethanol. Fixed gills were examined under a stereomicroscope using incident light and monogeneans removed. Another whipray (field number THC 17189), also identified as *M. toshi* (sex unknown), was collected off Peel Island, Queensland, Australia (27°30′S, 153°20′E) on January 15, 2016 as part of a comprehensive survey of the parasites occurring in the fishes of Moreton Bay. The gills of the ray were removed and placed in hot (60°C) sea water to relax and kill the attached monogeneans. The helminths were then collected and fixed and stored in 5% formalin.

Worms were stained with Semichon's carmine or Van Cleave's hematoxylin or left unstained, dehydrated in an ethanol series, cleared in cedarwood oil and mounted on microscope slides in Canada balsam. Preserved adult specimens were examined using a compound photomicroscope equipped with phase contrast optics and drawings were made with the aid of a drawing tube. Measurements were made using a computerised digitising system similar to that described by Roff & Hopcroft (1986). All measurements are given in micrometres as the range followed by the mean and the number of structures measured in parentheses. Type- and voucher specimens are deposited in the Australian Helminthological Collection (AHC) at SAMA, Adelaide, Australia and at the Queensland Museum (QM), Brisbane, Australia.

Monocotylidae Taschenberg, 1879

Heterocotylinae Chisholm, Wheeler & Beverley-Burton, 1995

Heterocotyle Scott, 1904

Heterocotyle whittingtoni n. sp.

Type-host: *Maculabatis toshi* (Whitley) (Elasmobranchii: Myliobatiformes: Dasyatidae).

Type-locality: Moreton Bay near Dunwich, Stradbroke Island (27°15′S, 153°15′E), Queensland, Australia.

Other localities: Moreton Bay off Peel island (27°30′S, 153°20′E), Queensland, Australia; Gulf of Carpentaria, near Weipa (12°35′11″S, 141°42′34″E), Queensland, Australia.

Type-specimens: Holotype AHC 36698, 25 Paratypes AHC 36699–36723, 8 Paratypes QM G238329–G238336.

Voucher specimens: AHC 36724 (4 slides), AHC 36725 (6 slides), AHC 36726 (4 slides).

Host tissue vouchers: ABTC 79232 (=SI-06), ABTC 79235 (=SI-09), ABTC 79236 (=SI-10), ABTC 79237 (=SI-11), ABTC 79238 (=SI-12).

Site on host: Monogeneans usually found in pairs at the ends of the gill filaments wedged between the secondary gill lamellae.

Prevalence and intensity: Data based on the five rays collected from Stradbroke Island in August 2002. Prevalence 100%; Intensity: > 200 (SI-06); 80 (SI-09); >200 (SI-10); 10 (SI-11); 17 (SI-12).

ZooBank registration: To comply with the regulations set out in Article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been

submitted to ZooBank. The Life Science Identifier (LSID) for *Heterocotyle whittingtoni* n. sp. is urn:lsid:zoobank.org:act:9D3038B5-2CF1-4776-B4E5-7C5D60F3AFD2.

Etymology: The species name "*whittingtoni*" is in honor of Ian Whittington and in recognition of his extensive work on the Monocotylidae and for his assistance collecting this new species.

Description

[Based on 31 whole-mounted flattened specimens and 3 live specimens from Moreton Bay off Dunwich; Figs. 1, 2.] Body (excluding haptor) 651–1,129 (934, n = 20) long, 212–418 (352, n = 20) wide at level of ovary (Fig. 1A). Haptor oval, 211–332 (268, n = 10) long, 309–358 (355, n = 20) wide, with one central and 8 peripheral loculi (Fig. 1A); single sinuous ridge on all haptoral septa (Fig. 1A). Hamuli (Fig. 1B) 44–52 (49, n = 20) long, associated with posterolateral septa (Fig. 1A). Fourteen hooklets distributed in marginal valve of haptor as illustrated (Fig. 1A); hooklet 9–11 (10, n = 13) long. Dorsal surface of haptor with 4 accessory structures situated dorsal to 4 posteriormost loculi; each accessory structure bearing sclerotised, striated anterior edge (Fig. 1A). Mouth ventral, subterminal. Anterior (cephalic) glands 3, containing granular secretion; 2 ducts from anteromedian gland and single duct from each anterolateral gland opening on anterior margin of head (Fig. 1A). Gland containing needle-like secretion present on either side of head just anterior to pharynx; three ducts arising from each gland opening on either side of anterior margin of head (Fig. 1A).

Eye-spots in the form of dispersed pigment granules lateral and anterior to pharynx. Pharynx 89–127 (109, n = 20) long, 76–107 (97, n = 20) wide; pharyngeal glands not seen. Intestinal caeca 2, lacking diverticula, terminating blindly in prehaptoral region. Testis single, oval in outline. Vas deferens arises from left side of testis, runs anteriorly dorsal to transverse vitelline duct and vagina and then inflates to form sigmoid seminal vesicle (Figs. 1A, 2). Seminal vesicle narrows to form ejaculatory duct that curves to enter ejaculatory bulb dorsally. Ejaculatory bulb 56–76 (66, n = 20) long, 29–54 (42, n = 20) wide. Male accessory glands present. Male copulatory organ 55–74 (67, n = 20) long, having diagonal proximal opening and slightly angled and flared distal end with distinctly thickened walls (Figs. 1A, C, 2). Ovary encircles right intestinal caecum dorsoventrally and narrows to form oviduct. Ovovitelline duct entering oötype. Oötype 71–137 (98, n = 20) long, opening at common genital pore. Mehlis' gland not observed. Vitellarium extends from level of mid-region of pharynx to anterior margin of haptor; transverse vitelline ducts as illustrated (Figs. 1A, 2). Common vitelline duct not seen. Vaginal pore unarmed, opens ventrally on left side of body at level of common genital pore (Figs. 1A, 2). Vagina sac-like distally then narrows and loops posteriorly and then anteriorly swelling to form seminal receptacle (Fig. 2) before joining oviduct. Vaginal walls not sclerotised; vaginal sclerites absent. Spermatophores not observed. Eggs tetrahedral (Fig. 1D); side of egg 61–85 (76, n = 9) long.

Remarks

The monocotylids collected from the gills of *M. toshi* belong to *Heterocotyle* because they have a haptor with one central and eight peripheral loculi, haptoral septa with a single sinuous ridge and four dorsal haptoral accessory structures on the dorsal side of the haptor each bearing a sclerotised striated anterior edge. The arrangement of the sinuous septal ridge(s) and the presence/absence of an accessory piece associated with the male copulatory organ are useful characters for easy identification of *Heterocotyle* species. Two configurations of the sinuous septal ridge(s) in species of the genus are known and the most common is the 1/2/3 arrangement (see Chisholm & Whittington, 1996). The sinuous ridge is single on all haptoral septa of our new species and six other valid species, including *H. armata* Timofeeva, 1983; *H. capricornensis* Chisholm & Whittington, 1996; *H. confusa* Timofeeva, 1983; *H. forcifera* Neifar, Euzet & Ben Hassine, 1999; *H. granulatae* Young, 1967 and *H. sulamericana* Santos, Santos, Cunha & Chisholm, 2012. An accessory piece associated with the male copulatory organ is lacking in *H. whittingtoni* n. sp., *H. capricornensis* and *H. sulamericana*, all of which have the single septal ridge configuration. *Heterocotyle whittingtoni* n. sp. can be distinguished from the latter two species by the morphology of distal portion of the male copulatory organ which is slightly flared with uniquely thickened walls.

A key to the species of *Heterocotyle* was recently published (see Chero et al., 2020) and therefore we do

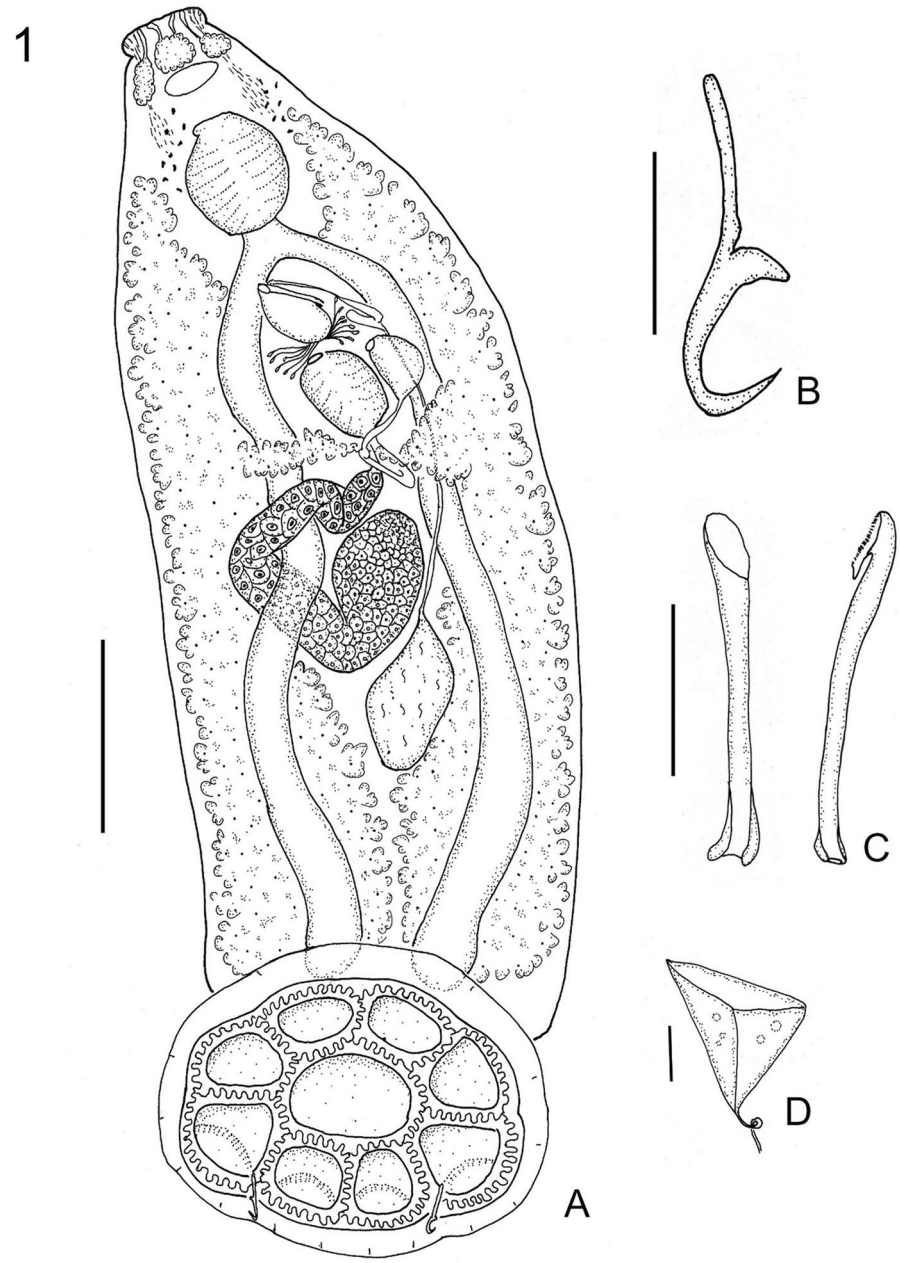

Fig. 1 *Heterocotyle whittingtoni* n. sp. A, Whole adult worm, ventral view; B, Hamulus; C, Sclerotised male copulatory organs showing thickened walls of distal ends from two specimens, lateral orientation depicted on right; D, Egg. *Scale-bars*: A, 200 μm; B, C, D, 25 μm

not provide one here. Chero et al. (2020) used the sinuous ridge configurations and presence/absence of the accessory piece in the copulatory complex in the first two couplets which resulted in *H. capricornensis* and *H. sulamericana* keying out first (as would *H. whittingtoni* n. sp.). They then use testis morphology to distinguish between the two species – three lobes in *H. capricornensis* and tubular forming a complete loop in *H. sulamericana*. The testis of *H. whittingtoni* n. sp. is a single oval-shaped mass.

The seminal receptacle in species of *Heterocotyle* is usually a distinct round or oblong structure joining to

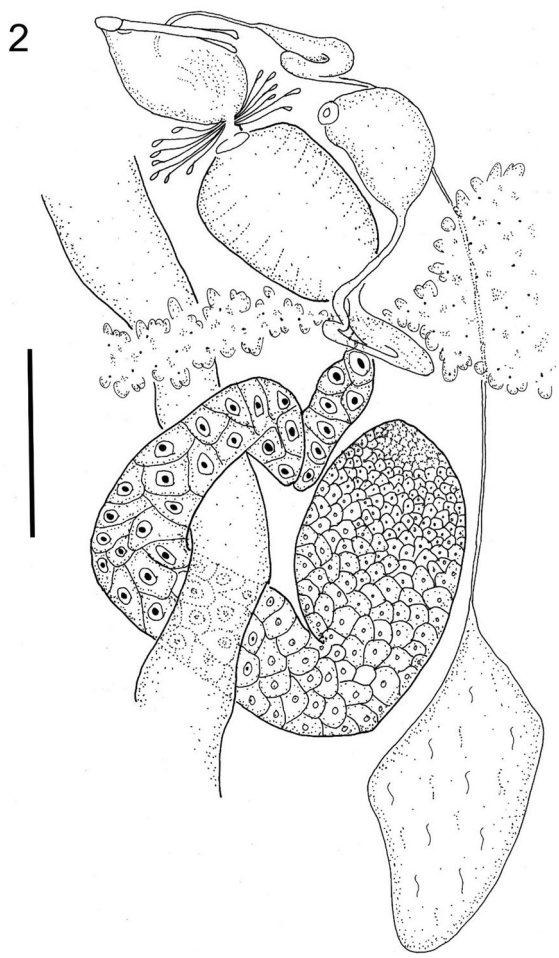

Fig. 2 Reproductive system of *Heterocotyle whittingtoni* n. sp., ventral view. *Scale-bar*: 100 µm

the proximal portion of the vagina (e.g. Neifar et al., 2000; Vaughan & Chisholm, 2010; Santos et al., 2012) but it was not obvious in *H. whittingtoni* n. sp. The sac-like portion of the vagina narrows, runs posteriorly and after it turns anteriorly, it swells before joining the oviduct (Fig 1A, 2). We consider that the dilated portion of the vagina that runs anteriorly is the seminal receptacle (Fig. 2).

Discussion

There are now 21 valid species of *Heterocotyle*. *Heterocotyle elliptica* Pillai & Pillai, 1976 and *H. robusta* (Johnston & Tiegs, 1922) Price, 1938 were considered by Chisholm & Whittington (1996) to be *species inquirendae* and this decision has been followed by others (e.g. Chero et al., 2020). Neither the presence of a sinuous ridge on the haptoral septa nor the four dorsal haptoral accessory structures were described or illustrated by Pillai & Pillai (1976) for *H. elliptica*, and types could not be located to verify the status of these characters in the species (see Chisholm & Whittington, 1996). Similarly, Johnston & Tiegs (1922) did not describe a sinuous ridge on the haptoral septa or the four dorsal haptoral accessory structures for *H. robusta* and the poor condition of the holotype precluded determination of their presence/absence in the species (see Chisholm & Whittington, 1996).

Four *Heterocotyle* species have now been described from Queensland, Australia, including *H. capricornensis* from the gills of *Pateobatis fai* (Jordan & Seale) from off Heron Island on the Great Barrier Reef (see Chisholm & Whittington, 1996) and three species from Moreton Bay, including *H. chinensis* from the gills of *Hemitrygon fluviorum* (Ogilby) and *Himantura uarnak* (Gmelin) (see Chisholm & Whittington, 1996), *Heterocotyle granulatae* from the gills of *Urogymnus granulatus* (Macleay) (see Young, 1967) and *H. whittingtoni* on the gills of *M. toshi* (present study). We also found *H. whittingtoni* on the gills of *M. toshi* collected in the Gulf of Carpentaria, near Weipa.

Batoids are regarded as the most taxonomically problematic of the elasmobranch groups. Nearly a quarter of the approximately 633 species of rays known worldwide have been described in the past 15 years (Last et al., 2016a). The Australian shark and ray fauna is almost 50% more diverse than that of other similar geographic regions of the world (Last & Stevens, 2009). One third of the 296 taxa of chondrichthyan fishes listed from Australia by Last & Stevens (1994) were only identified to genus with a letter code (i.e. sp. A) assigned for the species. These taxa were considered distinct and detailed descriptions/illustrations were provided by Last & Stevens (1994). In the second edition of the Sharks & Rays of Australia (Last & Stevens, 2009), the letter-coded species of Last & Stevens (1994) were updated to described species and another 26 species from Australian waters were also included, bringing the total to 322 at that time.

For the non-specialist, species determination within the Australian ray fauna can be particularly difficult, especially since characters such as colour patterns can vary within and between adult and juvenile animals.

Present specimens of *H. whittingtoni* were collected from the same ray hosts that harboured *Dendromonocotyle lasti* Chisholm & Whittington, 2005 and *Monocotyle caseyae* Chisholm & Whittington, 2005 (see Chisholm & Whittington, 2005). In that study, identification of the host ray was discussed in detail and images and descriptions of key diagnostic characters were provided. Chisholm & Whittington (2005) noted that the rays were most similar to *Himantura* sp. A of Last & Stevens (1994, p. 297) but that the morphology of the mouth and nostril region was not "consistent" with that illustrated and described by Last & Stevens (1994). Therefore, Chisholm & Whittington (2005) made the cautious decision to identify the host species only as *Himantura* sp. Last & Stevens (2009) subsequently identified *Himantura* sp. A as *H. toshi* Whitley. Last et al. (2016b) erected *Maculabatis* for a subgroup of *Himantura* and reassigned *H. toshi* to the new genus as *Maculabatis toshi*.

The present study prompted us to reassess the host identification made by Chisholm & Whittington (2005). Peter Last recently re-examined the original host images and descriptions provided in Chisholm & Whittington (2005) and confirmed the hosts were *M. toshi* (Last, personal communication). In addition, tissue (ABTC 85510) from a ray collected off Joseph Bonaparte Gulf, Western Australia, and identified morphologically as *M. toshi* was sequenced (mitochondrial *ND4*, nuclear *RAG1*, nuclear *POMC*) for a large phylogenetic study examining the evolution of stingrays (Bertozzi et al., 2016). While not included in the published work of Bertozzi et al. (2016), Bertozzi also sequenced (ND4) the tissues (ABTC 79236 (SI-10) and ABTC 79237(SI-11) collected by Chisholm & Whittington (2005) from *Himantura* sp. A. These ND4 sequences are identical to those of the ray identified as *M. toshi* in Bertozzi et al. (2016) (Bertozzi, personal communication). Thus, we are confident that the hosts we collected from Moreton Bay were *M. toshi*. Therefore, the monocotylids *D. lasti*, *M. caseyae* and *Heterocotyle whittingtoni* have all been described from this host species in Australia.

In addition to Moreton Bay, we also found *H. whittingtoni* on the gills of *M. toshi* in the Gulf of Carpentaria off Weipa, Queensland. The identification notes of this ray (CM03-81) were originally written as "*Himantura toshi* / sp. A" (= *M. toshi*) but they have since been updated to *Maculabatis astra* (Last, Manjaji-Matsumoto & Pogonoski) (see http://tapewormdb.uconn.edu/index.php/hosts/specimen_details/elasmobranch/1923/0). Recent data now show that *M. astra* is likely a junior synonym of *M. toshi* (Last et al., 2016b, Last, personal communication) and therefore we consider the host from Weipa to be *M. toshi* as originally identified by the collectors.

Acknowledgements We are grateful to Peter Fearnside (Dunwich, Queensland) for collecting the rays in Moreton Bay. The Moreton Bay study was funded by an Australian Research Council large grant (no. A00104437) awarded for 2001–2003. We thank Janine Caira (University of Connecticut) and Kirsten Jensen (University of Kansas) for providing us with the gill tissue from rays collected near Weipa, Queensland; their collections were funded in part by PEET grant DEB no. 0118882. The collection of *M. toshi* and its parasites during 2016 were made during the survey of the parasites of the fishes of Moreton Bay and supported by an Australian Biological Resources Study Grant (RF215-40) awarded to Tom Cribb and Scott Cutmore (The University of Queensland). We thank Vanessa Glennon (The University of Adelaide) who kindly mounted the helminth specimens and Peter Last for his advice and expert assistance in identifying the hosts.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guidelines on the care and use of laboratory animals.

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