Species Diversity **20**: 89–93
25 May 2015
DOI: 10.12782/sd.20.1.089

Heterocotyle chinensis (Monogenea: Monocotylidae) from the Whip Stingray *Dasyatis akajei* in the Seto Inland Sea, Japan

Masato Nitta[1,2] and Kazuya Nagasawa[1]

[1] *Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8528, Japan*
E-mail: licht.bsn.mono@gmail.com (MN)
[2] *Corresponding author*

(Received 24 October 2014; Accepted 13 April 2015)

Heterocotyle chinensis Timofeeva, 1983 (Monogenea: Monocotylidae) is reported from the gills of the whip stingray *Dasyatis akajei* (Müller and Henle, 1841) in the Seto Inland Sea, Hiroshima Prefecture, Japan, as a new country record. A morphological description and illustrations are provided, along with an emended key to the species of *Heterocotyle*. The present monogenean most likely has some tolerance for low salinity because one of the two infected fish examined was collected in brackish water.

Key Words: *Heterocotyle chinensis*, Monogenea, *Dasyatis akajei*, brackish water, new country record, redescription, Seto Inland Sea, Hiroshima, Japan, taxonomic key.

Introduction

Heterocotyle chinensis Timofeeva, 1983 (Monogenea: Monocotylidae) was originally described from the whip stingray *Dasyatis akajei* (Müller and Henle, 1841) (Myliobatiformes: Dasyatidae) in the Yellow Sea, China (Timofeeva 1983). Subsequently, *H. chinensis* has been reported from the estuary stingray *D. fluviorum* Ogilby, 1908 and the honeycomb stingray *Himantura uarnak* (Gmelin, 1789) (Myliobatiformes: Dasyatididae) in Moreton Bay, Australia (Chisholm and Whittington 1996) and from the blue-spotted stingray *D. kuhli* (Müller and Henle, 1841) and the blackish stingray *D. navarrae* (Steindachner, 1892) in Fujian Province, China (Zhang *et al.* 2001). In this paper we report *H. chinensis* parasitic on *D. akajei* in the Seto Inland Sea, Japan, as a new country record, with a morphological description of the specimens and a revised key to the species of *Heterocotyle*.

Materials and Methods

Two whip stingrays were collected at two sites in the Seto Inland Sea, Hiroshima Prefecture, Japan: the mouth of the Kamo River (34°19′34.8″N, 132°53′50.9″E) by hand net at the lowest tide on 28 May 2014, and off Ōsaki-kami-jima island (33°14′N, 132°48′E) by trawl fishing on 14 July 2014. The rays were brought alive to the laboratory and examined for parasites under a dissecting microscope. Monogeneans were picked from the gills using small needles and forceps and flattened on glass slides under slight coverslip pressure. The glands and ducts of the head and the posterior glands were observed in living specimens. Some monogeneans were fixed in ammonium picrate glycerin (Lim 1991) for observation of sclerotized structures, while others were fixed in 70% ethanol and stained in Heidenhain's iron hematoxylin. All specimens were dehydrated through a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Drawings were made with the aid of a drawing tube fitted on an Olympus BX51 light microscope. Measurements, in micrometers, are expressed as the mean±standard deviation followed in parentheses by the range and the number (n) of specimens examined. Fish identification was based on Yamaguchi *et al.* (2013), and common names of fishes used in this paper follow Froese and Pauly (2014). Specimens are deposited in the Platyhelminthes collection of the National Museum of Nature and Science, in Tsukuba, Ibaraki Prefecture, Japan (NSMT-Pl).

Heterocotyle chinensis Timofeeva, 1983
(Fig. 1)

Heterocotyle chinensis Timofeeva, 1983: 42–44, fig. 4; Timofeeva 1985: 54; Chisholm *et al.* 1995: 177; Chisholm and Whittington 1996: 1175–1176, figs 1B, 2B, 9, 12D; Zhang *et al.* 1999: 186–187, fig. 8-71; Zhang *et al.* 2001: 42–43, fig. 2-2; Zhang *et al.* 2003: 112.

Material examined. Twenty-five specimens (NSMT-Pl 6164) from the mouth of the Kamo River on 28 May 2014, and 6 specimens (NSMT-Pl 6165) from off Ōsaki-kami-jima island on 14 July 2014.

Description. Body (Fig. 1A) including haptor length 865±132.7 (671–1073; n=17), width at mid-body 326±56.1 (194–406; n=17). Two ducts from point of anterolateral glands exiting medial to two ducts from anteromedian gland present in anterior part of head. Trio of other ducts

opening on either side of front of head. Eyespots dispersed over dorsal body surface between mouth and pharynx. Mouth muscular, round to elliptical, length 62±11.6 (48–87; n=14), width 71±18.1 (34–108; n=14). Pharynx muscular, spherical to oval, with muscular fibers attached to both sides of its anterior part, length 138±36.2 (65–185; n=17), width 110±31.9 (46–113; n=17). Esophagus not present, bifurcate intestine extending to end of body. Pharyngeal glands present on either side of posterior part of pharynx. Testis single, posterior to ovary. Vas deferens arising from left anterior side of testis, extending along dorsal side of left intestine. Seminal vesicle located beneath branching point of intestine, extending to ejaculatory bulb, and connected to posterior part of ejaculatory bulb. Ejaculatory bulb muscular, oval to pyriform, length 97±9.0 (82–113; n=17), width 75±12.6 (37–98; n=17). Male accessory glands entering posterior part of ejaculatory bulb. Male copulatory organ (Fig. 1D) lacking accessory piece, sclerotized slightly curved tube, tip twisted slightly, length 78±4.9 (73–92; n=30) in chord straight line from base to tip. Ovary in mid-body, elongate, wrapping around right intestine. Oviduct arising from anterior part of ovary, continuing on as oötype. Mehlis' gland connected to middle of oötype. Vagina, without sclerotized structure, connecting between posterior part of oötype and seminal receptacle. Vaginal pore opening on left side of ventral body surface. Vitellaria approximately co-extensive with intestine. Transverse vitelline duct lying at level of oviduct. Pair of posterior glands located near either tip of intestine.

Haptor elliptical, length 232±24.1 (172–269; n=15), width 319±39.3 (223–383; n=15), ventral surface of haptor with 1 central and 8 peripheral loculi. Pair of hamuli (Fig. 1B), length 41±2.1 (38–46; n=31). Sinuous ridge single on 3 posterior radial septa, double on 2 lateral septa, triple on 3 anterior septa. Fourteen hooklets (Fig. 1C), length 9±0.7 (8–10; n=31), located in marginal valve as illustrated (Fig. 1A). Dorsal haptoral accessory structure U-shaped, located on dorsal surface of 4 posterior loculi.

Host. Whip stingray *Dasyatis akajei* (Myliobatiformes: Dasyatidae).

Localities. Mouth of the Kamo River, Takehara city, and off Ōsaki-kami-jima island, Ōsaki-kami-jima town, both in the Seto Inland Sea, Hiroshima Prefecture, Japan.

Site of infection. Gills.

Intensity. Two hundred and thirty-two individuals were found infecting the whip stingray (173 mm in disk width) collected at the mouth of the Kamo River, and 50 individuals were collected from the other whip stingray (323 mm in disk width) caught off Ōsaki-kami-jima island.

Remarks. *Heterocotyle chinensis* was originally described by Timofeeva (1983) and later redescribed by Chisholm and Whittington (1996). The morphology of the specimens collected in this study corresponds to the description by Chisholm and Whittington (1996) and measurements by Timofeeva (1983) and Zhang *et al.* (2001).

Currently, 19 species of the genus *Heterocotyle* Scott, 1904 are regarded as valid: *H. americana* Hargis, 1955, *H. armata* Timofeeva, 1983, *H. capapei* Neifar, Euzet and Ben Hassine, 2000; *H. capricornensis* Chisholm and Whittington, 1996; *H. chinensis* Timofeeva, 1983, *H. confusa* Timofeeva, 1983; *H. dasyatis* (Yamaguti, 1965); *H. forcifera* Neifar, Euzet and Ben Hassine, 1999; *H. granulatae* Young, 1967; *H. minima* (MacCllum, 1916), *H. mokhtarae* Neifar, Euzet and Ben Hassine, 1999; *H. pastinacae* Scott, 1904, *H. pseudominima* Hargis, 1955; *H. scotti* Neifar, Euzet and Ben Hassine, 1998; *H. similis* Neifar, Euzet and Ben Hassine, 1998; *H. striata* Neifar, Euzet and Ben Hassine, 1999; *H. sulamericana* Santos, Santos, Cunha and Chisholm, 2012; *H. taeniuropi* Cao, Ding, Zhang and Liu, 2010; *H. tokoloshei* Vaughan and Chisholm, 2010 (Chisholm and Whittington 1996; Cao *et al.* 2010; Santos *et al.* 2012). *Heterocotyle armata*, *H. capricornensis*, *H. confusa*, *H. forcifera*, *H. granulatae*, and *H. sulamericana* all differ from *H. chinensis* in having a haptor with a single sinuous ridge on all septa (Young 1967; Timofeeva 1983; Chisholm and Whittington 1996; Neifar *et al.* 1999; Santos *et al.* 2012). The accessory piece is absent in *H. chinensis*, while it is present in *H. americana*, *H.*

Table 1. Species of Monocotylidae reported from Japan.

Species	Host	Locality	Reference
Calicotyle japonica Kitamura, Ogawa, Shimizu, Kurashima, Mano, Taniuchi and Hirose, 2010	*Squalus mitsukurii* Jordan and Snyder, 1903 (Squaliformes: Squalidae)	Sagami Bay, Kanagawa and Shizuoka prefectures; Tōkyō Bay, Tōkyō Metropolis	Kitamura *et al.* (2009)
C. mitsukurii Goto, 1894	*Squatina japonica* Bleeker, 1858* (Squatiniformes: Squatinidae)	Mitsugahama, Ehime Prefecture	Goto (1894)
Dendromonocotyle akajei Ho and Perkins, 1980	*Dasyatis akajei* (Müller and Henle, 1841)	Toyama Bay, Toyama Prefecture	Ho and Perkins (1980)
Heterocotyle chinensis Timofeeva, 1983	*D. akajei*	Mouth of Kamo River and off Ōsaki-kami-jima island, Hiroshima Prefecture	Present study
Monocotyle ijimae Goto, 1894	*D. akajei**	Ujina Port, Hiroshima Prefecture	Goto (1894)
Triloculotrema japanicae Kearn, 1993	*Hemitriakis japanica* (Müller and Henle, 1839) (Carcharhiniformes: Triakidae)	Off Minabe, Wakayama Prefecture	Kearn (1993)

* Goto (1894) recorded the hosts as "*Rhina* sp.?" (Japanese name "Katasahi-zamé") and "*Trygon pastinaca*" ("Aka-ei"). The former Japanese name is an old local name for *Squatina japonica* Bleeker, 1858 (Tamura 1935), and the latter Japanese name means *Dasyatis akajei* (Yamaguchi *et al.* 2013). Only one species of shark of the genus *Rhina*, *R. ancylostoma* Bloch and Schneider, 1801 occurs in Japanese waters (Hatooka *et al.* 2013), but this species has not been reported from Ehime Prefecture (the Seto Inland Sea), where Goto (1894) collected *Calicotyle mitsukurii. Trygon pastinaca* is currently regarded as identical with *D. pastinaca* in the Atlantic Ocean and Mediterranean Sea (see Froese and Pauly 2014).

dasyatis, *H. minima*, and *H. tokoloshei* (Hargis 1955; Yamaguti 1965; Chisholm and Whittington 1996; Vaughan and Chisholm 2010). By the male copulatory organ length (79–95 μm: Timofeeva 1983; 83–107 μm: Zhang *et al.* 2001; 73–92 μm: present study), *H. chinensis* is differentiated from *H. capapei* (166–182 μm: Neifar *et al.* 2000), *H. mokhtarae* (145–165 μm: Neifar *et al.* 1999), *H. scotti* (115–155 μm: Neifar *et al.* 1998), *H. striata* (130–155 μm: Neifar *et al.* 1999), *H. taeniuropi* (193–330 μm: Cao *et al.* 2010), and *H. pseudominima* (36–54 μm: Hargis 1955). Although *H. pastinacae* has sclerotized spines in the proximal part of the vagina (Chisholm and Whittington 1995, 1996; Neifar *et al.* 1998) and *H. similis* possesses a crotchet-shaped male copulatory organ (Neifar *et al.* 1998), *H. chinensis* has no such features.

Discussion

Heterocotyle chinensis has so far been recorded from dasyatids caught at sea (Timofeeva 1983; Chisholm and Whittington 1996; Zhang *et al.* 2001), but we collected live specimens of this monogenean from a whip stingray collected in brackish water at the mouth of the Kamo River. Whip stingrays are known to sometimes enter brackish water from the sea (Kamohara 1961) and live in brackish-water lakes (Nakamura 2007). Therefore, like its host fish, *H. chinensis* may have some tolerance for low salinity.

Monogeneans of the family Monocotylidae are gill parasites of elasmobranchs (Chisholm *et al.* 1995), and six species of four genera have been reported from Japan (Table 1). Since the elasmobranch fauna of Japan is rich and consists of over 200 species (Nakabo 2013), many monocotylids may be unreported in Japan.

Emended key to species of the genus *Heterocotyle* (after Chisholm and Whittington 1996; Neifar *et al.* 1999)

1. Haptor with single sclerotized ridge on all septa..... 2
   Sinuous sclerotized ridge single on inner and outer ring septa and 3 posterior radial septa, double on 2 lateral radial septa, and triple on 3 anterior radial septa 7
2. Accessory piece of male copulatory organ absent..... 3
   Accessory piece of male copulatory organ present 4
3. Male copulatory organ long (77–106 μm); testis with 3 posteriorly-directed finger-like lobes..... *H. capricornensis*
   Male copulatory organ short (23–27 μm); testis long, tubular, forming complete loop ..... *H. sulamericana*
4. Accessory piece with spines 5
   Accessory piece without spines..... 6
5. Accessory piece branched into two at base ... *H. forcifera*
   Un-branched accessory piece with distinct spines distally ..... *H. armata*
6. Accessory piece associated with distal part of male copulatory organ ..... *H. confusa*
   Accessory piece not associated with distal part of male copulatory organ; male copulatory organ flared distally ..... *H. granulatae*
7. Accessory piece of male copulatory organ present 8
   Accessory piece of male copulatory organ absent..... 11
8. Accessory piece composed of about 10 long sclerotized spines ..... *H. dasyatis*
   Accessory piece composed one piece..... 9
9. Accessory piece articulating with proximal part of male copulatory organ..... *H. americana*
   Accessory piece associated with distal end of male copulatory organ 10
10. Proximal end of male copulatory organ straight, distal end with 20 small spines; accessory piece comb-like..... *H. tokoloshei*
    Proximal end of male copulatory organ curved, distal end without spine; accessory piece small and bell-shaped ..... *H. minima*
11. Distal part of male copulatory organ with long points... 12
    Distal part of male copulatory organ without points..... 13
12. Vagina with rows of sclerotized bars armed with spines ..... *H. pastinacae*
    Vagina without sclerotized structures..... *H. capapei*
13. Distal end of male copulatory organ curved, hook-shaped 14
    Distal end of male copulatory organ straight..... 15
14. Male copulatory organ long (193–330 μm); proximal end smooth..... *H. taeniuropi*
    Male copulatory organ short (85–105 μm); proximal end curved..... *H. similis*
15. Male copulatory organ with two rows of filaments..... *H. striata*
    Male copulatory organ without filaments 16
16. Ejaculatory bulb (300–400 μm) over twice as long as male copulatory organ (145–156 μm)..... *H. mokhtarae*
    Ejaculatory bulb (82–170 μm) 1–2 times as long as male copulatory organ (73–107 μm)..... *H. chinensis*
    Ejaculatory bulb of approximately same length as male copulatory organ..... 17
17. Male copulatory organ short (36–54 μm)..... *H. pseudominima*
    Male copulatory organ long (115–155 μm)..... *H. scotti*

Acknowledgments

We thank Yusuke Kondo and Shoma Okada, Takehara Marine Science Station, Hiroshima University, for assistance with fish sampling. We are also grateful to Pavel I. Gerasev, Russian Academy of Sciences, and Mark J. Grygier, Lake Biwa Museum for valuable comments on the manuscript.

References

Cao, S., Ding, X., Zhang, J., and Liu, L. 2010. Monogenea of Chinese

marine fishes. XVIII. Two monocotylids, including a new species, from the blotched fantail ray *Taeniurops meyeni* (Elasmobranchii: Dasyatidae) in the South China Sea. Folia Parasitologica 57: 169–172.

Chisholm, L. A., Wheeler, T. A., and Beverley-Burton, M. 1995. A phylogenetic analysis and revised classification of the Monocotylidae Taschenberg, 1879 (Monogenea). Systematic Parasitology 32: 159–191.

Chisholm, L. A. and Whittington, I. D. 1995. A redescription of *Heterocotyle pastinacae* Scott, 1904 (Monogenea: Monocotylidae) from *Dasyatis pastinaca* (Dasyatididae), with a neotype designation. Systematic Parasitology 36: 207–211.

Chisholm, L. A. and Whittington, I. D. 1996. A revision of *Heterocotyle* (Monogenea: Monocotylidae) with a description of *Heterocotyle capricornensis* n. sp. from *Himantura fai* (Dasyatididae) from Heron Island, Great Barrier Reef, Australia. International Journal for Parasitology 26: 1169–1190.

Froese, R. and Pauly, D. (Eds) 2014. FishBase. Available at www.fishbase.org (24 October 2014).

Goto, S. 1894. Studies on the ectoparasitic trematodes of Japan. Journal of the College of Science, Imperial University of Tokyo 8: 1–273.

Hargis, W. J. Jr. 1955. Monogenetic trematodes of Gulf of Mexico fishes. Part IV. The superfamily Capsaloidea Price, 1936. Revista Iberica de Parasitología Tomo Extraordinario: 1–16.

Hatooka, K., Yagishita, N., and Yamaguchi, A. 2013. Rhynchobatidae. Pp. 200, 1769. *In*: Nakabo, T. (Ed.) *Fishes of Japan with Pictorial Keys to the Species, Third Edition.* Tokai University Press, Hadano. [In Japanese]

Ho, J.-S. and Perkins, P. S. 1980. Monogenea from fishes of the Sea of Japan. Part 1. Order Monopisthocotylea. Annual Report of the Sado Marine Biological Station Niigata University 10: 1–10.

Kamohara, T. 1961. Coloured Illustrations of the Fishes of Japan Vol. I. Hoikusha Publishing Co., Ltd. Osaka, 158 pp. [In Japanese]

Kearn, G. C. 1993. *Triloculotrema japanicae* n. g., n. sp. (Monogenea: Monocotylidae) from the olfactory sacs of the Japanese topeshark *Hemitriakis japanica* (Müller & Henle, 1839) (Carcharhiniformes: Triakidae). Systematic Parasitology 26: 53–57.

Kitamura, A., Ogawa, K., Shimizu, T., Kurashima, A., Mano, N., Taniuchi, T., and Hirose, H. 2009. A new species of *Calicotyle* Diesing, 1850 (Monogenea: Monocotylidae) from the shortspine spurdog *Squalus mitsukurii* Jordan & Snyder and the synonymy of *Gymnocalicotyle* Nybelin, 1941 with this genus. Systematic Parasitology 75: 117–124.

Lim, L. H. S. 1991. Three new species of *Bychowskyella* Achmerow, 1952 (Monogenea) from Peninsular Malaysia. Systematic Parasitology 19: 33–41.

Nakabo, T. 2013. *Fishes of Japan with Pictorial Keys to the Species, Third Edition.* Tokai University Press, Hadano. 2428 pp. [In Japanese]

Nakamura, M. 2007. *Shinji-ko to Nakaumi no Sakana-tachi* [*Fishes of Lake Shinji and Lake Nakaumi*]. San-in Chuo Shimpo, Matsue. 211 pp. [In Japanese]

Neifar, L., Euzet, L., and Ben Hassine, O. K. 1998. Nouveaux Monocotylidae (Monogenea), parasites branchiaux de *Dasyatis pastinaca* (L.) (Euselachii, Dasyatidae). Compléments à la description de *Heterocotyle pastinacae* Scott, 1904. Systematic Parasitology 41: 197–208.

Neifar, L., Euzet, L., and Ben Hassine, O. K. 1999. Trois nouveaux *Heterocotyle* (Monogenea, Monocotylidae) parasites branchiaux de *Taeniura grabata* (Euselachii, Dasyatidae) en Tunisie. Zoosystema 21: 157–170.

Neifar, L., Euzet, L., and Ben Hassine, O. K. 2000. New species of the Monocotylidae (Monogenea) from the stingray *Dasyatis tortonesi* Capapé (Euselachii, Dasyatidae) off the Tunisian coast, with comments on host-specificity and the specific identities of Mediterranean stingrays. Systematic Parasitology 47: 43–50.

Santos, C. P., Santos, A. L., Cunha, R., and Chisholm, L. A. 2012. A new species of *Heterocotyle* Scott, 1904 (Monogenea: Monocotylidae) from the gills of *Dasyatis guttata* (Dasyatidae) in southwestern Atlantic waters off Rio de Janeiro, Brazil. Systematic Parasitology 81: 65–70.

Tamura, M. 1935. [Local names of fishes in Hiroshima Prefecture]. Hiroshima-ken Suisan Kaihō 13: 25–37. [In Japanese]

Timofeeva, T. A. 1983. New representatives of monocotylids (Monogenea, Monocotylidae) from cartilaginous fishes of the South-China and Yellow Seas. Trudy Zoologicheskogo Instituta 121: 35–47. [In Russian with English abstract]

Timofeeva, T. A. 1985. Morphological and ecological aspects of monocotylid's evolution (Monogenea, Monocotylidae). Parazitologicheskii Sbornik 33: 44–76. [In Russian with English abstract]

Vaughan, D. B. and Chisholm, L. A. 2010. *Heterocotyle tokoloshei* sp. nov. (Monogenea, Monocotylidae) from the gills of *Dasyatis brevicaudata* (Dasyatidae) kept in captivity at Two Oceans Aquarium, Cape Town, South Africa: description and notes on treatment. Acta Parasitologica 55: 108–114.

Yamaguchi, A., Aonuma, Y., Yagishita, N., and Yoshino, T. 2013. Dasyatidae. Pp. 220–226, 1775–1776. *In*: Nakabo, T. (Ed.) *Fishes of Japan with Pictorial Keys to the Species, Third Edition.* Tokai University Press, Hadano. [In Japanese]

Yamaguti, S. 1965. New monogenetic trematodes from Hawaiian fishes, I. Pacific Science 19: 55–95.

Young, P. C. 1967. A taxonomic revision of the subfamilies Monocotylinae Gamble, 1896 and Dendromonocotylinae Hargis, 1955 (Monogenoidea: Monocotylidae). Journal of Zoology, London 153: 381–422.

Zhang, J., Liu, L., Ding, X., Pan, J., and Pang, Q. 1999. [Monogenea parasitic on fishes and monogenean diseases.] Pp. 108–285. *In*: Zhang, J., Qiu, Z., and Ding, X. (Eds) *Parasites and Parasitic Diseases of Fishes.* Science Press, Beijing. [In Chinese]

Zhang, J., Yang, T., and Liu, L. 2001. *Monogeneans of Chinese Marine Fishes.* Agriculture Press, Beijing, 400 pp. [In Chinese]

Zhang, J., Yang, T., Liu, L., and Ding, X. 2003. A list of monogeneans from Chinese marine fishes. Systematic Parasitology 54: 111–130.