Three *Dendromonocotyle* species (Monogenea: Monocotylidae) reported from captive rays, including *D. lotteri* sp. n. from *Himantura gerrardi* (Elasmobranchii: Dasyatidae) in the public aquarium at the Atlantis resort, Dubai

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Abstract: *Dendromonocotyle lotteri* sp. n. is described from the dorsal skin surface of the stingray *Himantura gerrardi* (Gray) on exhibit in the public aquarium at the Atlantis resort in Dubai. It is differentiated from all other *Dendromonocotyle* species by the unique morphology of the distal portion of the sclerotised male copulatory organ. *Dendromonocotyle lotteri* is the second representative in the genus with 56 marginal haptor papillae having a papillae to loculus association represented numerically as 6–6–8–8. We found *Dendromonocotyle colorni* Chisholm, Whittington et Kearns, 2001 on the same host specimens at the Atlantis resort public aquarium and *Dendromonocotyle kuhlii* Young, 1967 on *Neotrygon kuhlii* (Müller et Henle) kept at Burgers' Zoo Aquarium in Arnhem, The Netherlands. Supplemental information is provided for both *D. colorni* and *D. kuhlii*. The presence of *Dendromonocotyle* infections in public aquaria and host specificity are discussed. A key to the 17 species of *Dendromonocotyle* is also provided.

Key words: Monogenea, Monocotylidae, *Dendromonocotyle*, public aquarium, *Himantura gerrardi*, *Neotrygon kuhlii*, Dubai

Members of *Dendromonocotyle* Hargis, 1955 (Monogenea: Monocotylidae) have been reported on the dorsal surface of stingrays from the Dasyatidae, Myliobatidae and Urolophidae. There are currently 16 known species of *Dendromonocotyle*, of which 7 are reported from stingrays in captivity (see Chisholm and Whittington 1995, 2009, Chisholm et al. 2001, 2004, Vaughan et al. 2008). It has become clear that *Dendromonocotyle* infections can cause health problems in stingrays kept in public aquaria. Monogeneans are generally considered to be host specific but increasing data indicate that some *Dendromonocotyle* species can infect more than one host stingray species. This is cause for concern in public aquaria where multi-species elasmobranch displays are common.

The public aquarium at the Atlantis resort in Dubai is one of the newest international public aquaria, opening its doors to the public in September 2008. We recently received specimens from a monogenean outbreak on four *Himantura gerrardi* (Gray) specimens housed at the new aquarium and describe them here as a new species of *Dendromonocotyle*. We also give supplemental data for *Dendromonocotyle colorni* Chisholm, Whittington et Kearns, 2001 which was present on the same host stingrays. Ad-

ditional data are provided for *Dendromonocotyle kuhlii* Young, 1967 found on the dorsal skin surface of *Neotrygon kuhlii* (Müller et Henle) at Burgers' Zoo Aquarium in Arnhem, The Netherlands.

MATERIALS AND METHODS

Four *Himantura gerrardi*, identified using Compagno (2003), were collected in the Arabian Gulf and kept on display at the public aquarium at the Atlantis resort, Dubai. The stingrays exhibited signs of monogenean infections and were removed to the quarantine facility for closer inspection. Numerous monogeneans were noted on the dorsal skin surface. The rays were given a freshwater bath for 1.5 min to give them immediate relief from the infection. All monogeneans collected after the freshwater bath were transferred directly to 70% ethanol and shipped to the senior author for identification. *Dendromonocotyle kuhlii* specimens collected from *N. kuhlii* at Burgers' Zoo Aquarium were fixed in 70% ethanol and sent to the senior author for species confirmation in March 2008. Upon arrival, monogeneans were re-hydrated in freshwater, stained in Alum Carmine, dehydrated in a graded ethanol series, cleared in cedarwood oil and mounted permanently in Canada balsam on glass microscope slides. Hamuli and marginal hooklets of some specimens were examined after total digestion of surrounding soft tissue using the modified

proteolytic digestion technique of Harris et al. (1999) as used by Vaughan et al. (2008).

Mounted specimens were examined using an Olympus SZ60 Stereo Zoom dissection microscope fitted with an eyepiece micrometer and an Olympus CX41 compound light microscope fitted with phase-contrast and dark-field condensers. Drawings were made with the aid of a drawing tube. Measurements, given in micrometres as the mean $\pm$ standard deviation followed in parentheses by the range and number measured, were taken using Olympus AnalySIS[®] software and Altra20 digital microscope camera except for total body length and width measurements of *D. colorni* which were calculated using an eyepiece micrometer on the dissecting microscope. Any curved male copulatory organs were measured using the poly-line function of the software, measuring their true lengths following the curvature.

Taxonomic terminology follows that of Chisholm et al. (2001). The type series of the new species and the voucher material of *D. colorni* are deposited in the Iziko South African Museum (SAMCTA) in Cape Town, 8000, South Africa, and the Australian Helminthological Collection (AHC) at the South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia. Two voucher specimens of *D. kuhlii* are deposited in SAMCTA.

RESULTS

Monocotylidae Taschenberg, 1879

Dendromonocotyle Hargis, 1955

Dendromonocotyle lotteri sp. n. Figs. 1–4

Description. Based on 5 flattened adult specimens. Total body (Fig. 1) 3052 ± 442.3 (2720–3680, $n = 5$) long. Body proper 2693 ± 422.7 (2233–3167, $n = 5$) long and 2128 ± 349.0 (1800–2567, $n = 5$) maximum width at level of testis. Haptor 1379 ± 230.1 (1187–1681, $n = 5$) in diameter, divided into 8 peripheral and 1 central loculus (Fig. 1). Haptor rim consisting of 56 marginal papillae armed with 4–5 papillary sclerites (Figs. 1, 2A, ps). Terminal papillary sclerite as illustrated (Fig. 2A, tps). Marginal papillae to loculus associations as follows: posterior and postero-lateral loculi associated with 8 marginal papillae each; antero-lateral and anterior loculi associated with 6 loculi each. Tripartite sclerites (Fig. 2A, tri) observed at junction of radial septa and inner and outer-ring septa in some, but not all specimens (Fig. 1). Radial and inner ring septal sclerites uniform (Fig. 2A, ss). Outer ring sclerites as illustrated (Fig. 2A, os). Hamuli present, 88 ± 2.0 (86–90, $n = 3$) long (Figs. 1, 2B). Fourteen marginal hooklets (Fig. 2C) 13 ± 0.6 (12–14, $n = 8$) long, distributed symmetrically in marginal valve between every 4 papillae (Fig. 1). Mouth ventral, subterminal. Subterminal groove anterior to mouth. Six pairs of antero-lateral gland openings anterior to, and on either side of mouth (Fig. 1); type of secretion they contain could not be determined. Pharynx 372 ± 43.4 (336–420, $n = 5$) long, 289 ± 30.1 (262–328, $n = 5$) wide. Eyespots not observed, possibly obscured by pigmentation of intestinal caecum. Intestinal

caeca highly dendritic and heavily pigmented; extending from head to posterior portion of body proper (Fig. 1).

Ovary bi-lobed with branch looping right intestinal caecum before terminating at base of oötype (Figs. 1, 3A). Mehlis' gland present. Oötype 391 ± 33.7 (362–428, $n = 3$) long (Figs. 1, 3A). Tetrahedral egg (in distal portion of oötype) side length, 96 ± 2.4 (94–98, $n = 2$); short appendage present at one pole (Fig. 4A). Vaginal pore ventral, inconspicuous, positioned left of anterior-most part of ejaculatory bulb. Vagina 734 ± 44.7 (671–776, $n = 4$) long, widens proximally, containing 2 spherical structures before terminating in a muscular arrowhead-shaped base (Figs. 1, 3A). Transverse vitelline duct anterior to ovary, passing over base of oötype or posterior to it (Figs. 1, 3A). Seminal receptacle not seen, possibly obscured by pigmented branches of intestinal caecum. Vitellarium concealed between pigmented branches of intestinal caeca. No spermatophores observed.

Testis single (Fig. 1), 271 ± 25.4 (237–297, $n = 5$) long, 526 ± 73.1 (444–612, $n = 5$) wide. Vas deferens originates from left side of testis, obscured by intestinal caecum, travels anteriorly, dorsal to vagina and anterodorsally to ejaculatory bulb before inflating to form seminal vesicle (Figs. 1, 3B). Seminal vesicle then narrows and enters ejaculatory bulb posteriorly. Ejaculatory bulb 353 ± 28.4 (334–400, $n = 5$) in diameter. Male copulatory organ (Figs. 1, 3B) 1126 ± 45.7 (1073–1192, $n = 5$) long, 15 ± 0.4 (14–15, $n = 5$) wide; extends to level of posterior portion of ovary. Distal portion of male copulatory organ with weak corresponding s-curve bends and spiral sclerotised filament (Figs. 3B, 4B). Distal tip cone-shaped, tapering to a point. Entire length of male copulatory organ surrounded by muscular sheath 105 ± 26.2 (69–126, $n = 5$) wide.

Type host: *Himantura gerrardi* (Gray), Dasyatidae.

Type locality: Atlantis resort public aquarium, Dubai.

Site on host: Dorsal skin surface.

Material deposited: SAMCTA 29469 (holotype and 2 paratypes), AHC 29678 and 29679 (2 paratypes).

Etymology: This species is named after Mr. Paul Lötter, Director of Large Exhibits, Atlantis resort public aquarium, Dubai, who collected the original material.

Remarks. The distal portion of the male copulatory organ is distinct following a series of corresponding weak s-curves and is surrounded by a sclerotised spiral filament, before tapering into a cone-like terminal section. This unique morphology distinguishes *D. lotteri* from all other *Dendromonocotyle* species. *Dendromonocotyle lotteri* is the second species together with *D. colorni* in the group with 56 marginal papillae with a papillae to loculus association represented numerically as 6–6–8–8 (Vaughan et al. 2008). All other *Dendromonocotyle* species with 56 marginal papillae conform to the papillae to loculus association represented numerically as 6–7–7–8 (Vaughan et al. 2008).

Fig. 1. *Dendromonocotyle lotteri* sp. n. Ventral view composite. *Abbreviations:* alg – antero-lateral gland duct openings; dmco – distal portion of male copulatory organ; eb – ejaculatory bulb; ham – hamulus; i – intestinal caecum; m – mouth; mco – male copulatory organ; mh – marginal hooklet; mp – marginal papilla; ms – muscular sheath surrounding male copulatory organ; mv – marginal valve; o – ovary; ob – ovarian branch; oot – oötype; p – pharynx; sg – subterminal groove; sv – seminal vesicle; t – testis; tps – terminal papillary sclerite; tvd – transverse vitelline duct; v – vagina; vd – vas deferens; vp – vaginal pore. Scale bar = 1000 µm.

Dendromonocotyle colorni Chisholm, Whittington et Kearn, 2001

Type host: *Himantura uarnak* (Forsskål), Dasyatidae.

Additional host: *Himantura gerrardi* (Gray), Dasyatidae.

Type locality: Eilat Underwater Observatory, Israel.

Additional localities: Sea World at uShaka Marine World, Durban, South Africa (Vaughan et al. 2008); Atlantis resort public aquarium, Dubai (present study).

Site on host: Dorsal skin surface.

Material deposited: SAMCTA 29473 (10 vouchers), AHC 29680 (5 vouchers).

Remarks. Fifteen flattened adult worms were examined from specimens of *H. gerrardi* from Atlantis resort, Dubai. These specimens conform morphologically in most aspects to those of the original description of Chisholm et al. (2001) and the redescription of Vaughan et al. (2008). However, they are the largest representatives

Fig. 2. *Dendromonocotyle lotteri* sp. n. Haptoral armature. **A** – papillary sclerites (ps), terminal papillary sclerite (tps) and radial and inner-ring septal sclerite (ss) including variations of outer-ring septal sclerites (os), and tripartite sclerite (tri) at the junction of inner and outer ring and radial septa; **B** – hamulus; **C** – marginal hooklet. Scale bars: A = 30 μ m; B = 20 μ m; C = 10 μ m.

of the species collected to date with the total body length 4504 ± 1103.6 (3000–6200, $n = 12$) and the maximum width at the level of the testis 3569 ± 1130.3 (1720–5067, $n = 15$). The vas deferens passes anterior to the ejaculatory bulb before swelling to form the seminal vesicle in both the new voucher specimens and in the specimens described by Chisholm et al. (2001). In the material collected from *H. gerrardi* and *H. uarnak* from uShaka Marine World, Durban, South Africa, the vas deferens runs dorsally across the posterior section of the ejaculatory bulb (Vaughan et al. 2008). Tripartite sclerites are observed at the junctions of the radial septa and the inner and outer-ring septa in some voucher specimens, and the marginal papillae are armed with between 3–4 papillary sclerites, as seen in those from South Africa. This is the third report

of *D. colorni* from stingrays in captivity and the second report from the Northern hemisphere.

Dendromonocotyle kuhlii Young, 1967

Supplemental information. Based on 2 flattened juvenile specimens. Haptoral rim consisting of 56 marginal papillae armed with 3 papillary sclerites. Marginal papillae to loculus association as follows: posterior loculi associated with 8 marginal papillae each, postero-lateral and antero-lateral loculi with 7 marginal papillae each and anterior loculi associated with 6 marginal papillae each. Fourteen marginal hooklets 10 ± 0.9 (10–11, $n = 3$) long, distributed symmetrically in the marginal valve between every 4 papillae.

Fig. 3. *Dendromonocotyle lotteri* sp. n. Reproductive system. **A** – female reproductive system; **B** – male reproductive system. Abbreviations as in Fig. 1. me – Mehlis' gland. Scale bars = 500 μ m.

Fig. 4. *Dendromonocotyle lotteri* sp. n. **A** – egg (inside oötype); **B** – distal portion of the male copulatory organ showing the sclerotised filament (sf). Scale bars = 20 μ m.

Vas deferens originates from left side of testis, travels anteriorly, runs towards middle of anterior body section before inflating to form seminal vesicle. Male copulatory organ tightly coiled and folded back on itself, sclerotised, 254 ± 3.9 (252–257, $n = 2$) long, 3 ± 0.6 (3–4, $n = 2$) wide. Distal portion of male copulatory organ tapers to a point; accessory filament absent. Muscular sheath 22 ± 3.8 (19–24, $n = 2$) wide, surrounds entire length of male copulatory organ.

Type host: *Neotrygon kuhlii* (Müller et Henle) Dasyatidae.

Type locality: Moreton Bay, Queensland, Australia.

Additional locality: Burgers' Zoo Aquarium, Arnhem, The Netherlands (host stingrays originally obtained from Jakarta).

Site on host: Dorsal skin surface.

Material deposited: SAMCTA 29474 (2 vouchers).

Remarks. Both voucher specimens are juveniles. Three haptoral papillary sclerites are clearly visible on the marginal papillae and not 2 as indicated in the illustration of Young (1967). The male copulatory organ which is straight in adults (see Young 1967, Chisholm and Whittington 1995) is highly coiled in these juvenile specimens. This permits the long male copulatory organ to be accommodated in the smaller body (e.g. Chisholm and Whittington 1995, Chisholm et al. 2001). The male copulatory organ then straightens as the body grows. In addition, the vas deferens in one of the vouchers can clearly be seen originating from the left side of the testis and not from the middle of its anterior-most section as illustrated by Young (1967). This is the first record of *D. kuhlii* from a public aquarium.

Key to *Dendromonocotyle* species

- 1 Male copulatory organ not extending beyond level of posterior portion of ejaculatory bulb 2
- Male copulatory organ extending beyond level of posterior portion of ejaculatory bulb 3
- 2 Male copulatory organ with donut-shaped structure midway along length *D. centrourae*
- Male copulatory organ lacking donut-shaped structure midway along length *D. pipinna*
- 3 Haptoral septal pairs 2 and 3 not joining inner ring septum; outer ring septal sclerites absent; hamuli present *D. californica*
- All haptoral septa joining inner ring septum; outer ring septal sclerites present; hamuli present or absent 4
- 4 Haptor with 38 or 42 marginal papillae 5
- Haptor with 56 marginal papillae 7
- 5 Haptor with 38 marginal papillae; spines present at distal end of muscular sheath surrounding male copulatory organ *D. lasti*

- Haptor with 42 marginal papillae 6
- 6 Distal end of male copulatory organ looping, accessory filaments present *D. ardea*
- Distal end of male copulatory organ not looping, accessory filaments absent; vaginal pore armed with spines *D. taeniurae*
- 7 Hamuli present 8
- Hamuli absent 11
- 8 Inner wall of vagina armed with spines *D. ukuthena*
- Inner wall of vagina unarmed 9
- 9 Proximal end of vagina a sclerotised tightly coiled duct; haptoral papillae armed with 3–4 sclerites *D. colorni*
- Proximal end of vagina not a sclerotised tightly coiled duct 10
- 10 Haptoral papillae armed with 7–8 sclerites including terminal papillary sclerite; male copulatory organ not extending to level of ovary *D. akajeii*
- Haptoral papillae armed with 4–5 sclerites including terminal papillary sclerite; male copulatory organ extends to level of ovary *D. lotteri*
- 11 Distal end of male copulatory organ lacking accessory filament 12
- Distal end of male copulatory organ with accessory filament(s) 14
- 12 Male copulatory organ with distinct spherical inflation midway along its length *D. urogynni*
- Male copulatory organ without distinct spherical inflation midway along its length 13
- 13 Male copulatory organ extending beyond level of ovary; distal end simple tube coming to a point; terminal papillary sclerite keyhole-shaped *D. kuhlii*
- Male copulatory organ extending to level just beyond posterior portion of ejaculatory bulb; distal end widens; ovary anchor-shaped *D. torosa*
- 14 Distal end of male copulatory organ with 2 criss-crossed sperm ducts *D. bradsmithi*
- Distal end of male copulatory with single sperm duct 15
- 15 Distal end of sperm duct within male copulatory organ ending subterminally *D. octodiscus*
- Distal end of sperm duct within male copulatory organ ending terminally 16
- 16 Distal end of sperm duct within male copulatory organ looping once *D. cortesi*
- Distal end of sperm duct within male copulatory organ not looping *D. citrosa*

DISCUSSION

There are now 17 known representatives of *Dendromonocotyle*. Nine of these have been reported from stingrays in captivity including *D. centrourae* Cheung et Whittaker, 1993 from *Dasyatis centrourae* (Mitchill), *D. citrosa* Vaughan, Chisholm et Christison, 2008 from *Dasyatis chrysonota* (Smith) and *Himantura gerrardi*, *D. colorni* from *Himantura uarnak* and *H. gerrardi*, *D. kuhlii* from *Neotrygon kuhlii*, *D. lotteri* from *H. gerrardi*, *D. octodiscus* Hargis, 1955 from *Urobatis jamaicensis* (Cuvier), *D. pipinna* Chisholm et Whittington, 2004 from *Taeniurops meyeri* (Müller et Henle), *D. torosa* Chisholm et Whittington, 2004 from *Aetobatus narinari* (Euphrasen) and *D. ukuthena* Vaughan, Chisholm et Christison, 2008 from *H. uarnak* and *H. gerrardi*. Reports of *Dendromonocotyle* infections are increasing internationally and some have been responsible for direct skin damage and disease in stingrays kept in captivity. It is likely that the ecology of the captive environment favours the direct life-cycle of these monogeneans. The absence of effective quarantine procedures, host overcrowding/stress and poor water quality in the exhibit, would certainly play important roles in the onset of sudden population explosions of *Dendromonocotyle*. Chisholm et al. (2004) indicated that warm water temperature combined with the likelihood of stress in juvenile *T. meyeri*, caused by interactions with the aquarium patrons at Underwater World, Queensland, Australia, favoured increasing intensities of *D. pipinna*. Chisholm et al. (2004) found that a proactive approach to their management, by reducing host stress, supported a sustainable equilibrium between host and parasite. Identifying and reducing stressors is paramount, but not always possible. Host stress likely contributed to the problematic infections of *Dendromonocotyle* species reported by Vaughan et al. (2008) at Sea World at uShaka Marine World in Durban, South Africa. The source of the stress could not be pinpointed, but may have been the combined result of poor water quality, territorial interactions and feeding competition between species in the exhibition. In our opinion the most effective strategy is to actively prevent the introduction of *Dendromonocotyle* by using adequate preventative quarantine protocols prior to the stingrays being put into the display tanks.

Chisholm et al. (2004) discussed the use of freshwater baths and chemicals to treat *Dendromonocotyle* infections. However, they noted that when infections are already established in an exhibit, these treatments only give temporary relief because the rays are quickly re-infected by larvae hatching from eggs (which are resistant to treatment), present in the display tank. If the parasite eggs hatch spontaneously, then the simplest method to eliminate infections is to keep the hosts out of the tank until

it is determined that all eggs in the system have hatched and the larvae (24 h lifespan on average; see Whittington et al. 2000) have died. However, this strategy will not work if the eggs are waiting for another cue to hatch such as mechanical disturbance or specific chemicals secreted by the host (see Whittington et al. 2000, Chisholm et al. 2004). Furthermore, since we have shown that some *Dendromonocotyle* species are not strictly host specific, it may not be practical or cost effective to remove all rays from a display tank. Alternatively, treatments can be timed strategically to remove worms from the host after re-infection but prior to the worms reaching maturity and laying more eggs. Thus, after a number of well-timed treatments all eggs could effectively be removed from the system. However, establishing the timing of these treatments is no small feat. Extensive biological data including egg development times, hatching triggers, larval and adult longevity and parasite development time to maturity must be collected at the various temperatures encountered in the display tank.

Dendromonocotyle colorni was originally reported from *H. uarnak* kept in an outside saltwater pond at Israel's Eilat Underwater Observatory (Chisholm et al. 2001). This species is now reported from three different public aquaria from both *H. uarnak* and *H. gerrardi*. *Dendromonocotyle octodiscus* has also been reported from multiple host species (see Chisholm et al. 2004). Until recently, it was thought that a single ray species was only parasitized by a single species of *Dendromonocotyle*. However, Vaughan et al. (2008) reported the first sympatric association of three *Dendromonocotyle* species parasitizing *H. gerrardi* on exhibit at uShaka Sea World. Here we report the second occurrence of a sympatric association between *D. lotteri* and *D. colorni* on *H. gerrardi* kept in captivity. This provides growing support that holding multiple ray species together in captivity may promote opportunistic host-switching in *Dendromonocotyle* species. There are currently no reports of sympatric associations of *Dendromonocotyle* species occurring on wild populations of stingrays.

Public aquaria continue to be developed and with the pressures to produce presentable exhibits quickly, the introduction of fishes into new exhibits without the necessary quarantine procedures is becoming more common. This in turn will undoubtedly have an influence on the future occurrence of *Dendromonocotyle* species on captive stingrays as well as other parasites of aquarium fishes.

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