***Prochristianella mattisi* sp. n. (Trypanorhyncha: Eutetrarhynchidae) from the wedgenose skate, *Dipturus whitleyi* (Rajiformes: Rajidae), from Tasmania (Australia)**

Bjoern C. Schaeffner and Ian Beveridge

Department of Veterinary Science, The University of Melbourne, Veterinary Clinical Centre, Werribee, Victoria, Australia

Abstract: A new species of *Prochristianella* Dollfus, 1946 is described from the spiral intestine of the wedgenose skate, *Dipturus whitleyi* (Iredale) (Rajiformes: Rajidae), off the north-western coast of Tasmania (Australia). *Prochristianella mattisi* sp. n. is characterised by an acraspedote scolex, two oval bothria, elongate, bent bulbs, a retractor muscle inserting at the base of each bulb and the presence of gland-cells within the bulbs and prebulbar organs. The tentacular armature is typical heteroacanthous, heteromorphous, with a characteristic basal oncotaxy and a metabasal armature with hooks first increasing and then decreasing in size along each principle row. It can be differentiated from other species of *Prochristianella* by a combination of morphological characters, such as the metabasal tentacular armature with eight hooks per principle row, a unique basal armature without enlarged hooks on the basal swelling and genital pores slightly posterior to the mid-line of the segment. The description of *P. mattisi* sp. n. increases the number of known species within *Prochristianella* to 20, eight of which occur in Australian waters. A key for the identification to species within *Prochristianella* is provided.

Keywords: tapeworms, Cestoda, taxonomy, parasites, *Prochristianella*, rays, identification key

The eutetrarhynchid genus *Prochristianella* Dollfus, 1946 accommodates species with a characteristic basal tentacular armature and a metabasal armature with an initial gradual increase of hook sizes followed by a gradual decrease along the principle rows (Dollfus 1946). Beveridge et al. (2004) provided the most recent generic diagnosis. However, a uniform diagnosis proved problematic, because intermediate forms now exist exhibiting morphological characteristics of the closely related genera *Dollfusiella* Campbell et Beveridge, 1994 and *Eutetrarhynchus* Pintner, 1913 (see Schaeffner and Beveridge 2012). The first key for the morphological identification of species within *Prochristianella* was compiled by Palm (2004), including 13 species. Since then, several species have been added. Friggens and Duszynski (2005) described *Prochristianella multum* Friggens et Duszynski, 2005 from Mexico, while Beveridge and Justine (2010) added two species from New Caledonia, *P. aciculata* Beveridge et Justine, 2010 and *P. omunae* Beveridge et Justine, 2010.

In the most recent study, Schaeffner and Beveridge (2012) described four additional species of *Prochristianella* from Australia and Borneo. These authors also synonymised *P. macracantha* Palm, 2004 with *P. butlerae* Beveridge, 1990, due to apparent morphological similarities, and provided new host and locality records

for three additional species. To date, 19 species of *Prochristianella* are considered valid, namely: *P. aciculata*; *P. butlerae*; *P. cairae* Schaeffner et Beveridge, 2012; *P. clarkeae* Beveridge, 1990; *P. fragilis* Heinz et Dailey, 1974; *P. glabra* (Dollfus, 1969); *P. heteracantha* Dailey et Carvajal, 1976; *P. hispida* (Linton, 1890); *P. jensenae* Schaeffner et Beveridge, 2012; *P. kostadinovae* Schaeffner et Beveridge, 2012; *P. minima* Heinz et Dailey, 1974; *P. mooreae* Beveridge, 1990; *P. multum*; *P. odonoghuei* Beveridge, 1990; *P. omunae*; *P. papillifer* (Poyarkoff, 1909) (type-species); *P. scholzi* Schaeffner et Beveridge, 2012; *P. thalassia* (Kovacs et Schmidt, 1980) and *P. tumidula* (Linton, 1890).

The present material represents a new species of *Prochristianella*, increasing the number of valid species within the genus to 20. *Prochristianella*, thus, represents one of the most species-rich genera within the Eutetrarhynchidae. For this reason, a key for the species of *Prochristianella* is provided.

MATERIALS AND METHODS

Specimens of this study were obtained from the spiral intestine of the wedgenose skate, *Dipturus whitleyi* (Iredale), examined from the Bass Strait off Stanley, Tasmania, Australia. Cestodes were fixed in 10% formalin for morphological observation and later placed in 70% ethanol. Selected specimens

were processed as described by Schaeffner et al. (2011). Line drawings of mounted specimens were made using an Olympus BH2 microscope with a drawing tube and Nomarski interference contrast. The terminology of morphological characteristics follows Dollfus (1942) and Campbell and Beveridge (1994). The attachment organs are referred to as bothria following Hyman (1951), Caira et al. (1999) and Jones et al. (2004). Microtrix terminology follows Chervy (2009). Measurements are in micrometres (μm), unless otherwise indicated, and are given as the range followed in parentheses by the mean, standard deviation and the number of features measured. Vitelline follicles are shown on the lateral margins of segments only, unless otherwise indicated. Three specimens were selected for scanning electron microscopy (SEM) and processed as described in Schaeffner et al. (2011). Scanning electron micrographs were taken using a Hitachi S-570 scanning electron microscope (Hitachi Ltd.). Type-specimens mounted on microscope slides have been deposited in the helminthological collections of the South Australian Museum, Adelaide, Australia (SAM), and the United States National Parasite Collection, Beltsville, Maryland, USA (USNPC). Host classification follows Naylor et al. (2012).

RESULTS

Prochristianella mattisi sp. n.

Figs. 1–7

Description. Based on 11 whole-mounts, two specimens in glycerol, three specimens used for SEM. Mature cestodes 4.2–5.5 (4.7 ± 0.7 ; $n = 3$) mm long, with 6–11 (9 ± 3 ; $n = 3$) segments in mature strobilae; strobila with 5–9 (7 ± 2 ; $n = 3$) immature segments and 1–2 (1 ; $n = 3$) mature segments; gravid segments not present. Scolex acraspedote (Figs. 1, 2), 1 500–2 030 ($1\,637 \pm 168$; $n = 9$) long; maximum width at level of pars bothrialis, 430–530 (487 ± 36 ; $n = 6$) wide; pars bothrialis 360–420 (395 ± 20 ; $n = 10$) long; bothria two in number, oval, 350–450 (390 ± 37 ; $n = 10$) long by 350–420 (397 ± 24 ; $n = 10$) wide, with free posterior margins, prominent posterior notch absent, V-shaped ridge seen in SEM specimens only; distal bothrial surface covered with columnar spinitriches; proximal bothrial surface covered with acicular filitriches; bothrial pits absent; pars vaginalis longer than pars bothrialis, 870–1 220 (972 ± 109 ; $n = 10$) long; tentacle sheaths thin, sinuous to irregularly coiled, 15–20 (19 ± 2 ; $n = 10$) in diameter; pars bulbosa 590–810 (654 ± 70 ; $n = 10$) long by 290–410 (347 ± 43 ; $n = 11$) wide; prebulbar organs present, small; bulbs elongate (Fig. 3), bent, thick-walled, 600–760 (649 ± 53 ; $n = 22$) long by 100–130 (117 ± 10 ; $n = 22$) wide, in some specimens reach to pars proliferans scolecis; bulb width : length ratio 1.0 : 4.6–7.5 (5.6 ± 0.7 ; $n = 22$); retractor muscle broad (Fig. 3), originates at base of bulb; gland-cells present, in posterior third to mid-region of bulb (Fig. 3); pars post-bulbosa absent; scolex ratio (pars bothrialis : pars vaginalis : pars bulbosa) 1.0 : 2.1–2.9 : 1.4–1.9 ($1.0 : 2.5 \pm 0.2 : 1.6 \pm 0.2$; $n = 9$).

Fully everted tentacle 940 ($n = 1$) long; tentacle diameter 28–33 (30 ± 2 ; $n = 6$) at base, 23–28 (25 ± 2 ; $n = 6$) in metabasal region, 20–23 (21 ± 1 ; $n = 6$) in distal re-

gion. Armature heteroacanthous, heteromorphous; hooks hollow, in ascending half spiral rows (Figs. 5, 6). Hook files begin on antiothrial surface of tentacle (Fig. 5), terminate on bothrial surface (Fig. 6); 8 hooks per principle row; hook files 1 and 1' slightly spaced (Fig. 5). Hooks 1 (1') uncinat, 10–13 (11 ± 1 ; $n = 15$) long, base 6–8 (7 ; $n = 15$) long; hooks 2 (2') uncinat, slightly larger, 10–14 (12 ± 1 ; $n = 9$) long, base 5–8 (7 ± 1 ; $n = 19$) long; hooks 3 (3') falcate, larger, 12–15 (13 ± 1 ; $n = 15$) long, base narrower, 4–5 (4 ; $n = 15$) long; hooks 4 (4') falcate, smaller, 6–9 (8 ± 1 ; $n = 15$) long, base 3–4 (4 ± 1 ; $n = 15$) long; hooks 5 (5') falcate, larger, 11–14 (12 ± 1 ; $n = 15$) long, base 4–5 (4 ; $n = 15$) long; hooks 6 (6')–8 (8') spiniform, of similar size, 6–7 (6 ; $n = 30$) long, base 1–3 (2 ± 1 ; $n = 30$) long.

Distinctive basal armature present (Fig. 7); basal hooks on external surface uncinat, 4–7 (6 ± 1 ; $n = 5$) long, base 2–4 (3 ± 1 ; $n = 5$) long; basal hooks on bothrial and antiothrial surfaces falcate, greatly enlarged, 8–12 (9 ± 1 ; $n = 15$) long, base 1–3 (2 ; $n = 15$) long; patch devoid of hooks on external surface; hooks anterior to patch falcate, small, 3–4 (3 ; $n = 11$) long, base *c.* 1 ($n = 11$) long; basal swelling weakly developed, with *c.* 11 rows of billhooks (Fig. 7); billhooks of almost similar size (Fig. 7), 3–5 (4 ± 1 ; $n = 20$) long, base *c.* 1 ($n = 20$) long; billhooks become uncinat towards bothrial surface anterior to hook row 11 on basal swelling (Fig. 7), 2–3 (2 ; $n = 7$) long, base 2–3 (2 ; $n = 7$) long; hooks of first principle rows uncinat, of almost similar size (Fig. 7), 5–6 (6 ± 1 ; $n = 6$) long, base 4–5 (4 ± 1 ; $n = 6$) long.

Segments acraspedote; immature segments 30–50 (35 ± 9 ; $n = 5$) long by 230–320 (262 ± 35 ; $n = 5$) wide; terminal mature segments 1 390–1 430 ($1\,410 \pm 28$; $n = 2$) long by 230–310 (270 ± 56 ; $n = 2$) wide. Genital pores slightly posterior to mid-line of segment (Fig. 4); pore inconspicuous; cirrus sac ovoid (Fig. 4), 88 ($n = 1$) long by 113 ($n = 1$) wide; cirrus unarmed; internal and external seminal vesicles absent. Vas deferens not observed. Testes arranged in two columns, in 1–2 layers, exclusively preovarian, absent in region of cirrus sac (Fig. 4), 58–68 (59 ± 3 ; $n = 10$) long by 33–38 (36 ± 2 ; $n = 10$) wide; total number of testes 58–71 (64 ± 7 ; $n = 3$), with 38–41 (39 ± 2 ; $n = 3$) preporal, and 20–30 (25 ± 5 ; $n = 3$) postporal. Vagina enters genital atrium posterior to cirrus-sac (Fig. 4); course of vagina not observed; seminal receptacle absent. Ovary at posterior margin of segment, bilobed in dorsoventral view; ovarian lobes 75–78 ($n = 2$) long by 35–40 ($n = 2$) wide. Mehli's gland between posterior parts of ovarian lobes; *c.* 55 ($n = 1$) in diameter. Vitelline follicles small, circumcortical, 10–15 (13 ± 2 ; $n = 10$) in diameter. Uterus median, tubular, simple, extending between rows of testes to anterior extremity of segment; uterine pore absent.

Type and only host: *Dipturus whitleyi* (Iredale) (Rajiformes: Rajidae).

Figs. 1–4. Line drawings of *Prochristianella mattisi* sp. n. **Fig. 1.** Scolex in lateral view. **Fig. 2.** Scolex in dorso-ventral view. **Fig. 3.** Bulb. **Fig. 4.** Mature segment. *Abbreviations:* c – cirrus; cs – cirrus-sac; mg – Mehlis' gland; ov – ovary; t – testis; va – vagina; vit – vitelline follicle.

Type locality: Bass Strait off Stanley, Tasmania, Australia (40°37'S, 17°43'E).

Additional localities: None.

Site of infection: Spiral intestine.

Specimens deposited: Holotype in SAM (AHC 35627); paratypes in SAM (AHC 35628–31) and USNPC (106132–33).

Etymology: This species is dedicated to Tom E. Mattis, who passed away at the end of 2012, for his efforts in elucidating the life-cycles of trypanorhynchs, many productive discussions and his lively interest in species of the genus *Prochristianella*.

Remarks. The species described above belongs to the eutetrarhynchid genus *Prochristianella*, due to its acraspedote, slender scolex, two oval bothria, bulbs with gland-cells, a retractor muscle inserting at the base of the bulb and the presence of prebulbar organs. The tentacular armature shows a basal swelling, with a characteristic basal armature and a heteroacanthous metabasal region, with hooks gradually increasing and then decreasing in size along each principle row. However, the present specimens show the gradation of hooks along each principle row in the metabasal armature being interrupted by

Figs. 5–7. Line drawings of tentacular armature of *Prochristianella mattisi* sp. n. **Fig. 5.** Metabasal armature, antibothrial surface. **Fig. 6.** Metabasal armature, bothrial surface. Note: opposing tentacular surface to tentacle illustrated in Fig. 5. **Fig. 7.** Basal armature, external surface.

much smaller hooks 4 (4') compared to the neighbouring hooks 3 (3') and 5 (5') and also possess only slight space between hooks 1 and 1'. These features differ from the generic diagnosis provided by Beveridge et al. (2004). The diagnosis provided by these authors (Beveridge et al. 2004), however, is not applicable for all species within *Prochristianella*, as discussed in detail by Schaeffner and Beveridge (2012).

Prochristianella mattisi is characterised by a relatively large scolex lacking enlarged microtriches covering its surface and segments with more than 50 testes, a basal tentacular armature with a basal swelling with similarly sized hooks and absence of enlarged billhooks, and eight hooks per principle row in the metabasal armature. Because of these morphological characteristics, we consider the present specimens to represent a new species, which

can be readily distinguished from all other congeners. Unfortunately, only very few, slightly convoluted mature segments were present and detailed features of the cirrus sac cannot be provided at present.

The new species is described from a single locality in Tasmania and was obtained from two specimens of the wedgenose skate, *Dipturus whitleyi*. Only two other trypanorhynch species are reported from this host, namely *Dollfusiella martini* (Beveridge) Beveridge et Jones and *P. clarkeae* - Beveridge (1990). The geographic distribution of *D. whitleyi* is limited to the eastern Indian Ocean, where it occurs exclusively in southern Australian waters. This relatively narrow distribution of the host and the occurrence of this parasite in a single host species lead to the assumption that *P. mattisi* might be a parasite endemic to the southern ocean with a high host specificity.

Key to the species of *Prochristianella*

This key is simplified, with only the most easily observed characteristics utilised. To confirm identifications, original species descriptions and/or re-descriptions should be consulted.

- 1 Scolex covered with enlarged, microscopically visible microtriches 2
 - Scolex lacking enlarged microtriches 6
- 2 Cestode longer than 4 mm 3
 - Cestode shorter than 4 mm 4
- 3 Short, ovoid bulbs; bulb ratio 1.0 : 5.4; 43–57 testes *P. cairae*
 - Long, slender bulbs; bulb ratio 1.0 : 10.6; 28–35 testes *P. thalassia*
- 4 Scolex larger than 1 mm; more than 40 testes 5
 - Scolex smaller than 1 mm; 30–37 testes *P. multidum*
- 5 Metabasal tentacular armature with greatly enlarged, uncinuate hook 1 and much smaller, falcate hook 1' ... *P. hispida*
 - Metabasal armature with uncinuate hooks 1 (1'); hooks 1 only slightly larger than hooks 1' *P. clarkeae*
- 6 Segments with fewer than 25 testes 7
 - Segments with more than 30 testes 8
- 7 Scolex 530–680 µm long; bulbs 180–350 µm long and 30 to 50 µm wide; metabasal tentacular armature with 9 hooks per principle row; hooks 1 (1') very small; enlarged hooks at base of tentacle absent *P. minima*
 - Scolex 692–1 200 µm long; bulbs 325–470 µm long and 54–75 µm wide; metabasal tentacular armature with 13–15 hooks per principle row; hooks 1 (1') large and robust; enlarged hooks at base of tentacle present *P. omunae*
- 8 Pre- and postporal testes in equal numbers; genital pores median; gland-cells within bulbs absent *P. jensenae*
 - Greater number of preporal testes than postporal ones; genital pores in posterior half of segment; gland-cells within bulbs present 9
- 9 Scolex longer than 3 mm; bulbs longer than 1 mm *P. glabra*
 - Scolex shorter than 2.2 mm; bulbs shorter than 0.85 mm 10
- 10 Hooks 1 (1') represent largest hooks within principle row in metabasal armature *P. heteracantha*
 - Hooks 1 (1') smaller than hooks 2 (2') 11
- 11 Gradation in hook sizes (increase and subsequent decrease) along principle row interrupted by smaller hook 12
 - Gradual increase and decrease of hook sizes along principle row 16
- 12 Bulbs shorter than 420 µm; fewer than 50 testes per segment 13
 - Bulbs longer than 450 µm; more than 50 testes per segment 14
- 13 Scolex 960–1 250 µm long; bulbs 330–420 µm long; hooks 1 (1') and 2 (2') present in proximal 4–6 rows of metabasal armature, absent anteriorly *P. mooreae*
 - Scolex 640–1 050 µm long; bulbs 250–340 µm long; hooks 1 (1') and 2 (2') persist along the tentacle *P. aciculata*
- 14 Bulbs longer than 600 µm; retractor muscle within bulb very wide; 8 hooks per principle row in metabasal armature *P. mattisi*
 - Bulbs shorter than 570 µm; retractor muscle not widened; 10 hooks per principle row 15
- 15 Gland-cells inconspicuous; enlarged hooks at base of tentacle present; 2 additional hooks posterior to hooks 1 (1') present in most proximal part of metabasal armature *P. scholzi*
 - Gland-cells large; enlarged hooks at base of tentacle absent; additional hooks posterior to hooks 1 (1') absent in metabasal armature *P. kostadinovae*
- 16 Additional, small hooks at commencement of principle rows in transition from basal to metabasal regions present 17
 - Additional hooks at commencement of principle rows absent 18
- 17 Total length 6.4–12.8 mm; bulbs 360–480 µm long and 60–90 µm wide; segments with 34–65 testes *P. papillifer*
 - Total length 3.5–4.2 mm; bulbs 360–370 µm long and 50–60 µm wide; segments with about 63 testes *P. tumidula*
- 18 Several, greatly enlarged, characteristic billhooks on basal swelling; 3 greatly enlarged, falcate hooks at base of tentacle *P. odonoghuei*
 - Greatly enlarged billhooks absent on basal swelling; basal hooks not or only slightly enlarged 19
- 19 Principle rows in metabasal armature with dissimilar number of hooks (i.e. 8 and 10 hooks); principle rows begin on antiothrial surface and terminate on bothrial surface *P. butlerae*
 - Principle rows in metabasal armature with equal number of hooks; principle rows begin on bothrial surface and terminate on antiothrial surface *P. fragilis*

DISCUSSION

The current description of a new species of *Prochrochianella* brings the total number of species within the genus to 20. It also allows a more precise definition of the genus based on morphological grounds. However, some species show morphological inconsistencies with the most recent generic diagnosis by Beveridge et al. (2004). Five species (i.e. *P. aciculata*, *P. kostadinovae*, *P. mattisi*, *P. mooreae* and *P. scholzi*), for instance, show a gradation in hook sizes along the principle rows (i.e. hook sizes first increasing and then decreasing), which is interrupted by a much smaller hook in-between two larger ones. In *P. heteracantha*, hooks 1 and 1' are much larger than the succeeding hooks of the principle row, which, by definition, would make its placement within *Prochrochianella* dubious. One species, *P. jensena*, however, represents the most remarkable species currently placed in the genus. It lacks both gland-cells within the muscular bulbs and a basal swelling on the tentacular armature. Although Schaeffner and Beveridge (2012) supported its placement within *Prochrochianella*, they admitted that subsequent molecular analyses might reveal an independent status of this species, which could then lead to the erection of a separate genus (see Schaeffner and Beveridge 2012). All species of *Prochrochianella*, which are currently considered valid, have been included in a key (see above).

In recent molecular phylogenetic studies performed by Palm et al. (2009) and Olson et al. (2010), species of *Prochrochianella* formed two distinct clusters, rendering the genus polyphyletic (see Schaeffner and Beveridge 2012). The first clade included species with solid hooks and microscopically visible, enlarged microtriches cover-

ing the scolex peduncle (e.g. *P. clarkeae* and *P. hispida*), whereas the second cluster grouped species with hollow hooks and lacking enlarged microtriches on their scoleces (e.g. *P. aciculata* and *P. scholzi*) (see Schaeffner and Beveridge 2012). The addition of species of *Prochrochianella* with solid or hollow hooks and with or without enlarged microtriches in future phylogenetic studies could reveal the importance of these morphological characters. This might subsequently split the genus into *Prochrochianella* (i.e. with hollow hooks and absence of enlarged microtriches) and a separate, closely related genus (i.e. for taxa with solid hooks and presence of enlarged microtriches). However, until further studies reveal a separation of species based on these features, we tentatively retain all species in the same genus.

Based on some morphological characteristics presented by several species of *Prochrochianella*, we emphasise the importance of ethanol-fixed material for molecular studies. Further phylogenetic studies might provide additional information on the interrelationships of species. Furthermore, new morphological characters are needed to confirm the existence and membership of monophyletic groups within the genus. Although an updated generic diagnosis might be necessary, we are unable to provide that information at present.

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