



A new species of *Dollfusiella* Campbell & Beveridge, 1994 (Cestoda: Eutetrarhynchidae), with remarks on *Halysiorhynchus macrocephalus* (Shiple & Hornell, 1906) (Cestoda: Mixodigmatidae) from the bowmouth guitarfish *Rhina ancylostoma* Bloch & Schneider (Rhinidae) in the Persian Gulf

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Abstract *Dollfusiella nimai* n. sp. (Cestoda: Eutetrarhynchidae) is described from the intestine of *Rhina ancylostoma* Bloch & Schneider in the Persian Gulf. The number of the hooks per half spiral row in the metabasal tentacular armature distinguishes the new species from its congeners, except for *D. vooremi* (São Clemente & Gomes, 1989) possessing approximately the same number of hooks per half spiral row. While the principle hooks 1(1′)–21(21′) were homeomorphous in the metabasal armature of *D. nimai* n. sp., the billhooks on the antibothrial surface and the uncinat hooks on the bothrial surface were the principle hooks 1(1′)–16(16′) in the metabasal armature of *D. vooremi*. *Dollfusiella nimai* n. sp. most closely resembles *D. michiae* (Southwell, 1929) in the tentacular armature as well as the morphology of the scolex and strobila but differs clearly in the number of the hooks

per half spiral row in the metabasal tentacular armature (25–26 vs 16 respectively). A detailed examination of the specimens of *Halysiorhynchus macrocephalus* (Shiple & Hornell, 1906) (Cestoda: Mixodigmatidae) ex *R. ancylostoma* from the Persian Gulf revealed intraspecific variability including the number of the principle hooks per half spiral row in the metabasal armature, the number of the hook rows in the basal armature, and the size of the basal hooks.

Introduction

Of the 26 eutetrarhynchoid genera, *Dollfusiella* Campbell & Beveridge, 1994 is the most specious genus with 30 species and *Halysiorhynchus* Pintner, 1913 is one of the nine monotypic genera (Beveridge et al., 2017). The members of *Dollfusiella* infect both the batoids, i.e. dasyatids, myliobatids, rhinobatids, rhinids, and arhynchobatids (see Palm, 2004; Campbell & Beveridge, 2009; Schaeffner & Beveridge, 2013; Menoret & Ivanov, 2014, 2015), as well as the triakid and hemiscylliid sharks (Palm, 2004; Schaeffner & Beveridge, 2013). The single species of *Halysiorhynchus*, *H. macrocephalus* (Shiple & Hornell, 1906), infects elasmobranchs belonging to the families Dasyatidae, Gymnuridae and Rhinidae (see Palm, 2004).

Dollfusiella is the most diverse genus in the Persian Gulf (Haseli et al., 2010; Haseli & Palm, 2015; Haseli

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et al., 2017), infecting seven host species of the families Dasyatidae, Myliobatidae, and Rhinobatidae. In contrast, the single species of *Halysiorhynchus* was reported from *Pastinachus* cf. *sephen* (Forsskål) (Dasyatidae) and *Rhynchobatus* sp. (Rhinidae) (Haseli et al., 2010). Among the members of the family Rhinidae in the Persian Gulf, there is no information on the trypanorhynch fauna of *Rhina ancylostoma* Bloch & Schneider. Nonetheless, outside this region, *D. michiae* (Southwell, 1929) off the Northern Territory coast, Australia (Campbell & Beveridge, 2009), *Halysiorhynchus macrocephalus* from off the coast of Borneo (Schaeffner & Beveridge, 2014), and *Mixonybelinia southwelli* (Palm & Walter, 1999) off Sri Lanka (Palm & Walter, 1999) were earlier reported from this host species.

In the present study, examination of the trypanorhynch fauna of *R. ancylostoma* from the Persian Gulf resulted in the description of a new species of *Dollfusiella* and a report of *H. macrocephalus*.

Materials and methods

In November 2014, the local fishermen caught two specimens of *R. ancylostoma* (one male and one female; total length 76–191 cm) from off the coast of Bandar Lengeh, north-eastern Persian Gulf, Iran. The intestine of each fish was removed, cut longitudinally along the ventral side, and placed into a plastic bag filled with 10% seawater-buffered formalin. The cestodes were isolated using the stereomicroscope, stored in 70% ethanol, stained with acetic carmine, dehydrated in an ethanol series, cleared in methyl salicylate, and mounted on slides in Canada balsam. The taxonomically important structures of the new species were drawn with the aid of a drawing tube attached to an Olympus CH2 microscope. The vitelline follicles are shown only on the lateral margins of the segment. The identification of the specimens of *H. macrocephalus* was carried out using Palm's (2004) monograph.

The specimens prepared for scanning electron microscopy were hydrated, stored in 1% osmium tetroxide for 20 hours at 4 °C, dehydrated in an ethanol series, dried in hexamethyldisilazane, mounted on stubs, coated with gold using a K450X carbon coater (Quorum Technologies) to a thickness of 5 nm, and examined using a Vega 2 LM scanning electron microscope (Tescan Orsay Holding) at 15 kv.

One of the mounted paratypes of the new species was selected for histology, demounted using xylene, and embedded in paraffin. The transverse serial sections were cut at a thickness of 6 µm. The sections on the slide were deparaffinized with xylene, washed with 100% ethanol, hydrated to 70% ethanol, stained with acetic carmine, dehydrated in an ethanol series, cleared in methyl salicylate, and mounted on slides in Canada balsam.

Measurements taken using an ocular micrometer are reported in micrometers and presented as the range followed by the mean, standard deviation (when $n \geq 15$), the number of the measured worms (N) and the total number of measurements for each character (n) in parentheses. In measuring the microtriches, ImageJ 1.46r (Wayne Rasband, NIH, USA) was used. We followed Chervy (2009) for the terminology of the microtriches.

The mounted type-specimens along with the histological cross-sections of the new species and the voucher specimens of *H. macrocephalus* have been deposited in the Muséum d'Histoire Naturelle, Geneva, Switzerland (MHNG).

Family Eutetrarhynchidae Guiart, 1927

Genus *Dollfusiella* Campbell & Beveridge, 1994

Dollfusiella nimai n. sp.

Type-host: *Rhina ancylostoma* Bloch & Schneider, 1801 (Rhinopristiformes: Rhinidae).

Type-locality: Off Bandar Lengeh (26°25'N, 54°57'E), Persian Gulf, Iran.

Site in host: Spiral intestine.

Prevalence: 50% (1 of 2 individuals examined, 66 worms per host).

Type-material: Holotype (MHNG-PLAT-121651; 1 slide); 25 paratypes (permanent mounts, MHNG-PLAT-121652; 31 slides); cross-sections of 1 strobila (MHNG-PLAT-121653; 4 slides); material prepared for SEM is retained in the personal collection of Mohammad Haseli.

Etymology: This species is named in honour of Nima Rasa, who helped the first author in sampling.

Description (Figs. 1–4)

[Based on whole mounts of 14 immature, 6 mature and 6 gravid specimens; 2 scoleces observed with SEM; cross-sections of 1 mature strobila.] Small worms,

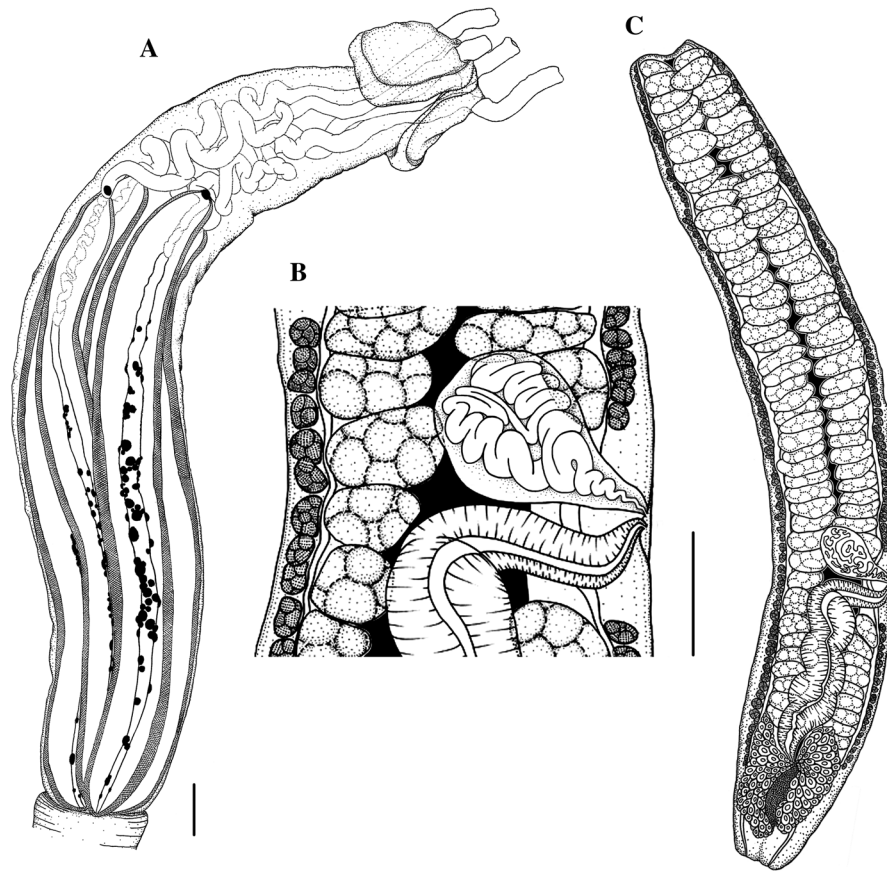


Fig. 1 *Dollfusiella nimai* n. sp. A, Scolex; B, Terminal genitalia (vitelline follicles shown only on the lateral margins of the segment); C, Mature segment (vitelline follicles shown only on the lateral margins of the segment). Scale-bars: A, C, 200 μ m; B, 100 μ m

10,187–22,008 (15,576, N = 8) long, with 21–27 (24 ± 2 , N = 11) segments. Scolex surface with or without microscopically visible microtriches; scolex, elongate, acraspedote (Fig. 1A), 3,029–4,188 ($3,501 \pm 266$, N = 23) long, with elongate tentacles (Fig. 2); scolex width 287–535 (400 ± 62 , N = 16) at level of pars bothrialis, 297–485 (409 ± 55 , N = 22) at level of pars vaginalis, 485–673 (567 ± 46 , N = 23) at level of pars bulbosa. Bothria 2 in number with free margins, oval, 327–505 (402 ± 44 , N = 22, n = 28) long, 239–436 (336 ± 41 , N = 15, n = 29) wide, with posterior notch in some specimens only (Fig. 1A); bothrial pits absent; distal bothrial surface covered with columnar spinitriches (Fig. 3D), 2.2–2.7 (2.4 , N = 1, n = 7) long. Scolex peduncle covered with trifid spinitriches (Fig. 3E, F), 7.2–10.4 (8.2 , N = 1, n = 10) long, possessing prongs comprising 3–8% (5 ± 1 , N = 1, n = 30) of spinitrix length. Pars bothrialis 346–535 (426 ± 47 , N = 24) long; pars vaginalis 1,158–1,990 (1,417

± 218 , N = 23) long; tentacle sheaths coiled posteriorly, straight to sinuous anteriorly (Fig. 1A), 39–80 (58 ± 9 , N = 23, n = 56) in diameter; pars bulbosa 1,960–2,386 ($2,223 \pm 113$, N = 23) long; prebulbar organs present (Fig. 1A); bulbs elongate (Fig. 1A), 1,900–2,366 ($2,146 \pm 121$, N = 23, n = 92) long, 149–288 (222 ± 24 , N = 23, n = 92) wide, bulb width: length ratio 1.0: 7.2–13.3 (9.8 ± 1.1 , N = 23, n = 92); retractor muscles originate at posterior extremity of bulbs (Fig. 1A); gland-cells attached to retractor muscle within bulb (Fig. 1A); pars post-bulbosa absent. Scolex ratio (pars bothrialis: pars vaginalis: pars bulbosa) 1.0: 1.1–2.1: 4.3–6.5 (1.0: 1.6: 5.3; N = 23).

Tentacles 3,473–4,405 (3,886, N = 5, n = 14) long, emerging from anterior bothrial margins; basal swelling present (Fig. 2D–F), with maximum width 48–87 (62 ± 7 , N = 15, n = 40), tentacle width 37–82 (52 ± 7 , N = 15, n = 24) at level of metabasal region, 31–57 (49

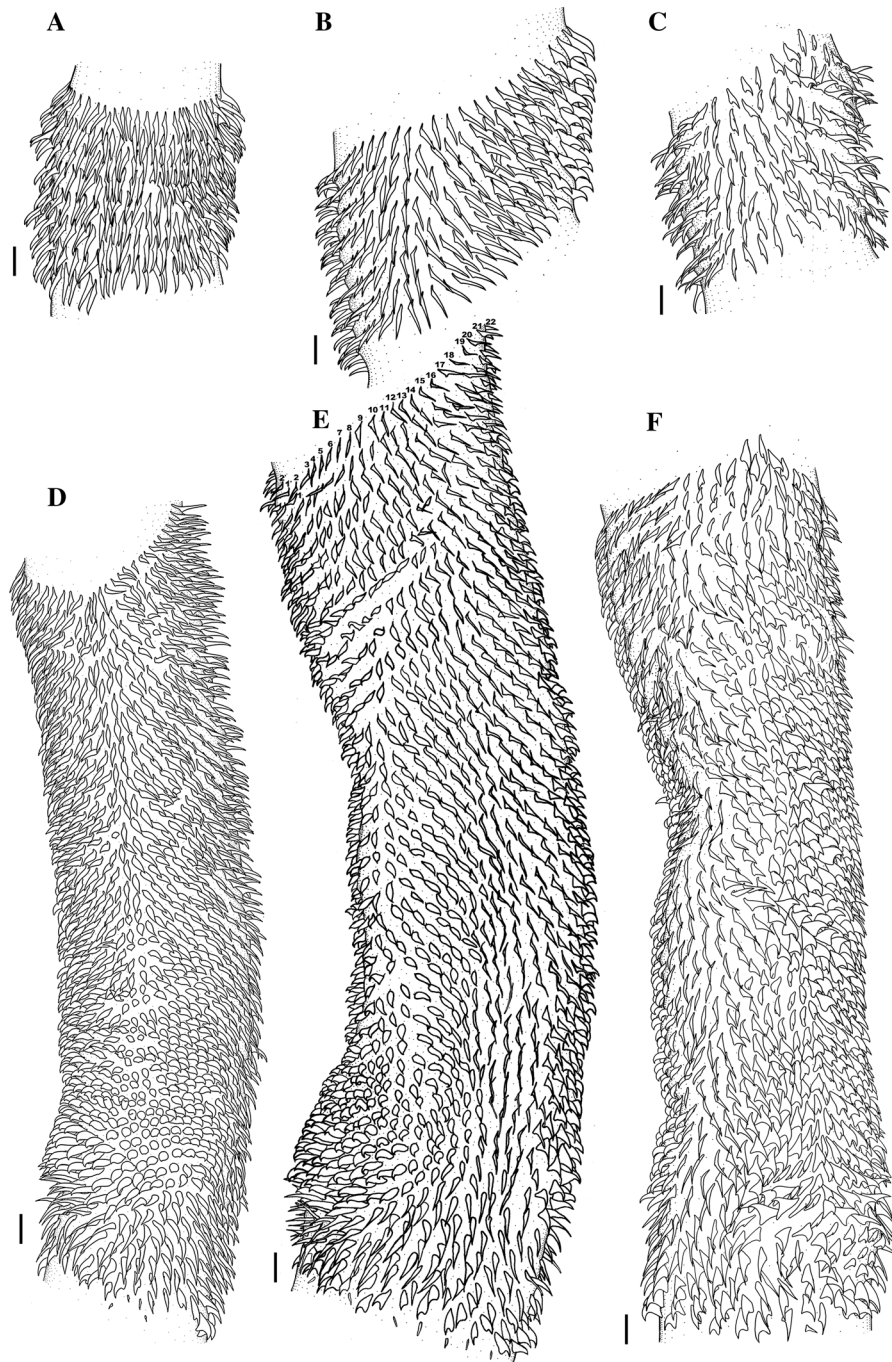


Fig. 2 Tentacular armature of *Dollfusiella nimai* n. sp. A, Antibothrial surface, metabasal armature; B, External surface, metabasal armature; C, Bothrial surface, metabasal armature; D, Antibothrial surface, basal armature; E, External surface, basal armature; F, Bothrial surface, basal armature. Scale-bars: 10 μ m

± 8 , $N = 5$, $n = 17$) at level of distal region of tentacle. Hooks hollow, tentacles with *c.*650 rows of hooks; hook files begin on antibothrial surface and terminate

on bothrial surface (Figs. 2A–F, 3A–C). Characteristic basal armature present with *c.*42 hook rows (Figs. 2D–F, 3A, B); initial 2 rows of hooks with

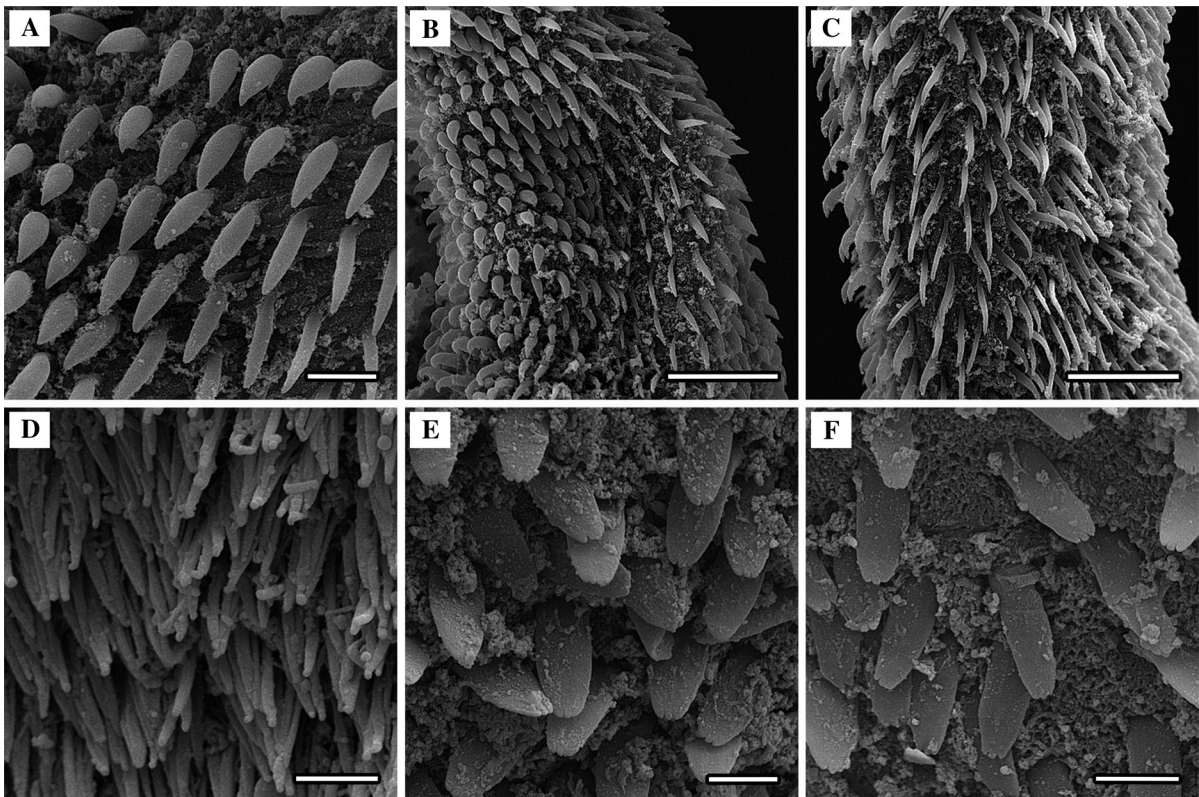


Fig. 3 Hooks and surface ultrastructure of *Dollfusella nimai* n. sp. A, Antibothrial surface, basal armature; B, External surface, basal armature; C, External surface, metabasal armature; D, Columnar spinitriches on distal bothrial surface; E, Trifid spinitriches on pars vaginalis; F, Trifid spinitriches on pars bulbosa. Scale-bars: A, E, F, 5 μ m; B, C, 20 μ m; D, 2 μ m

uncinate and falciform hooks (Fig. 2D–F), 7–12 (9 ± 1 , $N = 15$, $n = 38$) long, base 4–8 (6 ± 1 , $N = 15$, $n = 40$) long, tiny spiniform hooks present in some specimens only (Fig. 2D, E), 2–5 (3 ± 1 , $N = 7$, $n = 16$) long, base 1–4 (3 ± 1 , $N = 7$, $n = 15$) long; rows 3–5 with long spiniform hooks on antibothrial, internal and external surfaces (Fig. 2D, E), 8–14 (11 ± 1 , $N = 13$, $n = 47$) long, base 2–6 (4 ± 1 , $N = 13$, $n = 47$) long, terminating in falciform hooks on bothrial surface (Fig. 2F), 8–12 (10 ± 1 , $N = 13$, $n = 18$) long, base 4–7 (5 ± 1 , $N = 13$, $n = 16$) long; row 6 with long spiniform hooks on antibothrial, internal and external surfaces (Fig. 2D, E), 8–13 (11 , $N = 13$, $n = 13$) long, base 3–6 (4 , $N = 11$, $n = 11$) long, terminating in distinctive uncinata hook on bothrial surface (Fig. 2F), 7–10 (8 , $N = 11$, $n = 11$) long, base 5–8 (7 , $N = 11$, $n = 11$) long; spiniform hooks of rows 3–6 decrease in size towards bothrial surface (Fig. 2E, F); rows 7–42 with billhooks on antibothrial surface (Figs. 2D, 3A, B), spiniform hooks on internal/external surfaces (Figs. 2E, 3B),

falciform hooks with broad bases and then uncinata hooks on bothrial surface (Fig. 2F); row 7 with slender and long billhooks on antibothrial surface (Fig. 2D), 6–16 (12 , $N = 6$, $n = 12$) long, base 2–3 (3 , $N = 6$, $n = 12$) long, and spiniform hooks on internal/external surfaces (Fig. 2E), 8–9 (8 , $N = 6$, $n = 6$) long, base 2–4 (3 , $N = 6$, $n = 6$) long, terminating in falciform hooks with broad bases, 8–10 (9 , $N = 4$, $n = 4$) long, base 4–6 (5 , $N = 4$, $n = 4$) long, and uncinata hooks, 7–9 (8 , $N = 4$, $n = 4$) long, base 7–8 (8 , $N = 4$, $n = 4$) long, on bothrial surface (Fig. 2F); rows 8–9 with thick and long billhooks, of similar size, on antibothrial surface (Fig. 2D), 6–15 (10 ± 3 , $N = 6$, $n = 22$) long, base 2–5 (3 ± 1 , $N = 6$, $n = 25$) long, and spiniform hooks on internal/external surfaces (Fig. 2E), 8–9 (8 , $N = 6$, $n = 12$) long, base 2–4 (3 , $N = 6$, $n = 11$) long, terminating in falciform hooks with broad bases, 8–10 (9 , $N = 4$, $n = 7$) long, base 3–8 (5 , $N = 4$, $n = 7$) long, and uncinata hooks, 8–10 (8 , $N = 4$, $n = 4$) long, base 7–10 (8 , $N = 4$, $n = 8$) long, on bothrial surface (Fig. 2F); rows 10–12

with smaller billhooks on antiothrial surface (Fig. 2D), 4–14 (8 ± 3 , $N = 6$, $n = 37$) long, base 1–4 (2 ± 1 , $N = 6$, $n = 36$) long, and spiniform hooks on internal/external surfaces (Fig. 2E), 7–9 (8 ± 1 , $N = 6$, $n = 18$) long, base 2–4 (3 ± 1 , $N = 6$, $n = 18$) long, terminating in falciform hooks with broad bases, 8–10 (9 , $N = 4$, $n = 14$) long, base 4–6 (5 ± 1 , $N = 4$, $n = 15$) long, and uncinat hooks, 7–10 (8 , $N = 4$, $n = 13$) long, base 6–9 (8 , $N = 4$, $n = 13$) long, on bothrial surface (Fig. 2F); row 13 with smaller billhooks on antiothrial surface (Fig. 2D), 5–9 (7 , $N = 6$, $n = 12$) long, base 2–3 (2 , $N = 6$, $n = 12$) long, and spiniform hooks on internal/external surfaces (Fig. 2E), 6–9 (8 , $N = 6$, $n = 6$) long, base 2–4 (3 , $N = 6$, $n = 6$) long, terminating in falciform hooks with broad bases, 9–10 (9 , $N = 3$, $n = 3$) long, base 5–6 (5 , $N = 3$, $n = 3$) long, and uncinat hooks, 6–8 (7 , $N = 3$, $n = 4$) long, base 6–8 (6 , $N = 3$, $n = 5$) long, on bothrial surface (Fig. 2F); rows 14–42 with small billhooks on antiothrial surface (Fig. 2D), of similar size, 5–10 (7 ± 1 , $N = 6$, $n = 27$) long, base 2–4 (2 ± 1 , $N = 6$, $n = 27$) long, and spiniform hooks, of similar size, on internal/external surfaces (Fig. 2E), 6–10 (8 , $N = 6$, $n = 14$) long, base 2–4 (3 , $N = 6$, $n = 14$) long, terminating in falciform hooks with broad bases, of similar size, 7–13 (10 , $N = 4$, $n = 9$) long, base 4–6 (5 , $N = 4$, $n = 10$) long, and uncinat hooks, of similar size, 6–10 (8 , $N = 4$, $n = 9$) long, base 6–8 (7 , $N = 4$, $n = 9$) long, on bothrial surface (Fig. 2F).

Metabasal armature heteroacanthous typical, hook rows in ascending half spirals (Figs. 2A–C, 3C). Metabasal tentacular armature with heteromorphous hooks, 25–26 hooks per half spiral row (row 50) decreasing in number distally (Fig. 2A–C); hook files 1(1') not separated (Fig. 2A); hooks 1(1')–21(21') falciform (Fig. 2A, B), 8–13 (11 ± 1 , $N = 8$, $n = 330$) long, base 3–7 (5 ± 1 , $N = 8$, $n = 330$) long; hooks 22(22')–24(24') falciform with slightly broader bases (Fig. 2C), 10–12 (11 , $N = 4$, $n = 13$) long, base 4–8 (6 , $N = 4$, $n = 13$) long; hooks 25(25')–26(26') uncinat (Fig. 2C), 12 ($N = 2$, $n = 2$) long, base 7–8 ($N = 2$, $n = 2$) long. Distal tentacular armature with homeomorphous hooks, 15 falciform hooks per half spiral row (row 500); hooks 1(1')–12(12') 10–12 (11 , $N = 1$, $n = 12$) long, base 3–4 (4 , $N = 1$, $n = 12$) long; hooks 13(13')–15(15') 13 ($N = 1$, $n = 3$) long, base 5 ($N = 1$, $n = 3$) long.

Segments acraspedote (Fig. 1C), apolytic; immature segments 19–25 (22 ± 2 , $N = 12$) in number, initially wider than long, becoming longer than wide

with maturity; mature segments 1–4 (2 ± 1 , $N = 10$) in number, 1,702–3,445 ($2,597 \pm 563$, $N = 10$, $n = 18$) long, with maximum width 257–424 (334 ± 41 , $N = 10$, $n = 18$), width : length ratio 1 : 5–10 (8 ± 2 , $N = 10$, $n = 18$); gravid segments observed, 3,020–4,188 ($3,689$, $N = 5$, $n = 5$) long, with maximum width 376–427 (402 , $N = 5$, $n = 5$). Genital pore lateral, postequatorial (Fig. 1B, C), 315–1,256 (872 ± 214 , $N = 12$, $n = 21$) from posterior margin of segment; cirrus-sac ovoid, unipartite (Fig. 1B, C), 155–267 (212 ± 37 , $N = 10$, $n = 15$) long, 81–140 (107 ± 16 , $N = 10$, $n = 15$) wide; cirrus unarmed; seminal vesicles absent; vas deferens extensive (Fig. 4B, C), descends from level of cirrus-sac towards ovarian isthmus, coiling lateral and anterior to cirrus-sac, entering cirrus-sac at its antero-medial margin; testes oval, occupy intervacular space (Fig. 1C), 47–139 (87 ± 23 , $N = 9$, $n = 45$) long, 17–71 (42 ± 11 , $N = 9$, $n = 45$) wide, arranged in 2 columns in single layer, 81–110 (94 ± 8 , $N = 10$) in number, 41–53 (47 ± 4) antiporal, 34–46 (38 ± 4) prevaginal, 6–11 (8 ± 1) post-vaginal. Uterus median, thin-walled, extends anterior to cirrus-sac (Figs. 1C, B, 4A–C); uterine pore not observed; vagina thick-walled, muscular, relatively uniform in width (Figs. 1B, C, 4C), 62–102 (78 ± 11 , $N = 9$, $n = 18$), enters genital atrium at posterior level of cirrus-sac (Fig. 1B, C); seminal receptacle not observed. Ovary symmetrical, H-shaped in dorso-ventral view (Fig. 1C), tetralobed in cross-section (Fig. 4D), posterior, 280–451 (371 , $N = 8$, $n = 12$) long by 178–339 (242 , $N = 8$, $n = 12$) wide; ovarian isthmus anterior to centre of ovary; Mehlis' gland posterior to ovarian isthmus (Fig. 1C), 112–267 (171 , $N = 9$, $n = 12$) long, 66–105 (79 , $N = 9$, $n = 12$) wide; vitelline follicles circumcortical (Fig. 4), 18–41 (28 ± 1 , $N = 8$, $n = 39$) long by 14–33 (21 ± 4 , $N = 8$, $n = 39$) wide, interrupted at level of cirrus-sac and genital atrium (Figs. 1B, 4B). Two osmoregulatory canals per segment (Fig. 4), 13–54 (29 ± 10 , $N = 10$, $n = 48$) in diameter. Eggs ovoid, 15–22 (17 ± 2 , $N = 6$, $n = 15$) long, 13–20 (16 ± 2 , $N = 6$, $n = 15$) wide.

Remarks

The number of the hooks per half spiral row in the metabasal tentacular armature in *D. nimai* n. sp. (25–26) distinguishes it from *D. aculeata* Beveridge, Neifar & Euzet, 2004 (8–10), *D. acuta* Menoret & Ivanov, 2015 (7–8), *D. aetobati* (Beveridge, 1990)

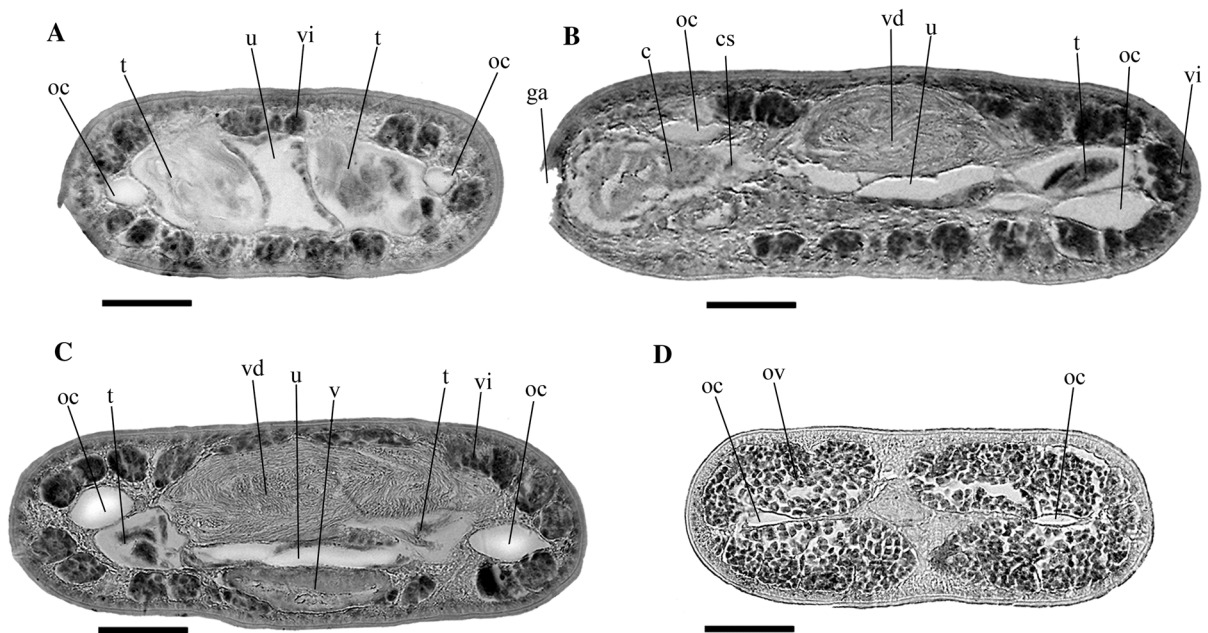


Fig. 4 Cross-sections through mature segments of *Dollfusiella nimai* n. sp. A, Cross-section through mature proglottid anterior to cirrus-sac; B, Cross-section through cirrus-sac; C, Cross-section between genital atrium and ovary; D, Cross-section through tetralobed ovary. *Abbreviations:* c, cirrus; cs, cirrus-sac; ga, genital atrium; oc, osmoregulatory canal; ov, ovary; t, testis; u, uterus; v, vagina; vd, vas deferens; vi, vitelline follicle. *Scale-bars:* 50 μ m

(10), *D. angustiformis* Schaeffner & Beveridge, 2013 (7), *D. australis* (Prudhoe, 1969) (14), *D. bareldsi* (Beveridge, 1990) (7–8), *D. carayoni* (Dollfus, 1942) (12), *D. cortezensis* (Friggens & Duszynski, 2005) (6–8), *D. geraschmidti* (Dollfus, 1974) (7–8), *D. elongata* Beveridge, Neifar & Euzet, 2004 (7), *D. hemispinosa* Schaeffner & Beveridge, 2013 (10), *D. imparispinis* Schaeffner & Beveridge, 2013 (12), *D. lineata* (Linton, 1909) (11–12), *D. martini* (Beveridge, 1990) (5–6), *D. micracantha* (Carvajal, Campbell & Cornford, 1976) (9), *D. musteli* (Carvajal, 1974) (9–13), *D. ocallaghani* (Beveridge, 1990) (7–8), *D. owensi* (Beveridge, 1990) (7–8), *D. parva* Schaeffner & Beveridge, 2013 (12–15), *D. schmidti* (Heinz & Dailey, 1974) (12), *D. spinifer* (Dollfus, 1969) (10), *D. spinosa* Schaeffner & Beveridge, 2013 (8), *D. spinulifera* (Beveridge & Jones, 2000) (8), *D. taminii* Menoret & Ivanov, 2014 (7–9), and *D. tenuispinis* (Linton, 1890) (8–10).

Based on the lack of a pars post-bulbosa, *D. nimai* n. sp. is different from *D. litocephala* (Heinz & Dailey, 1974) and *D. macrotrachela* (Heinz & Dailey, 1974), both of which have a long pars post-bulbosa (4,130–10,000 and 23,110–77,330 respectively). While the principle hooks 1(1')–21(21') are

homeomorphous in the metabasal armature of *D. nimai* n. sp., the principle hooks 1(1')–16(16') in the metabasal armature of *D. vooremi* (São Clemente & Gomes, 1989) are heteromorphous and different in shape. A prominent external seminal vesicle (c.250 μ m in length) and the homeomorphous hooks in the metabasal armature differentiate *D. qeshmiensis* Haseli & Palm, 2015 from *D. nimai* n. sp. in which the seminal vesicle is absent and the hooks are heteromorphous in the metabasal region.

Dollfusiella nimai n. sp. most closely resembles *D. michiae*, reported also from *R. ancyllostoma* (see Campbell & Beveridge, 2009), in the tentacular armature as well as the morphology of the scolex and strobila. Nonetheless, *D. nimai* n. sp. is easily differentiated from *D. michiae* by the number of the hooks per half spiral row in the metabasal tentacular armature (25–26 vs 16 respectively). Whereas the vagina of *D. nimai* n. sp. is relatively uniform in width for its entire length, there is only a thickening of the distal vagina in *D. michiae*. In addition, the heteromorphous hooks in the metabasal region of *D. nimai* n. sp. is another major feature distinguishing it from *D. michiae* possessing the homeomorphous hooks.

Family Mixodigmatidae Dailey & Vogelbein, 1982
Genus *Halysiorhynchus* Pintner, 1913

***Halysiorhynchus macrocephalus* (Shiple & Hornell, 1906) Pintner, 1913**

Host: *Rhina ancylostoma* Bloch & Schneider (Rhinopristiformes: Rhinidae).

Locality: Off the coast of Bandar Lengeh, Persian Gulf, Iran (26°25'N, 54°57'E).

Voucher material: Three immature specimens (MHNG-PLAT-121537; 3 slides).

Site in host: Spiral intestine.

Description

[Based on 3 immature specimens with partly everted tentacles.] Scolex 3,797–4,581 (4,181, N = 3) long; scolex width 735–857 (808, N = 3) at level of pars vaginalis, 710–882 (800, N = 3) at level of pars bulbosa. Pars bothriialis 490–563 (514, N = 3) long; pars vaginalis 1,788–2,474 (2,196, N = 3) long; pars bulbosa 1,837–1,984 (1,927, N = 3) long; bulbs 1,851–1,980 (1,937, N = 3, n = 3) long, 247–346 (297, N = 3, n = 3) wide, bulb width: length ratio 1.0: 5.7–7.9 (6.6, N = 3, n = 3). Scolex ratio (pars bothriialis: pars vaginalis: pars bulbosa) 1.0: 3.6–5.0: 3.5–4.0 (1.0: 4.3: 3.7; N = 3). Pars post-bulbosa 89–99 (92, N = 3) long.

Tentacle width 114–124 (119, N = 2, n = 2) at level of basal region, 121–126 (124, N = 2, n = 2) at level of metabasal region. Characteristic basal armature with 4–5 half spirals of hooks in different sizes; hooks of first basal row 29–49 (37, N = 3, n = 8) long, base 24–33 (29, N = 3, n = 9) long; hooks of second basal row 17–51 (32, N = 3, n = 10) long, base 10–36 (21, N = 3, n = 9) long; hooks of third basal row 24–63 (43, N = 3, n = 11) long, base 10–36 (21, N = 3, n = 10) long, hooks of fourth basal row 24–61 (49, N = 3, n = 6) long, base 12–36 (23, N = 3, n = 6) long, hooks of fifth basal row 24–73 (49, N = 1, n = 6) long, base 10–36 (20, N = 1, n = 6) long.

Metabasalar armature with 8–10 hooks per half spiral row; hooks 1(1') 61–73 (66, N = 3, n = 5) long, base 36–49 (41, N = 3, n = 5) long; hooks 2(2') 68–83 (76, N = 3, n = 3) long, base 29–34 (32, N = 3, n = 3) long; hooks 3(3') 70–85 (76, N = 3, n = 3) long, base 27–36 (30, N = 3, n = 3) long; hooks 4(4') 73–78 (75, N = 2, n = 2) long, base 29–32 (30, N = 2, n = 2) long; hooks

5(5') 49–75 (66, N = 3, n = 3) long, base 19–29 (25, N = 3, n = 3) long; hooks 6(6') 41–49 (45, N = 3, n = 3) long, base 19–22 (20, N = 3, n = 3) long; hooks 7(7') 24–29 (26, N = 3, n = 3) long, base 10–15 (12, N = 3, n = 3) long; hooks 8(8') 24–30 (26, N = 3, n = 3) long, base 8–12 (11, N = 3, n = 3) long; hooks 9(9'), occasionally present, 17 (N = 2, n = 2) long, base 5–9 (7, N = 2, n = 2) long; hooks 10(10'), occasionally present, 10 (N = 1, n = 1) long, base 5 (N = 1, n = 1) long; 11–12 chainette elements between each principle row of hooks on external surface of tentacle; chainette elements 27–29 (27, N = 3, n = 6) in width, 12–15 (13, N = 3, n = 4) in height; chainette elements commence at end of 5th–6th row of hooks.

Remarks

After the erection of the genus *Halysiorhynchus* for *Tetrarhynchus macrocephalus* Shiple & Hornell, 1906 by Pintner (1913), some host and locality records were reported by Zaidi & Khan (1976), Bilqeas (1985), Beveridge & Campbell (1992), Palm (2004) and Haseli et al. (2010). All the three specimens examined in this study had a small appendix at the end of the scolex and hence, it was not possible to evaluate the segment morphology. Although the scolex and tentacular armature of these specimens were consistent with the re-description of *H. macrocephalus* by Beveridge & Campbell (1992) based on the specimens off the Northern Territory coast of Australia, some intraspecific variation was observed in the specimens of the Persian Gulf. While the basal armature included approximately three ascending half spirals of hooks in the Australian specimens, 4–5 basal rows of hooks occurred in the posterior region of the tentacles of the Iranian specimens. Likewise, while each half spiral row of the basal armature in the Australian specimens contained the hooks of approximately the same size, each half spiral row of the basal armature in the Iranian specimens contained the hooks of different sizes such that the hooks on the internal surface of the tentacle were obviously larger than those on the external surface. It is worth mentioning that, unlike the re-description, the hooks of the basal armature are not the same in size in the drawings provided by Beveridge & Campbell (1992) (figures 2, 3; page 154).

Beveridge & Campbell (1992) described the principle hooks 9(9'), which occurred occasionally in the satellite position posterior to the hooks 8(8') more

often towards the base of the tentacle. This feature was also observed in the specimens of the Persian Gulf. Additionally, the 10th hook occurred asymmetrically in the satellite position posterior to the 9th hook at the posterior metabasal armature region. This spiniform hook observed only in one half spiral row in one specimen was very small in comparison to the ninth hook. We could not examine the distal tentacular armature in the specimens of the Persian Gulf, where the hook files 7(7') to 9(9') were missing in the Australian specimens.

Discussion

All species of *Dollfusiella* possess a heteroacanthous typical metabasal armature either with homeomorphous or heteromorphous hooks along the tentacle. There are no species within *Dollfusiella* in which the metabasal and distal tentacular armature include heteromorphous and homeomorphous hooks or vice versa respectively. Additionally based on the literature, of the 29 valid species of *Dollfusiella*, there are eight species, i.e. *D. spinulifera*, *D. lineata*, *D. litoccephala*, *D. australis*, *D. carayoni*, *D. geraschmidti*, *D. imparispinis* and *D. vooremi*, in which the metabasal hook number per half spiral row decreases slightly along the tentacle. Among these species, the maximum decrease in the hook number towards the distal part of the tentacle is four (see Palm, 2004; Schaeffner & Beveridge, 2013; Menoret & Ivanov, 2014, 2015). Since *D. nimai* n. sp. possesses a metabasal as well as a distal tentacular armature containing, respectively, heteromorphous and homeomorphous hooks and that the metabasal hook number decreases drastically towards the distal part of the tentacle (from 25–26 to 15), the generic diagnosis of *Dollfusiella* is in need of amendment. In addition, the description of the surface ultrastructure of *D. nimai* n. sp. necessitates also an extra amendment for the generic diagnosis of this genus.

The information on the surface ultrastructure of species in the genus *Dollfusiella* is available only for 12 species (Palm, 2004; Beveridge & Jones, 2000; Friggens & Duszynski, 2005; Schaeffner & Beveridge, 2013; Menoret & Ivanov, 2014, 2015) in which, the proximal bothrial surface and scolex peduncle are lined by gladiate (*D. spinulifera*), bifid (*D. vooremi* and *D. tamini*), trifid (*D. acuta*,

D. cortezensis, *D. nimai* n. sp., *D. spinosa*, *D. tamini* and *D. tenuispinis*), and palmate spinitriches (*D. angustiformis*, *D. hemispinosa*, *D. imparispinis* and *D. parva*). In spite of the variation in the microtriches of the scolex peduncle in *Dollfusiella* spp., the distal bothrial surface is mostly adorned with capilliform filitriches. Although the microtriches occurring on the scolex peduncle of *D. nimai* n. sp. have been reported earlier from its congeners, the columnar spinitriches of its distal bothrial surface are a unique feature within all the eutetrarhynchoids. Such microtriches did not occur in trypanorhynchids and have been reported from non-trypanorhynch genera (see Chervy, 2009), e.g. *Litobothrium* Dailey, 1969, *Phyllobothrium* van Beneden, 1850 and *Marsupiobothrium* Yamaguti, 1952.

Considering the oncotaxy as well as surface ultrastructure of *D. nimai* n. sp. and a need for the standardisation of the terminology of the microtriches based on Chervy (2009), some parts of the most recent generic diagnosis for *Dollfusiella* (see Palm, 2004) are amended as follows: scolex peduncle covered either with bifid, trifid, palmate or gladiate spinitriches, or a combination of bifid and trifid spinitriches, spinitriches of scolex peduncle interspersed sometimes with acicular filitriches; distal bothrial surface covered with capilliform filitriches or columnar spinitriches or a combination of coniform spinitriches and acicular filitriches; segments covered with capilliform filitriches. Metabasalar tentacular armature with homeomorphous or heteromorphous hooks or hooks heteromorphous in metabasal and homeomorphous in distal tentacular region. Number of metabasal hooks per half spiral row may drastically decrease distally along tentacle.

Unlike most of the specimens of *D. nimai* n. sp. in possessing the microscopically visible microtriches on the scolex peduncle, there were several specimens in which such structures were not observed. This case is in accordance with the difficulties discussed by Haseli & Palm (2015) in using the microscopically visible microtriches and their distribution on the scolex peduncle as the main characters for identification keys. Using the key presented by Haseli & Palm (2015) in which the microscopically visible microtriches on the scolex were neglected, the species of *Dollfusiella* are identified by the characters which are intraspecifically invariable. Accordingly in a closely related genus, Salmani & Haseli (2017) also proved

that the Iranian specimens of *Prochristianella clarkae* Beveridge, 1990, although being the same genetically, possessed different distributional patterns of microscopically visible microtriches on the scolex.

In the Persian Gulf, *H. macrocephalus* has been reported from three batoid host species, i.e. the adult specimens from *Pastinachus* cf. *sephen* and *Rhynchobatus* sp. (see Haseli, 2010) and the immature specimens ex *R. ancylostoma* from the present study, described as having a short appendix. In the specimens described by Haseli (2010), the scolex was smaller in the adults (3,134–3,667 μm) than in the present immature specimens (3,797–4,581 μm). All specimens were fixed and stained according to the same method. The same situation was also observed for *Callitetrarhynchus gracilis* (Rudolphi, 1819) both in the Persian Gulf (Haseli, 2010) and outside this region (see Palm, 1997, 2004). There are also some other similar cases within trypanorhynchids, for example, the larval specimens of *Progrillotia dasyatidis* Beveridge, Neifar & Euzet, 2004 off the Portuguese coast were larger than the conspecific adults off Tunisia and the Atlantic coast of France (see Beveridge et al., 2004; Marques et al., 2005). It seems that the physiological condition of the definitive host, the physiology of the intermediate host species, the occurrence of paratenic hosts, and the age of the larva, when it is transferred to the definitive host, can be considered as key factors in explaining this situation (see also Palm, 2004).

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Compliance with ethical standards

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

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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