

Laticola dae n. sp. (Monogenea: Diplectanidae) from *Epinephelus maculatus* (Perciformes: Serranidae) off New Caledonia

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Accepted for publication 14th September, 2005

Abstract

Laticola dae n. sp. is described from specimens collected from the gill-filaments of the highfin grouper *Epinephelus maculatus*, a coral reef fish caught off Nouméa, New Caledonia, South Pacific. The species is characterised by a spoon-shaped sclerotised male copulatory organ, with four thin walls and 73–108 µm in outer length, and a sclerotised vagina in form of a disc, 16–22 µm in diameter, with a smaller hemisphere on one side. *Laticola* Yang et al., 2006 was described to accommodate diplectanids from *Lates calcarifer* (Centropomidae); this is the first *Laticola* described from a serranid. Other diplectanids, including several species of *Pseudorhabdosynochus* Yamaguti, 1958, were also found on the same species of fish; specimens of *L. dae* represented about half of the diplectanids collected; all other species were rare.

Résumé

Laticola dae n. sp. est décrit à partir de spécimens collectés sur les filaments branchiaux de la loche grisette, *Epinephelus maculatus*, un poisson de récif corallien pêché au large de Nouméa, Nouvelle-Calédonie, Pacifique Sud. L'espèce est caractérisée par un organe copulateur mâle sclérifié en forme de cuiller, avec quatre parois fines, long de 73–108 µm, et un vagin sclérifié en forme de disque, de diamètre 16–22 µm, avec un hémisphère plus petit d'un côté. *Laticola* Yang et al., 2006 a été décrit pour rassembler des Diplectanidae de *Lates calcarifer* (Centropomidae); ceci est le premier *Laticola* décrit d'un Serranidae. D'autres Diplectanidae, y compris plusieurs espèces de *Pseudorhabdosynochus*, ont aussi été trouvés chez ce poisson; les spécimens de *L. dae* représentaient environ la moitié des Diplectanidae récoltés, toutes les autres espèces étaient rares.

Introduction

Diplectanids of groupers (Serranidae, Epinephelinae) mostly belong to the species-rich genus *Pseudorhabdosynochus* Yamaguti, 1958 (lists in Santos, Buchmann & Gibson, 2000; additional species are listed in Justine, 2005a, b, Hinsinger & Justine, 2006a, 2006b; Yang, Zeng & Gibson, 2005). A few species of *Diplectanum* Diesing, 1858 and *Lamellodiscus* Johnston & Tiegs, 1922 have

also been reported (Bu, Leong, Wang, Woo & Foo, 1999; Zhang, Yang & Liu, 2001), and Justine & Euzet (2006) recently proposed the new genus *Echinoplectanum* Justine & Euzet, 2006 for the diplectanids from the coralgroupers *Plectropomus* spp. Although each diplectanid genus seems associated with certain genera of epinephelines, the limits are not strict. *Pseudorhabdosynochus* spp. are mainly found in the numerous members of *Epinephelus*, but are also recorded from species of *Mycteroperca* (see Vidal-Martinez & Mendoza-Franco, 1998), *Variola* (see Justine, 2005b) and

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Table 1. Details of the hosts and numbers of diplectanids collected. Results show that *Laticola dae* n. sp. is the dominant species; all other diplectanids (nine species) are rare.

Fish	Stn	FL	W	<i>Laticola dae</i>	All diplectanids	Remark
JNC1061	F1	440	1150	27 (73%)	37	Both sides, not exhaustive
JNC1101	F2	385	780	21 (60%)	35	Only first right gill
JNC1131	F3	410	900	12 (32%)	38	Both sides, not exhaustive
JNC1150	F4	465	1200	77 (59%)	131	Both sides, not exhaustive
JNC1170	F5	430	925	84 (53%)	159	Both sides, not exhaustive
JNC1190	F6	435	1100	23 (35%)	65	Both sides, exhaustive
JNC1522	F7	450	1200	34 (56%)	61	Right side, not exhaustive
JNC1523	F7	395	800	40 (44%)	91	Right side, not exhaustive
JNC1524	F7	360	610	15 (38%)	40	Right side, not exhaustive
JNC1547	F9	365	600	42 (43%)	99	Right side, exhaustive
Total				375 (50%)	756	

FL, fork length (mm); W weight (g).

Cephalopholis (see Justine, unpublished observations). *Echinoplectanum* spp. are mainly found in *Plectropomus*, but a species was also found in a member of *Epinephelus* and this was interpreted as the result of host-switching (Justine & Euzet, 2006).

Laticola Yang, Kritsky, Sun, Zhang, Shi & Agrawal, 2006 was proposed to accommodate diplectanids from *Lates calcarifer* (Centropomidae), previously attributed to *Diplectanum* or *Pseudorhabdosynochus*. In the highfin grouper *Epinephelus maculatus* collected off New Caledonia, from which no diplectanid was previously described, we found several species of *Pseudorhabdosynochus*, but the majority of diplectanid individuals belonged to a single species of *Laticola*, which is described here.

Materials and methods

Specimens of *Epinephelus maculatus* (Bloch) ('high-fin grouper'; local name: 'loche grisette' or 'loche uitoé', see Laboute & Grandperrin, 2000) were caught with hand-lines on board the R/V 'Coris' (see list of stations). Live fish were kept in a container with seawater and immediately brought back to the laboratory. All fish were measured, weighed (Table 1) and photographed. A unique number (JNC) was assigned to each fish; for certain hosts (JNC1101, JNC1522–1524, JNC1547), only the right gills were collected and the fish specimens were deposited in the ichthyological collection of the Muséum National d'Histoire Naturelle, Paris

(MNHN). The parasitological material was then assigned a corresponding JNC linked to the respective fish host. In the lists of material examined (Table 1), measurements of hosts are indicated for possible future comparison of parasite prevalence and host age in other localities.

List of stations. Stations are along the barrier reef off Nouméa, New Caledonia, at a distance of about 20 km from the shore, with a depth of 20–40 m. Stn F1, Récif Le Sournois (RLS), 22°31'30"S, 166°26'30"E, 15 March, 2004; Stn F2, Récif To, 22°30'40"S, 166°26'00"E, 10 May, 2004; F3, RLS, 8 June, 2004; F4, RLS, 16 June, 2004; Stn F5, RLS, 29 June, 2004; F6, RLS, 8 July, 2004; F7, Récif Aboré (RA), 22°21'20"S 166°15'30", 21 April, 2005; F8, RA, 26 April, 2005; F9, RA, 31 May, 2005.

Gills were extracted and examined in seawater with a dissecting microscope. Live monogeneans were individually picked off the gills with fine needles and immediately prepared. Specimens were examined live in seawater, then processed for carmine staining, including an initial flattening in ethanol (referred to as 'Carmine' – see Justine, 2005a), according to Justine (2005a) or with ammonium picrate-glycerine according to Malmberg (1957), slightly modified (referred to as 'Picrate' – see Justine, 2005a). For three fish (JNC1522–1524), the gills were placed in hot 4% formalin and the monogeneans were later removed with fine needles and processed for carmine staining without flattening stage. All Carmine

slides were made with a single worm per slide for the study of the different monogenean species.

Monogeneans were drawn using a BH2 microscope equipped with a camera lucida and DIC optics. Measurements were taken on the pencil drawings with the help of a custom-made transparent rule, previously calibrated with a stage micrometer. Drawings were scanned and redrawn on a computer with Adobe Illustrator. Method of measurement of the haptor hard-parts are as in Justine (2005a); the means of the measurements of the right-hand haptor hard-parts and the left-hand equivalents were compared with Student *t*-tests to ascertain that no bilateral asymmetry was present, and measurements from both sides were then pooled. All measurements are given in micrometres as: holotype, mean of paratypes \pm standard deviation, with range and number in parentheses. Measurements in ammonium picrate preparations and in specimens flattened in ethanol vary for the hollow sclerotised male and female organs (Justine, 2005a); only measurements of carmine specimens are given in this article. "Unflattened" specimens fixed in hot formalin were not used for the description but were adequate for evaluating the relative abundance of the diplectanids.

Laticola dae n. sp.

Type-host: *Epinephelus maculatus* (Bloch) (Serranidae).

Type-locality: Reef off Nouméa, New Caledonia.

Site: Between secondary gill-lamellae.

Type-specimens: Holotype, slide JNC1170A12. Paratypes, 51 slides from hosts JNC1061, JNC1150 and JNC1170.

Material examined: About 400 specimens (see Table 1) stained with carmine in Canada balsam, or in picrate. Observations on live specimens from hosts JNC1523 and JNC1525, Stn F8, FL 355 mm, W 600 g.

Type-material: Muséum National d'Histoire Naturelle (MNHN), Paris, holotype, slide JNC1170A12, and 47 paratypes, from hosts JNC1061, JNC1150 and JNC1170; Natural History Museum, London, 2 paratypes, 2 slides BMNH 2004.9.15.10–11, from host JNC1170; United States National Parasite Collection, Beltsville, USA, 1 paratype, 1 slide USNPC 095080.00,

from host JNC1170; Institute of Aquatic Economic Animal, Zhongshan University, Guangzhou, China, 2 paratypes, 2 slides 2005080807, 2005080808, from hosts JNC1061 and JNC1170.

Prevalence: 100% (11/11).

Intensity: See Table 1. Total number of *L. dae* estimated to up to 100 per fish, among up to 200 diplectanids per fish. *L. dae* was always the dominant monogenean species and represented 32–73% (mean 50%) of the total number of diplectanids; all other diplectanid species were rare.

Remarks on the host: The highfin grouper *E. maculatus* is known from the coral reefs of the Eastern Indian Ocean and Western Pacific and is considered "not common, but probably of commercial interest in artisanal fisheries" (Heemstra & Randall, 1993). It is very abundant off New Caledonia (Laboute & Grandperrin, 2000) and often seen at the fish market in Nouméa, where, under the local name 'loche grisette', it is generally mixed with other less common species of reticulated groupers. *Etymology*: In an initial examination of the several species of diplectanids found on *E. maculatus*, this species was designated as "d.a.e". for French "Diplectanidae à anneau et entonnoir" (Diplectanids with ring and funnel). The acronym is here latinised as *dae* and used as an indeclinable noun in apposition.

Description (Figures 1–2)

Diplectanidae. Body length 480, 466 ± 75 (350–650, $n = 51$), width 290, 235 ± 41 (170–350, $n = 51$). Tegument smooth. Anterior region with 3 pairs of head organs and 2 pairs of eye-spots; distance between outer margins of anterior eye-spot pair 37, 32 ± 6.0 (23–42, $n = 38$), of posterior eye-spot pair 29, 25 ± 5.0 (15–37, $n = 48$).

Haptor differentiated from rest of body, width 185, 172 ± 15 (140–200, $n = 50$), provided with 2 similar squamodiscs, 2 pairs of lateral hamuli, 3 bars and 14 marginal hooklets. Squamodiscs round in shape, made up of rows of rodlets, with 2–4 central rows forming closed circles; rodlets interlocking and robust in 3 inner rows, becoming progressively thinner and separate in peripheral rows; ventral squamodisc, 50, 45 ± 4.9 (35–50, $n = 46$) long, 45, 41 ± 5.2 (30–55, $n = 46$) wide, with 10, 11 (9–12, $n = 45$) rows of scales including 2–4 ($n = 45$) closed rows; dorsal squamodisc, 45,

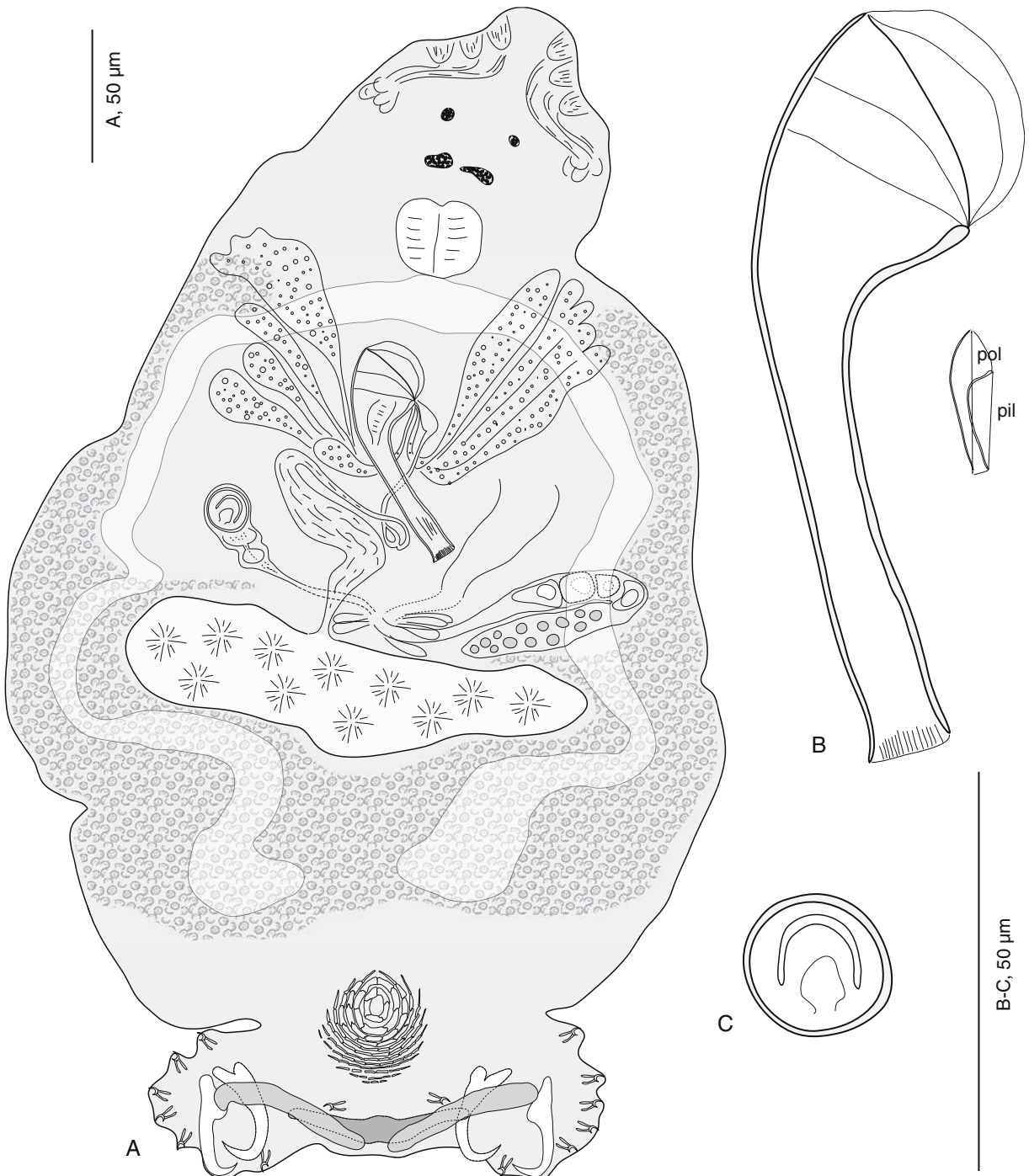


Figure 1. *Laticola dae* n. sp. A. Dorsal view, composite drawing from holotype and observations on live specimens; B. Sclerotised male copulatory organ; inset, measurements (pil, penis inner length; pol, penis outer length); C. Sclerotised vagina.

46 ± 4.6 (35–55, $n = 37$) long, 40, 38 ± 4.5 (30–53, $n = 37$) wide, with 11, 12 (10–12, $n = 39$) rows of scales including 1–3 ($n = 40$) closed rows. Ventral

hamulus with distinct handle and guard, outer length 39 ± 2.7 (30–47, $n = 105$), inner length 39 ± 2.9 (30–49, $n = 105$). Dorsal hamulus with

indistinct guard, outer length 39 ± 2.1 (32–45, $n = 105$), inner length 25 ± 1.9 (20–30, $n = 105$). Dorsal (lateral) bars with slightly flattened interior extremity and roughly cylindrical external extremities with posteriorly directed protuberance, length 61 ± 3.2 (55–70, $n = 106$), maximum width 8 ± 0.9 (5–10, $n = 106$). Ventral bar flat, bow-shaped, length 75 ± 4.3 (64–87, $n = 53$), maximum width 9 ± 1.2 (7–11, $n = 53$); groove visible on ventral side.

Pharynx subspherical, length 30, 31 ± 3.2 (22–40, $n = 52$), width 36, 31 ± 4.4 (22–43, $n = 52$). Oesophagus apparently absent, such that intestinal bifurcation immediately follows pharynx. Intestinal caeca simple, terminate blindly at level of posterior margin of vitelline field.

Testis generally wider than longer, intercaecal, length 30, 39 ± 16 (10–75, $n = 43$), width 112, 126 ± 31 (25–180, $n = 43$). Vas deferens emerges from anterior part of testis, forms seminal vesicle sinistro-submedially; duct from seminal vesicle reflexes toward body mid-line, bends and forms small ejaculatory bulb; duct from ejaculatory bulb directed anteriorly, thin, forms small, thin-walled ampulla which connects laterally with anterior part of penis. Prostatic glands conspicuous, form 2 large fields on both sides of body from pharynx to mid-length of penis, and join posteriorly as 2 ducts which reflex and finally connect laterally with anterior part of penis near sperm duct. Sclerotised male copulatory organ: a tubular penis, with no accessory part. Penis 'spoon-shaped': a curved funnel, made up of anterior cone and straight posterior tube with regular diameter, forming angle of $c.60^\circ$ with longitudinal axis of cone. Posterior limit of tube with minute parallel longitudinal striation (Figure 2A). Within penis anterior cone, 4 thin transversal walls limit 4 chambers. Thin cirrus inside penis from most anterior part of cone to end of tube. Posterior protruding cirrus and most anterior chamber both slightly mobile, seen to inflate or deflate in living specimens (dotted line in Figure 2B). Inner length of penis 62, 63 ± 5.6 (54–85, $n = 53$), outer length 94, 88 ± 6.6 (73–108, $n = 53$), diameter of cylinder 8 ± 1.1 (6–11, $n = 49$).

Ovary subequatorial, intercaecal, pre-testicular, encircles right caecum. Ovary width 75, 71 ± 16 (30–125, $n = 47$). Oviduct passes medially to form oötype, surrounded by Mehlis' gland; oötype short, opens into uterus. Uterus dextral. Vagina sinistral; unsclerotised vagina visible in live specimens

but inconspicuous in preserved slides, forms several valves and connects with inconspicuous duct; duct joins oötype. No seminal receptacle seen. Within unsclerotised vagina very characteristic sclerotised part present in form of disc, external diameter 18, 19 ± 1.4 (16–22, $n = 54$), with smaller hemisphere, generally oriented anteriorly, on one side; external margin of disc heavily sclerotised; within hemisphere, inconspicuous small chamber with anteriorly directed tube; aspect of sclerotised vagina changes slightly according to specimen and orientation (Figure 2); in perfectly polar orientation it resembles 2 concentric rings. Possible spermatophore, with homogeneous contents which stains well with carmine, sometimes visible close to sclerotised vagina (Figure 2I); its diameter corresponds well to both sclerotised vaginal hemisphere and extremity of penis. Vitelline fields extend posteriorly from posterior to pharyngeal level in 2 lateral bands, confluent in post-testicular region, terminate anterior to peduncle. Egg seen only *in utero*, oval, 112×32 (80–175 \times 27–39, $n = 8$).

Generic attribution

The new species described here is considered a member of *Laticola* because it has the following characteristics: a spoon-shaped penis; and an unsclerotised vagina with several valves. Among the three species of *Laticola* described, two of them, namely *L. lingaoensis* Yang, Kritsky, Sun, Zhang, Shi & Agrawal, 2006 and *L. paralatesi* (Nagibina, 1976) Yang et al., 2006, have a penis in which the anterior sclerotised wall is curved posteriorly, thus partly closing the anterior opening; but the other species, *L. latesi* (Tripathi, 1957) Yang et al., 2006, has a penis in which the anterior conical part is not curved but is open wide (figure 12 in Yang et al., 2006); this penis shape is very similar to that of *L. dae* n. sp.

Differential diagnosis

L. dae n. sp. is differentiated from all three previously known species of *Laticola* by the presence of a ring-shaped sclerotised vagina and the absence of tegumental scales. In addition, it is differentiated from *L. lingaoensis* and *L. paralatesi* by its penis, the anterior wall of which is not curved posteriorly. It is differentiated from *L. latesi* by its much longer penis (73–108 vs 52–72 μm) and the

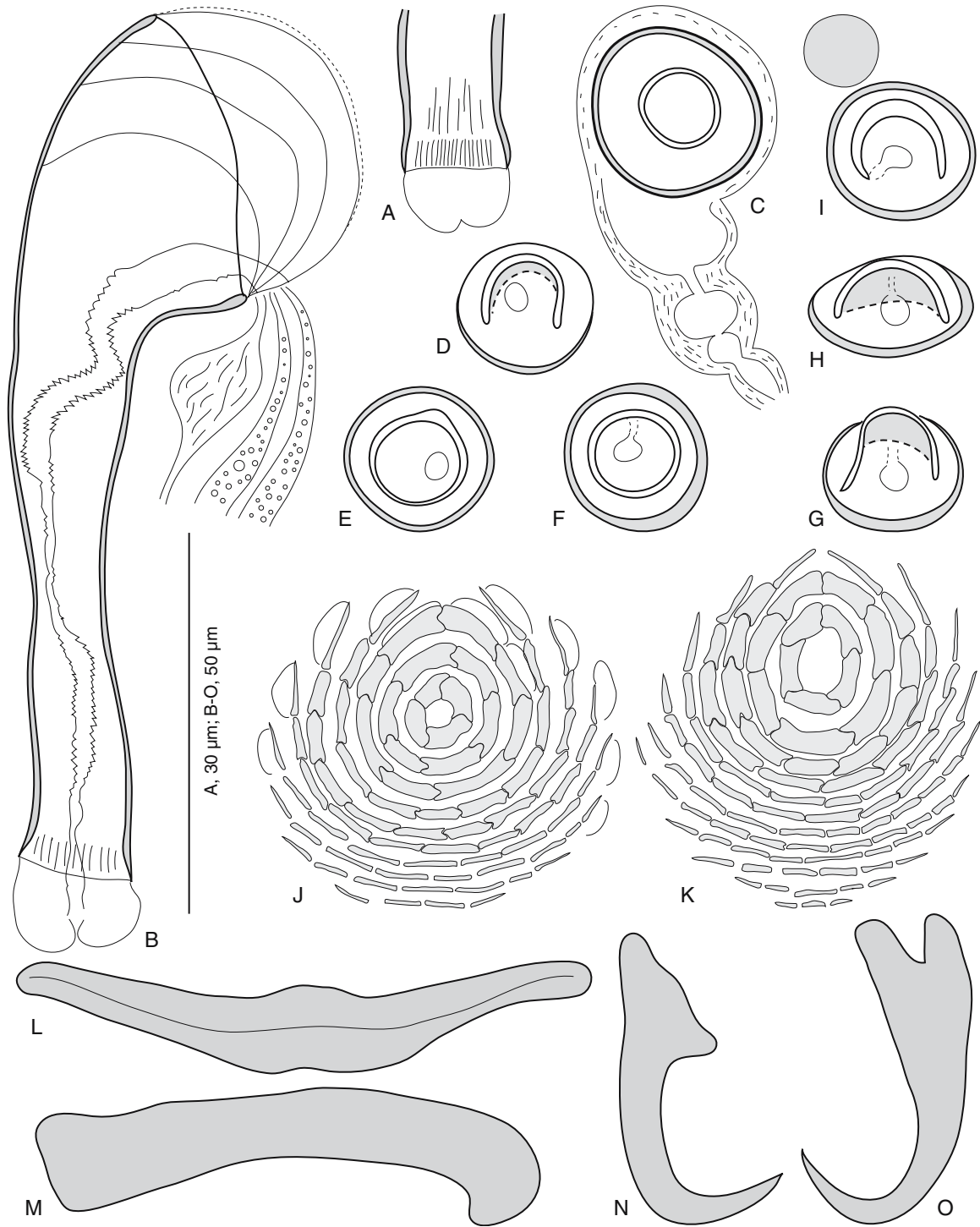


Figure 2. *Laticola dae* n. sp. A. Posterior extremity of penis, showing longitudinal parallel striations; B. Male copulatory organ, showing internal arrangement of cirrus, and connection of sperm duct and two prostatic ducts at same level (dotted line, movement of thin anterior wall visible in live specimens); C. Vagina, live specimen. D–I. Paratypes, slight variations of sclerotised vagina according to orientation and specimens; J. Ventral squamodisc, paratype; K. Dorsal squamodisc, paratype; L. Ventral bar; M. Dorsal (lateral) bar; N. Dorsal hamulus; O. Ventral hamulus.

shape of the dorsal bars, which are thinner than in *L. latesi*.

Discussion

Laticola dae n. sp. is the fourth species of *Laticola*, which was originally erected (Yang et al., 2006) to accommodate diplectanids parasitic on *Lates calcarifer*, a member of the Centropomidae. With the present description, *Laticola* is now found in two families of fish, centropomids and serranids.

Diplectanum grouperi Bu, Leong, Wong, Woo, & Foo, 1999 has a funnel-shaped penis with four transverse walls forming four chambers (Bu et al., 1999), and thus exhibits some similarities to members of *Laticola*. A detailed morphological comparison would be useful, and the generic attribution of this species should be re-evaluated. In groupers from New Caledonia, we found several species of diplectanids with a male copulatory organ similar to *D. grouperi*. Unfortunately, in all cases, including *D. grouperi*, the penis is very small, and comparison is difficult. A molecular study (Yang et al., 2006) did not indicate a close relationships between *D. grouperi* and *Laticola* spp.

Other species of gill parasites

The gills of *Epinephelus maculatus* harbour an exceptionally rich community of parasites. Eight species of *Pseudorhabdosynochus*, another diplectanid and two species of *Haliotrema* Johnston & Tiegs, 1922 (Ancyrocephalidae) were found and will be described in a separate paper. Three species of copepods, *Dissonus manteri* Kabata, 1966, *Hatschekia* sp. 1 and *Hatschekia* sp. 2 were also present (identification by G. Boxshall, Natural History Museum, London), as well as gnathiid isopod larvae. A total of 16 ectoparasitic species (12 monogeneans, three copepods and one isopod) were thus found on a single organ, the gill. In addition, a didymozoid digenean was found within the gill arch.

Acknowledgements

Geoffrey Boxshall (NHM, London, England) kindly agreed to study the copepods. Delane Kritsky generously communicated a manuscript

in press. Angelo di Matteo (IRD, Nouméa) and Jimmy Cassone (MNHN, Paris) provided technical help. Michel Pascal (INRA) occasionally participated in the fishing operations. Amandine Marie, Maya Robert, Eric Bureau, Damien Hinsinger and Violette Justine, all students, participated in the fishing operations and parasitological survey. Sam Tereua, Miguel Clarque, and Napoléon Colombani, captains of the R/V 'Coris', provided safe navigation, counsels and assistance. Dr Michel Kulbicki (UR CoRêUs, IRD, Nouméa) greatly helped with the fish identification.

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