Two new species of *Prochristianella* Dollfus, 1946 (Platyhelminthes, Cestoda) from the blue-spotted stingray, *Neotrygon kuhlii* (Müller & Henle, 1841) off New Caledonia

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Beveridge I. & Justine J.-L. 2010. — Two new species of *Prochristianella* Dollfus, 1946 (Platyhelminthes, Cestoda) from the from blue-spotted stingray, *Neotrygon kuhlii* (Müller & Henle, 1841) off New Caledonia. *Zoosystema* 32 (4): 643-652.

ABSTRACT
Prochristianella aciculata n. sp. and *P. omunae* n. sp. (Platyhelminthes, Cestoda, Trypanorhyncha, Eutetrarhynchidae) are described from the spiral valve of *Neotrygon kuhlii* (Müller & Henle, 1841) (Rajiformes, Dasyatidae) from Nouméa, New Caledonia, representing the first records of this cestode genus from the southern Pacific Ocean. *Prochristianella aciculata* n. sp. is distinguished from congeners by aciculate hooks in the principal rows of the metabasal tentacular armature and by the unique morphology of the first hooks in each row. *Prochristianella omunae* n. sp. is distinguished from congeners by its extended basal armature (20 rows of hooks) and the presence of enlarged hooks at the very base of the tentacle.

KEYWORDS
Platyhelminthes,
Cestoda,
Trypanorhyncha,
Prochristianella,
New Caledonia,
new species.

RÉSUMÉ
Deux nouvelles espèces de Prochristianella *Dollfus, 1946 (Platyhelminthes, Cestoda), parasites de la raie pastenague à points bleus,* Neotrygon kuhlii *(Müller & Henle, 1841), au large de la Nouvelle-Calédonie.*
Prochristianella aciculata n. sp. et *P. omunae* n. sp. (Platyhelminthes, Cestoda, Trypanorhyncha, Eutetrarhynchidae) sont décrits de la valvule spirale de *Neotrygon kuhlii* (Müller & Henle, 1841) (Rajiformes, Dasyatidae) de Nouméa, Nouvelle-Calédonie, et représentent les premières mentions de ce genre de cestode dans l'Océan Pacifique Sud. *Prochristianella aciculata* n. sp. se distingue de ses congénères par des crochets aciculés dans les rangées principales de l'armature tentaculaire métabasale et par la morphologie exceptionnelle des premiers crochets de chaque rangée. *Prochristianella omunae* n. sp. se distingue de ses congénères par son armature basale développée (20 rangées de crochets) et la présence de grands crochets à l'extrême base du tentacule.

MOTS CLÉS
Platyhelminthes,
Cestoda,
Trypanorhyncha,
Prochristianella,
Nouvelle-Calédonie,
espèces nouvelles.

INTRODUCTION

Prochristianella Dollfus, 1946 is an apparently cosmopolitan genus of trypanorhynch cestode currently comprising 14 species parasites from the spiral valves of rays (order Rajiformes) with individual taxa described from the coasts of Europe, North and South America, India, Australian and Indonesia (summary in Palm 2004; Friggins & Duszynski 2005). In spite of its apparently wide geographical distribution, there are no records of this genus from the southern Pacific Ocean. Examination of specimens of the blue-spotted stingray, *Neotrygon kuhlii* (Müller & Henle, 1841) (formerly *Dasyatis kuhlii* Müller & Henle, 1841), from the lagoon surrounding New Caledonia revealed the existence of two new species of the genus, which we report here, as well as the presence of *Prochristianella clarkeae* Beveridge, 1990.

MATERIALS AND METHODS

Two specimens of *N. kuhlii* were collected in waters off Nouméa, New Caledonia. Measurements (in mm) and weight (in g) are given for more precise specific identification when the taxonomy of *N. kuhlii* is better resolved: specimen MNHN JNC957: body length 360, span 400, tail 420, weight 3200; specimen MNHN JNC958: body length 370, span 410, tail 400, weight 2600. The spiral valves were removed and were injected immediately with 10% formalin. Subsequently, the content of the spiral valves was examined for parasites and cestodes were removed and stored in 70% alcohol. Cestodes were stained in celestine blue, dehydrated in ethanol, cleared in methyl salicylate and mounted in Canada balsam. In addition, a slide prepared by L. Euzet from a *N. kuhlii* collected off Nouméa in October 1998 (MNHN JNC1627) containing three species of *Prochristianella* was also examined.

Drawings were made using a drawing tube attached to an Olympus BH microscope. Measurements are in µm unless otherwise indicated, as is the range, followed by the mean and the number of specimens measured. Specimens have been deposited in the Muséum national d'Histoire naturelle, Paris (MNHN). Terminology for anatomical features follows Campbell & Beveridge (1994) with the exception of the use of the term "bothria" which follows Jones *et al*. (2004). Following Beveridge *et al.* (2004), hooks on the external surface of the tentacle are distinguished by a prime.

SYSTEMATICS

Family EUTETRARHYNCHIDAE Guiart, 1927
Genus *Prochristianella* Dollfus, 1946

Prochristianella aciculata n. sp.
(Figs 1; 2)

TYPE MATERIAL. — **New Caledonia**, Nouméa, Îlot Maître (166°40'E, 22°34'S), from spiral valve of *Neotrygon kuhlii* (Müller & Henle, 1841) (Rajiformes, Dasyatidae Jordan, 1888), 5.XI.2003, holotype (JNC 958). — Same data, 19 paratypes (MNHN JNC957-958).

OTHER MATERIAL EXAMINED. — **New Caledonia**, off Nouméa, X.1998, 1 specimen (MNHN JNC1627).

ETYMOLOGY. — The specific name relates to the aciculate nature of the larger hooks of the principal rows of the metabasal armature.

DESCRIPTION

Small cestodes, mature specimens 3.0-5.0 (3.5, n = 7) long with 7-9 (8, n = 9) segments, gravid specimens 3.5-6.5 (4.3, n = 7) long with 8-11 (9, n = 8) segments. Scolex acraspedote, surface smooth, 640-1050 (788, n = 10) long; width in region of pars bulbosa 140-190 (160, n = 10); 2 sub-circular bothria, pars bothrialis 190-240 (218, n = 10), bothrial width 190-200 (195, n = 4); pars vaginalis 390-740 (515, n = 10); sheaths sinuous; prominent transverse striations present in pars vaginalis of some specimens; bulbs elongate, extending to posterior margin of scolex or in some specimens extending slightly into pars proliferans scolecis; bulbs 250-340 (279, n = 10) long, 40-50 (45, n = 10) wide; bulb ratio 5.00-7.75 (6.26, n = 10); retractor muscle originates at base of bulb, origin surrounded by small number of gland cells; pre-bulbar organ present.

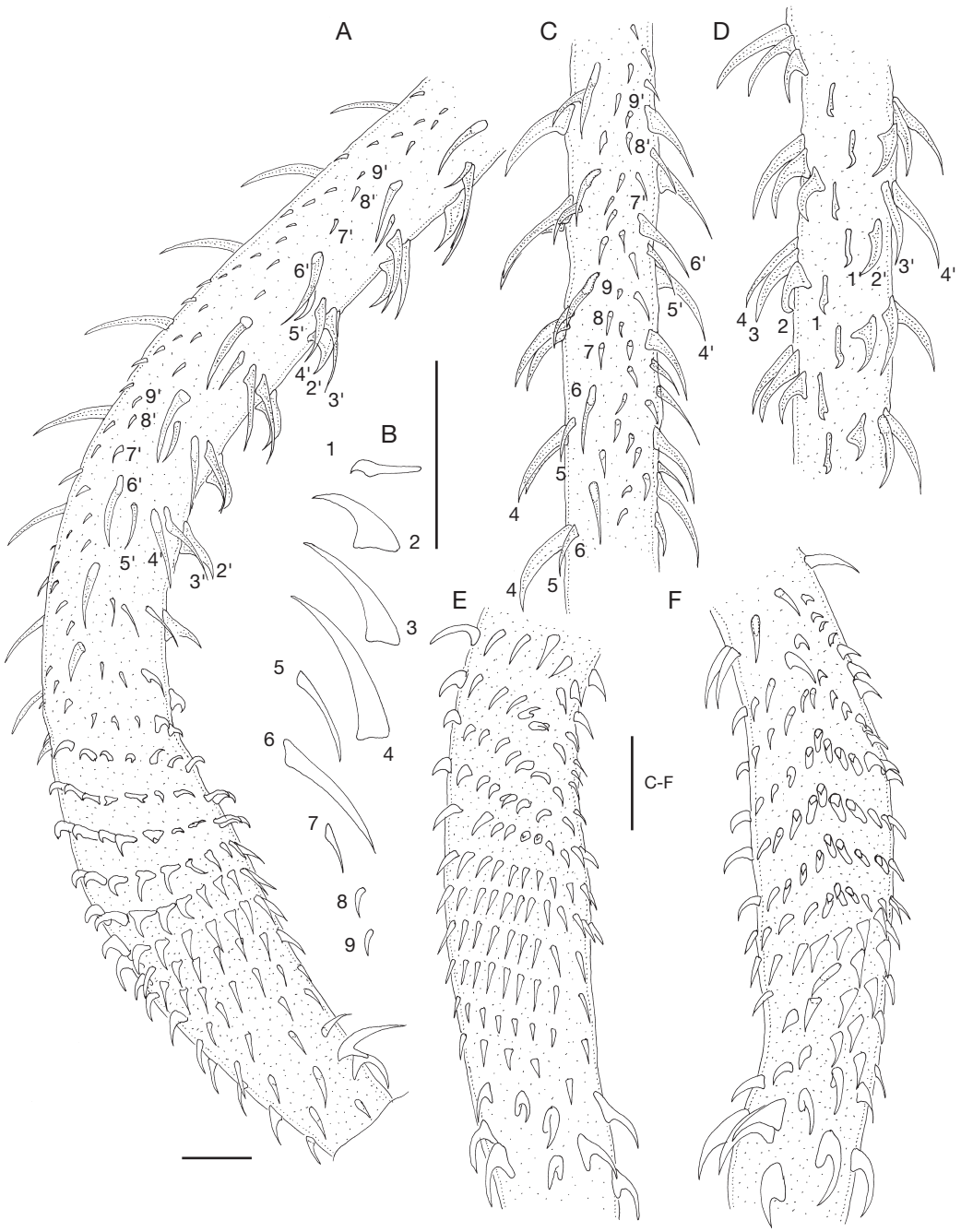

FIG. 1. — *Prochristianella aciculata* n. sp. from *Neotrygon kuhlii* (Müller & Henle, 1841): **A**, basal and metabasal region of tentacle, external surface, bothrial surface on left hand side; **B**, profiles of hooks 1-9; **C**, metabasal tentacular armature, bothrial surface, internal surface on left hand side; **D**, metabasal tentacular armature, antibothrial surface, internal surface on left hand side; **E**, basal armature, oblique view of antibothrial surface; **F**, basal armature, bothrial surface. Scale bars: A-F, 10 µm.

Partially everted tentacles up to 300 long, with prominent basal swelling; diameter in basal region 20-28 (25, n = 10), in metabasal region 15-20 (17, n = 10); hooks hollow. Basal armature: first 2-3 rows of hooks enlarged, falcate, 7-11 (10.7, n = 5) long, base 5-6 (5.4, n = 5); next 6-7 rows of hooks smaller, sagittate on antibothrial surface, tending to uncinate on bothrial surface, 3-5 (4.2, n = 10) long, base 1-4 (2, n = 10); rows 8-12 with elongate, recurved hooks on antibothrial surface changing to sagittate hooks on internal and external surfaces and then to bill hooks on bothrial surface, rows originating on antibothrial surface, terminating on bothrial surface; elongate, recurved hooks 3-6 (4.7, n = 10) long, base 0.5-1.0 (0.85, n = 10); bill hooks 3.5-5.0 (4.1) long, base 0.5-1.0 (0.9, n = 10) long; transition to metabasal armature occurs at about row 15.

Metabasal armature heteroacanthous, typical; hook rows begin on antibothrial surface of tentacle, terminate on bothrial surface. Distinct space between hooks 1 and 1' on antibothrial surface of tentacle. Hooks 1(1') small, almost linear with tiny, hooked extremity, 5-6 (5.5, n = 5) long, base 3.5-4.5 (4.2, n = 5), not clearly visible in many views of tentacle; hooks 2(2') robust, uncinate, 7-10 (8.7, n = 10) long, base 3-5 (4.3, n = 10) long; hooks 3(3') slender, falcate, 10-15 (12.4, n = 10) long, base 3-4 (3.5, n = 10) long; hooks 4(4') slender falcate, 10-16 (13.6, n = 10) long, base 3-5 (4.7, n = 10) long; hooks 5(5') much shorter than hooks 4(4') and 6(6'), slender, 4.5-8.9 (6.6, n = 10) long, base 0.5-2.0 (1.6, n = 10) long; hooks 6(6') larger, slender falcate, 9-14 (11.7, n = 10) long, base 2-5 (2.9, n = 10) long; hooks 7(7') small spiniform, 1.5-5.5 (3.8, n = 10) long, base 0.5-2.0 (1.1, n = 10); hooks 8(8')-10(10'), tiny, spiniform, 1-3 (2, n = 10) long, base 0.5-1.0 (0.6, n = 10) long.

Mature segments acraspedote, elongate, 750-1340 (950, n = 5) long, 160-200 (179, n = 5) wide; length/width ratio 4.63-7.88 (5.34, n = 5); genital pore irregularly alternating in posterior third of segment, 250-580 (364, n = 5) from posterior end. Cirrus sac sub-globular, 70-100 (82, n = 3) in diameter, cirrus unarmed, coiled within cirrus sac; testes 40-50 (45, n = 5) per segment arranged in 2 columns anterior to ovary; 5-7 (6, n = 5) testes posterior to genital pore, 14-19 (17, n = 7) anterior to genital pore, 21-25 (22, n = 5) on anti-poral side of segment; testis diameter 40-55 (49, n = 10). Ovary at posterior extremity of segment, with 4 lobes, each lobe 150-280 (172, n = 5) long, 40-60 (48, n = 5) wide, with multiple lobules; Mehlis' gland present between ovarian lobes; vitelline follicles circummedullary, 10-25 (19, n = 10) in diameter; uterus tubular, median, extending to anterior extremity of segment; uterine pore absent. Gravid segments 950-2030 (1268, n = 5) long, 390-570 (508, n = 5) wide; gravid uterus entirely filling medulla; eggs immature.

Prochristianella omunae n. sp.
(Figs 3; 4)

TYPE MATERIAL. — **New Caledonia**, Nouméa, Îlot Maître (166°40'E, 22°34'S), from spiral valve of *Neotrygon kuhlii* (Müller & Henle, 1841) (Rajiformes, Dasyatidae Jordan, 1888), 5.XI.2003, holotype (JNC 957). — Same data, 7 paratypes (MNHN JNC957-958).

OTHER MATERIAL EXAMINED. — **New Caledonia**, off Nouméa, X.1998, 1 specimen (MNHN JNC1627).

ETYMOLOGY. — The specific name is an anagram of the collection locality.

DESCRIPTION

Small cestodes, gravid specimens 3.39-4.90 (4.15, n = 2) long with 5 (n = 2) segments. Scolex acraspedote, surface smooth, 692-1200 (908, n = 6) long; width in region of pars bulbosa 167-208 (188, n = 6); 2 sub-circular bothria, pars bothrialis 208-156 (240, n = 6), bothrial width 163-196 (179, n = 4); pars vaginalis 396-813 (596, n = 6); sheaths sinuous, arranged spirally in contracted specimens; bulbs elongate, extending to posterior margin of scolex; bulbs 325-470 (408, n = 6) long, 54-75 (65, n = 6) wide; bulb ratio 5.28-7.85 (6.27, n = 6); retractor muscle originates at base of bulb, origin surrounded by small number of gland cells; pre-bulbar organ present.

Everted tentacles up to 400 long, with prominent basal swelling; diameter in basal region 21-29 (25, n = 10), in metabasal region 17-21 (19, n = 10); hooks hollow.

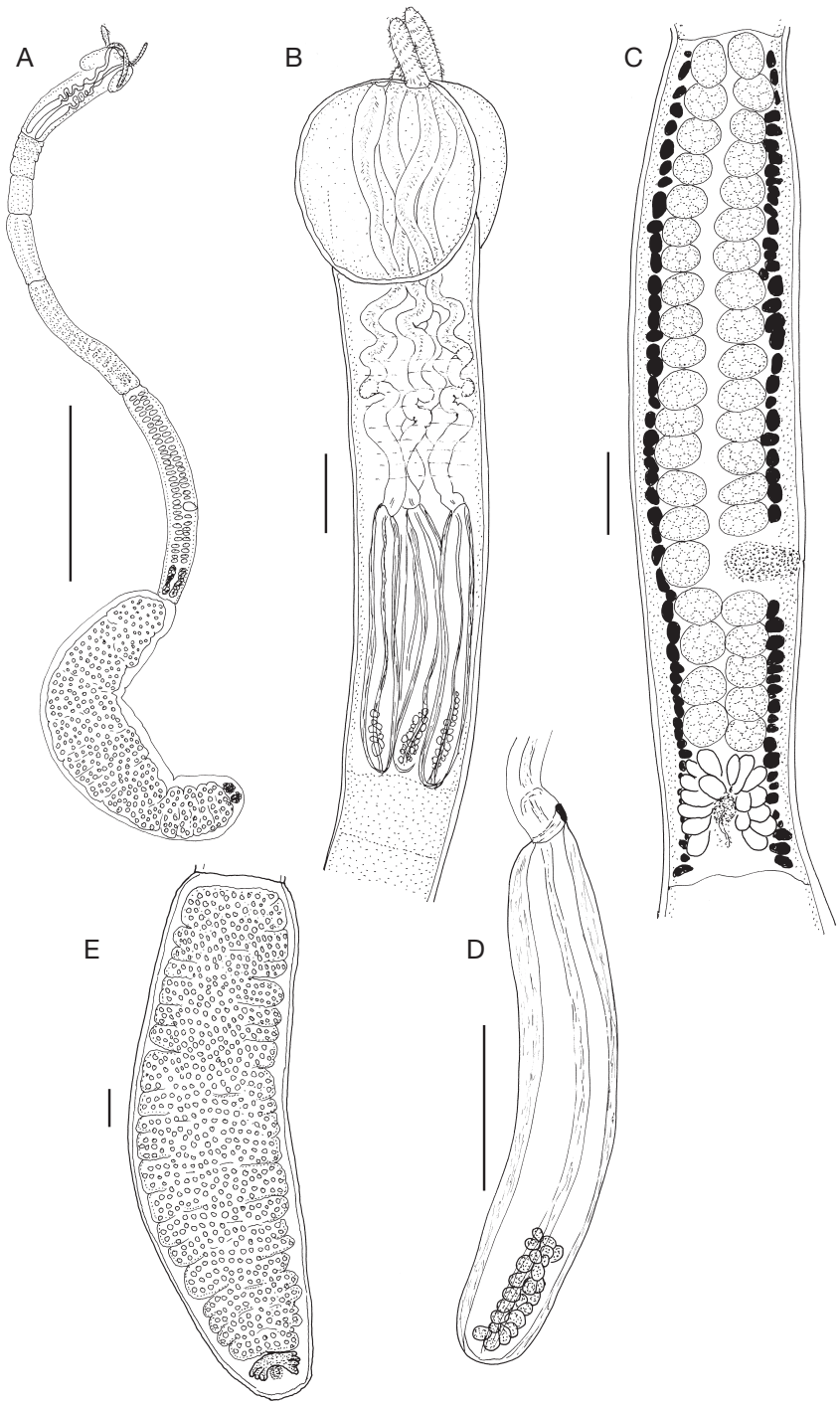

FIG.2. — *Prochristianella aciculata* n. sp. from *Neotrygon kuhlii* (Müller & Henle, 1841): **A**, entire gravid worm; **B**, scolex; **C**, mature segment; **D**, tentacular bulb; **E**, gravid segment. Scale bars: A, 1.0 mm; B-E, 0.1 mm.

Basal armature: first 2-3 rows of hooks enlarged, falcate, 8-11 (9.9, n = 10) long, base 5-9 (6.9, n = 10); next 18 rows of hooks originating on antibothrial surface, terminating on bothrial surface; hooks smaller, triangular on bothrial, internal and external surfaces, tending to spiniform towards antibothrial surface, 2-5 (3.4, n = 10) long, base 2.0-3.5 (2.7, n = 10); slender, erect hooks on antibothrial surface 3-5 (4.3, n = 10) long, base 0.5-1.0 (0.6, n = 10) long; transition to metabasal armature occurs at about row 20.

Metabasal armature heteroacanthous, typical; hook rows begin on antibothrial surface of tentacle, terminate on bothrial surface. Distinct space between hooks 1 and 1' on antibothrial surface of tentacle. Hook sizes increase from hooks 1(1') to 3(3') then gradually decrease in size; 13-15 hooks per principal row. Hooks 1(1') large, uncinate, 7-10 (8.4, n = 10) long, base 5-8 (6.5, n = 5); hooks 2(2') slender, falcate, 8-16 (11.8, n = 10) long, base 3-6 (4.7, n = 10) long; hooks 3(3') slender, falcate, 10-15 (12.2, n = 10) long, base 3-5.5 (4.8, n = 10) long; hooks 4(4') slender, falcate, 10-14 (11.9, n = 10) long, base 3-4 (3.4, n = 10) long; hooks 5(5') falcate, shorter than hooks 4(4'), slender, 7-10 (7.9, n = 10) long, base 1-2 (1.9, n = 10) long; hooks 6(6') larger, slender, spiniform, 4-7 (5.9, n = 10) long, base 1-1.5 (1.1, n = 10) long; hooks 7(7')-15(15') small, spiniform, 1.0-2.5 (1.8, n = 10) long, base 0.5-1.0 (1.8, n = 10).

Mature segments acraspedote, elongate, 562-1146 (792, n = 4) long, 146-229 (177, n = 4) wide; length/width ratio 3.85-5.00 (4.40, n = 4). Testes visible in single segment only, 20 in number, arranged in 2 columns anterior to ovary; 3 testes posterior to genital pore, 6 anterior to genital pore, 11 on antiporal side of segment. Ovary at posterior extremity of segment, with 4 lobes, each lobe 188-271 (222, n = 3) long, 50-75 (58, n = 3) wide, with multiple lobules; Mehlis' gland present between ovarian lobes; vitelline follicles circum- medullary, 25-68 (45, n = 10) in diameter; uterus tubular, median, extending to anterior extremity of segment; uterine pore absent. Gravid segments 1688-1825 (1754, n = 3) long, 279-438 (342, n = 3) wide; gravid uterus entirely filling medulla; eggs 16 in diameter, spherical, shell tanned.

DISCUSSION

The two species described above are allocated to *Prochristianella* Dollfus, 1946 because they possess two bothria, a typical heteroacanthous metabasal armature, elongate tentacular bulbs with a prebulbar organ and gland cells attached to the retractor muscle (features of the Eutetrarhynchidae Guiart, 1927) as well as a basal swelling bearing a distinctive basal armature and the hooks of the principal rows of the metabasal region of the tentacle increasing then decreasing in size along each row (features of *Prochristianella*) (Palm 2004).

Palm (2004) recognised 13 species in *Prochristianella* (*P. butlerae* Beveridge, 1990, *P. clarkeae* Beveridge, 1990, *P. fragilis* Heinz & Dailey, 1974, *P. glaber* (Dollfus, 1969), *P. heteracantha* Dailey & Carvajal, 1976, *P. hispida* (Linton, 1890), *P. macracantha* Palm, 2004, *P. minima* Heinz & Dailey, 1974, *P. mooreae* Beveridge, 1990, *P. odonoghuei* Beveridge, 1990, *P. papillifer* (Poyarkoff, 1909), *P. thalassia* (Kovaks & Schmidt, 1980), *P. tumidula* (Linton, 1890)) since which one additional species, *P. multidum* Friggins & Duszynski, 2005, has been described. The latter species was reported in the abstract of the paper as "*P. minima* sp. n.", a potential homonym for *P. minima* Heinz & Dailey, 1974. However, this appears to be an error as in the text, the new species is named *P. multidum*. An additional species, *Prochristianella bengalensis* Chandra & Rao, 1984 (orthography corrected), is unrecognisable from its description (Chandra & Rao 1984) and is here treated as a *species inquirenda*.

Palm (2004) distributed the species of *Prochristianella* phenetically among three groups: those with prominent microtriches (spines) on the surface of the scolex and those without, the latter being divided into two groups, those with prominently enlarged hooks in the first two rows of the basal region of the tentacle and those without enlarged hooks. *Prochristianella multidum* falls into the first group although this feature was not clearly indicated in the original description. However, figures 21 and 24 of Friggins & Duszynski (2005) clearly show the enlarged microtriches on the scolex.

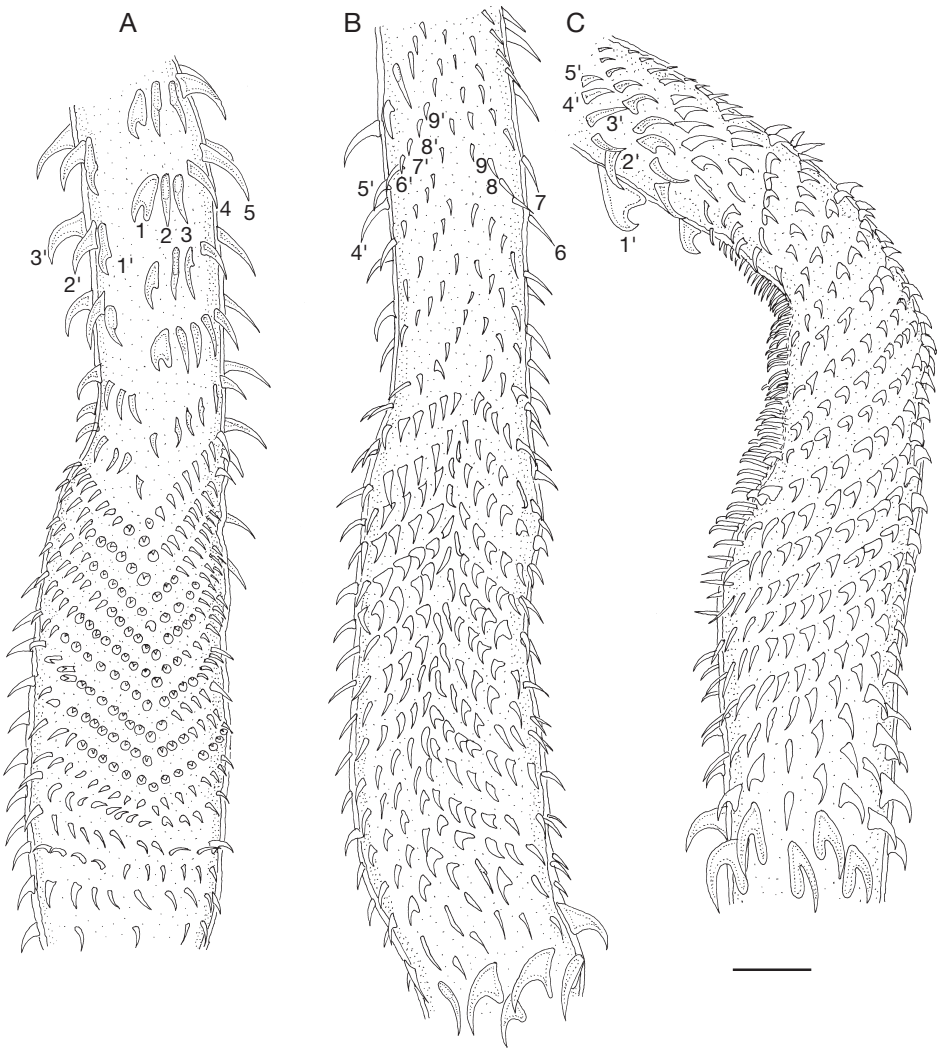

FIG. 3. — *Prochristianella omunae* n. sp. from *Neotrygon kuhlii* (Müller & Henle, 1841), basal and metabasal tentacular armature: **A**, antibothrial surface, internal surface on left hand side (enlarged basal hooks not shown); **B**, bothrial surface, internal surface on right hand side; **C**, external surface, bothrial surface on right hand side. Scale bar: 0.01 mm.

The first species described above, *P. aciculata* n. sp., lacks prominent microtriches on the scolex but has enlarged hooks at the base of the tentacle, thereby indicating similarities with *P. butlerae*, *P. fragilis*, *P. mooreae*, *P. odonoghuei* and *P. macracantha*. The species described here differs from *P. odonghuei* in that it lacks enlarged bill hooks on the bothrial surface of the basal armature, and from *P. macracantha* which has only two to three enlarged basal hooks rather than the two to three rows in *P. aciculata* n. sp. It differs from all of these species since hooks 1(1') are unique in shape, being almost linear with a small hook at their proximal extremity, in hooks 3(3') and 4(4') being slender and almost needle-like or aciculate, with hooks 5(5') much smaller than either hooks

4(4') or 6(6') and with hooks 7(7')-10(10') being tiny and spiniform. The tentacular armature is therefore clearly unique and for these reasons, the specimens described here are considered to represent a new species.

The morphology of the mature segment could not be described in detail as the vitelline follicules completely obscured all remaining features of the internal anatomy. However, the number of testes (40-50) also separates this species from *P. fragilis* (50-60) and *P. macracantha* (58-62).

There is some variation between species of *Prochristianella* in the hook arrangement in the metabasal region of the tentacle. In the type species, *P. papillifer*, hooks 1(1') are present in the first few principal rows, such that along most of the tentacle, the first hook observed in each row is a large, falcate, hook 2 (Beveridge *et al*. 2004). In *P. mooreae*, hooks 1 and 2 disappear in the metabasal region so that along most of the tentacle, the first hook observed in each row is a large, falcate, hook 3 (Beveridge 1990). In other species such as in *P. hispida* and *P. clarkeae*, hooks 1 differ in size from hooks 1' while in *P. odonoghuei*, *P. fragilis*, *P. butlerae*, *P. minima* and *P. tumidula*, hooks 1(1') are small and falcate. Thus, in species adequately described, the first hooks of the principal rows provide useful specific characters. In *P. aciculata* n. sp., hooks 1(1') persist along the tentacle but are unique in shape being almost linear and can be easily overlooked. In most views of the tentacle, the rows appear to commence with a large, uncinate hook, but this is in fact hook 2 and may obscure the much smaller and less conspicuous hook 1.

Prochristianella omunae n. sp. differs from all congeners apart from *P. minima* in having an extended basal armature of about 20 rows distal to the enlarged 2-3 rows at the base. In all the remaining species, there are up to 12 rows of hooks beyond the initial basal hooks (for summary see Palm 2004). However, *P. minima* lacks the rows of enlarged hooks at the base of the tentacle and has nine hooks per principal row compared with 13-15 in the present species. Heinz & Dailey (1974) did not give detailed measurements of the sizes of individual hooks but it appears from their figure 13, that hooks 1(1') are extremely small compared with the succeeding hooks, unlike the present species which has relatively large and robust hooks 1(1'). Unfortunately, in mature segments of the present species, internal organs were entirely obscured by the vitelline follicles and testes could be counted in one pre-mature segment only. The number found in the present species (20) is similar to that reported in *P. minima* (18-25). No features of the cirrus sac and vagina were visible in either mature or gravid segments, being obscured in the first and having involuted in the second.

Other cestode parasites found in the rays examined included *Prochristianella clarkeae* (MNHN JNC1627), a species extending from the Bay of Bengal to Australia, with its distribution now extended to New Caledonia and *Mecistobothrium pauciortesticulatum* Palm, 2004 (MNHN JNC957) a species previously known only from *Taeniura lymma* (Forsskål, 1775) from Indonesia (Palm 2004), thus substantially extending its known geographical range. Two other eutetrarhynchid species found were *Eutetrarhynchus leucomelanus* (Shipley & Hornell, 1906) (MNHN JNC958), the adults of which were previously known only from Sri Lanka and northern Australia (Beveridge 1990) and a single specimen of a species of *Dollfusiella* Campbell & Beveridge, 1994 (MNHN JNC958) was also found. The current record therefore also substantially expands the known geographical range of *E. leucomelanus*.

The host of the new species is here provisionally identified as *N. kuhlii*, with recognition that this species appears to be a complex in the south Pacific and that host nomenclature is likely to be revised in the future (Last & Compagno 1999; Last & Stevens 2009).

The eutetrarhynchid fauna of the southern Pacific region is very poorly known, almost certainly due primarily to a lack of collecting. The current paper reports three species of *Prochristianella* from this region, of which two are new. It also indicates that *Dollfusiella* occurs in the same region, although the material available is insufficient to describe or identify the species present. The data available suggest that additional collecting is likely to reveal additional species.

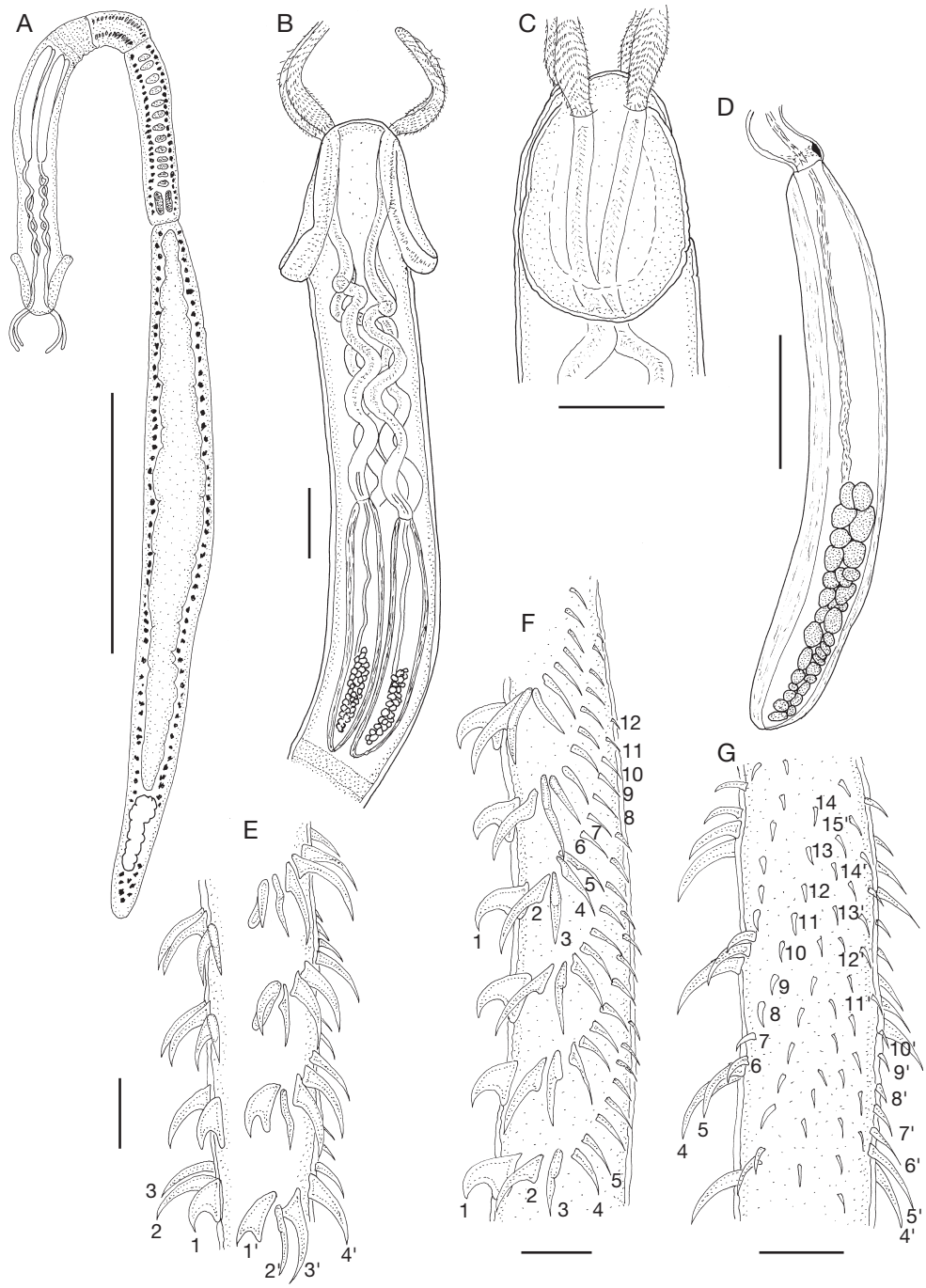

FIG. 4. — *Prochristianella omunae* n. sp. from *Neotrygon kuhlii* (Müller & Henle, 1841): **A**, entire gravid worm; **B**, scolex; **C**, bothrium; **D**, tentacular bulb; **E**, metabasal armature, antibothrial surface, internal surface on left hand side; **F**, metabasal armature, internal surface, antibothrial surface on left hand side; **G**, metabasal armature, bothrial surface, internal surface on left hand side. Scale bars: A, 1.0 mm; B-D, 0.1 mm; E-G, 0.01 mm.

Acknowledgements
Financial support to IB was provided by National Science Foundation PB&I grant nos. 0818696 and 0818823 (A survey of the tapeworms (Cestoda: Platyhelminthes)). Prof. Claude Chauvet (Université de Nouvelle-Calédonie, Nouméa, New Caledonia, UNC) and Jean-Louis Pagnon are gratefully thanked for collecting of fish on board the *Alcyon*. Soazig Le Mouellic (UNC) provided technical help. Prof. Louis Euzet collected parasites used in this report; Amandine Marie, student, and Angelo di Matteo (IRD Nouméa) provided valuable assistance.

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Submitted on 16 February 2010;
accepted on 3 August 2010.