

Research Article

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New species of *Guidus* Ivanov, 2006 (Cestoda: Phyllobothriidea) from *Bathyraja magellanica* (Philippi) from the Patagonian Continental Shelf of Argentina

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³This paper is dedicated to the memory of the late Verónica Ivanov with whom the first author started writing the manuscript.

Abstract: Specimens representing two new species of *Guidus* Ivanov, 2006 were collected from the Magellan skate (*Bathyraja magellanica* [Philippi]) in the Patagonian Continental Shelf of Argentina, Southwestern Atlantic Ocean. *Guidus francoi* sp. n. and *Guidus magellanicus* sp. n. differ from their congeners by a particular combination of features, including type of bothridia, worm length, number of testes, and distribution of vitelline follicles. *Guidus francoi* sp. n. is distinguished from *G. magellanicus* sp. n. by having fewer proglottids, fewer testes and a higher ratio between the cirrus sac length and the proglottid width. The microthrix pattern of species of *Guidus* from the Southwestern Atlantic is described, based on specimens of *G. francoi* sp. n., *Guidus magellanicus* sp. n., and newly collected specimens of *Guidus argentinense* Ivanov, 2006. These three species share the presence of wide aristate gladiate spinitriches on the proximal bothridial surface, narrow gladiate spinitriches on the bothridial rim, and filitriches on the distal bothridial surface. The diagnosis of *Guidus* is revised to include several features exhibited by the new species (i.e., presence of bothridial indentations and bothridial stalks, distribution of vitelline follicles, and eggs grouped in cocoons). The discovery of *G. francoi* sp. n. and *G. magellanicus* sp. n. from *B. magellanica* increases the number of species of *Guidus* collected from batoids in the Southwestern Atlantic from one to three. The specificity exhibited by the species herein described reinforces the tight association between rays in the genera *Guidus* and *Bathyraja*.

Key words: *Guidus francoi* sp. n., *Guidus magellanicus* sp. n., morphology, description, SEM, Magellan skate, Arhynchobatidae, Argentinian Continental Shelf, Southwestern Atlantic Ocean.

The order Phyllobothriidea Caira, Jensen, Waeschenbach, Olson et Littlewood, 2014 is known to be cosmopolitan in distribution, with the majority of records from the Northern Hemisphere (Ruhnke 2011, Moghadam and Haseli 2019, Caira et al. 2020, Maleki et al. 2020). The order has been found in a diversity of definitive hosts including sharks, batoids and chimaeras. To date, most of the genera (11/18) within the order are known to be associated to a particular species, genus or family of shark (Ruhnke 2011, Ruhnke et al. 2017, 2020, Moghadam and Haseli 2019, Caira et al. 2020, Maleki et al. 2020).

Unlike most phyllobothriideans, members of *Guidus* Ivanov, 2006 are typically distributed in temperate and cold waters in the Southern Hemisphere and parasitise arhynchobatid skates of the genus *Bathyraja* Ishiyama (see Wojciechowska 1991, Rocka and Zdzitowiecki 1998, Ivanov 2006). Among the three valid species, *Guidus awii*

(Rocka et Zdzitowiecki, 1998) and *Guidus antarcticus* (Wojciechowska, 1991) were reported from Antarctic waters and described from the type host *Bathyraja maccaini* Springer, whereas *Guidus argentinense* Ivanov, 2006 was reported from lower latitudes off Argentina from *Bathyraja brachyurops* (Fowler) (see Wojciechowska 1991, Rocka and Zdzitowiecki 1998, Ivanov 2006).

During a parasite survey of batoids off the Argentinian Patagonian Shelf in the Southwestern Atlantic Ocean between ~49°–54°S, tapeworms parasitising the Magellan skate, *Bathyraja magellanica* (Philippi), were recognised as members of the genus *Guidus*. Two new species of *Guidus* are herein described based on detailed morphology of adult worms using light and scanning electron microscopy (SEM), and histological sections. Additionally, the bothridial surfaces of *G. argentinense* are studied with SEM for the first time, based on newly collected specimens.

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MATERIALS AND METHODS

Specimens examined in this study were collected from the intestine of nine *Bathyrāja magellanica* and two *Bathyrāja brachyurops* (Fowler) (Rajiformes: Arhynchobatidae) taken from different localities along the Argentine Sea. The specimens of *B. magellanica* were caught off Río Grande, Tierra del Fuego Province at 54.5082S, 65.2328W (assigned unique host number PD4-001) and 54.4033S, 63.9625W (PD4-025) in March 2012, 54.0279S, 67.1134W (PD4-097) in April 2012, 53.9293S, 67.0834W (PD10-017) in March 2014, and 53.6042S, 67.6512W (PD10-070, PD10-071) and 53.9381S, 66.0790W (PD10-156) in April 2014; and off Puerto San Julián, Santa Cruz Province at 49.4938S, 66.1869W (PD7-105, PD7-107) in April 2013. Specimens of *B. brachyurops* were caught off Buenos Aires Province at 39.5713S, 56.2694W (PD3-285) in March 2011, and off the Namuncurá Marine Protected Area/Burdwood Bank at 54.7470S, 59.9476W (PD12-297) in April 2016. All hosts were caught with bottom trawls on board of the RV “*Puerto Deseado*” (CONICET).

All tapeworms were removed from the spiral intestine of their respective host, relaxed in seawater, fixed in 10% formalin and transferred to 70% ethanol for storage. The specimens prepared for light microscopy were hydrated in a graded ethanol series, stained with Harris haematoxylin, dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted in Canada balsam. The terminal portion of four strobilae of specimens from *B. magellanica* was embedded in paraffin and serial cross sections were cut at a thickness of 7 μ m. Sections were stained with Harris haematoxylin, counterstained with eosin and mounted in Canada balsam. Gravid proglottids of specimens from *B. magellanica* were opened with insect pins to release the cocoons. Cocoons were mounted in lactophenol and sealed with nail polish.

Whole mounts, non-permanent mounts and histological sections were examined and measured using Olympus BX 51 and Zeiss Axioscope compound microscopes. Drawings were made with the aid of a drawing tube attached to the Olympus BX 51 compound microscope. Measurements are expressed as the range, followed in parentheses by the mean and standard deviation and the number of worms from which the measurements were taken. Measurements of genitalia were taken from mature proglottids of mature and gravid worms. All measurements are in micrometres unless otherwise stated.

Worms prepared for scanning electron microscopy (SEM) were hydrated in a graded ethanol series, post-fixed in 1% osmium tetroxide overnight at room temperature, dehydrated in a graded ethanol series, and dried using hexamethyldisilazane. After drying, the specimens were mounted on stubs with carbon tape, coated with *c.* 40 nm of gold/palladium with a Thermo VG Scientific Polaron SC 7630 and examined in either a Philips XL 30 or a Carl Zeiss NTS-SUPRA 40 scanning electron microscope.

Terminology for microthrix shape and size follows Chervy (2009). Valid host names follow Froese and Pauly (2020). Marine regionalisation follows Spalding et al. (2007). Museum abbreviation used are as follows: IP-

CAS, Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic; MACN-Pa, Museo Argentino de Ciencias Naturales, Colección Parasitológica, Buenos Aires, Argentina.

RESULTS

Guidus francoi sp. n.

Figs. 1–3

ZooBank number for species:

[urn:lsid:zoobank.org:act:1A016D65-7CA8-40A3-A2C6-818D397EC5E0](https://zoobank.org/act:1A016D65-7CA8-40A3-A2C6-818D397EC5E0)

Description (based on 13 mature and 21 gravid whole worms, two mature and one gravid specimens examined with SEM, and cross sections of mature proglottids). Worms anapolytic; proglottids acraspedote. Specimens possessing mature proglottids 3.4–5.7 (4.5 ± 0.7 , $n = 13$) mm long, 8–17 (12 ± 3 , $n = 13$) proglottids per worm (Figs. 1A, 2A). Specimens possessing gravid proglottids 2.9–10.9 (5.9 ± 2.3 , $n = 21$) mm long, 8–24 (13 ± 4 , $n = 20$) per worm (Fig. 1B,E). Maximum worm width at level of scolex (Fig. 1A,B). Scolex composed of scolex proper and 4 stalked bothridia, 445–1,050 (689 ± 141 , $n = 30$) long, 450–1,087 (692 ± 137 , $n = 30$) wide (Figs. 1A–D, 3A). Bothridia globose, conspicuously muscular, 365–1,012 (530 ± 129 , $n = 31$) long, 175–345 (268 ± 48 , $n = 31$) wide; with anteriorly-oriented apical aperture, continuous muscular sphincter, and apical sucker; anterior bothridial margin indented (Figs. 1A–D, 3A). Bothridial aperture 160–355 (266 ± 53 , $n = 28$) diameter in apical view. Muscular sphincter encircling bothridial aperture, 12–25 (16 ± 3 , $n = 24$) thick. Apical sucker on anterior margin of bothridium 49–92 (69 ± 12 , $n = 10$) in diameter (Figs. 1C,D, 3B). Stalks short, 44–75 (59 ± 13 , $n = 4$) long, 151–192 (172 ± 16 , $n = 8$) wide (Figs. 1D, 3A,B). Cephalic peduncle absent. Neck, 1,170–4,430 ($2,224 \pm 804$, $n = 25$) long, 110–210 (157 ± 28 , $n = 25$) wide.

Apex of scolex proper covered with capilliform filitriches. Bothridial rim covered with narrow gladiate spinitriches interspersed with capilliform filitriches (Fig. 3B,I). Proximal bothridial surface covered with wide aristate gladiate spinitriches interspersed with capilliform filitriches; aristate gladiate spinitriches becoming shorter and wider more proximally (Fig. 3A,E,F,H). Distal bothridial surface covered with acicular to capilliform filitriches (Fig. 3G). Apical sucker surface covered with capilliform filitriches (Fig. 3J). Stalks covered with aristate gladiate spinitriches interspersed with capilliform filitriches, lacking spinitriches more proximally (Fig. 3B,D). Capilliform filitriches on neck and strobila arranged in scutes (Fig. 3A,C,K).

Specimens possessing mature proglottids with 7–16 (11 ± 3 , $n = 13$) immature proglottids, and single mature proglottid per worm (Fig. 1A). Terminal mature proglottid longer than wide, 610–865 (755 ± 82 , $n = 13$) long, 270–360 (325 ± 30 , $n = 13$) wide, length to width ratio 1.9–2.9 (2.3 ± 0.2 , $n = 13$): 1 (Figs. 1A, 2A). Specimens possessing gravid proglottids with 6–18 (12 ± 6 , $n = 20$) immature proglottids, 1–2 (1.5 ± 0.5 , $n = 20$) mature proglottids, and 1–5 (2 ± 1 , $n = 20$) gravid proglottids per

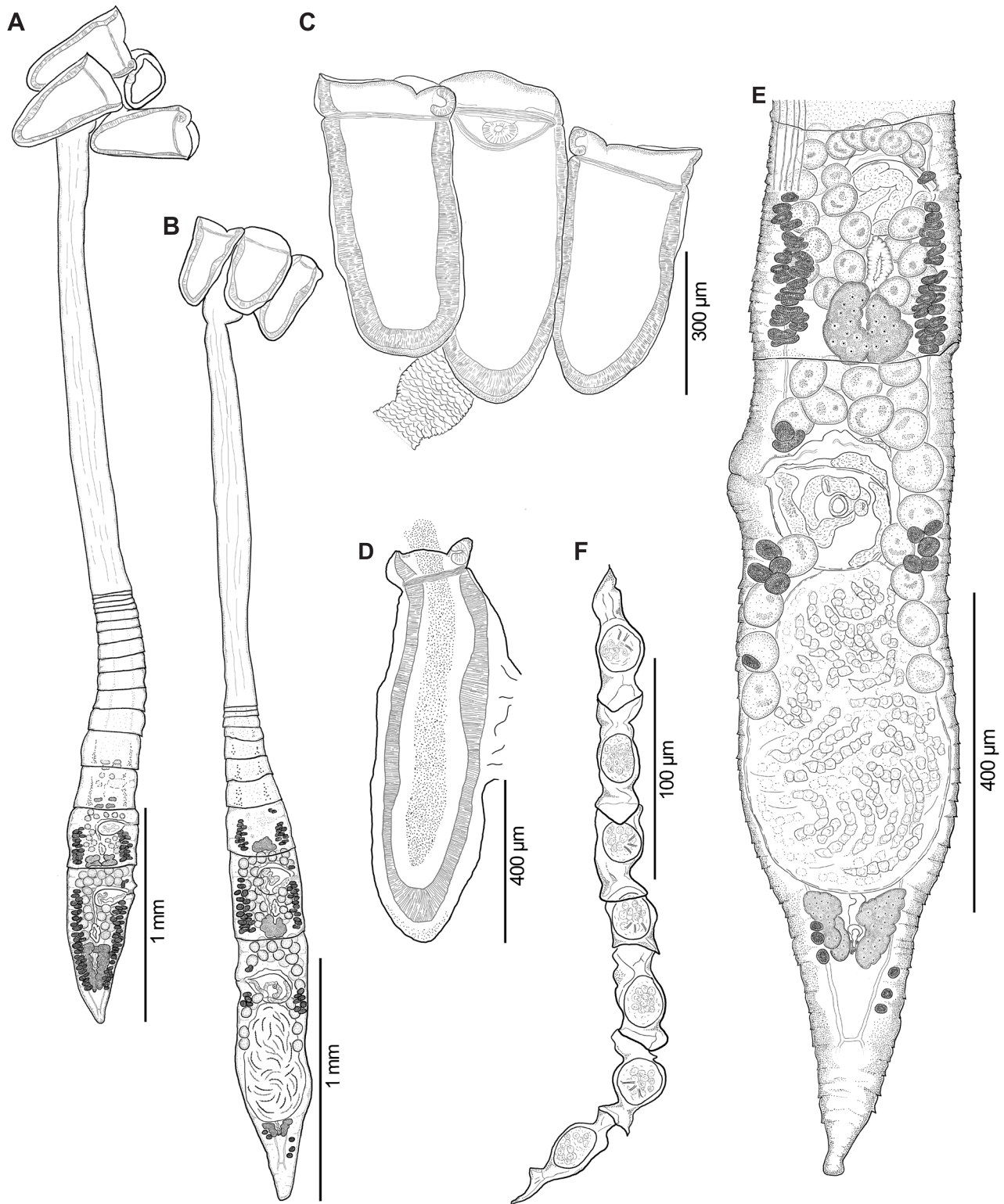


Fig. 1. *Guidus francoi* sp. n. from *Bathyraja magellanica* (Philippi), line drawings. **A** – entire mature worm (holotype MACN-Pa No. 739); **B** – entire gravid worm (paratype MACN-Pa No. 746/6); **C** – scolex (paratype MACN-Pa No. 743); **D** – bothridium attached to host tissue, muscular bothridial sphincter contracted (paratype MACN-Pa No. 741/1); **E** – terminal portion of gravid strobila, ventral view (paratype MACN-Pa No. 746/6), longitudinal muscles partially drawn to allow the view of internal organs; **F** – cocoon.

worm. Posterior-most mature proglottids wider than long to longer than wide, 240–490 (342 ± 63 , $n = 21$) long, 285–605 (406 ± 83 , $n = 21$) wide, length to width ratio 0.5–1.4 (0.9 ± 0.3 , $n = 21$): 1. Subterminal gravid proglottids wider than long to longer than wide, 405–610 (474 ± 70 , $n = 8$)

long, 422–608 (513 ± 72 , $n = 8$) wide, length to width ratio 0.7–1.3 (1.0 ± 0.2 , $n = 19$): 1. Terminal gravid proglottid longer than wide, 700–1,338 (993 ± 166 , $n = 13$) long, 300–550 (416 ± 67 , $n = 13$) wide, length to width ratio 1.8–3.4 (2.4 ± 0.4 , $n = 13$): 1 (Figs. 1B,E, 3K). Testes oval,

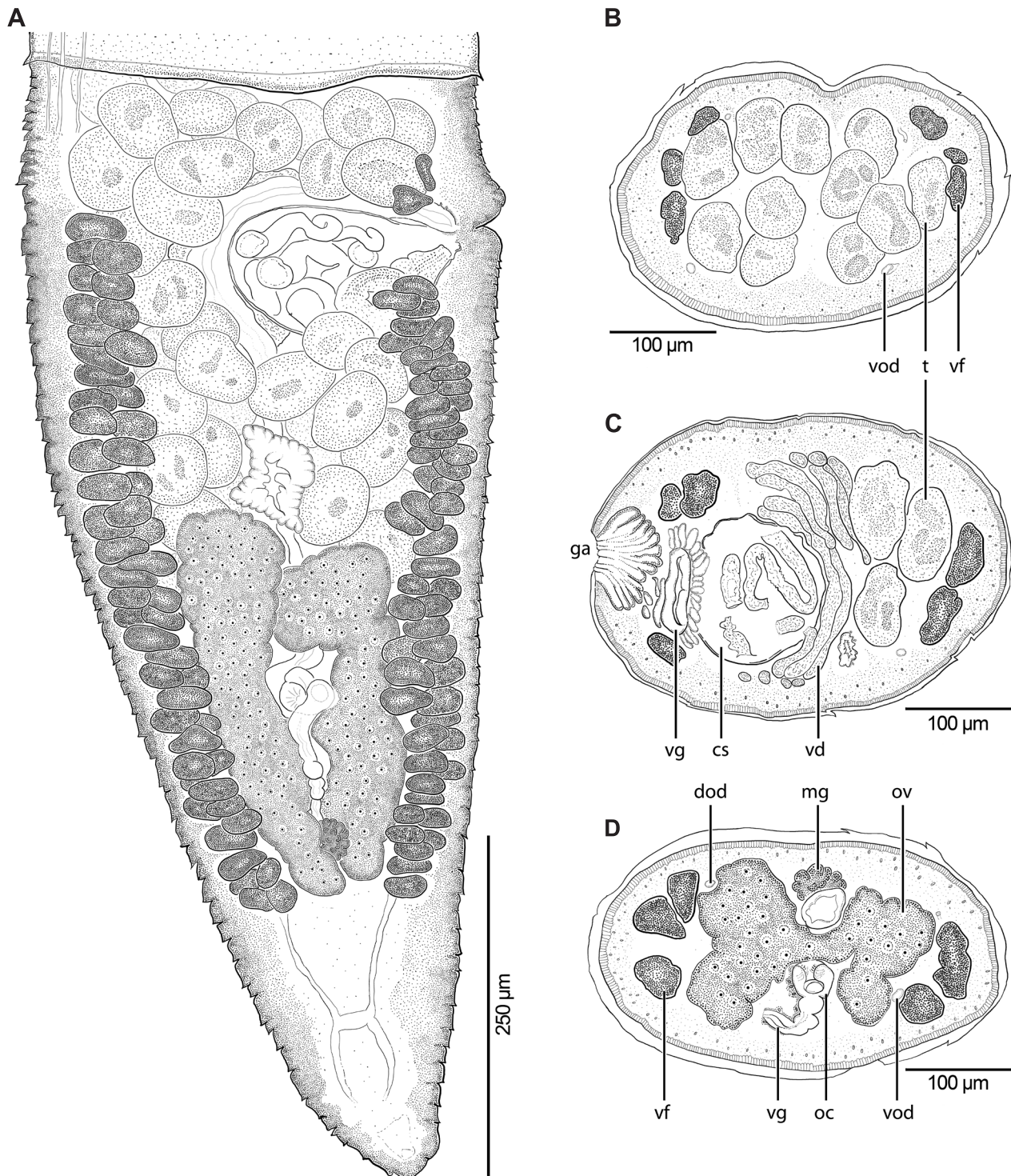


Fig. 2. *Guidus francoi* sp. n. from *Bathyraja magellanica* (Philippi), line drawings. **A** – terminal mature proglottid, ventral view (holotype MACN-Pa No 739), longitudinal muscles partially drawn to allow the view of internal organs; **B** – cross section of terminal mature proglottid at level of testes anterior to cirrus sac; **C** – cross section of terminal mature proglottid at level of genital atrium; **D** – cross section of terminal mature proglottid at level of ovarian isthmus. **Abbreviations:** cs – cirrus sac, dod – dorsal osmoregulatory duct, ga – genital atrium, mg – Mehlis' gland, oc – ovicapt, ov – ovary, t – testis, vd – vas deferens, vf – vitelline follicle, vg – vagina, vod – ventral osmoregulatory duct.

42–68 (52 ± 7 , $n = 31$) long, 38–51 (45 ± 5 , $n = 31$) wide, arranged in 4–5 columns dorsoventrally and in 2–3 layers deep in cross section; 42–68 (53 ± 6 , $n = 33$) in number; extending from anterior margin of proglottid to anterior margin of ovary (Figs. 1E, 2A–C). Cirrus sac oval, 118–230

(163 ± 25 , $n = 34$) long, 80–150 (116 ± 19 , $n = 34$) wide, cirrus sac length to proglottid width ratio 0.4–0.6 (0.5 ± 0.1 , $n = 34$): 1. Cirrus covered with minute spinitriches. Vas deferens coiled, extending from anterior margin of vagina to posterior margin of cirrus sac (Figs. 1E, 2A,C).

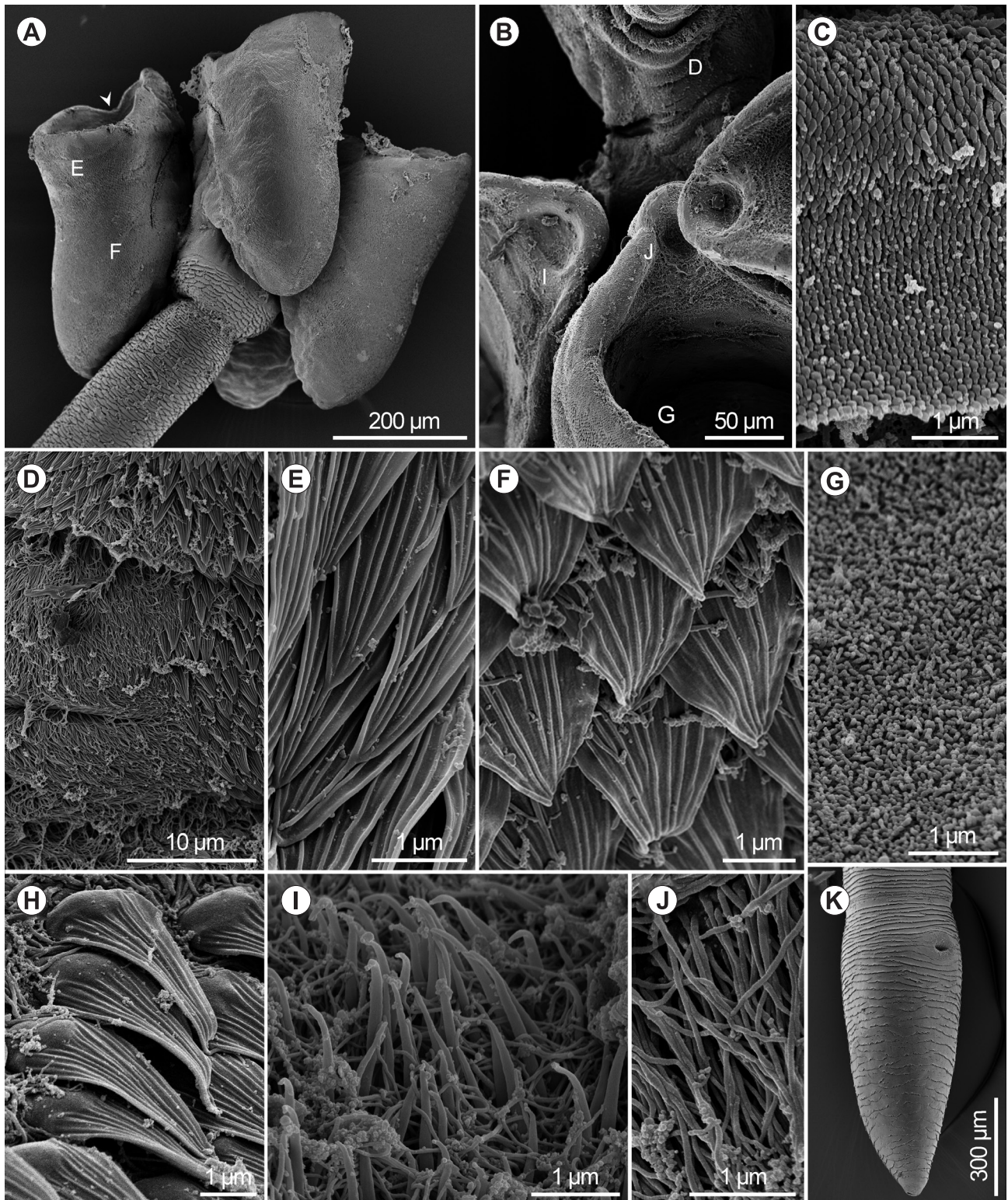


Fig. 3. *Guidus francoi* sp. n. from *Bathyraja magellanica* (Philippi), scanning electron micrographs. **A** – scolex, small letters indicate locations of detail shown in E and F, arrow indicates marginal indentation; **B** – partial apical view of scolex, small letters indicate locations of detail shown in D, G, I and J; **C** – partial view of strobila scutes; **D** – surface of bothridial stalk, apical view; **E** – proximal bothridial surface near the bothridial rim, wide aristate gladiate spinitriches; **F** – proximal bothridial surface in mid-region, wide and short aristate gladiate spinitriches; **G** – distal bothridial surface at base of internal cavity, acicular filitriches; **H** – detail of aristate gladiate spinitriches showing pad-shaped bases; **I** – bothridial rim, narrow gladiate spinitriches and capilliform filitriches; **J** – surface of apical sucker, capilliform filitriches; **K** – terminal gravid proglottid showing scutes.

Ovary weakly lobulated, H-shaped in dorsoventral view, tetralobed in cross section, 88–283 (179 ± 51 , $n = 23$) long, 100–305 (167 ± 54 , $n = 23$) wide (Figs. 1E, 2A,D). Vagina thick-walled, essentially straight, surrounded by darkly stained cells distally, extending from ootype region anteriorly, then laterally along anterior margin of cirrus sac to enter atrium anterior to cirrus (Figs. 1E, 2A,C). Genital pores alternate irregularly, 17–38% (26 ± 6 , $n = 34$) of proglottid length from anterior margin of proglottid. Vitelline follicles arranged in 2 irregular columns on each lateral margin of proglottid, 16–27 (20 ± 4 , $n = 11$) long, 19–39 (32 ± 5 , $n = 11$) wide, extending from level of genital on aporal side and from level slightly anterior to genital pore on poral side to posterior margin of proglottid, interrupted by terminal genitalia, uninterrupted by ovary; vitelline follicles degenerate in fully gravid proglottids (Figs. 1E, 2A–D). Uterus saccate, restricted to region between ovary and cirrus sac; uterine duct not observed. Osmoregulatory ducts 4, 1 dorsal and 1 ventral pair. Oncospheres arranged in linear cocoons, 229–314 (265 ± 44 , $n = 13$) long, 26–32 (29 ± 3 , $n = 13$) wide; 7–9 (8 ± 1 , $n = 26$) oncospheres per cocoon (Fig. 1B,E,F).

Type host: *Bathyraja magellanica* (Philippi), Magellan skate (Rajiformes: Arhynchobatidae).

Type locality: Off Río Grande, Tierra del Fuego Province, Argentina (53.9381S, 66.0790W) (host PD10-156).

Additional localities: Off Puerto San Julián, Santa Cruz Province, Argentina (49.4938S, 66.1869W) (hosts PD7-105, PD7-107), and Off Río Grande, Tierra del Fuego Province, Argentina (54.5082S, 65.2328W; 54.4033S, 63.9625W; 54.0279S, 67.1134W; 53.9293S, 67.0834W; 53.6042S, 67.6512W) (hosts PD4-001, PD4-025, PD4-097, PD10-017, PD10-070, PD10-071).

Deposited specimens: Holotype MACN-Pa No. 739 (one worm with mature proglottid), eight paratypes MACN-Pa Nos. 740/1–3, 741/1, 744, 745, 746/1–2 (eight worms with mature proglottids), 12 paratypes MACN-Pa Nos. 740/4, 741/2–3, 742/1–3, 743, 746/3–7 (12 worms with gravid proglottids), four paratypes IPCAS No. C-887 (four worms with mature proglottids), nine paratypes IPCAS No. C-887 (nine worms with gravid proglottids). Additional specimens (histological sections and specimens prepared for SEM) retained in the personal collection of Adriana Menoret.

Prevalence and intensity of infection: Prevalence 100% (nine host specimens infected out of nine specimens examined), 15–30 worms per host.

Etymology: This species is named for Franco Donati, the first author's nephew, in honour of his early enthusiasm for skates and sharks.

Remarks. *Guidus francoi* sp. n. is easily distinguished from the three valid species in the genus based on the scolex, distribution of the vitelline follicles, worm length, and number of testes. In *G. francoi* the scolex bears stalked bothridia, whereas in the other three species it is composed by sessile bothridia. The vitellarium in *G. francoi* is distributed in two lateral columns, each two follicles in width, extending anteriorly near the level of the genital pore, whereas the columns are composed by numerous vitelline follicles in width and reach the anterior margin of the pro-

glottid in the other three species. In addition, *G. francoi* is shorter than *Guidus antarcticus*, *Guidus argentinense* and *Guidus awii* (2.9–10.9 mm vs. 140 mm, 76–116 mm, and 30–90 mm, respectively) and has fewer testes (42–68 vs. 200–220, 89–153, and 120–150, respectively).

Guidus magellanicus sp. n.

Figs. 4–6

ZooBank number for species:

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Description (based on eight gravid whole worms, two gravid specimens examined with SEM, and cross sections of mature and gravid proglottids). Worms anapolytic, possessing gravid proglottids, 8.7–15.9 (12.4 ± 2.5 , $n = 8$) mm long, maximum width at level of scolex or terminal proglottid (Fig. 4A). Proglottids craspedote, 27–60 (41 ± 13 , $n = 8$) per gravid worm. Scolex composed of scolex proper and 4 stalked bothridia, 475–1,150 (792 ± 255 , $n = 7$) long, 600–1,075 (790 ± 154 , $n = 8$) wide (Figs. 4A,B,D,E, 6A). Bothridia globose, conspicuously muscular, 362–829 (544 ± 201 , $n = 7$) long, 203–300 (245 ± 32 , $n = 7$) wide; with anteriorly-oriented apical aperture, continuous muscular sphincter, and apical sucker; anterior bothridial margin indented (Figs. 4A,B,D,E, 6A). Bothridial aperture 112–300 (211 ± 65 , $n = 6$) diameter in apical view. Muscular sphincter encircling bothridial aperture, 20–25 (22 ± 2 , $n = 4$) thick. Apical sucker, 60–65 (63 ± 2 , $n = 4$) in diameter (Figs. 4B,D,E, 6C). Stalks short, 38–75 (53 ± 18 , $n = 4$) long, 200–290 (230 ± 52 , $n = 4$) wide (Figs. 4B,D,E, 6A,B). Cephalic peduncle absent. Neck 3,310–6,250 ($4,525 \pm 1,472$, $n = 4$) long, 112–260 (183 ± 62 , $n = 4$) wide.

Apex of scolex proper covered with capilliform filitriches (Fig. 6F). Bothridial rim covered with narrow gladiate spinitriches interspersed with capilliform filitriches (Fig. 6I). Proximal bothridial surface covered with wide aristate gladiate spinitriches interspersed with capilliform filitriches, aristate gladiate spinitriches becoming larger more proximally (Fig. 6A,D,E). Distal bothridial surface covered with acicular filitriches (Fig. 6A,H). Apical sucker surface covered with capilliform filitriches (Fig. 6C,G). Stalks covered with aristate gladiate spinitriches interspersed with capilliform filitriches, lacking spinitriches more proximally (Fig. 6B). Capilliform filitriches on neck and strobila arranged in scutes (Fig. 6A,J).

Worms with 20–52 (33 ± 12 , $n = 8$) immature proglottids, 1–3 (2 ± 1 , $n = 8$) mature proglottids, and 4–9 (6 ± 2 , $n = 8$) gravid proglottids per worm (Fig. 4A). Mature proglottids wider than long, 265–470 (344 ± 81 , $n = 8$) long, 520–830 (675 ± 129 , $n = 8$) wide, length to width ratio 0.3–0.9 (0.5 ± 0.2 , $n = 8$): 1. Subterminal gravid proglottids wider than long to longer than wide, 314–670 (479 ± 141 , $n = 8$) long, 555–837 (669 ± 105 , $n = 8$) wide, length to width ratio 0.4–1.2 (0.8 ± 0.2 , $n = 8$): 1. Terminal gravid proglottid, longer than wide, 810–1,387 ($1,049 \pm 197$, $n = 8$) long, 490–730 (592 ± 94 , $n = 8$) wide, length to width ratio 1.2–2.8 (1.8 ± 0.6 , $n = 8$): 1 (Figs. 4A, 5A, 6J). Testes oval, 38–58 (44 ± 8 , $n = 8$) long, 30–55 (49 ± 8 , $n = 8$) wide, arranged in 6–7 columns dorsoventrally and

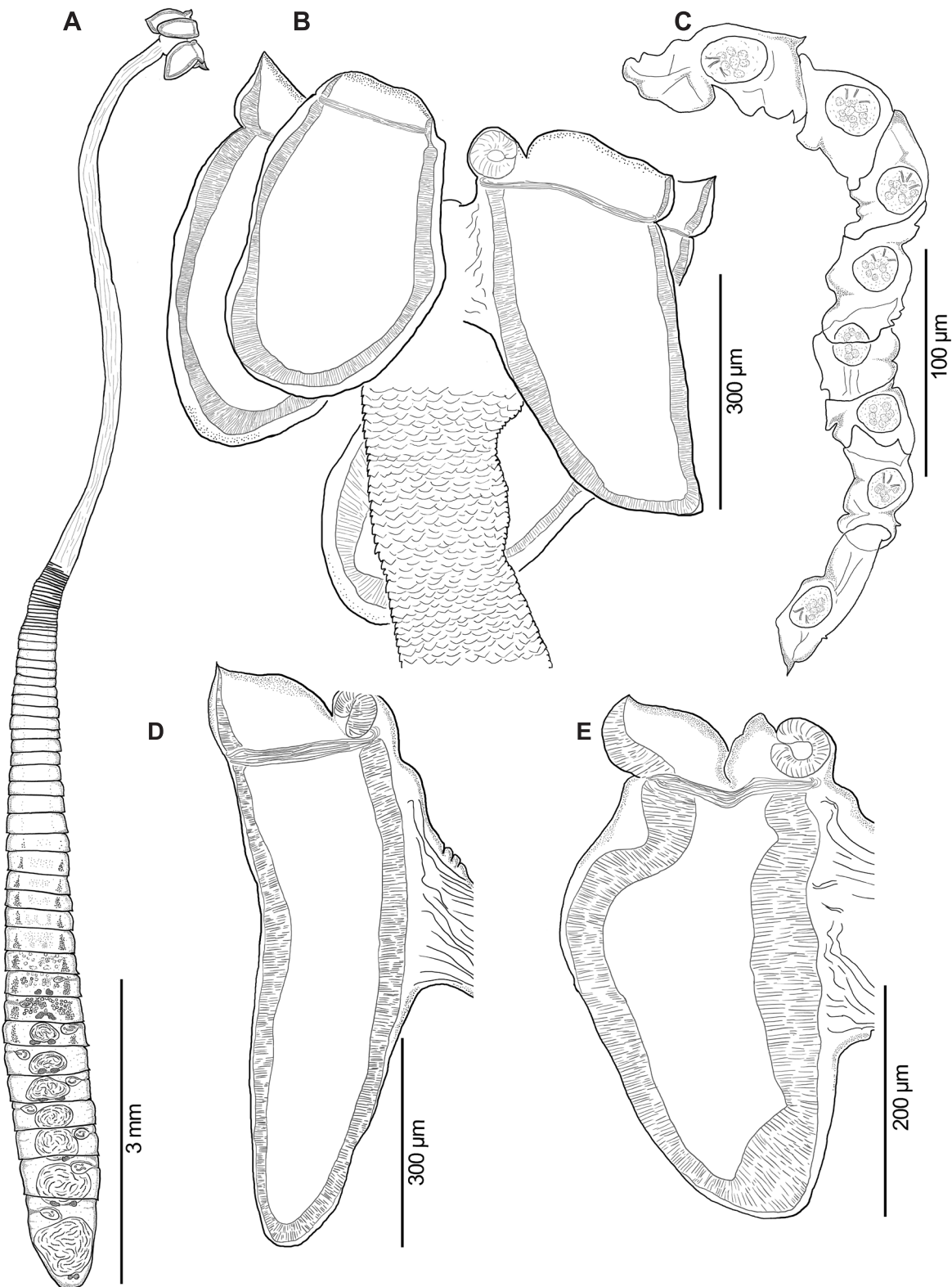


Fig. 4. *Guidus magellanicus* sp. n. from *Bathyraja magellanica* (Philippi), line drawings. **A** – entire gravid worm (holotype MACN-Pa No. 747); **B** – scolex (holotype MACN-Pa No. 747); **C** – cocoon; **D** – bothridium, muscular sphincter relaxed (paratype MACN-Pa No. 748/2); **E** – bothridium, muscular sphincter contracted (paratype IPCAS No. C-888).

4–5 layers deep in cross section; 71–105 (84 ± 7 , $n = 8$) in number, extending from anterior margin of proglottid to the level of ovarian isthmus (Fig. 5A–D). Cirrus sac oval, 140–185 (160 ± 15 , $n = 8$) long, 70–121 (90 ± 21 , $n = 8$) wide, cirrus sac length to proglottid width ratio 0.2–0.3

(0.2 ± 0.1 , $n = 8$) : 1. Cirrus covered with minute spinitriches. Vas deferens coiled, extending near anterior margin of proglottid to posterior margin of cirrus sac (Fig. 5A–C).

Ovary weakly lobulated, H-shaped in dorsoventral view, tetralobed in cross section, 82–105 (93 ± 11 , $n = 3$) long,

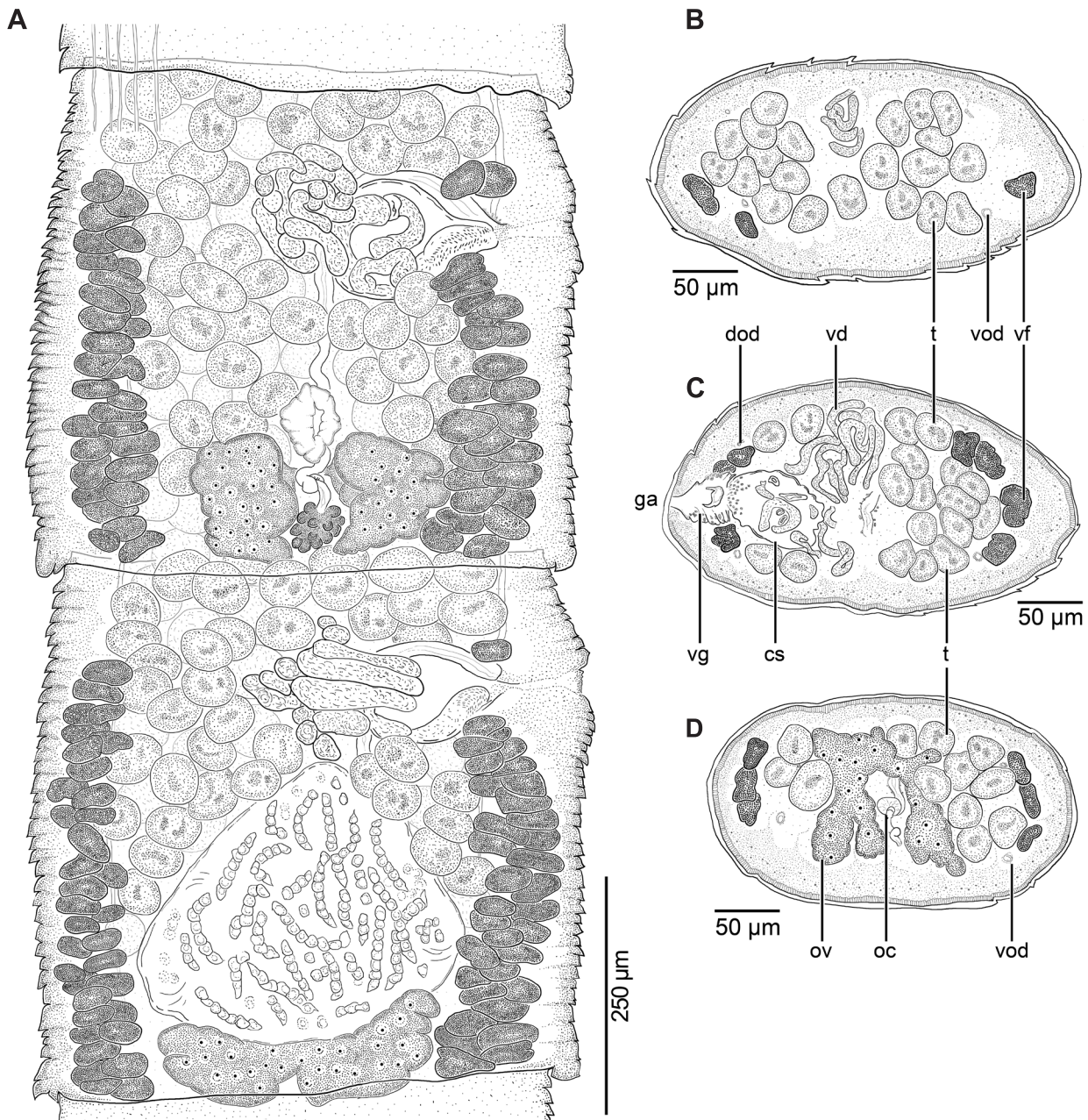


Fig. 5. *Guidus magellanicus* sp. n. from *Bathyraja magellanica* (Philippi), line drawings. **A** – subterminal portion of strobila including mature and gravid proglottids, dorsal view (paratype MACN-Pa No. 748/1); **B** – cross section of mature proglottid at level of testes anterior to cirrus sac; **C** – cross section of mature proglottid at level of genital atrium; **D** – cross section of mature proglottid at level of ovarian isthmus. *Abbreviations:* cs – cirrus sac, dod – dorsal osmoregulatory duct, ga – genital atrium, oc – ovicapt, ov – ovary, t – testis, vd – vas deferens, vf – vitelline follicle, vg – vagina, vod – ventral osmoregulatory duct.

285–350 (308 ± 36 , $n = 3$) wide (Fig. 5A,D). Vagina thick-walled, surrounded by darkly stained cells distally, essentially straight, extending from ootype region anteriorly, then laterally along anterior margin of cirrus sac to enter genital atrium anterior to cirrus (Fig. 5A,C). Genital pores alternate irregularly, 31–40% (35 ± 3 , $n = 6$) of proglottid length from anterior margin of proglottid. Vitelline follicles arranged in 2 irregular columns on each lateral margin of proglottid, 15–25 (19 ± 4 , $n = 6$) long, 27–39 (34 ± 4 , $n = 6$) wide, extending from level of genital pore on aporal side and from level slightly anterior to genital pore on poral

side to posterior margin of proglottid, interrupted by terminal genitalia, uninterrupted by ovary (Fig. 5A,D). Uterus saccate, restricted to region between ovary and cirrus sac; uterine duct not observed. Osmoregulatory ducts 4, 1 dorsal and 1 ventral pair. Oncospheres arranged in linear cocoons; cocoons 308–314 (311 ± 4 , $n = 12$) long, 31–33 (32 ± 1 , $n = 12$) wide; 7–8 oncospheres per cocoon (Figs. 4A,C, 5A).

Type host: *Bathyraja magellanica* (Philippi), Magellan skate (Rajiformes: Arhynchobatidae).

Type locality: Off Rio Grande, Tierra del Fuego Province, Argentina (54.0279S, 67.1134W) (host PD4-097).

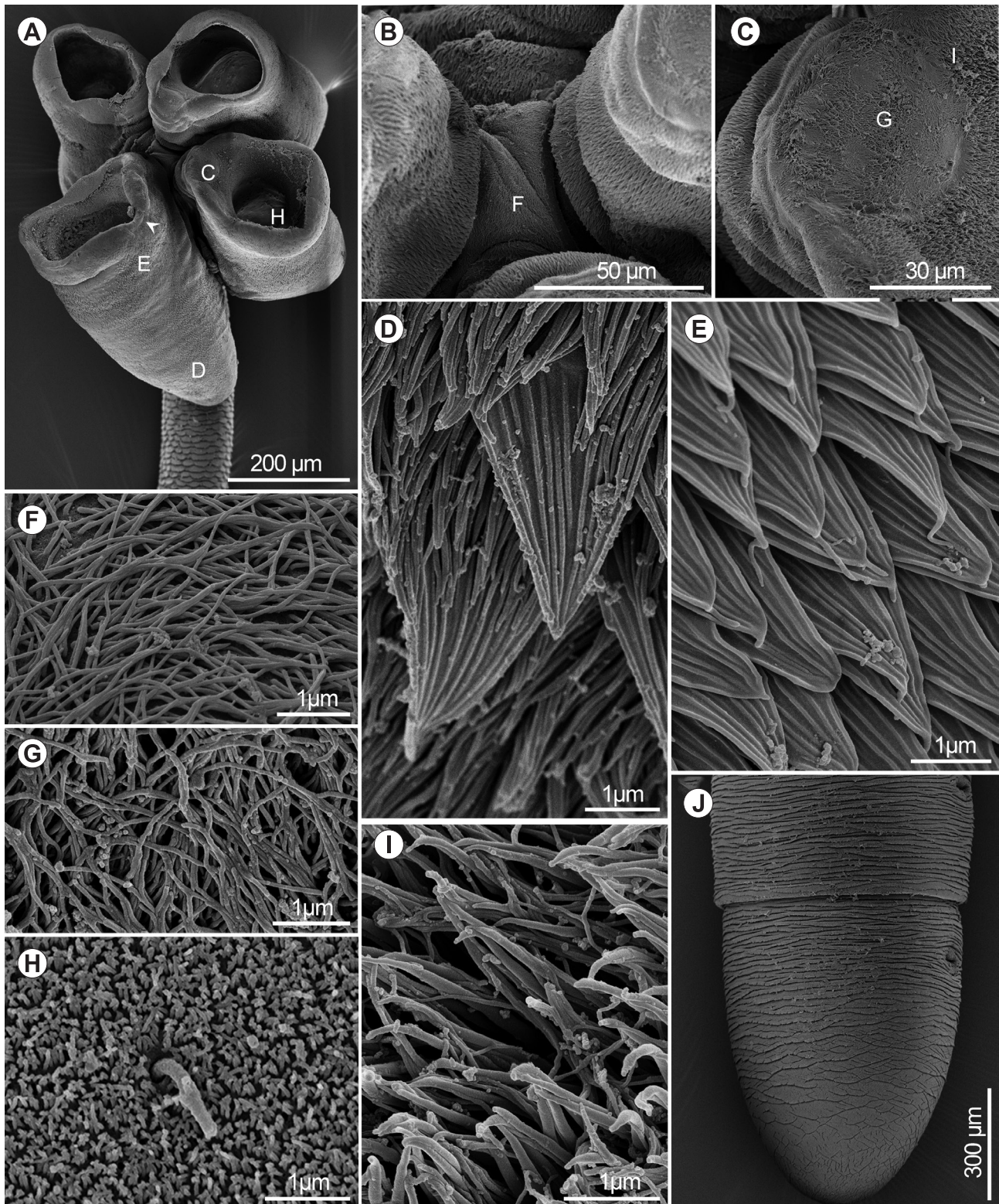


Fig. 6. *Guidus magellanicus* sp. n. from *Bathyraja magellanica* (Philippi), scanning electron micrographs. **A** – scolex, small letters indicate locations of detail shown in C–E and H, arrow indicates marginal indentation; **B** – partial apical view of scolex showing bothridial stalks, small letter indicates location of detail shown in F; **C** – apical sucker, small letter indicates location of detail shown in G; **D** – proximal bothridial surface near proximal extreme, enlarged wide aristate spinitriches and capilliform filitriches; **E** – proximal bothridial surface near bothridial rim, wide aristate spinitriches densely packed; **F** – apex of scolex proper, capilliform filitriches; **G** – surface of apical sucker, capilliform filitriches; **H** – distal bothridial surface near base of internal cavity, acicular filitriches and cilium; **I** – bothridial rim, narrow gladiate spinitriches and capilliform filitriches; **J** – terminal portion of strobila showing scutes.

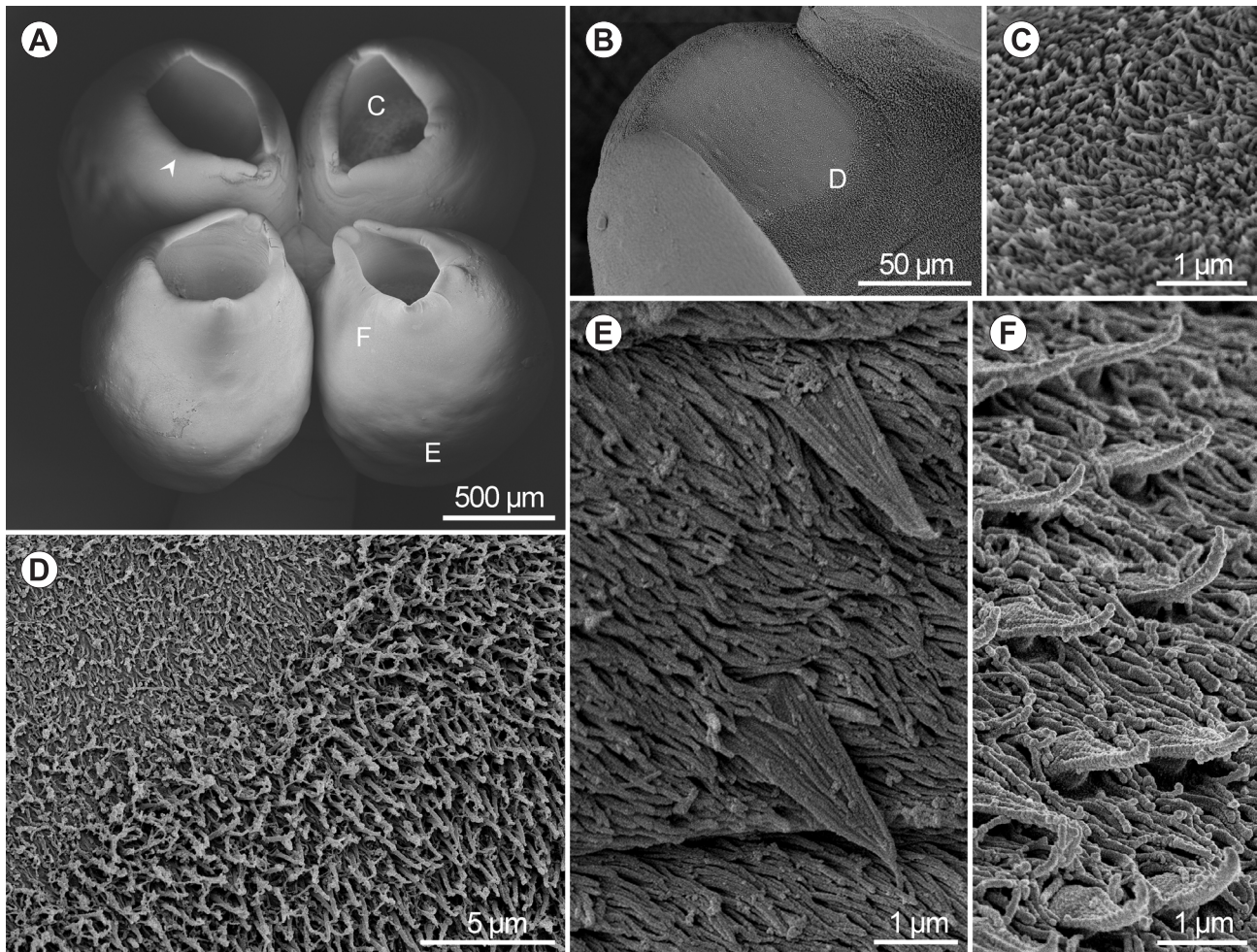


Fig. 7. *Guidus argentinense* Ivanov, 2006 from *Bathyraja brachyurops* (Fowler), scanning electron micrographs. **A** – scolex, small letters indicate locations of detail shown in C, E and F, arrow indicates marginal indentation; **B** – apical sucker, small letter indicates location of detail shown in D; **C** – distal bothridial surface, acicular filitriches; **D** – detail of apical sucker and surrounding area; **E** – proximal bothridial surface near proximal bothridial extreme, wide aristate gladiate spinitriches and capilliform filitriches; **F** – proximal bothridial surface near apical rim showing wide aristate gladiate spinitriches with pad-shaped bases.

Additional locality: Off Puerto San Julián, Santa Cruz Province (49.4938S, 66.1869W) (host PD7-107).

Site of infection: Spiral intestine.

Deposited specimens: Holotype MACN-Pa No. 747 (one worm with gravid proglottids), four paratypes MACN-Pa Nos. 748/1–2, 749/1–2 (four worms with gravid proglottids), three paratypes IPCAS No. C-888 (three worms with gravid proglottids). Additional specimens (histological sections and specimens prepared for SEM) retained in the personal collection of Adriana Menoret.

Prevalence and intensity of infection: Prevalence 22% (two host specimens infected out of nine specimens examined), 5–10 worms per host

Etymology: The specific name refers to its distribution along the Magellanic Province in the Argentine Sea.

Remarks. *Guidus magellanicus* sp. n. is easily distinguished from *G. antarcticus*, *G. argentinense* and *G. awii* by having bothridial stalks rather than sessile bothridia. In *G. magellanicus* the vitellarium is arranged in two lateral columns, consisting of two follicles in width, extending anteriorly near the level of the genital pore. In contrast, the columns are composed of numerous vitelline follicles

in width and reach the anterior margin of the proglottid in *G. antarcticus*, *G. argentinense* and *G. awii*. Finally, *G. magellanicus* has more proglottids per worm than *G. francoi* (8–24 vs. 27–60, respectively), a higher number of testes (71–105 vs. 42–68, respectively), with testes arranged in more columns in dorsoventral view (6–7 vs. 4–5, respectively) and in more layers deep in cross section (4–5 vs. 2–3 rows, respectively), and a lower ratio between the cirrus sac length and the proglottid width (0.2–0.3 vs. 0.4–0.6, respectively).

Guidus argentinense Ivanov, 2006

Fig. 7

Amended description of the scolex based on two scolexes prepared for SEM. Scolex composed of scolex proper and 4 sessile bothridia, lacking apical organ. Bothridia globose, conspicuously muscular with anteriorly-oriented apical aperture, continuous muscular sphincter, and apical sucker; anterior bothridial margin indented (Fig. 7A,B).

Bothridial rim covered with narrow gladiate spinitriches interspersed with capilliform filitriches (Fig. 7D). Proximal bothridial surface covered with wide aristate gladiate spinitriches interspersed with capilliform filitriches;

aristate gladiate spinitriches becoming less densely packed more proximally (Fig. 7 A,E,F). Distal bothridial surface covered with acicular filitriches (Fig. 7C). Capilliform filitriches on neck and strobila arranged in scutes.

Type host: *Bathyraja brachyurops* (Fowler), broadnose skate (Rajiformes: Arhynchobatidae).

Type locality: Southwestern Atlantic Ocean, coastal waters off Buenos Aires Province, Argentina at 37.1108S 54.3339W.

Site of infection: Spiral intestine.

Additional localities: Off Bahía Blanca, Buenos Aires Province at 39.5713S, 56.2694W (PD3-285) and off the Namuncurá Marine Protected Area/Burdwood Bank at 54.7470S, 59.9476W (PD12-297).

Specimens deposited: Holotype and two paratypes, MACN-Pa No. 432/1–7; one paratype (USNM No. 1393041).

Additional material: two voucher specimens MACN-Pa Nos. 750, 751 (two strobilae without scolex).

Remarks. The original description of *G. argentinense* is amended to include expanded observations of the scolex (e.g., the presence of a bothridial notch and microtrix morphology of the proximal and distal bothridial surfaces). *Guidus argentinense* has a shallower bothridial notch than the two species described herein from *B. magellanica*. The proximal and distal surfaces of the scolex studied for first time in *G. argentinense* exhibit a similar configuration of the microtriches to that observed in *G. francoi* and *G. magellanicus*. Capilliform filitriches arranged in scutes on the neck previously mentioned in the original description of *G. argentinense* were confirmed during this study. The distribution of this species is now extended from the known locality off Pinamar in Buenos Aires Province (given as geographic coordinates in the original description) to include Bahía Blanca and southern waters off the Namuncurá Marine Protected Area/Burdwood Bank.

DISCUSSION

Morphological characters

Specimens collected from *Bathyraja magellanica* were recognised as members of *Guidus* by having most of the diagnostic features of the genus *sensu* Ivanov (2006), which are revised to accommodate the new species described. Not all species in the genus have sessile bothridia as short bothridial stalks are present in *Guidus francoi* and *Guidus magellanicus*. Specimens of *Guidus* from *B. magellanica* have bothridia with a marginal indentation which is easily noticeable when the bothridial sphincter is contracted, allowing the formation of a conspicuous projection delimited by the notch (Figs. 1D, 4E) or more slightly perceptible when it is fully relaxed (Figs. 1C, 3A, 4D, 6A). In addition, the presence of this feature was also confirmed in *Guidus argentinense* (see fig. 4 in Ivanov 2006 and Fig. 7A in the present study). In fact, the conspicuous anterior projections in *Guidus antarcticus* and *Guidus awii* were defined as lappets by Ivanov (2006). We now include the possession of an indented bothridia as one of the diagnostic features of *Guidus*, and suggest to not consider the lappets for differentiation of species of *Guidus* because their pres-

ence depends on the contraction of the bothridial sphincter. Similarly, the shape of bothridia can also vary due to the effect of the bothridial muscles. In *G. francoi* and *G. magellanicus* they are essentially globose and conical (Figs. 1A–C, 3A, 4A,B,D and 6A) becoming cylindrical when specimens are strongly attached to host tissue (see Figs. 1D and 4E).

Guidus francoi and *G. magellanicus* have darkly stained cells surrounding the distal portion of the vagina (Figs. 2C, 5C) rather than having a vaginal sphincter as in the other three species. Two distinctive configurations of the vitellarium can be recognised among *Guidus* species. In *G. francoi* and *G. magellanicus* it consists of lateral narrow columns, each being two vitelline follicles in width with the field of follicles not reaching the anterior margin of the proglottid. In the other three species of *Guidus* the columns are broad (with numerous vitelline follicles in width in each column) and extend anteriorly to the anterior margin of the proglottid. Eggs shape among species of *Guidus* include fusiform and spherical unembryonated eggs, as described in *G. argentinense* and *G. awii*, respectively. Additionally, oncospheres are grouped in linear cocoons in *G. francoi* and *G. magellanicus*, and are described for the first time in *Guidus*. Among phyllobothriideans, egg cocoons are also present in *Trilocularia eberti* Pickering et Caira, 2012.

All three species of *Guidus* studied with SEM possess filitriches and gladiate spinitriches (aristate or unmodified). The proximal bothridial surface is covered by wide aristate gladiate spinitriches with pad-shaped bases, interspersed with capilliform filitriches. The distal bothridial surface is covered with acicular to capilliform filitriches, and the bothridial rim including the apical sucker is mostly covered with capilliform filitriches. Microtriches on the apex of scolex consist of capilliform filitriches in *G. francoi* and *G. magellanicus*, but remain unknown (or probably absent) in *G. argentinense* since microtriches were not observed in that surface in the two scoleces prepared for SEM in the present study.

A great diversity of spinitriches was described among phyllobothriideans and several genera of the order Phyllobothriidea were characterised by having scutes covering the cephalic peduncle, neck and/or strobila (Ivanov 2006, Caira et al. 2020, Maleki et al. 2020). Among them, *Scyphophyllidium haselii* (Caira, Malek et Ruhnke, 2011) and *Scyphophyllidium musculosum* (Subhadrappa, 1955) partially resemble the three species herein studied in having capilliform filitriches arranged in scutes on the neck and strobila, and gladiate spinitriches (serrate rather than aristate) covering the proximal surface of the globose bothridia, but differ from species of *Guidus* in the microtriches covering the distal bothridial surface (serrate aristate spinitriches vs. filitriches). Therefore, the homogeneous microtrix pattern exhibited by the three species of *Guidus* from the Argentine Sea can be useful for differentiating this subset of species from other phyllobothriidean genera.

Distribution patterns

Despite the cosmopolitan distribution of the order Phyllobothriidea, only a few species have been reported from

southern latitudes, mainly from coastal waters off Australia (Cutmore et al. 2011, Pickering and Caira 2012, Ruhnke and Workman 2013, Ruhnke et al. 2017, Caira et al. 2020). Particularly, only 10% of the phyllobothriideans (8 out of 81 valid species) belonging to *Crossobothrium* Linton, 1889, *Guidus*, *Orygmatobothrium* Diesing, 1863 and *Scyphophyllidium* Woodland, 1927 were known so far from the Southwestern Atlantic and adjacent areas (Rocka and Zdzitowiecki 1998, Brooks et al. 1999, Suriano and Labriola 2001, Ivanov 2006, 2008, 2009, Caira et al. 2020).

Among them, the genus *Guidus* is now known to include *G. argentinense*, *G. awii*, *G. antarcticus*, *G. francoi*, and *G. magellanicus* with a distribution extending from the Argentine Shelf to Antarctic waters between 37°S–73°S. *Guidus argentinense* has the greatest latitudinal range with northern records in the Warm Temperate Southwestern Atlantic Province and the southern distribution boundary in the Magellanic Province. The two new species are also present along the Magellanic Province, whereas *G. antarcticus* and *G. awii* are typically distributed in higher latitudes including the Scotia Sea and the Continental High Antarctic Provinces, respectively. However, species of the genus mainly occur in cold-temperate to polar waters of its geographic distribution. Unlike the members of *Guidus*, other phyllobothriideans registered at latitudes above ~30°S such as *Monorygma* Diesing, 1863 and *Trilocularia* Olsson, 1867 also occur in the Northern Hemisphere (Ruhnke 2011, Pickering and Caira 2012).

Host association

The two new species described from the Argentine Shelf follow the tight host association found among phyllobothriideans. However, all members of *Guidus* as well as New genus 20 of Ruhnke et al. (2017) exclusively parasitise skates restricted to temperate waters in South America. The description of the new species of *Guidus* from *B. magellanica* is the second record in the genus parasitising a single host species. Moreover, specimens of *B. magellanica* caught in the same sampling hauls (hosts PD4-097 and PD7-107) were found simultaneously infected by both *G. francoi* and *G. magellanicus*, in contrast to *Bathyraja*

maccaini which was found infected by *G. antarcticus* and *G. awii* in two different localities. It would be interesting to examine specimens of the Magellan skate along its geographical range to verify if *G. francoi* and *G. magellanicus* remain locally restricted to the southern Magellanic Province despite the wider distribution of their host.

The discovery of *G. francoi* and *G. magellanicus* from *B. magellanica* brings the total number of described species in the Southwestern Atlantic from one to three, and increases the number of species in the genus to five. Furthermore, finding these new species contributes substantially to our little knowledge of the cestode fauna in *B. magellanica*, considering that only *Acanthobothrium carolinae* Franzese et Ivanov, 2020 has previously been reported from that host in the studied area. It is expected that new species will be identified from *Bathyraja* skates based on the strict specificity exhibited by the members of *Guidus* and the high diversity of arhynchobatids of the genus *Bathyraja* inhabiting Southwestern Atlantic waters.

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