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# Two New Species of *Scalithrium* (Cestoda: Rhinebothriidea) from Rhinopristiform Elasmobranchs with a Revised Generic Diagnosis Based on Refined Generic Membership

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ABSTRACT: The genus *Scalithrium* is one of the most poorly known genera of rhinebothriidean tapeworms. It currently houses 10 morphologically heterogeneous species hosted by stingrays and guitarfishes. This study aimed to expand our understanding of this genus. Two new species, *Scalithrium healyae* n. sp. and *Scalithrium johnvolini* n. sp., are described from the giant guitarfish, *Glaucostegus typus*, off Australia and the lesser guitarfish, *Acroteriobatus annulatus*, off South Africa, respectively. The microtriches of members of this genus are characterized using scanning electron microscopy for the first time. Existing generic membership is critically assessed in the context of the morphology of the type species of the genus, *Scalithrium minutum*. As a result, *Scalithrium bilobatum*, *Scalithrium smitii*, and *Scalithrium trygonis* are considered *species inquirenda* owing to the limited amount of available information and material of both species. *Scalithrium palombii* and *Scalithrium rankini* are considered *incertae sedis* given the numerous morphological differences that exist between these species and the type and six other species in the genus. These differences include a greater length, more proglottids, and a much greater number of testes, the distribution of which extends to the ovary, rather than being restricted to the region anterior to the genital pore. The diagnosis of the genus is revised to accommodate these modifications in membership. We anticipate that the more cohesive concept of *Scalithrium* developed here will help advance future work circumscribing several of the other problematic genera of rhinebothriideans.

KEY WORDS: Cestoda, tapeworm, Rhinebothriidea, *Scalithrium healyae* n. sp., *Scalithrium johnvolini* n. sp., *Acroteriobatus annulatus*, *Glaucostegus typus*, scanning electron microscopy, lesser guitarfish, giant guitarfish, South Africa, Australia.

The rhinebothriidean cestode genus *Scalithrium* Ball, Neifar, and Euzet, 2003, was established by Ball et al. (2003) with *Scalithrium minimum* (van Beneden, 1850) Ball, Neifar, and Euzet, 2003, as its type species. Their goal in erecting this genus was to provide a home for a series of species originally assigned to *Rhinebothrium* Linton, 1890, that lacked the longitudinal septum seen on the bothridia of the type species of the genus, *Rhinebothrium flexile* Linton, 1890. At that time, Ball et al. (2003) also transferred 7 other species lacking a longitudinal septum to their new genus. These species were: *Scalithrium bilobatum* (Young, 1955) Ball, Neifar, and Euzet, 2003; *Scalithrium geminum* (Marques, Brooks, and Ureña, 1996) Ball, Neifar, and Euzet, 2003; *Scalithrium magniphallum* (Brooks, 1977) Ball, Neifar, and Euzet, 2003; *Scalithrium palombii* (Baer, 1948) Ball, Neifar, and Euzet, 2003; *Scalithrium rankini* (Baer, 1948) Ball, Neifar, and Euzet, 2003; *Scalithrium shipleyi* (Southwell, 1912) Ball, Neifar, and Euzet, 2003; and *Scalithrium trygonis* (Shipley and Hornell, 1906) Ball, Neifar, and Euzet, 2003. However, *Sc. shipleyi* was transferred to the genus *Barbeaucestus* Caira, Healy, Marques, and Jensen, 2017, as *Barbeaucestus shipleyi* (Southwell, 1912) Caira, Healy, Marques, and Jensen, 2017, by Caira et al. (2017).

The membership of *Scalithrium* was subsequently expanded by Coleman et al. (2019). These authors described *Scalithrium australiense* Coleman, Beveridge, and Campbell, 2019, and they transferred *Echeneibothrium filamentosum* Subhapradha, 1955 and *Echeneibothrium smitii* Shinde, Deshmukh, and Jadhav, 1981 (misspelled as *E. smitti*) to the genus as *Scalithrium filamentosum* (Subhapradha, 1955) Coleman, Beveridge, and Campbell, 2019 and *Scalithrium smitii* (Shinde, Deshmukh, and Jadhav, 1981) Coleman, Beveridge, and Campbell, 2019, respectively. Franzese and Ivanov (2021) further expanded the concept of the genus to include their 2 new species *Scalithrium ivanovae* Franzese, 2021, and *Scalithrium kirchneri* Franzese and Ivanov, 2021. They revised the diagnosis of *Scalithrium* to accommodate these 2 new species as well as the species recognized by Coleman et al. (2019) and Healy (2006, unpublished dissertation, University of Connecticut, Storrs, Connecticut, U.S.A.). However, inclusion of the latter species was

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inappropriate given that Healy (2006, unpublished dissertation, University of Connecticut) included the disclaimer recommended in Article 8 of the *International Code of Zoological Nomenclature* (Ride et al., 1999) indicating that the actions in her work were disclaimed for nomenclatural purposes.

Most recently, based on a combination of morphological and molecular data, Bueno et al. (2024) transferred *Sc. ivanovae* and *Sc. kirchneri* to *Semiorbiseptum* as *Semiorbiseptum ivanovae* (Franzese, 2021) Bueno, Trevisan, and Caira, 2024, and *Semiorbiseptum kirchneri* (Franzese and Ivanov, 2021) Bueno, Trevisan, and Caira, 2024, respectively. Although Bueno et al. (2024) revised the diagnosis of *Semiorbiseptum*, they did not revise the diagnosis of *Scalithrium* to reflect the removal of these 2 species.

This paper has three goals. The first is to provide descriptions of 2 new species of *Scalithrium* collected from guitarfish—one off South Africa and the other off Australia—to help expand our understanding of the genus. The second is to evaluate the appropriateness of membership in *Scalithrium* for the 9 species currently assigned to the genus beyond the type species, *Sc. minimum*. The final goal is to revise the diagnosis of *Scalithrium* to reflect the modifications in membership indicated by our findings.

## MATERIALS AND METHODS

In total, 7 individuals of the lesser guitarfish, *Acroteriobatus annulatus* (Smith), collected in the Indian Ocean off South Africa on the Department of Agriculture, Forestry, and Fisheries Fishing Vessel *Africana* using a bottom trawl in April and May of 2010, were examined for cestodes. Each guitarfish was assigned a unique specimen number consisting of a collection code and collection number (e.g., AF-88), and basic morphometric data and a series of digital images were obtained. Four of these specimens were female (AF-88, AF-141, AF-182, and AF-183) with a total length (TL) of 60–91 cm, and 3 specimens were male (AF-155, AF-156, and AF-176) with a TL of 64.5–94 cm. The tapeworm specimens examined from the giant guitarfish *Glaucostegus typus* (Anonymous [Bennett]) were provided to us by Malcom Jones. They consisted of both vialed material and whole mounts. They were collected in the Coral Sea off Heron Island, Queensland, Australia (23°27′S; 151°55′E) in February of 2002.

In the case of each freshly collected host specimen, a small sample of liver tissue was preserved in 95% ethanol for molecular verification of host identity. The spiral intestine was then removed through a midventral incision and opened with a longitudinal incision. A subset of tapeworms was removed and preserved in either 10% formalin buffered in seawater (1:9) for morphological work or 95% ethanol for molecular work. All 7 spiral intestines from *A. annulatus* were subsequently preserved in 10% formalin buffered in seawater. All samples preserved in formalin were transferred to 70% ethanol for storage after approximately 1 wk. Samples preserved in 95% ethanol were ultimately stored in a −20°C freezer.

Whole mounts were prepared for light microscopy as follows. Tapeworms were hydrated in a graded ethanol series, stained in Delafield's hematoxylin, destained in acid ethanol, neutralized in basic ethanol, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted on glass slides in Canada balsam. They were subsequently placed in an oven at 54°C for approximately 1 wk. Specimens were examined under a Zeiss Axioskop 2 Plus compound microscope (Zeiss, Thornwood, New York, U.S.A.). They were photographed and measured using a SPOT Diagnostic Instrument Digital Camera System and SPOT software (version 4.6; SPOT Imaging Solutions, Sterling Heights, Michigan, U.S.A.). All measurements are in micrometers unless otherwise stated and are presented as the range followed in parentheses by the mean, the standard deviation, the number of specimens measured, and the number of measurements taken when more than a single measurement was taken per worm. Shape nomenclature follows Clopton (2004).

The scoleces of 5 specimens of the species from *A. annulatus* and 4 specimens of the species from *G. typus* were examined with scanning electron microscopy (SEM). In each case, the scolex was removed, and the strobila was prepared as a whole mount as described above to serve as a voucher. Each scolex was hydrated in a graded ethanol series, placed in 1% osmium tetroxide overnight, dehydrated in a graded ethanol series, transferred to hexamethyldisilazane under a fume hood, and then allowed to air dry. Scoleces were mounted on a double-sided PELCO carbon tab (Ted Pella Inc., Redding, California, U.S.A.) on aluminum stubs and placed in a desiccator overnight. They were sputter coated with 25–30 nm of gold/palladium and examined with an FEI Nova NanoSEM 450 field emission SEM (FEI, Hillsboro, Oregon, U.S.A.) at the Bioscience Electron Microscopy Laboratory, University of Connecticut. Microthrix terminology follows Chervy (2009).

Histological sections were prepared as follows: Tapeworms were dehydrated in a graded ethanol series, cleared in xylene, and embedded in TissuePrep (Fisher Scientific, Fairlawn, New Jersey, U.S.A.), and 7-μm sections were cut using an Olympus CUT4060 retracting rotary microtome (Olympus, Center Valley, Pennsylvania, U.S.A.). Sections were attached to glass slides with a 3% solution of sodium silicate, stained in Delafield's hematoxylin, counterstained in eosin, differentiated in Scott's solution, dehydrated in a graded ethanol series, cleared in xylene, and mounted under glass slides in Canada balsam.

Museum abbreviations are as follows: Lawrence R. Penner Parasitology Collection (LRP), University of Connecticut, Storrs, Connecticut, U.S.A.; National Museum (NMB-P), Bloemfontein, South Africa, Parasite Collection; National Museum of Natural History (USNM), Smithsonian Institution, Washington, D.C., U.S.A.; and Queensland Museum (QM), South Brisbane, Queensland, Australia.

## RESULTS

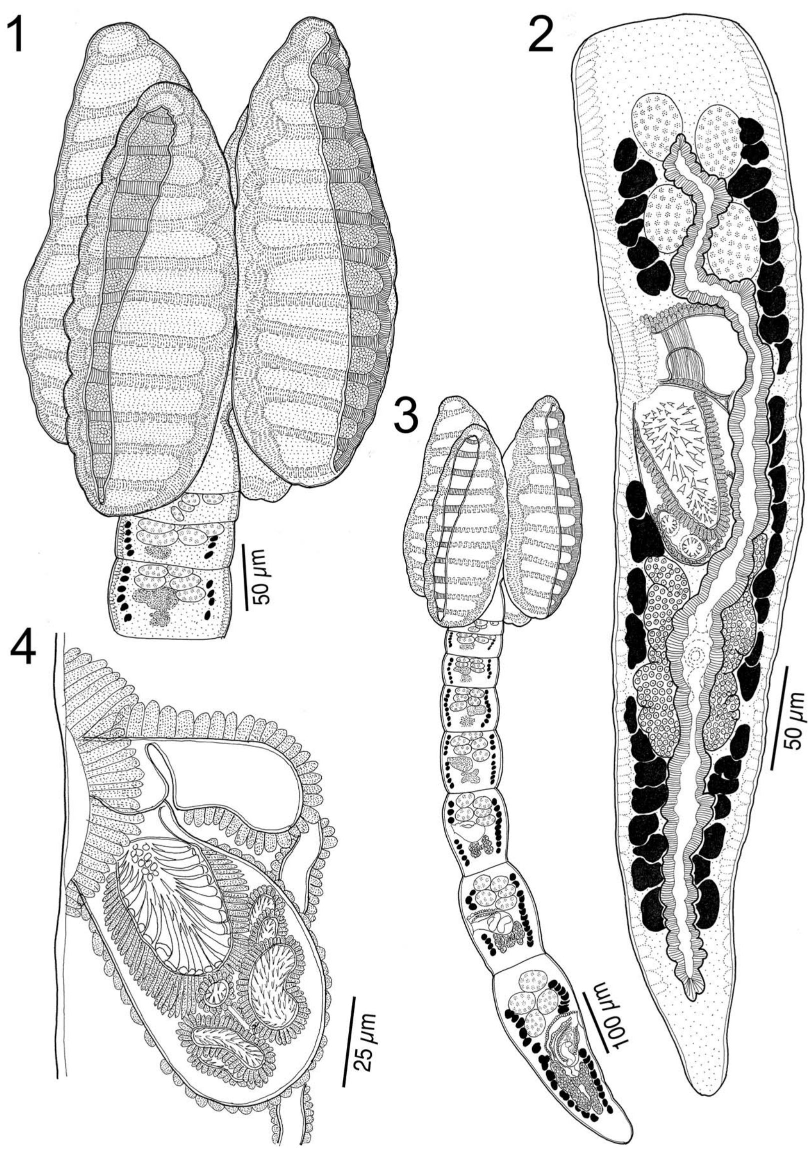
The following 3 species currently assigned to *Scalithrium* are so poorly known that they should be considered *species inquirenda* and are thus not considered in the comparisons made in the Remarks sections of either new species. *Scalithrium bilobatum* is known only from the single immature holotype specimen that Appy and Dailey (1977, p. 119) characterized as "badly fractured." Beyond Young (1955), who noted in the original description that there are 6 testes in a single row, the proglottid anatomy of this species is entirely unknown. It should remain a *species inquirendum* until such time as it can be more fully characterized through the collection of additional material. *Scalithrium smitii* was originally described by Shinde et al. (1981) from 2 specimens collected from *Pastinachus sephen* (Forsskål) (as *Trygon sephen*) off the west coast of India. The scolex was redescribed by Coleman et al. (2019) from 3 fragmented specimens collected from *Maculabatis toshi* (Whitley) off western Australia. However, several major inconsistencies exist between these 2 treatments. Most notably, Shinde et al. (1981) reported their worms to be 15 mm in length, but Coleman et al. (2019) reported their worms to be 3.2–6.9 mm in length. In addition, Shinde et al. (1981) reported the pedicels (i.e., bothridial stalks) of their specimens to be 350 μm long; however, Coleman et al. (2019) reported the pedicels of their material to be 1.8–4.7 mm long. Unfortunately, comparison of proglottid anatomy between the 2 treatments is not possible because the internal anatomy of the cestodes they identified as *Sc. smitii* was not characterized by Coleman et al. (2019). Based on the existing evidence, it seems likely that the Indian and Australian specimens are not conspecific. The confusion surrounding the details of the features of this unusual worm—which, based on illustrations provided in both works (Shinde et al., 1981, plate 1A, B; Coleman et al., 2019, fig. 8B), bears extremely long bothridial stalks—is sufficient for it to be considered a *species inquirendum* until additional material can be examined. Finally, as noted by Ball et al. (2003), *Sc. trygonis* is also poorly known. The original description by Shipley and Hornell (1906) is brief, and the accompanying illustration is superficial. Furthermore, no details of the proglottid anatomy were provided. Ball et al. (2003) suggested that new research on this species is required before its validity can be determined. We agree and recommend that it be formally considered a *species inquirendum* until such time as that additional work has been completed.

We have, however, included *Sc. palombii* and *Sc. rankini* in the Remarks sections of both new species here despite the fact that, as argued in the Discussion, we believe both of these valid species will ultimately be found to belong to a genus other than *Scalithrium*. We have done so in part because those comparisons help to emphasize the substantial morphological differences between the new species and these 2 members of the genus. However, this strategy also seems appropriate if we are incorrect in predicting a change in the future generic home for these species.

### *Scalithrium johnvolini* n. sp.
### (Figs. 1–10)

*Description (based on 14 complete mature worms and 5 scoleces observed with SEM):* Worms euapolytic, 0.95–1.7 mm (1.3 mm ± 0.6; 14) long, greatest width 188–284 (255 ± 33; 7) at level of scolex; 5–10 (7.5 ± 1.7; 14) proglottids per worm (Fig. 3). Scolex consisting of 4 stalked bothridia (Figs. 1, 5); cephalic peduncle lacking. Stalks extremely short. Bothridia 109–370 (302 ± 59; 14; 35) long by 82–213 (126 ± 36; 8; 10) wide, extending slightly posterior to germinative zone, eliptoidal, divided into 10–12 loculi by transverse septa, longitudinal septum and apical sucker lacking; bothridial rim narrow.

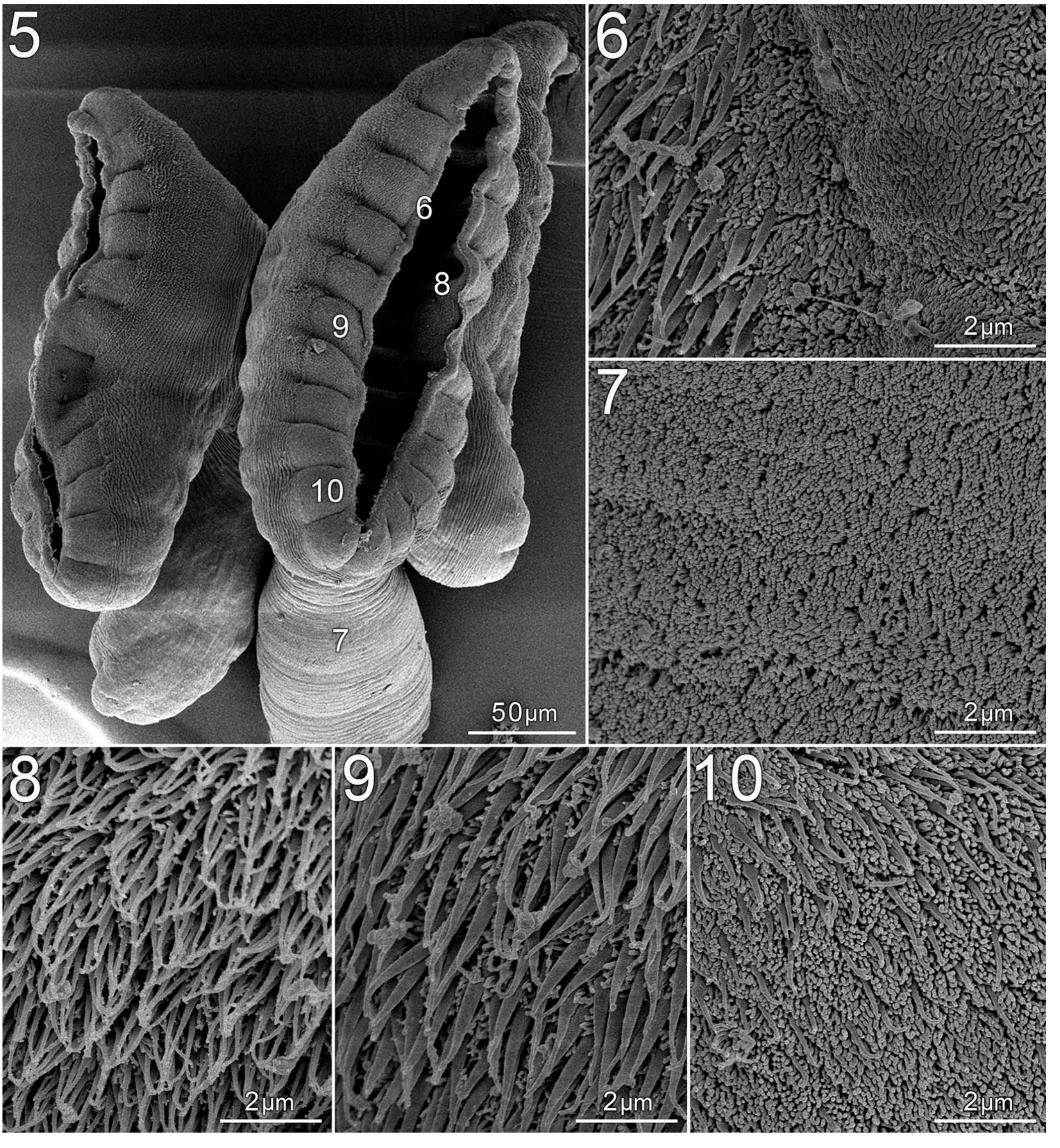
Distal bothridial surface bearing small gladiate spinitriches and acicular filitriches (Fig. 8). Proximal surface of bothridial rim bearing acicular filitriches (Fig. 6); proximal bothridial surface away




**Figures 1–4.** Line drawings of *Scalithrium johnvolini* n. sp. **1.** Scolex (holotype, NMB-P 1030). **2.** Mature terminal proglottid (paratype, USNM 1707626). **3.** Whole worm (holotype, NMB-P 1030). **4.** Terminal genitalia of mature terminal proglottid (paratype, LRP 11082).

from rim with small gladiate spinitriches and acicular filitriches (Fig. 9), spinitriches becoming more sparsely distributed posteriorly (Fig. 10). Surfaces of stalks not observed. Strobila with densely distributed capilliform filitriches (Fig. 7).

Proglottids acraspedote. Immature proglottids 4–9 (6.4 ± 1.5; 14) in number, initially wider than long, becoming longer than wide with maturity. Mature proglottids 1–2 (1.1 ± 0.4; 14) in number, conspicuously longer than wide. Terminal mature proglottid (Fig. 2) 301–500 (399 ± 66; 14; 14) long by 89–134 (116 ± 15; 14; 14) wide. Gravid proglottids not observed. Testes 3–6 (4.4 ± 0.8; 14) in number, 20–50 (30 ± 6; 14; 28) long by 23–44 (34 ± 6; 14; 28) wide, 1 layer deep, arranged in 2 irregular columns anterior to genital pore; postporal testes lacking. Vas deferens inconspicuous, extending anterior to cirrus sac. Cirrus sac obovoid (Fig. 4), tilted posteriorly, 48–




**Figures 5–10.** Scanning electron micrographs of *Scalithrium johnvolini* n. sp. **5.** Scolex, where labels indicate locations of Figures 6–10. **6.** Proximal surface of bothridial rim showing acicular filitriches. **7.** Strobilar surface showing densely distributed capilliform filitriches. **8.** Distal bothridial surface showing very densely distributed, small gladiate spinitriches and acicular filitriches. **9.** Proximal surface of central region of bothridium away from rim showing densely distributed, small gladiate spinitriches and acicular filitriches. **10.** Proximal surface of posterior region of bothridium showing less densely distributed, small gladiate spinitriches and acicular filitriches.

86 (70 ± 12; 14; 14) long by 34–52 (45 ± 5; 14; 14) wide, containing coiled cirrus; cirrus expanded at base, bearing conspicuous spinitriches. Internal and external seminal vesicles absent. Genital pores lateral, irregularly alternating, 62–70% (67% ± 3%; 13) from posterior margin of proglottid; genital atrium conspicuous. Ovary subterminal in mature proglottids, 45–85 (61 ± 12; 10; 10) wide, H-shaped in dorsoventral view, asymmetrical, aporal lobe often slightly longer than poral lobe, aporal lobe 72–121 (92 ± 13; 11) long, poral lobe 51–96 (17 ± 18; 11) long, with weakly lobulate margins, bilobed in cross section; ovarian bridge near midlevel of ovary. Mehlis' gland immediately posterior to ovarian bridge. Vagina relatively thick-walled,

weakly sinuous, extending medially in proglottid from ootype to anterior margin of cirrus sac, then laterally to open into genital atrium anterior to cirrus, highly expanded distally, with conspicuous sphincter. Seminal receptacle absent. Vitellarium follicular; vitelline follicles arranged in 2 lateral bands, each band consisting of 1 dorsal and 1 ventral column of follicles, extending from anterior margin of testicular field to near posterior margin of proglottid, interrupted by terminal genitalia. Uterus saccate, medial, ventral to vagina, extending from near anterior margin of proglottid to near posterior margin of proglottid. Excretory ducts in 2 lateral pairs. Eggs not observed.

*Type and only known host:* Lesser guitarfish, *Acroteriobatus annulatus* (Smith) (Rhinopristiformes: Rhinobatidae).

*Site of infection:* Spiral intestine.

*Type locality:* Indian Ocean off South Africa (33° 51.67′S; 26°14.82′E).

*Additional localities:* Indian Ocean off South Africa (34°10.12′S; 24°54.92′E) and (33°47.67′S; 26°5.12′E).

*Specimens deposited:* Holotype (NMB-P 1030), 5 paratypes (NMB-P 1031–1035); 4 paratypes (LRP 11079–11082), 5 paratype SEM vouchers (LRP 11083–11087); 4 paratypes (USNM 1707626–1707629).

*ZooBank registration:* urn:lsid:zoobank.org:act: 7C2D715B-8754-4115-9829-393FCFF9A517.

*Etymology:* The name of this species honors John C. Volin, the father of the first author, for his enthusiastic support of her academic pursuits.

*Provisional name: Scalithrium* sp. nov. 2 of Bueno et al. (2024).

## Remarks

*Scalithrium johnvolini* n. sp. is a smaller worm than *Sc. australiense*, *Sc. magniphallum*, *Sc. minimum*, *Sc. palombii*, and *Sc. rankini* (0.95–1.7 mm vs. 2.1 mm, 2.28–3.06 mm, 3.27 mm, 30–35 mm, and 13 mm, respectively). It further differs from *Sc. minimum*, *Sc. palombii*, and *Sc. rankini* in possessing fewer proglottids (5–10 vs. 10–20, ∼25, ∼46, and 30, respectively). The new species also differs from *Sc. australiense*, *Sc. magniphallum*, and *Sc. palombii* in that it has fewer loculi (10–12 vs. 20, 16–18, and 20–22, respectively), and from *Sc. filamentosum* in that it has a greater number of loculi (10–12 vs. 8). Its possession of a smaller number of testes further distinguishes *Sc. johnvolini* n. sp. from *Sc. australiense*, *Sc. geminum*, *Sc. magniphallum*, *Sc. palombii*, and *Sc. rankini* (3–6 vs. 15–17, 9–12, 10–15, 95–100, and 50–55, respectively).

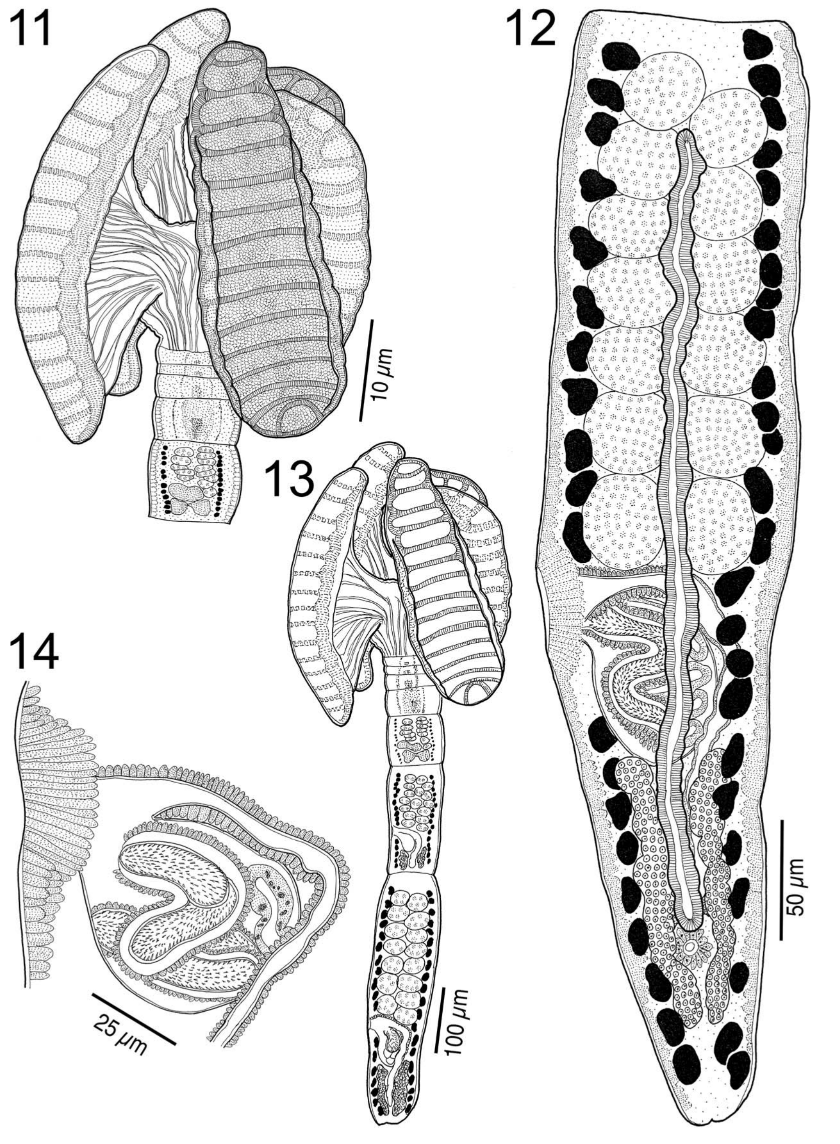
Examination of the voucher (LRP 11078) of the specimen collected from *A. annulatus* identified as *Scalithrium* sp. nov. 2 by Bueno et al. (2024) indicates that this specimen is conspecific with *Sc. johnvolini* n. sp.

### *Scalithrium healyae* n. sp.
### (Figs. 11–21)

*Description (based on 11 mature worms, cross sections of 2 specimens, and 4 specimens observed with SEM):* Worms euapolytic, 0.61–2.3 mm (1.1 mm ± 1.3; 11) long, greatest width 250–621 (404 ± 24; 11) at level of scolex; 5–9 (6 ± 0.4; 11) proglottids per worm (Fig. 13). Scolex consisting of 4 stalked bothridia (Figs. 11, 15); cephalic peduncle lacking. Stalks short, 64–140 (106 ± 7; 11; 11) long by 63–105 (79 ± 3; 11; 11) wide. Bothridia 325–528 (417 ± 15; 11; 16) long by 90–137 (110 ± 11.6; 9; 16) wide, extending posteriorly well past germinative zone, dolioform, divided into 16–17 (17 ± 0.1; 11; 14) loculi by transverse septa, longitudinal septum and apical sucker lacking; posteriormost septum semicircular; bothridial rim narrow.

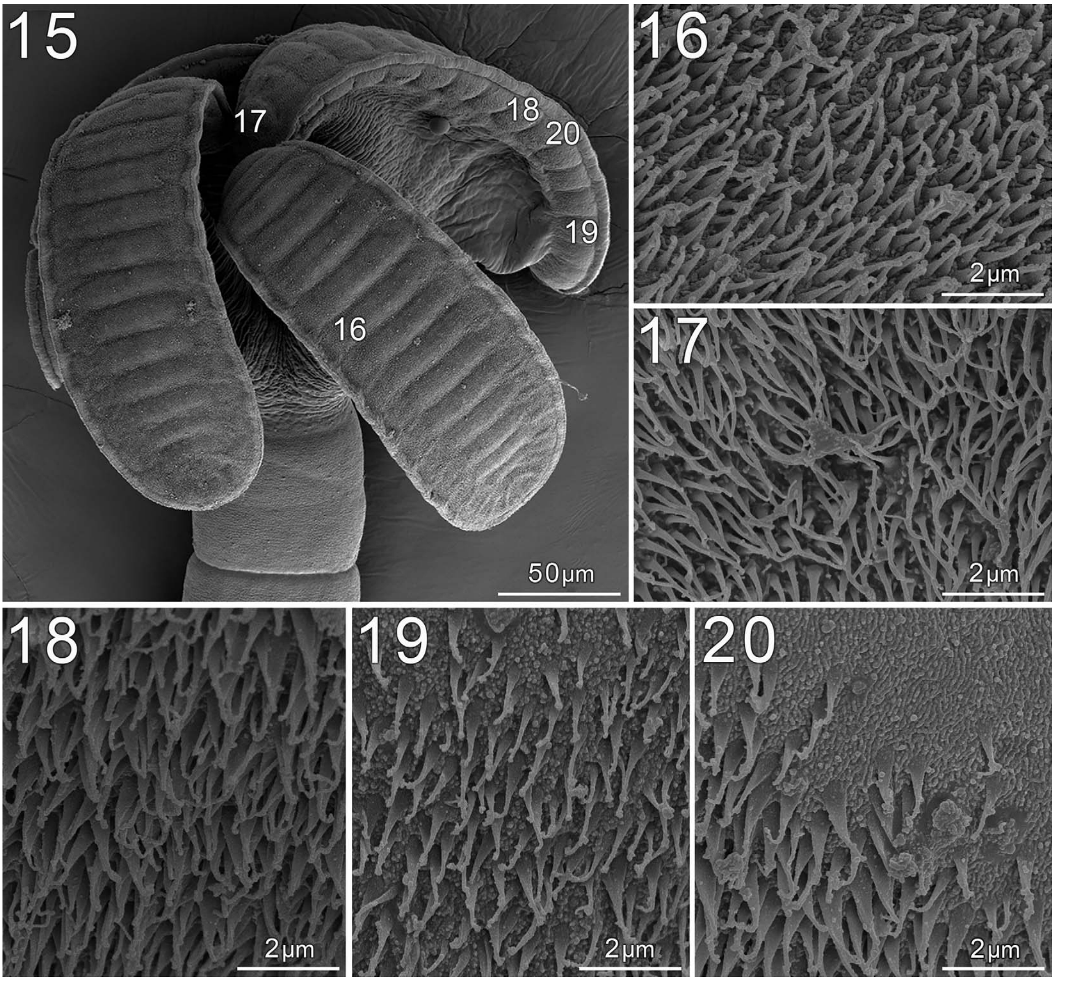
Distal bothridial surface bearing small aristate gladiate spinitriches and acicular filitriches anteriorly (Fig. 16) and small gladiate spinitriches and acicular filitriches more posteriorly (Fig. 17). Proximal bothridial surfaces bearing small gladiate spinitriches and papilliform filitriches more anteriorly (Fig. 18), gladiate spinitriches becoming more sparsely distributed posteriorly (Fig. 19), proximal surface of bothridial rim bearing papilliform filitriches (Fig. 20). Surfaces of stalks not observed. Strobila bearing capilliform filitriches.

Proglottids acraspedote. Immature proglottids 4–7 (5 ± 0.3; 11) in number, initially wider than long, becoming longer than wide with maturity. Mature proglottids 1–2 (1 ± 0.1; 11) in number, conspicuously longer than wide. Terminal mature proglottid (Fig. 12) 247–673 (391 ± 38; 11) long by 95–148 (121 ± 6; 11) wide. Gravid proglottids not observed. Testes 11–18 (14 ± 0.4; 11; 13) in number, 20–46 (31 ± 1; 11; 24) long by 29–57 (41 ± 1.5; 11; 22) wide, 1 layer deep, arranged in 2–3 (2 ± 0.1; 11; 13) columns anterior to genital pore; postporal testes lacking. Vas deferens inconspicuous, extending anterior to cirrus sac, joining cirrus at anterior margin of cirrus sac. Cirrus sac obovoid (Fig. 14), tilted posteriorly, 42–120 (73 ± 7; 11; 13) long by 34–61 (49 ± 2; 11; 13) wide, containing coiled cirrus; cirrus expanded at base, bearing inconspicuous spinitriches. Internal and external seminal vesicles absent. Genital pores lateral, irregularly alternating, 35–54% (44% ± 2; 11; 13) from posterior margin of proglottid; genital atrium




**Figures 11–14.** Line drawings of *Scalithrium healyae* n. sp. **11.** Scolex (holotype, QM G241278). **12.** Mature terminal proglottid (paratype, USNM 1707630). **13.** Whole worm (holotype, QM G241278). **14.** Terminal genitalia of mature terminal proglottid (paratype, LRP 11088).

inconspicuous. Ovary terminal, H-shaped in dorsoventral view, symmetrical, 61–228 (121 ± 15; 11; 14) long by 42–93 (63 ± 5; 11; 13) wide, with very weakly lobulate margins, bilobed in cross section (Fig. 21); lobes constricted medially by ventral excretory ducts; ovarian bridge slightly posterior to midlevel of ovary. Mehlis' gland immediately posterior to ovarian bridge. Vagina relatively thin-walled, weakly sinuous, extending medially in proglottid from ootype to anterior margin of cirrus sac, then laterally to open into genital atrium anterior to cirrus, slightly expanded distally, sphincter not observed. Seminal receptacle absent. Vitellarium follicular; vitelline follicles arranged in 2 lateral bands, each band consisting of 1 dorsal and 1 ventral column of follicles, extending from anterior margin of testicular field to near posterior margin of proglottid, interrupted by terminal genitalia. Uterus saccate, medial, ventral to vagina, extending from near anterior margin of proglottid to ovarian bridge. Excretory ducts in 2 lateral pairs. Eggs not observed.




**Figures 15–20.** Scanning electron micrographs of *Scalithrium healyae* n. sp. **15.** Scolex, where labels indicate locations of Figures 16–20. **16.** Distal bothridial surface at anterior of bothridium showing densely distributed, small gladiate spinitriches and acicular filitriches. **17.** Distal bothridial surface at middle of bothridium showing densely distributed, small gladiate spinitriches and acicular filitriches. **18.** Proximal surface of central region of bothridium showing densely distributed, small gladiate spinitriches and papilliform filitriches. **19.** Proximal surface of posterior region of bothridium showing less densely distributed, small gladiate spinitriches and papilliform filitriches. **20.** Proximal surface of bothridial rim showing papilliform filitriches.

*Type and only known host:* *Glaucostegus typus* (Anonymous [Bennett]) (Rhinopristiformes: Glaucostegidae).

*Site of infection:* Spiral intestine.

*Type locality:* Indian Ocean off Heron Island, Queensland, Australia (23°27′S; 151°55′E).

*Specimens deposited:* Holotype (QM G241278), 4 paratypes (QM G241279–G241282); 3 paratypes (LRP 11088–11090); cross sections of 2 paratypes (LRP 11091–11092); 3 paratypes (USNM 1707630–1707632).

*ZooBank registration:* urn:lsid:zoobank.org:act:8073813E-2F24-4820-A33E-F3CE5966FFAB.

*Etymology:* This species is named after Claire Healy in recognition of the foundational work she conducted on this and other species of *Scalithrium* (see Healy, 2006; see also Healy, 2006, unpublished dissertation, University of Connecticut, Storrs, Connecticut, U.S.A.).

## Remarks

*Scalithrium healyae* n. sp. is a much smaller worm than *Sc. palombii* and *Sc. rankini* (0.61–2.3 mm vs. 30–35 mm and 13 mm, respectively). It can be distinguished from *Sc. magniphallum* in its possession of

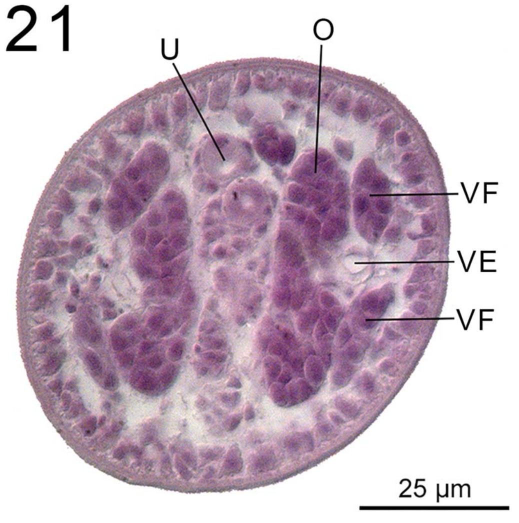



**Figure 21.** Cross section of *Scalithrium healyae* (LRP 11091) ovary showing bilobed configuration. Abbreviations: O, ovary; VE, ventral excretory duct; VF, vitelline follicle; U, uterus.

fewer proglottids (5–9 vs. 10–20). It bears more testes than *Sc. filamentosum*, *Sc. johnvolini*, and *Sc. minimum* (11–18 vs. 5, 3–6, and 3–6, respectively) and differs further from *Sc. palombii* and *Sc. rankini* in possessing conspicuously fewer proglottids (5–9 vs. ~46 and 30, respectively) and also fewer testes (11–18 vs. 95–100 and 50–55, respectively). *Scalithrium healyae* n. sp. differs from *Sc. australiense* in its possession of fewer loculi (16–17 vs. 20) and from *Sc. geminum* in possessing more loculi (16–17 vs. 12–14).

## DISCUSSION

We propose that *Sc. palombii* and *Sc. rankini* do not belong in *Scalithrium* and should be considered *incertae sedis* until a more appropriate generic home for them is found. Both species are much longer worms than the 7 other members of the genus (including our 2 new species) with total lengths that range from 13 to 35 mm (vs. 0.7–5 mm). Both species also possess many more proglottids than the 7 other members of the genus (30 to approximately 46 vs. 4–20). In addition, both *Sc. palombii* and *Sc. rankini* bear a considerably greater number of testes than the 7 other species with testis counts ranging from 50 to 100 (vs. 3–18). Perhaps most notably, in both species, the testicular field extends posteriorly to the level of the ovary, rather than being restricted to the region of the proglottid anterior to the genital pore.

As a result of the above 5 attributes, membership in *Scalithrium* can be characterized by a number of features beyond the presence of a single column of bothridial loculi, as originally suggested by Ball et al. (2003). However, the taxonomic actions outlined above require only slight modification of the revised diagnosis of the genus provided by Franzese and Ivanov (2021). This is because, although they included all 5 of the problematic taxa discussed above in the genus, their diagnosis (e.g., with respect to testes distribution, etc.) did not accommodate the features of those species. Additions to the diagnosis of Franzese and Ivanov (2021) are indicated in bold below. The features "craspedote proglottids" and "cephalic peduncle present" were removed as a result of the transfer of *Sc. ivanovae* and *Sc. kirchneri* to *Semiorbiseptum* by Bueno et al. (2024). The Rajiformes were also removed from the list of host orders as a result of the transfer of these two species. Finally, details of the configuration of the musculature of the transverse septa have also been removed until such time that this configuration has been confirmed to exist in all members of the genus. This brings the total number of valid species in *Scalithrium* to 7.

### *Scalithrium* Ball, Neifar, and Euzet, 2003, Emended from Franzese and Ivanov (2021)

Worms **small**, euapolytic, acraspedote. Scolex composed of 4 stalked bothridia, cephalic peduncle absent, myzorhynchus absent in adult stage. Distal bothridial surface divided by transverse septa into single column of loculi, longitudinal septum absent. Testes 1 layer deep in cross section, **arranged in single field anterior to genital pore**. Vas deferens entering anterior or medial margin of cirrus sac. Genital pores marginal, irregularly alternating. Vagina extending from ootype along midline of proglottid to anterior margin of cirrus sac, then laterally along the anterior margin of cirrus sac to common genital atrium, **weakly** recurving or not recurving anterior to cirrus sac, may be expanded proximally. Vaginal sphincter and seminal receptacle present or absent. Ovary near posterior end of proglottid, H- or inverted A-shaped in dorsoventral view, with symmetric or asymmetric lobes, bilobed or tetralobed in cross section. Vitellarium follicular; **vitelline follicles in 2 lateral bands**, relatively large. Uterus sacciform, ventral, occupying midline of proglottid. Parasites of Myliobatiformes and Rhinopristiformes.

*Type species: Scalithrium minimum* (van Beneden, 1850) Ball, Neifar, and Euzet, 2003.

*Additional species: Sc. australiense* Coleman, Beveridge, and Campbell, 2019; *Sc. filamentosum* (Subhapradha, 1955) Coleman, Beveridge, and Campbell, 2019; *Sc. geminum* (Marques, Brooks and Ureña, 1996) Ball, Neifar, and Euzet, 2003; *Sc. healyae* n. sp.; *Sc. johnvolini* n. sp.; and *Sc. magniphallum* (Brooks, 1977) Ball, Neifar, and Euzet, 2003.

*Scalithrium johnvolini* and *Sc. healyae* are the first of the 7 valid members of the genus to be examined with SEM. The microthrix pattern seen was consistent across both species. In both cases, the distal bothridial surfaces were covered with small gladiate spinitriches and acicular filitriches. However, the gladiate spinitriches were more densely arranged in *Sc. johnvolini* (Fig. 8) than they were in *Sc. healyae* (Fig. 17). In both species, the proximal bothridial surfaces were covered with small gladiate spinitriches and acicular or papilliform filitriches, with spinitriches becoming more sparsely distributed towards the posterior of the bothridium. The gladiate spinitriches of *Sc. healyae* typically exhibit much longer, slender tips (Fig. 18) than those of *Sc. johnvolini* (Fig. 9). However, the presence of gladiate spinitriches and acicular or papilliform filitriches on the bothridial surfaces is not unique to *Scalithrium*. This combination is found in 1 or more species in numerous other rhinebothriidean genera, including *Anindobothrium* (see Trevisan et al., 2017), *Anthocephalum* (see Ruhnke et al., 2015), *Barbeaucestus* (see Caira et al., 2017), *Divaricobothrium* (see Caira et al., 2017), *Mixobothrium* (see Herzog et al., 2023), *Rhinebothrium* (see Healy, 2006), *Semiorbiseptum* (see Bueno et al., 2024), *Stillabothrium* (see Ruhnke et al., 2022), and *Sungaicestus* (see Healy, 2006).

Based on its revised membership, *Scalithrium* now houses a relatively cohesive group of species. They are small, delicate worms that range in total length from 0.7 to 5 mm and bear 4 to 20 proglottids. Their testis count is modest at 3 to 18, and their testes are arranged in a single field anterior to the genital pore. Their cirrus sac is conspicuous; their vitelline follicles are relatively large. Their bothridia bear 8 to 20 facial loculi. Three species parasitize rhinopristiformes. More specifically, *Sc. johnvolini* parasitizes the rhinobatid *Acroteriobatis annulatus*, *Sc. healyae* parasitizes the glaucostegid *Glaucostegus typus*, and *Sc. filamentosum* parasitizes the glaucostegid *Glaucostegus granulatus* (Cuvier). The remaining 4 species parasitize myliobatiforms. *Scalithrium australiense* parasitizes the dasyatid *Himantura australis* Last, White, and Naylor, *Sc. minimum* parasitizes the dasyatid *Dasyatis pastinaca* (L.). *Scalithrium geminum* parasitizes the potamotrygonid *Styracura pacifica* (Beebe & Tee-Van), and *Sc. magniphallum* parasitizes the potamotrygonid *Styracura schmardae* (Werner).

In light of these host associations, other members of the rhinopristiform families Rhinobatidae and Glaucostegidae as well as of the myliobatiform family Dasyatidae would be the most promising sources of additional novel species of *Scalithrium*. We predict that the myliobatid family Potamotrygonidae is less likely to yield additional species. Not only has the extensive work conducted on the freshwater members of this family to date failed to yield species of *Scalithrium* (e.g., see Brooks et al., 1981; Reyda and Marques, 2011), but also, to date, there is no evidence that more than a single species of *Scalithrium* parasitizes the same species of host, and both species of *Styracura*—the only marine genus in this family—have already been reported to host a species of *Scalithrium*.

*Scalithrium* is global in distribution. *Scalithrium magniphallum* occurs in the western Atlantic Ocean off Colombia; *Sc. minimum* occurs in the eastern Atlantic Ocean off Belgium and Tunisia; *Sc. johnvolini* occurs off South Africa; *Sc. filamentosum* occurs in the northern Indian Ocean off Madras, India; *Sc. australiense* occurs in the eastern Indian Ocean off western Australia; *Sc. healyae* is found off the eastern coast of Australia; and *Sc. geminum* occurs in the eastern Pacific Ocean off Costa Rica.

Recent revisionary work that has included molecular data has done much to help resolve generic boundaries across rhinebothriidean cestode taxa (e.g., Caira et al., 2017; Trevisan et al., 2017; Herzog et al., 2023; Bueno et al., 2024). However, *Scalithrium* remains one of the few genera of rhinebothriideans that has not yet benefited from the focused application of such methods. To date, only 3 specimens of *Scalithrium* have been included in molecular phylogenetic analyses. These are a specimen identified as *Scalithrium* n. sp. (LRP 3895) from *Hypanus longus* (Garman) in the molecular analyses of Healy et al. (2009), a specimen identified as *Scalithrium* sp. 1 (LRP 8333), also from *H. longus*, in the analyses of Caira et al. (2014), and a specimen (LRP 11078) identified as *Scalithrium* sp. nov. 2 from *A. annulatus* by Bueno et al. (2024), which, as noted above, we believe is *Sc. johnvolini*. In fact, examination of the vouchers of the 2 specimens from *H. longus* leads us to believe these specimens are conspecific. Beyond the fact that these 2 species grouped together in the tree resulting from the molecular analyses of Bueno et al. (2024), not much can be said about the implications of the results of that analysis for our understanding of *Scalithrium*. Inclusion of additional representation of the genus—and ideally all 6 remaining valid species—will be very informative. It will also be extremely interesting to explore the affinities of the two *incertae sedis* species, *Sc. palombii*, from *Pteroplatytrygon violacea* (Bonaparte), and *Sc. rankini*, from *Bathytoshia centroura* (Mitchill), in future molecular analyses to help inform their appropriate generic placements.

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