A Puzzle No More:
The Identity of *Spirobranchus tetraceros* (Schmarda, 1861) (Annelida, Serpulidae) is Revealed

Elena K. Kupriyanova[1,2] ⓘD, Beth Flaxman[1,3] ⓘD, and Ingo Burghardt[1] ⓘD

[1] Australian Museum Research Institute,
Australian Museum, 1 William Street, Sydney NSW 2010, Australia

[2] Department of Biological Sciences,
Macquarie University, North Ryde NSW 2109, Australia

[3] School of Life and Environmental Sciences,
University of Sydney NSW 2006, Australia

Abstract. *Spirobranchus tetraceros* (Schmarda, 1861) originally briefly described from New South Wales, Australia was later reported as a widely distributed species of Indo-Pacific origin. The species was assumed to be a highly successful invasive Lessepsian migrant to the Mediterranean *via* the Suez Canal. However, recently, such wide distributions have been questioned and *S. tetraceros* was treated as a complex of morphologically similar species. Moreover, genetic evidence proved that the species that invaded the Mediterranean originated neither in warm temperate Australia nor in the Red Sea. This study examines the taxonomic status of *Spirobranchus tetraceros* populations along the east coast of Australia. Given the absence of the holotype, we re-described *Spirobranchus tetraceros* from New South Wales, designated the neotype supported by DNA sequence data, and fixed Port Botany as the type locality. The phylogenetic analysis revealed the existence of a sympatric cryptic species with a mean genetic distance of 36% (described here as *S. schmardai* sp. nov.) and proved that the tropical coral-associated specimens from Queensland belong to at least two distinct species. We also suggest resurrecting the name *S. multicornis* Grube, 1862 for the Red Sea population of the *Spirobranchus tetraceros* complex. This study calls for a worldwide revision of the complex.

Introduction

Morphological variation in opercular morphology within the genus *Spirobranchus* de Blainville, 1818 appears to be so large that earlier taxonomists (e.g., Fauvel, 1923, 1932; Rullier, 1972) lumped most of the tropical forms under *S. giganteus*. Ever since, many of the identifications of *"S. giganteus"* have included a number of different taxa. An initial morphological separation of the taxa within *Spirobranchus* was made by ten Hove (1970), who differentiated at least three geographically separated morphologies in the *Spirobranchus giganteus / corniculatus* complex (tropical forms with spiral radioles). He also separated forms with circular arrangement of radioles from those with spiral arrangements. However, following the cosmopolitan concept, which was dominant at the time
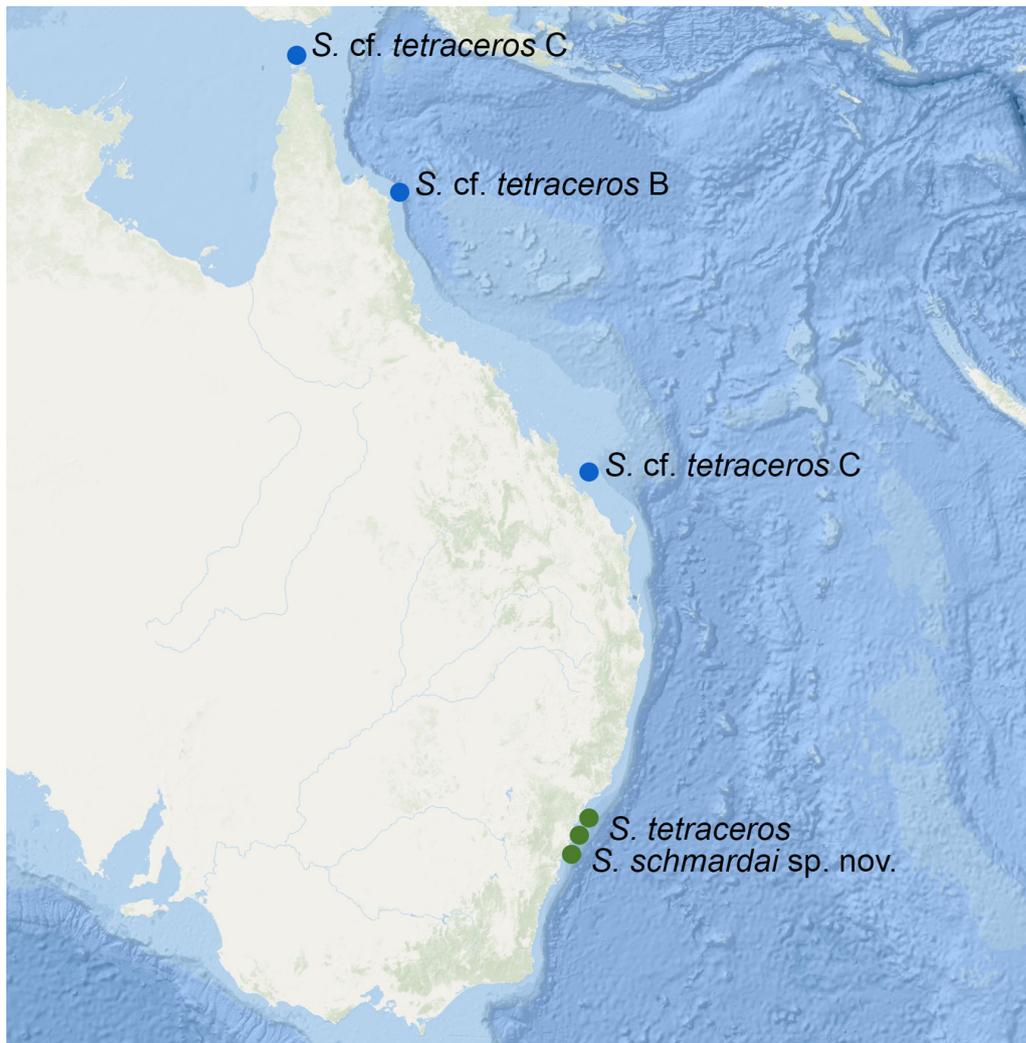
Figure 1. Map of localities for the specimens of the *S. tetraceros* complex collected along NSW and Qld coasts of Australia. Green points indicate *S. tetraceros sensu stricto* and *S. schmardai* sp. nov. (NSW), blue points indicate the *S.* cf. *tetraceros* complex (Qld).

(reviewed by Hutchings & Kupriyanova, 2018), ten Hove (1970) lumped 22 nominal taxa worldwide with circular radioles under the oldest available name *Spirobranchus tetraceros*. Morphologically all these taxa have radioles arranged in a circle (non-spiral), fringed peduncular wings, and a conical or flat opercular endplate bearing in the middle three ("*tricornis*"), four ("*tetraceros*") or six separate groups of antler-like spines branched at the tips. *Spirobranchus tetraceros* was considered as a species complex already by ten Hove (1994: 113); Fiege & ten Hove (1999: fig. 4); ten Hove & Kupriyanova (2009: 98); and Ben-Eliahu & ten Hove (2011: 91). Under the *Spirobranchus tetraceros* complex, for instance, ten Hove (1994: 113) states: "in 1970, I united various nominal taxa from circumtropical origin in a single species: *S. tetraceros*. Nowadays I realise that this has been an oversimplification, the taxon *tetraceros* probably contains a number of species". However, the name has been used for various populations around the world (see Palero *et al.*, 2019).

The first DNA sequence data for the *S. tetraceros* complex were provided by Perry *et al.* (2018) who examined a population of *S.* cf. *tetraceros* from the Red Sea (Eilat, Israel). The authors expressed doubt that the Red Sea population belongs to *S. tetraceros sensu stricto*. This issue

was resolved by Palero *et al.* (2019) who revealed that the Mediterranean specimens of *S.* cf. *tetraceros* recently newly recorded from Valencia were genetically identical to those from Heraklion, Crete, but differed from those collected in the Red Sea (Perry *et al.*, 2018) and Sydney, NSW, Australia. Thus, the results of Palero *et al.* (2019) for the first time confirmed, by means of molecular data, the long-standing morphology-based conclusion that *S. tetraceros* is a species complex.

The status of the *Spirobranchus tetraceros* complex in Australia remains unresolved. The nominal species was described (as *Pomatoceros tetraceros*) by Schmarda (1861) from Australia during an around the world voyage and the collection locality is not specified beyond New South Wales. The original description translated from German (by IB) is very brief: "Body of animal has general shape of the genus, as far as I can tell. Body colours are brick-red and blue-green. Length 5 mm. Operculum shaped like inverted cone, the upper part slightly protruding and ribbed. Edge denticulate. Centre of plain slightly depressed. Four short, antler-shaped extensions sitting in dent. Colour red (with the exception of ribs, which are green). Number of radioles about 20 per bunch. Lower part red, upper part green. Collar split on ventral side, somewhat folded back and with white edge;

Table 1. Terminals used in phylogenetic analysis with registration numbers and collection localities; *reg.* museum registration number; *loc.* collection locality; * data derived during the present study; ZRC—numbers for Zoological Record Collection of the Lee Kong Chian Natural History Museum, National University of Singapore; *VR*—Steinhardt Museum of Natural History, Tel Aviv, Israel; *ZUTC*—Zoological Museum of the University of Tehran, Tehran, Iran; *MUVHN*—Museu de la Universitat de València d'Història Natural. *Auckland_S3*, *PuysegurBank_2,* and *Quarantine_S2,* from New Zealand, are field numbers only.

species	reg.	loc.	cyt *b*	18S	source
S. cf. *tetraceros* sp. C	W.30500	Qld, Torres Strait	ON457550	ON228373	*
S. tetraceros	W.35308	NSW, Cape Three Points	ON457546	ON221916	*
S. cf. *tetraceros* sp. C	W.42374	Qld, Heron Island	ON457540	ON228374	*
S. schmardai sp. nov.	W.42389	NSW, Kurnell	MN631161	ON228372	*& Palero *et al.*, 2020
S. cf. *tetraceros* sp. C	W.42391	Qld, Heron Island	ON457541	ON228370	*
S. schmardai sp. nov.	W.42393	NSW, Kurnell	ON457552	ON221934	*
S. cf. *tetraceros* sp. B	W.45073	Qld, Lizard Island	ON457542	ON228371	*
S. tetraceros	W.49841	NSW, Port Kembla	ON457543	—	*
S. tetraceros	W.49844	NSW, Shellharbour	ON457544	—	*
S. tetraceros	W.49845	NSW, Shellharbour	ON457545	—	*
S. tetraceros	W.51856	NSW, Port Botany	ON457547	ON221936	*
S. schmardai sp. nov.	W.51857	NSW, Port Botany	ON457553	ON221935	*
S. tetraceros	W.51858	NSW, Port Botany	ON457548	ON221937	*
S. tetraceros	W.51859	NSW, Port Botany	ON457549	ON221938	*
S. corniculatus	VR.25267	Israel	MF319327	MF319293	Perry *et al.*, 2018
S. gardineri	VR.25319	Israel	MF319342	MF319300	Perry *et al.*, 2018
S. aloni	VR.25205	Israel	MF319307	MF319276	Perry *et al.*, 2018
S. kraussii	W.49976	South Africa	MK308657	MK308672	Simon *et al.*, 2019
S. sinuspersicus	ZUTC.6805	Iran	MN372439	MN372446	Pazoki *et al.*, 2020
S. cariniferus	Auckland_S3	New Zealand	JX144875	JX144819	Smith *et al.*, 2012
S. cf. *kraussii* sp. 3	W.48302	Qld, Australia	MK308648	MK308663	Simon *et al.*, 2019
S. cf. *kraussii* sp. 2	W.45327	Hawaii, USA	MK308655	MK308670	Simon *et al.*, 2019
S. akitsushima	W.49981	Japan	MK308654	MK308669	Simon *et al.*, 2019
S. latiscapus	PuysegurBank_2	New Zealand	JX144879	JX144821	Smith *et al.*, 2012
S. multicornis	VR.25311	Israel	MF319335	MF319295	Perry *et al.*, 2018
S. multicornis	VR.25312	Israel	MF319336	MF319296	Perry *et al.*, 2018
S. cf. *tetraceros*	MUVHN-ZK0002	Spain	MN631163	—	Palero *et al.*, 2020
S. cf. *tetraceros*	MUVHN-ZK0004	Greece	MN631162	—	Palero *et al.*, 2020
Galeolaria hystrix	Quarantine_S2	New Zealand	JX144859	JX144800	Smith *et al.*, 2012

dorsally with plural pointed extensions on the upper margin. Apart from six bundles of bristles attached to the mantle there are (as in other serpulids) two bundles of bristles attached to the collar. New South Wales." The description is accompanied by a colour drawing (Fig. 3A) of the single specimen (not retained) presumably used for the description. This single holotype has not been deposited either in Australia, or in the Museum Joanneum (now Universalmuseum Joanneum) where Ludwig Schmarda was head of the zoology department from 1849 to 1851 (U. Hausl-Hofstätter, pers. comm.)

The reported geographic distribution of the *S. tetraceros* complex in Australia is wide (NSW and Qld: Straughan, 1967, fig. 14b–d as *S. tricornis*, Qld: Kupriyanova *et al.*, 2015: 332, fig. 28A, B), WA: Johansson, 1918; Pillai, 2009: 158), while both morphology and ecological requirements reported for this nominal taxon are very variable. Straughan (1967) does not use the name *S. tetraceros*, but instead uses the name *S. semperi* for specimens with four distinct horns from Qld, and *S. tricornis* for specimens with three horns from both Qld and NSW. Smith (1985: 51–61) differentiates between rock and wooden pylons harbouring "sp. A" in NSW (thus probably the original *S. tetraceros*), and coral harbouring "sp. B" in the Great Barrier Reef. In the tropical Kimberley region, Western Australia, Pillai (2009) reports *S. tetraceros* mostly living in tubes covered by scleractinian corals, consequently referring to the former as coral

associates. Pillai (2009: 149) states: "The number of main horns in *S. tetraceros* from the Kimberleys is 3, and they are dichotomously branched". Apparently at least two species, a tropical and temperate could be present in the *S. tetraceros* complex in Australia.

Because the identity of the original *S. tetraceros* cannot be determined and the type does not exist, a designation of the neotype from NSW, Australia is needed to start resolving the complex worldwide. Thus, the goal of this paper was to re-describe *Spirobranchus tetraceros* from the type locality (NSW) and to designate the neotype supported by DNA sequence data. In addition, we aimed to determine the status of the species from the east coast of Australia, particularly from tropical Queensland.

Material and methods

The study was based on serpulids tentatively identified as *Spirobranchus tetraceros* collected along the east coast of Australia (NSW and Qld) and deposited in the collections of the Australian Museum (Fig 1). Although numerous specimens under the name *S. tetraceros* from NSW and Qld (156 registration lots) are available in the collection, we selected only 14 specimens that were either preserved in 95% ethanol or fixed in formalin but had tissue samples

fixed in ethanol and deposited in the Frozen Tissue Collection (FTC) of the Australian Museum. Australian Museum (AM) registered specimens are prefixed "W".

Specimens were photographed using a Canon EOS 7D with a Macro EF 100 mm and the Spot Flex CCD 15.2 fitted on a Leica MZ16 Stereo microscope at the Australian Museum. Two paratypes with missing opercula of *S. tetraceros* and *S. schmardai* sp. nov. were dehydrated in ethanol, critical point dried, coated with 20 nm of gold, and examined under the Scanning Electron Microscope (SEM) JEOL JSM-6480 at Macquarie University, Sydney.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from posterior parts of abdomens using the Bioline Isolate II genomic DNA kit according to the manufacturer's protocol. Stock DNA was diluted 1:10 with deionized water to produce template strength DNA for Polymerase Chain Reactions (PCR). A combination of mitochondrial (cytochrome b) and ribosomal (18S) genes was used to check for concordance between these types of markers. Each of these markers evolves at a different rate and thus provides a different level of resolution.

The 18S rRNA genes (approximately 1800 bp) were amplified in two overlapping fragments, one of approximately 1100 bp with the primers TimA (AMCTGGTTGATCCTGCCAG) and 1100R2 (CGGTATCTGATCGTCTTCGA) from Nóren and Jordelius (1999); the other of approximately 1300 bp using 18s2F (GTTGCTGCAGTTAAA) and 18s2R (ACCTTGTTAGCTGTTTTACTTCCTC) from Kupriyanova *et al*. (2006). The 18S fragments were combined using Geneious Prime 2019.0.4 (https://www.geneious.com). The cyt *b* gene fragments (approximately 350 bp) were amplified with the primer pair Cytb424F (GGWTAYGTWYTWCCWTGRGGWCARAT) and cobr825 (AARTAYCAYTCYGGYTTRATRTG) from Halt *et al*. (2009).

PCR conditions were as follows: an initial denaturation step at 94°C for 3 min (18S and cyt *b*), 40 cycles at 94°C for 30 s (18S) respectively 45 cycles at 94°C for 1 min (cyt *b*), 52°C for 30 s (18S) respectively 50°C for 30 s (cyt *b*), 72°C for 30 s (18S) respectively 72°C for 1 min (cyt *b*), with a final extension at 72°C for 5 min (18S) respectively 8 min (cyt *b*). PCR success was detected using gel electrophoresis (1 % agarose gel stained with gel red (Biotium TM, San Francisco)) and visualized using a Bio-Rad XR+ Gel Documentation System. Successful PCR products were sent to Macrogen TM, South Korea where they were purified and standard Sanger sequencing was performed. Sequences were edited using Geneious and were aligned in Geneious (cyt *b*) or using MAFFT (18S; Katoh & Standley, 2013). A BLAST search confirmed the correct gene regions had been amplified (Altschul *et al.*, 1990) and the new sequences were submitted to GenBank.

Two datasets were combined and analysed together. The first dataset included 13 cyt *b* sequences of *S. tetraceros* morphospecies from NSW and Qld and 15 sequences of *Spirobranchus* species downloaded from GenBank. The second dataset included 11 18S sequences of the same specimens, together with 12 sequences of the same *Spirobranchus* species from GenBank. *Galeolaria hystrix* Mörch, 1863 from New Zealand was used as the outgroup following Pazoki *et al.* (2020). Collection localities and GenBank accession numbers are found in Table 1.

Table 2. Mean pairwise distances (*p*-distance) for cyt *b* sequences between *Spirobranchus* species used for phylogenetic reconstruction (range in parentheses).

| species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 S. cf. tetraceros	—														
2 S. cf. tetraceros	0.372 (0.012)	—													
3 S. tetraceros	0.343 (0.015)	0.411 (0.011)	—												
4 S. multicornis	0.194 (0.008)	0.366 (0.001)	0.375 (0.011)	—											
5 S. kraussii	0.343 (0.001)	0.335 (0)	0.373 (0.013)	0.374 (0.002)	—										
6 S. sp. 2	0.398 (0.002)	0.388 (0)	0.366 (0.008)	0.414 (0.002)	0.210 (0)	—									
7 S. akitsushima	0.355 (0.006)	0.382 (0)	0.368 (0.008)	0.366 (0.001)	0.269 (0)	0.230 (0)	—								
8 S. sp. 3	0.401 (0.009)	0.352 (0)	0.370 (0.013)	0.394 (0.002)	0.235 (0)	0.160 (0)	0.233 (0)	—							
9 S. gardineri	0.391 (0.014)	0.408 (0)	0.423 (0.017)	0.431 (0.002)	0.423 (0)	0.411 (0)	0.360 (0)	0.349 (0)	—						
10 S. corniculatus	0.415 (0.010)	0.444 (0)	0.441 (0.013)	0.417 (0.002)	0.382 (0)	0.414 (0)	0.349 (0)	0.408 (0)	0.211 (0)	—					
11 S. cariniferus	0.376 (0.004)	0.408 (0)	0.406 (0.007)	0.385 (0.001)	0.305 (0)	0.320 (0)	0.266 (0)	0.309 (0)	0.439 (0)	0.415 (0)	—				
12 S. schmardai	0.426 (0.009)	0.451 (0.010)	0.357 (0.040)	0.398 (0.016)	0.418 (0.017)	0.403 (0.023)	0.377 (0.035)	0.391 (0.019)	0.423 (0.009)	0.441 (0.014)	0.388 (0.011)	—			
13 S. aloni	0.436 (0.011)	0.419 (0)	0.435 (0.012)	0.472 (0.002)	0.433 (0)	0.400 (0)	0.384 (0)	0.412 (0)	0.249 (0)	0.207 (0)	0.446 (0)	0.457 (0.010)	—		
14 S. sinuspersicus	0.419 (0.008)	0.385 (0)	0.377 (0.001)	0.437 (0.002)	0.302 (0)	0.293 (0)	0.296 (0)	0.301 (0)	0.489 (0)	0.449 (0)	0.337 (0)	0.514 (0.016)	0.461 (0)	—	
15 S. latiscapus	0.455 (0.011)	0.473 (0)	0.411 (0.010)	0.504 (0.002)	0.465 (0)	0.411 (0)	0.390 (0)	0.423 (0)	0.368 (0)	0.421 (0)	0.359 (0)	0.449 (0.016)	0.451 (0)	0.416 (0)	—
16 S. cf. tetraceros	0.211 (0.009)	0.420 (0.012)	0.359 (0.009)	0.273 (0.030)	0.361 (0.009)	0.375 (0.002)	0.383 (0.010)	0.398 (0.007)	0.399 (0.010)	0.379 (0.010)	0.312 (0.006)	0.422 (0.016)	0.430 (0.019)	0.414 (0.006)	0.418 (0.005)
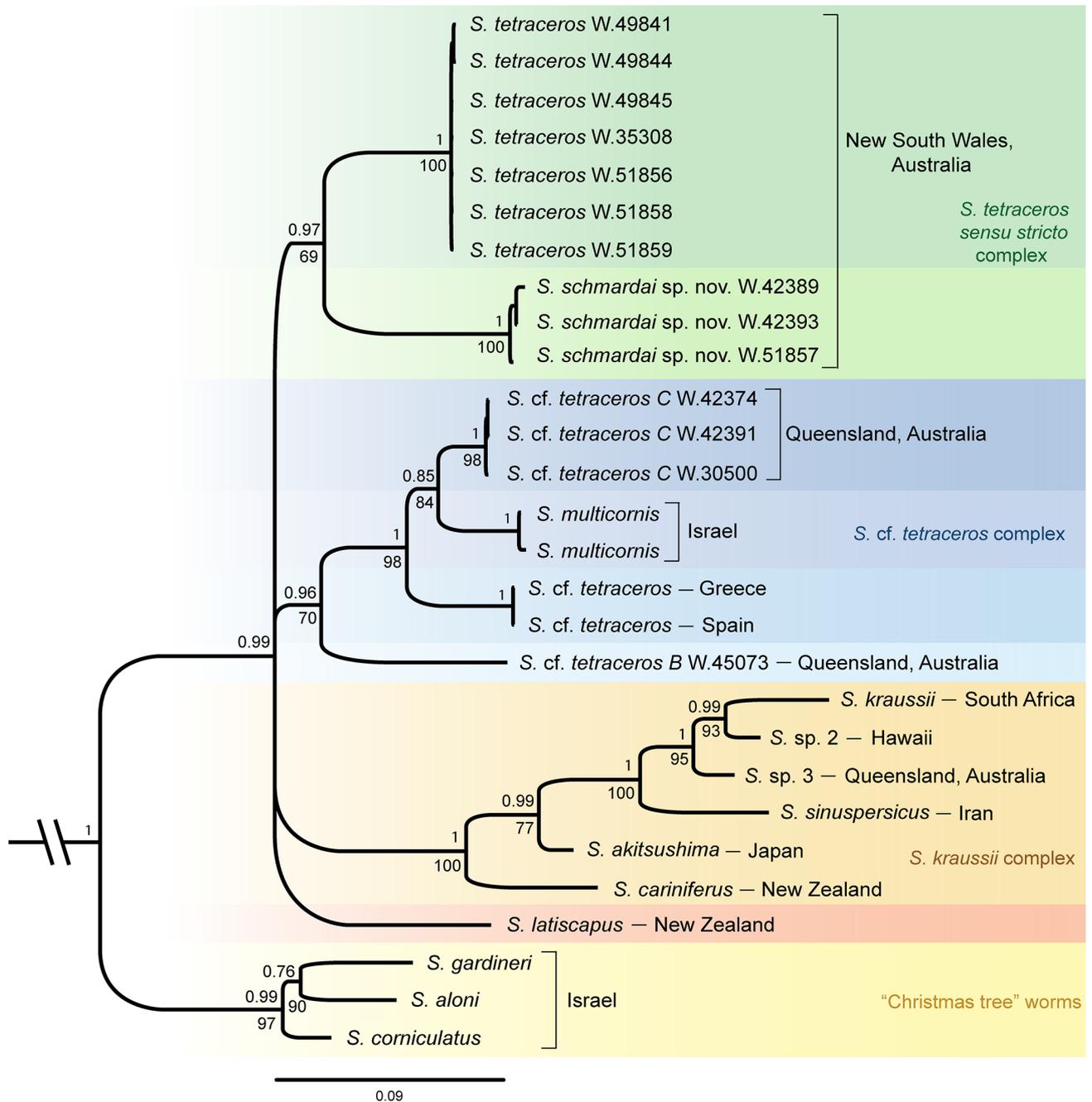
Figure 2. Bayesian majority rule consensus phylogram of the concatenated (18S + cyt *b*) sequence data set with congruent nodes indicated for maximum likelihood analysis. Numbers above branches are posterior probabilities obtained from the Bayesian analysis; numbers below branches are bootstrap values from ML analysis. Posterior probabilities >0.76 and bootstrap values >69 are shown.

Phylogenetic analyses

The concatenated analysed dataset included 1616 bp long 18S and 347 bp cyt *b* long gene fragments. The phylogenetic relationships were inferred using maximum-likelihood analysis in IQ-TREE (Minh *et al.*, 2020) and Bayesian inference in MrBayes (Ronquist *et al.*, 2012). Separate nucleotide substitution models selected using the Bayesian information criterion in ModelFinder (Kalyaanamoorthy *et al.*, 2017) for maximum likelihood analysis were HKY+F+I+G4 for cyt *b* and TNe+I for 18S. Branch support was estimated using 1000 ultrafast bootstraps (Hoang *et al.*, 2018). For Bayesian inference, independent GTR+I+G models were used for each marker and a Markov chain Monte Carlo analysis was run for 10 million generations,

with samples drawn every 1000 generations and the first 1000 samples removed as burn-in. Nodal support was indicated by posterior probabilities. Pairwise distances between all *Spirobranchus* cyt *b* sequences were calculated (Table 2) using the Kimura 2-parameter model in MEGA-X (Kumar *et al.*, 2018).

Results

The results of maximum-likelihood and Bayesian inference (BI) analyses using the concatenated dataset are presented in Fig. 2. Both analyses resulted in the same tree topology and inferred five major clades within *Spirobranchus*: *S. tetraceros sensu stricto* complex, *S.* cf. *tetraceros* complex,

S. kraussii complex, *S. latiscapus* and a "Christmas Tree worms" clade. The relationships among these major clades were poorly resolved, with the first four clades forming a four-way polytomy. The "Christmas Tree Worm" clade was recovered in sister-group relationships with the major polytomy.

Ten specimens of *S. tetraceros* from warm temperate localities (Sydney, NSW) formed a reasonably well supported (BI posterior probability 0.97, ML bootstrap 69) clade made of perfectly supported (pp 1, bs 100) sister clades *Spirobranchus tetraceros sensu stricto* and *S.* cf. *tetraceros* A (described here as *S. schmardai* sp. nov.). The second major clade (pp 0.96, bs 70) included monophyletic *S.* cf. *tetraceros* from Queensland, Australia (Heron Island and Torres Strait) forming a sister group with the specimens of *S. multicornis* from Eilat, the Red Sea (as *S. tetraceros* in Perry *et al.*, 2020), *S.* cf. *tetraceros* clade from the Mediterranean (Greece and Spain) and *S.* cf. *tetraceros* from Queensland (Lizard Island) forming a basal grade. Thus, the monophyly of the three "*S. tetraceros*" Australian clades was not supported.

Overall, this study recovered six distinct lineages (putative species) of the *S. tetraceros* complex (Table 2). Among Australian clades of the *S. tetraceros* complex (*S. tetraceros sensu stricto* and *S.* cf. *tetraceros*), intraspecific Kimura-2 parameter (K2P) distances ranged from 0.00 to 0.01 and interspecific distances ranged from 0.34 to 0.46 substitutions per site.

Taxonomy

Genus *Spirobranchus* de Blainville, 1818

Spirobranchus tetraceros (Schmarda, 1861)

Figs 3A–C, 4

Pomatoceros tetraceros Schmarda, 1861: 30, pl. 21, fig. 179. ["Neu-Süd-Wales" (New South Wales). Short description with a figure; Grube, 1862: 66. [Name only]

Galeolaria ?tetracerus (Schmarda) Mörch, 1863, p. 371 [no new data]; contrary to Halt *et al.* (2009, p. 217) neither Schmarda's original description (*operculum quadricorne*, repeated in German "vier kurze, geweihartige Fortsätze", in which four antler-like projections are visible in his fig. 179 as well), nor Mörch's interpretation of Schmarda's figure, can refer to *Galeolaria hystrix*, the taxon had been correctly attributed by Schmarda to *Pomatoceros,* nowadays *Spirobranchus*]

Vermilia tetraceros (Schmarda) Quatrefages, 1865, p. 520 [diagnosis of Schmarda].

In part *Pomatoceros elaphus* Haswell, 1885, pp. 663–665, pl. 31, fig. 7, pl. 3 2, figs 9–10 [Port Jackson, Sydney. Description; figure of operculum, peduncular wings and radioles].

In part *Spirobranchus giganteus*.—Dew, 1959, pp. 45–46, fig. 17 [several localities from Queensland to New South Wales (of the four specimens studied, two belonged to *S. corniculatus*)]

In part *Spirobranchus tricornis*.—Straughan, 1967, p. 244, fig. 14b–d [New South Wales—few characters]

In part *Spirobranchus tetraceros*.—Day & Hutchings, 1979, p. 147 [checklist of Australian records and specimens].

Material studied

Neotype: Australian Museum W.51859, New South Wales, Port Botany, off La Perouse Point, 33°59'36"S 151°13'39"E, 1 spec. without tube.

Additional materials: Australian Museum W.35308, NSW, Cape Three Points 33°31'55"S 151°24'57"E, 1 spec. without operculum (prepared for SEM); W.49841, Port Kembla, north east of Martin Island, 34°29'47"S 150°56'11"E, 1 spec. in tube; W.49844, NSW, Shellharbour, 34°36'12"S 150°53'42"E, 1 spec. without operculum; W.49845, NSW, Shellharbour, same as above, 1 spec. without operculum; W.51856, NSW, Port Botany, off La Perouse Point, 33°59'36"S 151°13'39"E, 1 spec. with simple conical operculum without spines; W.51858, NSW, Port Botany, La Perouse, same as above, 1 spec. without operculum.

Comparative material

Spirobranchus cf. *tetraceros* sp. B

Australian Museum W.45073 Qld, Lizard Island, 14°40'46"S 145°26'49"E, 1 spec. without operculum.

Spirobranchus cf. *tetraceros* sp. C

Australian Museum W.30500, Qld, Torres Strait, west of Hawkesbury Island, 10°21'44"S 142°7'4"E, 1 spec.; W.42374, Qld, Heron Island, 23°26'30"S 151°54'2"E, 1 spec.; AM W.42391, Qld, Heron Island, 23°26'30"S 151°54'2"E, 1 spec. without operculum.

Description

Tube: attached to substrate throughout their length; tube colour predominantly pink (some parts maybe white) outside, white inside, circular in cross-section, without a tooth over entrance (Fig. 3D, E). Rounded in cross-section, one distinct irregular higher median keel and two or three lower slightly denticulate lateral keels and some irregular transversal ridges.

Radiolar crown: radioles arranged in two circles. Radioles square-shaped in cross-section, external side smooth, internal sides with two rows of pinnules of the same length, becoming slightly shorter towards tips of radioles. Terminal filaments without pinnules. Stylodes absent.

Interradiolar membrane: high, connecting over half of radiolar length, without lappets (processes) between radioles (Figs 3C, 4A).

Peduncle: nearly twice as thick as normal radioles (4A); inserted on the left of median line (Figs 3C, 4A), pigmented with white and blue colours (Fig. 3A). Lateral distal wings elongated triangular, with pointed tips and crenulated inner margins.

Operculum: very variable, without prominent basal ampulla; endplate circular with thickened brim, slightly concave (AM W.51856 with simple conical operculum). In the centre opercular endplate bearing three groups of dichotomously branching (antler-like) spines (Fig. 3C–E); one group positioned medio-ventrally and two groups latero-dorsally. The most complex opercula showing one medio-ventral spine split thrice and two latero-dorsal spines split twice to thrice, with medial spinules irregularly placed.
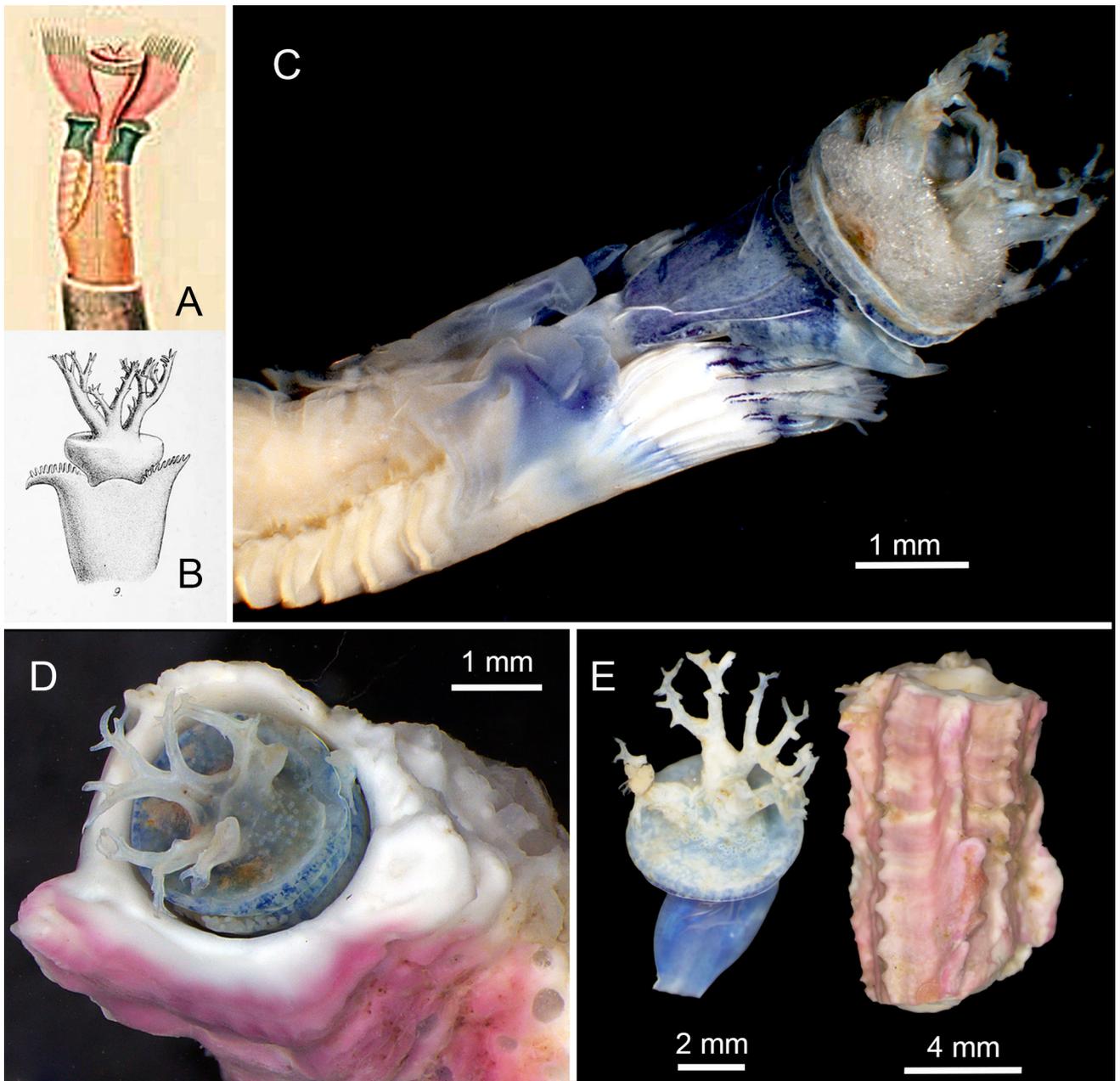
Figure 3. Photographs of the neotype and additional specimens of *Spirobranchus tetraceros sensu stricto*. (*A*) original drawings of *Pomatoceros tetraceros* by Schmarda (1861); (*B*) original drawing of *Pomatoceros elaphus* by Haswell (1885); (*C*) neotype of *S. tetraceros* W.51859; (*D*) specimen W.49841 in tube; and (*E*) specimen W.49844, operculum (left), a tube fragment (right).

Collar and thoracic membranes: short, covering only the bases of radioles (Fig. 3C); tri-lobed, with a larger ventral and two smaller lateral lobes. Tonguelets present between lateral and ventral lobes. Lateral lobes continuing into thoracic membranes producing a ventral apron reaching to the second abdominal chaetiger (Fig. 4A). Bundle of collar chaetae large, distinct, situated at some distance anterior to remaining thoracic chaetae (Fig. 4A). Collar chaetae of two types: special *Spirobranchus*-type bearing basal bosses covered with minute denticles and simple limbate (Fig. 4B).

Thorax: with seven thoracic chaetigers, including six uncinigerous (Fig. 4A). Thoracic chaetae simple limbate of two sizes (Fig. 4C), *Apomatus* chaetae absent. Uncini saw-shaped with 12–14 teeth in profile, anterior peg flat, nearly triangular-shaped (dorsal uncini of second thoracic chaetiger, Fig. 3D). Ventral ends of thoracic uncinigerous tori widely separated anteriorly, gradually approaching one another towards the end of thorax, thus leaving a triangular depression.

Abdomen: abdominal chaetae long (approximately the same length as thoracic chaetae) throughout the abdomen, not becoming significantly longer towards pygidium; true trumpet-shaped with two rows of teeth (Fig. 3F). Uncini saw-shaped throughout the length of abdomen, with 11–12 teeth per row, anterior pegs flat, nearly triangular (Fig. 4E).

Colour of preserved specimens: anterior end of thorax, distal ends of radioles, peduncle, and opercular endplate blue or white with blue specks (Fig. 3C–E).
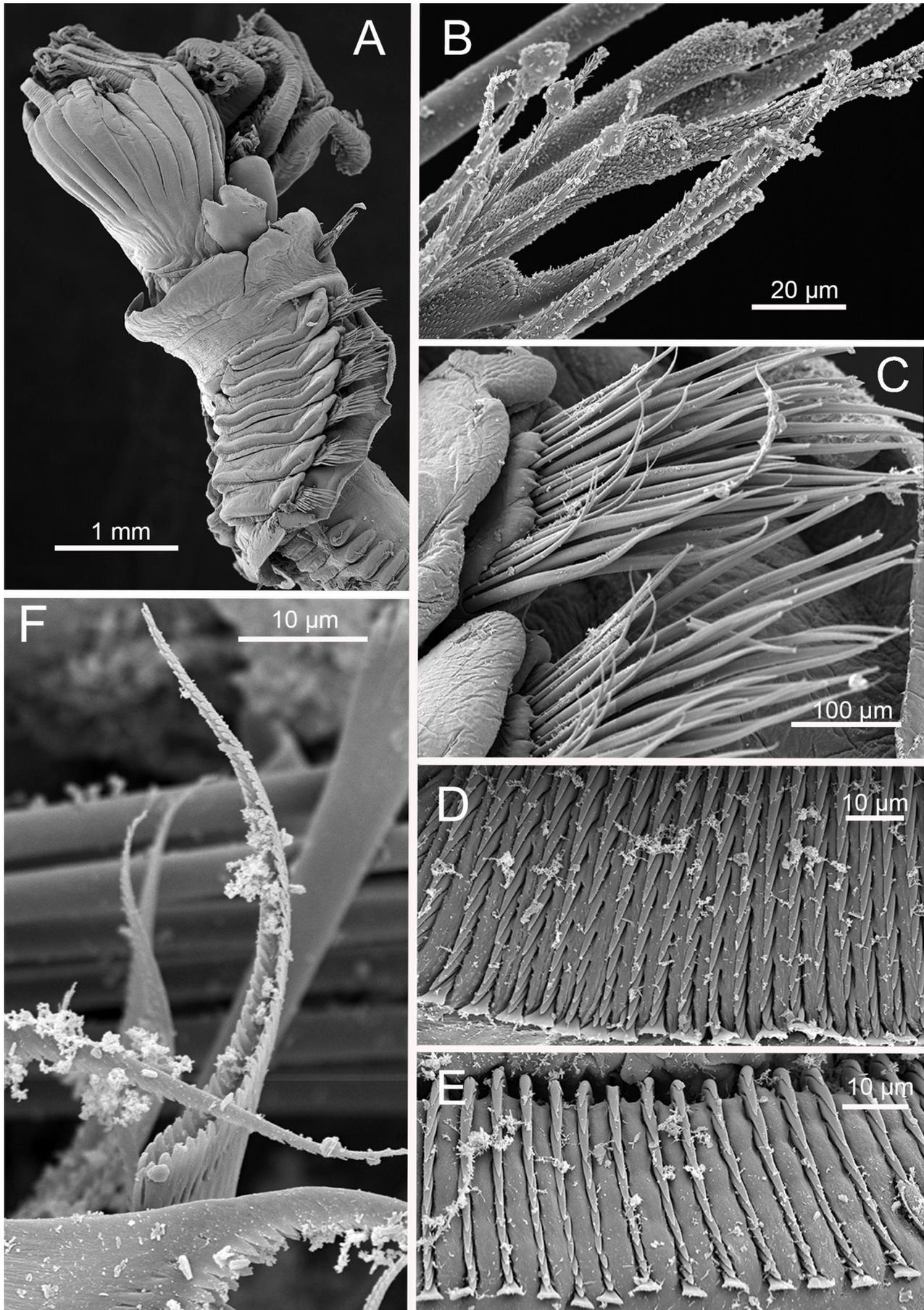
Figure 4. Scanning electron microscopic micrographs of *S. tetraceros sensu stricto* W.35308. (*A*) lateral view of the anterior part of the paratype; (*B*) collar chaetae bundle with special *Spirobranchus*-type chaetae; (*C*) thoracic chaetae; (*D*) thoracic uncini; (*E*) anterior abdominal uncini; (*F*) anterior abdominal chaetae.

Remarks

For a *Spirobranchus* cf. *tetraceros* population from Eilat, Perry *et al.* (2018) mention the inter-radiolar membrane with lappets between radioles and so does Palero *et al.* (2020) for the specimens from Spain. Moreover, ten Hove (1970) also included lappets (processes) between radioles in his composite description based on specimens from the Caribbean, Iranian Gulf, Banda Sea, and Philippines. The neotype and additional specimens of *Spirobranchus tetraceros*, however, lack such inter-radiolar lappets altogether. Ironically, although Schmarda (1861) named his species "*tetraceros*" because of four short, antler-shaped extensions (spines) in the centre of the operculum (Fig. 3A), none of the specimens examined in this study show such opercula, they rather have three groups of branching spines very similar to the operculum of *Pomatoceros elaphus* Haswell, 1885 (Fig. 3B).

Interestingly, while Schmarda's figure appears to illustrate four unbranching opercular spines figure, high magnification of the drawings shows at least three spines with a terminal bifurcation. Confusingly, Schmarda drew two spines turning away from the opercular endplate and crossing two others more or less parallel to the endplate, which might be interpreted as bifid. The total number in this figure would have been four, thus justifying the name "tetraceros". However, if the right "horizontal spine" is merely reflecting a shadow on the endplate seen by Schmarda, the remaining arrangement of two more or less erect spines turning away from the distal plate and one projecting in between conform the general observed pattern of two latero-dorsal and one medio-ventral groups of spines. Also, in Schmarda's figure the edge of the opercular ampulla is apparently shown as being "toothed". One wonders whether the colour patterns as in Fig. 3C, E might have led Schmarda, with either poor illumination or bad optics, to such an interpretation of the opercular edge shape.

As Schmarda did not leave any collections and thus the type material of *Pomatoceros tetraceros* is not available, here we designated a neotype in accordance with the International Code of Zoological Nomenclature (ICZN 1999), Article 75.3.

Spirobranchus schmardai sp. nov.

urn:lsid:zoobank.org:act:52EEB181-5C75-4AC3-95DD-DB7EE76F5FB4

Figs 5, 6

In part *Pomatoceros elaphus* Haswell, 1885, pp. 663–665, pl. 31, fig. 7, pl. 32 figs 9–10. [Port Jackson, Sydney. Description; figure of operculum, peduncular wings and radioles]

In part *Spirobranchus giganteus.*—Dew, 1959, pp. 45–46, fig. 17. [several localities in Australia, from New South Wales to Queensland]

In part *Spirobranchus tricornis.*—Straughan, 1967, p. 244, fig. 14b–d. [New South Wales]

In part *Spirobranchus tetraceros.*—Day & Hutchings, 1979, p. 147 [checklist of Australian records and specimens]

Holotype Australian Museum W.42389 without tube, northeast of Kurnell, "Anchor Reef", 34°00'33"S 151°13'51"E; 17.8 m. **Paratypes** W.42393, same data as for holotype, 1 spec. without tube and operculum prepared for SEM; W.51857, Port Botany, off La Perouse Point, 33°59'36"S 151°13'39"E, 1 spec. in tube.

Description

Tube: missing in the holotype and in paratype AM W.42393; in paratype AM W.51857 predominantly pink outside, white inside, circular in cross-section, without a tooth over entrance (Fig. 5). One distinct irregular higher median keel and two or three lower lateral keels and some transversal ridges (Fig. 5B).

Radiolar crown: radioles in two circles (Fig. 5A). Radioles square-shaped in cross-section, external side smooth, internal sides with two rows of pinnules of the same length, becoming slightly shorter towards tips of radioles. Terminal filaments without pinnules. Stylodes absent.

Interradiolar membrane: high, connecting over half of radiolar length without distinct lappets (processes), but some thickenings between radioles present (Figs 5A, 6A).

Peduncle: three times as thick as normal radioles, inserted on the left of median line, pigmented with white/blue colours (Fig. 5A). Lateral distal wings wide, with pointed tips bearing finger-like processes on their inner margins (Fig. 5A).

Operculum: with circular flat calcareous endplate bearing three groups of dichotomously branched (antler-like) spines, position of spines always the same: one group medio-ventrally and two latero-dorsally (Fig. 5A, B).

Collar and thoracic membranes: collar short, covering only the bases of radioles (Figs 5A, 6A), divided into one ventral and two lateral lobes. Tonguelets present between lateral and ventral lobes. Latero-dorsal lobes continuing into thoracic membranes (Figs 5A, 6A) producing a short ventral apron. Collar chaetae of two types: special *Spirobranchus*-type with basal bosses covered with minute denticles and simple limbate (Fig. 6B).

Thorax: with seven thoracic chaetigers, including six uncinigerous (Fig. 6A). Thoracic chaetae simple limbate of two sizes (Fig. 6C). Uncini saw-shaped 12-14 teeth with anterior peg flat, nearly triangular-shaped (Fig. 6D). Ventral ends of thoracic uncinigerous tori widely separated anteriorly, gradually approaching one another towards the end of thorax, thus leaving a triangular depression (Fig. 6A).

Abdomen: abdominal chaetae long (approximately of the same length as thoracic chaetae) throughout the abdomen, not becoming significantly longer posteriorly; true trumpet-shaped (Fig. 6F). Uncini saw-shaped throughout the abdomen, with 11–12 teeth per row, anterior pegs flat, nearly triangular (Fig. 6E)

Colour of preserved specimens: anterior end of thorax, distal ends of radioles, peduncle, and opercular endplate blue or white with blue specks (Fig. 5A–C).

Etymology. The species is named after Ludwig K. Schmarda, the author of *Pomatoceros tetraceros*.

Remarks

The holotype of *S. schmardai* sp. nov. has already been reported (along with the corresponding cyt *b* sequence) in Palero *et al.* (2020) as "*S. tetraceros sensu stricto* from NSW". Since the present study shows lack of obvious phenotypic differentiation between the sibling species, the description of morphological characters of *S. schmardai* sp. nov. follows that of *S. tetraceros* included above. Also, the reliable synonymy for the new species is problematic, as either of the two cryptic sympatric species could have been reported under the names *S. tetraceros*, *S. elaphus*, and *S. tricornis*. The only potentially important morphological
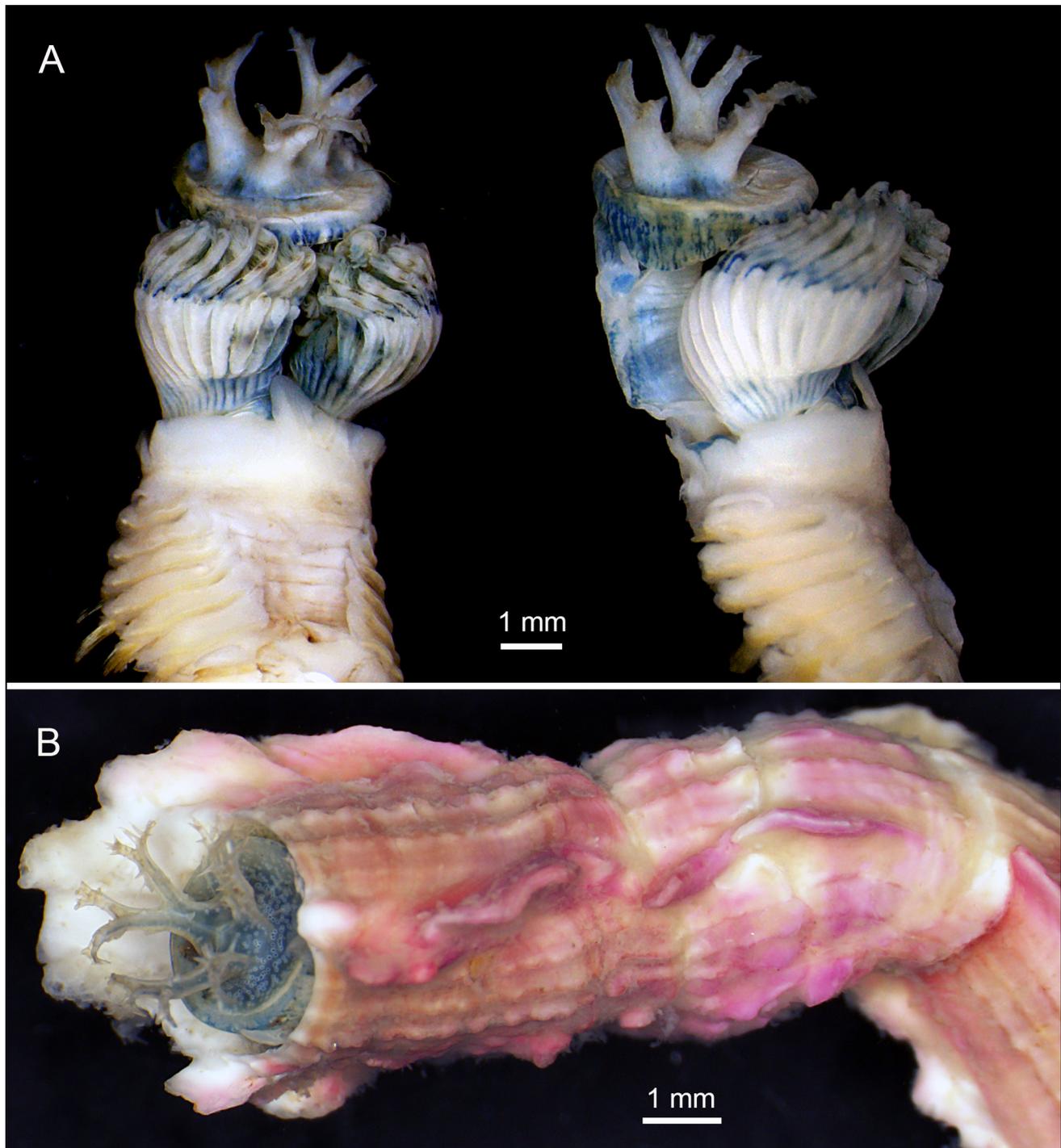
Figure 5. Photographs of *S. schmardai* sp. nov. holotype and paratype. (*A*) anterior part of the holotype W.42393, ventral view (left), lateral view (right); (*B*) paratype W.51857 in tube.

difference between the two species is the obvious lack of lappets between radioles in *S. tetraceros* and the presence of slight inter-radiolar thickenings in *S. schmardai* sp. nov. However, these subtle inter-radiolar characters have never been given consistent attention in previous descriptions and they might turn out to be common throughout the complex. Thus, following the example of Halt *et al.* (2009, for *Galeolaria caespitosa* Lamarck, 1818 and *G. geminoa* Halt *et al.*, 2009), we included the molecular diagnostic characters of *S. schmardai* sp. nov. (Table 3).

Discussion

Morphological species delimitation is notoriously difficult within the genus *Spirobranchus* because of their high intraspecific variability in opercular structures, traditionally considered the major taxonomic characters of the genus. Mainly because of this high opercular variation, ten Hove (1970) initially synonymized 22 nominal taxa under the name *S. tetraceros*. However, already 20 years later, mainly on biogeographic insights, ten Hove's synonymy was
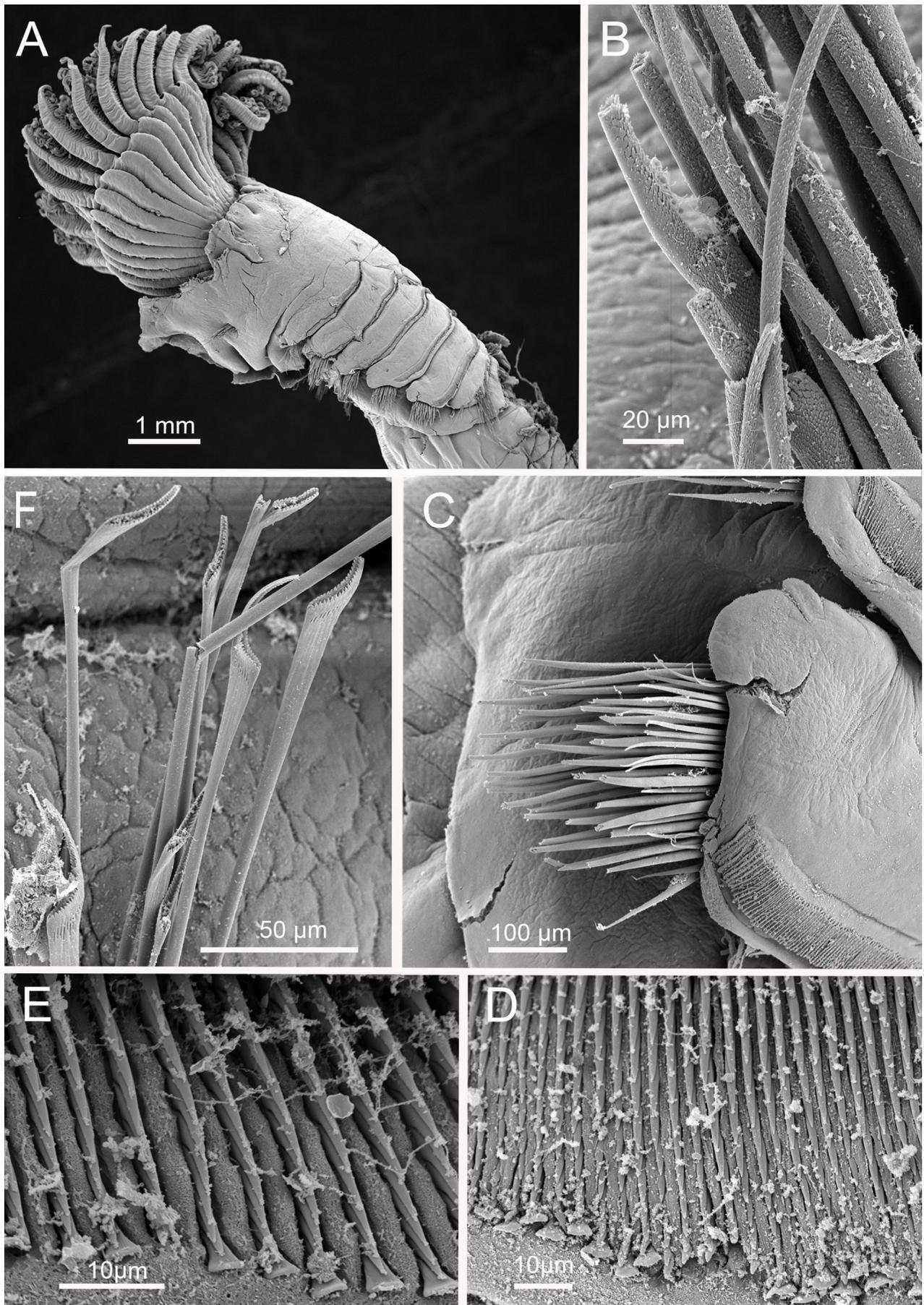
Figure 6. Scanning electron microscopic micrographs of *S. schmardai* sp. nov. paratype W.42393. (*A*) lateral view of the anterior part; (*B*) collar chaetae bundle with special *Spirobranchus*-type chaetae; (*C*) thoracic chaetae; (*D*) thoracic uncini; (*E*) anterior abdominal uncini; (*F*) anterior abdominal chaetae.

Table 3. The molecular diagnostic characters of *S. schmardai* sp. nov. in the following positions after primer in cyt *b*.

position	*S. tetraceros*	*S. schmardai* sp. nov.
45	G	A
48	G	C
65–67	TGC	GGT
81	A	G
87–93	GGGGTAC	AGGCTTT
102	A	G
108	A	T
112–120	CTTAATCGA	TTAAACCGG
125	A	T
132	C	T
144	T	C
147	A	G
151–154	GCAA	CTTT
157–165	GTGCCTGTG	ATTCCACTT
170–173	GTTT	TGGC
177–181	CCTAC	TTTAT
186–196	ACTTCATAGGC	CTTGCACAAAT
201	A	C
207–219	AAATCCATTAGGG	TAACCCGTTGGGT
228	A	T
231	T	C
234	T	C
244–246	GTA	ATT
252	C	T
255	T	C
258	T	G
263–266	CTAG	ACTC
271	G	A
280–282	GTG	ATT
286–291	GTCGTA	ATAATT
295–297	TCA	GTC
306–315	ATTGTCTATT	TGTTTTAGTG
324	A	T
330	T	C
334–337	CGTG	TACA
340–345	TTAGGG	CTTGGA
357–359	CTA	TTT
363–365	CCC	TCT
369–375	TAATCCT	AAACCCG
387	A	T

acknowledged as an oversimplification and ever since the name *S. tetraceros* has been regarded as a complex of species (e.g., Frank & ten Hove, 1992; ten Hove, 1994; Fiege & ten Hove, 1999; ten Hove & Kupriyanova, 2009; Ben-Eliahu & ten Hove, 2011; Perry *et al.*, 2018), in fact the taxon thus should be regarded to be a *species inquirenda*. Both genetic data in Palero *et al.* (2020) and the results of our study clearly support the morphology-based conclusion at the beginning of this discussion. Moreover, the molecular characterization of specimens from the Mediterranean by Palero *et al.* (2020), including specimens with either simple conical opercula or those with branching spines, confirms that this morphological variability is a result of intraspecific plasticity.

It is remarkable that although numerous studies worldwide have been using the name *Spirobranchus tetraceros* (reviewed in Perry *et al.*, 2018), this study is the first to describe and illustrate specimens collected from the type

locality of the original species. Schmarda (1861) collected the specimen (his entire diagnosis suggests a single specimen only) of what he described as *Pomatoceros tetraceros* during a voyage around the world (1853–1857). As type locality for this species, he only mentioned "New South Wales" without specifying any further. However, the only Australian localities mentioned in the introduction of his account of the voyage are Melbourne, Sydney, and the Blue Mountains. Moreover, all his other new Australian species described in the taxonomic part of the Schmarda (1861) monograph were from New South Wales. For eight of them the type locality was specified as "Port Jackson" (modern day Sydney) and one was from the "Coast of Illawarra" (just south of Sydney). Based on this information, we argue that Sydney was the type locality for his *Pomatoceros tetraceros*.

While we designated here a neotype for *S. tetraceros* based on topotypical material from Sydney, our phylogenetic analyses also provided compelling evidence for the existence of two sympatric well-supported clades within the *S. tetraceros sensu stricto* morphotype. These two clades show a mean interspecific p-distance of 36%, which actually exceeds those observed for the same cyt *b* fragment within the *Spirobranchus kraussii* complex (14.6–26.9%, see Nishi *et al.*, 2022) and other serpulid genera, such as, for example, *Ficopomatus* (19.2%, Styan *et al.*, 2017), *Galeolaria* (22.8–24.5%, Halt *et al.*, 2009), and *Hydroides* (15.8–23.1%, Sun *et al.*, 2016). Thus, although we detected no consistent morphological differences between the specimens of the two clades, we treated them as sympatric cryptic species and in addition to designating the neotype of *S. tetraceros,* we also described the sister species as *S. schmardai* sp. nov.

As expected on biogeographical grounds, specimens from NSW (Greater Sydney in this case) and tropical Queensland belong to different taxa and probably also to separate faunas. The clear difference between the northern tropical and southern temperate marine Australian faunas (Ekman, 1953; Briggs, 1974), with a transition zone on the East and West Coasts (Wilson & Allen, 1987) has been long recognized. Apparently, there is a strong historical and environmental influence in the composition of the Australian marine faunas. The northern tropical fauna is similar to a broad Indo-Pacific one that has developed in the Tertiary period, particularly with the emergence of coral reefs. The southern fauna in south-eastern Australia consists of a Palaeoaustral component that can be traced to the late Eocene–Mid Miocene (reviewed in Poore & O'Hara, 2007).

Moreover, the phylogenetic results of this study support this biogeography-based conclusion as the Australian species of the *S. tetraceros* complex do not form a monophyletic group. Instead, the species from tropical Queensland are shown to be more closely related to the Indo-Pacific *S. multicornis* and the Mediterranean introduced taxon of unknown origin from Palero *et al.* (2020). Unfortunately, our results do not provide unequivocal evidence whether the entire *S. tetraceros* complex constitutes a monophyletic group, so further phylogenetic studies of the complex are needed.

Based on the results of our study, the taxonomic status of S. *tetraceros* reported from Queensland (Kupriyanova *et al.*, 2015) and Western Australia (Johansson, 1918; Pillai, 2009) needs to be revised. The specimens from Queensland (Mackay, Gladstone, Burnet Heads, Pialba) reported as *Spirobranchus semperi* Mörch, 1863 by Straughan (1967: 246-247) likely belongs to either S. cf. *tetraceros* sp. B, S. cf. *tetraceros* sp. C of this study, or both. The type locality

of Mörch's species is the Philippines (unspecified further), so further studies are needed to determine the taxonomic status of the tropical Indo-Pacific populations of *S.* cf. *tetraceros* in Queensland and here no name is suggested for these specimens.

Here, however, we suggest resurrecting the older available name *S. multicornis* Grube, 1862 for the Red Sea population of *Spirobranchus* cf. *tetraceros* (examined in Perry *et al.* (2017, 2018)). The original description by Grube (1862) is very short: "*S. multicornis* n. sp. was brought in by Professor Ehrenberg from the Red Sea (see Fig. 3, p. 59). The opercular plate bearing a circle of six projections, of which only three were preserved, these are antler-like, every antler branched with multiple tines; the wide peduncle, with wings right and left, is inserted above the left radiolar lobe, the radioles in a simple circle" (translated by H. ten Hove).

Spirobranchus multicornis specimens associated with corals in the Red Sea (as *S.* cf. *tetraceros* in Perry *et al.*, 2017, fig. 3E; 2018, fig. 5A) have a circular opercular plate with three pairs of antler-like spines positioned in the middle of the plate and not arising from a common base; each spine is forked at the tip. Also, in *S. multicornis* the inter-radiolar membrane bears distinct lappets between radioles. In contrast, although the opercular structure is notoriously variable in this group, *S. tetraceros* and *S. schmardai* sp. nov. have opercular spines roughly arising from the common base and lack inter-radiolar lappets.

Specimens of another *Spirobranchus* cf. *tetraceros* recently discovered in the Mediterranean by Palero *et al.* (2020) are also characterized by the interradiolar membrane and anterior margin of peduncular wings bearing finger-like processes, thus are morphologically more similar to *S. multicornis* than to *S. tetraceros* or *S. schmardai* sp. nov. Genetic distance between the Red Sea (*S. multicornis*) specimens and Mediterranean *S.* cf. *tetraceros* is large enough to be considered as belonging to distinct taxa. Thus, the widely accepted hypothesis of Ben-Eliahu (1991) that *S.* cf. *tetraceros* is a Lessepsian migrant passively crossing the Suez Canal to the Mediterranean was not supported by Palero *et al.* (2020). The identity and origin of the introduced Mediterranean population will remain a mystery until the source population is found.

In summary, the results of this study call for a revision of the *Spirobranchus tetraceros* complex both in Australia and worldwide. In particular, it will help to determine the identity and origin of the introduced established population of *S.* cf. *tetraceros* from the western Mediterranean reported in Palero *et al.* (2020).

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