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Orbiniidae (Annelida: Errantia) from Lizard Island, Great Barrier Reef, Australia with notes on orbiniid phylogeny

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Abstract

The fauna of Orbiniidae (Annelida: Errantia) from the Lizard Island has been studied. Five species were found and each was redescribed and illustrated using light microscopy and SEM. *Scoloplos acutissimus* Hartmann-Schröder, 1991 and *Scoloplos dayi* Hartmann-Schröder, 1980 collected for the first time since their original descriptions and confirmed through re-examination of their type materials. Molecular analyses were carried out using nuclear 18S rDNA and mitochondrial 16S rDNA and CO1 gene sequences with evolutionary distances and the Neighbor-Joining Method. The molecular analyses did not support the monophyly of the genera *Scoloplos*, *Leitoscoloplos*, *Leodamas*, and *Naineris,* and its results are incongruent with morphological data.

Key words: *Scoloplos*, *Leitoscoloplos*, *Leodamas*, *Naineris*, 18S rDNA, 16S rDNA, CO1

Introduction

Species of Orbiniidae are moderate-sized elongate sediment-burrowing deposit feeders, inhabiting all depths from intertidal to abyssal. The main taxonomic revisions were done by Eisig (1914) and Hartman (1957). These publications redefined all genera and most of the common species, and Hartman (1957) arranged them in two subfamilies, Orbiniinae and Protoariciinae. Blake (2000) performed a cladistic analysis of the family based on morphological characters; the subfamily Protoariciinae was found to be an artificial group and consequently was synonymized with Orbiniinae, whereas the subfamilies Microorbiniidae and Methanoariciinae were erected. Subsequent molecular analyses (Bleidorn *et al.* 2005; Bleidorn *et al.* 2009) did not confirm this grouping and indicated that most of major orbiniid genera were paraphyletic.

Genera considered in present work—*Scoloplos* Blainville 1828, *Leitoscoloplos* Day, 1977, *Leodamas* Kinberg, 1866 and *Naineris* Blainville, 1828—belong to the subfamily Orbiniinae in both Hartman (1957) and Blake (2000) classifications. The main characteristic of *Naineris* is round to square shape of prostomium, which is pointed in other Orbiniinae genera. Three species of *Naineris* form a monophyletic sister group to *Protoaricia oerstedii* in molecular analysis by Bleidorn *et al.* (2009). Genus *Scoloplos* has four or less foot papillae plus stomach papillae in total, thoracic neuropodial hooks (also called spines or uncini) accompanied with capillary chaetae, and branchiae starting from chaetiger 8 or later. In the molecular analysis (Bleidorn *et al.* 2009) species of *Scoloplos* grouped with different clades of orbiniid phylogenetic tree. Genus *Leitoscoloplos* includes species that are generally similar to *Scoloplos*, but lack thoracic neuropodial hooks. The presence and number of thoracic hooks and hook-bearing segments may vary with size of the animal and some authors did not recognize this character as having generic significance (i.e., Pettibone 1957; Zhadan 1998; see Mackie 1987 for details of the history). Molecular analysis (Bleidorn *et al.* 2009) revealed that within Orbiniidae neuropodial hooks evolved independently or been lost few times. Diagnosis of *Leitoscoloplos* was emended by Eibye-Jacobsen (2002), who described *L. papillatus* with up to seven subpodal papillae. Recently species of *Leitoscoloplos* with up to 8 subpodal papillae

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and up to 14 stomach papillae has been described (Hernández-Alcántara & Solís-Weiss 2014). Genus *Leodamas* has been traditionally regarded as a subgenus of *Scoloplos* (Hartman 1957; Pettibone 1957; Day 1973, 1977; Eibye-Jacobsen 2002). Blake (2000) elevated *Leodamas* to the generic level; it was accepted by Bleidon *et al.* (2009) and we also considered *Leodamas* as a full genus in the present work. *Leodamas* differs from *Scoloplos* mainly by having numerous hooks in thoracic neuropodia accompanied with few or no capillary chaetae, single thick projecting aciculae in abdominal neuropodia, and branchiae starting before chaetiger 7. In phylogenetic tree (Bleidorn *et al.* 2009) species of *Leodamas* forms two separate clades.

Australian orbiniids were studied by Day (1977), Hartmann-Schröder (1979, 1980, 1991), Mackie (1987: *Leitoscoloplos*), Hutchings & Rainer (1979), Hutchings & Murray (1984), and Glasby (2000). There are 17 genera and about 150 species of Orbiniidae worldwide, and 27 species belonging to 9 genera are known in Australian waters (http://www.ala.org.au). The orbiniid fauna of Lizard Island has not been studied previously.

Material and methods

Samples were collected from the intertidal and upper subtidal zones in the vicinity of Lizard Island Research Station during the Polychaete workshop in August 2013. Locality data for material examined during the Lizard Island Polychaete Workshop (MI QLD xxxx) are provided in the Table of Ribas & Hutchings (2015, *Zootaxa* 4019) together with figures showing sampling sites. Living animals were photographed using a Canon 5d Mark 2 DSLR camera with a Canon MP-E 65 mm f2.8 (1-5x) Macro lens, and with two strobes fired from both sides. Specimens were preserved in 4% formaldehyde solution buffered with sea water, and after washing in fresh water were transferred to 80% alcohol. Some tissues were preserved in 96% alcohol for molecular studies. Preserved specimens were studied using a stereomicroscope; glycerol mounts of parapodia were studied with a compound microscope.

All microscope photographs were taken with a mounted Leica digital camera. Aqueous solution of methylene blue was used to add contrast to external structures, such as branchiae, papillae, parapodia, and to highlight segmental borders. All specimens collected at Lizard Island are registered in Australian Museum (AM) collections with numbers prefixed by "AM W." Also collections of Zoologisches Institut und Zoologisches Museum der Universität, Hamburg, Germany (HZM) were used with numbers prefixed by "P-". Number of specimens under each registration number is one unless otherwise specified.

TABLE 1. PCR primers used for amplification and sequencing.

Gene fragment	Primer	Sequence (5'-3')	Reference
16S	ArL	CGCCTGTTTATCAAAAACAT	Palumbi *et al.* 1991
	BrH	CCGGTCTGACTCAGATCACGT	Palumbi *et al.* 1991
	AnnF	GCGGTATCCTGACCGTRCWAAGGTA	Sjölin *et al.* 2005
	AnnR	TCCTAAGCCAACATCGAGGTGCCAA	Sjölin *et al.* 2005
CO1	polyLCO(F)	GAYTATWTTCAACAAATCATAAAG	Carr *et al.* 2011
	polyHCO(R)	TAMACTTCWGGGTGACCAAARAATCA	Carr *et al.* 2011
18S	1F	TACCTGGTTGATCCTGCCAGTAG	Giribet *et al.* 1996
	5R	CTTGGCAAATGCTTTCGC	Giribet *et al.* 1996
	3F	GTTCGATTCCGGAGAGGGA	Giribet *et al.* 1996
	*18S*bi	GAGTCTCGTTCGTTATCGGA	Giribet *et al.* 1999
	*18S*a2.0	ATGGTTGCAAAGCTGAAAC	Giribet *et al.* 1999
	9R	GATCCTTCCGCAGGTTCACCTAC	

DNA extraction, amplification, and sequencing

We used the Promega Wizard SV Genomic DNA Purification Kit and protocol (Promega Corporation, Madison, USA) for tissue lysis and DNA purification. Polymerase chain reaction (PCR) amplification of nuclear 18S rDNA, mitochondrial 16S rDNA and CO1 gene fragments was accomplished with the primers given in Table 1. The 18S rDNA gene was PCR amplified in three overlapping fragments of about 950, 900, and 850 bp each, using primer pairs 1F-5R, 3F-18Sbi and 18Sa2.0-9R, respectively (Table 1).

The universal primers 16Sar-L and 16Sbr-H did not work well. We therefore used the primer pair 16SAnnF and 16SAnnR to amplify these loci. The universal barcoding primers HC02198 and LCO1490 did not work either. We therefore used the primer pair designed especially by N.Ivanova (Carr, 2010) for polychaetes polyLCO and polyHCO (Table 1). All loci were amplified using the Encyclo PCR kit (Evrogen Joint Stock Company, Russia). We amplified a 25 µl reaction mix containing 1 x PCR buffer, 1 µl of 10 µM of primer pair mix, 1 µl of template, 0.2 mM of each dNTPs and 0.5 units of Taq polymerase. Reaction mixtures were heated on Veriti® Thermal Cycler to 94°C for 300 s, followed by 35 cycles of 15 s at 94°C, 30 s at a specific annealing temperature, and 45–60 s at 72°C, depending on the length of fragment, and then a final extension of 7 min at 72°C. Annealing temperature was set to 49°C for the 18S primer pairs 1F-5R and 18Sa2.0-9R, 52°C for the 18S primer pair 3F-18Sbi, 60°C for the 16S primer pair 16SAnnF and 16SAnnR, and 45°C for CO1 primer pair polyLCO and polyHCO. We used the Promega PCR Purification Kit and protocol (Promega) to purify our amplification products, which were sequenced in both directions. Each sequencing reaction mixture included 1 µl of BigDye (Applied Biosystems, Perkin-Elmer Corporation, Foster City, CA), 1 µl of 1 µM primer and 1 µl of DNA template, and was processed for 40 cycles of 96°C (15 s), 50°C (30 s), and 60°C (4 min). Sequences were purified by ethanol precipitation to remove unincorporated primers and dyes. Products were re-suspended in 12 µl formamide and electrophoresed in an ABI Prism 3500 sequencer (Applied Biosystems). GenBank accession numbers of sequences obtained in the present study are given in Table 2.

TABLE 2. Taxa included in phylogenetic analyses, voucher numbers, and GenBank accession numbers. **Bold**: species investigated in present work. Vouchers are deposited in Australian Museum, Sydney, Australia (W.xxxx), and Zoological Museum, Moscow State University, Moscow, Russia (WSxxxx).

Taxa	Authority	Voucher/ origin	Accession no.		
			16S	18S	CO1
Leitoscoloplos bifurcatus	(Hartman, 1957)	W.44938.001	KR349351	KR778793	KR781456
Leitoscoloplos fragilis	(Verrill, 1873)	GenBank	AY532341	AY532360	FJ612498
Leitoscoloplos pugettensis	(Pettibone, 1957)	GenBank	–	–	HM473437–HM473442
		GenBank	–	–	HM473769–HM473774
		GenBank	FJ612454	FJ612482	FJ612501
		GenBank	AY532342	AY532365	FJ612502
Leitoscoloplos robustus	(Verrill, 1873)	GenBank	FJ612455	–	FJ612499
		GenBank	FJ612456	FJ612480	FJ612500
		GenBank	FJ612457	–	–
Leitoscoloplos sp.		GenBank	–	–	HQ024063–HQ024066
Leodamas dubia	(Tebble, 1955)	W.45479.001	KR349348	KR778795	–
		W.45480.001	KR349347	KR778794	–
Leodamas johnstonei	(Day, 1934)	GenBank	AY532332	AF508126	–
Leodamas rubra	(Webster, 1879)	GenBank	FJ612460	FJ612478	FJ612497
Leodamas tribulosus	(Ehlers, 1897)	GenBank	FJ612458	FJ612476	FJ612496
		GenBank	FJ612459	FJ612477	–
		GenBank	FJ612467	–	–
Methanoaricia dendrobranchiata	Blake, 2000	GenBank	AY532333	AY532357	FJ612503

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TABLE 2. (Continued)

Taxa	Authority	Voucher/ origin	Accession no.		
			16S	18S	CO1
Naineris dendritica	(Kinberg, 1866)	GenBank	–	–	HM473473– HM473483
		GenBank	FJ612462	–	FJ612504
		GenBank	AY532345	AY532358	–
		GenBank	–	–	HM473486
Naineris grubei australis	Hartman, 1957	W.44763.001	KR920029	KR920031	KR920026
Naineris laevigata	(Grube, 1855)	GenBank	FJ612463	–	–
		GenBank	–	–	GU362690
Naineris quadricuspida	(Fabricius, 1780)	GenBank	–	–	FJ612506
		GenBank	AY532346	AY532361	–
		GenBank	–	–	KF815723
		GenBank	FJ612464	FJ612484	FJ612505
		WS0010	–	–	GU672621
		WS0033	–	–	GU670786
		WS0061	–	–	GU670784
Orbinia bioreti	(Fauvel, 1919)	GenBank	AY532334	AF448158	FJ612507
Orbinia cornidei	(Rioja, 1934)	GenBank	–	KC460270	–
Orbinia latreillii	(Audouin & Milne Edwards, 1833)	GenBank	AY961084	–	AY961084
		GenBank	AY532335	AY532355	
		GenBank	NC007933		NC007933
Orbinia swani	Pettibone, 1957	GenBank	–	DQ790087	–
		GenBank	AY532336	AY532363	FJ612509
Orbiniella plumisetosa	Buzhinskaya, 1993	GenBank	AY532348	AY532364	FJ612508
Pettibonella multiuncinata	Solis-Weiss & Fauchald, 1989	GenBank	AY532339	AY532359	FJ612510
Phylo felix	Kinberg, 1866	GenBank			AY583703
Phylo foetida	(Claparède, 1869)	GenBank	AY532337	AY532356	FJ612511
		GenBank	FJ612465	FJ612485	FJ612512
Phylo michaelseni	(Ehlers, 1897)	GenBank	AY532338	AY532362	FJ612513
Phylo norvegicus	(Sars, 1872)	GenBank	FJ612466	AY612619	FJ612514
Proscoloplos cygnochaetus	Day, 1954	GenBank	AY532340	AF448162	FJ612515
Protoaricia oerstedii	(Claparède, 1864)	GenBank	AY532347	AF508123	FJ612516
Protoariciella uncinata	Hartmann-Schröder, 1962	GenBank	–	AF508124	–
Scoloplos acmeceps	Chamberlin, 1919	GenBank	FJ612468	FJ612488	FJ612518
		GenBank	AY532344	AY532366	–
		GenBank	FJ612470	FJ612490	FJ612520
		GenBank	FJ612469	FJ612489	FJ612519
Scoloplos acutissimus	Hartmann-Schröder, 1991	GenBank	KR920027	–	KR920024
		W.44175.001	KR920028	KR920030	KR920025
Scoloplos acutus	(Verrill, 1873)	GenBank	–	–	HQ024223– HQ024230

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TABLE 2. (Continued)

Taxa	Authority	Voucher/ origin	Accession no.		
			16S	18S	CO1
		GenBank	–	–	GU670791– GU670792
		GenBank	–	–	GU670794
		GenBank	–	–	GU672579
Scoloplos* cf. *armiger intertidal clade	(Müller, 1776)	GenBank	AY532343	AY532367	–
		GenBank	FJ612471	FJ612491	–
		WS0018	–	–	GU670806
		WS0023	–	–	GU670811
		WS0025	–	–	GU670808
		WS0027	–	–	GU670807
		WS0028	–	–	GU670805
		WS0029	–	–	GU670802
		WS0030	–	–	GU670804
		WS0031	–	–	GU670803
		WS0034	–	–	GU670801
		WS0046	–	–	GU670799
		WS0048	–	–	GU670800
		WS0049	–	–	GU670797
		WS0050	–	–	GU670798
		WS0057	–	–	GU670795
		WS0058	–	–	GU670793
		WS0060	–	–	GU670796
		WS0065	–	–	GU672619
Scoloplos cf. *armiger* Malibu clade	(Müller, 1776)	GenBank	FJ612461		FJ612517
Scoloplos cf. *armiger* subtidal clade	(Müller, 1776)	GenBank	FJ612472	FJ612492	FJ612521
			–	–	DQ517436
		WS0019	–	–	GU670809
Scoloplos cf. *armiger* type locality clade	(Müller, 1776)	GenBank	AY340480	AY612493	–
		GenBank	FJ612473	FJ612493	–
Scoloplos dayi	Hartmann-Schröder, 1980	W.44764.001	KR349352	KR778796	KR781457
		W.45476.001	KR349350	KR778797	KR781458
		W.45477.001	KR778798	KR349349	KR781459
Scoloplos normalis	(Day, 1977)	GenBank	FJ612474	FJ612494	FJ612522
Outgroups					
Lumbricus terrestris	Linnaeus, 1758	GenBank	HE611667	–	–
		GenBank	JN869821	–	–
		GenBank	HQ691206	–	–
		GenBank	–	HQ691211	–
		GenBank	–	AJ272183	–

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TABLE 2. (Continued)

Taxa	Authority	Voucher/ origin	Accession no.		
			16S	18S	CO1
		GenBank	–		HQ024607
		GenBank	–		JQ909131
Ophelina acuminata	Örsted, 1843	GenBank	–	KF511828	–
		GenBank	–	HM746735	–
		GenBank	–	KF511825	–
		GenBank	–	KF511827	–
		GenBank	–	KF511826	–
		GenBank	–		KC164693
Platynereis dumerilii	(Audouin & Milne Edwards, 1834)	GenBank	EU221666	–	–
		GenBank	–	EF117897	–
		GenBank	–	AY894303	–
		GenBank	–	–	KF815726
Stygocapitella subterranea	Knöllner, 1934	GenBank	FJ612453	–	–

Data analysis

We used 23 of our own sequences (Table 2) and 171 sequences of Orbiniidae from GenBank (Table 2). Multiple alignments were performed using ClustalW algorithm (Wang & Jiang 1994). For the phylogenetic analysis, all the aligned sequences were trimmed according to the shortest sequence.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site.

The analysis involved 146 CO1, 58 18S and 72 16S gene fragments sequences (Table 2). All ambiguous positions were removed for each sequence pair. Evolutionary analyses (Neighbor-Joining) were conducted in MEGA6 (Tamura *et al*. 2013). The trees were rooted with the species *Lumbricus terrestris* Linnaeus, 1758, *Ophelina acuminata* Örsted, 1843, *Platynereis dumerilii* (Audouin & Milne Edwards, 1834), and *Stygocapitella subterranea* Knöllner, 1934.

Results

Taxonomic account

Genus *Scoloplos* Blainville, 1828

Scoloplos Blainville, 1828: 493.
Scoloplos (*Scoloplos).*—Hartman 1957: 280; Pettibone 1957: 160; Day 1973: 84; Mackie 1987: 20.

Type-species. *Lumbricus armiger* O. F. Müller, 1776: 215; by original designation.

Diagnosis. Prostomium pointed, conical; single achaetous peristomial ring. Thoracic neuropodia bearing crenulated capillaries and hooks arranged in one or more rows; abdominal furcate and flailed notochaetae present or absent. Abdominal neuropodia lack robust emergent aciculae. Branchiae simple or branched, from chaetiger 8 or later. 1–2 thoracic neuropodial podal papillae, 0–2 thoracic subpodal papillae, 0–4 abdominal subpodal papillae, stomach papillae absent.

Scoloplos acutissimus Hartmann-Schröder, 1991

(Figs 1, 2, 12A)

Scoloplos acutissimus Hartmann-Schröder, 1991: 48–49, figs 73–80.

Type material. Holotype: ZMH P–20562 (photographed).

Other material examined. AM W.44175, MI QLD 2366 (photographed); AM W.44175.001, tissue for molecular study; MI QLD 2340, unregistered posterior end used for molecular analysis; AM W.44248, MI QLD 2373; AM W.46090, MI QLD 2376 (3, 1 photographed); AM W.46091, MI QLD 2378 (6); AM W.46092, MI QLD 2422 (12); AM W.46096, MI QLD 2429 (20, 1 photographed using SEM); AM W.46097, MI QLD 2432 (3); AM W.44940, MI QLD 2433 (6); AM W.44942, MI QLD 2439 (3, 1 photographed).

Examination of type material. Holotype incomplete, thoracic width 0.8 mm, with flattened thorax. Eighteen thoracic chaetigers on right side and 19 thoracic chaetigers on left side. Branchiae starting from chaetiger 17. Three or four rows of hooks and one posterior row of capillary chaetae in thoracic neuropodia; thoracic neuropodia with one podal papilla, no subpodal papillae. Anterior abdominal neuropodia with very big subpodal flange and developed subpodal notch, well developed long subequal outer and inner lobes. Notopodial lobes narrow, digitiform. Branchiae longer than notopodia. In posterior abdominal segments neuropodia with shorter lobes, inner lobes round and wider than outer.

Description. (Lizard Island material) Body long, slender; thorax slightly flattened, abdomen cylindrical (Figs 1A, 2A). Colour in life pale yellowish-brown with red blood vessels and yellow gut content (Fig. 12 A). Thoracic width up to 0.9 mm. Prostomium sharply conical with drawn out tapering tip (Fig. 2B). Peristomium with pair of dorso-lateral nuchal organs (Fig. 2B). Thoracic chaetigers numbering 14–20 (usually 17–19) (Table 3). Branchiae starting from penultimate thoracic chaetiger (Fig. 1A, Table 3), usually chaetiger 16–18 (13 in smallest specimen). First branchiae small and digitiform; becoming larger and triangular in anterior abdomen; then long, strap-like, markedly wider and longer than notopodia, in middle and posterior abdomen (Figs 1G, H, 2I). Thoracic postchaetal lobes well developed from chaetiger 1 (neuropodia) or 2 (notopodia) (Figs 1D, E, 2B). Notopodial lobes short and papilliform in anterior thorax; gradually increasing in length, becoming digitiform, as long as branchiae in posterior thorax (Figs 1A–C, E–G, 2C, D, F). Lateral organs below notopodia of all segments well developed (Fig. 2B, D). Thoracic neuropodial postchaetal lobes round papilliform, in posterior thorax becoming elongated and arising from low ridge, more developed below papilla (mammiform shape) (Figs 1C, E–G, 2C, D, F). No subpodal or stomach papillae. Abdominal notopodial lobes narrow, lanceolate, shorter than branchiae (Figs 1H, 2I). Abdominal neuropodia supported one thin acicula and bilobed with subequal lobes; inner lobe rounded, slightly longer and thicker than outer one (Figs 1H, I, 2C, I). Parapodial flange well developed, with deep notch and round upper margin without flange papilla (Fig. 1H, I). Ciliated dorsal organs with two short ciliated strips present middorsally (Fig. 2I, J). Thoracic notopodia bearing only crenulate capillary chaetae; neuropodia with 3–4 anterior rows of hooks and one posterior row of capillaries, neuropodial lobe located on same level as capillary chaetae in middle of row (Figs 1C, E–G, 2B–G); hooks in anterior chaetigers slightly curved, serrated with 4 denticles; in posterior thoracic chaetigers hooks almost straight, smooth, hooded, very short in anterior row; in one or two last thoracic chaetigers hooks replaced by capillary chaetae (Figs 1C, E–G, 2B–G). In abdomen both rami bearing thin capillaries, besides forked chaetae present in notopodia (Fig. 2H) and flail chaetae in neuropodia (Fig. 1H, J). Pygidium with two long anal cirri (Fig. 2K).

Remarks. *Scoloplos acutissimus* was described by Hartmann-Schröder (1991) from Gladstone, Queensland and has not been recorded since this study. Re-examination of holotype revealed higher number of thoracic chaetigers (18/19 vs 17) than originally reported. Lizard Island material had up to 20 thoracic chaetigers (Table 3). The newly collected specimens correspond well with the original description and the type material which was examined. The variability in the number of thoracic chaetigers and in the location of the first pair of branchiae was

investigated for 26 specimens (Table 3). The pygidium and anal cirri are described for the first time.

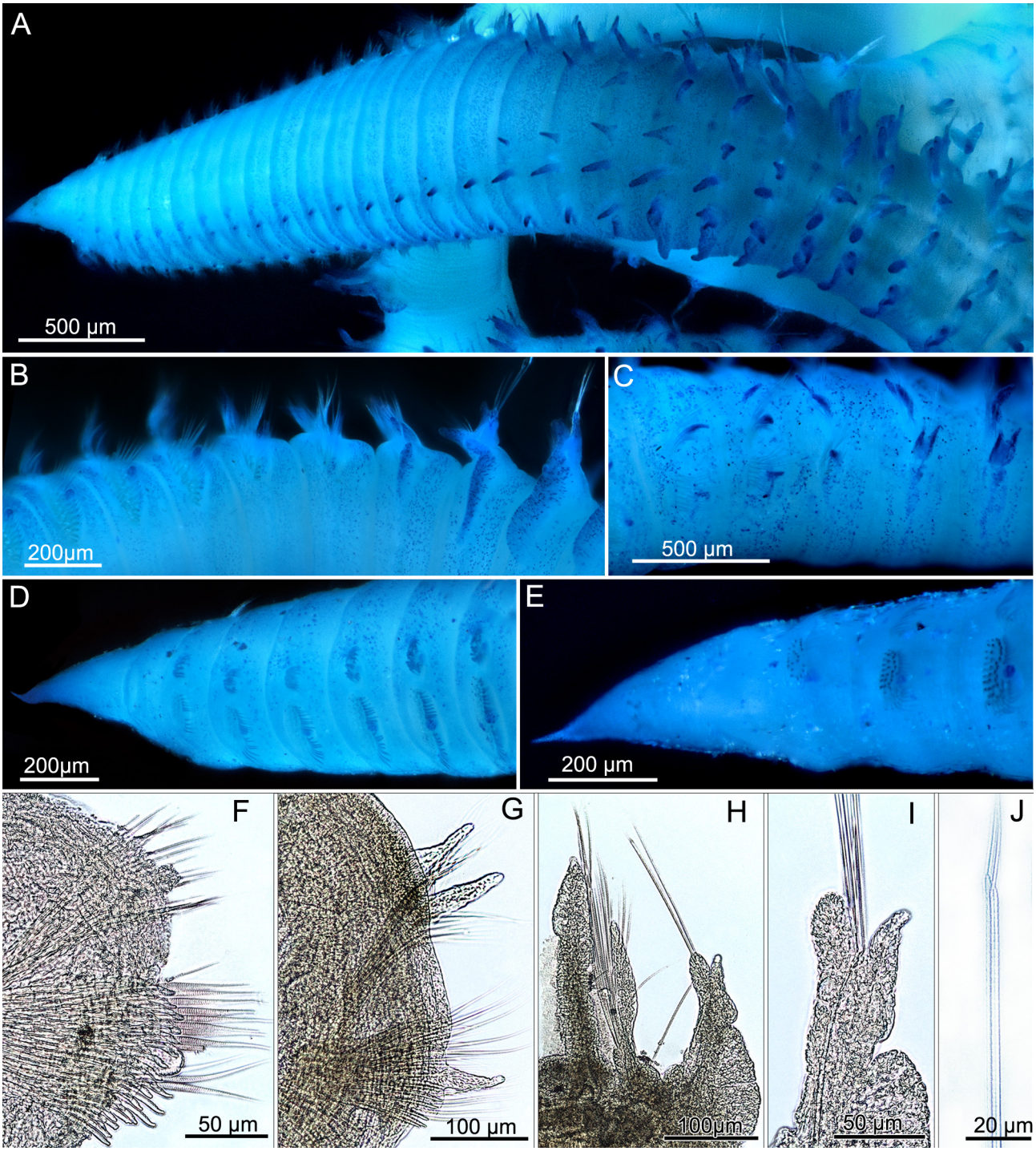

FIGURE 1. *Scoloplos acutissimus*, A–E: methylene blue staining. A–B: AM W.46090. A. Anterior end, left lateral view; B. Thorax-abdomen transition; C–D: AM W.44175. C. Thorax-abdomen transition; D. Anterior end, close-up, left lateral view; E. AM W.44942, anterior end, left lateral view. F–J: AM W.44175, glycerol mounts of parapodia. F. Parapodia of chaetiger 4; G. Parapodia of chaetiger 18; H. Abdominal parapodia; I. Abdominal neuropodia; J. Flail chaetae of abdominal neuropodia.

Type locality. Gladstone, Queensland.

Distribution. Gladstone, Lizard Island, Queensland.

Molecular analyses. The analysis of the sequence data for the 18S rRNA, 16S rRNA and CO1 gene has shown (with a good support for 18S and 16S) that all trees include the clade that contains *Scoloplos armiger, S.*

acmeceps, and *Leitoscoloplos pugettensis* (Figs 13–15). In CO1 analysis this clade also included *S. acutus.* In the CO1 and 16S analyses *Scoloplos acutissimus* joined the *Scoloplos armiger-S. acmeceps-Leitoscoloplos pugettensis* clade (Figs 14, 15)**.** However the analysis of the sequence data for 18S rRNA gene (Fig. 13) showed that *Scoloplos acutissimus* was not included in this clade, but with low support it was likely in the same clade as representatives of the genera *Nainereis*, *Orbinia*, *Leodamas*, *Phylo* and others instead; the same clade contains *Scoloplos dayi*, *S. normalis*, *Leitoscoloplos robustus* and *L. fragililis*.

TABLE 3. Variability in the number of thoracic chaetigers and first segment bearing branchiae in *Scoloplos acutissimus* from AM W.46096 and AM W.44940.

Registration number #	Number of thoracic chaetigers	Branchiae start from chaetiger number
AM W.46096	19	17
AM W.46096	19	16
AM W.46096	19	17
AM W.46096	18	17
AM W.46096	19	17
AM W.46096	17	15
AM W.46096	20	18
AM W.46096	19	18
AM W.46096	18	16
AM W.46096	18	16
AM W.46096	18	16
AM W.46096	18	16
AM W.46096	17	15
AM W.46096	19	17
AM W.46096	19	17
AM W.46096	17	15
AM W.46096	18	16
AM W.46096	17	15
AM W.46096	18	16
AM W.46096	18	16
AM W.44940	18	16
AM W.44940	17	15
AM W.44940	17	15
AM W.44940	18	16
AM W.44940	14	13
AM W.44940	17	15

Scoloplos dayi Hartmann-Schröder, 1980
(Figs 2, 3, 12B)

Scoloplos dayi Hartmann-Schröder, 1980: 67–68, figs 74–68.

Type material. Holotype: HZM P–16373 (photographed). Paratype: HZM P–16374 (photographed).

Other material examined. AM W.44249, MI QLD 2376 (2); AM W.45476, MI QLD 2440; AM W.45476.001, tissue for molecular studies; AM W.46093, MI QLD 2422 (3, 1 photographed on SEM); AM W.46095, MI QLD 2429 (9); AM W.44764, MI QLD 2429 (photographed); AM W.44764.001, tissue for molecular studies; AM

W.45477, MI QLD 2376 (photographed); AM W.45477.001, tissue for molecular studies.

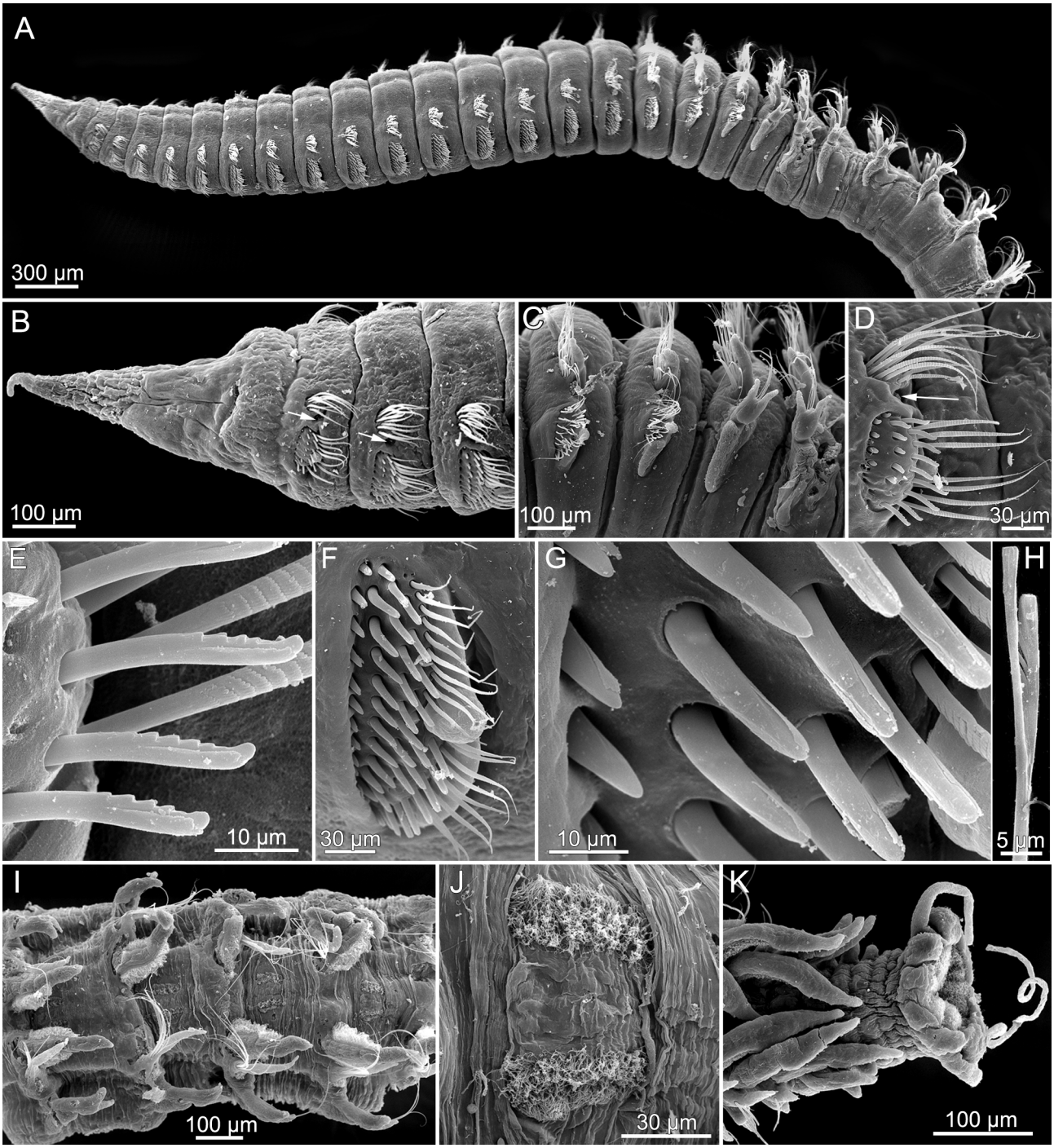

FIGURE 2. *Scoloplos acutissimus*, AM W.46096, SEM images. A. Anterior end, left lateral view; B. Same, close-up, arrows indicate lateral organs; C. Thorax-abdomen transition; D. Parapodia of chaetiger 1; E. Neuropodia hooks of chaetiger 1; F. Parapodia of chaetiger 11; G. Neuropodial hooks of chaetiger 11; H. Forked chaeta of abdominal segment; I. Middle part of abdomen, dorsal view; J. Dorsal organ; K. Posterior end of abdomen with pygidium.

Examination of type material. Holotype: complete specimen, with cylindrical body, 20 thoracic chaetigers. Branchiae from chaetiger 8, as minute papillae. Flange papillae present in all abdominal segments. Prostomium conical, acute. Bilobed neuropodia from chaetiger 14–16. Hooks very short and inconspicuous, only detectable after examination under compound microscope. Pygidium with 2 short cirri, attached ventrally. Paratype: anterior fragment with 25 thoracic chaetigers. Branchiae from chaetiger 8, well developed from first pair. Bilobed

neuropodia from chaetiger 11. Hooks easily observed. Flange papillae present in all abdominal segments.

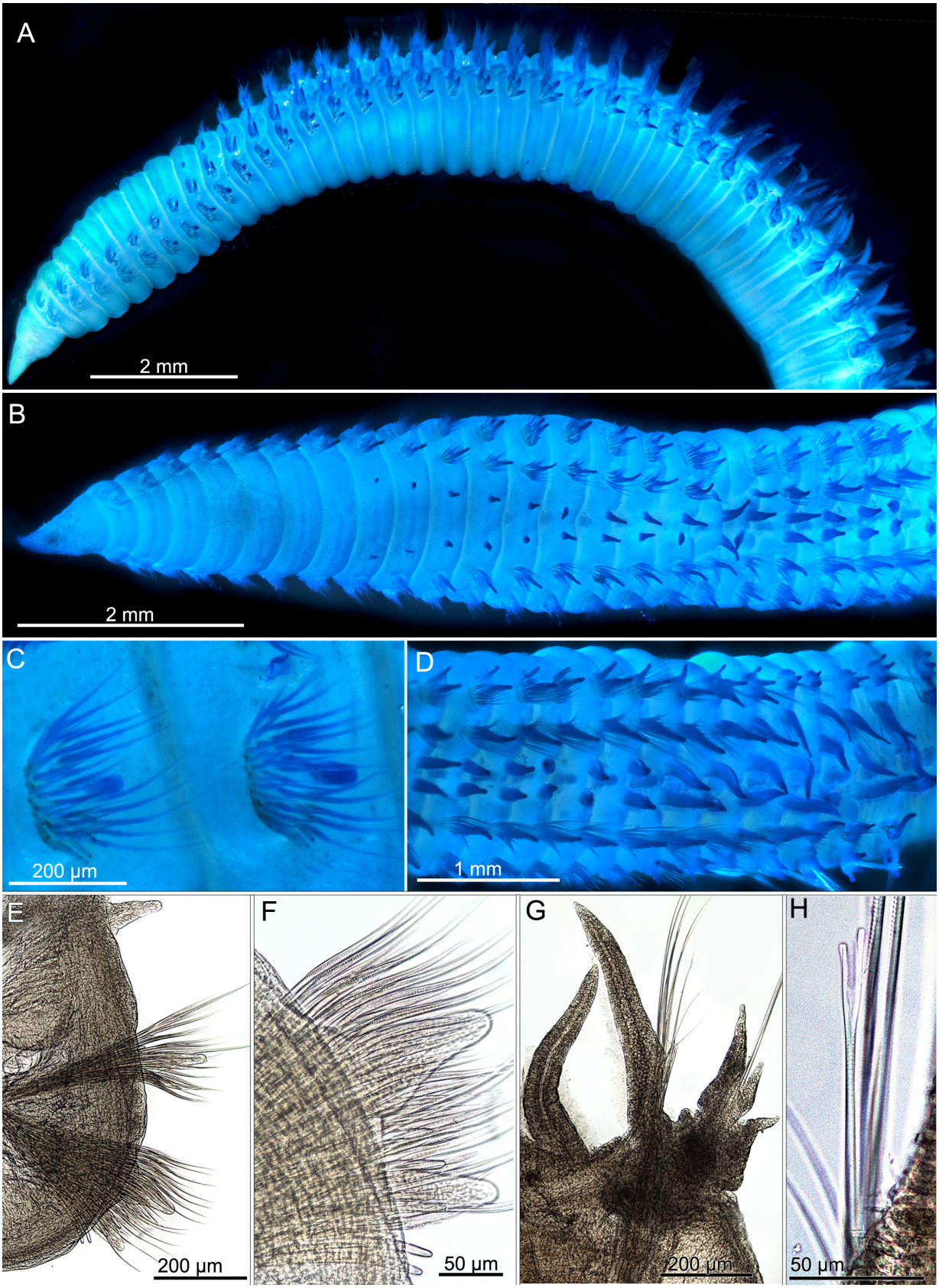

FIGURE 3. *Scoloplos dayi*, A–D: methylene blue staining. A–C: AM W.45477. A. Anterior end, left lateral view; B. Anterior end, dorsal view; C. Neuropodia of segments 3 and 4; D. AM W.44764, thorax-abdomen transition, dorsal view; E–H: AM W.45477, glycerol mounts of parapodia. E. Chaetiger 13; F. Close-up of neuropodia of chaetiger 13; G. Abdominal chaetiger;

H. Forked chaetae from abdominal neuropodia.

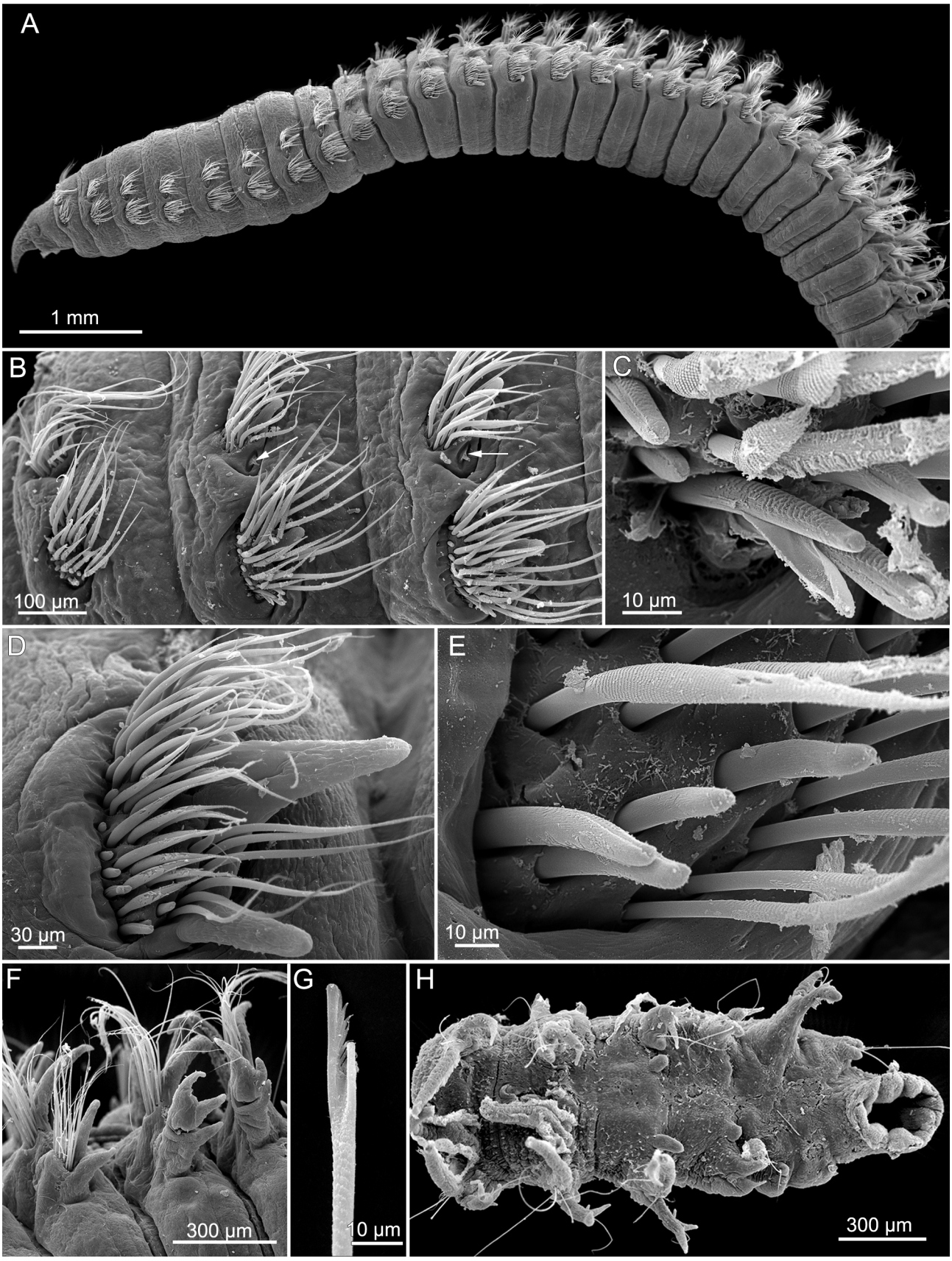

FIGURE 4. *Scoloplos dayi*, AM W.46093, SEM images. A. Anterior end, left lateral view; B. Parapodia of chaetigers 1–3, arrows indicate lateral organs; C. Neuropodia hooks of chaetiger 2; D. Neuropodia of chaetiger 12; E. Neuropodial hooks of chaetiger 18; F. Thorax-abdomen transition; G. Forked chaetae from abdominal neuropodia; H. Posterior end with pygidium,

anal cirri are broken.

Description. Body cylindrical; anterior thorax often swollen, posterior thorax slightly wider than abdomen (Figs 3A, 4A). Colour in life orange-brown with red blood vessels, ventral part of abdomen grey (Fig. 12B). Thoracic width up to 2.2 mm. Prostomium sharply conical. Peristomium with a pair of dorso-lateral nuchal organs. Thoracic chaetigers numbering 21–26 (usually 24–25). Branchiae starting from chaetiger 8 (rarely 9–11) as minute papillae, gradually increasing in size; in chaetiger 17–20 becoming large, triangular with tapered tips (Fig. 3B, D); in abdomen long narrow triangular, as long as notopodia or slightly longer (Fig. 3D, G). Thoracic post-chaetal lobes present from chaetiger 1, first neuropodial lobe very small; both notopodial and neuropodial lobes gradually increasing in size, becoming digitiform; in anterior thorax similar in size, in posterior thorax notopodial lobes longer (Figs 3A, B, 4B). Lateral organs developed at base of notopodia (Fig. 4B). Neuropodial lobes becoming bilobed from chaetiger 10–12, in some specimens 13–14 (Figs 3A, B, F, 4D). No subpodal or stomach papillae. Abdominal notopodial lobes narrowly foliaceous, with slightly swollen basal part (Fig. 3D, G). Abdominal neuropodial lobes supported by two thin aciculae and bilobed; with elongate triangular lobes, inner lobe 2–3 times longer than outer (Figs 3D, G, 4F). Subpodal flange well developed, upper edge forming flange papilla (ventral cirrus) in all abdominal segments (Figs 3D, G, 4F). Low interramal papilla present between rami in some specimens (Fig. 3G). Ciliated dorsal organs with two curved ciliated strips present mid-dorsally in each segment. Thoracic notopodia bearing only crenulated capillary chaetae; all thoracic neuropodia with an anterior J-shaped row of slightly curved hooded hooks and 3–4 rows of crenulated capillary chaetae (Figs 3C, E, F, 4B–E); hooks smooth or slightly serrated (Fig. 4C, E). Crenulated capillary chaetae accompanied by forked chaetae in abdominal notopodia (Figs 3H, 4G) and flail chaetae in abdominal neuropodia. Pygidium with two anal cirri (Fig. 4H).

Remarks. *Scoloplos dayi* was described in 1980 from Northwest coast of Australia (Exmouth: Town Beach) and this represents the first record since then. Specimens from Lizard Island agree with the original description and type material examined, although some variability occurred in the number of thoracic chaetigers (21–26 instead of 20–24 in original description), chaetiger where branchiae begin (8–11 vs 8–9), and chaetiger where bilobed neuropodia start (10–14 vs 14–15). *Scoloplos dayi* is very similar to *Leitoscoloplos bifurcatus* (Hartman, 1957), which co-occurs with this species in our samples from Lizard Island. The only differences between two species are the presence of thoracic hooks (sometimes very short and inconspicuous) and flange papillae in *S. dayi*. This species also has more thoracic chaetigers in general, but this character overlaps with *L. bifurcatus*.

Type locality. Northwest coast of Australia (Exmouth: Town Beach, Western Australia).

Distribution. Northwest coast of Australia, Lizard Island, Queensland.

Molecular analyses. According to data obtained for three specimens (Figs 13–15), *S. dayi* does not belong to the *Scoloplos armiger* - *S. acmeceps* - *Leitoscoloplos pugettensis* clade. The analysis showed that one of the three studied specimens genetically differed from the other two. The genetic distance between these specimens was 1.7% for 18S sequence fragments and 6.2% for both 16S and CO1 fragments. Two other specimens did not differ genetically and had no substitutions in any of the studied sequences. These results may be indicative of an unrecognized complex of cryptic sympatric species.

Genus *Leitoscoloplos* Day, 1977

Leitoscoloplos Day, 1977: 218, fig. 1a–g.
Leitoscoloplos.—Mackie 1987: 2; Eibye-Jacobsen 2002: 79; Hernández-Alcántara & Solís-Weiss 2014: 142–143.

Type-species. *Haploscoloplos bifurcatus* Hartman, 1957: 277–279; by original designation.

Diagnosis. Prostomium pointed, conical; one achaetous peristomial ring. Thoracic neurochaetae with only crenulated capillaries; abdominal furcate notochaetae present or absent. Branchiae simple or branched, either present from posterior thoracic, transitional or abdominal chaetigers, or absent. Interramal cirri present or absent. Posterior thoracic neuropodia with up to six podal papillae. Subpodal and stomach papillae absent, or with up to eight subpodal papillae per parapodium and with numerous stomach papillae in the posterior thorax / anterior abdomen.

Leitoscoloplos bifurcatus (Hartman, 1957)

(Figs 5, 6)

Haploscoloplos bifurcatus Hartman, 1957: 277–279
Leitoscoloplos bifurcatus.— Day 1977: 223–224; Hutchings & Rainer 1979: 760–761; Mackie 1987: 13–14, fig. 14a–f.

Material examined. AM W.46089, MI QLD 2376 (photographed); AM W.44761, MI QLD 2429 (4, 1 photographed on SEM); AM W.44299, MI QLD 2378 (2); AM W.44938, MI QLD 2439 (photographed); AM W.44938.001, tissue for molecular studies.

Description. Body cylindrical, thorax slightly wider than abdomen (Fig. 5A). Thoracic width up to 2.5 mm. Prostomium sharply conical. Peristomium bearing pair of dorso-lateral nuchal organs (Fig. 6A). 19–21 thoracic chaetigers (Fig. 5A). Branchiae starting from chaetiger 8 as minute papillae, well developed from chaetiger 15–16; in abdomen triangular, narrow lanceolate in posterior part of abdomen; slightly shorter or equal to notopodial lobes (Figs 5A, D, G, H, 6F). Thoracic post-chaetal lobes developed from chaetiger 1, gradually increase in size, narrow triangular in shape; in anterior thorax equal size or neuropodia lobes longer, in posterior thorax notopodial lobes longer (Figs 5C–F, 6A, D, E). Lateral organs developed at base of notopodia (Fig. 6A, E, F). Neuropodial lobes becoming bilobed from chaetiger 10–12 (Fig. 5A). No subpodal or stomach papillae. Abdominal notopodial lobes narrow lanceolate; neuropodial lobes bilobed with inner lobe longer than outer, with two or three aciculae; in anterior abdomen lobes short, subequal, in posterior abdomen inner lobe very long, 2–3 times longer than outer (Figs 5G, H, 6E, F, G). Subpodal notch and flange present, in first abdominal chaetiger subpodal flange can form papilla (ventral cirrus) in some specimens, but in other segments its upper margin rounded; all other abdominal neuropodia without flange papillae (Figs 5B, D, G, H, 6E, F, G). Elongate ciliated dorsal organs present mid-dorsally as two lateral curved ciliated strips (Fig. 6H) in all segments from posterior thorax. Chaetae crenulated capillaries in all parapodia (Figs 5C, E, F, 6B, C), forked chaetae in abdominal notopodia not found. Pygidium with two anal cirri (Fig. 6I).

Remarks. *Leitoscoloplos bifurcatus* has been described from South Australia and later reported also from Victoria, New South Wales, Queensland and Northern Territory (Hutchings & Rainer 1979). Specimens described in present study agree well with previous descriptions. *Leitoscoloplos bifurcatus* is very similar to *S. dayi* in general appearance, shape of thoracic neuropodia, and the first segment with branchiae. Differences between these two species are listed above.

Molecular analyses. The 18S and 16S sequence analyses (Figs 13, 14) support the inclusion of *Leitoscoloplos bifurcatus* in the clade that contains representatives of the genus *Leodamas*: *L. dubia, L. rubra* and *L. johnstonei*. This result strongly incongruent with the morphology indicate possible paraphyly of *Leitoscoloplos*. The analysis of CO1 sequences (Fig. 15) showed that *Leitoscoloplos bifurcatus* does not form a clade with *Leodamas* or with other *Leitoscoloplos species*. It is interesting that three clades including species assigned to *Leitoscoloplos* appeared to show agreement with three of the five morphological subgroups recognised by Mackie (1987). *Leitoscoloplos pugettensis* belongs to group 3 (species with more than 10 thoracic chaetigers, mammiform or mammiform/triangular thoracic neuropodial lobes, and strap-like branchiae; no interramal cirri or subpodal papillae); *L. bifurcatus* belongs to group 4 (species with more than 10 thoracic chaetigers, single triangular or triangular/bifurcate thoracic neuropodial lobes, and triangular branchiae; no interramal cirri or subpodal papillae), and *L. robustus* and *L.fragilis* to group 5 (species with more than 10 thoracic chaetigers, single triangular, triangular/bifurcate or mammiform/bifurcate thoracic neuropodial lobes, and triangular branchiae; interramal cirri and subpodal papillae present). Whether this would hold up with the inclusion of additional taxa is unknown.

Genus *Leodamas* Kinberg, 1866

Leodamas Kinberg, 1866: 252.
Scoloplos (*Leodamas*).—Hartman 1957: 284–285; Pettibone 1957: 160; Day 1973: 84; Eibye-Jacobsen 2002: 87.

Type-species. *Scoloplos (Leodamas) verax* Kinberg, 1866: 251–252; by monotypy.

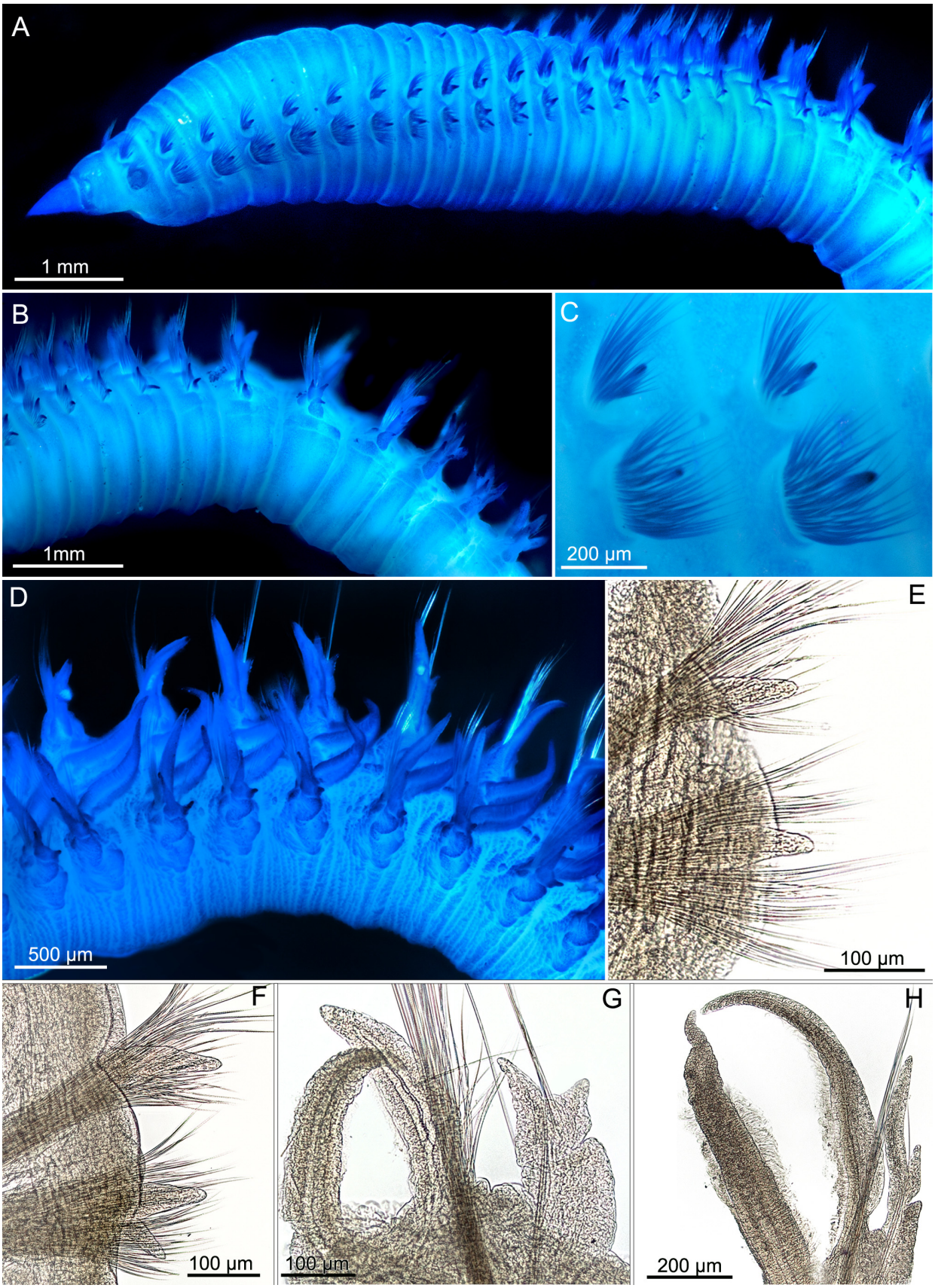

FIGURE 5. *Leitoscoloplos bifurcatus*, A–D: AM W.44938, methylene blue staining. A. Anterior end, left lateral view; B. Thorax-abdomen transition, left lateral view; C. Parapodia of chaetigers 2 and 3; D. Abdomen, lateral view. E–H: AM W.46089, glycerol mounts of parapodia. E. Chaetiger 4; F. Chaetiger 13; G. Anterior abdominal chaetiger; H. Posterior

abdominal chaetiger.

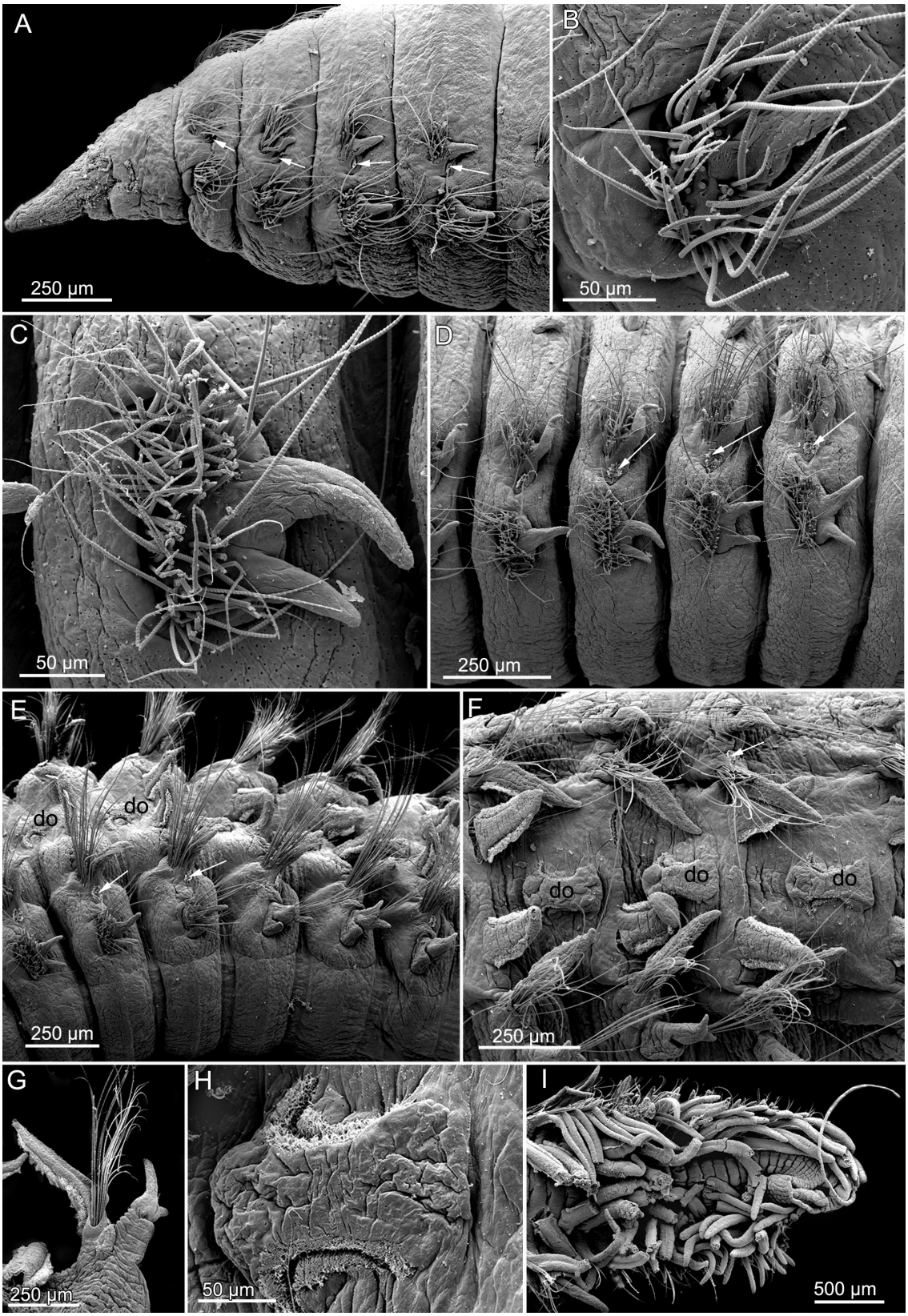

FIGURE 6. *Leitoscoloplos bifurcatus*, AM W.44761, SEM images. A. Anterior end, left lateral view, arrows indicate lateral organs; B. Neuropodium of chaetiger 2; C. Neuropodium of chaetiger 12; D. Parapodia of chaetigers 11–14, arrows indicate lateral organs between rami; E. Thorax-abdomen transition, dorsal organs (do) are seen mid-dorsally, arrows indicate lateral organs; F. Abdominal segments, dorsal view, dorsal organs (do) are seen mid-dorsally, arrow indicates lateral organ; G.

Abdominal parapodia, anterior view; H. Dorsal organ; I. Posterior end with pygidium, one anal cirrus is broken.

Diagnosis. Prostomium acutely pointed, usually prolonged; one achaetous peristomial ring. Thoracic neuropodia bearing large and numerous hooks accompanied by few or no crenulated capillaries, abdominal furcate notochaetae present or absent, abdominal neuropodia possess robust emergent aciculae. Branchiae simple or branched, first present from chaetiger 5–7. Thoracic neuropodial podal papillae, subpodial papillae, stomach papillae present or absent.

Leodamas dubia (Tebble, 1955)

(Figs 7, 8, 12C)

Scoloplos dubia Tebble, 1955: 123–124, fig. 26a–c.
Scoloplos (*Leodamas*) *rubra australiensis* Hartmann-Schröder, 1979: 131–132, figs 276–282.
Scoloplos (*Leodamas*) *dubia*—Eibye-Jacobsen 2002: 89–91, fig. 8A–D.

Material examined. AM W.44579, MI QLD 2422 (3); AM W.46094, MI QLD 2429 (10, 1 photographed); AM W.44762, MI QLD 2429 (5); AM W.44765, MI QLD 2429 (photographed); AM W.44765.001, tissue for molecular study; AM W.46098, MI QLD 2439 (7, 1 photographed); AM W.45478, MI QLD 2439; AM W.45478.001, tissue for molecular study; AM W.45479, MI QLD 2438 (photographed); AM W.45479.001, tissue for molecular study; AM W.45480, MI QLD 2376; AM W.45480.001, tissue for molecular study; AM W.44941, MI QLD 2438, posterior fragments.

Description. Body long, slender; thorax flattened dorso-ventrally; abdomen cylindrical (Figs 7A–C, 8A, B). Colour in life pale yellowish in thorax, in abdomen more saturated yellow, with red blood vessels, chaetae golden (Fig. 12C). Thoracic width up to 1.1 mm. 15–19 (usually 16–18) thoracic chaetigers. Branchiae starting from chaetiger 6, digitiform, gradually increasing in size; becoming triangular with drawn-up tip, longer and wider than notopodia in abdomen (Figs 7A, D, E, H, 8F, K). Thoracic post-chaetal lobes developed from chaetiger 2 (Fig. 7B). Notopodial lobes digitate, increasing in length along thorax; in abdomen becoming triangular or lanceolate, slightly shorter than branchiae (Figs 7A, B, D, E, G, H, 8C, F, K). Thoracic neuropodial postchaetal lobes represented by low ridges; triangular foot papilla of last one or two thoracic chaetigers shifted to upper side (Figs7B, C, F, G, 8A–D). Abdominal neuropodial lobes rectangular, bilobed, supported by thick, curved, projecting acicula; with digitate outer lobe and reduced inner lobe (Figs 7H, I, 8F, G). Parapodial flange not developed. No subpodal, stomach or flange papillae. Dorsal organs as short curved strips seen in each segment dorsally (Fig. 7D). Notopodia bearing crenulated capillary chaetae in both thorax and abdomen; abdominal notopodia additionally having forked chaetae with equal tines and pointed tips (Fig. 8J). Thoracic neuropodia with four rows of straight hooded slightly serrated hooks; hooks in anterior row shorter and thicker than in posterior ones; upper hooks thicker than lower (Figs 7B, C, F, G, 8C–E); two or three crenulated capillary chaetae located in upper part of posterior row (Figs 7B, C, F, G, 8C–E). Abdominal neuropodia with crenulated capillary chaetae and very thick projecting acicula; its shape vary from almost straight to bent almost 180° (Figs 7I–K, 8G–I). Pygidium with four short cirri, one pair ventrolateral and the other lateral (Fig. 8K).

Remarks. *Leodamas dubia* was originally described from the Gold Coast (Ghana, West Africa) as *Scoloplos dubia*. The species was characterized by having 21–23 thoracic chaetigers, branchiae starting from chaetiger 7, and hooked aciculae in abdominal neuropodia. Eibye-Jacobsen (2002) described numerous specimens from the Thai sector of the Andaman Sea, which he referred to this species. Thai specimens had 18–21 thoracic chaetigers and differed from original description by branchiae starting from chaetiger 6 and shape of abdominal notopodial lobes. Eibye-Jacobsen (2002) also synonymized *S.* (*L.*) *rubra australiensis* Hartmann-Schröder, 1979 with *S.* (*L.*) *dubia* based on the characteristic hooked shape of the neuropodial aciculae. Specimens reported here correspond well to descriptions by Eibye-Jacobsen (2002) and Hartmann-Schröder (1979) and differ by having a lower number of thoracic chaetigers (15–19 instead of 18–21 and 23). As noted for specimens from the Andaman Sea, worms from Lizard Island exhibit large variability in the shape of neuropodial aciculae; they can be almost straight, slightly bent or curved almost 180°. Hence, for correct identification of this species it is necessary to check parapodia from certain abdominal region (20^{th}–45^{th} abdominal chaetigers according to Eibye-Jacobsen (2002)). Otherwise specimens could be referred to other species of *Leodamas*, i.e., *L. rubra* (Webster, 1879) differing mainly by less curved aciculae.

There is a possibility that West-African and Indo-Pacific populations of *L. dubia* represent different species as

they show important morphological differences. To resolve this issue further investigations are needed.

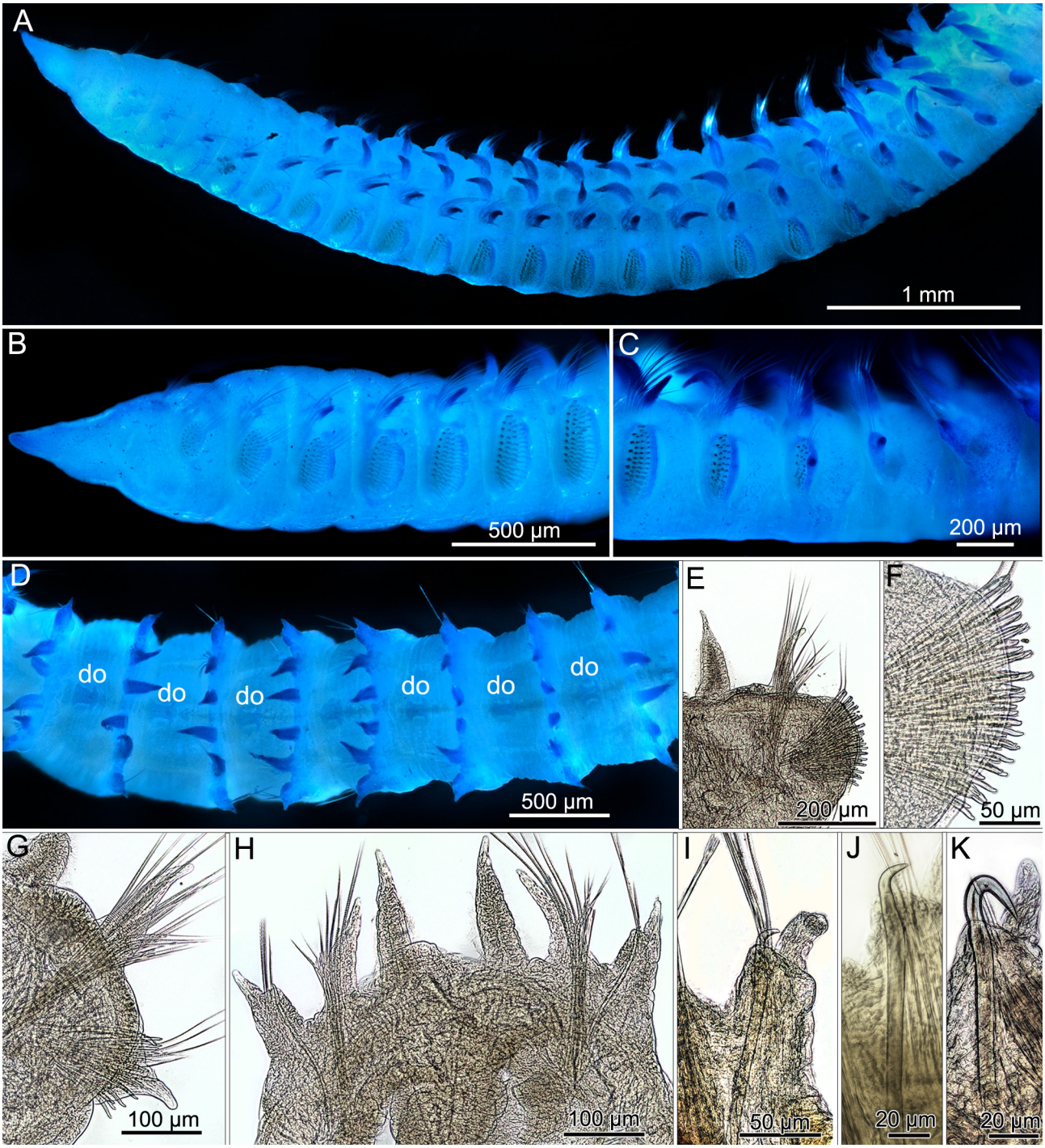

FIGURE 7. *Leodamas dubia*, A–D: methylene blue staining. A. AM W.44765, anterior end, left lateral view; B. AM W.45479, anterior end, left lateral view; C. AM W.45479, thorax-abdomen transition; D. AM W.44765, abdomen, dorsal view, note dorsal organs (do). E–K: Glycerol mounts of parapodia. E. AM W.46094, parapodium of chaetiger 17; F. Same, close-up of neuropodia; G. AM W.46094, parapodium of chaetiger 18; H. AM W.46098, abdominal parapodia; I. AM W.44765, abdominal neuropodia; J. AM W.44765, neuropodial acicula; K. AM W.45478, neuropodial acicula.

Molecular analyses. The 18S and 16S sequence analyses (Figs 13, 14) indicated that *Leodamas dubia* belongs in the clade that consists of *L. rubra* and *L. johnstonei*. This clade also includes *Leitoscoloplos bifurcatus.* As mentioned above, morphologically *Leodamas* and *Leitoscoloplos* are very different from each other and molecular analysis contradicts morphological data. The genetic distance between *L. rubra* and *L. dubia* is less than 2% for

18S sequences and approximately 10% for 16S sequences. The analysis of orbiniid phylogeny performed by Bleidorn *et al.* (2009) showed that *L. rubra* and *L. johnstonei* form a clade that is a sister clade to all other orbiniid species, but in our trees species of *Leodamas* did not form sister group to other orbiniids.

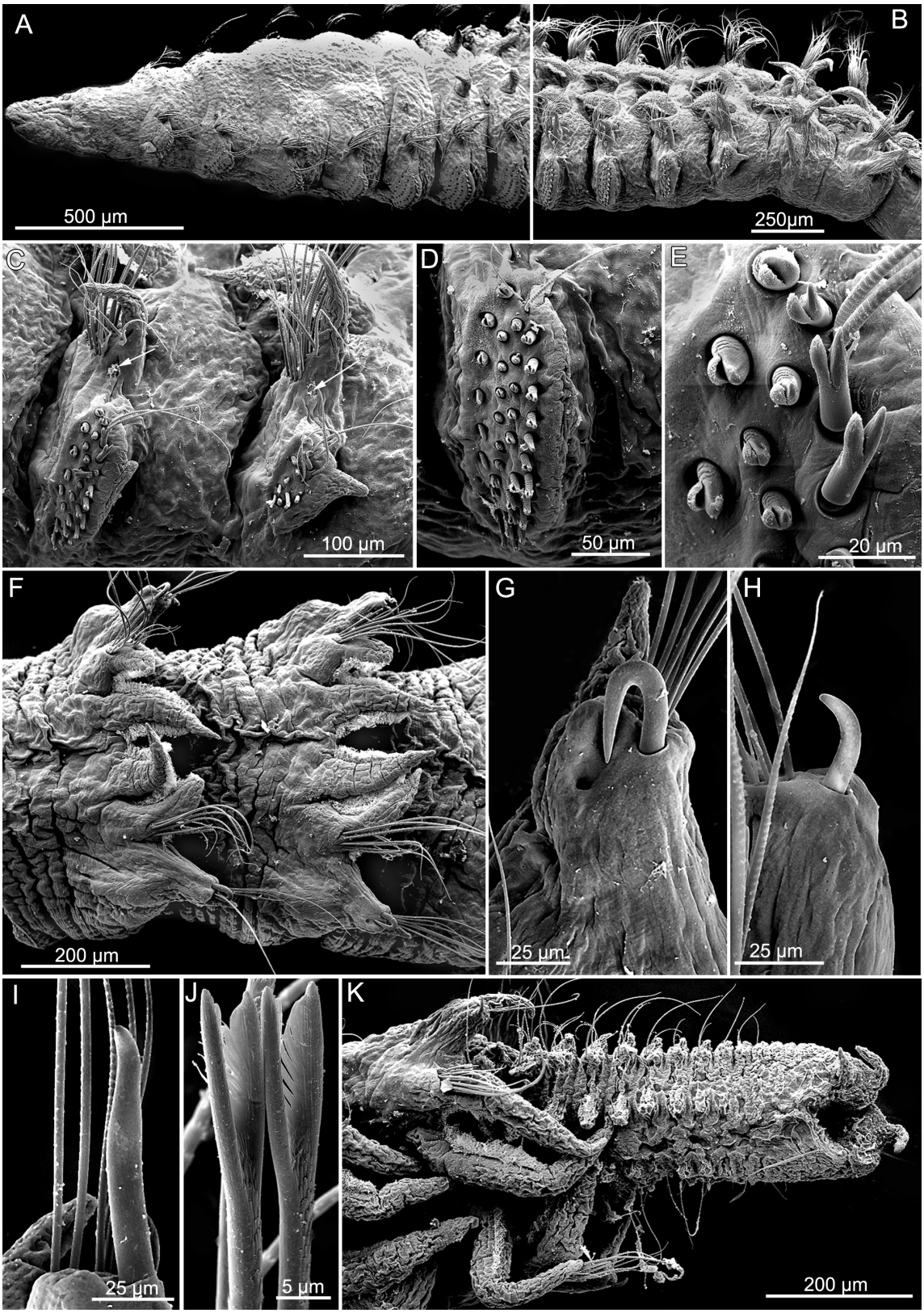

FIGURE 8. *Leodamas dubia*, AM W.46094, SEM images. A. Anterior end, dorso-ventral view; B. Thorax-abdomen transition; C. Neuropodia of chaetigers 16 and 17, arrows indicate lateral organs; D. Neuropodium of chaetiger 15; E. Neuropodial hooks of chaetiger 15; F. Abdomen, dorsal view; G–I. Abdominal neuropodia with a variety of aciculae shapes; J. Forked chaetae of

abdominal notopodia; K. Regenerating posterior end with pygidium.

Genus *Naineris* Blainville, 1828

Nais Fabricius, 1780: 315–316.
Naineris Blainville, 1828: 490–491.
Nainereis.—Hartman 1957: 296; Pettibone 1957: 160.6

Type-species. *Nais quadricuspida* Fabricius, 1780, by original designation.

Diagnosis. Prostomium rounded to square in front; one achaetous peristomial ring. Thoracic neurochaetae may include crenulate capillaries, hooks and subuluncini, or crenulate capillaries only, abdominal furcate notochaetae present or absent. First pair of branchiae starting on any thoracic chaetiger from 2 to 23, branchial bases widely separated mid-dorsally. Thoracic neuropodia with 0–2 podal papillae; subpodal papillae and interramal cirri absent.

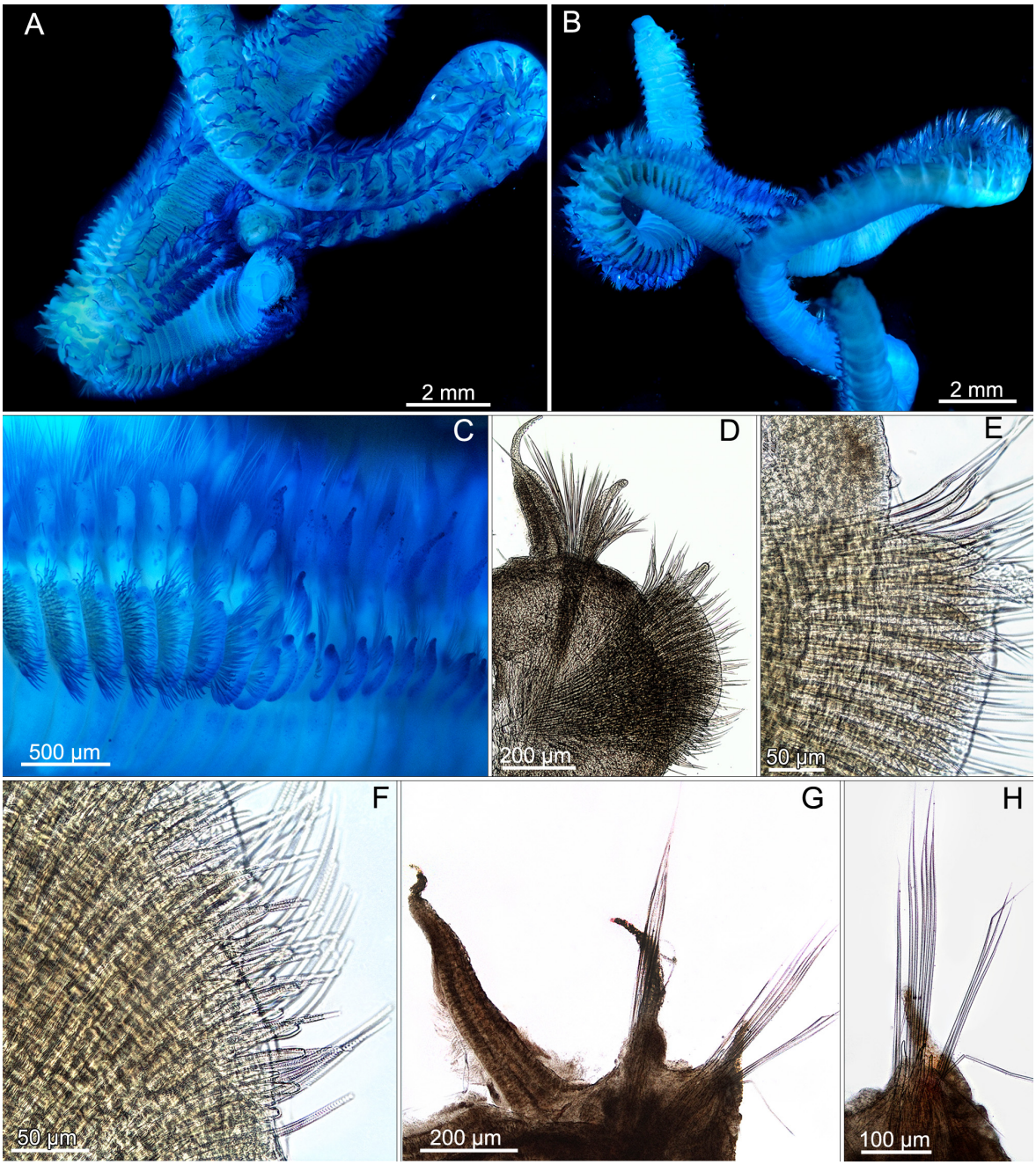

FIGURE 9. *Naineris grubei australis*, AM W.44763. A–C: methylene blue staining. A, B. General view; C. Thorax-abdomen transition; D–H: Glycerol mounts of parapodia. D. Chaetiger 13; E. Same, close-up of upper part with subuluncini; F. Same,

close-up of lower part with hooks and capillaries; G. Abdominal parapodia; H. Abdominal neuropodia.

Naineris grubei australis Hartman, 1957

(Figs 9–11, 12D)

Naineris grubei australis Hartman, 1957: 303–304, pl. 39, figs 1–4.
Naineris grubei australis.—Day 1977: 238; Hutchings & Rainer 1979: 761; Hartmann-Schröder 1980: 66.

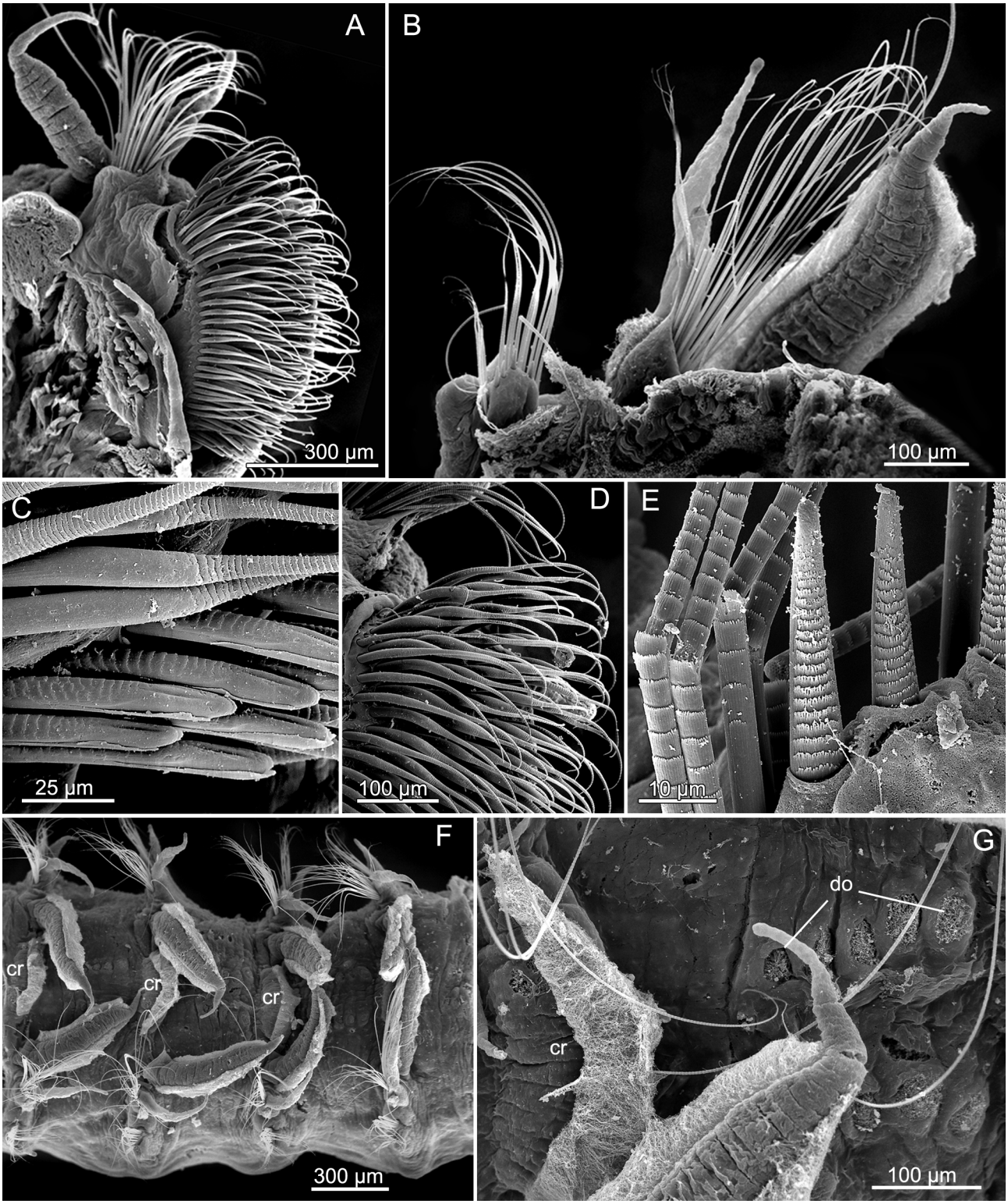

FIGURE 10. *Naineris grubei australis*, AM W.44763, SEM images. A. Thoracic parapodia, anterior view; B. Abdominal parapodia, anterior view of outer neuropodial lobe; C. Thoracic neuropodial hooks; D. Thoracic neuropodial subuluncini and capillary chaetae; E. Abdominal neuropodial aciculae and capillary chaetae; F. Abdomen, dorsal view, note ciliated ridge (cr)

between bases of branchiae; G. Close-up view of abdomen, note ciliated ridge (cr) and dorsal organs (do).

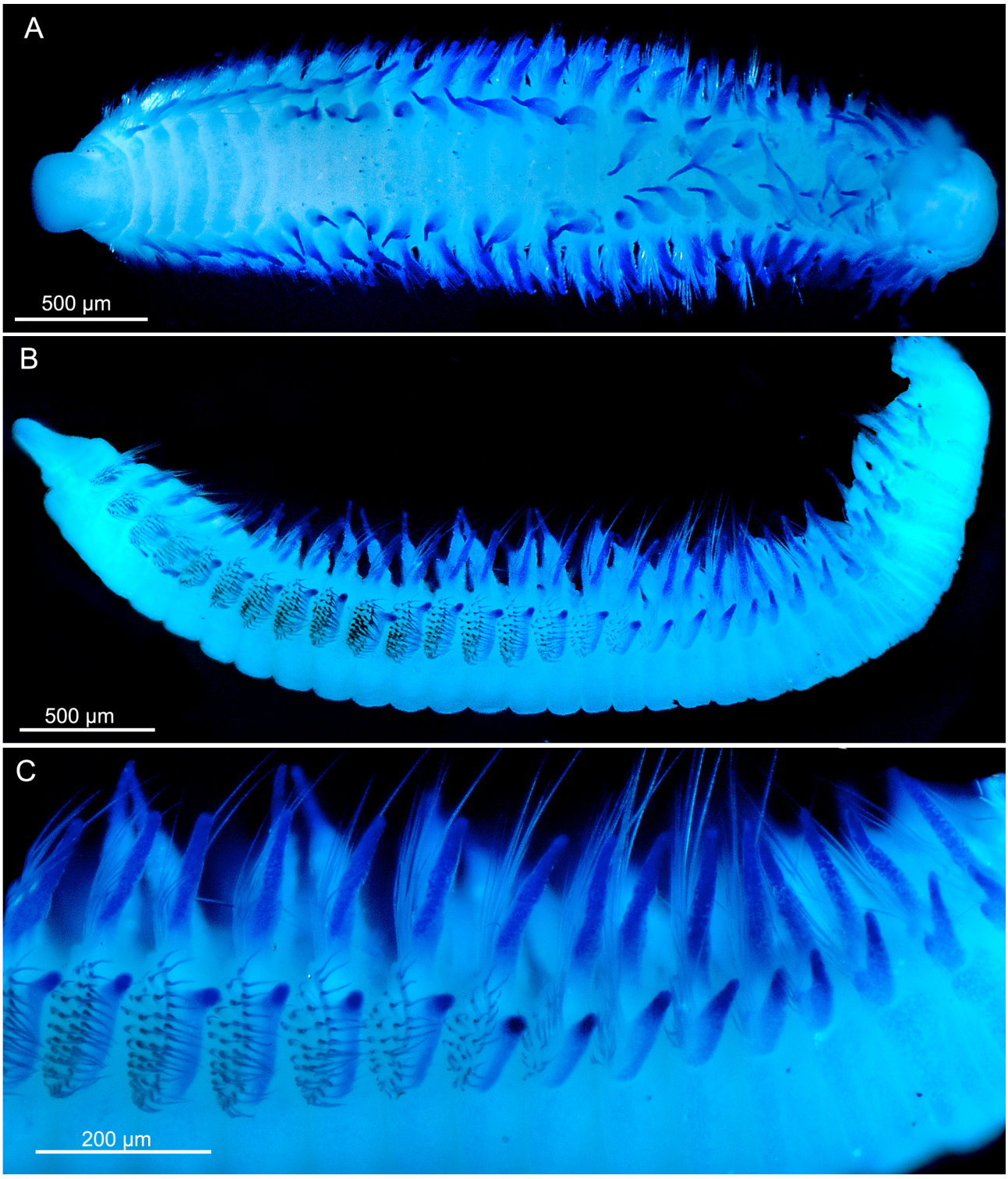

FIGURE 11. *Naineris grubei australis* (?), juvenile, AM W.44038, methylene blue staining. A. Dorsal general view; B. General lateral view; C. Thorax-abdomen transition.

Material examined. AM W.44763, MI QLD 2429 (photographed); AM W.44763.001, tissue for molecular study; AM W.44038, MI QLD 2340 (photographed, juvenile).

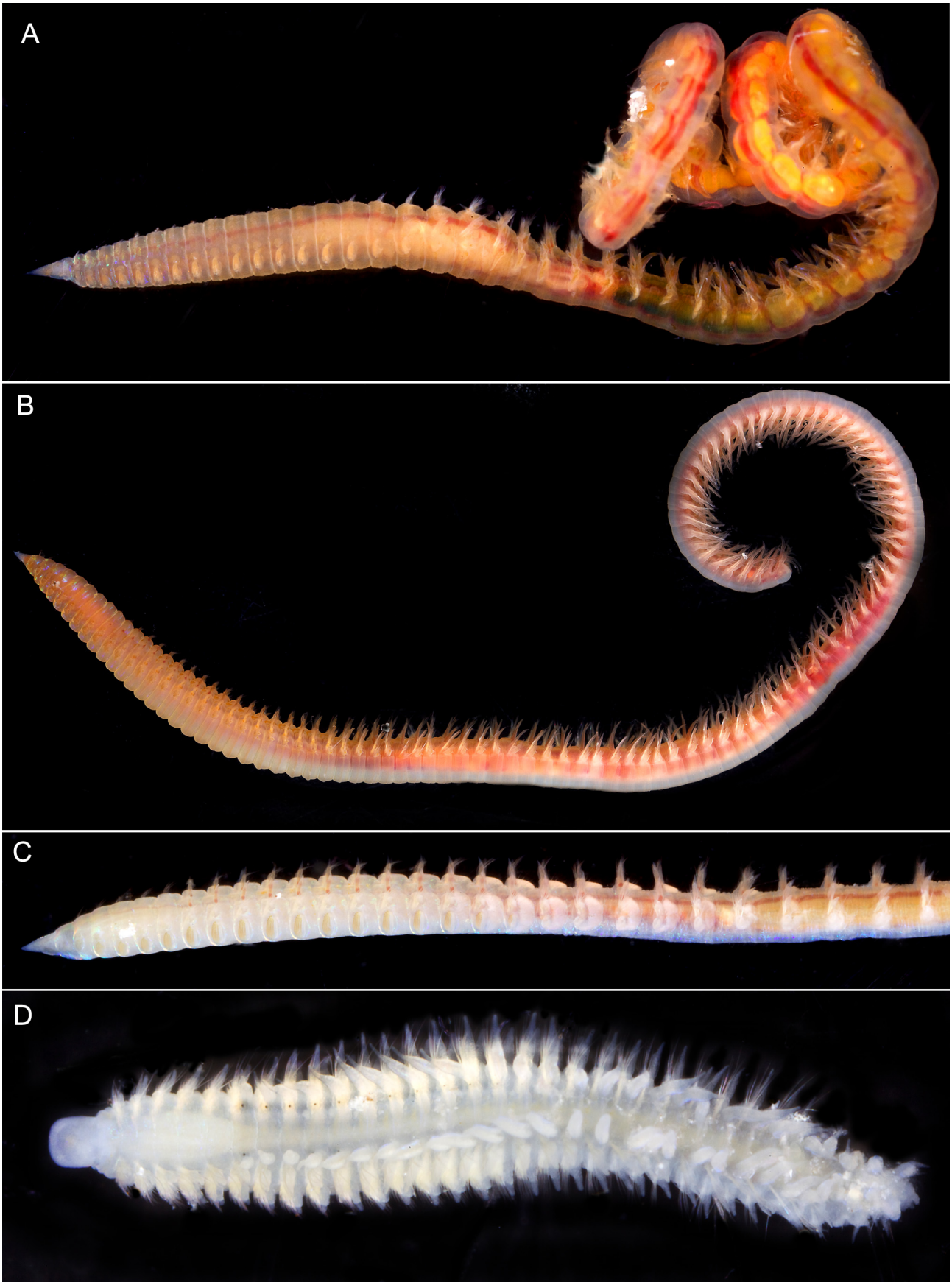

FIGURE 12. Photographs of live specimens. A. *Scoloplos acutissimus*; B. *Scoloplos dayi*; C. *Leodamas dubia*; D. *Naineris*

grubei australis (?), juvenile. Photo: Alexander Semenov.

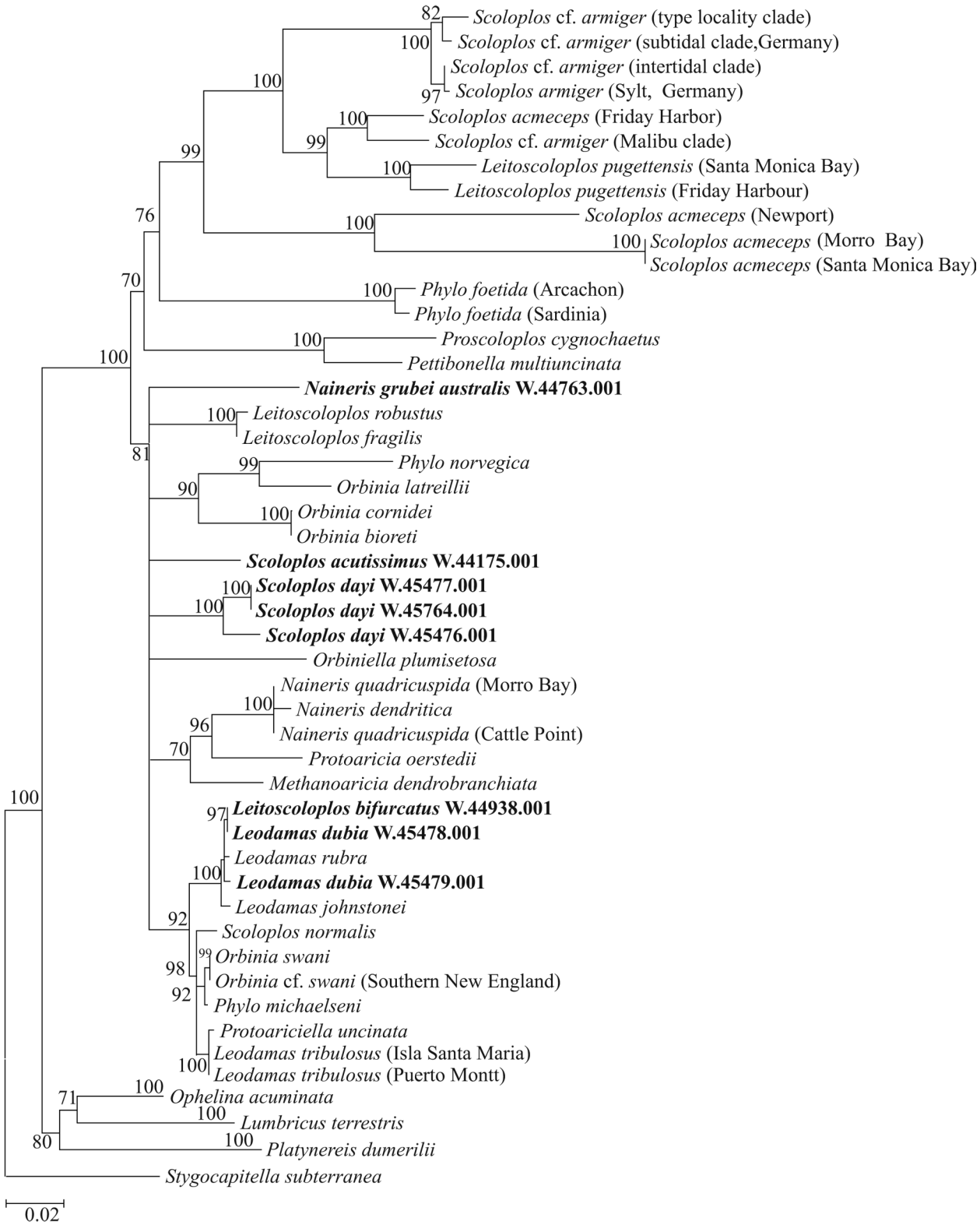

FIGURE 13. Neighbor-Joining phylogenetic tree of 53 specimens based on 18S gene sequences (Table 2). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Statistical support for clades in the tree was assessed using the bootstrap analysis with 1,000 replicates.

Description. Body long, cylindrical, thorax slightly flattened (Fig. 9A, B). Prostomium broad rectangular.

Thorax 1.6 mm wide for 43 thoracic chaetigers. Branchiae from chaetiger 6, well developed from beginning, triangular with narrow slender tips; in posterior thorax and abdomen becoming longer than notopodia (Figs 9A, C, G, 10A, B, F); on each segment two branchiae widely separated mid-dorsally and connected by raised ciliated ridge (Fig. 10F, G). Postchaetal parapodial lobes developed from chaetiger 1. Thoracic notopodial lobes triangular with narrow slender tips, in abdomen narrow digitiform (Figs 9D, G, 10A, F). Thoracic neuropodial lobes represented by postchaetal ridge with round or elongated papilla; in anterior thorax papilla located in middle of ridge, in middle and posterior part shifted dorsally (Figs 9C–F, 10A). Abdominal neuropodia with long triangular outer lobe and reduced round inner lobe, supported by 3–5 aciculae (Figs 9G, H, 10B). No subpodal flange, interramal cirri, subpodal or stomach papillae. Dorsal organs represented by five pairs of ciliated spots in each segment (Fig. 10G). All notochaetae crenulate capillaries, in both thorax and abdomen. Thoracic neurochaetae of three kinds: capillaries, subuluncini with thick base and sharply tapering tips, and straight, hooded hooks located mostly in lower part of neuropodia (Figs 9D–F, 10A, C, D). Abdominal neuropodial lobes supported by 3–4 projecting straight serrated aciculae (Figs 9H, 10E). Posterior end unknown.

Juvenile specimen (Fig. 11A–C) similar to the description above in prostomial shape, segment of branchiae start, shape of branchiae and podial lobes. It has only 18 thoracic chaetigers, and a thoracic width of 0.8 mm, and most probably belongs to the same species. Colouration in life white, pale yellowish, with brown dots (statocysts) near bases of each branchia in thorax (Fig. 12D).

Remarks. *Naineris grubei australis* was described from the vicinity of Adelaide, South Australia, and later was found in Victoria, New South Wales, and Western Australia (Hutchings & Rainer 1979; Hutchings & Murray 1984; Hartmann-Schröder 1980). Specimens reported herein agree well with the previous descriptions. The present study expands the distribution of this species to Queensland.

Molecular analyses. According to sequencing data for 18S rRNA (Fig. 13) and 16S rRNA gene fragments (Fig. 14) of *Naineris grubei australis*, this species belongs in an unresolved clade that contains all *Nainereis* species and also all *Phylo* and *Orbinia* species as well as *Orbiniella plumisetosa*, *Methanoaricia dendrobranchiata*, *Scoloplos normalis*, *Scoloplos dayi* and *Leodamas tribulosus*. The sequencing data for the CO1 fragment (Fig. 15) indicates that *Naineris grubei australis* belongs in a separate group with another *Naineris* species and *Protoaricia oerstedii*; however, this group lacks support (51%).

Discussion

Our results do not correlate well with the orbiniid phylogeny that was reconstructed by Bleidorn *et al.* (2009) based on sequence data for five mitochondrial genes and 18S rRNA, and we obtained trees with different topology. The possible explanation is that we added five new species, used different set of genes, and different algorithms (Neighbor-Joining vs. Maximum Likelihood and Bayesian Analyses). The topologies of our trees constructed from different genes are not identical either.

Results of our analyses are incongruent with morphology and do not support the monophyly of four investigated genera. Thus, the species of *Scoloplos* were found in four places on the tree (for 18S, 16S and CO1 analyses). The first is *Scoloplos armiger*-*S. acmeceps*- *Leitoscoloplos pugettensis* clade, which also present on tree reconstructed by Bleidorn *et al.* (2009); *S. acutissimus* and *S. dayi* formed the separate clades each, and finally, *S. normalis* joined the clade, which included *Orbinia swani* and *Phylo michaelseni*. Sister to this clade is the clade that includes *Leodamas tribulosus*. Species of the genus *Leitoscoloplos* were also located in different parts of the tree (this is true for all three trees constructed). *Leitoscoloplos bifurcatus,* the type species of the genus, in 18S and 16S trees unexpectedly grouped together with *Leodamas* spp. (*L. rubra*, *L. jonstonei,* and *L. dubia)*, which resembles the situation with *Scoloplos normalis*. The taxonomic position of *Leitoscoloplos bifurcatus* needs further investigation as we sequenced only one specimen. The *Leodamas* group is also divided into two clades that which coincide with the Bleidorn *et al.* (2009) data; sequences of *L. dubia* joined the clade that included *L. rubra* and *L. jonstonei,* while *L. tribulosus* formed a separate clade. In the analysis by Bleidorn *et al.* (2009) three species of the genus *Naineris* (*N. quadricuspida, N. dendritica,* and *N. laevigata*) formed a monophyletic group, however in our trees *N. grubei australus* did not join this clade.

The lack of monophyly, node support, and changing of tree topology depending on the set of taxa included, the method and genes used in the analysis demonstrate the necessity for further study of phylogenetic relationships within the family Orbiniidae using both a larger number of genes and more inclusive taxon sampling.

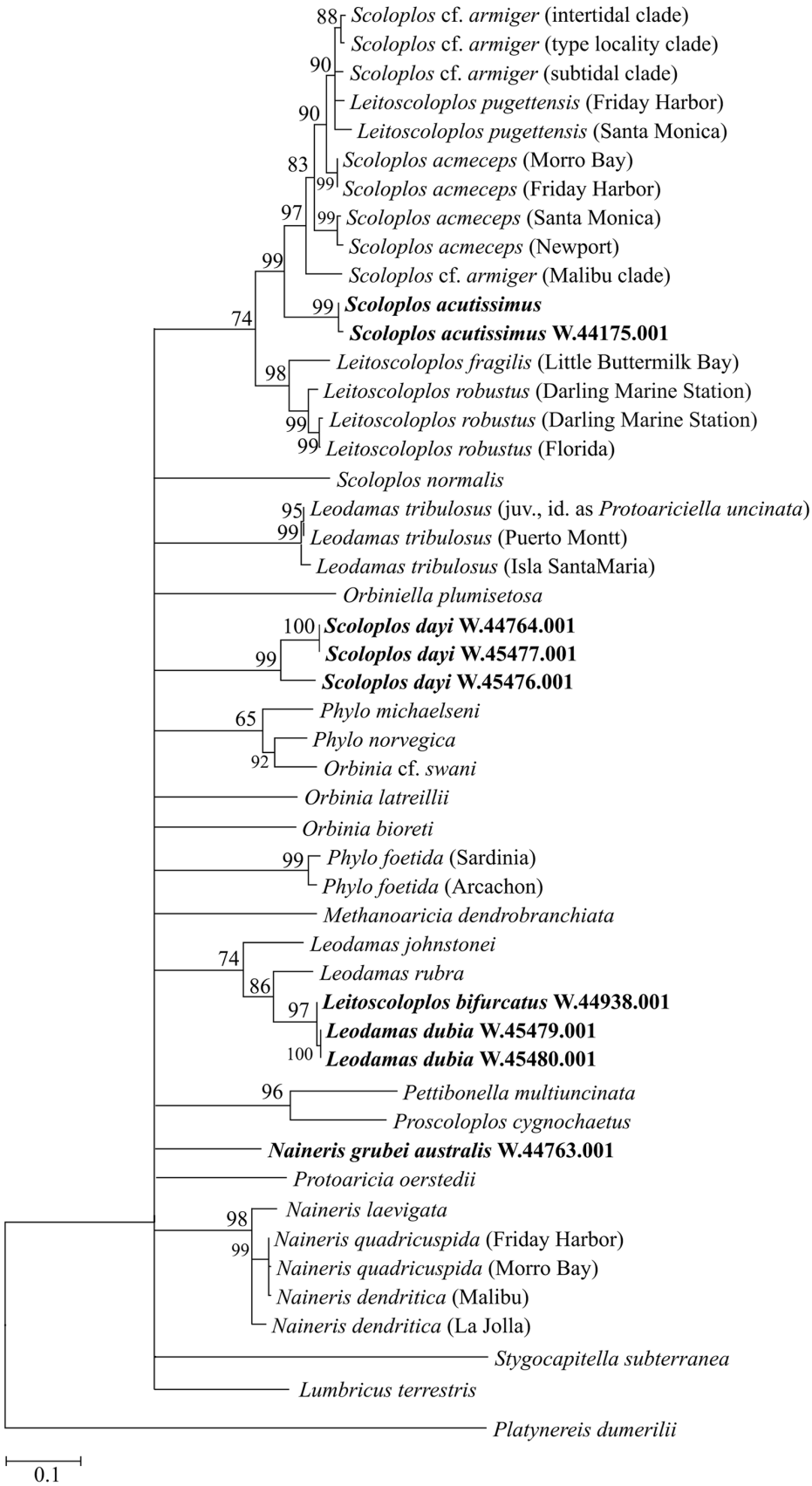

FIGURE 14. Neighbor-Joining phylogenetic tree of 55 specimens based on 16S gene sequences (Table 2). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Statistical support for clades in the tree was assessed using the bootstrap analysis with 1,000 replicates.

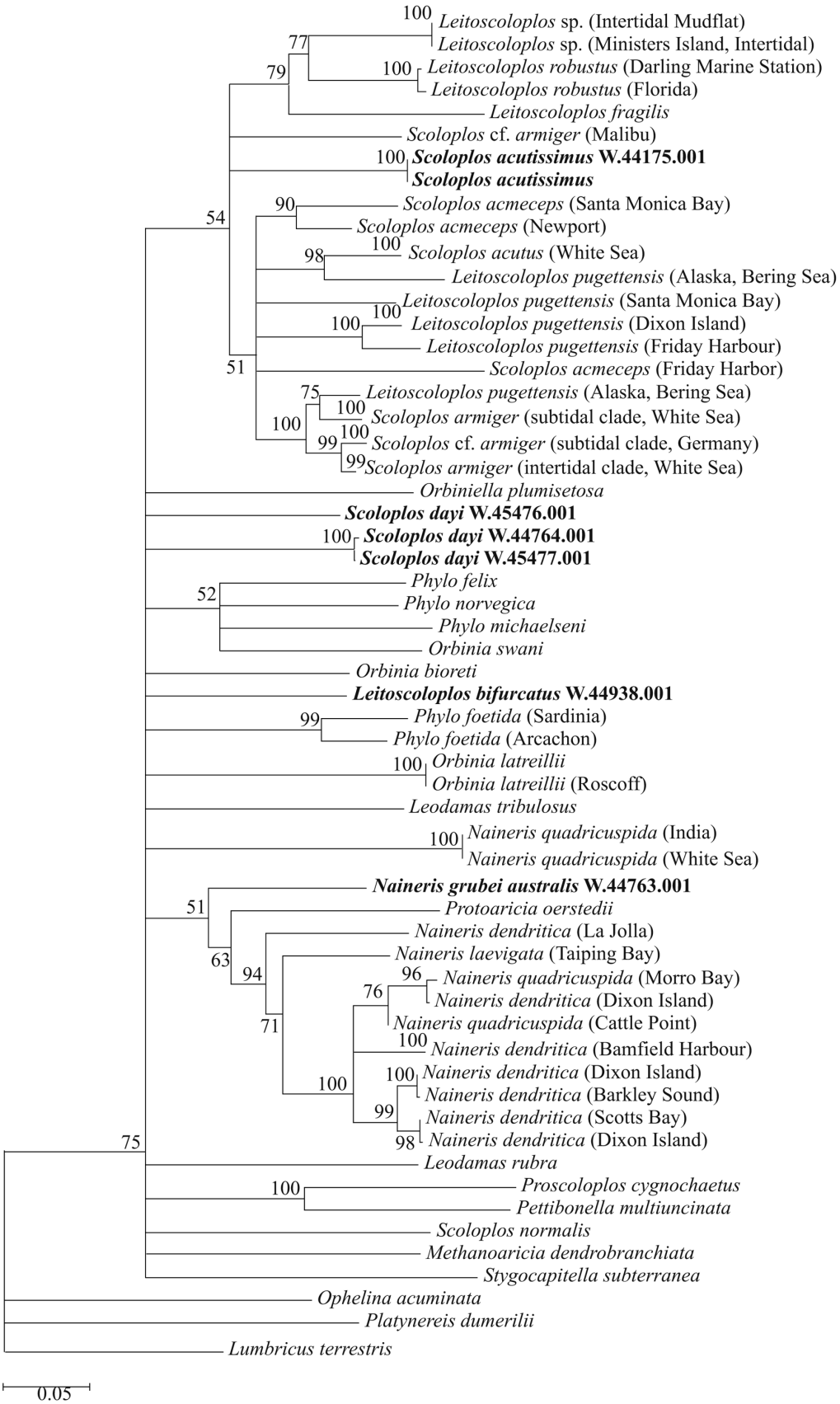

FIGURE 15. Neighbor-Joining phylogenetic tree of 106 specimens based on CO1 gene sequences (Table 2). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Statistical support for clades in the tree was assessed using the bootstrap analysis with 1,000 replicates.

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