

# Two New Deep-water Species of Ampharetidae (Annelida: Polychaeta) from the Eastern Australian Continental Margin

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**ABSTRACT.** Two new species, *Melinnopsis gardelli* sp. nov. and *Melinnopsis chadwicki* sp. nov. (Annelida, Ampharetidae, Melinninae), are described from deep waters off the east coast of Australia. One hundred and 11 specimens were collected during RV *Investigator* voyage IN2017\_V03 in May–June 2017 using a beam trawl at lower bathyal depths (1000–2500 m). This is the first record of *Melinnopsis* from the eastern Australian coast. The two new species are morphologically similar, but differ by methyl blue staining pattern, shape of thoracic uncini and pigmented glandular bands above the nuchal slits. *Melinnopsis gardelli* sp. nov. has a conspicuous stained band on the dorsum ending between chaetigers 9 and 10, uncini with three teeth above the rostral tooth and lacks glandular bands, while *M. chadwicki* sp. nov. has a faint stained band on the dorsum ending at chaetiger 5, uncini with two teeth above the rostral tooth and possesses glandular bands. They also show differences in bathymetric distribution as *M. gardelli* sp. nov. was collected around 2500 m and *M. chadwicki* sp. nov. around 1000 m depth. Phylogenetic relationships among the new species and other members of the family Ampharetidae were assessed using the nuclear 18S and the mitochondrial 16S and cytochrome oxidase subunit I (COI) gene fragments. The results revealed that *M. gardelli* sp. nov. and *M. chadwicki* sp. nov. form a monophyletic clade and are genetically distinct from each other and all other analysed species. This is the first time molecular data have been used to describe a species in the genus *Melinnopsis*.

## Introduction

Ampharetidae Malmgren, 1866 is a family of tubicolous annelids. They inhabit soft sediments from intertidal to abyssal depths in all oceans (Aguirrezabalaga & Parapar, 2014; Böggemann, 2009; Rouse & Pleijel, 2001) and can be well-represented and speciose in deep-sea benthic samples (Böggemann, 2009; Holthe, 2000; Saeedi & Brandt, 2020). The majority of recent species descriptions and molecular

data published on deep-sea ampharetids has focused on specimens from chemosynthetic hydrothermal vents and methane seeps (Kongsrud *et al.*, 2017; Reuscher *et al.*, 2009; Stiller *et al.*, 2013; Zhou *et al.*, 2019) as well as organic matter falls (Bennett *et al.*, 1994; Queirós *et al.*, 2017), habitats which represent a small fraction of the deep seafloor.

The family Ampharetidae comprises 312 species (Read & Fauchald, 2020) with high numbers (32 out of 62) of accepted monotypic genera. Currently, Ampharetinae and

**Keywords:** *Melinnopsis*; Ampharetidae; eastern Australia; lower bathyal; new species

**Zoobank registration:** urn:lsid:zoobank.org:pub:78FA352E-E590-4AA2-9C07-D963C36A7F5D

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**Received:** 20 March 2020 **Accepted:** 12 June 2020 **Published:** 12 August 2020 (in print and online simultaneously)

**Publisher:** The Australian Museum, Sydney, Australia (a statutory authority of, and principally funded by, the NSW State Government)

**Citation:** Gunton, Laetitia M., Elena Kupriyanova, and Tom Alvestad. 2020. Two new deep-water species of Ampharetidae (Annelida: Polychaeta) from the eastern Australian continental margin. *Records of the Australian Museum* 72(4): 101–121. <https://doi.org/10.3853/j.2201-4349.72.2020.1763>

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Melinninae are the only two accepted subfamilies, they differ morphologically by the presence of fine acicular chaetae in three to four anterior thoracic segments (III–V or III–VI), presence (sometimes) of dorsal hooks and transverse membrane and the lack of paleae in Melinninae, as opposed to the presence of paleae (sometimes) and absence of acicular chaetae, dorsal hooks and transverse membrane in Ampharetidae (Day, 1964; Ebbe & Purschke, 2019; Jirkov, 2011; Reuscher *et al.*, 2009). A historical overview of the family was given in Salazar-Vallejo & Hutchings (2012).

Ampharetinae with 261 described species is larger than Melinninae which includes only 49 species (two genera have not been assigned subfamilies) (Read & Fauchald, 2020). The five genera currently accepted within Melinninae are *Isolda* Mueller, 1858 (six species), *Melinantipoda* Hartman, 1967 (one species), *Melinna* Malmgren, 1866 (25 species), *Melinnopsides* Day, 1964 (one species) and *Melinnopsis* McIntosh, 1885 (16 species). Unfortunately, these genera are poorly defined (Jirkov, 2011), as characters typical for the type genus *Melinna* such as the presence of dorsal hooks and dorsal transverse membrane may be present or absent among other genera (Hilbig, 2005).

### Historical review of *Melinnopsis*

The genus *Melinnopsis* was erected for *Melinnopsis atlantica* McIntosh, 1885 collected from off Chesapeake Bay, Maryland in the Atlantic Ocean at around 3100 m (1700 fathoms) during the HMS *Challenger* expedition. McIntosh's original description is brief, it lacks images of the whole type specimen and is only illustrated with a line-drawing of a single uncinus (not labelled thoracic or abdominal). A clear justification for erecting a new genus was not provided, instead, McIntosh (1885) simply stated that dorsal hooks are not present, while the branchiae are "proportionally smaller" and chaetae shorter than those of *Melinna*.

A lack of clear generic definition has led to much confusion about the characters typical of the genus. The diagnosis of *Melinnopsis* has changed multiple times as authors synonymized genera and described new species (Day, 1964; Fauchald, 1977a; Jirkov, 1989, 2011; Reuscher *et al.*, 2015). Chamberlin (1919) even noted that due to the brevity of the original description the position of *M. atlantica* within the subfamily Melinninae was "somewhat doubtful".

*Melinnopsis capensis* Day, 1955 from South Africa was the second species described within the genus. It is characterized by three pairs of branchiae as opposed to four pairs in the type species. In his review of Ampharetidae Day (1964) transferred *Melinnopsis capensis* into a new monotypic genus *Melinnopsides* that he erected for species with three pairs of branchiae. Day (1964) also synonymized *Melinnexis* Annenkova, 1931 and *Melinnides* Wesenberg-Lund, 1950 with *Melinnopsis* without an explanation. His review includes the first formal generic diagnosis of *Melinnopsis* that is characterized by smooth buccal tentacles, four pairs of branchiae, acicular chaeta in segments III–V, 10–14 thoracic uncini, 25–30 abdominal segments, presence or absence of transverse membrane, and absence of dorsal hooks (Day, 1964). However, it is unclear which specimens he based this diagnosis on.

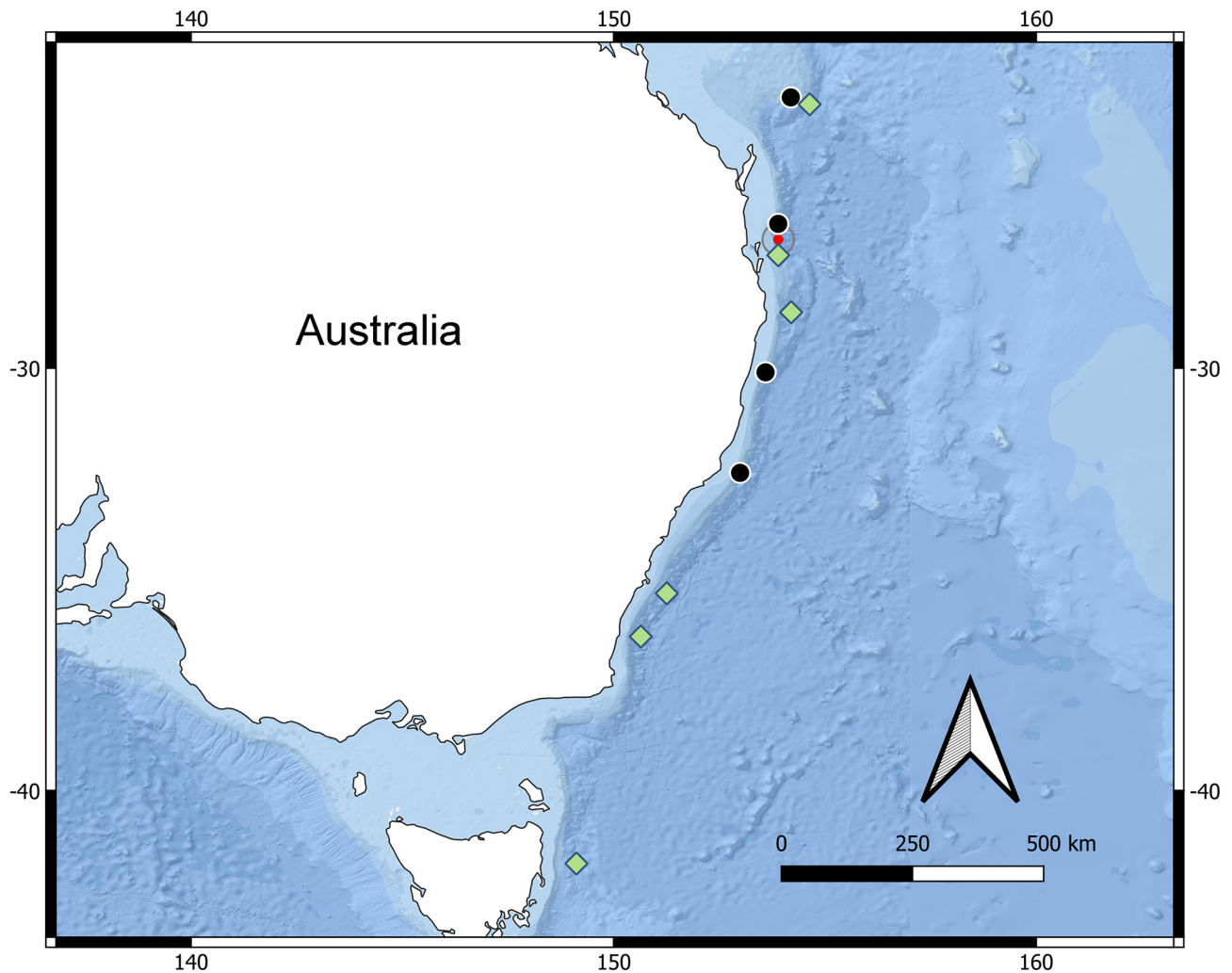
Fauchald (1977a) did not accept Day (1964)'s synonymization of *Melinnexis* and *Melinnides* with *Melinnopsis*, but instead moved *Melinnides* into *Melinnexis*, he also amended the generic definition of *Melinnopsis* given by Day (1964) to include four pairs of branchiae, ten thoracic uncini, similar buccal tentacles, notochaetae starting from segment V, and the absence of dorsal hooks and transverse membrane. In his revision of North Atlantic polychaetes (Holthe, 1986a) and in his catalogue of Terebellomorpha (Holthe, 1986b), Holthe followed Day (1964) and treated the genera *Melinnexis* and *Melinnides* (misspelt *Melinnides*) as synonyms of *Melinnopsis*. The generic diagnosis was changed again to include both "large and small" buccal tentacles, three to four pairs of branchiae, and 10–14 thoracic segments (Holthe, 1986a).

Jirkov (1989) synonymized *Amelinna* Hartman, 1969 with *Melinnexis* (now a synonym of *Melinnopsis*), arguing that *Amelinna* was established for species without dorsal hooks and transverse dorsal membrane, which is typical for *Melinnexis arctica*, the type species of *Melinnexis*. Jirkov (1989) also suggests synonymizing *Melinnopsides* with *Melinnopsis* stating that although the former has three pairs of branchiae compared with four in the latter, the number of branchiae varies between species, but this synonymy has not been widely accepted (Hilbig, 2005). Jirkov (1989) revised the generic diagnosis of *Melinnopsis* to "three to four pairs of smooth branchiae, no dorsal membrane and hooks". Jirkov (2011) again revised the diagnosis for *Melinnopsis* stating "there are neither hooks nor crest (= transverse membrane) behind the branchiae". The most recent generic diagnosis was given by Reuscher *et al.* (2015) to include, "large buccal tentacles occurring along with smaller ones. Four pairs of branchiae. Post branchial hooks absent. Brittle acicular neurochaetae in segments II–IV or II–V. Twelve to 14 thoracic uncini. Uncini with subrostral process."

### Molecular phylogenetic studies

The molecular phylogenetic studies on the family Ampharetidae (Bernardino *et al.*, 2017; Eilertsen *et al.*, 2017; Kongsrud *et al.*, 2017; Parapar *et al.*, 2018; Stiller *et al.*, 2013; Zhong *et al.*, 2011; Zhou *et al.*, 2019) have focused on species within Ampharetinae, only one (Bernardino *et al.*, 2017; Kongsrud *et al.*, 2017; Stiller *et al.*, 2017) or two species (Eilertsen *et al.*, 2017) of Melinninae were included in each dataset. Recently, Stiller *et al.* (2020) included six species of Melinninae in their phylogeny of all Terebelliformia. There are little molecular data available for the subfamily Melinninae. Data for the COI, 16S and 18S gene fragments commonly used in ampharetid phylogenetic studies (Kongsrud *et al.*, 2017) exist from only ten taxa, including eight named species (*Melinna cristata* (M. Sars, 1851), *M. maculata* Webster, 1879, *M. albicincta* Mackie & Pleijel, 1995, *M. palmata* Grube, 1870, *M. heterodonta* (Moore, 1923), *M. oculata* Hartman, 1969, *Isolda pulchella* Müller in Grube, 1858, and *Isolda bipinnata* Fauchald, 1977b).

In the present study we describe two new species belonging to the genus *Melinnopsis* from deep waters along the Australian eastern continental margin. The phylogenetic position of the new species within Ampharetidae is assessed using molecular data.



**Figure 1.** Map of eastern Australia showing records of the species *Melinnopsis gardelli* sp. nov. (green diamonds) and *Melinnopsis chadwicki* sp. nov. (black circles) collected during the RV *Investigator* expedition IN2017\_V03 “Sampling the Abyss”. The red circle represents point displacement where both species were found at sites close to one another.

## Methods

### Study area

All samples were collected during research vessel (RV) *Investigator* voyage “Sampling the Abyss” (IN2017\_V03), the first dedicated expedition to sample the biological fauna from the eastern Australian lower bathyal and abyssal environments. From 15 May–16 June 2017 samples were taken along a south to north latitudinal transect of 18 degrees along the east coast of Australia, from 42 to 24°S (Fig. 1). Samples were collected from 1000–4800 m depth using the CSIRO 4 m wide  $\times$  0.5 m high Beam Trawl (Lewis, 2010). Onboard, collected specimens were live sorted into higher taxa on ice in chilled (5°C) seawater, annelids were sorted into family, then fixed in either 95% ethanol or in 10% buffered formalin. In the laboratory, formalin-fixed specimens were rinsed in water and then fixed in 80% ethanol.

### Morphological investigations

The specimens were examined in ethanol using a dissecting microscope (OLYMPUS SZX7) and compound microscope

(OLYMPUS BX53). Specimens were extracted from their tubes, measured (length from prostomium to pygidium), stained with methyl blue and photographed using an OLYMPUS DP74 camera attached to the dissecting and compound microscopes with the imaging software OLYMPUS cellSens Standard 1.17. Some paratypes of *Melinnopsis gardelli* sp. nov. and *Melinnopsis chadwicki* sp. nov. were dehydrated in ethanol, critical point dried, coated with 20 nm of gold, and examined under the Scanning Electron Microscope JEOL JSM-6480 at Macquarie University. The type material is lodged at the Australian Museum, Sydney (AM); all Australian Museum registered specimens are prefixed “W.”.

### DNA extraction, amplification and sequencing

Tissue samples were obtained from six *Melinnopsis* specimens (Table 1). DNA extraction was performed using a Bioline Isolate II genomic DNA kit following the manufacture’s protocols. PCR amplification of the COI, 16S and 18S genes was conducted using six sets of primers (Table 2). Polymerase chain reaction (PCR) mixtures consisted of 0.4  $\mu$ l of each primer (forward and reverse), 1  $\mu$ l of template

**Table 1.** Ampharetidae, Terebellidae, Alvinellidae and Scalibregmatidae taxa used in molecular phylogenetic analysis with museum voucher number, sampling location, depth, GenBank accession numbers. Institutional abbreviations used RUB, Ruhr-Universität Bochum; SIO-BIC, Scripps Institution of Oceanography Benthic Invertebrate Collection; ZMBN, Department of Natural History, University Museum of Bergen; AM, Australian Museum. Dashes indicate no data available. Single asterisk (\*) indicates sequences omitted from analysis. Double asterisk (\*\*) in the voucher column indicates the entire specimen was used for sequencing.

species	voucher	location	depth (m)	GenBank or BOLD accession number	reference		
				COI	16S	18S	
<b>Melinninae</b>							
<i>Isolda pulchella</i> Müller in Grube, 1858	ZMBN 105698	Morocco	35	MG270119	MG253081	MG253135	Eilersten <i>et al.</i> , 2017
<i>Isolda pulchella</i> Müller in Grube, 1858	ZMBN 105697	Morocco	35	MG270120	MG253082	MG253136	Eilersten <i>et al.</i> , 2017
<i>Melinna albicincta</i> Mackie & Pleijel, 1995	SIO-BICA1113	Trondheimsfjord, Norway	230–280	JX423767	JX423679	JX423649	Stiller <i>et al.</i> , 2013
<i>Melinna cristata</i> (M. Sars, 1851)	ZMBN 95306	Skagerrak, Norway	212	MG270118	MG253102	MG253147	Eilersten <i>et al.</i> , 2017
<i>Melinna cristata</i> (M. Sars, 1851)	NTNU-VM 68699	Trondheimsfjord, Norway	40	—	MG253103	MG253148	Eilersten <i>et al.</i> , 2017
<i>Melinna maculata</i> Webster, 1879	—	Off North Carolina, USA	<200	KY972411	KY972391	—	Bernardino <i>et al.</i> , 2017
<i>Melinna palmata</i>	—	France	—	—	—	KJ182970	Cowart <i>et al.</i> , 2015
<i>Melinnopsis</i> sp.	RUB Msp_01	Antarctica	2057	RUMS096-09	—	—	unpublished
<i>Melinnopsis</i> sp.	RUB Msp_09	Antarctica	2057	RUMS104-09	—	—	unpublished
<i>Melinnopsis</i> sp.	RUB Msp_27	Antarctica	2057	RUMS122-09	—	—	unpublished
<i>Melinnopsis</i> sp.	RUB Msp_28	Antarctica	2057	RUMS123-09	—	—	unpublished
<i>Melinnopsis</i> sp.	RUB Msp_29	Antarctica	2057	RUMS124-09	—	—	unpublished
<i>Melinnopsis chadwicki</i> sp. nov.	AM W50414	off Moreton Bay, Australia	1071–1138	MT556172	MT556641	MT561568	this study
<i>Melinnopsis chadwicki</i> sp. nov.	AM W52949	Coral Sea Marine Park, Australia	1013–1093	MT556174	MT556643	MT561570	this study
<i>Melinnopsis chadwicki</i> sp. nov.	AM W52948	Coral Sea Marine Park, Australia	1013–1093	MT556173	MT556642	MT561569	this study
<i>Melinnopsis gardelli</i> sp. nov.	AM W52539	Jervis Marine Park, Australia	2650–2636	MT556177	MT556646	MT561573	this study
<i>Melinnopsis gardelli</i> sp. nov.	AM W50735	Jervis Marine Park, Australia	2650–2636	MT556175	MT556644	MT561571	this study
<i>Melinnopsis gardelli</i> sp. nov.	AM W51476	Freycinet Marine Park, Australia	2820–2751	MT556176	MT556645	MT561572	this study
<b>Ampharetinae</b>							
<i>Ampharete finnarchica</i> (Sars, 1865)	SIO-BIC A1100	Hornsunddjupet, Svalbard	291	JX423738	JX423670	JX423642	Stiller <i>et al.</i> , 2013
<i>Ampharete octocirrata</i> (Sars, 1835)	SIO-BIC A1109	Hornsunddjupet, Svalbard	271	JX423770	JX423682	JX423652	Stiller <i>et al.</i> , 2013
<i>Amphiteis nionae</i> Jirkov, 1985	ZMBN 95441	Svalbard	340	KX497038	KX513561	—	Kongsrud <i>et al.</i> , 2017
<i>Amphisamytha bioculata</i> (Moore, 1906)	SIO-BIC A2524	San Nicholas Island, CA, USA	400	JX423685	JX423654	JX423634	Stiller <i>et al.</i> , 2013
<i>Amphisamytha carladarei</i> Stiller <i>et al.</i> , 2013	SIO-BIC A2576-7	South Cleft, Juan de Fuca	—	JX423726	JX423664	JX423638	Stiller <i>et al.</i> , 2013
<i>Amphisamytha fauchaldi</i> Solis-Weiss & Hernández-Alcántara, 1994	SIO-BIC A2563	Hydrate Ridge, OR, USA	612	JX423699	JX423658	JX423636	Stiller <i>et al.</i> , 2013
<i>Amphisamytha galapagensis</i> Zottoli, 1983	**	German Flats, East Pacific Rise.	2216	JX423711	JX423662	JX423637	Stiller <i>et al.</i> , 2013
<i>Amphisamytha jacksoni</i> Stiller <i>et al.</i> , 2013	**	German Flats, East Pacific Rise.	2216	JX423758	JX423675	JX423646	Stiller <i>et al.</i> , 2013
<i>Amphisamytha julianae</i> Stiller <i>et al.</i> , 2013	**	North Fiji Basin, W. Pacific	1973	JX423763	JX423676	JX423647	Stiller <i>et al.</i> , 2013
<i>Amphisamytha luzi</i> (Desbruyères & Laubier, 1996)	SIO-BIC A2530	Rainbow, Mid-Atlantic Ridge	2330	JX423736	JX423667	JX423639	Stiller <i>et al.</i> , 2013
<i>Amphisamytha vanuatensis</i> Reuscher <i>et al.</i> , 2009	**	Lau Back-Arc Basin, West Pacific	2655	JX423741	JX423673	JX423645	Stiller <i>et al.</i> , 2013
<i>Anobothrus gracilis</i> (Malmgren, 1866)	SIO-BIC A1106	Trondheimsfjord, Norway	271	JX423739	JX423671	JX423643	Stiller <i>et al.</i> , 2013
<i>Eclysippe vanelli</i> (Fauvel, 1936)	SIO-BIC A1108	Trondheimsfjord, Norway	88	JX423766	JX423678	JX423648	Stiller <i>et al.</i> , 2013
<i>Grassleia</i> cf. <i>hydrothermalis</i> Solis-Weiss, 1993	SIO-BIC A6137	Pinkie's Vent, Gulf of California	1572	KX497032	KX513552	KX513568	Kongsrud <i>et al.</i> , 2017

Table 1 continued on next page ...

**Table 1** [continued from previous page]. Ampharetidae, Terebellidae, Alvinellidae and Scalibregmatidae taxa used in molecular phylogenetic analysis with museum voucher number, sampling location, depth, GenBank accession numbers. Institutional abbreviations used *RUB*, Ruhr-Universität Bochum, *SIO-BIC*, Scripps Institution of Oceanography Benthic Invertebrate Collection; *ZMBN*, Department of Natural History, University Museum of Bergen; *AM*, Australian Museum. Dashes indicate no data available. Single asterisk (\*) indicates sequences omitted from analysis. Double asterisk (\*\*) in the voucher column indicates the entire specimen was used for sequencing.

species	voucher	location	depth (m)	GenBank or BOLD accession number	reference		
				COI	16S	18S	
<i>Paramytha schanderi</i> Kongstrud <i>et al.</i> , 2017	ZMBN 87801	Loki's Castle Hydrothermal Vents	2350	—	KX513556	KX513572	Kongstrud <i>et al.</i> , 2017
<i>Paramytha schanderi</i> Kongstrud <i>et al.</i> , 2017	ZMBN 87820	Loki's Castle Hydrothermal Vents	2350	KX497035	KX513555	KX513571	Kongstrud <i>et al.</i> , 2017
<i>Paramytha schanderi</i> Kongstrud <i>et al.</i> , 2017	ZMBN 87821	Loki's Castle Hydrothermal Vents	2350	—	KX513559	KX513575	Kongstrud <i>et al.</i> , 2017
<i>Paramytha ossicola</i> Queirós <i>et al.</i> , 2017	ZMBN 107232	Setúbal Canyon, Portugal	1000	—	KX513547	KX513563	Kongstrud <i>et al.</i> , 2017
<i>Paramytha ossicola</i> Queirós <i>et al.</i> , 2017	ZMBN 107234	Setúbal Canyon, Portugal	1000	—	KX513549	KX513565	Kongstrud <i>et al.</i> , 2017
<i>Pavelius smileyi</i> Kongstrud <i>et al.</i> , 2017	ZMBN 87807	Loki's Castle Hydrothermal Vents	2350	KX497034	KX513554	KX513570	Kongstrud <i>et al.</i> , 2017
<i>Pavelius smileyi</i> Kongstrud <i>et al.</i> , 2017	ZMBN 87810	Loki's Castle Hydrothermal Vents	2350	KX497036	KX513558	KX513574	Kongstrud <i>et al.</i> , 2017
<i>Pavelius smileyi</i> Kongstrud <i>et al.</i> , 2017	ZMBN 87809	Loki's Castle Hydrothermal Vents	2350	—	KX513557	—	Kongstrud <i>et al.</i> , 2017
<i>Pavelius smileyi</i> Kongstrud <i>et al.</i> , 2017	ZMBN 87825	Loki's Castle Hydrothermal Vents	2350	KX497037	KX513560	KX513576	Kongstrud <i>et al.</i> , 2017
<i>Pavelius uschakovi</i> Kuznetsov & Levenstein, 1988	ZMBN 108241	Okhotsk Sea, Russia	800	KX497033	KX513553	KX513569	Kongstrud <i>et al.</i> , 2017
<i>Samythella neglecta</i> Wollbæk, 1912	ZMBN 99276	Norwegian Sea	823–809	MG270113	KX513551	KX513567	Eilertsen <i>et al.</i> , 2018
<i>Sosane wahrbergi</i> (Eliason, 1955)	SIO-BIC A1118	Gullmarsfjorden, Sweden	66	JX423768	JX423680	JX423650	Stiller <i>et al.</i> , 2013
<i>Sosane wirenti</i> (Hessle, 1917)	ZMBN 95447	Bergen, Norway	98	KX497039	KX513562	KX513577	Kongstrud <i>et al.</i> , 2017
<b>Terebellidae</b>							
<i>Polycirrus carolinensis</i> Day, 1973	SIO-BIC A1101	Curlew Bank, Belize	15–17	JX423769	JX423681	JX423651	Stiller <i>et al.</i> , 2013
<i>Terebella lapidaria</i> Linnaeus, 1767	SIO-BIC A1102	Plymouth, UK	low tide	JX423771	JX423683	JX423653	Stiller <i>et al.</i> , 2013
<b>Alvinellidae</b>							
<i>Alvinella caudata</i> Desbruyères & Laubier, 1986	SIO-BIC A1092	German Flats, E.P.R.	2216	JX423737	JX423669	JX423641	Stiller <i>et al.</i> , 2013
<b>Scalibregmatidae (outgroup)</b>							
<i>Scalibregma inflatum</i> Rathke, 1843	—	Helgoland, Germany	—	—	AY532331	AF448163	Bleidorn <i>et al.</i> , 2003; Bleidorn, 2005

**Table 2.** Primers used for PCR and sequencing.

gene primer	sequence 5'–3'	direction	reference
16S Ann16SF	GCGGTATCCTGACCGTRCWAAGGTA	forward	Sjölin <i>et al.</i> , 2005
16S BrH	CCGGTCTGAACTCAGATCACGT	reverse	Palumbi, 1991
18S 18e	CTGGTTGATCCTGCCAGT	forward	Hillis & Dixon, 1991
18L	GAATTACCGCGGCTGCTGGCACC	reverse	Halanych <i>et al.</i> , 1995
18F509	CCCCGTAATTGGAATGAGTACA	forward	Struck <i>et al.</i> , 2002
18R	GTCCCCTTCCGCAATTCTTTAAG	reverse	Passamaneck <i>et al.</i> , 2004
18F997	TTCGAAGACGATCAGATACCG	forward	Struck <i>et al.</i> , 2002
18R1843	GGATCCAAGCTTGATCCTTCTGCAGGTTACCTAC	reverse	Struck <i>et al.</i> , 2005
TimA	AMCTGGTTGATCCTGCCAG	forward	Norén & Jondelius, 1999
1100R2modified	CGGTATCTGATCGTCTTCCA	reverse	Kupriyanova <i>et al.</i> , 2006
COI polyLCO	GAYTATWTTCAACAAATCATAAAGATATTGG	forward	Carr <i>et al.</i> , 2011
polyHCO	TAMACTTCWGGGTGACCAARAATCA	reverse	Carr <i>et al.</i> , 2011

DNA, 2 µl Coral Load Qiagen PCR buffer, 1.5 µl MgCl<sub>2</sub>, 1.5 µl dNTPs, 0.1 MyTaq DNA Polymerase Bioline and 13.1 µl water, making a total mixture of 20 µl. PCRs were conducted in a Thermal Cycler with the following conditions; COI: 94°C/1 min, 5 cycles 94°/40 s, 45°/40 s, 72°/60 s, followed by 35 cycles 94°/40 s, 51°/40 s, 72°/60 s, and finally 72°/5 min 16S: 94°/3 min, 35 cycles of 94°/30 s, 50°/30 s, 72°/90 s, and finally 72°/7 min 18S: 94°/3 min, 40 cycles of 94°/30 s, 52°/30 s, 72°/30 s and finally 72°/5 min. The quantity of PCR products was detected using gel electrophoresis and visualized using a Bio-Rad XR+ Gel Documentation System. Successful PCR products were sent to Macrogen South Korea where they were purified and standard Sanger sequencing was performed.

### Sequence analysis

Overlapping fragments were assembled into consensus sequences and edited in Geneious Prime 2019.0.4 (<https://www.geneious.com>). A BLAST analysis (Altschul *et al.*, 1990) was performed to confirm the correct region had been amplified, to compare with other sequences on GenBank, and to check for contamination. New sequences were submitted to GenBank (Table 1). Additional sequences from the family Ampharetidae (Melinninae: 6 species and 21 sequences, Ampharetinae: 21 species and 74 sequences) Alvinellidae (1 species 3 sequences) and Terebellidae (2 species, 6 sequences) were downloaded from GenBank (Table 1). Sequences were selected from GenBank using the following criteria suggested by Hutchings & Kupriyanova (2018): voucher specimens were available, collection location information was available, specimens were collected near the type locality, sequences were from published literature and at least two gene fragments were available (except for *Melinnopsis* sp. from Antarctica the only *Melinnopsis* sequences available). One species of Scalibregmatidae (*Scalibregma inflatum*) was used as an outgroup.

Sequences were aligned using the Geneious plugins with the default settings: MAFFT (Katoh *et al.*, 2002) for 16S and 18S and MUSCLE (Edgar, 2004) for COI. Pairwise genetic distances for 16S and COI were calculated in Geneious. Concatenated sequences for all three genes were made in Geneious. JModelTest (Darriba *et al.*, 2012) was used to find the best model using the Akaike information criteria. The model GTR + I + G was selected as the best model for each

gene. Phylogenetic trees were constructed in MrBayes v3.2.6 (Ronquist *et al.*, 2012). The analysis was run for 2,000,000 generations, until the standard deviation of split frequencies was below 0.01 and potential scale reduction factor (PSRF) was 1.0 for all parameters, the first 25% of the generations were discarded as burn-in. Trees were visualized in FigTree v1.4.4 (Rambaut, 2018) and edited in Adobe Illustrator.

## Taxonomy

### *Melinnopsis* McIntosh, 1885

*Melinnopsis* McIntosh, 1885 (including *Amelinna* Hartman, 1969; *Melinnexis* Annenkova, 1931; and *Melinnides* Wesenberg-Lund, 1950) *sensu* Reuscher *et al.*, 2015.

**Type species.** *Melinnopsis atlantica* McIntosh, 1885 (type lodged at the Natural History Museum in London U.K., catalogue number 1885.12.1.330).

**Generic diagnosis.** Large buccal tentacles occurring along with smaller ones. Four pairs of branchiae. Post branchial hooks absent. Brittle acicular neurochaetae in segments II–IV or II–V. Twelve to 14 thoracic uncinigers. Uncini with subrostral process.

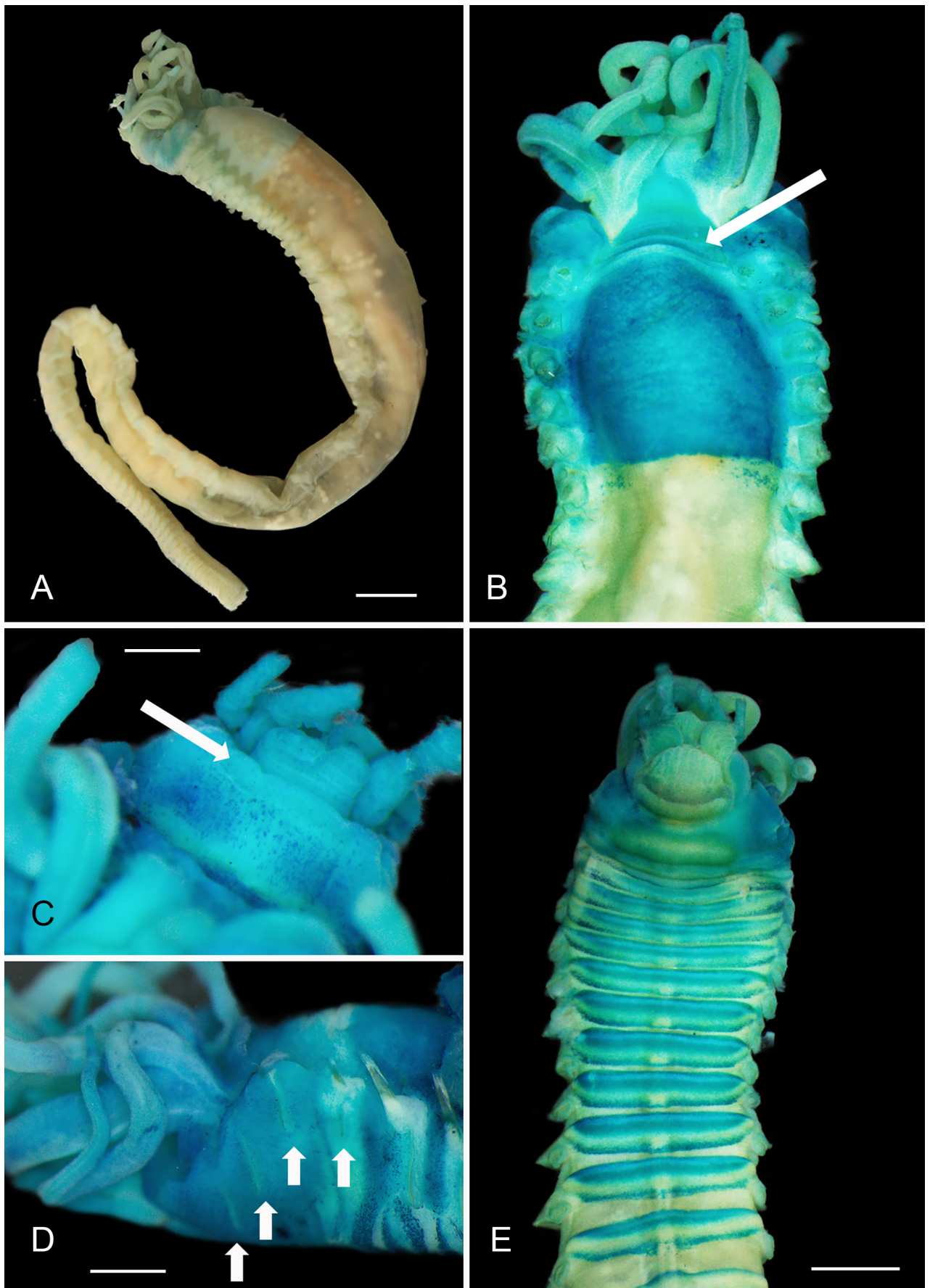
**Remarks.** Our species fit the generic diagnosis of Reuscher *et al.* (2015) well, one long buccal tentacle, four pairs of branchiae, acicular chaetae on segments II–V, 12 thoracic uncinigers, uncini with subrostral process and absence of post branchial hooks (dorsal hooks).

### *Melinnopsis* *gardelli* sp. nov.

urn:lsid:zoobank.org:act:D98E867B-B70F-42C3-AAEA-BD178B01D424

Figs 2–5

**Holotype** incomplete missing part of long buccal tentacle: AM W.50735, IN2017\_V03 operation (OPS) 056, Australia, New South Wales, Jervis Commonwealth Marine Reserve, Beam Trawl (start 35°19'58.8"S 151°15'28.8"E, 2650 m; end 35°19'55.2"S 151°12'50.4"E, 2636 m) 29/5/2017. **Paratypes:** AM W.53131 and AM W.52539 (mounted for SEM), OPS 056 Australia, New South Wales, Jervis Commonwealth Marine Reserve, Beam Trawl (start 35°19'58.8"S 151°15'28.8"E, 2650 m; end 35°19'55.2"S 151°12'50.4"E, 2636 m) 29/5/2017.



**Figure 2.** *Melinnopsis gardelli* sp. nov. light microscope images. (A) holotype (AM W.50735) lateral view of complete specimen; (B) holotype (W.50735) dorsal view of anterior region, arrow indicates postbranchial dorsal membrane; (C) holotype (W.50735) prostomium, arrow indicates slightly raised lip; (D) W.53107 lateral view of anterior region, arrows indicate acicular neurochaetae and (E) holotype (AM W.50735) ventral view of anterior region showing ventral shields. Scale bars: 1 mm.

**Other material examined.** Total 47 specimens. (2 specimens) AM W.51476, W.51480 OPS 004 Australia, Tasmania, Freycinet Commonwealth Marine Reserve, Beam Trawl (start 41°43'51.6"S 149°7'12"E, 2820 m; end 41°47'27.6"S 149°9'21.6"E, 2751 m) 18/05/2017. (2 specimens) AM W.50424 OPS 044 Australia, New South Wales, off Bermagui, Beam Trawl (start 36°21'18"S 150°38'38.4"E, 2821 m; end 36°18'54"S 150°39'3.6"E, 2687 m) 27/05/2017. (38 specimens) AM W.50395, W.50735, W.52987, W.52988, W.50370, W.50394, W.50396, W.53107, W.50398, OPS 056 Australia, New South Wales, Jervis Commonwealth Marine Reserve, Beam Trawl (start 35°19'58.8"S 151°15'28.8"E, 2650 m; end 35°19'55.2"S 151°12'50.4"E, 2636 m) 29/5/2017. (2 specimens) AM W.50736, W.50411 OPS 090 Australia, New South Wales, off Byron Bay, Beam Trawl (start 28°40'37.2"S 154°12'10.8"E, 2587 m; end 28°42'32.4"S 154°11'24"E, 2562 m) 07/06/2017. (1 specimen) AM W.50412, OPS 101 Australia, Queensland, off Moreton Bay, Beam Trawl (start 26°56'45.6"S 153°56'42"E, 2520 m; end 26°58'15.6"S 153°57'3.6"E, 2576 m) 09/06/2017. (2 specimen) AM W.50418, W.50419, OPS 122 Australia, Queensland, Coral Sea Commonwealth Marine Reserve Beam Trawl, (start 23°45'3.6"S 154°38'20.4"E, 2369 m; end 23°46'22.8"S 154°36'57.6"E, 2329 m) 13/06/2017.

**Description (based on holotype).** Holotype 40 mm length for more than 60 chaetigers (Fig. 2A), widest at post-branchial region 4 mm (Fig. 2B), thereafter gradually tapering to abdomen (1 mm width) and pygidium. Thorax with 16 chaetigers; neurochaetae as small acicular spines on segments II–V and uncini on remaining > 56 chaetigers.

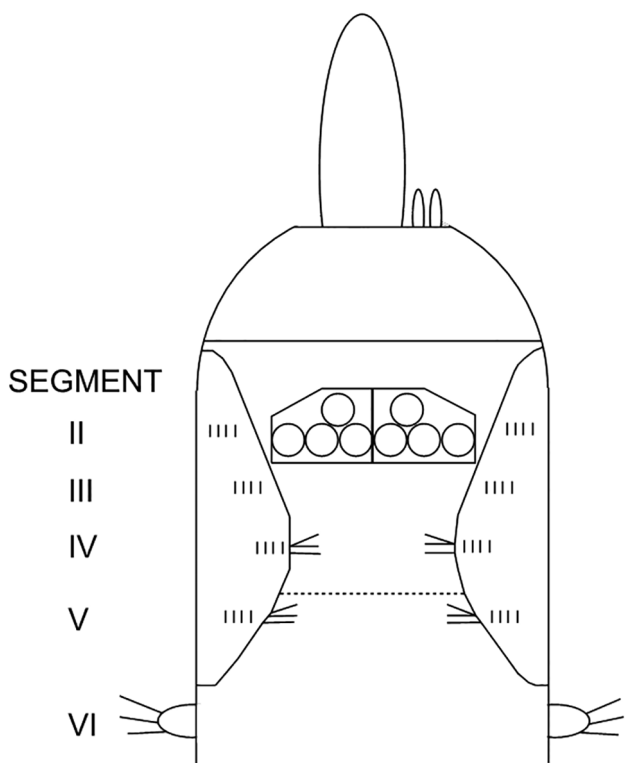
Prostomium with well-defined anterior and posterior sections separated by a pair of deep transverse nuchal slits meeting mid-dorsally (Fig. 2C). Anterior part of prostomium whole, without any distinct lobes, and with a slightly raised lip (Fig. 2C). No eyespots or pigmented glandular bands present. Segments I and II obscured by dorsal branchial ridge. Segment I continued ventrally forming lower margin of mouth with low crenulations on the ventral side, variation in other specimens no crenulations.

Buccal tentacles in holotype one large ridged stump, six damaged smaller tentacles arranged in three pairs arising from large membranous lip (Fig. 2C). Small tentacles smooth and grooved. Variation other specimens, one long buccal tentacle, ridged at base becoming smooth along length, measuring up to 34 mm and 6 smaller tentacles (3 pairs) length around 2 mm length.

Lateral wings of anterior body between prostomium and segment V highly arched (Fig. 2D).

Segment I collar-like, laterally and ventrally encompassing head region. Branchiae emerging together on dorsal branchial ridge at level of segment II–III, arranged in two basally fused groups of four, three branchiae in front and one situated slightly behind (towards the anterior) (Fig. 2B). Inner- and anteriormost branchia of each group joined by low membrane (less than 10% branchial length). Branchiae in cross-section slightly flattened smooth with central groove, gently tapering to filiform tips. Branchiae roughly one fifth the length of longest buccal tentacle. Outer pair of branchiae longest. Variation in other specimens, branchiae circular in cross section.

Postbranchial dorsal membrane low inconspicuous, located on chaetiger 4 (Fig. 2B). Postbranchial hooks absent. Segmentation visible dorsally in postbranchial area. No visible nephridial papillae.



**Figure 3.** Schematic drawing depicting the distinguishing features of the anterior end of *Melinnopsis gardelli* sp. nov.

Notochaetae from segment IV, neurochaetae from segment II (Fig. 3).

Capillary notochaetae present in 14 thoracic chaetigers starting from segment IV (Fig. 2D). In holotype, notopodial chaetae damaged along length of body, only stubs remaining on many chaetigers. In other specimens, chaetiger 3 (segment IV) with few fine notochaetal capillaries and chaetiger 4 (segment V) with more abundant fine notochaeta arising from small slightly projecting notopodia (Fig. 2D). Short cylindrical notopodia with thicker capillaries evident from chaetiger 5. Notochaeta arranged in double rows, those of anterior rows shorter.

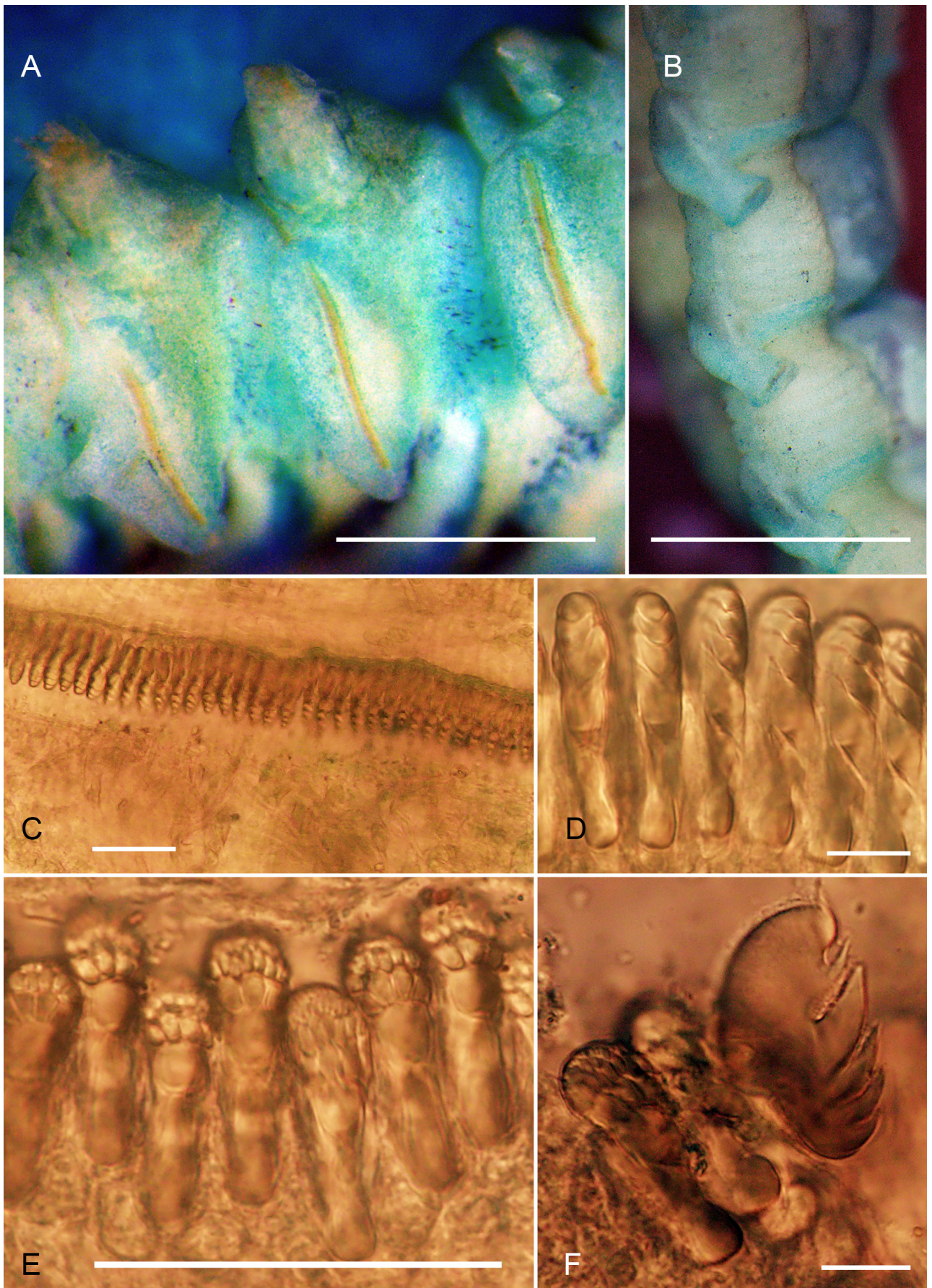
In holotype, abdominal notochaetae lacking. Small, rounded projections, no cilia observed, evident in notopodial positions decreasing in size to pygidium.

Neurochaetae as small acicular spines with lanceolate tips on segment II–V (Fig. 5A). Neuropodial uncini from chaetiger 5 (segment VI), present in 12 thoracic uncini (Fig. 4A). Holotype, incomplete, with more than 40 abdominal uncini. Variation in other specimens, up to 28 abdominal uncini.

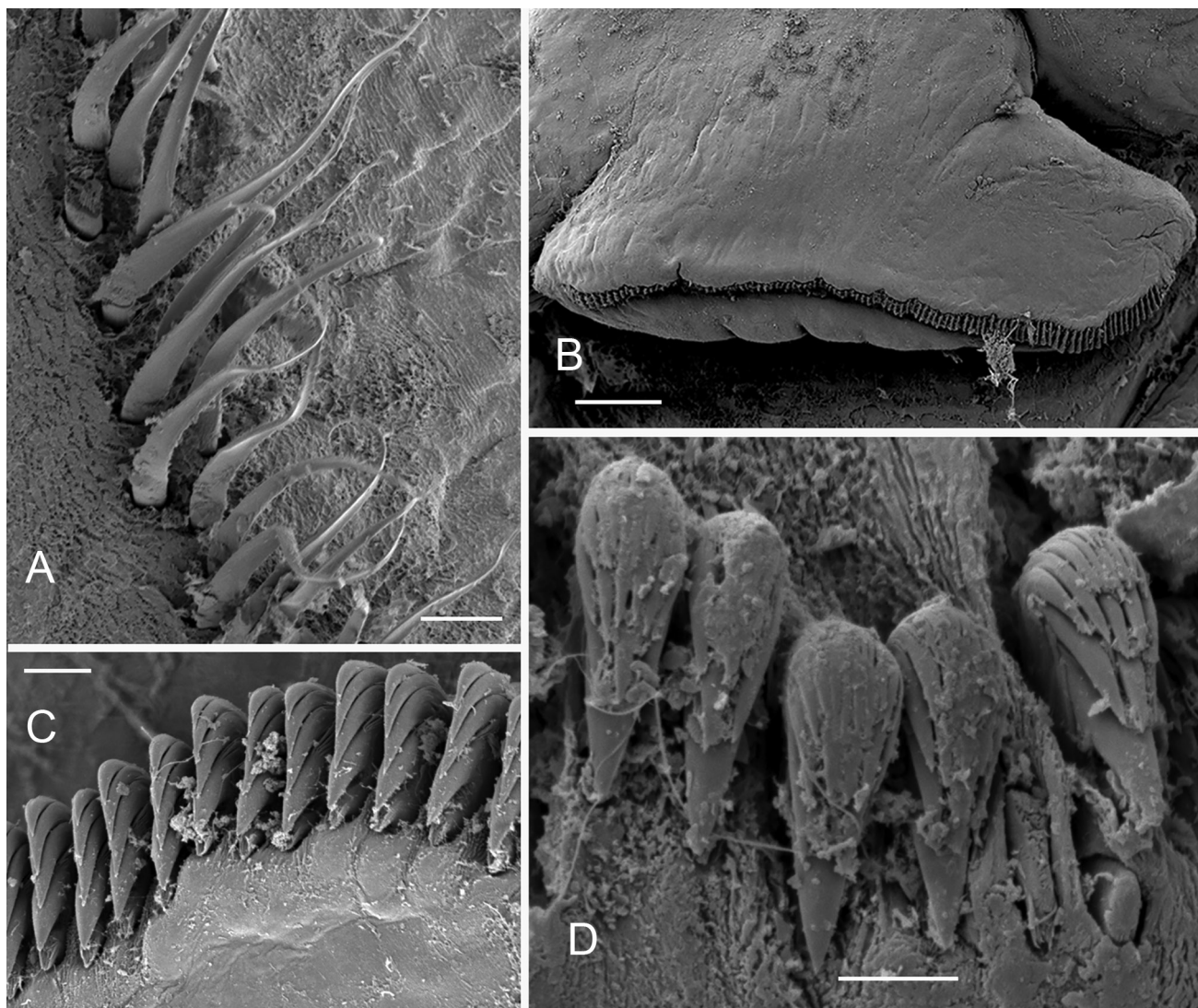
Thoracic uncini emerging subdistally on short flaps from chaetigers 5 to 14, distally more like narrow lappets on chaetigers 15 and 16. Abdominal uncini arranged on narrow lappets (Fig. 4B) decreasing in size until pygidium (minute at the end), similar to last two thoracic chaetigers (15 and 16).

Thoracic uncini in single line with around 65 uncini (Figs 4A, C, D, 5B). Abdominal uncini in single line with around 37 uncini (Fig. 4B, E, F). Uncini of thoracic uncini with three teeth in one vertical row over rostral tooth, subrostral process and basal prow (Figs 4D, 5C). Uncini of abdominal uncini with numerous teeth over rostral tooth, subrostral process and basal prow (Figs 4E, F, 5D).





**Figure 4.** *Melinnopsis gardelli* sp. nov. light microscope images of holotype (W.50735). (A) lateral view of thoracic uncini; (B) lateral view of abdominal uncini; (C) row of thoracic uncini; (D) thoracic uncini; (E) row of abdominal uncini; (F) abdominal uncini. Scale bars: A, B 1 mm; C, E 50 µm; D, F 10 µm.



**Figure 5.** *Melinnopsis gardelli* sp. nov. SEM micrographs of paratype (W.52539). (A) acicular chaetae; (B) thoracic uncinigerous pinnule (neuropodia); (C) thoracic uncini; (D) abdominal uncini. Scale bars: A 20  $\mu$ m; B 100  $\mu$ m; C, D 10  $\mu$ m.

Pygidium missing in holotype. Other specimens, terminal crenulated anus, bounded dorsolaterally by 8 small indistinct lobes. No anal cirri.

**Methyl blue staining pattern.** Use of methyl blue reveals in holotype strong staining of prostomium except nuchal slits, strong staining segments I to IV, branchiae lightly speckled along edges, postbranchial membrane (Fig. 2B). Conspicuous stained band immediately behind dorsal fold ending between chaetigers 9 and 10 (Fig. 2A, B). Stained band region shorter in other specimens. Speckled staining laterally between chaetiger 5 to end of thorax. Stained bands (anterior/ posterior direction) on prostomium ventral lobe (Fig. 2E). Strong staining around thirteen ventral shields, staining strong anterior section of ventral shield light staining posterior section of shield, shields cover entire ventral surface of the segment (Fig. 2E). Abdomen staining weak, mainly as light speckles on dorsal side of neuropodial lappets and on small rounded projections in notopodial position.

**Tube.** Missing in holotype. Some specimens have fine-grained sediment tube with some thin green veins running throughout others not, others with Foraminifera. The tube is lined with a thin, stiff clear membrane. Length of tube at least twice as long as specimens.

**Distribution.** Coral Sea Marine Park to Freycinet Commonwealth Marine Reserve, Tasmania. Eastern Australia.

**Etymology.** The new species is named *gardelli* after Rickard Gardell for his generous donation to the Australian Museum Research Institute.

**Remarks.** The new species has 12 thoracic uncinigers, like nine other species of *Melinnopsis* (*M. abyssalis*, *M. annenkovae*, *M. arctica*, *M. chadwicki* sp. nov., *M. collaris*, *M. dubita*, *M. monocera*, *M. rostrata* and *M. somovi*) (Table 3). Buccal tentacles of *M. abyssalis* are all free at base, those of *M. gardelli* sp. nov. are fused. The original description of *M. annenkovae* is brief (Uschakov, 1952) and it is difficult to

draw morphological comparisons between *M. annenkovae* and *M. gardelli* sp. nov. *Melinnopsis annenkovae* possesses a “well-developed glandular band” on the fourth dorsal segment, but no images or further details are provided, in *M. gardelli* sp. nov. a glandular band was absent, but a post-branchial dorsal membrane was present on chaetiger 4. *Melinnopsis annenkovae* has a triangular shaped buccal tentacle while that of *M. gardelli* sp. nov. is rounded, although tentacle shape may vary within species. *Melinnopsis gardelli* sp. nov. differs from *M. arctica* by the absence of papillae on the large buccal tentacle. *Melinnopsis collaris* is described as having “a large, thin, foliaceous collar about the sides and ventrum to conceal the peristomium” (Hartman, 1967), this foliaceous collar is not present in *M. gardelli* sp. nov. *Melinnopsis dubita* has multiple long buccal tentacles instead of one long one. *Melinnopsis monocera* aligns well with *M. gardelli* sp. nov., but differs by the shape of thoracic uncini, *M. monocera* has two teeth above the rostral tooth whereas *M. gardelli* sp. nov. has three. *Melinnopsis rostrata* possesses a denticulated transverse membrane which is absent in *M. gardelli*. *Melinnopsis somovi* has three pairs of branchiae, whereas *M. gardelli* sp. nov. has four pairs. The new species has a conspicuous stained band on the dorsal area when stained with methyl blue, which has not been noted in any other species.

### *Melinnopsis chadwicki* sp. nov.

urn:lsid:zoobank.org:act:FD5DA304-2BBB-4CB3-85F3-11D285CA149E

#### Figs 6–7

**Holotype** incomplete missing part of long buccal tentacle: AM W.52950, IN2017\_V03 operation (OPS) 104 Australia, Queensland, off Moreton Bay, Beam Trawl (start 26°57'39.6"S 153°50'52.8"E, 1071 m; end 26°59'27.6"S 153° 50'49.2"E, 1138 m) 10/06/2017. **Paratypes** mounted for SEM AM W.52537 and AM W.52538, OPS 104 Australia, Queensland, off Moreton Bay, Beam Trawl (start 26°57'39.6"S 153°50'52.8"E, 1071 m; end 26°59'27.6"S 153°50'49.2"E, 1138 m) 10/06/2017.

**Other material examined.** Total 58 specimens. (17 specimens) AM W.50417, W.50416, W.52980, W.52949, W.52979, W.50737, W.52948, W.52999, W.53000 OPS 121 Australia, Queensland, Coral Sea Commonwealth Marine Reserve, Beam Trawl (start 23°35'13.2"S 154°11'38.4"E, 1013 m; end 23°37'1.2"S 154°11'42"E, 1093 m) 13/06/2017. (4 specimens) AM W.50404, W.52997, W.50406, W.50405 OPS 080 Australia, New South Wales, Central Eastern Commonwealth Marine Reserve, Beam Trawl (start 30°5'56.4"S 153°35'45.6"E, 1257 m; end 30°7'40.8"S 153°34'15.6"E, 1194 m) 05/06/2017. (36 specimens) AM W.50415, W.50414, W.52981, W.52950, W.52951, W.52965, W.52975, W.52955, W.52961, W.52958, W.52973, W.52954, W.52956, W.52952, W.52953, W.52969, W.52976, W.52959 OPS 104 Australia, Queensland, off Moreton Bay, Beam Trawl (start 26°57'39.6"S 153°50'52.8"E, 1071 m; end 26°59'27.6"S 153°50'49.2"E, 1138 m) 10/06/2017. (1 specimen) W.50403 OPS 069 Australia, New South Wales, Hunter Commonwealth Marine Reserve, Beam Trawl (start 32°28'44.4"S 152°59'38.4"E, 1006 m; end 32°30'25.2"S 152°59'27.6"E, 1036 m) 03/06/2017.

**Description (based on holotype).** Holotype 22 mm length for more than 55 chaetigers, widest at post-branchial region 1 mm (Fig. 6A), thereafter gradually tapering to abdomen (0.5 mm width) and pygidium. Thorax with 16 chaetigers, neurochaetae as small acicular spines on segments II to V and uncini on remaining 46 chaetigers.

Prostomium with well-defined anterior and posterior sections separated by a pair of deep transverse nuchal slits that meet mid-dorsally (Fig. 6C). Pigmented glandular bands above nuchal slits (Fig. 6C). Anterior part of prostomium whole, without any distinct lobes, and with a slightly raised lip. No eyespots present. Segments I and II obscured by branchial ridge. Segment I continued ventrally to form lower margin of the mouth no crenulations.

Buccal tentacles in holotype one short stump, three small tentacles, arising from a large membranous lip (Fig. 6B). Small tentacles smooth and grooved. In other specimens, one long buccal tentacle, smooth along length, twisted and ventrally grooved (Fig. 7A), generally around 5 mm in length. In many specimens long buccal tentacle broken off, four smaller tentacles (three and one on each side) although probably six in total (two pairs of three).

Lateral wings of anterior body between prostomium and segment V highly arched (Fig. 6B).

Segment I collar-like, laterally and ventrally encompassing head region. Branchiae emerging together on dorsal branchial ridge at level of segment II–III (Fig. 7A), arranged in two basally fused groups of four, three branchiae in front and one pair slightly behind (towards anterior). Inner- and anteriormost branchia of each group not joined by membrane. Branchiae circular in cross section, slightly ridged (Fig. 7A), dorsal groove, gently tapering to filiform tips. Branchiae roughly one third the length of longest buccal tentacle. Outer pair of branchiae longest (Fig. 6B).

Postbranchial dorsal membrane not visible. Postbranchial hooks absent. Segmentation not visible dorsally in postbranchial area. No visible nephridial papillae.

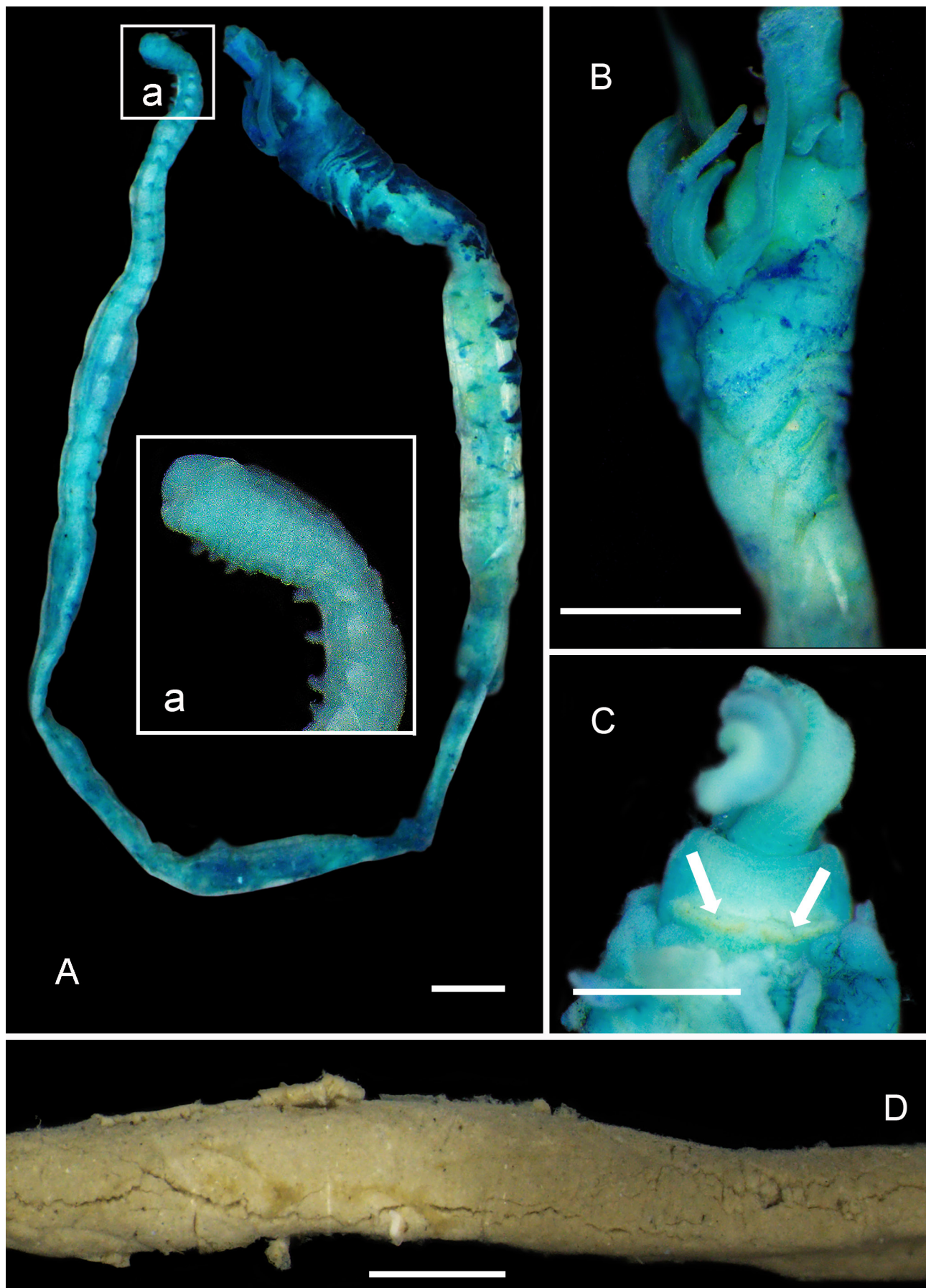
Capillary notochoetae present in 14 thoracic chaetigers, starting from segment IV. In holotype, anterior end notopodial chaetae damaged. In paratypes, chaetiger 3 (segment IV) with few fine notochoetal capillaries and chaetiger 4 (segment V) with more abundant fine notochoetae arising from body wall. Short, cylindrical notopodia with thicker capillaries starting from chaetiger 6. Notochoetae arranged in double rows, roughly the same length. Microfiber ends on notochoetae visible (Fig. 7C).

Abdominal notochoetae lacking. No small, papilliform projections evident in notopodial positions.

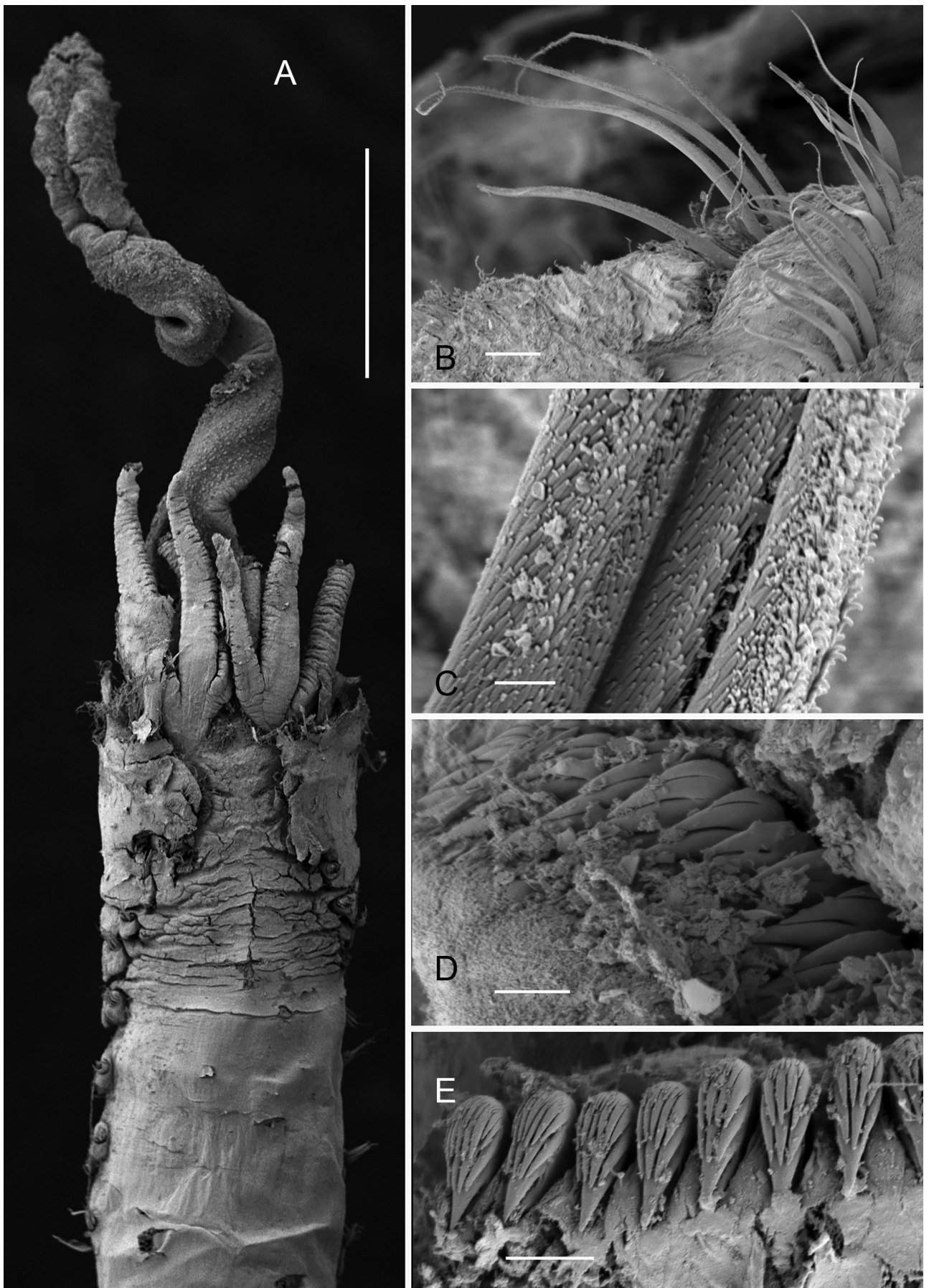
Neurochaetae as small acicular spines with lanceolate tips, on segments II to V (Fig. 7B). Neuropodial uncini from chaetiger 5 (segment VI) present in 12 thoracic uncinigers. Holotype complete with more than 40 abdominal uncinigers.

Holotype damaged at end of thorax beginning of abdomen. In other specimens, thoracic uncini emerge subdistally on short flaps until chaetiger 16, distally on narrow lappets on chaetigers 17 and 18. In holotype, abdomen uncini arranged on narrow lappets decreasing in size until pygidium, similar to last two thoracic chaetigers.

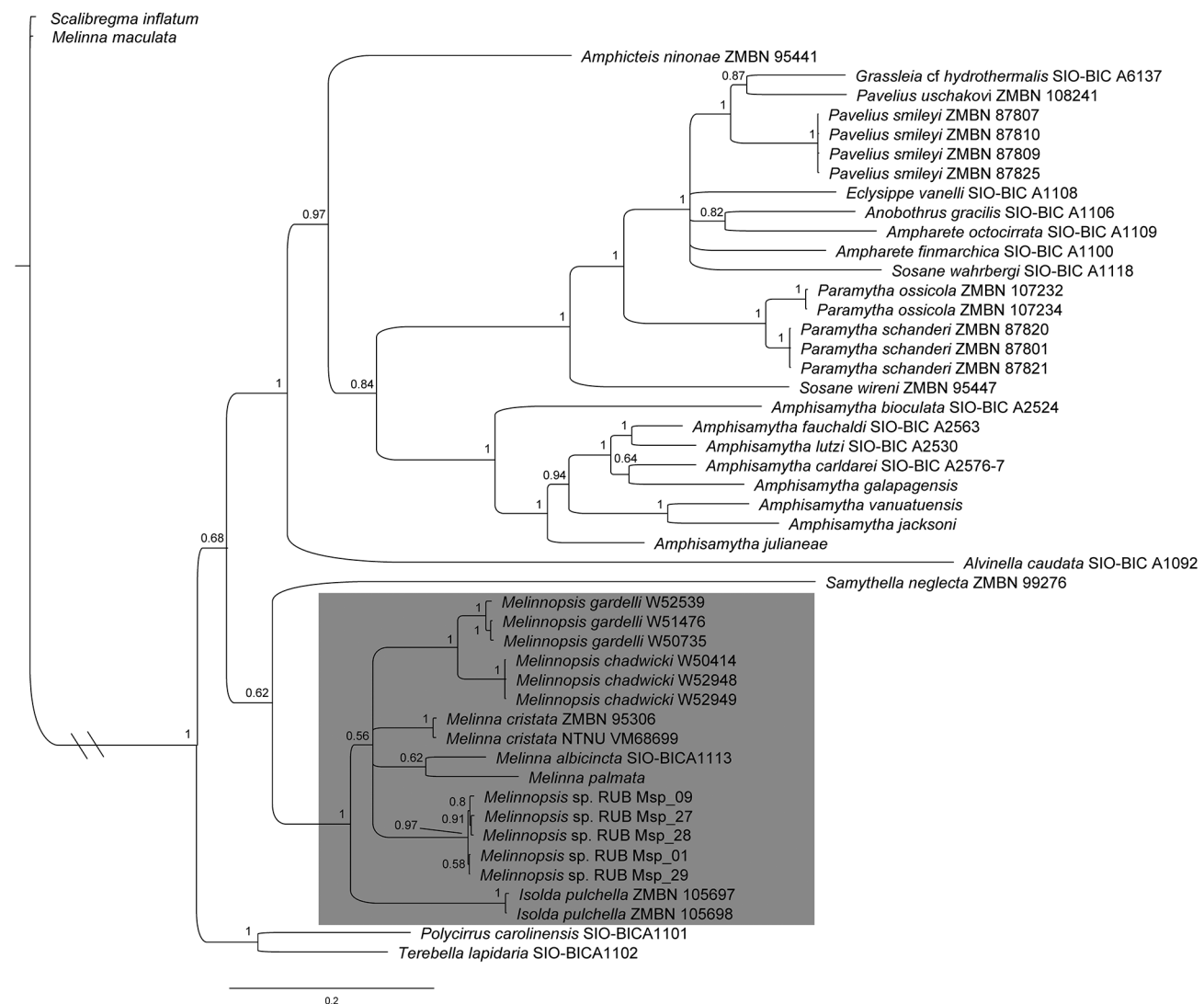
Thoracic uncini in single line of around 43 (Fig. 7D). Abdominal uncini in a single line of 14 (Fig. 7E). Uncini of thoracic uncinigers with two teeth in one vertical row over rostral tooth, subrostral process and basal prow (Fig. 7D). Uncini of abdominal uncinigers with numerous teeth over



**Figure 6.** *Melinnopsis chadwicki* sp. nov. light microscope images. (A) holotype (AM W.52950) lateral view of entire specimen, (a) pygidium; (B) holotype lateral view of anterior part; (C) AM W.52981 prostomium, arrows indicate transverse nuchal organs; (D) holotype sediment tube. Scale bars: A, B 1 mm; C 0.5 mm; D 2 mm.



**Figure 7.** *Melinnopsis chadwicki* sp. nov. SEM micrographs of paratypes (W.52537 and W.52538). (A) AM W.52538 dorsal view of anterior section; (B) W.52538 acicular chaetae segment 3 and 4; (C) W.52537 close up of notochaetae microfiber endings; (D) W.52537 thoracic uncini; (E) W.52537 abdominal uncini. Scale bars: A 1 mm, B 20  $\mu$ m, C 2  $\mu$ m, D 5  $\mu$ m, E 10  $\mu$ m.



**Figure 8.** Phylogeny obtained from Bayesian analysis of combined dataset of COI, 16S and 18S in MrBayes. Node labels show posterior probabilities. Scale bar represents 0.2 substitutions per site. The branch bearing double hatch marks indicates that it has been truncated and is not proportional to the rest of the diagram. The original length of the truncated branch was approximately 0.64 substitutions. Grey box highlights the sequences from species within the subfamily Melinninae.

rostral tooth, subrostral process and basal prow (Fig. 7E).

Pygidium with terminal crenulated anus, bounded by 4 small indistinct lobes. No anal cirri. (Fig. 6a).

**Methyl blue staining pattern.** Use of methyl blue in holotype revealed light staining of prostomium except nuchal slits. Stained bands (anterior/ posterior direction) on prostomium ventral lobe absent, however stained band along anterior edge of prostomium ventral lobe present. Strong staining in segments I to IV. Light staining branchiae. Variation, specimens occasionally have stained dorsal banded region as in *M. gardelli* sp. nov., but less distinct and until chaetiger 5. Light speckling between thoracic chaetigers. Stained ventral shields, shields cover entire ventral surface of the segment. Indistinct number of shields on holotype due to damage. Light speckles staining on abdomen. No staining on neuropodial lappets.

**Tube.** In holotype, sediment tube with small green veins

running throughout and Foraminifera attached (Fig. 6D). Tube similar length to the specimen. Tube varies among specimens, may not have green veins and Foraminifera attached.

**Distribution.** Coral Sea Marine Park to Hunter Commonwealth Marine Reserve. Eastern Australia

**Etymology.** This species is named after Clarence (Clarry) Chadwick for his endowment that supports the Chadwick Biodiversity Fellowship at the Australian Museum Research Institute.

**Remarks.** *Melinnopsis chadwicki* sp. nov. appears to be closely related to *M. gardelli* sp. nov. by acicular neurochaetae on segments II–V, one large buccal tentacle and multiple smaller ones, four branchiae and branchial arrangement and 12 thoracic uncini, however, it can be differentiated from the latter by the shape of the thoracic uncini which have 2 rather than 3 teeth above the rostral

tooth, the distinct presence of pigmented glandular bands above nuchal slits and the lack of a conspicuous stained band ending between chaetigers 9 and 10. The two species are found at different depths: *M. chadwicki* sp. nov. around 1000 m and *M. gardelli* sp. nov. around 2500 m. The difference between new species of *Melinnopsis* and others with 12 thoracic uncini are discussed in the remarks of *M. gardelli* sp. nov. above and in Table 3.

## Molecular results

Bayesian analysis of combined dataset of COI, 16S and 18S sequence data (Fig. 8) inferred two major poorly supported (posterior probability, pp 0.68) clades within Ampharetidae. The first strongly supported (pp 1.0) clade included taxa typical for Ampharetinae as a sister taxon to the alvinellid *Alvinella caudata*. Within the Ampharetinae clade, *Amphicteis ninona* is a sister taxon to a clade comprising two major ampharetin clades (pp 0.97). The monophyletic *Amphisamytha* (pp 1.0) clade is a sister group to the clade comprising other typical ampharetins (*Sosane-Paramytha-Ampharete-Anobothrus-Eclysippe-Pavelius-Grassleia*). Within the latter large ampharetin clade, *Paramytha* constitutes a well-supported monophyletic group (pp 1.0) which is sister to well-supported (pp 1.0), but poorly resolved *Sosane-Ampharete-Anobothrus-Eclysippe-Pavelius-Grassleia* clade. Within the latter clade there is a four-way polytomy comprising clades *Sosane-Ampharete*, (*Anobothrus-Ampharete*), *Eclysippe*, and non-monophyletic *Pavelius* that has *Grassleia* nested within.

The second major clade within Ampharetidae includes the terminals attributed to Melinninae (*Isolda*, *Melinna*, and *Melinnopsis*), but also includes an ampharetin *Samythella neglecta* Wollebaek, 1912 as clade that forms a sister group (pp 0.62) to a well-supported (pp 1.0) monophyletic, but poorly resolved clade comprising all other melinnins. Monophyletic *Isolda pulchella* (pp 1.0) forms a poorly supported (pp 0.56) clade with an unresolved melinnin clade. The latter clade is a four-way polytomy that includes clades *Melinna cristata* (pp 1.0), *Melinna albicincta* + *Melinna palmata* (pp 0.62), *Melinna* sp. (pp 0.91) and a well-supported (pp 1.0) clade comprising sister *Melinnopsis chadwicki* sp. nov. (pp 1.0) and *M. gardelli* sp. nov. (pp 1.0). The terebellid clade (*Polycirrus caroliensis* + *Terebella lapidaria*) (pp 1.0) was recovered as a sister to all ampharetids (including an alvinellid) with high support (pp 1.0). *Melinna maculata* was recovered as a taxon closely related to the outgroup *Scalibregma inflatum*.

The COI intraspecific genetic distances within *M. chadwicki* sp. nov. ranged 0.3–0.5% and within *M. gardelli* sp. nov. ranged 1.2–3.7%. The mean interspecific genetic distance between *M. chadwicki* sp. nov. and *M. gardelli* sp. nov. was 13.9%. The single closest COI sequence of both *Melinnopsis chadwicki* sp. nov. and *M. gardelli* sp. nov. was *Melinnopsis* sp. sequence RUMS122-09 (18.4% difference). The 16S intraspecific genetic distance between *M. chadwicki* sp. nov. was 0–0.3% and between *M. gardelli* sp. nov. 0–0.3%. The mean interspecific genetic distance between *M. chadwicki* sp. nov. and *M. gardelli* sp. nov. was 6.6%. The closest 16S sequence to both *M. chadwicki* sp. nov. and *M. gardelli* sp. nov. was *M. cristata* sequence NTNU-VM 68699 (18.7% difference).

## Discussion

*Melinnopsis gardelli* sp. nov. and *M. chadwicki* sp. nov. are the first two species of *Melinnopsis* described from Australian waters. Previously, two specimens of *Melinnopsis* sp. were reported from two localities sampled from 2000–3000 m during deep-water research voyages in 2013 (SS2013\_C02) and 2017 (RE2017\_C0I) to the Great Australian Bight (GAB), South Australia (MacIntosh *et al.*, 2018, *Atlas of Living Australia*). Unfortunately, we could not confirm whether these GAB specimens matched our species as the material was in poor condition and formalin fixed, meaning no further morphological or molecular studies could be conducted.

*Melinnopsis gardelli* sp. nov. and *M. chadwicki* sp. nov. appear to have distinct, non-overlapping bathymetric ranges, as *M. gardelli* sp. nov. is recorded from 2520–2821 m depth and *M. chadwicki* sp. nov. from 1006–1257 m depth. Thirteen of the currently accepted 16 *Melinnopsis* species were described from below 1000 m and all the other species were described from below 100 m (Table 3). Solis-Weiss (1993) suggested that Melinninae are generally restricted to deeper waters, this holds true for *Melinnopsis* but not for all genera because species of *Melinna* and *Isolda* are known from shallower depths (for example, *Melinna palmata* occurs in high densities around 10–15 m in the English Channel (Kempf *et al.*, 2002) and *Isolda albula* Mohammad, 1971 was described from intertidal areas in Kuwait).

The species reported here agree well morphologically with the most recent generic diagnosis of *Melinnopsis* by Reuscher *et al.* (2015), which includes the presence of one very long buccal tentacle. This important morphological feature was not reported, either broken off or never present in the original description of *M. atlantica*, and McIntosh (1885) only notes that a “proboscis protrudes”. It is in Holthe (1986a) that tentacle size is used for the first time to distinguish *Melinna* from *Melinnopsis*, the former having tentacles of uniform size and the latter having tentacles of two sizes. Holthe (1986a) goes on to mention “one very large and several small tentacles” in the key for *Melinnopsis arctica* (previously *Melinnexis arctica*) and *M. annenkovae* (previously *Melinnexis annenkovae*). This characteristic large buccal tentacle is likely a feature that has been incorporated into the generic diagnosis after the synonymization by Day (1964) of *Melinnexis* and *Melinnides* with *Melinnopsis* because it is not a feature of the genus *Melinnopsis* according to McIntosh’s (1885) original description of *Melinnopsis atlantica*. Examination of the holotype of *M. atlantica* lodged at the Natural History Museum in London revealed that the specimen was badly damaged and lacked a large buccal tentacle (M. Georgieva pers. comm.). A revised morphological description of *M. atlantica* is needed along with molecular data, which is not possible using the holotype due to its poor condition and the fact that it was collected over 100 years ago. Consequently, specimens from the type locality should be collected, a neotype designated, examined and sequenced, as performed e.g., for *Hydroides brachyacantha* Rioja, 1941 in Sun *et al.*, (2016). This will allow a revision of the generic diagnosis and eventually of the entire genus *Melinnopsis*.

The two new species are morphologically similar but display differences in the shape of thoracic uncini and

**Table 3.** Branchiae number and arrangement—comparison of all known species of *Melinnopsis* McIntosh, 1885 (after Hilbig, 2005: table 3). Key to footnotes on facing page.

species	no. of TU	dorsal membrane segment <sup>5</sup>	buccal tentacles	branchiae	no. of teeth above rostral tooth in TU	body size, length: width (mm)	tube	type locality
<i>M. abyssalis</i> (Hartman, 1969)	12	Absent	2 types: 1 large, many small	4 pairs: 1 anterior middle pair largest, 3 pairs in a crescent shape	3	52 mm : 3 mm	135–150 mm long, 3–4 mm wide, tapering slightly, smooth, dark silt	San Clemente basin, NE Pacific, 1920 m
<i>M. angolensis</i> Hilbig, 2005	13	Present: serrated (up to 21 teeth)	2 types: 4–6 large, 6 small	4 pairs: 1 anterior middle pair largest	2	21–50 mm : 3–4 mm	Up to 3 times length of worm, muddy with fine inner mucus lining	Angola Basin, SE Atlantic, 5385–5439 m
<i>M. annenkovae</i> (Uschakov, 1952)	12	Absent <sup>6</sup>	2 types: 1 large > 7 mm, 3–4 small	4 pairs: 1 median pair largest	3 (5 teeth in one row)	— : 3 mm	Sturdy silted tube	Arctic Ocean, 51–1900 m
<i>M. atlantica</i> * McIntosh, 1885	14?	Absent	— <sup>6</sup>	4 pairs: unclear <sup>c</sup>	3 (top tooth indistinct) <sup>0</sup>	35 mm : 3 mm	Stiff cylinder, fine grey mud, Foraminifera attached	Off Chesapeake Bay, NW Atlantic, 3109 m
<i>M. arctica</i> (Annekova, 1931)	12	Present/indistinct <sup>6</sup>	2 types: 1 large, 2 small	4 pairs: anterior (inner) larger than others	2	25 mm : 3 mm	Solid tube <sup>1</sup> covered in sand in the front part	Arctic Ocean 165–480 m
<i>M. arripatens</i> (Moore, 1923)	(13?) <sup>6</sup>	Absent/indistinct	2 types: 1 large (12 mm long 8 mm wide), few small (1 mm long)	4 pairs: anterior (largest by one-third)	3	31 mm : 1.3 mm	—	Santa Catalina Islands, NE Pacific, 4070 m
<i>M. augeneri</i> Reuscher et al. 2015	13	Indistinct, no serration	2 types: 4 long thick and annulated, 3 small	4 pairs: arranged in continuous arch	2	14 mm : 0.8 mm	—	Goto-Kasayama Bank, west off Kyushu, 185 m
<i>M. chaadwicki</i> sp. nov.	12	Absent/indistinct	2 types: 1 long, 6 small	4 pairs: 1 pair slightly anterior	2	22 mm : 1 mm	Fine-grained sediment sometimes with green veins and Foraminifera	Eastern Australia, 1006–1257 m
<i>M. collaris</i> (Hartman, 1967)	12	Absent	2 types: 1 large, many small	4 pairs: crescent shape, 1 pair anterior	—	46–51 mm : 4.6 mm	Long, tough, covered with silt, internal membrane	Mid-Pacific Basin, 4041–4813 m
<i>M. dubita</i> (Hoagland, 1920)	12?	Indistinct ridge	2 types: 6 tentacles up to 15 mm, many smaller 3–7 mm	4 pairs: unclear	3 <sup>H</sup>	15 mm : 7 mm	Fine brown mud	Mindanao, Philippines, 920 m
<i>M. gardelli</i> sp. nov.	12	Indistinct	2 types: 1 long 34 mm and shorter tentacles 2 mm	4 pairs: 1 pair anterior	3	40 mm : 4 mm	Fine-grained sediment sometimes with green veins and Foraminifera	Eastern Australia, 2520–2821 m
<i>M. mcintoshii</i> Reuscher, Fiege & Imajima, 2015	13	Present: smooth	2 types: 3 long thick, annulated, 4 thinner	4 pairs: 2 rows of 2	2	44 mm : 2 mm	—	Japan Pacific Ocean, 164–5600 m
<i>M. monocera</i> (Augener, 1906)	12	Indistinct	2 types: 1 long (length 26 anterior segments), 6 short	4 pairs: unclear	2	28–42 mm : 2 mm	—	Caribbean, 212–310 m
<i>M. moorei</i> (Hartman, 1960) using Moore, 1923	13?(17 TC)	Present: slightly serrated	—	4 pairs: cluster on each side	2	80 mm : —	Heavy mud walls	Off Santa Catalina and San Miguel Islands, NE Pacific, 495–3990 m
<i>M. rostrata</i> (Wesenberg-Lund, 1950)	12	Present: 15–17 teeth	2 types: 5 long curled, 3 shorter	4 pairs: 1 pair anterior	—	72 mm : —	—	West of Greenland, Arctic, 3229 m
<i>M. somovi</i> (Uschakov, 1957)	12	Absent	2 types: 1 large, some small	3 pairs: internal largest	2	15 mm : 1 mm	Silt with Foraminifera shells and small stones	Arctic, 1239–1694 m
<i>M. tentaculata</i> (Treadwell, 1906)	—	—	2 types: 1 large length of anterior region of body, 2 or 3 smaller	4 pairs: 2 rows, outer pair largest	3	9 mm : 1.5 mm	Thick mud tube with sponge spicules	Hawaii, 508–1358 m
<i>M. tetradentata</i> (Imajima, 2001)	13	Present: serrated 14 dentations	2 types: 1 long trihedral. Multiple shorter ones	4 pairs: 2 rows of 2	2	70 mm : 4 mm	Thick, dark, fine-grained mud particles, thin inner membrane	Tosa bay, Japan, 400–800 m



**Table 3 footnotes** (see facing page). Branchiae number and arrangement—comparison of all known species of *Melinnopsis* McIntosh, 1885 (after Hilbig, 2005: table 3).

A	Well-developed glandular band on segment four.
B	McIntosh, 1885 notes a “proboscis protrudes”.
C	McIntosh, 1885 states branchiae are “four in number, and arranged in a similar way to <i>Melinna</i> ” we interpret this to be two groups of four as in <i>Melinna cristata</i> . He also stated branchiae are proportionally shorter than in <i>Melinna</i> .
D	Assuming plate XXVIIA, fig. 18, of McIntosh, 1885, is thoracic.
E	In comparative remarks Annekova (1931) remarks an absence of dorsal fold in <i>M. monocera</i> , indicating dorsal fold is present in <i>M. artica</i> , “dorsal fold” may be the same as a dorsal membrane.
F	Tube like <i>Melinna cristata</i> .
G	TU number not given in original description of Moore, 1923, 13 TU in Hilbig (2005).
H	Assuming plate 51, fig. 5, of Hoegland, 1920, is thoracic.
TC	Thoracic chaetigers.
TU	Thoracic uncini.
*	Type species.
—	information not given in original description.

presence of glandular bands above nuchal slits, as well as in methyl blue staining patterns. *Melinnopsis gardelli* sp. nov. had a conspicuous stained band ending between chaetigers 9 and 10. Reuscher *et al.* (2015) used position of branchiae and lateral wings (collar-like extension of anterior segments from prostomium to segment V), number of teeth above rostral tooth (a new character suggested) and presence of abdominal type uncini in the last thoracic chaetiger to delineate species. We suggest that methyl blue or green staining pattern is a useful characteristic for species identification in *Melinnopsis*, a character that is already used for other ampharetid genera identification (Jirkov, 2011; Alvestad *et al.*, 2014; Kongsrud *et al.*, 2017; Mackie & Pleijel, 1995) and is reversible.

This is the first study to include molecular data in the description of a *Melinnopsis* species. The molecular data agree with the morphological finding that *M. gardelli* sp. nov. and *M. chadwicki* sp. nov. are two separate species and differ from all other species. The COI pairwise genetic distances between our two species (13.9%) are similar to those found in other studies between ampharetids. In Kongsrud *et al.* (2017), *Pavelius smileyi* Kongsrud, Eilertsen, Alvestad, Kongshavn & Rapp, 2016 and the closest related species, *Ampharete octocirrata* (Sars, 1835), had a COI pairwise genetic distance of 14.6%, Zhou *et al.* (2019) found COI GTR corrected distances between *Amphisamytha* species ranged 11.9–40.3%. In a study by Carr *et al.* (2011) on 1876 polychaetes across 333 provisional species from 36 families, including Ampharetidae, interspecific COI sequence divergence was slightly higher (average 16.5%) than our results. For 16S, genetic distances between our two new species (6.6%) were lower compared with the results of Kongsrud *et al.* (2017) (*Pavelius smileyi* and *Pavelius uschakovi* Kuznetsov & Levenstein, 1988—genetic distance 15%, *Paramytha schanderi* Kongsrud, Eilertsen, Alvestad, Kongshavn & Rapp, 2017 and *Paramytha* sp.—genetic distance 17.6–19.4%).

The results of our study support the inclusion of *Melinnopsis gardelli* sp. nov. and *M. chadwicki* sp. nov. within the group of annelids morphological defined as Melinninae. Unfortunately, the data do not provide enough resolution to support or reject the monophyly of the genera *Melinna* and *Melinnopsis*. Even if all available sequences of

*Melinnopsis* did form a well-supported clade, without data from the type species it would remain uncertain whether these taxa belong to *Melinnopsis*.

The subfamily Melinninae here was recovered as monophyletic. Our results are supported by a recent phylogeny of Terebelliformia which used five genetic markers, 90 morphological characters and a transcriptome phylogeny backbone to construct a maximum likelihood tree of 121 species (Stiller *et al.*, 2020). The study recovered all melinnins (6 species) as a monophyletic group and further suggested Melinninae become Melinnidae (Stiller *et al.*, 2020), however, the new family status has not yet been widely accepted (Ebbe & Purschke, 2019; Read & Fauchald, 2020). In our study, all sequences fell into a well-supported clade, except for the sequences for *Melinna maculata* (Melinninae) that was recovered as being closely related to the outgroup *Scalibregma inflatum* Rathke, 1843. While the sequences of *M. maculata* were sourced from GenBank (Table 1), the identity of the voucher specimen as belonging to *Melinna* sp. has been confirmed (K. Halanych, pers. comm.). However, BLAST searches on the COI and 16S gene fragments reveal the closest matching sequence on GenBank as *Scalibregma inflatum*, a species which is distantly related to ampharetids. It is likely this is a result of a contamination and we suggest additional *M. maculata* material needs to be sourced and re-sequenced.

The results of our phylogenetic analysis suggest Ampharetinae is paraphyletic as *Samytheta neglecta* is positioned outside the Ampharetinae clade. The position of *Samytheta* has been disputed in previous phylogenetic studies. Our results contradict Kongsrud *et al.* (2017) which recovered Ampharetidae as monophyletic with *Samytheta neglecta* nested within Ampharetinae (posterior probability 0.78 for the Ampharetinae clade from combined COI, 16S and 18S tree). Ampharetinae was also recovered as monophyletic with high support, in Bernardino *et al.* (2017) using protein-coding and mitochondrial genes and in Stiller *et al.* (2013) using the COI, 16S, 18S gene fragments, however, both studies did not include *Samytheta* sequences in their datasets. In Eilertsen *et al.* (2017), the position of *Samytheta neglecta* varied between gene trees: in the concatenated gene tree (COI, 16S, 18S, 28S) *Samytheta* was

recovered as the sister group to the rest of Ampharetidae and Avinellidae with high support (posterior probabilities (PP) = 1, bootstrap values (BS) = 83) also in the COI and 18S gene trees *Samythella* was recovered outside Ampharetinae and sister to Melinninae (COI: PP < 0.75/ BS < 50, 18S: PP 0.98/ BS 53), whilst in the 16S and 28S gene trees it was recovered within Ampharetinae (16S: PP 0.94/ BS 48, 28S: 0.57/ BS 76). In accordance with our results, a recent phylogenetic study on all Terebelliformia suggests that *Samythella* is the sister taxon to *Melinna* plus *Isolda* clade (Stiller *et al.*, 2020). A BLAST analysis on the *Samythella neglecta* sequence (MG270113) reveals the top result as *S. neglecta* (MG270114), however, the next closest sequences on GenBank were non-ampharetids, which again suggest a possibility of contamination. As with *M. maculata*, additional sequences are required to resolve this issue.

As a result of this study, nine ampharetid species, including three melinnins (*Isolda warnbroensis* Augener, 1914, *M. gardelli* sp. nov. and *M. chadwicki* sp. nov.), have been described from Australian waters (Alvestad & Budaeva, 2015; Hartmann-Schröder, 1981; Hutchings & Rainer, 1979; Hutchings, 1977; Quatrefages, 1866). *Isolda warnbroensis* from Western Australia was synonymized by Day (1963) with *Isolda pulchella* Müller in Grube, 1858 from Santa Catarina, Brazil, but this synonymy is unlikely valid on biogeographical grounds. Despite this seemingly low diversity, the *Atlas of Living Australia* lists over 1700 records of Ampharetidae from Australia, yet over half (1000) are not identified past family level, a clear indication the fauna of Australia contains many undescribed species.

**ACKNOWLEDGEMENTS.** The authors wish to thank the CSIRO Marine National Facility (MNF) for its support in the form of sea time on RV *Investigator*, support personnel, scientific equipment and data management. All data and samples acquired on the voyage are made publicly available in accordance with MNF Policy. We also thank all the scientific staff and crew who participated in voyage IN2017\_V03. Project funding was provided by the Marine Biodiversity Hub, supported through the Australian Government's National Environmental Science Program (NESP). We are grateful to Rickard Gardell and Clarence ('Clarry') Chadwick for their generous donations to the Australian Museum. We thank the collection staff at the Australian Museum Sydney for help with loan material, and Sue Lindsay at Macquarie University bioimaging laboratory who helped with SEM imaging. We also thank Nicolas Lavesque for his comments on a draft of a manuscript and an anonymous reviewer for their helpful comments. The study was funded by the Linnean Society of New South Wales Joyce W. Vickery Scientific Research Fund grant to PI. The PI was funded by the Chadwick Biodiversity fellowship at the Australian Museum, Sydney. Further financial support was provided by the Australian Biological Research Study (ABRS) grant RG18-21 to EK.

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### Note added in proof

After this manuscript had been reviewed, Stiller *et al.* (2020) published a revised status of subfamily Melinninae to family Melinnidae. The new status did not appear in the World Polychaeta database (Read & Fauchald, 2020) until the final stages of proofing of the current manuscript, unfortunately not in time to be included.

Gunton, Kupriyanova and Alvestad, 20 July 2020.

