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

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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., 2009). 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 Melinnopside 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 Melinmides with Melinnopsis, but instead moved Melinmides 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 Melinmides (misspelt Melinmides) 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 Melinmides 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 (Melinm is 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.
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 ×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 manufacturer’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 µl of each primer (forward and reverse), 1 µl 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-Universitat 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 |
|---------|---------|----------|-----------|----------------------------------|-----------|
| **Melinninae** | | | | | |
| *Isolda pulchella* Müller in Grube, 1858 | ZMBN 105698 | Morocco | 35 | MG270119 MG253081 MG253135 | Eilersten et al., 2017 |
| *Isolda pulchella* Müller in Grube, 1858 | ZMBN 105697 | Morocco | 35 | MG270120 MG253082 MG253136 | Eilersten et al., 2017 |
| *Melina albicincta* Mackie & Pleijel, 1995 | SIO-BIC A1113 | Trondheimsfjord, Norway | 230–280 | JX423767 JX423679 JX423649 | Stiller et al., 2013 |
| *Melina cristata* (M. Sars, 1851) | ZMBN 95306 | Skagerrak, Norway | 212 | MG270118 MG253102 MG253147 | Eilersten et al., 2017 |
| *Melina maculata* Webster, 1879 | — | Off North Carolina, USA | < 200 | KY972411 KY972391 | Bernardino et al., 2017 |
| *Melina palmata* | — | France | — | — | unpublished |
| *Melinopsis* sp. | RUB Msp_01 | Antarctica | 2057 | RUMS096-09 | — | unpublished |
| *Melinopsis* sp. | RUB Msp_09 | Antarctica | 2057 | RUMS104-09 | — | unpublished |
| *Melinopsis* sp. | RUB Msp_27 | Antarctica | 2057 | RUMS22-09 | — | unpublished |
| *Melinopsis* sp. | RUB Msp_28 | Antarctica | 2057 | RUMS23-09 | — | unpublished |
| *Melinopsis* sp. | RUB Msp_29 | Antarctica | 2057 | RUMS24-09 | — | unpublished |
| *Melinopsis* sp. nov. | AM W50414 | off Moreton Bay, Australia | 1071–1138 | MT556172 MT556641 MT561568 | this study |
| *Melinopsis* sp. nov. | AM W52949 | Coral Sea Marine Park, Australia | 1013–1093 | MT556174 MT556643 MT561570 | this study |
| *Melinopsis* sp. nov. | AM W52948 | Coral Sea Marine Park, Australia | 1013–1093 | MT556173 MT556642 MT561569 | this study |
| *Melinopsis* gardelli sp. nov. | AM W50735 | Jervis Marine Park, Australia | 2650–2636 | MT556175 MT556644 MT561571 | this study |
| *Melinopsis* gardelli sp. nov. | AM W51476 | Freycinet Marine Park, Australia | 2820–2751 | MT556176 MT556645 MT561572 | this study |
| **Ampharetinae** | | | | | |
| *Ampharete finmarchica* (Sars, 1865) | SIO-BIC A1100 | Hornsundjupet, Svalbard | 291 | JX423738 JX423670 JX423642 | Stiller et al., 2013 |
| *Ampharete octocirrata* (Sars, 1835) | SIO-BIC A1109 | Hornsundjupet, Svalbard | 271 | JX423770 JX423682 JX423652 | Stiller et al., 2013 |
| *Amphicteis ninonae* Jirkov, 1985 | ZMBN 95441 | Svalbard | 340 | KX497038 KX513561 — | Kongsrud et al., 2017 |
| *Amphisamytha bioculata* (Moore, 1906) | SIO-BIC A2524 | San Nicholas Island, CA, USA | 400 | JX423685 JX423654 JX423634 | Stiller et al., 2013 |
| *Amphisamytha carldarei* | SIO-BIC A2576-7 | South Cleft, Juan de Fuca | — | — | Stiller et al., 2013 |
| *Amphisamytha fauchaldi* Solís-Weiss & Hernández-Alcántara, 1994 | SIO-BIC A2563 | German Flats, East Pacific Rise. | 2216 | JX423699 JX423658 JX423636 | Stiller et al., 2013 |
| *Amphisamytha jacksoni* Stiller et al., 2013 | SIO-BIC A2576-7 | South Cleft, Juan de Fuca | — | — | Stiller et al., 2013 |
| *Amphisamytha julianeae* Stiller et al., 2013 | SIO-BIC A2563 | German Flats, East Pacific Rise. | 2216 | JX423711 JX423675 JX423646 | Stiller et al., 2013 |
| *Amphisamytha lutzi* (Desbruyères & Laubier, 1996) | SIO-BIC A2530 | Rainbow, Mid-Atlantic Ridge | 2330 | JX423736 JX423673 JX423645 | Stiller et al., 2013 |
| *Amphisamytha vanuatuensis* Reuscher et al., 2009 | SIO-BIC A1106 | Trondheimsfjord, Norway | 271 | JX423739 JX423671 JX423643 | Stiller et al., 2013 |
| *Anobothrus gracilis* (Malmgren, 1866) | SIO-BIC A1108 | Trondheimsfjord, Norway | 88 | JX423766 JX423678 JX423648 | Stiller et al., 2013 |
| *Grasslea* cf. *hydrothermalis* Solís-Weiss, 1993 | SIO-BIC A6137 | Pinksie’s Vent, Gulf of California | 1572 | KX497032 KX513552 KX513568 | Kongsrud et al., 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-Universitat 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 |
|---------|---------|----------|-----------|---------------------------------|-----------|
| **Paramytha schanderi** Kongsrud et al., 2017 | ZMBN 87801 | Loki’s Castle Hydrothermal Vents | 2350 | — | KX513556 KX513572 | Kongsrud et al., 2017 |
| **Paramytha schanderi** Kongsrud et al., 2017 | ZMBN 87820 | Loki’s Castle Hydrothermal Vents | 2350 | KX497035 | KX513555 KX513571 | Kongsrud et al., 2017 |
| **Paramytha schanderi** Kongsrud et al., 2017 | ZMBN 87821 | Loki’s Castle Hydrothermal Vents | 2350 | — | KX513559 KX513575 | Kongsrud et al., 2017 |
| **Paramytha ossicola** Queirós et al., 2017 | ZMBN 107232 | Setúbal Canyon, Portugal | 1000 | — | KX513547 KX513563 | Kongsrud et al., 2017 |
| **Paramytha ossicola** Queirós et al., 2017 | ZMBN 107234 | Setúbal Canyon, Portugal | 1000 | — | KX513549 KX513565 | Kongsrud et al., 2017 |
| **Pavelius smileyi** Kongsrud et al., 2017 | ZMBN 87807 | Loki’s Castle Hydrothermal Vents | 2350 | KX497034 | KX513554 KX513570 | Kongsrud et al., 2017 |
| **Pavelius smileyi** Kongsrud et al., 2017 | ZMBN 87810 | Loki’s Castle Hydrothermal Vents | 2350 | KX497036 | KX513558 KX513574 | Kongsrud et al., 2017 |
| **Pavelius smileyi** Kongsrud et al., 2017 | ZMBN 87809 | Loki’s Castle Hydrothermal Vents | 2350 | — | KX513557 — | Kongsrud et al., 2017 |
| **Pavelius smileyi** Kongsrud et al., 2017 | ZMBN 87825 | Loki’s Castle Hydrothermal Vents | 2350 | KX497037 | KX513560 KX513576 | Kongsrud et al., 2017 |
| **Pavelius uschakovi** Kuznetsov & Levenstein, 1988 | ZMBN 108241 | Okhotsk Sea, Russia | 800 | KX497033 | KX513553 KX513569 | Kongsrud et al., 2017 |
| **Samythella neglecta** Wollbæk, 1912 | ZMBN 99276 | Norwegian Sea | 823–809 | MG270113 | KX513561 KX513573 | Eilertsen et al., 2018 |
| **Sosane wahrbergi** (Eliason, 1955) | SIO-BIC A1118 | Gullmarsfjorden, Sweden | 66 | JX423768 | JX423680 JX423650 | Stiller et al., 2013 |
| **Sosane wireni** (Hessle, 1917) | ZMBN 95447 | Bergen, Norway | 98 | KX497039 | KX513562 KX513577 | Kongsrud et al., 2017 |
| **Terebellidae** | | | | | |
| **Polycirrus carolinensis** Day, 1973 | SIO-BIC A1101 | Curlew Bank, Belize | 15–17 | JX423769 | JX423681 JX423651 | Stiller et al., 2013 |
| **Terebella lapidaria** Linnaeus, 1767 | SIO-BIC A1102 | Plymouth, UK | low tide | JX423771 | JX423683 JX423653 | Stiller et al., 2013 |
| **Alvinellidae** | | | | | |
| **Alvinella caudata** Desbruyères & Laubier, 1986 | SIO-BIC A1092 | German Flats, E.P.R. | 2216 | JX423737 | JX423669 JX423641 | Stiller et al., 2013 |
| **Scalibregmatidae (outgroup)** | | | | | |
| **Scalibregma inflatum** Rathke, 1843 | — | Helgoland, Germany | — | — | — | — |

Species voucher location depth GenBank or BOLD accession number reference
Table 2. Primers used for PCR and sequencing.

| gene   | primer             | sequence 5′−3′ | direction | reference       |
|--------|--------------------|---------------|-----------|----------------|
| 16S    | Ann16SF            | GCGGTATCCCTCGACCCTGRCWAAGGTA | forward   | Sjölin et al., 2005 |
| 16SbrH |                    | CCGGTCTGAACTCGATGATCA | reverse   | Palumbi, 1991   |
| 18S    | 18e                | CTGGTGTGTCCTGGCCAGT | forward   | Hillis & Dixon, 1991 |
|        | 18L                | GAATACCGCGGTCTGCGACC | reverse   | Halanych et al., 1995 |
|        | 18F509             | CCCGTTAATGGTGAAGTATGA | forward   | Struck et al., 2002 |
|        | 18R                | TCCGAAAGCGCTGATCAGATACCG | reverse   | Passamaneck et al., 2004 |
|        | 18F997             | TCGCACTTCTGGCAATTTCTTAA | forward   | Struck et al., 2002 |
|        | 18R1843            | GATGCCAATCGTATCTCTGCGAGTCACCTAC | reverse   | Struck et al., 2005 |
|        | TimA               | AMCTGGTTGATCCTGGCA | forward   | Norén & Jondelius, 1999 |
|        | 11000R2modified    | CGGTATCTGACATCGTCTTCGA | reverse   | Kupriyanova et al., 2006 |
| COI    | polyLCO            | GAYTATWTTCAACACAACTCATCAAGATATTGG | forward   | Carr et al., 2011 |
| polyHCO|                    | TAMACTTCWGGGTGACCAAARAATCA | reverse   | Carr et al., 2011 |

DNA, 2 µl Coral Load Qiagen PCR buffer, 1.5 µl MgCl₂, 1.5 µl dNTPs, 0.1 µl MyTaq DNA Polymerase Biofil 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, 50°/30 s, 72°/60 s, and finally 72°/5 min 16S: 94°/3 min, 35 cycles 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 in 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 (Melininae: 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 Melinopsis sp. from Antarctica the only Melinopsis 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

Melinopsis McIntosh, 1885

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

Type species. Melinopsis 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 uncini. 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 uncini, uncini with subrostral process and absence of post branchial hooks (dorsal hooks).

Melinopsis gardelli sp. nov.

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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 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.
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 dubia* 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:FD5DA304-28BB-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°55’56.4”S 153°35’45.6”E, 1257 m; end 30°74’0.8”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 groove (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 notochaetae present in 14 thoracic chaetigers, starting from segment IV. In holotype, anterior end notopodial chaetigers damaged. In paratypes, chaetiger 3 (segment IV) with few fine notochaetal capillaries and chaetiger 4 (segment V) with more abundant fine notochaetae arising from body wall. Short, cylindrical notopodia with thicker capillaries starting from chaetiger 6. Notochaetae arranged in double rows, roughly the same length. Microfibre ends on notochaetae visible (Fig. 7C).

Abdominal notochaetae 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 uncini. Holotype complete with more than 40 abdominal uncini.

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 uncini with two teeth in one vertical row over rostral tooth, subrostral process and basal prow (Fig. 7D). Uncini of abdominal uncini with numerous teeth over

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**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 µm, C 2 µm, D 5 µm, E 10 µm.
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 ninonae* is a sister taxon to a clade comprising two major ampharetin clades (pp 0.97). The monophyletic *Amphisamithya* (pp 1.0) clade is a sister group to the clade comprising other typical ampharetins (*Sosane-Paramytha-Ampharetinae-Enobothrus-Eclyssipe-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-Ampharetinae-Enobothrus-Eclyssipe-Pavelius-Grassleia* clade. Within the latter clade there is a four-way polytomy comprising clades *Sosane-Ampharetinae, (Enobothrus-Ampharetinae), Eclyssipe, 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 melinins. 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 (*Polyclavus caroliensis + Terebella lapidaria*) (pp 1.0) was recovered as a sister to all ampharetids (including an alvinellid) with high support (pp 1.0). Within the latter large ampharetin clade, *Ampharetidae*. The first strongly supported (pp 1.0) clade is a sister taxon to a clade comprising other typical ampharetins (*Sosane-Paramytha-Ampharetinae-Enobothrus-Eclyssipe-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-Ampharetinae-Enobothrus-Eclyssipe-Pavelius-Grassleia* clade. Within the latter clade there is a four-way polytomy comprising clades *Sosane-Ampharetinae, (Enobothrus-Ampharetinae), Eclyssipe, and non-monophyletic Pavelius* that has *Grassleia* nested within.

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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 intraspecific 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 intraspecific 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 CO1) 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 *Mellinexis arctica*) and *M. annenkovaevae* (previously *Mellinexis annenkovaevae*). This characteristic large buccal tentacle is likely a feature that has been incorporated into the generic diagnosis after the synonymization by Day (1964) of *Melinexis* and *Mellinides* 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 brachycantha* 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
| species                     | no. of TU | dorsal membrane | buccal tentacles | branchiae | no. of teeth above rostral tooth in TU | body size, length: width (mm) | tube | type locality |
|-----------------------------|-----------|-----------------|------------------|-----------|----------------------------------------|-----------------------------|------|---------------|
| *M. abyssalis* (Hartman, 1969) | 12        | Absent          | 2 types: 1 large, many small | 4 pairs: 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 |
| *M. angolensis* 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 |
| *M. annenkoevae* (Uschakov, 1952) | 12        | Absent         | 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 |
| *M. atlantica* McIntosh, 1885 | 14?       | Absent          | 2 types: 1 large, many small | 4 pairs: unknown | 3 (top tooth indistinct) | 35 mm : 3 mm | Stiff cylinder, fine grey mud, Foraminifera attached | Off Chesapeake Bay, NW Atlantic, 3109 m |
| *M. arctica* (Anneckova, 1931) | 12        | Present/indistinct | 2 types: 1 large, 2 small | 4 pairs: anterior (inner) larger than others | 2 | 25 mm : 3 mm | Solid tube covered in sand in the front part | Arctic Ocean 165–480 m |
| *M. armipotens* (Moore, 1923) | (13?)     | 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 |
| *M. augeneri* 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-Kasaya Bank, west off Kyushu, 185 m |
| *M. Chadwicki* 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 |
| *M. collanis* (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 |
| *M. dubita* (Hoagland, 1920) | 12?       | Indistinct ridge | 2 types: 6 tentacles up to 15 mm, many smaller 3–7 mm | 4 pairs: unclear | 3 | 15 mm : 7 mm | Fine brown mud | Mindanao, Philippines, 920 m |
| *M. gorgelli* 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 |
| *M. mcintoshi* Reuscher, Fieg & 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 |
| *M. monocera* (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 |
| *M. moorei* (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 Miguelas Islands, NE Pacific, 495–3990 m |
| *M. rastrata* (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, 3229 m |
| *M. somovi* (Uschakov, 1957) | 12        | Absent          | 2 types: 1 large, many small | 3 pairs: Internal largest | 2 | 15 mm : 1 mm | Silt with Foraminifera shells and small stones | Arctic, 1339–1694 m |
| *M. tentaculata* (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 |
| *M. tetradentata* (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 |
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 prostormium 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 *Amphipsamithya* 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 uschkovii* 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 morphologically 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 *Samythella neglecta* is positioned outside the Ampharetinae clade. The position of *Samythella* has been disputed in previous phylogenetic studies. Our results contradict Kongsrud *et al.* (2017) which recovered Ampharetidae as monophyletic with *Samythella 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 *Samythella* sequences in their datasets. In Eilertsen *et al.* (2017), the position of *Samythella neglecta* varied between gene trees: in the concatenated gene tree (COI, 16S, 18S, 28S) *Samythella* 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 Ampharetidae and sister to Melininae (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 _Melinella_ 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 melinins (_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 Ampharetidae from Australia, yet over half (1000) are not identified past family level, a clear indication the fauna of Australia contains many undescribed species.

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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.
