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New species of *Ophelina* (Annelida: Opheliidae: Ophelininae) from northern Australia

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Abstract Three species of the genus *Ophelina* are described from northern Australian waters. *Ophelina fauveli* (Caullery, 1944) is reported for the first time in Australian waters and its description has been updated; the two other species are new to science and are formally described. The main diagnostic characters for the species are based on differences in the pygidial funnel. *Ophelina tessellata* sp. nov. is distinguished by having a club-shaped funnel with a distinctive tessellated pattern on the ventral edge. *Ophelina cyprophilia* sp. nov. has a more elongated pygidial funnel and fewer rim cirri. Recognition of these two morphologically similar species was supported by sequences of the cytochrome oxidase I and histone H3 genes.

Keywords Annelida · Taxonomy · Systematics · Cytochrome oxidase I · Histone H3

Introduction

Opheliidae are common, often locally abundant, members of sand and mud substrates from the intertidal to the abyss; some species also form part of the encrusting fauna of hard substrates (Hutchings 2000; Rouse 2001). In Australia the taxonomy and species composition of Opheliidae is poorly known; only five genera and 13 species have been reported and most species are thought to have localised distributions (Hutchings 2000), but this likely reflects the lack of comparative systematic studies. The genus *Ophelina* (Ophelininae) is represented in Australia by four species: *Ophelina acuminata* Ørsted, 1843 from Broome (Hartmann-Schröder 1979), *O. breviata* (Ehlers, 1913) from SE Australia, *O. gigantea* (Rullier, 1965) from Moreton Bay, and *O. longicirrata* Hartmann-Schröder & Parker, 1995 from the eastern Great Australian Bight. Although the record of *O. acuminata* from Broome is geographically the closest to those in the present study, the description by Hartmann-Schröder (1979) bears little resemblance to the present species (see Appendix) and so it is not considered further. Another 11 species of *Ophelina* have been reported from southern Asia and the Indo-Malay archipelago under the old name *Ammotrypane* Rathke (Caullery 1944; Horst 1919; Pillai 1961).

Recently, two morphologically similar forms of *Ophelina* were identified in an investigation into the utility of polychaetes in the assessment of marine ecosystem health (Neave et al. 2012a, b). The specimens came from subtidal sites in Melville Bay, Gove and Cullen Bay and nearby shores of Darwin Harbour. The Cullen Bay specimens were part of a depauperate polychaete assemblage in sediments containing high levels of copper resulting from the 1999 treatment of the Bay with copper sulphate to eradicate the Black Stripe Mussel, *Mytilopsis sallei* (Ferguson 2000). The Darwin and Gove forms were morphologically very similar so a molecular comparison was done using the cytochrome oxidase subunit I (COI) and histone H3 genes to determine whether they differed genetically. Based on the molecular results and small morphological differences found *a posteriori*, the two forms are herein described as two new species. Comparison with other specimens of *Ophelina* from northern Australia held in the collections of Museum & Art Gallery Northern Territory (NTM) yielded a third species in the genus, which was determined as *Ophelina fauveli* Caullery, 1944, which is known to date only from Gisser, eastern Indonesia. All three species of *Ophelina* are described and the new species are compared to other Australian and Indo-west Pacific species.

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Materials and methods

Collection sites and specimen preparation

This study is based on *Ophelina* specimens collected from several locations in northern Australia over the last 30 years (Fig. 1). Most specimens have been fixed in 10 % formaldehyde-seawater and preserved in 70 % ethanol solution; some recently collected specimens were fixed in 95 % ethanol for genetic study.

Morphological data

Light microscopy observations were made using a Nikon SMZ 1500 stereomicroscope and a Nikon Eclipse 80i compound microscope with Nomarsky optics (http://www.nikoninstruments.com). Photographs were taken using a Canon EOS 5D Mk II (http://www.canon.com/) with MPE-65 lens mounted on a Cognisys Stackshot automated rail (http://www.cognisys-inc.com). Image stacks were obtained using Zerene Stacker (http://zerenesystems.com) and post-processed using Adobe Lightroom (http://www.adobe.com).

Molecular data and analyses

Ophelina specimens were preserved in 95 % alcohol before molecular analysis. The two new species, *Ophelina tessellata* sp. nov. and *Ophelina cyprophilia* sp. nov., were collected from Darwin Harbour and Melville Bay. Reference numbers and GenBank accession numbers for the *Ophelina* specimens are given in Table 1. DNA was extracted from the specimens using the Promega Wizard SV Genomic DNA Purification System (Promega, Madison, WI), according to the manufacturers instructions. The COI gene was amplified from the polychaete samples using the forward primer, LCO1498: 5' GGTCAACAAATCATAAAGATATTGG (Folmer et al. 1994), and the reverse primer, COI-E: 5' TATACTTCTGGGTGTCCGAAGAATCA (Bely and Wray 2004). The histone H3 gene was amplified from the polychaete samples using the forward primer, H3F: 5' ATGGCTCGTACCAAGCAGACVGC, and the reverse primer, H3R: 5' ATATCCTTRGGCATRATRGTGAC (Colgan et al. 2000).

PCR reactions were compiled using the Kapa Biosystems Robust PCR Kit (Kapa Biosystems, Woburn, MA). Each PCR reaction was made up of 1 μl template DNA, 10 μl 5x KAPA2G Buffer A, 1 μl 10 mM dNTPs, 5 μl 4 μM forward and reverse primers, 1.5 μl 25 mM $MgCl_2$, 1 μl DMSO, 0.15 μl KAPA2G Robust DNA Polymerase and 30.35 μl dH_2O for a total volume of 50 μl. The COI and histone genes were amplified for 35 cycles of 94 °C for 50 s, 49 °C for 120 s, 72 °C for 90 s, then a final extension of 72 °C for 7 min.

The amplified PCR products were then purified using the Promega SV Gel and PCR Clean-up System, according to the manufacturer's instructions (Promega). COI and histone H3 fragments of sufficient quality and quantity were selected for sequencing. Sequencing reactions were compiled using the Big Dye Terminator Kit, version 3.1 (Applied Biosystems, Foster City, CA). The reactions contained 4 μl of either forward or reverse primer (0.8 pmol/μl), 1 μl big dye terminator enzyme, 3.5 μl of 5x sequencing buffer and 5–10 ng template DNA in a 20 μl reaction. The sequencing reactions were cycled through 94 °C for 300 s, followed by 30 cycles of 96 °C for 10 s, 50 °C for 5 s and 64 °C for 240 s. Products were then precipitated and sequenced in both directions using a Genetic Analyzer 3130XL (Applied Biosystems). The

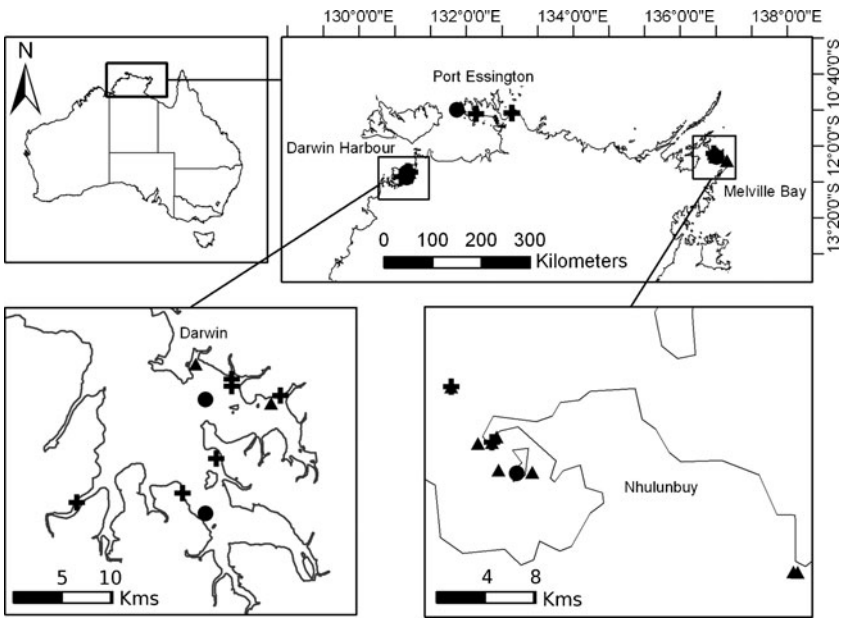

Fig. 1 Map of the study sites and collection location for each of the three *Ophelina* species. *Circles Ophelina fauveli*, *crosses Ophelina cyprophilia* sp. nov., *triangles Ophelina tessellata* sp. nov.

Table 1 Origin and reference data, including GenBank accession numbers, of sequenced specimens of *Ophelina cyprophilia* sp. nov. and *Ophelina tessellata* sp. nov., and others used in the phylogenetic analyses. *COI* Cytochrome oxidase I, *NTM* Northern Territory Museum, *CDU* Charles Darwin University

	Origin	NTM reference	CDU reference	*COI* GenBank accession no.	*Histone H3* GenBank accession no.
Sequences generated in this study					
Ophelina cyprophilia	Darwin Harbour		Pol 5	JN182653	JN182667
Ophelina cyprophilia	Darwin Harbour	204	Pol 49	JN182654	JN182668
Ophelina cyprophilia	Darwin Harbour		Pol 243	JN182655	JN182669
Ophelina cyprophilia	Darwin Harbour		Pol 244	JN182656	JN182670
Ophelina cyprophilia	Darwin Harbour		Pol 248	JN182657	JN182671
Ophelina cyprophilia	Darwin Harbour		Pol 249	JN182658	JN182672
Ophelina cyprophilia	Darwin Harbour		Pol 333	JN182659	JN182673
Ophelina cyprophilia	Darwin Harbour		Pol 334	JN182660	JN182674
Ophelina tessellata	Melville Bay	W23419	Pol 406	JN182661	JN182675
Ophelina tessellata	Melville Bay	W23420	Pol 407	JN182662	JN182676
Ophelina tessellata	Melville Bay	W23421	Pol 408	JN182663	JN182677
Ophelina tessellata	Melville Bay	W23422	Pol 409	JN182664	JN182678
Ophelina tessellata	Melville Bay	W23423	Pol 410	JN182665	JN182679
Ophelina tessellata	Melville Bay	W23426	Pol 413	JN182666	JN182680
Sequences from other studies					
Arenicola marina	Rousset et al. (2007)				DQ779718
Armandia bilobata	Rousset et al. (2007)				DQ779719
Armandia brevis	Paul et al. (2010)				HM746752
Armandia maculata	Paul et al. (2010)				HM746753
Cirratulus cirratus	Rousset et al. (2007)				DQ779724
Cossura candida	Paul et al. (2010)				HM746754
Euzonus ezoensis	Paul et al. (2010)				HM746755
Ophelia bicornis	Paul et al. (2010)				HM746762
Ophelia limacina	Carr et al. (2011)			GU672187	
Ophelia neglecta	Paul et al. (2010)				HM746764
Ophelina acuminata	Paul et al. (2010)				HM746761
Ophelina cylindricaudata	Paul et al. (2010)				HM746763
Polyophthalmus pictus	Brown et al. (1999)				AF185259

consensus for each individual was obtained by editing and reconciling the forward and reverse sequences using MacVector, version 10.5 (MacVector, Cary, NC).

The COI and histone H3 consensus sequences were aligned using ClustalW in MEGA (Molecular Evolutionary Genetics Analysis) software (Tamura et al. 2011). For the COI trees, we used GenBank sequences of *Ophelia limacina* (GU672187; Carr et al. 2011) as an outgroup. For the histone H3 trees, we aligned our sequences with selected Opheliidae sequences from GenBank (Table 1). This was done to see where our sequences fit into the current opheliid taxonomy. The program jmodeltest (Posada 2008) was used to determine the best fitting model for each of the alignments. For the COI alignment, both the AICc and BAC tests indicated HKY+I as the most appropriate model. For the histone H3 alignment, K80+I was the best model. Phylogenetic trees were computed in MEGA using maximum likelihood analysis with the appropriate model for each gene. Clade support was calculated using bootstrapping with 1,000 pseudoreplicates. Genetic distances were calculated in MEGA using the same model as previously for each gene and the variance was calculated using 1,000 bootstrap replications. The phylogenetic data are available in TreeBase at the following URL: http://purl.org/phylo/treebase/phylows/study/TB2:S12194

Results

Molecular

The partial nucleotide sequence of both the mitochondrial COI gene and the nuclear histone H3 gene were analysed in

14 individuals collected from northern Australia (Table 1). Nucleotide substitutions occurred at 129 positions within the 690-bp fragment of the COI gene (18.7 %) and at 18 positions within the 378-bp fragment of the histone H3 gene (4.8 %). Phylogenetic relationships among the specimens were analysed using maximum likelihood analysis. In both the COI (Fig. 2) and histone H3 (Fig. 3) trees, the specimens were divided into two clades, which have been designated *Ophelina tessellata* sp. nov. and *O. cyprophilia* sp. nov. These clades were supported in at least 98 % of bootstrap replicates for both genes. In addition, the histone H3 tree (Fig. 3) showed the new species as sister groups to *Ophelina cylindricaudata*, suggesting that the new species were placed correctly within *Ophelina.* On the other hand, *Ophelina acuminata* was further from the newly described species, although still within the Opheliniinae radiation. At higher taxonomic groupings, the data supported monophyly of the Opheliinae and Opheliniinae.

For the COI gene, the average distance between the two new species was 18.9±1.5 %. The variation was 0.4±0.2 % within *O. tessellata* sp. nov. and less than 0.1 % within *O. cyprophilia* sp. nov. The average distance between the two species using the histone H3 gene was 3.9±1.0 %, reflecting the higher conservation rates of this gene (Colgan et al. 2000). Within specimens of *O. tessellata* sp. nov., histone H3 variation was 0.4±0.2 %, and within *O. cyprophilia* sp. nov., histone H3 variation was 0.5±0.2 %.

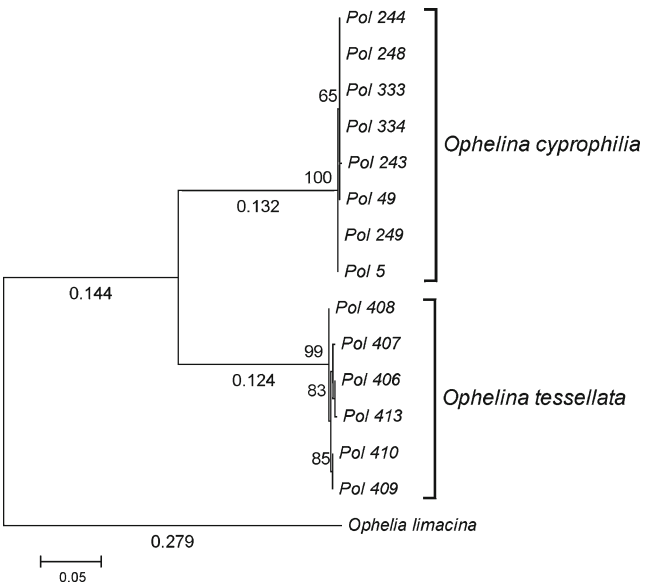

Fig. 2 Maximum likelihood tree of the cytochrome oxidase I (COI) dataset drawn using MEGA with the HKY+I model. Support from 1,000 bootstrap replicates is given at the nodes if greater than 50 %. Branch lengths are shown below the branches and were measured in the number of substitutions per site

Morphological characters

Members of *Ophelina* display many 'conservative' features, i.e., those that show little if any interspecific variation. The general body form is slender, smooth and glossy, cylindrical, pointed anteriorly, with deep mid-ventral and lateral grooves along the body. Colour in alcohol varies from white to various shades of brown, and secondary pigmentation appears to be absent. Primary segmentation is indistinct and secondary intra-segmental annulations are more- or less-well-developed. The prostomium is conical and usually bears a terminal palpode (rarely absent); a pair of eyespots may be present or absent. A pair of eversible nuchal organs is present at the base of the prostomium; when not everted they appear to be covered by a posterior lappet, as observed in the present specimens, but rarely the lappet is absent (Parapar et al. 2011). The peristomium is fused to the prostomium and includes the region around the mouth, which is transverse, slit-like; a large, lobate sac-like proboscis was rarely everted in the present preserved specimens, and described rarely in the literature. Simple cirriform, distally tapered, branchiae arise just above and behind the parapodia, beginning on chaetiger 2 and continuing posteriorly (rarely branchiae are completely absent). The branchiae, which typically arch over the dorsal surface, have two lateral rows of cilia along the posterior edge; however, the ciliation pattern is not observed easily under light microscopy, especially if the animal is not ideally fixed. Each parapodium bears a single type of capillary chaeta, which may be smooth (Figs. 4, 5) or sparsely hairy (hispid). The type of chaetae appears to be the same, both within an individual (i.e. along the length of the body) and within a species (Fig. 4). However, the relative length of the chaetae proved to be useful for distinguishing the present species.

The most useful taxonomic features are associated with the parapodia and funnel-shaped pygidium. The parapodia of *Ophelina* are small, rounded to pointed projections bearing two bundles of chaetae; an upper one, which most authors are calling notochaetae, and a lower bundle of neurochaetae. The parapodium comprises three main features: a pre-chaetal lobe situated between the noto- and neurochaetae (varies in shape from low and rounded to digitiform as in the present specimens); adjacent to the notochaetae may be a short 'dorsal cirrus' (e.g. Parapar et al. 2011, Fig. 6e), but this structure may be absent, as in the present material; a low, lingulate ventral lobe immediately ventral to the neurochaetae (Figs. 4, 5). In most descriptions of *Ophelina* no ventral lobe is mentioned; however, we suspect that a low ventral lobe may actually be present in many *Ophelina* species, as in our specimens. Similarly, few descriptions mention a dorsal cirrus, possibly because this structure is very small and its detection may require the use of scanning elcetron microscopy (SEM).

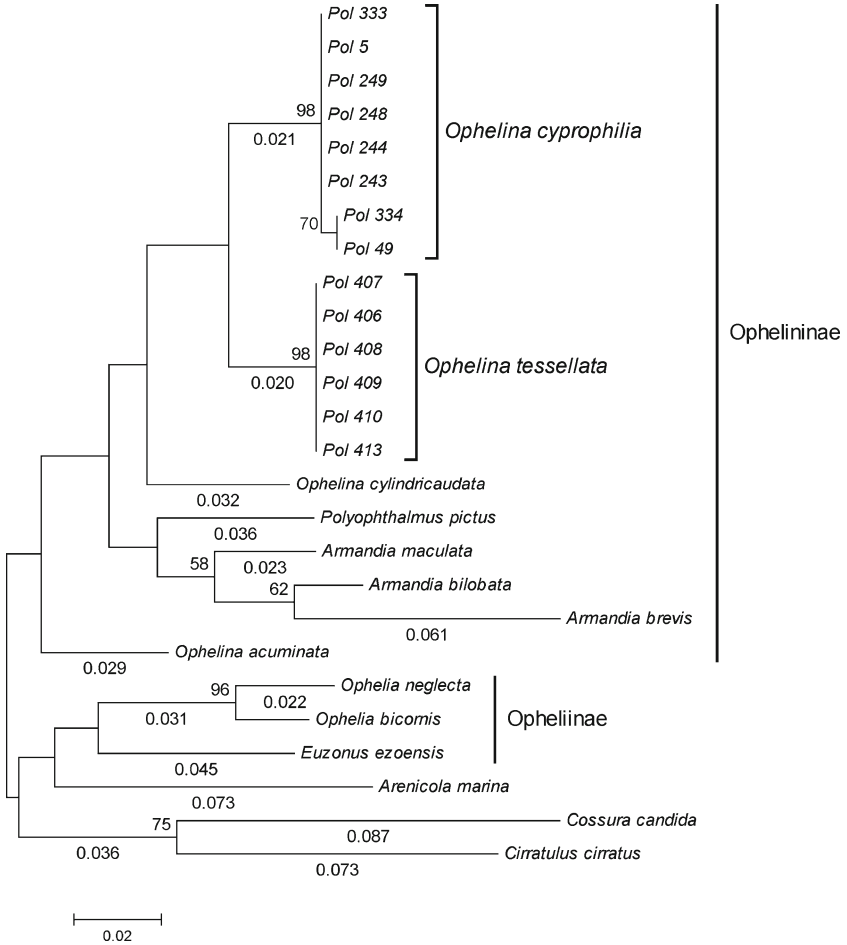

Fig. 3 Maximum likelihood tree of the histone H3 dataset drawn using MEGA with the K80+I model. Support from 1,000 bootstrap replicates is given at the nodes if greater than 50 %. Branch lengths are shown below the branches and were measured in the number of substitutions per site

The tubular, elongate pygidial funnel bears a single mid-ventral cirrus originating inside the funnel, a pair of external lateral cirri, and many marginal cirri located on the rim of the funnel (Fig. 6). Inside the funnel is a terminal anus. The morphologically complex pygidial funnel is the most diagnostic structure of *Ophelina*; species identification may not be possible in specimens where it is damaged or has fallen off.

Taxonomy

Ophelina Ørsted, 1843

Ophelina cyprophilia sp. nov.

(Figs. 6, 7)

Material examined HOLOTYPE, Australia, Northern Territory, Darwin Harbour, Bayview Haven, near entrance to lock, 12.44258S, 130.85900E, coll. M. Neave & C. Glasby, 21 February 2007 (NTM W23825). PARATYPES, Darwin Harbour, Hudson Creek, Stn HC MF1 Anox 1, 12.48216667S 130.9266667E, coll. M. Neave, 2 May 2007, 1 specimen (NTM W22279) (sequenced); Stn DW109a, 12.56466667S 130.8446667E, coll. MEU (Marine Ecology Unit), 18 March 1994, 1 specimen, NTM W13689; Stn DW132a, 12.53533333S, 130.8728333E, coll. MEU, 18 March 1994, 1 specimen, NTM W13666; Stn DW71a, 12.572S 130.755E, coll. MEU, 17 March 1994, 1 specimen, NTM W13649. NON-TYPES: Northern Territory, Darwin Harbour, Stn D158a, 12.4745S 130.8853333E, coll. MEU, 17 July 1993, 2 specimens, NTM W10483; Darwin Harbour, Stn DW155a, 12.46833333S 130.8858333E, coll. MEU, 23 March 1994, 1 specimen, NTM W13661; Darwin Harbour, 1 specimen, NTM W23824; Annesley Point, Stn AP/5, 11.40833333S 132.85E, coll. R. Hanley, P. Hutchings & C. Watson, 18 June 1984, 1 specimen, NTM W1951; Melville Bay, Cargo Wharf, Stn GVCW, 12.20416667S 136.6808333E, coll. K. Neil & party, 12 June 2001, 1 specimen NTM W19547, Melville Bay, 12.16666667S 136.65E, coll. MEU, November 1991–March 1992, 1 specimen NTM W8217, Melville Bay, 12.16666667S 136.65E, coll. MEU, November 1991–March 1992, 1 specimen, NTM W8216, West Bay, Port Essington, Stn CPV5, 11.41666667S 132.175E, coll. R. Hanley, M. Burke & C. Watson, 14 September 1985, 1 specimen, NTM W3582; West Bay, Port Essington, Stn CPV5, 11.41666667S 132.175E, coll. R. Hanley, M. Burke & C. Watson, 14 September 1985, 1 specimen, NTM W3580; West Bay, Port Essington, Stn CPV5, 11.41666667S 132.175E, coll. R. Hanley, M.

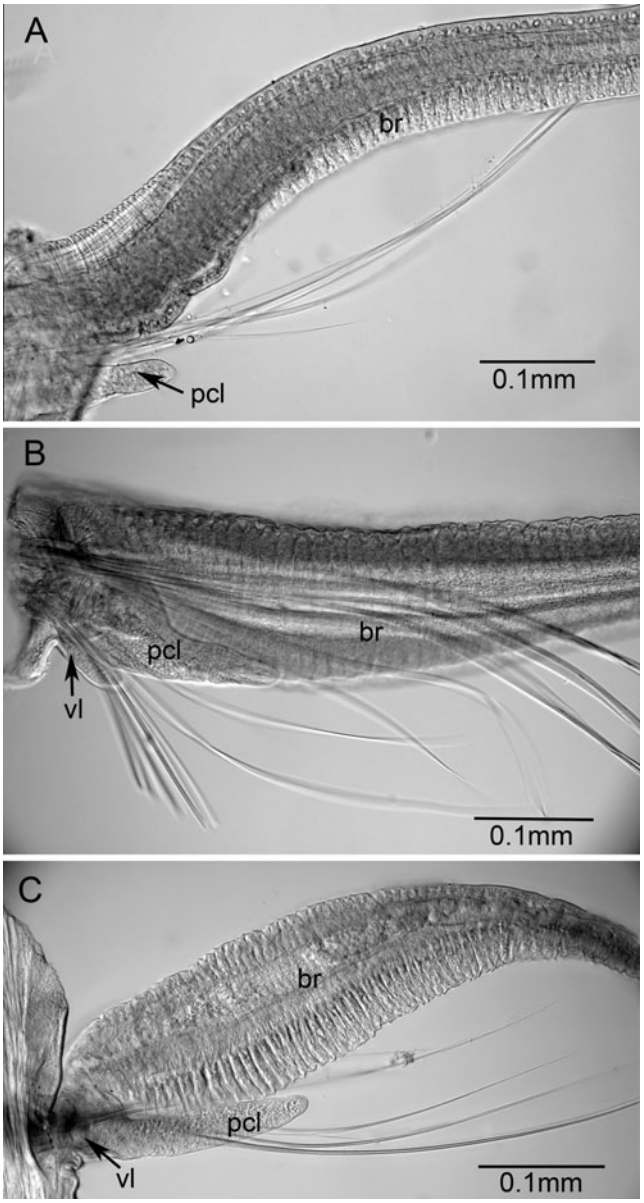

Fig. 4 **a–c** Light microscope photographs showing the parapodia of *Ophelina* species in this study. **a** *Ophelina cyprophilia* sp. nov. holotype, parapodium from posterior chaetiger (ventral lobe small, not visible); **b** *O. fauveli* NTM W13673, parapodium, chaetiger 7; **c** *O. tessellata* sp. nov. holotype, parapodium, chaetiger 10. *br* Branchiae, *pcl* prechaetal lobe, *vl* ventral lobe

Burke & C. Watson, 14 September 1985, 1 specimen, NTM W3543; Yankee Creek, Stn AP/4, 11.41666667S 132.8583333E, coll. R. Hanley, P. Hutchings & C. Watson, 17 June 1984, 3 specimens, NTM W1784.

Description (n=19; holotype values indicated, followed by variation in other material) Body 22.0 (13.5–22.0) mm long, for 58 (48–65) chaetigers. Prostomium 1.2 (1.2–1.4) times longer than wide; terminal palpode present. Prostomial eyes absent. Nuchal organs with posterior lappet (not readily visible in holotype but obvious in paratypes). Prechaetal lobe digitiform throughout; at chaetiger 3, 0.2 times length of branchiae; posteriorly 0.1 times length of branchiae. Dorsal cirri absent. Ventral cirri present, low, lingulate. Chaetae all smooth capillaries, those of anterior parapodia oriented laterally or posterolaterally. Notochaetae anteriorly 1.0 (0.9–1.2) times branchiae length; in midbody 0.6 (0.6–1.0) times branchiae length; posteriorly 0.6 (0.6–0.9) times branchiae length. Branchiae start on chaetiger 2 and end on the final chaetiger. Branchiae length anteriorly 0.8 (0.6–1.0) times body width; in mid body 0.9 (0.7–0.9) times body width, in posterior body 0.8 (0.6–1.0) times body width. Branchiae ciliated along entire posterior edge, most dense at the base. Pygidial funnel is laterally compressed; opening ventrally (hood shaped); 1.8 (1.7–2.0) times longer than deep; 40 (25–42) annulations present on funnel. Unpaired ventral anal papilla present, tapered and 0.9 (0.7–1.0) times funnel length. Paired ventral anal papillae present, tapered and 0.3 (0.2–0.3) times funnel length. Anal margin cirri present, 48 (42–58) cirri, weakly tapered and anterior cirri 2 (1.8–2.0) times longer than posterior cirri, anterior cirri 0.6 (0.6–1.0) times length of paired ventral papillae.

Distribution and habitat 'Top End' of northern Australia in mudflats from the intertidal to 10 m deep; maybe associated with mangroves. Sympatric with *Ophelina tessellata* [found together in a sample collected at Melville Bay (NTM W8216)].

Etymology The species name is derived from the Greek, *Kypros*, meaning copper, and *philia*, meaning fondness, referring to this species ability to live in sediments with high levels of copper.

Remarks The three *Ophelina* species described in the present study differed only slightly morphologically, with most differences associated with the pygidial funnel. *Ophelina cyprophilia* sp. nov. had an oval-shaped pygidial funnel that was neither especially long or club shaped, which distinguished it from the other species. In addition, *O. cyprophilia* sp. nov. had notochaetae in the anterior body that were only slightly longer than the branchiae (different to *O. tessellata* sp. nov.) and an unpaired anal cirrus that was approximately as long at the pygidial funnel (different to *Ophelina fauveli*). The branchiae of *O. cyprophilia* sp. nov. also tended to be more tapered compared to the other species. Compared to other species of *Ophelina* in the region, *O. cyprophilia* sp. nov. was most similar to *O. grandis* (Pillai, 1961) collected from Sri Lanka. However, *O. cyprophilia* sp. nov. differed by having shorter branchia and fewer anal rim cirri that were shorter. In addition, the anal funnel was spoon-shaped in *O. grandis*, while in *O. cyprophilia* sp. nov. the anal funnel was laterally compressed (see Appendix).

Ophelina fauveli Caullery, 1944

(Figs. 6, 8)

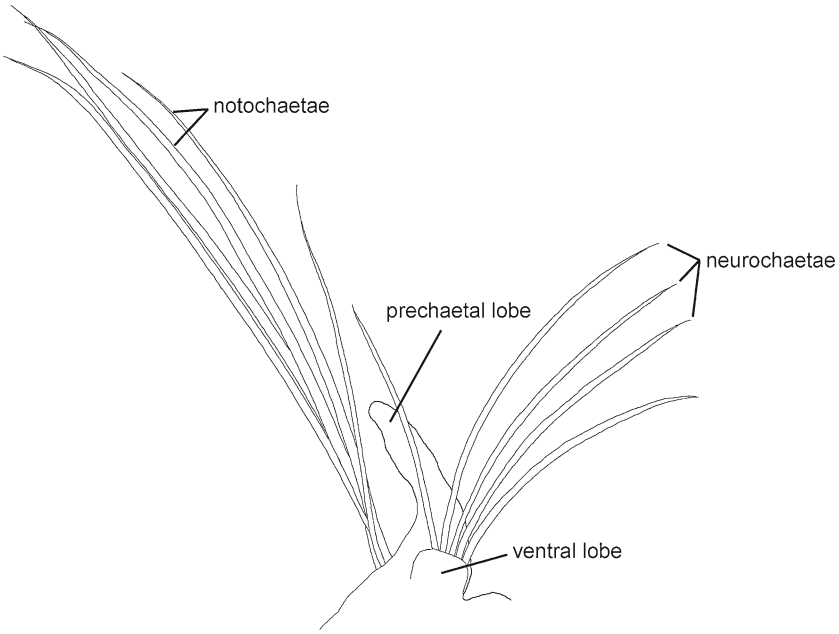

Fig. 5 *Ophelina fauveli* NTM W13673, right side parapodia from posterior body, ventral view. The branchia is not shown

Ammotrypane fauveli Caullery, 1944: 42, fig. 33. Type locality: Gisser, Indonesia

Material examined NON-TYPES: Australia, Darwin Harbour, Stn D114a, 12.58133333S 130.8628333E, coll. MEU, 13 July 1993, 1 specimen, NTM W10492; Stn DW143a, 12.48533333S 130.8636667E, coll. MEU, 23 March 1994, 2 specimens, NTM W13673; Melville Bay, Off Catalina Boat Ramp (=Catalina Bay), Stn GVCBS, 12.22583333S 136.6983333E, coll. K. Neil & party, June 2001, 1 specimen, NTM W19550; Port Essington, Cape Don, Stn CP/15, 11.33333333S 131.8166667E, coll. R. Hanley et al., 13 October 1981, 1 specimen NTM W1271.

Description (n=5) Body 19.0–35.0 mm long, for 54–65 chaetigers. Prostomium 1.2–1.9 times longer than wide; terminal palpode present. Prostomial eyes absent. Nuchal organs with posterior lappet. Prechaetal lobe digitiform throughout, at chaetiger 3, 0.15 times length of branchiae; posteriorly 0.1 times length of branchiae. Dorsal cirri absent. Ventral cirri present, low, lingulate. Chaetae all smooth capillaries, those of anterior parapodia oriented dorsolaterally. Notochaetae anteriorly 0.9–1.1 times branchiae length; in midbody 0.6–1.0 times branchiae length; posteriorly 0.7–1.0 times branchiae length. Branchiae start on chaetiger 2 and end on the final chaetiger. Branchiae length anteriorly 0.6–0.7 times body width; in mid body 0.7–0.8 times body width, in posterior body 0.7–1.0 times body width. Branchiae ciliated along entire posterior edge, evenly distributed. Pygidial funnel is slightly laterally compressed; opening ventrally (hood shaped); 1.8–2.2 times longer than deep; 20–30 annulations present on funnel. Unpaired ventral anal papilla present, tapered and 1.5–2.2 times funnel length. Paired ventral anal papillae present, cirriform and

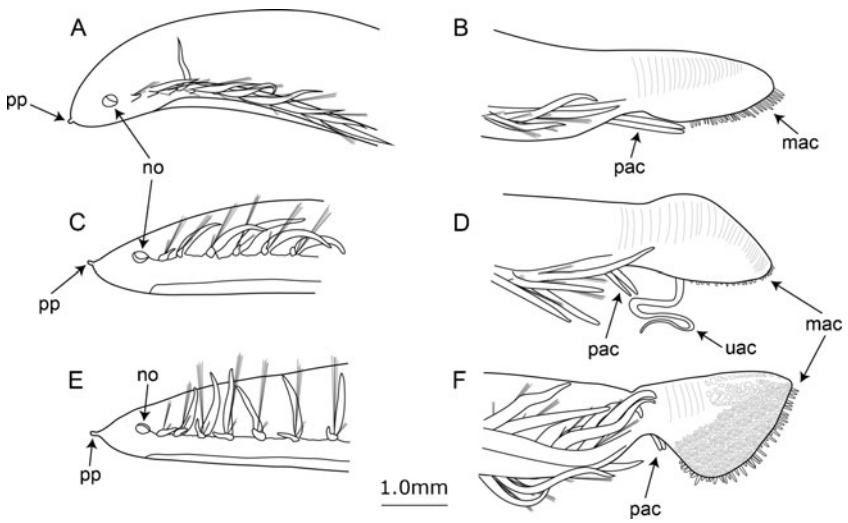

Fig. 6 *Ophelina cyprophilia* sp. nov. holotype, anterior (**a**) and posterior (**b**), *Ophelina fauveli* NTM W13673, anterior (**c**) and posterior (**d**), *Ophelina tessellata* sp. nov. holotype, anterior (**e**) and posterior (**f**). *no* Nuchal organ, *pp* palpode, *pac* paired anal cirri, *uac* unpaired anal cirri, *mac* margin anal cirri

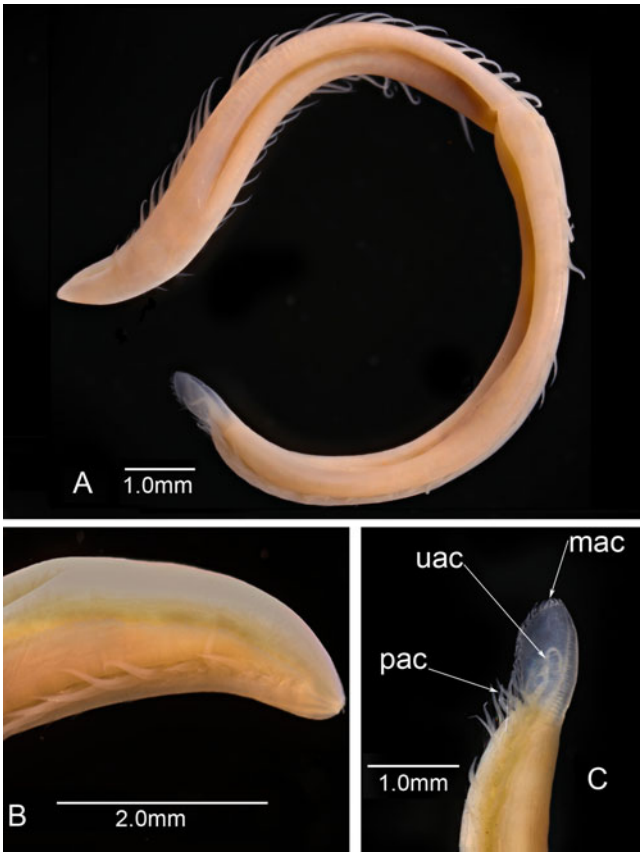

Fig. 7 *Ophelina cyprophilia* sp. nov. holotype, whole body (**a**), anterior (**b**) and posterior (**c**). *pac* Paired anal cirri, *uac* unpaired, *mac* margin anal cirri

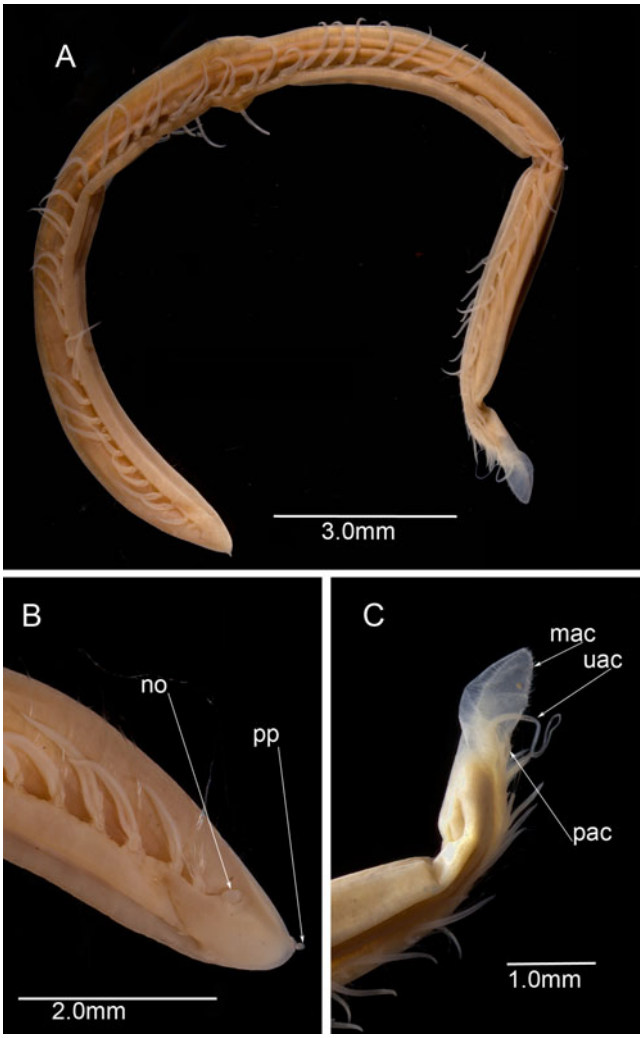

Fig. 8 *Ophelina fauveli* NTM W13673, whole body (**a**), anterior (**b**) and posterior (**c**). *no* Nuchal organ, *pp* palpode, *pac* paired anal cirri, *uac* unpaired anal cirri, *mac* margin anal cirri

slightly tapered, 0.1–0.2 times funnel length. Anal margin cirri present, 20–36 cirri, present only on posterior edge and of equal length, posterior cirri 0.1–0.5 times length of paired ventral papillae.

Distribution and habitat Eastern Indonesia and 'Top End' of northern Australia. Sand substrate, 10–21 m.

Remarks Ophelina fauveli was readily distinguished from the other described species by the presence of an unusually long unpaired anal cirrus, which was approximately two times longer than the pygidial funnel (the unpaired anal cirrus on the other species was approximately the same length as the pygidial funnel). In addition, the pygidial rim cirri were very short compared with the other species.

The present specimens agree in all features with the type description, except in the relative length of the anal margin cirri. In the present material they are much shorter (2–3 times) than described for the holotype of *O. fauveli*. Some of the cirri in the present material approach the clavate shape described by Caullery (1944). Although the length of the anal margin cirri is likely to increase in length allometrically (shown by Saito et al. 2000 for *Armandia amakusaensis*), we cannot explain the variation in this way because Caullery's holotype is within the size range of our specimens. We therefore attribute the difference to regional variation, but caution that molecular data is required to confirm species identity.

Ophelina tessellata sp. nov.

(Figs. 6, 9)

Material examined HOLOTYPE, Australia, Northern Territory, Melville Bay, Site 2, Export Wharf, Stn GVEX2, 12.205S 136.67E, coll. K. Neil & party, 12 June 2001, NTM W19553. PARATYPES: Melville Bay, Stn B7, 3 specimens, NTM W23821, Stn A34, 4 specimens NTM W23820, Stn B1, 1 specimen, NTM W23823, Stn B10, 4 specimens, NTM W23822; Melville Bay, Cargo Wharf, Stn GVCW, 12.20416667S 136.6808333E, coll. K. Neil & party, 12

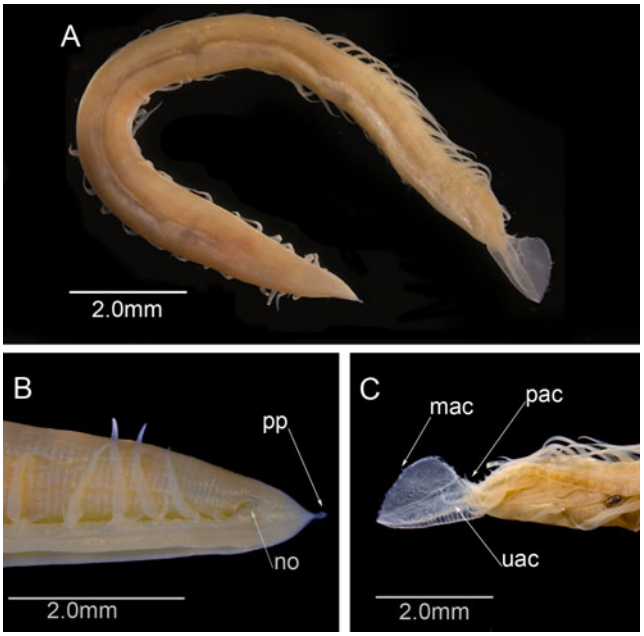

Fig. 9 *Ophelina tessellata* sp. nov. holotype, whole body (**a**), anterior (**b**) and posterior (**c**). *no* Nuchal organ, *pp* palpode, *pac* paired anal cirri, *uac* unpaired anal cirri, *mac* margin anal cirri

June 2001, 1 specimen, NTM W19554; E of Drimmie Peninsula, Stn CM2/1, 12.22433333S 136.7096667E, coll. M. Neave & C. Glasby, 26 Feb 2009, 1 specimen, NTM W22865 (sequenced). NON-TYPES: Darwin Harbour, Stn, DW139a, 12.45583333S 130.8558333E, coll. MEU, 23 March 1994, 2 specimens NTM W13667; Stn DW170a, 12.488S 130.918E, coll. MEU, 22 March 1994, 1 specimen, NTM W13669; Melville Bay, Stn B7, 1 specimen, NTM W23819, Stn B5E, 1 specimen NTM W23818; Melville Bay, E of Drimmie Peninsula, Stn D2, 12.221025S 136.681705E, coll. M. Neave, 12 August 2010, 2 specimens, NTM W23419 (sequenced); E of Drimmie Peninsula, Stn CM1, 12.28833333S 136.898055E, coll. M. Neave, 11–12 August 2010, 1 specimen, NTM W23420; E of Drimmie Peninsula, Stn CM1, 12.28833333S 136.898055E, coll. M. Neave, 11–12 Aug 2010, 1 specimen, NTM W23421; E of Drimmie Peninsula, Stn CM2, 12.22450333S 136.7097267E, coll. M. Neave, 11–12 Aug 2010, 1 specimen, NTM W23422 (sequenced); E of Drimmie Peninsula, Stn, CM2, 12.22450333S 136.7097267E, coll. M. Neave, 11–12 August 2010, 6 specimens NTM W23423 (sequenced); E of Drimmie Peninsula, Stn CM1, 12.28833333S 136.898055E, coll. M. Neave, 11–12 August 2010, 1 specimen, NTM W23426 (sequenced); Between Cargo Wharf and Perkins Wharf, Stn GVCW, 12.2S 136.6833333E, coll. K. Neil & party, 12 June 2001, 1 specimen, NTM W19551, Melville Bay, 12.16666667S 136.65E, coll. MEU, November 1991–March 1992, 2 specimens NTM W8216

Description (n=39; holotype values indicated, followed by variation in other material) Body 26.0 (15.0–26.0) mm long, for 44 (42–58) chaetigers. Prostomium 1.5 (1.1–1.6) times longer than wide; terminal palpode present. Prostomial eyes absent. Nuchal organs with posterior lappet. Prechaetal lobe digitiform throughout; at chaetiger 3, 0.2 times length of branchiae; posteriorly 0.15 times length of branchiae. Dorsal cirri absent. Ventral cirri present, low, lingulate. Chaetae all smooth capillaries, those of anterior parapodia oriented dorsolaterally. Notochaetae anteriorly 0.6 (0.6–2.0) times branchiae length; in midbody 0.7 (0.6–1.0) times branchiae length; posteriorly 0.5 (0.5–0.8) times branchiae length. Branchiae start on chaetiger 2 and end on the final chaetiger. Branchiae length anteriorly 0.8 (0.4–0.9) time body width; in mid body 0.9 (0.5–1.0) times body width, in posterior body 1.2 (0.8–1.2) times body width. Branchiae ciliated. Pygidial funnel is laterally compressed; opening ventrally (hood shaped); 1.2 (1.2–1.8) times longer than deep; 25 (23–70) incomplete annulations present on funnel dorsally, tessellated pattern ventrally. Unpaired ventral anal papilla present, tapered and 0.5 (0.2–1.0) times funnel length. Paired ventral anal papillae present, thick and not tapered, 0.1 (0.1–0.8) times funnel length. Anal margin cirri present, 40 (40–88) cirri, weakly tapered and of equal length, anterior cirri 0.7 (0.3–1.0) times length of paired ventral papillae.

Distribution and habitat 'Top End' of northern Australia in mudflats from the intertidal to 10 m deep; maybe associated with mangroves. Sympatric with *Ophelina cyprophilia* [found together in a sample collected at Melville Bay (NTM W8216)].

Etymology The species name is formed from the Greek, *tessella*, meaning 'small square', referring to the distinctive pattern on the pygidial funnel of this species.

Remarks Ophelina tessellata sp. nov. was distinguished from the other described species by the presence of a club-shaped pygidial funnel that was only 1.5 times longer than deep (the other species had pygidial funnels that were approximately 2 times longer than deep). *Ophelina tessellata* sp. nov. also had a distinctive pattern of tessellated annulations on the ventral part of the pygidial funnel, whereas in the other species the annulations extended more ventrally and were not accompanied by tessellations. In addition, the notochaetae of the anterior body of this species were up to 2 times longer than the branchiae length. In *O. cyprophilia* sp. nov. and *O. fauveli*, the notochaetae were only slightly longer than the branchiae. Compared to other species of *Ophelina* in the region, *O. tessellata* sp. nov. was most similar to *O. gigantea* (Rullier, 1965) from Moreton Bay on Australia's east coast. However, *O. tessellata* sp. nov. had shorter branchiae and a club-shaped

anal funnel. *O. gigantea* also lacked tessellated annulations on the anal funnel and had a more prominent palpode.

Discussion

Unfortunately most of the *Ophelina* species described from southern Asia and the Indo-Malay archipelago are uncertain. This is because descriptions are based on specimens that are damaged or on single specimens (e.g. *O. longicirrata* Hartman-Schröder and Parker 1995; *O. kampeni* (Horst, 1919); *O. ehlersi* (Horst, 1919); *O. buitendijki* (Horst, 1919); *O. fauveli* (Caullery, 1944); *O. longicaudata* (Caullery, 1944); *O. dubia* (Caullery, 1944); *O. profunda* (Caullery, 1944); *O. remigera* (Ehlers, 1916)). In addition, Saito et al. (2000) noted that most of the traditionally used morphological and morphometric characters of *Armandia* (Opheliniinae) are highly variable (many correlated positively with body size) so require data from a large number of different-sized specimens. To start to address these issues, we described three *Ophelina* species from northern Australia by morphologically characterising a larger number of specimens and supplementing these data with molecular indices.

Species of *Ophelina* have traditionally been identified based on the presence or absence and morphology of the pygidial funnel (Parapar et al. 2011); we also found that most of the diagnostic characters for *Ophelina* were associated with this structure. Recently, ultrastructural features have been used to distinguish species (e.g. Parapar et al. 2011). These include the form of the lateral organs, which are located on the prechaetal lobe, the form and distribution of the transverse ciliary bands on the venter and those of along the branchiae, and the distribution and form of the cilia and pores along the body surface. Unfortunately, information on the variability of these features within and between species for most opheliids is currently lacking, so their usefulness in discriminating species is limited at this stage. The material that we had available in this study was not suitable for SEM because the specimens were not relaxed appropriately or consistently (may affect form of nuchal organs and pores) or fixed for SEM (affects form of cilia). Basing species identification primarily on external structures, such as the pygidial funnel, which can be easily lost during collection and preservation, or features only observable using SEM, is not ideal. We used molecular data in conjunction with morphological characteristics to ensure correct species identification. The use of molecular data when describing species of the *Ophelina* may be more important than for other polychaete taxa due of the lack of practical morphological characters for the group; this is particularly true if the pygidial funnel is damaged or has fallen off.

According to the histone H3 data, the new species herein described were most similar to *Ophelina cylindricaudata*. This is consistent with the morphological findings and suggests that the new species were categorised correctly as *Ophelina*. The other *Ophelina* species (*Ophelina acuminata*), however, did not fall within this radiation. Paraphyly of the *Ophelina* was also found by Silva (2007) and Paul et al. (2010) and our data support these findings although, it should be noted that few sequences are available for *Ophelina* species and these relationships are likely to change with increased taxon sampling. At higher taxonomic classifications, our data supports the monophyly of Opheliinae and Opheliniinae as found by Paul et al. (2010).

The specimens examined in this study were collected from three coastal sites in northern Australia (Darwin Harbour, Port Essington and Melville Bay) bordering the Arafura Sea, which extends from northern Australia to Indonesia. We collected all three *Ophelina* species at all three sites, indicating that each species maintains a stable gene pool across the region despite being isolated geographically. This may have occurred through each *Ophelina* species sharing genetic information across northern Australia and, therefore, reducing divergence through gene flow. Although the reproductive cycle of *Ophelina* species has been poorly studied, they are likely to have a planktonic larval stage based on studies of closely related polychaetes, such as *Ophelia* and especially *Armandia* (Rouse 2001; Tamaki 1985). Planktonic larvae in the Arafura Sea are likely to be transported in an easterly direction during the monsoonal months but in a westerly direction during the dry season, according to recent oceanographic models (Condie 2011). This may result in the efficient dispersal of *Ophelina* larvae across the northern edge of the Northern Territory, reducing genetic drift and specialization.

The two new species herein described, *Ophelina tessellata* and *O. cyprophilia*, occurred sympatrically: they occurred not only at the same sampling site but also in the same samples. The other described species, *Ophelina fauveli*, occurred at the same sites but was collected only in deeper waters ranging from 10 to 21 m. Both *O. tessellata* and *O. cyprophilia* were collected from intertidal environments and at depths less than 10 m. Despite these two species occurring sympatrically and having similar morphologies, they were clearly separated into two clades based on sequences of the COI and histone H3 genes. This

maintenance of independent gene pools, despite living in sympatry, may be achieved through differences in reproductive timing or by specific gamete recognition systems and hybrid inviability (Maltagliati et al. 2004; Vacquier 1998). Species of *Ophelia* (Opheliinae) have also been found to live in sympatry while maintaining genetic differentiation (Maltagliati et al. 2004). It should also be noted that we cannot be sure that the two new species herein described are sister species. To explore this possibility, sequence data from other closely related *Ophelina* species, such as *O. fauveli, O. gigantea, O. grandis*, are needed. We also cannot rule out the possibility that these species evolved elsewhere and colonised the northern Australian sites at a later date.

One of the species described in this paper, *O. cyprophilia* sp. nov., was found as part of a depauperate polychaete assemblage in sediments containing high copper levels. This species was consistently found in the polluted sediments and appears to be tolerant to elevated copper concentrations. This ability may make *O. cyprophilia* sp. nov. a useful organism for toxicological studies in tropical coastal Australian environments, particularly those examining sub-lethal biomarkers. The use of opheliids for toxicology testing has some benefits. Opheliids are sub-surface deposit feeders (Fauchald and Jumars 1979), ensuring contact with sediment-bound contaminants. Moreover, opheliids have a distinctive locomotive pattern, facilitating rapid family-level identification in the field.

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Ethical standards and conflicts of interest The research contained within this manuscript complied with Australian law. The authors declare that they have no conflict of interest.

Appendix A. Selected key features of *Ophelina* species reported from Australia and the Indo-Malay-Philippine archipelago

Table 2 Character key for Tables 3, 4, 5, 6 and 7

#	Character	#	Character
1	Taxonomic data source	19	Branchiae length (anterior): relative to body width
2	Specimens examined	20	Branchiae length (mid): relative to body width
3	Worm length: millimeters (up to)	21	Branchiae length (posterior): relative to body width
4	Number of chaetigers (up to)	22	Cilliated branchiae: presence
5	Intrasegmental body anulations: presence	23	Cilliated branchiae: description
6	Prostomial length	24	Anal funnel shape: cylindrical or laterally compressed
7	Prostomial width	25	Anal funnel opening: ventrally (hood shaped) or terminally
8	Terminal palpode: presence	26	Anal funnel length: relative to depth
9	Prostomial eyes: number	27	Anulations on anal funnel: presence
10	Prechaetal lobe (chaetiger 3): relative length to the branchiae	28	Number of anulations on anal funnel
11	Prechaetal lobe (posterior): relative length to the branchiae	29	Anal papillae, unpaired ventral: presence
12	Postchaetal lobe: description (absent, fillet or cirrus)	30	Anal papillae, unpaired ventral: description
13	Chaetae length (anterior): relative to branchiae length	31	Anal papillae, paired ventral: presence
14	Chaetae length (mid): relative to branchiae length	32	Anal papillae, paired ventral: description
15	Chaetae length (posterior): relative to branchiae length	33	Anal papillae, rim cirri: number
16	Chaetae of anterior parapodia: orientation (forward or lateral)	34	Anal papillae, rim cirri: description
17	Branchiate chaetigers: start	35	Anal papillae, rim cirri: anterior length relative to paired ventral
18	Branchiate chaetigers: finish		

Table 3 Comparison of key features of *Ophelina* species from the Australian region

Character	*Ophelina acuminata* Ørsted, 1843	*Ophelina longicirrata* Hartman-Schröder and Parker, 1995	*Ophelina gigantea* (Rullier, 1965) [a]	*Ophelina kampeni* (Horst, 1919)	*Ophelina ehlersi* (Horst, 1919)
1	Hartmann-Schröder 1979	Hartmann-Schröder and Parker 1995	Rullier 1965	Horst 1919	Horst 1919
2	2	1	3	1	1
3	10	52	64	35	35
4	54	41	68	58	38
5		Strongly annulated	Present	Faintly annulated	Faintly annulated in posterior region
6	Approximately as long as high	Longer than wide at base			
7					
8		Present	Present	Present	Present
9	3: 1 forward and dorsal and 2 behind and ventral	0	0		
10		0.3 [a]	0.15		
11		0.1 [a]		0.15 [a]	
12	Cirriform	Long cirrus	Cirriform	Absent	Small cirrus, distally dilated
13	Cirri not obviously long	0.3: Nowhere obviously long [a]	0.5		
14		0.2 [a]	0.6		0.5
15				1.2 [a]	
16		Posterio-laterally [a]	Dorso-laterally		
17	2	2		2	First parapodia are without branchia
18	None on last 7	Last		Last	
19		1: long, cirriform to filiform [a]	1.2	0.5	
20		2 [a]	1	Longer	
21		1.5 [a]	1.2	0.8 [a]	
22					
23					
24		Cylindrical		Elongated	Elongated and oval
25	Ventrally	ventro-terminal	Ventrally	Ventrally	
26	Equal to the last 4 or 5 segments together	As long as the 5–6 last segments together	2	1.8 [a]	Short or broken
27	Rings present	Present	Present	Present	
28	21		45	30	
29	Present	Absent ?lost	Present	Present	Absent
30	Apparently contracted		Tapered, 0.4x the funnel length	0.5 times funnel length [a]	
31	Present		Present	Present	Couple of elongated papillae ventrally
32	long		Tapered, 0.15x funnel length	Much shorter than funnel (0.05 times) [a]	
33	10	11	52	44 [a]	
34	Flat or spoon forming of unequal length		Weakly tapered, anterior papillae slightly longer than posterior	Tapered, anterior papillae length same as posterior [a]	
35			0.3	0.4 [a]	

[a] Taxonomic data was obtained from figures

Table 4 Comparison of key features of *Ophelina* species from the Australian region continued

Character	*Ophelina grandis* (Pillai, 1961) [a]	*Ophelina grandis* (Pillai, 1961)	*Ophelina Sibogae* (Caullery, 1944) [a]	*Ophelina cf. sibogae* (Caullery, 1944)	*Ophelina kükenthali* (Horst, 1919)
1	Pillai 1961	Eibye-Jacobsen 2002	Caullery 1944	Eibye-Jacobsen 2002	Horst 1919
2	9	2	14	11	2
3	34.5	47	30	14.5	18
4	66	65	65	42	29
5			Present		
6	Triangular	Slightly longer than wide		Slightly longer than wide	
7					
8	Present	Present	Present	Present	Present
9	0	0	0	1 observed in 1 specimen	
10	0.1		0.1		
11	0.1				
12	Absent		Small cirrus		Small cirrus
13	0.6		0.4		
14	0.6		0.7		
15	0.4		Absent		
16	Anteriorally	Bent forwards and elongate	Posterio-laterally		
17	2	2	2	2	2
18	Last	Last	Last	Present to at least 7 setigers from posterior	Absent from last three parapodia
9	1.1	Relatively long	0.75	Relatively long	Rather long but not reaching median dorsal line
20	0.8		1		
21	1.2	Well developed	1.2		
22					
23					
24	Spoon-shaped				Gutter-shaped
25	Ventrally	Ventrally	Ventrally	ventrally	Ventrally
26		2 times as long as width at base	3	3.5-4 times as long as width at base	Not so high distally as proximally
27			Present		Faintly annulated
28			36		
29	Present	Present	Present	Present	Absent
30	Tapered, 0.8 times the funnel length	Blunt, 0.3 times funnel length [a]	Tapered, 0.8 times funnel length	0.75 times funnel length	
31	Present	Present	Present	Absent	Present
32	Tapered, 0.15 times funnel length	0.05 times funnel length [a]	Weakly tapered, 0.1 times funnel length		
33	31	5 pairs posteriorly plus a few ventrally	20	5 pairs concentrated posteriorly and up to 6 pairs along rest of margin	8 or 9 cirri posteriorly
34	Weakly tapered, anterior papillae 2 times longer than posterior papillae	Posterior margin with 5 pairs of cirriform papillae, 3 times longer than broad	Not tapered, anterior same length as posterior	Papillae cirriform, 4–6 times longer than broad	
35	1.2		0.9		

[a] Taxonomic data was obtained from figures

Table 5 Comparison of key features of *Ophelina* species from the Australian region continued

Character	*Ophelina buitendijki* (Horst, 1919)	*Ophelina bimensis* (Caullery, 1944) [a]	*Ophelina fauveli* (Caullery, 1944) [a]	*Ophelina cordiformis* (Caullery, 1944) [a]	*Ophelina cf. cordiformis* (Caullery, 1944)
1	Horst 1919	Caullery 1944	Caullery 1944	Caullery 1944	Eibye-Jacobsen 2002
2	1	3	1	1	10
3	40	15	20	22	23
4	64	35	31	50	51
5	Absent	Present	Present		
6					Slightly longer than wide
7					
8	Present	Present	Present		Present
9		0	0		1 observed in 1 specimen
10	0.1	0.15	< 0.1	0.3	
11	0.3		0.1		
12	Short cylindrical cirrus	Small cirrus			
13		0.6	0.3		
14	0.6	0.6		1	
15		0.7	0.3		
16		Posterio-laterally	Posterio-laterally		
17	2	2	2		2
18	Last	Last	Last		Second last
19	Long cirriform reaching the median dorsal line	0.8	0.8		Relatively long, 0.8 [a]
20		1.1			
21	Shorter	1	1.2		
22					
23					
24	Slender and gutter-shaped		Laterally compressed		
25		Ventrally	Ventro-terminally	Ventrally	Ventrally
26		2	Slightly longer than deep	2	1.5-2 times as long as width at base
27			Present	Present	
28			14	20	
29	Absent	Present	Present	Present	Present
30		Tapered, 1.5 times funnel length	Tapered, 2 times longer than funnel	Not tapered, 0.5 times funnel length	Blunt, 0.4 times funnel length [a]
31	Present	Absent	Present	absent / not shown	Absent
32			Tapered, similar length to funnel		
33	Long cirri on the border	19	18	Absent / not shown	6 pairs
34		Tapered, similar lengths	Blunt, some spoon-shaped, unequal lengths		Posterior margin with 6 pairs of irregular papillae, none ventrally
35		Much longer than usual	0.1-0.5		

[a] Taxonomic data was obtained from figures

Table 6 Comparison of key features of *Ophelina* species from the Australian region continued

Character	*Ophelina longicaudata* (Caullery, 1944) [a]	*Ophelina dubia* (Caullery, 1944) [a]	*Ophelina brevibranchiata* (Caullery, 1944) [a]	*Ophelina profunda* (Caullery, 1944) [a]	*Ophelina pygocirrata* (Ehlers, 1920)
1	Caullery 1944	Caullery 1944	Caullery 1944	Caullery 1944	Ehlers 1920
2	1	1	2	1	3
3	12	11	20	35	18.5
4	30	51	31	45	29
5					Absent, segment borders not well defined
6	Equal length and width	Equal length and width	Similar length and width	Wider than long	
7					
8	Present	Present	Present	Absent or reduced	Present
9	0	0	0	0	
10	0.25	0.2	0.2	0.15	
11	<0.1				
12				Absent	Present
13	0.3	0.7	2	0.8	
14					
15	0.5	2			
16	Posterio-laterally	Posterio-laterally	Laterally	Laterally	
17	2	1	3	2	
18	Last	Last			
19	0.9	1.2	0.2	0.5	
20				0.4	0.5
21	0.5	0.5			
22					
23					
24	Much more elongated	Missing from specimen		Laterally compressed	
25	Ventro-terminally		Terminally	Termanally along entire length	
26	6		1	2	
27	Present		Present	Present	
28	45		7	16	
29	Present			Present	Absent or broken off
30	Extends from the funnel end a further 0.75 times length			0.7	
31	Absent			Absent / not shown	Absent or broken off
32					
33	Present			5	10
34	8 on the posterior rim, absent elsewhere			Only at posterior end	
35	Very short			Uniform in length	

[a] Taxonomic data was obtained from figures

Table 7 Comparison of key features of *Ophelina* species from the Australian region continued

Character	*Ophelina remigera* (Ehlers, 1916)	*Ophelina langii* (Kükenthal, 1887)
1	Ehlers 1916	Kükenthal 1887
2	1	
3	40	23
4	37	50
5	Absent	Present, eight rings on each segement
6	Hardly as long as base width	
7		
8	Present but not obviously distinct	Present
9		
10		
11	0.1 [a]	<0.1 [a]
12	Cirrus distally dilated [a]	
13		
14	Dorsal chaetae longer than ventral	
15		Longer than usual
16		
17	2	
18	Last	
19		0.6 [a]
20		0.9 [a]
21		1.1 [a]
22		
23		
24	Nearly round	Very thin
25	Ventrally	Ventrally
26	Equal to last 4 segments	
27		Present
28		20
29	Present	
30	Longer than rim papillae	
31		
32		
33	Line of papillae round rim edge	Absent [a]
34	Second protrusion also contains papillae	
35	7 papillae along edge and 3 longer papillae posteriorly	

[a] Taxonomic data was obtained from figures

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