

Research Article

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Terrigenous leaf-utilizing life of the tube-bearing annelid *Anchinothria cirrobranchiata* (Annelida: Onuphidae) in the deep sea

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Abstract

Deep-sea ecosystems are generally oligotrophic because they lack photosynthesizing producers. On deep-sea slopes near land, however, various terrestrial plant remains flow to and are deposited on near-shore deep bottoms. From a depth of 300 m off the Pacific coast of Owase, central Japan, we collected an onuphid polychaete, *Anchinothria cirrobranchiata* (Annelida: Onuphidae), which lives in a dorsoventrally flattened portable tube. The polychaete tubes were made of sand, as well as leaves and twigs of terrestrial evergreen trees. The leaves glued on the portable tubes were chartaceous, blackish and tough; they belonged mainly to two genera of Fagaceae, *Castanopsis* and *Quercus*, which are dominant components of coastal evergreen oak forests. In aquaria, the polychaetes fed on the leaves on their tubes, as well as autochthonous sedimented leaves, suggesting utilization of terrigenous plant litter as food. As to the evolution of *Anchinothria*, a molecular phylogenetic analysis suggests that *Anchinothria* is monophyletic with other Hyalinoeciinae (*Nothria*, *Leptoecia*, *Hyalinoecia*).

Introduction

The deep sea lacks primary photosynthetic producers; it is often an oligotrophic environment compared with the shallow sea. In such oceanic deep environments, the sedimentation of marine snow (i.e. the mucus-laden remains and excretions of organisms living in the shallow sea) and the diurnal vertical movement of zooplankton provides a route for supplying photosynthetic products from the shallow sea to the deep sea (Wiebe *et al.*, 1979). Nutrients are also supplied to the deep sea through the influx of plant remains. There is constant runoff of seagrass from shallow-water communities to deeper ecosystems. During floods and storms, large amounts of seagrass flow from the communities and eventually sink to the deep-sea bottom (Menzies *et al.*, 1967; Menzies & Rowe, 1969). On these sea beds, abyssal sea urchins directly consume large amounts of eelgrass leaves at depth of 2500–4000 m; sea cucumbers ingest nutrients from the decomposed seagrass remains (Suchanek *et al.*, 1985).

On deep-sea bottoms near land, there are characteristic inflows of terrestrial plant remains, such as fallen leaves and twigs, and occasional large pieces of wood. These terrigenous plant remains are decomposed to differing degrees by fungi, bacteria and other microorganisms; they are utilized by diverse benthic invertebrates (Sridhar, 2012; Xu *et al.*, 2014). For example, *Xylophaga* bivalves bore into wood; after the death of these borers, the remaining holes are used by sipunculids, galatheoids and polychaetes belonging to Cirratulidae, Maldanidae, Capitellidae and Ampharetidae. Various sessile organisms (e.g. bivalves, bryozoans and barnacles) also use wood as a substrate (Wolff, 1979).

Terrestrial plant remains on deep-sea bottoms are also used by three described species of tube-bearing polychaetes belonging to the genus *Anchinothria* (Onuphidae), which live in portable tubes that are characteristically encrusted with submerged fallen leaves or twigs of land plants, spines of sea urchins or sponges, foraminiferas etc. (Table S1). While the use of fragments of fallen leaves/twigs as material for portable tubes is common among trichopteran larvae in inland waters (Thomas *et al.*, 2020), it is remarkable that deep-sea polychaetes have a similar lifestyle. Generally, polychaetes belonging to Onuphidae live on sea bottoms as settling semi-burrowers (*Onuphis*) or crawling tube bearers (Hyalinoeciinae: *Anchinothria*, *Hyalinoecia*, *Leptoecia*, *Nothria*). In Onuphinae, *Diopatra* is the most common leaf-utilizing species. *Diopatra* is a semi-burrowing species found in the intertidal and subtidal mud flat, which is known to utilize leaves, shells and seaweed to encrust the upper tube exposed on the sand (Paxton, 1998). As to Hyalinoeciinae, *Leptoecia* and *Hyalinoecia* worms live in rigid transparent tubes made of onuphic acid (Graham *et al.*, 1965; Budaeva *et al.*, 2014), while *Nothria* worms live in portable tubes encrusted with components such as sand, stones and shell fragments (e.g. Fauchald, 1982; Imajima, 1999). It is intriguing to consider how the use of fallen leaves/twigs as portable tube material evolved in an Onuphidae clade.

Anchinothria has been described in previous studies as morphologically sister to *Nothria* and *Hyalinoecia* (Budaeva *et al.*, 2016). On the other hand, ecological and molecular phylogenetic knowledge of *Anchinothria* is limited due to its deep-sea habitat and rare occurrence



which makes it difficult to collect. In the genus, *A. cirrobranchiata* is collected only in the seas around Japan, and known to have a tube with scattered pieces of leaves and wood (Table S1, Imajima, 1999). Because deep-sea life utilizing terrigenous plant remains is unique, it is intriguing to determine what plant species are used as tube materials, if the plant debris on portable tubes is also used as food, and when the utilization of land plant debris originated in the phylogeny of Onuphidae.

From the deep-sea bottom near land in the Japanese Archipelago, we collected the deep-sea polychaete *Anchinothria cirrobranchiata*, which uses fallen leaves of land plants as material for its portable tube. To address the above-mentioned questions, we observed the feeding biology of these polychaetes, investigated the composition of the materials encrusting their portable tubes, identified the plant species of the leaves on the tubes, and performed a molecular phylogenetic analysis of Onuphidae.

Materials and methods

Sample collection

Deep-sea benthic organisms were collected by trawling a dragnet at a depth of 200–300 m off the coast of Owase, Mie Prefecture (34°01'N 136°22'E), on 26 February 2017. Owase has a very high amount of precipitation and is surrounded by extensive evergreen forests. In addition to various benthic organisms, extensive terrigenous plant remains were collected in this bottom trawl. Eighteen *Anchinothria cirrobranchiata* were collected. The worms were identified based on Imajima (1999); branchiae first present from chaetiger 11 and branched with five filaments (Figure 1A), bidentate pseudocomposite hooks are seen in chaetiger 1–4 (Figure 1B), scoop-shaped pectinate chaetae are seen from chaetiger 2 (Figure 1C) and subacicular hooks start from chaetiger 9–11 (Figure 1D). Three of the collected *Anchinothria* worms were used for ecological observations and the others were fixed in 70% ethanol for detailed observation of tube materials and in 99% ethanol for molecular phylogenetic analysis; the voucher is stored at Seto Marine Biological Laboratory (specimen number: SMBL-V0637).

Ecological observations and identification of the leaves used for tubes

One of the *Anchinothria* polychaetes collected was kept in an aquarium at Toba Aquarium with filtered natural seawater regulated to 10°C, and its biological photograph were taken and behaviour was recorded with TG-4 (OLYMPUS, Tokyo, Japan) at 1.0–2.5×. Two polychaetes were kept in Petri dishes with artificial seawater at a room temperature of 25 ± 3°C for observation.

The individual kept in an aquarium was provided autochthonous fallen leaves collected with polychaetes and its behaviour was observed. The individuals in the Petri dishes were provided fallen land leaves *Quercus glauca*, *Quercus phillyraeoides*, *Zelkova serrata* and *Castanopsis sieboldii* soaked overnight in 1 litre of seawater to prevent changes in salinity and runoff of secondary metabolites into the culturing seawater, and time-lapse photography was performed from a vertical position for 24 h. To determine the structure and constitution of tube materials, a single tube was cut into thin slices and its cross-sectional structure was observed under a microscope at 10×. We examined all materials in each portable tube for the 15 fixed specimens; we identified the plant species of all leaf fragments based on the morphology of nervure and leaf margin. The area of all tube-encrusting materials was measured using ImageJ v. 1.53 and the percentages of area of each material against total area of materials were calculated.

Molecular phylogenetic analysis

We performed direct polymerase chain reaction using Ampdirect® Plus reagent (Shimadzu) to amplify DNA from ethanol-preserved skin (3–5 mg dry weight) of *A. cirrobranchiata*. The tissue was dried, soaked in 50 µl lysis buffer (20 mM Tris-HCl at pH 8.0, 5 mM ethylenediaminetetraacetic acid, 400 mM NaCl, 3% sodium dodecyl sulphate, and 200 µmol ml⁻¹ proteinase), and incubated at 60°C for 12 h. Polymerase chain reaction was used to amplify the 16S rRNA gene (~500 bp amplicon) using primers 16SarL and 16S-OnuR (Palumbi *et al.*, 1991; Budaeva *et al.*, 2016), and 18S rRNA gene (~1850 bp amplicon) using three primer pairs: 18e + 18L; 18F509 + 18R; 18F997 + 18D843 (Elwood *et al.*, 1985; Hillis *et al.* 1993; Struck *et al.*, 2002). The purified polymerase chain reaction products (≥50 ng µl⁻¹) were directly sequenced (Eurofins, Japan) using the above primers. The resulting sequences were deposited in the DDBJ/EMBL/GenBank databases under the accession numbers listed in Table S2. With the obtained sequences, the phylogenetic position of *Anchinothria* were analysed based on the phylogenetic tree derived in Budaeva *et al.* (2016). Our dataset includes the sequences of the representative OTUs of each major clade of Onuphinae used in this study. Eunicidae, which comes outside Onuphidae, was used as an out-group. The cited sequences are listed in Table S2. These sequences were aligned using the Muscle package (Edgar, 2004) implemented in SeaView software (Galtier *et al.*, 1996; Gouy *et al.*, 2010) with the default settings. We used Gblocks v. 0.91b (Castresana, 2000; Talavera & Castresana, 2007) to eliminate any ambiguously aligned regions of each sequence. The 16S and 18S gene sequence lengths before and after Gblocks treatment were 448 and 443 bp, 1774 and 1636 bp, respectively. Bayesian and maximum likelihood phylogenetic analyses were performed on the combined 16S + 18S dataset using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003) and RAxML v. 7.4.2 (Stamatakis, 2006) implemented in raxmlGUI v. 1.31 (Silvestro & Michalak, 2012). We selected the GTRGAMMA model for the RAxML analysis and used Kakusan4 software (Tanabe, 2011) to select appropriate models for the MrBayes analysis. The model selected for the MrBayes analysis was GTR_GAMMA for each gene. Two independent runs of the Metropolis-coupled Markov chain Monte Carlo procedure were performed simultaneously for 2,000,000 generations. The convergence of MCMC was confirmed with Tracer v1.7.2.

Results

Materials of portable tubes

The portable tube of *Anchinothria cirrobranchiata* was long, straight, and dorsoventrally flattened; it comprised a thin inner film and parchment-like layer with accretion glued on it (Figure 1E). Each tube had an opening at each end. The accretion comprised sand grains and fragments of fallen leaves and twigs; the fragments of fallen leaves and twigs mechanically reinforced the tube (Figure 1F–G). Table 1 shows the percentages of materials glued on the portable tubes. These glued-on leaves were characeous, blackish, tough, and generally fragmented. All plants were evergreen tree species, except one deciduous tree species, *Zelkova serrata*.

Moving and feeding behaviour

An *Anchinothria* worm bearing its portable tube advanced by protruding the anterior part of its body to a great extent from the tube, anchoring the body to the substrate with one to three parapodia, and drawing the posterior part of the body with the tube forward (Figure 1H). *Anchinothria* worms were also

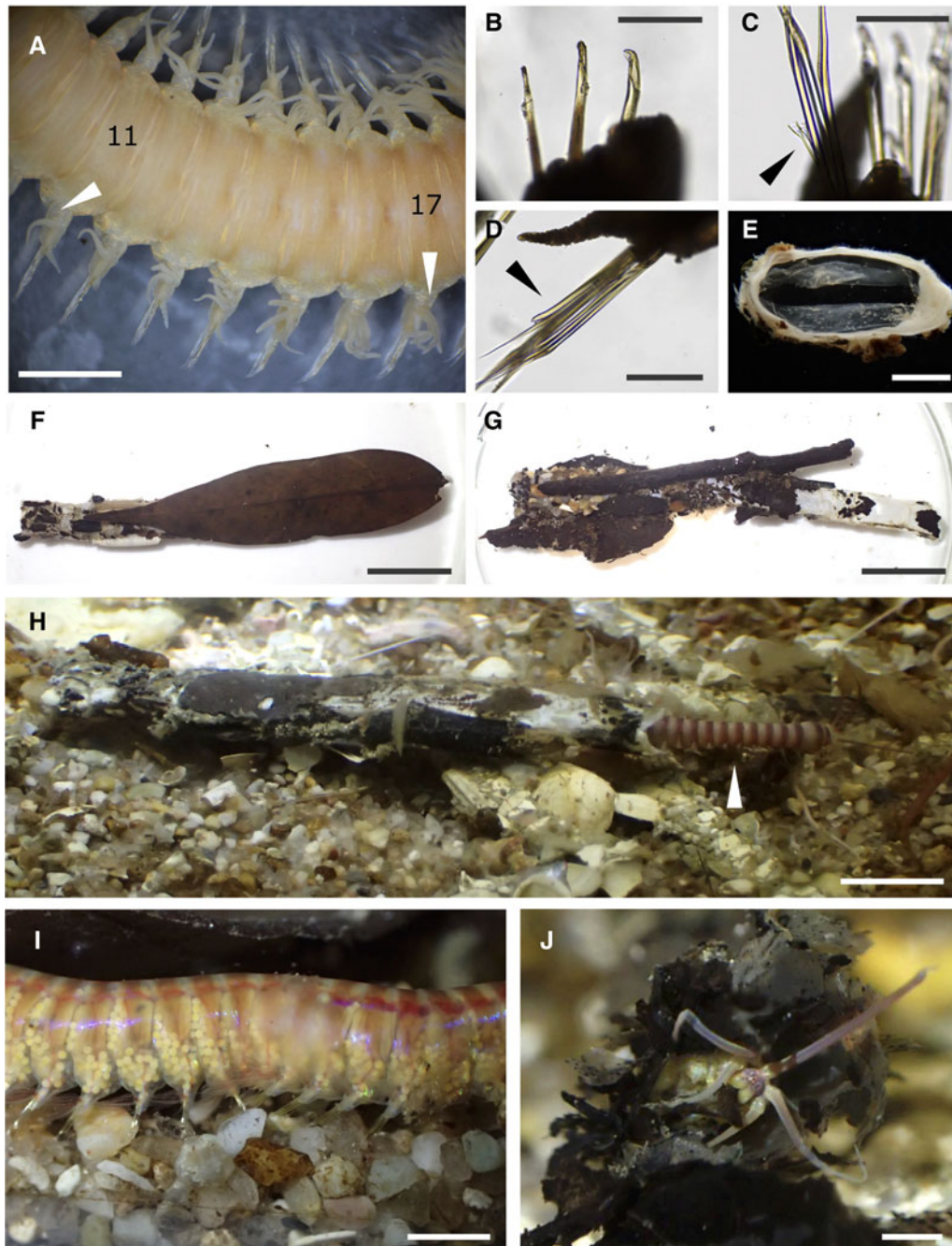


Fig. 1. Ecology and morphology of *Anchinothria cirrobranchiata*. (A) dorsal view which shows branchiae. Branchiae start from chaetiger 11, and branched with five filaments from chaetiger 17 (arrowhead); (B) bidentate pseudocomposite hooks in chaetiger 1; (C) scoop-shaped pectinate chaetae in chaetiger 3; (D) subacicular hooks in chaetiger 9; (E) a tube cross-section, showing a thin film stretched inside a parchment-like layer; (F–G) tubes encrusted with fallen leaves and twigs; (H) A worm moving forward by moving the front of its body (arrowhead) out of the tube; (I) body filled with numerous eggs; (J) front view of a worm foraging for fallen leaves; Scale bars: (A) 1 mm; (B–D) 200 μ m; (E) 1 mm; (F–H) 1 cm; (I, J) 1 mm.

observed to turn anterior-posteriorly and up-down within their portable tube; for example, when they were moving in one direction and their head hit the aquarium wall, they turned around in the portable tube, changed their body direction to the opposite side, and continued moving. We also observed worms turning up and down in the tube while foraging, reversing their body orientation against the tube. Eggs were held in the body cavity of the exposed anterior part (Figure 1I). The average diameter of the eggs was 150 μ m (\pm 14 μ m, N = 20).

The *Anchinothria* worms kept with autochthonous brownish fallen leaves in aquaria were observed to use their jaws to cut the leaves and glue them onto their tubes. The worms were also observed feeding on leaves of their own tube material by holding

them with the first and second parapodia, then cutting them with their jaws and taking them in (Figure 1J). However, the worms did not feed on the fallen leaves collected on land.

Molecular phylogenetic analysis

The phylogenetic tree derived from Bayesian method is shown in Figure 2. The tree topology was consistent with the ML and Bayesian methods except for specimens inside *Hyalinoecia* and *Mooreonuphis*. Hialinoeciinae including *Hyalinoecia*, *Leptoecia*, *Nothria* and *Anchinothria* was monophyletic (Bayesian posterior probability: PP = 1, bootstrap support value: BS = 100). Within this clade, *Anchinothria* was sister to the remaining genera,

Table 1. Percentage of materials glued on portable *Anchinothria cirrobranchiata* tubes (N = 15)

				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Tube length				38.4	41.3	41.7	52.3	52.6	52.9	53.7	55.0	55.5	55.8	56.5	58.5	59.7	66.3	68.7	
Tube materials	Plant species	Plant family	Leaf texture																
Leaf fragment	<i>Daphniphyllum teijsmannii</i>	Daphniphyllaceae	Evergreen	10.0															
	<i>Quercus glauca</i>	Fagaceae	Evergreen											15.8					
	<i>Quercus phillyraeoides</i>	Fagaceae	Evergreen	31.1				28.1		35.4			40.0		30.2		36.8		
	<i>Quercus myrsinifolia</i>	Fagaceae	Evergreen												46.9				
	<i>Castanopsis sieboldii</i>	Fagaceae	Evergreen	31.4				21.6		45.3		19.8		18.9		41.5			
	<i>Machilus thunbergii</i>	Lauraceae	Evergreen								11.3								
	<i>Litsea acuminata</i>	Lauraceae	Evergreen	52.4															
	<i>Cinnamomum yabunikkei</i>	Lauraceae	Evergreen	23.8											18.5				
	<i>Ternstroemia gymnanthera</i>	Pentaphylacaceae	Evergreen									13.5							
	<i>Pittosporum tobira</i>	Pittosporaceae	Evergreen	18.3					13.9										
	<i>Camellia japonica</i>	Theaceae	Evergreen	21.4															
	<i>Zelkova serrata</i>	Ulmaceae	Deciduous													40.8			
Sand grain				8.75	1.93	10.7	3.27	4.00	8.54	3.64	18.6	6.27	11.2	6.45	1.69	4.25	9.95	5.62	
Twig fragment				13.8	34.6	5.6	12.0	12.7	8.2	23.7	18.0	26.7	18.4	23.0	15.3	13.4	35.1	32.0	
	Unidentified leaf fragment	-	-	25.1	32.1	44.0	29.9	33.6	38.0	27.7	31.0	31.6	14.6	23.6	12.0	17.0	55.0	20.9	

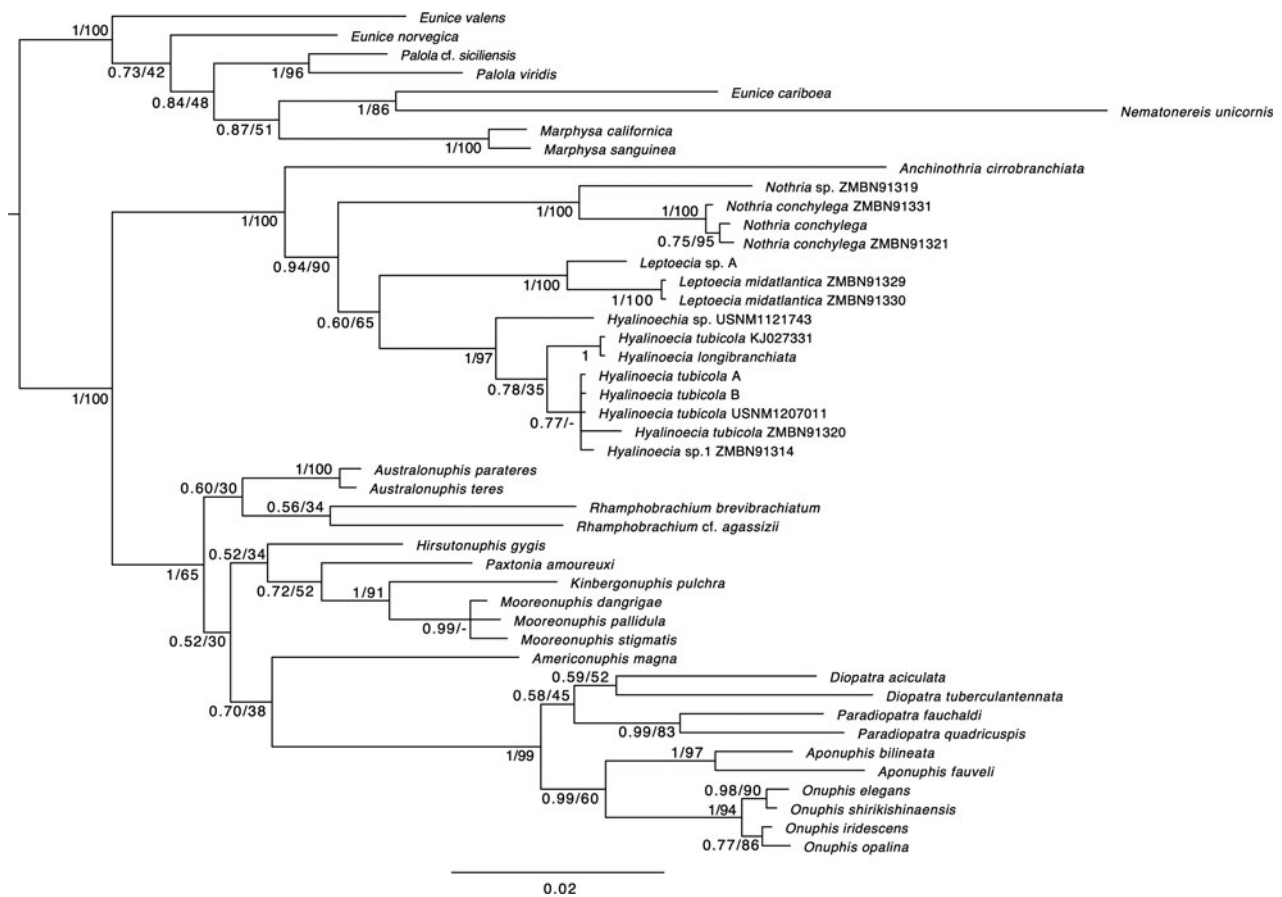


Fig. 2. Bayesian/ML tree of the combined 16S and 18S rRNA sequences. The numbers at the nodes are Bayesian posterior probability values and maximum likelihood bootstrap support values. See Table S2 for the list of accession numbers.

which were also monophyletic (PP = 0.94, BS = 90). Each of *Nothria*, *Leptoecia* and *Hyalinoecia* was monophyletic (Figure 2).

Discussion

Utilization of fallen leaves and twigs

The leaves and twigs glued on tubes presumably contribute to reinforcing the tube, and they serve as both camouflage and portable food. The dominant species belonged to two genera of Fagaceae, *Quercus* and *Castanopsis*, both of which are dominant genera in the natural forests around Owase. These results suggest that the life of *Anchinothria* is supported by the nearby terrestrial evergreen forest ecosystem via the substantial influx of forest litter caused by high precipitation.

The behavioural observations that the worms turn up and down, and reverse their body orientation against the tube suggested that the tube does not have a dorsoventral or anterior–posterior orientation. The moving behaviour with portable tube has been observed in detail in *Hyalinoecia artifex* (Meyer et al., 2016), and *Anchinothria cirrobranchiata* was found to move and change the body direction in a similar method. On the other hand, *A. cirrobranchiata* has thick tubes, unlike *Hyalinoecia* which builds fitted tubes.

From the observation in aquarium tanks, it is supposed that *Anchinothria* use fallen leaves deposited in the deep sea not only as tube-encrusting material but also as food. As to fallen leaves on land, it is possible that the small size of the water system in the Petri dish stressed the worms and prevented them from feeding. To determine whether *Anchinothria* will feed on undecayed fallen leaves, it is necessary to conduct another test in the same environment in which they fed on decayed fallen leaves.

Reproductive ecology

The eggs of *A. cirrobranchiata* were small-sized similar to many of tube-bearing onuphid species (Giangrande, 1997). Some Onuphid species, such as *Notonuphis antarctica*, are known to have eggs larger than 1 mm and nurse in adult tubes, but species with eggs as small as 200 µm are generally known to spawn eggs (Paxton, 1986). From this knowledge, it is assumed that *A. cirrobranchiata* spawn its eggs.

Evolution of tube-bearing life

The phylogenetic tree suggests that *Anchinothria* is sister to other Hyalinoeciinae genera: *Hyalinoecia*, *Leptoecia* and *Nothria*, which were also monophyletic. This result contradicts the morphological assumption in previous research that the subfamily Hyalinoeciinae was divided into two groups: *Hyalinoecia* group including *Hyalinoecia*, *Leptoecia* and *Hyalospinifera*, and *Nothria* group including *Nothria* and *Anchinothria* (Budaeva et al., 2016). Molecular phylogenetic trees do not provide well-supported estimates of phylogenetic relationships among four genera due to low support values in both previous and present studies. Further taxon sampling and analysis with more genetic markers may resolve their relationships.

Onuphinae have developed non-portable tubes encrusted with sediment materials such as shell fragments, algae, fallen leaves, sponge rods etc. (Fauchald, 1982; Paxton 1986), and in the Hyalinoeciinae, the accretion of sediment material onto portable tubes is only seen in the *Nothria* and *Anchinothria*. *Nothria* species build tubes with shells and gravel fragments, but *Anchinothria* has a diverse selection of tube-encrusting materials (Table S1). It is hoped that phylogenetic analysis of *Anchinothria* species which

utilize a variety of case-encrusting materials will elucidate the evolutionary path of case-encrusting materials selection and the ecology and morphology acquired in the use of each material.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0025315422000807>.

Data. Gene sequences analysed during this study are deposited to GenBank.

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Author contributions. LY designed the study; LY and TM carried out the study; LY and MK analysed the data; LY, TM and MK interpreted the findings and wrote the article.

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Conflict of interest. The authors declare none.

Ethical standards. Not applicable.

References

- Budaeva N, Pyataeva S and Meissner K (2014) Development of the deep-sea viviparous quill worm *Leptoecia vivipara* (Hyalinoeciinae, Onuphidae, Annelida). *Invertebrate Biology* **133**, 242–260.
- Budaeva N, Schepetov D, Zanol J, Neretina T and Willassen E (2016) When molecules support morphology: phylogenetic reconstruction of the family Onuphidae (Eunicida, Annelida) based on 16S rDNA and 18S rDNA. *Molecular Phylogenetics and Evolution* **94**, 791–801.
- Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**, 540–552.
- Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* **5**, 1–19.
- Elwood H, Olsen GJ and Sogin M (1985) The small-subunit ribosomal RNA gene sequences from the hypotrichous ciliates *Oxytricha nova* and *Stylonychia pustulata*. *Molecular Biology and Evolution* **2**, 399–410.
- Fauchald K (1982) Revision of Onuphis, *Nothria*, and *Paradiopatra* (Polychaeta: Onuphidae) based upon type material. *Smithsonian Contributions to Zoology*. Washington, DC: Smithsonian Institution Press.
- Galtier N, Gouy M and Gautier C (1996) SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Computer Applications in the Biosciences* **12**, 543–548.
- Giangrande A (1997) Polychaete reproductive patterns, life cycles and life histories: an overview. *Oceanography and Marine Biology* **35**, 323–386.
- Gouy M, Guindon S and Gascuel O (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* **27**, 221–224.
- Graham G, Kelly P, Pautard F and Wilson R (1965) Onuphic acid – a sugar phosphate polymer from the tube of *Hyalinoecia tubicola*. *Nature* **206**, 1256–1257.
- Hillis DM, Ammerman LK, Dixon MT and de Sá RO (1993) Ribosomal DNA and the phylogeny of frogs. *Herpetological Monographs* **7**, 118–131.
- Imajima M (1999) Onuphidae (Annelida, Polychaeta) from Japan, excluding the genus *Onuphis*. *National Science Museum Monographs* **16**, 1–115.
- Menzies RJ and Rowe GT (1969) The distribution and significance of detrital turtle grass, *Thalassia testudinata*, on the deep-sea floor off North Carolina. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* **54**, 217–222.
- Menzies RJ, Zaneveld JS and Pratt RM (1967) Transported turtle grass as a source of organic enrichment of abyssal sediments off North Carolina. *Proceedings of the Deep Sea Research and Oceanographic Abstracts*. Amsterdam: Elsevier, pp. 111–IN117.
- Meyer KS, Wagner JKS, Ball B, Turner PJ, Young CM and Van Dover CL (2016) *Hyalinoecia artifex*: field notes on a charismatic and abundant epifaunal polychaete on the US Atlantic continental margin. *Invertebrate Biology* **135**, 211–224.
- Palumbi S, Martin A, Romano S, McMillan W, Stice L and Grabowski G (1991) *The Simple Fool's Guide to PCR. Version 2*. University of Hawaii, Zoology Department, Honolulu, HI.
- Paxton H (1986) Generic revision and relationships of the family Onuphidae (Annelida: Polychaeta). *Records of the Australian Museum* **38**, 1–74.
- Paxton H (1998) The *Diopatra chiliensis* confusion – redescription of *D-chiliensis* (Polychaeta, Onuphidae) and implicated species. *Zoologica Scripta* **27**, 31–48.
- Ronquist F and Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.
- Silvestro D and Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* **12**, 335–337.
- Sridhar KR (2012) Decomposition of materials in the sea. In Jones EBG and Pang K-L (eds), *Marine Fungi and Fungal-Like Organisms*, Berlin/Boston: Walter de Gruyter GmbH & Co. KG, pp. 475–500.
- Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.
- Struck T, Hessler R and Purschke G (2002) The phylogenetic position of the Aeolosomatidae and Parergodrilidae, two enigmatic oligochaete-like taxa of the ‘Polychaeta’, based on molecular data from 18S rDNA sequences. *Journal of Zoological Systematics and Evolutionary Research* **40**, 155–163.
- Suchanek TH, Williams SL, Ogden JC, Hubbard DK and Gill IP (1985) Utilization of shallow-water seagrass detritus by Caribbean deep-sea macrofauna: $\delta^{13}C$ evidence. *Deep Sea Research Part A. Oceanographic Research Papers* **32**, 201–214.
- Talavera G and Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* **56**, 564–577.
- Tanabe AS (2011) Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Molecular Ecology Resources* **11**, 914–921.
- Thomas JA, Frandsen PB, Prendini E, Zhou X and Holzenthal RW (2020) A multigene phylogeny and timeline for Trichoptera (Insecta). *Systematic Entomology* **45**, 670–686.
- Wiebe PH, Madin LP, Haury LR, Harbison GR and Philbin LM (1979) Diel vertical migration by *Salpa aspera* and its potential for large-scale particulate organic matter transport to the deep-sea. *Marine Biology* **53**, 249–255.
- Wolff T (1979) Macrofaunal utilization of plant remains in the deep sea. *Sarsia* **64**, 117–143.
- Xu W, Pang K-L and Luo Z-H (2014) High fungal diversity and abundance recovered in the deep-sea sediments of the Pacific Ocean. *Microbial Ecology* **68**, 688–698.