Description of a new species of Eulepethus (Annelida, Eulepethidae) from the northern South China Sea, and comments on the phylogeny of the family

JINGHUAI ZHANG¹, YANJIE ZHANG², KAREN OSBORN³ & JIAN-WEN QIU²,⁴

¹South China Sea Environmental Monitoring Center, State Oceanic Administration, 155 Xingangxi Road, Guangzhou, P. R. China. E-mail: zhangjinghuai@sohu.com
²Department of Biology, Hong Kong Baptist University, Hong Kong, P. R. China. E-mail: yanjie5337@gmail.com; qiujw@hkbu.edu.hk
³Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D. C. 20013, U. S. A. E-mail: osbornk@si.edu
⁴Corresponding author. E-mail: qiujw@hkbu.edu.hk

Abstract

Eulepethidae is a family of scale-bearing polychaetes. Although members of this family are common inhabitants of tropical and subtropical coastal waters, their diversity is low, with only 22 recognized species in six genera. Here we describe Eulepethus nanhaiensis sp. nov. based on 12 specimens collected from the coastal waters of the northern South China Sea. This new species can be distinguished from Eulepethus hamifer, the only previously described species in this genus, by having up to two spade-shaped lateral processes in some of the anterior elytrae, a blunt-tipped acicular chaeta in the neuropodia of segment 3, and a pair of non-overlapping elytrae in each posterior segment. Phylogenetic analysis indicates that Grubeulepis and Mexieulepis are sister genera, and these two genera form the sister clade of Eulepethus.

Key words: South China Sea, Taxonomy, Polychaeta, Annelida, scale worm

Introduction

Eulepethidae is a family of scale-bearing polychaetes (Phyllodocida, Aphroditoidea) with the dorsal cirri alternating with elytrae in anterior chaetigers (Fauchald, 1977). Eulepethidae is distinguished from other scale-bearing polychaetes by their unique neuroaciculae, which are distally enlarged to form a hammer-head like structure supporting the distal margin of the neuropodia (Fauchald & Rouse, 1997; Glasby & Fauchald, 2000). There are six recognized genera in Eulepethidae, which are distinguished by a combination of characteristics including the number of pairs of elytrae, presence or absence of ventral lamellae on bases of neuropodia, and whether the lateral margin of elytrae is smooth, notched, or with lappets (Pettibone, 1969; 1986; Woolley & Wilson, 2011). This family is small, with only 22 recognized species in total, and one to eight species per genus. Norlinder et al. (2012) assessed the phylogeny of scale-worms based on data from 18S rRNA, 28Sr RNA, 16S rRNA, COI sequences and morphology, however, the phylogenetic relationships among the eulepethid genera could not be assessed because only two genera were included.

Only three species of Eulepethidae have been described from the Northwestern Pacific: Eulepethus hamifer (Grube, 1875) from the Philippines, Mexieulepis amioi (Imajima, 1974) from Japan, and Proeulepethus nanshaensis Sun, 1998 from the South China Sea. Among them, M. amioi and P. nanshaensis have not been recorded outside their respective type locality, but E. hamifer has been recorded widely from several tropical to temperate locations (Pettibone, 1969; Uschakov, 1972; Jiang et al., 1990; Imajima, 2001, 2003; Du et al., 2011). While identifying polychaetes collected from regular benthic ecology monitoring surveys along the coast of Guangdong Province in the northern South China Sea, we encountered 13 specimens of Eulepethus that are morphologically distinct from E. hamifer, the only currently recognized species in the genus. Based on these specimens, we describe a new species of Eulepethus in this paper. We also conduct a preliminary assessment of the phylogenetic relationship among the species of eulepethids with available gene sequences.
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<th>Body length (mm)</th>
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* This is an incomplete specimen with 20 segments that are 8.5 mm in length.
Material and methods

Sediment samples were collected from Daya Bay, the Pearl River Estuary and Shantou waters in the northern South China Sea along the coasts of Guangdong Province (Table 1). Sediment samples were taken using a 0.05 m² or 0.1 m² van Veen grab, and washed on board through a sieve with a 0.5 mm mesh size. Specimens on the sieve were picked up, fixed in 5% formalin and later transferred to 70–100% ethanol for morphological observation, or 100% ethanol for phylogenetic analysis. Observations were made under a Carl Zeiss StemI 2000-C dissecting microscope and a Carl Zeiss Axio Imager M2 compound microscope. Scaled photographs were taken using an AxioCam ICc 1 camera fitted to the microscopes. Based on the photographs, line-drawings of the body and chaetae were made using Adobe Illustrator CS4. Two paratypes were dehydrated using a Xiangyi CFD-10D freeze-dryer, gold coated with an EDT SC-150, and observed under a TESCAN CEGA 3 scanning electron microscope (SEM).

Morphological description was mainly based on the holotype, supplemented with information from compound microscope and SEM micrographs to show the structural details of the chaetae, and variations in morphological characters among the type specimens. A specimen of *Eulepethus hamifer* (Grube, 1875) (USNM 17483), collected from the east coast of Mindanao, Philippine Islands and described by Pettibone (1969), was examined for comparison.

Types are deposited in the Marine Biological Science Museum (MBM) of the Chinese Academy of Sciences, Qingdao, China and the Australian Museum (AM), Sydney, Australia.

The following abbreviations are used in figure legends: An, anus; Br, branchia; dC, dorsal cirrus; dTc, dorsal tentacular cirrus; Es, eyespots; lAC, left anal cirrus; lAn, lateral antenna; mAn, median antenna; Pa, palp; rAC, right anal cirrus; vC, ventral cirrus; vTc, ventral tentacular cirrus; s3, segment 3; s7, segment 7.

One specimen (catalog no. P20150708009) was preserved in 100% ethanol for phylogenetic analysis. Genomic DNA was extracted, sequenced at a low coverage using Illumina Hiseq 4000, and the reads were subject to quality control procedures detailed in Zhang et al. (2015). Specifically, COI, 16S, 18S and 28S rRNA sequences of *E. nanhaiensis* were found using Basic Local Alignment Search Tool (BLAST) V2.2.24. The available corresponding sequences of *Grubelepis mexicana* Berkeley & Berkeley, 1939 and *Mexieulepis weberi* Horst, 1922 were downloaded from GenBank (Table 2). *Laetmonice filicornis* Kinberg, 1856 (Aphroditidae) and *Pholoe pallida* Chambers, 1985 (Pholoidae) were chosen as outgroups and their corresponding gene sequences were also downloaded from GenBank (Table 2). Sequences for each gene were aligned by Mesquite (Maddison and Maddison, 2016). Sequences from the five species were concatenated using Sequence Matrix (Vaidya et al., 2011). The most suitable molecular evolution model for each gene and the concatenated sequences were determined using jModeltest2 (Darriba et al., 2012) based on the Akaike Information Criterion (AIC), resulting in the use of GTR+I for 16S, 18S and COI, and GTR+G for 28S and the concatenated data. Maximum Likelihood (ML) analysis and Maximum Parsimony (MP) analysis were conducted with RaXML GUI1.3 and PAUP* V4.0, respectively, using settings reported by Osborn et al. (2007).

**TABLE 2.** Information about scale-worms used for phylogenetic analysis.

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Results

**FAMILY Eulepethidae Chamberlin, 1919**
GENUS Eulepethus Chamberlin, 1919

Generic diagnosis. (modified after Pettibone, 1986). Body elongate, with up to 70 segments. Elytrae numerous pairs; first 12 pairs larger, with lateral notch only or leaf-like process inside the notch, on segments 2, 4, 5, 7, alternate segments to 21, 24; smaller elytrae with entire margins present on all posterior segments beginning from segment 28. Dorsal cirri on segments 3 and 6. Branchiae 12 pairs, on segments 8, 10, alternate segments to 22, 23, 25, 26, 27.

Type species. Eulepethus hamifer (Grube, 1875)

Eulepethus nanhaiensis sp. nov.
http://zoobank.org/5AC0DD1D-35C0-430A-8C6C-FC766F7DAA3F
(Figs 1–5, Table 1)

Material examined. 12 type specimens of Eulepethus nanhaiensis sp. nov., collected from the muddy bottom of the Pearl River Estuary, Daya Bay, and Shantou along the coasts of Guangdong Province at 13 to 30 m depth (Table 1). Holotype: MBM285075. Paratypes (11 specimens): MBM285076, MBM285084, MBM285078, MBM285079, MBM285080, MBM285077 (juvenile) and AMW.49068 (juvenile) fixed in 5% formalin and later transferred to 70% ethanol; MBM285081, MBM285082, MBM285083 and AMW.49067 were fixed in 100% ethanol directly. AMW.49067, a complete specimen, was dissected to remove three middle parapodia for use in phylogenetic analysis. MBM285078 is incomplete with 20 anterior segments. Others specimens are complete. A specimen of Eulepethus hamifer (Grube) (USNM 17483) was examined for comparison.

Etymology. The specific name nanhaiensis is derived from nai hai, latinized Chinese characters for the south sea, which refers to the type locality in the northern South China Sea.


Description. Body elongate (Figs 4A & 5A). Complete specimens 10.8–56 mm long (n = 11; holotype 28.4 mm), 2.5–6.8 mm wide including chaetae (holotype 5.0 mm), 39–70 segments (holotype 48 segments) (Table 1). Elytrae numerous pairs, surface smooth (Figs 1A, C–D & 5A–B). First 12 pairs of elytrae on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 24, more elongate towards posterior end, overlapping dorsally, with lateral margins notched or having 1–2 spade-shaped processes. In holotype left elytra 2, 3, 4, 6 and right elytra 3, 4 with 1 process; left elytra 5 and right elytra 2, 12 with 2 processes; others with a notch (Fig. 1D & Table 1). Branchiae 12 pairs on segments 8, 10, 12, 14, 16, 18, 20, 22, 23, 25, 26, 27, inflated in dorsal region of notopodia, with four ciliated ridges on under side and a distally attached branchial cirrus (Fig. 2E). Segments 3 and 6 without branchiae but with subulate dorsal cirrus (Fig. 2C). Posterior segments beginning on segment 28 each with a pair of elytrae with entire margins, non-overlapping dorsally (Figs 1C; 4E & 5A, I).

Prostomium covered laterally by parapodia of first two segments, and dorsally by first pair of elytrae (Fig. 1A). Three antennae present, all short (approximately 1/3 to 1/2 of the length of prostomium) and taper to a tip (Fig. 1B). Median antenna attached mid-dorsally, lateral pair attached anteriorly. A pair of ventral palps elongate-tapered, extending slightly beyond the first parapodia (Fig. 1A). Two pairs of black eyespot groups present, anterior pair mid-laterally, posterior pair near posterior border (Fig. 1B).

Parapodia of segment 1 with subulate tentacular cirrus, ventral cirrus slightly longer than dorsal one (Fig. 2A). Dorsal cirrus present on posterior side of upper margin of parapodia 3 and 6 (Fig. 2C). Ventral cirri on segments 2–7 slender, and taper to a tip (Figs 2B–D & 4B); on segment 8 and following segments ovoid, articulated, with a minute tip (Figs 2E–F & 4B).

Parapodia biramous, each ramus supported by an amber acicula. Notoacicula of median and posterior segments with hooked tips. Except for segment 1, neuroaciculae throughout expanded distally forming a hammer-shaped distal plate (Fig. 2B–E). Parapodia with capillaries (Figs 3A, G, K, D; 4C, G–I, L & 5F). Notopodial capillaries 2 types: smooth and serrate capillaries. Neuropodial capillaries all smooth. Parapodia of segment 1 only with 2 bundles of capillaries (Fig. 2A). Notopodia of segment 2 with a bundle of capillaries on posterior.
Neuropodia of segment 2 with 3 types of chaetae: 2 pectinate chaetae in dorsal-most position, an even fan-shaped array of smooth biliminate chaetae with an abruptly tapered tip, and a few capillaries in ventral-most position (Fig. 2B). Notopodia of segment 3 with 3 types of chaetae: dorsally two amber serrate hooks with a tapered tip, an amber serrate hook with a disciform tip ventrally, and a bundle of capillaries on posterior part (Figs 2C; 3B; 4C & 5D–E). Neuropodia of segment 3 with 4 types of chaetae: 1 pectinate chaeta in dorsal-most position (Figs 2C & 3H), a fan-shaped array of smooth biliminate chaetae with an abruptly tapered tip (Figs 2C; 3C, E–F; 4C & 5H), a non-limbate acicular chaeta with a blunt tip among the smooth biliminate chaetae (Figs 2C; 3D; 4C, M & 5H), and a few capillaries in ventral-most position (Fig. 2C). Notopodia of posterior segments with 3 types of chaetae: a dorsal array of amber serrate hooks with a disciform tip (Figs 3L–M & 4I), a ventral array of amber serrate hooks with a tapered tip (Figs 3N; 4H), and a bundle of capillaries on ventral posterior part (Fig. 2D–F). Neuropodia of posterior segments similar throughout the body, with 3 types of chaetae (Figs 2D–F; 3I–K; 4K–L & 5G): 1–2 pectinate in dorsal-most position, an even fan-shaped array of smooth biliminate chaetae abruptly tapering to a fine tip with the chaetae being thinner and shorter on dorsal and ventral position than middle ones, and a few capillaries in ventral-most position. No non-limbate acicular chaetae in posterior segments (Figs 2D–F & 4C).

Pygidium with two anal cirri (Figs 1C & 4D–E). Left cirrus short, smooth and taper to a point (Fig. 1C & 4F). Right cirrus long, minutely papillated on one side for most of the length, except the basal-most part which is smooth (Fig. 1C).

Variation in morphological characters. The complete specimens vary greatly in length (10.8–56 mm), width (2.5–6.8 mm) and the number of segments (39–70) (Table 1). Juveniles are substantially smaller, with fewer segments on posterior part of the body. The body width (BW) has significant positive correlation with the body length (BL) and the number of segments (NS) (BL = 9.99 BW = 15.38, R² = 0.76, P < 0.001, n = 11; NS = 6.95 BW + 22.63, R² = 0.81, P < 0.001, n = 11). In all specimens there are elytrae with spade-shaped processes in the anterior part of the body, but the number and position of the processes are irregular. Juveniles have fewer processes than the adults, and there is no elytra bearing more than one process. Body width has significant positive correlation with the number of elytrae (NE) with 1–2 processes (NE = 2.02 BW - 1.29, R² = 0.40, P = 0.001, n = 11). In the two juveniles, lateral margin of the second and fifth elytrae has a process having a digitiform lappet with a constriction at the base, and a process in other pairs of elytrae (Fig. 5B–C).

Distribution and habitat. Currently only Daya Bay and Shantou in the northern South China Sea in depths 10–30 m. Some of the specimens identified as E. hamifer for specimens collected from locations off Vietnam and mainland China could be E. nanhaiensis sp. nov.

Molecular analysis. Partial sequences of COI (617 bp), 16S rRNA (505 bp), 18S rRNA (1895 bp) and 28S rRNA (1010 bp) and their concatenated sequences (4027 bp) were used for phylogenetic analyses using the ML and MP methods. The results from the 16S, 18S, and concatenated sequences (Fig. 6) show that the three species of Eulepethidae form a well-supported clade. Within Eulepethidae, Eulepethus nanhaiensis sp. nov. is the sister taxon of a clade including Mexievalpes weberi and Grubeulepis mexicana. For the phylogenetic trees based on COI and 28S that include only two species of Eulepethidae, Grubeulepis mexicana and Eulepethus nanhaiensis sp. nov. form a well-supported clade that is distinct from the two outgroups.

Remarks. Eulepethus nanhaiensis sp. nov. can be distinguished from Eulepethus hamifer (Grube, 1875) by several features. First, a conspicuous blunt-tipped non-limbate acicular chaeta is present in the neuropodia of segment 3 in E. nanhaiensis but absent in E. hamifer. Second, in E. nanhaiensis each posterior segment has a pair of elytrae that are widely separated and the dorum is exposed, but in E. hamifer the posterior elytrae are “fused medially” (Figure 3d, Pettibone, 1969). Third, the anterior elytrae of E. nanhaiensis can have a notch only or 1–2 spade-shaped lateral processes inside the notch, but those in E. hamifer can have a notch or only a single spade-shaped process in the notch: there is a single spade-shaped process inside the lateral notch in the third elytra. No other elytra of E. hamifer has the spade-shaped process. Nevertheless, it should be kept in mind that all elytra anterior to the third are missing on the E. hamifer specimen.

Eulepethus hamifer has been reported along the coasts of the Philippines (Grube 1878; Pettibone, 1969, 1986), Tonkin Gulf (Uschakov, 1972), Daya Bay (Jiang et al., 1990; Du et al., 2011) and Japan (Imajima, 2001; 2003). However, only in Uschakov (1972) and Imajima (2001, 2003) were the specimens described. Figure 1 of Uschakov (1972) and Figure 44 of Imajima (2003) both clearly showed that the elytrae of the posterior segments are widely separated mid-dorsally, therefore the specimens they described as E. hamifer should not be that species. However, it is not sure whether they are E. nanhaiensis sp. nov. due to the following observations. Uschakov (1972)
FIGURE 1. Eulepethus nanhaiensis sp. nov., holotype MBM285075. A, anterior region, dorsal view, first and second elytron removed to show the prostomium; B, dorsal view of prostomium; C, dorsal view of pygidium and posterior segments, left and right cirrus present; D, 1st–12th right elytron.
A NEW SPECIES OF POLYCHAETOUS SCALE WORM

FIGURE 2. Eulepethus nanhaiensis sp. nov., holotype MBM285075. A, dorsal view of right tentacular parapodium, segment 1. Note that the parapodium was rotated clockwise so that the left side faces up to show the two bundles of ventral chaetae; B, posterior view of right parapodium, segment 2; C, anterior view of right parapodium, segment 3; D, anterior view of right parapodium, segment 4; E, posterior view of right parapodium from segment 16; F, posterior view of right parapodium, segment 42.
FIGURE 3. Eulepethus nanhaiensis sp. nov., holotype MBM285075. A, capillary notochaeta from segment 1; B–H chaetae from segment 3; B, hooked notochaeta; C, dorsal neurochaeta; D, non-limbate acicular neurochaeta; E, middle bilimbate neurochaeta; F, ventral bilimbate neurochaeta; G, capillary neurochaeta; H, pectinate neurochaeta; I–O chaetae from segment 42; I, dorsal neurochaeta; J, middle neurochaeta; K, ventral neurochaeta; L, dorsal hooked notochaeta; M, middle hooked notochaeta; N, ventral hooked notochaeta; O, capillary notochaetae.
A NEW SPECIES OF POLYCHAETOUS SCALE WORM

FIGURE 4. Eulepethus nanhaiensis sp. nov., Paratype MBM285084. A, lateral view of the whole worm; B, ventral view of anterior end; C, right parapodia, segments 2–4; D E, ventral and dorsal view of pygidium and posterior segments; F, dorsal view of pygidium and Anal cirrus; G, capillary notochaetae, segment 28; H, ventral hooked notochaeta, segment 48; I, dorsal hooked notochaeta, segment 48; J, pectinate neurochaeta, segment 11; K, dorsal neurochaeta, segment 48; L, ventral capillary neurochaeta, segment 11. Holotype MBM285075. M, middle neurochaeta, segment 3.
described that the specimens he examined had two pairs of small eyes on the prostomium, while Imajima (2003) did not mention the presence of eyes on the specimen he examined. Since pigments may fade in ethanol preserved specimens, it is not sure whether the lack of eye spots was due to the loss of pigment during preservation.
Uschakov (1972) did not describe segment 3 and his specimens are not available for examination, therefore it is not known whether the neuropodia of this segment bears a non-limbate acicular chaeta, a distinct feature of *E. nanhaiensis*. Imajima (2003) provided a drawing of segment 3 (Fig. 44c) and described the chaetae of this segment, but in his drawing and description there is no non-limbate acicular chaeta in this segment. It should be noted that such stout non-limbate acicular chaetae have been reported from segment 3 of several other species of eulepethids, also among a fan-shaped array of neuropodial limbate chaetae: e.g., *Grubeulepis mexicana* (Fig. 25E, I in Pettibone, 1969), *Lamelleulepethus orensaz* (Fig. 8E, G in Pettibone, 1986), and *Proeulepethus clarki* (Fig. 16F, G in Pettobone, 1986).

The *Eulepethus* specimen described by Imajima (2003) has either a notch or process on the lateral margin of the anterior elytrae, which also indicates that it is *E. nanhaiensis*, not *E. hamifer*. Imajima (2003) noticed this difference from Pettibone's description of *E. hamifer* (Pettibone, 1969), but he thought that it might be accounted for by body size and maturity. Examination of our juvenile and adult *E. nanhaiensis* specimens collected from different seasons (Table 1) showed that the anterior elytrae have a notch with either one or two spade-shaped processes. The consistency of this feature within both juveniles and adults and throughout the seasons indicates that the presence of processes on elytrae is independent of age or season.

Despite the great similarity, except for size, between the adults and juveniles, the two juveniles have an enigmatic feature – the lateral margin of two elytral processes (i.e., segments 2 and 5) has a digitiform lappet with a constriction at the base. Since this feature is absent in the adults and present only on two pairs of elytrae, we suggest that the long lappet may detach from the constriction during the development. Observation of some eulepethids species with long fringes shows that even on the same elytra, some fringes are bilobed with a constriction between the lobes and others are unilobed (e.g., Figs 2, 6, 15 in Pettibone, 1986), indicating the terminal lobe could drop off, providing evidence to show that the detachment of some of the terminal lobes from the elytrae may be a common phenomenon in the family.

**FIGURE 6.** Phylogenetic trees of Eulepethidae generated by two methods of analysis (ML/MP). Tree A, B, C, D, E shows results based on COI and 16S, 18S, 28S rRNA, and their concatenated sequences, respectively. Numbers represent ML/MP bootstrap values based on 1,000 replicates. Asterisks stand for highest possible support 100.
Due to the limited number of gene sequences available, we were only able to conduct a preliminary phylogenetic analysis of three species of eulepethids (Mexieulepis weberi, Grubeulepis mexicana and Eulepethus nanhaiensis sp. nov.) with scale worms in two other families as outgroups. The eulepethids form a well-supported clade, which is congruent with the conclusion about the monophyly of the family by a precious study (Norlinder et al., 2013). Among the eulepethids, Mexieulepis weberi has a closer relationship to Grubeulepis mexicana than to Eulepethus nanhaiensis. Morphologically, the lateral borders of elytrae in the anterior region of both Mexieulepis and Grubeulepis are fimbriated with many flattened leaflike processes, whereas those of Eulepethus have only a notch or one or two flattened processes. Eulepethus and Mexieulepis have 12 pairs of larger anterior elytrae and numerous pairs of smaller posterior elytrae, whereas Grubeulepis has 12 pairs of anterior elytrae only. Therefore, our analysis shows that having fimbriated elytrae is a synapomorphic character for Mexieulepis and Grubeulepis, whereas the presence of numerous pairs of elytrae posteriorly is a plesiomorphic character in Eulepethus and Mexieulepis. Nevertheless, more taxon sampling at the species and genus level are required to fully resolve the phylogenetic relationship among eulepethids.

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