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Rediscovery and augmented description of the HMS 'Challenger' acorn worm (Hemichordata, Enteropneusta), *Glandiceps abyssicola*, in the equatorial Atlantic abyss

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A 2009 oceanographic expedition of the Russian Academy of Sciences collected the anterior region of a single acorn worm (phylum Hemichordata, class Enteropneusta) by trawling at a depth of 5560 m in the Romanche Trench (equatorial Atlantic). The specimen was a ripe female with numerous, relatively small oocytes in each ovary. Phylogenetic analysis of rDNA sequences robustly placed the worm in the family Spengelidae. In addition, morphological features of the proboscis, collar, and anterior trunk region indicated that the worm was Glandiceps abyssicola*, a species previously represented solely by the holotype, which had been dredged from the equatorial Atlantic in 1873 by the HMS 'Challenger' and subsequently sent to Germany for description by Spengel (1893). The holotype was presumably destroyed by World War II bombing; therefore, we here designate the Romanche Trench specimen as the neotype of* G. abyssicola *and supply an augmented species diagnosis.*

Keywords: Hemichordata, Enteropneusta, Spengelidae, *Glandiceps abyssicola*, neotype, rDNA sequence, phylogenetic analysis, histology

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INTRODUCTION

Eleven species of deep-sea enteropneusts have so far been described by Spengel (1893), Belichov (1971), Woodwick & Sensenbaugh (1985), Holland *et al.* (2005, 2009, 2012a, b), Osborn *et al.* (2012), and Priede *et al.* (2012). The present paper concerns the first of these, *Glandiceps abyssicola* (Spengel, 1893), originally represented by one incomplete animal dredged by the HMS 'Challenger' in 1873 in the Sierra Leone–Liberia Basin (Figure 1A, triangle). The specimen was sent to Germany, where it was described by Spengel and later, presumably, destroyed during World War II. Now, 140 years after the collection of the holotype, a second individual of what we determined to be *G. abyssicola* has been captured by a Russian oceanographic expedition dredging in the Romanche Trench in the equatorial Atlantic. Like the holotype, the recently-collected worm was incomplete, comprising only the proboscis, collar and most anterior portion of the trunk. Nevertheless, the material served for both rDNA-based phylogenetic analysis and histological description, sufficing to identify the specimen to genus and species. In the light of the new information, we here designate the Romanche Trench specimen as the neotype of *G. abyssicola*.

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MATERIALS AND METHODS

On 5 December 2009, RV 'Akademik Ioffe' of the Russian Academy of Sciences deployed a trawl at 5560 m in the equatorial Atlantic (cruise 29, Station 2169; latitude +0.3853, longitude −16.3928). The trawling site (Figure 1A, filled circle) was near the eastern end of the Romanche Trench, named for 'La Romanche', the French ship that discovered that topographic feature. The specimens brought up in the trawl included the anterior end of an acorn worm (phylum Hemichordata, class Enteropneusta). The freshly collected worm was photographed in colour under xenon strobe illumination and then divided into two portions: the first was fixed in 90% ethanol for molecular sequence analysis, and the second was fixed in 10% formalin–seawater for histological investigation.

Genomic DNA was extracted using the standard AutoGenprep965 proteinase K/phenol extractions method (AutoGen, Inc., Holliston, MA). Amplification and sequencing of partial 16S and complete 18 rDNA were as described in Osborn *et al.* (2012). The phylogenetic analysis was based on approximately 500 bp of 16S rDNA and 1700 bp of 18S rDNA (the GenBank Accession numbers are, respectively, KC776732 and KC776731). These new sequences were added to the hemichordate sequences in Osborn *et al.* (2012), Priede *et al.* (2012), and Worsaae *et al.* (2012). The echinoderm outgroup comprised *Parastichopus californicus*, *Aspidodiadema jacobi*, *Asterias forbesi*, *Ophioderma brevispinum*,

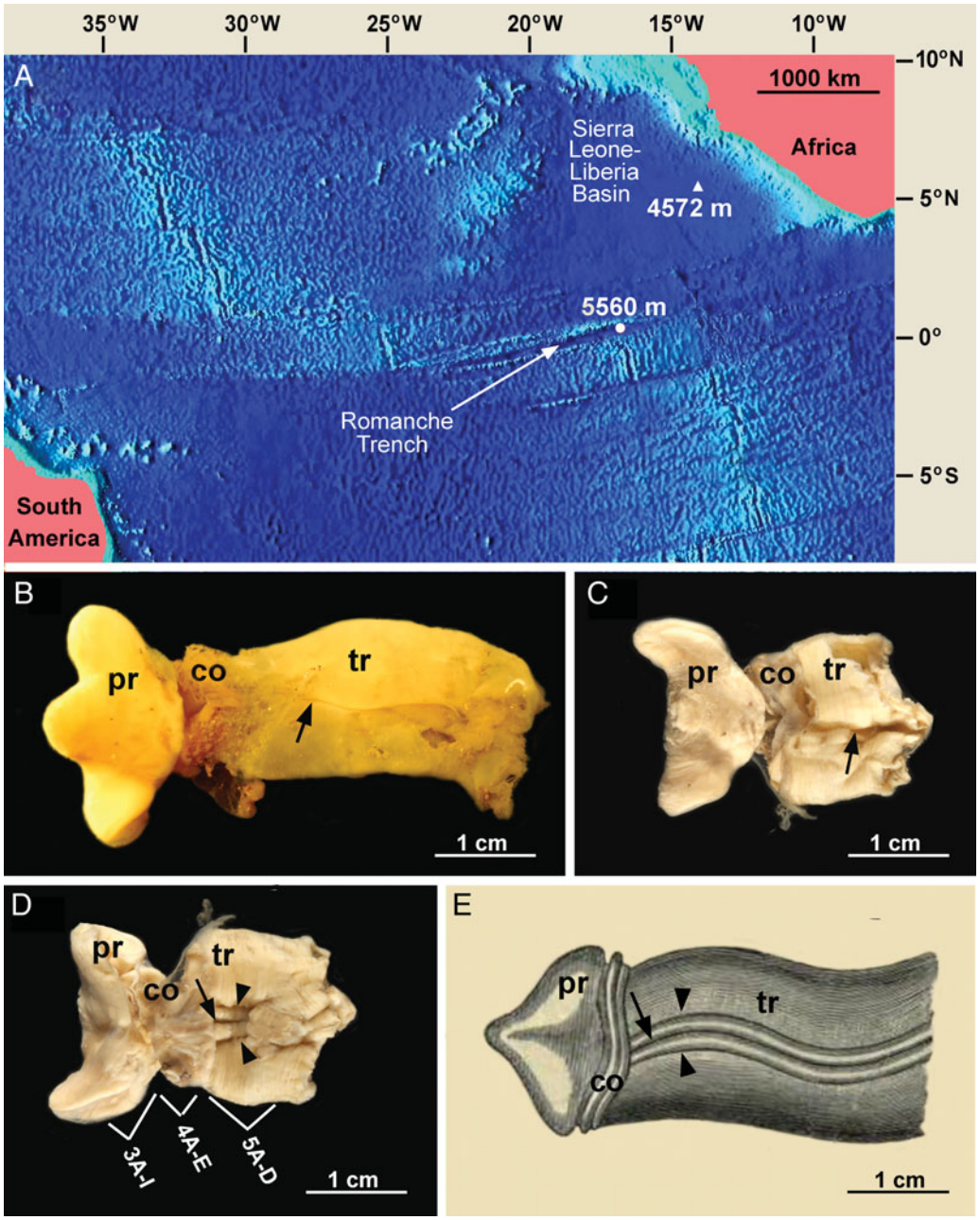

Fig. 1. *Glandiceps abyssicola*. (A) Map of the equatorial Atlantic Ocean with the triangle indicating where the holotype was dredged by the HMS 'Challenger' on 19 August 1873 and the filled circle indicating where the neotype was trawled by the RV 'Akademik Ioffe' on 5 December 2009; (B–E) anterior fragments of neotype and holotype (anterior toward left) showing proboscis (pr), collar (co) and portion of trunk (tr); (B) ventral view of freshly-collected neotype with the ventral furrow indicated by the arrow; (C) ventral view of formalin-fixed neotype with the ventral furrow indicated by the arrow; (D) dorsal view of formalin-fixed neotype with median sagittal furrow and flanking parasagittal grooves indicated by the arrow and arrowheads, respectively. At the bottom of (D) the approximate levels of the histological cross-sections in Figures 3–5 are indicated; (E) drawing of freshly collected holotype in dorsal view reproduced from p. 195 of Thomson & Murray (1885) after removal of original labels; arrow and arrowheads indicate, respectively, the median sagittal furrow and parasagittal grooves running along the anterior region of the trunk.

Neogymnocrinus richeri and *Bathycrinus* sp. To select a best-fit model of nucleotide substitution, we used jModelTest (Posada, 2008). A 95% majority rule consensus tree for hemichordates (40 million generations) was constructed from concatenated but unlinked nucleotide sequences in MrBayes 3.2.1 run on the Smithsonian Institution's Hydra cluster in replicate. Node support was expressed as posterior probabilities; poorly supported nodes (<0.95 posterior probability) were collapsed.

For morphological study, the formalin-fixed portion of the worm was photographed in dorsal view and in ventral view. The specimen was then embedded in paraplast wax and prepared as 12 μm serial cross-sections that were stained in 0.1% aqueous azure-A. Because the wax-embedded gonads tended to shatter during sectioning, several gonads were dissected free from most of the surrounding trunk tissues, embedded in Spurr's resin, sectioned at 4 μm with a glass knife, and stained in aqueous 0.1% azure-A with 0.1% sodium borate.

RESULTS

Neotype designation and augmented diagnosis

On basis of molecular phylogenetic analysis and morphological data (described later in the present paper), we here designate the Romanche Trench enteropneust as the neotype of *Glandiceps abyssicola*.

SYSTEMATICS
Phylum HEMICHORDATA Bateson, 1885
Class ENTEROPNEUSTA Gegenbaur, 1870
Family SPENGELIDAE Willey, 1899
Genus *Glandiceps* Spengel, 1891
Glandiceps abyssicola Spengel, 1893%
(Figures 1; 3–5 in present paper)

TYPE MATERIAL

Holotype: adult female (missing most of posterior region of trunk); HMS 'Challenger' Station 101. Latitude +5.80, longitude −14.33; depth: 4572 m; coll. R. von Willemoes-Suhm, 19 August 1873. Fresh colour: proboscis yellow, collar red, anterior trunk region reddish yellow. Prepared as histological sections on microscope slides at the University of Giessen, Germany; evidently destroyed by bombs in 1944.

Neotype (hereby designated): adult female (missing most of posterior region of trunk) RV 'Akademik Ioffe' Cruise 29, station 2169. Latitude +0.3853, longitude −16.3928; depth 5560 m; coll. A. Rogacheva, 5 December 2009. Fresh colour: proboscis yellow, collar red-tinged yellow, anterior trunk region yellow. Serial histological sections of the neotype are deposited in the Scripps Institution of Oceanography Benthic Invertebrate Collection as SIO-BIC-H28.

Augmented diagnosis: gill bars limited to dorsal third of pharyngeal circumferance in holotype and neotype of *Glandiceps abyssicola*. By contrast, in all other known species in the genus *Glandiceps*, the gill bar region occupies the dorsal half or more of the pharyngeal circumference.

PHYLOGENETIC ANALYSIS

Figure 2 shows the results of the phylogenetic analysis based on the 16S and 18S rDNA from hemichordate sequences available to date plus echinoderm sequences serving as the outgroup. The Romanche Trench enteropneust proved to be in the family Spengelidae, most closely related to the East Asian *Glandiceps hacksi*, which had been included in the earlier phylogenetic analyses of Osborn *et al.* (2012), Priede *et al.* (2012), and Worsaae *et al.* (2012).

GROSS ANATOMY

The freshly collected specimen (Figure 1B) consisted of a proboscis (yellow), collar (yellow with a reddish tinge), and anterior extremity of the trunk (yellow). The proboscis, which measured 1.4 cm long, was dome-shaped anteriorly, but abruptly broadened out on either side posteriorly, reaching a maximum width of 2.4 cm. The collar, which had been conspicuously abraded during capture, was about 0.5 cm long by 1.2 cm wide, and the anterior trunk fragment was about 3 cm long by 1.2 cm wide. Judging from the dimensions of the recovered portion of the freshly collected specimen, one can estimate that the living worm would have had an intact length on the order of 10 cm.

During fixation, the anterior part of the proboscis had been artifactually bent back dorsally (Figure 1C, D). As seen in the fixed specimen, all three body regions were somewhat flattened; the ratio of dorso-ventral dimensions to side-to-side dimensions was about 40% for the proboscis and 60% for the collar and anterior trunk. The ventral side of the anterior end of the trunk was characterized by a median furrow (Figure 1B, C, arrow), and the dorsal side was grooved by a median sagittal furrow accompanied on either side by parasagittal grooves (Figure 1D, E, arrow and arrowheads, respectively). These anatomical features are shared by other species in the family Spengelidae (Spengel, 1893, 1907; Willey, 1898; Horst, 1940).

HISTOLOGICAL DESCRIPTION

Like the overall morphology, several structures at the histological level of organization were also damaged during collection. The epidermis was missing from about 95% of the proboscis, collar, and anterior part of the trunk (examples of undamaged and damaged epidermis are illustrated in Figure 3A, B, respectively). Moreover, the collar had been even more deeply abraded, especially on the ventral side. Thus, our histological description of the recovered part of the body will be incomplete (for example, because of loss of most of the epidermis, nothing definite can be said about possible coelomopores). The description, which will mainly cover relatively durable features like the proboscis complex and the collar nerve cord, will proceed generally from anterior to posterior.

The proboscis, from outside to inside, comprises an epidermis including mucous cells, a neurite layer of the intra-epidermal nervous system, and a well-developed basal lamina (Figure 3A). Immediately beneath the basal lamina, there is often a thin layer of muscle cells running parallel to one another in a generally circular direction (Figure 3B), but the deeper tissues of the proboscis consist of scattered muscle cells not obviously oriented in any particular direction and associated with a meshwork of fibrous connective tissue (Figure 3A–C).

More posteriorly in the proboscis, a proboscis coelom opens up (Figure 3D, asterisk). The space appears single, although it is likely that mid-sagittal membranes dividing it into right and left portions were destroyed by the trauma of collection. The most anterior tissues encountered within the coelom are projections of a median and two lateral lobes of the glomerulus (Figure 3D). Slightly more posteriorly (Figure 3E), one encounters the anterior extremities of the pericardial vesicle dorsally and the stomochord ventrally. In this region, a lateral group of glomeruli on either side is associated with the wall of the pericardial vesicle. In contrast to the situation in some other spengelids, no vermiform process (a strand-like anterior extension of the stomochord) is detectable, although such a delicate feature could well have been lost along with the mid-sagittal membranes of the proboscis. Further posteriorly in the proboscis, the pericardial vesicle and stomochord become closely apposed to each other, with glomeruli running along either side of the zone of apposition (Figure 3F, G). The heart, which is sandwiched between the pericardial vesicle and the stomochord, is not conspicuous due to the paucity of haemal fluid in the specimen.

As one proceeds posterior-ward, the pericardial vesicle and glomeruli terminate, whilst the stomochord continues posteriorly just dorsal to the proboscis skeleton (Figure 3H). Near its

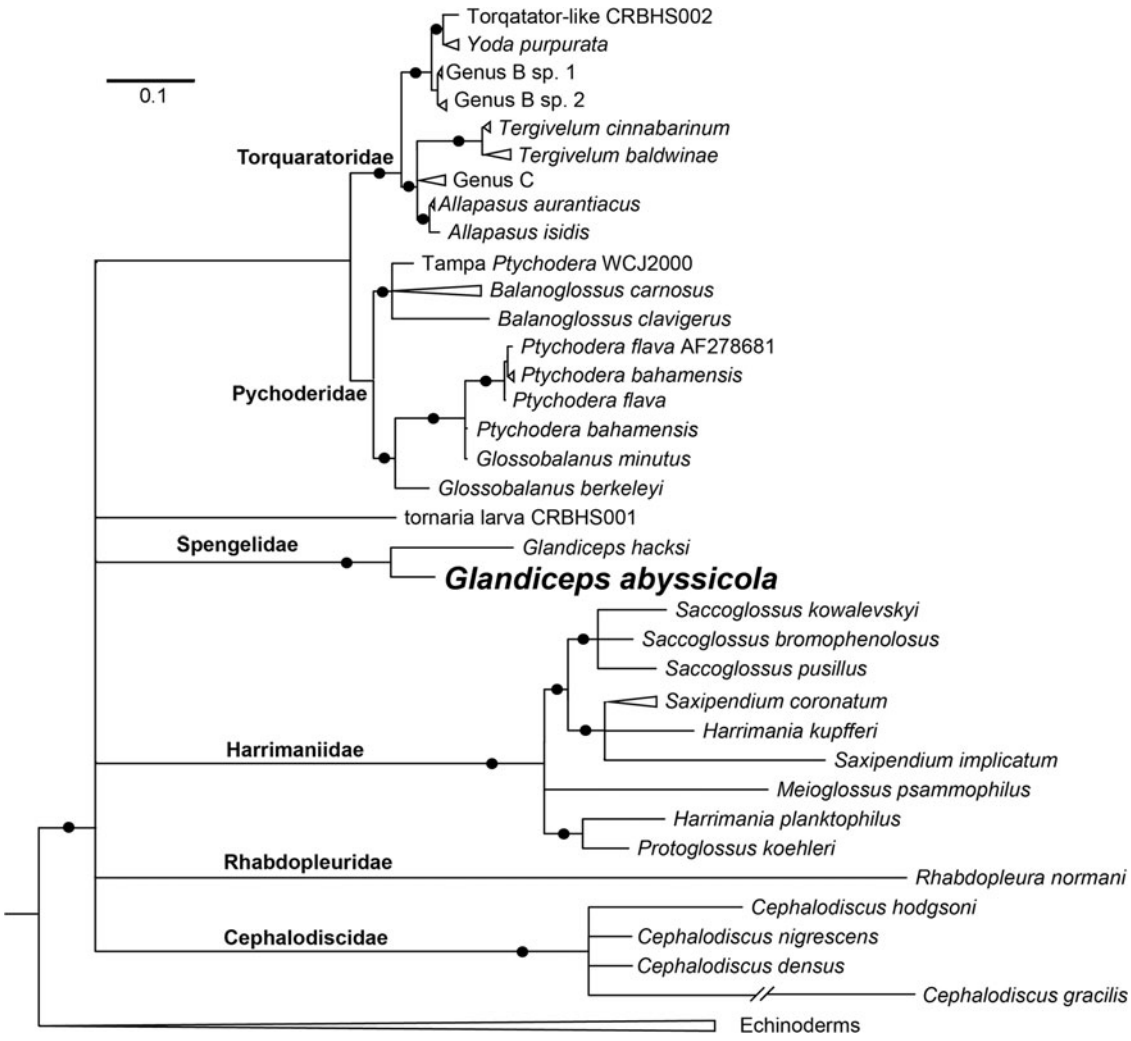

Fig. 2. Phylogenetic analysis of Hemichordata based on concatenated 18S and 16S rDNA sequences. Taxa used are those of Osborn *et al.* (2012) and additional sequences provided by Worsaae *et al.* (2012). This is a 95% majority rule consensus tree from final 40 million generation Bayesian analysis. The scale bar at the top left indicates the number of substitutions per nucleotide site. Branches with less than 0.95 posterior probability were collapsed. The filled circles at nodes indicate at least 0.99 posterior probability support.

blunt anterior end, the proboscis skeleton comprises a dense medial region ('primary skeleton' in the terminology of Harmer, 1904) intimately flanked by a mass of chondroid tissue on either side. This striking chondroid tissue comprises a relatively homogeneous extracellular matrix in which run anastomosing channels lined by numerous small cells (Figure 3I). The spaces within the channels are continuous with the coelomic space in the proboscis, and the cells within the channels are evidently continuous with the peritoneum lining of the proboscis coelom. By the criteria of Cole & Hall (2004), such chondroid tissue is probably not homologous to vertebrate cartilage.

Both the chondroid tissue and the primary skeleton are indented by two pairs of relatively voluminous caeca (Figures 3 H, 4A, B, arrowheads and arrows, respectively) continuous with the surrounding coelomic space. Further posteriorly, at about the level where the proboscis skeleton gives off its posterior horns (Figure 4C), the anterior end of the collar nerve cord appears. The roof and sides of the cord are thin, contrasting markedly with its thick floor. The cord, which has a continuous lumen, is not united with the overlying epidermis via dorsal nerve roots, but is underlain by paired perihaemal coeloms crowded with longitudinal muscles (Figure 4C–E). The horns of the proboscis skeleton continue posteriorly throughout the collar (Figure 4D, E) and do not terminate until they reach the anterior extremity of the pharynx (Figure 5A). Due to extensive tissue damage in the collar region, it was not possible to judge whether peribuccal spaces were present.

The dorsal side of the pharyngeal region of the trunk is characterized by primary (septal) and secondary (tongue) gill bars not connected by synapticles (Figure 5A). In histological cross-sections (Figure 5B), the gill bars resemble those of enteropneusts generally, as described by Benito & Prados (1997). Therefore, we do not agree with Spengel (1893, p. 267) that the gill bars of *G. abyssicola* are structurally deviant (*abweichend*). The gill bars are associated with gill pores that debouche into the parasagittal grooves on either side of the dorsal midline. The total number of such pores could not be determined, because the posterior ones in the series were unavailable for study. As seen in cross-sections of the anterior pharyngeal region, the gill bars occupy only

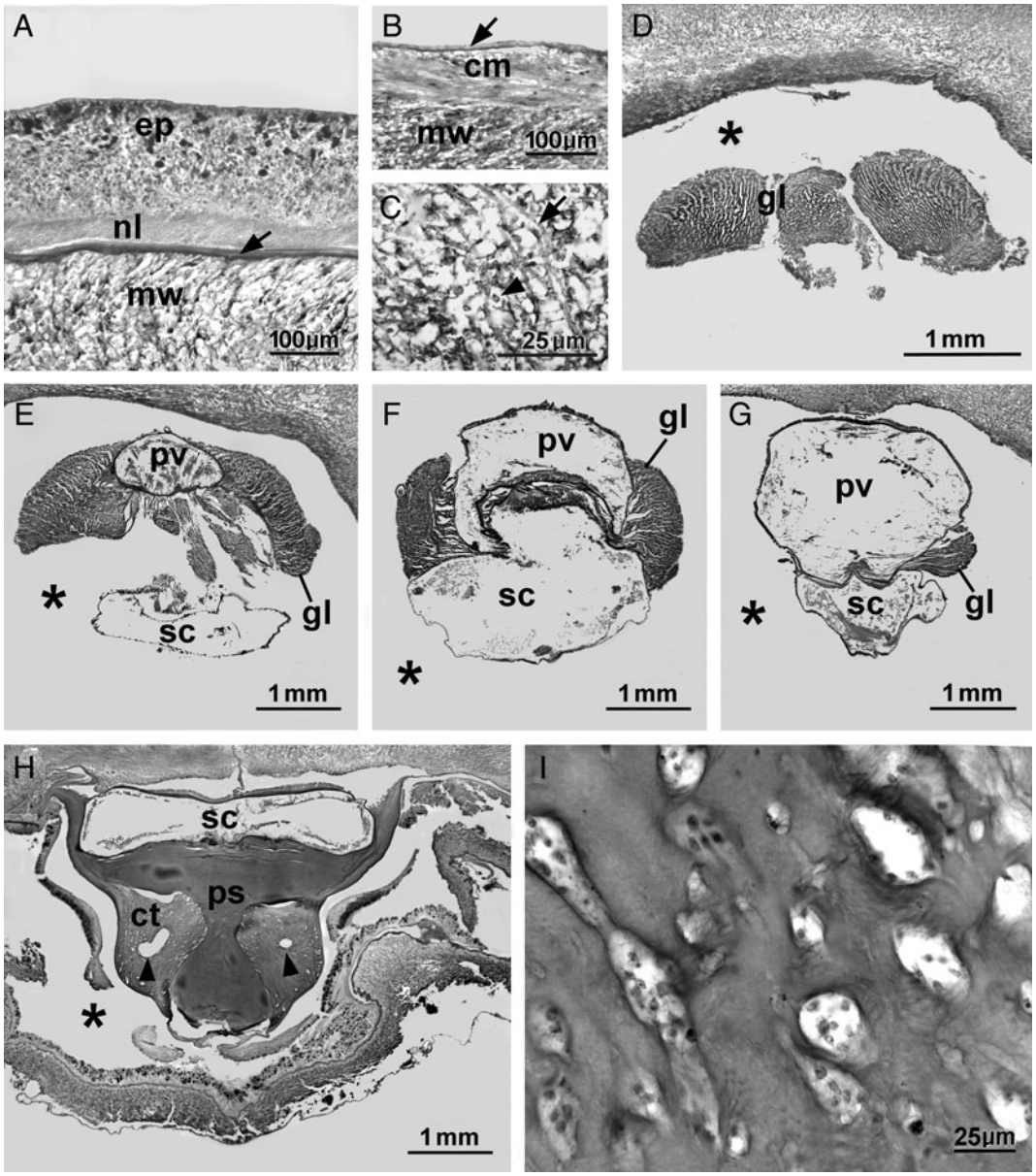

Fig. 3. *Glandiceps abyssicola* neotype; histological cross-sections. (A) Periphery of proboscis in a region showing epidermis (ep) underlain by neurite layer (nl) of the intraepidermal nervous system; the arrow indicates the basal lamina overlying a meshwork (mw) of muscle and connective tissue fibres; (B) periphery of proboscis where the epidermis and neurite layer have been artifactually torn away from the underlying basal lamina (arrow); a layer of circular muscles (cm) overlies a meshwork (mw) of fibrous connective tissue mixed with scattered muscle fibres; (C) enlargement of the meshwork in A and B, showing muscle cells cut across and longitudinally (arrowhead and arrow, respectively); (D) proboscis coelom (asterisk) near the anterior extremity of the proboscis complex, where only the glomerulus (gl) is visible; (E) short distance posterior to (D), showing proboscis coelom (asterisk), pericardial vesicle (pv), glomerulus (gl), and stomochord (sc); (F) short distance posterior to (E), with same structures labelled; (G) short distance posterior to (F), with same structures labelled; (H) section posterior to (G), showing stomochord (sc), and proboscis skeleton (ps); the latter is flanked by chondroid tissue (ct) and is penetrated by caeca (arrowheads) opening anteriorly into the proboscis coelom (asterisk); (I) enlargement of the chondroid tissue in (H).

about the dorsal third of the circumference of the gut. The more ventral tissues of the available part of the trunk are too badly disrupted to determine whether the pharynx is partially divided into a dorsal, respiratory half and a ventral, digestive half.

In the pharyngeal region, numerous spherical ovaries, each up to about 1 mm in diameter, are embedded in the body wall near the parasagittal grooves. Each ovary (Figure 5C) comprises a thin germinal epithelium enclosing hundreds of primary oocytes of various sizes, each containing a large germinal vesicle (nucleus) with a single, dark staining nucleolus. Most of the oocytes in the ovaries of the neotype are about 95 µm in diameter and have presumeably attained their maximum size. Each large oocyte is surrounded by a jelly later about 10–20 µm thick (Figure 5D) that stains metachromatically with azure A, indicating an abundance of sulphated acid mucopolysaccharides (Spicer, 1963). Although nutrient-rich accessory cells are not conspicuous in the ovary, they might have been abundant at earlier stages of ovarian maturation—as described by Hadfield (1975) for a shallow-water enteropneust species known to spawn numerous, relatively small eggs.

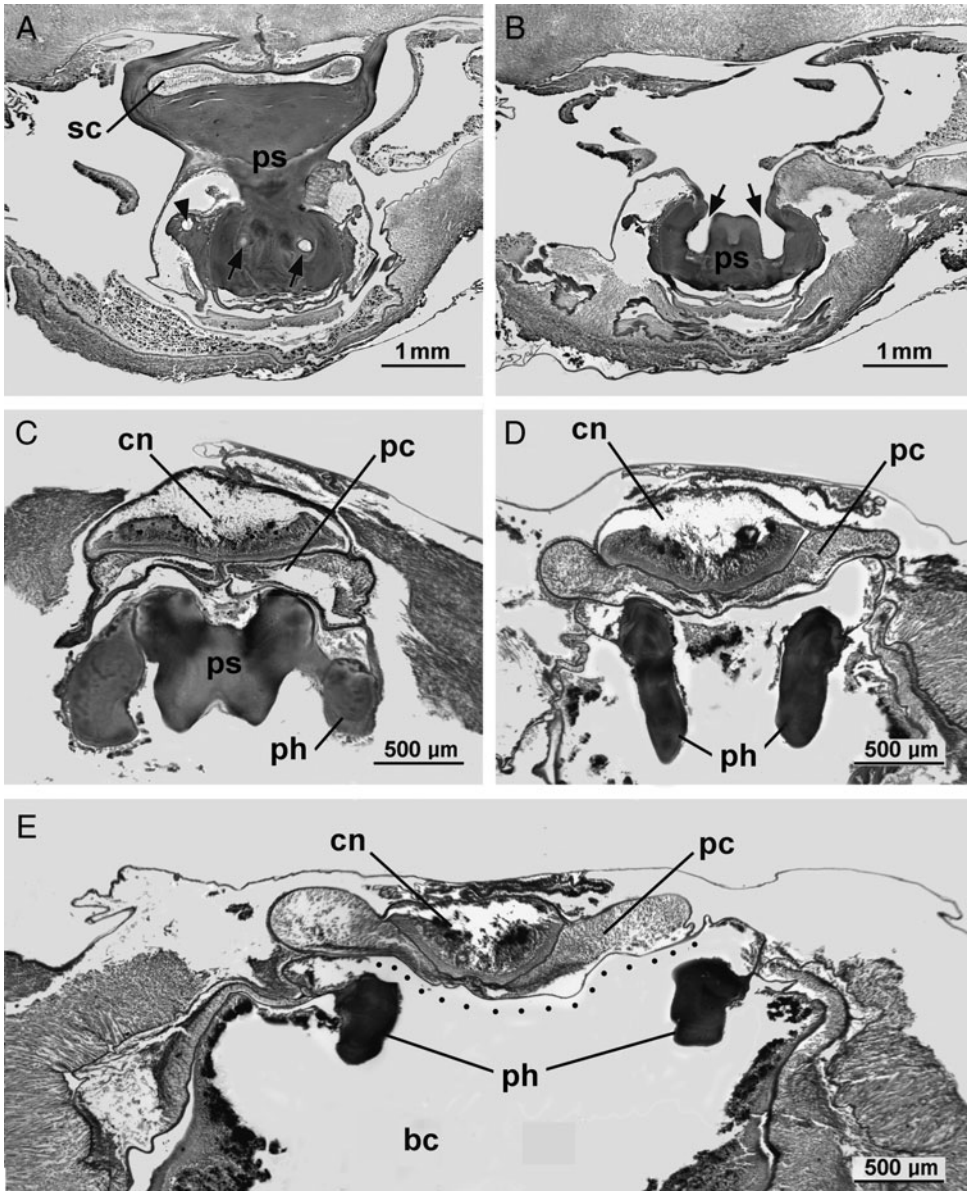

Fig. 4. *Glandiceps abyssicola* neotype; histological cross-sections. (A) Caeca (arrows) in the proboscis coelom (ps) are just beginning to appear; conversely, the caeca in the chondroid tissue (arrowhead) are just disappearing; (B) proboscis skeleton (ps) posterior to A; the connections of the caeca with the proboscis coelom are indicated by arrows; due to disruption of several tissues, it is not clear if the stomochord is present at this level; (C) the anterior end of the collar showing the collar nerve cord (cn) underlain by a pair of perihaemal coeloms (pc); the proboscis skeleton (ps) gives rise to its posterior horns (ph); (D) short distance posterior to (C), showing collar nerve cord (cn) underlain by perihaemal coeloms (pc); the posterior horns (ph) are present, but the main body of the proboscis skeleton is no longer visible; (E) posterior end of the collar showing the buccal cavity (bc), posterior horns (ph), collar nerve cord (cn), and perihaemal coeloms (pc); the row of dots indicates where the dorsal epithelium of the buccal cavity is artifactually missing.

DISCUSSION

History of the holotype of *Glandiceps abyssicola*

Rudolf von Willemoes-Suhm was a naturalist on the HMS 'Challenger'. Even before embarking, he was familiar with enteropneusts, since he had already written a short description of what is now recognized as *Harrimania kupfferi* (von Willemoes-Suhm, 1871). Relatively early during the voyage, he took the following notes (reproduced on pp. 195–196 of Thomson & Murray (1885)): 'Station 101, 19th August 1873, 2500 fathoms. Among the worms there is a fragment of *Balanoglossus* [sic] . . . . The one we got today was probably of considerable length, but owing to the extreme softness of the tissues, only the anterior part remained in the dredge when hauled on board; it was distinguished by very lively colours [yellow proboscis, red collar, reddish-yellow trunk] . . . . From this fragment it would hardly be permissible to establish a new species.' The same naturalist decorated his notes with a sketch of the freshly collected specimen (reproduced here without its original labelling as our Figure 1E). He later amended his notes to mention the subsequent capture of even smaller fragments of deep-sea acorn worms at Atlantic Station 106 and at Pacific Station 147 (there is no further record of these fragments, presumably because they were

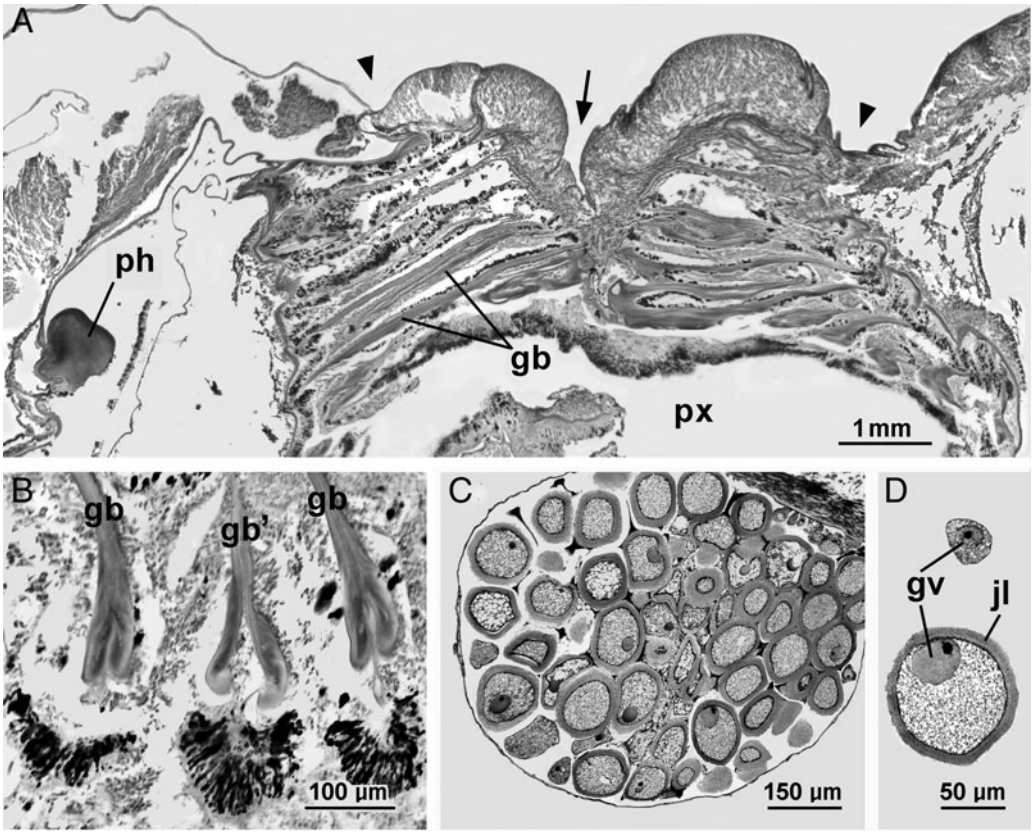

Fig. 5. *Glandiceps abyssicola* neotype; histological cross-sections. (A) The anterior extremity of the trunk, showing the pharynx (px), longitudinally-cut gill bars (gb), dorsal furrow (arrow), and parasagittal grooves (arrowheads); posterior horns (ph) are still present, one of which is visible. Note that the dorsal epidermis (including the dorsal nerve cord of the trunk) is completely missing; (B) cross-sections of primary and secondary gill bars (gb and gb', respectively; (C) ovary filled with primary oocytes; part of body wall is at top right; (D) details of a small primary oocyte (top) and a primary oocyte of maximum size (bottom) showing the germinal vesicle (gv) and jelly layer (jl).

discarded). Tragically, von Willemoes-Suhm did not survive the voyage. He died suddenly from erysipelas at age 28 when the 'Challenger' was in the South Pacific and was buried at sea the following day (Thomson, 1875).

At some point before Thomson himself died in 1882, he sent the 'Challenger' acorn worm to Johann Wilhelm Spengel at the University of Giessen, Germany. Spengel had accepted a professorship at Giessen in 1887 and remained there for the rest of his career. By the time the worm reached Germany, most of the proboscis was missing, the trunk was bent, and the ventral side was ripped open. Spengel (1893), who prepared histological sections of the worm, gave a brief written account of some features of the proboscis stalk and collar, but limited his illustrations to tissues in the pharyngeal region of the trunk (his plate 21, figures 54–57).

In spite of the opinion of von Willemoes-Suhm that the specimen was inadequate for the naming of a new species, Spengel (1893) proceeded to do exactly that. First he argued, reasonably, that the worm belonged in the genus *Glandiceps* on the basis of the relative shortness of the collar region, the detailed distribution of muscle bundles in its body wall and the exceptional prominence of chondroid tissue flanking the (primary) proboscis skeleton. Next, and somewhat sketchily, he differentiated his specimen from its known congeners and named it *G. abyssicola* on the basis of the shortness of the gill-slit region of the pharynx, the position of the gonads relative to the gill slits, and the deviant form of the gill bar skeleton (as mentioned above, we think he was mistaken about this last character). Spengel never explicitly mentioned a repository for the histological sections constituting the holotype, but he presumably retained them in the Zoological Institute of the University of Giessen. The Zoological Institute and its collections, which were directly across the street from the railroad station in Giessen, were completely demolished by bombs on 6 December 1944 (Ankel, 1957).

The Romanche Trench specimen as neotype of *Glandiceps abyssicola*

As discussed above, we have designated the Romanche Trench enteropneust as the neotype for *G. abyssicola*. Cameron & Perez (2012) reviewed morphological characters important for placing an acorn worm in the spengelid genus *Glandiceps*; unfortunately several of these are missing or damaged in our specimen. Even so, this difficulty was overcome, because rDNA-based phylogenetic analysis robustly identified the Romanche Trench specimen as a spengelid (Figure 2).

An & Li (2005) published a dichotomous morphological key for distinguishing among the *Glandiceps* species that had been described by Marion (1886), Spengel (1903, 1907), and An & Li (2005). Unfortunately, navigating the key requires information on some features in the posterior body regions often lost during collection. Therefore, we sought a

distinguishing morphological feature that would remain after the loss of most of the posterior part of the worm. We found *G. abyssicola* could be distinguished from its congeners by the limitation of its gill bars to the dorsal third of the pharyngeal circumferance. Spengel (1893), who had the entire gill bar region of the holotype available for study, illustrates this feature in figure 56 on his plate 21, and we illustrate it here for the neotype in our Figure 5A. In all other known *Glandiceps* species, the gill bars occupy between one half and two-thirds of the dorsal circumference of the pharynx. By this criterion, the 'Challenger' and Romanche Trench specimens match, and we consider both to represent *G. abyssicola.*

It is only fair to mention here two features that might argue against considering the 'Challenger' and Romanche Trench enteropneusts to be conspecific. First, the anterior limit of the gonads in the pharyngeal region extends more anteriorly in the neotype than in the holotype; however, the extent of gonadal tissue in the body could be sensitive to the degree of sexual maturity. Second, the 'Challenger' worm has a bright red collar and a yellowish-red trunk, whereas the Romanche Trench worm has a yellowish-red collar and a purely yellow trunk; even so, part of the colour difference may be due to the stripping away of almost all the epidermis and some of the deeper tissues during the collection of the neotype. In sum, we think that important aspects of the morphology outweigh these discrepancies. In addition, conspecificity of the two specimens is consistent with their bathymetric and geographical distribution: the holotype and neotype were collected at depths of 4572 m and 5560 m, respectively, at equatorial Atlantic stations without any intervening shallow water barriers.

Aspects of the natural history of *Glandiceps abyssicola*

At 'Challenger' collecting Station 101, where the holotype of *G. abyssicola* was collected, the substratum was predominantly mud, and the bottom temperature was 1.7°C (Théel, 1882). In the Romanche Trench, where the neotype was collected, the substratum has variously been described as erosional sands (Heezen & Laughton, 1963) or ooze (Cifelli, 1967), and the minimum bottom temperature was about 0.7°C (Morozov *et al.,* 2010). Because both the holotype and neotype were broken open during collection, all the gut contents were lost. However, from what is known about other deep-sea enteropneusts (Smith *et al.,* 2005; Holland *et al.,* 2009, 2012a, b; Anderson *et al.,* 2011; Priede *et al.*, 2012), *G. abyssicola* is very likely a deposit feeder, although nothing is yet known about how selective it might be.

Because the holotype and neotype of *G. abyssicola* were collected by dredging or trawling, it is not possible to know whether these worms typically inhabit shallow burrows or lie entirely exposed on the abyssal sea-floor. Alternatively, like another relatively muscular deep-sea enteropneust, *Allapasus aurantiacus* (Holland *et al.*, 2012a), *G. abyssicola* might alternate between a burrowing and an epifaunal existence. Additionally, it is possible that *G. abyssicola*, with its relatively muscular trunk, might occasionally swim by undulating the body, as described by Urata *et al.* (2012) for its relatively shallow-living congener, *G. hacksi.*

Both the holotype and neotype of *G. abyssicola* are females, but presumably the species has separate sexes, even though no male has yet been collected. Each ovary of the neotype contains numerous oocytes, most of which had a uniform diameter about 95 μm and were enveloped with a jelly layer. This strongly suggests that the female gametes were ready for spawning. Moreover, the small size of the oocytes of *G. abyssicola* indicates that development is indirect, with a swimming (and possibly feeding) larval stage. In contrast, the relatively large (300–1500 μm diameter) oocytes of other deep-sea enteropneusts studied to date (Holland *et al.*, 2005, 2009, 2012a, b; Priede *et al.*, 2012) indicate that these species develop directly without passing through a larval stage—although Young (1994) has pointed out that predictions about life history strategy based solely on female gamete size can sometimes be unreliable.

Shallow-living marine invertebrates that produce numerous female gametes of small size are often broadcast spawners. This reproductive strategy is most effective when numerous conspecific males and females live close together. Unless individuals of *G. abyssicola* are similarly gregarious (which seems unlikely), males and females probably require special behaviour to bring them into close proximity at spawning time. It seems likely that a spawning episode would involve either the temporary retention of shed male and female gametes in a common mucous cocoon or an even more elaborate copulatory behaviour to increase the likelihood of fertilization success.

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