First record of the family Spengeliidae (Hemichordata: Enteropneusta) from Chinese waters, with description of a new species

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Abstract
Six species belonging to two families of Hemichordata have previously been recorded in Chinese waters. This paper records the discovery and description of a new species of the genus Glandiceps found in Jiaozhou Bay, Qingdao, Shandong Province, named Glandiceps qingdaoensis. The new species has a long proboscis with dorsal and ventral grooves, a stomochord with a long vermiform process, a proboscis cavity with a dorsal median, right and left glomeruli, right and left glomeruli very large and encircling the stomochord, a proboscis skeleton in the cavity extends into the median posterior of the collar, a well-developed dorsal ventral muscular septum in the proboscis cavity dividing the cavity completely into two separate parts. The collar cord is without giant nerve roots. The trunk with four distinct regions that can be recognized externally: branchial-genital region, genital region, hepatic region, and intestinal region. The dorsal pharynx is large and the gill pores are small. The tongue bars are encircled by vesicles, and the first gonad commences at the level of the second or third gill slit.

Keywords: Hemichordata, Enteropneusta, new species, Glandiceps qingdaoensis, stomochord.

Introduction
Since Eschscholtz (1825) described the first enteropneust species, Ptychodera flava, about 76 species belonging to 13 genera of this group have been reported (Cameron nd). The Enteropneusta is divided into four families, i.e. Ptychoderaidae, Saxipendiidae, Harrimaniidae, and Spengeliidae. Spengel (1891) reported that the Spengeliidae is distinguished from other families by the stomochord with a vermiform process. The Spengeliidae consists of four genera, Spengelia Willey, 1897, Schizocardium Spengel, 1891, Willeya Punnett, 1903, and Glandiceps Spengel, 1891. Sixteen species of the family have been described. According to Cameron’s (2002) report, Glandiceps is distinguished from...
Willeyia by lacking peribuccal coelom, from Spengelia by lacking synapticles and nerve roots, and from Schizocardium by lacking liver sacs.

In a review of Enteropneusta species in Chinese waters, Liang (1984) recorded six species, one belonging to the Harrimaniidae, i.e. Saccoglossus hwangtauensis (Tchang and Koo, 1935), and the remaining five belonging to Ptychoderidae, including Glossobalanus mortenseni Horst, 1932, Glossobalanus polybranchioporus Tchang and Liang, 1965, Balanoglossus carnosus (Willey, 1899), B. misakiensis Kuwano, 1902, and Pthchodera flava Eschscholtz, 1825. Saccoglossus hwangtauensis was found from Jiaozhou Bay, Qingdao, Shandong Peninsula; G. mortenseni was collected from Sanya, Hainan Island; G. polybranchioporus was found in the southern Yellow Sea and northern Bohai Bay besides its type locality Jiaozhou Bay; B. carnosus was found in Xinying and Xincun, Hainan Island; B. misakiensis was found in Jiaozhou Bay, and also Hepu, Guangxi, and Beibu Bay, and P. flava was found in southern Hainan Island and the Xisha Islands.

When the hemichordate material from Jiaozhou Bay, deposited in the Marine Biological Museum of the Institute of Oceanology Chinese Academy of Science (MBMCAS) was checked, four specimens belonging to the genus Glandiceps, family Spengeliidae, were separated. It is therefore believed that a new species has been discovered and it is the first time this genus and family have been described from Chinese waters.

Material and methods

Four specimens were collected from Jiaozhou Bay, Qingdao, Shandong Peninsula, Yellow Sea, between November 1999 and February 2004 and were preserved in 70% ethanol. Two of the four specimens for histology investigation were fixed in Bouin’s solution and dehydrated in a graded series of ethanol; after being dehydrated in 100% ethanol, specimens were dissected into small pieces, transferred to xylene, and infiltrated with paraffin wax. Sections (8 μm thickness) were cut from specimens and stained with Ehrlich’s haematoxylin and eosin, then viewed and photographed with a Zeiss Photomicroscope II compound microscope. Some larger sections were photographed with a dissection microscope (Zeiss Stemi SVIIApo). Figures 4–19 were photographed with the software Axivision 4.1. All materials examined are deposited in MBMCAS.

The following abbreviations are used in the figures: br, branchial genital region; bv, blood vessel; bph, branchial pharynx; cc, collar coelom; cco, collar cord; cm, circular muscles; cn, collar nerve cord; co, collar; cpk, cornua of proboscis skeleton; ct, chondroid tissue; dic, digestive canal; dlm; dense longitudinal muscles; dm, dorsal mesentery; dph, digestive pharynx; dv, dorsal vessel; dvs, dorsal ventral septum; ep, epidermis; ev, efferent vessel of proboscis; gb, gill bar; gc, central gland cells; gl, glomerulus; go, gonad; gp, Gill pore; gr, genital region; gs, gill slit; gu, gut lumen; hr, hepatic region; hs, heart sinus; ir, intestine region; lm, longitudinal muscles; lps, lateral pouch of stomochord; m, mouth; mc, medullary cavity; mgl, medial glomerulus; nl, nerve layer; pc, proboscis coelom; pe, pericardium; peh, perihaemal coelom; ph, pharynx; pk, proboscis skeleton; po, proboscis; reb, respiratory portion of branchial genital region; s, stomochord; slm, sparse longitudinal muscles; spn, splanchnic nerve-fibres layer; tb, tongue bar; tc, trunk coelom; tn, trunk nerve-fibres layer; tr, trunk; v, vesicle; vf, vermiform process of stomochord; vm, ventral mesentery; vs, ventral septum; vv, ventral vessel; wdc, wall of digestive canal.
Systematic account

**Phylum HEMICHORDATA**
**Class ENTEROPNEUSTA**
**Family SPENGELIIDAE** Willey, 1899
**Genus Glandiceps** Spengel, 1893

*Glandiceps qingdaoensis*, new species
(Figures 1–19)

**Material examined**

Holotype: adult female, sectioned, Jiaozhou Bay, 36°05′30″N, 120°10′48″E, 15 m depth, 29 May 2001, collected by Mr Baolin Zhang (MBM HE 0001). Paratypes: (1) male, sectioned, Jiaozhou Bay, 36°06′00″N, 120°15′00″E, 14 m depth, 18 February 2004, collected by Jianmei An (MBM HE 0002); (2) male, fresh specimen, Jiaozhou Bay, 36°09′30″N, 120°15′00″E, 16 m depth, 17 January 2004, collected by Shaoqing Wang (MBM HE 0003); (3) male, preserved specimen, Jiaozhou Bay, 36°02′12″N, 120°14′00″E, 18 m depth, 29 May 2000, collected by Baolin Zhang (MBM HE 0004).

**Diagnosis**

Adult animals are distinguished by vesicles encircling the tongue bar; the stomochord with a long vermiform process, median glomerulus; a proboscis skeleton which extends into the median posterior of the collar, and a proboscis with a well-developed dorsal-ventral muscular septum that completely divides the proboscis into two parts. The first gonad commences at the level of the second or third gill slit.

**Description**

*External form, measurement and colour.* Body size large, body fragile and easily broken. Proboscis subconical, 19.1 mm in one of the paratypes, about three times as long as the collar, basal width 7.1 mm in vertical direction, 10.1 mm in transverse direction; with one middorsal and one midventral groove, which extend three-quarters of the length of the proboscis from the posterior margin; one proboscis pore on the left dorsal lateral proboscis. The collar is 6.1 mm in length and 10.5 mm wide, with a distinctive groove in the median posteriorly; posterior part behind the groove covered with creases; anterior lip broader than posterior lip. Trunk subcylindrical and divided into four regions: anterior branch genital region, flat genital region, very short hepatic region and slender intestinal region. The branchial genital region is sub-cylindrical and faintly annulated with grooves, 42.45 mm long, the vertical diameter is 7.05 mm, with transverse diameter of 11.15 mm. This region has a less deep ventral and a deep dorsal groove, and paired dorsal ridges parallel to the dorsal groove. The gill pores (mean number: 56 pairs) are small and invisible without magnification. The genital region of the paratype not dissected is broken into five fragments, with total length about 222.3 mm, vertical diameter is 3.8 mm, transverse diameter is 13.8 mm and the epidermis is almost transparent with the inner gonads visible; the dorsal ridges become more slender and the ventral ridges prominent. The hepatic region is 39 mm in length. The intestinal region is incomplete in the preserved paratype,
without external liver sacs, dorsal ridges turgid. The intestinal region is missing in the holotype and three paratypes.

In fresh specimens the body colour is yellow, with irregular brown patches on the surface (Figure 1). The preserved specimens faded to a pale yellow (Figures 2, 3). The genital region of the holotype faded to a dull yellowish white (Figure 2) and the paratypes are yellowish white (Figure 3). The hepatic region is dark green (Figure 3).
Anatomical and histological characters

Proboscis. The circular musculature of the proboscis is thickest anteriorly (0.68 mm) (Figure 4). The epidermis of the proboscis is 0.53 mm, and the nerve plexus is between 0.06 and 0.08 mm thick. The longitudinal muscles occur in dense and sparse layers (Figure 5). Anterior to the heart–kidney complex the proboscis coelom is separated completely into right and left cavities by a median dorsal-ventral muscular plate that extends to the tip of the proboscis (Figure 5). Many irregular stained gland cells occur in the walls of the proboscis cavities (Figure 5).

The stomochord is comprised of a central pouch and a pair of lateral pouches (Figure 7). There is a long vermiform process similar to that described in other species by Spengel (1907). The ventral septum of the proboscis commences at the position below the junction
Figures 7–12. *Glandiceps qingdaoensis*, new species, histological characteristics. (7) Transverse section through base of proboscis passing through the pouched region of the stomochord, behind the glomerulus, showing the lateral pouch and median pouch of stomochord. The pericardium is above the median pouch. (8) Longitudinal section through median septum of the proboscis to show the vermiform process of the stomochord and its varied calibre (diameter of vermiform process tube). (9) Transverse section through anterior end of the stomochord showing small pericardial auricles and pericardium wedged in the longitudinal muscles. (10) Transverse section through commencement of the heart–kidney complex (include stomochord, glomerulus, and pericardium). The section passes immediately in front of the anterior end of the pericardium, and shows the right and left halves of the glomerulus on either side of the stomochord and distinct median glomerulus. (11) Longitudinal section through the heart–kidney complex showing large left half part of glomerulus, which envelops the anterior stomochord. (12) Transverse section through the junction between proboscis and collar showing chondroid tissue and nuchal skeleton which is strongly developed and occupying a large portion of the section.
Figures 13–19. *Glandiceps qingdaoensis*, new species, anatomical and histological characteristics. (13) Transverse section through the median region of collar showing perihaemal coelom and mesentery. (14) Longitudinal section through median-posterior region of collar showing small disconnected medullary cavities arranged in a line and collar dorsal nerve strand. (15) Transverse section through the branchial region showing well-developed digestive portion and branchial portion. (16) Magnified upper part of Figure 15, showing dorsal septum, dorsal vessel, and splanchnic nerve-fibre layer. (17) Magnified lower part of Figure 15, showing ventral septum, ventral vessel, and nerve-fibre layer. (18) Longitudinal section through the branchial genital region showing gill bar, tongue bar, and distinct vesicle encircling every tongue bar. (19) Transverse section through intestine, showing the thin wall of digestive canal.
of the vermiform process and the body of the stomochord. The vermiform process, lying in the median septum of the proboscis, consists of a solid cord composed of undifferentiated cells. The process extends distally and is coextensive with the median septum to about one-third of the length of the proboscis. Its anterior portion is vacuolar, with one or two actual discontinuities (Figure 8); calibre (diameter of tube) of the vermiform process is varied.

The pericardium is wedged in the longitudinal muscles and the cavity of the pericardium contains a mass of loose cellular tissue; pericardial auricles are very small (Figure 9). A median, right and left glomeruli occur in the proboscis (Figure 10). The right and left are large and extend beyond the end, enveloping the tip of the stomochord (Figure 11). The cornua of the nuchal skeleton are large and extend posteriorly to the median posterior region of the collar (Figure 4). Relations between the substance of the nuchal skeleton and the chondroid tissue are intimate (Figure 12).

Collar. The collar cord is without giant nerve roots. The peribuccal coelom is absent. The perihæmal coelom is divided into two parts by dorsal mesentery (Figure 13). The collar cord contains some small disconnected medullary cavities arranged in a line (Figure 14), and the nerve root is absent.

Trunk. The branch genital region has a well-developed lower digestive pharynx, sub-equal in size with the dorsal branchial pharynx portion (Figure 15). The splanchnic nerve-fibre layer is distinct, and at the base of the pharynx epithelium, the dorsal septum and dorsal vessel are distinct (Figure 16), and the ventral pharyngeal epithelium (Figure 17) is thickest anteriorly. Peribuccal coelom and synapticula absent in the region. Every tongue-bar encircled by a distinct vesicle (Figure 18). The gill bars are larger than the tongue bars. The first gonad containing many ripe ova commences at the level of the second or third gill slit in the branchial-genital region (Figure 4). In the genital region, dermal pits and medial gonads absent, the wall of digestive canal becomes thin (Figure 19).

Etymology

The new species is named after its type locality Qingdao, Shandong Peninsula.

Remarks

Including the new species, there are seven species in the genus *Glandiceps*. The new species, *Glandiceps qingdaoensis*, is distinguished from the other six species by having vesicles encircling every tongue bar, a proboscis cavity with a dorsal median, and large right and left glomeruli enveloping the stomochord; the proboscis skeleton in the cavity extending into the median posterior of the collar; a well-developed dorsal-ventral muscular septum is present in the proboscis cavity dividing the cavity completely into two parts; the collar cord has no nerve roots; trunk with four distinct regions that can be recognized externally, i.e. branchial genital region, genital region, hepatic region, and intestinal region; the dorsal pharynx is large although gill pores are small; the first gonad commences at the level of the second or third gill slit; branchial genital region with a well-developed lower sub-rectangular digestive portion, which is smaller than the branchial portion; the gill skeleton lacks synapticles. The new species has a strong odour resembling that of iodoform and the smell remains overpowering and persistent after years in ethanol.
Furthermore, this new species is distinguished from *G. abyssicola* Spengel, 1893 which was dredged during the Challenger expedition in the Atlantic Ocean off the coast of Africa at a depth of 2500 fathoms, the type species of the genus, by the large proboscis, the absence of median gonads, and the gonads commencing from the pharyngeal region. In *G. abyssicola*, the proboscis is shorter than its collar, the genital region has median gonads and they commence from behind the pharyngeal region. It also differs from *G. eximius* Spengel, 1907 which was reported from Japanese waters by a proboscis cavity with a median dorsal-ventral septum which divides the cavity into two separate parts. *G. eximius*, however, has the dorsal-ventral septum in the proboscis cavity which does not reach the anterior proboscis coelom. As the median gonad is absent and the tongue bar is smaller than the gill bar, it can also be distinguished from *G. talaboti* (Marion, 1876) which is found from Madras and the Mediterranean, as the latter has median gonads, and the tongue bar is larger than the gill bar. Liver sacs are absent in both the new species and *G. talaboti*. In addition, the new species can be distinguished from *G. hacksi* (Marion, 1885) which are found from Yokohama, Japan as the gonads of *G. qingdaoensis* commence from about the second or third gill slit and the proboscis cavity has a median and a pair of large left and right glomeruli. *G. qingdaoensis* also lacks liver sacs in the hepatic region. *G. hacksi*, however, has coextensive gonads and gill slits, no median glomerulus or median gonads, glomeruli paired and small liver sacs in the hepatic region. Differentiated also from *G. malayanus* Spengel, 1907 which are reported from Java and Indonesia, the new species has a distinct median glomerulus in the proboscis cavity, but *G. malayanus* lacks this median glomerulus. The body colour of *G. malayanus* is usually white, without easily discernible pigments, the proboscis measures $5 \times 3.5 \times 3$ mm, the pharyngal region is very narrow, the groove of the collar is situated at the posterior end of the collar, and the dorsal groove of the proboscis extends one-quarter of the proboscis length; all features which differ from *G. qingdaoensis*. Finally, the new species can be also distinguished from *G. coromandelicus* Spengel, 1907 which was found from Madras (India) by the existence of nerve pores on the collar cord and a vermiform process and collar longer than the latter. *G. coromandelicus* has only a short vermiform process of about 0.1 mm, and a proboscis of $6.5 \times 4$ mm, collar $2.5 \times 4.5$ mm and no nerve pores on the posterior of the collar cord.

Key to the seven species of the genus *Glandiceps*

1. Median gonad present .................................................. 2
   – Median gonad absent .................................................. 3

2. Tongue bar longer than its gill bar .................................. *G. talaboti* (Marion, 1876)
   – Tongue bar shorter than its gill bar .............................. *G. malayanus* Spengel, 1907

3. Proboscis cavity with median glomerulus ............................ 4
   – Proboscis cavity without median glomerulus ..................... 5

4. Proboscis cavity separated incompletely by the median dorsal-ventral septum .................................................. *G. eximius* Spengel, 1907
   – Proboscis cavity separated completely by the median dorsal-ventral septum .................................................... *G. qingdaoensis*, n. sp.

5. Hepatic region with small liver sacs .................................. *G. hacksi* (Marion, 1885)
   – Hepatic region without liver sacs .................................. 6
6. Gonads commence behind the branchial region. . .  G. abyssicola Spengel, 1893
– Gonads commence with the branchial region. . .  G. coromandelicus Spengel, 1907

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