Comparative morphology of the hammer-shaped penises of two species of opisthobranch molluscs of the genus *Philine* Ascanius, 1772 (Opisthobranchia: Cephalaspidea: Philinidae)

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Penises of two species of opisthobranch mollusks of *Philine* were first examined with use of a scanning electron microscope: *Ph. aperta* (Linnaeus, 1767) and *P. finmarchica* M. Sars, 1858. This study revealed significant morphological differences. The morphology of male copulatory apparatus of these species is discussed.

Key words: Opistobranchia, Philine, copulatory system, penial morphology

INTRODUCTION

Structure of the copulatory apparatus of Philinidae Gray, 1850 is the most morphologically diverse among the Cephalaspidea. It is described for many species of the family (Marcus & Marcus, 1969; Rudman, 1972; Bouchet, 1975; Gosliner, 1988). The copulatory apparatus of the type species of the genus Philine, Ph. aperta (Linnaeus, 1767), one of model species of Cephalaspidea, is well studied (Vayssière, 1880; Guiart, 1901; Bergh, 1908; Pruvot-Fol, 1930; Brown, 1934). Nevertheless, its descriptions differ in some publications (Guiart, 1901; Thompson, 1976; Gosliner, 1994). Philine aperta is an Atlantic boreal species absent from the fauna of Russia. Philine finmarchica M. Sars, 1858 is a common species of Russian Arctic, from the Barents Sea to the Laptev Sea. Both species have significant morphological differences but the descriptions of their penises were rather similar. The penis of *Ph*. aperta is described as being hammer-shaped with an orifice on the posterior tip, smooth with pointed tips (Vayssière, 1880; Guiart, 1901; Bergh, 1908; Pruvot-Fol, 1930; Brown, 1934). The penis of Ph. finmarchica

was firstly noted by Marcus and Marcus (1969) as being verrucose hammer-shaped and then as the smooth one (Marcus, 1974). We studied the penis and copulatory apparatus of *Ph. finmarchica* using a light microscope (Chaban, 1999) and now a scanning electron microscope. We compare the structure of the penis in both species and define their copulatory apparatus more accurately.

MATERIAL AND METHODS

Specimens of Philine finmarchica were collected by some Russian expeditions in the Barents Sea in 1901-1929. Philine aperta was collected in the Mediterranean Sea by Milashevitch in the Sea of Marmara and by Bogdanov near Naples. All specimens are kept in the Zoological Institute, Russian Academy of Sciences, St. Petersburg (ZISP). A scanning electron microscope HITACHI S-570 and a light microscope Leica DMLS-2 were used. Whole penises were mounted in balsam without staining using a standard procedure for examining with a light microscope or they were dehydrated in alcohol and then dried with hexamethyldisilazane (HMDS) during 20 minutes.

RESULTS

Philine aperta (Linnaeus, 1767)

(Figs 1a, b, 2a-f, 4a, b, 5a-e)

Material. Three specimens in alcohol; Sea of Marmara; coll. & det. K.O. Milashevitch; 3 specs in alcohol; Mediterranean Sea near Naples; coll. & det. Bogdanov; 8 specs in alcohol; Mediterranean Sea, Vigo-Bay, 2.7-9.1m, stone, mud, sand; 23 Oct. 1908; "Krasniy Bogatyr", coll. B. Bachinsky, det. E. Chaban.

Description of male copulatory apparatus (Figs 1a, b). The penis pocket is with thick muscular walls. It narrows anteriorly and has a small appendix (penis coecum) distally. The penis is hammer-shaped (Figs 2b, c). The anterior arm lies nearer the mouth of the pocket. The end of the posterior arm lies in the appendix of the penis pocket. Two ducts begin from the opposite side of the penis pocket. Duct 1 is short, slightly S-shaped. It ends in a small seminal vesicle (seminal sac). Duct 2 is doubled, much more with shining dense walls. One end of it connects with penis pocket, another with seminal vesicle. The thick long convoluted prostate runs from seminal vesicle. Its tail connects with the appendix of the penis pocket by a ligamentous strand. The duct 1 is the continuation of the internal seminal groove of the penis pocket, it is the incurrent sperm groove. The duct 2 is a ejaculatory duct, it leads to the base of the penis.

The penis is covered with cuticle. Its anterior arm is short and triangular with a fold at the base (Figs 2c, d). Its upper side is slightly wavy (Fig. 2e). The other arm is longer, slender with an orifice at its point (Figs 4b, f). The orifice is closed with muscular folds. This arm appears smooth in an light microscope, but in the reality it is covered with small warts and short longitudinal folds (Fig. 2f). The appendix of the penis pocket is a sheath for this tip of the penis.

The SEM photography was made of a specimen 29 mm long, the shell was 14.5 mm high and 11.0 mm wide. Light photography was made of a specimen 11.0 mm long, the shell was 8.5 mm high and 7.0 mm wide.

Philine finmarchica M. Sars, 1858 (Figs 1c-g, 3a-f, 4c, d)

Material. Six specs (from 25 in the sample); Barents Sea, 78°21'N, 38°16'E, R/V "Persey", stat 1244, 182 m depth, mud; 29 Aug. 1929; 1 spec (from 6 in the sample); Barents Sea, R/V "Andrey Pervozvannij", stat 480.

The animals are 10 to 15 mm long, its shell were 6 to 9 mm height.

Description of male copulatory apparatus (Figs 1c-g). The penis pocket with thick muscular wall has almost rectangular form. An appendix is on the lower angular of the penis pocket. The penis is hammer-shaped. The anterior arm lies nearer the mouth of the pocket. The end of the posterior arm lies in the appendix of the penis pocket. Two ducts begin from the opposite to the appendix side of the penis pocket. The duct 1 is short, slightly S-shaped, it ended by seminal vesicle. It is clearly seen on ventral side of penis pocket (Fig. 1e). The duct 1 is the continuation of the internal seminal groove of the penis sheath; it is incurrent sperm groove (Fig. 1g). The duct 2 is doubled. strongly convoluted, not much more than duct 1, with shining dense walls. It is clearly seen on dorsal side of penis pocket (Fig. 1d). One end of it connects with penis pocket and the second with the seminal vesicle. It consists of two tubes (Fig. 1f). The inner tube has small circle in cross section with thick muscular walls. It doesn't lie free but its one side is united with outer wall. Another tube is moon shaped. The cells lining both tubes bear large cilia. Thick long convoluted prostate runs from seminal vesicle. Its tail connects with the appendix of penis pocket by strand of conjunctive tissue. The middle section of prostate is knobby. The anterior part of copulatory system has strongly winding of conjunctive strands (Figs 1d, e).

The anterior (upper) arm of the penis is long, smooth with tapering end (Fig. 3e). The posterior arm is smooth on dorsal view but on ventral side has wing like extension (Fig. 3c). This extension bears knobs and slanting crests from the tip of the arm on



Fig. 1. Penial morphology. *Philine aperta*: a – dorsal view, b – ventral view, the part, enlarged. *Philine finmarchica*: c – penis with sac opened, dorsal view; d – enlarged, dorsal view, e – enlarged, ventral view, f – cross section of ejaculatory duct, g – penis sac opened, enlarged.

Abbreviations: ej.d – ejaculatory duct, in – incurrent sperm groove, m.m – muscle mass around penis, p.c – coecum of penis sheath, p.p – penial papilla, pr – prostate, p.s – penis sac (sheath), s.s – seminal vesicle ("reservoir spermatique" or seminal sac), t.c – tissue conjunctive strand. Scale bar: 1.0mm (a-e, g), 0.1mm (f).



Fig. 2. *Philine aperta*: a - shell, ventral view; b-f - penial papilla morphology on SEM: <math>b - penial papilla, dorsal view; <math>c - penial papilla, ventral view; d - upper arm; e - upper arm enlarged; <math>f - lower arm. Scale bar: 1.0 mm (a), 400 µm (b, c, d), 120 µm (f), 75 µm (e).



Fig. 4. Penial papilla morphology in light microscope. *Philine aperta*: a - total; b - lower arm enlarged.*Philine finmarchica*: <math>c - total; d - lower arm enlarged. Orifices of seminal duct noted with pointers. Scale bar: 100 µm (a-d).



Fig. 3. *Philine finmarchica*: a – shell, ventral view; b-f – penial papilla morphology (SEM): b – penial papilla, dorsal view; c – penial papilla, ventral view; d – lower arm; e – upper arm; f – lower arm enlarged. Scale bar: same data as for Fig. 2.

spiral to the dorsal side of the penis where is the orifice of seminal duct (a continuation of the duct 2) (Fig. 3f). This orifice is et the base of posterior arm, not et its tip. The orifice is open, it gapes. The arms of the penis are covered with cuticle which continues inside of the orifice of the penis (Fig. 4b). The base of the penis is short and has a large spoon-shaped muscular flap (muscular muss) (Figs 1g, 3c, 4c). The border of the flap bears small knobs (Fig. 3c). Both arms are of almost equal length.

DISCUSSION

According to Thompson (1976: Fig. 69e) a penis pocket of *Philine aperta* has three components: a prostate, a short duct ending with a seminal vesicle and an ap-



Fig. 5. Schematized structure of male copulatory system: a - from Vayssière, 1880; b - from Guiart, 1901; c - from Pruvot-Fol, 1930 and our data; d - Thompson, 1976; e - Gosliner, 1994. *Abbreviations: c.pr, c.pr'* – "canaux prostatiques" on Guiart, *ej.d* – ejaculatory duct, *in* – incurrent sperm groove, *pr* – prostate, *ps* – penis sac (sheath), r.s = s.s – seminal vesicle ("reservoir spermatique" or seminal sac), t.c – tissue conjunctive strand. Gray is a part of the copulatory system that corresponds to the ejaculatory duct in modern sense as it follows from original descriptions.

pendix. All three parts do not communicate with one another (Fig. 5d). According to Gosliner (1994: Fig. 50d) all three parts connect with one another by ducts (Fig. 5e). According to Pruvot-Fol (1930) (Fig. 5c), Brown (1934), Bergh (1908) and Guiart (1901) (Fig. 5b) duct 1 and duct 2 are connected with each other at the point where the prostate begins. Pruvot-Fol noted that: 1) the point of connection of duct 1 and duct 2 is a seminal vesicle from where prostate begins; 2) the end of the prostate is connected to the appendix of penis pocket with strands of connective tissue ("une bride conjonctive"); 3) duct 2 is doubled, it divided lengthways into two equal parts by a septum; the septum persists nearly to the orifice on the penis.

We agree with Pruvot-Fol (1930) and Brown (1934). Thompson (1976) reproduced drawing from Vayssière (1880: tab.8, Fig. 71) but he made a mistake separating the ejaculatory duct and the prostate. Gosliner's (1994) drawing is not correct in connecting the appendix sac of the penis with a prostate channel rather than a connective ligament. This structure of the copulatory apparatus is common for some species of *Philine: Ph. approximans* Dautzenberg & Fischer, 1896, *Ph. azorica* Bouchet, 1975, *Ph. monilifera* Bouchet, 1975, *Ph. monterosati* Vayssière, 1885 (Bouchet, 1975); *Ph. argentata* Gould, 1859, *Ph. finmarchica*, *Yokoyamaia ornatissima* (Yokoyama, 1927) (Chaban, 1999).

Because of having the crests, the penis of Ph. finmarchica under light microscope looks knobby that was noticed by Marcus & Marcus (1969) for this species. But in their description the muscular mass was not noticed. We explain it by the differences of size of ours and Marcus & Marcus' specimens: ours were 10-15 mm long but Marcus & Marcus' - 1.3-10.0 mm. Latter Marcus (1974) identified 2 specimens collected on 07°37'N, 55°22'W, 1220-1335 m depth as Ph. finmarchica. The specimens have the same morphology of shell, radula and gizzard plate but the penis had no muscular muss and its penial papilla was smooth. These specimens were 18 mm long and its differences can not be explained by differences of its sizes. We decide that these 2 specimens belong to another species close to Ph. finmarchica.

Hammer-shaped penises of *Philine aperta* and *Ph. finmarchica* also look similar under light microscope. But SEM photography represents their differences very clear. The penis of *Ph. aperta* has smooth, tapering end, while the penis of *Ph. finmarchica* has end bearing crests; the orifice of seminal duct of *Ph. aperta* is on the tip of the arm but of *Ph. finmarchica* is on the base of the arm on the dorsal side.

Currently, systematics of the Philinidae at the generic level has not been settled. But the two species have significant morphological differences in structure of the digestive system, and their penises are not similar. Therefore, we believe that *Philine finmarchica* belongs to another, perhaps a new genus.

ACKNOWLEDGEMENTS

We are thankful to T.K. Tzogoyev for his help during our work on the SEM, to A.B. Shatrov for possibility of our work with light microscope Leica DMLS-2, and to P.V. Kijashko for his help with photography of the shells.

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Received 10 February 2009 / Revised 12 May 2009 / Accepted 20 May 2009