

Mating and morphology of the copulatory apparatus in *Planorbarius corneus* (Linnaeus, 1758) (Gastropoda: Pulmonata)

Копуляция и морфология копулятивного аппарата у *Planorbarius corneus* (Linnaeus, 1758) (Gastropoda: Pulmonata)

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This paper examines mating behaviour and copulatory mechanics (including insemination) in *Planorbarius corneus* (Linnaeus, 1758) and presents a revised description of its copulatory apparatus. The taxonomic position of the genus *Planorbarius* within the Basommatophora is discussed. The study shows that although *P. corneus* is similar to *Planorbella* (*Seminolina*) *duryi* (Wetherby, 1879) in the structure of the shell, copulatory apparatus, mating behaviour and even mating positions, they have different mechanisms of intromission. The analysis of morphology and mating behaviour does not support a close phylogenetic affinity of *Planorbarius* to the family Bulinidae P. Fischer et Crosse, 1880 and Planorbidae Rafinesque, 1815.

В статье приведено описание репродуктивного поведения и механизма копуляции, включая механизм передачи спермы, и уточнено строение копулятивного аппарата у *Planorbarius corneus* (Linnaeus, 1758). Обсуждается таксономическое положение рода *Planorbarius* в пределах Basommatophora. Показано, что, несмотря на сходство этого вида с *Planorbella* (*Seminolina*) *duryi* (Wetherby, 1879) по строению раковины, копулятивного аппарата, репродуктивному поведению и даже позе спаривания, механизмы передачи спермы у них несколько различны. Анализ морфологии и репродуктивного поведения не подтверждает близкое филогенетическое родство *Planorbarius* с представителями семейств Bulinidae P. Fischer et Crosse, 1880 и Planorbidae Rafinesque, 1815.

Key words: molluscs, morphology, insemination, mating behaviour, *Planorbarius corneus*

Ключевые слова: моллюски, морфология, механизм передачи спермы, процесс копуляции, *Planorbarius corneus*

INTRODUCTION

This study continues a series of publications on mating behaviour and morphology of copulatory organs in freshwater molluscs (Soldatenko & Petrov, 2009b, 2012) and examines *Planorbarius corneus* (Linnaeus, 1758), one of the most common and largest members of Palearctic freshwater snails. The taxonomy of the basommatophoran pulmonates is to a large extent based on

the characters of reproductive organs (e.g.: Baker, 1956; Hudec, 1967; Starobogatov 1967; Hubendick, 1948, 1978; Burch, 1989; Nordsieck, 1990) and therefore the study of reproduction in *Planorbarius* Dumeril, 1806 may help resolve the existing disagreement about the taxonomic position of this genus. The mating process of *Planorbarius* is of special interest in this regard, because the identification of homologies between the copulatory structures depends heav-

ily on correct interpretation of their functional role during copulation. Surprisingly enough, despite the large body size and successful attempts to rear *P. corneus* in the laboratory (Nekrassow, 1928; Precht, 1936; Bondesen, 1950; Frömring, 1956; Kruglov & Frolenkova, 1980; Berezkina & Starobogatov, 1988; Maksimova, 1995), no one has yet been able to observe the whole complex of mating behaviour, and a few authors who studied the copulation (Hazay, 1881; Maksimova & Yakovleva, 1991) did not describe the intromission or observe the copulatory structures during insemination.

The morphology of copulatory structures in *Planorbarius* has been studied by several authors (Moquin-Tandon, 1855; Baudelot, 1863; Taylor, 1900; Germain, 1931; Baker, 1945; Hubendick, 1955; Starobogatov, 1958; Maksimova and Yakovleva, 1991). The phylogenetic interpretation of this information eventually led to three different views on the taxonomic position of this genus. Some authors have placed *Planorbarius* in the family Planorbidae Rafinesque, 1815, either within the subfamily Helisomatinae F.C. Baker, 1928 (Baker, 1928, 1945) or within the subfamily Planorbinae Rafinesque, 1815 (Hubendick, 1978; Glöer, 2002), while others have included it in the family Bulinidae Fischer & Crosse, 1880 (Starobogatov 1967; Maksimova, 1995; Starobogatov et al., 2004). Recent molecular phylogenetic analyses (Morgan et al., 2002; Jørgensen et al., 2004; Walther et al., 2006; Albrecht et al., 2007) confirmed the separation of *Planorbarius* from the other genera of Planorboidea, but could not resolve its phylogenetic position unambiguously.

The three possible candidates for being close phylogenetic relatives of *Planorbarius* differ from one another both in the structure of copulatory organs and in mating behaviour, providing enough distinction for comparison with the same characters in *Planorbarius*. The copulatory apparatus in the first candidate group, the Bulinidae, has a unique morphology, due to the presence

of the ultrapenis. The copulation has been studied in several species of *Bulinus* (Larabergue, 1939; Wu, 1972; Kuma; 1975; Rudolph, 1979). The snails mate only unilaterally with distinct stereotypic behaviour and the mating process (when the snails do not form copulatory chains) lasts 40–120 min. The passive partner (“female actor”) usually does not stop feeding or moving during intromission. The male actor may deposit a copulatory plug, whose function is unclear (Rudolph, 1979).

The second candidate group, the subfamily Helisomatinae, and, specifically, the genus *Planorbella* Haldeman, 1842, have both unilateral and simultaneously reciprocal mating. The duration of copulation is approximately 1–2 hours and mating is preceded by a complex, stereotypic courtship process. During intromission, the partners are not firmly attached to each other and penetration can easily be observed directly. The mates use the preputial organ to hold the partner; the preputial organ may function as a holdfast and probably also as an excitatory organ (Abdel-Malek, 1952; Pace, 1971; Soldatenko & Petrov, 2012).

A significant number of species in the third candidate group, the subfamily Planorbinae, are specialised forms with long copulatory stylets. Since *Planorbarius* does not have a stylet, it seems reasonable to compare it with those forms in the Planorbinae that have a small cap-shaped stylet (*Planorbis* Geoffroy, 1767) (Soldatenko & Petrov, 2009a; Soldatenko & Shatrov, 2013) or lack a stylet (*Biomphalaria* Preston, 1910). The copulatory apparatuses in these two genera do not have any accessory structures, such as flagellum, preputial organ, preputial gland or accessory duct (Trigwell et al., 1997; Soldatenko & Petrov, 2012). Copulation in both genera is quite similar and can be both unilateral and reciprocal. The duration of copulation is about 1–2 hours and intromission is always preceded by shell circling. During insemination, the mates are so closely attached to each other that penetration cannot be directly observed.

The aim of this paper is to describe mating behaviour and copulatory mechanics in *Planorbarius corneus* (Linnaeus, 1758) and provide new information about its taxonomic status. The paper also examines the morphology of copulatory organs in the snails killed and fixed during intromission and supplements these data with the description of musculature of the ejaculatory duct stained with phalloidin-TRITC for confocal microscopy.

MATERIAL AND METHODS

Collection sites

Sexually mature individuals of *Planorbarius corneus* were collected by the senior co-author in 2010–2013 (June through October). Collections were made from the following localities in the European part of Russia:

1. Smolensk Province, Demidovskiy District, Smolenskoye Poozerye National Park, town Przhevalskoye, channel Sapsho-Svjatec (N 55°29'59'' E 31°49'06'', altitude 154 m), June 2010, July 2011, July 2012, July 2013;

2. Smolensk, a section of the Dubrovenka river artificially expanded for recreational use (N 54°47'48'' E 31°56'96'', altitude 209 m), June 2010, August 2011, May 2012, August 2012, June 2013.

The individuals collected in nature were reared in the laboratory for 4 years; in total, 9 groups were observed, 3 of which (the groups collected in July 2011, August 2012, and July 2013) are still maintained in the laboratory.

Behavioural study

Mature individuals of *P. corneus* were placed in containers with the water column of 15 cm in groups of 10–12 individuals. Each month the water was heated with fluorescent tube lamps to 25–28 °C (Duncan, 1975; Smith, 1981) and was maintained at this temperature for a week to induce mating. Mating pairs were photographed and time intervals were recorded for all stages of the mating process. Some individuals were marked with coloured nail polish to keep track of the formation of mating pairs in the containers. Only an approximate estimate was made for the beginning of precopulation, because this species does not show any distinct behavioural transition from grazing to the pursuit of the prospective partner. The entire precopulatory phase was observed only for one pair, after both snails in this pair were isolated in separate containers for 9 days. Time intervals for the stages of the mating process are shown in Table 1.

Two of the mating pairs were separated during mating to study the mechanism of insemination. In many freshwater molluscs, including *Anisus vortex* (Linnaeus, 1758), *Bathyomphalus contortus* (Linnaeus, 1758), *Choanomphalus riparius* (Westerlund, 1865), *Planorbella (Seminolina) duryi* (Wetherby, 1879), *Planorbis planorbis* (Linnaeus, 1758), and *Segmentina oelandica* (Westerlund, 1885), such intervention causes an immediate disintegration of the pair. By contrast, the forcefully separated pairs of *P. corneus* keep exchanging the sperm at the outstretched copulatory ap-

Table 1. Time intervals (minutes) for mating of *P. corneus*; *n* is a number of observed mating encounters.

Species	t °C	Pre-copulation	Copulation	Post-copulation	Mating period
		n = 1	n = 5 mean ± SD (min–max)	n = 5 mean ± SD (min–max)	n = 1
<i>P. corneus</i>	20–25	27.00	85.24 ± 12.07 (70.00–107.00)	19.12 ± 6.06 (12.00–28.00)	130.00

paratuses for over an hour allowing a more detailed examination of the insemination process.

Morphological study

Nine individuals of *P. corneus* were dissected to study the anatomy of the copulatory organs. Three pairs were allowed to intromit for no less than 10 minutes and then were killed by immersion in boiling-hot water for at least 5 minutes. This method of immobilization prevented the snails from retracting the copulatory organs during fixation. The snails were fixed in 70% ethanol; their male copulatory apparatuses and distal portions of the female reproductive system were dissected out and examined as whole-mount preparations to study the functional morphology of the copulatory structures during insemination. Whole-mount preparations were examined and photographed under a MBS-9 stereomicroscope equipped with a CCD camera; the line drawings were copied from photographs to scale.

For confocal microscopy, two specimens of each species were fixed for 1 h in 4% formaldehyde buffered with 0.1 M phosphate-buffered saline (PBS) at room temperature, rinsed in 0.1 M PBS (3×15 min), permeabilised for 1 h in PBS containing 0.2% Triton X-100, rinsed again shortly in 0.1 M PBS and then transferred to phalloidin-TRITC (Sigma-Aldrich) for 1 h. After that, the specimens were washed again for 3×15 min in 0.1 M PBS, mounted in 80% glycerol on glass slides and viewed with a Leica TCS SP5 microscope.

Terminology

This paper uses the conventional terminology for gender roles (e.g. Smith, 1981; Jordaens et al., 2009). Mating is reciprocal and therefore the individual that exhibits a more active mating behaviour and initiates mating is referred to as the “active partner” and the other individual as the “passive partner”. Mating is divided into three consecutive phases (precopulation, copulation, and

postcopulation) following the terminology of Trigwell et al. (1997). The period from the beginning of precopulation to the end of postcopulation is designated as the mating period. Both definitive and embryonic shells of *P. corneus* are sinistral. The coiling of the shell is the same as that of the body, which is unusual in planispiral Palearctic freshwater snails. To make this paper terminologically consistent with our previous publications, we use the term “upper” for the side of the shell that is facing upward when the snail moves and the term “basal” for the opposite side of the shell. It should be noted, however, that in *P. corneus* the “upper” shell side corresponds to the umbilical side and the “basal” to the apical side.

RESULTS

Mating behaviour

The active individual stops feeding and starts moving erratically over the shell of the passive individual, probing it with the tentacles. During this phase the passive partner crawls over the substrate, feeds or sits motionlessly on the side of the container and begins to display interest in the partner only during the second phase of precopulation. The second phase is marked by the change in the behaviour of the active individual: it starts moving along the shell of the partner towards its head (Fig. 1a). During this phase the snails do not seem to exhibit any distinct precopulatory behaviour; only one pair showed a more complex behaviour that may be considered stereotypic. In this pair the active individual gripped the outer margin of the partner's shell with its foot, rotated the shell of the passive individual and forced the partner to detach from the substrate, making the pair float up to the water surface.

At the end of the precopulatory phase the basal sides of the partners' shells are facing one another. The entire precopulatory phase was observed only in one pair: in this case, precopulation lasted approximately 27 min, but in the other observed pairs



Fig. 1. *Planorbarius corneus*, photographs of copulating snails, precopulation: **a**, the active partner crawls over the shell of the passive partner; **b**, copulation, the active partner is on the right, the black arrow points to its foot; **c**, intromission, snails are detached from one another, the active partner is on the top, the arrow points to its everted preputium with the preputial organ; **d**, postcopulation, the passive partner is retracting its copulatory apparatus, the black arrow points to its atrial organ, the white arrow points to the preputial organ. Scale bars: 1.0 cm (a–c); 0.5 cm (d).

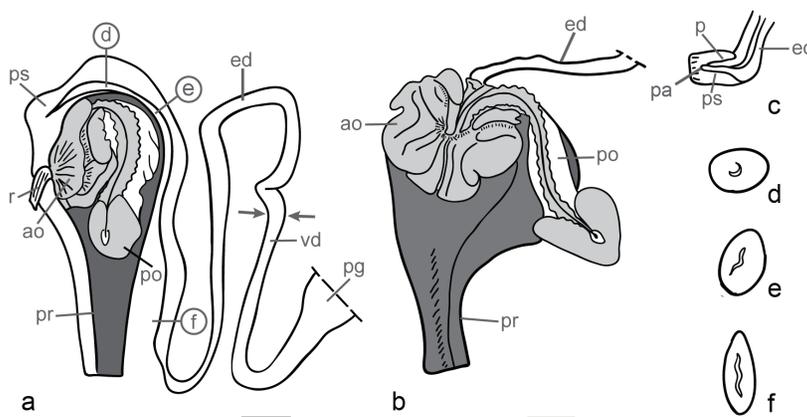


Fig. 2. *Planorbarius corneus*, schematic diagrams of copulatory apparatuses: **a**, general diagram of copulatory apparatus with anterior wall removed; **b**, preputium unfolded and spread out; **c**, penis sheath; **d–f**, cross-sections through ejaculatory duct (d–f; cross-sections made at levels shown with circled letters in Fig. 2a). Light grey: areas of glandular epithelium; dark grey: internal lumen of copulatory apparatus. Arrows showing border between ejaculatory duct and vas deferens. Abbreviations: *ao*, atrial organ; *ed*, ejaculatory duct; *p*, penis; *pa*, papilla; *pg*, prostate gland; *po*, preputial organ; *pr*, preputium; *ps*, penis sheath; *r*, retractor muscle; *vd*, vas deferens. Scale bars: 1.0 mm .

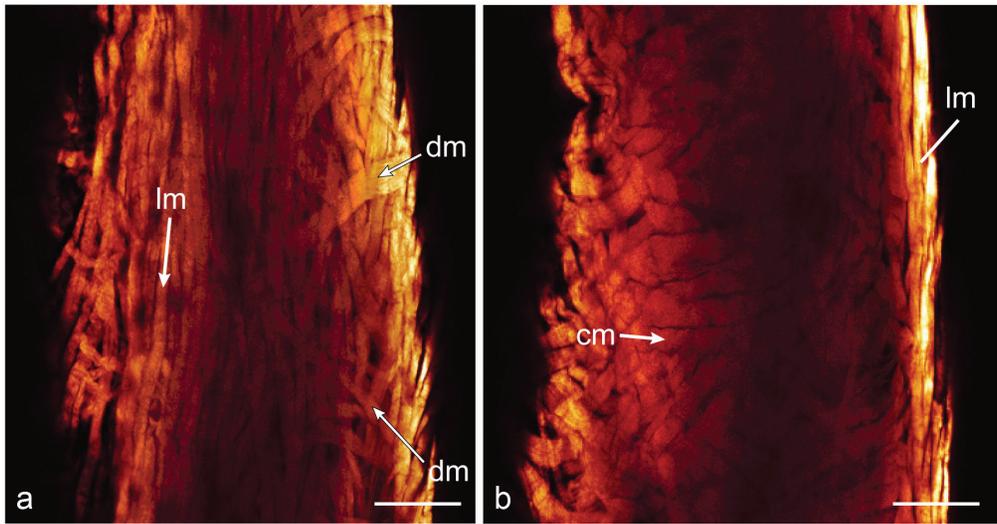


Fig. 3. *Planorbarius corneus*, maximum intensity projections through musculature of ejaculatory duct. Phalloidin TRITC: **a**, projection close to surface of wall showing outer longitudinal muscles (lm) and underlying diagonal muscles (dm); **b**, projection deeper inside wall showing circular muscles (cm). Scale bars: 50 μ m.

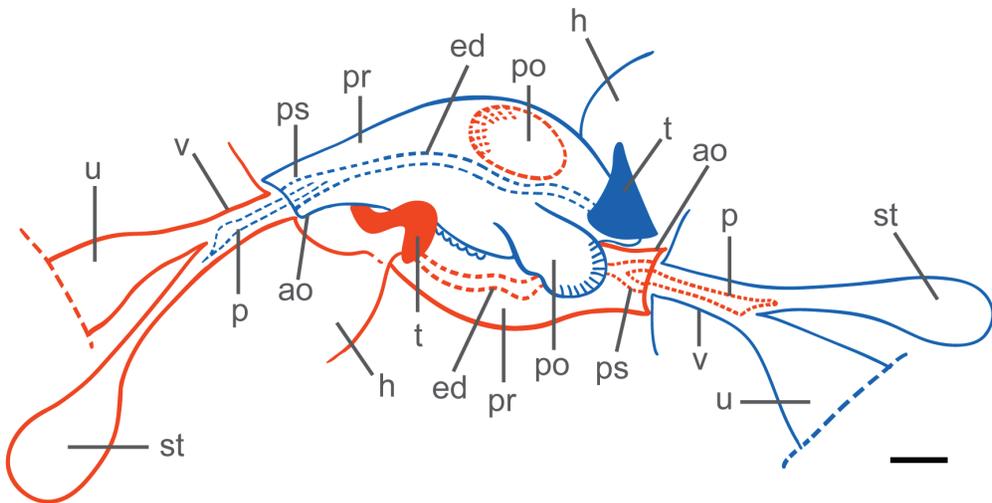


Fig. 4. Schematic diagrams of intromission in *Planorbarius corneus*. Blue: copulatory organs of active partner; orange: copulatory organs of passive partner. Abbreviations: ao, atrial organ; ed, ejaculatory duct; h, head; p, penis; po, preputial organ; pr, preputium; ps, penis sheath; st, spermatheca; t, tentacle; u, uterus; v, vagina. Scale bar: 1 mm.

this period seems to be comparably long. The passive individual attaches its foot to the partner's shell near the outer lip of the aperture. The foot of the active partner grows significantly in size (about 2–3 times its initial size) and looks like a bump-like swelling; this distinction makes it easier to identify the individual that initiates mating (Fig. 1b). Once the partners are firmly attached to each other, the active individual everts its copulatory apparatus that penetrates into the space under the partner's shell and reaches the vaginal opening. The passive partner repeats the manoeuvres of the active individual after 7–10 minutes of delay and reaches the vaginal opening of the active individual with its copulatory apparatus. During intromission, the movements of the copulatory structures can be observed directly, if the partners are taken apart without breaking the connection of the copulatory organs (Fig. 1c). All copulations that we have observed were reciprocal. The partners remain completely motionless throughout the copulatory phase, except for waving of tentacles. The active individual stops copulating first, detaches and retracts the copulatory apparatus, and then loosens its foothold on the partner's shell (the foot shrinks to its normal size). The active individual does not make any abrupt movements allowing the passive partner to finish the copulation. The passive individual retracts its copulatory apparatus only when the pair has disintegrated (Fig. 1d); at this point, it may discharge the surplus sperm that can be visible as a dense white thread. Despite keeping the snails for almost 5 years in the laboratory and despite observations in nature, the copulation was observed only in 5 pairs (Table 1). Maintaining the snails at low temperature (10–12 °C) for one week with subsequent warming to 20–25 °C did not induce mating.

Copulatory apparatus

The copulatory apparatus of *Planorbarius corneus* has no glandular appendages and is divided into 3 parts: ejaculatory duct,

penis sheath and preputium (Fig. 2a, b). We consider the ejaculatory duct as a major anatomical division of the copulatory apparatus based on its function during copulation (see below). In the literature (Baker, 1945; Hubendick, 1955; Starobogatov, 1958; Maksimova & Yakovleva, 1991) the ejaculatory duct is called spermoduct. In fact, the actual spermoduct is a very short portion of the duct, immediately adjacent to the prostate gland. The ejaculatory duct is milky white or yellowish in colour, has a thick muscular wall and is connected to the penis sheath (Fig. 2a, d–f). The muscular wall of the ejaculatory duct is composed of two layers of muscles (Fig. 3a, b). The outer layer consists of 1 or 2 rows of longitudinal muscles (Fig. 3a, b, *lm*). The inner layer is composed of 6–8 rows of diagonal or circular muscles. The outermost 2 or 3 rows in this layer are diagonal muscles arranged in an alternating criss-crossing pattern (Fig. 3a, *dm*). The muscles in the next 2–4 rows gradually change their direction from diagonal to circular and 2 innermost rows are arranged in a perfectly circular fashion, i.e. perpendicular to the longitudinal muscles (Fig. 3b, *cm*). The penis sheath is the most compact and thin-walled part of the copulatory apparatus that houses a small penis (Fig. 2c). The preputium is the distal, most massive, portion of the copulatory apparatus, containing the preputial and atrial organs (Fig. 2a). The preputial organ is a musculo-glandular organ that forms as an outgrowth of the preputium wall. The atrial organ (see Alaphilippe, 1959 for discussion of this term) is a rosette-shaped musculo-glandular structure in the proximal portion of the preputium connected to the preputial organ. We use the term “atrial organ” instead of the “preputial gland”, which was widely used in the Russian literature (Starobogatov, 1958; Stadnichenko, 1990; Maksimova & Yakovleva, 1991). There are no significant differences in histology of the preputial and atrial organs (Alaphilippe, 1959; Maksimova & Yakovleva, 1991) and therefore it would be incorrect to use the

term “gland” for the atrial organ, because its main structural component is muscles and its main functional role (attachment to the partner’s vagina) is accomplished primarily by muscular force. The structures previously described as “a canal in the penial gland” (Baudelot, 1863; Baker, 1945; Hubendick, 1955) or “sperm groove” (Maksimova & Yakovleva, 1991) are, in fact, the folds of the preputial and atrial organs used for fixation.

Insemination

The insemination is reciprocal. The active partner everts the preputium first, bringing the preputial organ and then the atrial organ to the outside. All structures become bright red because of the afflux of the hemolymph. The proximal portion of the preputium with the atrial organ takes the shape of a rosette and grips the surface around the vaginal opening. The fixation of the partners is so strong that the copulatory apparatus remains attached even if the partners are forcefully separated (Fig. 1c). The preputial organ of the active partner attaches to the everting preputium of the passive partner and probably guides the partner’s preputium towards the vaginal opening. The eversion of the preputium draws the penis sheath and ejaculatory duct inside the distal part of the copulatory apparatus. Active contraction of the duct expels very dense sperm from the genital tracts of the snail through the penis that distends to become about 3–4 times its normal size. It should be noted that the length of the penis during insemination matches the length of the vagina. The opening of the ejaculatory duct is subterminal and is tilted towards the uterus. The insemination is shown in Fig. 4. The sperm volume is about 0.3 cm³ per individual. Once the sperm transfer is completed, the active partner starts slowly retracting its copulatory apparatus; it first pulls in the penis and detaches the rosette of the atrial organ from the partner’s vagina and then retracts the preputium with the preputial organ. The passive individual

repeats these manoeuvres (Fig. 1d) almost immediately, as soon as the active partner has finished retracting its copulatory organ.

DISCUSSION

Anatomy and histology of the copulatory organs in *Planorbarius corneus* were extensively studied by a number of authors (Moquin-Tandon, 1855; Baudelot, 1863; Taylor, 1900; Germain, 1931; Baker, 1945; Hubendick, 1955; Starobogatov, 1958; Alaphilippe, 1959; Maksimova and Yakovleva, 1991), but the lack of information on mating behaviour led to incorrect interpretations of the functional role of some copulatory structures. It was widely accepted that sperm passes through the preputial organ and insemination is accomplished by contraction of its muscular walls, whereas the penis has lost its original function and is rudimentary (Moquin-Tandon, 1855; Baudelot, 1863; Taylor, 1900; Germain, 1931; Hubendick, 1955; Starobogatov, 1958; Alaphilippe, 1959; Maksimova & Yakovleva, 1991). It was also suggested that the preputium is unable to evert due to its firm attachment to the roof and to the body surface of the mantle cavity (Maksimova & Yakovleva, 1991). Our observations of the copulation in *P. corneus* show that these views must be revised. The preputial organ does not participate directly in sperm transfer and functions as a holdfast that holds the partners’ preputia firmly together. The preputium everts completely during the copulation. The penis is a fully functional and very extensible structure, which is, however, unlikely to be able to accumulate and transfer a relatively large amount of dense sperm without significant muscular assistance from other copulatory structures, especially from the ejaculatory duct. The ejaculatory duct is the structure that is directly responsible for sperm propulsion during insemination. Our confocal microscopy analysis shows that the ejaculatory duct has a complex muscular wall composed of several rows of antagonistic

circular and longitudinal muscles. This arrangement of muscle layers suggests that the ejaculatory duct is capable of coordinated propulsive or peristaltic movement. The ejaculatory duct therefore plays an essential role in copulation and must be recognised as the third major anatomic division of the copulatory apparatus together with the penis sheath and preputium. The atrial organ is a very extensible portion of the proximal wall of the preputium that attaches to the everted preputium of the partner and to the partner's body near the vagina. It should be noted that the atrial and preputial organs are composed of both glandular and muscular tissues and include two different types of secretory cells (Maksimova & Yakovleva, 1991) and therefore their role is probably not limited only to fixation. Baker (1945) assumed that these structures can expand the opening of the diaphragm between the penis sheath and preputium, secrete a lubricating fluid and function as excitatory organs. We think that secretion of the atrial organ can enhance the effect of fixation, while secretion of the preputial organ can stimulate the partner and help synchronise the copulation, in the same way as it was previously observed in *P.(S.) duryi* (Soldatenko & Petrov, 2012). The copula-

tory apparatuses of *P.(S.) duryi*, *Planorbella (Pierosoma) trivolvis* (Say, 1817) and *P. corneus* are therefore similar both in morphology and function, which supports the Baker's view about a close phylogenetic affinity of these species (Baker, 1945).

P. corneus has the longest mating process compared to the planorbids studied by the authors (*A. vortex*, *B. contortus*, *C. riparius*, *P.(S.) duryi*, *P. planorbis*, *S. oelandica*). The mean duration of copulation is close to those of *P.(S.) duryi*, *P. planorbis* and *A. vortex* (Soldatenko & Petrov, 2012), but precopulation and postcopulation are significantly longer (Table 1). *P. corneus* mate face-to-face, and therefore the passive individual stops feeding and moving and the partners intromit reciprocally (Table 2). Some authors (Berezkina & Starobogatov, 1988; Maksimova & Yakovleva, 1991) mention the possibility of unilateral copulation, but we never observed this type of mating. Given the characteristics of insemination in this species, we can assume that aforementioned authors came to this conclusion, because they observed the copulatory structures during the postcopulatory phase, when the active partner had already retracted its organ and the passive individual was about to finish the same process. The preputial and atrial

Table 2. Mating behaviour traits and associated morphological characters in *P. corneus* compared to some other basommatophoran species (Wu, 1972; Kuma, 1975; Rudolph, 1979; Soldatenko & Petrov, 2009, 2012). Characters 2, 3, and 4 are based on Jordaens et al. (2009).

Species	Characters							
	1	2	3	4	5	6	7	8
<i>B. globosus</i>	NS	M	SM	R	up	–	–	FP
<i>P. planorbis</i>	S	Re	SM/FF	U/R	–	+	–	FP
<i>P.(S.) duryi</i>	S	O	FF/SM	R/U	po	+	*	SH?
<i>P. corneus</i>	S	O	FF	R	ao+po	–	*	SH

Characters: (1) female copulatory activity: S – stops feeding and crawling; NS – continues feeding and crawling. (2) shell orientation: M – mounted, i.e. umbilicus of upper shell is over spire of the lower shell; P – planar, i.e. both shells lie on substrate, with their spires away from substrate; O – obverse, i.e. shells are oriented with their spires toward each other; Re – reverse, i.e. shells are oriented with their umbilica toward each other. (3) mating position: FF – face-to-face; SM – shell mounting. (4) penis (ultrapenis) usage during copulation: R – reciprocal; U – unilateral. (5) morphology of the penial complex: ao – atrial organ; po – preputial organ, up – ultrapenis. (6) stylet: + present; – absent; (7) copulatory stimulation: * – copulation is accompanied by mechanical or chemical stimulation; – stimulation not observed. (8) gender expression: SH – simultaneous hermaphrodites; FP – functional protandry.

organs in *P. corneus* function as suckers and excitatory organs; the stylet is absent. It is possible that *P. corneus* is a simultaneous hermaphrodite because mating is reciprocal and simultaneous and occurs only between partners of equal body size.

Table 2 summarises the characteristics of copulation for the species (*B. globosus*, *P. planorbis*, and *P. (S.) duryi*) that are traditionally thought to be closely related to *Planorbarius*. Comparison shows that the characters of *B. globosus* (unilateral copulation, morphology of the copulatory apparatus and the absence of stimulating organs) are quite different from those of the other three species. Apparently, the inclusion of the genus *Planorbarius* in the family Bulinidae resulted from an erroneous interpretation of the morphology of the copulatory apparatus in *P. corneus*. Likewise, there are very few shared traits between *P. planorbis* and *P. corneus*. These traits are limited only to face-to-face mating and the possibility of mutual sperm transfer, whereas the significant differences in the structure of copulatory apparatus and other morphological characteristics have long raised doubts about the phylogenetic affinity of the genera *Planorbis* Geoffroy, 1767 and *Planorbarius* (Baker, 1945; Hubendick, 1978; Starobogatov, 1967). For this reason, there is no sufficient evidence to include *P. corneus* in the subfamily Planorbinae Rafinesque, 1815 (family Planorbidae) and even in the family Planorbidae as defined by Starobogatov (1967). The characteristics of copulation (Table 2) and the structure of the copulatory apparatus in *P. corneus* are most similar to that in *P. (S.) duryi*, but in the latter species the mating process does not seem to be so strictly determined and allows certain variability (in characters 3 and 4) depending on the physiological condition of the partners. The similarity in the anatomy of these species was noted by Baker (1945), who placed the genus *Planorbarius* in the subfamily Helisomatinae within the family Planorbidae. It should be noted that these species differ significantly in the

structure and development of their copulatory apparatuses (Soldatenko, 2011), with differences probably accumulated as a result of the long period of geographical isolation of these related forms. Observations of the mating process together with the studies of morphology (Baker, 1945; Hubendick, 1955; Alaphilippe, 1959; Maksimova & Yakovleva, 1991) and development of the copulatory structures (Soldatenko, 2011) allowed us to rule out the family Bulinidae P. Fischer et Crosse, 1880 and Planorbidae Rafinesque, 1815 as possible close relatives of *Planorbarius* and emphasise the close affinity of this genus to the family Helisomatidae. However, the taxonomic status of this genus (the inclusion into either Helisomatidae F.C. Baker, 1928 or Camptoceratidae Dall, 1870 or separation into a new family) can be clarified only when new or revised information on the anatomy, cytology and physiology of other species within these groups becomes available.

ACKNOWLEDGEMENTS

This work was supported by the Russian Foundation for Basic Research (grant 13-04-97514). Confocal microscopy was performed at the Shared Services Centre "Taxon".

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Received October 14, 2013 / Accepted November 17, 2013