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Macro- and microscopic morphology of the reproductive system of the terrestrial snail *Macrochlamys indica* (Godwin-Austen, 1883) (Eupulmonata, Stylommatophora, Ariophantidae)

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ABSTRACT

The morpho-anatomical and morphometrical characteristics of the reproductive system, together with the spermatophore and dart apparatus, of *Macrochlamys indica* are described and the results compared with available data of other *Macrochlamys* species. Histology of some reproductive structures such as the ovotestis, hermaphrodite duct, albumen gland, penis and dart shaft of *M. indica* are also studied. A muscular, brownish capsule is developed between the free oviduct and the vagina, here named as vaginoviducal capsule and its location is species-specific. The distal end of the vaginoviducal capsule bifurcates into a free oviduct and the duct of the gametolytic organ. The gametolytic sac of the gametolytic organ is a whitish, tubular structure that becomes pinkish and club-shaped in inseminated snails. Two to seven spermatophores are found in the pinkish gametolytic sac. The spermatophore is a whip-like, translucent structure. The epiphallus possesses a coiled epiphallic caecum to which a penial retractor muscle is attached. The penis is a short, muscular structure with a distinct penial caecum. The dart apparatus is well-developed, having four distinct parts. The proximal part of the dart apparatus possesses a non-disposable muscular dart. The dart shaft includes a central core which is encircled by many layers of circular muscle cells.

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Introduction

Stylommatophoran gastropods (Panpulmonata, Eupulmonata) are fully terrestrial and hermaphroditic (Tompa 1984; Bouchet et al. 2017; Teasdale 2017). Some stylommatophorans show functional protandry, although self-fertilisation and parthenogenesis occur in this group (Tompa 1984). The morphology and functional interpretation of the reproductive structures are important in systematics and determining the phylogenetic relationships among terrestrial gastropods (Panha 1997; Hyman and Ponder 2010, 2016; Pholyotha et al. 2018). Additionally, the spermatophore and the dart apparatus (an auxiliary copulatory organ) have significant value in systematics and in the evolution of successful fertilisation and sperm competition in molluscs (Tompa 1984; Hausdorf 1998; Gómez 2001; Koene and Schulenburg 2005; Kimura et al. 2015). The development of the spermatophore and dart apparatus in an individual is species-specific (Tompa 1984). The spermatophore is developed in the cavity of the epiphallus and its morphological characteristics depend on the structure of epiphallus cavity of an individual (Tompa 1984; Gómez 2001; Pholyotha et al. 2018). The dart apparatus comprises a shooting part termed a 'dart' or 'love dart' (Tompa 1984). Stylommatophoran snails stab their dart into the body of the opposite partner during mating, with a great influence on the paternity success of individuals (Adamo and Chase 1990; Rogers and Chase 2001; Kimura et al. 2015; Lodi et al. 2017). In some mating events, snails [e.g. *Cornu aspersum* (O.F. Müller, 1774)] can lose their dart, which is regenerated before the next mating event (Tompa 1982), whereas other snails (e.g. *Philomycus carolinianus* Bosc, 1802) retract the dart into the dart sac for reuse (Tompa 1980). The anatomy of the genitalia of stylommatophorans has been rather well studied (Mead 1950; Duncan 1960; Gómez 2001), but the details of the dart apparatus and spermatophore for many taxa are less well known (Ghose 1962; Deshmukh et al. 2012; Hyman et al. 2017).

Macrochlamys (Gray 1847) (Eupulmonata, Stylommatophora) is a common terrestrial snail and one of the most speciose genera in the family Ariophantidae (Godwin-Austen 1883). The structural characteristics of the genitalia in several *Macrochlamys* species were published recently (Deshmukh et al. 2012; Pholyotha et al. 2018; Sajan et al. 2019), but there is very little information regarding the structural and functional characteristics of the whole reproductive system, and

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especially the spermatophore and dart apparatus. Furthermore, none of the previous studies attempted to describe the morphometrics of the genitalia and other components of the reproductive system in *Macrochlamys* (Ghose 1962; Panha 1997; Deshmukh et al. 2012; Pholyotha et al. 2018).

The present study aims to investigate the detailed morpho-anatomical and morphometrical characteristics of the reproductive system along with the spermatophore and dart apparatus of *Macrochlamys indica* (Godwin-Austen 1883). The results from *M. indica* are compared with the available data for other species of *Macrochlamys* as well as characteristics of their reproductive strategies.

Materials and methods

Fifty live specimens of *Macrochlamys indica* were collected from fields around Kolkata (22.5726° N, 88.3639° E), West Bengal, India, during the months of June and July. The species was identified by Zoological Survey of India (ZSI) and voucher specimens have been deposited at ZSI, Kolkata in the National Zoological Collections (NZC). The specimens were 2.1 ± 0.5 cm (mean ± SD) in shell diameter and of 5 – 6 whorls. They were acclimatised in earthen pots and provided with leafy vegetables. Water was sprayed regularly to maintain a moist environment.

Anatomical study

Twenty-five live specimens were killed by prolonged drowning using standard methods (Medeiros et al. 2013). Specimens slightly hardened with 70% alcohol for a few days were most suitable for dissection. Eighteen to twenty well-extended specimens were used for dissection as well as descriptions of components of the reproductive system, spermatophore and dart apparatus. The dissecting tray was filled with a thick layer of paraffin wax. The reproductive system and the auxiliary copulatory organ were photographed using a digital photographic camera (Canon SX700 HS) and a stereomicroscope (Leica DMC4500). The spermatophore was removed from the gametolytic sac, cleaned of extraneous tissue with fine forceps and rinsed gently in water. A few spermatophores were mounted on a clean slide and examined under a light microscope (NIKON 50i). In descriptions of the reproductive system, 'proximal' refers to the region closest to the genital opening or the mouth and 'distal' refers to the region closest to the apex of the shell coil. The terminology of the genitalia and the various reproductive organs follows Blanford and Godwin-Austen (1908), Tompa (1984), Gómez et al. (1996), Gómez (2001) and Pholyotha et al. (2018). The measurements (mean value ± SD, $N = 5$) of various reproductive structures were analysed with 'ImageJ 1.51t' software (Wayne Rasband, NIH, USA) using photomicrographs.

Histological study

For histological analysis, some parts of the reproductive system such as the hermaphroditic gonad (ovotestis), hermaphrodite duct, albumen gland, penis and the dart apparatus were dissected out from the living specimens ($N = 10$), immediately fixed in aqueous Bouin's solution for 12 h, subsequently dehydrated in ethanol and embedded in paraffin for 4 – 6 µm thick sections. These sections were examined under a light microscope (NIKON 50i) after staining with Delafield's haematoxylin and eosin.

Results

The reproductive system of *Macrochlamys indica* is a complex structure including the distally located hermaphrodite gonad (ovotestis), hermaphrodite duct, albumen gland, and fertilisation pouch-spermatheca complex, and proximally, a penis, epiphallus with flagellum, vas deferens, gametolytic organ (bursa copulatrix), free oviduct, vagina and genital atrium. The spermoviduct (oviducal channel and prostate gland together) is the connection between the proximal and the distal part of the reproductive system (Figures 1–5A, B, 2A–C, 3A–D, 4A–C, 5A–C, and Tables 1–4). The dart apparatus is a major auxiliary copulatory organ and opens to the exterior through the genital atrium (Figures 1A, B, 3A, 5A, 5C and Table 5). The genital opening and most of the reproductive organs are on the right side of the mouth (Figures 1A, B, 3A, 5A).

The ovotestis

The ovotestis is whitish, multilobate and located towards the apex of the coiled shell (approximately in the third and fourth whorls). It consists of seven to ten cuboidal (or flask-like) lobes (white asterisks in Figure 2C) which are firmly embedded in the digestive gland and cannot be separated cleanly or completely (Figures 1A, B, 2C). The ovotestis occupies a large area (8.70 ± 1.2 mm in length) in the digestive gland towards the inner border (or concave border) of its spiral (Figure 1A). It is covered with a thin, slightly pigmented, transparent layer through which the whitish ovotestis lobes are visible (Figure 2C). The size of the lobes becomes gradually smaller towards the apex of the digestive glands. The size of the most proximal lobe (first lobe) is 1.55 × 1.63 mm and that of the distal-most lobe (last lobe) is 0.55 × 0.96 mm. The narrow space (0.19 ± 0.04 mm) between two adjacent ovotestis lobes, especially at the basal area, is filled by the digestive gland tissue (Figures 1A, B, 2C). The ovotestis lobes are each connected to the hermaphrodite duct by a thin efferent duct (white

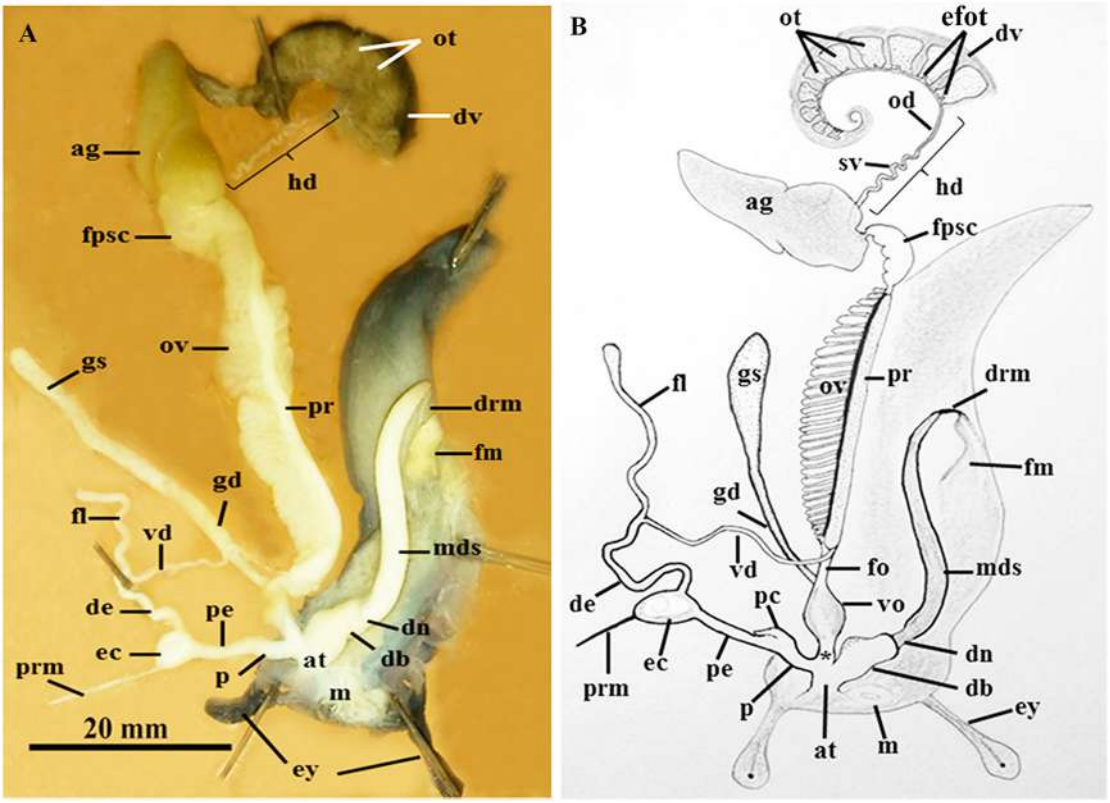

Figure 1. Reproductive system of *Macrochlamys indica*. **A**, A complete reproductive system with associated dart apparatus; **B**, Schematic diagram of the reproductive system and its association with the dart apparatus. Abbreviations: ag = albumen gland; at = genital atrium; db = dart bag; de = distal epiphallus; dn = dart neck; drm = dart retractor muscle; dv = digestive gland; ec = epiphallic caecum; efot = efferent ovotestis duct; ey = eye stalk; fl = flagellum; fpsc = fertilisation pouch-spermatheca complex; gd = gametolytic duct; gs = gametolytic sac; hd = hermaphrodite duct; m = mouth; mds = median dart shaft; od = ovotestis duct; ot = ovotestis lobes; ov = oviduct; p = penis; pc = penial caecum; pe = proximal epiphallus; pr = prostate gland; prm = penial retractor muscle; sv = seminal vesicle; vd = vas deferens; vo = vaginoviducal capsule; Asterisk = vagina.

arrowhead in Figure 2C) that comprises several efferent ductules of acini.

Each lobe of the ovotestis comprises numerous, small, ovoid acini. The whole space in each acinus is divided into an acinar cortex and an acinar medulla or acinar lumen. Spermatogenesis and oogenesis occur simultaneously in the same acinus (Figure 6). The developing spermatogenic cells are distributed throughout the acinar space and most of the acinar lumen is occupied by the spermatozoa. The spermatogenesis occurs in the vicinity of the Sertoli cells. Oogenesis is strictly restricted to the area of the acinar cortex. Each acinus consists of only one oval shaped oocyte (3.60 × 2.39 µm) with a prominent nucleus (1.64 ± 0.12 µm in diameter). The oocyte is surrounded by the follicular layer (f, in Figure 6) which acts as a physical barrier between developing female gametes (oc, in Figure 6) and developing male gametes (i.e. spermatocyte, spermatids, and etc.) (Figure 6). Developing sperm are transported to the hermaphrodite duct through fine acinar ductules.

The hermaphrodite duct

The hermaphrodite duct is a creamy white, elongated (29.12 ± 1.3 mm) tubular structure that connects the ovotestis to the spermoviduct (Figures 1A, B, 2C). It is divisible into three morpho-functionally differentiated regions, although these are lacking sharp external demarcations (Figures 1B, 2C).

(i) The first region is the ovotestis duct, being the distalmost part of the hermaphrodite duct and very close to the ovotestis (Figures 1B, 2C). The ovotestis duct is a thin membrane-bound, clearly visible, free-running, elongated (16.34 ± 1.40 mm in length) duct and devoid of spermatozoa (Figures 2C, 7A). Histologically the ovotestis duct (3.18 ± 0.27 µm in diameter) has a narrow H-shaped lumen (1.23 ± 0.27 µm in diameter) and folded inner wall. The lumen is surrounded by a layer of highly ciliated, nucleated cuboidal endothelial cells along with many small endothelial folds (Figure 7A).

(ii) The second region is known as the seminal vesicle. It is the clearly differentiated middle of the hermaphrodite duct and is comparatively thicker than the ovotestis duct (Figures 1B, 2C). The seminal vesicle is a moderately elongated (9.76 ± 0.53 mm in length), tubular, highly convoluted, distended duct (Figure 2C). Histologically it is about 5.88 ± 0.30 µm in diameter and possesses a central lumen (3.73 ± 0.20 µm in diameter) that

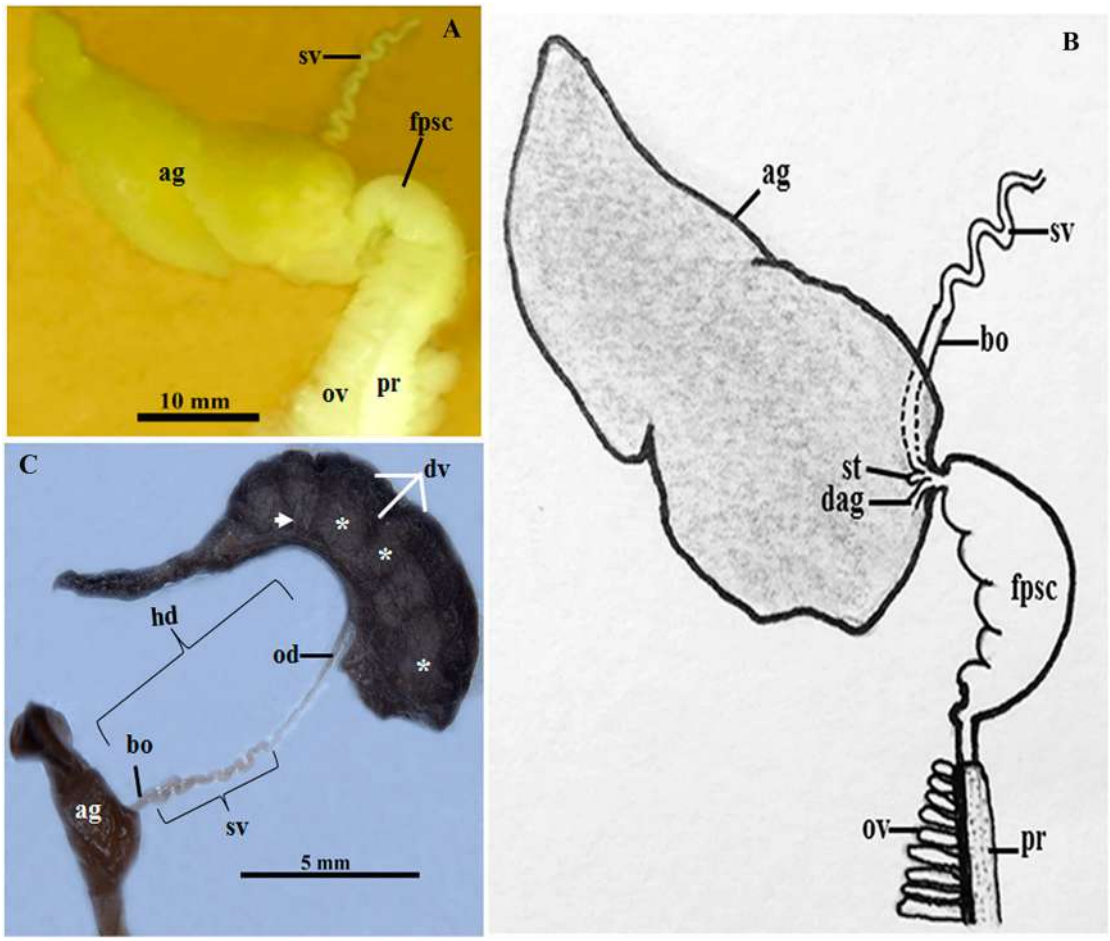

Figure 2. Photomicrographs and schematic diagram of some parts of the reproductive system of *Macrochlamys indica*. **A**, Enlarged view of the distal part of the spermoviduct and its association with various adjacent structures; **B**, Schematic drawing showing the anatomical association near both ends of fertilisation pouch-spermatheca complex (fpsc). Note the spermatheca and a part (dotted line) of the basal ovotestis duct are embedded by the albumen gland; **C**, Magnified view of ovotestis lobes and various parts of hermaphrodite duct. Abbreviations: ag = albumen gland; bo = basal ovotestis duct; dag = duct of albumen gland; db = dart bag; dv = digestive gland; fpsc = fertilisation pouch-spermatheca complex; hd = hermaphrodite duct; od = ovotestis duct; ov = oviduct; pr = prostate gland; st = spermatheca; sv = seminal vesicle. Asterisk = lobe of ovotestis. White arrow = efferent duct of ovotestis lobe.

is encircled with a layer of ciliated, nucleated cuboidal endothelial cells and is loaded with spermatozoa. Endothelial folds are absent or very poorly developed in the lumen (Figure 7B).

(iii) The third region is the basal ovotestis duct, being the proximal-most of the hermaphrodite duct and is very close to the albumen gland. It is a short (1.40 ± 0.05 mm in length), thick, muscular tube which is partly embedded in the albumen gland (Figure 2C). This proximal portion of the hermaphrodite duct opens to the distal end of the fertilisation pouch-spermatheca complex (or talon) at the base of the albumen gland (Figures 1A, B, 2A-C). Histologically the basal ovotestis duct is about 4.32 ± 0.07 µm in diameter and comprises a wide H-shaped lumen (2.32 ± 0.69 µm in diameter). The folds of the inner wall reappear in this region and are enclosed with a highly folded, ciliated, nucleated endothelial layer (Figure 7C). Like the distal ovotestis duct, the basal ovotestis duct lacks spermatozoa (Figures 7A, C). The comparative characteristics of different regions of the hermaphrodite duct are documented in Table 1.

The fertilisation pouch-spermatheca complex

The fertilisation pouch-spermatheca complex (fpsc) is a small, bean-shaped, bulbous structure which is where the duct of the albumen gland, hermaphrodite duct and the spermatheca open s (Figure 2A). The fpsc complex is 3.17 ± 0.44 mm in length and 1.05 ± 0.25 mm in diameter. The proximal end of the fpsc complex joins with the distal end of the spermoviduct (Figures 1B, 2A, B). Comparisons of the fpsc complex of *M. indica* with other *Macrochlamys* species are documented in Table 2. The spermatheca is a very small diverticulum of the basal ovotestis duct at its proximal end and is fully embedded in the albumen gland and not visible externally.

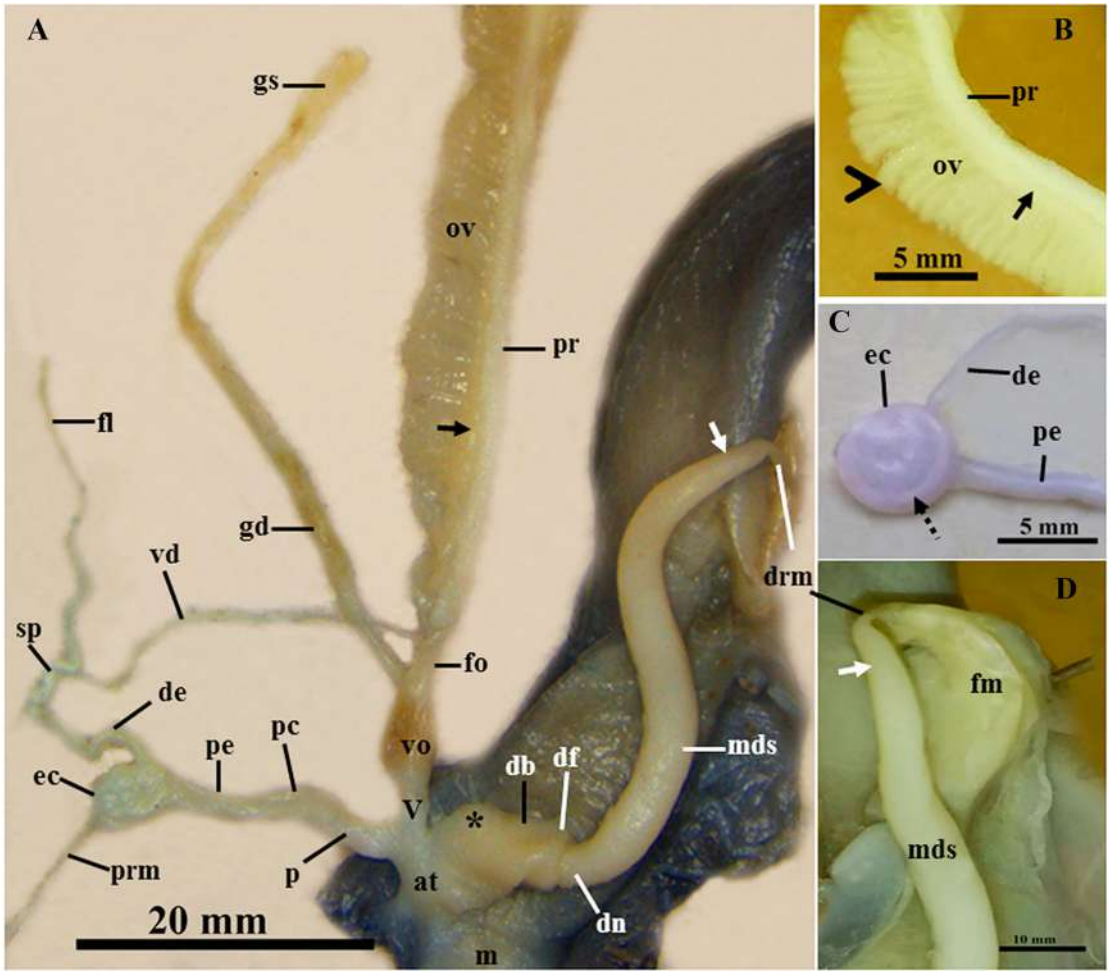

Figure 3. Proximal portion of the reproductive system of the *Macrochlamys indica*. **A**, Positions of genitalia with the association of spermoviduct, gametolytic organ (gametolytic duct and gametolytic sac together), vaginoviducal capsule and dart apparatus; **B**, Enlarged view of a part of spermoviduct. Note the arrangement of lateral lamellae (arrowhead) on the compressed basal part (black arrows) of the oviduct adjacent to the prostate gland; **C**, Magnified view of the epiphallic caecum with crescent shaped coiling (dashed arrow); **D**, Enlarged view of distal part of the dart apparatus showing its connection with body muscle. Abbreviations: at = genital atrium; db = dart bag; de = distal epiphallus; df = dart flange; dn = dart neck; drm = dart retractor muscle; ec = epiphallic caecum; fl = flagellum; fm = foot muscle; fo = free oviduct; gd = gametolytic duct;; gs = gametolytic sac; m = mouth; mds = median dart shaft; ov = oviduct; p = penis; pc = penial caecum; pe = proximal epiphallus; pr = prostate gland; prm = penial retractor muscle; sp = spermatophore; V = vagina; vd = vas deferens; vo = vaginoviducal capsule. White arrows = brownish distal part of dart shaft. Asterisk indicates tissue casting.

The albumen gland

The albumen gland is a creamy white or yellowish, smooth-surfaced, moderately elongated (10.27 ± 0.37 mm in length) tongue-shaped structure (Figures 1A, B, 2A–C) which lies near the concave surface of the digestive gland. The size of the gland increases with body size. The diameter at the free distal end (1.07 ± 0.07 mm) of the albumen gland is comparatively smaller than that of the proximal end (3.50 ± 0.21 mm). A small duct from the proximal end of the albumen gland opens into the fertilisation pouch-spermatheca complex (fpsc). The albumen gland consists of numerous small tubules (0.78 ± 0.03 µm in diameter), each of which is surrounded by a thin, smooth, single cell layer (tl in Figure 8). The lumens of the tubules contain a homogeneous matrix of secretory materials (asterisk in Figure 8). Several (approximately 15–20) albumen tubules are very closely packed and form a large lobular structure (bold arrow in Figure 8) which is encircled by an irregular layer of nucleated epithelial cells (arrowheads in Figure 8). The lobules of the albumen gland have no definite shape or size. The junctions of the adjacent lobules of the albumen gland possess a prominent blood vessel (bv in Figure 8).

The spermoviduct

The spermoviduct is an elongated structure, comprised of a tubular prostate gland and a highly folded oviducal channel (Figures 1A, B, 2A–C, 3A). It is about 20.42 ± 0.28 mm in length and 3.01 ± 0.18 mm in width. The oviducal channel (2.35 ± 0.19 mm in width) is almost equal in length to the spermoviduct (20.42 ± 0.28 mm) while the prostate gland (0.65 ± 0.16 mm in

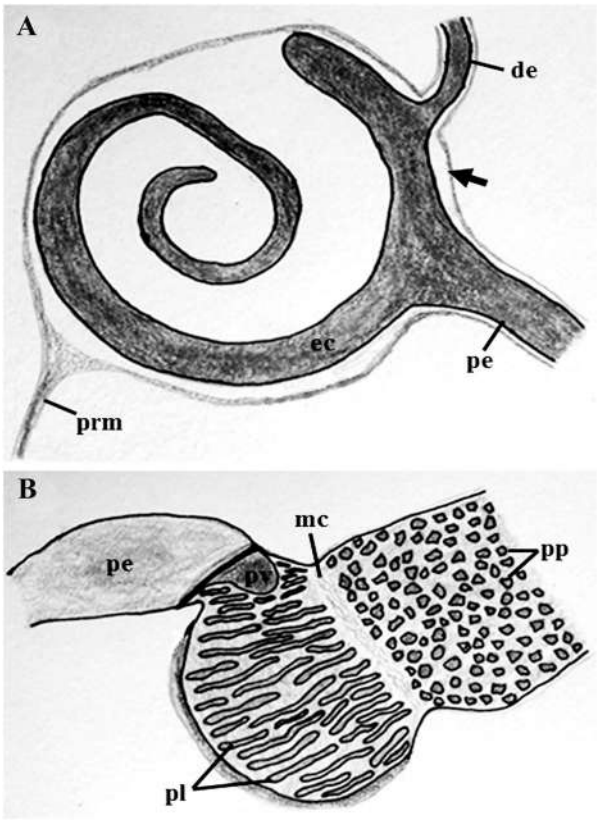

Figure 4. Schematic diagram of the epiphallic caecum and the inner penial wall of *Macrochlamys indica*. **A**, Epiphallic caecum and associated structures; **B**, Internal structure of penis with penial verge. Abbreviations: de = distal epiphallus; dp = penial pilasters; ec = epiphallic caecum; mc = median constriction of penis; pe = proximal epiphallus; pl = penial pilasters; pp = penial pustules; prm = penial retractor muscle; pv = penial verge. Bold arrow = outer membranous sheath.

diameter) is slightly shorter (20.08 ± 0.31 mm). The oviducal channel comprises a stack of small plate-like, numerous lamellae (arrowhead in Figure 3B) which are laterally arranged like the pages of a folded book against the prostate gland (Figures 1A, B, 3A, B). These lateral lamellae individually open into a dorsoventrally compressed basal part (0.27 ± 0.07 mm in width) of the oviducal channel (black arrow in Figure 3 A, B). Each lamella is free and slightly bulbous at the distal end. In the middle region of the oviducal channel, the size of the lamellae (2.09 ± 0.14 mm in length, 0.15 ± 0.04 mm in width) is slightly greater than those in its proximal and distal ends. The male and female gametes are transported in separate channels from the proximal end of the fertilisation pouch-spermatheca complex (fpsc) along the spermoviduct (Figures 1B, 2A, B). The spermoviduct proximally divides into a slim, tubular vas deferens and a short, thick free oviduct (Figures 2B, 3A, 5A).

The free oviduct and the vagina

The free oviduct is a short, tubular structure (0.81 ± 0.02 mm in length, 0.69 ± 0.04 mm in diameter) that is extended proximally as a thick, muscular vagina lying between the tubular complex of the penial sheath and the dart apparatus (Figures 1B, 3A, 5A). The vagina (approximately 0.88 × 0.72 mm in size) opens anteriorly into the genital atrium and hence the genital pore. The interspecific characters of the free oviduct and the vagina in different *Macrochlamys* species are documented in Tables 2 and 4. A brownish, muscular capsule developed at the junction of the free oviduct, the gametolytic organ and the vagina is here named the vaginoviducal capsule (Figures 1B, 3A, 5A). It is about 2.67 (± 0.22) × 1.36 (± 0.11) mm in size. The distal end of the vaginoviducal capsule opens to the free oviduct and the duct of the gametolytic organ and proximally continues as the vagina (Figures 1B, 3A, 5A, C). The differences in the vaginoviducal capsules, along with the reproductive system of different *Macrochlamys* species, are documented in Table 4.

The gametolytic organ

The gametolytic organ (or bursa copulatrix) is an almost straight, elongated, tubular structure comprised of a proximal gametolytic duct and a distal cylindrical, blind gametolytic sac. The gametolytic organ arises below the brownish vaginoviducal capsule (Figures 1B, 3A, 5A, 5C). In the virgin snails, the gametolytic sac is a cylindrical, much more elongated (13.50 ± 3.61 mm in length), blunt tube, usually filled with a creamy white or dirty white coloured secretion and lacking spermatophores (Figures 1A, 3A). This gametolytic sac is just slightly thicker (0.56 ± 0.08 mm in diameter) than its proximal gametolytic duct (0.35 mm in diameter). In some individuals the gametolytic organ is a club-shaped structure including a typical distal spheroidal sac (9.89 × 2.43 mm in size), filled with pinkish secretion and an elongated whitish gametolytic duct (Figure 9A). The membranous wall of the gametolytic sac in inseminated snails is much more transparent than that of virgin snails so that the spermatophores within the pinkish secretion are slightly visible through the membrane (asterisks in Figure 9A). Two to seven spermatophores are found in the pinkish gametolytic sac of the inseminated snails and contain many sperm (including exogenous sperm or allosperm). The gametolytic ducts in inseminated snails (approximately 7.13 × 0.55 mm in size) are comparatively thicker and more elongated than those in virgin snails, which are approximately 2.31 × 0.35 mm in size (Figures 1A, 3A, 9A). The comparative characteristics of different parts of the gametolytic organ among the *Macrochlamys* species are documented in Table 3.

The vas deferens and flagellum

The vas deferens is a long, narrow (0.15 ± 0.04 mm in diameter), whitish, slightly coiled, tubular structure

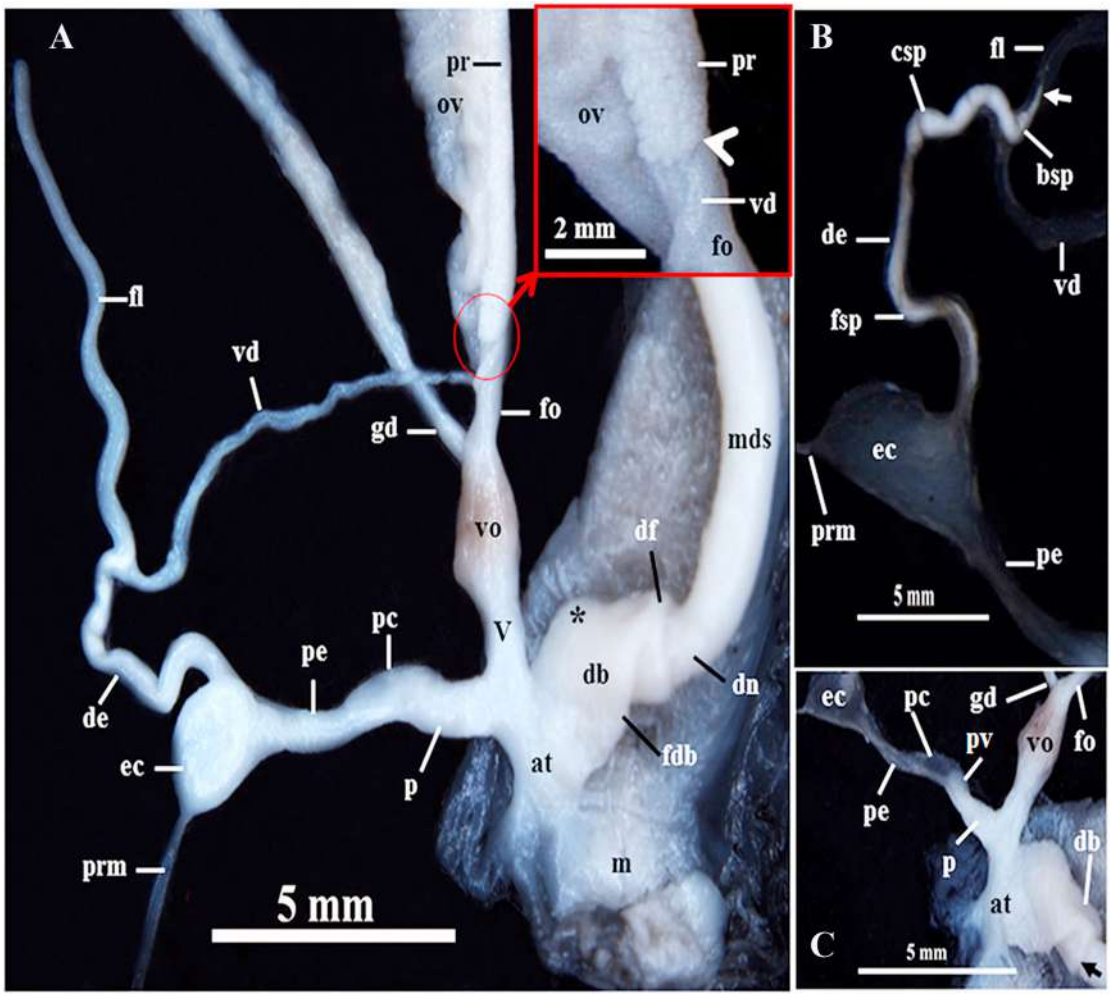

Figure 5. Enlarged view of the proximal portion of the reproductive system of *Macrochlamys indica*. **A**, The connection of various genital ducts and dart apparatus at proximal region. Proximal end (red circular mark) of the spermoviduct is more enlarged in the inset showing the origin (white arrowhead) of the vas deferens at the junction of the free oviduct and prostate gland. Note the membranous folds of the dart bag (fdb) and tissue casting (asterisk); **B**, Casting of spermatophore in the lumen of distal epiphallus is visible through membranous sheath. Note the pointed peg-like structure (white arrow) is developed from the end of the conical base of spermatophore; **C**, Genital atrium with associated structures. Abbreviations: at = genital atrium; bsp = base of spermatophore; csp = median cylindrical sac of spermatophore; db = dart bag; de = distal epiphallus; df = dart flange; dn = dart neck; ec = epiphallic caecum; fdb = fold of dart bag; fl = flagellum; fo = free oviduct; fsp = anterior filament of spermatophore; gd = gametolytic duct; m = mouth; mds = median dart shaft; ov = oviduct; p = penis; pc = penial caecum; pr = prostate gland; pe = proximal epiphallus; prm = penial retractor muscle; pv = penial verge; V = vagina; vd = vas deferens; vo = vaginoviducal capsule. Asterisk = tissue casting. Black arrow = dart flange (neck) of the dart apparatus.

(9.37 ± 0.35 mm in length). It arises from the proximal part of the prostate gland, near the connection (white arrowhead in inset of Figure 5A) with the distally free oviduct and connects horizontally to the distal end of the epiphallus (Figures 1A, B, 3A, 5A). The vas deferens opens near the distal end of the epiphallus (Figures 1B, 3A, 5A). The vas deferens is slightly thicker near the epiphallus-flagellum junction (Figure 5A) and does not insert terminally into the epiphallus. A long, slender flagellum is also developed (Figures 3A, 5A, B). The autosperm are transported to the epiphallus through the vas deferens. The flagellum is 7.41 ± 2.14 mm in length and 0.32 ± 0.09 mm in diameter. The distal free end of the flagellum is developed as a blind tube and is slightly less in diameter than that of the proximal end (Figures 1A, 5A). The inner wall of the flagellum is almost smooth and the lumen lacks any filamentous structure. The comparative flagellar features in the different *Macrochlamys* species are documented in Table 4.

The genital atrium

The genital atrium is wider (0.94 ± 0.02 mm) than the vagina and is a slightly elongated (1.66 ± 0.06 mm) muscular structure. The lumen of the genital atrium provides a common terminal passage of the male and female conduits and the dart bag of the dart apparatus (Figures 1A, B, 3A, 5A, C, Table 2).

The epiphallus

The epiphallus is a slender tubular structure, about five to six times longer than the penis. The epiphallus has

Table 1. Comparative characteristics of three regions of the hermaphrodite duct in *Macrochlamys indica*. The measurement is mean value ± SD. $N = 5$.

	Characters	First region (Ovotestis duct)	Second region (Seminal vesicle)	Third region (Basal ovotestis duct)
1.	Location	Very close to ovotestis	Middle part of the duct	Very close to albumen gland
2.	Visibility	Visible externally	Visible externally	Partially embedded by albumen gland
3.	Folding of duct	Slightly folded	Highly folded	Mostly linear
4.	Length	More elongated (16.34 ± 1.40 mm)	Moderately elongated (9.76 ± 0.53 mm)	Short (1.40 ± 0.05 mm)
5.	Duct diameter (after cross section)	Lower (3.18 ± 0.27 µm)	Higher (5.88 ± 0.30 µm)	Moderate (4.32 ± 0.07 µm)
6.	Lumen diameter of the duct	Smaller (1.23 ± 0.68 µm)	Larger (3.73 ± 0.20 µm)	Medium (2.32 ± 0.69 µm)
7.	Endothelial folds around lumen	Highly folded (approx. 3–4 in number)	No folds are found along the endothelial layer	Highly folded (approx. 4–5 in numbers)
8.	Cilia of endothelial cells	Medium (0.18 ± 0.03 µm in height)	Larger (0.26 ± 0.04 µm in thickness)	Lower (0.16 ± 0.01 µm in thickness)
9.	Cell size in endothelial layer	Medium; 0.90 (± 0.11) × 0.25 (± 0.05) µm	Larger; 1.05 (± 0.19) × 0.22 (± 0.08) µm	Smaller; 0.40 (± 0.08) × 0.09 (± 0.04) µm
10.	Sperm load	Nil	Sperm fills the lumen of seminal vesicle	Nil

three distinct regions; the proximal epiphallic tube, epiphallic caecum, and distal epiphallic tube (Figures 1A, B, 3A, 4A, 5A).

i The proximal epiphallic tube (3.26 × 0.51 mm in size) is the immediate continuation of the penis and opens into the epiphallic caecum. It extends from the distal end of the penial verge to the epiphallic caecum. (Figures 1A, 3A, 4A).

ii The epiphallic caecum is a membrane-bound, small, spherical to circular sac about 1.87 × 1.28 mm in size (Figures 1A, 3A, 4A, 5A–C). It is located at one third the length of the epiphallus and coiled (crescent-like) for about two complete circles (Figures 3C, 4A). A penial retractor muscle is developed from the lateral side of the epiphallic caecum and is attached to the body muscle near the columella; it assists then extrusion and retraction of the penis (Figures 1A, 3A, 4A, 5A).

iii The distal epiphallic tube is extended from the proximal epiphallic caecum to a distally located junction of the vas deferens and flagellum. It is much more elongated (5.26 ± 0.36 mm) than the proximal epiphallic tube (3.26 ± 0.51 mm). Its lumen is responsible for moulding the

Table 2. Relative characteristics of the ovotestis and other proximally and distally located major reproductive structures of *M. indica* and some other *Macrochlamys* species based on published figures and descriptions. (* = species described by Pholyotha et al. 2018; # = species described by Deshmukh et al. 2012; † = species described by Sajan et al. 2019; ? = not adequately described).

	Features	*Macrochlamys* species							
		M. indica	*M. aurantia**	*M. coleus**	*M. caverna**	*M. lemma**	*M. tanymentula**	*M. kelantanensis**	*M. petrosa#*	*M. laggeae†*
1.	Ovotestis	7–10 lobes, embedded in digestive gland	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	5–8 lobes, embedded in digestive gland	Unknown
2.	Spermatheca	Small	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Small	Unknown
3.	Fertilization pouch-spermatheca complex (fpsc)	Large, bean shaped, bulbous sac	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Bulbous sac	Unknown
4.	Free oviduct	Short, thick tube	Long slender tube	Short thick tube	Long thick tube	Long thick tube	Long slender tube	Very short, triangular, entirely enclosed by vaginoviductal tissue	Long thick tube/?	Long slender tube
5.	Genital atrium	Wider, elongated muscular	Short	Short	Short	Large, slightly elongated	Very short	Very short	Short	Short
6.	Tissue casting	Well developed	Absent	Present	Absent	Present	Absent	Absent	Unknown	Unknown

Table 3. Comparative features of the gametolytic organ and spermatophore of *M. indica* and some other species of *Macrochlamys* based on figures and descriptions by the authors. (* = species described by Pholyotha et al. 2018; # = species described by Deshmukh et al. 2012; † = species described by Sajan et al. 2019).

		Macrochlamys species								
	Features	*M. indica*	*M. aurantia**	*M. coleus**	*M. caverna**	*M. lemma**	*M. tanymentula**	*M. kelantanensis**	*M. petrosa#*	*M. laggeae†*
1.	Gametolytic organ	Unbent, prolonged, cylindrical or club shaped	Slightly bent, prolonged, club shaped	Slightly bent, prolonged, bulbous	Unbent, prolonged, bulbous	Unbent, short, club shaped	Slightly bent, prolonged, bulbous	Slightly bent, prolonged, club shaped	Unbent, prolonged, club shaped	Unbent, prolonged, bulbous
2.	Origin of gametolytic duct	From the junction of distal end of vaginoviductal capsule and proximal end of free oviduct	From the thick proximal end of free oviduct	From the junction of distal end of vagina and proximal end of free oviduct	From the junction of distal end of vagina and proximal end of free oviduct	From the junction of distal end of vagina and proximal end of free oviduct	From the distal end of vagina	From the distal end of vagina	From the distal end of vagina	From the distal end of vagina
3.	Gametolytic sac	White slender tube (in virgin snails), pinkish bulbous (in inseminated snails)	Prolonged, bulbous	Elongated, bulbous	Prolonged, bulbous	Short, bulbous	Long, bulbous	Prolonged, bulbous	Bulbous reddish brown colour	Prolonged, bulbous
4.	Spermatophore	Whip-like, translucent with cylindrical median capsule	Needle-shaped, translucent with cylindrical median capsule	Needle-shaped, translucent with cylindrical median capsule	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
5.	Anterior filament of spermatophore	Very long, fully spinous with a slim central canal	Very long, spines only present at proximal and distal ends	Very long, only both ends and few portions of middle region contain spines	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown

Table 4. Comparative characteristics of the flagellum, epiphallus, penis and the vagina of *M. indica* and some other species of *Macrochlamys* based on published figures and descriptions by the authors. (* = species described by Pholyotha et al. 2018; # = species described by Deshmukh et al. 2012; † = species described by Sajan et al. 2019; ? = not adequately described).

		Macrochlamys species								
	Features	*M. indica*	*M. aurantia**	*M. coleus**	*M. caverna**	*M. lemma**	*M. tanymentula**	*M. kelantanensis**	*M. petrosa#*	*M. laggeae†*
1.	Flagellum	Long, slender, about 2/3 of epiphallic length, distally ended as a blind tube	Long, irregular, coiled about 1/2 of the epiphallic length, distally bulbous	Short, cylindrical, about 1/2 epiphallic length, distally globular	Short, cylindrical about 1/3 epiphallic length, distally cylindrical	Long, bulbous, slightly folded about 1/2 epiphallic length, distally rounded	Very long, slender about 1/2 epiphallic length, distally ended as a blind tube	Very long, cylindrical, about same as epiphallic length, distally cylindrical	Slender/?	Elongated, distally ended as a blind tube
2.	Epiphallus	Very long, five times length of penis	Short, twice length of penis	Short, twice length of penis	Short, twice length of penis	Long, two & half times length of penis	Very long, about five times length of penis	Very long, about four times length of penis	Unknown	Long, slender tube
3.	Epiphallic caecum	Two circles	One circle	One circle	One circle	One circle	One circle	Two circles	Unknown	One circle
4.	Penial caecum	Prolonged	Slightly prolonged	Slightly prolonged	Short, swollen	Short, swollen	Ill developed	Ill developed	Unknown	Unknown
5.	Penis	Unbent, prolonged with a median constriction, distally round	Slightly bent, prolonged cylindrical, distally cylindrical	Slightly bent with a median constriction, distally round	Slightly curved distally, prolonged tubular, distally cylindrical	Slightly curved distally, prolonged cylindrical, distally cylindrical	Unbent, short, enlarged cylindrical, distally round	Unbent, short, cylindrical, distally round	Unbent, short, tubular, distally swollen	Unbent, short, cylindrical
6.	Penial verge	Short, thick, cylindrical shaped	Small, thick	Very small, short	Short, triangular shaped	Small, thick	Slightly small, cylindrical shaped	Small, cylindrical shaped	Sack like/?	Cylindrical/?
7.	Inner wall of penis (proximal/distal)	Small, irregular pustules/ longitudinal, thin pilasters	Both with very small, oblique, wrinkled pilasters	Small, irregular, thin longitudinal wrinkled folds	Oblique, trapezoid /long, narrow pilasters	Oblique, trapezoid/ long, transverse folds	Both with small, wrinkled, longitudinal pilasters	Both with very small, irregular pilasters	Unknown	Unknown
8.	Vagina	Short, tubular	Long, tubular	Slightly long, tubular	Long, tubular	Slightly long, tubular	Short, tubular	Very short, globular	Short, globular	Short, cylindrical
9.	Vaginoviductal capsule	Brownish, well developed capsule, at the junction of free oviduct & vagina	Brownish globular tissue encircles the proximal end of free oviduct	Brownish globular tissue at the very near of proximal end of free oviduct	Brownish globular tissue at 1/4 position on free oviduct from proximal end	Brownish globular tissue at the middle position on free oviduct	Brownish, well developed capsule, near the proximal end of free oviduct	Whitish tissue, encircles the entire free oviduct	Absent	Unknown

Table 5. Relative features of the dart apparatus of *M. indica* and some other species of *Macrochlamys* based on published figures and descriptions by the authors. (* = described by Pholyotha et al. 2018; # = described by Deshmukh et al. 2012; † = described by Sajan et al. 2019; ? = not properly described).

		Macrochlamys species								
	Features	*M. indica*	*M. aurantia**	*M. coleus**	*M. caverna**	*M. lemma**	*M. tanymentula**	*M. kelantanensis**	*M. petrosa#*	*M. laggeae†*
1.	Dart apparatus	Well developed, elongated S-shaped, open at the proximal end of vagina	Well developed, slender, open at the proximal end of vagina	Well developed, cylindrical, open at the proximal end of vagina	Well developed, cylindrical, open at the proximal end of vagina	Well developed, slender, open near the proximal end of vagina	Well developed, cylindrical, open at the proximal end of vagina	Well developed, cylindrical, open at the proximal end of vagina	Unknown	Well developed, cylindrical, open at the proximal end of vagina
2.	Dart retractor muscle	Short, thick tubular muscle thread	Elongated, thin muscular thread/?	Elongated, thin muscular thread/?	Short, muscular thread/?	Elongated, thin muscular thread/?	Elongated, thin muscular thread/?	Elongated, thick muscular thread/?	Unknown	Present/?
3.	Dart bag	Short, bound with muscular membrane & encircles only love dart	Prolonged/?	Short/?	Prolonged/?	Prolonged/?	Very long/?	Long/?	Unknown	Cylindrical/?
4.	Dart	Nondisposable, chitinous, pointed tubular tip	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
5.	Location of dart flange	Short distance from the proximal end of the dart bag	Very long distance from the proximal end of the dart bag/?	Short distance from the proximal end of the dart bag/?	Very long distance from the proximal end of the dart bag/?	Long distance from the proximal end of the dart bag/?	Very long distance from the proximal end of the dart bag/?	Long distance from the proximal end of the dart bag/?	Unknown	Unknown

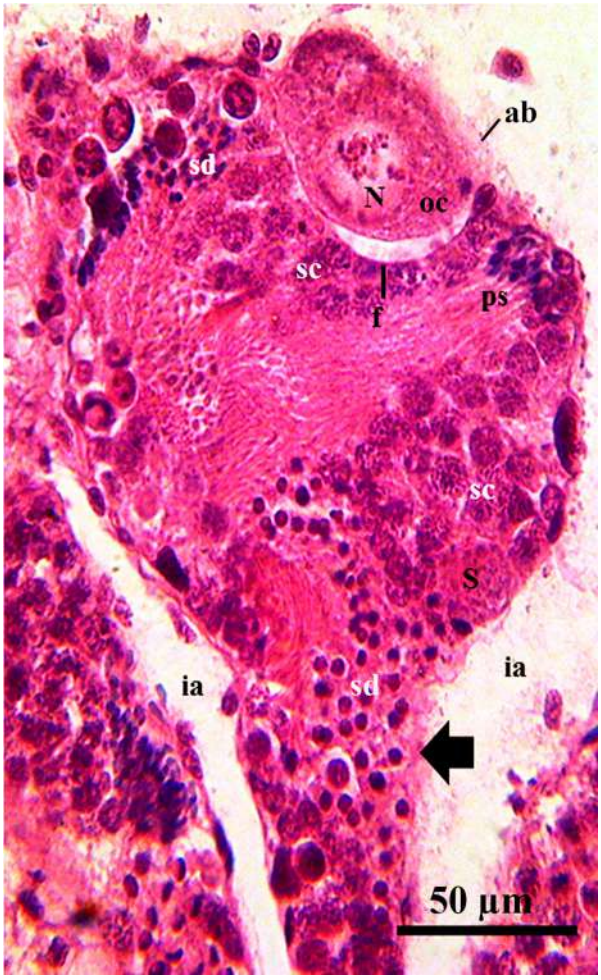

Figure 6. Photomicrograph of the section of an acinus of the ovotestis of *Macrochlamys indica*. The spermatogenic cells are developed and distributed throughout the acinar space and the oocytes are restricted only in the peripheral acinar area. Abbreviations: ab = acinar boundary; f = follicular layer; ia = interacinar space; N = nucleus; oc = oocyte; S = Sertoli cell; sc = spermatocyte; sd = spermatid; ps = sperm. Bold arrow = an acinus.

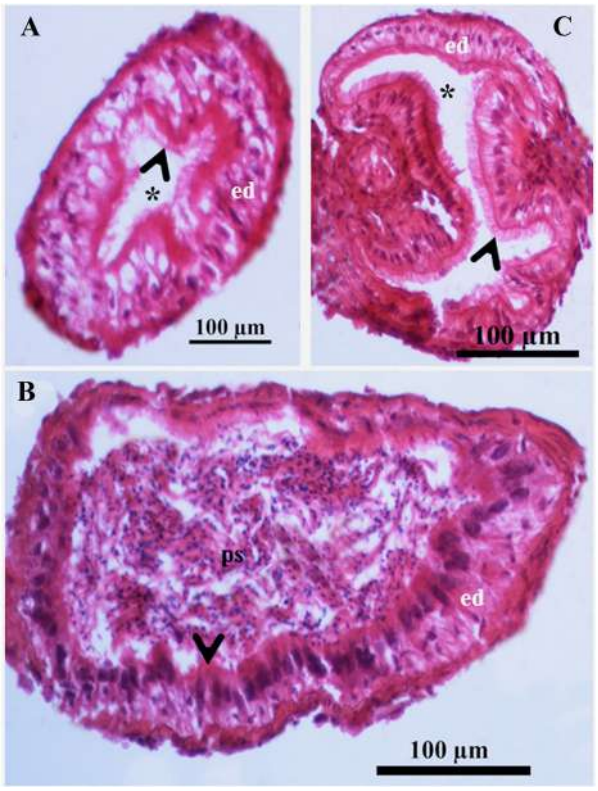

Figure 7. Photomicrograph of a section of the hermaphrodite duct of *Macrochlamys indica*. **A**, Section of ovotestis duct (first region of the hermaphrodite duct); **B**, Section of seminal vesicle (second region of the hermaphrodite duct); **C**, Section of basal ovotestis duct (third region of the hermaphrodite duct). Abbreviations: ed = nucleated endothelial layer; ps = sperm. Asterisk = lumen of the first and third region of the hermaphrodite duct, showing lack of sperm. Arrowhead = cilia of columnar epithelial cells in hermaphrodite duct.

spermatophores. The spermatophore is often just visible through the translucent membranous sheath of the distal epiphallic tube (Figures 3A, 4A, 5B).

The morpho-anatomical characteristics of the epiphallus of *M. indica* are compared to those of the other *Macrochlamys* species in Table 4.

The spermatophore

The spermatophore is a whip-like translucent structure (24.48 ± 0.60 µm in length), surrounded by a semitransparent membranous sheath and is developed in the lumen of the distal epiphallic tube and the proximal part of the flagellum (Figures 3A, 5B). The spermatophore comprises an anterior filament, a cylindrical medial body, and a conical base (Figure 9A, B). The anterior filament (5.1 × 0.23 µm in size) is approximately 1/5 of the length of the whole spermatophore and possesses small spines. This anterior filament is located in the lumen of the distal epiphallic tube and nearby epiphallic caecum (fsp in Figure 5B). The medial cylindrical body (16.09 × 1.29 µm in size) is packed with spermatozoa (i.e. autosperm) and is located distally in the epiphallic tube, near the junction of the epiphallus and the vas deferens (Figure 5B). The posterior conical base is very short (0.30 × 0.40 µm in size), usually located in the lumen of a small stretch of the flagellar proximal tube (Figure 5B). A smooth, slightly curved, peg-like structure (2.67 ± 0.2 µm in length) is developed outwardly from the conical base of some spermatophores (arrowhead in Figure 9C). The proximal portion of this peg-like structure is relatively wider (0.64 ± 0.15 µm in diameter) than that of the distal end (0.19 ± 0.02 µm in diameter). The spermatophores are transferred into the gametolytic sac or bursa copulatrix of the female (i.e. sperm-recipient mating partner) after copulation. The spermatophores remain coiled in the pinkish secretion of the gametolytic sac (asterisks in Figure 9A). The comparative characteristics of spermatophores among the *Macrochlamys* species are documented in Table 3.

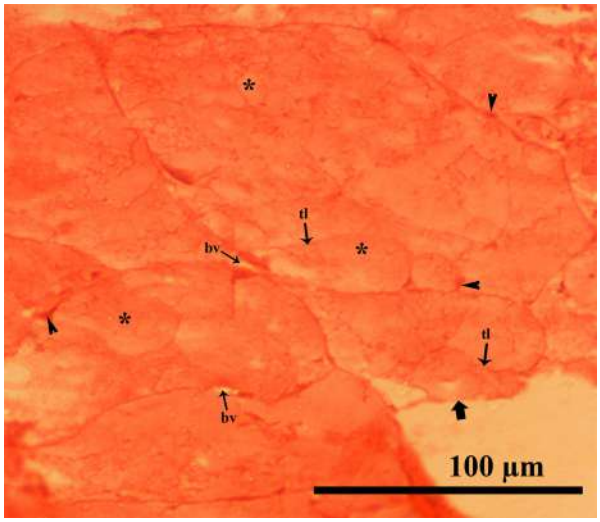

Figure 8. Photomicrograph of a section of the albumen gland of *Macrochlamys indica* showing numerous tubules with their secretions in the lumen (asterisk). Abbreviations: bv = blood vessel; tl = tubular layer. Bold arrow = lobule; arrowhead = nucleated cell in the outer layer of the lobule.

The penis

The penis is an elongated, almost straight, muscular structure and is surrounded by a semi-transparent penial sheath. The penis is 1.74 ± 0.05 mm in length with a median constriction (0.36 ± 0.03 mm in diameter) and distally rounded in the region that houses the penial verge (pv, in Figure 5C). The diameter at the proximal part (0.62 ± 0.04 mm in diameter) of the penis is slightly greater than that of the rounded distal end (0.58 ± 0.03 mm in diameter). The penial caecum is slightly elongated (Figures 2A, 3A, 4A). The inner wall of the penis possesses numerous, small, irregular pustules proximally (pp, in Figure 4B) and distally near the penial caecum has longitudinal, thin pilasters around the penial verge (pl, in Figure 4B) (Table 4). The interior sculpture at the median constricted region of the penis comprises some small stretches of very thin, circularly arranged and interlaced, wrinkled folds (mc in Figure 4B). The penis extrudes into the bursa copulatrix of the opposite mating partner and deposits the autospermatozoa encased in spermatophores. The penial retractor muscle (4.16 ± 0.38 mm in length) is a well-developed, thick muscle, which is involved in ejection and retraction of the penis (Figures 1A, B, 3A, 4A, 5A and Table 4). Histologically, the penis is comprised of layers (2.93 ± 0.97 µm in thickness) of circular muscles which encircle a central lumen and a blood vessel (Figure 9D). The central lumen is spheroidal, approximately 2.24 ×1.05 µm in size and enclosed by a non-ciliated, non-glandular endothelial layer (0.24 ± 0.06 µm in thickness) (Figure 9D). The blood vessel is thin (0.53 ± 0.14 µm in diameter) and found below the central lumen in the penis (Figure 9D).

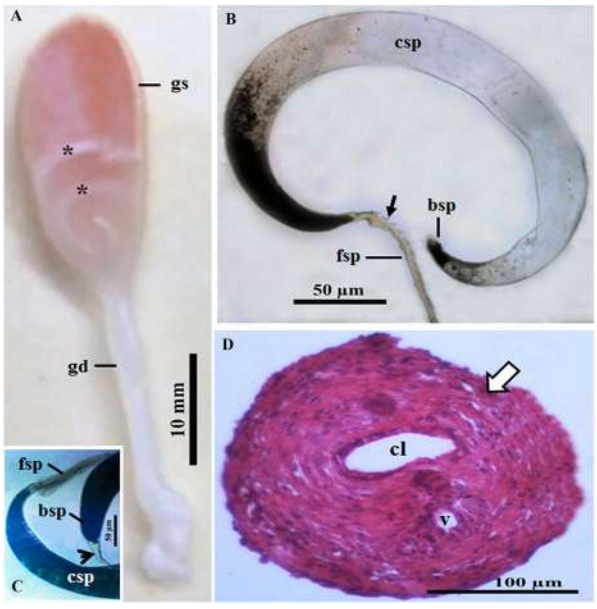

Figure 9. Photomicrograph of the gametolytic organ, spermatophores of an inseminated individual, and section of the penis of *Macrochlamys indica*. **A**, Club shaped, pinkish gametolytic organ showing slightly visible spermatophores (asterisk) in the gametolytic sac; **B**, Whip-like spermatophore; **C**, Peg-like development (arrowhead) from the base of the spermatophore; **D**, Section of the penis. Abbreviations: bsp = base of spermatophore; cl = central lumen; csp = median cylindrical sac of spermatophore; fsp = anterior filament of the spermatophore; gd = gametolytic duct; gs = gametolytic sac; v = blood vessel. Arrow = spine on the anterior filament; bold arrow = circular muscle layer.

The dart apparatus

The dart apparatus is a whitish, smooth, elongate, S-shaped tubular structure and opens into the genital atrium at the proximal end of the vagina (Figures 1A, B, 3A, D, 5A). A thick, globular tissue casting is developed near the proximal end of the dart apparatus (asterisk in Figure 3A) and differs among species of *Macrochlamys* (Table 2). The dart apparatus is comprised of four distinguishable regions: dart bag, dart neck, median dart shaft, and dart retractor muscle.

i The dart bag is a short, proximal sac-like, slightly folded structure (3.26 ± 0.56 mm in length and 1.83 ± 0.32 mm in diameter) and is comprised of a non-disposable (reusable), pointed, muscular dart (2.48 ± 0.41 mm in length) (Figures 3A, 5A). The dart represents about one eighth of the total dart apparatus (16.21 ± 1.61 mm in length including the dart retractor muscle).

ii The dart neck is a thin, short muscular tube (0.74 ± 0.05 mm in length) between the dart bag and the median dart shaft (Figures 1B, 3A, 5A). It is comparatively thinner (0.59 ± 0.02 mm in diameter) than the dart bag and the dart median shaft. The dart neck is separated from the dart bag by a distinct dart flange (1.64 ± 0.21 mm in

diameter) and continues with the median shaft (Figures 3A, 5A).

iii The medial dart shaft is the cylindrical main body (10.35 ± 0.10 mm in length and 1.24 ± 0.20 mm in diameter) of the dart apparatus and is surrounded by a thin membranous sheath. The medial dart shaft narrows gradually toward the distal end. The distal part of dart shaft is slightly brownish and tapering (Figures 1A, 3A, D). This brownish distal part of the dart shaft is 1.71 ± 0.26 mm in length and 0.70 ± 0.14 mm in diameter.

iv The dart retractor muscle is small (0.77 ± 0.44 mm in length and 0.03 ± 0. 02 mm in diameter when retracted) and tubular. It connects the tapering distal end of the dart shaft to the foot muscle, and helps in extrusion and retraction of the love dart during mating (Figures 1A, 3A, D).

Histologically, the dart shaft possesses a solid, muscular central core (cr, in Figure 10), surrounded by modified cell layers of epithelial cells. The central core (1.06 ± 0.11 µm in diameter) of the dart shaft is encircled by a thin epithelial layer (0.18 ± 0.03 µm in width) comprised of a single layer of cells which is surrounded by a circular, smooth muscle layer (1.78 ± 0.18 µm in width). Several thin fibrous connections (0.39 ± 0.03 µm in length and 0.04 - 0.05 µm in thickness) radiate from the central core to the epithelial layer (Figure 10). A layer (1.21 ± 0.28 µm in width) of several nucleated epithelial cells (ml, in Figure 10) is developed in the middle of an inward smooth muscle layer (smu, in Figure 9) and an outward membranous sheath (0.15 ± 0.02 µm in width) (Figure 10). The

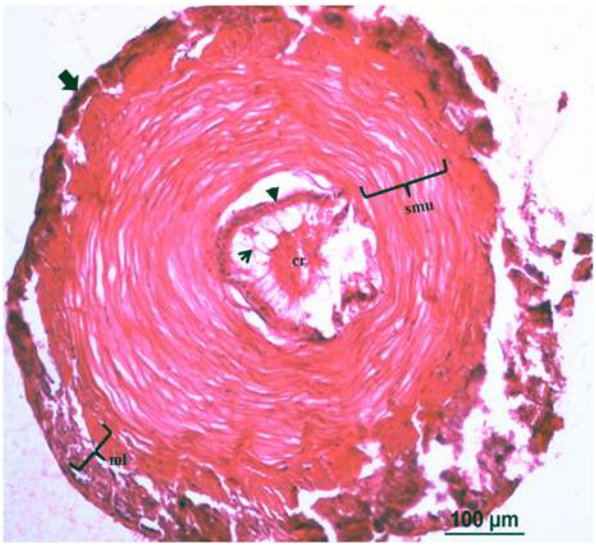

Figure 10. Photomicrograph of a section through the dart shaft of the dart apparatus of *Macrochlamys indica*. It includes layers of modified epithelial cells. Abbreviations: cr = central core; ml = medial layer with numerous epithelial cells; smu = smooth muscle layer. Arrowhead = single cell epithelial layer; thin arrow = thin fibrous connection; bold arrow = outer membranous sheath.

characteristics of the dart apparatus in various *Macrochlamys* species are compared in Table 5.

Discussion

Stylommatophoran gastropods have morphologically and functionally complex reproductive systems, exhibiting a variety of reproductive strategies and complex mating behaviour (Duncan 1975; Tompa 1984; Gómez 2001; Jordaens et al. 2009). The comprehensive morphological diversity of the reproductive system enables the distinction between species and genera and its morphofunctional complexity reflects mating strategies as well as life history (Leonard 1991; Jordaens et al. 2007, 2009; Hyman & Ponder 2010, 2016; Pholyotha et al. 2018).

The present study describes morpho-anatomical as well as morphometrical characteristics of the reproductive system with the spermatophore and dart apparatus of *M. indica* and compares these characteristics with other species of *Macrochlamys*. In *Macrochlamys*, the morphological features of the penial structures (e.g. penis, penial caecum, penial verge, and interior sculpture of penis), spermatophore, epiphallus, epiphallic caecum, flagellum, and gametolytic organ are the most useful for species-level taxonomy (Blanford and Godwin-Austen 1908; Pholyotha et al. 2018). Furthermore, the characteristics of the dart apparatus are useful for both the genus-level and species-level taxonomy (Blanford and Godwin-Austen 1908; Tompa 1984; Gómez et al. 1996; Schileyko 2003; Deshmukh et al. 2012; Pholyotha et al. 2018). The penis in *M. indica* includes a distinct median constriction which is present but slight in *Macrochlamys coleus* (Pholyotha et al. 2018) and absent in other *Macrochlamys* species (Deshmukh et al. 2012; Pholyotha et al. 2018; Sajan et al. 2019). There are some characteristic variations of the interior sculpture in the penis among *Macrochlamys* (Pholyotha et al. 2018) (Table 4). The inner wall at the median constriction of the penis of *M. indica* contains thin, horizontal, wrinkled folds which are unknown in other *Macrochlamys*. However, the sculpture of the inner penial wall is species-specific and is the most powerful characteristic at species-level, particularly for those land snails that lack the dart apparatus or hard spermatophore (Tompa 1984; Barker 2001).

The ovotestis of *M. indica* is multilobate (Roy et al. 2016) resembling that of other stylommatophoran snails (Tompa 1984; Stewart et al. 2016). In most *Macrochlamys*, the morphoanatomical features of the ovotestis are unknown (Pholyotha et al. 2018; Sajan et al. 2019), while the ovotestis of *Macrochlamys petrosa* (Hutton, 1834) contains five to eight lobes (Deshmukh et al. 2012). Probably, the number of ovotestis lobes may be species-specific and vary among *Macrochlamys* taxa (Deshmukh et al. 2012). The hermaphrodite duct

of *M. indica* has three morphofunctionally discriminated regions (Table 1) without any strong demarcations and thus resembles most other pulmonates (Ghose 1962; Tompa 1984; Barker 1999). In *M. indica*, both the proximal and distal regions of the hermaphrodite duct comprise several well-developed endothelial folds with a smaller lumen whereas the middle region (seminal vesicle) possesses an almost smooth endothelial layer probably due to the larger lumen for storing autospermatozoa. The ciliary movements of the endothelial layer of the hermaphrodite duct may help in movement of sperm into the epiphallus through the spermoviduct and vas deferens. In the epiphallus the autosperm are housed in a whip-like spermatophore that is transferred to the gametolytic organ of the partner during copulation (Tompa 1984; Gómez 2001).

The fertilisation pouch-spermatheca complex (fpsc) of *M. indica* is a bean shaped, bulbous, pouch that is very small in size compared to other terrestrial pulmonates (Ghose 1962; Lind 1973; Tompa 1984; Stewart et al. 2016; Hyman et al. 2017). The spermoviduct is a combined structure of the spermiduct, with a prostate gland, and the oviducal channel, with the albumen gland, as reported in other *Macrochlamys* species and other stylommatophorans (Gómez 2001; Deshmukh et al. 2012; Hyman et al. 2017; Pholyotha et al. 2018). The prostate gland and the oviducal channel of *M. indica* are slightly unequal in length, as also reported in other *Macrochlamys* species (Pholyotha et al. 2018). The oviducal channel of *Macrochlamys* species is ornamented with numerous lateral lamellae whereas in other stylommatophoran snails this oviduct is much more folded (Ghose 1962; Hyman et al. 2017). The spermoviduct in the *Macrochlamys* species is, as in other Stylommatophora, proximally modified into a vas deferens and a free oviduct (Ghose 1962; Gómez 2001; Stewart et al. 2016; Hyman et al. 2017; Pholyotha et al. 2018). The albumen gland of *M. indica* comprises numerous small tubules and secretory materials of the gland store in its lumen and these are also found in other stylommatophoran snails (Egonmwan 2007). As in other pulmonates, the luminal materials in the albumen gland of *M. indica* may help in development of oogenesis and vitellogenesis in its ovotestis (Bride and Gomot 1995; Xiong et al. 2017; Roy et al. 2018).

A brownish, muscular capsule is developed at the junction of the gametolytic gland, free oviduct, and the vagina of *M. indica*. Pholyotha et al. (2018) studied the genitalia of various *Macrochlamys* species and described the development of a thick, brownish tissue on the free oviduct (*cf.* Figures 4A, F, 5A, C, 9A). Based on their location between the vagina and the free oviduct, the thick, brownish tissues in *Macrochlamys* species could be better interpreted as the vaginoviducal capsule. Hyman et al. (2017) previously described the development of a variously-shaped capsular gland on the distal portion of the free oviduct in some helicarionid snails. This capsular gland possibly has some structural relationships with the vaginoviducal capsule of *Macrochlamys* and needs further investigation. The free oviduct and the gametolytic organ are united proximally to the vaginoviducal capsule in *M. indica*. The topographical development of the vaginoviducal capsule along with reproductive system may be species-specific (Table 4) and is easily found in species of *Macrochlamys* (Deshmukh et al. 2012; Pholyotha et al. 2018). However, this vaginoviducal capsule is very poorly-developed in *M. kelantanensis* Möllendorff, 1902 (*cf.* Figure 9C; Pholyotha et al. 2018) and is absent in *M. petrosa* (Deshmukh et al. 2012) and in some other stylommatophoran snails (Ghose 1962; Gómez 2001; Stewart et al. 2016).

The gametolytic organ of *M. indica* possesses a distal transparent gametolytic sac and an elongated proximal gametolytic duct which is a genus-specific distinctive feature of *Macrochlamys* (Schileyko 2003; Pholyotha et al. 2018) having little interspecific structural or topographical variations among the species (Table 3). Furthermore, it is reported that in stylommatophoran snails, the gametolytic organ is either digitiform (e.g. *Flammulina festiva* Scott, 1970; Cádiz and Gallardo 2008), has a well-developed diverticulum (e.g. *Cornu aspersum*; Koene and Chase 1998) or is absent [in the family Rhytididae Pilsbry, 1893 (Climo 1977)]. It is assumed that the morpho-anatomical diversity of the gametolytic organ in stylommatophoran snails is taxon specific (Tompa 1984; Holyoak et al. 2013). Repeated mating in *M. indica* was previously reported by Raut and Ghose (1984) however, some terrestrial snails mate repeatedly and storage of unused exogenous spermatozoa in the spermatheca of females is very common and may increase the fecundity of individuals (Tompa 1984; Chen and Baur 1993; Madec and Daguzan 1993; Baur 1994; Dillen et al. 2009; Nakadera et al. 2017). The gametolytic sac in some *M. indica* was pinkish and swollen and contained two to seven spermatophores. It is not known whether the pinkish secretion in the sac is acquired along with the spermatophores from the mate or secreted from the membranous wall of the gametolytic organ. However, further investigations need to determine the source of all these spermatophores.

The presence of an elongated epiphallus with a coiled epiphallic caecum is a characteristic feature of species of *Macrochlamys* (Blanford and Godwin-Austen 1908; Schileyko 2003; Pholyotha et al. 2018). However, there are small interspecific coiling differences in the epiphallic caecum among species of *Macrochlamys* (Table 4). The presence of a penial caecum is also a distinct feature in species of *Macrochlamys* and differs slightly among them (Table 4). The flagellar lumen of *M. indica* is almost smooth and

devoid of any filamentous structure, while an axial filament was found in the lumen of other stylommatophoran snails (Hyman et al. 2017).

The spermatophores in species of *Macrochlamys* are elongated, whip-like or needle-like structures developed in the lumen of the epiphallus and the proximal part of the flagellum. However, due to the differential configuration of the epiphallic and flagellar lumen, the moulding of the spermatophore results in a species-specific shape (Table 3) and surface characteristics (Lind 1973; Wiktor 1987; Gómez 2001; Hyman et al. 2017). A number of workers described the slim filamentous part of the spermatophore as the tail (Tompa 1984; Hyman et al. 2017) but their orientation during construction in the epiphallic lumen and while being transferred to the gametolytic organ of the mate indicates that the filamentous part is anterior, hence it is most appropriately called the anterior filament (Barker 1999; Gómez 2001). The anterior filament of the spermatophore of *M. indica* is fully spinose whereas in the case of *M. aurantia* Pholyotha & Panha, 2018 and *M. coleus* only some of the anterior filament of the spermatophore is spinose (Pholyotha et al. 2018). The middle region of the anterior filament of the spermatophore of *M. aurantia* lacks spines while *M. coleus* contains five rows of long spines on that organ (Pholyotha et al. 2018). There is controversy as to whether the anterior filament of the spermatophore of the stylommatophoran snails is formed in the flagellar lumen or epiphallic lumen. Earlier studies on the spermatophore in some Stylommatophora (e.g., *Parmavitrina maculosa* Hyman et al. 2017, *Mysticarion obscurior* Hyman et al. 2017) had reported that the lumen of flagellum moulds the anterior filament of the spermatophore (Hyman et al. 2017). In the present study, it is observed that the lumen of the distal epiphallic tube of *M. indica* possesses the anterior filament of the spermatophore and also reported in some other Stylommatophora such as *Helix pomatia*, Linnaeus, 1758 (Meisenheimer 1907; Lind 1973; Tompa 1984; Wiktor 1987; Gómez 2001), *Parmarion pupillaris* Humbert, 1864 (Hoffman 1940), and *Arion ater* Linnaeus, 1758 (Smith 1965). After copulation, the spermatophore of the male snail is transferred to the gametolytic organ of the partner. During fertilisation, the exogenous sperm are released from the medial cylindrical body of spermatophore via the anterior filament with the help of peristaltic waves of the gametolytic duct (Lind 1973; Tompa 1984). The exogenous sperm in the spermatophore are temporarily stored in the spermatheca and finally released into the fpsc to fertilise the ova (Meisenheimer 1907; Lind 1973; Tompa 1984).

The dart apparatus of *M. indica* is an elongated, tubular auxiliary copulatory organ with a retractor muscle resembling that of the other species of *Macrochlamys* (Pholyotha et al. 2018). The dart shaft of *M. indica* possesses four distinct regions here described for the first time in a stylommatophoran snail. The dart shaft of *M. indica* comprises a central core surrounded by many layers of modified epithelial cells also described for first time. The dart is a non-disposable muscular structure and may be coated with mucus secreted by the muscular dart bag. The mucus is transferred to the body cavity of the mate when it is stabbed by the dart during mating, perhaps increasing the likelihood of paternity of the sperm donor (Gómez et al. 1996; Kimura et al. 2015; Stewart et al. 2016). A prominent dart flange separates the dart from the rest of the dart body. A dart retractor muscle at the distal end of the elongated dart apparatus may support the eversion and retraction of the dart during mating.

The overall results of the present study suggest that *M. indica* possesses many unique interspecific characteristics in the different parts of the reproductive system (i.e., the penis and its interior sculptures, coiling of epiphallic caecum, oviducal channel of spermoviduct with lamellae, origin of gametolytic organ, location of the vaginoviducal capsule, structure of the fertilisation pouch-spermatheca complex), spermatophore, and dart apparatus. All these characteristics of *M. indica* justify its recognition as a separate species within *Macrochlamys* (Tables 2–5). The reproductive strategies of *M. indica* may be influenced by the unique position of the gametolytic organ with the vaginoviducal capsule, and the morpho-structural characteristics of the epiphallic caecum, penis, spermatophores, vagina, fertilisation pouch-spermatheca complex (fpsc), and dart apparatus.

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Disclosure statement

No potential conflict of interest was reported by the author.

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