Malacopedia

São Paulo, SP, Brazil ISSN 2595-9913
Volume 7(2): 7-18 April/2024

The average higher heterobranch genital system

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Abstract

This study explores the genital structures of higher Heterobranchia. It describes and explains the typical structures of the Stylommatophora, which are among the most complex, outlining the pathways, including schematic illustration, taken by autosperm, allosperm, ovules, comprising the fertilization and egg formation processes. The paper presents various examples and discusses evolutionary aspects in stylommatophorans and other higher heterobranchs.

Keywords: anatomy, adaptation, taxonomy, phylogeny, evolution, reproduction.

Introduction

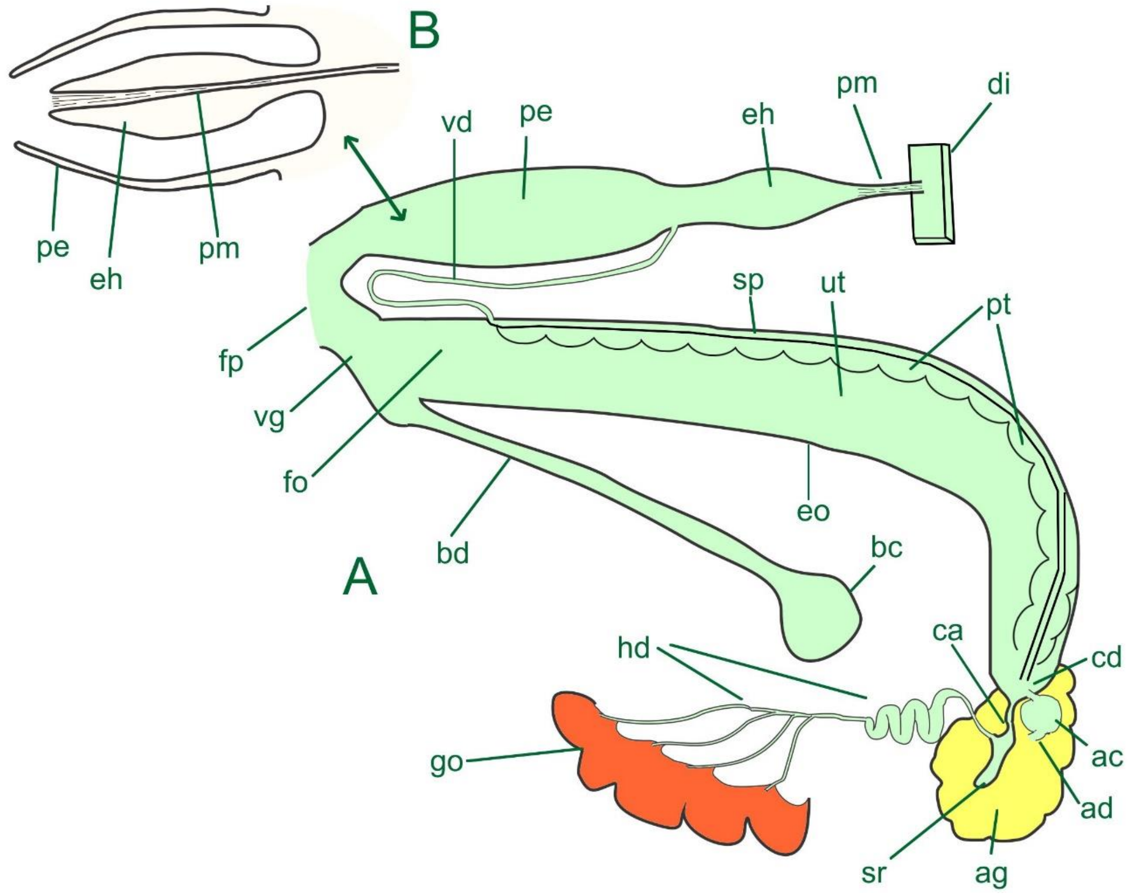
The subclass Heterobranchia is a megadiverse branch of mollusk gastropods that has adapted to various habitats. Regarding their reproductive system, a wide range of configurations is found, particularly among the more basal taxa. However, at least two characteristics are considered synapomorphic for this group: (1) simultaneous hermaphroditism, and (2) the detachment of genital structures from the mantle and integument, typically running free in the haemocoel without attachments (Simone, 2011).

This schema is more apparent in higher heterobranchs, specifically within the infraclass Euthyneura, which includes most Ringipleura species such as nudibranchs and pleurobranchs, as well as Tectipleura, which encompasses the eupulmonates. This section discusses their genital structures, including the typical arrangement observed in most groups. Additionally, the function of each structure, common to many of them, is also described.

The genital structures in heterobranchs have multiple functions. These range from the production of gametes (ovules and sperm), fertilization, and egg formation, to copulation and mating stimuli in some cases. Some structures even serve to prevent interspecific mating.

The common structures in the genital system of higher heterobranchs are presented first, followed by an overview of the function of each structure during a hypothetical reproductive phase. This section concludes with some examples of structural variations, bearing in mind that numerous other variations exist. These variations in reproductive structures are highly valuable for comparative studies, including taxonomy and phylogeny, which will be discussed later.

Main components of a high heterobranch genital system



1: schematic representation of genital structures found in most stylommatophorans as example of higher heterobranch (no scales or proportions)**. A**, whole ventral view of uncoiled and expanded structures; **B**, penis during the copula, transversely sectioned. Lettering: ac, albumen chamber; ad, albumen gland duct; ag, albumen gland; bd, bursa copulatrix duct; ca, carrefour; cd, duct of albumen chamber; di, diaphragm or integument or columellar muscle; eh, epiphallus; eo, spermoviduct; fo, free oviduct; fp, genital aperture; go, gonad; hd, hermaphrodite duct; mp, penis muscle; pe, penis; pt, prostate; sp, spermgroove(s); ut, uterus; vd, vas deferens; vg, vagina.

Stylommatophora, a highly derived and diverse taxon within heterobranchs, serves as a useful example for understanding the complexity of genital systems. Its gonad (Fig. 1: go) is the structure that produces gametes during reproductive phases. Both ovules and sperm are generated in mixed acini (Fig. 2), although each individual acinus only produces one type of gamete—either ovules or sperm. Based on observations, ovules and sperm are not released together, but are instead secreted one at a time via the hermaphrodite duct (hd). In shelled forms, the gonad is located in the first whorls of the visceral mass, typically situated in the posterior lobe of the digestive gland, facing the columella.

The hermaphrodite duct (Fig. 1: hd) starts as several narrow branches that converge into a single duct, which is also initially quite narrow. Its central region is often highly convoluted or zigzagging, with thickened walls. This swollen section of the hermaphrodite duct serves as a storage area for endogenous gametes. The duct narrows again before its junction with the carrefour (ca), also known as the fertilization complex or 'crossing,' which is where fertilization occurs.

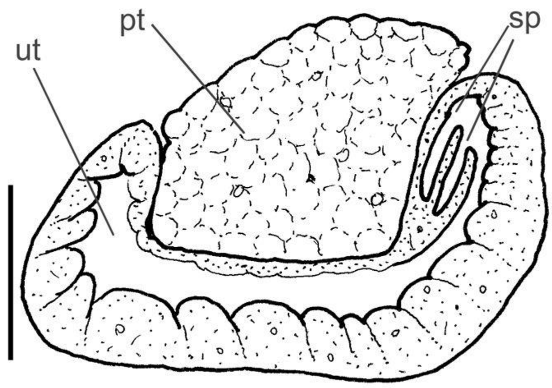
In this region, there is typically a seminal receptacle (Fig. 1: sr). It is generally shaped as a long, narrow, and slender sac, serving the function of storing exogenous sperm (see details below).

2: histological slide of *Glaucus atlanticus* (Gastropoda Nudibranchia) showing portion of the gonad. Cason, 3 µm, scale= 500 µm (photo by Gemany C.R. Santos). Lettering: oy, ovary; ts, testis.

The structures at the distal end of the hermaphrodite duct are situated within the hilar region of a large albumen gland (Fig. 1: ag). In shelled forms, this gland is located in the final visceral whorl, compressing the stomach and kidney, as well as the anterior lobe of the digestive gland. The albumen gland has a duct (ad) that drains albumen to the distal end of the spermoviduct (eo). Typically, there is an albumen chamber (Fig. 1: ac) in this area, which stores albumen and has its own duct (cd).

The organization and composition of structures in the hilar region of the albumen gland can vary greatly, making them valuable for comparative biology. Because accessing these structures requires dissection and partial removal of the albumen gland, they are seldom included in taxonomic descriptions, even in studies that consider anatomical features.

The spermoviduct (Fig. 1: eo) is typically the largest part of the genital structures. It forms from the sudden widening of the carrefour duct and extends as a long, hollow cylinder, running from the last whorl of the visceral mass along the haemocoel. The spermoviduct consists of three portions (Fig. 3): (1) the uterus (ut), which is the largest part and is hollow, with walls that range from weakly to thickly glandular and often very folded; (2) the spermgroove(s) (sp), which can be one or two and run longitudinally along one side of the spermoviduct; if there are two spermgrooves, one is designated for autosperm (self-produced sperm) and the other for allosperm (sperm from other individuals); if there's only one spermgroove, this separation does not occur anatomically; and (3) the prostate (pt), the only solid portion of the spermoviduct, which runs parallel to the spermgroove.



3: Drawing of transverse section in spermoviduct. *Cratracca uhlei*, middle region, scale= 0.5 mm (from Simone, 2022). Lettering: pt, prostate, sp, spermgrooves, ut, uterus.

In the region before the genital pore (fp), the spermgroove—or the autosperm spermgroove if there are two—detaches from the spermoviduct to form the vas deferens (Fig. 1: vd). The vas deferens is a narrow tube that runs along the outer surface of the other genital structures, including the penis (pe), usually connecting to the tip of the penis.

Once separated from the male portion, the proximal part of the spermoviduct is referred to as the free oviduct (Fig. 1: fo). It generally has the same width as the preceding region of the spermoviduct but is much shorter in length.

At the base of the free oviduct, the duct of the bursa copulatrix inserts (Fig. 1: bd). The section from this point to the genital pore (fp) is called the vagina (vg). Both the free oviduct and the vagina usually have muscular walls, and in some taxa, they can also be exteriorized or everted during copulation.

The other branch of the genital system is entirely masculine, with the primary component being the retractile penis (Fig. 1: pe). The penis typically has muscular walls and can vary from stubby to very long and slender. In its retracted state (Fig. 1A), the penis resides within the haemocoel, running along the mid-dorsal region, covering the buccal mass and esophagus. During copulation, the penis is everted like a proboscis (Fig. 1B), which is significant because the internal surface in its retracted state becomes the external surface during copulation. The penis usually has various folds, specialized glands, projections, flagella, chambers, and other features. These structures are crucial for recognizing partners and facilitating or maintaining copulation. They are internal when retracted but become prominent and external during mating.

At the tip of the penis, there's the epiphallus (Fig. 1: eh). The epiphallus typically extends from the distal end of the penis (Fig. 1A) but remains inside the penis during copulation (Fig. 1B). Its primary function is to produce the spermatophore, a sperm-containing envelope that will be transferred to the mating partner.

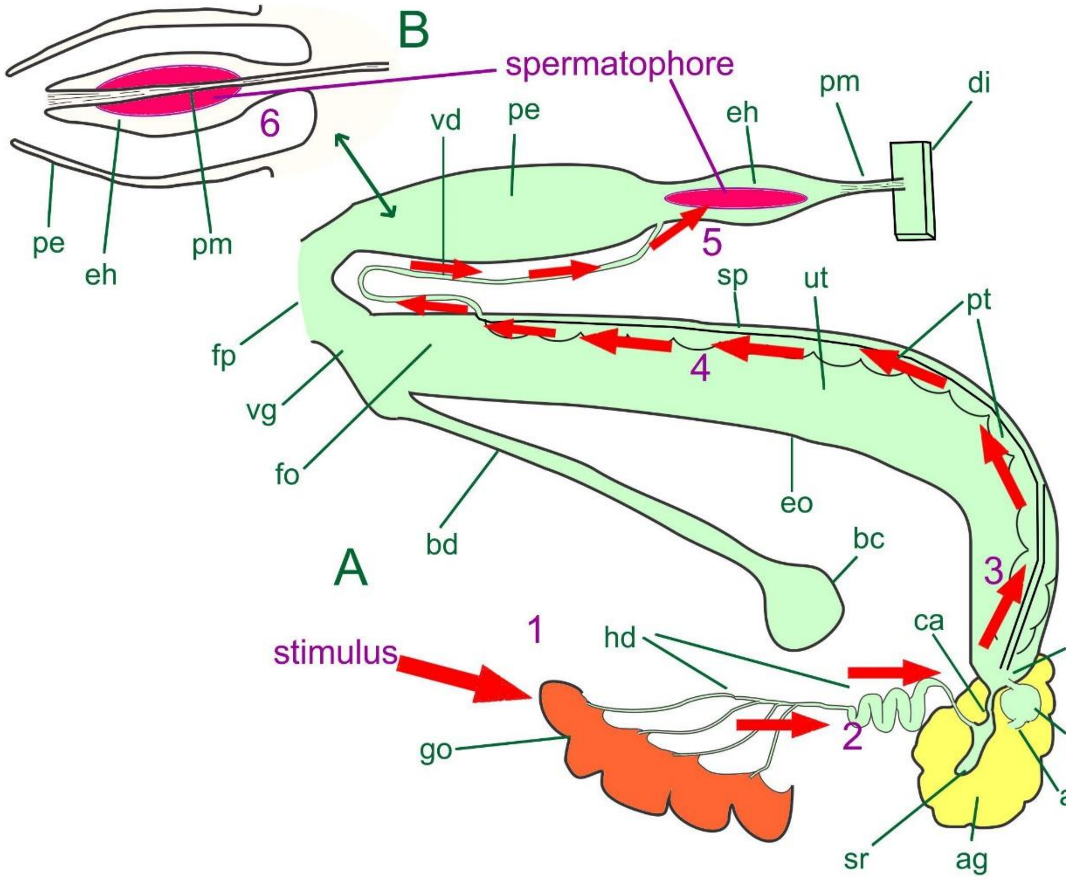
The eversion of the penis is driven by hydraulic pressure, while its retraction back into the haemocoel is accomplished through the contraction of the penis muscle (Fig. 1: pm). In stylommatophorans, the penis muscle usually originates in the anterior region of the diaphragm (pallial floor membrane), but some groups have variations where the muscle originates from the columellar muscle or even from parts of the integument.

The insertion points of the vas deferens and the penis muscle vary among different taxa, providing valuable information for comparative analysis. The vas deferens can insert at the tip of the penis or at various points along the epiphallus. The same applies to the insertion of the penis muscle. Another aspect valuable for comparative studies is the arrangement of structures on the inner surface of the penis when retracted; each species has its unique pattern.

Examples of variations and functions of these genital structures will be discussed in the following sections.

The way the genital system of stylommatophorans works

Production of autosperm



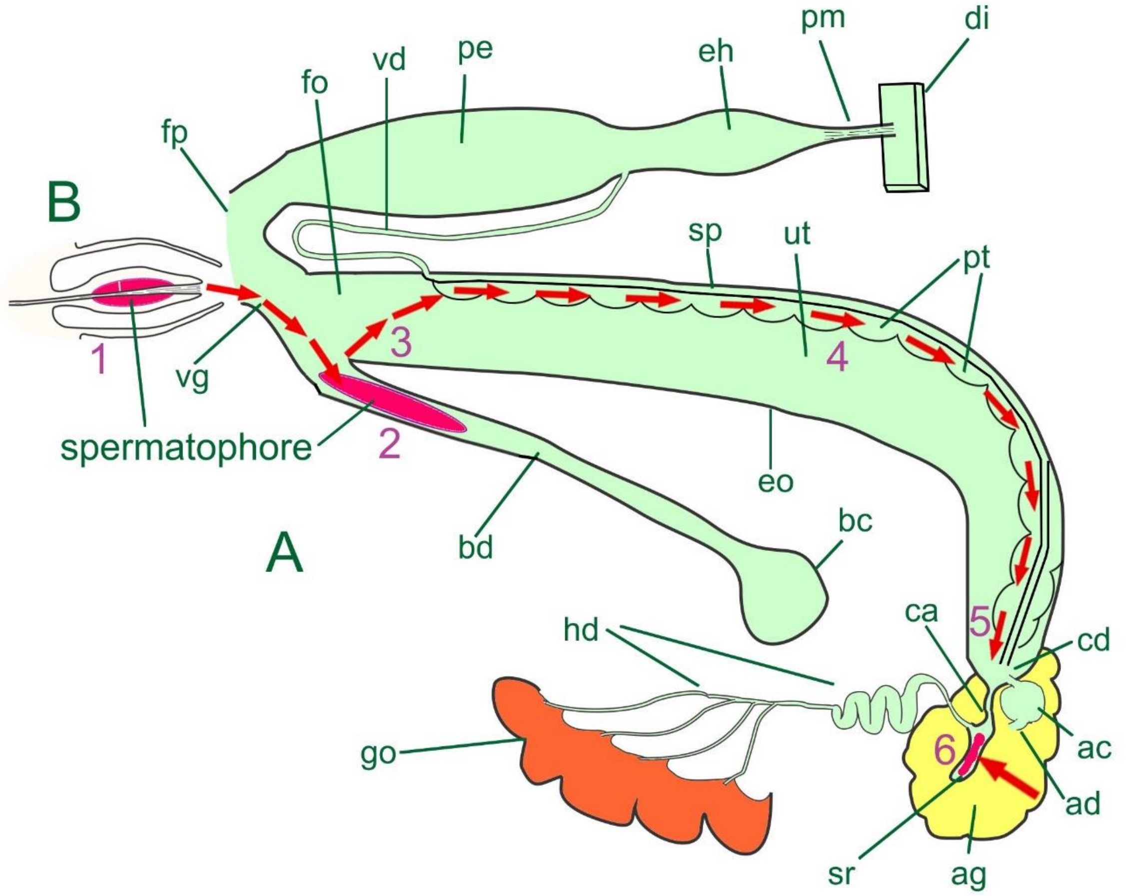
4: same schematic representation as Fig. 1 with concern to autosperm process. Red arrows indicating way the sperm runs along the structures, stages from 1 to 6 (see text for details). Lettering: ac, albumen chamber; ad, albumen gland duct; ag, albumen gland; bc, bursa copulatrix; bd, bursa copulatrix duct; ca, carrefour; cd, duct of albumen chamber; di, diaphragm or integument or columellar muscle; eh, epiphallus; eo, spermoviduct; fo, free oviduct; fp, genital aperture; go, gonad; hd, hermaphrodite duct; mp, penis muscle; pe, penis; pt, prostate; sr, seminal receptacle; sp, spermgroove(s); ut, uterus; vd, vas deferens; vg, vagina.

Sperm, or spermatozoa, is produced in the testis portion (Fig. 2: ts) of the hermaphrodite gonad (Figs. 1, 4: go), also known as the ovotestis. It is referred to as 'autosperm' because it is produced by the individual itself. Environmental and/or hormonal stimuli trigger the production of sperm (Fig. 4: stage 1). The sperm then moves through the hermaphrodite duct (hd) and can be stored in its swollen, convoluted section (Fig. 4: stage 2).

In the carrefour (ca), sperm is transferred through a narrow duct to the spermoviduct (eo). In this area, sperm travels along the spermgroove (sp), where it is enriched by secretions from the prostate (pt), which runs parallel to it (Fig. 4: stage 3). At the anterior end of the spermoviduct (Fig. 4: stage 4), the sperm traveling along the spermgroove is directed into a tube that forms the vas deferens (vd). The vas deferens flanks the remaining female portion, crossing into the male portion, and runs along the outer surface of the penis (in its retracted state) (pe) (Fig. 4: stage 5). When the vas deferens connects, the sperm enters the lumen of the penis-epiphallus. The epiphallus (eh) encases the sperm in a protein structure called the spermatophore (Fig. 4: stage 6).

The spermatophore, which varies among different taxa from a simple structure to a complex and elaborate element, represents the final stage. It is transferred to the mating partner during copulation, as described below.

Reception and process of the allosperm



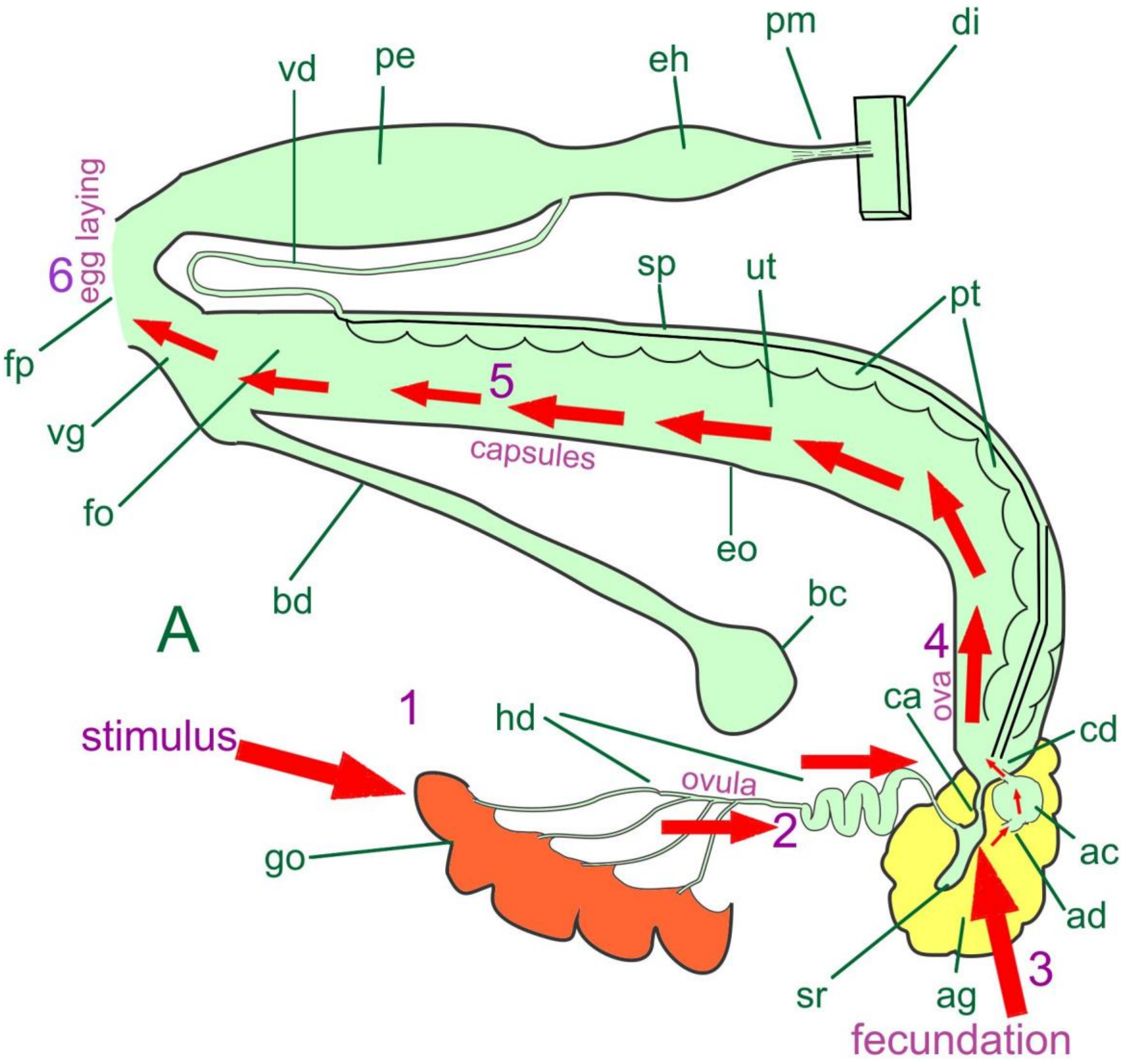
5: same schematic representation as Fig. 1 with concern to allosperm process. A, entire genital structures just after copula; **B**, mate's penis in pre-copula that will be introduced to deposit the spermatophore in the duct of the bursa. Red arrows indicating way the allosperm runs along the structures, stages from 1 to 6 (see text for details). Lettering: ac, albumen chamber; ad, albumen gland duct; ag, albumen gland; bc, bursa copulatrix; bd, bursa copulatrix duct; ca, carrefour; cd, duct of albumen chamber; di, diaphragm or integument or columellar muscle; eh, epiphallus; eo, spermoviduct; fo, free oviduct; fp, genital aperture; go, gonad; hd, hermaphrodite duct; mp, penis muscle; pe, penis; pt, prostate; sr, seminal receptacle; sp, spermgroove(s); ut, uterus; vd, vas deferens; vg, vagina.

Allosperm, also known as allogenic sperm or allospermia, refers to sperm from a mate that is not genetically related to the recipient. This sperm, delivered via the spermatophore (Fig. 5B, stage 1), is transferred from the partner's penis through the vagina into the duct of the bursa copulatrix (Fig. 1: bd) (Fig. 5: stage 2).

The bursa copulatrix (bc) is a digestive structure within the genital system, designed to break down anything within it and its duct. The spermatophore and sperm have a specific chemical property that inhibits or slows down the digestive action of the bursa, allowing the sperm to move further through the genital tubes. This mechanism acts as a safeguard against interbreeding among species, preventing interspecific reproduction, as different species lack the same chemical key to counteract the bursa's digestive process. If copulation occurs between different species, the spermatophore and sperm are entirely digested, preventing undesirable hybridization.

When the digestive function of the bursa is blocked or slowed down, the sperm can leave the spermatophore. It travels along the spermoviduct (eo), following the spermgroove (sp) (Fig. 5: stage 3). At this stage, the sperm moves through the entire length of the spermoviduct to its distal end. It then passes through the narrow duct of the carrefour (ca), ultimately being stored in the seminal receptacle (sr) (Fig. 5: stage 6). The allosperm remains in the receptacle until the fertilization of the ovules, which is discussed in the following section.

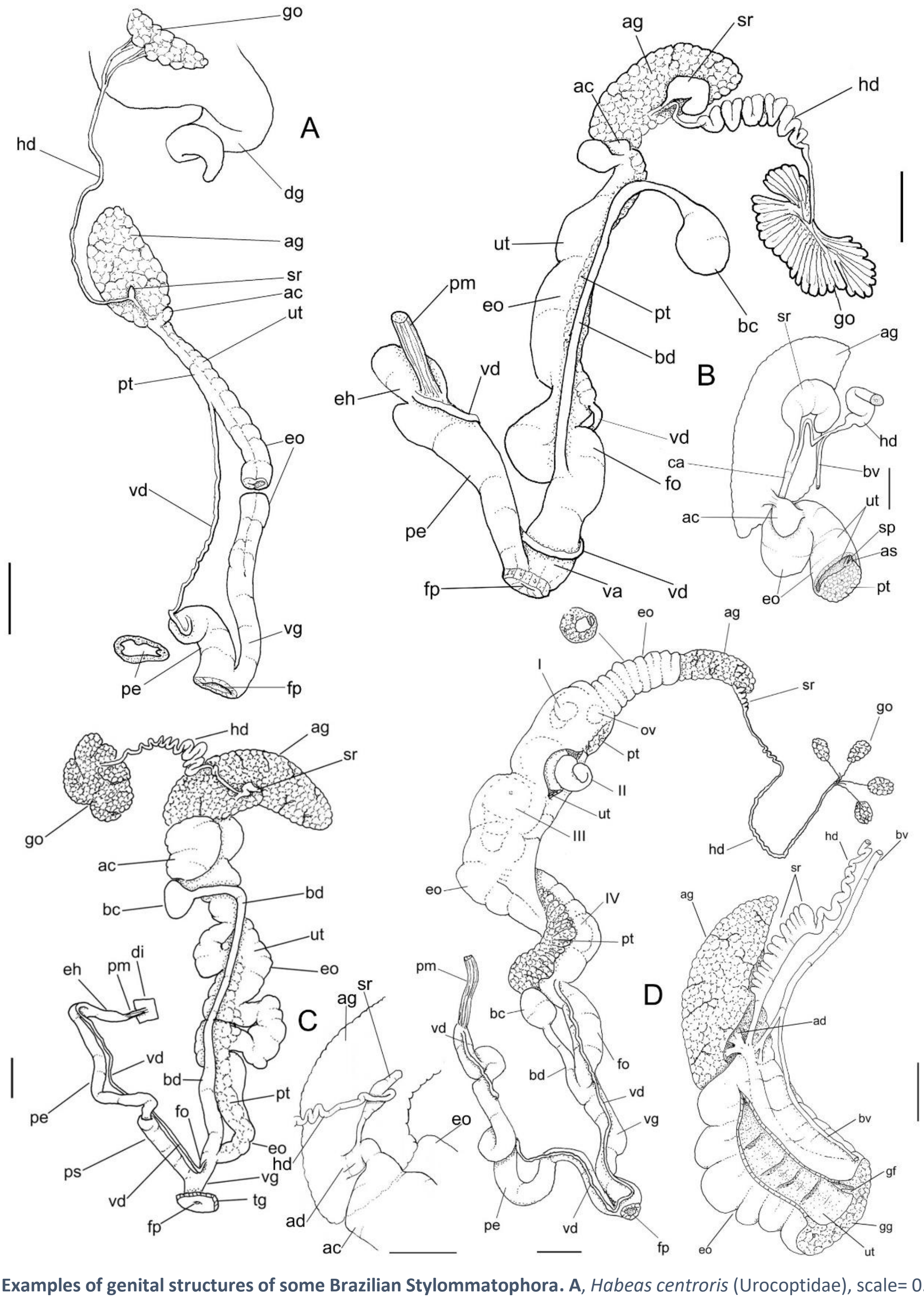
Ovula, fecundation and ova processes



6: same schematic representation as Fig. 1 with concern to ovula production and process. entire genital structures showing by red arrows the way the feminine gametes are formed (ovula), are fertilized (ova), and become eggs (capsules), stages from 1 to 6 (see text for details). Lettering: ac, albumen chamber; ad, albumen gland duct; ag, albumen gland; ; bc, bursa copulatrix; bd, bursa copulatrix duct; ca, carrefour; cd, duct of albumen chamber; di, diaphragm or integument or columellar muscle; eh, epiphallus; eo, spermoviduct; fo, free oviduct; fp, genital aperture; go, gonad; hd, hermaphrodite duct; mp, penis muscle; pe, penis; pt, prostate; sr, seminal receptacle; sp, spermgroove(s); ut, uterus; vd, vas deferens; vg, vagina.

The female gametes, called ovules, are produced in the ovarian part of the gonad (Fig. 2: oy) following an environmental or hormonal stimulus (Fig. 6: go; stage 1). The ovules travel through the hermaphrodite duct (hd) (Fig. 6: stage 2) alongside the sperm, but in much smaller numbers and one at a time. They are not stored along the duct.

In the carrefour region (ca), the ovules are fertilized by allosperm stored in the seminal receptacle (Fig. 6: sr; stage 3), turning them into ova. These ova then move through the narrow duct of the carrefour to the spermoviduct (eo), where they receive a coating of albumen. The albumen comes from the albumen gland (Fig. 6: ag) and is either delivered directly or stored in the albumen chamber (ac) (Fig. 6: stage 4).



7: Examples of genital structures of some Brazilian Stylommatophora. A, *Habeas centroris* (Urocoptidae), scale= 0.5 mm (from Simone, 2022b); **B**, *Anthinus multicolor* (Odontostomidae), scale= 3 mm, with a detail of carrefour region, scale= 1 mm (from Simone, 2022a); C, *Drymaeus castilhensis* (Bulimulidae), with a detail of carrefour region, scales= 2 mm (from Simone & Amaral, 2018); **D**, *Lavajatus moroi* (Achatinidae), scale= 1 mm, with a detail of carrefour region, scale= 0.5 mm (from Simone, 2018a).Lettering: ac, albumen chamber; ad, albumen gland duct; ag, albumen gland; as, accessory genital gland; ; bc, bursa copulatrix; bd, bursa copulatrix duct; bv, blood vessel; ca, carrefour; cd, duct of albumen chamber; dg, digestive gland; di, diaphragm; eh, epiphallus; eo, spermoviduct; fo, free oviduct; fp, genital aperture; gf, spermo fold; gg, genital gland; go, gonad; hd, hermaphrodite duct; mp, penis muscle; ov, ova; pe, penis; ps, penis shield; pt, prostate; sp, spermgroove(s); sr, seminal receptacle; tg, integument; ut, uterus; vd, vas deferens; vg, vagina.

In the uterine section (Fig. 6: ut) of the spermoviduct (eo), the combined ova and albumen begin to form eggs or capsules (Fig. 6: stage 5). These capsules can resemble typical bird eggs in some groups (such as Megalobuliminae), or appear as small white dots in others. They contain embryos and albumen, protected by an eggshell. This shell can be chitinous, calcified, or a combination of both. These eggs or capsules are designed to withstand environmental conditions (Fig. 6: stage 6). In some respects, the heterobranch uterus is similar to the capsule gland found in other gastropod branches.

Examples of genital structures in higher Heterobranchia

Some Stylommatophora

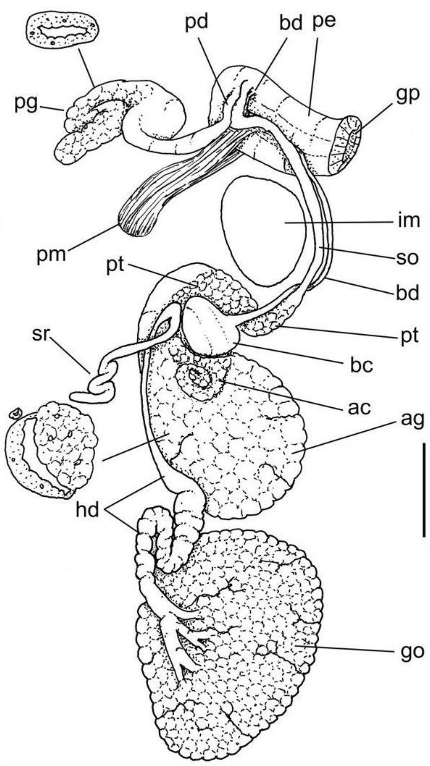
Figure 7 presents examples of genital structures in stylommatophorans, selected from Brazilian taxa. Despite being fully mature, the urocoptid *Habeas centroris* (Fig. 7A) has a remarkably simple genital system (Simone, 2022b). This simplicity has also been observed in other congeners (Simone, 2022c). The structures are narrow, small, and weakly coiled. The vas deferens (vd) detaches from the spermoviduct (eo) quite far to the anterior region. The penis (pe) is highly reduced and lacks an epiphallus. Additionally, the bursa copulatrix is absent.

Another example selected was the odontostomid *Anthinus multicolor* (Fig. 7B), which has a more typical genital system (Simone, 2022a). An interesting feature is the intense convolution of the hermaphrodite duct (hd) and the narrow carrefour (ca). This species also has an accessory genital gland (as) located parallel to the prostate, which may play a role in forming the calcareous eggshell. The penis (pe) is quite short and muscular, along with its epiphallus.

The bulimulid *Drymaeus castilhensis* is another example (Fig. 7C), characterized by ordinary but notably elongated genital structures (Simone & Amaral, 2018). A unique feature is the carrefour, which connects to a double duct of the albumen gland (ad). The spermoviduct (eo) is heavily coiled. This species also has a penis shield (ps), a muscular covering at the base of the penis.

The final example among stylommatophorans is the achatinid (subulinine) *Lavajatus moroi* (Fig. 7D), which, like other achatinoideans, incubates young specimens within the uterus. As depicted in Fig. 7D, there are three young specimens (labeled I, II, and III) at various stages of development, along with an ovum (ov) in the middle section of the uterus (ut). The distal part of the spermoviduct is highly glandular (Fig. 7D: eo at tip), potentially providing nourishment to the developing young (Simone, 2018a). As is common for this group, they are ovoviviparous, meaning the young are born already able to crawl away.

A Tectipleura siphonariid

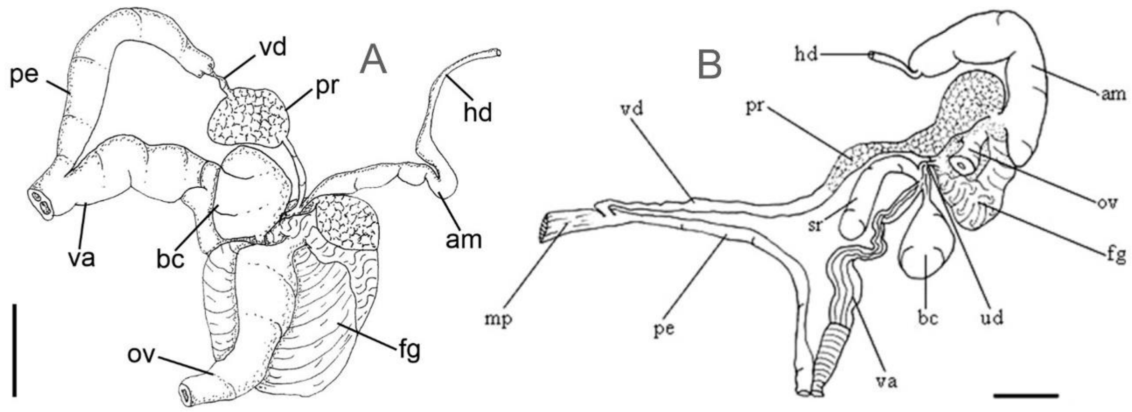


8: *Siphonaria pectinata* **genital system.** Whole view, with transverse section of 2 indicated regions also shown, scale= 2 mm (from Simone & Seabra, 2017). Lettering: ac, albumen chamber; ag, albumen gland; bc, bursa copulatrix; bd, bursa copulatrix duct; go, gonad; gp, genital pore; hd, hermaphrodite duct; im, isolated portion of shell muscle; mp, penis muscle; pe, penis; pg, penis gland; pt, prostate; *so*, spermoviduct; sr, seminal receptacle.

Siphonariids were once considered basal pulmonates, part of the Basommatophora. However, more recently, they have been reassigned to a separate taxon, Siphonarimorpha. Molecular studies have indicated that they are closely related to sacoglossans (e.g., Dayrat et al., 2011). An anatomical study of *Siphonaria pectinata* revealed further similarities with sacoglossans, specifically in the localization of the genital system (Fig. 8) (Simone & Seabra, 2017). The key observation was the arrangement of the genital structures around an isolated part of the shell muscle (Fig. 8: im). This configuration is not found in other higher heterobranchs, suggesting an evolutionary divergence. Sacoglossans are among the few groups with a specialized muscle in the same area, used to close the flexible shell aperture. Additionally, some haemocoelic structures surround this muscle, resembling the structure observed in siphonariids.

Another interesting feature of the siphonariid is the configuration of the penis. It has feminine ducts at the tip and masculine ones at its base, creating what could be described as a feminine penis.

Some Nudibranchia



9: Examples of nudibranch genital structures. **A**, *Platydoris angustipes*, Brazil, scale= 2 mm (from Lima & Simone, 2018); **B**, *Doris verrucosa*, Europe, scale= 1 mm (from Lima & Simone 2015). Lettering: am, ampulla; bc, bursa copulatrix; fg, female gland; hd, hermaphrodite duct; mp, penis muscle; ov, oviduct; pe, penis; pr, prostate; sr, seminal receptacle; ud, uterine duct; va, vagina; vd, vas deferens.

Similar to pulmonates, nudibranchs are a highly derived branch of higher heterobranchs. They are notable for lacking a shell and for exhibiting the maximum degree 2 of limacization (Simone, 2018b).

Generally, nudibranchs have similar components in their genital system as those found in stylommatophorans, but they are much shorter, stubby, and more compact (Fig. 9). The hermaphrodite duct, for example, has a long, swollen region known as the ampulla (Fig. 9: am). What corresponds to the spermoviduct in other species is a tangled, spherical glandular structure called the female gland (fg). Instead of being an elongated structure along the spermoviduct, the prostate in nudibranchs is typically a spherical, isolated gland (pr).

Final comments

Higher heterobranchs are known for their intricate egg-laying processes, complex mating behaviors, and strategies for egg protection or concealment. These factors are closely related to the interactions between the central nervous system, genital structures, and the head-foot's actions. The complexity of the genital system, the focus of this paper, reflects these varied functions. For example, some stylommatophorans, like helicids, possess a spine and a corresponding gland at the tip of the eversible vagina, used to stimulate the sexual partner by causing a mild injury. Similarly, some nudibranchs, like certain glaucids and doridids, have penises equipped with spines. This function might be analogous to that in helicids.

The evolution and inference of homology among the genital structures of the Heterobranchia are not fully understood. This process is relatively straightforward in higher heterobranchs, but in the basal branches—especially those at the triganglionate grade (e.g., Rissoellidae, Ommalogyridae, Ammatinidae, Architectonicidae, Pyramidellidae)—our understanding is limited. Some of these taxa still have genital structures attached to the integument or mantle. A comprehensive phylogeny based on morphological features is lacking for these groups, hindering further comparative analysis.

Acknowledgements

A special thanks to Gemany Caetano Rosa dos Santos for the Fig. 2. To Patricia O. V. Lima for the permission of publishing the images of the Fig. 9.

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