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**Anatomical characters for easy identification between *Biomphalaria pfeifferi*, *Helisoma duryi* and *Indoplanorbis exustus* during field surveys**

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## **Abstract**

Accurate identification of the freshwater snails that are responsible for schistosome transmission is needed in order to settle the best control strategy. We propose herein a description of the anatomical characters that allow distinguishing between the three planorbids snails: *Biomphalaria pfeifferi*, the main intermediate host of *Schistosoma mansoni* in Africa, *Helisoma duryi* and *Indoplanorbis exustus*. *B. pfeifferi* has a few prostatic diverticulae arranged in a row; the penis sheath is narrower and a little smaller than the preputium. *H. duryi* has a few prostatic diverticulae branched repeatedly giving the organ a cauliflower-like appearance; the penis sheath is pear-shaped and the preputium shows a characteristic lateral swelling produced by a preputial organ. In *I. exustus*, the prostatic diverticulae are arranged in a compact fan-shaped organ. The penial complex lacks the conspicuous preputial organ of *H. duryi*; it is of the bulinid type with a penis sheath four times longer than the preputium.

Key words: *Biomphalaria pfeifferi*, *Helisoma duryi*, *Indoplanorbis exustus*, morphology, reproductive system

## INTRODUCTION

Identification and estimation of the density and distribution of the snail intermediate hosts of schistosomiasis represent important issues for epidemiological studies on schistosomes and are central to public health-control decisions. Malacological surveys are regularly conducted in order to ensure a good follow-up of the potential intermediate snail hosts associated with the local freshwater habitats and to estimate potential risks for schistosomiasis transmission. Thus, accurate identification of the species of freshwater snails that are responsible for schistosome transmission in the area are needed.

Transmission of schistosomiasis is increasing in Africa due to the construction of water resources schemes and *Schistosoma mansoni* is now endemic in 42 countries (Chitsulo et al., 2000). In the majority of the endemic countries in Africa, *Schistosoma mansoni* uses the planorbid *Biomphalaria pfeifferi* (Krauss, 1848) as intermediate host snail. However, there is a risk to confuse this species with two other planorbid snails, *Helisoma duryi* (Wetherby, 1879) and *Indoplanorbis exustus* (Deshayes, 1834) because they share, morphologically, a discoidal shell (Kristensen and Ogunnowo, 1987; Brown, 1994). Indeed, snail identification is generally made in the field exclusively using the morphology of the shell and a misidentification is often possible. One way to avoid any confusion is to use the different anatomical characters of the reproductive system of these snails. However, the data available on the reproductive system of *B. pfeifferi*, *H. duryi* and *I. exustus* make comparison difficult because they are either diluted in papers providing exhaustive descriptions of the whole anatomy of *B. pfeifferi* (Mandhal-Barth, 1957; Schutte and Van Eeden, 1960), *H. duryi* (Paraense, 1976) and *I. exustus* (Larambergue, 1939; Rao, 1923) or disconnected from the general anatomy of the reproductive system of the snails (Madsen, 1984; Kristensen and Ogunnowo, 1987). We propose, in this paper, to provide comparative drawings of the whole anatomy of the reproductive systems of *B. pfeifferi*, *H. duryi* and *I. exustus* and to provide

anatomical tools in order to make sure the identification of these snails.

## **MATERIAL AND METHODS**

Sampling of *B. pfeifferi* and *I. exustus* were carried out since 2006 from Toho-Todougba lake (6°23'N, 2°12'E), Benin and Pahou sand quarry (6°23'N, 2°11'E), Benin respectively. We also used *H. duryi* collected in 2005 from an artificial reservoir, Bel Air (16°15'N, 61°37'W), Guadeloupe, West Indies. Snails were transferred alive in the laboratory in Perpignan where they were maintained in culture.

Thirty large specimens of each species were allowed to relax overnight using menthol. They were then immersed for one minute in 70°C water and transferred to water at room temperature. The soft parts were drawn from the shell with a small forceps and fixed in slightly modified Railliet-Henry solution (distilled water 930ml, sodium chloride 6g, formalin 50ml, glacial acetic acid 20ml). Dissection of the reproduction system was made under the stereoscopic microscope and drawings of the reproductive system were made using a camera lucida attachment. The shells were cleaned with chlorex. Voucher specimens were deposited in the Museum National d'Histoire Naturelle de Paris (MNHN) and in the Laboratoire de Biologie et d'Ecologie Tropicale et Méditerranéenne (UMR 5244 CNRS-EPHE-UPVD) of the University of Perpignan.

## **RESULTS**

The discoid shells of *B. pfeifferi*, *H. duryi* and *I. exustus* are difficult to distinguish for a non-malacologist (Figures 1, 2 and 3, respectively) but the anatomy of the reproductive system shows several reliable characters (Figures 4, 5 and 6, respectively).

The hermaphrodite part does not present great differences between the three species as well as the female reproductive system; however the duct of the spermatheca is very short in *I.*

*exustus* compared to those in *B. pfeifferi* or *H. duryi*. The differences among the three species are mainly found in the male reproductive system, especially the prostate gland and the penial complex. *Biomphalaria pfeifferi* has a few prostatic diverticulae arranged in a row (Figure 4); the penis sheath is narrower and a little smaller than the preputium. *Helisoma duryi* has a few prostatic diverticulae branched repeatedly giving the organ a cauliflower-like appearance (Figure 5) ; the penis sheath is pear-shaped and the preputium shows a characteristic lateral swelling produced by a preputial organ. In *I. exustus* (Figure 6), the prostatic diverticulae are arranged in a compact fan-shaped organ. The penial complex lacks the conspicuous preputial organ of *H. duryi*; it is of the bulinid type with a penis sheath four times longer than the preputium.

## DISCUSSION

The results presented in this paper provide anatomical characters in order to make a clear distinction between *B. pfeifferi*, *H. duryi* and *I. exustus*. They will be useful to any epidemiological field research in Africa because they will help to detect which waterbodies may be considered at risk for intestinal schistosomiasis. *Biomphalaria pfeifferi* is widely distributed in Africa where it is known to act naturally as the most important intermediate host of *Schistosoma mansoni* (Dejong et al., 2003). *Helisoma duryi* is known to occur in Africa since many years; it has been introduced there for biological control purposes (Frandsen and Madsen, 1979) and is not an intermediate host for schistosomes. To our knowledge, no specimen of this snail was ever collected in Benin. *Indoplanorbis exustus* is known to act naturally in Asia as an intermediate host of the Asiatic schistosomes, *S. spindale*, *S. nasale* and *S. indicum*, but experimental infection trials by *S. mansoni* always failed (Wright, 1971; Ibikounlé et al., 2006). In the last decades, it has been reported from West and Central Africa in Ivory Coast (Mouchet et al., 1987), Nigeria (Kristensen and Ogunnowo, 1987) and more

recently in Benin (Ibikounlé et al., 2006).

In Africa, the waterbodies may harbour only one of the three species, either *B. pfeifferi*, *H. duryi* or *I. exustus* and the consequences on schistosome transmission will be different according to the species: while the presence of *B. pfeifferi* should activate control measures, the presence of *H. duryi* or *I. exustus* should not. However, the possible implication of *I. exustus* in transmission of some species of schistosomes as those of the *indicum* group (Rollinson and Southgate, 1987) should be regularly checked.

The co-occurrence of *B. pfeifferi* with *H. duryi* seems to be unlikely because *H. duryi* cannot be considered as an invasive species as it is limited to specific habitats or/and displays poor competitive abilities (Pointier et al., 2005). On the other hand, the co-occurrence of *B. pfeifferi* with *I. exustus* seems to be more likely due to the invasive capacity of the latter (Pointier et al., 2005). However, no data is available in the literature, except our finding of such a co-occurrence in only 1 among the 35 waterbodies (distributed in 9 Departments) we prospected in 2004 in Benin. The site harbouring this snail was Parakou city (Ibikounlé, 2006). Anyhow, the co-occurrence of *B. pfeifferi* with one or more species of snails should lead to analyse the impact of these co-occurrent snails on schistosome transmission. Such a schistosome transmission enhancement was shown in the presence of different species of snails in the host-parasite system *B. glabrata-S. mansoni* (Moné, 1991; Moné et al., 1986).

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## Legends

Figure 1 Shell of *Biomphalaria pfeifferi* from Toho-Todougba, Benin (9.5 mm).

Figure 2 Shell of *Helisoma duryi* from Bel Air, Guadeloupe, West Indies (12.6 mm).

Figure 3 Shell of *Indoplanorbis exustus* from Pahou, Benin (13.4 mm).

Figure 4 Anatomy of the reproductive system of *Biomphalaria pfeifferi* from Toho-Todougba, Benin. : ca = carrefour ; cc = collecting canal ; ng = nidamental gland ; od = ovispermiduct ; ot = ovotestis ; ov = oviduct ; po = oviduct pouch ; pp = preputium ; pr = prostate ; ps = penis sheath ; sd = spermiduct ; sp = spermatheca ; sv = seminal vesicle ; ut = uterus ; va = vagina ; vd = vas deferens.

Figure 5 Anatomy of the reproductive system of *Helisoma duryi* from Bel Air, Guadeloupe, West Indies : ca = carrefour ; dp = duct of preputial organ ; ng = nidamental gland ; od = ovispermiduct ; ot = ovotestis ; ov = oviduct ; pp = preputium ; pr = prostate ; ps = penis sheath ; sd = spermiduct ; sp = spermatheca ; sv = seminal vesicle ; ut = uterus ; va = vagina ; vd = vas deferens.

Figure 6 Anatomy of the reproductive system of *Indoplanorbis exustus* from Pahou, Benin : ca = carrefour ; cc = collecting canal ; ng = nidamental gland ; od = ovispermiduct ; ot = ovotestis ; ov = oviduct ; po = oviduct pouch ; pp = preputium ; pr = prostate ; ps = penis sheath ; rm = retractor muscle ; sd = spermiduct ; sp = spermatheca ; sv = seminal vesicle ; ut = uterus ; va = vagina ; vd = vas deferens.

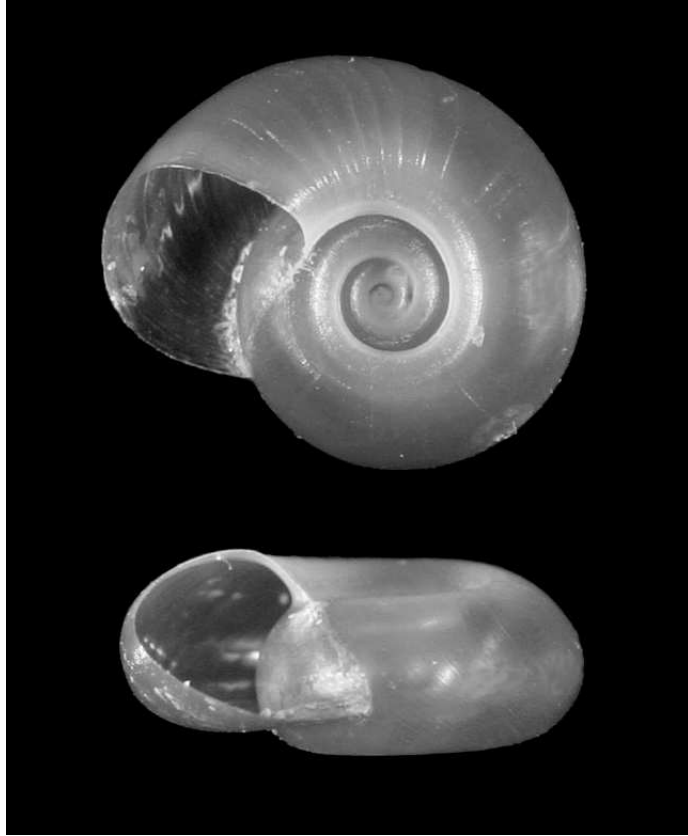


Figure 1



Figure 2

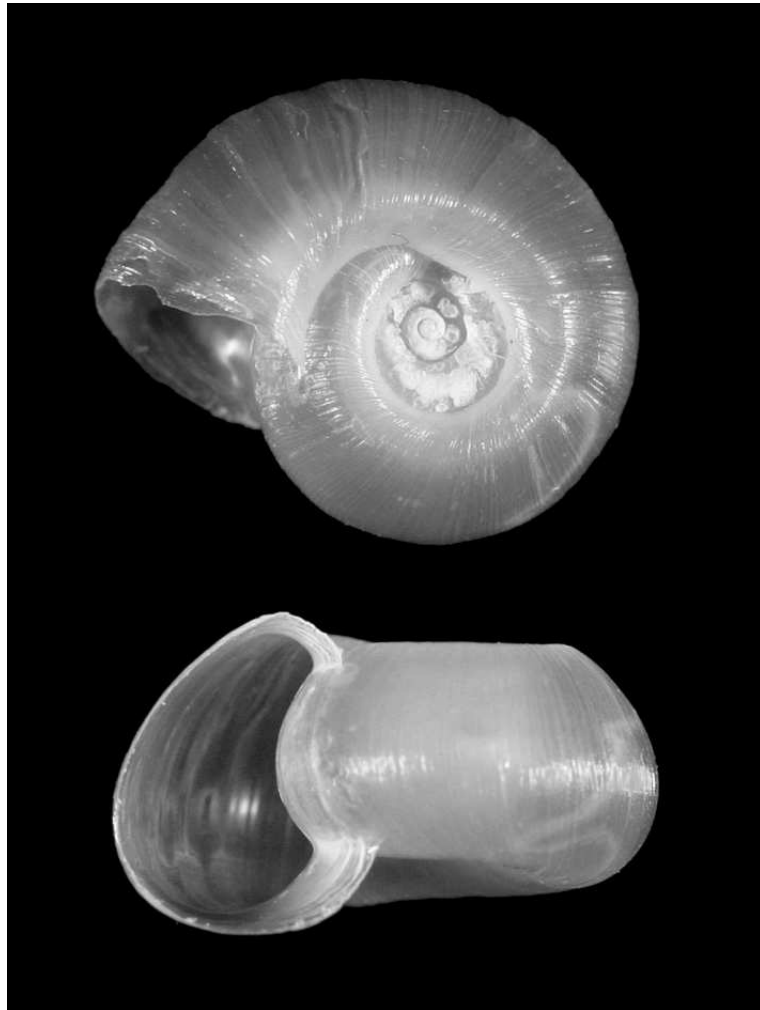


Figure 3

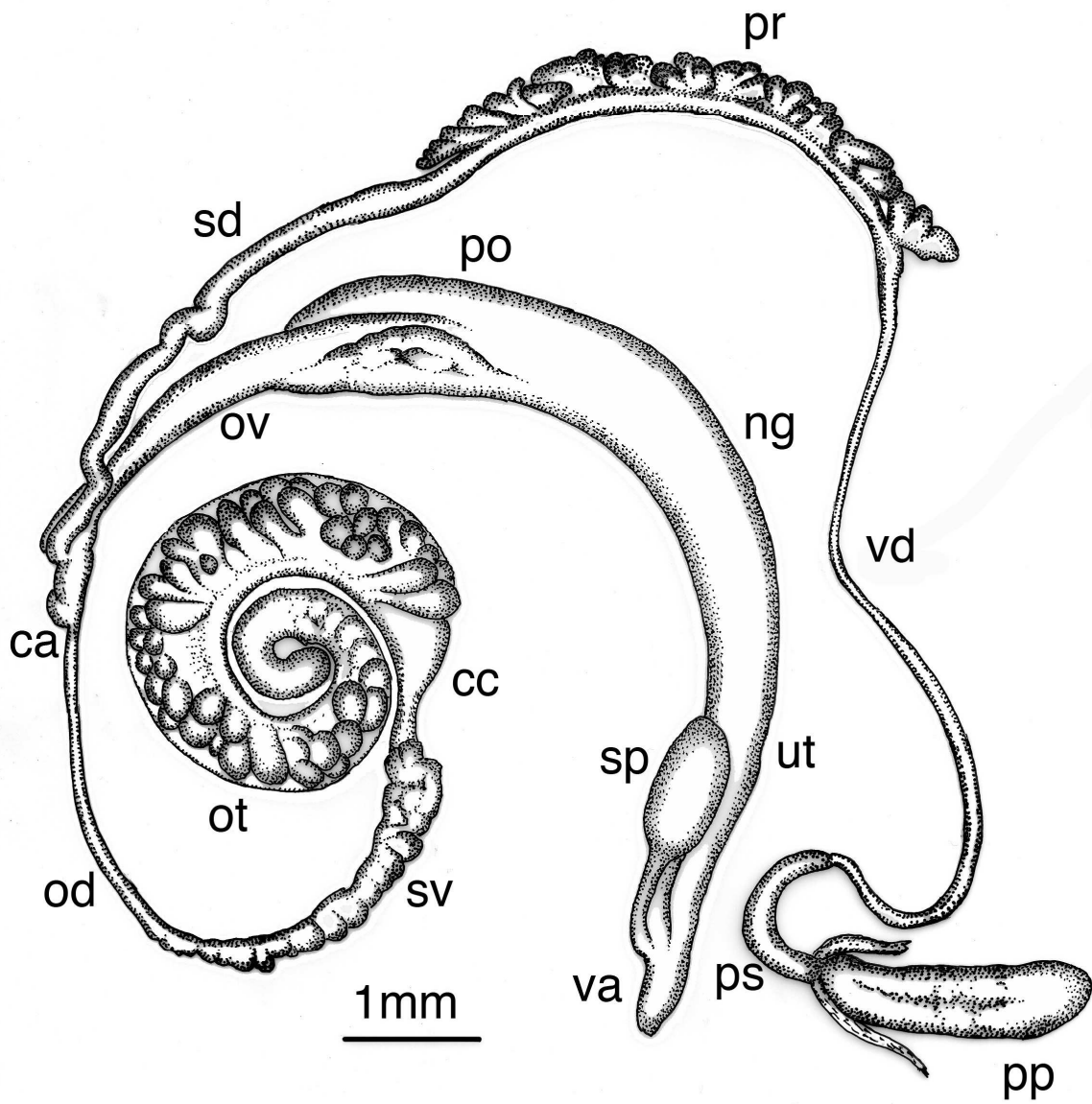


Figure 4

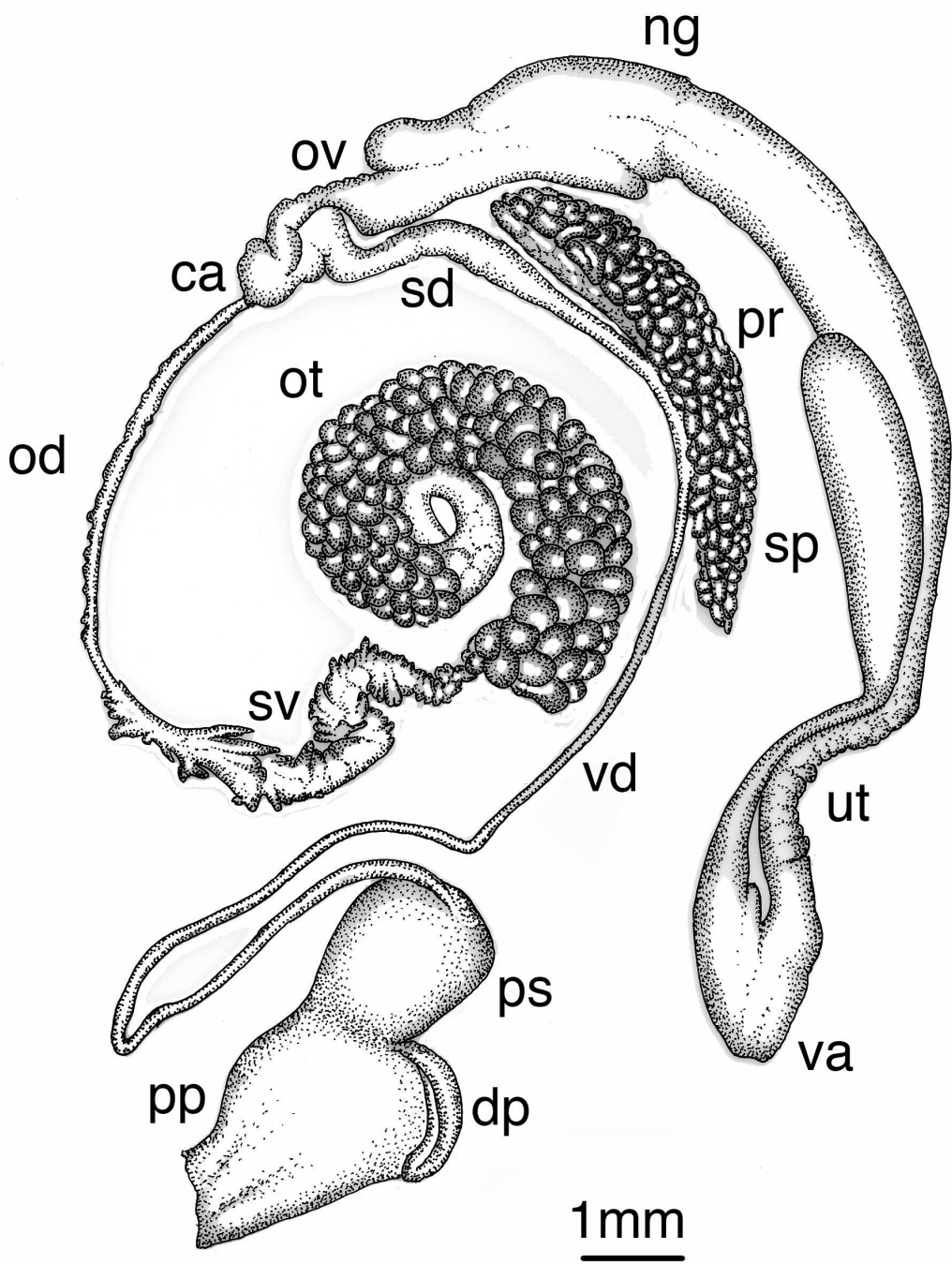


Figure 5



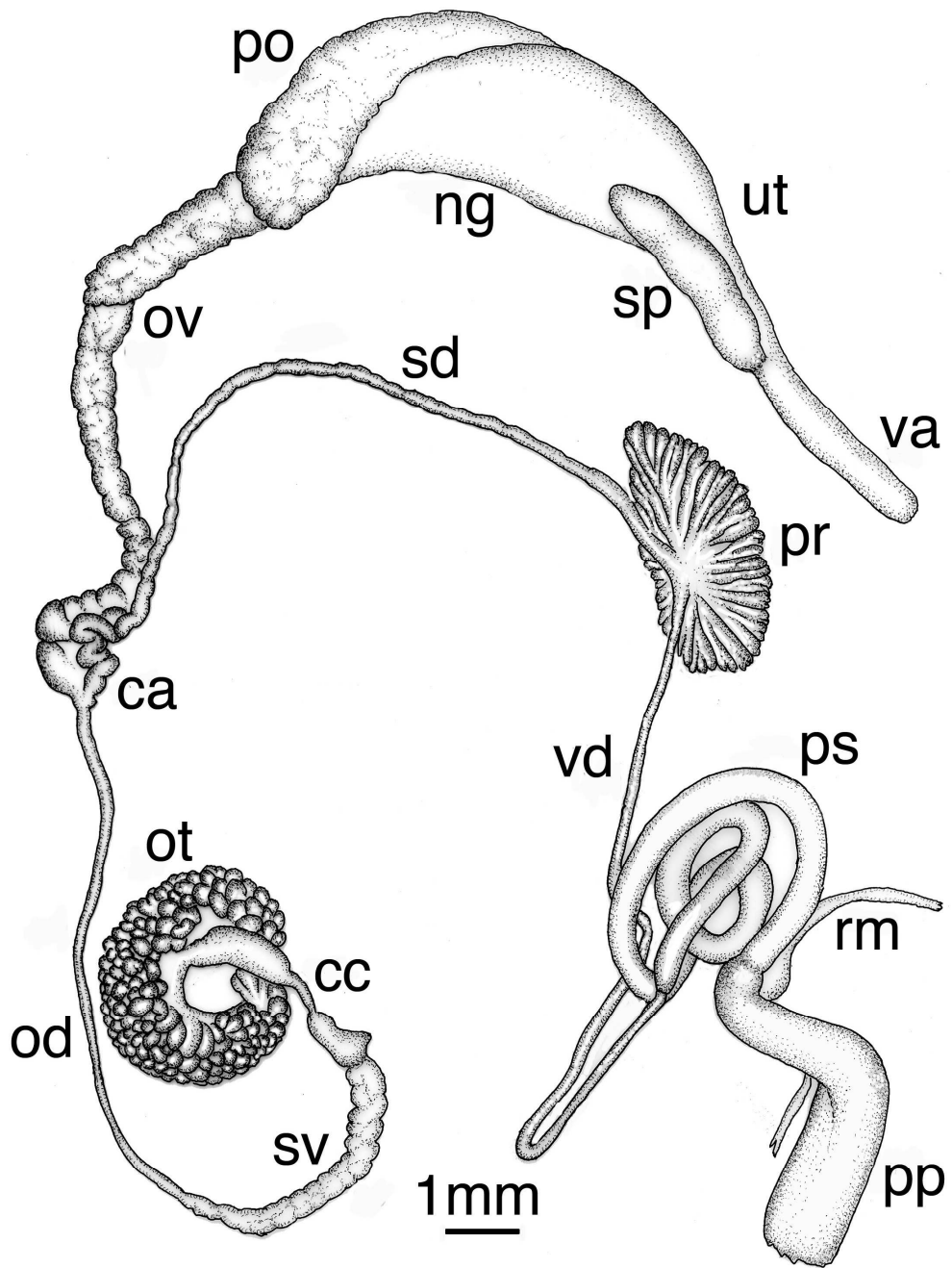


Figure 6