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Morphology of the male reproductive tract in the water scavenger beetle *Tropisternus collaris* Fabricius, 1775 (Coleoptera: Hydrophilidae)

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ABSTRACT

Members of the Hydrophilidae, one of the largest families of aquatic insects, are potential models for the biomonitoring of freshwater habitats and global climate change. In this study, we describe the morphology of the male reproductive tract in the water scavenger beetle *Tropisternus collaris*. The reproductive tract in sexually mature males comprised a pair of testes, each with at least 30 follicles, *vasa efferentia*, *vasa deferentia*, seminal vesicles, two pairs of accessory glands (a bean-shaped pair and a tubular pair with a forked end), and an ejaculatory duct. Characters such as the number of testicular follicles and accessory glands, as well as their shape, origin, and type of secretion, differ between Coleoptera taxa and have potential to help elucidate reproductive strategies and the evolutionary history of the group.

Introduction

Coleoptera is the most diverse group of insects in the current fauna, with about 400,000 described species and still thousands of new species waiting to be discovered (Slipinski et al., 2011; Kundrata et al., 2019). Beetles have conquered several ecological niches in the terrestrial environment (Toussaint and Short, 2017) and are the most abundant group of aquatic insects, with about 13,000 species, most of them representatives of Dytiscidae and Hydrophilidae (Short, 2018). Coleoptera species richness is greatest in the Neotropical region, particularly in South America (Jach and Balke, 2008; Short, 2018).

Aquatic beetle faunas have been widely sampled in ecological studies focused on biomonitoring and species richness. These relatively large insects are sensitive to environmental changes, show wide distribution, can be easily sampled, and occupy different guilds in aquatic and semi-aquatic ecosystems (Short and Fikáček, 2011; Short, 2018). Because of these characteristics, aquatic beetles have been proposed as potential bioindicators of water quality and global climate change impacts (Guareschi et al., 2012; Kaboré et al., 2016).

The evolutionary history of Coleoptera diversity (Lawrence et al., 1995; Lawrence, 2016) has been grounded in phylogenies with characters derived from external and molecular morphology (e.g., Lawrence et al., 2011; Zhang et al., 2018). Although hydropilid form a diverse group it is one of the few families of Polyphaga beetle with robust phylogenetical data (Short and Fikáček, 2013; Nasserzadeh et al., 2017; Toussaint et al., 2017).

The morphology of the reproductive tract and aspects of spermatogenesis have been described in several species of Coleoptera (Sharp, 1912; Happ, 1992), including representatives of Bruchidae (Sigh, 1978; Kasap and Crowson, 1979), Carabidae (Sasakawa, 2007; Hodgson et al., 2013; Kruger et al., 2013; Schubert et al., 2017), Chrysomelidae (Kasap and Crowson, 1979; Wang et al., 2007), Curculionidae (Alzahrani et al., 2013; Wu et al., 2017), Dryophthoridae (Paoli et al., 2014), Scarabaeidae (Carrilo-Ruiz et al., 2008; Martínez and Trotta-Moreu, 2010), Scolytidae (Cerezke, 1964), and Tenebrionidae (Dias et al., 2013, 2015). In Hydrophilidae, a highly diverse family with about 3,100 aquatic species (Madaric et al., 2013; Short and Fikáček, 2013; Nasserzadeh et al. 2017), some studies on different taxa focus on the morphology of internal organs (eg Gundevia

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and Ramamurty, 1977; Bameul, 1996; 1997; Nasserzadeh et al., 2005; Nasserzadeh and Komarek, 2017; Angus et al., 2020; Nasserzadeh, 2020).

Although those studies provide good data about the structure and function of some organs, there are few comparative data from reproductive tract, which might reveal differences between groups, such as presence/absence of structures and shape variations in reproductive organs and spermatozoa (Araújo et al., 2012, 2020, 2021; Paoli et al., 2014; Munhoz et al., 2020; Nasserzadeh, 2020). This study aimed to describe the anatomy and histology of the male reproductive system in the hydrophilid *Tropisternus collaris* Fabricius, 1775, in order to contribute to the knowledge of reproductive biology and evolutionary history of the Coleoptera.

Material and methods

Adult males of *T. collaris* (n = 13) were collected in the Pampulha lagoon (19°50'S 43°59'"W) in the municipality of Belo Horizonte, state of Minas Gerais, Brazil. The collections were carried out with entomological nets and sieves.

The insects were immobilized at -5 ^{0}C for 10 min and the reproductive tract was dissected in sodium cacodylate buffer, 0.1 M, pH 7.2 and transferred to 2.5% glutaraldehyde in the same buffer for 2 hours at room temperature. Then, the samples were washed in the same buffer, dehydrated in a graded ethanol series (50, 70 90 and 95%) and embedded in historesin (Leica Historesin). Semithin sections (2 µm thick) were obtained with a glass knife in a rotary microtome (Leica RM 2255, Germany), stained with 1% toluidine blue sodium borate, analyzed and photographed with light microscopy (Olympus BX-60, Japan).

Results

The reproductive tract of sexually mature *T. collaris* males comprised a pair of testes, *vasa efferentia*, *vasa deferentia*, seminal vesicles, two pairs of accessory glands (ag1 = a tubular pair with a forked end; ag2 = a bean-shaped pair), and an ejaculatory duct (Fig. 1A, 1B). Accessory glands and seminal vesicles fused at their extremities, opening into the ejaculatory duct (Fig. 1A).

Testes had a pectinate shape and contained at least 30 follicles each (Figs. 1A, 2A). Spermatozoa moved in bundles from testicular follicles through the short *vas efferens* into the *vas deferens*, where bundles were dissociated (Fig. 2A, 2B). Testis follicles showed cells at three different stages of spermatogenesis (Fig. 2A–D). The growth zone was characterized by groups of spermatogonia forming cysts, the maturation zone contained sperm bundles (Fig. 2C), and the differentiation zone comprised elongated spermatids and spermatozoa (Fig. 2D).

Tubular accessory glands (ag1) were parallel to the *vas deferens* in the vicinity of the bean-shaped accessory gland (ag2) (Fig. 3A, 3B). The *vas deferens* was dilated near the glandular region, opening into a seminal vesicle, which showed the lumen filled with spermatozoa, a simple squamous epithelium, and an external muscle layer (Fig. 3C, 3D). Tubular accessory glands were composed of a single epithelial layer (Fig. 4A). The most distal portion of the tubular gland (ag1) showed a prismatic epithelium and apocrine-type secretion with many granules in the lumen (Fig. 4B, 4C). Bean-shaped accessory glands (ag2) had a folded wall with a monolayered prismatic epithelium and lumen full of granular secretion (Figs. 3A, 3B, 4A, 4B). The region of the bean-shaped gland (ag2) where cisternae were found was lined with thick muscle fibers (Figs. 3A, 4A, 4B). Near the center of the bean-shaped gland (ag2), tubular accessory glands (ag1) and *vasa deferentia* fused and opened into the ejaculatory duct (Figs. 4A, 4B, 5A).

The ejaculatory duct was long and dilated in the median region, formed by simple cuboidal epithelium with basal nuclei, lined internally by a thin cuticle and externally by several layers of longitudinal and circular muscles (Fig. 5B, 5C).

Discussion

The anatomy of the reproductive tract of *T. collaris* is similar to that of most Coleoptera families, which, in general, is composed of testes, *vasa deferentia*, a pair of seminal vesicles, and a single ejaculatory duct, varying mainly in the number of testicular follicles and type of accessory glands (Cerezke, 1964; Nasserzadeh et al., 2005; Dias et al., 2013; Kruger et al., 2013; Paoli et al., 2014; Schubert et al., 2017; Wu et al., 2017; Nasserzadeh, 2020). For beetles, the morphological characters from reproductive tract are potential sources for phylogenetical hypotheses in higher taxa (Dettner et al., 1986; Opitz, 2014; Nasserzadeh, 2020). In fact, Imms (1964) investigated characters derived from testicular morphology and identified two general anatomical patterns of the male reproductive tract in the two largest suborders of Coleoptera, Adephaga and Polyphaga. In Adephaga, testes are composed of a singular, spiral follicle surrounded by a conjunctive capsule (Will et al., 2005; SasaKawa,

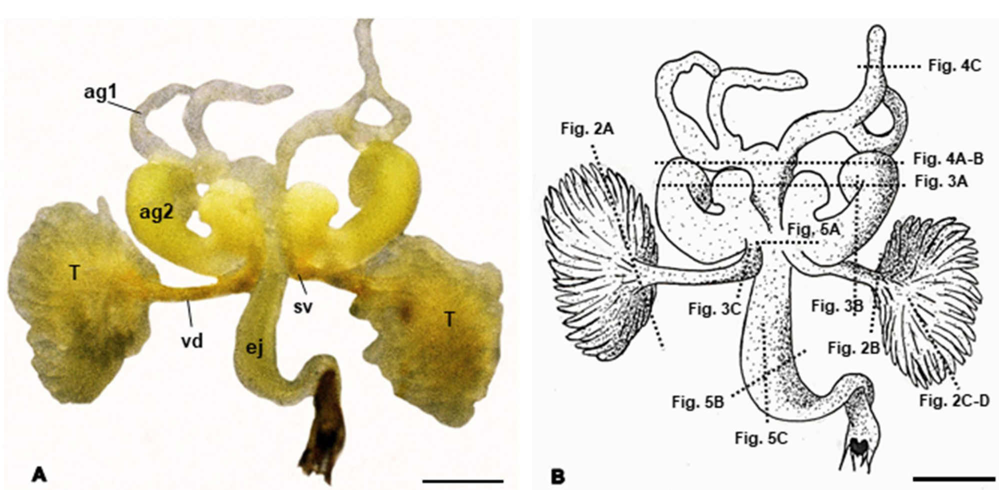

Figure 1 Anatomy of the male reproductive tract of *T. collaris*. (A) Photograph of the anatomy of the male reproductive tract showing the testes (T), *vasa deferentia* (dd), seminal vesicles (sv), tubular accessory glands (ag), bean-shaped accessory glands (pg), and ejaculatory duct (ej). (B) Schematic drawing of the reproductive tract showing the position and direction of histological sections presented in Figures 2–5. Bars: 0.4 cm.

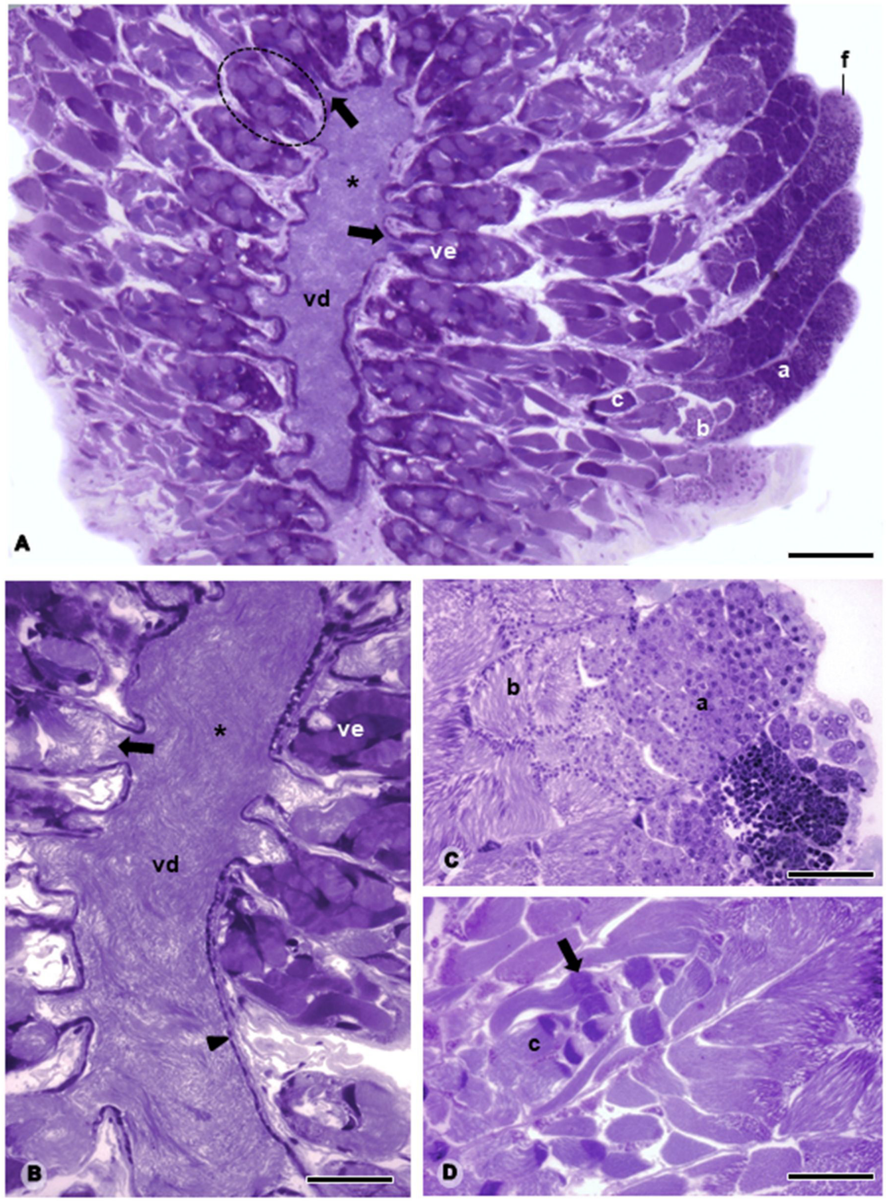

Figure 2 Histology of the male reproductive tract of *T. collaris*. (A) Longitudinal section of a testis showing the follicles (f); growth (a), maturation (b), and differentiation (c) zones; and *vas efferens* (ve) opening (arrow) into a *vas deferens* (vd) with lumen full of spermatozoa (asterisk). (B) Longitudinal section showing detail of the *vasa efferentia* (ve) in the confluence zone (black arrow) with the *vas deferens* (vd). The white arrow points to sperm bundles in the *vas efferens*. The asterisk indicates the lumen of a *vas deferens* with dissociated bundles. The arrowhead points to the epithelial of the *vas deferens*. (C and D) Details of the stages of spermatogenesis, showing zones of cell growth (a), maturation (b), and differentiation (c). The arrow points to a sperm bundle. Bars: A and B = 600 µm, C and D = 200 µm.

2007; Schubert et al., 2017). In Polyphaga, the number of testicular follicles varies, even within families: 6–60 in Tenebrionidae (Doyen and Tschinkel, 1982; Dias et al., 2013), 6 in Geotrupidae (Martínez and Trotta-Moreu, 2010) and Scarabaeidae (Carrilo-Ruiz et al., 2008), 8–20 in Curculionoidea (Calder, 1990; Goldson and Emberson 2012; Wu et al., 2017), 20 in Chrysomelidae (Simões, 2012), and 50–60 in Dryophthoridae (Paoli et al., 2014). In *T. collaris* adults, each testis contained at least 30 follicles and cells at different stages of spermatogenesis, indicating continuous sperm production, a common characteristic of insects that copulate several times during adulthood (Moreira et al., 2008; Wu et al., 2017; Munhoz et al., 2020).

In Hydrophilinae, morphological differences in the testes were reported among genera (Nasserzadeh, 2020). The accessory gland (ag2) size was used as a feature for the testis size. In this sense, in the representatives of *Hydrochara* and *Hidrophilus*, the testes are smaller than the accessory gland (ag2). However, in *Hydrochara* the testis follicles are distinguishable, short and narrowed, whereas in *Hydrophilus* the testes are compact and bean-shaped and the follicles are hard to be distinguished. Testes larger or equal to the accessory gland (ag2) occur in *Hydrobius fuscipes* and in *Sternolophus testis*, the latter similar to here observed for *T. collaris*, in which the testis follicles are tubular and clustered. Short et al. (2017) have pointed out that *Tropisternus* as a sister group of *Sternolophus,* which may be supported by similar testis morphology between the closely related genera of Hydrophilini.

As in *T. collaris* (Hydrophilidae), the seminal vesicles are well developed in other Coleoptera, including the curculionoid families (Calder, 1990; Hoffman and Raffa, 1992; Goldson and Emberson, 2012; Wu et al., 2017), and Carabidae (Schubert et al., 2017). However, males of *Rhynchophorus ferrugineus* Heyne, A.-Taschenberg & O., 1908 (Dryophthoridae) do

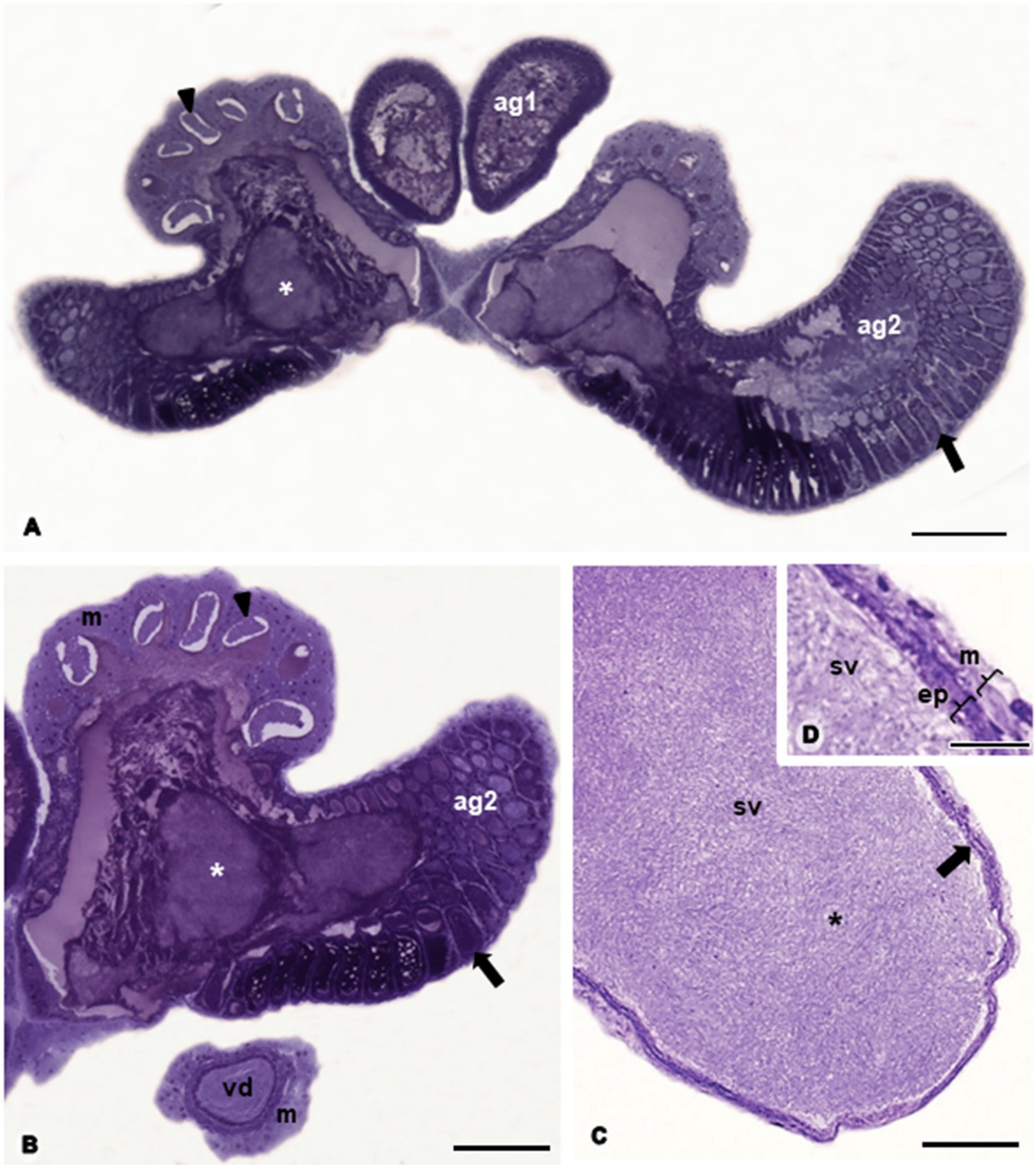

Figure 3 Histology of the male reproductive tract of *T. collaris*. (A and B) Cross section of the glandular region showing the tubular accessory gland (ag1) and bean-shaped gland (ag2). The arrowhead indicates the epithelial folds, and the arrows point to the region of the secretory epithelium. Note the secretion accumulated in the lumen of the bean-shaped gland (asterisk). (C and D) Cross section of a seminal vesicle (sv) with simple epithelium (arrow), and lumen full of spermatozoa (asterisk). In (D) detail of the epithelium (ep) and the external muscular layer (m). Bars: A = 200 µm, B and C = 100 µm, D = 25 µm.

not have a typical seminal vesicle, a large number of spermatozoa can be found in the proximal region of *vasa deferentia* (Paoli et al., 2014).

We observed two pairs of accessory glands in *T. collaris*. The number varies among Coleoptera families, being used as an auxiliary character in the taxonomy of Bruchidae (Sigh, 1978). Scarabaeidae males have a pair of accessory glands (Carrilo-Ruiz et al., 2008) and Curculionidae, one or two pairs (Burke, 1959; Cerezke, 1964; Wu et al., 2017). Tubular accessory glands (ag1), such as those found in *T. collaris*, are common in many coleopterans (Kaulenas, 1992), including Chrysomelidae (Anderson, 1950), Scarabaeidae (De Loof and Lagasse, 1972), Cleridae, Carabidae (Opitz, 2003), and Dryophthoridae (Paoli et al., 2014). In addition to tubular accessory glands (ag1), *T. collaris* has a pair of bean-shaped glands (ag2), as also described in *Hydrochara* spp. (Nasserzadeh et al., 2005). In *Hydrochara* ssp., the bean-shaped gland (ag2) contains individual globules, where secretions are produced and stored (Nasserzadeh et al., 2005). Such secretions are mainly associated with sperm maintenance and activation, induction and acceleration of oviposition in females, spermatophore formation, and mechanisms to ensure fidelity, such as formation of a barrier to prevent further mating (Chen, 1984; Gillott, 2003; King et al., 2011; Polat et al., 2020).

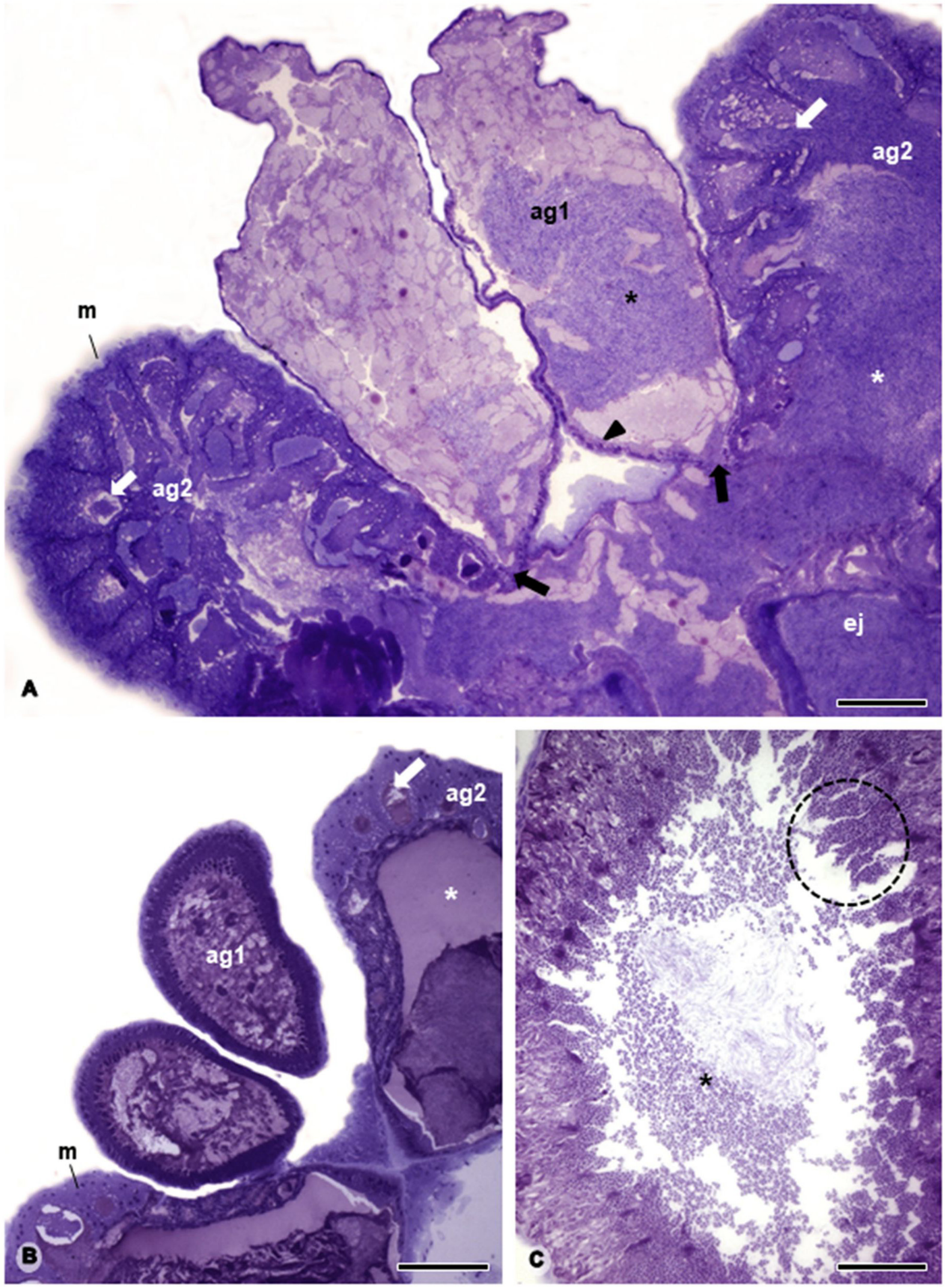

Figure 4 Histology of the male reproductive tract of *T. collaris*. (A and B) Longitudinal section of the glandular region showing a tubular gland (ag1) with lumen full of secretion (black asterisk). The arrowhead points to the nucleus of the epithelium, and the black arrows to the opening of the common duct into the bean-shaped gland (ag2) in a region that precedes the ejaculatory duct (ej). Note the multilobed secretion (white arrow), lumen with secretion (white asterisk), and muscle layer (m) of the bean-shaped gland (ag2). (C) Cross section of the apical portion of the tubular gland showing apocrine secretion (dashed circle) and secretion granules in the lumen (asterisk). Longitudinal section of the ejaculatory duct (ej) showing the lumen (asterisk), simple cuboidal epithelium (arrow), and thick muscle layer (m). Bars: A = 200 µm, B = 150 µm, and C = 100 µm.

Like most insects, *T. collaris* has a single, median ejaculatory duct covered by a cuticle, evidence of its ectodermal origin (Bushrow et al., 2006; Araújo et al., 2010). The cuticle helps stabilize the duct against the action of intrinsic muscles during ejaculation. The dilated region in the median portion of the ejaculatory duct appears to be where spermatozoa are pumped to the female bursa copulatrix through the action of a strong muscle sheath surrounding the duct epithelial wall (Nasserzadeh et al., 2005; Wu et al., 2017).

In this study, we described the anatomy and histology of the male reproductive tract of *T. collaris*. The size and shape of the testis, number of testis follicles and accessory glands have differences between the species of the tribe already studied. These results may contribute to

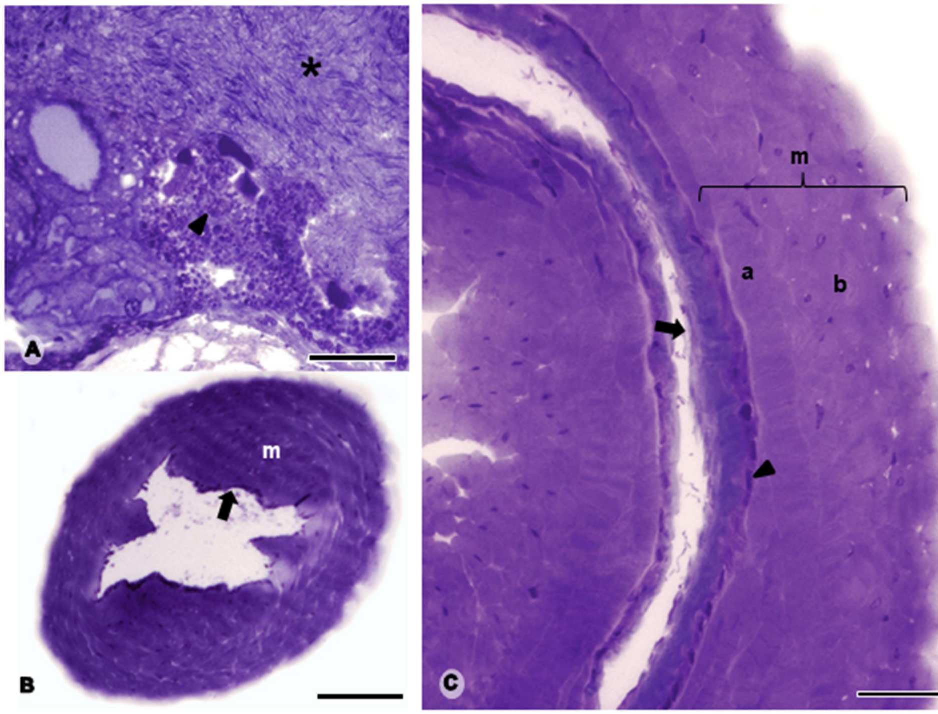

Figure 5 Histology of the male reproductive tract of *T. collaris*. (A) Cross section of the confluence region of spermatic ducts and glands showing the presence of secretion (arrowhead) and spermatozoa (asterisk). (B) Cross and (C) longitudinal sections of the ejaculatory duct, indicating the cuboidal epithelium with basal nuclei (arrowhead), cuticle (arrowhead), and thick muscle layer (a = circular muscles, b = longitudinal muscles). Bars: A and B = 200 µm, C = 400 µm.

elucidating reproductive strategies in Hydrophilidae and the evolutionary history of aquatic Coleoptera.

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Conflicts of interest

The authors declare no conflict of interest.

Author contribution statement

VAA and ILAM conceived the research, dissections, photographs and the reproductive morphology work. ILAM realized illustrations and VAA the preparation of plates. VAA and JES improved the introduction, results and discussion of the document. All authors participated in the final redaction and critically reviewed the manuscript and accepted the final version of the paper.

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