

Gonad morphology of *Rhyacichthys aspro* (Valenciennes, 1837), and the diagnostic reproductive morphology of gobioid fishes

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Abstract

Rhyacichthys aspro is a “basal” taxon in the Suborder Gobioidi of the teleost order Gobiiformes. We provide detailed descriptions of the reproductive morphology of adult males and females to assess the diagnostic reproductive morphological characters of this speciose clade of bony fishes. Female *R. aspro* are asynchronous spawners: they are able to spawn more than once in a breeding season. Oocytes are inferred to have short attachment filaments. A conspicuous feature of the external anatomy of the reproductive system (RSy) of female *R. aspro* is an ornate fimbriate pad upon which the urogenital papilla rests. The male reproductive system is characterized by an intralobar collection system in both the testicular and secretory lobes, termed the “sperm-collecting canal” and “milt-collecting canal,” respectively. These may provide additional storage for sperm and milt. The spermatogenic lobe, or testis, is that portion of the male gobioid RSy comprising seminiferous lobules and separate from other RSy components. The secretory lobe is that portion of the male gobioid reproductive system that consists of secretory lobules and is separated from other components of the male RSy. The secretory lobe has also been called, in English, the sperm-duct gland, accessory gonadal structure, or seminal vesicle, and is endorsed as a synapomorphy of gobioid fishes.

KEYWORDS

fimbriate pad, fish ovary, fish testes, sperm-duct gland, urogenital papilla

1 | INTRODUCTION

Gobioids are a highly diverse group of mostly small, marine, and freshwater teleost fishes. With over 2500 valid species, they comprise one of the largest clades of acanthomorphs: the suborder Gobioidi in the order Gobiiformes (Betancur-R et al., 2017; Nelson et al., 2016). They inhabit nearly all pantropical and temperate aquatic habitats, except for the northernmost and southernmost latitudes. A majority are inconspicuous, tropical coastal marine species, a large portion of the

overlooked and understudied cryptobenthic fauna that plays a critical ecological role on coral reefs (Brandl et al., 2018; Brandl et al., 2019; Depczynski & Bellwood, 2003). Some so-called terrestrial gobioid species spend much of their time out of water on mudflats (Jaafar & Murdy, 2017; Martin, 2014; Sayer, 2005; Zhang et al., 2003). One species lives in small pools high in the splash zone (Greenfield & Randall, 2004); another has been recorded from a depth of over 1100 meters in the western Pacific (Murdy, 2011). An unknown number of species of gobioids are amphidromous, a specialized type of diadromy

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in which adults live and breed in freshwater and larvae are carried passively to marine waters where they transform then migrate back upstream, often to their natal habitat. Many of these species are excellent waterfall climbers, with one species able to climb a vertical drop of 480 m in Hawaii (Englund & Filbert, 1997; Ford & Kinzie, 1982).

Two families are, by consensus, the most “basal” of the gobioids: the western Pacific Rhyacichthyidae, comprising two genera and three species (*Rhyacichthys aspro* [*R. aspro*], *R. guilberti*, and *Protogobius*

attiti); and the East and Southeast Asian Odontobutidae, with some 15 to 22 species in six genera (Li et al., 2018). The families are allopatric (Parenti, 2019: fig. 12). Adult rhyacichthyids are modified for life in fast-flowing streams; they are dorsoventrally flattened and their pectoral and pelvic fins are broad and used to maintain their position (Figure 1).

The genus *Rhyacichthys* is recovered as “basal” to all other gobioid taxa in some molecular phylogenies (Agorreta et al., 2013; Larson, 2011; Thacker & Hardman, 2005). Morphologists concur that

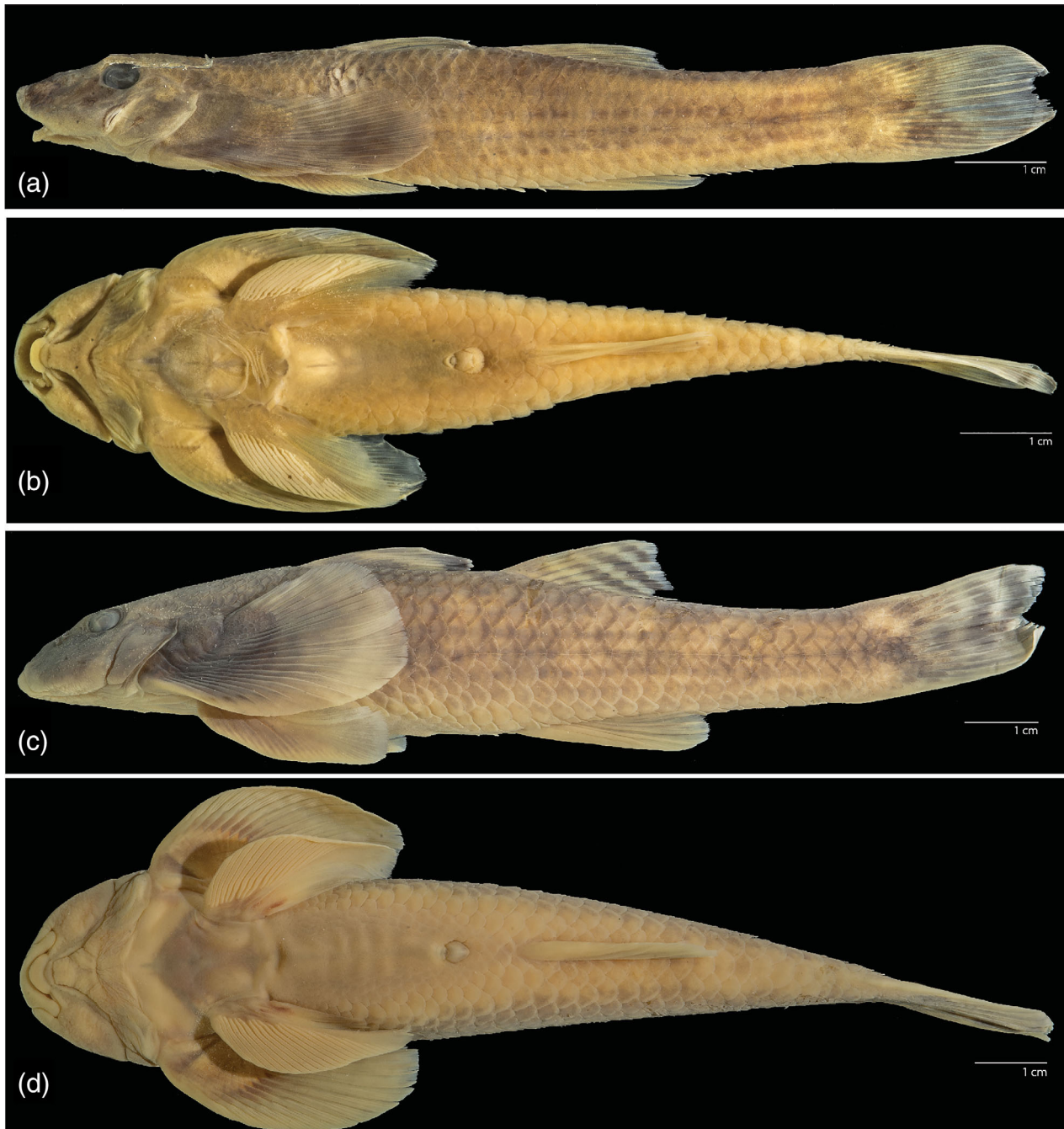


FIGURE 1 (a) and (b) *Rhyacichthys aspro*, adult female, USNM 320018. (a) Lateral view; (b) ventral view. Top of head dissected as part of another study. (c) and (d) *R. aspro*, adult male, USNM 390006. (c) Lateral view; (d) ventral view. Bar = 1 cm. Source: Photo by S. J. Raredon

Rhyacichthys has the inferred plesiomorphic states of anatomical characters that differentiate it from all other gobioids, such as two rather than one infraorbital bones, and three rather than one or two epural bones (see Miller, 1973; Springer, 1983, pp. 35–36). In a morphological phylogenetic hypothesis, Hoese and Gill (1993), fig. 9) placed the Rhyacichthyidae at the base of the gobioid tree, with the Odontobutidae sister to all remaining gobioids. More recent molecular hypotheses of Li et al. (2018), Kuang et al. (2018), and McCraney et al. (2020) propose that these two families are sister taxa, which together are sister to all other gobioids.

The phylogenetic position of *Rhyacichthys* has focused studies on its morphology to assess gobioid monophyly and search for possible close relatives (e.g., Miller, 1973; Springer, 1983; Winterbottom, 1993). The anatomy of the reproductive system of *R. aspro* was described by Miller (1992) who proposed the sperm-duct gland as a gobioid synapomorphy. Comparative anatomy of the sperm-duct gland of gobies was studied in detail by Eggert (1931). Variation in form of the sperm-duct gland among species of Japanese gobioids was examined by Egami (1960) and Arai (1964), although none of these studies included specimens of *Rhyacichthys*. Miller's (1992) study of *R. aspro* was limited as he had just one mature male specimen and he did not discuss or illustrate histology in detail.

The reproductive system (abbreviated herein as RSy) of male teleosts in its simplest form includes a bilateral pair of testicular lobes comprising seminiferous lobules having lumina lined with a germinal epithelium sensu Grier (2000, 2002), Grier and Lo Nostro (2000) and Grier et al. (2016). Posteriorly, the two testicular lobes unite and typically have a single gonoduct for sperm egress via the papilla. Reproductive secretory materials may be produced throughout the male RSy (Cinquetti, 1997; Patzner et al., 1991).

The RSy of male gobioids typically includes testes (seminiferous lobules) that comprise a spermatogenic region and secretory lobules that comprise a secretory region (Fishelson, 1989; Miller, 1984). These secretory tissues may occupy separate lobes or, less commonly, a restricted region within the testis (Fishelson, 1991; Miller, 1984). The spatial association of the spermatogenic and secretory portions varies among gobioid taxa (Egami, 1960; Arai, 1964; Cole, 2010a, 2010b; Fishelson, 1991; Miller, 1984). Some taxa lack secretory tissues (Mazzoldi et al., 2011). Although RSy morphology is known to be highly diverse among gobioids (Miller, 1984; Fishelson, 1991; Cole, 2010a, 2010b), much of the information available is narrow in scope and taxonomically scattered, thereby limiting our understanding of RSy evolution.

To infer the evolutionary pattern(s) of reproductive morphology in the Gobioidae, we undertook a detailed examination of the internal and external reproductive morphology of adult male and female *R. aspro*. We have two goals: (1) to assess the diagnostic reproductive morphological characters of the Gobioidae; and (2) to develop a clear and precise terminology for the various portions of the RSy of *R. aspro* that may be applied across gobioid taxa.

2 | MATERIALS AND METHODS

Twelve specimens of *R. aspro* (Valenciennes, 1837) from the Smithsonian's National Museum of Natural History fish collection (USNM) were used in this study (Table 1). Each specimen is identified by a USNM catalog number, sex, standard length (SL), preparation, and notes on its state of preservation. Additional collection data on these specimens are available from the online catalog of the Division of Fishes: <http://collections.mnh.si.edu/search/fishes/>

TABLE 1 Twelve specimens of *Rhyacichthys aspro* used in this study

Catalog no.	Sex	Size (SL)	Preparation/notes
USNM 385607	♂	120.0 mm	Histology of RSy and urogenital papilla/paraffin embedded/specimen straight
USNM 432483	♀	95.0 mm	Histology of RSy and urogenital papilla/paraffin embedded
USNM 432483	♀	106.0 mm	External morphology, urogenital papilla
USNM 371845	♂	55.0 mm	Histology of whole specimen/paraffin embedded/immature
USNM 385607	♂	120.0 mm	Histology of RSy and urogenital papilla/plastic embedded/specimen curved
USNM 390005	♀	87.5 mm	Histology of RSy and urogenital papilla/plastic embedded
USNM 320018	♀	91.2 mm	External morphology, urogenital papilla
USNM 390006	♂	115.0 mm	External morphology, urogenital papilla
USNM 355578	♀	118.3 mm	External morphology, urogenital papilla
USNM 432490	♂	155.0 mm	External morphology, urogenital papilla
USNM 346645	♀	103 mm	External morphology, urogenital papilla
USNM 390047	♀	105 mm	External morphology, urogenital papilla

Five specimens were sectioned to study histology: three embedded in paraffin and two in plastic (Table 1). Specimens were fixed originally in 10% formalin and maintained in 75% ethanol. Each specimen was sexed using urogenital papilla morphology (Egami, 1960; Miller, 1992): males have a pointed papilla whereas females have a more broad, rounded papilla. In two specimens, an adult male (USNM 385607) and a female (USNM 432483), a ventral longitudinal incision was begun at the mid-point between the pectoral fins, bisected the pelvic girdle, and continued to the anus to remove the entire reproductive system (RSy, used here to avoid confusion with RS, a common abbreviation for reproductive success). The line of cut subsequently diverged to either side of the anus and continued caudad to encompass the urogenital papilla and the integument immediately surrounding it. Additional lateral incisions on each side were made from the ventral midline dorsad toward the vertebral column to permit the retraction of the lateral musculature to access the abdominal organs. Viewed through a stereo microscope (Zeiss STEMI SV8), the components of the RSy including the urogenital papilla and adjacent body tissues were removed along with portions of the adjacent and partially attached gut and urinary system tissues and maintained in 75% ethanol prior to histological processing.

When different body sections from the same specimen were processed independently, each section was further coded to indicate its relative position within the body. All histology was carried out in the Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland.

Following standard dehydration procedures (Coolidge & Howard, 1979), the reproductive complex consisting of the RSy and associated urogenital papilla of each specimen was embedded in paraffin (Surgipath ParaplastPlus, Leica). Paraffin embedding allows for the sectioning of continuous ribbons of tissue to reveal finely detailed sequential sections of morphology. The paraffin-embedded tissues were sectioned transversely at 6 μm (Leica RM2255 microtome) and mounted on glass microscope slides (Fisherbrand

Superfrost Plus pre-cleaned, 25 \times 75 \times 1.0 mm) followed by application of DPX mounting medium and cover slipped for subsequent examination with a light microscope (Leitz, SM-LUX). Digital images were obtained using an Olympus BX63F Compound Fluorescent Microscope (Olympus Corporation, Tokyo, Japan) equipped with the

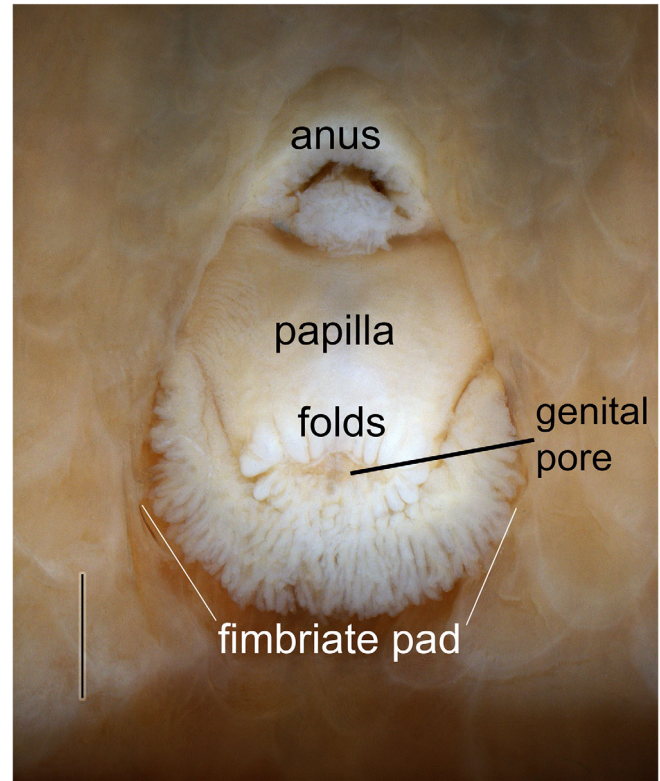


FIGURE 3 *Rhyacichthys aspro*, female urogenital region showing pleated folds at the posterior margin of the subtriangular papilla, terminating at the genital pore, lying on the fimbriate pad. The margin of the anus is also folded. Anterior is toward the top. USNM 320018. Bar = 1 mm. Source: Photo by D. N. Lumbantobing

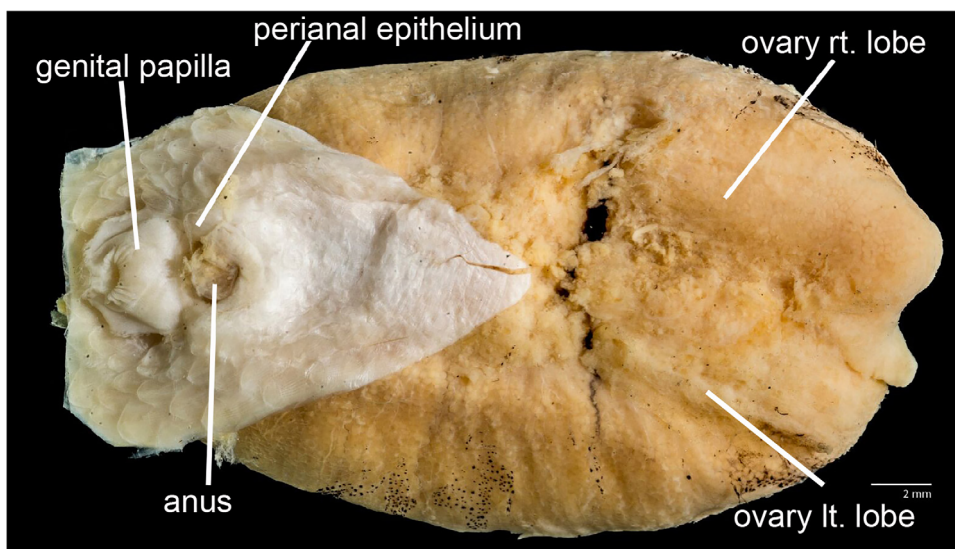


FIGURE 2 *Rhyacichthys aspro*, female reproductive system, ventral view, gross morphology. Anterior is to the right. USNM 432483. Bar = 2 mm. Source: Photo by S. J. Raredon

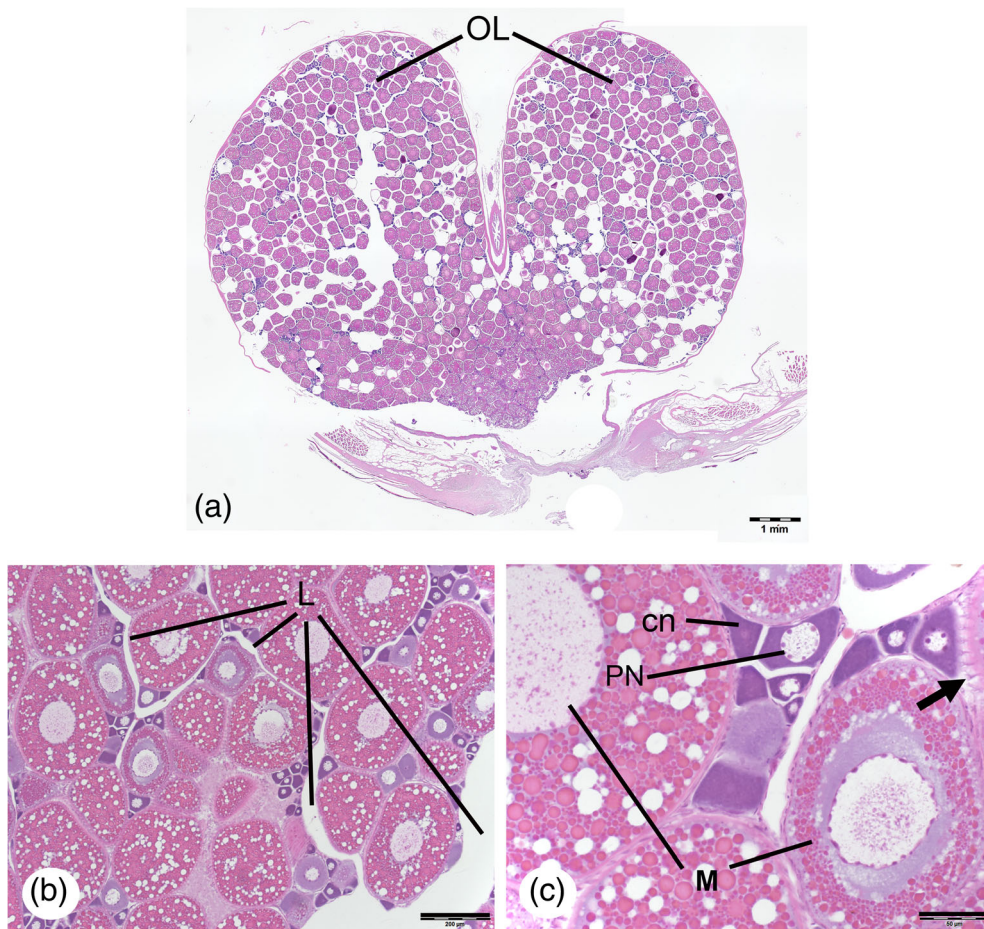


FIGURE 4 *Rhyacichthys aspro*, female reproductive system, histology. (a) Transverse section of adult ovary through the right and left lobe. The central ovarian lumen is surrounded by slightly to deeply folded ovigerous tissue. The lumen of the left lobe is visible whereas that of the right lobe is mostly occluded, being visible as a narrow gap between ovarian lamellae. Plastic-compatible hematoxylin and eosin (H&E) stain. USNM 432483. Bar = 1 mm. OL, ovarian lobe. (b) Detail of transverse section of Figure 4a showing ovarian lamellae outlined by a narrow gap demarking a portion of the ovarian lumen. H&E stain. USNM 385607. Bar = 200 μm . L, ovarian lumen. (c) Stages of developing oocytes showing chromatin nucleolar, perinucleolar, and secondary growth, maturing vitellogenic oocytes. Small filaments are noted on the zona pellucida (arrow). H&E stain. USNM 385607. Bar = 50 μm . L, ovarian lumen; CN, chromatin nucleolar oocytes; PN, perinucleolar oocytes; M, maturing, vitellogenic oocytes

Olympus imaging software package cellSens in the National Museum of Natural History Scientific Imaging Laboratory. Approximately every third slide in the series was stained with hematoxylin and eosin (H&E). Some of the remaining unstained slides were stained subsequently with Mallory Trichrome, Luxol Fast Blue or Alcian blue/Periodic acid-Schiff (AB-PAS).

A third specimen, an immature male *R. aspro* (USNM 371845) was paraffin-embedded and transverse-sectioned with the RSY in situ. Following sectioning and slide mounting as described above, the tissues were stained with Masson's Trichrome and examined using a light microscope.

The RSY of an additional adult male (USNM 385607) and female (USNM 390005) specimen of *R. aspro* were dissected as described above and each RSY was embedded in plastic resin JB-4 (JB-4[®] Embedding Kit, PolySciences Inc.). JB-4 is a glycol methacrylate-based medium that provides reliable preservation of tissue morphology without

attendant artifacts from the dehydration process used with paraffin. To facilitate complete perfusion of the embedding solution throughout the tissues of each section, prior to embedding the entire reproductive complex was cut transversely into disc-shaped sections along its length. Each section was then placed in a numbered cassette in the order of its sequential location in the body and in the same orientation (caudal-most surface down). JB-4 protocols were used to infiltrate tissues, transfer them to embedding molds, then to a standard refrigerator until sectioning. The embedded tissue was sectioned on a microtome (Leica RM2255) at 5 μm thickness and the tissue sections were placed on microscope slides (Corning 75 \times 25 double-frosted, precleaned, \times 0.96–1.06 mm) and cover slipped using DPX mounting medium. Every third slide was stained with plastic-compatible H&E. Some of the remaining slides were selected to demonstrate tissue features and stained with plastic-compatible toluidine blue. Additional specimens were used to illustrate *R. aspro* morphology (Table 1).

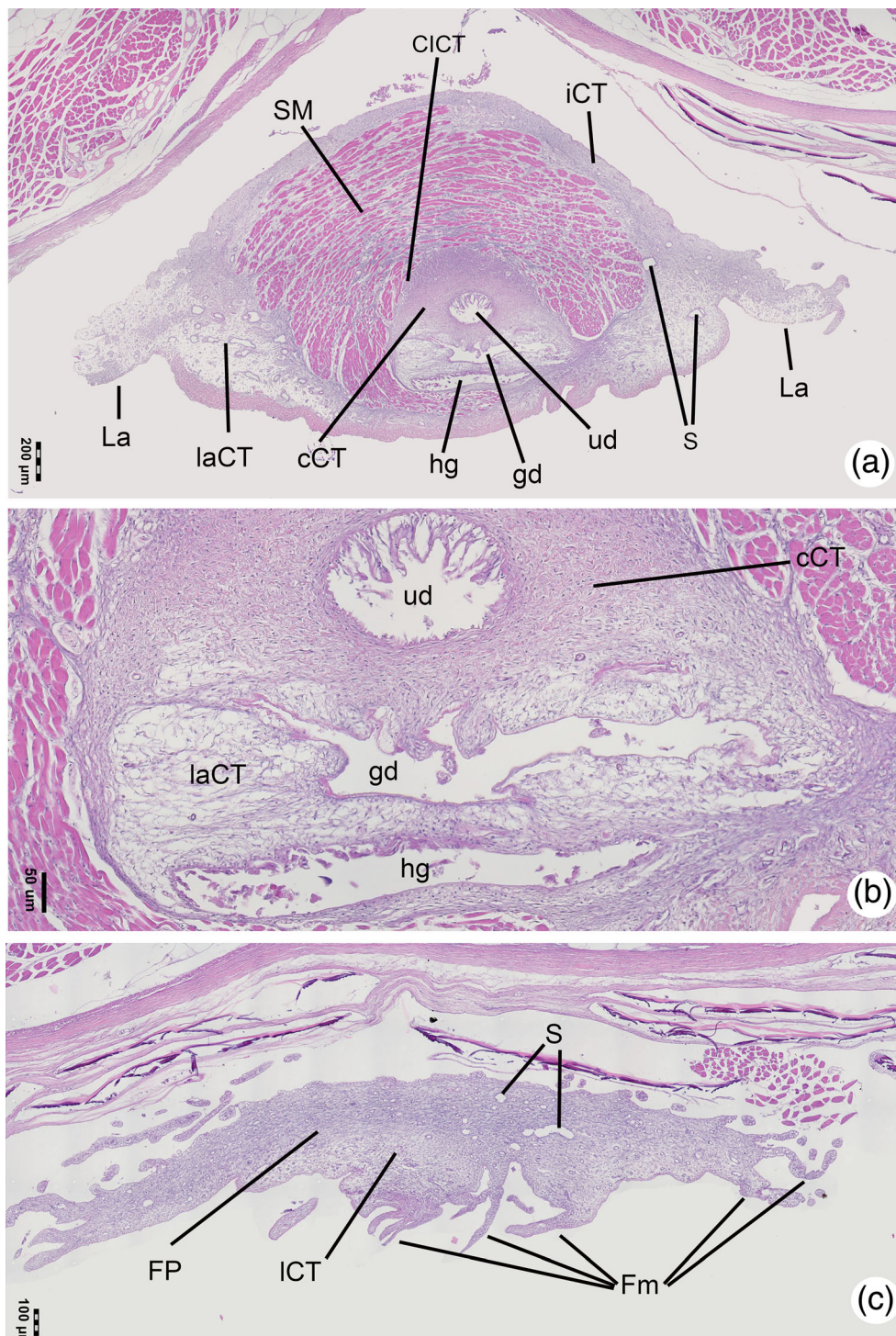


FIGURE 5 *Rhyacichthys aspro*, female reproductive system, histology, continued.

(a) Transverse section through the female papilla showing the laterally extended lappets, core of circular smooth muscle, ventral loose areolar tissue fenestrated with sinuses, and dorsal compact irregular connective tissue layer. Compact connective tissue surrounds the urinary duct, gonoduct, and hindgut. H&E stain. USNM 432483.

Bar = 200 μ m. cCT, compact connective tissue; CICT, compact irregular connective tissue; gd, gonoduct; hg, hindgut; laCT, Loose areolar tissue; La, Lappets; S, sinuses; SM, smooth muscle; ud, urinary duct. (b) Detail of papilla core showing hindgut, gonoduct surrounded by loose areolar connective tissue, and urinary duct with highly folded epithelium. H&E stain. USNM 432483. Bar = 50 μ m. cCT, compact connective tissue; gd, gonoduct; hg, hindgut; laCT, loose areolar tissue. (c) Transverse section of portion of the female *R. aspro* fimbriate pad caudal to the papilla and free of the body, comprised of irregular connective tissue with sinuses and numerous fimbriae ventrally and laterally and fewer dorsally. H&E stain. USNM 432483. Bar = 100 μ m. Fm, fimbria; FP, fimbriate pad; ICT, irregular connective tissue; S, sinuses

3 | RESULTS

3.1 | *R. aspro* female reproductive system

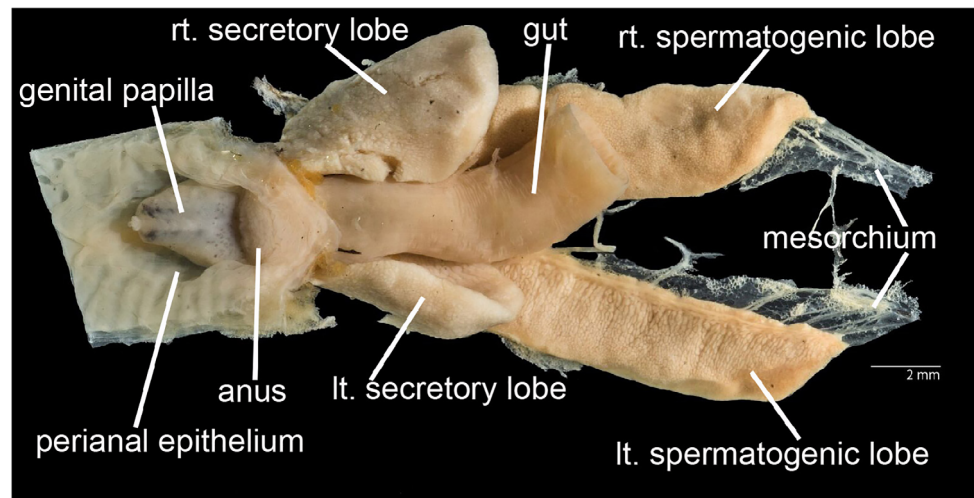
3.1.1 | Ovarian morphology

The RSy of female *R. aspro* is comparable to that of most teleosts. The external portion of the female reproductive system consists of the urogenital papilla (Figures 1b, 2, and 3) located directly posterior to

the anus. Internally, paired ovarian lobes lie on either side of the mid-line (Figures 2 and 4a) with each lobe connected to the papilla via a singular gonoduct. The ovarian wall is relatively thin. Within the ovarian lobes are numerous lamellar folds of ovigerous tissue that are separated by narrow, inter-lamellar extensions of the ovarian lumen (Figure 4b). Short filaments are noted on the zona pellucida (Figure 4c).

The germinal epithelium sensu Grier (2002) contains all stages of developing oocytes including chromatin nucleolar, perinucleolar,

FIGURE 6 *Rhyacichthys aspro*, male reproductive system, ventral view, gross morphology. Anterior is to the right. USNM 385607. Bar = 2 mm. Photo by S. J. Raredon



cortical alveolar, and maturing vitellogenic stage oocytes (Figure 4b,c). The identification of oocyte stages reflects Cole (2002), Grier et al. (2009), Brown-Peterson et al. (2011), and Grier (2012). Posteriorly, the two ovarian lobe lumina unite to form a single chamber, the common genital sinus (Cole & Robertson, 1988), into which ova pass just after ovulation. The common genital sinus becomes attenuate posteriad to form an elongate passageway, the gonoduct, an uninterrupted passage through the papilla that terminates at the genital pore. The gonoduct and the surrounding tissue constitute part of a tract, that we refer to here as the gastro-urogenital (GU) tract and that has several distinct regions.

3.1.2 | Female papilla

The papilla of female *R. aspro* is relatively short with a broad base and a wide urogenital pore, the margin of which is pleated by small folds (Figure 3). In transverse section, conspicuous lateral wing-like extensions, or lappets, extend from the base of the papilla to just before the papilla tip (Figure 5a). Lappets increase the ventral width of the papilla by approximately one-third and give the papilla a subtriangular outline (Figures 3 and 5a). The papilla is covered by a thin, squamous epithelium. Below the epithelium lies a compact layer of irregular connective tissue (iCT) that extends dorsally and laterally and transitions to loose areolar CT with numerous sinuses more ventrally. Much of the central core of the papilla comprises a robust layer of smooth muscle surrounding the gonoduct (Figure 5a).

The gonoduct and urinary duct have a central position within the papilla (Figure 5a). The urinary duct is surrounded by a robust, compact layer of CT and its lumen has a thin epithelium that is highly folded dorsally (Figure 5a,b). The gonoduct, which lies ventral to the urinary duct, has a lumen lined with a thin, unspecialized epithelium and is surrounded by a well-developed layer of loose areolar CT (Figure 5b).

3.1.3 | Fimbriate pad

A rugose, fimbriate tissue pad lies between the body and papilla and extends beyond, and independent of, the papilla terminus (Figure 3). The pad has numerous elongate, thin extensions, or fimbriae, which form a highly irregular outline in transverse section (Figure 5c). The pad and its fimbriae are covered with a thin squamous epithelium and composed primarily of loose areolar CT with numerous small blood vessels and cell-lined sinuses (Figure 5c). There are no muscle layers, individual muscle fibers, sensory cells, or secretory structures associated with the fimbriate pad or associated fimbriae. There is variation in the width of the triangular papilla and the extent of the fimbriae; a larger female adult specimen (USNM 355578; Table 1) has a somewhat broader papilla than that of the smaller female illustrated (Figure 3).

3.2 | *R. aspro* male reproductive system

3.2.1 | Gross morphology

The RSy of male *R. aspro* has confluent testicular (spermatogenic) and secretory regions in the form of individual lobes that occupy much of the abdominal cavity (Figure 6 and 7a). The paired secretory lobes are united most posteriorly and then separate more anteriorly on either side of the midline. The two testicular lobes arise from the approximate midpoint of the associated secretory lobe as a second branching lobe (see Figure 7a, left side). Each testicular lobe extends anteriorly beyond the secretory lobe terminus (Figures 6 and 8). In cross section, each testicular lobe is notably smaller than its associated secretory lobe (Figure 7a).

3.2.2 | Testicular lobe morphology

The testicular or spermatogenic lobe comprises seminiferous lobules in a curvilinear array, radiating out from the dorsomedial

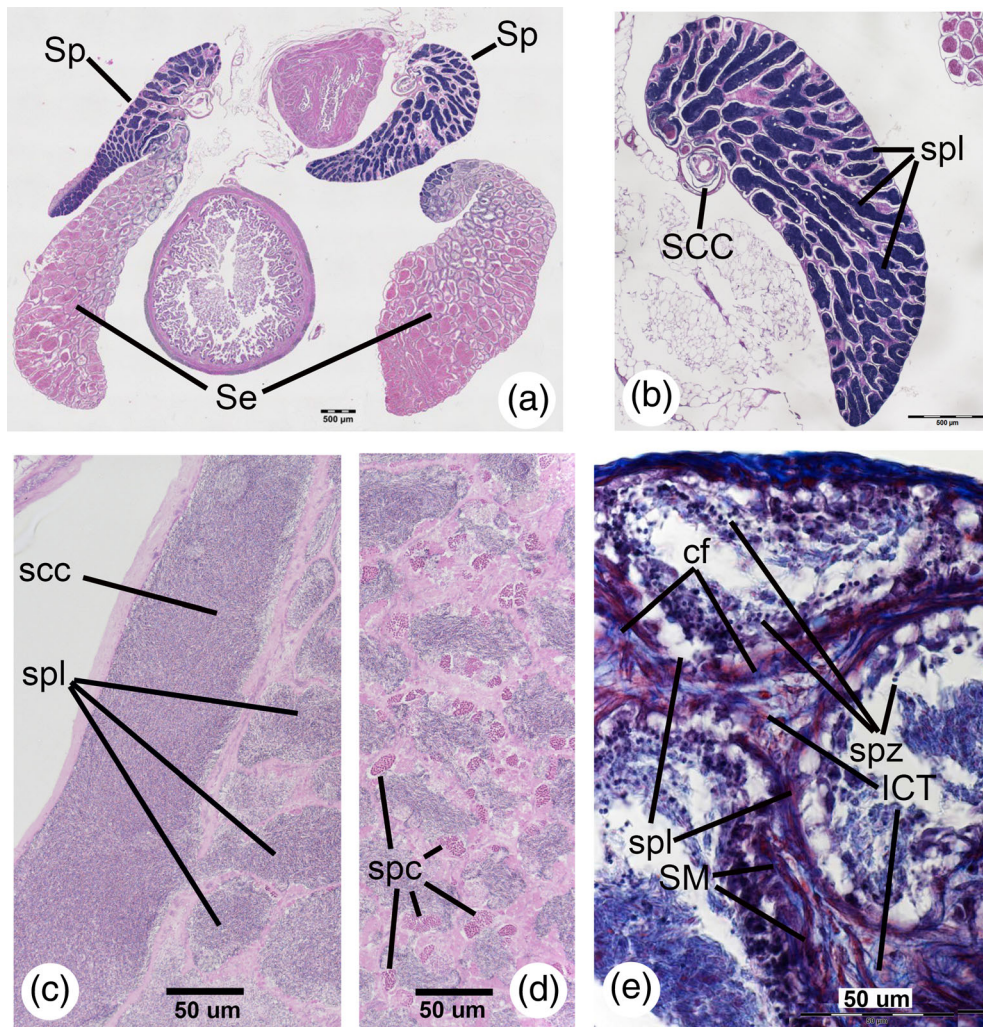


FIGURE 7 *Rhyacichthys aspro*, male reproductive system, histology, transverse and longitudinal sections. (a) Transverse section of the male *R. aspro* reproductive system in situ showing the point of merger of the testicular (spermatogenic) and its associated secretory lobe (left side of image) and point just anterior to the point of merger (right side of image). H&E stain. USNM 385607. Bar = 500 μm . Sp, spermatogenic lobe; Se, secretory lobe. (b) Transverse section of a single *R. aspro* testicular lobe showing the curvilinear arrangement of the seminiferous lobules radiating away from the dorsomedial portion of the spermatogenic lobe. The seminiferous lobule lumina here filled with spermatozoa are blind ended peripherally and continuous with a longitudinal channel, the sperm-collecting canal, the sperm-collecting canal is located dorsomedially and runs along the length of the spermatogenic lobe. H&E stain. USNM 385607. Bar = 500 μm . SCC, sperm-collecting canal; spl, seminiferous lobules filled with darkly staining spermatozoa. (c) Longitudinal section of male *R. aspro* showing the sperm-collecting canal and a direct connection between the lumina of the sperm-collecting canal and spermatogenic lobule providing open passage for sperm. H&E stain. USNM 385607. Bar = 50 μm . SCC, sperm-collecting canal; spl, testicular (seminiferous) lobules. (d) Seminiferous lobules showing a discontinuous distribution of spermatocysts in the lobule germinal epithelium. H&E stain. USNM 385607. Bar = 50 μm . Spc, spermatocysts. (e) Transverse section of male *R. aspro* showing interstitial connective tissue separating adjacent seminiferous lobules and highlighting the presence of muscle cells (staining red) and collagen fibers (staining blue) within the interstitial connective tissue. Mallory trichrome stain. Bar = 50 μm . cf, collagen fibers; ICT, interstitial connective tissue; SM, smooth muscle; spl, testicular (seminiferous) lobules; spz, spermatozoa

region of the lobe (Figure 7b). Each seminiferous lobule is blind-ended peripherally and open-ended medially where sperm can pass into a dorsomedial channel (Figure 7c) that extends the length of the testicular lobe.

The lumen of a seminiferous lobule is lined with a germinal epithelium consisting of intermittent clusters of spermatocysts filled with spermatocytes along the length of the lobule (Figure 7d). Interstitial connective tissue between the seminiferous lobules contains smooth muscle cells and collagen fibers (stained red and blue, respectively,

with Mallory Trichrome) as well as fibroblasts, Leydig cells, and small blood vessels (Figure 7e).

The dorsomedial channel that extends the length of the testicular lobe and connects to the medially located open ends of the seminiferous lobules constitutes a sperm collection system, referred to here as a “sperm-collecting canal,” abbreviated SCC (Figure 7c). The SCC provides a pathway for sperm to exit the seminiferous lobules and pass to the posterior region of the testicular lobe (Figure 8). The SCC lumen is lined with a thin, non-

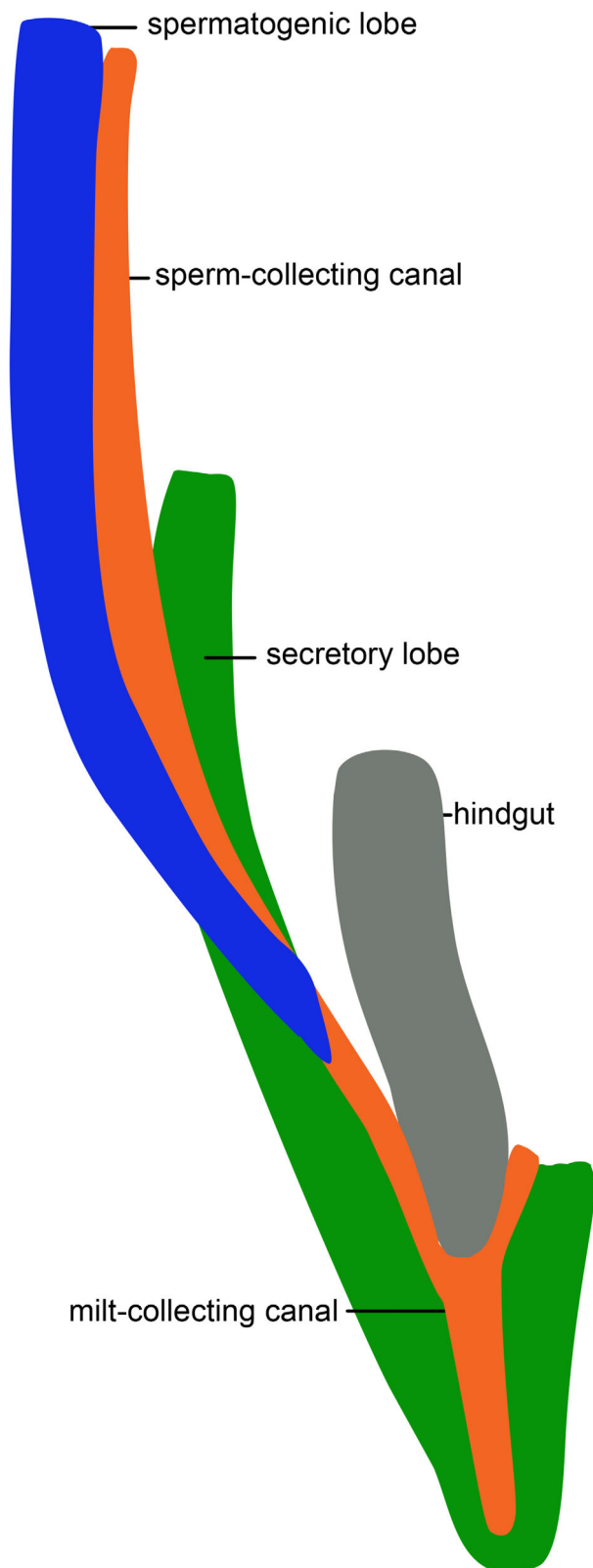


FIGURE 8 Idealized illustration of a portion of the reproductive canal collection system of an adult male *R. aspro*. The hindgut is gray. The secretory lobe is green. The testicular (spermatogenic) lobe that arises from the approximate mid-portion of the secretory lobe is blue. The internal collection system consisting anteriorly of the sperm-collecting canal (abbreviated SCC in the text) and posteriorly of the milt-collecting canal (abbreviated MCC in the text) is orange

germinal epithelium overlying collagenic connective tissue and circular smooth muscle.

3.2.3 | Transitional region at the spermatogenic-secretory lobe junction

Where the testicular and secretory lobes meet marks a transitional region, consisting of lobules that have a thin, non-germinal, non-secretory epithelium (Figure 9a,b). The lobules of the transitional region contained both sperm and secretory material.

3.2.4 | Secretory lobe morphology

The two secretory lobes originate posteriorly in the R_{Sy} and extend anteriorly on either side of the midline. Each is composed of numerous secretory lobules. The lobule lumina are lined with a tall columnar secretory epithelium containing numerous secretory cells (Figure 9c, d). The secretory material within the secretory lobules is eosinophilic (H&E stain, Figure 9a,b) and PAS⁺ (Alcian blue/Periodic acid-Schiff [AB-PAS] stain, not shown).

The secretory lobe is morphologically like the testicular lobe, consisting of secretory lobules that open dorsomedially into a channel similar to, and continuous with, the SCC. This channel contains both sperm from the SCC and secretions from the secretory lobules, which forms a milt, and is termed here a “milt-collecting canal,” abbreviated MCC. The MCC lumen, unlike that of the SCC lumen, is lined with a secretory epithelium (Figure 9c).

Caudally, the two secretory lobes are united medially: the connective tissue associated with the left and right MCC merge to form a single structure, the beginning of the gastro-urogenital (GU) tract. The lumina of the two MCCs become partitioned by infolding of the lumen wall (Figure 9d), to form numerous, smaller channels. These channels merge to form a single passageway, the gonoduct, which is surrounded by a robust layer of loose areolar CT, solitary and grouped adipocytes, blood vessels, and smooth muscle fibers. These supporting tissues constitute the wall of the GU tract.

3.2.5 | Gastrointestinal-urogenital tract

The GU tract originates at the anterior end of the gonoduct and increases in width as the gastrointestinal, urinary, and reproductive systems become progressively bound together by surrounding muscle and connective tissue (Figure 10a). We recognize four regions of the GU tract based on the form of the passageways. From anterior to posterior, the regions are: genital; gastro-genital; gastro-urogenital; and urogenital. In the genital region, only the gonoduct is present. The gastro-genital region begins where the hindgut merges with the GU tract. The gastro-urogenital region starts where the urinary duct joins the GU tract (Figure 10a). The urogenital region follows the gastro-urogenital region after the hindgut ends at the anus.

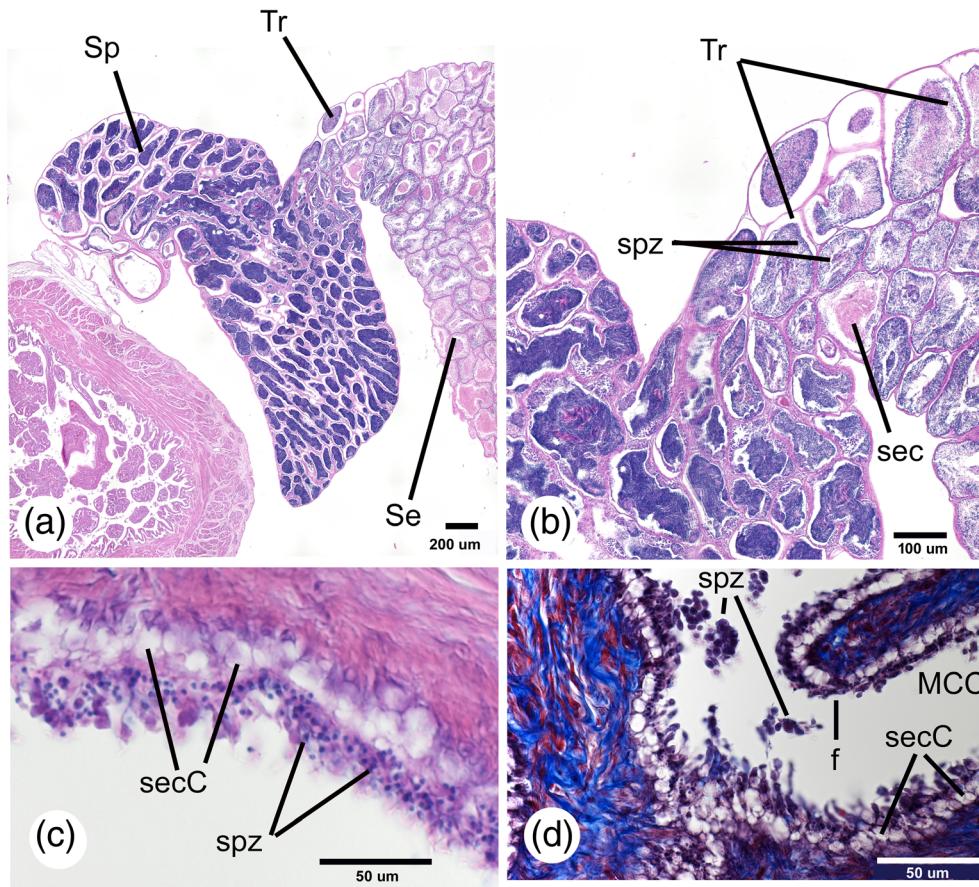


FIGURE 9 Male *Rhyacichthys aspro* reproductive system, histology, transverse sections. (a) Transverse section of junction of the testicular (spermatogenic) lobe, the secretory lobe, and the linking transitional region. USNM 385607. Bar = 200 μ m. Se, secretory lobe; Sp, testicular (spermatogenic) lobe; Tr, transitional region. (b) Closeup of transitional region showing the unspecialized epithelium of the transitional lobules the latter containing a mixture of spermatozoa and secretions. H&E stain. USNM 385607. Bar = 100 μ m. sec, secretions; spz, spermatozoa; Tr, transitional region. (c) Detail of epithelium of the secretory lobule lumen showing an abundance of secretory cells with enlarged vacuoles filled with secretions, and aggregations of spermatozoa directly adjacent. H&E stain. USNM 385607. Bar = 50 μ m. secC, secretory cells; spz, spermatozoa. (d) Transverse section of the milt-collecting canal epithelium where the two milt collection canals are united by the merger of their medial walls, showing spermatozoa, secretory cells, and infolding by which the milt-collecting canal is partitioned into smaller channels. Collagen (blue) and smooth muscle (red) fibers make up much of the MCC wall. Mallory trichrome stain. USNM 385607. Bar = 50 μ m. f, Infolding of milt-collecting canal; MCC, milt-collecting canal; secC, secretory cells; spz, spermatozoa

Within the gastro-urogenital region, each of the three passages comprising the gonoduct, the hindgut, and the urinary duct is surrounded by a layer of smooth circular muscle which is particularly robust around the hindgut (Figure 10a). The gonoduct lumen is notably larger than that of the urinary duct and hindgut and is surrounded by a layer of loose areolar connective tissue that does not surround the urinary duct and hindgut (Figure 10a). At the termination of the hindgut at the anus, the remaining tissue-bound common genital and urinary ducts comprise the urogenital region of the GU tract, which extends for a short distance to the base of the urogenital papilla.

3.2.6 | Male papilla

The male papilla is elongate, broad at its base, and narrow distally; it lies in a shallow depression of perianal epithelium that we term the genital

groove (Figures 2b and 6). In cross-section, the central region of the papilla is flanked on each side by a lateral wing-like extension, or lappet, that originates at the papilla base and runs along most of the length of the papilla (Figure 10b). These lappets are much larger than those of the female papilla. The papilla epidermis consists of a stratified squamous epithelium interspersed with mucus cells (Figure 10b,c). Below the epidermis lies a band of irregular CT, which is most prominent in the ventral and lateral regions, including the lappets (Figure 10b,c). The aligned gonoduct and urinary duct extend the length of the papilla and open separately at their respective urinary and genital pores. The gonoduct continues ventral to the urinary duct. The urinary duct is lined with a tall columnar epithelium and has prominent infolding forming a stellate lumen whereas the gonoduct is lined with a low columnar epithelium resting on an irregular, sponge-like layer of loose areolar CT (Figure 10d). Loose reticular CT provides a large proportion of the support tissue within the urogenital papilla (Figure 10b-d).

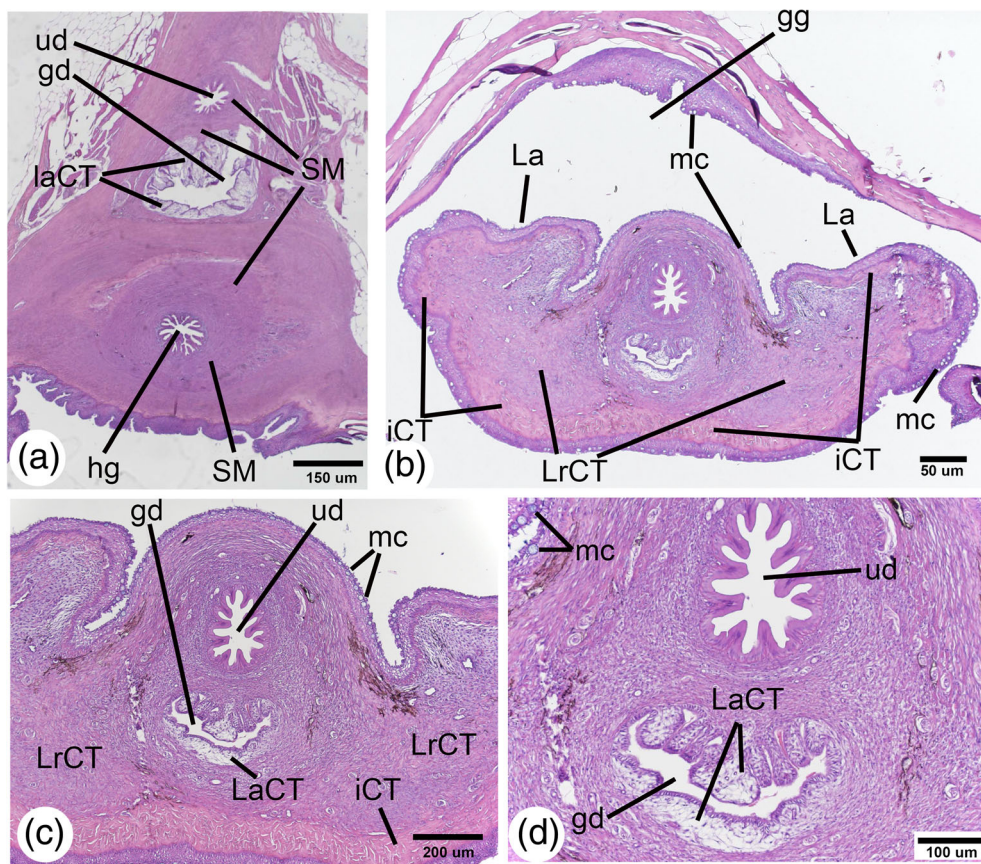


FIGURE 10 Male *R. aspro* gastro-urogenital tract and papilla, transverse sections. (a) Gastro-urogenital tract showing smooth muscle surrounding the urinary duct, gonoduct and hindgut and loose areolar connective tissue underlying the gonoduct lumen epithelium. H&E stain. USNM 385607. Bar = 150 μm. gd, gonoduct; hg, hindgut; laCT, loose areolar connective tissue; SM, smooth muscle; ud, urinary duct. (B) Section through the papilla partially surrounded by a genital groove, showing mucus cells in the epithelium surrounding the papilla and lining the central portion of the genital groove, lappets formed by lateral extensions of the papilla, and the body of the papilla consisting of an inner core of loose reticular connective tissue and an outer layer of irregular connective tissue. H&E stain. USNM 385607. Bar = 50 μm. gg, genital groove; iCT, Irregular connective tissue; La, lappets; LaCT, loose areolar connective tissue; LrCT, loose reticular connective tissue (LrCT); mc, mucus cells. (c) Detail of the central body of the papilla showing loose areolar connective tissue surrounding the lumen of the gonoduct, mucus cells of the papilla epithelium, an inner core of loose reticular connective tissue and peripheral band of irregular connective tissue. H&E stain. USNM 385607. Bar = 200 μm. gd, gonoduct; iCT, irregular connective tissue; LaCT, loose areolar connective tissue; LrCT, loose reticular connective tissue; ud, urinary duct; mc, mucus cells. (d) Close-up of the central region of the papilla section shown in (c). Abbreviations are as in (c). H&E stain. USNM 385607. Bar = 100 μm

4 | DISCUSSION

The descriptions of the male and female specimens of *R. aspro* examined and reported on here provide the first detailed information on RSy morphology in a “basal” gobioid. We have identified characters that are unique to, hence diagnostic of the species *R. aspro*. Further, we compare these characters to those known for other gobioid fishes and summarize our findings in a comprehensive terminology for the reproductive system of *R. aspro*.

4.1 | Female reproductive system

The reproductive system of female *R. aspro* is similar to that of other teleosts. Paired ovarian lobes are united posteriorly and the lobe

lumina merge to form an enlarged chamber, the common genital sinus. This chamber is reduced in size more posteriorly and attenuated to form a single gonoduct through which ova pass to the genital pore and exit the body. The common genital sinus leads to the anterior origin of the gastrointestinal-urogenital (GU) tract which continues caudad to the base of the urogenital papilla.

A conspicuous feature of the external anatomy of the RSy of female *R. aspro* is a fimbriate pad upon which the papilla rests (Figure 3). This thick tissue pad between the base of the papilla and the body is independent posterior to the papilla terminus. Internally, most of the pad is composed of loose areolar CT. Externally, the pad has numerous slender projections, or fimbriae, on its ventral surface. The fimbriae also consist primarily of loose areolar CT. These fimbriae cannot have a sensory function as they have no specialized sensory structures. In addition, without muscle tissue, the fimbriae are not

capable of intrinsic movement. The function of the fimbriate pad is unknown. Yet, the fimbriae are positioned to both surround and brush over the eggs during oviposition. To our knowledge this is the first report of such a reproductive morphological feature of a gobioid female papilla.

Female *R. aspro* are asynchronous spawners (i.e., able to spawn more than once in a breeding season). This is confirmed by the presence of all stages of oocytes within the ovary (Figure 4). Each of the adult females we dissected has ovaries with mature ova, indicating that they were collected during the breeding season (which includes February in the Philippines and July in Indonesia). Length of the breeding season and how variable it may be between years and across geographical ranges are unknown. This is the first report of information on the spawning mode and seasonal breeding of *R. aspro*.

The urogenital papilla of female *R. aspro* is typical of many gobioids, being short, truncate, and broad at its base with slight narrowing at its terminus. The urogenital pore is relatively wide, with a pleated margin that can expand with egg release during spawning. A prominent band of dense irregular CT surrounds the ventral and lateral periphery of the female papilla, including the lappets, of *R. aspro*. This form of CT, composed principally of densely packed collagen fibers, can translate stretching forces in multiple directions to provide strength and resist tearing (Krause & Cutts, 1994). In *R. aspro*, the ventral margin is where the female papilla directly contacts the spawning surface and experiences the greatest internal deformation from ova release during spawning. A similar prominent band of dense irregular CT is absent from the dorsal region of the papilla supporting a possible role for this tissue band to protect the papilla from external pressure and counter contact pressure and abrasion during oviposition. An oocyte of *R. aspro* has what we identify as a tuft of short filaments on one end. We have not been able to examine ovulated eggs.

4.2 | Male reproductive system

Arrangement of the seminiferous lobules within the testicular lobe is distinctive in male *R. aspro*. Each lobule is blind-ended at the lobe periphery and open-ended at the opposite side of the lobe; the lobule follows a curved path paralleled by adjacent lobules. The open end of each lobule connects with a dorsomedial channel that extends from the anterior to the posterior end of the lobe. This channel forms a collection system for sperm and may aid in the rapid expression of sperm during spawning by providing additional space to store sperm between spawning events. The presence of an unspecialized epithelium lining the channel lumen supports this conjecture. We call this intralobar channel a SCC. The SCC provides a pathway through which sperm from the seminiferous lobules exit the spermatogenic lobe. The SCC wall consists of abundant compact CT that can resist deformation from internal pressure. The robust smooth muscle layer likely provides contractile force to propel sperm out of the SCC.

The secretory lobes of the male *R. aspro* RSy have an intralobar collection system formed by a channel like the SCC of the associated testicular lobe. Mirroring the arrangement of the seminiferous lobules,

the secretory lobules are blind-ended at the lobe periphery and open-ended medially where they are confluent with the channel. The lumen of the secretory lobe channel is continuous with the secretory lobule lumina, and secretions can pass from the latter to the former. The lumen of the secretory lobe channel is also continuous with the lumen of the SCC of the testicular lobe which provides a continuous exit pathway for sperm. The sperm from the SCC, in combination with the secretory material produced by the secretory lobules, meet in the MCC and together form milt.

The different histological characteristics of the MCC and SCC indicate that they are not identical in function. The SCC has an unspecialized epithelium more suitable for containment than for other functions, whereas the MCC has a pronounced secretory epithelium. The secretions produced within the MCC lumen may provide an additional component to the milt, enhance milt movement, or both. The increased robustness of the MCC wall relative to that of the SCC suggests a greater resistance to deformation in the former, possibly to accommodate the greater volume and density of milt compared with sperm alone.

4.3 | Comparisons with other gobioid fishes

R. aspro lives in high order, freshwater streams with a steep gradient and swift-to-torrential water flow that far exceeds that of any other known gobioid species (Allen, 1991; Miller, 1973). The position of the fimbriae of the female papilla provides a soft shield for it and the eggs during oviposition. This could reinforce egg attachment and/or prevent eggs from being swept away in strong currents. The egg of gobioid fishes is subovoid, not spherical, and characterized by a small tuft of attachment filaments at one end, as described and illustrated for the odontobutid *Micropercops swinhonis* (see Iwata et al., 2001: Figure 2), the subovoid egg with its tuft of filaments is considered as a potential gobioid synapomorphy (Miller, 1986: Figure 3). We infer the presence of a tuft of filaments in *R. aspro*, but this needs confirmation.

Some gobioid species are also known for the male production of sperm trails (Marconato et al., 1996; Mazzoldi et al., 2011). These are strings of sperm-laden mucus laid down by the male prior to female oviposition. The sperm trail mucins, produced by the secretory lobules, are eosinophilic and PAS+ (Mazzoldi et al., 2011), as are the secretions produced in the secretory lobules of male *R. aspro*. If *R. aspro* use sperm trails, the fimbriate pad may function to secure additional sperm while passing over the sperm-rich mucin trail prior to brushing over the newly oviposited eggs, potentially increasing fertilization under fast waterflow conditions. The papilla of male *R. aspro* is elongated, narrowed distally, and extends to the base of the first anal-fin ray. Much of the papilla rests within a parallel groove on the body that we call the genital groove. A similar groove has been reported in the gobioid *Valenciennesia* (as *Eleotriodes*) *muralis* (see Har, 2000).

In transverse section, both the male and female papilla of *R. aspro* are depressed in outline and have laterally projecting wing-like structures, or lappets. This character has not been reported in other gobioids and may be unique to the species. Among males, the lappets

may act to reduce water turbulence alongside the papilla, thereby slowing the dispersal and/or dilution of male products released under strong water current conditions. The smaller lappets of the female papilla could reflect the larger size and sticky nature of the eggs, making rapid water flow less critical during oviposition. Lateral flaps or projections on the urogenital papilla of females have been reported as present or absent in species of the gobioid genus *Callogobius* (Akihito & Ikeda, 2021; Delventhal et al., 2016; Delventhal & Mooi, 2013), but none described as ornate as those of *R. aspro*.

Miller (1992) described two openings in the male *R. aspro* papilla: one for the urinary duct and one for the gonoduct. In both female and male *R. aspro* examined here, the urinary duct is dorsal to the gonoduct within the papilla; the two remain independent of one another and open to separate pores at the papilla tip. In the sand gobies *Pomatoschistus minutus* and *P. lozanoi*, the urinary duct is also dorsal to the gonoduct and the gonoduct and urinary duct open via pores (Kirby et al., 2003).

In the two *R. aspro* females we examined histologically, mucus cells were absent from the papilla epithelium. In two males of *R. aspro*, mucus cells were present and distributed predominantly on the ventral papilla surface, including that of the lappets. Additionally, a small number of mucus cells were present in the epithelium of the dorsal-most region of the genital groove. This may provide protection against tissue damage if males lay sperm trails against rock surfaces.

The term “fimbriae” has been used in the gobioid literature to describe ornamentation on the posterior margin of the urogenital papilla (e.g., Miller, 1986). Females of the third species of Rhyacichthyidae, *Protogobius attiti* Watson and Pöllabauer (1998), p.150, Figure 2c,d), have an ornate urogenital papilla with a “...distal tip with three projections of tissue, two laterally and one dorsally, all three projections with numerous fimbriate projections.” The papilla of female *P. attiti* extends beyond the ventral outline of the body, but it does not rest on a separate and distinct fimbriate pad as in *R. aspro*. The sole congener of *R. aspro*, *Rhyacichthys guilberti* Dingerkus & Séret, 1992, lives in gently sloping rivers and wide streams (Keith, 2002) as does *P. attiti* (see Larson, 2012; Lord & Keith, 2006). The description of *R. guilberti* did not include morphology of the papilla and the unique holotype is likely a male.

Among gobioids, secretory lobes typically merge posteriorly with the testicular lobes such that the products of both pass into a shared compartment, the common genital sinus (e.g., Cole & Robertson, 1988). Along its length, the common genital sinus becomes attenuate and forms a narrow channel, the gonoduct, through which sperm and secretory products exit the body (Cole, 2010b; Mata-Cortés et al., 2004; McMillan, 2007). In contrast in *R. aspro*, the paired secretory lobes arise caudally near the origin of the genital papilla and the conjoined region of the two lobes represents the merged left and right MCC. The paired testicular lobes originate more anteriorly, uniting with the secretory lobes at the approximate midpoint of the associated secretory lobe. A similar arrangement has been reported for another gobioid species, *Pomatoschistus pictus* (see figures in Fishelson, 1991 and Miller, 1984), but the absence of detail in these descriptions prevents direct comparison with *R. aspro*. Based on our

current knowledge, this arrangement of testicular and secretory lobes in *R. aspro* is atypical.

The spermatogonia in the seminiferous lobules in *R. aspro* are distributed along the length of the lobules, a common male reproductive character of non-atherinomorph neoteleosts (Grier, 1981; Grier & Lo Nostro, 2000). The distribution of developing spermatocysts in the specimens we examined form isolated clusters in what is called a discontinuous germinal epithelium (Grier, 2000) and has been reported for several gobioid species (Cinquetti & Dramis, 2003; Parenti & Grier, 2004; Thacker & Grier, 2005). Among some fishes, this discontinuous germinal epithelium is related to seasonal reproductive classes of gonad maturation (Grier, 1993, 2002; Parenti & Grier, 2004). Uribe et al. (2014) described changes in the germinal epithelium during the annual reproductive cycle in bony fishes and reported that throughout the breeding season, the germinal epithelium is characterized by areas in which only Sertoli cells are present (i.e., spermatocyst distribution is clustered rather than continuous). A discontinuous germinal epithelium in *R. aspro* may also be seasonally dependent as in other fishes.

In some male gobioids, a condensed body of steroidogenic (Leydig) cells is located within the spermatogenic lobe. This was first described for the black goby, *Gobius niger* (as *Gobius jozo*) by Colombo and Burighel (1974) and Colombo et al. (1980) and called a mesorchial gland. The mesorchial gland provides a steroid conjugate (conjugated 5 β -reduced androgens) that acts as a pheromonal attractant (Colombo et al., 1977). Such an aggregate of steroid-producing cells has also been described for *Gobius paganellus* (see Stanley et al., 1965), *Neogobius melanostomus* (see Arbutckle et al., 2005), *Padogobius martensi* (see Cinquetti & Rinaldi, 1987), *Glossogobius olivaceus* (see Asahina et al., 1983; Asahina et al., 1985), and some species of *Pomatoschistus* (Miller, 1984). The genera *Gobius*, *Neogobius*, and *Padogobius* are all classified in the *Gobius* clade (Horvatić et al., 2016; Macali, 2017). But the *Gobius*, *Glossogobius* and *Pomatoschistus* clades, all of which have one or more species with a mesorchial gland, are not each other's closest relative (Agorreta et al., 2013). This suggests possible independent evolution of the gobioid mesorchial gland. It is not a general feature of gobioids as it is not present in the two adult male *R. aspro* examined in this study.

The intralobar channel system of *R. aspro* has not, to our knowledge, been reported for any other gobioids. In the grass goby, *Zosterisessor ophiocephalus*, the spermatic duct sensu Patzner et al. (1991) is internal to the testicular lobe wall cranially but at the mid-point becomes external to the lobe and continues posteriad as an extralobar duct. The paired spermatic ducts fuse into a single structure which is continuous with the secretory lobules (referred to as chambers of the vesicle by Patzner et al. (1991) and we infer equivalent to the common genital sinus as used here). Lahnsteiner and Patzner (2009) described the seminiferous tubules (= lobules) of the paired testes of *Z. ophiocephalus* as coalesced to form “spermatic ducts” on the ventral side of the testis, but did not specify an extralobar or intralobar location. Miller (1992): Figure 1) described the “sperm duct” of the male *R. aspro* as superficial and running the length of each testicular lobe. In our examination, the SCC does protrude from the spermatogenic lobe proper in some regions (see Figures 7a,b

and 9a), but is an integral, not superficial, part of the lobe and continuous with the seminiferous lobules along its length as illustrated in Figure 7c. Thus, the “sperm duct” of gobioids, sensu Miller (1992), is part of the SCC. The morphology of the SCC and MCC described in detail here for *R. aspro* may provide useful characters to test phylogenetic relationships among basal gobioids.

4.4 | Terminology of the reproductive system

Our second goal was to develop a precise terminology for the RSy of *R. aspro*. This terminology may be used to compare RSy anatomy across all gobioid taxa and contribute to the identification of proposed

homologous and novel characters (Table 2). We sought to develop a terminology that is unambiguous, anatomically appropriate, location- and structural relationship-based, and useful as a guide for terminology of features/structures not included here or yet described. The proposed terminology may encourage the use of reproductive characters in the reconstruction of gobioid evolutionary and phylogenetic history.

The term “testis” in descriptions of male gobioid RSy has been applied variously to the spermatogenic portion, the spermatogenic and secretory portion, or the entire male reproductive complex. Because of this inconsistency, we recommend limiting the term testis to the spermatogenic portion among gobioids, as did Miller (1984). The “spermatogenic lobe” is that portion of the male gobioid RSy

TABLE 2 Anatomical terminology for the reproductive system of the gobioid, *Rhyacichthys aspro*

Term	Description
Testis	A portion of the male reproductive system (RSy) distinct from other RSy components and comprising seminiferous lobules. Testes or spermatogenic lobes are typically paired and lie on either side of the midline.
Secretory lobe	A portion of the male RSy distinct from other RSy components and comprising secretory lobules. Secretory lobes are typically paired and lie on either side of the midline but are absent in some taxa. Also called the AGS (accessory gonadal structure) or sperm duct gland.
Seminiferous lobules	Elongate structures that comprise a spermatogenic lobe. Each has an epithelium (lining a central lumen) that is made up of Sertoli and germ cells and is the site of sperm production.
Secretory lobules	Elongate structures that comprise a secretory lobe. Each has an epithelium (lining a central lumen) that is made up of secretory cells and constitutes sites of secretion production within the RSy.
Transitional lobule	The transitional region that bridges the testes and secretory lobes. Transitional lobules have an unspecialized epithelium but their lumina may contain sperm and secretions.
Sperm-collecting canal (SCC)	An open, intralobar sperm collection system within the testis characterized by a cranio-caudal running channel with unspecialized epithelium and thick walls relative to seminiferous lobule walls. Seminiferous lobules empty into the SCC.
Milt-collecting canal (MCC)	An open, intralobar milt collection system within the secretory lobe characterized by a cranio-caudal running channel with unspecialized epithelium. Secretory lobules empty into the MCC.
Testicular duct	An extralobar, closed passageway lying outside and running for some portion of the length of the spermatogenic lobe for sperm egress from the spermatogenic lobe to the genital duct formed by merger of left and right testicular ducts. Not present in <i>R. aspro</i> .
Common genital sinus	A shared chamber to which both testes and secretory lobes are directly connected, which receives both sperm and secretory lobe-generated secretions. Not present in <i>R. aspro</i> .
Gonoduct	The singular, closed channel within the genital tract that provides an egress for gametes and other RSy products, between the posterior terminus of the gonadal and/or secretory lobes and the genital pore at the distal end of the genital papilla.
Gastro-urogenital (GU) tract	The posterior portion of the RSy, from the union of the left and right MCC, to the base of the urogenital papilla and consisting of layers of smooth muscle and connective tissue (CT) surrounding one or more internal passageways.
Urogenital mesenteries	Left and right mesenteries that connect the urinary bladder to the genital tract along its length.
Gastrogenital mesenteries	Left and right mesenteries that connect the hind gut to the anterior region of the genital tract prior to the inclusion of the gut within the tract.
Genital region of GU tract	The anterior region of the GU tract containing only the gonoduct.
Gastro-genital region of GU tract	The region of the GU tract posterior to the genital region and including both the gonoduct and hindgut.
Genital groove	An anterior-posterior groove on the ventral surface of the body in which the male genital papilla rests.
Gastro-urogenital region of the GU tract	The portion of the GU directly posterior to the gastro-genital region, beginning where the urinary ducts join the genital tract and ending where the hindgut terminates at the anus. This region includes the hindgut, gonoduct, and urinary duct.
Urogenital region of the GU tract	The most posterior region of the GT located just posterior to the anus and ending at the base of the urogenital papilla, which includes the urinary duct and gonoduct.

consisting of seminiferous lobules and distinctly separate from other RSy components. Likewise, the “secretory lobe” is that portion of the male gobioid reproductive system that consists of secretory lobules and is separated from other components of the male RSy. In some gobioid taxa the secretory lobe is extremely reduced, or absent (Mazzoldi et al., 2005). In species in which the male RSy has spermatogenic and secretory functions that are not in separate lobes, we propose the term “gonadal lobe,” which includes a spermatogenic region and secretory region. The term “gonadal lobe” is also most appropriate for hermaphroditic gobioid species in which the function of the gametogenic tissue can change with a shift in sex.

The term “seminiferous lobule” is used for an elongate sperm-generating compartment located within the testicular lobe among functioning male teleosts, including gobioids. The majority of male gobioid taxa also have a secretory lobe or secretory region which is composed of numerous elongate, secretion-generating compartments, referred to as “secretory lobules.” In the adult male specimens of *R. aspro* we examined, the secretory lobules contained both secretions and sperm, suggesting that the secretory lobules of adult male *R. aspro* may also be sites of temporary sperm storage. The secretory lobe of gobioids has also been called seminal vesicles (e.g., Arai, 1964; Egami, 1960; Weisel, 1949; Young & Fox, 1937), accessory gonadal structures or AGS (it is found in males and also hermaphrodites, e.g., Cole & Hoese, 2001), or sperm-duct gland (Miller, 1986, 1992). We prefer the term “secretory lobe” because it specifies the secretory function of this part of the RSy. The secretory lobe may be a gobioid synapomorphy, as proposed by Miller (1992).

The collection and transport system(s) associated with the testicular and secretory lobes of male gobioids have been described using differing terminology. The sperm transport system has been variously referred to as “sperm” or “spermatogenic” duct(s) (Arbuckle et al., 2005; Cinquetti, 1997; Patzner et al., 1991), “main testicular ducts” (Grier et al., 1978; Mazzoldi et al., 2011) or “main ducts” (Grier et al., 1978). Patzner et al. (1991) restricted the use of “sperm duct” in *Z. ophiocephalus* to the channel formed by the union of the two gonadal lobes that continues to the genital papilla, whereas Thacker and Grier (2005) used the same term to describe both the internal and external gamete transport system in the paedomorphic gobioid *Schindleria praematura*.

The collection systems of the spermatogenic and secretory lobe of *R. aspro* consist of a longitudinal, medially located, internal, or intralobar, channel that is open to its associated lobules. To clarify the inconsistency in terminology associated with the channels through which sperm exits the gonad proper, and then the body, we refer to the intralobar sperm and secretory collection systems of *R. aspro* as a SCC and MCC.

No dedicated intralobar collection channel for sperm and secretion transport, as described for *R. aspro*, is reported for other gobioid RSy (see Cole, 2010a; Cole, 2010b). Where described for other gobioids, the secretory and spermatogenic lobes arise in parallel, are mostly independent of one another, have lobule lumina that coalesce in the posterior region of the lobe, and have the lobule contents pass directly into a shared chamber. For this anatomical arrangement, the

term common genital sinus has been used (Cole, 1990; Cole & Robertson, 1988) and is proposed for the male gobioid RSy to describe a shared chamber to which both spermatogenic and secretory lobes are directly connected and into which sperm and secretions pass prior to coming into contact with one another. A common genital sinus is not present in *R. aspro*.

In many bony fish taxa, the duct through which ova or sperm pass from the gonadal lobes to the genital pore has been referred to as the oviduct or sperm duct, respectively. In hermaphroditic gobioids, both ova and sperm typically pass-through this duct. Therefore, we endorse using the non-sex-specific term gonoduct to identify the (usually) single passageway through which gametes pass from the spermatogenic lobe or common genital sinus, to the genital pore, in gobioids. The term gonoduct has been used for other teleost fishes (e.g., Campuzano-Caballero & Uribe, 2014; McMillan, 2007; Wourms, 1981).

Posterior to the gonadal lobes, the gonoduct is surrounded by a robust layer of connective tissue (CT) and forms the gastro-urogenital (GU) tract. The GU tract extends from the anterior union of the left and right MCC to the origin of the papilla. At various points, the GU tract consists of posterior portions of the urinary system, the digestive system, and the reproductive system. Consequently, the GU tract does not have a uniform morphology along its length. We thus name the sections of the GU tract to reflect landmark morphological changes in *R. aspro*.

The anterior portion of the GU tract, comprising the partitioned channels of the remnant MCCs and the single gonoduct, marks the “genital region” of the tract as it contains only gametes and other RSy-produced materials. The hindgut, in joining the tract, marks the beginning of the “gastro-genital region.” The subsequent addition of the urinary duct marks the beginning of the “gastro-urogenital region.” With the separation of the hindgut at the anus, only the urinary duct and gonoduct remain within the tract to form the “urogenital region” which extends to the origin of the urogenital papilla. These sections may be compared across gobioid taxa. For example, in two species of *Pomatoschistus* examined by Kirby et al. (2003) and 11 species of Indian goby examined by Har (2000), the urinary duct and gonoduct remain separate for the length of the urogenital papilla and exit via independent pores. This is the same as in *R. aspro* and may be a diagnostic character of all gobioids.

Terminology for the components of the female RSy of gobioids is less controversial than that of the male RSy due to the relative simplicity of the female morphology. The female gobioid RSy has four components. The central lumen of each of the paired ovarian lobes is lined with a germinal epithelium, the source of ova. The common genital sinus, comprising the posteriorly confluent region of the two ovarian lobes, has no germinal or otherwise specialized epithelium and is a receptacle for ovulated eggs, filling with ova at ovulation. The genital tract extends from the common genital sinus to the base of the urogenital papilla and includes the gonoduct which extends throughout the GU tract length and the full length of the urogenital papilla. It terminates at the genital pore at the terminus of the papilla.

5 | CONCLUSIONS

We here provide the first detailed description including histology of the morphology of the reproductive system of female and male *R. aspro*, representing a “basal” species of gobioids. We report several novel features. One is a fimbriate tissue pad associated with the female genital papilla that may shield eggs during oviposition and ensure fertilization by facilitating the application of sperm to the eggs. Another is the presence of an intralobar collection system within the male RSy. This collection system is in both the testicular (spermatogenic) and secretory lobes, termed the SCC and MCC, respectively, and may provide additional storage for sperm and milt. Lastly, we propose a standardized terminology for gobioid reproductive system morphology based on the RSy of *R. aspro*. We anticipate that broader application of this terminology will advance our understanding of gobioid evolution and phylogenetic relationships.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Kathleen S. Cole: Conceptualization, dissection and selection of materials for histology, interpretation and photography of histology, writing and editing manuscript and preparing figures and tables. **Lynne R. Parenti:** Conceptualization, interpretation of histology, writing and editing manuscript, preparing figures and tables, providing resources and acquisition of financial support for the project.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/jmor.21440>.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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