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A new genus and two new species of land planarians (Platyhelminthes: Tricladida: Geoplanidae) from Southern Chile

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ABSTRACT

The paper describes the new land planarian genus *Mapuplana* gen. nov. from Chile, on the basis of the two new species *Mapuplana guttulata* sp. nov. and *M. fjordica* sp. nov. The genus *Mapuplana* is mainly characterised by two putative apomorphies: a subneural parenchymal musculature consisting of diagonal decussate muscle fibres; and a blind duct opening sideways into the female atrium. The new species are very similar to each other in their general anatomy and differ only in details in the pattern of dorsal colouration, the relative distance between mouth and gonopore, the relative thickness of the ventral cutaneous musculature, the orientation of the gonoduct, and the shape of the female atrium.

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Introduction

Unlike most Neotropical species, the taxonomy of Brazilian representatives of the subfamily Geoplaninae has been reviewed in some detail, which resulted in the recognition of several new genera that were supported by both morphological and molecular data (Carbayo et al. 2013). As a result, the Brazilian Geoplaninae taxa are much better understood in terms of their diversity and interrelationships than those from other South American countries. For example, Chilean land planarians remain poorly studied, despite the fact that the overall diversity of the Chilean fauna presumably comprises many more species than are known at present (Grau and Carbayo 2010; unpublished data).

Presently, 28 species of land planarians have been described for Chile. Most of these Chilean land planarians were described or reported by Darwin (1844), Blanchard (1845), Gay (1849), Von Graff (1899), Marcus (1954) and Froehlich (1978), while more recent studies authored by Grau and Carbayo (2010, 2011), Bulnes et al. (2018), Almeida et al. (2021) and Negrete et al. (2021) have contributed to this list of native land planarians from Chile.

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Currently, nominal Chilean land planarian species belong to three subfamilies, viz. Geoplaninae Stimpson, 1858, Rhynchodeminae Graff, 1896, and Timyminae Almeida and Carbayo, 2021, the latter recently proposed as an endemic subfamily of Chile. Geoplaninae represent exclusively Neotropical species, with 25 of them being known from Chile. However, only a few of these species have been properly classified according to modern standards, while some are considered species *incertae sedis* because the genera to which they were originally assigned were later discovered to be polyphyletic (and thus are herein referred to as '*Geoplana*' or '*Pasipha*'), or because the anatomy of the species is poorly known (species currently placed in the collective genus *Pseudogeoplana* Ogren and Kawakatsu, 1990) (Carbayo et al. 2013).

Recent studies (Grau and Carbayo 2010, 2011; Bulnes et al. 2018) have attempted to further unveil the Chilean taxonomic diversity of the Geoplaninae, and our present contribution also aims to achieve more insight into the diversity of this group of land planarians by describing two new species from southern Chile, for which we propose also a new genus.

Materials and methods

Specimens of land planarians were collected by hand in the field and photographed alive, and a description was made of their external appearance. Subsequently, the animals were killed by immersion in 97% ethanol. In the laboratory, the hardened body of one specimen (MNHNCL PLAT-15045) was immersed in Sandison solution for three days in order to rehydrate it (Sandison 1955). Preserved worms were transversally cut into several pieces, including the cephalic extremity, the ovarian region, the pre-pharyngeal region, the pharynx and the copulatory apparatus. Each of these parts was dehydrated in a graded series of ethanol, treated with pure isopropyl alcohol and de-alcoholised in clove oil before embedding into Paraplast tissue-embedding medium (melting temperature 56–58°C). Serial histological sections were made at intervals of 7 or 9 µm and were stained with Mallory-Cason (see Cason 1950; Winsor and Sluys 2018) and Azocarmine and Aniline Blue (AZAN) (see Gabe 1976). The ratio of sub-cutaneous musculature thickness:body height was calculated at the pre-pharyngeal region, after Froehlich (1954). Reconstruction drawings were prepared using a compound microscope fitted with a drawing tube. Colour descriptions of the body follow online RAL palette colours (© RAL gemeinnützige GmbH, available at https://www.ral-farben.de/uebersicht-ral-classic-farben.html?&L=1). Drawings and photomicrographs of sagittal and horizontal views are oriented with the anterior region to the left. Maps for Figure 1 were generated with Simplemappr (Shorthouse 2010). Type specimens are deposited in the Museo Nacional de Historia Natural Chile (MNHNCL) and Museum für Naturkunde Berlin, Germany (ZMB).

Abbreviations used in the figures

bd, blind duct; **ci**, circular cutaneous muscle; **cl**, clump; **cm**, common muscle coat; **dc**, diagonal cutaneous muscle; **dd**, dorsal double diagonal parenchymal muscle; **de**, dorsal epithelium; **e**, eye; **ed**, ejaculatory duct; **f**, fold; **fa**, female genital atrium; **fd**, female genital duct; **g**, gonopore; **gl**, gland; **gn**, gonoduct; **in**, intestine; **le**, subepidermal longitudinal cutaneous muscle; **ls**, sunken portion of the ventral longitudinal cutaneous muscle; **lu**, lumen; **m**, muscle; **ma**, male genital atrium; **mo**, mouth; **od**, ovovitelline duct; **pg**, prostatic vesicle glands; **ph**, pharynx; **pn**, peripheral nerve plexus; **po**, pharyngeal pouch; **pp**, penis papilla; **pv**, prostatic vesicle; **sb**, subintestinal parenchymal muscle

Figure 1. Sampling localities of *Mapuplana guttulata* and *M. fjordica*.

layer; **sd**, sperm duct; **sg**, shell glands; **sn**, subneural parenchymal muscle of diagonal decussate fibres; **sp**, supraintestinal parenchymal muscle layer; **st**, spermatophore; **t**, testis; **ve**, ventral epithelium; **vi**, vitellaria; **vn**, ventral nerve plate.

Systematic account

Order **TRICLADIDA** Lang, 1881

Family **GEOPLANIDAE** Stimpson, 1858

Subfamily **GEOPLANINAE** Stimpson, 1858

Genus ***Mapuplana*** gen. nov.

Diagnosis

Geoplaninae with a flattened, slightly lanceolate body, ranging between 40 and 50 mm in length. Monolobulated eyes surround the entire cephalic region. Sensory depressions present. Thickness of cutaneous muscle relative to body height: 12.5–27%. Ventral longitudinal cutaneous musculature partially sunken beneath the peripheral nervous plexus

and below the main nerve plate. Musculature in the cephalic region thicker. Subneural parenchymal musculature consisting of diagonal decussate fibres, intermingled with the sunken portion of the ventral longitudinal cutaneous musculature. Prostatic vesicle receives the secretion of tubular, branched glands. Eversible penis type; penis papilla small and conical. A blind duct opens into the left side of the female atrium. Female genital duct projects postero-ventrally from the postero-dorsal region of the female atrium.

Type species

Mapuplana guttulata sp. nov.

Distribution

Purén (Región de La Araucanía) and Chonos Archipelago (Región de Aisén), Chile.

Etymology

The generic epithet refers to the native Mapuche nation of Southern South America, plus *plana*, meaning flat.

Mapuplana guttulata sp. nov.

Material examined

Holotype. MNHNCL PLAT-15045 (Field code, F4906). Monumento Natural Contulmo, Purén, Región de La Araucanía, Chile, (38.000°S, 73.183°W), coll. F. Carbayo, 12 December 2010. Transverse sections of the cephalic and ovarian region on 31 slides; horizontal sections of a portion behind the cephalic region on 25 slides; transverse sections of the pre-pharyngeal region on 17 slides; sagittal sections of the pharynx and copulatory apparatus on 62 slides.

Diagnosis

Species of *Mapuplana* measuring about 50 mm in length; yellowish dorsum with a dark, minutely reticulated pattern. Ventral cutaneous musculature is thickest in parasagittal planes. Male atrium twice as long as the female atrium. Entire length of the female genital duct receives openings of shell glands. Gonoduct vertical. Female atrium ovoid.

Type locality

Monumento Natural Contulmo, Southern Chile. This small natural reservation (82 ha) is one of the few protected fragments of native forest in the region. The area is dominated by the *Nothofagus* temperate rainforest, receiving up to 1500 mm of rain per year.

Etymology

The specific epithet is derived from the Latin *guttula*, meaning droplet, and alludes to the dots and marks adorning the dorsum.

Description

External appearance. The live specimen measured approximately 50 mm in length and 5 mm in width, while preserved it was 30.5 mm long, 6 mm wide, and 2.8 mm thick. The body is lanceolate, with the dorsum convex and the ventral side being only slightly convex. From about halfway along its length, the body tapers towards the front end, giving rise to a narrow head with a rounded anterior margin. Posteriorly, the body first widens and then tapers, thus acquiring the shape of a broad arrowhead (Figure 2(a)). In resting position, the dorsal surface is more or less corrugated, ie provided with wrinkles and folds. The ground colour of the dorsum is pastel yellow (RAL 1034), and it is adorned with scarce yellow-orange (RAL 2000) specks and numerous, small red-orange (RAL 2001) marks forming a minutely reticulated pattern (Figure 2(a,b)). A broad mid-dorsal stripe (11% of body width) grades from yellow-orange at mid-body to red-orange at the extremities of the body. This median stripe is composed of two thinner lines in some regions of the body. The ventral surface bears the same colour pattern as the dorsal surface, albeit the median band is lighter (Figure 2(c)).

The eyes are monolobated, ranging between 45 and 82 µm in diameter. The eyecups are distributed in a single-to-double marginal row, contouring the very anterior body margin and extending on either side of the body to the posterior margin. Sensory pits are absent. Instead, spots of cilia, housed in slight depressions, are located at the ventro-lateral portion of the cephalic region (Figure 3(a)). These sensory depressions are rare and inconspicuous, and were not observed in the anterior-most body region.

The width of the creeping sole was difficult to measure in the pre-pharyngeal region because of the abundant erythrophil secretion adhering to the ventral surface, but it occupies 57% of the body width in the cephalic region. Relative position of the mouth: body length is 61% in relation to the anterior tip of the body; that of the gonopore:body length is 78%.

Figure 2. *Mapuplana guttulata*. Photographs of the live holotype in dorsal (a, b) and ventral (c) views. Scale bars not available.

Epidermis and its secretions. The epidermis is pierced by openings of three types of gland cells, producing erythrophil, cyanophil and xanthophil granules, respectively, with the erythrophil type being very abundant ventrally. In addition, rhabditogen cells pierce the dorsal and marginal portions of the epidermis. All types of glands are scarcer in the cephalic region. A glandular margin is absent (Figure 3(b)).

Cutaneous musculature. In the pre-pharyngeal region, the cutaneous musculature comprises three layers, namely a subepidermal circular layer (5–7.5 µm thick), followed by a double layer (12.5–20 µm) with decussate fibres, and a strong, innermost longitudinal muscle layer (Figure 3(c)). The longitudinal musculature is 80 µm thick dorsally and 400 µm ventrally; dorsally, the fibres are gathered in large bundles (Figure 3(c)). Ventrally the longitudinal musculature is divided into a subepidermal portion, which is about 65 µm thick and composed of small bundles, and a sunken dense portion, located beneath the cutaneous nerve plexus that measures 335 µm in thickness (Figure 3(d,f)). In the pre-pharyngeal region of the body the cutaneous musculature thickness relative to the body height (abbreviated CMI) corresponds to 12.5% in the mid-sagittal plane. This value increases to 20% in parasagittal planes, due to an increase in the thickness of this musculature (Figure 3(d,f)).

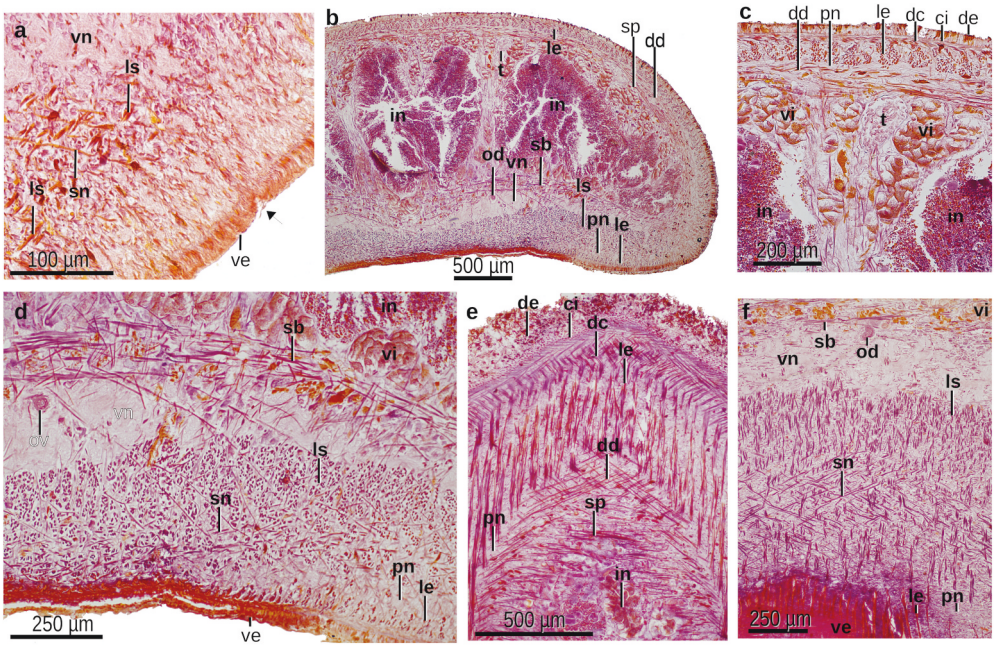

Figure 3. *Mapuplana guttulata*. Photomicrographs of histological sections. (a) Transverse section of the cephalic region, showing the sensory depression (arrowhead); (b–d) transverse sections of the pre-pharyngeal region; (e, f) horizontal sections showing cutaneous and parenchymal muscles (anterior at the top).

Parenchymal musculature. There are four parenchymal muscle layers, viz. a dorsal layer of decussate fibres (30 µm thick, 1.0% of the body height), a supraintestinal layer (70–80 µm) of transverse fibres, a subintestinal transverse muscle layer (65–83 µm), and a fourth, subneural layer with diagonal decussate fibres (90–120 µm); the subneural layer is embedded in the insunk ventral cutaneous muscle layer (Figure 3(d)).

Musculature in cephalic region. In the cephalic region, the musculature is relatively stronger than in the pre-pharyngeal region and it is also organised differently (Figure 4). At 1 mm from the anterior tip of the body, the ventral sunken longitudinal musculature is concentrated along the longitudinal body axis, so that it occupies about 67% of the body width, while the subepidermal portion represents 63% (Figure 4(a)); in this region CMI is 35%. At 460 µm from the anterior tip of the body, the width of the subepidermal and sunken longitudinal muscle layers relative to body width and CMI decreases to 58% and 34%, respectively (Figure 4(c,d)); in this region of the body the transverse muscle fibres are relatively more abundant than in the pre-pharyngeal region, while the subneural muscle fibres are restricted to mid-body. In this region, the sunken fibres are apparently oriented obliquely towards the dorsal body surface and to the body margins, but the precise path followed by these fibres was not discernible beyond the central nervous system (Figure 4 (c)). At 100 µm from the anterior tip, the sunken portion of the ventral longitudinal cutaneous musculature is lacking, while the subepidermal portion is still present.

Pharynx. The cylindrical pharynx is located at a short distance behind the middle of the body and projects out of the mouth opening, the latter situated at the posterior end of the pharyngeal pouch (Figure 5(a)). The oesophagus is 0.5 mm long. The pharyngeal pouch musculature is composed of subepithelial longitudinal muscle fibres, followed by circular fibres. The outer pharyngeal musculature consists of a subepithelial longitudinal muscle layer (8 µm thick), followed by a layer of circular muscles (20–25 µm thick). Underneath this coat of muscles, there is a 200 µm thick layer of intermingled longitudinal and circular muscle fibres. The inner pharyngeal musculature consists of a single, subepithelial layer of intermingled circular and longitudinal fibres (120–225 µm thick) (Figure 5(b)). Radial muscle fibres are also present. Two types of glands, producing granular erythrophil and xanthophil secretions, respectively, run through the parenchyma of the pharynx and discharge their contents at the tip of the pharynx.

Male reproductive system. The globular testes, measuring 210–320 µm in diameter, are distributed in two rows on either side of the body between the supra-intestinal transversal parenchymatic muscle layer and the intestinal diverticula (Figure 3(b,c)). The anterior-most testes are located at a distance from the anterior tip of the body equivalent to 22% of the body length; the posterior-most testes are located at the equivalent of 50% of body length, as measured from the anterior margin, ie they are distributed anteriorly to the root of the pharynx.

The sperm ducts run immediately above the sub-intestinal parenchymal musculature and slightly laterally to the oviducts. Posterior to the pharynx, these ducts are sinuous and dilated, thus forming spermiducal vesicles, which are packed with spermatozoa.

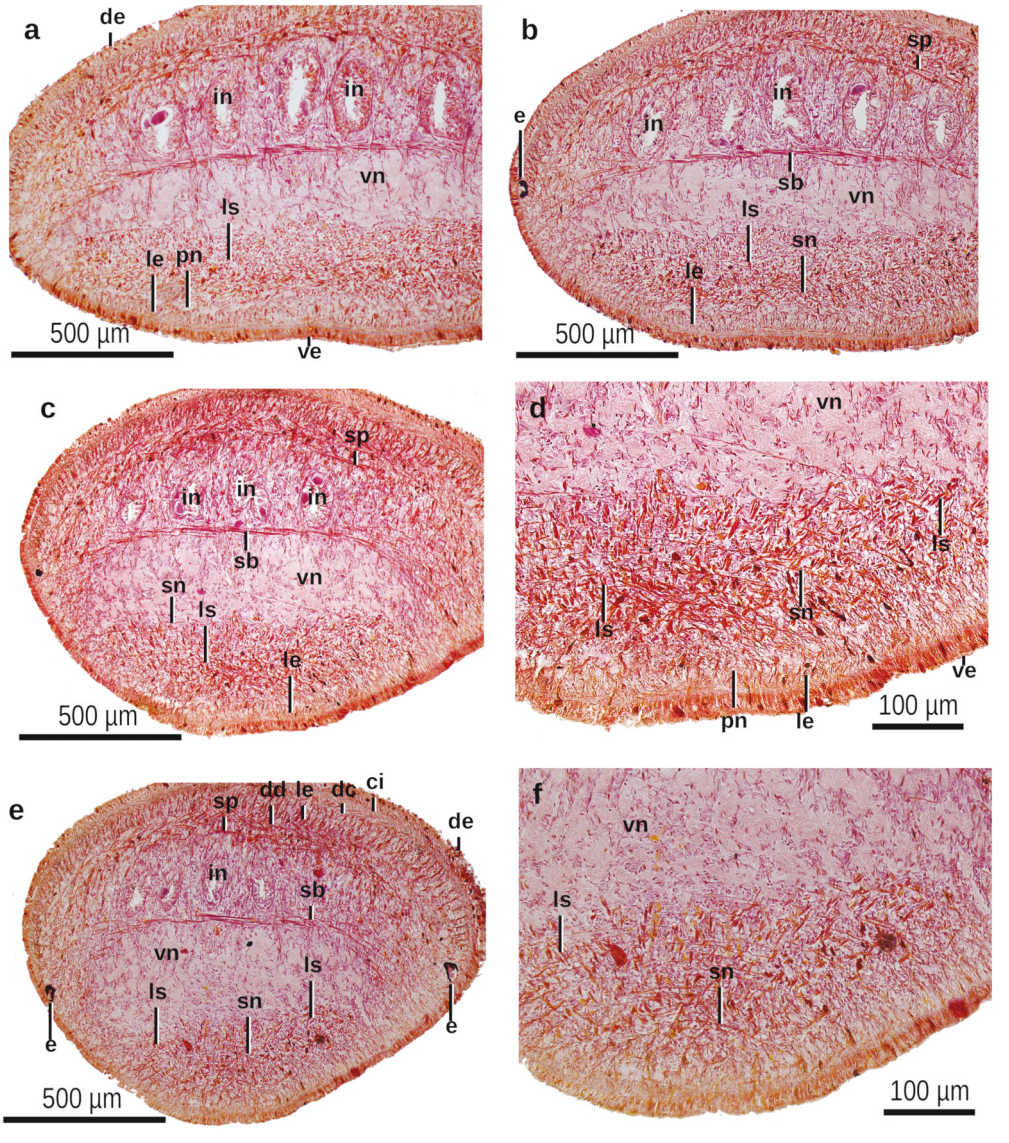

Figure 4. *Mapuplana guttulata*. Photomicrographs of transverse sections of the anterior region of the body, located at (a) 1000 µm, (b)900 µm, (c, d) 500 µm, and (e, f) 400 µm from the anterior extremity of the body.

The posterior portions of the spermiducal vesicles narrow considerably while curving postero-dorsad and, subsequently, communicate with a branch of one of the highly ramified glands of the tubular prostatic vesicle. This communication of the sperm ducts with the ducts of the prostatic glands takes place via a transitional canal lined with a low epithelium, which is surrounded by a 10 µm thick layer of circular muscle. These two glands consist of numerous ramified ducts, which measure 15–37 µm in diameter and are lined with a 37–40 µm high, ciliated epithelium. These ducts collect erythrophil and

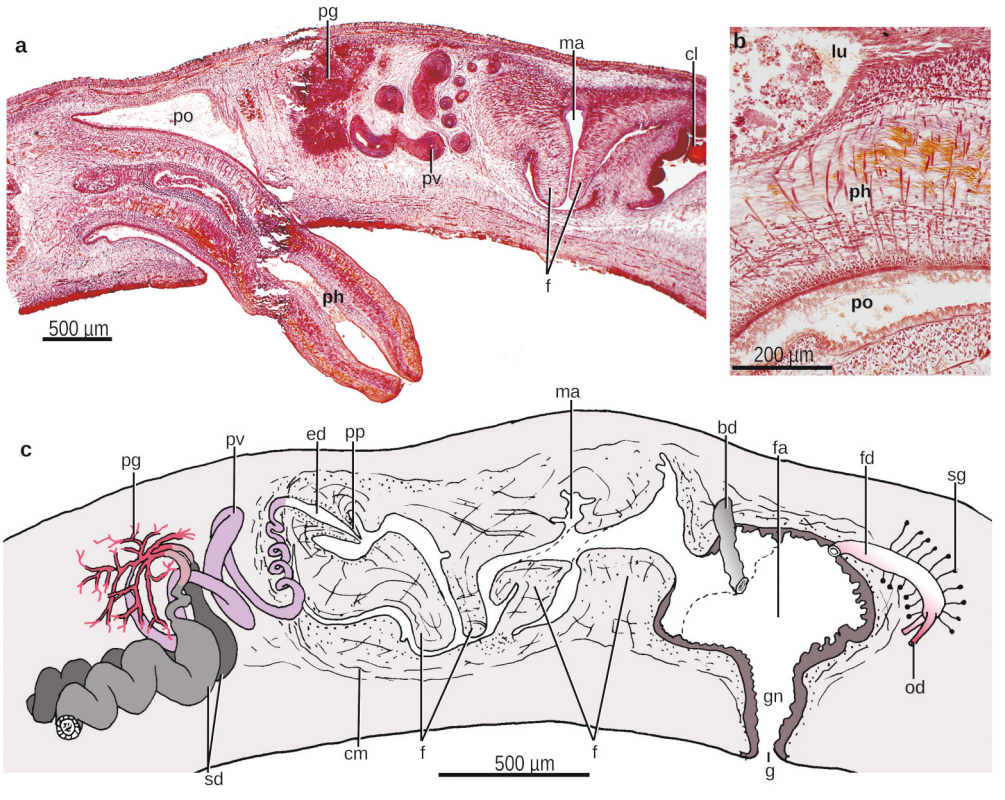

Figure 5. *Mapuplana guttulata*. (a) Photomicrograph of a sagittal section of the pharynx and male copulatory organ; (b) photomicrograph of outer and inner pharyngeal musculature of the paratype; (c) diagrammatic reconstruction of the copulatory apparatus.

xanthophil granular secretions, produced by gland cells located all around the tubules (Figures 5(c) and 6(c,d)). The tubular glands join before opening into the very proximal, posterior portion of the prostatic vesicle (Figure 5(c)).

The unpaired tubular prostatic vesicle follows a spiralling trajectory (Figures 5(c) and 6 (b,c)) before it penetrates the ventral musculature of the common muscle coat and, subsequently, ascends to open into the ejaculatory duct. The proximal, anterior portion of the prostatic vesicle measures 35–50 µm in diameter, while its distal section measures 10–18 µm (Figures 5(c) and 6(b,c)). The prostatic vesicle is lined with a cuboidal, ciliated epithelium and is surrounded by a 50–75 µm thick layer of circular muscle.

On its way through the penis papilla, this duct doubles its diameter to 60 µm before narrowing again to open at the tip of the papilla through an opening with a diameter of about 5 µm. The ejaculatory duct is lined with a ciliated epithelium and is surrounded by a 30 µm thick layer of circular musculature.

The small penis papilla is conical, with its length corresponding to about 9% of the length of the male atrium (Figures 5(c) and 6(e)). This papilla projects from the antero-dorsal portion of the male atrium and points postero-ventrally; it is covered with an infranucleated epithelium, which is pierced by two types of gland cells, producing

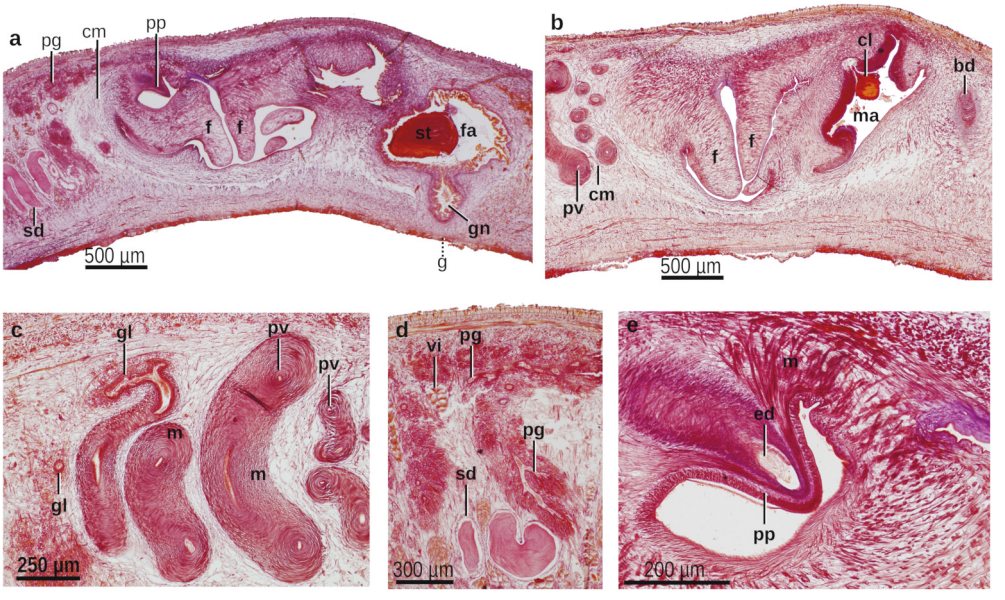

Figure 6. *Mapuplana guttulata*. Photomicrographs, sagittal sections. (a) Copulatory apparatus; (b) male atrium; (c) prostatic vesicle; (d) tubulous gland of the prostatic vesicle; (e) penis papilla.

xanthophil and cyanophil granules, respectively. The epithelium of the penis papilla is underlain by a 17–20 µm thick, subepithelial layer of circular muscle, followed by a 15–33 µm thick layer of longitudinal fibres.

The male atrium is long, provided with distinct folds and lined with a low epithelium. (Figures 5(c) and 6(a,b)). Two large, transverse flap-shaped folds occupy the anterior half of the male atrium. One flap is ventral to the penis papilla, the other posterior to it. The posterior half of the male atrium is occupied by two oblique folds, the anterior one being narrower than the posterior one. The basement membrane underlying the epithelium that is located between the flaps, as well as that between the oblique folds, is 4–8 times thicker than in any other region of the atrium. The entire epithelium of the male atrium is pierced by openings of gland cells producing erythrophil granules, while it is underlain by a 18–35 µm thick layer of circular muscle fibres. Transverse and longitudinal muscle fibres are abundant in the flaps and oblique folds. Additionally, the posterior oblique and narrow fold is reinforced with a 75–85 µm thick coat of intermingled circular and longitudinal muscle fibres. A strongly xanthophil clump, 250 × 350 µm in size, is attached to the wall of the atrium between the two oblique folds; the atrial surface where the clump is attached lacks epithelium (Figures 6(b) and 7(a)).

Female reproductive system. A single pair of ovaries is located at a distance from the anterior tip of the body corresponding to 23% of the body length, with the gonads being situated on top of the ventral nerve plate and underneath the transverse sub-intestinal parenchymal muscle layer. The ovaries are approximately globular, measuring about 250 µm in diameter. The ovovitelline ducts emerge from the dorsal wall of the ovaries and, thereafter, run above the nerve plate. Posteriorly to the gonopore, the ducts ascend to open into the female genital duct, which is

about 120 µm wide and curves antero-dorsally to communicate with the female atrium (ie proflex with anterior approach; Figure 5(c)). Approximately two-thirds of the length of the female genital duct receives the openings of shell glands; at the point of communication with the female atrium, this duct diminishes somewhat in diameter (Figures 5(c) and 7(b,c)). The female genital duct is lined with a columnar epithelium, with the apical portion of its cells containing xanthophil granules.

The gonoduct originates from the mid-ventral wall of the female atrium, the latter being spacious and more or less ovoid in shape, with its length being about half of that of the male atrium (Figures 5(c) and 7(b,c)). The female atrium is lined with a 40–100 µm high, somewhat irregular, nucleated epithelium, which exhibits some recesses that are sunken into the underlying parenchyma (Figure 7(d)). The cytoplasmic membrane of the epithelial cells could not be recognised. Most of the cytoplasm of these cells has low affinity for stain, while the apical portion of the cells is provided with a bright, xanthophil secretion. The female atrium is surrounded by a 25 µm thick, subepithelial, layer of circular musculature, followed by a loose coat of longitudinal fibres.

A blind duct, measuring about 140 µm in width and 520 µm in length, opens into the antero-lateral region of the female atrium (Figures 5(c) and 7(e,f)). This duct is lined with a cuboidal epithelium, which is pierced by the openings of two types of gland cells, producing erythrophil and cyanophil granules, respectively. The blind duct is surrounded by a 20 µm thick layer of circular muscle.

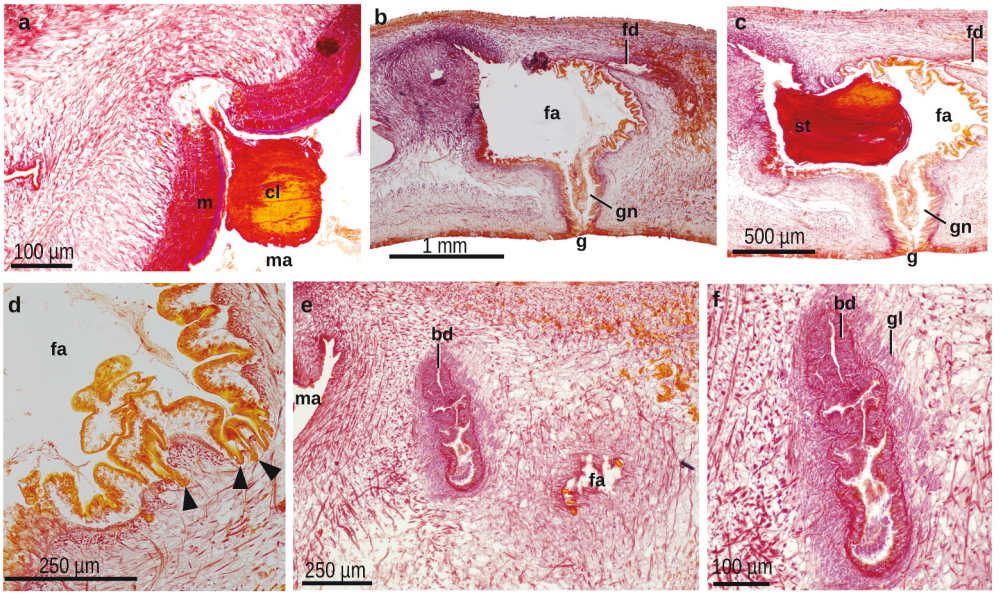

Figure 7. *Mapuplana guttulata*. Photomicrographs, sagittal sections. (a) Male atrium and the xanthophil clump; (b–c) female atrium; (d) epithelium of female atrium (arrowheads indicate epithelial recesses sunken into the underlying parenchyma); (e, f) blind duct opening into the female atrium.

The female atrium houses a spermatophore, with a shape between ovate and quadrate (Figures 6(a) and 7(c)), about 550 × 300 µm in size. It is composed of a strongly xanthophil substance – similar to that of the clump in the male atrium – and is amorphous in its central portion, whereas it is fibrous at its periphery. Small portions at the innermost region of the spermatophore contain sperm.

The common muscular coat consists of longitudinal and oblique muscle fibres, and surrounds the distal region of the prostatic vesicle, the male and female atria, and the female genital duct.

Mapuplana fjordica sp. nov.

Material examined

Holotype ZMB 11512. Puerto Gaviota, Magdalena Island, Chonos Archipelago, Southern Chile (44°40′0″S, 73°8′0″W), coll. J.H. Grau, 27 January 2007. Transverse sections of the cephalic and ovarian region on 14 slides; horizontal sections of a portion behind the cephalic region on seven slides; transverse sections of the pre-pharyngeal region on four slides; sagittal sections of the pharynx and copulatory apparatus on 34 slides.

Type locality

Puerto Gaviota, Magdalena Island, Chonos Archipelago, Southern Chile. The worm was collected from beneath fallen wood near Puerto Gaviota in Magdalena Island, Southern Chile (44°40′0″S, 73°8′0″W). This region is characterised by the Valdivian temperate rain-forest and receives up to 4000 mm of rain per year.

Etymology

The specific epithet refers to the word *fjord*, alluding to the landscape of the Chonos Archipelago.

Diagnosis

Species of *Mapuplana* about 40 mm in length with yellowish dorsum with numerous short, longitudinal dark striae. Ventral cutaneous musculature thickest in the median region of the body. Male atrium 2.4 times longer than female atrium. Proximal portion of the female genital duct lacks openings of shell glands. Gonoduct obliquely oriented, with postero-dorsal inclination. Female atrium with irregular shape.

Description

External appearance. The live specimen measured about 40 mm in length and 3 mm in width; preserved it measured 25 × 6 mm, with a thickness of 1.4 mm. The body is lanceolate, dorsally convex and ventrally flat. The anterior tip is rounded and the posterior one obtusely pointed (Figure 8). At rest, the dorsum is corrugated (Figure 8(a)). The ground colour of the dorsal body surface is yellow-orange (RAL 2000), while it is provided with a pair of thin, mid-dorsal, orange-brown (RAL 8023) longitudinal stripes. Furthermore, numerous short and anastomosing longitudinal stripes, with the same orange-brown colour, are spread all over the dorsum, except the paramedian region

(Figure 8(a)). The ventral side of the preserved specimen exhibited the same pattern of pigmentation as the dorsum, albeit paler, while the anterior extremity was greyish (Figure 8(d)).

The eyes are monolobated, and measure approximately 40 µm in diameter. The eye-cups contour the anterior tip of the body and extend in a single row along the entire lateral body margin. Sensory pits are absent. The creeping sole is about 80% of the body width (Figure 9(a)). The relative position of the mouth:body length is 77% in relation to the anterior tip of the body, while that of the gonopore:body length is 83%.

Epidermis and its secretions. The dorsal and ventral epithelia are about 27 µm in height and are pierced by the openings of gland cells, producing an erythrophil, granular secretion. Rhabditogen cells open onto the latero-dorsal surface, as well as the lateral body margins. A glandular margin is absent.

Cutaneous musculature. The cutaneous musculature consists of a subepidermal layer of circular muscle, followed by a double diagonal muscle layer and a layer of longitudinal fibres. The longitudinal muscle layer consists of large bundles and is about 85 µm thick dorsally, while it measures 240 µm ventrally (Figures 9 and 10(a,b)). A 30 µm thick portion of the ventral longitudinal muscle is subepidermal and consists of fibres that are gathered into bundles of 10–15 fibres, while a 210 µm thick portion of the ventral longitudinal muscle is composed of fibres joined into bundles of 8–25 fibres, and is sunken into the parenchyma. The cutaneous musculature thickness relative to the body height in the pre-pharyngeal region corresponds to 27%.

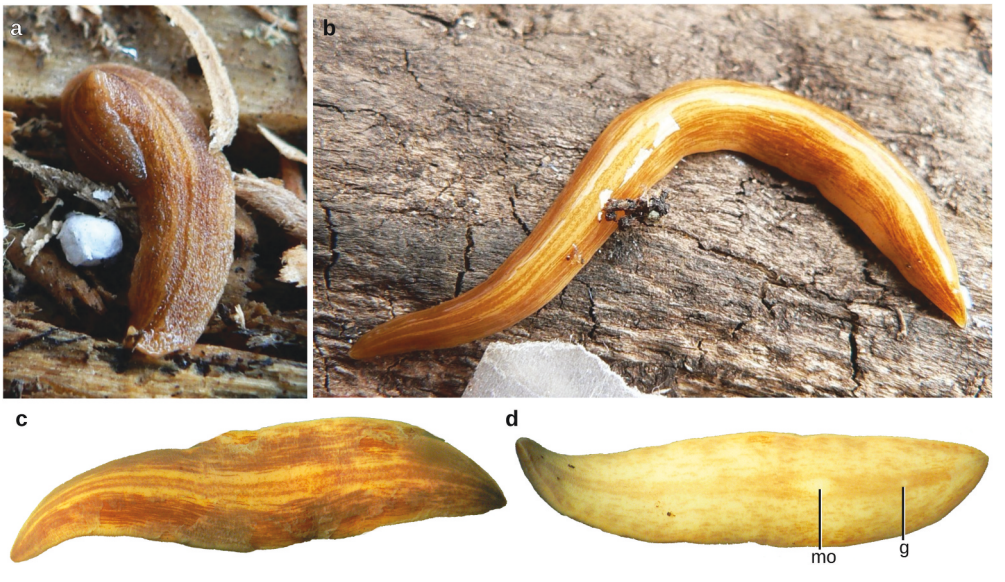

Figure 8. *Mapuplana fjordica*. External features. (a) Dorsal view of holotype during rest; (b) dorsal view of holotype while creeping; (c) dorsal view of preserved holotype; (d) ventral view of preserved holotype. Scale bars not available.

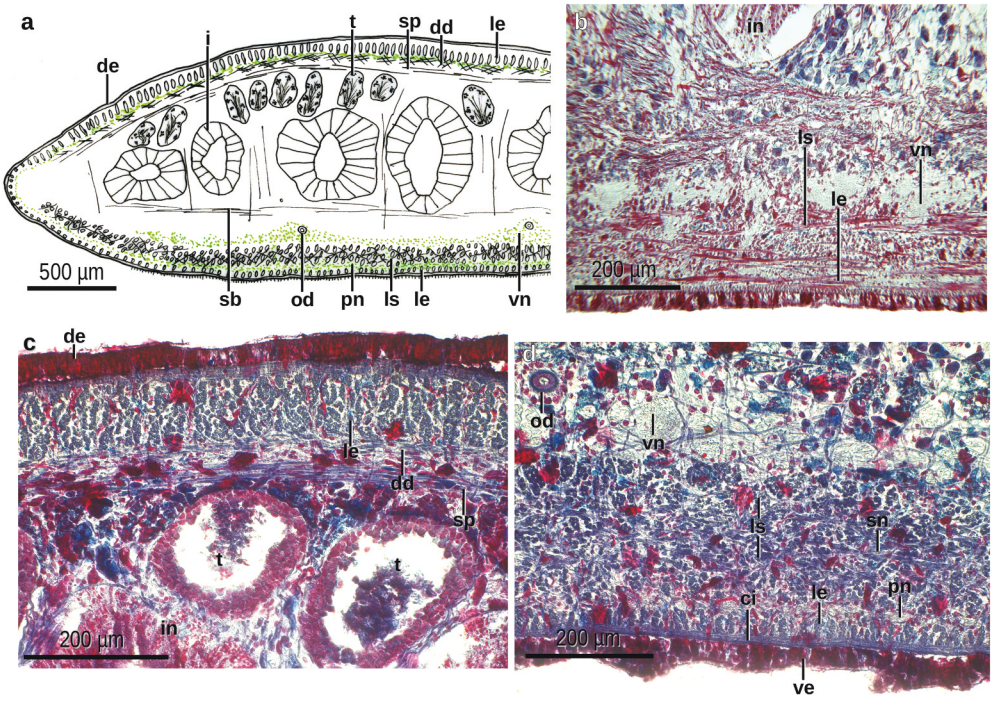

Figure 9. *Mapuplana fjordica*. (a) Diagrammatic reconstruction of a transverse section of the pre-pharyngeal region; (b) photomicrograph of a sagittal section of the body, showing cutaneous and parenchymal musculature; (c, d) photomicrographs of transverse sections of the pre-pharyngeal region.

Parenchymal musculature. There are four parenchymal muscle layers, namely a dorsal layer of decussate fibres (28 µm thick, 2.0% of the body height), a supraintestinal transverse muscle layer (40 µm), a subintestinal transverse muscle layer (30 µm), and a layer of subneural muscle with diagonal decussate fibres (170 µm); the decussate fibres and those of the insunk ventral cutaneous muscle layer are intermingled (Figures 9(d) and 10(a,b)).

Musculature in cephalic region. Towards the anterior tip of the body the muscle layers gradually strengthen, so that the CMI here reaches a value of 28% (Figure 10(c,d)). Unfortunately, similar stain affinities of muscle fibres and gland cells hinder clear visualisation of the arrangement of the fibres.

Pharynx. The mouth is located at a distance from the anterior region of the pharyngeal pouch equivalent to 64% of its length (Figure 11(a)). The oesophagus is about 15% of the length of the pharynx, which is cylindrical and occupies most of the pharyngeal pouch. The epithelium of the pharyngeal pouch is underlain by a layer of circular muscle. The outer pharyngeal epithelium is underlain by a layer of subepidermal longitudinal muscle (6 µm thick), followed by a layer of circular muscle (12 µm thick). Immediately underneath this circular muscle layer is located a 100–110 µm thick layer of intermingled longitudinal and circular muscle fibres that projects from the pharynx anteriorly into the adjacent parenchyma. The inner pharyngeal epithelium is underlain by a layer of intermingled

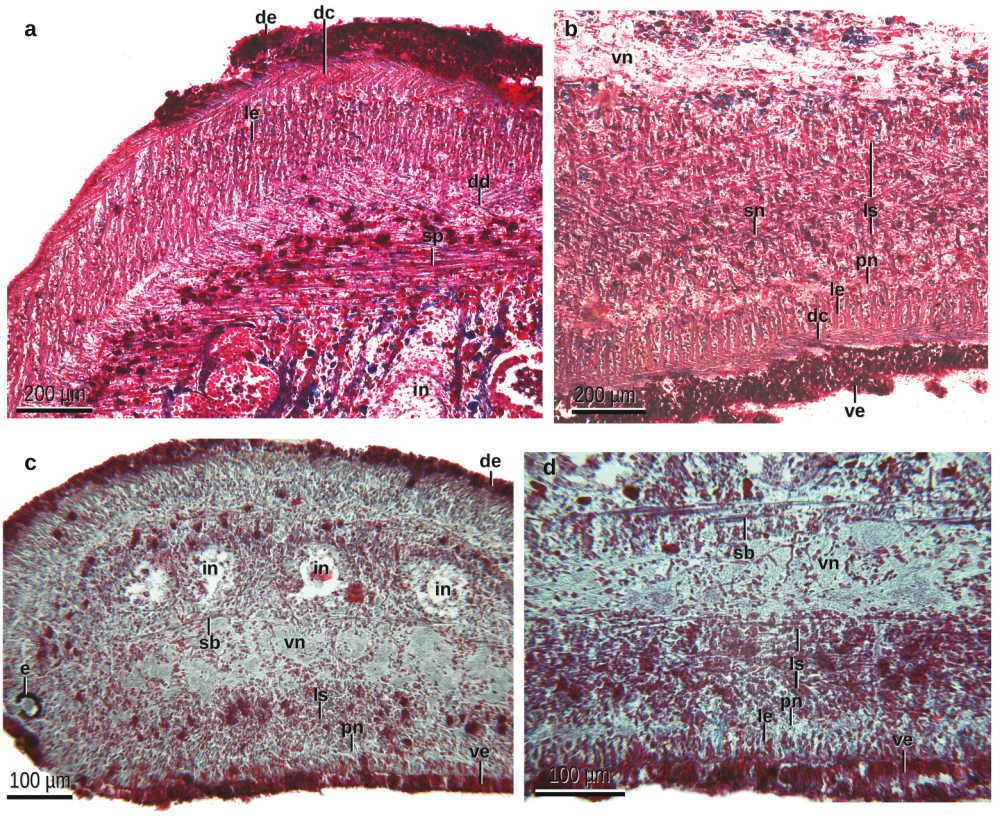

Figure 10. *Mapuplana fjordica*. Photomicrographs. (a, b) Horizontal sections of a portion behind the cephalic region, showing (a) dorsal and (b) ventral regions; (c, d) transverse sections of the cephalic region, showing the musculature.

circular and longitudinal muscle fibres (130–145 µm thick) (Figure 11(a,b)). Radial muscle fibres are also present. Erythrophil and cyanophil granular secretions run through the parenchyma of the pharynx and are discharged at its tip.

Male reproductive apparatus. The testes are ovoid, measuring 150–170 µm in diameter; the follicles are located dorsally between the intestinal branches and the supra-intestinal transversal parenchymal muscle layer. They are arranged in multiple irregular rows on either side of the body. The testes are prepharyngeal, with the anterior-most ones being located at a distance of 2934 µm from the anterior tip of the body and the posterior-most follicles positioned just anterior to the root of the pharynx.

The sperm ducts run immediately above the sub-intestinal parenchymal muscle layer and slightly laterally to the oviducts. Behind the pharyngeal region, the sperm ducts gradually expand to form spermiducal vesicles, which are packed with spermatozoa. Shortly behind the pharynx these ducts curve dorsally and open separately into one of the tubules of the prostatic vesicle glands (Figure 11(c)). The latter communicate with the tubular prostatic vesicle by means of several connections, which are very difficult to discern and reconstruct. In point of fact, one of the sperm ducts communicates at

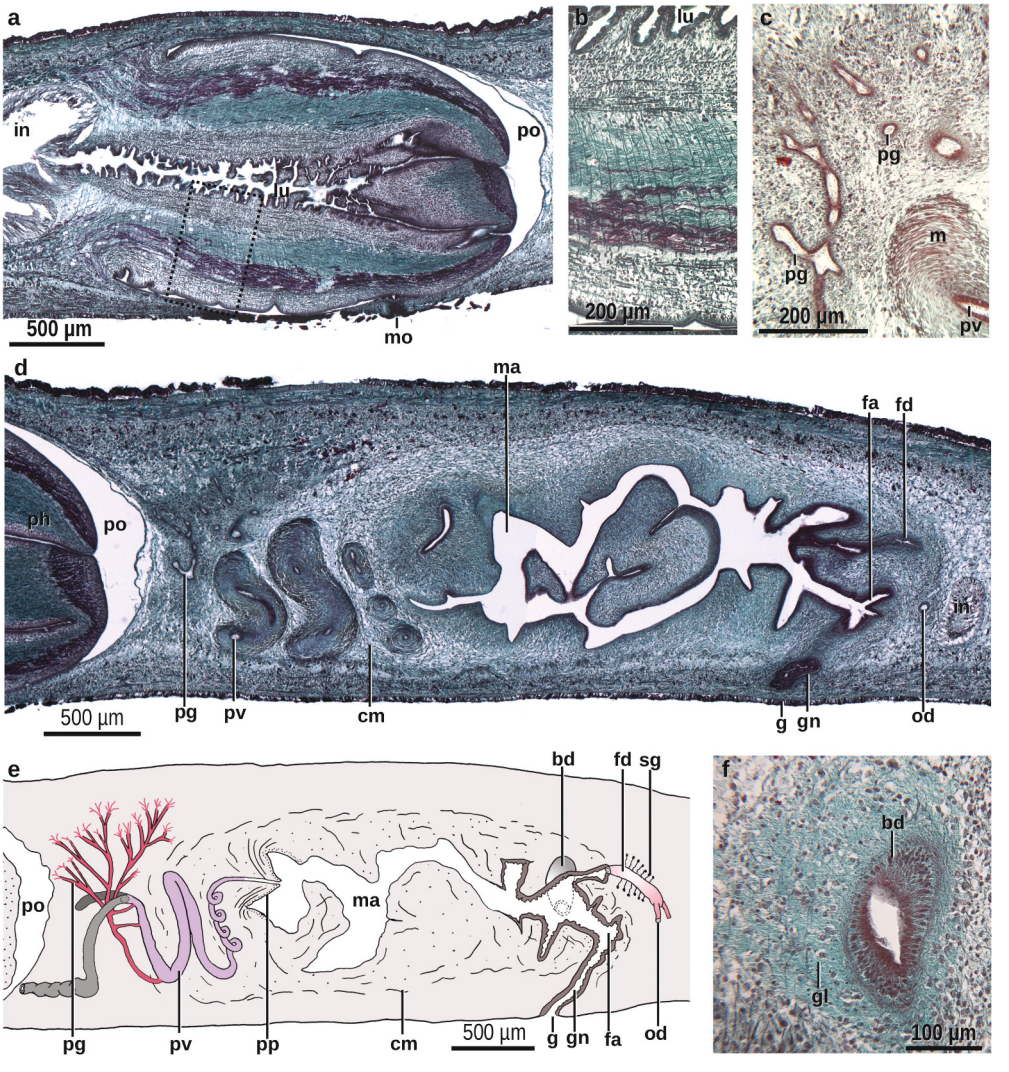

Figure 11. *Mapuplana fjordica*. Pharynx and copulatory apparatus. (a, b) Photomicrographs of sagittal section of (a) complete pharynx and (b) detail of pharyngeal musculature; (c) photomicrograph of sagittal section of tubules of prostatic vesicle glands; (d) photomicrograph of sagittal section of copulatory complex; (e) diagrammatic reconstruction of the copulatory apparatus; (f) photomicrograph of sagittal section of the blind duct.

a rather ventral position with a tubule of the prostatic vesicle gland and for this gland we were unable to find its connection with the prostatic vesicle. The latter consists of a winding, extra-bulbar duct with a diameter of about 25 µm, while it is considerably narrower at its anterior and posterior extremities. The prostatic vesicle penetrates the antero-ventral region of the penis bulb and, thereafter, ascends vertically in a coiled fashion to communicate with the ejaculatory duct (Figure 11(e)). This prostatic vesicle is lined with a cuboidal, nucleated and ciliated epithelium and is surrounded by a coat of circular muscle between 40 and 100 µm in diameter. This layer of circular muscle is

followed by a muscle web that surrounds the entire prostatic vesicle and attaches to the penis bulb. In some histological sections, this muscle web seems to be a continuation of the common muscle coat.

There are two prostatic vesicle glands, one on either side of the body, consisting of highly branched tubules, lined with a cuboidal, nucleated and ciliated epithelium (Figure 11(c,e)). The numerous branching tubules, which are surrounded by a thin layer of circular muscle, collect the erythrophil, granular secretion produced by the surrounding gland cells.

The ejaculatory duct horizontally traverses the penis papilla to exit at its tip through a narrow opening (Figure 11(e)); the duct is lined with a ciliated, cuboidal epithelium and is surrounded by a 25 µm thick layer of circular musculature.

The small, conical penis papilla is horizontally oriented, projects from the antero-dorsal wall of the male atrium and measures about 10% of the length of the atrium. The musculature of the penis papilla consists of a subepithelial layer of circular muscle, followed by a layer of longitudinal fibres.

The male atrium is ample and provided with 2–3 large transverse folds, and is about 2.4 times as long as the female atrium. The epithelium of the male atrium is cuboidal and is pierced by openings of scarce glands, producing an amorphous, cyanophil secretion. This epithelium is underlain by a 20–30 µm thick layer of circular muscle, which is continuous with a net of abundant muscle fibres without any definite orientation.

Female reproductive system. A single pair of ovaries is located at the posterior end of the anterior third of the body, with the gonads being placed immediately above the ventral nerve plate and directly underneath the transversal sub-intestinal parenchymal muscle layer. The globular ovaries measure about 200 µm in diameter. The ovovitelline ducts arise from the dorsal surface of the ovaries and run posteriorly immediately above the ventral nerve plate. Posteriorly to the gonopore, the oviducts ascend to open into the female genital duct (Figure 11(e)). The latter receives the openings of shell glands along approximately 60% of its anterior length, while it curves antero-dorsad to open into the female atrium.

The gonoduct originates at the postero-ventral wall of the female atrium, the latter being rather small and having an irregular shape, while it is surrounded by a layer of circular muscle fibres. The female atrium is lined with columnar cells with basal nuclei in which an erythophil, granular secretion accumulates in the apical portions of the cells. A blind duct originates from the lateral wall of the female atrium on the left side of the animal, but quickly assumes a vertical orientation when it projects into the adjacent parenchyma, while being embedded in the musculature of the female atrium (Figure 11 (e,f)). This duct measures about 200–300 µm in length and about 50–100 µm in diameter. Basically, the histology of this blind duct is the same as that of the female atrium, including the erythrophil secretion accumulating in the apical portions of the cells. However, in contrast to the female atrium, the epithelium of the blind duct is pierced by abundant openings of gland cells lying around the duct, producing an amorphous cyanophil secretion.

The common muscular coat surrounds the distal ascending portion of the prostatic vesicle, the male atrium, female atrium, and female genital duct and consists of longitudinal and obliquely running muscle fibres.

Discussion

Although the broad creeping sole, dorsal testes and strong cutaneous musculature strongly suggest that the two new species belong to the Geoplaninae, their anatomical features prevent taxonomic assignment to any of the presently known genera within this subfamily. In particular, several features set these two species apart from all other Geoplaninae genera, namely a subneural parenchymal musculature consisting of diagonal decussate fibres, being intermingled with those of the insunk ventral cutaneous longitudinal musculature, and a blind duct opening laterally into the female atrium.

Besides this, *Mapuplana* presents a stronger cephalic musculature, while the sunken fibres are oriented obliquely towards the dorsal body surface and to the body margins, at least in *M. guttulata*. In most geoplanin species the cephalic musculature does not differ from that in the pre-pharyngeal region, except that the muscle layers become thinner until they disappear in the anterior tip of the body. In *Geobia subterranea* (Müller, 1856, in Schultze and Müller 1856) the cutaneous and parenchymal muscle layers are extraordinarily strong, but without other specialisations (Froehlich 1954). In *Cephaloflexa* Carbayo and Leal-Zanchet, 2003 and *Choeradoplana* von Graff, 1896, the fibres of the ventral longitudinal cutaneous musculature form the retractor muscle of the cephalic region, with the fibres running parallel to the longitudinal body axis, which differs from the situation in *M. guttulata*. The genera *Pichidamas*, *Issoca* Froehlich, 1954, *Supramontana* Carbayo and Leal-Zanchet, 2003, *Luteostriata* Carbayo, 2010, and *Winsoria* Negrete et al., 2019 also present a cephalic retractor muscle that is mainly derived from the ventral longitudinal cutaneous musculature, with the fibres of this retractor running obliquely towards the dorsum and the lateral portions of the body (cf. Froehlich 1954; Carbayo and Leal-Zanchet 2003; Carbayo 2010; Bulnes et al. 2018; Negrete et al. 2019), thus resembling the situation in *M. guttulata*. However, in these genera the subneural muscle fibres run from one side of the body to the other, whereas in *M. guttulata* they are restricted to mid-body.

Although the two species of *Mapuplana* exhibit a number of other, rather rare characters, these structures do occur also in a number of other geoplanin genera, notably (a) epithelial sensory depressions, which are present also in *Pichidamas* and *Wallmapuplana ruca* (cf. Bulnes et al. 2018; Negrete et al. 2021); and (b) glands with branched ducts associated with the prostatic vesicle, which are exhibited also by *Wallamapuplana ruca* (Marcus, 1954) (see Negrete et al. 2021). Furthermore, these branched glands have also recently been found to be present in *Pichidamas piru* Bulnes et al., 2018. When the latter species was described, the suboptimal quality of the sections prevented full appreciation of the ductal nature of these glands. It was not until the same type of glands were observed in the new species of *Mapuplana* that some photomicrographs taken from the holotype of *P. piru* could be reinterpreted as documenting the presence of branched glands (FC pers. obs.). These two rare characters suggest a close relationship between the genera *Mapuplana, Pichidamas* and *Wallmapuplana*.

Differences in the relative development of the muscular components of the cephalic region suggest that the cephalic retractor musculature in the abovementioned genera is not homologous to the muscular modification observed in *M. guttulata*. For this reason, we referred to the modifications in the cephalic musculature as simply being stronger. The short time during which the live animals of *Mapuplana* could be observed at the time of sampling did not reveal any noticeably different movement of the cephalic region.

Anatomically, the two new species are very similar and share several characteristic features, such as the highly coiled intrabulbar section of the prostatic vesicle; the branched and tubular glands of the prostatic vesicle, communicating in a complex manner with the vesicle; the presence of a blind duct communicating with the female atrium and receiving the openings of glands; an erythophil, granular secretion accumulating in the apical portions of the cells lining the female atrium; small, conical penis papilla; and the wall of the male atrium giving rise to large, irregular folds.

The two new species can be distinguished from each other mainly by (a) the dorsal colour pattern, which in *M. guttulata* exhibits a dark, minutely reticulated pattern, while in *M. fjordica* it shows numerous short, longitudinal dark striae; (b) the relative distance between mouth and gonopore, being larger in *M. guttulata* (equivalent to 17% of the body length) than in *M. fjordica* (6%); (c) the relative thickness of the ventral cutaneous musculature, which is thickest in parasagittal planes in *M. guttulata*, while in *M. fjordica* it is evenly thick along the median region of the body; (d) the gonoduct, which is vertical in *M. guttulata* (vs oblique in *M. fjordica*); (e) the female atrium being ovoid in *M. guttulata* (vs irregular in *M. fjordica*); and (f) the penis papilla being oriented somewhat obliquely in postero-ventral direction in *M. guttulata*, while it is oriented horizontally (albeit slightly ventral) in *M. fjordica*, although the slightly more oblique orientation in *M. guttulata* may well be due to a more distinct preservation and contraction artefact.

The blind canal in the two species of *Mapuplana* resembles the atrial diverticulum of the caenoplanin *Platydemus manokwari* Beauchamp, 1962 in its general shape and position, and in the presence of a weak coat of muscle. However, in *P. manokwari* the blind canal does not open through the lateral wall of the female atrium, but opens through the posterior atrial wall into the common genital atrium. Nevertheless, in both *Mapuplana* and *P. manokwari* the canal is lined by a cuboidal or columnar epithelium that is pierced by gland cells, while its lumen lacks sperm (cf. Winsor 1998). On account of these similarities, it could be inferred that the blind canal in *Mapuplana* functions also as a viscid mucus gland, producing a cocoon adhesive, as demonstrated for the atrial diverticulum of *P. manokwari* through histochemical observations (Winsor 1998).

It is interesting to note that in *Arthurdendyus triangulatus* (Dendy, 1896) the vasa deferentia consist of a series of wide, branched and convoluted tubules that have several openings into the seminal vesicle (Fyfe 1937). *Arthurdendyus vegrandis* Winsor and Stevens, 2005 (Subfamily Caeoplaninae), and *Luteostriata ceciliae* (Leal-Zanchet and Froehlich, 2003) and *L. subtilis* Boll, Amaral and Leal-Zanchet, 2019 (Subfamily Geoplaninae) also have branched vasa deferentia, albeit less developed. These branched vasa deferentia are reminiscent of the situation in *Mapuplana*, with its intricate relationship between the tubular glands of the prostatic vesicle, the vasa deferentia and the prostatic vesicle, but the condition in *A. triangulatus* is structurally different.

The two new species of *Mapuplana* from Chile suggest that a morphologically and evolutionarily distinct group of land planarians may be distributed west of the Andes mountains, especially south of the Atacama Desert.

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Disclosure statement

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References

Almeida AL, Francoy TM, Álvarez-Presas M, Carbayo F. 2021. Convergent evolution: a new subfamily for bipaliin-like Chilean land planarians (Platyhelminthes). Zool Scr. 50(4):500–508. doi:10.1111/zsc.12479

Blanchard E. 1845. Recherches anatomiques et zoologiques faites pendent un voyage sur les côtes de la Sicile et sur divers points du littoral de la France, par MM. H. Milne Edwards, A. de Quatrefages et E. Blanchard, troisième partie. Vol. 4. Paris; p. 1–353 + 25 pls.

Boll P, Do Amaral SV, Leal-Zanchet AM. 2019. Two new land planarian species (Platyhelminthes: Tricladida: Geoplanidae) from protected areas in southern Brazil. Zootaxa 4664(4):535–550. doi:10.11646/zootaxa.4664.4.5

Bulnes VN, Grau JH, Carbayo F. 2018. A new Chilean genus and species of land planarian (Platyhelminthes: Tricladida, Geoplaninae) with cephalic retractor muscle and adenodactyl. J Nat Hist. 52(39–40):2553–2566. doi:10.1080/00222933.2018.1538468

Carbayo F, Leal-Zanchet AM. 2003. Two new genera of geoplaninid land planarians (Platyhelminthes: Tricladida: Terricola) of Brazil in the light of cephalic specialisations. Invertebr Syst. 17(3):449–468. doi:10.1071/IT01035

Carbayo F. 2010. A new genus for seven Brazilian land planarian species, split off from *Notogynaphallia* (Platyhelminthes, Tricladida). Belg J Zool. 140(Suppl.):91–101.

Carbayo F, Álvarez-Presas M, Olivares CT, Marques FPL, Froehlich EM, Riutort M. 2013. Molecular phylogeny of Geoplaninae (Platyhelminthes) challenges current classification: proposal of taxonomic actions. Zool Scr. 42(5):508–528. doi:10.1111/zsc.12019

Cason JE. 1950. A rapid one-step Mallory-Heidenhain stain for connective tissue. Stain Technol. 25 (4):225–226. doi:10.3109/10520295009110996

Darwin C. 1844. Brief descriptions of several terrestrial Planariæ, and of some remarkable marine species, with an account of their habits. Ann Mag Nat Hist. 14(91):241–251. doi:10.1080/037454809495165

de Beauchamp P. 1962. Platydemus manokwari n. sp., planaire terrestre de la Nouvelle-Guinée Hollandaise. Bull Soc Zool Fr. 87:609–615.

Froehlich CG. 1954. Sôbre a morfologia e taxonomia das Geoplanidae. Bol Fac Fil Ciênc Letr Zool. 19:195–279.

Froehlich EM. 1978. On a collection of Chilean land planarians. Bol Zool. 3(3):7–80. doi:10.11606/.2526-3358.bolzoo.1978.121651

Fyfe ML. 1937. The reproductive system of the planarian *Artioposthia triangulata* (Dendy). Q J Microsc Sci. 80:99–126 + 3 pls.

Gabe M. 1976. Histological techniques. Paris: Masson.

Gay C. 1849. I. Planarianos. In: Gay C, editor. Historia Fisica y Politica de Chile, Zoologia. Tomo Tercero. Paris; p. 69–72.

Grau JH, Carbayo F. 2010. Panorama de la diversidad de planarias terrestres (Platyhelminthes: Tricladida) de Chile. Bol Biodivers Chile. 2:41–54.

Grau JH, Carbayo F. 2011. A new land planarian species of *Geoplana* (Platyhelminthes, Tricladida, Geoplanidae) from the Valdivian temperate rainforest of Southern Chile. Zoosyst Evol. 87 (2):327–334. doi:10.1002/zoos.201100010

Lang A. 1881. Der Bau von *Gunda segmentata* und die Verwandtschaft der Plathelminthen mit Coelenteraten und Hirudineen. Mittheilungen aus der Zoologischen Station zu Neapel. 3:187–251.

Leal-Zanchet AM, Froehlich EM. 2003. A new species of terrestrial planarian of the genus *Notogynaphallia* Ogren & Kawakatsu (Platyhelminthes, Tricladida, Terricola) from south Brazil and some comments on the genus. Rev Bras Zool. 20(4):745–753. doi:10.1590/S0101-81752003000400030

Marcus E. 1954. Reports of the Lund University Chile expedition 1948-1949. 11 Turbellaria. Lunds Univ Årsskr NF Avd 2. 49(13):1–114.

Negrete L, Álvarez-Presas M, Riutort M, Brusa F. 2021. Integrative taxonomy of land planarians (Platyhelminthes: Geoplanidae) from the Andean-Patagonian forests from Argentina and Chile, with the erection of two new genera. J Zool Syst Evol Res. 59(3):588–612. doi:10.1111/jzs.12444

Negrete L, Do Amaral SV, Ribeiro GG, Gonçalves JW, Valiati VH, Damborenea C, Brusa F, and Leal-Zanchet AM. 2019. Far away, so close! Integrative taxonomy reveals a new genus and species of land flatworm (Platyhelminthes: Geoplanidae) from southern South America. Zool J Linn Soc. 189 (3):722–744. doi:10.1093/zoolinnean/zlz131

Ogren RE, and Kawakatsu M. 1990. Index to the species of the family Geoplanidae (Turbellaria, Tricladida, Terricola) part I: Geoplaninae. Bull Fuji Women's Coll. no. 28, ser. II:79–166.

Sandison AT. 1955. The histological examination of mummified material. Stain Technol. 30 (6):277–283. doi:10.3109/10520295509114479

Schultze M, Müller F. 1856. Beiträge zur Kenntniss der Landplanarien nach Mittheilungen des Dr. Fritz Müller in Brasilien und nach eigenen Untersuchungen. Abhandlungen der naturforschenden Gesellschaft zu Halle, Bd. 4:19–38.

Shorthouse DP. 2010. SimpleMappr, an online tool to produce publication-quality point maps. [accessed 2022 Feb 17]. https://www.simplemappr.net.

Stimpson W. 1858. Prodromus descriptionis animalium evertebratorum quae in Expeditione ad Oceanum Pacificum Septentrionalem, Johanne Rodgers Duce a Republica Federate missa, observavit et descripsit. Proc Acad Nat Sci Philadelphia 9:19–31.

von Graff L. 1896. Über das System und die geographische Verbreitung der Landplanarien. Verh Dtsch Zoolog Ges. VI:61–75.

Von Graff L. 1899. Monographie der Turbellarien. II. Tricladida Terricola (Landplanarien). I-XII + 574 pp. Atlas von Achtundfünfzig Tafeln zur Monographie der Turbellarien II. Tricladida Terricola (Landplanarien). Pls. I-LVIII. Leipzig: Wilhelm Engelmann.

Winsor L. 1998. The role of the atrial diverticulum in the copulatory apparatus of the terrestrial flatworm *Platydemus manokwari* de Beauchamp (Tricladida: Terricola). Hydrobiologia. 383(1/3):83–89. doi:10.1023/A:1003451829795

Winsor L, Sluys R. 2018. Basic histological techniques for planarians. In: Rink JC, editor. Planarian regeneration: Methods and protocols. Methods in Molecular Biology. Vol. 1774. New York: Springer Science+Business Media; p. 285–351.