

A new genus and two new species of land planarians (Platyhelminthes, Tricladida, Geoplanidae) from Southern Chile, including the Chonos archipelago

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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 characterized by two putative apomorphies, namely a subneural parenchymal musculature constituted by 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 thickness of the ventral cutaneous musculature, the orientation of the gonoduct, and the shape of the female atrium.

KEYWORDS

Chile, land flatworm, new genus, new species, *Mapuplana*

INTRODUCTION

The taxonomy of Neotropical land planarians (Platyhelminthes, Geoplanidae) has advanced considerably in the last decade, mainly due to the inclusion of genetic data as well as detailed morphological information, which led to more highly resolved phylogenetic trees (Carbayo et al., 2013; Álvarez-Presas and Riutort 2014; Sluys and Riutort 2018). 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 diversity and interrelationships of Brazilian Geoplaninae taxa are much better understood 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diversity of the Chilean fauna presumably comprises many more species than known at present (Grau and Carbayo, 2010; unpublished data).

Presently, approximately 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 (1889), Marcus (1954), and Froehlich (1978), while more recent studies authored by Grau and Carbayo (2010, 2011), Bulnes et al. (2018), Negrete et al. (2021), and Almeida et al. (2021) have contributed to this list of native land planarians in Chile.

Currently, nominal Chilean land planarian species belong to three subfamilies, viz., Geoplaninae Stimpson, 1858, Rhynchodeminae Graff, 1896, and Timyminae Almeida & Carbayo, 2021, the latter recently proposed as an endemic subfamily of Chile. Geoplaninae represents 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 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 & Kawakatsu, 1990) (Carbayo et al. 2013).

Recent studies already attempted to further unveil the Chilean taxonomic diversity of the Geoplaninae (Grau and Carbayo 2010, 2011; Bulnes et al. 2018) and our present contribution also aims at achieving 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

Both specimens of land planarians were collected by hand in the field, photographed alive, while also 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 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 isopropyl alcohol and, thereafter, dealcoholized in clove oil before embedding into synthetic wax. Serial histological sections were made at intervals of 7 µm or 9 µm and were stained with Mallory-Cason (see Cason 1950, Winsor and Sluys 2018) and Azan (see Gabe 1976). Ratio of sub-cutaneous musculature thickness:body height was calculated at the pre-pharyngeal region, after Froehlich (1955). Reconstruction drawings were prepared by 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 Fig. 1 were generated with Simplemappr (Shorthouse 2010). Type specimens were 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; **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; **pvs**, prostatic vesicle; **sb**, subintestinal parenchymal muscle layer; **sd**, sperm duct; **sg**, shell glands; **sn**, subneural parenchymal muscle of diagonal decussate fibres; **sp**, suprainintestinal parenchymal muscle layer; **st**, spermatophore; **t**, testis; **ve**, ventral epithelium; **vi**, vitellaria; **vn**, ventral nerve plate

RESULTS

Systematic account

Order Tricladida Lang, 1881

Family Geoplanidae Stimpson, 1858

Subfamily Geoplaninae Stimpson, 1858

Genus *Mapuplana* gen. nov.

Diagnosis. Geoplaninae of flattened, slightly lanceolate body, ranging between 40-50 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 reinforced. Subneural parenchymal musculature constituted by diagonal decussate fibres, intermingled with the sunken portion of the ventral longitudinal cutaneous

musculature. Prostatic vesicle receives the secretion of tubular, branched glands. Penis papilla small and conical. A blind duct opens sideways into 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°0'00"S, 73°11'00" W), coll. F. Carbayo, December 12th, 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 in shape.

Type locality. Monumento Natural Contulmo, Southern Chile.

Etymology. The specific epithet is derived from the Latin *guttula*, 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 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, after which it tapers, thus acquiring the shape of a broad arrowhead (Fig. 2A). In resting position, the dorsal surface is more or less corrugated, i.e., provided with wrinkles and folds. The ground colour of the dorsum is pastel yellow (RAL 1034), and is adorned with scarce yellow-orange (RAL 2000) specks and numerous, red-orange (RAL 2001) small marks forming a minutely reticulated pattern (Fig. 2A-B). A broad mid-dorsal stripe (1/9th 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 that the median band is lighter (Fig. 2C).

The eyes are monolobated, ranging between 45-82 μm in diameter. The eye cups are distributed in a single-to-double marginal row, extending on either side of the body from the very anterior tip 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 (Fig. 3A). 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. The mouth and gonopore are located posterior to mid-body. Relative position of the mouth:body length is 60.6% in relation to the anterior tip of the body; that of the gonopore:body length is 77.7%.

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 (Fig. 3B).

Cutaneous musculature. The cutaneous musculature comprises three layers, namely a subepidermal circular layer (5-7.5 μm thick in the pre-pharyngeal region), followed by a double layer (12.5-20 μm) with decussate fibres, and a strong, innermost longitudinal muscle layer (Fig. 3C-F). The longitudinal musculature is 80 μm thick dorsally and 400 μm ventrally; dorsally, the fibres are gathered in large bundles (Fig. 3C). In the ventral portion of the body 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 (Fig. 3D, F). In the pre-pharyngeal region of the body the cutaneous musculature thickness relative to the body height (abbreviated, MCI) corresponds to 12.5% in the mid-sagittal plane. This value increases to 20% in para-sagittal planes, due to an increase in the thickness of this musculature (Fig. 3D, F).

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 suprainestinal 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 (Fig. 3D).

Musculature in cephalic region. In the cephalic region, the musculature is relatively stronger than in the pre-pharyngeal region and it is also organized differently (Fig. 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, whereas the subepidermal portion represents 63% (Fig. 4A); in this region MCI 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 MCI decreases to 58% and 34%, respectively (Fig. 4C-D); in this region of the body the transverse muscle fibres are relatively more abundant than in the pre-pharyngeal region, whereas 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 (Fig. 4C-F). At 100 μm from the anterior tip, the sunken portion of the ventral longitudinal cutaneous musculature is lacking, whereas 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 (Fig. 5A). The esophagus 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) (Fig. 5B). 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 (Fig. 3B-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, i.e., 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, dilated, thus forming spermiducal vesicles, which are packed with spermatozoa.

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 xanthophil granular secretions, produced by gland cells located all around the tubules (Figs 5C, 6C-D). The tubular glands join before opening into the very proximal, posterior portion of the prostatic vesicle (Fig. 5C).

The unpaired tubular prostatic vesicle follows a spiralling trajectory (Figs. 5C, 6B-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 (Fig. 5C, 6B-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 (Figs 5C, 6E). 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 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 and provided with distinct folds and is lined with a low epithelium. (Figs 5C, 6A-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 x 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 (Figs 6B, 7A).

Female reproductive system. The ovaries are approximately globular, measuring about 250 μm in diameter. These ovaries are located at a distance from the anterior tip of the body corresponding to 23% of the body length and are situated on top of the ventral nerve plate, and underneath the transverse sub-intestinal parenchymal muscle layer. 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 (Fig. 5C). Approximately 2/3th 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 (Figs 5C, 7B-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 (Figs 5C, 7B-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 (Fig. 7D). The cytoplasmic membrane of the epithelial cells cannot be recognized. 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 (Figs 5C, 7E-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.

The female atrium houses a spermatophore, with a shape between ovate and quadrate (Figs 6A, 7C). The spermatophore measures about 550 x 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 is constituted by 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, January 27th, 2007.

Transverse sections of the cephalic and ovarian region on 14 slides; horizontal sections of a portion behind the cephalic region on 7 slides; transverse sections of the pre-pharyngeal region on 4 slides; sagittal sections of the pharynx and copulatory apparatus on 34 slides.

Type locality. Puerto Gaviota, Magdalena Island, Chonos Archipelago, Southern Chile.

Etymology. The specific epithet refers to the word *fjord*, thus 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, while in preserved condition it measured 25 x 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 (Fig. 8). At rest, the dorsum is corrugated (Fig. 8A). 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, spread all over the dorsum, excepting the paramedian region (Fig. 8A-C). The ventral side of the preserved specimen exhibited the same pattern of pigmentation as the dorsum, albeit paler, while the anterior extremity was greyish (Fig. 8D).

The eyes are monolobated, and measure approximately 40 μ m 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 (Fig. 9A). 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 μ m 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 is constituted of a subepidermal layer of circular muscle, followed by a double diagonal muscle layer and a layer of longitudinal fibres. The longitudinal muscle layer is constituted of large bundles and is about 85 μ m thick dorsally, while it measures 240 μ m ventrally (Fig. 9, 10A-B). A 30 μ m thick portion of the ventral longitudinal muscle is subepidermal and is constituted 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%.

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 suprainestinal 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 (Fig. 9D, 10A-B).

Musculature in cephalic region. Towards the anterior tip of the body the muscle layers gradually strengthen, so that the MCI reaches here a value of 28% (Fig. 10C-D). Unfortunately, similar stain affinities of muscle fibres and gland cells hinder clear visualization 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 (Fig. 11A). The esophagus 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 circular and longitudinal muscle fibres (130-145 μm thick) (Fig. 11A-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 anteriormost ones being located at a distance of about 2934

µm from the anterior tip of the body and the posteriormost 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 (Fig. 11C-E). 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 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 (Fig. 11E). This prostatic vesicle is lined with a cuboidal, nucleated and ciliated epithelium and is surrounded by a coat of circular muscle ranging between 40 - 100 µm in diameter. Additionally, 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 from 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 (Fig. 11C, 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 traverses horizontally the penis papilla to exit at its tip through a narrow opening (Fig. 11E); 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 and 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, cyanophilic 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. The globular ovaries measure about 200 μm in diameter. The gonads are located at the posterior end of the anterior third of the body and are located immediately above the ventral nerve plate and directly underneath the transversal sub-intestinal parenchymal muscle layer. 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 (Fig. 11E). 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 with 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 erythrophil, granular secretion accumulates in the apical portions of the cells. A blindly ending duct originates from the lateral wall of the female atrium, but quickly assumes a vertical orientation when it projects into the adjacent parenchyma, while being embedded in the musculature of the female atrium (Fig. 11E-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 is constituted by longitudinal and obliquely running muscle fibres.

Habitat. 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 characterized by the Valdivian temperate rainforest and receives up to 4000 mm of rain per year.

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 constituted by diagonal decussate fibres, being intermingled with those of the insunk ventral cutaneous longitudinal musculature, as well as a blind duct opening sideways into the female atrium.

Besides this, *Mapuplana* presents a reinforced 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 only become thinner until they disappear in the anterior tip of the body. In *Geobia subterranea* (Müller, 1856, in Schultze & Müller, 1856) the cutaneous and parenchymal muscle layers are extraordinarily strong, but without other specializations (Froehlich 1954). In *Cephaloflexa* Carbayo & 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 & 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. Bulnes et al. 2018; Froehlich 1954; Carbayo and Leal-Zanchet 2003; Carbayo 2010; 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 *Wallmapuplana 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 (F.C. 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 constituting a reinforcement. The short time during which the live animals of *Mapuplana* could be observed at the time of sampling, did not reveal any particularly 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; 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; 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 thickness of the ventral cutaneous musculature, which is thickest in para-sagittal planes in *M. guttulata*, while in *M. fjordica* it is evenly thick along the median region of the body; (c) the gonoduct, which is vertical in *M. guttulata* (vs. oblique in *M. fjordica*); and (d) the female atrium is ovoid in *M. guttulata* (vs. irregular in *M. fjordica*), (e) the penis papilla is oriented somewhat obliquely in postero-ventral direction in *M. guttulata*, while it is oriented horizontally (albeit slightly ventral) in *M. fjordica*, albeit that the slightly more oblique orientation in *M. guttulata* may well be due to a more distinct preservation and contraction artefact.

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). Although this is reminiscent of the situation in *Mapuplana*, with its intricate relationship between the tubular glands of the prostatic vesicle, vasa deferentia and the prostatic vesicle, 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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LEGENDS TO THE FIGURES

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

Figure 2. *Mapuplana guttulata*. Photographs of the live holotype in dorsal (A-B) and ventral (C) views. Scale bars not available.

Figure 3. *Mapuplana guttulata*. Photomicrographs of histological sections. (A) transverse section of the cephalic region, showing the sensory depression (arrow); (B-D) transverse sections of the pre-pharyngeal region; (E-F) horizontal sections showing cutaneous and parenchymal muscles (anterior at the top).

Figure 4. *Mapuplana guttulata*. Photomicrographs of transverse sections of the anterior region of the body, located at 1000 μm (A), 900 μm (B), 500 μm (C-D), and 400 μm (E-F) from the anterior extremity of the body.

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.

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.

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.

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.

Figure 10. *Mapuplana fjordica*. Photomicrographs. (A-B) Horizontal sections of a portion behind the cephalic region, showing dorsal (A) and ventral (B) regions; (C-D) Transverse sections of the cephalic region, showing the musculature.

Figure 11. *Mapuplana fjordica*. Pharynx and copulatory apparatus. (A-B) Photomicrographs of sagittal section of complete pharynx (A) and detail of pharyngeal musculature (B); (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.





















