

1 **Integrative taxonomy of land planarians (Platyhelminthes: Geoplanidae) from the**  
2 **Andean-Patagonian Forests from Argentina and Chile, with the erection of two new**  
3 **genera**

4  
5 Two new genera of land planarians

6  
7 **Lisandro Negrete<sup>1,2</sup>, Marta Álvarez-Presas<sup>3,4</sup>, Marta Riutort<sup>4</sup>, Francisco Brusa<sup>1,2,\*</sup>**

8 <sup>1</sup>División Zoología Invertebrados, Facultad de Ciencias Naturales y Museo (UNLP),  
9 Boulevard 120 & 61, B1900CHX, La Plata, Buenos Aires, Argentina

10 <sup>2</sup>CONICET – Consejo Nacional de Investigaciones Científicas y Técnicas, La Plata, Buenos  
11 Aires, Argentina

12 <sup>3</sup>School of Biological Sciences, University of Bristol, Bristol, United Kingdom

13 <sup>4</sup>Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia and Institut de  
14 Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Av Diagonal, 643 08028  
15 Barcelona, Spain

16

17 \*Corresponding author: [fbrusa@fcnym.unlp.edu.ar](mailto:fbrusa@fcnym.unlp.edu.ar)

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19 **KEYWORDS**

20 Terrestrial flatworms, Geoplaninae, Argentinean and Chilean Patagonia, *Inakayalia*,  
21 *Wallmapuplana*

22

23 **Abstract**

24 Our knowledge about the diversity of land planarians (Platyhelminthes: Geoplanidae) from  
25 the Andean-Patagonian Forests is scarce as compared with other forested biomes. These cold-  
26 temperate forests are located in southern Chile and western Patagonia in Argentina, at the  
27 southern end of South America. Many species of land planarians from this region are known  
28 from descriptions based on a single or only a few specimens. That is why the finding of new  
29 material can reveal cryptic species, as well as anatomical or histological differences among  
30 specimens due to different maturation stages, physiological states, or intra-specific variation.  
31 In this paper, we focus on two geoplaninid species, *Geoplana valdiviana* and *Amaga ruca*,  
32 with the main goal of offering detailed re-descriptions of both species (previously known  
33 from Chilean Patagonia) from new material recently found in Argentinean Patagonia. In this  
34 contribution, we added new information on histological and anatomical features of these  
35 species. After assessing taxonomically relevant anatomical characters together with molecular  
36 data (mitochondrial *COI* gene and *18S* nuclear DNA), we took three taxonomic decisions: (1)  
37 *G. valdiviana* was removed from *Geoplana* and re-allocated in the new genus *Inakayalia*; (2)  
38 *Geoplana chanca* and *Geoplana tirua* were also included in this new genus; (3) *A. ruca* was  
39 split off from the genus *Amaga* and placed in the new monotypic genus *Wallmapuplana*. In  
40 addition, the known distribution of these planarian species was expanded to include the  
41 Argentinean portion of the Andean-Patagonian Forests.

42

## 43 1 INTRODUCTION

44 The Andean-Patagonian Forests, also known as Subantarctic forests, extend over a narrow  
45 strip on both sides of the Andes Mountains, in southern South America (Veblen, 2007). These  
46 are the southernmost forests in the world and have their origin 45 million years ago on the  
47 Gondwana supercontinent. The Andean-Patagonian Forests represent a large biogeographic  
48 ‘green’ island, separated from climatically similar areas by extensive ocean barriers and  
49 deserts. The connection of these cold-temperate forests with other forest ecosystems in South  
50 America was probably broken up by the end of the Tertiary and has remained severed during  
51 the entire Quaternary (Axelrod, Arroyo, & Raven, 1991; Villagrán & Hinojosa, 1997). The  
52 main biological value of these forests resides in their high degree of endemism. The large  
53 percentage of endemic species, e.g. almost 90% of the seed plants, suggests geological  
54 antiquity, long isolation, and high rates of extinction during the cooler Pleistocene (Villagrán  
55 & Hinojosa, 1997).

56 Land planarians are free-living flatworms (Platyhelminthes, Geoplanidae) usually present  
57 in humid forested areas. Since they lack water retention mechanisms, the animals spend most  
58 of the time below fallen logs and leaf litter during daylight to avoid insolation and  
59 desiccation. Although terrestrial flatworms form an important component of the soil fauna,  
60 their diversity remains underestimated, partly due to their cryptic behaviour. This applies  
61 especially to land planarians from the Andean-Patagonian Forests, which have received very  
62 little attention over time from biologists. Darwin (1844) was the first naturalist to describe  
63 land planarians from the Chilean portion of the Andean Forests during his voyage around the  
64 world on board the HMS Beagle (1831–1836). This was the starting point of a series of  
65 publications in which new species were sporadically described for the Chilean region, such as  
66 *Polycladus gayi* Blanchard, 1845, one of the largest planarians in the world. The last work of  
67 great relevance on the terrestrial planarians from Chile was carried out over 40 years ago by  
68 EM Froehlich (1978), who described seven new species and three new genera.

69 There are currently 27 land planarian species described for Chilean Patagonia, which are  
70 placed in eight genera within the subfamily Geoplaninae and two genera in the subfamily  
71 Rhynchodeminae (tribe Caenoplanini) (Darwin, 1844; Graff, 1899; Marcus, 1954; Froehlich,  
72 1978; Grau & Carbayo, 2010, 2011; Bulnes, Grau, & Carbayo, 2018). The only record known  
73 for Argentinean Patagonia is *Polycladus gayi* Blanchard, 1847, of which there are only  
74 sightings and photographic records (Semenas, 2013).

75 With the main goal of contributing to knowledge on the diversity of land planarians in the  
76 Andean-Patagonian Forest, we studied new material of *Geoplana valdiviana* Grau & Carbayo,

77 2011 and *Amaga ruca* (Marcus, 1954), collected in Argetina, species previously known only  
78 from Chilean Patagonia. We performed molecular phylogenetic analyses and examined  
79 anatomical and histological features of taxonomic relevance for these species. Our results  
80 pointed to the need of a taxonomic revision, which we performed in the present work by re-  
81 describing and re-allocating the two species into two new genera. In consequence, their  
82 known distribution was expanded towards the eastern side of the Andes Mountains. In  
83 addition, the systematic position of other two Chilean species was discussed, viz., *Geoplana*  
84 *chanca* Froehlich, 1978 and *Geoplana tirua* Froehlich, 1978.

85

86

## 87 **2 MATERIALS AND METHODS**

88

### 89 **2.1 Study area**

90 Specimens were collected from the southern, Patagonian portion of Argentina, including the  
91 provinces of Neuquén (Villa La Angostura), Rio Negro (El Bolsón), and Chubut (Los Alerces  
92 National Park) (Figure 1). The land planarians from Villa La Angostura were found in the  
93 Laguna Verde reserve (40°46'29'' S, 71°39'42'' W). This reserve harbours different floristic  
94 representatives of the Andean-Patagonian Forests, e.g. mountain cypress *Austrocedrus*  
95 *chilensis* (D. Don) Pic. Serm. & Bizzarri, coihue *Nothofagus dombeyi* (Mirb.) Oerst., Chilean  
96 myrtle *Luma apiculata* (DC.) Burret, radial *Lomatia hirsuta* (Lam.) Diels, and other native  
97 species. The land planarians from El Bolsón (41°53'19'' S, 71°38'8.5'' W) were sampled  
98 from a trail inside a native forest with an abundance of the lenga beech *Nothofagus pumilio*  
99 (Poepp. & Endl.) Krasser. In Los Alerces National Park (42°42'59'' S, 71°43'18'' W), the  
100 land planarians were found in paths where the dominant plant species was *Fitzroya*  
101 *cupressoides* (Molina) I. M. Johnst, a widely-extended native plant which, together with other  
102 native flora, is protected by this reserve.

103

### 104 **2.2 Morphological analysis**

105 Land planarians were manually collected during daylight by searching under fallen logs in the  
106 native forests mentioned above. Photographs of some live specimens were taken *in situ*.  
107 Specimens were euthanized by pouring boiling water over them or by direct immersion in  
108 absolute ethanol. In the former case, before throwing boiling water, a small portion of the  
109 posterior region of the body of some specimens was removed for molecular analysis. After  
110 euthanasia, they were fixed with 10 % formaldehyde and, subsequently, preserved in 70 %

111 ethanol. Different portions of some fixed specimens were embedded in Paraplast©, serially  
112 sectioned at intervals of 4–6 µm with a microtome and stained with a modified Masson's  
113 trichrome stain (Negrete, Díaz Gira, & Brusa, 2019). The relative thickness of the cutaneous  
114 musculature in relation to body height (CMI, cutaneous muscular index) was measured at the  
115 pre-pharyngeal region, following Froehlich (1955). The nomenclature of colours follows the  
116 RAL Classic colour standard chart (<https://www.ral-farben.de/>).

117 We examined also photographs of histological preparations of *Amaga ruca* specimens, and  
118 from the type material of *Geoplana chanca* and *Geoplana tirua* studied by Froehlich (1978),  
119 stored at the Museu de Zoologia da Universidade de São Paulo (MZUSP).

120

### 121 **2.3 DNA extraction, amplification and sequencing**

122 A small piece of tissue was preserved in absolute alcohol for DNA extraction. The nucleic  
123 acids were extracted following the protocol in Álvarez-Presas, Carbayo, Rozas, & Riutort  
124 (2011). DNA was used as template for the amplification of two genes with the Polymerase  
125 Chain Reaction (PCR): the small ribosomal subunit RNA gene (*18S* type II), using the  
126 primers 18S1F, 18S4F, 18S7R and 18S9R (Carranza, Giribet, Ribera, Baguña, & Riutort,  
127 1996); and the mitochondrial Cytochrome Oxidase I (*COI*) gene, using the primers BarS  
128 (Álvarez-Presas et al., 2011) and COIR (Lázaro et al. 2009). PCR products were purified  
129 using a vacuum manifold (Millipore, SA) and then used as the template for the sequencing in  
130 both directions at Macrogen Europe Inc. (Amsterdam).

131

### 132 **2.4 Molecular analysis**

133 DNA sequence chromatograms were revised and contigs obtained using Geneious v10  
134 software ([www.geneious.com](http://www.geneious.com); Kearse et al. 2012). COI sequence alignment was performed in  
135 the software BioEdit (Hall, 1999), using amino acid sequences as guidance (translation table  
136 number 9) since this is a coding gene. On the other hand, the ribosomal gene sequences were  
137 aligned using MAFFT v7 online (Katoh & Standley, 2013) with the G-INS-i iterative  
138 refinement method and all the other options set as default. Misaligned regions from the *18S*  
139 alignment were deleted using the software GBlocks v0.91 (Talavera & Castresana, 2007) with  
140 half allowed gap positions and the minimum length of a block set to 3. Three different  
141 datasets were used to infer phylogenies: 1) *COI* dataset, 2) *18S* dataset, and 3) *concatenated*  
142 dataset. For the *concatenated* dataset only those specimens having both gene sequences were  
143 selected, so that, there is no missing data in this dataset.

144 Phylogenies were inferred following two different criteria: 1) Bayesian Inference (BI),  
145 using the software MrBayes v3.2.2 (Ronquist et al., 2012) with two independent runs and 6  
146 chains producing 5 million generations each and storing 5000 trees; 25% default burn-in was  
147 applied and the convergence of the two runs was checked by the average standard deviation of  
148 split frequencies value below 0.1; 2) Maximum Likelihood inference (ML) using the software  
149 IQ-TREE v 1.6.1 (Nguyen, Schmidt, Von Haeseler, & Minh, 2015) with the best-fit  
150 substitution model automatically selected by the programme according to the Akaike  
151 information criterion (AIC) and 10000 ultrafast bootstrap replicates for inferring bootstrap  
152 supports (BP). For the *concatenated* dataset we used partitions selected with PartitionFinder 2  
153 (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2017) as implemented in CIPRES gateway  
154 (Miller, Pfeiffer, & Schwartz, 2010). Trees were visualized with FigTree v 1.4.4. software  
155 (Rambaut, 2007-2017) and edited with iTOL v5.5 (Letunic & Bock, 2019).

156 Additionally, a ML phylogenetic tree based on the *COI* gene was inferred using sequences  
157 from *Amaga ruca* (obtained for this study), from other representatives of the subfamily  
158 Geoplaninae, and from all the extant subfamilies of terrestrial planarians for which sequences  
159 were available in GenBank. This analysis was conducted in order to test the possibility that *A.*  
160 *ruca* belongs to another subfamily.

161

162

### 163 **3 RESULTS**

164

#### 165 **3.1 Molecular results and phylogenetic analyses**

166 The sequences of each gene included in our analyses are listed in Table 1. The *18S* dataset  
167 comprised 53 ingroup sequences (35 species) and two outgroups with a final length of  
168 alignment of 1411 bp. The *COI* dataset included 96 ingroup sequences (65 species) of the  
169 subfamily Geoplaninae and two outgroup sequences (2 species) belonging to the subfamily  
170 Rhynchodeminae. The final length of the alignment was 795 bp. The *concatenated* dataset  
171 (alignment length of 2206 bp) was formed by 50 ingroup sequences of Geoplaninae and two  
172 of Rhynchodeminae as outgroup. The lower number of sequences for the *18S* dataset is due to  
173 the lesser representation in the GeneBank database of this gene, notably in the Geoplaninae  
174 species. The tree built with the latter dataset (Figure S1) locates *Geoplana valdiviana* outside  
175 the genus *Geoplana* Stimpson, 1857, constituting an independent clade as sister group to  
176 *Polycladus* Blanchard, 1845. We recognize this clade as a new genus, *Inakayalia* gen. nov.  
177 (changing the species name to *Inakayalia valdiviana* comb. nov., see “Systematics” section

178 below). The remaining specimens studied here also constitute a different clade, represented by  
179 the new genus *Wallmapuplana* gen. nov., housing the species *Wallmapuplana ruca* comb.  
180 nov. (see “Systematics” section), which appears as the sister group to the rest of ingroup  
181 genera. The tree inferred from the *COI* gene dataset (Figure S2) shows that, although most  
182 groups are recovered in agreement with a previous report for the same data (Carbayo et al.,  
183 2013), some relationships present weak statistical support. According to our results, only the  
184 *Luteostriata* Carbayo, 2010, *Paraba* Carbayo et al., 2013 and *Geoplana* genera would be  
185 polyphyletic. Regarding the two new genera proposed in this work, the two specimens of  
186 *Inakayalia* are grouped into a single clade, as the sister group to *Paraba multicolor* (Graff,  
187 1899). Further, all representatives of *Wallmapuplana* also form a monophyletic group, sister  
188 to the clade constituted by *Inakayalia*, *Paraba multicolor* and the genus *Gusana* Froehlich,  
189 1978. The ML tree based on the *COI* gene, including data of Geoplanidae subfamilies,  
190 supported the inclusion of the new genus *Wallmapuplana* into the subfamily Geoplaninae  
191 (Figure S3).

192 The phylogeny inferred from the *concatenated* dataset represents an intermediate situation  
193 between datasets described above, where *Inakayalia* appears as the sister group to *Polycladus*  
194 and *Wallmapuplana* as the sister group to the rest of the ingroup sequences (Figure 2). This  
195 analysis has a strong support of the terminal clusters, but has weak supports at some basal  
196 nodes. Both ML and BI analyses show the same supported groups, differing only in the  
197 position of the genus *Geobia* Diesing, 1862. This genus appears as a more basal clade in the  
198 BI tree and as a more internal clade in the ML tree. All known genera are monophyletic,  
199 except *Luteostriata*. There is a major well-supported clade including the genera *Cephaloflexa*  
200 Carbayo & Leal-Zanchet, 2003 and *Choeradoplana* Graff, 1896, as the sister group to a clade  
201 constituted by *Nothogynaphallia* Ogren & Kawakatsu, 1990 and *Xerapoa* Froehlich, 1955  
202 (although this last relationship lacks statistical support), the entire cluster being the sister  
203 group of the genus *Matuxia* Carbayo et al., 2013. There is also a well-supported clade  
204 constituted by the genera *Luteostriata*+*Supramontana* Carbayo & Leal-Zanchet, 2003 and  
205 *Issoca* Froehlich, 1955, which is the sister group of the genus *Pasipha* Ogren & Kawakatsu,  
206 1990. This clade constitutes the sister group of a lineage including the genera *Cratera*  
207 Carbayo et al., 2013, *Geobia*, *Obama* Carbayo et al., 2013 and *Paraba* (*Geobia* belongs to  
208 this clade only in the ML tree, as mentioned above), but the relationships between these  
209 genera are not well supported. In a basal position, there is a clade including the new genus  
210 *Inakayalia*, together with the genera *Polycladus* and *Gusana*, however, their internal  
211 relationships were unresolved. Finally, the new genus *Wallmapuplana* is situated next to the

212 root of the tree as the sister group to all the other Geoplaninae genera included as ingroups in  
213 the phylogenetic analyses (Figure 2).

214

215

### 216 **3.2 Systematics**

217 Order Tricladida Lang, 1884

218 Suborder Continenticola Carranza, Littlewood, Clough, Ruiz-Trillo, Bagueña & Riutort, 1998

219 Family Geoplanidae Stimpson, 1857

220 Subfamily Geoplaninae Stimpson, 1857

221 **Genus *Inakayalia* gen. nov.**

222

223 *Diagnosis.* Geoplaninae with medium-sized slender body with nearly parallel margins; dorsal  
224 surface convex and ventral body surface flat or slightly concave; monolobulated eyes  
225 extending dorsally along the body with large clear halos; bell-shaped pharynx; extrabulbar,  
226 voluminous, horizontal prostatic vesicle; penis papilla nearly dome-shaped with irregular  
227 walls; distal ascending portions of ovovitelline ducts with expanded diameter; common  
228 ovovitelline duct dorsal to female atrium; short, antero-dorsally flexed female genital canal,  
229 ascending from the postero-dorsal region of female atrium; female atrium with narrow lumen.

230

231 *Etymology.* The generic name honours of the great cacique (chief) Antonio Modesto Inakayal,  
232 who led the indigenous people from the Huilliche ethnic group during the late nineteenth  
233 century. This group was a member of the "Mapuche nation", in the region of Villa La  
234 Angostura (Neuquén province, Argentina), where the new specimens were found.

235

236 ***Inakayalia valdiviana* (Grau & Carbayo, 2011) comb. nov.**

237 *Geoplanea valdiviana* Grau & Carbayo, 2011

238

239 *Type Locality.* Teja Island (39° 48' S; 73° 15' W), Valdivia, Chile.

240 *Distribution.* Valdivia (Chile) and Neuquén (Argentina) provinces.

241

242 Material examined

243 **MLP He-7661:** February 4, 2018, Laguna Verde reserve, Villa La Angostura city  
244 (40°46'29.13'' S, 71°39'41.95'' W), Neuquén province, Argentina; transverse sections of the  
245 cephalic region on 17 slides; sagittal sections of the anterior region at the level of ovaries and



246 anterior-most testes on 26 slides; transverse sections of the pre-pharyngeal region on 10  
247 slides; sagittal sections of the pharyngeal region on 18 slides; and sagittal sections of the  
248 copulatory apparatus on 20 slides.

249 **MLP He-7662:** February 4, 2018, Laguna Verde reserve, Villa La Angostura city  
250 (40°46'29.13" S, 71°39'41.95" W), Neuquén province, Argentina; cephalic region on 14  
251 slides; sagittal sections of the anterior region at the level of ovaries and anterior-most testes on  
252 21 slides; transverse sections of the pre-pharyngeal region on 10 slides; sagittal sections of the  
253 pharyngeal region on 18 slides; and sagittal sections of the copulatory apparatus on 20 slides.

254 **MLP He-7663:** February 5, 2019, Laguna Verde reserve, Villa La Angostura city  
255 (40°46'29.13" S, 71°39'41.95" W), Neuquén province, Argentina; whole specimen preserved  
256 in absolute ethanol.

257

## 258 Description

259 *External morphology.* *In vivo* body size up to ~40 mm in length and ~5 mm in maximum  
260 width when crawling. After fixation, body size was 30–35 mm in length, 2.8–4.5 mm in  
261 maximum width, and 1.4–1.6 mm in height. Body margins nearly parallel, with a blunt  
262 anterior tip, and rounded posterior tip ending abruptly (Figure 3A, B). Convex dorsum and  
263 almost flat ventral side. Dorsal surface with black pigmentation and a transversal ‘saffron-  
264 yellow’ band (~1 mm wide) with irregular edges in the cephalic region (Figure 3A, B). This  
265 band extends forward as a thin median stripe, whose pigment gradually changes to ‘light  
266 ivory’, disappearing 1 mm before the anterior tip. “Ivory”-coloured ventral side, being ‘oyster  
267 white’ along the median region, and ventro-marginally bordered by black dots (Figure 3B, D).  
268 After fixation, the dorsum colour is slightly faded, and a ‘light ivory’ median band (occupying  
269 1/5th of body width), which is difficult to recognize in live specimens since it is covered by  
270 black dots, is discernible, and the transverse band becomes paler (Figure 3C, D).

271 Mono-lobulated eyes marginally located in a single to double row in the anterior 1/7th of the  
272 body length (Figures 3C, D, 4), and then spreading dorsally in 3–5 irregular rows on either  
273 side of the body (Figures 3C, 4). At about half-way along the body length, rows of eyes  
274 increase to 6–9, thus reaching its maximum extension and occupying 30 % of the body width  
275 on either side (Figures 3C, 4). Hereafter, eyes gradually become less numerous and isolated,  
276 albeit staying dorsal until the posterior end of the body (Figure 4). Eyes with conspicuous  
277 clear halos (Figure 3A–C, E).

278 In preserved specimens, mouth and gonopore located at 63–64 % and 83 % of the total  
279 body length as measured from the anterior tip, respectively.

280

281 *Internal morphology*

282 *Sensory organs, musculature and glandular secretions.* Sensory pits, as simple invaginations  
283 of the ventral epidermis (25–35 µm deep; Figure 5A), contour the anterior tip and body  
284 margins in a single row, extending posteriorly until 1/5th–1/8th of the body length. Ventral  
285 epidermis of the pre-pharyngeal region ciliated on the creeping sole (80–90 % of body width),  
286 with small rhabdites, and receiving secretion of two types of secretory cells: one producing  
287 fine granular erythrophil secretion and the other fine granular cyanophil secretion (Figure  
288 5D). Dorsal epidermis containing rhabdites and the same type of secretions as the ventral  
289 epidermis (Figure 5E). Glandular margin with coarse erythrophil granules and a small amount  
290 of fine cyanophil granules (Figure 5C). Ventral epidermis of the cephalic region provided  
291 with small rhabdites and receiving abundant secretion from cells producing coarse erythrophil  
292 granules and less abundant fine cyanophil granules (Figure 5A). Dorsal epidermis of the  
293 cephalic region pierced by the same secretory cells as the pre-pharyngeal region. Cutaneous  
294 muscle layers with the typical arrangement of the subfamily Geoplaninae: a circular layer (5  
295 µm thick) followed by a diagonal layer (10–20 µm thick), and a subjacent longitudinal layer,  
296 which is arranged in bundles, being ventrally thicker (60–85 µm thick) than dorsally (25–40  
297 µm thick) (Figure 5D, E). CMI ranges between 8 % and 10 %. Parenchymatic musculature  
298 with three layers: a dorsal layer with decussate fibres (15–25 µm thick), supra-intestinal and  
299 sub-intestinal transverse layers (25–30 µm thick) (Figure 5D, E). Parenchymatic muscle  
300 layers decreasing in thickness towards the cephalic region (Figure 5A). Abundant dorso-  
301 ventral muscle fibres traverse between the intestinal branches (Figure 5E).

302 *Pharynx.* The pharynx is bell-shaped (1.7–2 mm in length), with the mouth nearly at the  
303 middle of the pharyngeal pouch, which measures 2–2.6 mm in length (Figure 6A). Dorsal  
304 insertion of pharynx at the level of the mouth or posteriorly to it (Figure 6A). Outer  
305 pharyngeal epithelium cuboidal and ciliated, underlain by a longitudinal muscle layer (5 µm  
306 thick) and a subjacent circular layer (10 µm thick) (Figure 6B). Inner epithelium columnar  
307 and ciliated, underlain by thick muscle layer of circular fibres (100–150 µm thick), followed  
308 by a longitudinal layer (10–20 µm thick) (Figure 6C). Distal portion of inner pharyngeal  
309 epithelium pierced by glandular secretion of two types: abundant fine erythrophil granules  
310 and scarce cyanophil amorphous secretion (Figure 6A). Oesophagus absent.

311 *Male reproductive system.* Round-to-oval testes, located dorsally to intestine, and just  
312 below the supra-intestinal parenchymatic muscle layer (Figure 5E). Testes arranged in four or  
313 five irregular rows on either side of the body (Figure 5B). Testicular follicles extend from

314 posteriorly to the ovaries (26–29 % of the body length) to nearly the dorsal insertion of the  
315 pharynx (62–63 % of the body length). Sperm ducts dorsal to the ovovitelline ducts and  
316 located above the sub-intestinal parenchymatic muscle layer (Figure 5B, D). Lumen of distal  
317 portion of the sperm ducts expanded, at the level of pharynx, forming spermiducal vesicles.  
318 Posteriorly to the pharyngeal pouch, spermiducal vesicles bend medially to open laterally into  
319 the proximal portion of the prostatic vesicle, which is located just behind the pharynx (Figures  
320 6A, 7, 8A). Extrabulbar portion of the prostatic vesicle almost horizontal, and with folded  
321 walls. Short, tubular, highly tortuous intrabulbar portion (Figures 7, 8A, 9B). The intrabulbar  
322 prostatic vesicle continues as an ejaculatory duct, which traverses the penis papilla (Figures 7,  
323 8). The penis papilla is nearly dome-shaped, but with irregular walls, occupying nearly half of  
324 the total length of the male atrium. The ventral insertion of the penis papilla is slightly  
325 posteriorly displaced in comparison with the dorsal one (Figures 7, 8). An ample oblique fold  
326 extends from the dorsal wall of the male atrium towards the female atrium (Figures 7, 8).

327 Sperm ducts lined with ciliated, cuboidal epithelium and surrounded by a one-fibre-thick  
328 longitudinal muscle layer (2.5  $\mu\text{m}$  thick). Extra- and intrabulbar prostatic vesicle lined with a  
329 columnar, ciliated epithelium, pierced by abundant fine granular erythrophil secretion (Figure  
330 9A, B), and covered by circular muscle fibres intermingled with some longitudinal fibres (60–  
331 80  $\mu\text{m}$  thick). Epithelial lining penis papilla and ejaculatory duct columnar, non-ciliated and  
332 receiving abundant fine granular, strongly erythrophil secretion (Figure 9C, D). Dorsal  
333 surface of penis papilla also receiving abundant fine granular cyanophil secretion (Figures 8,  
334 9D), the gland cells of which are located latero-dorsally to the penis (Figure 10). Male atrium  
335 lined with columnar and non-ciliated epithelium (Figure 9D), and surrounded by circular  
336 muscle fibres intermingled with oblique fibres ( $\sim 10$   $\mu\text{m}$  thick); epithelium pierced by coarse  
337 xanthophil granules, fine cyanophil granules and scarce erythrophil amorphous secretion.

338 *Female reproductive system.* Ovaries oval-elongated, measuring 400–500  $\mu\text{m}$  along  
339 antero-posterior axis and 250–300  $\mu\text{m}$  in diameter (Figure 9G). The ovaries are located below  
340 the sub-intestinal parenchymatic muscle layer, at a distance from the anterior end  
341 corresponding to 16–22% of the body length. Vitellaria are distributed around intestine  
342 (Figure 5B, D, E). Ovovitelline ducts emerge from the mid-dorsal part of the ovaries (Figure  
343 9G). Laterally to the penis bulb, the ovovitelline ducts gradually turn dorso-medially and joint  
344 to form a common duct above the proximal region of the female atrium, nearly at the level of  
345 the gonopore (Figures 7, 10). The distal portions of the ovovitelline ducts are greatly  
346 expanded in diameter, while their lumen is reduced due to large folds (Figures 9E, 10D).  
347 Common ovovitelline duct (0.7–1 mm in length) located above the female atrium, posteriorly

348 slightly downwards inclined (Figures 7, 8). Short female genital canal (~200 µm in length)  
349 dorso-anteriorly flexed, communicating with the female atrium (Figures 7, 8B). Female  
350 atrium connected with the male atrium. A fold projecting from the bottom of the female  
351 atrium reduces its lumen (Figures 7, 8).

352 Ovovitelline ducts lined with a ciliated, cuboidal epithelium and surrounded by a one-  
353 fibre-thick longitudinal muscle layer (2.5 µm thick). Distal portions of the ovovitelline ducts  
354 lined with a tall columnar and ciliated epithelium of stratified appearance (75–85 µm in  
355 height) (Figure 9E), surrounded by a muscle layer consisting of circular fibres intermingled  
356 with some oblique ones (20 µm thick). Lining epithelium of common ovovitelline duct  
357 columnar and ciliated (50 µm in height), pierced by fine erythrophil granules produced by  
358 shell glands (Figure 9F). Common ovovitelline duct surrounded by circular and oblique  
359 muscle fibres (30–50 µm thick). Female canal and female atrium lined with non-ciliated  
360 columnar epithelium (~50 µm height) (Figure 9H), which progressively diminishing in height  
361 towards the gonopore, where it is about 20 µm in height. Muscle coat of female atrium with  
362 circular and some oblique fibres (20–25 µm thick). Epithelium of female atrium pierced by  
363 glands producing fine cyanophil and erythrophil granules (Figure 9H). Common muscular  
364 coat surrounding male and female atria consisting of longitudinal and oblique muscle fibres  
365 (60–75 µm thick).

366

367

368 **Genus *Wallmapuplana* gen. nov.**

369

370 *Diagnosis.* Geoplaninae of small-sized body; mono-lobulated eyes along body margins;  
371 cylindrical pharynx; intra-penial papilla; male atrium with highly folded walls; musculo-  
372 glandular organs of adenocyst type in the male atrium; highly dilated proximal tract of  
373 ovovitelline ducts; distal portion of the ovovitelline ducts ventro-lateral to female atrium;  
374 common ovovitelline duct posterior to female atrium and with two portions, receiving shell  
375 glands only distally; female canal opening dorsally into female atrium.

376

377 *Etymology.* Name alludes to the ‘Mapuche Nation’ (*Wallmapu*), originally occupied by the  
378 Mapuche ethnic group. This indigenous Nation inhabits regions that extend on both sides of  
379 the Andes Mountains, in Argentinean (*Puelmapu*) and Chilean (*Ngulumapu* or *Gulumapu*)  
380 Patagonia, and where the new species is distributed.

381

382 *Wallmapuplana ruca* (Marcus, 1954) comb. nov.

383 *Microplana ruca* Marcus, 1954

384 *Geoplana ruca* (Marcus, 1954) Froehlich 1978 (p. 23–25)

385 *Amaga ruca* (Marcus, 1954) Ogren & Kawakatsu 1990 (p. 94)

386

387 *Type Locality.* Petrohué (41° 07' S; 72° 23' W), Llanquihue province, Chile.

388 *Distribution.* Llanquihue and Malleco provinces (Chile), and Neuquén, Rio Negro, and  
389 Chubut provinces (Argentina).

390

391 Material examined

392 Locality: Laguna Verde reserve, Villa La Angostura city, Neuquén province, Argentina  
393 (40°46'29'' S, 71°39'42'' W), February 4, 2018: **MLP He-7664**: transverse sections of the  
394 cephalic region and anterior region at the level of ovaries and anterior-most testes on 23  
395 slides; transverse sections of the pre-pharyngeal region on 9 slides; sagittal sections of the  
396 pharynx and copulatory apparatus on 12 slides. **MLP He-7665**: transverse sections of the  
397 cephalic region on 12 slides; sagittal sections of the anterior region at the level of ovaries up  
398 to pre-pharyngeal region on 8 slides; sagittal sections of the pharynx and copulatory apparatus  
399 on 14 slides. **MLP He-7666**: transverse sections of the cephalic region on 7 slides; sagittal  
400 sections of the anterior region at the level of ovaries up to pre-pharyngeal region on 7 slides;  
401 sagittal sections of the pharynx and copulatory apparatus on 10 slides. **MLP He-7667**:  
402 transverse sections of the cephalic region on 9 slides; sagittal sections of the anterior region at  
403 the level of ovaries up to pre-pharyngeal region on 14 slides; sagittal sections of the pharynx  
404 and copulatory apparatus on 12 slides. **MLP He-7668**: sagittal sections of the cephalic region,  
405 anterior region at the level of ovaries, and pre-pharyngeal region on 9 slides; longitudinal  
406 sections of the pharynx and copulatory apparatus on 8 slides. **MLP He-7669**: transverse  
407 sections of the cephalic region on 8 slides; sagittal sections of the anterior region at the level  
408 of ovaries, and pre-pharyngeal region on 7 slides; sagittal sections of the pharynx and  
409 copulatory apparatus on 7 slides. **MLP He-7670**: sagittal sections of the pharynx and  
410 copulatory apparatus on 6 slides. **MLP He-7671**: sagittal sections of the pharynx and  
411 copulatory apparatus on 5 slides. **MLP He-7672**: sagittal sections of the pharynx and  
412 copulatory apparatus on 4 slides. **MLP He-7673**: sagittal sections of the pharynx and  
413 copulatory apparatus on 5 slides. **MLP He-7674**: sagittal sections of the pharynx and  
414 copulatory apparatus on 4 slides. **MLP He-7675**: sagittal sections of the pharynx and  
415 copulatory apparatus on 5 slides.

416 Locality: El Bolsón city, Rio Negro province, Argentina (41°53'19'' S, 71°38'8.5'' W),  
417 February 11, 2019: **MLP He-7676**: transverse sections of the cephalic region on 7 slides;  
418 longitudinal sections of the anterior region at the level of ovaries on 5 slides; transverse  
419 sections of the pre-pharyngeal region on 3 slides; sagittal sections of the pharynx and  
420 copulatory apparatus on 7 slides. **MLP He-7677**: transverse sections of the cephalic region on  
421 14 slides; longitudinal sections of the anterior region at the level of ovaries and pre-  
422 pharyngeal region on 6 slides; sagittal sections of the pharynx and copulatory apparatus on 9  
423 slides.

424 Locality: Los Alerces National Park, Chubut province, Argentina (42°42'59'' S, 71°43'18''  
425 W), February 14, 2019: **MLP He-7678**: transverse sections of the cephalic region on 10  
426 slides; transverse sections of the anterior region at the level of ovaries and pre-pharyngeal  
427 region on 11 slides; sagittal sections of the pharynx and copulatory apparatus on 7 slides.  
428 **MLP He-7679**: transverse sections of the cephalic region and anterior region at the level of  
429 ovaries on 12 slides; transverse sections of the pre-pharyngeal region on 3 slides; sagittal  
430 sections of the pharynx and copulatory apparatus on 8 slides.

431

#### 432 Description

433 *External morphology.* Body margins nearly parallel, anterior tip blunt and posterior end  
434 pointed (Figure 12A, B). When animals are in rest the general aspect is chubbier, with the  
435 posterior end being rounded (Figure 11). While crawling, they reach ~20 mm in maximum  
436 length, and ~2 mm in width. After fixation, body sizes are 10–16 mm in length, 1.2–1.5 mm  
437 in maximum width, and 0.85–1.1 mm in height (Figure 12A, B, Table 2). Dorsal surface  
438 black, bordered by narrow dirty white marginal stripes on either side of the body that  
439 converge at both ends of the body (Figures 11, 12A, B). In preserved specimens, a thin  
440 whitish median stripe is visible only under the stereomicroscope (Figure 12A). In some  
441 animals this median stripe runs incompletely over the dorsum, fading at the posterior quarter  
442 of the body. Creeping sole ashy-coloured in the cephalic region and whitish on the rest of the  
443 ventral surface, bordered by fine, black-pigmented stripes (Figure 12B).

444 Mono-lobulated eyes bordering the cephalic end (Figures 13, 14A) and extending  
445 uniserially onto the body margins, except for the anterior 1/8th of the body length in which  
446 they are bi-serial (Figures 12C, 13). In preserved specimens, mouth and gonopore are located  
447 at a relative distance, as measured from the anterior tip, of 51–61 % and 65–78 % of the body  
448 length, respectively (Figure 12B, Table 2).

449

450 *Internal morphology*

451 *Sensory organs, musculature and glandular secretions.* Sensory pits absent in the cephalic  
452 region. Instead, there are epidermal cells on the latero-ventral margins of the body devoid of  
453 rhabdites and with larger cilia than those from the creeping sole, which could represent a  
454 sensory margin (Figure 14B, C). Ventral epidermis ciliated on the creeping sole, occupying  
455 ~50 % of body width in the cephalic region, and 70–80 % of body width in the pre-  
456 pharyngeal region. Ventral epidermis with small rhabdites and receiving two types of  
457 secretion: fine granular erythrophil secretion and fine granular cyanophil secretion (Figure  
458 14C, D). Dorsal epidermis containing rhabdites and the same type of secretions as the ventral  
459 epidermis, but cyanophil granules less abundant (Figure 14B). Glandular margin absent.  
460 Cutaneous musculature with the three typical layers of Geoplaninae: a circular layer (2.5  $\mu\text{m}$   
461 thick), an intermediate diagonal layer (2.5–5  $\mu\text{m}$  thick), and a subjacent longitudinal layer  
462 arranged in discrete bundles. The ventral longitudinal layer (25–40  $\mu\text{m}$  thick) is nearly twice  
463 as thick as the dorsal one (10–25  $\mu\text{m}$  thick). The relative thickness of the cutaneous  
464 musculature in relation to body height, at pre-pharyngeal region, ranges between 5 % and 8 %  
465 (Table 2). Parenchymatic musculature with supra-intestinal and sub-intestinal transverse  
466 layers (2–4 fibres in thickness per layer), and a subneural muscle layer (2–3 fibres in  
467 thickness) (Figure 14B, D). Dorso-ventral muscle fibres are also present.

468 *Pharynx.* The pharynx cylindrical; mouth opening located at the posterior third of the  
469 pharyngeal pouch (Figure 15A). The outer pharyngeal epithelium is cuboidal and ciliated,  
470 underlain by a thin longitudinal muscle layer (2.5  $\mu\text{m}$  thick) and a subjacent circular layer (10  
471  $\mu\text{m}$  thick) (Figure 15B). The inner pharyngeal epithelium is columnar and ciliated, underlain  
472 by a thick muscle layer composed of circular fibres and some intermingled longitudinal fibres  
473 (40–50  $\mu\text{m}$  thick) (Figure 15C). A short oesophagus (100  $\mu\text{m}$  in length) is present (Figure  
474 15A). Distal portion of inner pharyngeal epithelium pierced by glandular secretion of three  
475 types: abundant fine erythrophil and cyanophil granules and less abundant xanthophyl  
476 amorphous secretion (Figure 15A–C).

477 *Male reproductive system.* Three or four irregular rows of round-to-oval testes, arranged on  
478 either side of the body, are located below the supra-intestinal parenchymatic fibres (Figures  
479 14B, 16A). The testes extend from anteriorly to ovaries to nearly the root of the pharynx  
480 (16 $\pm$ 4 % to 49 $\pm$ 4 % of the body length), lying in the interstices between the intestinal  
481 branches. Sperm ducts dorsal to the ovovitelline ducts (Figure 14D). Laterally and posteriorly  
482 to pharynx, sperm ducts are expanded forming spermiducal vesicles whose courses are highly  
483 tortuous (Figures 16B–D, 17). The sperm ducts open into an extrabulbar, tubular prostatic

484 vesicle that receives numerous openings of profusely branched small ducts of prostatic glands  
485 (Figures 16B–D, 17). The prostatic vesicle continues with a tortuous and muscularized  
486 common vas deferens which penetrates the common muscle coat to become the ejaculatory  
487 duct (Figures 16B–D, 17, 18A). The ejaculatory duct is dilated forming an ejaculatory cavity  
488 which concentrically traverses a small conical penis papilla (150–250  $\mu\text{m}$  in length). The  
489 penis papilla is obliquely upwards oriented (Figures 17, 18A, B, G, 19), and is encircled by  
490 lateral folds of the ental part of the male atrium which conform a penis sheath (Figures 17,  
491 18A, B, G, 19). The highly folded walls of the male atrium, which is two or three times longer  
492 than the female one (Table 3), greatly reduce its lumen (Figures 17, 19). Numerous type III  
493 musculo-glandular organs or adenocysts (see Winsor 1998) are embedded in the inner walls  
494 of the male atrium (Figure 18C–I). Some specimens show secretions of these adenocysts  
495 being released into the atrial lumen (see below) (Figure 18D–I).

496 Sperm ducts lined with ciliated, cuboidal epithelium, and devoid of any surrounding  
497 musculature. Spermiducal vesicles lined with squamous epithelium. Prostatic vesicle lined  
498 with ciliated and cuboidal epithelium, and surrounded by a thin muscle coat composed of  
499 circular and oblique fibres (10  $\mu\text{m}$  thick). The prostatic vesicle receives abundant coarse  
500 granular erythrophil secretion from glands whose small ducts anastomose and converge in  
501 glandular ducts (lined with ciliated, cuboidal epithelium), which open through the prostatic  
502 epithelium (Figures 16B–D, 17, 19). Cell bodies of prostatic glands located in the proximity  
503 of the prostatic vesicle as well as distant from it, i.e., laterally to the pharynx (Figures 15,  
504 16B–D, 17, 19). The common vas deferens, lined with ciliated, squamous epithelium, is  
505 covered by a thick muscle coat consisting of longitudinal fibres (50–75  $\mu\text{m}$  thick) (Figures  
506 16B–D, 18A, 19). This muscle coat diminishes its thickness (25–30  $\mu\text{m}$  thick) in the distal  
507 part of the vas deferens. The ejaculatory cavity is lined with columnar and ciliated epithelium  
508 pierced by fine granular erythrophil secretion (Figure 18A–C). Near the penis tip, the  
509 epithelial lining of the ejaculatory duct becomes squamous and non-ciliated and receives a  
510 fine granular cyanophil secretion (Figure 18A–C). The musculature of the ejaculatory cavity  
511 is composed of circular and oblique fibres (30–40  $\mu\text{m}$  thick), diminishing in thickness toward  
512 its distal part, near the tip of the penis (5  $\mu\text{m}$  thick). The penis papilla is covered with  
513 squamous epithelium, followed by a circular muscle coat (10  $\mu\text{m}$  thick) and a subjacent  
514 longitudinal muscle coat (30  $\mu\text{m}$  thick) (Figure 18B). The ental part of the male atrium is  
515 lined with non-ciliated, squamous epithelium, while the rest of atrium is lined with cuboidal  
516 epithelium, strongly erythrophil due to abundant fine granular erythrophil secretion (Figure  
517 18C–I). The adenocysts consist of ‘spherical bodies’ embedded in the atrial walls provided



518 with densely packed fine erythrophil granules, surrounded by circular and some longitudinal  
519 muscle fibres (Figure 18C). The epithelial lining of the male atrium in which these musculo-  
520 glandular organs are located is somewhat higher than the surrounding epithelium and it is also  
521 intensely erythrophil. Some adenocysts show abundant erythrophil secretion protruding into  
522 the atrial lumen (Figure 18F–I). Musculature of male atrium consisting of a circular layer (10–  
523 20  $\mu\text{m}$  thick) and a subjacent layer with longitudinal fibres intermingled with some oblique  
524 ones (5–10  $\mu\text{m}$  thick). There is no common muscle coat enfolding both atria. Instead, a  
525 muscular bulb, organized by circular and some oblique and longitudinal fibres (15–25  $\mu\text{m}$   
526 thick), enfolds penis papilla and male atrium.

527 *Female reproductive system.* Oval-shaped ovaries (~500  $\mu\text{m}$  in length in antero-posterior  
528 axis, and 100–125  $\mu\text{m}$  in diameter) (Figure 20A–C), located among the sub-intestinal  
529 parenchymatic muscle fibres, at a distance from the anterior end corresponding to 14–23 % of  
530 the body length (Table 3). Vitellaria scattered among intestinal branches (Figures 14D, 15A,  
531 16A, 20B–C). Ovovitelline ducts arise laterally from the ovaries (Figure 20A–C). The lumen  
532 of the proximal part of the ovovitelline ducts, at the point of their emergence from the ovaries,  
533 was expanded and full of spermatozoa in all specimens examined (Figure 20A–C). In the pre-  
534 pharyngeal region, ovovitelline ducts are located beneath the sub-intestinal parenchymatic  
535 muscle fibres (Figure 14D). Posteriorly, the ducts run ventro-laterally to the copulatory  
536 apparatus and posterior to the female atrium they curve to the median plane and unit to form a  
537 common ovovitelline duct (Figures 17, 20E). The latter has an ascending tortuous path and  
538 distally it slightly curves forward to connect with a short female canal (Figures 17, 20D, E).  
539 The female atrium shows highly folded walls and measures approximately one-third of the  
540 length of the male atrium (Table 3). Female atrium laterally displaced from the male one,  
541 therefore both open separately into the gonoduct (Figure 19).

542 Ovovitelline ducts lined with a ciliated, cuboidal epithelium and surrounded by a one-  
543 fibre-thick longitudinal muscle layer (2.5  $\mu\text{m}$  thick). Common ovovitelline duct lined with  
544 columnar, ciliated epithelium followed by circular and oblique muscle fibres (5–10  $\mu\text{m}$  thick);  
545 shell glands with fine erythrophil granules, though cyanophil in some specimens, discharge  
546 onto the epithelium of the distal portion of the common ovovitelline duct, which lacks cilia  
547 (Figure 20D, E). Female atrium lined with tall columnar, ciliated epithelium (Figure 20D). It  
548 is surrounded by a thin muscle coat composed of circular and oblique fibres (5  $\mu\text{m}$  thick). The  
549 atrial epithelium is pierced by abundant coarse cyanophil granules, whose glandular cells are  
550 located laterally to the atrium (Figures 19, 20D, E). Muscle coat surrounding female atrium  
551 composed of loose longitudinal fibres (4–6 fibres in thickness). Gonoduct straight or slightly

552 curved backward (Figure 19), lined with ciliated, columnar epithelium, pierced by fine  
553 erythrophil granules, and surrounded by circular muscle layer (5  $\mu\text{m}$  thick) and a subjacent  
554 longitudinal layer (10  $\mu\text{m}$  thick).

555

556 *Remarks.* Specimens MLP He-7670, MLP He-7671, MLP He-7672, MLP He-7673, MLP He-  
557 7674, and MLP He-7675 show evidences of contraction and some distortion in the general  
558 anatomy due to an improper fixation method (they were directly stored in 100% ethanol). In  
559 these animals, the pharynx is retracted, and in the copulatory apparatus of the majority of  
560 them, the common vas deferens is located below the penis papilla and the proximal part of the  
561 male atrium (Figure S4).

562

563

#### 564 **4 DISCUSSION**

565

566 Firstly, we will justify the taxonomic decisions taken above for *Geoplana valdiviana* and  
567 *Amaga ruca*, according to the molecular support together with morphological evidence.  
568 Thereafter, we will discuss the re-descriptions of these species in the light of our observations  
569 of the external morphology of live specimens and the histological and anatomical features of  
570 the new material from Argentina, as compared to the published descriptions of Chilean  
571 specimens.

572

#### 573 **4.1 Phylogenetic support and morphological evidence for the updated systematics**

574 Under the more recent molecular phylogeny of the Geoplaninae (Carbayo et al., 2013),  
575 supported by anatomical characters, the genus *Geoplana* was reduced to 13 species, while  
576 originally counting more than 60 species (Almeida, Marques, & Carbayo, 2019). The  
577 remaining species were transferred to new genera, while others were considered *incertae sedis*  
578 for being morphologically poorly known or for exhibiting features contrasting with the  
579 current diagnosis of the genus *Geoplana*, as was the case with *G. valdiviana* (Carbayo et al.,  
580 2013). Therefore, *G. valdiviana* cannot be assigned to *Geoplana* due to several anatomical  
581 features inconsistent with the genus, namely, bell-shaped pharynx, absence of longitudinal  
582 parenchymatic muscle fibres, penis papilla not extending to the level of the gonopore and  
583 neither projecting into the entire female atrium, presence of extrabulbar prostatic vesicle.

584 The phylogenetic analyses based on the *COI*, *18S* and *concatenated* datasets support  
585 exclusion of *G. valdiviana* from *Geoplana*, and induced us to coin for this species a new

586 genus, viz., *Inakayalia* gen. nov. However, the sister-group of *Inakayalia valdiviana* comb.  
587 nov. remains a mystery, since none of our datasets gives enough statistical support to shed  
588 light on this issue.

589 The genus *Amaga* Ogren & Kawakatsu, 1990 groups land flatworms with a large and  
590 flattened body, male copulatory system with an intra-antral papilla, ovovitelline ducts  
591 approaching antero-dorsally to female atrium, female canal opening dorsally into the female  
592 atrium, and absence of adenodactyls or musculo-glandular organs (Ogren & Kawakatsu,  
593 1990). More recently, Grau, Sluys, Froehlich, & Carbayo (2012), after studying type material  
594 of *Amaga amagensis* (Fuhrmann, 1914), the type species of the genus, re-evaluated its  
595 diagnostic characters and proposed an emendation for this genus. They added some  
596 anatomical features, such as the unusual location of testes (above the supra-intestinal  
597 parenchymal muscle layer), and recognized that the so-called small intra-antral papilla is truly  
598 an eversible penis. Several anatomical characters mentioned above, notably the very dorsal  
599 position of testes, presence of an eversible penis, absence of adenodactyls, and ovovitelline  
600 ducts antero-dorsally approaching the female atrium (Grau et al., 2012), preclude assignment  
601 of *Amaga ruca* to the genus *Amaga*. Its position in the phylogenetic analyses, as the sister  
602 group to the rest of representatives of Geoplaninae (based on the concatenated and *18S* trees),  
603 suggests that this clade represents a new taxon for which we coined the new genus  
604 *Wallmapuplana* gen. nov. Unfortunately, lack of molecular data for species of *Amaga*  
605 prevented us to test this hypothesis.

606

#### 607 **4.2 New observations on the morphology of *Inakayalia valdiviana* comb. nov. and** 608 **taxonomic actions for *Geoplana chanca* and *Geoplana tirua***

609 In the present work, we added information about the *in vivo* pigmentation pattern of  
610 *Inakayalia valdiviana* comb. nov., since the description by Grau & Carbayo (2011) was based  
611 only on the external morphology one preserved specimen from Valdivia, Chile. The main  
612 discrepancy between the specimens concerns the colour of the transverse stripe at the cephalic  
613 region of the body. It was originally described as white for the preserved Chilean specimen,  
614 whereas it is saffron, yellow (orange to the naked eye) in the live specimens from Argentina, a  
615 colour that remains (although a bit paler) even after fixation and preservation. Additionally,  
616 Grau & Carbayo (2011) mentioned the presence of a median yellowish band with scattered  
617 small dark dots on the dorsal surface. This band is virtually indistinguishable in living  
618 specimens from Argentina, but it is noticeable once the specimens are preserved. The Chilean  
619 specimen was collected in 2007, some years before its publication, so perhaps the pigment

620 had already faded at the moment it was studied. Therefore, the observed differences could  
621 simply be due to intraspecific variation. Regarding the internal anatomy, the Argentinean  
622 specimens mostly agree with the description of the Chilean one, except for some details in the  
623 copulatory apparatus. In both the Chilean and Argentinean specimens, the penis papilla has its  
624 dorsal insertion located anterior to the ventral one (in sagittal view). However, in the  
625 specimen from Valdivia the penis papilla occupies most of the male atrium, whereas in both  
626 specimens here studied it is significantly shorter. The folded walls of the female atrium of the  
627 Argentinean specimens are absent in the Chilean specimen, which has a funnel-shaped atrium.  
628 We presume that these small differences regarding the anatomy are due to intraspecific  
629 variation or even differences in maturity since the Chilean specimen measured 24 mm in  
630 length (after preservation), while the Argentinean ones reached 30–35 mm. Absence of  
631 molecular information on the Chilean specimen prevented us from testing possible co-  
632 specificity with the Argentinean animals.

633 The presence of extremely widened distal ovovitelline ducts in *I. valdiviana*, lined with a  
634 tall columnar epithelium of stratified appearance, is an uncommon feature within the  
635 Geoplaninae. In point of fact, it has only been described for the Chilean species *Geoplana*  
636 *chanca* Froehlich, 1978 and *Geoplana tirua* Froehlich, 1978, which currently are species  
637 *incertae sedis* (Carbayo et al., 2013). Although the specimen of *G. tirua* studied by Froehlich  
638 (1978) was incompletely mature, she mentioned the increase in diameter during the ascending  
639 course of the ovovitelline ducts, which also occurs in *G. chanca*. We corroborated these  
640 observations after observing photomicrographs of the sectioned type material of both species  
641 (Figures S5, S6). These species also resemble *I. valdiviana* in that they possess a medium-  
642 sized body (40–50 mm in length), dorsal eyes with large clear halos (not mentioned by  
643 Froehlich but evident from the original illustrations), bell-shaped pharynx, long extrabulbar  
644 prostatic vesicle with sperm ducts opening laterally into it, protrusible penis, common  
645 ovovitelline duct dorsal to female atrium, and a short, antero-dorsally flexed female canal. In  
646 view of these similarities, we have assigned both species to the new genus *Inakayalia*,  
647 namely, *Inakayalia chanca* comb. nov., and *Inakayalia tirua* comb. nov.

648

#### 649 **4.3 New observations on the morphology of *Wallmapuplana ruca* comb. nov.**

650 *Wallmapuplana ruca* comb. nov. was originally described by Marcus (1954) from material  
651 collected during the Lund University expedition to Chile (1948–1949). Later, Froehlich  
652 (1978) re-described this species as *Geoplana ruca* including observations based on new

653 specimens. Subsequently, Ogren & Kawakatsu (1990) re-allocated the species in the genus  
654 *Amaga*.

655 The external appearance of the Argentinean specimens agrees, in general, with the  
656 descriptions provided by Marcus (1954) and Froehlich (1978). However, Marcus (1954) did  
657 not provide a comprehensive account of the internal anatomy and only described several  
658 features enabling identification of the species. In the male reproductive system, a small  
659 prostatic vesicle receives the openings of the sperm ducts as well as those of several ramified  
660 canals of glands producing an abundant granular secretion. These glands, which we here  
661 designate as prostatic glands, surround the prostatic vesicle, discharging via their narrow  
662 ducts a great quantity of erythrophil granular secretion into the latter. Marcus (1954) also  
663 described a spiralling common vas deferens with a constant diameter throughout most of its  
664 length and widening only at its distal portion within the penis papilla. It should be noted that  
665 Marcus (1954) referred to this common vas deferens as the "männlicher Genitalkanal", or  
666 male genital canal. The Argentinean specimens also show this highly spiralling common vas  
667 deferens, while the dilated part matches with the ejaculatory cavity inside the penis. Marcus  
668 (1954, fig. 81) provided a reconstruction of the copulatory apparatus with a detail of the male  
669 system showing the papilla with the ejaculatory cavity lined with ciliated columnar  
670 epithelium, except near the tip of the papilla where the cilia are lacking. This arrangement  
671 fully agrees with the condition in the Argentinean specimens. The fold of the wall of the male  
672 atrium surrounding the small protrusible penis papilla, called penis sheath by Marcus (1954),  
673 is present also in all specimens studied here. Judging from Marcus drawing of the female  
674 system, the female atrium seems to be tubular-shaped, whereas in the Argentinean specimens  
675 it is an irregularly-walled cavity, even in small-sized specimens. Otherwise, Marcus (1954)  
676 noted that the common ovovitelline duct is divided into two portions, a tubular proximal  
677 portion and an expanded distal portion receiving secretion from the shell glands. This  
678 arrangement was also observed in the Argentinean specimens. These morphological  
679 characteristics were also confirmed by Froehlich (1978), who noted that the female atrium is  
680 dislocated, "opening laterally into the common atrium". The specimens from Argentina also  
681 show this trait, with both atria opening independently into the gonoduct. Neither Marcus  
682 (1954) nor Froehlich (1978) reported the presence of adenodactyls in the male system or the  
683 extremely wide ovovitelline ducts when these emerge from the ovaries. In fact, the ovarian  
684 region was not describe at all by these authors. It should be noted that not all specimens from  
685 Argentina had adenodactyls, so their presence could depend on the physiological state or  
686 maturity of the animals. Except for this discrepancy, the specimens from Argentina fit well

687 with the previous descriptions (Marcus 1954, Froehlich 1978), and therefore we presume that  
688 the animals are con-specific.

689 The gross anatomy of the copulatory apparatus of *W. ruca* resembles that of *Pichidamas*  
690 *piru* Bulnes, Grau, & Carbayo, 2018, recently described from Chilean Patagonia. *Pichidamas*  
691 *piru* also shares with *W. ruca* the uncommon absence of sensory pits and presence of an  
692 adenodactyl in the male atrium. However, the small penis papilla of *P. piru* is not surrounded  
693 by a penis sheath, as in *W. ruca*. In addition, *P. piru* has a cephalic retractor muscle (Bulnes,  
694 Grau, & Carbayo, 2018), which is absent in *W. ruca*. Another species, *Gusana cruciata*  
695 (Graff, 1899) shows also similarities with *W. ruca* in the gross morphology its copulatory  
696 apparatus, but in *G. cruciata* both the penis sheath and adenodactyls are absent. The prostatic  
697 vesicle is also different between these two species, being tubular and voluminous in *G.*  
698 *cruciata* (Froehlich 1978), while it is a small chamber in *W. ruca*.

699 The type III of musculo-glandular organ (MGO) present in *W. ruca*, viz., the adenocyst in  
700 the classification of MGOs of Winsor (1998), are small, spherical and weakly muscularised,  
701 with a short canal surrounded by glands secreting erythrophilic granules. Similar MGOs have  
702 been described for several land planarians, such as *Gigantea sandersoni* (Prudhoe, 1949),  
703 *Australopacifica hamiltoni* (Dendy, 1894), and some representatives of Bipaliinae (L. Winsor,  
704 pers. comm.). However, whether these structures are comparable to each other remains  
705 uncertain. The function of these MGOs is also unclear, although it is supposed that the  
706 secretions of these structures are concerned with the cocoon formation and facilitate adhesion  
707 of the capsules to the substrate (Winsor, 1998).

708

709

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724

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826

827 **Figure legends**

828

829 **Figure 1.** Map showing the distribution of *Inakayalia valdiviana* comb. nov. (triangles) and  
830 *Wallmapuplana ruca* comb. nov. (circles) in Chilean Patagonia (in yellow) and the new  
831 records in Argentinean Patagonia (in red). Numbers represent the following localities: (1)  
832 Teja Island (type-locality of *I. valdiviana*), (2 and 5) Villa La Angostura, (3) Nahuelbuta, (4)  
833 Petrohué (type-locality of *W. ruca*), (6) El Bolsón, (7) Los Alerces National Park. Map  
834 modified from an original of Google Earth.

835

836 **Figure 2.** Maximum Likelihood (ML) tree inferred from concatenated dataset (i.e. including  
837 both genes, *COI* and *18S* rDNA type II). Numbers at the nodes correspond to the bootstrap  
838 values of the ML analysis expressed as percentage (above the branch) and the Bayesian  
839 Posterior Probability (under the branch). Scale bar represents number of substitutions per site.

840

841 **Figure 3.** External aspect of *Inakayalia valdiviana* comb. nov. (A) Dorsal view of live  
842 specimen MLP He-7662; (B) dorsal and partially ventral view of live specimen MLP He-  
843 7661; (C) dorsal view of specimen MLP He-7661 after preservation; (D) detail of the cephalic  
844 region of C in dorsal (left) and ventral (right) views (MLP He-7661); (E) detail of eyes on the  
845 dorsum (MLP He-7662). Scale bars: 5 mm.

846

847 **Figure 4.** Schematic drawing, in dorsal view, of the eyes pattern of *Inakayalia valdiviana*  
848 comb. nov. (MLP He-7661). The eyes were enlarged for clarity. Scale bar: 5 mm.

849

850 **Figure 5.** Photomicrographs of transverse sections of the cephalic and the pre-pharyngeal  
851 body regions of *Inakayalia valdiviana* comb. nov. (MLP He-7661). (A) Cephalic region; (B)  
852 pre-pharyngeal region; (C–E) details of transverse sections of the pre-pharyngeal region: (C)  
853 body margin, (D) ventral to the intestine, and (E) dorsal to the intestine. Scale bars: (A, B)  
854 200  $\mu$ m, (C–D) 100  $\mu$ m. Abbreviations: cc, circular cutaneous muscle layer; cs, creeping sole;  
855 dc, diagonal cutaneous muscle layer; dp, dorsal parenchymatic muscle layer; dv, dorso-ventral  
856 parenchymatic muscle fibres; gm, glandular margin; i, intestine; lc, longitudinal cutaneous  
857 muscle layer; n, nerve plate; od, ovovitelline duct; rb, rhabditogen glands; s, sensory pit; sb,  
858 sub-intestinal parenchymatic muscle layer; sd, sperm duct; sp, supra-intestinal parenchymatic  
859 muscle layer; t, testis; vt, vitellaria.

860

861 **Figure 6.** Photomicrographs of sagittal sections of the pharynx of *Inakayalia valdiviana*  
862 comb. nov. (MLP He-7661) at the level of the mouth (A), and details of the outer (B) and  
863 inner (C) musculature of the pharynx. Scale bars: (A) 500  $\mu\text{m}$ , (B, C) 100  $\mu\text{m}$ . Abbreviations:  
864 di, dorsal insertion of the pharynx; i, intestine; im, inner musculature of pharynx; mo, mouth;  
865 om, outer inner musculature of pharynx; ph, pharynx; pl, pharyngeal lumen; pp, pharyngeal  
866 pouch; pv, prostatic vesicle; vi, ventral insertion of the pharynx.

867

868 **Figure 7.** Schematic reconstruction from sagittal sections of the copulatory apparatus of  
869 *Inakayalia valdiviana* comb. nov. (MLP He-7661). Scale bar: 500  $\mu\text{m}$ . Abbreviations: cod,  
870 common ovovitelline duct; ej, ejaculatory duct; fa, female atrium; fc, female genital canal; go,  
871 gonopore; ma, male atrium; od, ovovitelline duct; p, penis papilla; pg, prostatic glands; pp,  
872 pharyngeal pouch; pv, prostatic vesicle; sg, shell glands; sv, spermiducal vesicle.

873

874 **Figure 8.** Photomicrographs of sagittal sections of the copulatory apparatus of *Inakayalia*  
875 *valdiviana* comb. nov. (MLP He-7661). Scale bars: 500  $\mu\text{m}$ . Abbreviations: cod, common  
876 ovovitelline duct; ej, ejaculatory duct; fa, female atrium; fc, female genital canal; go,  
877 gonopore; ma, male atrium; p, penis papilla; pp, pharyngeal pouch; pv, prostatic vesicle; sg,  
878 shell glands.

879

880 **Figure 9.** Photomicrographs of sagittal sections of the reproductive system of *Inakayalia*  
881 *valdiviana* comb. nov. (MLP He-7661). (A) detail of the prostatic vesicle; (B) detail of the  
882 intrabulbar part of the prostatic vesicle; (C) detail of the ejaculatory duct; (D) glands  
883 discharging into the epithelium of penis papilla; (E) distal ascending portion of the  
884 ovovitelline duct; (F) common ovovitelline duct; (G) ovary; (H) detail of the female atrium.  
885 Scale bars: (A–D, H) 50  $\mu\text{m}$ , (E–G) 100  $\mu\text{m}$ . Abbreviations: cod, common ovovitelline duct;  
886 ej, ejaculatory duct; fa, female atrium; lc, longitudinal cutaneous muscle layer; ma, male  
887 atrium; n, nerve plate; od, ovovitelline duct; ov, ovary; p, penis papilla; pv, prostatic vesicle;  
888 sb, sub-intestinal parenchymatic muscle layer; sg, shell glands; asterisk shows sperm inside  
889 prostatic vesicle.

890

891 **Figure 10.** Photomicrographs of sagittal sections of the reproductive system of *Inakayalia*  
892 *valdiviana* comb. nov. (MLP He-7661) showing the course of one of the ovovitelline ducts  
893 before opening into the common ovovitelline duct. Scale bars: (A–D) 500  $\mu\text{m}$ . Abbreviations:

894 go, gonopore; i, intestine; lc, ma, male atrium; od, ovovitelline duct; p, penis papilla; sg, shell  
895 glands; vt, vitellaria. The arrows indicate cyanophil glands of the penis papilla.

896

897 **Figure 11.** Dorsal view of live specimens of *Wallmapuplana ruca* comb. nov. Scale bar: 10  
898 mm.

899

900 **Figure 12.** External aspect of *Wallmapuplana ruca* comb. nov. after preservation (MLP He-  
901 7664). (A) Dorsal view; (B) ventral view, showing mouth (mo) and gonopore (go); and (C)  
902 lateral view, showing the eyes. Scale bars: 2 mm.

903

904 **Figure 13.** Schematic drawing, in dorso-lateral view, of the eye pattern of *Wallmapuplana*  
905 *ruca* comb. nov. (MLP He-7668). The eyes were enlarged for clarity. Scale bar: 3 mm.

906

907 **Figure 14.** Photomicrographs of transverse sections of cephalic and pre-pharyngeal body  
908 regions of *Wallmapuplana ruca* comb. nov. (MLP He-7676). (A) Cephalic region, near the  
909 tip; (B) combined cephalic (left) and pre-pharyngeal (right) regions; (C–D) details of  
910 transverse sections of the cephalic region (C) and pre-pharyngeal region (D). Scale bars: (A)  
911 100  $\mu\text{m}$ , (B) 200  $\mu\text{m}$ , (C–D) 50  $\mu\text{m}$ . Abbreviations: cs, creeping sole; e, eyes; i, intestine; lc,  
912 longitudinal cutaneous muscle layer; n, nerve plate; od, ovovitelline duct; rb, rhabditogen  
913 glands; s, sensory margin; sb, sub-intestinal parenchymatic muscle layer; sd, sperm duct; sn,  
914 sub-neural parenchymatic muscle layer; sp, supra-intestinal parenchymatic muscle layer; t,  
915 testis; ve, ventral epidermis; vt, vitellaria.

916

917 **Figure 15.** Photomicrographs of sagittal sections of the pharynx of *Wallmapuplana ruca*  
918 comb. nov. (MLP He-7664), at the level of the mouth (A), and details of the outer (B) and  
919 inner (C) musculature of the pharynx. Scale bars: (A) 250  $\mu\text{m}$ , (B, C) 50  $\mu\text{m}$ . Abbreviations:  
920 di, dorsal insertion of the pharynx; i, intestine; im, inner musculature of pharynx; mo, mouth;  
921 oe, oesophagus; om, outer inner musculature of pharynx; pg, prostatic glands; ph, pharynx; pl,  
922 pharyngeal lumen; pp, pharyngeal pouch; sv, spermiducal vesicle; vi, ventral insertion of the  
923 pharynx; vt, vitellaria.

924

925 **Figure 16.** Photomicrographs with details of the male reproductive system of *Wallmapuplana*  
926 *ruca* comb. nov. (A) Longitudinal section of the anterior region of the body (MLP He-7676);  
927 (B–D) sagittal sections of the copulatory apparatus (MLP He-7664). Scale bars: (A–D) 200

928  $\mu\text{m}$ . Abbreviations: i, intestine; p, penis papilla; pg, prostatic glands; ph, pharynx; pv,  
929 prostatic vesicle; t, testes; sv, spermiducal vesicle; vd, common vas deferens; vt, vitellaria.  
930 Arrowheads indicate the small ducts of the prostatic glands.

931

932 **Figure 17.** Schematic reconstruction from sagittal sections of the copulatory apparatus of  
933 *Wallmapuplana ruca* comb. nov. (MLP He-7664). Scale bar: 500  $\mu\text{m}$ . Abbreviations: cod,  
934 common ovovitelline duct; ej, ejaculatory duct; fa, female atrium; fc, female genital canal; go,  
935 gonopore; ma, male atrium; od, ovovitelline duct; p, penis papilla; pg, prostatic glands; pp,  
936 pharyngeal pouch; pv, prostatic vesicle; sg, shell glands; vd, common vas deferens; sv,  
937 spermiducal vesicle.

938

939 **Figure 18.** Photomicrographs with details of the male reproductive system of *Wallmapuplana*  
940 *ruca* comb. nov. (A–C) Penis papilla (A: MLP He-7677, B–C: MLP He-7669); (D–F) male  
941 atrium of specimen MLP He-7669; (G–I) male atrium of specimen MLP He-7670. Scale bars:  
942 (A–C) 100  $\mu\text{m}$ , (D–G) 250  $\mu\text{m}$ , (H, I) 50  $\mu\text{m}$ . Abbreviations: ad, adenodactyl; ej, ejaculatory  
943 duct; ma, male atrium; p, penis papilla; vd, common vas deferens. Arrow indicates the  
944 strongly erythrophilic secretion (also shown in D–I) of the adenodactyl.

945

946 **Figure 19.** Photomicrographs of sagittal (A, B) and horizontal (C) sections of the copulatory  
947 apparatus of *Wallmapuplana ruca* comb. nov. (A) MLP He-7661, (B) MLP He-7677, (C)  
948 MLP He-7668. Scale bars: 500  $\mu\text{m}$ . Abbreviations: cod, common ovovitelline duct; ej,  
949 ejaculatory duct; fa, female atrium; go, gonopore; i, intestine; ma, male atrium; mo, mouth;  
950 od, ovovitelline duct; p, penis papilla; pg, prostatic glands; ph, pharynx; pv, prostatic vesicle;  
951 sg, shell glands; sv, spermiducal vesicle; vd, common vas deferens; vt, vitellaria.

952

953 **Figure 20.** Photomicrographs with details of the female reproductive system of  
954 *Wallmapuplana ruca* comb. nov. (A–C) Anterior region at the level of the ovaries; (A)  
955 longitudinal (MLP He-7676), (B) transverse (MLP He-7678), and (C) sagittal (MLP He-  
956 7665) sections; (D) sagittal section of the female atrium (MLP He-7661); (E) female ducts  
957 (MLP He-7661). Scale bars: (A–C) 200  $\mu\text{m}$ , (D) 100  $\mu\text{m}$ , (E) 50  $\mu\text{m}$ . Abbreviations: cod;  
958 common ovovitelline duct; fa, female atrium; fc, female genital canal; go, gonopore; i,  
959 intestine; n, nerve plate; od, ovovitelline duct; ov, ovary; sb, sub-intestinal parenchymatic  
960 muscle layer; sg, shell glands; t, testis; vd, common vas deferens; vt, vitellaria.

961

962 **Legends of Supporting information**

963

964 **Figure S1.** Maximum Likelihood tree showing the phylogenetic relationships based on the  
965 *18S* dataset. Values at the nodes correspond to percentage bootstrap support. Scale bar:  
966 number of substitutions per site.

967

968 **Figure S2.** Maximum Likelihood tree showing the phylogenetic relationships based on the  
969 *COI* dataset. Values at the nodes correspond to percentage bootstrap support. Scale bar:  
970 number of substitutions per site.

971

972 **Figure S3.** Maximum Likelihood tree showing the phylogenetic relationships among all  
973 subfamilies of the Geoplanidae, inferred with *COI* sequences. Values at the nodes correspond  
974 to percentage bootstrap support. Scale bar: number of substitutions per site.

975

976 **Figure S4.** Sagittal sections of the copulatory apparatus of *Wallmapuplana ruca* comb. nov.  
977 (A) MLP He-7675, (B) MLP He-7672. Abbreviations: cod, common ovovitelline duct; fa,  
978 female atrium; go, gonopore; i, intestine; ma, male atrium; mo, mouth; od, ovovitelline duct;  
979 p, penis papilla; ph, pharynx; sg, shell glands; sv, spermiducal vesicle; vd, common vas  
980 deferens.

981

982 **Figure S5.** Sagittal sections of the pharynx (A) and the copulatory apparatus (B, C) of  
983 *Geoplana chanca*. (A) Sagittal section of the pharynx of the holotype; (B) sagittal sections of  
984 the copulatory apparatus (a–h) of the holotype; (C) sagittal sections of the copulatory  
985 apparatus (a–c) of a paratype. Abbreviations: cg, common ovovitelline duct; di, dorsal  
986 insertion of the pharynx; ej, ejaculatory duct; fa, female atrium; go, gonopore; i, intestine; ma,  
987 male atrium; mo, mouth; od, ovovitelline duct; p, penis papilla; ph, pharynx; pl, pharyngeal  
988 lumen; pp, pharyngeal pouch; pv, prostatic vesicle; sg, shell glands; sv, spermiducal vesicle;  
989 vi, ventral insertion of the pharynx.

990

991 **Figure S6.** Sagittal sections of the pharynx (A) and the copulatory apparatus (B, a–d) of  
992 *Geoplana tirua* (holotype). Abbreviations: cod, common ovovitelline duct; di, dorsal insertion  
993 of the pharynx; ej, ejaculatory duct; fa, female atrium; go, gonopore; i, intestine; ma, male  
994 atrium; mo, mouth; od, ovovitelline duct; p, penis papilla; ph, pharynx; pl, pharyngeal lumen;  
995 pp, pharyngeal pouch; pv, prostatic vesicle; vi, ventral insertion of the pharynx.



996 **Table 1.** List of samples used in this study, with molecular code, voucher code and GenBank  
 997 Accession numbers.

Species	Molecular code	Museum Code	GenBank Accession	
			Cox1	18S
<i>Cephaloflexa araucariana</i>	F3387	MZUSP PL 1073	KC608316	KC608550
	F3426	MZUSP PL 1076	KC608319	KC608553
<i>Cephaloflexa bergi</i>	F1034	MZUSP PL 303	KC608238	KC608470
	F1038	MZUSP PL 305	KC608240	KC608472
<i>Choeradoplana iheringi</i>	F0365	MZUSP PL 651	MF802642	MF802591
	F3355	MZUSP PL 512	MF802643	MF802592
	F3481	MZUSP PL 539	MF802662	MF902611
<i>Choeradoplana pucupucu</i>	F2844	MZUSP PL 541	MF802666	MF802614
<i>Cratera pseudovaginuloides</i>	F1244	MZUSP PL 670	KC608251	KC608483
	F1245	MZUSP PL 671	KC608252	KC608484
<i>Cratera</i> sp.1	F2807	MZUSP PL 1050	KC608285	KC608516
<i>Cratera</i> sp.2	F2783	MZUSP PL 1048	-	KC608513 <sup>b</sup>
<i>Geobia subterranea</i>	F0358	MZUSP PL 650	KC608225	KC608457
	F1355	MZUSP PL 673	KC608255	KC608487
<i>Geoplana apua</i>	F5236	MZUSP PL 2072	MG653226 <sup>a</sup>	-
<i>Geoplana boraceia</i>	F4351	MZUSP PL 1086	KC608329	KC608563
<i>Geoplana cambara</i>	F1614	MZUSP PL 1009	KC608262	KC608494
<i>Geoplana cananeia</i>	F1635	MZUSP PL 2063	MG653229 <sup>a</sup>	-
<i>Geoplana caraguatatuba</i>	F6976	MZUSP PL 2126	MG653251 <sup>a</sup>	-
<i>Geoplana ibiuna</i>	F3562	MZUSP PL 2066	MG653232 <sup>a</sup>	-
<i>Geoplana iporanga</i>	F3166	MZUSP PL 2065	MG653231 <sup>a</sup>	-
<i>Geoplana paranapiacaba</i>	F6660	MZUSP PL 2079	MG643246 <sup>a</sup>	-
<i>Geoplana piratininga</i>	F0509	MZUSP PL 2060	MG653222 <sup>a</sup>	-
	F1163	MZUSP PL 666	KC608247	KC608479
<i>Geoplana pulchella</i>	F6528	MZUSP PL 2075	MG653240 <sup>a</sup>	-
<i>Geoplana</i> sp. 1	F2934	MZUSP PL 2127	MG653241 <sup>a</sup>	-
<i>Geoplana</i> sp. 2	F5145	MZUSP PL 2130	MG653234 <sup>a</sup>	-
<i>Geoplana</i> sp. 3	F2638	MZUSP PL 2133	MG653230 <sup>a</sup>	-
<i>Geoplana</i> sp. 4	F1707	MZUSP PL 2134	MG653252 <sup>a</sup>	-
<i>Geoplana</i> sp.	F4409	MZUSP PL 1087	KC608330	KC608564
<i>Geoplana vaginuloides</i>	F6387	MZUSP PL 2074	MG653228 <sup>a</sup>	-
<i>Gusana</i> sp.	F4421	MZUSP PL 1088	KC608331	KC608565
<i>Gusana</i> sp. 1	F4428	MZUSP PL 1089	KC608332	KC608566
<i>Imbira guaiana</i>	F0432	MZUSP PL 653	HQ542894	KC608461

	F3361	MZUSP PL 1071	HQ542896	KC608548
<i>Imbira marcusii</i>	F1041		KC608241	KC608473
	F1704	MZUSP PL 264	HQ026402 <sup>a</sup>	-
	F2051	MZUSP PL 290	HQ026406 <sup>a</sup>	-
	F2833	MZUSP PL 404	KC608291	KC608523
<i>Imbira</i> sp.	IPP 2479	MZU PL.1689	KY073285 <sup>a</sup>	-
	IPP 2473	MZU PL.00215	KY073286 <sup>a</sup>	-
<i>Inakayalia valvidiana</i> *	9-5F	MLP He-7661	MT649093*	MT648393*
	9-6F	MLP He-7662	MT649094*	MT648394*
<i>Issoca rezendei</i>	F1679	MZUSP PL 1010	KC608263	KC608495
	F1182	MZUSP PL 667	KC608248	KC608480
<i>Luteostriata abundans</i>	F0205	MZUSP PL 646	KC608223	KC608455
	F0238	MZUSP PL 648	KC608224	KC608456
<i>Luteostriata ernesti</i>	F3358	MZUSP PL 1070	KC608313	KC608547
<i>Luteostriata muelleri</i>	F3268	MZUSP PL 1199	KC608311	KC608545
<i>Matuxia matuta</i>	F2184	MZUSP PL 1021	KC608276	KC608508
<i>Matuxia</i> sp.		MZU PL 00166	KJ690054 <sup>a</sup>	-
<i>Matuxia tuxaua</i>	F1275	MZUSP PL 1192	KC608253	KC608485
<i>Notogynaphallia plumbea</i>	F3072	MZUSP PL 1060	KC608303	KC608537
<i>Notogynaphallia sexstriata</i>	F0792	MZUSP PL 656	KC608232	KC608464
	F1413	MZUSP PL 680	KC608257	KC608489
<i>Obama anthropophila</i>	F0422	MZUSP PL 1236	KP962397	KP962343
<i>Obama apeva</i>	F3848		KU564146 <sup>a</sup>	-
	F3850		KU564147 <sup>a</sup>	-
<i>Obama applanata</i>	F1262		KU564126 <sup>a</sup>	-
<i>Obama argus</i>	F1242		KU564125 <sup>a</sup>	-
<i>Obama braunsi</i>	F1081		KU564123 <sup>a</sup>	-
<i>Obama burmeisteri</i>	F1126		KP962354 <sup>a</sup>	-
<i>Obama eudoxiamariae</i>	F4065		KU564213 <sup>a</sup>	-
<i>Obama ficki</i>	840		KU564118 <sup>a</sup>	-
<i>Obama fryi</i>	F2550		KU564133 <sup>a</sup>	-
	F2554		KU564134 <sup>a</sup>	-
<i>Obama nungara</i>	GEO27		KM053226 <sup>a</sup>	-
	GEO28		KM053227 <sup>a</sup>	-
	F3191	MZUSP PL 1066	KC608308	KC608542
	F6101		KT714107 <sup>a</sup>	-
	F6102		KT714108	KT714090
<i>Obama</i> sp.	GEO26A		MF155855 <sup>a</sup>	-
	GEO71B		MF155888 <sup>a</sup>	-

<i>Obama tribalis</i>	O_trib	MZU PL 00288	MH378885 <sup>a</sup>	-
<i>Paraba multicolor</i>	F2101	MZUSP PL 1017	KC608271	KC608503
	F2997	MZUSP PL 1055	KC608299	KC608532
<i>Paraba rubidolineata</i>	F5482		KC608333	KC608567
<i>Pasipha albicaudata</i>	P_albi	MZU PL 227	MF375271 <sup>a</sup>	-
<i>Pasipha brevilineata</i>	P_brev	MZU PL 264	MF375272 <sup>a</sup>	-
<i>Pasipha hauseri</i>	P_haus1	MZU PL 261	MF375273 <sup>a</sup>	-
	P_haus2	MZU PL 262	MF375274 <sup>a</sup>	-
<i>Pasipha paucilineata</i>	P_pauc	MZU PL 239	MF375287 <sup>a</sup>	-
<i>Pasipha pinima</i>	F1714	MZUSP PL 1011	KC608264	KC608496
	F2585	MZUSP PL 717	-	KC608512 <sup>b</sup>
<i>Pasipha</i> sp.	GEO15A		MF155840 <sup>a</sup>	-
<i>Pasipha</i> sp.	Pasip	MZU PL 256	MF375303 <sup>a</sup>	-
<i>Pasipha tapetilla</i>	F2878	MZUSP PL 732	KC608296	KC608528
	F5631	MZUSP PL 938	KC608336	KC608570
<i>Pasipha varistriata</i>	P_varis	MZU PL 252	MF375299 <sup>a</sup>	-
<i>Polycladus</i> sp.	F0397	MZUSP PL 1186	KC608228	KC608460
<i>Supramontana irritata</i>	F3453	MZUSP PL 772	-	KC608556 <sup>b</sup>
	F5483	MZUSP PL 937	KC608237	KC608568
<i>Wallmapuplana ruca</i> *	9-7F	MLP He-7670	MT649095*	MT648395*
	9-8F	MLP He-7671	MT649096*	-
	9-9F	MLP He-7672	short*	-
	9-10F	MLP He-7673	MT649097*	-
	9-11F	MLP He-7674	short*	-
	9-12F	MLP He-7675	MT649098*	MT648392*
<i>Xerapoa pseudorhynchodemus</i>	F1013	MZUSP PL 1189	KC608237	KC608469
<b>Outgroup</b>				
<i>Dolichoplana striata</i>	F0379	MZUSP PL 1005	KC608226	KC608458
<i>Endeavouria septemlineata</i>	F0988	MZUSP PL 657	KC608233	KC608465

998 \* This study

999 <sup>a</sup> Only for the Cox1 Dataset

1000 <sup>b</sup> Only for the 18S Dataset

1001 short= sequence too short to be accepted in GenBank

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1003

1004 **Table 2.** Measurements (mm) of the sectioned specimens of *Wallmapuplana ruca* comb. nov.  
1005 and thickness of dorsal and ventral cutaneous musculature (DCM, VCM) (µm). All  
1006 measurements were obtained from preserved specimens. CS: width of the creeping sole in  
1007 relation to body width (%); DG: distance of gonopore from anterior end; DM: distance of

1008 mouth from anterior end. Percentages represent the position relative to body length, measured  
 1009 from the anterior tip. The ratio of the height of cutaneous musculature to the height of the  
 1010 body (cutaneous muscular index, CMI) was measured at the pre-pharyngeal region.

	MLP	MLP	MLP	MLP	MLP	MLP	MLP	MLP	MLP	MLP
	P1	P2	P3	P4	P5	P6	B13	B14	A43	A44
Body length	16	16	13.8	12	10.7	12.2	12.8	16.2	14.1	13
Body width	1.4	1.5	1.2	1.5	1.5	-	1.6	1.6	1.8	2.2
Body height	0.85	0.95	0.95	0.9	-	0.9	0.9	1	1.1	1
DM	8.6	8.8	7.4	7.3	5.5	6.8	7.3	8.7	8.2	7.2
	54%	55%	54%	61%	51%	56%	57%	54%	58%	55%
DG	11.2	10.9	9.8	9.4	7.2	8.8	8.3	11	10.3	9.3
	70%	68%	71%	78%	67%	72%	65%	68%	73%	71%
CS	70%	-	-	-	-	-	70%	-	70%	80%
DCM	30	15	-	30	-	17.5	20	20	20	20
VCM	30	40	-	40	-	30	45	37.5	45	40
CMI	8%	6%	-	8%	-	5%	7%	6%	6%	6%

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1012

1013 **Table 3.** Measurements (mm) of the reproductive system of sectioned specimens of  
 1014 *Wallmapuplana ruca* comb. nov. LFA: length of female atrium; LMA: length of male atrium;  
 1015 LPP: length of penis papilla. Percentages between parentheses represent the position relative  
 1016 to body length, measured from the anterior tip.

	MLP	MLP	MLP	MLP	MLP	MLP	MLP	MLP	MLP	MLP	MLP
	P1	P2	P3	P4	P5	P6	B13	B14	A43	A44	
Anterior-most testes	1.9	2.8	2.1	2.2	1.4	2.5	2.4	2.5	2.4	1.8	
	(12%)	(17%)	(15%)	(18%)	(13%)	(20%)	(19%)	(15%)	(17%)	(14%)	
Posterior-most testes	7.3	7.8	6.9	6.4	4.8	5.6	6.7	7.8	7.4	6	
	(46%)	(49%)	(50%)	(53%)	(45%)	(46%)	(52%)	(48%)	(52%)	(46%)	
LMA	1.5	1.25	1.4	1.3	1.2	1.2	0.9	0.9	1.2	1.25	
LPP	0.25	0.25	0.3	0.25	0.2	0.25	0.2	0.2	0.15	0.15	
Location of ovaries	2.5	3.5	2.5	2.5	1.5	2.8	3	3	3	2.1	
	(16%)	(22%)	(18%)	(21%)	(14%)	(23%)	(23%)	(18%)	(21%)	(16%)	
LFA	0.4	0.45	0.5	0.4	0.4	0.45	0.5	0.45	0.45	0.4	

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