NEOTROPICAL BIOLOGY AND CONSERVATION

Neotropical Biology and Conservation
17(3): 185–203 (2022)
doi: 10.3897/neotropical.17.e82779

REVIEW ARTICLE

Current state of knowledge on freshwater planarians (Platyhelminthes, Tricladida, Dugesiidae) from Chile

Constanza Vásquez-Doorman[1,2], Javiera Escobedo[1], Miguel L. Allende[1,2]

1 *Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile*
2 *Millennium Institute Center for Genome Regulation (CRG), Facultad de Ciencias, Universidad de Chile, Santiago, Chile Las Encinas 3370, 7800024, Santiago, Chile*

Corresponding author: Constanza Vásquez-Doorman (rdoorman@uchile.cl)

Academic editor: Piter Boll | Received 25 February 2022 | Accepted 2 May 2022 | Published 5 August 2022

Citation: Vásquez-Doorman C, Escobedo J, Allende ML (2022) Current state of knowledge on freshwater planarians (Platyhelminthes, Tricladida, Dugesiidae) from Chile Neotropical Biology and Conservation 17(3): 185–203. https://doi.org/10.3897/neotropical.17.e82779

Abstract

The unique geography of Chile encompasses a wide diversity of ecosystems and a rich biodiversity. However, the platyhelminth fauna has been poorly studied. The aim of this work is to compile the historical record of freshwater planarians described for this country. We accessed worldwide databases and published articles to provide a comprehensive review of their discovery history, morphological characteristics and their localities. Freshwater planarians have been collected mainly in central and southern Chile, while in the northern region a single species has been described. The discovery of new species of freshwater triclads has the potential to reveal novel animal models to study regeneration and/or biological adaptations, as some species are suitable for culture in the laboratory. We discuss the many reasons why further research is needed for this animal group, which should include genomic and molecular genetic studies.

Keywords

biodiversity, dugesiids, neotropical region, triclads

Introduction

The phylum Platyhelminthes comprehends a diverse group of free-living and parasitic flatworms. Free-living Tricladida flatworms are divided into three suborders based on their habitat: Maricola (marine), Cavernicola (cave), and Continenticola (freshwater and terrestrial) (Sluys et al. 2009). The three main freshwater families

Planariidae, Dugesiidae and Dendrocoelidae are distributed worldwide. However, only Dugesiidae has been reported in the southern hemisphere (Sluys and Riutort 2018; Vila-Farré and Rink 2018). The most represented Dugesiidae genera are *Girardia* Ball, 1974, *Neppia* Ball, 1974, *Cura* Strand, 1942, *Schmidtea* Ball, 1974 and *Dugesia* Girard, 1850. Freshwater triclads vary in size, coloration, reproductive strategies, and regenerative capacity. Body size varies from millimeters to several centimeters and body pigmentation varies from light brown and gray to darker colors such as black. Head shape is characteristic of each genus: *Cura* and *Schmidtea* have rounded heads, while *Dugesia* species have a triangular head, with a pair of rounded or more pointed auricles, also present in all species of *Girardia*, but which have auricles that tend to be much more protuberant (Sluys and Riutort 2018). The presence of pigmentation of the outer pharyngeal wall is considered a defining characteristic of *Girardia*, as proposed by de Vries and Sluys (1991). Although the mentioned morphological characteristics are important when classifying species, the description of the reproductive apparatus is an essential feature for correct identification.

The reproductive system's complexity and its diversity makes it ideal for taxonomic classification as a diagnostic character because it offers numerous characters for taxonomic and systematic studies. Sexual triclads are hermaphrodites, each animal has male and female reproductive organs and mate by cross-fertilization. Testes appear as discrete follicular structures distributed ventrally or dorsally along the body, and a pair of ovaries is usually located closely posterior to the brain. Other anatomical structures to consider are the vitellarian follicles or yolk glands distributed along the body and that generally occupy the entire dorsoventral space, as well as, the shape of the penis papilla, muscle types, routes of ovovitelline and seminal ducts, glands, and characteristics of the copulatory bursa.

For many years, the freshwater planarian fauna in Chile was known only by the studies of Borelli and Böhmig in the early 1900s (Borelli 1898, 1901; Böhmig 1902). However, our current knowledge is still very limited. Additional descriptions during the 20th century were published (Marcus 1954; Hyman 1959; Kawakatsu et al. 1984a; Curino and Cazzaniga 1993; Moretto 1996). Based on worldwide triclad diversity, one could estimate that a large number of species from Chile remains to be described. In this work, we present the state of knowledge on freshwater planarian diversity motivated by use of these organisms as animal models to study regeneration and biological adaptations, especially because some wild species are suitable for culturing under laboratory conditions. We obtained data from the World Register of Marine Species (WoRMS 2022), the Turbellarian Taxonomic Database (TTD) (Tyler et al. 2006–2020), check list publications (Ball 1969; Kenk 1974; Brusa et al. 2020), and others, several of which are old literature written in German, French, Italian, and Spanish. We list and summarize the main characteristics of each species and provide a general map indicating the approximate geographic position recorded for the seven freshwater specimens known from this country, generally concentrated within central (30°S) and southern (55°S) Chile.

Methods

Bibliographic search and databases

To search on the WoRMS database, we selected the distribution tab and chose "Chile (Nation)" under geounit. Then, we limited taxa to "Dugesiidae Ball, 1974" and asked to include all records, unchecking "extant only" and "marine taxa". To search within the TTD (Tyler et al. 2006–2020), we selected "Chile" under the geographic distribution search and generated a list to find freshwater species. We complemented the information following the original publications available.

Figure making

The map was generated using QGIS Hannover version 3.16 incorporating the coordinates as delimited text layers (.csv) for each species. Then, we edited the images on Adobe Photoshop 2021 to add location numbers. Planarian drawings and copulatory apparatus sketches were recreated using Adobe Illustrator and Photoshop 2021.

Results

The freshwater family Dugesiidae was coined by Ball (1974). Presently, it comprises twelve genera: *Bopsula*, *Cura*, *Dugesia*, *Eviella*, *Girardia*, *Neppia*, *Recurva*, *Reynoldsonia*, *Romankenkius*, *Schmidtea*, *Spathula*, and *Weissius* (Sluys and Riutort 2018), three of which are present in Chile (*Girardia*, *Dugesia* and *Romankenkius*) with a total of seven species: *Dugesia ambigua* Böhmig, 1902; *Dugesia similis* Böhmig, 1902; *Girardia canai* Curino & Cazzaniga, 1993; *Girardia chilla* Marcus, 1954; *Girardia festae* Borelli, 1898; *Girardia rincona* Marcus, 1954; *Romankenkius patagonicus* Borelli, 1901.

The Italian zoologist Alfredo Borelli described species collected by Enrico Festa, providing the first description of two species from Ecuador and Argentina, but also present in Chile: *Planaria festae* (*G. festae*) and *Planaria patagonica* (*R. patagonicus*) (Borelli 1898, 1901). The Austrian zoologist Ludwig Böhmig contributed with descriptions of triclads collected by the German colleague Johann W. Michaelsen in South America during the Hamburg Magalhaensian collecting trip, called the Michaelsen collection. Thus, two new species were collected in Chile: *Planaria ambigua* (*D. ambigua*) and *Planaria similis* (*D. similis*) (Böhmig 1902). Later, the German zoologist Ernst Marcus, who worked in Brazil, described two species from Chile: *Dugesia rincona* (*G. rincona*) and *Dugesia chilla* (*G. chilla*) (Marcus 1954). Finally, and more recently, the Argentinean researchers Alejandro Curino and Nestor Cazzaniga described *Girardia canai* (Curino and Cazzaniga 1993). Redescription of previously prepared and/or freshly collected samples were also essential contributions made by the American zoologist Libbie Hyman (1959), the Argentinean biologist Humberto Moretto (1996), the Dutch biologist Ronald Sluys (1992; Sluys et al.

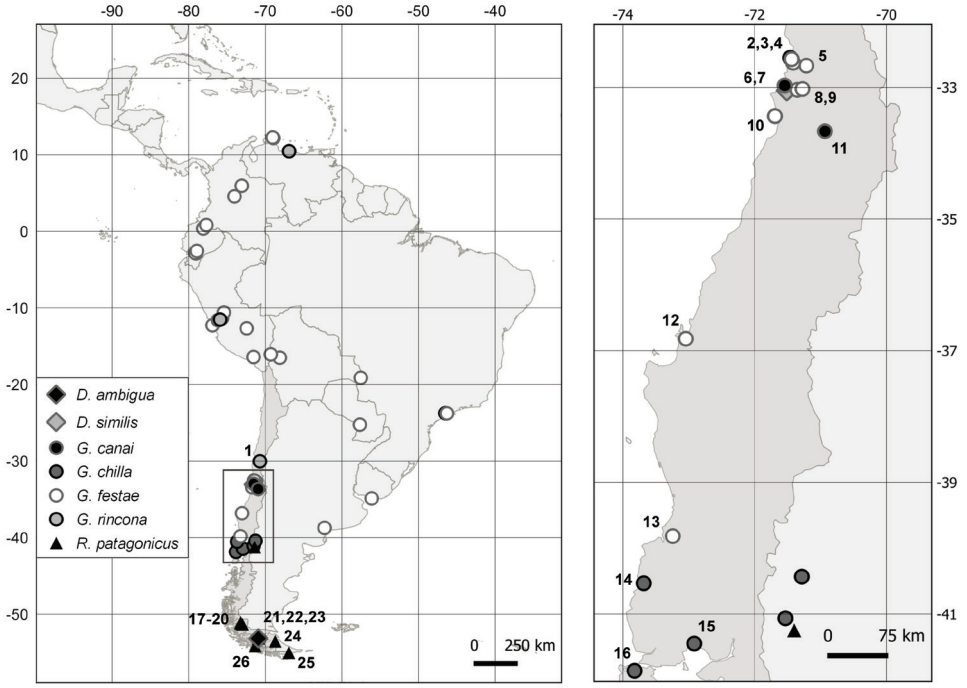

Figure 1. Distribution of freshwater planarians described from Chile and their known distribution in Latin America. We have listed the records (spots 1–26) with the corresponding coordinates in Table 1. Localities outside Chile were extracted from other publications (Borelli 1898; Fuhrmann 1914; de Beauchamp 1939; du Bois-Reymond Marcus 1953; Martins 1970; Kawakatsu and Mitchell 1984; Kawakatsu et al. 1984b; Sluys 1992, 1996; Sluys et al. 2005; Iannacone and Tejada 2007). Rhombus = genus *Dugesia*, circles = genus *Girardia*, triangles = genus *Romankenkius*. Zoomed-in rectangle is shown on the right.

2005), the Japanese zoologist Masaharu Kawakatsu (Kawakatsu and Mitchell 1984; Kawakatsu et al. 1984a, 1984b), among others.

Overall, the described species occur in central (30–40°S) and southern (50–55°S) Chile (Fig. 1, Table 1). The genus *Girardia* has representatives distributed mainly in the central regions, inhabiting rivers, estuaries, and brooks in Mediterranean or continental Mediterranean climates, while the only *Romankenkius* species is concentrated in the cold waters of the south in temperate-rainy Oceanic climate. Both *Dugesia* species were collected over 120 years ago in central and southern Chile and remain inquirenda due to their immature sexual organs. *G. festae* and *G. chilla* co-exist in two locations (spots 3 and 10 in Fig. 1).

Because most Chilean specimens were collected many years ago, only a few of them have been deposited in international museums. For example, *G. festae* was deposited in the American Museum of Natural History (AMNH), New York City (Hyman 1959); *G. canai* in the Museo Argentino de Ciencias Naturales Bernardino Rivadavia (MACN) in Buenos Aires and the AMNH (Curino and Cazzaniga 1993);

Table 1. Localities of freshwater planarian species from Chile. Indicating locality, coordinates, spot map number, collector and/or year of sampling and associated reference for each species.

Sp. Locality (Chile)	Coordinates	Spot	Collector	Reference
***Dugesia* Girard, 1850**				
D. ambigua				
A mountain lake, Punta Arenas, Magallanes	53°09'50"S, 70°55'02"W	22	J. Michaelsen, 1893	Böhmig 1902
D. similis				
A brook, Salto, Valparaíso	33°02'56"S, 71°30'42"W	9	J. Michaelsen, 1893	Böhmig 1902
***Girardia* Ball, 1974**				
G. canai				
Reñaca Estuary, Concón, Valparaíso	32°58'23"S, 71°32'38"W	6	Unknown	Curino and Cazzaniga 1993
Mapocho River, Talagante, Metropolitana	33°40'00"S, 70°56'00"W	11	P. Sánchez, 1986	Curino and Cazzaniga 1993
G. chilla				
Cadillos and Cajon Brooks, Zapallar, Valparaíso	32°33'04"S, 71°27'38"W	2	Unknown	Hyman 1959
El Tigre Brook, Zapallar, Valparaíso	32°34'20"S, 71°26'17"W	3	Unknown	Hyman 1959
Huallilemu Estuary, Punta de Tralca, Valparaíso	33°26'13"S, 71°41'29"W	10	P. Sánchez	Hyman 1959
Near Pucatrihue Beach, Osorno, Los Lagos	40°41'00"S, 73°32'00"W **	14	1991	Sluys et al. 2005
Pichi Pilluco Estuary, Llanquihue, Los Lagos	41°27'00"S, 72°55'00"W *	15	1948	Marcus 1954
A stream, Ancud, Chiloé Island, Los Lagos	41°51'50"S, 73°49'20"W *	16	1948	Marcus 1954
A stream, Cancha Ski, Punta Arenas, Magallanes	53°11'00"S, 70°58'00"W *	23	1949	Marcus 1954
G. festae				
El Tigre Brook, Zapallar, Valparaíso	32°34'20"S, 71°26'17"W	3	Unknown	Hyman 1959
Catapilco Estuary, La Laguna, Valparaíso	32°37'36"S, 71°24'58"W	4	Unknown	Hyman 1959
El Cobre Estuary, Nogales, Valparaíso	32°40'17"S, 71°12'56"W	5	Unknown	Hyman 1959
Reñaca Estuary, Concón, Valparaíso	32°58'23"S, 71°32'38"W	6	Unknown	Hyman 1959
Limache Estuary, Valparaíso	33°01'28"S, 71°16'23"W	7	Unknown	Hyman 1959
A ditch, Peñablanca, Quilpué, Valparaíso	33°02'14"S, 71°21'10"W	8	J. Michaelsen, 1893	Böhmig 1902
Huallilemu Estuary, Punta de Tralca, Valparaíso	33°26'13"S, 71°41'29"W	10	Unknown	Hyman 1959
Mapocho River, Talagante, Metropolitana	33°40'00"S, 70°56'00"W	11	P. Sánchez	Hyman 1959
Mapocho River, Talagante, Metropolitana	33°40'00"S, 70°56'00"W *	11	M. Sánchez	Moretto 1996
Concepción, Biobío	36°49'09"S, 73°02'40"W	12	I. Hermosilla, T. Cekalocic, 1976	Benazzi 1978
A flow, Valdivia, Los Ríos	39°49'02"S 73°14'33"W	13	J. Michaelsen, 1893	Böhmig 1902
G. rincona				
Elqui River, Vicuña, Coquimbo	30°02'00"S, 70°42'00"W ***	1	1949	Marcus 1954
***Romankenkius* Ball, 1974**				
R. patagonicus				
Stream by Pehoe Lake, Torres del Paine, Magallanes	51°04'00"S, 73°14'00"W *	17	M. Haga, 1966	Kawakatsu et al. 1984a
Pehoe Lake, Paine, Magallanes	51°04'00"S, 73°14'00"W	17	S. Inoue, 1971	Kawakatsu et al. 1984a
Serrano River, del Toro Lake, Magallanes	51°11'00"S, 73°12'00"W *	18	S. Inoue, 1971	Kawakatsu et al. 1984a
Serrano River, near its confluence with the Grey River, Magallanes	51°14'00"S, 73°13'00"W *	19	S. Inoue, 1971	Kawakatsu et al. 1984a
Stream by del Toro Lake with Porteño Lake, Magallanes	51°19'00"S, 72°59'00"W *	20	S. Inoue, 1971	Kawakatsu et al. 1984a
de las Minas River, Punta Arenas, Magallanes	53°08'00"S, 70°51'00"W *	21	M. Haga, 1966	Kawakatsu et al. 1984a
Daly Estuary, Grande Island Tierra del Fuego, Magallanes	53°33'38"S, 68°42'27"W ****	24	M. Haga, 1966	Kawakatsu et al. 1984a
Picton Island, Cabo de Hornos, Magallanes	54°10'00"S, 71°20'00"W	25	J. Michaelsen, 1892	Böhmig 1902
Aracena Island, Cockburn Channel, Magallanes	55°02'19"S, 66°55'33"W	26	1932	Sluys et al. 2005

* coordinates provided in reference. Others, inferred. All rounded off to the nearest second. ** original coordinates were 73°41'S, 40°32'W. *** original coordinates were 30°02'S, 69°36'W. **** original coordinates were 53°55'S, 61°05'W.

G. chilla in the Zoological Museum of the University of Amsterdam (ZMA) (Sluys et al. 2005); and *R. patagonicus* in the British Museum of Natural History of London (BMNH) (Sluys et al. 2005).

We have summarized the external aspect and some morphological characteristics of each species in Fig. 2 and Table 2. As other triclads, Chilean species are of brownish, grayish and blackish colors, with intraspecific variation, such as paler midline or spotty pattern. Sizes are also variable, some are long and thin, others shorter.

Regarding the copulatory apparatus, we summarized sketches based on reported information to highlight the differences between the species (Fig. 3).

Dugesia ambigua Böhmig, 1902, species inquirenda

Synonyms. *Planaria ambigua* Böhmig, 1902; *Curtisia ambigua* Marcus 1954.

Distribution. Southern Chile (Magallanes Region).

The original description of the species was made by Böhmig (1902). The specimens were collected in 1893 during the Hamburg Magalhaensian collecting trip to Patagonia, from a large lake in Punta Arenas (Fig. 1, spot 22), at approximately 300 m above sea level (masl). No details are provided regarding the lake. The dorsal surface of the specimen is of deep brown, blackish or dirty olive-green color. A whitish or yellowish color appears on the side margins, the midline and on the ventral side, on which brownish spots are occasionally noticeable. The head tip is blunt; the posterior end is bluntly pointed (Fig. 2A, Table 2).

The largest organism collected was about 7 mm long and 3 mm wide after fixation, but its copulatory organs were immature and the definitive configura-

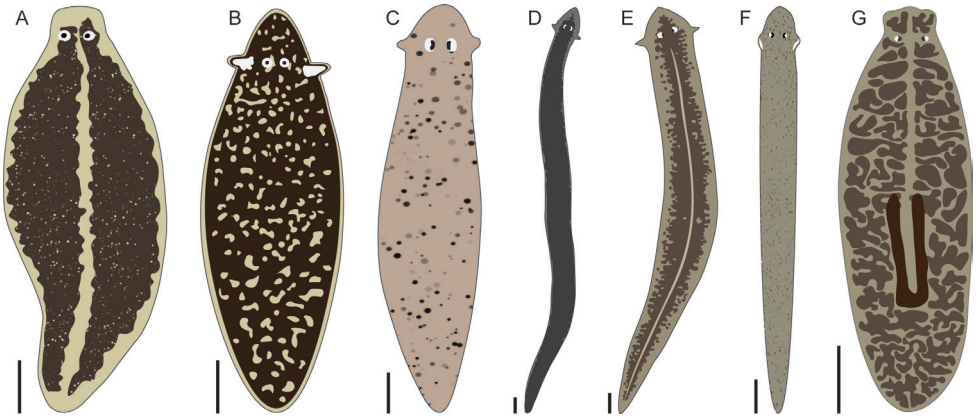

Figure 2. External appearance of freshwater planarians described from Chile. (A) *Dugesia ambigua*; (B) *Dugesia similis*; (C) *Girardia canai*; (D) *Girardia chilla*; (E) *Girardia festae*; (F) *Girardia rincona*; (G) *Romankenkius patagonicus*. Scale bars = 1 mm. Illustrations redrawn based on publications (Borelli 1901; Böhmig 1902; Marcus 1954; Hyman 1959).

Table 2. Summary of morphological characteristics of freshwater triclads from Chile. 1 = number of studied specimens, 2 = animal size in mm (length × width), 3 = presence of mature sexual organs, 4 = testes distribution along the dorsoventral axis, 5 = signs of asexual reproduction, 6 = pharynx pigmentation, ? = information not provided.

1	2	3	4	5	Dorsal coloration	Ventral coloration	6
D. ambigua							
?	7 × 3	No	D	?	Deep brown, blackish or dirty olive-green; whitish or yellowish on the margins and midline.	Whitish or yellowish, occasional brownish spots.	?
D. similis							
many	1.5–7 × 1.5–2.5	No	?	Yes	Isolated or confluent dark-brown, almost blackish spots covering the yellow pale background.	Evenly colored light gray or blackish spotted.	?
G. canai							
16	9–10 × 2–4	Yes	V	?	Light brown, with dark brown spots that might form a median stripe.	?	Yes
G. chilla							
50+	9–25 × 3	Yes	DV	Yes	Uniformly dark, with grayish, hassle and black hues.	Pale/light bluish gray.	Yes
G. festae							
100+	5–20 × 1.5–4	Yes	V	Yes	Brown, grayish or yellow tones, with or without a clear midline; uniform granular pigmentation or distributed laterally.	Uniformly grayish-yellowish.	Yes
G. rincona							
9	12 × 1.5	Yes	V	?	Grayish brown or grayish yellow, sometimes with a pale midline.	White.	No
R. patagonicus							
65+	3–6 × 1–3.5	Some	D	?	Yellowish with black-brown in broad confluent spots/ uniform dark brown-black.	White with darker blurry spots/uniform dark brown-black/pale brown.	No

tion could not be concluded, thus the specimen was named *Planaria ambigua*. The mouth lies at the beginning of the posterior third and the genital gonopore is about 0.5 mm away from it. The ovaries are found in the anterior region and the testes lie dorsal between the third and fourth branches. Marcus (1946, 1954) proposed that this species belongs to the genus *Cura* Strand, 1942, because the testes do not extend beyond the base of the pharynx. In concordance, because no mature reproductive organ was available for analysis, he did not include *C. ambigua* in his list of turbellaria from Chile (Marcus 1954). Later, Ball (1974) considered that the species was inadequately described and suggested it probably belongs to the genus *Dugesia*. Finally, after examining several specimens collected in the area, Kawakatsu et al. (1984a) commented that *Pl. ambigua* is very similar to *R. patagonicus*, the predominant triclad in southern Patagonia. This triclad remains as species inquirenda.

Dugesia similis Böhmig, 1902, species inquirenda

Synonyms. *Planaria similis* Böhmig, 1902.

Distribution. Central Chile (Valparaíso Region).

Very little information has been published on *Dugesia similis*. Many specimens were collected in 1893 in Salto brook (Fig. 1, spot 9), near Valparaíso, and

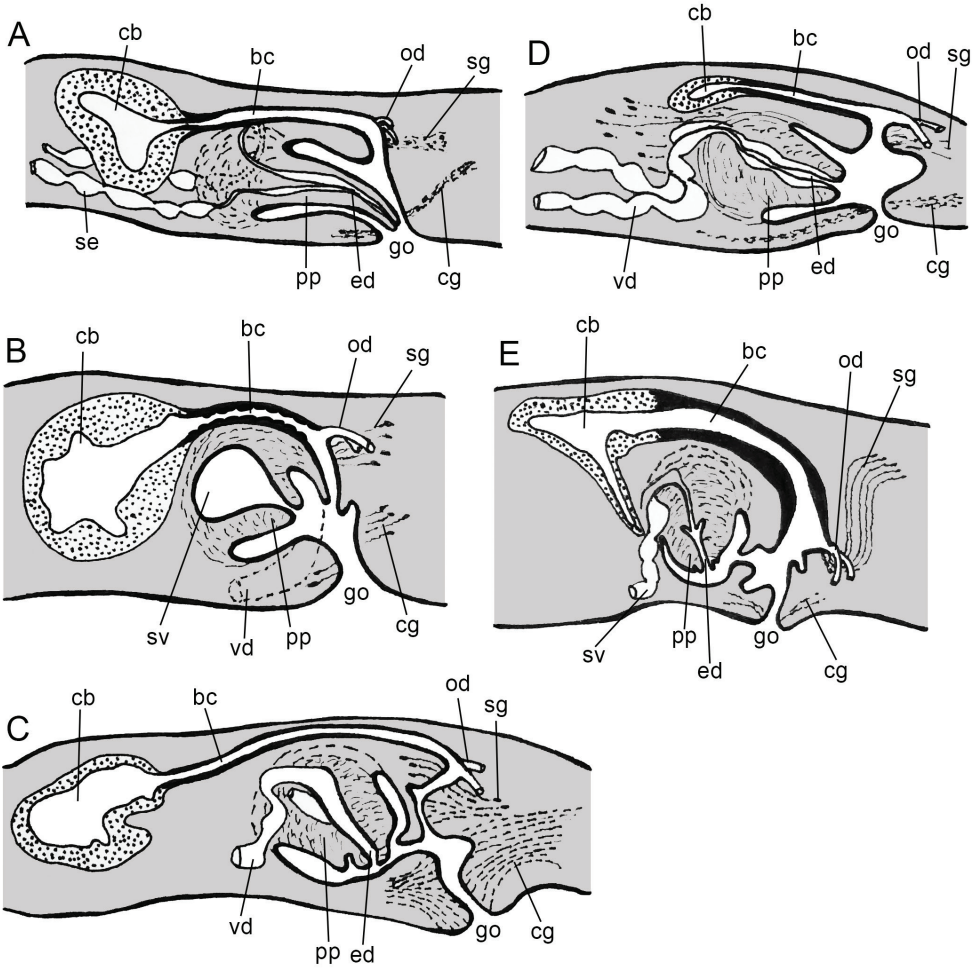

Figure 3. Reconstruction of the copulatory apparatus of species recorded for Chile. (A) *G. canai*; (B) *G. chilla*; (C) *G. festae*; (D) *G. rincona*; (E) *R. patagonicus*. Labels: bc = bursal canal, cb = copulatory bursa, cg = cement glands, ed = ejaculatory duct, go = gonopore, od = ovovitelline duct, pp = penis papilla, sd = sperm duct, se = false seminal vesicle, sg = shell glands, sv = seminal vesicle, vd = vas deferens. Sketches redrawn based on publications (Marcus 1954; Curino and Cazzaniga 1993; Sluys 1996; Kawakatsu et al. 1984a; Sluys et al. 2005; others) and simplified to highlight the main differences between the species.

described by Böhmig (1902). The climate in this region is Mediterranean, characterized by dry warm summers and wet mild winters. Most specimens showed signs of asexual reproduction by fission. Accordingly, size varied between 1.5–7.0 mm in length and 1.5–2.5 mm in width, and the number of secondary intestinal posterior branches was very variable, probably because body fission takes place at different distances from the mouth opening. When possible, Böhmig represented genital organs with special attention to the glands connected with

them, which play a main role in identifying individual species. However, specimens of *Planaria similis* had no mature reproductive organs and therefore could not be truly identified.

The author commented that the animals are very similar in body conformation to the North American *Planaria maculata* Leidy, 1847 (a synonym of *Girardia tigrina* Girard, 1850): a slender anterior, acute-triangular head shape and pointed rear end (Fig. 2B, Table 2). The dorsal side was colored dark brown, almost blackish, caused by isolated or confluent pigment spots covering the pale-yellow background. The ventral side was either evenly colored light gray or blackish spotted. The auricles were clearly recognizable in all individuals, conical, whitish and are very sharply defined from the body.

de Beauchamp (1939) assigned the species to *Dugesia*. However, because no reproductive organ was available for analysis, Marcus did not include this species in his list of turbellaria from Chile (Marcus 1946, 1954). He also highlighted that *D. similis* differs in coloration from *G. chilla*, a species collected in the same area (Hyman 1959). *D. similis* remains as species inquirenda.

Girardia canai Curino & Cazzaniga, 1993

Synonyms. none.

Distribution. Central Chile (Metropolitana and Valparaíso Regions).

The discovery of this species dates back to 1986. The authors' particular interest was to find *G. festae* in the locations previously described by Hyman (1959). Instead, *Girardia canai* was collected under stones in backwater areas of the Mapocho River near the province of Talagante and at the mouth of the Reñaca Estuary, Valparaíso (Fig. 1, spots 6 and 11) (Curino and Cazzaniga 1993). The Mapocho flows from the Andes through Santiago and is the principal river of the capital. The authors named the species under the genus *Girardia*, due to its morphology and the characteristics of its reproductive system, and "*canai*" which means "friend" in Mapudungun, the native language of the Mapuche ethnic group, in honor of the friendship provided by Patricio Sánchez, a Professor at Universidad Católica at the time, who helped in the collection of the specimens. Fixed specimens measured 9–10 mm long and 2–4 mm wide. The external morphology showed the typical triangular shape of the genus *Girardia*, with visible rounded auricles and a pair of eye cups. The dorsal pigmentation was mainly light brown, with dark brown spots that might form a longitudinal mid stripe. The number of spots and the presence of the stripe were very variable (Fig. 2C, Table 2). The pharynx was pigmented.

The authors examined sixteen mature specimens and thus provided a very detailed description of the reproductive apparatus (Fig. 3A). Testes run on both sides of the ventral nerve cords, extending along the length of the body. The extrapenial seminal vesicles or "false seminal vesicles" (se) are well developed, forming two wide tubes filled with sperm that originate from the anterior half of the pharynx. The course of the sperm ducts is asymmetrical and variable within the specimens,

and the authors describe three types of patterns for their course, but in general they run into the penis bulb without forming intrabulbar enlargements. The penis papilla (pp) of *G. canai* is notably long. This species can be distinguishable from *G. chilla* (Fig. 3B), which has an enormous cavity in the penial bulb. Ovoid ovaries are located directly posterior to brain branches. The ovovitelline ducts (od) open separately at a minimum distance between them and run dorsally until before the gonopore (go) where they curve and run towards a mid-sagittal plane and then curve again and open in the bursal canal (bc). Furthermore, this species presents two muscle layers in the angled bursal canal, characteristic of the genus *Girardia*.

Girardia chilla Marcus, 1954

Synonyms. *Dugesia chilla* Marcus, 1954; *Dugesia veneranda* Martins, 1970.

Distribution. Central and South Chile (Valparaíso, Los Lagos and Magallanes Regions), Brazil, Argentina.

G. chilla has some peculiar characteristics, including a stubby cone or ball-shaped penis papilla and dorsal or dorsoventral testes. The first description of this species was made by Marcus (1954) under the name of *Dugesia chilla*, after the Lund University expedition in 1948–1949, when forty-nine specimens were collected and analyzed. The author provided a general description based on the external morphology and reproductive system of specimens collected from three localities in southern Chile: Llanquihue, Chiloé Island and Punta Arenas (Fig. 1, spots 15, 16 and 23). Later, Hyman (1959) complemented the description, and provided Sánchez' notes on the species collected from three places in Valparaíso Region, central Chile (Fig. 1, spots 2, 3 and 10). More recently, Sluys (1996) confirmed that *Dugesia veneranda*, originally described from Brazil by Martins (1970), is a junior synonym of *G. chilla*, as suggested earlier (Ball 1974; Kenk 1974).

The specimens are slender, measuring approximately 9–25 mm long and 2–4.5 mm wide. The head is large, narrow, and pointy with protruding flexible auricles. Dorsal pigmentation is uniformly dark, dense, with grayish, hassle and black hues (Fig. 2D, Table 2). The ventral side is much paler with light bluish gray color. The pharynx is 4 mm long with gray pigment, typical of the genus *Girardia*. Eye size varies among specimens, suggesting that the worms might be at different regeneration stages after reproducing by fission. In concordance, Hyman (1959) mentioned that when the species is in an asexual state, it undergoes fission behind the pharynx and, in its sexual state during spring through summer, it lays cocoons with peduncles on the lower sides of the stones from which several juveniles hatch three to five weeks later.

Marcus's specimens from Pichi Pilluco Estuary, Llanquihue, were sexually mature, but the samples were contracted, and reconstruction of its copulatory apparatus was criticized by Hyman (1959). She provided a detailed description of relaxed samples (Fig. 3B), supplementing the histological description that coincides with several aspects, including the characteristic globular penis (pp) and ball-shaped copulatory bursa (cb). Sluys et al. (2005) redescribed the species by comparing

specimens from Argentina and Chile. The bursal canal (bc) is characteristic as it frequently shows constrictions (Marcus 1954; Sluys et al. 2005), which were not seen in samples from Valparaíso or São Paulo (Hyman 1959; Sluys 1996). This difference was attributed to the age of the animals (Sluys 1996). The bursal canal receives the opening of the unified ovovitelline ducts (od) at the posterior section and the openings of shell glands (sg) immediately ventral to them.

The species seems to be distributed across South America. *G. chilla* has also been found in two locations in Southern Argentina: Moreno Lake at 765 masl and Ñireco River (Sluys et al. 2005). Thus, the range of *G. chilla* extends from 32°S to 53°S. The species coexists with *G. festae* in Huallilemu Estuary and El Tigre Brook. However, *G. chilla* is less suitable for culturing in the laboratory.

Girardia festae Borelli, 1898

Synonyms. *Planaria festae* Borelli, 1898; *Planaria dimorpha* Böhmig, 1902; *Planaria polyorchis* Fuhrmann, 1914; *Euplanaria aurita* de Beauchamp, 1939; *Dugesia titicana* Hyman, 1939; *Dugesia polyorchis* Kenk, 1941; *Dugesia dimorpha* Marcus, 1954; *Dugesia festai* Marcus, 1954; *Dugesia* (*Girardia*) *sanchezi* Hyman, 1959; *Dugesia* (*Girardia*) *polyorchis* Ball, 1974; *Girardia festai* Sluys, 1992.

Distribution: Central and Southern Chile (Valparaíso, Metropolitana, Biobío and Los Ríos Regions), Argentina, Peru, Ecuador, Bolivia, Venezuela, Colombia, Curaçao, Brazil, Uruguay, Paraguay.

Girardia festae is one of the planarians present in Chile with the largest distribution and several synonyms. It has been found in springs, streams and lakes at altitudes up to 4800 masl. Its Latin American distribution has been summarized elsewhere (Kawakatsu and Mitchell 1984; Sluys 1992; Brusa et al. 2012). The first description dates back to the 19th century by Borelli (1898), where he described specimens found in Ecuador under the name *Planaria festae*. Four years later, Böhmig (1902) described *Planaria dimorpha* based on specimens found in Quilpué and Valdivia, Chile (Fig. 1, spots 8 and 13). Marcus (1954) briefly mentioned that *Pl. dimorpha* and *Pl. festae* should be included in the *Dugesia* genus, thus renaming them *Dugesia dimorpha* and *Dugesia festai*. Then, Hyman (1959) published a description as a new species under the name of *Dugesia sanchezi* in honor of Patricio Sánchez, who found the specimens in different locations in the Metropolitana and Valparaíso Regions (Fig. 1, spots 3–7, 10 and 11). Marcus (1960) described the species from Curaçao and Sluys (1992) offered a redescription under the name of *G. festae* comparing with the information provided by Böhmig (1902) and Hyman (1959), and proposing that *G. dimorpha* is a junior synonym of *G. festae*. Benazzi (1978, 1981) published articles in which he described the reproductive biology of *G. festae* from specimens obtained near Concepción, Chile (Fig. 1, spot 12). Then, Moretto (1996) redescribed the planarian collected in the Mapocho River (Fig. 1, spot 11) and reared in his laboratory. Finally, Sluys et al. (2005) redescribed specimens collected from Brazil, Paraguay and Argentina under the genus *Girardia*, remaining as *Girardia festae*.

The species reaches dimensions of 10–20 mm long and 1.5–4 mm wide. It has a triangular head with pointed auricles, typical of the genus *Girardia*. The body has the same width up to the rear third of the body, then narrows into a pointy posterior end. The dorsal pigmentation is variable, generally brown that varies towards grayish or yellow tones, with or without a clear longitudinal midline, plus a pattern of granular pigmentation that can be uniform throughout the body or distributed in the lateral regions (Fig. 2E, Table 2). While on the ventral side it presents a uniform gray-yellowish pigmentation. *G. festae* cohabits with *G. chilla* at Huallilemu Estuary and El Tigre Brook, Valparaíso.

The reproductive system of *G. festae* (Fig. 3C) consists of two ovaries in an anterior position between the second and third intestinal diverticula. Ovovitelline ducts run beyond the gonopore (go) and turn dorsally to connect with the bursal canal (bc), while numerous testes are located ventral to the ovaries and extend posteriorly through the lateral regions. In some specimens, testes extend dorsally, occupying the dorsoventral space. The penis papilla (pp) is characteristic as it has two lobes and does not have much muscle. The sperm ducts join to the penis bulb laterodorsally. Regarding the reproductive biology of *G. festae*, the species can reproduce both sexually and asexually by fission. These are diploid organisms with eight pairs of chromosomes (Benazzi 1981; Puccinelli and Deri 1991). Egg capsules are round with a dark-brown pigmentation and the peduncle is amber in color (Moretto 1996).

Girardia rincona Marcus, 1954

Synonyms. *Dugesia rincona* Marcus, 1954; *D.* (*G.*) *rincona* Ball, 1974; *Girardia rincona* Kawakatsu et al. 1984b.

Distribution. Northern Chile (Coquimbo Region), Peru, Venezuela.

Marcus (1954) was the first to describe *Girardia rincona* based on nine specimens found in Coquimbo Region (Fig. 1, spot 1). The largest specimen measured 12 mm long and 1.5 mm wide. The head was almost round, characteristic of the *Dugesia* genus and small white protruding auricles. The body was equally broad or expanded in the middle and the posterior end pointed. The dorsal side was grayish brown or grayish yellow, sometimes with a pale midline; the ventral side is white (Fig. 2F, Table 2); the unpigmented pharynx measured up to 2.5 mm long.

A detailed description of the reproductive apparatus (Fig. 3D) was given by Marcus (1954). In summary, numerous testes were located ventrally; sperm ducts were dilated, convoluted and located posterior to the pharynx. Contrary to other *Girardia* species, no bicuspid seminal vesicle was found. The ovaries were located at the level of the fourth and fifth intestinal branches; ovovitelline ducts (od) unite to form a common duct that opens to the bursal canal (bc), which characterizes the species. However, in this specimen, the female organs were not fully mature.

The species was redescribed by Kawakatsu and coworkers based on material from Peru and Venezuela (Kawakatsu and Mitchell 1984; Kawakatsu et al. 1984b). In these specimens, the sperm ducts give rise to a small seminal vesicle, continuing

as a unique and narrow ejaculatory duct (ed). The penis papilla (pp) is short and conical, and accompanied by many eosinophilous glands. The epithelium of the ejaculatory duct is similar to that of *G. canai*, found in central Chile. However, it differs significantly in other characteristics: the copulatory bursa (cb) of *G. rincona* is smaller, there are preovaric testicular follicles, extrapenial seminal vesicles run upward to the anterior dorsal side of the penis bulb, among others. Even though the rounded head shape and unpigmented pharynx are characteristic of *Dugesia*, Ball (1974) listed the species under the genus *Girardia*, considering the absence of seminal vesicles and the ventral testes.

Romankenkius patagonicus Borelli, 1901

Synonyms. *Planaria patagonica* Borelli, 1901; *Planaria michaelseni* Böhmig, 1902; *Curtisia patagonica* Kenk, 1930; *Curtisia michaelsoni* Kenk, 1930; *Cura patagonica* Ball, 1974; *Cura michaelseni* Ball, 1974; *Dugesia patagonica* Ball, 1974.

Distribution. Southern Chile (Magallanes Region), Argentina.

The first description of *Romankenkius patagonicus* dates from the beginning of the 20th century. Borelli (1901) described this triclad found in the Argentinean Patagonia near Santa Cruz River and named it *Planaria patagonica*. He also mentioned that Bergendal (1899) had found a similar sexually immature planarian from a forest stream with almost no vegetation near Punta Arenas, Chile, during the Swedish expedition to Tierra del Fuego (1895–1896). Based only on external description and geography, Borelli considered both to belong to the same species. A year later, Böhmig (1902) described *Planaria michaelseni* from Picton Island in Tierra del Fuego, Magallanes (Fig. 1, spot 25). Two specimens were collected from under rocks in a freshwater lake, of which only one was sexually mature. The length of the animals was about 5.5 mm, the width 3.5 mm. Böhmig contrasted anatomical characteristics to Borelli's planarian and suggested that the specimens likely correspond to *Planaria torva* or *Pl. polychroa*. Later, the species was renamed under the genus *Curtisia* (Kenk 1930), and de Beauchamp (1939) suggested that all these specimens correspond to one, *Curtisia michaelseni*, which was agreed by other authors (Marcus 1954; Kawakatsu et al. 1984a; Sluys et al. 2005). Ball (1974) placed the species in the genus *Cura*.

Subsequently, expeditions by Haga in 1966 and Inoue in 1971 resulted in the collection of specimens of this species in seven locations in Torres del Paine, Tierra del Fuego (Fig. 1, spots 17–21, and 24), leading to a publication that provided a detailed redescription of the species (Kawakatsu et al. 1984a). Based on differences in the connection between male and female systems, Sluys (1997) determined that *C. patagonica* and *C. michaelseni* should be included in the genus *Romankenkius*. These specimens have ovovitelline ducts (od) uniting to form a common duct that opens into the proximal region of the bursal canal (bc), at the posterior section of the common atrium (Fig. 3E). Later, Sluys et al. (2005) published an article focused on the biogeography of the genus *Girardia* describing new species, detailing old records and redescribing some species. Despite being focused on *Girardia*, it offers

images, a description and discussion about *R. patagonicus* comparing samples from Aracena Island, Magallanes (Fig. 1, spot 26), and Gutierrez Lake, Argentina, and contributes to the current understanding of the species. Thus, the range of *R. patagonicus*, which inhabits cold waters (6–11 °C), extends from Gutierrez Lake, Argentina (41°S) to Cockburn Channel (55°S).

Out of the sixty-five specimens collected in the description of Kawakatsu et al. (1984a), some were fixed in formalin and included small, darkly pigmented individuals. Sexual and non-sexual specimens were obtained, where the sexual ones had dimensions of approximately 6 mm long and 2–3 mm wide, while the sexually immature ones had dimensions of approximately 3 mm long and 1 mm wide, in agreement with Böhmig (1902). The authors mention they found fragments but do not discuss the possibility of asexual reproduction. The head of living animals is moderately rounded with broad ends and white, short, blunt auricular sense organs (Böhmig 1902; Kawakatsu et al. 1984a; Sluys et al. 2005).

The dorsal surface of most of the specimens is covered by spots of a very dark brown or gray color, except for a pale colored median longitudinal line, which starts from the top of the eyes and extends almost to the posterior end of the body (Fig. 2G, Table 2) (Borelli 1901; Böhmig 1902; Kawakatsu et al. 1984a). The ground color is gray or brownish-gray, with preserved animals showed pale-brown or olive-green hues (Böhmig 1902; Sluys et al. 2005). The ventral surface is light gray or whitish with some spots of dark pigment scattered on the surface but very numerous in the anterior cephalic part (Borelli 1901; Böhmig 1902) or uniformly blackish brown with numerous dark colored spots and a short, narrow, whitish, midventral line at the head level (Kawakatsu et al. 1984a). The pharynx is large and unpigmented.

The specimens have a pair of small ovaries located ventrally between the second and third intestinal diverticula, while the testes are located mainly dorsally and pre-pharyngeally (Kawakatsu et al. 1984a). The penis has a moderately large bulb embedded in the parenchyma and a short conical papilla (pp), while the sperm ducts come from mature spermiducal vesicles from the mouth to the anterior level of the penis. The copulatory bursa (cb) is large. The common genital atrium is a wide cavity that goes towards the male antrum in front and towards the female canal dorsally and opens to the genital pore (go) ventrally by a narrow tubular fragment of the antrum.

Discussion

In 1954, Marcus wrote "Chile is one of the places whose Turbellaria fauna has become known much more through the collective exploitation of traveling zoologists, than through the activities of workers resident in the country itself". Almost 70 years later, this statement remains valid. The information compiled for the species summarized in this paper, with records dating from the end of the 19th century and sparsely reaching the present, and mostly provided by non-Chilean scientists, reflects on the lack of specialists, interest, and/or resources in studying the fresh-

water planarians in this country. Furthermore, none of the reported specimens are deposited in Chilean museums. Herein, we provide a map with the record locations (Fig. 1) as a general view of their distribution, and representative illustrations (Fig. 2 and 3) of freshwater planarians in Chile, along with detailed tables listing the location coordinates of specimen acquisition and summarizing morphological characteristics (Tables 1 and 2).

We have found some inconsistencies. The WoRMS database finds five matching records for Dugesiidae in Chile, but two of those actually correspond to one species (*D. michaelsoni* = *R. patagonicus*) and it lacks the records of *D. ambigua*, *D. similis*, and *G. canai*. We have revised some coordinates (Table 1). Furthermore, it remains unclear if *D. ambigua* corresponds to *R. patagonicus*, as suggested by Kawakatsu et al. (1984a). Most of the described freshwater species are concentrated in central and southern Chile, with only one described for the northern zone. Remarkably, records of the composition of benthic macroinvertebrates present in the springs of the Ascotán salt pan (3800 masl, in the Atacama Desert) includes the finding of triclads (Vila et al. 2020). Although the authors do not provide a characterization of the specimens, this is a very interesting extreme environment to study.

Chile represents a great source of biodiversity that should be carefully studied due to its different climates and biogeography; several hot spots of endemicity are found and must be protected. It could be estimated that there are many planarian species that have not yet been described for marine, terrestrial and freshwater environments, including cave planarians, which are surely present in Chile but have not been recorded so far. Records of Chilean land planarians were compiled by Grau and Carbayo (2010). They present 25 species in total that are concentrated in the Atacama and Aysén Regions and agree with the lack of interest of zoologists in the area. Regarding the marine planarians, it is worth mentioning the contributions of Bergendal (1899), Böhmig (1902) and Marcus (1954).

In the future, we aim to welcome new investigators to resample type localities and carry out a thorough sampling to extend the knowledge of the species listed here. The correct determination of species in the family Dugesiidae is difficult due to similarities in both the external morphology and reproductive apparatus. Therefore, new studies should integrate the traditional techniques (morphological, anatomical and reproductive system description) with molecular analyses of nuclear and mitochondrial markers, such as ribosomal subunit 18 (18S), ribosomal subunit 28 (28S), cytochrome c oxidase subunit 1 (COI), elongation factor 1α (EF), and internal transcribed spacer 1 (ITS-1) sequencing (Álvarez-Presas et al. 2008, 2015; Álvarez-Presas and Riutort 2014; Negrete et al. 2020; Lenguas Francavilla et al. 2021). To these sequences, additional markers are being developed from next-generation sequencing (NGS), such as DOM5, Tnuc813, and Dugesia nuclear (Dunuc), which have been incorporated into planarian identification pipelines (Araujo et al. 2020; Leria et al. 2020). Such molecular profiling provides more accurate specimen identification, increases the amount of available information, and allows more accurate prediction of the phylogenetic relationships between species.

Conclusions

We have summarized the characteristics and brief sampling history of the seven freshwater triclads known from Chile and hypothesize that there are many more to discover. Interestingly, Chile, Argentina and Brazil share at least two species: *G. chilla* and *G. festae* (Borelli 1901; Sluys et al. 2005), opening an opportunity to evaluate biogeographic and evolutionary processes. Along with the relevance of knowing and protecting our wildlife, freshwater planarians are characterized by their great regenerative capacity, and constitute a pioneering model in the study of stem cells that contribute greatly to the area of biomedicine. Furthermore, we might face a great opportunity to study biological adaptations using planarians that inhabit extreme Chilean environments, such as the salt pans in the North and the cold waters of Patagonia.

Acknowledgements

We are thankful to Francisco Brusa, Universidad Nacional de La Plata, for providing information and support. We thank the World Register of Marine Species (WoRMS) and the Turbellarian Taxonomic databases. We also thank the reviewers for their constructive comments. This work was funded by the Agencia Nacional de Investigación y Desarrollo de Chile (ANID)/FONDECYT Postdoctoral Grant number 3180580 to CVD. MLA and CVD received funding from ANID - MILENIO - ICN2021_044.

References

Álvarez-Presas M, Riutort M (2014) Planarian (Platyhelminthes, Tricladida) diversity and molecular markers: A new view of an old group. Diversity (Basel) 6(2): 323–338. https://doi.org/10.3390/d6020323

Álvarez-Presas M, Baguñà J, Riutort M (2008) Molecular phylogeny of land and freshwater planarians (Tricladida, Platyhelminthes): From freshwater to land and back. Molecular Phylogenetics and Evolution 47(2): 555–568. https://doi.org/10.1016/j.ympev.2008.01.032

Álvarez-Presas M, Amaral SV, Carbayo F, Leal-Zanchet AM, Riutort M (2015) Focus on the details: Morphological evidence supports new cryptic land flatworm (Platyhelminthes) species revealed with molecules. Organisms, Diversity & Evolution 15(2): 379–403. https://doi.org/10.1007/s13127-014-0197-z

Araujo APG, Carbayo F, Riutort M, Álvarez-Presas M (2020) Five new pseudocryptic land planarian species of *Cratera* (Platyhelminthes: Tricladida) unveiled through integrative taxonomy. PeerJ 8: e9726–e9742. https://doi.org/10.7717/peerj.9726

Ball IR (1969) An annotated checklist of the freshwater Tricladida of the Nearctic and Neotropical Regions. Canadian Journal of Zoology 47(1): 59–64. https://doi.org/10.1139/z69-013

Ball IR (1974) A contribution to the phylogeny and biogeography of the freshwater triclads (Platyhelminthes: Turbellaria). Biology of the Turbellaria. New York, USA, 339–401.

Benazzi M (1978) Karyological and genetic data on the planarian *Dugesia sanchezi* from Chile. Rendiconti 64: 299–301. https://eudml.org/doc/289949

Benazzi M (1981) Reproductive biology of *Dugesia sanchezi*, a fresh-water planarian from Chile. Hydrobiologia 84(1): 163–165. https://doi.org/10.1007/BF00026175

Bergendal D (1899) Uber drei Tricladen aus Punta Arenas und umliegender Gegend. Zoologischer Anzeiger 22: 521–524.

Böhmig L (1902) Turbellarien, Rhabdocoeliden und tricladiden. Hamburg Magalhaensische Sammelreise Hamburg 3: 1–30.

Borelli A (1898) Viaggio del Dr Enrico Festa nell' Ecuador e regioni vicine, IX: Planarie d'acqua dolce. Bollettino cel Musei di Zoologia ed Anatomia Comparata della R. Università di Torino 13: 1–6. https://doi.org/10.5962/bhl.part.16811

Borelli A (1901) Di una nuova planaria d'acqua dolce della Republica Argentina. Bollettino del Musei di Zoologia ed Anatomia comparata della R. Università di Torino 16: 1–5.

Brusa F, Herrera-Martínez Y, Negrete L, Herrando-Pérez S (2012) *Girardia festae* (Borelli, 1898) (Platyhelminthes: Tricladida: Dugesiidae): distribution extension in a high-altitude lake from Colombia. Check List 8(2): 276–279. https://doi.org/10.15560/8.2.276

Brusa F, Leal-Zanchet AM, Noreña C, Damborenea C (2020) Thorp and Covich's Freshwater Invertebrates. Phylum Platyhelminthes. 5: 101–120. https://doi.org/10.1016/B978-0-12-804225-0.00005-8

Curino A, Cazzaniga N (1993) A new species of freshwater planarian from Chile (Platyhelminthes: Tricladida), with a nomenclatural note on *Girardia festae* (Borelli, 1898). Proceedings of the Biological Society of Washington 106: 633–644.

de Beauchamp P (1939) The Percy Sladen Trust Expedition to Lake Titicaca in 1937. Rotiferes et Turbellaries. Transactions of the Linnean Society of London 1: 51–79. https://doi.org/10.1111/j.1096-3642.1939.tb00005.x

de Vries EJ, Sluys R (1991) Phylogenetic relationships of the genus *Dugesia* (Platyhelminthes, Tricladida, Paludicola). Journal of Zoology 223(1): 103–116. https://doi.org/10.1111/j.1469-7998.1991.tb04752.x

du Bois-Reymond Marcus E (1935) Some South American Triclads. Anais da Academia Brasileira de Ciências 25: 65–78.

Fuhrmann O (1914) Turbellaries d'eau douce de Colombie. In: Voyage dexploration scientifique en Colombie par Dr O Fuhrmann et Dr Eug Mayor. Mémoires de la Société neuchâteloise des Sciences naturelles 5: 793–804. http://doi.org/10.5169/seals-100139

Grau JH, Carbayo F (2010) Panorama de la diversidad de planarias terrestres (Platyhelminthes: Tricladida) de Chile. Boletín de Biodiversidad de Chile 2: 41–54. https://repositorio.usp.br/item/001997978

Girard C (1850) A brief account of the freshwater Planariae of the United States. Proceedings of the Boston Society of Natural History 3: 264–265.

Hyman L (1939) New species of flatworms from North, Central, and South America. Proceedings of the United States National Museum 86(3055): 419–439. https://doi.org/10.5479/si.00963801.86-3055.419

Hyman L (1959) On Two Fresh-Water Planarians from Chile. American Museum Novitates 1932: 1–11. https://digitallibrary.amnh.org/handle/2246/4434

Iannacone J, Tejada M (2007) Empleo de la regeneración de la planaria de agua dulce *Girardia festae* (Borelli, 1898) (Tricladida: Dugesiidae) para evaluar la toxicidad del carbofurano. Neotropical Helminthology 1: 7–13. https://10.24039/rnh2007111147

Kawakatsu M, Mitchell RW (1984) Redescription of *Dugesia festai* (Borelli, 1898) based upon material from Venezuela and Peru (Turbellaria, Tricladida, Paludicola). Nihon Seibutsu Chiri Gakkai Kaiho 39: 1–12.

Kawakatsu M, Mitchell RW, Inoue S (1984a) A freshwater planarian from South Chile collected by the members of two Japanese limnobiological expeditions into Chile: *Cura patagonica* (Borelli, 1901) (Turbellaria, Tricladida, Paludicola). Journal of the Faculty of Agriculture 61: 377–398. https://eprints.lib.hokudai.ac.jp/dspace/bitstream/2115/12999/1/61(4)_p377-398.pdf

Kawakatsu M, Mitchell W, Kishida Y (1984b) A freshwater planarian from Central Peru collected by members of Kanazawa University Expedition into the Peruvian Andes: *Dugesia rincona* Marcus, 1954 (Turbellaria: Tricladida, Paludicola). Biology of Inland Waters 3: 1–18.

Kenk R (1930) Beiträge zum System der Probursalier (Tricladida Paludicola). Zoologischer Anzeiger 89: 289–302. http://planarias.each.usp.br/system/artigos/158/original/Kenk1930-Versuch_einer_nat__rlichen_Gruppierung_der_Probursalier.pdf

Kenk R (1974) Index of the genera and species of the freshwater triclads (Turbellaria) of the world. Smithsonian Contributions to Zoology (183): 1–90. https://doi.org/10.5479/si.00810282.183

Lenguas Francavilla M, Negrete L, Martínez-Aquino A, Damborenea C, Brusa F (2021) Two new freshwater planarian species (Platyhelminthes: Tricladida: Dugesiidae) partially sympatric in Argentinean Patagonia. Canadian Journal of Zoology 99(4): 269–278. https://doi.org/10.1139/cjz-2020-0169

Leria L, Vila-Farré M, Álvarez-Presas M, Sánchez-Gracia A, Rozas J, Sluys R, Riutort M (2020) Cryptic species delineation in freshwater planarians of the genus *Dugesia* (Platyhelminthes, Tricladida): Extreme intraindividual genetic diversity, morphological stasis, and karyological variability. Molecular Phylogenetics and Evolution 143: e106496. https://doi.org/10.1016/j.ympev.2019.05.010

Marcus E (1946) Sôbre Turbellaria brasileiros. Boletim Faculdade Filosofia, Ciencias e Letras; Universidade São Paulo. Zoologia 11: 5–254. https://doi.org/10.11606/issn.2526-4877.bsffclzoologia.1946.125301

Marcus E (1954) Reports of the Lund University Chile expedition 1948–1949. Turbellaria. Lunds Universitets Årsskrift 49: 3–115.

Marcus E (1960) Turbellaria from Curaçao and other Caribbean Islands. Studies on the fauna of Curaçao and other Caribbean islands 10: 41–51. http://repository.naturalis.nl/pub/506180

Martins MEQP (1970) Two new species of *Dugesia* (Tricladida Paludicola) from the State of São Paulo, Brazil. Anais da Academia Brasileira de Ciências 42: 113–118.

Moretto HJ (1996) La planaria chilena *Dugesia sanchezi* (Platyhelminthes: Turbellaria) y *Dugesia bonaerensis* n. sp., planaria de la pampa húmeda de Buenos Aires, Argentina. Revista Chilena de Historia Natural 69: 213–230. http://rchn.biologiachile.cl/pdfs/1996/2/Moretto_1996.pdf

Negrete L, Álvarez-Presas M, Riutort M, Brusa F (2020) Integrative taxonomy of land planarians (Platyhelminthes: Geoplanidae) from the Andean-Patagonian Forests from Argentina and Chile, with the erection of two new genera. Journal of Zoological Systematics and Evolutionary Research 59(3): 588–612. https://doi.org/10.1111/jzs.12444

Puccinelli I, Deri P (1991) Comparative karyological analysis of some American planarians belonging to the genus *Dugesia* (subgenus *Girardia*) (Platyhelminthes, Tricladida). Caryologia 44(3–4): 225–232. https://doi.org/10.1080/00087114.1991.10797189

Sluys R (1992) Synopsis of the freshwater triclads of the Caribbean (Platyhelminthes, Tricladida, Paludicola). Studies on the Natural History of the Caribbean Region 71: 1–23. https://repository.naturalis.nl/pub/503170

Sluys R (1996) Reconsiderations of The species status of some South American planarians (Platyhelminthes: Tricladida: Paludicola). Proceedings of the Biological Society of Washington 109: 229–235. https://www.biodiversitylibrary.org/page/34644834#page/251/mode/1up

Sluys R (1997) An old problem in a new perspective: The enigmatic evolutionary relationships of some Australian freshwater planarians (Platyhelminthes, Tricladida, Paludicola). Canadian Journal of Zoology 75(3): 459–471. https://doi.org/10.1139/z97-056

Sluys R, Riutort M (2018) Planarian diversity and phylogeny. Planarian Regeneration: Methods and Protocols, Methods in Molecular Biology vol. 1774, Springer Science + Business Media, 1–56. https://doi.org/10.1007/978-1-4939-7802-1_1

Sluys R, Kawakatsu M, Ponce de León R (2005) Morphological stasis in an old and widespread group of species: Contribution to the taxonomy and biogeography of the genus *Girardia* (Platyhelminthes, Tricladida, Paludicola). Studies on Neotropical Fauna and Environment 40(2): 155–180. https://doi.org/10.1080/01650520500070220

Sluys R, Kawakatsu M, Riutort M, Baguñà J (2009) A new higher classification of planarian flatworms (Platyhelminthes, Tricladida). Journal of Natural History 43(29–30): 1763–1777. https://doi.org/10.1080/00222930902741669

Tyler S, Schilling S, Hooge M, Bush LF [comp.] (2006–2020) Turbellarian taxonomic database. Version 1.7. http://turbellaria.umaine.edu

Vila I, Hermosilla V, Gonzalez F, Sobarzo G, Rojas P (2020) Macroinvertebrate community structure in an extreme altiplanic environment from Chile: The Ascotán salt pan. Global Ecology and Conservation 24: e01260. https://doi.org/10.1016/j.gecco.2020.e01260

Vila-Farré M, Rink JC (2018) The ecology of freshwater planarians. Planarian Regeneration: Methods and Protocols. Methods in Molecular Biology (Clifton, N.J.) 1774: 173–205. https://doi.org/10.1007/978-1-4939-7802-1_3

WoRMS (2022) World Register of Marine Species. https://www.marinespecies.org at VLIZ. https://doi.org/10.14284/170 [Accessed 2022-02-16]