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Research Article

An update on morphology and distribution of *Ruppia filifolia* (Philippi) Skottsberg in the Magellan region, southern Chile

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ABSTRACT. Vegetative and reproductive morphology of *Ruppia filifolia* (Philippi) Skottsberg is updated and completed by examining natural specimens, reviewing the literature, comparing to specimens from different locations and generating new iconography of the species from waters across the Magellan region, Subantarctic Chile. We expand current knowledge on morphology (rhizome-root, branching, foliage, inflorescence, fruit, habits) and geographical distribution of *R. filifolia* in Austral South America, with the addition of six new localities for the taxon in Chile. Our results are used to identify this macrophyte and to distinguish it from *R. maritima* after some authors reported their co-occurrence in the southern tip of South America, leading to taxonomic confusion. Also, the new information on *R. filifolia* distribution in the Magellan region broadens the research on a neglected seagrass. In the Southern Hemisphere, contrary to global trends of seagrass ecosystems decline, new niche distributions are opening up in southernmost Chile, reflecting environmental changes.

Keywords: seagrass, iconography, exsiccate, geographic allocation, Subantarctic waters, Magellan region.

INTRODUCTION

The taxonomy of *Ruppia* species is not fully resolved because of an inherent high plasticity in morphology that results from the frequent occurrence of hybrids and polyploids (Mannino *et al*., 2015). *Ruppia* L., a cosmopolitan genus of aquatic plants, is classified either in the euryhaline family of Potamogetonaceae (Gamerro, 1968; Den Hartog, 1981; Jacobs & Brock, 1982) or in the monogeneric group of the Ruppiaceae (Cronquist, 1981; Les *et al*., 1997). Taxonomic confusion is also driven by the morphological plasticity resulting from acclimation to local environmental conditions (Mannino & Graziano, 2014) and by the prevalence of tradition when naming *Ruppia* plants in regional flora (Triest & Sierens, 2013), often without complete morphological descriptions and comparisons to specimens from other locations (Den Hartog, 1981).

In Chile, *Ruppia filifolia* (Philippi) Skottsberg is distributed in freshwater ponds and rivers along the Andean mountain range, from 26°S in River Basins of the Atacama Desert (Pell *et al*., 2013) to 55°S in the austral region (Ramírez *et al*., 1979). Brackish water coastal ponds, estuaries, lagoons, and fjords are also habitats, where *R. filifolia* has been found from 44°S down to Tierra del Fuego and Falkland Islands (Moore, 1973, 1983; Álvarez *et al*., 2010; San Martín *et al*., 2011). Recent studies show that biomass and productivity of some *R. filifolia* perennial meadows in Chile´s Subantarctic region, are quite significant (Murcia *et al*., 2015), suggesting this species might play ecosystem roles as relevant as those played by seagrasses in other coastal areas. Seagrass communities worldwide are paramount to coastal, benthic ecosystem function, owing to their contribution to biological productivity, maintenance of biodiversity, nursery habitat, nutrient cycling, carbon sequestration, production and export of organic carbon, shoreline protection, and as bio-engineers, sediment stabilizers and bioindicators of environmental and coastal conditions (Terrados & Duarte 2000; Orth *et al.*, 2006). Their distribution worldwide has rapidly declined in the last 60 years (Hemminga & Duarte, 2000; Orth *et al.*, 2006),

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mainly due to anthropogenic developments (*e.g.*, dredging, aquaculture, boat anchoring) deteriorating water quality in coastal areas (Orth *et al.*, 2006; Waycott *et al.*, 2009; Abadie *et al.*, 2016). Yet, in the Southern Hemisphere, seagrass distribution appears to be expanding southward with newly identified communities of *Ruppiaceae* in Subantarctic waters (this study).

The name *Ruppia filifolia* was proposed by Skottsberg (1916) and is the combination commonly used to refer to *Ruppia*-like plants in the Patagonian Fjords Ecosystem of southernmost Chile. Older synonyms are *Potamogeton filifolius* (Philippi, 1860), *Ruppia andina* (Philippi, 1896 in Skottsberg, 1916) and *Ruppia obtusa* (Hagström, 1911). The ancient morphological descriptions by Philippi (1860) and Hagström (1911) did not include an iconography of the taxon. Later morphological descriptions (Moore, 1973, 1983) provided an illus-tration of plant habits only with details of the inflorescence and the fruit. The available information did not prevent the use of the name *Ruppia maritima* for plants found near Punta Arenas, southern Chile (Dusén, 1900) and only the genus name for plants in the nearby Skyring Sound (Mazzella & Gambi, 1993), surprisingly the locality studied by Hagström (1911), and Skottsberg (1916).

Short *et al.* (2007) identify "*Ruppia maritima"* as the seagrass with the world's southernmost distribution. Tierra del Fuego Island had plants with a mixture of characters from *R. maritima* and *R. cirrhosa* according to Gamerro´s (1968) descriptions and suggested *R. filifolia* as a separate taxon until further studies would clarify its status. Molecular phylogenetic studies of *Ruppia*, including material from Falkland Islands identified as *R. filifolia*, separate this entity from other *Ruppia* species but within the "*R. maritima* complex," and suggest that *R. filifolia* might result from hybridization among yet unknown entities of the complex (Ito *et al.*, 2010, 2013). Hence, the available evidence supports the status of *R. filifolia* as a distinct taxon, but additional knowledge on the morphology and distribution of the taxon in the Subantarctic tip of South America is needed. In fact, there is a large knowledge gap on this species, which is classified as "data deficient" by the IUCN (2014).

The objective of this study was to update and complete the morphological description of *Ruppia filifolia* in the Subantarctic tip of South America by providing new iconography of key morphological characters that would facilitate the identification of the taxon. We used material collected in Skyring Sound (Hagström, 1911; Skottsberg, 1916) for this purpose. Additional collections of plant material from new localities where the taxon was unknown to be present were also considered, as well as those archived in national and regional herbaria.

MATERIALS AND METHODS

More than 100 samples of *Ruppia filifolia* were collected over the years in several sites in the north and south shores of Skyring Sound (52°32'S, 71º56'W; Fig. 1), a brackish (18) water body (Kilian *et al*., 2007) from the Magellan region, where this taxon is abundant according to ancient (Hagstrom, 1911; Skottsberg, 1916) and recent reports (Mazzella & Gambi, 1993, Mansilla *et al*., 2013, Murcia *et al*., 2015). These collections of *R. filifolia* include also 20 plants sampled in Tierra del Fuego areas reported by Moore (1983), as well as non-systematic collections of 31 plants in total from different shallow water bodies throughout the archipelago (see Results). Water salinity of the surveyed sites was recorded *in situ* using a digital refractometer (Hannah Instruments Inc., HI 96822). Meadow location was registered in a handheld GPS (Garmin GPSMAP 64st).

Samples were carefully collected by hand to obtain complete plants (*i.e.*, with all plant organs: rhizome, roots, vertical stem, leaves, flowers, fruits), most obtained by SCUBA diving, or walking into shallow areas with neoprene waders. The collected material was placed in plastic bags for examination and measurements and maintained fresh, at ambient air temperature, within the following <24 h. Direct observation and measurements, of vegetative and reproductive structures, were performed with dissecting and optical micros-copes equipped with micrometers to examine the morphological characters of the collected specimens and to compare them with the morphological descriptions of the taxon in the literature and in herbarium collections. Fernald & Wigand (1914), Gamerro (1968), Haynes (1978), Novelo & Lot (1994) and Ramírez-García (2013) were used for the general description of the genus *Ruppia* in the American continent. Philippi (1860), Hagström (1911), Skottsberg (1916) and Moore (1973, 1983) were used for the description of *R. filifolia*.

More than fifty specimens of *R. filifolia* were pressed and dried into exsiccates, and five samples of flowers and fruits were preserved in 70% ethanol. Ten exsiccates were added to the collection in the Herbarium of the Institute de la Patagonia (HIP #15065$_{(4)}$, 15066$_{(3)}$, 15067$_{(3)}$) at the University of Magallanes (UMAG), five were sent to the Herbarium of the University of Concepción (CONC #184295-184297 two duplicates), Concepción; five were taken to the National Herbarium at the National Museum of Natural

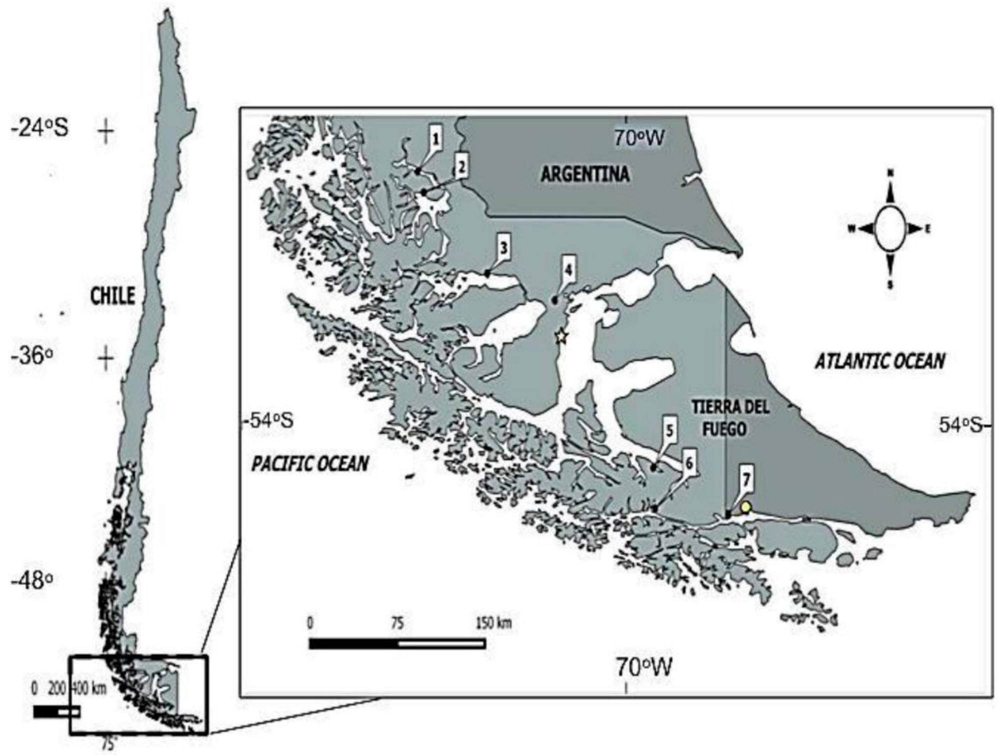

Figure 1. Sampling localities in Subantarctic waters of the Magellan region, and of Argentina, at the southern tip of South America, where *Ruppia filifolia* was found in this study. 1-2 Última Esperanza Sound; 3 Skyring Sound, mark refers to one of several sites from prior studies (*e.g.*, Murcia *et al.* 2015); 4 Cabeza de Mar; 5 Ainsworth Pond in Ainsworth Fjord; 6 Pía Pond, at the base of Pía Glacier; 7 Laguna Verde Pond in Lapataia, Argentina. The star marks the Magellan regional capital city of Punta Arenas; open circle marks the Argentinean regional capital city of Ushuaia on Tierra del Fuego Island.

History in Santiago de Chile (SGO #167991-167995), three were sent to Mexico's National Herbarium (MEXU #820, 890, 891) at the University Nacional Autónoma de Mexico (UNAM) and earlier exsiccates of *R. filifolia* remain in the herbarium of the Laboratory of Antarctic and Subantarctic Marine Ecosystems of UMAG, Punta Arenas, Chile.

RESULTS

Morphological description

Ruppia filifolia has terete stems of dimorphic branching (Fig. 2a) that remain above the substrate, erect, with foliage and floral structures present. The stems within the substrate are rhizome-type with unbranched roots. The leaves are sessile, alternate, with a stipule adnate to the leaf base, which wraps the stem (Fig. 2b). The leaf blade is linear with a margin, the apex acute, obtuse or rounded with unicellular trichomes (Fig. 2d). The inflorescence is present at the terminal or axillary spikes, stalked (up to 8 cm in length, first straight but curved after fruiting, often spiral), covered by a hyaline spathe 1 cm in length. Two flowers without bracts or perianth, and two sessile stamens with bilocular anthers, 1.6 to 1.2 mm long by 0.9 to 0.8 mm wide (Fig. 3); four sessile carpels (Fig. 2c). The fruits (1 to 6) are asymmetrical, dorsally rounded, 2.4 to 2.6 mm long by 1.9 to 2.1 mm wide, with pedicel of 0.1 to 0.2 mm or absent, with no rostellum, sometimes with a remnant capitated stigma (Figs. 2e, 3i), and one seed.

Habitat and habits

Ruppia filifolia is a perennial, rooted, submerged aquatic plant, inhabiting fresh to hyposaline, and shallow, water bodies, in soft, fine-sediment substrates at 0.5 to 6.0 m depth range in southern Chile (Murcia *et al.*, 2015). Areas of *R. filifolia* presence in Tierra del Fuego (Fig. 30h in Moore, 1983) were surveyed with little success. We found *R. filifolia* in Laguna Verde Pond, Lapataia Bay of the Argentinean sector (see below), which we consider a likely, or close to, one of the localities identified by Moore (1983). Additional habitats likely to host *R. filifolia* were surveyed with success and this plant was collected anew in six other localities (Fig. 1):

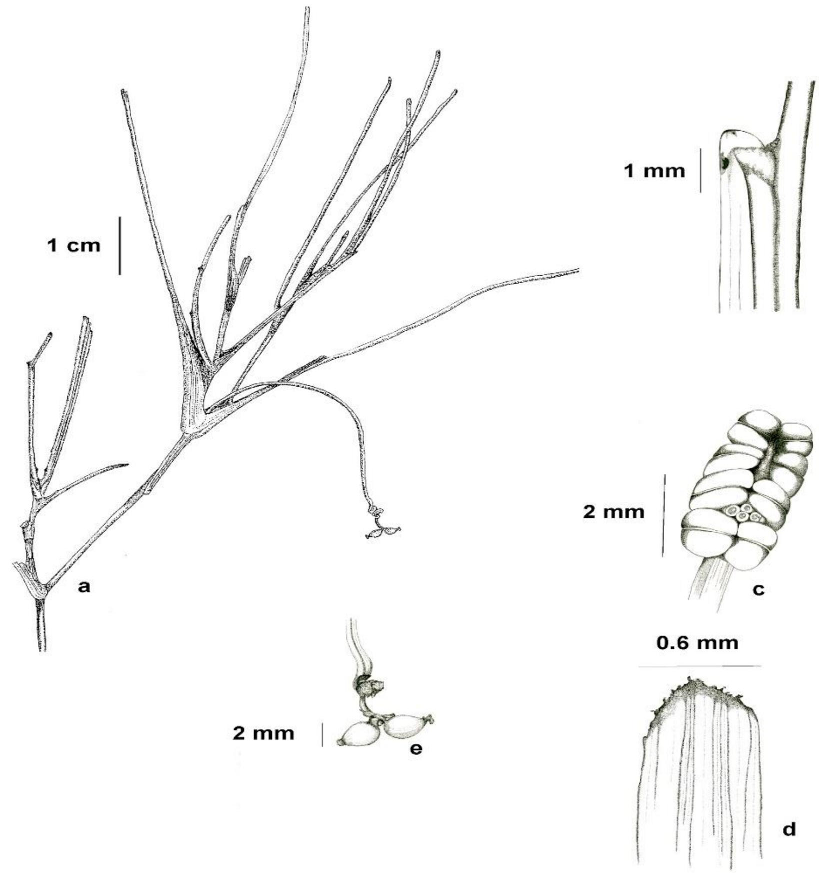

Figure 2. *Ruppia filifolia* from Skyring Sound, Magellan region. a) Vertical stem with infructescence, b) stipule at the leaf base, c) inflorescence with two flowers, the anthers are observed, as well as the four carpels of a flower (flowers in opposite position), d) the leaf apex showing its trichomes or strands on its edge, and e) two fruits, in one of which the remnant capitate stigma is observed.

1. Última Esperanza (Lit. *Last Hope*) Sound, Antonio-Varas Peninsula, Puerto Natales, Chile, 250 km North of the regional capital city of Punta Arenas (Fig. 1); 51°33'43.80"S, 72°55'30.80"W; depth 2-6 m, patchy meadows, seawater (18) fjord, 7 May 2016 [fr].
2. Última Esperanza Sound, Antonio-Varas Peninsula, Puerto Natales, Chile, 250 km north of the regional capital city of Punta Arenas (Fig. 1); 51º45'41.68"S, 72º50'29.71"W; depth 3-4 m, patchy meadows, seawater (19) fjord, 8 May 2016 [fr].
3. Skyring Sound (described above, Fig. 1)
4. Cabeza de Mar (Lit. *Sea Head*), Brunswick Peninsula, Chile; 52°48'05.80"S, 70°59'58.03"W (Fig.1). Shallow (1-2 m depth), patchy meadows, seawater (21) inlet connected to the Strait of Magellan, 45 km north of Punta Arenas, 16 April 2016 [fl, fr].
5. Ainsworth Pond, Marinelli Fjord, Tierra del Fuego Island (Chile´s side); 54°24'16.1"S, 69°37'22.3"W. Shallow (1 m depth), brackish water pond (20) in glacial moraine by the Marinelli Glacier, Magellan region, 13 March 2016 [fl, fr].
6. Pía Pond, Pía Fjord, Tierra del Fuego Island (Chile´s side); 54°47'28"S, 69°35'46"W. Shallow (<1 m depth), brackish water (11) pond in glacial moraine of Kalv/Pía Glacier, north-east arm of Pía Fjord, Magellan region, 14 March 2016 [fl, fr].
7. Verde (Lit. *Green*) pond, Lapataia Bay, Tierra del Fuego Island (Argentina´s side); 54°50'42.00"S, 68°34'39.36"W. Shallow (0.5-1 m depth) freshwater pond draining into Lapataia Bay and the Beagle Channel, 26 March 2012 [fl, fr].

DISCUSSION

The morphology of *Ruppia filifolia* specimens examined is consistent with former descriptions of the taxon by Philippi (1860), Hagström (1911) and Moore (1973, 1983). Moore (1983) considered *R. filifolia* a

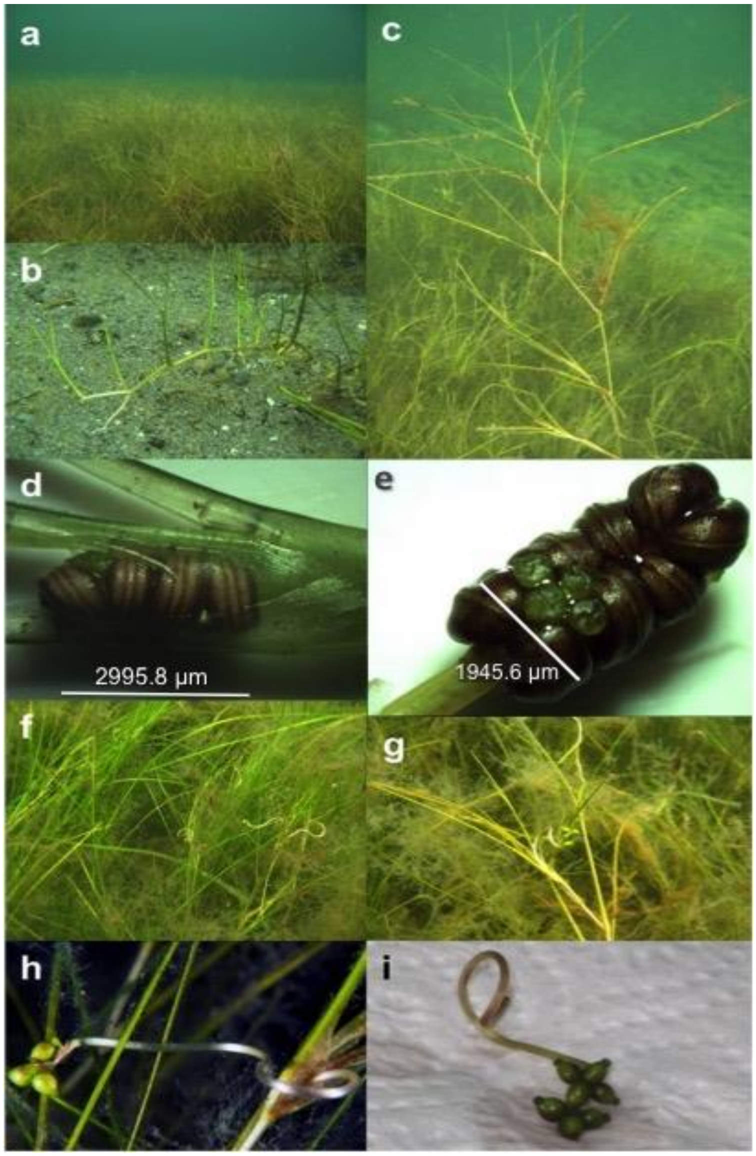

Figure 3. *Ruppia filifolia* in Skyring Sound, Magellan region. a) Natural meadow, b) apical section of horizontal rhizome, c) vertical stem, d) inflorescence partly wrapped by leaf sheaths, e) inflorescence with two flowers, four peltate stigmas are seen between the two reniform anthers of the distal flower, f-g) infructescence with curved stalk, h-i) infructescence with ovoid fruits without pedicle.

separate taxon with a mixture of characters from *R. cirrhosa* (Petag.) Grande and *R. maritima* L. according to the descriptions provided by Gamerro (1968) for those taxa. The molecular phylogenies of *Ruppia* (Ito *et al*., 2010, 2013) reveal that *R. filifolia* is close to *R. maritima* albeit maintaining significant differences with other taxa of the genus. Consequently, we support Moore´s (1983) proposal of maintaining the status of *R. filifolia* as a separate taxon until in-depth morphological, caryological and phylogenetic analyses are completed on a comprehensive collection of specimens from all localities where this taxon is found. Meanwhile, our results are useful to identify *R. filifolia* and particularly to distinguish it from *R. maritima* given that some authors consider that they co-occur in the southern tip of South America (Ramírez *et al*., 1979) and that taxonomic confusion remains (Mazzella & Gambi, 1993; Short *et al*., 2007). The main differences we found between *R. maritima* and *R. filifolia* are in the leaf's apex and the fruit's pedicel. The leaf apex in *R. filifolia* shows no pluricellular denticles, but unicellular trichomes, and the fruit pedicel is tiny or absent. These key characters were included in the taxon descriptions by Hagström (1911), and Moore (1983).

Morphological, in-depth comparison of the new specimen and iconographic data on Subantarctic *Ruppia filifolia* with those in local and national herbaria, revealed consistency with former descriptions of the taxon and confirmed the identity of our samples. Analyses of herbaria specimen of *R. filifolia* under 8-10x stereomicroscope verified our morphological description of plant parts (*e.g.*, inflorescence, fruit, long spiral stalk, stem branching, rhizome, leaf base). Although there was only one exsiccate (#0253, col. E. Pisano, 1973) of *R. filifolia* in the local HIP at UMAG, its collection locality coincides with our site 7, Lapataia Bay in the Argentinean portion of Tierra del Fuego Island (Fig. 1). This is the coastal, brackish pond-habitat type of *R. filifolia* described by Moore (1983) for Subantarctic environments. Likewise, the 35 exsiccate of *R. filifolia* in the CONC herbarium, and 17 exsiccate in the National Herbarium SGO, further confirm the taxonomy of our plant samples, but especially, the high phenotypic plasticity of the species. Only one specimen in the CONC (#179894, col. E. Teneb & E. Yañez, 2015) came from Última Esperanza Sound and three in the SGO (#058835 col. C. Skottsberg 1908; 154427/28 col. M. Ramírez, 2002) came from Skyring Sound, which coincides with our sites 1, 2, 3 (Fig. 1). Moreover, 47 (90%) of these 52 exsiccate of *R. filifolia* came from high-elevation (>3200 m a.s.l) ponds of brackish-to-saline waters similar to our pond sites 5, 6 and 7 (albeit at sea level). This further echoes the cosmopolitan nature of the species and validates our description of its habits and habitat types in the Magellan region. Before this study, only four specimens of high-latitude *R. filifolia* were part of the herbaria collections in CONC, SGO and the only one in the HIP. Except for these, distribution latitudes of all herbarium exsiccates examined ranged between 20ºS and 29ºS, including those from the Loa River Basin in the Atacama Desert (Pell *et al.*, 2013) and the Andean, high-elevation salt flats in northern Chile (*e.g.*, SGO samples).

Ruppia filifolia has also been described in marine, hyposaline waters of the Patagonian Fjords Ecosystem in the Magellan region of Chile's Subantarctic archipelago, including the species phenology and biomass (Mansilla *et al.*, 2013; Murcia *et al.*, 2015). But continued monitoring and assessment of new niche distributions for Ruppiaceae communities in the region, and their molecular phylogenies is paramount to gather baseline information to identify future environmental and community changes (*e.g.*, Coles *et al.*, 2011) in the Magellan region. Seagrass communities overall receive relatively little scientific and media attention compared to other coastal ecosystems (Duarte *et al.*, 2008) despite their ranking amongst the most productive ecosystems on Earth (Duarte & Chiscano, 1999) and are rapidly lost worldwide.

Hence the added relevance of the present study. The new records of *Ruppia filifolia* in coastal ponds of "recently" deglaciated moraines in Subpolar fjords (Pía and Marinelli) suggest expanding seagrass distributions, contrary to worldwide declining trends for seagrass communities (Orth *et al.*, 2006; Short *et al.*, 2007). Together with the records of *R. filifolia* in Laguna Verde (Argentina), Cabeza de Mar and Última Esperanza Sound, our results also aid in reducing the current taxonomic uncertainty that characterizes most records of this taxon in its Subantarctic range. Indeed, *Ruppia*-like plants that did not conform to *R. filifolia* morphology were never observed during our collection surveys. Interestingly, the finding of *R. filifolia* in ponds of recent formation after glacier retreat (Arróniz-Crespo *et al*., 2014) suggests that the geographical distribution of the taxon is expanding throughout the Subantarctic tip of South America likely mediated by bird transport. Further efforts are underway to improve the resolution of the geographical distribution and genetics of the taxon in the region. The complex coastline in Chile's Patagonian Fjords Ecosystem makes access to sites rather challenging in the short-term but under consideration in the authors' ongoing research on aquatic macrophytes in the region.

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