

Geographic distribution modeling and taxonomy of *Stephadiscus lyratus* (Cothouny in Gould, 1846) (Charopidae) reveals potential distributional areas of the species along the Patagonian Forests

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Background: *Stephadiscus lyratus* is an endemic charopid land snail species in southern South America. This, and most of the remaining South American charopids, was originally described by few dry shells. The lack of species anatomical data in Charopidae constitutes a barrier for intra family taxonomy. In the same way, the species distribution is known on scatter points of occurrences, mainly from material deposited in Museum collections. We provide here new information on the taxonomy, anatomy, habitat, and microhabitat preferences and estimate the potential geographic distribution of the species to test if *S. lyratus* is exclusively endemic to the Subpolar Magellanic Forest.

Methods: Fieldwork was carried out in National Parks of the Patagonian Forests. Adult snails were photographed, measured, and dissected for anatomical studies. We compiled a total of 60 geographic species records from different sources, from which 20 are new sites for *S. lyratus*. Estimation of the species geographical distribution (EDG) was obtained through correlative ecological niche modeling (ENM). We designed a calibration area a priori with known species points of occurrence in the Magellanic Subpolar Forests and the southern portion of Patagonian steppe. Seven non correlated bioclimatic variables of WorldClim database were used for the analyses. The best ENMs were calibrated and selected using a maximum entropy method with Maxent v3.3.3K through the R package "kuenm". We evaluated candidate model performance based on significance (partial ROC), omission rates ($E = 5\%$), and model complexity (AICc). From the best models obtained, a final model was transferred to a region "G" consisting of the calibration area plus the Valdivian Temperate Forests and whole Patagonian steppe, where we hypothesize that the species could be present. Finally, we obtained binary presence-absence maps. We quantified the proportion of the distribution range of *S. lyratus* in different land cover categories. To explore the degree of protection of *S. lyratus*' EGD, we quantified the proportion of its distributional range within protected areas.

Results: The presence of a be-lobed kidney, a close secondary ureter, the terminal portion of the spermoviduct forming a compact glandular mass and the vas deferens with a dilatation at its point of insertion are new anatomical characters provided for the species. *S. lyratus* inhabit cold native forests, mainly living on or under humid logs in contact with the ground. These preferred microhabitats are less frequent in disturbed forests. Main constraining variables to explain *S. lyratus* distribution in the EGD are variables of temperature and Annual Precipitation. The potential area of distribution obtained almost duplicates their original range (140,454 Km²), extending to the Valdivian temperate forests mainly in Chile. However, only 14.7% of the potential area occurs within current protected areas from both, Argentina and Chile.

Introduction

Stephadiscus Hylton Scott, 1981 is a genus of the diverse Charopidae, a family of Punctoidea land gastropods, having an extensive distribution that includes South America, South Africa, Australia, New Zealand, and Oceania (Salvador et al., 2020). Southern Argentina and Chile are particularly rich in endemic species of Charopidae. Hylton Scott (1964, 1968, 1970, 1973, 1981) was the researcher that most studied and described Charopidae species in South America. Unfortunately, most of her descriptions were based on a single or few dry shells, which resulted in the absence of intraspecific shell variability studies. The lack of species anatomical data constitutes a barrier for intra family taxonomy. Also, adequate fieldwork to estimate the current distributional range of genera and species has not been done.

Stephadiscus initially included some species that are currently classified in *Stephanoda* Albers, 1860, and *Stephacharopa* Miquel & Araya, 2013 (Miquel & Barker, 2009; Miquel & Araya, 2013). At present, the genus is formed by six species, *Stephadiscus lyratus*, *S. celinae* (Hylton Scott, 1969), *S. mirabilis* (Hylton Scott, 1968), *S. perversus* (Hylton Scott, 1969), *S. rumbolli* (Hylton Scott, 1973), and *S. stuardoi* Miquel & Barker, 2009. The taxonomic position of *Stephadiscus striatus* Hylton Scott, 1981 from northeast Argentina and Venezuela, will have to be reconsidered since it seems to belong to Punctidae (Miquel & Barker, 2009). The distribution of the *Stephadiscus* is restricted to Patagonia at both sides of the Andes from S 36° towards the southernmost portion of the continent, including Malvinas islands and southern archipelagos (Miquel & Barker, 2009; Miquel & Araya, 2013).

Biogeographically, southern South America belongs to the Andean region, proposed among others by Morrone (2018), who divided this region into three subregions, the Subantarctic, Central Chilean and Patagonian. The Andean region has a closer relationship to the Austral region in the Austral kingdom (Morrone, 2018). Current distributional range of *Stephadiscus lyratus* (Couthouy in Gould, 1846), designated as the genus type species, had been established on scattering points of occurrences taken from its original description, and subsequent species mentions (Gould, 1845-1948; Hylton Scott, 1972, 1981; Miquel & Araya, 2013, see "Species remarks" section). Erroneous taxonomic identified material from Museum Collections suggested the presence of this species in the forests of northern Patagonia, even though Hylton Scott (1981) stated that *S. lyratus* could be a strictly Magellanic species. While *Stephadiscus celinae*, *S. perversus*, and *S. mirabilis* also occur in the Valdivian rainforest sub-ecoregion of northern

Patagonia, *S. rumbolli* is exclusively from the southern sub-ecoregions. For this reason, there are doubts as to whether the distribution of *S. lyratus* extends naturally to the Valdivian rainforest or it is restricted to southern areas. Modern methodologies to estimate a species distributional area involves the ecological niche models (ENMs) that relate the species distribution data (species occurrence at known locations) with information about the environmental and/or spatial characteristics of the locations (abiotic factors) (Medina, Ponssa & Aráoz, 2016). The environmental variables of the species localities of occurrence are also informative about the species' potential distribution. In this sense, estimates of the geographic range obtained by ENM techniques when data are scarce or when species are rare have proved to be more successful than those obtained by traditional methods (e.g., minimum convex polygon) (Marcer et al., 2013; Syfert et al., 2014; Pena et al., 2014). These also allow avoiding the potential subjective bias of experts (Fourcade et al., 2014). Furthermore, identifying scenopoetic variables related to the species offers the opportunity to discover populations isolated by unsuitable environments (Wiens & Graham, 2005). On the other hand, modeling species potential areas of distribution may also provide information on the geographic distribution of unknown sister species (Peterson et al., 2011).

The objective of this research is first to provide new information on the anatomy and the ultrastructure of the shell of *Stephadiscus lyratus*, using recently collected material, and second to characterize the microhabitat where it is found. Finally, we estimate its potential geographic distribution to test whether *S. lyratus* is also distributed in the Valdivian Temperate Forest or if this species is endemic exclusively to the Subpolar Magellanic Forest.

Material and Methods

Study area (Fig. 1)

Fieldwork was carried out in the Patagonian Forests that extends in a narrow strip over the Andes mountain ranging from 35 ° to 55 ° south latitude. In Argentina, this region occupies the western zone from the provinces of Neuquén to Tierra del Fuego and islands of the southern Atlantic. The Patagonian Forests are divided into two different areas, the northern Valdivian Temperate and the southern Magellanic Subpolar Forest ecoregions *sensu* Olson & Dinnerstein (1998), Olson et al. (2001) or sub-ecoregions *sensu* Dos Santos et al. (2020). We will followed here this last mentioned classification. The Valdivian Temperate Forest (VTF) covers a narrow area

running from 35° to 48° south latitude between Chile and Argentina. Annual precipitation varies between 1,000 mm in the north and more than 6,000 mm per year in the southern part of the sub-ecoregion. This seasonal precipitation decreases significantly on the eastern slope of the Andes in Argentina, where rainfall of less than 200 mm is recorded only 100 km east of the Andean peaks. Maximum annual average temperatures vary between 21 °C and 13 °C in the northern and southern ends of the sub-ecoregion. Minimum annual average temperatures range from 7 °C to 4 °C (<https://www.worldwildlife.org/ecoregions/nt0404>). The high rainfall in Valdivian Forest favors the formation of dense primary forests, and for this reason, it is commonly called a jungle. Biogeographically, these forests share floristic similarities with other temperate forests in the southern hemisphere located in Australia and New Zealand. There is a high degree of endemism not only in the vegetation but also in animal species. The southern areas of the Magellanic Subpolar Forests (MSF) are well represented in Tierra del Fuego, where they occupy the entire south of the province (Fig. 1A-C). However, dominant vegetation is forests of less species diversity in comparison to the Valdivian rainforests due to the low temperatures and rainfall. Tierra del Fuego or Fueguia is the archipelago located south of the Strait of Magallanes between 52° 28'S and 55° 03'S; it occupies about 66,000 km² (Fig. 1A). The main island is Isla Grande, with 48,000 km² representing 70% of the surface of the archipelago, from which 21,263 km² belongs to Argentina (Frangi et al., 2004). The vegetation of the island is mainly formed by a Patagonian steppe of grasslands and shrubs located at the northern part of the island, and humid deciduous and evergreen forests plus peat bogs located in the center and southern portion of the island. The trees of the genus *Nothofagus* dominate the forest composition in Tierra del Fuego (Fig. 1B, C) and constitute the most austral forest in the world as part of the Magellanic Subpolar forest sub-ecoregion. The highest rainfall is recorded in the south of the island, decreasing to the east and center of it. The average annual temperature on the island is 5.5 °C to the north and 5.9 °C to the south. Above the mountains, the temperature decreases with elevation; these gradients determine temperatures below zero in the winter months (Frangi et al., 2004).

Fieldwork and specimen collections

Fieldwork was carried out in the Magellanic Subpolar Forests of Tierra del Fuego National Park (DRPA 146/2019) and other non-preserved areas in Isla Grande of Tierra del Fuego, Argentina, during December 2018 - January 2019. In the Valdivian Temperate Forests, fieldwork was done

within Los Alerces National Park and Puerto Blest in Nahuel Huapi National Park (DRPA 1674, DFyFS1/19) during January 2020. We qualitatively searched for land snails along transects for half an hour in each collecting site. Searching was mainly focused on microhabitats that seem to be most favorable for snails, such as between exposed roots of trees, under the bark of trees, under rocks, or under tree trunks lying on the forest floor in contact with soil. In each collecting site, we recorded altitude and geographic coordinates. We also took samples of 50x50 cm quadrats of leaf litter plus 2 cm of topsoil from moist microhabitats. Samples were placed in plastic bags and posteriorly sieved through three decreasing mesh widths (3, 1.5, and 0.5 mm) in the laboratory of the Centro Austral de Investigaciones Cientificas (CADIC-CONICET, Tierra del Fuego) under a stereoscopic microscope. All snails collected were photographed alive before relaxing them in water for 24 hours, posteriorly fixed in ethanol 96%, and preserved in ethanol 70% for anatomical studies. Several specimens were fixed directly in alcohol without relaxation in water for future molecular studies. All the material collected was deposited in the Malacological Collection of the Instituto de Biodiversidad Neotropical (IBN, CONICET-UNT, Tucumán, Argentina).

Morphology

Ten adult shells were photographed using a Zeiss Stemi 508 with ActionCam and measured using the software ImageJ 1.49 (Schneider, Rasband & Eliceiri, 2012). Shell measurements selected, on dorsal and lateral views, are: major shell diameter (DM), shell minor diameter (Dm), shell height (H), apertural height (Hap), and apertural diameter (Dap). The number of shell whorls was calculated following the Kerney & Cameron (1979) methodology. Photographs and shell measurements were carried out at the CADIC in Tierra del Fuego. For anatomical information, dissections of 7 adult specimens were studied under a Leica MZ6 stereoscope. Illustrations of the dissected organs/systems were carried out with the aid of a camera lucida. The terms proximal and distal refer to the position of an organ or part of an organ in relation to the gamete flow from ovotestis (proximal) to genital pore (distal) as in previous works (Cuezzo et al., 2018). The distinction of the limits between the epiphallus and penis is based on the internal sculpture of their inner wall. Shell ultrastructure was studied and described using a SEM Zeiss Supra 55VP at the Integral Center of Electron Microscopy (CIME) of the National University of Tucumán, Argentina (UNT).

Occurrence records

We compiled a total of 60 geographic records of *Stephadiscus lyratus* from field surveys, museum collections, and scientific articles (Fig. 1, Table S1). Twenty new reliable records as part of the 60 were obtained through fieldwork in Tierra del Fuego. To avoid primary taxonomic sources of error from specimens deposited at different Museums, we corroborated the taxonomic identification of specimens according to their shell morphology. The malacological collections of IBN (Instituto de Biodiversidad Neotropical, Tucumán, Argentina), MACN-In (Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina), and MLP (Museo de La Plata, Buenos Aires, Argentina) were accordingly revised. Other sources of information were electronic databases from the Smithsonian National Museum of Natural History (NMNH), Museum of Comparative Zoology (MCZ), and Academy of Natural Sciences in Philadelphia (ANSP). We used Google Earth to georeferenced localities of occurrences that lacked geographic coordinates. From the total number of records, we removed duplicate records, which left a total of 37 unique sites with trustable geographic information. To reduce spatial autocorrelation between presence points, and to avoid over-representation of certain environmental combinations, we spatially filtered records based on a 5 km radius, which left 24 localities.

Estimations of potential geographic distributions (EGDs)

Estimates of the geographical distribution (EGD) of *S. lyratus* were obtained through correlative ecological niche modeling (ENM). To adequately model the species niche, we used the theoretical Biotic-Abiotic-Mobility framework (Soberón & Peterson, 2005). Only abiotic and mobility factors were taken into account because the biotic components (i.e., biotic interactions) are virtually impossible to spatially quantify thoroughly at regional scales (Peterson et al., 2011). As for the mobility component, we designed a calibration area a priori “M” (Barve et al., 2011) considering sub-ecoregions with known species points of occurrence, i.e., in the MSF and the southern portion of Patagonian steppe. To delineate abiotic components, we used 15 bioclimatic variables of the WorldClim database (Hijmans et al., 2005) at a spatial resolution of 30 arc seconds (~1 km²), excluding the four variables that combine temperature and precipitation owing to be known artifacts (Escobar et al., 2014). We clipped the environmental data layers to the

calibration area defined. To eliminate one variable per pair of highly correlated variables ($r \geq 0.85$), we performed a correlation analysis through the "ntbox" package (Osorio-Olvera et al., 2020). Thus, seven non correlated bioclimatic variables were selected for the analyses: BIO1 = Annual Mean Temperature, BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp)), BIO3 = Isothermality (BIO2/BIO7) (* 100), BIO4 = Temperature Seasonality (standard deviation *100), BIO6 = Min Temperature of Coldest Month, BIO12 = Annual Precipitation, and BIO15 = Precipitation Seasonality (Coefficient of Variation).

The best ENMs were calibrated and selected using a maximum entropy method with Maxent v3.3.3K (Phillips, Anderson & Schapire, 2006) through the R package "kuenm" (Cobos et al., 2019). Then, candidate models were created by combining 4 values of regularization multiplier (0.1, 0.5, 1, 2), and all possible combinations of 3 feature classes (linear = l, quadratic = q, product = p). We evaluated candidate model performance based on significance (partial ROC, with 100 iterations and 50 percent of data for bootstrapping), omission rates ($E = 5\%$), and model complexity (AICc). Best models were selected according to the following criteria: (1) significant models with (2) omission rates $\leq 5\%$. From among this set, models with delta AICc values of ≤ 2 were chosen as final models. The final model was created using the full set of occurrences and the selected parameterizations. We produced 100 iterations with five replicates by bootstrap, with logistic outputs. We ran the models with no extrapolation or clamping to avoid artificial projections based on extreme values of the bioclimatic variables (Owens et al., 2013; Guevara et al., 2018). Then, the final model was transferred to a region "G" consisting of the calibration area plus the VTF and Patagonian steppe regions, where we hypothesize that the species could be present. To identify extrapolation risk areas in model transfers, we performed MOP analyses (Owens et al., 2013). This function calculates multivariate environmental distances between sites across the transfer region (G) and the nearest portion of the calibration region to identify areas that have a condition of strict or combinational extrapolation. Finally, to obtain binary presence-absence maps, we used a minimum presence threshold, modified to consider presence data errors (Peterson, Papeş & Eaton, 2007). This modified threshold included 100% of the presence points minus the dataset error (E) (Phillips & Dudík, 2008); we assumed $E = 5\%$ based on our experience of obtaining the presence data. This conservative method minimizes the commission error rate.

Spatial analysis

To spatially characterize the potential distribution of *S. lyratus*, we quantified the proportion of its extent in the different categories of the land cover of the Globcover 2009 dataset (Arino et al., 2012). The 22 land cover categories (e.g., Closed to open (>15%) broadleaved evergreen or semi-deciduous forest (> 5m), Mosaic forest or shrubland (50-70%) / grassland (20-50%)) are according to the UN Land Cover Classification System (LCCS) (Di Gregorio, 2005). To explore the degree of protection of *S. lyratus*' EGD, we quantified the proportion of its range within protected areas. The protected areas categories I to VI assigned by the International Union for Conservation of Nature (IUCN) (Dudley, 2008) and the National Parks were taken into account, even though these are not included in any IUCN category. Shapefiles of the protected areas were obtained from the World Database of Protected Areas (IUCN & UNEP-WCMC, 2020) and <http://mapas.parquesnacionales.gob.ar/>.

Results

Systematic account

Superfamily Punctoidea Morse, 1864

Family Charopidae Hutton, 1884

Subfamily Charopinae Hutton, 1884

Stephadiscus Hylton Scott, 1981

Type species: Helix lyratus Couthouy "in" Gould, 1846, by original designation.

Species description (Figs 2, 3, 4)

Stephadiscus lyratus (Couthouy "in" Gould, 1846)

Helix lyrata Couthouy "in" Gould, 1846: 167; 1852: 39.

Patula rigophila Mabilie, 1886: 123.

Amphidoxa lirata Pilsbry, 1894: 41.

Amphidoxa (Stephanoda) lyrata Smith, 1905: 339

Stephanoda lyrata Pilsbry, 1911, 518; Hylton Scott, 1972: 67.

Stephadiscus lyratus Hylton Scott, 1981: 124; Miquel and Araya, 2013: 230.

Syntype: MCZ 88297 MCZ: Museum of Comparative Zoology, Harvard University
Type locality: Orange Harbor, Tierra del Fuego [according to Johnson (1964): located at the west side of Nassau Bay].

Morphology (Figs 2-4)

External body morphology (Fig. 2A-B)

Animal black with lighter foot and mantle collar around shell aperture. A deep longitudinal furrow, the pedal groove, runs parallel to the foot edge on each side and above it. Dark irregular spots are seen for transparency through the shell, although some specimens are lighter. Foot short, triangular pointed, not to slightly surpassing the shell diameter.

Shell (Figs. 2 C-E; 3A-E): Discoidal, 3 ½ to 4 convex regularly expanded whorls, low spire depressed, not planispiral as apex elevated, fragile (DM=4,247-5,041mm; Dm=3,444 - 4,487mm; H= 2,283-2,432mm) with brown caramel to light whitish color (Figs. 2 A-E).

Protoconch with 35 - 40 axial delicate, smooth, elevated ribs separated at regular intervals (Fig. 3A). Spaces between protoconch ribs with thinner axial costulae. Protoconch not clearly delimited from the teleoconch, and similarly sculptured. Teleoconch surface with major axial ribs (Figs. 3B-D), interspace between them of 4-7 µm filled with 5 to 7 micro radial costulas in between major ribs, nodules at regular intervals supporting ribs, giving the appearance of radial cords (Figs. 3B-C). Deep irregular suture (Fig. 3D). Circular aperture, not descendent, with sharp peristome (Hap=1,861-2,110; Dap= 1,809-1,998). Umbilicus 1/3 or slightly less of body whorl major diameter, with same sculpture as nepionic portion of the shell (Fig. 3E).

Pallial system (Fig. 4A): Pulmonary roof dark with black spots and whitish small granules over the surface. Spots and granules visible through shell. Pulmonary sac short occupying 1/4 of body whorl. Kidney triangular, bilobated, with pericardial side lobe overlapping pericardium. Rectal side kidney lobe bigger than pericardial arm. Principal pulmonary vein short, slender, not branched, smoothly marked. Remaining pulmonary roof smooth with no other veins. Secondary ureter present, close along its length, parallel to rectum. Pallial gland absent.

Jaw and radula: Jaw consist on narrow vertical plates, slightly arched, cream colored. Radula as described by Hylton Scott (1970).

Reproductive system (Figs. 4B-E): Albumen gland shapeless, roughly globular, rounded margins. Sperмовiduct formed by prostate and uterus fused together. Uterus divided in two portions,

proximal short, cylindrical, continuous to albumen gland, distal expanded in rounded glandular chamber (Fig. 4B, C). Free oviduct cylindrical, short. Bursa copulatrix sac round, resting over uterus distal portion. Duct of bursa copulatrix basally thickened, progressively decreasing in diameter towards the sac (Fig. 4D). Vagina as long as bursa copulatrix duct, distally widened, featuring three thick, longitudinal pilasters on the interior wall. Vas deferens cylindrical, narrow in diameter, short, running from basal prostate towards peni-oviducal angle, inserting into epiphallus. Penial retractor thin, inserted in penis. Penis cylindrical, thicker than epiphallus, with a sac-like appendix in upper portion (Fig. 4C-E). Short verge in upper penial chamber, inner penial wall with short pilaster noticeable towards middle penis length. Epiphallus reflected over penis, shorter, thinner ending in a short finger-like flagellum (Fig. 4E). Vas deference dilated before inserting into epiphallus anteriorly of penial retractor insertion, dilatation as long as flagellum, giving appearance of fork ending epiphallus.

Microhabitat characterization

Rainforests provide a variety of living spaces where snails can feed, crawl, and live. Although micro snails are usually associated with leaf litter, *Stephadiscus lyratus* was mainly found living on or under the bark of fallen trees or under humid logs in contact with the ground (Fig. 1B, C). We found actively crawling snails only on tree barks or on moss logs. This species is not considered to be arboreal or semi-arboreal. Specimens were not found on leaves of trees or shrubs but are always associated with tree logs, not leaf litter. During the hibernation period in wintertime, these microhabitats in contact with soil can act as a buffer and help the species to survive during extreme freezing conditions. Dry shells were recovered from soil samples, but in general, they were worn out. Feeding habits are unknown. Species of the genus *Radiodiscus* occur in sympatry with *S. lyratus*. In places outside protected areas where the forest shows some degree of alteration, living snails were scarce or difficult to find.

Species Remarks

Helix lyrata was discovered by Joseph Pitty Couthouy, the named conchologist of the "United States Exploring Expedition" to the South Pacific and Antarctic seas between 1838 and 1842, commanded by Charles Wilkes. The Expedition made base in Tierra del Fuego during 1838 and 1839, while Wilkes and part of the crew travel to Antarctica. Couthouy made careful and

suggestive notes of all the interesting species and especially of the new ones, intending to amplify them upon his return (Johnson, 1964). Wilkes had antagonized with Couthouy during the trip when he refused to give Wilkes his notes and graphics from the trip. For this reason, Wilkes suspended him for "disobedience of orders" and order him home (Johnson, 1964). Gould (1852) said that "Cothouy's numerous notes were extremely valuable, especially those relating to the land snails...". Captain Couthouy had drawn up full notes on the external characters of the soft parts, habits, geographical distribution, and other essential points (Wyman, 1903). The collection of the Expedition upon arrival was sent initially to Peale's Museum in Philadelphia, later removed and sent to Washington, wherein 1856 was deposited at the Smithsonian Institution. When Couthouy returned to USA in 1840, he found that part of the material was lost or misplaced. Some of the collected specimens had been sent to prominent conchologists, as a favor, before any naturalist of the Expedition could work with them. Augustus Addison Gould was hired to study and publish all about the shells collected during the Expedition. He requested that the specimens were sent to Boston. Gould recognized Couthouy's work as a collector, as well as his notes describing species such as *Helix lyrata*. Then, the species description appeared in Gould's work on the shells of the Expedition (Gould, 1845-1848). Some of the shells collected during the Exploring Expedition are housed at the Museum of Comparative Zoology, Boston, while others are at the Smithsonian Institution. Duplicates and paratypes were exchanged with other Museums.

Comparison with species of the same genus: *S. lyratus* has the largest shell diameter (DM= 5.5 mm) among all species classified in *Stephadiscus*. It is a very conspicuous species regarding its shell coloration and sculpture. Although *S. perversus* is similar in shell coloration, it cannot be confused with *S. lyratus* because it is sinistrorse and has a smaller shell (DM=2, 8; H= 1.5mm). *S. lyratus* is also similar in shell sculpture and general shape to *S. stuardoi* (DM= 2.0-2.02, H= 0.85), but clearly differs from this species in its larger shell diameter and the absence of weak spiral threads in the teleoconch. In the original description, Couthouy in Gould mentioned that *S. lyratus* could be a synonym of *Helix costellata* d'Orbigny, 1835 (now *Zilchogyra costellata* (d'Orbigny, 1835)). However, *H. costellata* is distributed in the Humid Pampa ecoregion in Buenos Aires, Argentina, an extra Patagonian area with completely different ecological

requirements. *H. costellata* (DM=4 mm, H= 2 mm) is smaller in shell diameter and height than *S. lyratus* and its protoconch is a smooth surface.

Stephadiscus lyrata and *S. mirabilis* are the only species of the genus with known anatomies. Both show a rounded glandular mass, identified as a "dilated sac" by Hylton Scott (1970) at the base of the spermiduct, in the distal genitalia. A similar structure is also present in *Stephacharopa testalba* (Hylton Scott, 1970). Most of the described species of the genus have been originally established only on single or two dry shells (Table S2), and after their discovery, rarely fresh specimens have been collected in their area of distribution.

Comparison with related genera: *Stephadiscus* is defined by having plane-convex whorls, presence of a protoconch, and teleoconch with similar ornamentation consisting of thin, nodulose ribs, without a marked limit between the protoconch and the teleoconch (Hylton Scott, 1981; Miquel & Araya, 2013). Therefore, the transition of protoconch towards teleoconch is barely distinguished. These ribs increase in height towards the body whorl, with thinner costula in the interspaces. *Stephadiscus* is different from *Stephacharopa* Miquel & Araya, 2013 because in the latter genus, the sculptured protoconch is dissimilar to the teleoconch ornamentation while in the former, the sculptured protoconch is similar to the one present in the teleoconch. *Stephadiscus* differs from *Stephanoda* mainly in the sculpture of the protoconch, since the latter possesses a reticulated pattern. Differences in anatomy are not possible to be established for the lack of studies on these genera. Other South American charopid genera, such as *Lilloiconcha* Weyrauch, 1965 and *Zylchogyra* Weyrauch, 1965 can reach similar shell sizes but differ from *Stephadiscus* in general shell shape and in that their protoconch is smooth (Miquel & Araya, 2013). *S. lyratus* shows a specialized vas deferens-epiphallus junction as other Charopinae from the Pacific Islands described by Solem (1982), marking a difference with subfamilies Semperdoninae, Trukcharopinae, and Rotadiscinae.

Estimations of potential geographic distributions (EGDs)

We obtained 24 candidate models statistically significantly better than null expectations (i.e., predictions from the models coincided with testing occurrence data more frequently than would be expected by random association of points and a prediction of that areal extent). From these, only one final model was selected that was statistically significant and met the AICc criteria (two

of three selection criteria). The chosen settings were linear, quadratic, and product features and 0.1 of regularization multiplier. The bioclimatic variables that most contribute to the model were BIO3 = Isothermality (BIO2/BIO7) ($\times 100$), BIO12 = Annual Precipitation, BIO6 = Minimum Temperature of Coldest Month, and BIO4 = Temperature Seasonality (standard deviation of mean month temperature $\times 100$) (variables with percent of contribution higher than 10%).

Spatial analysis

The known area of distribution of *S. lyratus* was approximately 72,672 km², mainly coincident in the southern portion of the MSF (below -51° of latitude), at both sides of the Andes and marginally in the southern part of Patagonian steppe in Tierra del Fuego (Fig. 5 A). The MOP analysis (Fig. S1) indicated that areas with the most dissimilar variables conditions (i.e., where one or more environmental variables are outside the range present in the training data) were found beyond the potential distributional areas predicted by models in the “G” area. When the final model is transferred to region “G”, we found that the potential area of distribution almost duplicates their original range (140,454 Km²). This new region extends mainly to the VTF between -40 and -46 latitude, mostly in Chile and a small portion of the Argentina, while towards the Patagonian steppe increase marginally (Fig. 5 A-C). Natural and semi-natural terrestrial vegetation was predominant in the potential area of distribution of *S. lyratus*, with 48% of overlap with woody trees (closed to open ($>15\%$) broadleaved evergreen or semi-deciduous forest ($>5m$)), followed by 21% of overlap with shrub (closed to open ($>15\%$) (broadleaved or needle-leaved, evergreen or deciduous shrubland) (Table 1).

The proportion of the potential distribution area in protected areas was 14.7%, occurring in 16 protected areas, from which six of them are located in Argentina while the remaining are from Chile. The higher proportional area protected is due to National Parks “Alberto D'Agostini” and “Yendegaia” (Chile), and Multiple Use Provincial Reserve “Corazon de la Isla Tierra del Fuego (Argentina). Taking into account the category of management of IUCN, nine are National Parks and a Wilderness Nature Reserve, which IUCN category are II, Ib respectively; the six remaining are Forest Reserve and Multiple Use Provincial Reserve which IUCN category are IV and VI respectively (Table 2).

Discussion

Taxonomy and Species Morphology

Features of the gastropod shell have always been an essential and convenient source of taxonomic information. However, it is well recognized that shell characters such as shape, coiling patterns, and ribbing can be convergent and often mask crucial differences in anatomy (Barker, 2001). Hylton Scott, understanding the value of anatomical information, was the only researcher to provide anatomical descriptions of some South American charopids such as *Stephacharopa testalba*, *Stephadiscus lyratus*, *S. mirabilis*, *Zilchogyra leptotera* (Mabille, 1886). Recently, a study on Punctoidea phylogeny provided molecular information on some charopid species (Salvador et al., 2020). *S. lyratus*, as the type species of *Stephadiscus*, has similar ornamentation in the shell protoconch as well as in the teleoconch, without a marked limit between them. The type of ornamentation and similitude between protoconch and teleoconch differences *Stephadiscus* from all other South American charopid genera (Miquel & Barker, 2009; Miquel & Araya, 2013). Anatomical information provided proves to be an essential source of characters relevant for future taxonomic and phylogenetic studies. The presence of a be-lobed kidney with the rectal side lobe bigger than the pericardial portion, plus the presence of a close secondary ureter, clearly indicate that *S. lyratus* belongs to Charopidae, differencing it from the Endodontidae. Solem (1982) raised these characters as the main differences between both families. The terminal portion of the uterus (spermoviduct), forming a compact glandular mass, is also a striking character that has only been mentioned before for *Stephacharopa testalba*. Along with this, the insertion of the vas deferens into the epiphallus through a dilatation constitutes unique structures of *S. lyratus*.

Estimation of the potential geographical distribution (EGD) and spatial analysis.

The new records obtained were associated with native areas of the Magellanic Subpolar Forest sub-ecoregion in Argentina. The transferred model shows a potential distribution of *S. lyratus* to the Valdivian Temperate Forest, spreading the known area to a new sub-ecoregion, corresponding mainly to the area in Chile. Although in previous campaigns, no specimens of *S. lyratus* have been found in the Valdivian forest areas in Argentina (Nahuel Huapi and Los Alerces National Parks), these surveyed areas are outside to the east of the potential area predicted by the model. Thus, the potential area of distribution of *S. lyratus* is coincident with the MSF and the VTF (more towards Chile) together with its boundaries with the Patagonian Steppe

ecoregion. The obtained hypothesis of the potential distribution of *S. lyratus* will direct the next exploratory surveys with the expectation to find new populations in the future.

The biogeographic Valdivian Forest province according to Kuschel (1960) and Morrone (2018) has faunistic relationships with Magellanic Forests, probably due to the fact that both regions have been remained isolated from other South American forests since the Neogene (Axelrod, Kalin Arroyo & Raven, 1991). In addition, the cooling cycles followed by warmer periods in the Quaternary caused the contractions and expansions of the temperate forests (Villagrán & Hinojosa, 1997), and some areas in the coastal range remained free of ices and may have been the source for the recovery of the forest biota (Smith, 2017). From the seven environmental variables used in the EGD analysis of *S. lyratus*, the main constraining variables are the temperature (Isothermality, Minimum Temperature of Coldest Month, and Seasonality) and Annual Precipitation. Thus, temperature and precipitation were the most important abiotic factors to explain *S. lyratus* occurrence. Other studies using native snails in South America that analyzed the role of abiotic factors in relation to their distribution are scarce for macro snail species, e.g., the case of *Megalobulimus sanctipauli* (Ihering & Pilsbry, 1900), but are virtually nonexistent for micro snails. *Megalobulimus sanctipauli*, known to inhabit the Atlantic Forest, showed temperature and rainfall as the determinant factors of their geographic distribution (Beltramino et al., 2015).

Stephadiscus lyratus, a vulnerable species to protect

Stephadiscus lyratus inhabit mostly woody and shrubland areas of native forests. The preferred microhabitats of the species are sites on or under the bark of fallen trees or under humid logs in contact with the soil. These microhabitats are less frequent in disturbed forest areas with high human pressure located in Tierra del Fuego outside the National Park, where specimens were difficult to find alive.

Land gastropods are suffering habitat loss and competition from introduced species (more numerous and prolific every year), although are regarded as non-charismatic groups for conservation purposes. In the case of Orthalicoidean land snail in Argentina, only 3% of their average species distribution ranges are safeguarded within current protected areas, showing that the existing protected areas system is not effective at all for the protection of this invertebrate group (Ovando et al., 2019). In the present study, we found that 14,5 % from a total current

distributional area of 72,672 km² of *S. lyratus* is inside the system of protected areas. However, it is important to emphasize the threats and pressures of land-use change such as tourism, logging and frequent fires that compromise not only areas inside the southern National Parks but also the matrix that surrounds them. Intensive harvesting over the native forest started in the middle of the XX Century in southern Patagonia with the increase of the human population (Gea-Izquierdo et al., 2004). In fact, 700–1000 ha are logged each year (from 1980 to 2003) in Argentina, Tierra del Fuego, which the main consumer has been the sawmill industry (Gea-Izquierdo et al., 2004). In addition, extensive livestock holdings, aquaculture, and tourism are advancing into formerly undisturbed areas. In the Chilean Magellanic forests between 53.1 % and 68.1 % of this land is influenced by human activity in some way (Inostroza, Zasada & König, 2016). In this context, habitat loss for land gastropod conservation is very worrying. Moreover, if we consider that the protected areas in Argentina were created for the protection of plants (Ortega-Baes et al., 2012) and/or vertebrates (Arzamendia & Giraudo, 2004; Tognelli et al., 2011), ignoring the invertebrates even when their importance has been proven (Chehébar et al., 2013). The under evaluation of invertebrates is also seen in the IUCN Red List, although they constitute 97 % of all animals on the planet (Karam-Gemael et al., 2020). Cardoso et al. (2011) sustained that the IUCN Red List has a taxonomic tendency to exclude species with small body sizes, narrow distribution ranges and low dispersal abilities, which constitute the vast majority of the planet's biota, particularly local invertebrate endemic species. This situation is a worldwide problem since there are more than a million invertebrate described species but only 3,500 are protected (Brooks et al., 2006; Nieto et al., 2017).

Empirical data on the life history of small land snail species in their natural habitat are hardly available worldwide, and most studies are carried out on macro snails under laboratory conditions. There are also very few data on gastropod cold hardiness, especially on land snail species of minute sizes. Ansart & Vernon (2003) sustained that two alternatives exist for organisms living in areas that will freeze in winter, such as the southern forest in Patagonia: move to an unfrozen habitat or face freezing conditions. For this last category, avoid freezing by extensive supercooling (freezing avoidance) or survive freezing of the body fluids (freezing tolerance) are the only possibilities. Supercooling point refers to the temperature at which spontaneous freezing of body water occurs during the gradual cooling of the snail at a constant cooling rate (Ansart & Vernon, 2003; Ansart et al., 2010). On the other hand freezing avoidance,

involves the choice of a hibernation site, which buffers the temperature differences, and which permits avoidance of inoculative freezing (e.g., by contact with ice). During hibernation, the snails rapidly suppress their metabolism and minimize water loss using a discontinuous gas-exchange pattern (Košťál et al., 2013). Most land snails find favorable overwintering microhabitat in soil or under stones in contact with soil, which is well buffered from temperature and moisture fluctuations allowing them to survive during winter freezing or extreme dryness periods. The combination of the ectothermic physiological traits of this species, low dispersal capacity, probable low fecundity producing only few eggs, and its narrow habitat requirements (forest specialist) turns *S. lyratus* into a vulnerable species, not only because of the land-use change but also for the climate change we are suffering. Along with habitat loss through human land use, climate change is a major contributor to biodiversity loss in the 21st century. However, terrestrial gastropods have so far received little attention regarding the potential impact of current rapid climate change on their distribution, compared with other taxa (Lee et al., 2015). The climate is changing rapidly, and terrestrial ectotherms are expected to be particularly vulnerable to an increase in extreme weather events in temperate regions (Nicolai & Ansart, 2017). Projected global warming is likely to reach 1.5 ° C between 2030 and 2052 (IPCC, 2018), also including rises in the frequency and duration of heatwaves and the occurrence of extreme precipitation events. Also, they will be affected seasonality by more frequent hot temperature extremes and fewer cold temperature extremes over most land areas (IPCC, 2014). Temperatures follow the same trends in southern South America (Christensen et al., 2007). Meanwhile, the projected precipitation changes show reductions for the dry area in the central-western region and for the whole of Patagonia (Barros et al., 2015). A reduction in the available water from rivers in northern Patagonia potentially would influence both irrigation for regional crops (vegetables and fruits) (Barros et al., 2015) and native forests. Thus, the quality and availability of habitat for *S. lyratus* could be compromised by the effect of both land uses changes and global warming. In this context, these regional changes in climate and land use put *S. lyratus* populations at serious risk of extinction that must be taken into consideration for future conservation actions.

Conclusions

1. Here we confirm that the shell ultrastructure of *S. lyratus* has a protoconch and teleoconch with similar ornamentation, not showing a marked limit between them. We provide new anatomical information highlighting the presence of a be-lobed kidney, a close secondary ureter, and the terminal portion of the uterus (spermoviduct) forming a compact glandular mass, the vas deferens inserting into the epiphallus through a dilatation as the most notable anatomical characters of *S. lyratus*.
2. The potential distribution obtained shows that *S. lyratus* can be found beyond the Magellanic Subpolar Forests into the Valdivian Temperate Forest, incrementing its known distribution area to a new sub-ecoregion, mainly within Chile. Thus, our initial hypothesis that *S. lyratus* is endemic of the Magellanic Subpolar forest is not supported.
3. From seven environmental abiotic variables used, the main constraining ones to explain *S. lyratus* occurrence in the EGD are the temperature (Isothermality, Minimum Temperature of Coldest Month, and Seasonality) and Annual Precipitation.
4. *S. lyratus* inhabits cold native forest areas where it is found mainly on or under the bark of fallen trees or under damp trunks in contact with the ground. This microhabitat allows them overwintering, buffered from temperature and moisture fluctuations, and survive during winter, probably as a strategy to avoid freezing.
5. The combination of the ectothermic physiological traits of this species, low dispersal capacity, and its narrow habitat requirements (forest specialist) turns *S. lyratus* into a vulnerable species, not only because of current land-use change but also to climate change.

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Figure 1

Study area.

(A) Pink circles are new records obtained for the present study and blue circles are historic records from Museums. (B) Native *Nothofagus* forest in Tierra del Fuego National Park. (C) Forest in the south eastern part of Tierra del Fuego in Moat. Photo credit: MG Cuezco.

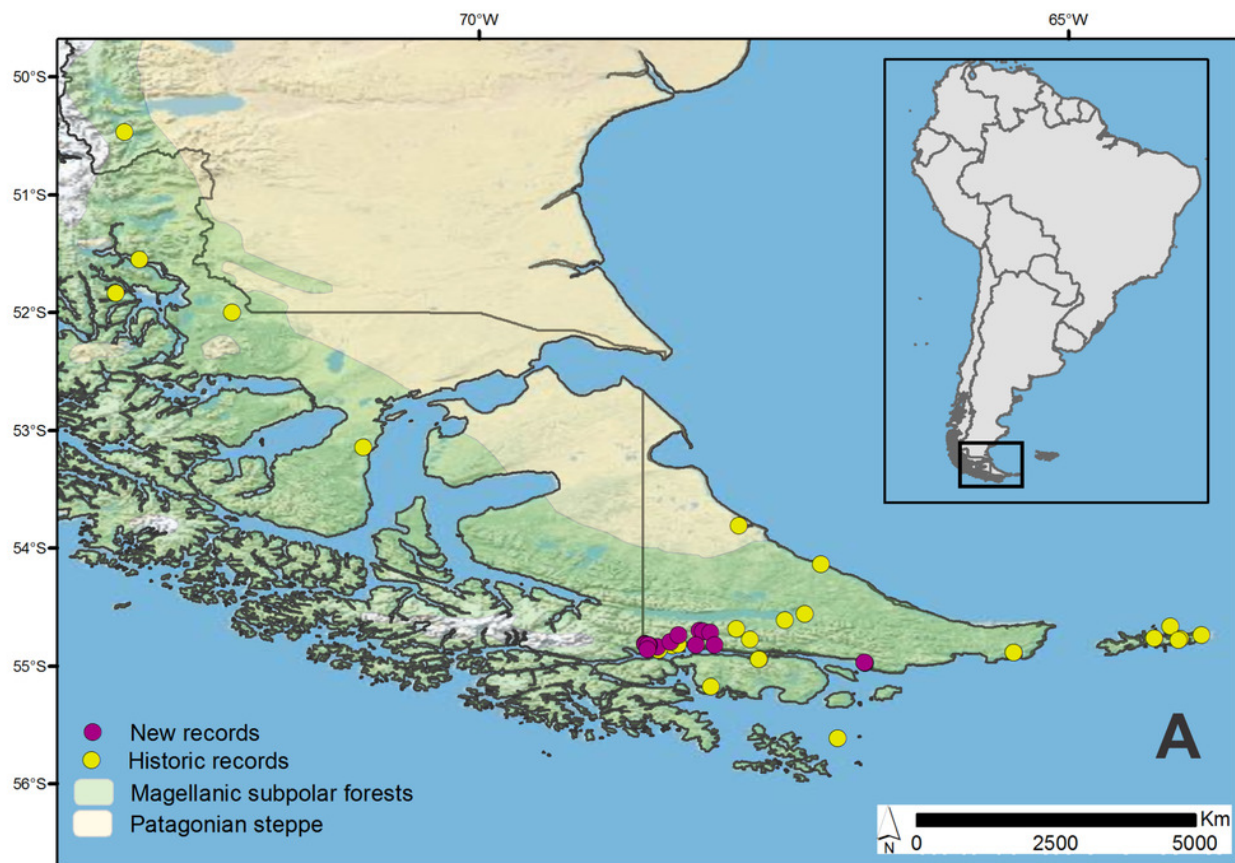


Figure 2

External morphology of *Stephadiscus lyratus* shell and live animal.

(A) (B) Live animal from Tierra del Fuego National Park showing natural shell coloration and by transparency, the irregular spots of the lung. Note black animal body with lighter basal foot and mantle collar. (C) Dorsal, (D) ventral and (E) lateral views of shell, scale bar= 1mm (IBN 951). Photo credit: MG Cuezco.

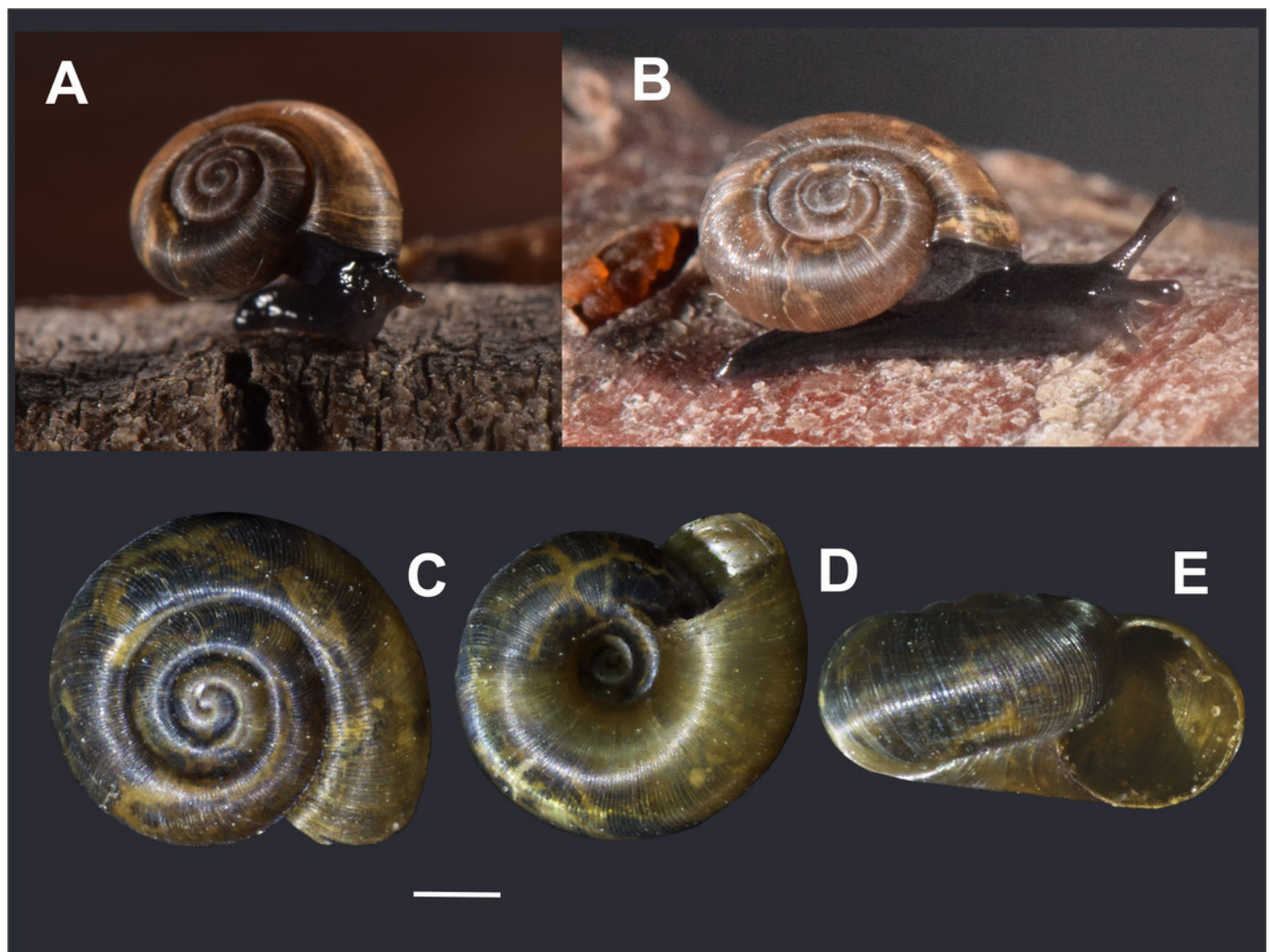


Figure 3

Shell ultrastructure of *Stephadiscus lyratus*.

(A) Ultrastructure of protoconch showing the radial disposition of major ribs, scale bar= 100 μm . (B) (C) details of the body whorl sculpture with major ribs separated at regular spaces, and micro radial ribs. Note nodules supporting ribs, scale bar= 10 μm . (D) Deep suture between body whorls and penultimate whorl, scale bar= 10 μm . (E) Shell umbilicus with sculpture, scale bar=20 μm . Photo credit: MG Cuezco.

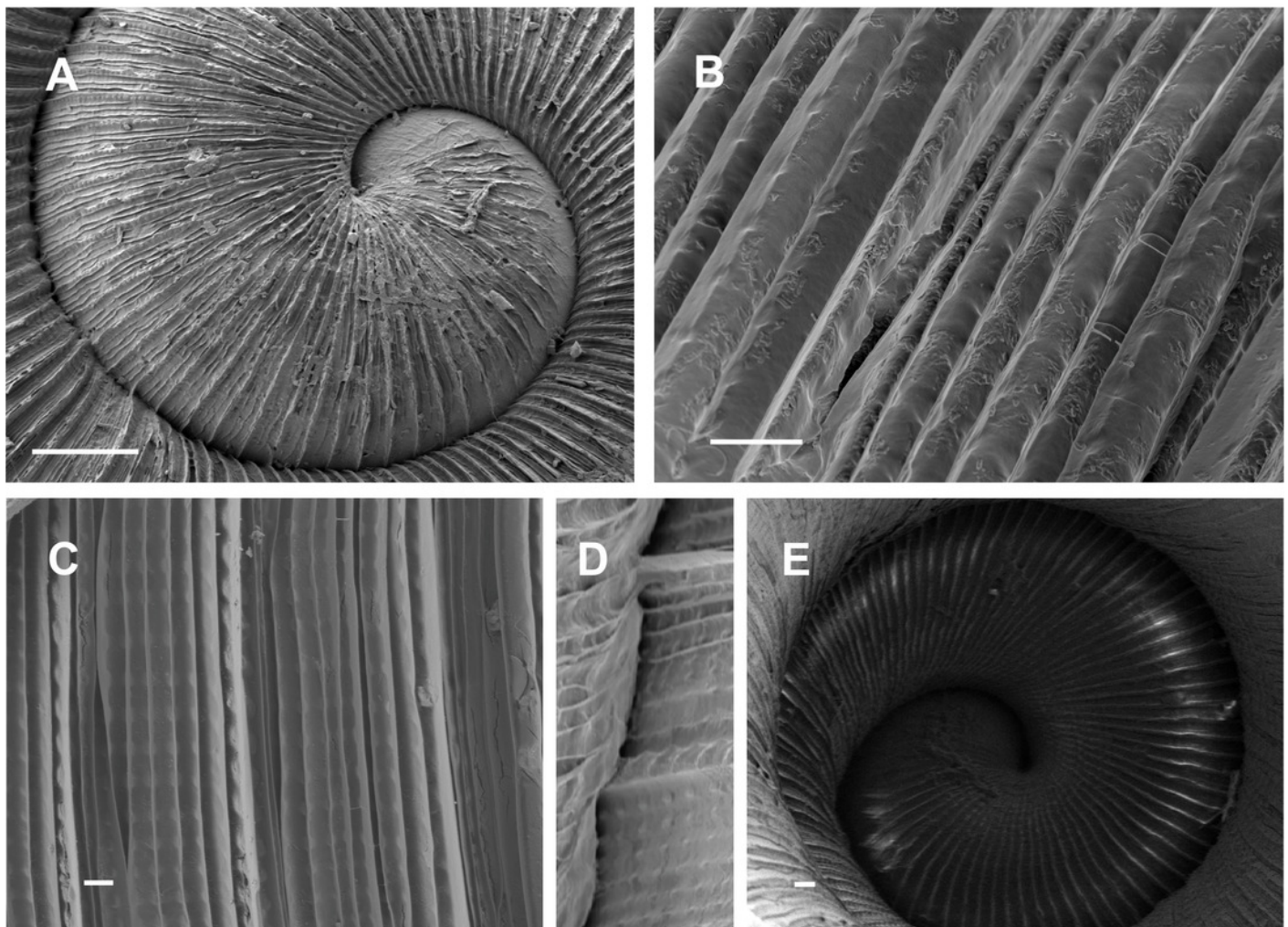


Figure 4

Morphology of pallial and reproductive systems of *Stephadiscus lyratus*.

(A) Pallial system with pulmonary and pericardial cavity. Note the beloved kidney overlapping the pericardium. Small whitish concretion are scatter along the pulmonary roof. (B) General shape of reproductive system showing large, rounded albumen gland and spermoviduct divided in two portions, proximal short, cylindrical, continuous to albumen gland, distal expanded in rounded glandular chamber, scale bar= two mm. (C) Detail of the uterus chamber in distal spermoviduct, free oviduct cylindrical, short, scale bar= two mm. (D) Duct of bursa copulatrix with basal widening, sac rounded. (E) Detail of the penial complex showing penis with a sac-like appendix. The epiphallus reflected over penis, is continuous with a short finger-like flagellum. Note that the vas deference is delated before inserting into epiphallus and that the dilatation is as long as the flagellum, giving appearance of fork ending epiphallus, scale bar= two mm. Abbreviations: ag, albumen gland; bc, bursa copulatrix; dbc, bursa copulatrix duct; e, epiphallus; f, flagellum; fo, free oviduct; hd, hermaphroditic duct; kl1, pericardial side kidney lobe; kl2, rectal side kidney lobe; mc, mantle collar; p, penis, pr, penial retractor; r, rectum; s, Spermoviduct; su, secondary ureter; u, uterus; v, vagina; vd, vas deferens.

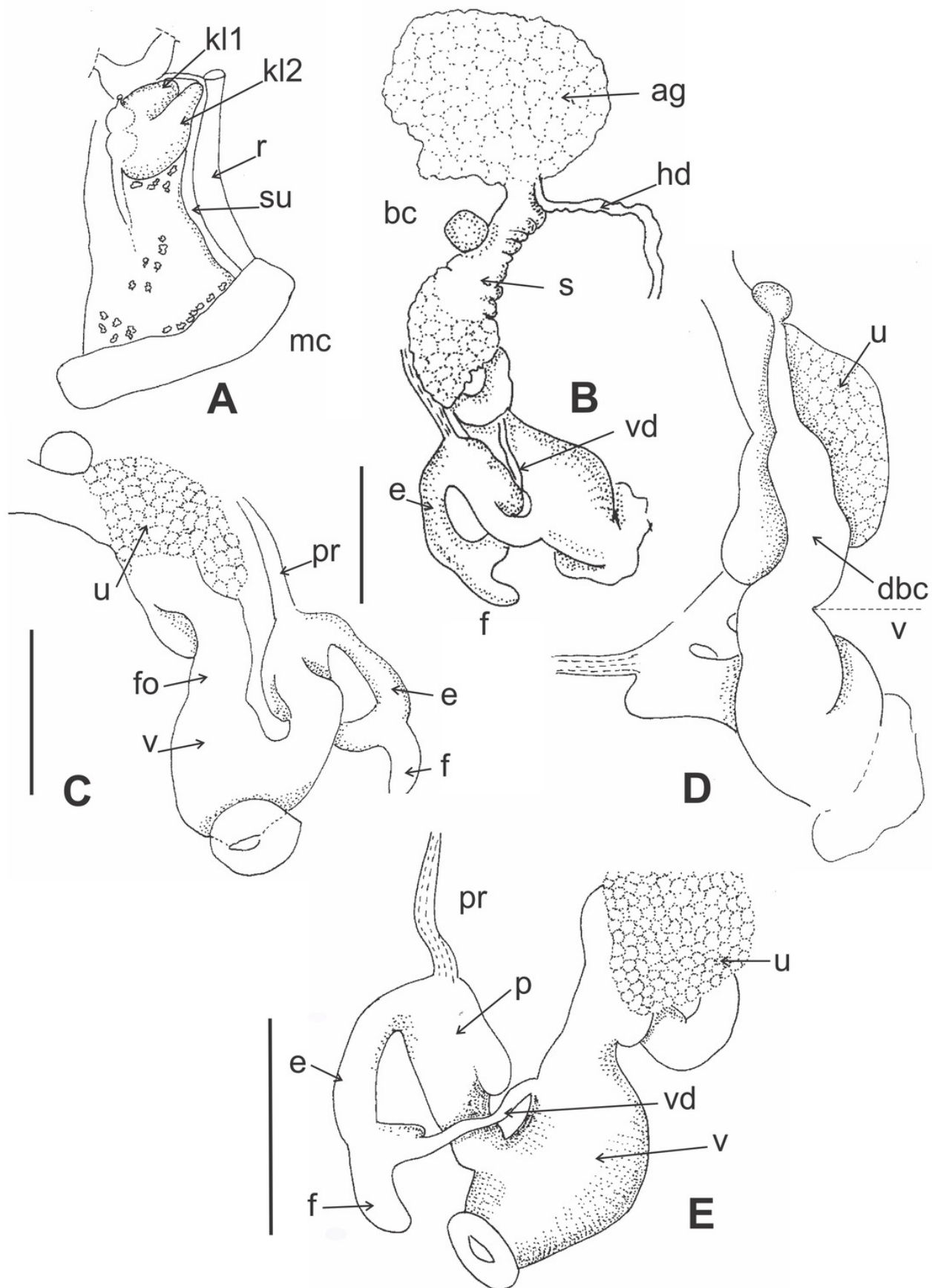


Figure 5

Distribution models of *Stephadiscus lyratus*.

(A) Geographic position of sub-ecoregions considered to the distribution model. (B) Estimation of geographic distribution using the known distribution in Magellanic subpolar forests as a calibration area ("M" region). (C) Projected model to an area that include Valdivian temperate forests and Patagonian steppe ("G" region).

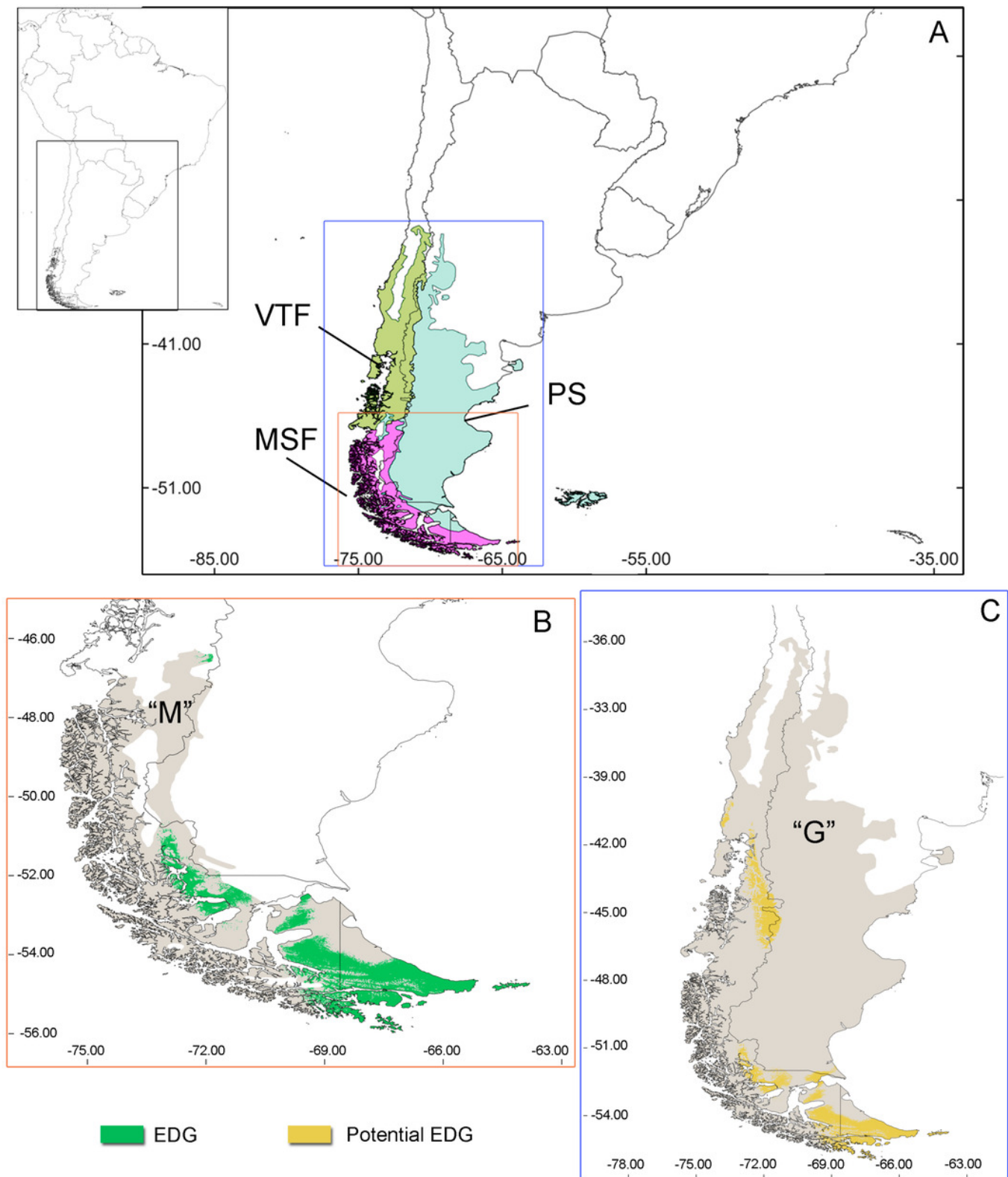


Table 1(on next page)

Percentages of overlap of the distribution model of *S. lyratus* with land cover categories from Globcover 2009 dataset (UCLouvain & ESA Team).

LANDCOVER	OVERLAPPING (%)
Closed to open (>15%) broadleaved evergreen or semi-deciduous forest (>5m)	48.67
Closed to open (>15%) (broadleaved or needleleaved, evergreen or deciduous) shrubland (<5m)	21.04
Closed (>40%) broadleaved deciduous forest (>5m)	5.77
Closed to open (>15%) herbaceous vegetation (grassland, savannas or lichens/mosses)	4.41
Mosaic cropland (50-70%) / vegetation (grassland/shrubland/forest) (20-50%)	2.66
Mosaic vegetation (grassland/shrubland/forest) (50-70%) / cropland (20-50%)	2.02
Rainfed croplands	1.95
Open (15-40%) broadleaved deciduous forest/woodland (>5m)	1.75
Others	11.72

Table 2 (on next page)

Protected areas overlapped with distribution model of *Stephadiscus lyratus*. Names, IUCN categories, country of origin and number of *S. lyratus* EGD pixels within protected areas.

1

Name of protected area	Design Type	IUCN Category	Country	Pixels (n°)
Alberto D'Agostini	National Park	II	Chile	 3065
Bernardo O'Higgins	National Park	II	Chile	 56
Cabo de Hornos	National Park	II	Chile	 694
Corazón de la Isla	Multiple Use Provincial Reserve	VI	Argentina	 1599
Isla de los Estados y Archipiélago de Año Nuevo	Wilderness Nature Reserve	Ib	Argentina	 703
Kawésqar	National Park, National Reserve	II, IV	Chile	 868
Laguna Negra	Multiple Use Provincial Reserve	VI	Argentina	 27
Laguna Parrillar	Forest Reserve	IV	Chile	 9
Los Glaciares	National Park, National reserve and World Heritage Site	II, VI, Not Applicable*	Argentina	 41
Magallanes	Forest Reserve	IV	Chile	 16
Patagonia	National Park	II	Chile	 11
Rio Valdez	Multiple Use Provincial Reserve	VI	Argentina	 57
Seno Almirantazgo	Marine and Coastal Protected Area	IV	Chile	 102
Tierra del Fuego	National Park	II	Argentina	 932
Torres del Paine	National Park	II	Chile	 836
Yendegaia	National Park	II	Chile	 1687

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