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Range extension of the poorly known and Critically Endangered freshwater snail, *Heleobia transitoria* (Biese, 1947) (Gastropoda, Cochliopidae), in the Atacama Desert, northern Chile

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Abstract
The range distribution of *Heleobia transitoria* (Biese, 1947) is restricted to the type locality, Quebrada Cachina, Antofagasta Region, northern Chile. This paper reports the occurrence of this species in the Atacama Region, northern Chile, after the study of morphological characteristics using light and scanning electron microscopy. This new record is important because *H. transitoria* is classified as "En Peligro Crítico" according to Ministry of the Environment of Chile, a category equivalent to Critically Endangered of the International Union for Conservation of Nature Red List of Threatened Species.

Keywords
Endangered species, endemism, minute snails, taxonomy, spring snails.

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Introduction

The family Cochliopidae comprise minute marine, brackish and freshwater snails distributed worldwide, mainly in the New World (Hershler and Thompson 1992). Among several genera, *Heleobia* Stimpson, 1865 is the most specious genus of the family, with more than 100 species, mainly occurring in the New World (Hershler and Thompson 1992; Collado 2015a; Collado et al. 2016a, 2016b; Martin and Díaz 2016). In South America, these snails constitute one of the most representative groups of mollusks in freshwater ecosystems, considering that more than 90 species have been described (Hershler and Thompson 1992; Cazzaniga, 2011; Collado 2015a; Collado et al. 2016a, 2016b). Although in Chile *Heleobia* is represented by about 30 species, it has been reported that there are several candidate species that require formal description (Collado et al. 2013, 2016a, 2016b; Collado 2015a), so this number will possibly increase through future research.

In the Atacama Desert and Chilean Altiplano, ten species and seven subspecies of *Heleobia* have been described mainly using conchological characters: *H. ascotanensis* (Courty, 1907), with five subspecies; *H. atacamensis* (Philippi,1860); *H. carcotensis* Collado, Valladares & Méndez, 2016; *H. chimbaensis* (Biese, 1944), with one subspecies; *H. copiapoensis* (Biese 1944), with one subspecies; *H. deserticola* Collado, 2015; *H. loaensis* (Biese 1947); *H. opachensis* (Biese, 1947); *H. striata* (Biese, 1944); and *H. transitoria* (Biese, 1947).

Heleobia transitoria was originally described from Quebrada Cachina, a ravine located on the Atacama Desert coast, northern Chile. Considering the extreme endemism of the species, the reduced occupancy area, and the decreased habitat quality, in addition to serious threats such as prolonged drought or habitat destruction associated with road works, the species was classified as "En Peligro Crítico" according to Ministry of the Environment of the Republic of Chile (Collado 2015b), a category equivalent to Critically Endangered of the International Union for Conservation of Nature Red List of Threatened Species.

Collado et al. (2011) studied the holotype of *H. transitoria* housed in the Malacology section of the Museo Nacional de Historia Natural, Santiago, Chile (MNHNCL–200617), and examined for the first time the penis of two topotype specimens of the species. Subsequently, Collado et al. (2013) examined the phylogenetic relationship of *Heleobia* populations from the Atacama Desert and Chilean Altiplano based on 12S/16S gene markers, including samples of *H. transitoria* from its type locality and a population from Quebrada El León, treated as undescribed species. In a more inclusive phylogenetic analysis using the cytochrome c oxidase subunit 1 (COI) mitochondrial gene, Collado et al. (in press) included several species of *Heleobia*, including *H. transitoria*. In the present study we compared the morphological characteristics of topotype specimens of *H. transitoria* with specimens from Quebrada El León to assess the taxonomic position of this last population.

Methods

Samples were taken in 2011 from two sites along the Atacama Desert coast, northern Chile: Quebrada Cachina, Antofagasta Region, and Quebrada El León, Atacama Region. The snails from Quebrada Cachina live in small pools of water that emerges on the side of a dirt road (Fig. 1). In Quebrada El León, the water emerges from the base of the rocks in a small ravine, where it accumulates forming a small pool. In both sites the specimens were collected using a sieve of 0.5 mm mesh width, then anesthetized in menthol crystals, and stored in absolute ethanol. The shell was photographed using a Motic SMZ-168 stereomicroscope equipped with a Moticam 2000 digital camera and a millimeter ruler with which the animals were measured. For the morphological study, we performed microdissections using the same equipment. To isolate the soft body, the shell was put between the sheets of a small tweezer and then broken gently pressing on it. The penis, opercula and radulae were isolated from the soft body using a scalpel. The last two structures were washed in a diluted hypochlorite solution (1:3) for 3–10 min to remove organic material attached, and then observed using scanning electron microscopy (SEM) (Jeol JSM–6610LV and Hitachi SU3500). A similar method was used for the protoconchs, which were measured directly using the SEM.

Voucher specimens are deposited at the Laboratorio de Malacología y Sistemática Molecular, Universidad del Bío-Bío, Chillán, Chile (LMSM-UBB), and the Museo de Ciencias Naturales y Arqueología Profesor Pedro Ramírez Fuentes, Chillán, Chile (MCNAPPRF-CC).

Results

Superfamily Truncatelloidea
Family Cochliopidae
Genus *Heleobia* Stimpson, 1865

***Heleobia transitoria* (Biese, 1947)**
Figures 1–5

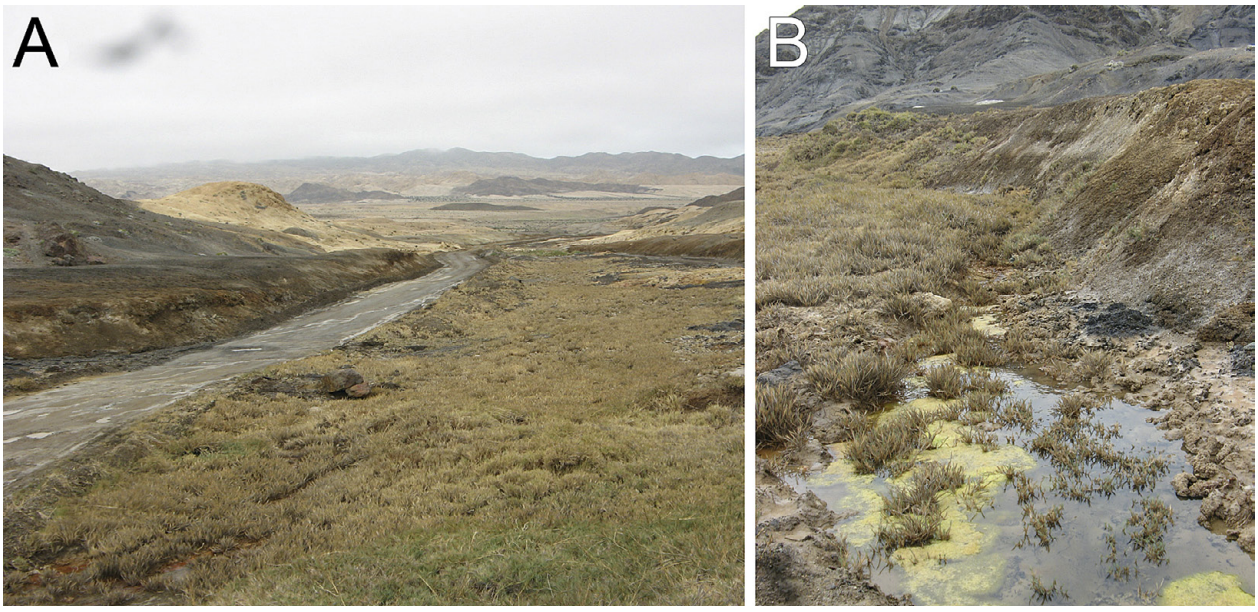

Figure 1. Quebrada Cachina, Antofagasta Region, Chile, type locality of *Heleobia transitoria*. **A.** Panoramic view of the ravine, with a dirt road in the middle. **B.** Habitat of the species, a small spring on the side of the road.

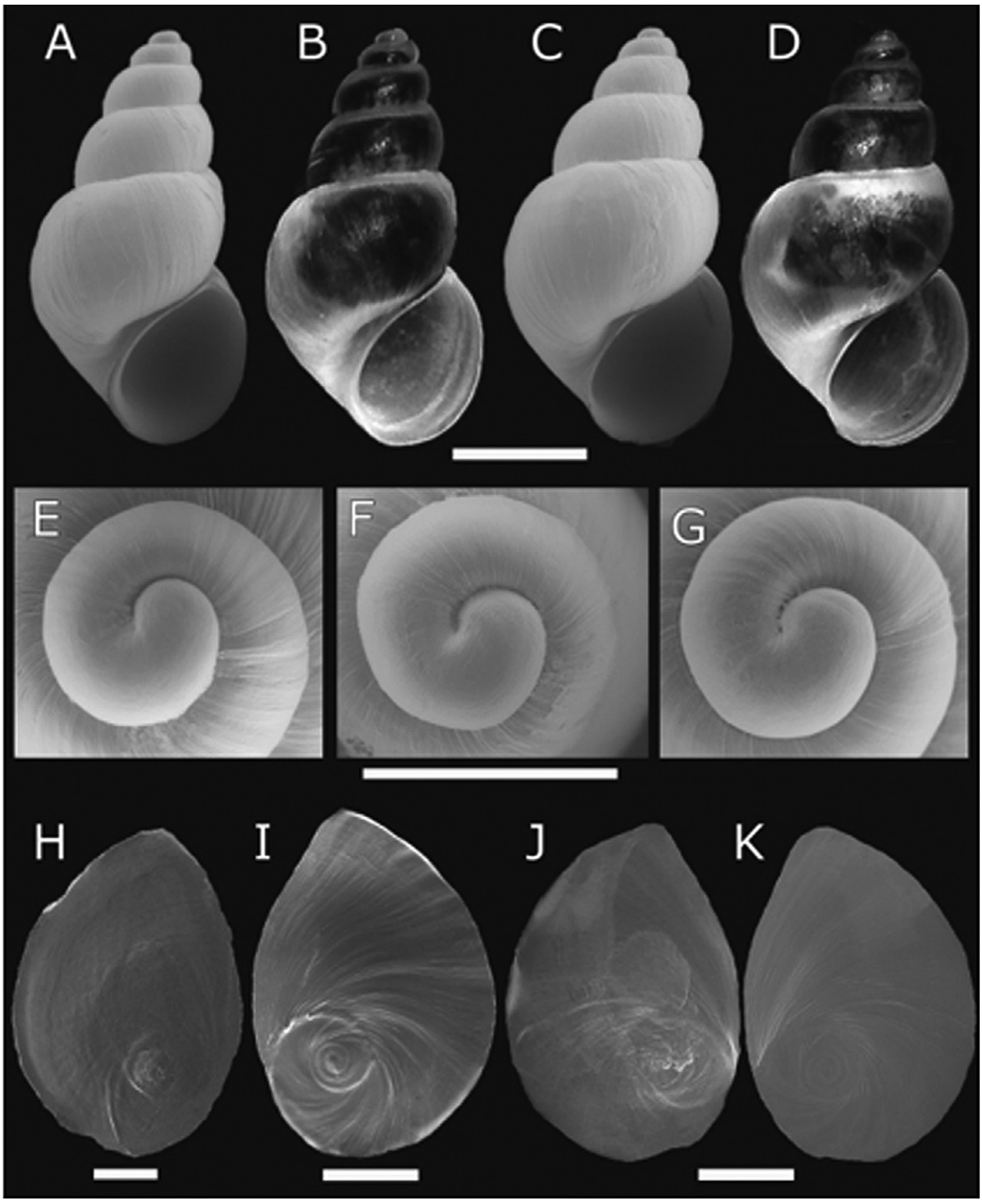

Figure 2. Morphology of the topotypes of *Heleobia transitoria* from Quebrada Cachina, Antofagasta Region. **A, C.** Scanning electron microscopy images. **B, D.** Stereomicroscope images. **A, B.** Shell, LMSM-UBB QC8k/MCNAPPRF-CC 156–1. **C–D.** Shell, LMSM-UBB QC9k/MCNAPPRF-CC 156–2. **E–G.** Protoconchs. **E.** LMSM-UBB QC5k/MCNAPPRF-CC 156–3. **F.** LMSM-UBB QC6k/MCNAPPRF-CC 156–4. **G.** LMSM-UBB QC7k/MCNAPPRF-CC 156–5. **H–K.** Opercula. **H, J.** Inner surface. **I, K.** Outer surface. **H.** LMSM-UBB QC 15k. **I.** LMSM-UBB QC10k/MCNAPPRF-CC 156–7. **J, K.** LMSM-UBB QC7k/MCNAPPRF-CC 156–6. Scale bars: 1 mm (A–D), 300 µm (E–G), 200 µm (H), 250 µm (I–K).

Littoridina transitoria Biese 1947: 70, 72–73 (fig. 2); Stuardo (1961: 180), Valdovinos (1999: 128, 2006: 90), Sielfeld (2001: 3).

Heleobia transitoria—Hershler and Thompson (1992: 56), Collado et al. (2011: 51, 52, 54, 56, figs 1K–L, 3A, B), Collado et al. (2013: 2, 6–11, fig. 2E).

Heleobia sp.—Collado et al. (2013: 3, 4, 8–10; from Quebrada El León).

Material examined. CHILE • 1♂, 4.2 mm; Antofagasta Region, Quebrada Cachina; 25°54′03″S, 70°36′47″W; 321 m a.s.l.; 28 Nov. 2011; G.A. Collado leg.; LMSM-UBB 1QC (soft body and broken shell) • 2♂, 3.9 mm; same collection data as for preceding; LMSM-UBB 2 QC (soft body and broken shell) • 3♂, 3.6 mm; same collection

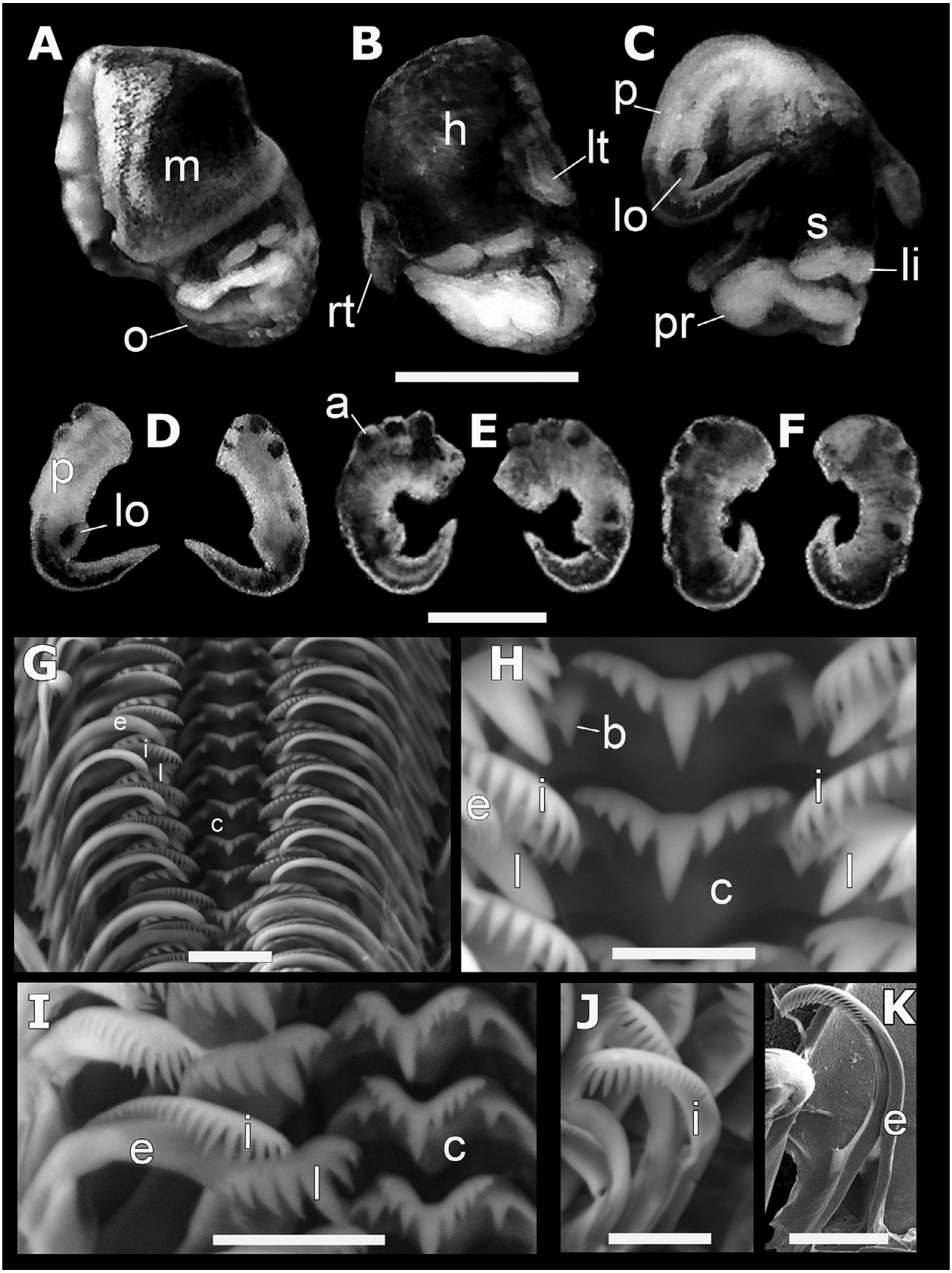

Figure 3. Anatomy of the topotypes of *Heleobia transitoria* from Quebrada Cachina, Antofagasta Region. **A–C.** Soft parts from stereomicroscope images of the anterior region of the body. **A.** Unsexed specimen, LMSM-UBB QC2k/MCNAPPRF-CC 156–8. **B.** Female, LMSM-UBB QC5k/MCNAPPRF-CC 156–9. **C.** Male, LMSM-UBB QC6k/MCNAPPRF-CC 156–10. **D–F.** Penises photographed by both sides using a stereomicroscope. **D.** LMSM-UBB QC6k/MCNAPPRF-CC 156–11. **E.** LMSM-UBB QC10k/MCNAPPRF-CC 156–12. **F.** LMSM-UBBQC13k. **G–K.** Radulae from scanning electron microscopy images. **G.** Anterior section, LMSM-UBBQC12k/MCNAPPRF-CC 156–13. **H–I.** LMSM-UBB QC5k/MCNAPPRF-CC 156–14. **H.** Central tooth. **I.** Radula teeth. **J–K.** Marginal teeth. **J.** Inner marginal tooth; LMSM-UBB QC5k/MCNAPPRF-CC 156–14. **K.** Outer marginal tooth; LMSM-UBB QC14k. Abbreviations: a, apocrine gland; b, basal cusp; c, central tooth; e, outer marginal tooth; h,head; i, inner marginal tooth; l, lateral tooth; li, lip; lo, lobe; lt, left tentacle; m, mantle; o, operculum; p, penis; pr, propodium; rt, right tentacle; s, snout. Scale bars: 1 mm (A–C); 500 µm (D–F); 20 µm (G, I, K); 10 µm (H); 15 µm (J).

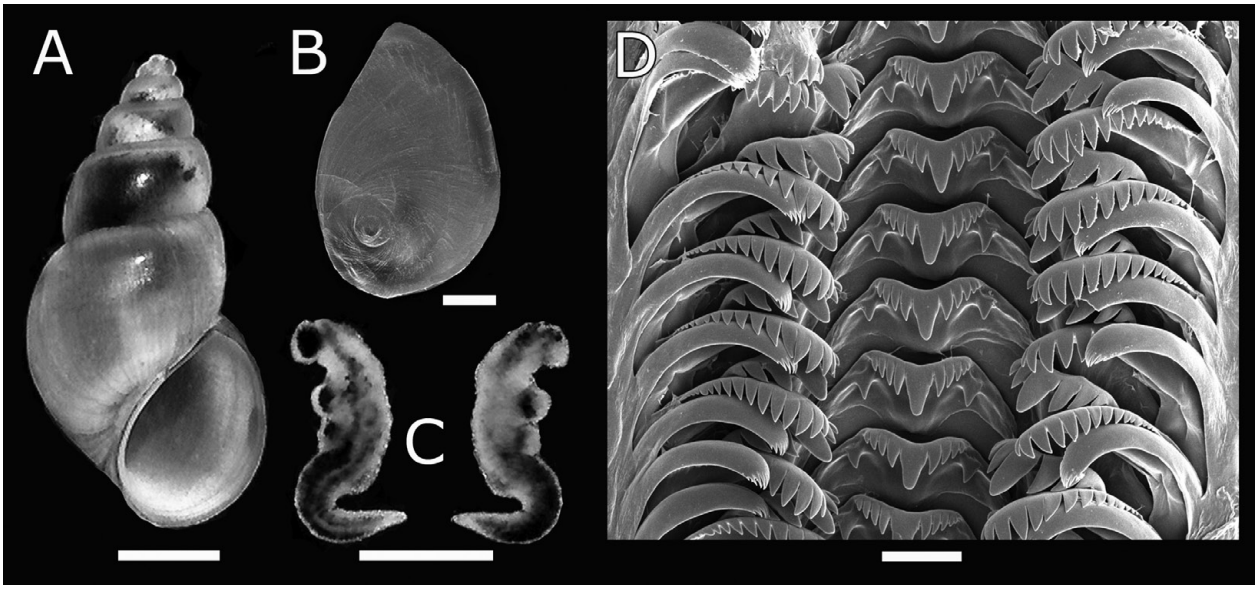

Figure 4. Morphology of *Heleobia transitoria* from Quebrada El León, Atacama Region. **A, C.** Stereomicroscope images. **B, D.** Scanning electron microscopy images. **A.** Shell, LMSM-UBB QEL3. **B.** Operculum, LMSM-UBB QEL32. **C.** Penis, LMSM-UBB QEL1. **D.** Radula, LMSM-UBB QEL35. Scale bars: 1 mm (A); 200 µm (B); 500 µm (C); 20 µm (D).

data as for preceding; LMSM-UBB QC13k (soft body and broken shell) • 4♀, 3.5 mm; same collection data as for preceding; LMSM-UBB QC14k (soft body and broken shell) • 5♀, 3.3 mm; same collection data as for preceding; MCNPPRF-CC 156–8 (head-foot), LMSM-UBB QC2k (broken shell) • 6♂, 3.3 mm; same collection data as for preceding; MCNPPRF-CC 156–7 (operculum), MCNPPRF-CC 156–12 (penis), LMSM-UBB QC10k (broken shell) • 7♀, 3.2 mm; same collection data as for preceding; MCNPPRF-CC 156–15 (head), LMSM-UBB QC1k (broken shell) • 8 unsexed, 3.2 mm; same collection data as for preceding; MCNPPRF-CC 156–2 (shell) • 9 unsexed, 3.1 mm; same collection data as for preceding; MCNPPRF-CC 156–1 (shell) • 10♂, 3.0 mm; same collection data as for preceding; MCNPPRF-CC 156–4 (protoconch), MCNPPRF-CC 156–10 (head), MCNPPRF-CC 156–11 (penis), LMSM-UBB QC6k (broken shell) • 11♂, 2.9 mm; same collection data as for preceding; LMSM-UBB QC3k (soft body and broken shell) • 12♀, 2.9 mm; same collection data as for preceding; LMSM-UBB QC15k (broken shell) • 13♀, 2.6 mm; same collection data as for preceding; MCNPPRF-CC 156–3 (protoconch), MCNPPRF-CC 156–9 (head-foot), MCNPPRF-CC 156–14 (radula), LMSM-UBB QC5k (broken shell); • 14♀, 2.5 mm; same collection data as for preceding; LMSM-UBB QC4k (broken shell) • 15♀, 2.4 mm; same collection data as for preceding; MCNPPRF-CC 156–13 (radula), LMSM-UBB QC12k (broken shell) • 16♀, 2.0 mm; same collection data as for preceding; MCNPPRF-CC 156–5 (protoconch), MCNPPRF-CC 156–6 (operculum), LMSM-UBB QC7k (broken shell) • 17♀, 2.0 mm; same collection data as for preceding; LMSM-UBB QC11k (soft body and broken shell) • 1♂, 5.8 mm; Atacama Region, Quebrada El León; 26°57′34″S, 70°44′15″W; 243 m a.s.l.; 28 Nov. 2011; G.A. Collado leg.; LMSM-UBB QEL1 (soft body and broken shell) • 2♂, 5.3 mm; same collection data as for preceding; LMSM-UBB QEL2 (soft body and broken shell) • 3♀, 5.3 mm; same collection data as for preceding; LMSM-UBB QEL30 (soft body) • 4♂, 5.0 mm; same collection data as for preceding; LMSM-UBB QEL29 (soft body) • 5♂, 4.8 mm; same collection data as for preceding; LMSM-UBB QEL27 (soft body) • 6♂, 4.8 mm; same collection data as for preceding; LMSM-UBB QEL32 (soft body) • 7(unsexed), 4.7 mm; same collection data as for preceding; LMSM-UBB QEL3 (shell) • 8♂, 4.3 mm; same collection data as for preceding; LMSM-UBB QEL35 (soft body).

Identification. The snails from Quebrada El León were identified as *H. transitoria* after comparison with the holotype (Biese 1947) and the topotypes of the species (Collado et al. 2011). The morphological study (Figs 2–4) provided the following results: shell small (2.8 mm length, 1.4 mm width for specimen LMSM-UBB QC8k/ MCNAPPRF-CC 156–1), conic-elongated, thin, light brown translucent, umbilicus nearly closed, sculpture smooth, suture depth (Figs 2A–D, 4A). Aperture oval, outer lip thin, with a light brown line. Protoconch approximately one whorl (Fig. 2E–G); it is differentiated from the teleoconch. The mean protoconch length is 343.3 ±11.2 µm (n = 4). Operculum paucispiral (Figs 2H–K, 4B), oval, thin, light brown in the central area and almost diaphanous on the outside. Foot grayish-black, propodium white. Head and snout black with brown tones in the central part, thick white lips, tentacles black interrupted by patches brown and gray (Fig. 3A–C). Penis with a broad base that extends to about two-thirds of the organ, followed by an elongated hook-shaped glans (Figs 3C–F, 4C). The color of this last portion is black on a gray background while the columnar is grayish. Two to five rounded apocrine glands, gray or black, are developed on the convex side of the proximal medial part of the organ or sometimes

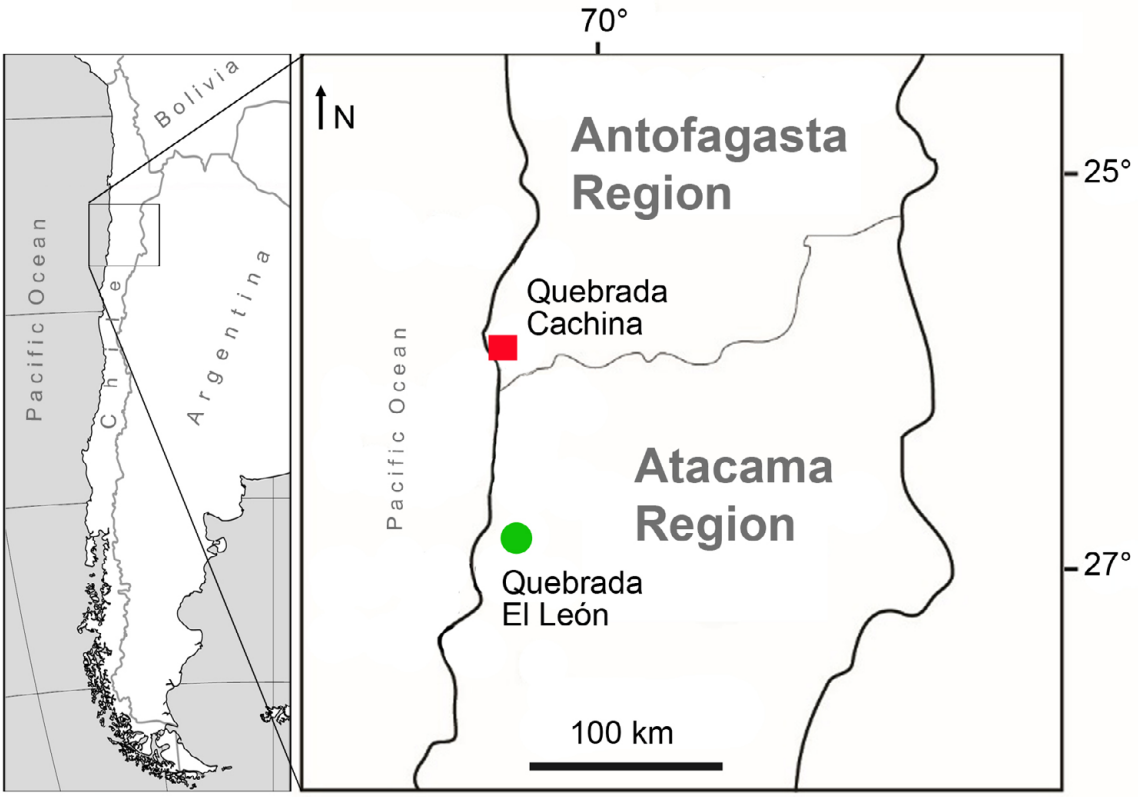

Figure 5. Distribution of *Heleobia transitoria* on the Atacama Desert coast, northern Chile. Red square indicates the type locality of the species, Quebrada Cachina, Antofagasta Region, and green circle represents the new record from Quebrada El León, Atacama Region, Chile.

near the base. A conspicuous single lobe develops in the concave side of the penis where the columnar portion and the glans meet. Radula taenioglossate (39–42 rows of teeth), with seven teeth in a transverse row: a central (or rachidian) tooth and three teeth on each side of it, a lateral and two marginal (Figs 3G–K, 4D). Central tooth with 5 or 6 lateral cusps on each side of the large and pointed median cusp and one pair of basal cusps, one on each side of the basal tongue. Lateral tooth with 4 or 5 external cusps and three internal on each side of the median cusp, which is well-developed and is pointed. Inner marginal teeth with about 22–25 cusps. Outer marginal teeth with about 21–23 cusps.

Remarks. *Heleobia transitoria* is endemic to Chile. It has been recorded only from Quebrada Cachina (Biese 1947; Collado et al. 2011, 2013) and Quebrada El León (Collado et al. 2013; present study). As a gilled snail, its life cycle must be carried out in the small pools of water where it lives.

Discussion

Heleobia transitoria was originally described under the genus *Littoridina* Souleyet, 1852 (Biese 1947), which was accepted by several authors (Stuardo 1961; Valdovinos 1999, 2006, 2008; Sielfeld 2001). Meanwhile, Hershler and Thompson (1992) transferred all Chilean species of *Littoridina*, including *L. transitoria,* to the genus *Heleobia*, although without reviewing biological material. The systematic position of the species within the genus *Heleobia* was clarified based on penis morphology and molecular phylogenetic investigations (Collado et al. 2011, 2013; present study).

Biese (1947) related *H. transitoria* to *H. parchappii* (d'Orbigny, 1835) (Argentina) and *H. copiapoensis* (Copiapó River, Chile) based on shell size, and to *H. chimbaensis* based on shell aperture. However, in the phylogenetic analysis performed by Collado et al. (2013), the sequences of *H. transitoria* from Quebrada Cachina and Quebrada El León were recovered as sister of a subclade formed by *H. deserticola*, from Aguada de Chorrillos, and *Heleobia* sp., from Carrera Pinto, whereas *H. chimbaensis* and several populations of *Heleobia* from Copiapó River were positioned in a different clade, which is indicative of character convergence. In the COI molecular analysis, Collado et al. (in press) recovered again *H. transitoria* as sister of *H. deserticola,* whereas *H.* cf. *cumingii* (d'Orbigny, 1835) from Aconcagua River (Chile) and *Heleobia kuesteri* (Strobel, 1874) (Argentina) were inferred as consecutive sister groups to this clade. In this analysis, Collado et al. (in press) recovered *H. parchappii* as sister of *H. occidentalis* (Doering, 1885), and this subclade as sister of the subclade that include *H. transitoria*.

The general shell shape of *H. transitoria* is similar to those of other species of *Heleobia* from northern Chile (e.g. *H. atacamensis*, *H. chimbaensis*, *H. deserticola*) and Bolivian Altiplano (e.g. *H. languiensis* (Haas, 1955),

H. saracochae (Haas, 1955), *H. vestita* (Haas, 1955)). *Heleobia transitoria* can be differentiated from *H. carcotensis*, a congener recently described from the Carcote Saltpan, Chilean Altiplano, considering the shape of shell aperture, oval in the first species and strongly angled adapically in the last one (Collado et al. 2016b).

The conic-elongated shell of *H. transitoria* can be also distinguishable of species from the Bolivian Altiplano which are, for example: more elongate-turriforms in *H. andicola* (d'Orbigny, 1835), *H. berryi* (Pilsbry, 1924) and *H. profunda* (Haas, 1955); globose-conics in *H. aperta* (Haas, 1955), *H. carinifera* (Haas, 1955), and *H. umbilicata* (Haas, 1955); pyramidals in *H. magna* (Haas, 1955), *H. ortoni* (Pilsbry, 1924), and *H. stiphra* (Haas, 1955); and spiral-thin in *H. mirum* (Haas, 1957) (see Haas 1955; Hershler and Thompson 1992; Kroll et al. 2012).

The shape of the operculum of *H. transitoria* is similar to that of *H. andicola andicola* and *H. carcotensis* illustrated by Hershler and Thompson (1992) and Collado et al. (2016b), respectively, but it is clearly distinguishable of that of *H. robusta* Silva & Veitenheimer-Mendes, 2004, which is elongate-helicoidal. The gray pigmentation of the foot, head and tentacles of *H. transitoria* differs from *H. compacta* (Haas, 1955), *H. cumingii* and *H. aperta* from Titicaca Lake, which are almost completely black, and *H. neveui* (Bavay, 1904), which is less pigmented (Hubendick 1955).

The penis morphology has been studied in several species of *Heleobia* proving to be of taxonomic usefulness in most cases (Hubendick 1955; Gaillard and de Castellanos 1976; Cazzaniga 1980, 1982a, 1982b; Silva 1993; Silva and Veitenheimer-Mendes 2004, Ovando and De Francesco 2011; Collado et al. 2013, 2016b; Collado 2015a; present study). The general shape of the penis of *H. transitoria* closely resemble that of *H. parchappii*, *H. occidentalis* and *Heleobia* sp. 1 (Gaillard and de Castellanos 1976; Cazzaniga 1980, 1982a; Ovando and De Francesco 2011), especially regarding the position of apocrine glands, the black pigmentation of these structures, and the presence and position of the lobe located in the concave side. However, there are also some conspicuous differences, mainly regarding the glans, which is hook-shaped in *H. transitoria*, but much longer in *H. parchappii*, thick and blunt in *H. occidentalis*, and shaped like a duck's head and beak in *Heleobia* sp. 1. Moreover, compared to *H. transitoria*, all of these species have more apocrine glands (Gaillard and de Castellanos 1976; Cazzaniga 1980, 1982a; Ovando and De Francesco 2011). It is important to mention that the hook shape of the *H. transitoria* glans also distinguishes it from other congeners.

For poorly known species of minute size and those threatened with extinction, is important to know the basic morphological attributes that allow identification of populations. This paper contributes significantly to fill this gap in *H. transitoria*. Similarly, it is also important to know the geographic distribution of the imperiled species. The new record performed in Quebrada El León acquires a high relevance considering that until now *H. transitoria* was known only from its type locality, Quebrada Cachina. Currently, the distribution of the species is restricted to these two small springs, which are located in two different regions of the country (Fig. 5). Despite the antecedents presented in this study, *H. transitoria* continues to be a poorly known species, so the time and mode of reproduction, habitat preference, type of diet, population sizes, as well as other population and ecological aspects together with other features of the life cycle, still need to be investigated.

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Authors' Contributions

GAC conceived the paper, collected data and took photographs. CGF prepared voucher specimens. GAC wrote the manuscript with input of CGF.

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