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Orestias gloriae, a new species of cyprinodontid fish from saltpan spring of the southern high Andes (Teleostei: Cyprinodontidae)

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Orestias gloriae, new species, is described from the isolated springs that drain into the Carcote saltpan in the southern high Andes, in the Chilean Altiplano, at an altitude of 3706 m a.s.l. (21°16'58.6"S 68°19'28.4"W). This is the only fish species found in these saltpan springs characterized by scarce vegetation, aquatic insects, crustaceans, and mollusks. The new species is separated from other species of *Orestias* by several unique characters such as a truncated cephalic lyre-like pattern of neuromasts represented only by the rostral series followed by few and large neuromasts irregularly placed; discontinuous series of infraorbital and preopercle-mandibular neuromasts; thick and irregularly-shaped ornamented cycloid scales covering the skull roof; scales covering the posterior part of the skull roof ankylosed into a plate. Additional characters are the presence of a protractile mouth and a characteristic karyotype. Although differences in sizes is a common sexually dimorphic feature found in *Orestias*, differences in the dentition on the fifth ceratobranchial distinsguish males and females of *O. gloriae*.

Introduction

The southern high Andean systems of the Altiplano range from 17° to 22°S and from 3000 to about 4500 m in altitude. This region is hypothesized to represent the remnants of remarkable Plio-Pleistocene lake and river systems, which, with the subsequent lack of rainfall, have evolved into ponds and saltpans due to evaporation mainly as the result of high temperatures in the region and additionally, by human water use. Currently, the Chilean Altiplano is represented by many important water bodies such as Chungará Lake in the north and Ascotán Salar in the south (about 600 km between both; Chong, 1988; Keller & Soto, 1998; Risacher et al., 2003; Placzek et al., 2006).

The endemic ichthyofauna that inhabits the aquatic systems of the Altiplano belongs to two genera: the killifish *Orestias* and the catfish *Trichomycterus*. The genus *Orestias* is known from 45 species, 23 of which live in Lake Titicaca where

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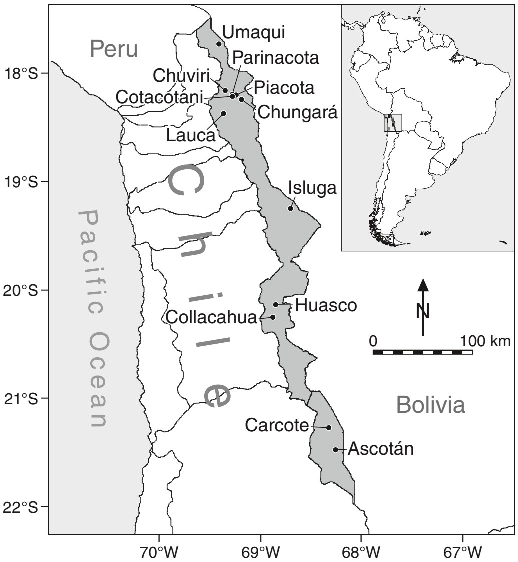

Fig. 1. Location of the Altiplano in South America. Left image: Southern Altiplano systems with Carcote Salar (21°16'58.6"S 68°19'28.4"W), showing no connections with other systems.

it has had an explosive species radiation (Lauzanne, 1981; Parenti, 1984a–b; Loubens, 1989). In addition to the Bolivian and Peruvian species, the *Orestias* of the southern Altiplano in Chile were first described by Arratia (1982) and Parenti (1984a) in a revision of the genus. Within *Orestias*, Parenti (1984a) defined four species complexes: *cuvieri*, *mulleri*, *gilsoni* and *agassii*. The last complex is characterized by (1) ontogenetic changes in color pattern; (2) squamation of head and median dorsal ridge with enlarged and smooth or granulated scales (these scales are larger in comparison to lateral flank scales); (3) presence of a lateral shield formed by large and smooth or granulated lateral scales positioned between opercle and a point at about the posterior extent of the pectoral fin; and (4) caudal peduncle relatively deep in adults, reaching over 22 % of standard length (SL).

At present, the *agassii*-complex includes 26 species, six of which have been described from the southern Altiplano (Arratia, 1982; Parenti, 1984a–b; Vila & Pinto, 1986; Vila, 2006). Lüssen et al. (2003) divided the *agassii*-complex species group into two clades: one that lives exclusively in Titicaca Lake basin (17°–20° S) and a second clade that lives in the southern Altiplano region (21°–22° S). Among the six species found in the southern Altiplano, *O.* cf. *agassii* is the most broadly distributed within the genus.

Recent fieldwork in the southern Altiplano has revealed the presence of *Orestias* in the Carcote saltpan (Fig. 1), a region that has not been previously investigated. The Carcote saltpan is a remnant of a Pleistocene lake (Ochsenius, 1974) at an altitude of 3706 m a.s.l. (21°16'58.6"S 68°19' 28.4"W). Due to evaporation, this aquatic system evolved into a saltpan, but keeping small ponds fed by springs in which live small populations of *Orestias* of an unnamed species. Here, we describe this new species and provide information about its habitat.

Material and methods

Specimens were captured with hand nets and a number of them were kept alive to obtain samples used in chromosome studies. Specimens were euthanized using 100 mg · l^{-1} tricaine methanosulfonate. Morphometric and meristic data for all the specimens were taken following the standard methodology outlined in previous descriptions of *Orestias* (e. g., Lauzanne, 1981; Parenti, 1984a; Vila, 2006). All measurements were taken on straight-line distances recorded with a precision caliper reading to the nearest 0.1 mm. Cleared-and-stained specimens were prepared following the protocol of Taylor & Van Dyke (1985). They were used in osteological studies of jaws, branchial skeleton, and vertebrae and fin-rays counts. The branchial apparatus was dissected and the gill rakers of the left gill arches I to IV were counted in 11 specimens. The terminology of the head neuromast lines follows Coombs et al. (1988) and Arratia & Huaquín (1995), with the exceptions noted below.

The specimens were sexed by direct examination of the gonads under a stereomicroscope. The number and morphology of chromosomes were studied in four females and three males following Vila et al. (2010). Institutional abbreviations follow Leviton et al. (1985) except for: LLFS-UCH, Laboratory of Limnology, Faculty of Sciences, Universidad de Chile, Santiago.

Orestias gloriae, new species
(Fig. 2)

Holotype. MNHNC 7424, female, 70.4 mm SL; Chile: Loa Province: springs of Carcote saltpan, 21°16'58.6" S 68°19'28.4" W, altitude 3706 m a.s.l.; I. Vila & S. Scott, April 2006.

Paratypes. MNHNC 7425, 1 male, 21.9 mm SL; MNHNC 7426, 1 female, 59.0 mm SL; LLFS-UCH 2006, 10 females 38.2–60.1 mm SL; LLFS-UCH, 7 males, 20.3–38.2 mm SL; collected with holotype.

Diagnosis. A relatively slender-bodied species, *Orestias gloriae* is clearly distinguished from all other species of *Orestias* by the following unique characters: (1) The lyre-shaped pattern of dorsal neuromasts, which is characteristics of *Orestias* is reduced to the rostral and anterior supraorbital lines formed by small neuromasts followed caudally by few and large isolated neuromasts, which are irregularly placed. (2) The infraorbital line of neuromasts is represented by a discontinuous series of few small neuromasts. (3) The supraorbital line of neuromasts is not connected with both the infraorbital and preopercle-mandibular lines. (4) The skull roof is covered by thick, irregularly-shaped cycloid scales some of which bear marked ridges, whereas other lack ornamentation. (5) The large, irregularly-shaped scales covering the posterior part of the skull roof are ankylosed to each other into a thin, rigid plate.

In addition, *O. gloriae* presents a protractile upper jaw reaching one-third of the head length when extended. This value is higher than those found in other species of *Orestias* with protactibility and inhabiting the southern Chilean Altiplano. The new species has 29–31 vertebrae, a value that represents the lowest range among species of *Orestias*.

Description. Morphometric data for the holotype, and paratypes are summarized in Table 1. Size not reaching beyond 80 mm SL, with a head moderately large, length 26–31 % SL. Orbits not reaching dorsal profile of head. Gently convex dorsal profile between head and dorsal fin. Slightly convex ventral profile between origin of anal and caudal fins. Lateral profile notably expanded just posterior to pectoral fin, decreasing slightly posteriorly up to anal-fin origin. Caudal peduncle relatively short and slender (see Table 1). 29–31 vertebrae.

Fig. 2. *Orestias gloriae*; Chile: Loa Province: springs of Carcote saltpan. **a,** MNHNC 7424, holotype, 70.4 mm SL; **b,** MNHNC 7425, paratype, 59.0 mm SL.

Dorsal series of neuromasts represented by rostral and anterior section of supraorbital lines (Fig. 3). Rostral line formed by continuous series of approximately 8 to 10 relatively small and rounded neuromasts on each side. No posterior section of lyre-like pattern of dorsal neuromasts. Infraorbital and preopercle-mandibular lines formed by few, small and irregularly placed neuromasts. Both infraorbital and preopercle-mandibular lines of neuromasts not connected to supraorbital line. Pores of both supraorbital and infraorbital sensory canals difficult to observed.

Upper jaw protractile. Premaxilla as well as dentary bearing mainly conical teeth, slightly recurved medially, and with conspicuous acrodin cup. Some teeth with slightly blunt tips. Premaxilla with three main rows of teeth, and dentary with two.

Preopercle and opercle laterally prominent.

Table 1. Morphometric characteristics of *Orestias gloriae*.

	holotype	paratypes
Standard length (mm)	70.4	21.9–59.0
In percent of standard length		
Head length	30.6	26.5–26.9
Head depth	21.9	20.1–21.9
Predorsal length	59.5	56.1–59.6
Preanal length	62.5	58.4–63.0
Caudal-peduncle length	28.9	22.2–23.7
Caudal-peduncle depth	15.0	13.3–18.3
Body depth	23.3	22.0–23.4
Eye diameter	9.2	7.3–9.7
Interorbital distance	9.3	4.4–9.0
Mouth width	7.0	7.0–7.1

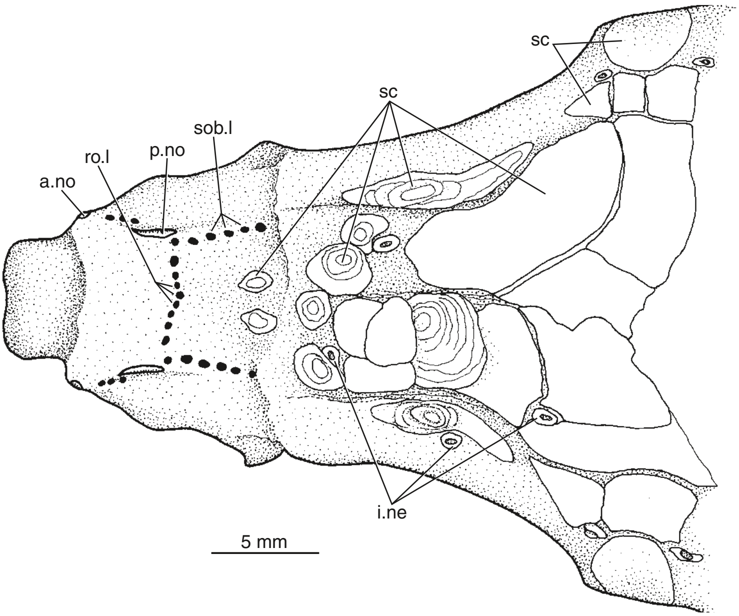

Fig. 3. *Orestias gloriae*, LLFS-UCH 2006, female, 50.9 mm SL; head in dorsal view. Thick lines and arrows between scales represent area with ankylosed scales. Abbreviations: **a.no**, anterior nostril; **i.ne**, isolated neuromasts; **p.no**, posterior nostril; **ro.l**, rostral line of neuromasts; **sc**, scales; **sob.l**, supraorbital line of neuromasts.

Preopercle bordered anteriorly by thick, large, and slightly rounded or oval scales.

Branchial apparatus (Fig. 4) characterized by marked asymmetry between left and right sides, otherwise branchial bones morphologically similar to other *Orestias*, with following exceptions. Fifth ceratobranchials forming a large isosceles triangle and unfused at midline. Females (Fig. 4a) with dorsal surface of ceratobranchial 5 densely covered with two kinds of teeth; most teeth long and conic, ending in a small conic acrodin cup usually darker than base of tooth; most posterior series of teeth formed by large, molariform teeth, each bearing a small conic acrodin tip, characterized by its dark color. In males fifth ceratobranchials bearing scarce teeth, discontinuously distributed on oral surface of bone. Most teeth molariform type (Fig. 4b).

Simple, partially ossified gill rakers present on both sides of each arch (Fig. 4b). Anterior and posterior margins of ceratobranchial 1 bearing gill rakers. Only anterior row of gill rakers present on anterior margins of hypobranchial and ceratobranchials 2 and 3. Ceratobranchial 5 bearing few gill rakers on anterior margin. Number of gill rakers on gill arches 1 to 4 ranging from 104 to 125.

Pectoral fin rounded, with 16–19 rays, both first and last rays shorter than middle ones and uniformly pigmented. Dorsal fin rather small, with 14 or 15 rays, first principal rays (terminology after Arratia, 2008) longer than remaining rays giving fin a pointed shape. Anal fin pointed, with 13–16 rays, anterior principal rays longer than following ones. Caudal fin truncate, with 23–27 total rays.

Squamation. Head and trunk covered with cycloid scales. Skull roof covered with scales (Fig. 3) of different shapes and sizes, showing strong bilateral asymmetry. Most anterior scales being the smallest, generally slightly round- or oval-shaped and with a few marked concentric striae. Most posterior scales thick, large, irregularly shaped and ankylosed between them forming a thin, rigid plate covering skull roof. One elongate, large scale positioned above each orbital margin of skull. Each scale with concentric striae at its mid section. Nine to 12 large and slightly rounded scales arranged in three rows positioned below orbit, and preceded by a small dorso-anterior cycloid scale with marked and protruding circuli. Nine to 12 large, thick and rounded scales positioned below orbit, on the cheek region, a

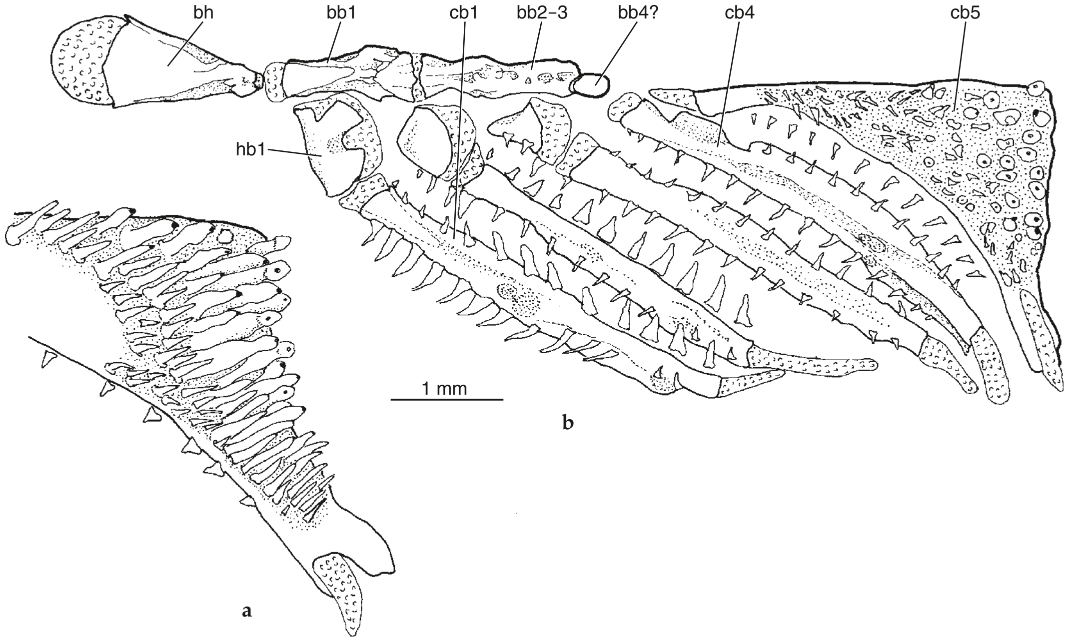

Fig. 4. *Orestias gloriae*, LLFS-UCH 2006; branchial arches in dorsal view. **a,** female, 50.9 mm SL; **b,** male, 38.2 mm SL (posterior part of ceratobranchial 5 teeth). Abbreviations: **bb1–3**, basibranchials 1–3; **bb4?**, possible basibranchial 4; **bh**, basihyal; **cb1, cb4, cb5**, ceratobranchials 1, 4 and 5; **hb1**, hypobranchial 1.

row of 6–8 bordering the preopercle. Commonly, 6–8 scales found at anterior preopercular margin.

Juveniles as well as adults fully scaled and covered with cycloid scales small and of uniform size over entire body. Ventral body region naked. Median dorsal ridge scales barely visible and few. Median dorsal ridge scales slightly larger than those of anterior section of flank.

Scales begining at posttemporal region arranged in 3–5 horizontal rows, increasing from 8 to 10 in middle flank area, and decreasing to 3 or 4 on caudal peduncle. Flank scales decreasing in size from rostrad to caudad. Exposed fields of lateral body scales with striae and sclerites. 31–37 scales forming lateral series of middle flank in males and females (see Table 2).

Karyotypes. Male and females karyotypes with diploid chromosome number equal 48, consisting of one pair of metacentric (m), three pairs of submetacentric (sm), 20 pairs of subtelocentric (st), and 11 pairs of telocentric (t) (Fig. 5). Fundamental arm number (NF) equal 56 (Fig. 5; Table 3). Pair number 3 heteromorphic in all individuals. No morphologically differentiated sexual chro-

Table 2. Meristic and karyotype characteristics of species of *Orestias* from the southern Altiplano. **1**, present results; **2**, Vila, pers. obs.; **3**, Arratia (1982); **4**, Vila & Pinto (1986); **5**, Parenti (1984a); **6**, Vila (2006).

	size range mm SL	2n (NF)	vertebrae	dorsal-fin rays	anal-fin rays	pectoral fin rays	caudal fin rays	scales in lateral series
O. gloriae	21.9–58.9	48 (56)	29–31	14–15	13–16	16–19	23–27	31–37
O. cf. *agassii*[2]	36.7–53.7	48 (54)	31–35	11–16	11–16	14–20	22–26	30–37
O. parinacotensis[2,3]	29.1–56.4	48 (54)	31–32	13–16	14–16	16–20	19–25	30–37
O. laucaensis[2,3]	41.7–90.0	50 (54)	33–35	14–15	14–15	16–17	24–28	31–39
O. chungarensis[2,4]	39.5–82.1	55 (54)	31–32	11–14	11–14	16–18	24–27	33–35
O. ascotanensis[2,5]	58.6–75.0	48 (54)	31–32	12–15	12–14	17–19	21–25	29–34
O. piacotensis[2,6]	37.4–71.7	52 (54)	32–34	12–14	12–14	14–16	21–27	30–34

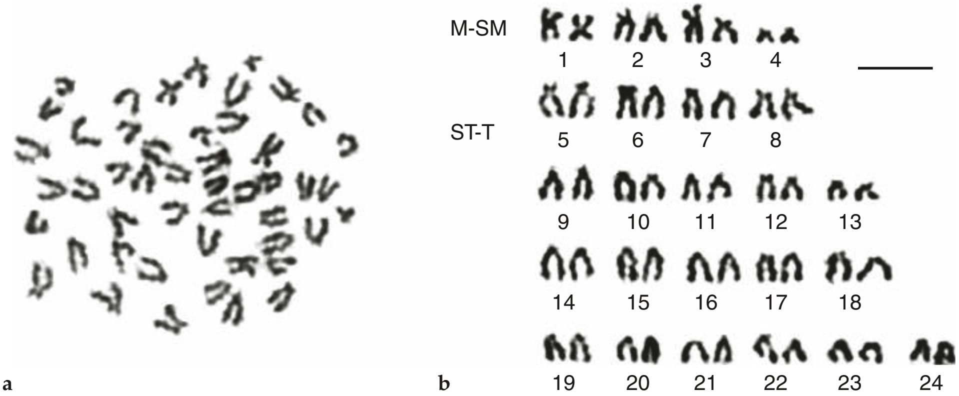

Fig. 5. *Orestias gloriae*. **a,** chromosome metaphase plate; **b,** karyotype. Scale bar 10 µm.

mosomes. Karyotype of *O. gloriae* differentiating from other southern *Orestias* in chromosomal formula and presence of four biarmed pairs of chromosomes (for comparison see Vila et al., 2010: table 4).

Table 3. Relative length (%), Centromeric Index ± S.D. and chromosome type of karyotype of *Orestias gloriae*. **M,** metacentric; **Sm**, submetacentric; **St**, subtelocentric; **T**, telocentric.

pair	relative length	centromeric index	chromosome type
1	4.53	45.65 ± 2.92	M
2	4.81	28.79 ± 2.32	Sm
3	5.90	36.03 ± 5.83	Sm
4	2.59	32.88 ± 3.60	Sm
5	5.27	19.91 ± 4.52	St
6	4.95	18.42 ± 4.21	St
7	4.68	18.60 ± 3.80	St
8	4.51	19.53 ± 4.10	St
9	4.32	19.23 ± 3.36	St
10	4.14	19.45 ± 3.82	St
11	3.91	18.83 ± 3.37	St
12	3.60	19.18 ± 3.25	St
13	3.00	20.70 ± 2.84	St
14	5.32	0	T
15	4.80	0	T
16	4.42	0	T
17	4.25	0	T
18	4.06	0	T
19	3.94	0	T
20	3.76	0	T
21	3.61	0	T
22	3.47	0	T
23	3.30	0	T
24	2.86	0	T

Sexual dimorphism. Females reaching greater lengths than males, a plesiomorphic condition in killifishes after Parenti (1984a). Longest examined female 70.4 mm SL, male 38.2 mm SL.

In females, oral surface of ceratobranchial 5 covered almost completely by elongate conic teeth, and by massive molariform teeth placed at its posterior border (Fig. 4a); conical teeth scarce but molariform teeth more abundant in males (Fig. 4b). Two or three projections or spiculae present at posterior margin of scales covering mid-flank lateral series of males. Spiculae absent in females.

Coloration in life. Males and females with same color pattern throughout ontogeny, and not sexually dichromatic. Sexual dichromatism observed only at spawning periods when males present a yellow coloration in anal fin.

Individuals with uniform dark gray color on head and flank because of numerous malanophores covering exposed field of scales. Dark gray color turning into pale yellow in ventral region of head and trunk. Pectoral, dorsal and anal fins uniformly pigmented due to small but abundant, star-shaped melanophores.

Habitat and reproduction. Habitat made of small ponds and creeks formed by springs of the Carcote saltpan near to Calama city (Fig. 1) at 3706 m altitude, and representing an endorrheic isolated system in the southern Altiplano that includes a few endemic forms (Arratia, 1982; Parenti, 1984a; Costa, 1997, 1998, 2003; Vila, 2006; Vila et al., 2007). Water of small ponds with high salinity due to concentrations of sodium chloride (Marquez et al., 2009). Fauna of Carcote saltpan represented by small populations of insects (Elmidae, Chironomidae, and Hydroptilidae), crustaceans (Boeckellidae, Chidoridae, and Hyalellidae), and mollusk *(Biomphalaria)*.

Shallow waters of Carcote saltpan springs inhabited by *O. gloriae* where small populations are distributed among macrophyte mats. Observations in situ along with analyses of stomach contents showed larvae of aquatic insects, amphipods and mollusks inside stomachs of *O. gloriae*.

Eggs moderately large, 1.9–2.3 mm in diameter and surrounded completely by filaments. Fishes protecting themselves from bird predation by fixing eggs to vegetation in a manner similar to that of other species of *Orestias* (Vila & Pinto, 1986; Pinto & Vila, 1987; Martínez et al., 1999; Vila, 2006).

Etymology. The specific epithet *gloriae* honors the research work of Gloria Arratia on Chilean fishes, and especially those of the Andean region.

Discussion

As described by Parenti (1981; 1984a,b), the genus *Orestias* underwent an explosive sympatric speciation in Lake Titicaca. Nevertheless, most species described for the southernmost region of the Altiplano in Chile indicate a high degree of endemism with unique species in each hydrographic system suggesting allopatric speciation (Fig. 1). This may be a result of the geographic isolation caused by the separation of the southwestern region from the rest of South America by the lifting of the Andes during the mid Oligocene (Placzek et al., 2006; Moreno et al., 2009). During recent years salt content and temperature have been increasing, as is revealed by the number and extension of the saltpans in northern Chile (Chong, 1988). The historical formation of the saltpans is accelerated by climatic changes and water extraction, two factors that diminish freshwaters and cause isolation of small populations of *Orestias* in the springs that feed the salars (Chong, 1988; Aceituno, 1997; Risacher et al., 2003; Morales et al., 2011) and indirectly increases the salinity of ponds associated to salars.

Lüssen et al. (2003) performed a phylogeographic analysis using haplotypes of mitochondrial D-loop, and reported that the species of the *O. agassii* complex comprises two groups, one corresponding to Lake Titicaca endemics and a second one made mainly of the species inhabiting the southern Altiplano, *O. tschudii* (from Huatajata, Bolivia and Puno, Perú) and *O. agassii* (from Lake Titicaca). Recently, Scott (2010), using three mitochondrial markers (D-Loop, ND2 and Cytb) and meristic characters, completed a phylogenetic study of the Chilean species of the *agassii*-complex, including the individuals from Carcote saltpan studied herein. In this analysis, Scott proposed the monophyly of the species of the Chilean Altiplano and found that individuals from Carcote belong to a lineage independent from the other species, suggesting that they correspond to a new species. The present study supports Scott's hypothesis that populations living in Carcote saltpan represent a new species of *Orestias*. As shown here, this new species is supported by several morphological features and its characteristic karyotype.

According to Parenti, 1984a, one of the defining characters of *Orestias* is its unique squamation and head pore pattern. The so-called "pore" pattern does not correspond to pores of the cephalic sensory canals but to series of neuromasts that are present on the skull roof, around the orbit, and on the preopercular and mandibular regions. The neuromasts on the roof of the skull are organised in a lyre pattern (Fig. 3; Parenti, 1984a). The unique morphology of the incomplete lyre pattern of supraorbital neuromasts, reduced to only the anterior portion of the supraorbital line and the rostral line (Fig. 3), is a unique feature of *O. gloriae*. In addition, in all species of *Orestias* there is a continuous series of neuromasts around the orbit (infraorbital line) and another in the skin covering the mandible and preopercle (preopercle-mandibular line) (Parenti, 1984a). *Orestias gloriae* is unique in having both the infraorbital and preopercle-mandibular series formed by few and irregularly placed neuromasts.

According to Parenti (1984a: 123-124), *Orestias* has a unique squamation "and the dorsal surface of the head is incompletely covered by irregularly distributed thickened scales that have been referred to as scutes in *Orestias* (Tchernavin, 1944a, 1946) although the term scutes is considered inappropriate here". *Orestias gloriae* presents a specialized squamation on the surface of the head (Fig. 3) that agrees with the general features mentioned by Parenti (1984a), but differs from other species of the genus in that the posterior-most scales covering the dorsal surface of the head are unornamented and are ankylosed to each other forming a rigid plate.

The degree of protractibility of the premaxilla in *O. gloriae* is the largest among species with protractile jaws among southern species such as *O.* cf. *agassii*, *O. ascotanensis*, and *O. laucaensis*. Other species of the southern Altiplano such as *O. chungarensis* do not have protractile mouths.

Comparative material. *Orestias* cf. *agassii*: MNHNC P7394–P7395, 20, 36.7–53.7 mm SL; Chile: Tamarugal Province: Huasco salt flat, 20°05' S 68°15' W.

O. ascotanensis: MNHNC P7401–402, 20, 58.6–75.0 mm SL; Chile: Loa Province, Ascotán salt flat, 21°29' S 68°19' W.

O. chungarensis: MNHNC P7399–4000, 20, 39.5–82.1 mm SL; Chile: Parinacota Province: Chungará Lake, 18°15' S 69°07' W.

O. laucaensis: MNHNC P7393, 20, 41.7–90.0 mm SL; Chile: Parinacota Province: Lauca river, 18°05' S 69°15' W. *O. parinacotensis*: MNHNC P7396–P7397, 20, 29.1–56.4 mm SL; Chile: Parinacota Province: Parinacota wetland, 18°10' S 69°20' W.

O. piacotensis: MNHNC P7398, 20, 37.4–71.7 mm SL; Chile: Parinacota Province: Piacota Lake, 18°11' S 69°15' W.

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