A new species of *Telmatobufo* (Schmidt 1852) (Anura, Calyptocephalellidae) from a remnant of the Maulino Forest, central Chile

Una nueva especie de *Telmatobufo* (Schmidt 1852) (Anura, Calyptocephalellidae) en un remanente de bosque Maulino, Chile Central

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ABSTRACT

A new species, *Telmatobufo ignotus*, from the Reserva Nacional Los Queules, Cauquenes Province, central Chile is described herein. The new taxon is hypothesized based on a new combination of adult, larval, and chromosomal characters. The new species present the more septentrional distribution on the coastal range and a very restrictive distribution area compared to its congeners; because of this, taxonomic and conservation comments are discussed and advanced.

KEYWORDS: New species, anura, *Telmatobufo*, morphology, chromosomes, central Chile.

RESUMEN

Se describe una nueva especie, *Telmatobufo ignotus*, de la reserva Nacional Los Queules en Chile Central. El nuevo taxón se hipotetiza en base a una nueva combinación de caracteres del adulto, larvas y cromosomas. La nueva especie al contrario de sus congéneres, presenta la distribución más septentrional en la Cordillera de la Costa y un área de distribución muy restringida: por esto, se discuten algunos aspectos sobre su taxonomía y conservación.

PALABRAS CLAVE: Nueva especie, anura, *Telmatobufo*, morfología, cromosomas, Chile Central.

INTRODUCTION

The genus *Telmatobufo* Schmidt 1952 is a rare ancient group of South American frogs containing up to day three valid allopatric species, occurring all of them in a wide geographical range, but each one in a different forest ecosystem throughout the Chilean territory (Formas *et al.* 2001). Thus, *T. venustus* (Philippi 1899), is endemic of the Andean hygrophite forest of central Chile (ca. 35° 28' - 37° 47' S); *T. bullocki* Schmidt 1952, microendemic in the forest of the Nahuelbuta coastal range (ca. 37° - 38° S); and *T. australis* Formas 1972 has been documented distributed in the temperate forest of *Nothofagus* in Southern Chile. This is the only species occurring in both, Andes and coastal range (ca. 41° - 39° S).

Since the description of this genus (Schmidt 1952), only 20 adults' specimens of the three species have been recovered from Cabrerías, Llancahue, Cerro Púschel (*T. australis*); Parque Nacional Nahuelbuta, Rucapuhén (*T. bullocki*); Altos de Vilches (*T. venustus*) (see Formas *et al.* 2001; Ortiz & Díaz-Páez 2006) and therefore they are considered as "rare" species (Formas 2005; Ortiz & Ibarra-Vidal 2005). However, despite of the paucity of both, biological material and listing localities; precious knowledge concerning adult and larval morphology, cytogenetics, taxonomy, systematic and some ecological notes about these frogs (see Péfaur 1971; Formas 1972; Díaz *et al.* 1983; Formas 1988; Formas & Cuevas 2000; Núñez & Formas 2000; Formas *et al.* 2001), has been published. Nevertheless, many aspects of its reproductive biology, ethology and biogeography are still unknown, and because of increasing threat of extinction that amphibians suffer worldwide today (mainly due to the loss of habitat) (Beebe & Griffiths 2005; IUCN 2008), perhaps

we will never achieve that knowledge.

In this context, there is a need for freshly taxonomic knowledge related to Chilean frogs to get an accurate species inventories and basic biological and ecological information, given the scarce number of species and the high degree of endemism and vulnerability that characterizes the anurans of the forest ecosystem of South America (Formas 1979, Bustamante & Simonetti 2005, Cuevas & Cifuentes 2009). During successive fieldwork campaigns conducted between summer 2007 and autumn 2009, in the National Reserve Los Queules (coastal range in central Chile), 28 frog's tadpoles, a juvenile and an adult male of the genus *Telmatobufo* were collected. This finding was very striking given the zoogeographic connotation of this genus (San Mauro *et al.* 2005; Frost 2009). Thus, bearing in mind that a morphological species is a hypothesis based on a new combination of characters (Dayrat 2005); we hypothesized that the new sample and locality belong to a new species of *Telmatobufo*, based on adult and larval characteristics. This new species is named encompassed with ecological notes, life history traits and chromosome attributes herein. Finally, the new record of *Telmatobufo* is discussed and assessed in view of bio-geographical hypothesis related with gondwanic frogs, and the possible negative consequences of the habitat degradation on his viability given the larval strategies of the *Telmatobufo* species.

MATERIAL AND METHODS

Both, adult specimen and tadpole samples were stored in Alcohol 70% and formaldehyde 10%, respectively. Abbreviations used in the measurements of the adult specimens are SVL (snout-vent length), HL (head length), HW (head width), IDi (internareal distance), ED (eye distance), ThL (thigh length), TL (tibia length), FL (foot length) following Wiens (1993). Formulae for toes webbing are those of Savage and Heyer (1967), as modified by Myers & Duellman (1982). Measurements were made with a digital calliper to the nearest 0.01 mm. The tadpoles were staged according to Gosner (1960), and their morphology was characterized following Altig (2007) and Altig & Johnson (1989). Measurements included total length (TL), body length (BL), body width (BW), maximum tail width (MTW), snout eye distance (SED), eye distance (Edi), and oral disc width (ODW). To reduce bias, all the measurements were done by the first author.

To characterize metaphasic chromosomes, a section of the tail of each tadpole was excised and submerged in Colchicine 0.05% for 2 h. The fragments were treated with distilled water during 30 m, fixed in acetic alcohol (1:3), and then placed in 45% acetic acid. Metaphasic plates were

obtained by squashing fragments of tail epithelium and then dipping them in liquid nitrogen (Cuevas & Formas 1996). Chromosomes were stained with acetic Orcein 50% to determine the diploid number and the position of secondary constrictions (Schmid 1978). The best five metaphasic plates were selected to determine the relative length of chromosomes (Bogart 1970). Centromeric ratio and type of chromosome were determined according to Green & Sessions (1991). Photograph of metaphasic chromosome plates and specimens (adult and tadpoles) were deposited in the Amphibian Collection of the Instituto de Zoología, Universidad Austral de Chile (IZUA).

RESULTS

Telmatobufo ignotus sp. nov.

HOLOTYPE: IZUA 3542, one adult male of *Telmatobufo* with well developed sexual secondary attributes collected by José Núñez, César Cuevas and Felipe Rabanal, on May of 2009 (Fig. 1).

TYPE LOCALITY: Los Queules National Reserve (35°59'19"S, 72°41'25" W; at 187 m.a.s.l.), a remnant of the Maulino forest in central Chile; 22 km NW, 15 minutes by road from Curanipe city), Cauquenes Province, Western slopes of the coastal range (Fig. 2).

PARATYPES: A group of twenty eight tadpoles (lot IZUA 3543). Same data as holotype.

DIAGNOSIS: *Telmatobufo ignotus* sp. nov. can be distinguished from its congeners by the following combination of characters: 1) maximum snout-vent length in male (SVL) 70 mm, 2) snout slightly truncated in dorsal and lateral profile, 3) Soft yellow spots with orange tints colour over both eyes, joined by a more orange inter-ocular spot, 4) Dorsal part of the arms (near to the body) yellowish in colour and orange in the forearms, 5) two median ovoid's parotids glandules, longitudinally located and, smaller than ocular globes, 6) iris rounded, brilliant bronze-yellowish with reticulations, 7) minutes dark spines (20) present under the parotids, 8) flanks and dorsum with moderate amount of warts, which are bigger in the vertebral line region, 9) Inner palmar tubercle oval and enlarged, bigger than the outer, 10) four to five small vomerine teeth on each side, in a prominent elevated patches behind the level of the choanae, 11) dorsum of legs smooth and unbarred, 12) tips of fingers and toes whitish with rounded points, 13) toes very thin, long with well developed webbing present, 14) mountain stream tadpole with two teeth rows on the upper labium, 15) $2n = 26$ (all biarmed) chromosomes), $FN = 52$, 16) SC on pair 5.

COMPARISON: *Telmatobufo ignotus* differs clearly from *T. australis* and *T. venustus* in dorsum colour patterns (grey with yellowish longitudinal lines in *T. australis*, dark with orange spots in *T. venustus*, and slightly brown with cooperish hints in *T. ignotus* n. sp.). It also differs with *T. bullocki* in the size of the parotids (median sized, smaller than ocular globe in *T. ignotus*; larger sized, bigger than

ocular globes in *T. bullocki*), in the form and colour of a distinctive mark over the head (slightly orange-yellowish spots in form over both ocular globes in *T. ignotus* (Fig.1); clearly yellowish highlighted, transversally located over and between the ocular globes in *T. bullocki*). *Telmatobufo ignotus* present a higher amount, but smaller sized warts in the flanks and dorsum than *T. bullocki*, and also differs from this species in vomerine teeth number; 4-5 in *T. ignotus*, 6-7

FIGURE 1. Adult male of *Telmatobufo ignotus* from Los Queules. (a) Lateral view; Bar = 2 cm, (b) Dorsal view; Bar = 1.7 cm.

FIGURA 1. Macho adulto de *Telmatobufo ignotus* de Los Queules. (a) Vista lateral; Barra = 2 cm, (b) Vista dorsal; Barra = 1.7 cm.

FIGURE 2. Maps of Chile: (a) showing study area in crossed off., (b) distributional geographic dots of *Telmatobufo* species in Central South of Chile and Los Queules locality with grey circle. Forest classification is explained in the inferior left frame.

FIGURA 2. Mapas de Chile: (a) mostrando el área de estudio en achurado, (b) puntos de distribución geográfica de las especies de *Telmatobufo* en la zona centro y sur de Chile y Los Queules en círculo gris. En recuadro inferior izquierda se explica la clasificación de los bosques.

in *T. bullocki* (Schmidt 1952, Péfaur 1971).

When we compare tadpoles in similar Gosner (1960) stage of the four *Telmatobufo* species (see table 2), those of *T. ignotus* (stage 26) are clearly larger than those of *T. australis* (stage 29), *T. bullocki* (26) and *T. venustus* (27). Tadpoles of *T. ignotus* are markedly larger sized than those of *T. australis*, *T. bullocki* and *T. venustus* (Table 2). Furthermore, they differ

in pattern of colouring from the other *Telmatobufo* species. The tadpoles from Los Queules present a broad variety of colours that varies between dark blue, brown with gray spot, and brown with orange spot. Tadpoles known from *T. bullocki* from Nahuelbuta Range were always gray in colour with the belly whitish and translucent (Formas *et al.* 2001). From the chromosomal point of view *T. ignotus* present a formula 7m+ 4sm+ 2st (Table 3) differing from those of *T. australis* (8m+ 2sm+ 2st), *T. bullocki* (7m+ 3sm+ 3st), and

TABLE 1. Measurement (mm) of the *Telmatobufo ignotus* tadpoles from Los Queules National Reserve (Mean $\pm$ standard deviation). Number of replicates in bracket. Abbreviations as given in material and methods.

TABLA 1: Medidas (mm) de las larvas de *Telmatobufo ignotus* de la Reserva Nacional Los Queules (Media $\pm$ desviación típica). Número de réplicas entre paréntesis. Abreviaciones como las indicadas en material y métodos.

stages characters	25 (2)	34 (1)	35 (3)	36 (3)	37 (2)
TL	41.95 – 41.10	64.40	64.93 $\pm$ 3.98	69.35 $\pm$ 4.51	75.20 – 73.90
BL	17.55 – 18.40	27.80	28.33 $\pm$ 1.46	36.23 $\pm$ 3.66	33.20 – 35.60
BW	7.25 – 7.65	12.20	10.40 $\pm$ 0.85	13.77 $\pm$ 4.55	11.80 – 18.55
MTW	6.95 – 7.60	12.30	11.97 $\pm$ 1.97	11.72 $\pm$ 3.01	13.60 – 8.35
SED	7.10 – 6.15	11.90	11.13 $\pm$ 1.27	12.23 $\pm$ 0.84	11.10 – 13.95
EDi	1.70 – 1.80	2.00	2.63 $\pm$ 0.49	4.03 $\pm$ 2.06	2.30 – 6.35
ODW	7.25 – 8.00	13.80	11.47 $\pm$ 1.93	9.87 $\pm$ 5.91	13.50 – 15.55

TABLE 2: Comparative morphometrics of tadpoles in similar Gosner's stage of four species of *Telmatobufo*.

TABLA 2: Morfometría comparativa de larvas en similar estado de Gosner de las cuatro especies de *Telmatobufo*.

	<i>T. australis</i>	<i>T. bullocki</i>	<i>T. ignotus</i>	<i>T. venustus</i>
Characteristics	Stage 29 (3)	Stage 26 (2)	Stage 26 (14)	Stage 27 (2)
TL	38.00	35.3 $\pm$ 2.08	48.93 $\pm$ 4.96	43.05 $\pm$ 51.00
BL	16.00	15.5 $\pm$ 0.86	21.56 $\pm$ 2.93	20.70 $\pm$ 20.00
BH	7.00	5.7 $\pm$ 0.25	9.93 $\pm$ 3.20	6.25 $\pm$ 8.80
FH	6.70	7.8 $\pm$ 0.05	8.00 $\pm$ 1.23	7.10 $\pm$ 8.70
SED	5.50	4.7 $\pm$ 0.15	8.16 $\pm$ 1.27	5.00 $\pm$ 6.10
SND	3.00	2.8 $\pm$ 6.70	3.95 $\pm$ 1.36	3.80 $\pm$ 4.00
EDi	1.40	1.3 $\pm$ 0.00	2.34 $\pm$ 1.77	0.90 $\pm$ 1.70
ODW	8.80	7.1 $\pm$ 0.36	10.04 $\pm$ 2.07	9.40 $\pm$ 11.40

T. venustus (7m+ 3sm+ 3st) (Formas & Cuevas 2000).

DESCRIPTION OF THE HOLOTYPE Mature adult male (Fig. 1). Morphometric measurements are: SVL 70 mm, HL 23.4 mm, HW 25.25 mm, IDi 6.25 mm, ED 6.35 mm, ThL 36.8 mm, TL 30.30 mm, FL 50.75 mm.

Head 1.2 times wider than long, and 2.77 times the snout vent length distance. *Canthus rostralis* slightly distinct, loreal region plane in frontal view. Lips thick. Low lip with a neckline in the half. Superior lip slightly concave in the half. Mouth reaches the first part (1/3) of the parotids in lateral and vertical view. Parotids ovoid, medians in size (7.27 mm) (clearly smaller than antero-posterior ocular globe diameter) and longitudinally located (Fig. 3b). Tympanum absent. Tongue rounded free behind. Snout rounded in dorsal view, slightly truncated in lateral profile (Fig. 3b). The anterior part of the throat presents a file of small thorns following the internal border of the inferior lips. Nostrils slightly ovoid, fringed and the inter-narial distance 1.2 times the inter-orbital distance. Inter-narial region slightly concave. Nostrils nearer to snout terminus than anterior part of the eyes (Fig. 1b). Moderate amount of warts (15), but numerous small spines below the parotids at the end of the mandible (Fig. 3b). Two slight orange-yellowish spots over the ocular globes are present; they are joined by an inter-orbital orange area. The same orange yellowish colour is noted on the arms proximally to the body, and also ventrally in the forearms (Fig. 1b). Body skin free. Forelimbs well developed. Tarsal fold thin, extending 85% of tarsus length, confluent distally with the fringe along inner margin of Toe I. Fingers thin, shorts and possessing whitish and rounded points (Fig. 1a). First finger with a patch of spines on it. Relative lengths of fingers: III>IV>II>I (Fig. 3a, c). Feet with a wide inter digital webbing. Webbing formula: I 1 3/4-1 3/4 II 3/4-1 3/4 III 3/4-1 3/4 IV 3/4-1 3/4 V. Toes thin, slender and with single whitish rounded points. Relative lengths of toes: IV>III>V>II>I (Fig. 3 a, d). Well developed post femoral cutaneous fringe and gross tarsal fold present (Fig. 3a). Dorsum brown in colour, with orange cooperish hints near the head; possessing moderate darker warts with almost imperceptible yellowish reticulations among them. Belly greyish with yellowish irregular's minutes spot, extending on the ventral part of the arms, thighs and feet.

FEMALE: unknown.

Tadpole (Stage 36): Lateral view (Fig. 4a): Tip of the snout blunt. Inter-narial distance minor than inter-ocular one (0.8 times). Body ovoid, approximately 0.8 times the tail length, and 2.6 longer than deep. Nostril, small dorso-lateral in position closer to the eyes (5.0 mm) than to the snout tip (7.5 mm). Eyes rounded, small, antero-dorso-laterally oriented, located in the first third of the head. Spiracle sinistral, distal extreme with 2 mm free from the body; located on

the inferior half of the body directed up and backward (40°) and nearer the body end than the tip of the snout. Oral disc extended to eye position in lateral view. Tail muscle is robust; musculature segmentation is visible from the end of the body backward. Fin tale symmetrical and present only in the last third of total length of the tadpole. Tail tip lanceolate, slightly rounded, paddle-shaped caudal fin. Anal fold is extended under tail muscle. Dorsal view (Fig. 4b): Anterior portion of the head blade shaped, wider in the anterior than posterior part. Anterior part of the oral disc visible in dorsal profile. The inter-ocular distance is longer than inter-narial separation. Altogether the head and body present an oval shape with maximum width in the anterior part. Tail muscle starts in the first half of the body. Dorsal fin begins in the first half of the tail and represents 1/3 the total tadpole length. Oral disc details (Fig. 4c): Oral disc wide, with sucks in form, and two rows of papillae on the anterior labium (AL), and papillae in random distribution on the posterior labium (PL), each of them with minute dark spot of pigmentation. In the commissures only the external row is presented. The upper rows of teeth reach the commissures. Oral disc is emarginated. Mandibles strong, crescent shaped, and internal borders smooth. Each mandible possesses long and thin teeth at the base. Tooth bilaterally located and decreases in size towards the centre. Tooth formula (2)/(1-1) (2). The inner row of teeth of the posterior labium presents a gap in the half that coincides with the vertices of the inferior mandible. Ventral region (Fig. 4d): Body opaque and prolonged backward in a sheath of skin that also covers the minute hind limbs. Two muscular cords with evident transverse stretch marks. Anal overture is dextral. Anal fold completely sticks to the tail muscle. Segmentation of tail muscle visible.

Colour in formalin. Ocular globe transparent and prominent, black pupils and white irises. Ventral region of the body whitish with orange irregular spot. Dorsum brown, with minute dark orange irregular, dark guanophores spots, and orange-reddish spots which are less abundant towards the tail. Ventral region presents two white cordons, which start after the oral disc and extend backward converging in the whitish anal sheath. Tadpole staging and measurements are presented in Table 1.

STANDARD KARYOTYPE

The karyotype of *T. ignotus* from the Los Queules National Reserve showed 26 chromosomes, each of them biarmed. Based on their relative length, pairs 1-5 were large (> 100 units), pair 6 was intermediate (among 80 to 100 units), and pairs 7 to 13 were small (< 80 units). Chromosome pairs 1, 7, 8, and 10-13 were metacentrics, pairs 2, 4, 6 and 9 were submetacentrics and pairs 3 and 5 were subtelocentrics (Fig. 5). A secondary constriction was detected on the short arm of pair 5. A large difference in size between pairs 6 and 7 was observed. Measurements are presented

FIGURE 3. Morphological details of the adult of *Telmatobufo ignotus*, (a) Ventral view, (b) Lateral view of the head, (c) Left hand with sexual asperities, (d) Right foot with webbing. Bar in (a) 1.75 cm, in (b), (c) and (d) 0.5 cm.

FIGURA 3. Detalles morfológicos del adulto de *Telmatobufo ignotus*, (a) vista ventral, (b) vista lateral de la cabeza, (c) mano izquierda con asperezas sexuales, (d) pata izquierda con membranas. Barra en (a) = 1,75 cm, en (b), (c), y (d) = 0,5 cm.

FIGURE 4. Tadpole of *Telmatobufo ignotus* from Los Queules, (a) Lateral view, (b) Dorsal view, (c) Oral disc details, (d) Ventral region. Bar equal 5mm.

FIGURA 4. Larvas de *Telmatobufo ignotus* de Los Queules, (a) Vista lateral, (b) Vista Dorsal, (c) Detalles del disco oral, (d) Región ventral. Barra = 5 mm.

TABLE 3. Relative length, arm radio, and types of chromosomes of *Telmatobufo ignotus*, from Los Queules National Reserve.
TABLA 3. Longitud relativa, razón de los brazos, y tipos de cromosomas de *Telmatobufo ignotus*, de la Reserva Nacional Los Queules.

Chromosomes													
Pairs	1	2	3	4	5	6	7	8	9	10	11	12	13
RL ^a	143.85±1.19	140.67±7.70	102.74±3.79	106.13±2.43	100.32±4.16	86.51±8.76	51.36±5.22	51.99±1.11	48.29±4.39	48.25±5.76	42.27±5.12	41.31±5.18	35.71±7.91
AR ^b	1.14±0.06	1.77±0.04	3.52±0.45	1.73±0.04	3.53±0.04	2.47±0.09	1.51±0.20	1.36±0.36	1.73±0.02	1.36±0.33	1.44±0.17	1.37±0.17	1.28±0.16
CC ^c	m	sm	st	sm	st	sm	m	m	sm	m	m	m	m

^aAccording to Bogart 1970, ^b long/short arm, and ^c assignments to type were metacentric (m = 1.00 – 1.70); submetacentric (sm = 1.71 – 3.00); subtelocentric (st = 3.01 – 7.00); telocentric (t = 7.01 - ∞) (Green & Sessions 1991). * Secondary constriction.
^a De acuerdo a Bogart 1970, ^b brazo largo/brazo corto, y ^c asignación de los tipos cromosómicos fueron metacéntrico (m = 1.00 – 1.70); submetacéntrico (sm = 1.71 – 3.00); subtelocéntrico (st = 3.01 – 7.00); telocéntrico (t = 7.01 - ∞) (Green & Sessions 1991). * Constricción secundaria.

FIGURE 5. Standard karyotype of *Telmatobufo ignotus*. Arrows indicate secondary constrictions position. Bar = 10 μ m.

FIGURA 5. Cariotipo estándar de *Telmatobufo ignotus*. La Flecha indica la posición de las constricciones secundarias. Barra = 10 μ m.

in Table 3.

DISTRIBUTION AND ECOLOGY

The Maulino forest is located at the coastal range of south-central Chile. These woody ecosystems have been severely disrupted by intense human activity and at present, the landscape is dominated by large extensions of Monterrey pine (*Pinus radiata*) cultures, where scarce remnants of native forest persist (Bustamante & Simonetti 2005). The new collection site is located within a remnant of the Maulino forest dominated by *Nothofagus alessandrii* (Ruiz) and *Gomotergera queule* (Queule). The National Reserve Los Queules is a small area of 147 ha inserted in a matrix of *Pinus radiata* and *Eucalyptus globulus*. Weather data were collected at Chanco station near of Los Queules National Reserve. From 2003 to 2006, mean annual rainfall was 857 mm.

Both, the adult and the juvenile of *Telmatobufo ignotus* were collected during the austral autumn under rocks at the border of the stream Ramadillas in the year 2009, which has a maximum wide of 5 m. The juvenile was more vivid coloured with orange and yellowish tints over a brown colour and was 40 – 50 mm in large. Both specimens were kept in a cooler where the juvenile was eaten by the adult during the night (cannibalism). A froglet was collected in the middle of May 2008 at the border of the same stream. Tadpoles of *T. ignotus* were collected during the summer season (December to February 2007 and 2008) in the same place. *Eupsophus septentrionalis* Ibarra-Vidal, Ortiz & Torres-Pérez 2004 and *Alsodes* sp. are species of frogs that also occur in this locality but the tadpoles of these species differs from *Telmatobufo* larvae in their size (i.e. 25 mm in *Eupsophus queulensis*, 44 mm in *Alsodes* sp., 75 mm in *T.*

ignotus).

ETIMOLOGY The name *ignotus* is a Latin adjective and refers to the fact that this species during long time remaining in the anonymity and ignored.

DISCUSSION

Schmidt (1952), and Formas *et al.* (2001) suggested that the following presumptive morphological synapomorphies may support the genus *Telmatobufo*: 1) Larger sized animals (60.4 – 83.0 mm) with bufoniform aspect; 2) parotid glandules present; 3) humeral glandule well marked; 4) well developed foot webbing; 5) wide xiphisternum; 6) stream mountain tadpole with mouth-like sucker in form, 7) strong and wide tail; and 8) 26 chromosomes (Formas & Cuevas 2000). The new frog material collected is considered to be a member of *Telmatobufo* because it has at least seven of the eight aforementioned characters; although, its distribution at National Reserve Los Queules precludes for the species' inclusion in *Telmatobufo*, this is cited as a new record for the genus herein.

Concerning the specific status, at first sight the adult morphological appearance of *Telmatobufo* from Los Queules (brown with orange-yellowish spot over the eyes, and an orange area between ocular globes) resemble *T. bullocki* from Nahuelbuta Range (*sensu* Schmidt 1952), however, other morphologic details observed in the adult, tadpoles and chromosomes (see comparison section) drive to propose a new morphological species hypothesis herein: *Telmatobufo*

ignotus sp. nov.. Furthermore, results on chromosome gross morphology of the specimens from Los Queules showed some auto-apomorphic attributes, such as its chromosome formula, and secondary constriction position. Actually, in the karyotype of new specimens from Los Queules, a single secondary constriction is present on the short arm of pair 5 (st). In each of the other three species, a secondary constriction occurs on the short arm of pair 12. In *T. australis* and *T. venustus*, an additional secondary constriction also occurs on the short arm the pair 6. Formas & Cuevas (2000) proposed the number of secondary constrictions as specific markers to the genus. Thus, chromosomal characteristics distinguish the specimens from Los Queules and the specimens of *T. bullocki* from Nahuelbuta Range. Further analyses (e.g. chromosome banding and ADN sequences) will added more evidences to support the specific taxonomic status of the new species from Los Queules.

Telmatobufo ignotus occurs only in his type locality, being this place the more septentrional distribution in the coastal range brought for *Telmatobufo* until now. Recently, Donoso et al. (2010) determined as member of *T. bullocki* two specimens detected in Los Queules Reserve (type locality of *T. ignotus*). However, their conclusion is supported on two weak arguments; a) The examination of a female specimen (not specified by the authors), being males characters more important in *Telmatobufo* species determination (Formas et al. 2001); and b) A comparison of the 16s ribosomal mtDNA sequence between the specimen from Los Queules and *T. bullocki* from Caramavida River, being also this argument controversial since this molecular marker has been suggested just as a complement of the COI (Cytochrome oxidase I) in vertebrates DNA bar-coding (Vences et al. 2005). Moreover, this approach has not yet been implemented in Chilean anurans and its reliability is widely debated at present. Based on these arguments, that specimen is primarily suggested as a member of *T. ignotus* herein. These new data extend the northern distribution of *Telmatobufo* in 50 km from Quirihue (Escobar et al. 2005) and 200 km from the Mountain range of Nahuelbuta (Ramadillas), being both localities the most recent records known for the genus (Cuevas & Cifuentes 2009). However, because the geographical notes published by Escobar et al. (2005) were not accompanied by a morphological description or photography of the animals detected, at the light of the new species description, the taxonomic status of that material will remain undetermined, until a detailed examination of an adult specimen, since it might correspond to another locality of *T. ignotus*.

The taxonomic status of the previously knew species of *Telmatobufo* (*T. australis*, *T. bullocki* and *T. venustus*) has been well established (Formas et al. 2001). Nevertheless its phylogenetic relationships have been recently revised (Frost et al. 2006). For a long time *Telmatobufo* remained

grouped in the tribe Calyptocephalellini of the family Leptodactylidae beside *Caudiverbera* (Lynch 1978). But recently, Frost (2009) “upgraded” the tribe status to Calyptocephalellidae to imbed *Telmatobufo* and *Caudiverbera*; following chromosomal arguments given by Formas & Espinoza (1975), Córdova & Descailleaux (2005) which suggest that *Batrachophrynus* is not phylogenetically close to *Caudiverbera* and discarding Batrachophrynidae (*Batrachophrynus*, *Caudiverbera*, *Telmatobufo*) sensu Frost et al. (2006). The present hypothesis of classification (Calyptocephalellidae) agree with San Mauro et al. (2005) whom suggest 120 my of a split of *Calyptocephalella* from Australian Myiobatrachids. San Mauro et al. (2005) also suggest that the current presence of *Calyptocephalella* (= *Caudiverbera*) restricted to geographic refuges indicates “that these early neobatrachians may have been more widespread but were outcompeted by the more modern hyloid and ranoid radiations in large parts of their original distribution area”. The occurrence of *Telmatobufo* (a member of Calyptocephalellidae) in Los Queules agrees with this biogeographic hypothesis. The wider *Telmatobufo* dispersion in coastal range could be explained by the association of this ancient fauna with a broad distribution of *Nothofagus* forest towards the north (*Caudiverbera* reach the 32° LS in their northern range), which could have provided refuge for the amphibians during the height of glaciations in the south of Patagonia (Vuilleumier 1968).

Nowadays, the global amphibian declining is a well-known issue (Beebe & Griffith 2005) and has been attributed to several causes, especially habitat loss and disease. Becker et al. (2007), identified a further factor, namely “habitat split” (defined as human-induced disconnection between habitats used by different life history stages of a species) which forces forest-associated amphibians with aquatic larvae to make risky breeding migrations between suitable aquatic and terrestrial habitats. The habitats of the Chilean frogs such as the Chilean winter Valdivian rainy forest have suffered a rapid deterioration in the last 30 years. Because of the loss of at least 70 % of plants species and the increasing menace experienced by its fauna (including frog species), this ecosystem is considered one of 25 planet’s diversity hotspot (Conservation International 2007). Unfortunately scarce knowledge exists concerning the adaptation of amphibians to this new distressing habitat status (Bustamante & Simonetti 2005, Cuevas & Cifuentes 2009). According to Formas et al. (2001) specimens of *Telmatobufo* were collected at the border of a road (under a rock), and Escobar et al. (2005) detected specimens within a plantation of *Pinus radiata*, 90 meters distant from native forest. This suggests a wide home range to these species and a series of additional problems distressing them, such as, border effect and refuge loss. Furthermore, *Telmatobufo* species possess a free life tadpole of mountain stream environment, with a mouth-

like sucker (Formas *et al.* 2001, Cuevas & Cifuentes 2009), implying that larvae develop preferably in streams with big stone substratum (Ortiz & Ibarra-Vidal, 2005) and very strong water flowing, where they stay on at least two years before reaching the metamorphosis. This life strategy makes it a very vulnerable species (Formas *et al.* 2001, Cuevas & Cifuentes 2009). Conservation efforts until now have been focused in more emblematic species like *Rhinoderma darwinii* or *Rhinoderma rufum*, leaving other species without awareness. The type locality of *Telmatobufo ignotus* is a very small remnant of Maulino forest, and constitutes an immersed island in a matrix of *Pinus* and *Eucalyptus* (Ortiz & Ibarra-Vidal, 2005). The stream flowing through this remnant shows a high grade of sediment accumulation (dragged by the rain from nearby pines plantations), putting in danger the reproductive habitat of these animals and its viability as a species because of habitat split.

Taxonomy can have a profound and instant impact on conservation planning and decisions and may even be able to force some taxonomists to consider concealing locality data to prevent the exploitation of species habitat (Stuart *et al.*, 2006). The new species described herein, is a new record within a National Reserve (SNASPE= National Service of Wild Protected Areas of the State) for one of the more primitive Gondwanic genera of frog of South America. Considering the progressive habitat degradation and the fact that most of the *Telmatobufo* population occurs in unprotected areas, this information represent an important argument in support of protecting remnants of native forest in areas where this ecosystems has been seriously altered.

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Examined material

- Telmatobufo australis* (1 adult male) San Pablo de Tregua, Panguipulli.
- Telmatobufo australis* (3 tadpoles) Chaiguin, Corral.
- Telmatobufo bullocki* (1 adult female). El Vergel, Angol.
- Telmatobufo venustus* (1 adult male). Altos de Vilches, Talca.
- Telmatobufo venustus* (1 tadpole). Altos de Vilches, Talca.

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