

## A new species of *Alsodes* (Amphibia: Anura: Leptodactylidae) from southern Chile

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*Abstract.*—A new species of frog, *Alsodes kaweshkari*, is described from the temperate *Nothofagus* forests of South America in Southern Chile. From the karyological point of view this species is included in the *monticola* group (2N = 26) of the genus *Alsodes*.

The genus *Alsodes* Bell 1843, is distributed in central and southern Chile and along the eastern slope of the Andes, in Argentina (south of Mendoza city). Frost (1985) included the following species in the genus: *A. barrioi*, *A. gargola*, *A. illotus*, *A. laevis*, *A. montanus*, *A. monticola*, *A. nodosus*, *A. pehuenche*, *A. tumultuosus*, *A. vanzolinii*, and *A. verrucosus*. Formas (1989) studied the identity and synonymy of the Chilean frog *Eupsophus vittatus* and concluded that this species must be included in the genus *Alsodes* as *A. vittatus*. The taxonomic status of *A. illotus* is not clear (Ceï 1980) and according to Frost (1985) *A. laevis* has not been collected since its description by Philippi (1902). The most remarkable characteristic of the males of this genus is the presence of thorny structures on the fingers and round spiny patches on the chest.

During the course of collecting frogs in southern Chile we found a new species of *Alsodes* which is here described. Moreover, we describe the karyotype and C-banded chromosomal pattern of the new taxon. The bands of hemoglobin of the new species were characterized electrophoretically and compared with those of *A. monticola* (type species of the genus).

### Methods and Materials

Specimens were deposited in the Instituto de Zoología, Universidad Austral de Chile (IZUA). Adults were measured with a dial

caliper to the nearest 0.1 mm according to Ceï (1962). The following measurements were taken: snout–vent length (SVL), head length, head width, nostril–snout distance, tibia length, and foot length. Internarial distance was measured according to Ceï (1980) and eye diameter according to Duellman (1970).

The chromosomal characteristics were obtained from the holotype (IZUA 1624) according to the following methods: the animal was treated with 0.1% colchicine for two hours, then anaesthetized with diethyl ether, cut open ventrally under sterile conditions and its intestine carefully removed. Metaphase plates were obtained by squashing intestinal epithelium fragments that were hypotonically treated with distilled water, then fixed in acetic-alcohol (1:3), and finally placed in 45% acetic acid. Small tissue fragments were squashed between a glass slide and cover slip and dipped in liquid nitrogen; thereafter, the cover slip was removed with a razor blade to allow chromosomes to air dry. After 3 days, the chromosomes were stained for 15 minutes in Sorensen's phosphate buffer (pH 6.8), containing 4% Giemsa solution (Formas 1991). Centromeric positions were determined according to Levan et al. (1964). Secondary constrictions were included in the measurements. Chromosomes were stained to reveal C-band pattern position (Sumner 1972). Microscopic slides and the specimen (IZUA

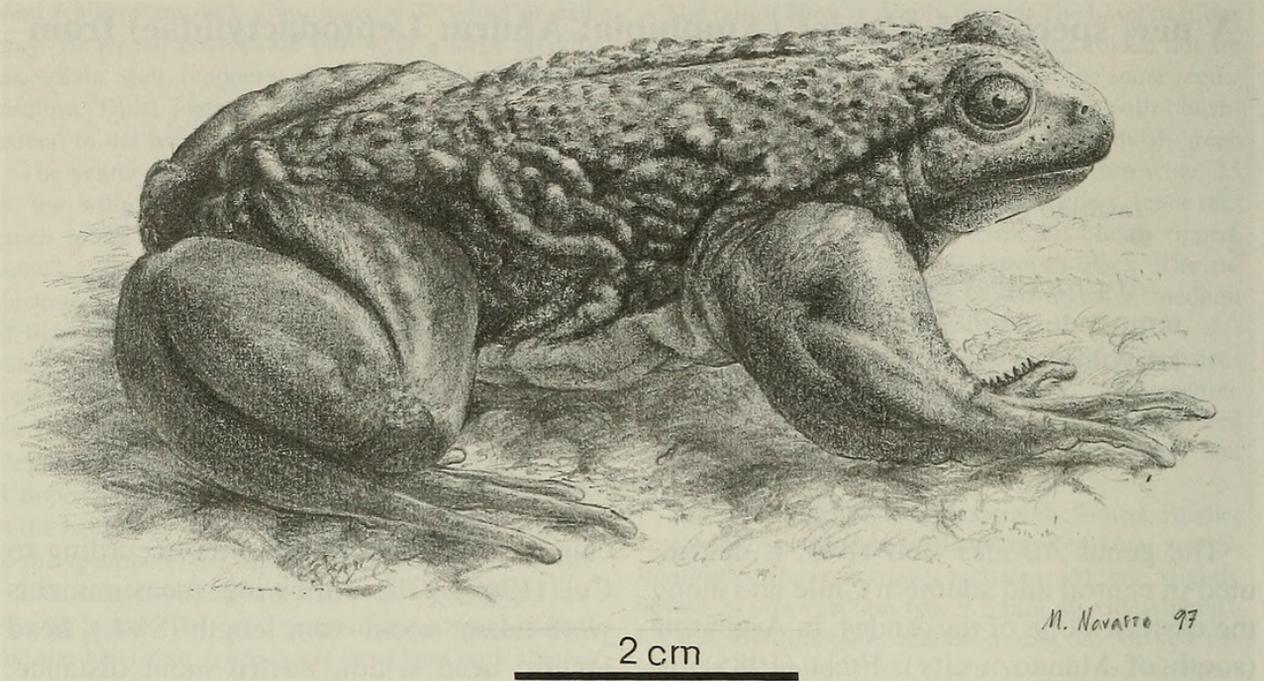


Fig. 1. *Alsodes kaweshkari* Holotype, IZUA 1624.

1624) were deposited in the amphibian collection of the Instituto de Zoología, Universidad Austral de Chile.

Hemoglobin was obtained according to the following methodology: blood was collected by cardiac puncture from the holotype and a specimen of *Alsodes monticola* (control species) using a sterile syringe with 3.8% of sodium citrate as anticoagulant; the red cells were washed 3 times with 0.85% NaCl and centrifuged at 700 g for 5 minutes at room temperature (20°C). The packed cells were hypotonically treated with distilled water (10 volumes) for 30 minutes and centrifuged at 3500 g for 10 minutes. The supernatant was stored at -70°C. The hemolyzate was treated according to the method described by Muir (1981) with modifications. We used a minicolumn (5 × 70 mm) of Sephadex G-50 equilibrated with 1.0 mM of potassium phosphate (pH 7.0). Fractions of 500 µl were collected and their absorbances measured at 410 nm, the wave length at which the porphyrin ring of the hemoglobin molecule absorbs specifically. Those fractions with the highest absorbance value were used for the electrophoretic analysis of the hemoglobin of the

new species and *A. monticola*. To proceed with the electrophoresis the samples were denatured by heating (100°C, 5 minutes) in presence of 2% of betamercaptoethanol. Electrophoresis of the hemoglobins from *Alsodes kaweshkari* and *A. monticola* was carried out individually in vertical slab gels of polyacrylamide (15%) in denaturant conditions at 20 mA for 20 hours (Laemmli 1970). Gels were stained with Coomassie Blue R-250 for 2 hours and destained with acetic acid (7%). The molecular weight of the hemoglobin bands was estimated using a protein standard for molecular weight (Sigma Co).

#### Systematics

##### *Alsodes kaweshkari*, new species.

##### Fig. 1

*Holotype*.—IZUA 1624, an adult male collected by J. Ramón Formas on 8 March 1995, at Puerto Edén (49°8'S, 74°25'W, 10 m), Wellington Island, Ultima Esperanza Province, Magallanes and Chilean Antarctic Region (XII Región), Chile (Fig. 2).

*Paratype*.—IZUA 1625, an adult male collected by Pablo Corti on 21 Oct 1995,

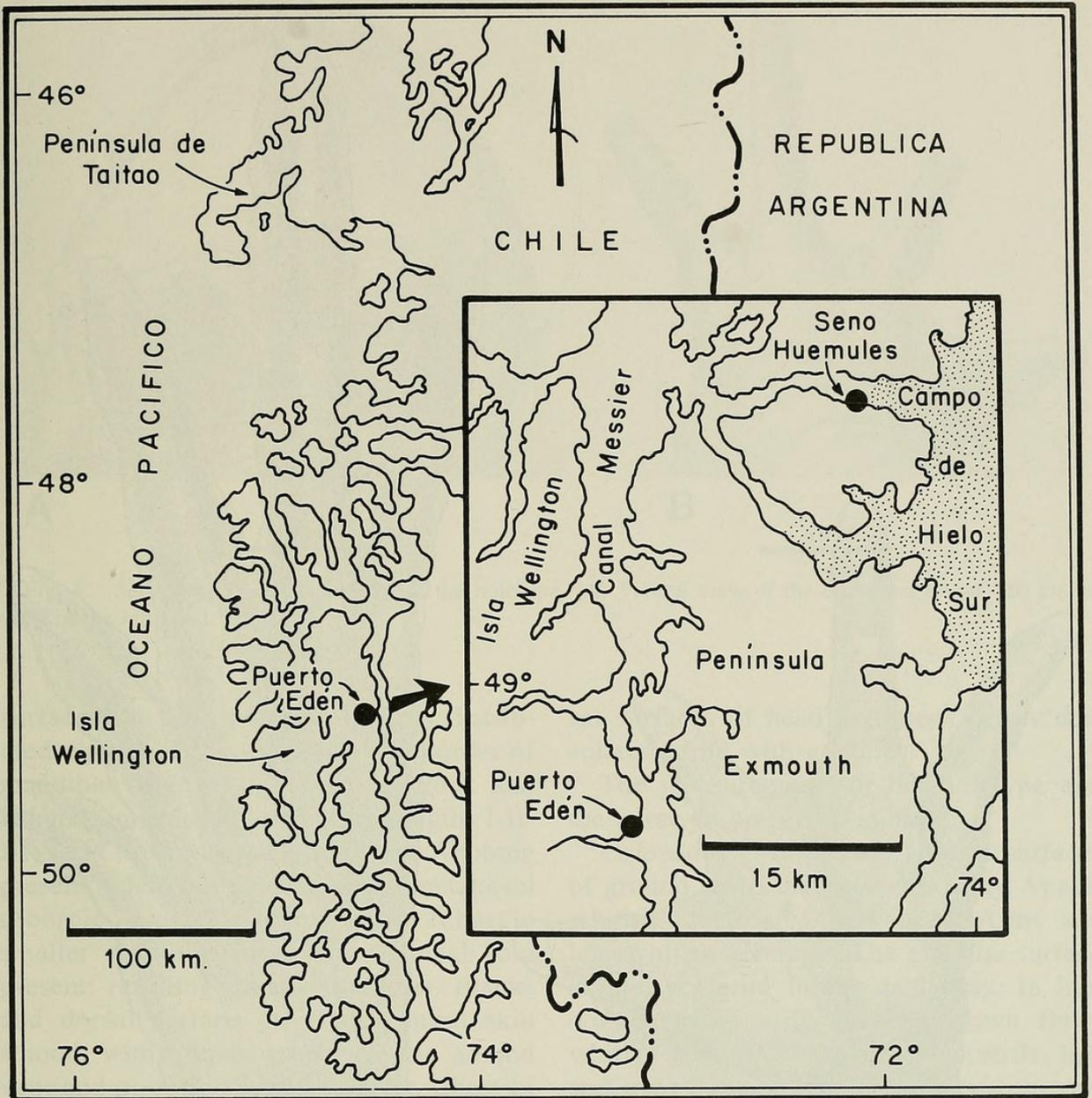


Fig. 2. Type locality of *Alsodes kaweshkari*.

at Seno Huemules ( $48^{\circ}43'5S$ ,  $74^{\circ}25'W$ , 8 m), Ultima Esperanza Province, Magallanes and Chilean Antarctic Region (XII Región), Chile (Fig. 2).

*Diagnosis.*—*Alsodes kaweshkari* can be distinguished from its congeneric species by the following combination of characters: SVL 56.5–62.2 mm; toes totally fringed; webbing of feet present between all toes, but reduced; dorso-lateral surfaces granular; skin around vent and posterior thighs granular.

*Description.*—Based on the type series.

Body robust, arms and legs well developed. Head depressed, slightly wider than long, its length 29% of snout–vent length. Snout truncated from above, rounded in lateral profile; canthus rostralis slightly rounded, loreal region concave in cross section; nostrils anterolateral, midway between tip of snout and anterior border of eye; eye diameter greater than distance between eye and nostril; internarial distance less than interorbital distance. Tympanum absent; postocular fold evident, reaching insertion of arm. Tongue rounded, without notch at tip.

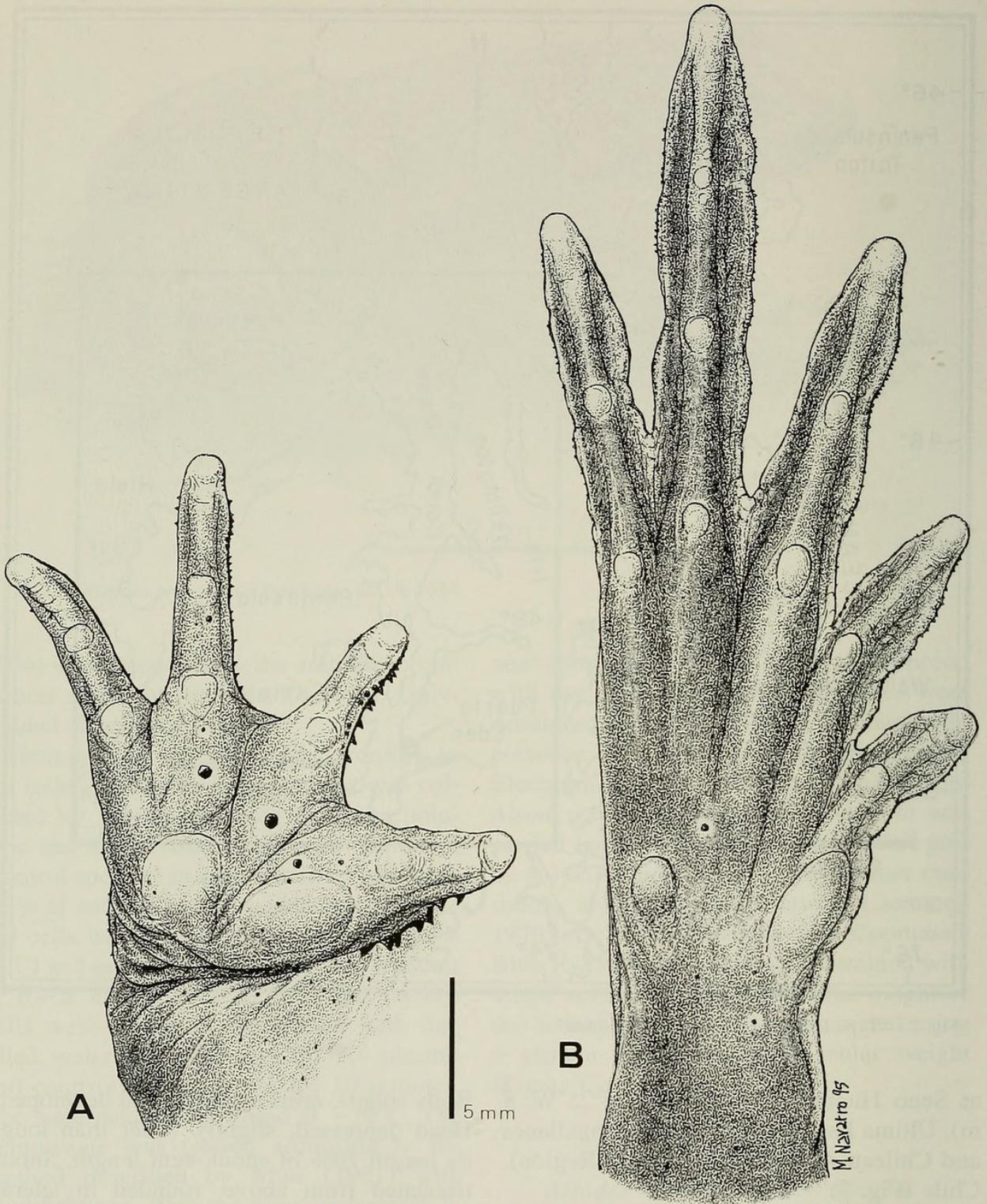


Fig. 3. Morphological details of the holotype. (A) Palmar view of the right hand. (B) Plantar view of the right foot.

Choanae oval-shaped; dentigerous processes of vomer between choanae. Forelimbs of males robust. Fingers in order of increasing length: II, I, IV, III. Webbing of hand absent. Fingers long with moderately globular

tips. Inner palmar tubercle ovoid, outer palmar tubercle rounded; subarticular tubercles rounded, (Fig. 3A). First finger with strong thorny excrescences; second finger with narrow band of spines (Fig. 4B). Palmar

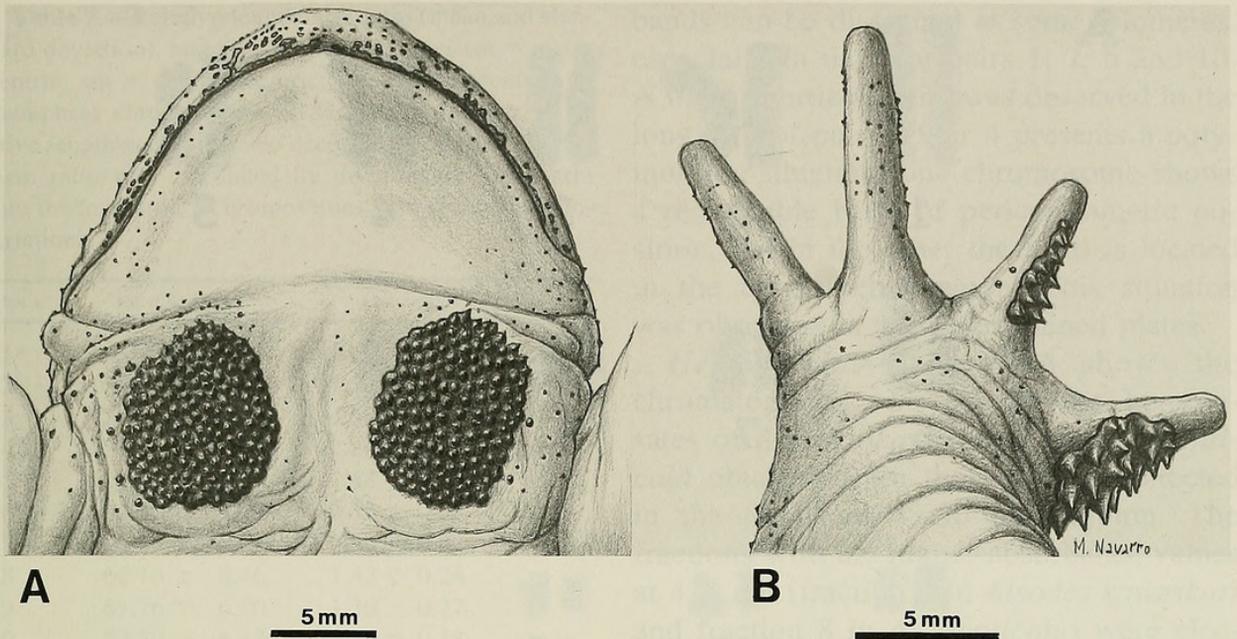


Fig. 4. Secondary sexual characters of the holotype. (A) Ventral view of the chest and throat. (B) Dorsal view of the left hand.

surface with few thorns irregularly distributed. Spines also present at lower border of mandible (Fig. 4A). Toes long, thick and fringed; in order of increasing length: I-II-III-V-IV; tips moderately rounded. Webbing present, thick, but reduced. Inner metatarsal tubercle oval, elongate; outer tubercle smaller than the inner, oval. Tarsal fold present; reaching middle of tarsus. Flanks and dorsal surface granular; ventral skin smooth with minute granules; skin around vent and posterior thighs granular. Chest of males with two bilateral rounded black patches of keratinous spines (Fig. 4A). Dor-

sal surfaces of head and areas below dorsolateral fold with granules.

The measurements of the holotype and the paratype are given in Table 1.

*Coloration.*—In alcohol, dorsal surfaces of ground, arms and legs dark gray. Venter whitish, gular area dark gray. Arms and legs whitish ventrally. The granular surface around posterior thighs dark gray. In life, dorsal surface, arms and legs brown. Belly whitish yellow. Areas around nostrils, lips and eyes light brown. The iris with a coppery reticulum.

*Distribution and ecology.*—The species is known from two Chilean localities (Puerto Edén and Seno Huemules) (Fig. 2). The type locality (Puerto Edén) is a small beach of the Wellington Island, near a small fishing town. There are small forests (*Nothofagus betuloides*, *Embothrium coccineum*, *Maytenus magellanicus*, and *Drymis winteri*) surrounded by typical tundra with plants of the genera *Donatia*, *Astelia*, and *Azorella* and rushes of the families Cyperaceae and Juncae. This area is situated in the oceanic cold temperate region (di Castri 1968). The annual mean temperature of this region is 8.8°C; the relative humidity is

Table 1.—Measurements (mm) of the type series of *Alsodes kaweshkari*.

Character	Holotype IZUA 1624 male	Paratype IZUA 1625 male
Snout-vent length	62.2	59.9
Head length	17.7	16.4
Head width	24.2	22.8
Eye diameter	7.0	6.7
Nostril-snout distance	4.0	3.6
Internarial distance	5.7	5.4
Tibia length	27.7	27.4
Foot length	33.8	30.9

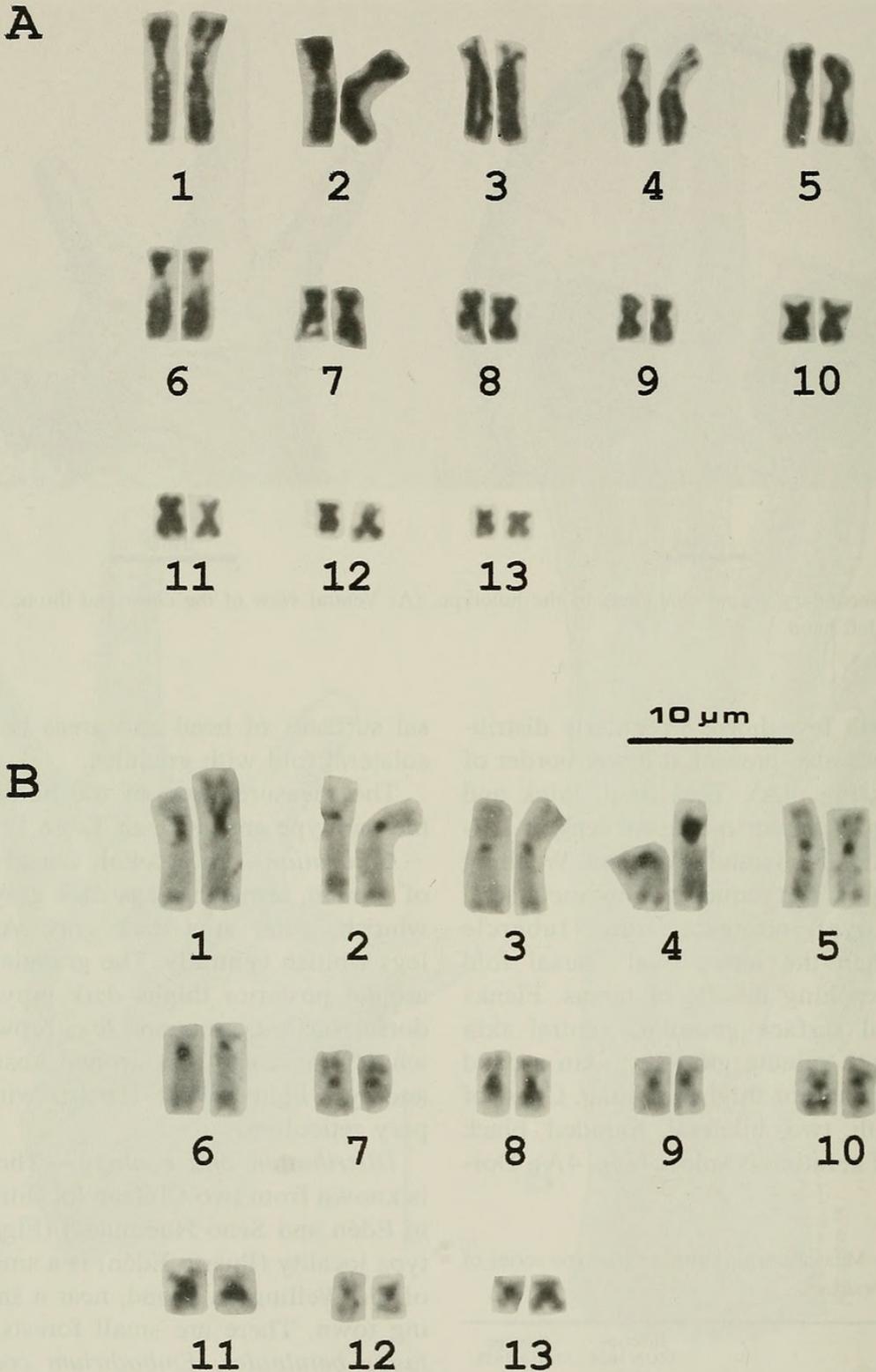


Fig. 5. Chromosomes of the holotype. (A) Karyotype. (B) C-banded karyotype.

87%, and the rain fall ranges from 2000–2500 mm per annum. The frog was collected under a log between the forest and the tundra. The male holotype shows its remarkable thorny excrescences on the chest

and fingers (Fig. 4A, B). The following amphibians were also collected in this area: *Bufo variegatus*, *Batrachyla antartandica* and *Eupsophus calcaratus*. Lynch (1975) pointed out the presence of *Atelognathus*

Table 2.—Relative length, arm ratio (mean and standard deviation), and type of chromosomes (m = metacentric; sm = submetacentric; st = subtelocentric) of metaphase chromosomes of *Alsodes kaweshkari*. Relative length was calculated according to Bogart (1970). Arm ratio was calculated by dividing the short arm into the long arm. \*Chromosomes with secondary constrictions.

Pair n°	Relative length	Arm ratio	Type
1*	148.72 ± 18.32	1.97 ± 0.20	sm
2	135.58 ± 18.76	1.71 ± 0.63	sm
3	126.80 ± 10.50	4.69 ± 0.48	st
4*	116.74 ± 12.13	2.28 ± 0.74	sm
5	102.27 ± 12.27	1.43 ± 0.50	m
6*	92.39 ± 7.21	2.05 ± 0.60	sm
7	74.03 ± 8.03	2.10 ± 0.32	sm
8	66.16 ± 6.46	1.42 ± 0.24	m
9	61.76 ± 6.00	1.30 ± 0.27	m
10	57.29 ± 6.43	1.20 ± 0.18	m
11	51.95 ± 5.54	1.90 ± 0.15	sm
12	48.73 ± 3.71	1.50 ± 0.27	m
13	39.75 ± 5.59	1.40 ± 0.12	m

*grandisonae* and Diaz & Nuñez (1988) reported *Alsodes verrucosus* (adult and tadpoles). The other locality where *Alsodes kaweshkari* was collected is Seno Huemules. This area has the same ecological characteristics as Puerto Edén. The male paratype, collected at a border of a cold stream, also has the nuptial asperities on fingers and chest.

**Chromosomes.**—Examination of 10 metaphase plates from the holotype revealed a diploid number of  $2N = 26$ . All chromosomes are bi-armed and the fundamental number (NF) is 52. Pairs 5, 8–10, 12, 13 are metacentric, pairs 1, 2, 4, 6, 7, 11 are submetacentric, and pair 3 is subtelocentric. Pairs 1 and 4 have secondary constrictions on the smaller arm, and pair 6 exhibits secondary constrictions on the longer arm. The karyotype of *Alsodes kaweshkari* is shown in Fig 5A. A summary of the relative length, arm ratio, and type of chromosomes is presented in Table 2.

The C-banded karyotype, based on five plates (Fig 5B) shows constitutive heterochromatin in the pericentromeric region of all chromosomes. Thin heterochromatic

bands can be discerned at some telomeres, especially in those of pairs 1, 2, 6 and 10. A thin interstitial band was observed in the long arm of pair 5. Pair 4 presents a polymorphic situation: one chromosome shows a remarkable band of pericentromeric position, but in the other the band is located in the centromeric region. This situation was observed in all the examined plates.

**Hemoglobin.**—Figure 6A shows the chromatographic profiles of the hemolysates of *Alsodes kaweshkari* and *A. monticola* obtained from the fractions collected in the Sephadex G-50 minicolumn. The fractions with the higher absorbance values at 410 nm (fraction 9 in *Alsodes kaweshkari* and fraction 8 in *A. monticola*) were electrophoretically analyzed under the experimental conditions used in this study. Figure 6B indicates the electrophoretical patterns obtained for these species. Both taxa share a similar two-banded pattern with an anodal band (Hb<sub>1</sub>) of 14.2 KDa and another cathodal one (Hb<sub>2</sub>) of 16.8 KDa.

**Etymology.**—The new taxon is named for the Kaweshkar indians, a brave people, hunters of sea-lions, who still live in Puerto Edén, the type locality of the new species.

### Comparisons

When the adult snout–vent length of *Alsodes kaweshkari* (males, 59.9–62.2 mm) is compared with that of the other *Alsodes* species, it is observed that this frog is as large as *A. nodosus* (58.5 mm), *A. barrioi* (59.9 mm) and *A. tumultuosus* (61.5 mm). Other members of the genus are small [*A. vittatus* (37.4 mm), *A. gargola* (40.8 mm), and *A. verrucosus* (42.4 mm)] and medium-sized [*A. monticola* (49.1 mm), *A. montanus* (50.2 mm), *A. vanzolinii* (51.1 mm), and *A. pehuenche* (52.3 mm)]. Among the large-sized frogs, the external morphology of the foot provides useful characteristics to identify each taxon. One example of this is *A. nodosus*, a species characterized by the absence of lateral fringes (present on the toes of *A. barrioi*, *A. kaweshkari*, and *A.*

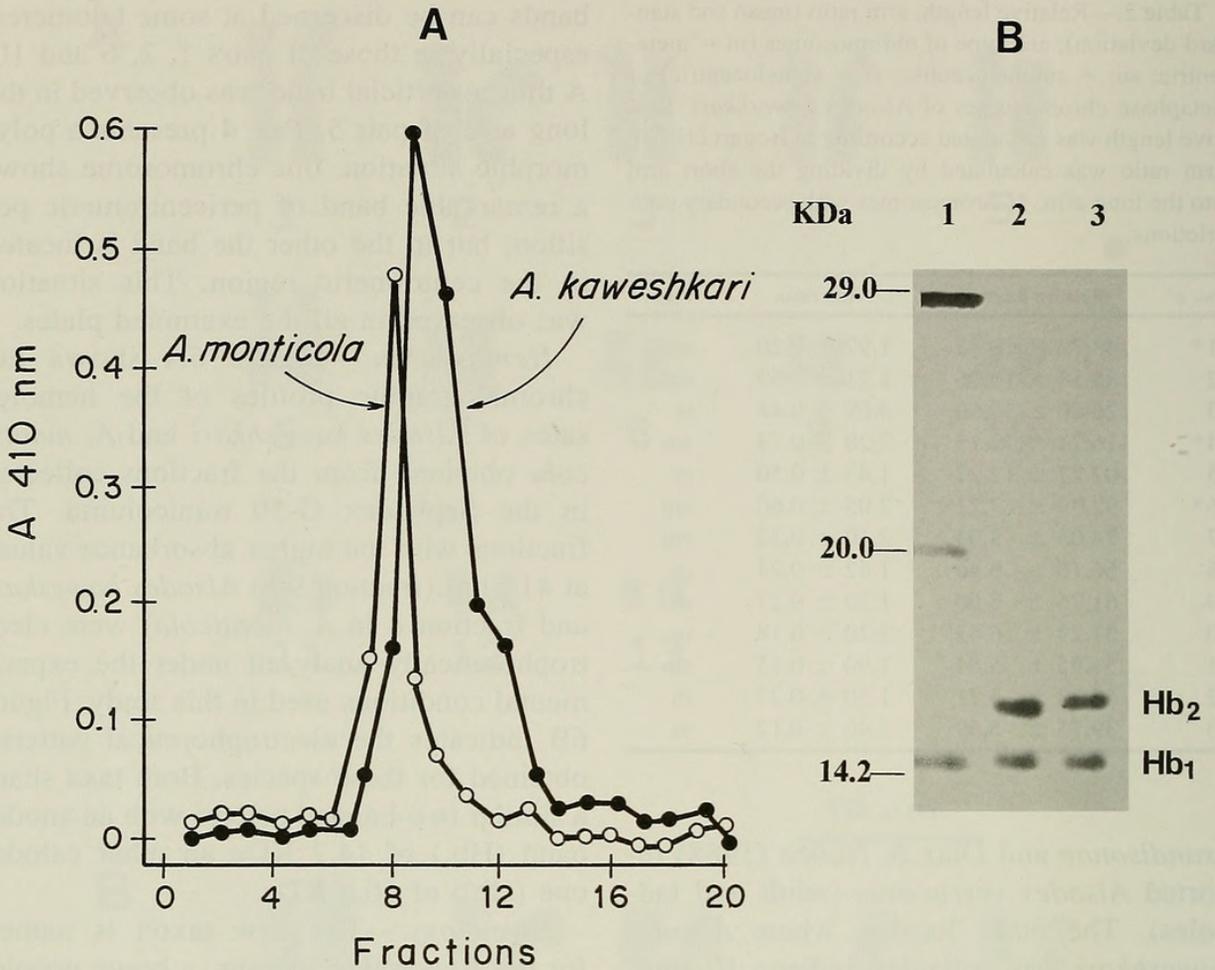


Fig. 6. Hemoglobins of *Alsodes kaweshkari* and *A. monticola*. (A) Chromatographic elution profiles of *A. monticola* and *A. kaweshkari* in Sephadex G-50 minicolumn (B) Electrophoretical patterns of *Alsodes kaweshkari* and *A. monticola*. (1) Standard of molecular weight, (2) *A. monticola*, (3) *Alsodes kaweshkari*.

*tumultuosus*). In the case of this latter species, its toes are long and thin, whilst in *Alsodes kaweshkari* and *A. tumultuosus* they are thick and strongly fringed. These frogs also differ in characteristics of the outer metatarsal tubercle (large in *Alsodes kaweshkari* and small in *A. tumultuosus*). On the other hand, both species differ in webbing; reduced in *Alsodes kaweshkari* and moderately developed in *A. tumultuosus* (Fig. 7A, B).

Formas & Vera (1983) studied the karyological relationships among the member of the genus *Alsodes*. They recognized three groups within the genus: the *barrioi* group ( $2N = 34$ ) (*A. barrioi*), the *monticola* group ( $2N = 26$ ) (*A. gargola*, *A. monticola*, *A. tumultuosus*, *A. vanzolinii*, and *A. verrucosus*), and the *nodosus* group ( $2N = 22$ ) (*A.*

*nodosus*). The presence of 26 chromosomes in *Alsodes kaweshkari* allows its inclusion in the *monticola* group.

Muir (1981) studied the electrophoretical patterns of the hemoglobin molecule of 14 taxa of the frogs of the genus *Xenopus*. The species and subspecies were characterized and grouped according the number of bands obtained in the electrophoretical profiles. *Xenopus borealis*, *X. fraseri*, *X. muelleri*, *X. ruwenzoriensis*, *X. tropicalis*, *X. vestitus* and *X. witteii* show an electrophoretical pattern of two bands (Hb<sub>1</sub>, Hb<sub>2</sub>). *Alsodes kaweshkari* and *A. monticola* show a similar two-banded electrophoretical pattern like that found in some *Xenopus* species. Though our biochemical study was unable to differentiate *Alsodes kaweshkari* and *A. monticola*, this is the first attempt to char-

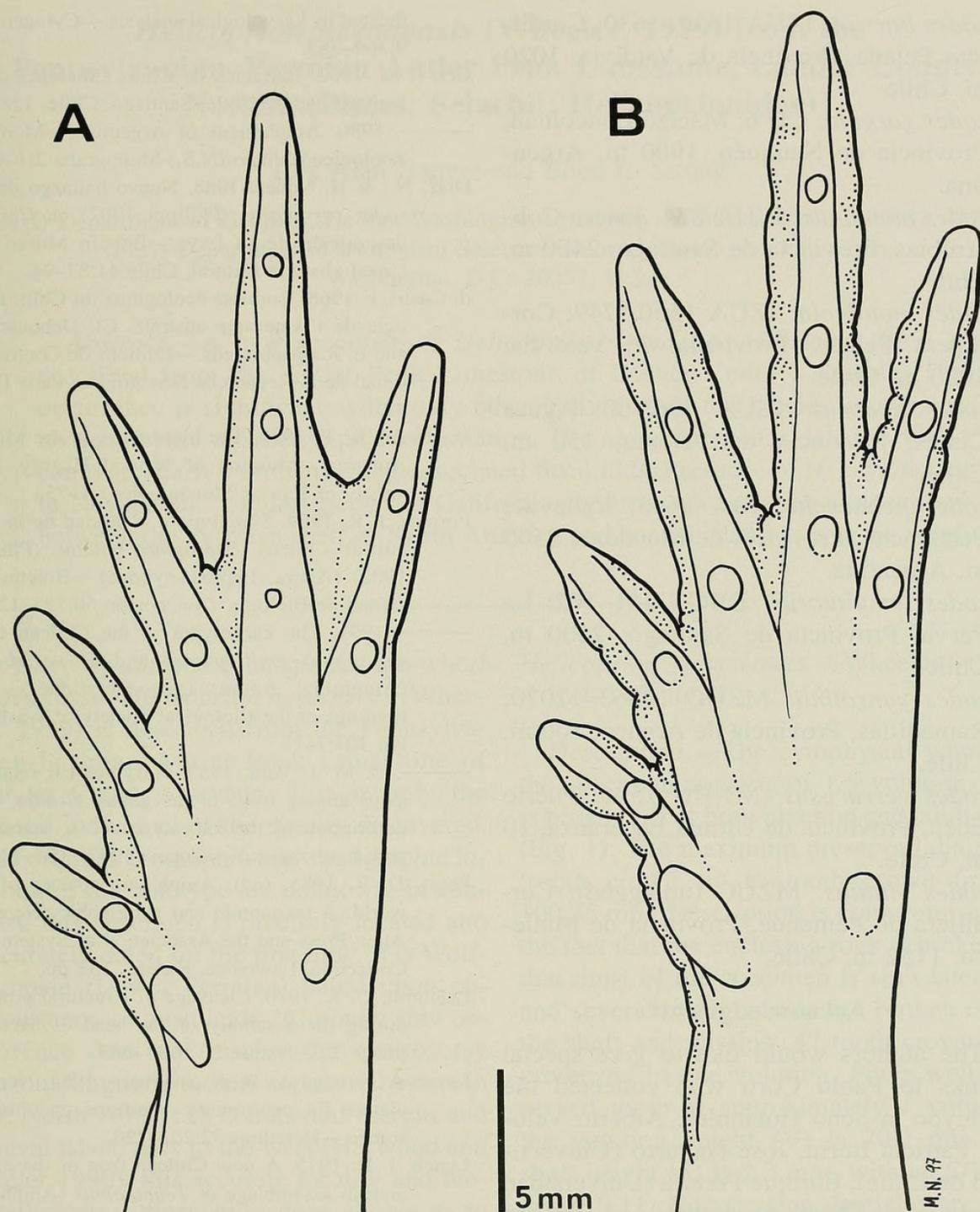


Fig. 7. Schematic plantar views of the left foot of *Alsodes kaweshkari* (A) and *A. tumultuosus* (B).

acterize the molecule of hemoglobin of frogs of the genus *Alsodes*.

#### Specimens Examined

*Abbreviations.*—Carmen Ubeda (personal collection) (CU), Argentina; Departamento de Biología Celular y Genética, Universidad

de Chile (DBCG), Chile; Instituto de Biología Animal, Universidad Nacional de Cuyo (IBA), Argentina; Instituto de Zoología, Universidad Austral de Chile (IZUA), Chile; Museo Nacional de Historia Natural (MNHN), Chile; Museo de Zoología, Universidad de Concepción (MZUC), Chile.

- Alsodes barrioi*: IZUA 1629–1630; Cordillera Pelada, Provincia de Valdivia, 1020 m, Chile.
- Alsodes gargola*: CU 6; Macizo Loncoluán, Provincia de Neuquén, 1900 m, Argentina.
- Alsodes montanus*: IZUA 824; Estero Cobarrubias, Provincia de Santiago, 2400 m, Chile.
- Alsodes monticola*: IZUA 1550,1749; Cordillera Pelada, Provincia de Valdivia, 1020 m, Chile.
- Alsodes nodosus*: IZUA 756,700; Aguas Claras, Provincia de Petorca, 150 m, Chile.
- Alsodes pehuenche*: IBA 1643; Valle del Pehuenche, Provincia de Mendoza, 2500 m, Argentina.
- Alsodes tumultuosus*: DBCG 161–162; La Parva, Provincia de Santiago, 2600 m, Chile.
- Alsodes vanzolinii*: MZUC 12063–12070; Ramadillas, Provincia de Arauco, 100 m, Chile.
- Alsodes verrucosus*: MNHN 1506; Puerto Edén, Provincia de Ultima Esperanza, 10 m, Chile.
- Alsodes vittatus*: MZUC (untagged); Cordillera de Pemehue, Provincia de Malleco, 1152 m, Chile.

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