RESEARCH ARTICLE



# Molecular species delimitation and description of a new species of *Phenacogaster* (Teleostei, Characidae) from the southern Amazon basin

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### Abstract

*Phenacogaster* is the most species-rich genus of the subfamily Characinae with 23 valid species broadly distributed in riverine systems of South America. Despite the taxonomic diversity of the genus, little has been advanced about its molecular diversity. A recent molecular phylogeny indicated the presence of undescribed species within *Phenacogaster* that is formally described here. We sampled 73 specimens of *Phenacogaster* and sequenced the mitochondrial cytochrome c oxidase subunit I (COI) gene in order to undertake species delimitation analyses and evaluate their intra- and interspecific genetic diversity. The results show the presence of 14 species, 13 of which are valid and one undescribed. The new species is known from the tributaries of the Xingu basin, the Rio das Mortes of the Araguaia basin, and the Rio Teles Pires of the Tapajós basin. It is distinguished by the incomplete lateral line, position of the humeral blotch near the pseudotympanum, and shape of the caudal-peduncle blotch. Meristic data and genetic differentiation relative to other *Phenacogaster* species represent strong evidence for the recognition of the new species and highlight the occurrence of an additional lineage of *P. franciscoensis*.

### Keywords

Biodiversity, Characinae, mitochondrial DNA, Neotropical freshwater fishes, Phenacogasterini

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# Introduction

The Neotropical fish subfamily Characinae encompasses small- to mediumsized tetras found across South America and in Panama and Costa Rica (Lucena and Menezes 2003; Mattox et al. 2018). Most members of this subfamily have the anterodorsal region of the body with a gibbosity (except for *Acestrocephalus* Eigenmann, 1910 and *Phenacogaster* Eigenmann, 1907) and diverse trophic strategies, including carnivory, omnivory, and lepidophagy (Géry 1977; Sazima 1984). The subfamily sensu Souza et al. (2022) currently comprises 85 valid species distributed among seven genera: *Acanthocharax* Eigenmann, 1912, *Acestrocephalus*, *Charax* Scopoli, 1777, *Cynopotamus* Valenciennes, 1850, *Galeocharax* Fowler, 1910, *Phenacogaster*, and *Roeboides* Günther, 1864. *Phenacogaster* stands out as the largest and most taxonomically complex genus within Characinae, with 23 species distributed across cis-Andean South American riverine habitats (Fricke et al. 2023). They are small fishes measuring up to 6 cm standard length (SL) and are often known as "lambaris", "glass tetras", "mojaritas", and "yaya" (Lucena and Malabarba 2010).

Relative to other Characinae genera, *Phenacogaster* possesses two longitudinal series of elongate and imbricated scales producing a zigzag pattern in a flat preventral region, as well as the outer premaxillary tooth row divided into a medial and a lateral section separated by a diastema (Eigenmann 1917; Malabarba and Lucena 1995; Mattox and Toledo-Piza 2012). Lucena and Malabarba (2010) presented the most comprehensive taxonomic revision of the genus with descriptions of nine species of *Phenacogaster*, nearly doubling the species diversity, and an identification key for the species, with the exception of the so-called *Phenacogaster pectinata* complex with *P. pectinata* (Cope, 1870), *P. microstictus* Eigenmann, 1909, *P. beni* Eigenmann, 1911 and *P. suborbitalis* (Ahl, 1936). Recently, three more species from the Brazilian Shield have been described: *P. naevata* Antonetti, Lucena & Lucena, 2018; *P. eurytaenia* Antonetti, Lucena & Lucena, 2019 from the Rio São Francisco (Lucena and Lucena 2019).

No study has been conducted to assess the interspecific genetic diversity of *Phenacogaster*, although species delimitation methods have been used for such purposes in other Characidae (Rossini et al. 2016; García-Melo et al. 2019; Brito et al. 2021; Malabarba et al. 2021; Mattox et al. 2023). A recent molecular phylogeny of Characinae revealed the presence of the two clades in *Phenacogaster*, the *P. pectinata* clade and the *P. franciscoensis* clade, as well as an undescribed species of *Phenacogaster* from the Xingu basin (Souza et al. 2022). To further investigate this question, we used mitochondrial data and species delimitation techniques to estimate intra- and interspecific genetic diversity within the genus. The results confirmed the presence of a new species in the upper Rio Xingu of the Amazonian Brazilian Shield, which is formally described in this paper.

# Materials and methods

# Taxon sampling

The molecular analysis encompassed 74 taxa (Suppl. material 4), including 73 specimens of *Phenacogaster* and *Tetragonopterus carvalhoi* Melo, Benine, Mariguela & Oliveira, 2011 as an outgroup. Seventy sequences were generated, and four were retrieved from BOLD/GenBank: one *Tetragonopterus carvalhoi*, two *P. wayana*, and one *P. calverti* (Suppl. material 4). We used *Phenacogaster* specimens collected or received from ichthyological collections, which were identified morphologically using identification key (Lucena and Malabarba 2010). All fishes were collected in accordance with Brazilian law through SISBIO/MMA permit no. 3,245, and collection, maintenance, and analyses procedures were conducted in accordance with international guidelines for animal experiments via CEEAA IBB/UNESP protocol no. 304.

## DNA amplification and sequencing

DNA was extracted from muscles or gills using the extraction method of Ivanova et al. (2006). The cytochrome c oxidase subunit I (COI) gene was amplified by polymerase chain reaction (PCR) using the FishF1/FishR1 and FishF2/FishR2 primers (Ward et al. 2005) or the L6252-Asn/H7271-COXI primers (Melo et al. 2011). PCR amplifications were performed in a total volume of 12.5 µl that included 1.25 µl of 10X buffer, 0.25 µl of MgCl<sub>2</sub> (50 mM), 0.2 µl dNTPs (2 mM), 0.5 µl of each primer (5 mM), 0.1 µl of PHT Taq DNA polymerase (*Phoneutria*), 1.0 µl of genomic DNA (200 ng) and 8.7 µl ddH<sub>2</sub>O. The PCR conditions included an initial denaturation (5 min at 94 °C), 30 cycles of chain denaturation (50 s at 94 °C), primer hybridization (45 s at 50–54 °C), and nucleotide extension (1 min at 68°C), and a final extension (10 min at 68 °C). All PCR products were checked on 1% agarose gels and then purified using ExoSap-IT (USB Corporation) according to the manufacturer's instructions. The purified PCR products were subjected to sequencing procedures with the BigDye Terminator v. 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and purified by ethanol precipitation. PCR products were loaded onto an ABI 3130 DNA Analyzer automatic sequencer (Applied Biosystems).

# Molecular data analysis

Forward and reverse sequences were assembled using Geneious v. 7.1.9 (Kearse et al. 2012) and contigs aligned with MUSCLE (Edgar 2004) using the default parameters. Substitution saturation was determined using Xia et al. (2003)'s approach in DAMBE v5.3.38 (Xia 2013). Nucleotide variation, substitution patterns, and the best-fit model of nucleotide evolution were estimated in MEGA v. 10 (Kumar et al. 2018).

The maximum likelihood (ML) analysis was conducted using RAxML HPC-PTHREADS-SSE3 (Stamatakis 2014) with five random parsimony trees and the GTRGAMMA model on the *Zungaro* server at LBP/UNESP. The neighbor-joining (NJ) tree was estimated with the K2P+G model (Kimura 1980) and 1,000 bootstrap replicates in MEGA v10 (Kumar et al. 2018). Two species delimitation methods were performed: the Assemble Species by Automatic Partitioning (ASAP) analysis (Puillandre et al. 2021) via the webserver (https://bioinfo.mnhn.fr/abi/public/asap/asapweb. html) with Kimura (K80; 2.0); and the Poisson Tree Process (PTP; Zhang et al. 2013) using the ML tree as input, 100,000 generations, and other parameters at default in the PTP webserver (http://species.h-its.org/ptp/). MEGA v. 10 computed K2P+G distances across groups based on their morphological identification. The ASAP, PTP, and genetic distance analyses were conducted without the outgroup.

# Morphological analysis

Morphometric and meristic data were collected on the left side of every specimen whenever possible, following Malabarba and Lucena (1995) and Lucena and Gama (2007). Point-to-point measurements were taken with a precision of 0.1 mm using a digital caliper. Counts of vertebrae, supraneurals, gill rakers, and teeth were obtained from cleared and stained (c&s) specimens prepared in accordance with Taylor and Van Dyke's (1985) methodology. Vertebral counts include the four centra of the Weberian apparatus as separate elements and the compound ural centrum as a single vertebra. Except for head subunits, which are reported as a percentage of head length (HL), other measurements are expressed as a percentage of standard length (SL). In the description, the frequency of each count is mentioned in parenthesis, and the holotype count is indicated with an asterisk. Institutional acronyms follow Sabaj (2020). Specimens from the Xingu basin were determined as types and specimens from Araguaia and Tapajós are listed as non-types. Examined material is organized by acronym and collection number, number of specimens, range of SL, locality, collection date, and collectors. Comparative material is classified according to the alphabetical order of species, and, within a species, it follows the same order as examined material.

# Results

### Molecular species delimitation

Partial COI gene sequences were obtained from 68 specimens representing 13 of the 23 valid species of *Phenacogaster* (56.2%), and for five specimens that represent the species described in this study. The matrix consisted of 678 bp (153 variable sites) and had a nucleotide composition of 24.6% adenine, 27.5% cytosine, 18% guanine, and 30% thymine. In both asymmetrical (Iss.cAsym) and symmetrical (Iss.cSym) topologies, neither transitions nor transversions were found to be saturated by DAMBE. Both ML and NJ trees recovered similar topologies and supported the recognition of *P. lucenae* as a new species (Fig. 1, Suppl. material 1). The best partition provided

by ASAP identified 14 species (1.00 asap-score) (Fig. 1, Suppl. material 2) and supported *P. lucenae* as new species. The PTP analysis defined 15 species and recognized the new species as a distinct lineage (Fig. 1, Suppl. material 3). Both methods recovered the same species limits, except for *P. franciscoensis* which was split in two by the PTP method. The overall mean K2P genetic distance was  $0.077 \pm 0.007$ . Interspecific genetic distances were between  $0.026 \pm 0.007$  and  $0.143 \pm 0.020$ , and intraspecific genetic distances ranged between  $0.000 \pm 0.000$  and  $0.010 \pm 0.003$  (Table 1).

**Table 1.** Pairwise K2P genetic distances and intraspecific genetic variation of *Phenacogaster* species included in this study. Numbers below the diagonal represent the interspecific distance, while the numbers above the diagonal represent the relative standard deviation.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Intraspecific
															genetic
															distances
1. P. microstictus		0.007	0.011	0.012	0.011	0.011	0.016	0.012	0.015	0.016	0.014	0.016	0.016	0.016	-
2. P. prolata	0.026		0.009	0.011	0.009	0.010	0.017	0.013	0.015	0.017	0.013	0.016	0.017	0.016	0
3. P. capitulata	0.050	0.042		0.008	0.010	0.011	0.021	0.015	0.018	0.019	0.017	0.017	0.022	0.020	0
4. P. pectinata	0.076	0.066	0.028		0.010	0.011	0.017	0.016	0.017	0.017	0.016	0.019	0.020	0.018	$0.004 {\pm} 0.001$
5. P. beni	0.058	0.052	0.045	0.061		0.008	0.016	0.014	0.016	0.017	0.014	0.016	0.018	0.018	0
6. P. tegata	0.058	0.053	0.046	0.069	0.039		0.016	0.013	0.015	0.015	0.014	0.017	0.016	0.017	$0.003 \pm 0.001$
7. P. wayana	0.102	0.111	0.123	0.125	0.111	0.110		0.014	0.012	0.016	0.013	0.017	0.014	0.016	0
8. P. maculoblonga	0.071	0.079	0.091	0.113	0.090	0.081	0.086		0.010	0.011	0.009	0.010	0.010	0.010	$0.007 {\pm} 0.006$
9. P. calverti	0.102	0.106	0.106	0.123	0.108	0.101	0.067	0.052		0.011	0.008	0.012	0.011	0.012	0
10. P. franciscoensis	0.114	0.118	0.123	0.131	0.125	0.114	0.106	0.068	0.069		0.010	0.008	0.012	0.012	$0.010 {\pm} 0.003$
11. P. eurytaenia	0.092	0.088	0.103	0.118	0.097	0.093	0.073	0.049	0.042	0.051		0.008	0.010	0.012	$0.001 {\pm} 0.001$
12. P. naevata	0.094	0.094	0.107	0.120	0.100	0.098	0.085	0.050	0.063	0.034	0.029		0.013	0.015	0
13. P. retropinna	0.110	0.110	0.129	0.143	0.126	0.110	0.080	0.053	0.058	0.073	0.050	0.058		0.008	$0.005 \pm 0.002$
14. P. lucenae	0.107	0.104	0.116	0.131	0.123	0.111	0.100	0.051	0.067	0.076	0.061	0.067	0.038		$0.003 {\pm} 0.001$

# Taxonomy

## Phenacogaster lucenae sp. nov.

https://zoobank.org/22F1F5CC-705D-406D-BA0F-7386E434C963 Fig. 2, Table 2

*Phenacogaster* sp. Xingu: Souza et al. 2022: 9, figs 3, 5 [molecular phylogeny; cited in figures also as *Phenacogaster* sp. Xingu].

**Type material.** *Holotype.* MZUSP 126754, 26.7 mm SL, Brazil, Pará, Novo Progresso, Amazon basin, Rio Xingu, stream affluent of Rio Curuá, 08°29'59"S, 54°58'06.1"W, 08 Aug 2015, F.C.P Dagosta, M.M.F. Marinho, P. Camelier, V. Giovannetti.

*Paratypes*: All from Brazil, Amazon basin, Rio Xingu. LBP 15807, 2, 21.5–28.1 mm SL, Mato Grosso, Querência, Rio Feio, 12°33'20.5"S, 52°16'16.1"W, 31 Jul 2012, C. Oliveira, M. Taylor, G.J.C. Silva, J.H.M. Martinez. LBP 15835, 2, (tissue: 64949) 19.3–26.9 mm SL, Mato Grosso, Querência, Rio Suiá-Missu, Rio Feio, 12°31'55.7"S, 52°20'29.8"W, 31 Jul 2012, C. Oliveira, M. Taylor, G.J.C. Silva, J.H.M. Martinez.



**Figure 1.** Maximum likelihood tree based on the cytochrome oxidase c subunit 1 gene (678 bp) of *Phenacogaster*. Vertical bars represent the number of species delimited by ASAP (14) and PTP (15). Gray bars represent the new species. Black bars indicate the other examined *Phenacogaster* species. Numbers near nodes represent bootstrap support for relevant nodes; values < 50% are not shown. Codes before tip names are tissue or database accession numbers.

LBP 16061, 9, 22.9–35.4 mm SL, Mato Grosso, Primavera do Leste, Rio Culuene, Córrego Xavante, 14°38'24"S, 53°55'38"W, 05 Aug 2012, C. Oliveira, M. Taylor, G.J.C. Silva, J.H.M. Martinez. LBP 25217, 1, (tissue: 94032) 30.5 mm SL, Pará, Altamira, Rio Treze de Maio, 08°39'06.9"S, 55°02'09.1"W, 24 Sep 2017, A.C. Dias, C.S. Souza, C. Souto, N. Flausino Jr, R. Devidé. LBP 30738, 1, 38.0 mm SL, Mato Grosso, Primavera do Leste, Rio Culuene, Córrego Xavante, 14°38'24"S, 53°55'38"W, 23 Aug 2021, C.S. Souza, L. Reia, G.S.C. Silva, E.V. Ywamoto. LBP 32224, 1, 30.3 mm SL, Pará, Altamira, Castelo dos Sonhos, Rio Iriri, waterfall in Rio Curuá, 08°19'07"S, 55°05'22"W, 23 Aug 2022, T. Faria, G.S.C. Silva. MZUSP 97621, 49, 21.6–33.6 mm SL (7 c&s, 22.0–32.5 mm SL), Pará, Altamira, Amazon basin, Rio Curuá-Iriri, 08°15'17"S, 55°06'40"W, 27 Oct 2007, J.L.O. Birindelli, L.M. Souza, A.L. Netto-Ferreira, M.H. Sabaj, N.K. Lujan. MZUSP 120058, 28, 20.4–34.9 mm SL (5 c&s, 22.7–29.9 mm SL), collected with holotype.

**Non-type specimens.** LBP 32258, 1, 28.3 mm SL, Mato Grosso, Sinop, Rio Tapajós, Rio Teles Pires, Arroio Sáo José, Tujá, 11°36'04.47"S, 55°25'37.79"W, 25 Aug 2022, T. Faria, G.S.C. Silva. LBP 32321, 14, 19.0–25.7 mm SL (4 c&s, 18.8–22.7 mm SL), Mato Grosso, Guarantá do Norte, Rio Tapajós, Rio Teles Pires, igarapé of Rio Braço Norte, 09°56'54"S, 55°01'50"W, 24 Aug 2022, T. Faria, G.S.C. Silva. LBP 32332, 1, 22.6 mm SL, Mato Grosso, Sinop, Rio Tapajós, Rio Teles Pires, Arroio Sáo José, Tujá, 11°36'04.47"S, 55°25'37.79"W, 27 Aug 2022, T. Faria, G.S.C. Silva. MZUSP 97708, 8, 27.3–32.4 mm SL, Mato Grosso, Santo Antonio do Leste, Araguaia basin, Rio das Mortes, Rio Suspiro, 14°52'30.0"S, 54°05'0.0"W, 18 Jan 2002, N.A. Menezes, O.T. Oyakawa, G.M. Guazzelli, R. Quevedo. MZUSP 118678, 8, 26.9–29.6, Mato Grosso, Santo Antonio do Leste, Araguaia basin, Rio das Mortes, Rio Suspiro, 14°52'30.92"S, 54°05'1.47"W, 17 Nov 2014, F.C.P. Dagosta, W.M. Ohara, V. Giovannetti.

Diagnosis. Phenacogaster lucenae is distinguished from all congeners except P. tegata (Eigenmann, 1911), P. carteri (Norman, 1934), P. napoatilis Lucena & Malabarba, 2010, and P. capitulata Lucena & Malabarba, 2010 by having an incomplete lateral line (vs. complete lateral line). It differs from P. tegata by the presence of a round or slightly longitudinal oval humeral blotch near the pseudotympanum and distant from the vertical through dorsal-fin origin (vs. humeral blotch longitudinally elongated distant from pseudotympanum, closer to vertical through dorsal-fin origin). The new species differs from *P. carteri* by having a humeral blotch in males and females (vs. absence of humeral blotch in both sexes) and from *P. napoatilis* and *P. capitulata* by having a humeral blotch in both sexes (vs. absence of humeral blotch in males). In addition to the incomplete lateral line (vs. complete), P. lucenae differs from P. retropinna Lucena & Malabarba, 2010 by the anal-fin origin at vertical through base of first or second dorsal-fin branched ray (vs. anal-fin origin located posteriorly to that point), and from P. ojitata Lucena & Malabarba, 2010 by the round caudal peduncle blotch slightly reaching over the middle caudal-fin rays (vs. a diamond-shaped caudal peduncle blotch and further extending over the middle caudal-fin rays).



**Figure 2.** *Phenacogaster lucenae* A MZUSP 126754, holotype, 26.7 mm SL, Brazil, Pará, Novo Progresso, Xingu basin, stream affluent of Rio Curuá B LBP 30738, paratype, 38.1 mm SL, Brazil, Mato Grosso, Primavera do Leste, Xingu basin, Rio Culuene, Córrego Xavante C LBP 25217, paratype, 30.6 mm SL, Brazil, Pará, Altamira, Xingu basin, Rio Treze de Maio.

**Description.** Morphometric data summarized in Table 2. Body compressed. Dorsal profile convex from anterior tip of upper jaw to origin of dorsal fin with a slight concavity in occipital region; slightly straight from dorsal-fin base to origin of

	Holotype	Range	Mean	SD
Standard length (SL) (mm)	26.7	24.1-38	29.5	-
Percentages of standard length				
Greatest body depth	31.5	29.4-36.2	32.6	1.8
Snout to dorsal-fin origin	53.3	50.6-55.3	53.1	1.1
Snout to pectoral-fin origin	26.7	26.6-31.5	28.5	1.3
Snout to pelvic-fin origin	42.7	39.1-44.	41.6	1.2
Snout to anal-fin origin	53.8	51.3-58.9	55.4	2.0
Dorsal-fin origin to hypural joint	51.5	48-54.3	51.2	1.6
Dorsal-fin origin to anal-fin origin	31.2	30.2-36.9	33.0	1.7
Dorsal-fin origin to pelvic-fin origin	32.3	31.9-37.6	34.4	1.5
Dorsal-fin origin to pectoral-fin origin	38.9	36-41.3	38.4	1.4
Caudal-peduncle depth	9.1	8.6-11.4	9.8	0.7
Pectoral-fin length	16.6	16.5-22.8	20.2	1.8
Pelvic-fin length	15.2	14.1-20.4	17.5	1.6
Head length	28	24-29.5	27.1	1.2
Percentages of head length				
Snout length	26.5	23.4-31.4	26.8	1.9
Orbital diameter	36.2	34-42.9	37.7	2.1
Interorbital width	27	24.4 - 31.5	27.5	1.9

**Table 2.** Morphometric data of *Phenacogaster lucenae* (*n* = 32 including holotype and paratypes). All from the Rio Xingu. Range includes holotype. SD = standard deviation.

adipose fin and slightly concave from that point to base of dorsal procurrent caudal-fin rays. Ventral profile of body convex from tip of lower jaw to anal-fin origin, straight along anal-fin base, straight to slightly concave from that point to ventral procurrent caudal-fin rays. Preventral area flattened with two longitudinal series of elongate scales overlapping; scales different in shape from remaining body scales and forming zigzag pattern in ventral view. Pseudotympanum triangular extending from region of rib of fifth vertebra to anterior border of rib of sixth vertebra.

Mouth terminal, lower jaw slightly shorter than upper jaw; posterior tip of maxilla reaching vertical at midpoint of second infraorbital. Premaxillary teeth in two rows. Outer row with 6(4), 7(3), 8(4), 9(4), or 10(1) total teeth, divided in medial and lateral sections by gap; medial section with 2(6), 3(9) or 4(1) tricuspid teeth; lateral section with 3(1), 4(4), 5(5), or 6(6) conical teeth. Inner row with 8(1), 9(2), 10(6), 11(4), or 12(3) teeth, 3(2), 4(6), or 5(8) tricuspid teeth followed by 4(3), 5(1), 6(7), 7(3), or 8(2) conical teeth. Maxilla with 20(1), 21(2), 22(1), 23(1), 24(1), 25(1), 26(2), 27(5), or 29(1) conical teeth. Dentary with single row of 14(1), 15(1), 16(1), 17(3), 18(5), 19(3), 20(1), or 21(1) teeth, with 4(2), 5(1), 6(7), 7(5), or 8(1) tricuspid teeth followed by 10(3), 11(5), 12(5), 13(1), or 14(2) conical teeth (Fig. 3).

Dorsal-fin rays ii,8(7) or  $9^{*}(17)$ . Anal-fin rays iii-v,29(2), 30(8), 31(3), 32<sup>\*</sup>(4), 33(6), or 34(1). Pectoral fin rays i,11<sup>\*</sup>(13) or i,12(12). Pelvic-fin rays i,7<sup>\*</sup>(28); its tip reaching beyond anal-fin origin. Lateral line incomplete. Longitudinal line of scales 32(2), 33(2), 34<sup>\*</sup>(19), or 36(4). Pored scales 8(6), 9<sup>\*</sup>(9), 10(3), 11(5), 12(4), 13(1), 14 (3), or 16(1); some specimens with 2(3) or 3(3) perforated scales anterior



**Figure 3.** *Phenacogaster lucenae*, MZUSP 97621, 24.9 mm SL, c&s paratype, lateral view, right side, image flipped. Abbreviations: Aa, anguloarticular; Cm, coronomeckelian; De, dentary; Mx, maxilla; Pmx, premaxilla; Ra, retroarticular. Scale bar: 1 mm.

to last vertical series of scales. Scale series between lateral line and dorsal-fin origin 5(3),  $6^*(22)$ , or 7(5). Scale series between lateral line and anal-fin origin 4(12),  $5^*(14)$ , or 6(4). Gill rakers on upper limb of first gill arch 3(10) or 4(6); gill rakers on lower limb 7(10) or 8(6). Total vertebrae 33(1), 35(9), 36(1), or 37(1): precaudal 14(1), 15(11), or 16(1), caudal 19(1), 20(9), or 21(3). Supraneurals 3(1), 4(13), or 5(2).

**Color in alcohol.** Overall ground coloration pale yellow (Fig. 2A). Dorsolateral region of body with melanophores along margins of scales. Ventrolateral region less pigmented. Thin lines of melanophores accompanying myosepta along flanks, more evident in the hypaxial musculature. Females and males with rounded or slightly lon-gitudinally oval humeral blotch immediately posterior to pseudotympanum, covering roughly three to five scale rows vertically and two to five scales longitudinally. Caudal peduncle with circular patch of melanophores covering whole caudal peduncle depth and reaching base of caudal-fin middle rays. Thin line of melanophores extending along horizontal septum between humeral and caudal peduncle blotches. Anal, pelvic, pectoral, and dorsal fins scattered by small melanophores. Adipose fin hyaline (Fig. 2).

**Color in life.** Overall ground coloration yellowish to golden on slightly translucent background (Fig. 2B, C). Dorsolateral body region with melanophores along margins of scales. Ventrolateral area less pigmented. Humeral blotch rounded or oval with anterior portion black and posterior edge iridescent yellow to orange. Round black blotch on middle portion of caudal peduncle extending vertically over entire caudal peduncle depth and extending posteriorly to proximal portion of caudal-fin middle rays. Some specimens with bright golden or white patches on posterior portion of caudal peduncle blotch, covering base of caudal-fin rays in upper and lower lobes. Thin line of melanophores between humeral and caudal peduncle blotches. Abdominal cavity, opercular series and portion of infraorbitals covered with guanine. All fins orange to yellowish coloration, with anterior halves of caudal-fin lobes more intensely colored. Posterior tip of caudal and dorsal fins scattered by small dark chromatophores (Fig. 2B, C).

**Sexual dimorphism.** Our samples consist of two adult males (MZUSP 97621, 30.4–34.4 mm SL) with hooks on pelvic- and anal-fin rays (Fig. 4). Four to six lateral-most branched pelvic-fin rays with five to nine curved hooks on medial edge of rays, one hook per segment towards the tips and more hooks per segment toward the base of the rays. Hooks more developed and frequent on medial regions of branched rays (Fig. 4A). Anal-fin rays with four to nine curved hooks on the posterior edges of the last unbranched and the first to eleventh branched fin rays. Fin hooks more developed and abundant on anterior branched rays. In most cases, one pair of small hooks per segment, but occasionally two pairs per segment. Hooks in some cases incipient in the form of bumps. A few rays with additional single hook on the anterior edge of distal portion (Fig. 4B).

**Distribution and habitat.** *Phenacogaster lucenae* is known from tributaries of the Rio Curuá-Iriri, Rio Culuene, and Rio Suiá-Miçu (upper Xingu basin), tributaries of Rio das Mortes (upper Araguaia basin), and Rio Teles Pires (upper Tapajós basin), Amazon basin, Pará and Mato Grosso states, Brazil (Fig. 5). The new species was found in association with marginal vegetation (Fig. 6).

**Etymology.** *Phenacogaster lucenae* is named in honor of Dr. Zilda Margarete Seixas de Lucena, an eminent ichthyologist who has significantly contributed to our knowledge of *Phenacogaster* taxonomy. A noun in genitive case.



**Figure 4.** *Phenacogaster lucenae*, MZUSP 97621, 30.4 mm SL, c&s paratype, male **A** pelvic-girdle, ventral view, distal tip of rays damaged **B** anal-fin rays in lateral view. Scale bars: 2 mm.

**Conservation status.** *Phenacogaster lucenae* is found in the upper Xingu, Araguaia, and Tapajós basins, where specimens were collected during focused expeditions. Although deforestation and hydroelectric plants have affected the region, 18 specimens of *P. lucenae* have been collected in recent years (2021–2022), demonstrating a likely high resilience to anthropogenic impacts. Therefore, we suggest the categorization Least Concern (LC) according to the International Union for Conservation of Nature criteria (IUCN 2014, Standards and Petitions Subcommittee).



**Figure 5.** Map showing the distribution of *Phenacogaster lucenae* in the upper Xingu, Tapajós, and Araguaia basins. White star represents the type locality at tributary of Rio Curuá, Xingu basin.

**Comparative material examined.** *Phenacogaster capitulata*: LBP 17802 (6, 27.38–31.97 mm SL), Peru, Pucallpa, Coronel Portillo, Ucayali basin, 08°35'44.2"S, 74°48'04.3"W, 18 Jun 2013, R. Britzke. *P. napoatilis*: MZUSP 38667 (9, 21.5–35 mm SL), Equador, Napo, Napo basin, Rio Jatuncocha, 2km above Laguna Jatuncocha, 1°3'0.00"S, 75°31'4.0"W, 26 Oct 1981, D. Stewart & M. Ibarra. *P. ojitata*: MZUSP 30551 (36.3 mm SL), Brazil, Pará, Rio Curuá, Serra do Cachimbo, rodovia Santarém-Cuiabá, poço de cachoeira, 09°22'0.0"S, 54°52'0.0"W, 15 Aug 1984, M Goulding. MZUSP 97588 (9, 30.5–48.8 mm SL), Brazil, Pará, Altamira, Xingu basin, Rio Curuá, na ponte da BR163, 08°53'54"S, 55°59'20"W, 29 Oct 2007, J. Birindelli, L. Sousa, A.



**Figure 6.** Habitats of *Phenacogaster lucenae* **A** Rio Treze de Maio, Xingu basin, Altamira, Pará, Brazil, 08°39'06.9"S, 55°02'09.1"W (LBP 25217) **B** Córrego Xavante, Rio Culuene, Xingu basin, Mato Grosso, Primavera do Leste, Brazil, 14°38'24"S, 53°55'38"W (LBP 30738). Photographs by CS Souza.

Netto-Ferreira, M. Sabaj, N. Lujan. MZUSP 100922 (22, 28.4–33.5 mm SL; 2 d&c, 31.2-30.5 mm SL), Brazil, Pará, Rio Curuá, Serra do Cachimbo, rodovia Santarém-Cuiabá, poco de cachoeira. *P. retropinna*: LBP 15676 (105, 21.5–43.3 mm SL), Brazil, Mato Grosso, Ribeirão Cascalheira, Xingu basin, Córrego do Gato, 13º09'13.6"S, 51°55'18.7"W, 30 Jul 2012, C. Oliveira, M. Taylor, G. Silva, J. Henriques. LBP 25926 (2, 33.6-36.4 mm SL), Brazil, Mato Grosso, Paranatinga, Xingu basin, Rio Culuene, 13°50'50.8"S, 53°15'40.2"W, 24 Jan 2018, N.F. Junior, N. Estevão, F.A. Machado. MZUSP 30550 (12, 18.5-30.5 mm SL), Brazil, Mato Grosso, Gaúcha do Norte, Rio Xingu, mouth of Rio Culuene and Sete de Setembro, 12°56'0.0"S, 52°49'0.0"W, 23 Jul 1984, M. Goulding, Portugal & Carvalho. MZUSP 81267 (17, 32.9-39.2 mm SL), Brazil, Amazonas, Rio Negro, 00°16'22.0"N, 69°54'3.0"W, 7 Nov 2002, F. Lima et al. MZUSP 99771 (14, 32.0-40.3 mm SL), Brazil, Mato Grosso, Aripuanã, Madeira basin, Rio Aripuanã, Balneário Primavera, a jusante do salto de Dardanelos, 10°09'54"S, 59°26'55"W, 12 Dec 2004, F. Machado, C. Leite, N. Silva, R. Rosa. P. tegata: LBP 7606 (16, 21.7–31.8 mm SL), Brazil, Mato Grosso, Barão de Melgaço, Paraguay basin, Lagoa Marginal rio Cuiabá, 16°11'39.5"S, 55°48'25.1"W, 29 Jan 2021, C. Oliveira, G.A. Lopez, R. Britzke. LBP 7641 (6, 35.8-39.1 mm SL), Brazil, Mato Grosso, Santo Antonio do Leverger, Paraguay basin, 15°46'03.8"S, 55°30'44.5"W, 01 Mar 2009, M. Mehanna, P.A. Campos. MZUSP 35889 (5, 26.9-37.9 mm SL), Brazil, Mato Grosso, Itiquira, Paraguay basin, Rio Piquiri, faz. Santo Antônio do Paraíso, 17°12'0.0"S, 54°9"0.0"W, J.H.B. Medeiros, J.C. Oliveira. MZUSP 96694 (10, 24.8–27.7 mm SL), Brazil, Mato Grosso, Barão do Melgaço, Paraguay basin, Rio Mutum.16°19'30"S, 55°49'59"W, F.A. Machado et al.

# Discussion

This is the first molecular delimitation using barcode sequences of the genus *Phenacogaster* spanning more than half of the known species diversity and supplements the phylogenetic study of the Characinae recently published including 16 species of *Phenacogaster* (Souza et al. 2022). Based on the application of the species delimitation methods, results identified 14 or 15 species (ASAP and PTP) and both supported *P. lucenae* as a new species (Fig. 1). The minor difference of ASAP and PTP results may be attributable to the range of algorithms and implementations, population size, species delimitation methods are useful tools that, when combined with other types of information such as morphological data, may constitute solid evidence for species delimitation (Melo et al. 2016; Mateussi et al. 2020; Lozano et al. 2022).

The *Phenacogaster pectinata* complex (*P. pectinata*, *P. microstictus*, *P. suborbitalis* and *P. beni*) was proposed for widely distributed species characterized by humeral blotch present only in females, humeral blotch absent or restricted to a few chromatophores in males, complete lateral line, and 32–42 branched anal-fin rays

(Lucena and Malabarba 2010). Phylogeny based on genomic evidence supports the group (*P. pectinata* clade) and adds *P. capitulata*, *P. megalostictus*, *P. prolata*, *P. suborbitalis*, and *P. tegata* (Souza et al. 2022). Both topologies of our study concur with the molecular phylogeny (Fig. 1, Suppl. material 1) and adds *P. microstictus* from the Rupununi River as another member of the clade closer to *P. prolata* from the Negro basin (Fig. 1).

*Phenacogaster lucenae* belongs to the *P. franciscoensis* clade (Souza et al. 2022). In fact, these authors sequenced ultraconserved elements for *P. lucenae* (identified there as *Phenacogaster* sp. Xingu) and discovered that it is the sister species of *P. retropinna* (Tapajós and Xingu) (Souza et al. 2022). Lucena and Malabarba (2010) described *P. retropinna* from the Amazonian rivers Negro, Madeira, Xingu, and Araguaia. Here, both molecular and morphological evidence support the distinction between *P. lucenae* and *P. retropinna*. The mitochondrial data analysis revealed a reasonably high genetic divergence (0.038±0.008) between these species (Table 1, Fig. 1).

Lucena and Malabarba (2010) described the endemic *Phenacogaster ojitata* from the Rio Curuá, a left tributary of the Xingu. Unfortunately, there are currently no tissues of *P. ojitata* for molecular analyses. Morphologically, *P. lucenae* can be recognized from *P. ojitata* by the round caudal peduncle blotch slightly reaching over the middle caudal-fin rays and a larger orbital diameter (34–42.9% of HL; see diagnosis). In addition, *P. lucenae* can be distinguished from other *Phenacogaster* with incomplete lateral line by the presence of humeral blotch (vs. absence of humeral blotch in *P. carteri*), presence of humeral blotch in males and females (vs. absence of humeral blotch in males of *P. napoatilis* and *P. capitulata*); humeral blotch near pseudotympanum and distant from vertical line through dorsal-fin origin in males and females (vs. humeral blotch distant from pseudotympanum and near dorsal-fin origin in males and females of *P. tegata*).

Reduction or lack of pores in the laterosensory system is a classic reductive trait in fishes (Myers 1958) and most likely results from the loss of terminal developmental stages as consequence of the body size reduction (Marinho et al. 2021). As stated previously, the incomplete lateral line is present in four of the currently 23 valid species of *Phenacogaster* (Lucena and Malabarba 2010) in addition to *P. lucenae* described here. Although we did not have access to tissue samples from all these species, our results indicate that reduction of the lateral line independently evolved three times in the phylogeny (Souza et al. 2022). We detected incomplete lateral lines in both juveniles and adults of *P. lucenae*, with only six of 32 specimens exhibiting scale interruptions along the lateral line (i.e., incomplete pored series with two or three pored scales towards the end of the scale series, and a long gap of non-pored scales between them). The evolutionary significance for this modification still needs additional research as well as the investigation of sympatric occurrence of species with completely and incompletely developed laterosensory system.

Additional research on *Phenacogaster* can concentrate on taxa that have not been sampled and additional gene sampling. The presence of two distinct lineages of *P. franciscoensis*, one in the Rio São Francisco and another in the Rio Parnaíba is under investigation. Additional undescribed species are also expected for the genus as we increase taxon sampling in research projects. Finally, further research is required to understand the historical biogeographic processes that contributed to the disjunct distribution of the *Phenacogaster* species across the Brazilian Shield.

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# References

- Ahrens D, Fujisawa T, Krammer HJ, Eberle J, Fabrizi S, Vogler AP (2016) Rarity and incomplete sampling in DNA-based species delimitation. Systematic Biology 65(3): 478–494. https://doi.org/10.1093/sysbio/syw002
- Antonetti DA, Lucena CAS, Lucena ZMS (2018) Two new species of *Phenacogaster* (Actinopterygii: Characidae) from the rio Tocantins drainage, Brazil. Ichthyological Exploration of Freshwaters 1075: 1–10. http://doi.org/10.23788/IEF-1075
- Brito PS, Guimarães EC, Guimarães KL, Rodrigues LR, Anjos MR, Katz AM, Carvalho-Costa LF, Ottoni FP (2021) Cryptic speciation in populations of the genus *Aphyocharax* (Characiformes: Characidae) from eastern Amazon coastal river drainages and surroundings revealed by single locus species delimitation methods. Neotropical Ichthyology 19(4): 1–18. https://doi.org/10.1590/1982-0224-2021-0095
- Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340
- Eigenmann CH (1917) The American Characidae, part 1. Memoirs of the Museum of Comparative Zoology 43: 1–102.
- Fricke R, Eschmeyer WN, van der Laan R (2023) Eschmeyer's catalog of fishes: genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp
- García-Melo JE, Oliveira C, Da Costa Silva GJ, Ochoa-Orrego LE, Garcia Pereira LH, Maldonado-Ocampo JA (2019) Species delimitation of neotropical Characins (Stevardiinae): Implications for taxonomy of complex groups. PLoS ONE 14(6): e0216786. https://doi. org/10.1371/journal.pone.0216786
- Géry J (1977) Characoids of the World. TFH Publications, Neptune City, New Jersey.
- IUCN [International Union for Conservation of Nature] (2014) Standards and Petitions Subcommittee. Guidelines for using the IUCN Red List categories and criteria, Version 14 [Internet], Cambridge, 2019. https://www.iucnredlist.org/resources/redlistguidelines
- Ivanova NV, Dewaard JR, Hebert PD (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes 6(4): 998–1002. https://doi. org/10.1111/j.1471-8286.2006.01428.x
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious

Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2): 111–120. https://doi.org/10.1007/BF01731581
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096
- Lozano AP, Lasso-Alcalá OM, Bittencourt PS, Taphorn DC, Perez N, Farias IP (2022) A new species of *Astronotus* (Teleostei, Cichlidae) from the Orinoco River and Gulf of Paria basins, northern South America. ZooKeys 1113: 111–152.https://doi.org/10.3897/zookeys.1113.81240
- Lucena ZMSD, Gama CDS (2007) Phenacogaster apletostigma, nova espécie de peixe do Estado do Amapá, Brasil (Characiformes, Characidae). Revista Brasileira de Zoologia 24: 151–156. https://doi.org/10.1590/S0101-81752007000100018
- Lucena ZMSD, Lucena CASD (2019) A new glass tetra species of *Phenacogaster* from the rio Salitre, rio São Francisco drainage, Brazil (Characiformes: Characidae). Neotropical Ichthyology 17(1): 1–6. https://doi.org/10.1590/1982-0224-20180134
- Lucena ZMSD, Malabarba LR (2010) Descrição de nove espécies novas de *Phenacogaster* (Ostariophysi: Characiformes: Characidae) e comentários sobre as demais espécies do gênero. Zoologia (Curitiba) 27: 263–304. https://doi.org/10.1590/S1984-46702010000200014
- Lucena CAS, Menezes NA (2003) Subfamily Characinae. In: Reis RE, Kullander SO, Ferraris Jr CJ (Eds) Checklist of the Freshwater Fishes of South and Central America. Edipucrs, Porto Alegre, 200–208.
- Malabarba LR, Chuctaya J, Hirschmann A, de Oliveira EB, Thomaz AT (2021) Hidden or unnoticed? Multiple lines of evidence support the recognition of a new species of *Pseudocorynopoma* (Characidae: Corynopomini). Journal of Fish Biology 98(1): 219–236. https://doi.org/10.1111/jfb.14572
- Malabarba LR, Lucena ZMS (1995) *Phenacogaster jancupa*, new species, with comments on the relationships and a new diagnosis of the genus (Ostariophysi: Characidae). Ichthyological Exploration of Freshwaters 6(4): 337–344.
- Marinho MMF, Ohara WM, Dagosta FCP (2021) A new species of *Moenkhausia* (Characiformes: Characidae) from the rio Madeira basin, Brazil, with comments on the evolution and development of the trunk lateral line system in characids. Neotropical Ichthyology 19(2): 1–20. https://doi.org/10.1590/1982-0224-2020-0118
- Mateussi NTB, Melo BF, Oliveira C (2020) Molecular delimitation and taxonomic revision of the wimple piranha *Catoprion* (Characiformes: Serrasalmidae) with the description of a new species. Journal of Fish Biology 97(3): 668–685. https://doi.org/10.1111/jfb.14417
- Mattox GMT, Toledo-Piza M (2012) Phylogenetic study of the Characinae (Teleostei: Characiformes: Characidae). Zoological Journal of the Linnean Society 165(4): 809–915. https://doi.org/10.1111/j.1096-3642.2012.00830.x
- Mattox GMT, van der Sleen P, Toledo-Piza M (2018) Subfamily Characinae. In: van der Sleen P, Albert JS (Eds) Field guide to the fishes of the Amazon, Orinoco, and Guianas (Vol. 115). Princeton University Press, 102–106. https://doi.org/10.1515/9781400888801

- Mattox GMT, Britz R, Souza CS, Casas AL, Lima FC, Oliveira C (2023) Two new species of miniature tetras of the fish genus *Priocharax* from the Rio Juruá drainage, Acre, Brazil (Teleostei: Characiformes: Characidae). Canadian Journal of Zoology 101(4): 248–266. https://doi.org/10.1139/cjz-2022-0136
- Melo BF, Benine RC, Mariguela TC, Oliveira C (2011) A new species of *Tetragonopterus* Cuvier, 1816 (Characiformes: Characidae: Tetragonopterinae) from the rio Jari, Amapá, northern Brazil. Neotropical Ichthyology 9: 49–56. https://doi.org/10.1590/S1679-62252011000100002
- Melo BF, Benine RC, Silva GS, Avelino GS, Oliveira C (2016) Molecular phylogeny of the neotropical fish genus *Tetragonopterus* (Teleostei: Characiformes: Characidae). Molecular Phylogenetics and Evolution 94: 709–717. https://doi.org/10.1016/j.ympev.2015.10.022
- Myers GS (1958) Trends in the evolution of teleostean fishes. Stanford Ichthyol Bulletin 7: 27–30.
- Puillandre N, Brouillet S, Achaz G (2021) ASAP: Assemble species by automatic partitioning. Molecular Ecology Resources 2(2): 609–620. https://doi.org/10.1111/1755-0998.13281
- Rossini BC, Oliveira CA, Melo FA, Bertaco VD, Astarloa JM, Rosso JJ, Foresti F, Oliveira C (2016) Highlighting *Astyanax* species diversity through DNA barcoding. PLoS ONE 11(12): e0167203. https://doi.org/10.1371/journal.pone.0167203
- Sabaj MH (2020) Codes for natural history collections in ichthyology and herpetology. Copeia 108(3): 593–669. https://doi.org/10.1643/ASIHCODONS2020
- Sazima I (1984) Scale-eating in characoids and other fishes. In: Zaret TM (Ed.) Evolutionary ecology of neotropical freshwater fishes. Springer, Dordrecht 9–23. https://doi. org/10.1007/978-94-015-7682-6\_1
- Souza CS, Melo BF, Mattox GM, Oliveira C (2022) Phylogenomic analysis of the Neotropical fish subfamily Characinae using ultraconserved elements (Teleostei: Characidae). Molecular Phylogenetics and Evolution 171: 107462. https://doi.org/10.1016/j. ympev.2022.107462
- Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033
- Taylor WR, Van Dyke GC (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium, Paris 9: 107–119.
- Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PD (2005) DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences 360(1462): 1847–1857. https://doi.org/10.1098/rstb.2005.1716
- Xia X (2013) DAMBE5: A comprehensive software package for data analysis in molecular biology and evolution. Molecular Biology and Evolution 30(7): 1720–1728. https://doi. org/10.1093/molbev/mst064
- Xia X, Xie Z, Salemi M, Chen L, Wang Y (2003) An index of substitution saturation and its application. Molecular Phylogenetics and Evolution 26(1): 1–7. https://doi.org/10.1016/ S1055-7903(02)00326-3
- Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29(22): 2869–2876. https://doi.org/10.1093/bioinformatics/btt499

# Supplementary material I

### NJ tree of species of *Phenacogaster*

Authors: Camila S. Souza, George M. T. Mattox, George Vita, Luz E. Ochoa, Bruno F. Melo, Claudio Oliveira

Data type: phylogenetic

- Explanation note: NJ tree of species of *Phenacogaster*, based on the COI gene. Numbers on branches represent bootstrap support (> 50%) based on 1000 bootstrap pseudoreplicates. Note the position of the new species *Phenacogaster lucenae*.
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Link: https://doi.org/10.3897/zookeys.1164.102436.suppl1

# Supplementary material 2

Best-score results of Assemble Species by Automatic Partitioning (ASAP) delimitation of species of *Phenacogaster* 

Authors: Camila S. Souza, George M. T. Mattox, George Vita, Luz E. Ochoa, Bruno F. Melo, Claudio Oliveira

Data type: analysis

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Link: https://doi.org/10.3897/zookeys.1164.102436.suppl2

# Supplementary material 3

#### Poisson Tree Processes (PTP) delimitation of species of *Phenacogaster*

Authors: Camila S. Souza, George M. T. Mattox, George Vita, Luz E. Ochoa, Bruno F. Melo, Claudio Oliveira

Data type: analysis

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Link: https://doi.org/10.3897/zookeys.1164.102436.suppl3

# Supplementary material 4

# List of the specimens included in the species delimitation analyses

Authors: Camila S. Souza, George M. T. Mattox, George Vita, Luz E. Ochoa, Bruno F. Melo, Claudio Oliveira

Data type: table (docx file)

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# A revision of the Chilean water penny genus Tychepsephus Waterhouse, 1876 (Coleoptera, Psephenidae, Eubriinae), with description of a second species and two larval morphotypes, and notes on other Chilean Psephenidae

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### Abstract

The Chilean water penny genus *Tychepsephus* Waterhouse, 1876 is revised, with descriptions and photographic illustrations of life stages including two larval morphotypes, the pupa of one morphotype, and adults of two species. The pupa of *Tychepsephus* has not been reported previously. *Tychepsephus cekalovici* **sp. nov.** is described, and *Ectopria (Chilectopria) grandis* Pic, 1947, **syn. nov.** is proposed as a new synonym of *Tychepsephus felix* Waterhouse, 1876, which is redescribed. Taxonomic treatment of the adults of both species includes images of the habitus of males and females, morphological variation, and male and female genitalia. Males and females are sexually dimorphic. Information on the habitat of *Tychepsephus* is provided and illustrated with photographs, and the known geographic distribution of the two species is mapped. The occurrence of *Tychepsephus* in Argentina is reported; therefore, the genus no longer can be considered endemic to Chile. The taxonomic status and geographic distribution in South America of other species of Psephenidae, particularly members of the subfamily Eubriinae, is reviewed.

### Keywords

Aquatic beetles, biology, distribution, habitat, life stages, neotropical, sexual dimorphism, South America, synonym

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# Introduction

Members of the family Psephenidae, commonly called water penny beetles, occur on all continents except Antarctica, and are absent from many islands including New Zealand, Hawaii, and Ireland (Lee et al. 2016). The larvae are found in diverse aquatic habitats and mature over multiple years. The adults are mostly terrestrial in nearby riparian vegetation or debris, do not feed, and are short-lived. Pupae are almost always terrestrial.

The subfamily Eubriinae is cosmopolitan and presently consists of 15 described genera. Larval eubriines, depending on larval morphology, live in a variety of habitats ranging from sluggish seeps to moderately swift streams and small rivers. However, the majority, which are not strongly flattened and streamlined, inhabit slowly flowing water. *Tychepsephus* Waterhouse, 1876, until now a monotypic genus, has been known only from Chile. Larvae have been found on the substrates of very small streams to small rivers with moderate to fast current; adults have been collected from adjacent vegetation.

The taxonomy of *Tychepsephus* has been muddled. Waterhouse (1876) described the genus *Tychepsephus* and the species *T. felix* from "Chili" and placed them in the family Psephenidae. Philippi (1887) misspelled the genus as "*Tychepselaphus*" and placed the genus in the family Pselaphidae. Blackwelder (1944) misspelled the genus as "*Tychepsephenus*" and listed *T. felix* under Psephenidae: Psepheninae in his catalog of neotropical Coleoptera, as did Moroni (1985) in his list of Chilean aquatic beetles. The generic name was misspelled as "*Tychepsephenus*" by many authors, but not all, for more than one hundred years dating from Zaitzev (1910) through Passos et al. (2018).

Elgueta and Arriagada (1989) added two species to the list of Chilean Psephenidae, *Ectopria* (*Chilectopria*) grandis Pic, 1947 and *Eubrianax luteosignatus* Pic, 1947, that had been placed in Dascillidae (Pic 1947). Jerez and Moroni (2006) listed *T. felix* and *Ec.* (*C.*) grandis in Psephenidae: Eubriinae, and *Eu. luteosignatus* in Psephenidae: Eubrianacinae, currently accepted subfamily placements. They also cited *Tychepsephus* as a faunal connection of Chile to Australia and New Zealand, and reported the presence of larval *Tychepsephus* specimens in the Museo de Zoologia de la Universidad de Concepción. Zarges et al. (2019) listed three psephenid genera (*Tychepsephus, Ectopria* LeConte, 1853 and *Eubrianax* Kiesenwetter, 1874), and noted that the larvae are found in areas with "high slope, with high speeds of currents, low and stable temperatures, and high concentrations of oxygen," citing *T. felix* as an example. Ashworth and Hoganson (1987) collected adults of *T. felix* in the Central Valley adjacent to Parque Nacional de Puyehue by "trampling water marginal vegetation."

There has been a question of how closely related *Tychepsephus* is to the Australian eubriine *Sclerocyphon* Blackburn, 1892. In her revision of *Sclerocyphon*, Davis (1986) considered the two genera to be potentially congeneric. Lee et al. (2007) constructed a phylogeny of Psephenidae based on morphological characters of the larvae, pupae, and adults. Their phylogenetic tree placed the two genera as distinct but sister genera near the base of the Eubriinae. In their analysis of the Eubriinae, there was a basal trichotomy with the pair (*Sclerocyphon* + *Tychepsephus*) sister to Eubriinae genus A (since

described as *Neoeubria* Shepard & Barr, 2014) and the rest of the Eubriinae genera. When this phylogeny was constructed, the pupae of *Tychepsephus* and Eubriinae genus A (*Neoeubria*) were unknown.

The larva of *Tychepsephus* has also been taxonomically misinterpreted and the generic name incorrectly spelled. Lataste (1897a) published a fairly extensive and accurate description of a "crustacéiforme" larva from near Peñaflor, Chile, that she assigned to the "Pseudo-Névroptère" *Prosopistoma* Latreille, 1833 (now in Ephemeroptera: Prosopistomatidae). However, before that description was published, Lataste discovered that what she described was actually a coleopteran associated with the "Elmides et de Parnides" and issued a correction (Lataste 1897b), but she never associated a specific name with her larva. Later, Artigas (1963) described and illustrated a psephenid larva from the south-central zone of Chile which he believed to be the larva of *T. felix*, although he misspelled the genus as "*Tychepsephenus*." A key to the genera of neotropical psephenid larvae was published by Passos et al. (2018) in which the name was also misspelled as "*Tychepsephenus*."

The initial description of larval *Tychepsephus* (Lataste 1897a) as being "crustacéiforme" is similar to the initial description of the larvae of *Psephenus herricki* (DeKay, 1844), the type species of *Psephenus*, which was originally described as an isopod crustacean (Brown 1983: 1). Other aquatic insect larvae which may be mistaken for *Tychepsephus* larvae, and that have been labelled as such, include various cased Trichoptera larvae and the larvae of Diptera (Blephaceridae: *Edwardsina chilota* Edwards, 1929; Psychodidae: *Maruina* Müller, 1895).

In 2005, at a meeting of the Sociedad Chilena de Entomologia, Elgueta and Guerrero presented a talk entitled "Nuestro Conocimiento de Psephenidae (Coleoptera) en Chile" in which they reviewed the taxonomy, morphology, and biology of the family in general, and of the Chilean taxa in particular. The state of knowledge of Chilean species and future directions for research were discussed (Elgueta and Guerrero 2005).

We conducted a survey of the Chilean aquatic Dryopoidea during 2002–2008, and in the process, discovered an undescribed species of Psephenidae. The primary objectives of this paper are to further clarify the taxonomy of *Tychepsephus*, including the proposal of a new synonymy, to describe the new species and redescribe the type species, and to provide new biological, ecological, and geographical information.

# Materials and methods

Institutional acronyms and other abbreviations

CASC	California Academy of Sciences, San Francisco, California, USA
CONOCET	CONICET-UNPSJB, Chubut, Argentina
EMEC	Essig Museum of Entomology, University of California, Berkeley,
	California, USA
MNHN	Muséum National d'Histoire Naturelle, Paris, France

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MNNC	Museo Nacional de Historia Natural, Santiago, Chile
MCZC	Museum of Comparative Zoology, Harvard University, Cambridge,
	Massachusetts, USA
NHMUK	The Natural History Museum, London, UK
UMC	Enns Entomological Museum, University of Missouri, Columbia,
	Missouri, USA

The following abbreviations are used in the text: **morph** = morphotype; **AB** = larval abdominal segment; *Ec.* = *Ectopria*; *Eu.* = *Eubrianax*; lin. = lines (obsolete, small English unit of length, varying from 1/10-1/40 in.); **Pte.** = Puente (Spanish for "bridge"); **WDS** = William D. Shepard.

# Aquatic sampling

Aquatic sampling of small rivers and streams was conducted during four trips to Chile during the austral summer (December through March) in 2002, 2002–2003, 2007, and 2008. Larvae were collected from the substrate of watercourses by disturbing the gravel and cobbles upstream from an aquatic net. Adult specimens were swept or beat-en from adjacent riparian vegetation. Specimens were preserved in vials of ethanol.

# Study material

In total, 103 adult specimens of *Tychepsephus* were examined during the study and are, or will be, deposited in the various institutions listed above. The larval specimens were not counted, but several hundred were collected. Only one pupa was examined. All larvae and the pupa will be deposited in the EMEC. The types of *Ectopria* (*Chilectopria*) grandis and Eubrianax luteosignatus were borrowed from the MNHN for examination. The type of *Tychepsephus felix* and other specimens of the species at the NHMUK were examined in London, and photographs were later supplied by the museum.

# Laboratory procedures

An American Optical Spencer stereo microscope fitted with a calibrated ocular grid was used for examination and measurement of specimens, as well as a Leica MZ 125 stereomicroscope fitted with a micrometer. Measurements of total body length represent the length of the pronotum plus the length of the elytra, excluding the head and the variable space between the pronotum and elytra. Measurements of body width include both elytra at their widest point. Specimens with separations between the elytra were not measured for width; therefore, the reported length and width measurements may have different "n" numbers. Genitalia from selected male and female specimens were dissected, examined, placed in genitalia vials containing a drop of glycerin and pinned beneath the point-mounted specimens. The terminology used to describe the larvae and pupa follows that of Lee et al. (2007, 2016).

# Specimen label data

Complete label data, not necessarily verbatim, are reported in the "Material examined" sections. Additional clarifying details not found on the labels are provided within square brackets "[]". More complete locality data for all samples containing *Tychepsephus* are presented in Appendix 1. Some of the geographic coordinates and elevations, which were taken in the field in 2002 and 2003 using a hand-held GPS unit, were subsequently discovered to be somewhat inaccurate. In the years following our fieldwork, governmental changes were made to some of the Chilean regional designations. Two regions that were split affect some of our label data: Región Ńuble (XVI) was formed from the northern part of Región Bío Bío (VIII), and Región Los Ríos (XIV), from the northern part of Región Los Lagos (X). Our specimen labels and the data in Appendix 1 reflect the situation at the time of collection.

# Specimen imaging and distribution mapping

Habitus images by the authors were taken using a Visionary Digital BK Plus Lab System fitted with a Canon EOS 7D camera. Images of holotypes were provided by The Natural History Museum, London, UK and the Muséum National d'Histoire Naturelle, Paris, France, as indicated in figure legends. Rachael Diaz-Bastin (California Academy of Sciences) took the genitalic images using a Syncroscopy AutoMontage system. Images were prepared and assembled into plates using Adobe PhotoShop Elements. Additional digital photographs were provided by Nicolás Román (CONI-CET), Mario Elgueta, Marcelo Guerrero (MNNC) and Robert Sites (UMC).

SimpleMappr, a free internet program (Shorthouse 2010), was used to create the adult distribution map. Geographical coordinates taken in the field were verified with Google Earth Pro for use in low-resolution mapping in the SimpleMappr format. These data were obtained from Google Earth Pro imagery containing the following attribution: "Data SIO, NOAA, U.S. Navy, NGA, GEBCO; Image Landsat / Copernicus; Imagery Date: 12/13/2015."

# Taxonomy

# Subfamily Eubriinae Lacordaire, 1857

### Type genus. Eubria Latrielle, 1829

**Diagnosis.** The following characters, in combination distinguishing the Eubriinae from the other four psephenid subfamilies, Afroeubriinae, Eubrianacinae, Psepheninae and Psephenoidinae, are for the most part taken from Lee et al. (2007, 2016). **Adults:** 1) dorsally convex body (flattened in other subfamilies except Afroeubriinae); 2) anterior margin of pronotum truncate or emarginate with an exposed head (Eubrianacinae with pronotum rounded anteriorly and head entirely concealed); 3) maxillary palpus

with apex not tapering (i.e., truncate, rounded or bifurcate) (tapering in Afroeubriinae); 4) apex of the mesosternal process truncate or emarginate (Afroeubriinae with process acute; Psephenoidinae with process tapered); and 5) five abdominal ventrites (Psepheninae with seven ventrites in males and six in females). **Larvae:** 1) abdominal paratergites VII not lengthened to reach abdominal segment IX (reaching anterolateral angles of IX in Afroeubriinae and Psepheninae; surrounding IX in Psephenoidinae); 2) ventral external gills absent (present in Eubrianacinae and Psepheninae); and 3) mature larvae metapneustic with a pair of spiracles near bases of abdominal paratergites VIII (Afroeubriinae metapneustic with spiracles at apices of paratergites VIII; Eubrianacinae and Psepheninae amphineustic with exposed ventral gills; Psephenoidinae apneustic).

**Geographic distribution.** The Eubriinae occur almost worldwide except in Antarctica and on some islands, including New Zealand. The subfamily is represented by 15 genera and 144 species (Lee et al. 2016; Barr and Shepard 2017), with the greatest diversity in Asia. The genera *Dicranopselaphus* Guérin-Méneville, 1861, *Eubria, Neoeubria*, and *Tychepsephus* occur in the Neotropics. Of these, only *Neoeubria* and *Tychepsephus* are known from South America, although *Dicranopselaphus* possibly occurs there as well.

**Habitat and biology.** See Lee et al. (2016) for an overview of the subfamily. See Shepard and Barr (2014) and Barr and Shepard (2017) for habitat descriptions of two neotropical species in the genera *Neoeubria* and *Eubria*, respectively.

**Remarks.** In Chile, the only known psephenids are eubriines in the genus *Tychepse-phus*, and the species currently named *Eubrianax luteosignatus*. The type of *Eu. luteo-signatus* appears to be a eubriine, so it is likely misplaced in *Eubrianax* which is in the subfamily Eubrianacinae. Elgueta and Guerrero (2005) stated that all known Chilean psephenids are eubriines.

### Genus Tychepsephus Waterhouse, 1876

Tychepsephus Waterhouse, 1876: 15. Ectopria (Chilectopria) Pic, 1947: 3–4, syn. nov. Tychepselaphus: Philippi, 1887: 665, lapsus calami. Tychepsephenus: Zaitzev, 1910: 4, lapsus calami. Tychepsephenus: Blackwelder, 1944: 274, lapsus calami. Tychepsephenus: Artigas, 1963: 6, lapsus calami. Tychepsephenus: Moroni, 1985: 173, lapsus calami. Tychepsephenus: Ashworth & Hoganson, 1987: 879, lapsus calami. Tychepsephenus: Passos et al., 2018: 598, lapsus calami.

Type species. Tychepsephus felix Waterhouse, 1876, by monotypy.

**Etymology.** Waterhouse (1876) did not explain the etymology of the genus. However, *Tyche* (Gr.) refers to the goddess of fortune; and the suffix, *–psephus*, from *psephenos* (Gr.), meaning "dark, obscure," has been used as the stem for multiple psephenid genera. Adult diagnosis. The following characters used to distinguish *Tychepsephus* from the neotropical eubrine genera *Dicranopselaphus*, *Eubria*, and *Neoeubria* are for the most part taken from Lee et al. (2007, 2016): 1) antenna of the male weakly serrate (pectinate in *Neoeubria*); 2) pronotum with serrate lateral margins (not serrate in *Dicranopselaphus* and *Eubria*); 3) mesoventrite with a median longitudinal sulcus (absent in *Eubria* and some *Dicranopselaphus*); and 4) metaventrite with a transverse suture (vestigial in other eubrines except *Sclerocyphon*). For the original characterization of adults of *Tychepsephus*, see Waterhouse (1876: 15–16).

*Tychepsephus* and the Australian genus *Sclerocyphon* are closely related sister genera which, in the adult stage, do not have many good characters to separate them. One obvious difference is that in *Tychepsephus* the apical margins of abdominal ventrites 2–4 are entire, whereas in *Sclerocyphon* they are serrate. Of course, there is also the geographical difference with each occurring on different continents: *Tychepsephus* in South America and *Sclerocyphon* in Australia.

**Adult description.** Waterhouse (1876) described the genus *Tychepsephus* in English, unlike the type species description which is in Latin. His description is adequate for identifying the genus. However, since Waterhouse had only a female specimen, he did not know that his species, *T. felix*, is sexually dimorphic, with males having a distinctive patch of setae on the abdominal venter. This character is also present in *T. cekalovici* sp. nov. The dorsal patterning also differs between the sexes of both species.

**Remark, adult males.** A new morphological note is that males have a sperm pump composed of a heavily muscularized ejaculatory duct which is situated medially, anterior to the aedeagus, bent around itself in an S-shape and coming from the juncture of the paired testes. The sperm pump is as short as or shorter than the aedeagus.

**Larval diagnosis.** The following characters used to distinguish *Tychepsephus* from the neotropical eubrine genera *Dicranopselaphus*, *Eubria*, and *Neoeubria* are for the most part taken from Lee et al. (2007, 2016): 1) antennomere 1 subequal to antennomere 2 (much shorter in other eubriines except *Sclerocyphon*); 2) maxillary and labial palpi with 4 and 3 palpomeres, respectively (*Dicranopselaphus* and *Eubria* with 3 and 2, respectively); 3) longitudinal medial suture from thorax to abdominal segment VII (not in other eubriines except *Sclerocyphon*).

Larval characters separating *Tychepsephus* from its Australian sister genus *Sclero-cyphon* include the following: 1) setae on the posterior margin of the thoracic and abdominal tergites hair-like in *Tychepsephus* and absent in *Sclerocyphon*; 2) paired longitudinal rows of setae or sensillae near the dorsal midline in *Sclerocyphon*, but not in *Tychepsephus*; and 3) gin traps on the abdominal tergites of *Sclerocyphon*, but not on *Tychepsephus*.

Larval descriptions. *Tychepsephus* larvae were previously well-described by Lataste (1897a) and Artigas (1963). Our collections revealed the presence of two distinct larval morphotypes (Figs 1, 2) which have not been associated with adults and are therefore unnamed. Artigas (1963) illustrated a larva that corresponds to our larval morph 2, below.



**Figures 1, 2.** *Tychepsephus* larvae **I** morph 1 **a** dorsal view **b** ventral view **2** morph 2 **a** dorsal view **b** ventral view.

*Tychepsephus* larval morph 1 (Fig. 1). Body shape elongate-oval; color brown and red-brown with variable yellow patterning; tergites darker than paratergites. Body margined with a long, dense fringe of golden yellow, white-tipped setae. Dorsal surface with very small, scattered cuticular beads. Longitudinal medial suture from middle of pronotum to AB VIII tergite. Mesothoracic tergite to AB VII tergite each with a pair of large, prominent, dark tubercles straddling the medial suture. Abdominal tergite VIII clasping AB IX laterally; AB VIII with a pair of large, spiracular tubercles on posterior margin at bases of paratergites. Abdominal tergite IX subquadrate, flattened, apex rounded. Paratergites generally rectangular, more than twice as wide as long, anterolateral margin curved. Ventral surfaces lacking tubercles and cuticular beads. Abdominal ventrites I–VIII with sternopleural sutures. Operculum as long as wide, widest just anterior to apex; apex broadly rounded.

*Tychepsephus* larval morph 2 (Fig. 2). Body shape elongate-oval; color brown to red-brown with yellow areas of variable size, shape, and position, often along midline of tergites and base of paratergites. Body margined with a long, dense fringe of yellow-brown, white-tipped setae. Dorsal surface sculptured with shallow, irregularly shaped depressions of varying sizes; cuticle with numerous, round, dark, flat-topped tubercles arranged in curvilinear shapes often encircling the depressions. Longitudinal medial suture from middle of pronotum to AB VII tergite. Tergites without pairs of prominent tubercles at the midline. Abdominal tergites each with an irregular, transverse line of tubercles; paratergites often with a longitudinal line of tubercles near the midline. Abdominal tergite IX subelliptical, convex, sometimes sculptured and laterally angulate. Paratergites I–VIII paddle-shaped, twice as wide as long, narrower at base than apex, anterolateral margin curved. Abdominal ventrites with anterior and posterior transverse lines of small, faint, brown tubercles. Abdominal ventrites I–VIII with sternopleural sutures. Operculum nearly circular from midline to apex.

**Remarks, larvae.** The most obvious differences between the larval morphs are: 1) the presence on the dorsum of numerous dark tubercles, some in curvilinear patterns (morph 2); 2) the presence of pairs of large, prominent tubercles at the midline (morph 1); 3) the shape of AB IX tergite; and 4) the shape of the operculum. The dorsal morphology of larval morph 2 is quite variable in regard to the number and arrangement of tubercles and the amount of sculpturing. Because of this, it would not be surprising if another undescribed species is discovered with further sampling of the adult habitat.

Some larvae have an abundance of peritrich protozoans attached to the venter in a scattered fashion on both sclerites and membranes.

**Pupal description (pupa of larval morph 2) (Figs 3, 4).** Pupa (Fig. 3) under exuvium of last larval instar (Fig. 4). Exuvium 8.3 mm long; entire dorsum intact; venter with abdominal ventrites anterior to AB II separated from tergum and reflexed, remainder of exuvium intact. Pupa 7.1 mm long, exarate, unsclerotized, color golden yellow. Pronotum projecting anteriorly, covering head; entire margin with very long setae. Elytra and abdominal segments I–IX with long setae on lateral margins.



**Figures 3, 4.** *Tychepsephus* pupa and larval exuvium **3** pupa **a** dorsal view **b** ventral view **4** exuvium of last larval instar, ventral view.

Abdominal tergites I–VIII each with two faint, transverse rows of round tubercles, at anterior 1/3 and near posterior margin, lateral to midline on each side. Abdominal tergite IX with apical margin nearly truncate, each posterolateral angle with a short spine. Paratergites separate; I reduced; II larger, projecting weakly anteriorly; III–VI each longer than II, projecting posteriorly, each with an anterobasal spiracular tubercle; VII similar but with a lateral spiracular tubercle. Ventrally, AB II–VI with sternopleural sutures; II–VIII each with a faint, transverse row of round tubercles near posterior margin.

**Remarks, pupa.** This rare specimen was provided to WDS by Tomás Čekalović who collected it as a larva at Estero Nonguén in Concepción Province (Región VIII, Bío Bío) on 27 January 1996. He kept it alive for more than nine months until it pupated on 10 November 1996.

In comparison with sister genus *Sclerocyphon*, pupal characters separating the two genera include the following: 1) gin traps in *Sclerocyphon* but absent in *Tychepsephus*; 2) spiracle on abdominal paratergite II reduced in *Tychepsephus* but not in *Sclerocyphon*; 3) spiracles on all paratergites located at the anterior base in *Tychepsephus* but mid-dor-sally in *Sclerocyphon*; 4) paratergites in *Sclerocyphon* much longer than in *Tychepsephus*; and 5) apex of abdominal segment IX with a median projection in *Sclerocyphon* but lacking in *Tychepsephus*. The latter two characters probably aid the pupa of *Sclerocyphon* in crawling out from under the larval exuvium (Smith 1981; Davis 1986). The pupa of *Tychepsephus* remains under the larval exuvium until the adult emerges.

# Tychepsephus geographic and seasonal distribution

In Chile, published localities describe *Tychepsephus* occurring from Peñaflor (Región Metropolitana) in central Chile (Lataste 1897a), south to Chillán, Tomé and Arauco (Región VIII, Bío Bío; Región XVI, Ńuble) (Artigas 1963), and west of Puerto Varas (Región X, Los Lagos) (Ashworth and Hoganson 1987). Data records from the MNNC include adult specimens from the Andes and foothills in Reserva Nacional Altos de Lircay (Región VII, del Maule), Cordillera Chillán (Región XVI, Ňuble), Parque Nacional Conguillío (Región IX, Araucanía) and Parque Nacional Vicente Pérez Rosales (Región X, Los Lagos), as well as specimens from near the Pacific Coast in Quirihue (Región XVI, Ňuble) and Valdivia (Región XIV, Los Ríos) (M. Elgueta, in litt.). See Appendix 2 for detailed locality information regarding the above records. Collections by the authors of adult and larval *Tychepsephus*, from regions VIII (Bío Bío) through XI (Aysén), plus regions XIV (Los Ríos) and XVI (Ňuble), are listed in Appendix 1.

Larval records from Provincia del Neuquén, Argentina, indicate that *Tychepsephus* also occurs on the east front of the Andes. Wais (1990, 1995) reported *Tychepsephus* (listed as *Chilectopria grandis*) from the Rio Meliquina in the Rio Negro Basin north of San Carlos de Bariloche. Nicolás Román (N. Román, in litt.) has more recently reported finding a larva (morph 2) (Fig. 5) near San Martin de los Andes. More surprisingly, in the EMEC there is a larval eubriine from French Guiana (Fig. 6) that greatly resembles the larva of *Tychepsephus*. If this is actually a *Tychepsephus* larva, the geographic distribution of the genus would be significantly expanded (see Discussion).



**Figures 5, 6.** Non-Chilean larvae **5** *Tychepsephus* larva from Argentina **a** dorsal view **b** ventral view. Images provided by Nicolás Román (CONICET) **6** larva from French Guiana, possibly *Tychepsephus* **a** dorsal view **b** ventral view. Images provided by Robert Sites (UMC).

A summation of the locality data for adults and larvae reveals a geographic distribution of *Tychepsephus* in the middle third of Chile, including both the Andes and the Coast Range, and on the eastern front of the central Andes in Neuquén Province, Argentina (Appendices 1, 2). The distribution map (Fig. 7) represents species-verified adult records only.

The adults examined for this study were collected from November through January, during the austral summer. Larvae are present year-round. Tomás Čekalović collected larvae in January and August, and Fierro et al. (2012) reported collecting larval *T. felix* in all seasons except winter.

## Tychepsephus habitat

We have collected larvae and/or adults of *Tychepsephus* across an elevational range of 15–1685 m at streams and rivers in Chile. In general, these watercourses were small to medium-sized and rather shallow, with moderate current, and with clear or often brown, tannin-stained water. This is in contrast to Zarges et al. (2019) who reported them from areas with "high slope and high water currents." Label data from specimens collected by Tomás Čekalović show larvae living in temperatures of 10–14 °C. Surprisingly, some them were found in humus under *Chusquea quila* [Kunth (Poaceae) bamboo], 2–3 m from a river.

Examples of the small to medium-sized streams and small, shallow rivers in which adult *Tychepsephus* have been collected by the authors, described below, include Río Colegual, Río Contaco, Río Oroco, and an unnamed tributary of the Río Blanco (Figs 8, 9, 11).

**Río Colegual** (Fig. 8), located west of Puerto Varas at an elevation of ~ 200 m, is a medium-sized stream with moderate to slow flow over cobble and gravel coated with brown algae. Pools with laminar water flow are interrupted by areas of shallow riffles. Riparian vegetation overhangs some of the banks. At the time of sampling, the water was cool, clear, and brown-stained (Fig. 8b). The watercourse is situated in mostly flat terrain bordered by cleared fields (Fig. 8a) and is partly shaded at the collection site near the bridge. Adults of two species were readily collected by sweeping and beating the riparian vegetation, which consisted of willows, streamside grasses and forbs. Blacklight sampling yielded no specimens. Many larvae were present in the substrate of the riffles, including both morphs.

**Río Contaco (= Río Tranallaquín)** (Fig. 9), located west of Osorno at an elevation of ~ 160 m, is a medium-sized stream with clear water, moderate flow, and a substrate of sand, gravel and rubble with submerged mosses. Larvae were very abundant in the substrate of the riffles, as they also were at a small tributary of the Río Contaco at Puente El Avion (Fig. 10).

**Río Oroco** at Puente Hondo, located east of Puerto Montt at an elevation of ~ 30 m, is small and shallow, with cool, brown-stained water and a sand and cobble substrate with aquatic moss.



**Figure 7.** Map of Chile with enlarged section showing the geographic distribution of species-verified adult *Tychepsephus* specimens: *T. cekalovici*, yellow triangles; *T. felix*, violet squares; both species, red circles.

An unnamed tributary of Río Blanco (Fig. 11), located between Caleta Gonzalo and Caleta Santa Bárbara (north of Chaitén) at an elevation of ~ 160 m, yielded one adult and a small number of larvae. The stream is small, clear, and very cold with moderate current, shallow riffles, and knee-deep pools. It has a substrate of cobbles and sandy gravel with some aquatic moss present.


**Figures 8–11.** *Tychepsephus* habitat **8** Río Colegual, *T. cekalovici* type locality **a** stream with riffle-pool morphology flowing through a mixture of forest and pasture land **b** streamside shrubby vegetation, adult habitat **9** Río Contaco **10** stream at Puente El Avion **11** tributary Río Blanco.

## Tychepsephus felix Waterhouse, 1876

Figs 7, 8, 12–17

*Tychepsephus felix* Waterhouse, 1876: 16 (original description). Philippi (1887: 665, catalog); Blackwelder (1944: 274, catalog); Artigas (1963: 8, larval description); Moroni (1985: 173, taxonomy, distribution); Ashworth and Hoganson (1987: 879, habitat, distribution); Elgueta and Arriagada (1989: 16, literature review);

Jerez and Moroni (2006: 76, taxonomy, checklist); Lee et al. (2007: 527, phylogenetics), Zarges et al. (2019: 16, habitat).

Ectopria (Chilectopria) grandis Pic, 1947: 4, syn. nov.

**Type locality.** The type locality was listed as only "Chili" on both the type specimen and in the species description. The female holotype specimen is housed in the NHMUK.

**Type material.** *Tychepsephus felix, Holotype* female, pinned. CHILE: "Type [white, circular label with red border] // Chili [blue, circular label // 668 // *Tychepsephus felix,* C. Waterh. (Type.) // NHMUK015011475" (Fig. 12). Deposited in the NHMUK. *Ectopria (Chilectopria) grandis* syn. nov., *Holotype* female, pinned. "Chili [green label] // Dascillide ? [pale brown label] // type [pale brown label] // Museum Paris / Coll. M.Pic [blue-green label] // Chilectopria / s.g. grandis / n sp [pale brown label] // Chilectopria / grandis Pic // TYPE [red label] // HOLOTYPE [red label] // Tychepsephus / felix ♀ / W D Shepard // MNHN, Paris / EC17127" (Fig. 17). Deposited in the MNHN.

Other material examined. Non-types (33). CHILE: Region X, 3 km W of Nueva Braunau, Rio Colegual, 30 XII 2002 (WDS-A-1502) [on reverse], William D. Shepard, leg. (EMEC, 5, 2∂ 3♀); CHILE: Region X, 9 km E Loncotoro, Pte. Colegual 2, 650' [198 m], 8 I 2003 (WDS-A-1519) [on reverse], William D. Shepard, leg. (11; EMEC, 7, 3 42; MNNC, 4, 2 22; CHILE: Región X Lagos, Río Colegual 8 rd. km W Llanquehue, elev.700' [213 m], 41°16.51'S, 73°06.52'W, 8 Jan. 2003, C. B. Barr, sweeping willows and other riparian vegetation (EMEC, 5,  $4^{\circ}_{\circ}$  1 $^{\circ}_{\circ}$ ); CHILE: Region X, 8 km SW Correntoso, Pte. Hondo [Río Oroco], 420' [128 m], 31 XII 2002 (WDS-A-1504) [on reverse], William D. Shepard, leg. (EMEC, 1♀); CHILE: Corral, Dec. 1905, R. Thaxter, MCZ (MCZC, 1♂); CHILE: F. C. Bowditch Coll., "Bradytoma", MCZ (MCZC, 13; CHILE: Concepción Pr, Estero Nonguen, 11 Noviembre 1996, Tomas Cekálovic (EMEC, 1<sup>Q</sup>); CHILE: Chili, Germain, Sharp Coll. 1905-313, NHMUK015011806 (NHMUK, 1); CHILE: Sharp Coll. 1905-313, Tycepsephus [sic] felix, C.O. Waterh., Chili - Germain, NHMUK015011809 (NHMUK, 1); CHILE: Puerto Varas, 16.xii.1926, S. Chile: Llanguihue prov., F. & M. Edwards, B.M.1927-63, NHMUK015011807 (NHMUK, 1); CHILE: Casa Pangue, 4-10.xii.1926, S. Chile: Llanquihue prov., F. & M. Edwards, B.M.1927-63, Tychepsephus felix Waterh., M.I. Russell det. 1973, NHMUK015011808 (NHMUK, 1); CHILE: Ancud. 17–19.xii.1926, S. Chile: Chiloe I., F. & M. Edwards, B.M.1927-63, NHMUK015011810 (NHMUK, 1); CHILE: as above, NHMUK015011811 (NHMUK, 1); CHILE: Ancud, 19.xii.1926, S. Chile: Chiloe I., F. & M. Edwards, B.M.1927-63, NHMUK015011812 (NHMUK, 1); as above, Tychepsephus felix Waterh., M.I. Russell det. 1973, NHMUK015011813 (NHMUK, 1).

**Differential diagnosis.** Males of *T. felix* (Figs 13, 14) are much larger (4.6–5.2 mm long) than those of *T. cekalovici* sp. nov. (3.3–3.9 mm long); the pronotal cuticle is dark brown to black with pale lateral margins, with no yellow markings on disc; the elytral cuticle is dark brown or red-brown, with setal patterning only; the depressed frontal area between the eyes does not have an inverted Y-shaped sulcus; and abdominal ventrite 3 has a median, golden yellow setal patch that is not distinctly raised and does not extend the entire length of the ventrite.

In contrast, males of *T. cekalovici* sp. nov. (Figs 18–20) are considerably smaller than males of *T. felix*; the pronotal cuticle is dark brown to black with pale lateral and basal margins, and a mediobasal yellow spot anterior and adjacent to the scutellar shield; the elytral cuticle is usually yellow-brown with dark markings in a zig-zag pattern, but may be mostly plain, without patterning; the depression between the eyes has a narrow, inverted Y-shaped sulcus; and abdominal ventrite 3 has a prominent, raised, golden yellow setal patch extending the full length of the ventrite.

The aedeagi (Figs 14, 19) of the two species are clearly different. In *T. felix* (Fig. 14), the parametes have straight lateral margins and only slightly curved medial margins, and the apices are narrow. In *T. cekalovici* sp. nov. (Fig. 19) the parametes have curved lateral and medial margins, and the apices are broad.

Females of *T. felix* (Figs 15, 16) are much larger (4.3–5.7 mm long) than those of *T. cekalovici* (3.3–3.9 mm long); the elytral cuticle is brown, without yellow patterning except for variable, slightly paler areas near the base; and the pronotal disc has no yellow markings. *Tychepsephus cekalovici* sp. nov. females (Figs 21–23) usually have elytra with transverse yellow bands in a zig-zag pattern, but those without may be distinguished by a mediobasal yellow spot on the pronotum anterior to the scutellar shield. The ovipositors are similar.

**Original type description, female (Fig. 12).** Waterhouse (1876) described the species in Latin, as was the custom at the time. An English translation follows:

Ovate, convex, glossy, dark pitch black, bronzy; fine, short, grey pubescence. Head yellow, rather wide, narrow between antennae, eyes prominent, antennal bases yellow. Thorax slightly convex, densely finely punctate, length twice width, suddenly narrowed anteriorly, frontal margin slightly lobed in middle, both sides sinuate, anterior angles somewhat prominent, sides slightly rounded behind middle, posterior angles almost right-angled, edges narrowly yellow. Scutellum yellow, apex acute. Elytral bases slightly wider than thorax, enlarged posteriorly, arched at apex, narrowed, convex, more clearly finely punctured, dorsum depressed; humeri obtuse, with edges narrowly yellow. Venter with densely grey pubescence, tarsi pitch black-yellow. Length 2.75 lin., width 2 lin.

Waterhouse (1876) added two sentences, in English: "The thorax is at the base nearly straight next to the scutellum, but is broadly sinuate on each side, so that at first sight it appears only bisinuate. Epipleural fold of the elytra is broad at the base, gradually narrowing to the apex, channeled posteriorly."

**Redescription based on new material. Male** (Figs 13, 14). *Body:* covered with short black setae and thick, moderately long yellow setae; yellow setae forming patterns on elytra. Cuticle with closely spaced punctures, punctures finer ventrally. Pronotum black with yellow margins. Elytra dark brown or red-brown with yellow lateral margins. Length 4.6–5.2 mm (n = 6), width 2.8–3.5 mm (n = 5). *Head:* covered with moderately long, yellow setae. Vertex between eyes wider than diameter of an eye. Frons deflexed at 90° angle from vertex, with a contiguous pair of broad, moderately deep depressions between eyes. Frontoclypeal suture absent. Clypeus trapezoidal, longer than wide, widest apically; clypeus noticeably raised; anterolateral angles curved beneath antennal bases. Maxillary palpus with four palpomeres; palpomeres 1–3 yellow to yellow-



**Figure 12.** *Tychepsephus felix*, female type specimen **a** dorsal view **b** ventral view **c** specimen data labels. Images provided by Keita Matsumoto (NHMUK).

brown; 4 yellow-brown to dark brown, obliquely hatchet-shaped, weakly curved at apex. Labial palpus with three palpomeres; palpomeres 1–2 yellow; 3 yellow-brown to dark brown, with apex truncate to weakly curved; glossae and paraglossae split, apically acicular. Antenna weakly serrate, with 11 antennomeres; 1 longest, cylindrical, yellow;



Figures 13, 14. *Tychepsephus felix*, male 13 habitus **a** dorsal view **b** ventral view; length 5.0 mm 14 aedeagus **a** dorsal view **b** lateral view **c** ventral view.

2 shortest, yellow-brown; 3-11 dark brown, each widest apically. Antennal base encircled by raised margin. Eye large, bulbous, finely faceted. Pronotum: twice as wide as long, widest just anterior to base; anterior margin convex between anterior angles; anterior angles obtuse, broadly rounded, projecting anteriorly, clasping eyes; lateral margins finely sculptured with shallow notches, narrowly explanate, straight from anterior angles to basal 1/3 then curved to posterior angles; posterior angles square; posterior margin crenulate, nearly straight between posterior angles almost to scutellar shield, then curved posteriorly, straight adjacent to scutellar shield; disc depressed near anterior angles, convex at middle, flattened basomedially. Scutellar shield: pentagonal, flat, depressed between elytra; densely covered by moderately long, yellow setae; anterior margin crenulate; apex acute. *Elytra*: conjointly longer than wide, widest at ~ 1/3 the distance from apices, wider than pronotum; each with anterior margin crenulate; lateral margin smooth, narrowly explanate; humerus prominent with adjacent medial depression; area adjacent to lateral margin in anterior 1/3 with a wide, moderately deep depression; disc much more convex at posterior 1/2. Setae largely absent from oval area posterior to middle and round area at apical 1/4 near suture; setae sparse near base. Epipleuron widest below humerus, narrowed posteriorly; channeled adjacent to abdomen, channel becoming deeper towards apex. Metathoracic wings: macropterous. Prosternum: wider than long; cuticle brown, densely setose; anterior margin projecting to cover mouthparts with a short chin piece; prosternal process long, narrow, extending well past procoxae to anterior third of mesosternum, elevated above rest of prosternum; disc with shallow, longitudinal, median ridge. *Mesoventrite*: brown; wider than long, extending between mesocoxae; mesoventral cavity deep to receive prosternal process. *Metaventrite*: wider than long, cuticle black; disc convex lateral to discrimen; deep fossa at junction of discrimen and metakatepisternal suture; posterior margin weakly sinuate anterior to metacoxae. Legs: completely covered with dense, yellow setae; similar, except procoxa and mesocoxa globular, metacoxa widely transverse; femur and tibia usually darker than tarsus; tibia and tarsus long and narrow, tarsus longer than tibia; tarsus densely covered with pale setae; claws long, slender. Abdomen: brown, densely setose; with five weakly convex ventrites; ventrites 1-4 short, ventrite 5 longest; ventrites 1–3 with lateral margins notched medially; ventrite 3 with a dense patch of coarse, golden yellow setae covering the posteromedial 1/2-2/3 of the disc. Aedeagus: (Fig. 14) more than twice as long as wide; phallobase vase-shaped, narrow at base, wide at apex; parameres stout, much longer than penis, lateral margins straight at basal 2/3 then gradually curved inward at apical 1/3, medial margins only slightly curved, parameres each narrowed toward a rounded apex; penis tapered, apex broadly rounded, basally with two apophyses. Female (Figs 15, 16) similar to male except: frons with an inverted Y-shaped suture between eyes; setation of dorsum less dense except for scutellar shield; cuticle of humeri and elytral bases sometimes paler than disc; tarsi less densely setose; abdominal ventrite 3 without a dense patch of yellow setae. **Ovipositor:** (Fig. 16) with baculus nearly twice as long as gonocoxite; baculus almost twice as long as wide, strap-like, wider apically; each proximal gonocoxite triangular, distal gonocoxites separate basally then converging to meet apically, apices obliquely truncate; each gonostylus long and narrow, half as long as distal gonocoxite.



Figures 15, 16. *Tychepsephus felix*, female 15 habitus **a** dorsal view **b** ventral view; length 4.2 mm 16 ovipositor, dorsal view.

**Variation.** The sizes of males and females overlap: males, 4.6–5.2 mm long (n = 6), 2.8–3.5 mm wide (n = 5); females 4.3–5.7 mm long (n = 10), 2.9–3.7 wide (n = 5); but the largest specimens are female. Most males (Fig. 13a) have an elytral pattern with

moderately dense setae surrounding mostly glabrous areas; most females (Fig. 15a) have no pattern and are more sparsely setose. Females (Fig. 15b) lack the dense patch of yellow setae on abdominal ventrite 3 that is present in males (Fig. 13b).

**Etymology.** Waterhouse (1876) did not explain the trivial name. However, *felix* (L.) means happy or prosperous.

**Geographic distribution.** *Tychepsephus felix* is known only from Chile. Adults have been collected mainly in Región X (Los Lagos), but also in regions VIII (Bío Bío), IX (Araucanía), and XIV (Los Ríos) (M. Elgueta, in litt.), in both the Andean and the coastal mountain areas (Fig. 7). The greatest number of adults collected by the authors was at Río Colegual west of Puerto Varas (Fig. 8).

Habitat. *Tychepsephus felix* adults were found in habitats as described for the genus. Specimens were collected by sweeping marginal vegetation along streams during the austral summer (Fig. 8b).

Associated dryopoid taxa. Elmidae: Larainae: *Hydora annectans* Spangler & Brown, 1981, *H. lenta* Spangler & Brown, 1981; Elminae: *Austrolimnius* Carter & Zeck, 1929, *Luchoelmis* Spangler & Staines, 2004, *Neoelmis sissicollis* (Germain, 1892). Both *T. felix* and *T. cekalovici* sp. nov. occurred at Río Colegual.

**Type remarks.** The species was described by Waterhouse (1876) from a single female type (Fig. 12) which is in the NHMUK along with eight non-type specimens subsequently acquired. The type was originally pinned, breaking the right elytron just posterior to the pin. Subsequently, the specimen was removed from the pin and card-mounted. Missing parts include the following: left antenna without antennomeres 3–11; left foreleg; right hind leg; tarsi on all legs except left hind leg; and tarsomere 5 on the left hind leg. One leg, without the tarsus, is in a gelatin capsule pinned below the carded specimen.

The female type of *Ectopria* (*Chilectopria*) grandis Pic, 1947 (Fig. 17), housed at the MNHN, was examined and found to be synonymous with *Tychepsephus felix* Waterhouse, 1876. Pic described *Chilectopria* as a subgenus of *Ectopria*: "*Chilectopria* sometimes has been cited incorrectly as a genus, rather than a subgenus, likely because the heading and description occur on different pages (Pic 1947: 3–4). Contributing to this error, the type specimen also lacks "*Ectopria*" on the identification label. The genus *Ectopria* is a Holarctic element.

#### Tychepsephus cekalovici sp. nov.

https://zoobank.org/BEB4F7FE-9984-419A-9D9C-1A08A5255B1E Figs 7–9, 11, 18–23

**Type locality.** Chile: Región X (Los Lagos), 3.5 rd. km W of Nueva Braunau, Puente Colegual, Río Colegual, -41.3264°, -73.1225°, 158 m, sweeping riparian vegetation, 8 January 2003, William D. Shepard leg. (Fig. 8).

**Type material.** *Holotype* male, pinned. "CHILE: Region X / 9 km E Loncotoro / 8 I 2003 650' / Pte Colegual 2 [Río Colegual] (WDS-A-1519) [on reverse] // William D. Shepard, leg. // [genitalia in vial below specimen] // HOLOTYPE / Tychepsephus / cekalovici / Shepard & Barr [red handwritten label]". Deposited in the MNNC.



**Figure 17.** *Chilectopria grandis*, female type specimen **a** dorsal view **b** lateral view **c** ventral view **d** specimen data labels. Images provided by Christophe Rivier (MNHN).

Other material examined. Paratypes (67). CHILE: Region X, 3 km W Nueva Braunau, Rio Colegual, 30 XII 2002 (WDS-A-1502) [on reverse], William D. Shepard, leg. (9; EMEC, 6, 5% 1%; MNHN, 1%; MNNC, 1%; NHMUK, 1%); CHILE: Región X Lagos, Río Colegual 3.5 rd. km W Nueva Braunau, elev.160' [49 m], 41°19.58'S, 73°07.35'W, 30 Dec. 2002, C. B. Barr, sweeping willows and other riparian vegetation (11; EMEC, 9, 8 $\overrightarrow{\partial}$ , 1 $\overrightarrow{Q}$ ; MNNC, 1 $\overrightarrow{\partial}$ ; NHMUK, 1 $\overrightarrow{\partial}$ ); data as above, 31 Dec. 2002 (EMEC, 5Å); data as above, 8 Jan. 2003 (14; EMEC, 10, 8Å 2°; MNHN, 2Å; MNNC, 1♂; NHMUK, 1♀); CHILE: Region X, 9 km E Loncotoro, Pte. Colegual 2, 650' [198 m], 8 I 2003 (WDS-A-1519) [on reverse], William D. Shepard, leg. (10; EMEC, 6, 432; MNHN, 12; MNNC, 2, 1312; NHMUK, 13; CHILE: Región X Lagos, Río Colegual, 8 rd. km W Llanquehue, elev.700' [213 m], 41°16.51'S, 73°06.52'W, 8 Jan. 2003, C. B. Barr, sweeping willows & other riparian vegetation (10; EMEC, 9, 6 $\bigcirc$  3 $\bigcirc$ ; MNNC, 1 $\bigcirc$ ); CHILE: 8 mi W of Puerto Varas, 1-16-51, Ross and Michelbacher, CAS (CASC, 1); CHILE: Region X, 20 km N Chaitén, unnamed stream [trib. Río Blanco], 520' [158 m], 3 I 2003 (WDS-A-1511) [on reverse], William D. Shepard, leg. (EMEC, 13); CHILE: Region X, Rio Contaco, 520' [158 m], 9 I 2003 (WDS-A-1521) [on reverse], William D. Shepard, leg. (EMEC, 3,  $1^{\uparrow}_{\circ} 2^{\circ}_{\circ}$ ); CHILE: Corral, Dec 1905, R. Thaxter, MCZ (MCZC, 3).

**Differential diagnosis.** Males of *T. cekalovici* sp. nov. (Figs 18–20) are considerably smaller (3.3–3.9 mm long) than males of *T. felix* (4.6–5.2 mm long); the pronotal cuticle is dark brown to black with pale lateral and basal margins, and an elongate yellow spot anterior and adjacent to the scutellar shield; the elytral cuticle is usually yellow-brown with dark markings in a zig-zag pattern, but may be mostly plain, without patterning; the depressed frontal area between the eyes has a narrow, inverted Yshaped sulcus; and abdominal ventrite 3 has a prominent, raised, golden yellow setal patch extending the full length of the ventrite (Fig. 18b).

In contrast, males of *T. felix* (Figs 13, 14) are much larger than those of *T. cekalo-vici* sp. nov.; the pronotal cuticle is dark brown to black with pale lateral margins and no yellow discal markings; the elytral cuticle is dark brown or red-brown, with setal patterning only; the depression between the eyes does not have an inverted Y-shaped sulcus; and abdominal ventrite 3 has a median, golden yellow setal patch that is not distinctly raised and does not extend the entire length of the ventrite (Fig. 13b).

The aedeagi (Figs 14, 19) of the two species are clearly different. In *T. cekalovici* sp. nov. (Fig. 19) the parameres have curved lateral margins and curved medial margins, and the apices are broad. In *T. felix* (Fig. 14), the parameres have straight lateral margins and only slightly curved medial margins, and the apices are narrow.

Like the males, females of *T. cekalovici* sp. nov. (Figs 21, 22) are much smaller (3.3–3.9 mm long) than females of *T. felix* (4.3–5.7 mm long), and most have elytra with transverse yellow bands in a zig-zag pattern. Individuals of *T. cekalovici* without elytral patterning usually may be distinguished by a mediobasal yellow or yellow-brown spot, sometimes faint, on the pronotum anterior to the scutellar shield. In contrast, females of *T. felix* (Fig. 15) have brown elytra without yellow banding, and do not have a mediobasal yellow spot on the pronotum.

Description. Male (Figs 18–20). Body: dorsally and ventrally covered with moderately long, coarse, yellow setae and shorter, thinner, silky, black setae; cuticle with closely spaced punctures, punctures finer ventrally. Cuticle of head, pronotum and venter dark brown to black; pronotum with yellow margins; elytra usually yellow-brown with dark brown patterns. Length 3.3–3.9 mm, width 2.0–2.6 mm (n = 19). *Head*: covered with moderately long, yellow setae. Vertex between eyes slightly wider than diameter of an eye. Frons deflexed at 90° angle from vertex; moderately deep depression between eyes containing a narrow, inverted Y-shaped sulcus, arms of Y deeply incised. Frontoclypeal suture absent. Clypeus wider than long, narrow at base; anterolateral angles curved beneath antennal bases. Maxillary palpus with four palpomeres; palpomeres 1-3 yellowbrown; 4 dark brown, obliquely hatchet-shaped, weakly curved at apex. Labial palpus light to dark brown, with three palpomeres, palpomere 3 truncate to weakly curved; glossae and paraglossae apically bifid and acicular. Antenna weakly serrate, with 11 antennomeres; 1 longest, cylindrical, yellow; 2 half as long as 1, yellow-brown; 3-11 dark brown, each wider apically. Antennal base encircled by raised margin. Eye large, bulbous, finely faceted. **Pronotum:** a little more than twice as wide as long, widest just anterior to base; all margins yellow; anterior margin slightly sinuate, convex between anterior angles; anterior angles prominent, broadly rounded, projecting anteriorly, clasping eyes; lateral margins finely sculptured with shallow notches, margins straight to basal 1/3 then curved to posterior angles; posterior angles slightly obtuse; posterior margin crenulate, straight laterally then curved to scutellar shield, straight anterior to scutellar shield; disc convex at middle, depressed near anterior angles; disc anterior to scutellar shield with an oblong, subbasal vellow spot and a short, shallow, median sulcus. Scutellar shield: pentagonal, as long as wide; anterior margin crenulate; disc flat, depressed, densely covered with coarse yellow setae. *Elytra*: conjointly longer than wide, widest ~ 1/3 the distance from apices, wider than pronotum; each elytron with anterior margin crenulate; lateral margin yellow, smooth, narrowly explanate; humerus moderately prominent; elytral base depressed between humerus and scutellar shield; area adjacent to lateral margin in anterior 1/2 with a wide, moderately deep depression; disc more convex in posterior 1/2. Disc densely punctate, punctures small, separated by less than own width; setose, setae less dense near base and along suture. Cuticle variably patterned, usually yellow with transverse, zig zag bands of dark brown spots near the base and at the apical 1/3, sometimes lacking the apical band. Epipleuron widest at humeri, narrowing at abdominal ventrite 1, grooved adjacent to abdomen. Metathoracic wings: macropterous. Prosternum: wider than long; anterior margin projecting to cover mouthparts with a chin piece; prosternal process extending past procoxae, medially with weak longitudinal carina, lateral margins carinate. Mesoventrite: strongly transverse; mesoventral process triangular, extending beyond mesocoxae; mesoventral cavity deep to receive prosternal process. *Metaventrite*: wider than long, dark brown to black; disc flattened at midline and strongly convex laterally; deep fossa at junction of discrimen and metakatepisternal suture. *Legs*: similar, except procoxa and mesocoxa globular, metacoxa very short and transverse; femur and tibia usually darker than tarsus; tibia and tarsus long and narrow, tarsus longer than tibia; tarsus usually with dense, short, pale setae; claws short, slender. Abdomen: mottled yellow-brown and



Figures 18, 19. *Tychepsephus cekalovici* sp. nov., male 18 habitus **a** dorsal view **b** ventral view; length 3.2 mm 19 aedeagus **a** dorsal view **b** lateral view **c** ventral view.



**Figure 20.** *Tychepsephus cekalovici* sp. nov., male dorsal habitus showing color pattern variation **a** length 3.3 mm **b** length 3.5 mm **c** length 3.5 mm.



**Figures 21–23.** *Tychepsephus cekalovici* sp. nov., female **21** habitus **a** dorsal view **b** ventral view; length 4.4 mm **22** dorsal color pattern variation; length 4.3 mm **23** ovipositor, dorsal view.

dark brown, densely setose; with five ventrites, 1 shortest, 5 longest; ventrites 1–3 very convex in middle; ventrite 3 with prominent, raised, oval, medial patch of dense, coarse, golden yellow setae. *Aedeagus*: (Fig. 19) 2.5X as long as wide; phallobase narrow basally then widening apically; parameres stout, much longer than penis and enclosing it, apices

with inner margins truncate; penis much narrower than a paramere, elongate, parallelsided, broadly rounded at apex, with broad basal apophyses. **Female** (Figs 21–23) larger than male and darker. Elytra red-brown or dark brown, usually patterned with several yellow, transverse, zig zag bands; less often with yellow markings only at base. Pronotum with a small, triangular, yellow or yellow-brown subbasal spot just anterior to scutellar shield, sometimes obscure. Tarsi not densely setose. Abdominal ventrite 3 lacking a prominent, raised, medial patch of golden yellow setae. **Ovipositor:** (Fig. 23) with baculus nearly twice as long as gonocoxite; baculus almost twice as long as wide, strap-like, wider apically; each proximal gonocoxite triangular, distal gonocoxites separate basally then converging to meet apically, apices obliquely truncate; each gonostylus long and narrow, half as long as distal gonocoxite. Very similar to that of *T. felix* (Fig. 16).

**Variation.** Males are smaller than females: males,  $3.3-3.9 \text{ mm} \log 2.0-2.6 \text{ mm}$  wide (n = 19); females,  $4.3-4.7 \text{ mm} \log 2.1-3.0 \text{ mm}$  wide (n = 7). The elytral cuticle of males is yellow, yellow-brown, or brown with dark brown patterning (Figs 18, 20), while that of females is red-brown to dark brown with yellow patterning (Figs 21, 22). The pronotal yellow, oblong, subbasal spot of the males is usually yellow-brown and reduced to a triangle or less in the females. The tibiae of males are more setose than those of females. Females (Fig. 21b) lack the prominent, raised patch of golden yellow setae on abdominal ventrite 3 that is present in males (Fig. 18b).

**Egg description.** Eggs spherical, 0.2 mm diameter (n = 10); flattened on one side; chorion with tiny dimples.

**Etymology.** The trivial name, *cekalovici*, honors the late Tomás Čekalović, who was an outstanding coleopterist, arachnologist, and field biologist from Concepción, Chile (Urbina Burgos 2013). The name is a noun in the genitive case.

**Geographic distribution.** *Tychepsephus cekalovici* sp. nov. is known only from Chile. Adults have been collected in Región VII (del Maule), Región VIII (Bío Bío) (M. Elgueta, in litt.), Region XIV (Los Ríos), and Región X (Los Lagos), in both the Andean and the coastal mountain areas (Fig. 7). The greatest number of adults collected by the authors was at Río Colegual west of Puerto Varas (Fig. 8).

**Habitat.** *Tychepsephus cekalovici* sp. nov. adults were found in habitats as described under the genus *Tychepsephus* (see above). Adults were collected by sweeping marginal vegetation along streams and small, shallow rivers during the austral summer (Figs 8, 9, 11).

Associated dryopoid taxa. Elmidae: Larainae: *Hydora annectans, H. lenta*; Elminae: *Austrolimnius, Luchoelmis, Neoelmis sissicollis.* Both *T. cekalovici* sp. nov. and *T. felix* occurred at Río Colegual.

# Eubrianax luteosignatus Pic, 1947

Fig. 24

#### Type locality. "Chili".

Material examined. *Holotype*. CHILE: "Chili // type // Eubrianax luteosignatus nsp // 314 // coll Germain [lavender label] // TYPE [red label]" (Fig. 24). Deposited in the MNHN.

**Remarks.** *Eubrianax* is a Holarctic element that is not expected to occur in Chile. When the type of *Eubrianax luteosignatus* (Fig. 24) from the MNHN was examined, it was found to be very dirty and glued on a point with the ventral side obscured. We did not seek permission to clean and remount it so our observations were limited. However, it appears that the type actually belongs in the Eubriinae, rather than in the Eubrianacinae where *Eubrianax* is placed. The elytral markings are very reminiscent of the eubriine genus *Dicranopselaphus* which, in the New World, is known from the eastern USA south through Central America. Pic (1947) described *Eubrianax luteosignatus* in the Dascillidae.

#### Eubriinae: unknown genus and species of larvae (Chile)

We know of two larval specimens of an unknown genus and species of Eubriinae from Chile, one in the EMEC and one in the MNNC. The EMEC specimen (Fig. 25) is unfortunately in marginal condition. It was collected by Tomás Čekalović who lived near Concepción, Chile. The locality, Estero Nonguén, flows from Parque Nacional Nonguén through an urban area of Concepción. The other larva (Fig. 26), from the MNNC, is from Reserva Nacional Los Ruiles near the coast northwest of Cauquenas (Elgueta and Guerrero 2005). These two specimens may be the larva of the species currently known as *Eubrianax luteosignatus*.



**Figure 24.** *Eubrianax luteosignatus*, type specimen **a** dorsal habitus **b** specimen data labels. Images provided by Christophe Rivier (MNHN).



**Figures 25, 26.** Unknown eubrine larvae from Chile **25** specimen from Estero Nonguén, Concepción **a** dorsal view **b** ventral view **26** specimen from Reserva Nacional Los Ruiles, northwest of Cauquenes **a** dorsal view **b** ventral view. Fig. 26 images provided by Mario Elgueta and Marcelo Guerrero (MNNC).

#### Eubriinae: unknown genus and species of larva (French Guiana)

One larva resembling *Tychepsephus* (larval morph 2) (Fig. 6) was collected in a stream in French Guiana (FRENCH GUIANA: Sinnamary, Carbet Mouche, Crique "Salle de Bains", 04.648440°N, 052.94082°W, elevation 37 m). If this actually is a species of *Tychepsephus*, it would represent a range extension of approximately 4500 km to the northeast of the known distribution of the genus in Chile. Verification would require collection of adult specimens.

#### Discussion

#### Sampling results and larval identification

To verify the taxonomic identity of larvae, one must either collect associated adults, conduct DNA studies, or rear larvae to adulthood. Larvae, if present, are present continuously, but adults are short-lived so the timing of sampling is important if one is to collect adults. Although we visited Chile during the summer when adults are present, our sampling regimen was weighted towards aquatics, and the riparian habitat was not as well-collected. During the 120 collection events, we collected hundreds of larvae in approximately one third of the events (44 times), but adults at only six events at five localities (Fig. 7). The literature and museum records reflect this disparity as well, with mostly larval and few adult records. Timely sampling of the adult habitat would yield more adult specimens, and perhaps additional species of *Tychepsephus* may be discovered. The variability of larval morph 2 points to that possibility.

Larval morph 2 (Fig. 2) was by far the most commonly collected of the two morphotypes. Both morphs co-occurred at only three localities. Of these, Río Colegual (Fig. 8) was the only one where both larval morphs and adults of both species were collected. Adults of both species were collected previously at Corral by another researcher. *Tychepsephus cekalovici* adults were three times more common than those of *T. felix* in our samples.

Association of specific larval morphs of *Tychepsephus* with particular species could be accomplished by either DNA barcoding or by rearing late-instars through to adulthood. Rearing would require holding late-instars (probably collected in November) until they pupate and emerge as adults (likely in December or January). No special equipment would be required. They could be reared in sealable plastic containers that retain humidity, along with some suitable substrate for pupation.

#### Remaining questions

The Chilean psephenid fauna currently includes three, or perhaps four, eubriine species: two species of *Tychepsephus*, *T. felix* (including *Ec.* (*Chilectopria*) grandis syn. nov.) and *T. cekalovici* sp. nov., one species currently known as *Eubrianax luteosignatus*, and one unidentified eubriine larva (Figs 25, 26). Possibly, this unidentified larva is that of *Eu. luteosignatus*. Because Pic sometimes described sexually dimorphic males and females of one species in different genera, it is not surprising that his *Eu. luteosignatus* is probably a eubriine rather than a eubrianacine, and that his *Ec. (Chilectopria) grandis* is synonymous with *T. felix*. Both *Eubrianax* and *Ectopria* are now considered to be Holarctic genera. Care must be taken when interpreting the literature on *Tychepsephus* because of the uncertainty about which species is being discussed.

*Tychepsephus* has been thought to be endemic to Chile, but older literature records and a recent larval collection from Argentina (Fig. 5) have shown otherwise. Furthermore, an enigmatic larva from French Guiana (Fig. 6), which resembles the larvae of *Tychepsephus*, is particularly important as it potentially represents a very large geographic extension for the genus. Additional specimens, particularly adults, are needed for verification and description of the latter. And sampling in previously uncollected areas would fill in distributional gaps.

The Psephenidae of South America are poorly known and problematic. The monospecific eubrine genus Neoeubria occurs in Colombia (W. D. Shepard, unpublished data), Ecuador, and Costa Rica (Shepard and Barr 2014), yet very few adult specimens exist. The more speciose psephenine genera Pheneps Darlington, 1936, Psephenops Grouvelle, 1898, and Psephenus Haldeman, 1853, all have species described from South America. Seven of the nine Pheneps species are South American, five of them from Brazil. The Brazilian Bertrandi bicoloripes Pic, 1943, is actually a species of *Pheneps* (W. D. Shepard, unpublished data). Of the nine valid species of Psephenops, only two are known to occur in South America; the remainder are from Mexico, Central America, and the Caribbean. However, the South American species described in *Psephenus* (four from Brazil and Peru) are actually species of *Psephenops*; Psephenus occurs only in North and Central America. Additionally, in South America, several undescribed psephenid taxa are known only from larvae. In Colombia, there are undescribed genera and species of Eubriinae and Psepheninae (L. Alvarez, in litt.), in Peru undescribed Psepheninae, and in French Guiana undescribed Eubriinae (W. D. Shepard, unpublished data). South America is a truly fertile territory for further research on the Psephenidae.

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#### References

- Artigas J (1963) Descripción de la larva de un psephenido Chileno (Coleoptera Psephenidae). Gayana (Concepción) 1963(8): 3–11.
- Ashworth AC, Hoganson JW (1987) Coleoptera bioassociations along an elevational gradient in the Lake Region of southern Chile, and comments on the postglacial development of the fauna. Annals of the Entomological Society of America 80(6): 865–895. https://doi. org/10.1093/aesa/80.6.865
- Barr CB, Shepard WD (2017) Eubria mesoamericana Barr and Shepard (Coleoptera: Psephenidae: Eubriinae), a new psephenid species from Central America with an enigmatic generic distribution. Coleopterists Bulletin 71(3): 437–444. https://doi.org/10.1649/0010-065X-71.3.437
- Blackwelder RE (1944) Checklist of the Coleopterous Insects of Mexico, Central America, the West Indies, and South America. Part 2. Bulletin of the United States National Museum 185. United States Government Printing Office, Washington, D. C., 189–341. https://doi. org/10.5479/si.03629236.185.2
- Brown HP (1983) A Catalog of the Coleoptera of America North of Mexico. Family Psephenidae. United States Printing Office, Washington, D. C., 8 pp.
- Davis JA (1986) Revision of the Australian Psephenidae (Coleoptera): Systematics, phylogeny and historical biogeography. Australian Journal of Zoology. Supplementary Series (119): 1–97. https://doi.org/10.1071/AJZS119
- Elgueta DM, Arriagada SG (1989) Estado actual del conocimiento de los Coleopteros de Chile (Insects: Coleoptera). Revista Chilena de Entomologia 17: 5–60.
- Elgueta M, Guerrero M (2005) Nuestro conocimiento de Psephenidae (Coleoptera) en Chile "peniques de aqua." Oral presentation. Sociedad Chilena de Entomologia, 14 Sept. 2005.
- Fierro P, Bertrán C, Mercado M, Peña-Cortés F, Tapia J, Hauenstein E, Vargas-Chacoff L (2012) Benthic macroinvertebrate assemblages as indicators of water quality applying a modified biotic index in a spatio-seasonal context in a coastal basin of southern Chile. Revista de Biología Marina y Oceanografía 47(1): 23–33. https://doi.org/10.4067/S0718-19572012000100003
- Jerez V, Moroni J (2006) Diversidad de Coleopteros acuaticos en Chile. Gayana (Concepción) 70(1): 72–81. https://doi.org/10.4067/S0717-65382006000100012
- Lataste F (1897a) Présentation d`une larve de Pseudo-Névroptère d`aspect crustacéen. Actes de la Société Scientifique du Chili 7: 103–106.

- Lataste F (1897b) La larve crustacéiforme de Peñaflor est de Coléoptère et non de Pseudo-Névroptère. Actes de la Société Scientifique du Chili 7: 107–108.
- Lee C-F, Satô M, Shepard WD, Jäch MA (2007) Phylogeny of Psephenidae (Coleoptera: Byrrhoidea) based on larval, pupal and adult characters. Systematic Entomology 32(3): 502– 538. https://doi.org/10.1111/j.1365-3113.2006.00374.x
- Lee C-F, Jäch MA, Beutel RG (2016) Psephenidae Lacordaire, 1854. In: Beutel RG, Leschen RAB (Eds) Handbook of Zoology Coleoptera, Beetles (Archostemata, Adephaga, Myx-ophaga, Polyphaga partim): Morphology and Systematics. 2<sup>nd</sup> edn. Walter de Gruyter, Berlin, 615–628. https://doi.org/10.1515/9783110373929
- Moroni BJC (1985) Addenda y corrigenda al elenco sistematico, sinonimico y distribucion de Coleopteros acuaticos Chilenos. Sociedad Chilena de Entomologia 12: 169–175.
- Passos MI, Manzo V, Maier CA (2018) Chapter 15.6. Families Dryopidae, Elmidae, and Psephenidae. In: Hamada N, Thorp J, Rogers DC (Eds) Thorp and Covich's Freshwater Invertebrates - Volume III - Keys to Neotropical Hexapoda. 4<sup>th</sup> edn. Academic Press, Salt Lake City, 583–598. https://dx.doi.org/10.1016/B978-0-12-801223-6.00027.5
- Philippi F (1887) Catálogo de los Coleópteros de Chile. Anales de la Universidad de Chile 71(1): 79–674, 675–806.
- Pic M (1947) Diversités entomologiques (10 Juin 1947). Les Impremeries Réunies. Moulins 1: 1–16. https://doi.org/10.5962/bhl.title.107929
- Shepard WD, Barr CB (2014) *Neoeubria inbionis* Shepard & Barr, a new genus and new species of neotropical water penny beetle (Coleoptera: Psephenidae: Eubriinae), with a key to the adult Eubriinae of the Neotropical Zone. Zootaxa 3811(4): 553–568. https://doi.org/10.11646/zootaxa.3811.4.7
- Shorthouse DP (2010) SimpleMappr, an online tool to produce publication-quality point maps. https://www.simplemappr.net [Accessed on 2.24.2023]
- Smith JA (1981) Two Tasmanian species of *Sclerocyphon* Blackburn (Coleoptera: Psephenidae) with notes on their life history and distribution. Journal of the Australian Entomological Society 20(4): 277–288. https://doi.org/10.1111/j.1440-6055.1981.tb01048.x
- Urbina Burgos M (2013) Obituario † Tomás Nicolás Čekalović Kušcević (1928–2012). Anales del Instituto de la Patagonia (Chile) 41(1): 165–168. https://doi.org/10.4067/S0718-686X2013000100018
- Wais IR (1990) A checklist of the benthic macroinvertebrates of the Negro River Basin, Patagonia, Argentina, including an approach to their functional feeding groups. Acta Limnologica Brasiliensia 3: 829–845.
- Wais IR (1995) El alimento presa que encontraron las truchas en la Argentina. Boletín Mosquero 19: 27–33.
- Waterhouse CO (1876) III. On various new genera and species of Coleoptera. Transactions of the Royal Entomological Society 1876(1): 11–25. https://doi.org/10.1111/j.1365-2311.1876. tb01122.x
- Zaitzev P (1910) Coleopterorum Catalogus. Pars 17: Dryopidae, Cyathoceridae, Georyssidae, Heteroceridae. W. Junk, Berlin, 47 pp.
- Zarges CV, Fierro P, Olmos V (2019) Freshwater invertebrates of southwestern South America: Diversity, biogeography, and threats. In: Devlin A, Pan J, Shah MM (Eds) Inland Waters - Dynamics and Ecology. IntechOpen, London, 1–30. https://doi.org/10.5772/intechopen.89077

# Appendix I

Chilean localities where *Tychepsephus* was collected by the authors. Sites where adult specimens were collected are marked with an asterisk, \*. Region designations are as at the time of the collection.

Región VIII, Bío Bío (some of these are now in Región XVI, Nuble)

- Termas de Chillan area (road to ski lift), unnamed stream, 5530 ft, 10 I 2002, 36°55'02"S, 71°25'45"W, larvae (WDS-A-1432)
- Termas de Chillan area (across road to Cueva Los Pincheira), Estero Renegado, 4100 ft, 10 I 2002, 36°53'46"S, 71°33'03"W, larvae (WDS-A-1434)
- Cueva Las Pincheira, unnamed stream, 4100 ft, 10 I 2002, 36°53'46"S, 71°33'03"W, larvae (WDS-A-1435)
- Puente San Jorge, 3 km NW Predio San Jorge, Río Culenco, 1780 ft, 11 I 2002, 37°15'30"S, 72°56'00"W, larvae (WDS-A-1438)

## Región IX, Araucanía

- Parque Nacional Nahuelbuta, picnic area, Estero Pehuenco, 3656 ft, 12 I 2002, 37°49'47"S, 73°00'32"W, larvae (WDS-A-1439)
- Parque Nacional Nahuelbuta, picnic area, Estero Pehuenco, 3659 ft, 5 I 2003, 37°49.702'S, 73°00.622'W, larvae (WDS-A-1732)
- Parque Nacional Nahuelbuta, Estero Los Gringos, 4167 ft, 12 I 2007, 37°48'21"S, 73°00'45"W, larvae (WDS-A-1440)
- Parque Nacional Nahuelbuta, Estero Los Gringos, 4043 ft, 5 I 2007, 37°48.517'S, 73°01.000'W, larva (WDS-A-1733)
- 14 road km east of P. N. Nahuelbuta, Puente El Manzana, Río Piculquén, 2850 ft, 12 I 2002, 37°48'42"S, 72°52'41"W, larva (WDS-A-1441)
- 1 road km NE Puente El Manzana, unnamed trib. to Río Piculquén,2960 ft, 12 I 2002, 37°47'51"S, 72°51'20"W, larvae (WDS-A-1442)
- 19 road km E Victoria, Puente Quino, Río Quino, 2080 ft, 13 I 2002, 38°17'21"S, 72°10'00"W, larvae (WDS-A-1443)
- 33 road km E Victoria, Puente Huillinlebu, 2720 ft, 13 I 2002, 38°19'05"S, 72°00'49"W, larvae (WDS-A-1444)
- 42 km E Victoria, Puente Rari Ruca, 2740 ft, 13 I 2002, 38°20'13"S, 71°58'15"W, larvae (WDS-A-1445)
- Parque Nacional Huerquehue, Refugio Tinquilco parking area, unnamed stream, 3640 ft, 15 I 2002, 39°09'27"S, 71°42'52"W, larva (WDS-A-1452)
- 3–4 km S Paillaco, Puente Huepil, Río Liucura, 2440 ft, 15 I 2002, 39°13'57"S, 71°45'20"W, larvae (WDS-A-1454)

Región X, Los Lagos (some of these are now in Región XIV, Los Ríos)

- 4 km SE Coñaripe, Río Llancahue (tributary of Lago Calafquen), ~ 39°34'52"S, 71°57'28"W, 16 I 2002, larva
- Parque Nacional Puyehue, 7 km S Aguas Calientes, Río Nauto, 3130 ft, 17 I 2002, 40°45'25"S, 72°17'42"W, larvae (WDS-A-1460)
- ca. 3 km SW Ensenada at Highway 225, unnamed stream, 1340 ft, 18 I 2002, 41°14'03"S, 72°36'03"W, larvae (WDS-A-1462)
- 3 km W Nueva Braunau, 13 km west Puerto Varas, Puente Colegual, Río Colegual, 1200 ft, 18 I 2002, 41°19'34"S, 73°07'20"W, larvae (WDS-A-1463)
- \*3 km W Nueva Braunau, Río Colegual, 30 XII 2002, 41°19.58'S, 73°07.35"W, adults (WDS-A-1502)
- \*3 km W Nueva Braunau, Río Colegual, 8 I 2003, 41°19.58'S, 73°07.35"W, adults
- 8 km W Loncotoro, unnamed stream, 1270 ft, 19 I 2002, 41°18'01"S, 73°18'27"W, larvae (WDS-A-1464)
- 9 km E Loncotoro, Puente Colegual 2, Río Colegual, 700 ft, 19 I 2002, 41°16'30"S, 73°06'31"W, larvae (WDS-A-1465)
- \*9 km E Loncotoro, Puente Colegual 2, Río Colegual, 700 ft, 8 I 2003, 41°16.51'S, 73°06.52'W, adults (WDS-A-1519)
- Isla Chiloé, 13 road km W Chacao, Río Huicha, 1000 ft, 19 I 2002, 41°52'51"S, 73°39'29"W, larvae (WDS-A-1466)
- Isla Chiloé, 5 km N Lago Tarahuín, unnamed stream, 180 ft, 20 I 2002, 42°40'30"S, 73°47'46"W, larva (WDS-A-1467)
- Isla Chiloé, 1 road km E Cucao, Puente Curahuelvo, unnamed stream, 50 ft, 21 I 2002, 42°38'31"S, 74°05'38"W, larvae (WDS-A-1469)
- Isla Chiloé, Parque Tepuhueico, Río Bravo, 68 ft, 3 III 2008, 42°44.392'S, 73°57.611'W, larvae (WDS-A-1781)
- Corral, 2 km SE Aguada, Estero La Aguada, 160 ft, 22 I 2002, 39°54'07"S, 73°25'16"W, larvae (WDS-A-1471)
- 14 road km SE Corral, Puente Central, Estero Los Llanos, 120 ft, 22 I 2002, 39°57'21"S, 73°22'28"W, larvae (WDS-A-1472)
- 20 road km SE Corral, Puente Las Romazas, Estero de la Romaza, 60 ft, 22 I 2002, 39°57'41"S, 73°19'33"W, larvae (WDS-A-1473)
- \*10 km NE Chamiza, Puente Hondo (Río Oroco), 420 ft, 31 XII 2002, 41°28.83'S, 72°48.20'W, adults (WDS-A-1504)
- 5 km NE Chamiza, Río Chamiza, 360 ft, 31 XII 2002, 41°26.57'S, 72°49.19'W, larvae (WDS-A-1506)
- 1 km N Contao, Puente Zambo, 440 ft, 1 I 2003, 41°48.30'S, 72°42.72'W, larvae (WDS-A-1507)
- \*4 km S Contao, Puente Puñon, 850 ft, 1 I 2003, 41°49.84'S, 72°41.95'W, adult elytron (WDS-A-1508)

- \*6 km E Contaco, Puente Contaco, Río Contaco, 520 ft, 9 I 2003, 40°35.91'S, 73°29.67'W, adult and larvae (WDS-A-1521)
- 6 km W Contaco, Puente El Avion, 470 ft, 9 I 2003, 40°35.04'S, 73°37.52'W, larvae (WDS-A-1522)
- 8 km E Puerto Austral, Puente Queche, 640 ft, 1 I 2003, 41°58.58'S, 72°39.72'W, larvae (WDS-A-1510)
- \*12 km S Caleta Gonzalo, unnamed stream (trib. Río Blanco), 520 ft, 3 I 2003, 42°49.34'S, 72°42.73'W, adult and larvae (WDS-A-1511)
- 13 km S Caleta Gonzalo, Puente Camahueto No. 1, 600 ft, 3 I 2003, 42°49.55'S, 72°43.44'W, larvae (WDS-A-1512)
- 3 km S El Amarillo, unnamed tributary of Río Yelcho, 4 I 2003, ~260 ft, 43°02.29'S, 72°28.23'W, larvae
- Chaihuin, Reserva Costera Valdiviana, unnamed stream, 686 ft, 12 I 2007, 39°59.884'S, 73°38.901'W, larva (WDS-A-1734)
- Chaihuin, Reserva Costera Valdiviana, unnamed stream, 686 ft, 26 II 2008, 39°58.179'S, 73°34.225'W, larvae (WDS-A-1780)

## Región XI, Aysén

- 6 km N La Junta, unnamed stream, 590 ft, 4 I 2003, 43°55.34'S, 72°22.66'W, larvae (WDS-A-1515)
- 3 km S La Junta, unnamed stream, 610 ft, 4 I 2003, 43°59.69'S, 72°24.74'W, larvae (WDS-A-1516)

## Appendix 2

These *Tychepsephus* locality records are from the literature and museum specimen data, not including that cited in the Material examined sections. Where available, citations include geographic location, date, collector, life stage, and source of information. Region designations are as at the time of collection.

## CHILE

#### Región Metropolitana

Chile, Peñaflor, 24 Sep 1896, (larva) (crustacéiforme de Pseudo-Névroptère) (Lataste 1897a, 1897b)

## Región VII, Maule

Chile, RN Altos del Lircay, Las Majadillas, 6–9 Dic. 2005, Alejandro Vera (*T. cekalovici*) (MNNC)

Chile, Fundo El Rosal, Constitución, 16–30 Oct 1994, G. Arriagada (*T. cekalovici*) (MNNC)Chile, Parque Nacional Los Ruiles (larva) (Elgueta and Guerrero 2005)

Chile, Tregualemu (larva) (Elgueta and Guerrero 2005)

## Región VIII, Bío Bío

- Chile, Arauco, Laraquete, Río "Los Cruces", 11 Oct 1958, Fidel Jeldis (larva) (Artigas 1963)
- Chile, Cordillera de Chillán, Cueva de los Pincheira, 22 Jul 1962, J. Stuardo y G. Sanhuea (larvae) (Artigas 1963)
- Chile, Cordillera Chillán, Pte. Marchant, 15 Ene 1978, Vidal-Taima (*T. cekalovici*) (MNNC)

Chile, Tomé, Río Collén, 21 Abr 1960, André Hulot (larvae) (Artigas 1963)

Chile, Chile, Nuble, Quirihue, Ene 1988, G. Moreno (T. felix) (MNNC)

Chile, Parque Nacional Tolhuaca (larva) (Elgueta and Guerrero 2005)

## Región IX, Araucanía

Chile, Malleco, PN Conguillío, 20 Dic 1924, F. Ledesma (Ectopria grandis) (MNNC)

## Región XIV, Los Ríos

Chile, Valdivia, 6 Dic 1980, E. Krahmer (*T. felix*) (MNNC) Chile, Valdivia, 8 Dic 1980, E. Krahmer (*T. cekalovici*) (MNNC) Chile, Valdivia, Sto. Domingo, 13 Dic 1981, E. Krahmer (*T. cekalovici*) (MNNC) Chile, Valdivia, Sto. Domingo, 16 Dic 1984, E. Krahmer (*T. cekalovici*) (MNNC) Chile, Valdivia, Santo Domingo, 1/15 Dic 1981, E. Krahmer (*T. felix*) (MNNC)

## ARGENTINA

- Argentina, Provincia del Neuquén, Río Negro basin (larva) (*Ectopria* (*Chilectopria*) grandis) (Wais 1990)
- Argentina, Provincia del Neuquén, Río Meliquina (larva) (*Chilectopria grandis*) (Wais 1995)

RESEARCH ARTICLE



# A new species of the Cyrtodactylus brevipalmatus group (Squamata, Gekkonidae) from Tak Province, northwestern Thailand

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#### Abstract

An integrative taxonomic analysis was used to delimit and diagnose a new species of the *Cyrtodactylus* brevipalmatus group from Tak Province in western Thailand. Although Bayesian phylogenetic analyses place *C. denticulatus* **sp. nov.** within the brevipalmatus group, the new species is neither nested within nor is it the sister species of any other species in the brevipalmatus group. Furthermore, based on the mitochondrial NADH dehydrogenase subunit 2 gene (ND2) and adjacent tRNAs, it bears an uncorrected pairwise sequence divergence of 7.87-21.94% from all other species in the brevipalmatus group. *Cyrtodactylus denticulatus* **sp. nov.** is differentiated from all other species in the brevipalmatus group by having a number of unique charateristics such as denticulate ventrolateral body folds and ventrolateral subcaudal ridges, characters not seen in any other species of the group (n = 51 individuals). Additionally, based on a multiple factor anlaysis, *C. denticulatus* **sp. nov.** underscores the unrealized diversity of upland ecosystems across Thailand and the urgent need for increased exploration and conservation of these unique imperiled montane refugia, especially in this era of climate change.

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#### **Keywords**

Bent-toed gecko, conservation, Indochina, integrative taxonomy, Southeast Asia, systematics

## Introduction

The *Cyrtodactylus brevipalmatus* group (sec. Grismer et al. 2021a, b) of Indochina and northern Sundaland has recently become the focus of a number of systematic reviews (Grismer et al. 2021c, 2022a, b, 2023; Le et al. 2021) following more than a decade-long hiatus of investigation (Grismer 2008). Grismer et al. (2022b) demonstrated that the gross underestimate of this group's biodiversity resulted from a lack of phylogenetic data which forced the conflation of morphological diagnoses and species delimitations. Doing so resulted in the erroneous placement of 11 currently recognized species (sec. Grismer et al. 2022b) in the synonymy of just two species, *C. interdigitalis* Ulber (Ulber 1993) and *C. brevipalmatus* (Smith). The *brevipalmatus* group as currently constituted extends from northern Vietnam through Laos and central Thailand and southward along the Thai-Malay Peninsula to southern Peninsular Malaysia (Grismer et al. 2021a). Nearly all species of this group are specialized for an arboreal lifestyle and bear a prehensile tail carried in a tightly coiled position, cryptic color patterns of different shades of brown, closely matching the vegetative substrate upon which they frequent, and generally slow, deliberate "chameleon-like" movements (Grismer et al. 2020).

We present herein the description of another new species of the *brevipalmatus* group from the Chao Doi waterfall, Mae Meoi, Tha Song Yang District, Tak Province, Thailand (Fig. 1). The existence of this species was first reported by Chomdej et al. (2021) in a molecular phylogeny of Thai *Cyrtodactylus* but due to a lack of morphological data, it was not described but reported as *Cyrtodactylus* sp. 10. This nomen was followed in subsequent works by Grismer et al. (2021c, 2022a, b, 2023). We now have morphological data from that specimen and comparing these data to those of all other species in the *brevipalmatus* group (n = 51 individuals) revealed it possesses a suite of unique morphological characters putatively separating it from all other species in the group. Based on these data, the most recent phylogeny of the group (Grismer et al. 2023), and an uncorrected pairwise sequence divergence from all other species ranging from 7.87–21.94% based on the mitochondrial NADH dehydrogenase subunit 2 gene (ND2) and adjacent tRNAs, we hypothesize this specimen represents a new species and thus we describe it herein.

## Materials

#### Species delimitation

Under the general lineage concept (GLC: de Queiroz 2007) adopted herein, the molecular phylogenies recovered monophyletic mitochondrial lineages of individuals



**Figure 1.** Distribution of the species of the *Cyrtodactylus brevipalmatus* group and the localities of the specimens used in this analysis. Stars denote type localities and white circles represent the localities of unsampled populations photographed in social media.

(populations) that were used to develop initial species-level hypotheses, equivalent to the grouping stage of Hillis (2019). A multivariate analysis of morphometric, meristic, and categorical data were then used to search for characters and morphospatial patterns consistent with the tree-designated species-level hypotheses (the construction of boundaries representing the hypothesis-testing step of Hillis 2019) thus providing independent diagnoses to complement the delimitations of the molecular analyses. In this way, delimiting (phylogeny) and diagnosing (taxonomy) species are not conflated (Frost and Hillis 1990; Frost and Kluge 1994; Hillis 2019).

## Genetic data

Methods for DNA extraction, sequencing, and editing followed Grismer et al. (2021c) and resulted in a 1,386 base pair segment of the mitochondrial NADH dehydrogenase subunit 2 gene (ND2) and adjacent tRNAs. All material examined and GenBank accession numbers are listed in Table 1 of Grismer et al. (2022b). The GenBank accession number for *Cyrtodactylus* sp. 10 is MT468902.

## Morphological data

The morphological data included 15 meristic, 16 normalized morphometric, and eight categorical characters. The data were taken using the protocol of Le et al. (2021) and Grismer et al. (2022a). All data were taken on the left side of the body (when possible) and morphometric characters were measured to the nearest 0.1 mm using digital calipers under a Nikon SMZ745 stereomicroscope. Morphometric data taken were: snout-vent length (SVL), taken from the tip of the snout to the vent; tail length (TL), taken from the vent to the tip of the tail, original or partially regenerated; tail width (TW), taken at the base of the tail immediately posterior to the postcloacal swelling (TL and TW were too variable between sexes and condition of the tail to be used in the analyses); humeral length (HumL), taken from the proximal end of the humerus at its insertion point in the glenoid fossa to the distal margin of the elbow while flexed 90°; forearm length (ForL), taken on the ventral surface from the posterior margin of the elbow while flexed 90° to the inflection of the flexed wrist; femur length (FemL), taken from the proximal end of the femur at its insertion point in the acetabulum to the distal margin of the knee while flexed 90°; tibia length (TibL), taken on the ventral surface from the posterior margin of the knee while flexed 90° to the base of the heel; axilla to groin length (AG), taken from the posterior margin of the forelimb at its insertion point on the body to the anterior margin of the hind limb at its insertion point on the body; head length (HL), the distance from the posterior margin of the retroarticular process of the lower jaw to the tip of the snout; head width (HW), measured at the angle of the jaws; head depth (HD), the maximum height of head measured from the occiput to base of the lower jaw posterior to the eyes; eye diameter (ED), the greatest horizontal diameter of the eye-ball; eye to ear distance (EE), measured from the anterior edge of the ear opening to the posterior edge of the bony orbit; eye to snout distance or snout length (ES), measured from anteriormost margin of the bony orbit to the tip of snout; eye to nostril distance (EN), measured from the anterior margin of the bony orbit to the posterior margin of the external

quence divergence (p-distances) among the putative species of the Cyrtodactylus brevipal-	se subunit 2 gene (ND2) and adjacent tRNAs. Intraspecific p-distance are in bold font.
percentages of uncorre	f mitochondrial NADI
Table I. Mean (minimum-maximum)	matus group based on 1,386 base pairs o.

cf. brevi- uthaiens																												l n/a			19.48 n/a
s sp.																										n/a		19.61			6.58
denticulatus sp.nov.																							n/a			10.45		18.84			8 30
thongp- haphumensis.																					0.22	(0.00-0.52)	8.06		(7.87-8.13)	8.96	(8.77–9.03)	19.60		(19.48-19.87)	7 80
rukhadeva																			1.55		13.25	(12.52-13.94)	14.32		(13.68-14.97)	15.61	(14.97 - 16.26)	20.00		(19.48-20.52)	13 0.6
rivularis																	0.52		4.65	(3.61 - 5.68)	13.12	(12.77–13.42)	13.94		(13.68–14.19)	15.35	(15.10–15.61)	19.74		(19.48 - 20.00)	13 0/i
ngati															0.84	(0.00-1.55)	15.03	(14.71–15.48)	15.23	(14.19–16.23)	8.81	(8.13-9.68)	10.21		(10.06 - 10.45)	8.28	(8.00-8.65)	20.52		(20.26-20.65)	6 07
kochangensis													n/a		14.58	(14.45 - 14.84)	12.26	(12.00 - 12.52)	13.10	(12.52-13.68)	13.22	(13.03-13.29)	13.68			14.58		19.10			12 0/
interdigitalis											n/a		15.23		8.13	(7.74-8.65)	15.48	(15.23-15.74)	16.00	(15.35-16.65)	8.96	(8.77-9.03)	10.19			3.87		20.13			11
fluvicavus									0.10	(0.00-0.26)	12.02	(12.00-12.13)	12.31	(12.26-12.31)	11.34	(11.10 - 11.87)	12.57	(12.26 - 13.03)	12.25	(11.61 - 13.03)	9.75	(9.55-9.94)	10.12		(10.06 - 10.32)	11.12	(11.10-11.23)	18.34		(18.32-18.45)	10.12
elok							n/a		20.15	(20.13-20.26)	22.84		20.90		21.11	(20.90-21.42)	21.61	(21.42-21.81)	21.61	(21.16-22.06)	22.02	(21.81-22.32)	21.94			22.19		20.00			21.16
cf. ngati2					n/a		21.42		11.02	(10.97-11.23)	9.16		14.71		3.71	(3.35-4.26)	15.03	(14.84-15.23)	15.48	(14.84 - 16.13)	9.51	(9.42-9.55)	10.84			8.90		20.65			9.13
cf. ngatil			n/a		4.39		22.58		10.64	(10.58 - 10.84)	6.97		14.58		3.30	(2.84 - 4.00)	15.87	(15.61 - 16.13)	15.42	(14.84 - 16.00)	7.93	(7.74-8.00)	9.29			7.23		20.90			5 81
brevipalmatus	n/a		21.03		21.68		20.77		18.86	(18.84-18.97)	20.77		19.35		20.70	(20.65-20.90)	20.00	(19.74-20.26)	20.65	(20.13-21.16)	20.34	(20.13-20.65)	19.87			20.39		6.45			10.74
~	brevipalmatus	n = 1	cf. ngati1	n = 1	cf. ngati2	n = 1	elok	n = 1	Auvicavus	<i>n</i> = 7	interdigitalis	n = 1	kochangensis	n = 1	ngati	<i>u</i> = 7	rivularis	n = 2	rukhadeva	n = 2	thongphaphu- mencic	1 = 9	denticulatus	sp. nov.	n = 1	sp. 11	<i>n</i> = 1	ff	brevipalmatus	n = 1	uthatencis

nares; interorbital distance (**IO**), measured between the dorsomedial-most edges of the bony orbits; internarial distance (**IN**), measured between the external nares across the rostrum; and ear length (**EL**), greatest oblique length across the auditory meatus. Normalization of the morphometric characters in order to prevent allometric biasing follows Chan and Grismer (2022).

Evaluated meristic characters were the number of supralabial scales (SL), counted from the largest scale at the corner of the mouth or posterior to the eye, to the rostral scale; infralabial scales (IL), counted from termination of enlarged scales at the corner of the mouth to the mental scale; number of paravertebral tubercles (PVT) between the limb insertions counted in a straight line immediately left of the vertebral column; number of longitudinal rows of body tubercles (LRT) counted transversely across the body midway between the limb insertions from one ventrolateral body fold to the other; number of longitudinal rows of ventral scales (VS) counted transversely across the abdomen midway between limb insertions from one ventrolateral fold to the other; number of transverse rows of ventral scales (VSM) counted along the midline of the body from the postmentals to just anterior to the cloacal opening, stopping where the scales become granular; number of expanded subdigital lamellae on the fourth toe proximal to the digital inflection (TL4E) counted from the base of the first phalanx where it contacts the body of the foot to the largest scale on the digital inflection; the large contiguous scales on the palmar and plantar surfaces were not counted; number of small, generally unmodified subdigital lamellae distal to the digital inflection on the fourth toe (TL4U) counted from the digital inflection to the claw including the claw sheath; total number of subdigital lamellae (TL4T) beneath the fourth toe (i.e. TL4E + TL4U = TL4T); number of expanded subdigital lamellae on the fourth finger proximal to the digital inflection (FL4E) counted the same way as with TL4E; number of small generally unmodified subdigital lamellae distal to the digital inflection on the fourth finger (FL4U) counted the same way as with TL4U; total number of subdigital lamellae (FL4T) beneath the fourth toe (i.e. FL4E + FL4U = FL4T); total number of enlarged femoral scales (FS) from each thigh combined as a single metric; number of enlarged precloacal scales (PCS); number of precloacal pores (PP) in males; number of femoral pores (FP) in males from each thigh combined as a single metric; number postcloacal tubercles (PCT) on each side of the base of the tail (this character was not used in the analyses); and the number of dark body bands (BB) between the dark band on the nape and the hind limb insertions on the body. A post-sacral or sacral band when present, was not counted. Categorical characters evaluated were the presence or absence of tubercles on the flanks (FKT); ventrolateral body fringe denticulate (VFD); slightly enlarged medial subcaudals (SC1); single enlarged, unmodified, medial subcaudal scales (SC2); enlarged medial subcaudals intermittent, medially furrowed, posteriorly emarginated (SC3); small longitudinal ventrolateral subcaudal ridge (SC4); large or small dorsolateral caudal tubercles (DCT) forming a narrow or wide ventrolateral caudal fringe (VLF1); ventrolateral caudal fringe scales generally homogenous or not (VLF2); and the cross-section of the tail round or square (TLcross).

## Phylogenetic analyses

An input file implemented in BEAUti (Bayesian Evolutionary Analysis Utility) v. 2.4.6 was run in BEAST (Bayesian Evolutionary Analysis Sampling Trees) v. 2.4.6 (Drummond et al. 2012) on CIPRES (Cyberinfrastructure for Phylogenetic Research; Miller et al. 2010) in order to generate a BEAST phylogeny. Data were partitioned by codon and a lognormal relaxed clock with unlinked site models and linked trees and clock models were employed. bModelTest (Bouckaert and Drummond 2017), implemented in BEAST, was used to numerically integrate over the uncertainty of substitution models while simultaneously estimating phylogeny using Markov chain Monte Carlo (MCMC). MCMC chains were run using a birth-death prior for 40,000,000 million generations and logged every 4,000 generations. The BEAST log file was visualized in Tracer v. 1.7.0 (Rambaut et al. 2018) to ensure effective sample sizes (ESS) were well-above 200 for all parameters. A maximum clade credibility tree using mean heights at the nodes was generated using TreeAnnotator v. 1.8.0 (Rambaut and Drummond 2013) with a burn-in of 1,000 trees (10%). Nodes with Bayesian posterior probabilities (BPP) of 0.95 and above were considered strongly supported (Huelsenbeck et al. 2001; Wilcox et al. 2002). Uncorrected pairwise sequence divergences were calculated in MEGA 11 (Tamura et al. 2021) using the complete deletion option to remove gaps and missing data from the alignment prior to analysis.

## Statistical analyses

All statistical analyses were conducted using R Core Team (2018). Morphometric characters used in statistical analyses were SVL, AG, HumL, ForL, FemL, TibL, HL, HW, HD, ED, EE, ES, EN, IO, EL, and IN. Tail metrics were not used due to the high degree of incomplete sampling (i.e., regenerated, broken, or missing). In order to most successfully remove the effects of allometry (sec. Chan and Grismer 2022), size was normalized using the following equation:  $X_{adj} = log(X) - \beta[log(SVL) - log(SVL_{mean})]$ , where  $X_{adj}$  = adjusted value; X = measured value;  $\beta$  = unstandardized regression co-efficient for each population; and SVL<sub>mean</sub> = overall average SVL of all populations (Thorpe 1975, 1983; Turan 1999; Lleonart et al. 2000), accessible in the R package GroupStruct (available at https://github.com/chankinonn/GroupStruct). The morphometrics of each species were normalized separately and then concatenated so as not to conflate potential intra- with interspecific variation (Reist 1986; McCoy et al. 2006). The juvenile Cyrtodactylus ngati (HNUE-R00112) was removed from the data so as not to skew the normalization results. All data were scaled to their standard deviation to ensure they were analyzed on the basis of correlation and not covariance. Meristic characters analyzed were SL, IL, PVT, LRT, VS, VSM, TL4E, TL4U, TL4T, FL4E, FL4U, FL4T, FS, PCS, and BB. Precloacal and femoral pores were omitted from the multivariate analyses due to their absence in females. Categorical characters analyzed were DCT, VLF1, VLF2, TLcross, SC1, SC2, and SC3.

Morphospatial clustering and positioning among the species/populations and individuals was analyzed using multiple factor analysis (MFA) on a concatenated data set comprised of 15 meristic characters, 16 normalized morphometric characters, and eight categorical characters (Suppl. material 1). The MFA was implemented using the mfa() command in the R package FactorMineR (Husson et al. 2017) and visualized using the Factoextra package (Kassambara and Mundt 2017). MFA is a global, unsupervised, multivariate analysis that incorporates qualitative and quantitative data (Pagès 2015), making it possible to analyze different data types simultaneously in a nearly total evidence environment. In an MFA, each individual is described by a distinct set of variables (i.e., characters) which are structured into different data groups in a global data frame, in this case, quantitative data (i.e., meristics and normalized morphometrics) and categorical data (i.e., scale, tubercle, and caudal morphology). In the first phase of the analysis, separate multivariate analyses are conducted for each set of variables: principal component analyses (PCA) for each quantitative data set and a multiple correspondence analysis (MCA) for the categorical data. The data sets are then normalized separately by dividing all their elements by the square root of their first eigenvalues. For the second phase of the analysis, these normalized data sets are concatenated into a single matrix for a global PCA of the normalized data. Standardizing the data in this manner prevents one data type from overleveraging another. In other words, the normalization of the data in the first phase prevents data types with the highest number of characters or the greatest amount of variation from outweighing other data types in the second phase. This way, the contribution of each data type to the overall variation in the data set is scaled to define the morphospatial distance between individuals as well as calculating each data type's contribution to the overall variation in the analysis (Pagès 2015; Kassambara and Mundt 2017).

## Results

## Phylogenetic analysis

The BEAST analysis recovered *C*. sp. 10 as being deeply nested within the *brevipalmatus* group on a long branch that was not embedded within, nor sister to any other species (Fig. 2). *Cyrtodactylus* sp. 10 was strongly recovered (BPP 1.00) recovered as the sister species to a lineage composed of *Cyrtodactylus thongphaphumensis*, *C. uthaiensis*, *C. interdigitalis*, *C.* sp. 11, *C.* cf. *ngati*1, *C.* cf. *ngati*2, *C. ngati*3, *C. ngati*4, and *C. ngati*. *Cyrtodactylus* sp. 10 and has an uncorrected pairwise sequence divergence from all other species ranging from 7.87–21.94% (Table 1).

## Multiple factor analysis

The MFA recovered *C*. sp. 10 as well-separated from all other species of the *brevipalmatus* group along the ordination of the first two dimensions (Dim) in that the specimen did not cluster near or within the convex hull of any other species (Fig. 3A). Dim-1



**Figure 2.** Maximum clade credibility BEAST phylogeny of the *Cyrtodactylus brevipalmatus* group highlighting the new species described herein. Bayesian posterior probabilities (BPP) are listed at the nodes.

accounted for 16.6% of the variation in the data set and loaded most heavily for the meristic characters FL4U, FL4T, TL4U, TL4T, PCS, FL4E, LRT, and the morphometric character HW in that they contributed a greater than average amount of variation along Dim-1 (Fig. 3B). The meristic characters VS and BB and the morphometric character HumL contributed a greater than average amount of variation along Dim-2 (Fig. 3C). Dim-1 and Dim-2 collectively accounted for a total of 38% of the variation in the data set. The first five dimensions account for a total of 60.7% of the variation in the data set.

## Taxonomy

Based on the phylogenetic position of *C*. sp. 10, its high percentage value of pairwise sequence divergence from all other species, and its unique morphology, we hypothesize



**Figure 3.** MFA analyses of the *Cyrtodactylus brevipalmatus* group **A** MFA of the species-level lineages based on the BEAST phylogeny (Fig. 2) **B** percent contribution of morphometric and meristic characters to Dimension 1 of the MFA. The red dotted line represents the average contribution **C** percent contribution of morphometric and meristic characters to Dimension 2 of the MFA. The red dotted line represents the average contribution.

*C.* sp. 10 represents a diagnosable evolutionarily distinct population at the northwestern extent of the range of the *brevipalmatus* group that should be recognized as a new species. As such, it is described below.

#### Cyrtodactylus denticulatus sp. nov.

https://zoobank.org/5052D212-71D3-43D5-B324-01F8BE8DF56C Figs 4–6 Suggested common name: Spiny-tailed bent-toed gecko Suggested Thai common name: ดุักกายพื้นเลือย Tuk Kay Fhun Leuy

*Cyrtodactylus* sp. 10: Chomdej et al. 2021: 11; Grismer et al. 2021: 725, 2022a: 247, 2022b: 111; 2023: 95.

**Type material.** *Holotype.* Adult male AUP-00680 collected on 8 March 2019 by Parinya Pawangkhanant and Chatmongkon Suwannapoom from a bamboo forest near a rocky stream at the Chao Doi waterfall, Mae Meoi, Tha Song Yang District, Tak Province, Thailand at 17°30.000'N, 98°03.000'E (DDM) and 610 m a.s.l.

**Diagnosis.** *Cyrtodactylus denticulatus* sp. nov. is tentatively separated (see below) from all other species of the *brevipalmatus* group by the combination of having nine supralabials, nine infralabials, 20 paravertebral tubercles, 19 rows of longitudinally arranged tubercles, 42 transverse rows of ventrals, 158 longitudinal rows of ventrals, nine


**Figure 4.** Photograph of the living holotype of *Cyrtodactylus denticulatus* sp. nov. AUP-00680 from the Chao Doi waterfall, Mae Meoi, Tha Song Yang District, Tak Province, Thailand.

expanded subdigital lamellae on the fourth finger, 11 unexpanded subdigital lamellae on the fourth toe, 19 total subdigital lamellae on the fourth toe; nine expanded subdigital lamellae on the fourth finger, 19 total subdigital lamellae on the fourth finger, 16 total number of enlarged femoral scales, 20 total number of femoral pores in the male specimen; 13 precloacal pores in the male specimen; 16 enlarged precloacals; enlarged femorals and enlarged precloacals not continuous; proximal femorals smaller than distal femorals; tubercles on forelimbs and flanks nearly same size as those on body; ventrolateral body fold weakly denticulate; spinose paravertebral rows; row of large dorsolateral caudal tubercles; wide ventrolateral caudal fringes; ventrolateral subcaudal ridges; tail square in cross-section; no slightly enlarged unpaired medial subcaudals; subcaudals not posteromedially furrowed; SVL 69.5 mm; three dark transverse body bands (Tables 2–4).

**Description of holotype (Figs 5, 6).** Adult male SVL 69.5 mm; head moderate in length (HL/SVL 0.27), width (HW/HL 0.68), depth (HD/HL 0.38), distinct from neck, triangular in dorsal profile; lores slightly concave anteriorly, weakly inflated posteriorly; prefrontal region weakly concave; canthus rostralis rounded; snout elongate (ES/HL 0.42), rounded in dorsal profile; eye large (ED/HL 0.21); ear opening oblique-ly elliptical, small; eye to ear distance greater than diameter of eye; rostral rectangular, divided by a dorsal furrow, bordered posteriorly by large left and right supranasals and one smaller azygous internasal, bordered laterally by first supralabials; external nares



**Figure 5.** Head of the holotype of *Cyrtodactylus denticulatus* sp. nov. AUP-00680 from the Chao Doi waterfall, Mae Meoi, Tha Song Yang District, Tak Province, Thailand **A** ventral view **B** dorsal view **C** right lateral view.

bordered anteriorly by rostral, dorsally by large supranasal, posteriorly by two unequally sized smaller postnasals, bordered ventrally by first supralabial; 9R/9L rectangular supralabials, second through seventh supralabials nearly same size as first, then tapering below eye; 9R/9L infralabials tapering smoothly to just below and slightly past posterior margin of eye; scales of rostrum and lores flat to slightly domed, larger than granular scales on top of head and occiput; scales of occiput intermixed with distinct, small tubercles; superciliaries subrectangular, largest anterodorsally; mental triangular, bordered laterally by first infralabials and posteriorly by large left and right trapezoidal postmentals contacting medially for 50% of their length posterior to mental; one row of enlarged, square to rectangular sublabials extending posteriorly to first (L) and fourth (R) infralabial; gular and throat scales small, granular, grading posteriorly into slightly larger, flatter, smooth, imbricate, pectoral, and ventral scales.

Body relatively short (AG/SVL 0.46) with well-defined denticulate ventrolateral folds; dorsal scales small, granular interspersed with larger, conical, semi-regularly arranged, weakly keeled tubercles; tubercles extend from occipital region onto base of tail and slightly beyond as paravertebral rows; smaller tubercles extend anteriorly onto



**Figure 6.** Holotype of *Cyrtodactylus denticulatus* sp. nov. AUP-00680 from the Chao Doi waterfall, Mae Meoi, Tha Song Yang District, Tak Province, Thailand **A** ventral view of the cloacal and femoral regions **B** ventral view of the left hand **C** ventral view of the left foot **D** dorsal view of the anterior section of the tail **E** ventral view of the tail showing the ventrolateral subcaudal ridge.

phometric data used in the analyses of specimens in the <i>Cyrtodactylus brevipalmatus</i> group. $\delta$ = male; $\varphi$ = female;	s denote characters that potentially differentiate C. denticulatus sp. nov. from the other species.
ategorical, and morphometric data	vailable. Shaded cells denote charae
Table 2. Sex, raw meristic, c	R/L = right/left; / = data unav
	н

Species	denticulatus sp. nov.	brevipalmatus	cf. brevipal- matus	cf. brevipal- matus	brevipal- matus	brevipal- matus	elok	elok	elok	elok	interdigi- talis	interdigi- talis	interdigi- talis	interdigi- talis	sp. 11
Institutional catalog number	AUP-00680	LSUHC 1899	LSUHC 15076	LSUHC 11788	02901 10670	THNHM 14112	LSUHC 8238	LSUHC 12180	LSUHC 12181	ZMMU R-16144	THNHM 20226 paratype	THNHM 20228 paratype	THNHM 20229 paratype	THNMH 20227 paratype	ZMMU R-16492
Sex	40	40	0+	0+	0+	0+	0+	60	60	0+	0+	0+	0+	0+	60
					Meristic	c data									
supralabials (SL)	6	11	12	10	14	12	11	8	13	6	14	12	11	12	11
infralabials (IL)	6	∞	10	6	11	11	11	8	11	6	6	8	8	7	6
paravertebral tubercles (PVT)	20	39	37	38	37	37	0	0	0	0	32	33	33	33	30
longitudinal rows of tuberdes (LRT)	19	15	16	17	16	14	6	7	4	4	19	20	19	19	18
ventral scales (VS)	42	38	38	38	36	39	45	45	47	36	42	40	42	43	34
ventral scales along middle of the body (VSM)	158	176	170	182	154	160	190	225	234	192	187	170	187	178	160
expanded subdigital lamellae on 4 <sup>th</sup> toe (TLAE)	6	~	~	6	8	8	10	6	6	6	12	10	10	11	6
unmodified subdigital lamellae on $4^{\rm th}$ toe (TLAU)	11	13	11	11	Π	12	11	10	11	6	14	13	12	14	10
total subdigital lamellae 4 <sup>th</sup> toe (TL4T)	20	20	19	20	19	20	21	19	20	18	26	23	22	23	19
expanded subdigital lamellae on 4 <sup>th</sup> finger (FL4E)	6	×	~	∞	7	8	×	6	6	6	6	∞	6	6	10
unmodified subdigital lamellae on 4 <sup>th</sup> finger (FL4U)	10	6	11	10	10	10	12	13	6	∞	12	11	12	13	6
total subdigital lamellae 4th finger (FL4T)	19	17	19	18	17	18	20	22	18	17	21	21	21	22	19
enlarged femoral scales (R/L)	8	0	0	0	8R/8L	7R/7L	0	0	0	0	11R/8L	10R/9L	8R/8L	9R/10L	9R/8L
total enlarged femoral scales (FS)	16	16	10	11	16	14	0	0	0	0	14	19	19	19	17
total femoral pores in males (FP)	20	7	/	_	/	/	_	0	0	-	_	_	/	_	17
enlarged precloacal scales (PCS)	13	7	7	7	8	7	∞	∞	∞	7	14	15	13	19	13
precloacal pores in males (PP)	13	7	/	/	1	/	/	8	8	/	0	/	0	0	13
postcloacal tubercles (PCT)	3	я	3	2	3	3	3	2	3	3	3	2	3	3	3
Body bands (BB)	3	4	9	3	5	5	2	2	e,	ŝ	Ś	5	5	5	3
					Categoric	cal data									
small tubercles on flank (FKT)	present	present	present	present	present	present	absent	absent	absent	absent	present	present	present	present	present
denticulate ventrolateral body folds (VFD)	present	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
dorsolateral caudal tubercles (DCT)	small	small	small	small	-	small	large	large	large	large	small	~	small	small	large

Species	denticulatus sp. nov.	brevipalmatus	cf. brevipal- matus	cf. brevipal- matus	brevipal- matus	brevipal- matus	elok	elok	elok	elok	interdigi- talis	interdigi- talis	interdigi- talis	interdigi- talis	sp. 11
Institutional catalog number	AUP-00680	LSUHC 1899	LSUHC 15076	LSUHC 11788	THNHM 10670	THNHM 14112	LSUHC 8238	LSUHC 12180	LSUHC 12181	ZMMU R-16144	THNHM 20226 paratype	THNHM 20228 paratype	THNHM 20229 paratype	THNMH 20227 paratype	ZMMU R-16492
Sex	50	6	0+	0+	0+	0+	0+	60	60	0+	0+	0+	0+	0+	50
ventrolateral caudal fringe narrow or wide (VLF1)	wide	narrow	narrow	narrow	~	narrow	wide	wide	wide	wide	narrow	-	narrow	narrow	wide
ventrolateral caudal fringe scales generally homogenous (VLF2)	ou	ou	ou	ou	~	ou	ou	ou	ou	QU	yes	yes	yes	yes	yes
tail cross-section (TLcross)	square	circular	circular	circular	/	circular	square	square	square	square	circular	/	circular	circular	square
slightly enlarged medial subcaudals (SC1)	absent	present	present	present	/	absent	absent	absent	absent	absent	absent	/	absent	absent	present
single enlarged medial subcaudal (SC2)	absent	absent	absent	absent	_	absent	absent	absent	absent	absent	absent	-	absent	absent	absent
enlarged medial subcaudals intermittent, medially furrowed, posteriorly emarginate (SC3)	ou	ou	ou	00	~	QU	ou	ou	ou	оп	yes	/	yes	yes	по
small ventrolateral subcaudal ridge of scales (SC4)	yes	Ю	ou	ou	оп	ou	ou	ou	ou	ou	ou	ou	ou	ou	по
					Morphome	tric data									
TAS	69.5	68.8	70.8	64.1	65.95	63.79	80.2	78.2	84.8	78.6	81.19	74.80	78.56	59.70	68.1
AG	83.0	35.7	33.4	30.1	30.0	26.5	39.7	37.8	41.5	36.2	34.5	33.7	32.7	24.6	34.6
HumL	9.1	9.7	9.3	8.0	9.6	9.5	10.2	9.1	10.1	1.7	9.8	10.2	11.2	7.4	10.3
ForL	10.2	9.9	9.8	8.9	8.2	8.7	11.5	11.7	11.8	10.2	10.6	10.5	11.1	8.4	8.5
FenL	12.8	12.0	12.6	11.5	11.7	9.8	12.9	14.2	14.6	13.1	14.7	13.2	12.7	10.2	12.6
TibL	6.5	11.6	12.2	10.5	9.7	8.2	13.5	14.0	13.8	12.3	13.1	11.9	12.9	10.2	11.4
HL	18.8	19.3	19.3	19.0	17.9	18.2	21.8	21.6	21.9	21.7	20.8	19.9	21.7	16.7	18.4
MH	12.7	13.2	13.8	12.3	12.3	12.0	15.6	16.1	15.9	15.1	14.0	13.4	14.2	11.4	13.1
HD	7.2	8.0	7.6	7.6	7.3	7.0	9.6	9.8	10.4	9.8	3.4	8.6	8.7	9.9	8.3
ED	4.0	5.2	4.5	4.3	5.3	4.4	4.8	5.0	5.7	5.0	5.3	5.5	5.9	4.4	4.4
EE	5.4	5.7	5.9	4.9	5.7	5.7	6.4	7.1	7.0	6.8	5.8	6.2	6.4	4.8	6.2
ES	7.9	7.4	7.6	7.0	7.0	7.2	8.6	8.7	9.5	8.6	8.3	7.8	9.1	6.8	7.7
EN	5.8	5.7	5.4	4.9	5.3	5.4	6.0	6.2	6.5	6.2	6.0	5.5	6.8	5.1	5.5
O	7.1	5.4	4.7	4.7	4.2	5.2	5.7	5.4	5.4	3.9	4.8	4.7	5.5	4.3	2.9
EL	0.7	1.0	1.4	1.1	1.3	1.0	1.9	1.4	1.5	1.4	1.3	1.3	1.6	1.2	0.9
NI	2.4	1.7	2.1	2.3	2.1	2.2	2.7	2.6	2.5	3.1	2.1	2.2	2.5	1.8	2.3

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Species	denticulatus sp. nov.		nga	tti			ngati3		ngati4	cf. ngatil	cf. ng	ati2
Institutional catalog number	AUP-00680	HNUE-R00111	IEBR 4829	VNUF R.2020.12	HNUE-R00112	FMNH 255454	FMNH 270493	FMNH 270492	FMNH 265806	NCSM 79472	ZMMU R-14917	NCSM 80100
Sex	60	60	0+	0+	0+	0+	۴O	50	60	0+	0+	0+
				Meristic d	ata							
supralabials (SL)	6	10	10	10	10	13	13	13	10	14	6	12
infralabials (IL)	6	6	6	6	6	10	6	11	8	11	10	12
paravertebral tubercles (PVT)	20	39	40	38	40	28	27	26	27	28	32	29
longitudinal rows of tubercles (LRT)	19	18	18	17	22	19	18	17	19	18	24	19
ventral scales (VS)	42	38	36	35	32	37	36	36	33	33	36	35
ventral scales along middle of the body (VSM)	158	168	164	178	158	159	166	156	158	164	166	165
expanded subdigital lamellae on 4 <sup>th</sup> toe )TL4E(	6	8	10	6	6	10	10	8	10	6	8	10
unmodified subdigital lamellae on 4th toe )TL4U(	11	11	10	11	10	11	11	11	11	12	10	10
total subdigital lamellae $4^{\rm th}$ toe (TL\$T)	20	13	16	17	16	21	21	19	21	21	18	20
expanded subdigital lamellae on 4 <sup>th</sup> finger (FL4E)	6	6	6	7	6	8	8	8	8	6	7	6
unmodified subdigital lamellae on 4th finger (FL4U)	10	6	6	6	6	10	10	10	10	8	6	10
total subdigital lamellae 4 <sup>th</sup> finger (FL4T)	19	15	15	18	15	18	18	18	18	17	16	19
enlarged femoral scales (R/L)	8	10R/10L	9R/8L	10R/9L	8R/9L	9R/7L	8R/9L	9R/9L	8R/8L	9R/8L	7R/8L	7R/8L
total enlarged femoral scales (FS)	16	20	17	19	17	16	17	18	16	17	15	15
total femoral pores (FP)	20	14	_	-	/	-	14	15	13	/	/	
enlarged precloacal scales (PCS)	13	13	13	13	13	15	13	13	13	12	13	13
precloacal pores (PP)	13	13	_	/	/	13	13	13	13	/	_	_
postcloacal tubercles (PCT)	3	3	2		2	-	-	/	/	2	3	4
Body bands (BB)	3	6	6	9	9	6	4	3	3	3	33	3
				Categorical	data							
small tubercles on flank (FKT)	present	present	present	present	present	present	present	present	present	present	present	present
denticulate ventrolateral body folds (VFD)	present	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
dorsolateral caudal tubercles (DCT)	small	small	small	small	small	small	small	small	small	small	small	small
ventrolateral caudal fringe narrow or wide (VLF1)	wide	narrow	narrow	narrow	narrow	narrow	narrow	narrow	narrow	narrow	narrow	narrow
ventrolateral caudal fringe scales generally homogenous (VLF2)	ou	no	ou	оп	no	yes	yes	yes	yes	yes	yes	yes
tail cross-section (TLcross)	square	circular	circular	circular	circular	circular	circular	circular	circular	circular	circular	circular
slightly enlarged medial subcaudals (SC1)	absent	present	present	present	present	/	present	present	present	present	present	present

Species	denticulatus sp. nov.		าชิน	ii.			ngati3		ngati4	cf. ngatil	cf. ng	ati2
Institutional catalog number	AUP-00680	HNUE-R00111	IEBR 4829	VNUF R.2020.12	HNUE-R00112	FMNH 255454	FMNH 270493	FMNH 270492	FMNH 265806	NCSM 79472	ZMMU R-14917	NCSM 80100
Sex	67	60	0+	0+	0+	0+	67	60	67	0+	0+	0+
single enlarged medial subcaudal (SC2)	absent	absent	absent	absent	absent	_	absent	absent	absent	absent	absent	absent
enlarged medial subcaudals intermittent, medially furrowed, posteriorly emarginate (SC3)	ou	ou	ou	no	no	-	ou	ou	ou	ou	ou	no
small ventrolateral subcaudal ridge of scales (SC4)	yes	ou	ou	no	ou	_	no	no	ou	ou	no	no
				Morphometr	ic data							
SVL	69.5	66.5	68.1	69.3	46.6	83.6	70.2	74.1	73.8	78.0	87.1	77.7
AG	83.0	28.8	29.8	30.2	19.7	41.3	35.4	37.0	31.3	38.2	41.9	36.8
HumL	9.1	7.9	8.1	8.5	5.6	8.6	8.7	8.6	6.9	8.7	11.5	9.2
ForL	10.2	9.2	10.0	10.1	6.5	10.2	9.3	10.4	10.0	10.3	10.4	10.7
FemL	12.8	11.5	11.5	11.5	7.6	13.7	12.7	13.0	13.1	13.1	15.2	14.2
TibL	6.5	10.8	11.1	11.8	7.8	12.5	11.8	11.2	11.1	12.8	12.6	12.7
HL	18.8	20.1	20.4	20.7	16.1	21.7	20.6	20.3	20.7	21.2	22.1	21.4
MH	12.7	12.6	12.0	11.8	8.8	13.8	12.5	13.0	12.3	12.7	14.8	13.5
HD	7.2	7.4	7.2	6.6	5.1	9.2	8.4	9.1	7.6	8.3	8.7	9.2
ED	4.0	3.8	4.1	3.4	2.6	4.9	4.9	4.9	4.8	6.5	4.6	6.0
EE	5.4	5.8	5.5	5.9	4.4	6.9	6.1	6.2	5.7	5.3	6.5	6.2
ES	7.9	5.7	7.6	6.9	5.0	9.0	8.3	8.3	8.2	8.7	8.8	8.4
EN	5.8	6.7	6.3	6.2	4.5	6.5	6.2	6.1	6.2	6.2	6.6	6.0
IO	7.1	5.6	5.4	5.6	4.2	6.6	5.6	5.4	5.1	4.9	3.5	5.7
EL	0.7	0.8	0.8	0.7	0.3	1.3	1.1	1.2	1.0	1.5	1.2	0.9
IN	2.4	2.8	2.6	2.6	2.0	2.8	2.5	2.5	2.3	2.7	2.2	2.5

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Species	denticulatus sp. nov.				thongphapl	umensis				sp.	13
Institutional catalog number	AUP-00680	ZMKU R 00950 paratype	ZMKU R 00951 paratype	ZMKU R 00952 paratype	ZMKU R 00953 holotype	ZMKU R 00954 paratype	ZMKU R 00955 pratype	ZMKU R 00956 paratype	ZMKU R 00957 paratype	THNHM 00104	THNHM 27821
Sex	50	0+	60	0+	50	50	0+	60	0+	0+	0+
		-		Meristic data							
supralabials (SL)	6	12	13	13	14	13	13	13	13	12	15
infralabials (IL)	6	∞	8	10	10	6	10	10	6	10	11
paravertebral tubercles (PVT)	20	32	33	34	34	36	36	30	30	33	29
Iongitudinal rows of tubercles (LRT)	19	21	19	20	20	21	21	19	19	18	20
ventral scales (VS)	42	34	33	33	34	30	33	32	33	37	36
ventral scales along middle of the body (VSM)	158	173	158	156	166	159	159	150	169	159	165
expanded subdigital lamellae on 4 <sup>th</sup> toe (TL4E)	6	6	10	6	8	10	8	6	6	6	7
unmodified subdigital lamellae on 4th toe (TL4U)	11	12	14	13	12	13	12	11	13	12	12
total subdigital lamellae 4th toe (TL\$T)	20	21	24	22	20	23	20	20	22	21	19
expanded subdigital lamellae on 4 <sup>th</sup> finger (FL4E)	6	8	7	7	8	8	8	8	8	8	8
unmodified subdigital lamellae on 4th finger (FL4U)	10	10	12	12	11	12	12	11	12	11	10
total subdigital lamellae 4th finger (FL4T)	19	18	19	19	19	20	20	19	20	19	18
enlarged femoral scales (R/L)	8	5R/7L	8R/8L	8R/8L	7R/8L	8R/8L	7R/8L	7R/6L	8R/8L	9R/9L	7R/10L
total enlarged femoral scales (FS)	16	12	16	16	15	16	15	13	16	18	17
total femoral pores (FP)	20	0	16	0	14	15	14	12	0	0	0
enlarged precloacal scales (PCS)	13	17	15	15	15	15	15	15	15	14	16
precloacal pores (PP)	13	0	15	0	15	15	15	15	0	0	0
postcloacal tubercles (PCT)	3	2	2R/3L	3	3	2R/3L	2R/3L	я	2	6	3
Body bands (BB)	3	3	4	3	4	3	5	4	4	ŝ	_
				Categorical data	_						
small tubercles on flank (FKT)	present	present	present	present	present	present	present	present	present	present	present
denticulate ventrolateral body folds (VFD)	present	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
dorsolateral caudal tubercles (DCT)	small	large	large	large	large	large	large	/	large	small	small
ventrolateral caudal fringe narrow or wide (VLF1)	wide	wide	wide	wide	wide	wide	wide	_	wide	narrow	narrow
ventrolateral caudal fringe scales generally homogenous (VLF2)	ou	оп	ou	ou	no	ou	ou	/	ou	yes	yes
tail cross-section (TLcross)	square	square	square	square	square	square	square	/	square	circular	circular

Species	denticulatus sp. nov.				thongphaph	umensis				sp.	13
Institutional catalog number	AUP-00680	ZMKU R 00950 paratype	ZMKU R 00951 paratype	ZMKU R 00952 paratype	ZMKU R 00953 holotype	ZMKU R 00954 paratype	ZMKU R 00955 pratype	ZMKU R 00956 paratype	ZMKU R 00957 paratype	THNHM 00104	THNHM 27821
Sex	60	0+	50	0+	60	60	0+	60	0+	0+	0+
slightly enlarged medial subcaudals (SC1)	absent	present	present	present	present	present	present	/	present	present	present
single enlarged medial subcaudal (SC2)	absent	absent	absent	absent	absent	absent	absent	_	absent	absent	absent
enlarged medial subcaudals intermittent, medially furrowed, posteriorly emarginate (SC3)	ou	оц	ou	ou	ou	yes	ou	-	no	ou	no
small ventrolateral subcaudal ridge of scales (SC4)	yes	ou	no	no	no	ou	no	/	no	ou	ou
			We	orphometric da	ta			-			
SVL	69.5	73.1	73.5	73.7	73.2	64.4	76.6	76.6	74.2	63.7	72.9
AG	83.0	34.8	33.9	35.4	33.6	28.5	37.1	33.2	35.1	25.8	30.6
HumL	9.1	8.4	7.2	9.0	9.0	7.2	8.0	8.1	8.6	7.6	10.1
ForL	10.2	9.5	9.1	9.2	9.8	9.2	10.0	8.6	9.8	8.1	9.6
FemL	12.8	12.8	11.6	12.3	12.5	10.9	13.7	10.8	12.5	10.7	12.8
TibL	6.5	10.5	10.1	10.6	10.6	6.6	11.1	10.0	11.4	10.1	10.2
HL	18.8	19.9	20.9	20.1	20.0	17.6	20.4	19.3	20.0	17.6	19.9
ММ	12.7	14.5	14.3	15.7	13.9	12.8	14.7	14.4	14.1	11.9	13.8
HD	7.2	7.8	7.7	7.9	7.7	7.0	8.2	7.8	7.6	7.7	8.4
ED	4.0	5.0	5.1	5.0	5.0	4.8	5.6	5.3	4.9	4.1	5.3
EE	5.4	5.9	5.9	6.0	5.9	5.3	6.1	6.0	6.0	4.9	6.3
ES	7.9	7.9	8.5	7.9	7.9	7.3	8.2	7.9	7.9	7.2	8.0
EN	5.8	6.0	6.1	6.0	5.8	5.4	6.1	6.0	5.9	5.6	5.9
IO	7.1	5.4	5.5	5.8	5.5	4.9	5.7	5.6	5.3	4.8	6.1
EL	0.7	1.1	1.5	1.5	1.2	1.2	1.0	1.2	1.3	1.4	1.4
IN	2.4	2.3	2.4	2.2	2.0	2.0	2.3	2.2	2.2	2.1	2.3

nape and occiput, diminishing in size anteriorly; approximately 19 longitudinal rows of tubercles at midbody; approximately 20 paravertebral tubercles; small tubercles on flanks; 42 longitudinal rows of flat, imbricate, ventral scales much larger than dorsal scales; 158 transverse rows of ventral scales; 13 large, pore-bearing, precloacal scales; no deep precloacal groove or depression; and three rows of enlarged post-precloacal scales on midline.

Forelimbs moderate in stature, relatively short (HumL/SVL 0.13; ForL/SVL 14.7); granular scales of forearm larger than those on body, interspersed with slightly larger tubercles; palmar scales rounded, slightly raised, subimbricate; digits well-developed, relatively short, inflected at basal interphalangeal joints; digits narrower distal to inflections; subdigital lamellae wide, transversely expanded proximal to joint inflections, narrower lamellae distal to inflections; claws well-developed, claw base sheathed by a dorsal and ventral scale; 9R/9L expanded and 11R/11L unexpanded lamellae beneath the fourth finger; hind limbs longer and thicker than forelimbs, moderate in length (FemL/SVL 18.4; TibL/SVL 0.09), covered dorsally by granular scales interspersed with moderately sized, conical tubercles dorsally and posteriorly and anteriorly by flat, larger, subimbricate scales; ventral scales of thigh flat, imbricate, larger than dorsals; subtibial scales flat, imbricate; one row of 8R/8L enlarged pore-bearing femoral scales not continuous with enlarged pore-bearing precloacal scales, terminating distally at knee; proximal femoral scales smaller than distal femorals, the former forming an abrupt union with much smaller, rounded, ventral scales of posteroventral margin of thigh; plantar scales raised, subimbricate; digits relatively long, well-developed, inflected at basal interphalangeal joints; 9R/9L wide, transversely expanded subdigital lamellae on fourth toe proximal to joint inflection extending onto sole, and 11R/11L unexpanded lamellae beneath the fourth toe distal to joint inflection; and claws welldeveloped, claw base sheathed by a dorsal and ventral scale.

Posterior one-fifth of tail regenerated, 83.0 mm long (TL/SVL 1.19), 6.5 mm in width at base, tapering to a point; nearly square in cross-section; dorsal scales flat, intermixed with large tubercles forming spinose paravertebral rows; row of large dor-solateral caudal tubercles; large, posteriorly directed, spinose tubercles forming wide ventrolateral caudal fringe; much larger scales of ventrolateral fringe occur at regular intervals; medial subcaudals slightly enlarged, no enlarged single medial subcaudal longitudinal row; subcaudals, larger than dorsal caudals; small longitudinal ventrolateral subcaudal ridges; base of tail bearing hemipenal swellings; 3R/3L conical postcloacal tubercles at base of hemipenal swellings; and postcloacal scales flat, imbricate.

**Coloration in life (Fig. 4).** Ground color of the head body, limbs, and tail tan; diffuse darker mottling on the top of the head; wide, cream-colored postorbital stripe extends across the occipital region from one eye to the other; faint whitish canthal markings; dark-brown, nuchal band bearing two posteriorly directed projections; paired dark-brown paravertebral blotches between forelimb insertions; three wide, dark brown, irregularly shaped and deeply emarginated body bands edged in slightly darker brown between the limb insertions; band interspaces bearing irregularly shaped scattered dark-brown markings; limbs generally unicolor tan; digits darkly banded;

six wide pale-brown caudal bands separated by six paler colored bands; bands do not encircle tail; ventral surfaces of body and limbs beige, generally immaculate, subcaudal region generally darker; iris orange-gold in color bearing black vermiculations.

**Distribution.** *Cyrtodactylus denticulatus* sp. nov. represents the northwesternmost species of the *brevipalmatus* group. At present, it is known only from the type locality at Chao Doi waterfall, Tha Song Yang District, Tak Province, western Thailand (Fig. 1).

**Etymology.** The specific epithet *denticulatus* is given as a noun in apposition, meaning "denticulate" or with small teeth, a reference to bearing small tooth-like dorsolateral and ventrolateral caudal tubercles and denticulate ventrolateral body folds.

Comparisons of categorical data (Tables 2-4). Cyrtodactylus denticulatus sp. nov. can be separated from all other species of the *brevipalmatus* group by having denticulate ventrolateral body folds and small ventrolateral subcaudal ridges. These characters were not observed in any other individuals of the *brevipalmatus* group (n = 51). The presence or absence of the following characters showed no intrapopulational variation. Cyrtodactylus denticulatus sp. nov. can be separated from C. brevipalmatus, C. cf. ngati1, C. cf. ngati2, C. fluvicavus, C. interdigitalis, C. ngati3, C. rukhadeva, and C. sp. 13 by have large as opposed to small dorsolateral caudal tubercles and a ventrolateral caudal fringe. It differs from C. cf. ngati1, C. cf. ngati2, C. interdigitalis, C. ngati3, C. rivularis, C. rukhadeva, C. sp. 11, and C. sp. 13 by having ventrolateral heterogeneous ventrolateral caudal fringe scales. It differs from all species except C. kochangensis, C. rivularis, C. rukhadeva, C. sp. 11, and C. thongphaphumensis by having a square cross-section of the tail. It differs from all species except C. brevipalmatus, C. cf. ngati1, C. cf. ngati2, C. fluvicavus, C. ngati, C. ngati3, C. thongphaphumensis and C. uthaiensis in having slightly enlarged medial subcaudal scales. Cyrtodactylus denticulatus sp. nov. can be differentiated from C. interdigitalis, C. rivularis, and C. rukhadeva by lacking an enlarged longitudinal row of medial subcaudals. From C. interdigitalis and C. uthaiensis it differs by lacking intermittently enlarged, medially furrowed, and posteriorly emarginate medial subcaudals. Being that this species is known from only one specimen we acknowledge that some of the meristic differences in Tables 2-4 may eventually prove not be diagnostic with the addition of more specimens just as other meristic characters may prove to be statistically diagnosable. Until then, we refrain from including metric characters in the diagnosis. Potential diagnostic meristic characters are highlighted (Tables 2-4).

**Natural history.** The holotype was collected at night between 19.00–23.00 hours in a bamboo forest near a rocky stream. The lizard was found on a bamboo branch 4 m above a large granite boulder (Fig. 7). The habitat was composed of large Bamboo (*Dendrocalamus copelandii*) Myrtaceae (*Syzygium* sp.) and dipterocarp trees (*Anisoptera costata*) (Fig. 5). We speculate *C. denticulatus* sp. nov. is an arboreal specialist that generally resides in the upper canopy similar to *C. brevipalmatus* and *C. elok* (Grismer 2011). At the type locality, *C. denticulatus* sp. nov. was recorded in sympatry with *Cyrtodactylus* sp. 1 (Chomdej et al. 2021), *Trimeresurus guoi, Ansonia inthanon, Rhacophorus rhodopus*, and *Theloderma albopunctatum*.



**Figure 7.** Habitat of *Cyrtodactylus denticulatus* sp. nov. AUP-00680 from the Chao Doi waterfall, Mae Meoi, Tha Song Yang District, Tak Province, Thailand.

# Discussion

The data above clearly demonstrate the unique phylogenetic and morphological properties of the individual described above as Cyrtodactylus denticulatus sp. nov. However, describing a species on the basis of a single specimen can be potentially misleading, especially in the absence of genetic data. Here we provide compelling genetic evidence for the unique phylogenetic position of this specimen wherein it resides on its own long branch (pairwise sequence divergence of 7.87-21.94%) that is not embedded within that of any other species nor sister to any other species as with C. kochangensis Grismer, Aowphol, Yodthong, Ampai, Temprayon, Aksornneam & Rujirawan, 2022 and C. uthaiensis Grismer, Aowphol, Yodthong, Ampai, Temprayon, Aksornneam & Rujirawan, 2022. Its phylogenetic position is what delimits it as a distinct evolutionarily independent lineage, not its morphology. The morphological diagnosis of a species provides evidence of how different or similar a species may be to other closely related species but has no bearing on whether or not it is a new species (see discussion in Grismer et al. 2022b). Many cryptic species defy discrete morphological diagnoses, as the designation of "cryptic" implies. The morphological diagnosis of a species based on a single specimen is incomplete, as it does not capture the range of variation of the species of which it represents. However, given the general difficulty in finding cryptic arboreal species, waiting for the acquisition of additional material that does not bear on its species status, in our opinion, is not the best option. A formal description of the species can begin the process of levying legal conservation measures and does not relegate undescribed species as "ecological ghosts" with no legal protection. This is particularly germane to tropical upland endemics whose ecosystems are some of the most imperiled in the world.

The addition of *Cyrtodactylus denticulatus* sp. nov. brings the total number of described species in the *brevipalmatus* group to ten. Based on photographs in social media, there are as many as five potentially undescribed species from unsampled areas (Fig. 1). Given that the members of this group are cryptic species generally restricted to isolated upland habitats, we are reasonably certain there are more populations to be discovered and described. This becomes particularly important in this era of climate change where some of its greatest negative impacts occur in tropical upland ecosystems.

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## References

- Bouckaert RR, Drummond AJ (2017) bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC Evolutionary Biology 17(1): e42. https://doi.org/10.1186/ s12862-017-0890-6
- Chan KO, Grismer LL (2022) GroupStruct: An R package for allometric size correction. Zootaxa 5124(4): 471–482. https://doi.org/10.11646/zootaxa.5124.4.4
- Chomdej S, Pradit W, Suwannapoom C, Pawangkhanant P, Nganvongpanit K, Poyarkov NA, Che J, Gao Y, Gong S (2021) Phylogenetic analyses of distantly related clades of benttoed geckos (genus *Cyrtodactylus*) reveal an unprecedented amount of cryptic diversity in northern and western Thailand. Scientific Reports 11(1): e2328. https://doi.org/10.1038/ s41598-020-70640-8
- de Queiroz K (2007) Species concepts and species delimitation. Systematic Biology 56(6): 879–886. https://doi.org/10.1080/10635150701701083

- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and BEAST 1.7. Molecular Biology and Evolution 29(8): 1969–1973. https://doi. org/10.1093/molbev/mss075
- Frost DR, Hillis DM (1990) Species in concept and practice: Herpetological application. Herpetologica 46: 86–104.
- Frost DR, Kluge AG (1994) A consideration of the epistemology in systematic biology, with special reference to species. Cladistics 10(3): 259–294. https://doi.org/10.1111/j.1096-0031.1994.tb00178.x
- Grismer LL (2008) On the distribution and identification of *Cyrtodactylus brevipalmatus* Smith, 1923 and *Cyrtodactylus elok* Dring, 1979. The Raffles Bulletin of Zoology 56: 177–179.
- Grismer LL, Wood Jr PL, Le MD (2020) Quah ESH, Grismer JL. (2020) Evolution of habitat preference in 243 species of Bent-toed geckos (Genus *Cyrtodactylus* Gray, 1827) with a discussion of karst habitat conservation. Ecology and Evolution 10(4): 1–14. https://doi.org/10.1002/ece3.6961
- Grismer LL, Wood Jr PL, Poyarkov NA, Le MD, Kraus F, Agarwal I, Oliver PM, Nguyen SN, Nguyen TQ, Karunarathna S, Welton LJ, Stuart BL, Luu VQ, Bauer AM, O'Connell KA, Quah ESH, Chan KO, Ziegler T, Ngo H, Nazarov RA, Aowphol A, Chomdej S, Suwannapoom C, Siler CD, Anuar S, Tri NV, Grismer JL (2021a) Phylogenetic partitioning of the third-largest vertebrate genus in the world, *Cyrtodactylus* Gray, 1827 (Reptilia; Squamata; Gekkonidae) and its relevance to taxonomy and conservation. Vertebrate Zoology 71: 101–154. https://doi.org/10.3897/vertebrate-zoology.71.e59307
- Grismer LL, Wood Jr PL, Poyarkov NA, Minh DL, Karunarathna S, Chomdej S, Suwannapoom C, Qi S, Liu S, Che J, Quah ESH, Kraus F, Oliver PM, Riyanto A, Pauwels OSG, Grismer JL (2021b) Karstic landscapes are foci of species diversity in the World's Third-Largest Vertebrate genus *Cyrtodactylus* Gray, 1827 (Reptilia: Squamata; Gekkonidae). Diversity 13(5): e183. https://doi.org/10.3390/d13050183
- Grismer LL, Suwannapoom C, Pawangkhanant P, Nazarov RA, Yushchenko PV, Naiduangchan M, Le MD, Luu VQ, Poyarkov NA (2021c) A new cryptic arboreal species of the *Cyrtodac-tylus brevipalmatus* group (Squamata: Gekkonidae) from the uplands of western Thailand. Vertebrate Zoology 71: 723–746. https://doi.org/10.3897/vz.71.e76069
- Grismer LL, Rujirawan A, Yodthong S, Stuart BL, Le MD, Le DT, Chuaynkern Y, Wood Jr PL, Aowphol A (2022a) The taxonomy and phylogeny of the *Cyrtodactylus brevipalmatus* group (Squamata: Gekkonidae) with emphasis on *C. interdigitalis* and *C. ngati*. Vertebrate Zoology 72: 245–269. https://doi.org/10.3897/vz.72.e80615
- Grismer LL, Aowphol A, Yodthong S, Ampai N, Termprayoon K, Aksornnean A, Rujirawan A (2022b) Integrative taxonomy delimits and diagnoses cryptic arboreal species of the *Cyrtodactylus brevipalmatus* group (Squamata, Gekkonidae) with descriptions of four new species from Thailand. ZooKeys 1129: 109–162. https://doi.org/10.3897/zookeys.1129.90535
- Grismer LL, Rujirawan A, Chomdej S, Suwannapoom C, Yodthong S, Aksornneam A, Aowphol A (2023) A new species of the *Cyrtodactylus brevipalmatus* group (Squamata, Gekkonidae) from the uplands of western Thailand. ZooKeys 1141: 93–118. https://doi. org/10.3897/zookeys.1141.97624

- Hillis DM (2019) Species delimitation in herpetology. Journal of Herpetology 53(1): 3–12. https://doi.org/10.1670/18-123
- Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294(5550): 2310–2314. https://doi. org/10.1126/science.1065889
- Husson F, Josse J, Le S, Mazet J (2017) FactoMine R: exploratory data analysis and data mining. R package, version 1.36.
- Kassambara A, Mundt F (2017) Factoextra: extract and visualize the result of multivariate data analyses. R package, version 1.0.5.999.
- Le DT, Sitthivong S, Tran TT, Grismer LL, Nguyen TQ, Le MD, Ziegler T, Luu VQ (2021) First record of the *Cyrtodactylus brevipalmatus* group (Squamata: Gekkonidae) from Vietnam with description of a new species. Zootaxa 4969(3): 492–510. https://doi.org/10.11646/ zootaxa.4969.3.3
- Lleonart J, Salat J, Torres GJ (2000) Removing allometric effects of body size in morphological analysis. Journal of Theoretical Biology 205(1): 85–93. https://doi.org/10.1006/ jtbi.2000.2043
- McCoy MW, Bolker BM, Osenberg CW, Miner BG, Vonesh JR (2006) Size correction: Comparing morphological traits among populations and environments. Oecologia 148(4): 547–554. https://doi.org/10.1007/s00442-006-0403-6
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Gateway Computing Environments Workshop (GCE), New Orleans (USA), November 2010, IEEE, 8 pp. https://doi.org/10.1109/ GCE.2010.5676129
- Pagès J (2015) Multiple Factor Analysis by Example Using R. CRC Press, New York, 272 pp. https://doi.org/10.1201/b17700
- R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna. http://www.R-project.org [Accessed 1 June 2022]
- Rambaut A, Drummond AJ (2013) TreeAnnotator version 1.8.0 MCMC Output Analysis. https://beast.community/treeannotator [Accessed 1 June 2022]
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5): 901–904. https://doi. org/10.1093/sysbio/syy032
- Reist JD (1986) An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. Canadian Journal of Zoology 64(6): 1363–1368. https://doi. org/10.1139/z86-203
- Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7): 3022–3027. https://doi.org/10.1093/ molbev/msab120
- Thorpe RS (1975) Quantitative handling of characters useful in snake systematics with particular reference to intraspecific variation in the Ringed Snake *Natrix natrix* (L.). Biological Journal of the Linnean Society. Linnean Society of London 7(1): 27–43. https://doi. org/10.1111/j.1095-8312.1975.tb00732.x

- Thorpe RS (1983) A review of the numerical methods for recognising and analysing racial differentiation. In: Felsenstein J (Ed.) Numerical Taxonomy. NATO ASI Series (Vol. 1). Springer, Berlin, 404–423. https://doi.org/10.1007/978-3-642-69024-2\_43
- Turan C (1999) A note on the examination of morphometric differentiation among fish populations: The Truss System. Turkish Journal of Zoology 23(3): 259–263.
- Ulber T (1993) Bemerkungen über cyrtodactyline Geckos aus Thailand nebst Beschreibungen von zwei neuen Arten (Reptilia: Gekkonidae). Mitteilungen aus dem Museum für Naturkunde in Berlin 69(2): 187–200. https://doi.org/10.1002/mmnz.19930690202
- Wilcox TP, Zwickl DJ, Heath TA, Hillis DM (2002) Phylogenetic relationships of the Dwarf Boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Molecular Phylogenetics and Evolution 25(2): 361–371. https://doi.org/10.1016/S1055-7903(02)00244-0

#### Supplementary material I

#### Dataset of meristic, normalized morphometric, and categorical characters

Authors: Siriwadee Chomdej, Chatmongkon Suwannapoom, Waranee Pradit, Apichaya Phupanbai, L. Lee Grismer

Data type: table (.csv file)

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