

# An illustrated key to the fiddler crabs (Crustacea, Decapoda, Ocypodidae) from the Atlantic coast of Brazil

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## Abstract

Fiddler crabs are one of the most notable animal groups in Brazilian estuarine environments, due to their high density and characteristic waving of males. An illustrated key to the ten species recorded as far in the country is provided using only clearly visible characters of males. Furthermore, additional recognition characters, information about geographic distribution and biology of each species are presented. Most examined crabs were collected in Guaratuba Bay, southern Brazil.

## Keywords

Biological notes, distribution, mangrove, recognition characters, tidal flats

## Introduction

Studies on fiddler crabs began more than 300 years ago, certainly because the observers were attracted to the immense claw of males that were tirelessly waving in a typical movement and rhythm. Fiddler crabs are semi-terrestrial decapod crustaceans and inhabit shaded substrates of mangrove forest or sunny tidal flats adjacent to it. During high tides they hide in individual burrows that are the center of a fiddler crab life (Crane 1975).

Ten species of fiddler crabs are known from the Brazilian Atlantic coast (Melo 1996), with the newest species described more than 30 years ago by von Hagen (1987).

Brazilian population studies, however, began only at the beginning of the present century, and a great deal of knowledge was generated since then (see partial review in Ribeiro and Bezerra 2014).

Despite the valuable identification keys for Brazilian fiddler crabs elaborated by Crane (1975), Melo (1996) and Bezerra (2012), the number of misidentifications found in various scientific collections is remarkable (see these three authors). The aim of this paper is to present an illustrated identification key for the species of fiddler crabs occurring at the Brazilian Atlantic coast in order to aid undergraduate students and early researchers.

## Materials and methods

Ten species of fiddler crabs recorded along Brazilian coast were analyzed, each one represented by numerous individuals. Most of them (seven species) were collected in various habitats of Guaratuba Bay, municipality of Guaratuba, southern Brazil: *Minuca burgersi* (Holthuis, 1967), *M. mordax* (Smith, 1870), *M. rapax* (Smith, 1870), *Leptuca leptodactyla* (Rathbun, 1898), *L. thayeri* (Rathbun, 1900), *L. uruguayensis* (Nobili, 1901), and *Uca maracoani* (Latreille, 1802–1803). These crabs were deposited in the Natural Museum of Natural History of Capão da Imbuia located in Curitiba, Paraná State, southern Brazil. The remaining three species were obtained from other locations in Brazil: *L. cumulanta* (Crane, 1943) from Natal (Rio Grande do Norte state, northeastern Brazil), *M. vocator* (Herbst, 1804) from Cananea (São Paulo state, southeastern Brazil) and *M. victoriana* (von Hagen, 1987) from Guarapari (Espírito Santo state, southeastern Brazil); the specimens of the latter species were deposited at the Museum of Zoology of University of São Paulo.

The illustrated key was elaborated as simple as possible, and only clearly visible characters were selected. The key is exclusively based on adult male individuals, as they are provided with the diagnostic characters of the species. As fiddler crabs have gregarious habits, male individuals are hardly absent in the populations.

Line drawings were prepared using a drawing tube attached to a stereoscopic microscope. The systematic nomenclature was based on Shih et al. (2016) and morphological terminology follows Crane (1975). Additional practical characters were added in the item “Recognition characters”. Information on geographical distribution of the species (Table 1) was based on Crane (1975), Mendes and Couto (2001), Koch et al. (2005), Baptista and Calado (2007), Bezerra (2012), Thurman et al. (2013), Pillon (2014), Martins (2018), and Silva (2019). Furthermore, the occurrence of *Uca maracoani* at coast of Santa Catarina state was based on the observation of S.B. Martins (pers. comm.).

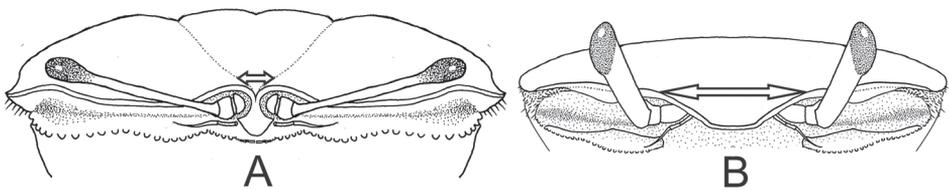
The pile, an important morphological feature, is a woolly pubescence on the surface of carapace and ambulatory legs of some species. It is a somewhat difficult to be recognized by beginners, especially in crabs that were preserved in liquids. Drying the specimens in the open air is a practical clue to facilitate the visualization: the piles appear as clear and rough patches on the darkened surface of the carapace or ambulatory legs. It is highly recommended that beginners learn to distinguish these piles, since some morphologically similar species can be easily identified by observing the distribution of these patches.

**Table I.** Geographic distribution of the fiddler crab species along the Atlantic coast of Brazil. The states were organized by increasing southern latitudes (from left to right), except Amapá that is located northern to Equator. Abbreviations: AL = Alagoas, AP = Amapá, BA = Bahia, CE = Ceará, ES = Espírito Santo, MA = Maranhão, PA = Pará, PB = Paraíba, PE = Pernambuco, PI = Piauí, PR = Paraná, RJ = Rio de Janeiro, RN = Rio Grande do Norte, RS = Rio Grande do Sul, SC = Santa Catarina, SE = Sergipe, SP = São Paulo. Black circle = occurrence recorded; white circle = probable occurrence but not officially recorded; black square = presence of mangrove.

States of Brazil	AP	PA	MA	PI	CE	RN	PB	PE	AL	SE	BA	ES	RJ	SP	PR	SC	RS
Mangrove	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Minuca mordax</i>	•	•	•	○	•	•	○	•	•	•	•	•	•	•	•	•	•
<i>Minuca rapax</i>	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
<i>Uca maracoani</i>	•	•	•	○	•	•	•	•	•	•	•	•	•	•	•	•	•
<i>Leptuca leptodactyla</i>		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
<i>Leptuca thayeri</i>		•	•	○	•	•	•	•	•	•	•	•	•	•	•	•	•
<i>Minuca burgersi</i>		•	•	○	•	•	•	•	•	•	•	•	•	•	•	•	•
<i>Minuca vocator</i>	•	•	•	○	•	○	•	•	•	•	•	○	•	•	•	•	•
<i>Leptuca cumulanta</i>	•	•	•	○	•	•	•	•	•	•	•	○	•	•	•	•	•
<i>Minuca victoriana</i>					•			•			•	•	•	•	•	•	•
<i>Leptuca uruguayensis</i>													•				•

**Key to the species of fiddler crabs from Brazil**

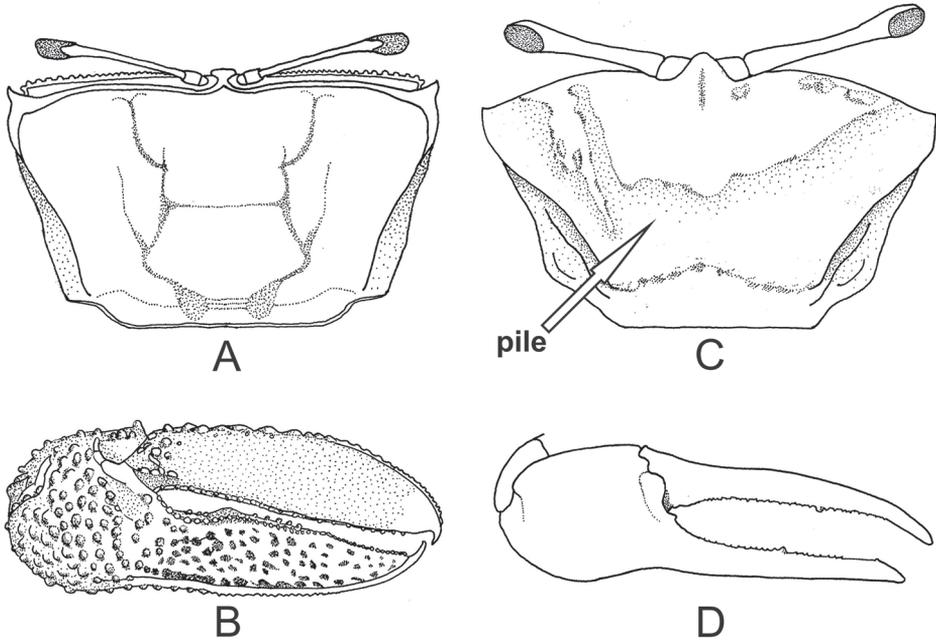
- 1 Narrow front, width less than or equal to 15% of front-orbital breadth (Fig. 1) (big and medium-sized crabs) ..... **2**
- Wide and triangular front, width more than 15% of front-orbital breadth (Fig. 1B) (medium-sized and small crabs) ..... **3**



**Figure 1.** Carapace, frontal view. **A** *Uca maracoani*: spatulate and narrow front (seta) **B** *Minuca burgersi*: triangulate and wide front (seta).

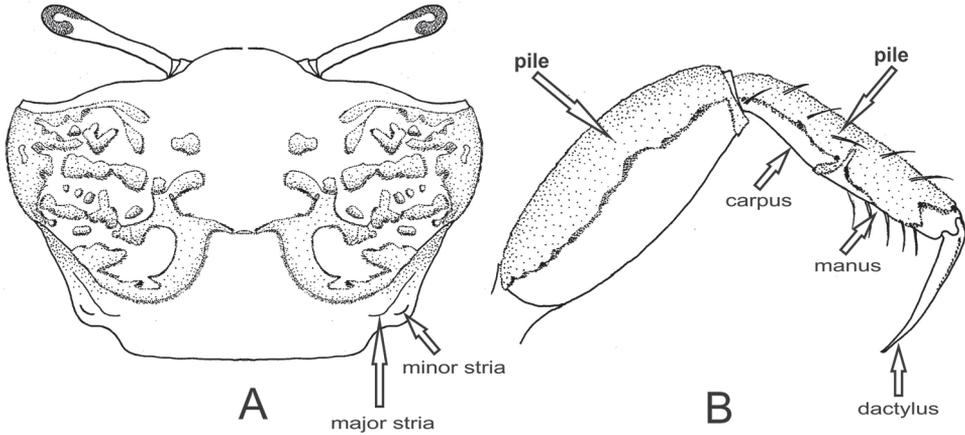
- 2 Spatulate front (Fig. 1A), width equal to or less than 4% of front-orbital breadth; carapace with bare dorsal surface (Fig. 2A); male major claw with flat fingers like two blades (Fig. 2B) (big crabs, adults can reach up to 45.0 mm carapace width CW) ..... *Uca maracoani*
- Triangular front, base ca. 15% of front-orbital breadth; patches of pile (= woolly pubescence, easily detached) on dorsal surface of carapace (Fig. 2C)

and on ambulatory legs; major claw of males with cylindrical fingers (Fig. 2D) (medium-sized crabs, adult males can reach up to 28.4 mm carapace width).....*Leptuca thayeri*



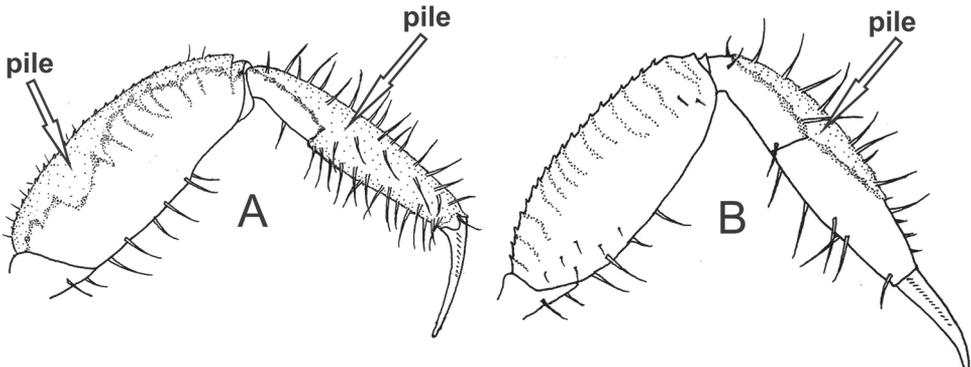
**Figure 2.** *Uca maracoani*. **A** carapace with bare surface, dorsal view **B** male major claw with flat fingers, frontal view. *Leptuca thayeri* **C** carapace mostly covered with pile (seta) **D** male major claw with cylindrical fingers, frontal view.

- 3 Carapace provided with major and minor pairs of postero–lateral striae (Figs 3A, 5B, 6A, 8A, C) (medium-sized crabs, adult males with maximum CW 19.0–29.0 mm) ..... 4
- Carapace provided with a single pair of postero–lateral striae (Figs 10A, 11A, C) (small crabs, adult males with maximum of 15.0 mm CW) ..... 8
- 4 Exuberant pile on the dorsal surface of the carapace forming a typical pattern (Fig. 3A) and on all segments of ambulatory legs except dactyl (Fig. 3B) ..... *Minuca vocator*
- Carapace with discrete pile or without any, but segments of ambulatory legs (at least from 1<sup>st</sup> to 3<sup>rd</sup> pairs) with pile (Figs 4A, B) ..... 5

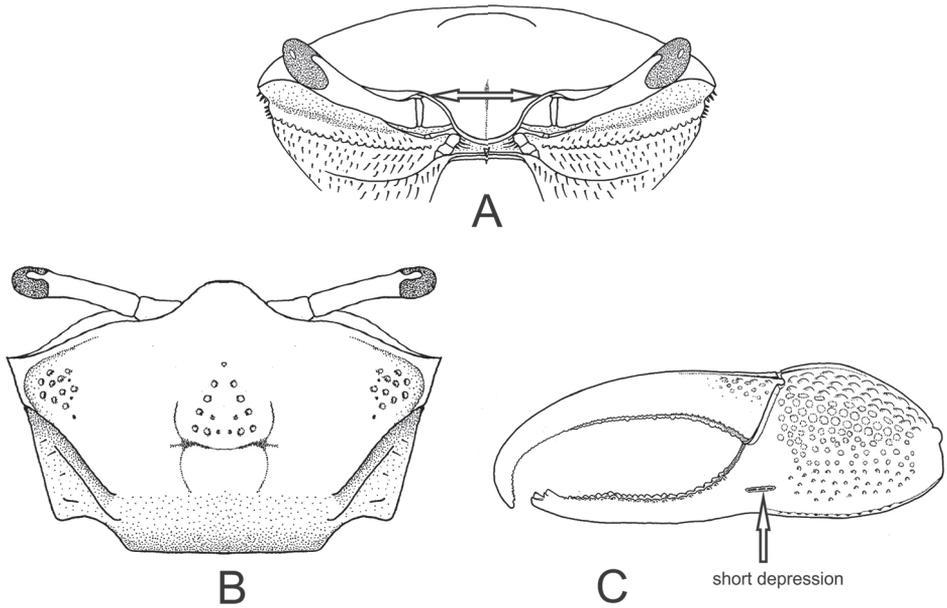


**Figure 3.** *Minuca vocator*. **A** carapace with pile forming a typical pattern, dorsal view **B** third ambulatory leg with pile on dorsal surface of all segments except dactylus (setae), posterior view.

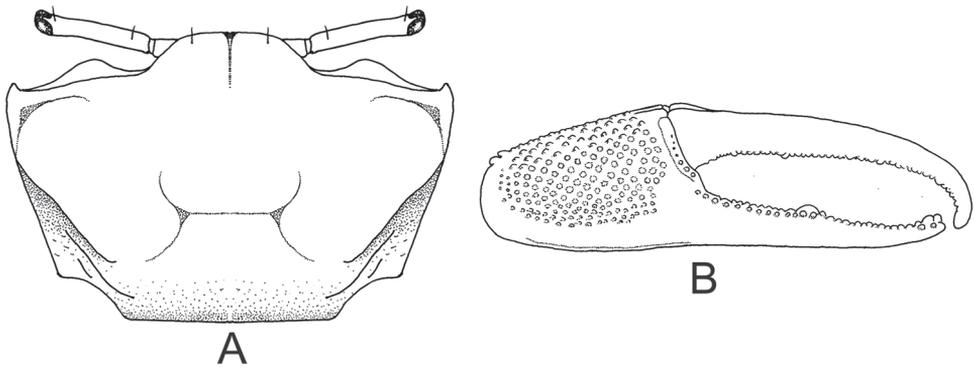
- 5 Male major chela provided with a short and straight depression filled with pile at the base of pollex (Fig. 5C); discrete pile on carpus and manus (adult male CW maximum 19.1 mm).....*Minuca victoriana*
- Male major chela without any depression at the base of pollex (Fig. 6B) (adult male CW up to 29.0 mm) ..... **6**
- 6 Pile on dorsal surface of carpus and merus and around the entire surface of manus of ambulatory legs (1<sup>st</sup> to 3<sup>rd</sup> pairs) (Fig. 4A).....*Minuca mordax*
- Pile limited to dorsal surface of carpus and manus of ambulatory legs (1<sup>st</sup> to 3<sup>rd</sup> pairs); merus without pile (Fig. 4B) ..... **7**



**Figure 4.** Third ambulatory leg, posterior view. **A** *Minuca mordax* with pile on dorsal surface of merus (seta) and carpus and all around surfaces of manus (seta) **B** *Minuca burgersi* with pile limited to dorsal surface of carpus and manus (seta).

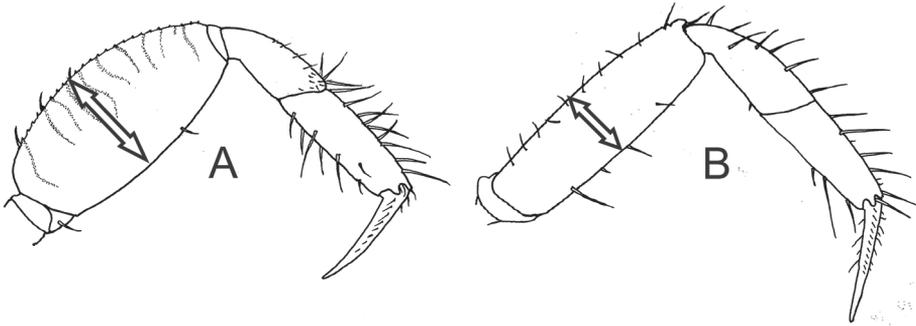


**Figure 5.** *Minuca victoriana*. **A** carapace with moderately large front, frontal view **B** carapace with two pairs of postero-lateral striae, dorsal view **C** male major claw with a short depression at the base of pollex (seta), frontal view.

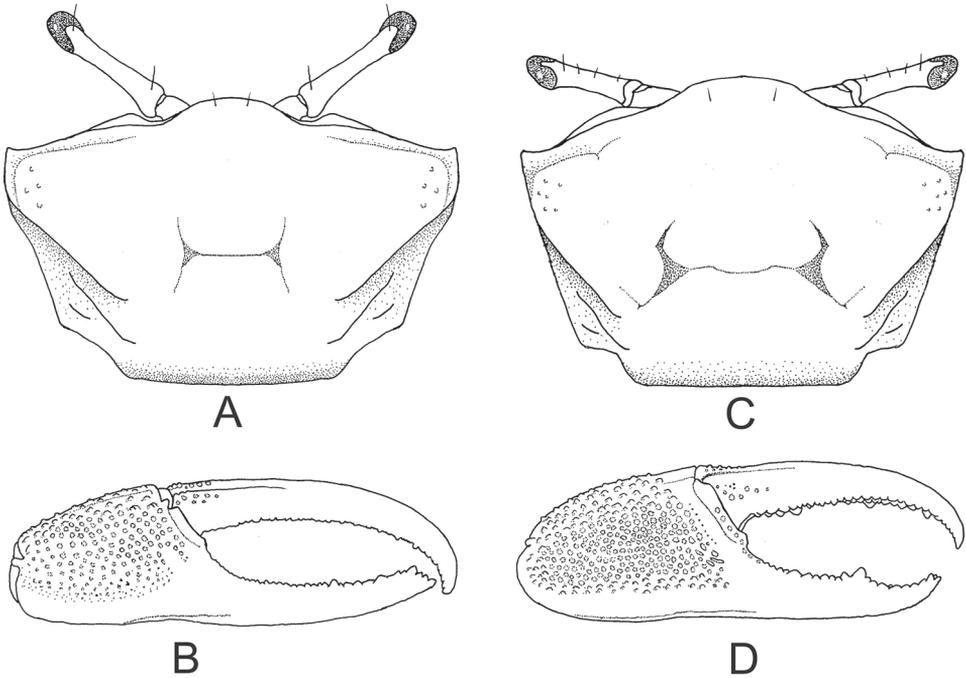


**Figure 6.** *Minuca mordax*. **A** carapace without pile and with two pairs of postero-lateral striae, dorsal view **B** male major claw, frontal view.

- 7 Ambulatory legs with wide merus and dorsal margin convex; merus of last pair of ambulatory legs more than two times wider than respective carpus in its maximum breadth (Fig. 7A) ..... *Minuca rapax*
- Ambulatory legs with narrow merus and dorsal margin almost straight; merus of last pair of ambulatory legs less than two times wider than respective carpus in its maximum breadth (Fig. 7B) ..... *Minuca burgersi*

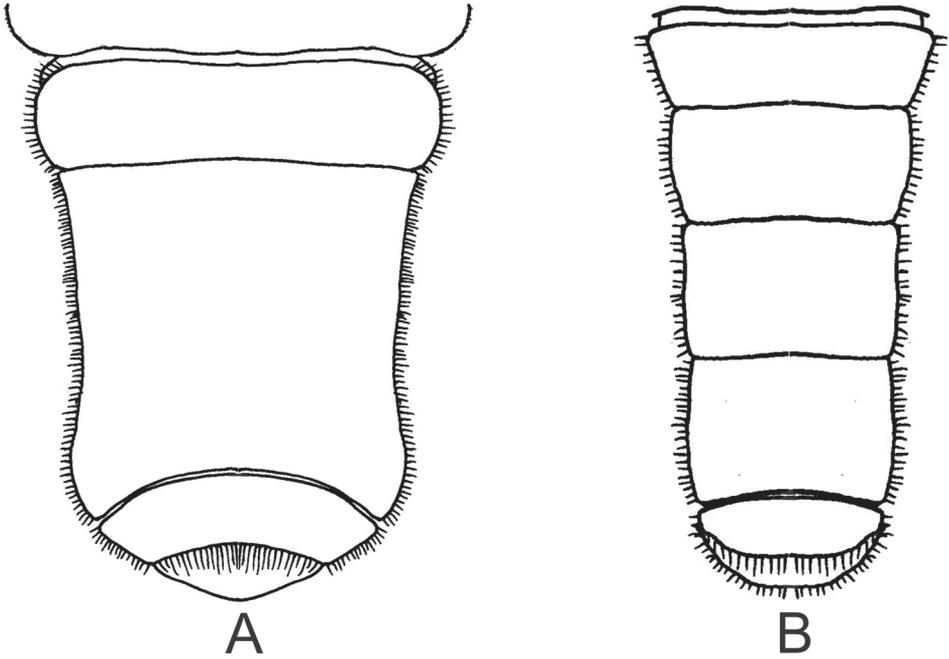


**Figure 7.** Last ambulatory legs, posterior view. **A** *Minuca rapax*, wide merus (seta). **B** *Minuca burgersi*, narrow merus (seta).

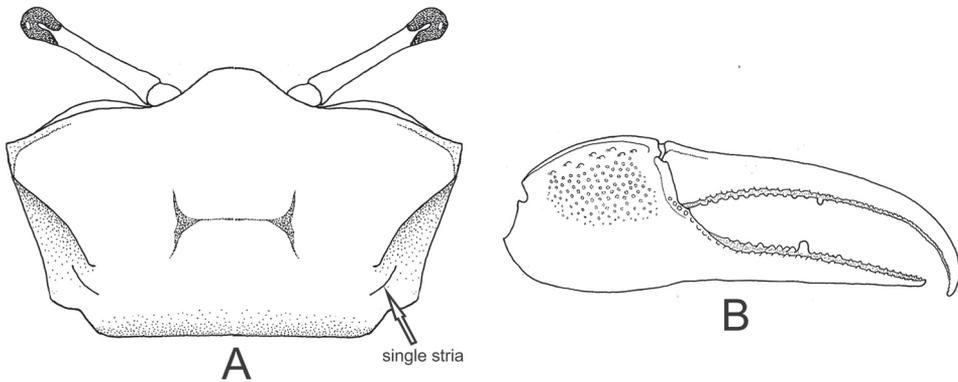


**Figure 8.** *Minuca rapax*. **A** carapace with two pairs of postero-lateral striae, dorsal view **B** male major claw, frontal view. *Minuca burgersi* **C** Carapace with two pairs of postero-lateral striae, dorsal view **D** male major claw, frontal view.

- 8 Abdomen with middle somites fused (Fig. 9A) .....9
- Abdomen with all somites distinct (Fig. 9B) .....
- ..... *Leptuca cumulanta* (Fig. 10A, B)

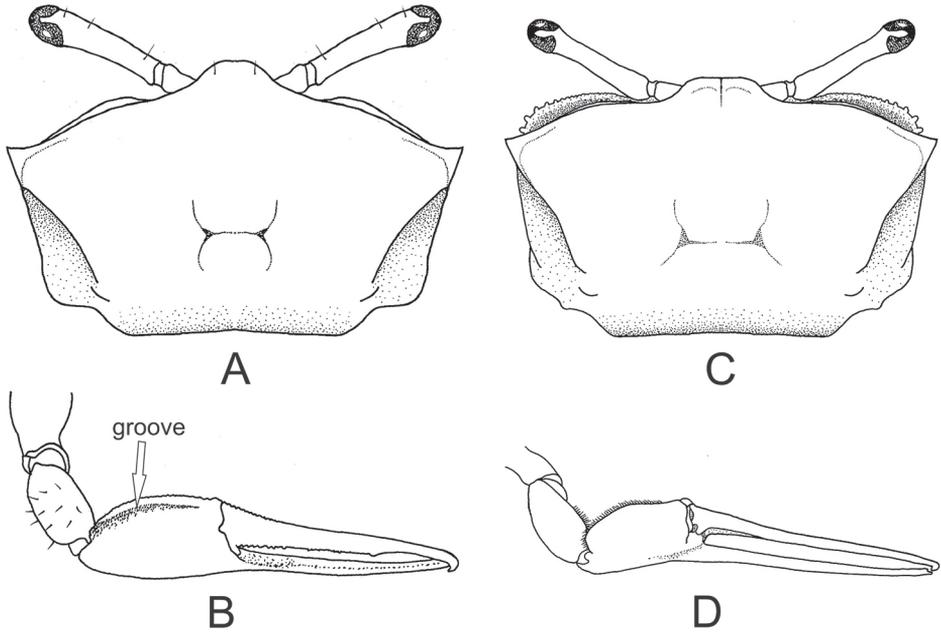


**Figure 9.** Abdomen of male, ventral view. **A** *Leptuca leptodactyla*, middle somites fused **B** *Leptuca cumulanta*, all somites distinct.



**Figure 10.** *Leptuca cumulanta*. **A** carapace with a pair of postero-lateral striae (seta), dorsal view **B** male major claw, frontal view.

- 9 Manus of major claw provided with a long groove on dorsal surface following its margin, mostly filled with dirt (Fig. 11B); length of major claw fingers ca. 1.3 times longer than manus.....*Leptuca uruguayensis*
- Manus of major claw without any groove on dorsal surface (Fig. 11D); length of major claw fingers at least 1.6 times longer than manus .....*Leptuca leptodactyla*



**Figure 11.** *Leptuca uruguayensis*. **A** carapace with a pair of postero-lateral striae, dorsal view **B** male major claw with dorsal groove (seta), dorso-frontal view. *Leptuca leptodactyla* **C** carapace with a pair of postero-lateral striae, dorsal view **D** male major claw without dorsal groove, dorso-frontal view.

**Taxonomy**

**Subfamily Gelasiminae**

***Minuca burgersi* (Holthuis, 1967)**

Figures 1B, 4B, 7B, 8C, D

**Recognition characters.** Carapace pentagonal moderately arched in the anteroposterior direction and dorsal surface without pile (Fig. 8C). Dorso-lateral margins well-marked and converging posteriorly; major and minor pairs of postero-lateral striae clearly visible (Fig. 8C). Front triangular and very wide making up from 36% to 41% of the front-

orbital breadth (Fig. 1B). Male major claw manus covered by small tubercles and provided with a strong groove (sometimes filled with dirt) on dorsal surface following the dorsal margin; fingers thick and slightly flattened; dactyl little longer than manus; pollex and dactyl curved forming a large gap (Fig. 8D). First three ambulatory legs with pile (= woolly pubescence) limited to dorsal surface of carpus and manus (Fig. 4B, setae), absent in ventral margin; all ambulatory legs with narrow merus and dorsal margin almost straight; last pair of ambulatory legs without piles and merus less than two 1.5 times wider than respective carpus in its maximum breadth (Fig. 7B). Male abdominal segments never fused. Medium-sized species and one of the smallest in the genus; males' carapace width (CW) up to 19.0 mm in a population from Fortaleza, CE, Brazil (Crane 1975).

**Biological notes.** The species reproduces year-round in the population of Ubatuba, southeastern Brazil (Benetti et al. 2007). It occurs in oligohaline and mesohaline areas and on sandy substrate although in low densities (Masunari 2006; Thurman et al. 2013).

**Remarks.** The species is morphologically close to its congeners *M. rapax* and *M. mordax*; the distinguishing characters among these species are treated in the subsequent items.

### Subfamily Gelasiminae

#### *Minuca mordax* (Smith, 1870)

Figures 4A, 6A, B

**Recognition characters.** Carapace pentagonal moderately arched and with naked dorsal surface, without pile (Fig. 6A). Dorso-lateral margins well marked and strongly convergent posteriorly; major and minor pairs of postero-lateral striae clearly visible (Fig. 6A). Front triangular and very wide making up between 34% to 38% of the front-orbital breadth. Male major claw with manus covered by tubercles and with strong groove on dorsal surface; fingers thick and slightly flattened; dactyl ca. 1.5 times the manus length; pollex almost straight with tip curved upwards; dactyl strongly arched ending in a curved downward tip; fingers form a wide gap (Fig. 6B). Ambulatory legs with narrow merus and dorsal margin almost straight; 1<sup>st</sup> to 3<sup>rd</sup> ambulatory legs with pile limited to dorsal surface of merus (weakly) and carpus (strongly), but all around the manus (strongly) (Fig. 4A); last pair with scant pile on merus, carpus and manus. Male abdominal segments never fused. Medium-sized crab: males' CW up to 26.1 mm in a population from mangrove of Itajaí River, southern Brazil (Scalco et al. 2016).

**Biological notes.** Oviparous females were collected inside burrows that were ornamented with poorly structured chimney at Guaratuba Bay, southern Brazil, during a warm month (November) (Martins et al. 2016). The duration of the reproductive period of this species, however, is still unknown. The species dispersal is larval retention type: after larval period in the pelagic environment of the bay, megalopae return to terrestrial areas, by colonizing mats of red algae that grow on humid substrates. Early juveniles seek shelter among entangled thalli of these red algae, and after molting they migrate to soft muddy substrate (S.B. Martins, pers. comm.). Adults live on consolidated sandy banks of rivers flowing into Guaratuba Bay, forming large populations (Masunari 2006).

**Remarks.** The most conspicuous morphological character of *M. mordax* is the presence of piles covering the entire surface (dorsal, lateral and ventral) of the manus of 1<sup>st</sup> to 3<sup>rd</sup> ambulatory legs. This feature allows to easily distinguish *M. mordax* from two other closely related species, *M. burgersi* and *M. rapax*. As no piles are present on carapace of these three species, they hardly will be confused with *Leptuca thayeri* or *Minuca vocator*. In the field, these species can also be distinguished by its respective habitat: while *M. mordax* is mostly found in freshwater or oligohaline areas such as river banks and tidal flats near river mouth, *M. rapax* and *M. burgersi* are mainly found in mesohaline tidal flats, often in co-occurrence.

### Subfamily Gelasiminae

#### *Minuca rapax* (Smith, 1870)

Figures 7A, 8A, B

**Recognition characters.** Carapace pentagonal moderately arched and provided with small and scarce tubercles in the antero-lateral corner (Fig. 8A); some individuals have pile on H-form depression. Dorso-lateral margins well marked and strongly convergent posteriorly (more pronounced in males); major and minor pairs of postero-lateral striae clearly visible (Fig. 8A). Front triangular and very wide making up 30% to 36% of the front-orbital breadth. Male major claw with manus covered with tubercles and provided with strong groove dorsally; fingers thick and slightly flattened; dactyl ca. 1.5 times longer than manus; pollex and dactyl strongly curved forming a large gap (Fig. 8B). Pile limited the dorsal surface of carpus and manus in the first three ambulatory legs; these legs with enlarged merus (especially the 2<sup>nd</sup> and 3<sup>rd</sup>), dorsal margin convex and dorsal surface with striated ornaments; last leg without piles and merus more than two times wider the respective carpus in their maximum breadth (Fig. 7A, seta). Male abdominal segments never fused. Medium-sized crabs, male CW up to 28.3 mm and female up to 27.3 mm in a population from Itamambuca mangrove, Ubatuba, south-eastern Brazil (Castiglioni and Negreiros-Fransozo 2004).

**Biological notes.** The species reproduces year-round in the populations from northern and southeastern Brazil (Koch et al. 2005, Castiglioni and Negreiros-Fransozo 2006; Costa and Soares-Gomes 2009). It prefers mesohaline to euhaline areas but it can be found in a wide range of salinities, from oligohaline to euhaline; the preferred substrate is firm sandy to silty clay with humus or clayed silt (Thurman et al. 2013).

**Remarks.** Morphologically very similar to *M. burgersi* and *M. mordax*. *Minuca rapax* can be distinguished from *M. mordax* in not having a pile around the entire surface of manus of 1<sup>st</sup> to 3<sup>rd</sup> ambulatory legs. The distinction between *M. rapax* and *M. burgersi*, however, requires an extra attention: both species have piles limited to the dorsal surface of carpus and manus of 1<sup>st</sup> to 3<sup>rd</sup> ambulatory legs. The easiest way to distinguish these two species is to compare the last ambulatory leg: while *M. rapax* has a wide merus with convex dorsal margin (Fig. 7A), that of *M. burgersi* is narrow and its margins are almost parallel (Fig. 7B).

## Subfamily Gelasiminae

### *Minuca victoriana* (von Hagen, 1987)

Figure 5A–C

**Recognition characters.** Carapace pentagonal moderately arched and provided with few tubercles on the surface of antero-lateral corners and on mesogastric area (Fig. 5A). Dorso-lateral margins well marked and strongly convergent posteriorly; major and minor pairs of postero-lateral striae clearly visible (Fig. 5B). The discrete pile on the carapace described by von Hagen (1987) was not observed in the specimens examined in the present study. Front triangular and moderately large making up ca. 22 % of front-orbital breadth (Fig. 5A, seta). Male major claw with manus covered with tubercles and provided with strong groove on dorsal margin filled with pile; fingers thick and slightly flattened; dactyl ca. 1.8 times longer than manus; pollex and dactyl strongly curved forming a large gap; a short and straight depression filled with pile at the base of pollex (Fig. 5C, seta). Scant pile on dorsal surface of carpus and manus of the first three pair of ambulatory legs. Male abdomen somites not fused. Medium-sized species and one of the smallest in the genus; males' carapace width (CW) up to 19.1 mm in a population from Vitória, Espírito Santo state, southeastern Brazil (von Hagen 1987).

**Biological notes.** Although with a wide geographical distribution, the species forms sparse populations constituted by small individuals in impacted mangroves of southeastern Brazil (Bedê et al. 2008). In tropical mangroves, these crabs form relatively dense populations, reaching larger CW than in southern population and preferring muddy substrates. The recruitment of juveniles occurs continuously; however, the reproductive period of the species is still unknown (Castiglioni et al. 2010).

**Remarks.** The easiest way to recognize this species is to examine the presence of a short and straight depression filled with a pile at the pollex base in the male major claw (Fig. 5C, seta). This character is unique among Brazilian fiddler crabs. Otherwise, the general shape of carapace of *M. victoriana* is similar to *M. rapax*, *M. burgersi* and *M. mordax*.

## Subfamily Gelasiminae

### *Minuca vocator* (Herbst, 1804)

Figure 3A, B

**Recognition characters.** Carapace pentagonal moderately arched; profuse pile on dorsal surface forming a characteristic pattern mostly on hepatic and branchial regions (Fig. 3A). Dorso-lateral margins well marked even covered by pile, and strongly convergent posteriorly; major and minor pairs of postero-lateral striae clearly visible (Fig. 3A, setae). Front triangular and very wide measuring from 36% to 38% of the front-orbital breadth. Male major claw with manus covered with small tubercles dorsally and frontally and provided with a strong groove on dorsal margin usually filled with dirt; fingers thick and slightly flattened, and a little longer than manus; pollex and dactyl slightly curved forming a gap as wide as the fingers in their base. Exuberant piles on

dorsal surface of merus, carpus and manus of all ambulatory legs; these piles can extend to ventral side of manus (Fig. 3B). Male abdomen somites never fused. Medium-sized crabs, males with CW up to 27.0 mm in a population from Itamambuca mangrove, Ubatuba, southeastern Brazil (Colpo and Negreiros-Fransozo 2004).

**Biological notes.** The species forms one of the densest populations composed by large crabs, and its reproductive period coincides with the rainy period in northern Brazil (Koch et al. 2005). The southeast populations, however, have continuous reproduction (Colpo and Negreiros-Fransozo 2003). On the other hand, in impacted mangroves of southeastern Brazil, populations are not dense and crabs are smaller than in other populations (Bedê et al. 2008). The species prefers muddy substrates (Colpo and Negreiros-Fransozo 2003; Thurman et al. 2013). Large and well-constructed chimneys at the entrance of burrows were observed in a population from Venezuela, but there is no record of this ornamentation in any other population including those from Brazilian coast (Crane 1975).

**Remarks.** Characteristic pubescence pattern on the carapace and dense piles on dorsal surface of ambulatory legs are the best diagnostic characters for distinguishing it from other *Minuca* species recorded in Brazil. Another Brazilian fiddler crab that has an exuberant pile on the carapace surface is *Leptuca thayeri*, easily distinguishable from *M. vocator* by a very narrow front of the former species (compare Figs 2C and 3A). Although Melo (1996) considered Santa Catarina State as the southernmost limit of the geographic distribution of the species, currently the species has been reported only in the states from Amapá to São Paulo (Table 1).

## Subfamily Gelasiminae

### *Leptuca cumulanta* (Crane, 1943)

Figures 9B, 10A, B

**Recognition characters.** Carapace semi-cylindrical, width ca. 1.6 times the length; strongly arched and dorsal surface without pile; lateral margins almost parallel (Fig. 10A). Dorso-lateral margins well marked and strongly converging posteriorly; single pair of postero-lateral striae clearly visible (Fig. 10A, seta). The discrete pile on the carapace described by von Hagen (1987) was not observed in the specimens examined in the present study. Front triangular and moderately wide measuring from 25% to 29% of the front-orbital breadth. Manus of male major claw with dorso-lateral surface covered by tubercles except along the strong dorsal groove (mostly filled with dirt); smooth surface in the submarginal longitudinal area; dorsal surface with sparse tubercles while dorso-lateral one with small and dense tubercles; fingers ca. 1.7 times the manus length; pollex almost straight but dactyl strongly arched ending in a curved downward tip, forming a very wide gap (Fig. 10B). Ambulatory legs without pile. Male abdomen somites distinct (Fig. 9B). Small crabs, males' CW up to 12.5 mm in a population from Caeté mangrove, Pará state, northern Brazil (Koch et al. 2005).

**Biological notes.** The species reproduces year-round in populations of northern Brazil and the crabs reach the largest CW among all other populations (Koch et al.

2005). In the impacted mangroves, *L. cumulanta* is the fourth most abundant fiddler crab species, but crabs' CW is the smallest among these populations (Bedê et al. 2008). The species prefers muddy substrates (Thurman et al. 2013). Hoods at the entrance of male burrows were observed in some populations in Venezuela and Curaçao, but there is no such record from populations of the Brazilian coast (Crane 1975).

**Remarks.** In sympatric area of Brazilian coast *L. cumulanta* can be confused with *L. leptodactyla* (from Pará to Rio de Janeiro) and *L. uruguayensis* (Rio de Janeiro state): they are similar in size and the major male claw is provided with a very curved dactyl forming a wide gap with the pollex. The best ways to distinguish these three species is described when referring to *L. leptodactyla* and *L. uruguayensis* (see below).

## Subfamily Gelasiminae

### *Leptuca leptodactyla* (Rathbun, 1898)

Figures 9A, 11C, D

**Recognition characters.** Carapace semi-cylindrical, width ca. 1.7 times the length; strongly arched and dorsal surface without any pile or other ornaments; lateral margins almost parallel (Fig. 11C). Front triangular and moderately wide making up 20% to 23% of the front-orbital breadth. Dorso-lateral margins well marked and converging posteriorly; short and single pair of postero-lateral striae clearly visible (Fig. 11C). Male major claw smooth, manus with dorsal margin lined up with minute tubercles; fingers very slender and long, dactylus ca. 1.7 times the manus length (Fig. 11D); pollex almost straight and dactyl strongly arched ending in a curved downward tip; very wide gap between fingers. Ambulatory legs with narrow segments and devoid of pile or other ornaments. Male abdomen with 3<sup>rd</sup> to 6<sup>th</sup> somites fused (Fig. 9A). Small crabs: male CW maximum 14.29 mm in a population from Itacuruçá mangrove, Sepetiba Bay, southeastern Brazil (Bedê et al. 2008).

**Biological notes.** One of the most common fiddler crabs in sandy substrate of estuarine intertidal zone, *L. leptodactyla* reproduces year-round in the population of Ceará state (Bezerra and Matthews-Cascon, 2007), but only in warmer months at the southern coast (Masunari 2012). During the reproductive period (September-March in Guaratuba Bay, Paraná state), males construct a typical hood by piling up sand beside the burrow entrance where they stay for usual waving (Masunari 2012). The species shows strong preference for sandy substrate of polyhaline areas (Masunari 2006), and its young individuals can find shelter in the shadow of cordgrasses (S. Masunari, pers. obs.).

**Remarks.** *Leptuca leptodactyla* may be confused with *L. cumulanta* at the coast from Pará to Rio de Janeiro states. These two species, however, can be distinguished by features of the male abdomen: the middle somites are fused in the former species (Fig. 9A), while in *L. cumulanta* all somites are distinct (Fig. 9B). Furthermore, *L. leptodactyla* may also be confused with *Leptuca uruguayensis* in the sympatric area (from Rio de Janeiro to Santa Catarina state), especially among juvenile specimens. The male major claw of *L. uruguayensis*, however, is provided with a strong groove parallel to the dorsal margin of the manus (even in juvenile specimens) (Fig. 11B, seta), while in *L. leptodactyla* this groove is absent (Fig. 11D).

### Subfamily Gelasiminae

#### *Leptuca thayeri* (Rathbun, 1900)

Figure 2C, D

**Recognition characters.** Carapace trapezoidal weakly arched and covered with exuberant pile (pubescence easily detached) (Fig. 2C, seta) and strongly converging posteriorly. Dorso-lateral margins well marked and also strongly converging posteriorly; major and minor pairs of postero-lateral striae clearly visible (Fig. 2C). Front triangular and narrow making up ca. 15% of the front-orbital breadth. Male major claw with manus provided with a strong groove on the dorsal surface; fingers cylindrical and smooth; dactyl almost straight in the proximal two-thirds and curving down toward pollex tip and provided with a short but strong groove on the dorsal surface usually filled with dirt; moderate gap between fingers (Fig. 2D). Ambulatory legs with wide merus ca. 3.3 times the width of proximal end of carpus; posterior surface of all segments (except dactyl) of ambulatory legs covered by pile. Male abdomen segments not fused. Medium-sized species: male CW measures up to 28.4 mm in the population from Formoso River mangrove, Pernambuco state, northeastern Brazil (Farias et al. 2014).

**Biological notes.** Populations living in the northeastern Brazilian coast reproduce only in the rainy season (Ceará state) (Bezerra and Matthews-Cascon 2007) or continuously (Pernambuco state) (Farias et al. 2014), and those from southeastern Brazil during the warmer months (São Paulo state) (Costa et al. 2006). The species prefers typically muddy mangrove substrates in mesohaline areas, and it is the only fiddler crab in Brazil that forms large populations in shaded areas of the mangrove forest in Guaratuba Bay, southern Brazil (Masunari 2006). Males and females of *L. thayeri* can construct year-round highly structured chimneys around the entrance of the burrows; among the burrow with chimneys recorded in the mangrove of Guaratuba Bay, 53.3 % contained non-ovigerous females, 37.7 % ovigerous females and only 9.0 % males. Furthermore, the chimneys belonging to males had always a larger diameter and were lower than those of females' (T.F. Moreto, pers. comm.).

**Remarks.** This species is hardly confused with other species of Brazilian fiddler crabs, as it has a very narrow triangular front (see Fig. 2C). Furthermore, the carapace and the ambulatory legs are heavily covered with pile.

### Subfamily Gelasiminae

#### *Leptuca uruguayensis* (Nobili, 1901)

Figure 11A, B

**Recognition characters.** Carapace semi-pentagonal strongly arched and dorsal surface without pile or other ornaments (Fig. 11A). Dorso-lateral margins well marked and converging posteriorly; short and single pair of postero-lateral striae clearly visible (Fig. 11A). Front triangular and moderately wide making up from 20.0 % to 23.6% of the front-orbital breadth. Manus of male major claw with dorso-lateral surface covered by small

tubercles except along the strong submarginal groove, mostly filled with dirt (Fig. 11B, seta); both edges of dorsal margin armed with lined up by tubercles; dactyl moderately long ca. 1.4 times the manus length; pollex almost straight but dactyl strongly arched ending in a curved downward tip, forming a wide gap. Ambulatory legs with narrow segments and without pile. Male abdomen with 4<sup>th</sup> to 6<sup>th</sup> somites fused. Small crabs: males with CW up to 12.0 mm in a population from Itacuruçá mangrove, Sepetiba Bay, southeastern Brazil (Bedê et al. 2008). *Leptuca uruguayensis*, however, can attain up to 19.5 mm CW in the population from Solís Grande River, Uruguay (Masunari et al. 2017).

**Biological notes.** The species reproduces year-round in southeastern (Costa et al. 2006) and southern coast (Martins and Masunari 2013). It tolerates a wide range of salinities and is recorded in sandy substrates with a high degree of organic matter (Masunari 2006). In environments where the mangrove forest is absent (such as in the Uruguayan coast), *L. uruguayensis* occurs in marginal lowlands of rivers that flow into estuaries.

**Remarks.** *Leptuca uruguayensis* can be confused with *L. cumulanta* and *L. leptodactyla* in Rio de Janeiro state coast (these species are sympatric) due to the small size attained by these three species. The easiest way to separate them is by observing the male abdominal segments: among these three species only *L. cumulanta* has all somites distinct (see Fig. 9B) while the other two species have middle somites fused (Fig. 9A). On the other hand, *L. uruguayensis* can be distinguished from *L. leptodactyla*, by having a deep groove (filled with dirt) on dorsal granulated surface of manus of male major claw (Fig. 11B); in contrast, *L. leptodactyla* has the major claw manus with bare surface (Fig. 11D). Furthermore, the carapace of *L. uruguayensis* is semi-pentagonal with dorso-lateral margins moderately converging posteriorly (Fig. 11A), while *L. leptodactyla* has a cylindrical carapace and dorso-lateral margins weakly converging posteriorly (Fig. 11C).

## Subfamily Ocypodinae

### *Uca maracoani* (Latreille, 1802–1803)

Figures 1A, 2A, B

**Recognition characters.** Carapace trapezoidal moderately arched and naked dorsal surface, without any ornaments. Dorso-lateral margins well marked, long and weakly converging posteriorly; postero-lateral striae absent (Fig. 2A). Front spatulate and very narrow making up ca. 4% of front-orbital breath (Fig. 1A). Male major claw extremely large, with flat fingers and ornamented with tubercles, granules and small patches; narrow gap (Fig. 2B). Ambulatory legs without pile. Male abdomen somites distinct. Large fiddler crab: male CW up to 45.0 mm and female 40.2 mm in Paraty Bay, Rio de Janeiro state, southeastern Brazil (Hirose and Negreiros-Fransozo 2008).

**Biological notes.** The species reproduces year-round in northern (Azevedo et al. 2016), northeastern (Silva et al. 2016), southeastern (Hirose and Negreiros-Fransozo 2008) and southern (Benedetto and Masunari 2009) regions, but only during the dry season in northern region (Koch et al. 2005). Well-established populations are typically recorded in muddy substrates of polyhaline areas of estuaries, where no other

fiddler crab species was seen sharing this space (Masunari 2006). Genetic analysis revealed a lack of discernible genetic subdivision among populations of *Uca maracoani* along Brazilian coast; however, geometric morphometric technique showed statistically significant morphological differentiation that would indicate a strong phenotypic plasticity (Wieman et al. 2014).

**Remarks.** In the field, these crabs are unmistakable recognizable by the flattened fingers of the male major claw. Furthermore, they are visibly larger than any other Brazilian fiddler crab species.

## Distribution

Most Brazilian fiddler crabs occur along the coastal estuaries in environments closely related to mangroves that are distributed in the country from Amapá state to Laguna do Imaruí in the municipality of Laguna (20°30'S), Santa Catarina state (Vale and Schaeffer-Novelli 2018). Only *Minuca mordax* and *Leptuca uruguayensis* exceed southwards into estuarine areas where mangroves do not grow (Table 1). In Rio Grande do Sul state, where mangroves are absent, *M. mordax* lives on marginal banks of streams (S.B. Martins, pers. comm.), while in Uruguayan estuaries *L. uruguayensis* inhabit stream lowlands (Masunari et al. 2017).

We hypothesized that in Piauí State and others, where records of some common fiddler crab species are missing (Table 1, open circles), future collections will certainly fill the gaps. Only four species occur continuously in all states from Amapá to Santa Catarina (*M. mordax*, *M. rapax*, *Uca maracoani* and *L. thayeri*) while another two species are reported from Pará to Santa Catarina (*L. leptodactyla* and *M. burgersi*). Three species do not follow the entire distribution of mangroves (*M. vocator* from Amapá to São Paulo; *L. cumulanta* from Amapá to Rio de Janeiro, and *L. uruguayensis* from Rio de Janeiro to Rio Grande do Sul). The only remaining species (*M. victoriana*) has a restricted distribution and infrequent occurrence (Table 1).

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# Two Paradoxostomatidae (Ostracoda) species from South Korea with a key to genera of the family

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## Abstract

*Cytherois gajinensis* **sp. nov.** is described and *Violacytherois sargassicola* (Hiruta, 1976) is redescribed. The species have been collected from littoral and interstitial waters in South Korea. They belong to Cytheroisinae, one of the three Paradoxostomatidae subfamilies. Both species are the first taxonomic records of the subfamily in Korea. Taxonomic keys to the living Paradoxostomatidae genera are provided in an attempt to clarify the position of some of the currently included genera as well as a key to East Asian Cytheroisinae species in order to facilitate further biodiversity research in the region.

## Keywords

Biodiversity, Cytheroisinae, East Asia, taxonomy

## Introduction

The family Paradoxostomatidae comprises ostracods with a fragile, elongated, and laterally compressed carapace (Cohen et al. 2007). They are mostly algal dwellers

(Insafitri and Kamiya 2014), but several genera have been reported living commensally (see Tanaka and Arai 2017; Tanaka and Hayashi 2019). Paradoxostomatidae is the only ostracod taxon in which the upper and lower lips are fused into a suctorial disk, functioning as piercing and/or sucking organs (Athersuch et al. 1989). This enables animals to stick to the surface of seaweeds and, depending on the size of mouthparts, they specialize to different types of algae (Insafitri and Kamiya 2014).

According to the World Ostracoda Database (Brandão et al. 2020), the family comprises 25 genera, but the list does not include recently described commensal genus, *Chelonocytherois* Tanaka & Hayashi, 2019. The family's main distinguishing character is a very reduced maxillular palp, mandibular palp, and mandibular coxa. Based on the level of these reductions the family is divided into three subfamilies: Cytheroisinae, Paracytheroisinae, and Paradoxostomatinae (see Schornikov 1993). Paracytheroisinae comprises only one genus, *Paracytherois* Müller, 1894 characterized by a long styliform mandibular coxa and its palp reduced into a long, whip-like seta (see Athersuch et al. 1989). Members of Cytheroisinae are on the opposite end of the reduction of mandibula, with more robust coxa and the palp consisting of at least two segments and several setae. This subfamily includes the following genera: *Cytherois* Müller, 1884; *Chelonocytherois*, *Flabellicytherois* Schornikov, 1993, and *Violacytherois* Schornikov, 1993. Furthermore, *Cytherois* is subdivided into two subgenera, the nominal and *Orientocytherois* Schornikov, 1993. Representatives of Paradoxostomatinae have mandibular palp similar to Cytheroisinae, while the coxa is similar to Paracytheroisinae. Paradoxostomatinae includes the rest of 20 Paradoxostomatidae genera, although position of many is doubtful (see discussion). Its most diverse genus, *Paradoxostoma* Fischer, 1855, has been revised several times, and most recently by Schornikov and Keyser (2004) who erected five genera to mirror morphological diversity of this taxon.

Although South Korean cytheroids are poorly studied in general (see Karanovic et al. 2017), with 52 species described/reported so far (Yoo et al. 2019), Paradoxostomatidae, and in particular *Paradoxostoma*, with eleven species, is by far the best studied genus from this country. In addition to those 52 named species, Lee et al. (2000) list another 400-plus cytheroids; however, they are mostly unidentified as their research was related to studying water pollution, rather than biodiversity. Their list includes 25 unnamed *Cytherois* species and one provisionally identified, *C. cf. megapoda* Schornikov, 1993.

Here we report on two Cytheroisinae species from South Korea. One is a new species of *Cytherois* and the other is *Violacytherois sargassicola* (Hiruta, 1976). *Cytherois* is by far the most diverse genus in the subfamily comprising about 60 species (see Brandão et al. 2020). Of those, more than 1/3 are known only after the shell, either because they are subfossil/fossil species, or because of an insufficient description. The following species have been reported or described from East Asia (species known only after their shells are marked with asterisk): *C. asamushiensis* Ishizaki, 1971\*; *C. decorata*

Okubo, 1980; *C. ikeyai* Nakao & Tsukagoshi, 2002; *C. leizhouensis* Gou and Huang in Gou, Zheng & Huang, 1983\*; *C. megapoda* Schornikov, 1993; *C. marginalis* Hu, 1984\*; *C. tosaensis* (Ishizaki, 1968); *C. uranouchiensis* Ishizaki, 1968\*; *C. wangchieni* Hu & Tao, 2008\*; and *C. zosteræ* (Schornikov, 1975). *Cytherois asamushiensis*, *C. decorata*, *C. ikeyai*, *C. tosaensis*, *C. uranouchiensis*, and *C. zosteræ* are all known from Japan (Ishizaki 1968, 1971; Okubo 1980; Schornikov 1975; Nakao and Tsukagoshi 2002); *C. leizhouensis* was described from China (Gou et al. 1983); *C. marginalis* and *C. wangchieni* from Taiwan (Hu 1984; Hu and Tao 2008); and *C. megapoda* from Russia (Schornikov 1993).

Both *Flabellicytherois* and *Chelonocytherois* are monospecific and endemic to East Asia (Okubo 1980; Schornikov 1993; Tanaka and Hayashi 2019). *Violacytherois sargassicola* was originally described from Hokkaido Island (Hiruta 1976) and later found in the Russian Far East (Schornikov 1993). It is one of the only three species known so far, all endemic to East Asia as well.

Beside the description and redescription of two Cytheroisinae species, we also provide a key to all living genera of Paradoxostomatidae and living East Asian species of Cytheroisinae.

## Materials and methods

Samples were collected by scientific scuba diving (Pardo 2014) and by algal rinsing (hand-net mesh size is 62 µm), as described by Giere (2009). Samples were fixed in 99% ethanol on site. Sorting was done under a stereomicroscope (Olympus SZX12) in the Laboratory at Hanyang University. Specimens were dissected, and soft parts mounted on slides in CMC-10 Mounting Media (Masters Company, Inc.), while carapaces were kept on the micro-palaeontological slides. All drawings were prepared using a drawing tube, attached to the Olympus BX51 microscope. For observations under the scanning electron microscope (SEM), carapaces were coated with platinum. SEM photographs were taken at Eulji University with the Hitachi S-4700 electron microscope. All specimens are deposited in the invertebrate collection of the National Institute of the Biological Resources (NIBR) in South Korea.

Abbreviations used in text and figures:

<b>A1</b>	Antennula;	<b>LV</b>	Left valve;
<b>A2</b>	Antenna;	<b>L5-7</b>	Leg 5-7;
<b>GF</b>	Genital field;	<b>Md</b>	Mandibula;
<b>H</b>	Height;	<b>Mxl</b>	Maxillula;
<b>Hp</b>	Hemipenis;	<b>RV</b>	Right valve.
<b>L</b>	Length;		

## Results

### Systematics

**Order Podocopida Sars, 1866**

**Superfamily Cytheroidea Baird, 1850**

**Family Paradoxostomatidae Brady & Norman, 1889**

**Genus *Cytherois* Müller, 1884**

***Cytherois gajinensis* sp. nov.**

<http://zoobank.org/75B1179A-7333-4570-9178-570AA30B4106>

Figures 1–3

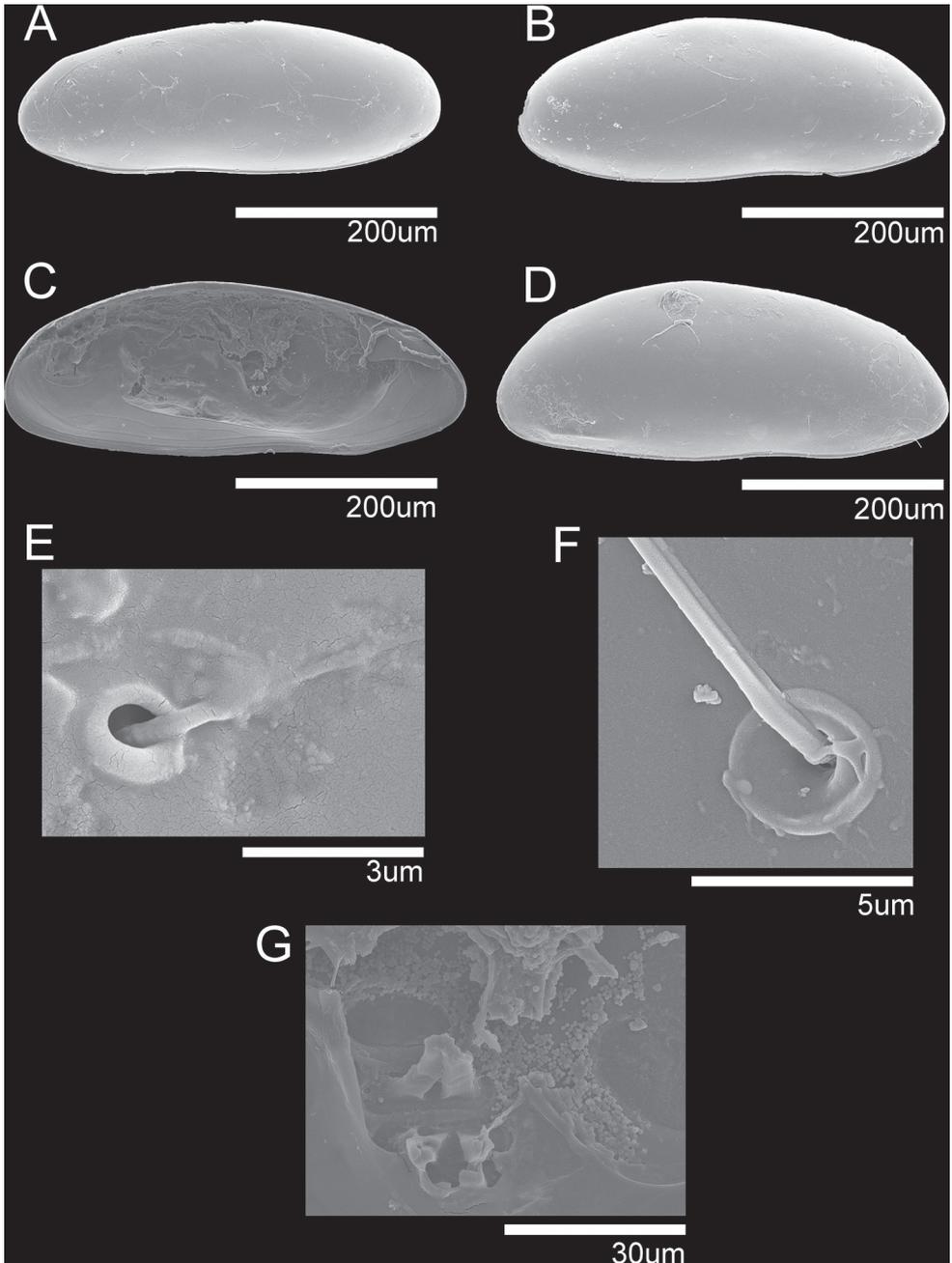
**Material examined.** *Holotype*, male, dissected on one slide (NIBRIV0000813439) and shell on micropalaeontological slide; allotype, female, dissected on one slide and shell on micropalaeontological slide; *paratypes*: two males dissected on each slides and shell on micropalaeontological slides, one female dissected on one slide and shell on micropalaeontological slide and five specimens kept in a 2 ml vial.

**Type locality.** South Korea, Gangwon-do, Goseong-gun, Jugwang-myeon, Gajin-ri; 38°18.16'N, 128° 34.36'E, 25 m, sandy bottom; 29 Aug. 2016, collected by Rae-hyuk Jeong and Wonchoel Lee.

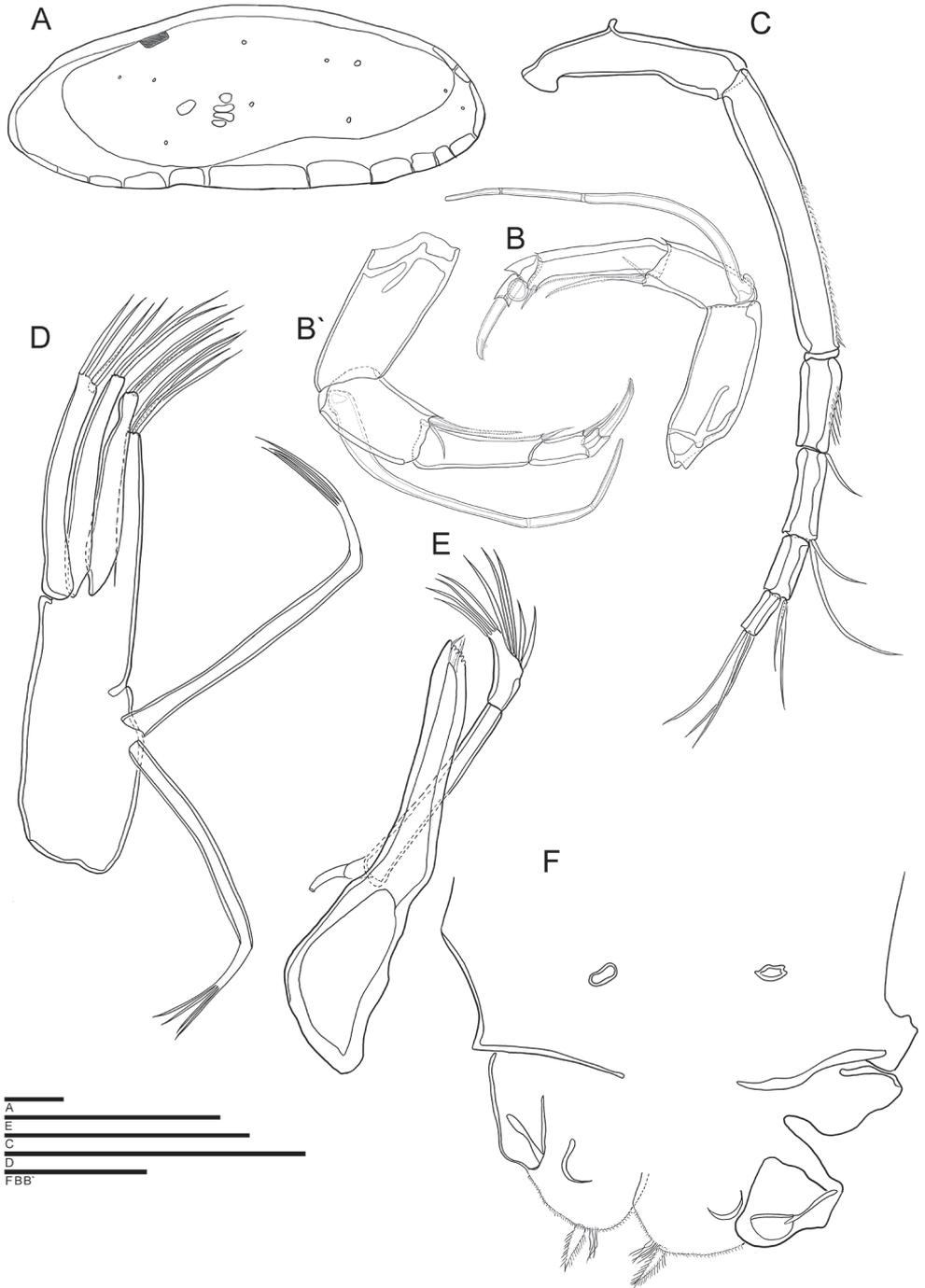
**Etymology.** The species is named after the beach from where it was collected.

**Description of male. Carapace** (Figs 1A–C, E–G, 2A). Relatively small, with L approximately 422 µm, H approximately 154 µm. LV overlapping RV. Carapace elongated ellipsoidal in lateral view (Fig. 1A). Dorsal margin slightly arched, antero-dorsal and postero-dorsal margins evenly curved, ventral margin slightly sinusoid around mouth region. Anterior and posterior margins rounded, with anterior margin being slightly narrower than posterior one. Greatest H situated slightly behind the middle. Eye present. Surface of the carapace smooth with few simple type setae distributed (Fig. 1E, F). Marginal pore canals noticeable along ventral and posterior margins (Fig. 2A), relatively short and not branched. Inner lamella equally wide anteriorly and posteriorly. Muscular scar imprints consisting of a row of four vertical scars and one frontal scar present (Figs 1G, 2A). Hinge adont (Fig. 1C).

**AI** (Fig. 2C). Six-segmented. First segment without setulae and setae. Second segment with setule along anterior to distal margin. Third segment with visible setulae along anterior to distal margin and one bare seta on antero-distal margin, not reaching end of fourth segment. Fourth segment with two bare setae on antero-distally, one reaching end of fifth segment and another twice longer than the fifth segment. Fifth segment with two bare setae on antero-distal part, one 1.5 times longer than terminal segment and the other twice as long as terminal segment. Terminal segment with three long bare setae on distal margin, almost 2.5 times longer than terminal segment. L ratio between six segments 4.1: 5.6: 1.7: 1.7: 1.36: 1.



**Figure 1.** SEM photographs of *Cytherois gajinensis* sp. nov. **A-C, E-G** male **D** female: **A** LV external view (holotype) **B** RV, external view (paratype) **C** LV, internal view (paratype) **D** RV, external view (allotype) **E, F** surface pores (holotype) **G** muscular scar print (paratype).



**Figure 2.** *Cytheroïs gajinensis* sp. nov. **A–E** male (holotype) **F, B'** female (allotype): **A** RV internal view **B** A2 **C** A1 **D** Mx1 **E** Md **F** GF. All scale bars: 50 µm.

**A2** (Fig. 2B). Five-segmented. Exopod transformed into three-segmented spinneret seta. First endopodal segment without setulae and seta. Second segment with two setae postero-distally: one plumose, seta reaching end of third segment, another bare, reaching 1/3 the third segment. Third segment with setule along posterior to distal margin, and one short, strong, bare seta postero-distally reaching distal end of terminal segment. Penultimate segment with seta transformed into sucking organ. Terminal segment very short and carrying only one strong claw on distal margin. L ratio between five distal segments: 6: 3.1: 4.3: 1: 1.

**Md** (Fig. 2E). Coxa with six tiny teeth and three thin, bare, setae on distal margin. Exopod with one seta; endopod 2-segmented. First endopodal segment elongated but not carrying any seta. Second segment with nine setae, five of which arise from central margin, four from distal margin. First segment almost four times longer than second segment.

**Mxl** (Fig. 2D). Palp 1-segmented carrying four bare setae on distal margin, all setae almost half as long as the palp. Two long mop-shaped setae (“aberrant setae”) present at the distal end of vibratory plate. Masticatory process with three endites, first and second endites each with three bare setae, third endite with four bare setae on distal margin.

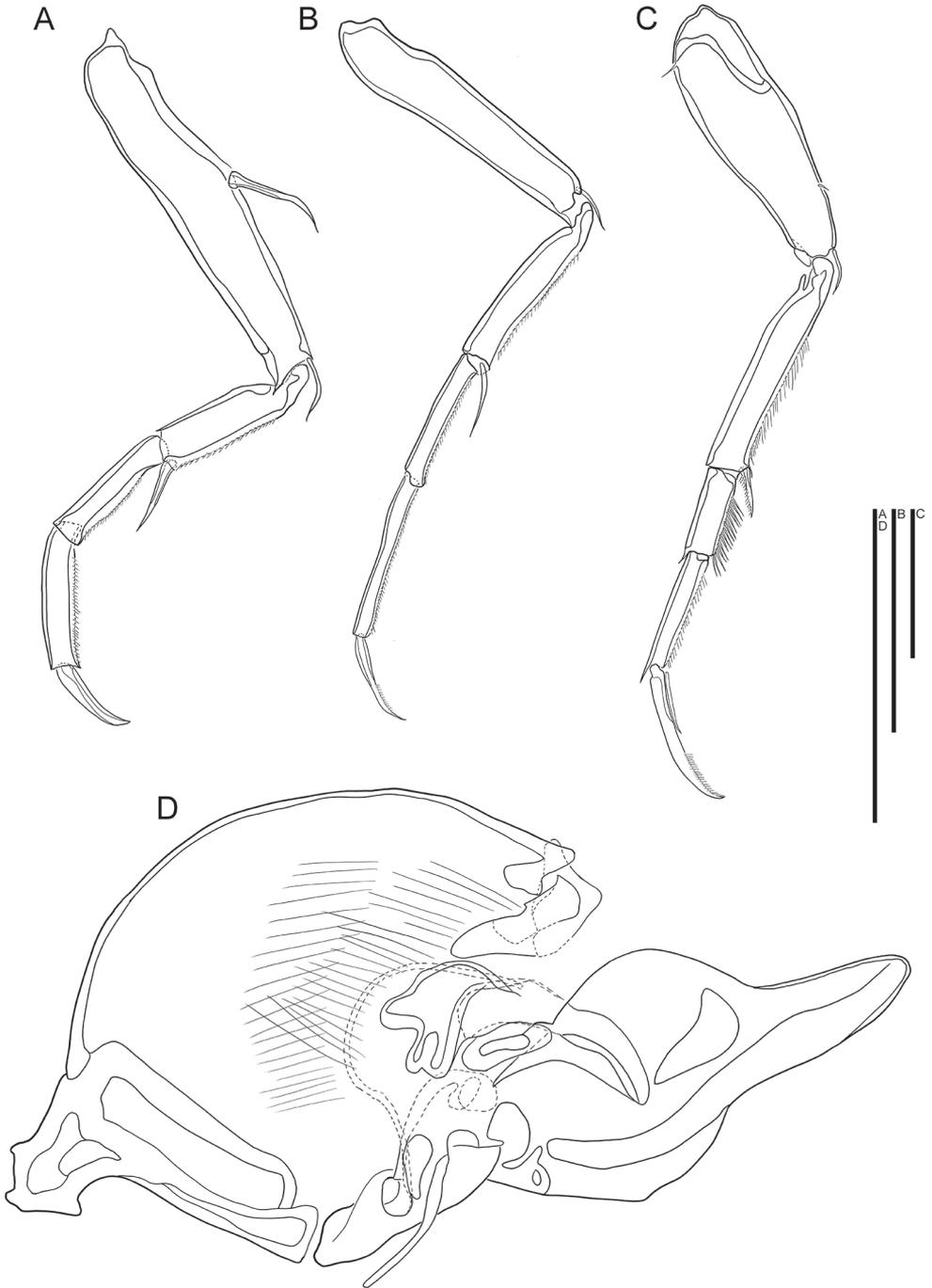
**L5** (Fig. 3A). Four-segmented. First segment with two bare setae, one on antero-medial margin, not reaching end of first segment, and another on antero-distally, reaching 1/3 of second segment. Second segment with one bare seta antero-distally, not reaching end of third segment. Penultimate segment without any seta. Terminal segment with one claw like seta on distal margin. Last three segments with setulae along posterior to distal margin. L ratio between four segments 2.7: 1.24: 1: 1.06.

**L6** (Fig. 3B). Four-segmented. First segment with one bare seta antero-distally, reaching 1/4 of second segment. Second segment with one bare seta antero-distally, reaching half of third segment. Following segment without any setae. Terminal segment with one claw like seta on distal margin. Last three segments with setulae along posterior to distal margin. L ratio between four segments 2.2: 1.4: 1: 1.3. In comparison to L5, L6 has more elongated segments.

**L7** (Fig. 3C). Four-segmented. First segment with tiny setule postero-proximally and, antero-medially, and one bare seta on antero-distal margin, reaching 1/4 of second segment. Second segment with one plumose seta on antero-distal margin reaching almost half length of the third segment. Third segment with long, almost spine-like setulae along anterior to distal margin. Terminal segment with one strong claw and one bare seta on distal margin, almost half as long as same segment. Second and terminal segment with setulae along posterior to distal margin. L ratio between four segments 2.9: 2.5: 1: 1.25. Segments of L7 are more elongated than on L5, but less than on L6.

**Hp** (Fig. 3D). Basal plate ovate. Distally Hp carrying a large lobe in a shape of eagle beak, dorsally to which a much smaller lobe-like process with triangular, but dull tip present.

**Description of female. Carapace** (Fig. 1D). Slightly larger than males. L approximately 451  $\mu\text{m}$ , H approximately 182  $\mu\text{m}$ . Shape and all other morphological features similar to male.



**Figure 3.** *Cytheroïs gajinensis* sp. nov. male (holotype) **A** L5 **B** L6 **C** L7 **D** Hp. All scale bars: 50  $\mu$ m.

**A2** (Fig. 2B'). Penultimate segment with one seta instead of sucking organ, and same segment longer than in male. L ratio between five distal segments of female A2. 9: 5.7: 6.5: 3.5: 1.

**GF** (Fig. 2F). Basal part rectangular. Two caudal rami present and long setulae cover the surface. End of the body seta not observed.

All other appendages same as in male.

### Genus *Violacytherois* Schornikov, 1993

#### *Violacytherois sargassicola* (Hiruta, 1976)

Figures 4–6

*Cytherois sargassicola* Hiruta, 1976: 24, figs 1–3.

*Violacytherois sargassicola* (Hiruta): Schornikov, 1993: 181, figs 7, 8; pl II, figs 7–10.

**Material examined.** Male, dissected on one slide (NIBRIV0000813440) and shell on micropalaeontological slide; Female, dissected on one slide and shell was broken; two males dissected on one slide each, shell broken; one female dissected on one slide, shell broken; one juvenile dissected on one slide; shell on micropalaeontological slide and 12 specimens kept in 2 ml vial in alcohol.

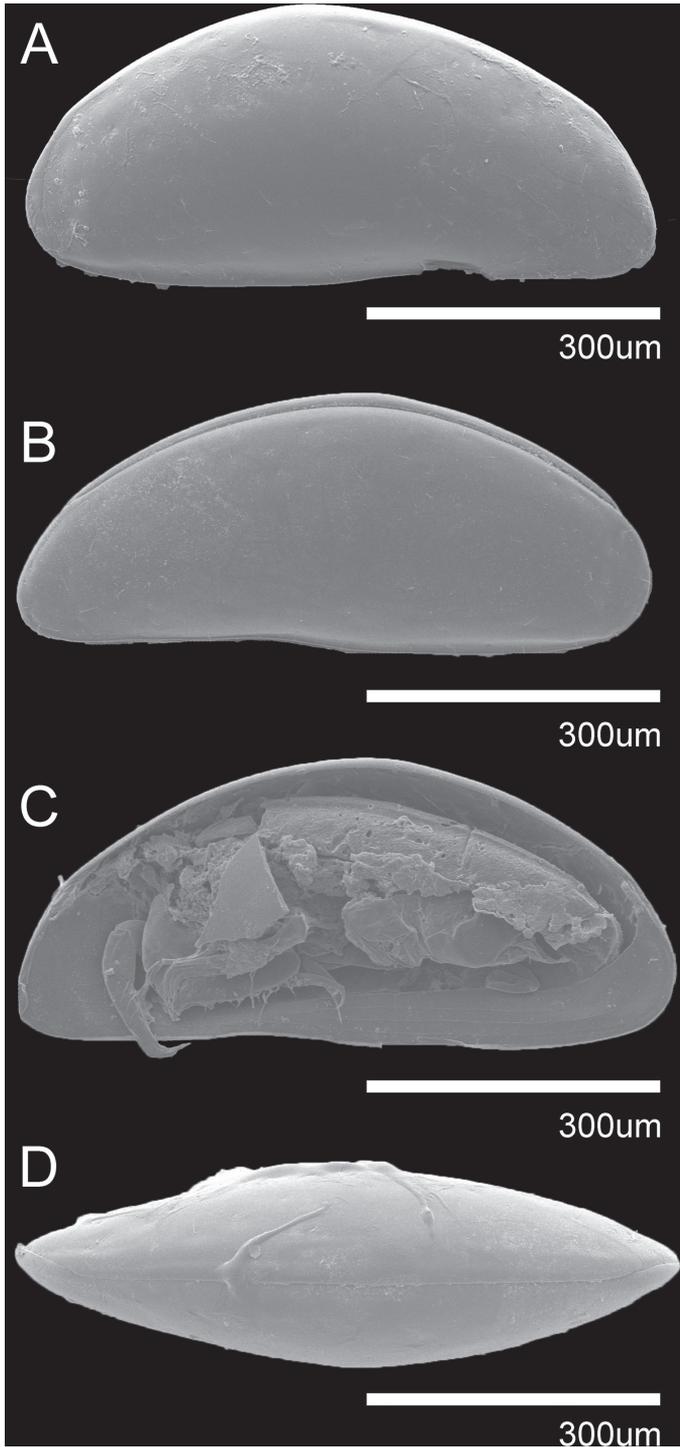
**Locality.** South Korea, Gyeongsangnam-do, Goseong-gun, Donghae-myeon, Dongdong beach; 34°59.63'N, 128°26.02'E, 0.5 m depth; 04 Apr. 2012; collected by Tomislav Karanovic and Ivana Karanovic.

**Description of female. Carapace** (Figs 4A, 5A). L approximately 647  $\mu\text{m}$ , H approximately 295  $\mu\text{m}$ . Carapace ellipsoidal in lateral view (Figs 4A, 5A). Dorsal margin arched, antero-dorsal margin slightly curved, ventral margin almost straight with weak curve point near the middle, the greatest H which is situated slightly behind the middle. Eye absent. LV overlapping RV on anterior and posterior margin, conversely RV overlapping LV on dorsal margin (Fig. 4D). Surface of the carapace smooth with few simple setae. Pore canals sparse, straight and distributed along the margin (Fig. 5A) not branched. Inner lamella wide at anterior margin and increasingly wider ventral, while almost the same with posteriorly. Muscular scar imprints consisting of a row of four vertical scars and one frontal scar present (Fig. 5A). Hinge adont (Fig. 4C).

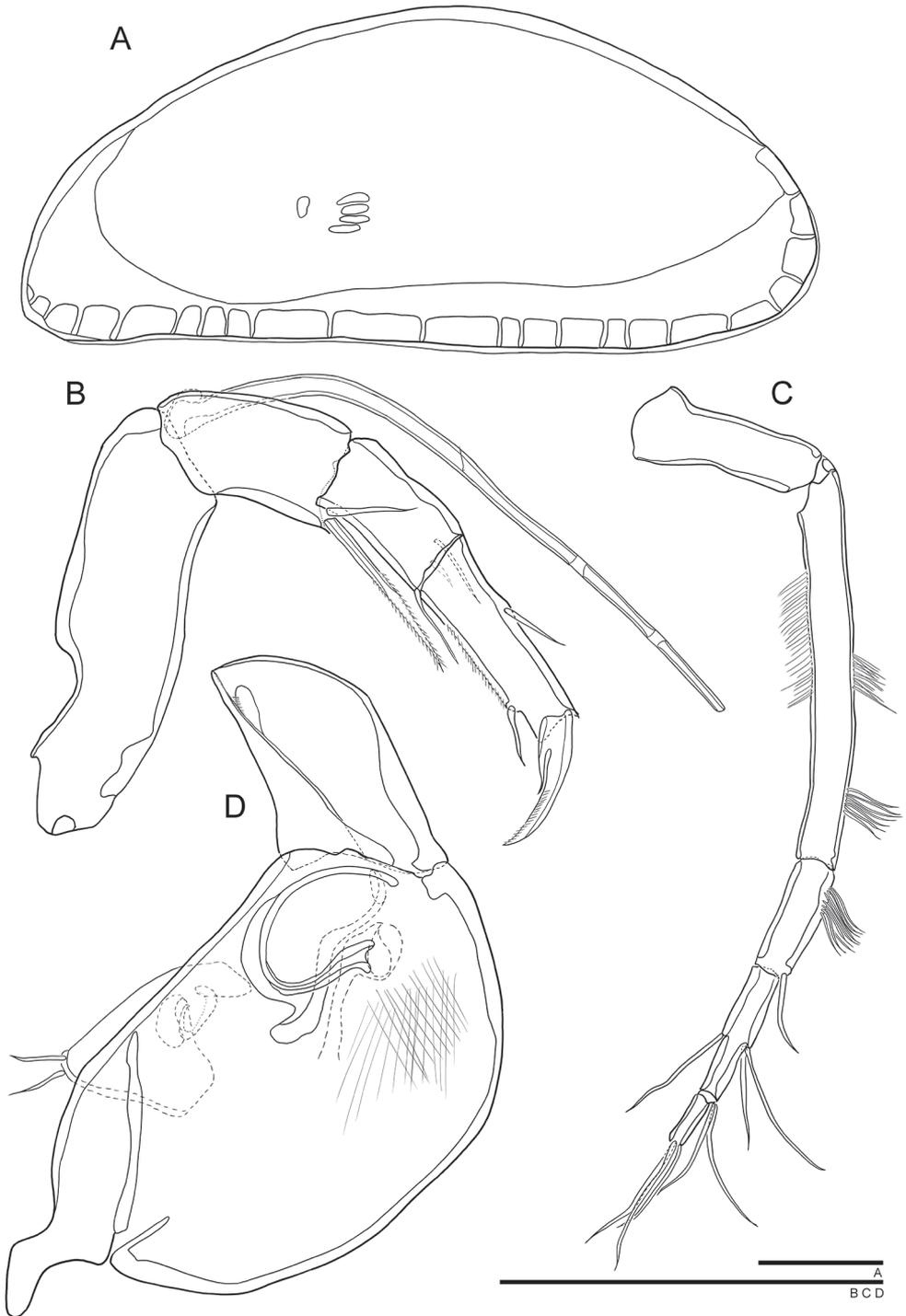
**A1 and A2** same as in male (see description below).

**Md** (Fig. 6F). Coxa with five small teeth and one strong tooth on distal margin, one bare seta antero-medially not reaching end of the antero-distal margin. Palp with two-segmented endopodite and exopodite carrying one bare seta (broken). First endopodal segment without any seta, almost three times as long as second segment. Second segment with ten setae, one plumose and one bare seta on antero-distally, eight bare setae on distal margin.

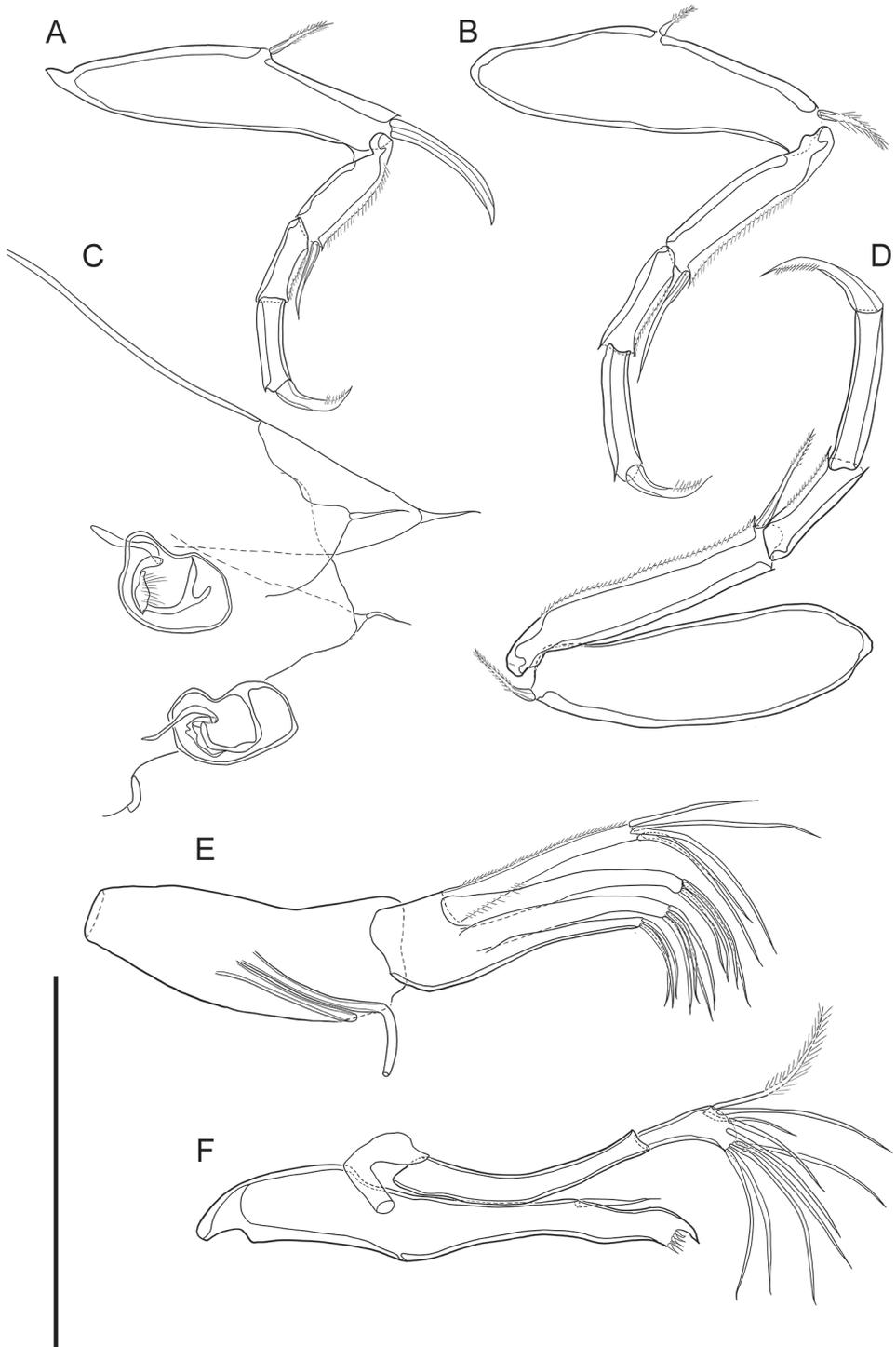
**Mxl** (Fig. 6E). Palp present with five bare setae on distal margin almost same length as the palp segment, setulae present along anterior to distal margin and poste-



**Figure 4.** SEM photographs of *Violacytherois sargassicola* (Hiruta, 1976) **A, D** female **B, C** male: **A** RV external view **B** LV external view **C** RV internal view with soft parts **D** dorsal view.



**Figure 5.** *Violacytherois sargassicola* (Hiruta, 1976) **A** female **B–D** male: **A** RV internal view **B** A2 **C** A1 **D** Hp. All scale bars: 100  $\mu$ m.



**Figure 6.** *Violacytherois sargassicola* (Hiruta, 1976): Female **A** L5 **B** L6 **C** GF **D** L7 **E** Mxl **F** Md. All scale bars: 100  $\mu$ m.

rior-proximally. Two long setae at the middle of vibratory plate (aberrant setae). Masticatory process with three endites, first and second endites each with four bare setae almost half length of palp segment, third endite with three bare setae almost 1/3 length of palp segment.

**L5** (Fig. 6A). Four-segmented. First segment with one plumose seta antero-medially not reaching end of the same segment, one claw-like seta on antero-distal margin. Second segment with setulae along anterior to distal margin, one bare seta antero-distally, reaching 1/5 of terminal segment. Third segment with setule along anterior to distal margin. Terminal segment with claw on distal margin. L ratio between four segments 4: 1.6: 1: 1.1.

**L6** (Fig. 6B). Four-segmented. First segment with one plumose seta antero-medially, not reaching end of the same segment, one plumose seta antero-distally reaching 1/3 of second segment. Second segment with setulae along anterior to distal margin, one bare seta antero-distally, reaching 1/4 of terminal segment. Third segment with setulae along anterior to distal margin. Terminal segment with claw-like seta on distal margin. L ratio between four segments 2.9: 1.7: 1: 1.08.

**L7** (Fig. 6D). Four-segmented. First segment with one plumose seta antero-distally reaching 1/7 of the second segment. Second segment with setulae along anterior to distal margin, one plumose seta antero-distally reaching end of third segment. Third segment with setulae along anterior to distal margin. Terminal segment with one claw like seta on distal margin. L ratio between four segments 2.8: 2.6: 1: 1.36.

**GF** (Fig. 6C). Basal part subtriangular. Ovary kidney-bean shaped, situated at the middle of the basal capsule. One caudal ramus seta present. One end of the body seta present.

**Description of male. AI** (Fig. 5C). Six-segmented. Fourth and penultimate segment fused. First segment without any seta. Second segment with setulae postero-medially and along anterior to distal margin. Third segment with setulae antero-proximally, one bare seta antero-distally, reaching end of fourth segment. Fourth segment with two bare setae antero-distally, one reaching end of terminal segment, another reaching half of same segment, one bare seta postero-distally, reaching end of terminal segment. Fifth segment with two bare setae antero-distally almost twice as long as terminal segment. Terminal segment with three bare setae on distal margin almost 2.5 times as long as same segment. L ratio between five segments 3.25: 6.5: 2.25: 2.42 (fused segment): 1.

**A2** (Fig. 5B). Five-segmented. Exopod transformed into three-segmented spinneret seta. First segment without any seta. Second segment with two setae postero-distally, one plumose seta reaching slightly over half of terminal segment. Third segment with one bare seta postero-distally not reaching half of the terminal segment, two bare setae situated medio-distally, not reaching half of the terminal segment. Fourth segment with setulae along antero-distal margin; one bare seta on posterior-distal margin, reaching slightly over distal end of the same segment; one bare seta antero-medially, not reaching distal end of the same segment. Terminal segment with one claw and one short seta fused with it. L ratio between three segments (excluding terminal segment) 2.5: 1.1: 1: 1.3.

**Hp** (Fig. 5D). Basal part subrectangular form with two bare setae on anterior medially. Distal lobe subtriangular with slightly cuneiform distal tip. Same lobe also vertically subdivided.

Other appendages same as in female.

### Key to living Paradoxostomatidae genera

1	Md-palp transformed into whip-like seta .....	<b><i>Paracytherois</i> Müller, 1894</b>
–	Md-palp with distinct segments .....	<b>2</b>
2	Md-coxa styliiform .....	<b>3</b>
–	Md-coxa with distinct teeth .....	<b>16</b>
3	Terminal claw on A2 as well as claws on all walking legs very short and hook-shaped .....	<b>4</b>
–	Terminal claw on A2 as well as claws on all walking legs not so short and hook-shaped .....	<b>6</b>
4	Mxl with only one endite.....	<b><i>Asterositus</i> Tanaka &amp; Arai, 2017</b>
–	Mxl with two prominent endites.....	<b>5</b>
5	Terminal segment of A2 reduced (i.e. completely fused with terminal claw)....	<b><i>Echinophilus</i> Schornikov, 1973</b>
–	Terminal segment of A2 not reduced (i.e. there is a clear division between the segment and the claw).....	<b><i>Echinositus</i> Schornikov, 1973</b>
6	Terminal segment of A2 with 2 claws.....	<b>7</b>
–	Terminal segment with one claw .....	<b>10</b>
7	Hinge lophodont .....	<b><i>Boreostoma</i> Schornikov, 1993</b>
–	Hinge adont .....	<b>8</b>
8	Carapace with a postero-ventral spinula .....	<b><i>Calcarostoma</i> Schornikov &amp; Keyser, 2004</b>
–	No postero-ventral spinula present.....	<b>9</b>
9	Mxl palp completely absent .....	<b><i>Lanceostoma</i> Schornikov &amp; Keyser, 2004</b>
–	Mxl palp reduced into a seta .....	<b><i>Paradoxostoma</i> Fischer, 1855</b>
10	Hinge adont .....	<b>11</b>
–	Hinge lophodont .....	<b>13</b>
11	Posterior end of carapace with extension situated slightly above middle, anterior margin cuneiform .....	<b><i>Austroparadoxostoma</i> Hartmann, 1979</b>
–	Both anterior and posterior margins rounded .....	<b>12</b>
12	Mxl palp reduced into a medium size seta.....	<b><i>Pontostoma</i> Schornikov &amp; Keyser, 2004</b>
–	Mxl palp absent .....	<b><i>Brunneostoma</i> Schornikov, 1993</b>
13	Terminal segment of A2 carrying a seta, at least half as long as the claw .....	<b><i>Obesostoma</i> Schornikov, 1993</b>
–	If present, seta is tiny .....	<b>14</b>
14	First endite on the Mxl at least ½ as long as the other two .....	<b><i>Bradystoma</i> Schornikov &amp; Keyser, 2004</b>
–	First endite on the Mxl much shorter.....	<b>15</b>

- 15 Anterior margin of the carapace cuneiform, and antero-ventral surface flattened.....*Acetabulastoma* Schornikov, 1970
- Anterior margin of the carapace rounded and antero-ventral surface not flattened..... *Triangulastoma* Schornikov & Keyser, 2004
- 16 Carapace with sieve-pores present ..... 17
- No sieve-pores present ..... 18
- 17 Terminal segment of Md-palp with a strong claw.....*Redekea* de Vos, 1953
- Terminal segment of Md-palp without a claw .....  
.....*Chelonocytherois* Tanaka & Hayashi, 2019
- 18 A2 with two strong terminal claws.....*Flabellicytherois* Schornikov, 1993
- A2 with one terminal claw ..... 19
- 19 L5 with claw-like postero-distal seta, and A2 not sexually dimorphic.....  
.....*Violacytherois* Schornikov, 1993
- L5 with seta-like postero-distal seta, A2 sexually dimorphic.....  
.....*Cytherois* Müller, 1884

### Key to East Asian species of Cytheroisinae

- 1 Carapace with sieve-pores present .....  
.....*Chelonocytherois omutai* Tanaka & Hayashi, 2019
- Carapace without sieve-pores ..... 2
- 2 Terminal segment of A2 with 2 claws.....  
.....*Flabellicytherois bingoensis* (Okubo, 1990)
- Terminal segment of A2 with one claw and at the most 1 seta ..... 3
- 3 L5 with claw-like postero-distal seta..... 4
- L5 with seta-like postero-distal seta..... 5
- 4 A1 5-segmented (4<sup>th</sup> and 5<sup>th</sup> segments fused).....  
.....*Violacytherois sargassicola* (Hiruta, 1976)
- A1 6-segmented..... *Violacytherois violacea* (Schornikov, 1974) and *V. flavoviolacea* Schornikov, 1993
- 5 Terminal segment of L7 beside a claw carrying one additional seta (clearly visible) ..... 6
- Terminal segment of L7 carrying only one claw ..... 7
- 6 A1 5-segmented (4<sup>th</sup> and 5<sup>th</sup> segments fused).....  
.....*Cytherois megapoda* Schornikov, 1993
- A1 6-segmented.....*Cytherois gajinensis* sp. nov.
- 7 Dorsal margin of the carapace highly arched.....  
.....*Cytherois decorata* Okubo, 1980
- Carapace more elliptical in lateral view ..... 8
- 8 Fourth and 5<sup>th</sup> A1 segments lacking any seta posteriorly (but carrying 2 setae each anteriorly) .....*Cytherois ikeyai* Nakao & Tsukagoshi, 2002
- Fourth and 5<sup>th</sup> A1 segments carrying one seta each posteriorly (in addition to 2 setae each anteriorly)..... *Cytherois zosterae* Schornikov, 1975

## Discussion

With the addition of *Cytheroïs gajinensis* there have been eleven *Cytheroïs* species described from East Asia, half of which are known from the shell only. Nevertheless, the shell shape of the new species is distinctly different from the fossil/subfossil ones. In addition, one of the subfossil species, *C. asamushiensis* from Aomori Bay in Japan (Ishizaki 1971), has been transferred to *Paracytheroma* Juday, 1907 by Schornikov (2006). Although the above key to species does not consider sexual characters, in order to avoid misidentification in a case that only one sex is available for study, the largest differences between not only East Asian but all living *Cytheroïs* species are in the morphology of the hemipenis. Additionally, the species differ in the presence of a sucker-type seta on the penultimate segment of the male A2. Among the East Asian species, only *C. ikeyai* seems to possess a seta (Nakao and Tsukagoshi 2002) like *C. gajinensis* does. The second antenna is sexually dimorphic in this genus, but this dimorphism in most of the species is related to the length of the penultimate segment in relation to other segments, and in females it is longer than in males. Of all other representatives of the genus that have the second antenna described/illustrated, males of the following species have a brush-like seta on the A2: *C. australis* Hartmann, 1989; *C. lignicola* Maddocks & Steineck, 1987; *C. vitrea* (Sars, 1866); and *C. neogracilis* Hartman & Peterson, 1985 (see Sars 1866; Hartmann 1964, 1989; Maddocks and Steineck 1987). It has to be pointed out that in these species the morphology of the transformed seta is quite different from the sucker-type seta found in the new species and *C. ikeyai*, and also its position is not on the penultimate segment (4<sup>th</sup>), but rather on the third. This, with the discrepancies in the number of claws on the terminal segment of A2, with few species having two instead of one (for example, *C. neogracilis*), suggests that the genus should be revised with the purpose of clarifying phylogenetic relationships between species.

The second species reported here, *Violacytheroïs sargassicola*, seems to be relatively widely distributed in East Asia, since it has been reported from Hokkaido (Hiruta 1976), Peter the Great Bay in Russia (Schornikov 1993), and Korea. There are no differences between the Korean and the other two records. *Violacytheroïs sargassicola* is morphologically very similar (both carapace and soft body parts) to *V. violacea* and *V. flavoviolacea*. Beside minute differences in the morphology of the hemipenis, the species mainly differ in the number of A1 segments. This needs to be taken with caution, because the division between segments can sometimes be obscure or partial. In the above key to species, *V. violacea* and *V. flavoviolacea* could not be distinguished based on their descriptions/illustrations (Schornikov 1974, 1993), and it is likely that the latter is junior synonym of *V. violacea*.

The following three genera currently included in the family Paradoxostomatidae (see Brandão et al. 2020) are not part of the above key, because they are known only after the carapace morphology: *Caribbella* Teeter, 1975, *Glyphidocythere* Ayress, Corregge & Whatley, 1993, and *Neopellucistoma* Ikeya & Hanai, 1982. We also excluded *Nodoconcha* Hartmann, 1989, *Paracythere* Müller, 1894, and *Pseudeucythere* Hartmann, 1989. In contrary to all other Paradoxostomatidae, those genera have much more ro-

bust A1, stronger mandibular coxa, robust Md-palp and well-developed Mxl-palp. In fact, Hartmann (1989) placed both of his genera in *incertae sedis* cytheroids and they have been included in WoRMS database erroneously. Müller (1894) considered *Paracythere* a member of Cytheridae, while Martin and Davis (2001) placed it into Paradoxostomatidae. Despite our attempt to provide a key to Paradoxostomatidae, it has to be used with caution as many of the genera are in need of revision. We based our key on the most typical representatives of each genus although large genera (such as *Paradoxostoma* and *Cytherois*) include species that are morphologically, and thus probably also phylogenetically, very distinct.

## Acknowledgements

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# *Neoamphitrite undevigintipes*, a new terebellid species from South Korea (Annelida, Terebellida, Terebellidae)

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## Abstract

A detailed description and illustrations of a new terebellid species are provided, and molecular information based on partial sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene are included. The new species, *Neoamphitrite undevigintipes* **sp. nov.**, is described from the deep sea off the eastern coast of South Korea. It is similar to *Neoamphitrite groenlandica* (Malmgren, 1866) in that the thorax has 19 notopodial chaetigers. However, *Neoamphitrite undevigintipes* **sp. nov.** is clearly distinguishable from *N. groenlandica* in having the uncini of the first abdominal chaetiger arranged in a single row and in having 12 ventral shields. A taxonomic key to all known *Neoamphitrite* species is also included.

## Keywords

*Amphitrite*, COI, new species, Polychaeta, polychaete, systematics, taxonomy

## Introduction

The genus *Neoamphitrite* Hesse, 1917 is a terebellid polychaete assigned to the subfamily Terebellinae Johnston, 1846 (Fauchald 1977; Londoño-Mesa 2009). Members of this genus usually have distinct lateral lobes on anterior segments, three pairs of dichotomous branchiae, distally hirsute notochaetae, and the uncini beginning at segment 5 (Fauchald 1977; Reuscher et al. 2012). This genus has been confused with *Amphitrite* Müller, 1771 due to controversial morphological differences between the two

genera (Hessel 1917; Fauvel 1927; Hutchings and Glasby 1988; Reuscher et al. 2012). Hessel (1917) considered that *Neoamphitrite* is distinguishable from *Amphitrite* by the branchial stem and nephridial papillae. *Neoamphitrite* has dichotomous branchiae with a well-developed stem and nephridial papillae with a free tube distinctly projecting from the body, while *Amphitrite* has filiform branchiae with a reduced stem and nephridial papillae with a fused tube retracted into the body. However, Fauvel (1927) and Hutchings and Glasby (1988) regarded that these differences were vague and not particularly useful in distinguishing *Neoamphitrite* from *Amphitrite*, and they considered *Neoamphitrite* to be a junior synonym of *Amphitrite*. Hessel's (1917) classification was re-accepted and currently the genus *Neoamphitrite* is recognized in recent taxonomic works (Hilbig 2000; Londoño-Mesa and Carrera-Parra 2005; Reuscher et al. 2012). Here, the definition of *Neoamphitrite*, as described by Hessel (1917), is followed.

To date, 12 species of *Neoamphitrite* are known (Hessel 1917; Fauvel 1927; Caullery 1944; Hutchings and Glasby 1988; Hilbig 2000; Londoño-Mesa and Carrera-Parra 2005; Reuscher et al. 2012). Among the described species, *N. edwardsi* (Quatrefages, 1865), *N. ramosissima* (Marenzeller, 1884), and *N. vigintipes* (Grube, 1870), have been recorded from East Asia (Hessel 1917; Imajima and Hartman 1964; Paik 1989). While studying polychaetes from Korean waters, a new species belonging to the genus *Neoamphitrite* was found in the deep sea off the eastern coast of South Korea. In this study, a detailed description and illustrations of the new species are provided, and molecular information pertaining to the barcoded regions of mitochondrial cytochrome c oxidase subunit I (COI) gene are included. This study also includes a taxonomic key to all *Neoamphitrite* species and is based on the literature (Hessel 1917; Fauvel 1927; Caullery 1944; Hutchings and Glasby 1988; Hilbig 2000; Londoño-Mesa and Carrera-Parra 2005; Reuscher et al. 2012).

## Materials and methods

### Sampling and morphological observation

Samples were collected from the benthos of the deep sea (500–1000 m depth). Specimens were sorted using sieves with a mesh size of 0.5 mm, initially fixed with 5% formaldehyde-seawater solution, and transferred to 85% ethyl alcohol. Characteristics of the whole body were observed with appendages dissected in a petri dish using dissection forceps or surgical knives and needles under a stereomicroscope (SMZ1500; Olympus, Tokyo, Japan). Dissected specimens were mounted onto temporary slides using glycerol or permanent slides using polyvinyl lactophenol solution. Drawings were made under the stereomicroscope and light microscope (LABOPHOT-2; Nikon, Tokyo, Japan) with the aid of drawing tubes. Photographs were taken of appendages mounted on a permanent slide. Images of appendages were captured using an imaging system (LAS V4.7, Leica Microsystems, Heerbrugg, Switzerland). Specimens for scanning electron microscopy (SEM) were dehydrated using a t-BuOH freeze dryer (VFD-21S; Vacuum Device, Ibaraki, Japan). They were mounted onto stubs and coated with gold-palladium. SEM observations were

conducted using a scanning electron microscope (SU3500; Hitachi, Tokyo, Japan). Type material and additional material examined were deposited at the National Institute of Biological Resources (NIBR) in Incheon, Korea and the National Marine Biodiversity Institute of Korea (MABIK) in Seocheon, Chungcheongnam-do, Korea, respectively.

## Molecular analysis

Genomic DNA was extracted from posterior segments of three specimens selected among additional materials using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. Amplifications of partial sequences of mitochondrial cytochrome c oxidase subunit I (COI) from gDNA were conducted by polymerase chain reaction (PCR) method using a set of primers: LCO 1490 5'-GGT-CAACAAATCATAAAGATATTGG-3' and HCO 2198 5'-TAAACTTCAGGGT-GACCAAAAAATCA-3' in COI amplification (Folmer et al. 1994). PCR amplification was conducted in a total volume of 20  $\mu$ L: 10  $\mu$ L of 2 $\times$  DyeMIX-Tenuto (Enzynomics), 0.5  $\mu$ L of each primer, 1  $\mu$ L of gDNA, and 8  $\mu$ L of sterile water. Touch-down-PCR was conducted according to the following cycling program: 94  $^{\circ}$ C for 5 min, 94  $^{\circ}$ C for 1 min, 50  $^{\circ}$ C for 1 min and 72  $^{\circ}$ C for 1 min, followed by 20 cycles at decreasing annealing temperatures in decrements of 0.5  $^{\circ}$ C per cycle, followed by 1 min. at 94  $^{\circ}$ C, 15 cycles of 1 min. at 40  $^{\circ}$ C, 1 min. at 72  $^{\circ}$ C, and final extension at 72  $^{\circ}$ C for 7 min. PCR products were purified with a QIAquick PCR Purification Kit (Qiagen, Chatsworth, CA, USA). Sequences for the new species were obtained with an Applied Biosystems 3730 DNA sequencer, and deposited in the GenBank under accession numbers MN306311 to MN306313. Sequences were aligned with those of other terebellid species and outgroup taxa using Geneious Pro v.9.1.8 (Biomatters, Auckland, New Zealand). Genetic distances between the new species and other species and the phylogenetic tree were produced by MEGA v.6.06 (Tamura et al. 2013).

## Systematic account

Family Terebellidae Johnston, 1846

Genus *Neoamphitrite* Hesse, 1917

**Type species.** *Amphitrite affinis* Malmgren, 1866 subsequently designated by Hesse (1917).

***Neoamphitrite undevigintipes* sp. nov.**

<http://zoobank.org/2CDD716F-CBAD-4B22-8A8A-3D51A9BB2322>

Figures 1–3

**Type locality.** South Korea, East Sea (Sea of Japan), 36 $^{\circ}$ 35'08.0"N, 130 $^{\circ}$ 08'19.7"E, 500–1000 m in depth.

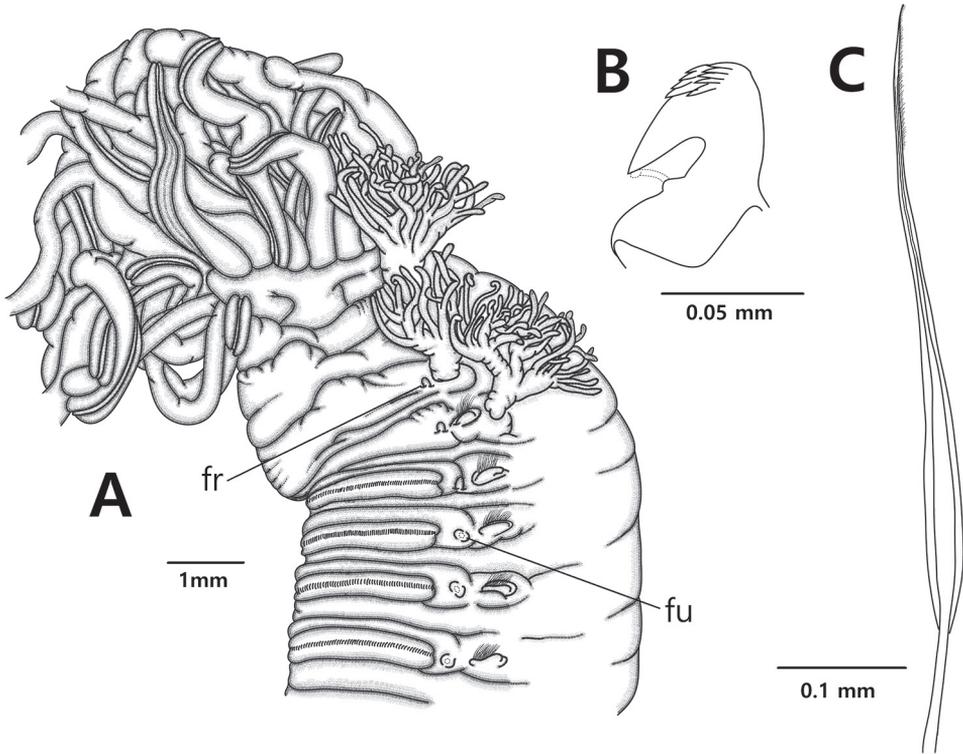


**Figure 1.** *Neoamphitrite undevigintipes* sp. nov., paratype (MABIKNA00156356), lateral view. Scale bar: 1.0 cm.

**Material examined. Holotype:** complete specimen (NIBRIV0000753905). **Paratypes:** one complete specimen (MABIKNA00156356); one complete specimen (MABIKNA00156357); one complete specimen (MABIKNA00156358); one complete specimen (MABIKNA00156359); one complete specimen (MABIKNA00156360). **Non-type material:** 16 specimens (all complete specimens). All materials examined were collected from the type locality, 13 April 2018 using the benthic trawl mounted on RV Tamgu 21 of National Institute of Fisheries Science (NIFS) from Korea.

**Diagnosis.** Body with distinct thoracic and abdominal region. Tentacular lobe collar-like. Peristomium with fleshy ridge on ventral side. Upper lip distinct and undulate with free margin. Lower lip well developed, projecting forward. Buccal tentacles filiform with ventral groove. Lateral lappets present on segments 2–4, well developed on segments 2 and 3, reduced on segment 4. 12 ventral shields from segment 3. Branchiae dichotomous with distinct stalk, 3 pairs, and present on segments 2–4. Nephridial papillae small, oval on segments 3–15, located between noto- and neuropodia. Notopodia present on 19 chaetigers on segment 4. Notochaetae medially winged and distally serrated. Neuropodia beginning at segment 5. Uncini avicular, short-handled, arranged in single row on segments 5–10, in double rows of beak to beak arrangement from segments 11–22, and in single row on all abdominal segments. Dental formula MF: 4–5: 5–6: 7–8. Pygidium reduced with 10 papillae.

**Description.** Holotype: complete, 11.0 cm long, 1.5 cm wide at segments 10, and with approximately 74 segments. Body uniformly light beige in alcohol, without



**Figure 2.** *Neoamphitrite undevigintipes* sp. nov. **A** holotype (NIBRIV0000753905) **B, C** paratype (MBIKI-NA00156359). **A** Anterior end, lateral view **B** uncinus **C** notochaeta. Scale bars: 1.0 mm (**A**), 0.05 mm (**B**), 0.1 mm (**C**). Abbreviations: **fr** – nephridial papillae with free tube, **fu** – nephridial papillae with fused tube.

pigmentation pattern, and consist of thorax with 19 chaetigers and abdomen; anterior thoracic segments compact until about 13 and then segments slightly narrower and longer than 13 anterior segments (Figs 1, 3A, B, E). Tentacular lobe short and collar-like. Peristomium with fleshy ridge on ventral side, separated anteriorly from lower lip by groove. Upper lip distinct and undulate with free margin. Lower lip well-developed, projecting forward. Buccal tentacles filiform with ventral groove. Lateral lappets paired on segments 2–4, distinct thickness flaps, protruding forwards, and with weakly developed glandular margin; first and second lappets well-developed, but third lappets reduced in length, located on nearby base of notopodia. Branchiae paired on segments 2–4, dichotomous, with 3 tiers of branches and weakly annulated stalk distinct. Nephridial papillae small, oval, present on segments 3–15, and located between noto- and neuropodia; those with fused tube retracted into body on segments 6–8 and other with free tube distinctly projecting from the body. Ventral shields trapezoidal, broader than longer, present on segments 3–14; first shield on segment 3 with glandular margin and others with smooth margin; thereafter shields replaced by mid-ventral groove extending to pygidium (Figs 2A, 3A, B, E). Notopodia short, rectangular, present on seg-

ments 4–22 (chaetigers 1–19); last 2 or 3 pairs becoming much shorter. Notochaetae slightly curved, medially winged and distally serrated, types of 2 lengths; chaetae on anterior row at least half as long as those on posterior row (Figs 2C, 3E–H). Neuro-podia beginning from segment 5 as low rectangular ridges, and with uncini arranged in single rows on segments 5–10 (chaetigers 2–7), uncini in double rows beak to beak arrangement on segments 11–22 (chaetigers 8–19), and in single row on all abdominal segments. Uncini avicular, short-handled with short triangular heel, distally pointed prow, minute dorsal button, and 5 rows of secondary teeth on main fang with subrostral guard. Dental formula MF: 4–5: 5–6: 7–8 (Figs 2B, 3C, D). Pygidium reduced with encircling 10 papillae.

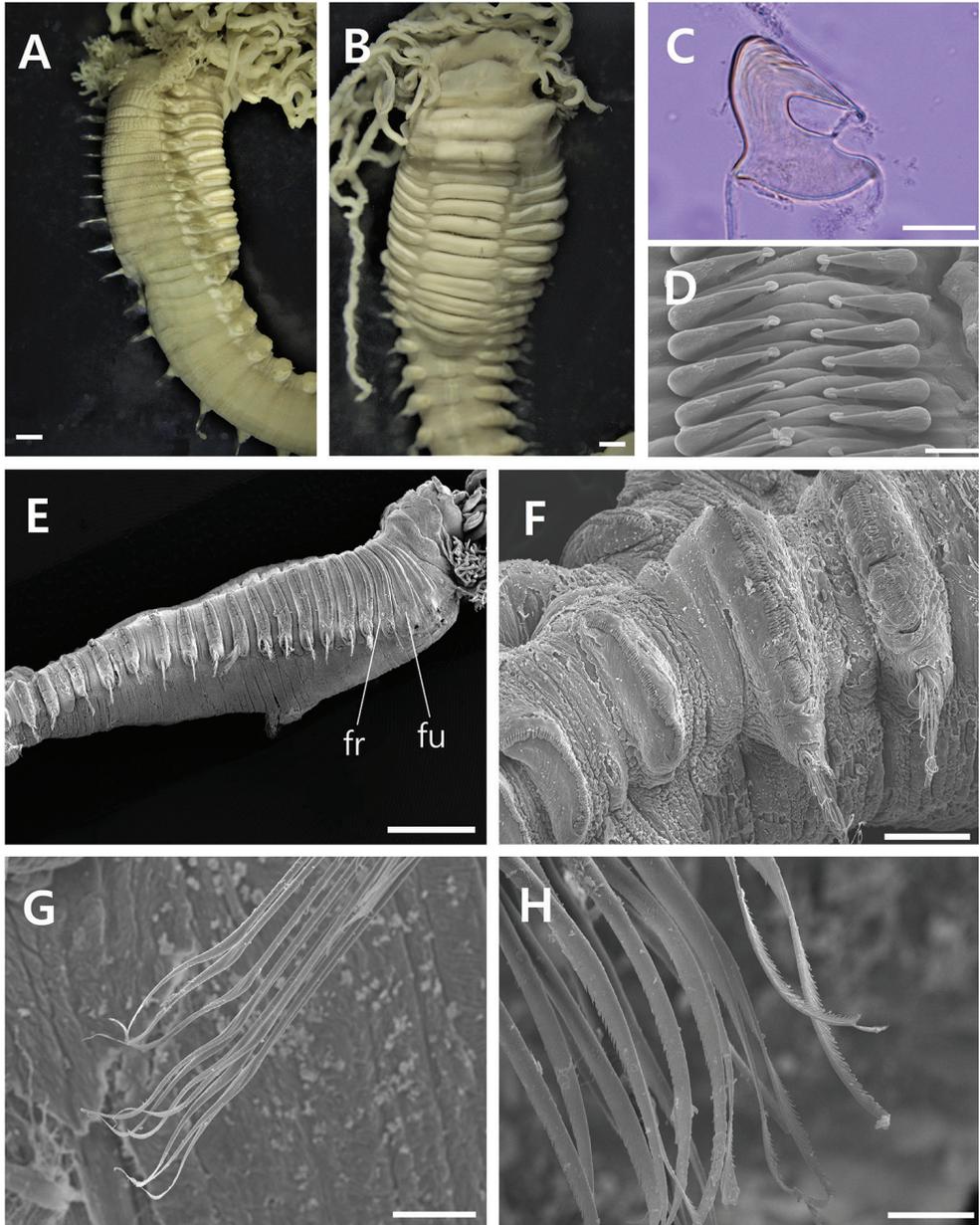
**Etymology.** A combination of the Latin *undeviginti* and *pes*. This name means ‘nineteen feet’, referring to the 19 pairs of notopodia on the thoracic segments.

**Habitat.** This species is found on the soft bottom of deep waters (500–1000 m depth) and lives in a mud tube.

**Remarks.** In *Neoamphitrite* taxonomy, the number of notopodia is a key character for the identification of species (Hutchings and Glasby 1988; Hilbig 2000; Londoño-Mesa and Carrera-Parra 2005; Reuscher et al. 2012). *Neoamphitrite undevigintipes* sp. nov. has 19 pairs of notopodia on the thoracic segments regardless of body size and number of segments. In this respect, the new species is most similar to *Neoamphitrite groenlandica* (Malmgren, 1866), which was originally described from the Atlantic Ocean and also has 19 pairs of notopodia (Malmgren 1866; Hessle 1917; Fauvel 1927). However, the new species is clearly differentiated from *N. groenlandica* by two characteristic features. The uncini in the first abdominal chaetiger are arranged in a single row in *N. undevigintipes* sp. nov., but in double rows in *N. groenlandica*, and the new species has 12 ventral shields, but *N. groenlandica* has 14 (Malmgren 1866; Hessle 1917; Fauvel 1927).

In East Asia, three *Neoamphitrite* species, *N. edwardsi* (Quatrefages, 1865), *N. ramosissima* (Marenzeller, 1884), and *N. vigintipes* (Grube, 1870) from Japan, have been recorded (Hessle 1917; Imajima and Hartman 1964; Paik 1989). *Neoamphitrite undevigintipes* sp. nov. shows several differences from these species as follows: notopodia of *N. undevigintipes* sp. nov. are present on 19 chaetigers, compared to 17 in *N. ramosissima* and *N. edwardsi*. *Neoamphitrite undevigintipes* sp. nov. has uncini arranged in a single row in all abdominal chaetigers, while *N. vigintipes* has the uncini arranged in double rows in abdominal chaetigers except for some final chaetigers. The new species has 13 pairs of nephridial papillae, whereas six, nine, and 12 pairs are present in *N. ramosissima*, *N. edwardsi*, and *N. vigintipes*, respectively (Hessle 1917; Imajima and Hartman 1964; Paik 1989).

Hessle (1917) suggested that *Neoamphitrite* species are distinguished from *Amphitrite* species by having nephridial papillae with free tubes distinctly projecting from the body rather than fused tubes retracted into the body. However, in several specimens of *N. undevigintipes* sp. nov., the nephridial papillae have fused tubes in two or three of all nephridial papillae pairs. Hutchings and Glasby (1988) mentioned that the form of nephridial papillae is difficult to use as a generic diagnostic feature because it can be variable according to the state of specimens. This character was overlooked in diagnoses of the terebellid genera (Fauvel 1927; Caullery 1944; Fauchald 1977; Hilbig 2000; Hutchings



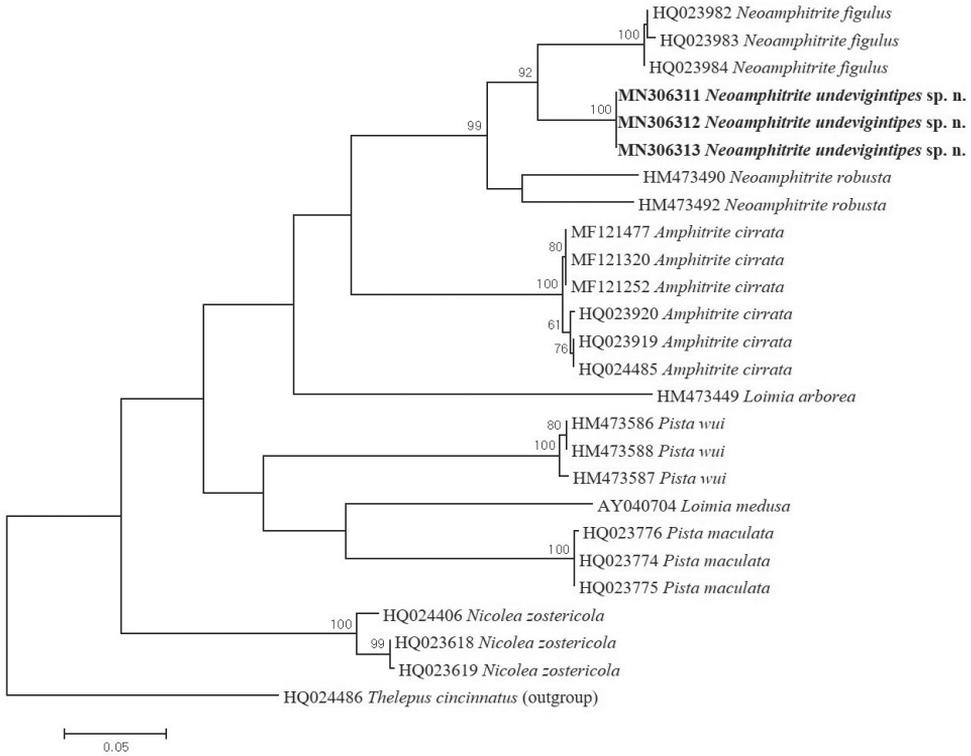
**Figure 3.** *Neoamphitrite undevigintipes* sp. nov. **A** paratype (MABIKNA00156357) **B** paratype (MABIKNA00156358) **C** (MABIKNA00156359) **D–H** (MABIKNA00156360). **A** Anterior end, lateral view **B** anterior end, ventral view **C** notopodial uncinus, lateral view **D** uncini arranged in double rows **E** thorax with 19 notopodia **F** last thoracic segment with uncini arranged in double rows and first abdominal segment with uncini arranged in single row **G** notochaetae **H** distal region of notochaetae. Scale bars: 1.0 mm (**A, B**), 0.025 mm (**C, D**), 5.0 mm (**E**), 0.5 mm (**F**), 0.1 mm (**G**), 0.025 mm (**H**). Abbreviations: **fr** – nephridial papillae with free tube, **fu** – nephridial papillae with fused tube.

and Glasby 1988; Londoño-Mesa and Carrera-Parra 2005; Reuscher et al. 2012). In this respect, we think that the form of nephridial papillae is not yet a useful diagnostic character and that its taxonomic value should be re-examined in detail using as many species as possible. We provide a key to the species presently regarded as members of *Neoamphitrite*.

**Genetic information.** In this study, partial COI sequences, each measuring 658 bp, were obtained from three specimens for genetic analysis of *Neoamphitrite undevigintipes* sp. nov. They are deposited in the GenBank under accession numbers MN306311 to MN306313. All COI sequences obtained were identical. Using data available from the GenBank (Carr et al. 2011; Siddall et al. 2011; Telfer and Dewaard 2017), we genetically compared the new species with two *Neoamphitrite* species, *N. figulus* (Dalyell, 1853) and *N. robusta* (Johnson, 1901), as well as six species belonging to the other terebelline genera: *Amphitrite cirrata* Müller, 1776, *Nicolea zostericola* Örsted, 1844, *Pista maculata* (Dalyell, 1853), *Pista wui* Safronova, 1988, *Loimia arborea* Moore, 1903, and *Loimia medusa* (Savigny, 1822). *Thelepus cincinnatus* (Fabricius, 1780) was used as the outgroup. GenBank accession numbers are represented in Table 1. Inter-specific genetic distances between the new species and two *Neoamphitrite* species, as measured by Kimura-2-parameter model, were distinct and ranged from 9.2 to 13.7%. The genetic distances between the new species and the six species in other genera ranged from 21.8 to 29.9%. In the maximum likelihood (ML) tree based on these genetic data (Fig. 4), all terebellid species showed specific validity. The new species was contained in a clade with *N. figulus* and *N. robusta*. At the generic level, the *Neoamphitrite* clade, including the new species, was closely related to *A. cirrata*, agreeing with the taxonomic view that *Neoamphitrite* and *Amphitrite* share many morphological features except for differences in the morphology of branchiae (Hessel 1917; Fauvel 1927; Hutchings and Glasby 1988; Reuscher et al. 2012). However, *Neoamphitrite* was monophyletic and clearly distinguishable from *A. cirrata* in the ML tree, supporting the known morphological differences between two genera (Hessel 1917; Fauvel 1927; Hutchings and Glasby 1988; Reuscher et al. 2012). Despite our results, further genetic studies with additional data and including more species of *Neoamphitrite* and *Amphitrite* are needed to confirm the phylogenetic relationship between the two genera.

**Table 1.** GenBank accession numbers for COI sequences obtained in the present study.

Species	Genbank accession number	Data source
<i>Neoamphitrite undevigintipes</i> sp. nov.	MN306311–MN306313	Present study
<i>Neoamphitrite figulus</i>	HQ023982–HQ023984	Carr et al. 2011
<i>Neoamphitrite robusta</i>	HM473490, HM473492	Carr et al. 2011
<i>Amphitrite cirrata</i>	HQ023919, HQ023920, HQ024485 MF121320, MF121320, MF121477	Carr et al. 2011 Telfer and Dewaard 2017
<i>Nicolea zostericola</i>	HQ023618, HQ023619, HQ024406	Carr et al. 2011
<i>Pista maculata</i>	HQ023774–HQ023776	Carr et al. 2011
<i>Pista wui</i>	HM473586–HM473588	Carr et al. 2011
<i>Loimia arborea</i>	HM473449	Carr et al. 2011
<i>Loimia medusa</i>	AY040704	Siddall et al. 2011
<i>Thelepus cincinnatus</i> (outgroup)	HQ024486	Carr et al. 2011



**Figure 4.** Maximum likelihood (ML) tree showing phylogenetic relationship based on COI sequences of three *Neoamphitrite* species with seven related species assigned to other genera and a outgroup species. Numbers above the branch indicate ML bootstrap values from 1000 replication.

### Key to known species of the genus *Neoamphitrite* Hesse, 1917

- 1 Notopodia present on first 15 chaetigers ..... *N. hydrothermalis* Reuscher, Fiege & Wehe, 2012
- Notopodia present on more than first 15 chaetigers ..... 2
- 2 Notopodia present on first 17 chaetigers ..... 3
- Notopodia present on more than first 17 chaetigers ..... 7
- 3 Thoracic neuropodial tori extending to ventral shield or mid-ventral groove ... 4
- Thoracic neuropodial tori not extended ..... 5
- 4 Nephridial papillae on segments 3–11 ..... *N. robusta* (Johnson, 1901)
- Nephridial papillae on segment 3 only ..... *N. sibogae* (Caullery, 1944)
- 5 Segments 4 with small lateral lappets ..... 6
- Segments 4 without lateral lappets ..... *N. ramosissima* (Marenzeller, 1884)
- 6 Nephridial papillae 6 pairs on segments 3–8 ... *N. affinis* (Malmgren, 1866)
- Nephridial papillae 9 pairs on segments 3–11 ..... *N. edwardsi* (Quatrefages, 1865)

- 7 Thorax with 39 notopodial chaetigers; lateral lappets on segment 2 inconspicuous..... *N. glasbyi* Londoño-Mesa & Carrera-Parra, 2005
- Thorax with less than 30 notopodial chaetigers; lateral lappets on segment 2 conspicuous ..... 8
- 8 Abdominal neuropodial tori with uncini arranged in double rows present on almost all chaetigers ..... *N. vigintipes* (Grube, 1870)
- Abdominal neuropodial tori with uncini arranged in double rows absent, or present on first and second abdominal chaetigers only ..... 9
- 9 Notopodia present on 19 thoracic chaetigers..... 10
- Notopodia present on more than 20 thoracic chaetigers..... 11
- 10 First abdominal chaetiger with uncini arranged in single row.....  
..... *N. undevigintipes* sp. nov.
- First abdominal chaetiger with uncini arranged in double rows.....  
..... *N. groenlandica* (Malmgren, 1866)
- 11 Nephridial papillae 7 pairs .... *N. pachyderma* (Hutchings & Glasby, 1988)
- Nephridial papillae at least 10 pairs..... 12
- 12 Ventral shields 13; nephridial papillae 10 pairs... *N. grayi* (Malmgren, 1866)
- Ventral shields 14; nephridial papillae 12 pairs..... *N. figulus* (Dalyell, 1853)

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# New data on the aphid (Hemiptera, Aphididae) fauna of New Caledonia: some new biosecurity threats in a biodiversity hotspot

## Nouvelles données sur la faune des Pucerons (Hemiptera, Aphididae) de Nouvelle-Calédonie: De nouvelles menaces biosécuritaires dans un hotspot de biodiversité

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### Abstract

Thirty-three species of aphids are now established in New Caledonia. All species appear to have been introduced accidentally by human activity in the last century. Here, 17 aphid species are recorded for the first time: *Aphis eugeniae*, *Aphis glycines*, *Aphis odinae*, *Aulacorthum solani*, *Brachycaudus helichrysi*, *Cerataphis orchidearum*, *Greenidea psidii*, *Hyperomyzus carduellinus*, *Hysteroneura setariae*, *Lipaphis pseudo-brassicae*, *Micromyzus katoi*, *Myzus ornatus*, *Pentalonia caladii*, *Rhopalosiphum nymphaeae*, *Rhopalosiphum rufiabdominale*, *Schizaphis rotundiventris*, and *Tetraneura fusiformis*. Thirteen more species are also more or less regularly intercepted at the borders through biosecurity surveys, without further establishment. This demonstrates that aphids represent a major biosecurity threat, including a threat as potential plant virus vectors. The reinforcement of biosecurity is a priority for such biodiversity hotspots, from the perspectives of both agriculture and the native environment. Prioritisation and promotion of local development

of vegetable and fruit production, rather than their risky importation from abroad, is desirable. Such an approach also should be promoted and extended to other Pacific islands, which all share the lack of native aphid fauna and their associated plant disease vector risks.

### Résumé

Trente trois (33) espèces de pucerons sont aujourd'hui recensées de Nouvelle-Calédonie. Toutes ces espèces sont exotiques et ont été introduites accidentellement par les activités humaines. Dix-sept (17) espèces y sont ainsi recensées pour la première fois : *Aphis eugeniae*, *Aphis glycines*, *Aphis odinae*, *Aulacorthum solani*, *Brachycaudus helichrysi*, *Cerataphis orchidearum*, *Greenidea psidii*, *Hyperomyzus carduellinus*, *Hysteroneura setariae*, *Lipaphis pseudobrassicae*, *Micromyzus katoi*, *Myzus ornatus*, *Pentalonia caladii*, *Rhopalosiphum nymphaeae*, *Rhopalosiphum rufiabdominale*, *Schizaphis rotundiventris* et *Tetraneura fusiformis*. Par ailleurs, au moins 13 autres espèces sont régulièrement interceptées par la biosécurité sans établissement actuel de populations. Les pucerons apparaissent donc comme une menace croissante pour la biosécurité de l'archipel. Aussi, le renforcement des mesures de biosécurité aux frontières apparaît prioritaire en association à une promotion du développement local de productions maraîchères et fruitières. Ainsi, la limitation de ces importations à risque, devrait contribuer à une meilleure protection des productions agricoles et de la biodiversité. Une telle approche devrait également être promue dans les pays insulaires du Pacifique, qui se caractérisent par la même disharmonie de peuplements, l'absence de communautés natives de pucerons et du risque associé de vectorisation de maladies phytopathogènes.

### Keywords

Aphids, biocontrol, biosecurity, invasive species, pests

### Mots-clefs

Biosécurité, espèces envahissantes, lutte biologique, pucerons, ravageurs

## Introduction

On a worldwide scale, aphids are currently represented by 5,558 valid species in 703 genera placed in 30 subfamilies (Favret 2018). Wegierek et al. (2017) state that aphids are known since the Permian, and appear more abundantly in the fossil records from the Early Cretaceous. But today, among this rich aphid fauna, only 250 species are considered as economically significant pests (Blackman and Eastop 2006; van Emden and Harrington 2007). Aphids are able to cause *direct damage* (through sap sucking and honeydew production) on all plant parts, and to cause *indirect damage* by transmission of plant viruses, which often has a greater impact on host plants. As they can be moved easily on commodities such as fresh fruits or ornamental plants, they are considered major quarantine insects on a world scale. Dissemination of exotic phytophagous insects among countries is an expected and significant side-effect of increased trade in fresh fruits, vegetables, and ornamental plants, and of tourist travel (Work et al. 2005; Hulme 2009). Establishment of exotic aphid species presents new threats to local agriculture (including the introduction of new plant viruses as aphids are well known as vectors). Adventive aphids also can result in significant restrictions in export trade (Batabyal and Beladi 2006; Dawson et al. 2017; Lohr et al. 2017; Turbelin et al. 2017). In this context, the continuation and expansion of international plant trade and human travel require sound and scientifically

based phytosanitary protocols. Associated phytosanitary protocols rely on accurate and up-to-date pest species checklists, which are also essential for pest control research programs, especially for integrated pest management (IPM). Such lists also provide tools for biosecurity policies and managers (Charles and Henderson 2002; Beauvais et al. 2006). This is a particularly major issue for islands, where such introductions may have higher impacts and more serious ecological consequences, as they are depauperate of such pests and often have vacant ecological niches. The last list of aphids from New Caledonia was published in 1986 (Brun and Chazeau 1986) and an update was published by Jourdan and Mille (2006). The present checklist accounts for all encountered aphid species as well as known interceptions on fresh imported fruits and vegetables.

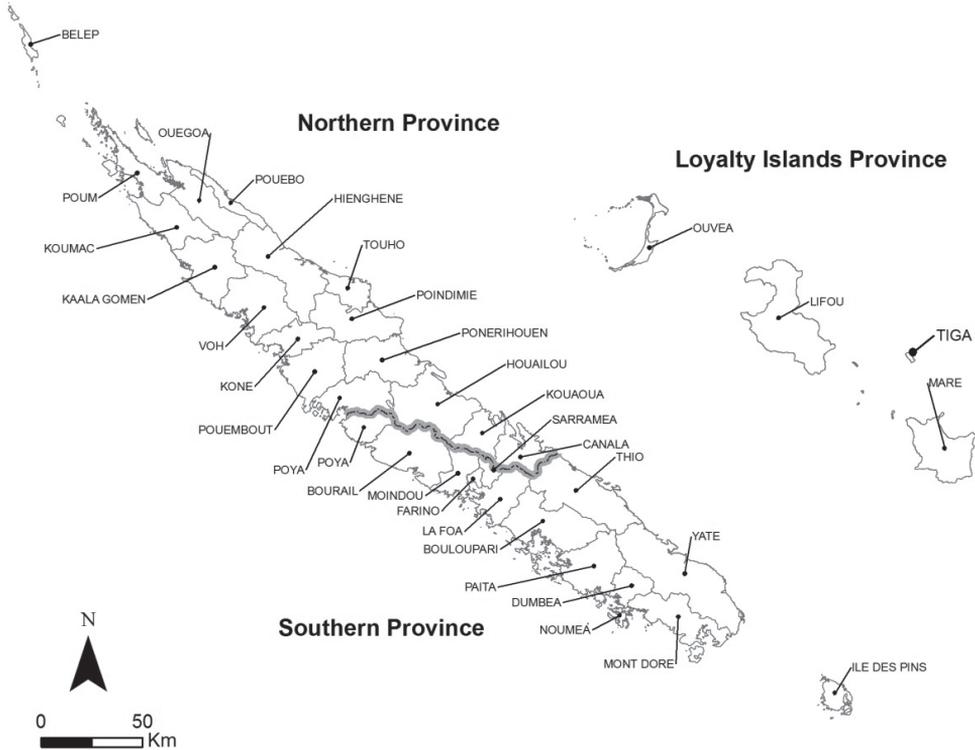
## Materials and methods

The essential data of this work were compiled from scattered scientific literature and checklists, and from studies of curated specimens in the Collection de Référence des Invertébrés Terrestres de Nouvelle-Calédonie – Xavier Montrouzier, **CXMNC** (New Caledonia Terrestrial Invertebrate Reference Collection – Xavier Montrouzier), hosted at the Institut Agronomique néo-Calédonien (**IAC**, New Caledonian Agronomic Institute), and the identification of intercepted species to update the present list. Identifications were generally achieved by the late Mrs. Rosa C. Henderson (New Zealand Arthropod Collection, **NZAC**, Landcare Research, Auckland, New Zealand) and Mr. Eric Maw (Canadian National Collection of Insects, Arachnids & Nematodes, **CNC**, Canadian National Collection of Insects, Agriculture & Agri-Food Canada, Ottawa, Ontario, Canada). This updated and annotated checklist summarizes all recorded species from New Caledonia including the main island (Grande Terre) and adjacent inhabited islands; the Loyalty Islands (Lifu, Ouvéa, Maré, and Tiga), Belep Archipelago, and Isles of Pines (Figure 1). Currently valid species names are listed alphabetically, and subfamily and tribe are noted below each species. For each species, the original name of description, with author and year of description, are given. General geographic distributions are taken from the literature. Full synonymies are available on the Aphid Species File (Favret 2018). Literature records of the species in New Caledonia, host plants from literature records and local observations, biological control agents recorded in New Caledonia and observations on local distributions and economic importance are given.

**Abbreviations used:** coll. collector, det. determiner, dep. depositories.

## Results

Five tribes within four subfamilies are represented in New Caledonia: Aphidinae (Aphidini and Macrosiphini), Greenideinae (Greenideini), Hormaphidinae (Cerataphidini), and Eriosomatinae (Eriosomatini). Species names preceded by an asterisk are new records for New Caledonia.



**Figure 1.** Map of New Caledonia showing administrative delimitations (provinces and counties).

## Current alphabetical aphid species list of New Caledonia

### Aphidinae

#### Aphidini

#### *Aphis (Toxoptera) aurantii* Boyer de Fonscolombe, 1841

Black Citrus Aphid, Camelia Aphid, Puceron noir des Agrumes.

**Material examined.** On *Citrus* sp. (Rutaceae), in June 2000, R.C. Henderson det. (NZAC); La Foa County (IAC-SRFP), on leaves of *Citrus* sp. and same loc. on leaves of *Eugenia* sp. (Myrtaceae), 14.V.2003, S. Cazères coll., R.C. Henderson det. (NZAC); Sarraméa County (Réserve du Col d'Amieu) on unknown plant with orange and red young leaves, 18.X.2006, S. Cazères coll., R.C. Henderson det. (NZAC), dep. CXMNC; Tribe of Moméa, Moindou County, on unknown plant, 12.IV.2012, S. Cazères coll., E. Maw det. (CNC), dep. CXMNC; Yaté County (South of the Grande Terre) in Vale-Inco Plant Nursery, 21.IV.2016 on young plant of *Dodonea viscosa* (L.) Jacq. (Sapindaceae), R.-M. M'Bouéri & C. Martin coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** This species was first recorded by Cohic (1956) and Brun and Chazeau (1986) on *Citrus* spp.

It is distributed throughout the tropics and subtropics including Pacific islands, as well as in glasshouses in temperate climates (CABI 2019). This species is particularly important on citrus, cacao, coffee and tea, but also on sugar apple, fig, mango, ornamentals and some native plants (Blackman and Eastop 2000). Larval and adult ladybirds (Coccinellidae) such as *Coccinella transversalis* (Fabricius, 1781) or *Menochilus sexmaculatus* (Fabricius, 1781), both present in New Caledonia (Nattier et al. 2015), are known to feed on the species (Agarwala and Ghosh 1988; Roy and Rahman 2014). Aphidiinae wasps (Braconidae) like *Aphidius colemani* Vierek, 1912 present in New Caledonia can also parasitize this aphid (Kavallieratos et al. 2004).

This species is considered as a vector for the Citrus tristeza virus (CTV), but it is not a particularly efficient one. As regulatory measures already cover the protection of citrus, a strong surveillance is needed to prevent *Aphis* (*Toxoptera*) *citricidus* (Kirkaldy, 1907), the Tropical Citrus Aphid, from becoming established, as it is present in all Oceania around New Caledonia (CABI 2019). With *Aphis gossypii* (see below), *A. citricidus* is the most efficient vector of CTV (O'Connor 1969; Vogel 1978). The introduction of *A. citricidus* could compromise the ongoing eradication of CTV, which fortunately has not yet become pandemic in New Caledonia (Stéphane Lebegin, pers. comm. 13 January 2014). CTV was mostly spread by grafting of Washington Navel oranges (François Mademba-Sy, pers. comm. 7 March 2014), a cultivar imported with the pathogen from Australia during the late 1960's. Most of the infected scions, with or without symptoms, which were distributed to the orchardists have now been destroyed, and CTV is considered as almost eradicated from New Caledonia. This statement is of course important for the New Caledonian citrus industry. But it is also significant for the conservation of biodiversity, as New Caledonia possess some early *Citrus* taxa (Bayer et al. 2009; Wu et al. 2018). The failure of the establishment of the CTV in New Caledonia could be explained by the presence of a "mild strain" of the virus, as suggested by some authors (e.g., Lee and Keremane 2013) and more likely to the absence of *A. citricidus*.

### ***Aphis craccivora* Koch, 1854**

Groundnut Aphid, Puceron noir de la Luzerne.

**Material examined.** Tribe of Mucaweng, Lifu County (Loyalty Islands), 14.IV.2010 on an unknown leguminous plant in a forestry garden, H. Jourdan coll., dep. CXMNC; Pouembout County, 18.IV.2012 on *Solanum nigrum*, C. Mille coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** This aphid was first recorded in New Caledonia as '*Aphis dolichis* Montrouzier, 1861'. Montrouzier (1861, p 74) found it in Lifu Island on a Fabaceae, a *Dolichos* which turned out to be *Vigna unguiculata* (L.) Walp., introduced from China. '*Aphis dolichis*' was then synonymized with *A. craccivora* (Renaudière and Renaudière 1997). Bordat and Daly (1995) recorded this species from New Caledonia. An *Aphis* species was mentioned in Cohic (1956) and Brun and Chazeau's (1986) catalogue which was probably in part *A. craccivora* based on the host plant species given: straw-

berry (*Fragaria vesca* L.), tomatoes (*Solanum lycopersicum* L.), beans (*Phaseolus* spp.), garden peas (*Pisum sativum* L.), eggplants (*Solanum melongena* L.) and wheat (*Triticum aestivum* L.). *Aphis craccivora* was also recently found on the European black nightshade, *Solanum nigrum* L. (Solanaceae).

Common in warm temperate and tropical regions, this highly polyphagous species can colonise young growths of numerous plants, mainly on Fabaceae, and including occasional records on Poaceae. It can be also found living on Araceae, Amaranthaceae, Asteraceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Convolvulaceae, Cucurbitaceae, Cupressaceae, Ebenaceae, Euphorbiaceae, Lamiaceae, Lauraceae, Liliaceae, Malpighiaceae, Malvaceae, Moringaceae, Myrtaceae, Nyctaginaceae, Oleaceae, Orchidaceae, Pedaliaceae, Rubiaceae, Rutaceae, Sterculiaceae, and Zingiberaceae (CABI 2018).

Several natural enemies can control this species which is preyed by larvae and adults of various ladybirds (Coccinellidae) of which *Coccinella transversalis*, *Harmonia octomaculata* (Fabricius, 1850) and *Menochilus sexmaculatus* (Agarwala and Ghosh 1988; Sarma et al. 1996), present in New Caledonia (Nattier et al. 2015). This aphid is also known to be preyed by larvae of hoverflies (Syrphidae), especially *Ischiodon scutellaris* (Fabricius, 1805) (Sarma et al. 1996) and *Melanostoma univittatum* (Wiedemann, 1824) both present in New Caledonia (Hull 1937). *Aphis craccivora* is also known to be parasitized by aphidiine wasps (Braconidae) probably *Aphidius colemani*, which is the only species known to be present in New Caledonia, and is found on several aphid species (Starý 1975).

This species is a known vector of more than 30 plant viruses (Blackman and Eastop 1984) and must therefore be regarded as an important threat to New Caledonian crops.

### \**Aphis eugeniae* van der Goot, 1917

**Material examined.** Mont-Dore County, Saint-Louis in IAC-SRMH, 23.II.2013, under the leaves of *Glochidion billardieri* (Baill.) Müll. Arg. (Myrtaceae), G. Karnadi coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** Originating from Southeast Asia, it is recorded eastward to Pakistan (Naumann-Etienne and Remaudière 1995), it is also known from Australia (Eastop 1966), Florida in 2011 (Skvarla et al. 2017) and Hawai'i (on Apocynaceae and Rosaceae; Foottit et al. 2012). It occurs most commonly on woody Euphorbiaceae, e.g., *Glochidion*, but has been recorded from plants in at least six other families (Blackman and Eastop 2006, 2020).

### \**Aphis glycines* Matsumura, 1917

Soybean Aphid, Puceron du Soja.

**Material examined.** Boulouparis County (La Ouenghi) in an Adecal Technopole experimental plot, 23.II.2012, on *Glycine max* (L.) Merr. (Soybean, Fabaceae), S. Cazères & C. Mille coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** Originating from Asia, this almost cosmopolitan species is now present in the USA (Voegtlin et al. 2004), in Canada and in eastern Australia since 2000 (M. J. Fletcher, pers. comm. 2000). It is mainly on soybean and wild *Glycine* spp. and other Fabaceae.

Some ladybirds, of which *Harmonia octomaculata* and/or *Coccinella transversalis*, appear to be very active against this aphid (personal observation in September 2012 by one of us, CM).

### *Aphis gossypii* Glover, 1877

Cotton Aphid, Melon Aphid, Puceron du Cotonnier.

**Material examined.** La Foa County (IAC-SRFP), on leaves of *Citrus* sp. 22.IV.2003, S. Cazères coll., R.C. Henderson det. (NZAC); same loc. on young leaves of *Psidium guajava* L. (Myrtaceae), 1.IV.2004, S. Cazères coll., R.C. Henderson det. (NZAC); same loc. on *Cucurbita pepo* L. (Cucurbitaceae), 2.VIII.2006, J. Marin coll., R.C. Henderson det. (NZAC); same loc. on leaves of *Euphorbia hirta* L. (Euphorbiaceae), 29.XI.2008, C. Mille coll., R.C. Henderson det. (NZAC); Nouméa County, on unknown plant (round leaves), 21.I.2009, J. Marin coll., Rosa Henderson det. (NZAC); Nouméa County (Ouémo), on *Ocimum basilicum* L. (Lamiaceae), 16.VI.12, M. Cazères coll., E. Maw det. (CNC), dep. CXMNC; Pouembout County, on Cucurbitaceae, 11.VII.2013, C. Mille coll., E. Maw det. (CNC); Ouégoa County (North of the Grande Terre), on *Colocasia esculenta* (L.) Schott leaves, 17.I.2014, E. Kastavi coll., E. Maw det. (CNC); La Foa County (Fonwhary), on *C. esculenta* leaves, 20.I.2014, L. Nemebreux coll., same loc. and same plant, 23.I.2013, S. Cazères & J. Brinon coll., E. Maw det. (CNC); Nouméa County (Ouémo), on *O. basilicum* leaves, 15.VI.2015, H. Jourdan coll., E. Maw det. (CNC); La Foa County (IAC-SRAP), on *O. basilicum* leaves, 3.VIII.2015, L. Marchal coll., E. Maw det. (CNC); Yaté County (South of the Grande Terre) in Vale-Inco Plant Nursery, on *Myodocarpus fraxinifolius* Brongn. & Gris (Myodocarpaceae), *Hibbertia pancheri* (Pancher & Sebert) Briquet (Dilleniaceae) and young plants of *Tarenna* sp. (Rubiaceae), 10.IX.2015, C. Mille coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** Brun and Chazeau (1986) first recorded this species in New Caledonia. This highly polyphagous species is mainly found on Cucurbitaceae, Rutaceae, and Malvaceae. In addition to the plants given above, there are New Caledonian records from Apiaceae (*Apium graveolens* L., *Daucus carota* L.), Apocynaceae (*Catharanthus roseus* (L.) G. Don), Araceae (*Alocasia macrorrhizos* (L.) G. Don, *Caladium bicolor* (Aiton) Vent., *Xanthosoma sagittifolium* (L.) Schott), Asteraceae (*Dahlia* spp., *Leucanthemum vulgare* Lam.), Cucurbitaceae (*Citrullus lanatus* (Thunb.) Matsumura and Nakai, *Cucumis* spp., *Sechium edule* (Jacq.) Swartz), Malvaceae (*Gossypium* spp., *Hibiscus rosa-sinensis* L.) and Rutaceae (*Citrus* spp.) (Brun and Chazeau 1986).

This cosmopolitan species is very common in the tropics and the Pacific region (Blackman and Eastop 2007). It is a major pest of cotton and cucurbits. *Aphis gossypii*

transmits at least 76 plant viruses (Chan et al. 1991). Its natural enemies are larvae and adults of ladybirds (Coccinellidae) such as *Menochilus sexmaculatus* and *Coccinella transversalis* (Agarwala and Ghosh 1988). The ladybird *Diomus notescens* (Blackburn, 1888) is known to prey on *Aphis gossypii* (Hopkinson et al. 2016) and also by *Micraspis frenata* (Erichson, 1842) and *Coelophora inaequalis* (Fabricius, 1775), all three being present in New Caledonia (Nattier et al. 2015). It can be also controlled by hoverfly larvae (Syrphidae) and Aphidiinae wasps (Braconidae), especially *Aphidius colemani*.

This species regularly causes local heavy damage on various cultivated plants in New Caledonia and is therefore the most important pest aphid species in the country. This is also the main aphid species regularly surveyed for virus transmission in New Caledonia. *Aphis gossypii* also is considered as a good CTV vector (Cambra et al. 2000) although its efficiency is estimated between 6 and 25 times less effective than *Aphis citricidus* (Halbert and Brown 1998). Thus, the occurrence of *Aphis gossypii* and *A. aurantii* in New Caledonia poses an important threat for New Caledonian citrus crops which represent 53% of all perennial fruit species grown in the country (Anonymous 2010), and to the ongoing CTV eradication program. However, preventing the establishment of *Aphis citricidus* (see above, under *A. aurantii*) is the most important issue with regards to spread of CTV.

### ***Aphis nerii* Boyer de Fonscolombe, 1841**

Oleander Aphid, Puceron du Laurier rose.

**Material examined.** Farino County, 29.IV.2004 on *Asclepias physocarpus* Schlechter (Apocynaceae), S. Cazères coll., R.C. Henderson det. (NZAC).

**Remarks.** Cohic (1956) first recorded this species in New Caledonia, and Brun and Chazeau (1986) found it on the Tropical Milkweed, *Asclepias curassavica* L. It was also collected on *Asclepias physocarpus* by one of us (SC).

Widely distributed through the tropical to warm temperate regions or subtropical areas including many Pacific islands. Its main hosts are Apocynaceae, especially *Nerium oleander* L., but it can also be found on Asteraceae, Convolvulaceae, Euphorbiaceae and Solanaceae. In Florida, it is occasionally observed on citrus (Rutaceae) without any damage (S. Halbert, pers. comm. 10 December 2019).

As for other aphids, predatory insects such as larvae and adults of the ladybirds (Coccinellidae) *Menochilus sexmaculatus* (Agarwala and Ghosh 1988), the hoverfly larvae of *Ischiodon scutellaris* (Syrphidae) and lacewings of which two the two widespread species *Eumicromus tasmaniae* (Walker, 1860) and *Mallada basalis* (Walker, 1853) (respectively Hemerobiidae and Chrysopidae) can control the populations in New Caledonia. *Aphidius colemani* wasps (Aphidiinae, Braconidae) are also known to parasitize the colonies of this aphid (Messing and Rabasse 1995).

The Oleander Aphid is able to transmit several viruses including SMV and PRSV which are respectively the Sugarcane mosaic potyvirus and the Papaya ringspot poty-

virus (McAuslane 2017). However, the main concern with this species is its large and unsightly outbreaks on milkweeds. The damage caused by its colonies is mainly aesthetic due to the large amounts of sooty mould produced on plants.

**\**Aphis odinae* (van der Goot, 1917)**

Mango Aphid, Puceron du Manguier.

**Material examined.** On *Schinus terebinthifolius* Raddi (Anacardiaceae), 9.IX.2011, C. Mille coll., E. Maw det. (CNC), dep. CXMNC; Nouméa County 4.XI.2015, on *Mangifera indica* L. (Anacardiaceae), F. Gimat coll., E. Maw det. (CNC).

**Remarks.** This species feeds on the undersides of leaves along main veins in dense colonies, attended by ants. It is commonly observed throughout the Old World tropics and subtropics on numerous plant species especially of the families Anacardiaceae, Araliaceae, Caprifoliaceae, Ericaceae, Rubiaceae, and Rutaceae. *Aphis odinae* is commonly grey-brown to rust-brown in colour, especially in Old World Tropics and in subtropics (Blackman et al. 2011) such as in New Caledonia. However, some much darker forms occur in Asia and a dark green form is found in Japan (Blackman et al. 2011). It has not yet been implicated in the transmission of any plant virus (Blackman and Eastop 1984).

***Aphis spiraeicola* Patch, 1914**

Spirea Aphid, Green Citrus Aphid, Puceron des Spirées.

**Material examined.** Mont-Dore County (Saint-Louis) in IAC-SRA, 3.VIII.2004 on *Pittosporum coccineum* (Montrouz.) Beauvis. (Pittosporaceae), G. Gâteblé coll., R.C. Henderson det. (NZAC), dep. CXMNC; same loc. 31.I.2006 on *Artia balansae* (Baill.) Pichon (Apocynaceae), G. Gâteblé coll., R.C. Henderson det. (NZAC); same loc. 7.VIII.2013 on *Ixora cauliflora* Montrouz. (Rubiaceae), E. Maw det. (CNC).

**Remarks.** This polyphagous species was first recorded in New Caledonia on *Citrus* spp. by Jourdan and Mille (2006). It is recorded from Araceae, Araliaceae, Convolvulaceae, Fabaceae, Lythraceae, Magnoliaceae, Nyctaginaceae, Rutaceae, Solanaceae and Verbenaceae, but occurs especially on Asteraceae, Caprifoliaceae, and Rosaceae. It has an almost cosmopolitan distribution.

Most predatory insects of this aphid are the adults and larvae of the ladybird *Harmonia octomaculata* (Coccinellidae) and the larvae of the hoverfly *Ischiodon scutellaris* (Syrphidae). Also, *Aphidius colemani* (Braconidae, Aphidiinae) parasitizes this aphid (Tomanović et al. 2009).

In many countries, the Green Citrus Aphid is the most damaging species to the citrus fruit industry. In addition to direct damage and the production of honeydew,

which favors the development of sooty moulds, this pest constitutes also a potential vector of the CTV (Kalaitzaki et al. 2019).

## Hormaphidinae

### Cerataphidini

#### *Astegopteryx bambusae* (Buckton, 1893)

Bamboo leaf Aphid, Puceron des feuilles du Bambou.

**Remarks.** This species was first recorded in New Caledonia by Dr Paul Cochereau in the 60's on *Bambusa* spp. (Brun and Chazeau 1986). *Astegopteryx bambusae* occurs throughout East and South-East Asia, generally colonising the undersides of the leaves of bamboos.

Known natural enemies are the coccinellids *Anisolemnia dilatata* (Fabricius) and *Synonycha grandis* (Thunberg), both absent from New Caledonia (Nattier et al. 2015).

## Aphidinae

### Macrosiphini

#### \**Aulacorthum solani* (Kaltenbach, 1843)

Foxglove Aphid, Puceron à taches vertes de la Pomme de terre.

**Material examined.** La Foa County (IAC-SRFP), 11.V.2007 on leaves and fruits of *Capsicum annuum* L. (Solanaceae), P. Caplong coll.; Poindimié County (Wagap), 17.VII.2007 on *Ipomoea batatas* (L.) Lam. (Convolvulaceae), D. Varin coll., both identified by R.C. Henderson det. (NZAC), dep. CXMNC.

**Remarks.** This cosmopolitan and very polyphagous aphid is present on many different families of plants and it is a common pest in glasshouses. *Aulacorthum solani* transmits at least 45 plant viruses (Chan et al. 1991). Further investigations of this species in New Caledonia are needed with respect to plant virus transmission.

#### \**Brachycaudus helichrysi* (Kaltenbach, 1843)

Leaf-curling Plum Aphid, Puceron vert du Prunier.

**Material examined.** La Foa County (IAC-SRFP), 16.VI.2015 on leaves of *Ageratum conyzoides* L. (Asteraceae), S. Cazères coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** Today, this species is globally distributed. Its primary hosts are *Prunus* spp. (Rosaceae), and its secondary hosts are numerous species of Asteraceae, Boraginaceae and sometimes Fabaceae, as well as many ornamental plants.

The only ladybird cited to feed on this aphid and present in New Caledonia is *Menochilus sexmaculatus* (Agarwala and Ghosh 1988). Regarding syrphid flies, *Melanostoma univittatum*, present in New Caledonia (Hull 1937), is known to prey on this aphid. This aphid is also parasitized by *Aphidius colemani*, also present in New Caledonia (Starý 1975).

It is involved in the transmission of several plant viruses, including the Cucumber mosaic virus (Blackman and Eastop 1984).

### ***Brevicoryne brassicae* (Linnaeus, 1758)**

Cabbage Aphid, Puceron cendré du Chou.

**Remarks.** Restricted to the members of the Brassicaceae, Cohic (1956) and Brun and Chazeau (1986) recorded this species in New Caledonia on cabbage (*Brassica* spp.) and on radish (*Raphanus sativus* L.). This species is distributed in all temperate and warm parts of the world.

As predators of this aphid species, Agarwala and Ghosh (1988) cite several ladybird species but the only one present in New Caledonia is *Coccinella transversalis* (Coccinellidae). Joshi and Ballal (2013) indicate that *Ischiodon scutellaris* is a good predator of this aphid. *Diaeretiella rapae* (M'Intosh, 1855) (Braconidae, Aphidiinae) is known to parasitize *B. brassicae* but is absent from New Caledonia (Lopez et al. 2016); this beneficial species could be a good candidate to enhance the biological control of this aphid.

It is an important pest on Brassicaceae and has been involved in the transmission of at least 20 plant viruses (Blackman and Eastop 1984).

### ***Capitophorus elaeagni* (del Guercio, 1894)**

Artichoke Aphid, Puceron vert de l'Artichaut.

**Remarks.** Brun and Chazeau (1986) first recorded this species in New Caledonia on the Artichoke thistle (*Cynara cardunculus* L.) and on the Barberton daisy (*Gerbera* spp.), both Asteraceae. Widely distributed through the temperate and warm temperate regions of the world (Blackman and Eastop 2000). The populations are mainly on undersides of leaves, but some are also observed on upper sides of young leaves. It is also reported on Polygonaceae.

Some entomopathogenic fungi can limit the importance of the colonies (Jouda et al. 2010).

## Hormaphidinae

### Cerataphidini

#### *Cerataphis lataniae* (Boisduval, 1867)

Latania Aphid, Puceron du Latanier.

**Remarks.** Brun and Chazeau (1986) first recorded this species in New Caledonia only on palms of *Cocos nucifera* (Arecaceae). Outside of tropical regions, *Cerataphis lataniae* is known in most of Europe (*Areca* and *Musa* spp., respectively Arecaceae and Musaceae), in Asia region, in North, Central and South America and Oceania. It appears to be widespread on palms, especially *Latania* spp. and other fan-palms, *Raphia* spp. and the coconut tree, through the tropics and in glasshouses. Pérez Hidalgo et al. (2000) signal this species as introduced in the Canary Isles, where it also colonizes *Strelitzia alba* (L.f.) Skeels (Strelitziaceae).

This species is not a major phytosanitary problem, but it has spread globally.

However, there is much confusion in the literature between this species and its close relative *Cerataphis brasiliensis* (Hempel, 1901), which is also widely distributed and colonises various palms including coconuts, so the identity of the species in New Caledonia needs further verification.

#### \**Cerataphis orchidearum* (Westwood, 1879)

Orchid Aphid, Puceron des Orchidées.

**Material examined.** Lifu Island County (Loyalty Islands), 23.III.2012 on *Vanilla* sp. (Orchidaceae), J-P. Lolo coll., E. Maw det. (CNC), dep. CXMNC; Maré Island County (Loyalty Islands), 6.VIII.2012 on *Vanilla* sp., C. Mille coll., det. S. Cazères, dep. CXMNC; Maré Island County (Loyalty Islands), 3.IV.2013 on *Vanilla* sp., J. Drouin coll., det. S. Cazères, dep. CXMNC.

**Remarks.** This pantropical species is found on various Orchidaceae in the tropics, and in European and North American glasshouses.

## Greenideinae

### Greenideini

#### \**Greenidea psidii* van der Goot, 1917

Asian Guava Aphid, Puceron asiatique du Goyavier.

**Material examined.** Tribe of Moméa, Moindou County, 12.IV.2012 by beating an unknown myrtle plant (Myrtaceae), S. Cazères coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** This aphid is an invasive pest that feeds on young shoots and undersides of young leaves of ecologically and economically important plants of the family Myrtaceae: *Psidium guajava* L., *Rhodomyrtus* spp., *Eugenia* spp., *Melaleuca* spp., *Plinia* spp. Originating from the Indo-Asian region, this species is now widely distributed in temperate and tropical regions, including Australia in the vicinity of New Caledonia (Blackman and Eastop 1994). It is also reported in Hawai'i (Beardsley 1993).

Potential natural enemies of this invasive aphid include Chrysopidae, Coccinellidae and Braconidae (Culik et al. 2016).

## Aphidinae

### Macrosiphini

#### *\*Hyperomyzus carduellinus* (Theobald, 1915)

Asian Sowthistle Aphid.

**Material examined.** Tribe of Hnae, Tiga (Loyalty Islands), 11.IV.2017, collected on *Sonchus oleraceus* L. (Asteraceae) in a garden, R.-M. M'Bouéri coll., E. Maw det. (CNC).

**Remarks.** This species is widely distributed in warm temperate and subtropical parts of the world including Australia, Fiji Islands and Hawai'i, and colonises many genera of Asteraceae.

The fungus *Pandora neoaphidis* is known to infect up to 70% of *Hyperomyzus carduellinus* populations in Argentina (Manfrino et al. 2013) but is absent from New Caledonia.

#### *Hyperomyzus lactucae* (Linnaeus, 1758)

Currant-sowthistle Aphid, Puceron des feuilles du Groseillier et de la Laitue.

**Remarks.** Brun and Chazeau (1986) first recorded this species in New Caledonia on *Sonchus* spp., (Asteraceae). It is now distributed all over the world except Southern Africa, feeding on new shoots and undersides of young leaves of *Sonchus* spp., which curl slightly and show yellow spots. It is occasionally found on other Asteraceae.

The ladybird *Coelophora mulsanti* is known to prey on this aphid in New Caledonia (Sallée and Chazeau 1985). There are no New Caledonian records of hoverflies (Diptera, Syrphidae) or hymenopterous parasitoids attacking this aphid.

It is the vector of approximately 12 non-persistent viruses (Boakye and Randles 1974).

## Aphidinae

### Aphidini

#### \**Hysteroneura setariae* (Thomas, 1878)

Rusty Plum Aphid, Puceron brun du Prunier.

**Material examined.** Nouméa County in PANC (Port Autonome de Nouvelle-Calédonie), 19.V.2015, on *Paspalum digitatum* (Sw.) Kunth (Poaceae), F. Gimat coll., E. Maw det. (CNC), dep. CXMNC; same loc. on an unknown plant, 7.III.2016, L. Sariman coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** Detected on *Paspalum digitatum* in large numbers in New Caledonia, elsewhere it is also known on many other Poaceae species such as rice (*Oryza sativa* L.), sugarcane (*Saccharum officinarum* L.), *Sorghum* spp. and some species of Cyperaceae. It is native of North America but is now distributed in many countries and regions of the world after a rapid spread as in Europe (Coeur d’acier et al. 2010). It is also present in regions near New Caledonia, such as Australia, Fiji Islands, Papua New Guinea, Solomon Islands, and Indonesia (Nasruddin 2013).

If this newly arrived species becomes a pest, larvae and adults of ladybirds (Coccinellidae) present in New Caledonia such as *Coccinella transversalis* or *Menochilus sexmaculatus* (Nattier et al. 2015) are known to feed on this species and are used in banker plant systems to maintain some predator populations near to crops for protection (Rattanapun 2017).

## Aphidinae

### Macrosiphini

#### \**Lipaphis pseudobrassicae* (Davis, 1914)

Turnip Aphid, Puceron de la Moutarde.

**Material examined.** La Foa County (Nili), 16.VIII.2011 on “rocket” (*Eruca sativa* Mill., Brassicaceae) on a hydroponic kit, C. Mille coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** The Turnip Aphid is also recorded on many genera and species of Brassicaceae and is widespread in the world (Blackman and Eastop 2000).

#### *Macrosiphum euphorbiae* (Thomas, 1878)

Potato Aphid, Puceron vert et rose de la Pomme de terre.

**Remarks.** Cohic (1958a) first recorded this species in New Caledonia on tomato (*Solanum lycopersicum* L., Solanaceae). This cosmopolitan and polyphagous species feeds

on over 200 plant species and can transmit at least 67 plant viruses (Chan et al. 1991). It should be monitored closely in New Caledonia because of its potential to become a serious pest.

The known beneficial agents against this aphid are not recorded from New Caledonia, but some of the present ones probably play an important role in its control. Some entomopathogenic fungi have also shown some promising clues for the biological control in greenhouses (Fournier and Brodeur 1999).

Cohic (1958a) rated this species as a very important pest on tomato during dry seasons, but there is no current information. The most dangerous activity of this aphid is the transmission of phytopathogenic viruses, especially the Potato Y virus (PYV) and the Beet yellow virus (BYV). Fortunately, the Potato Aphid does not transmit the Tomato yellow leafcurl virus (TYLCV) recently detected in New Caledonia (Péréfarres et al. 2012).

### ***Macrosiphum rosae* (Linnaeus, 1758)**

Rose Aphid, Puceron vert du Rosier.

**Material examined.** Bourail County (Gouaro), 19.X.2006 on young leaves of *Rosa* spp. S. Cazères coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** Cohic (1956) and Brun and Chazeau (1986) first recorded this species in New Caledonia on *Rosa* spp. (Rosaceae). This species is widespread in most of the world on cultivated roses, except Japan and southeast Asia. Secondary hosts are Dipsacaceae and Valerianaceae. Blackman and Eastop (2000) also recorded it on other Rosaceae (*Fragaria* spp., *Geum* spp., *Pyrus* spp., *Malus* spp., *Rubus* spp.) and on Onagraceae (*Chamaenerion* spp., *Epilobium* spp.).

In New Caledonia, the known present beneficial agents are *Coccinella transversalis* (Coccinellidae), *Eumicromus tasmaniae* (Neuroptera, Hemerobiidae) and the entomopathogenic fungus *Lecanicillium lecanii*.

This species is able to transmit at least 12 plant viruses including the persistent Strawberry mild yellow edge virus and should therefore be regularly checked in the New Caledonian context, but it is not a vector of the Rose mosaic virus (Blackman and Eastop 1984).

### **\**Micromyzus katoii* (Takahashi, 1925)**

**Material examined.** Lifu Island County (Loyalty Islands), 14.IV.2010, on ferns (Polypodiaceae) in an agro-forestry garden, H. Jourdan coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** This species is recorded from Australia, Hawai'i, Indonesia, and Taiwan. It is observed on undersides of fronds of several genera of Polypodiaceae ferns of the genera *Microsorium*, *Platyserium*, and *Polypodium*.

**\**Myzus ornatus* Laing, 1932**

Ornate Aphid, Puceron orné.

**Material examined.** La Foa County (Nili), 1.VII.2015 on the Liliaceae *Lilium* sp. (orange flower), Z. Lemerre Desprez coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** *Myzus ornatus* is widespread throughout the world, probably because of commercial trade in ornamental plants. It is a very polyphagous species. Besides the Liliaceae, it infests plant species in Apiaceae, Asteraceae, Bignoniaceae, Brassicaceae, Caryophyllaceae, Lamiaceae, Polygonaceae, Primulaceae, Rosaceae, Solanaceae and Violaceae.

It is regarded as a pest of various plants because it transmits at least 20 plant viruses, including Potato leaf roll virus.

***Myzus persicae* (Sulzer, 1776)**

Green Peach Aphid, Puceron vert du Pêcher.

**Material examined.** La Foa County (Pocquereux), 31.VIII.2015 on *Solanum tuberosum* L. (Red Pascal cv, Solanaceae), N. Hugot coll., E. Maw det. (CNC), dep. CXMNC; La Foa County, 15.IX.2015 on *Solanum tuberosum*, N. Hugot coll., E. Maw det. (CNC); Dumbéa County at Nondoué Farm, 12.IX.2016 on *Brassica oleracea* L. (Brassicaceae), C. Mille coll., E. Maw det. (CNC).

**Remarks.** Cohic (1956) and Brun and Chazeau (1986) first recorded this species in New Caledonia on *Brassica* spp., *Citrus* spp., *Prunus persica*, and *Solanum melongena* (respectively Brassicaceae, Rutaceae, Rosaceae, and Solanaceae). This almost cosmopolitan species is highly polyphagous, recorded from more than 40 plant families.

Sharanabasappa et al. (2007) have shown the potential of predation of this aphid by the hoverfly *Ischiodon scutellaris*. The two species of ladybirds cited as predators of *M. persicae* by Agarwala and Ghosh (1988) and present in New Caledonia are *Harmónia octomaculata* and *Menochilus sexmaculatus* (Nattier et al. 2015). *Aphidius colemani* (Braconidae, Aphidiinae) is known to be a very effective parasitoid against *M. persicae* (Messing and Rabasse 1995). Some predatory midges (Cecidomyiidae) have been collected in 2000 but without a formal identification to date. In greenhouse-grown vegetables in Europe, there has been considerable success using the entomopathogenic fungus *Verticillium* sp. (Mackauer 1968).

The Green Peach Aphid is the most important virus vector as it is able to transmit at least 182–200 plant viruses (Kennedy et al. 1962; Chan et al. 1991). In 2017, this species was the most intercepted aphid species, with 89 specimens among 192 intercepted aphids during 20 events of interceptions on fresh fruits and vegetables imported into New Caledonia (Cazères and Mille 2018).

**\**Pentalonia caladii* van der Goot, 1917**

Cardamom Aphid, Puceron de la Cardamome.

**Material examined.** Dumbéa County at Nondoué Farm, 12.IX.2016 on *Colocasia esculenta* (L.) Schott (Araceae), C. Mille coll., E. Maw det. (CNC).

**Remarks.** *Pentalonia caladii* was discovered in New Caledonia following the advice of Ross Miller of the University of Guam (R. Miller, pers. comm. 10 December 2010). This species is found on plants in the families of Zingiberaceae and Araceae, or occasionally on plants in other families (Heliconiaceae, Musaceae). It is widely distributed throughout the South Pacific, and is also known in China (Hong Kong), Australia and in glasshouses of the Northern Hemisphere.

Its known natural enemies are ladybird larvae and adults (Coccinellidae) and hoverfly larvae (Syrphidae).

It is known to be a Banana bunchy top virus (BBTV) vector (Watanabe et al. 2013). These authors add that the role played by *P. caladii* in the BBTV transmission would need some further studies. This species has been long regarded as a synonym of *P. nigronervosa*, or as a “form” of that species, but molecular and multivariate studies by Footitt et al. (2010) have shown that it should be treated as a distinct species.

***Pentalonia nigronervosa* Coquerel, 1859**

Banana Aphid, Puceron noir du Bananier.

**Material examined.** Poindimié County (North East of Grande Terre), 28.XI.2011 on *Musa* sp. (Musaceae), I. Murcia coll., R.C. Henderson det. (NZAC), dep. CXMNC; Dumbéa County at Nondoué Farm, 12.IX.2016 on *Musa* sp., C. Mille coll., E. Maw det. (CNC).

**Remarks.** It was recorded officially by Jourdan and Mille (2006) but was first identified in 1991 by François Leclant from specimens collected in La Foa County (Pocquereux Valley). This pantropical species is widespread through all tropical and subtropical parts of the world, and is introduced into glasshouses in Europe and North America. It occurs on other members of the families Musaceae (*Musa* spp.) and possibly Heliconiaceae (*Heliconia* spp.), although some records may be due to past confusion with *P. caladii*.

This relatively recent arrival is unexpected, as banana trees have been moved around in the Pacific Region for the last 3,000 years. It only took ten years after the discovery of the aphid for the outbreak of the BBTV that was not detected in the original population of banana aphids. This sad example illustrates the high importance of sound quarantine policy regarding allowance of plants that might harbor viruses that are absent, when a potential vector already is present. The importance of this species

in New Caledonia dramatically increased in 2001 with the discovery of BBTv (Kagy et al. 2001). It also is able to transmit Banana mosaic, Abaca bunchy top, and Cardamom mosaic viruses. Importantly, it is the sole vector of BBTv in Australia, Africa and Asia. The aphids can be found living under the old leaf bases, sometimes ant-attended (Blackman and Eastop 2000). *Pentalonia nigronervosa* was not recorded in Brun and Chazeau's catalogue (1986). It was identified formally in 1991 in the Pocquereux Fruit Research Station (Mille 2000). Prior to 1999, the date of the discovery of the BBTv in New Caledonia (Kagy et al. 2001), *P. nigronervosa* was not significant to banana crops, but then it became an important pest, as it was partly responsible of the spread of the BBTv throughout the country.

## Aphidinae

### Aphidini

#### *Rhopalosiphum maidis* (Fitch, 1856)

Maize Aphid, Corn Leaf Aphid, Puceron vert du Maïs.

**Material examined.** Boulouparis County (La Ouenghi) in an Adecap Technopole experimental plot, 23.II.2012, on maize (*Zea mays* L., Poaceae), S. Cazères & C. Mille coll., E. Maw det. (CNC), dep. CXMNC.

**Remarks.** Brun and Chazeau (1986) first recorded this species in New Caledonia on the Poaceae *Sorghum bicolor* (L.) Moench and maize (*Zea mays* L.). This cosmopolitan species is found on young leaves of grasses including the genera *Avena*, *Hordeum*, *Oryza*, *Saccharum*, *Secale*, *Sorghum*, *Triticum*, *Zea*, and occasionally Cyperaceae and Typhaceae (Blackman and Eastop 2000).

This aphid is preyed on by larval and adult ladybird species *Coccinella transversalis* and *Menochilus sexmaculatus* (Coccinellidae) (Agarwala and Ghosh 1988), and by the hoverfly larvae *Ischiodon scutellaris* (Syrphidae) (Ghorpadé 1981). It is also parasitized by the wasp *Aphidius colemani* (Braconidae, Aphidiinae).

This is probably the most important pest of cereals in tropical and warm climates because it can transmit the pathogens in the yellow dwarf virus complex and at least five other viruses (Blackman and Eastop 2000). It causes a longitudinally rolling of the last leaf during growth and secretes abundant honeydew on which sooty mould develops.

#### \**Rhopalosiphum nymphaeae* (Linnaeus, 1761)

Water Lily Aphid, Puceron noir du Nénuphar.

**Material examined.** Mont-Dore County (Saint-Louis) in IAC-SRMH/Biofabrique D.D.R. (Beneficials rearing factory, Direction du Développement Rural, Southern

Province), 12.XI.2013 and 5.XII.2013 on the invasive waterfern *Salvinia molesta* D. S. Mitch. (Salviniaceae), B. Gatimel coll., E. Maw det. (CNC); Bourail County (Gouaro), 28.III.2016 on *Nymphaea* sp. (purple flower, Nymphaeaceae), S. Cazères coll., E. Maw det. (CNC).

**Remarks.** This almost cosmopolitan species forms colonies which occur on a large variety of water plant genera as *Alisma*, *Butomus*, *Callitriche*, *Echinodorus*, *Juncus*, *Nelumbo*, *Nuphar*, *Nymphaea*, *Potamogeton*, *Sagittaria*, *Sparganium*, *Triglochin*, *Typha*, etc. Its primary hosts are *Prunus* spp. (Rosaceae), but in the tropics it is probably entirely anholocyclic.

The two genera *Coccinella* and *Harmonia* represented in New Caledonia (Nattier et al. 2015) are known to prey on this aphid species (Agarwala and Ghosh 1988), which is also parasitised by *Aphidius colemani* (Braconidae, Aphidiinae) (Tomanović et al. 2012).

*Rhopalosiphum nymphaeae* has been used for biological control of water weeds in rice plots (Oraze and Grigarick 1992).

### ***Rhopalosiphum padi* (Linnaeus, 1758)**

Bird Cherry-Oat Aphid, Puceron du Merisier à grappes.

**Remarks.** Brun and Chazeau (1986) first recorded this species in New Caledonia living on the two Poaceae species *Sorghum bicolor* (L.) Moench (“*Sorghum vulgare*”) and maize (*Zea mays* L.). This species is now virtually cosmopolitan. Primary hosts are *Prunus* spp. and secondary hosts are numerous species of Poaceae, including all the major cereals and pasture grasses.

Sallée and Chazeau (1985) studied the New Caledonian endemic ladybird *Coeleophora mulsanti* (Montrouzier, 1861) (Coleoptera, Coccinellidae) as a predator of *Rhopalosiphum padi*. *Aphidius colemani* is also known to control this aphid species (Elliott et al. 1994; Hullé et al. 2006).

This aphid is known to transmit pathogens in the yellow dwarf virus complex (D’Arcy et al. 1981), which is absent from New Caledonia, but present in Australia and New Zealand (Smith 1964).

### **\**Rhopalosiphum rufiabdominale* (Sasaki, 1899)**

Rice Root Aphid, Red Rice Root Aphid.

**Material examined.** Tribe of Mou, Lifu Island (Loyalty Islands), 03.XI.2017, on roots of some hydroponic lettuce (*Lactuca sativa* L., Asteraceae) D. Pastou coll., E. Maw det. (CNC).

**Remarks.** This species appears to be Oriental and was first described from Japan, but it is now almost cosmopolitan. Remaudière and Etienne (1988) documented its

presence on Réunion Island, which could explain a potential pathway of the species for its arrival in New Caledonia. This pathway is already observed for some scale insects (Mille et al. 2016a). It is known to be on underground parts of numerous species of Poaceae (sugarcane, oats, barley, millet, and wheat), Cyperaceae and some dicotyledons, particularly Solanaceae (eggplant, potato, tomato, tobacco and capsicum), also marrow and cotton.

The entomopathogenic fungus *Verticillium lecanii*, known to be present in New Caledonia (Mille 2011; Mille et al. 2016a), has been recorded on this aphid (Etzet and Pettitt 1992). Some predators and parasitoids are cited (Yano et al. 1983) but do not seem to be efficient against this species.

The Rice Root Aphid has a very broad host plant range, having been recorded from 22 plant families. Like the previous aphid species, this one uses *Prunus* spp. (Rosaceae) as primary hosts in east Asia (Blackman and Eastop 2000) and in Southern Europe (Rakauskas et al. 2015). It is known to be a vector of Barley yellow dwarf virus (Paliwal 1980), Cereal yellow dwarf virus (Hadi et al. 2011), Maize mosaic virus in India (Singh 1977), and Sugarcane mosaic virus also in India (Shukla and Sinha 2009). It is thought to be a non-persistent vector of the Cucumber mosaic virus, causing serious damages on tobacco in Taiwan (Chen and Weng 1969). The present development of grain crops, especially rice and wheat, in New Caledonia, in order to minimize imports, could be threatened by these viruses. Strong phytosanitary regulations are needed to avoid their introduction.

### \**Schizaphis rotundiventris* (Signoret, 1860)

Oil Palm Aphid.

**Material examined.** Tribe of Hwadrilla, Ouvéa Island (Loyalty Islands), 13.III.017, from Winkler traps in a garden, E. Bourguet coll., E. Maw det. (CNC).

**Remarks.** This species is considered nearly cosmopolitan (Skvarla et al. 2018). Its origin is uncertain, but sexual forms occur on pear trees (*Pyrus communis*) on the southern flanks of the Himalayan Mountains (Naumann-Etienne and Remaudière 1995). In other parts of the world *Schizaphis rotundiventris* lives all year around on secondary hosts, mainly on Cyperaceae but sometimes on other monocotyledons (Remaudière and Etienne 1988).

*Toxares macrosiphophagum* Shuja-Uddin, 1974 (Hymenoptera, Braconidae, Aphidiinae) and an unknown species of *Aphidius* are known from India on this aphid (Stary and Ghosh 1983). These two species could be candidates for the development of a biological control measure if *S. rotundiventris* becomes a pest in New Caledonia.

As this species was caught in Winklers traps during an invasive insect survey in the Loyalty Islands, its presence on the Grande Terre should be investigated.

## Eriosomatinae

### Eriosomatini

#### \**Tetraneura fusiformis* Matsumura, 1917

Root Aphid, Puceron des racines.

**Material examined.** Mont-Dore County, Rivière des Pirogues (South of the Grande Terre) at the Champalou Farm, 8.V.2007 on the roots of *Paspalum paniculatum* L. (Poaceae), P. Caplong coll., R.C. Henderson det. (NZAC), dep. CXMNC.

**Remarks.** It is also recorded in Africa, Central and South America, Australia, Fiji Islands, South and East Asia and Tonga (Footitt et al. 2012). *Tetraneura fusiformis* is known in colonies on roots of many genera and species of Poaceae (*Agropyron*, *Axonopus*, *Cenchrus*, *Chloris*, *Cynodon*, *Dactyloctenium*, *Echinochloa*, *Eleusine*, *Eragrostis*, *Oryza*, *Panicum*, *Paspalum*, *Pennisetum*, *Saccharum*, *Setaria*, *Sorghum*). Its presence is often indicated by a reddish-purple discoloration of the leaves.

We have recorded 33 species from New Caledonia. To date, all these species appear exotic to the archipelago. Among them, 17 are formally recorded for the first time in New Caledonia.

An overview of all species is compiled in Table 1. The aphid species recorded from endemic New Caledonian plants are listed in Table 2, and Table 3 summarizes information about the beneficial species mentioned in the text.

## Discussion

The paucity of native aphids reflects a general property of New Caledonian fauna as already pointed out by previous authors (Zimmerman 1948; Gressitt 1971; Chazeau 1993; Grandcolas et al. 2008), which is a lack of groups that are well represented around the world, and especially with respect to Stenorrhyncha, as already noted (Mille et al. 2016a) for the Coccoomorpha. In addition, related predators such as Coccinellidae, especially the coccidivorous and aphidivorous ones also are lacking as native species (Nattier et al. 2015). Native predaceous Coccinellidae are rather specialised in mite predation. The long isolation of New Caledonia can explain such a disharmonic faunal distribution as stated by recent studies (Anso et al. 2016; Nattier et al. 2017).

Comparing the aphid fauna of New Caledonia to that of other analogous island countries (Table 5), the Fiji Islands and Vanuatu have only 13 and 11 species respectively (Sunde et al. 1987; Wilson and Evenhuis 2007), all introduced. In French Polynesia, a list of 23 species was established eleven years ago (Nishida 2008), of which six are significant pest species (Grandgirard 2010). In the Hawaiian Islands, 104 aphid

**Table 1.** List of the 33 aphid species in New Caledonia. First records are in bold.

Subfamilies	Tribes	Species	Biogeographic region of origin and record
Aphidinae	Aphidini	<i>Aphis aurantii</i> (Boyer de Fonscolombe, 1841)	Probably Oriental, Brun and Chazeau (1986)
		<i>Aphis craccivora</i> Koch, 1854	Palaeartic, Bordat and Daly (1995), Jourdan and Mille (2006)
		<b><i>Aphis eugeniae</i> van der Goot, 1917</b>	<b>Oriental</b>
		<b><i>Aphis glycines</i> Matsumura, 1917</b>	<b>Oriental</b>
		<i>Aphis gossypii</i> Glover, 1877	Oriental, Brun and Chazeau (1986)
		<i>Aphis nerii</i> Boyer de Fonscolombe, 1841	Eastern Palaeartic, Brun and Chazeau (1986)
		<b><i>Aphis odinae</i> (van der Goot, 1917)</b>	<b>Oriental and in South Africa</b>
		<i>Aphis spiraeicola</i> Patch, 1914	Eastern Palaeartic, Jourdan and Mille (2006)
		<b><i>Hysteronera setariae</i> (Thomas, 1878)</b>	<b>Nearctic</b>
		<i>Rhopalosiphum maidis</i> (Fitch, 1856)	Central Palaeartic, Brun and Chazeau (1986)
		<b><i>Rhopalosiphum nymphaeae</i> (Linnaeus, 1761)</b>	<b>Palaeartic</b>
		<i>Rhopalosiphum padi</i> (Linnaeus, 1758)	Nearctic, Brun and Chazeau (1986)
		<b><i>Rhopalosiphum rufiabdominale</i> (Sasaki, 1899)</b>	<b>Eastern Palaeartic, in the Loyalty Islands only</b>
	<b><i>Schizaphis rotundiventris</i> (Signoret, 1860)</b>	<b>Cryptogenic, in the Loyalty Islands only</b>	
	Macrosiphini	<b><i>Aulacorthum solani</i> (Kaltenbach, 1843)</b>	<b>Western Palaeartic</b>
		<b><i>Brachycaudus helichrysi</i> (Kaltenbach, 1843)</b>	<b>Palaeartic</b>
		<i>Brevicoryne brassicae</i> (Linnaeus, 1758)	Palaeartic, Brun and Chazeau (1986)
		<i>Capitophorus elaeagni</i> (del Guercio, 1894)	Palaeartic, Brun and Chazeau (1986)
		<b><i>Hyperomyzus carduellinus</i> (Theobald, 1915)</b>	<b>Eastern Palaeartic (Asia), in the Loyalty Islands only</b>
		<i>Hyperomyzus lactucae</i> (Linnaeus, 1758)	Palaeartic, Brun and Chazeau (1986)
		<b><i>Lipaphis pseudobrassicae</i> (Davis, 1914)</b>	<b>Western Palaeartic</b>
		<i>Macrosiphum euphorbiae</i> (Thomas, 1878)	Nearctic (North America), Cohic (1958a)
		<i>Macrosiphum rosae</i> (Linnaeus, 1758)	Western Palaeartic, Brun and Chazeau (1986)
<b><i>Micromyzus katoii</i> (Takahashi, 1925)</b>		<b>Oriental</b>	
<b><i>Myzus ornatus</i> Laing, 1932</b>		<b>Palaeartic</b>	
<i>Myzus persicae</i> (Sulzer, 1776)		Eastern Palaeartic, Brun and Chazeau (1986)	
<b><i>Pentalonia caladii</i> van der Goot, 1917</b>		<b>Oriental</b>	
<i>Pentalonia nigronervosa</i> Coquerel, 1859		Oriental, first detected in 1991, Jourdan and Mille (2006)	
Greenideinae	Greenideini	<b><i>Greenidea psidii</i> van der Goot, 1917</b>	<b>Oriental</b>
Hormaphidinae	Cerataphidini	<i>Asteopteryx bambusae</i> (Buckton, 1893)	Oriental, first detected in the sixties, Brun and Chazeau (1986)
		<i>Cerataphis lataniae</i> (Boisduval, 1867)	Oriental, Brun and Chazeau (1986)
		<b><i>Cerataphis orchidearum</i> (Westwood, 1879)</b>	<b>Oriental</b>
Eriosomatinae	Eriosomatini	<b><i>Tetraneura fusiformis</i> Matsumura, 1917</b>	<b>Eastern Palaeartic</b>

**Table 2.** List of New Caledonian endemic hostplants and their associated aphid species.

Aphid species	Host-plants species (families)
<i>Aphis aurantii</i>	<i>Dodonea viscosa</i> (Sapindaceae)
<i>Aphis eugeniae</i>	<i>Glochidion billardieri</i> (Myrtaceae)
<i>Aphis gossypii</i>	<i>Hibbertia pancheri</i> (Dilleniaceae)
	<i>Myodocarpus fraxinifolius</i> (Myodocarpaceae)
	<i>Tarenna</i> spp. (Rubiaceae)
<i>Aphis spiraeicola</i>	<i>Artia balansae</i> (Apocynaceae)
	<i>Ixora cauliflora</i> (Rubiaceae)
	<i>Pittosporum coccineum</i> (Pittosporaceae)

**Table 3.** Aphid natural enemies in New Caledonia (after Mille 2011; Nattier et al. 2015; Starý 1975).

Orders	Families	Species	Origins	Preys/Hosts
Coleoptera	Coccinellidae	<i>Apolimus lividigaster</i> (Mulsant, 1853)	Australasian (only known from Australia, New Zealand and New Caledonia)	An aphid predator specialist
		<i>Coccinella transversalis</i> Fabricius, 1781	Australasian	A polyphagous predator of Aphididae, Psyllidae and Coccoidea
		<i>Coelophora inaequalis</i> (Fabricius, 1775)	Australasian	A polyphagous predator of Aphididae and Coccoidea
		<i>Coelophora mulsanti</i> (Montrouzier, 1861)	Australasian	A polyphagous predator in natural habitats but also in rangeland and disturbed areas, its preys are psyllids, aphids ( <i>Cerataphis</i> spp.), and lepidopterous eggs
		<i>Harmonia octomaculata</i> (Fabricius, 1781) = <i>Harmonia arcuata</i>	Australasian, also known from South Africa	A polyphagous predator of hemiptera including cicadellidae, Aphididae ( <i>Rhopalosiphum maidis</i> ) and Psyllidae
		<i>Menochilus sexmaculatus</i> (Fabricius, 1781)	Australasian (known from India to Japan and from Western Australia to Lord Howe Island)	Known to prey on <i>Aphis gossypii</i> and <i>Myzus persicae</i>
		<i>Micraspis frenata</i> (Erichson, 1842)	Australasian	A hemipterous predator including psyllids, aphids and cicadellids
Diptera	Syrphidae	<i>Ischiodon scutellaris</i> (Fabricius, 1805)	Oriental	Numerous species of aphids
Hymenoptera	Braconidae	<i>Aphidius colemani</i> Viereck, 1912*	Oriental and Australasian	Aphidinae aphids
Neuroptera	Chrysopidae	<i>Mallada basalis</i> (Walker, 1853)	Australasian	Numerous species of aphids
	Hemerobiidae	<i>Eumicromus tasmaniae</i> (Walker, 1860)	Australasian	Numerous species of aphids

\* This species was omitted in Mille (2011).

species are present, indicating a much greater influence of commerce compared with South Pacific islands. The situation in New Zealand is quite distinct, with the presence of 12 indigenous recorded aphid species, and a very important introduced fauna of 110 species (Teulon and Stufkens 2002), totalling at least 122 species (Table 5). The larger number of introduced species in New Zealand is probably because the ecology and climate of that country are more similar to that of their areas of origin, than to that of other Pacific or Indian ocean islands. In Réunion Island (Indian Ocean), Remaudière and Etienne (1988) established a list of 45 species, a higher number probably due to the proximity of Africa and Madagascar. Also, ancient and important commercial routes may have played a significant role in the introduction of exotic species in Réunion Island.

It is unique in the regional context that some endemic species are recorded from both Australia (ABRS 2009) and New Zealand (Teulon and Stufkens 1998; Teulon et al. 2010). In New Zealand, two lineages of Aphidina have been found, and Von Dohlen and Teulon (2003) hypothesized that Aphidinae originated in the Southern Hemisphere during the Tertiary and were then able to colonize the Northern Hemisphere, which is controversial if we regard the New Caledonian situation. Conversely,

**Table 4.** List of the aphid species intercepted by the Biosecurity Services (DAVAR-SIVAP) in New Caledonia but still considered unestablished.

Subfamilies	Tribes	Species / Common names / Noms communs	Intercepted commodity	Country of origin	Biogeographic region of origin
Aphidinae	Aphidini	<i>Aphis</i> sp.	Parsley ( <i>Petroselinum crispum</i> , Apiaceae)	Australia	–
	Macrosiphini	<i>Acyrtosiphon lactucae</i> (Passerini, 1860)	Lettuce ( <i>Lactuca sativa</i> , Asteraceae)	Australia	Palaeartic
		<i>Cavariella aegopodii</i> (Scopoli, 1763) Willow-carrot Aphid / Puceron de la Carotte	Parsley ( <i>Petroselinum crispum</i> , Apiaceae)	Australia	Western Palaeartic
		<i>Chaetosiphon fragaefolii</i> (Cockerell, 1901) Strawberry Aphid / Puceron jaune du Fraisier	Strawberry ( <i>Fragaria</i> spp., Rosaceae)	USA	Nearctic (North America)
		<i>Dysaphis apiifolia</i> (Theobald, 1923)	Fennel ( <i>Foeniculum vulgare</i> , Apiaceae)	New Zealand	Palaeartic
		<i>Dysaphis foeniculi</i> (Passerini, 1860)	Fennel ( <i>Foeniculum vulgare</i> , Apiaceae)	Australia	Western Palaeartic
		<i>Dysaphis lappae</i> (Koch, 1854) Thistle Root Aphid	Artichoke ( <i>Cynara scolymus</i> , Asteraceae)	Australia	Palaeartic
		<i>Hyadaphis coriandri</i> (Das, 1918) Coriander Aphid / Puceron de la Coriandre	Fennel ( <i>Foeniculum vulgare</i> , Apiaceae)	New Zealand	Palaeartic
		<i>Hyadaphis passerini</i> (Del Guercio, 1911) Honeysuckle Aphid / Puceron du Chèvrefeuille	Fennel ( <i>Foeniculum vulgare</i> , Apiaceae)	New Zealand	Palaeartic
		<i>Myzus ascalonicus</i> Doncaster, 1946 Shallot aphid / Puceron de l'Échalote	Fennel ( <i>Foeniculum vulgare</i> , Apiaceae) and Celery ( <i>Apium graveolens</i> , Apiaceae) but this species is highly polyphagous	Australia	Unknnonwn
<i>Nasonovia ribisnigri</i> (Mosley, 1841) Lettuce Aphid / Puceron de la Laitue	Lettuce ( <i>Lactuca sativa</i> , Asteraceae)	New Zealand	Western Palaeartic		
Eriosomatinae		<i>Eriosoma lanigerum</i> (Hausmann, 1802) Woolly Apple Aphid / Puceron lanigère du Pommier	Apple ( <i>Malus pumila</i> , Rosaceae)	France	Palaeartic?
Lachninae	Eulachnini	<i>Cinara tujafilina</i> (Del Guercio, 1909) Cypress Pine Aphid / Puceron du Thuya	On two cypress trees	Unknown	Palaeartic

Kim et al. (2011) provided evidence that four endemic Australasian aphidine species originated after divergence from European lineages.

The species most recently discovered in New Caledonia are *Pentalonia caladai*, *Hyperomyzus carduellinus*, *Rhopalosiphum rufiabdominale*, and *Schizaphis rotundiventris*, the last three species being found in the Province of the Loyalty Islands, respectively in Tiga, Lifu, and Ouvéa (Figure 1). These new records show the need for a comprehensive survey of aphids within the whole archipelago. From an environmental perspective, a study of aphid impacts on the rich New Caledonian endemic flora should be undertaken in order to evaluate their influence on the ecology of these plants. It is known that aphids cause some environmental issues in Hawai'i for instance as they feed on 64

**Table 5.** Comparison of aphid fauna (excl. Adelgidae and Phylloxeridae) between seven island countries (after Wilson and Evenhuis (2007) for Fiji Islands, Nishida (2008) and Grandgirard (2010) for French Polynesia, Footitt et al. (2012) and Messing et al. (2012) for Hawai'i, Macfarlane et al. (2010), Teulon et al. (2010, 2013) for New Zealand, Remaudière and Etienne (1988) for Réunion Island, Sunde et al. (1987) for Vanuatu).

Aphid subfamilies	Island countries						
	Fiji Islands	French Polynesia	Hawaii (USA)	New Caledonia	New Zealand	Réunion Island	Vanuatu
Aphidinae	11	20	80	28	78	38	8
Calaphidinae	0	0	3	0	13	0	0
Chaitophorinae	0	0	2	0	3	0	0
Eriosomatinae	1	0	4	1	10	2	0
Greenideinae	0	0	1	1	0	0	0
Hormaphidinae	1	3	5	3	2	3	3
Lachninae	0	0	7	0	7	2	0
Phyllaphidinae	0	0	0	0	1	0	0
Neophyllaphidinae	0	0	2	0	2	0	0
Saltusaphidinae	0	0	0	0	1	0	0
Taiwanaphidinae	0	0	0	0	1	0	0
<b>Total taxa</b>	<b>13</b>	<b>23</b>	<b>104</b>	<b>33</b>	<b>&gt;122*</b>	<b>45</b>	<b>11</b>

\* This approximative number does not comprise the four species of Adelgidae, the three of Phylloxeridae, and the 18 native species, making a total of 154 species of Aphidoidea for New Zealand (David Teulon, pers. comm. 19 July 2018).

native Hawaiian plants within 32 botanical families (Mondor et al. 2006; Messing et al. 2007, 2012). It also would be worthwhile to study the influence of aphids on predators and parasitoids, prey and host relationships, and their relationships with other invasive species. However, a related increase of predators (mostly introduced, such as ladybird beetles and lacewings) could jeopardize ecological balances in both agro- and natural ecosystems, although some authors advance the opinion that such environmental impacts are less quantifiable (Teulon and Stufkens 2002). Finally, the presence of these hemipterous insects in the wild can also facilitate the colonization by invasive ants (Le Breton et al. 2005; Idechiil et al. 2007), but could also enhance the spread of beneficial insects from agro-systems. Introduced aphids might disturb existing equilibria between native phytophagous and entomophagous insects. The recent spread of this faunal group may also have been helped by ants, as most invasive ant species are able to tend aphids, resulting in a strong protection for the aphids against predators and parasitoids. The recent arrival of at least 32 exotic ants (Jourdan *in prep.*) during the last century is probably also an important factor promoting the spread of aphids in New Caledonia, as already pointed out for the scale insects (Mille et al. 2016a).

With 33 exotic species introduced during a period of 165 years (1853–2018, counting from the incorporation of the archipelago in France in 1853 to the present), the average rate of introduction is 0.20 species per year. In comparison, in the Hawaiian archipelago (discovered in 1778), 105 species of Aphidoidea (incl. one species of Adelgidae) have become established with an average rate of introduction of 0.82 species per year—four times the rate in New Caledonia—from 1910 to 2012 (Footitt et al. 2012). Like New Caledonia, Hawai'i does not have any native aphid species (Footitt

et al. 2012). The closeness of the climates of these two archipelagos shows that New Caledonia potentially could host many other species of aphids. New Zealand has an introduced fauna of 110 species, but differs in that there are more than a dozen endemic species (Teulon and Stufkens 2002). There, the rate of introduction is estimated at 0.85 aphid per year. The low rate of introduction for New Caledonia can be explained mainly because the archipelago was not on major commercial routes until recently. In the last decade the number of interception events in New Caledonia has greatly increased (Figure 2, Table 4). In New Zealand, the rate of introduction of alien aphid species has declined dramatically in recent years (Teulon and Stufkens 2002), probably because of the strong biosecurity policy and efforts that are deployed at ports of entry to New Zealand, as also observed earlier in North America during the thirties (Skvarla et al. 2017). Increased biosecurity scrutiny is obviously a major tool to prevent the spread of these economically important pests.

The biogeographic origins of introduced aphid species in New Caledonia are mainly distributed between Oriental (52%, incl. Eastern Palaearctic) and Palaearctic (36%), only 9% being of Nearctic origin, plus one cryptogenic species (*Schizaphis rotundiventris*) (Figure 3). This compares with Hawai'i, where introduced aphids are 35% Oriental (incl. Eastern Palaearctic), 35% West Palaearctic, and 21% Nearctic (Footitt et al. 2012). One can assume that different patterns of trade affect the probability that species from certain biogeographic regions are introduced. However, the low rate of establishment in New Caledonia might also be partly explained by climatic mismatching between the countries involved. Biogeographic connections may also help to explain the low numbers of introduced aphid species in more tropical islands such as Fiji Islands, French Polynesia, and Vanuatu, although the pattern of trade may also differ according to lifestyle (lower or no import of fresh commodities such as vegetables or fruits).

The recent increase in imports of fresh commodities from two large neighboring countries (Australia and New Zealand) increases the risk of accidental introductions of new species. This is illustrated by Figure 2 and Table 4 showing the increase of interception events and intercepted specimens from 2008 to the present, particularly from these two countries. Table 4 shows that 80% of the regularly intercepted species are originally from the Palaearctic and the remaining 20% are from the Nearctic, most of them being in the tribe Macrosiphini. Some of the intercepted species originating from the Northern Hemisphere may not be able to adapt to the New Caledonian environments, but several examples show that some Northern Hemisphere aphids can adapt to New Caledonia biotopes. Eighty percent of aphids originating from Palaearctic or Western Palaearctic regions seem unfitted to colonise New Caledonia (Table 4), but repeated incursions may lead to introduction of more adapted strains, as we already have observed settlement of some Palaearctic species in the archipelago (Figure 3). This applies especially to species such as *Nasonovia ribisnigri*, which has been repeatedly intercepted in imports from New Zealand to New Caledonia since at least 2008. One can note the recent interceptions of *Dysaphis apiifolia* from Australia and New Zealand in 2017 and 2018, *Myzus ascalonicus* from Australia and New Zealand in March, June,

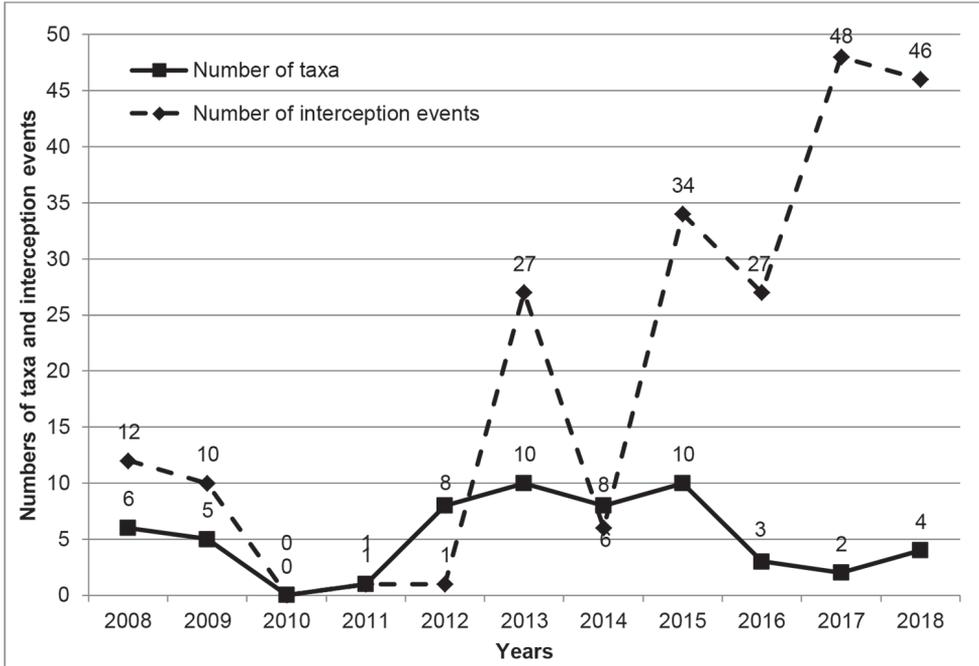


Figure 2. Aphid interceptions in New Caledonia from 2008 to 2018 on fresh imported fruits and vegetables.

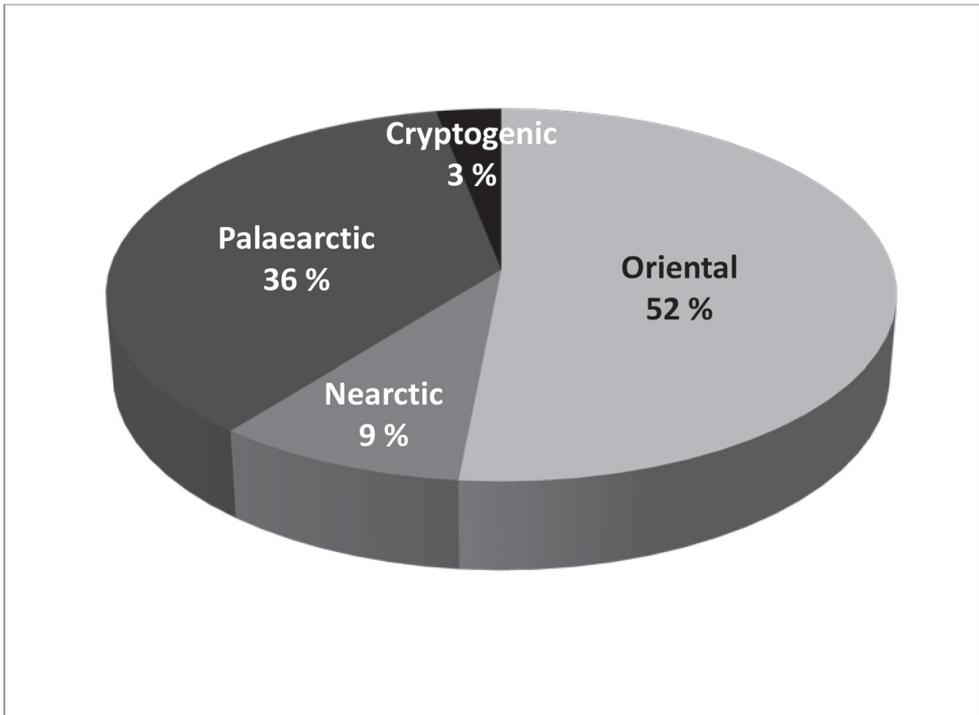


Figure 3. Biogeographic origin of the 33 aphid species present in New Caledonia.

and October 2019, and *Eriosoma lanigerum* from France in February 2018. In 2015, the Strawberry Aphid, *Chaetosiphon fragaefolii* was intercepted on strawberries imported from the USA. Establishment of *C. fragaefolii* in New Caledonia would bring a new pest in this crop, already attacked by many other pests and diseases. At the moment, these 13 species are not established in New Caledonia, but their recurrent interceptions might result in a future settlement, especially in the case of *Myzus ascalonicus*, because it is highly polyphagous and is known on potatoes which are cultivated in New Caledonia. Obviously, continued and enhanced surveillance of imported commodities is needed. Finally, *Cinara tujafilina* was discovered on the 12th October 2018 on two cypress trees in a garden of Nouméa. An eradication program was subsequently launched by the Biosecurity Services (DAVAR-SIVAP). This species can be considered as a potential and significant threat to endemic and endangered species of Cupressaceae, especially species of the genera *Callitris* and *Libocedrus*.

## Conclusions

To our present knowledge, no aphids occurred in New Caledonia before European settlements. The present updated species list is an important step to better secure the trade in fresh commodities. It is imperative to set up some strict regulations concerning the movement of fresh commodities, especially from the countries where the regularly intercepted species are present. In New Zealand, Teulon and Stufkens (2002) reminded us that “Aspects of aphid biology, such as small size, parthenogenetic reproduction, high reproductive rates, short generation time, rapid dispersal and eruptive population dynamics, pose particularly difficult challenges for aphid biosecurity in New Zealand”. This statement also is highly relevant for a subtropical “biodiversity-hotspot” country such as New Caledonia, where there are no endemic aphids.

Apart from virus transmissions (chiefly BBTV and CTV), direct damage by aphids does not constitute a major problem in New Caledonian orchards, probably because of the significant activities of predators and parasitoids. However, damage due to virus transmission in field crops, especially in squash (*Cucurbita pepo*) and several other crops, can be economically significant (Bordat and Daly 1995).

All 33 species appear to have been introduced accidentally by human activity in the last 100 years. Thirteen more species also are intercepted more or less regularly at the borders through biosecurity surveys, without further establishment. This demonstrates that aphids represent a major biosecurity threat, including the one as potential plant virus vectors. Consequently, the reinforcement of biosecurity is a priority for such biodiversity hotspots, from both the perspective of agriculture and of the native environment. Of course, these measures cannot guard against the long-distance dispersal of such low-weight insects as aphids on air currents, as stated by some authors (Johnson 1967). Even some heavier insects are already known to fly over several hundred to thousand kilometers over the sea, with *Calligrapha pantherina* Stål (Coleoptera, Chrysomelidae) as a recent example for New Caledonia (Mille et al. 2016b).

Furthermore, prioritization and promotion of local development of vegetable and fruit crops, rather than their risky importation from abroad, is desirable. Such an approach also should be promoted and extended to other Pacific islands which all share the lack of native aphid fauna and associated plant virus vector risks. Also, as a consequence of global climate change, the regularly intercepted species could find their ecological requirements, settle and dramatically change the fragile ecological balance in this insular biodiversity hotspot. There is an urgent need for a plant quarantine facility in New Caledonia (Cohic 1958b; Mille et al. 2016a), accompanied by some strict regulations against these and other quarantined insects.

## **Dedication**

We dedicate this article to the late Professor François Leclant (22 July 1934–14 January 2001), INRA, Montpellier, France, who trained one of us (CM) in the study of aphids, and more widely, in Agricultural Entomology, and to the late Mrs. Rosa C. Henderson (1 June 1942–13 December 2012) who trained one of us (SC) in the preparation of slides of aphids, other soft insects, and mites. We openly thank her, who encouraged two of us (SC and CM) to write the present article about aphids of New Caledonia.

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# A new species of the Asian leaf litter toad genus *Leptobrachella* (Amphibia, Anura, Megophryidae) from southwest China

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## Abstract

A new species of the Asian leaf litter toad genus *Leptobrachella* from Guizhou Province, China is described based on molecular phylogenetic analyses, morphological comparisons, and bioacoustics data. Phylogenetic analyses based on the mitochondrial 16S rRNA gene sequences supported the new species as an independent clade nested into the *Leptobrachella* clade and sister to *L. bijie*. The new species could be distinguished from its congeners by a combination of the following characters: small body size (SVL 30.8–33.4 mm in seven adult males, and 34.2 mm in one adult female); dorsal skin shagreened, some of the granules forming longitudinal short skin ridges; tympanum distinctly discernible, slightly concave; internasal distance longer than interorbital distance; supra-axillary, femoral, pectoral and ventrolateral glands distinctly visible; absence of webbing and lateral fringes on fingers; toes with rudimentary webbing and shallow lateral fringes, relative finger lengths  $II < IV < I < III$ ; heels overlapped when thighs are positioned at right angles to the body; and tibia-tarsal articulation reaches the tympanum.

## Keywords

Guizhou, molecular phylogenetic analyses, morphology, new species, taxonomy

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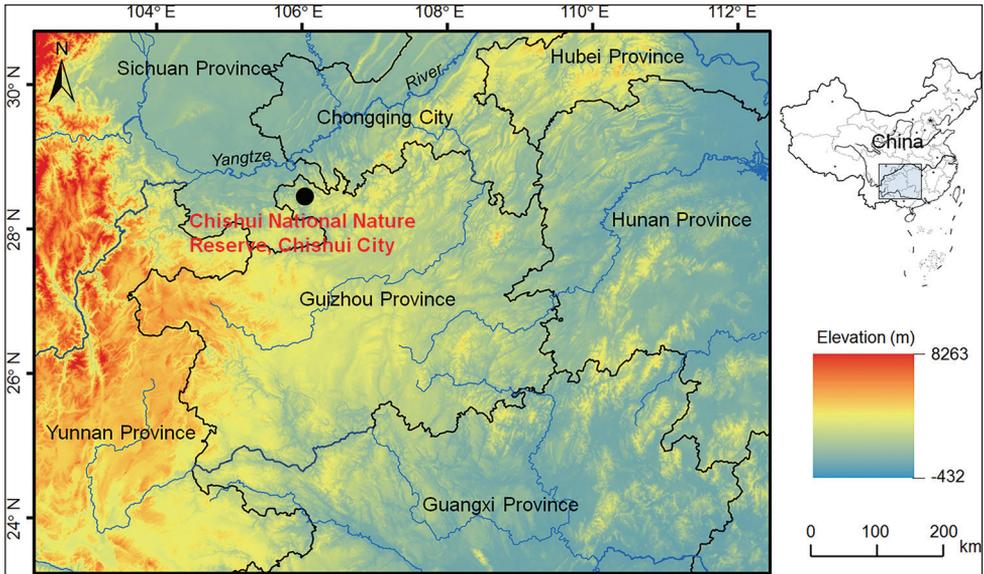
## Introduction

The Asian leaf litter toads of the genus *Leptobrachella* Smith, 1925 (Anura, Megophryidae) are widely distributed from southern China west to northeastern India and Myanmar, through mainland Indochina to peninsular Malaysia and the island of Borneo (Frost 2020). Many species in this genus had been ever classified into *Leptolalax* Dubois, 1983 (e.g., Fei et al. 2009, 2012), and Chen et al. (2018) placed *Leptolalax* as a junior synonym of *Leptobrachella* based on large-scale molecular analyses. Currently, the genus *Leptobrachella* contains 76 species, of which 44 species have been described in the past ten years (Frost 2020). Currently, 21 species of the genus *Leptobrachella* are known from China: *Leptobrachella alpina* (Fei, Ye & Li, 1990) and *L. bourreti* (Dubois, 1983) from Yunnan and Guangxi; *L. eos* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011) and *L. nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011) from Yunnan; *L. laui* (Sung, Yang & Wang, 2014) and *L. yunkaiensis* Wang, Li, Lyu & Wang, 2018 from southern Guangdong, including Hong Kong; *L. liui* (Fei & Ye, 1990) from Fujian, Jiangxi, Guangdong, Guangxi, Hunan, and Guizhou; *L. oshanensis* (Liu, 1950) from Gansu, Sichuan, Chongqing, Guizhou, and Hubei; *L. purpuraventra* Wang, Li, Li, Chen & Wang, 2019, *L. bijie* Wang, Li, Li, Chen & Wang, 2019, and *L. suiyangensis* Luo, Xiao, Gao & Zhou, 2020 from Guizhou; *L. purpurus* (Yang, Zeng & Wang, 2018), *L. pelodytoides* (Boulenger, 1893), *L. tengchongensis* (Yang, Wang, Chen & Rao, 2016), and *L. yingjiangensis* (Yang, Zeng & Wang, 2018) from Yunnan; *L. ventripunctata* (Fei, Ye & Li, 1990) from Guizhou and Yunnan; *L. mangshanensis* (Hou, Zhang, Hu, Li, Shi, Chen, Mo & Wang, 2018) from southern Hunan; and *L. sungi* (Lathrop, Murphy, Orlov & Ho, 1998), *L. maershanensis* (Yuan, Sun, Chen, Rowley & Che, 2017), *L. shangsiensis* Chen, Liao, Zhou & Mo, 2019, and *L. wuhuangmontis* Wang, Yang & Wang, 2018 from Guangxi (Sung et al. 2014; Li et al. 2016; Yang et al. 2016, 2018; Yuan et al. 2017; Chen et al. 2018, 2019; Hou et al. 2018; Wang et al. 2018, 2019; Wang et al. 2019; Luo et al. 2020). Even more, a series of cryptic species in the genus were still proposed in Chen et al. (2018).

In recent years, we carried out a series of biodiversity surveys in Chishui City, Guizhou Province, China, and collected some specimens of the genus *Leptobrachella*. Molecular phylogenetic analyses, morphological comparisons, and bioacoustics comparisons consistently indicated these specimens as an undescribed species of *Leptobrachella*. Hence, we describe it herein as a new species.

## Materials and methods

**Specimens.** Seven adult males and one adult female of the undescribed species were collected from the mountain streams in Chishui National Nature Reserve, Chishui City, Guizhou Province, China (for voucher information see Table 1; Fig. 1). After taking photographs, they were euthanized using isoflurane, and then the specimens were



**Figure 1.** Location of the type locality of *Leptobrachella chishuiensis* sp. nov., Chishui National Nature Reserve, Chishui City, Guizhou Province, China.

fixed in 10% buffered formalin. Before fixing, muscle tissue was taken and preserved separately in 95% ethanol. Specimens were deposited in Chengdu Institute of Biology, Chinese Academy of Sciences (CIB, CAS).

**Molecular phylogenetic analyses.** All eight specimens of the new taxon were included in the molecular analyses (Table 1). For phylogenetic analyses, the corresponding gene sequences for all those related species for which comparable sequences were available were also downloaded from GenBank (Table 1) mainly based on previous studies (Chen et al. 2018; Wang et al. 2019; Luo et al. 2020). Corresponding sequences of *Leptobrachium tengchongensis*, one *Leptobrachium huashen*, and one *Megophrys major* were also downloaded from GenBank, and used as outgroups according to previous phylogenetic works (Chen et al. 2018; Wang et al. 2019; Luo et al. 2020).

Total DNA was extracted using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). The mitochondrial 16S rRNA gene (16S) sequences were amplified, and the primers P7 (5'-CGCCTGTTTACCAAAAACAT-3') and P8 (5'-CCG-GTCTGAAGTCTCAGATCACGT-3') were used following Simon et al. (1994). Gene fragments were amplified under the following conditions: an initial denaturing step at 95 °C for 4 min; 36 cycles of denaturing at 95 °C for 30 s, annealing at 51 °C for 30 s and extending at 72 °C for 70 s. Sequencing was conducted using an ABI3730 automated DNA sequencer in Shanghai DNA BioTechnologies Co., Ltd. (Shanghai, China). New sequences were deposited in GenBank (for GenBank accession numbers see Table 1).

Sequences were assembled and aligned using the Clustalw module in BioEdit v. 7.0.9.0 (Hall 1999) with default settings. Phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI) methods, implemented in

**Table 1.** Information for samples used in molecular phylogenetic analyses in this study.

ID	Species	Voucher	Locality	GenBank accession number
1	<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518047	Chishui National Nature Reserve, Chishui City, Guizhou Province, China	MT117053
2	<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518042	Chishui National Nature Reserve, Chishui City, Guizhou Province, China	MT117054
3	<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518043	Chishui National Nature Reserve, Chishui City, Guizhou Province, China	MT117055
4	<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518049	Chishui National Nature Reserve, Chishui City, Guizhou Province, China	MT117056
5	<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518046	Chishui National Nature Reserve, Chishui City, Guizhou Province, China	MT117057
6	<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518045	Chishui National Nature Reserve, Chishui City, Guizhou Province, China	MT117058
7	<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518044	Chishui National Nature Reserve, Chishui City, Guizhou Province, China	MT330118
8	<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518048	Chishui National Nature Reserve, Chishui City, Guizhou Province, China	MT330119
9	<i>Leptobranchella bijie</i>	SYS a007313/ CIB110002	Mt. Zhaozi Nature Reserve, Bijie City, Guizhou Province, China	MK414532
10	<i>Leptobranchella bijie</i>	SYS a007314	Mt. Zhaozi Nature Reserve, Bijie City, Guizhou Province, China	MK414533
11	<i>Leptobranchella bijie</i>	SYS a007315	Mt. Zhaozi Nature Reserve, Bijie City, Guizhou Province, China	MK414534
12	<i>Leptobranchella purpuraventra</i>	SYS a007081	Wujing Nature Reserve, Bijie City, Guizhou Province, China	MK414517
13	<i>Leptobranchella purpuraventra</i>	SYS a007277/ CIB110003	Wujing Nature Reserve, Bijie City, Guizhou Province, China	MK414518
14	<i>Leptobranchella purpuraventra</i>	SYS a007278	Wujing Nature Reserve, Bijie City, Guizhou Province, China	MK414519
15	<i>Leptobranchella suiyangensis</i>	GZNU20180606002	Huoqiuba Nature Reserve, Suiyang County, Guizhou, China	MK829648
16	<i>Leptobranchella suiyangensis</i>	GZNU20180606006	Huoqiuba Nature Reserve, Suiyang County, Guizhou, China	MK829649
17	<i>Leptobranchella suiyangensis</i>	GZNU20180606005	Huoqiuba Nature Reserve, Suiyang County, Guizhou, China	MK829650
18	<i>Leptobranchella purpurus</i>	SYS a006530	Yingjiang County, Yunnan Province, China	MG520354
19	<i>Leptobranchella alpina</i>	KIZ046816	Huangcaoling, Yunnan Province, China	MH055866
20	<i>Leptobranchella bourreti</i>	AMS R 177673	Lao Cai Province, Vietnam	KR018124
21	<i>Leptobranchella oshanensis</i>	KIZ025776	Emei Shan, Emei Shan City, Sichuan Province, China	MH055895
22	<i>Leptobranchella eos</i>	MNHN:2004.0278	Phongsaly Province, Laos	JN848450
23	<i>Leptobranchella tengchongensis</i>	SYS a004598	Tengchong County, Yunnan Province, China	KU589209
24	<i>Leptobranchella mangshanensis</i>	MSZTC201701	Mt. Mang, Yizhang County, Hunan Province, China	MG132196
25	<i>Leptobranchella liui</i>	SYS a001597	Mt. Wuyi, Wuyishan City, Fujian Province, China	KM014547
26	<i>Leptobranchella laui</i>	SYS a001507	Mt. Wutong, Shenzhen City, Guangdong Province, China	KM014544
27	<i>Leptobranchella yunkaiensis</i>	SYS a004664 / CIB107272	Dawuling Forest Station, Maoming City, Guangdong Province, China	MH605585
28	<i>Leptobranchella maershanensis</i>	KIZ019385	Mt. Maoer Nature Reserve, Ziyuan County, Guangxi Province, China	KY986930
29	<i>Leptobranchella khasiorum</i>	SDBDU 2009.329	East Khasi Hills, Meghalaya, India	KY022303
30	<i>Leptobranchella yingjiangensis</i>	SYS a006532	Yingjiang County, Yunnan Province, China	MG520351
31	<i>Leptobranchella petrops</i>	AMS:R184826	Vietnam	KY459997
32	<i>Leptobranchella puhoatensis</i>	AMS:R184852	Pu Hoat Nature Reserve, Nghe An Province, Vietnam	KY849588
33	<i>Leptobranchella namdongensis</i>	VNUF A.2017.37	Thanh Hoa Provincen, Vietnam	MK965389

ID	Species	Voucher	Locality	GenBank accession number
34	<i>Leptobrachella isos</i>	VNMN A 2015.4/ AMS R 176480	Gia Lai Province, Vietnam	KT824769
35	<i>Leptobrachella firthi</i>	AMS R 176524	Kon Tum Province, Vietnam	JQ739206
36	<i>Leptobrachella minimus</i>	KUHE:19201	Thailand	LC201981
37	<i>Leptobrachella ventripunctata</i>	SYS a004536	Zhushihe, Yunnan Province, China	MH055831
38	<i>Leptobrachella aerea</i>	ZFMK 86362	Quang Binh Province, Vietnam	JN848409
39	<i>Leptobrachella wuhuangmontis</i>	SYS a003500 / CIB107274	Mt. Wuhuang, Pubei County, Guangxi Zhuang minority Autonomous Region, China	MH605581
40	<i>Leptobrachella pluvialis</i>	MNHN:1999.5675	Mt. Fan Si Pan, Lao Cai Province, Vietnam	JN848391
41	<i>Leptobrachella shangsiensis</i>	NHMG1704003	Shangsi County, Guangxi Zhuang minority Autonomous Region, China	MK095463
42	<i>Leptobrachella nahangensis</i>	ROM 7035	Na Hang Nature Reserve, Tuyen Quang, Vietnam	MH055853
43	<i>Leptobrachella nyx</i>	AMNH A163810	Ha Giang Province, Vietnam	DQ283381
44	<i>Leptobrachella zhangyapingi</i>	KIZ07258	Pang Num Poo, Chiang Mai Province, Thailand	MH055864
45	<i>Leptobrachella sungi</i>	ROM 20236	Tam Dao, Vinh Phuc, Vietnam	MH055858
46	<i>Leptobrachella tuberosa</i>	ZMMU-NAP-02275	Kon Ka Kinh National Park, Gia Lai, Vietnam	MH055959
47	<i>Leptobrachella boisfordi</i>	VNMN 03682	Fansipan, Lao Cai, Vietnam	MH055953
48	<i>Leptobrachella pallida</i>	UNS00510	Lam Dong Province, Vietnam	KR018112
49	<i>Leptobrachella kalonensis</i>	IEBR A.2015.15	Binh Thuan Province, Vietnam	KR018114
50	<i>Leptobrachella bidoupensis</i>	NAP-01453	Lam Dong Province, Vietnam	KP017573
51	<i>Leptobrachella tadungensis</i>	UNS00515	Dak Nong Province, Vietnam	KR018121
52	<i>Leptobrachella maculosa</i>	AMS R 177660	Ninh Thuan Province, Vietnam	KR018119
53	<i>Leptobrachella pyrrops</i>	ZMMU ABV-00148	Loc Bao, Lam Dong Province, Vietnam	KP017575
54	<i>Leptobrachella macrops</i>	IEBR A.2017.9	Hon Den Mt., Phu Yen Province, Vietnam	MG787990
55	<i>Leptobrachella melica</i>	MVZ 258197	Virachey National Park, Ratanakiri Province, Cambodia	HM133599
56	<i>Leptobrachella applebyi</i>	AMS R171704	Song Thanh, Quang Nam, Vietnam	HM133598
57	<i>Leptobrachella rowleyae</i>	ITBCZ 2783	Son Tra, Da Nang City, Vietnam	MG682552
58	<i>Leptobrachella ardens</i>	AMS R 176463	Gia Lai Province, Vietnam	KR018110
59	<i>Leptobrachella crocea</i>	AMS R 173740	Kon Tum, Vietnam	MH055954
60	<i>Leptobrachella melanoleuca</i>	KUHE 23840	Srat Thani, Thailand	LC201997
61	<i>Leptobrachella fuliginosa</i>	KUHE:20172	Thailand	LC201985
62	<i>Leptobrachella itiokai</i>	KUHE:55897	Mulu NP, Sarawak, Borneo, Malaysia	LC137805
63	<i>Leptobrachella brevicrus</i>	ZMH A09365	Sarawak: Gunung Mulu National Park: Small stream of the Sungei Tapin, Malaysia	KJ831302
64	<i>Leptobrachella parva</i>	KUHE 55308	Mulu NP, Sarawak, Borneo, Malaysia	LC056791
65	<i>Leptobrachella baluensis</i>	SP 21604	Tambunan, Sabah, Borneo, Malaysia	LC056792
66	<i>Leptobrachella mjobergi</i>	KUHE 17064	Gading NP, Sarawak, Borneo, Malaysia	LC056785
67	<i>Leptobrachella juliandringi</i>	SRC 00230/KUHE 49815	Mulu NP, Sarawak, Borneo, Malaysia	LC056779
68	<i>Leptobrachella arayai</i>	BORNEENSIS 22931	Liwagu, Kinabalu, Borneo, Malaysia	AB847558
69	<i>Leptobrachella bamidi</i>	KUHE 17545	Borneo, Malaysia	AB969286
70	<i>Leptobrachella marmorata</i>	KUHE 53227	Annah Rais, Padawan, Kuching Division, Sarawak, Malaysia	AB969289
71	<i>Leptobrachella maura</i>	SP 21450	Kinabalu, Sabah, Malaysia	AB847559
72	<i>Leptobrachella gracilis</i>	KUHE 55624	Camp 1, Gunung Mulu, Borneo, Malaysia	AB847560
73	<i>Leptobrachella sabahmontana</i>	BORNEENSIS 12632	Borneo, Malaysia	AB847551
74	<i>Leptobrachella dringi</i>	KUHE 55610	Camp 4 of Gunung Mulu, Malaysia	AB847553
75	<i>Leptobrachella picta</i>	UNIMAS 8705	Borneo, Malaysia	KJ831295
76	<i>Leptobrachella fritiniensis</i>	KUHE 55371	Headquarters, Gunung Mulu, Malaysia	AB847557
77	<i>Leptobrachella sola</i>	KUHE 23261	Hala Bala, Thailand	LC202007
78	<i>Leptobrachella heteropus</i>	KUHE 15487	Larut, Peninsular, Malaysia	AB530453
79	<i>Leptobrachella kecil</i>	KUHE 52440	Malaysia	LC202004
80	<i>Leptobrachella kajangensis</i>	LSUHC 4439	Tioman, Malaysia	LC202002
81	<i>Leptobrachium tengchongense</i>	SYSa004604d	Yunnan Province, China	KX066880
82	<i>Leptobrachium huashen</i>	KIZ049025	Yunnan Province, China	KX811931
83	<i>Megophrys major</i>	AMS R 173870	Kon Tum, Vietnam	KY476333

PhyML v. 3.0 (Guindon et al. 2010) and MrBayes v. 3.12 (Ronquist and Huelsenbeck 2003), respectively. We ran JMODELTEST v. 2.1.2 (Darriba et al. 2012) with Akaike and Bayesian information criteria on the alignment, resulting in the best-fitting nucleotide substitution models of GTR + I + G for the data used in ML and BI analyses. For the ML analysis, branch supports were drawn from 10,000 nonparametric bootstrap replicates. In BI analysis, the parameters for each partition were unlinked, and branch lengths were allowed to vary proportionately across partitions. Two runs each with four Markov chains were simultaneously run for 60 million generations with sampling every 1,000 generations. The first 25% trees were removed as the “burn-in” stage followed by calculations of Bayesian posterior probabilities and the 50% majority-rule consensus of the post burn-in trees sampled at stationarity. Finally, genetic distance between *Leptobrachella* species based on uncorrected *p*-distance model was estimated on 16S gene using MEGA v. 6.06 (Tamura et al. 2013).

**Morphological comparisons.** All eight adult specimens (Table 2) of the new taxon were measured. The terminology and methods followed Fei et al. (2005), Mahony et al. (2011), and Wang et al. (2019). Measurements were made with a dial caliper to the nearest 0.1 mm (Watters et al. 2016) with digital calipers. Corresponding measurements of *L. bijie* and *L. purpuraventra* were retrieved from Wang et al. (2019). Twenty-three morphometric characters of adult specimens were measured:

- ED** eye diameter (distance from the anterior corner to the posterior corner of the eye);
- FIL** first finger length (distance from base to tip of finger I);
- FIIL** second finger length (distance from base to tip of finger II);
- FIIL** third finger length (distance from base to tip of finger III);
- FIVL** fourth finger length (distance from base to tip of finger IV);
- FL** foot length (distance from tarsus to the tip of the fourth toe);
- HDL** head length (distance from the tip of the snout to the articulation of jaw);
- HDW** head width (greatest width between the left and right articulations of jaw);
- HLL** hindlimb length (distance from tip of fourth toe to vent);
- IND** internasal distance (minimum distance between the inner margins of the external nares);
- IOD** interorbital distance (minimum distance between the inner edges of the upper eyelids);
- LAL** length of lower arm and hand (distance from the elbow to the distal end of the Finger IV);
- LW** lower arm width (maximum width of the lower arm);
- ML** manus length (distance from tip of third digit to proximal edge of inner palmar tubercle);
- SL** snout length (distance from the tip of the snout to the anterior corner of the eye);
- SVL** snout-vent length (distance from the tip of the snout to the posterior edge of the vent);
- TYD** maximal tympanum diameter;
- TEY** tympanum-eye distance (distance from anterior edge of tympanum to posterior corner of eye);

- TFL** length of foot and tarsus (distance from the tibiotarsal articulation to the distal end of the toe IV);
- THL** thigh length (distance from vent to knee);
- TL** tibia length (distance from knee to tarsus);
- TW** maximal tibia width;
- UEW** upper eyelid width (greatest width of the upper eyelid margins measured perpendicular to the anterior-posterior axis).

In order to reduce the impact of allometry, the correct value from the ratio of each character to SVL was calculated and then was log-transformed for the following morphometric analyses. Mann-Whitney *U* tests were conducted to test the significance of differences on morphometric characters between the undescribed species, *L. bijie* and *L. purpuraventra*. The significance level was set at 0.05. Furthermore, principal component analyses (PCA) were conducted to highlight whether the different species were separated in morphometric space. Due to only the measurements SVL, HDL, HDW, SL, IND, IOD, ED, TYD, TEY, LAL, ML, TL, HLL, and FL of male *L. bijie* and *L. purpuraventra* being available from Wang et al. (2019), the morphometric analyses were conducted only based on these 14 morphometric characters for male group.

The new taxon was also compared with all other congeners of *Leptobranchella* based on morphological characters. Comparative morphological data were obtained from literatures (Table 3).

**Bioacoustics analyses.** The advertisement calls of the new taxon were recorded from the holotype specimen CIBCS20190518047 in the field on 18 May 2019 in Chishui National Nature Reserve, Chishui City, Guizhou Province, China. The advertisement call of the new species was recorded in the stream at ambient air temperature of 20 °C and air humidity of 87%. SONY PCM-D50 digital sound recorder was used to record within 20 cm of the calling individual. The sound files in wave format were resampled at 48 kHz with sampling depth 24 bits. Calls were recoded and examined as described by Wijayathilaka and Meegaskumbura (2016). Call recordings were visualized and edited with SoundRuler v. 0.9.6.0 (Gridi-Papp 2003–2007) and Raven Pro v. 1.5 software (Cornell Laboratory of Ornithology, Ithaca, NY, USA). Ambient temperature of the type locality was taken by a digital hygrothermograph.

## Results

Aligned sequence matrix of 16S contained 537 bps. ML and BI analyses based on the 16S matrix resulted in essentially identical topologies (Fig. 2). All six samples of the new taxon were clustered into one monophyletic group (node supports in ML and BI: 94 and 0.95) nested into *Leptobranchella*, and was a sister taxon to *L. bijie* (node supports in ML and BI: 92 and 1.00). The genetic distance between the new taxon and its closest relatives *L. bijie* was 2.1%, at the same level with that between *L. alpina* and *L. purpurus* (2.1%; Suppl. material 1: Table S1).

**Table 2.** Measurements of *Leptobranchella chishuiensis* sp. nov. Units in mm. See abbreviations for characters in the Materials and methods section.

Species	Voucher number	Sex	SVL	HDL	HDW	SL	IND	IOD	UEW	ED	TYD	LAL	LW	THL	TW	TL	TFL	FL	FIL	FIIL	FIIL	FIVL
<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518047	male	32.4	12.3	11.8	5.1	3.8	3.1	3.3	4.6	2.6	17.0	3.2	16.0	4.3	16.2	22.3	15.6	3.4	3.1	5.0	3.2
<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518042	male	32.7	12.2	11.9	5.8	3.5	3.1	3.1	5.0	2.2	15.4	3.1	15.3	3.6	15.5	22.3	14.7	3.6	3.4	5.5	3.5
<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518043	male	33.0	11.9	11.7	5.1	3.5	2.8	3.0	4.0	2.2	15.3	3.1	15.2	4.2	15.5	22.2	15.3	3.3	3.0	4.9	3.2
<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518049	male	30.9	11.9	10.8	5.0	3.5	3.0	3.1	4.1	2.2	14.9	2.6	13.9	3.4	15.3	21.1	14.4	3.0	2.8	5.1	2.9
<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518044	male	33.4	11.1	11.6	5.4	3.8	3.1	3.3	4.4	2.2	16.3	2.8	17.1	3.8	16.8	22.1	15.9	3.8	3.0	5.0	3.5
<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518045	male	30.8	11.8	11.4	4.8	3.6	3.0	3.0	4.1	2.0	15.5	3.0	14.2	4.1	15.2	21.2	15.1	3.6	3.1	5.0	3.1
<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518048	male	31.6	11.5	10.6	5.0	3.7	2.7	3.3	4.2	2.6	14.7	2.9	13.7	3.3	14.9	20.9	15.0	3.2	2.8	5.0	2.9
<i>Leptobranchella chishuiensis</i> sp. nov.	CIBCS20190518046	female	34.2	12.7	12.0	5.3	3.4	2.7	3.0	4.4	2.4	16.3	3.3	15.3	4.2	16.0	22.2	16.3	3.4	3.0	5.6	3.3

**Table 3.** References for morphological characters for congeners of the genus *Leptobrachella*.

ID	<i>Leptobrachella</i> species	Literature obtained
1	<i>L. aerea</i> (Rowley, Stuart, Richards, Phimmachak & Sivongxay, 2010)	Rowley et al. 2010c
2	<i>L. alpina</i> (Fei, Ye & Li, 1990)	Fei et al. 2009
3	<i>L. applebyi</i> (Rowley & Cao, 2009)	Rowley and Cao 2009
4	<i>L. arayai</i> (Matsui, 1997)	Matsui 1997
5	<i>L. ardens</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
6	<i>L. baluensis</i> Smith, 1931	Dring 1983; Eto et al. 2016
7	<i>L. bidoupensis</i> (Rowley, Le, Tran & Hoang, 2011)	Rowley et al. 2011
8	<i>L. bijie</i> Wang, Li, Li, Chen & Wang, 2019	Wang et al. 2019
9	<i>L. bondangensis</i> Eto, Matsui, Hamidy, Munir & Iskandar, 2018	Eto et al. 2018
10	<i>L. botsfordi</i> (Rowley, Dau & Nguyen, 2013)	Rowley et al. 2013
11	<i>L. bourreti</i> (Dubois, 1983)	Ohler et al. 2011
12	<i>L. brevicrus</i> Dring, 1983	Dring 1983; Eto et al. 2015
13	<i>L. crocea</i> (Rowley, Hoang, Le, Dau & Cao, 2010)	Rowley et al. 2010a
14	<i>L. dringi</i> (Dubois, 1987)	Inger et al. 1995; Matsui and Dehling 2012
15	<i>L. eos</i> (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
16	<i>L. firthi</i> (Rowley, Hoang, Dau, Le & Cao, 2012)	Rowley et al. 2012
17	<i>L. fritinniensis</i> (Dehling & Matsui, 2013)	Dehling and Matsui 2013
18	<i>L. fuliginosa</i> (Matsui, 2006)	Matsui 2006
19	<i>L. fusca</i> Eto, Matsui, Hamidy, Munir & Iskandar, 2018	Eto et al. 2018
20	<i>L. gracilis</i> (Günther, 1872)	Günther 1872; Dehling 2012b
21	<i>L. hamidi</i> (Matsui, 1997)	Matsui 1997
22	<i>L. heteropus</i> (Boulenger, 1900)	Boulenger 1900
23	<i>L. isos</i> (Rowley, Stuart, Neang, Hoang, Dau, Nguyen & Emmett, 2015)	Rowley et al. 2015a
24	<i>L. itiokai</i> Eto, Matsui & Nishikawa, 2016	Eto et al. 2016
25	<i>L. juliandrungi</i> Eto, Matsui & Nishikawa, 2015	Eto et al. 2015
26	<i>L. kajangensis</i> (Grismer, Grismer & Youmans, 2004)	Grismer et al. 2004
27	<i>L. kalonensis</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
28	<i>L. kecil</i> (Matsui, Belabut, Ahmad & Yong, 2009)	Matsui et al. 2009
29	<i>L. kbasiorum</i> (Das, Tron, Rangad & Hooroo, 2010)	Das et al. 2010
30	<i>L. lateralis</i> (Anderson, 1871)	Anderson 1871; Humtsoe et al. 2008
31	<i>L. laui</i> (Sung, Yang & Wang, 2014)	Sung et al. 2014
32	<i>L. liui</i> (Fei & Ye, 1990)	Fei et al. 2009; Sung et al. 2014
33	<i>L. macrops</i> (Duong, Do, Ngo, Nguyen & Poyarkov, 2018)	Duong et al. 2018
34	<i>L. maculosa</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
35	<i>L. mangshanensis</i> (Hou, Zhang, Hu, Li, Shi, Chen, Mo, & Wang, 2018)	Hou et al. 2018
36	<i>L. maershanensis</i> (Yuan, Sun, Chen, Rowley & Che, 2017)	Yuan et al. 2017
37	<i>L. marmorata</i> (Matsui, Zainudin & Nishikawa, 2014)	Matsui et al. 2014b
38	<i>L. maura</i> (Inger, Lakim, Biun & Yambun, 1997)	Inger et al. 1997
39	<i>L. melanoleuca</i> (Matsui, 2006)	Matsui 2006
40	<i>L. melica</i> (Rowley, Stuart, Neang & Emmett, 2010)	Rowley et al. 2010b
41	<i>L. minima</i> (Taylor, 1962)	Taylor 1962; Ohler et al. 2011
42	<i>L. mjobergi</i> (Smith, 1925)	Eto et al. 2015
43	<i>L. namdongensis</i> (Hoang, Nguyen, Luu, Nguyen & Jiang, 2019)	Hoang et al. 2019
44	<i>L. nahangensis</i> (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
45	<i>L. natunae</i> (Günther, 1895)	Günther 1895
46	<i>L. nokrekensis</i> (Mathew & Sen, 2010)	Mathew and Sen 2010
47	<i>L. nyx</i> (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
48	<i>L. oshanensis</i> (Liu, 1950)	Fei et al. 2009
49	<i>L. pallida</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
50	<i>L. palmata</i> Inger & Stuebing, 1992	Inger and Stuebing 1992
51	<i>L. parva</i> Dring, 1983	Dring 1983
52	<i>L. pelodytoides</i> (Boulenger, 1893)	Boulenger 1893; Ohler et al. 2011
53	<i>L. petrops</i> (Rowley, Dau, Hoang, Le, Cutajar & Nguyen, 2017)	Rowley et al. 2017a
54	<i>L. picta</i> (Malkmus, 1992)	Malkmus 1992

ID	<i>Leptobranchella</i> species	Literature obtained
55	<i>L. platycephala</i> (Dehling, 2012)	Dehling 2012a
56	<i>L. pluvialis</i> (Ohler, Marquis, Swan & Grosjean, 2000)	Ohler et al. 2000, 2011
57	<i>L. puhoatensis</i> (Rowley, Dau & Cao, 2017)	Rowley et al. 2017b
58	<i>L. purpuraventra</i> Wang, Li, Li, Chen & Wang, 2019	Wang et al. 2019
59	<i>L. purpurus</i> (Yang, Zeng & Wang, 2018)	Yang et al. 2018
60	<i>L. pyrrophos</i> (Poyarkov, Rowley, Gogoleva, Vassilieva, Galoyan & Orlov, 2015)	Poyarkov et al. 2015
61	<i>L. rowleyae</i> (Nguyen, Poyarkov, Le, Vo, Ninh, Duong, Murphy & Sang, 2018)	Nguyen et al. 2018
62	<i>L. sabahmontana</i> (Matsui, Nishikawa & Yambun, 2014)	Matsui et al. 2014a
63	<i>L. serasanae</i> Dring, 1983	Dring 1983
64	<i>L. shangsiensis</i> Chen, Liao, Zhou & Mo, 2019	Chen et al. 2019
65	<i>L. sola</i> (Matsui, 2006)	Matsui 2006
66	<i>L. sungi</i> (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
67	<i>L. suiyangwmsi</i> (Luo, Xiao, Gao & Zhou, 2020)	Luo et al. 2020
68	<i>L. tadungensis</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
69	<i>L. tamdil</i> (Sengupta, Sailo, Lalremsanga, Das & Das, 2010)	Sengupta et al. 2010
70	<i>L. tengchongensis</i> (Yang, Wang, Chen & Rao, 2016)	Yang et al. 2016
71	<i>L. tuberosa</i> (Inger, Orlov & Darevsky, 1999)	Inger et al. 1999
72	<i>L. ventripunctata</i> (Fei, Ye & Li, 1990)	Fei et al. 2009
73	<i>L. wuhuangmontis</i> Wang, Yang & Wang, 2018	Wang et al. 2018
74	<i>L. yingjiangensis</i> (Yang, Zeng & Wang, 2018)	Yang et al. 2018
75	<i>L. yunkaiensis</i> Wang, Li, Lyu & Wang, 2018	Wang et al. 2018
76	<i>L. zhangyapingi</i> (Jiang, Yan, Suwannapoom, Chomdej & Che, 2013)	Jiang et al. 2013

In PCA for male group, the total variation of the first two principal components was 64.6%. In males on the two-dimensional plots of PC1 vs. PC2, the undescribed species could be distinctly separated from *L. bijie* and *L. purpuraventra* (Fig. 3). The results of Mann-Whitney *U* tests indicated that in males, the new taxon was significantly different from *L. bijie* and *L. purpuraventra* on many morphometric characters (all *p*-values < 0.05; Table 4).

There were many differences in sonograms and waveforms of calls between the new species *L. bijie*, and *L. purpuraventra*. Firstly, a call contains 1–4 notes in the new species and only contains two notes of each call in *L. bijie* and *L. purpuraventra*. Secondly, the dominant frequency of the new species is higher than *L. bijie* and *L. purpuraventra*.

Based on the molecular, morphological, and bioacoustics differences, the specimens from Chishui City, Guizhou Province, China represent a new species which is described as follows.

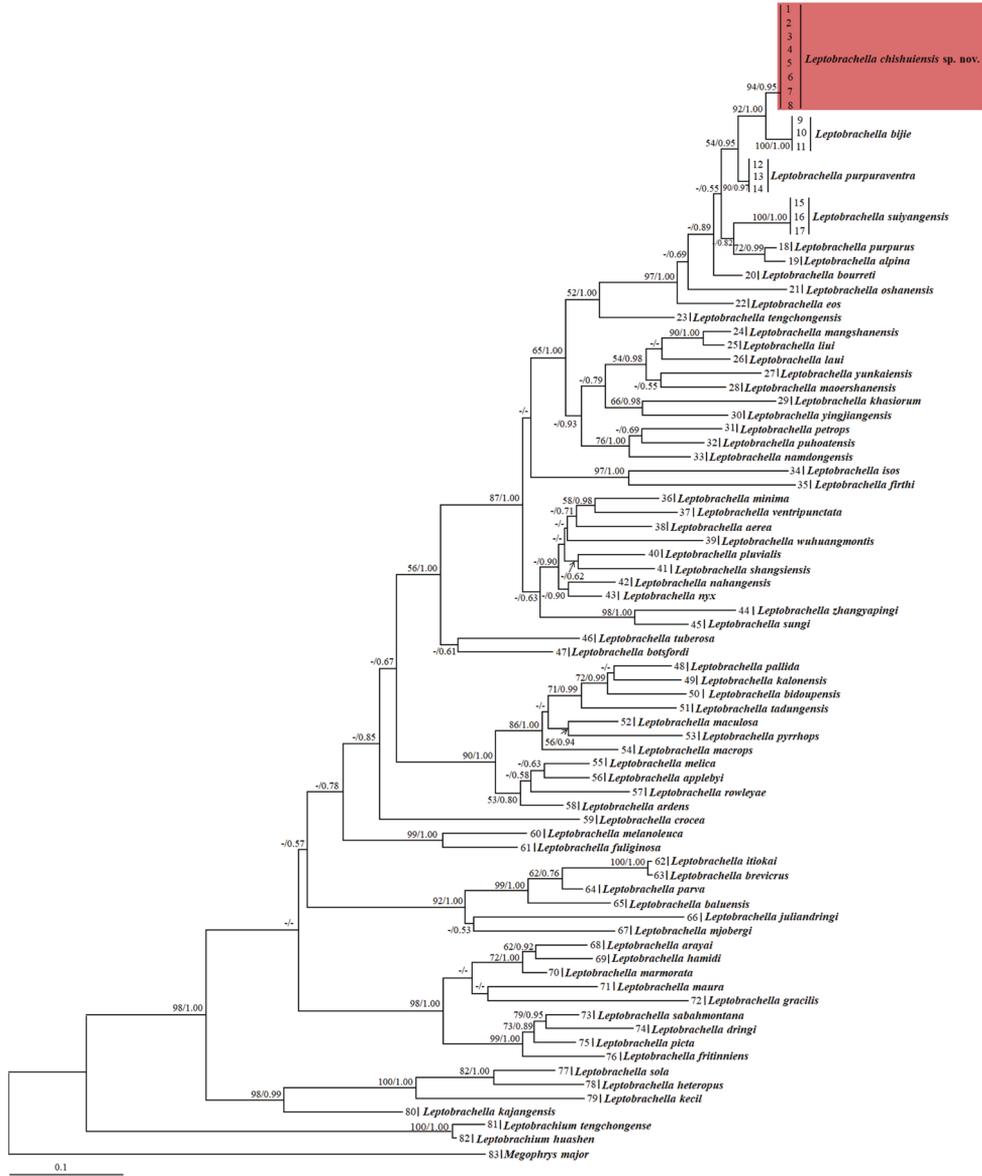
## Taxonomic account

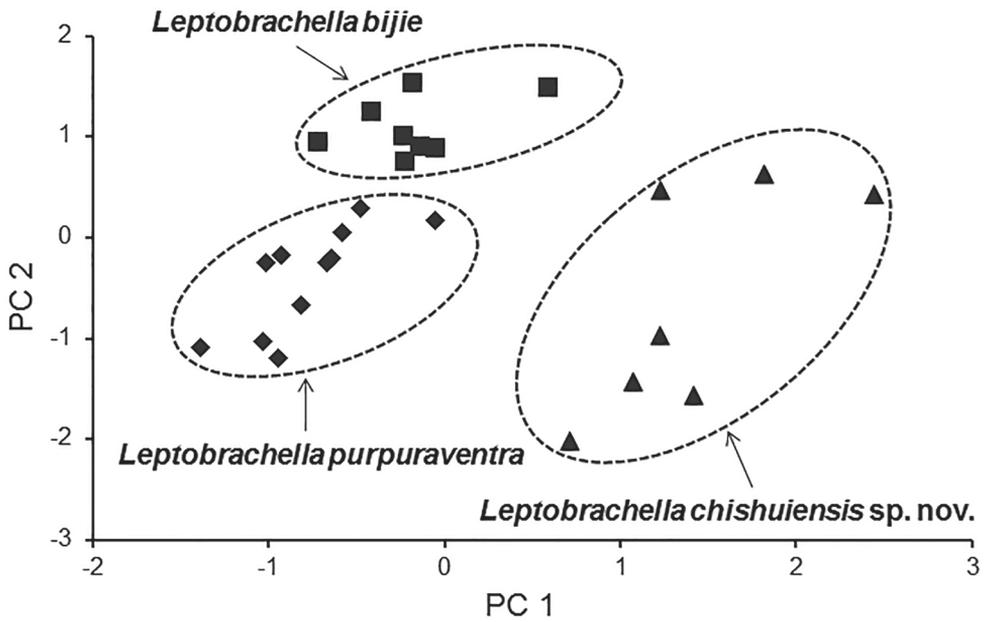
### *Leptobranchella chishuiensis* sp. nov.

<http://zoobank.org/DE8BA5C5-CB7B-4872-B489-61E7EFCF9B8C>

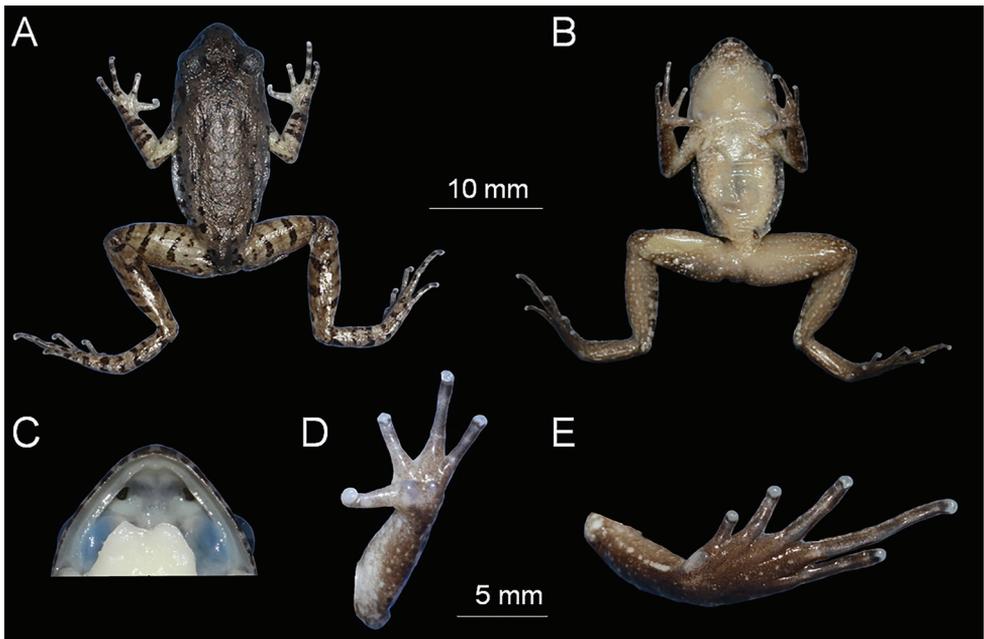
Figs 4–6; Tables 1–5

**Type material. Holotype.** CIBCS20190518047, adult male (Figs 4, 5), collected by Shi-Ze Li in Chishui National Nature Reserve (28.436708N, 105.997794E, ca. 465 m a. s. l.), Chishui City, Guizhou Province, China on 18 May 2019.





**Figure 3.** Plots of the first principal component (PC1) versus the second (PC2) for *Leptobrachella chishuiensis* sp. nov., *L. bijie*, and *L. purpuraventra* in males from a principal component analysis.



**Figure 4.** The holotype specimen CIBCS20190518047 of *Leptobrachella chishuiensis* sp. nov. in preservative **A** dorsal view **B** ventral view **C** frontal view of tongue **D** ventral view of hand **E** ventral view of foot.

**Diagnosis.** *Leptobranchella chishuiensis* sp. nov. is assigned to the genus *Leptobranchella* based on molecular phylogenetic analyses and the following morphological characters: small body size; having an elevated inner metacarpal tubercle; having macro-glands on body (including supra-axillary, femoral and ventrolateral glands); lacking vomerine teeth; having small tubercles on eyelids; anterior tip of snout with whitish vertical bar (Dubois 1983; Matsui 1997, 2006; Lathrop et al. 1998; Delorme et al. 2006; Das et al. 2010; Luo et al. 2020).

*Leptobranchella chishuiensis* sp. nov. could be distinguished from its congeners by a combination of the following characters: (1) small body size (SVL 30.8–33.4 mm in seven adult males, and 34.2 mm in one adult female); (2) dorsal skin shagreened, some of the granules forming longitudinal short skin ridges; (3) tympanum distinctly discernible, slightly concave; (4) internasal distance longer than interorbital distance; (5) supra-axillary, femoral, pectoral and ventrolateral glands distinctly visible; (6) absence of webbing and lateral fringes on fingers; (7) toes with rudimentary webbing and shallow lateral fringes; (8) relative finger lengths  $II < IV < I < III$ ; (9) heels overlapped when thighs are positioned at right angles to the body; and (10) tibia-tarsal articulation reaches the tympanum.

**Description of holotype.** Measurements in mm. Adult male (CIBCS20190518047). SVL 32.4. Head length slightly longer than head width (HDL/HDW ratio 1.04); snout slightly protruding, projecting slightly beyond margin of the lower jaw; nostril closer to snout than eye; canthus rostralis gently rounded; loreal region slightly concave; interorbital space flat, internarial distance longer than interorbital distance (IND/IOD ratio 1.23); pineal ocellus absent; vertical pupil; snout length larger than eye diameter; tympanum distinct, rounded, and slightly concave, diameter smaller than that of the eye (TMP/ED ratio 0.57); upper margin of tympanum in contact with supratympanic ridge; distinct black supratympanic line present; vomerine teeth absent; tongue notched behind; supratympanic ridge distinct, extending from posterior corner of eye to supra-axillary gland.

Tips of fingers rounded, slightly swollen; relative finger lengths  $II < IV < I < III$  (FIL/FIIL ratio 1.1, FIVL/FIIL ratio 1.03); absence of webbing; nuptial pad and sub-articular tubercles absent; inner palmar tubercle large, rounded separated from small, round outer palmar tubercle.

Hindlimbs slender, tibia 49% of snout-vent length; heels overlapped when thighs are positioned at right angles to the body, tibiotarsal articulation reaching tympanum when leg stretched forward; tibia length slightly longer than thigh length; relative toe lengths  $I < II < V < III < IV$ ; tips of toes rounded, slightly dilated; subarticular tubercle small, distinct at the base of each toes; toes without webbing; narrow lateral fringes present on all toes; inner metatarsal tubercle present, large, oval, outer metatarsal tubercle absent; dorsal surface shagreened and granular, some of the granules forming short longitudinal folds on the flank of dorsal; ventral skin smooth; dense tiny granules present on surface of chest and ventral surface of thigh and tibia; pectoral gland and

femoral gland oval, distinctly visible. Ventrolateral gland distinctly visible and forming an incomplete line.

**Colouration of holotype in life.** Dorsum brown, with small, distinct darker brown markings and spots and scattered with irregular light orange pigmentation. A dark brown inverted triangular pattern between anterior corner of eyes. Tympanum brown, a dark brown bar above tympanum, and a dark brown bar under the eye; transverse dark brown bars on dorsal surface of limbs; distinct dark brown blotches on flanks from groin to axilla, longitudinally in two rows; elbow and upper arms with dark bars and distinct coppery orange coloration; fingers and toes with distinct dark bars. Ventral surface of throat grey purple, chest and belly white, presence of distinct nebulous greyish speckling on flanks; ventral surface of limbs grey purple. Supra-axillary gland, femoral, pectoral and ventrolateral glands white (Fig. 5).

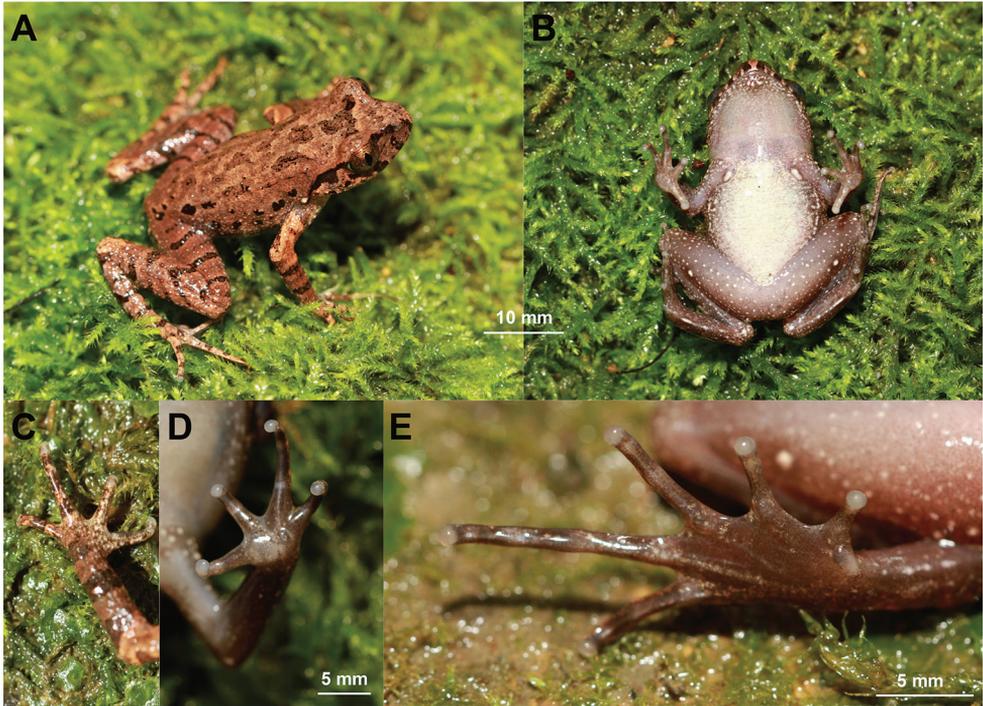
**Preserved holotype colouration.** Dorsum of body and limbs fade to dark brown; transverse bars on limbs become more distinct ventral surface of body and limbs fade to greyish white. Supra-axillary, femoral, pectoral and ventrolateral glands fade to greyish white (Fig. 4).

**Variations.** Morphological measurements were showed in Table 2. All specimens were similar in morphology but some individuals different from the holotype in color pattern. In some adult males, a dark brown inverted triangular pattern between anterior corner of eyes, in connected to the dark brown W-shaped marking on interorbital region (Fig. 6A); in adult female, the color of dorsum is blacker (Fig. 6B) and some patchiness on the chest and the flank of belly (Fig. 6C); in some adult males, the throat and bell creamy and white patchiness sparse on the ventral surface of limbs (Fig. 6D); in some specimens, the tibiotarsal articulation reaching tympanum to eye when leg stretched forward.

**Advertisement call.** A total of 32 advertisement calls of *Leptobrachella chishuiensis* sp. nov. were recorded in Chishui City, Guizhou Province, China on 18 May 2019 between 21:00–22:00. The call description is based on recordings of the holotype CIBCS20190518047 (Fig. 7) from a branch of bush nearby a stream. Each call contains 1–4 notes (mean  $2.34 \pm 0.827$ ,  $N = 32$ ). Call duration was 75–353 ms (mean  $200 \pm 67$ ,  $N = 32$ ). Call interval was 8–98 ms (mean  $60 \pm 21$ ,  $N = 31$ ) with a peak frequency was  $6140.15 \pm 69.35$  (6064–6284 Hz,  $N = 32$ ). Each note had a duration of 52–950 ms (mean  $104 \pm 107$ ,  $N = 69$ ), and the intervals between notes had a duration of 0.1–25 ms (mean  $5.3 \pm 8.5$ ,  $N = 37$ ). Amplitude modulation within note was apparent, beginning with high energy pulses then decreasing towards the end of each note.

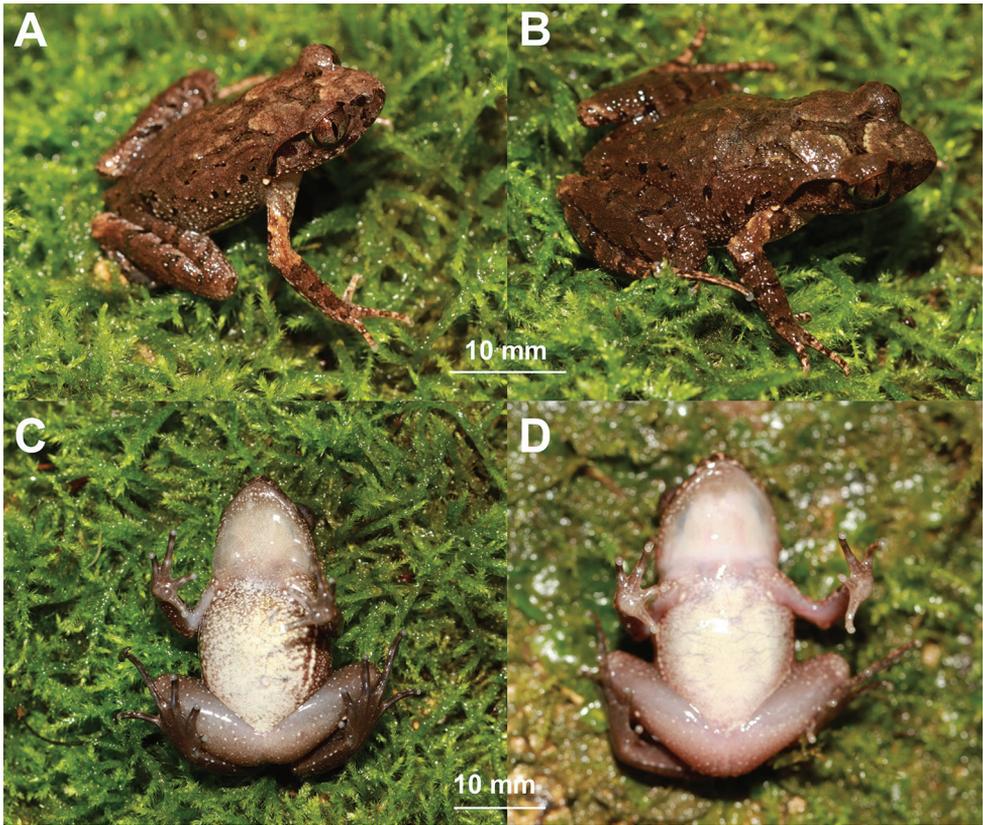
**Secondary sexual characteristics.** Adult males with a large subgular vocal sac, and nupital pads and spines absent.

**Comparisons.** The new species was compared with 52 congeners on morphology (Table 4). By having small body size (SVL 30.8–33.4 mm in seven adult males, and 34.2 mm in one adult female), *Leptobrachella chishuiensis* sp. nov. differs from the larger *L. bourreti* (42.0–45.0 mm in females), *L. eos* (33.1–34.7 mm in males and 40.7 in female), *L. lateralis* (36.6 mm in females), *L. nahangensis* (40.8 mm in male), *L. nyx* (37.0–41.0 mm in females), *L. platycephalus* (35.1 mm in male), *L. sungi* (48.3–



**Figure 5.** Photos of the holotype CIBCS20190518047 of *Leptobrachella chishuiensis* sp. nov. in life **A** dorsal view **B** ventral view **C** dorsal view of hand **D** ventral view of hand **E** ventral view of foot.

52.7 mm in males and 56.7–58.9 mm in females), and *L. zhangyapingi* (45.8–52.5 mm in males), and differs from the smaller *L. aerea* (25.1–28.9 mm in males), *L. alpina* (24.0–26.4 mm in males), *L. applebyi* (19.6–22.3 mm in males), *L. ardens* (21.3–24.7 mm in males), *L. baluensis* (14.9–15.9 mm in males), *L. bidoupensis* (18.5–25.4 mm in males), *L. bijie* (29.0–30.4 mm in males), *L. bondangensis* (17.8 mm in male), *L. brevicrus* (17.1–17.8 mm in males), *L. crocea* (22.2–27.3 mm in males), *L. frthi* (26.4–29.2 mm in males), *L. fuliginosa* (28.2–30.0 mm in males), *L. fusca* (16.3 mm in male), *L. isos* (23.7–27.9 mm in males), *L. itiokai* (15.2–16.7 mm in males), *L. julian-dringi* (17.0–17.2 mm in males and 18.9–19.1 mm in females), *L. khasiorum* (24.5–27.3 mm in males), *L. lateralis* (26.9–28.3 mm in males), *L. laui* (24.8–26.7 mm in males), *L. liui* (23.0–28.7 mm in males), *L. macrops* (28.0–29.3 mm in males), *L. maculosa* (24.2–26.6 mm in males), *L. mangshanensis* (22.22–27.76 mm in males), *L. melica* (19.5–22.8 mm in males), *mjobergi* (15.7–19.0 mm in males), *L. natunae* (17.6 mm in male), *L. pallida* (24.5–27.7 mm in males), *L. palmate* (14.4–16.8 mm in males), *L. parva* (15.0–16.9 mm in males), *L. petrops* (23.6–27.6 mm in males), *L. pluvialis* (21.3–22.3 mm in males), *L. purpuraventra* (27.3–29.8 mm in males), *L. puhoatensis* (24.2–28.1 mm in males), *L. purpura* (25.0–27.5 mm in males), *L. rowley-ae* (23.4–25.4 mm in males), *L. shangsiensis* (24.9–29.4 mm in males), *L. suiyangensis* (28.7–29.7 mm in males), *L. tadungensis* (23.3–28.2 mm in males), *L. tengchongensis*



**Figure 6.** Colour variation in *Leptobranchella chishuiensis* sp. nov. **A** dorsal view of the male specimen CIBCS20190518042 **B** dorsal view of the female specimen CIBCS20190518046 **C** ventral view of the female specimen CIBCS20190518046 **D** ventral view of the female specimen CIBCS20190518049.

(23.9–26.0 mm in males), *L. tuberosa* (24.4–29.5 mm in males), *L. ventripunctata* (25.5–28.0 mm in males), *L. wuhuangmontis* (25.6–30.0 mm in males), *L. yingjiangensis* (25.7–27.6 mm in males), and *L. yunkaiensis* (25.9–29.3 mm in males).

By supra-axillary and ventrolateral glands present, *Leptobranchella chishuiensis* sp. nov. differs from *L. arayai*, *L. dringi*, *L. fritinniensis*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*, *L. picta*, *L. platycephala*, *L. sabahmontana*, and *L. sola* (vs. absent in the latter).

By having black spots on flanks, *Leptobranchella chishuiensis* sp. nov. differs from *L. aerea*, *L. botsfordi*, *L. frithi*, and *L. tuberosa* (vs. lacking in the latter).

By toes with rudimentary webbing, *Leptobranchella chishuiensis* sp. nov. differs from *L. kalonensis* and *L. oshanensis* (vs. lacking webbing on toes in the latter), and differs from *L. pelodytoides* (vs. toes with wide webbing in the latter).

By having shallow lateral fringes on toes, *Leptobranchella chishuiensis* sp. nov. differs from *L. aerea*, *L. frithi*, *L. liui*, and *L. yunkaiensis* (vs. having prominently wide

lateral fringes on toes in the latter), and differs from *L. kalonensis*, *L. macrops*, *L. minima*, *L. nyx*, *L. oshanensis*, *L. pyrrhops*, and *L. tuberosa* (vs. lacking lateral fringes on toes in the latter).

By having dorsal surface shagreened and granular, lacking enlarge tubercles or warts, *Leptobranchella chishuiensis* sp. nov. differs from the following species: *L. bourreti* (dorsum smooth with small warts), *L. fuliginosa* (dorsum smooth with fine tubercles), *L. liui* (dorsum with round tubercles), *L. macrops* (dorsum roughly granular with large tubercles), *L. maoershanensis* (dorsum shagreened with tubercles), *L. minima* (dorsum smooth), *L. nyx* (dorsum with round tubercles), *L. pelodytoides* (dorsum with small, smooth warts), *L. tamdil* (dorsum weakly tuberculate, with low, oval tubercles), *L. tuberosa* (dorsum highly tuberculate), *L. yunkaiensis* (dorsum with raised warts), and *L. wuhuangmontis* (dorsum rough with conical tubercles).

By the finger II < I, *Leptobranchella chishuiensis* sp. nov. differs from *L. tamdil* (vs. II > I in the latter).

By head length slightly longer than wide, *Leptobranchella chishuiensis* sp. nov. differs from *L. namdongensis* (vs. head wider than long in the latter).

Six *Leptobranchella* species were reported to be distributed in Guizhou Province, China, they are: *L. liui*, *L. oshanensis*, *L. purpuraventra*, *L. bijie*, *L. ventripunctata*, and *L. suiyangensis* (Fei et al. 2012; Li et al. 2016; Wang et al. 2019; Luo et al. 2020). We make a comparative note between them and the new species as follows. *Leptobranchella chishuiensis* sp. nov. differs from *L. liui* by having shallow lateral fringes on toes (vs. wide lateral fringes on the toes in the latter), dorsal surface shagreened with small granules, lacking enlarge tubercles or warts (vs. dorsum with round tubercles in the latter); from *L. oshanensis* by having rudimentary webbing on the toes (vs. lack webbing on the toes in the latter), having shallow lateral fringes on toes (vs. lacking lateral fringes on the toes in the latter), from *L. suiyangensis* by heels overlapping when thighs are positioned at right angles to the body (vs. just meeting in the latter), tibia-tarsal articulation reaches tympanum or tympanum to eye (vs. reaches to the anterior corner of eye in the latter); from *L. ventripunctata* by bigger body size (SVL 30.8–33.4 mm in adult males vs. SVL 25.5–28.0 mm in males in the latter), chest and belly without large dark brown spots (vs. with large dark brown spots in the latter).

*Leptobranchella chishuiensis* sp. nov. is genetically closer to *L. bijie* and *L. purpuraventra*. The new species differs from *L. bijie* by the following characters: larger body size (SVL 30.8–33.4 mm in males vs. SVL 29.0–30.4 mm in males in the latter), internasal distance longer than interorbital distance (vs. equal to interorbital distance in the latter), heels overlapping (vs. just meeting in the latter), tibia-tarsal articulation reaches the tympanum or tympanum to eye (vs. reaching the region between middle of eye to anterior corner of eye in the latter), one call contains 1–4 notes (vs. 2 notes in each call in the latter), having shorter call interval ( $60 \pm 21$ ,  $N = 31$  in the new species vs.  $101.9 \pm 6.4$ ,  $N = 33$  in the latter), having significantly higher value of SVL in males, and having significantly higher value of HDL, HDW, SL, IND, IOD, TEY, TL and FL to SVL in males (all P-values < 0.05; Table 5).

**Table 4.** Diagnosis characters on morphology of *Leptobrachella chishuiensis* sp. nov. from other congeners.

ID	Species	Male SVL (mm)	Black spots on flanks	Toes webbing	Fringes on toes	Ventral coloration	Dorsal skin texture
1	<i>Leptobrachella chishuiensis</i> sp. nov.	30.8–33.4	Yes	Rudimentary	Narrow	White with distinct nebulous greyish speckling on chest and ventrolateral flanks	Shagreened and granular
2	<i>L. aerea</i>	25.1–28.9	No	Rudimentary	Wide	Near immaculate creamy white, brown speckling on margins	Finely tuberculate
3	<i>L. alpina</i>	24.0–26.4	Yes	Rudimentary	Wide in males	Creamy-white with dark spots	Relatively smooth, some with small warts
4	<i>L. applebyi</i>	19.6–22.3	Yes	Rudimentary	No	Reddish brown with white speckling	Smooth
5	<i>L. ardens</i>	21.3–24.7	Yes	No	No	Reddish brown with white speckling	Smooth- finely shagreened
6	<i>L. bidupensis</i>	18.5–25.4	Yes	Rudimentary	Weak	Reddish brown with white speckling	Smooth
7	<i>L. bijie</i>	29.0–30.4	Yes	Rudimentary	Narrow	White with distinct nebulous greyish speckling on chest and ventrolateral flanks	Shagreened and granular
8	<i>L. bousfordi</i>	29.1–32.6	No	Rudimentary	Narrow	Reddish brown with white speckling	Shagreened
9	<i>L. bourreti</i>	28.0–36.2	Yes	Rudimentary	Weak	Creamy white	Relatively smooth, some with small warts
10	<i>L. crocea</i>	22.2–27.3	No	Rudimentary	No	Bright orange	Highly tuberculate
11	<i>L. eos</i>	33.1–34.7	No	Rudimentary	Wide	Creamy white	Shagreened
12	<i>L. fritzi</i>	26.4–29.2	No	Rudimentary	Wide in males	Creamy white	Shagreened with fine tubercles
13	<i>L. fuliginosa</i>	28.2–30.0	Yes	Rudimentary	Weak	White with brown dusting	Nearly smooth, few tubercles
14	<i>L. isos</i>	23.7–27.9	No	Rudimentary	Wide in males	Creamy white with white dusting on margins	Mostly smooth, females more tuberculate
15	<i>L. kalomenis</i>	25.8–30.6	Yes	No	No	Pale, speckled brown	Smooth
16	<i>L. bhastorum</i>	24.5–27.3	Yes	Rudimentary	Wide	Creamy white	Isolated, scattered tubercles
17	<i>L. lateralis</i>	26.9–28.3	Yes	Rudimentary	No	Creamy white	Roughly granular
18	<i>L. laui</i>	24.8–26.7	Yes	Rudimentary	Wide	Creamy white with dark brown dusting on margins	Round granular tubercles
19	<i>L. lini</i>	23.0–28.7	Yes	Rudimentary	Wide	Creamy white with dark brown spots on chest and margins	Round granular tubercles with glandular folds
20	<i>L. macrops</i>	28.0–29.3	Yes	Rudimentary	No	Greyish-violet with white speckling	Roughly granular with larger tubercles
21	<i>L. maculosa</i>	24.2–26.6	Yes	No	No	Brown, less white speckling	Mostly smooth
22	<i>L. mangshanensis</i>	22.22–27.76	Yes	Rudimentary	Weak	White speckles on throat and belly	Nearly smooth
23	<i>L. maershanensis</i>	25.2–30.4	Yes	Rudimentary	Narrow	Creamy white chest and belly with irregular black spots	Longitudinal folds
24	<i>L. marmorata</i>	32.3–38.0	Yes	Rudimentary	No	Chest and belly immaculate white	Nearly smooth, scattered with small tubercles of varying sizes
25	<i>L. melica</i>	19.5–22.8	Yes	Rudimentary	No	Reddish brown with white speckling	Smooth
26	<i>L. ninima</i>	25.7–31.4	Yes	Rudimentary	No	Creamy white	Smooth
27	<i>L. nabanensis</i>	40.8	Yes	Rudimentary	No	Creamy white with light speckling on throat and chest	Smooth
28	<i>L. namdongensis</i>	30.9	Yes	Rudimentary	No	Creamy white with brown dusting on margins	Finely tuberculate

ID	Species	Male SVL (mm)	Black spots on flanks	Toes webbing	Fringes on toes	Ventral coloration	Dorsal skin texture
29	<i>L. nobrekensis</i>	26.0–33.0	Yes	Rudimentary	unknown	White with distinct nebulous greyish speckling on chest and ventrolateral flanks	Tubercles and longitudinal folds
30	<i>L. nyx</i>	26.7–32.6	Yes	Rudimentary	No	Creamy white with white with brown margins	Rounded tubercles
31	<i>L. oshanensis</i>	26.6–30.7	Yes	No	No	Whitish with no markings or only small, light grey spots	Smooth with few glandular ridges
32	<i>L. pallida</i>	24.5–27.7	No	No	No	Reddish brown with white speckling	Tuberculate
33	<i>L. pelodyoides</i>	27.5–32.3	Yes	Wide	Narrow	Whitish	Small, smooth warts
34	<i>L. petrops</i>	23.6–27.6	No	No	Narrow	Immaculate creamy white	Highly tuberculate
35	<i>L. pluvialis</i>	21.3–22.3	Yes	Rudimentary	No	Dirty white with dark brown marbling	Smooth, flattened tubercles on flanks
36	<i>L. puboatensis</i>	24.2–28.1	Yes	Rudimentary	Narrow	Reddish brown with white dusting	Longitudinal skin ridges
37	<i>L. purpurus</i>	25.0–27.5	Yes	Rudimentary	Wide	Dull white with indistinct grey dusting	Shagreen with small tubercles
38	<i>L. purpuraventra</i>	27.3–29.8	Yes	Rudimentary	Narrow	Grey purple with distinct nebulous greyish speckling on chest and ventrolateral flanks	Shagreened and granular
39	<i>L. pyrhopis</i>	30.8–34.3	Yes	Rudimentary	No	Reddish brown with white speckling	Slightly shagreened
40	<i>L. rouleyae</i>	23.4–25.4	Yes	No	No	Pinkish milk-white to light brown chest and belly with numerous white speckles	Smooth with numerous tiny tubercles
41	<i>L. sabahmontanus</i>	25–28	Yes	Rudimentary	Narrow	Cream-coloured with dark brown speckling	with tiny tubercles, weakly wrinkled
42	<i>L. shangienensis</i>	24.9–29.4		Rudimentary	Narrow	Ventral surface yellowish creamy-white with marble texture	Smooth
43	<i>L. sangi</i>	48.3–52.7	No or small	Wide	Weak	White	Granular
44	<i>L. suiyoungensis</i>	28.7–29.7	Yes	Rudimentary	Narrow	Yellowish creamy-white with marble texture chest and belly or with irregular light brown speckling	Shagreen with small granules
45	<i>L. taadangensis</i>	23.3–28.2	Yes	No	No	Reddish brown with white speckling	Smooth
46	<i>L. tamiil</i>	32.3	Yes	Wide	Wide	White	Weakly tuberculate
47	<i>L. tengchongensis</i>	23.9–26.0	Yes	Rudimentary	Narrow	White with dark brown blotches	Shagreened with small tubercles
48	<i>L. tuberosa</i>	24.4–29.5	No	Rudimentary	No	White with small grey spots/streaks	Highly tuberculate
49	<i>L. ventripunctata</i>	25.5–28.0	Yes	Rudimentary	No	Chest and belly with dark brown spots	Longitudinal skin ridges
50	<i>L. wuhuaugamontis</i>	25.6–30.0	Yes	Rudimentary	Narrow	Greyish white mixed by tiny white and black dots	Rough, scattered with dense conical tubercles
51	<i>L. yingjiaugensis</i>	25.7–27.6	Yes	Rudimentary	Wide	Creamy white with dark brown flecks on chest and margins	Shagreened with small tubercles
52	<i>L. yunkaimensis</i>	25.9–29.3	Yes	Rudimentary	Wide	Belly pink with distinct or indistinct speckling	Shagreened with short skin ridges and raised warts
53	<i>L. zhangyapingi</i>	45.8–52.5	No	Rudimentary	Wide	Creamy-white with white with brown	Mostly smooth with distinct tubercles

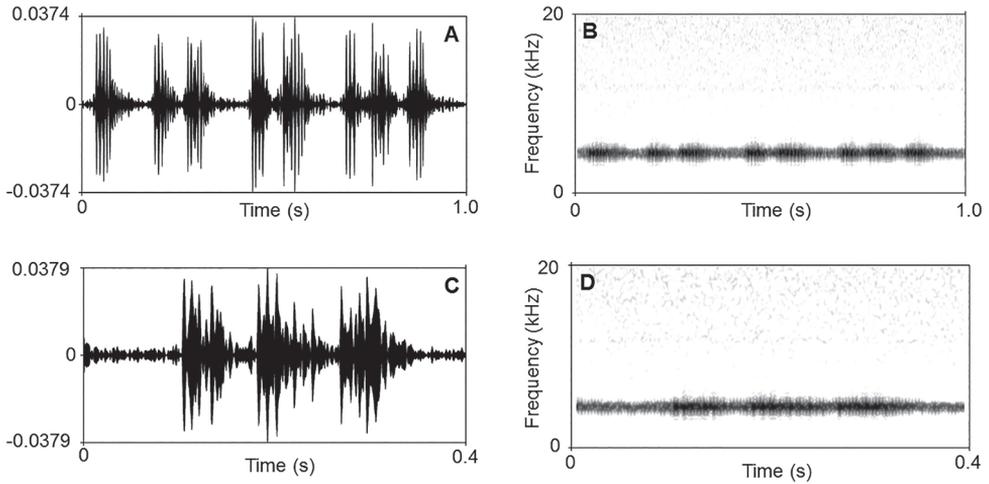
**Table 5.** Morphometric comparisons between *Leptobranchella chishuiensis* sp. nov and its relatives. Units in mm. See abbreviations for morphometric characters in Materials and methods section.

	<i>Leptobranchella chishuiensis</i> sp. nov.			<i>L. bijie</i>		<i>L. purpuraventra</i>		<i>P</i> -value from Mann-Whitney	
	Male ( <i>N</i> = 7)		Female ( <i>N</i> = 1)	Male ( <i>N</i> = 8)		Male ( <i>N</i> = 11)		<i>U</i> test	
	Range	Mean ± SD		Range	Mean ± SD	Range	Mean ± SD	<i>L. chishuiensis</i> vs. <i>L. bijie</i>	<i>L. chishuiensis</i> vs. <i>L. purpuraventra</i>
SVL	30.8–33.4	32.1 ± 1.0	34.2	29.0–30.4	29.7 ± 0.6	27.3–29.8	28.9 ± 0.8	0.001	0.000
HDL	11.1–12.3	11.8 ± 0.4	12.7	10.0–10.6	10.2 ± 0.2	9.6–10.3	9.9 ± 0.3	0.021	0.013
HDW	10.6–11.9	11.4 ± 0.5	12.0	9.5–10.2	9.8 ± 0.3	9.3–9.8	9.6 ± 0.2	0.001	0.001
SL	4.8–5.8	5.2 ± 0.3	5.3	4.0–4.7	4.2 ± 0.2	3.5–4.1	3.8 ± 0.2	0.002	0.000
IND	3.5–3.8	3.7 ± 0.1	3.4	2.8–3.4	3.1 ± 0.2	2.7–3.5	3.1 ± 0.2	0.003	0.094
IOD	2.7–3.1	3.0 ± 0.2	2.7	2.8–3.4	3.1 ± 0.2	2.6–3.2	2.9 ± 0.2	0.008	0.016
UEW	3.0–3.3	3.2 ± 0.1	3.0	/	/	/	/	/	/
ED	4.0–5.0	4.4 ± 0.4	4.4	3.6–4.1	3.8 ± 0.2	3.1–3.6	3.4 ± 0.2	0.064	0.001
TYD	2.0–2.6	2.3 ± 0.2	2.4	1.9–2.2	2.0 ± 0.1	1.7–1.9	1.8 ± 0.1	0.247	0.000
TEY	1.2–1.6	1.4 ± 0.2	1.2	0.9–1.1	1.0 ± 0.1	1.1–1.3	1.2 ± 0.1	0.002	0.751
LAL	14.7–17.0	15.6 ± 0.8	16.3	14.0–14.8	14.3 ± 0.3	12.6–14.0	13.3 ± 0.4	0.643	0.016
LW	2.6–3.2	3.0 ± 0.2	3.3	/	/	/	/	/	/
ML	7.9–8.8	8.2 ± 0.39	8.7	7.4–8.3	7.8 ± 0.3	7.0–7.7	7.4 ± 0.2	0.247	0.964
FIL	3.0–3.8	3.4 ± 0.3	3.4	/	/	/	/	/	/
FIIIL	2.8–3.4	3.0 ± 0.2	3.0	/	/	/	/	/	/
FIIIL	4.9–5.5	5.1 ± 0.2	5.6	/	/	/	/	/	/
FIVL	2.9–3.5	3.2 ± 0.2	3.3	/	/	/	/	/	/
HLL	43.3–49.7	49.7 ± 2.7	49.4	43.0–45.5	43.7 ± 0.8	39.0–44.6	41.4 ± 2.2	0.487	0.113
THL	13.7–17.1	15.1 ± 1.2	15.3	/	/	/	/	/	/
TW	3.3–4.3	3.8 ± 0.4	4.2	/	/	/	/	/	/
TL	14.9–16.8	15.6 ± 0.6	16.0	13.5–14.4	13. ± 0.3	12.5–14.0	13.1 ± 0.5	0.005	0.001
TFL	20.9–22.3	21.7 ± 0.6	22.2	/	/	/	/	/	/
FL	14.4–15.9	15.1 ± 0.5	16.3	13.0–13.8	13.3 ± 0.2	12.1–13.2	12.6 ± 0.4	0.004	0.000

*Leptobranchella chishuiensis* sp. nov. differs from *L. purpuraventra* by larger body size (SVL 30.8–33.4 mm in seven adult males vs. SVL 27.3–29.8 mm in eleven adult males in the latter), tibia-tarsal articulation reaches the tympanum or tympanum to eye (vs. reaching the middle of eye in the latter), the call contains 1–4 notes (vs. 2 notes in each call in the latter), having longer call duration ( $200 \pm 67$ ,  $N = 32$  vs.  $192.2 \pm 13.0$  as the longest call duration in *L. purpuraventra*), shorter call interval ( $60 \pm 21$ ,  $N = 31$  vs.  $90.8 \pm 5.6$ ,  $N = 20$  as the shortest call interval in *L. bijie*), having significantly higher value of SVL in males, and having significantly higher value of SVL, HDL, HDW, SL, IOD, ED, TYD, LAL, TL and FL to SVL in males (all *P*-values < 0.05; Table 5).

**Ecology.** *Leptobranchella chishuiensis* sp. nov. is known from the type locality, Chishui National Nature Reserve (28.383333–28.45N, 105.05–109.75E), Chishui City, Guizhou Province, China at elevations between 270–604 m a. s. l. This new species is found in bamboo forest nearby the streams (Fig. 8), and four sympatric amphibian species, i.e. *Megophrys omeimontis*, *Odorrana margaratae* (Liu, 1950), *Zhangixalus omeimontis* (Stejneger, 1924), and *Rana omeimontis* Ye & Fei, 1993 were found nearby.

**Etymology.** This specific name *chishuiensis* refers to the distribution of this species, Chishui City, Guizhou Province, China. We propose the common English name “Chishui leaf litter toads” (English) and its Chinese as “Chi Shui Zhang Tu Chan (赤水掌突蟾)”.



**Figure 7.** Advertisement calls of the holotype CIBCS20190518047 of *Leptobrachella chishuiensis* sp. nov. **A** waveform showing one second contains 4 calls **B** sonogram showing one second contains 4 calls **C** waveform showing 0.4 second contains a call **D** sonogram showing 0.4 second contains a call.



**Figure 8.** Habitats of *Leptobrachella chishuiensis* sp. nov. in the type locality Chuishui National Nature Reserve, Chishui City, Guizhou Province, China **A** landscape of montane forests in the type locality **B** a mountain stream in the type locality (*insert* holotype CIBCS20190518047 in life in the field).

## Discussion

The Asian leaf litter toads of *Leptobrachella* have low vagility and are in exclusive association with montane forests, and their populations are often highly structured. Underestimation of species diversity occurs in the genus, which suggests a high degree of localized diversification and micro-endemism (Fei et al. 2012; Chen et al. 2018). Many cryptic species were proposed by molecular analyses in areas where surveys are weak (Chen et al. 2018), but in Guizhou Province the investigation into the genus was poor although this area was likely to be an important transition zone for many clades or lineages (Chen et al. 2018). Additionally, in Guizhou Province, many new amphib-

ian species has been described in recent years (Zhang et al. 2017; Li et al. 2018a, b; Li et al. 2019a, b; Lyu et al. 2019; Wang et al. 2019; Wei et al. 2020), including two species of *Leptobranchella*, indicating the underestimated species diversity of amphibians in this region. To date, in Guizhou Province, seven *Leptobranchella* species were recorded, i.e., *Leptobranchella chishuiensis* sp. nov., *L. liui*, *L. oshanensis*, *L. purpuraventra*, *L. bijie*, *L. ventripunctata*, and *L. suiyangensis* (Fei et al. 2012; Li et al. 2016; Wang et al. 2019; Luo et al. 2020). It is expected that in this area, the species diversity of *Leptobranchella* may be underestimated, and more investigation should be conducted for detecting richness of the toad species.

The new species is found along clear water rocky streams from Chishui County, Guizhou Province, China, and little is known about the population status of the new species. Thus, further research on the true distribution, population size and trends, and conservation actions are required.

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## Supplementary material I

### Table S1. Uncorrected *p*-distance between *Leptobranchella* species on the 16S rRNA gene

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Data type: genetic distance

Explanation note: Mean value of genetic distance is given in the lower half of the table.

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Link: <https://doi.org/10.3897/zookeys.943.51572.suppl1>

# A new species of the horned toad *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from southwest China

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## Abstract

A new species of the genus *Megophrys* is described from Guizhou Province, China. Molecular phylogenetic analyses based on mitochondrial DNA and nuclear DNA sequences all strongly supported the new species as an independent clade sister to *M. minor* and *M. jiangi*. The new species could be distinguished from its congeners by a combination of the following characters: body size moderate (SVL 43.4–44.1 mm in males, and 44.8–49.8 mm in females; vomerine teeth absent; tongue not notched behind; a small horn-like tubercle at the edge of each upper eyelid; tympanum distinctly visible, rounded; two metacarpal tubercles on palm; relative finger lengths  $II < I < V < III$ ; toes without webbing; heels overlapping when thighs are positioned at right angles to the body; tibiotarsal articulation reaching the level between tympanum and eye when leg stretched forward; in breeding males, an internal single subgular vocal sac in male, and the nuptial pads with black spines on dorsal surface of bases of the first two fingers.

## Keywords

Taxonomy, molecular phylogenetic analysis, morphology

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\* Contributed equally as the first authors.

## Introduction

The Asian horned toad *Megophrys* Kuhl & Van Hasselt, 1822 (Anura: Megophryidae Bonaparte, 1850) is widely distributed in eastern and central China, throughout south-eastern Asia, and extending to the islands of the Sunda Shelf and the Philippines (Frost 2020). The taxonomic arrangements especially on generic assignments of the group have been controversial for a long time (e.g., Tian and Hu 1983; Dubois 1987; Lathrop 1997; Rao and Yang 1997; Jiang et al. 2003; Delorme et al. 2006; Fei et al. 2009; Chen et al. 2016; Fei and Ye 2016; Deuti et al. 2017; Mahony et al. 2017; Frost 2019). Nevertheless, all molecular phylogenetic studies revealed this group as a monophyletic group which corresponds to the family (Chen et al. 2016; Mahony et al. 2017; Liu et al. 2018; Li et al. 2018; Liu et al. 2020; Wang et al. 2020), and thus many researchers considered it as a large genus *Megophrys sensu lato* (Mahony et al. 2017; Li et al. 2018; Liu et al. 2018, 2020; Frost 2020; Wang et al. 2020) although several studies divided the taxa of the group into different genera and subgenera, thus introducing better resolution of relationships within the family (Chen et al. 2016; Fei and Ye 2016; Deuti et al. 2017; Liu et al. 2018).

The large genus *Megophrys* currently contains 98 species, of which 41 species were described in the last decade (Frost 2020; Liu et al. 2020). Many cryptic species in the genus are indicated by molecular phylogenetic analyses (Chen et al. 2016; Liu et al. 2018) of which several have been described recently (e.g., Wang et al. 2019; Liu et al. 2020). Obviously, more cryptic species need to be verified and described in detail.

During field surveys in the Chishui National Nature Reserve, Chishui City, Guizhou Province, China, we collected a series of *Megophrys* specimens. Our molecular phylogenetic analyses and morphological comparisons support it as an undescribed species, and it is described herein as a new species.

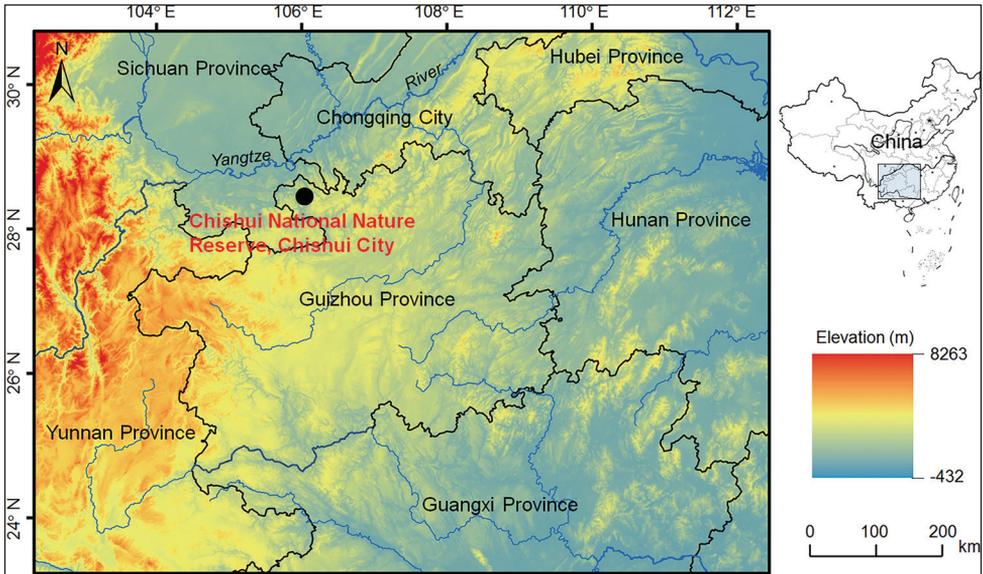
## Materials and methods

### Sampling

Three adult males and five adult females of the undescribed species were collected in Chishui National Nature Reserve, Chishui City, Guizhou Province, China (Suppl. material 1: Table S1; Fig. 1). In the field, the toads were euthanized using isoflurane, and the specimens were fixed in 75% ethanol. Tissue samples were taken and preserved separately in 99% ethanol prior to fixation. The specimens were deposited in Chengdu Institute of Biology, Chinese Academy of Sciences (CIB, CAS).

### Molecular data and phylogenetic analyses

Six specimens of the undescribed species were included in the molecular analyses (Suppl. material 2: Table S2). Total DNA was extracted using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). Two fragments of the mitochondrial genes encoding 16S rRNA and cytochrome oxidase subunit I (COI) were amplified



**Figure 1.** Geographical location of the type locality, Chishui National Nature Reserve, Chishui City, Guizhou Province, China, of *Megophrys chishuiensis* sp. nov.

using the primers in Simon et al. (1994) and Che et al. (2012), respectively. PCR were under the following conditions: 37 cycles at 94 °C for 4 min, 95 °C for 1 min, 53 °C (for 16S rRNA)/47 °C (for COI) for 30 sec, and 72 °C for 1 min followed by a 8-min extension at 72 °C. The nuclear gene sequences encoding brain-derived neurotrophic factor (BDNF) and recombination activating gene 1 (RAG1) were amplified using the primers and protocols in Vieites et al. (2007) and Shen et al. (2013), respectively (Suppl. material 3: Table S3). All PCR products were purified with spin columns, and then were sequenced with primers same as used in PCR. Sequencing was conducted using an ABI3730 automated DNA sequencer in Shanghai DNA BioTechnologies Co., Ltd. (Shanghai, China). All sequences were deposited in GenBank (for accession numbers see Suppl. material 2: Table S2).

For molecular analyses, the available sequence data for congeners of *Megophrys* were downloaded from GenBank (Suppl. material 2: Table S2), primarily from previous studies (Chen et al. 2017; Liu et al. 2018). For phylogenetic analyses, corresponding sequences of one *Leptobranchella oshanensis* (Liu, 1950) and one *Leptobranchium boringii* (Liu, 1945) were also downloaded (Suppl. material 2: Table S2), and used as outgroups according to Mahony et al. (2017). Sequences were assembled and aligned in BioEdit v. 7.0.9.0 (Hall 1999) with default settings. Alignments were checked by eye and revised manually if necessary. To avoid bias in alignments, GBLOCKS v. 0.91.b (Castresana 2000) with default settings was used to extract regions of defined sequence conservation from the length-variable 16S gene fragments. Non-sequenced fragments were defined as missing loci. For phylogenetic analyses, two datasets were obtained, i.e., two-mitochondrial genes concatenated dataset of 16S+COI and two-nuclear genes concatenated dataset of RAG1+BDNF.

Phylogenetic relationships were reconstructed based on the mitochondrial DNA data and nuclear DNA data, respectively. Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian Inference (BI) methods, implemented in PhyML v. 3.0 (Guindon et al. 2010) and MrBayes v. 3.12 (Ronquist and Huelsenbeck 2003), respectively. To avoid under- or over-parameterization (Lemmon and Moriarty 2004; McGuire et al. 2007), the best partition scheme and the best evolutionary model for each partition were chosen for the phylogenetic analyses using PARTITION-FINDER v. 1.1.1 (Robert et al. 2012). In the analyses, 16S, each codon position of the protein-coding genes (COI, RAG1 and BDNF) were defined, and Bayesian Inference Criteria (BIC) was used. As a result, the analyses selected the best partition scheme (i.e., 16S gene/each codon position of COI gene) and the GTR+ G + I model for each partition for mitochondrial DNA dataset, and as well, selected the best partition scheme (i.e., each codon position of RAG1 and BDNF genes) and the GTR+ G + I as the best model for all codon position of RAG1 and BDNF genes. For the ML tree, branch supports were drawn from 10000 non-parametric bootstrap replicates. In BI analyses, two runs each with four Markov chains were run for 40 million generations with sampling every 1000 generations. The first 25% of generations were removed as the “burn-in” stage followed by calculation of Bayesian posterior probabilities and the 50% majority-rule consensus of the post burn-in trees sampled at stationarity. Finally, genetic distance between species under uncorrected *p*-distance model was estimated on 16S gene sequences using MEGA v. 6.06 (Tamura et al. 2011).

## Morphological comparisons

All adult specimens of the undescribed species were measured. The terminology and methods followed Fei et al. (2009). Measurements were taken with a dial caliper to 0.1 mm. Seventeen morphometric characters of adult specimens were measured:

- ED** eye diameter (distance from the anterior corner to the posterior corner of the eye);
- FL** foot length (distance from tarsus to the tip of fourth toe);
- HDL** head length (distance from the tip of the snout to the articulation of jaw);
- HDW** maximum head width (greatest width between the left and right articulations of jaw);
- HLL** hindlimb length (maximum length from the vent to the distal tip of the Toe IV);
- IND** internasal distance (minimum distance between the inner margins of the external nares);
- IOD** interorbital distance (minimum distance between the inner edges of the upper eyelids);
- LAL** length of lower arm and hand (distance from the elbow to the distal end of the Finger IV);
- LW** lower arm width (maximum width of the lower arm);
- SL** snout length (distance from the tip of the snout to the anterior corner of the eye);

<b>SVL</b>	snout-vent length (distance from the tip of the snout to the posterior edge of the vent);
<b>TFL</b>	length of foot and tarsus (distance from the tibiotarsal articulation to the distal end of the Toe IV);
<b>THL</b>	thigh length (distance from vent to knee);
<b>TL</b>	tibia length (distance from knee to tarsus);
<b>TYD</b>	maximal tympanum diameter;
<b>TW</b>	maximal tibia width;
<b>UEW</b>	upper eyelid width (greatest width of the upper eyelid margins measured perpendicular to the anterior-posterior axis).

We compared morphological characters of the undescribed species with *Megophrys* congeners. Comparative data were obtained from related species as described in literature (Table 2).

### Bioacoustics notes

Ten advertisement calls from two individuals of the new species were recorded on 18 May 2018 between 21:00–23:00 in Chishui City, Guizhou Province, China in the field. SONY PCM-D50 digital sound recorder was used to record within 20 cm of the calling individuals. The sound files in wave format were resampled at 48 kHz with sampling depth 24 bits. The sonograms and waveforms were generated by WaveSurfer software (Sjöander and Beskow 2000) from which all parameters and characters were measured. Ambient temperature was taken by a digital hygrothermograph.

**Table 1.** Measurements of the adult specimens of *Megophrys chishuiensis* sp. nov. Units are given in mm. See abbreviations for the morphological characters in Materials and methods section.

	Male ( <i>N</i> = 3)		Female ( <i>N</i> = 5)	
	Range	Mean ± SD	Range	Mean ± SD
SVL	43.4–44.1	43.6 ± 0.4	44.8–49.8	47.8 ± 2.0
HDL	11.4–11.9	11.7 ± 0.3	11.2–12.7	11.7 ± 0.6
HDW	13.0–13.9	13.5 ± 0.5	13.8–15.4	14.7 ± 0.6
SL	4.2–5.3	4.8 ± 0.6	4.3–5.4	4.9 ± 0.4
IND	5.0–5.2	5.1 ± 0.1	4.5–5.8	5.1 ± 0.6
IOD	3.1–3.5	3.3 ± 0.2	3.1–4.3	3.5 ± 0.5
ED	4.4–5.0	4.7 ± 0.3	4.9–5.7	5.4 ± 0.3
UEW	4.1–4.9	4.4 ± 0.4	4.1–5.2	4.7 ± 0.4
TYD	2.8–3.5	3.2 ± 0.4	2.2–3.1	2.7 ± 0.3
LAL	18.4–20.0	19.0 ± 0.9	20.3–22.0	21.3 ± 0.7
LW	4.5–4.7	4.6 ± 0.1	3.2–3.6	3.4 ± 0.2
HLL	59.4–65.1	63.0 ± 3.1	64.2–75.6	70.7 ± 4.1
THL	17.2–21.3	19.8 ± 2.2	20.4–23.8	22.1 ± 1.3
TL	18.0–21.7	20.1 ± 1.9	22.0–24.0	23.2 ± 0.8
TW	4.6–5.1	4.9 ± 0.3	5.0–5.8	5.3 ± 0.3
TFL	28.0–30.2	28.9 ± 1.2	30.1–33.0	31.3 ± 1.1
FL	18.5–19.2	18.9 ± 2.3	18.8–22.1	21.0 ± 1.4

**Table 2.** References for morphological characters for congeners of the genus *Megophrys*.

Species	Literature
<i>M. aceras</i> Boulenger, 1903	Taylor 1962
<i>M. acuta</i> Wang, Li & Jin, 2014	Li et al. 2014
<i>M. ancræ</i> Mahony, Teeling & Biju, 2013	Mahony et al. 2013
<i>M. angka</i> Wu, Suwannapoom, Poyarkov, Chen, Pawangkhanant, Xu, Jin, Murphy & Che, 2019	Wu et al. 2019
<i>M. auralensis</i> Ohler, Swan & Daltry, 2002	Ohler et al. 2002
<i>M. baluensis</i> (Boulenger, 1899)	Boulenger 1899
<i>M. baolongensis</i> Ye, Fei & Xie, 2007	Ye et al. 2007
<i>M. binchuanensis</i> Ye & Fei, 1995	Ye and Fei 1995
<i>M. binlingensis</i> Jiang, Fei & Ye, 2009	Fei et al. 2009
<i>M. boettgeri</i> (Boulenger, 1899)	Fei et al. 2012
<i>M. brachykolos</i> Inger & Romer, 1961	Inger and Romer 1961
<i>M. carinense</i> (Boulenger, 1889)	Fei et al. 2009
<i>M. caobangensis</i> Nguyen, Pham, Nguyen, Luong & Ziegler, 2020	Nguyen et al. 2020
<i>M. caudoprocta</i> Shen, 1994	Fei et al. 2012
<i>M. cheni</i> (Wang & Liu, 2014)	Wang et al. 2014
<i>M. chuannanensis</i> (Fei, Ye & Huang, 2001)	Fei et al. 2012
<i>M. damrei</i> Mahony, 2011	Mahony 2011
<i>M. daweimontis</i> Rao & Yang, 1997	Fei et al. 2012
<i>M. dongguanensis</i> Wang & Wang, 2019	Wang et al. 2019
<i>M. dringi</i> Inger, Stuebing & Tan, 1995	Inger et al. 1995
<i>M. eduardinae</i> Inger, 1989	Inger 1989
<i>M. elfina</i> Poyarkov, Duong, Orlov, Gogoleva, Vassilieva, Nguyen, Nguyen, Nguyen, Che & Mahony, 2017	Poyarkov et al. 2017
<i>M. fansipanensis</i> Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018	Tapley et al. 2018
<i>M. faec</i> Boulenger, 1887	Fei et al. 2009
<i>M. feii</i> Yang, Wang & Wang, 2018	Yang et al. 2018
<i>M. flavipunctata</i> Mahony, Kamei, Teeling & Biju, 2018	Mahony et al. 2018
<i>M. gerti</i> (Ohler, 2003)	Ohler 2003
<i>M. gigantea</i> Liu, Hu & Yang, 1960	Fei et al. 2012
<i>M. glandulosa</i> Fei, Ye & Huang, 1990	Fei et al. 2012
<i>M. hansii</i> (Ohler, 2003)	Ohler 2003
<i>M. himalayana</i> Mahony, Kamei, Teeling & Biju, 2018	Mahony et al. 2018
<i>M. hoanglienensis</i> Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018	Tapley et al. 2018
<i>M. huangshanensis</i> Fei & Ye, 2005	Fei et al. 2012
<i>M. insularis</i> (Wang, Liu, Lyu, Zeng & Wang, 2017)	Wang et al. 2017a
<i>M. intermedia</i> Smith, 1921	Rao and Yang 1997
<i>M. jiangi</i> Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020	Liu et al. 2020
<i>M. jingdongensis</i> Fei & Ye, 1983	Fei et al. 2012
<i>M. jinggangensis</i> (Wang, 2012)	Wang et al. 2012
<i>M. jiulianensis</i> Wang, Zeng, Lyu & Wang, 2019	Wang et al. 2019
<i>M. kalimantanensis</i> Munir, Hamidy, Matsui, Iskandar, Sidik & Shimada, 2019	Munir et al. 2019
<i>M. kobayashii</i> Malkmus & Matsui, 1997	Malkmus and Matsui 1997
<i>M. koui</i> Mahony, Foley, Biju & Teeling, 2017	Mahony et al. 2017
<i>M. kuatunensis</i> Pope, 1929	Fei et al. 2012
<i>M. lancip</i> Munir, Hamidy, Farajallah & Smith, 2018	Munir et al. 2018
<i>M. leishanensis</i> Li, Xu, Liu, Jiang, Wei & Wang, 2018	Li et al. 2018
<i>M. lekaguli</i> Stuart, Chuaynkern, Chan-ard & Inger, 2006	Stuart et al. 2006
<i>M. liboensis</i> (Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017)	Zhang et al. 2017
<i>M. ligayae</i> Taylor, 1920	Taylor 1920
<i>M. lini</i> (Wang & Yang, 2014)	Wang et al. 2014
<i>M. lishuiensis</i> (Wang, Liu & Jiang, 2017)	Wang et al. 2017b
<i>M. longipes</i> Boulenger, 1886	Taylor 1962
<i>M. major</i> Boulenger, 1908	Mahony et al. 2018
<i>M. mangshanensis</i> Fei & Ye, 1990	Fei et al. 2012
<i>M. maosonensis</i> Bourret, 1937	Bourret 1937
<i>M. medogensis</i> Fei, Ye & Huang, 1983	Fei et al. 2012
<i>M. megacephala</i> Mahony, Sengupta, Kamei & Biju, 2011	Mahony et al. 2011
<i>M. microstoma</i> (Boulenger, 1903)	Fei et al. 2012
<i>M. minor</i> Stejneger, 1926	Fei et al. 2012
<i>M. montana</i> Kuhl & Van Hasselt, 1822	Kuhl and Van Hasselt 1822

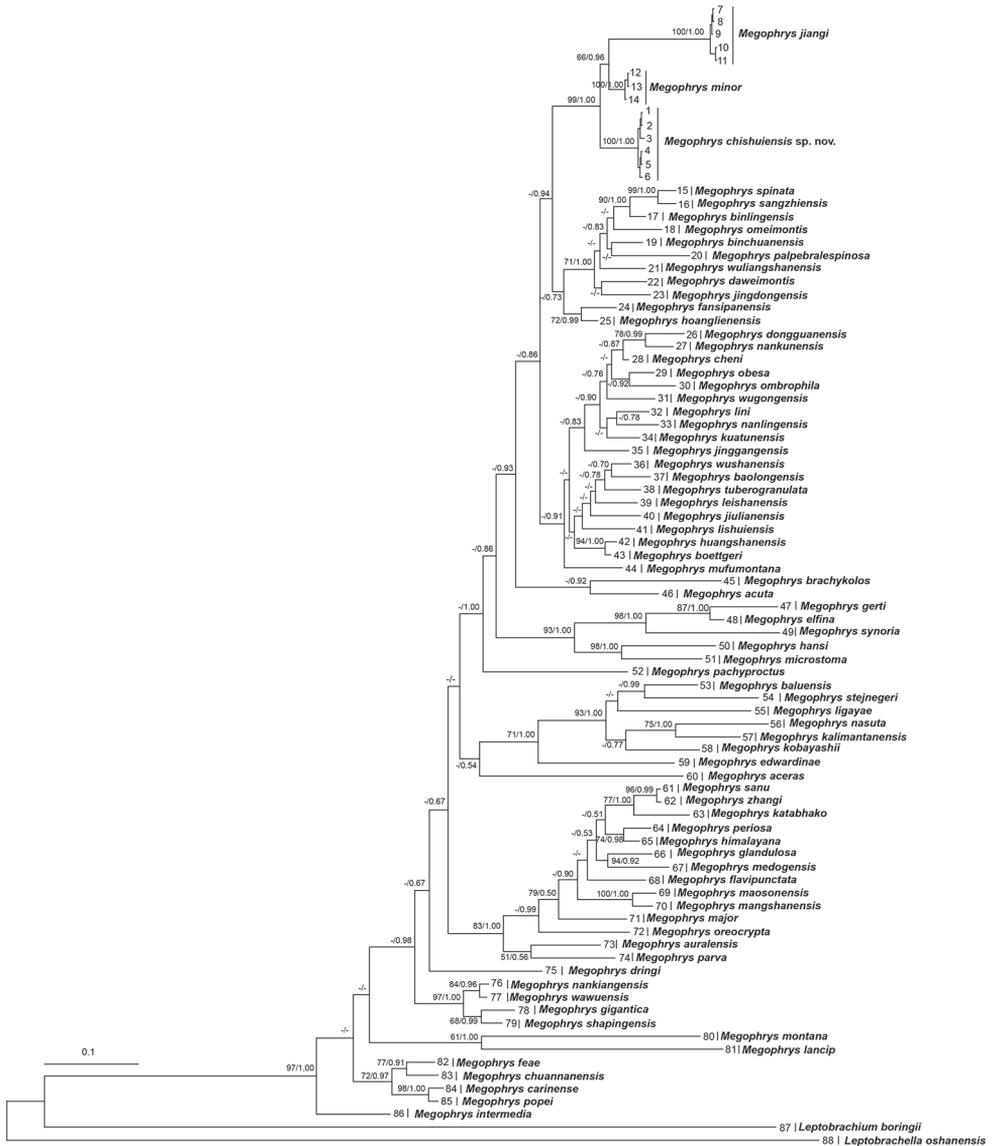
Species	Literature
<i>M. monticola</i> (Günther, 1864)	Mahony et al. 2018
<i>M. mufumontana</i> Wang, Lyu & Wang, 2019	Wang et al. 2019
<i>M. nankiangensis</i> Liu & Hu, 1966	Fei et al. 2012
<i>M. nankunensis</i> Wang, Zeng & Wang, 2019	Wang et al. 2019
<i>M. nanlingensis</i> Lyu, Wang, Liu & Wang, 2019	Wang et al. 2019
<i>M. nasuta</i> (Schlegel, 1858)	Taylor 1962
<i>M. obesa</i> Wang, Li & Zhao, 2014	Wang et al. 2014
<i>M. ombrophila</i> Messenger & Dahn, 2019	Munir et al. 2019
<i>M. omeimontis</i> Liu, 1950	Fei et al. 2009
<i>M. oreocrypta</i> Mahony, Kamei, Teeling & Biju, 2018	Mahony et al. 2018
<i>M. oropedion</i> Mahony, Teeling & Biju, 2013	Mahony et al. 2013
<i>M. orientalis</i> Li, Lyu, Wang & Wang, 2020	Li et al. 2020
<i>M. pachyproctus</i> Huang, 1981	Fei et al. 2009
<i>M. palpebralespinosa</i> Bourret, 1937	Fei et al. 2012
<i>M. parallela</i> Inger & Iskandar, 2005	Inger and Iskandar 2005
<i>M. parva</i> (Boulenger, 1893)	Fei et al. 2009
<i>M. periosa</i> Mahony, Kamei, Teeling & Biju, 2018	Mahony et al. 2018
<i>M. popei</i> (Zhao, Yang, Chen, Chen & Wang, 2014)	Zhao et al. 2014
<i>M. robusta</i> Boulenger, 1908	Mahony et al. 2018
<i>M. rubrimera</i> Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley, 2017	Tapley et al. 2017
<i>M. sangzhiensis</i> Jiang, Ye & Fei, 2008	Jiang et al. 2008
<i>M. serchhipii</i> (Mathew & Sen, 2007)	Mathew and Sen 2007
<i>M. shapingensis</i> Liu, 1950	Fei et al. 2009
<i>M. shuichengensis</i> Tian & Sun, 1995	Fei et al. 2009
<i>M. shunhuangensis</i> Wang, Deng, Liu, Wu & Liu, 2019	Wang et al. 2019a
<i>M. spinata</i> Liu & Hu, 1973	Fei et al. 2009
<i>M. stejnegeri</i> Taylor, 1920	Taylor 1920
<i>M. synoria</i> (Stuart, Sok & Neang, 2006)	Stuart et al. 2006
<i>M. takensis</i> Mahony, 2011	Mahony 2011
<i>M. tuberogranulata</i> Shen, Mo & Li, 2010	Fei et al. 2012
<i>M. vegrandis</i> Mahony, Teeling & Biju, 2013	Mahony et al. 2013
<i>M. wawuensis</i> Fei, Jiang & Zheng, 2001	Fei et al. 2012
<i>M. wugongensis</i> Wang, Lyu & Wang, 2019	Wang et al. 2019b
<i>M. wuliangshanensis</i> Ye & Fei, 1995	Fei et al. 2012
<i>M. wushanensis</i> Ye & Fei, 1995	Fei et al. 2012
<i>M. xianjuensis</i> Wang, Wu, Peng, Shi, Lu & Wu, 2020	Wang et al. 2020
<i>M. zhangj</i> Ye & Fei, 1992	Fei et al. 2012
<i>M. zunbebotoensis</i> (Mathew & Sen, 2007)	Mathew and Sen 2007

## Results

### Phylogenetic analyses

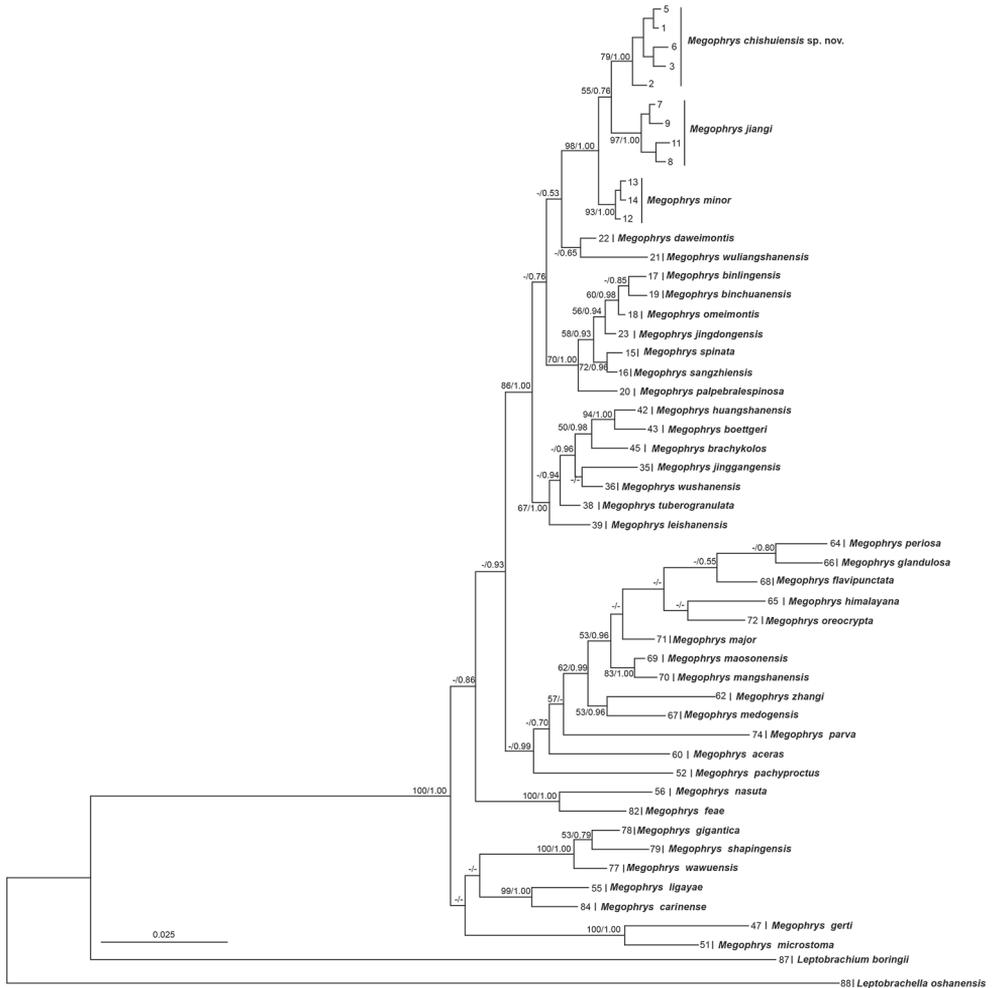
Aligned sequence matrix of 16S+COI and RAG1+BDNF contains 1104 bp and 1582 bp, respectively. ML and BI trees of the mitochondrial DNA dataset presented almost consistent topology (Fig. 2), and as well, ML and BI trees of the nuclear DNA dataset showed almost identical topology (Fig. 3), though relationships of many lineages were unresolved (Figs 2, 3). In mitochondrial DNA trees, the undescribed species was clustered as an independent clade sister to a clade in comprising of *M. minor* Stejneger, 1926 and *M. jiangi* Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020, but in nuclear DNA trees, the undescribed species clade was sister to *M. jiangi*, and then was clustered together with *M. minor*.

Genetic distances on 16S gene with uncorrected *p*-distance model between samples of the undescribed species were below 0.2%. The genetic distance between the undescribed species and its closest related species *M. minor* was 2.2% on 16S gene, which was higher or at the same level with those among many pairs of congeners, for



**Figure 2.** Maximum likelihood (ML) tree of the genus *Megophrys* reconstructed based on the 16S rRNA and COI gene sequences. Bayesian posterior probability/ML bootstrap supports were denoted beside each node. Samples 1–88 refer to Suppl. material 2: Table S2.

example, 1.7% between *M. spinata* Liu & Hu, 1973 and *M. sangzhiensis* Jiang, Ye & Fei, 2008, 2.1% between *M. omeimontis* Liu, 1950 and *M. binlingensis* Jiang, Fei & Ye, 2009, and 2.2% between *M. cheni* (Wang & Liu, 2014) and *M. nankunensis* Wang, Zeng & Wang, 2019; Suppl. material 4: Table S4).



**Figure 3.** Maximum likelihood (ML) tree of the genus *Megophrys* reconstructed based on the nuclear DNA sequences of RAG1 and BDNF genes. Bayesian posterior probability/ML bootstrap supports were denoted beside each node. Samples 1–88 refer to Suppl. msterial 2: Table S2.

## Taxonomic accounts

### *Megophrys chishuiensis* sp. nov.

<http://zoobank.org/20B6A80B-E937-4443-88A2-E357B77DB6CA>

Figures 4–8

**Type material. Holotype.** CIBCS20190518031 (Figs 4, 5), adult male, from Chishui National Nature Reserve, Chishui City, Guizhou Province, China (28.436708N, 105.997794E, ca. 460 m a. s. l.), collected by Shi-Ze Li on 18 May 2019.

**Paratype.** Two adult males and five adult females from the same place as holotype, collected by Shi-Ze Li and Jing Liu. Two females CIBCS20190518022 and CIBCS20190518023 collected by Jing LIU on 18 May 2019, two adult males CIBCS20190518019 and CIBCS20190518021 and three adult females CIBCS20190518025, CIBCS20190518027 and CIBCS20190518030 collected by Shi-Ze Li on 18 May 2019.

**Diagnosis.** *Megophrys chishuiensis* sp. nov. is assigned to the genus *Megophrys* based on molecular phylogenetic analyses and the following generic diagnostic characters: snout shield-like; projecting beyond the lower jaw; canthus rostralis distinct; chest glands small and round, closer to the axilla than to midventral line; femoral glands on rear part of thigh; vertical pupils (Fei et al. 2009).

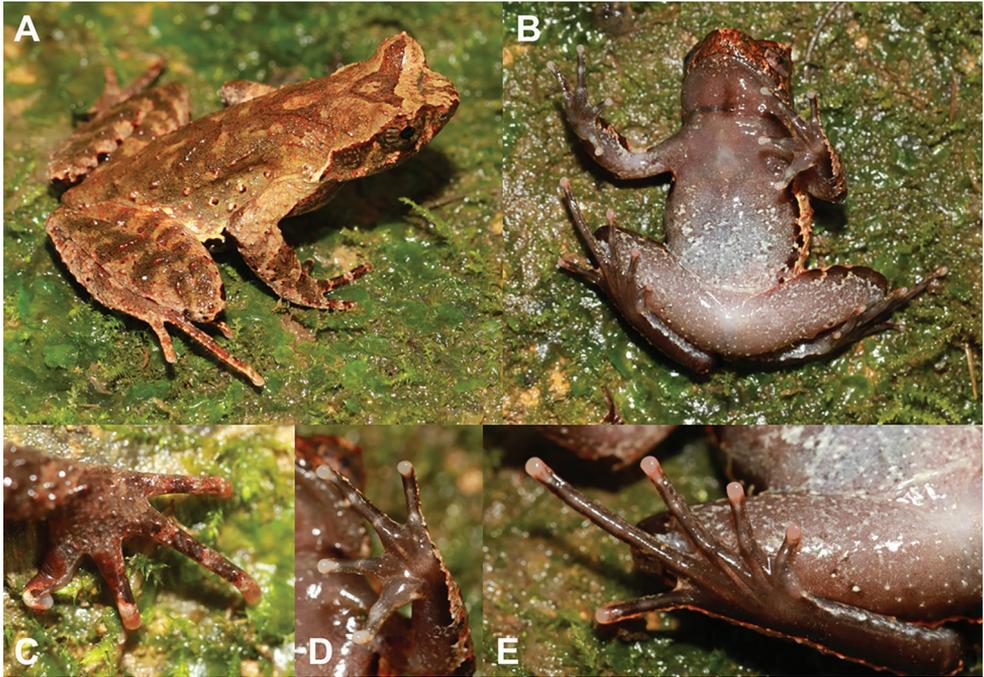
*Megophrys chishuiensis* sp. nov. could be distinguished from its congeners by a combination of the following morphological characters: (1) body size moderate (SVL 43.4–44.1 mm in males, and 44.8–49.8 mm in females); (2) vomerine teeth absent; (3) tongue not notched behind; (4) a small horn-like tubercle at the edge of each upper eyelid; (5) tympanum distinctly visible, rounded; (6) two metacarpal tubercles on palm; (7) relative finger lengths  $II < I < V < III$ ; (8) toes without webbing; (9) heels overlapping when thighs are positioned at right angles to the body; (10) tibiotarsal articulation reaching the level between tympanum and eye when leg stretched forward. In breeding male, (11) an internal single subgular vocal sac; (12) nuptial pads with black spines on dorsal surface of bases of the first two fingers.

**Description of holotype.** (Figs 4, 5). SVL 43.4 mm; head width larger than head length (HDW/HDL ratio about 1.2); snout obtusely pointed, protruding well beyond the margin of the lower jaw in ventral view; loreal region vertical and concave; canthus rostralis well-developed; top of head flat in dorsal view; a small horn-like tubercle at the edge of the upper eyelid; eye large, eye diameter 43.9% of head length; pupils vertical; nostril orientated laterally, closer to snout than eye; tympanum distinct, TYP/EYE ratio 0.64; vomerine ridges and vomerine teeth absent; margin of tongue smooth, not notched behind.

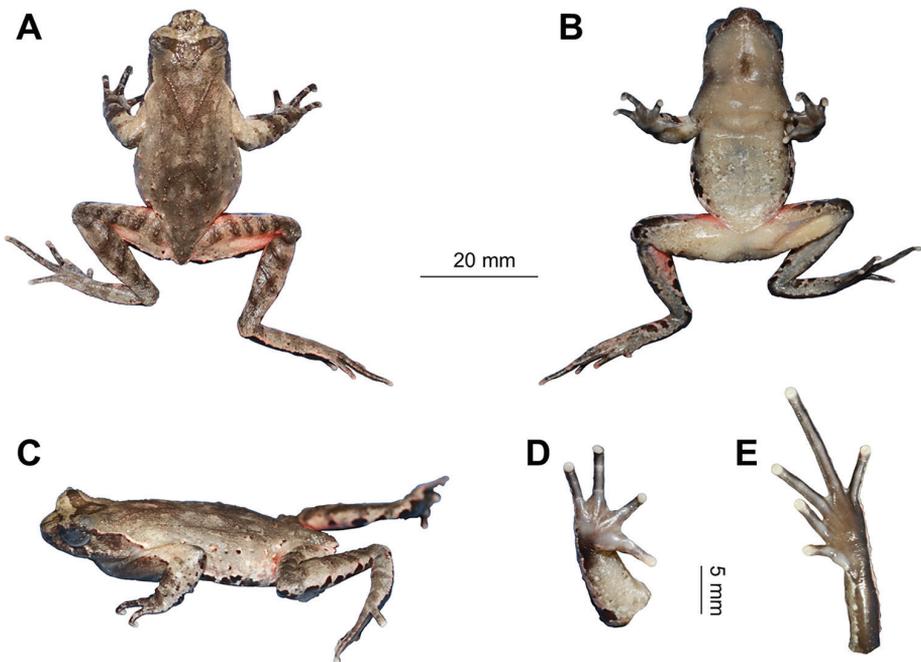
Forelimbs slender, the length of lower arm and hand 42.4% of SVL; fingers slender, relative finger lengths:  $II < I < V < III$ ; tips of digits globular, without lateral fringes; subarticular tubercle distinct at the base of each finger; two metacarpal tubercles, prominent, the outer one long and thin, the inner one oval-shaped.

Hindlimbs slender, 1.48 times SVL; heels overlapping when thighs are positioned at right angles to the body, tibiotarsal articulation reaching tympanum to eye when leg stretched forward; tibia length longer than thigh length; relative toe lengths  $I < II < V < III < IV$ ; tips of toes round, slightly dilated; subarticular tubercles absent; toes without webbing; no lateral fringe; inner metatarsal tubercle oval-shaped; outer metatarsal tubercle absent.

Dorsal skin rough, with numerous granules; several large warts scattered on flanks; a small horn-like tubercle at the edge of each upper eyelid; tubercles on the dorsum forming a weak X-shaped ridge, the V-shaped ridges disconnect; two discontinuous dorsolateral parallel ridges on either side of the X-shaped ridges; an inverted triangular brown speckle between two upper eyelids; several tubercles on the flanks and dorsal



**Figure 4.** Photos of the holotype CIBCS20190518031 of *Megophrys chishuiensis* sp. nov. in life **A** dorsal view **B** ventral view **C** dorsal view of hand **D** ventral view of hand **E** ventral view of foot.



**Figure 5.** The holotype specimen CIBCS20190518031 of *Megophrys chishuiensis* sp. nov. **A** dorsal view **B** ventral view **C** lateral view **D** ventral view of hand **E** ventral view of foot.

surface of thighs and tibiae and forming four transverse tubercle rows; supratympanic fold distinct.

Ventral surface smooth; chest with small and round glands, closer to the axilla than to midventral line; femoral glands on rear of thighs, numerous white granules on outer thighs; posterior end of the body distinctly protruding and forming an arc-shaped swelling above the anal region.

**Coloration of holotype in life.** (Fig. 4). An inverted triangular brown speckle between the eyes; X-shaped ridges on the dorsum, four transverse bands on the dorsal surface of the thigh and shank; several dark brown and white vertical bars on the lower and upper lip; venter purple grey, some white spots on the ventral surface of body and limbs; palms and soles uniform purple grey, tip of digits pinkish; pectoral and femoral glands white.

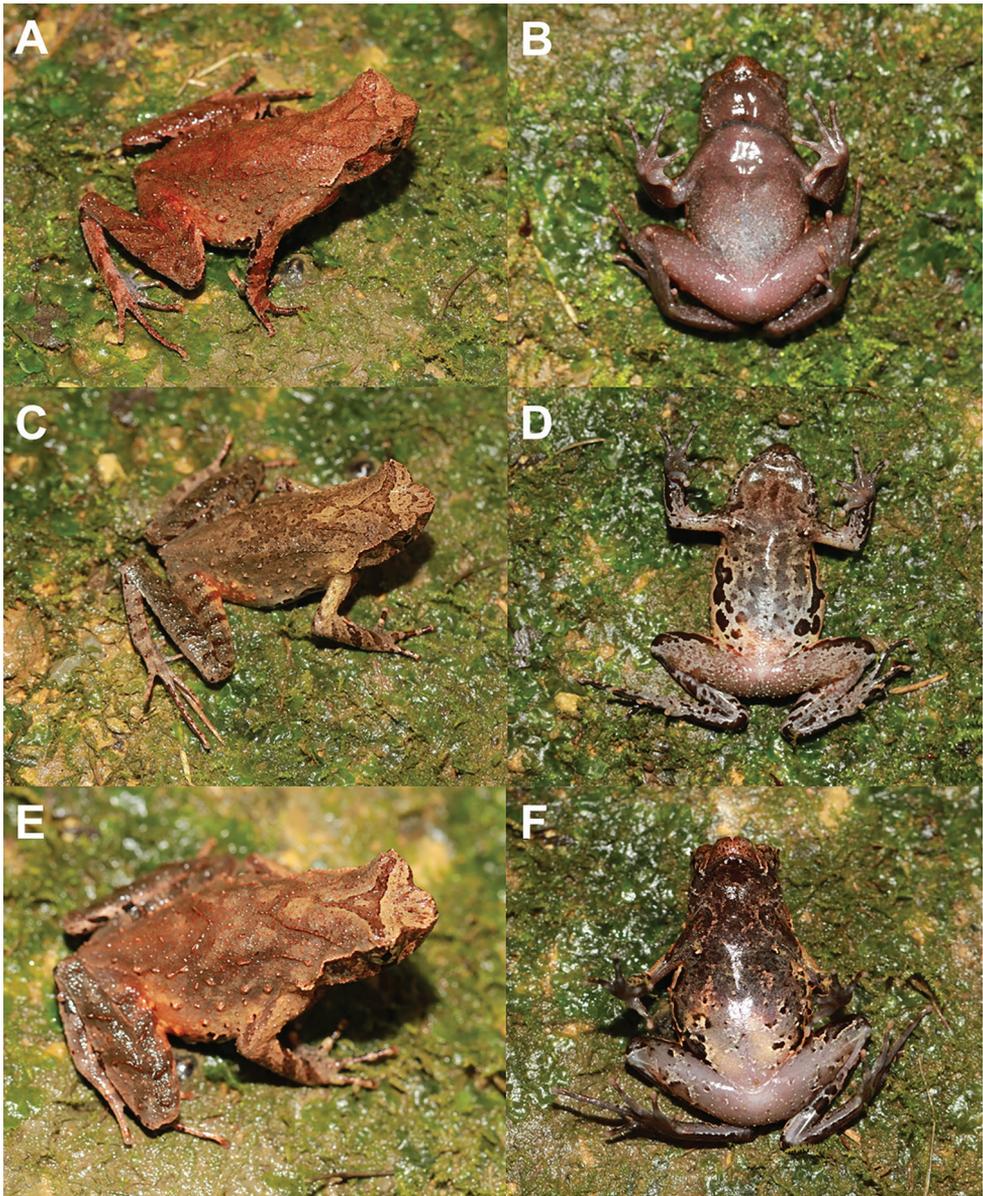
**Coloration of holotype in preservation.** (Fig. 5). Color of dorsal surface fades to olive; the inverted triangular brown speckle between the eyes, X-shaped ridges on dorsum and transverse bands on limbs and digits distinct; ventral surface greyish white; creamy-white substitutes the pinkish on tip of digits; the posterior of ventral surface of body, inner of thigh and upper of tibia light red.

**Variations.** In CIBCS20190518027, the back is brown with some brick-red granules (Fig. 6A); in CIBCS20190518030, the X-shaped marking on back of trunk consists of a ridge with brown spots (Fig. 6B), and the throat and anterior belly are purplish, with grey spots on the posterior belly and black spots on the flank belly (Fig. 6E); in CIBCS20190518025, the marking on the back consists of a V-shaped ridge (Fig. 6C), and the anterior belly is brownish with some black spots on flank and belly, and posterior belly is beige (Fig. 6F); in CIBCS20190518019, the whole ventrum is purplish except the posterior belly that shows white blotches (Fig. 6D).

**Advertisement call.** The call description is based on recordings of the holotype CIBCS20190518031 (Fig. 7) from the shrub leaf near the streamlet, and the ambient air temperature was 24.5 °C. Each call consists of 14–20 (mean  $16.14 \pm 1.95$ ,  $N = 10$ ) notes. Call duration was 2.10–3.18 second (mean  $2.51 \pm 0.33$ ,  $N = 7$ ). Call interval was 0.92–1.32 seconds (mean  $1.13 \pm 0.15$ ,  $N = 6$ ). Each note had a duration of 0.07–0.12 seconds (mean  $0.98 \pm 0.01$ ,  $N = 113$ ) and the intervals between notes 0.038–0.085 seconds (mean  $0.056 \pm 0.011$ ,  $N = 106$ ). Amplitude modulation within note was apparent, beginning with moderately high energy pulses, increasing slightly to a maximum by approximately mid note, and then decreasing towards the end of each note. The average dominant frequency was  $5859 \pm 118.02.61$  (5733–6064 Hz,  $N = 7$ ).

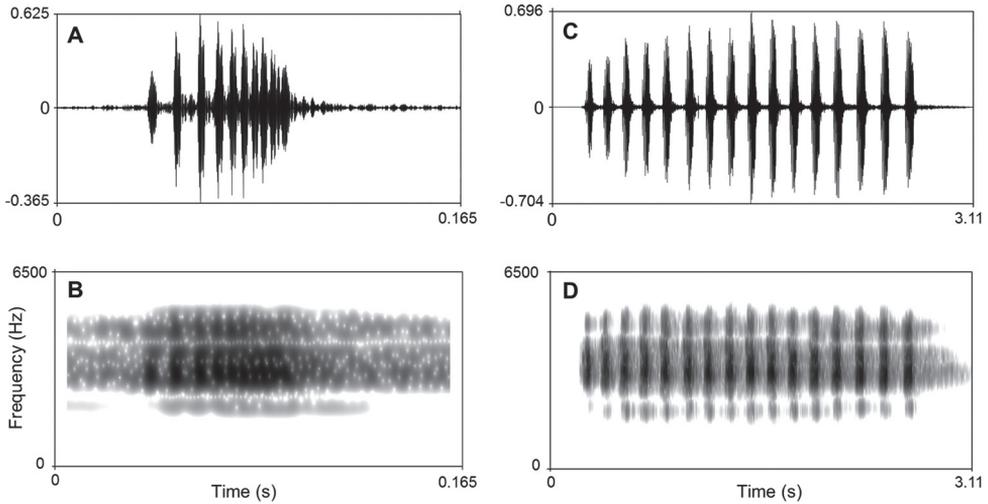
**Secondary sexual characters.** Adult females with SVL 44.8–49.8 mm, larger than adult males with 43.4–44.1 mm. Adult males have a single subgular vocal sac. In breeding males, brownish red nuptial pads are present on dorsal surface of the bases of the first and second fingers with black spines obvious under microscope.

**Comparisons.** By having medium body size, *Megophrys chishuiensis* sp. nov. differs from *M. aceras* Boulenger, 1903, *M. auralensis* Ohler, Swan & Daltry, 2002, *M. carinense* Boulenger, 1889, *M. caudoprocta* Shen, 1994, *M. chuannanensis* (Fei, Ye & Huang, 2001), *M. damrei* Mahony, 2011, *M. edwardinae* Inger, 1989, *M. feae* Boulenger, 1887, *M. flavipunctata* Mahony, Kamei, Teeling & Biju, 2018, *M. gigantica* Liu, Hu



**Figure 6.** Color variation in *Megophrys chishuiensis* sp. nov. **A** dorsolateral view of the female specimen CIBCS20190518027 **B** dorsolateral view of the female specimen CIBCS20190518030 **C** dorsal view of the female specimen CIBCS20190518025 **D** ventral view of the male specimen CIBCS20190518019 **E** ventral view of the female specimen CIBCS20190518030 **F** ventral view of the female specimen CIBCS20190518025.

& Yang, 1960, *M. glandulosa* Fei, Ye & Huang, 1990, *M. himalayana* Mahony, Kamei, Teeling & Biju, 2018, *M. intermedia* Smith, 1921, *M. jingdongensis* Fei & Ye, 1983, *M. kalimantanensis* Munir, Hamidy, Matsui, Iskandar, Sidik & Shimada, 2019, *M. lekaguli* Stuart, Chuaynkern, Chan-ard & Inger, 2006, *M. liboensis* (Zhang, Li, Xiao,



**Figure 7.** Visualization of advertisement calls of *Megophrys chishuiensis* sp. nov. **A** waveform showing one note **B** sonogram showing one notes **C** waveform showing 16 notes of one call **D** sonogram showing 16 notes of one call.

Li, Pan, Wang, Zhang & Zhou, 2017), *M. major* Boulenger, 1908, *M. mangshanensis* Fei & Ye, 1990, *M. maosonensis* Bourret, 1937, *M. medogensis* Fei, Ye & Huang, 1983, *M. omeimontis* Liu, 1950, *M. oreocrypta* Mahony, Kamei, Teeling & Biju, 2018, *M. orientalis* (Li, Lyu, Wang & Wang, 2020), *M. periosa* Mahony, Kamei, Teeling & Biju, 2018, *M. popei* (Zhao, Yang, Chen, Chen & Wang, 2014), *M. sangzhiensis* Jiang, Ye & Fei, 2008, *M. shapingensis* Liu, 1950, *M. shuichengensis* Tian & Sun, 1995, and *M. takensis* Mahony, 2011 (maximum SVL < 49.8 mm in the new species vs. minimum SVL > 53 mm in the latter), and differs from *M. acuta* Wang, Li & Jin, 2014, *M. angka* (Wu, Suwannapoom, Poyarkov, Chen, Pawangkhanant, Xu, Jin, Murphy & Che, 2019), *M. caobangensis* Nguyen, Pham, Nguyen, Luong & Ziegler, 2020, *M. damrei* Mahony, 2011, *M. dongguanensis* Wang & Wang, 2019, *M. cheni*, *M. jiangi*, *M. jinggangensis* (Wang, 2012), *M. jiulianensis* Wang, Zeng, Lyu & Wang, 2019, *M. kuatunensis* Pope, 1929, *M. lini* (Wang & Yang, 2014), *M. lishuiensis* (Wang, Liu & Jiang, 2017), *M. mufumontana* (Wang, Lyu & Wang, 2019), *M. minor*, *M. nanlingensis* (Lyu, Wang, Liu & Wang, 2019), *M. obesa* Wang, Li & Zhao, 2014, *M. pachyproctus* Huang, 1981, *M. palpebralespinosa* Bourret, 1937, *M. serchhipii* Mathew & Sen, 2007, *M. shunhuangensis* Wang, Deng, Liu, Wu & Liu, 2019, *M. vegrandis* Mahony, Teeling & Biju, 2013, *M. wuliangshanensis* Ye & Fei, 1995, *M. wushanensis* Ye & Fei, 1995, *M. zunhebotensis* Mathew & Sen, 2007, *M. xianjuensis* Wang, Wu, Peng, Shi, Lu & Wu, 2020, and *M. zhangii* Ye & Fei, 1992 (vs. maximum SVL < 42 mm in the latter).

By the absence of vomerine teeth, *Megophrys chishuiensis* sp. nov. differs from *M. aceras*, *M. ancræ* Mahony, Teeling & Biju, 2013, *M. carinense*, *M. baluensis* (Boulenger, 1899), *M. caudoprocta*, *M. chuannanensis*, *M. damrei*, *M. daweimontis* Rao & Yang, 1997, *M. dongguanensis*, *M. fansipanensis* Tapley, Cutajar, Mahony, Nguyen,

Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018, *M. flavipunctata*, *M. glandulosa*, *M. hoanglienensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018, *M. himalayana*, *M. insularis* (Wang, Liu, Lyu, Zeng & Wang, 2017), *M. intermedia*, *M. jingdongensis*, *M. jinggongensis*, *M. jiulianensis*, *M. kalimantanensis*, *M. kobayashii* Malkmus & Matsui, 1997, *M. lancip* Munir, Hamidy, Farajallah & Smith, 2018, *M. lekaguli*, *M. liboensis*, *M. ligayae* Taylor, 1920, *M. longipes* Boulenger, 1886, *M. major*, *M. mangshanensis*, *M. maosonensis*, *M. medogensis*, *M. megacephala* Mahony, Sengupta, Kamei & Biju, 2011, *M. montana* Kuhl & Van Hasselt, 1822, *M. nasuta* (Schlegel, 1858), *M. nankunensis*, *M. nanlingensis*, *M. omeimontis*, *M. oropedion* Mahony, Teeling & Biju, 2013, *M. oreocrypta*, *M. palpebralespinosa*, *M. parallela* Inger & Iskandar, 2005, *M. parva* (Boulenger, 1893), *M. periosa*, *M. popei*, *M. robusta* Boulenger, 1908, *M. rubrimer*a Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley, 2017, *M. sangzhiensis*, *M. stejnegeri* Taylor, 1920, *M. takensis*, *M. zhang*i, and *M. zunhebotensis* (vs. present in the latter).

By having a small horn-like tubercle at the edge of each upper eyelid, *Megophrys chishuiensis* sp. nov. differs from *M. binchuanensis* Ye & Fei, 1995, *M. binlingensis*, *M. damrei*, *M. gigantea*, *M. minor*, *M. monticola* (Günther, 1864), *M. nasuta*, *M. nankiangensis* Liu & Hu, 1966, *M. oropedion*, *M. pachyproctus*, *M. spinata*, *M. stejnegeri*, *M. takensis*, *M. wuliangshanensis*, *M. wushanensis*, *M. zhang*i, and *M. zunhebotensis* (vs. lacking tubercle in the latter), and differs from *M. carinense*, *M. feae*, *M. gerti* (Ohler, 2003), *M. hans*i (Ohler, 2003), *M. intermedia*, *M. kalimantanensis*, *M. kou*i Mahony, Foley, Biju & Teeling, 2017, *M. latidactyla*, *M. liboensis*, *M. microstoma* (Boulenger, 1903), *M. palpebralespinosa*, *M. popei*, *M. shuichengensis*, and *M. synoria* (Stuart, Sok & Neang, 2006) (vs. having a prominent and elongated tubercle in the latter).

By having a tongue not notched behind, *Megophrys chishuiensis* sp. nov. differs from *M. ancr*ae, *M. baolongensis* Ye, Fei & Xie, 2007, *M. binlingensis*, *M. boettgeri* (Boulenger, 1899), *M. carinense*, *M. cheni*, *M. chuannanensis*, *M. damrei*, *M. dringi* Inger, Stuebing & Tan, 1995, *M. fansipanensis*, *M. feae*, *M. fei*i Yang, Wang & Wang, 2018, *M. flavipunctata*, *M. gerti*, *M. glandulosa*, *M. hoanglienensis*, *M. huangshanensis* Fei & Ye, 2005, *M. insularis*, *M. jiulianensis*, *M. jingdongensis*, *M. kalimantanensis*, *M. kuatunensis*, *M. liboensis*, *M. mangshanensis*, *M. maosonensis*, *M. medogensis*, *M. minor*, *M. nankiangensis*, *M. nanlingensis*, *M. omeimontis*, *M. oropedion*, *M. pachyproctus*, *M. parallela*, *M. popei*, *M. robusta*, *M. sangzhiensis*, *M. shapingensis*, *M. shuichengensis*, *M. spinata*, *M. vegr*andis, *M. wawuensis* Fei, Jiang & Zheng, 2001, *M. zhang*i, and *M. zunhebotensis* (vs. tongue notched behind in the latter).

By lacking lateral fringes on the toes, *Megophrys chishuiensis* sp. nov. differs from *M. acuta*, *M. auralensis*, *M. baolongensis*, *M. binchuanensis*, *M. boettgeri*, *M. carinense*, *M. cheni*, *M. chuannanensis*, *M. elfina* Poyarkov, Duong, Orlov, Gogoleva, Vassilieva, Nguyen, Nguyen, Nguyen, Che & Mahony, 2017, *M. feae*, *M. fei*i, *M. flavipunctata*, *M. gigantea*, *M. glandulosa*, *M. hans*i, *M. intermedia*, *M. jingdongensis*, *M. jinggongensis*, *M. kuatunensis*, *M. latidactyla*, *M. lini*, *M. major*, *M. maosonensis*, *M. nankiangensis*, *M. omeimontis*, *M. palpebralespinosa*, *M. popei*, *M. rubrimer*a, *M. sangzhiensis*, *M. serchhipii*,

*M. shapingensis*, *M. shuichengensis*, *M. spinata*, *M. vegrandis*, *M. xianjuensis*, *M. zhangii*, and *M. zunhebotoensis* (vs. present in these species).

By having toes without webs at bases, *Megophrys chishuiensis* sp. nov. differs from *M. brachykolos* Inger & Romer, 1961, *M. carinense*, *M. flavipunctata*, *M. jingdongensis*, *M. jinggangensis*, *M. lini*, *M. major*, *M. palpebralespinosa*, *M. popei*, *M. shuichengensis*, *M. spinata* (vs. at least one-fourth webbed).

By heels overlapping when thighs are positioned at right angles to the body, *Megophrys chishuiensis* sp. nov. differs from *M. acuta*, *M. brachykolos*, *M. dongguanensis*, *M. huangshanensis*, *M. kuatunensis*, *M. nankunensis*, *M. obesa*, *M. ombrophila* Messenger & Dahn, 2019, and *M. wugongensis* Wang, Lyu & Wang, 2019 (vs. not meeting).

With tibiotarsal articulation reaching to the level between tympanum and eye when leg is stretched forward, *Megophrys chishuiensis* sp. nov. differs from *M. baolongensis*, *M. nankiangensis*, *M. pachyproctus*, *M. shuichengensis* and *M. tuberogranulata* Shen, Mo & Li, 2010 (vs. just reaching posterior corner of the eye in the latter); differs from *M. daweimontis*, *M. glandulosa*, *M. lini*, *M. major*, *M. medongensis*, *M. obesa*, and *M. sangzhiensis* (vs. reaching the anterior corner of the eye or beyond eye or nostril and tip of snout in the latter); differs from *M. leishanensis* Li, Xu, Liu, Jiang, Wei & Wang, 2018 (vs. reaching middle part of eye in this group of species); and differs from *M. mufumontana* (vs. reaching tympanum in males and to the eye in females).

By having an internal single subgular vocal sac in male, *Megophrys chishuiensis* sp. nov. differs from *M. caudoprocta*, *M. shapingensis*, and *M. shuichengensis* (vs. vocal sac absent).

By having nuptial pads and nuptial spines on dorsal surface of the base of the first two fingers in breeding males, *Megophrys chishuiensis* sp. nov. differs from *M. acuta*, *M. feii*, *M. shapingensis*, and *M. shuichengensis* (vs. lacking in these species).

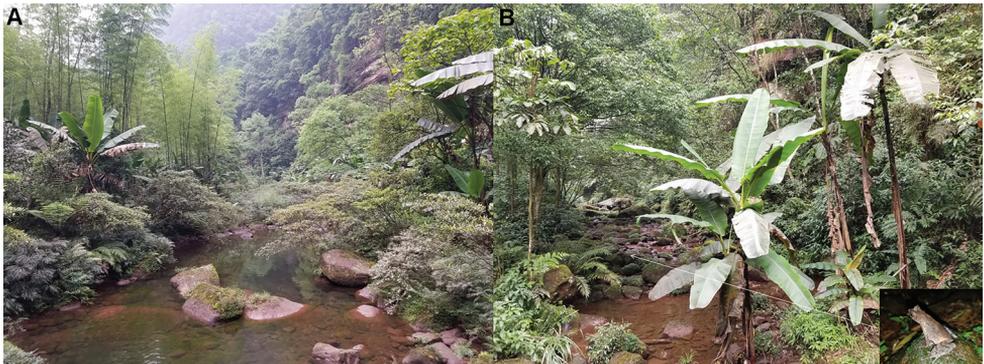
The congeners *M. carinense*, *M. jiangii*, *M. leishanensis*, *M. liboensis*, *M. shuichengensis*, and *M. spinata* have sympatric distribution with *Megophrys chishuiensis* sp. nov. (Fei et al. 2012; Zhang et al. 2017; Li et al. 2018; Liu et al. 2020). The new species can be distinguished from these species by a series of morphological characters as follows. The new species vs. *M. carinense*: body size smaller (adult males with 43.4–44.1 mm and adult females with SVL 44.8–49.8 mm vs. adult males with 92–123 mm and adult females with SVL 137 mm), vomerine teeth absent (vs. present), horn-like tubercle at the edge of each upper eyelid small (vs. prominent), tongue not notched behind (vs. notched behind), lacking lateral fringe in toes (vs. present), and toes without webs at bases (vs. one-fourth webbed). The new species vs. *M. jiangii*: body size bigger (adult males with 43.4–44.1 mm and adult females with SVL 44.8–49.8 mm vs. adult males with 34.4–39.2 mm and adult females with SVL 39.5–40.4 mm), and relative finger lengths  $II < I < V < III$  vs.  $I < II < V < III$ . The new species vs. *M. leishanensis*: body size bigger (adult males with 43.4–44.1 mm and adult females with SVL 44.8–49.8 mm vs. adult males with 30.4–38.7 mm and adult females with SVL 42.3 mm), and tibiotarsal articulation reaching forward to the region between tympanum and eye when hindlimb is stretched along the side of the body vs. reaching middle part of eye. The new species vs. *M. liboensis*: body size smaller in adult females (adult females with SVL 44.8–49.8 mm vs. adult females with SVL 60.8–70.6 mm), vomerine teeth absent vs. vomerine teeth present, and horn-like tubercle at the edge of each upper eyelid is small vs. prominent. The new

species vs. *M. shuichengensis*: body size smaller (adult males with 43.4–44.1 mm and adult females with SVL 44.8–49.8 mm vs. adult males with 102.0–118.3 mm and adult females with SVL 99.8–115.6 mm), horn-like tubercle at the edge of each upper eyelid is small vs. prominent, tongue not notched behind vs. tongue notched behind, lacking lateral fringe in toes vs. present, toes without webs at bases vs. one-fourth webbed, having an internal single subgular vocal sac in male vs. absent, and having nuptial pads and nuptial spines on the dorsal base of the first two fingers in breeding male vs. lacking. The new species vs. *M. spinata*: body size is smaller (adult males with 43.4–44.1 mm and adult females with SVL 44.8–49.8 mm vs. adult males with 47.2–54.4 mm and adult females with SVL 54.0–55.0 mm), horn-like tubercle at the edge of each upper eyelid is small vs. lacking tubercle, tongue not notched behind vs. notched behind, lacking lateral fringe in toes vs. present, and toes without webs at bases vs. one-fourth webbed.

*Megophrys chishuiensis* sp. nov. is phylogenetically closest to *M. minor*, and this new species could be identified from the latter distinctly by having larger body size (SVL 43.4–44.1 mm in males vs. 34.5–41.2 mm in males of *M. minor*), having a small horn-like tubercle at the edge of each upper eyelid (vs. absent in the latter), tongue not notched behind (vs. notched in the latter), tibiotarsal articulation reaching the level between tympanum to eye when leg stretched forward (vs. reaching the level between eye and tip of snout in the latter), and having two metatarsal tubercles in each hand (vs. absent in the latter).

**Distribution and habitats.** *Megophrys chishuiensis* sp. nov. is known from the type locality, Chishui National Nature Reserve (28.38–28.45N, 106.05–109.75E), Chishui City, Guizhou Province, China at elevations between 270–604 m. The individuals of the new species were frequently found in bamboo forest nearby the streams (Fig. 8), and five sympatric amphibian species were also found: *Megophrys omeimontis*, *Odorrana margaratae* (Liu, 1950), *Zhangixalus omeimontis* (Stejneger, 1924), and *Rana omeimontis* Ye & Fei, 1993.

**Etymology.** The specific name *chishuiensis* refers to the distribution of this species, Chishui City, Guizhou Province, China. We propose the common name “Chishui horned toad” and its Chinese name as Chi Shui Jiao Chan (赤水角蟾).



**Figure 8.** Habitats of *Megophrys chishuiensis* sp. nov. in the type locality, Chishui National Nature Reserve, Chishui City, Guizhou Province, China **A** landscape of montane forests **B** a mountain stream (the inset illustrates the holotype on stone).

## Discussion

The new species, *Megophrys chishuiensis* sp. nov., resembles *M. minor* and *M. jiangi*, and detailed comparison with different data sets are important for recognizing them. Our molecular phylogenetic data on mitochondrial DNA and nuclear DNA, and morphological comparisons both separated the new species from the two closely related species. *Megophrys minor* were reported to be distributed widely through the provinces of Sichuan, Guizhou, Chongqing, Yunnan, Guangxi, Jiangxi and north of Vietnam (Fei et al. 2012), but detailed investigations with multiple data suggested that several populations of the species should contain cryptic species (including *Megophrys chishuiensis* sp. nov. and *M. jiangi*). In recent years, a lot of new species of the genus *Megophrys* have been gradually described, of which, a large part of number of species were found in China (Frost 2020). To now, among the 97 species of *Megophrys*, 51 species were discovered in China. Even so, dozens of cryptic species need to be described (Chen et al. 2016; Liu et al. 2018) just in China. Obviously, we should conduct more investigations on the differentiation of the populations and explore the species identity in the wide range.

*Megophrys chishuiensis* sp. nov. with a narrow distribution also fits the “micro-endemism” model like many other congeners (Liu et al. 2018; Wang et al. 2019). Besides, the new species is likely to be threatened by several factors, i.e., developing tourism in Chishui National Nature Reserve, constructions in this area and increasing pollution from tourists. Reasonable managements of tourism in this area may probably facilitate the protection of the populations of the toad and other animal species.

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## Supplementary material 1

### Table S1

Authors: Ning Xu, Shi-Ze Li, Jing Liu, Gang Wei, Bin Wang

Explanation note: Measurements of the adult specimens of *Megophrys chishuiensis* sp. nov.

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Link: <https://doi.org/10.3897/zookeys.943.50343.suppl1>

## Supplementary material 2

### Table S2

Authors: Ning Xu, Shi-Ze Li, Jing Liu, Gang Wei, Bin Wang

Explanation note: Localities, voucher information, and GenBank accession numbers for molecular samples used in this study.

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### Supplementary material 3

#### Table S3

Authors: Ning Xu, Shi-Ze Li, Jing Liu, Gang Wei, Bin Wang

Explanation note: Primer sequences used in this study.

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### Supplementary material 4

#### Table S4

Authors: Ning Xu, Shi-Ze Li, Jing Liu, Gang Wei, Bin Wang

Explanation note: Uncorrected p-distances between the *Megophrys* species based on the 16S gene sequences.

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# A partial molecular phylogeny of *Rhadinaea* and related genera (Squamata, Dipsadidae) with comments on the generic assignment of *Rhadinaea eduardoi*

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## Abstract

The genus *Rhadinaea* is a diverse clade of New World dipsadid snakes, with 22 species arranged in six recognized species groups. The most recently described species, *Rhadinaea eduardoi*, was described based on a unique specimen collected in the Santa Catarina Juquila municipality in the Sierra Madre del Sur of southern Oaxaca, Mexico. Here, based on a reexamination of the holotype and the results of a phylogenetic analysis of the holotype of *Rhadinaea eduardoi* and representatives of several genera closely related to *Rhadinaea*, we reassessed the generic assignment of *Rhadinaea eduardoi*. In our phylogenetic hypothesis, *R. eduardoi* was nested within a strongly supported clade of *Coniophanes fissidens* samples, thus making *Rhadinaea* paraphyletic with respect to *Coniophanes*. Additionally, our reexamination of the holotype of *Rhadinaea eduardoi* revealed that the alleged presence of a subpreocular scale is only true on the right side of the head, and that this scale appears to be a malformed preocular scale; also, a reduction in dorsal scale rows is present; and posterior enlarged maxillary teeth are grooved. Herein we consider that *Rhadinaea eduardoi* should be placed in the synonymy of *Coniophanes fissidens*. Consequently, we recognized only five species groups within the genus *Rhadinaea*.

**Keywords**

*Coniophanes*, generic assignment, synonymization, taxonomy

**Introduction**

Dipsadid snakes are the most speciose family of snakes in the Western Hemisphere, with new species descriptions and taxonomic changes frequently modifying the current composition (García-Vázquez et al. 2018; Mata-Silva et al. 2019). Snakes of the genus *Rhadinaea* Cope, 1863 (Squamata: Dipsadidae) are distributed throughout Mesoamerica, ranging from the Sierra Madre Occidental of southern Sinaloa and Sierra Madre Oriental of northern Nuevo León in Mexico to northwestern Ecuador in South America, with an isolated species, *R. flavilata* (Cope, 1871), in the southeastern USA (García-Vázquez et al. 2018). *Rhadinaea* was formerly considered one of the most diverse New World snake genera, but after several taxonomic changes (see Myers 2011), only 22 species arranged in six species groups are currently recognized. These groups are (number of species in each group in parentheses) the *Rhadinaea calligaster* (1), *R. decorata* (12), *R. eduardoi* (1), *R. flavilata* (2), *R. taeniata* (3) and *R. vermiculaticeps* (3) groups (Myers 1974; García-Vázquez et al. 2018; Mata-Silva et al. 2019). The most recently described species, *Rhadinaea eduardoi* Mata-Silva, Rocha, Ramírez-Bautista, Berriozabal-Islas and Wilson, 2019 is known only from one specimen collected in the municipality of Santa Catarina Juquila in the Sierra Madre del Sur of southern Oaxaca, Mexico. According to the authors, *R. eduardoi* is most closely related to *R. laureata* (Günther, 1868), and is the only representative of its own species group (Mata-Silva et al. 2019). Herein, we present a phylogenetic analysis of *Rhadinaea* and related genera involving species (such as *R. eduardoi*) that were not previously included in the snake phylogeny. Together with a morphological analysis, we use this phylogeny to reassess the taxonomic status of the newly described *R. eduardoi*.

**Materials and methods****Molecular procedures**

To investigate the phylogenetic position of *Rhadinaea eduardoi*, we sequenced a fragment of the mitochondrial gene coding for Cytochrome b (*cyt b*) from 13 individuals including the holotype of *R. eduardoi* (Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Hidalgo, CIB5457); six samples of the remaining three Mexican species groups of *Rhadinaea*, including *Rhadinaea decorata* (Günther, 1858) (3), *R. taeniata* (Peters, 1863) (2) and *R. laureata* (Günther, 1868) (1); three samples of *Rhadinella* Smith, 1941, including one sample each of *R. hempsteadae* (Stuart & Bailey, 1941), *R. lachrymans* (Cope, 1870) and *R. stadelmani* Stuart & Bailey, 1941, previous-

ly *Rhadinaea godmani* group; and four samples of *Coniophanes* Hallowell, 1860 (Table 1). Additionally, we obtained sequences from GenBank of an additional sample of *Coniophanes fissidens* (Günther, 1858) and single samples of *Amastridium* Cope, 1860; *Pliocercus* Cope, 1860; *Synophis* Peracca, 1896; and *Tantalophis* Duellman, 1958. All of these genera are considered closely related to *Rhadinaea* by previous authors (Myers 1974, 2011; Pyron et al. 2013). Finally, we used *Hypsiglena jani* Dugès, 1865 to root the tree (Table 1). This region of *cyt b* has been successfully employed to elucidate phylogenetic relationships within Dipsadidae (Lawson et al. 2005; Daza et al. 2009; Pyron et al. 2013). We extracted genomic DNA from muscle or liver tissue using the standard phenol-chloroform method (Hillis et al. 1996), and utilized polymerase chain reaction (PCR) to amplify the aforementioned fragment with the primers L14919, H16064 (Burbrink et al. 2000), L15584 (de Queiroz et al. 2002), and H15716 (Slowinski and Lawson 2002). We sequenced DNA templates with an ABI 3730xl DNA analyzer (Applied Biosystems, Inc.), using primers L14919 and H16064 (Burbrink et al. 2000).

**Table 1.** Collection and voucher data for colubrid genetic samples used in this study. Acronyms for herpetological collections follow Sabaj (2019). RICB, JCSG, OFV, and UOGV are field identifiers for uncatalogued specimens being deposited in the MZFC-HE and UTA.

No.	Voucher number	Taxa	Locality	GenBank accession number
1	CAS228960	<i>Hypsiglena torquata</i>	USA: Texas: Culberson Co.	EU728592
2	KU289798	<i>Coniophanes fissidens</i> (1)	El Salvador: San Salvador	EF078538
3	RICB521	<i>Coniophanes fissidens</i> (2)	Mexico: Chiapas: Road to La Encrucijada	MT308775
4	MZFC-HE34715	<i>Coniophanes fissidens</i> (3)	Mexico: Guerrero: Arenal de Gómez	MT308776
5	RICB260	<i>Coniophanes fissidens</i> (4)	Mexico: Veracruz: Ocotepc, Los Reyes	MT308777
6	MZFC-HE15533	<i>Coniophanes imperialis</i>	Mexico: Oaxaca: Santa Maria Chimalapa, Cofradía	MT308778
7	CIB5457	<i>Rhadinaea eduardoi</i>	Mexico: Oaxaca: El Obispo, Santa Catarina Juquila	MT308779
8	UTAR44718	<i>Rhadinaea decorata</i> (1)	Guatemala: Huehuetenango: Barillas, Finca Chiblac Buena Vista	MT308780
9	JCSG58	<i>Rhadinaea decorata</i> (2)	México: Veracruz: Sierra de Otontepec	MT308781
10	OFV1109	<i>Rhadinaea decorata</i> (3)	Mexico: Oaxaca: San Felipe Jalapa de Díaz	MT308782
11	UOGV2181	<i>Rhadinaea taeniata</i> (1)	México: Estado de México: Valle de Bravo	MT308787
12	MZFC-HE23859	<i>Rhadinaea taeniata</i> (2)	Mexico: Oaxaca Santa Maria Yavesia	MT308788
13	MZFC-HE21661	<i>Rhadinaea laureata</i>	México: Morelos: Huitzilac	MT308785
14	UTAR42473	<i>Rhadinella stadelmani</i>	Guatemala: Huehuetenango: 3.2 km WSW Patagal	MT308786
15	UTAR42470	<i>Rhadinella hemsteadae</i>	Guatemala: Quiché: Uspantán, road El Chimel-San Pablo	MT308783
16	UTAR42335	<i>Rhadinella lachrymans</i>	Guatemala: San Marcos: San Rafael Pie de La Cuesta, Finca America El Vergel	MT308784
17	EBUAP1853	<i>Tantalophis discolor</i>	México: Oaxaca: Sierra de Monte Flor	EF078589
18	UTAR46905	<i>Amastridium sapperi</i>	Guatemala: Izabal	GQ334479
19	QCAZ9175	<i>Synophis zamora</i>	Ecuador: Zamora Chinchipe: Las Orquídeas	KT345376

## Phylogenetic relationships

We aligned the obtained sequences using the Muscle algorithm (Edgar 2004) included in the software MEGA 7 (Kumar et al. 2016). The best-fitting substitution models and partitioning schemes were selected jointly using the Bayesian Information Criterion in the software PARTITIONFINDER 1.1.1 (Lanfear et al. 2012). We performed a Bayesian phylogenetic analysis with the software MRBAYES 3.2 (Ronquist et al. 2012). We ran the analysis for 50,000,000 generations with the default settings and tree sampling every 1000 generations. Output parameters were visualized using TRACER 1.4 (Rambaut and Drummond 2007) to verify stationarity and convergence. After discarding the first 25% as burn-in, we summarized parameter values of the samples from the posterior distribution on the maximum clade credibility tree using TREEANNOTATOR 1.4.8 (Drummond and Rambaut 2007) with the posterior probability limit set to 0.1 and mean node heights summarized. We considered clades with posterior probabilities (Pp)  $\geq 0.95$  as significantly supported (Huelsenbeck and Rannala 2004).

## Genetic distances

To obtain an estimate of genetic distances, we computed pairwise genetic mean distances between *Coniophanes*, *Rhadinaea*, and *R. eduardoi*. We calculated the corrected pairwise genetic distances using the K2P model with MEGA 7 (Kimura 1980; Kumar et al. 2016).

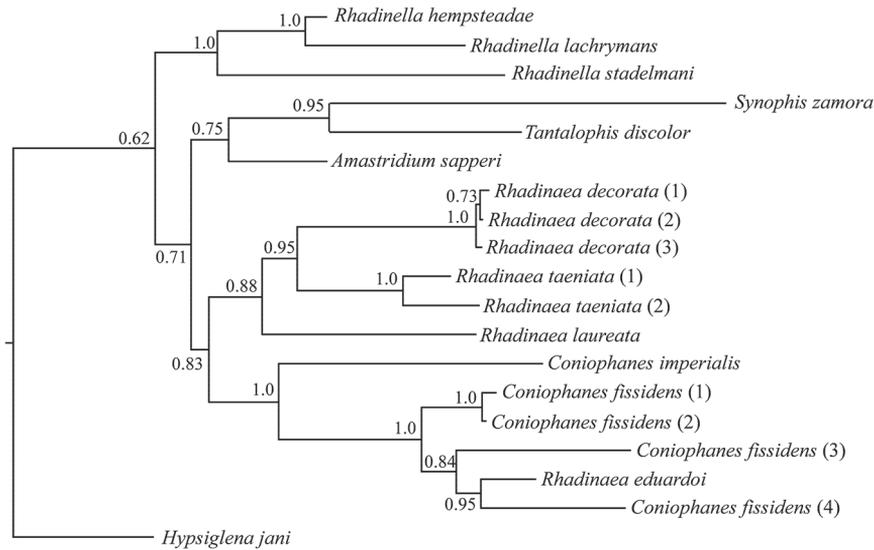
## Morphological comparisons

We compared the holotype of *Rhadinaea eduardoi* (CIB5457) with a series of *Coniophanes* specimens deposited at the Museo de Zoología “Alfonso L. Herrera”, Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC-HE). Scale nomenclature and ventral scale counts follow Myers (1974). To score the dorsal scale rows, we made three separate counts: the first located one head-length posterior to the head, the second located at midbody, and the third located four ventral scales anterior to the anal plate. We counted ventral scales as suggested by Dowling (1951a). Dorsal scale reduction formula is based on Dowling (1951b). Other scutellation characters that we scored were the number of preoculars, postoculars, supralabials, infralabials and subcaudals. We examined the maxillary dentition of the holotype in situ.

## Results

### Phylogenetic relationships

The final alignment consisted of 1055 bp. The partitions and models that best fit the data were GTR+G for the first and second codon positions, and GTR+G+I for the third codon position. In the Maximum Credibility Tree (Fig. 1), the haplotypes of *Rhadinella* (*R. hempsteadae*, *R. stadelmani*, and *R. lacrymans*) formed the sister taxon



**Figure 1.** Phylogenetic relationships and phylogenetic position of holotype of *Rhadinaea eduardoi* based on partial sequences of the mitochondrial gene Cytochrome b (*cyt b*). Numbers indicate the Bayesian posterior probabilities for each node.

to all the remaining haplotypes. Except for the haplotype of *R. eduardoi*, the haplotypes of *Rhadinaea* comprised a clade where *R. decorata* was strongly supported as sister taxon to *R. taeniata* and these two taxa formed the sister taxon to *R. laureata*, the supposedly closest relative of *R. eduardoi* (Mata-Silva et al. 2019), although this relationship was not significantly supported. The *Rhadinaea* clade was the sister taxon to a significantly supported clade comprised of all the haplotypes of *Coniophanes*. The haplotype of *R. eduardoi* was nested within a significantly supported clade composed of all the haplotypes of *C. fissidens*, with *C. imperialis* (Baird & Girard, 1859) as the sister taxon to this clade.

## Genetic distances

Genetic distances between species of *Rhadinaea* and *R. eduardoi* ranged from 18.9–22.4%, whereas distances between species of *Coniophanes* and *R. eduardoi* were much smaller (10.1–11.7%).

## Morphology

*Rhadinaea eduardoi* was originally assigned to *Rhadinaea* due to the presence of a small subpreocular scale, the absence of dorsal scale row reduction, supralabial counts, and dorsal color pattern (Mata-Silva et al. 2019). Our reexamination of the holotype (CIB 5457) verified most of the meristic data presented by Mata-Silva et al. (2019), such as ventral and subcaudal counts. However, two characters differed notably compared

to our reexamination: 1) the subpreocular is actually present only on the right side of the head and, furthermore, it appears to be a malformed preocular scale, and 2) dorsal scale row reduction is present, the arrangement being 17-17-15 with counts reduced by fusion of dorsal scale rows 8 + 9, according to the formula:

$$17 \frac{8+9(75)}{8+9(75)} 15(120)$$

Of additional diagnostic importance, the maxillary teeth posterior to the diastema are enlarged and grooved. Among other features, the genus *Rhadinaea* is characterized by having a small subpreocular inserted between the corners of two supralabials at the antero-ventral edge of the orbit; the same number of dorsal scale rows throughout the body; and not grooved maxillary teeth posterior to the diastema (Myers 1974, 2011). The characters present in the holotype of *R. eduardoi* are thus inconsistent with the current diagnosis of the genus *Rhadinaea*. Together with the molecular results presented above, this leads us to conclude that the generic allocation of *R. eduardoi* was erroneous.

## Discussion

The phylogenetic relationships obtained in this study are generally consistent with previous phylogenies that suggested a close relationship between *Amastridium*, *Coniophanes*, *Rhadinaea*, and *Tantalophis* (Daza et al. 2009; Pyron et al. 2013), and that supported the separation of *Rhadinella* from *Rhadinaea* (Myers 2011). Pyron et al. (2013) found a strong relationship between *Rhadinaea* and *Coniophanes*, and both with *Tantalophis discolor* (Günther, 1860) and *Amastridium veliferum* Cope, 1860. These relationships are similar to our result, but with the inclusion of *Synophis zamora* Torres-Carvajal, Echevarría, Venegas, Chávez & Camper, 2015 and *Pliocercus elapoides* Cope, 1860 in the same clade of *Amastridium* and *Tantalophis*. Furthermore, we resolved *Rhadinella* as the sister clade of *Rhadinaea* + *Coniophanes*. *Synophis*, *Pliocercus* and *Rhadinella* were not included in the phylogeny of Pyron et al. (2013). Additionally, Daza et al. (2009) found a supported clade formed by *Amastridium sapperi* (Werner, 1903), *Rhadinaea fulvivitis* and *Coniophanes fissidens*, however, *Tantalophis discolor* appear basal to these taxa, plus another dipsadids in an unsupported clade. None of the other genera considered in our study were included by Daza et al. (2009). Although the phylogenetic relationships of *Rhadinaea* with the remaining genera included here were recovered with low support (< 0.95), it is evident that the clade containing *C. fissidens* and *R. eduardoi* is not closely related to the genus (Fig. 1). This result, in addition to the genetic distinctiveness, leads us to consider that the generic allocation of *R. eduardoi* was erroneous. Our revision of morphological characters agrees with this assessment. Hence, we also propose the recognition of only five species groups within *Rhadinaea*.

Bailey (1939) defined the genus *Coniophanes* as consisting of medium sized snakes with enlarged, grooved posterior teeth; posterior dorsal scale reduction through fusion of paravertebral rows; and one or two preocular scales – all characters present on the holotype of *Rhadinaea eduardoi*. The color pattern (diffuse and poorly defined lateral

and middorsal stripes on body) and key characters (i.e., posterior dorsal scale reduction by fusion of paravertebral rows; and enlarged, grooved teeth posterior to the diastema) of the holotype clearly allocate it as a representative of *Coniophanes fissidens* (Fig. 2). However, some scutellation characters of the holotype merit discussion. The holotype shows a dorsal scale arrangement in 17-17-15 longitudinal rows, which is very rare in *C. fissidens* (see Smith 1941; and Campbell 1989 for a discussion on the variation exhibited by this species). Of over 100 specimens of *C. fissidens* (Palacios-Aguilar et al. in prep) examined from throughout its range in Mexico, only one specimen (MZFC-HE17791) from Santa María Huatulco, Cuenca del Río Magdalena, Oaxaca exhibited a similar arrangement. The presence of 17 scale rows at midbody is rarely seen in *Coniophanes*, but common in most *Rhadinaea* (Bailey 1939; Myers 1974), likely being one of the factors that led Mata-Silva et al. (2019) to a wrong generic allocation of *R. eduardoi*. The presence of a subpreocular scale is also rare in the genus *Coniophanes*, being consistently present only in the *Coniophanes piceivittis* species group (Bailey 1939; Flores-Villela and Smith 2009). *Coniophanes fissidens* is the most broadly distributed species within the genus, with many subspecies having been described (Smith 1941), and some authors considering it as a species complex (e.g. McCranie 2011).

Based on morphology and geographic distribution, *Rhadinaea eduardoi* is perhaps best considered a junior synonym of *C. f. dispersus*, a subspecies distributed on the Pacific versant of Mexico west of the Isthmus of Tehuantepec from Jalisco to Oaxaca (*sensu* Smith 1941). However, our phylogenetic tree shows a close relationship between the holotype of *R. eduardoi* and a sample from Veracruz, Mexico (*C. f. fissidens*) which together are the sister clade of a nearly topotypic sample of *C. f. dispersus* (Fig. 1, Table 1). The inclusion of additional samples would help to elucidate this interesting issue. For now, we refrain from recognizing subspecies within *C. fissidens*, pending the acquisition of more samples spanning the species' wide distribution and the inclusion of additional molecular markers in a more comprehensive study. Hence, we simply suggest the synonymization of *R. eduardoi* with *Coniophanes fissidens* Günther, 1858.

Following the monographic treatment of the genus *Rhadinaea* by Myers (1974), scientific understanding of the composition of the genus has been further modified. The former *brevirostris* and *lateristriga* groups were accommodated in the resurrected genera *Taeniophallus* Cope, 1895 and *Urotheca* Bibron, 1840, respectively (Myers and Cadle 1994). Subsequently, Savage and Crother (1989), and Myers (2011) resurrected *Rhadinella* to include the former *Rhadinaea godmani* group. To date, no large-scale molecular phylogeny has included more than two taxa of *Rhadinaea*, nor any representatives of the genera mentioned above (e.g., Figueroa et al. 2016; Zaher et al. 2019), so the validity of this taxonomy (based only on morphological evidence) remains to be tested in a more comprehensive way. Also, while many authors have agreed that a close relationship between *Rhadinaea* and *Coniophanes* is likely, only a study by Cadle (1984) based on immunological data presented rigorous evidence to support this hypothesis. The present work thus provides the first insights into the phylogenetic relationships of these Neotropical snake genera, supporting the reciprocal monophyly of *Rhadinaea* and *Rhadinella*, and a close relationship between the former genus and *Coniophanes* as sister groups. Only a few morphological characters (dorsal scale re-



**Figure 2.** Adult male *Coniophanes fissidens* (MZFC-HE34194) from East of Río Santiago, Guerrero, Mexico. This specimen was obtained approximately 60 kilometers WNW of the type locality of *C. f. dispersus*. Compare this specimen with images 2, 3, and 4 from Mata-Silva et al. (2019).

ductions, number of preoculars, and teeth grooving) have been considered useful for differentiating *Rhadinaea* and *Coniophanes* (Bailey 1939; Cadle 1989; Myers 1974). As such, additional work including more comprehensive sampling of groups, the use of more molecular markers, and detailed revision of morphology is needed to explore their monophyly and evolutionary history.

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## Supplementary material I

### Table S1. Specimens examined

Authors: Ricardo Palacios-Aguilar, Uri Omar García-Vázquez

Data type: Specimens examined

Explanation note: All of the specimens are *Coniophanes fissidens* from Mexico. Acronyms for herpetological collections follow Sabaj (2016).

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