# Six new species of Diostracus Loew (Diptera, Dolichopodidae) from Tibet 

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#### Abstract

Six species of Diostracus from Tibet are described as new to science: D. concavus sp. nov., D. fasciculatus sp. nov., $D$. laetus sp. nov., $D$. polytrichus sp. nov., $D$. strenus sp. nov., and $D$. translucidus sp. nov. A key to the species from Tibet of the genus is provided. The distribution of the genus in Tibet is also discussed.


## Keywords

Key, long-legged fly, morphology, The Himalayan region

## Introduction

Diostracus belongs to the subfamily Hydrophorinae of Dolichopodidae. These flies are usually stout and larger than other dolichopod flies. They prefer to live on the vertical or oblique surfaces of rocks at altitudes 1000 m to 3500 m , with slow water flow, or a thin water layer on the surface, or just wet, but they do not like those rocks behind streams or waterfalls.

Before our study, 101 species of the genus had been reported (Zhu 2006; Zhu et al. 2007a, b; Grichanov 2013, 2015, 2017; Pusch 2014; Wang et al. 2015). The first Diostracus, D. prasinus Loew, was named in 1861 from the Nearctic Region, whereas most species of the genus were reported from the Palaearctic and Oriental realms.

Remarkably, Diostracus shows great diversity in the Himalayas, which is the junctions of the two realms, and 39 species have been reported from this area (Zhu 2006; Wang et al. 2015). Tibet is located in the east of the Himalaya Mountains. However, there were only three species of Diostracus known from Tibet: D. nebulosus Takagi, 1972 is widely distributed in the Himalayas, while D. acutatus Wang et al., 2015, and D. tibetensis Wang et al., 2015 were reported in Nyingchi, Tibet.

Here we provide an investigation of the diversity of Diostracus in Tibet and six new species are reported.

## Materials and methods

This work is based on the material collected by sweep netting from Tibet in 2013 and 2018. The main locality is Yadong County ( $88^{\circ} 52^{\prime}-89^{\circ} 30^{\prime} \mathrm{E}, 27^{\circ} 23^{\prime}-28^{\circ} 18^{\prime} \mathrm{N}$ ), located on the southern slope of the Himalaya Mountains. All the altitudes of localities are approximately 3000 m a.s.l. The specimens are deposited in the Entomological Museum of China Agricultural University, Beijing (CAU). The information about the specimens studied in the paper are presented in Table 1. Morphological terminology for adult structures mainly follows Cumming and Wood (2017).

The following abbreviations are used:

| acr | acrostichal, | MSSC | male secondary sexual characters, |
| :--- | :--- | :--- | :--- |
| ad | anterodorsal bristle $(s)$, | pvt | postrovertical bristle (s), |
| av | anteroventral bristle $(s)$, | npl | notopleural bristle $(s)$, |
| dc | dorsocentral bristle $(s)$, | oc | ocellar bristle(s), |
| CI | fore coxa, | pd | posterodorsal bristle(s), |
| CII | mid coxa, | ph | postohumeral bristle, |
| CIII | hind coxa, | psa | postosupraalar bristle, |
| FI | fore femur, | pv | postoventral bristle(s), |
| FII | mid femur, | TI | fore tibia, |
| FIII | hind femur, | TII | mid tibia, |
| It | fore tarsomeres, | TIII | hind tibia, |
| h | humeral bristle, | sa | supraalar bristle, |
| IIt | mid tarsomeres, | sc | scutellar bristle(s), |
| IIIt | hind tarsomeres, | t | tarsomeres, |
| LI | fore leg, | vt | vertical bristle(s). |

Each holotype male was submitted to barcode sequencing, using the primers LCO1480/ HCO2198 (Folmer et al. 1994), under the following experimental procedures: 3 min at $95^{\circ} \mathrm{C}$ for the first cycle, reactions were amplified through 35 cycles at following paraments, 30 s at $95^{\circ} \mathrm{C}, 30 \mathrm{~s}$ at $55^{\circ} \mathrm{C}$, and 90 s at $72^{\circ} \mathrm{C}$, then elongation for 1 cycle at $72{ }^{\circ} \mathrm{C}$ for 10 min . The sequences were uploaded to GenBank (Table 2). Females were also sequenced to pair them to males, in addition to examination of their morphological characters.

Table I. List of materials studied in this paper.

| Species name | Number and sex | Locality | Altitude | Geographical coordinates | Type or other material |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Diostracus acutatus |  | Tibet, Shigatse, Yatung County | $2700-3200 \mathrm{~m}$ | $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ | Other material |
|  | 2 人才 1 ¢ | Tibet, Shigatse, Yatung County, Pamaimang | ca 3350 m | $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ | Other material |
| D. concavus | $1{ }^{1}$ | Tibet, Bomi, Gagela Mt. | 3026 m | 1 | Holotype |
| D. fasciculatus | $1{ }^{1}$ | Tibet, Shigatse, Yatung County | $2700-3200 \mathrm{~m}$ | $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ | Holotype |
|  |  | Tibet, Shigatse, Yatung County | $2700-3200 \mathrm{~m}$ | $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ | Paratypes |
|  | $\begin{gathered} 11 \text { रु } 4 \\ \text { Y } 4 \\ \hline \end{gathered}$ | Tibet, Shigatse, Yatung County, Pamaimang | ca 3350 m | $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ |  |
| D. laetus | $1{ }^{\text {® }}$ | Tibet, Shigatse, Yatung County | $2700-3200 \mathrm{~m}$ | $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ | Holotype |
| D. polytrichus | $1{ }^{\text {® }}$ | Tibet, Shigatse, Yatung County, Pamaimang | ca 3350 m | $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ | Holotype |
|  | 2 ôd 2 우 | Tibet, Shigatse, Yatung County, Pamaimang | ca 3350 m | $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ | Paratypes |
| D. strenus | $1{ }^{1}$ | Tibet, Shigatse, Yatung County | $2700-3200 \mathrm{~m}$ | $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ | Holotype |
|  | 10 | Tibet, Shigatse, Yatung County, Pamaimang | ca 3350 m | $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ | Paratype |
| D. translucidus | $1{ }^{1}$ | Tibet, Medog | 1 | 1 | Holotype |
|  | 2 우 | Tibet, Medog | 1 | 1 | Paratypes |

Table 2. Barcode label data for Diostracus species reported in this paper.

| Species | Specimen catalog code | Sex | GenBank code | GenSeq |
| :--- | :---: | :---: | :---: | :---: |
| D. acutatus | Di03M | male | MT4474559 | Genseq-5 COI |
|  | Di03F | female | MT462596 | Genseq-5 COI |
| D. concavus | Di06M | male | MT452300 | Genseq-1 COI |
| D. fasciculatus | Diostracus sp1 | male | MT080656 | Genseq-2 COI |
|  | Di01F | female | MT462594 | Genseq-2 COI |
| D. laetus | Di05M | male | MT452307 | Genseq-1 COI |
| D. polytrichus | Di02M | male | MT438694 | Genseq-2 COI |
|  | Di02F | female | MT462595 | Genseq-2 COI |
| D. strenus | Di04M | male | MT447458 | Genseq-2 COI |
| D. translucidus | Diostracus sp7 male | male | OP249496 | Genseq-1 COI |
|  | Diostracus sp7 female | female | OP249495 | Genseq-2 COI |

## Taxonomic accounts

## Genus Diostracus Loew, 1861

Diostracus Loew, 1861: 44. Type species: Diostracus prasinus Loew, 1861 (monotypy). Sphyrotarsus Mik, 1874: 342. Type species: Sphyrotarsus argyrostomus Mik, 1874 (monotypy).
Asphyrotarsus Oldenberg, 1916: 193. Type species: Liancalus leucostomus Loew, 1861 (original designation).
Takagia Negrobov, 1973: 1520 (as subgenus of Sphyrotarsus Mik, 1874) (not Matsumura, 1942). - Negrobov 1978: pl. CLXI (as genus, in error). Type species: Sphyrotarsus stackelbergi Negrobov, 1965 (original designation).

Lagodechia Negrobov \& Tsurikov, 1996: 632. Type species: Diostracus spinulifer Negrobov \& Tsurikov, 1988 (monotypy).
Ozmena Özdikmen, 2010: 265 (new name for Takagia Negrobov, 1973, not Matsumura, 1942) (as subgenus of Sphyrotarsus Mik, 1874).

Diagnosis. Medium to huge dolichopodid flies (males body length $3.4-7.6 \mathrm{~mm}$, usually larger in females). Body stout, metallic green, always with pollinosity. Vertex weakly concave; upper occiput slightly concave. Scape with or without dorsal seta; arista subapical or sub-basal. Palpus rather large and loosely applied on proboscis, sometimes elongated, beyond the apex of proboscis in males, and relatively smaller in females. Proboscis bulky. Acr absent; four or six pairs of dc; one or two strong npl; scutellum with two strong sc, sometimes with marginal hairs. Crossvein $m$-cu longer than final section of $5^{\text {th }}$ longitudinal vein. Legs and wings are often modified in males, which are usually the identical characters for groups or species. Abdomen cylindrical, with five visible segments; Sternite I or IV sometimes with produced process, and Sternite V usually split into pair of sclerites in males.

## Key to species (males) of Diostracus from Tibet

1 Palpus normal, not reaching apex of proboscis; scutellum with pair of sc and four or five pairs of marginal hairs; CI with row of anterior hairs and two strong recurved spines at extreme apex; FI with a deep hollow at base; wing indistinctly tinged grayish, apically with three translucent windows; FII with row of dense ad on apical $2 / 5$
D. translucidus sp. nov.

- Palpus prolonged, reaching apex of proboscis; scutellum with pair of sc, without marginal hairs; other characters variable2
2 Empodium and pulvilli reduced into minute protuberance (fenestratus group).... ..... 5
- Empodium and pulvilli distinct ..... 33 Wing with a dark square marking on vein $M$ near crossvein; five dc.D. nebulosus Takagi
- Wing with a small round black nodule at middle of crossvein; six dc ..... 4
4 Cercus finger-like, straight, with long yellow hairs D. tibetensis Wang et al.
- Cercus lamellate with broad basal half.. D. polytrichus sp. nov.5 Wing with dark and yellow markings at middle; discal crossvein strongly sinuate,S-shaped; anterodistal corner of discal cell with an accessory cellula (pulchripennissubgroup)D. laetus sp. nov.- Wing without distinct marking; discal crossvein nearly straight; anterodistalcorner of discal cell without accessory cellula (flex subgroup)6
6 Posterior margin of wing somewhat prolonged along vein $\mathrm{CuA}_{1}$; apex of TIIswollen with two rows of narrow flat willow leaf-like ventral bristles, row oflong av (anterior ones somewhat curved), rows of pale long ventral hairs ( $2-3 \times$longer than TII depth, curved), row of erect pv along whole length (as long asTII depth), apically with three long bristles.................D. fasciculatus sp. nov.
- Posterior margin of wing normal; TII normal, not swollen. ..... 7

7 Crossvein m-cu elongated, strongly bent, margined with black on long anterior portion, and with blackish spot at short posterior portion; FI slightly thickened; $\mathrm{It}_{1}$ with acute apicoventral corner, $\mathrm{It}_{2}$ with an acute ventral process near extreme base; abdominal sternite I with a nearly acute process at middle; sternite IV medially with an obtuse anterior process and two short thin, contiguous posterior processes bearing bundle of brown hairs
D. acutatus Wang et al.

- Crossvein m-cu not elongated, acutely and deeply arched to vein $M_{1}$, forming a ' h '-shaped curve, with a jet-black brand inside curve; FI distinctly thickened; $\mathrm{It}_{1}$ shortened, without acute apicoventral corner; abdominal sternite without such appendage8

8 Propleuron with two or three sparse short pale hairs on upper portion and one or two short pale hairs on lower portion; $\mathrm{It}_{3-5}$ normal, without ventral suture ...
D. strenus sp. nov.

- Propleuron with group of seven long pale hairs on upper portion and group of nearly 20 long pale hairs on lower portion; $\mathrm{It}_{3-5}$ with a ventral suture
D. concavus sp. nov.


## Diostracus acutatus Wang, Wang \& Yang, 2015

Figs 1, 2, 31A, B, 32A, B
Diostracus acutatus Wang, Wang \& Yang, 2015: 96, figs 1-6.
Material examined. China $\cdot 3$ đ $6 q$ q, Tibet, Shigatse, Yatung County; $27^{\circ} 48^{\prime} \mathrm{N}$, $88^{\circ} 90^{\prime}$ E; 13 VII. 2018; 2700-3200 m; leg. Yajun Zhu; • 2 ठ ${ }^{\top} 1$ q, same data as for preceding; Pamaimang; 14 VII. 2018; ca 3350 m .

Diagnosis. The species belongs to the fenestratus group, characterized by specialized $\mathrm{It}_{1}$ and $\mathrm{It}_{2}$ (MSSC). $\mathrm{It}_{1}$ distinctly shortened, with a nearly acute apicoventral process; $\mathrm{It}_{2}$ basally bent with a short finger-like ventral process near extreme base. Females of $D$. acutatus are characterized by the apical or subapical antenna, oblique crossvein, $\mathrm{m}-\mathrm{cu}$ oblique, and brownish trochanters.

Male (Fig. 1A). Same as description of Wang et al. (2015).
Female (Fig. 1B). Body length 6.0-6.4 mm; wing length $6.9-7.8 \mathrm{~mm}$. Antennal scape with two short dorsal bristles, first flagellomere somewhat prolonged, $1.2 \times$ longer than width, arista apical or subapical, $4.6 \times$ longer than first flagellomere (Fig. 31A, B). Proboscis yellowish brown with blackish edge; palpus relatively smaller than males, not reaching apex of proboscis. six dc, anterior four short and weak, $5^{\text {th }}$ long and weak. Propleuron with a single pale hair on upper portion, and group of 5-7 long pale hairs on lower portion. Legs black, except trochanters yellowish brown. CI without distinctive bristle or hair, but with short pale anterior hairs on lower portion; FI without distinct bristles; TI with an ad at basal $1 / 4$, four pd, apically with one bristle and comb of anterior bristles; FII with two anterior bristles on apical $1 / 3$; TII with three ad, two pd, apically with five bristles; FIII with three anterior bristles on apical $1 / 3$; TIII with four


Figure I. Diostracus acutatus A male; lateral view B female, lateral view. Scale bars: 1 mm .


Figure 2. D. acutatus female, abdomen $\mathbf{A}$ dorsal view $\mathbf{B}$ lateral view $\mathbf{C}$ ventral view. Scale bar: 1 mm .
ad, two pd, 4-6 weak v, apically with two bristles. Wing (Fig. 32A, B): m-cu somewhat curved, forming right angle with $\mathrm{CuA}_{1}$. Halter yellow.

Female terminalia (Fig. 2): Abdominal tergite VIII split into pair of sclerites; epiproct split into pair of triangular hemitergites, apically with row of seven strong curved spines; dorsal lobes of cercus rounded in lateral view, with yellow bristles; ventral lobes of cercus membranous.

Remarks. Diostracus acutatus is similar to D. nishidai Saigusa, in that they both have acute apico-ventral corners of $\mathrm{It}_{1}$ and $\mathrm{It}_{2}$ and the shapes of wings and the appendages on abdominal sternite IV are nearly identical. But for males, they are different in the shapes of the main lobe of surstylus, and the apicoventral corner of $\mathrm{It}_{1}$ in D. acutatus is sharper.

## Diostracus concavus sp. nov.

https://zoobank.org/916C3BDC-2FA0-4862-807F-B799C54D4A06
Figs 3-6
Type material. Holotype: China - ठ̃, Tibet, Nyingchi, Bomi, Gagela Mountain, 3026 m, 2013. VII. 13, leg. Xiaoyan Liu.

Diagnosis. MSSC: first flagellomere $1.5 \times$ longer than wide; propleuron with group of seven long pale hairs on upper portion and group of $\sim 20$ long pale hairs on lower portion; $\mathrm{It}_{1}$ shortened and expanded, concave ventrally, forming a hollow with an expanded $\mathrm{It}_{2}$. Wing (Fig. 4B) hyaline, m-cu acutely and deeply arched to vein $\mathrm{M}_{1}$, forming an ' $h$ '-shaped hairpin with a slender jet-black mark inside.

Description. Male (Fig. 3). Body length 5.7 mm ; wing length 6.8 mm .
Head (Fig. 4A) dark metallic green with pale gray pollinosity. Eyes separated; face widened towards clypeus. Hairs and bristles on head black; lower postocular bristles including posteroventral hairs pale. Ocellar tubercle distinct, with pair of strong oc, without posterior hairs; vt short, $0.7 \times$ as long as oc, nearly as long as pvt. Antenna black; scape without dorsal bristle; first flagellomere subtriangular, $1.5 \times$ longer than wide; arista subapical, $5.2 \times$ as long as first flagellomere, nearly bare. Proboscis blackish with pale hairs; palpus lobate, $3 \times$ as long as broad, blackish with a purple luster, without distinctive bristle.

Thorax dark metallic green with pale gray pollinosity. Hairs and bristles on thorax black; six mostly hair-like dc except posterior most one dc longest and thick; acr absent; two h, one ph, two npl, one sa, one psa; scutellum with pair of long sc. Propleuron with group of seven long pale hairs on upper portion and group of nearly 20 long pale hairs on lower portion.

Legs nearly entirely black except fore trochanter dark yellow; claws well developed, empodium and pulvilli reduced. Hairs and bristles on legs black except those on coxae pale. CI with cluster of anterior dense, erect, long, pale hairs on apical $1 / 4$ (nearly as long as CI) and comb of pale hairs along anterior margin; CII and CIII nearly bare. Fore trochanter with rows of tiny ventral spines and a hook-like posterior process.


Figure 3. D. concavus, male; lateral view. Scale bar: 1 mm .


Figure 4. $D$. concavus male $\mathbf{A}$ head, lateral view $\mathbf{B}$ apex of wing $\mathbf{C}$ apex of TI and $\mathrm{It}_{1-2,}$, posterior view D apex of FI TI and $\mathrm{It}_{1-2}$, anterior view. Scale bars: 1 mm .

FI distinctly thickened, with cluster of four or five erect ventral bristles at extreme base; TI distinctly thickened, weakly curved, with 3 ad , two pd , row of six long pv on apical $1 / 4$; $\mathrm{It}_{1}$ shortened and expanded, concave ventrally, forming a hollow with expanded $\mathrm{It}_{2}$, anterior margin expanded into two dentiform lobes with row of four or five bristles, ventral margin expanded into a lobe at base (corresponding to the lobe of $\mathrm{It}_{2}$ ), with a subapical pv (Fig. 4C, D); basal half of $\mathrm{It}_{2}$ expanded and concave ventrally, anterior margin expanded into a pale dentiform lobate and a rectangular lobate, posterior ventral margin expanded into a finger-like lobe at extreme base, apical half with rows of erect ventral bristles, apical half of $\mathrm{It}_{2}, \mathrm{It}_{3-5}$ with a ventral suture. FII somewhat flattened laterally, with three ad on apical $1 / 3$; TII with three weak ad, three weak pd, apical $1 / 5$ with two rows of long pale anteroventral hairs (longest ones nearly as long as $1 / 4$ of TII) and row of erect short pv (nearly as long as TII depth), and row of long pale posteroventral hairs along whole length (nearly as long as FII depth), apically with three long bristles. FIII with an anterior bristle and two curved av subapically; TIII with four ad and four pd, apically with two strong long bristles. Relative lengths of tibia and five tarsomeres: LI 5.0: 0.7: 2.5: 2.0: 1.0: 1.3; LII 9.0: 6.1: 2.2: 1.3: 0.7: 1.0; LIII 10.3: 5.2: 3.0: 1.5: 0.7: 1.0.


Figure 5. D. concavus male $\mathbf{A}$ abdominal Sternite IV and V, male genitalia removed, ventral view $\mathbf{B}$ male genitalia, lateral view $\mathbf{C}$ male genitalia, ventral view. Scale bar: 1 mm .


Figure 6. D. concavus male $\mathbf{A}$ abdominal Sternite IV and V, male genitalia removed, ventral view $\mathbf{B}$ male genitalia, lateral view $\mathbf{C}$ male genitalia, ventral view. Scale bar: 1 mm .

Wing (Fig. 4B) hyaline, indistinctly tinged grayish; veins dark brown; crossvein m -cu not elongated, acutely and deeply arched to vein $\mathrm{M}_{1}$, forming a ' $h$ '-shaped curve with $M_{1}$, with a slender jet-black brand inside hairpin curve; posterior margin somewhat prolonged along vein $\mathrm{CuA}_{1}$. Squama brown with brown hairs. Halter pale (somewhat faded).

Abdomen nearly as long as head and thorax combined, dark metallic green with pale gray pollinosity. Abdomen with pale pubescence. Sternite IV medially with an obtuse anterior process and a tubercle bearing bundle of brown bristles, each lateroposterior corner with a tubercle bearing bundle of brown bristles (Figs 5A, 6A). Sternite V split into pair of sclerites, each sclerite ginkgo leaf-like in shape (Figs 5A, 6A).

Male genitalia (Figs 5B, C, 6B, C): Epandrium slightly longer than wide. Epandrial lobe long, wavy, band-like, with an acute basal process, a short bristle at tip. Surstylus thick, lamellated, with two short spines. Hypandrium short thick, apically with a shallow, V-shaped apical incision. Cercus rather short ( $1 / 3$ as long as epandrium), spoon-shaped, with dark yellow hairs on outer surface, apical one long (nearly as long as cercus), and subapically with group of dense erect dark yellow bristles on inner surface.

Female. Unknown.
Distribution. China (Tibet).
Remarks. The new species belongs to the flexus subgroup of $D$. fenestratus group. This new species has wing characteristics similar to that of $D$. strenus sp. nov., but the latter can be separated from $D$. concavus by $\mathrm{It}_{3-5}$, which is normal and has no ventral suture (MSSC).

Etymology. New species name refers to the concave $\mathrm{It}_{1}$ of males.

## Diostracus fasciculatus sp. nov.

https://zoobank.org/49078BA3-AAB9-4799-B295-B557A671788D
Figs 7-11, 31C, 32C
Type material. Holotype: China • $\widehat{\lambda}$, China: Tibet, Shigatse, Yatung County ( $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ ), 2700-3200 m, 2018. VII. 13, leg. Yajun Zhu. Paratypes: • 3 ठす 2 $Q$, same data as for holotype; $11 \delta^{\top}, 4 q$, same data as for preceding, but Pamaimang, 3350 m, 2018. VII. 14.

Diagnosis. MSSC: posterior margin of wing somewhat prolonged along vein $\mathrm{CuA}_{1}$. TII and TIII prolonged; apex of TII swollen with two rows of narrow, flat, willow leaf-like ventral bristles, row of long av and pale curve ventral hairs, row of erect pv along whole length, apically with three long bristles; $\mathrm{It}_{1}$ swollen at extreme base, with rows of long curved posterior bristles.

Description. Male (Fig. 7A). Body length 5.0-5.4 mm; wing length $5.8-6.2 \mathrm{~mm}$.
Head (Fig. 8A) dark metallic green with pale gray pollinosity. Eyes separated; face widened towards clypeus. Hairs and bristles on head black; lower postocular bristles including posteroventral hairs pale. Ocellar tubercle distinct, with pair of strong oc,


Figure 7. D. fasciculatus A male; lateral view B female, lateral view. Scale bars: 1 mm .


Figure 8. D. fasciculatus male $\mathbf{A}$ head, lateral view $\mathbf{B}$ thorax, lateral view, show the bristles $\mathbf{C}$ wing $\mathbf{D}$ FI, anterior view $\mathbf{E}$ part of TII and IIt $_{1}$, anterior view. Scale bars: 1 mm .
without posterior hairs; vt rather short, $0.5 \times$ as long as oc, nearly as long as pvt. Antenna black; scape without dorsal bristle; first flagellomere subtriangular, $1.5 \times$ longer than wide; arista apicodorsal, $4.5 \times$ as long as first flagellomere, nearly bare. Proboscis blackish with pale hairs; palpus lobate, $4 \times$ as long as broad, blackish with a purple luster, without distinctive bristle.

Thorax (Fig. 8B) dark metallic green with pale gray pollinosity. Hairs and bristles on thorax black; six mostly hair-like dc except posterior most one dc longest and thick; acr absent; one h , one ph , two npl , one sa, one psa; scutellum with pair of long sc. Propleuron with two or three sparse short pale hairs on upper portion and one or two short pale hairs on lower portion.

Legs nearly entirely black except fore trochanter dark yellow; claws well developed, empodium and pulvilli reduced. Hairs and bristles on legs black except those on coxae pale. CI without distinctive bristle, but with dense erect anterior pale hairs on apical 1/4; CII nearly bare; CIII with blackish bristle at extreme apex. Fore trochanter elongated, with hook-like posterior process (Fig. 8D). FI distinctly thickened (Fig. 8D); TI slightly thickened, weakly curved, with one ad at basal $1 / 3$, two pd (one at apical $1 / 3$ outstanding), row of weak pv along whole length; $\mathrm{It}_{1}$ shortened, concave ventrally, anterior ventral margin expanded into a lobe and recurved, with short bristles apically
(Fig. 8D); $\mathrm{It}_{2}$ with a finger-like lobe at extreme base, corresponding to the cavity of $\mathrm{It}_{1}$, apical half thickened with short dense pv. FII somewhat flattened laterally, with three ad on apical half; apex of TII swollen with two rows of narrow flat willow leaflike ventral bristles, row of long av (anterior ones somewhat curved), rows of pale long ventral hairs ( $2-3 \times$ longer than TII depth, curved), row of erect pv along whole length (as long as TII depth), apically with three long bristles (Fig. 8E); $\mathrm{It}_{1}$ swollen at extreme base, with rows of long curved posterior bristles (Fig. 8E). FIII with two av on apical $1 / 3$, rows of sparse pale ventral hairs along whole length (less than FIII depth); TIII with four ad, three pd, without outstanding ventral bristle, apically with two strong long bristles. Relative lengths of tibia and five tarsomeres: LI 5.3: 0.8: 1.5: 1.8: 0.7 : 0.75; LII 7.3: 2.9: 1.4: 0.85: 0.45: 0.75; LIII 8.9: 3.3: 2.3: 1.2: 0.65: 0.85 .

Wing (Fig. 8C) hyaline, indistinctly tinged grayish; veins dark brown, $\mathrm{R}_{4+5}$ curved at apical $1 / 3$; crossvein $m-c u$ acutely and deeply arched to vein $M_{1}$, forming a hairpin curve, with a slender jet-black brand inside hairpin curve; posterior margin somewhat prolonged along vein $\mathrm{CuA}_{1}$. Squama brown with brown hairs. Halter brown.

Abdomen (Fig. 9A) nearly as long as head and thorax combined, dark metallic green with pale gray pollinosity. Abdomen with pale pubescence. Sternite IV medially with pair of obtuse anterior process (very close), bearing bundle of brown hairs (Fig. 10A). Sternite V split into pair of sclerites, each sclerite ginkgo leaf-like (Fig. 10A).

Male genitalia (Figs 9B, C, 10B, C): Epandrium slightly longer than wide. Epandrial lobe pale and lamellated, band-like, elongated, apically with short hairs. Surstylus thick, bent inwards, apically furcated. Hypandrium thick, apically with a deep, U-shaped incision in ventral view. Cercus broad, leaf-like, nearly as long as epandrium, with long dark yellow hairs along margins.

Female (Fig. 7B). Body length 4.9-6.7 mm; wing length $6.1-7.9 \mathrm{~mm}$. Nearly as same as male, but: antenna (Fig. 31C) scape with two short dorsal bristles, first flagellomere somewhat prolonged, $1.2 \times$ longer than width, arista apicobasal, $4.6 \times$ longer than first flagellomere. Proboscis yellowish brown with blackish edge; palpus relatively smaller than males, not reaching apex of proboscis. Six weak dc, except posterior most dc longest and thicken. Propleuron with one or two pale curved hairs on lower portion. Legs black, except trochanters yellowish brown. CI without distinctive bristle or hair, but with short pale anterior hairs on lower position; FI without distinct bristles; TI with one ad at basal $1 / 4$, four pd, apically with a bristle and comb of anterior bristles; FII with two anterior bristles on apical $1 / 3$; TII with three ad, two pd, apically with five bristles; FIII with three anterior bristles on apical $1 / 3$; TIII with four ad, two pd, 4-6 weak v, apically with two bristles. Wing (Fig. 32C): m-cu straight, forming acute angle with $\mathrm{CuA}_{1}$. Halter yellow.

Female terminalia (Fig. 11): Abdominal tergite VIII divided into two sclerites; epiproct split into pair of triangular hemitergites, apically with row of nine strong curved spines; dorsal lobes of cercus triangulated, with dark yellow bristles; ventral lobes of cercus short, apex rounded, with long yellow hairs.

Distribution. China (Tibet).


Figure 9. D. fasciculatus male $\mathbf{A}$ abdomen, lateral view $\mathbf{B}$ male genitalia, lateral view $\mathbf{C}$ male genitalia, ventral view. Scale bars: 1 mm .


Figure 10. D. fasciculatus male $\mathbf{A}$ abdominal Sternite IV and V, male genitalia removed, ventral view $\mathbf{B}$ male genitalia, lateral view $\mathbf{C}$ male genitalia, ventral view $\mathbf{D}$ surstylus, ventral view. Scale bars: 0.2 mm .

Remarks. The new species belongs to the flex subgroup D. fenestratus group. The species is unique for the shape of wings and $\mathrm{It}_{1-2}$, and the prolonged TII and TIII which have relatively short $\mathrm{IIt}_{1}$ and $\mathrm{IIIt}_{1}$. Females are characterized by an arched crossvein $\mathrm{m}-\mathrm{cu}$, and the crossvein vertical adjunct to vein $\mathrm{CuA}_{1}$; the trochanters are black.

Etymology. The new species name refers to the cluster of bristles on apex of TII of males.


Figure II. D. fasciculatus female, abdomen $\mathbf{A}$ dorsal view $\mathbf{B}$ lateral view $\mathbf{C}$ ventral view. Scale bar: 1 mm .

## Diostracus laetus sp. nov.

https://zoobank.org/43CC31E5-3F0A-4277-8734-A41164D50B41
Figs 12-15
Type material. Holotype: China - §̉, China: Tibet, Shigatse, Yatung County ( $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ ), $2700-3200 \mathrm{~m}, 2018$. VII. 13, leg. Yajun Zhu.

Diagnosis. MSSC: Wings with dark and yellow markings at middle. CI with a brown curved anterior spine at extreme apex; CII with row of four brown anterior spines along apical edge; fore trochanter with row of upwards curved bristles along basal edge. FI with short upwards curved ventral bristles on basal 1/4; TI thickened, with rows of pale ventral hairs on apical 3/4, and apically with two long wavy posterior bristles; FII with row of long av and posteroventral hairs.

Description. Male (Fig. 12). Body length 7.4 mm ; wing length 8.2 mm .
Head (Fig. 13A) dark metallic green with pale brown pollinosity. Eyes separated; face widened towards clypeus. Hairs and bristles on head black; lower postocular bristles including posteroventral hairs pale. Ocellar tubercle distinct, with pair of oc (broken), pair of posterior hairs; vt weak, slightly shorter than pvt. Antenna black; scape without dorsal bristle; first flagellomere subrectangular, $1.3 \times$ longer than wide; arista apicodorsal, $5.3 \times$ as long as first flagellomere, nearly bare. Proboscis yellowish brown with blackish edge; palpus lobate, $2.3 \times$ as long as broad, blackish with a purple luster, without distinctive bristle.


Figure I2. D. laetus, male, lateral view. Scale bar: 1 mm .


Figure 13. D. laetus male $\mathbf{A}$ head, lateral view $\mathbf{B}$ abdomen, lateral view $\mathbf{C}$ base of FII, anterior view $\mathbf{D}$ LI, anterior view $\mathbf{E}$ part of wing. Scale bars: 1 mm .

Thorax dark metallic green with pale brown pollinosity. Hairs and bristles on thorax black; six mostly hair-like dc except posterior most one dc longest and thick; acr absent; one h , one ph , two npl , one sa, one psa ; scutellum with pair of long sc. Propleuron with two or three sparse short pale curved hairs on upper portion and two long pale curved hairs on lower portion.

Legs nearly entirely black except extreme apexes of coxae and trochanters brownish yellow; claws well developed, empodium and pulvilli reduced. Hairs and bristles on legs black. CI with short sparse pale hairs on anterior surface, and a brown curved anterior spine at extreme apex (Fig. 13D); CII with row of four brown anterior spines along apical edge; CIII nearly bare. Fore trochanter with ridge and row of upwardly curved bristles along basal edge (Fig. 13D). FI distinctly thickened, with short upwards curved ventral bristles on basal $1 / 4$; TI thickened, with four ad, one pd at middle, rows of pale ventral hairs on apical 3/4, and apically with two long wavy posterior bristles (Fig. 13D); $\mathrm{It}_{1}$ shortened, apex expanded, concave ventrally, anterior ventral margin expanded into a lobate, with comb of bristles on anterior surface (Fig. 13D); $\mathrm{It}_{2}$ with a finger-like lobe at extreme base, with a posteroventral ridge on basal half (Fig. 13D). FII thickened, with row of seven short av spines, row of three long anteroventral hairs


Figure 14. D. laetus male $\mathbf{A}$ abdominal Sternite IV and V, male genitalia removed, lateral view $\mathbf{B}$ abdomen, male genitalia removed, ventral view $\mathbf{C}$ male genitalia, lateral view $\mathbf{D}$ male genitalia, ventral view. Scale bar: 1 mm .


Figure 15. D. laetus male $\mathbf{A}$ abdominal Sternite IV and V, male genitalia removed, ventral view $\mathbf{B}$ male genitalia, lateral view $\mathbf{C}$ male genitalia, ventral view. Scale bar: 1 mm .
and row of long posteroventral hairs (as long as FII depth, somewhat curved on basal 2/3) (Fig. 13C); TII with two ad, apically with two long bristles. FIII without conspicuous hairs and bristles; TIII with three ad, two pd, without outstanding ventral bristle. Relative lengths of tibia and five tarsomeres: LI 5.5: 1.2: 3.0: 3.3: 1.8: 1.0; LII 10.2: 6.4: 3.2: 1.8: 0.9: 1.0; LIII 11.8: 6.2: 4.2: 1.8: 0.9: 1.0 .

Wing (Fig. 13E) hyaline, anterior half and area around crossvein $\mathrm{m}-\mathrm{cu}$ yellowish, with dark cloud on middle of cell $R_{2+3}$, and a stripe of dark cloud along $M_{1}$ and expanding along posterodistal corner of distal cell, dark cloud in subapical portion of distal cell prominent; veins dark brown, $\mathrm{R}_{4+5}$ curved at middle, $\mathrm{M}_{1}$ curved at apical $1 / 3$; crossvein m -cu acutely and deeply arched to vein $\mathrm{M}_{1}$, forming a hairpin curve, with a slender jet-black brand inside hairpin curve, accessory cellula $1.3 \times$ longer than width. Squama brown with brown hairs. Halter yellow with blackish apex.

Abdomen (Fig. 13B) nearly as long as head and thorax combined, dark metallic green with pale brown pollinosity, except edge of sternites, apex of epandrium and base of cercus pale. Abdomen with short sparse pale pubescence. Posterior edge of sternite IV forwards recurved with row of short curve spines (Figs 14A, B, 15A). Lateroposterior corner of tergite V elongated into a triangular process. Sternite V split into pair of sclerites, each sclerite ginkgo leaf-like (Figs 14A, B, 15A).

Male genitalia (Figs 14C, D, 15B, C): Epandrium slightly longer than wide. Epandrial lobe and surstylus pale and lamellated. Epandrial lobe short, band-like, apically with one long and one short bristles. Surstylus irregular in shape, with irregular processes and bristles. Process of subepandrial sclerite exceeding epandrium margin, with short fine pubescence. Hypandrium thick, apically with a shallow, U-shaped incision. Cercus band-like, somewhat bent, with long dark yellow hairs around cercus.

Female. Unknown.
Distribution. China (Tibet).
Remarks. The new species belongs to pulchripennis subgroup and is quite similar to D. emotoi. Both species have same chaetotaxy on FII and they are similar in wing style. However, the new species has no posterior bristles on FI, long ventral hairs and two wavy bristles on TI, and relatively smaller wing accessory cellula ( $2.5 \times$ as long as wide).

Etymology. The name of new species refers to the bright coloration of male wings.

## Diostracus polytrichus sp. nov.

https://zoobank.org/4318BDD9-0AE4-4A15-96DE-549267680311
Figs 16-20, 31D, 32E
Type material. Holotype: China • §, China: Tibet, Shigatse, Yatung County ( $27^{\circ} 48^{\prime} \mathrm{N}, 88^{\circ} 90^{\prime} \mathrm{E}$ ), Pamaimang, $3350 \mathrm{~m}, 2018$. VII. 14, leg. Yajun Zhu. Paratypes: - 2 ठ $^{\top} 0^{\top} 2 q$, same data as for holotype.

Diagnosis. MSSC: palpus black; wing crossvein m-cu nearly straight, with jetblack nodule; halter yellow, with blackish apex. Abdomen with dense ventral hairs.

Description. Male (Fig. 16A). Body length $6.6-7.0 \mathrm{~mm}$; wing length $9.4-9.8 \mathrm{~mm}$.


Figure 16. D. polytrichus $\mathbf{A}$ male; lateral view $\mathbf{B}$ female, lateral view. Scale bars: 1 mm .

Head (Fig. 17A) dark metallic green with pale gray pollinosity. Eyes separated; face widened towards clypeus. Hairs and bristles on head black; lower postocular bristles including posteroventral hairs brownish. Ocellar tubercle distinct, with pair of strong oc, without posterior hairs; vt rather short, $0.3 \times$ as long as oc, sometimes hair-like; pvt slight shorter than oc. Antenna black; scape bare, or with three or four dorsal bristles; first flagellomere semicircular to subtriangular, 1.2-1.6 $\times$ longer than wide; arista apico-dorsal, $4.2-7.5 \times$


Figure 17. D. polytrichus male $\mathbf{A}$ head, lateral view $\mathbf{B}$ abdomen, lateral view $\mathbf{C}$ wing $\mathbf{D} \mathrm{It}_{1}$, anterior view. Scale bars: 1 mm .
as long as first flagellomere, nearly bare. Proboscis blackish with pale hairs; palpus lobate, $2.2-2.5 \times$ as long as broad, blackish with a purple luster, without distinctive bristle.

Thorax dark metallic green with pale gray pollinosity; mesoscutum with two dark brown longitudinal stripes. Hairs and bristles on thorax black; six weak dc except posterior most one dc longest and thick, occasionally with excess dc; acr absent; one weak and one strong h , one ph , two npl , one sa, one psa; scutellum with pair of long sc. Propleuron with one or two sparse long pale hairs on upper portion and group of long pale hairs on lower portion.

Legs nearly entirely black except fore trochanter dark brown; claws well developed, empodium and pulvilli reduced. Hairs and bristles on legs black except those on coxae pale. CI with group of pale curved anterior hairs on basal $1 / 3$, upper ones long, and cluster of erect bristles on apical $1 / 4$; CII nearly bare; CIII with blackish bristle at extreme apex. FI thickened, with two rows of ventral hairs (as long as FI depth), basal ones pale, and one posterior bristle at extreme base; TI with two pd, three d, row of six or seven long pv on apical half, apically with two bristles and comb of anterior bristles; $\mathrm{It}_{1-2}$ with rows of pd and pv , ventral surface with short dense fine hairs, $\mathrm{It}_{1}$ with row


Figure 18. D. polytrichus male $\mathbf{A}$ abdominal Sternite IV and V, male genitalia removed, ventral view B male genitalia, lateral view $\mathbf{C}$ male genitalia, ventral view. Scale bars: 1 mm .


Figure 19. D. polytrichus male $\mathbf{A}$ abdominal Sternite IV and V, male genitalia removed, ventral view $\mathbf{B}$ abdominal Sternite IV and V, male genitalia removed, lateral view with some angles $\mathbf{C}$ male genitalia, lateral view $\mathbf{D}$ male genitalia, ventral view. Scale bars: 1 mm .
of av spines, extending to $\mathrm{It}_{2}$ (Fig. 17D); FII with two rows of av on basal half, one distinct $v$ at middle, row of short av on apical $1 / 3$, row of pale long pv on basal $2 / 3$ (longest ones $2.5 \times$ longer than FII depth); TII with two ad, two pd, apically with four bristles. FIII with four av on middle $1 / 3$, apically with one av; TIII with four ad, four pd, four short ventral bristles, apically with three long bristles and comb of short anterior bristles. Relative lengths of tibia and five tarsomeres: LI 6.9: 3.1: 3.2: 1.7: 1.1: 1.0; LII 5.8: 3.2: 1.4: 0.9: 0.5: 0.8; LIII 7.8: 3.4: 2.5: 1.5: 0.8: 0.9 .

Wing (Fig. 17C) hyaline, indistinctly tinged grayish; veins dark brown; crossvein $\mathrm{m}-\mathrm{cu}$ nearly straight, curved around nodule, with a jet-black nodule. Squama brown with brown hairs. Halter yellow with blackish apex.

Abdomen (Fig. 17B) nearly as long as thorax, dark metallic green with pale gray pollinosity, bent upwards medially. Abdomen with dense long pale pubescence. Sternite V split into pair of sclerites (Figs 18A, 19A, B).


Figure 20. $D$. polytrichus female, abdomen $\mathbf{A}$ dorsal view $\mathbf{B}$ lateral view $\mathbf{C}$ ventral view. Scale bar: 1 mm .

Male genitalia (Figs 18B, C, 19C, D): Epandrium swollen, slightly longer than wide. Epandrial lobe tiny and pale, lamellate, apically with two pale bristles. Surstylus bifurcated, dorsal lobe broad and lamellated, inner surface with dense dentiform bristles, ventral lobe elongated, broad and leaf-like, apical margin pale, with one long inner bristle at middle. Hypandrium relatively small, apically with a deep U-shaped incision in ventral view. Cercus band-like, apical $1 / 3$ becoming narrower, with long yellow hairs along margins.

Female (Fig. 16B). Body length $5.4-6.0 \mathrm{~mm}$; wing length $6.8-7.9 \mathrm{~mm}$. Nearly as same as male, but: ocellar tubercle without posterior hairs, antenna (Fig. 31D) scape with two short dorsal bristles, first flagellomere semicircular, nearly as long as width, arista apicobasal, $5.6 \times$ longer than first flagellomere. Proboscis blackish; palpus relatively smaller than males, not reaching apex of proboscis. Seven weak dc, except posterior most dc longest and thicken. Propleuron with two or three sparse short pale curved hairs on upper portion and group of five or six sparse long pale curved hairs on lower portion. Legs black. CI with erect pale hairs on anterior surface, ones on lower portion black and thick; FI with one preapical pv; TI with four pd, two pv, apically with three bristles and comb of short anterior bristles; FII with one strong preapical av and one weak preapical pv; TII with three ad, two pd, apically with four strong bristles; FIII with one strong preapical av and one weak preapical pv; TIII with three ad, apically with three bristles. Wing (Fig. 32E): m-cu straight, forming obtuse angle with $\mathrm{CuA}_{1}$; area around m-cu tingled with blackish ash. Halter yellow with blackish apex.

Female terminalia (Fig. 20): Abdominal segments VII and VIII slender; tergite VIII divided into two sclerites; epiproct split into pair of hemitergites, apically with row of five strong curved spines; dorsal lobes of cercus finger-like, somewhat elongated, with dark yellow bristles; ventral lobes of cercus short, apex rounded.

Distribution. China (Tibet).
Remarks. The new species is quite similar to $D$. tibetensis, but the cerci of new species are lamellate with broad base. Females of the new species are characterized by the semicircular first flagellomere of antenna, the straight crossvein $\mathrm{m}-\mathrm{cu}$, and the blackish apex of halter.

Etymology. The name of the new species refers to the dense abdominal ventral hairs.

## Diostracus strenus sp. nov.

https://zoobank.org/DD9B81DF-05D1-485A-81E8-572D667BA73F
Figs 21-24
Type material. Holotype: China - ${ }^{\lambda}$, Tibet, Shigatse, Yatung County $\left(27^{\circ} 48^{\prime} \mathrm{N}\right.$, $88^{\circ} 90^{\prime}$ E), 2700-3200 m, 2018. VII. 13, leg. Yajun Zhu. Paratype: • 1 §, same data as for holotype but Pamaimang, $3350 \mathrm{~m}, 2018$. VII. 14.

Diagnosis. MSSC: dark and robust fly; FI and TI distinctly thickened; posterior ventral margin of $\mathrm{It}_{1}$ and anterior ventral margin of $\mathrm{It}_{2}$ expanded into auriform lobes; crossvein m-cu acutely and deeply arched to vein $M_{1}$, forming a ' $h$ '-shaped curve, with a jet-black mark inside curve.


Figure 21. D. strenus, male; lateral view. Scale bar: 1 mm .

Description. Male (Fig. 21). Body length 6.0 mm ; wing length 8.0 mm .
Head (Fig. 22A) dark metallic green with pale brown pollinosity. Eyes separated; face widened towards clypeus. Hairs and bristles on head black; lower postocular bristles including posteroventral hairs pale. Ocellar tubercle distinct, with pair of strong oc, without posterior hairs; vt short, $0.7 \times$ as long as oc, nearly as long as pvt. Antenna black; scape with a weak dorsal bristle at basal $1 / 3$; first flagellomere subtriangular, 1.5 $\times$ longer than wide; arista apicodorsal, $3 \times$ as long as first flagellomere, nearly bare. Proboscis blackish with pale hairs; palpus lobate, $3.5 \times$ as long as broad, blackish with a purple luster, without distinctive bristle.

Thorax dark metallic green with pale brown pollinosity. Hairs and bristles on thorax black; six mostly hair-like dc except $1^{\text {st }}$ and $6^{\text {th }}$ dc long and thick; acr absent; one $h$, one ph, two npl, one sa, one psa; scutellum with pair of sc. Propleuron with two or three sparse, short, pale hairs on upper portion and one or two short pale hairs on lower portion.

Legs nearly entirely black except fore and mid trochanters dark yellow; claws well developed, empodium and pulvilli reduced. Hairs and bristles on legs black except


Figure 22. D. strenus male $\mathbf{A}$ head, lateral view $\mathbf{B}$ abdomen, lateral view $\mathbf{C}$ wing $\mathbf{D}$ TI and It, anterior view E TI and It, posterior view. Scale bars: 1 mm .
those on coxae pale. CI without distinctive bristle, but with dense, erect, pale, anterior hairs on apical $1 / 4$; CII with cluster of black bristles at extreme apex; CIII nearly bare. Fore trochanter elongated, with lobate posterior process. FI distinctly thickened, with group of pale hairs on apical $1 / 5$ (less than FI depth) (Fig. 22D, E); TI distinctly thickened, curved, with three ad on basal half, two pd on apical $1 / 3$, row of five long pv on apical $1 / 3$, apically with comb of short pale av spines (Fig. 22D, E); $\mathrm{It}_{1}$ shortened, concave ventrally, posterior ventral margin expanded into a auriform lobe (Fig. 22D, E); $\mathrm{It}_{2}$ thickened and recurved, somewhat flattened dorsoventrally, with anterior ventral margin at extreme base expanded into an auriform lobe, corresponding to the auriform lobe of $\mathrm{It}_{1}$, a spine-like lobe at basal 1/4, and two rows of short pv spines (Fig. 22D, E). FII with rows of pale postoventral hairs (as long as FII depth), and one ad at apical 1/8; TII with rows of curved ventral hairs on basal $2 / 3$ (longest ones $2 \times$ longer than TII depth), three weak ad, two weak pd, apically with two long bristles. FIII with two ad on apical $1 / 6$, rows of sparse pale ventral hairs on basal half (less than FIII depth); TIII with five ad, three pd, without outstanding ventral bristle, apically with two bristles. Relative lengths of tibia and five tarsomeres: LI 5.7: $0.6: 2.8: 2.1: 1.0: 1.0$; LII 8.8: 5.2: 2.2: 1.3: 0.8: 1.0; LIII 10.3: 4.7: 3.0: 1.4: 0.7: 1.0.


Figure 23. D. strenus male $\mathbf{A}$ abdominal Sternite IV and $V$, male genitalia removed, ventral view $\mathbf{B}$ male genitalia, lateral view $\mathbf{C}$ male genitalia, ventral view. Scale bar: 1 mm .


Figure 24. D. strenus male $\mathbf{A}$ abdominal Sternite IV and $V$, male genitalia removed, ventral view $\mathbf{B}$ male genitalia, lateral view $\mathbf{C}$ male genitalia, ventral view. Scale bar: 1 mm .

Wing (Fig. 22C) hyaline, indistinctly tinged grayish; veins dark brown; crossvein $m$-cu acutely and deeply arched to vein $M_{1}$, forming a ' $h$ '-shaped curve, with a jet-black mark inside curve. Squama brown with brown hairs. Haltere blackish with pale knob.

Abdomen (Fig. 22B) nearly as long as head and thorax combined, dark metallic green with pale gray pollinosity. Abdomen with pale pubescence. Sternite IV medially with an obtuse process, and one tubercle bearing bundle of brown bristles, lateroposterior corner with brown bristles (Figs 23A, 24A). Sternite V split into pair of sclerites, each sclerite ginkgo leaf-like (Figs 23A, 24A).

Male genitalia (Figs 23B, C, 24B, C): Epandrium slightly longer than wide. Epandrial lobe elongated, lamellated, apically with two short bristles. Surstylus thick, apex dark, bending inwards, with a finger-like inner process. Hypandrium thick, apically with a shallow, V-shaped incision. Cercus straight and clavated, inner surface somewhat hollow, with long pale hairs along margin, apical half with dense, erect, dark yellow bristles on inner surface.

Female. Unknown.
Distribution. China (Tibet).
Remarks. The new species belongs to $D$. fenestratus group. It looks like $D$. flexus, but can be separated from the latter by the following features of males: the weak acute ventral process near extreme base of $\mathrm{It}_{2}$, the swollen apex of $\mathrm{It}_{2}$, the row of erect dense strong long posterior, and the anterior ventral bristles on apex of TII.

Etymology. The name of new species refers to the strongly thickened legs.

## Diostracus translucidus sp. nov.

https://zoobank.org/19498122-29A8-4F0E-8ADB-9318C2FD6101
Figs 25-30, 31E, 32D
Type material. Holotype: China • ठ, Tibet, Nyingchi, Medog, 80 k, 2013. IX. 13, leg. Gang Yao. Paratypes: • $2 q$, same data as for holotype.

Diagnosis. MSSC: palpus normal, not reaching apex of proboscis. Scutellum with pair of sc and four or five pairs of marginal hairs; CI with row of anterior hairs and two strong recurved spines at extreme apex; FI with a deep hollow at base; wing indistinctly tinged grayish; FII with row of dense ad on apical $2 / 5$; wing apically with three translucent windows between vein $C$, vein $R_{2+3}$, vein $R_{4+5}$, and vein $M_{1}$.

Description. Male (Fig. 25A). Body length 5.8 mm ; wing length 6.0 mm .
Head (Fig. 26A) dark metallic green with pale gray pollinosity. Eyes separated; face widened towards clypeus. Hairs and bristles on head black; lower postocular bristles including posteroventral hairs pale. Ocellar tubercle distinct, with pair of oc (lost), without posterior hairs; vt rather short, $0.2 \times$ as long as pvt. Antenna brownish black; scape prolonged, without dorsal bristle; first flagellomere subtriangular, $1.3 \times$ longer than wide; arista apicodorsal, $4.8 \times$ as long as first flagellomere, nearly bare. Proboscis huge and brown, apex blackish with pale hairs; palpus lobate, not reaching apex of proboscis, without distinctive bristle.


Figure 25. D. translucidus $\mathbf{A}$ male; lateral view $\mathbf{B}$ female, lateral view. Scale bar: 1 mm .


Figure 26. D. translucidus male $\mathbf{A}$ head, lateral view $\mathbf{B}$ apex of wing $\mathbf{C} L I$, anterior view $\mathbf{D}$ abdomen, lateral view. Scale bars: 1 mm .

Thorax dark metallic green with pale gray pollinosity; mesoscutum with pair of dark brown longitudinal stripe. Hairs and bristles on thorax black; six mostly hair-like dc except posterior most one dc longest and thick; acr absent; one h, one ph, one npl, one sa, one psa; scutellum with pair of sc (lost) and four or five pairs of marginal hairs (MSSC). Postnotum well developed, and convex. Propleuron with two or three sparse short pale hairs on lower portion.

Legs nearly entirely black except fore trochanter dark yellow, mid- and hind trochanters brownish black; claws prolonged (MSSC), empodium and pulvilli present. Hairs and bristles on legs black. CI depressed laterally, without distinctive bristle, but with row of anterior hairs and two strong recurved spines at extreme apex (MSSC); CII and CIII with clusters of anterior bristles at extreme apex. FI distinctly thickened, with a deep hollow at base, and row of three or four curved ventral spines and row of dense anterior bristles long the edge of the hollow (MSSC), basal $2 / 3$ with row of four long yellow av (nearly as long as FI depth) (MSSC), apically with long thin pale hairs (MSSC) (Fig. 26C); TI slightly thickened, with row of ad along whole length, ventral surface nearly bare (MSSC); $\mathrm{It}_{1}$ somewhat thicken, with row of long ad and pd along whole length (nearly as long as $\mathrm{It}_{1}$ depth), apical half flattened ventrally with two rows of short curved spines and rows of bristles along the edge, apically with a strong curved pv spine (MSSC) (Fig. 26C); $\mathrm{It}_{2}$ with row of long curved anteroventral hairs along the


Figure 27. D. translucidus male, apex of abdomen $\mathbf{A}$ dorsal view $\mathbf{B}$ ventral view $\mathbf{C}$ lateral view. Scale bar: 1 mm .


Figure 28. $D$. translucidus male $\mathbf{A}$ abdominal Sternite IV and V , male genitalia removed, ventral view B male genitalia, lateral view $\mathbf{C}$ male genitalia, ventral view. Scale bar: 1 mm .


Figure 29. D. translucidus male $\mathbf{A}$ abdominal Sternite IV and V, male genitalia removed, ventral view $\mathbf{B}$ male genitalia, lateral view $\mathbf{C}$ male genitalia, ventral view. Scale bar: 1 mm .


Figure 30. $D$. translucidus female, abdomen $\mathbf{A}$ dorsal view $\mathbf{B}$ lateral view $\mathbf{C}$ ventral view. Scale bar: 1 mm .


Figure 31. Female antenna, lateral view $\mathbf{A} D$. acutatus, show apical arista B $D$. acutatus, show subapical arista $\mathbf{C}$. fasciculatus $\mathbf{D}$. polytrichus $\mathbf{E}$. translucidus. Scale bars: 1 mm .
whole length, apical half with two rows of long posterior bristles (MSSC); $\mathrm{It}_{3}$ elongated, base and apex somewhat swollen, nearly bare, except with row of three or four posteroventral hairs at base and four or five dorsal bristles at apex (MSSC); $\mathrm{It}_{4}$ with long ventral bristles apically (MSSC). FII with row of dense ad on apical $2 / 5$ (MSSC); TII with two ad, 8 pd. FIII without outstanding bristle; TIII with row of long thin ad and long erect thin pd; IIIt with row of thin ad long whole length. Relative lengths of tibia and five tarsomeres: LI 5.3: 1.2: 2.2: 2.6: $0.4: 0.7$; LII 7.3: 5.0: 2.4: 1.2: 0.6: 0.8 ; LIII 8.5: 4.8: 3.4: 1.6: 0.8: 0.8 .

Wing (Fig. 26B) hyaline, indistinctly tinged grayish, apically with three translucent windows between vein $C$, vein $R_{2+3}$, vein $R_{4+5}$, vein $M_{1}$ (MSSC); vein $M$ with brown strip on middle section (MSSC); veins dark brown; crossvein m-cu somewhat curved. Squama yellow with yellow hairs. Halter brown.

Abdomen (Fig. 26D) nearly as long as head and thorax combined, dark metallic green with pale gray pollinosity. Abdomen with sparse pale pubescence. Tergites II-V


Figure 32. Apex of female wing A, B D. acutatus $\mathbf{C}$ D. fasciculatus $\mathbf{D}$ D. translucidus $\mathbf{E}$ D. polytrichus. Scale bar: 1 mm .
with triangular hyaline area on posterior margin (MSSC); Sternite II medially with a digitiform anterior process (MSSC) (Fig. 26D), Sternite IV with a pair of long brown band-like sclerites (Figs 27, 28A, 29A); Sternite V longer than Sternite IV, split into pair of sclerites (Figs 27, 28A, 29A). Hypandrium not distinctly swollen.

Male genitalia (Figs 28B, C, 29B, C): Epandrium slightly longer than wide. Epandrial lobe long, wavy, band-like, with an acute basal process, one short bristle at tip. Surstylus thick, lamellated, with two short spines. Hypandrium short thick, apically with a shallow, V-shaped apical incision. Cercus rather short ( $1 / 3$ as long as epandrium length), spoon-shaped, with dark yellow hairs on outer surface, apical ones long (nearly as long as cercus length), and subapically with group of dense erect dark yellow bristles on inner surface.

Female (Figs 25B, 31E). Body length $7.4-7.6 \mathrm{~mm}$; wing length $7.8-8.0 \mathrm{~mm}$. Same as male, except MSSC. FI without distinct bristles; TI with three ad, two pd; FII with two ad; TII with one ad, one pd, apically with three bristles; FIII bare; TIII with four ad, apically with two bristles. Wing (Fig. 32D) hyaline, indistinctly tinged grayish, crossvein m -cu straight, forming acute angle with $\mathrm{CuA}_{1}$.

Female genitalia (Fig. 30): Abdominal segments VII and VIII slender; tergite VIII divided into two sclerites; epiproct split into pair of hemitergites, apically with row of eight strong curved spines; hypoproct semicircular; dorsal lobes of cercus somewhat elongated, with dark yellow bristles; ventral lobes of cercus short, apex rounded.

Distribution. China (Tibet).
Remarks. The new species is unique. It has prolonged scapes, small palpus, and convex postnotum. But the huge proboscis, stout body and specialized structures of legs indicate that the new species belongs to Diostracus.

Etymology. New species name refers to the translucent windows on male wing.

## Discussion

Including the species described in this work, the number of worldwide species of Diostracus has increased to 107, of which nine species occur in Tibet (Zhu 2006; Zhu et al. 2007a, b; Grichanov 2013, 2015, 2017; Pusch 2014; Wang et al. 2015). The six new species of Diostracus were found in Nyingchi and Shigatse areas of Tibet. Most of them were found at Yatung in Shigatse, which is located on the southern slope of the Himalaya Mountains (Fig. 33). As well as these two sites, we performed a five-year survey in Lhasa, Nyingchi, Shannan, and Qamdo, but there were no Diostracus found, although it does not mean Diostracus only occur in Nyingchi and Shigatse in Tibet. Due to the special habitat of the genus, it can only be collected with sweep nets after finding the flies using the naked eyes. Malaise traps collect the majority of insects in


Figure 33. Distribution of new Diostracus species of Tibet: black circle: D. fasciculatus, D. laetus, D. polytrichus, and D. strenus; black square: D. translucidus; black star: D. concavus.

Tibet, as its environment is often too complex to walk through, but no Diostracus were found in them during these surveys.

Diostracus in the Oriental realm shows great diversity in MSSC, especially in modified FI and wing. Complex structures of FI are usually associated with modified wing. The most bizarre MSSC was shown in D. fenestratus group, with It distinctly shortened, almost triangular in shape, expanded portion concaved, and $\mathrm{It}_{2}$ sinuous, with a basal denticle (Saigusa 1984). The wing in this group is always ornamented, crossvein m -cu either in S -shape or running posteriorly parallel to $\mathrm{M}_{1}$. This species group includes 14 species all of which are distributed in the Himalayan Mountains. The species groups with partially thickened $\mathrm{It}_{1}$ and modified wings (mainly the D. unisetosus group) and the species groups with simple FI and modified wings (with a jet-black nodule besides crossvein $\mathrm{m}-\mathrm{cu}$, mainly the $D$. unipunctatus group) are distributed also mainly in the Himalayan Mountains. The species groups with partially thickened $\mathrm{It}_{1}$ and simple wings, mainly the $D$. nebulosus group, are distributed in the Himalayan Mountains and the Chinese mainland. Finally, the species groups with simple FI and simple wing is found in the Chinese mainland and Taiwan. Himalayan Mountains is the diversity center for the genus Diostracus, and species in this area show great diversity ( 53 species out of 107) and abundant specialized characters. The species in the Chinese mainland and Taiwan usually have low diversity ( 20 species of 107) and simple structures.

Besides the morphological characters, mitochondrial COI genes of females have also been sequenced to pair them with males. As a result, some females could not be matched to males, and the characters of these females are obviously different from known species found in our investigation. Therefore, we believe that more new species will be discovered in the Himalayan region in the future.

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## References

Cumming J, Wood DM (2017) Adult morphology and terminology. In: Kirk-Spriggs AH, Sinclair BJ (Eds) Manual of Afrotropical Diptera. Volume I. Introductory chapters and keys to Diptera families. Suricata 4. South African National Biodiversity Institute, Pretoria, 89-134. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome coxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294-299. [DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates - PubMed ] [nih.gov]

Grichanov IY (2013) West Palaearctic species of the genus Diostracus Loew, 1861 (Diptera: Dolichopodidae). European Journal of Taxonomy 61(61): 1-14. https://doi.org/10.5852/ ejt.2013.61
Grichanov IY (2015) A new species of Diostracus Loew (Diptera: Dolichopodidae) from the Yunnan Province of China. Far Eastern Entomologist 300: 12-16.
Grichanov IY (2017) A new species of Diostracus Loew, 1961 (Diptera: Dolichopodidae) from China with a key to species from the Yunnan Province. Caucasian Entomological Bulletin 13(1): 127-130. https://doi.org/10.23885/1814-3326-2017-13-1-127-130
Pusch MHE (2014) Diostracus lemavajulorum spec. nov. (Diptera: Dolichopodidae) - a second European Diostracus s. str. species from Corsica, France. Studia Dipterologia 21(1): 161-169.
Saigusa T (1984) The genus Diostracus from Nepal (Diptera, Dolichopodidae). Bulletin of the Kitakyushu Museum of Natural History 5: 1-74.
Wang N, Wang B, Yang D (2015) Two new species of the genus Diostracus Loew from Tibet, with a key to the Himalayan fauna (Diptera, Dolichopodidae). ZooKeys 488: 91-104. https://doi.org/10.3897/zookeys.488.8919
Zhu Y (2006) Genus Diostracus Loew, 1861. In: Yang D, Zhu Y, Wang M, Zhang L (Eds) World catalog of Dolichopodidae (Insecta: Diptera). China Agricultural University Press, Beijing, 229-234.
Zhu Y, Masunaga K, Yang D (2007a) New species of Diostracusfrom Yunnan, China (Diptera: Doli-chopodidae).AquaticInsects29(3):219-224.https://doi.org/10.1080/01650420701411192
Zhu Y, Yang D, Masunaga K (2007b) Two new species of Diostracus from China (Diptera: Dolichopodidae). Transactions of the American Entomological Society 133(1): 133-142. https://doi.org/10.3157/0002-8320(2007)133[133:TNSODF]2.0.CO;2

# New species of the dancing semislug Cryptosemelus Collinge, 1902 (Eupulmonata, Ariophantidae) from Loei Province, northeastern Thailand with a key to genera of mainland Southeast Asian semislugs and a key to species of the genus 

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#### Abstract

In this study, we describe a new dancing semislug from a limestone hill area in northeastern Thailand. Cryptosemelus niger sp. nov. differs from the three recognized congener species from western and southern Thailand, due to differences in their body and shell lobes coloration, appearance of penial caecum, shape and surface texture of penis and epiphallus, and radula formula and morphology.


## Keywords

Genital system, karst topography, limestone hill, mantle extensions, protective behavior, shell lobes

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## Introduction

Cryptosemelus Collinge, 1902 is a member of family Ariophantidae. Its common characters comprise a shell with reduced number of whorls, body with well-developed mantle extensions, tail without caudal horn and genitalia without flagellum and amatorial organ. This genus differs from other long-tail semislug genera described from the Malay Peninsula. It differs from Apoparmarion due to more shell whorls and the absence of a caudal horn, flagellum and dart apparatus; and, it differs from Paraparmarion by the presence of both right and left shell lobes, whereas, the latter genus presents only a right shell lobe (Collinge 1902; Pholyotha et al. 2021a) (Table 1).

Cryptosemelus has been referred to as a 'dancing semislug' because of its dance-like movement that it makes when it is disturbed or attacked (Collinge 1902). This protective behavior has also been reported in other species, including Laocaia simori Dedov \& Schneppat, 2019 in Dedov et al. (2019), Cryptaustenia altatorial Wiktor, 2002 and Cryptaustenia mirabilis Wiktor, 2002, Muangnua arborea Tumpeesuwan \& Tumpeesuwan, 2019; Cryptosemelus gracilis Collinge, 1902, C. betarmon Pholyotha, 2021 and C. tigrinus Pholyotha, 2021 in Pholyotha et al. (2021a), Ibycus spp. and Helicarion spp. (Junn Kitt Foon, pers. comm.)

According to a recent study by Pholyotha et al. (2021a), the type species of the genus, Cryptosemelus gracilis, and the recently named species C. betarmon and C. tigrinus were studied and described from their genital anatomy and radula morphology. These three species are characterized by differences in the anatomical details of their penis, epiphallus and spermatophore. We discovered and examined this lovely semislug from a limestone hill in Loei Province, Thailand, and it possesses distinct characters of external shell morphology, mantle lobes coloration, radula and genital organs. Thus, we describe it here as a new species of Cryptosemelus.

## Material and methods

Sixty specimens were collected from November to December 2012, June to September 2013, and $12^{\text {th }}$ October 2018 in the Phu Pha Lom limestone area $\left(17^{\circ} 33^{\prime} 62^{\prime \prime} \mathrm{N}\right.$, $101^{\circ} 52^{\prime} 31^{\prime \prime} \mathrm{E}$ ), elevation about 380-390 m above mean sea level, in the Mueang Loei District, Loei Province, northeastern Thailand (Fig. 1). The specimens were composed of fifty empty shells and 10 living specimens, which were collected from both leaf litter and the ground surface. The living specimens were photographed in their natural habitat (Figs 2B, 3), and then euthanized and preserved in $70(\mathrm{v} / \mathrm{v})$ ethanol for morphological and anatomical studies. Specimens were classified and identified from the literature, such as Collinge (1902), Blanford and Godwin-Austen (1908), Solem (1966), Schileyko (2003) and Pholyotha et al. (2021a). For the descriptive study, adult shells were measured for size using a vernier caliper and the number of whorls were counted. Photomicrographs were taken using a scanning electron microscope (JEOL, JSM-6460 LV) housed at the Central Laboratory, Faculty of Science, Mahasarakham University, Thailand. Eight specimens were dissected and examined under a stereoscopic light microscope.

Table I. Comparison of morphological characters among three semislug genera, Cryptosemelus, Apoparmarion and Paraparmarion (data from Collinge 1902; Schileyko 2003; Pholyotha et al. 2021a).

| Characters | Cryptosemelus | Apoparmarion | Paraparmarion |
| :--- | :---: | :---: | :---: |
| Number of shell whorls | $3-4$ | 2 | $3-4$ |
| Left shell lobe | Present | Present | Absent |
| Caudal horn | Absent | Present | Absent |
| Flagellum | Absent | Present | No information |
| Dart apparatus | Absent | Present | No information |

Descriptions of the new species herein are attributed to the first and the third authors, as indicated below. Type specimens and other voucher specimens were deposited in the Natural History Museum of Mahasakham University, Maha Sarakham, Thailand (NHMSU).

The abbreviations used were as defined by Blanford and Godwin-Austen (1908), Solem (1966), Pholyotha et al. (2020) and Pholyotha et al. (2021a, b): ag, albumen gland; at, atrium; e1, portion of epiphallus nearer to penis; e2, portion of epiphallus nearer to retractor muscle; fo, free oviduct; gd, gametolytic duct; gs, gametolytic sac; $\mathbf{h d}$, hermaphroditic duct; $\mathbf{l d} \mathbf{l}$, left dorsal lobe; $\mathbf{l s l}$, left shell lobe; ovt, ovotestes; $\mathbf{p}$, penis; prm, penial retractor muscle; pro, prostate gland; rsl, right shell lobe; $\mathbf{v}$, vagina; $\mathbf{v d}$, vas deferens; ut, uterus.

For the description of the genital system; 'proximal' refers to the region closest to the genital opening and 'distal' refers to the region outermost from the genital opening.

## Results

## Systematic description

Superfamily Helicarionoidea Bourguignat, 1877
Family Ariophantidae Godwin-Austen, 1883
Subfamily Ostracolethinae Simroth, 1901

## Genus Cryptosemelus Collinge, 1902

Cryptosemelus Collinge, 1902: 76. Blanford and Godwin-Austen 1908: 180. Thiele 1931: 640. Zilch 1959: 326. Vaught 1989: 97. Schileyko 2003: 1332. Bank 2017: 53. Inkhavilay et al. 2019: 75. Pholyotha et al. 2021a: 43-65.

Type species. Cryptosemelus gracilis Collinge, 1902. Cryptosemelus has a reduced shell of three to four whorls, well-developed mantle extensions with two dorsal lobes and right shell lobe covering the apex and larger than the left shell lobe. Caudal horn absent. Genital system without flagellum and dart apparatus.


Figure I. Map of type locality of Cryptosemelus niger S. Tumpeesuwan \& C. Tumpeesuwan, sp. nov.

## Cryptosemelus niger S. Tumpeesuwan \& C. Tumpeesuwan, sp. nov. https://zoobank.org/FA7C7967-4A62-4A6E-992C-4BE2E6DC9B36 Figs 1-4

Cryptaustenia sp. Tanmuangpak 2016: 109-110.
Cryptaustenia sp. Chimsaeng 2019: 41-42.

Type material. Holotype. NHMSU-00054 (Fig. 3). Phu Pha Lom limestone area, Mueang Loei District, Loei Province, northeastern Thailand, coll. Kitti Tanmuangpak, Nov. 2012. Paratypes. NHMSU-00055. Same locality and same date as holotype.

Type locality. Phu Pha Lom limestone area, Mueang Loei District, Loei Province, Thailand.

Diagnosis. Animal with blackish body, shell lobes with blackish reticulated skin (Figs 2, 3). Genitalia with very short cylindrical vagina, smooth elongated cylindrical epiphallus, without penial caecum (Fig. 4). Radula with bicuspid lateral teeth (Fig. 5B, C).

Description. (empty shells $=8$, living specimen $=4$ ) Shell (Fig. 2A). Shell globose, small size (shell height $6.03 \pm 0.71 .00 \mathrm{~mm}$, shell width $9.72 \pm 1.32 \mathrm{~mm}$ ), shell imperforate, thin, smooth, dark brown color; transparence, aperture large (aperture height $5.28 \pm 0.68 \mathrm{~mm}$ and aperture width $5.71 \pm 0.81 \mathrm{~mm}$ ).

Genital system $(N=3)$ (Fig. 4). Atrium (at) short. Penis rather long and cylindrical, with thin penial sheath covering entire penis. Penial retractor muscle (prm) present, short, thin and attached at junction of e1 and e2. Epiphallus (e1+e2) length is slightly equal to penis length, surface smooth, el cylindrical and gradually smaller in diameter, e2 cylindrical and larger than e1. Flagellum absent. Vas deferens long. Vagina is shorter than penis, cylindrical. Gametolytic duct (gd) thickened at base, gametolytic sac (gs) swollen gland at distal end. Free oviduct (fo) is shorter than vagina. Uterus and prostate gland long and stout.

Radula ( $N=3$ ) (Fig. 5). Teeth arranged in a wide V-shape with half row formula: 1-7-70+teeth. Central teeth symmetric tricuspid. Lateral teeth and marginal teeth gradually changing from broad to narrow bicuspid.

External appearance (Figs 2B, 3). Living semislug with reticulated skin, blackish to dark body marked by conspicuous grooves running downward. Four mantle extensions well developed and same color body. Shell lobes do not cover entire shell. Caudal horn absent.

Etymology. The species name "niger" is derived from Latin word, meaning "black" referring to blackish body and mantle extensions.

Distribution, habitat and behavior observations. Cryptosemelus niger sp. nov. is a species restricted to the dry evergreen forest that covers the Phu Pha Lom limestone area, Loei Province, Thailand. We searched after rain and found the specimens normally hiding on the ground and in the leaf litter. When the snails were disturbed, they escaped by quickly flipping and waging their tail. Information on its natural predators is unknown, but the carnivorous land snails, Discartemon sp., Oophana sp. (Streptaxidae), and Sinoennea loeiensis Tanmuangpak \& S. Tumpeesuwan, 2015 (Diapheridae) were found in the same locality (Tanmuangpak et al. 2012; Tanmuangpak et al. 2015; Tanmuangpak 2016).


Figure 2. Cryptosemelus niger S. Tumpeesuwan \& C. Tumpeesuwan, sp. nov. Photograph by Kitti Tanmuangpak A shell morphology of holotype NHMSU-00054 B living snail.


Figure 3. Cryptosemelus niger S. Tumpeesuwan \& C. Tumpeesuwan, sp. nov. Living snail in natural habitats. Photograph by Chanidaporn Tumpeesuwan 12 December 2018 A C. niger crawling on soil in limestone crevice (white circle) B close-up view in living position C,D semislug was moved to green leaf for taking photo $\mathbf{C}$ left side represents left shell lobe (lsl) D right side represents right shell lobe (rsl), left shell lobes (lsl), and left dorsal lobe (ldl).


Figure 4. Genital system of Cryptosemelus niger $S$. Tumpeesuwan \& C. Tumpeesuwan, sp. nov. (paratype NHMSU-00055) A photograph and B drawing by Kitti Tanmuangpak.


Figure 5. Radula morphology of Cryptosemelus niger $S$. Tumpeesuwan \& C. Tumpeesuwan, sp. nov. (paratype NHMSU-00055) $\mathbf{A}$ radula plate, teeth rows arranged in wide $V$-shape $\mathbf{B}$ close-up view of middle part of radula $\mathbf{C}$ close-up view of right side of radula $\mathbf{D}$ close-up view of right side of radula showing marginal teeth. Central teeth indicated by ' $C$ '; lateral teeth indicated by 'L'; marginal teeth indicated by ' M '.

Remarks. Cryptosemelus niger sp. nov. differ from other Cryptosemelus species by its black body and mantle lobes. The shell seems to have a more rapidly descending whorl than in C. gracilis and C. betarmon, but is similar to C. tigrinus. Cryptosemelus gracilis also lack a penial caecum but differs in the undulated surface patch on the proximal part of e2. The epiphallus and penis are cylindrical without a caecum, diverticulum, and granulate surface. The radula of the new species differs from all Cryptosemelus in having bicuspid lateral teeth, whereas other species have tricuspid lateral teeth (Table 2, Figs 2A, B, 3A-D, 4, 5B-D).

## Discussion

The absence of a caudal horn is the unique character shared between Cryptosemelus and Paraparmarion (Collinge 1902; Blanford and Godwin-Austen 1908; Solem 1966; Schileyko 2002, 2003; Pholyotha et al. 2021a). Cryptosemelus differs from Paraparmarion in that the left shell lobe is well developed, whereas it is missing in Paraparmarion (Collinge 1902; Pholyotha et al. 2021a).

Table 2. Comparison of shell, genital system and radula of Cryptosemelus spp. (data for C. gracilis, C. betarmon and C. tigrinus based on Pholyotha et al. 2021a).

| Characters | C. gracilis | C. betarmon | C. tigrinus | C. niger sp. nov. |
| :---: | :---: | :---: | :---: | :---: |
| Shell: |  |  |  |  |
| Shape | Less, globose | Depressed, subglobose | Globose | Globose |
| Shell width | Up to 6.6 mm | Up to 7.4 mm | Up to 10.7 mm | Up to 11.04 mm |
| Shell height | Up to 4.2 mm | Up to 4.1 mm | Up to 7.6 mm | Up to 6.74 mm |
| Whorls number | $3^{1 / 2}-4$ | $3^{1 / 2}-4$ | 4-41/2 | 3-4 |
| Shell color | Pale golden amber | Pale yellowish with olive tinge | Pale yellowish with olive tinge | Dark brown transparent |
| Living snails: |  |  |  |  |
| Shell lobes coloration | Monochrome bluegray to blackish | Monochrome pale to dark-grayish | Pale yellowish-orange banded | Blackish reticulated skin |
| Radula: |  |  |  |  |
| Radula formula | 1-(19-20)-38 | 1-(27-28)-37 | 1-(38-39)-44 | 1-7-70+ |
| Central tooth | Tricuspid | Tricuspid | Tricuspid | Tricuspid |
| Lateral teeth | Tricuspid | Tricuspid | Tricuspid | Bicuspid |
| Marginal teeth | Tricuspid | Bicuspid | Bicuspid | Bicuspid |
| Genital system: |  |  |  |  |
| Epiphallus e2: <br> Proximal part | Enlarged with irregularly undulated surface patch | Enlarged and with undulated surface | Cylindrical smooth surface | Long cylindrical with smooth surface |
| Epiphallus e2: <br> Distal part | Smooth surface | Cylindrical and gradually tapering to distal end, smooth surface | Cylindrical with prominently granulated surface | Long cylindrical with smooth surface |
| Penial caecum | Absent | Present | Present | Absent |
| Vagina | Larger and shorter than penis length | Smaller and shorter than half of penis length | Long slender and longer than half of penis length | Smaller and shorter than penis length |

All previously described species of Cryptosemelus show no epiphallic caecum, flagellum, and dart apparatus. Shell lobes coloration, appearance of the penial caecum, shape and surface sculpture of the epiphallus, and radula morphology are considered as taxonomically informative and these can be used to distinguish the new species from all recognized Cryptosemelus species.

We have improved the key to the genera of mainland Southeast Asian slug-like semislugs provided by Tumpeesuwan and Tumpeesuwan (2019b) for identifying slug-like semislugs in mainland Southeast Asia and provide a key to species of genus Cryptosemelus below.

## Key to genera of mainland Southeast Asian slug-like semislugs

1 Finger nail or triangular-shaped shell, always covered by shell lobes .............. 2

- Ear-shape, subglobose, or globose-shaped shell, frequently covered by shell lobe. 3
2 Finger nail-shaped shell; gametolytic sac long, cylindrical tube ..... Muangnua
- Triangular-shaped shell; gametolytic sac stalk, short and stout or moderately long and slender.
3 Ear-shape shell; caudal horn present; dart apparatus and flagellum present ..... 4
- Subglobose; or globose-shaped shell; caudal horn absent; dart apparatus andflagellum absent or no information5
4 Penis length shorter than half of dart apparatus length Parmarion
- Penis length longer than dart apparatus length Apoparmarion
5 Left shell lobe present only; dart apparatus no information Paraparmarion
- Both shell lobes present, dart apparatus absent Cryptosemelus
Key to species of genus Cryptosemelus
1 Shell lobes monochrome ..... 2
- $\quad$ Shell lobes with black reticulated stripes on pale colour ..... 32 Shell globose; body blue-gray; penial caecum absent; vagina large cylindrical.....

$\qquad$
C. gracilis- Shell depressed subglobose; body grayish, penial caecum present; vagina cylin-drical.C. betarmon3 Body color brownish; vagina long slender; penial caecum present; distal part ofepiphallus with prominently granulated surfaceC. tigrinus

- Body color dark brown to blackish; vagina short; penial caecum absent;epiphallus with smooth surfaceC. niger sp. nov.

Since 2007, an intensive survey on land snail diversity in limestone and non-limestone hills in northeastern Thailand has been continuously conducted and published (Tumpeesuwan 2007; Tumpeesuwan and Tumpeesuwan 2010a, 2010b; Srihata et al. 2010; Tanmuangpak et al. 2012; Jumlong et al. 2013 Tumpeesuwan et al 2014; Tanmuangpak 2016; Sasang 2019; Nahok 2020). In total, 16 species have been described as new to science, comprising 11 species from limestone hills, four species from sandstone hills, and one species from volcanic hills (Tumpeesuwan and Tumpeesuwan 2014, 2017, 2019a, 2019b; Tanmuangpak et al. 2015, 2017; Nahok et al. 2020, 2021a, 2021b; Deeprom et al. 2022; Tanmuangpak and Dumrongrojwattana 2022; Tongkerd et al. 2023). Future studies on the malacofauna of the northeastern part of Thailand require more surveys in overlooked and isolated natural areas.

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## References

Bank RA (2017) Classification of the Recent terrestrial Gastropoda of the World. Last update: July $16^{\text {th }}, 2017$.
Blanford WT, Godwin-Austen HH (1908) The Fauna of British India, including Ceylon and Burma. Mollusca. Testacellidae and Zonitidae. Taylor and Francis, London, 311 pp. https:// doi.org/10.5962/bhl.title. 13103
Chimsaeng N (2019) Reproductive system anatomy and radula moprphology of some land snail genera Dugella, Cryptaustenia and Aenigmatoconcha. Bachelor degree Science's project. Mahasarakham University, Maha Sarakham, Thailand. [In Thai with English abstract]
Collinge WE (1902) On the non-operculated land- and freshwater molluscs collected by the members of the "Skeat Expedition" in the Malay Peninsula, 1899-1900. The Journal of Malacology 9(3): 71-95. https://doi.org/10.1111/j.1469-7998.1902.tb08186.x
Dedov I, Schneppat U, Vu MQ, Huy NQ (2019) A new semislug of the genus Laocaia (Gastropoda, Pulmonata, Helicarionidae) from Vietnam. ZooKeys 846: 19-30. https:// doi.org/10.3897/zookeys.846.34372
Deeprom K, Tumpeesuwan C, Tumpeesuwan S (2022) A new species of the genus Quantula Baker, 1941 (Eupulmonata: Dyakiidae) from the southern part of north-eastern Thailand. The Raffles Bulletin of Zoology 70: 519-533.
Inkhavilay K, Sutcharit C, Bantaowong U, Chanabun R, Siriwut W, Srisonchai R, Pholyotha A, Jirapatrasilp P, Panha S (2019) Annotated checklist of the terrestrial molluscs from Laos (Mollusca, Gastropoda). ZooKeys 834: 1-166. https://doi.org/10.3897/zookeys.834.28800
Jumlong P, Tumpeesuwan C, Tumpeesuwan S (2013) Species diversity and abundance of land snails in sandstone and volcanic hills in Surin. Burapha Science Journal 18(1): 67-81. [In Thai with English abstract]
Nahok B (2020) Systematics and Taxonomy of Land Snails Genera Aegista, Thaitropis, Landouria, and Pseudobuliminus in Thailand. Unpublished PhD Thesis, Mahasarakham University. Maha Sarakham. Thailand.
Nahok B, Tumpeesuwan S, Tumpeesuwan C (2020) Anceyoconcha, a replacement name for the preoccupied tree snail genus Giardia Ancey, 1907 (Pulmonata: Helicoidea: Camaenidae). The Raffles Bulletin of Zoology 68: 80-90.
Nahok B, Tumpeesuwan C, Tumpeesuwan S (2021a) Two new species of genus Anceyoconcha S. Tumpeesuwan \& C. Tumpeesuwan, in Nahok et al. 2020 (Gastropoda: Pulmonata: Camaenidae), from northeastern Thailand. Raffles Bulletin of Zoology 69: 555-569.
Nahok B, Tumpeesuwan S, Tumpeesuwan C (2021b) Five new species of the land snail genus Landouria Godwin-Austen, 1918 (Gastropoda, Camaenidae) from northeastern Thailand, with note on genitalia and radula morphology of Landouria diplogramma (Möllendorff, 1902). European Journal of Taxonomy 767: 142-166. https://doi.org/10.5852/ejt.2021.767.1495

Pholyotha A, Sucharit C, Tongkerd P, Panha S (2020) Integrative taxonomic revision of the land snail genus Sarika Godwin-Austen, 1907 in Thailand, with descriptions of nine new species (Eupulmonata, Ariophantidae). ZooKeys 976: 1-100. https://doi.org/10.3897/zookeys.976.53859
Pholyotha A, Sucharit C, Panha S (2021a) Rediscovering the dancing semislug genus Cryptosemelus Colling, 1902 (Eupulmonata, Ariophantidae) from Thailand with description of two new species. ZooKeys 1076: 43-65. https://doi.org/10.3897/zookeys.1076.75576
Pholyotha A, Sucharit C, Tongerd P, Panha S (2021b) Systematic revision of the limestone karts-restricted land snail genus Aenigmatoconcha (Eupulmonata: Helicarionidae), with description of a new species. European Journal of Taxonomy 767: 55-82. https://doi. org/10.5852/ejt.2021.767.1487
Sasang C (2019) Species diversity and abundance of land snails in Si SaKet Province. Unpublished Master Thesis, Mahasarakham University, Maha Sarakham, Thailand. [In Thai with English abstract]
Schileyko AA (2002) Treatise on recent terrestrial pulmonate mollusks. Part 9. Helicarionidae, Gymnarionidae, Rhysotinidae, Ariophantidae. Ruthenica. Supplement 2(10): 1167-1307.
Schileyko AA (2003) Treatise on recent terrestrial pulmonate mollusks. Part 10. Ariophantidae, Ostracolethidae, Ryssotidae, Milacidae, Dyakiidae, Staffordiidae, Gastrodontidae, Zonitidae, Daudebardiidae, Parmacellidae. Ruthenica (Supplement 2): 1309-1466.
Solem A (1966) Some non-marine mollusks from Thailand, with notes on classification of the Helicarionidae. Spolia Zoologica. Musei Hauniensis 24: 103-114.
Srihata S, Tumpeesuwan C, Tumpeesuwan S (2010) Species diversity, abundance, and habitats of land snails in a square kilometer on Phu No, Kalasin Province. Journal of Science and Technology MSU 29(4): 359-371. [In Thai with English abstract]
Tanmuangpak K (2016) Species diversity, abundance, distribution and habitat relationship of land snail in Phu Pha Lom limestone area, Loei Province. Unpublished PhD Thesis, Mahasarakham University, Maha Sarakham, Thailand.
Tanmuangpak K, Dumrongrojwattana P (2022) Gyliotrachela cultura, a new species of terrestrial microsnail (Gastropoda: Eupulmonata: Vertiginidae) from Thailand. Raffles Bulletin of Zoology 70: 407-416.
Tanmuangpak K, Tumpeesuwan C, Tumpeesuwan S (2012) Diversity of Land Snails in Phu Pha Lom Forest Park at Muang District, Loei Province. Journal of Science and Technology Mahasarakham University 8(Special Issue): 15-26. [In Thai with English abstract]
Tanmuangpak K, Dumrongrojwattana P, Tumpeesuwan C, Tumpeesuwan S (2015) Sinoennea loeiensis, a new species of diapherid microsnail (Pulmonata: Streptaxoidea: Diapheridae) from Phu Pha Lom Limestone Hill, Loei Province, Northeastern Thailand. The Raffles Bulletin of Zoology 63: 293-300.
Tanmuangpak K, Tumpeesuwan C, Tumpeesuwan S (2017) A new species of Sesara Albers, 1860 from northeastern Thailand (Stylommatophora: Helicarionidae) A new species of Sesara Albers, 1860 from northeastern Thailand Stylommatophora Helicarionidae). Molluscan Research 37(1): 1-7. https://doi.org/10.1080/13235818.2016.1155431
Thiele J (1931) [1929-1935] Handbuch der systematischen Weichtierkunde. Erster Band, Tiel 2, Gastropoda: Ophisthobranchia and Pulmonata. [English translation: Bieler and Mikkelson (1992-1998)]

Tongkerd P, Tumpeesuwan S, Inkhavilay K, Prasankok P, Jeratthitikul E, Panha S, Sutcharit C (2023) Systematic revision of the snorkel snail genus Rhiostoma Benson, 1860 (Gastropoda, Caenogastropoda, Cyclophoridae) with descriptions of new species. ZooKeys 1142: 1-144. https://doi.org/10.3897/zookeys.1142.90097
Tumpeesuwan C (2007) Species diversity distribution and habitat relationship of terrestrial snails on the Phu Phan Range of Northeastern Thailand. Unpublished Ph D Thesis, Chulalongkorn University, Bangkok, Thailand.
Tumpeesuwan C, Tumpeesuwan S (2010a) Species diversity and abundance of land snails in Phu Thok Noi, Nong kai Province. Journal of Science and Technology MSU 29(3): 298307. [In Thai with English abstract]

Tumpeesuwan C, Tumpeesuwan S (2010b) Species diversity and abundance of land snails in Limestone Hills in Nong Bua Lamphu Province. Burapha Science Journal 15(2): 10-19. [In Thai with English abstract]
Tumpeesuwan C, Tumpeesuwan S (2014) Phuphania costata, a new species of dyakiid snail (Pulmonata: Dyakiidae) from Phu Pha Lom limestone area, Loei Province, northeastern Thailand. The Raffles Bulletin of Zoology 62: 352-357.
Tumpeesuwan C, Tumpeesuwan S (2017) Discovery of an overlooked Helicarionid land snail (Helicarionidae: Durgellinae) from northeastern Thailand, with description of a new genus and new species, and a note on radula morphology and genital system. The Raffles Bulletin of Zoology 65: 174-181.
Tumpeesuwan C, Tumpeesuwan S (2019a) First verified record of the genus Landouria God-win-Austen, 1918 from Thailand (Gastropoda: Stylommatophora: Camaenidae) with description of a new species. The Raffles Bulletin of Zoology 67: 298-305.
Tumpeesuwan C, Tumpeesuwan S (2019b) Muangnua arborea, a new semislug (Gastropoda, Stylommatophora, Helicarionidae, Durgellininae) from Loei Province, northeastern Thailand. ZooKeys 894: 19-32. https://doi.org/10.3897/zookeys.894.38327
Tumpeesuwan S, Tawinkarn N, Tumpeesuwan C (2014) Species diversity, abundance and habitat relationship of land snails in Phu Peng, Kalasin Province. In the $10^{\text {th }}$ Mahasarakham University Research Conference. Mahasarakham University: 498-506 [In Thai with English abstract]
Vaught KC (1989) A classification of the living Mollusca. American Malacologist, Inc., Melbourne, 189 pp.
Wiktor A (2002) Terrestrial gastropods of the Province of Madang in Papua-New Guinea (Part II) - Two species of Cryptaustenia Cockerell, 1898 (Pulmonata: Helicarionidae) new to the science. Folia Malacologica 10(4): 225-231. https://doi.org/10.12657/folmal.010.014
Zilch A (1959) Gastropoda, Euthyneura. In: Schindewolf OH (Ed.) Handbuch der Paläozoologie, Band 6, Gastropoda. Gebrüder Borntraeger, Berlin, 400 pp.

# Description of the male of Erromyrma Bolton \& Fisher, 2016 (Hymenoptera, Formicidae) 

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#### Abstract

The male of the myrmicine genus Erromyrma is described for the first time on the basis of two specimens of Erromyrma latinodis (Mayr, 1872) collected in northern Madagascar. We used COI barcoding to confirm the identification of the male specimens as conspecific with Erromyrma latinodis. We provide an illustrated male-based key to the four Myrmicinae tribes (Attini, Crematogastrini, Solenopsidini, Stenammini) and to the Solenopsidini genera (Adelomyrmex, Erromyrma, Solenopsis, Syllophopsis and Monomorium) for the Malagasy region.


## Keywords

Erromyrma, Madagascar, male ants, morphology, Myrmicinae, Solenopsidini

## Introduction

Within the Malagasy region, Myrmicinae is one of the largest and most diverse subfamilies of Formicidae (Hymenoptera), with 30 genera in four tribes (Fisher and Peeters 2019; Fisher 2022). The genus Erromyrma Bolton \& Fisher, 2016 (Solenopsidini), is represented by one species in the Malagasy region, Erromyrma latinodis (Mayr, 1872).

[^1]The species has been introduced in many countries, including the Malagasy region, and is thought to have originated in India (Sharaf et al. 2018). The global distribution also includes Indomalaya, the Southeastern Palearctic and Oceania bioregions. Erromyrma latinodis was originally placed in Monomorium but was shown to be a distinct lineage within the Solenopsidini based on molecular phylogenetic evidence (Ward et al. 2015) and placed in the newly described genus Erromyrma. Here we present the first description of the previously unknown male of Erromyrma based on E. latinodis, collected in northern Madagascar. We provide a male-based key to the Myrmicinae tribes and to genera for the tribe Solenopsidini of the Malagasy region.

## Material and methods

This study is based on two male ant specimens (unique specimen identifiers: CASENT0788835 and CASENT0801166) collected in northern Madagascar in the town of Antsohihy $(-14.89385,47.98261)$ in the Region of Sofia, at c. 11 m above sea level on April 23, 2017, by Brian L. Fisher and the Madagascar Biodiversity Center team (Team Vitsika). Two males along with workers and queens were collected by hand under the bark of a mango tree along a dirt road 1 km outside of the town of Antsohihy (collection code identifiers: BLF40204, BLF40205). The mango tree was 1.5 m in diameter and approximately 5 m tall. The ants were found under bark flakes before the first branch at about 1 m in height.

Terminology for general morphology follows Bolton (1994) and Boudinot (2013, 2015). The terminology of the wing venation follows Yoshimura and Fisher (2007). When referring to the presence or absence of veins in the descriptions, a vein is considered present regardless of whether it is tubular, nebulous, or spectral (Mason 1986).

## Imaging

Digital color montage images were created using a JVC KY-F75 digital camera and Syncroscopy Auto-Montage software (ver. 5.0), or a Leica DFC 425 camera in combination with the Leica Application Suite software (ver. 3.8). These images are available online through AntWeb.org (2022) and are accessible using the unique specimen identifier code.

## Mapping

The distribution map was generated by importing specimen distribution records into the Diva-GIS program (Hijmans et al. 2011).

## Morphological study

Morphological observations and measurements were carried out under Leica stereoscopic microscopes (MZ9.5). All measurements (see Fig. 1) and indices are expressed in millimeters.


Figure I. Illustration of measurements of Erromyrma latinodis (CASENT0788835) A head in full-face view $\mathbf{B}$ segment abdominal in dorsal view $\mathbf{C}$ segment abdominal in lateral view.

The following characters were recorded:
EL: Maximum eye length measured in full-face view along its maximum vertical diameter.
F1: Maximum length of the pedicel ( $1^{\text {st }}$ funicular segment, $2^{\text {nd }}$ antennal segment) measured in a straight line.
HL: Maximum head length in full-face view, measured in a straight line, from the anterior clypeal margin to the midpoint of a straight line drawn across the occipital margin.

HW1: Head width at the level of the posterior margin of compound eyes, measured in full-face view.
HW2: Maximum head width including the compound eyes, measured in full-face view.
IOD: Inner ocellar distance. Minimum distance between the inner edges of the two lateral ocelli, measured in full-face view.
LOD: Lateral ocellar distance. Minimum distance between the inner edge of the median and lateral ocelli, measured in full-face view.
OOD: Ocular-ocellar distance. Minimum distance from the outer edge of a lateral ocellus to the compound eyes, measured in full-face view.
PL: Petiole length, measured in profile view from the anterior margin of the peduncle to posterior most point of the petiolar tergite.
PW: Petiolar width. Maximum petiole width, measured in dorsal view.
PPW: Postpetiolar width. Maximum postpetiole width, measured in dorsal view.
SL: Scape length. Maximum length of the antennal scape measured in a straight line, excluding the basal constriction and condylar bulb.

## Indices

CI: Cephalic index. HW $1 / \mathrm{HL} \times 100$.
SI: $\quad$ Scape index. SL/HW $1 \times 100$.
EI: $\quad$ Eye index. EL/HW $1 \times 100$.
PI: Petiolar index. PL/PPL.

## DNA sampling

After searching for the males in colonies across Madagascar for six years, we wanted to confirm that these males did represent the first males of $E$. latinodis even though they were collected along with queens and workers. We sequenced 658 base pairs (bp) of mitochondrial cytochrome oxidase I (COI) gene from one of the males to evaluate similarity with CO1 sequenced from 33 workers of $E$. latinodis across the region. The distribution of the specimens sequenced is shown in Fig. 10. DNA extraction and COI sequencing were performed at University of Guelph (Ontario, Canada), following the protocol described in Fisher and Smith (2008).

Abbreviation of depositories:
BMNH British Museum of Natural History, London, UK;
CASC California Academy of Sciences, San Francisco, CA, USA;
MCZ Museum of Comparative Zoology Cambridge, MA, USA;
MHNG Muséum d'histoire naturelle, Genève, Switzerland;
MSNG Museo Civico di Storia Naturale 'Giacomo Doria', Genova, Italy;
NHMW Naturhistorisches Museum, Wien (= Vienna), Austria.

## Results

The 34 specimens sequenced (see Table 1) had a within-species sequence divergence of $0.00 \%$. Thus, based on CO1, the male specimen sequenced is conspecific with the workers from throughout the region.

Table I. Erromyrma latinodis Specimens sequenced for mitochondrial cytochrome oxidase I (COI) gene, including Genbank accession number, and caste. All voucher specimens are housed at the California Academy of Sciences.

| Specimen Identifier | Collection Event identifier | BOLD Process ID | COI-5P <br> GenBank | sequence length | Country | Caste |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CASENT0010900-D01 | R.J.1.765 | ASANR501-09 | HQ925412 | 590 | Mayotte | worker |
| CASENT0107528-D01 | BLF11668 | JDWAM495-05 | OP442963 | 654 | Madagascar | worker |
| CASENT0107541-D01 | BLF11664 | JDWAM503-05 | OP442956 | 654 | Madagascar | worker |
| CASENT0123018-D01 | BLF16532 | ASANP672-09 | GU710443 | 596 | Madagascar | worker |
| CASENT0123025-D01 | BLF16539 | ASANP673-09 | GU710442 | 625 | Madagascar | worker |
| CASENT0123498-D01 | BLF16507 | ASANP676-09 | HQ925385 | 618 | Madagascar | worker |
| CASENT0132440-D01 | BLF18832 | ASANO176-09 | GU709833 | 658 | Mayotte | worker |
| CASENT0134112-D01 | BLF19142 | ASANP692-09 | GU710444 | 658 | Madagascar | worker |
| CASENT0134329-D01 | BLF19879 | ASANO717-09 | GU709835 | 658 | Comoros | worker |
| CASENT0134937 | BLF18804 | ASIMB817-09 | OP442961 | 654 | Mayotte | worker |
| CASENT0134955 | BLF18810 | ASIMB824-09 | OP442957 | 654 | Mayotte | worker |
| CASENT0134970 | BLF18809 | ASIMB832-09 | OP442962 | 654 | Mayotte | worker |
| CASENT0136510-D01 | BLF19801 | ASANO766-09 | GU709838 | 658 | Comoros | worker |
| CASENT0136519-D01 | BLF19811 | ASANO769-09 | GU709837 | 658 | Comoros | worker |
| CASENT0136656-D01 | BLF19846 | ASANO786-09 | GU709840 | 658 | Comoros | worker |
| CASENT0136764-D01 | BLF19700 | ASANO809-09 | GU709839 | 658 | Comoros | worker |
| CASENT0136784 | BLF18809 | ASIMB886-09 | OP442959 | 654 | Mayotte | worker |
| CASENT0136900-D01 | BLF20364 | ASANR766-09 | GU711159 | 658 | Madagascar | worker |
| CASENT0136902-D01 | BLF20384 | ASANP695-09 | GU710446 | 658 | Madagascar | queen |
| CASENT0136903-D01 | BLF20384 | ASANP696-09 | GU710445 | 645 | Madagascar | worker |
| CASENT0137058-D01 | BLF19947 | ASANO840-09 | GU709842 | 658 | Comoros | worker |
| CASENT0137059-D01 | BLF19947 | ASANO841-09 | GU709841 | 658 | Comoros | worker |
| CASENT0137334-D01 | BLF19951 | ASANO912-09 | GU709844 | 658 | Comoros | worker |
| CASENT0137487-D01 | BLF19767 | ASANO967-09 | GU709836 | 658 | Comoros | worker |
| CASENT0145999-D01 | BLF21147 | ASANQ049-09 | GU710903 | 658 | Comoros | worker |
| CASENT0146463-D01 | BLF21164 | ASANQ138-09 | GU710902 | 658 | Comoros | worker |
| CASENT0146468-D01 | BLF21187 | ASANQ140-09 | GU710905 | 658 | Comoros | worker |
| CASENT0146475-D01 | BLF21160 | ASANQ144-09 | GU710904 | 658 | Comoros | worker |
| CASENT0146479-D01 | BLF21176 | ASANQ146-09 | GU710907 | 658 | Comoros | worker |
| CASENT0146495-D01 | BLF21188 | ASANQ150-09 | GU710906 | 658 | Comoros | worker |
| CASENT0147204-D01 | BLF20835 | ASANQ268-09 | GU710909 | 658 | Comoros | worker |
| CASENT0189653 | BLF18804 | ASIMB946-09 | OP442955 | 654 | Mayotte | worker |
| CASENT0189654 | BLF18810 | ASIMB947-09 | OP442960 | 654 | Mayotte | worker |
| CASENT0788835-D01 | BLF40204 | BFANT381-22 | OP442958 | 658 | Madagascar | male |

## Taxonomic synopsis

## Erromyrma latinodis (Mayr, 1872)

Monomorium latinode Mayr, 1872: 152 (w.). Lectotype worker (designated by Heterick 2006: 108): Malaysia ("Borneo"), Sarawak, 1865-66 (J. Doria \& O. Beccari), unique specimen identifier: CASENT0010941, examined [BMNH]. Paralectotype with same data as lectotype, unique specimen identifier: CASENT0905756, examined [MSNG]. [Combination in Erromyrma: Fisher and Bolton 2016: 276].
Monomorium latinode var. bruneum Emery, 1893: 243 (w.). Lectotype worker (designated by Heterick 2006: 108): Sri Lanka ("Ceylon"), Kandy, i.-ii. 1892 (E. Simon), unique specimen identifier: CASENT0008632, examined [MSNG]. [Junior synonym of latinode: Heterick 2006: 108].
Monomorium latinode var. voeltzkowi Forel, 1907: 78 (w.). Lectotype worker (designated by Heterick 2006: 108): Tanzania ("Ostafrika"), Pemba I., Chake-Chake (A. Voeltzkow) [MCZC]. Paralectotype with same data as lectotype, unique specimen identifier: CASENT0101928, examined [MHNG]. [Junior synonym of latinodis: Bolton 1987: 429].
Monomorium latinodoides Wheeler, 1928: 17 (w.). Syntype worker: China: Hong Kong, Kowloon (F. Silvestri) unique specimen identifier: MCZ-ENT00727982, examined [MCZC]. comb nov., syn. n.

Note. The type series at MCZ was examined. The syntypes series are labeled "Kowloon" (F. Silvestri): one pin with 3 workers (MCZ-ENT00020883) and 2 workers and one dealate queen on a second pin (MCZ-ENT00727982). The workers match the description and diagnosis (see below) of worker of E. latinodis. We formally combine the species in Erromyrma and synonymize the species with E. latinodis. However, we exclude the queen (on MCZ-ENT00727982) from the syntype series; it belongs to the genus Carebara (Westwood, 1840).

Diagnosis. Erromyrma workers [modified from Heterick (2006) and Fisher and Bolton (2016)]:

1. Worker caste polymorphic
2. Palp formula 3,3
3. Mandible triangular, smooth, and shiny
4. Five mandibular teeth
5. Antenna with 12 segments, with 3 -segmented apical club
6. Scape short, failing to reach occipital margin
7. Compound eyes present and conspicuous
8. Clypeus with a distinct unpaired seta at the midpoint of the anterior margin
9. Frontal carinae short and parallel
10. Antennal scrobe absent
11. Head without raised nuchal (= occipital) carina
12. Tibial spurs absent from meso- and metatibia
13. Promesonotal suture not distinct in dorsal view
14. Metanotal groove present
15. Propodeum unarmed
16. Propodeal dorsum with strong transverse striolae
17. Petiole pedunculated
18. Subpetiolar process absent
19. Sting developed

## Erromyrma latinodis males:

The following combination of characters diagnose males of Erromyrma latinodis.

1. Palp formula 5,3
2. Mandible triangular with distinct basal and masticatory margins (Fig. 2B)
3. Four mandibular teeth (Fig. 2B)
4. Antenna short and filiform, with 13 segments; pedicel subglobular (Fig. 2B)
5. Scape short (SI < 33, Fig. 2B)
6. Compound eyes large (EL/HW1 0.58, Fig. 1A)
7. Ocelli present and similar in size (Fig. 1A)
8. Clypeus with anterior margin convex. (Fig. 2B)
9. Frontal carinae absent (Fig. 2B)
10. Antennal scrobe absent (Fig. 2B)
11. Head without raised nuchal (= occipital) carina (Fig. 2C)
12. Notauli absent (Fig. 2C)
13. Single spur present on meso- and metatibia (Fig. 2A)
14. Pterostigma present on the forewing (Fig. 3)
15. First median-cubital cross-vein ( $1 \mathrm{~m}-\mathrm{cu}$ ) present on the forewing (Fig. 3)
16. Cubital vein of the forewing fused with the median vein $(\mathrm{M}+\mathrm{Cu})$ and forms an angle higher than $45^{\circ}$ with M (Fig. 3)
17. Forewing cross-vein 2rs-m absent (Fig. 3)
18. Petiole pedunculate (Fig. 2A)
19. Postpetiole elongated anteriorly, subglobose in lateral view (Fig. 2A)
20. Abdominal segment IV elongate and not shouldered (Fig. 2A)
21. Pygostyles present (Fig. 2A)

Male measurements $(N=2)$. HL $0.60-0.62$, HW1 0.48-0.5, HW2 0.62-0.65, EL 0.28-0.29, EW 0.21-0.23, IOD 0.20-0.21, LOD 0.06-0.07, OOD 0.16-0.18, SL $0.12-0.14$, F1 $0.09-0.10$, PL $0.51-0.54$, PW $0.20-0.21$, PPW $0.33-0.34$, CI 81-82, SI 28, EI 0.58.

## Description. Erromyrma latinodis males:

Structure. In full-face view, the head including compound eyes slightly wider than long. Posterior head margin relatively rounded; head with three large, rounded ocelli of the same size, situated on the frontal face of the head, lateral ocelli directed $45^{\circ}$ toward lateral sides. Compound eye large, strongly bulging. Anterior margin of clypeus


Figure 2. Erromyrma latinodis (CASENT0788835) A body in lateral view B head in full-face view C body in dorsal view.
convex. Mandible well developed and elongate triangular; masticatory margin with four teeth, the basal and masticatory margins are distinct.

Antennae 13 -segmented short and filiform, pedicel subglobular. The mesoscutum is strongly convex and bulging, in the dorsal view much broader than the head at the level of the forewing insertion. In dorsal view, pronotum short in the median portion; notauli absent on the mesoscutum; mesoscutellum broader than long and smaller than mesoscutum. Metapleural gland bulla present with metapleural lobe closed. In profile, propodeal angle rounded, without spines or teeth. Hind femora longer than tibia.

In lateral view, petiole distinctly pedunculated; subpetiolar process absent (Fig. 2A). In dorsal view, abdominal segment III (postpetiole) elongated anteriorly; abdominal segment IV not shoulder and broader than the remaining tergites.


Figure 3. Male forewing veins of Erromyrma latinodis (CASENT0788835). Abbreviations: Pt pterostigma; $\mathbf{S c}$ subcosta; $\mathbf{R}, \mathbf{r}$ radius; $\mathbf{R s}$, rs radial sector; $\mathbf{M}, \mathbf{m}$ media; $\mathbf{C u}$ cubitus; A, a, anal.

Sculpture. Clypeus, dorsum, lateral face, and venter of head weakly smooth and shiny. Pro- and mesothorax extensively smooth or very superficially sculptured and shiny, with posterolateral area of mesoscutum and posterior zone of mesopleuron unsculptured with shiny area. Metanotum and metapleuron unsculptured and matte. Apical area of anterior slope of petiole, coxae, femora, and tibiae of all legs smooth to superficially sculptured and shiny; tarsi entirely microsculptured. Gaster (abdominal segments IV to the apex) entirely smooth to superficially sculptured and shiny.

Color. Body and mandible largely brownish yellow except the ocellar region and the abdominal segments IV to the apex, brown.

Pilosity. Anterior margin of clypeus with a pair of stout setae and without a distinct unpaired seta at its midpoint. Mandible covered with standing hairs. Antennal scape and pedicel with short and decumbent whitish hairs; the flagellomeres densely hairy. Hairs on head and body moderately abundant, erect, short, and stout. Pronotum, mesoscutum, and mesoscutellum with many obliquely standing hairs; hairs on mesopleuron much sparser; metanotum and propodeum with erect hair. Femora and tibiae with appressed hairs; tarsi covered with short appressed hairs. Posterior margins of each abdominal tergite and sternite with long and suberect hairs. Parameres covered with stout hair.

Wings. (Fig. 3) Forewing with four closed cells. Costal vein (C) absent. Pterostigma pigmented, visible on the leading edge of the forewing. Radial vein (R) fused proximally to constitute $\mathrm{Sc}+\mathrm{R}+\mathrm{Rs}$ before reaching the pterostigma.

Radial sector (Rs). Past the separation from $\mathrm{Sc}+\mathrm{R}+\mathrm{Rs}$, Rs usually short free abscissa down curved and never reaching to the costal margin, the radial sector connects to the pterostigma via the second radial-radial sector cross-vein (2r-rs). Then merging with median vein ( $M$ ) and continuing fused (Rs+M).

Median vein (M). Further away from the leading wing margin is the median vein, proximally fused with cubital vein $(\mathrm{M}+\mathrm{Cu})$, following separation continuing as a free abscissa $M$ before joining with radial sector to form Rs+M. Median vein ( $M$ ) is fused with radial sector and present in past the junction of the radial sector.

Cubital vein $(\mathrm{Cu})$. Proximally the cubital vein is fused with median vein $(\mathrm{M}+\mathrm{Cu})$, the cubital vein $(\mathrm{Cu})$ divided by median-cubital cross-vein $(1 \mathrm{~m}-\mathrm{cu})$ the cubital vein does not connect to the distal wing margin.

Anal vein (A). A longitudinal vein running near the posterior wing margin. Consists of a free abscissa fused to cubital-anal cross-vein (cu-a), and continuing past cu-a.

Comments. The tribe Solenopsidini is separated from other Malagasy myrmicine tribes by the following combination of characters: with the head in full-face view, mandibles with masticatory margin less than five teeth; antennal scrobe reduced to absent; pedicel not more elongated than the remaining segments; ocelli present and same size situated on the frontal face of the head, lateral ocelli directed toward oblique front sides; occipital carina not visible in full-face view; head (including compound eyes) slightly wider than long with occipital margin of head rounded. In lateral view, the anterodorsal margin of mesopleuron lower than the highest point of the wing process, pronotum and mesonotum from a smooth convexity, pronotal furrow less marked; forewing venation: cross-vein $2 \mathrm{rs}-\mathrm{m}$ absent, costal vein absent, radial sector down curved and never reaching to the costal margin; propodeal spines absent; pygostyle present; abdominal segment III attached anteriorly to abdominal segment IV; peduncle of abdominal segment III is distinctly longer than that of the petiole; single tibial spur present on the front leg. In dorsal view, notauli absent.

Erromyrma can be distinguished from three other genera, Adelomyrmex (Emery, 1897), Monomorium (Mayr, 1855) and Syllophopsis (Santschi, 1915), by its subglobular pedicels. It can be separated from the genus Solenopsis (Westwood, 1840) by the number of its antennal segment.

## Key to the tribes of subfamily Myrmicinae based on males in the Malagasy region

The subfamily of Myrmicinae is represented by four tribes in the Malagasy region: Attini, Crematogastrini, Solenopsidini, Stenammini.

Attini: Cyphomyrmex (introduced), Eurhopalothrix, Pheidole, Pilotrochus, Strumigenys.
Crematogastrini: Calyptomyrmex, Cardiocondyla, Carebara, Cataulacus, Crematogaster, Dicroaspis, Eutetramorium, Malagidris, Melissotarsus, Meranoplus, Metapone,

Nesomyrmex, Pristomyrmex, Royidris, Terataner, Tetramorium, Trichomyrmex, Vitsika, Vollenhovia (introduced?).

Solenopsidini: Adelomyrmex, Erromyrma (introduced), Monomorium, Solenopsis, Syllophopsis.

## Stenammini: Aphaenogaster.

1 In profile, occipital carina strongly developed (Fig. 4A); mesoscutellum strongly elevated above metanotum; in dorsal view, scutellum smooth and convex (Fig. 4C); petiole distinctly pedunculate. With the head in full-face view, mandible always triangular $\qquad$ Stenammini (Aphaenogaster)

- In profile, occipital carina not forming a sharp ridge (Fig. 4B); mesoscutellum slightly convex to flat; in dorsal view, scutellum with or without sculptured (Fig. 4D); petiole sessile to shortly pedunculate. With the head in full-face view, the mandible broadly triangular to reduce 2


Figure 4. In profile view showing occipital carina A, B Aphaenogaster bressleri (CASENT0495103). In dorsal view form mesoscutellum C, D Cyphomyrmex minitus (CASENT0264488).

2 In profile, posterodorsal margin of head almost straight from the base of the lateral ocelli to the midpoint of the occipital carina. (Fig. 5A)

Attini (part)

- In profile, posterodorsal margin of head gradually rounded from the base of the lateral ocelli to the midpoint of the occipital margin. (Fig. 5B)


Figure 5. Head in profile view A Strumigenys chilo (CASENT0145240) B Tetramorium silvicola (CASENT0494732).
$3 \quad$ Cross-vein 2rs-m present on forewing (Fig. 6A) ........................................... 4


Figure 6. Forewing A Pheidole mgs006 (CASENT0135889) B Carebara drm03 (CASENT0143975).

4 Mandible strongly developed; masticatory margin with 7 large teeth which increase in size from apex to base; between each tooth is a minute denticle (Fig. 7A) (Attini) Pilotrochus

- Mandible normal to reduced; edentate to multidentate with many acute teeth which decrease in size from apex to base; without denticle between the teeth (Fig. 7B)


Figure 7. Mandible A Pilotrochus besmerus (CASENT0057183) B Malagidris sofina (CASENT0906626).

5 In lateral view, anterior margin of promesonotum forms a continuous outline, pronotal furrow not breaking outline (Fig. 8A) Solenopsidini

- In lateral view, anterior margin of promesonotum interrupted by an impressed pronotal furrow that breaks the outline (Fig. 8B) or mesonotum strongly produced anterodorsally (Fig. 8C)

Crematogastrinii


Figure 8. In profile view A Monomorium termitobium (CASENT0135952) B Meranoplus mayri (CASENT0062813) C Crematogaster hazolava (CASENT0317643).

## Male-based key to genera of the tribe Solenopsidini in the Malagasy region

1 Antennae 12-segmented.............................................................. Solenopsis

- Antennae 13-segmented.............................................................................. 2

2 In full-face view, pedicel subglobular; posteromedian margin of clypeus effaced so that clypeus and frons form a continuous surface (Fig. 9A); mandible triangular with distinct basal angle, masticatory margin with exactly 4 teeth
$\qquad$

- In full-face view, pedicel not globular, more cylindrical; posteromedian margin of clypeus visible (Fig. 9B); mandible spatulate to triangular, but its basal angle always indistinct, masticatory margin with 1 to 4 teeth...................... 3


Figure 9. Head in full-face view showing the pedicel, mandible, postero-median margin of clypeus $\mathbf{A} E r$ romyrma latinodis (CASENT0788835) B Syllophopsis cryptobia (CASENT0103340).

3 Forewing with five closed cells, $1 \mathrm{~m}-\mathrm{cu}$ cross-vein present (Fig. 10A). In profile, petiolar peduncle longer than postpetiolar length (Fig. 10C) Syllophopsis

- Forewing with four closed cells, $1 \mathrm{~m}-\mathrm{cu}$ cross-vein absent (Fig. 10B). In profile, petiolar peduncle absent or shorter than postpetiolar length (Fig. 10D)


Figure 10. In profile view showing forewing, petiole and post petiole A, C Syllophopsis modesta (CASENT0135642) B Monomorium termitobium (CASENT0135673) D Monomorium termitobium (CASENT0135952).

4 With the head in full-face view, antennal scape short, barely reaching the posterior ocular margin; mandible long, curved, masticatory margin with 3 to 4 teeth (Fig. 11A)

Monomorium

- With the head in full-face view, antennal scape long reaching the occipital margin; mandible short, spatulate, basal margin linear, unidentate (Fig. 11B) $\qquad$ Adelomyrmex (Seychelles)


Figure II. Head in full-face view showing mandible and scape A Monomorium exiguum (CASENT0135614) B Adelomyrmex sc01 (CASENT0160764).

## Discussion

In the Malagasy region, Erromyrma latinodis was collected from Comoros, Madagascar and Mayotte (see Fig. 12). The species inhabits montane rainforest, mangrove, Uapaca woodland, dry forest, and anthropogenic habitats from elevations of 2 to 1726 m . Workers were collected from a range of microhabitats and methods including foraging on low vegetation, on the ground, in ground nests, sifted litter, under stones, rotten wood or from dead twigs above ground. The males were collected along with workers and queens under the bark on the main trunk of a mango tree along a village road.

The males for this species were only collected after six expeditions. They are not collected by traditional means for example: malaise traps sampling or UV light samples from the region. Initial expeditions to known localities in northern Madagascar did not find the males. Colonies were kept alive for over a year without the production of males. Two males were finally found at one of the known collection sites.

The C01 data confirms the identification of the males and also shows a pattern of $0 \%$ sequence divergence between the samples from Madagascar, Comoros, and Mayotte. The lack of sequence divergence across island systems supports the hypothesis that this species is introduced in the region. Low sequence diversity could also be explained by other factors such as reproductive systems. The difficulty of finding males could be linked to a reproductive system that would reduce sequence divergence.


Figure 12. Distribution of Erromyrma latinodis in the Malagasy region. Red indicates localities of sequenced specimens.

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## References

AntWeb.org (2022) AntWeb, California Academy of Sciences, San Francisco, California, USA. http://www.antweb.org

Bolton B (1987) A review of the Solenopsis genus-group and revision of Afrotropical Monomorium Mayr (Hymenoptera: Formicidae). Bulletin of the British Museum (Natural History). Entomology 54: 263-452.
Bolton B (1994) Identification guide to the ant genera of the world. Mass.: Harvard University Press, Cambridge, 222 pp.
Boudinot BE (2013) The male genitalia of ants: Musculature, homology, and functional morphology (Hymenoptera, Aculeata, Formicidae). Journal of Hymenoptera Research 30: 29-49. https://doi.org/10.3897/jhr.30.3535
Boudinot BE (2015) Contributions to the knowledge of Formicidae (Hymenoptera, Aculeata): A new diagnosis of the family, the first global male-based key to subfamilies, and a treatment of early branching lineages. European Journal of Taxonomy 120(120): 1-62. https:// doi.org/10.5852/ejt. 2015.120
Emery C (1893) Voyage de M. E. Simon à Île de Ceylan (Janvier-Février 1892) Formicides. Annales de la Société Entomologique de France 62: 239-255.
Fisher BL (2022) Hymenoptera: Formicidae, ants, Vitsika. In: Goodman SM (Ed.) The New Natural History of Madagascar. Princeton University Press: Princeton, New Jersey, 11731188, 1202-1244. https://doi.org/10.2307/j.ctv2ks6tbb. 153
Fisher BL, Bolton B (2016) Ants of Africa and Madagascar, A Guide to the Genera. Berkeley: University of California Press, 512 pp. https://doi.org/10.1525/9780520962996
Fisher BL, Peeters C (2019) Ants of Madagascar: a guide to the 62 genera. Antananarivo, Madagascar: Association Vahatra, 9782953892383: 260 pp.
Fisher BL, Smith MA (2008) A revision of Malagasy Species of Anochetus Mayr and Odontomachus Latreille (Hymenoptera: Formicidae). https://doi.org/10.1371/journal.pone. 0001787
Forel A (1907) Ameisen von Madagaskar, den Comoren und Ostafrika. Wissens chaftliche Ergebnisse. Reise in Ostafrika 2: 75-92.
Heterick BE (2006) A revision of the Malagasy ants belonging to genus Monomorium Mayr, 1855. Proceedings of the California Academy of Sciences 57: 69-202.

Hijmans RJ, Guarino L, Mathur P (2011) DIVA-GIS. Version 7.5. A geographic information system for the analysis of species distribution data. http://www.diva-sig.org
Mason WRM (1986) Standard drawing conventions and definitions for venation and other features of wings of Hymenoptera. Proceedings of the Entomological Society of Washington 88: 1-7.
Mayr G (1872) Formicidae Borneenses collectae a J. Doria et O. Beccari in territorio Sarawak annis 1865-1867. Annali del Museo Civico di Storia Naturale 2: 133-155.
Sharaf MR, Fisher BL, Dhafer MA, Polaszek A, Aldawood AS (2018) Additions to the ant fauna (Hymenoptera: Formicidae) of Oman: an updated list, new records and a description of two new species. Asian Myrmecology 10: e010004. [1-38] https://doi.org/10.20362/am. 010004
Ward PS, Brady SG, Fisher BL, Schultz TR (2015) The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). Systematic Entomology 40(1): 61-81. https://doi.org/10.1111/syen. 12090
Wheeler WM (1928) Ants collected by Professor F. Silvestri in China. Bollettino del Laboratorio di Zoologia Generale e Agraria della Reale Scuola Superiore d'Agricoltura. Portici 22: 3-38.
Yoshimura M, Fisher BL (2007) A revision of male ants of the Malagasy region (Hymenoptera: Formicidae): Key to subfamilies and treatment of the genera of Ponerinae. Zootaxa 1654(1): 21-40. https://doi.org/10.11646/zootaxa.1654.1.2

# Tapeworms (Platyhelminthes, Cestoda) from marine chondrichthyans of the Southwestern Atlantic Ocean, and the sub-Antarctic and Antarctic islands: a checklist 

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#### Abstract

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#### Abstract

A parasite-host list of cestodes parasitizing chondrichthyans in the Southwest Atlantic off Argentina and surrounding waters of Antarctica is compiled based on the available literature. The list is based on published descriptions and redescriptions of species, and newly collected worms during the current study. A total of 57 valid species belonging to 28 genera of the orders Cathetocephalidea, Diphyllidea, Gyrocotylidea, Lecanicephalidea, Onchoproteocephalidea, Phyllobothriidea, Rhinebothriidea, "Tetraphyllidea", and Trypanorhyncha is listed. Information on hosts, localities, specimens in collections and comments on tapeworms are also included. A host-parasite list including chimaeras ( 1 order, 1 genus), batoids ( 4 orders, 10 genera), and sharks ( 3 orders, 5 genera) is provided. Tapeworm diversity, distribution range, and host associations are discussed. The cestodes orders Phyllobothriidea and Rhinebothriidea exhibit the highest species richness, with 13 and 12 species, respectively. Onchoproteocephalideans and rhinebothriideans have the broadest geographic distribution in the study area. Regarding hosts, arhynchobatid skates are the group most frequently associated with cestodes. However, further collecting efforts are necessary to understand whether this data reflect the real diversity and host association of these parasites or is a result of a bias in sampling.


[^2]
## Keywords

Batoids, biodiversity, parasites, sharks, tapeworms, taxonomy

## Introduction

According to Froese and Pauly (2022), more than 500 species of fishes have been registered along the Southwestern Atlantic off Argentina and the sub-Antarctic and Antarctic islands (including South Georgia, Elephant, and Joinville islands), including 100 chondrichthyan species (Table 1) (Gabbanelli et al. 2018; Concha et al. 2019; Froese and Pauly 2022). Since these cartilaginous fishes are the definitive hosts of a great diversity of adult cestodes (Caira and Jensen 2014), it is not uncommon to find a large variety of taxa of tapeworms along the Southwestern Atlantic and the Southern seas.

Complete and accurate species lists are essential for many biological disciplines such as ecology, conservation, and biogeography. Particularly, comprehensive fish cestodes datasets are necessary if we consider the usefulness of these parasites as biological tags for stock identification of their elasmobranch hosts in the context of overfishing and habitat degradation have profoundly altered the populations of marine elasmobranch (Dulvy et al. 2014; Irigoitia et al. 2017; Irigoitia et al. 2022). To date, cestodes from the Southwestern Atlantic, sub-Antarctic, and Antarctic regions were listed only in a few articles. These included a list of fishes and their tapeworms from South America (Alves et al. 2017), a compilation of marine invertebrates from the Argentine Sea focusing on taxonomic information at the generic level only (Bigatti and Signorelli 2018), and a few works about cestodes of Antarctic fishes (Rocka and Zdzitowiecki 1998; Rocka 2003, 2017). The analysis of the endoparasites in Antarctic fishes showed significantly higher values of diversity indices compared to the sub-Antarctic ichthyofauna (Muñoz and Cartes 2020); it would be interesting to consider the diversity of cestodes in a wider context, especially including the Southwestern Atlantic and southern latitudes off Antarctica in a single study. However, no complete work about cestodes from chondrichthyans, with detailed distributional ranges and host associations in this particular area of the Southern Hemisphere has been compiled so far.

In order to facilitate further studies, the main goal of this work is to elaborate a complete checklist of cestodes in chondrichthyan hosts based on summarizing references. The study area includes the Southwestern Atlantic Ocean off Argentina, Río de la Plata estuary, and the surrounding waters of South Georgia and the Elephant and Joinville islands by surrounding waters of South Georgia, Elephant and Joinville islands. This list includes information on localities, specimens in collections, and comments about the parasites and their hosts reported in previous works. We have also incorporated information about cestodes described in the last years, which included numerous new records and new localities (Menoret et al. 2017; Franzese and Ivanov 2018, 2020a, b, 2021; Menoret and Ivanov 2021; Franzese et al. 2022; this study).

Table I. Chondrichthyans reported from the Southwestern Atlantic Ocean off Argentina, Río de la Plata estuary, and the sub-Antarctic and Antarctic islands (including South Georgia, Elephant, and Joinville islands).

| Subclass | Order | Family | Genera | Species | Sampled chondrichthyan species |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Holocephalii | Chimaeriformes | Callorhinchidae | 1 | 1 | 1 |
|  |  | Chimaeridae | 1 | 1 | 0 |
| Subtotals | 1 | 2 | 2 | 2 | 1 |
| Batoidea | Myliobatiformes | Dasyatidae | 2 | 3 | 0 |
|  |  | Mobulidae | 1 | 1 | 0 |
|  |  | Myliobatidae | 1 | 3 | 2 |
|  | Rajiformes | Arhynchobatidae | 5 | 31 | 19 |
|  |  | Rajidae | 2 | 8 | 3 |
|  | Rhinopristiformes | Pristidae | 1 | 1 | 0 |
|  |  | Rhinobatidae | 1 | 1 | 0 |
|  |  | Trygonorrhinidae | 1 | 1 | 1 |
|  | Torpediniformes | Narcinidae | 2 | 3 | 1 |
|  |  | Torpedinidae | 1 | 1 | 0 |
| Subtotals | 4 | 10 | 17 | 53 | 26 |
| Selachii | Carcharhiniformes | Carcharhinidae | 2 | 7 | 1 |
|  |  | Galeocerdonidae | 1 | 1 | 0 |
|  |  | Scyliorhinidae | 2 | 2 | 0 |
|  |  | Sphyrnidae | 1 | 3 | 0 |
|  |  | Triakidae | 2 | 4 | 3 |
|  | Echinorhiniformes | Echinorhinidae | 1 | 1 | 0 |
|  | Hexanchiformes | Hexanchidae | 3 | 3 | 1 |
|  | Lamniformes | Alopiidae | 1 | 1 | 0 |
|  |  | Carchariidae | 1 | 1 | 0 |
|  |  | Cetorhinidae | 1 | 1 | 0 |
|  |  | Lamnidae | 3 | 3 | 0 |
|  | Squaliformes | Dalatiidae | 2 | 2 | 0 |
|  |  | Etmopteridae | 2 | 6 | 0 |
|  |  | Somniosidae | 3 | 4 | 0 |
|  |  | Squalidae | 1 | 3 | 0 |
|  | Squatiniformes | Squatinidae | 1 | 3 | 1 |
| Subtotals | 6 | 16 | 27 | 45 | 6 |
| Totals | 11 | 28 | 46 | 100 | 33 |

## Materials and methods

After an exhaustive bibliographical search, an annotated and revised parasite-host checklist was generated for the adult cestodes from marine chondrichthyans reported between $35^{\circ} \mathrm{S}-63^{\circ} \mathrm{S}$. The geographical area considered covers the Southwestern Atlantic Ocean off Argentina (from $35^{\circ} \mathrm{S}$ southward), Río de la Plata estuary, and surrounding waters off South Georgia, Elephant, and Joinville islands. The cestode species are arranged according to taxonomic categories and are presented alphabetically, followed by data on their hosts, including valid species name, order, family, and synonymous species name used in literature (if available) in parentheses. The information for localities includes location, coordinates in degrees and minutes (if available in the literature), province, and country (where applicable) only for the type locality. The type-host and the type locality refer to data included in the original descriptions of cestodes species.

Other hosts and other localities only refer to the records within the study area, including those in the original descriptions as well as those mentioned in redescriptions, other papers and newly collected materials sampled during the present study. Specimens in collections include type material from original descriptions, voucher specimens from redescriptions and new voucher specimens prepared during the present study. Information about the new voucher specimens is in bold.

For the preparation of the figures, estimated coordinates were assigned to those records that lacked such information in the original publication.

Based on the information from the parasite-host checklist, the host-parasite data were subdivided into two inventories, one for batoids and chimaeras and another for sharks. The host species are arranged according to taxonomic categories and presented alphabetically, followed by the data on their parasites.

New vouchers of cestodes were obtained from the spiral intestines of chondrichthyans that had been caught by commercial trawlers between 2009 and 2017. The spiral intestines were fixed in $10 \%$ formalin and transferred to $70 \%$ ethanol for storage in the Laboratorio de Sistemática y Biología de Parásitos de Organismos Acuáticos (SIBIPOA) of Instituto de Biodiversidad y Biología Experimental y Aplicada (IBBEA, CONICET-UBA). Cestodes were hydrated in a graded ethanol series, stained with Harris' hematoxylin, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted in Canada balsam (Menoret and Ivanov 2021; Franzese et al. 2022).

The accession numbers of the available molecular sequences were taken from the GenBank database, considering only those specimens whose identification is not doubtful.

The classification and valid cestodes names follow Caira and Jensen (2017) and Caira et al. (2022). The classification and valid host names follow Menni and Lucifora (2007), Naylor et al. (2012), Weigmann (2016), Gabbanelli et al. (2018), Concha et al. (2019), Stehmann et al. (2021), and Froese and Pauly (2022). Abbreviations of the collection names used are listed in Table 2.

Table 2. Museum abbreviations.

| AHC | Australian Helminthological Collection, South Australian Museum, Adelaide, Australia |
| :--- | :--- |
| CHIOC | Coleção Helmintológica do Instituto Oswaldo Cruz, Rio de Janeiro, Brazil |
| BMNH/NHMUK | Natural History Museum, London, United Kingdom |
| HWML | Harold W. Manter Laboratory of Parasitology, University of Nebraska <br> State Museum, Nebraska, United States of America |
| IPCAS | Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic |
| LRP | Lawrence R. Penner Parasitology Collection, Department of Ecology and Evolutionary Biology, University of <br> Connecticut, Connecticut, United Stated of America |
| MACN-Pa | Museo Argentino de Ciencias Naturales, Colección Parasitológica, Buenos Aires, Argentina |
| MLP | Colección de Invertebrados, Museo de La Plata, La Plata, Argentina |
| MNHNC | Museo Nacional de Historia Natural de Chile, Santiago, Chile |
| MNHNF | Muséum National d'Histoire Naturelle, Paris, France |
| MZPW | Museum and Institute of Zoology, Polish Academy of Science, Warsaw, Poland |
| NMW | Naturhistorisches Museum Wien, Vienna, Austria |
| USNM | National Museum of Natural History of the Smithsonian Institution, Washington, United States of America |
| USNPC | U. S. National Parasite Collection, Maryland, United States of America, currently incorporated in the UNNM |

## Results

Parasite-host checklist
Order Cathetocephalidea Schmidt \& Beveridge, 1990
Family Cathetocephalidae Dailey \& Overstreet, 1973
Genus Cathetocephalus Dailey \& Overstreet, 1973

Cathetocephalus australis Schmidt \& Beveridge, 1990
Type host. Carcharhinus brachyurus (Günther) (Carcharhiniformes: Carcharhinidae).
Type locality. Goolwa, South Australia.
Other locality. Off Argentina.
Specimens in collections. AHC No. V4123 (holotype); AHC Nos. 17535, 17536 (paratypes).

References. Schmidt and Beveridge (1990), Suriano and Labriola (2001a).

Order Diphyllidea Carus, 1863
Family Echinobothriidae Perrier, 1897
Genus Coronocestus Caira, Marques, Jensen, Kutcha \& Ivanov, 2013
Coronocestus notoguidoi (Ivanov, 1997)
Echinobothrium notoguidoi Ivanov, 1997. Syn.
Type host. Mustelus schmitti Springer (Carcharhiniformes: Triakidae).
Type locality. Mar del Plata ( $38^{\circ} 00^{\prime} \mathrm{S}, 57^{\circ} 33^{\prime} \mathrm{W}$ ), Buenos Aires Province, Argentina.
Specimens in collections. MLP No. 3893C (holotype); MLP Nos. 3894C (paratypes); USNPC No. 87169 (paratypes).

GenBank Acc. No. DQ088034.
References. Ivanov (1997), Alarcos et al. (2006), Tyler (2006), Caira et al. (2013b).
Comments. Tyler (2006) modified the original description of Ivanov (1997) and added new morphological information based on type material.

Genus Echinobothrium Van Beneden, 1849
Echinobothrium acanthocolle Wojciechowska, 1991
Type host. Amblyraja georgiana (Norman) (Rajiformes: Rajidae) (Raja georgiana).
Type locality. Shelf near South Georgia, South Atlantic Ocean.
Specimens in collections. No specimens were deposited in a public collection.
References. Wojciechowska (1991a), Rocka (2003).
Comments. Holotype and paratype are in Wojciechowska's personal collection.

Genus Halysioncum Caira, Marques, Jensen, Kutcha \& Ivanov, 2013
Halysioncum megacanthum (Ivanov \& Campbell, 1998)
Echinobothrium megacanthum Ivanov \& Campbell, 1998. Syn.
Type host. Myliobatis goodei Garman (Myliobatiformes: Myliobatidae).
Type locality. San Antonio Oeste, San Matías Gulf ( $40^{\circ} 44^{\prime} \mathrm{S}, 64^{\circ} 56^{\prime} \mathrm{W}$ ), Río Negro Province, Argentina.

Specimens in collections. MLP No. 3958 (holotype); IPCAS No. C-288 (paratypes); USNM No. 1382674 (paratypes).

References. Ivanov and Campbell (1998a), Tyler (2006), Caira et al. (2013b).
Comments. Tyler (2006) modified the original description of Ivanov and Campbell (1998a) and added new morphological information based on type material.

## Halysioncum pigmentatum (Ostrowski de Núñez, 1971)

Echinobothrium pigmentatum Ostrowski de Núñez, 1971. Syn.
Type host. Zapteryx brevirostris (Müller \& Henle) (Rhinopristiformes: Trygonorrhinidae).
Type locality. Mar del Plata, Buenos Aires Province, Argentina.
Specimens in collections. No specimens were deposited in a public collection.
References. Ostrowski de Núńez (1971), Tyler (2006), Caira et al. (2013b).
Comments. Holotype and paratypes remain in Ostrowski de Núnez's personal collection. Tyler (2006) modified the original description of Ostrowski de Núñez (1971) and added new morphological information based on material from the author's personal collection.

## Order Gyrocotylidea Poche, 1926 <br> Genus Gyrocotyle Diesing, 1850

## Gyrocotyle maxima Mac Donagh, 1927

Type host. Probably Callorhinchus callorynchus (Linnaeus) (Chimaeriformes: Callorhinchidae) (Mustelus asterias).

Type locality. Probably off Mar del Plata, Buenos Aires Province, Argentina.
Specimens in collections. Instituto Bacteriológico, Buenos Aires.
Reference. Mac Donagh (1927).

## Gyrocotyle rugosa Diesing, 1850

Type host. Callorhinchus callorynchus (Chimaeriformes: Callorhinchidae).

Type locality. Portum Natalensem, South Africa.
Other locality. Necochea, Buenos Aires Province.
Specimen in collections. NMW No. 2502 (neotype).
GenBank Acc. Nos. MW587267, MW587258, MW581656.
References. Mac Donagh (1927), Barčák et al. (2021).
Comments. Gyrocotyle rugosa has a wide distribution including coastal waters of South America, South Africa, and New Zealand.

## Order Lecanicephalidea Hyman, 1951

Family Aberrapecidae Jensen, Caira, Cielocha, Littlewood \& Waeschenbach, 2016 Genus Aberrapex Jensen, 2001

Aberrapex arrhynchum (Brooks, Mayes \& Thorson, 1981)
Discobothrium arrhynchum Brooks, Mayes \& Thorson, 1981. Syn.
Type host. Myliobatis goodei (Myliobatiformes: Myliobatidae).
Type locality. Río de la Plata estuary near Montevideo, Uruguay.
Specimens in collections. USNPC No. 75722 (holotype); USNPC No. 75723 (paratype); HWML No. 21003 (paratypes).

References. Brooks et al. (1981), Jensen (2001).

## Aberrapex ludmilae Menoret, Mutti \& Ivanov, 2017

Type host. Myliobatis goodei (Myliobatiformes: Myliobatidae).
Type locality. San Matias Gulf ( $40^{\circ} 58^{\prime} \mathrm{S}, 64^{\circ} 56^{\prime} \mathrm{W}$ ), Río Negro Province, Argentina.

Specimens in collections. MACN-Pa No 616-1 (holotype); MACNPa Nos. 616/2-5 (paratypes); IPCAS No. C-755/1-2 (paratypes); LRP No. 9239 (paratypes).

Reference. Menoret et al. (2017).

## Aberrapex sanmartini Menoret, Mutti \& Ivanov, 2017

Type host. Myliobatis goodei (Myliobatiformes: Myliobatidae).
Type locality. Off Carmen de Patagones ( $40^{\circ} 42^{\prime} \mathrm{S}, 62^{\circ} 00^{\prime} \mathrm{W}$ ), Buenos Aires Province, Argentina.

Specimens in collections. MACN-Pa No. 617/1 (holotype); MACN-Pa Nos. 617/2-12 (paratypes); IPCAS Nos. C-756/1-2 (paratypes); LRP Nos. 9242, 9243 (paratypes).

Reference. Menoret et al. (2017).

## Aberrapex vitalemuttiorum Menoret, Mutti \& Ivanov, 2017

Type host. Myliobatis ridens Ruocco, Lucifora, Díaz de Astarloa, Mabragaña \& Delpiani (Myliobatiformes: Myliobatidae).

Type locality. Off Villa Gesell ( $37^{\circ} 29^{\prime} \mathrm{S}, \quad 56^{\circ} 45^{\prime} \mathrm{W}$ ), Buenos Aires Province, Argentina.

Other locality. Punta Negra, Necochea ( $38^{\circ} 37^{\prime} \mathrm{S}, 58^{\circ} 51^{\prime} \mathrm{W}$ ), Buenos Aires Province.
Specimens in collections. MACN-Pa No 618/1 (holotype); MACN-Pa Nos. 618/2-10 (paratypes); IPCAS Nos. C-757/1-2 (paratypes); LRP Nos. 9240, 9241 (paratypes).

Reference. Menoret et al. (2017).

## Family Paraberrapecidae Jensen, Caira, Cielocha, Littlewood \& Waeschenbach, 2016 Genus Paraberrapex Jensen, 2001

Paraberrapex atlanticus Mutti \& Ivanov, 2016
Type host. Squatina guggenheim Marini (Squatiniformes: Squatinidae).
Type locality. Off Puerto Quequén ( $38^{\circ} 53^{\prime} \mathrm{S}, 58^{\circ} 27^{\prime} \mathrm{W}$ ), Buenos Aires Province, Argentina.

Other localities. Near Río de la Plata estuary ( $36^{\circ} 21^{\prime} \mathrm{S}, 54^{\circ} 32^{\prime} \mathrm{W}$ ), off Villa Gesell ( $37^{\circ} 17^{\prime}$ S, $56^{\circ} 27^{\prime} \mathrm{W}$ ), off Carmen de Patagones ( $40^{\circ} 58^{\prime} \mathrm{S}, 62^{\circ} 00^{\circ} \mathrm{W}$ ), Buenos Aires Province. San Matías Gulf ( $41^{\circ} 03^{\prime} \mathrm{S}, 64^{\circ} 06^{\prime} \mathrm{W}$ ), Río Negro Province.

Specimens in collections. MACN-Pa No 618/1 (holotype); MACN-Pa Nos. 618/2-10 (paratypes); IPCAS Nos. C-757/1-2 (paratypes); LRP Nos. 9240, 9241 (paratypes).

Reference. Mutti and Ivanov (2016).

Order Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson \& Littlewood, 2014
Family Onchobothriidae Braun, 1900
Genus Acanthobothrium Blanchard, 1848

## Acanthobothrium carolinae Franzese \& Ivanov, 2020

Type host. Bathyraja magellanica (Philippi) (Rajiformes: Arhynchobatidae).
Type locality. Coastal waters off Puerto San Julián ( $49^{\circ} 29^{\prime} \mathrm{S}, 66^{\circ} 11^{\prime} \mathrm{W}$ ), Santa Cruz Province, Argentina.

Other localities. Coastal waters off Río Grande ( $54^{\circ} 01^{\prime} \mathrm{S}, 67^{\circ} 06^{\prime} \mathrm{W}$ ), Tierra del Fuego Province. Namuncurá Marine Protected Area/Burdwood Bank (54³2'S, $60^{\circ} 01^{\prime} \mathrm{W}$ ).

Specimens in collections. MACN-Pa No. 716 (holotype); MACN-Pa Nos. 717/1-4, 718/1-3, 719/1-2 (paratypes); IPCAS No. C-838 (paratypes); LRP Nos. 10179-10184 (paratypes).

Reference. Franzese and Ivanov (2020a).

Table 3. Cestodes and their respective hosts collected for this study.

| Taxon | Host | Capture coordinates | New locality |
| :---: | :---: | :---: | :---: |
| Onchoproteocephalidea |  |  |  |
| Onchobothriidae |  |  |  |
| Acanthobothrium |  |  |  |
| A. domingae | Dipturus brevicaudatus | $38^{\circ} 00^{\prime} \mathrm{S}, 56^{\circ} 04^{\prime} \mathrm{W}$ | Mar del Plata, Buenos Aires |
| A. marplatensis | Atlantoraja castelnaui | $38^{\circ} 46^{\prime} \mathrm{S}, 57^{\circ} 56^{\prime} \mathrm{W}$ | Puerto Quequén, Buenos Aires |
| A. stefaniae | Discopyge tschudii | $38^{\circ} 46^{\prime} \mathrm{S}, 57^{\circ} 56^{\prime} \mathrm{W}$ | Puerto Quequén, Buenos Aires |
| Rhinebothriidea |  |  |  |
| Echeneibothriidae |  |  |  |
| Echeneibothrium |  |  |  |
| E. williamsi | Dipturus brevicaudatus | $38^{\circ} 46^{\prime} \mathrm{S}, 57^{\circ} 56^{\prime} \mathrm{W}$ | Puerto Quequén, Buenos Aires |
| Notomegarhynchus |  |  |  |
| $N$. navonae | Atlantoraja castelnaui | $38^{\circ} 46^{\prime} \mathrm{S}, 57^{\circ} 56^{\prime} \mathrm{W}$ | Puerto Quequén, Buenos Aires |

Acanthobothrium domingae Franzese \& Ivanov, 2020
Type host. Dipturus brevicaudatus (Marini) (Rajiformes: Rajidae).
Type locality. Coastal waters off Santa Teresita ( $36^{\circ} 35^{\prime} \mathrm{S}, 54^{\circ} 54^{\prime} \mathrm{W}$ ), Buenos Aires Province, Argentina.

Other localities. Coastal waters off Río Grande ( $53^{\circ} 35^{\prime} \mathrm{S}, 66^{\circ} 37^{\prime} \mathrm{W}$ ), Tierra del Fuego Province. Coastal waters off Mar del Plata ( $\mathbf{3 8}^{\circ} \mathbf{0 0}$ 'S, $5^{\circ}{ }^{\circ} 4^{\prime}$ 'W), Buenos Aires Province (Table 3).

Specimens in collections. MACN-Pa No. 720 (holotype); MACN-Pa Nos. 721/1-3, 722/1-9, 723(paratypes); IPCAS No. C-839 (paratypes); LRP Nos. 1018510195 (paratypes); MACN-Pa No. 770 (voucher).

Reference. Franzese and Ivanov (2020a).

## Acanthobothrium marplatensis Ivanov \& Campbell, 1998

Type host. Atlantoraja castelnaui (Miranda Ribeiro) (Rajiformes: Arhynchobatidae) (Rioraja castelnaui).

Type locality. Mar del Plata ( $38^{\circ} 00^{\prime} \mathrm{S}, 57^{\circ} 33^{\prime} \mathrm{W}$ ), Buenos Aires Province, Argentina.
Other locality. Puerto Quequén (38ㄴ́́S, $\left.57^{\circ} 56^{\prime} \mathrm{W}\right)$, Buenos Aires Province (Table 3).

Specimens in collections. MLP No. 4025 (holotype); MLP No 4026 (paratype); USNM No. 1382675 (paratypes); BMNH No 1998.2.10.1-2 (paratypes); MACN-Pa
No. 771 (voucher).
Reference. Ivanov and Campbell (1998b).

## Acanthobothrium stefaniae Franzese \& Ivanov, 2018

Type host. Discopyge tschudii Heckel (Torpediniformes: Narcinidae).

Type locality. Coastal waters off Mar Chiquita City ( $37^{\circ} 46^{\prime} \mathrm{S}$, $56^{\circ} 56^{\prime} \mathrm{W}$ ), Buenos Aires Province, Argentina.

Other localities. Coastal waters off Villa Gesell $\left(37^{\circ} 29^{\prime} \mathrm{S}, 56^{\circ} 45^{\prime} \mathrm{W}\right)$, off San Clemente del Tuyú ( $35^{\circ} 50^{\prime}$ S, $56^{\circ} 18^{\prime} \mathrm{W}$ ), off Puerto Quequén ( $38^{\circ} \mathbf{4 6}$ 'S, $57^{\circ} 56^{\prime} \mathrm{W}$ ) (Table 3), Buenos Aires Province. Coastal waters off Camarones ( $45^{\circ} 08^{\prime} \mathrm{S}, 65^{\circ} 19^{\prime} \mathrm{W}$ ), Chubut Province.

Specimens in collections. MACN-Pa No 624 (holotype); MACN-Pa Nos. 625/1-6, 626/1-3, 627/1, 628/1-2 (paratypes); IPCAS No. C-786 (paratypes); LRP Nos. 9403-9410 (paratypes); MACN-Pa No. 772 (voucher).

Reference. Franzese and Ivanov (2018).

## Acanthobothrium zapterycum Ostrowski de Núñez, 1971

Type host. Zapteryx brevirostris (Rhinopristiformes: Trygonorrhinidae).
Type locality. Mar del Plata, Buenos Aires Province, Argentina.
Other localities. Coastal waters off Villa Gessel ( $37^{\circ} 29^{\prime} \mathrm{S}, 56^{\circ} 45^{\prime} \mathrm{W}$ ), La Lucila del Mar ( $36^{\circ} 38^{\prime} \mathrm{S}, 56^{\circ} 15^{\prime} \mathrm{W}$ ), Puerto Quequén ( $38^{\circ} 46^{\prime} \mathrm{S}$, $57^{\circ} 56^{\prime} \mathrm{W}$ ), Buenos Aires Province. Puerto Pirámides ( $42^{\circ} 05^{\prime} \mathrm{S}, 62^{\circ} 50^{\prime} \mathrm{W}$ ), Chubut Province.

Specimens in collections. MACN-Pa No. 214/1 (holotype); MACN-Pa No. 214/1-5 (paratypes); MACN-Pa Nos. 629/1, 630/1-3, 631/1-4, 632/1-4 (vouchers); IPCAS No. C-787 (vouchers); LRP Nos. 9411-9417 (vouchers).

Reference. Ostrowski de Núñez (1971), Franzese and Ivanov (2018).

## Acanthobothrium sp.

Hosts. Bathyraja cousseauae Díaz de Astarloa \& Mabragaña, Bathyraja magellanica (Rajiformes: Arhynchobatidae); Myliobatis goodei (Myliobatiformes: Myliobatidae); Zapteryx brevirostris (Rhinopristiformes: Trygonorrhinidae).

Localities. Río de La Plata estuary, Uruguay; Mar del Plata, Buenos Aires Province, Argentina; Malvinas Islands, Southwestern Atlantic Ocean.

Specimens in collections. HWML Nos. 20999, 21000.
References. Ostrowski de Núñez (1971), Brooks et al. (1981), Beer et al. (2019).
Comments. Ostrowski de Núñez (1971) registered Acanthobothrium sp. from Z. brevirostris in Mar del Plata. Brooks et al. (1981) reported two specimens of Acanthobothrium sp. from M. goodei at Río de la Plata, which could be a different species. They pointed out that one of these specimens could correspond to the same species reported by Ostrowski de Núñez (1971) in Z. brevirostris. Beer et al. (2019) reported Acanthobothrium sp. from B. cousseauae and B. magellanica off Malvinas Islands. The deposited material only corresponds to the specimens studied by Brooks et al. (1981).

## Genus Onchobothrium de Blainville, 1828

## Onchobothrium antarcticum Wojciechowska, 1990

Type host. Bathyraja eatonii (Günther) (Rajiformes: Arhynchobatidae).
Type locality. shelf around Joinville Island in Bransfield's Strait, Antarctica.
Specimens in collections. MZPW No. 1805 (holotype); MZPW No. 1806 (paratype); BMNH 1989.4.19.1 (paratype).

References. Wojciechowska (1990a), Rocka (2003, 2017).

Order Phyllobothriidea Caira, Jensen, Waeschenbach, Olson \& Littlewood, 2014
Family Phyllobothriidae Braun, 1900
Genus Crossobothrium Linton, 1889
Crossobothrium antonioi Ivanov, 2009
Type host. Notorynchus cepedianus (Péron) (Hexanchiformes: Hexanchidae).
Type locality. Puerto Quequén (38.32'S, $\left.58^{\circ} 42^{\prime} \mathrm{W}\right)$, Buenos Aires Province, Argentina.

Specimens in collections. MACN-Pa No. 493/1 (holotype); MACN-Pa Nos. 493/2-6 (paratypes).

Reference. Ivanov (2009).

Crossobothrium pequeae Ivanov, 2009
Type host. Notorynchus cepedianus (Hexanchiformes: Hexanchidae).
Type locality. Puerto Quequén ( $38^{\circ} 32^{\prime} \mathrm{S}, 58^{\circ} 42^{\prime} \mathrm{W}$ ), Buenos Aires Province, Argentina.

Specimens in collections. MACN-Pa No. 494/1 (holotype); MACN-Pa Nos. 494/2-6 (paratypes).

Reference. Ivanov (2009).

Genus Guidus Ivanov, 2006
Guidus antarcticus (Wojciechowska, 1991)
Marsupiotbothrium antarcticum Wojciechowska, 1991. Syn.
Type host. Bathyraja maccaini Springer (Rajiformes: Arhynchobatidae).
Other host. Bathyraja eatonii (Rajiformes: Arhynchobatidae).

Type locality. Shelf around Joinville Island, Antarctica.
Specimens in collections. MZPW No. 1817 (holotype); BMNH No. 1992.1.6.31 (paratype).

References. Wojciechowska (1991a), Rocka (2003), Ivanov (2006).

## Guidus argentinense Ivanov, 2006

Type host. Bathyraja brachyurops (Fowler) (Rajiformes: Arhynchobatidae).
Type locality. Coastal waters off Buenos Aires Province $\left(37^{\circ} 06\right.$ 'S, $\left.54^{\circ} 20^{\prime} \mathrm{W}\right)$, Argentina.

Other localities. Off Bahía Blanca ( $39^{\circ} 34^{\prime} \mathrm{S}, 56^{\circ} 16^{\prime} \mathrm{W}$ ), Buenos Aires Province. Namuncurá Marine Protected Area/Burdwood Bank ( $54^{\circ} 44^{\prime} \mathrm{S}, 59^{\circ} 56^{\prime} \mathrm{W}$ ).

Specimens in collections. MACN-Pa No. 432/1 (holotype); MACN-Pa Nos. 432/2-7 (paratypes); USNM No. 1393041 (paratypes); MACN-Pa Nos. 750751 (vouchers).

References. Ivanov (2006), Menoret and Ivanov (2021).

## Guidus francoi Menoret \& Ivanov, 2021

Type host. Bathyraja magellanica (Rajiformes: Arhynchobatidae).
Type locality. Off Río Grande ( $\left.53^{\circ} 56^{\prime} \mathrm{S}, 66^{\circ} 04^{\prime} \mathrm{W}\right)$, Tierra del Fuego Province, Argentina.

Other localities. Off Puerto San Julián (49 $\left.29^{\prime} \mathrm{S}, 66^{\circ} 11^{\prime} \mathrm{W}\right)$, Santa Cruz Province. Off Río Grande ( $54^{\circ} 30^{\prime} \mathrm{S}, 65^{\circ} 13^{\prime} \mathrm{W} ; 54^{\circ} 24^{\prime} \mathrm{S}, 63^{\circ} 57^{\prime} \mathrm{W} ; 54^{\circ} 01^{\prime} \mathrm{S}, 67^{\circ} 06^{\prime} \mathrm{W} ; 53^{\circ} 55^{\prime} \mathrm{S}$, $\left.67^{\circ} 05^{\prime} \mathrm{W} ; 53^{\circ} 36^{\prime} \mathrm{S}, 67^{\circ} 39^{\prime} \mathrm{W}\right)$, Tierra del Fuego Province.

Specimens in collections. MACN-Pa No. 739 (holotype); MACN-Pa Nos. 740/1-3, 741/1, 744, 745, 746/1-2, 740/4, 741/2-3, 742/1-3, 743, 746/3-7 (paratypes); IPCAS No. C-887 (paratypes).

Reference. Menoret and Ivanov (2021).

## Guidus magellanicus Menoret \& Ivanov, 2021

Type host. Bathyraja magellanica (Rajiformes: Arhynchobatidae).
Type locality. Off Río Grande ( $54^{\circ} 01^{\prime} \mathrm{S}, 67^{\circ} 06^{\prime} \mathrm{W}$ ), Tierra del Fuego Province, Argentina.

Other localities. Off Puerto San Julian ( $\left.49^{\circ} 29^{\prime} \mathrm{S}, 66^{\circ} 11^{\prime} \mathrm{W}\right)$, Santa Cruz Province.
Specimens in collections. MACN-Pa No. 747 (holotype); MACN-Pa Nos. 748/1-2, 749/1-2 (paratypes); IPCAS No. C-888 (paratypes).

Reference. Menoret and Ivanov (2021).

## Guidus sp.

Host. Bathyraja multispinis (Norman) (Rajiformes: Arhynchobatidae).
Locality. Malvinas Islands Shelf, Southwestern Atlantic Ocean.
Reference. Beer et al. (2019).
Comments. These specimens were studied by Beer et al. (2019) at a molecular rather than morphological level, without reaching an identification at the specific level.

## Genus Orygmatobothrium Diesing, 1863

Orygmatobothrium juani Ivanov, 2008
Type host. Mustelus fasciatus (Garman) (Carcharhiniformes: Triakidae).
Typelocality.Puerto Quequén ( $38^{\circ} 32^{\prime} \mathrm{S}, 58^{\circ} 42^{\prime} \mathrm{W}$ ), BuenosAires Province, Argentina.
Specimens in collections. MACN-Pa No. 445/1 (holotype); MACN-Pa Nos. 445/2-6 (paratypes).

Reference. Ivanov (2008).

## Orygmatobothrium schmitti Suriano \& Labriola, 2001

Type host. Mustelus schmitti (Carcharhiniformes: Triakidae).
Type locality. Mar del Plata ( $38^{\circ} 00^{\prime} \mathrm{S}, 57^{\circ} 33^{\prime} \mathrm{W}$ ), Buenos Aires Province, Argentina.
Other locality. Puerto Quequén ( $38^{\circ} 32^{\prime} \mathrm{S}, 58^{\circ} 42^{\prime} \mathrm{W}$ ), Buenos Aires Province.
Specimens in collections. MACN-Pa Nos. 382/1-2 (holotype and paratype); MNHN 20HG:158 CIX, MNHN 20HG:159 CIX (paratypes); MACN-Pa Nos. 444/1-5 (vouchers).

References. Ostrowski de Núñez (1973), Suriano and Labriola (2001b), Alarcos et al. (2006), Ivanov (2008).

Comments. Ostrowski de Núñez (1973) redescribed O. velamentum based on material collected in Mar del Plata. Later, Ivanov (2008) reassigned these specimens to $O$. schmitti.

## Genus Phyllobothrium Van Beneden, 1850

## Phyllobothrium sp.

Hosts. Sympterygia bonapartii Müller \& Henle (Rajiformes: Rajidae) (as Psammobatis microps in Ostrowski de Núńez [1971]), Myliobatis goodei (Myliobatiformes: Myliobatidae), Zapteryx brevirostris (Rhinopristiformes: Trygonorrhinidae).

Localities. Mar del Plata, Argentina. Río dela Plata estuary nearMontevideo, Uruguay.
Specimen in collections. HWML 21001.

References. Ostrowski de Núñez (1971), Brooks et al. (1981).
Comments. Ostrowski de Núñez (1971) registered Phyllobothrium sp. from S. bonapartii and Z. brevirostris in Mar del Plata. Brooks et al. (1981) reported ten specimens of Phyllobothrium sp. from M. goodei at the Río de la Plata estuary. The deposited material only corresponds to the specimens studied by Brooks et al. (1981).

## Genus Rockacestus Caira, Bueno \& Jensen, 2021

## Rockacestus arctowskii (Wojciechowska, 1991)

Phyllobothrium arctowskii Wojciechowska, 1991, Anthocephalum arctowskii Rocka \& Zdzitowiecki, 1998. Syns.

Type host. Bathyraja arctowskii (Dollo) (Rajiformes: Arhynchobatidae) (Bathyraja sp. 2).
Type locality. Admiralty Bay, environs of the South Shetlands, Antarctica.
Other locality. Shelf near Elephant Island, Antarctica.
Specimens in collections. MZPW No. 1814 (holotype); BMNH No. 1992.1.6.30 (paratypes).

References. Wojciechowska (1991b), Rocka (2003, 2017), Caira et al. (2021).
Comments. Rocka (2017) established the name Rajicestus Rocka \& Laskowski, 2017 for cestodes from Antarctic and sub-Antarctic skates described originally in Wojciechowska (1991b) as members of Phyllobothrium. Unfortunately, no generic diagnosis or type species was designated; therefore, the name Rajicestus is unavailable. Regarding host identification, Stehmann et al. (2021) assigned specimens of Bathyraja sp. 2 to Bathyraja arctowskii, a wide-ranging, circum-Antarctic species locally common in the Atlantic sector of the Southern Ocean.

## Rockacestus conchai Caira, Bueno \& Jensen, 2021

Type host. Bathyraja albomaculata (Norman) (Rajiformes: Arhynchobatidae).
Type locality. Malvinas Islands ( $48^{\circ} 39^{\prime} \mathrm{S}, 60^{\circ} 44^{\prime} \mathrm{W}$ ), Southwestern Atlantic Ocean.
Other locality. Malvinas Islands ( $49^{\circ} 38^{\prime} \mathrm{S}, 59^{\circ} 50^{\prime} \mathrm{W}$ ).
Specimens in collections. NHMUK No. 2020.12.17.1 (holotype); USNM Nos. 1638654, 1638655 (paratypes); LRP Nos. 10293, 10294 (paratypes); LRP Nos. 10279-10281 (SEM vouchers).

GenBank Acc. No. MW419959.
Reference. Caira et al. (2021).

## Rockacestus georgiensis (Wojciechowska, 1991)

Phyllobothrium georgiense Wojciechowska, 1991, Anthocephalum georgiense Rocka \& Zdzitowiecki, 1998. Syns.

Type host. Amblyraja georgiana (Rajiformes: Rajidae) (Raja georgiana).
Type locality. Shelf around South Georgia, South Atlantic Ocean.
Specimens in collections. MZPW No. 1812 (holotype); No. BMNH No. 1992.1.6.27 (paratype).

References. Wojciechowska (1991b), Rocka (2003, 2017), Caira et al. (2021).
Comments. Rocka (2017) established the name Rajicestus for cestodes from Antarctic and sub-Antarctic skates described originally in Wojciechowska (1991b) as members of Phyllobothrium. Unfortunately, no generic diagnosis or type species was designated; therefore, the name Rajicestus is unavailable.

## Rockacestus rakusai (Wojciechowska, 1991)

Phyllobothrium rakusai Wojciechowska, 1991, Anthocephalum rakusai Rocka \& Zdzitowiecki, 1998. Syns.

Type host. Bathyraja maccaini (Rajiformes: Arhynchobatidae).
Type locality. Shelf around Elephant Island and Joinville Island in Bransfield Strait, Antarctica.

Specimens in collections. MZPW No. 1816 (holotype); BMNH No. 1992.1.6.28 (paratype).

References. Wojciechowska (1991b), Rocka (2003, 2017), Caira et al. (2021).
Comments. Rocka (2017) established the name Rajicestus for cestodes from Antarctic and sub-Antarctic skates described originally in Wojciechowska (1991b) as members of Phyllobothrium. Unfortunately, no generic diagnosis or type species was designated; therefore, the name Rajicestus is unavailable.

## Rockacestus siedleckii (Wojciechowska, 1991)

Phyllobothrium siedleckii Wojciechowska, 1991, Anthocephalum siedleckii Rocka \& Zdzitowiecki, 1998. Syns.

Type host. Bathyraja eatonii (Rajiformes: Arhynchobatidae).
Type locality. Shelf around Elephant Island and Joinville Island in Bransfield Strait, Antarctica.

Specimens in collections. MZPW No. 1815 (holotype); BMNH No. 1992.1.6.29 (paratype).

References. Wojciechowska (1991b), Rocka (2003, 2017), Caira et al. (2021).
Comments. Rocka (2017) established the name Rajicestus for cestodes from Antarctic and sub-Antarctic skates described originally in Wojciechowska (1991b) as members of Phyllobothrium. Unfortunately, no generic diagnosis or type species was designated; therefore, the name Rajicestus is unavailable.

## Phyllobothriidea gen. sp.

Hosts. Amblyraja doellojuradoi (Pozzi), Bathyraja albomaculata, B. brachyurops, B. cousseauae, B. macloviana (Norman), B. magellanica, B. multispinis, B. scaphiops (Norman), Dipturus chilensis (Guichenot), Psammobatis sp. 3, Psammobatis sp. 2.

Locality. Malvinas Islands Shelf, Southwestern Atlantic Ocean.
Reference. Beer et al. (2019).
Comments. Beer et al. (2019) studied these specimens at the molecular rather than the morphological level, without reaching generic or specific identification. Caira et al. (2021) noted that the specimens of Phyllobothriidea gen. sp. found by Beer et al. (2019) could correspond to the genus Rockacestus; however, further molecular and morphological studies are necessary to identify them at the specific level. Beer et al. (2019) also pointed out the presence of Phyllobothriidea gen. sp. parasitizing D. chilensis. Nevertheless, the distribution of D. chilesis is restricted to the Pacific Ocean; therefore, this record is based on a misidentification of the host (Concha et al. 2019).

Order Rhinebothriidea Healy, Caira, Jensen, Webster \& Littlewood, 2009
Family Echeneibothriidae de Beauchamp, 1905
Genus Echeneibothrium van Beneden, 1850
Echeneibothrium cristinae Franzese, 2022

Type host. Bathyraja cousseauae (Rajiformes: Arhynchobatidae).
Type locality. Isla de los Estados ( $54^{\circ} 25^{\prime} \mathrm{S}, 65^{\circ} 18^{\prime} \mathrm{W}$ ), Tierra del Fuego Province, Argentina.

Specimens in collections. MACN-Pa No. 734 (holotype); MACN-Pa Nos. 735/1-5, 736/1-23 (paratypes).

Reference. Franzese et al. (2022).

## Echeneibothrium multiloculatum Carvajal \& Dailey, 1975

Type host. Dipturus chilensis (Rajiformes: Rajidae) (Raja chilensis).
Other host. Dipturus brevicaudatus (Rajiformes: Rajidae).
Type locality. Between Papudo and Talcahuano (between 32 ${ }^{\circ} 28^{\prime} \mathrm{S}$ and $37^{\circ} 15^{\prime}$ S), Chile.

Other localities. Mar de Ajó ( $36^{\circ} 34^{\prime} \mathrm{S}, 54^{\circ} 39^{\prime} \mathrm{W}$ ), Mar del Plata ( $38^{\circ} 05^{\prime} \mathrm{S}$, $\left.56^{\circ} 58^{\prime} \mathrm{W}\right)$, Quequén ( $38^{\circ} 35^{\prime} \mathrm{S}, 58^{\circ} 39^{\prime} \mathrm{W}$ ), Buenos Aires Province. San Jorge Gulf ( $46^{\circ} 13^{\prime} \mathrm{S}, 66^{\circ} 26^{\prime} \mathrm{W}$ ), Santa Cruz Province. Tolhuin ( $54^{\circ} 29^{\prime} \mathrm{S}, 65^{\circ} 59^{\prime} \mathrm{W}$ ), Río Grande ( $53^{\circ} 31^{\prime} \mathrm{S}, 67^{\circ} 48^{\prime} \mathrm{W}$ ), Tierra del Fuego Province.

Specimens in collections. USNM No. 1368523 (holotype); USNM No. 1368524 (paratypes); MACN-Pa Nos. 737/1-10, 738, 739, 740/1-8 (vouchers).

GenBank Acc. Nos. MZ594651, MH688748, KY569546, KY569547, KY569548, KY569549.

References. Carvajal and Dailey (1975), Franzese et al. (2022).

## Echeneibothrium williamsi Carvajal \& Dailey, 1975

Type host. Dipturus chilensis (Rajiformes: Rajidae) (Raja chilensis).
Other host. Dipturus brevicaudatus (Rajiformes: Rajidae).
Type locality. Between Papudo and Talcahuano (between $32^{\circ} 28^{\prime} \mathrm{S}$ and $37^{\circ} 15^{\prime}$ S), Chile.
Other localities. San Jorge Gulf ( $46^{\circ} 13^{\prime} \mathrm{S}, 66^{\circ} 26^{\prime} \mathrm{W}$ ), Santa Cruz Province. Tolhuin ( $54^{\circ} 29^{\prime}$ S, $65^{\circ} 59^{\prime} \mathrm{W}$ ), Río Grande ( $53^{\circ} 31^{\prime} \mathrm{S}, 67^{\circ} 48^{\prime} \mathrm{W}$ ), Tierra del Fuego Province. Puerto Quequén ( $38^{\circ} \mathbf{4} 6^{\prime}$ S, $57^{\circ} 56^{\prime} \mathrm{W}$ ), Buenos Aires Province (Table 3).

Specimens in collections. USNM No. 1368521 (holotype); USNM No. 1368522 (paratypes); MACN-Pa Nos. 741/1-14, 742/1-4, 743, 773 (vouchers).

GenBank Acc. Nos. MZ594641, MH688742, KY569542, KY569543, KY569544, KY569545.

References. Carvajal and Dailey (1975), Franzese et al. (2022).

## Echeneibothrium sp.

Hosts. Bathyraja albomaculata, B. brachyurops, B. cousseauae, B. griseocauda (Norman), B. macloviana, B. multispinis, B. scaphiops (Rajiformes: Arhynchobatidae).

Locality. Malvinas Islands, Southwestern Atlantic Ocean.
Reference. Beer et al. (2019).
Comments. These specimens were studied by Beer et al. (2019) at a molecular rather than morphological level and did not manage to reach an identification at a specific level. Franzese et al. (2022) noted that the specimens of Echeneibothrium sp. found in B. cousseauae by Beer et al. (2019) at Malvinas Islands could correspond to E. cristinae. Considering that the remaining species of Bathyraja have not been recorded as hosts for Echeneibothrium and that most marine rhinebothriideans show a high degree of specificity to their definitive hosts, Franzese et al. (2022) supposed that some Echeneibothrium specimens reported by Beer et al. (2019) could be new species. However, further morphological studies are necessary to identify them at a specific level. Beer et al. (2019) also pointed out the presence of Echeneibothrium and Echeneibothrium sp. 2 parasitizing D. chilensis at Malvinas Islands; however, the distribution of $D$. chilesis is restricted to the Pacific Ocean, i. e. this record has been based on a host misidentification.

## Genus Notomegarhynchus Ivanov \& Campbell, 2002

Notomegarhynchus navonae Ivanov \& Campbell, 2002

Type host. Atlantoraja castelnaui (Rajiformes: Arhynchobatidae).
Type locality. Mar del Plata ( $38^{\circ} 00^{\prime} \mathrm{S}, 57^{\circ} 33^{\prime} \mathrm{W}$ ), Buenos Aires Province, Argentina.

Other locality. Puerto Quequén ( $\left.38^{\circ} 46^{\prime} \mathrm{S}, 57^{\circ} 56^{\prime} \mathrm{W}\right)$, Buenos Aires Province (Table 3).

Specimens in collections. MACN-Pa No. 404/1 (holotype); MACN-Pa Nos. 404/2-3 (paratypes); USNM No. 1387025 (paratypes); MACN-Pa No. 774 (voucher).

Reference. Ivanov and Campbell (2002).

Notomegarhynchus shetlandicum (Wojciechowska, 1990)

Pseudanthobothrium shetlandicum Wojciechowska, 1990. Syn.

Type host. Bathyraja eatonii (Rajiformes: Arhynchobatidae).
Other host. Bathyraja maccaini (Rajiformes: Arhynchobatidae).
Specimens in collections. MZPW No. 1810 (holotype); MZPW No. 1811 (paratypes); BMNH No. 1989.4.19.3 (paratypes).

Type locality. South Shetlands region, Joinville shelf, Elephant Island Shelf, and Admiralty Bay, Antarctica.
References. Wojciechowska (1990b), Ivanov and Campbell (2002), Rocka (2003, 2017).

Genus Pseudanthobothrium Baer, 1956
Pseudanthobothrium notogeorgianum Wojciechowska, 1990
Type host. Amblyraja georgiana (Rajiformes: Rajidae) (Raja georgiana).
Type locality. South Georgia area, South Atlantic Ocean.
Specimens in collections. MZPW No. 1807 (holotype); MZPW Nos. 18081809 (paratypes); BMNH No. 1989.4.19.2 (paratypes).

References. Wojciechowska (1990b), Rocka (2003, 2017).

## Pseudanthobothrium minutum Wojciechowska, 1991

Type host. Bathyraja eatonii (Rajiformes: Arhynchobatidae).
Type locality. Elephant Island, Antarctica.
Specimens in collections. No specimens were deposited in a public collection.
References. Wojciechowska (1991a), Rocka (2003, 2017).
Comments. Type specimens are in Wojciechowska's personal collection.

## Pseudanthobothrium sp.

Host. Amblyraja doellojuradoi (Rajiformes: Rajidae).

Locality. Malvinas Islands, South Atlantic Ocean.
Reference. Beer et al. (2019).
Comments. Beer et al. (2019) indicated the presence of Pseudanthobothrium sp. and Pseudanthobothrium sp. 2 parasitizing $A$. doellojuradoi at Malvinas Islands.

## Family Rhinebothriidae Euzet, 1953

Genus Rhinebothrium Linton, 1890
Rhinebothrium chilensis Euzet \& Carvajal, 1973
Type host. Sympterygia lima (Poeppig) (Rajiformes: Arhynchobatidae) (Psammobatis lima).

Other host. Sympterygia bonapartii (Rajiformes: Arhynchobatidae).
Type locality. North coast of Chile.
Other localities. Estuary of Bahía Blanca ( $38^{\circ} 45^{\prime} \mathrm{S}, 62^{\circ} 15^{\prime} \mathrm{W}$ ), Villa Gesell, Necochea, El Rincón, Buenos Aires Province, Argentina. San Matías Gulf, Río Negro Province, Argentina. San Jorge Gulf, Santa Cruz Province, Argentina. Río de La Plata estuary, Uruguay.

Specimens in collections. MNHNC No. 20005 (holotype); MNHNF Nos. Sb 267, Sb 268 (paratypes).

References. Euzet and Carvajal (1973), Tanzola et al. (1998), Irigoitia et al. (2017).

Genus Scalithrium Ball, Neifar \& Euzet, 2003
Scalithrium ivanovae Franzese, 2021

Type host. Atlantoraja platana (Günther) (Rajiformes: Arhynchobatidae).
Type locality. San Matías Gulf ( $41^{\circ} 11^{\prime} \mathrm{S}, 64^{\circ} 03^{\prime} \mathrm{W}$ ), Río Negro Province, Argentina.
Specimens in collections. MACN-Pa No. 762 (holotype); MACN-Pa Nos. 763/1-4, 764/1-7, 765/1-3 (paratypes); IPCAS No. C-897 (paratypes).

Reference. Franzese and Ivanov (2021).

## Scalithrium kirchneri Franzese \& Ivanov, 2021

Type host. Rioraja agassizii (Müller \& Henle) (Rajiformes: Arhynchobatidae).
Type locality. Continental shelf waters off San Clemente del Tuyú ( $36^{\circ} 12^{\prime} \mathrm{S}$, $\left.55^{\circ} 20^{\prime} \mathrm{W}\right)$, Buenos Aires Province, Argentina.

Other locality. Continental shelf waters off Quequén ( $39^{\circ} 56^{\prime} \mathrm{S}, 58^{\circ} 20^{\prime} \mathrm{W}$ ), Buenos Aires Province.

Specimens in collections. MACN-Pa No. 757 (holotype); MACN-Pa Nos. 758/1-13, 759, 760/1-3, 761 (paratypes); IPCAS No. C-896 (paratypes).

Reference. Franzese and Ivanov (2021).

Genus incertae sedis and other forms with uncertain family allocations
Genus Semiorbiseptum Franzese \& Ivanov, 2020

## Semiorbiseptum alfredoi Franzese \& Ivanov, 2020

Type host. Psammobatis normani McEachran (Rajiformes: Arhynchobatidae).
Type locality. Coastal waters off Mar de Ajó (36³4'S, $\left.54^{\circ} 39^{\prime} \mathrm{W}\right)$, Buenos Aires Province, Argentina.

Other localities. Coastal waters off Pinamar ( $\left.37^{\circ} 12^{\prime} \mathrm{S}, 54^{\circ} 53^{\prime} \mathrm{W}\right)$, Buenos Aires Province. Caleta Olivia ( $46^{\circ} 23^{\prime} \mathrm{S}, 64^{\circ} 20^{\prime} \mathrm{W}$ ), Santa Cruz Province.

Specimens in collections. MACN-Pa No. 706 (holotype); MACN-Pa Nos. 707/1-5, 708/1-3, 709, 710, 711/1-2 (paratypes); IPCAS No. C-837/1 (paratypes).

Reference. Franzese and Ivanov (2020b).

Semiorbiseptum mariae Franzese \& Ivanov, 2020
Type host. Psammobatis rudis Günther (Rajiformes: Arhynchobatidae).
Other host. Psammobatis normani (Rajiformes: Arhynchobatidae).
Type locality. Coastal waters off Isla de los Estados ( $54^{\circ} 30^{\prime} \mathrm{S}, 65^{\circ} 13^{\prime} \mathrm{W}$ ), Tierra del Fuego Province, Argentina.

Other localities. Coastal waters off Río Grande ( $53^{\circ} 34^{\prime} \mathrm{S}, 66^{\circ} 32^{\prime} \mathrm{W}$ ), Tierra del Fuego Province. Coastal waters off Miramar ( $39^{\circ} 34^{\prime} \mathrm{S}, 56^{\circ} 16^{\prime} \mathrm{W}$ ), Buenos Aires Province.

Specimens in collections. MACN-Pa No. 701 (holotype); MACN-Pa Nos. 702/1-4, 703, 704/1-13, 705 (paratypes); IPCAS No. C-836/1 (paratypes).

Reference. Franzese and Ivanov (2020b).

## Rhinebothriidea gen. sp.

Hosts. Psammobatis sp. 1, Psammobatis sp. 2, Psammobatis sp. 3 (Rajiformes: Arhynchobatidae).

Locality. Malvinas Islands, Southwestern Atlantic Ocean.
References. Beer et al. (2019).
Comments. These cestode specimens were studied by Beer et al. (2019) at a molecular rather than morphological level.

## Order "Tetraphyllidea" van Beneden, 1850

Clade 2
Genus Anthobothrium van Beneden, 1850

Anthobothrium galeorhini Suriano, 2002
Type host. Galeorhinus galeus (Linnaeus) (Carcharhiniformes: Triakidae).
Type locality. Puerto Madryn ( $42^{\circ} 43^{\prime} \mathrm{S}, 65^{\circ} 00^{\prime} \mathrm{W}$ ), Chubut Province, Argentina. Specimens in collections. MLP No. 4942 (holotype); MNHN No. 37G (paratype). Reference. Suriano (2002).

## Anthobothrium sp.

Host. Bathyraja arctowskii (Rajiformes: Arhynchobatidae) (Bathyraja sp. 2).
Locality. Drake Strait near King George Island and environs of Elephant Island, Antarctica.

Reference. Wojciechowska (1991a, b).
Comments. Specimens remain in Wojciechowska's personal collection. Regarding host identification, Stehmann et al. (2021) assigned specimens of Bathyraja sp. 2 to Bathyraja arctowskii, a wide-ranging, circum-Antarctic species locally common in the Atlantic sector of the Southern Ocean.

Family Calliobothriidae Perrier, 1897
Genus Calliobothrium van Beneden, 1850
Calliobothrium australis Ostrowski de Núñez, 1973

Type host. Mustelus schmitti (Carcharhiniformes: Triakidae).
Type locality. Mar del Plata, Buenos Aires Province, Argentina.
Other locality. Puerto Quequén ( $38^{\circ} 32^{\prime}$ S, $58^{\circ} 42^{\prime} \mathrm{W}$ ), Provincia de Buenos Aires.
Specimens in collections. MACN No. 409/1 (holotype); MACN Nos. 405/1-4 (vouchers); USNPC No. 92398 (voucher).

GenBank Acc. Nos. KP128030, KP128031.
References. Ostrowski de Núñez (1973), Ivanov and Brooks (2002), Alarcos et al. (2006).

Comments. Ivanov and Brooks (2002) redescribed C. australis based on the material studied originally by Ostrowski de Núñez (1973), who considered this species a subspecies of $C$. verticillatum.

## Genus Symcallio Bernot, Caira \& Pickering, 2015

Symcallio barbarae (Ivanov \& Brooks, 2002)
Calliobothrium barbarae Ivanov \& Brooks, 2002. Syn.
Type host. Mustelus schmitti (Carcharhiniformes: Triakidae).
Type locality. Puerto Quequén ( $38^{\circ} 322^{\prime} \mathrm{S}, 58^{\circ} 42^{\prime} \mathrm{W}$ ), Buenos Aires Province, Argentina.

Other locality. Mar del Plata ( $38^{\circ} 00^{\prime} \mathrm{S}, 57^{\circ} 33^{\prime} \mathrm{W}$ ), Buenos Aires Province.
Specimens in collections. MACN No. 410/1 (holotype); MACN No. 410/2 (paratypes); USNPC No. 92399 (paratypes).

GenBank Acc. Nos. KP128023.
References. Ivanov and Brooks (2002), Alarcos et al. (2006), Bernot et al. (2015).
Comments. Specimens of Calliobothrium eschrichti van Beneden, 1850, identified by Ostrowski de Núñez (1973), were considered by Ivanov and Brooks (2002) as C. barbarae. Later, Bernot et al. (2015) transferred C. barbarae to the new genus Symcallio.

Symcallio lunae (Ivanov \& Brooks, 2002)

Calliobothrium lintoni Euzet, 1954, Calliobothrium lunae Ivanov \& Brooks, 2002. Syns.
Type host. Mustelus schmitti (Carcharhiniformes: Triakidae).
Type locality. La Paloma ( $34^{\circ} 40^{\prime} \mathrm{S}, 54^{\circ} 10^{\prime} \mathrm{W}$ ), Rocha, Uruguay.
Other locality. Mar del Plata ( $38^{\circ} 00^{\prime} \mathrm{S}, 57^{\circ} 33^{\prime} \mathrm{W}$ ), Buenos Aires Province.
Specimens in collections. MACN No. 411/1 (holotype); MACN Nos. 411/2-5 (paratypes); USNPC No. 92400 (paratypes).

References. Ivanov and Brooks (2002), Alarcos et al. (2006), Bernot et al. (2015).

## Clade 4 <br> Genus Caulobothrium Baer, 1948

Caulobothrium ostrowskiae Brooks, Mayes \& Thorson, 1981

Type host. Myliobatis goodei (Myliobatiformes: Myliobatidae).
Type locality. Río de La Plata estuary, near Montevideo, Uruguay.
Specimens in collections. USNM No. 75726 (holotype); USNM No. 75727
(paratype), Univ. Nebraska State Museum No. 21004 (paratype).
Reference. Brooks et al. (1981).

## Caulobothrium uruguayense Brooks, Mayes \& Thorson, 1981

Type host. Probably Myliobatis goodei (Myliobatiformes: Myliobatidae) (Myliobatis uruguayensis).

Type locality. Río de la Plata estuary, Uruguay.
Specimens in collections. USNM No. 75724 (holotype); USNM No. 75725 (paratype); Univ. Nebraska State Museum No. 21002.

Reference. Brooks et al. (1981).
Comments. Caulobothrium uruguayense was originally described by Brooks et al. (1981) from Myliobatis uruguayensis. However, this batoid's name is invalid. Considering original article's title, the type host of this cestode species is probably M. goodei.

## Order Trypanoryncha Diesing, 1863

Suborder Trypanobatoida Olson, Caira, Jensen, Overstreet, Palm \& Beveridge, 2010
Superfamily Eutetrarhynchoidea Guiart, 1927
Genus Dollfusiella Campbell \& Beveridge, 1994

## Dollfusiella acuta Menoret \& Ivanov, 2015

Type host. Sympterygia acuta Garman (Rajiformes: Arhynchobatidae).
Other hosts. Atlantoraja castelnaui, Atlantoraja platana, Sympterygia bonapartii (Rajiformes: Arhynchobatidae).

Type locality. Off Punta Mejillón ( $41^{\circ} 11^{\prime} \mathrm{S}, 64^{\circ} 03^{\prime} \mathrm{W}$ ), Río Negro Province, Argentina.
Other localities. off Puerto Quequén ( $38^{\circ} 37^{\prime} \mathrm{S}, 58^{\circ} 53^{\prime} \mathrm{W}$ ), off Río Colorado (3955'S, $\left.62^{\circ} 03^{\prime} \mathrm{W}\right)$, Bahía Blanca, Buenos Aires Province. San Matías Gulf, Río Negro/Chubut Provinces.

Specimens in collections. MACN-Pa No. 575/1 (holotype); MACN-Pa Nos. 575/2-4 (paratypes); IPCAS No. C-700 (paratypes).

References. Menoret and Ivanov (2015), Irigoitia et al. (2017).

## Dollfusiella taminii Menoret \& Ivanov, 2014

Type host. Psammobatis bergi Marini (Rajiformes: Arhynchobatidae).
Type locality. Puerto Quequén ( $38^{\circ} 37^{\prime} \mathrm{S}$, $58^{\circ} 53^{\prime} \mathrm{W}$ ), Buenos Aires Province, Argentina.

Other locality. off Necochea ( $38^{\circ} 46^{\prime}$ S, $57^{\circ} 56^{\prime} \mathrm{W}$ ), Buenos Aires Province.
Specimens in collections. MACN-Pa No. 544/1 (holotype); MACN-Pa Nos. 544/2-4 (paratypes); IPCAS No. C-661 (paratypes).

Reference. Menoret and Ivanov (2014).

## Dollfusiella vooremi (São Clemente \& Gomes, 1989)

Eutetranychus vooremi São Clemente \& Gomes, 1989. Syn.
Type host. Mustelus canis (Mitchill) (Carcharhiniformes: Triakidae).
Other hosts. Mustelus schmitti (Carcharhiniformes: Triakidae).
Type locality. Southern Brazilian coast ( $30^{\circ} 40^{\prime} \mathrm{S}, 53^{\circ} 20^{\prime} \mathrm{W}-50^{\circ} 40^{\prime} \mathrm{W}$ ).
Other localities. Off San Antonio Oeste ( $40^{\circ} 50^{\prime} \mathrm{S}, 64^{\circ} 58^{\prime} \mathrm{W}$ ), Río Negro Province. Off Mar del Plata ( $38^{\circ} 00^{\prime} \mathrm{S}, 57^{\circ} 33^{\prime} \mathrm{W}$ ), Buenos Aires Province.

Specimens in collections. CHIOC No. 32.566e (holotype); CHIOC Nos. 32.566a-d (paratypes); MACN-Pa Nos. 543/1-2 (vouchers).

References. São Clemente and Gomes (1989), Tanzola et al. (1998), Alarcos et al. (2006), Menoret and Ivanov (2014).

Genus Mecistobothrium Heinz \& Dailey, 1974
Mecistobothrium oblongum Menoret \& Ivanov, 2015
Type host. Myliobatis goodei (Myliobatiformes: Myliobatidae).
Typelocality. OffPuntaMejillón ( $41^{\circ} 11^{\prime} \mathrm{S}, 64^{\circ} 03^{\prime} \mathrm{W}$ ), RíoNegroProvince, Argentina.
Specimens in collections. MACN-Pa No. 576/1 (holotype); MACN-Pa Nos. 576/2-3 (paratypes).

Reference. Menoret and Ivanov (2015).

Genus Parachristianella Dollfus, 1946

## Parachristianella damiani Menoret \& Ivanov, 2014

Type host. Myliobatis goodei (Myliobatiformes: Myliobatidae).
Type locality. Playa Punta Negra ( $38^{\circ} 36^{\prime} \mathrm{S}, 58^{\circ} 48^{\prime} \mathrm{W}$ ), Necochea, Buenos Aires Province, Argentina.

Specimens in collections. MACN-Pa No. 545/1 (holotype); MACN-Pa No. 545/2 (paratypes), IPCAS No. C-660 (paratypes).

Reference. Menoret and Ivanov (2014).

Superfamily Tentacularoidea Poche, 1926
Genus Heteronybelinia Palm, 1999
Heteronybelinia mattisi Menoret \& Ivanov, 2012

Type host. Sympterygia bonapartii (Rajiformes: Arhynchobatidae).
Typelocality. Puerto Quequén ( $38^{\circ} 37^{\prime} \mathrm{S}, 58^{\circ} 53^{\prime} \mathrm{W}$ ), BuenosAiresProvince, Argentina.
Specimens in collections. MACN-Pa No. 537/1 (holotype); MACN-Pa Nos. 537/2-4 (paratypes); NHMUK Nos. 2012.9.11.1-2 (paratypes).

Reference. Menoret and Ivanov (2012a).
Comments. Larval stages (plerocercoids) of H. mattisi were reported from teleosts from coastal waters off Buenos Aires Province (Menoret and Ivanov 2012a).

## Suborder Trypanoselachoida Olson, Caira, Jensen, Overstreet, Palm \& Beveridge, 2010

Superfamily Lacistorhynchoidea Guiart, 1927
Genus Grillotia Guiart, 1927
Grillotia (Christianella) carvajalregorum Menoret \& Ivanov, 2009

Progrillotia dollfusi Carvajal \& Rego, 1983, Grillotia (Progrillotia) dollfusi (Carvajal \& Rego, 1983) Palm 2004, Grillotia carvajalregorum Menoret \& Ivanov, 2009. Syns.

Type host. Cynoscion striatus (Cuvier) (Perciformes: Sciaenidae).
Other host. Squatina guggenheim (Squatiniformes: Squatinidae).
Type locality. Coast of Brazil.
Other locality. Puerto Quequén ( $38^{\circ} 37^{\prime} \mathrm{S}$, $58^{\circ} 53^{\prime} \mathrm{W}$ ), Buenos Aires Province.
Specimens in collections. CHIOC No. 32.018a (holotype); CHIOC Nos. 32.018b-d (paratypes); MACN-Pa Nos. 487/1-2 (vouchers).

References. Carvajal and Rego (1983), Menoret and Ivanov (2009, 2012b), Beveridge and Campbell (2010).

Comments. Grillotia (C.) carvajalregorum was originally described from plerocercoids from C. striatus at coasts off Brazil (Carvajal and Rego 1983). Later, Menoret and Ivanov (2009) described adults of G. (C.) carvajalregorum from S. guggenheim at coasts of Argentina. This species was found in a wide range of teleost fishes (Menoret and Ivanov 2009, 2012b).

## Grillotia (Grillotia) patagonica Menoret \& Ivanov, 2012

Type host. Psammobatis rudis (Rajiformes: Arhynchobatidae).
Other host. Sympterygia bonapartii (Rajiformes: Arhynchobatidae).
Type locality. Off Puerto San Julián ( $48^{\circ} 59^{\prime} \mathrm{S}, 65^{\circ} 15^{\prime} \mathrm{W}$ ), Santa Cruz Province, Argentina.

Other locality. San Jorge Gulf, Santa Cruz Province.
Specimens in collections. MACN-Pa No. 534/1 (holotype); MACN-Pa Nos. 534/2-4 (paratypes).

References. Menoret and Ivanov (2012b), Irigoitia et al. (2017).
Comments. Grillotia (G.) patagonica was originally described from adults and plerocercoids caught at different localities along the Patagonian Shelf of Argentina (Menoret and Ivanov 2012b). Other reports in the area include this cestode in S. bonapartii at San Jorge Gulf (Irigoitia et al. 2017).

## Grillotia sp.

Hosts. Amblyraja doellojuradoi, Bathyraja brachyurops, B. cousseauae, B. griseocauda, Bathyraja magellanica, Dipturus chilensis, Psammobatis sp. 2, Psammobatis sp. 3.

Locality. Malvinas Islands Shelf, Southwestern Atlantic Ocean.
Reference. Beer et al. (2019).
Comments. These specimens were studied by Beer et al. (2019) at a molecular rather than a morphological level, without reaching a specific identification. Beer et al. (2019) noted the presence of Grillotia sp. parasitizing D. chilensis. However, the distribution of $D$. chilesis is restricted to the Pacific Ocean. Therefore, this record was based on a misidentified host (Concha et al. 2019).

Species incertae sedis (at the ordinal level)

## Phyllobothrium myliobatidis Brooks, Mayes \& Thorson, 1981

Type host. Myliobatis goodei (Myliobatiformes: Myliobatidae).
Type locality. Río de la Plata estuary, Uruguay.
Specimens in collections. USNM No. 1371266 (holotype); USNM No. 1371267 (paratype).

Reference. Brooks et al. (1981).
Comments. Ruhnke (2011) considers P. myliobatidis as a possible member of the order Rhinebothriidea.

## Host-parasite checklist: chimaeras and batoids

## Order Chimaeriformes

Family Callorhinchidae
Callorhinchus callorynchus
Gyrocotyle maxima (Gyrocotylidea)
Gyrocotyle rugosa (Gyrocotylidea)

## Order Myliobatiformes

Family Myliobatidae
Myliobatis goodei
Aberrapex arrhynchum (Lecanicephalidea)
Aberrapex ludmilae (Lecanicephalidea)
Aberrapex sanmartini (Lecanicephalidea)
Acanthobothrium sp. (Onchoproteocephalidea)
Caulobothrium ostrowskiae ("Tetraphyllidea")
Caulobothrium uruguayense ("Tetraphyllidea")
Halysioncum megacanthum (Diphyllidea)
Mecistobothrium oblongum (Trypanorhyncha)
Parachristianella damiani (Trypanorhyncha)Phyllobothrium myliobatidis (Incertae sedis)Phyllobothrium sp. (Phyllobothriidea)
Myliobatis ridens
Aberrapex vitalemuttiorum (Lecanicephalidea)
Order Rajiformes
Family Arhynchobatidae
Atlantoraja castelnaui
Acanthobothrium marplatensis (Onchoproteocephalidea)
Dollfusiella acuta (Trypanorhyncha)
Notomegarhynchus navonae (Rhinebothriidea)
Atlantoraja platana
Dollfusiella acuta (Trypanorhyncha)
Scalithrium ivanovae (Rhinebothriidea)
Bathyraja albomaculata
Echeneibothrium sp. (Rhinebothriidea)
Rockacestus conchai (Phyllobothriidea)
Phyllobothriidea gen. sp. (Phyllobothriidea)
Bathyraja arctowskii
Anthobothrium sp. ("Tetraphyllidea")
Rockacestus arctowskii (Phyllobothriidea)
Bathyraja brachyurops
Echeneibothrium sp. (Rhinebothriidea)
Grillotia sp. (Trypanorhyncha)
Guidus argentinense (Phyllobothriidea)
Phyllobothriidea gen. sp. (Phyllobothriidea)
Bathyraja cousseauae
Acanthobothrium sp. (Onchoproteocephalidea)
Echeneibothrium cristinae (Rhinebothriidea)
Echeneibothrium sp. (Rhinebothriidea)
Grillotia sp. (Trypanorhyncha)
Phyllobothriidea gen. sp. (Phyllobothriidea)
Bathyraja eatonii
Guidus antarcticus (Phyllobothriidea)
Notomegarhynchus shetlandicum (Rhinebothriidea)
Onchobothrium antarcticum (Onchoproteocephalidea)
Pseudanthobothrium minutum (Rhinebothriidea)
Rockacestus siedleckii (Phyllobothriidea)
Bathyraja griseocauda
Echeneibothrium sp. (Rhinebothriidea)
Grillotia sp. (Trypanorhyncha)
Bathyraja maccaini
Guidus antarcticus (Phyllobothriidea)

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    Notomegarhynchus shetlandicum (Rhinebothriidea)
    Rockacestus rakusai (Phyllobothriidea)
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## Bathyraja macloviana

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Echeneibothrium sp. (Rhinebothriidea)
Phyllobothriidea gen. sp. (Phyllobothriidea)
Bathyraja magellanica
Acanthobothrium carolinae (Onchoproteocephalidea)
Acanthobothrium sp. (Onchoproteocephalidea)
Grillotia sp. (Trypanorhyncha)
Guidus francoi (Phyllobothriidea)
Guidus magellanicus (Phyllobothriidea)
Phyllobothriidea gen. sp. (Phyllobothriidea)
Bathyraja multispinis
Echeneibothrium sp. (Rhinebothriidea)
Guidus sp. (Phyllobothriidea)
Phyllobothriidea gen. sp. (Phyllobothriidea)
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## Bathyraja scaphiops

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Echeneibothrium sp. (Rhinebothriidea)
Phyllobothriidea gen. sp. (Phyllobothriidea)
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## Psammobatis bergi

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Dollfusiella taminii (Trypanorhyncha)
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## Psammobatis normani

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Semiorbiseptum alfredoi (Rhinebothriidea)
Semiorbiseptum mariae (Rhinebothriidea)
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## Psammobatis rudis

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Grillotia (G.) patagonica (Trypanorhyncha)
Semiorbiseptum mariae (Rhinebothriidea)
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## Psammobatis sp. 1

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Rhinebothriidea gen. sp. (Rhinebothriidea)
Psammobatis sp. 2
Grillotia sp. (Trypanorhyncha)
Phyllobothriidea gen. sp. (Phyllobothriidea)
Rhinebothriidea gen. sp. (Rhinebothriidea)
Psammobatis sp. 3
Grillotia sp. (Trypanorhyncha)
Phyllobothriidea gen. sp. (Phyllobothriidea)
Rhinebothriidea gen. sp. (Rhinebothriidea)
Rioraja agassizii
Scalithrium kirchneri (Rhinebothriidea)
Sympterygia acuta
Dollfusiella acuta (Trypanorhyncha)
Sympterygia bonapartii
Dollfusiella acuta (Trypanorhyncha)
Grillotia (G.) patagonica (Trypanorhyncha)
```Heteronybelinia mattisi (Trypanorhyncha)Phyllobothrium sp. (Phyllobothriidea)Rhinebothrium chilensis (Rhinebothriidea)
Family Rajidae
Amblyraja doellojuradoi
Grillotia sp. (Trypanorhyncha)
Phyllobothriidea gen. sp. (Phyllobothriidea)
Pseudanthobothrium sp. (Rhinebothriidea)
Amblyraja georgiana
Echinobothrium acanthocolle (Diphyllidea)Pseudanthobothrium notogeorgianum (Rhinebothriidea)Rockacestus georgiensis (Phyllobothriidea)
Dipturus brevicaudatus
Acanthobothrium domingae (Onchoproteocephalidea)
Echeneibothrium multiloculatum (Rhinebothriidea)
Echeneibothrium williamsi (Rhinebothriidea)
Order Rhinopristiformes
Family Trygonorrhinidae
Zapteryx brevirostris
Acanthobothrium zapterycum (Onchoproteocephalidea)
Acanthobothrium sp. (Onchoproteocephalidea)
Halysioncum pigmentatum (Diphyllidea)
Phyllobothrium sp. (Phyllobothriidea)
Order Torpediniformes
Family Narcinidae
Discopyge tschudii
Acanthobothrium stefaniae (Onchoproteocephalidea)
Host-parasite checklist: sharks
Order CarcharhiniformesFamily CarcharhinidaeCarcharbinus brachyurus
Cathetocephalus australis (Cathetocephalidea)
Family Triakidae
Galeorbinus galeus
Anthobothrium galeorhini ("Tetraphyllidea")
Mustelus fasciatus
Orygmatobothrium juani (Phyllobothriidea)
Mustelus schmitti
Calliobothrium australis ("Tetraphyllidea")
Coronocestus notoguidoi (Diphyllidea)
Dollfusiella vooremi (Trypanorhyncha)

Orygmatobothrium schmitti (Phyllobothriidea)
Symcallio barbarae ("Tetraphyllidea")
Symcallio lunae ("Tetraphyllidea")

\section*{Order Hexanchiformes}

Family Hexanchidae
Notorynchus cepedianus
Crossobothrium antonioi (Phyllobothriidea)
Crossobothrium pequeae (Phyllobothriidea)

\section*{Order Squatiniformes}

Family Squatinidae
Squatina guggenheim
Grillotia (C.) carvajalregorum (Trypanorhyncha)
Paraberrapex atlanticus (Lecanicephalidea)

\section*{Geographical distribution of the cestode orders}

The tapeworm orders reviewed in this study show different geographical ranges. These distributions are represented in Fig. 1A for the Phyllobothriidea, Fig. 1B for the Onchoproteocephalidea, Fig. 2A for the Rhinebothriidea, Fig. 2B for "Tetraphyllidea" and Gyrocotylidea, Fig. 3A for the Diphyllidea and Lecanicephalidea, and Fig. 3B for Trypanorhyncha. Geographical sites of the order Cathetocephalidea could not be rep-


Figure I. Distribution of representatives of the orders Phyllobothriidea and Onchoproteocephalidea A order Phyllobothriidea B order Onchoproteocephalidea. Insets show records in the sub-Antarctic and Antarctic regions.


Figure 2. Distribution of representatives of the orders Rhinebothriidea, "Tetraphyllidea" and Gyrocotylidea A order Rhinebothriidea B red dot Orders "Tetraphyllidea" and black star Gyrocotylidea. Insets show records in the sub-Antarctic and Antarctic regions.
resented since the only existing record reports Cathetocephalus australis in Argentina, without specifying the locality or coordinate.

The orders with the broadest geographic distributions are Onchoproteocephalidea (Fig. 1B) and Rhinebothriidea (Fig. 2A), with representatives in the Río de la Plata estuary, along the Argentine Sea, and the southern islands. In addition, the phyllobothriideans (Fig. 1A) show a similar distribution, although without records between the \(40^{\circ} \mathrm{S}-47^{\circ} \mathrm{S}\) latitudes, in the central region of the Argentine Sea. On the other hand, the cestodes with the narrowest distribution are those of the order Gyrocotylidea (Fig. 2B), being recorded only in two locations in the Buenos Aires Province.

\section*{Discussion}

\section*{Cestode diversity}

Cestodes as parasites of chondrichthyans have been mostly recorded in the Northern Hemisphere (Caira et al. 2022). However, reports in southern latitudes have remarkably increased in the last decades due to focused sampling efforts in the area (Menoret and Ivanov 2012a, b, 2014, 2015, 2021; Pickering and Caira 2012; Caira et al. 2013a; Abbott and Caira 2014; Mutti and Ivanov 2016; Menoret et al. 2017; Franzese and


Figure 3. Distribution of the orders Diphyllidea, Lecanicephalidea and Trypanorhyncha \(\mathbf{A}\) black star Diphyllidea and red dot Lecanicephalidea B order Trypanorhyncha. Inset shows records in the sub-Antarctic region.

Ivanov 2018, 2020a, b, 2021; Palm et al. 2019; Oosthuizen et al. 2021; Franzese et al. 2022; Van Der Spuy et al. 2022). The present annotated checklist comprises 57 valid cestode species of 28 genera in nine orders, registered in the Río de la Plata estuary, Southwestern Atlantic off Argentina and the surrounding waters off Antarctica. The orders Phyllobothriidea and Rhinebothriidea show the highest richness at the specific level, with 13 and 12 valid species, respectively; they are followed by the order Trypanorhyncha, with a total of eight species. In addition, the list includes cestodes without an identification up to the generic or the specific level, as in the case of the Onchoproteocephalidea (i.e., Acanthobothrium sp.), Phyllobothriidea (i.e., Genus sp., Guidus sp., Phyllobothrium sp.), Rhinebothriidea (i.e., Genus sp., Echeneibothrium sp., Pseudanthobothrium sp.) and Trypanorhyncha (i.e., Grillotia sp.) (Ostrowski de Núñez 1971; Brooks et al. 1981; Beer et al. 2019). Several of the comments made in this work are intended to aid in future morphological and molecular studies addressing the lower taxonomic resolution of these entities.

In view of the high degree of specificity of adult cestodes to their marine hosts (Reyda and Marques 2011; Caira and Jensen 2017) and that only \(33 \%\) (33/100) of marine chondrichthyans in the study area have been sampled for cestodes (Table 1),
this fauna is probably underestimated. We can speculate that more than 60 species of cestodes have not yet been discovered in this area. Future taxonomic surveys will be essential to increase the knowledge of the diversity of these parasites in the region.

\section*{Taxonomic resolution}

The poor taxonomic resolution of several taxa listed in the present study is probably a consequence of the lack of use of multiple tools to develop an integrative taxonomy, such as morphological and molecular studies used as evidence to delineate species boundaries. Some of the previous works cited here lacked modern morphological tools, e.g., scanning (SEM) and transmission electron microscopy (TEM) and the molecular tools necessary for the development of an integrative approach (Ostrowski de Núnez 1971; Brooks et al. 1981). In contrast, the recent work by Beer et al. (2019) recorded in the Argentine Sea numerous specimens of cestodes belonging to different orders but without achieving a specific identification for many of them, using molecular sequences as the only identification tool. The development and use of molecular tools have allowed the detection of cryptic species in some cestode groups (Scholz et al. 2014; Choudhury and Scholz 2020). Of the 57 valid species recorded in this work, only seven have been sequenced, so it is still unknown whether cryptic species will be discovered in this particular region. In addition to molecular sequences, the use of modern morphological tools, such as SEM and TEM, might be helpful in discovering new characters that complement traditional morphological studies, which could contribute to solve species identification problems (Franzese et al. 2023; Mutti et al. 2023). The development of the integrative taxonomy, including the use of all available tools, will allow resolving the poor taxonomic resolution observed in several taxa registered in our study area.

\section*{Host association}

Rajiform batoids represent the most frequent hosts for adult cestodes in the study area. In particular, the family Arhynchobatidae has been found parasitized by \(42 \%(24 / 57)\) of the recorded cestode species (Ivanov and Campbell 1998b, 2002; Rocka 2003; Menoret and Ivanov 2012a, 2014, 2021; Irigoitia et al. 2017; Franzese and Ivanov 2020a, b; Caira et al. 2021; Franzese et al. 2022). This percentage could be higher since many species of arhynchobatids of the region, such as Atlantoraja cyclophora (Regan), Bathyraja meridionalis Stehmann, Bathyraja papilionifera Stehmann, Bathyraja schroederi (Krefft), Psammobatis extenta (Garman), Psammobatis lentiginosa McEachran, Psammobatis parvacauda McEachran, Psammobatis rutrum Jordan, and Psammobatis scobina (Philippi), have not yet been sampled for cestodes. The association between tapeworms and this host family is not surprising if we consider that arhynchobatids have the highest species number, with \(31 \%(31 / 100)\) of the chondrichthyan fauna recorded in the area (Table 1) (Menni and Lucifora 2007; Froese and Pauly 2022). Upcoming studies should focus on sampling arhyncobatids that have not yet been reported as hosts for tapeworms.

Considering that the major number of cestode species from this checklist are hosted by the myliobatiform Myliobatis goodei (Brooks et al. 1981; Ivanov and Campbell 1998;; Menoret and Ivanov 2014, 2015; Menoret et al. 2017), it would be interesting to sample M. freminvillei Lesueur, the only species of myliobatid that has not been yet examined for cestodes in the region. On the other hand, only \(13 \%(6 / 45)\) of the species of sharks have been reported as hosts in this area (Table 1). Host species with a relatively low occurrence or a particular bathymetric distribution are likely to host an undiscovered and exciting cestode fauna.

More collecting efforts are necessary to conclude if this data reflects the actual biodiversity of cestodes in the different groups of chondrichthyans or is a result of a bias in sampling. Although this list shows the substantial advances in taxonomical surveys in the last decades, expanding the number of sampled hosts is essential to increase the knowledge of the current cestode fauna of chondrichthyans in the region.

\section*{Studied area and newly collected material}

Five species of cestodes have been recorded in new localities of the Southwestern Atlantic Ocean (Table 3). New material (voucher) identified, processed, and deposited in the MACN parasitological collection corresponds to three onchoproteocephalideans (i.e., Acanthobothrium domingae, \(A\). marplatensis, \(A\). stefaniae) and two rhinebotriideans (i.e., Echeneibothrium williamsi, Notomegarhynchus navonae). One of these records has extended until the Buenos Aires Province the northern limit of the known geographic distribution in the Argentine Sea of E. Williamsi, which, prior to this work, ranged from Santa Cruz Province to Tierra del Fuego Province (Franzese et al. 2022). The remaining new records have added new localities within the province of Buenos Aires for \(A\). domingae, \(A\). marplatensis, \(A\). stefaniae, and \(N\). navonae. Previously, these four species had been reported off Buenos Aires, although in different locations (Ivanov and Campbell 1998b, 2002; Franzese and Ivanov 2018, 2020a).

Several of the original descriptions of cestode species are based on material collected from a single locality. However, this probably reflects the absence of a more exhaustive sampling. The present checklist shows that about half of the species included in this region have additional localities. Among these, Rhinebothrium chilensis and Echeneibothrium williamsi show the highest number with 7 and 6 localities, respectively (Tanzola et al. 1998; Irigoitia et al. 2017). It is likely that as the intensity of sampling increases, new localities will be discovered for several known cestode species.

The localities with the most significant number of cestodes species are Puerto Quequén and Mar del Plata, with 17 and 11 species reported to date, respectively. A strong sampling effort could explain these numbers since both sites are commercial ports from the Buenos Aires Province close to the facilities of the main Argentinean research taxonomic cestodes groups (Luque and Poulin 2007; Randhawa and Poulin 2019).

\section*{Conclusions}

Some difficulties concerning the understanding of chondrichthyan cestode diversity are:
1) many works have a poor taxonomic resolution or are outdated, with incomplete drawings and without the use of modern tools such as transmission electron microscopy, scanning electron microscopy and molecular approaches;
2) the existence of cryptic species underestimates the actual number of cestodes;
3) less than half of the marine chondrichthyans have been examined for cestodes in the area covered in this work.

A modern taxonomic approach for future characterizations should be made by combining descriptive tools (e.g., TEM and SEM, molecular data, histological sections, and histochemical techniques). It would also be desirable that all the voucher material could be available in public parasitological collections to facilitate its study to the entire community of taxonomists. Regarding sampling effort, it is likely that the higher the number of chondrichthyans examined in parasitological surveys, the higher the number of parasite-host associations will be identified. We have critically compiled as much detailed information as possible including valuable comments, providing a complete list of references and information from the deposited material. We hope this list may help future studies and contributes to correctly estimating the cestode biodiversity that inhabits this underexplored region of the Southern Hemisphere.

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\section*{References}

Abbott LM, Caira JN (2014) Morphology meets molecules: A new genus and two new species of diphyllidean cestodes from the yellowspotted skate, Leucoraja wallacei, from South Africa. The Journal of Parasitology 100(3): 323-330. https://doi.org/10.1645/13-414.1

Alarcos AJ, Ivanov VA, Sardella NH (2006) Distribution patterns and interactions of cestodes in the spiral intestine of the narrownose smooth-hound shark, Mustelus schmitti Springer, 1939 (Chondrichthyes, Carcharhiniformes). Acta Parasitologica 51(2): 100-106. https:// doi.org/10.2478/s1 1686-006-0015-7
Alves PV, de Chambrier A, Scholz T, Luque JL (2017) Annotated checklist of fish cestodes from South America. ZooKeys 650: 1-205. https://doi.org/10.3897/zookeys.650.10982
Barčák D, Fan CK, Sonko P, Kuchta R, Scholz T, Orosová M, Chen HW, Oros M (2021) Hidden diversity of the most basal tapeworms (Cestoda, Gyrocotylidea), the enigmatic parasites of holocephalans (Chimaeriformes). Scientific Reports 11(1): 1-11. https://doi. org/10.1038/s41598-021-84613-y
Beer A, Ingram T, Randhawa HS (2019) Role of ecology and phylogeny in determining tapeworm assemblages in skates (Rajiformes). Journal of Helminthology 93(6): 738-751. https://doi.org/10.1017/S0022149X18000809
Bernot JP, Caira JN, Pickering M (2015) The dismantling of Calliobothrium (Cestoda: Tetraphyllidea) with erection of Symcallio n. gen. and description of two new species. The Journal of Parasitology 101(2): 167-181. https://doi.org/10.1645/14-571.1
Beveridge I, Campbell RA (2010) Validation of Christianella Guiart, 1931 (Cestoda: Trypanorhyncha) and its taxonomic relationship with Grillotia Guiart, 1927. Systematic Parasitology 76(2): 111-129. https://doi.org/10.1007/s11230-010-9239-x
Bigatti G, Signorelli J (2018) Marine invertebrate biodiversity from the Argentine Sea, South Western Atlantic. ZooKeys 791: 47-70. https://doi.org/10.3897/zookeys.791.22587
Brooks DR, Mayes MA, Thorson TB (1981) Cestode parasites in Myliobatis goodei Garman (Myliobatiformes: Myliobatidae) from Río de la Plata, Uruguay, with a summary of cestodes collected from South American elasmobranchs during 1975-1979. Proceedings of the Biological Society of Washington 93: 1239-1252.
Caira JN, Jensen K (2014) A digest of elasmobranch tapeworms. The Journal of Parasitology 100(4): 373-391. https://doi.org/10.1645/14-516.1
Caira JN, Jensen K (2017) Planetary biodiversity inventory (2008-2017): Tapeworms from vertebrate bowels of the earth. Natural History Museum, University of Kansas, Kansas, 463 pp.
Caira JN, Pickering M, Schulman AD, Hanessian IV NJ (2013a) Two new species of Echinobothrium (Cestoda: Diphyllidea) from batoids off South Africa. Comparative Parasitology 80(1): 22-32. https://doi.org/10.1654/4598.1
Caira JN, Marques FPL, Jensen K, Kuchta R, Ivanov V (2013b) Phylogenetic analysis and reconfiguration of genera in the cestode order Diphyllidea. International Journal for Parasitology 43(8): 621-639. https://doi.org/10.1016/j.ijpara.2013.03.001
Caira JN, Bueno V, Jensen K (2021) Emerging global novelty in phyllobothriidean tapeworms (Cestoda: Phyllobothriidea) from sharks and skates (Elasmobranchii). Zoological Journal of the Linnean Society 193(4): 1336-1363. https://doi.org/10.1093/zoolinnean/zlaa185
Caira JN, Jensen K, Barbeau E [Eds] (2022) Global Cestode Database. World Wide Web electronic publication. http://www.tapewormdb.uconn.edu [Accessed 1 December 2022]
Carvajal J, Dailey MD (1975) Three new species of Echeneibothrium (Cestoda: Tetraphyllidea) from the skate, Raja chilensis Guichenot, 1848, with comments on mode of at-
tachment and host specificity. The Journal of Parasitology 61(1): 89-94. https://doi. org/10.2307/3279115
Carvajal J, Rego AA (1983) Progrillotia dollfusi sp. n. (Cestoda: Trypanorhyncha) parasito de pescada do litoral brasileiro. Memorias do Instituto Oswaldo Cruz 78(2): 231-234. https://doi.org/10.1590/S0074-02761983000200012
Choudhury A, Scholz T (2020) Ex uno plures? Morphotype and lineage diversity of Bothriocephalus (Cestoda: Bothriocephalidea) in North American freshwater fishes. The Journal of Parasitology 106(5): 589-602. https://doi.org/10.1645/17-98
Concha FJ, Caira JN, Ebert DA, Pompert JHW (2019) Redescription and taxonomic status of Dipturus chilensis (Guichenot, 1848), and description of Dipturus lamillai sp. nov. (Rajiformes: Rajidae), a new species of long-snout skate from the Falkland Islands. Zootaxa 4590(5): 501-524. https://doi.org/10.11646/zootaxa.4590.5.1
Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM, Harrison LR, Carlson JK, Davidson LNK, Fordham SV, Francis MP, Pollock CM, Simpfendorfer CA, Burgess GH, Carpenter KE, Compagno LJV, Ebert DA, Gibson C, Heupel MR, Livingstone SR, Sanciangco JC, Stevens JD, Valenti S, White WT (2014) Extinction risk and conservation of the world's sharks and rays. eLife 3: e00590. https://doi.org/10.7554/eLife. 00590
Euzet L, Carvajal J (1973) Rhinebothrium (Cestoda, Tetraphyllidea) parasites de raies du genre Psammobatis au Chili. Bulletin du Muséum National d'Histoire Naturelle 101: 779-787.
Franzese S, Ivanov VA (2018) Hyperapolytic species of Acanthobothrium (Cestoda: Onchoproteocephalidea) from batoids off Argentina. Parasitology International 67(4): 431-443. https://doi.org/10.1016/j.parint.2018.04.001
Franzese S, Ivanov VA (2020a) Two new species of Acanthobothrium Blanchard, 1848 (Cestoda: Onchoproteocephalidea) from rajiform batoids off Argentina. Folia Parasitologica 67: 016. https://doi.org/10.14411/fp. 2020.016
Franzese S, Ivanov VA (2020b) A new genus of Rhinebothriidea from species of Psammobatis (Rajiformes: Arhynchobatidae) off Argentina. Zootaxa 4803(2): 355-372. https://doi. org/10.11646/zootaxa.4803.2.7
Franzese S, Ivanov VA (2021) Two new species of Scalithrium (Cestoda: Rhinebothriidea) from rajiform batoids of the Argentine Sea. Zootaxa 5005(1): 62-76. https://doi.org/10.11646/ zootaxa.5005.1.4
Franzese S, Mutti LD, Tropea C, Ivanov VA (2022) Morphological study of members of the genus Echeneibothrium (Cestoda: Rhinebothriidea: Echeneibothriidae) from rajiform skates of the Argentine Sea and analysis of the phylogenetic relationships within the family Echeneibothriidae. Zoologischer Anzeiger 299: 1-20. https://doi.org/10.1016/j. jcz.2022.05.002
Franzese S, Mutti LD, Battista AG, Ivanov VA (2023) Morphology and glandular composition of the myzorhynchus and the remnant apical organ in adult cestodes of the order Rhinebothriidea from batoids off Argentina. Journal of Morphology 284(4): e21573. https://doi. org/10.1002/jmor. 21573
Froese R, Pauly D [Eds] (2022) FishBase. World Wide Web electronic publication. http://www. fishbase.org [Accessed 30 November 2022]

Gabbanelli V, Diaz de Astarloa JM, González-Castro M, Vazquez DM, Mabragaña E (2018) Almost a century of oblivion: Integrative taxonomy allows the resurrection of the longnose skate Zearaja brevicaudata (Marini, 1933) (Rajiformes; Rajidae). Comptes Rendus Biologies 341(9-10): 454-470. https://doi.org/10.1016/j.crvi.2018.10.002
Irigoitia MM, Incorvaia IS, Timi JT (2017) Evaluating the usefulness of natural tags for host population structure in chondrichthyans: Parasite assemblages of Sympterygia bonapartii (Rajiformes: Arhynchobatidae) in the Southwestern Atlantic. Fisheries Research 195: 8090. https://doi.org/10.1016/j.fishres.2017.07.006

Irigoitia MM, Levy E, Canel D, Timi JT (2022) Parasites as tags for stock identification of a highly exploited vulnerable skate Dipturus brevicaudatus (Chondrichthyes: Rajidae) in the south-western Atlantic Ocean, a complementary tool for its conservation. Aquatic Conservation 32(10): 1634-1646. https://doi.org/10.1002/aqc. 3869
Ivanov VA (1997) Echinobothrium notoguidoi n. sp. (Cestoda: Diphyllidea) from Mustelus schmitti (Chondrichthyes: Carcharhiniformes) in the Argentine Sea. The Journal of Parasitology 83(5): 913-916. https://doi.org/10.2307/3284288
Ivanov VA (2006) Guidus n. gen. (Cestoda: Tetraphyllidea), with description of a new species and emendation of the generic diagnosis of Marsupiobothrium. The Journal of Parasitology 92(4): 832-840. https://doi.org/10.1645/GE-767R. 1
Ivanov VA (2008) Orygmatobothrium spp. (Cestoda: Tetraphyllidea) from triakid sharks in Argentina: Redescription of Orygmatobothrium schmitti and description of a new species. The Journal of Parasitology 94(5): 1087-1097. https://doi.org/10.1645/GE-1482.1
Ivanov VA (2009) New species of Crossobothrium (Cestoda: Tetraphyllidea) from the broadnose sevengill shark, Notorynchus cepedianus, in Argentina. The Journal of Parasitology 95(6): 1479-1488. https://doi.org/10.1645/GE-2096.1
Ivanov VA, Brooks DR (2002) Callliobothrium spp. (Eucestoda: Tetraphyllidea: Onchobothriidae) in Mustelus schmitti (Chondrichthyes: Carcharhiniformes) from Argentina and Uruguay. The Journal of Parasitology 88(6): 1200-1213. https://doi.org/10.1645/00223395(2002)088[1200:CSETOI]2.0.CO;2
Ivanov VA, Campbell RA (1998a) Echinobothrium megacanthum sp. n. (Cestoda: Diphyllidea) from the eagle ray Myliobatis goodei (Chondrichthyes: Rajoidei) from the Patagonian shelf of Argentina. Folia Parasitologica 45: 225-229.
Ivanov VA, Campbell RA (1998b) A new species of Acanthobothrium van Beneden, 1849 (Cestoda: Tetraphyllidea) from Rioraja castelnaui (Chondrichthyes: Rajoidei) in coastal waters of Argentina. Systematic Parasitology 40(3): 203-212. https://doi. org/10.1023/A:1006049404646
Ivanov VA, Campbell RA (2002) Notomegarhynchus navonae n. gen. and n. sp. (Eucestoda: Tetraphyllidea), from skates (Rajidae: Arhychobatinae) in the Southern Hemisphere. The Journal of Parasitology 88(2): 340-349. https://doi.org/10.1645/0022-3395(2002)088[0340:NN NGAN]2.0.CO;2
Jensen K (2001) Four new genera and five new species of lecanicephalideans (Cestoda: Lecanicephalidea) from elasmobranchs in the Gulf of California, Mexico. The Journal of Parasitology 87(4): 845-861. https://doi.org/10.1645/0022-3395(2001)087[0845:FNG AFN]2.0.CO;2

Luque JL, Poulin R (2007) Metazoan parasite species richness in Neotropical fishes: Hotspots and the geography of biodiversity. Parasitology 134(6): 865-878. https://doi.org/10.1017/ S0031182007002272
MacDonagh J (1927) Parasitos de peces comestibles III. Dos cestodarios: Gyrocotyle rugosa del "Pez gallo" y Gyrocotyle maxima n. sp. del "Gatuso". La Semana Médica Buenos Aires 34: 1232-1235.
Menni RC, Lucifora LO (2007) Condrictios de la Argentina y Uruguay: lista de trabajo. Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, 15 pp.
Menoret A, Ivanov VA (2009) New name for Progrillotia dollfusi Carvajal et Rego, 1983 (Cestoda: Trypanorhyncha): description of adults from Squatina guggenheim (Chondrichthyes: Squatiniformes) off the coast of Argentina. Folia Parasitologica 56(4): 284-294. https:// doi.org/10.14411/fp.2009.033
Menoret A, Ivanov VA (2012a) A new species of Heteronybelinia (Cestoda: Trypanorhyncha) from Sympterygia bonapartii (Rajidae), Nemadactylus bergi (Cheilodactylidae) and Raneya brasiliensis (Ophidiidae) in the south-western Atlantic, with comments on host specificity of the genus. Journal of Helminthology 87(4): 467-482. https://doi.org/10.1017/ S0022149X12000545
Menoret A, Ivanov VA (2012b) Description of plerocerci and adults of a new species of Grillotia (Cestoda: Trypanorhyncha) in teleosts and elasmobranchs from the Patagonian shelf off Argentina. The Journal of Parasitology 98(6): 1185-1199. https://doi.org/10.1645/GE-3107.1
Menoret A, Ivanov VA (2014) Eutetrarhynchid trypanorhynchs (Cestoda) from elasmobranchs off Argentina, including the description of Dollfusiella taminii sp. n. and Parachristianella damiani sp. n., and amended description of Dollfusiella vooremi (Sáo Celemente et Gomes, 1989). Folia Parasitologica 61(5): 411-431. https://doi.org/10.14411/fp. 2014.056

Menoret A, Ivanov VA (2015) Trypanorhynch cestodes (Eutetrarhynchidae) from batoids along the coast of Argentina, including the description of new species in Dollfusiella Campbell et Beveridge, 1994 and Mecistobothrium Heinz et Dailey, 1974. Folia Parasitologica 62: 058. https://doi.org/10.14411/fp. 2015.058
Menoret A, Ivanov VA (2021) New species of Guidus Ivanov, 2006 (Cestoda: Phyllobothriidea) from Bathyraja magellanica (Philippi) from the Patagonian Continental Shelf of Argentina. Folia Parasitologica 68: 011. https://doi.org/10.14411/fp.2021.011
Menoret A, Mutti L, Ivanov VA (2017) New species of Aberrapex Jensen, 2001 (Cestoda: Lecanicephalidea) from eagle rays of the genus Myliobatis Cuvier (Myliobatiformes: Myliobatidae) from off Argentina. Folia Parasitologica 64: 009. https://doi.org/10.14411/fp.2017.009
Muñoz G, Cartes FD (2020) Endoparasitic diversity from the Southern Ocean: Is it really low in Antarctic fish? Journal of Helminthology 94: e180. https://doi.org/10.1017/ S0022149X20000590
Mutti LD, Ivanov VA (2016) A new species of Paraberrapex Jensen, 2001 (Cestoda: Lecanicephalidea) from Squatina guggenheim Marini (Squatiniformes: Squatinidae) off Argentina. Folia Parasitologica 63: 007. https://doi.org/10.14411/fp.2016.007
Mutti LD, Franzese S, Ivanov VA, Arredondo NJ (2023) Ultrastructure and histochemistry of the scolex of two Neotropical proteocephalidean species (Cestoda: Onchoproteocephalidea). Journal of Morphology 284(5): e21580. https://doi.org/10.1002/jmor. 21580

Naylor GJP, Caira JN, Jensen K, Rosana KAM, Straube N, Lakner C (2012) Elasmobranch phylogeny: A mitochondrial estimate based on 595 species. In: Carrier JC, Musick JA,Heithaus MR (Eds) The Biology of Sharks and Their Relatives (2 \(2^{\text {nd }}\) ed.). CRC Press, Boca Raton, 31-56. https://doi.org/10.1201/b11867-4
Oosthuizen G, Acosta AA, Smit NJ, Schaeffner BC (2021) A new species of Grillotia Guiart, 1927 (Cestoda: Trypanorhyncha) from the spotted skate, Raja straeleni Poll, in South Africa. Parasitology International 82: e102307. https://doi.org/10.1016/j.parint.2021.102307
Ostrowski de Nuñez M (1971) Estudios preliminares sobre la fauna parasitaria de algunos elasmobranquios del litoral bonaerense, Mar del Plata, Argentina. I. Cestodes y trematodes de Psammobatis microps (Günther) y Zapteryx brevirostris (Müller y Henle). Physis 30(81): 425-446.
Ostrowski de Núñez M (1973) Estudios preliminares sobre la fauna de parasitaria de algunos elasmobranquios del litoral bonaerense, Mar del Plata, Argentina. Physis 32: 1-14.
Palm HW, Palm N, Haseli M (2019) Tentaculariid trypanorhynchs (Platyhelminthes: Cestoda) from Mobula japanica (Müller \& Henle) from Indonesia, with the description of two new species. Parasitology Research 118(12): 3307-3313. https://doi.org/10.1007/s00436-019-06497-2
Pickering M, Caira JN (2012) A new hyperapolytic species, Trilocularia eberti sp. n. (Cestoda: Tetraphyllidea), from Squalus cf. mitsukurii (Squaliformes: Squalidae) off South Africa with comments on its development and fecundity. Folia Parasitologica 59(2): 107-114. https://doi.org/10.14411/fp.2012.016
Randhawa HS, Poulin R (2019) Tapeworm discovery in elasmobranch fishes: Quantifying patterns and identifying their correlates. Marine and Freshwater Research 71(1): 78-88. https://doi.org/10.1071/MF18418
Reyda FB, Marques FPL (2011) Diversification and species boundaries of Rhinebothrium (Cestoda; Rhinebothriidea) in South American freshwater stingrays (Batoidea; Potamotrygonidae). PLoS ONE 6(8): 1-26. https://doi.org/10.1371/journal.pone. 0022604
Rocka A (2003) Cestodes of the Antarctic fishes. Polish Polar Research 24: 3-4.
Rocka A (2017) Cestodes and nematodes of Antarctic fishes and birds. In: Klimpel S, Kuhn T, Mehlhorn H (Eds) Biodiversity and Evolution of Parasitic Life in the Southern Ocean. Springer International, Basel, 77-107. https://doi.org/10.1007/978-3-319-46343-8_6
Rocka A, Zdzitowiecki K (1998) Cestodes in fishes of the Weddell Sea. Acta Parasitologica 2(43).
Ruhnke TR (2011) Tapeworms of elasmobranchs (Part III). A monograph on the Phyllobothriidae (Platyhelminthes, Cestoda). Bulletin of the University of Nebraska State Museum 25(i-xii), Nebraska, 205 pp.
Sáo Clemente SC, Gomes DC (1989) Trypanorhyncha from sharks of southern Brazilian coast: Eutetranychus vooremi sp. n. and two other species parasites of Mustelus (Pisces, Triakidae). Memorias do Instituto Oswaldo Cruz 84(4, suppl 4): 475-481. https://doi.org/10.1590/ S0074-02761989000800083
Schmidt GD, Beveridge I (1990) Cathetocephalus australis n. sp. (Cestoidea: Cathetocephalidae) from Australia, with a proposal for Cathetocephalidea n. ord. The Journal of Parasitology 76(3): 337-339. https://doi.org/10.2307/3282661

Scholz T, Oros M, Bazsalovicsová E, Brabec J, Waeschenbach A, Xi B-W, Aydogdu A, Besprozvannykh V, Shimazu T, Králová-Hromadová I, Littlewood DTJ (2014) Molecular evidence of cryptic diversity in Paracaryophyllaeus (Cestoda: Caryophyllidea), parasites of loaches (Cobitidae) in Eurasia, including description of P. vladkae n. sp. Parasitology International 63(6): 841-850. https://doi.org/10.1016/j.parint.2014.07.015
Stehmann MF, Weigmann S, Naylor GJ (2021) First complete description of the dark-mouth skate Raja arctowskii Dollo, 1904 from Antarctic waters, assigned to the genus Bathyraja (Elasmobranchii, Rajiformes, Arhynchobatidae). Marine Biodiversity 51(1): 1-27. https:// doi.org/10.1007/s12526-020-01124-1
Suriano DM (2002) Anthobothrium galeorhini n. sp. (Eucestoda: Tetraphyllidea) a parasite of Galeorhinus galeus (Triakidae) from the Argentine coast. Parasite (Paris, France) 9(2): 121125. https://doi.org/10.1051/parasite/2002092121

Suriano DM, Labriola JB (2001a) Redescription of Cathetocephalus australis Schmidt et Beveridge, 1990 [Cestoda, Cathetocephalidae] parasite of Carcharhinus brachyurus [Gunther] [Pisces, Carcharhiniformes] from the Southwestern Atlantic Ocean. Acta Parasitologica 46(4): 276-279.
Suriano DM, Labriola JB (2001b) A new Orygmatobothrium Diesing, 1863 (Eucestoda: Tetraphyllidea) parasite of Mustelus schmitti Springer, 1939 (Carcharhiniformes, Triakidae) from the southwestern Atlantic Ocean. Zoosystema 23: 669-673.
Tanzola RD, Guagliardo SE, Brizzola SM, Arias MV, Botte SE (1998) Parasite assemblage of Sympterygia bonapartii (Pisces: Rajidae), an endemic skate of the southwest Atlantic. Helminthologia 35(3): 123-129.
Tyler GA II (2006) A Monograph on the Diphyllidea (Platyhelminthes, Cestoda). Bulletin of the University of Nebraska State Museum 25(i-xii), Nebraska, 142 pp.
Van Der Spuy L, Smit NJ, Schaeffner BC (2022) Threatened, host-specific affiliates of a redlisted host: Three new species of Acanthobothrium van Beneden, 1849 (Cestoda: Onchoproteocephalidea) from the endangered white skate, Rostroraja alba (Lacépède). International Journal for Parasitology. Parasites and Wildlife 17: 114-126. https://doi.org/10.1016/j. ijppaw.2021.12.010
Weigmann S (2016) Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity. Journal of Fish Biology 88(3): 837-1037. https://doi.org/10.1111/jfb. 12874
Wojciechowska A (1990a) Onchobothrium antarcticum sp. n. (Tetraphyllidea) from Bathyraja eatonii (Günther, 1876) and a plerocercoid from Notothenioidea (South Shetlands, Antarctic). Acta Parasitologica Polonica 35(2): 113-117.
Wojciechowska A (1990b) Pseudanthobothrium shetlandicum sp. n. and P. notogeorgianum sp. n. (Tetraphyllidea) from rays in the regions of the South Shetlands and South Georgia (Antarctic). Acta Parasitologica Polonica 35(3): 181-186.
Wojciechowska A (1991a) Some tetraphyllidean and diphyllidean costodes from Antarctic batoid fishes. Acta Parasitologica Polonica 36(2): 69-74.
Wojciechowska A (1991b) New species of the genus Phyllobothrium (Cestoda, Tetraphyllidea) from Antarctic batoid fishes. Acta Parasitologica Polonica 36(2): 63-68.

\title{
A zoogeographical analysis of true bugs (Insecta, Heteroptera) from Uzbekistan
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\begin{abstract}
The purpose of this study is to perform a zoogeographic analysis of terrestrial true bugs (Heteroptera) in the Lower Amudarya Territory, Uzbekistan. According to the findings of a literature review, there are 149 species of terrestrial hemipterans in the Territory. All hemipteran species, with the possible exception of five, have most likely been misidentified. Until now, reliable information on the zoogeography of true bug species in Uzbekistan's north-western region has not been published. The composition of species, diversity, and the proportion of endemism vary greatly across the country's zoogeographic regions. The Heteroptera fauna of the Khorezm and Karakalpakstan can be divided into four groups: most species belonging to the Palaearctic region, with 125 species constituting \(83.89 \%\) of the fauna; the second group of Holarctic origin is characterized by no more than ten species, which constitutes \(6.71 \%\); the third consists of endemics, 13 species or \(8.72 \%\); and one species ( \(0.67 \%\) ) is cosmopolitan. Much more research is needed to investigate distributions in a more northern climate. The introduction of invasive Heteroptera to the north-western part of Uzbekistan will increase and deserves further consideration.
\end{abstract}

\section*{Keywords}

Abundance, distribution, faunistics, geography range, Karakalpakstan, Khorezm, Lower Amudarya, occurrence

\section*{Introduction}

Heteroptera or true bugs are a large group with more than 40,000 species in approximately 50 families distributed across the world (Weirauch and Schuh 2011; Henry 2017a). In Russia, 760 species in 285 genera, and 35 families, are recorded (Vinokurov et al. 2010), however, more than 1250 species are distributed in Central Asia (Esenbekova 2013), and 700 species of true bugs are distributed in Uzbekistan (Animal World of Uzbekistan 2023).

The study of the fauna of true bugs by Central Asia region has been occurring for more than 170 years (Saprykin 2013). Many individuals have studied regional true bugs from 1995-2013 using the large, published Catalogue of Palaearctic Heteroptera (Catalogue of Palaearctic true bugs 2013).

The geographical distribution of Heteroptera from around the world has always been of interest to researchers (Latreille 1810; Leach 1815; Panizzi and Grazia 2015; Schuh and Weirauch 2020). Many research papers have been published recently, including Chandra and Kushwaha (2013); Samra et al. (2015); Vinokurov et al. (2015); Yasunaga (2016); Drapolyuk (2017); Henry (2017b); Oh et al. (2017); Kim and Jung (2018); Kuzhuget and Vinokurov (2018); Gapon (2019); Yazici (2020); Gandjaeva (2011, 2012a, b, 2020); Gandjaeva and Abdullaeva (2022a, b); Gandjaeva and Allabergenova (2022); Gandjaeva et al. (2019, 2020a, b, c, d, e, 2021, 2022a, b, c); Abdullaev et al. (2020a, b); Allabergenova and Gandzhaeva (2022); Yusupova and Gandjaeva (2022);Yusupova et al. (2022); Iskandarov et al. (2022).

Since the second half of the \(19^{\text {th }}\) century, new descriptions of Central Asian species have been published regularly in the works of Yakovlev (1890); Oshanin (1891) and others. These researchers conducted route surveys in the Fergana Valley, Turkestan Ridge, Alay Range, and Alay Valley, as well as in Samarkand and Djizzakh. Approximately 384 species of true bugs were identified during expeditions, and their zoogeography was studied in Central Asia by prominent zoologists such as Oshanin (1891), who was the first scientist to investigate Heteropteran zoogeography and listed more than 530 species. In the \(21^{\text {st }}\) century, many American scientists studied regional Heteroptera including Rider (2006, 2016); Hoebeke and Carter (2003); Bundy and McPherson (2018); Schuh and Weirauch (2020).

The literature on the fauna of terrestrial true bugs in different habitats of the Republic of Uzbekistan is meager. This lack of study also includes true bugs of Central Asia, mainly in the southern regions, which cover the territories of Samarkand, Bukhara, Tashkent, Andijan, Fergana, Kashkadarya, and Surkhandarya.

The purpose of the current paper is to explain database entries for the Lower Amudarya Heteroptera species, including brief geographic histories and original references. Every database should be a living document, with the ability to track changes regularly. Additional information on newly studied species is being added continuously (Gandjaeva 2011, 2012a, b, 2020; Gandjaeva et al. 2019, 2020a, b, c, d, e, 2021, 2022a, b, c; Abdullaev et al. 2020a, b; Allabergenova and Gandzhaeva 2022; Gandjaeva and Abdullaeva 2022a, b; Gandjaeva and Allabergenova 2022; Iskandarov et al. 2022; Yusupova and Gandjaeva 2022; Yusupova et al. 2022).

The goals of this study include classifying species ranges and conducting a zoogeographical analysis of the nation's actual true bug fauna, as well as determining species compositions and distributions in various belts of the Khorezm region and Karakalpakstan Republic.

\section*{Materials and methods}

The study was conducted in a lowland area in the northwestern part of Uzbekistan along the lower sections of the Amudarya River: between 60' and 61' longitude and 41 ' and 42 ' latitude, at an altitude of \(113-138 \mathrm{~m}\) above sea level. The vegetative cycle of plants lasts 200-210 days. The climate is continental, with an average annual precipitation of \(80-90 \mathrm{~mm}\), and average temperature ranges from \(-5^{\circ} \mathrm{C}\) in January to \(40^{\circ} \mathrm{C}\) in July. The climate has been changing, and the temperature has risen in summer, reaching \(50^{\circ} \mathrm{C}\) in July (Gandjaeva 2019; Abdullaev et al. 2022; Ruzmetov et al. 2022). The usual alkali soils are meadow, meadow-marsh, and marsh-sandy. The area is located in the steppe zone, as well as in the southern portion of the Aral Sea and the western part of the Khorezm oasis. The historic Amudarya delta is made up of river sediments. Sand can be found on the sections connecting with Karakum in the west and southwest. Minerals include limestone, sand, clay, and other building materials (Khamraev 2003).

For the analysis, we used zoogeographical categories of the heteropteran species that had been recorded earlier. Approximately 180 specimens of Heteroptera indexed in the territory of the Lower Amudarya River and were identified to 149 species in 89 genera, and two infraorders. These species were deposited in the Zoological collections of the Zoology Institute (ZIN) of the Academy of Sciences of the Republic of Uzbekistan.

The study was carried out between 2007 and 2020 (see Gandjaeva 2011, 2012a, b, 2020; Gandjaeva et al. 2019, 2020a, b, c, d, e, 2021, 2022a, b, c; Abdullaev et al. 2020a, b; Allabergenova and Gandzhaeva 2022; Gandjaeva and Abdullaeva 2022a, b; Gandjaeva and Allabergenova 2022). Terrestrial Heteroptera were collected from various fields, including the agricultural farms "Odilbek," "Amir Temur," "Gulrukhbegim," and "Oltin Kal'a" located in the Urgench district, "Dildora Bojimon" and "Buz Os Yep" agricultural farms, as well as the educational-experimental station of UrSU named "Uchkhoz" in Yangibazar district, "Ziroat-21" agricultural farm of Kushkupir district, "Raximbergan Xoji Anbar" in Khiva district, "Otabek garchak" and "Gulkand Istikbolli bog'i" in Khanka district and natural landscapes in the Khorezm region, as well as "Zaripboy," "Kilchinok," and "Yangiyer" agricultural farms in Ellikkala district of the Republic of Karakalpakstan and "Badai Tugai Nature Reserve," Karatau mountain in the Beruniy district of the Republic of Karakalpakstan (Gandjaeva et al. 2021). The geographical locations of the sites are shown in Fig. 1.

The zoogeographic analysis of identified species in the Lower Amudarya was based on zoogeographical nomenclature by Emelyanov (1974). In brief, geographic longitude was used to establish the zone along its meridional boundaries.


Figure I. Geographical locations of the collection sites of terrestrial Heteroptera specimens in the Lower Amudarya Territory.

The descriptive area nomenclature utilized in this work uses the concepts of physical geography and applies two axis coordinates: the latitudinal axis runs from north to south and is critical because it is used to determine climatic conditions of the distributed species, especially temperature; the longitudinal axis runs from west to east. In some species, the range coincides with the boundaries of the landscape zone and is labeled as Arctic (polar deserts, tundra), boreal (taiga), subboreal (broad-leaved forests), subtropical and tropical (evergreen forests) (Lopatin and Meleshko 2016).

We used the basic data on the geographic distribution of these species from the Catalogue "Heteroptera of the Palaearctic" Volumes I-VI, published by the Netherlands Entomological Society, Amsterdam (NES) (1995-2013) (Catalogue of Palaearctic true bugs 2013) to describe the analysis of the zoogeographic areas of terrestrial Heteroptera (Aukema et al. 2013) the database is continually updated.

An analysis of the occurrence and abundance of species on cultivated and wild plants were carried out by observing 50-100 plant specimens every day along the diagonal of the fields. The number of adult bugs, larvae of all ages, and egg clutches was recorded (Gandjaeva et al. 2021).

The number of species and their occurrence was calculated using the formula devised by Dajoz (2000):
\[
\mathrm{F}(\%)=100 \times(\mathrm{Pi} / \mathrm{P})
\]
where Pi refers to the species that was found; P is an absolute number.

Species are divided into four groups based on their frequency of occurrence:
Constantly occurring species: \(\mathrm{F} \geq 50 \%\)
Often occurring species: \(25 \%<\mathrm{F}<50 \%\)
Additional occurring species: \(5 \% \leq \mathrm{F}<25 \%\)
Rarely occurring species: \(\mathrm{F}<5 \%\)

The dynamics of the abundance of species was calculated using the formula of Zaime and Gautier (1989):
\[
\operatorname{Ar}(\%)=100 \times(\mathrm{Ni} / \mathrm{N})
\]
where Ni is the coefficient of special observable species; N is the absolute number of all observable species.

The analyses of the dynamics of the numbers of species are also divided into four groups:
Abundant: \(\mathrm{Ar} \geq 10\)
Frequent: \(5 \leq \mathrm{Ar}<10\)
Some: \(1 \leq \mathrm{Ar}<5\)
Few: \(\mathrm{Ar}<1\)

\section*{Results and discussion}

Checklists of Heteroptera for the Khorezm region and Karakalpakstan Republic were published more than 20 years ago. Khamraev (2003) and Kulumbetova (1998a, b, c, 1999) listed several species found to the north of Uzbekistan and, respectively, but some species have yet to be discovered while others are rare or migratory.

We carried out a comparative analysis of the lists of regional faunas using the data from Khamraev (2003) in the Khorezm Region and Kulumbetova (1998a, b, c, 1999) in the Republic of Karakalpakstan, which allowed us to determine regional features of the fauna in the Lower Amudarya (Table 1). Based on taxonomic distribution, this method enables the collection of data about species complexes with various zoogeographical characteristics (Table 1; Fig. 2). To classify the areas of the Lower Amudarya, information from Gandjaeva et al. (2021) was used (Fig. 2).

Entomologists (Kulumbetova 1998a, b, c, 1999; Khamraev 2003) discovered five new species: Tarajala brevicornis (Reuter, 1879), Emblethis dilaticollis (Jakovlev, 1874), Aethus nigronervosus (Melichar, 1906), Microporus virgata (Fabricius, 1974), and Sciocoris helferi (Fieber, 1851), which were indexed between 1998-2003 (Kulumbetova 1998a, b, c, 1999; Khamraev 2003) for the Lower Amudarya. These could be rare or migratory species, or are probably misidentified. These five species, shaded in Table 1, have not yet been verified and these records are not used in the distributional and zoogeographical analyses of the group; they are only mentioned in the checklist of the heteropterans found in the Khorezm region and Karakalpakstan.


Khorezm region
Republic of Karakalpakstan
Figure 2. Numbers of terrestrial Heteroptera recorded in the regions of the northern part of Uzbekistan.

Table I. Checklist of the terrestrial Heteroptera from the Lower Amudarya (2007-2020).
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline & Taxon & Family & Occurrence & Abundance & Distribution & Zoogeographic categories & References \\
\hline 1 & Anthocoris pilosus (Jakovlev, 1877) & \multirow{4}{*}{Anthocoridae Fieber, 1837} & + & F & *** & SA & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 2 & Orius niger (Wolff, 1811) & & ++ & FR & - & TP & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 3 & Orius ribauti (Wagner, 1952) & & + & F & *** & P & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 4 & Orius albidipennis (Reuter, 1884) & & + & S & ** & TP & \begin{tabular}{l}
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 5 & \begin{tabular}{l}
Nabis ferus \\
(Linnaeus, 1758)
\end{tabular} & \multirow{7}{*}{\begin{tabular}{l}
Nabidae \\
Costa, 1852
\end{tabular}} & ++ & FR & - & TP & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 6 & \begin{tabular}{l}
Nabis palifer \\
(Seidenstücker, 1954)
\end{tabular} & & + & F & *** & TS & \begin{tabular}{l}
Khamraev (2003); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 7 & \begin{tabular}{l}
Nabis viridis \\
(Brullé, 1839)
\end{tabular} & & + & F & *** & SA & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 8 & \begin{tabular}{l}
Nabis rugosus \\
(Linnaeus, 1758)
\end{tabular} & & ++ & FR & *** & SA & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 9 & Nabis remanei (Kerzhner, 1962) & & + & F & ** & ChCA & \[
\begin{array}{|l|}
\hline \text { Kulumbetova (1999); } \\
\text { Gandjaeva et al. (2021) }
\end{array}
\] \\
\hline 10 & Nabis sareptanus (Dohrn, 1862) & & + & F & *** & TP & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 11 & Prostemma sanguineum (Rossi, 1790) & & + & F & ** & PA & \[
\begin{array}{|l|}
\hline \text { Kulumbetova (1999); } \\
\text { Gandjaeva et al. (2021) }
\end{array}
\] \\
\hline
\end{tabular}

\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline & Taxon & Family & Occurrence & Abundance & Distribution & Zoogeographic categories & References \\
\hline 37 & \begin{tabular}{l}
Orthotylus flavosparsus \\
(Sahlberg, 1841)
\end{tabular} & \multirow{13}{*}{\begin{tabular}{l}
Miridae \\
Hahn, 1833
\end{tabular}} & ++ & FR & ** & TP & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 38 & Campylomma annulicorne (Signoret, 1865) & & ++ & FR & ** & P & Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 39 & Campylomma diversicornis (Reuter, 1878) & & +++ & A & *** & NS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 40 & Campylomma verbasci (Meyer-Dur, 1843) & & +++ & A & ** & PA & Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 41 & \begin{tabular}{l}
Camptotylidea alba \\
(Reuter, 1879)
\end{tabular} & & ++ & FR & *** & TNT & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 42 & Camptotylus meyeri (FreyGessner, 1863) & & ++ & FR & *** & NS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 43 & Europiella alpina (Reuter, 1875) & & ++ & FR & **** & TP & Gandjaeva et al. (2021) \\
\hline 44 & Heterocapillus tigripes (Meyer \& Dur, 1852) & & + & F & * & SA & Gandjaeva et al. (2021) \\
\hline 45 & Macrotylus herrichi (Reuter, 1873) & & + & F & * & SA & Gandjaeva et al. (2021) \\
\hline 46 & Tuponia elegans (Jakovlev, 1867) & & ++ & FR & *** & SA & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 47 & \begin{tabular}{l}
Tuponia pallida \\
(Jakovlev, 1867)
\end{tabular} & & ++ & FR & *** & & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 48 & Tuponia roseipennis (Reuter, 1889) & & ++ & FR & *** & ChCA & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 49 & Tarajala brevicornis (Reuter, 1879) & & - & - & - & - & Khamraev (2003) \\
\hline 50 & \begin{tabular}{l}
Monosteira discoidalis \\
(Jakovlev, 1883)
\end{tabular} & \multirow{3}{*}{\begin{tabular}{l}
Tingidae \\
Laporte, 1832
\end{tabular}} & + & F & - & SA & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 51 & \begin{tabular}{l}
Stephanitis pyri \\
(Fabricius, 1775)
\end{tabular} & & + & F & **** & P & Gandjaeva et al. (2021) \\
\hline 52 & \begin{tabular}{l}
Tingis leptochila \\
(Horvath, 1906)
\end{tabular} & & + & F & *** & ITCA & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 53 & \[
\begin{aligned}
& \text { Stenolemus bogdanovi } \\
& \text { (Oshanin, 1896) }
\end{aligned}
\] & \multirow{11}{*}{\begin{tabular}{l}
Reduviidae \\
Latreille, 1807
\end{tabular}} & + & F & *** & TS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 54 & \begin{tabular}{l}
Coranus aegyptius \\
(Fabricius, 1775)
\end{tabular} & & ++ & FR & *** & SA & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 55 & \begin{tabular}{l}
Coranus subapterus \\
(De Geer, 1773)
\end{tabular} & & ++ & FR & ** & NS & Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 56 & Rhynocoris monticola
(Oshanin, 1870) & & ++ & FR & *** & TS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 57 & \begin{tabular}{l}
Rhinocoris nigronitens \\
Reuter, 1881
\end{tabular} & & ++ & FR & *** & TS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 58 & Vachiria deserta (Becker, 1867) & & + & F & **** & ITCA & Gandjaeva et al. (2021) \\
\hline 59 & \begin{tabular}{l}
Ectomocoris ululans \\
(Rossi, 1807)
\end{tabular} & & + & F & *** & ETPE & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 60 & \[
\begin{aligned}
& \text { Reduvius testaceus } \\
& \text { (Herrich-Schäffer, 1845) }
\end{aligned}
\] & & + & S & *** & TS & Gandjaeva et al. (2021) \\
\hline 61 & \begin{tabular}{l}
Reduvius disciger \\
(Horváth, 1896)
\end{tabular} & & + & F & *** & TS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 62 & Reduvius christophi (Jakovlev, 1874) & & + & S & \(\bigcirc\) & TS & Khamraev (2003); Kulumbetova (1999); Gandjaeva et al. (2021) \\
\hline 63 & Reduvius fedtschenkianus (Oshanin, 1871) & & + & F & - & TNT & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline & Taxon & Family & Occurrence & Abundance & Distribution & Zoogeographic categories & References \\
\hline 64 & \begin{tabular}{l}
Reduvius semenovi \\
(Jakovlev, 1885)
\end{tabular} & \multirow{4}{*}{\begin{tabular}{l}
Reduviidae \\
Latreille, 1807
\end{tabular}} & + & F & *** & TNT & Khamraev (2003); Gandjaeva et al. (2021) \\
\hline 65 & \begin{tabular}{l}
Reduvius elegans \\
(Jakovlev, 1885)
\end{tabular} & & ++ & FR & *** & TNT & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 66 & Oncocephalus brachymerus (Reuter, 1882) & & ++ & FR & *** & TS & \begin{tabular}{l}
Khamraev (2003); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 67 & Oncocephalus termezanus (Kiritshenko, 1914) & & ++ & FR & ** & ITCA & \begin{tabular}{l}
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 68 & Camptopus lateralis (German, 1817) & \multirow[t]{2}{*}{Alydidae Amyot \& Serville, 1843} & + & F & - & SA & \begin{tabular}{l}
Khamraev (2003); \\
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 69 & Megalotomus ornaticeps (Stål, 1858) & & + & F & ** & NS & \begin{tabular}{l}
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 70 & Centrocoris volxemi (Puton, 1878) & \multirow{6}{*}{\begin{tabular}{l}
Coreidae \\
Leach, 1815
\end{tabular}} & + & F & *** & TS & \[
\begin{gathered}
\text { Khamraev (2003); } \\
\text { Gandjaeva et al. (2021) }
\end{gathered}
\] \\
\hline 71 & Coreus marginatus (Linnaeus, 1758) & & + & S & *** & TP & Khamraev (2003); Gandjaeva et al. (2021) \\
\hline 72 & Enoplops eversmanni (Jakovlev, 1881) & & + & F & *** & T & Khamraev (2003); Gandjaeva et al. (2021) \\
\hline 73 & Bathysolen nubilus (Fallen, 1807) & & + & F & ** & TS & \begin{tabular}{l}
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 74 & Bothrostethus annulipes (Herrich-Schäffer, 1835) & & + & S & ** & TS & \begin{tabular}{l}
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 75 & Coriomeris vitticollis (Reuter, 1900) & & + & F & - & TS & \begin{tabular}{l}
Khamraev (2003); \\
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 76 & Brachycarenus tigrinus (Schilling, 1829) & \multirow{10}{*}{Rhopalidae Amyot \& Serville, 1843} & ++ & FR & O & TP & \begin{tabular}{l}
Khamraev (2003); \\
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 77 & Chorosoma schillingi (Schilling, 1829) & & ++ & FR & *** & SA & \[
\begin{gathered}
\text { Khamraev (2003); } \\
\text { Gandjaeva et al. (2021) }
\end{gathered}
\] \\
\hline 78 & Corizus limbatus (Rey, 1887) & & +++ & A & - & SA & \begin{tabular}{l}
Khamraev (2003); \\
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 79 & Corizus tetraspilus (Horvath, 1917) & & +++ & A & ** & NS & \begin{tabular}{l}
Khamraev (2003); \\
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 80 & \begin{tabular}{l}
Corizus hyoscyami \\
(Linnaeus, 1758)
\end{tabular} & & +++ & A & *** & TP & \begin{tabular}{l}
Khamraev (2003); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 81 & Maccevethus persicus (Jakovlev, 1882) & & ++ & FR & *** & TS & Khamraev (2003); Gandjaeva et al. (2021) \\
\hline 82 & Liorhyssus hyalinus (Fabricius, 1794) & & ++ & FR & - & C & \begin{tabular}{l}
Khamraev (2003); \\
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 83 & Rhopalus parumpunctatus (Schilling, 1829) & & ++ & FR & *** & TP & \[
\begin{gathered}
\text { Khamraev (2003); } \\
\text { Gandjaeva et al. (2021) }
\end{gathered}
\] \\
\hline 84 & Rhopalus distinctus (Signoret, 1859) & & ++ & FR & *** & TS & Khamraev (2003); Gandjaeva et al. (2021) \\
\hline 85 & Stictopleurus unicolor (Jakovlev, 1873) & & ++ & FR & *** & W & Khamraev (2003); Gandjaeva et al. (2021) \\
\hline 86 & \begin{tabular}{l}
Dicranocephalus \\
marginatus \\
(Ferrari, 1874)
\end{tabular} & \multirow[b]{2}{*}{\begin{tabular}{l}
Stenocephalidae \\
Dallas, 1852
\end{tabular}} & + & F & O & TS & \begin{tabular}{l}
Khamraev (2003); \\
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 87 & Dicranocephalus ferghanensis (Horváth, 1887) & & + & F & O & TS & \begin{tabular}{l}
Khamraev (2003); \\
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline & Taxon & Family & Occurrence & Abundance & Distribution & Zoogeographic categories & References \\
\hline 88 & Artheneis alutacea (Fieber, 1861) & Artheneidae Stål, 1872 & + & S & *** & W & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 89 & Geocoris ater (Fabricius, 1787) & \multirow{9}{*}{\begin{tabular}{l}
Geocoridae \\
Baerensprung, 1860
\end{tabular}} & ++ & FR & ** & TP & Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 90 & Geocoris arenarius (Jakovlev, 1867) & & + & F & ** & NS & \[
\begin{aligned}
& \text { Kulumbetova (1999); } \\
& \text { Gandjaeva et al. (2021) }
\end{aligned}
\] \\
\hline 91 & \[
\begin{aligned}
& \text { Geocoris dispar } \\
& \text { (Waga, 1839) }
\end{aligned}
\] & & ++ & FR & ** & W & \begin{tabular}{l}
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 92 & Geocoris lapponicus (Zetterstedt, 1838) & & + & F & **** & P & Gandjaeva et al. (2021) \\
\hline 93 & Geocoris fedtschenkoi (Reuter, 1885) & & + & F & *** & NS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 94 & \[
\begin{aligned}
& \text { Geocoris scutellatus } \\
& \text { (Montandon, 1907) }
\end{aligned}
\] & & + & F & *** & KNTIT & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 95 & Engistus salinus (Jakovlev, 1874) & & + & F & *** & TS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 96 & Engistus exsanguis (Stál, 1872) & & ++ & FR & *** & TS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 97 & Henestaris halophilus (Burmeister, 1835) & & + & F & *** & W & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 98 & \begin{tabular}{l}
Lygaeus equestris \\
(Linnaeus, 1758)
\end{tabular} & \multirow{6}{*}{\[
\begin{gathered}
\text { Lygaeidae } \\
\text { Schilling, } 1829
\end{gathered}
\]} & ++ & FR & - & TP & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 99 & \begin{tabular}{l}
Spilostethus rubriceps \\
(Horvath, 1899)
\end{tabular} & & + & F & - & TS & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 100 & Spilostethus pandurus (Scopoli, 1763) & & + & F & ** & TS & \begin{tabular}{l}
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 101 & Nysius graminicola (Kolenati, F.A., 1845) & & ++ & FR & *** & SA & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 102 & \begin{tabular}{l}
Oxycarenus pallens \\
(Herrich-Schäffer, 1850)
\end{tabular} & & + & S & *** & SA & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 103 & Ortholomus punctipennis (Herrich-Schäffer, 1850) & & ++ & FR & *** & P & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 104 & Beosus quadripunctatus (Muller, 1766) & \multirow{9}{*}{\begin{tabular}{l}
Rhyparochromidae \\
Amyot \& Serville, 1843
\end{tabular}} & ++ & FR & ** & SA & \begin{tabular}{l}
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 105 & Bleteogonus beckeri (FreyGessner, 1863) & & + & F & ** & TS & \begin{tabular}{l}
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 106 & Emblethis griseus (Wolff, 1802) & & + & F & \(\bigcirc\) & SA & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 107 & Emblethis verbasci (Fabricius, 1803) & & + & F & - & SA & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 108 & Emblethis ciliatus (Horváth, 1875) & & + & F & \(\bigcirc\) & SA & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 109 & \begin{tabular}{l}
Emblethis denticollis \\
(Horváth, 1878)
\end{tabular} & & + & F & *** & P & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 110 & Emblethis dilaticollis (Jakovlev, 1874) & & - & - & - & - & Kulumbetova (1999) \\
\hline 111 & Hyalocoris pilicornis (Jakovlev, 1874) & & + & S & - & TS & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 112 & \begin{tabular}{l}
Lamprodema maura \\
(Fabricius, 1803)
\end{tabular} & & ++ & FR & - & W & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline & Taxon & Family & Occurrence & Abundance & Distribution & Zoogeographic categories & References \\
\hline 113 & Aethus pilosulus (Klug, 1845) & \multirow{8}{*}{Cydnidae Billberg, 1820} & + & F & - & TS & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al.
(2021) \\
\hline 114 & Aethus nigronervosus (Melichar, 1906) & & - & - & - & - & Khamraev (2003) \\
\hline 115 & \begin{tabular}{l}
Byrsinus fossor \\
(Mulsant \& Rey, 1866)
\end{tabular} & & + & F & - & TP & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 116 & Microporus virgata (Fabricius, 1794) & & - & - & - & - & Khamraev (2003) \\
\hline 117 & Microporus nigrita (Fabricius, 1794) & & + & F & ** & ETPE & Gandjaeva et al. (2021) \\
\hline 118 & Stibaropus hoblbecki (Kiritshenko, 1912) & & + & F & ** & TNT & Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 119 & Sehirus morio (Linnaeus, 1761) & & + & F & *** & W & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 120 & Amaurocoris candidus (Horváth, 1889) & & + & F & *** & TS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 121 & Aelia acuminata (Linnaeus, 1758) & \multirow{15}{*}{\begin{tabular}{l}
Pentatomidae \\
Leach, 1815
\end{tabular}} & +++ & A & ** & W & Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 122 & Aelia furcula (Fieber, 1868) & & +++ & A & *** & TS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 123 & Aelia melanota (Fieber, 1868) & & +++ & A & ** & TS & Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 124 & Brachynema germari (Kalenati, 1846) & & ++ & FR & - & TP & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al.
(2021) \\
\hline 125 & Carpocoris pudicus (Poda, 1761) & & ++ & FR & *** & P & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 126 & \begin{tabular}{l}
Carpocoris fuscispinus \\
(Boheman, 1851)
\end{tabular} & & ++ & FR & \(\bigcirc\) & W & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al.
(2021) \\
\hline 127 & Palomena prasina (Linnaeus, 1761) & & +++ & A & **** & SA & Gandjaeva et al. (2021) \\
\hline 128 & Dolycoris penicillatus (Horváth, 1904) & & +++ & A & \(\bigcirc\) & TS & Khamraev (2003); Kulumbetova (1999); Gandjaeva et al. (2021) \\
\hline 129 & Desertomenida quadrimaculata (Horváth, 1892) & & +++ & A & *** & NS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 130 & Desertomenida albula (Kiritshenko, 1914) & & +++ & A & *** & TS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 131 & Derula longipennis (Oshanin, 1871) & & + & F & **** & TP & Gandjaeva et al. (2021) \\
\hline 132 & \begin{tabular}{l}
Apodiphus integriceps \\
(Horváth, 1888)
\end{tabular} & & +++ & A & \(\bigcirc\) & TS & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 133 & Cellobius abdominalis (Jakovlev, 1885) & & ++ & FR & *** & NS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 134 & Codophila varia (Fabricius, 1787) & & ++ & FR & *** & SA & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 135 & Holcostethus nitidus (Kiritshenko, 1914) & & ++ & FR & *** & TNT & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline & Taxon & Family & Occurrence & Abundance & Distribution & Zoogeographic categories & References \\
\hline 136 & Holcostethus strictus vernalis (Wolff, 1804) & \multirow{14}{*}{\begin{tabular}{l}
Pentatomidae \\
Leach, 1815
\end{tabular}} & ++ & FR & ** & P & \begin{tabular}{l}
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 137 & Menaccarus deserticola (Jakovlev, 1900) & & ++ & FR & *** & TS & Khamraev (2003); Gandjaeva et al. (2021) \\
\hline 138 & Eurydema ornata (Linnaeus, 1758) & & +++ & A & *** & SA & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 139 & \begin{tabular}{l}
Eurydema oleracae \\
(Linnaeus, 1758)
\end{tabular} & & +++ & A & **** & SA & Gandjaeva et al. (2021) \\
\hline 140 & Eurydema wilkinsi (Distant, 1879) & & +++ & A & * & NS & Gandjaeva et al. (2021) \\
\hline 141 & Eurydema ventralis (Kolenati, 1846) & & +++ & A & **** & SA & Gandjaeva et al. (2021) \\
\hline 142 & Eurydema maracandica (Oshanin, 1871) & & +++ & A & ** & NS & \begin{tabular}{l}
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline 143 & Graphosoma lineatum (Linnaeus, 1758) & & ++ & FR & *** & SA & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 144 & Graphosoma consimile (Horvath, 1903) & & ++ & FR & *** & TS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 145 & Tarisa elevata (Reuter, 1901) & & ++ & FR & *** & TS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 146 & Tarisa subspinosa (Germar, 1839) & & ++ & FR & *** & TP & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 147 & Tarisa virescens (HerrichSchäffer, 1851) & & ++ & FR & *** & NS & \[
\begin{gathered}
\text { Khamraev (2003); } \\
\text { Gandjaeva et al. (2021) }
\end{gathered}
\] \\
\hline 148 & Tarisa pallescens (Jakovlev, 1871) & & ++ & FR & *** & TS & Khamraev (2003);
Gandjaeva et al. (2021) \\
\hline 149 & Sciocoris helferi (Fieber, 1851) & & - & - & - & - & Kulumbetova (1999) \\
\hline 150 & Eurygaster integriceps (Puton, 1881) & \multirow{3}{*}{\begin{tabular}{l}
Scutelleridae \\
Leach, 1815
\end{tabular}} & ++ & FR & o & P & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 151 & Odontotarsus impictus (Jakovlev, 1886) & & + & F & - & TS & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 152 & Odontotarsus angustatus (Jakovlev 1883) & & + & F & *** & TS & \[
\begin{gathered}
\text { Khamraev (2003); } \\
\text { Gandjaeva et al. (2021) }
\end{gathered}
\] \\
\hline 153 & \begin{tabular}{l}
Scantius aegyptius \\
(Linnaeus, 1758)
\end{tabular} & \multirow[t]{2}{*}{Pyrrhocoridae Amyot \& Serville, 1843} & + & F & o & NS & Khamraev (2003);
Kulumbetova (1999);
Gandjaeva et al. (2021) \\
\hline 154 & Pyrrhocoris apterus (Linnaeus, 1758) & & ++ & FR & - & W & \begin{tabular}{l}
Khamraev (2003); \\
Kulumbetova (1999); \\
Gandjaeva et al. (2021)
\end{tabular} \\
\hline
\end{tabular}

Total number of species: 154
Symbols and abbreviations used in the table
Occurrence: constantly occurring species (CO): ++++; often occurring species (OO): +++; additional occurring species (AO): ++;
Rarely occurring species (RO): +.
Abundance: Abundant: A; frequent: FR; some: S; few: F.
Distribution: o - species presence;
- species presence not confirmed
* - previously unregistered species for Uzbekistan;
** - previously unregistered species for the Khorezm region;
*** - previously unregistered species for the Republic of Karakalpakstan;
** ** - previously unregistered species for the Khorezm region and the Republic of Karakalpakstan.

\section*{Zoogeographical categories}

C - Cosmopolitan; TP - Trans-Palaearctic; P - Pancontinental; ETPE - Ethiopia - Trans-Palaearctic - Eastern; SA - Super-Atlantic; W - The Western; PA - Pan-Atlantic; NC - Narrow continental; NS - The North Seitan; TS - Tethyan-Siberian; ChCA - ChineseCentral Asian endemics; TNT - Turkestanian-Northern Turanian endemics; ITCA - Irano-Turanian-Central Asian endemics; KNTIT - Kazakh-Northern Turanian, Irano-Turanian; T - Turanian endemics.

Khamraev (2003) identified 110 species for the Khorezm, and Kulumbetova (1998a, b, c, 1999) 62 species for the Republic of Karakalpakstan.

The analysis of terrestrial Heteroptera in the Lower Amudarya by Gandjaeva (20072020) represented 39 species, which were first studied for the fauna of the Khorezm region and 87 species for the Republic of Karakalpakstan. According to the data, there are currently 149 species of terrestrial Heteroptera recorded in the Lower Amudarya (Fig. 2).

During 2007-2020, 149 species of terrestrial heteropterans were recorded in the Lower Amudarya territory as represented in Table 1.

From the surveys, it has been established that approximately 30 species are abundant and numerous. Sixty-two species are frequent, eight are sometimes encountered, and 49 were recorded as few. They belong to 17 families, 89 genera and the most numerous are Miridae - 37 species and Pentatomidae - 28 species, followed by Reduviidae - 15; Rhopalidae - 10; Geocoridae - 9; Rhyparochromidae - 8; Nabidae - 7; Coreidae, Lygaeidae, Cydnidae - 6, Anthocoridae - 4. Other families are represented by not more than two or three species (Table 2).

Recorded species belong to 11 types according to sector ranges, and 28 groups of areas according to belt ranges (Table 3). The Lower Amudarya's hemipteran species were divided into four large groups: Wide Areas, Holarctic Areas, Palaearctic Areas, and Endemic Areas (Fig. 3).
- Broad areas - extend beyond the Holarctic;
- Cosmopolitan areas - occur on at least three continents;
- Holarctic areas - cover the Palaearctic and the Nearctic region;
- Palaearctic areas - cover parts of Europe, Asia, and North Africa;
- Nearctic areas - cover North America, Mexico, and Greenland;
- The Ethiopia - Trans-Palaearctic - Eastern areas - this complex combines the Palaearctic, Ethiopia and Eastern regions;
- Trans-Palaearctic areas - cover the entire Palaearctic;
- Super-Atlantic areas - cover from the Atlantic sectors to the Eastern transitional sectors;
- The Western areas - cover the part of the Palaearctic Realm from the Eastern Atlantic to the Western Eucontinental sectors;
- Pan-Atlantic areas - encompass the Atlantic sector as well as the western subcontinental subsectors;
- Pancontinental areas - located from the sub-Atlantic to the eastern continental sectors inclusive;
- Narrow Continental areas - cover the Sahara-Gobi Desert area, the Mediterranean and the Irano-Turanian sub-areas.
- The North-Setian areas - cover the Trans-Scythian, the Western-Scythian, and the Eastern-Scythian sub-regions;
- Tethyan-Siberial areas - cover the Tethyan Subkingdom, Scythian, Setian, and European, Mediterranean, and Irano-Turanian subregions;
- Endemic areas - occur only in a certain area and nowhere else.

Table 2. Distribution of the number of genera, species within families, as well as their percentage (\%) in the fauna of terrestrial heteropterans.
\begin{tabular}{|c|c|c|c|c|}
\hline Family & Number of genera & \% & Number of species & \% \\
\hline Anthocoridae & 2 & 2.27 & 4 & 2.68 \\
\hline Nabidae & 2 & 2.27 & 7 & 4.70 \\
\hline Miridae & 20 & 21.59 & 37 & 24.16 \\
\hline Tingidae & 3 & 3.41 & 3 & 2.01 \\
\hline Reduviidae & 7 & 7.95 & 15 & 10.07 \\
\hline Alydidae & 2 & 2.27 & 2 & 1.34 \\
\hline Coreidae & 6 & 6.82 & 6 & 4.03 \\
\hline Rhopalidae & 7 & 7.95 & 10 & 6.71 \\
\hline Stenocephalidae & 1 & 1.14 & 2 & 1.34 \\
\hline Artheneidae & 1 & 1.14 & 1 & 0.67 \\
\hline Geocoridae & 3 & 3.41 & 9 & 6.04 \\
\hline Lygaeidae & 5 & 5.68 & 6 & 4.70 \\
\hline Rhyparochromidae & 5 & 5.68 & 8 & 5.37 \\
\hline Cydnidae & 6 & 6.82 & 6 & 4.03 \\
\hline Pentatomidae & 15 & 17.05 & 28 & 18.79 \\
\hline Scutelleridae & 2 & 2.27 & 3 & 2.01 \\
\hline Pyrrhocoridae & 2 & 2.27 & 2 & 1.34 \\
\hline Total: & 89 & 100 & 149 & 100 \\
\hline
\end{tabular}


Figure 3. Species numbers of true bugs by area grouping.

In the northern part of Uzbekistan, only one species ( \(0.67 \%\) ) is cosmopolitan. The group of the Holarctic range is characterized by no more than ten species, which constitutes \(6.71 \%\) of the total, and most species belong to the Palaearctic group, which is most diverse. The group contains 125 species ( \(83.89 \%\) ), with 38 from the TethyanSiberian type constituting \(25.50 \%\). Approximately 15 species account for \(10.07 \%\) of the Irano-Turanian range, while ten species constitute \(6.71 \%\) of the Euro-MediterraneanTuranian range. In the Super-Atlantic range, 28 species account for \(18.79 \%\), with eight

Table 3. Percentage of the terrestrial Heteroptera by area grouping.
\begin{tabular}{|c|c|c|c|c|}
\hline Type area & The sector and belt range & Number of species & Species & Percentage \\
\hline I. Groups of wide areas & I.1. Cosmopolitan & 1 & Liorhyssus hyalinus; & 0.67 \\
\hline \multirow[t]{7}{*}{II. Holarctic} & II.1. Trans-Palaearctic & 9 & & 6.04 \\
\hline & \begin{tabular}{l}
a) Extratropical, \\
Nearctic
\end{tabular} & 3 & Lygus rugulipennis, Orius albidipennis, Derula longipennis; & 2.01 \\
\hline & b) Boreal-subtropical, Nearctic & 3 & Agnocoris rubicundus, Lygus punctatus, Polymerus cognatus; & 2.01 \\
\hline & c) Boreal-subtropical & 2 & Polymerus vulneratus, Orthotylus flavosparsus; & 1.34 \\
\hline & d) Boreal - subboreal & 1 & Stenodema tripsinosa; & 0.67 \\
\hline & II.2. Pancontinental & 1 & & 0.67 \\
\hline & a) Extratropical & 1 & Deraeocoris punctulatus; & 0.67 \\
\hline \multirow[t]{31}{*}{III. Palaearctic} & \begin{tabular}{l}
III.1. Ethiopia - Trans- \\
Palaearctic - Eastern
\end{tabular} & 2 & & 1.34 \\
\hline & a) Southern & 2 & Ectomocoris ululans, Microporus nigrita; & 1.34 \\
\hline & III.2. Trans-Palaearctic & 16 & & 10.74 \\
\hline & a) Extratropical & 1 & Europiella alpina; & 0.67 \\
\hline & b) Arctic & 3 & Brachynema germari, Byrsinus fossor, Tarisa fraudatrix; & 2.01 \\
\hline & c) Boreal & 2 & Nabis ferus, Nabis sareptanus; & 1.34 \\
\hline & d) Boreal-subtropical & 9 & Orius niger, Adelphocoris lineolatus, Lygus gemellatus gemellatus, Stenodema calcaratum, Geocoris ater, Coreus marginatus, Brachycarenus tigrinus, Corizus hyoscyami hyoscyami, Rhopalus parumpunctatus; & 6.04 \\
\hline & e) Boreal-subboreal & 1 & Lygaeus equestris; & 0.67 \\
\hline & III.3. Super-Atlantic & 28 & & 18.79 \\
\hline & a) Arcto-Subboreal & 7 & Tuponia elegans, Tuponia pallida, Coranus aegyptius, Nysius graminicola graminicola, Emblethis griseus, Emblethis verbasci, Corizus limbatus; & 4.70 \\
\hline & b) Boreal-subboreal & 1 & Orthops basalis; & 0.67 \\
\hline & c) Boreal-subtropical & 7 & Lygus pratensis, Notostira elongata, Eurydema ornata, Eurydema oleracae, Palomena prasina, Orthops kalmi, Chorosoma schillingi; & 4.70 \\
\hline & d) Subboreal & 8 & Nabis rugosus, Nabis viridis Brullé, Heterocapillus tigripes, Macrotylus herrichi, Monosteira discoidalis, Beosus quadripunctatus, Codophila varia, Camptopus lateralis; & 5.37 \\
\hline & e) Subboreal-subtropical & 2 & Eurydema ventralis, Graphosoma lineatum; & 1.34 \\
\hline & f) Southern & 3 & Anthocoris pilosus, Oxycarenus pallens, Emblethis ciliatus; & 2.01 \\
\hline & III. 4. The Western & 13 & & 8.72 \\
\hline & a) Boreal & 2 & Deraeocoris serenus, Adelphocoris seticornis; & 1.34 \\
\hline & b) Boreal-subtropical & 5 & Lamprodema maura, Stictopleurus unicolor, Sehirus morio, Aelia acuminata, Carpocoris fuscispinus; & 3.36 \\
\hline & c) Boreal-subboreal & 2 & Pyrrhocoris apterus, Megaloceroea recticornis; & 1.34 \\
\hline & d) Subboreal & 3 & Artheneis alutacea, Brachycoleus decolor, Geocoris dispar; & 2.01 \\
\hline & e) Southern & 1 & Henestaris halophilus; & 0.67 \\
\hline & III. 5. Pan-Atlantic & 4 & & 2.68 \\
\hline & a) Boreal-subtropical & 2 & Stenodema laevigata, Campylomma verbasci; & 1.34 \\
\hline & b) Boreal-subboreal & 1 & Trigonotylus ruficornis; & 0.67 \\
\hline & c) Subboreal-subtropical & 1 & Prostemma sanguineum; & 0.67 \\
\hline & III. 6. Pancontinental & 10 & & 6.71 \\
\hline & a) Northern & 1 & Geocoris lapponicus; & 0.67 \\
\hline & b) Boreal-subtropical & 5 & Ortholomus punctipennis, Emblethis denticollis, Holcostethus strictus vernalis, Carpocoris pudicus, Trigonotylus pulchellus; & 3.36 \\
\hline & c) Subboreal & 1 & Orius ribauti; & 0.67 \\
\hline & d) Subboreal-subtropical & 1 & Eurygaster integriceps; & 0.67 \\
\hline & e) Southern & 2 & Campylomma annulicorne, Stephanitis pyri; & 1.34 \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|c|}
\hline Type area & The sector and belt range & Number of species & Species & Percentage \\
\hline & III. 7. Narrow & 2 & & 1.34 \\
\hline & Continental & & & \\
\hline & a) Eastern & 1 & Stenodema turanica; & 0.67 \\
\hline & Mediterranean Gobian & & & \\
\hline & b) Mediterranean-IranoTuranian & 1 & Geocoris fedtschenkoi; & 0.67 \\
\hline & III. 8. The North & 12 & & 8.05 \\
\hline & Setian & & & \\
\hline & a) Trans-Scythian & 1 & Geocoris arenarius; & 0.67 \\
\hline & b) Western Scythian & 3 & Coranus subapterus, Campylomma diversicorne, Camptotylus meyeri; & 2.01 \\
\hline & c) Eastern Scythian & 8 & Corizus tetraspilus, Megalotomus ornaticeps, Desertomenida quadrimaculata, Cellobius abdominalis, Eurydema wilkinsi, Eurydema maracandica, Tarisa virescens, Scantius aegyptius; & 5.37 \\
\hline & III. 9. Tethyan-Siberian & 38 & & 25.50 \\
\hline & a) Western-Scythian-Saharo-Gobian & 1 & Stenolemus bogdanovi; & 0.67 \\
\hline & \begin{tabular}{l}
b) Euro-Mediterranean \\
- Turanian
\end{tabular} & 10 & Spilostethus pandurus, Tarisa pallescens, Reduvius testaceus, Centrocoris volxemi, Bathysolen nubilus, Coriomeris vitticollis, Rhopalus distinctus, Engistus exsanguis, Aelia furcula, Graphosoma consimile; & 6.71 \\
\hline & c) Irano-TuranianGobian & 4 & Megacoelum brevirostre, Orthotylus eleagni, Oncocephalus brachymerus, Bothrostethus annulipes; & 2.68 \\
\hline & d) Irano-Turanian & 15 & Reduvius disciger, Reduvius christophi, Engistus salinus, Tarisa elevata, Desertomenida albula, Odontotarsus impictus, Odontotarsus angustatus, Amaurocoris candidus, Aelia melanota, Dolycoris penicillatus, Apodiphus integriceps, Menaccarus deserticola, Maccevethus corsicus persicus, Dicranomerus marginatus, Dicranomerus ferghanensis; & 10.07 \\
\hline & \begin{tabular}{l}
e) Kazakh-Northern \\
Turanian, IranoTuranian
\end{tabular} & 6 & Nabis palifer, Rhynocoris monticola monticola, Rhynocoris nigronitens, Spilostethus rubriceps, Bleteogonus beckeri, Geocoris scutellatus; & 4.03 \\
\hline & f) Tethys-Ethiopian & 2 & Hyalocoris pilicornis, Aethus pilosulus; & 1.34 \\
\hline \multirow[t]{6}{*}{IV. Endemics} & IV. Endemics & 13 & & 8.72 \\
\hline & a) Chinese-Central Asian & 2 & Nabis remanei, Tuponia roseipennis; & 1.34 \\
\hline & b) Chinese-IranoCentral Asian & 1 & Reduvius fedtschenkianus; & 0.67 \\
\hline & \begin{tabular}{l}
c) Turkestanian- \\
Northern Turanian
\end{tabular} & 6 & Stibaropus hohlbecki, Holcostethus nitidus, Lygus pachycnemis, Camptotylidea alba, Reduvius semenovi, Reduvius elegans; & 4.03 \\
\hline & d) Irano-TuranianCentral Asian & 3 & Vachiria deserta, Tingis leptochila, Oncocephalus termezanus; & 2.01 \\
\hline & e) Turanian & 1 & Enoplops eversmanni; & 0.67 \\
\hline Total: & & 149 & & 100 \\
\hline
\end{tabular}
species making up \(5.37 \%\) of subboreal and seven species accounting for \(4.70 \%\) of bore-al-subtropical species recorded. Sixteen Trans-Palaearctic species (10.74\%) have been recorded, followed by 13 Western (8.72\%), 12 North Setian (8.05\%), ten Pancontinental ( \(6.71 \%\) ), and four Pan-Atlantic ( \(2.68 \%\) ) species. The number of species with Ethiopia-Trans-Palaearctic-Eastern distributions and Narrow Continental is only two for each area or \(1.34 \%\). It can be seen that the prevailing part of the group, 125 species ( \(83.89 \%\) ), were found in wider areas of the Holarctic, and 13 are endemic species ( \(8.72 \%\) ).

The endemics are divided into Chinese-Central Asian, Chinese-Irano-Central Asian, Turkestanian-Northern Turanian, Irano-Turanian-Central Asian, and Turanian (found in Central Asia only). For the assessment of any territory, endemics have a high conservation value since they indicate the distinctive nature of the fauna.

\section*{Conclusions}

In this study, we collected new 39 species for the Khorezm region and 87 species for the Republic of Karakalpakstan during 2007-2020. In addition, we compare our collections with reports of Khamraev (2003) and Kulumbetova (1998a, b, c, 1999) and a total of 154 species ( 17 families) of terrestrial Heteroptera (Fig. 2, Table 1) were analyzed.

Khamraev (2003) identified 110 species for the Khorezm, and Kulumbetova (1998a, b, c, 1999) 62 species for the Republic of Karakalpakstan. There are currently 149 species of terrestrial Heteroptera in the Lower Amudarya. The results show that 62 species are highly abundant at the site, divided into 17 families and 89 genera, with the Miridae and Pentatomidae having most species ( 37 and 28 , respectively), followed by Reduviidae (15), Rhopalidae (10), Geocoridae (9), Rhyparochromidae (8), Nabidae (7), Coreidae, Lygaeidae, Cydnidae (6 each) (Table 2).

The Heteroptera fauna of Khorezm and Karakalpakstan can be divided into four groups: Cosmopolitan with one species ( \(0.67 \%\) ); Holarctic, with no more than ten species, or \(6.71 \%\); Palaearctic, with most of species ( 125 species, or \(83.89 \%\) ); and endemic with 13 species, or \(8.72 \%\).

An understanding of the fauna is important, as the productivity of crops is currently being negatively impacted by invasive species from neighboring countries. For example, recently we recorded (Gandjaeva et al. 2022b) the brown marmorated stink bug Halyomorpha halys (Stål, 1855) (Heteroptera: Pentatomidae) from Uzbekistan for the first time. Several adults and immatures were found in the Khorezm and Ferghana provinces. This species is native to East Asia (China, Korea, Japan, and Taiwan) (Rider et al. 2002; Hoebeke and Carter 2003; Rider 2006, 2016) and is a dangerous pest of many agricultural plants. Therefore, more study is required to examine the impacts of dispersion in a northern environment. In the north-western region of Uzbekistan, an increase in the number of invasive Heteroptera is expected, which will require careful monitoring.

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\section*{References}

Abdullaev II, Gandjaeva LA, Allabergenova KS, Kurbondurdiev ZJ, Razzakova RS (2020a) Biology and ecology of true bugs of the genus Eurydema Lap. Bulletin of the Khorezm Mamun Academy 10: 11-13. http://www.mamun.uz/bulletin/archive/2020/10 [In Russian]
Abdullaev II, Gandjaeva LA, Allabergenova KS (2020b) The cabbage bugs in the territory of the Amu Darya River (Heteroptera, Pentatomidae, Eurydema). Electronic Journal of Actual Problems of Modern Science. Education + Training 3: 266-278. http://khorezmscience.uz/public/upload/2020/3/26.pdf
Abdullaev U, Abdullaev I, Gandjaeva L (2020) The Social WASP Fauna of Riparian Tuqai Forest in Khorezm Region of Uzbekistan (Hymenoptera, Vespidae). International Journal of Current Research and Review 12 (14): 96-99. https://doi.org/10.31782/IJCRR.2020.121420
Abdullaev I, Gandjaeva I, Matyakubov Z, Doschanova M, Kholmatov B, Ruzmetov R, Iskandarov A, Otaev O, Ibragimov Sh, Atajanova Sh (2022) Survival of \(A\). abngerianus (Isoptera: Hodotermitidae) Under the Influence of External Factors (Relative Humidity and Temperature) and Use of Different Water Sources. WSEAS Transactions on Environment and Development 18: 1208-1215. https://doi.org/10.37394/232015.2022.18.113
Allabergenova KS, Gandzhaeva LA (2022) Mating, fecundity and egg development of the true bugs (Heteroptera, Pentatomidae). Scientific Review. Biological Sciences 3: 5-10. https:// doi.org/10.17513/srbs. 1288 [In Russian]
Animal World of Uzbekistan (2023) Animal World of Uzbekistan. http://www.orient-tracking. com/Fauna.htm [Accessed on 01.10.2023]
Aukema B, Rieger C, Rabitsch W (2013) Catalogue of the Heteroptera of the Palaearctic Region 6: Supplement. Netherlands Entomological Society, Amsterdam, 653 pp. https:// www.pemberleybooks.com/product/catalogue-of-the-heteroptera-of-the-palaearctic-re-gion-6-supplement/25071/
Bundy CS, McPherson JE (2018) Life history of Mecidea major with descriptions of nymphal instars (Hemiptera, Heteroptera, Pentatomidae). In: Wheeler Jr AG (Ed.) A Festschrift Recognizing Thomas J. Henry for a Lifetime of Contributions to Heteropteran Systematics. ZooKeys 796: 335-346. https://doi.org/10.3897/zookeys.796.21325
Catalogue of Palaearctic true bugs (2013) Catalogue of the palaearctic heteropteran. https:// catpalhet.linnaeus.naturalis.nl [Accessed on 01.10.2023]
Chandra K, Kushwaha S (2013) Distribution and diversity of Hemiptera fauna of Singhori Wildlife Sanctuary, Raisen District, Madhya Pradesh, India. Munis Entomology \& Zoology 8(2): 677-681. https://www.munisentzool.org/yayin/vol8/issue2/vol8issue2-431788.pdf
Dajoz R (2000) Précis d'écologie ( \(7^{\circ}\) edn.). Dumond, Paris, 615 pp. [In French]
Drapolyuk IS (2017) Ecological and zoogeographical analysis of capsid bug family (Insecta, Heteroptera, Miridae) of the Caucasus. South of Russia: ecology, development 12(1): 184190. https://doi.org/10.18470/1992-1098-2017-1-184-190 [In Russian]

Emelyanov AF (1974) Proposals on classification and nomenclature of ranges. Entomological Review 53(3): 497-522. https://www.zin.ru/animalia/coleoptera/rus/palearct.htm [In Russian]
Esenbekova PA (2013) True bugs (Heteroptera) of Kazakhstan. Nur-Print, Almaty, 349 pp. https://zool.kz/wp-content/uploads/2020/04/esenbekova2013_poluzhestkokrylye_kz.pdf [In Russian]

Gandjaeva LA (2011) Geographical distribution of cruciferous bugs (Eurydema maracandica Osh. and E. wilkinsi Dist.). Scientific conference of the Institute of Zoology, Tashkent, 109-110. [In Russian]
Gandjaeva LA (2012a) Dynamics of the number of larvae of cruciferous bugs on cultivated and wild cruciferous plants. Actual problems of the humanities and natural sciences 4(39): 25-26. https://cyberleninka.ru/article/n/dinamika-chislennosti-lichinok-krestotsvetnyh-klopov-na-kulturnyh-i-dikih-krestotsvetnyh-rasteniyah [In Russian]
Gandjaeva LA (2012b) Influence of the feeding plant on the rate of puberty, fertility and lifespan of bugs. FAN-Science journal of scientific works, 11-12. [In Russian] https://www. elibrary.ru/item.asp?id=17844710
Gandjaeva L (2019) Effect of sowing date on yield of winter wheat cultivars Grom, Asr and Kuma in Khorezm region. Bulgarian Journal of Agricultural Science 25(3): 474-479. https://www.agrojournal.org/25/03-07.pdf
Gandjaeva LA (2020) Effect of temperature on embryonic development of the Central Asian Cabbage bug. I International Multidisciplinary Conference "Recent Scientific Investigation". Primedia E-launch LLC. Shawnee, USA, 6-9. https://www.internauka.org/authors/ lola-gandjaeva
Gandjaeva LA, Abdullaeva SI (2022a) Species composition of cruciferous bugs on cabbage. Research of ways of improvement of scientific and technical potential of society in strategic period: Collection of articles of the International scientific-practical conference (Russia), May 2022. Magnitogorsk, Ufa, 22-24. https://os-russia.com/SBORNIKI/KON-436-2. pdf\#page=24 [In Russian]
Gandjaeva LA, Abdullaeva SI (2022b) The cruciferous bugs (Miridae). Conceptions for sustainable development of science in modern conditions: Proceedings of the International Scientific and Practical Conference (Novosibirsk), Part 2. June 2022. Ufa, 15-17. https:// os-russia.com/SBORNIKI/KON-437-2.pdf\#page=15 [In Russian]
Gandjaeva LA, Allabergenova K (2022) Ecological groups of cruciferous bugs. Bulletin of the Khorezm Mamun Academy 7(1): 42-45. http://www.mamun.uz/bulletin/archive/2022/7 [In Russian]
Gandjaeva LA, Nurullaeva MSh, Jumanazarova NR, Ruzmatova MI, Boltaeva S (2019) Geographical distribution of cruciferous bugs (E. maracandica Osh. and E. wilkinsi Dist). Materials of the International Scientific and Practical Conference Actual issues of theory and practice of scientific research development, 4, Ufa, 61-63. https://www.elibrary.ru/ item.asp?id=41576582 [In Russian]
Gandjaeva LA, Abdullaev II, Allabergenova K (2020a) Study of ranges of Cruciferous Bugs. Bulletin of the Khorezm Mamun Academy 8: 36-38. http://www.mamun.uz/bulletin/archive/2020/8 [In Russian]
Gandjaeva LA, Abdullaev II, Abdullaeva SB (2020b) Analysis of population dynamics of the Central Asian Bugs on agricultural crops in the territory of the Lower Amudarya River (Heteroptera, Pentatomidae, Eurydema). Scientific Review. Biological Sciences 3: 94-100. https://doi.org/10.17513/srbs. 1203 [In Russian]
Gandjaeva LA, Abdullaev II, Razzakov KB (2020c) Characteristic of Identified Insect species on Cabbage in the Conditions of Khorezm Region. Scientific Review. Biological Sciences 4: 5-11. https://doi.org/10.17513/srbs. 1206 [In Russian]

Gandjaeva LA, Abdullaev I, Razzakov K, Allabergenova K (2020d) Climate impact on the population dynamics of Cruciferae Bugs (Heteroptera, Pentatomidae, Eurydema). EurAsian Journal of BioSciences 14: 3349-3358. http://www.ejobios.org/download/climate-impact-on-the-population-dynamics-of-cruciferae-bugs-heteroptera-pentatomidae-eu-rydema-7940.pdf
Gandjaeva LA, Ismayilova I, Saidova S (2020e) The Central Asian Cabbage Bugs. Tendenze attuali della moderna ricerca scientifica: der Sammlung wissenschaftlicher Arbeiten « \(\Lambda\) О́ГО \(\Sigma » ~ z u ~ d e n ~ M a t e r i a l i e n ~ d e r ~ i n t e r n a t i o n a l e n ~ w i s s e n s c h a f t l i c h-p r a k t i s c h e n ~ K o n f e r e n z . ~\) 2, Stuttgart, Germany, 122-123. https://doi.org/10.36074/05.06.2020.v2.50
Gandjaeva LA, Abdullaev II, Allabergenova K (2021) True Bugs in the Lower Amu Darya. Bulletin of the Khorezm Mamun Academy 5: 42-46. http://www.mamun.uz/bulletin/archive/2021/5 [In Russian]
Gandjaeva LA, Abdullayev II, Bobojonova XM, Iskandarov AI (2022a) Occurrence of the true bugs (Heteroptera) in a variety of habitats. Scientific Review. Biological Sciences 3: 10-15. https://doi.org/10.17513/srbs. 1277 [In Russian]
Gandjaeva LA, Hudayberdieva MO, Abdullaev II, Mirzayeva GS, Yusupboev EK (2022b) First record of Halyomorpha halys (Heteroptera: Pentatomidae) from Uzbekistan. Zoosystematica Rossica 31(2): 329-331. https://doi.org/10.31610/zsr/2022.31.2.329
Gandjaeva LA, Yusupova SK, Doschanov JS (2022c) Trophic relationships of bread bugs (Heteroptera). Bulletin of the Khorezm Mamun Academy 7(1): 45-48. http://www.mamun. uz/bulletin/archive/2022/7 [In Russian]
Gapon DA (2019) The brown marmorated stink bug Halyomorpha halys (Stål, 1855) (Heteroptera: Pentatomidae): expansion of its range in the European part of Russia, description of the imago and larvae, and the diagnostics of the species. Caucasian Entomological Bulletin 15(2): 241-247. https://doi.org/10.23885/181433262019152-241247
Henry TJ (2017a) Biodiversity of Heteroptera. In: Foottit RG, Adler PH (Eds) Insect Biodiversity. Science and Society (Vol. I. 2nd edn.). Wiley-Blackwell Press, Oxford, 904 pp. https:// doi.org/10.1002/9781118945568.ch10
Henry TJ (2017b) First record of the Palaearctic plant bug Rhabdomiris striatellus (Fabricius) (Heteroptera: Miridae: Mirinae) in North America. Proceedings of the Entomological Society of Washington 119(4): 575-579. https://doi.org/10.4289/00138797.119.4.575

Hoebeke ER, Carter ME (2003) Halyomorpha halys (Stal) (Heteroptera: Pentatomidae): A polyphagous plant pest from Asia newly detected in North America. Proceedings of the Entomological Society of Washington 105: 225-237. https://www.cabdirect.org/cabdirect/ abstract/20033040299
Iskandarov A, Abdullaev I, Gandjaeva L, Musaev D, Mirzayeva G, Kholmatov B, Jumanazarov H, Jangabaeva A, Razzakov K, Abdullaev U (2022) Updated Checklist of the Pentatomidea (Heteroptera: Pentatomomorpha) of Uzbekistan. WSEAS Transactions on Environment and Development 18: 1283-1295. https://doi.org/10.37394/232015.2022.18.121
Khamraev ASh (2003) Soil organisms and entomocomplexes in Khorezm and Karakalpakstan (Uzbekistan). Tashkent. ZEF Work Papers for Sustainable Development in Central Asia, 63 pp. https://www.zef.de/fileadmin/webfiles/downloads/projects/khorezm/downloads/ Publications/wps/ZEF-UZ-WP06-Khamraev1.pdf [In Russian]

Kim J, Jung S (2018) Three new records of the subfamily Mirinae (Hemiptera: Heteroptera: Miridae) from the Korean Peninsula. Journal of Asia-Pacific Biodiversity 11(2): 255-258. https://doi.org/10.1016/j.japb.2018.04.003
Kulumbetova TT (1998a) Insects of the Southern Aral Sea (part 1). Bulletin of the Karakalpakstan 1: 66-68. [In Russian]
Kulumbetova TT (1998b) Insects of the Southern Aral Sea (part 2). Bulletin of the Karakalpakstan 4: 64-66. [In Russian]
Kulumbetova TT (1998c) Insects of the Southern Aral Sea (part 3). Bulletin of the Karakalpakstan 1:37-39. [In Russian]
Kulumbetova TT (1999) Insects of the Southern Aral Sea (part 4). Bulletin of the Karakalpakstan 1: 62-69. [In Russian]
Kuzhuget SV, Vinokurov NN (2018) Types of areal of Heteroptera from Tuva, Russia. Evraziatskii Entomologicheskii Zhurnal 17(2): 103-109. https://doi.org/10.15298/euroasentj.17.2.05 [In Russian]
Latreille PA (1810) Ordre III. Hemipteres. Section Premiere. In: Heteropteres. Considerations generales des Crustaces, Arachnides et Insectes sur l'ordre naturel des animaux composant les classes des Crustaces des Arachnides et des Insectes, avec un tableau methodique de leurs genres, disposes en families. F. Schoell, Paris, 250-434. https://doi.org/10.5962/bhl. title. 34917 [In French]
Leach WE (1815) Entomology. The Edinburgh Encyclopedia 9 (1): 57-172. https://zoobank. org/NomenclaturalActs/6B4353F6-7682-4CB6-97D0-8548009000D3
Lopatin IK, Meleshko JE (2016) Zoogeography (with electronic application). BSU, Minsk, 53-55. https://elib.bsu.by/handle/123456789/158509 [In Russian]
Oh M, Yasunaga T, Lee S (2017) Taxonomic review of Phytocoris Fallén (Heteroptera: Miridae: Mirinae: Mirini) in Korea, with one new species. Zootaxa 4232(2): 197-215. https://doi. org/10.11646/zootaxa.4232.2.4
Oshanin VF (1891) Zoogeographical character of the fauna of True bugs of Turkestan. SanktPeterburg 23(1): 116. [In Russian]
Panizzi AR, Grazia J (2015) Introduction to True Bugs (Heteroptera) of the Neotropics. In: Panizzi A, Grazia J (Eds) True Bugs (Heteroptera) of the Neotropics. Entomology in Focus, 2: 3-20. https://doi.org/10.1007/978-94-017-9861-7_1
Rider DA (2006) Family Pentatomidae Leach, 1815, 233-402. In: Aukema B, Rieger Ch (Eds) Catalogue of the Heteroptera of the Palaearctic Region. The Netherlands Entomological Society, 500-550.
Rider DA (2016) Aeliavuori linnacostatus, a new genus and species of Pentatomidae from the Democratic Republic of the Congo (Hemiptera: Heteroptera: Pentatomidae: Pentatominae: Carpocorini). Entomologica Americana 122(1-2): 212-219. https://doi.org/10.1664/15-RA-038
Rider DA, Zheng LY, Kerzhner IM (2002) Checklist and nomenclatural notes on the Chinese Pentatomidae (Heteroptera). II. Pentatomina. Zoosystematica Rossica 11(1): 135-153. https://doi.org/10.31610/zsr/2002.11.1.135
Ruzmetov R, Abdullaev I, Gandjaeva L, Matyakubov Z, Razzakov K, Iskandarov A, Otaev O, Ibragimov Sh (2022) Fundamentals of using Geographical Information Systems in predicting the distribution of Helicoverpa armigera (Lepidoptera: Noctuidae). Biodiversitas 23(6): 3251-3256. https://doi.org/10.13057/biodiv/d230653

Samra S, Ghanim M, Protasov A, Mendel Z (2015) Development, reproduction, host range and geographical distribution of the variegated caper bug Stenozygum coloratum (Hemiptera: Heteroptera: Pentatomidae).EJE 112(2):362-372.https://doi.org/10.14411/eje.2015.041
Saprykin MA (2013) Ecological and faunistic analysis of water bugs and water striders (Heteroptera: Nepomorpha, Gerromorpha) of the Northwestern Caucasus. PhD thesis, Kuban State university, Krasnodar city, 253 pp. https://doi.org/10.13140/2.1.4042.7523
Schuh RT, Weirauch C (2020) True Bugs of the World (Hemiptera: Heteroptera): Classification and Natural History (2 \(2^{\text {nd }}\) edn.). (Monographs Series, vol. 8). Siri Scientific Press, Manchester, 800 pp . https://www.nhbs.com/true-bugs-of-the-world-hemiptera-heteroptera-book
Vinokurov NN, Kanyukova EV, Golub VB (2010) Catalogue of true bugs insects (Heteroptera) of Asian part. Nauka, Novosibirsk, 317 pp. [In Russian]
Vinokurov NN, Golub VB, Zinovieva AN (2015) Plant bugs (Heteroptera, Miridae) of the South Urals State Natural Reserve. I. Bryocorinae, Deraeocorinae, Mirinae. Bulletin of Belgorod State University. Series Natural Sciences, BGNIU 15 (212): 84-93. https://cy-berleninka.ru/article/n/klopy-slepnyaki-heteroptera-miridae-yuzhno-uralskogo-gosudarst-vennogo-prirodnogo-zapovednika-i-bryocorinae-deraeocorinae-mirinae [In Russian]
Weirauch C, Schuh RT (2011) Systematic and Evolution of Heteroptera: 25 years of progress. Annual Review of Entomology 56(1): 487-510. https://doi.org/10.1146/annurev-ento-120709-144833
Yakovlev VE (1890) Fauna of True bugs of Russia and neighboring countries. Russian Entomology Society 24: 311-348. [In Russian]
Yasunaga T (2016) A new mirine plant bug genus (Heteroptera: Miridae: Mirinae: Mirini), with two confirmed species from Nepal and Taiwan. Tijdschrift voor Entomologie 159(3): 209-216. https://doi.org/10.1163/22119434-15903002
Yazici G (2020) Overview of the Zoogeographical Distribution of Aquatic and Semi-Aquatic Heteroptera (Hemiptera) in Turkey. Journal of Insect Biodiversity and Systematics 6(2): 135-155. https://doi.org/10.52547/jibs.6.2.135
Yusupova SK, Gandjaeva LA (2022) Species composition of bread bugs (Heteroptera) on triticale. Research of ways of improvement of scientific and technical potential of society in strategic period: Collection of articles of the International scientific-practical conference (Magnitogorsk), Part 2, May 2022. Ufa, 20-22. https://www.elibrary.ru/item. asp?id=48728365 [In Russian]
Yusupova SK, Gandzhaeva LA, Doschanov ZhS (2022) True bugs in triticale agrocenoses. Scientific Review. Biological Sciences 3: 57-62. https://doi.org/10.17513/srbs. 1285 [In Russian]
Zaime A, Gautier JY (1989) Comparaison des régimes alimentaires de trois espèces sympatriques de Gerbillidae en milieu saharien au Maroc. Revue d'Ecologie (Terre et vie) 44(2): 153-163. https://hal.science/hal-01320881

\title{
Two new species of Dixonius from Vietnam and Laos with a discussion of the taxonomy of Dixonius (Squamata, Gekkonidae)
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\begin{abstract}
Integrated analyses using maximum likelihood (ML), Bayesian inference (BI), principal component analysis (PCA), discriminate analysis of principal components (DAPC), multiple factor analysis (MFA), and analysis of variance (ANOVA) recovered two new diagnosable species of gekkonid lizards in the genus Dixonius, one from the Central Highlands, Gia Lai Province, Vietnam and another from the Vientiane Province, Laos. Phylogenetic analyses based on the mitochondrial NADH dehydrogenase subunit 2 gene (ND2) and adjacent tRNAs showed that Dixonius gialaiensis sp. nov. is the sister species of \(D\). minhlei from Dong Nai Province, Vietnam and is nested within a clade that also includes the sister species \(D\). siamensis and \(D\). somchanhae. Dixonius muangfuangensis sp. nov. is the sister species to \(D\). lao from Khammouane Province, Laos and is embedded in a clade with \(D\). vietnamensis, \(D\). taoi, and undescribed species from Thailand. Multivariate (PCA, DAPC, and MFA) and univariate (ANOVA) analyses using combina-
\end{abstract}
tions of 15 meristic (scale counts), six morphometric (measurements), and five categorical (color pattern and morphology) characters from 44 specimens encompassing all eight species of Dixonius from Vietnam and Laos clearly illustrate Dixonius gialaiensis sp. nov. and Dixonius muangfuangensis sp. nov. are statistically different and discretely diagnosable from all closely related species of Dixonius. These integrative analyses also highlight additional taxonomic issues that remain unresolved within Dixonius and the need for additional studies. The discovery of these new species further emphasizes the underappreciated herpetological diversity of the genus Dixonius and illustrates the continued need for field work in these regions.

\section*{Keywords}

Gekkota, Indochina, integrative taxonomy, molecular phylogeny, morphology, new species, Southeast Asia

\section*{Introduction}

The genus Dixonius was established by Bauer et al. (1997) to contain two species, D. melanostictus (Taylor, 1962) and D. siamensis (Boulenger, 1898), with a distribution range through Myanmar, Thailand, Laos, Vietnam, and Cambodia. Currently, thirteen species have been recognized worldwide (Nguyen et al. 2020, 2021; Pauwels et al. 2021; Uetz et al. 2022). In Vietnam, six species of Dixonius have been documented, including four originally described from the country, i.e., D. vietnamensis (Das 2004) from Khanh Hoa and Binh Thuan provinces, D. aaronbaueri (Ngo and Ziegler 2009) from Ninh Thuan and Binh Thuan provinces, D. taoi (Botov, Phung, Nguyen, Bauer, Brennan \& Ziegler, 2015) from Binh Thuan Province, D. minhlei (Ziegler, Botov, Nguyen, Bauer, Brennan, Ngo \& Nguyen, 2016) from Dong Nai Province, and two from outside Vietnam, D. siamensis from Thailand and Cambodia and D. melanostictus from Thailand (Uetz et al. 2022). Lastly, in Laos, there are three species (D. siamensis, D. lao (Nguyen, Sitthivong, Ngo, Luu, Nguyen, Le \& Ziegler, 2020), D. somchanhae (Nguyen, Luu, Sitthivong, Ngo, Nguyen, Le \& Ziegler, 2021)) two of which, D. lao from Vientiane Capital and \(D\). somchanhae from Khammouane Province, were described within the last five years (Fig. 1).

During a recent herpetofaunal surveys in Chu Se Mountain Pass, Hbong Commune, Gia Lai Province in Vietnam and Vientiane Province in Laos, new populations of Dixonius were found at each location (Fig. 1). Based on phylogenetic evidence from the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene and adjacent tRNAs, morphometric, meristic, and color pattern data, neither can be ascribed to any known species and as such they are described below as new species.

\section*{Materials and methods}

A total of six Dixonius specimens were caught by hand from Gia Lai Province, Vietnam and Vientiane Province, Laos. The specimens were fixed in approximately \(80 \%\) ethanol and then transferred to \(70 \%\) ethanol for permanent storage. Tissue samples taken before the specimens were preserved were stored separately in \(95 \%\) ethanol.


Figure I. Location of the type localities of all known species of Dixonius. The inset delimits the study area. 1 Dixonius aaronbaueri from Ninh Thuan Province, Vietnam; 2 D. dulayaphitakorum from Ranong Province, Thailand; \(\mathbf{3}\) D. mekongensis from Ubon Ratchathani Province, Thailand; 4 D. hangseesom from Kanchanaburi Province, Thailand; 5 D. kaweesaki from Prachuap Khiri Khan Province, Thailand; 6 D. pawangkhananti from Phetchaburi Province, Thailand; 7 D. lao from Khammouane Province, Laos; 8 D. melanostictus from Nakhon Ratchasima Province, Thailand; 9 D. minhlei from Dong Nai Province, Vietnam; \(\mathbf{1 0}\) D. siamensis from SaraBuri and Nakhon Ratchasima provinces, Thailand; \(\mathbf{1 1}\) D. somchanhae from Vientiane Capital, Laos; 12 D. taoi from Binh Thuan Province, Vietnam; 13 D. vietnamensis from Khanh Hoa Province, Vietnam; \(\mathbf{1 4}\) D. muangfuangensis sp. nov. from Vientiane Province, Laos; 15 D. gialaiensis sp. nov. from Gia Lai Province, Vietnam.

The specimens have been deposited in the collection of the Vietnam National University of Forestry (VNUF), Hanoi, Vietnam and the National University of Laos (NUOL), Vientiane, Laos.

\section*{Species delimitation}

The general lineage concept (GLC: de Queiroz 2007) adopted herein proposes that a species constitutes a population of organisms evolving independently from other such populations owing to a lack of, or limited gene flow. By "independently," it is meant that new mutations arising in one species cannot spread readily into another species (Barraclough et al. 2003; de Queiroz 2007). Molecular phylogenies recovered multiple monophyletic mitochondrial lineages of individuals (populations) that were used to develop initial species-level hypotheses, the grouping stage of Hillis (2019). Discrete color pattern data and univariate and multivariate analyses of morphological data were then used to search for characters and morphospatial patterns consistent with the tree-designated species-level hypotheses, the construction of boundaries representing the hypothesistesting step of Hillis (2019), thus providing independent diagnoses to complement the molecular analyses. In this way, delimiting (phylogeny) and diagnosing (taxonomy) species are not conflated (Frost and Hillis 1990; Frost and Kluge 1994; Hillis 2019).

\section*{Molecular data and phylogenetic analyses}

Four samples of the newly collected specimens were analyzed, two from Gia Lai Province, Vietnam (VNUF R. 2020.22 - field number GL.02, VNUF R. 2020.33 - field number GL.03) and two from Vientiane Province, Laos (VNUF R. 2022.42 - field number MF.02, VNUF R. 2022.52 - field number MF.03). We used the protocols of Nguyen et al. (2021) for DNA extraction, amplification, and sequencing. The complete NADH dehydrogenase subunit 2 (ND2) gene with six partial or complete adjacent tRNAs, approximately 1200 bp long, respectively, were amplified and sequenced using the primer pair, MetF1(5'-AAGCTTTCGGGCCCATACC-3') and COIR1(5'-AGRGTGCCAATGTCTTTGTGRTT-3’) (Macey et al. 1997). Genomic DNA was extracted from all liver tissues stored in ethanol following the standard protocols of DNeasy blood and tissue kit, Qiagen (California, USA). The PCR volume consisted of \(20 \mu \mathrm{l}\) ( \(1 \mu \mathrm{l}\) each primer, \(7 \mu \mathrm{l}\) water, \(10 \mu \mathrm{l}\) of Taq mastermix and \(1 \mu \mathrm{l}\) DNA template). PCR conditions were: \(95^{\circ} \mathrm{C}\) for 5 min , followed by 42 cycles: \(95^{\circ} \mathrm{C}\) for \(30 \mathrm{~s}, 50^{\circ} \mathrm{C}\) for 45 s and \(72^{\circ} \mathrm{C}\) for 60 s with a final elongation step for 6 min at \(72^{\circ} \mathrm{C}\). PCR products were visualized using electrophoresis through a \(1.2 \%\) agarose gel, marker \(100 \mathrm{bp}, 1 \mathrm{X}\) TAE and stained with RedSafe Nucleic Acid Staining Solution and photographed under UV light of Geldoc system (Quantum CX5, Villber, France). Successful amplifications were purified using innuPREP Gel Extraction Kit (Analytik Jena, Germany). Cleaned PCR products were sent to \(1^{\text {st }}\) Base (Malaysia) for sequencing in both directions.

We obtained 1,444 base pairs of NADH dehydrogenase subunit 2 gene (ND2) sequence data and the flanking tRNAs from 29 ingroup individuals of Dixonius
representing 13 nominal species including the new samples from Vietnam and Laos. Heteronotia spelea was used as an outgroup to root the tree based on the phylogenetic results generated by Gamble et al. (2015). Sequence data for other species were acquired from GenBank. Newly generated sequences were deposited in GenBank (Table 1).

Maximum likelihood (ML) and Bayesian inference (BI) were used to estimate phylogenetic trees. Best-fit models of evolution determined in IQ-TREE (Nguyen et al. 2015) using the Bayesian information criterion (BIC) implemented in ModelFinder (Kalyaanamoorthy et al. 2017) indicated that \(\mathrm{F} 81+\mathrm{F}\) was the best-fit model of evolution for the tRNAMET and K2P+I, and HKY+F+G4 were the best models of evolution for tRNAs2 and ND2, respectively. The ML analysis was performed using the IQ-TREE webserver (Trifinopoulos et al. 2016) with 1000 bootstrap pseudoreplicates using the ultrafast bootstrap (UFB) analysis (Minh et al. 2013; Hoang et al. 2018). The BI analysis was performed on CIPRES Science Gateway (Miller et al. 2010) using MrBayes v. 3.2.4 (Ronquist et al. 2012). Two independent runs were performed using Metropolis-coupled Markov Chain Monte Carlo (MCMCMC), each with four chains: three hot and one cold. The MCMCMC chains were run for \(80,000,000\) generations with the cold chain sampled every 8000 generations and the first \(10 \%\) of each run being discarded as burn-in. The posterior distribution of trees from each run was summarized using the sumt function in MrBayes v. 3.2.4 (Ronquist et al. 2012). Stationarity was checked with Tracer v. 1.6 (Rambaut et al. 2014) to ensure effective sample sizes (ESS) for all parameters were well above 200. We considered Bayesian posterior probabilities (BPP) of 0.95 and above and ultrafast bootstrap support values (UFB) of 95 and above as an indication of strong nodal support (Huelsenbeck et al. 2001; Minh et al. 2013). Uncorrected pairwise sequence divergences (p-distance) were calculated in MEGA 11 (Kumar et al. 2016) using the complete deletion option to remove gaps and missing data from the alignment prior to analysis.

A time-calibrated Bayesian phylogenetic tree was estimated using BEAST 2 (Bayesian Evolutionary Analysis by Sampling Trees) v. 2.7.3 (Drummond et al. 2012) implemented in CIPRES (Cyberinfrastructure for Phylogenetic Research; Miller et al. 2010) where the ingroup node subtending the split between Dixonius aaronbaueri and the remaining species was given a 24.04 mya prior with an offset range of 20.23-27.68 mya following Gamble et al. (2015). The split between Heteronotia and Dixonius was set at 45.0 mya with an offset range of 33.3-56.8 mya (Gamble et al. 2015). An input file was constructed in BEAUti (Bayesian Evolutionary Analysis Utility) v. 2.7.3. An optimized relaxed clock with unlinked site models, linked clock models and linked trees, and a calibrated Yule prior were employed for the species level. BEAST Model Test (Bouckaert and Drummond 2017), implemented in BEAST, was used to numerically integrate over the uncertainty of substitution models while simultaneously estimating phylogeny using Markov chain Monte Carlo (MCMC). MCMC chains were run for 80 million generations and logged every 8,000 generations. The BEAST log file was visualized in Tracer v. 1.7.2 (Rambaut et al. 2014) to ensure effective sample sizes (ESS) were above 200 for all parameters. A maximum clade credibility tree using mean heights at the nodes was generated using TreeAnnotator v. 2.7.3 (Rambaut and

Table I. List of specimens used for the phylogenetic analyses.
\begin{tabular}{|c|c|c|c|}
\hline Species & Catalog no. & Location & GenBank no. \\
\hline Dixonius aaronbaueri & ZFMK87274 & Nui Chua NP, Ninh Thuan Province, southern Vietnam & HM997152 \\
\hline \multirow[t]{2}{*}{Dixonius gialaiensis sp. nov.} & \begin{tabular}{l}
VNUF R. 2020.22 \\
(Field no. GL.02)
\end{tabular} & Chu Se District, Gia Lai Province, Vietnam & OQ819041 \\
\hline & \begin{tabular}{l}
VNUF R. 2020.33 \\
(Field no. GL.03)
\end{tabular} & Chu Se District, Gia Lai Province, Vietnam & OQ8190412 \\
\hline \multirow[t]{3}{*}{Dixonius lao} & VNUF R.2016.2 & Khammouane Province, Laos & MT024681 \\
\hline & IEBR A.2019.5 & Khammouane Province, Laos & MT024683 \\
\hline & IEBR A. 2019.6 & Khammouane Province, Laos & MT024682 \\
\hline Dixonius melanostictus & VU 022 & Captive, Thailand & HM997153 \\
\hline Dixonius minhlei & ZFMK 97745 & Vinh Cuu, Dong Nai Province, Vietnam & KX379194 \\
\hline \multirow[t]{2}{*}{Dixonius muangfuangensis sp. nov.} & \begin{tabular}{l}
VNUF R. 2020.42 \\
(Field no. MF02)
\end{tabular} & Muangfuang District, Vientiane Province, Central Laos & OQ818586 \\
\hline & \begin{tabular}{l}
VNUF R. 2020.52 \\
(Field no. MF03)
\end{tabular} & Muangfuang District, Vientiane Province, Central Laos & OQ818587 \\
\hline Dixonius cf. siamensis & VU 023 & Captive, Thailand & KX379195 \\
\hline \multirow[t]{3}{*}{Dixonius siamensis} & LSUHC 7328 & Phnom Aural, Purset Province, Cambodia & EU054299 \\
\hline & FMNH 263003 & Keo Seima District, Mondolkiri- Province, Cambodia & EU054298 \\
\hline & LSUHC 7378 & Phnom Aural, Purset Province, Cambodia & KP979732 \\
\hline \multirow[t]{7}{*}{Dixonius somchanhae} & VNUF R. 2020.2 & Nasaithong District, Vientiane Capital, Laos & MW605166 \\
\hline & VNUF R.2020.1 & Nasaithong District, Vientiane Capital, Laos & MW605165 \\
\hline & VNUF R.2020.3 & Nasaithong District, Vientiane Capital, Laos & MW605167 \\
\hline & \begin{tabular}{l}
VNUF R. 2020.55 \\
(Field no. VT05)
\end{tabular} & Vientiane Capital, Laos & OQ818589 \\
\hline & \begin{tabular}{l}
VNUF R. 2020.54 \\
(Field no. VT04)
\end{tabular} & Vientiane Capital, Laos & OQ818588 \\
\hline & \begin{tabular}{l}
VNUF R.2020.59 \\
(Field no.VT09)
\end{tabular} & Vientiane Capital, Laos & OQ818591 \\
\hline & \begin{tabular}{l}
VNUF R. 2020.56 \\
(Field no. VT0T06)
\end{tabular} & Vientiane Capital, Laos & OQ818590 \\
\hline Dixonius sp. & LSUHC 9466 & Sai Yok, Kanchanaburi Province, Thailand & KX379196 \\
\hline \multirow[t]{4}{*}{Dixonius taoi} & ZFMK 96680 & Phu Quy Island, Binh Thuan Province, Vietnam & KP979733 \\
\hline & CAS 257300 & Phu Quy Island, Binh Thuan Province, Vietnam & KP979734 \\
\hline & IEBR A 2014-26 & Phu Quy Island, Binh Thuan Province, Vietnam & KP979735 \\
\hline & IEBR A 2014-27 & Phu Quy Island, Binh Thuan Province, Vietnam & KP979736 \\
\hline Dixonius cf. vietnamensis & ZFMK 87273 & Nui Chua, Ninh Thuan Province, Vietnam & KX379201 \\
\hline Dixonius vietnamensis & IEBR R. 20163 & Nha Trang, Khánh Hòa Province, Vietnam & KX379198 \\
\hline
\end{tabular}

Drummond 2013) with a burn-in of the first \(10 \%\) of each run. Nodes with Bayesian posterior probabilities (BPP) of 0.95 and above were considered strongly supported (Huelsenbeck et al. 2001; Wilcox et al. 2002).

\section*{Morphological data and analysis}

The morphological data set comprised six closely related species including six type specimens of Dixonius minhlei from Dong Nai Province, Vietnam (IEBR A.0801-02, VNMN R.2016.1-2, ZFMK 97745-46), three type specimens of D. lao from Khammouane Province, Laos (VNUF R.2016.2, IEBR A.2016.5-6), eight specimens of D. siamensis from Pursat Province, Cambodia (LSUHC 07328, 07378, 08420, 08487,

08491, 08522, 09284, 09289), five type specimens of D. somchanhae from Vientiane Capital, Laos (VNUF R.2020.1-5), four specimens of D. sp. from Gia Lai Province, Vietnam, and 12 specimens of D. vietnamensis from Nha Trang Province, Vietnam (ZRC 2.6024-27, IEBR R.2016.1, 2016.3, 2016.4, VNMN R.2016.3-4, ZFMK 97747-49).

Morphological data included both meristic and morphometric characters. Morphological characters were taken from the 44 specimens following Bauer et al. (2004) and Ngo and Ziegler (2009). Morphometric characters were taken after preservation with a digital caliper to the nearest 0.1 mm under a zoom stereomicroscope on the right/left of the body. Recorded data included: SVL: snout-vent length (taken from the tip of the snout to the vent), TaL: tail length (taken from the vent to the tip of the tail, original or partially regenerated), TW: tail width (taken at the base of the tail immediately posterior to the postcloacal swelling), BW: body width (greatest width of torso, taken at the level of midbody), HL: head length (the distance from the posterior margin of the retroarticular process of the lower jaw to the tip of the snout), HW: head width (the distance from the posterior margin of the retroarticular process of the lower jaw to the tip of the snout), HD: head depth (the maximum height of head measured from the occiput to base of the lower jaw), EL: ear length (greatest oblique length across the auditory meatus), TBL: Tibia length (taken on the ventral surface from the posterior surface of the knee while flexed \(90^{\circ}\) to the base of the heel), AG: axilla to groin length (taken from the posterior margin of the forelimb at its insertion point on the body to the anterior margin of the hind limb at its insertion point on the body), FA: forearm length (taken on the ventral surface from the posterior margin of the elbow while flexed \(90^{\circ}\) to the inflection of the flexed wrist), ED: eye diameter (the greatest horizontal diameter of the eye-ball), EN: eye nostril distance (measured from the anterior margin of the bony orbit to the posterior margin of the external nares), ES: eye snout distance (measured from anteriormost margin of the bony orbit to the tip of snout), EE: eye ear distance (measured from the anterior edge of the ear opening to the posterior edge of the bony orbit), IN: internarial distance (measured between the external nares across the rostrum), IO: interorbital distance (measured between the dorsal-most edges of the bony orbits).

Meristic data taken were: \(\mathbf{V}\) : ventral scales (counted transversely across the abdomen midway between limb insertions from one ventrolateral fold to the other), DTR: longitudinal rows of dorsal tubercles (counted transversely across the body midway between the limb insertions from one ventrolateral body fold to the other), PV: paravertebral scales (counted in a paravertebral row from first scale posterior to parietal scale to last scale at the level of vent opening), PV': paravertebral scales (counted in a row between limb insertions), T4: lamellae under fourth toe (counted from the distal scale containing claw to basal scale that broadly contacts adjacent fragmented scales), IOS: Interorbital scales (counted at narrowest point between orbits), ICS: interciliary scales (counted between supraciliaries at midpoint of orbit), SPL: supralabials (counted from the largest scale at the corner of the mouth to the rostral scale), IFL: infralabials (counted from termination of enlarged scales at the corner of the mouth to the mental scale), MO: number of supralabial at midorbital position, PP: precloacal pores in males.

Color pattern on dorsum including the presence or absence of canthal stripes (CanthStrp), the presence or absence of strong darkly barred lips (LipBar), the presence or absence of dark-colored round blotches on the top of the head (RdHdBlch) and dorsum (RdBodBlch), and the presence or absence of two regularly arranged whitish tubercles on flanks (Tub). The raw morphological data for all characters and specimens are presented in Tables 2, 3.

All statistical analyses were performed using R v. 4.2.1 (R Core Team, 2021). Morphometric characters used in the statistical analyses were SVL, BW, HL, HW, HD, EL, ED, EN, ES, EE, IN, IO, FAr, TBLr, and AGr. Tail metrics were not used due to the high degree incomplete sampling (i.e., regenerated, broken, or missing). To remove potential effects of allometry on morphometric traits (sec. Chan and Grismer 2022), we used the following equation: \(\mathrm{Xadj}=\log (\mathrm{X})-\beta[\log (\mathrm{SVL})-\log (\) SVLmean \()]\), where Xadj = adjusted value; \(X=\) measured value; \(\beta=\) unstandardized regression coefficient for each population; and SVLmean = overall average SVL of all populations (Thorpe 1975, 1983; Turan 1999; Lleonart et al. 2000, accessible in the R package GroupStruct (available at https://github.com/chankinonn/ GroupStruct). The morphometrics of each species were normalized separately and then concatenated into a single data set so as not to conflate potential intra- with interspecific variation (Reist 1986; McCoy et al. 2006). All data were scaled to their standard deviation to ensure they were analyzed on the basis of correlation and not covariance. Meristic characters (scale counts) used in statistical analyses were SPLr/l, IFLr/l, MO, IOS, ICS, V, DTR, and T4r/l. Precloacal and femoral pores were omitted from the analyses due to their absence in females. Categorical characters analyzed were CanthStrp, LipBar, RdHdBlch, RdBodBlch, and Tub.

A Levene's test for normalized morphometric and meristic characters was conducted to test for equal variances across all groups. Analyses of variance (ANOVA) were conducted on meristic and normalized morphometric characters (see below) with statistically similar variances to search for the presence of statistically significant mean differences ( \(\mathrm{p}<0.05\) ) among species across the data set. Characters bearing statistical differences were subjected to a TukeyHSD test to ascertain which species pairs differed significantly from each other for those particular characters. Boxplots were generated for discrete meristic characters in order to visualize the range, mean, median, and degree of differences between pairs of species bearing statistically different mean values and violin plots were generated for continuous morphometric characters to visualize the same.

Morphospatial positions were visualized using principal component analysis (PCA) from the ADEGENET package in R (Jombart et al. 2010) to determine if their positioning was consistent with the putative species boundaries delimited by the molecular phylogenetic analyses and defined by the univariate analyses (see above). PCA, implemented using the "prcomp()" command in R, is an indiscriminate analysis plotting the overall variation among individuals (i.e., data points) while treating each individual independently (i.e., not coercing data points into pre-defined groups). Subsequent to the PCA, a discriminant analysis of principle components (DAPC) was used to test

Table 2. Sex and raw meristic and categorical data used in the analyses from specimens of Dixonius from Vietnam and Laos. \(m=\) male; \(f=\) female; \(j=j u v e n i l e ; ~ r / l=r i g h t / l e f t . ~\)
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline \multirow[t]{2}{*}{Species} & \multirow[t]{2}{*}{Museum no.} & \multirow[t]{2}{*}{Sex} & \multicolumn{6}{|c|}{Meristic data} & \multicolumn{5}{|c|}{Categorical data} \\
\hline & & & \[
\begin{gathered}
\mathrm{SPL} \\
\mathrm{r} / \mathrm{l}
\end{gathered}
\] & \[
\begin{array}{|c|}
\hline \text { IFL } \\
\text { r/l }
\end{array}
\] & MO & IOS & V & \[
\begin{aligned}
& \mathrm{T} 4 \\
& \text { r/l }
\end{aligned}
\] & Canthal stripe & Lips strong barred & Blotches on the head round & Blotches on dorsum round & Two regularly disposed whitish tubercles on each side of the flanks \\
\hline \multirow[t]{6}{*}{minhlei} & IEBR A. 0802 & m & 8 & 6 & 6 & 10 & 22 & 14 & present & no & yes & yes & absent \\
\hline & ZFMK 97746 & m & 8 & 6.5 & 6 & 10 & 23 & 14.5 & present & no & yes & yes & absent \\
\hline & IEBR A. 0801 & f & 8.5 & 7 & 6 & 10 & 22 & 12 & present & no & yes & yes & absent \\
\hline & ZFMK 97745 & f & 7.5 & 6 & 5.5 & 10 & 23 & 13 & present & no & yes & yes & absent \\
\hline & VNMN R.2016.1 & f & 8 & 6 & 5.5 & 8 & 23 & 15 & present & no & yes & yes & absent \\
\hline & VNMN R.2016.2 & f & 8 & 6.5 & 6 & 7 & 20 & 13 & present & no & yes & yes & absent \\
\hline \multirow[t]{3}{*}{gialaiensis sp. nov.} & VNUF R.2020.22 & m & 7.5 & 6 & 6 & 7 & 21 & 14 & present & yes & yes & yes & present \\
\hline & VNUF R.2020.33 & f & 7 & 6 & 6 & 7 & 19 & 14 & present & yes & yes & yes & present \\
\hline & VNUF R.2020.44 & mj & 8 & 7 & 6 & 7 & 21 & 14.5 & present & yes & yes & yes & present \\
\hline \multirow[t]{12}{*}{vietnamensis} & ZRC 2.6024 & m & 5 & 6 & 5 & 10 & 20 & 13 & present & no & no & no & present \\
\hline & ZRC 2.6025 & m & 5 & 6 & 5 & 9 & 20 & 13 & present & no & no & no & present \\
\hline & ZRC 2.6026 & j & 5 & 6 & 6 & 8 & 20 & 13 & present & no & no & no & present \\
\hline & ZRC 2.6027 & j & 6 & 7 & 6 & 8 & 20 & 13 & present & no & no & no & present \\
\hline & IEBR R.2016.3 & m & 8 & 6 & 5.5 & 10 & 19 & 13.5 & present & no & no & no & present \\
\hline & VNMN R.2016.3 & m & 7.5 & 6 & 5.5 & 9 & 19 & 13.5 & present & no & no & no & present \\
\hline & IEBR R.2016.1 & f & 7 & 6 & 5.5 & 8 & 18 & 13.5 & present & no & no & no & present \\
\hline & VNMN R.2016.4 & f & 7.5 & 7 & 6 & 9 & 20 & 13 & present & no & no & no & present \\
\hline & ZFMK 97748 & f & 7.5 & 6 & 6 & 8 & 20 & 14 & present & no & no & no & present \\
\hline & ZFMK 97747 & mj & 7.5 & 6 & 5.5 & 10 & 15 & 13.5 & present & no & no & no & present \\
\hline & IEBR R.2016.4 & fj & 8 & 7 & 6 & 7 & 21 & 12.5 & present & no & no & no & present \\
\hline & ZFMK 97749 & f & 7 & 6.5 & 5.5 & 8 & 19 & 13.5 & present & no & no & no & present \\
\hline \multirow[t]{4}{*}{sp.} & VNUF R. 2022.81 & m & 8 & 6.5 & 6 & 9 & 24 & 14 & present & no & no & yes & present \\
\hline & VNUF R.2022.82 & f & 7.5 & 5.5 & 6 & 8 & 23 & 14.5 & present & no & no & yes & present \\
\hline & VNUF R.2022.83 & f & 8 & 7 & 6 & 8 & 23 & 14 & present & no & no & yes & present \\
\hline & VNUF R. 2022.84 & fj & 8.5 & 6 & 6 & 8 & 22 & 13.5 & present & no & no & yes & present \\
\hline \multirow[t]{5}{*}{somchanhae} & VNUF R.2020.3 & m & 7 & 5 & 6 & 8 & 24 & 14 & present & yes & no & no & present \\
\hline & VNUF R.2020.2 & m & 8 & 6 & 6 & 8 & 23 & 15 & present & yes & no & no & present \\
\hline & VNUF R.2020.1 & m & 8 & 5.5 & 6 & 8 & 23 & 15 & present & yes & no & no & present \\
\hline & VNUF R.2020.4 & f & 8 & 5.5 & 6 & 8 & 23 & 15 & present & yes & no & no & present \\
\hline & VNUF R.2020.5 & f & 8 & 6 & 6 & 7 & 26 & 13 & present & yes & no & no & present \\
\hline \multirow[t]{8}{*}{siamensis} & LSUHC09284 & f & 8 & 7 & 6 & 9 & 19 & 14 & absent & yes & no & yes & present \\
\hline & LSUHC08522 & f & 8 & 6.5 & 6 & 10 & 22 & 14.5 & absent & yes & no & yes & present \\
\hline & LSUHC08487 & f & 8 & 7 & 6 & 10 & 20 & 14.5 & absent & yes & no & yes & present \\
\hline & LSUHC08420 & m & 8.5 & 7 & 6 & 10 & 21 & 13 & absent & yes & no & yes & present \\
\hline & LSUHC08491 & f & 8 & 7 & 6 & 9 & 20 & 14.5 & absent & yes & no & yes & present \\
\hline & LSUHC07328 & j & 7.5 & 6 & 5.5 & 9 & 22 & 14 & absent & yes & no & yes & present \\
\hline & LSUHC07378 & m & 8 & 6 & 6 & 10 & 20 & 14.5 & absent & yes & no & yes & present \\
\hline & LSUHC09289 & m & 7.5 & 6 & 6 & 10 & 21 & 16 & absent & yes & no & yes & present \\
\hline \multirow[t]{3}{*}{muangfuangensis sp. nov.} & NUOL R. 2022.01 & m & 7 & 6.5 & 6 & 7 & 21 & 15 & absent & yes & no & no & present \\
\hline & VNUF R.2020.42 & m & 8 & 7 & 6 & 7 & 20 & 15 & absent & yes & no & no & present \\
\hline & VNUF R.2020.52 & f & 8 & 6.5 & 6 & 7 & 21 & 15 & absent & yes & no & no & present \\
\hline \multirow[t]{3}{*}{lao} & VNUF R.2016.2 & m & 9.5 & 8 & 7.5 & 9 & 23 & 15 & absent & yes & no & no & absent \\
\hline & IEBR A. 2019.5 & f & 8.5 & 8 & 7 & 8 & 23 & 15 & absent & yes & no & no & absent \\
\hline & IEBR A.2019.6 & f & 9 & 7.5 & 8 & 8 & 24 & 15 & absent & yes & no & no & absent \\
\hline
\end{tabular}

\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline Species & Museum no. & Sex & SVL & BW & HL & HW & HD & EL & ED & EN & ES & EE & IN & IO & FAr & TBLr & AGr \\
\hline \multirow[t]{5}{*}{siamensis} & LSUHC08420 & m & 46.9 & 8.8 & 13.1 & 9.1 & 5.3 & 1.3 & 2.7 & 3.7 & 5.3 & 3.9 & 1.5 & 3.7 & 6.7 & 7.3 & 20.7 \\
\hline & LSUHC08491 & f & 45.2 & 10.2 & 13 & 8.2 & 5.7 & 1.4 & 2.8 & 3.3 & 4.7 & 4.2 & 2 & 3.7 & 6.2 & 6.9 & 19 \\
\hline & LSUHC07328 & j & 28.6 & 5.8 & 8.4 & 5.5 & 3 & 0.7 & 2.1 & 2.4 & 3.3 & 2.8 & 1.5 & 2.8 & 3.8 & 5 & 12 \\
\hline & LSUHC07378 & m & 36.7 & 6.5 & 10.9 & 7.3 & 4.5 & 1.3 & 2.6 & 3.1 & 4.6 & 3.4 & 1.6 & 3.4 & 6 & 6.6 & 16.1 \\
\hline & LSUHC09289 & m & 45.3 & 9.1 & 12.7 & 8.6 & 5.1 & 1.6 & 2.6 & 3.7 & 5 & 3.6 & 2 & 3.5 & 7 & 7.3 & 18.9 \\
\hline \multirow[t]{3}{*}{muangfuangensis sp. nov.} & NUOL R.2022.01 & m & 38.3 & 7.83 & 10.5 & 7.2 & 4.3 & 0.8 & 2.4 & 2.8 & 3 & 3.4 & 1.3 & 1.7 & 4.3 & 4.9 & 16.5 \\
\hline & VNUF R.2020.42 & m & 55.6 & 11.93 & 15.2 & 10.8 & 6.9 & 2.3 & 3 & 3.8 & 5.9 & 5.1 & 1.6 & 2.3 & 6.8 & 7.2 & 23.1 \\
\hline & VNUF R. 2020.52 & f & 56.7 & 12.23 & 16.7 & 10.7 & 6.9 & 2.1 & 3.5 & 3.8 & 5.8 & 5.1 & 1.7 & 2.4 & 7.1 & 7.3 & 27.4 \\
\hline \multirow[t]{3}{*}{lao} & VNUF R.2016.2 & m & 50.1 & 9.7 & 14.1 & 9.2 & 5.3 & 1.4 & 3.6 & 4.4 & 5.6 & 4.1 & 1.7 & 1.7 & 6.9 & 7.6 & 20.6 \\
\hline & IEBR A.2019.5 & f & 55.4 & 11.5 & 14.3 & 9.7 & 6.2 & 1.7 & 3.6 & 4 & 5.5 & 4.4 & 1.8 & 1.5 & 7.1 & 8.5 & 22.2 \\
\hline & IEBR A.2019.6 & f & 35.8 & 7.2 & 9.9 & 7 & 4 & 1.1 & 2.7 & 2.8 & 3.6 & 2.6 & 1.1 & 1.1 & 4.6 & 5.9 & 15.2 \\
\hline
\end{tabular}
Table 4. Mean percentages of uncorrected pairwise sequence divergence (p-distances) among the species of Dixonius. Intraspecific p-distance are in bold font, \(n / a\) = data not applicable.
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline & Dixonius sp. & cf. siamensis & aaronbaueri & taoi & vietnamensis & cf. vietnamensis & muangfuangensis sp. nov. & lao & minhlei & gialaiensis sp. nov. & siamensis &  & melanostictus \\
\hline Dixonius sp. & n/a & & & & & & & & & & & & \\
\hline cf. siamensis & 6.33 & n/a & & & & & & & & & & & \\
\hline aaronbaueri & 18.52 & 18.37 & n/a & & & & & & & & & & \\
\hline taoi & 11.49 & 13.16 & 16.07 & 0.01 & & & & & & & & & \\
\hline vietnamensis & 12.12 & 13.67 & 18.84 & 6.58. & n/a & & & & & & & & \\
\hline cf. vietnamensis & 12.12 & 12.43 & 18.31 & 7.36 & 2.57 & n/a & & & & & & & \\
\hline muangfuangensis sp. nov. & 10.78 & 8.17 & 18.17 & 11.36 & 12.79 & 12.50 & 0.00 & & & & & & \\
\hline lao & 8.46 & 9.26 & 16.66 & 10.90 & 11.90 & 11.39 & 3.10 & 0.00 & & & & & \\
\hline minhlei & 13.97 & 15.33 & 17.56. & 13.35 & 14.13 & 13.92 & 13.23 & 13.24 & n/a & & & & \\
\hline gialaiensis sp. nov. & 13.51 & 14.27 & 15.73 & 11.78 & 13.40 & 13.18 & 13.11 & 10.90 & 3.60 & 0.00 & & & \\
\hline siamensis & 13.71 & 14.83 & 16.14 & 11.74 & 12.33 & 12.22 & 12.70 & 11.96 & 12.54 & 10.56 & 0.00 & & \\
\hline somchanbae & 13.31 & 12.66 & 17.73 & 12.90 & 12.57 & 12.40 & 12.40 & 12.27 & 12.24 & 10.63 & 9.07 & 0.00 & \\
\hline melanostictus & 13.30 & 13.04 & 15.16 & 11.23 & 13.12 & 13.01 & 11.99 & 10.70 & 14.09 & 11.53 & 12.10 & 11.09 & n/a \\
\hline
\end{tabular}
for corroboration and further discrimination of morphospatial differences among the putative species. DAPC a priori groups the individuals of each predefined population inferred from the phylogeny into separate clusters (i.e., plots of points) bearing the smallest within-group variance that produce linear combinations of centroids having the greatest between-group variance (i.e., linear distance; Jombart et al. 2010). DAPC relies on standardized data from its own PCA as a prior step to ensure that variables analyzed are not correlated and number fewer than the sample size. Principal components with eigenvalues accounting for \(90-95 \%\) of the variation in the data set were retained for the DAPC analysis according to the criterion of Jombart et al. (2010).

To test and further corroborate the PCA and DAPC analyses, we conducted a multiple factor analysis (MFA) on the above-mentioned morphological characters plus the categorical color pattern differences for a near total evidence data set (see Tables 5, 6). The MFA was implemented using the \(m f a()\) command in the R package FactorMineR (Husson et al. 2017) and visualized using the Factoextra package (Kassambara and Mundt 2017). MFA is a global, unsupervised, multivariate analysis that incorporates qualitative and quantitative data (Pagès 2015), making it possible to analyze different data types simultaneously in a nearly total evidence environment. In an MFA, each individual is described by a different set of variables (i.e., characters) which are structured into different data groups in a global data frame, in this case, quantitative data (i.e., meristics and normalized morphometrics) and categorical data (i.e., color pattern). In the first phase of the analysis, separate multivariate analyses are carried out for each set of variables, principal component analyses (PCA) for the quantitative data sets and multiple correspondence analysis (MCA) for categorical data. The data sets are then normalized separately by dividing all their elements by the square root of their first eigenvalues. For the second phase of the analysis, the normalized data sets are concatenated into a single matrix for a global PCA of the data. Standardizing the data in this manner prevents one data type from overleveraging another. In other words, the normalization of the data in the first phase prevents data types with the highest number of characters or the greatest amount of variation from outweighing other data types in the second phase. This way, the contribution of each data type to the overall variation in the data set is scaled to define the morphospatial distance between individuals as well as calculating each data type's and each character's contributions to the overall variation in the data set (Pagès 2015; Kassambara and Mundt 2017).

\section*{Results}

\section*{Molecular results}

The results of ML, BI, and BEAST analyses produced trees with identical topologies and strong support at nearly every node (Figs 2, 3). The molecular analyses suggest that Dixonius aaronbaueri is the sister species to a clade containing all other species of Dixonius. Additionally, all analyses recovered the newly discovered population from

Table 5. Summary statistics of the principal component analysis of Dixonius species. Abbreviations are listed in the Materials and methods.
\begin{tabular}{lccccccc}
\hline & & PC1 & PC2 & PC3 & PC4 & PC5 & PC6
\end{tabular} PC7 9
\begin{tabular}{lcccccccc}
\hline & PC15 & PC16 & \(\mathbf{P C 1 7}\) & PC18 & PC19 & PC20 & PC21 \\
\hline \multicolumn{1}{c}{ Standard deviation } & \(\mathbf{0 . 3 7 6 1 9 9 7 2 1}\) & \(\mathbf{0 . 3 6 5 4 7 7 4 7 5}\) & \(\mathbf{0 . 3 3 9 1 7 9 7 5 2}\) & \(\mathbf{0 . 2 8 2 9 1 6 6 2 6}\) & \(\mathbf{0 . 2 3 6 1 8 7 0 3 7}\) & \(\mathbf{0 . 1 7 1 1 4 9 6 8 5}\) & \(\mathbf{0 . 1 4 9 4 8 0 1 8 8}\) \\
\hline Proportion of Variance & \(\mathbf{0 . 0 0 6 7 4}\) & \(\mathbf{0 . 0 0 6 3 6}\) & \(\mathbf{0 . 0 0 5 4 8}\) & \(\mathbf{0 . 0 0 3 8 1}\) & \(\mathbf{0 . 0 0 2 6 6}\) & \(\mathbf{0 . 0 0 1 3 9}\) & \(\mathbf{0 . 0 0 1 0 6}\) \\
\hline Cumulative Proportion & \(\mathbf{0 . 9 7 9 2 3}\) & \(\mathbf{0 . 9 8 5 5 9}\) & \(\mathbf{0 . 9 9 1 0 7}\) & \(\mathbf{0 . 9 9 4 8 8}\) & \(\mathbf{0 . 9 9 7 5 4}\) & \(\mathbf{0 . 9 9 8 9 4}\) & \(\mathbf{1}\) \\
\hline eigen & 0.14152623 & 0.133573785 & 0.115042904 & 0.080041817 & 0.055784316 & 0.029292215 & 0.022344327 \\
SVL & -0.083499418 & 0.052323475 & -0.106015238 & 0.003344968 & -0.031996278 & 0.03916795 & -0.019457555 \\
BW & -0.178247777 & -0.058497317 & 0.597846907 & -0.255954135 & 0.379366422 & -0.199217481 & 0.221418054 \\
HL & 0.08265144 & -0.13083192 & -0.024132742 & 0.014837636 & 0.131660321 & 0.479388229 & 0.2891919 \\
HW & 0.244724903 & -0.059011694 & 0.113956101 & -0.074931386 & 0.04577337 & -0.069612504 & -0.732492773 \\
HD & 0.050630806 & 0.316234183 & 0.16660119 & 0.299515683 & -0.49781424 & 0.046244883 & 0.219619924 \\
EL & 0.296740909 & 0.376559691 & -0.260809485 & -0.089676204 & -0.027290387 & -0.071210438 & 0.140852117 \\
ED & -0.231235089 & -0.168155577 & -0.115371244 & 0.284151752 & 0.361474786 & 0.202917841 & -0.049017145 \\
EN & 0.022603597 & 0.420837275 & -0.236893263 & 0.02900181 & 0.436157574 & 0.238920063 & -0.045962367 \\
ES & 0.248024801 & -0.355459446 & -0.140327076 & 0.068202164 & 0.014098586 & -0.168841736 & 0.325882917 \\
EE & -0.393037351 & -0.425564191 & -0.418495874 & -0.066368342 & -0.173649977 & -0.04666024 & -0.080329 \\
IN & -0.081626216 & 0.152259274 & -0.192312604 & -0.041856001 & 0.036286384 & -0.306664296 & 0.054524547 \\
IO & -0.115305892 & 0.069986307 & 0.310505175 & 0.23842857 & -0.096262685 & 0.459593261 & -0.180632462 \\
FAr & -0.116757716 & 0.126720851 & 0.055407627 & 0.30600813 & 0.081267972 & -0.470097472 & -0.064137815 \\
TBLr & -0.021197578 & -0.178071358 & 0.100589652 & -0.310374261 & -0.331807871 & 0.142300685 & 0.047913831 \\
AGr & 0.334353255 & -0.016725971 & -0.026314358 & -0.397995653 & -0.105887039 & 0.100382932 & -0.137085205 \\
SPLr.l & -0.184269598 & 0.036899566 & 0.194841372 & 0.163667167 & -0.204455175 & -0.121267207 & -0.156153276 \\
IFLr.l & 0.428551026 & -0.165166993 & 0.155004291 & 0.142458525 & 0.008969802 & -0.065554124 & 0.111490515 \\
MO & -0.391157106 & 0.255079755 & -0.004834981 & -0.342258191 & -0.175496507 & 0.081476947 & 0.028734251 \\
IOS & -0.059743664 & -0.04851249 & 0.20733712 & -0.095515858 & 0.057436279 & -0.015186946 & 0.173384569 \\
V & 0.002458015 & -0.062420243 & -0.039680685 & 0.361422477 & -0.145522506 & 0.02608135 & 0.040083497 \\
T4r.l & 0.117972701 & -0.206198025 & 0.0622901 & 0.17118706 & 0.012651195 & 0.069479213 & -0.122746338 \\
\hline & & & & & & & & \\
\hline
\end{tabular}

Chu Se District, Gia Lai Province, Vietnam as the strongly supported (1.00/100) sister species of \(D\). minhlei and the newly discovered population from Muangfuang District, Vientiane Province, Laos as the strongly supported (1.00/100) sister species of D. lao (Figs 2, 3). Uncorrected pairwise sequence divergences among Dixonius species ranged from 2.57-18.84\% (Table 4). Ranges for the new species described (see below) are as follows: new species from Vietnam 3.60-15.73\%, being most similar to D. minhlei and most distant to \(D\). aaronbaueri and new species from Laos 3.10-18.17\%, being most similar to \(D\). lao and most distant to \(D\). aaronbaueri.

The time-calibrated BEAST analysis places the divergence between Dixonius aaronbaueri and the remaining species of Dixonius at approximately 24.04 mya (20.2327.68 highest posterior density [HPD]). Within the Vietnam's lineages, D. gialaiensis sp. nov. and \(D\). minhlei diverged from each other at approximately 3.19 mya (0.795.78 HPD ) and within the Lao lineages, \(D\). muangfuangensis sp. nov. and \(D\). lao diverged approximately 3.47 mya (1.37-6.16 HPD) (Fig. 3).

\section*{Statistical analyses}

The first two principal components ( PC 1 and PC 2 ) of the PCA analysis recovered \(56.6 \%\) of the variation in the morphometric and meristic data set (Fig. 4A) and loaded most heavily for body width (BW), head width (HW), eye nostril distance (EN), eye snout distance (ES), and eye ear distance (EE) along PC1 and interorbital dis-


Figure 2. Maximum likelihood topology of the Dixonius species from Vietnam and Laos with ultrafast bootstrap values (UFB) and Bayesian posterior probabilities (BPP) at the nodes, respectively.


Figure 3. BEAST chronogram of the Dixonius species from Vietnam and Laos. Numbers at the nodes are mean ages in millions of years. Bars represent \(95 \%\) highest posterior densities.
tance (IO), supralabials (SPLr/l), number of supralabial at midorbital position (MO), and ventral scales (V) along PC2 (Table 5). The PCA recovered D. gialaiensis sp. nov. and \(D\). muangfuangensis sp. nov. to be widely separated from most other species with \(D\). muangfuangensis sp. nov. only overlapping with the distantly related \(D\). siamensis. The two distantly related new species are well-separated from most other species in the DAPC but each overlaps with one other species in their 67\% inertia ellipses (Fig. 4B).

The MFA analysis recovered all species to be separated from one another including Dixonius muangfuangensis sp. nov. and \(D\). siamensis (Fig. 5A). The morphometric data
Table 6. Summary statistics of morphometric and meristic characters among the Dixonius species.
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline Species & SVL & BW & HL & HW & HD & EL & ED & EN & ES & EE & IN & IO & FAr & TBLr & AGr & SPLr. 1 & IFLr. 1 & MO & IOS & V & T4r.1 \\
\hline \multicolumn{22}{|l|}{Dixonius gialaiensis sp. nov. ( \(n=3\) )} \\
\hline Mean & 1.62 & 0.93 & 1.07 & 0.89 & 0.73 & 0.04 & 0.47 & 0.50 & 0.63 & 0.51 & 0.14 & 0.11 & 0.75 & 0.83 & 1.23 & 7.5 & 6.33 & 6 & 7 & 20.33 & 14.17 \\
\hline SD & 0.060 & 0.007 & 0.003 & 0.001 & 0.007 & 0.005 & 0.002 & 0.007 & 0.002 & 0.005 & 0.018 & 0.029 & 0.034 & 0.013 & 0.025 & 0.5 & 0.577 & 0 & 0 & 1.155 & 0.289 \\
\hline Lower & 1.56 & 0.92 & 1.06 & 0.89 & 0.72 & 0.04 & 0.47 & 0.49 & 0.63 & 0.51 & 0.12 & 0.08 & 0.73 & 0.82 & 1.20 & 7 & 6 & 6 & 7 & 19 & 14 \\
\hline Upper & 1.68 & 0.93 & 1.07 & 0.89 & 0.73 & 0.05 & 0.47 & 0.50 & 0.64 & 0.52 & 0.15 & 0.13 & 0.79 & 0.84 & 1.25 & 8 & 7 & 6 & 7 & 21 & 14.5 \\
\hline \multicolumn{22}{|l|}{D. lao ( \(n=3\) )} \\
\hline Mean & 1.67 & 0.98 & 1.11 & 0.94 & 0.71 & 0.15 & 0.52 & 0.57 & 0.69 & 0.57 & 0.18 & 0.16 & 0.79 & 0.87 & 1.29 & 9 & 7.83 & 7.5 & 8.33 & 23.33 & 15 \\
\hline SD & 0.099 & 0.014 & 0.017 & 0.005 & 0.013 & 0.022 & 0.016 & 0.042 & 0.028 & 0.012 & 0.013 & 0.047 & 0.017 & 0.007 & 0.003 & 0.5 & 0.289 & 0.5 & 0.577 & 0.577 & 0 \\
\hline Lower & 1.55 & 0.96 & 1.09 & 0.93 & 0.70 & 0.12 & 0.51 & 0.54 & 0.67 & 0.56 & 0.17 & 0.12 & 0.78 & 0.86 & 1.29 & 8.5 & 7.5 & 7 & 8 & 23 & 15 \\
\hline Upper & 1.74 & 0.99 & 1.13 & 0.94 & 0.72 & 0.17 & 0.54 & 0.62 & 0.72 & 0.58 & 0.20 & 0.21 & 0.81 & 0.87 & 1.29 & 9.5 & 8 & 8 & 9 & 24 & 15 \\
\hline \multicolumn{22}{|l|}{D. minhlei \((n=6)\)} \\
\hline Mean & 1.65 & 0.97 & 0.86 & 0.82 & 0.67 & 0.12 & 0.45 & 0.55 & 0.68 & 0.56 & 0.17 & 0.57 & 0.80 & 0.86 & 1.33 & 7.75 & 6.42 & 5.83 & 7.67 & 21.33 & 14.33 \\
\hline SD & 0.025 & 0.012 & 0.008 & 0.037 & 0.022 & 0.044 & 0.022 & 0.021 & 0.025 & 0.022 & 0.023 & 0.025 & 0.020 & 0.017 & 0.060 & 0.418 & 0.376 & 0.258 & 1.211 & 1.366 & 1.033 \\
\hline Lower & 1.61 & 0.95 & 0.85 & 0.79 & 0.65 & 0.08 & 0.42 & 0.52 & 0.64 & 0.53 & 0.14 & 0.53 & 0.78 & 0.85 & 1.28 & 7 & 6 & 5.5 & 7 & 20 & 13 \\
\hline Upper & 1.68 & 0.98 & 0.87 & 0.89 & 0.71 & 0.18 & 0.49 & 0.57 & 0.70 & 0.59 & 0.21 & 0.60 & 0.83 & 0.89 & 1.44 & 8 & 7 & 6 & 10 & 23 & 15 \\
\hline \multicolumn{22}{|l|}{Dixonius muangfuangensis sp. nov. ( \(n=3\) )} \\
\hline Mean & 1.69 & 1.03 & 1.15 & 0.99 & 0.78 & 0.21 & 0.47 & 0.54 & 0.68 & 0.66 & 0.19 & 0.33 & 0.78 & 0.81 & 1.35 & 8.17 & 6.5 & 6 & 10 & 22.33 & 13.5 \\
\hline SD & 0.096 & 0.001 & 0.016 & 0.006 & 0.005 & 0.031 & 0.030 & 0.003 & 0.011 & 0.005 & 0.011 & 0.006 & 0.004 & 0.001 & 0.032 & 0.289 & 0.5 & 0 & 0 & 0.577 & 1.323 \\
\hline Lower & 1.58 & 1.03 & 1.13 & 0.97 & 0.77 & 0.18 & 0.44 & 0.54 & 0.67 & 0.65 & 0.18 & 0.32 & 0.78 & 0.81 & 1.31 & 8 & 6 & 6 & 10 & 22 & 12 \\
\hline Upper & 1.75 & 1.03 & 1.16 & 0.99 & 0.78 & 0.25 & 0.50 & 0.54 & 0.69 & 0.66 & 0.20 & 0.34 & 0.78 & 0.81 & 1.38 & 8.5 & 7 & 6 & 10 & 23 & 14.5 \\
\hline \multicolumn{22}{|l|}{D. siamensis ( \(n=8\) )} \\
\hline Mean & 1.62 & 0.93 & 1.09 & 0.90 & 0.70 & 0.13 & 0.43 & 0.54 & 0.68 & 0.58 & 0.25 & 0.54 & 0.80 & 0.84 & 1.27 & 7.94 & 6.56 & 5.94 & 9.63 & 20.63 & 14.38 \\
\hline SD & 0.077 & 0.0357 & 0.009 & 0.014 & 0.029 & 0.048 & 0.034 & 0.044 & 0.021 & 0.031 & 0.046 & 0.016 & 0.028 & 0.017 & 0.021 & 0.320 & 0.496 & 0.177 & 0.518 & 1.061 & 0.835 \\
\hline Lower & 1.61 & 0.95 & 0.85 & 0.79 & 0.65 & 0.08 & 0.42 & 0.52 & 0.64 & 0.53 & 0.14 & 0.53 & 0.78 & 0.85 & 1.23 & 7 & 6 & 5.5 & 7 & 20 & 13 \\
\hline Upper & 1.68 & 0.987 & 0.87 & 0.89 & 0.71 & 0.18 & 0.49 & 0.57 & 0.70 & 0.59 & 0.21 & 0.60 & 0.83 & 0.89 & 1.44 & 8 & 7 & 6 & 10 & 23 & 15 \\
\hline \multicolumn{22}{|l|}{D. somchanbae ( \(\boldsymbol{n}=\mathbf{6}\) )} \\
\hline Mean & 1.62 & 0.97 & 1.07 & 0.91 & 0.70 & 0.17 & 0.46 & 0.48 & 0.65 & 0.50 & 0.18 & 0.23 & 0.73 & 0.83 & 1.26 & 7.75 & 5.67 & 6 & 8.17 & 23.33 & 14.67 \\
\hline SD & 0.045 & 0.033 & 0.013 & 0.009 & 0.026 & 0.034 & 0.043 & 0.041 & 0.024 & 0.023 & 0.073 & 0.150 & 0.050 & 0.016 & 0.027 & 0.418 & 0.408 & 0 & 0.983 & 1.633 & 1.033 \\
\hline Lower & 1.55 & 0.93 & 1.05 & 0.90 & 0.66 & 0.10 & 0.40 & 0.42 & 0.62 & 0.47 & 0.098 & 0.13 & 0.68 & 0.80 & 1.24 & 7 & 5 & 6 & 7 & 21 & 13 \\
\hline Upper & 1.67 & 1.01 & 1.08 & 0.92 & 0.74 & 0.20 & 0.51 & 0.55 & 0.68 & 0.54 & 0.29 & 0.53 & 0.82 & 0.84 & 1.31 & 8 & 6 & 6 & 10 & 26 & 16 \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline Species & SVL & BW & HL & HW & HD & EL & ED & EN & ES & EE & IN & IO & FAr & TBLr & AGr & SPLr. 1 & IFLr. 1 & MO & IOS & V & T4r.1 \\
\hline \multicolumn{22}{|l|}{D. sp. \((n=4)\)} \\
\hline Mean & 1.55 & 0.85 & 1.03 & 0.81 & 0.54 & 0.05 & 0.33 & 0.45 & 0.55 & 0.49 & 0.11 & 0.21 & 0.66 & 0.75 & 1.18 & 8 & 6.25 & 6 & 8.25 & 23 & 14 \\
\hline SD & 0.083 & 0.033 & 0.009 & 0.037 & 0.049 & 0.068 & 0.017 & 0.033 & 0.087 & 0.033 & 0.007 & 0.015 & 0.032 & 0.032 & 0.020 & 0.408 & 0.645 & 0 & 0.5 & 0.816 & 0.408 \\
\hline Lower & 1.48 & 0.81 & 1.02 & 0.76 & 0.49 & -0.03 & 0.31 & 0.41 & 0.44 & 0.44 & 0.11 & 0.19 & 0.62 & 0.72 & 1.16 & 7.5 & 5.5 & 6 & 8 & 22 & 13.5 \\
\hline Upper & 1.66 & 0.88 & 1.04 & 0.84 & 0.61 & 0.13 & 0.35 & 0.48 & 0.62 & 0.52 & 0.12 & 0.22 & 0.70 & 0.80 & 1.20 & 8.5 & 7 & 6 & 9 & 24 & 14.5 \\
\hline \multicolumn{22}{|l|}{D. vietnamensis ( \(n=12\) )} \\
\hline Mean & 1.56 & 0.83 & 0.83 & 0.82 & 0.65 & -0.03 & 0.41 & 0.47 & 0.61 & 0.48 & 0.10 & 0.29 & 0.67 & 0.78 & 1.20 & 6.75 & 6.29 & 5.63 & 8.67 & 19.25 & 13.25 \\
\hline SD & 0.088 & 0.055 & 0.016 & 0.028 & 0.049 & 0.054 & 0.030 & 0.031 & 0.035 & 0.055 & 0.070 & 0.165 & 0.063 & 0.035 & 0.051 & 1.177 & 0.450 & 0.377 & 0.985 & 1.545 & 0.399 \\
\hline Lower & 1.41 & 0.72 & 0.80 & 0.77 & 0.58 & -0.13 & 0.35 & 0.42 & 0.55 & 0.36 & 0.02 & 0.12 & 0.55 & 0.73 & 1.12 & 5 & 6 & 5 & 7 & 15 & 12.5 \\
\hline Upper & 1.66 & 0.92 & 0.85 & 0.86 & 0.72 & 0.05 & 0.46 & 0.51 & 0.68 & 0.55 & 0.28 & 0.53 & 0.76 & 0.85 & 1.27 & 8 & 7 & 6 & 10 & 21 & 14 \\
\hline
\end{tabular}
contributed to approximately \(40 \%\) of the variation along Dim-1 followed by the categorical and meristic data. For Dim-2, the categorical data contributed \(80 \%\) of the variation followed by morphometric and meristic data. Dim-3 showed that meristic data contributed \(70 \%\) of the variation followed by morphometric and categorical data (Fig. 5B).

The ANOVAs and subsequent TukeyHDS tests demonstrated that Dixonius gialaiensis sp. nov. bears statistically different mean values between it and all other species in various combinations of characters (Tables 6, 7) and differs significantly from its sister species \(D\). minhle \(i\) in head length (HL mean \(=1.07\) vs. \(0.86, p=0.000\), respectively), in head width (HW mean \(=0.89\) vs. \(0.82, p=0.005\), respectively), and in axilla to groin length, (AGr mean \(=1.23\) vs \(1.32 p=0.022\) ) (Fig. 5; Tables 6, 7). Dixonius muangfuangensis sp. nov. also differed significantly from all other species in various combinations of characters and from its sister species \(D\). lao it differs in head length (HL mean \(=1.15\) vs. \(1.11, p=0.004\), respectively) and numbers of infralabials (IFL mean \(=6.50\) vs. 7.83, \(p=0.026\), respectively), and in numbers of supralabial at midorbital position (MO mean \(=6.00\) vs. \(7.50, p=0.00001\), respectively) (Fig. 5, Tables 6, 7). Variation in all metric characters are visualized in Figs 6, 7.

\section*{Taxonomy}

\section*{Dixonius gialaiensis sp. nov.}
https://zoobank.org/10BF67E1-8059-47CE-891C-B219BD7AA9C1
Fig. 8
Gialai leaf-toed gecko
Material examined. Holotype. Adult male, VNUF R. 2020.22 (Field no. GL02) in Chu Se Mountain Pass, H'Bong Commune, Chu Se District, Gia Lai Province ( \(13^{\circ} 34^{\prime} 44.3^{\prime \prime} \mathrm{N}, 108^{\circ} 13^{\prime} 55.7^{\prime \prime} \mathrm{E}\); 330 m a.s.l.), collected by Oanh Van Lo and Khanh Quoc Nguyen on 15 February 2020. Paratypes. VNUF R. 2020.44 (Field No. GL04), juvenile male, and VNUF R.2020.33 (Field No. GL03), adult female; the same data as the holotype.

Diagnosis. Dixonius gialaiensis sp. nov. can be separated from all other species of Dixonius by possessing the unique combination of having a maximum SVL of \(47.4 \mathrm{~mm} ; 19\) longitudinal rows of dorsal tubercles at midbody; 19-21 longitudinal rows of ventrals across the abdomen; 7 or 8 supralabials, sixth in at midorbital position; 6 or 7 infralabials; 7 interorbital scales; 7 or 8 precloacal pores in males, femoral pores lacking; precloacal and femoral pores absent in female; 13-15 lamellae on fourth toe; dorsum olive grey color with more round brown blotches; canthal stripe continues behind orbit to back of head; lips with dark bars; two regularly disposed whitish tubercles along the sides near the flanks to tail tip. These characters are scored across all Dixonius species from Vietnam and Laos in Tables 6, 7.

Description of the holotype. Adult male, SVL 41.2 mm ; head moderate in length (HL/SVL 0.28), wide (HW/HL 0.66), depressed (HD/HL 0.44), distinct from

Table 7. Significant \(p\)-values from the results of the ANOVA and TukeyHDS analyses comparing all combinations of species pairs. Character abbreviations are listed in the Materials and methods.
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline Morphometric characters & BW & HL & HW & HD & EL & ED & EN & ES & FAr & TBLr & AGr \\
\hline lao vs. gialaiensis sp. nov. & & 0.007 & & & & & & & & & \\
\hline minhlei vs. gialaiensis sp. nov. & & 0.00 & 0.005 & & & & & & & & 0.022 \\
\hline muangfuangensis sp. nov. vs. gialaiensis sp. nov. & 0.040 & <0.001 & 0.001 & & 0.001 & & & & & & 0.023 \\
\hline \multicolumn{12}{|l|}{siamensis vs. gialaiensis sp. nov.} \\
\hline somchanhae vs. gialaiensis sp. nov. & & & & & 0.016 & & & & & & \\
\hline sp. vs. gialaiensis sp. nov. & & 0.021 & 0.001 & < 0.001 & & < 0.001 & & & & 0.006 & \\
\hline vietnamensis vs. gialaiensis sp. nov. & 0.005 & 0.00 & < 0.001 & 0.030 & & 0.040 & & & & 0.036 & \\
\hline minhlei vs. lao & & 0.00 & < 0.001 & & & 0.017 & & & & & \\
\hline muangfuangensis sp. nov. vs. lao & & 0.003 & & & & & & & & & \\
\hline siamensis vs. lao & & & & & & < 0.001 & & & & & \\
\hline somchanhae vs. lao & & < 0.001 & & & & & 0.002 & & & & \\
\hline sp. vs. lao & 0.002 & <0.001 & < 0.001 & < 0.001 & & < 0.001 & < 0.001 & < 0.001 & 0.005 & < 0.001 & 0.018 \\
\hline vietnamensis vs. lao & < 0.001 & 0.00 & < 0.001 & & < 0.001 & < 0.001 & < 0.001 & 0.017 & 0.001 & < 0.001 & 0.023 \\
\hline muangfuangensis sp. nov. vs. minhlei & & 0.00 & < 0.001 & 0.002 & & & & & & & \\
\hline siamensis vs. minhlei & & 0.00 & < 0.001 & & & & & & & & \\
\hline somchanhae vs. minhlei & & 0.00 & < 0.001 & & & & 0.006 & & 0.035 & & \\
\hline sp. vs. minhlei & < 0.001 & 0.00 & & < 0.001 & & < 0.001 & 0.001 & < 0.001 & < 0.001 & < 0.001 & < 0.001 \\
\hline vietnamensis vs. minhlei & < 0.001 & <0.001 & & & < 0.001 & & < 0.001 & 0.007 & < 0.001 & < 0.001 & < 0.001 \\
\hline siamensis vs. muangfuangensis sp. nov. & 0.016 & <0.001 & 0.001 & 0.030 & & & & & & & \\
\hline somchanhae vs. muangfuangensis sp. & & <0.001 & 0.006 & & & & & & & & \\
\hline \multicolumn{12}{|l|}{nov.} \\
\hline sp. vs. muangfuangensis sp. nov. & < 0.001 & < 0.001 & < 0.001 & < 0.001 & 0.001 & < 0.001 & 0.016 & 0.001 & 0.013 & & < 0.001 \\
\hline vietnamensis vs. muangfuangensis sp. nov. & <0.001 & 0.00 & \(<0.001\) & <0.001 & <0.001 & 0.021 & 0.019 & 0.038 & 0.004 & & <0.001 \\
\hline somchanhae vs. siamensis & & 0.031 & & & & & 0.010 & & 0.018 & & \\
\hline sp. vs. siamensis & 0.016 & <0.001 & < 0.001 & < 0.001 & & < 0.001 & 0.002 & < 0.001 & <0.001 & < 0.001 & 0.012 \\
\hline vietnamensis vs. siamensis & < 0.001 & 0.00 & < 0.001 & & < 0.001 & & < 0.001 & < 0.001 & <0.001 & < 0.001 & 0.007 \\
\hline sp. vs. somchanhae & < 0.001 & 0.017 & < 0.001 & \(<0.001\) & 0.013 & < 0.001 & & 0.017 & & 0.003 & 0.032 \\
\hline vietnamensis vs. somchanhae & < 0.001 & 0.00 & < 0.001 & & < 0.001 & 0.003 & & & & 0.013 & 0.038 \\
\hline vietnamensis vs. sp. & & 0.00 & & < 0.001 & & < 0.001 & & & & & \\
\hline Morphometric characters & SPLr.l & IFLr.l & MO & IOS & V & T4r.l & & & & & \\
\hline lao vs. gialaiensis sp. nov. & & 0.008 & < 0.001 & & & & & & & & \\
\hline \multicolumn{12}{|l|}{minhlei vs. gialaiensis sp. nov.} \\
\hline muangfuangensis sp. nov. vs. gialaiensis & & & & < 0.001 & & & & & & & \\
\hline sp. nov. & & & & & & & & & & & \\
\hline siamensis vs. gialaiensis sp. nov. & & & & < 0.001 & & & & & & & \\
\hline somchanhae vs. gialaiensis sp. nov. & & & & & 0.011 & & & & & & \\
\hline \multicolumn{12}{|l|}{sp. vs. gialaiensis sp. nov.} \\
\hline vietnamensis vs. gialaiensis sp. nov. & & & & 0.041 & & & & & & & \\
\hline minhlei vs. lao & & 0.003 & < 0.001 & & & & & & & & \\
\hline muangfuangensis sp. nov. vs. lao & & 0.026 & < 0.001 & & & & & & & & \\
\hline siamensis vs. lao & & 0.007 & < 0.001 & & & & & & & & \\
\hline somchanhae vs. lao & & <0.001 & < 0.001 & & & & & & & & \\
\hline sp. vs. lao & & 0.002 & < 0.001 & & & & & & & & \\
\hline vietnamensis vs. lao & < 0.001 & <0.001 & < 0.001 & & < 0.001 & 0.015 & & & & & \\
\hline muangfuangensis sp. nov. vs. minhlei & & & & 0.004 & & & & & & & \\
\hline siamensis vs. minhlei & & & & 0.001 & & & & & & & \\
\hline somchanhae vs. minhlei & & & & & 0.045 & & & & & & \\
\hline \multicolumn{12}{|l|}{sp. vs. minhlei} \\
\hline vietnamensis vs. minhlei & & & & & 0.038 & & & & & & \\
\hline siamensis vs. muangfuangensis sp. nov. & & & & & & & & & & & \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline Morphometric characters & SPLr.l & IFLr. 1 & MO & IOS & V & T4r. 1 \\
\hline somchanhae vs. muangfuangensis & & & & 0.010 & & \\
\hline sp. nov. & & & & & & \\
\hline sp. vs. muangfuangensis sp. nov. & & & & & & \\
\hline vietnamensis vs. muangfuangensis & & & & & 0.011 & \\
\hline sp. nov. & & & & & & \\
\hline somchanhae vs. siamensis & & 0.019 & & & 0.002 & \\
\hline sp. vs. siamensis & & & & & 0.065 & \\
\hline vietnamensis vs. siamensis & 0.022 & & & & & 0.035 \\
\hline sp. vs. somchanhae & & & & & & \\
\hline vietnamensis vs. somchanhae & & & & & \(<0.001\) & \\
\hline vietnamensis vs. sp. & & & & & < 0.001 & \\
\hline
\end{tabular}


Figure 4. A principal component analysis (PCA) of Dixonius species showing their morphospatial relationships along the first two components based on normalized morphometric and meristic characters B discriminant analysis of principal components (DAPC) based on retention of the first five PCs with 67\% inertia ellipsoids.


Figure 5. A MFA scatter plot showing the morphospatial relationships among the Dixonius species based on normalized morphometric, meristic, and color pattern characters B bar graphs showing the percent contribution of each data type to the overall variation in the data dimensions \(1-4\). The dashed red line in the bar graphs indicates the expected average value if the contributions of each data type were equal.
neck; prefrontal region concave; canthus rostralis rounded; snout elongate (ES/HL 0.37 ), rounded in dorsal profile; eye moderate size (ED/HL 0.25); ear opening oval, obliquely oriented, moderate in size; diameter of eye slightly smaller than eye to ear distance (ED/EE 0.88); rostral rectangular, partially divided dorsally by straight rostral groove, bordered posteriorly by large left and right supranasals, bordered laterally by first supralabials; external nares bordered anteriorly by rostral, dorsally by large supranasal, posteriorly by two smaller postnasals, bordered ventrally by first supralabial; 8,7 (R,L) rectangular supralabials extending to below and slightly past posterior margin of


Figure 6. Boxplot comparisons of meristic characters among the Dixonius species where interspecific statistical differences were recovered (see Table 7). Pale blue circles are means and the black horizontal bars are medians.
eye, sixth in midorbital position; \(6,6(\mathrm{R}, \mathrm{L})\), infralabials tapering smoothly to just below midpoint of eye, decreasing gradually in size; scales of rostrum and lores flat to domed, larger than granular scales on top of head and occiput; scales of occiput intermixed with distinct, small, conical tubercles; superciliaries elongate, largest anteriorly; mental triangular, bordered laterally by first infralabials and posteriorly by large left and right trapezoidal postmentals contacting medially for \(60 \%\) of their length posterior to mental; gular and throat scales small, granular, grading anteriorly into slightly larger, flatter, smooth, imbricate, pectoral and ventral scales.

Body relatively short (AG/SVL 0.38); dorsal scales small, granular interspersed with larger, conical, regularly arranged, keeled tubercles; tubercles extend from top


Figure 7. Violin plots of the normalized morphometric characters overlain with box plots showing the range, frequency, mean (white dot), and \(50 \%\) quartile (black rectangle) of characters where interspecific statistical differences were recovered (see Table 7). New species in bold italics.


Figure 8. Dorsal views of Dixonius gialaiensis sp. nov. A adult male holotype VNUF R. 2020.22 (Field no. GL02) B adult female paratype VNUF R. 2020.33 (Field No. GL03) C juvenile male paratype VNUF R. 2020.44 (Field No. GL04) in Chu Se Mountain Pass, Hbong Commune, Chu Se District, Gia Lai Province.
of head onto posterior haft of tail forming longitudinal rows, terminating at last portion of tail; smaller tubercles extend anteriorly onto nape and occiput, diminishing in size and distinction on top of head; 19 longitudinal rows of tubercles at midbody; 33 paravertebral scales, number of scales in a paravertebral row from first scale posterior to parietal scale to last scale at the level of vent opening; 23 paravertebral scales in a row between limb insertions; 21 flat, imbricate, ventral scales much larger than dorsal scales; 7 enlarge, pore-bearing, precloacal scales in an angular series; and no deep precloacal groove or depression.

Forelimbs moderate in stature, relatively short (FA/SVL 0.15); granular scales of forearm slightly larger than those on body, interspersed with small tubercles; hind limbs more robust than forelimbs, moderate in length (TBL/SVL 0.17), covered dorsally by granular scales interspersed with large, and small conical tubercles; ventral scales of thigh flat, imbricate, larger than dorsals; subtibial scales flat, imbricate; proximal femoral scales smaller than distal femorals; femoral pores absent; digits relatively long with 14 lamellae on fourth toe; and claws well developed.

Tail 108.4 mm in length, 4.5 mm in width at base, tapering to a point; dorsal scales of flat, square with conical, keeled tubercles in anterior part; median row of transversely expanded subcaudal scales, significantly larger than dorsal caudal scales on original portion; base of tail bearing hemipenal swellings; and postcloacal scales flat, imbricate.

Coloration in life (Fig. 8). Ground color of dorsal head and dorsum grey brown with rounded black-brown blotches, decreasing gradually in size from head to body; canthal stripe continues behind orbit to back of head; dark bars on the lips; uneven light spots running from postorbital along the flanks to tip tail; upper surface of foreand hindlimbs uniformly light grey with black-brown spots; dorsum of tail covered with some small rounded black-brown blotches; ventral surface beige uniformly as the belly and the throat.

Variation (Fig. 8). The female paratype (VNUF R.2020.33) generally has more dark brown blotches on head and dorsum, and uniformly black on the new regenerated tail. The dorsum of the of head and body of the juvenile male paratype (VNUF R.2020.44) pale brown with pale-colored blotches on granulose skin arranged along its sides extending from the flanks to the tail tip. Further measurements are summarized in Tables 2-4, Suppl. material 1: table S1.

Distribution. Dixonius gialaiensis sp. nov. currently is only known from the type locality of Chu Se Mountain Pass, H'Bong Commune, Chu Se District, Gia Lai Province, Central Highlands, Vietnam (Fig. 1).

Natural history. The specimens were found at night, between 19:45 and 21:00 \(h\), on the ground in an area along the National Highway 25. The surrounding habitat was secondary montane forest with woody trees. The temperature and humidity were approximately \(32.6^{\circ} \mathrm{C}\) and \(57 \%\) (Fig. 9).

Etymology. The new species is named after the type locality of Gia Lai Province, Central Highlands, Vietnam.

Comparisons. Dixonius gialaiensis sp. nov. is the sister species to \(D\). minhlei (Fig. 2) from which it differs by an uncorrected pairwise sequence divergence of \(3.60 \%\) (Table 4). It is differentiated from it morphologically by having a significantly higher


Figure 9. Habitat of Dixonius gialaiensis sp. nov. HBong Commune, Chu Se District, Gia Lai Province, Central Highlands, Vietnam.
mean number of head length (HL), head width (HW), and axilla to groin length (AG). In addition, it differs from \(D\). minblei in color pattern (grey-brown dorsum with more round black-brown blotches versus olive gray dorsum with round brownish olive blotches). Statistically significant and discrete categorical differences between Dixonius gialaiensis sp. nov. and all other species and populations are presented in Tables 5-7.

\section*{Dixonius muangfuangensis sp. nov.}
https://zoobank.org/A447EC01-F653-4FE5-A616-5FBD25F027C6
Fig. 10
Muangfuang leaf-toed gecko
Material examined. Holotype. Adult male, VNUF R. 2020.42 (Field no. MF.02) in Sinxay Temple, Nadan Village, Muangfuang District, Vientiane Province, Central Laos ( \(18^{\circ} 32^{\prime} 52^{\prime \prime N}, 101^{\circ} 58^{\prime} 31^{\prime \prime E} ; 276 \mathrm{~m}\) a.s.l.), collected by Saly Sitthivong and Thuong Huyen Nguyen on 05 December 2020. Paratypes. NUOL R.2022.01 (Field no. MF. 01), juvenile male, and VNUF R.2020.52 (Field no. MF. 03), adult female; the same data as given for the holotype.

Diagnosis. Dixonius muangfuangensis sp. nov. can be separated from all other species of Dixonius by possessing the unique combination of having a maximum SVL of \(56.7 \mathrm{~mm} ; 21-23\) longitudinal rows of dorsal tubercles at midbody; 20 or 21 longitudinal rows of ventrals across the abdomen; 7 or 8 supralabials, sixth in at midorbital position; 6 or 7 infralabials; 7 interorbital scales; 7 or 8 precloacal pores in males, femoral pores lacking; precloacal and femoral pores absent in female; 15 lamellae on fourth toe;
dorsum olive grey color with numerous small and irregular black blotches; head with brown spots; light spots irregularly arranged from the back of the head to base of tail; lips with dark bars; two regularly disposed whitish tubercles on each side on each side. These characters are scored across all Dixonius species from Vietnam and Laos in Tables 6, 7.

Description of the holotype. Adult male, SVL 55.6 mm ; head moderate in length (HL/SVL 0.28), wide (HW/HL 0.71), depressed (HD/HL 0.45), distinct from neck; prefrontal region concave; canthus rostralis rounded; snout elongate (ES/HL 0.39), rounded in dorsal profile; eye moderate size (ED/HL 0.20); ear opening oval, obliquely oriented, moderate in size; diameter of eye much smaller than eye to ear distance (ED/EE 0.59); rostral rectangular, partially divided dorsally by straight rostral groove, bordered posteriorly by large left and right supranasals, bordered laterally by first supralabials; external nares bordered anteriorly by rostral, dorsally by large supranasal, posteriorly by two smaller postnasals, bordered ventrally by first supralabial; 8,8 (R,L) rectangular supralabials extending to below midpoint of eye, sixth in midorbital position; 7,7 (R,L), infralabials tapering smoothly to be just slightly past posterior below midpoint of eye, decreasing gradually in size; scales of rostrum and lores flat to domed, larger than granular scales on top of head and occiput; scales of occiput intermixed with distinct, small, conical tubercles; superciliaries elongate, largest anteriorly; mental triangular, bordered laterally by first infralabials and posteriorly by large left and right parallelogram postmentals contacting medially for \(60 \%\) of their length posterior to mental; gular and throat scales small, granular, grading anteriorly into slightly smaller, flatter, smooth, imbricate, pectoral and ventral scales.

Body relatively short (AG/SVL 0.42 ) with well-defined ventrolateral folds; dorsal scales small, granular interspersed with moderate, conical, regularly arranged, keeled tubercles; tubercles extend from top of head onto interior haft of tail forming longitudinal rows, terminating at regenerated portion of tail; smaller tubercles extend anteriorly onto nape and occiput, diminishing in size and distinction on top of head; 23 longitudinal rows of tubercles at midbody; 45 paravertebral scales, number of scales in a paravertebral row from first scale posterior to parietal scale to last scale at the level of vent opening; 24 paravertebral scales in a row between limb insertions; 20 flat, imbricate, ventral scales much larger than dorsal scales; 8 enlarge, pore-bearing, precloacal scales in an angular series; and no deep precloacal groove or depression.

Forelimbs moderate in stature, relatively short (FA/SVL 0.12); granular scales of forearm slightly larger than those on body, interspersed with small tubercles; hind limbs more robust than forelimbs, moderate in length (TBL/SVL 0.13), covered dorsally by granular scales interspersed with large, and small conical tubercles; ventral scales of thigh flat, imbricate, larger than dorsals; subtibial scales flat, imbricate; proximal femoral scales smaller than distal femorals; femoral pores absent; digits relatively long with 15 lamellae on fourth toe; and claws well developed.

Tail 37.8 mm in length, first 17.1 mm original, 6.1 mm in width at base, tapering to a point; dorsal scales of flat, square with conical, keeled tubercles, regenerated portion covered with small, smooth subcircular scales; median row of transversely expanded subcaudal scales, significantly larger than dorsal caudal scales on original portion; base of tail bearing hemipenal swellings; and postcloacal scales flat, imbricate.


Figure IO. View of Dixonius muangfuangensis sp. nov. A adult male holotype VNUF R. 2020.42 (Field no. MF.02) B adult female paratype VNUF R. 2020.52 (Field no. MF. 03) \(\mathbf{C}\) juvenile male paratype NUOL R. 2022.01 (Field no. MF. 01) in Nadan Village, Muangfuang District, Vientiane Province, Central Laos.


Figure II. Habitat of Dixonius muangfuangensis sp. nov. Nadan Village, Muangfuang District, Vientiane Province, Central Laos.

Coloration in life (Fig. 10). Ground color of dorsal head and dorsum dark grey with numerous small and irregular black blotches; lips with dark bars; two regularly disposed whitish tubercles on each side on each side running from postorbital along the flanks to tail, terminating at regenerated portion of tail; upper surface of fore and hind limbs uniformly dark brown with round black-brown spots; dorsum of tail covered with some large black-brown blotches; ventral surface beige uniformly as the belly and the throat.

Variation (Fig. 10). The female paratype (VNUF R.2020.52) generally matches that of the holotype in all characteristics. The juvenile male paratype (NUOL R.2022.01) has fewer black blotches on head and dorsum and two regularly disposed whitish tubercles on each side on each side of the head extending from the postorbital region, along the flanks, to the tail tip. Further measurements are summarized in Tables 2-4 and Suppl. material 1: table S2.

Distribution. Dixonius muangfuangensis sp. nov. currently is only known from the type locality of Nadan Village, Muangfuang District, Vientiane Province, Central Laos (Fig. 1).

Etymology. The specific epithet of the new species refers to the type locality of the new species in Muangfuang District, Vientiane Province, Central Laos.

Natural history. The type series was collected between 19:10 and 19:30 h, on the ground inside Sinxay Temple, at an elevation of 276 m a.s.l. The surrounding habitat was disturbed lowland karst forest (Fig. 11).

Comparisons. Dixonius muangfuangensis sp. nov. is the sister species to \(D\). lao (Fig. 2) from which it differs by an uncorrected pairwise sequence divergence of 3.10\% (Table 4). It is differentiated morphologically by having a significantly higher mean number of head length (HL), infralabials (IFL), and numbers of supralabial at midorbital position (MO). In addition, it differs from \(D\). lao in dorsal pattern (dorsal pebble brown versus dorsal dark gray with black blotches). Statistically significant and discrete categorical differences between Dixonius muangfuangensis sp. nov. and all other species and populations are presented in Tables 5-7.

\section*{Discussion}

Morphological comparisons indicated that Dixonius gialaiensis sp. nov. is most similar to its sister species \(D\). minhlei, but can be differentiated from the latter species by the number of dorsal tubercle scale rows and differences in color pattern. The results of the molecular analysis show the uncorrected pairwise sequence divergence between the two taxa is \(3.60 \%\). Additionally, the two species are widely separated geographically being in different mountain systems and separated by the Dong Nai River system (Fig. 1). Collectively, these data suggest these are separate and distinct species.

Dixonius gialaiensis sp. nov. was discovered in a protected forest near the National Highway 25. The construction of new infrastructure at this site strongly impacts the habitat of \(D\). gialaiensis sp. nov., including range fragmentation and forest degradation. Further investigations on conservation status is urgently required to develop effective conservation measures.

Dixonius muangfuangensis sp. nov. is most closely related to \(D\). lao, but can be distinguished from it by head shape and color pattern differences. The molecular analysis indicated these two species differ by a \(3.1 \%\) uncorrected pairwise genetic distance. In addition, the two species evolved separately in geographically isolated regions. The type locality of \(D\). muangfuangensis sp. nov. is approximately 500 km south of the type locality of \(D\). lao and the type localities are separated by the Nam Ngiap and Xebangfai river network systems (Fig. 1).

The BEAST analysis indicates that the divergence between Dixonius gialaiensis sp. nov. and \(D\). minhlei and that between \(D\). muangfuangensis sp. nov. and \(D\). lao may have been the result of cyclical climatic events during the recent interglacial periods of the Pliocene as noted for several other Indochinese species (see Grismer and Grismer 2017 and references therein). \(D\). muangfuangensis sp. nov. and \(D\). lao diverged from one another at approximately 3.47 mya. Relatively soon after, at approximately 3.19 mya, Dixonius gialaiensis sp. nov. and D. minblei separated from one another, thus allowing sufficient time for them to evolve significant differences between them in a number of characteristics. During this time period, the formation of separate karstic habitats and granitic mountains and hills may have prevented gene flow between these populations, placing each species on separate evolutionary trajectories (Grismer and Grismer 2017).

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\section*{References}

Barraclough TG, Birky Jr CW, Burt A (2003) Diversification in sexual and asexual organisms. Evolution; International Journal of Organic Evolution 57: 2166-2172. https://doi. org/10.1554/02-339
Bauer AM, Branch WR, Good DA (1997) The taxonomy of the southern African leaf-toed geckos (Squamata: Gekkonidae), with a review of old world. Proceedings of the California Academy of Sciences 49(14): 447-497.
Bauer AM, Sumontha M, Grossmann W, Pauwels OSG, Vogel G (2004) A new species of Dixonius (Squamata: Gekkonidae) from Kanchanaburi Province, western Thailand. Current Herpetology 23(1): 17-26. https://doi.org/10.5358/hsj.23.17
Botov A, Phung TM, Nguyen TQ, Bauer AM, Brennan IG, Ziegler T (2015) A new species of Dixonius (Squamata: Gekkonidae) from Phu Quy Island, Vietnam. Zootaxa 4040(1): 48-58. https://doi.org/10.11646/zootaxa.4040.1.4
Bouckaert RR, Drummond AJ (2017) bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC Evolutionary Biology 17(1): 1-42. https://doi.org/10.1186/ s12862-017-0890-6
Boulenger GA (1898) Third report on additions to the lizard collection in the Natural History Museum. Proceedings of the Zoological Society of London 1898(4): 912-923. https://doi. org/10.1111/j.1096-3642.1898.tb03194.x [pls LV-LVII.]
Chan KO, Grismer LL (2022) GroupStruct: An R package for allometric size correction. Zootaxa 5124(4): 471-482. https://doi.org/10.11646/zootaxa.5124.4.4
Das I (2004) A new species of Dixonius (Sauria: Gekkonidae) from southern Vietnam. The Raffles Bulletin of Zoology 52(2): 629-634. https://doi.org/10.5358/hsj.23.63

De Queiroz K (2007) Species concepts and species delimitation. Systematic Biology 56(6): 879-886. https://doi.org/10.1080/10635150701701083
Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian Phylogenetics with BEAUti and BEAST 1.7. Molecular Biology and Evolution 29(8): 1969-1973. https://doi. org/10.1093/molbev/mss075
Frost DR, Hillis DM (1990) Species in concept and practice: Herpetological application. Herpetologica 46: 87-104.
Frost DR, Kluge AG (1994) A consideration of the epistemology in systematic biology, with special reference to species. Cladistics 10(3):259-294. https://doi.org/10.1111/j.1096-0031.1994. tb00178.x
Gamble T, Greenbaum E, Jackman TR, Bauer AM (2015) Into the light: Diurnality has evolved multiple times in geckos. Biological Journal of the Linnean Society. Linnean Society of London 115(4): 896-910. https://doi.org/10.1111/bij. 12536
Grismer LL, Grismer JL (2017) A re-evaluation of the phylogenetic relationships of the Cyrtodactylus condorensis group (Squamata; Gekkonidae) and a suggested protocol for the characterization of rock-dwelling ecomorphology in Cyrtodactylus. Zootaxa 4300(4): 486-504. https://doi.org/10.11646/zootaxa.4300.4.2
Hillis DM (2019) Species delimitation in herpetology. Journal of Herpetology 53(1): 3-12. https://doi.org/10.1670/18-123
Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35(2): 518-522. https://doi.org/10.1093/molbev/msx281
Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294(5550): 2310-2314. https://doi. org/10.1126/science. 1065889
Husson F, Josse J, Le S, Mazet J (2017) FactoMine R: Exploratory Data Analysis and Data Mining. R package version 1.36.
Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. BMC Genetics 11(1): 1-15. https://doi.org/10.1186/1471-2156-11-94
Kalyaanamoorthy S, Minh BQ, Wong TK, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587-589. https://doi.org/10.1038/nmeth. 4285
Kassambara A, Mundt F (2017) factoextra: Extract and Visualize the Result of Multivariate Data Analyses. R package version 1.0.5.999.
Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870-1874. https:// doi.org/10.1093/molbev/msw054
Lleonart J, Salat J, Torres GJ (2000) Removing allometric effects of body size in morphological analysis. Journal of Theoretical Biology 205(1): 85-93. https://doi.org/10.1006/jtbi.2000.2043
Macey JR, Larson A, Ananjeva NB, Fang Z, Papenfuss TJ (1997) Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. Molecular Biology and Evolution 14(1): 91-104. https://doi.org/10.1093/oxfordjournals.molbev.a025706

McCoy MW, Bolker BM, Osenberg CW, Miner BG, Vonesh JR (2006) Size correction: Comparing morphological traits among populations and environments. Oecologia 148(4): 547-554. https://doi.org/10.1007/s00442-006-0403-6
Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, Louisiana, 14 November 2010, 8 pp. https://doi. org/10.1109/GCE.2010.5676129
Minh Q, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30(5): 1188-1195. https://doi.org/10.1093/ molbev/mst024
Ngo VT, Ziegler T (2009) A new species of Dixonius from Nui Chua National Park, Ninh Thuan Province, southern Vietnam (Squamata, Gekkonidae). Zoosystematics and Evolution 85(1): 117-125. https://doi.org/10.1002/zoos. 200800018
Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution 32(1): 268-274. https://doi.org/10.1093/molbev/msu300
Nguyen TH, Sitthivong S, Ngo HT, Luu VQ, Nguyen TQ, Le MD, Ziegler T (2020) A new species of Dixonius (Squamata: Gekkonidae) from the karst forest of Khammouane Province, central Laos. Zootaxa 4759(4): 530-542. https://doi.org/10.11646/zootaxa.4759.4.4
Nguyen TH, Luu VQ, Sitthivong S, Ngo HT, Nguyen TQ, Le MD, Ziegler T (2021) A new species of Dixonius (Squamata: Gekkonidae) from Vientiane Capital, Laos. Zootaxa 4965(2): 351-362. https://doi.org/10.11646/zootaxa.4965.2.8
Pagès J (2015) Multiple Factor Analysis by Example Using R. CRC Press, New York, 272 pp. https://doi.org/10.1201/b17700
Pauwels OSG, Panitvong N, Kunya K, Sumontha M (2021) A new sandstone-dwelling leaf- toed gecko (Gekkonidae: Dixonius mekongensis) from the Thai-Lao border. Zootaxa 4969(3): 526-538. https://doi.org/10.11646/zootaxa.4969.3.5
R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna. 2018. http://www.R-project.org [Accessed 1 December 2022]
Rambaut A, Drummond AJ (2013) TreeAnnotator. Version 1.7.3 MCMC Output Analysis. https://beast. community/treeannotator [Accessed 15 January 2023]
Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer. Version 1.7. http://tree.bio. ed.ac.uk/ software/tracer/ [Accessed 15 January 2023]
Reist JD (1986) An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. Canadian Journal of Zoology 64(6): 1363-1368. https://doi. org/10.1139/z86-203
Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539-542. https://doi.org/10.1093/sysbio/sys029
Taylor EH (1962) New oriental reptiles. The University of Kansas Science Bulletin 43: 209263. https://doi.org/10.5962/bhl.part. 13346

Thorpe RS (1975) Quantitative handling of characters useful in snake systematics with particular reference to interspecific variation in the Ringed Snake Natrix natrix (L.). Biologi-
cal Journal of the Linnean Society. Linnean Society of London 7(1): 27-43. https://doi. org/10.1111/j.1095-8312.1975.tb00732.x
Thorpe RS (1983) A review of the numerical methods for recognizing and analyzing racial differentiation. In: Felsenstein J (Ed.) Numerical Taxonomy. NATO ASI Series (Series G: Ecological Sciences) (Vol. 1). Springer-Verlag, Berlin, 404-423. https://doi.org/10.1007/978-3-642-69024-2_43
Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44(W1): W232-W235. https://doi.org/10.1093/nar/gkw256
Turan C (1999) A note on the examination of morphometric differentiation among fish populations: The Truss System. Turkish Journal of Zoology 23: 259-263.
Uetz P, Freed P, Aguilar R, Reyes F, Hošek J [Eds] (2022) The Reptile Database. http://www. reptile-database.org [Accessed 14 January 2023]
Wilcox TP, Zwickl DJ, Heath TA, Hillis DM (2002) Phylogenetic relationships of the Dwarf Boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Molecular Phylogenetics and Evolution 25(2): 361-371. https://doi.org/10.1016/S1055-7903(02)00244-0
Ziegler T, Botov A, Nguyen TT, Bauer AM, Brennan IG, Ngo HT, Nguyen TQ (2016) First molecular verification of Dixonius vietnamensis Das, 2004 (Squamata: Gekkonidae) with the description of a new species from Vinh Cuu Nature Reserve, Dong Nai Province, Vietnam. Zootaxa 4136(3): 553-566. https://doi.org/10.11646/zootaxa.4136.3.7

\section*{Supplementary material I}

Measurements and morphological characters of the type series of Dixonius gialaiensis sp. nov.
Authors: Vinh Quang Luu, Thuong Huyen Nguyen, Minh Duc Le, Jesse L. Grismer, Hong Bich Ha, Saly Sitthivong, Tuoi Thi Hoang, L. Lee Grismer
Data type: tables (Excel spreadsheet)
Explanation note: table S1: Measurements (in mm) and morphological characters of the type series of Dixonius gialaiensis sp. nov. (for abbreviations see Material and methods). Measurements taken on right side; FA is given in the left side; SPL/IFL/ T4 given in right/ left order; -absence; * tail regenerated; table S2: Measurements (in mm ) and morphological characters of the type series of Dixonius muangfuangensis sp. nov. (for abbreviations see material and methods). Measurements taken on right side; FA and T4 are given in the left side; SPL/IFL given in right/ left order; -absence, \({ }^{*}\) tail regenerated; \({ }^{* *}\) tail lost.
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Link: https://doi.org/10.3897/zookeys.1163.101230.suppl1

\title{
Review of the pill millipede genus Hyperglomeris Silvestri, 1917 (Diplopoda, Glomerida, Glomeridae) with description of two new species from Laos
}

\author{
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\begin{abstract}
The pill millipede genus Hyperglomeris Silvestri, 1917 is reported from Laos for the first time. Two new species, namely H. bicaudata Likhitrakarn, sp. nov. and H. inkhavilayi Likhitrakarn, sp. nov., from Houaphanh and Khammouane provinces, northern Laos, are described and illustrated based on morphological characters and molecular analyses. Sequences of COI gene were used as DNA barcoding markers, and successfully supported the accurate identification of other Glomeridae species. Interspecific divergence of the COI uncorrected p-distance between these new species and other Hyperglomeris species ranged from \(7.84-13.07 \%\), while the intraspecific divergence was \(0.45 \%\) in H. inkhavilayi sp. nov. and \(5.3 \%\) in H. bicaudata sp. nov. The updated status of Hyperglomeris, a map of its distribution, and identification keys for all species are given.
\end{abstract}

\section*{Keywords}

Distribution map, key, Laos, morphology, molecular phylogeny, new species

\section*{Introduction}

The pill millipede genus Hyperglomeris Silvestri, 1917 belongs to the family Glomeridae, order Glomerida. Members of this family and others in the order are capable of complete volvation, where the head and collum are tucked within the rolled-up body segments or ball-like shape when threatened. For this reason they are commonly referred to as 'pill millipedes'. Prior to this study, the genus Hyperglomeris consisted of only seven species, all narrowly endemic to northern Vietnam (Nguyen et al. 2019). Species of this genus are relatively small, ranging in length from 5.5 to 24 millimeters, and have a distinct body color. However, there is one colorless species, H. depigmentata Golovatch et al., 2013, which was found to inhabit a cave (Golovatch et al. 2013).

Hyperglomeris is classified within the subfamily Haploglomerinae, which has nine genera, the majority of which have only one or two species. The classification of these genera is still complicated due to few distinctive morphological characteristics, such as the presence of trichosteles on the prefemur or femur of the telopods, the number of apical cones on the antennae, and the number of striae on the thoracic shield (Wesener 2015a), and it is uncertain whether these characteristics represent species-level or ge-nus-level distinctions. Therefore, it is essential to incorporate genetic information (as in our case, DNA barcoding based on COI gene sequences) in order to delineate species boundaries within this group. It can also be used as a foundation for further classification of the family.

Laos (or the Lao PDR) is a Southeast Asian country that shares borders with Myanmar and China to the north, Vietnam to the east, Cambodia to the south, and Thailand to the west. Laos is well-known for its beautiful mountains and forests, as well as its diverse ecosystems, which include tropical rainforests, dry lowlands, and hilly regions (ADB 2000). Previously, a total of 34 species of millipedes from 20 genera, 13 families, and seven orders have been recorded from Laos (Likhitrakarn et al. 2014). Among these, the Glomerida has remained represented by only four species of the genus Hyleoglomeris Verhoeff, 1910.

In this study, we were fortunate to discover two new species of the genus Hyperglomeris from Laos. These two new species are investigated using an approach of integrative taxonomy, combining both morphological characters and a common DNA barcoding fragment of the COI gene. In addition, we have revised the scope of the genus, also providing its distribution map and an identification key to all nine species.

\section*{Materials and methods}

\section*{Morphological studies}

Specimens were collected from Laos under the Animal Care and Use Protocol Review No. 1723018. Locations of collecting sites were recorded by GPS using a Garmin GPSMAP 60 CSx based on the WGS 84 datum, and all coordinates and elevations
were double-checked with Google Earth. Photographs of live animals were taken using a Nikon 700D digital camera with a Nikon AF-S VR 105 mm macro lens. The specimens collected were euthanized by a two-step method following AVMA Guidelines for the Euthanasia of Animals (AVMA 2013). Specimens were then preserved in 90\% ethanol for morphological and molecular studies. After 24 hr , ethanol was replaced with new \(95 \%\) ethanol to prevent their defensive chemicals from interfering with future DNA extraction.

The holotype and all paratypes are housed in the Chulalongkorn University Museum of Zoology (CUMZ), Bangkok, Thailand. The specimens were examined, measured, and photographed under a Nikon SMZ 745T trinocular stereo microscope equipped with a Canon EOS 5DS R digital SLR camera. The acquired digital photos were processed and modified with Adobe Photoshop CS5. Line drawings were based on photographs taken under the stereo microscope equipped with a digital SLR camera.

The terminology used to describe morphological structures is consistent with the most recent publications (Golovatch et al. 2006; Golovatch 2017; Nguyen et al. 2019). In the catalogue sections, \(\mathbf{D}\) stands for the original description; \(\mathbf{K}\) for appearance in a key; \(\mathbf{L}\) for appearance in a species list; \(\mathbf{M}\) for a mere mention; and \(\mathbf{M I}\) for molecular information. The following abbreviations listed below are used in the figures:
\begin{tabular}{ll}
\(\mathbf{c x}\) & coxa; \\
\(\mathbf{c x l}\) & coxal lobe; \\
\(\mathbf{f e}\) & femur; \\
\(\mathbf{f p}\) & femoral process; \\
\(\mathbf{N P}\) & national park; \\
pf & prefemur; \\
pfc & prefemoral cone of telopod; \\
pft & prefemoral trichostele of telopod; \\
\(\mathbf{s h}\) & syncoxital horn of telopod; \\
\(\mathbf{s l}\) & syncoxital lobe of telopod; \\
\(\mathbf{s n}\) & syncoxite notch; \\
\(\mathbf{s x}\) & syncoxite; \\
\(\mathbf{t a}\) & tarsus; \\
\(\mathbf{t c}\) & tibial cone; \\
\(\mathbf{t i}\) & tibia; \\
\(\mathbf{t p}\) & tibial process.
\end{tabular}

\section*{DNA extraction, PCR amplification, and sequencing}

Total genomic DNA was extracted from the legs and part of thoracic tissue of the paratype using the DNA extraction kit for animal tissue (NucleoSpin Tissue extraction kit, Macherey-Nagel, Germany), following the standard procedure of the manual. Fragments with size of 660 bp of the mitochondrial cytochrome \(c\) oxidase subunit I gene (COI) were amplified using LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3';

Folmer et al. 1994) and HCOoutout (5'-GTAAATATATGRTGDGCTC; Schulmeister et al. 2002) or Nancy (5'-CCCGGTAAAATTAAAATATAAACTTC-3'; Bogdanowicz et al. 1993).

The PCR amplification was performed using a T100 thermal cycler (BIO-RAD) with a final reaction volume of \(20 \mu \mathrm{~L}(15 \mu \mathrm{~L}\) of EmeraldAmp GT PCR Master Mix, \(1.5 \mu \mathrm{~L}\) of each primer, 10 ng of template DNA and distilled water up to \(20 \mu \mathrm{~L}\) total volume). Thermal cycling was performed at \(94^{\circ} \mathrm{C}\) for 3 min , followed by 35 cycles of \(94{ }^{\circ} \mathrm{C}\) for 30 s , annealing at \(42-56^{\circ} \mathrm{C}\) (depending on samples and the primer pair) for 60 s , extension at \(72{ }^{\circ} \mathrm{C}\) for 90 s , and a final extension at \(72^{\circ} \mathrm{C}\) for 5 min . Amplification of PCR products was confirmed through \(1.5 \%(\mathrm{w} / \mathrm{v})\) agarose gel electrophoresis before purification by MEGAquick-spinTM plus (Fragment DNA purification kit) and sequencing in both directions (forward and reverse) using an automated sequencer (ABI prism 3730XL).

All nucleotide sequences obtained in this study were deposited in the GenBank Nucleotide sequences database under accession numbers OQ661871-OQ661874. The collecting localities and GenBank accession numbers of each nominal species are listed in Table 1.

\section*{Phylogenetic analyses}

The sequences were aligned using MEGA7 (Kumar et al. 2016). The final aligned dataset included 660 bp of 23 COI sequences. All species of Hyperglomeris for which sequences are available in Genbank were included, along with members of some other genera of Glomeridae (i.e., Peplomeris Silvestri, 1917, Tonkinomeris Nguyen, Sierwald \& Marek, 2019, Rhopalomeris Verhoeff, 1906, Hyleoglomeris, Glomeris Latreille, 1802, and Trachysphaera Heller, 1857); the genera Sphaerobelum and Zephronia were used as outgroups (Table 1).

Two phylogenetic methods were used in this study. Firstly, the maximum likelihood (ML) method was performed using RAxML v. 8.2.10 (Stamatakis, 2014), with GTRGAMMA as the nucleotide substitution model and 1,000 ML bootstrap replicates to assess topology bootstrap support (bp). Secondly, Bayesian Inference (BI) analysis was performed by MrBayes 3.2.6 (Ronquist et al. 2012) using the Markov chain Monte Carlo technique (MCMC), and with the best-fit model of nucleotide substitution as suggested by PartitionFinder2 v. 2.3.4 (Lanfear et al. 2016). The selected best-fit models for the three COI codon positions were SYM \(+\mathrm{G}, \mathrm{F} 81+\mathrm{I}\), and GTR+G, respectively. The BI trees were run for ten million generations with a random starting tree. The resultant trees were sampled every 1,000 generations and the values were used to estimate consensus tree topology; bipartition posterior probability (bpp), and branch lengths, after the first \(25 \%\) of obtained trees were discarded as burn-in. All average effective sample size (ESS) values sampled from the MCMC analysis were greater than 2,000 in all parameters. Both phylogenetic analyses were implemented through the on-line CIPRES Science Gateway (Miller et al. 2010). The obtained tree was drawn by FigTree v. 1.4 .3 (http://tree.bio.ed.ac.uk/software/ figtree/, accessed on 28 February 2023). In addition, genetic divergence based on

Table I. List of species used for molecular phylogenetic analyses and relevant information. \({ }^{*}=\) paratype.
\begin{tabular}{|c|c|c|c|c|c|}
\hline Voucher number & Species & Locality & Geographical coordinates & GenBank accession number COI & Reference \\
\hline CUMZ-GLO004* & Hyperglomeris bicaudata Likhitrakarn, sp. nov. & Ban Tham Na Tan, Houaphanh, Laos & \(20^{\circ} 27^{\prime} 28\) "N, 104*08'43"E & OQ661871 & This study \\
\hline CUMZ-GLO007* & Hyperglomeris bicaudata Likhitrakarn, sp. nov. & Limestone mountain area near vocational-technical school, Houaphanh, Laos & \(20^{\circ} 24^{\prime} 15^{\prime \prime} \mathrm{N}, 104^{\circ} 15^{\prime} 4^{\prime \prime} \mathrm{E}\) & OQ661872 & This study \\
\hline CUMZ-GLO095/1* & Hyperglomeris inkhavilayi Likhitrakarn, sp. nov. & Orchard, Ban Phawong, Khammouane, Laos & \(17^{\circ} 32^{\prime} 244^{\prime \prime} \mathrm{N}, 105^{\circ} 25^{\prime} 18^{\prime \prime} \mathrm{E}\) & OQ661873 & This study \\
\hline CUMZ-GLO095/2* & Hyperglomeris inkhavilayi Likhitrakarn, sp. nov. & Orchard, Ban Phawong, Khammouane, Laos & \(17^{\circ} 32^{\prime} 244^{\prime \prime N}, 105^{\circ} 25^{\prime} 18^{\prime \prime} \mathrm{E}\) & OQ661874 & This study \\
\hline FMNH-SVE102 & \begin{tabular}{l}
Hyperglomeris simplex \\
Nguyen et al., 2019
\end{tabular} & Tam Dao NP, Vinh Phuc, Vietnam & & MT749410 & Nguyen et al. 2021 \\
\hline IEBR-Myr 605 & \begin{tabular}{l}
Hyperglomeris simplex \\
Nguyen et al., 2019
\end{tabular} & Tam Dao NP, Vinh Phuc, Vietnam & & MT749403 & Nguyen et al. 2021 \\
\hline IEBR-Myr 674 & Hyperglomeris sp. 1 & Pu Mat NP, Nghe An, Vietnam & & MT749409 & Nguyen et al. 2021 \\
\hline FMNH-SVE176 & Peplomeris magna Golovatch, 1983 & Cuc Phuong NP, Ninh Binh, Vietnam & & MT749407 & Nguyen et al. 2021 \\
\hline IEBR-Myr 677 & Peplomeris magna Golovatch, 1983 & Cuc Phuong NP, Ninh Binh, Vietnam & & MT749405 & Nguyen et al. 2021 \\
\hline IEBR-Myr 656 & Peplomeris magna Golovatch, 1983 & Cuc Phuong NP, Ninh Binh, Vietnam & & MT749408 & Nguyen et al. 2021 \\
\hline FMNH-SVE204 & Hyleoglomeris lobus Nguyen et al., 2019 & Cuc Phuong NP, Ninh Binh, Vietnam & & MT749391 & Nguyen et al. 2021 \\
\hline IEBR-Myr 653 & \begin{tabular}{l}
Hyleoglomeris lobus \\
Nguyen et al., 2019
\end{tabular} & Cuc Phuong NP, Ninh Binh, Vietnam & & MT749402 & Nguyen et al. 2021 \\
\hline IEBR-Myr 678 & \begin{tabular}{l}
Hyleoglomeris lobus \\
Nguyen et al., 2019
\end{tabular} & Cuc Phuong NP, Ninh Binh, Vietnam & & MT749406 & Nguyen et al. 2021 \\
\hline IEBR-Myr 533 & Rhopalomeris sauda Nguyen et al., 2019 & Ba Be NP, Bac Kan, Vietnam & & MT749404 & Nguyen et al. 2021 \\
\hline IEBR-Myr 654 & \begin{tabular}{l}
Rhopalomeris sauda \\
Nguyen et al., 2019
\end{tabular} & Me Linh District, Vinh Phuc, Vietnam & & MT749401 & Nguyen et al. 2021 \\
\hline IEBR-Myr 706 & \begin{tabular}{l}
Rhopalomeris sauda \\
Nguyen et al., 2019
\end{tabular} & Khao Ca NR, Ha Giang, Vietnam & & MT749400 & Nguyen et al. 2021 \\
\hline IEBR-Myr 801 & \begin{tabular}{l}
Rhopalomeris sauda \\
Nguyen et al., 2019
\end{tabular} & Cham Chu NR, Tuyen Giang, Vietnam & & MT749398 & Nguyen et al. 2021 \\
\hline IEBR-Myr 804a & Tonkinomeris napoensis Nguyen et al., 2019 & Bac Me NR, Ha Giang, Vietnam & & MT749397 & Nguyen et al. 2021 \\
\hline IEBR-Myr 804b & Tonkinomeris napoensis Nguyen et al., 2019 & Bac Me NR, Ha Giang, Vietnam & & MT749396 & Nguyen et al. 2021 \\
\hline Tcost8-MK & Trachysphaera costata (Waga, 1857) & Slovakia & & KX467622 & Mock et al. 2016 \\
\hline GBOL33714 & Glomeris marginata (Villers, 1789) & Königshütte, Wernigerode, SachsenAnhalt, Germany & \(51.743^{\circ} \mathrm{N}, 10.767^{\circ} \mathrm{E}\) & MG892112 & Reip and Wesener 2018 \\
\hline ZFMK1634 & Glomeris marginata (Villers, 1789) & Bockswiese Goslar, Niedersachsen, Germany & \(51.841^{\circ} \mathrm{N}, 10.326^{\circ} \mathrm{E}\) & MG892119 & Reip and Wesener 2018 \\
\hline \multicolumn{6}{|l|}{Outgroup Sphaerotheriida: Zephroniidae} \\
\hline \[
\begin{aligned}
& \hline \text { FMNH-INS } 0000 \\
& 072 \text { 674) }
\end{aligned}
\] & Sphaerobelum truncatum Wongthamwanich, 2012 & Pang Hi Village, Nan, Thailand & \(19^{\circ} 23^{\prime} 46.3\) " \(\mathrm{N}, 100^{\circ} 41^{\prime} 42.4^{\prime \prime} \mathrm{E}\) & JN885184 & Wongthamwanich et al. 2012 \\
\hline ZFMK Myr3502 & \begin{tabular}{l}
Zephronia laotica \\
Wesener, 2019
\end{tabular} & Garden of Erawan Riverside Hotel, Champasak, Laos & \(15^{\circ} 6^{\prime} 27.0{ }^{\prime \prime} \mathrm{N}, 105^{\circ} 49^{\prime} 14.3\) "E & MK330977 & Wesener 2019 \\
\hline
\end{tabular}
the COI sequence was evaluated using uncorrected p-distances as implemented in MEGA7 (Kumar et al. 2016). The missing data in pairwise comparison were treated with pairwise deletion method.

\section*{Taxonomy}

\section*{Family Glomeridae Leach, 1815}

\section*{Genus Hyperglomeris Silvestri, 1917}

Hyperglomeris Silvestri, 1917: 145 (D, K).
Hyperglomeris-Golovatch, 1983a: 110 (M); 2017: 196 (M); Golovatch et al. 2013: 202 (M); Nguyen et al. 2019: 274 (M, K); 2021: 257 (M); Kuroda et al. 2022a: 162 (M); 2022b: 117 (M).
Dinoglomeris Silvestri, 1917: 147 (D, K), synonymized by Golovatch (1983b: 180).
Diagnosis. Pill millipedes with four apical cones on the antennae; the caudal margins of the pygidium are sometimes modified into small paramedian lobes, but are mostly emarginate or slightly concave medially; leg-pair 18 devoid of any evident mesal outgrowths on the femur or tibia; the posterior telopods are rather stout, with prefemoral trichosteles reduced or only present as a small cone; and the femoral trichosteles are strongly reduced or absent.

Type species. Hyperglomeris lamellosa Silvestri, 1917, by original designation.
Species included. Hyperglomeris lamellosa Silvestri, 1917, H. dirupta (Silvestri, 1917), H. conspicua Golovatch, 1983, H. maxima Golovatch, 1983, H. depigmentata Golovatch, Geoffroy \& VandenSpiegel, 2013, H. nigra Golovatch, 2017, H. simplex Nguyen, Sierwald \& Marek, 2019, H. bicaudata Likhitrakarn, sp. nov., H. inkhavilayi Likhitrakarn, sp. nov.

Remarks. The genus Hyperglomeris was established by Silvestri (1917), who designated \(H\). lamellosa Silvestri, 1917 as the type species and provided a detailed description and excellent illustrations. At the same time, he created a new genus and species, Dinoglomeris dirupta, which only superficially differed from Hyperglomeris. Both species were discovered on Mount Mẫu Sơn, Vietnam, but at different altitudes. Subsequently, Golovatch (1983b) investigated the scope of the genus using his material from Vietnam and proposed that the two genera be combined into one. Afterwards, Golovatch published two new species, synonymizing the name Dinoglomeris with Hyperglomeris.

\section*{Hyperglomeris lamellosa Silvestri, 1917}

Hyperglomeris lamellosa Silvestri, 1917: 147 (D); Golovatch 1983a: 110 (M, K); 1983b: 180 (L); 2017: 196 (M, K); Golovatch et al. 2013: 201 (M); Enghoff et al. 2004: 31 (L); Nguyen et al. 2019: 263 (L, M).

Remarks. This species was described from Mount Mẫu Sơn, 2000-3000 feet a.s.l., Langson Province, Vietnam (Silvestri 1917). Endemic to Vietnam.

\section*{Hyperglomeris dirupta (Silvestri, 1917)}

Dinoglomeris dirupta Silvestri, 1917: 147 (D).
Hyperglomeris dirupta-Golovatch, 1983a: 110 (M, K); 1983b: 180 (L); 2017: 196 (M, K); Golovatch et al. 2013: 201 (M); Enghoff et al. 2004: 31 (L); Nguyen et al. 2019: 263 (L, M).

Remarks. This species was described from Mount Mẫu Sơn, 200-300 feet a.s.l., Langson Province, Vietnam (Silvestri 1917). Endemic to Vietnam.

\section*{Hyperglomeris conspicua Golovatch, 1983}

Hyperglomeris conspicua Golovatch, 1983a: 110 (D, K); Golovatch 1983b: 180 (L); 2017: 197 (M, K); Golovatch et al. 2013: 201 (M); Enghoff et al. 2004: 31 (L); Nguyen et al. 2019: 262 (L, M).

Remarks. This species was described from Vạn Mai, Mai Châu District, Hòa Bình Province, Vietnam (Golovatch 1983a). Endemic to Vietnam.

\section*{Hyperglomeris maxima Golovatch, 1983}

Hyperglomeris maxima Golovatch, 1983a: 108 (D, K); Golovatch, 1983b: 180 (L); 2017: 197 (M, K); Golovatch et al. 2013: 201 (M); Enghoff et al. 2004: 31 (L); Nguyen et al. 2019: 263 (L, M).

Remarks. This species was described from Vạn Mai, Mai Châu District, Hòa Bình Province, Vietnam (Golovatch 1983a). Endemic to Vietnam.

\section*{Hyperglomeris depigmentata Golovatch, Geoffroy \& VandenSpiegel, 2013}

Hyperglomeris depigmentata Golovatch et al., 2013: 206 (D); Golovatch 2017: 197 (M,
K); Nguyen et al. 2019: 262 (L, M); Kuroda et al. 2022a: 162 (M, K).

Remarks. This species was described from Cave Hang Doi, \(20.496176^{\circ} \mathrm{N}\), \(105.137465^{\circ}\) E, Lang Kho Muong, Than Son, Thanh Hoa Province, Vietnam (Golovatch et al. 2013). Endemic to Vietnam.

\section*{Hyperglomeris nigra Golovatch, 2017}

Hyperglomeris nigra Golovatch, 2017: 195 (D, K); Nguyen et al. 2019: 263 (L, M).
Remark. This species was described from Xuan Son National Park, \(21^{\circ} 07^{\prime} 52^{\prime \prime N}\), \(104^{\circ} 57^{\prime} 07^{\prime \prime} \mathrm{E}, 400-470 \mathrm{~m}\) a.s.l., ca. 90 km northwest of Hanoi, Phu Tho Province, Vietnam (Golovatch 2017).

Hyperglomeris simplex Nguyen, Sierwald \& Marek, 2019
Hyperglomeris simplex Nguyen et al., 2019: 276 (D).
Hyperglomeris simplis (sic!)-Nguyen et al. 2021: 258 (MI, M).
Remark. This species was described from Me Linh Station for Biodiversity, \(21.3850^{\circ} \mathrm{N}\), \(105.7119^{\circ}\) E, Ngoc Thanh Commune, Phuc Yen Town, Vinh Phuc Province, Vietnam (Nguyen et al. 2019).

\section*{Hyperglomeris bicaudata Likhitrakarn, sp. nov.}
https://zoobank.org/FB13C74A-496A-45F0-BB73-C710A6C5123D
Figs 1-3A, B
Material examined. Holotype: Laos - Houaphanh • (CUMZ-GLO006); Viengxay District, Limestone mountain area near Kaysone Phomvihane Cave; elev. 890 m a.s.l.; \(20^{\circ} 20^{\prime} 24^{\prime \prime N}\), \(104^{\circ} 13^{\prime} 44^{\prime \prime E}\); 6 Jul. 2014; R. Srisonchai, C. Sutcharit, K. Inkhavilay leg.; CUMZ; Paratypes: Laos - Houaphanh • 1 q; same collection data as holotype; • 3 웅 (CUMZ-GLO004); Viengxay District, Ban Tham Na Tan, Limestone mountain area; elev. 860 m a.s.1.; \(20^{\circ} 27^{\prime} 28^{\prime \prime} \mathrm{N}, 104^{\circ} 08^{\prime} 43^{\prime \prime} \mathrm{E}\); 5 Jul. 2014; R. Srisonchai, C. Sutcharit, K. Inkhavilay leg.; CUMZ; OQ661871 • 1 §', 2 앙 (CUMZ-GLO007); Viengxay District, Limestone mountain area near vocational-technical school around kilometre 31; elev. 840 m a.s.l.; \(20^{\circ} 24^{\prime} 15^{\prime \prime N}\) N, \(104^{\circ} 15^{\prime} 4^{\prime \prime E}\) E; 6 Jul. 2014; R. Srisonchai, C. Sutcharit, K. Inkhavilay leg.; CUMZ; OQ661872.

Name. To emphasize the caudal margin of the anal shield being more ( \(\delta^{\top}\) ) or less ( \(q\) ) strongly bisinuate medially; adjective in feminine gender.

Diagnosis. Its unique color pattern is similar to that of H. nigra Golovatch, 2017, from Vietnam (Golovatch, 2017), but the two species differ by the thickness of the contrasting paler bands at the lateral and caudal edges of all tergites (ca. 1/3 vs. \(1 / 5 \times\) as high as tergite height), the number of striae at the lateral edge of midbody tergites ( 2 vs. 3 ), the number of ommatidia ( \(10+1(2)\) vs. \(8+1\) ), coupled with two tibial processes (one large process and one small cone vs. two small tibial cones), and the caudal edge of the anal shield (two strongly bisinuate medially vs. slightly emarginate medially).


Figure I. Hyperglomeris bicaudata sp. nov. A-D đ̉ paratype (CUMZ-GLO006), habitus, live coloration E-G \({ }^{\lambda}\) holotype (CUMZ-GLO006) habitus in dorsal, ventral, and lateral views \(\mathbf{A}, \mathbf{C}\) unrolled, sublateral views \(\mathbf{B}, \mathbf{D}\) rolled, sublateral and subdorsal views, respectively. A-D not to scale.

Description. Body length of stretched holotype 13.2 mm , width 8.3 mm . Body length of stretched paratypes \(13.5 \mathrm{~mm}\left(\delta^{\top}\right), 13.5-15.5 \mathrm{~mm}\) ( ( ) , width 9.5 ( \(\mathrm{O}^{\top}\) ), \(8.5-\) 9.5 mm ( \(q\) ).

Coloration of live animals (Fig. 1A-D): body blackish, with contrasting pale yellow to orange yellow, rather broad bands at the lateral and caudal edges of all tergites, ca. \(1 / 3 \times\) as high as each tergite height, including collum, thoracic and anal shields.


Figure 2. Hyperglomeris bicaudata sp. nov. A, C, E, F \(\oslash\) holotype (CUMZ-GLO006) B \(q\) paratype (CUMZ-GLO006) D \({ }^{\text {® }}\) paratype (CUMZ-GLO007) A, B anal shield edge in venter view, male and female, respectively \(\mathbf{C}\) leg 17, anterior view \(\mathbf{D}\) leg 18, anterior view \(\mathbf{E}, \mathbf{F}\) telopod, posterior and anterior views, respectively \(\mathbf{G}\) tip of syncoxital lobes (not to scale). Scale bars: \(1 \mathrm{~mm}(\mathbf{A}-\mathbf{F})\). Abbreviations: cx coxa, cxl coxal lobe, fe femur, \(\mathbf{f p}\) femoral process, \(\mathbf{p f}\) prefemur, \(\mathbf{p f c}\) prefemoral cone of telopod, sh syncoxital horn of telopod, sl syncoxital lobe of telopod, sn syncoxite notch, sx syncoxite, ta tarsus, tc tibial cone, ti tibia, tp tibial process.

Head and antennae black, only labrum and Tömösváry's organ yellowish. Venter and legs dark brown to brown with a pale yellowish claw and the posterior part of each tarsus; coloration in alcohol faded after eight years of preservation (Fig. 1E-G), body pale black to charcoal, with contrasting pale yellow to whitish bands. Head and antennae grey to blackish. Venter and legs pale brown to brownish.

Labrum sparsely setose (Fig. 1F). Gnathochilarium with \(2+2\) palps of subequal length. Ocular fields whitish, \(10+1(2)\) ommatidia, cornea convex, oval in shape, translucent. Antennae with four evident apical cones, segment \(6 \mathrm{ca} .2 .1-2.4 \times\) as long as high. Organ of Tömösváry typical, horseshoe-shaped, oblong-oval, elongate, ca. 1.5\(1.8 \times\) as long as broad (Fig. 1F).

Collum as usual, with two transverse striae (Fig. 1F). Thoracic shield with a small hyposchism field not projecting caudad past tergal margin. Striae 4-6, mostly


Figure 3. Leg 18 A, B Hyperglomeris bicaudata sp. nov., ô paratype (CUMZ-GLO007), left, anterior and posterior views, respectively \(\mathbf{C}, \mathbf{D}\) Hyperglomeris inkhavilayi sp. nov., ô paratype, right, anterior, and posterior views, respectively. Scale bars: 1 mm .
superficial, only lower 3 or 4 lying above schism, one level with schism, remaining 1 or 2 below schism, with 4 and 5 complete, crossing the dorsum (Fig. 1G). Terga 3 and 4 rather broadly rounded laterally (Fig. 1G). Following terga in front of pygidium faintly concave medially at caudal edge and with two striae starting above lateral edge, sometimes first stria fading away towards midway. Caudal edge of anal shield more ( \(\sigma^{\text {, }}\) Figs 1C, E, F, G, 2A) or less ( , Fig. 2B)) strongly bisinuate medially.

Male legs 17 (Fig. 2C) strongly reduced, with a rather high, often irregularly rounded coxal lobe (cxl) and a 4 -segmented telopodite.

Male legs 18 (Figs 2D, 3A, B) simple, rather strongly reduced, without any evident outgrowths; syncoxite membranous, on either side with a simple, small, and narrowly ogival syncoxite notch (sn) and a 4-segmented telopodite.

Telopods (= male legs 19) (Fig. 2E-G) with a very large, broad and roundly subtrapeziform syncoxital lobe (sl) flanked by two short, spiniform, obliquely truncate, setose syncoxital horns (sh), level with syncoxital lobe (Fig. 2F). Telopodite 4-segmented, with a spine apically. Prefemur subellipsoid, with an evident, rather small, distad tapering, tuberculiform, distomesal prefemoral cone (pc) (a reduced trichostele), ca. \(1 / 4-1 / 5 \times\) as long as femur. The latter in caudal view with a prominent, stout, fingershaped, distomesal femoral process ( fp ) devoid of a trichostele, produced apically to ca. 3/4 tibia. Tibia elongate, gently tapering distad and curved apically basad towards process on femur, with an evident, caudad curved, distolateral tibial process (tp) and
a small, short and pointed distomesal tibial cone (tc). Tarsus smallest, subcylindrical, moderately sigmoid, strongly curved, narrowly rounded apically.

Remarks. Unique to this species is that the caudal margin of the anal shield shows two more ( \(\widehat{\text { § }}\), Figs 1C, E, F, G, 2A) or less ( \(\uparrow\), Fig. 2A) pronounced paramedian knobs. That the male is equipped with such modifications is quite usual in various lineages of Glomerida (e.g., Liu and Golovatch 2020), but their presence in the female, albeit not as strongly as in the male, is really striking.

This distinguishing character can be hypothesized as possibly playing an important role in a courtship process or being associated with courtship behavior. Certain male structures dedicated to interactions with females during courtship have often diverged relatively quickly during evolution, causing these features to change into species-specific differences (Eberhard 2004). Noteworthy examples of such characters are antennae, legs and heads in springtails (Collembola: Bourletiellidae) (Kozlowski and Aoxiang 2006) and stridulation organs in giant pill millipedes (Sphaerotheria) (Wesener et al. 2011) that may not be involved directly in sperm transfer but are associated with mating behavior. In order to understand the relationship between these types of traits and their function in the glomerids, it is essential to examine the mating behavior of this species.

\section*{Hyperglomeris inkhavilayi Likhitrakarn, sp. nov.}
https://zoobank.org/60149C6A-59F8-4AD9-82EC-7A8A1CCBD7EA
Figs 3C, D, 4, 5
Material examined. Holotype: LaOs - Khammouane - § (CUMZ-GLO095); Nhommalath District, Ban Phawong, orchard; elev. 190 m a.s.l.; \(17^{\circ} 32^{\prime} 24^{\prime \prime N} \mathrm{~N}\), \(105^{\circ} 25^{\prime} 18^{\prime \prime}\) E; 25 Aug. 2014; R. Srisonchai, C. Sutcharit, K. Inkhavilay leg.; CUMZ; Paratypes: Laos - Khammouane • \(2 \widehat{\sigma}^{\lambda}, 3\) q \(q\); same collection data as holotype; OQ661873, OQ661874.

Name. To honor Dr. Khamla Inkhavilay, the director of the Center of Excellence in Biodiversity at National University of Laos, Vientiane, Laos, who participated in collecting the type series.

Diagnosis. Although its color pattern seems to be similar to that of H. simplex Nguyen, Sierwald \& Marek, 2019 (Nguyen et al. 2019), it differs by the coloration of the collum, thoracic and anal shields (mostly pale yellowish to brownish vs. dark brown to blackish), leg-pair 18 (with a simple, subtriangular syncoxital notch (sn) vs. an evident pronounced syncoxital tubercles), coupled with a longer prefemoral trichostele (pt) (more than \(2 / 3\) vs. \(1 / 2\) of femur).

Description. Body length of stretched holotype 10.7 mm , width 6.9 mm . Body length of stretched paratypes \(9.3 \mathrm{~mm}\left(\delta^{\top}\right), 13.5-15.5 \mathrm{~mm}(\) Q \()\), width 5.6 ( \(\left.{ }^{\top}\right), 5.2-\) 8.5 mm ( \(q\) ) .

Coloration in alcohol faded after eight years of preservation (Fig. 4), body mostly yellowish, with contrasting black paramedian spots flanking the midline. Mid-dorsal spots on each of tergites 3-11 usually subtriangular (Fig. 4A, D), or parallel-sided


Figure 4. Hyperglomeris inkhavilayi sp. nov., habitus in dorsal, ventral, and lateral views \(\mathbf{A}-\mathbf{C} q\) paratype

(Fig. 4E), with smaller and detached patches at caudal edge of tergite 2 and at anterior edge of tergite 12. Lateral sides of each of tergites 2-11 also with a pair of large, sublateral, yellow to marbled blackish spots beside the triangles, normally not reaching the translucent caudal and lateral edges (Fig. 4A, C-E). Head and collum pale yellowish to dark brownish with darker color laterally. Antennae black to dark brown, only tip of antennae yellowish. Legs and venter pale yellowish to pale brown (Fig. 4B).

Labrum sparsely setose (Fig. 4B). Gnathochilarium with \(2+2\) palps of subequal length. Ocular fields blackish, \(8(7)+1\) ommatidia, cornea very convex, translucent. Antennae with four evident apical cones, segment \(6 \mathrm{ca} .2 .1-2.4 \times\) as long as high


Figure 5. Hyperglomeris inkhavilayi sp. nov. A \({ }^{\lambda}\) paratype (CUMZ-GLO095) B-F \({ }^{\lambda}\) holotype (CUMZP085) A,B \(\operatorname{leg} 17\), anterior views \(\mathbf{C} \operatorname{leg} 18\), anterior view \(\mathbf{D}, \mathbf{E}\) telopod, posterior and anterior views \(\mathbf{F}\) tip of syncoxital lobes (not to scale). Scale bars: \(1 \mathrm{~mm}(\mathbf{A}-\mathbf{E})\). Abbreviations: \(\mathbf{c x}\) coxa, \(\mathbf{c x l}\) coxal lobe, fe femur, \(\mathbf{f p}\) femoral process, \(\mathbf{p f}\) prefemur, \(\mathbf{p f t}\) prefemoral trichostele of telopod, sh syncoxital horn of telopod, \(\mathbf{s l}\) syncoxital lobe of telopod, \(\mathbf{s n}\) syncoxite notch, \(\mathbf{s x}\) syncoxite, ta tarsus, \(\mathbf{t c}\) tibial cone, \(\mathbf{t i}\) tibia, tp tibial process.
(Fig. 4B). Organ of Tömösváry typical, horseshoe-shaped, oblong-oval, elongate, ca. \(1.3-1.5 \times\) as long as broad (Fig. 4B).

Collum as usual, with two transverse striae. Thoracic shield with a small hyposchism field not projecting caudad to nearly reaching the tergal margin. Striae 5-7, mostly superficial, only lower 2 or 3 lying above schism, one level with schism, remaining 3 or 4 below schism, with 5 or 6 complete, crossing the dorsum. Terga 3 and 4 broadly rounded laterally (Fig. 4C, F). Following terga in front of anal shield rather clearly concave medially at caudal edge and with 2-4 striae starting above lateral edge. Male anal shield slightly concave medially at caudal edge (Fig. 4D, E).

Male legs 17 (Fig. 5A, B) strongly reduced, with a rather large, often irregularly rounded coxal lobe (cxl) and a 4 -segmented telopodite.

Male legs 18 (Figs 3C, D, 5C) simple, rather strongly reduced, without any evident outgrowths; syncoxite membranous, on either side with a broad, subtriangular syncoxital notch (sn) and a 4-segmented telopodite.

Telopods (= male legs 19) (Fig. 5D-F) with a large, rather roundly subtrapeziform syncoxital lobe (sl) with a small notch medially (Fig. 5E), flanked by two very long, spiniform, obliquely truncate, setose syncoxital horns (sh), two \(\times\) as high as syncoxital lobe (Fig. 5D, E). Telopodite 4 -segmented, with a spine apically. Prefemur subquadrangular, with a long, rather stout, distad tapering, slightly curved prefemoral trichostele (pt) carrying a short seta, ca. \(2 / 3 \times\) as long as femur. The latter on caudal face with a prominent, stout, finger-shaped, gently tapering distad, distomesal femoral process (fp) devoid of a trichostele, produced apically to ca. 3/4 tibia. Tibia elongate, gently tapering distad and curved apically basad towards process on femur, with an evident, caudad curved, caudal tibial process (tp). Tarsus smallest, subcylindrical, moderately sigmoid, strongly curved, narrowly rounded apically.

Remarks. Unfortunately, the claw on the male legs 18 tarsus could not be observed, being broken off in both available male specimens, of which only one leg is available for examining the entire tarsus. Consequently, additional specimens are required to determine tarsal claws.

Key to the known species of Hyperglomeris based on adults, modified after Golovatch (2017)

1 Body completely unpigmented, pallid................................H. depigmentata
- Body at least partly pigmented (Figs 1, 4)................................................... 2

2 Dorsum entirely blackish (except for narrow pale lateral and caudal margins of tergites) (Fig. 1) ...................................................................................... 3
- Dorsum with evident pale markings (Fig. 4) ................................................ 4

3 Caudal margins \(1 / 3\) as high as tergite height; caudal edge of anal shield evidently bisinuate medially (Figs 1C, F, 2A, B). 10+1(2) ommatidia. Tibia of telopod with a large process and a small tibial cone (tc) (Fig. 2E)
.H. bicaudata sp. nov.
- Caudal margins \(1 / 5\) as high as tergite height. Caudal edge of anal shield slightly emarginate medially. 8+1 ommatidia. Tibia of telopod with two small tibial cones.
4 Anal shield entirely, tergum 2 partly or entirely unpigmented; tergum 2 without fine striae, but with a conspicuous sulcus anterodorsad of schism
H. conspicua
- Both anal shield and tergum 2 at least partly with dark pigment................. 5

5 Dorsum without a dark axial line, background coloration either black or redyellow
- Dorsum with a contrasting and brighter axial line against a darker side background (Fig. 4) .7
6 Telopod syncoxital lobe trapeziform and truncate, its ventral margin straight.
H. dirupta
- Telopod syncoxital lobe semi-circular, its ventral margin rounded.

7 Prefemoral cone very short, less than \(1 / 5\) femur. Syncoxital horns (sh) as long as syncoxital lobe (sl)
H. maxima
- Prefemoral trichostele (pt) longer than \(1 / 2\) femur. Syncoxital horns (sh) higher than syncoxital lobe (sl). 8

8 Collum, thoracic and anal shields mostly dark brown to blackish. Male legpair 18 with an evidently pronounced tubercles on syncoxite. Prefemoral trichostele (pt) of telopod longer than \(1 / 2\) femur. \(\qquad\) H. simplex - Collum, thoracic and anal shields mostly pale yellowish to brownish (Fig. 4). Leg-pair 18 with a simple, subtriangular syncoxital notch (sn) (Figs 3C, D, 5C). Prefemoral trichostele (pt) of telopod longer than \(2 / 3\) femur (Fig. 5E) ....
\(\qquad\) H. inkhavilayi sp. nov.

\section*{Phylogenetic analysis}

The COI alignment (Table 1) was 660 bp in length and contained 23 individuals, including 21 taxa from the Glomeridae as ingroup and two taxa from the Zephroniidae as outgroup. All ten pill millipede species from seven genera of the family Glomeridae in this study were retrieved as monophyletic with strong support values (Fig. 7) (77\(100 \%\) bp for ML and \(0.96-1 \mathrm{bpp}\) for BI). However, most relationships among these species were still unresolved ( \(<80 \%\) bootstrap values and \(<0.95\) bpp for BI).

The COI tree demonstrated that the genus Hyperglomeris is at least paraphyletic, because of the inclusion of Peplomeris magna in the same clade with H. bicaudata sp. nov., Hyperglomeris sp. 1, and H. inkhavilayi sp. nov., although with moderate nodal support; and the exclusion of \(H\). simplex, which was placed distantly at the basal position to all Glomeridae. Each of the three Hyperglomeris species in this study (H. bicaudata sp. nov., H. inkhavilayi sp. nov., and H. simplex) was retrieved as a distinct clade/species with significant support (99-100\% for ML; and 0.99 bpp for BI, except 0.58 in \(H\). simplex).

The interspecific divergence based on COI uncorrected p-distance among the glomerid species in this study ranged from 8.81 to \(16.45 \%\), with an average of \(13.07 \%\) (Table 2), and among Hyperglomeris species ranged from 8.81 to \(12.48 \%\), with an average of \(11.23 \%\). This analysis also demonstrated that the intraspecific divergence for H. bicaudata sp. nov. was \(5.30 \%\) and for \(H\). inkhavilayi sp. nov. was \(0.45 \%\).

\section*{Discussion and conclusion}

This study has revealed two new species of Hyperglomeris, a genus new to the fauna of Laos. In addition, we have also refined the scope of the genus and the species distributions. These new records have increased the number of species of the order Glomerida in Laos from four (all in Hyleoglomeris) to a total of six. At present, the genus Hyperglomeris comprises nine species, mostly recorded from Vietnam (seven species), now also from Laos (two species) (Fig. 6). All Hyperglomeris species appear to be highly localized and

Table 2. Matrix of the average uncorrected p-distance (\%) based on \(660-\mathrm{bp}\) COI barcoding region between Hyperglomeris species and some related glomerid and sphaerotheriid taxa. Interspecific divergence is below diagonal and intraspecific divergence is in bold.
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline Taxa & 1. & 2. & 3. & 4. & 5. & 6. & 7. & 8. & 9. & 10. & 11. & 12. \\
\hline 1. Hyperglomeris bicaudata sp. nov. & \[
\begin{gathered}
5.30 \pm \\
0.85
\end{gathered}
\] & & & & & & & & & & & \\
\hline 2. Hyperglomeris inkhavilayi sp. nov. & \[
\begin{gathered}
10.76 \pm \\
1.14
\end{gathered}
\] & \[
\begin{gathered}
0.45 \pm \\
0.27
\end{gathered}
\] & & & & & & & & & & \\
\hline 3. Hyperglomeris sp. 1 & \[
\begin{gathered}
11.93 \pm \\
1.21
\end{gathered}
\] & \[
\begin{gathered}
8.81 \pm \\
1.10
\end{gathered}
\] & n/a & & & & & & & & & \\
\hline 4. Hyperglomeris simplex & \[
\begin{gathered}
12.48 \pm \\
1.15
\end{gathered}
\] & \[
\begin{gathered}
11.40 \pm \\
1.20
\end{gathered}
\] & \[
\begin{gathered}
11.96 \pm \\
1.24
\end{gathered}
\] & \[
\begin{gathered}
4.86 \pm \\
0.84
\end{gathered}
\] & & & & & & & & \\
\hline 5. Peplomeris magna & \[
\begin{gathered}
10.87 \pm \\
1.16
\end{gathered}
\] & \[
\begin{gathered}
10.17 \pm \\
1.16
\end{gathered}
\] & \[
\begin{gathered}
10.01 \pm \\
4.21
\end{gathered}
\] & \[
\begin{gathered}
12.47 \pm \\
1.25
\end{gathered}
\] & \[
\begin{gathered}
0.47 \pm \\
0.28
\end{gathered}
\] & & & & & & & \\
\hline 6. Hyleoglomeris lobus & \[
\begin{gathered}
12.89 \pm \\
1.22
\end{gathered}
\] & \[
\begin{gathered}
11.65 \pm \\
1.21
\end{gathered}
\] & \[
\begin{gathered}
13.85 \pm \\
1.31
\end{gathered}
\] & \[
\begin{gathered}
11.98 \pm \\
1.17
\end{gathered}
\] & \[
\begin{gathered}
12.73 \pm \\
1.27
\end{gathered}
\] & \[
\begin{gathered}
3.24 \pm \\
0.55
\end{gathered}
\] & & & & & & \\
\hline 7. Tonkinomeris napoensis & \[
\begin{gathered}
12.76 \pm \\
1.17
\end{gathered}
\] & \[
\begin{gathered}
12.00 \pm \\
1.22
\end{gathered}
\] & \[
\begin{gathered}
14.25 \pm \\
1.36
\end{gathered}
\] & \[
\begin{gathered}
13.43 \pm \\
1.24
\end{gathered}
\] & \[
\begin{gathered}
12.91 \pm \\
1.30
\end{gathered}
\] & \[
\begin{gathered}
13.58 \pm \\
1.27
\end{gathered}
\] & \[
\begin{gathered}
2.16 \pm \\
0.57
\end{gathered}
\] & & & & & \\
\hline 8. Rhopalomeris sauda & \[
\begin{gathered}
13.84 \pm \\
1.22
\end{gathered}
\] & \[
\begin{gathered}
11.99 \pm \\
1.15
\end{gathered}
\] & \[
\begin{gathered}
13.53 \pm \\
1.23
\end{gathered}
\] & \[
\begin{gathered}
12.15 \pm \\
1.11
\end{gathered}
\] & \[
\begin{gathered}
12.52 \pm \\
1.15
\end{gathered}
\] & \[
\begin{gathered}
12.25 \pm \\
1.12
\end{gathered}
\] & \[
\begin{gathered}
13.16 \pm \\
1.17
\end{gathered}
\] & \[
\begin{gathered}
6.84 \pm \\
0.69
\end{gathered}
\] & & & & \\
\hline 9. Trachysphaera costata & \[
\begin{gathered}
14.46 \pm \\
1.45
\end{gathered}
\] & \[
\begin{gathered}
13.35 \pm \\
1.41
\end{gathered}
\] & \[
\begin{gathered}
14.46 \pm \\
1.51
\end{gathered}
\] & \[
\begin{gathered}
13.44 \pm \\
1.36
\end{gathered}
\] & \[
\begin{gathered}
13.86 \pm \\
1.45
\end{gathered}
\] & \[
\begin{gathered}
13.10 \pm \\
1.35
\end{gathered}
\] & \[
\begin{gathered}
14.29 \pm \\
1.45
\end{gathered}
\] & \[
14.16 \pm
\] & n/a & & & \\
\hline 10. Glomeris marginata & \[
\begin{gathered}
16.45 \pm \\
1.40
\end{gathered}
\] & \[
\begin{gathered}
14.17 \pm \\
1.42
\end{gathered}
\] & \[
\begin{gathered}
16.28 \pm \\
1.48
\end{gathered}
\] & \[
\begin{gathered}
15.50 \pm \\
1.41
\end{gathered}
\] & \[
\begin{gathered}
14.84 \pm \\
1.40
\end{gathered}
\] & \[
\begin{gathered}
15.22 \pm \\
1.40
\end{gathered}
\] & \[
\begin{gathered}
14.23 \pm \\
1.33
\end{gathered}
\] & \[
\begin{gathered}
15.06 \pm \\
1.31
\end{gathered}
\] & \[
\begin{gathered}
12.93 \pm \\
1.41
\end{gathered}
\] & \[
\begin{gathered}
3.04 \pm \\
0.69
\end{gathered}
\] & & \\
\hline 11. Sphaerobelum truncatum & \[
\begin{gathered}
28.79 \pm \\
1.68
\end{gathered}
\] & \[
\begin{gathered}
28.64 \pm \\
1.72
\end{gathered}
\] & \[
\begin{gathered}
29.50 \pm \\
1.82
\end{gathered}
\] & \[
\begin{gathered}
28.43 \pm \\
1.70
\end{gathered}
\] & \[
\begin{gathered}
28.36 \pm \\
1.72
\end{gathered}
\] & \[
\begin{gathered}
29.02 \pm \\
1.70
\end{gathered}
\] & \[
\begin{gathered}
28.96 \pm \\
1.73
\end{gathered}
\] & \[
\begin{gathered}
28.64 \pm \\
1.65
\end{gathered}
\] & \[
\begin{gathered}
28.57 \pm \\
1.87
\end{gathered}
\] & \[
\begin{gathered}
29.80 \pm \\
1.72
\end{gathered}
\] & n/a & \\
\hline 12. Zephronia laotica & \[
\begin{gathered}
30.42 \pm \\
1.77 \\
\hline
\end{gathered}
\] & \[
\begin{gathered}
28.91 \pm \\
1.74 \\
\hline
\end{gathered}
\] & \[
\begin{aligned}
& 28.75 \\
& \pm 1.79 \\
& \hline
\end{aligned}
\] & \[
\begin{gathered}
28.54 \pm \\
1.77 \\
\hline
\end{gathered}
\] & \[
\begin{gathered}
29.63 \pm \\
1.77 \\
\hline
\end{gathered}
\] & \[
\begin{gathered}
30.58 \pm \\
1.74
\end{gathered}
\] & \[
\begin{gathered}
31.91 \pm \\
1.76 \\
\hline
\end{gathered}
\] & \[
\begin{gathered}
29.55 \pm \\
1.72 \\
\hline
\end{gathered}
\] & \[
\begin{gathered}
29.13 \pm \\
1.85 \\
\hline
\end{gathered}
\] & \[
\begin{gathered}
31.35 \pm \\
1.76 \\
\hline
\end{gathered}
\] & \[
\begin{gathered}
22.39 \pm \\
1.65 \\
\hline
\end{gathered}
\] & n/a \\
\hline
\end{tabular}
endemic, with H. depigmentata probably a troglobiont, found exclusively in Hang Doi Cave. At two of these localities (Mount Mẫu Sơn and Vạn Mai), a coexistence of two species has been documented (Fig. 6). In addition to reporting these two new species, this study presents the southernmost record of Hyperglomeris in southern Laos. The distribution patterns (Fig. 6) clearly indicate that further new species of the genus can be expected from Laos, southern China and northern and/or eastern Thailand in the future.

The interspecific COI uncorrected p-distances among Hyperglomeris species in this study ( \(8.81-16.45 \%\) ) are comparable to those of European Glomeris species (11.517.1\%; Wesener 2015b) and among the Vietnamese glomeridan genera (Peplomeris, Hyperglomeris, Hyleoglomeris, and Tonkinomeris) (13-15.8\%; Nguyen et al. 2021). The smallest difference was retrieved between \(H\). inkhavilayi sp. nov. and Hyperglomeris sp. 1 ( \(8.81 \%)\), and the distance between these two species' localities is around 180 kilometers. Thus, it is reasonable to believe that they are highly close congeners. Likewise, this result is consistent with the re-calculated interspecific distances between European Glomeris species, which ranged from \(6.7 \%\) to \(15.9 \%\), and where the least distance (6.7-9.0\%) was between G. primordialis and G. klugii (Wesener and Conrad 2016).

The intraspecific distance within the new species ranged from 0.45 to \(5.3 \%\). This is comparable to the range of the Vietnamese glomerid species, Peplomeris magna ( \(0.2 \%\) ) and Rhopalomeris sauda (7.7\%) (Nguyen et al. 2021). The mean intraspecific distance of \(R\). sauda was rather high ( \(6.84 \%\) ) because of its extensive distribution (Nguyen et al. 2021), and the fact that the analyzed samples were gathered from multiple localities,


Figure 6. Distribution of nine Hyperglomeris species. Crossed square H. lamellosa Silvestri, 1917 and H. dirupta (Silvestri, 1917) Crossed circle H. simplex Nguyen, Sierwald \& Marek, 2019 Open diamond H. nigra Golovatch, 2017 Inverted triangle H. conspicua Golovatch, 1983 and H. maxima Golovatch, 1983 Circle H. depigmentata Golovatch, Geoffroy \& VandenSpiegel, 2013 Triangle H. bicaudata Likhitrakarn, sp. nov. Square H. inkhavilayi Likhitrakarn, sp. nov.
whereas for the other species, a single locale was selected. Similarly, the relatively high value of H. bicaudata sp. nov. (5.3\%) was obtained from samples collected from two sites for analysis; thus, there are greater differences between the two population groups compared to \(H\). inkhavilayi sp. nov., which had a low value \((0.45 \%)\) due to the selection of study specimens from a single population.

The COI tree clustered both new species with Hyperglomeris sp. 1 and Peplomeris magna with a supported clade (Fig. 7). Peplomeris Silvestri, 1917 and Hyperglomeris are closely related genera found in the same country and classified in the same subfamily Haploglomerinae. Morphological characteristics of the genus Peplomeris are extremely similar to those of the genus Hyperglomeris, including the basic structure of posterior


Figure 7. Maximum likelihood tree (ML) of pill millipedes in the family Glomeridae based on 660 bp of COI gene. Clades of new species in this study are highlighted in blue. Numbers on nodes are bootstrap values (bs) from ML analysis and bipartition posterior probability (bpp) from Bayesian inference analysis (BI), and are shown as ML/BI.
telopods with reduced or eliminated prefemoral and femoral trichosteles. However, Hyperglomeris has only four apical cones on their antennae, whereas Peplomeris has numerous apical cones (Wesener 2015a; Nguyen et al 2019). Consequently, it is not surprising that the genetic relationship between the members in these two genera is very close. Despite this, it is premature to make conclusions about the relationships among the two genera based on the results of this study; additional persuasive evidence (i.e., more taxa and genetic markers) is needed to clarify the taxonomic status of both genera.

DNA sequence analysis was previously introduced and has provided a more reliable identification of glomerid species (Nguyen et al. 2019, 2021; Liu and Golovatch 2020). The present paper provides a phylogenetic analysis of ten members of seven genera within the family Glomeridae, including three new sequences from two recently discovered species. Unfortunately, the phylogenetic relationships among these genera is still not supported, which is consistent with studies by Liu and Golovatch (2020) and Nguyen et al. (2021). Hence, data from the COI gene alone are not sufficient to confirm the relationship between genera within this millipede family. We recommend including more genes such as \(16 \mathrm{~S}, 28 \mathrm{~S}\) ribosomal RNA or other advanced molecular techniques (i.e.,
transcriptomic and phylogenomic data) in future studies to clarify phylogenetic relationships (Means et al. 2021; Benavides et al. 2023). Nonetheless, our findings regarding Hyperglomeris demonstrate that the sequencing of the COI gene is still beneficial for species delimitation and facilitates accurate identification among glomerid species.

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\section*{References}

ADB (2000) Environments in transition: Cambodia, Lao PDR, Thailand, Vietnam. Asian Development Bank, Manila, 144 pp .
AVMA (2013) AVMA guidelines for the euthanasia of animals. https://www.avma.org/KB/ Policies/Documents/euthanasia.pdf [Accessed on 3 March 2023]
Benavides LR, Edgecombe GD, Giribet G (2023) Re-evaluating and dating myriapod diversification with phylotranscriptomics under a regime of dense taxon sampling. Molecular Phylogenetics and Evolution 178: e107621. https://doi.org/10.1016/j.ympev.2022.107621
Bogdanowicz SM, Wallner WE, Bell J, Odell TM, Harrison RG (1993) Asian gypsy moths (Lepidoptera: Lymantriidae) in North America: evidence from molecular data. Annals of the Entomological Society of America 86(6): 710-715. https://doi.org/10.1093/aesa/86.6.710
Eberhard WG (2004) Rapid Divergent Evolution of Sexual Morphology: Comparative Tests of Antagonitic Coevolution and Traditional Female Choice. Evolution; International Journal of Organic Evolution 58(9): 1947-1970. https://doi.org/10.1111/j.0014-3820.2004. tb00482.x
Enghoff H, Golovatch SI, Nguyen DA (2004) A review of the millipede fauna of Vientam (Diplopoda). Arthropoda Selecta 13(1-2): 29-43.
Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294-299.
Golovatch SI (1983a) On several new Glomeridae (Diplopoda) from Indochina. Annales His-torico-Naturales Musei Nationalis Hungarici 75: 107-116.
Golovatch SI (1983b) Millipedes (Diplopoda) of the fauna of Vietnam. In: Medvedev LN (Ed.) Fauna and animal ecology of Vietnam. Nauka, Moscow 1983: 1-207. [In Russian]
Golovatch SI (2017) Another two new species of the millipede family Glomeridae from Vietnam, with a new record of Rhopalomeris variegata Golovatch et Semenyuk, 2016
from southern Vietnam (Diplopoda: Glomerida). Russian Entomological Journal 26(2): 195-202. https://doi.org/10.15298/rusentj.26.2.10
Golovatch SI, Geoffroy JJ, Mauriès JP (2006) Review of the millipede genus Hyleoglomeris Verhoeff, 1910 (Diplopoda, Glomerida, Glomeridae), with descriptions of new species from caves in Southeast Asia. Zoosystema 28(4): 887-915.
Golovatch SI, Geoffroy JJ, VandenSpiegel D (2013) On several new species of the millipede family Glomeridae from Vietnam (Diplopoda: Glomerida). Arthropoda Selecta 22(3): 201-206. https://doi.org/10.15298/arthsel.22.3.02
Kozlowski MW, Aoxiang S (2006) Ritual behaviors associated with spermatophore transfer in Deuterosminthurus bicinctus (Collembola: Bourletiellidae). Journal of Ethology 24(2): 103-109. https://doi.org/10.1007/s10164-005-0162-6
Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33(7): 1870-1874. https://doi.org/10.1093/molbev/msw054
Kuroda M, Eguchi K, Oguri E, Nguyen AD (2022a) Two new cave Hyleoglomeris species (Glomerida, Glomeridae) from northern Vietnam. ZooKeys 1108: 161-174. https://doi. org/10.3897/zookeys. 1108.85423
Kuroda M, Susukida M, Sakamoto K, Tsukamoto S, Nguyen AD, Oguri E, Eguchi K (2022b) A new species of the genus Hyleoglomeris Verhoeff 1910 from Central Japan (Diplopoda: Glomerida: Glomeridae). Acta Arachnologica 71(2): 115-124. https://doi.org/10.2476/asjaa.71.115
Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2016) PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34(3): 772-773. https://doi. org \(/ 10.1093 / \mathrm{molbev} / \mathrm{msw} 260\)
Likhitrakarn N, Golovatch SI, Panha S (2014) A checklist of the millipedes (Diplopoda) of Laos. Zootaxa 3754(4): 473-482. https://doi.org/10.11646/zootaxa.3754.4.8
Liu W, Golovatch S (2020) The first representatives of the millipede family Glomeridellidae (Diplopoda, Glomerida) recorded from China and Indochina. ZooKeys 954: 1-15. https://doi.org/10.3897/zookeys.954.54694
Means JC, Hennen DA, Tanabe T, Marek PE (2021) Phylogenetic systematics of the millipede family Xystodesmidae. Insect Systematics and Diversity 5(2): 1. [1-26] https://doi. org/10.1093/isd/ixab003
Miller M, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, 8 pp. https://doi.org/10.1109/ GCE. 2010.5676129
Mock A, Tajovský K, Žurovcová M, Jarošová A, Kocourek P, Gruber J, Angyal D, Spelda J (2016) Hungarosoma bokori Verhoeff, 1928 (Diplopoda: Chordeumatida): new insights into its taxonomy, systematics, molecular genetics, biogeography and ecology. Zootaxa 4178(2): 234-256. https://doi.org/10.11646/zootaxa.4178.2.4
Nguyen AD, Sierwald P, Marek PE (2019) The pill millipedes of Vietnam: a key to genera and descriptions of five new species (Diplopoda: Glomerida: Glomeridae). The Raffles Bulletin of Zoology 67: 260-297. https://doi.org/10.26107/RBZ-2019-0020

Nguyen AD, Nguyen SG, Eguchi K (2021) A new Rhopalomeris species (Diplopoda: Glomerida: Glomeridae), and notes on the phylogenetic relationships between glomeridans in Vietnam. Zootaxa 4927(2): 257-264. https://doi.org/10.11646/zootaxa.4927.2.5
Reip HS, Wesener T (2018) Intraspecific variation and phylogeography of the millipede model organism, the Black Pill Millipede Glomeris marginata (Villers, 1789) (Diplopoda, Glomerida, Glomeridae). In: Stoev P, Edgecombe GD (Eds) Proceedings of the \(17^{\text {th }}\) International Congress of Myriapodology, Krabi, Thailand. ZooKeys 741: 93-131. https://doi. org/10.3897/zookeys.741.21917
Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Systematic Biology 61(3): 539-542. https://doi.org/10.1093/sysbio/sys029
Schulmeister S, Wheeler WC, Carpenter JM (2002) Simultaneous analysis of the basal lineages of Hymenoptera (Insecta) using sensitivity analysis. Cladistics 18(5): 455-484. https://doi. org/10.1111/j.1096-0031.2002.tb00287.x
Silvestri F (1917) Contributions to a knowledge of the oriental Diplopoda Oniscomorpha. I. The family Glomeridae. Records of the Indian Museum 13(3.9): 103-151. https://doi. org/10.26515/rzsi/v13/i3/1917/163604
Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312-1313. https://doi.org/10.1093/bioinformatics/btu033
Wesener T (2015a) Pentazonia. In: Minelli A (Ed.) The Myriapoda (Vol. 2). Treatise on Zoology. Brill, 370-381.
Wesener T (2015b) No millipede endemics north of the Alps? DNA-Barcoding reveals Glomeris malmivaga Verhoeff, 1912 as a synonym of G. ornata Koch, 1847 (Diplopoda, Glomerida, Glomeridae). Zootaxa 3999(4): 571-580. https://doi.org/10.11646/zootaxa.3999.4.7
Wesener T (2019) First records of giant pill-millipedes from Laos (Diplopoda, Sphaerotheriida, Zephroniidae). Zootaxa 4563(2): 201-248. https://doi.org/10.11646/zootaxa.4563.2.1
Wesener T, Conrad C (2016) Local Hotspots of Endemism or Artifacts of Incorrect Taxonomy? The Status of Microendemic Pill Millipede Species of the Genus Glomeris in Northern Italy (Diplopoda, Glomerida). PLoS ONE 11(9): e0162284. https://doi.org/10.1371/journal. pone. 0162284
Wesener T, Köhler J, Fuchs S, VandenSpiegel D (2011) How to uncoil your partner-"mating songs" in giant pill-millipedes (Diplopoda: Sphaerotheriida). Naturwissenschaften 98(11): 967-975. https://doi.org/10.1007/s00114-011-0850-8
Wongthamwanich N, Panha S, Sierwald P, Wesener T, Thirakhupt K (2012) A new species of the giant pill-millipede genus Sphaerobelum Verhoeff, 1924 from northern Thailand, with an extensive description and molecular characters (Diplopoda: Sphaerotheriida: Zephroniidae). Zootaxa 3220(1): 29-43. https://doi.org/10.11646/zootaxa.3220.1.2```


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