

# *Polyura inopinatus* Röber, 1940; a remarkable butterfly mystery resolved

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## Abstract

The most distinctive species of *Polyura*, *P. inopinatus*, described from a single specimen said to be from North Sulawesi, Indonesia, has been a great mystery since it was first described by Röber, in 1940. The holotype, originally illustrated in monochrome in the journal *Deutsche Entomologische Zeitschrift, Iris*, was lost very soon after it was described, almost certainly destroyed during allied bombing of Dresden in the 1940s. No other specimen was known for almost eight decades. We suggest that the type locality (Sulawesi) is incorrect and that the holotype was more likely to have been collected in the Baining Mountains, East New Britain Province, Papua New Guinea. We report the recent discovery of several male *P. inopinatus* from West New Britain Province, and describe and illustrate specimens. A neotype is designated.

## Keywords

Taxonomy, Lepidoptera, Nymphalidae, Charaxinae, *Charaxes*, *Polyura*, neotype, Bismarck Archipelago, Sulawesi

## Introduction

### Background

Johannes Karl Max Röber (known as Julius) (1861–1942) was a Dresden entomologist. He became a prolific author from the age of 25, describing many new butterfly taxa between 1886 and 1940. His final paper concerning the Lepidoptera was a detailed (annotated) checklist of the 406 butterfly species known to occur on Celebes (now Sulawesi), Indonesia, which included description of three new taxa: *Appias melania kalisi* (Pieridae) (Röber, 1940: 93); *Charaxes inopinatus* (Nymphalidae) (Röber, 1940: 102) and *Celaenorrhinus chamunda subconcolor* (Hesperiidae) (Röber, 1940: 113).

There is some divergence of opinion regarding the publication date of Röber's paper. Authors of the Sulawesi checklist (Vane-Wright and de Jong 2003) and the most recent treatment of *Polyura* (Turlin 2017 a, b, c), used the year 1939 for the name *inopinatus*, but we note that Vane-Wright and Ackery (2003: 27, 187) used 1940 for *subconcolor* (Vane-Wright and de Jong 2003: 61). Yata et al. (2010: 796, supporting information, 13) subsequently used the year 1940 for the name *kalisi*. The journal itself is clearly dated 1939, and was the final issue of that year. However, it was actually published on the 10<sup>th</sup> of April 1940 (Matthias Nuss, in litt., 2018).

Of the three new taxa described in his paper, *inopinatus* (*inopinatus* means 'unexpected' in Latin) was clearly – and rightly – regarded by Röber as the most important / impressive, since it was the only butterfly to be illustrated in his paper (Fig. 1). Röber's checklist was primarily based on the previous work of Ludwig Martin (1858–1929) (see references), who lived on Sulawesi between 1914 and 1929, and collected butterflies there for some of this period. However, the solitary specimen of *inopinatus* was not from Martin, but was said to have been “captured in North Celebes (Tondano) and comes from the stores of Dr. Staudinger and A. Bang-Haas in Dresden-Blasewitz. It is probable that this new species has its home in the mountains, and in the lowlands only specimens blown by the wind are encountered. As yet no mountain of the island of Celebes is sufficiently explored, many surprises are to be expected in the future” (Röber 1940: 104 [translation]).

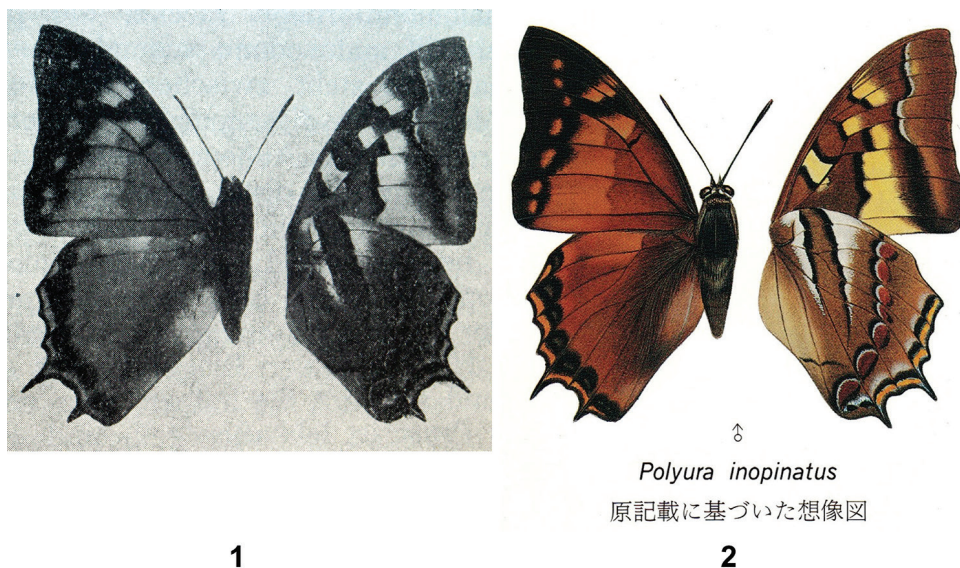
An English language translation of relevant sections from Röber's rather long original German language description, is provided here:

“*inopinatus* sp. n. is the latest and most remarkable discovery of the island of Celebes. This new species has no nearer kinship in the whole genus, and only a few resemblances on the underside of the wings with *cognatus*, also from Sulawesi, which is also without a close relative. The upper side is light reddish-brown with black markings...”

At the middle cell is a large black spot, which connects with the black margin; The anterior is approximately 1mm wide, black and the discocellulars are narrowly black sculpted, the remaining ribs are narrowly black.

Posterior wing parts are wider and diffuse yellowish.

The outside edge is monochrome brownish; the proximal wing part is brown; in the centre of the cell there is a large yellowish-yellow spot, on both sides a broad black spot, the cell is closed by a black stripe. The hind wings are grey-brown, and in the



**Figures 1, 2.** *Polyura inopinatus* holotype: **1** as illustrated by Röber (1940) **2** as prepared by Toshitsugu Endo and reproduced in colour in Tsukada (1991).

centre there is a darker band, about 3 mm wide, bounded by silvery white; This white band widens distally to an oblong triangular patch which runs into the anterior margin; distally 7 reddish-brown spots, partly semicircular, which are proximally bluish-silver-white, and then narrowly black.

The antennae are black, the palps are black, the abdomen is yellow, the thorax is brighter and the legs are black, yellowish below. Wing span 50 mm.”

### Where is the *inopinatus* holotype?

The unique specimen from which Röber described *inopinatus* is lost. Turlin’s rather subjective critical assessment (Turlin 2017b: 15) that the specimen was lost as a result of “the terrible bombings of 13/14 February 1945”, whilst fundamentally accurate, is ultimately unhelpful. The true story is more complex. Bombing of Dresden in February 1945 has been offered as the explanation for the wholesale destruction of materials including natural history collections at what is now the Senckenberg Museum für Tierkunde in Dresden. This has provided anecdotes – for example, the only specimens to escape the bombing were those out on loan at the time – that are clearly nonsense.

An objective, interesting and factual account of the history of the collections before and during the war was presented by Robert Reichert (1954, 1956), a member of the museum staff who felt moved to make an historical record covering major changes from the period 1937 until almost a decade after the end of the war, when his papers were published.

The Nationalsozialistische Deutsche Arbeiterpartei (NSDAP – usually referred to in English as the Nazi Party) was formed in 1920, and rose to prominence in the following decades, before being declared illegal in 1945, immediately following the end of the Second World War. This was a dark period in European history, but prevalent attitudes of those times may also be relevant to our story of what may have happened to the type (holotype) of *Polyura inopinatus* described by Röber in 1940 at the beginning of the war.

Dresden was the most important cultural centre in Saxony, with the world famous Semper Oper House and a number of other important and magnificent buildings which included the Zwinger building – part of the city of Dresden when it was constructed in the early 18<sup>th</sup> century – which now houses the Old Masters Picture Gallery, the Dresden (Meissen) Porcelain Collection, and a museum of Mathematical and Physical instruments.

Until the 1930s, the perimeter buildings (the Zwinger is in effect a series of buildings linked by high walls, enclosing a large open area) housed ‘museums’ (collections) including ethnology, zoology and mineralogy. Under the influence of the Nazi regime, the museums of ethnology and zoology were re-organised to the “Staatliches Museum Tierkunde, Völkerkunde und Rassenkunde” (State Museum of Zoology, Ethnology and [Race Theory]) and the Nazis ordered the movement of the contents of this Museum from the Zwinger to another building in the Ostra-Alle 15, which was completed by the year 1937. In 1939, some valuable books and type specimens of birds were secured there.

With a likelihood of allied bombing as early as 1940, movement of museum objects for safe keeping was ordered on 15<sup>th</sup> February 1940 to 16 different ‘safe’ localities in castles and other major buildings throughout the State of Saxony – although some of these buildings were also severely damaged during the war, with resultant loss of museum material. The 7<sup>th</sup> of October 1944 was a bleak day for the Dresden Museum, where much of the entomology collections remained. The building suffered a direct hit around mid-day, causing a fire in the museum that burned for 12 hours. The bulk of the Entomology Department was completely destroyed, including the larger part of the butterflies, the moths, all Hymenoptera, one cabinet of Coleoptera and the important collection of Heinrich Wilhelm Calberla (1839–1916).

Most of what remained of the zoology collections in Dresden was destroyed by intensive bombing on 13<sup>th</sup>-15<sup>th</sup> February 1945, which caused massive destruction of the City Centre. Most of the Museum documentary records were also destroyed as a result of the October 1944 and February 1945 bombings.

Immediately following the war, problems continued to beset the surviving residue of the entomology collections. Staff members visited all the ‘safe’ localities in the summer of 1945, to find extensive local damage. At one locality 17 insect cabinets were found to have been destroyed, at another locality fire had destroyed a large proportion of the library. Return of surviving collections to Dresden following the war was, predictably, problematic since most of the buildings suitable to house collections in the centre of Dresden were either destroyed or rendered uninhabitable. Space was at a premium, and much of the returning material remained packed in boxes for many years.



In summary, a significant volume of entomological material, including we presume the unique specimen of *inopinatus* perished as a direct influence of war. Concurrent loss of museum records leaves us with no explanation of what actually happened to the specimen, and the truth cannot now be established. But what can be presumed, with a high degree of certainty, is that any specimen that might be expected to have been in Dresden and which is now neither in the Senckenberg Museum für Tierkunde (Museum of Zoology) in Dresden nor in the Museum für Naturkunde (Museum of Natural History) in Berlin, was destroyed during these raids.

### Subsequent references to *inopinatus*

Subsequent published history, comment and opinion regarding *inopinatus* is sufficiently fascinating to be worth recording in detail. The butterfly was described by Röber in the genus *Charaxes* but note that we continue to use *Polyura* in the sense of Smiles (1982) for the group of Indo-Australian Charaxini with an open hindwing discal cell.

There seems to have been no further mention of *inopinatus* until the spectacularly illustrated work of Tsukada (1991). Smiles (1982) revised *Polyura*, but overlooked *inopinatus*; D'Abrera (1985) – undoubtedly using Smiles as a primary reference – also neglected to acknowledge its existence.

### Tsukada

Tsukada's series of *Butterflies of the South East Asian islands* is a five volume monographic work which was intended to include all the butterflies of the region, although the lycaenids and hesperiids were never published. But *Polyura inopinatus* was presented with a magnificent colour illustration (Tsukada 1991: 236) (Fig. 2) with the legend/text "*Polyura inopinatus*, imaginary picture, drawn based upon its original description [translation]" (but see below). The drawing / painting was made by Toshitsugu Endo (Tsukada 1991: 518) and rather artistically superimposed over the German text from Röber's description.

### Vane-Wright and de Jong

In a comprehensive checklist of Sulawesi Region butterflies, Vane-Wright and de Jong (2003: 27) noted "The endemic species that Smiles unfortunately overlooked, *Polyura inopinatus*, is very distinctive. It is known only from the unique holotype, described from northern Sulawesi in 1939 [sic], and its place within Smiles' scheme has not been determined. *P. inopinatus* was not encountered during Project Wallace" (see below for discussion on 'Project Wallace') and later (Vane-Wright and de Jong 2003: 187) "Described by Röber (1940) from a single male from Tondano, this distinctive taxon was

overlooked by Smiles (1982). Tsukada (1991: 236) reproduces the original colour illustration; so far as we are aware, no further material of this beautiful species has come to light”.

Reference to “the original colour illustration” is erroneous. Röber’s paper was published at the beginning of the Second World War, when it was difficult to obtain issues of German-published journals. The volume in the library of the Natural History Museum in London is a photocopy, and it might reasonably be assumed that the original was actually in colour. However, the second author, during a visit to the Senckenberg Museum für Tierkunde in Dresden in November 2017, examined an original copy of the journal, and found the illustration to be monochrome.

We know now that Tsukada’s colour illustration was prepared by Toshitsugu Endo from a combination of a Japanese translation of Röber’s original German description and the monochrome illustration. It is noted that the size of the specimen – in reality very small indeed in comparison to other Indo-Australian species of *Polyura* or *Charaxes* – was apparently overlooked. Although Röber (1940: 104) did provide a wing-span (“Flügelspannweite 50 mm”), Tsukada’s illustration (Tsukada 1991: 236, between plates 202, 203) depicted the upperside and underside ‘halved’, each half of which was *ca* 30 mm; a space between the two halves gave the impression of a butterfly with a span of approximately 70 mm. Tsukada noted ‘upper side *Charaxes*-like, under side *Polyura*-like, looking like a monster’ (Tsukada 1991: 518 [translation]), which we take to have meant that it seemed aberrant in some way.

### Toussaint, Turlin and a ‘hybrid origin’

More recently, Toussaint et al. (2015) presented a phylogeny of *Polyura* sensu stricto (Indo-Australian region) that included all known taxa with the exception of *inopinatus*, for the obvious reason that no specimen was available to the authors, although Tsukada’s coloured illustration was included (Toussaint et al. 2015: 198), without acknowledging the source. It was accompanied by the comment (Toussaint et al. 2015: 197) “*P. inopinatus* is highlighted in a red rectangle to indicate its likely extinction *in natura*. A drawing of this species is presented since the monotype was destroyed during World War II”. The possibility of a hybrid origin for *inopinatus* was suggested: “... Our dataset includes 205 specimens representing all described species except for the dubious Sulawesi endemic *P. inopinatus* which is known only from the lost holotype and may be a hybrid” (Toussaint et al. 2015: 195).

A modern treatment of *Polyura* was presented by Turlin (2017a, b, c). He said of *inopinatus*: “...the single male specimen, reputed to have come from Tondano, near Manado, was deposited in Dresden Museum collection just before the beginning of the 2<sup>nd</sup> world war. It unfortunately disappeared at that time, presumably destroyed during the terrible bombings of 13 and 14 February 1945, which destroyed most of Dresden. Only the original description, with a black and white photo, remains as proof of its existence ... Tsukada managed to publish a colour painting from the original de-

scription text ...” (Turlin 2017a: 21). As will be seen from our previous section, above, the specimen was almost certainly destroyed during the World War II allied bombing of Dresden, although this was not as straightforward as Turlin suggested. Turlin went on to say (Turlin 2017b: 15) that he considered a Sulawesi origin may be erroneous and that *inopinatus* “... could have come from another island, maybe a very small one [presumably near Sulawesi], which was visited only once and never again since the discovery and still remains unidentified ... the pity is that the only specimen is lost forever. This case will remain a mystery for a long time!”.

Potential for a ‘hybrid origin’, raised by Toussaint et al. (2015: Turlin was a co-author of that paper) and Turlin (2017b: 15), as an explanation for a lack of available specimens, was unconvincing. Such a scenario would require the presence, in the same place, of suitable parents and an expectation that a hybrid offspring would display characters of both. Such potential parents were and are not present on Sulawesi – or anywhere else – and although a hybrid possibility was investigated in some detail by Turlin (2017b: 15), it is not pursued here, in part because there is no evidence whatever for a hybrid origin, but also because of the recent discovery of the species in Papua New Guinea.

## Materials and methods

Adult specimens were photographed using a Nikon D300s Digital SLR Camera with a Nikon AF-S VR Micro-Nikkor 105mm f/2.8G IFED Macro lens and Nikon R1C1 Close-up Kit Flashes Speedlights. Male genitalia were photographed in glycerol using the fore-mentioned camera body adapted to a Meiji Techno EMZ-5TR-P-FOI Trinocular Stereozoom Microscope, with OPTTEK FL95E Fibreoptic Illuminator and twin arm optical fibre, after being extracted following maceration of abdomens in 10% KOH at room temperature for 36 hours. Individual images were taken with the remote acquisition software DIYPhotoBits Camera Control 5.2. Sliced genitalia photographs were stacked and concatenated using the software Helicon Focus 6.0 and edited in Adobe Photoshop CS6. Image plates were designed in Adobe InDesign CS6. Genitalia were stored in small glycerol-filled vials pinned beneath the specimen.

## Results

### The re-discovery of *Polyura inopinatus*

During early September, 2015, whilst carrying out unrelated research into the lycaenid genus *Hypochrysops* on West New Britain, in the Bismarck Archipelago, the first author glimpsed what appeared initially to be a particularly small species of black and orange *Doleschallia* butterfly (Nymphalidae) alighting briefly on an isolated branch some 20 metres above the ground. Some days later, a second butterfly observed in the same

place was examined through binoculars; it appeared to have an under surface similar in appearance to that of a species of *Polyura* and an upper surface that appeared to be largely orange. With some difficulty, over a period of several days, five male specimens were collected.

It was clear from the outset that, despite its presence some 3,000 kilometres to the east of its published type locality on North Sulawesi, this was *Polyura inopinatus*. Specimens from West New Britain are very similar in shape and maculation to the specimen illustrated in Röber (1940) (see figs). The diminutive (for a *Polyura*) wing span (50 mm) given by Röber is also accurate when compared to our specimens. There can be little doubt that the specimens from New Britain are the same as that supposedly from Sulawesi, and that the original claim for a Sulawesi origin is at best highly dubious. The female was not seen; males of *P. inopinatus* were observed to be very high flying, establishing territories in the canopy close to the summit of a tall volcanic hill. The locality was completely destroyed by fire towards the end of the severe drought (El Niño) of July–December 2015.

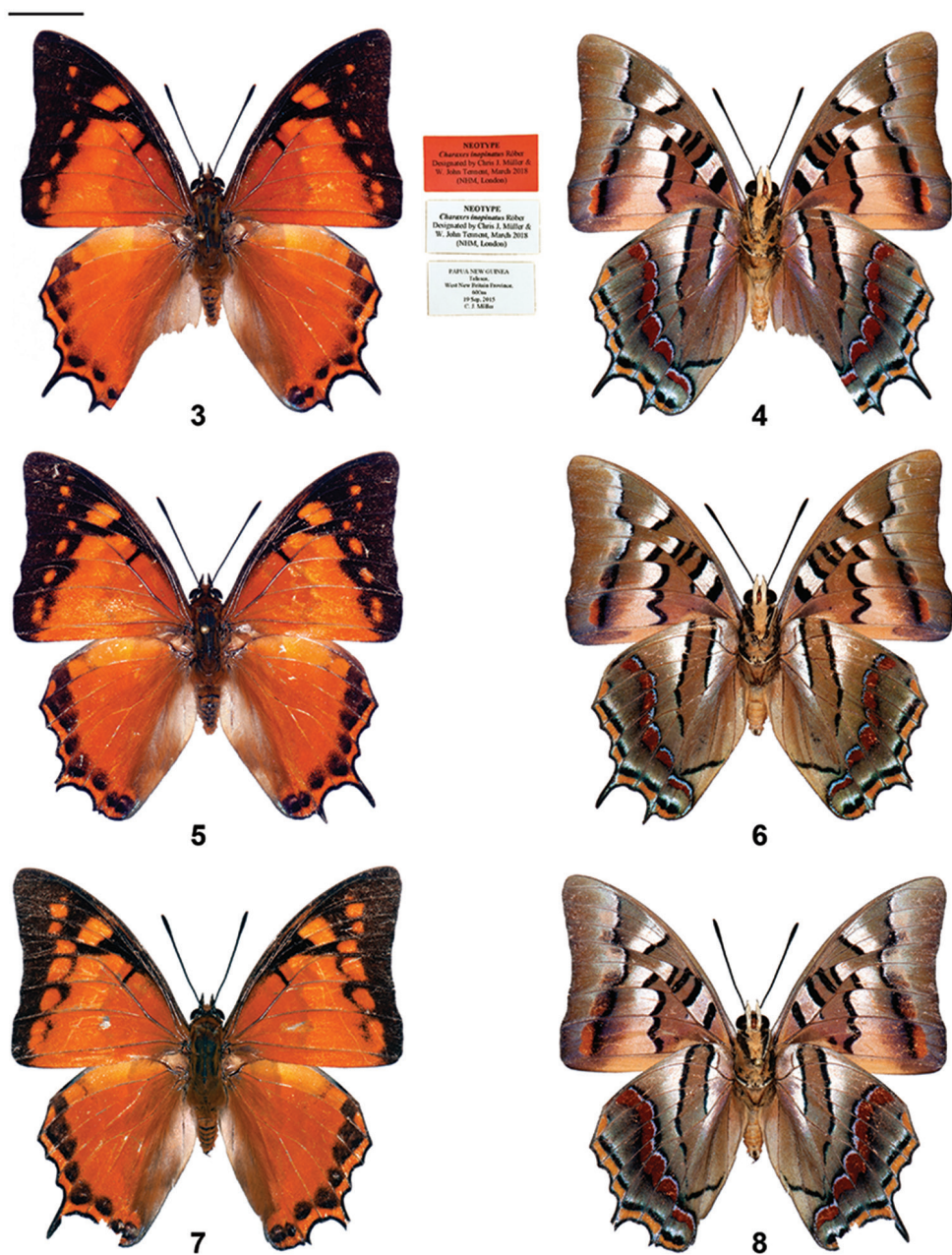
The second author has examined every nymphalid drawer in the Senckenberg Museum für Tierkunde in Dresden and both authors have examined the nymphalid drawers of the Staudinger and general collections in the Museum für Naturkunde, Berlin. Others have also searched for the specimen in previous years. There is no doubt that the holotype of *P. inopinatus* is lost, almost certainly associated with the bombing of Dresden during World War II. As will be clear from our reproduction of Röber's monochrome picture, Tsukada's colour representation, and fresh specimens, there is equally no doubt (cf Figs 3–8) that the butterflies found on West New Britain represent *inopinatus*.

The International Code of Zoological Nomenclature (The Code) requires that a neotype designation should not be made for “curatorial convenience”, but that factors including taxonomic status or the type locality of a nominal taxon should be considered. In addition to fascinating historical aspects of the “re-discovery” of *inopinatus*, we are confident that the unusual and unique circumstances we have outlined clearly warrant designation of a neotype. We also note that such a designation customarily includes a comparison with closely associated (similar) taxa; in this case, as previous authors have also noted, *Polyura inopinatus* has no obvious association with any other *Polyura* species. A Type Locality of West New Britain is established. The neotype has been deposited in the Natural History Museum (NHM), London.

## Taxonomy

### Designation of neotype for *inopinatus* Röber, 1940

A neotype of *Charaxes inopinatus* Röber, 1940, is hereby designated. Male (Figs 3, 4), with the following labels: (1) Papua New Guinea, Talasea, West New Britain Province, 600m, 19 Sep, 2015, Chris J. Müller; printed: NEOTYPE / *Charaxes inopinatus* Röber, 1940 / designated by Chris J. Müller & W. John Tennent, March 2018 (NHM, London).



**Figures 3–8.** *Polyura inopinatus* males, Talasea (PNG) (odd numbers upperside, even numbers underside): **3, 4** Neotype male, with labels **5, 6** additional male **7, 8** additional male. Scale bar = 10 mm.

Here we also redescribe *P. inopinatus*, including the male genitalia, since various important diagnostic features were omitted from Röber's original description. Figs 3–8 depict male specimens of *P. inopinatus*.



Description. ♂ Forewing length 31.5mm (Neotype), Antenna length 14.1mm (Neotype). Head orange-brown; antenna black tipped with orange; thorax and abdomen deep orange-brown on upperside, light orange-brown beneath; legs light-medium brown, with black along the anterior margin of the femur.

Forewing with costa slightly bowed, termen concave and inner margin straight.

Forewing upperside bright orange, a deeper shade in basal area; costa, apex and termen broadly black; discocellulars black, forming a black stripe perpendicular to costa at end of cell; a small postmedian patch of bright orange occupying spaces 5 and 6; a subterminal row of small bright orange spots (of variable shape) in spaces 6–1b, those in the latter space are duplicate; cilia black.

Forewing beneath deep grey-brown with indistinct purple suffusion and chestnut coloured in basal half; three broad (1–2 mm wide) black bars in cell, perpendicular to costa, that in the middle extending into space 2; discocellulars broadly black; the basal and middle cell bars joined with broad diffuse cream borders; a broad cream median area spanning spaces 4 to the inner margin, grading to orange at inner margin, basally thickly (1.5 mm) bordered with black; a small postmedian patch of cream in spaces 5 and 6, also thickly bordered with black basally; an irregular black subterminal band approximately parallel with the termen and widening towards inner margin, bordered narrowly with bluish-white and orange progressively on outer margin at costa and broadly with bright orange at inner margin; cilia brown.

Hindwing with a sharp narrow tail approximately 6mm long at vein 4 and a shorter (2 mm) tail at vein 2; termen scalloped.

Hindwing above bright orange, a slightly deeper shade basally but very pale orange in median area of costa; termen bordered with black (approximately 1mm thick); a row of subterminal black elongated spots, that closest to tornus bifurcated and narrowly dusted basally with purple and white, all subterminal spots basally diffused with black dusting; tornus narrowly blue-white; inner margin broadly orange-brown, white-brown near base; cilia black.

Hindwing beneath with ground colour rich chestnut in basal half, grey-green in distal half; two broad (approximately 1mm wide) black sub-parallel bars in basal and median area, approximately parallel with inner margin and tapering towards tornus, bordered on outer edges with cream (broadest in median area nearest to costa); a postmedian band of crescent-shaped rich red-maroon spots, with purple-white and progressively black basally; a broad (1 mm) black postmedian bar in spaces 1a and 1b, roughly parallel with costa, connecting these spots with inner margin; a row of orange-yellow elongated spots along termen, set in background sky blue terminal area, basally thickly bordered with black and progressively light blue; termen border black (approximately 1mm thick); cilia black.

♂ genitalia (Fig. 9). Tegumen elongate (a), oval-shaped dorsally (b) and ventrally (c); sociuncus hooded posteriorly, with prominent dorsal saddle anteriorly at uncus, which is broad dorsally, with fine setae at apex; gnathos brachia short and sharp, plunging downwards; valva broad laterally (a), with pronounced hook at apex; juxta long and narrow, sclerotised apically (c); aedeagus elongate (d, e), with hooked apical spine (d).



**Figure 9.** *Polyura inopinatus* male genitalia (Talasea, PNG): **a** genitalia lateral view, aedeagus removed **b** genitalia dorsal view, aedeagus removed **c** genitalia ventral view, aedeagus removed **d** aedeagus lateral view **e** aedeagus dorsal view. Scale bar = 1 mm.

### A New Britain connection – and a Sulawesi disconnection?

It seems rather unlikely that such a distinctive species occurs from west of the Moluccas on Sulawesi to the Bismarck Archipelgo, but we believe that Röber's original specimen also came from New Britain, and not from Sulawesi.

Röber (1940: 104) stated that, unlike most of the material he examined for his Sulawesi checklist, which came from Ludwig Martin, his *inopinatus* came from the stores of Staudinger and Bang-Haas; this was a very large and flourishing business started by Otto Staudinger (1830–1900) in the late 1850s. Andreas Bang-Haas (1846–1925) married Staudinger's daughter in 1880 and Staudinger and Bang-Haas ran the business together under their joint names until the former's death in 1900. In 1913 Bang-Haas' son Otto (1882–1948) took over the business and ran it until his own death. The business was dissolved in September 1948. The Staudinger collection now resides in Berlin; the Bang-Haas commercial material and collections are deposited in Dresden.

Otto Bang-Haas described a number of Lepidoptera species, including two butterflies: *Chilasa moernereri mayrhoferi* and *Delias mayrhoferi* in different issues of the *Entomologische Zeitschrift*, Stuttgart, in 1940. We believe it is significant that both these taxa were described from material collected by "A. Mayrhofer" in the Baining Mountains of East New Britain. These taxa were published without illustrations and, like *P. inopinatus*, were overlooked for many years although, unlike *inopinatus*, type specimens remain extant in the Dresden and Berlin collections. *Delias mayrhoferi* appears to have been completely overlooked – and was redescribed as a new species more than half a century later (*Delias shunichii* Morita, 1996), a synonymy which was resolved by Häuser et al. (2009). For the record, butterfly taxa occurring in East New Britain invariably also occur in West New Britain.

Häuser et al. were unable to establish any biographical data concerning "A. Mayrhofer" (Häuser et al. 2009: 122), and Christoph Häuser (pers comm. to both authors,

2018) has confirmed that this remains the case, although the authors believe Mayrhofer's given name may have been 'Alfrons'. The first author carried out research into Mayrhofer and happened across a book by Gail Pool entitled "*Lost Among the Baining: Adventure, Marriage, and Other Fieldwork*" (Pool 2015). In the late sixties, Gail Pool and her husband set off for an adventure in New Guinea. He was a graduate student in anthropology; she an aspiring writer. They met a 'Father' Mayrhofer on New Britain and, although there is no direct evidence that this was the Mayrhofer who collected the *Delias* and *Chilasa* that now bear the Mayrhofer name, this may well have been so. Similarly, we have no knowledge that it was Mayrhofer who collected *P. inopinatus* on New Britain – although the period when *Delias mayrhoferi* and *Chilasa moeneri mayrhoferi* were collected (*ca* 1939) and locality (New Britain), and the fact that all passed through the hands of Bang-Haas, raises the distinct possibility that this might have been the case. All three taxa, now known to be from the Bismarck mountains, may well have come from the same locality and source on New Britain.

So Bang-Haas, who provided the *inopinatus* specimen to Röber, had access to material from the mountains of New Britain in exactly the same period. Why he sent the *inopinatus* specimen to Röber is not known: perhaps it was mis-labelled as being from Sulawesi and he knew Röber was working on a checklist; although if he had realised it was an undescribed taxon, he would presumably have retained it and described it himself. On the other hand, Bang-Haas was fundamentally a dealer in butterflies, and may even have sold individuals or a batch of butterflies to Röber, without intimate knowledge of the contents. There is no direct evidence to support such a conclusion, but we believe it is well within the realms of possibility that the solitary *inopinatus* specimen was actually collected by Mayrhofer in the Baining (or somewhere else) on New Britain, and not on the island of Sulawesi as Röber believed.

The other obvious question now raised is whether there is any direct evidence to suggest that the butterfly was *not* collected on Sulawesi. The short answer is no, but it might be considered relevant that one of the well-known features of butterflies from many families from Sulawesi is a propensity for acutely angled forewings and a distinctly concave forewing outer margin. A good example is that of *Polyura cognatus* Vollenhoven, 1861, which is endemic to Sulawesi and its immediate satellites. *P. inopinatus* does not display this feature. Also, as Vane-Wright and de Jong correctly pointed out (2003: 27), *P. inopinatus* was not encountered during Project Wallace, a year long scientific expedition to the Dumoga Bone National Park on the north-eastern arm of Sulawesi in 1985. The expedition was organised by the Royal Entomological Society and supported by the British Armed Services; various research projects concerning butterflies were undertaken by international scientists. The second author was part of that expedition, spending four months on Sulawesi from May to August 1985, including visiting all the high sub-camps; *P. cognatus* was encountered frequently when it was attracted to sap on trees near the base camp and to baited traps in various localities. It is hard to believe that the distinctive *P. inopinatus* would not have been sighted at all, had it been present.

The Japanese text of Tsukada (1991), which so far as we know has not previously been translated by western researchers, also shows an unsuccessful but concerted ef-

fort by Tsukada himself to rediscover the species in its stated locality on Sulawesi: “On April 9, 1991, I still cannot get this rarest species at all. [I] imagine that there are no specimens preserved in the world. It is lost, and nobody can tell where it is. I sent catchers, totally for more than 50 man-days, to the locality to find the butterfly, only in vain. No information was sent back to me from them. A monochrome picture is given by Röber with his original detailed description. I asked Prof Asao Okada to translate the original Deutsch description into Japanese ...”. (Tsukada, 1991: 518 [translation]). The first author visited Lake Tondano (the supposed type locality of *P. inopinatus*) on Sulawesi during 1996 and 2003, specifically in search of *P. inopinatus* but without a glimpse of the insect.

On balance, we believe that the butterfly is not very widespread (*i.e.* from Sulawesi to the Bismarcks) but is instead a Bismarck endemic, wrongly reported to be from Sulawesi when it was described by Röber in 1940.

## Discussion

The enigma of *Polyura inopinatus* has occupied nymphalid specialists and Lepidopterists for decades, due in large part to the fact that the solitary specimen known – the most distinctive representative of the genus – was long believed to have been destroyed during one of the worst international conflicts of our recent history. It may be the case that the actual specimen was seen by very few people – perhaps only Bang-Haas, Röber and whoever took the original monochrome photograph. Entomological research would have been a low priority on the eve of World War II, there would have been no entomological research visitors to Dresden during the war, and by the end of the war the specimen was destroyed. To re-discover the species after more than seven decades, 3,000 kilometres from the published type locality, which was almost certainly erroneous, is as remarkable as it is serendipitous.

Tsukada’s unusual step in presenting a colour illustration of the butterfly from a monochrome photograph and a written description would have been quite a challenge. It is a tribute to the artist, Toshitsugu Endo, that its acceptance (without a translation from the Japanese) as the ‘real thing’, aided by a lack of access to the original journal issued on the eve of World War II, was accomplished so easily. The colours and markings are not perfect, of course; for example, in referring to the underside of the forewings, Röber (1940) said “Posterior wing section wider and diffuse yellowish [translation]”, when the reality is creamy-orange. Also, Röber (1940) declared “The upper side is light reddish-brown”, when the upperside ground colour is bright orange. Another distinctive feature of *inopinatus* is the bright orange distal border to the underside forewing postmedian black band, predictably omitted in Tsukada’s painting since the feature is not mentioned in Röber’s written description. But by and large the painting is fairly accurate. There is some minor variation in the few specimens now seen (*cf* figs); for example in the line of terminal spots on the upper surface of the hindwing, which may be straight or slightly irregular.

One might question why Tsukada went to the trouble of preparing a colour picture of a butterfly he had never seen and of which a specimen was not available. The answer lies in the Japanese text; he made a concerted effort to obtain the species, and in sending local collectors to what he thought was the type locality, would have needed a colour picture to show them what to look for.

The orange colouration of *P. inopinatus* is like no other *Polyura*, and in this regard it resembles several species of *Charaxes*. The bright orange spots along the subtermen of the underside of the forewing are unlike any other *Polyura*; it is one of the smallest known species of the genus, similar in size to the diminutive *P. athamas* and its siblings from Sundaland. Specimens were observed flying in company with larger numbers of its congener, *P. jupiter* (Butler, 1869), in comparison with which they appeared dwarfed.

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# Updated checklist of the extant Chondrichthyes within the Exclusive Economic Zone of Mexico

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## Abstract

The checklist presented in this study includes the latest taxonomic and systematic modifications and updates (early 2018) for the Chondrichthyes that inhabit the Exclusive Economic Zone (EEZ) of Mexico. The list is based on a literature review of field-specific books, scientific publications and database information from collections and museums worldwide available online such as, the Ocean Biogeographic Information System (OBIS), Global Biodiversity Information Facility (GBIF), Encyclopedia of Life (EOL), iSpecies, FishBase and the National Biodiversity Information System (SNIB–CONABIO). Information was cross-referenced with digital taxonomic systems such as the Catalog of Fishes of the California Academy of Sciences, the World Register of Marine Species (WoRMS), and the Integrated Taxonomic Information System (ITIS). There is a total of two subclasses two divisions, 13 orders, 44 families, 84 genera, and 217 species that represent approximately 18% of all living and described species of chondrichthyans worldwide. For the Mexican Pacific and the Gulf of California, 92 species of chondrichthyans are listed compared to 94 species for the Gulf of Mexico and the Caribbean Sea. Additionally, 31 species listed occur on both coasts of Mexico. The species richness of the Mexican chondrichthyans will surely continue to increase, due to the exploration of deep-water fishing areas in the EEZ.

## Keywords

chimaeras, elasmobranchs, rays, sharks, systematics, taxonomy

## Introduction

The natural history of the Chondrichthyes (chimaeras, sharks, skates, and rays) inhabiting the waters of the maritime territory and the adjacent and oceanic zones of Mexico, (referred hereinafter as the Exclusive Economic Zone (EEZ)) has always been a difficult issue to address. The causes of the underestimation or overestimation of these species can be diverse (Del Moral-Flores et al. 2015a) and are typically attributed to i) incorrect taxonomic identifications, ii) undetected synonyms, iii) inaccurate or incorrect curatorial records (e.g., date of collection, location, coordinates), iv) use of obsolete information and, v) absence of updated taxonomic lists.

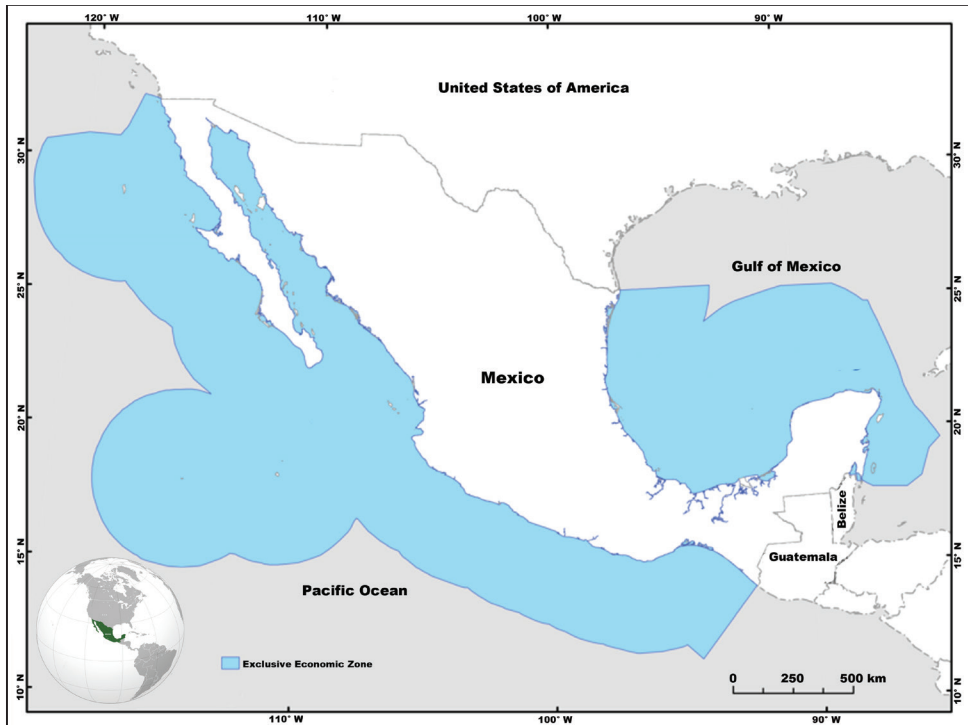
Recent chondrichthyans studies have led to various taxonomic and systematic readjustments, name substitutions, new gender-specific combinations and description of new species. New taxonomic arrangements are based on combined conventional morphology, geometric morphometrics and DNA studies (e.g., Del Moral-Flores et al. 2015b, Acero et al. 2016, Last et al. 2016a, Last et al. 2016b, Last et al. 2016c, Last et al. 2016d, White and Naylor 2016).

The current systematic checklist of chimaeras, sharks, rays, and skates from the Mexican EEZ, incorporates the newest taxonomic and systematic proposals for chimaeras and sharks (Ebert et al. 2013, Weigmann 2016, 2017), skates and rays (Last et al. 2016a, b, c, d, Weigmann 2016, 2017) and follows, with some nomenclatural modifications, the Chondrichthyes classifications made by Weigmann 2016. The present inventory also includes information provided since last century until last year regarding the chondrichthyans of Mexico and Mexican endemic species (e.g., Castro-Aguirre 1965, Castro-Aguirre et al. 1996, Espinosa-Pérez et al. 2004, Palacios-Salgado et al. 2012, Del Moral-Flores and Pérez-Ponce de León 2013, Del Moral-Flores et al. 2015a, Del Moral-Flores et al. 2016).

In Mexico, an updated inventory of the natural resources relevant to Chondrichthyes is necessary, and many ecological relationships are also unknown. This information is imperative for developing environmental or biogeographic theory and for explaining scientific implications of the availability of resources (Siqueiros-Beltrones and De La Cruz-Agüero 2004). Systematic lists are thus a fundamental requirement for the decision-making process in the evaluation of the changes in biodiversity derived from anthropogenic factors and consequently, for the establishment of regional systems and priority areas with regard to the conservation of Mexican chondrichthyans. Due to the constant updating of taxonomic and systematic information for Chondrichthyes, the primary objective of this study is to provide an updated list (early 2018) of the chimaeras, sharks, skates, and rays, living in the EEZ of Mexico.

## Materials and methods

First, the Weigmann taxonomic checklists (2016, 2017) were consulted to select the species that were registered in the areas defined by the latter author (see also IHO 1953)



**Figure 1.** Map showing the Exclusive Economic Zone (EEZ) of Mexico. The zone comprises, including islands and territorial sea, approximately 3,150,000 km<sup>2</sup>. Modified from CONABIO (2011), available at: [http://www.conabio.gob.mx/informacion/gis/layouts/contdv250\\_zeemgw.png](http://www.conabio.gob.mx/informacion/gis/layouts/contdv250_zeemgw.png)

as northeastern Pacific (NEP, Canada to Panama) and northwestern Atlantic (NWA, eastern United States of America to the southern Caribbean Sea). In this geographical framework, all chondrichthyan species with a distribution identified within the Mexican EEZ were included. The selected taxocenoses were contrasted and compared with the biological stocks recorded in the Ichthyological Collection (IC) (<http://coleccion.cicimar.ipn.mx/>) of the Centro Interdisciplinario de Ciencias Marinas (marked with an asterisk in Table 1). The Mexican EEZ is the area of ocean extending 200 nautical miles (370.4 km) from the coast (DOF 1976). This geographic boundary refers to a political delineation rather than an ecological boundary (Figure 1).

Field-specific books were consulted (e.g., Compagno 1999, Castro 2011a, Ebert et al. 2013, Last et al. 2016a) for comparative purposes and several recent scientific publications were reviewed in a deliberate manner (e.g., Ruiz-Campos et al. 2010, Hoyos-Padilla et al. 2013, Del Moral-Flores et al. 2015a, Del Moral-Flores et al. 2016, Villalobos et al. 2016, Weigmann 2016, 2017). In this way, a clear, cumulative database was compiled with all existing records for species within the Mexican EEZ. This database was cross-referenced with information available online from biological collections and museums worldwide. Global databases consulted included the Ocean Biogeographic Information System (OBIS 2017), the Global Biodiversity Informa-



**Table 1.** A systematic listing of the chondrichthyans inhabiting the Exclusive Economic Zone of Mexico (EEZ). The checklist was depurated from references, field books, catalogued specimens and online databases (see text) about the distribution of Chondrichthyes in the littoral areas of the Pacific Ocean (including the Gulf of California) and the Gulf of Mexico (including the Caribbean Sea). The systematic arrangement is based on Weigmann (2016), with some modifications for the batomorphs cited by Last et al. (2016a). The species are listed in alphabetical order. (\*) = Species with specimens cataloged in the Ichthyological Collection (IC) of CICIMAR–IPN (<http://coleccion.cicimar.ipn.mx/>). (†) = New valid taxa according to Weigmann (2017) and Last et al. (2016a), taking into account that when a genus is designated, all species are included. (?) = Species whose validity requires further investigation. (West C.) = west coast of the EEZ of Mexico. (East C.) = east coast of the EEZ of Mexico.

Subclass	Order	Family	Genus	Species	West C.	East C.
Holocephali	Chimaeriformes	Rhinochimaeridae	<i>Harriotta</i>	<i>Harriotta haeckeli</i> Karret, 1972	✓	
				<i>Harriotta raleighana</i> Goode & Bean, 1895	✓	
		Chimaeridae	<i>Rhinochimaera</i>	<i>Rhinochimaera atlantica</i> Holte & Byrne, 1909		✓
				<i>Hydrolagus alberti</i> Bigelow & Schroeder, 1951		✓
				<i>Hydrolagus collei</i> * (Lay & Bennett, 1839)	✓	
				<i>Hydrolagus macrophthalmus</i> De Buen, 1959	✓	
				<i>Hydrolagus melanophasma</i> * James, Ebert, Long & Didier, 2009	✓	
	<i>Hydrolagus mirabilis</i> (Collett, 1904)		✓			
Euselachii						
Infraclass						
Elasmobranchii						
Division	Order	Family	Genus	Species	West C.	East C.
Selachii	Heterodontiformes	Heterodontidae	<i>Heterodontus</i>	<i>Heterodontus francisci</i> * (Girard, 1855)	✓	
				<i>Heterodontus mexicanus</i> *Taylor & Castro-Aguirre	✓	
	Orectolobiformes	Ginglymostomatidae	<i>Ginglymostoma</i>	<i>Ginglymostoma cirratum</i> (Bonnaterre, 1788)		✓
				<i>Ginglymostoma unanui</i> * Del Moral-Flores, Ramírez-Antonio, Angulo & Pérez-Ponce de León, 2015	✓	
				<i>Rhincodon typus</i> Smith, 1828	✓	✓
	Lamniformes	Rhincodontidae	<i>Rhincodon</i>	<i>Alopius pelagicus</i> Nakamura, 1935	✓	
				<i>Alopius superciliosus</i> Lowe, 1841	✓	✓
<i>Alopius vulpinus</i> (Bonnaterre, 1788)				✓	✓	

Subclass	Order	Family	Genus	Species	West C.	East C.
Selachii	Lamniformes	Cetorhinidae	<i>Cetorhinus</i>	<i>Cetorhinus maximus</i> (Gunnerus, 1765)	✓	✓
			<i>Carcharodon</i>	<i>Carcharodon carcharias</i> (Linnaeus, 1758)	✓	✓
		Lamnidae	<i>Isurus</i>	<i>Isurus oxyrinchus</i> Rafinesque, 1810	✓	✓
				<i>Isurus paucus</i> Guitart, 1966	✓	✓
			<i>Lamna</i>	<i>Lamna ditropis</i> Hubbs & Follett, 1947	✓	✓
		Megachasmidae	<i>Megachasma</i>	<i>Megachasma pelagios</i> Taylor, Compagno & Struhsaker, 1983	✓	✓
		Odontaspidae	<i>Carcharias</i>	<i>Carcharias taurus</i> Rafinesque, 1810	✓	✓
			<i>Odontaspis</i>	<i>Odontaspis ferox</i> (Risso, 1810)	✓	✓
				<i>Odontaspis noronhai</i> (Maul, 1955)	✓	✓
		Pseudocarchariidae	<i>Pseudocarcharias</i>	<i>Pseudocarcharias kamoharui</i> * (Matsubara, 1936)	✓	✓
	Carcharhiniformes	Carcharhinidae	<i>Carcharhinus</i>	<i>Carcharhinus acronotus</i> (Poey, 1860)	✓	✓
				<i>Carcharhinus albimarginatus</i> (Rüppell, 1837)	✓	✓
				<i>Carcharhinus altimus</i> (Springer, 1950)	✓	✓
				<i>Carcharhinus brachyurus</i> (Günther, 1870)	✓	✓
				<i>Carcharhinus brevipinna</i> (Müller & Henle, 1839)	✓	✓
				<i>Carcharhinus cerdale</i> Gilbert, 1898	✓	✓
				<i>Carcharhinus falciformis</i> (Müller & Henle, 1839)	✓	✓
				<i>Carcharhinus galapagensis</i> (Snodgrass & Heller, 1905)	✓	✓
				<i>Carcharhinus isodon</i> (Müller & Henle, 1839)	✓	✓
				<i>Carcharhinus leucas</i> (Müller & Henle, 1839)	✓	✓
				<i>Carcharhinus limbatus</i> * (Müller & Henle, 1839)	✓	✓
				<i>Carcharhinus longimanus</i> (Poey, 1861)	✓	✓
				<i>Carcharhinus melanopterus</i> (Quoy & Gaimard, 1824)	✓	✓
				<i>Carcharhinus obscurus</i> (Lesueur, 1818)	✓	✓
				<i>Carcharhinus perezii</i> (Poey, 1876)	✓	✓
				<i>Carcharhinus plumbeus</i> (Nardo, 1827)	✓	✓
				<i>Carcharhinus porosus</i> * (Ranzani, 1839)	✓	✓
				<i>Carcharhinus signatus</i> (Poey, 1868)	✓	✓
			<i>Galeocerdo</i>	<i>Galeocerdo cuvier</i> (Péron & Lesueur, 1822)	✓	✓
			<i>Nasolamia</i>	<i>Nasolamia velox</i> * (Gilbert, 1898)	✓	✓

Subclass	Order	Family	Genus	Species	West C.	East C.
Selachii	Carcharhiniformes	Carcharhinidae	<i>Negaprion</i>	<i>Negaprion brevirostris</i> (Poey, 1868)	✓	✓
			<i>Prionace</i>	<i>Prionace glauca</i> * (Linnaeus, 1758)	✓	✓
			<i>Rhizoprionodon</i>	<i>Rhizoprionodon longurio</i> * (Jordan & Gilbert, 1882)	✓	
				<i>Rhizoprionodon porosus</i> (Poey, 1861)		✓
				<i>Rhizoprionodon ternaenovae</i> (Richardson, 1836)		✓
			<i>Triacnodon</i>	<i>Triacnodon obesus</i> (Rüppell, 1837)	✓	
		<i>Apristurus</i>	<i>Apristurus brunneus</i> (Gilbert, 1892)	✓		
			<i>Apristurus kampae</i> Taylor, 1972	✓		
			<i>Apristurus laurussonii</i> (Saemundsson, 1922)		✓	
			<i>Apristurus nasutus</i> de Buen, 1959	✓		
			<i>Apristurus parvipinnis</i> Springer & Heemstra, 1979		✓	
			<i>Apristurus riveri</i> Bigelow & Schroeder, 1944		✓	
		<i>Cephalurus</i>	<i>Cephalurus cephalus</i> (Gilbert, 1892)	✓		
		<i>Galeus</i>	<i>Galeus anae</i> (Nichols, 1927)		✓	
		<i>Parmaturus</i>	<i>Galeus piperatus</i> Springer & Wagner, 1966	✓		
			<i>Parmaturus campechiensis</i> Springer, 1979		✓	
			<i>Parmaturus xaniurus</i> * (Gilbert, 1892)	✓		
		<i>Cephaloscyllium</i>	<i>Cephaloscyllium ventriosum</i> * (Garman, 1880)	✓		
		<i>Scyliorhinus</i>	<i>Scyliorhinus hesperius</i> Springer, 1966		✓	
			<i>Scyliorhinus meadi</i> Springer, 1966		✓	
			<i>Scyliorhinus retifer</i> (Garman, 1881)		✓	
		<i>Sphyrna</i>	<i>Sphyrna corona</i> Springer, 1940	✓		
			<i>Sphyrna lewini</i> * (Griffith & Smith, 1834)	✓	✓	
	<i>Sphyrna media</i> Springer, 1940		✓			
	<i>Sphyrna mokarran</i> (Rüppell, 1837)		✓	✓		
	<i>Sphyrna tiburo</i> (Linnaeus, 1758)		✓	✓		
	<i>Galeorhinus</i>	<i>Sphyrna zygaena</i> * (Linnaeus, 1758)	✓	✓		
		<i>Galeorhinus galeus</i> (Linnaeus, 1758)	✓			
		<i>Mustelus</i>	<i>Mustelus albipinnis</i> * Castro-Aguirre, Antuna-Mendiola, González-Acosta & De La Cruz-Agüero, 2005	✓		

Subclass	Order	Family	Genus	Species	West C.	East C.
Selachii	Carchariniiformes	Triakidae	<i>Mustelus</i>	<i>Mustelus californicus</i> * Gill, 1864	✓	
				<i>Mustelus canis</i> (Mitchill, 1815)		✓
				<i>Mustelus dorsalis</i> Gill, 1864	✓	
				<i>Mustelus henlei</i> * (Gill, 1863)	✓	
				<i>Mustelus higmani</i> Springer & Lowe, 1963		✓
				<i>Mustelus lunulatus</i> * Jordan & Gilbert, 1882	✓	
				<i>Mustelus norrisi</i> Springer, 1939		✓
				<i>Mustelus sinuotenuis</i> Heemstra, 1997		✓
			<i>Triakis</i>	<i>Triakis semifasciata</i> * Girard, 1855	✓	
		Chlamydoselachidae	<i>Chlamydoselachus</i>	<i>Chlamydoselachus anguineus</i> Garman, 1884	✓	✓
Selachii	Hexanchiiformes	Hexanchidae	<i>Heptranchias</i>	<i>Heptranchias perlo</i> (Bonnaterre, 1788)		✓
			<i>Hexanchus</i>	<i>Hexanchus griseus</i> * (Bonnaterre, 1788)	✓	
				<i>Hexanchus nakamunai</i> Teng, 1962		✓
				<i>Hexanchus vitulus</i> Springer & Waller, 1969		✓
			<i>Notorynchus</i>	<i>Notorynchus cepedianus</i> (Péron, 1807)	✓	
		Centrophoridae	<i>Centrophorus</i>	<i>Centrophorus granulosus</i> (Bloch & Schneider, 1801)		✓
				<i>Centrophorus uyato</i> (Rafinesque, 1810)		✓
		Dalatidae	<i>Dalatias</i>	<i>Dalatias licha</i> (Bonnaterre, 1788)		✓
			<i>Euprotomiscus</i>	<i>Euprotomiscus bispinatus</i> (Quoy & Gaimard, 1824)		✓
			<i>Isistius</i>	<i>Isistius brasiliensis</i> (Quoy & Gaimard, 1824)	✓	✓
				<i>Isistius plutodus</i> Garrick & Springer, 1964		✓
			<i>Squaliolus</i>	<i>Squaliolus laticaudus</i> Smith & Radcliffe, 1912		✓
Selachii	Squaliiformes	Etmopteridae	<i>Centroscyllium</i>	<i>Centroscyllium nigrum</i> * Garman, 1899		✓
			<i>Etmopterus</i>	<i>Etmopterus bullisi</i> Bigelow & Schroeder, 1957		✓
				<i>Etmopterus billyianus</i> (Poey, 1861)		✓
				<i>Etmopterus schultzei</i> Bigelow, Schroeder & Springer, 1953		✓
				<i>Etmopterus virens</i> Bigelow, Schroeder & Springer, 1953	✓	
		Oxynoridae	<i>Oxymotus</i>	<i>Oxymotus caribbaeus</i> Cervigón, 1961		✓
		Somniosidae	<i>Centroscyrmnus</i>	<i>Centroscyrmnus coadolepis</i> Barbosa du Bocage & de Brito Capello, 1864		✓

Subclass	Order	Family	Genus	Species	West C.	East C.
Selachii	Squaliformes	Somniosidae	<i>Centroscymnus</i>	<i>Centroscymnus austonii</i> Garman, 1906		✓
			<i>Somniosus</i>	<i>Somniosus microcephalus</i> (Bloch & Schneider, 1801)		✓
				<i>Somniosus pacificus</i> Bigelow & Schroeder, 1944	✓	
		Squalidae	<i>Zameus</i>	<i>Zameus squamulosus</i> (Günther, 1877)		✓
			<i>Cirrhigaleus</i>	<i>Cirrhigaleus asper</i> (Merrett, 1973)		✓
				<i>Squalus acanthias</i> * Linnaeus, 1758	✓	✓
				<i>Squalus cubensis</i> Howell Rivero, 1936		✓
			<i>Squalus</i>	<i>Squalus mitsukurinii</i> Jordan & Snyder, 1903		✓
				<i>Squalus suckleyi</i> (Girard, 1855)	✓	
	Echinorhiniformes	Echinorhinidae	<i>Echinorhinus</i>	<i>Echinorhinus brucus</i> (Bonnaterre, 1788)		✓
				<i>Echinorhinus cookei</i> Pletschmann, 1928	✓	
				<i>Squatina californica</i> * Ayres, 1859	✓	
Batomorphi	Rajiformes	Squatinae		<i>Squatina dumeril</i> Lesueur, 1818		✓
			<i>Squatina</i>	<i>Squatina heteroptera</i> ? Castro-Aguirre, Espinosa Pérez & Huidobro Campos, 2007		✓
				<i>Squatina mexicana</i> ? Castro-Aguirre, Espinosa Pérez & Huidobro Campos, 2007		✓
		Anacanthobatidae	<i>Springeria</i> †	<i>Springeria foliostris</i> (Bigelow & Schroeder, 1951)		✓
				<i>Springeria longirostris</i> Bigelow & Schroeder, 1962		✓
				<i>Bathyraja abyssicola</i> (Gilbert, 1896)	✓	
			<i>Bathyraja</i>	<i>Bathyraja interrupta</i> (Gill & Townsend, 1897)	✓	
				<i>Bathyraja spinosissima</i> (Beebe & Tee-Van, 1941)	✓	
				<i>Bathyraja trachura</i> (Gilbert, 1892)	✓	
		Gurgestiellidae †	<i>Pseudonaja</i>	<i>Pseudonaja fischeri</i> Bigelow & Schroeder, 1954		✓
			<i>Cruriraja</i>	<i>Cruriraja poeyi</i> Bigelow & Schroeder, 1948		✓
				<i>Cruriraja rugosa</i> Bigelow & Schroeder, 1958		✓
				<i>Fenestraraja ishijamai</i> (Bigelow & Schroeder, 1962)		✓
			<i>Fenestraraja</i>	<i>Fenestraraja plutonia</i> (Garman, 1881)		✓
				<i>Fenestraraja sinuomexicanus</i> (Bigelow & Schroeder, 1950)		✓



Subclass	Order	Family	Genus	Species	West C.	East C.
Batomorphi	Rajiformes	Rajidae	<i>Gurgesiella</i>	<i>Gurgesiella atlantica</i> (Bigelow & Schroeder, 1962)		✓
			<i>Amblyraja</i>	<i>Amblyraja badia</i> (Garman, 1899)	✓	
				<i>Amblyraja hyperborea</i> (Collett, 1879)	✓	
			<i>Beringaja</i>	<i>Beringaja binoculata</i> (Girard, 1855)	✓	
				<i>Beringaja cortezensis</i> † (McEachran & Miyake, 1988)	✓	
				<i>Beringaja inornata</i> †* (Jordan & Gilbert, 1881)	✓	
				<i>Beringaja rhina</i> † (Jordan & Gilbert, 1880)	✓	
				<i>Beringaja stellulata</i> † (Jordan & Gilbert, 1880)	✓	
			<i>Brevinaja</i>	<i>Brevinaja colesi</i> Bigelow & Schroeder 1948		✓
				<i>Brevinaja spinosa</i> Bigelow & Schroeder, 1950		✓
		<i>Dactylobatus</i>		<i>Dactylobatus armatus</i> Bean & Weed, 1909		✓
				<i>Dactylobatus clarkii</i> (Bigelow & Schroeder, 1958)		✓
		<i>Dipturus</i>		<i>Dipturus bullisi</i> (Bigelow & Schroeder, 1962)		✓
				<i>Dipturus garricki</i> (Bigelow & Schroeder, 1958)		✓
				<i>Dipturus olseni</i> (Bigelow & Schroeder, 1951)		✓
				<i>Dipturus oregoni</i> (Bigelow & Schroeder, 1958)		✓
				<i>Dipturus teevani</i> (Bigelow & Schroeder, 1951)		✓
		<i>Leuconaja</i>		<i>Leuconaja garmani</i> (Whitley, 1939)		✓
				<i>Leuconaja lentiginosa</i> (Bigelow & Schroeder, 1951)	✓	
				<i>Leuconaja yucatanensis</i> (Bigelow & Schroeder, 1950)		✓
		<i>Rajella</i>		<i>Rajella fuliginea</i> (Bigelow & Schroeder, 1954)		✓
				<i>Rajella purpuriventralis</i> † (Bigelow & Schroeder, 1962)		✓
		<i>Rostronaja</i> †		<i>Rostronaja ackleyi</i> (Garman, 1881)		✓
				<i>Rostronaja eglanteria</i> (Bosc, 1800)		✓
				<i>Rostronaja equatorialis</i> * (Jordan & Bollman, 1890)	✓	
				<i>Rostronaja texana</i> (Chandler, 1921)		✓
		<i>Dipllobatis</i>		<i>Rostronaja velezi</i> (Chirichigno, 1973)	✓	
				<i>Dipllobatis ommata</i> * (Jordan & Gilbert, 1890)	✓	
				<i>Narcine bancroftii</i> (Griffith & Smith, 1834)		✓
	Torpediniformes	Narcinidae	<i>Narcine</i>	<i>Narcine brasiliensis</i> (Olfers, 1831)		✓

Subclass	Order	Family	Genus	Species	West C.	East C.
Batomorphi	Torpediniformes	Narcinidae	<i>Narcine</i>	<i>Narcine entemedor</i> * Jordan & Starks, 1895	✓	
				<i>Narcine vermiculatus</i> Breder, 1928	✓	
		Torpedinidae	<i>Tetronarce</i>	<i>Tetronarce californica</i> (Ayres, 1855)	✓	
				<i>Tetronarce nobiliana</i> (Bonaparte, 1835)		✓
				<i>Torpedo andersoni</i> Bullis, 1962		✓
	Rhinopristiformes †	Platyrrhinidae	<i>Platyrrhinoidis</i>	<i>Platyrrhinoidis riseriana</i> * (Jordan & Gilbert, 1880)	✓	
		Pristidae	<i>Pristis</i>	<i>Pristis pectinata</i> Latham, 1794		✓
				<i>Pristis pristis</i> (Linnaeus, 1758)	✓	✓
		Rhinobatidae	<i>Pseudobatos</i> †	<i>Pseudobatos glaucostigmus</i> * (Jordan & Gilbert, 1883)	✓	
				<i>Pseudobatos lentiginosus</i> (Garman, 1880)		✓
				<i>Pseudobatos leucorhynchus</i> * (Günther, 1866)	✓	
				<i>Pseudobatos perciliens</i> (Walbaum, 1792)		✓
				<i>Pseudobatos planiceps</i> (Garman, 1880)	✓	
	Trygonorhinidae †	Actobatidae †	<i>Zapteryx</i>	<i>Pseudobatos prahli</i> (Acero & Franke, 1995)	✓	
				<i>Pseudobatos productus</i> * (Ayres, 1854)	✓	
				<i>Pseudobatos spinosus</i> ? (Günther, 1870)	✓	
				<i>Zapteryx exasperata</i> * (Jordan & Gilbert, 1880)	✓	
				<i>Zapteryx xyster</i> Jordan & Evermann, 1896	✓	
	Myliobatiformes	Dasyatidae	<i>Hypanus</i> †	<i>Actobatus narinari</i> (Euphrasen, 1790)		✓
				<i>Actobatus laticeps</i> * † (Gill, 1865)	✓	
				<i>Hypanus americanus</i> (Hildebrand & Schroeder, 1928)		✓
				<i>Hypanus dipterurus</i> * (Jordan & Gilbert, 1880)	✓	
				<i>Hypanus guttatus</i> (Bloch & Schneider, 1801)		✓
	Potamotrygonidae		<i>Pteroplatytrigon</i>	<i>Hypanus longus</i> (Garman, 1880)	✓	
				<i>Hypanus sabinus</i> (Lesueur, 1824)		✓
				<i>Hypanus sayi</i> (Lesueur, 1817)		✓
				<i>Pteroplatytrigon violacea</i> (Bonaparte, 1832)	✓	✓
				<i>Syracura pacifica</i> (Beebe & Tee-Van, 1941)	✓	
				<i>Syracura schmandae</i> (Werner, 1904)	✓	✓

Subclass	Order	Family	Genus	Species	West C.	East C.
Batomorphi	Myliobatiformes	Gymnuridae	<i>Gymnura</i>	<i>Gymnura crebripunctata</i> * (Peters, 1869)	✓	
				<i>Gymnura marmorata</i> * (Cooper, 1864)	✓	
				<i>Gymnura lessae</i> Yokota & De Carvalho, 2017		✓
		Mobulidae	<i>Mobula</i>	<i>Mobula alfredi</i> † (Krefft, 1868)		✓
				<i>Mobula birostris</i> † (Walbaum, 1792)	✓	✓
				<i>Mobula hypostoma</i> (Bancroft, 1831)		✓
				<i>Mobula mobular</i> (Bonnaterre, 1788)	✓	
				<i>Mobula munkiana</i> Notarbartolo Di Sciara, 1987	✓	
				<i>Mobula tarapacana</i> (Philippi, 1892)	✓	
		Myliobatidae	<i>Myliobatis</i>	<i>Mobula thurstoni</i> (Lloyd, 1908)	✓	
				<i>Myliobatis californica</i> * Gill, 1865	✓	
				<i>Myliobatis longirostris</i> * Applegate & Fitch, 1964	✓	
		Rhinopteridae	<i>Aetomylaeus</i>	<i>Aetomylaeus asperimus</i> (Gilbert, 1898)	✓	
				<i>Rhinoptera bonasus</i> (Mitchill, 1815)		✓
				<i>Rhinoptera brasiliensis</i> Müller, 1836		✓
		Urotrygonidae	<i>Urobatis</i>	<i>Rhinoptera steindachneri</i> * Evermann & Jenkins, 1891	✓	
				<i>Urobatis concentricus</i> * Osburn & Nichols, 1916	✓	
				<i>Urobatis halleri</i> * (Cooper, 1863)	✓	
				<i>Urobatis jamaicensis</i> (Cuvier, 1816)		✓
				<i>Urobatis maculatus</i> * Garman, 1913	✓	
				<i>Urotrygon aspidura</i> * (Jordan & Gilbert, 1882)	✓	
		Urotrygonidae	<i>Urotrygon</i>	<i>Urotrygon chilensis</i> * Günther, 1872)	✓	
				<i>Urotrygon cimar</i> López & Bussing, 1998	✓	
				<i>Urotrygon munda</i> Gill, 1863	✓	
				<i>Urotrygon nana</i> Miyake & McEachran, 1988	✓	
				<i>Urotrygon rogersi</i> * (Jordan & Starks, 1895)	✓	
				<i>Urotrygon simulatrix</i> Miyake & McEachran, 1988	✓	

tion Facility (GBIF 2017), the Encyclopedia of Life (EOL 2017), iSpecies (2017), the FishBase Project (Froese and Pauly 2017) and the National Biodiversity Information System (SNIB–CONABIO, <https://goo.gl/9aNLSv>).

Chondrichthyes taxa were verified using published references from online resources (i.e., the Internet) such as the Catalog of Fishes of the California Academy of Sciences (<https://goo.gl/S792vp>), World Register of Marine Species (<http://www.marine-species.org/>), Integrated Taxonomic Information System (<https://www.itis.gov/>), and Chondrichthyan Tree of Life (<https://sharksrays.org/>). Online database searches were carried out between June 22 and November 15 of 2017. The systematic arrangement (Table 1) is organized in a manner based on the proposal in Weigmann (2016) with some modifications for the group of skates and rays species (Last et al. 2016a). Finally, the spellings in the citations of the common names of the species (sensu Froese and Pauly 2017) follows Nelson et al. (2002).

## Results

The detailed literature review involving the species of chimaeras, sharks, skates, and rays that currently exist worldwide includes 1,212 species (Weigmann 2017). Taxa delineations are in accordance with the phylogenetic system published by Weigmann (2016) for the Chondrichthyes and include two subclasses (Holocephali and Euselachii), one infraclass (Elasmobranchii) with two divisions (Selachii and Batomorphi), 14 orders, 64 families, and 215 genera. The phylogeny includes one order, three families, six genera, and 49 species of chimaeras; nine orders, 34 families, 105 genera, and 517 species of sharks; and four orders, 27 families, 104 genera, and 646 species of skates and rays. For a comparison of the number of species of Chondrichthyes recognized worldwide between 2005 and 2015, see Table 1 of Weigmann (2016).

It should be noted that the specific taxonomic richness of the Chondrichthyes of Mexico may vary slightly within the recent literature (e.g., Weigmann 2016, Last et al. 2016a, Del Moral-Flores et al. 2015a) (approximately ten or fewer species). However, at the family level for the batomorphs, there are important taxonomic implications with a difference of up to nine families. Thus, the proposed classification for the skates and rays by Last et al. (2016a) was included in this systematic listing for the Mexican EEZ, regarding the newest taxa in the Dasyatidae family (Last et al. 2016b), the resurrection of the Aetobatidae family (White and Naylor 2016), the new order Rhinopristiformes (Last et al. 2016c), and nomenclatural changes made in the order Rajiformes (Last et al. 2016d). For a detailed description of the taxonomic changes in Chondrichthyes in general, see Weigmann (2017).

The dynamism (or uncertainty) of the classification of Chondrichthyes can be exemplified by the Thornback guitarfish, *Platyrrhinoidis triseriata* (Jordan & Gilbert, 1880), which is currently recognized in two families, Rhinobatidae (sensu Froese and Pauly 2017) and Platyrrhinidae (sensu Last et al. 2016a), and in four different orders such as, Rajiformes (Froese and Pauly 2017), Rhinobatiformes (Weigmann, 2016), Torpediniformes (Last et al. 2016a) and Myliobatiformes (Nelson et al. 2016). Herein,

for the species mentioned, we follow the criteria for family allocation of Naylor et al. (2012) based on DNA sequences, which concurs with the criteria that the American zoologist Samuel W. Garman suggested more than a century ago (Garman 1913: 290). At the order level, we adopted the proposal depicted in the phylogenetic tree by Yang et al. (unpublished) cited in Last et al. (2016a) followed too, in the Chondrichthyan Tree of Life Project (<https://sharksrays.org/>).

Finally, according to the worldwide species richness of Chondrichthyes, in Mexico, there are no records of one family of Holocephali, the Plownose chimaeras, Callorhynchidae (distributed in the south of the American continent); neither for 11 families of sharks: Parascylliidae, Brachaeluridae, Orectolobidae, Hemiscylliidae, Stegostomatidae, Leptochariidae, Hemigaleidae, Pseudotriakidae, Pristiophoridae, Proscylliidae, and Mitsukurinidae (the last three families having species that inhabit the American continent and are likely to be found in the future within the Mexican EEZ, such as the Goblin shark *Mitsukurina owstoni* Jordan, 1898); and finally not for eight families of skates and rays: Glaucostegidae, Hexatrygonidae, Hypnidae, Rhinidae, Narkidae, Plesiobatidae, Urolophidae and Zanobatidae (none of which are distributed in the American continent).

### **The Mexican Exclusive Economic Zone (EEZ)**

A total of 217 species of chimaeras, sharks, skates, and rays was recorded and classified into two subclasses, one infraclass, two divisions, 13 orders, 44 families, and 84 genera (Table 1). This species richness corresponds to 17.9% of the total of current species of Chondrichthyes worldwide, according to Weigmann (2017). The Euselachii subclass was the most representative in terms of species richness at 96.3%, while the Holocephali subclass was the least represented at only 3.7%. The chimaeras were represented in the Mexican EEZ exclusively by a single order (Chimaeriformes) with two families. Of these, Rhinochimaeridae was the best represented with two genera, while *Hydrolagus* (Chimaeridae) was the genus that had the largest number of species (five species). In terms of sharks, the Carcharhiniformes and Squaliformes orders were represented by six families each. Within Carcharhiniformes, the Carcharhinidae family contained seven genera, and *Carcharhinus* had 18 species resulting with the highest species diversity. Finally, for rays and skates, the order Myliobatiformes was represented by eight families, where the genera *Pseudobatos* (eight species), *Mobula* (seven species) and *Urotrygon* (seven species) were the most diverse at the species level. On the other hand, the family Rajidae (order Rajiformes) contained eight different genera with 27 species (Table 1).

### **The Pacific Ocean and the Gulf of California (west coast)**

For this area of the Mexican EEZ, Chondrichthyes was represented by 92 species found only in this area and 31 species distributed on both coasts (i.e., with an ampho-Amer-

ican distribution), for 123 species. These species belong to two subclasses (Table 1), with sharks (Selachii division) representing 51.2% of the species (63 species), followed by rays (Batomorphi division) representing 44.7% of the species (55 species) and finally chimaeras (subclass Holocephali), with 4.1% of the species (five species). Considering both coasts of Mexico, the species richness for this zone represents 49.59% of the total of Chondrichthyes currently recorded for the EEZ (of a total of 248 species including the amphi-American species).

The order Chimaeriformes was represented by two families (Chimaeridae and Rhinochimaeridae) with two genera and five species, representing 62.5% of the chimaera species reported for Mexico (Table 1). The two species of chimaeras from the genus *Harriotta* (Rhinochimaeridae) were distributed exclusively on this basin of the Mexican EEZ.

For the group of sharks, eight different orders were recorded, including 22 families, 36 genera, and 63 species. The Carcharhiniformes were the best represented with six families. Specifically, Carcharhinidae was the most taxonomically diverse with seven genera and 17 species. Of these species, eleven belong to the genus *Carcharhinus*. The Triakidae family includes three genera and seven species, which five species belonged to the genus *Mustelus* (Table 1). The horn sharks, Heterodontidae family, were distributed exclusively within the Pacific Mexican EEZ. On the other hand, the batomorphs were represented by four orders, 16 families, 23 genera, and 55 species (Table 1). The genus *Urotrygon* (Myliobatiformes: Urotrygonidae) contained the highest diversity of batomorph, with seven species (Table 1). The Banded guitarfish *Zapteryx exasperata* (Jordan & Gilbert, 1880) and the Witch guitarfish *Z. xyster* (Jordan & Evermann, 1896) are now members of the new family Trygonorrhinidae (sensu Last et al. 2016c), and they are exclusively distributed in the western slope of the Mexican EEZ.

In this area, the following species were identified as endemic: the Whitemargin smoothhound *Mustelus albipinnis* Castro-Aguirre, Antuna-Mendiola, González-Acosta & De La Cruz-Agüero, 2005 (Carcharhiniformes: Triakidae), the Spotted round ray *Urobatis maculatus* Garman, 1913 (Myliobatiformes: Urotrygonidae), the Cortez skate *Beringraja cortezensis* (McEachran & Miyake, 1988) (Rajiformes: Rajidae) and the Spiny guitarfish *Pseudobatos spinosus* (Günther, 1870) (Rhinopristiformes: Rhinobatidae). For the latter species, see the Discussion.

### The Gulf of Mexico and the Caribbean Sea (east coast)

The Exclusive Economic Zone of the eastern slope of Mexico was represented by 94 chondrichthyans species that occur only in this area and 31 amphi-American species (125 in total), belonging to two subclasses (Table 1). Of these, 75 species belong to sharks (60.0%), 47 (37.6%) to skates and rays, and three (2.4%) to chimaeras. Considering both coasts of Mexico, the species richness for this zone represents 50.41% of the total chondrichthyans currently recorded (of 248 species including the amphi-Americans species).



The Holocephali were grouped into one order (Chimaeriformes), two families, two genera, and three species that represent approximately 40% of the species recorded to date for the Mexican EEZ (Table 1). Within the sharks, the Squaliformes and Lamniformes orders contained the most number of families with six families. However, the Carcharhinidae family (order Carcharhiniformes) had the highest number of genera with five genera, and the *Carcharhinus* genus had the highest species richness with 14 species (Table 1). The sharks belonging to the family Centrophoridae were distributed exclusively in this slope of the EEZ of Mexico. For the batomorphs, the order Myliobatiformes contained the largest number of families with seven, where Rajidae with six genera was the most diverse, as its genus *Dipturus* is the taxon with the highest species richness with five species (Table 1).

For the Gulf of Mexico and the Caribbean Sea there were three endemic species of sharks: Campeche catshark *Parmaturus campechiensis* Springer 1979 (Carcharhiniformes: Scyliorhinidae), the Disparate angel shark *Squatina heteroptera* Castro-Aguirre, Espinosa Pérez & Huidobro Campos, 2007 the Mexican angel shark *S. mexicana* Castro-Aguirre, Espinosa Pérez & Huidobro Campos, 2007 (Squatiniiformes: Squatinidae) and one species of skate, the Yucatan skate *Leucoraja yucatanensis* (Bigelow & Schroeder, 1950). However, see in Discussion about these Angel shark species.

### Amphi-American Chondrichthyes in the EEZ of Mexico

Thirty-one species of chondrichthyans were recorded on both oceanic basins of the EEZ of Mexico. Sharks, 27 species, constituted approximately 87% of the total of species followed by four species of rays. Amphi-American chimaeras were not recorded.

Among the sharks, the genus *Carcharhinus* (Carcharhiniformes: Carcharhinidae) is the richest with seven species, the Bull shark *C. leucas* (Müller & Henle, 1839), the Oceanic whitetip shark *C. longimanus* (Poey, 1861), the Blacktip shark *C. limbatus* (Müller & Henle, 1839), the Silky shark *C. falciformis* (Müller & Henle, 1839), the Dusky shark *C. obscurus* (Lesueur, 1818), the Smalltail shark *C. porosus* (Ranzani, 1839) and the Bignose shark *C. altimus* (Springer, 1950). The hammerhead sharks of the Sphyrnidae family with distributions on both coasts are the Scalloped hammerhead *Sphyrna lewini* (Griffith & Smith, 1834), the Great hammerhead *S. mokarran* (Rüppell, 1837), the Bonnethead *S. tiburo* (Linnaeus, 1758) and the Smooth hammerhead *S. zygaena* (Linnaeus, 1758).

In the case of the batomorphs, only four species are distributed on both coasts of the country, the Pelagic stingray *Pteroplatytrygon violacea* (Bonaparte, 1832), the Chupare stingray *Styracura schmardae* (Werner, 1904), the Giant manta *Mobula birostris* (Walbaum, 1792) and the Common sawfish *Pristis pristis* (Linnaeus, 1758). The amphi – American distribution of the species *Ginglymostoma cirratum* Bonnaterre, 1788 (Nurse shark) and *Aetobatus narinari* (Euphrasen, 1790) (the Spotted eagle ray) is not considered in the present study because of the reasons stated in the Discussion.

## Discussion

The species richness of Mexican chimaeras, sharks, skates, and rays, when compared to other Latin American countries, is above the 165 species reported for Brazil (Rosa and Gadig 2014); the 117 from Colombia (62 Pacific coast species and 75 for the Caribbean Sea; Navia et al. 2016); the 99 for Costa Rica (12 Caribbean Sea, 75 Pacific, and 12 amphi-American; Bussing and López 1999, 2010, Espinoza et al. 2018); the 98 for Venezuela (60 sharks, 37 rays, and one chimera; Cervigón and Alcalá 1999, Tavares and López 2009), and the 38 species for Ecuador (Jiménez-Prado and Beárez 2004, Estupiñán-Montaña et al. 2016). This diversity of Chondrichthyes inhabiting the EEZ of Mexico (approximately 3,000,150 km<sup>2</sup>) makes it a megadiverse country for this group of species.

The total numbers of chondrichthyans fishes herein reported to the species, genus, family, and order levels in this study are 217, 84, 44, and 13, respectively. These numbers are similar to those reported for Mexico by Del Moral-Flores et al. (2015a) and Del Moral-Flores et al. (2016). However, some of the discrepancies can be attributed to recent taxonomic readjustments, especially for the Division Batomorphi.

Sharks, in general, are the group with the highest diversity of species in the EEZ of Mexico with 111 species (51%). These results are consistent with a previous study by Del Moral-Flores et al. (2016), who reported the same number of species; however, the systematic inventories are not equivalent because the present study does not include records of species reported by those authors as *aff. sp.* or species herein recognized as synonyms (e.g., *Sphyrna vespertina* Springer, 1940; *Negaprion fronto* (Jordan & Gilbert, 1882); *Centrophorus niaukang* Teng, 1959) or subspecies (e.g. *Leucoraja garmani caribbaea* (McEachran, 1977)).

The group of the skates and rays contained 98 species and constituted 45% of the total diversity recorded for the EEZ of Mexico. This figure is very similar to that reported by Del Moral-Flores et al. (2016), who listed 95 species. The species additions to the Mexican chondrichthyans for this group are the Longsnout butterfly ray *Gymnura crebripunctata* (Peters, 1869), the Lessa's butterfly ray *Gymnura lessae* Yokota & De Carvalho, 2017 (see below), the Brazilian cownose ray *Rhinoptera brasiliensis* Müller, 1836, the Fake round ray *Urotrygon simulatrix* Miyake & McEachran, 1988, and the Pacific guitarfish *Pseudobatos planiceps* (Garman, 1880) (the latter species is placed in a new genus, sensu Last et al. 2016c). The eight species of chimaeras did not present any differences among the bibliographic sources and the databases consulted.

Although the Batomorphi constitutes approximately 53% (633) of the total living species of the Chondrichthyes class worldwide, with an addition of at least fifty detected species and yet to be described (Last et al. 2016a), it is not surprising that sharks are the most representative group in the present study. This phenomenon is considered to be related to a historical preference for studying sharks, due to their charismatic characteristics (sensu Isasi-Catalá 2011), and a greater interest in fishing for them, in comparison with the rays and skates. However, currently, the number of scientists referencing batomorphs is increasing, allowing us to consider a scenario where skates

and rays species might be studied with a greater emphasis at national and international levels (see Last et al. 2016e).

The new families identified and restored by Last et al. (2016c) and White and Naylor (2016) (Trygonorrhinidae and Aetobatidae, respectively) are represented within the EEZ of Mexico, with the records of the species of guitarfish *Zapteryx exasperata* and *Z. xyster* (Rhinopristiformes: Trygonorrhinidae) for the coasts of the Pacific Ocean and the Eagle rays *Aetobatus narinari* and *A. laticeps* (Gill, 1865) (Myliobatiformes: Aetobatidae) for the Atlantic and Pacific Oceans, respectively. Recently, the existence of genetic and morphometric variability in a latitudinal gradient for the guitar species of the *Zapteryx* genus has been identified and should be studied in more detail (Castillo-Páez et al. 2017).

According to the information in OBIS (2017) and GBIF (2017), the Reef stingray *Urobatis concentricus* Osburn & Nichols, 1916 (Myliobatiformes: Urotrygonidae) is exclusive to the western Mexican seas of the Mexican EEZ except for three records from Costa Rica. We considered that these latter records are misidentification or a data capture error in the computer platforms previously mentioned. Thus, in the study conducted by Palacios-Salgado et al. (2012), they do not consider this species (or any other batomorph species) as endemic to the Cortez biogeographic province (eastern Pacific Ocean Region, sensu Briggs (1974)). Nevertheless, other authors consider this species as endemic to the western coast of Mexico (Ehemann et al. 2017).

In the case of the Bat ray *Myliobatis californica* (Gill, 1865) (Myliobatiformes: Myliobatidae), a situation similar to that previously reported for *U. concentricus* occurred, because Palacios-Salgado et al. (2012) also make no reference to this species as endemic to the Cortez biogeographic province as there are records outside the study area of the authors. The source of the data consulted (i.e., OBIS 2017) reviewed all the records for this species ( $n = 73$ ) within the continental shelf of Mexico, which may be considered as an endemic species. However, another source of data consulted (i.e., GBIF 2017) presented more than 500 records, with a few dozen registered for the area of the United States of America, and three records for Indonesia, the Maldives and Panama, which could be attributed, again, to misidentification or a capture error within the GBIF platform, which would corroborate this hypothesis with the one record of this species in the Gulf of Mexico.

The Spiny guitarfish *Pseudobatos spinosus* (Günther, 1870) (Rhinopristiformes: Rhinobatidae) had a single record in the databases consulted (i.e., OBIS 2017), which referred to the collection location of the holotype. Excluding the original description and some of the checklist and computer databases references (e.g., Del Moral-Flores et al. 2015a, Del Moral-Flores et al. 2016, Froese and Pauly 2017), there is no other information available for this species. According to F. Del Moral-Flores (UNAM–Campus Iztacala, pers. comm.), the record could be related to an anomalous specimen (i.e., the holotype) of *Pseudobatos* spp., described as *Rhinobatos spinosus* by Günther (1870: 518). Another possibility that has been cited for this record is that it may be a juvenile specimen of another Rhinobatidae species (Compagno 1999: 471–498). Taking into consideration the conditions of the holotype (a dissected and unrecognizable specimen

of 33 cm in length, deposited in the British Museum, BMNH: 1870.6.20.2) and the total absence of records since its diagnosis, the exclusion of this species from the EEZ of Mexico could be considered. Thus, the taxonomic lists and identification guides would avoid, to some extent, the overestimation of the chondrichthyans species from the EEZ of Mexico.

For the Disparate angel Shark (*Squatina heteroptera*) and the Mexican angel shark (*S. mexicana*) (both referred as endemic species from the east coast of Mexico), recently Weigmann (2016) and Vaz and De Carvalho (2018), have treated these species as junior synonyms of *Squatina dumeril* Lesueur, 1818. Despite the fact that those authors came to the same preliminary conclusion, in this manuscript each *Squatina* species mentioned, are retained as valid species. Nevertheless, it is highly recommended to do further investigation to demonstrate its taxonomic validity. Such as the Spiny guitarfish, these two species are marked in the checklist with a question mark (i.e. species whose validity requires further investigation).

At present, there are recent publications that support the separation of a species considered to have an amphi-American distribution, which is the case for the Nurse shark *Ginglymostoma cirratum* Bonnaterre, 1788 and the UNAM's nurse shark *Ginglymostoma unami* Del Moral-Flores, Ramírez-Antonio, Angulo & Pérez-Ponce de León, 2015. The latter species was described from specimens collected in the tropical eastern Pacific Ocean initially identified as *G. cirratum* and recorded as this new species (*G. unami*) and as endemic to this region, excluding the presence of *G. cirratum* for the Pacific Ocean (Del Moral-Flores et al. 2015b).

Another similar case is for the species the Spotted eagle ray *Aetobatus narinari* (Euphrasen, 1790), considered as an amphi-American species (in fact cosmopolitan species). Currently, based on DNA sequences (White and Naylor 2016, Last et al. 2016a), the Pacific eagle ray *Aetobatus laticeps* is recognized as distributed exclusively on the eastern Pacific coast, while its congener *A. narinari* is an inhabitant of the western Atlantic coast including the Gulf of Mexico and the Caribbean Sea. However, Last et al. (2016e) consider “that it is necessary to do more work to distinguish morphologically the two forms”. Currently, the species is cited as an ambiguous synonym for *A. narinari* in Froese and Pauly (2017).

According to the recent morphometric and molecular results obtained by De Carvalho et al. (2016), the subfamily Styracurinae was described and relocated within the family Potamotrygonidae, which are freshwater batomorphs known only in South America until these recent results. The species *Styracura scharmadae* (Werner, 1904) and the Pacific chupare ray *S. pacifica* (Beebe & Tee-Van, 1941) were removed from *Himantura* in Dasyatidae (sensu Last et al. 2016c, De Carvalho et al. 2016) and are thus the only representatives of the potamotrygonids within the EEZ of Mexico; the first species is cited an inhabitant of both coasts, and the second species is restricted to the western basin.

The recent taxonomic relocation of the two species of the genus *Manta*, the Reef manta ray *M. alfredi* (Kreff, 1868) and the Giant oceanic manta ray *M. birostris* (Walbaum, 1792) within the genus *Mobula* (see Last et al. 2016a), and the consideration

of the Spinetail mobula *Mobula japanica* (Müller & Henle, 1841) as synonymous with the Devil fish *M. mobular* (Bonnaterre, 1788), are considered by some specialists as a taxonomic decision subject to discussion (Guy Stevens, The Manta Team: <https://goo.gl/KYbtZO>; com. pers.).

Finally, a recent taxonomic and morphological revision of butterfly rays (Gymnuridae) has limited the distribution of the Smooth butterfly ray *Gymnura micrura* (Bloch & Schneider, 1801) to the southwestern Atlantic and the new species the Lessa's butterfly ray *Gymnura lessae* Yokota & De Carvalho, 2017 occurring in the Gulf of Mexico, north, and central western Atlantic, substituting *G. micrura* records in that area (see Yokota and De Carvalho 2017).

With the increasing use of various techniques and the analysis tools currently available (e.g., molecular sequences, mitogenome analysis, geometric morphometrics), the future of the biological classification of Chondrichthyes may have higher stability, predictability, and robustness (sensu Crisci and López-Armengol 1983). Due to its geographical location, the extension of its patrimonial sea, and the increase in studies on its chondrichthyans, Mexico will undoubtedly continue to contribute to the knowledge for this group of cartilaginous fishes. At present, for the country, there are at least four species of sharks and two batomorphs that need to be formally described, which have been previously mentioned by various authors (e.g., Castro-Aguirre et al. 1996, Castro 2011a, 2011b, Del Moral-Flores et al. 2015a).

As a corollary to the above, recently published works or studies in progress can be cited. Thus, Fields et al. (2016) proposed the existence of populations with possible cryptic speciation among hammerhead sharks (Sphyrnidae) from the Gulf of Mexico and the Caribbean Sea. Another study conducted by Hinojosa-Alvarez et al. (2016) indicates the possibility of a third species of manta ray within the genus *Mobula* (sensu lato *Manta*, see Last et al. 2016a) for the Yucatan Peninsula. Finally, the mitochondrial divergence between the populations of the Cownose ray *Rhinoptera steindachneri* Evermann & Jenkins, 1891, of the Gulf of California (see also Sandoval-Castillo and Rocha-Olivares 2011) is currently being investigated (Christian Jones, NOAA-SFSC Mississippi, pers. comm.), as is the case of *Urotrygon* spp. in the southern Gulf of California by the present authors, what could result in the description of new species in the EEZ of Mexico.

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article. This paper is in honor of the late José Luis “Doc” Castro-Aguirre (1943–2011), a mainstay of contemporary ichthyology in Mexico. At the time of his death, “Doc” Castro-Aguirre was the co-author of the first and the last species of chondrichthyans described in Mexico by national scientists, i.e., the Mexican hornshark *Heterodontus mexicanus* Taylor & Castro-Aguirre, 1972, and the Whitemargin smoothhound *Mustelus albiginnis* Castro-Aguirre, Antuna-Mendiola, González-Acosta and De La Cruz-Agüero, 2005. AJE (<https://www.aje.com/>) performed the English language editing of the manuscript.

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# Checklist of ladybirds of Algeria with two new recorded species (Coleoptera, Coccinellidae)

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## Abstract

An updated and corrected checklist of species of ladybird beetles (Coleoptera: Coccinellidae) known in Algeria now contains 75 species belonging in ten tribes. New country records include the European species *Oenopia conglobata* and the invasive Asian species *Harmonia axyridis*. Sampling data is provided for 14 species found during a faunistic survey performed mostly in agroecosystems, together with host plant and prey species.

## Keywords

Africa, aphids, *Coccinelloidea*, distribution, ladybug, taxonomy

## Introduction

Family Coccinellidae (ladybirds) is the most species-rich family in the recently recognized beetle superfamily Coccinelloidea (Robertson et al. 2015) with approximately 6000 species described worldwide (Vandenberg 2002). They are mostly beneficial in-

sects, their larvae and adults feeding on pests, especially on scale insects and aphids (Hodek et al. 2012, Giorgi et al. 2009).

Among beetle families, ladybird beetles (Coccinellidae) of individual countries are relatively well known, and the fauna of Algeria is also relatively well documented (Saharaoui and Gourreau 2000, Kovář 2007, Saharaoui et al. 2014). During a recent relatively limited survey, we found two species recorded for the first time in Algeria which need to be added to the list. In preparing the checklist, we also found many taxonomical errors in the previous species lists or old taxonomy that was recently changed mainly due to molecular phylogenetic studies. Thus, we provide an updated and corrected checklist of species of the family Coccinellidae in Algeria.

During the faunistic survey performed mostly in agroecosystems, we found 12 species reported before and therefore we provide details of their localities and dates of sampling as well as their host plants and prey species.

## Materials and methods

Literature records were reviewed to set up up-to-date list of species of Coccinellidae recorded from Algeria. Our sampling was performed to confirm species occurrence and their host/food relationships as a part of study on the natural enemies of aphids. Survey has been carried out in agroecosystems in distant localities within the country – name of locality, geographic coordinates, date of sampling and host plant are given in Table 1. Beetles were sampled from plants using sweeping net (the most effective method for Coccinellidae found by Kherbouche et al. 2015) and the Japanese umbrella. We also sampled plant fragments infested with aphids for their identification. Besides adults, also larvae of the ladybirds were collected for identification. Samples were preserved in 70% ethanol, adult beetles were subsequently allowed to dry. Insects were photographed by digital camera Lumenera Infinity 2 mounted on stereomicroscope Nikon SMZ 1500, operated by QuickPHOTO CAMERA software. Series of images was stacked using Zerene Stacker 1.04. Species were identified using various available keys, such as Iablokoff-Khnzorian (1982), Nedvěd (2015). The specimens are deposited in Agronomic National School Superior of El Harrach, Algeria.

## Results

The updated checklist of Coccinellidae species of Algeria now includes the following 75 species assigned in ten tribes in the sense of Seago et al. (2011). Species taxonomy and synonymy follow Kovář (2007) and Nedvěd (2015). Species collected by the authors are marked with asterisk (\*). The details of sampling regimes are listed in Table 1. Presence of herbivorous insects that may serve as food for the ladybirds is indicated in Table 2.



**Table 1.** Original records of the species of Coccinellidae in Algeria. Developmental stages, host plants or habitat, region of sampling, date of sampling, and coordinates are provided. The two species in bold are new records for Algeria.

Species	Adult	Larva	Plant/habitat	Region	Date	Coordinates
<i>Adalia bipunctata</i> (Linnaeus, 1758)	+	–	peach orchard	Mouzaia, BLIDA	10/04/2017	36°32'49"N, 2°41'47"E
<i>Adalia decempunctata</i> (Linnaeus, 1758)	3	0	<i>Ficus retusa</i>	El Harrach, ALGER	29/04/2017	36°43'02"N, 3°09'16"E
<i>Coccinella septempunctata</i> Linnaeus, 1758	+	–	pear orchard	Mouzaia, BLIDA	10 and 15/04/2017	36°32'51"N, 2°41'54"E
	+	–	peach orchard	Mouzaia, BLIDA	10 and 15/04/2017	36°32'49"N, 2°41'47"E
	+	+	wheat field	Mouzaia, BLIDA	01/05/2017	36°32'55"N, 2°41'32"E
	+	–	alphalpa	(university) OUARGLA	04/04/2017	31°56'28"N, 5°18'20"E
	+	–	wheat field	ITDAS OUARGLA	02/04/2017	32°0'13"N, 5°27'58"E
	+	–	<i>Aristida</i> sp	Oued en Nsa, OUARGLA	04/04/2017	32°36'46"N, 4°57'43"E
	+	+	<i>Nerium oleander</i>	Mouzaia, BLIDA	10/05/2017	36°28'13"N, 2°41'29"E
	+	–	<i>Malva parviflora</i>	Mouzaia, BLIDA	08/05/2018	36°28'14"N, 2°41'29"E
	+	–	<i>Anthemis</i> sp.	Mouzaia, BLIDA	08/05/2018	36°28'14"N, 2°41'29"E
<i>Harmonia axyridis</i> (Pallas, 1773)	<b>1</b>	<b>0</b>	<b>peach orchard</b>	<b>Mouzaia, BLIDA</b>	<b>10/04/2017</b>	<b>36°32'49"N, 2°41'46"E</b>
	<b>1</b>	<b>0</b>	–	El Harrach, ALGER	<b>05/12/2017</b>	<b>36°43'01"N, 3°09'16"E</b>
	<b>20</b>	<b>16</b>	<i>Malva parviflora</i>	Mouzaia, BLIDA	<b>08/05/2018</b>	<b>36°28'14"N, 2°41'29"E</b>
	<b>12</b>	<b>13</b>	<i>Notobasis syriaca</i>	Mouzaia, BLIDA	<b>08/05/2018</b>	<b>36°28'14"N, 2°41'29"E</b>
<i>Hippodamia variegata</i> (Goeze, 1777)	+	+	alphalpa field	(university) OUARGLA	04/04/2017	31°56'28"N, 5°18'20"E
	+	+	wheat field	ITDAS OUARGLA	03/04/2017	32°0'13"N, 5°27'58"E
	+	+	wheat field	Mouzaia, BLIDA	01/05/2017	36°32'54"N, 2°41'32"E
	+	–	wheat field	El Harrach, ALGER	17/04/2017	36°43'11"N, 3°09'03"E
	+	–	<i>Nerium oleander</i>	El Harrach, ALGER	05/05/2017	36°43'16"N, 3°9'5"E
	+	+	<i>Nerium oleander</i>	Mouzaia, BLIDA	10/05/2017	36°28'13"N, 2°41'29"E
	+	–	<i>Nerium oleander</i>	Mouzaia, BLIDA	08/05/2018	36°28'14"N, 2°41'29"E
	+	–	<i>Malva parviflora</i>	Mouzaia, BLIDA	08/05/2018	36°28'14"N, 2°41'29"E
	+	–	<i>Anthemis</i> sp.	Mouzaia, BLIDA	08/05/2018	36°28'14"N, 2°41'29"E

Species	Adult	Larva	Plant/habitat	Region	Date	Coordinates
<i>Hyperaspis duvergeri</i> Füersch, 1985	+	–	conifers	El Harrach, ALGER	09/03/2017	36°43'19"N, 3°08'58"E
<i>Hyperaspis marmottani</i> (Fairmaire, 1868)	+	–	peach orchard	Mouzaia, BLIDA	10/04/2017	36°32'49"N, 2°41'47"E
<i>Nephus (Bipunctatus)</i> <i>peyerimhoffi</i> (Sicard, 1923)	+	–	<i>Ficus retusa</i>	El Harrach, ALGER	29/04/2017	36°43'02"N, 3°09'16"E
<b><i>Oenopia conglobata</i></b> (Linnaeus, 1758)	1	0	<i>Quercus ilex</i>	El Harrach, ALGER	13/04/2017	36°43'14"N, 3°8'58"E
	1	0	<i>Salpichroa origanifolia</i>	El Harrach, ALGER	09/03/2017	36°43'13"N, 3°8'58"E
	1	0	<i>Malva parviflora</i>	Mouzaia, BLIDA	08/05/2018	36°28'13"N, 2°41'29"E
	1	0	<i>Malva parviflora</i>	Mouzaia, BLIDA	08/05/2018	36°28'14"N, 2°41'26"E
	1	0	<i>Nerium oleander</i>	Mouzaia, BLIDA	08/05/2018	36°28'14"N, 2°41'29"E
<i>Oenopia doublieri</i> (Mulsant, 1846)	+	–	<i>Pittosporum tobira</i>	El Harrach, ALGER	16/03/2017	36°43'10"N, 3°09'00"E
<i>Psyllobora vigintiduopunctata</i> (Linnaeus, 1758)	+	–	<i>Salpichroa origanifolia</i>	El Harrach, ALGER	13/04/2017	36°43'14"N, 3°08'58"E
	+	–	<i>Pittosporum tobira</i>	El Harrach, ALGER	16/03/2017	36°43'10"N, 3°09'00"E
	+	–	<i>Citrus</i> sp.	Boufarik, Blida	18/03/2017	36°35'39"N, 2°55'8"E
<i>Rodolia cardinalis</i> (Mulsant, 1850)	+	+	<i>Pittosporum tobira</i>	El Harrach, ALGER	02/05/2017	36°43'05.2"N, 3°09'13"E
<i>Scymnus suffrianioides</i> Sahlberg, 1913	+	+	<i>Pittosporum tobira</i>	El Harrach, ALGER	17/04/2017	36°43'15"N, 3°8'59"E
<i>Stethorus pussilus</i> (Herbst, 1797)	+	–	<i>Pittosporum tobira</i>	El Harrach, ALGER	02/05/2017	36°43'05"N, 3°09'13"E

**Table 2.** Occurrence of aphid species on host plants that were visited by coccinellid predators.

Species	<i>Pyrus communis</i>	<i>Prunus persica</i>	<i>Triticum durum</i>	<i>Medicago sativa</i>	<i>Pittosporum tobira</i>	<i>Nerium oleander</i>	<i>Ficus retusa</i>	<i>Citrus</i> sp.	<i>Capsicum annuum</i>	<i>Malva parviflora</i>
<i>Acyrtosiphon pisum</i>				+						
<i>Aphididae</i> sp.							+			
<i>Aphis craccivora</i>				+						
<i>Aphis fabae</i>	+		+		+					
<i>Aphis gossypii</i>									+	
<i>Aphis nerii</i>						+				
<i>Aphis spiraeicola</i>					+			+		
<i>Aphis umbrellae</i>										+
<i>Dysaphis pyri</i>	+									
<i>Myzus persicae</i>	+	+							+	
<i>Rhopalosiphum padi</i>			+							

### Chilocorini

- Chilocorus bipustulatus* (Linnaeus, 1758) (not *C. bipunctatus* as misspelled by Saharaoui<sup>1</sup> and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)
- Exochomus ericae* Crotch, 1874 (syn. *E. anchorifer* Bedel, 1885; syn. *Parexochomus anchorifer* (Allard, 1870) used by Saharaoui<sup>1</sup> and Gourreau 2000 and Saharaoui et al. 2014; Kovář 2007)
- Exochomus quadripustulatus* (Linnaeus, 1758) (source: Saharaoui<sup>1</sup> and Gourreau 2000; missing in Kovář 2007; syn. *Brumus quadripustulatus* used by Saharaoui et al. 2014)
- Parexochomus nigripennis* (Erichson, 1843) (syn. *Exochomus nigripennis* used by Saharaoui<sup>1</sup> and Gourreau 2000 and Saharaoui et al. 2014; Kovář 2007)
- Parexochomus pubescens* (Küster, 1848) (syn. *Exochomus pubescens* used by Saharaoui<sup>1</sup> and Gourreau 2000 and Saharaoui et al. 2014; Kovář 2007)

### Coccidulini

- Rhyzobius chrysomeloides* (Herbst, 1793) (source: Saharaoui<sup>1</sup> and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)
- Rhyzobius litura* (Fabricius, 1787) (Kovář 2007)
- Rhyzobius lophantae* (Blaisdell, 1892) (source: Saharaoui<sup>1</sup> and Gourreau 2000; occurrence confirmed by Kherbouche et al. 2015; Kovář 2007; Saharaoui et al. 2014)
- Tetrabrachys cordicollis* (Guérin-Méneville, 1844) (Kovář 2007)
- Tetrabrachys cribratellus* (Fairmaire, 1876) (Kovář 2007)
- Tetrabrachys volkonskyi* (Peyerimhoff, 1943) (Kovář 2007)

### Coccinellini

- Adalia bipunctata* (Linnaeus, 1758) (source: Saharaoui<sup>1</sup> and Gourreau 2000; missing in Kovář 2007; Saharaoui et al. 2014) \*
- Adalia decempunctata* (Linnaeus, 1758) (not *A. decimpunctata* as misspelled by Saharaoui<sup>1</sup> and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014) \*
- Bulaea lividula* Mulsant, 1850 (Kovář 2007)
- Calvia quatuordecimguttata* (Linnaeus, 1758) (source: Saharaoui<sup>1</sup> and Gourreau 2000; missing in Kovář 2007; Saharaoui et al. 2014)
- Ceratomegilla notata* (Laicharting, 1781) (syn. *Semiadalia notata* used by Frah et al. 2009; missing in Kovář 2007)
- Ceratomegilla undecimnotata* (Schneider, 1792) (syn. *Hippodamia* (*Semiadalia*) *undecimnotata* used by Saharaoui<sup>1</sup> and Gourreau 2000; missing in Kovář 2007; Saharaoui et al. 2014)
- Cheilomenes propinqua* (Mulsant, 1850) (Kovář 2007)
- Coccinella septempunctata* Linnaeus, 1758 (syn. *C. algerica* Kovář 1977 used by Saharaoui<sup>1</sup> and Gourreau 2000; Kovář 2007; occurrence confirmed by Frah et al. 2009; Saharaoui et al. 2014) \*
- Coccinella undecimpunctata* Linnaeus, 1758 (source: Saharaoui<sup>1</sup> and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)
- Harmonia axyridis* (Pallas, 1773) (**new record**) \*

*Harmonia quadripunctata* (Pontoppidan, 1763) (Kovář 2007)

*Hippodamia tredecimpunctata* (Linnaeus, 1758) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)

*Hippodamia variegata* (Goeze, 1777) (as *H. (Adonia) variegata* by Saharaoui‘ and Gourreau 2000; Kovář 2007; occurrence confirmed by Frah et al. 2009; Saharaoui et al. 2014) \*

*Myrrha octodecimpunctata* (not *M. octodecimpunctata* as misspelled by Saharaoui‘ and Gourreau 2000 and Saharaoui et al. 2014; Kovář 2007)

*Myrrha thuriferae* (Sicard, 1923) (Kovář 2007)

*Oenopia conglobata* (Linnaeus, 1758) (**new record**) \*

*Oenopia doublieri* (Mulsant, 1846) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014) \*

*Oenopia lyncea* (Olivier, 1808) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)

*Propylea quatuordecimpunctata* (Linnaeus, 1758) (not *P. quatuordecimpunctata* as misspelled by Saharaoui‘ and Gourreau 2000; missing in Kovář 2007; Saharaoui et al. 2014)

*Psyllobora vigintiduopunctata* (Linnaeus, 1758) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014) \*

*Tytthaspis phalerata* (Costa, 1849) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)

## Epilachnini

*Chnootriba elaterii* (Rossi, 1794) (syn. *Henosepilachna elaterii* used by Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)

*Henosepilachna angusticollis* (Reiche, 1862) (Kovář 2007)

*Henosepilachna argus* (Geoffroy, 1785) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)

## Hyperaspidini

*Hyperaspis algerica* Crotch, 1874 (not *H. algerica* as misspelled by Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)

*Hyperaspis duvergeri* Fürsch, 1985 (Kovář 2007) \*

*Hyperaspis guttulata* Fairmaire, 1870 (Kovář 2007)

*Hyperaspis marmottani* (Fairmaire, 1868) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014) \*

*Hyperaspis pseudopustulata* Mulsant, 1853 (Kovář 2007)

*Hyperaspis teinturieri* Mulsant & Godart, 1869 (Kovář 2007)

## Noviini

*Novius cruentatus* Mulsant, 1846 (Kovář 2007)

*Rodolia cardinalis* (Mulsant, 1850) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014) \*

## Platynaspidini

*Platynaspis luteorubra* (Goeze, 1777) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)

## Scymnini

*Clitostethus arcuatus* (Rossi, 1794) (source: Saharaoui‘ and Gourreau 2000; missing in Kovář 2007; Saharaoui et al. 2014)

*Diomus rubidus* (Motschulsky, 1837) (Kovář 2007)

*Nephus (Bipunctatus) bicinctus* (Mulsant & Godart, 1870) (Kovář 2007)

*Nephus (Bipunctatus) bipunctatus* (Kugelann, 1794) (Saharaoui et al. 2014; missing in Kovář 2007)

*Nephus (Bipunctatus) conjunctus* (Wollaston, 1870) (Kovář 2007)

*Nephus (Sidis) hiekei* (Fürsch, 1965) (Kovář 2007)

*Nephus (Sidis) levaillanti* (Mulsant, 1850) (syn. *Scymnus levaillanti* used by Saharaoui‘ and Gourreau 2000; missing in Kovář 2007)

*Nephus (Nephus) ludyi* (Weise, 1879) (Kovář 2007)

*Nephus (Bipunctatus) peyerimhoffi* (Sicard, 1923) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014) \*

*Nephus (Nephus) quadrimaculatus* (Herbst, 1783) (source: Saharaoui‘ and Gourreau 2000; missing in Kovář 2007; Saharaoui et al. 2014)

*Nephus (Nephus) redtenbacheri* (Mulsant, 1846) (Kovář 2007)

*Scymniscus splendidulus* (Stenius, 1952) (Kovář 2007)

*Scymnus (Scymnus) apetzi* Mulsant, 1846 (source: Saharaoui‘ and Gourreau 2000; missing in Kovář 2007; Saharaoui et al. 2014)

*Scymnus (Scymnus) bivulnerus* Baudi di Selve, 1894 (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)

*Scymnus (Mimopullus) fulvicollis* Mulsant, 1846 (syn. *Pullus fulvicollis* used by Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)

*Scymnus (Scymnus) interruptus* (Goeze, 1777) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)

*Scymnus (Scymnus) laetificus* Weise, 1879 (Kovář 2007)

*Scymnus (Scymnus) marginalis* (Rossi, 1794) (Kovář 2007)

*Scymnus (Mimopullus) marinus* (Mulsant, 1850) (syn. *Mimopullus mediterraneus* Iablokoff-Khznorian, 1972 used by Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)

*Scymnus (Scymnus) nubilus* (Mulsant, 1850) (Saharaoui et al. 2014; missing in Kovář 2007)

*Scymnus (Scymnus) pavesii* Canepari, 1983 (Kovář 2007)

*Scymnus (Scymnus) rufipes* (Fabricius, 1798) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)

*Scymnus (Pullus) subvillosus* (Goeze, 1777) (syn. *Pullus subvillosus* used by Saharaoui‘ and Gourreau 2000; Kovář 2007 Saharaoui et al. 2014)

*Scymnus* (*Scymnus*) *suffrianioides* Sahlberg, 1913 (syn. *S. pallipediformis* Günther, 1958 used by Saharaoui‘ and Gourreau 2000 and Saharaoui et al. 2014; missing in Kovář 2007) \*  
*Scymnus* (*Pullus*) *suturalis* Thunberg, 1795 (syn. *Pullus suturalis* used by Saharaoui‘ and Gourreau 2000; Kovář, 2007; Saharaoui et al. 2014)

**Stethorini**

*Stethorus pussilus* (Herbst, 1797) (syn. *S. punctillum* (Weise, 1891) used by Saharaoui‘ and Gourreau 2000 and Saharaoui et al. 2014; occurrence confirmed by Idder and Pintureau 2008; missing in Kovář 2007) \*

**Sticholotidini**

*Coelopterus salinus* Mulsant & Rey, 1852 (Kovář 2007)  
*Pharoscymnus numidicus* (Pic, 1900) (not *P. numidicus* as misspelled by Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)  
*Pharoscymnus ovoideus* Sicard, 1929 (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)  
*Pharoscymnus setulosus* (Chevrolat, 1861) (source: Saharaoui‘ and Gourreau 2000; Kovář 2007; Saharaoui et al. 2014)  
*Pharoscymnus sexguttatus* (Pic, 1926) (Kovář 2007)

**Discussion**

*Chilocorus cacti* was introduced in Algeria but probably did not establish itself (Smirnoff 1957). *Coccinella algerica* Kovář, 1977 was described based on small morphological differences of North African populations originally thought to be *C. septempunctata*. Marin et al. (2010) demonstrated that these two species do not form genetically distinct lineages and synonymized *C. algerica* with *C. septempunctata*.

*Adalia decempunctata* was previously known from Algeria. Specimens of *Adalia decempunctata* found during our survey bear a mixture of characters of *A. decempunctata* and *A. conglomerata* (see Table 3 and Fig. 1). The former lives on a wide variety

**Table 3.** Character states for *Adalia decempunctata*, *A. conglomerata*, and the specimens from El Harrach from 29 April 2017.

Character	<i>Adalia conglomerata</i>	<i>Adalia decempunctata</i>	Specimen 29/4/2017
Subapical elytral keel	absent	usually present	absent
Elytral background	yellow	variable	yellow
Shape of spots	deltoid	variable	deltoid
Length to width ratio	1.5	1.4	1.4
Tarsal claws	with tiny tooth	with large tooth	with large tooth
Body to scutellum ratio	25–30	15–18	25
Host plant	conifers	trees	<i>Ficus</i>





**Figure 1.** *Adalia decempunctata* found on *Ficus retusa* in El Harrach, Alger, 29 April 2017.



**Figure 2.** *Harmonia axyridis* found on *Prunus persica* (peach) in Mouzaia, Blida, 10 April 2017.

of woody plants, while *A. conglomerata* is a specialist on conifers, mainly spruce in Central Europe. Differences in the shape of male genitalia are generally small within *Adalia* to be used for clear species identification.

The occurrence of the invasive alien species *H. axyridis* in Algeria confirms predictions of its potential distribution made by Poutsma (2008) using a CLIMEX model. Although meanwhile it has been found in a few countries with wet tropical climate (Kenya: Nedvěd et al. 2011; Tanzania: Nedvěd and Háva 2016), and in dry tropical desert (Biranvand et al., in press) not predicted by the model, it probably did not establish itself there. The climate and host plants present in north Algeria and the occurrence of prey species and other predator ladybirds found during our study suggested establishment and future spread of *H. axyridis* in Algeria. The first specimen found was a male (Fig. 2), the second a female, both with well-developed elytral ridge, belonging to the form *succinea*. The establishment of the species was confirmed by occurrence of



**Figure 3.** *Oenopia conglobata* found on *Quercus ilex* in El Harrach, 13 April 2017.

many larvae and pupae in 2018. All adults found in 2018 were of form *succinea*, which is the most common colour form in the native Chinese as well as in most invasive populations (Roy et al. 2016).

*Oenopia conglobata* is a common tree inhabiting predatory ladybird living in most European countries and as a subspecies in large parts of Asia. The specimen collected in Algeria has yellow elytral background (Fig. 3), while it is usually pink or beige in Europe. Additionally, the spots are rather small, while in many European individuals, at least some spots fuse together (Nedved 2015).

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# Freshwater bryozoans of Lithuania (Bryozoa)

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## Abstract

Nine species of freshwater bryozoans were recorded in Lithuania in a survey of 18 various types of freshwater bodies. Eight species were assigned to the Class Phylactolaemata and families Plumatellidae and Cristatellidae (*Plumatella repens*, *Plumatella fungosa*, *Plumatella fruticosa*, *Plumatella casmiana*, *Plumatella emarginata*, *Plumatella geimermassardi*, *Hyalinella punctata* and *Cristatella mucedo*). The ninth species, *Paludicella articulata*, represented the Class Gymnolaemata.

*Plumatella geimermassardi* and *P. casmiana* were recorded for the first time in Lithuania. For the plumatellids, species identification was achieved partly by analysing statoblasts' morphological ultrastructures by scanning electron microscopy.

## Keywords

Phylactolaemata, *Plumatella*, statoblasts, bryozoa, Lithuania

## Introduction

Freshwater bryozoans grow in colonies of minute tentacle-bearing clones (zooids) that feed upon microscopic plankton. They are often found in ponds, lakes, and rivers, forming a cryptic but often a significant part of the aquatic fauna (Bushnell 1966). Bryozoans are important to ecosystems as filter feeders (Wood et al. 2006), extracting phytoplankton from the water and producing faecal pellets that nourish benthic mei-

ofauna (Bushnell and Rao 1974). The colony structure also creates important habitat and shelter for other organisms: protozoans, rotifers, ostracods, nematodes and chironomids (Ricciardi and Reisinger 1994). Bryozoans hosting certain myxozoan parasites can spread proliferative kidney disease in fish, which is often fatal in farmed and wild fish populations (Grabner and El-Matbouli 2008; Bartošová-Sojtková et al. 2014).

In their natural habitat freshwater bryozoans are easily overlooked and, in many areas, there is little information on the identity or distribution of species.

Fundamental studies of freshwater bryozoans in Europe were launched with a pioneering monograph by Allman (1856), which established the Class Phylactolaemata as an exclusively freshwater group and named Plumatellidae as the largest family within that class. A second monograph by Jullien (1885) described species from France following Allman's taxonomy. Shortly afterwards a third monograph appeared from Germany (Kraepelin 1887) proposing a new taxonomic scheme for the plumatellids which has by now fallen out of use. Since those early years there have been bryozoan surveys from a number of European countries, including the Netherlands (Lacourt 1949), Sweden (Borg 1941), Italy (Viganó 1965, Luxembourg (Geimer and Massard 1986), Belgium (Loppens 1906), Bulgaria (Gruncharova 1968), Ireland (Smyth 1994), and Britain (Mundy 1980). In most of these works only 5–8 species were documented.

Until recently freshwater bryozoans in the Baltic region were known only from a brief paper from Latvia (Trauberg 1940). Six species were listed using the Kraepelin taxonomic scheme. They included *Plumatella polymorpha* Krpln. var. *repens* (L.), Krpln., *Plumatella polymorpha* Krpln. var. *appressa* Krpln., *Plumatella polymorpha* Krpln. var. *fungosa* (Pall.) Krpln., *Plumatella princeps* Krpln. var. *emarginata* Allm., *Cristatella mucedo* Cuv., and *Paludicella ehrenbergi* Bened. Trauberg (1940) provided measurements of certain colonies and statoblasts and included some ecological information as well.

In 2015 an old master's thesis was uncovered in Lithuania with a detailed account of bryozoans from the area (Šatkauskienė et al. 2018). Written by Bronė Pajiedaitė (1933) the work covered a period of 1931–1933. Pajiedaitė collected bryozoans from widely scattered locations in Lithuania (five districts and approximately eleven localities) including lakes, ponds, and rivers. From microscopic examinations, she illustrated colonies and statoblasts, prepared notes on associations with other organisms, and described substrata on which common bryozoans were found. During her studies, Bronė Pajiedaitė prepared at least 12 bottles of fixed whole specimens and 70 high quality microscope slides (Šatkauskienė et al. 2018). Unfortunately, the whole specimens were apparently destroyed during World War II (1941–1945), but the surviving microscopic slides are now deposited in Vilnius University. Altogether Pajiedaitė described seven species of freshwater bryozoans in detail: *Paludicella articulata* (Ehrenberg, 1831), *Cristatella mucedo* (Cuvier, 1798), *Plumatella fungosa* (Pallas, 1768), *Plumatella repens* (Linnaeus, 1758), *Plumatella emarginata* (Allman, 1844), *Plumatella fruticosa* (Allman, 1844), and *Hyalinella punctata* (Hancock, 1850).

In recent years the number of freshwater bryozoans documented from Europe has grown to 19 (Massard and Geimer 2005, 2008a; Wood and Okamura 2004). Meanwhile from seven to eleven freshwater bryozoan species have been reported in countries neighbouring Lithuania: Latvia, Poland, and Belarus (Kaminski 1984, Fauna Euro-

pea 2013). Based on the species diversity in other European countries, we can expect a more diverse list of bryozoans in Lithuania as well.

The present work describes freshwater bryozoans studied in 18 freshwater bodies in Lithuania.

## Materials and methods

### Climate of Lithuania

Lithuania is distinguished by a highly diverse geography: plains, hills, abundant forests, lakes, wetlands, and Baltic Sea. The climate of the Lithuania can be described as typical European with strong continental influence providing warm summers and fairly severe winters. The weather is often windy and humid due to the proximity of the Baltic Sea.

The average air temperature is 7.2 °C. July is the warmest month with an average temperature of 18 °C. January and February are the coldest months with average temperatures around -3.35 °C, but sometimes winter days can be much colder with temperatures about -32.4 °C. Annual precipitation ranges from 560 to 700 mm. Snow cover can last from 60 to 90 days. The flat landscape retains much of the precipitation, which leads to a relatively high water level (Lithuanian Hydrometeorological Service under the Ministry of Environment).

### Characteristic of sampling sites

Our bryozoan survey was conducted during April through October 2015–2017. We investigated localities that included different types of water bodies: lakes, ponds, lagoons and lotic habitats (streams and rivers). Figure 1 shows regions in Lithuania that were surveyed. Geographical details and descriptions of collecting sites are listed below and summarized in Table 1.

### Region 1

**Pond in Kaunas botanical garden** (located in Kaunas city). Small eutrophic pond with abundant macro- and microalgae. The bottom is sludge. Water pH is 7.89.

**Linksmakalnis pond** (Kaunas district). Large artificial pond, what shorelines are overgrown by *Phragmites* sp. The bottom is sandy. Water pH is 7.18.

**Raudondvaris, Rokai and Tribalė ponds.** All these ponds located in Kaunas district and have similar characteristics: the bottom is sand mixed with sludge, the littoral is overgrown by *Acorus calamus* and *Phragmites* sp. in Raudondvaris and Tribalė ponds. Vegetation on the shores of Rokai pond are rare, water birds are common here.





**Figure 1.** Map of the investigated regions in Lithuania. Triangles mark the approximate locations of collecting sites, which are further identified by name abbreviations (see Table 1).

Water pH varies from 7.51–7.85 (Rokai and Tribalė ponds respectively) to pH 8.35 in Raudondvaris pond.

**Lampėdžiai lake** (located in Kaunas city). Relative large (1,252 km<sup>2</sup>), semi-artificial lake. The bottom is sandy. Shorelines are without the trees, only *Phragmites* sp. occurs occasionally in the littoral. Water pH is 8.06.

**Maišia stream** (located in the outskirts of Kaunas). One side of shore is overgrown by deciduous trees, *Phragmites* sp. and *Typha angustifolia*. Water is polluted by sewage. Water pH is 7.48.

**Veršvio stream** (located in western part of Kaunas city). Small and shallow stream, that dries up in the summer. Shore is lined by trees and shrubs. Bottom is sandy. Water pH is 7.79.

## Region 2

**Šventupė pond** (Ukmergė district). The shoreline is overgrown by shrubs and other vegetation. *Phragmites* sp., *Acorus calamus*, *Lemna minor* dominates in littoral. A small stream enters in one end of pond. Another end of the pond is connected with Šventoji River. Water pH 7.40.

**Mūša pond** (Ukmergė district). Mūšia stream enters in this artificial pond. *Phragmites* sp., *Acorus calamus* and *Nymphaea lutea* occur in the littoral. Water pH is 7.81.

**Table 1.** Summary of collecting sites, their locations, abbreviation of locations names, and the bryozoan species collected. Bryozoan species are expressed by the first letter of the genus followed by the first three letters of the species.

	District	Site and name abbreviation	Coordinates	Species
1	Kaunas City	Pond, Kaunas Botanical Garden, BG	54°52'20.5"N, 23°54'45.4"E	PREP; PFUN; PFRU; PGEI; CMUC
1	Kaunas	Linksmakalnis pond, LP	54°45'31.3"N, 23°55'20.5"E	PREP; PFUN; PFRU; PCAS
1	Kaunas	Raudondvaris pond, RP	54°59'10.7"N, 23°46'12.5"E	PREP
1	Kaunas	Rokai pond, ROP	54°50'01.2"N, 23°57'21.9"E	PREP; PFRU; CMUC
1	Kaunas	Tribalė pond, TP	54°50'23.3"N, 23°51'30.0"E	PREP; PFRU
1	Kaunas	Lampėdžiai lake, LL	54°54'54.3"N, 23°49'29.7"E	PREP; PCAS; PGEI; CMUC
1	Kaunas	Maišia stream, MS	54°49'47.8"N, 23°52'13.4"E	PREP; PFUN; PCAS; PGEI
1	Kaunas	Veršvio stream, VS	54°55'39.8"N, 23°52'09.6"E	PREP; HPUN
2	Ukmergė	Šventupė pond, ŠP	55°19'20.4"N, 24°53'07.4"E	PREP; PFRU; PEMA
2	Ukmergė	Mūša pond, MP	55°18'33.5"N, 24°49'38.6"E	PREP; PFRU; PCAS; PFUN
3	Plungė	Plateliai Lake, PL	56°02'41.9"N, 21°51'35.5"E	PREP; PFRU; PCAS; PGEI
3	Plungė	Skyplaičiai Lake, SL	56°00'46.2"N, 21°56'15.9"E	PREP; PFRU; PFUN; PGEI
4	Trakai	Škilietai Lake, ŠL	54°37'01.5"N, 24°30'33.5"E	PREP; PFUN; PFRU;
4	Vilnius and Trakai	Elektrėnai Reservoir, EL	54°45'34.4"N, 24°40'16.5"E	PREP; PFUN; PCAS; PGEI; CMUC
4	Trakai and Kaunas	Strėva river, SR	54°35'03.8"N, 24°41'57.4"E	PREP; PCAS; PGEI
5	Utena	Saterečius pond, SP	55°39'55.7"N, 25°42'00.3"E	PREP; PFRU; PGEI
5	Utena	Žvirgždelis Lake, ŽL	55°42'02.1"N, 25°41'34.9"E	PREP; PFRU
6	Lazdijai	Snaigynas Lake, SNL	54°05'35.4"N, 23°44'03.0"E	PREP; PFRU; CMUC; PART

### Region 3

**Plateliai Lake** (Plungė district) is the large lake covering about 12 km<sup>2</sup> with a maximum depth of 47 m. Water is contributed by seventeen small streams. The Bottom is sandy in the collecting sites.

**Skyplaičiai Lake** (Plungė district) covers 0.068 km<sup>2</sup> and is surrounded by a mixed deciduous forest. The bottom is muddy; shorelines are overgrown by *Phragmites* sp. According to the EU Habitats Directive, this lake is notable for its Charophyta communities.

## Region 4

**Škilietai Lake** (Trakai district) covers about 0.033 km<sup>2</sup> with maximum depth of 12 m. The lake is surrounded by pine forest.

**Elektrėnai Reservoir** (Vilnius district and Trakai district) is the third largest artificial lake in Lithuania. The reservoir measures about 0.0126 km<sup>2</sup>. The lake is fed by inflows from the Strėva River, and nine other rivulets.

**Strėva River** (Trakai and Kaunas district). Average current velocity is 0.1–0.3 m/s. The bottom is sandy mixed with silt. *Phragmites* sp. and *Nymphaea lutea* grow at the edges of the river. Water pH was 7.80 in the sampling site.

## Region 5

**Saterečius Pond** (Utena district). The pond is surrounded by marsh and mixed deciduous forest dominated by *Alnus* sp. In summer the pond is almost overgrown with macro-algae and such macrophytes as *Nymphaea lutea* and *Stratiotes aloides*. Water pH is 6.78.

**Žvirgždelis Lake** (Utena district) covers an area of 0.027 km<sup>2</sup>; the bottom is silt and *Phragmites* sp. dominates in littoral. Water pH is 7.03.

## Region 6

**Snaigynas Lake** (Lazdijai district). The lake covers an area of 2 km<sup>2</sup>, with an average depth of 3 m. The shores are low and overgrown by shrubs and trees. The lake bottom is sandy in littoral. A small shallow stream flows out from this lake into Trikojis Lake.

## Sampling and observations

Statoblasts were taken by net from the surface of water and aquatic plants. Bryozoan colonies were collected from submerged branches, stones, and aquatic plants in the littoral of the water bodies. Statoblasts and bryozoan colonies were stored in 70% ethanol.

Identification of most species was based on morphology of statoblasts and colony (when colonies were available) using light and scanning electron microscopy (SEM) (Hitachi S-3400N).

Statoblasts characters included overall length and width, length and width of the fenestrae, and surface micro-sculpture of statoblasts. Abbreviations used for measurements are as follows:

**L/W** ratio of the statoblast,  
**VfL** ventral fenestra length;  
**VfW** ventral fenestra width;

**DfL** dorsal fenestra length;  
**DfW** dorsal fenestra width.

Measurements were taken from SEM images with software Original Hitachi S-3400N Scanning Electron Microscope software ver 7.3.

Statoblasts were rinsed with distilled water several times then treated by KOH in order to remove any debris and cleaned using vortex for a few minutes. Statoblasts were prepared for scanning electron microscopy by simple drying without sputtering. The identification keys by Wood and Okamura (2005) were used.

### Material examined

The authors collected specimens during April through October 2015–2017. In total, 53 statoblasts and 8 colonies collected from 18 localities in Lithuania were examined. In addition, some data collected by Bronė Pajedaitė (1932–1934) were included in this study for comparison. The representative specimens are deposited in the zoological collection of Biology Department of Vytautas Magnus University.

### Results

The survey of 18 water bodies yielded nine species of freshwater bryozoans (Table 1). Eight of these are classified with the Class Phylactolaemata: *Cristatella mucedo*, *Hyalinella punctata*, *Plumatella casmiana* Oka, 1907, *Plumatella fungosa*, *Plumatella fruticosa*, *Plumatella geimermassardi* Wood & Okamura, 2004 *Plumatella repens*, and *Plumatella emarginata*. The ninth species, *Paludicella articulata*, belongs to the Class Gymnolaemata.

### Taxonomy

**Class Phylactolaemata Allman, 1856**

**Order Plumatellida Allman, 1856**

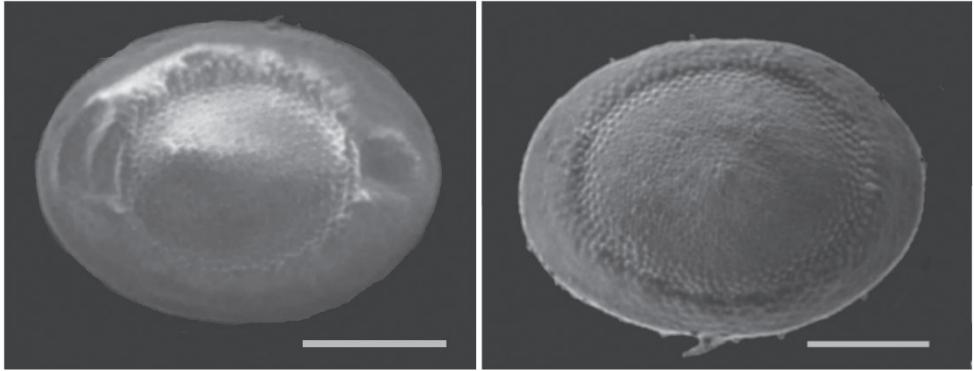
**Family Plumatellidae Allman, 1856**

***Plumatella repens* (Linnaeus, 1758)**

Fig. 2

**Material examined.** Ten floatoblasts collected from ponds of Kaunas Botanical garden, Raudondvaris pond in April 2015, and Skyplaičiai lake, collected in June 2015; colonies collected from Raudondvaris and Rokai pond in June 2015 and July 2016 respectively. Sessoblasts were not found.

**Description.** Colonies were about 5–8 cm size. The transparent branches of colonies were attached to the substratum for almost whole of their length. Floatoblasts were identified by the broadly oval shape and the absence of tubercles on the statoblast



**Figure 2.** *Plumatella repens*. Scanning electron micrograph of floatoblasts showing dorsal valve (left) and ventral valve (right). Scale bars: 200  $\mu\text{m}$ .

annulus (Fig. 2). Floatoblasts were 315–341 ( $325 \pm 3$ )  $\mu\text{m}$  long by 226–270 ( $252 \pm 4$ )  $\mu\text{m}$  wide; L/W ratio was 1.3; VfL 144–245 ( $187 \pm 14$ )  $\mu\text{m}$ ; VfW 126–212 ( $168 \pm 10$ )  $\mu\text{m}$  ( $n=10$ ); DfL 135–258 ( $178 \pm 14$ )  $\mu\text{m}$ ; DfW 126–212 ( $163 \pm 9$ )  $\mu\text{m}$  ( $n=10$ ). Fenestra of floatoblasts circular, covered with rounded tubercles that become less prominent towards the centre of fenestra. The annular nodules often described for this species have not yet been observed in Lithuanian material.

**Distribution in Europe.** According to Økland and Økland (2005), Wood and Okamura (2005), *P. repens* is common in Britain, Ireland and Europe. Kaminski (1984) described *P. repens* as most common species in the studied lakes in Poland.

**Remarks on habitat and ecology in Lithuania.** *Plumatella repens* has been the most commonly encountered species, with floatoblasts occurring in all surveyed sites, include lentic and stagnant habitats. Although colonies were found in only two ponds from listed sites, we have since become aware of colonies occurring in other lakes and ponds not listed here.

Pajiedaitė (1933) described *P. repens* as most common species in Lithuania, which can grow in various freshwater bodies. On the other hand, the exact locations of her collecting sites were not listed in her thesis. In addition, because of early difficulty in identifying this species, distribution reports prior to the mid-1980s are not necessarily reliable (Wood and Okamura 2005).

**Remarks.** Lacourt (1968) postulated close relationship between *Plumatella repens* and *P. fungosa* based on a “short oval statoblasts” and molecular studies confirmed a close relationship between these species (Hirose et al. 2011). *Plumatella repens* can be confused with young colonies of *P. fungosa* (Wood and Okamura 2005). In addition, statoblasts of *P. repens* are similar to those of its congeners *P. nitens* Wood, 1996, *P. nodulosa* Wood, 2001, *P. orbisperma* (Kellicott, 1882), *P. reclusi* Smith, 1992, and *P. rugosa* Wood, Wood, Geimer & Massard, 1998 (Massard and Geimer 2008a).



***Plumatella geimermassardi* Wood & Okamura, 2004**

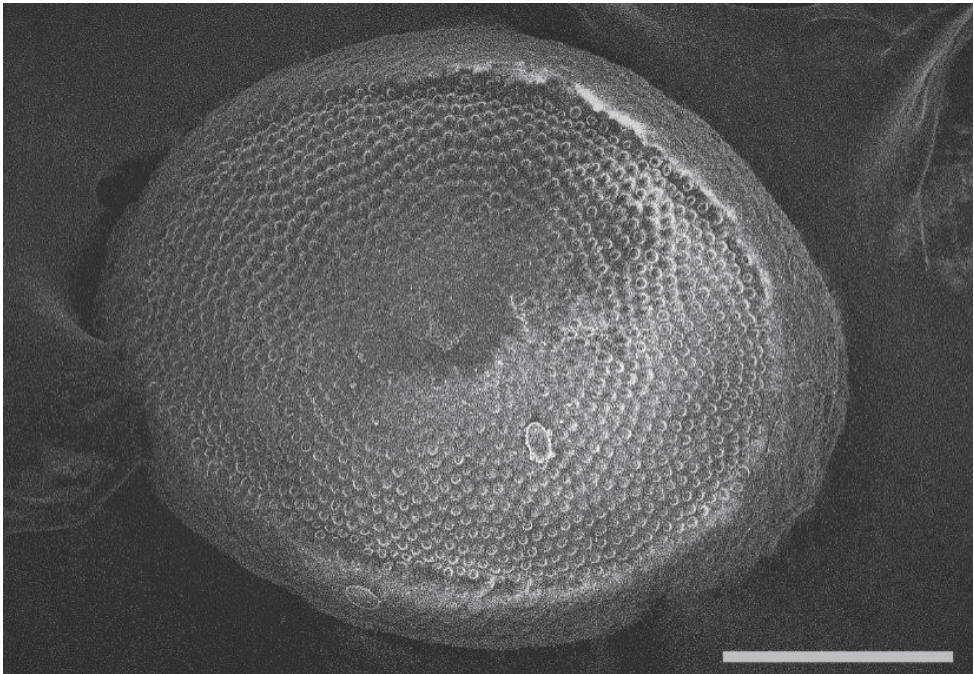
Fig. 3

**Material examined.** A few floatoblasts from Lampėdžiai Lake in April 2016. *P. geimermassardi* were recorded in Lithuania for the first time. However, the species is so far represented only by statoblasts.

**Description.** Floatoblasts were identified by the large dorsal fenestra with tubercles and narrow annulus. The annulus at the poles is mostly as large as laterally and is covered by weakly visible tubercles (Fig. 3). Length and width of floatoblast were 311–325 ( $317 \pm 4$ )  $\mu\text{m}$  and 221–273 ( $244 \pm 15$ )  $\mu\text{m}$  ( $n=3$ ) respectively. L/W ratio 1.3; DfL 199–205 ( $202 \pm 3$ )  $\mu\text{m}$ ; DfW 174–201 ( $187 \pm 13$ )  $\mu\text{m}$  ( $n=3$ ); VfL 200–254 ( $227 \pm 26$ )  $\mu\text{m}$  and VfW 185–198 ( $192 \pm 6$ )  $\mu\text{m}$  ( $n=3$ ).

**Distribution in Europe.** *Plumatella geimermassardi* is known from England, Ireland, Belgium, southern Norway, northern Germany, Italy and Finland (Wood and Okamura 2005).

**Remarks.** Floatoblasts of *P. geimermassardi* are among the smallest floatoblasts among all European plumatellids with an average length of around 320  $\mu\text{m}$  (Wood and Okamura 2004). The uniformly narrow annulus offers an easy identifying feature characteristic for broad floatoblasts in this species. The relatively large area of dorsal and ventral fenestrae is matched only by those of *P. nitens* or *Stephanella hina* on other continents (Wood 1996; Toriumi 1955).



**Figure 3.** *Plumatella geimermassardi*. Scanning electron micrograph of the floatoblast ventral valve showing the uniformly narrow annulus. Scale bar: 100  $\mu\text{m}$ .

***Plumatella fungosa* (Pallas, 1768)**

Fig. 4

**Material examined.** A floatoblasts collected from Linksmakalnis pond (June 2015) and Maišia stream (April 2015). Colony from Aristava pond (locates in Kėdainiai district 55°17'07.1"N, 24°04'28.6"E and it is not included in general list of studied sites during this survey) was taken in June 2017 (Fig. 4).

**Description.** The colony dark, spindle shaped, and large (15–17 cm), formed on stems of reeds (*Phragmites*). Examined floatoblasts exhibited characteristic tubercles on the floatoblast annulus (Fig. 4) and a ridge-like suture between the dorsal and ventral valves. Dorsal floatoblast tubercles were larger on the fenestra than on the annulus. The length of floatoblasts was 324–368 (339±5) µm; width 220–290 (254±8) µm (n=8), L/W ratio 1.3; DfL 130–160 (147±4) µm (n=6); DfW 125–161 (144±5) µm (n=6); VfL 214–250 (227±4) µm and VfW 205–228 (214±2) µm (n=6). However, dimensions of *P. fungosa* floatoblasts provided by Pajiedaitė (1933), were slightly larger: 470 µm × 290 µm. Pajiedaitė also recorded the variability in sessoblast dimensions from different localities: 790 µm × 470 µm in Nevėžis river (Kaunas district); 480 µm × 370 µm in Lake Aukštadvaris (Trakai district), and 580 µm × 420 µm in Snaigynas Lake (Lazdijai district) (Pajiedaitė 1933). During current study sessoblasts were not found.

**Distribution in Europe.** According to Wood and Okamura (2005) *P. fungosa* is widespread in Europe. It has been recorded from several places in southern Sweden and Finland; it is common in Denmark and has been reported from Iceland (Økland and Økland 2005) and Poland (Kaminski 1984).

**Remarks on habitat and ecology in Lithuania.** During this survey, floatoblasts of *P. fungosa* were found in seven water bodies from 18 surveyed, with prevalence in stagnant water, with neutral to slightly alkaline pH 7.01–8.15 (Table 1). Pajiedaitė (1933) described the colonies in Kaunas Lagoon, Nevėžis River (Kaunas district) and Dubysa River (Šiauliai district). She noted that *P. fungosa* often occurred in polluted water and described colonies, found in old port of Kaunas city, where water was polluted by oil of ships and trash. A similar observation has been made by other authors (Bushnell 1966; Geimer and Massard 1986). Based on the available data, we consider *P. fungosa* to be prevalent in Lithuania.

**Remarks.** Large bulky colonies of *P. fungosa* are easily recognizable freshwater bryozoan species in Europe (Wood and Okamura 2004). Floatoblasts of *P. fungosa* are laterally asymmetrical and distinct from the symmetrical floatoblasts of *P. repens* and *P. rugosa*. Molecular studies showed a close relationship between *P. repens* and *P. fungosa* (Hirose et al. 2011).

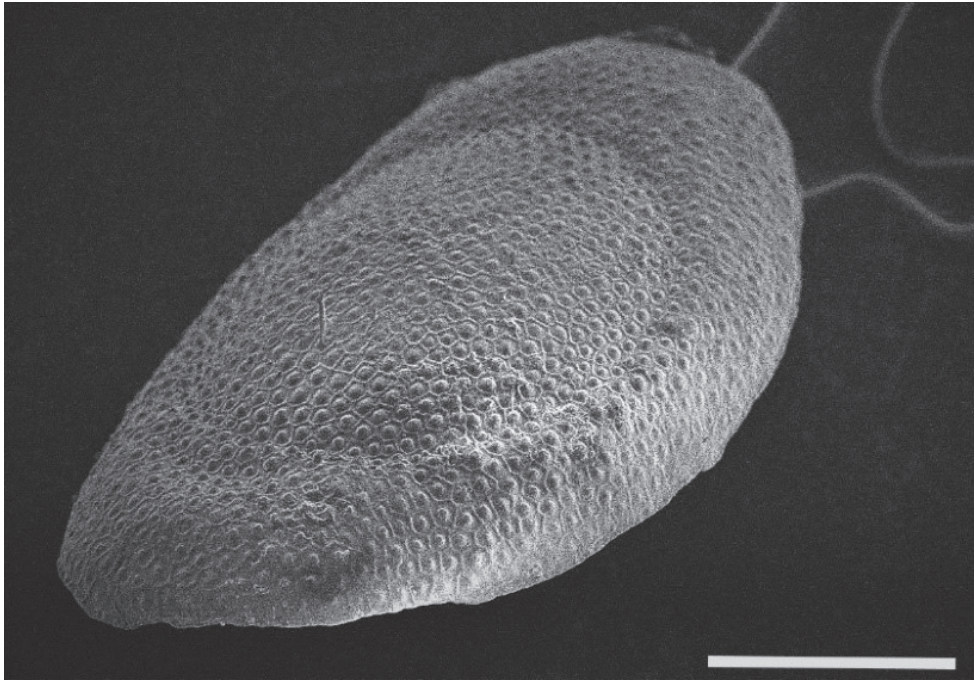
***Plumatella emarginata* (Allman, 1844)**

Fig. 5

**Material examined.** A few floatoblasts from Šventupė pond were collected in July 2016.

**Description.** Floatoblasts elongated in shape, with a circular ventral fenestra and small dorsal fenestra, covered by tubercles. Floatoblasts were 357–489 (407±18) µm





**Figure 4.** *Plumatella fungosa*. Scanning electron micrograph showing characteristic tubercles on the floatoblast annulus. Scale bar: 100  $\mu\text{m}$ .

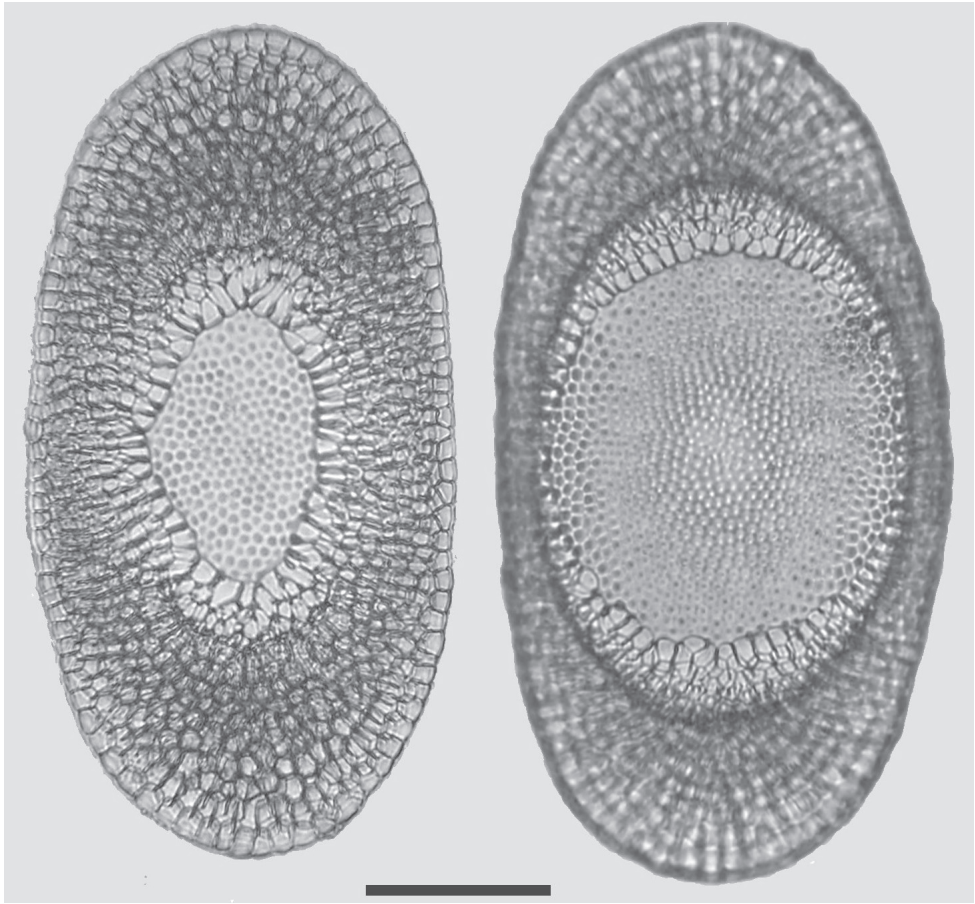
long and 197–235 ( $216 \pm 6$ )  $\mu\text{m}$  ( $n=6$ ) wide, L/W ratio 1.9; DfL 97–125 ( $107 \pm 9$ )  $\mu\text{m}$ ; DfW 60–82 ( $68 \pm 7$ )  $\mu\text{m}$  ( $n=3$ ); VfL 101–184 ( $149 \pm 9$ )  $\mu\text{m}$  and VfW 110–162 ( $133 \pm 4$ )  $\mu\text{m}$  ( $n=6$ ). The approximate size of statoblasts provided by Pajiedaitė (1933) was 560  $\mu\text{m}$  long and 260  $\mu\text{m}$  wide.

**Distribution in Europe.** Geimer and Massard (1986) defined the range of this species to include most of Europe. Økland and Økland (2005) considered *P. emarginata* to be a southern species, with limited distribution in Norway and Sweden.

**Remarks on habitat and ecology in Lithuania.** Pajiedaitė (1933) described morphology of *P. emarginata* colonies, but her text is not clear about the location of collection sites. However, the statoblasts she found were recorded from Paštys Lake ( $55^{\circ}42'36''\text{N}$ ,  $25^{\circ}41'48''\text{E}$ ), Satarečius pond and Dubysa River (Kaunas district,  $55^{\circ}12'12''\text{N}$ ,  $23^{\circ}30'28''\text{E}$ ).

In our survey only a few statoblasts were found in Šventupės pond (Table 1). Wood and Okamura (2005) noted that *P. emarginata* is particularly tolerant of rapidly-flowing water. The occurrence of floatoblasts in the Neris River (Kaunas district - not included in this study) is consistent with this observation, although colonies were not found. From our data *P. emarginata* would be considered uncommon in Lithuania, although this should be verified through further surveys.

**Remarks.** The species is widely distributed throughout the Holarctic (Wood and Okamura 2005), although some reports may have confused it with similar species, *P. mukaii* or *P. reticulata* (Massard and Geimer 2008a).



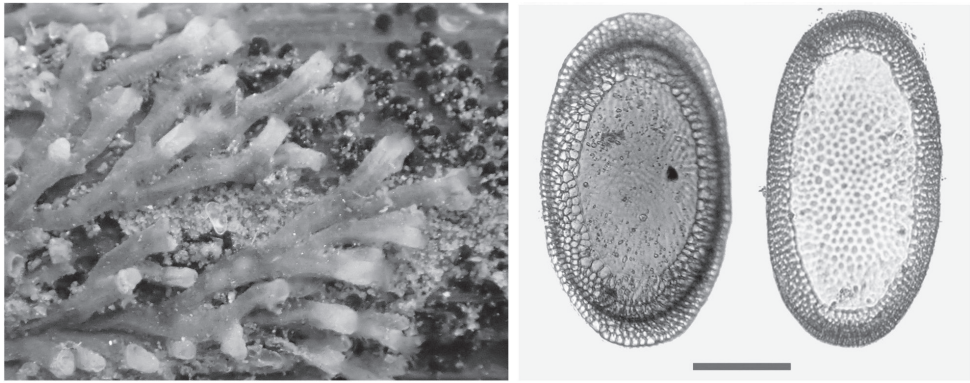
**Figure 5.** *Plumatella emarginata* floatblast valves. The dorsal valve (left) has a characteristically small central fenestra; in the ventral valve (right) the fenestra is nearly circular. Scale bar: 100  $\mu\text{m}$ .

***Plumatella casmiana* (Oka, 1908)**

Fig. 6A, B

**Material examined.** Floatoblasts, leptoblasts, and colony from Linksmakalnis pond collected from submerged branches in 20 July 2016.

**Description.** Colony was about 5–6 cm long. Branches of colony are short, almost entirely attached to the substrate. The terminal parts of branches are semi-transparent and whitish. The floatoblasts were recognized by the distinctly elongated shape of the fenestra on both valves. Both capsuled floatoblasts and the distinctive leptoblasts were found, along with associated colonies (Fig. 6A, B). The surface fenestra of capsuled floatoblasts was almost smooth. Length of floatoblasts 345–432 ( $397 \pm 15$ )  $\mu\text{m}$ ; width 188–260 ( $214 \pm 14$ )  $\mu\text{m}$ ; L/W ratio 1.8; DfL 112–198 ( $154 \pm 15$ )  $\mu\text{m}$ ; DfW 90–135 ( $113 \pm 7$ )  $\mu\text{m}$ ; VfL 174–236 ( $205 \pm 12$ )  $\mu\text{m}$ ; VfW 150–195 ( $167 \pm 8$ ) ( $n=5$ ). Leptoblasts



**Figure 6.** *Plumatella casmiana*. **A** Portion of a colony showing crowded zooids almost entirely attached to the substratum **B** Dorsal valve of floatoblast (left) and leptoblast (right). Scale bars: 1 mm (**A**), 100  $\mu\text{m}$  (**B**).

(Fig. 6B, right side) have a uniformly narrow annulus and extensive oval fenestrae; which length was at least 1.5 times its width.

**Distribution in Europe.** *Plumatella casmiana* is currently known throughout most of Europe (Massard and Geimer 1995b).

**Remarks on habitat and ecology in Lithuania.** This is the first reported occurrence of *P. casmiana* in Lithuania. Floatoblasts of *P. casmiana* were recorded in almost half of the investigated water bodies (Table 1). However, colonies were found in Linksmakalnis Pond only.

**Remarks.** Beyond Europe *P. casmiana* is widely distributed through Asia, North America, Africa, and very likely other continents as well (Wood and Okamura 2005). A unique feature is the appearance of floatoblasts lacking the inner capsule (Figure 6B, right side). This so-called leptoblast is capable of hatching immediately after release from the colony, enabling populations to grow very rapidly each season. Colonies also produce conventional capsuled floatoblasts (Figure 6B, left side) which retain the obligatory dormancy period.

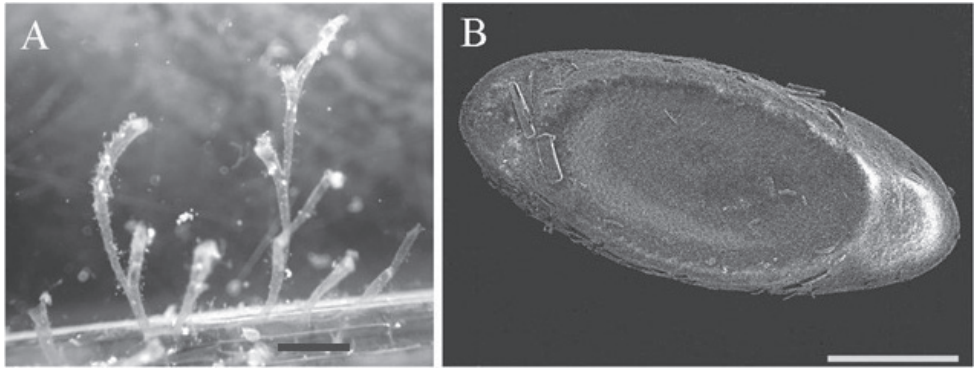
### *Plumatella fruticosa* (Allman, 1844)

Fig. 7A, B

**Material examined.** Colony from Rokai pond (Kaunas district) found in June 2016; floatoblasts from pond of Kaunas Botanical garden collected in July and August 2016.

**Description.** The colony measured approximately 3 x 4 cm and had sparse, narrow and upright branches. Free statoblasts are long and narrow, exhibiting a length at least twice the width: 432–496 ( $459 \pm 8$ )  $\mu\text{m}$  long and 187–220 ( $203 \pm 4$ )  $\mu\text{m}$  ( $n=10$ ) wide; L/W ratio 2.2; DfL 120–320 ( $197 \pm 19$ ) ( $n=10$ )  $\mu\text{m}$ ; DfW 56–100 ( $75 \pm 6$ ) ( $n=6$ )  $\mu\text{m}$ ; VfL 211–313 ( $266 \pm 21$ ) ( $n=4$ )  $\mu\text{m}$  and VfW 74–128 ( $108 \pm 17$ ) ( $n=3$ ). Sessoblasts were not found during this study. According to Pajiedaitė (1933) the average size of the floatoblasts was 590  $\mu\text{m}$  long and 230  $\mu\text{m}$  wide.





**Figure 7.** *Plumatella fruticosa*. **A** Colony showing upright zooids and branches **(B)** Scanning electron micrograph showing floatoblast ventral valve with characteristic long, narrow shape. Scale bars: 3 mm **(A)**; 200  $\mu$ m **(B)**.

**Distribution in Europe.** *Plumatella fruticosa* is considered to be widespread, especially in northern portion of Europe (Økland and Økland 2005). It is considered common in Poland (Kaminski 1984).

**Remarks on habitat and ecology on Lithuania.** Pajiedaitė collected colonies in Dubysa river (Šiauliai district) and Satarečius pond (Utena district) (Pajiedaitė 1933). At first glance we could state that *P. fruticosa* is common in Lithuania, since during this survey statoblasts were found in most water bodies. However, we found colonies only in Rokai pond with sandy-mud bottom and stones in the littoral (Table 1, Fig. 7). Thus, it is possible, that statoblasts are spread by waterfowl among various ponds and lakes, but these may not be the preferred environment for growing colonies (Økland and Økland 2005).

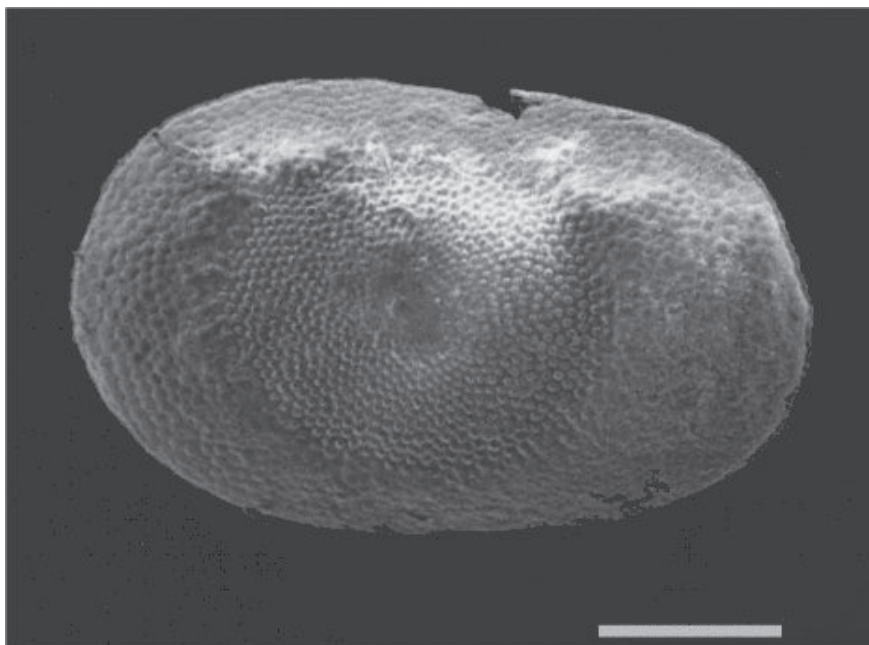
**Remarks.** The combined statoblast characteristics (large length/width ratio, strong asymmetry of floatoblast and sessoblast, narrow fenestra on dorsal floatoblast valve) distinguish *P. fruticosa* from all other plumatellid species (Ricciardi and Reising 1994). Molecular results provided by Hartikainen (Hartikainen et al. 2013) imply that *P. fruticosa* is not a plumatellid and provide evidence for polyphyly in *Plumatella*. However, the position of *P. fruticosa* remains unresolved (Hartikainen et al. 2013).

### *Hyalinella punctata* (Hancock, 1850)

Fig. 8

**Material examined.** A few statoblasts from Veršvio stream were found in August 2015. Unfortunately, these were later lost before critical dimensions could be taken.

**Description.** Colonies were not observed, and species was identified according floatoblasts. The statoblasts are larger than any other plumatellid species and show crowded tubercles on the fenestrae of both valves. Pajiedaitė (1933) described floatoblasts by oval shape, with length 440  $\mu$ m and width 230  $\mu$ m. These dimensions were slightly smaller than 500  $\mu$ m and 350  $\mu$ m suggested by Wood and Okamura (2005).



**Figure 8.** *Hyalinella punctata*. Scanning electron micrograph showing the floatoblast ventral valve. Scale bar: 300  $\mu\text{m}$ .

Length and width of measured statoblasts during current study was 425–459 ( $444 \pm 7$ ) and 280–299 ( $290 \pm 4$ )  $\mu\text{m}$ , respectively ( $n=5$ ).

**Distribution in Europe.** *Hyalinella punctata* has been widely reported worldwide, including neighbouring Poland (Kaminski 1984), but verified specimens are known only from Britain, Ireland, Europe, North America and northern Asia (Wood and Okamura 2005).

**Remarks on habitat and ecology in Lithuania.** Few small colonies of *H. punctata* were described on *Nymphaea lutea* leaves in small lakes in the Zarasai district ( $55^{\circ}44'50''\text{N}$ ,  $25^{\circ}50'4''\text{E}$ ) and Dubysa river (Šiauliai district;  $55^{\circ}51'29''\text{N}$ ,  $23^{\circ}08'31''\text{E}$ ) by Pajiedaitė (1933). During the present survey, floatoblasts of *H. punctata* were recorded only in the Veršvio stream (Table 1). The available data are not sufficient to estimate the prevalence and frequency of this species in Lithuania.

**Remarks.** Hancock (1850) described colonies of *H. punctata* as “thick and transparent with less profuse branching than in *Plumatella* and produce only floatoblasts, while individual zooids are indistinct, usually arranged linearly and lack interzooidal septa”. In fact, features distinguishing *Hyalinella* from *Plumatella* are not clear-cut (Hirose and Mawatari 2011), because the diagnosis of *Hyalinella* is based on the transparency and thickness of the colony wall (ectocyst), but the condition of the ectocyst depends to some extent on environmental factors (Wood and Okamura 2005, Hirose and Mawatari 2011). Generic placement of some species between *Plumatella* and *Hyalinella* has remained unstable (Hirose and Mawatari 2011).

**Family Cristatellidae Allman, 1856*****Cristatella mucedo* (Cuvier, 1798)**

Fig. 9

**Material examined.** Colony from Snaigynas lake (Lazdijai district) collected in July 2016, floatoblasts from Rokai pond found in September 2016.

**Description.** Colonies of *C. mucedo* are recognized by their elongated shape and colourless, transparent body wall. The length of colonies found varied from 5 to 10 cm (Pajiedaitė 1933; this study). The large statoblasts are easily recognized by circular form with hooked spines radiating from the edges of the fenestrae on both valves (Fig. 9). Diameter of statoblasts was about 1 mm.

**Distribution in Europe.** *Cristatella mucedo* is a common species in Lithuania, with a Holarctic distribution, occurring in Britain, Ireland, Europe, Asia and North America (Økland and Økland 2000; Wood and Okamura 2005).

**Remarks on habitat and ecology in Lithuania.** During this survey a few colonies of *C. mucedo* occurred in South Lithuania (Snaigynas lake), but statoblasts were found in various water bodies of different regions of the country (Table 1). Pajiedaitė (1933) noted that *C. mucedo* more often occurred in South Lithuania. She found colonies of *C. mucedo* without statoblasts in June/July and noted that statoblasts inside colonies appeared in first part of August. Numerous colonies with statoblasts were found at the end of September and they died late autumn once the water temperature dropped to 3 °C in November 1932 (Pajiedaitė 1933).

**Remarks.** A more detailed discussion of the ecology and life history of *C. mucedo* can be found in Okamura (1997).

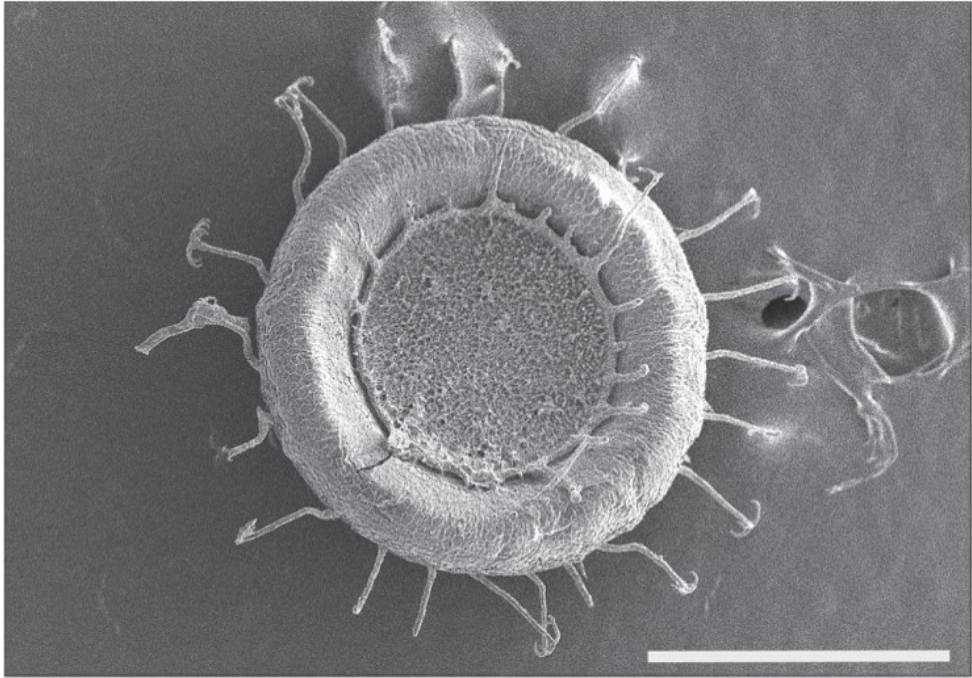
**Class Gymnolaemata Allman, 1856****Order Ctenostemata Busk, 1852****Family Paludicellidae Allman, 1844*****Paludicella articulata* (Ehrenberg, 1831)**

Fig. 10

**Material examined.** Two colonies from the outlet of Snaigynas Lake (Lazdijai district) were found in May 2017. Hibernaculae were not found.

**Description.** The species was recognized by the slender colony branches forking at wide angles and often growing free from the substratum. Colonies were small, about 2–3 cm. Branches of colony were transparent and shiny. Zooids 1.0–1.5 mm in length with 16 tentacles on a circular lophophore were described by Pajiedaitė (1933).

**Distribution in Europe.** *Paludicella articulata* is known worldwide (Wood and Okamura 2005). However, the species has not been found in Poland (Kaminski 1984).



**Figure 9.** *Cristatella mucedo*. Scanning electron micrograph showing floatoblast with characteristic spines. Scale bar: 500  $\mu$ m.

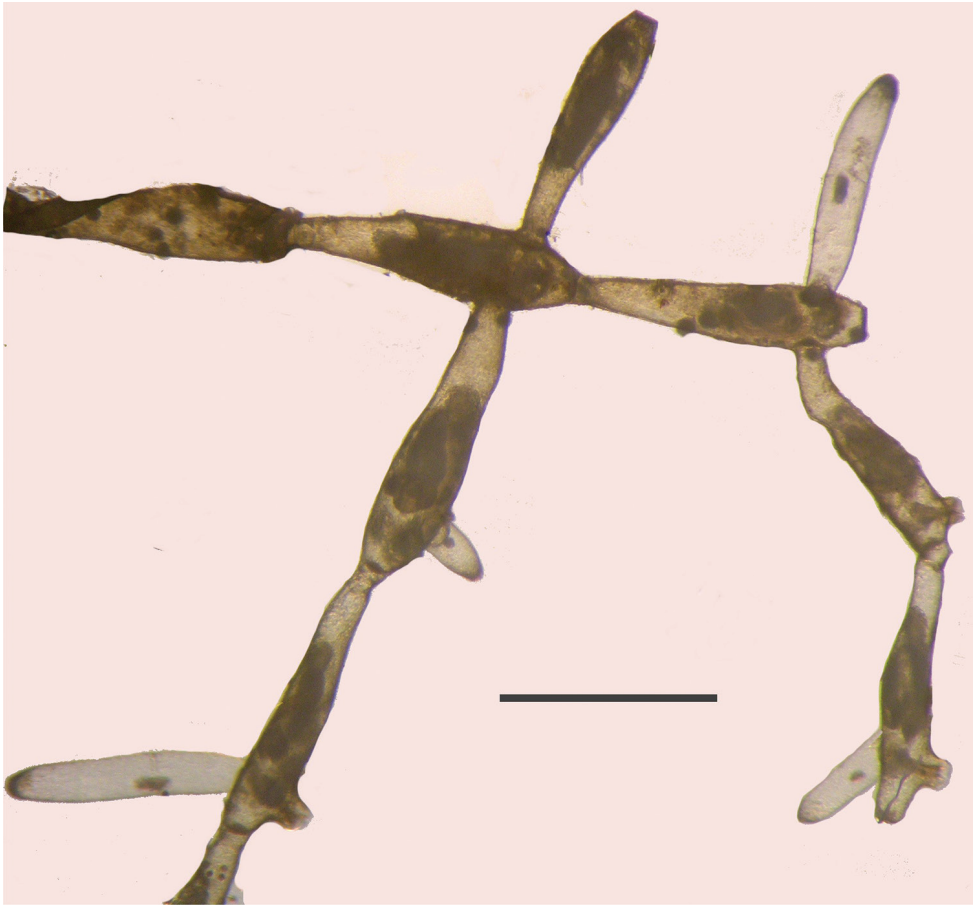
**Remarks on habitat and ecology in Lithuania.** *Paludicella articulata* was recorded by Pajiedaite (1933) in only two localities: Paštys Lake (Utena district) (55°42'36"N, 25°41'48"E) and Satarečius pond (Utena district). Since *P. articulata* tolerates cold temperatures (Økland and Økland 2005) and prefers flowing water (Wood and Okamura 2005) it was surprising finding of this species in stagnant Satarečius pond together with *C. mucedo*. Coexistence of the two species was also noted by Pajiedaitė (1933), who explained it by different local conditions in the same pond; colonies of *P. articulate* were observed only near a small stream flowing into the pond. Otherwise, she noted that *C. mucedo* was mostly observed in the warmer waters of Central and South Lithuania.

Økland and Økland (2005) showed positive co-occurrence of these two species in Norway.

During this survey *P. articulata* was found in the outlet of Snaigynas lake, which is of glacial origin and characterised by low temperature.

**Remarks.** Colonies of *P. articulata* consist of sometimes creeping but more often elongated, mostly erect, slender zooids. There are normally three adjacent zooids: one distal and two lateral ones (Davenport 1891) The contiguous arrangement of the zooids and the subterminal 4-sided zooecial orifice readily distinguish the species from its closest relative, *Pottsiella erecta* (Ricciardi and Reiswig 1994).





**Figure 10.** Fragment colony of *Paludicella articulata*. Scale bar: 1 mm.

## Discussion

Overall nine species of freshwater bryozoans are now known from Lithuania. This contrasts with about 19 species reported from Europe and about 13 species from the Baltic area (Massard-Geimer and Massard-Geimer 2004; Nikulina 2006). Given the fact that only a relatively few water bodies of Lithuania have been investigated so far it is likely that the final tally of species will be higher.

The majority of surveyed pools were stagnant, neutral or slightly alkaline (Table 1) and should have been suitable for bryozoans to grow successfully. However, while intact colonies were found only in few sites, statoblasts were widely distributed. The rarity of colonies in water pools might be explained by fluctuating climatic conditions, especially the alternation of drought and rainfall. The similar process under Lithuanian conditions was described by Pajiedaitė (1933), who found

*P. articulata* and *P. repens* colonies in Paštys Lake in 1931, but completely absent the following year. Pajiedaitė (1933) suggested this disappearance may have been due to rising of water level after rainfall in Paštys Lake. She wrote that bryozoans are sensitive “creatures” and cannot survive such drastic environmental change. She went on to describe a similar situation in Nevėžis River in 1932, where after week of rainfall, nearly all bryozoan colonies had died (Pajiedaitė 1933). Jong-Yun-Choi et al. (2015) documented the negative effects of heavy rainfall in Korea on colonies of *Pectinatella magnifica* (Leidy, 1851).

Another possible reason of finding small number of colonies could be the lack of suitable substratum for the attachment of colonies. Because bryozoans are sessile organisms, they absolutely require a solid, inert substratum on which to grow (Ryland 1970). For example, we have found statoblasts of five bryozoan species in a pond at the Kaunas botanical garden (Table 1). However, colonies have never been observed there, possibly due to lack of solid substratum to which bryozoan colonies can attach. The presence of statoblasts could be the result of waterfowl, which are known to transport them from one site to another (Wood and Okamura 2005).

With this study, we have now recorded 13 species of freshwater bryozoans recorded in Baltic area: *C. mucedo*, *P. magnifica*, *Fredericella indica* Annandale, 1909, *Fredericella sultana* (Blumenbach, 1779), *Lophopus crystallinus* (Pallas, 1768), *H. punctata*, *P. casmiana*, *P. emarginata*, *P. fruticosa*, *P. fungosa*, *P. geimermassardi*, *P. repens*, *Stolella indica* Annandale, 1909, and *P. articulata* (Massard-Geimer and Massard-Geimer 2004; Nikulina 2006).

All bryozoan species documented in Lithuania are common and widely distributed through Europe. The composition of species found through this survey was similar to that recorded 86 years ago, with the exception of finding two additional species for Lithuania. The status of *P. repens* and *P. fungosa* as common freshwater bryozoan species, and *P. articulata* as rare, have not changed for almost a century.

Curiously, this survey did not encounter *F. sultana*, which is otherwise well known in northern Europe, Britain and Ireland (Geimer and Massard 1986; Wood and Okamura 2005). Also absent was the large gelatinous species, *P. magnifica*, which is currently expanding its range across Europe and is already reported from areas including Hungary (Szekeres et al. 2013), Germany (Grabow 2005), Czech Republic (Rodriguez and Vergon 2002; Balounova et al. 2011), and Poland (Balounová et al. 2011). Additional bryozoan species that might be expected in Lithuania include *L. crystallinus*, which is recorded in neighbouring countries as Belarus, Kaliningrad and Poland (Nikulina 2006); also, *P. rugosa*, *P. reticulata* Wood, 1988, and *F. indica*. Since the brackish species, *Victorella pavidula* Saville Kent, 1870, is known in neighbouring Latvia, it is likely to be found also in Lithuania.

In summary, we believe that the list of freshwater bryozoa presented here is not final. Lithuania is an extremely watery region; there are more than 3000 lakes of a wide variety of sizes and many rivers flow across the country. Therefore, it is very likely, that further research will reveal additional species.

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# *Labiobaetis* from the Kingdom of Saudi Arabia (Insecta, Ephemeroptera, Baetidae)

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## Abstract

Mayfly larvae and imagoes were collected at approximately 50 localities of the Kingdom of Saudi Arabia (KSA). Included in this material, three species of *Labiobaetis* Novikova & Kluge, 1987 are recorded, two of them being new to science. *Labiobaetis potamoticus* Gattolliat & Al Dhafer, **sp. n.** is described from both larvae and adults, whereas *Labiobaetis alahmadii* Gattolliat & Al Dhafer, **sp. n.** is only known from the larval stage. The two species are compared morphologically with Palearctic and Afrotropical species of *Labiobaetis*. A third species, *Labiobaetis glaucus* (Agnew, 1961) is reported for the first time from the Arabian Peninsula. The species was originally described from South Africa and subsequently reported from the east and northeast of Africa. A molecular reconstruction including 18 Afrotropical and Palearctic species of *Labiobaetis* was performed using 658 bp of the mitochondrial gene CO1. The reconstruction highly supported the validity of the two new species and confirmed the occurrence of *L. glaucus* in KSA.

## Keywords

Arabian Peninsula, mayflies, new species, new records

## Introduction

*Labiobaetis* Novikova & Kluge, 1987 is a species-rich genus with an almost worldwide distribution (only absent in Neotropical Region); it is mainly diversified in Afrotropical (28 species) and Oriental realms (23 species) (Gattolliat and Nieto 2009). The status and validity of the genus has often been the subject of controversy during the last two decades (Fujitani 2008; Fujitani et al. 2003; Gattolliat 2001; Gattolliat and Staniczek 2011; Kluge 2012; Kluge and Novikova 2016; Kubendran et al. 2015; Lugo-Ortiz and McCafferty 1997; Lugo-Ortiz et al. 1999). Molecular reconstructions indicated that the concept of *Labiobaetis* is most probably at least diphyletic (Gattolliat et al. 2008; Monaghan et al. 2005). Larvae of *Labiobaetis* generally colonize the more lentic portion of streams and rivers with rich aquatic vegetation where it can be the most abundant mayfly.

The distribution of the genus in Arabian Peninsula seems restricted to the southwestern Kingdom of Saudi Arabia (KSA) and Yemen despite potential suitable habitats present in Oman, Jordan and the United Arab Emirates (Gattolliat and Sartori 2008; Gattolliat et al. 2012).

Relatively little information is available on the freshwater habitats and faunas of the Arabian Peninsula and, in particular, of the KSA. The vast landscape of KSA is one of the driest and hottest countries in the world and has almost no runoff, surface water or perennial rivers (Alshareef 1995, Alkolibi 2002, Al-Rashed and Sherif 2000). Other than the Midwestern Region and the southwestern mountains, the average annual precipitation in KSA ranges from 80 mm to 140 mm and maximum summer temperatures often exceeds 45 °C (Alkolibi 2002). Seasonal rainfall occurs more frequently and in greater quantity in the southwestern Hejaz and Asir mountains, therefore most permanent and semi-permanent lotic drainages or wadis occur in this region of KSA (Crosskey and Büttiker 1982, Alkahem and Behnke 1983, Whitton et al. 1986, Sorman and Abdulrazzak 1993, Al-Ghamdi and Abu-Zinadah 1998). These wadis are usually the only habitats for mayflies. Many of these drainages however, have been dammed to capture surface water. More than 230 dams now store annual runoff in reservoirs (Kingdom of Saudi Arabia Ministry of Foreign Affairs 2015). This water is used primarily for agriculture and is distributed through thousands of kilometers of irrigation canals, which can also support mayfly populations. Habitats such as oases (Edgell 2006) in KSA have not been adequately sampled for mayflies.

The first systematic studies of the mayflies of KSA were published more than 25 years ago. Six species belonging to three families (Baetidae, Caenidae and Leptophlebiidae) were mentioned from KSA (Sartori 1991; Sartori and Gillies 1990; Thomas and Sartori 1989). Within Baetidae, *Baetis balcanicus* Müller-Liebenau & Soldán, 1981, *Centroptilum dimorphicum* Soldán & Thomas, 1985 and *Cloeon saharense* Soldán & Thomas, 1983 were reported (Sartori 1991; Sartori and Gillies 1990; Thomas and Sartori 1989). *Baetis balcanicus* is now considered as a *Labiobaetis* (Lugo-Ortiz and McCafferty 1997; McCafferty and Waltz 1995). The validity of this identification is debated below in the discussion section. *Centroptilum dimorphicum* is now assigned

to the genus *Cheleocloeon* Wuillot & Gillies, 1993 (Wuillot and Gillies 1993). The attribution of the specimens collected in the Arabian Peninsula to a species previously known only from North-West Africa appears now highly questionable, especially as a new species of *Cheleocloeon*, *C. soldani* Gattolliat & Sartori, 2008, was described from United Arab Emirates (Gattolliat and Sartori 2008). We therefore consider the above specimens of *Centroptilum dimorphicum* reported from KSA as belonging to *Cheleocloeon* cf. *soldani*. Close morphological analysis is required before a definitive species assignment can be decided. At least two species of *Cloeon* Leach, 1815 occur in KSA and they appear both morphologically and genetically more related to Afrotropical species of *Cloeon* rather than to North African species such as *Cloeon saharensense* (Salles et al. 2014).

## Material and methods

The majority of the material was collected in November 2012 during a scientific expedition organised by King Saud University Museum of Arthropods, College of Food and Agriculture Sciences, Department of Plant Protection, King Saud University, KSA. Mayflies were collected from approximately 50 localities mainly along the southern coast of KSA. Other specimens were collected during different fieldtrips led by the same institution mainly in 2010 and 2012. Larvae were collected primarily by kick netting in stream vegetation along edges of wadis. Imagos were collected by light traps using black light with a white sheet. Mature larvae were reared in rearing chambers; larval and subimaginal exuviae were collected and stored with the corresponding imago. All material is stored in 80% ethanol. Holotypes and part of the paratypes are deposited in the King Saud University Museum of Arthropods, Riyadh, Saudi Arabia (KSU); other paratypes are housed in the Museum of Zoology, Lausanne, Switzerland (MZL). Each GBIFCH code refers to a tube with group of specimens in or a slide with a single specimen (sequenced or not).

The association of the ontogenetic stages was easily made for reared material. For specimens obtained by light trapping and kick sampling, we used sequence divergence of the fragment of mitochondrial cytochrome c oxidase subunit I (CO1) gene. Specimens belonging to the different “morphospecies” and collected in the same localities were selected for genetic analysis. The CO1 gene was sequenced using LCO1490 and HCO2198 primers (Folmer et al. 1994). We followed the laboratory procedures, edition and alignment of sequences as described in Vuataz et al. (2011). The final data matrix included 41 CO1 sequences of 658 bp representing all the *Labiobaetis* taxa from Palearctic and Afrotropical regions for which sequences are available (Table 1). Analyses were conducted in MEGA7 (Kumar et al. 2016).

Tree topology was reconstructed using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches (Bootstrap with 1000 replicates). We used 3.5%

**Table 1.** Specimens used for the phylogenetic analysis of the mitochondrial gene CO1.

Species	Locality	Specimen catalog #	GenBank # (COI)	GenSeq Nomenclature
<i>Labiobaetis glaucus</i>	South Africa	GBIFCH00517537	MH070310	genseq-4 COI
	South Africa	GBIFCH00517539	MH070321	genseq-4 COI
	South Africa	GBIFCH00517538	MH070319	genseq-4 COI
	Mayotte	GBIFCH00517531	MH105069	genseq-4 COI
	Mayotte	GBIFCH00521580	MH070315	genseq-4 COI
	Mayotte	GBIFCH00517530	MH070318	genseq-4 COI
	Saudi Arabia	GBIFCH00465151	MH070288	genseq-4 COI
	Saudi Arabia	GBIFCH00235741	MH070311	genseq-4 COI
	Saudi Arabia	GBIFCH00235750	MH105068	genseq-4 COI
	Saudi Arabia	GBIFCH00235731	MH070317	genseq-4 COI
	Saudi Arabia	GBIFCH00517523	MH070320	genseq-4 COI
<i>Labiobaetis potamoticus</i> sp. n.	Saudi Arabia	GBIFCH00517520	MH070306	genseq-2 COI
	Saudi Arabia	GBIFCH00517521	MH070308	genseq-2 COI
	Saudi Arabia	GBIFCH00235735	MH070312	genseq-2 COI
	Saudi Arabia	GBIFCH00235732	MH070316	genseq-2 COI
	Saudi Arabia	GBIFCH00465152	MH070289	genseq-2 COI
	Saudi Arabia	GBIFCH00465154	MH070290	genseq-2 COI
	Saudi Arabia	GBIFCH00517527	MH070307	genseq-2 COI
	Saudi Arabia	GBIFCH00235747	MH070313	genseq-2 COI
	Saudi Arabia	GBIFCH00235757	MH070314	genseq-2 COI
	Saudi Arabia	GBIFCH00517526	MH070322	genseq-2 COI
	Saudi Arabia	GBIFCH00465155	MH070291	genseq-2 COI
<i>Labiobaetis boussoulii</i>	Ivory Coast	GBIFCH00517528	MH070309	genseq-4 COI
<i>Labiobaetis</i> sp.	Ivory Coast	GBIFCH00465136	MH070294	
<i>Labiobaetis</i> sp.	Ivory Coast	GBIFCH00465137	MH070295	
<i>Labiobaetis</i> sp.	Ivory Coast	GBIFCH00465138	MH070296	
<i>Labiobaetis</i> sp.	South Africa	GBIFCH00465153	MH070305	
<i>Labiobaetis</i> sp.	South Africa	GBIFCH00465135	MH070303	
<i>Labiobaetis piscis</i>	South Africa		IBOL CED 150U	genseq-4 COI
<i>Labiobaetis latus</i>	South Africa	GBIFCH00465142	MH070297	genseq-4 COI
<i>Labiobaetis vinosus</i>	South Africa	GBIFCH00465143	MH070304	genseq-4 COI
<i>Labiobaetis dambrensis</i>	Madagascar	GBIFCH00465144	MH070293	genseq-2 COI
<i>Labiobaetis nigrocercus</i>	Madagascar	GBIFCH00465145	MH070300	genseq-4 COI
<i>Labiobaetis longicercus</i>	Madagascar	GBIFCH00465146	MH070298	genseq-4 COI
	Madagascar	GBIFCH00465147	MH070299	genseq-4 COI
<i>Labiobaetis punctatus</i>	Madagascar	GBIFCH00465148	MH070301	genseq-4 COI
	Madagascar	GBIFCH00465149	MH070302	genseq-4 COI
<i>Labiobaetis atrebatinus</i>	France	GBIFCH00465150	MH070292	genseq-4 COI
<i>L. atrebatinus orientalis</i>	Japan		KF563032	genseq-4 COI
<i>Labiobaetis tricolor</i>	Hungary		JN164313	genseq-4 COI
<i>Baetis rhodani</i>	Switzerland		HG935037	genseq-4 COI

sequence divergence (measured by Kimura 2-parameter (K2P)) as the maximal value for intraspecific divergence (Ball et al. 2005; Gattolliat et al. 2015; Rutschmann et al. 2014; Rutschmann et al. 2017; Webb et al. 2012). As part of the sequenced specimens came from the same population, the intraspecific divergence is expected to be even clearly lower than the limit.

## Taxonomy

### *Labiobaetis potamoticus* Gattolliat & Al Dhafer, sp. n.

<http://zoobank.org/EAAD82B8-5680-49C0-879C-8B4F204FF351>

Figs 1–19

*Baetis balcanicus* Müller-Liebenau & Soldán, 1981 in Thomas and Sartori 1989: 87.

**Type material. Holotype:** Male larva (GBIFCH00521578): Saudi Arabia (AR44); Wadi Shahadan; 17°28'36"/ 42°42'50"; Alt. 190m; 13.XI.2012; Coll. J-L Gattolliat.

**Paratypes:** 4 larvae (GBIFCH00235716 + GBIFCH00235735 (Genetics)), 1 male imago (GBIFCH00235732 (Genetics)) + 3 larvae (KSU: GBIFCH00526192); same data as holotype.

42 larvae (GBIFCH00235729 + 3 slides GBIFCH00235758, GBIFCH00235733, GBIFCH00235760): Saudi Arabia (AR01); Al Jiwah, Thee Aine; 19°55'32"/ 41°26'17"; Alt. 752m; 13.X.2010; Coll. B. Kondratieff.

14 larvae (GBIFCH00235721): Saudi Arabia (AR20); Wadi Baqrah; 18°47'29"/ 41°56'19"; Alt. 490m; 13.III.2012; Coll. Al Dhafer, H. & Kondratieff, B.

5 larvae (GBIFCH00235722): Saudi Arabia (AR28); Thee Ain, Al-Baha; 19°55'46"/ 41°26'34"; Alt. 760m; 3.VI.2012; Coll. Al Dhafer, H. & Kondratieff, B.

2 larvae (GBIFCH00235714): Saudi Arabia (AR31); Thee Ain, Al-Baha; 19°55'46"/ 41°26'34"; Alt. 760m; 8.XI.2012; Coll. J-L Gattolliat.

58 larvae (GBIFCH00235706, GBIFCH00235728, GBIFCH00235717 + 2 slides GBIFCH00235749, GBIFCH00235759 + GBIFCH00517520 (Genetics), GBIFCH00517521 (Genetics)) + 11 larvae (KSU: GBIFCH00526173): Saudi Arabia (AR32); Wadi Elarj, near Adam; 20°27'11"/ 40°48'56"; Alt. 440m; 9.XI.2012; Coll. J-L Gattolliat.

23 larvae (GBIFCH00235726, GBIFCH00235719 + 3 slides GBIFCH00235753, GBIFCH00465152 (Genetics), GBIFCH00465155 (Genetics)) + 4 larvae (KSU: GBIFCH00526224): Saudi Arabia (AR43a); Wadi Shahadan; 17°28'36"/ 42°51'25"; Alt. 460m; 12.XI.2012; Coll. J-L Gattolliat.

3 larvae (KSU: GBIFCH00526237): Saudi Arabia (AR43b); Wadi Shahadan; 17°28'17"/ 42°51'14"; Alt. 455m; 12.XI.2012; Coll. J-L Gattolliat.

**Additional material.** 1 larva (on slide), Saudi Arabia, Wadi Buwah, 1340m, 20°47'N / 41°12'E, 20.IX.1980, Leg. W. Büttiker. Coll. Naturhistorisches Museum Basel, Switzerland. (Previously identified as *Baetis balcanicus*, det. A. Thomas).

**Differential diagnosis. Larva:** Tergites I–X medium brown with peculiar pattern formed of six ecru dots (Fig. 19). Scape of antenna without distolateral process (Fig. 13). Segment II of the maxillary palp without a distomedial concavity (Fig. 7). Segment II of labial palp with a slender triangular distomedial projection (Fig. 8). Dorsal margin of femur with a few medium setae, not abundant proximally; ventral margin with abundant medium setae (Fig. 10). Dorsal margin of tibia almost bare (Fig. 9). Paraproct with numerous distal spines (Fig. 15). **Male imago:** Genitalia with inner margin of segments I and II without expansion; segment III almost globular (Fig. 18).

**Description. Larva.** *Length:* fully grown female: Body 5.1–7.7 mm, cerci 3.6–4.0 mm, terminal filament 2.5–2.6 mm. Fully grown male: Body 5.0–5.3 mm, cerci 3.4–3.6 mm, terminal filament 2.5 mm.

*Colouration* (Fig. 19): Head almost uniformly brown with ecru vermiform marking on vertex and frons, border of sclerites yellow. Prothorax medium brown, lighter laterally; mesothorax medium brown with a central yellow dot and a transversal yellow stripe; metathorax medium brown. Legs: ecru except a brown central dot on femora; dorsal and ventral margin of femora brown, dorsal margin of tibiae and tarsi brown. Tergites I–X medium brown with peculiar pattern formed of six ecru dots sometimes fused. Abdominal sternites brown sometimes with four ecru dots. Cerci ecru brown getting progressively ecru towards apex.

*Head:* scape of antenna without distolateral process (Fig. 13).

*Labrum* (Fig. 1) rounded with a small anteromedial emargination, dorsally with a distolateral row of approx. eight feathered setae, without a submedian seta; short, thin, simple setae scattered on dorsal surface of labrum; distal margin bordered with feathered setae.

*Right mandible* (Fig. 2): canine with two almost fused incisivi each with four denticles, outer denticle much shorter than others, inner margin of inner incisive with a row of very thin setae; stout prostheca apically with small pointed denticles (Fig. 3); margin between prostheca and mola slightly convex, smooth, without setae; tuft of setae at apex of mola absent.

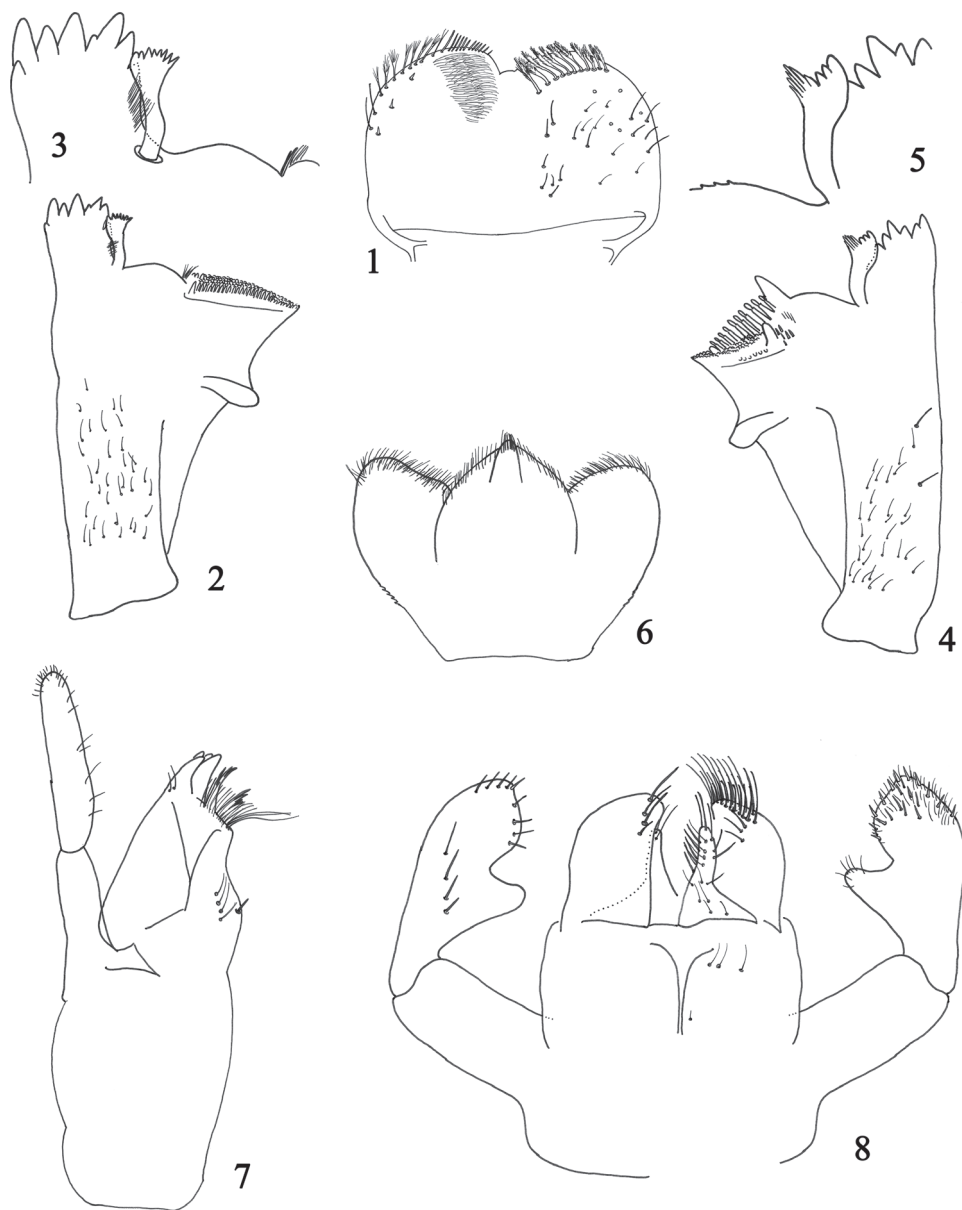
*Left mandible* (Fig. 4): canine with two almost fused incisivi each with four denticles, outer denticle much shorter than others; stout prostheca apically with small denticles and a comb-shaped structure (Fig. 5); margin between prostheca and mola slightly concave, without crenulations; tuft of setae at apex of mola absent.

*Hypopharynx* as illustrated in Fig. 6.

*Maxilla* (Fig. 7) with a medioapical row of medium setae, basal end of row with a few long setae; posterior side of lacinia mediobasally with a row of four medium-sized setae, a single small seta close to the medial margin of lacinia; palp 2-segmented, longer than galea-lacinia, segment II without distomedial concavity.

*Labium* (Fig. 8) with glossae shorter than paraglossae; glossae inner margin with two rows of approx. six long setae, apically with a few simple setae; paraglossae stout, apically flattened, with three rows of long simple setae; labial palp with segment I slender, quadrangular, shorter than segments II and III combined; segment II with a

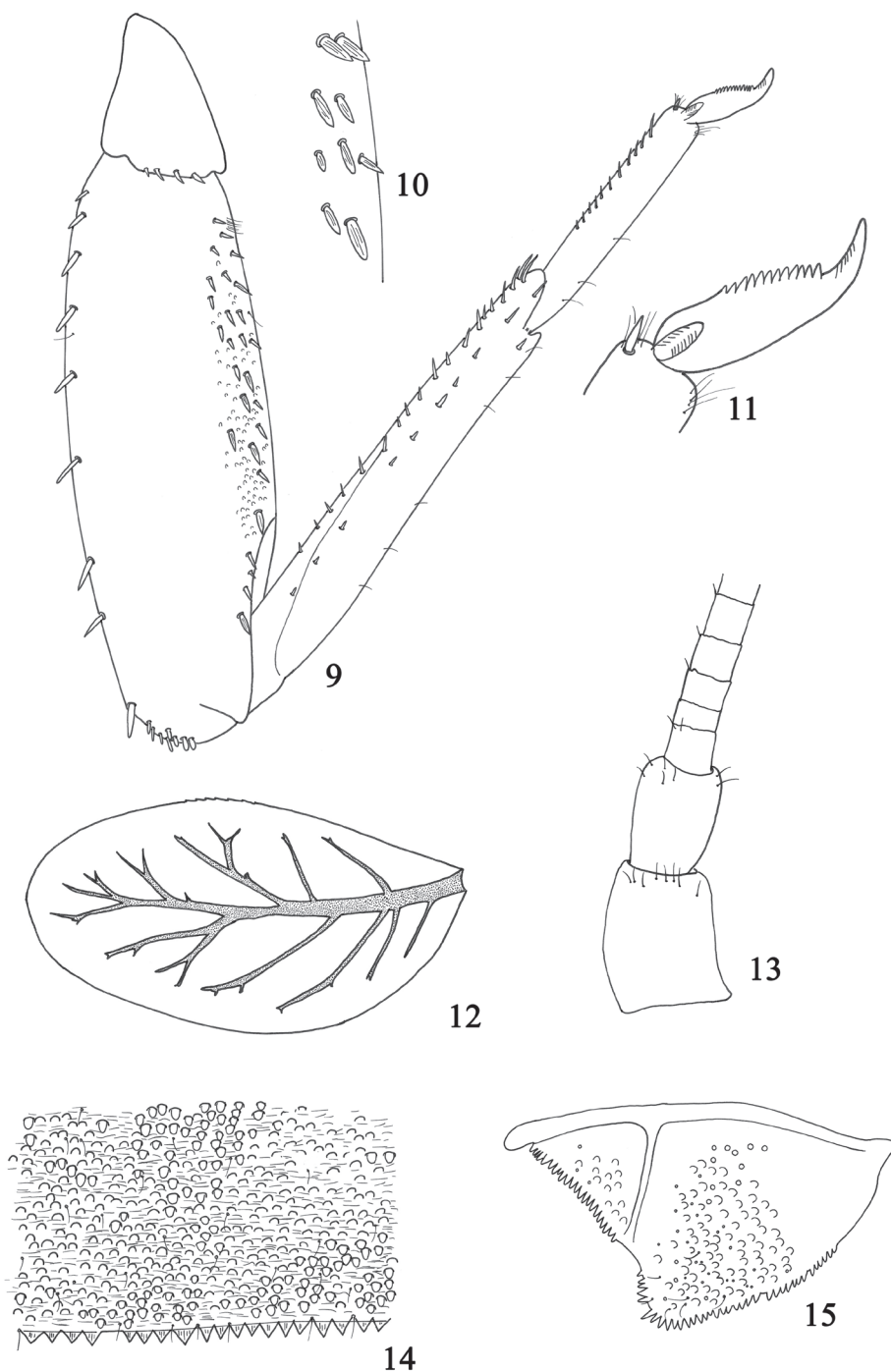




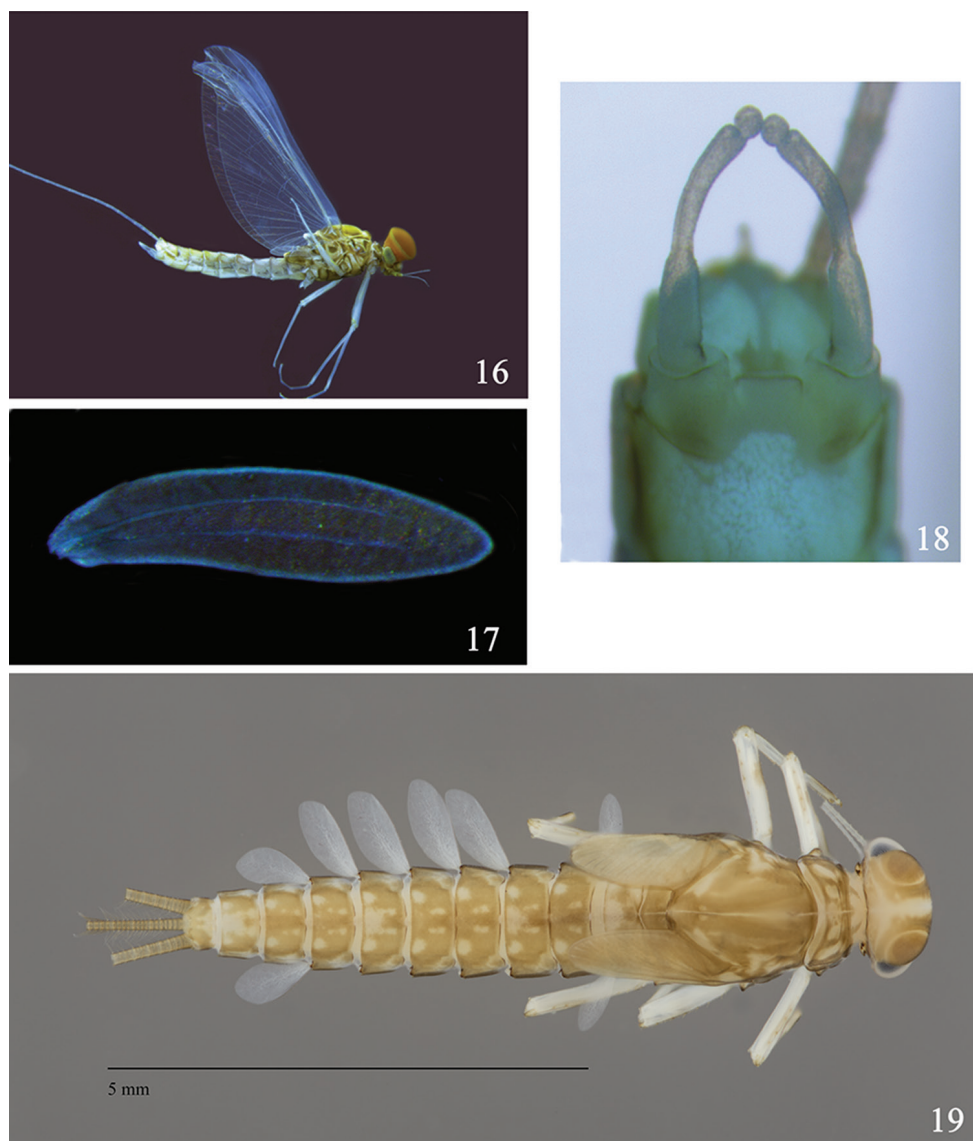
**Figures 1–8.** Larval structures of *Labiobaetis potamoticus* sp. n.: **1** labrum (left : ventral; right : dorsal) **2** right mandible **3** detail of right mandible: canines and prostheca **4** left mandible **5** detail of left mandible: prostheca **6** hypopharynx **7** right maxilla **8** labium.

slender, elongated distomedial projection with few thin setae apically, on posterior side with a row of four long setae increasing in length; segment III subconical, inner margin apically slightly concave, with abundant scattered short thin setae and stouter setae.





**Figures 9–15.** Larval structures of *Labiobaetis potamoticus* sp. n.: **9** foreleg **10** detail of ventral margin of forefemora **11** tarsal claw **12** fourth gill **13** base of antenna **14** posterior margin of fourth abdominal tergite **15** paraproct.



**Figures 16–19.** Larval and imaginal structures of *Labibaetis potamoticus* sp. n.: **16** male imago (lateral view) **17** hindwing **18** male genitalia **19** male larva (dorsal view).

*Thorax:* hind wing pads present.

*Legs* (Fig. 9): forefemur dorsally with a row of approx. eight medium-sized, apically rounded setae; apex with one medium stout seta and several short flattened setae; ventral margin with a poorly developed villopore and abundant, medium, stout setae (Fig. 10). Foretibia dorsally almost bare; ventrally with short setae, only slightly longer apically. Foretarsus almost bare dorsally; ventral margin with a row of pointed setae slightly increasing in length toward apex; tarsal claw (Fig. 11) with a single row of ap-

prox. twelve pointed teeth; without subapical setae. Middle and hind legs similar to foreleg but with reduced setation.

**Abdomen:** tergites (Fig. 14) shagreened with numerous scales and scale bases, with a few setae; posterior margin with triangular spination as long as broad. Sternites with scales and scale bases; posterior margin smooth, without spines.

**Gills** present on abdominal segments I–VII (Fig. 12), poorly serrated, tracheation brown, with abundant ramifications.

**Paraproct** (Fig. 15) with scale bases and a few setae, margin with numerous small triangular spines regular in size; posterolateral extension with a few scale bases, spines along the margin of the same size as those of the paraproct.

### Male imago

**Length.** Body 4.8 mm; forewing 4.4 mm; hindwing 0.8 mm.

**Colouration:** head brown; antenna ecru except base of scape and pedicel brown. Facetted surface of turbinate eyes dark orange brown, shaft orange brown, lighter apically (Fig. 16). Thorax yellowish brown with margin of sclerites generally dark brown. Legs: yellowish without marks or pattern. Wings hyaline, hyaline venation. Abdomen: tergites I to X ecru without pattern. Sternites I to IX ecru. Cerci ecru. Genitalia (Fig. 18) ecru except inner margin of segment I medium brown.

**Forewing:** pterostigma with approx. two cross-veins not reaching subcostal vein; double intercalary veins shorter than distance between corresponding main veins.

**Hindwing** (Fig. 17) without costal spur; two longitudinal veins almost reaching margin, none of them bifurcated, without incomplete veinlets between main longitudinal veins.

**Genitalia** (Fig. 18): basal segment with inner margin not expanded apically; segments I and II almost completely fused; constriction at basis of segment II; segment III globular.

**Distribution and habitat.** *Labiobaetis potamoticus* appears to be the most widespread species of *Labiobaetis* in KSA. It colonizes aquatic vegetation in still reaches of small to medium-sized streams with a sandy substrate (Figs 48, 52). This species was also collected in a secondary channel with *Typha* sp. (Fig. 49). This species seems less rheophilic than the two following taxa.

**Etymology.** The specific name *potamic-* was given in reference to the ecological preferences of the species for still and standing water.

### *Labiobaetis alahmadii* Gattolliat & Al Dhafer, sp. n.

<http://zoobank.org/605BFF9A-A54E-419F-912B-462B1E9FAAF9>

**Type material. Holotype:** Female larva (GBIFCH00521579): Saudi Arabia (AR43a); Wadi Shahadan; 17°28'36"/ 42°51'25"; Alt. 460m; 12.XI.2012; Coll. J-L Gattolliat.

**Paratypes:** 151 larvae (GBIFCH00235715, GBIFCH00235720 + GBIFCH00517525 + 3 slides GBIFCH00235737, GBIFCH00235755, GBIFCH00235757 (Genetics), GBIFCH00235737) + 8 larvae (KSU: GBIFCH00526208): same data as holotype.

47 larvae (GBIFCH00235710) + 10 larvae (KSU: GBIFCH00526227): Saudi Arabia (AR43b); Wadi Shahadan; 17°28'17"/ 42°51'14"; Alt. 455m; 12.XI.2012; Coll. J-L Gattolliat.

29 larvae (GBIFCH00235727) + 6 larvae (KSU: GBIFCH00526223): Saudi Arabia (AR39); Wadi Damad; 17°12'21"/ 43°01'35"; Alt. 260m; 11.XI.2012; Coll. J-L Gattolliat.

15 larvae (GBIFCH00235709 + 3 slides GBIFCH00235744, GBIFCH00235747 (Genetics), GBIFCH00465155 (Genetics) + GBIFCH00517526 (Genetics), GBIFCH00517527 (Genetics)) + 4 larvae (KSU: GBIFCH00526179): Saudi Arabia (AR44); Wadi Shahadan; 17°28'36"/ 42°42'50"; Alt. 190m; 13.XI.2012; Coll. J-L Gattolliat.

**Differential diagnosis. Larva:** *Colouration:* mesothorax medium brown with a W-shaped yellow pattern; tergites I–VIII medium brown with two broad ecru spots, tergites IX and X yellow (Figs 32, 33). Scape of antenna without distolateral process (Fig. 28). Segment II of the maxillary palp with a small distomedial concavity (Fig. 23). Segment II of labial palp with a broad apically rounded triangular distomedial projection (Fig. 24). Dorsal margin of femur with regularly spaced setae; ventral margin almost bare (Fig. 25). Dorsal margin of tibia with a row of small spatulate setae (Fig. 26). Paraproct with approximately eight stout, pointed spines increasing in length toward the apex (Fig. 31).

**Description. Larva.** *Length:* fully grown female: Body 9.5–10.6 mm, cerci 4.0–4.2 mm, terminal filament 2.9–3.1 mm. Fully grown male: Body 8.8–9.9 mm, cerci 3.8–4.1 mm, terminal filament 2.8–2.9 mm.

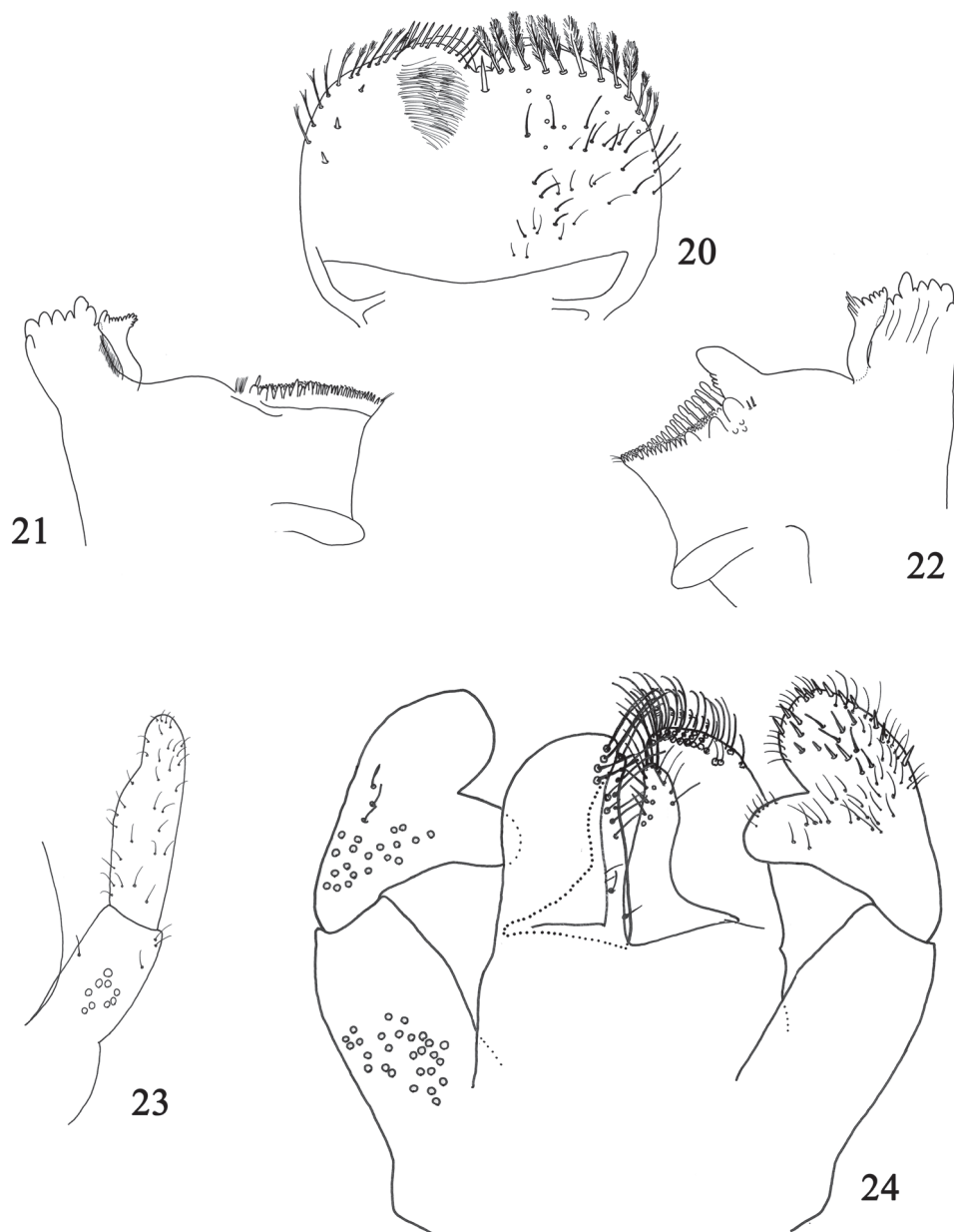
*Colouration* (Figs 32, 33): head almost uniformly medium brown, with darker, vermiform marking on vertex and frons, border of sclerites yellow. Prothorax ecru with proximal margin medium brown and a brown dot medio-apically; mesothorax medium brown with a double V-shaped yellow pattern; metathorax medium brown. Legs: ecru except femora with a central brown spot and apex of femora, tibiae and tarsi brown. Tergites I–VIII medium brown with two broad ecru spots sometimes fused medially, tergites IX and X yellow. Abdominal sternites ecru getting darker and brownish after sternite VI. Cerci ecru without dark stripe.

*Head:* scape of antenna without distolateral process (Fig. 28).

*Labrum* (Fig. 20) rounded, with a small anteromedial emargination, dorsally with a relatively short submedian seta and a distolateral row of approx. ten feathered setae; short, thin, simple setae scattered on dorsal surface of labrum; distal margin bordered with feathered setae.

*Right mandible* (Fig. 21): canine with two almost fused incisivi each with four denticles, outer denticle much shorter than others, inner margin of inner incisive with a row of very thin setae; stout prostheca apically with numerous small rounded denticles, also covering distoapical corner; margin between prostheca and mola slightly convex, smooth, without setae; tuft of setae at apex of mola present.

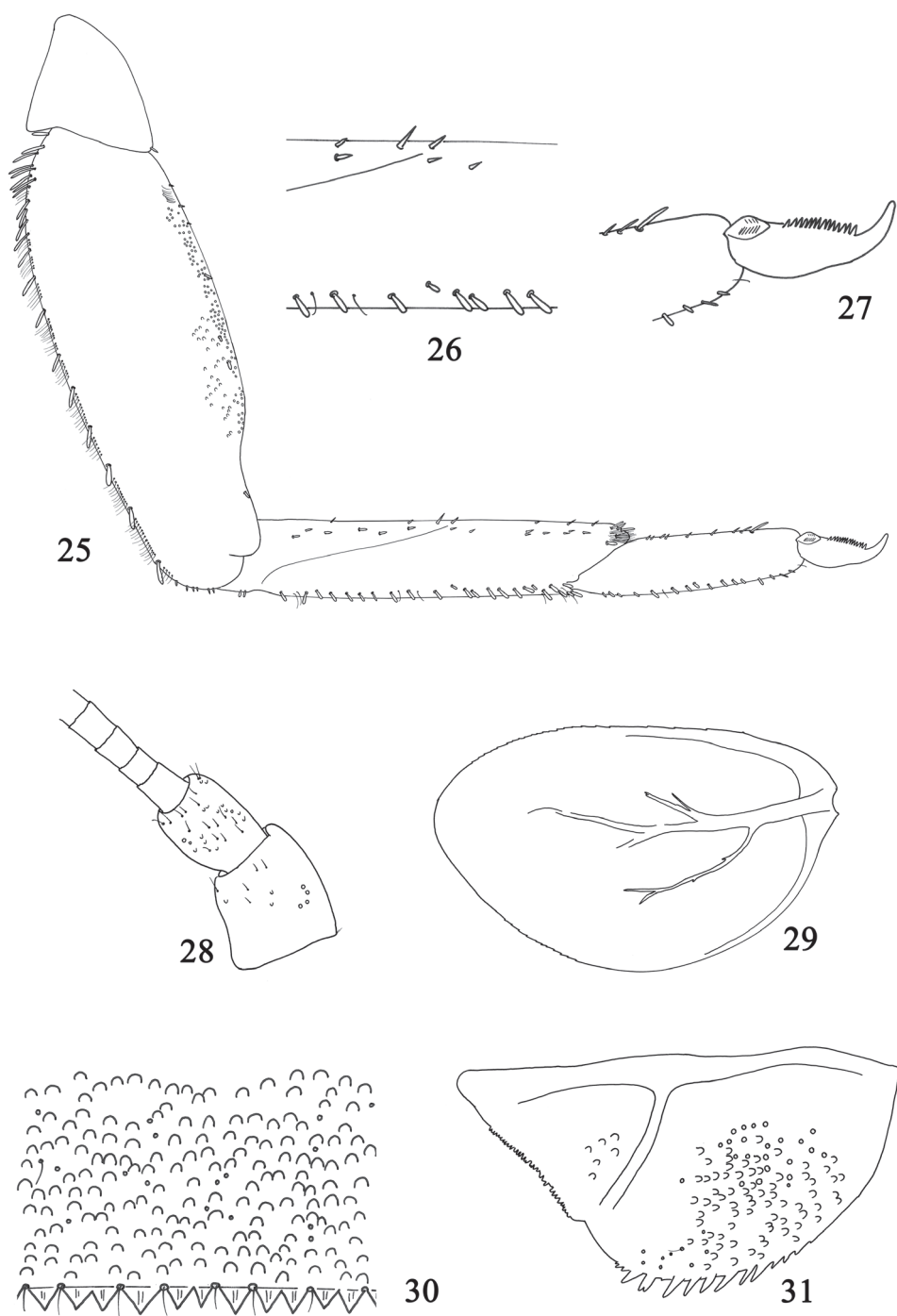
*Left mandible* (Fig. 22): canine with two almost fused incisivi each with four denticles, outer denticle much shorter than others; stout prostheca apically with small den-



**Figures 20–24.** Larval structures of *Labiobaetis alahmadii* sp. n.: **20** labrum (left : ventral; right: dorsal) **21** right mandible **22** left mandible **23** detail of maxilla **24** labium.

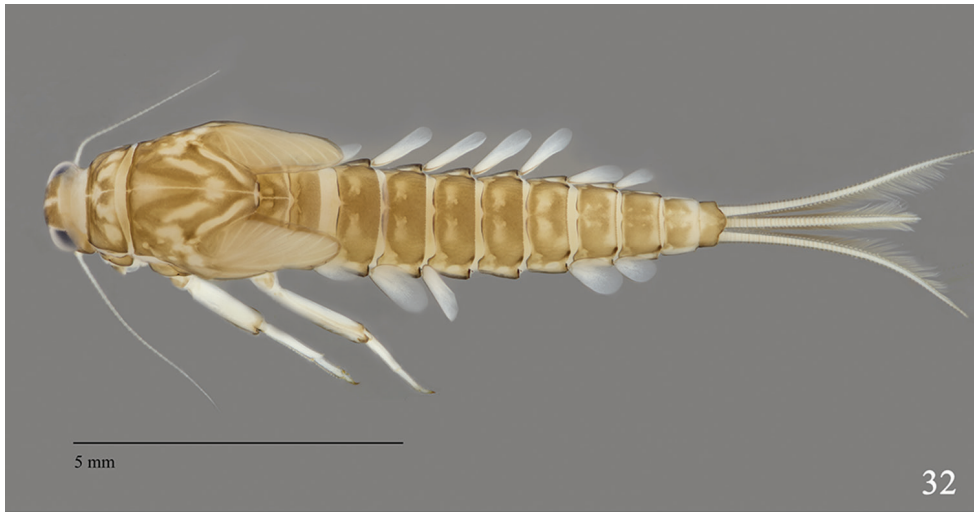
ticles and a comb-shaped structure; margin between prostheca and mandible slightly concave, without crenulations; tuft of setae at apex of mola present.

*Hypopharynx* similar to Fig. 6.



**Figures 25–31.** Larval structures of *Labiobaetis alahmadii* sp. n.: **25** foreleg **26** detail of ventral margin of foretibia **27** tarsal claw **28** base of antenna **29** fourth gill **30** posterior margin of fourth abdominal tergite **31** paraproct.





**Figures 32–33.** Larvae of *Labiobaetis alahmadii* sp. n.: **32** female larva (dorsal view) **33** larvae in vivo.

*Maxilla* with a medioapical row of medium setae, basal end of row with a few long setae; posterior side of lacinia mediobasally with a row of four medium-sized setae, a single small seta close to the medial margin of lacinia; palp 2-segmented, as long as galea-lacinia, segment II with distomedial concavity (Fig. 23).

*Labium* (Fig. 24) with glossae shorter than paraglossae; glossae with medium to long simple setae in apical half; paraglossae stout, apically rounded, with three rows of setae: two rows with simple setae and one row with setae feathered on one side; labial palp with segment I slender, quadrangular, as long as segments II and III combined; segment II with an elongated triangular distomedial projection with few scattered thin setae, on posterior side with a row of three medium setae; segment III subconical, with abundant scattered short thin setae and stouter setae.

*Thorax*: hind wing pads present.

*Legs* (Fig. 25): forefemur dorsally with a row of medium-sized, apically rounded setae, abundant proximally; apex with one short stout seta and minute setae; ventral margin with a poorly developed villopore and scarce minute setae. Foretibia dorsally with a row of short spatulate setae (Fig. 26); ventrally with short setae, not longer apically, apex with a patch of numerous flattened short setae. Foretarsus dorsally with a row of short spatulate setae; ventral margin with a row of minute pointed setae slightly increasing in length toward apex; tarsal claw (Fig. 27) with a single row of approx. 14 pointed teeth; without subapical setae. Middle and hind legs similar to foreleg but with reduced setation.

*Abdomen*: tergites (Fig. 30) with numerous scale bases with a few setae; posterior margin with triangular spination as long as broad. Sternites with numerous scales and scale bases; sternites I–VII with posterior margin smooth without spines, sternites VIII and IX with small triangular spines.

*Gills* present on abdominal segments I–VII, margins serrated, tracheation poorly marked and poorly divided (Fig. 29).

*Paraproct* (Fig. 31) with scale bases, almost bare, margin with approx. eight stout, pointed spines and bordered by few small spines; posterolateral extension with a few scale bases, minute spines along the margin.

**Imagos:** Unknown

**Distribution and habitat.** We collected this species in only three different localities in close proximity, in extreme southwestern KSA close to the Yemen border. The larvae occur in medium-size streams with stony substrates (Figs 50, 51, 52). Larvae apparently prefer relatively fast flowing reaches. Larvae were even collected at the base of small waterfalls. *Labiobaetis alahmadii* appeared to be an abundant species when the above ecological conditions are optimal. The new species was always sympatric with *L. glaucus*.

**Etymology.** This species is dedicated to the memory of Professor Ahmed Ziad Al Ahmadi, the well-known Syrian entomologist who passed away few months ago.

### ***Labiobaetis glaucus* (Agnew, 1961)**

Figs 34–47

*Baetis glaucus* Agnew, 1961: 14.

*Pseudocloeon glaucum*, Lugo-Ortiz et al. 2000: 281.

*Labiobaetis glaucus*, Kluge and Novikova 2016: 32–33.

**Specimens examined.** 18 larvae (GBIFCH00235711 + 4 slides GBIFCH00235741 (Genetics), GBIFCH00235746, GBIFCH00235750 (Genetics), GBIFCH00235756: Saudi Arabia (AR01); Al Jiwah, Thee Aine; 19°55'32"/ 41°26'17"; Alt. 752m; 13.X.2010; Coll. B. Kondratieff.

3 larvae (GBIFCH00235708): Saudi Arabia (AR19); Wadi Khat; 19°05'22"/41°58'16"; Alt. 490m; 13.III.2012; Coll. Al Dhafer, H.

1 larva (GBIFCH00235712): Saudi Arabia (AR28); Thee Ain, Al-Baha; 19°55'43"/41°26'34"; Alt. 760m; 3.VI.2012; Coll. Al Dhafer, H. & Kondratieff, B.

3 larvae (GBIFCH00235713): Saudi Arabia (AR31); Thee Ain, Al-Baha; 19°55'43"/41°26'34"; Alt. 760m; 8.XI.2012; Coll. J-L Gattolliat.

1 larva (GBIFCH00235707): Saudi Arabia (AR43a); Wadi Shahadan; 17°28'36"/42°51'25"; Alt. 460m; 12.XI.2012; Coll. J-L Gattolliat.

2 larvae GBIFCH00465151 (Genetics): Saudi Arabia (AR43b); Wadi Shahadan; 17°28'17"/42°51'14"; Alt. 440m; 12.XI.2012; Coll. J-L Gattolliat.

7 larvae (GBIFCH00235723 + 1 slide GBIFCH00235738), 3 male imagos (GBIFCH00235724 + 1 slide GBIFCH00235731 (Genetics)): Saudi Arabia (AR44); Wadi Shahadan; 17°28'36"/42°42'50"; Alt. 190m; 13.XI.2012; Coll. J-L Gattolliat.

**Differential diagnosis.** *Larva*: abdominal pattern (Fig. 47) with tergites I, VI and X lighter (in some specimens tergites V and IX also lighter). Scape of antenna without distolateral process (Fig. 41). Segment II of the maxillary palp without a distomedial concavity (Fig. 38). Segment II of labial palp with a broad apically rounded triangular distomedial projection (Fig. 39). Dorsal margin of femur (Fig. 40) with numerous setae proximally and rarely any distally; ventral margin with a few scattered setae. Dorsal margin of tibia with a few minute setae. Paraproct with approx. ten stout, pointed spines increasing in length towards apex (Fig. 44). **Male imago**: Genitalia with inner margin at the apex of segment I and base of segment II with a triangular well-marked expansion; segment III almost globular (Fig. 46).

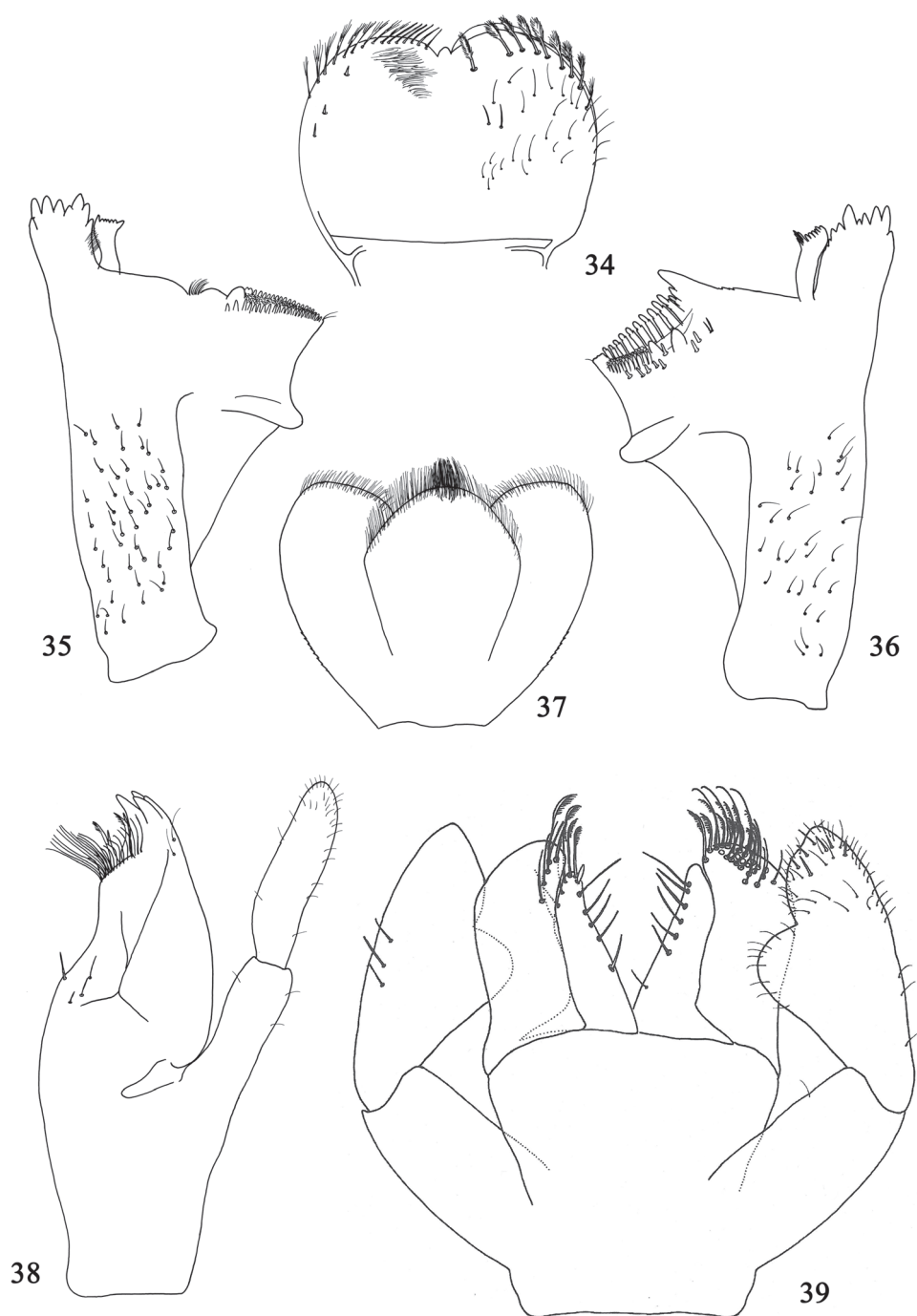
**Description.** *Larva*. Length: fully grown female: Body 6.2–8.0 mm, cerci 3.6–4.0 mm, terminal filament 2.4–2.8 mm. Fully grown male: Body 4.8–7.3 mm, cerci 3.3–3.6 mm, terminal filament 1.7–1.9 mm.

*Colouration* (Fig. 47): head almost uniformly medium brown, with darker, faint vermiform marking on vertex and frons, border of sclerites yellow. Prothorax medium brown with poorly marked yellowish pattern; mesothorax medium brown with a V-shaped yellow pattern; metathorax medium brown. Legs ecru except femora with a central brown spot and apex of femora, tibiae and tarsi brown. Tergites medium brown with small ecru spot except tergites I, V, VI, IX and X yellow, tergites V and VI generally with a dark M-shaped mark. Abdominal sternites ecru. Cerci ecru without dark stripe.

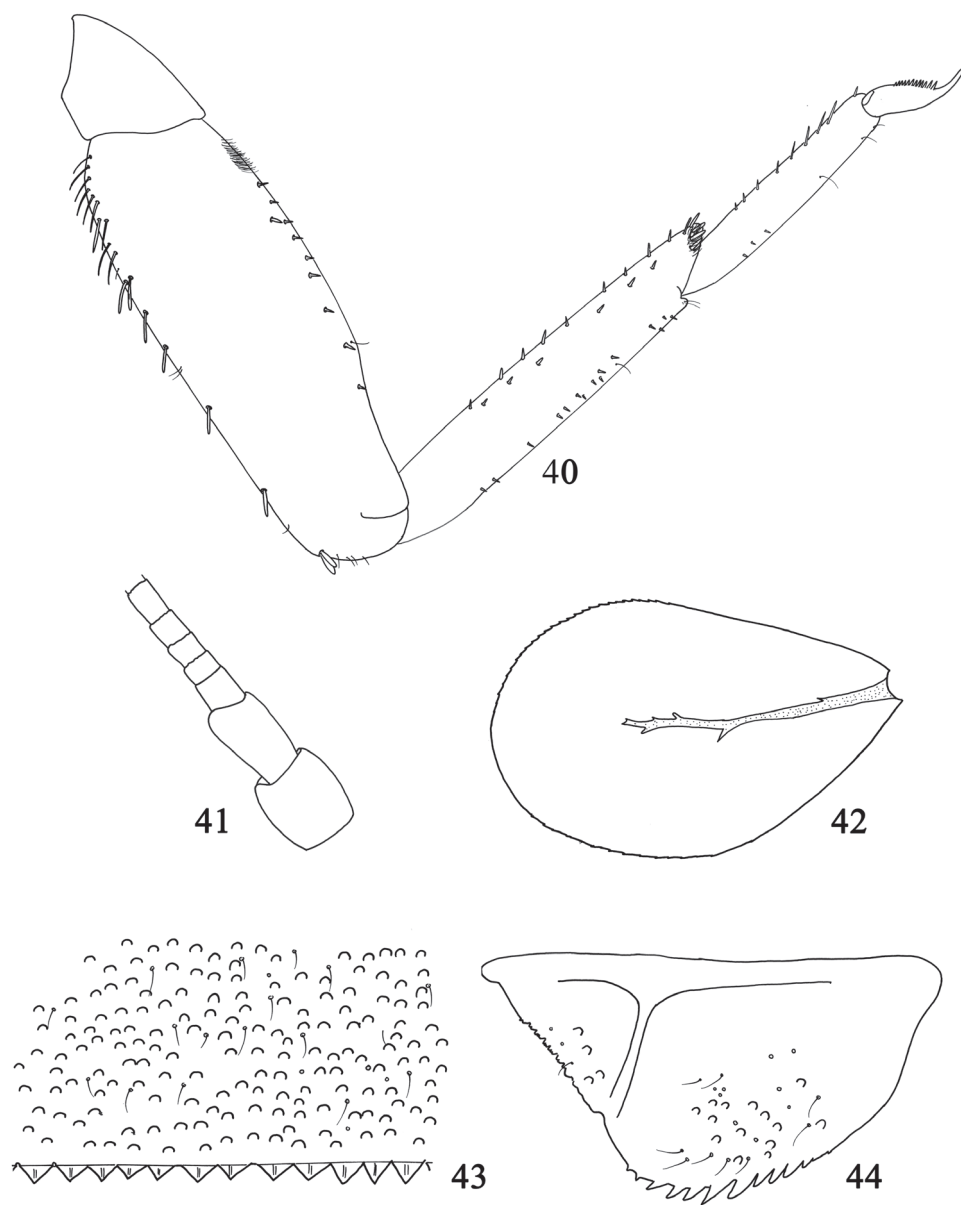
*Head*: scape of antenna without distolateral process (Fig. 41).

*Labrum* (Fig. 34) rounded, with a small anteromedial emargination, dorsally with one feathered submedian seta, and a distolateral row of approx. eight feathered setae; short, thin, simple setae scattered on dorsal surface of labrum; distal margin bordered with feathered setae.

*Right mandible* (Fig. 35): canine with two almost fused incisivi each with four denticles, outer denticle much shorter than others, inner margin of inner incisive with a row of very thin setae; stout prostheca apically with small rounded denticles; margin between prostheca and mola straight, smooth, without setae; tuft of setae at apex of mola reduced to two small setae.



**Figures 34–39.** Larval structures of *Labiobaetis glaucus*: **34** labrum (left: ventral; right: dorsal) **35** right mandible **36** left mandible **37** hypopharynx **38** left maxilla **39** labium.



**Figures 40–44.** Larval structures of *Labiobaetis glaucus*: **40** foreleg **41** base of antenna **42** fourth gill **43** posterior margin of fourth abdominal tergite **44** paraproct.

*Left mandible* (Fig. 36): canine with two almost fused incisivi each with four denticles, outer denticle much shorter than others; stout prostheca apically with small denticles and a comb-shaped structure; margin between prostheca and mola straight, distally with crenulations; tuft of setae at apex of mola reduced to a single seta.



**Figures 45–47.** Larval and imaginal structures of *Labiobaetis glaucus*: **45** male imago (lateral view) **46** male genitalia **47** male and female larvae (dorsal view).

*Hypopharynx* as in Fig. 37.

*Maxilla* (Fig. 38) with a medioapical row of relatively short setae, basal end of row with approx. seven long setae; posterior side of lacinia mediobasally with a row of three medium-sized setae, a single small seta close to the medial margin of lacinia; palp 2-segmented, segment II without distomedial concavity.





**Figures 48–52.** General aspects of *Labiobaetis* larval habitats: **48** AR32; Wadi Elarj, near Adam (Type locality of *Labiobaetis potamoticus*) **49** AR32; Wadi Elarj, near Adam: lateral channel with *Typha* sp. **50** AR43b; Wadi Shahadan: small waterfall **51** AR43a; Wadi Shahadan (type locality of *Labiobaetis alahmadii*) **52** AR44; Wadi Shahadan.

*Labium* (Fig. 39) with glossae slightly shorter than paraglossae; glossae inner margin with two rows of approx. six long setae, apically with a few setae feathered on one side; paraglossae stout, apically rounded, with three rows of long setae, part of them feathered on one side; labial palp with segment I slender, shorter than segments II and III combined; segment II with a broad apically rounded triangular distomedial projection covered with thin setae, on posterior side with a row of three long setae; segment III subconical, inner margin apically slightly concave, with scattered short thin setae and a few stouter setae.

*Thorax*: hind wing pads present.

*Legs* (Fig. 40): Forefemur dorsally with a row of medium-sized, apically rounded setae, numerous proximally and rare distally; apex with two short flattened setae; ventral margin with a well-developed villopore and scarce, short, stout setae. Foretibia dorsally with a row of scarce tiny, stout setae; ventrally with a few short setae, not longer apically, apex with a patch of numerous flattened short setae. Foretarsus almost bare dorsally; ventral margin with a row of pointed setae slightly increasing in length toward apex; tarsal claw with a single row of approx. twelve pointed teeth; subapical setae absent. Middle and hind legs similar to foreleg but with reduced setation.

*Abdomen*: tergites (Fig. 43) with numerous scale bases, with a few setae; posterior margin with short and broad triangular spination. Sternites with a few setae, without scales and scale bases; sternites I-VII with posterior margin smooth without spines, sternites VIII and IX with small triangular spines.

*Gills* present on abdominal segments I-VII, distally serrated, tracheation brown, poorly developed (Fig. 42).

*Paraproct* (Fig. 44) with scale bases and a few setae, margin with approx. ten stout, pointed spines increasing in length; posterolateral extension with a few scale bases, minute spines along the margin.

**Male imago.** *Length.* Body 4.4–4.5 mm; forewing 4.2–4.3 mm; hindwing 0.8 mm.

*Colouration*: head dark brown; antenna ecru. Facetted surface of turbinate eyes orange brown, shaft orange brown (Fig. 45). Thorax yellowish brown with margin of sclerites generally dark brown. Legs: yellowish without marks or pattern. Wings hyaline except costal and subcostal area apically white, with brown venation. Abdomen: tergites I to X light brown without mark or pattern. Sternites I and II light brown; sternites III to VII uniformly ecru without marks or pattern; sternites VIII and IX light brown. Cerci ecru. Genitalia (Fig. 46) ecru except inner margin of segment I medium brown. Forewing (Fig. 45): pterostigma with approx. four cross-veins not reaching subcostal vein; double intercalary veins shorter to almost equal to distance between corresponding main veins. Hindwing similar to Fig. 17 except two longitudinal veins reaching margin. Genitalia (Fig. 46): basal segment with inner margin not expanded apically; segment I and II almost completely fused; inner margin at the apex of segment I and base of segment II with a triangular well-marked expansion; segment III almost globular.

**Distribution and habitat.** This species was collected in three different wadis at altitudes between 200 m and 750 m. Larvae occur in small streams, generally very shallow (a few centimeters to 20 cm) with moderate current. The substrate was a mix of sand, cobbles and rocks (Figs 50, 51, 52). This species was sympatric with the two other species of *Labiobaetis* and *C. cf. soldani*, but generally less abundant than other species.

**Table 2.** Estimates of evolutionary divergence between major haplogroups of *Labiobaetis* species (using corrected p distances). In brackets are indicated the minimum and maximum distances.

	<i>Labiobaetis glaucus</i>	<i>Labiobaetis glaucus</i>	<i>Labiobaetis glaucus</i>	<i>Labiobaetis alahmadii</i>	<i>Labiobaetis potamoticus</i>
<i>Labiobaetis glaucus</i>	0 (0.0–0.004)				
<i>Labiobaetis glaucus</i>	0.02 (0.06–0.021)	0 (0.0–0.012)			
<i>Labiobaetis glaucus</i>	0.023 (0.021–0.025)	0.012 (0.012)	0		
<i>Labiobaetis alahmadii</i>	0.196 (0.194–0.199)	0.173 (0.173)	0.183 (0.183)	0	
<i>Labiobaetis potamoticus</i>	0.179 (0.173–0.183)	0.182 (0.173–0.189)	0.168 (0.168)	0.168 0.162–0.173	0.003 (0.00–0.008)
<i>Labiobaetis</i> spp.	(0.178–0.254)	(0.178–0.250)	(0.173–0.255)	(0.153–0.293)	(0.042–0.256)

Molecular results

The mitochondrial reconstruction clearly recovers *L. potamoticus* and *L. alahmadii* as monophyletic clades (BS (Bootstrap support) of 83% and 100% respectively), with intraspecific K2P distances below 1% (Table 2). *Labiobaetis glaucus* is also highly supported as a monophyletic clade (BS of 100%), with the three populations (KSA, Mayotte, and South Africa) supported as monophyletic sister-clades (BS of 87%, 81% and 90% respectively). The sister group of *Labiobaetis potamoticus* is an undescribed species from South Africa; the distance between the two taxa is slightly higher than intraspecific distance (between 4.2 and 5.1%). *Labiobaetis potamoticus* possesses high distances to all the other species included in the study (16.2 to 25.5%). The relationships of *L. alahmadii* and *L. glaucus* with other species of Afrotropical and Palearctic origins also are unclear and have no molecular support (Fig. 11). Both species are highly distant from any other taxa (Table 2).

Discussion

*Labiobaetis alahmadii* is morphologically similar to *L. glaucus* as well as with the Palearctic species *L. cleopatrae* (Thomas & Soldán, 1989) and *L. balcanicus* and the Afrotropical species *L. bous-soulius* (Gillies, 1993). The shape of the distolateral process of the second segment of the labial palp is of taxonomic importance to separate the different species: more elongated and curved in *L. potamoticus* (Fig. 8) and *L. balcanicus* whereas shorter and more rounded in *L. glaucus* (Fig. 39), *L. cleopatrae*, and *L. bous-soulius*. *L. alahmadii* differs from these four species by the presence of short spatulate setae on the dorsal margin of the tibia and tarsi (Fig. 26) (Gillies 1993; Lugo-Ortiz et al. 2000; Müller-Liebenau and Soldán 1981; Thomas and Soldán 1989).

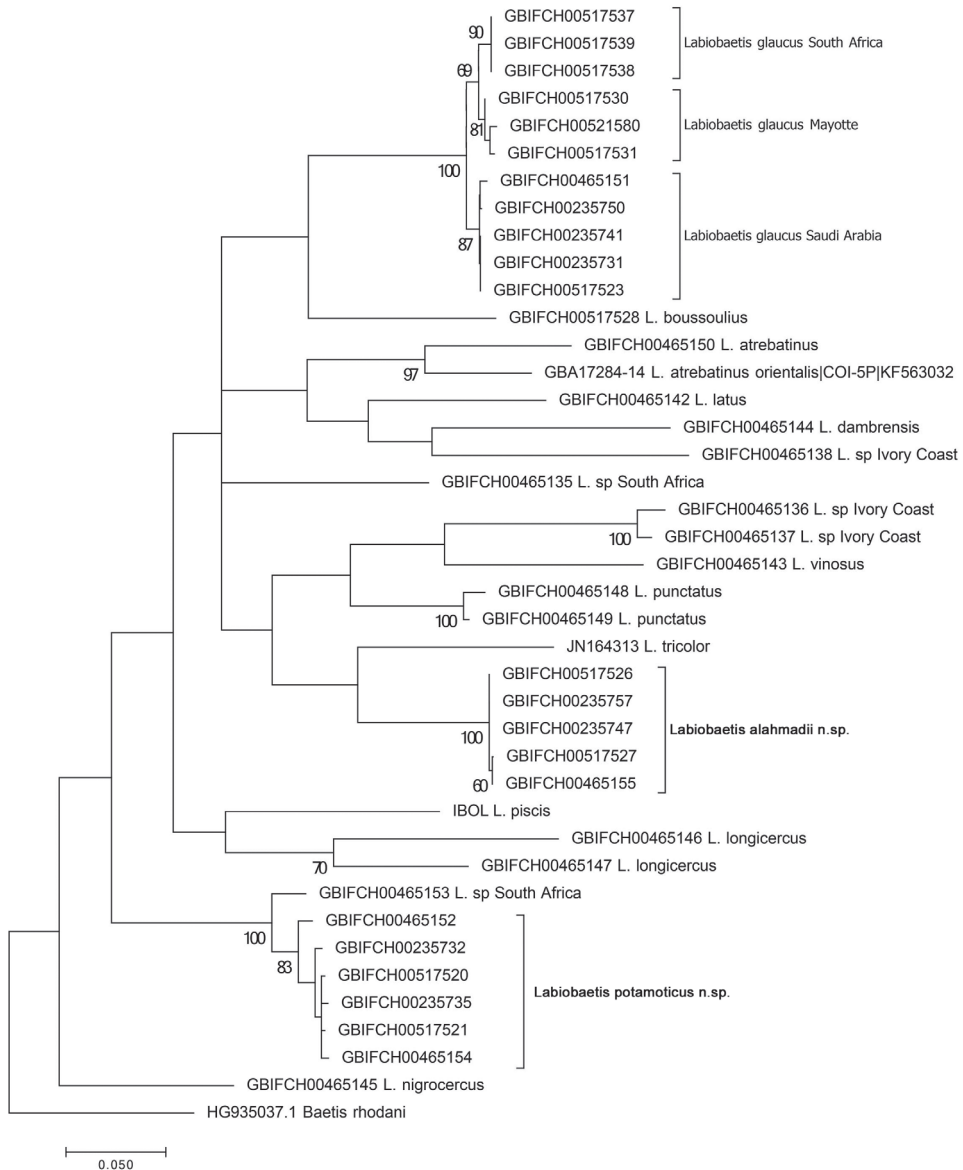
*Labiobaetis glaucus* is rather similar to *L. cleopatrae*. The two species differ by minor characters such as: the shape of the margin between prostheca and mola of the right and



left mandibles (straight in *L. glaucus* (Fig. 35) and curved in *L. cleopatrae*), the shape of the apex of the segment II of the maxillary palp (simple in *L. glaucus* (Fig. 38) and slightly curved with a small apical protuberance in *L. cleopatrae*), distal margin of tergites (triangular and pointed simple spines in *L. glaucus* (Fig. 43) and double triangular slightly worn spines in *L. cleopatrae*), tarsal claw less curved and less stout in *L. glaucus* (Fig. 40) than in *L. cleopatrae*. These statements are based on the comparison of material of *L. glaucus* stored in the collection of the MZL with the original description of *L. cleopatrae*. According to the variability already noticed in *L. glaucus* and the known synonyms of the species (Lugo-Ortiz et al. 2000; Lugo-Ortiz and McCafferty 1997), we cannot exclude that *L. cleopatrae* is a possible junior synonym of *L. glaucus*.

*Labiobaetis potamoticus* and *L. piscis* Lugo-Ortiz & McCafferty, 1997 share several important synapomorphies: shape of the distomedial projection of segment II of the labial palp (Fig. 8); spines of the paraprocts (Fig. 15) and setation of the inner margin of the femur (Lugo-Ortiz and McCafferty 1997). These two species differ primarily by the shape of segment III of the labial palp (more slender and with the inner margin more concave in *L. piscis*) and the setation of the ventral margin of femora (villopore reduced but always present in *L. potamoticus*, absent in *L. piscis*; setae almost as long in ventral margin than dorsal margin in *L. piscis*, much shorter in *L. potamoticus*). *Labiobaetis potamoticus* and *L. piscis* show a peculiar pattern on the abdominal tergites (Fig. 19). A similar pattern is also present in *L. tripunctatus* Gillies, 1994 and *L. punctatus* Gattolliat, 2001 (Gattolliat 2001; Gillies 1994). *Labiobaetis tripunctatus* has no hindwings and only six pairs of gills (Gillies 1994), while *L. punctatus* is strictly endemic to Madagascar and clearly differs by the excavation of the maxillary palp segment II, the spination of the distal margin of tergites and the setation of the labrum (Gattolliat 2001). Genetically, *L. potamoticus* does not appear related to either *L. piscis* or *L. punctatus*. No sequences are available for *L. tripunctatus*. The sister group of *Labiobaetis potamoticus* is an undescribed species from South Africa. The two species are morphologically similar but also exhibit some differences especially in the femoral setation. This tends to confirm that the two sister taxa are closely related species but are not conspecific. The status of this undescribed species will be discussed in a revision of *Labiobaetis* from the Afrotropical Region (Kaltenbach, comm. pers.). *Labiobaetis potamoticus* was initially identified as *L. balcanicus* (Thomas and Sartori 1989). The two species share important characters (paraproct, antenna, labrum, mandibles, abdominal pattern), but also clearly differ by the shape of the spines of the distal margin of abdominal tergites (apically pointed in *L. potamoticus* (Fig. 14) whereas apically rounded in *L. balcanicus*) and the shape of the distomedial projection of the segment II of the labial palp (shorter and more slender in *L. potamoticus* (Fig. 8)).

The imago of the different species of *Labiobaetis* are generally similar. The presence or absence of the hindwings and the shape of the genital plates are the main characters to separate the species (Gattolliat 2001). The male imago of *Labiobaetis potamoticus* cannot be separated from most other species of *Labiobaetis* with hindwings and broad, apically flat genital plates. *Labiobaetis glaucus* and *L. boussoulii* differ from most other species of the genus by the presence of a well-marked triangular expansion on the inner margin of the gonopods (Fig. 3 in Gillies 1993). A similar triangular expansion on



**Figure 53.** Maximum Likelihood (ML) consensus tree reconstructed for 40 haplotypes of *Labiobaetis* spp. Tree drawn to scale, branch lengths measured in number of substitutions per site, deeper nodes labelled above branches with Maximum Likelihood bootstrap support (>50%).

the inner margin of the gonopods is present, but even more pronounced, in *L. tricolor* Tshernova, 1928 (fig. 111a in Müller-Liebenau 1969).

The discovery of *L. glaucus* in KSA is rather unexpected despite that this species is widely distributed in Afrotropical Region (South Africa, Angola, Kenya, Lesotho, Namibia, and Zimbabwe (de Moor et al. 2000; Lugo-Ortiz et al. 2000); Comoros islands

(N. Mary unpublished data)). We examined specimens of populations from South Africa, Mayotte (Comoros Islands) and KSA. We found no clear morphological differences between them and they fully correspond to the original description and subsequent re-descriptions (Agnew 1961; Lugo-Ortiz and McCafferty 1997; Lugo-Ortiz et al. 2000). As already mentioned (Lugo-Ortiz et al. 2000), segment II of the labial palp may have a more or less developed distomedial projection and the abdominal pattern may differ between populations. Genetically, populations from South Africa, Mayotte and KSA form a highly supported monophyletic clade. Genetic distances between the three populations are clearly of intraspecific range especially if we consider that intermediate populations from East and North-East Africa are not included in the analysis.

### Key to the larvae of *Labiobaetis* known from KSA

- 1 Ventral margin of femora with abundant pointed setae (Fig. 10); labial palp with a moderately developed, relatively slender distolateral process (Fig. 8); margin of paraproct with abundant regular spines (Fig. 15); abdominal segments I–X brown with six ecru dots (Fig. 19) ..... ***Labiobaetis potamoticus* sp. n.**
- Ventral margin of femora with scarce short setae (Figs 25, 40); labial palp with a well-developed distolateral process (Figs 24, 39); margin of paraproct with 8–10 spines increasing in length (Figs 31, 44); abdominal segments I–X with different patterns..... **2**
- 2 Dorsal margin of tibiae and tarsi with a complete row of spatulate setae (Fig. 26); mesothorax medium brown with a double V-shaped yellow pattern; tergites I–VIII medium brown with two broad ecru spot; tergites IX and X yellow (Figs 32, 33)..... ***Labiobaetis alahmadii* sp. n.**
- Dorsal margin of tibiae and tarsi with scattered minute pointed setae; mesothorax (Fig. 40) uniformly brown (Fig. 47); tergites II–IV and VII–VIII medium brown with small ecru spot, other tergites yellow ..... ***Labiobaetis glaucus***

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# Systematics of Neotropical microteiid lizards (Gymnophthalmidae, Cercosaurinae), with the description of a new genus and species from the Andean montane forests

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## Abstract

Cercosaurine lizards (subfamily Cercosaurinae of the family Gymnophthalmidae) represent a substantial component of the reptile fauna in the Neotropics. Several attempts have been made to reconstruct the phylogenetic relationships within this group, but most studies focused on particular genera or regions and did not cover the subfamily as a whole. In this study, material from the montane forests of Peru was newly sequenced. In combination with all cercosaurine sequences available on GenBank, an updated phylogeny of Cercosaurinae is provided. Monophyly was not supported for three of the currently recognised genera (*Echinosaura*, *Oreosaurus*, and *Proctoporus*). The genus *Proctoporus* is formed by five monophyletic groups, which should be used in future taxonomic revisions as feasible entities. Recognition of two previously identified undescribed clades (Unnamed clades 2 and 3) was supported and yet another undescribed clade (termed here Unnamed clade 4), which deserves recognition as an independent genus, was identified herein. *Selvasaura brava*, a new genus and new species of arboreal gymnophthalmid lizard is described from the montane forests of the Pui Pui Protected Forest, Provincia de Chanchamayo, Región Junín, Peru. The new species is characterised by its small size (SVL 42.1–45.9 mm), slender body, smooth head shields, presence of paired prefrontal shields, fused anteriormost supraocular and anteriormost superciliary

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shields, transparent not divided lower palpebral disc, slightly rugose subimbricate rectangular dorsal scales in adults (slightly keeled in juveniles), distinctly smaller but non-granular lateral scales, smooth squared to rectangular ventral scales, and hemipenial lobes large, distinct from the hemipenial body. Phylogenetic affinities of the new genus to the other cercosaurine genera, as well as basal phylogenetic relationships between the other cercosaurine genera remain unresolved.

### Keywords

Andes, arboreality, phylogeny, reptile diversity, *Selvasaura* gen. n., *Selvasaura brava* sp. n., taxonomy

## Introduction

Gymnophthalmid lizards (family Gymnophthalmidae) represent a substantial component of the reptile fauna in the Neotropics. They are traditionally divided into subfamilies (sometimes referred to as tribes; Pellegrino et al. 2001a; Goicoechea et al. 2016), of which the cercosaurines (Cercosaurinae), with approximately 140 species, form the most species-rich clade. This subfamily is distributed throughout South America and the Andes represent the main centre of its diversity.

Phylogenetic analyses of cercosaurines based on genetic data started appearing after 2000 (Pellegrino et al. 2001a) and have since progressed considerably with respect to taxon sampling. Recent phylogenetic studies have brought new findings that resulted in taxonomic changes at the level of genera: some genera were synonymised, others resurrected, and new genera described. For example, Doan and Castoe (2005) proposed the new generic names *Potamites*, *Petracola*, and *Riama* for some species formerly ranked under *Neusticurus* and *Proctoporus*. Subsequently, Torres-Carvajal et al. (2016) described *Gelanesaurus* for some species that had formerly belonged to *Potamites*, after they found the latter to be paraphyletic. Most recently, Sánchez-Pacheco et al. (2017b) described *Andinosaura* and resurrected *Oreosaurus* to accommodate the polyphyly of *Riama*. At the moment, Cercosaurinae consists of 15 formally described genera: *Anadia*, *Andinosaura*, *Cercosaura*, *Echinosaura*, *Euspondylus*, *Gelanesaurus*, *Macropholidus*, *Neusticurus*, *Oreosaurus*, *Petracola*, *Pholidobolus*, *Placosoma*, *Potamites*, *Proctoporus*, and *Riama*. Recently, Torres-Carvajal et al. (2016) identified three more evolutionary lineages within cercosaurines at the level of genera, some of which are still awaiting formal descriptions.

Despite the undeniable advances in untangling the cercosaurine tree, there are still genera and species for which monophyly has failed to be proven (Torres-Carvajal et al. 2016). Moreover, basically every new phylogenetic study published to date brought evidence for cryptic species being present (Goicoechea et al. 2012; Torres-Carvajal et al. 2015, 2016; Sánchez-Pacheco et al. 2017b). The amount of overall cryptic diversity within all cercosaurines is not straightforward to gauge because most previous phylogenies had a narrow focus on particular genera (Doan et al. 2005; Goicoechea et al. 2012; Torres-Carvajal and Maffa-Endara 2013; Aguirre-Peñafiel et al. 2014) or regions (Kok et al. 2012), or had species represented by a single sample (Pellegrino et al. 2001b; Kok 2015). The most comprehensive phylogenetic reconstructions of higher

clades were published recently by Torres-Carvajal et al. (2015, 2016), Goicoechea et al. (2016), and Sánchez-Pacheco et al. (2017b). The aim of this study was to contribute to the phylogeny of Cercosaurinae by inferring the phylogenetic placement of new material collected during recently conducted surveys with a special emphasis on the Pui Pui Protected Forest in Peru and its surroundings.

The Pui Pui Protected Forest (Bosque de Protección Pui Pui, hereafter PPPF) is located in the Selva Central of Peru and covers 60,000 hectares (30% montane forest, 70% puna habitats) between 1700 and 4500 m a.s.l. (SERNANP 2010). We surveyed the herpetofauna of the PPPF in upper montane forests and high Andean grasslands (puna) in 2012, 2013, and 2014 in order to record the amphibian and reptile species richness and to evaluate their conservation status. We have discovered several new species of amphibians and reptiles, e.g., frogs of the genera *Pristimantis*, and *Phrynopus*, and lizards of the genera *Euspondylus*, *Potamites*, and *Proctoporus* (Lehr and Moravec 2017; Lehr et al. 2017a, b; work in progress), which suggests that biodiversity of this region is still far from being fully inventoried. Additionally, the material collected in PPPF contained a new gymnophthalmid lizard that was morphologically difficult to assign to the currently recognised genera.

## Materials and methods

### Material for phylogenetic analyses

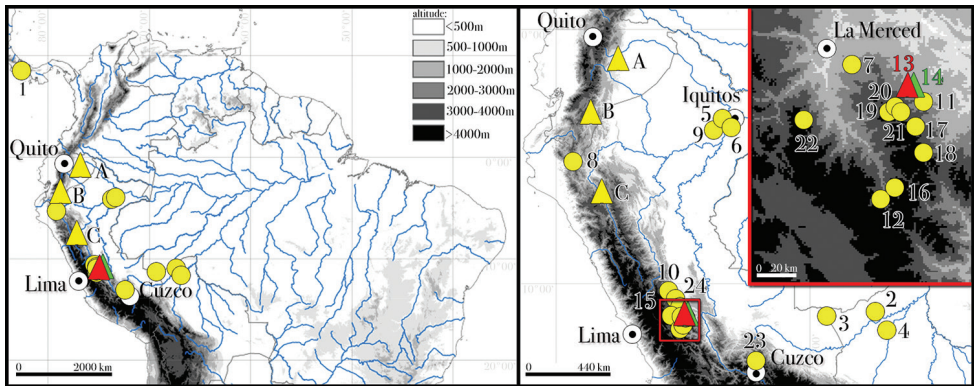
We assembled a genetic dataset that included sequences for the subfamily Cercosaurinae available on GenBank. Additionally, we newly sequenced 38 samples of nine genera (*Anadia*, *Cercosaura*, *Euspondylus*, *Pholidobolus*, *Potamites*, *Proctoporus*, the new genus described herein and two unnamed genera; Table 1, Suppl. material 1: Fig. S1) deposited in the SMF, NMP, IWU, JCM, and MUSM collections (for collection acronyms see below). All genera that are presently recognised to form the content of the subfamily (Sánchez-Pacheco et al. 2017b) were included in the dataset, represented as follows: *Anadia* (3 of 18 described species included), *Andinosaura* (9 of 11 described species included), *Cercosaura* (10 of 14 described species included), *Echinosaura* (5 of 7 described species included), *Euspondylus* (1 of 11 described species included), *Gelanesaurus* (2 of 2 described species included), *Macropholidus* (3 of 4 described and one yet undescribed species included), *Neusticurus* (2 of 5 described species included), *Oreosaurus* (4 of 6 described and one yet undescribed species included), *Petracola* (2 of 4 described species included), *Pholidobolus* (8 of 9 described and one yet undescribed species included), *Placosoma* (2 of 4 described species included), *Potamites* (6 of 7 described species included), *Proctoporus* (15 of 17 described and eight yet undescribed species included), *Riama* (14 of 15 described and three yet undescribed species included). We also included the unnamed clades identified by Torres-Carvajal et al. (2016) that could not be assigned to any described genus.



**Table 1.** List of material newly sequenced for this study. Sample codes are those shown in tree figures. Locality numbers refer to those in Fig. 1. Upper indexes H and P denote holotype and paratypes, respectively.

Species	Sample code	Voucher code	GenBank Accession				Locality (Locality no.)	Lat	Long
			12S	16S	cytb	cmos			
<i>Anadia ocellata</i>	SMF90095	SMF 90095	MH579588	MH579625	MH579659	-	Panama, Chiriquí, Santa Clara (1)	8.833	-82.782
<i>Cercosaura argulus</i>	184	NMP6V 72184	MH579589	MH579626	MH579660	MH579686	Bolivia, Pando, Nacebe (2)	-11.000	-67.417
<i>Cercosaura eigenmanni</i>	609	NMP6V 72609	MH579590	MH579627	-	-	Bolivia, Pando, Biocanica (3)	-11.133	-69.367
<i>Cercosaura eigenmanni</i>	112	NMP6V 73112	MH579591	MH579628	MH579661	-	Bolivia, Pando, Canada (4)	-11.750	-67.133
<i>Cercosaura oshaughnesyi</i>	155/2	NMP6V 71155/2	MH579592	-	-	-	Peru, Loreto, 21 km W of Iquitos (5)	-3.787	-73.429
<i>Cercosaura oshaughnesyi</i>	160/1	NMP6V 71160/1	MH579593	MH579629	MH579662	MH579687	Peru, Loreto, Puerto Almendras (6)	-3.829	-73.376
<i>Euspondylus excelsum</i>	IWU234	IWU 234	MH579594	MH579630	-	MH579688	Peru, Junín, B.P. Pui Pui surr. (7)	-11.096	-75.228
<i>Pholidobolus</i> sp.1	JCM238	JCM 238	MH579595	MH579631	MH579663	MH579689	Peru, Cajamarca, S.N. Tabaconas (8)	-5.157	-79.273
<i>Pholidobolus</i> sp.1	JCM239	JCM 239	MH579596	MH579632	MH579664	MH579690	Peru, Cajamarca, S.N. Tabaconas (8)	-5.157	-79.273
<i>Pholidobolus</i> sp.1	843	MUSM 31843	MH579597	MH579633	MH579665	MH579691	Peru, Cajamarca, S.N. Tabaconas (8)	-5.157	-79.273
<i>Pholidobolus ulisei</i>	JCM310	JCM 310	MH579598	MH579634	MH579666	MH579692	Peru, Cajamarca, S.N. Tabaconas (8)	-5.157	-79.273
<i>Potamites ecleopus</i>	186/1	NMP6V 73186/1	MH579599	MH579635	-	-	Peru, Loreto, Anguilla (9)	-3.913	-73.661
<i>Potamites ecleopus</i>	186/2	NMP6V 73186/2	MH579600	MH579636	-	-	Peru, Loreto, Anguilla (9)	-3.913	-73.661
<i>Proctoporus chasqui</i>	IWU24	MUSM 31108	MH579601	MH579637	MH579667	MH579693	Peru, Pasco, N.P. Yanachaga Chemillen (10)	-10.395	-75.482
<i>Proctoporus chasqui</i>	IWU25	MUSM 31109	MH579602	MH579638	MH579668	MH579694	Peru, Pasco, N.P. Yanachaga Chemillen (10)	-10.395	-75.482
<i>Proctoporus chasqui</i>	IWU50	MUSM 31123	MH579603	MH579639	MH579669	MH579695	Peru, Pasco, N.P. Yanachaga Chemillen (10)	-10.395	-75.482
<i>Proctoporus chasqui</i>	IWU82	MUSM 31142	MH579604	MH579640	MH579670	MH579696	Peru, Pasco, N.P. Yanachaga Chemillen (10)	-10.395	-75.482
<i>Proctoporus chasqui</i>	IWU133	MUSM 31172	MH579605	MH579641	MH579671	MH579697	Peru, Junín, B.P. Pui Pui (11)	-11.255	-74.892
<i>Proctoporus spinalis</i>	IWU119	MUSM 31162	MH579607	MH579643	-	-	Peru, Junín, B.P. Pui Pui surr. (12)	-11.712	-75.089
<i>Proctoporus spinalis</i>	IWU120	IWU 120	MH579608	MH579644	-	MH579699	Peru, Junín, B.P. Pui Pui surr. (12)	-11.712	-75.089
<i>Proctoporus</i> sp.4	IWU358	MUSM 32727	MH579606	MH579642	MH579672	MH579698	Peru, Junín, B.P. Pui Pui, Rio Bravo (13)	-11.211	-74.958
<i>Selvasaura braui</i> gen. et. sp. n.	IWU339	MUSM 32718 <sup>P</sup>	MH579609	MH579645	MH579673	MH579700	Peru, Junín, B.P. Pui Pui, Rio Bravo (14)	-11.208	-74.955

Species	Sample code	Voucher code	GenBank Accession				Locality (Locality no.)	Lat	Long
			12S	16S	cytb	cmos			
<i>Selvasauna brava</i> gen. et. sp. n.	IWU340	NMP6V 75655 <sup>P</sup>	MH579610	MH579646	MH579674	MH579701	Peru, Junín, B.P. Pui Pui, Rio Bravo (14)	-11.208	-74.955
<i>Selvasauna brava</i> gen. et. sp. n.	IWU380	NMP6V 75653 <sup>P</sup>	MH579611	MH579647	MH579675	MH579702	Peru, Junín, B.P. Pui Pui, Rio Bravo (13)	-11.211	-74.958
<i>Selvasauna brava</i> gen. et. sp. n.	IWU381	MUSM 32738 <sup>H</sup>	MH579612	MH579648	MH579676	MH579703	Peru, Junín, B.P. Pui Pui, Rio Bravo (13)	-11.211	-74.958
<i>Selvasauna brava</i> gen. et. sp. n.	IWU382	NMP6V 75654 <sup>P</sup>	MH579613	MH579649	MH579677	MH579704	Peru, Junín, B.P. Pui Pui, Rio Bravo (13)	-11.211	-74.958
Unnamed clade 2	IWU57	MUSM 31127	-	MH579650	MH579678	MH579705	Peru, Pasco, Bosque de Shollet (15)	-10.676	-75.322
Unnamed clade 2	IWU114	MUSM 31160	MH579614	-	MH579679	MH579706	Peru, Junín, B.P. Pui Pui surr. (16)	-11.665	-75.037
Unnamed clade 2	IWU165	MUSM 31188	MH579615	MH579651	MH579680	MH579707	Peru, Junín, B.P. Pui Pui, Tarhuish (17)	-11.378	-74.937
Unnamed clade 2	IWU287	MUSM 32973	MH579616	-	-	MH579708	Peru, Junín, Toldopampa (18)	-11.484	-74.891
Unnamed clade 2	IWU288	NMP6V 75084	MH579617	MH579652	MH579681	MH579709	Peru, Junín, Toldopampa (18)	-11.484	-74.891
Unnamed clade 2	IWU296	MUSM 31978	MH579618	MH579653	MH579682	-	Peru, Junín, B.P. Pui Pui, Hatunpata (19)	-11.302	-75.026
Unnamed clade 2	IWU320	MUSM 31991	MH579619	MH579654	MH579683	MH579710	Peru, Junín, B.P. Pui Pui, Trancapampa (20)	-11.297	-75.013
Unnamed clade 2	IWU325	MUSM 31994	MH579620	MH579655	MH579684	MH579711	Peru, Junín, B.P. Pui Pui, Antuyo Bajo (21)	-11.315	-74.993
Unnamed clade 2	90	NMP6V 75090	MH579621	MH579656	-	-	Peru, Junín, Maraynioc (22)	-11.346	-75.445
Unnamed clade 2	91	NMP6V 75091	MH579622	MH579657	-	-	Peru, Junín, Maraynioc (22)	-11.346	-75.445
Unnamed clade 4	EL409	MUSM 27610	MH579623	-	-	-	Peru, Cusco, Alfamayo (23)	-13.066	-72.416
Unnamed clade 4	ML1352	MUSM 25345	MH579624	MH579658	MH579685	-	Peru, Pasco, N.P. Yanachaga Chemillen surr. (24)	-10.658	-75.298



**Figure 1.** Map showing localities of samples newly sequenced for this study. Locality numbers correspond to those in Table 1. Localities of the new genus described here, *Selvasaura* gen. n., are marked with triangles; red triangle indicates the type locality of its type species, *S. brava* sp. n.; green triangle locality of paratypes MUSM 32718 and NMP6V 75655; yellow triangles localities published by Torres-Carvajal et al. (2016): **A** Provincia de Napo, Wildsumaco Wildlife Sanctuary, Ecuador **B** Provincia de Zamora Chinchipe, El Pangui, Ecuador **C** region San Martin, Provincia Mariscal Cáceres, Laurel, Peru. White circles denote major cities.

To avoid confusion, sequences of all loci were matched with the sample or museum code of the specimen to which they belonged as was used in the original reference (when available). As Cercosaurinae still contains non-monophyletic taxa (see below), we avoided combining sequences of more individuals into chimeric samples, even if they putatively belonged to the same species. As a result, each terminal in the tree represents an existing voucher specimen or tissue sample (Suppl. material 1: Table S1). For those species whose monophyly has been previously confirmed, we included only one (or a few) samples (e.g., *Cercosaura ocellata*; Sturaro et al. 2017).

## DNA extractions, amplifications, and sequencing

Genomic DNA was extracted from ethanol-preserved tissue samples using a Geneaid kit. We PCR-amplified up to four loci, three from the mitochondrial DNA (mtDNA): 12S rRNA (12S), 16S rRNA (16S), cytochrome b (cytb), and the oocyte maturation factor MOS (cmos) from the nuclear DNA. Sanger sequencing of both the forward and reverse strands was carried out at Macrogen (Amsterdam, The Netherlands) using the same primers as for the PCRs. Details on the primers and amplification conditions are given in Table 2. Newly produced sequences were edited and contigs assembled in Geneious v.6 (Kearse et al. 2012). MAFFT v.7 (Katoh and Standley 2013) was used to align all loci individually with the ‘auto’ option selected for all. The Q-INS-I option that considers the secondary structure of RNA and that would therefore have been more suitable for the 12S and 16S datasets could not be used as the number of sequences in both datasets (332, respectively 343) exceeded the allowed limit. The alignments of cytb and cmos were translated into amino acids and no stop codons were detected, suggesting we did not amplify pseudo-

**Table 2.** Primers and PCR conditions used in this study. Amplicon length refers to the length of the fragment amplified. PCR cycle shows temperatures and times of steps in the cycle itself and not the initial denaturation (94 °C for 5 min) and final elongation (72 °C for 5–10 min) steps.

Gene	Primer	Primer sequence	Amplicon length (bp)	PCR cycle	Primer source
12S rRNA	12Sa	AAACTGGGATTAGATACCCCACTAT	370–381	94 °C (30sec), 48 °C (45sec), 72 °C (1min), 35 cycles	Kocher et al. (1989)
	12Sb	GAGGGTGACGGGCGGTGTGT			
16S rRNA	16SL1	CGCCTGTTTAAACAAAAACAT	449–455	94 °C (1min), 47 °C (45sec), 72 °C (1min), 40 cycles	Palumbi et al. (1991)
	16SH1	CCGGTCTGAACTCAGATCACGT			
cytb	Cytb1	CCATCCAACATCTCAGCATGATGAAA	307	94 °C (35sec), 45–46 °C (35sec), 72 °C (1min 30sec), 30 cycles	Kocher et al. (1989)
	Cytb2	CCCTCAGAATGATATTTGTCTCTCA			
cmos	FUF	TTTGGTTCKGTCTACAAGGCTAC	415	94 °C (30sec), 53 °C (45sec), 72 °C (1min 30sec), 35 cycles	Gamble et al. (2008)
	FUR	AGGGAACATCCAAAGTCTCCAAT			

genes. We applied Gblocks (Castresana 2000) to the 12S and 16S alignments to trim regions that aligned ambiguously. We trimmed the tRNA-end of the ND4 and used only its coding part for the analyses. The final concatenated dataset for the phylogenetic analyses consisted of 2217 bp composed of the following loci with lengths given in parentheses: 12S (325 bp), 16S (454 bp), cytb (307 bp), ND4 (694 bp), and cmos (437 bp).

Phylogenetic analyses

The dataset was partitioned by gene. Models of sequence evolution were assessed for each partition by Partition Finder v.1.1 (Lanfear et al. 2012) with the following settings: branch lengths linked, models available in BEAST evaluated, model selection based on BIC. The models identified as most suitable were as follows: GTR+I+Γ for the 12S, 16S, and ND4, SYM+I+Γ for cytb and HKY+I+Γ for cmos. As outgroups, we used 21 species representing the genera *Rhachisaurus*, *Gymnophthalmus*, *Alopoglossus*, *Riolama*, *Ecpleopus*, and *Bachia* that are known to be closely related to the cercosaurine genera but not being part of the subfamily (Pyron et al. 2013; Kok 2015). The outgroup species are also listed in Suppl. material 1: Table S1. In total the dataset consisted of 357 samples, of which 26 represented the outgroup taxa.

Phylogenetic analyses were conducted by means of maximum likelihood (ML) and Bayesian inference (BI). The ML analysis was conducted using RAxML-HPC2 v.8.2.9 (Stamatakis 2014) with a heuristic search that included 100 random addition replicates and 1000 thorough bootstrap pseudoreplications. We applied the GTR+CAT model to all partitions as the CAT model has been shown to be a faster and computa-

tionally less demanding alternative to the  $\Gamma$  model (Stamatakis 2006; Stamatakis et al. 2008). We skipped the +I parameter because the 25 default rate categories of the CAT model account for potentially invariant sites (Stamatakis 2006).

The Bayesian analyses were conducted using MrBayes v.3.2 (Ronquist et al. 2012) and BEAST 2.4.5 (Bouckaert et al. 2014). MrBayes settings were the following: GTR as the preferred substitution model for the mtDNA genes and HKY for cmos, invariable proportions of among-site rate variation and a gamma-distributed rate parameter applied, ploidy set to haploid for the mtDNA genes, four parallel runs ran each with four chains, number of generations set to  $10^8$  with a  $10^5$  sampling frequency, 10% of trees discarded as burnin. Stationarity was confirmed by the value of average standard deviations of the split frequencies being lower than 0.01. Convergence of the four runs was confirmed by the values of PSRF (potential scale reduction factor) reaching 1.00. Estimated parameter values were inspected and a 50% majority-rule consensus tree was generated in MrBayes.

The second Bayesian analysis was run in BEAST. In order to avoid over-parameterisation caused by the large size of the dataset, we applied the HKY model for all partitions instead of the GTR as preferred by PartitionFinder. The  $\Gamma$  parameter was selected to have four categories and shape estimated. We applied the Yule process tree prior with uniformly distributed birth rate (lower: 0, upper: 1000) and an independent relaxed uncorrelated lognormal clock prior for each partition. Ambiguities in the cmos alignment coded by the IUPAC ambiguity codes were accounted for. Clock rates were set to have lognormal distributions with the mean = 1 and st. dev. = 1.25 for the mtDNA genes and mean = 0 and st. dev. = 1.0 for the cmos relative to the first partition of the concatenated alignment, which was the 12S. Standard deviation of the clock parameter (among-lineage rate heterogeneity) was for all partitions estimated with an exponential distribution with the mean = 1. Four independent runs were made, each for  $2.5 \times 10^8$  MCMC generations and parameters logged every  $10^5$  generation. 10% of sampled trees were discarded from each analysis as burnin. Stationarity, convergence of the runs, and effective sample sizes (ESS) of all parameters were inspected in Tracer v.1.5 (Rambaut and Drummond 2007). Post-burnin posterior trees were combined using LogCombiner v.2.4.5 and the maximum clade credibility (MCC) tree with mean node heights was identified and posterior probabilities calculated using TreeAnnotator v.1.7.5.

All phylogenetic analyses were run through the CIPRES Science Gateway (Miller et al. 2010). Tree nodes were considered strongly supported when they received ML bootstrap support  $\geq 70\%$  and posterior probability (pp) values inferred by the two BI analyses  $\geq 0.95$ .

Genetic distances between the clades of cercosaurines were calculated for all genetic markers analysed except cytb using MEGA 6 (Tamura et al. 2013) with the pairwise deletion option selected.

## **Morphological characters**

The format of the descriptions and terminology of the morphological characters follow mostly Oftedal (1974), Chávez et al. (2017), and Sánchez-Pacheco et al. (2017b).

Specimens were fixed in 96% and stored in 70% ethanol. Sex and maturity of specimens were identified through dissection of gonads. Specimens with SVL  $\leq 30.2$  mm were considered juveniles. The following metric characters were taken using a digital caliper and dissecting microscope (to the nearest 0.1 mm):

<b>SVL</b>	snout-vent length – distance from the snout tip to cloaca;
<b>HL</b>	head length – distance from the snout tip to the angle of jaw;
<b>HW</b>	head width – greatest width of the head;
<b>HD</b>	head depth – greatest depth of the head;
<b>TL</b>	tail length – distance from cloaca to the tail tip, if original;
<b>E–N</b>	eye-snout distance – straight distance from the snout tip to anterior corner of eye;
<b>FLL</b>	forelimb length – from axilla to tip of distal claw;
<b>HLL</b>	hindlimb length – from groin to tip of distal claw;
<b>AGD</b>	axilla-groin distance – distance between limbs;
<b>hemipenis length</b>	distance from hemipenial base to distal margin of hemipenial lobes.

Meristic and qualitative pholidotic characters were counted and evaluated as follows: number of supralabials from the rostral to the mouth corner, last labial defined by its considerably larger size compared with the posteriorly adjacent shields; dorsal scales by the number of transverse rows of dorsal scales from the third row behind the interparietal to the level of the rear edge of the hindlimb; ventral scales, the number of transverse rows of ventral scales (from collar to the anterior row of anal scales); lateral scales, the number of longitudinal rows of considerably smaller lateral scales lying between larger dorsal and ventral scales at midbody; scales around midbody; preanal plates are the number of large plates in the posterior row of anal scales; number of lamellae under Finger IV including the number of single and divided lamellae (left/right, lamellae divided into segments counted as one individual lamella); number of lamellae under Toe IV refers to the number of single and divided lamellae (left/right, lamellae divided into segments counted as one individual lamella); number of preanal pores (left/right).

Description of colouration in life was based on field notes and photographs. Collection acronyms are: **MUSM** Museo de Historia Natural Universidad Nacional Mayor de San Marcos, Lima, Peru; **NMP6V** National Museum Prague, Prague, Czech Republic; **SMF** Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt, Germany. Field number codes are: **IWU** Illinois Wesleyan University; **JCM** Juan Carlos Cusi collection. Threat status was evaluated using the IUCN criteria (2016). High-resolution versions of photographs presented in this article and additional pictures of the type specimens have been uploaded to MorphoBank (project number: 3136; <http://www.morphobank.org>) where they are available for download.



## Drawings and maps

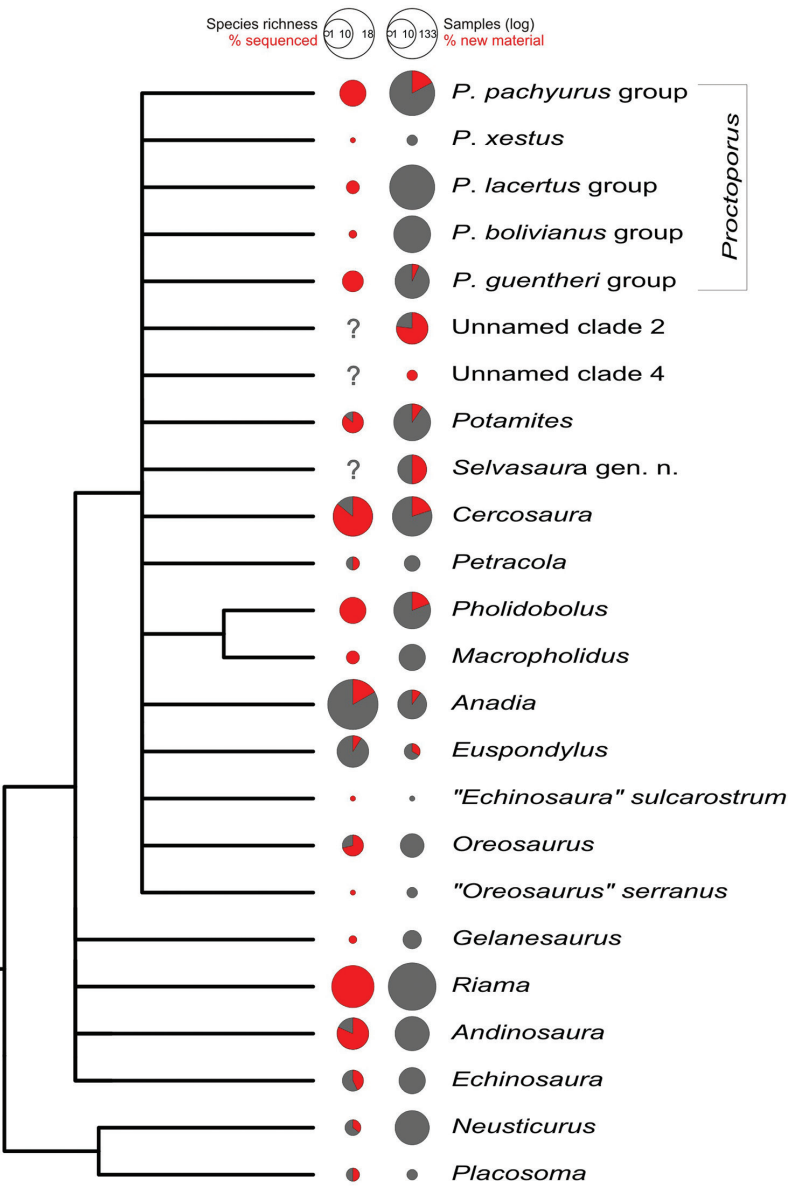
All drawings were made by the senior author using a stereomicroscope and a camera lucida. Maps were made with QGIS (Quantum GIS Development Team 2014).

## Results

### Phylogenetic analyses

All three analyses performed here resulted in topologies concordant with previous studies (Torres-Carvajal et al. 2016, Sánchez-Pacheco et al. 2017b). The subfamily Cercosaurinae was monophyletic, although the pp support from the BEAST analysis did not exceed the 0.95 threshold (ML bootstrap: 100; MrBayes pp: 1; BEAST pp: 0.92; nodal support values in the same order hereafter; Suppl. material 1: Figs S1–S3). Most of the cercosaurine genera were strongly supported in all the analyses: *Anadia* (100, 1, 1); *Andinosaura* (86, 1, 1); *Cercosaura* (78, 1, 1); *Euspondylus* (98, 1, 1); *Gelanesaurus* (100, 1, 1); *Macropholidus* (92, 1, 1); *Neusticurus* (98, 1, 1); *Petracola* (93, 1, 1); *Pholidobolus* (63, 0.97, 0.96); *Placosoma* (100, 1, 1); *Potamites* (93, 1, 1); *Riama* (100, 1, 1). Two undescribed clades recovered by Torres-Carvajal et al. (2016) and referred to as Unnamed clade 2 and 3 were also strongly supported here: Unnamed clade 2 (100, 1, 1), Unnamed clade 3 (96, 1, 1), the latter being part of the ramification of the new genus described herein. Additionally, we identified yet another clade that may deserve recognition as an independent genus and that we term tentatively Unnamed clade 4 (support 91, 1, 1) and whose phylogenetic affinities to the other genera remained unclear due to low support (Figs 2, 3). Unnamed clade 4 consisted of two samples collected by EL and collaborators in cloud forests in the surroundings of Alfamayo (Region of Cuzco, Peru) and in a montane forest close to the National Park Yanachaga-Chemillén (Region Pasco, Peru).

Monophyly was not supported for three of the described genera (Fig. 3). First, one species of the genus *Echinosaura*, *E. sulcarostrum*, did not cluster with the remaining four species of the genus included in the dataset and which formed a clade (100, 1, 1; as previously found by Torres-Carvajal et al. 2016). It was instead topologically closest to *Euspondylus*, although support of this sister relationship was low in two of the analyses (35, 0.96, 0.92). Second, monophyly of the recently resurrected genus *Oreosaurus* (Sánchez-Pacheco et al. 2017b) was also questionable. Although the topology of the BEAST tree shows all *Oreosaurus* species to form one group, monophyly of this group was not supported in any of the analyses and the phylogenetic position of *O. serranus* was unstable across the analyses. The other four *Oreosaurus* species that were included in the dataset formed a clade (80, 1, 1). Third, in concordance with Torres-Carvajal et al. (2016), but contrary to Goicoechea et al. (2012) and Sánchez-Pacheco et al. (2017b), monophyly of *Proctoporus* was found to be questionable as the genus was supported only in the



**Figure 2.** Phylogenetic tree showing relationships between cercosaurine genera or, in cases when genera were not recovered as monophyletic, their major lineages. The tree is a strict consensus tree based on the results of three analytical approaches undertaken: ML, MrBayes, BEAST. The 24 lineages shown were supported in all three phylogenetic analyses. Relationships between genera are shown as dichotomies only for nodes that were strongly supported in all three analyses; otherwise, nodes were collapsed into polytomies to emphasise how little we can tell about the phylogeny of the subfamily Cercosaurinae with the data currently available. Outgroups are not depicted. For a tree that shows variability within genera see Fig. 3, for full trees see Fig. S1–S3. The pie charts on the right show i) species richness of the genera indicated by circle size with the proportion of species included in the analyses highlighted in red (left column), and ii) number of samples (log scale) available for each genus indicated by circle size with the proportion of material newly sequenced in this study in red (right column).

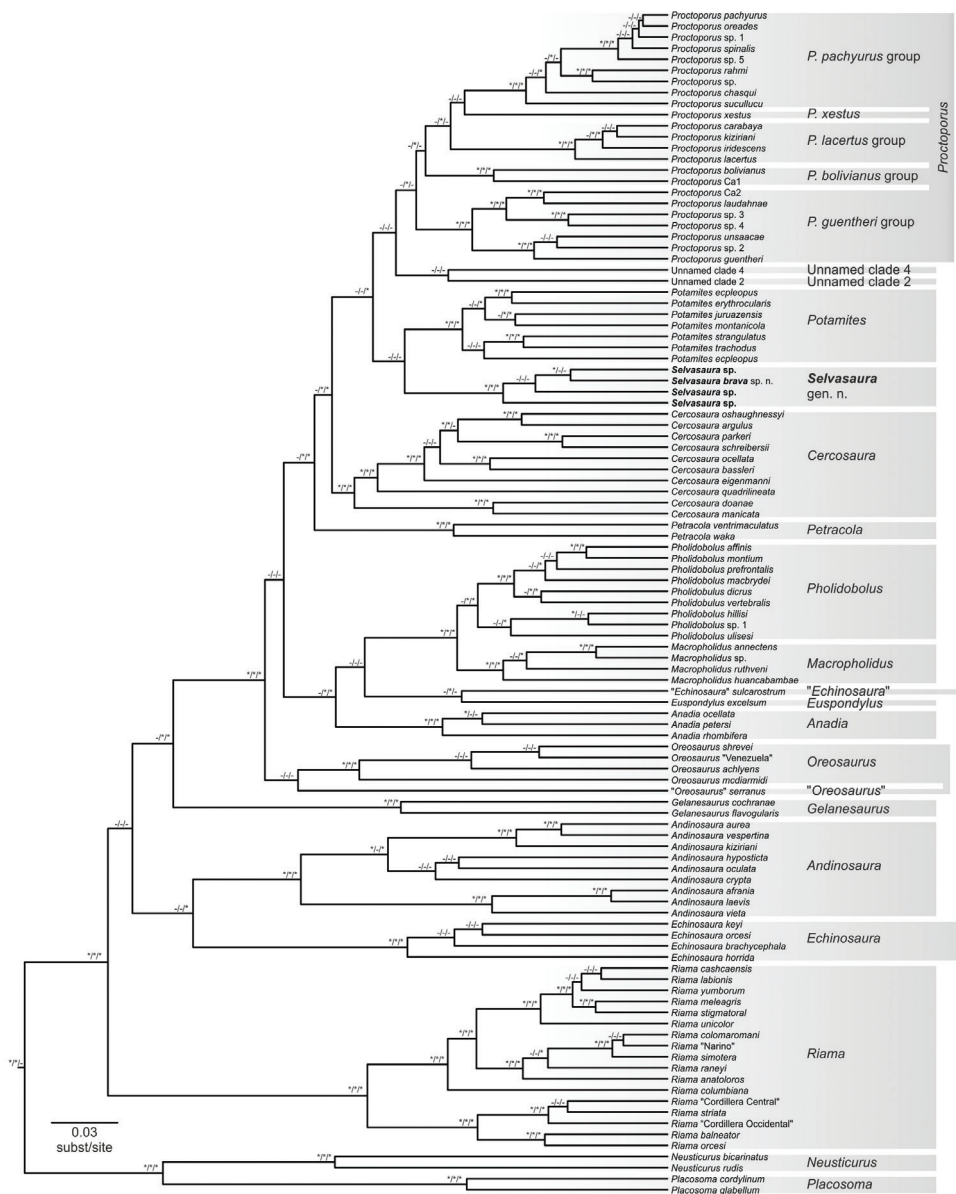
MrBayes analysis while it received no support in the ML and BEAST analyses (13, 0.95, 0.51). The *Proctoporus* species formed five well supported groups: i) a clade of *P. pachyurus*, *P. oreades*, *P. spinalis*, *P. rahmi*, *P. chasqui*, *P. sucullucu*, and three yet undescribed species (support 88, 1, 1; termed *Proctoporus pachyurus* group); ii) a clade comprising a single species, *P. xestus* (98, 1, 1); iii) a clade of *P. carabaya*, *P. kiziriani*, *P. iridescens*, *P. lacertus* (98, 1, 1; termed *Proctoporus lacertus* group); iv) a clade of *P. bolivianus* and one undescribed species (93, 1, 1; termed *Proctoporus bolivianus* group); v) a clade of *P. unsaaciae*, *P. guentheri*, *P. laudahnae*, and four undescribed species (96, 1, 1; termed *Proctoporus guentheri* group). Mutual relationships between these five *Proctoporus* groups as well as their relationships to the other cercosaurine genera remained unresolved.

Higher-level relationships between the cercosaurine genera were difficult to infer for the generally low node support at this phylogenetic depth. Only a few clades could be identified that were common to the three different phylogenetic analyses undertaken (Fig. 2). In all analyses, *Neusticurus* was sister to *Placosoma* (100, 1, 1) and the clade of these two was sister to all the remaining genera of Cercosaurinae (76, 0.99, 1). Of the remaining genera, only the sister pair *Pholidobolus*/*Macropholidus* was recovered in all analyses with strong support (100, 1, 1) and a large clade comprising the five *Proctoporus* groups, *Potamites*, *Cercosaura*, *Petracola*, *Pholidobolus*, *Macropholidus*, *Anadia*, *Euspondylus*, “*Echinosaura*” *sulcarostrum*, both *Oreosaurus* lineages, Unnamed clades 2 and 4, and the new genus described herein (100, 1, 1). Otherwise, no other genera clustered into clades that would be supported by all three phylogenetic approaches.

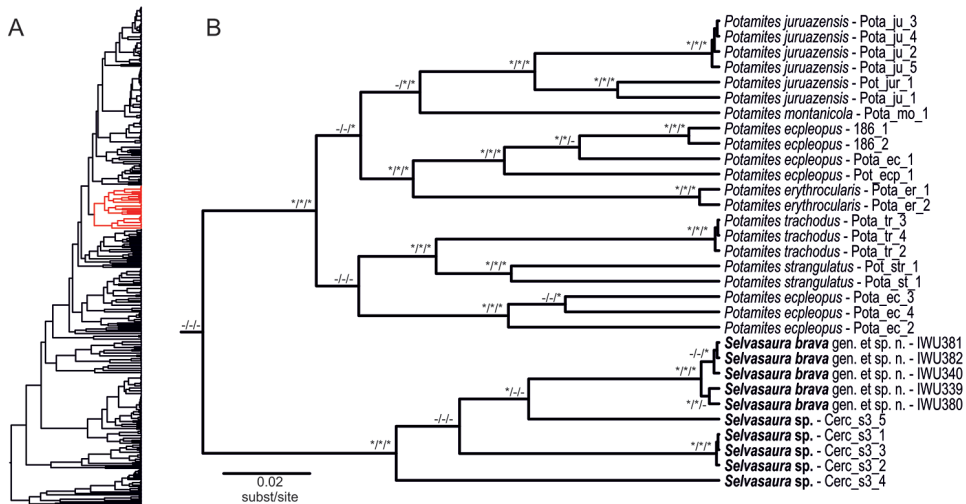
Phylogenetic affinities of the new genus described herein to the other cercosaurine genera remained unresolved (Fig. 3). Although it was reconstructed as a sister lineage to the genus *Potamites* in all analyses, the topology was not supported in any of them (47, 0.55, 0.89). Within the genus, all analyses unambiguously identified four well genetically differentiated lineages (Fig. 4), albeit their mutual relationships remained unresolved due to the lack of nodal support. The first was a cluster of five samples from the PPPF, Peru that represent the new species described below, the second of one sample from El Panguí, Zamora-Chinchipe Province, Ecuador (voucher QCAZ 12891; sample code Cerc\_s3\_5), the third of three samples from Laurel, Mariscal Cáceres Province, Peru (vouchers CORBIDI 15117–19; sample codes Cerc\_s3\_1–3), and the fourth of one sample from Wildsumaco Wildlife Sanctuary, Napo Province, Ecuador (voucher QCAZ 12798; sample code Cerc\_s3\_4) (Fig. 1). The three latter lineages were published by Torres-Carvajal et al. (2016), who also found the weakly supported sister relationship between *Potamites* and the new genus.

## Morphological characters

The examined morphological characters were used for comparisons with other genera and for the formal descriptions of the new genus and species provided below.



**Figure 3.** Maximum clade credibility tree for 107 species (both described and candidate) of the subfamily Cercosaurinae from the BEAST analysis. The dataset for the analysis contained 357 samples with most species being represented by multiple samples, but for visual purposes only one sample was retained for each species in this tree. Nodal support is shown in the ML/MrBayes/BEAST order; supported nodes are marked with asterisks, unsupported with dashes. Monophyletic groups at the genus level are highlighted by grey rectangles. Vertical grey bars connect species that supposedly belong to one genus, but whose monophyly was not supported in any of the phylogenetic analyses: the genera *Proctoporus*, *Echinosaure*, and *Oreosaurus*. Outgroups are not depicted. For a full BEAST tree see Fig. S3.



**Figure 4.** **A** Maximum clade credibility tree of Cercosaurinae based on the BEAST analysis with the position of *Selvasaura* gen. n. and *Potamites* highlighted in red. **B** A close-up of the red part of the tree in the left showing the phylogenetic relationships between and within *Selvasaura* gen. n. and *Potamites*. Nodal support is shown in the ML/MrBayes/BEAST order; supported nodes are marked with asterisks, unsupported with dashes. Note that the basal node in the inset is not supported and that the sister relationship of the two genera may not be real.

## Taxonomy

### Family Gymnophthalmidae Fitzinger, 1826

#### Subfamily Cercosaurinae Gray, 1838

#### Genus *Selvasaura* gen. n.

<http://zoobank.org/71A0F024-36F5-4420-BEEF-7222AE7B9534>

Unnamed clade 3 (in Torres-Carvajal et al. 2016)

**Type species.** *Selvasaura brava* sp. n.

**Diagnosis.** Phenotypic synapomorphies are not known for this genus. Morphologically, *Selvasaura* gen. n. can be distinguished from all other genera of Cercosaurinae by the combination of the following characters: lower palpebral disc transparent, not divided (divided in *Andinosaura*, *Euspondylus*, *Gelanesaurus*, *Oreosaurus*, *Petracola*, *Riama*, and most *Anadia* and *Placosoma* species; opaque in *Pholidobolus*); dorsal scales slightly rugose (smooth in *Anadia*; keeled in *Cercosaura*; strongly keeled and tuberculate in *Echinosaura*, *Gelanesaurus*, *Neusticurus*, *Potamites*; minute tubercles on posterior dorsal scales in *Placosoma*); lateral scales distinctly smaller than dorsal scales (lateral scales not distinctly reduced in size in *Macropholidus*); lateral scales adjacent to ventrals

non-granular (granular in *Proctoporus*) (see e.g., Oftedal 1974; Cadle and Chuna 1995; Altamirano-Benavides et al. 2013; Kok et al. 2013; Torres-Carvajal and Mafla-Endara 2013; Echevarría et al. 2015; Borges-Nojosa et al. 2016; Chávez et al. 2017; Sánchez-Pacheco et al. 2017b). Genetically, the genus is differentiated from the other cercosaurines by distances given in Table 3 and 4.

**Definition.** (1) head shields smooth; (2) frontoparietal and parietal shields paired; (3) frontonasal, frontal and interparietal shields single; (4) prefrontal shields present; (5) lower palpebral disc transparent, not divided; (6) loreal shield present; (7) scale organs on labials present; (8) anteriormost supraocular and anteriormost superciliary shields fused; (9) dorsal surface of the tongue covered by scale-like papillae; (10) nuchal scales smooth; (11) dorsal scales rectangular, slightly rugose; (12) ventral scales squared to rectangular, smooth; (13) limbs pentadactyl, digits clawed; (14) femoral pores present in males, absent in females; (15) hemipenial lobes large, distinct from the hemipenial body.

**Content.** *Selvasaura brava* sp. n. and undescribed species of Unnamed clade 3 (sensu Torres-Carvajal et al. 2016) whose formal descriptions are underway (see Torres-Carvajal et al. 2016).

**Distribution.** Peru: Región Junín, Provincia de Chanchamayo, Pui Pui Protected Forest (*Selvasaura brava* sp. n.); Región San Martín, Provincia Mariscal Cáceres, Laurel (Cercosaurinae sp. 3; Torres-Carvajal et al. 2016). Ecuador: Provincia de Zamora Chinchipe, El Pangui (Cercosaurinae sp. 3; Torres-Carvajal et al. 2016); Provincia de Napo, Wildsumaco Wildlife Sanctuary (Cercosaurinae sp. 3; Torres-Carvajal et al. 2016).

**Etymology.** The generic name *Selvasaura* is derived from the Spanish noun 'selva' (forest) and the Greek noun σαύρα (lizard; *saura* is the feminine form) and refers to the habitat (montane rainforest) of the type species.

### ***Selvasaura brava* sp. n.**

<http://zoobank.org/88FAD0FE-8FBC-41BD-BCD2-334715157340>

Suggested English name: Brave forest microtegu

Suggested Spanish name: Microtegu selva brava

**Holotype.** (Figs 5, 6). MUSM 32738 (sample code IWU 381; MorphoBank pictures: M485668–M485671), an adult male from the border of the Pui Pui Protected Forest (11.211S, 74.958W; WGS84), 1700 m elevation, Distrito Pichanaqui, Provincia Chanchamayo, Región Junín, Peru, collected on 19 May 2014 by Edgar Lehr and Jiří Moravec.

**Paratypes.** (Fig. 7). Five: two adult males: NMP6V 75653 (sample code IWU 380; MorphoBank pictures: M485674–M485678), NMP6V 75654 (sample code IWU 382) and one juvenile MUSM 32739 (not included in the genetic analyses), all collected at the type locality on 19 May 2014 by Edgar Lehr and Jiří Moravec; one adult female MUSM 32718 (sample code IWU 339; MorphoBank pictures: M485672–M485673) and one juvenile NMP6V 75655 (sample code IWU 340; MorphoBank

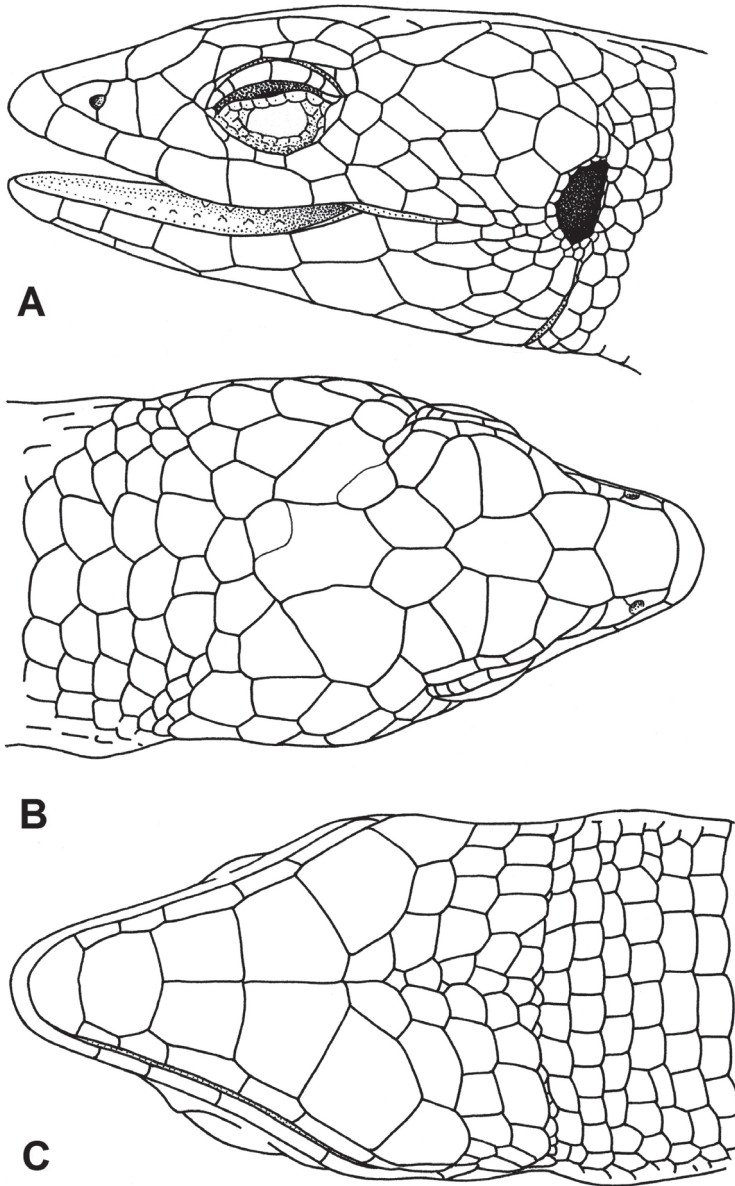


**Table 3.** Mean uncorrected genetic distances ( $p$ -distances, in %) between cercosaurine genera or their monophyletic clades if the genus' monophyly was not supported. Below diagonal are values based on the 12S alignment, above on the 16S. Values for *Selvasaura* gen. n. are in bold.

	<i>Anadia</i>	<i>Andinosaura</i>	<i>Cercosaura</i>	<i>Echinosaura</i>	<i>"Echinosaura" sulcarostrum</i>	<i>Euspondylus</i>	<i>Gelanesaurus</i>	<i>Macropholidus</i>	<i>Neusticurus</i>	<i>Oreosaurus</i>	<i>"Oreosaurus" serranus</i>	<i>Petracola</i>	<i>Pholidobolus</i>	<i>Placosoma</i>	<i>Potamites</i>	<i>Proctoporus bolivianus</i> group	<i>Proctoporus guentheri</i> group	<i>Proctoporus lacerus</i> group	<i>Proctoporus pachynus</i> group	<i>Proctoporus xestus</i>	<i>Riama</i>	<i>Selvasaura</i> gen. n.	Unnamed clade 2	Unnamed clade 4
<i>Anadia</i>																								
<i>Andinosaura</i>	10.44																							
<i>Cercosaura</i>	9.88	11.88																						
<i>Echinosaura</i>	13.77	13.79	12.84																					
<i>"Echinosaura" sulcarostrum</i>	9.21	12.82	10.74	12.65																				
<i>Euspondylus</i>	7.42	9.59	8.73	11.61	6.06																			
<i>Gelanesaurus</i>	11.74	12.76	12.99	14.71	13.13	11.18																		
<i>Macropholidus</i>	8.97	8.71	8.54	13.1	7.53	7.09	13.03																	
<i>Neusticurus</i>	11.04	14.67	13.66	14.33	14.24	11.41	13.6	11.89																
<i>Oreosaurus</i>	8.48	11.33	8.74	12.48	9.57	6.65	11.4	7.64	13.04															
<i>"Oreosaurus" serranus</i>	10.06	11.62	10.57	14.1	12	9.03	12.54	8.2	14.86	8.13														
<i>Petracola</i>	8.27	11.82	9.11	13.42	10.27	7.19	11.94	8.44	13.95	8.06	8.52													
<i>Pholidobolus</i>	8.59	9.37	8.93	12.61	7.86	6.16	11.7	4.31	11.85	6.99	8.62	8.14												
<i>Placosoma</i>	16.73	18.61	17.04	17.98	18.92	16.9	17.68	16.65	15.87	16.36	18	16.84	17.47											
<i>Potamites</i>	8.44	10.17	7.87	12.39	7.08	6.86	12	7.77	12.81	6.91	8.86	6.69	7.55	15.61										
<i>Proctoporus bolivianus</i> group	9.39	12.04	7.68	12.08	9.37	7.63	12.5	8.39	14.53	7.62	10.5	6.28	8.1	17.38	5.71									
<i>P. guentheri</i> group	8.86	11.14	8.4	12.55	8.02	7.67	12.41	8.09	12.98	7.24	8.92	5.66	8.17	16.17	6.26	5.26								
<i>P. lacerus</i> group	7.83	10.72	7.67	12.01	7.95	5.83	11.25	7.3	13.51	6.3	8.98	4.85	6.92	17.47	5.03	3.96	3.99							
<i>P. pachynus</i> group	7.91	10.48	7.4	11.66	7.94	7.15	11.44	6.73	12.95	6.52	8.46	5.28	6.79	16.29	5.17	4.74	4.32	3.4						
<i>P. xestus</i>	9.16	12.03	8.48	11.63	8.78	7.21	12.39	7.89	13.8	8.4	10.48	7.1	7.78	18.72	6.57	5.61	5.78	4.41	4.45					
<i>Riama</i>	10.36	11.13	10.59	11.91	11.51	9.31	13.05	9.13	13.67	9.37	11.46	10.18	9.1	18.55	9.08	9.13	9.11	8.29	7.93	9.48				
<i>Selvasaura</i> gen. n.	<b>7.88</b>	<b>9.86</b>	<b>7.37</b>	<b>11.86</b>	<b>8.46</b>	<b>7.04</b>	<b>11.92</b>	<b>7.01</b>	<b>12.42</b>	<b>7.13</b>	<b>8.83</b>	<b>5.75</b>	<b>7.58</b>	<b>15.78</b>	<b>5.37</b>	<b>5.23</b>	<b>4.75</b>	<b>4.21</b>	<b>3.8</b>	<b>5.35</b>	<b>8.22</b>		<b>4.35</b>	<b>3.89</b>
Unnamed clade 2	8.57	11.55	8.05	11.45	9.16	7.44	11.78	7.03	13.43	7.24	8.82	5.76	7.92	16.52	5.95	5.39	4.88	4.17	3.79	5.75	8.56	<b>4.81</b>		3.65
Unnamed clade 4	9.47	11.69	8.15	13.04	9.54	8.05	12.37	8.7	14.94	7.82	8.15	6.52	8.76	15.31	5.47	6.57	6.08	5.52	5.08	7.47	10.31	<b>5.82</b>	6.23	

**Table 4.** Mean uncorrected genetic distances ( $p$ -distances, in %) between cercosaurine genera or their monophyletic clades if the genus' monophyly was not supported. Below diagonal are values based on the ND4 alignment, above on the cmos. Values for *Selvasaura* gen. n. are in bold. Note that compared to Table 3 some clades are missing here because they did not have the ND4 and cmos sequences available ("*Echinosaura*" *sulcarostrum*, Unnamed clade 4).

	<i>Anadia</i>	<i>Andinosaura</i>	<i>Cercosaura</i>	<i>Echinosaura</i>	<i>Euspondylus</i>	<i>Gelanesaurus</i>	<i>Macropholidus</i>	<i>Neusticurus</i>	<i>Oreosaurus</i>	<i>"Oreosaurus"</i> <i>serrius</i>	<i>Petracola</i>	<i>Pholidobolus</i>	<i>Placosoma</i>	<i>Potamites</i>	<i>Proctoporus bolivianus</i> group	<i>Proctoporus guentheri</i> group	<i>Proctoporus lacertus</i> group	<i>Proctoporus pachyurus</i> group	<i>Proctoporus xestus</i>	<i>Riama</i>	<i>Selvasaura</i> gen. n.	Unnamed clade 2
<i>Anadia</i>		3.73	5.61	3.57	2.26	4.23	1.26	4.31	3.2	3.03	3.21	1.49	6.85	2.91	3.96	4.37	4.02	3.65	3.64	4.59	<b>3.15</b>	4.21
<i>Andinosaura</i>	23.1		7.33	2.73	4.49	4.68	3.65	3.63	4.95	5.04	5.08	3.66	6.69	4.68	5.66	5.85	5.73	5.49	5.35	3.33	<b>4.91</b>	5.75
<i>Cercosaura</i>	20.8	22.3		6.82	4.86	7	4.98	6.76	5.59	5.63	5.5	5.09	9.52	3.28	3.6	4.49	3.7	3.39	3.3	6.57	<b>3.43</b>	4.7
<i>Echinosaura</i>	25.3	23.5	24.6		4.02	4.15	3.09	2.74	4.45	4.5	4.32	3.17	6.21	4.13	5.19	5.35	5.31	4.92	4.92	3.05	<b>4.34</b>	5.08
<i>Euspondylus</i>	17.5	21.2	19.7	25		4.18	1.22	4.22	2.85	2.68	2.83	1.33	7.46	2.73	3.79	4.2	3.85	3.11	3.48	4.41	<b>2.99</b>	4.06
<i>Gelanesaurus</i>	23.8	23.3	23.1	26.2	24.4		3.76	4.41	4.61	4.41	4.73	3.85	7.19	4.28	5.38	5.12	5.41	5.07	5.07	4.37	<b>4.54</b>	5.45
<i>Macropholidus</i>	19.8	22.5	20.6	26.3	17.7	24.6		3.79	2.67	2.67	2.86	0.44	6.85	2.39	3.42	3.86	3.48	3.16	3.11	4.06	<b>2.62</b>	3.7
<i>Neusticurus</i>	25	23.3	23.1	24.9	24	24.2	26		4.51	4.81	4.9	3.83	5.6	4.24	5.38	5.52	5.46	4.72	5.07	3.08	<b>4.04</b>	5.45
<i>Oreosaurus</i>	20.8	22.9	21.3	25.6	20.2	23.3	21.3	24.1		3.61	3.67	2.8	7.74	3.14	4.18	4.57	4.24	3.76	3.87	4.83	<b>3.5</b>	4.62
" <i>Oreosaurus</i> " <i>serrius</i>	22.2	24.6	21.4	26.3	22.9	23.8	23.5	25.8	21.3		2.84	2.86	7.49	3.36	4.32	4.8	4.36	4.09	4.01	4.45	<b>3.58</b>	4.41
<i>Petracola</i>	20.4	21.3	17.7	24.1	20.9	23.2	19.8	22.4	20.6	22		2.89	8.04	2.84	3.7	4.23	3.85	3.54	3.47	4.16	<b>3.01</b>	3.65
<i>Pholidobolus</i>	19.4	22.4	20	26.2	17.5	24.1	17.7	25.1	20.5	22.7	20.6		7.19	2.49	3.43	3.94	3.5	3.24	3.12	4.08	<b>2.68</b>	3.74
<i>Placosoma</i>	25.7	25.2	24.3	25.6	23.4	25.2	24.8	22.3	24.4	25.8	23.7	25.1		7.42	8.71	8.32	8.75	7.96	8.4	5.84	<b>7.33</b>	8.56
<i>Potamites</i>	21	23.2	19.7	25.6	20.7	24.1	21.7	23.1	21.3	21.4	19.9	20	25.2		1.49	1.99	1.6	1.19	1.19	3.89	<b>0.77</b>	2.33
<i>Proctoporus bolivianus</i> group	20.7	22.2	19.1	24.7	19.6	23	20.4	22.9	20.2	19.2	18.2	19.7	25.1	19.2		2.69	1.23	1.8	0.84	4.93	<b>1.54</b>	3.06
<i>Proctoporus guentheri</i> group	19.7	21.9	18.7	24.2	19.6	22.9	19.9	23.4	20.5	19.4	18.6	19.4	24.8	18.1	16.5		2.77	2.43	2.38	5.13	<b>1.99</b>	2.93
<i>Proctoporus lacertus</i> group	20.6	23.1	19.5	25	19.6	23.2	20.8	24.5	20.1	20.8	19.4	19.5	25.2	18.3	16.8	15.3		1.82	0.41	5.03	<b>1.63</b>	3.14
<i>Proctoporus pachyurus</i> group	19.6	22.2	18.4	24.7	19.3	21.9	20.5	22.3	19.1	20.1	17.9	19.1	24.7	17.8	15.9	15.7	15.5		1.42	4.35	<b>0.89</b>	2.78
<i>Proctoporus xestus</i>	22.7	23.5	21.1	25.7	20.9	24.5	23.8	25.5	21.2	21	20.6	21.8	27.3	20.9	20	18.6	19	18.2		4.64	<b>1.23</b>	2.77
<i>Riama</i>	24.1	23.6	24.4	25.8	23.3	22.7	24.3	24.6	23.2	24.9	23.9	24.1	25	24.2	24.1	23.3	23.9	23	25.4		<b>3.69</b>	4.74
<i>Selvasaura</i> gen. n.	<b>20.7</b>	<b>22.4</b>	<b>19.8</b>	<b>24.4</b>	<b>19.6</b>	<b>23</b>	<b>21.3</b>	<b>23.6</b>	<b>21.4</b>	<b>19.7</b>	<b>20.1</b>	<b>19.8</b>	<b>24.7</b>	<b>19.5</b>	<b>18.7</b>	<b>17.5</b>	<b>18.4</b>	<b>17.3</b>	<b>19.5</b>	<b>23.7</b>		<b>2.27</b>
Unnamed clade 2	21.3	20.8	19.9	24.4	17.8	23.2	19.6	23.3	20.3	19.4	19	20.2	23.7	20.3	17.4	18	18.6	18.6	19.6	23.7		<b>19</b>



**Figure 5.** Drawing of the head of the holotype of *Selvasaura brava* sp. n. (MUSM 32738). **A** lateral, **B** dorsal **C** ventral view. Scale bar: 5 mm. Drawing by J. Moravec.

pictures: M485679–M485680), both collected at the border of the Pui Pui Protected Forest (11.208S, 74.955W; WGS84), 1678 m elevation, Distrito Pichanaqui, Provincia Chanchamayo, Región Junín, Peru, on 12 May 2014 by Edgar Lehr and Jiří Moravec.

**Diagnosis.** A small gymnophthalmid (SVL 42.1–45.9 mm,  $n = 4$ ), which can be characterised by the following combination of characters: 1) body slender, slightly depressed, maximum SVL 45.9 mm in males, 42.1 mm in a single female; 2) head relatively short, pointed, about 1.5 times longer than wide; 3) ear opening distinct, moderately recessed; 4) nasals separated by undivided frontonasal; 5) prefrontals, frontal, frontoparietals, parietals, postparietals and interparietal present; 6) parietals slightly longer than wide; 7) supraoculars four, anteriormost fused with anteriormost superciliar; 8) superciliar series complete, consisting of four scales; 9) nasal shield divided above and below or behind the nostril; 10) loreal separated or in contact with second supralabial; 11) supralabials seven; 12) genials in four pairs, first and second pair in contact; 13) collar present, containing 9–11 enlarged scales; 14) dorsals in 33–36 transverse rows, rectangular, nearly twice as long as wide, subimbricate, rugose in adults, slightly keeled in juveniles; 15) ventrals in 22–25 transverse rows, squared to rectangular, smooth, juxtaposed; 16) scales around mid-body 32–34; 17) lateral scales at mid-body reduced in 4–7 lines; 18) limbs pentadactyl, all digits clawed, forelimb reaching anteriorly to third supralabial; 19) subdigital lamellae under Finger IV 14–16, under Toe IV 18–22; 20) femoral pores in males 7–9; 21) four large preanal plate scales; 22) tail about 1.5–1.7 times longer than body (in juveniles); 23) caudals subimbricate, rugose to slightly keeled dorsally in adults, slightly keeled in juveniles, smooth ventrally; 24) lower palpebral disc transparent, undivided; 25) in life, dorsal surface of head, body and limbs light brown with fine dark brown speckling, dorsal surface of tail light brown with a reddish tint or reddish-brown markings; a tan or yellowish brown vertebral stripe bordered laterally by dark brown, vertebral stripe extends on head anteriorly and on tail caudally (inconspicuous in the female); a narrow dirty white to tan dorsolateral line extending on each side from above the tympanum to pelvic region (discontinuous caudally from the level of forelimbs in adults, reaching posterior edge of orbit in some individuals); a narrow dirty white to tan stripe running from above the orbit across parietals and first postparietals up to the neck (connected with the dorsolateral line in some individuals); a narrow white stripe extending from below of orbit to insertion of forelimbs (bordered dorsally by black in juveniles and some adults); minute ocelli-like white spots on flanks (most conspicuous at forearm insertion, absent in some adults); ventrolateral parts of flanks whitish brown; throat and belly creamy white with fine dark grey speckling inside the individual scales (yellowish white with black speckling in juveniles); ventral surfaces of limbs, anal area and tail yellowish white in males and juveniles, white in the female; iris tan with orange tint in males, tan in the female.

**Description of the holotype.** Body slender; legs moderately long, tail regenerated; head length 22.0% of SVL, head width 14.6% of SVL; snout pointed, moderately long, eye-nose distance 34.7% of HL; neck distinct, collar present; head scales smooth; rostral scale wider than long, slightly higher than adjacent supralabials, in contact with frontonasal, nasals, and first supralabials; frontonasal slightly wider than long, prefron-

tals present, in wide contact medially; frontal longer than wide, in contact with second and third supraoculars; frontoparietals in contact with third and fourth supraoculars, parietals and interparietal; supraoculars four, none in contact with ciliaries; superciliary series complete, consisting of four shields; anteriormost superciliary fused with anteriormost supraocular, in contact with prefrontal and loreal anteriorly; parietals (left divided) in contact with frontoparietal, fourth supraocular, dorsalmost postocular (separated by small interstitial shield on the left side), one temporal and two postparietals; interparietal longer than wide (divided posteriorly), in contact with three postparietals posteriorly; postparietals six; nasal shield divided above and below the nostril, in contact with first and second supralabial; frenocular triangular, in contact with loreal and second, third and fourth (at one point) supralabial ventrally on the left side and with loreal, nasal (at one point) and second and third supralabial on the right side; palpebral disc oval, translucent, undivided; postoculars three; temporals polygonal, supratympanic temporal one; supralabials seven, fifth below the centre of eye; infralabials six; mental wider than long, in contact with first infralabials; postmental single, in contact with first and second infralabials; genials in four pairs, first and second pair in contact medially, first pair in contact with second and third infralabials, second pair in contact with third and fourth infralabials, third pair in contact with fourth and fifth infralabials, fourth pair in contact with fifth and sixth infralabials; gulars 14; plates in collar 11; dorsal scales homogenous, rectangular, longer than wide, subimbricate, rugose, in 34 transverse rows; dorsals (enlarged scales) across body at fifth transverse ventral scale row 10, at 10<sup>th</sup> transverse ventral scale row 16, at 15<sup>th</sup> transverse ventral scale row 16; laterals (smaller lateral scales) at fifth transverse ventral scale row 8–9, at 10<sup>th</sup> transverse ventral scale row 4–5, at 15<sup>th</sup> transverse ventral scale row 4–5; ventrals squared to rectangular, juxtaposed, in 23 transverse rows; ventrals across belly at midbody 10; scales around midbody 32; anterior preanal plate scales two; posterior preanal plate scales four; scales on tail rectangular, subimbricate, slightly keeled dorsally at tail base, smooth and juxtaposed ventrally; subdigital lamellae under Finger IV 14/15 (4/5 distal lamellae single and smooth, remaining lamellae divided in two subconical segments); subdigital lamellae under Toe IV 19/18 (4/4 distal lamellae single and smooth, remaining lamellae divided in two subconical segments); femoral pores 9/7.

**Measurements of the holotype (in mm).** SVL 45.9; TL (tail regenerated) 38.5; HL 10.1; HW 6.7; HD 5.4; EN 3.5; FLL 11.5; HLL 16.5; AGD 25.0.

**Colouration of the holotype in life.** (Fig. 6). Head, body, and limbs light brown dorsally with fine dark brown speckling, dorsal surface of tail light brown with reddish brown markings; a tan to yellowish brown vertebral stripe bordered laterally by dark brown, the vertebral stripe is about two dorsal scales wide and extends on the head anteriorly and the tail caudally; a nearly inconspicuous tan dorsolateral line extending on each side from above the tympanum to pelvic region, the line becomes discontinuous and barely visible from the level of forelimbs; a barely visible narrow tan stripe bordered by dark brown ventrally running from above the orbit across parietals and first postparietals and disappearing before reaching the neck; a narrow white stripe bordered by dark brown dorsally extending from below of orbit to





**Figure 6.** Holotype of *Selvasaura brava* sp. n. (MUSM 32738) in life. Photographs by E. Lehr.

insertion of forelimbs; ocelli-like spots on flanks absent; ventrolateral parts of flanks whitish brown; throat and belly creamy white with fine dark grey speckling inside the individual scales; ventral surfaces of limbs, anal area and tail yellowish white; iris tan with an orange tint.

**Colouration of the holotype in preservative.** General colouration pattern is as described for the holotype in life. The dorsal colouration has a bronze-brown tint, the



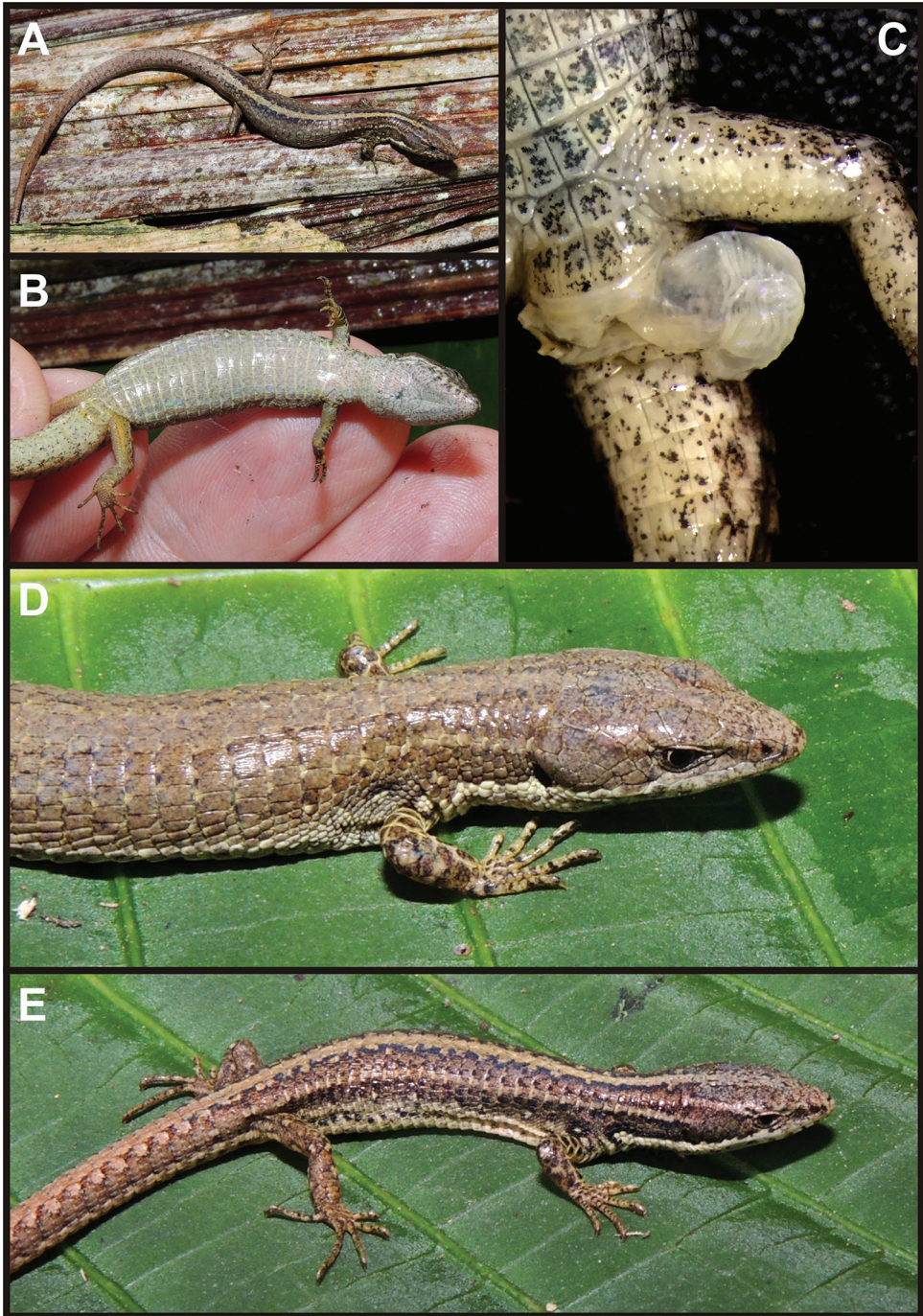
reddish brown markings on the tail disappeared. Ventral surfaces dirty white with fine dark grey speckling.

**Hemipenial morphology.** (Fig. 7c; MorphoBank pictures: M485676–M485677). The hemipenes of the paratype NMP6V 75653 were everted during preservation and fixed in alcohol. The completely everted organs measure approximately 5 mm. The hemipenial body has a conical shape with proximal region distinctly thinner than the distal region with lobes. The hemipenial lobes are relatively large, ovoid, distinct from the hemipenial body and do not possess filiform appendages. The flounces on the asulcate side form about 14 discontinuous, but nearly complete, more or less horizontal lines expanding widely on the lateral sides of the distal part of the hemipenial body. There are about seven isolated nearly horizontal flounces on the proximal-central region of the asulcate side. Flounce ornamentation consists of subtle, barely visible denticulation. The sulcus spermaticus begins at the hemipenial base and proceeds in a straight central line towards the lobes. It is edged by lateral fleshy nude areas, which expand in two lateral wings covering the area of lobular division. In that area, the sulcus spermaticus forks into two arms separated by a central fold, which has about eight horizontal ribs. The sulcate arms terminate among lobes and lateral fleshy wings in the apical area of the hemipenis.

**Variations.** Measurements and scutellation data of the type series are given in Table 5. Colour variation is described in the species diagnosis. In juveniles, the colour pattern is generally brighter than in adults and consists of distinct vertebral and dorso-lateral lines and ocelli-like spots on flanks. In the single female, the dorsal colouration is nearly uniformly light brown and the vertebral and dorsolateral lines as well as the ocelli-like spots are poorly developed (Fig. 7).

**Etymology.** The species epithet *brava* is derived from the Spanish adjective *bravo* (brave, courageous, wild; *brava* the feminine form) and refers to Río Bravo, the largest river in the area of occurrence of the new species, as well as to the fearless nature of the lizard to share shelter with people.

**Distribution, natural history, and threat status.** *Selvasaura brava* sp. n. is known from two localities lying at the northeastern border of the Pui Pui Protected Forest, ca. 18 km (straight airline distance) NW of the town of Satipo (Fig. 1). Both localities are located in the valley of the tributary of Río Bravo (on opposite banks of the tributary) about 500 m (straight distance) from each other. The valley and its slopes are covered by a primary montane rainforest characterized by 15–20 m high canopy and frequent occurrence of bromeliads, ferns, and epiphytic mosses (see also Lehr and Moravec (2017). All specimens of *S. brava* sp. n. were collected during the day within roofs of provisional camp shacks consisting of dried palm leaves and built by locals on small forest clearings (Fig. 8; MorphoBank picture: M485681). The roofs of the shacks were placed on 1.5–4 m pillars made of tree trunks and stood in an open space fully exposed to sun. The activity of all observed specimens seemed correlated with the intensity of solar radiation. During the sunny hours, the animals emerged from their shelters in the leaf layer, climbed and basked on the roof surface and searched for prey. As agile climbers, the lizards were able to climb up thin vertical tree trunks and jump



**Figure 7.** Paratypes of *Selvasaura brava* sp. n. Dorsal (A) and ventral (B) view of adult male (NMP6V 75653) with a detail of an everted hemipenis (C) D adult female (MUSM 32718) E – juvenile (NMP6V 75655). Note the generally uniform colouration of the female compared to the male and juvenile specimens. Photographs by J. Moravec.





**Figure 8.** Type locality of *Selvasaura brava* sp. n. The lizards were active during the day basking and foraging in the leaves of the roof and on the shack pillars. They used the leaves on the roof as a refuge to hide in. Photograph by J. Moravec.

between the palm leaves. These observations indicate that *S. brava* sp. n. represents an arboreal heliothermic species. Other gymnophthalmid species found at the type locality in sympatry with *S. brava* sp. n. included *Potamites* sp. (not included in the genetic analyses), which inhabited banks of small forest brooks, and *Proctoporus* sp. 4 (sensu this publication, Fig. 3) collected on the ground in the open clearing. With respect to the sparse data available, we suggest classifying *S. brava* as “Data Deficient” according to the IUCN red list criteria.

## Discussion

In this study, we used an unprecedented dataset of nearly all DNA sequences for the cercosaurine lizards available to date to infer a robust phylogeny of the subfamily and to contribute to the knowledge of the biodiversity of the little surveyed montane forests of central Peru. Although more species are being included in the phylogenetic analyses of cercosaurines every year and new phylogenetic hypotheses are being presented, our understanding of the systematics of the subfamily is still far from settled. New genetic data often bring unexpected results that reshuffle the taxonomy of cercosaurines, such as

**Table 5.** Morphological characters of the type specimens of *Selvasaura brava* sp. n.

Character	MUSM 32738 (holotype)	NMP6V 75653	NMP6V 75654	MUSM 32718	MUSM 32739	NMP6V 75655
Sex	M	M	M	F	Juv	Juv
SVL	45.9	43.9	45.3	42.1	26.8	30.2
TL	—	—	—	—	45.5	44.0
HL	10.1	10.0	10.6	9.8	6.5	6.9
HW	6.7	6.7	7.1	6.6	4.6	4.7
HD	5.4	5.5	5.3	4.8	3.5	3.6
E-N	3.5	3.2	3.6	3.4	2.4	2.4
FLL	11.5	10.5	11.5	10.5	7.5	7.5
HLL	16.5	15.0	16.5	14.5	10.5	10.5
AGD	25.0	22.5	24.4	22.2	13.2	16.5
Supralabials	7/7	7/7	7/7	7/7	7/7	7/7
Scales in collar	11	10	10	11	10	9
Transverse rows of dorsals	34	35	33	34	34	36
Laterals at midbody	6	7	6	6	6	6
Scales around midbody	32	34	34	34	32	33
Transverse rows of ventrals	23	22	24	23	22	25
Ventrals across belly	10	10	10	10	10	10
Prenal plate scales	4	4	4	4	4	4
Lamellae under Finger IV	14/15	15/14	16/15	15/14	14/15	15/16
Lamellae under Toe IV	19/18	19/20	21/21	21/20	21/21	21/22
Femoral pores	9/7	9/8	8/8	—	8/8	—

reassignments of species to different genera (Kok 2015; Sánchez-Pacheco et al. 2017b), resurrections of generic names that had once been synonymised (Goicoechea et al. 2012; Chávez et al. 2017), identification of new clades at the genus level (this study; Torres-Carvajal et al. 2016), recognition of cryptic species (Goicoechea et al. 2013), or detection of paraphyletic species or genera (this study; Goicoechea et al. 2012; Torres-Carvajal et al. 2016). Therefore, it is critical to build the phylogenetic trees on extensive taxon sampling, as otherwise many of the above listed issues may go unnoticed.

In concert with previous studies, our results show generally low support for the relationships between the *Cercosaurinae* genera (Fig 2). One possibility of the low resolution of the basal nodes is that the group experienced a rapid initial radiation that left few genetic traces that would indicate the actual branching pattern of the *cercosaurine* evolution. An alternative explanation, and one we find more likely, is that the genetic data currently available for the subfamily (i.e., four mtDNA genes and a single nDNA locus sequenced) are not sufficient for inferring deep-level relationships. We believe that having more nuclear genes sequenced (either by Sanger or next generation sequencing approaches) would improve resolving these basal nodes and shed further light on the monophyly/paraphyly of the questionable genera (see below).

Our results raise many important issues regarding the systematics and taxonomy of the *Cercosaurinae* that we discuss in detail below.

## Unexpected diversity of genera and species

Recent studies that examined the phylogeny and systematics of the Cercosaurinae on the basis of thorough sampling of taxa (Goicoechea et al. 2012; Torres-Carvajal et al. 2016; Sánchez-Pacheco et al. 2017b) detected previously unknown evolutionary lineages being present in the cercosaurine tree. While most of them could be assigned to currently existing genera (and some have been taxonomically revised since (Goicoechea et al. 2013; Sánchez-Pacheco et al. 2017a)), there were clades whose high levels of genetic divergence and morphological disparity indicated towards the existence of yet unknown clades at the level of genera (Torres-Carvajal et al. 2016). Similarly, our phylogenetic analyses also identified several previously undetected evolutionary lineages.

By formally describing the genus *Selvasaura* we extend the list of currently recognised genera of Cercosaurinae to 16 (Sánchez-Pacheco et al. 2017b). Apart from the formally named genera, there are two clades within cercosaurines that merit genus-level distinction and that are termed here in accordance with previous studies, Unnamed clades 2 and 4. The phylogenetic position of neither of them could be inferred with certainty. Results of both studies in which Unnamed clade 2 was included, this work and Torres-Carvajal et al. (2016), vary in its placement depending on the method of phylogenetic inference. Although all analyses tend to show it close to *Proctoporus* (at least to some groups), lack of support hampers any definitive conclusions regarding its evolutionary origins. We herein provide additional material of ten voucher specimens for Unnamed clade 2 from the PPPF (Table 1), which extends the range of the clade further north compared to previously published localities (Mantaro Valley and Colcabamba-Quintao District, Peru; Torres-Carvajal et al. 2016).

This study is the first to identify a clade that is termed here Unnamed clade 4. As in the case of Unnamed clade 2, the phylogenetic affinities of this clade remain obscure as a result of the low support of deeper level relationships within cercosaurines. Two of the analyses (ML, MrBayes) placed it as sister to *Selvasaura* gen. n. plus *Potamites*, while BEAST placed it as a sister lineage to Unnamed clade 2 (Suppl. material 1: Figs S1–S3). However, none of the topologies was supported. The two specimens that form Unnamed clade 4 represented arboreal lizards caught in cloud forests of southern and central Peru. For the moment, no other data such as morphology, ecology, and natural history are available for the clade as they are being collected and will be published with the formal description of the genus (work in progress).

Besides the newly discovered genera, there are currently 19 unnamed lineages at the level of species in the Cercosaurinae (Fig. 3). They are either morphologically disparate from existing taxa, represent unique genetic lineages or have been delimited as candidate species (this study; Doan et al. 2005; Goicoechea et al. 2012; Torres-Carvajal et al. 2016; Sánchez-Pacheco et al. 2017b) and their systematics and taxonomy should be revised. The presence of so many unidentified species points at the disturbing fact of how little we know about the real diversity of cercosaurine lizards and, perhaps, Neotropical biota in general.

## Paraphyly and polyphyly of genera and species

Some previous studies have already pointed out problems with certain genera not being monophyletic when samples of more species of that genus were included in a phylogenetic analysis. Although many of these issues have been resolved, some still persist or were identified in our study and are to be addressed.

One of the recent examples is paraphyly of the genus *Echinosaura*. As Torres-Carvajal et al. (2016) revealed and we confirmed herein, *E. sulcarostrum* does not cluster with the other species of the genus. The phylogenetic position of this species varies across different phylogenetic analyses. Torres-Carvajal et al. (2016) reconstructed it in their BEAST analysis as a strongly-supported sister lineage to a large clade containing *Anadia*, *Euspondylus* (a name resurrected by Chávez et al. [2017] for the Unnamed clade 1 of Torres-Carvajal et al. [2016]), *Macropholidus*, *Pholidobolus*, *Petracola*, *Cercosaura*, *Selvasaura* (termed Unnamed clade 3 in their paper), *Potamites*, *Proctoporus*, and Unnamed clade 2; their ML analysis recovered it as sister to *Proctoporus xestus*. Subsequently, the genetic analysis of Sánchez-Pacheco et al. (2017b) recovered it as sister to a clade of *Oreosaurus*, *Potamites*, *Petracola*, *Cercosaura*, and *Proctoporus*, although with a limited taxon sampling. Finally, our analyses yielded it as sister to *Euspondylus*, although the topology was supported only in the MrBayes analysis. Regardless, none of the analyses found it close to the other *Echinosaura* species including *E. horrida*, the type species of the genus. As a result, its taxonomy, as well as proper phylogenetic placement, remain to be revised.

Another possible case of paraphyly is the genus *Oreosaurus* that was recently resurrected by Sánchez-Pacheco et al. (2017b). While the authors found the genus monophyletic, our analyses yielded no support for the clade as a whole with *O. serranus* placed separately from the other species. It was found sister to the remaining species only in our BEAST analysis, but support for the basal node was very low (pp = 0.3) indicating that 70% of the posterior trees actually had a different topology. Such a discrepancy between our and Sánchez-Pacheco et al. (2017b) results may stem from the difference in the analytical approaches undertaken. All our phylogenetic analyses were model-based, i.e., we assumed the sequences to evolve under evolutionary models that take into account the variation of substitution rates among sites in the alignments and the possibility of recurrent mutations at one site. On the contrary, Sánchez-Pacheco et al. (2017b) performed a maximum parsimony analysis that reconstructs the phylogeny based on the smallest number of evolutionary events necessary to explain the sequence data. Both methods may under some circumstances result in different topologies, especially when analysing relatively distant taxa where long branch attraction can occur (Felsenstein 1978; Alfaro et al. 2003). This may be the case here considering that we deal with a group whose origin has been estimated to have taken place in the early Tertiary (Zheng and Wiens 2016). Regarding the morphology, Sánchez-Pacheco et al. (2017b) found the genus *Oreosaurus* being clearly different from *Andinosaura* and *Riama* by lacking a narrow band of differentiated granular lateral scales. On the other hand, Sánchez-Pacheco et al. (2017a) show that *Oreosaurus serranus* can be distinguished morphologically from all other *Oreosaurus* species by having only one pair



of genial scales. Therefore, taking into account the above-mentioned discrepancies in the results of molecular analyses, and the morphological distinctiveness of *O. serranus* from other *Oreosaurus* species, the character of nucleotide divergence between *O. serranus* and the other *Oreosaurus* species should be examined in detail in order to trace the inconsistency in the phylogenetic reconstructions.

At the species level, recent taxonomic advances made possible by the tremendous effort of many authors are making considerable progress in stabilising the taxonomy of cercosaurines (e.g., Goicoechea et al. 2013; Torres-Carvajal et al. 2014; Venegas et al. 2016; Sánchez-Pacheco et al. 2017a; among many others), yet non-monophyletic species are still present in the phylogeny. For example, the seven samples of *Potamites ecpleopus* used in this study form two groups, one is distributed in eastern Ecuador and the other in northeastern and southern Peru. Paraphyly of this species was already noted by Torres-Carvajal et al. (2016). Because the type locality of the species lies approximately in the centroid of the crescent delineated by the sampled localities, it is impossible to assign the species name to either of the groups with certainty until a comprehensive revision with specimens from the type locality is undertaken. Another example are two *Cercosaura* species, *C. parkeri* and *C. schreibersii*, which are paraphyletic with respect to each other as also noted by Sturaro et al. (2017). *Cercosaura parkeri* was originally described as a subspecies of *C. schreibersii* (Ruibal 1952) and elevated to species status by Tedesco and Cei (1999), but a further taxonomic revision of the species complex is apparently needed to resolve the remaining issues.

### Species groups in *Proctoporus*

Yet another genus in which between-species relationships have proven difficult to infer is *Proctoporus* (Doan 2003; Doan et al. 2005; Torres-Carvajal et al. 2016), and this study supports this notion (but see Goicoechea et al. 2012). These semi-fossorial lizards inhabit primarily montane forests between 1000 m and 4000 m of altitude from central Peru to central Bolivia (Uzzell 1970; Goicoechea et al. 2012). Although certain species groups have traditionally been identified, mutual relationships between them and to other cercosaurine genera remain poorly resolved. Given the amount of cryptic species present within the genus (8 undescribed or candidate species; Fig. 3) indicating that future taxonomic revisions are to be expected, we herein propose the following terminology of the species groups in order to facilitate addressing this issue in future studies. The species groups are: (1) *Proctoporus pachyurus* group that contains *P. chasqui*, *P. oreades*, *P. pachyurus*, *P. rahmi*, *P. spinalis*, *P. sucullucu*, and three yet undescribed species (labelled *P. sp.*, *P. sp. 1*, *P. sp. 5*); (2) *Proctoporus lacertus* group that consists of four recently described or resurrected species (*P. carabaya*, *P. iridescens*, *P. kiziriani*, *P. lacertus*), which were formerly considered part *P. bolivianus* (Goicoechea et al. 2012; 2013); (3) *Proctoporus bolivianus* group of two species, *P. bolivianus* and a confirmed candidate species (labelled *P. Ca1* following Goicoechea et al. [2012, 2013]); (4) *Proctoporus guentheri* group, which contains the highest proportion of undescribed species (four, labelled *P. sp. 2*, *P. sp. 3*, *P.*

sp. 4, *P. Ca2* following Goicoechea et al. [2012, 2013]) besides three described species (*P. guentheri*, *P. laudahnae*, *P. unsaaca*); and (5) a clade of a single species, *P. xestus*. Most recent phylogenetic reconstructions of the genus were based on identical sampling of loci (three mitochondrial [12S, 16S, ND4] and one nuclear marker [cmos]) and our study has added sequences of one additional mtDNA marker (cytb) for only two species. The congruence of results obtained across studies and showing little support for the basal nodes is thus not surprising. We believe that getting a better resolution of the relationships between the *Proctoporus* groups is a matter of better sampling of loci and that more nuclear markers sequenced would shed more light on this subject.

### Phylogenetic placement of generic type species

The above problems with non-monophyletic genera raise an important nomenclatural issue regarding the application of generic names. Generic names apply to clades that contain the type species of the genus. In cases when genera are formed by more unrelated evolutionary lineages (e.g., *Echinosaura*) inferring the phylogenetic position of the type species is the only way to determine which of the lineages will bear the genus name; the other has to be renamed. In cercosaurines, most type species have been sequenced and placed in the phylogenetic context of the subfamily (for type species see Uetz et al. 2018), and this study provides an important addition to it.

For the first time, we sequenced the type species of the genus *Anadia* (*A. ocellata*). The sample clusters with other congeneric species in the dataset and thus fixes the name *Anadia* to this clade. Most species of *Anadia* have not been sequenced yet (Fig. 2), and it cannot be ruled out that once they are included in phylogenetic analyses, more cases of paraphyly will be detected. Similar situations have occurred in *Anadia* before when *A. mcdiarmidi* was found not to cluster with other species of the genus (Torres-Carvajal et al. 2016) and was subsequently reassigned to the genus *Oreosaurus* (Sánchez-Pacheco et al. 2017b).

Currently, the only cercosaurine genera with type species missing from the phylogenetic trees presented here are *Euspondylus* (type species *E. maculatus*) and *Oreosaurus* (type species *O. luctuosus*). Obtaining DNA sequences of the latter is particularly desired, as including its samples in phylogenetic analyses should help resolving the issue with the potential paraphyly in *Oreosaurus* (see above).

### Montane forests of Peru

Montane forests (región yunga or selva alta) are found in the eastern Andes roughly between 800 and 3500 m a.s.l. (Perú, Ministerio del Ambiente 2015) and are known for their high biodiversity and an increasing endemism with increasing elevation (Young and León 2000). Yet, montane forests are among the least studied and least understood ecosystems (Ledo et al. 2012). In a recent vegetation map of Peru, 12 different types of

montane forests were recognized within the región yunga, covering in total 9.58% of the national territory (Perú, Ministerio del Ambiente 2015). Its dense vegetation and steep slopes make the herpetofauna of montane forests relatively difficult to survey, and the canopy herpetofauna is probably the least known. However, exciting discoveries often happen by accident (e.g., arboreal species found in bromeliads on a fallen tree, Duellman et al. 2004).

All specimens of *Selvasaura brava* sp. n. were found in secondary forests, hiding in the roofs of simple wooden shacks where specimens could be easily seen and caught, whereas not a single specimen was found in primary forests. Our discovery of the new cercosaurine clade of arboreal lizards (Unnamed clade 4) together with a recent description of a new arboreal *Euspondylus* from central Peru (Chávez et al. 2017) indicate that arboreal species of cercosaurines may be much more diverse than previously thought and further research will be necessary to fully understand their diversity and ecology.

Some members of several cercosaurine genera (*Anadia*, *Euspondylus*, *Selvasaura*, Unnamed clade 4) are adapted to life in the above-ground vegetation (Ofstedal 1974; Chávez et al. 2017; this study) and certain species of some other genera also show tendency to arboreality (e.g., *Cercosaura*; Vitt et al. 2003). Repeated convergent adaptation to arboreality in Neotropical lizards has been well documented and studied in anoles (Losos 1990; 1992; Kolbe et al. 2011). If such is the case for Cercosaurinae can only be answered when we have a better-resolved phylogeny. Furthermore, no such studies have been conducted to compare different arboreal lifestyles and arboreal locomotion (see Fischer et al. (2010) for definitions) in Cercosaurinae, nor have different ecomorphs, and their adaptations to their arboreal niches been described. We note the small size of *Selvasaura brava* (SVL = 42.1–45.9 mm, n = 4) and the relatively short front and hind limbs, yet detailed observations of their locomotory behaviour in nature are missing.

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## Supplementary material I

### Supplementary phylogenetic trees and table of analysed samples

Authors: Jiří Moravec, Jiří Šmíd, Jan Štundl, Edgar Lehr

Data type: molecular data

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# Widespread continental mtDNA lineages prevail in the bumblebee fauna of Iceland

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## Abstract

Origins of the fauna in Iceland is controversial, although the majority of modern research supports the postglacial colonization of this island by terrestrial invertebrates rather than their long-term survival in glacial refugia. In this study, we use three bumblebee species as a model to test the hypothesis regarding possible cryptic refugia in Iceland and to evaluate a putative origin of recently introduced taxa. *Bombus jonellus* is thought to be a possible native Icelandic lineage, whereas *B. lucorum* and *B. hortorum* were evidently introduced in the second half of the 20<sup>th</sup> century. These phylogeographic analyses reveal that the Icelandic *Bombus jonellus* shares two COI lineages, one of which also occurs in populations on the British Isles and in mainland Europe, but a second lineage (BJ-02) has not been recorded anywhere. These results indicate that this species may have colonized Iceland two times and that the lineage BJ-02 may reflect a more ancient Late Pleistocene or Early Holocene founder event (e.g., from the British Isles). The Icelandic populations of both *Bombus lucorum* and *B. hortorum* share the COI lineages that were recorded as widespread throughout Eurasia, from the European countries across Russia to China and Japan. The findings presented here highlight that the bumblebee fauna of Iceland comprises mainly widespread ubiquitous lineages that arrived via natural or human-mediated dispersal events from the British Isles or the mainland.

## Keywords

Dispersal, Hymenoptera, invertebrate introduction, island biogeography, North Atlantic islands

## Introduction

Iceland is a large North Atlantic island, the fauna of which is mostly of Palaearctic origin, with very few lineages that arrived from the Nearctic Region (Gíslason 2005, Gíslason et al. 2015, Novichkova et al. 2014, Pálsson et al. 2016, Bolotov et al. 2017). The majority of recent phylogeographic research supports the *tabula rasa* hypothesis, which suggests the allochthonous origin of freshwater and terrestrial fauna in Iceland since the last glaciation (Pálsson et al. 2016, Bolotov et al. 2017). However, the possibility of the long-term survival of several cold-adapted lineages on this island could not be excluded, at least for inhabitants of specific environments, e.g., groundwater (Kornobis et al. 2010).

Bumblebees (Hymenoptera: Apidae: *Bombus* spp.) are an appropriate model for biogeographic reconstructions because these insects are associated with flowering plants and are poorly equipped for dispersal across large water barriers (Bolotov et al. 2013, Potapov et al. 2017). The faunistic research of bumblebees in Iceland has a long history (Prÿs-Jones et al. 1981, 2016, Kristjánsson 2013, Kratochwil 2016). In summary, the fauna of Iceland comprises seven species, but only the *Bombus jonellus* (Kirby, 1802) is thought to be a native inhabitant of this island (Prÿs-Jones et al. 1981, 2016, Kratochwil 2016). This species appears to be at risk of decline due to the spread of invasive plant species such as Nootka lupine (*Lupinus nootkatensis*) and cow parsley (*Anthriscus sylvestris*) (Willow 2017). However, Prÿs-Jones et al. (1981, 2016) have suggested that it probably originated with a historical founder event via the arrival of hibernating queens on ships carrying Irish monks (8<sup>th</sup>–9<sup>th</sup> centuries) or Vikings (9<sup>th</sup>–10<sup>th</sup> centuries), or even later. *Bombus lucorum* (Linnaeus, 1761) and *B. hortorum* (Linnaeus, 1761) appear to have arrived in Iceland in the second half of the 20<sup>th</sup> century, whereas *B. hypnorum* (Linnaeus, 1758), *B. pascuorum* (Scopoli, 1763), and *B. pratorum* (Linnaeus, 1761) appeared at the beginning of the 21<sup>st</sup> century. Finally, *B. terrestris* (Linnaeus, 1758) is actively utilized as a pollinator in greenhouses and may have become naturalized in the country (Prÿs-Jones et al. 2016, Kratochwil 2016).

In spite of the fact that there have been multiple colonizations of Iceland by bumblebees, the origin of certain lineages has not been studied using a molecular approach, and only a single barcode sequence of *Bombus lucorum* from Iceland is currently available (Williams et al. 2012, Prÿs-Jones et al. 2016). Our objective herein is to test the hypothesis regarding possible cryptic refugia in Iceland and to evaluate a putative origin of artificially introduced taxa based on molecular sequence data inferred from three bumblebee species, i.e. *Bombus jonellus* (putative native lineage), *B. lucorum*, and *B. hortorum* (recently introduced species).

## Materials and methods

### Data sampling

The bumblebee samples were collected with an entomological net (Ivan N. Bolotov leg.) in western and northern Iceland (Fig. 1). In summary, 64 individuals of three

species were collected from five localities (Table 1). Specimens were deposited at the Russian Museum of the Biodiversity Hotspots (RMBH) of the Federal Center for Integrated Arctic Research of the Russian Academy of Sciences (Arkhangelsk, Russian Federation). Bumblebee species were identified following Løken (1973) and Rasmont and Terzo (2010). The species names are given in accordance with Williams (2018).

## Laboratory protocols and sequence data set

We obtained new *cytochrome c oxidase subunit I* (COI) gene sequences from 12 Icelandic bumblebee specimens (Table 2). Molecular analysis (purification and PCR) was performed at the Federal Center for Integrated Arctic Research of the Russian Academy of

**Table 1.** Collecting localities and samples of bumblebees in Iceland.

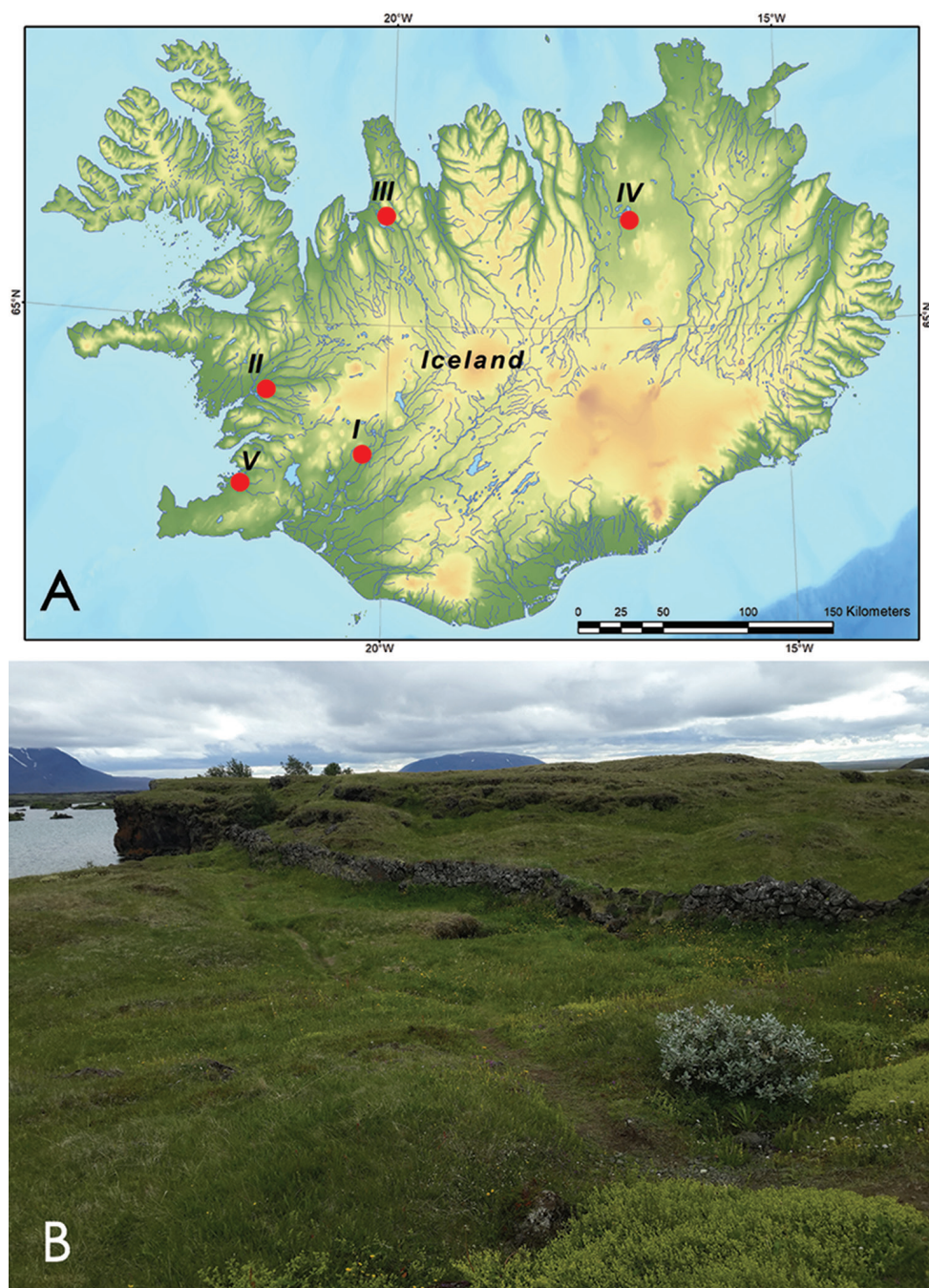
Code	Localities	Coordinates	Date	Habitats	Species and samples
I	Geysir	64°18'50.9"N, 20°17'58.7"W	12.VII.2013	Mountain herb-dwarf shrub tundra assemblages on lava fields and geysirite	<i>Bombus lucorum</i> (2 ♀)
II	Flókadalsá River	64°37'4.2"N, 21°30'9.4"W	16.VII.2013	Roadside, Nootka lupine assemblages	<i>B. lucorum</i> (4 ♀); <i>B. jonellus</i> (2 ♀)
III	Blanda River	65°34'52.7"N, 20°2'59.7"W	17.VII.2013	Herb meadows	<i>B. lucorum</i> (19 ♀); <i>B. jonellus</i> (1 ♀, 7 ♂)
IV	Mývatn Lake	65°34'12.4"N, 16°57'12.7"W	17.VII.2013	Mountain herb-dwarf shrub tundra assemblages	<i>B. lucorum</i> (2 ♀); <i>B. jonellus</i> (11 ♀, 1 ♂)
V	Reykjavík	64°7'44.7"N, 21°47'12.6"W	18.VII.2013	Herb meadows	<i>B. lucorum</i> (2 ♀); <i>B. jonellus</i> (7 ♀, 4 ♂); <i>B. hortorum</i> (2 ♀)

**Table 2.** List of COI barcode sequences for bumblebee specimens from Iceland

Species	COI lineage	COI GenBank acc. no.	Specimen Voucher*	Locality
<i>Bombus jonellus</i>	BJ-01	MH168019	BMB35	Blanda River
	BJ-01	MH168020	BMB50	Mývatn Lake
	BJ-01	MH168022	BMB54	Mývatn Lake
	BJ-01	MH168027	BMB71	Reykjavík
	BJ-01	MH168028	BMB75	Reykjavík
	BJ-01**	MH168025	BMB66	Reykjavík
<i>B. lucorum</i>	BJ-02	MH168021	BMB52	Mývatn Lake
	BL-01	MH168017	BMB19	Blanda River
	BL-01	MH168024	BMB64	Reykjavík
	BL-01	MH168018	BMB28	Blanda River
<i>B. hortorum</i>	BL-01	MH168023	BMB63	Reykjavík
	BH-01	MH168026	BMB70	Reykjavík

\*Deposited in the collection of the Russian Museum of Biodiversity Hotspots, Federal Center for Integrated Arctic Research of the Russian Academy of Sciences, Arkhangelsk, Russia. \*\*This specimen shares a specific singleton, which differs from the other haplotype in lineage BJ-01 (497 A vs. 497 T).





**Figure 1.** Map of study sites and typical habitat of *Bombus jonellus* in Iceland. **A** Map of study sites (see Table 1 for details). Red circles indicate sampling locations. **B** Shore of Mývatn Lake, a site with sympatric occurrences of the two lineages of *Bombus jonellus* (BJ-01 and BJ-02) in mountain herb-dwarf shrub tundra assemblages. Photograph by Mikhail Yu. Gofarov.

Sciences. A total DNA was extracted from a head capsule of each dried specimen using a standard phenol-chloroform procedure (Sambrook et al. 1989). The COI gene was amplified and sequenced using primer pairs C1-J-1718 and C1-N-2329R (Simon et al. 1994). The PCR mix contained approximately 200 ng of total cell DNA, 10 pmol of each primer, 200 µmol of each dNTP, 2.5 µl of PCR buffer (with  $10 \times 2$  mmol  $\text{MgCl}_2$ ), and 0.8 units Taq DNA polymerase (SibEnzyme Ltd.);  $\text{H}_2\text{O}$  was added for a final volume of 25 µl. Temperature cycling was as follows: 95 °C (4 min), 40 cycles of 95 °C (45 sec), 48–53 °C (40 sec), 72 °C (50 sec) and a final extension at 72 °C (5 min). The sequencing was carried out at the Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences (Moscow) using the ABI PRISM® BigDye Terminator v. 3.1 reagent kit. Reaction products were analyzed using an automatic sequencer, ABI PRISM 3730 (Applied Biosystems). The obtained results were analyzed using BioEdit version 7.2.5 (Hall 1999). Additionally, 165 COI sequences were obtained from the NCBI GenBank and the Barcode of Life Database (BOLD) (Suppl. material 1).

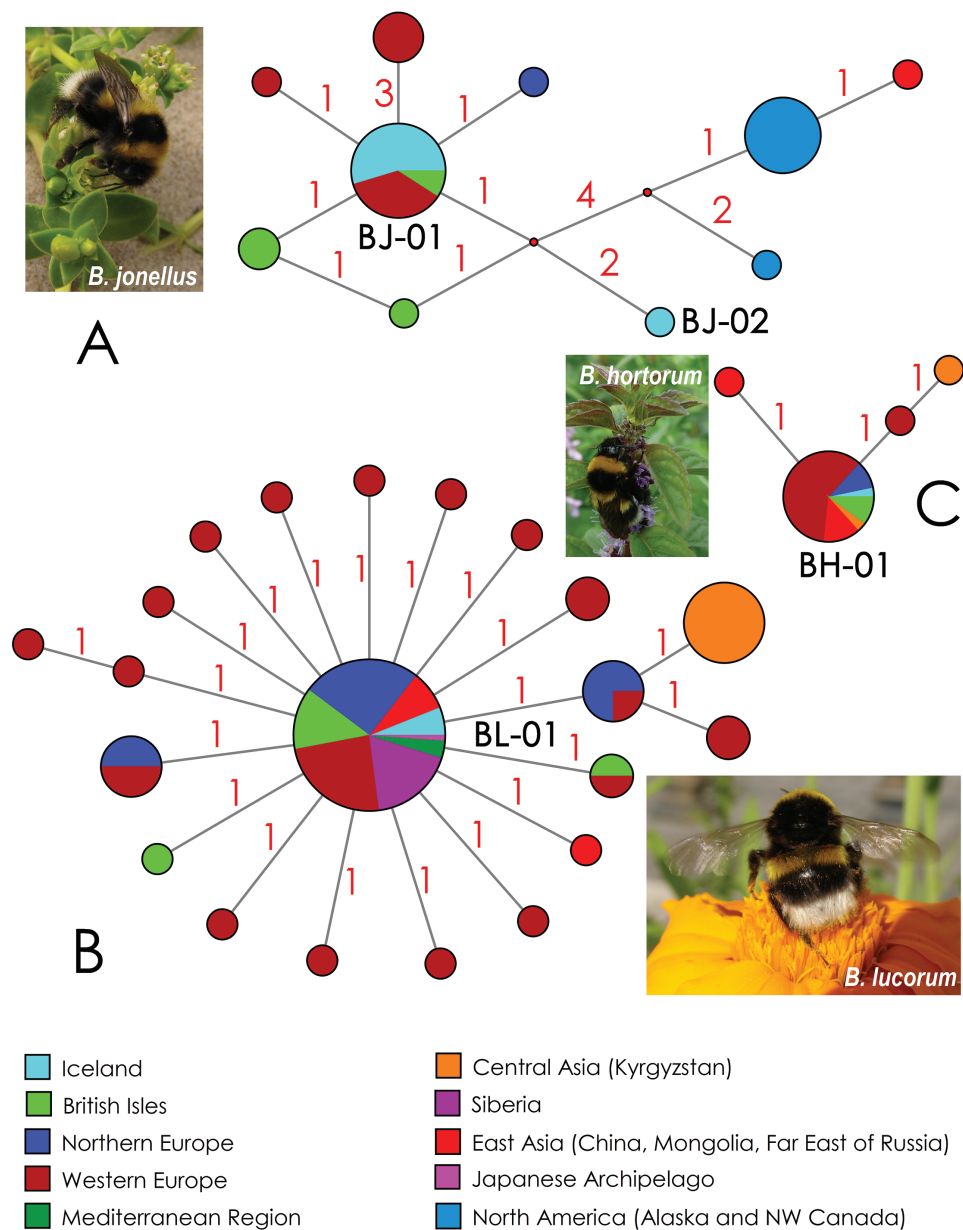
### Sequence alignment and phylogeographic analyses

The alignment of COI sequences was performed using the ClustalW algorithm implemented in MEGA6 (Tamura et al. 2013). Each COI sequence of the aligned datasets was trimmed, leaving a 455-bp fragment for *Bombus jonellus*, 448-bp for *B. lucorum*, and 423-bp for *B. hortorum*. The phylogeographic analyses were performed based on a median-joining network approach using Network version 4.6.1.3 software with default settings (Bandelt et al. 1999). Genetic divergences and nucleotide substitutions were estimated in MEGA6 (Tamura et al. 2013).

### Results

Three bumblebee species were recorded in our new samples from Iceland, i.e., *Bombus jonellus*, *B. lucorum* and *B. hortorum* (Table 1). The first two species were common and widespread, while *B. hortorum* appears to be rare and has been collected from only the Reykjavík area.

We found that the sequenced *Bombus jonellus* specimens from Iceland share three COI haplotypes belonging to two different lineages (Fig. 2). The first lineage (BJ-01) appears to be more common in Iceland, as it was found in six sequenced specimens, one of which shares a specific singleton, with a non-synonymous substitution in pos. no. 497 (A instead of T) (Table 2). This singleton is not shown on the network illustrated in Fig. 2 because it was calculated on the basis of a short-sequence dataset (see Materials and methods). The lineage BJ-01 has also been recorded from populations on the British Isles (Ireland) and in mainland Europe (Germany) (Figs 2, 3). In summary, eleven specimens belong to this lineage (37%



**Figure 2.** Median-joining haplotype networks of the available COI sequences of bumblebee species inhabiting Iceland. The circle size is proportional to the number of available sequences belonging to a certain haplotype. The small red dots indicate hypothetical ancestral haplotypes. Red numbers near branches indicate the number of nucleotide substitutions between haplotypes. Black codes indicate the COI lineages inhabiting Iceland. **A** *Bombus jonellus* (N = 29) **B** *B. lucorum* (N = 115) **C** *B. hortorum* (N = 33). Photographs by Yulia S. Kolosova.

of the total sample of the species [ $N = 29$ ]; see Suppl. material 1). The second *Bombus jonellus* lineage (BJ-02) appears to be rare and was found in a single specimen collected from the shore of Lake Mývatn. This lineage has not previously been recorded anywhere. It differs from the lineage BJ-01 in three non-synonymous nucleotide substitutions (218 T vs. 218 A, 284 T vs. 284 C, and 383 T vs. 383 C). The mean uncorrected COI p-distance between the lineages BJ-01 and BJ-02 is  $0.5 \pm 0.3\%$ . The haplotype network of *Bombus jonellus* reveals two shallow but geographically distinct clades, i.e. the European (including Iceland) and Nearctic – Northeast Asian haplogroups (Fig. 2) that may reflect two cryptic glacial refugia.

The Icelandic *Bombus lucorum* specimens belong to a single COI lineage (BL-01) that occurs in populations from Russia, China, Mongolia, Northern Europe (Denmark, Finland, Sweden, and Latvia), Western Europe (Austria and Germany), the British Isles (Ireland and United Kingdom), and Turkey, and in an invasive population from Hokkaido, Japan (Takahashi et al. 2017) (Figs 2, 3). In general, 83 specimens belong to this lineage (72% of the total sample of the species [ $N = 115$ ]; see Suppl. material 1). The star-shaped network may indicate a sudden population expansion in this species, most likely since the Last Glacial Maximum.

A single sequenced specimen of *Bombus hortorum* from Iceland also belongs to a widespread COI lineage (BH-01) that occurs in populations from China, European Russia, Northern Europe (Denmark and Norway), Western Europe (France and Germany), Southern Europe (Italy), and the British Isles (United Kingdom) (Figs 2, 3). In general, 30 specimens belong to this lineage (90% of the total sample of the species [ $N = 33$ ]; see Suppl. material 1). The network with four haplotypes is too simple, but it has a rather star-like shape, most likely indicating a rapid postglacial expansion or recent human-mediated dispersal of *Bombus hortorum*.

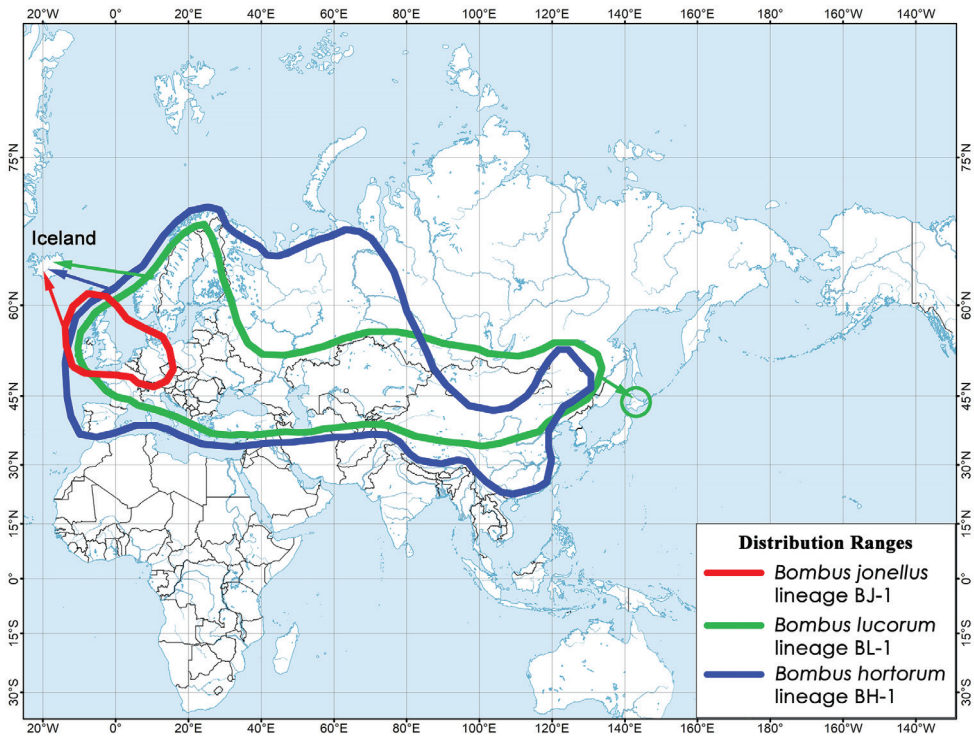
## Taxonomy

### *Bombus (Pyrobombus) jonellus* Kirby, 1802

= *Bombus (Pyrobombus) jonellus subborealis* Richards, 1933, **syn. n.**

**Remarks.** This subspecies is thought to be distributed in Norway and Iceland (Richards 1933, Løken 1973, Kratochwil 2016). However, our molecular data (Fig. 2) clearly indicate that this geographic race should be considered a synonym of *Bombus jonellus*. There are three other subspecies of *Bombus jonellus* from Northern Europe, i.e. *B. j. vogtii* Richards, 1933 from Shetland, *B. j. hebridensis* (Wild, 1931) from Hebrides, and *B. j. suecicus* (Friese, 1911) from Sweden (Kratochwil 2016). However, the close geographic proximity of the type localities of the named taxa raises questions about their validity and deserves further research.





**Figure 3.** Approximate distribution ranges of the widespread ubiquistic COI lineages recorded in bumblebee populations from Iceland (see Suppl. material 1 for details). Northern range boundaries of *Bombus lucorum* and *B. hortorum* lineages are in accordance with published sources (Williams et al. 2012, Kolosova et al. 2016, Potapov and Kolosova 2016). The map was created using ESRI ArcGIS 10 software ([www.esri.com/arcgis](http://www.esri.com/arcgis)). The topographic base of the map was created with Natural Earth Free Vector and Raster Map Data ([www.naturalearthdata.com](http://www.naturalearthdata.com)).

## Discussion

### Origin of recent immigrants in bumblebee fauna of Iceland

The modern bumblebee fauna of Iceland with seven species is close to species-poor assemblages on boreal European islands (e.g. the Solovetsky Archipelago in Northern European Russia: Bolotov et al. 2013) but is entirely different from those on the Arctic Ocean Islands, the faunas of which are dominated by cold-adapted Arctic species (Kolosova and Potapov 2011, Potapov et al. 2017). Based on long-term collection data, Prŷs-Jones et al. (2016) have suggested that *Bombus hortorum* and *B. lucorum* invaded Iceland around the middle of the 20<sup>th</sup> century (in the 1950s and in the 1970s, respectively). We discovered that the most common and widespread mtDNA lineages are found in the Icelandic populations of both species (Fig. 2). However, *Bombus hortorum* appears to have had a restricted range in southwest Iceland (Reykjavik and surrounding towns) until the present time, which aligns with the findings of Prŷs-Jones et al. (1981, 2016) and Kratochwil (2016).

We are unable to discuss the putative places of founders' origin for Icelandic *Bombus hortorum* and *B. lucorum* populations due to extensive distribution ranges of the founding lineages, and they may have originated from the British Isles and from anywhere on the mainland (Fig. 3). Such lineages have more opportunities to invade remote island areas from a statistical perspective, e.g. via the arrival of a queen or queens hibernating in cargo or via intentional introductions (Prŷs-Jones et al. 2016). Additionally, widespread ubiquitous lineages appear to be adapted to a broad range of habitats and foraging sources, which could help them to establish permanent populations within an island environment (Bolotov et al. 2013, Bolotov 2014). We can assume that the three other bumblebee species, i.e. *Bombus hypnorum*, *B. pascuorum*, and *B. pratorum*, that have colonized Iceland in the beginning of the 21<sup>st</sup> century may also share the most widespread and ubiquitous mtDNA lineages, like their predecessors, but this preliminary hypothesis has to be examined in a future study. Interestingly, an expansion of *Bombus pratorum* to Iceland in 2010 coincided with its appearance and establishment on the Faroe Islands (Madsen and Jensen 2011, Jensen and Madsen 2013, Witaliński and Jensen 2017), suggesting an intense natural dispersal event from the mainland. Kratochwil (2016) has shown that the bumblebee species turn-over in Iceland is driven primarily by global warming and the introduction of non-native species.

### Phylogeographic pattern in populations of *Bombus jonellus* and a prospective scenario of its expansion into Iceland

At first glance, a global phylogeographic pattern in *Bombus jonellus* may reflect its survival in two distant glacial refugia, i.e., in Europe and in Beringia, although no sequences of this species from Siberia are available, and may narrow the current gap between European and Nearctic haplogroups (Fig. 2). Based on our preliminary survey, we assume that the only nominative subspecies of *Bombus jonellus* is ranged in Europe because all of the European COI haplotypes of this species are quite similar and belong to a single compact haplogroup (Fig. 2).

The presence of putative unique haplotypes in Norway, the United Kingdom, and Iceland could indicate a rapid northwestern expansion of this species from glacial refugia in Southern and Central Europe in the Late Pleistocene or Early Holocene. Our data set is very limited, and it is highly likely that the unique lineage BJ-02 from Iceland can be found somewhere on the British Isles, Shetland, and Hebrides or in mainland Europe. However, our results indicate that *Bombus jonellus* may have colonized Iceland two times and that the lineage BJ-02 may reflect a more ancient, Late Pleistocene or Early Holocene founder event (e.g. from the British Isles), albeit more sampling efforts are necessary to obtain a fully resolved biogeographic model for this species. We agree with Prŷs-Jones et al. (1981, 2016) that the first expansion of *Bombus jonellus* to Iceland was most likely caused by a historical, human-mediated dispersal event. The Viking period, when large numbers of cargo ships could have supported long-distance dispersal processes in several species, e.g. the Orkney house mouse lineage (Searle et al. 2009), appears to be the most probable time of this expansion.



Indeed, our results inferred from the Icelandic bumblebees correspond well to the *tabula rasa* hypothesis. Such a phylogeographic pattern has been discovered in several other taxa, and a slowly growing body of molecular research indicates that invertebrate faunas on the North Atlantic Islands have had postglacial allochthonous origin (Pálsson et al. 2016, Bolotov et al. 2017, Vinarski et al. 2017). The Icelandic subterranean amphipods, the only known exception, were able to survive in groundwater reservoirs under glaciers during the Last Glacial Maximum (Kornobis et al. 2010). Finally, we could conclude that environmental conditions supporting the survival of freshwater and terrestrial invertebrates were lacking in Iceland during the LGM, and they may have arrived on the island after its deglaciation (pond snails: Bolotov et al. 2017, caddisflies: Pálsson et al. 2016, bumblebees: this study). This phylogeography-based conclusion is in agreement with paleogeographic modelling that suggests the existence of a continuous, thick ice sheet covering the entire island (Bingham et al. 2003, Ingólfsson et al. 2010). More interestingly, a phylogeographic pattern has recently been discovered on the Novaya Zemlya Archipelago that is thought to have served as a cryptic glacial refugium for bumblebees during the Late Pleistocene epoch (Potapov et al. 2017).

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## Supplementary material I

### Table S1

Authors: Grigory S. Potapov, Alexander V. Kondakov, Yulia S. Kolosova, Alena A. Tomilova, Boris Yu. Filippov, Mikhail Yu. Gofarov, Ivan N. Bolotov

Data type: Dataset of sequences

Explanation note: List of additional COI sequences of *Bombus* species obtained from GenBank or BOLD, including taxon, accession number, specimen code, locality information and data source.

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