# Description of a novel mating plug mechanism in spiders and the description of the new species Maeota setastrobilaris (Araneae, Salticidae) 

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#### Abstract

Reproduction in arthropods is an interesting area of research where intrasexual and intersexual mechanisms have evolved structures with several functions. The mating plugs usually produced by males are good examples of these structures where the main function is to obstruct the female genitalia against new sperm depositions. In spiders several types of mating plugs have been documented, the most common ones include solidified secretions, parts of the bulb or in some extraordinary cases the mutilation of the entire palpal bulb. Here, we describe the first case of modified setae, which are located on the cymbial dorsal base, used directly as a mating plug for the Order Araneae in the species Maeota setastrobilaris sp. n. In addition the taxonomic description of $M$. setastrobilaris $\mathbf{s p}$. $\mathbf{n}$. is provided and based on our findings the geographic distribution of this genus is extended to the Northern hemisphere.


## Keywords

Reproduction, Neotropics, taxonomy, sperm priority

## Introduction

Arthropod reproductive systems are an interesting topic of research where several mechanisms of sexual selection have evolved. In this context females seek to maximize sperm diversity throughout polyandry, while males try to maximize parenthood by preventing the female from being inseminated by other males. One possibility of preventing subsequent inseminations is the production of mating plugs which might be a result of an arms race or male-male competition (Eberhard 1985; Eberhard and Huber 2010), or as part of sperm competition (Uhl et al. 2010). In any case, mating plugs are perfect examples of structures that block the female copulatory ducts (Eberhard 1985) regardless of the hypotheses that have been postulated to explain their evolution.

Production of mating plugs in Araneae have mainly evolved in Entelegynae reproductive systems characterized by the presence of two different pairs of ducts, one specialized for insemination and the other for fertilization. Mating plugs are only found on the copulatory duct openings and have been classified as defensive traits to avoid sperm competition, in contrast to offensive traits that introduce secretions in the female genital track detering the quality of previously deposited sperm. Both mechanism were explained by the sperm competition hypothesis; however, alternative non-exclusive functions under natural selection such as retaining sperm in an advantageous position for fertilization, preventing sperm leakage or desecration have been postulated (Uhl et al. 2010).

Mating plugs can be either chemical or physical. The former are secretions produced by structures such as bulb glands, the spermatic duct, glands in the male mouth area or plugs produced by secretion of female origin (Eberhard 1983; Uhl et al. 2010). Plugs in zodariid spiders have even been attributed to specialized setae on the dorsal area of the cymbium, i.e., in Storenomorpha reinholdae Jocqué \& Bosmans, 1989 and Cicynethus sp. (Jocqué 1991), but thorough documentation of these cases are still in progress as more evidence is adquired. Plugs may also be formed by male genital structures like the embolus, embolus and conductor, paracymbium, and there are extraordinary cases where the complete palpal bulbs or even the whole male (Tidarren spp.) are used as plugs after insemination (Uhl et al. 2010). Detachment of tibial setae has been suggested in Paccius cf. scharff Platnick, 2000; although this observation has not been thoroughly tested (Uhl et al. 2010).

The genus Maeota Simon, 1901 has currently four described species inhabiting South America: M. dichrura Simon, 1901, M. dorsalis Zhang \& Maddison, 2012, M. flava Zhang \& Maddison, 2012 and M. simoni Zhang \& Maddison, 2012 (World Spider Catalog 2015). The monophyly of this genus is currently supported only by molecular evidence (Zhang and Maddison 2012). These authors provided a diagnosis for Maeota, comparing it to the known diversity of other Euophryinae, as the following combination of features: medium small-sized spiders (ca. 2.7-4.3 mm), chelicerae with two promarginal and one retromarginal teeth, first tibia with three pairs of ventral macrosetae and first metatarsus with two pairs, plane of the embolic spiral more or less
perpendicular to the longitudinal axis of the bulb and tegulum with a proximal lobe (Zhang and Maddison 2012). This diagnosis may change as more Maeota species are discovered and these diagnostic character hypotheses tested.

The present study describes the new species Maeota setastrobilaris sp. n., which possesses a new kind of physical mating plug mechanism in the Order Araneae, which functions by the detachment of modified cymbial setae, referred hereafter as strobilate setae.

## Methods

The specimens here described were collected as part of a spider inventory in a tropical forest remnant located near the city of Xilitla from August 2011 to June 2012. This inventory was made following standardized protocols (Coddington et al. 1991). All specimens were collected inside a one hectare plot (with central coordinates $21^{\circ} 23^{\prime} 50.9^{\prime \prime} \mathrm{N}, 98^{\circ} 59^{\prime} 38.2^{\prime \prime} \mathrm{W}$, elev. 626 m ) mainly on vegetation either with beating trays or by direct collecting. More information regarding this inventory and additional views of specimens can be found at www.unamfcaracnolab.com (Alvarez-Padilla Laboratory 2014).

Specimens were collected and stored in $96 \%$ ethanol. The female genitalia were dissected and digested following the protocol by Alvarez-Padilla and Hormiga (2008) and mounted using semi-permanent mounts (Coddington 1983). Photographs were taken with a Nikon DS-Fi2 camera, external anatomy images were taken using a Nikon SMZ1000 Stereomicroscope. Images of the cleared genitalia were taken with a Nikon E200 Microscope. Drawings were done with the respective drawing tubes for both microscopes. The images were captured using NIKON NIS ELEMENTS 4.0 software and multiple focal plane images were combined into montages using HELICON FOCUS 5.3.14. SEM samples were coated with platinum and images taken with a Zeiss EVO 55 SEM microscope. All measurements were performed using a micrometric ocular and are given in millimeters. High resolution versions of the images are available at www.unamfcaracnolab.com (Alvarez-Padilla Laboratory 2014). Mating plugs were documented by checking the available material from two collections: Museum of Comparative Zoology (MCZ) and the Arachnology lab from Facultad de Ciencias UNAM. A total of 127 specimens were examined documenting the presence of modified setae in both sexes, i.e., cymbium with broken and complete seta and epigynal genital openings either free or plugged (Table 1).

Abbreviations used in text and figures: (As) adjacent minor setae, (B) basis of seta, (BD) breakage disc, (bH) basal hematodocha, (CASC) California Academy of Sciences, (CO) copulatory opening, (dH) distal hematodocha, (E) embolus, (EF) epigynal flaps, (Fd) fertilization Ducts, (ip) irregular cuticular pigmentation, (M) thin cuticular layer between articles, (MCZ) Museum of Comparative Zoology at Harvard University, (RTA) retrolateral tibial apophysis, (Sp) spermathecae, ( $\mathrm{s} S \mathrm{p}$ ) secondary spermathecae, (stS) strobilate seta.

## Systematics

Maeota Simon, 1901
Type species. M. dichrura Simon, 1901

## Maeota setastrobilaris sp. n.

http://zoobank.org/2D91AD1F-47B1-438F-9E13-A12E621BAB93
Figures 1-23

Type materials. Holotype: male from Jardín Escultórico Edward James, Xilitla, San Luis Potosí, Mexico 26-30 March 2012 (Alvarez-Padilla col.). Allotype: female with the same locality data and collected 10-15 June 2012 (Gonzáles-Contreras col.). Both specimen deposited at MZC, Harvard University.

Etymology. The species epithet is a noun in apposition referring to the anatomy of the cymbial strobilate seta used as a mating plug, which resembles a strobilar gymnosperm cone.

Diagnosis. M. setastrobilaris differs from M. simoni by the embolus coiled less than two times. It differs from M. flava and M. dorsalis by larger proximal tegular lobe and the finger shaped RTA extended ventrally (Fig. 13, 19). This species differs from $M$. dichrura by the shorter PLS and the absence of a lateral abdominal patch of enlarged setae in males. Unique features of $M$. setastrobilaris also include: epigynum copulatory duct openings covered by drop-shaped flaps (Fig. 23), embolus coiled 1.2 times and the cymbium dorso-basal edge with modified setae used as mating plugs.

Description. Male total length 3.58 mm . Cephalothorax: length 1.83 mm , width 1.29 mm . Carapace dorsal surface dark-orange covered with scattered white scales and a glabrous longitudinal paler area posterior to the PLE. Carapace lateral surfaces and clypeus dark-orange with a reticulated pattern darker in color and concentrated towards the carapace edges (Fig. 18). Ocular anterior region: AME encircled with several scales, white at the center and orange at the sides. Ocular region 2/3 wider than long. PLE 0.5 , PME 0.1 and ALE 0.5 times the diameter of AME. PME closer to PLE than ALE (Fig. 8). Chelicera paturon base dark-brown turning lighter distally, retromarginal tooth bifurcated. Endites pale-yellow distally, darker at their base and slightly longer than wide. Labium with same color pattern as the endites and triangular in shape. Sternum pale-yellow and longer than wide (Figs 11). Abdomen: surface background dark yellow covered with white or iridescent scales. Dorsal pattern with two brown longitudinal lines and a posterior broken pattern of dark chevrons (Fig. 8). Lateral surface almost covered by an additional dark reticulated thick line that extends towards the spinnerets. Ventral pattern with a dark gray rectangle that extends behind both sides of the epigastric furrow. Spinnerets gray and encircled with black pigmentation. PMS twice as thick as the PLS. PLS $2 / 3$ longer than PMS with the distal segment darker in coloration and its ventral surface cov-
ered by thick black setae. Legs pale-yellow, I and II similar in size and smaller than III and IV. Leg IV the longest. Femora lighter than the other articles. Macroseta patterns based on voucher specimen JAM327. Femora with a 1-1-1 dorsal macroseta pattern. The distal spination has up to three subdorsal shorter macrosetae, two prolateral and one retrolateral. Patella with two subdorsal macrosetae, one on each side. Retrolateral tibia surfaces I to IV with six macrosetae distributed at 2-2-1-1 pattern. Tibia prolateral surfaces with the following macrosetae numbers and distributions: I, II 3-1-1-1; III-IV 3-2-1-1. Metatarsus prolateral surfaces: I 1-1-1-3; II 1-1-0-3; III-IV 2-0-0-3. Tarsi without macrosetae. Pedipalp: Tegulum with a proximal dropshaped lobe and spermatic duct coils visible (Figs 14, 21). An additional sclerite present between the prolateral cymbial edge and the tegulum (Fig. 21 arrow). Embolus base circular, flat and attached to the embolus. Embolus heavily sclerotized, thick and coiling 1.2 times around embolus base edge (Fig. 21). Cymbium ventral surface notched distally near embolus, dorsal surface hirsute with four modified basal seta on the edge, the largest used as a mating plug (Fig. 19, stS). Female as in male except as noted. Total length 3.87 mm . Cephalotorax: length 1.62 mm , width 1.25 mm . Carapace lighter in coloration, reticular lateral pattern absent (Fig. 2). Abdomen: pattern as in male but lighter in coloration. Spinnerets: PLS 1.2 times longer than PMS. Legs: macroseta patterns based on voucher specimen JAM326. Retrolateral tibia surfaces: I-III 1-2-1-1; IV 2-1-1-1. Prolateral tibia surfaces: I, III-IV 1-2-1-1; II 1-1-1-1. Epigynum: with two comma-shaped, concentric cuticular flaps covering the genital opening entrance (Figs 6, 7, 20, 23). Spermathecae peanut-shaped with two lobes communicating by a thick channel, fertilization ducts tiny and located on the middle section of posterior lobes (Fig. 22).

Variation: Male size: total length $2.76-3.58 \mathrm{~mm}$, carapace $1.29-1.61 \mathrm{~mm} . \mathrm{Fe}$ males total length $2.75-4.29 \mathrm{~mm}$, carapace $1.20-1.70 \mathrm{~mm}$. Spermathecal lobes vary considerably in orientation and the length of the channel. Several specimens presented asymmetric spermathecae. Flaps covering the copulatory ducts also vary in shape and in orientation relative to the middle longitudinal axis of the genital plate (Fig. 20). Specimen coloration varies from pale-yellow to dark-brown (Figs 1-5, 8-11).

Distribution. Mexico from the Eastern Cordillera to the Southwestern States. (Fig. 16).

Records: $\mathrm{N}=139$. Mexico: Campeche: Chicana ruins ca. 8 km W of Xpujil $18^{\circ} 32^{\prime} \mathrm{N}, 89^{\circ} 31^{\prime} \mathrm{W} 270 \mathrm{~m}$. Jul. 14, 1983, W. Maddison col., 19, MCZ. Chiapas; 76 $\mathrm{km} S$ on road from Palenque to Ocosingo $17^{\circ} 01^{\prime} \mathrm{N}, 92^{\circ} 02^{\prime} \mathrm{W} 1377 \mathrm{~m}$. Jul. 26-29, 1983, W. Maddison col., 1 , $10^{\top}, \mathrm{MCZ}$; Palenque ruins $17^{\circ} 29^{\prime} \mathrm{N}, 92^{\circ} 01^{\prime} \mathrm{W} 118 \mathrm{~m}$. Jun. 02-11, 1983, W. Maddison \& R.S. Anderson col., 4中, 3 ${ }^{\text {® }}$, MCZ (paratypes); Jul. 31, 1983, W. Maddison col., 2q, MCZ. Nuevo León: 29 E Linares along highway km $6024^{\circ} 08^{\prime} \mathrm{N}, 99^{\circ} 08^{\prime} \mathrm{W} 197 \mathrm{~m}$. Jun. 03-05, 1983, W. Maddison col., $10^{\text {on, }}$ MCZ. Oaxaca: 2 km S El Tule $17^{\circ} 02^{\prime} \mathrm{N}, ~ 96^{\circ} 40^{\prime} \mathrm{W} 1868 \mathrm{~m} .1983$, W. Maddison $\&$ R.S. Anderson col., 1q, MCZ. Quintana Roo: 31 NE Felipe Carrillo Puerto on highway km $30719^{\circ} 48^{\prime} \mathrm{N}, 87^{\circ} 52^{\prime} \mathrm{W} 13 \mathrm{~m}$. Jul. 17, 1983, W. Maddison \& R.S. Anderson col., $10^{\top}$, MCZ. San Luis Potosí: Xilitla, Cueva de Salitre $21^{\circ} 23^{\prime} \mathrm{N}, 98^{\circ} 59^{\prime} \mathrm{W}$


Figures I-7. M. setastrobilaris female anatomy. I habitus live specimen $\mathbf{2}$ habitus of specimen in dorsal view $\mathbf{3}$ same lateral view $\mathbf{4}$ prosoma anterior view $\mathbf{5}$ habitus ventral view $\mathbf{6}$ epigynum ventral view $\mathbf{7}$ epigynum dorsal view (cleared). Scale bars $0.5 \mathrm{~mm}(\mathbf{2 - 5}) ; 0.2 \mathrm{~mm}(\mathbf{6})$; and $0.1 \mathrm{~mm}(\mathbf{7})$.


Figures 8-I 5. M. setastrobilaris male anatomy. $\mathbf{8}$ habitus dorsal view 9 same lateral view $\mathbf{1 0}$ prosoma anterior view II habitus ventral view $\mathbf{I} \mathbf{2}$ pedipalp prolateral view $\mathbf{I} \mathbf{3}$ same retrolateral view $\mathbf{I} \mathbf{4}$ same ventral view $\mathbf{I} 5$ pedipalp expanded retrolateral view. Scale bars: $0.5 \mathrm{~mm}(\mathbf{8}-\mathbf{I I}) ; 0.2 \mathrm{~mm}(\mathbf{I} \mathbf{2} \mathbf{I} \mathbf{5}) .(\mathrm{bH}, \mathrm{dH})$, basal and distal hematodochae, the larger arrow points to the conductor-like structure.


Figures 16-23. M. setastrobilaris distribution map and illustrations. 16, Distribution map. 17, female prosoma lateral view. 18, male prosoma lateral view. 19, male pedipalp retrolateral view. 20, epigynum ventral view. 21, male pedipalp ventral view 22 cleared epigynum dorsal view. 23, same ventral view. Scale bars $1 \mathrm{~mm}(\mathbf{1 7}, \mathbf{1 8}) ; 0.2 \mathrm{~mm}$ ( $\mathbf{1 9 - 2 I}$ ); $0.1 \mathrm{~mm}(\mathbf{2 2 , 2 3})$. (ip), irregular cuticular pigmentation, (stS) strobilate seta, (RTA) retrolateral tibial apophysis, (E) embolus, (EF) epigynal flaps, (Cd, Fd) copulatory and fertilization ducts, $(s S p)$ secondary spermathecae, $(S p)$ spermathecae.


Figures 24-30. M. setastrobilaris mating plug SEM images. 24 cymbium at retrolateral view showing the seta $\mathbf{2 5}$ same view without seta $\mathbf{2 6}$ epigynal genital openings plugged by seta $\mathbf{2 7}$ close up of mating plug 28 strobilate seta brakeage disk 29 strobilate setae attached to cymbial membrane $\mathbf{3 0}$ detached strobilate seta. Scale bars 10 microns in all Figures. (B) basis of seta, (BD) breakage disc, (M) thin cuticular layer between articles, (As) adjacent minor setae, (stS) Strobilar seta, (CO) copulatory opening.

Table I. Specimen strobilate setae presence or absence counts.

| SEX | Total | Without seta | With seta | Left side | Right side | Both sides |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| MALE | 47 | 18 | 29 | 24 | 24 | 19 |
| FEMALE | 80 | 38 | 42 | 25 | 20 | 3 |
| Total | 127 | 56 | 71 | 49 | 44 | 22 |

576 m. Jun. 13, 1983, W. Maddison col., 2 ${ }^{\top}$, MCZ; Las Pozas (Jardín escultórico de Edward James) $21^{\circ} 23^{\prime} 50.9^{\prime \prime N} 98^{\circ} 59^{\prime} 38.2^{\prime \prime} \mathrm{W} 626 \mathrm{~m}$. Arachnology team col. Aug. 27-31, 2011, 13q, 7 §; N. 14-18, 2011, 27q, 11 §; Mar. 26-30, 2012, 25q, 12 §; Jun. 10-15, 2012, 13q, 12§, Spider collection Arachnology Lab. Facultad de Ciencias. Supplementary images for paratypes deposited at CASC available at http://www.unamfcaracnolab.com with voucher and collection code numbers: N. 14-18, 2011, 1中, JAM326 and CASENT 9051538; 1才, JAM327 and CASENT 9051537. Additional specimens at CASC 1 q, $1 \widehat{O}^{\wedge}$, CASENT 9051536; 1q, CASENT 9051539; 2q, CASENT 9051540; 1q, CASENT 9051541. Tabasco: 2.4 km. E Teapa, Grutas de Corona $17^{\circ} 33^{\prime} \mathrm{N}, 92^{\circ} 56^{\prime} \mathrm{W} 55 \mathrm{~m}$. Jul. 7, 1983, W. Maddison col., $10^{\lambda}$, MCZ. Veracruz: Estacion Biología Tropical Los Tuxtlas near La Palma N of Catemaco $18^{\circ} 36^{\prime} \mathrm{N}, 95^{\circ} 07^{\prime} \mathrm{W} 366 \mathrm{~m} .1983$, W. Maddison \& R.S. Anderson col., 1 , MCZ.

Behavioral notes. The dorsal base of cymbium presents a cluster of modified setae on its edge used during copulation as a mating plug. They are located over a pit between the membrane joining tibia-tarsus articles (Fig. 25: M). Inside the pit are three to four strobilate setae, the largest used as a mating plug while the others remain attached to the tibia-tarsus joint (Fig. 25). The largest of these strobilate setae is detached in several specimens and was found blocking the copulatory ducts entrance (Figs 24,29: stS; 27, 28: CO). Evidence supporting the use of this strobilate seta as a mating plug lies in their rupture from a basal breakage disc (Fig. 28: BD) attached to weak setal basis (Figs 27, 30: B) and the texture that makes it difficult to mechanically extract the setae from the genital openings. The function of the smaller setae is unknown, but they could work either as pressure indicators controlling the detachment of the larger seta, or guiding it throughout the mating plug function. Total sample consisted of 127 specimens (Table 1) where $62 \%$ were females. Almost $50 \%$ of these females had the seta at least in one of their genital openings, while $60 \%$ of the males lost at least one seta from both pedipalps. Absence of both setae in males was higher than the presence of two setae in females with a ratio of $10: 1$. Accidental loss of setae from specimens after preservation is unlikely: exemplars collected 30 years ago that were examined in MCZ presented the structures in the same proportions as exemplars captured in 2011, suggesting that handling does not lead to accidental loss of these setae. The low proportion of females with both copulatory ducts plugged suggests a negative response to the insertion of a second seta.

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# Systematics of the Madagascar Anelosimus spiders: remarkable local richness and endemism, and dual colonization from the Americas 

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#### Abstract

Despite the alarming rates of deforestation and forest fragmentation, Madagascar still harbors extraordinary biodiversity. However, in many arthropod groups, such as spiders, this biodiversity remains mostly unexplored and undescribed. The first subsocial Madagascan species of the theridiid spider genus $A n$ elosimus were described in 2005 when six new species were found to coexist in the Périnet forest fragment within Andasibe-Mantadia NP. However, this discovery was based only on a few specimens and the extent of this Madagascan radiation has remained unknown. We here report on a thorough survey of >350 colonies from Périnet, and three pilot surveys into additional Madagascar forests (Ambohitantely, Ranamofana, and Montagne d'Ambre). The morphological, molecular and natural history data from these surveys facilitated a revised taxonomy and phylogenetic hypothesis of Madagascan Anelosimus. This subsocial clade currently comprises six previously known (A. andasibe Agnarsson \& Kuntner, 2005, A. may Agnarsson, 2005, A. nazariani Agnarsson \& Kuntner, 2005, A. sallee Agnarsson \& Kuntner, 2005, A. salut


#### Abstract

Agnarsson \& Kuntner, 2005, A. vondrona Agnarsson \& Kuntner, 2005) and 10 new species: A. ata sp. n., A. buffoni sp. n., A. darwini sp. n., A. hookeri sp. n., A. buxleyi sp. n., A. lamarcki sp. n., A. moramora $\mathbf{s p} . \mathbf{n}$., $A$. tita sp. n., A. torfi sp. n., A. wallacei sp. n.. With the exception of $A$. may and $A$. vondrona, all other species appear to be single forest endemics. While additional sampling is necessary, these data imply a much higher local richness and endemism in Madagascan forests than in any other comparable area globally. The phylogenetic results establish a sister clade relationship between the subsocial Anelosimus in Madagascar and the American 'eximius group', and between the solitary $A$. decaryi on Madagascar and a solitary American clade. These findings imply duplicate colonizations from America, an otherwise rare biogeographical pattern, calling for more detailed investigation of Anelosimus biogeography.


## Keywords

Cobweb spiders, subsocial, Theridiidae, biogeography, colonization, radiation, congener coexistance

## Introduction

Madagascar is a biodiversity hotspot that has undergone extreme deforestation during the last century. What once was near continuous and vast forest along the eastern slopes of Madagascar is now fragmented, often into discontinuous patches. Many such isolated patches harbor unique diversity of species, with particularly well known vertebrate examples including endemic lemurs, chameleons, geckoes, snakes, frogs, and others (Agnarsson et al. 2014; Agnarsson and Kuntner 2012; Karanth et al. 2005; Raxworthy et al. 2007, 2008; Vences et al. 2003, 2004; Vidal et al. 2010; Yoder and Nowak 2006). However, for many groups of organisms, including notably diverse arthropod lineages such as hemipterans and beetles (Krishnankutty 2012; Rainio 2012), knowledge of species and their distribution in Madagascar remains very limited. Because such groups are critical for holistic understanding of the major events and fac-tors-biotic and abiotic-responsible for diversification of lineages in Madagascar (Agnarsson et al. 2014; Agnarsson and Kuntner 2012; Dijkstra 2007; Fisher 1997) and other ancient islands (Gillespie and Roderick 2002), further taxonomic species discovery of arthropods in Madagascar is essential.

In spider research, Madagascar has been relatively neglected with the most notable exception being the four year inventory by the California Academy of Sciences under the leadership of Dr. Charles E. Griswold, and a few additional efforts (see the works of Agnarsson 2006; Agnarsson et al. 2010b; Alvarez-Padilla et al. 2012; Griswold 1997, 2000; Griswold and Ledford 2001; Jocqué 1994; Kuntner 2006; 2007; Kuntner and Agnarsson 2011; Wood et al. 2007). For example, Darwin's bark spider exhibiting the web with the longest bridgelines and toughest silk of all spiders (Agnarsson et al. 2010a), was only discovered in the recent years (Kuntner and Agnarsson 2010), and despite lively recent research into their biology (Gregoric et al. 2011a-b), the Madagascar radiation of bark spiders remains largely undescribed (Gregorič et al. 2015).

Similarly, the first subsocial species of the spider genus Anelosimus Simon, 1891 were described from Madagascar only 10 years ago (Agnarsson and Kuntner 2005). This initial discovery of six related subsocial species in a single forest fragment (Périnet) -
more than any other locality on Earth-indicated that Madagascar may be home to a rich radiation of these spiders. Here, we report on a thorough survey of Périnet, and three pilot surveys of additional forest patches, from the highly isolated fragments of Ambohitantely and Montagne d'Ambre National Parks, and from a National Park at the heart of the remaining continuous eastern slope forests, Ranamofana National Park. We describe the newly discovered species, offer basic information on their natural history, and place them in a global phylogenetic context.

## Methods

Spiders were collected in the field from Ambohitantely Special Reserve, Ankazobe district, 28.iv. 2008 (Agnarsson and Kuntner), Montagne d’Ambre National Park, Antsiranana district, 4.iv. 2008 (Agnarsson and Kuntner), Périnet Special Reserve, 3-20. iv. 2008 and 13-26.xi. 2008 (Agnarsson, Kuntner, Hanitriniaina and Rabarison), and Ranamofana National Park, Fianarantsoa district, 27.iv.-2.v. 2013 (Suppl. material 1). Colonies were located in the forest along trails and rivers at tips of branches, often containing one or more dead leaves, which were webbed together as a retreat. Entire colonies were sampled into zip-lock bags and contents were subsequently sorted into sampling vials. In both Ambohitantely and Montagne d'Ambre sampling was opportunistic and rapid as only a few hours were available for sampling at each site. However, in Ranamofana basic observations were also made on the biology of the species, and in Perinét a systematic survey of $>350$ colonies was made.

Specimens were identified to species (Agnarsson and Kuntner 2005) and/or morphospecies under a Leica MZ16 dissecting microscope and photographed using the Visionary Digital BK lab system equipped with a Canon 5D camera and a 65 mm macro 5x zoom lens. Multiple images taken at different focal planes were combined with Helicon Focus 5.3 and processed with Photoshop CS6 to adjust contrast and sharpness, and to create a white background. For photography, specimens were temporarily mounted in alcohol-based hand sanitizer (GermX containing 65\% ethanol and no added perfumes or other chemicals), then covered with $75 \%$ ethanol. Most measurements were made directly from photographs using the 'analyses' tools in Photoshop, with additional measurements made, as needed, using a micrometer eye-piece in the Leica microscope. All measurements are in millimeters. Prosoma and abdomen length and height were measured in lateral view, the width in dorsal view, all measured at widest points. Leg segments were measured without the detachment of legs from the prosoma and are thus approximations. Female genitalia were excised using sharp forceps and digested using KOH for photography of internal genitalia. Photographs were assembled into plates and labeled in Illustrator CS6.

DNA was isolated from one to multiple individuals per putative morphospecies depending on specimen availability using the QIAGEN DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA). We targeted fragments of two mitochondrial (cytochrome coxidase subunit 1-COI, 16S rRNA) and two nuclear (Internal transcribed spacer-ITS2 and 28S
rRNA) loci previously demonstrated to be effective phylogenetic markers at low taxonomic levels for spiders (Agnarsson et al. 2007, 2010b, 2013). We amplified COI with the LCO1490 (Folmer et al. 1994), and C1-N-2776 primers (Hedin and Maddison 2001). We used 16SA and B primers (Simon et al. 1994) to amplify 16S, the ITS-5.8S (FITS) and ITS-28S (RITS, or ITS 4) primers for ITS2 (White et al. 1990), and 28Sc and 28 So primers for 28 S (Whiting et al. 1997). Sequencing was done at the University of Arizona. Sequences were submitted to GenBank (accession numbers: KR909226KR909300, KT174673-KT175005).

Chromatograms were interpreted employing Phred and Phrap (Green 2009; Green and Ewing 2002) through the Chromaseq 1.01 module (Maddison and Maddison 2010) in the evolutionary analysis program Mesquite 2.75 (Maddison and Maddison 2014) with default parameters. The sequences were then proofread by examining chromatograms by eye. The data were aligned in MAFFT (Katoh 2013) through the online portal EMBL-EBI, using default settings and increasing the tree rebuilding and maxiterate settings to 100 . Gaps were treated as missing data. For Bayesian analyses, the appropriate substitution model was selected with jModelTest 2.1.4 (Posada 2008) using the AIC criterion to select among the 24 models implemented in MrBayes (Huelsenbeck and Ronquist 2001). The best model for $28 S$, COI and 16 S was GTR+G+I, for ITS2 was GTR+G, and for previously published ND1 sequences HYK+G. We ran Bayesian analysis of the five loci concatenated in MrBayes V3.2.2 (Ronquist and Huelsenbeck 2003) through the CIPRES online portal. The concatenated analysis was partitioned by locus. We ran the Markov chain Monte Carlo with four chains for $10,000,000$ generations, sampling the Markov chain every 1,000 generations. The results were examined in Tracer 1.5 (Rambaut and Drummond 2007) to verify proper mixing of chains and that stationarity had been reached, and to determine adequate "burnin". Trees were edited in Mesquite and then exported as PDF files and all figures were compiled and finalized in Adobe Illustrator.

Species were delimited based on a combination of molecular and morphological diagnosability. Specimens from different areas showing identical or near identical DNA barcoding (COI) haplotypes and sharing detailed genital morphology were treated as conspecific. Specimens differing distinctly in DNA barcodes, generally over $3 \%$ sequence divergence (e.g. Hebert et al. 2004) but in some cases as little as $2 \% ~(2 \%$ is close to minimal distance between closely related previously described Anelosimus species pairs, e.g. Agnarsson 2007, 2012a, b) from other individuals in the analysis, and showing genitalic differences, albeit subtle, were treated as heterospecific. Paucity of specimens of some species prevented establishing a 'barcoding gap' (e.g. Čandek and Kuntner 2015) but for the most closely related species that have multiple specimens available all are separated by a gap of $>10$ times greater inter- than intraspecific genetic distances (Table 1). All types are deposited in the National Museum of Natural History, Smithsonian Institution (NMNH).

In addition to describing new species, we also redescribe known species to include first descriptions of males, or to correct previous taxonomic errors, and include illustrations and DNA barcodes of all Madagascan Anelosimus to facilitate future identification from a single source.
Table I. Estimates of divergence (Genetic distances) within and among species based on averaging substitutions per site over all sequence pairs under a Maximum Likelihood model with gamma distributed rate variation. The analyses were done including 1011 COI positions for 324 individuals in MEGA6 (Tamura et al. 2013 ).

|  |  | Intraspecific | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | A. moramora | $\mathrm{n} / \mathrm{a}$ | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 | A. hookeri | $\mathrm{n} / \mathrm{a}$ | n/a | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 3 | A. lamarcki | 0.002 | 0.111 | 0.032 | X |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 4 | A. tita | n/a | 0.149 | 0.060 | 0.053 | X |  |  |  |  |  |  |  |  |  |  |  |  |
| 5 | A. torfi | $\mathrm{n} / \mathrm{a}$ | 0.162 | 0.051 | 0.076 | 0.078 | X |  |  |  |  |  |  |  |  |  |  |  |
| 6 | A. andasibe | 0.000 | 0.118 | 0.038 | 0.022 | 0.054 | 0.080 | X |  |  |  |  |  |  |  |  |  |  |
| 7 | A. wallacei | 0.002 | 0.113 | 0.028 | 0.022 | 0.055 | 0.070 | 0.023 | X |  |  |  |  |  |  |  |  |  |
| 8 | A. buffoni | 0.001 | 0.119 | 0.029 | 0.023 | 0.048 | 0.074 | 0.021 | 0.019 | X |  |  |  |  |  |  |  |  |
| 9 | A. ata | 0.002 | 0.129 | 0.055 | 0.058 | 0.056 | 0.076 | 0.056 | 0.055 | 0.052 | X |  |  |  |  |  |  |  |
| 10 | A. darwini | 0.000 | 0.128 | 0.055 | 0.055 | 0.057 | 0.059 | 0.053 | 0.048 | 0.046 | 0.052 | X |  |  |  |  |  |  |
| 11 | A. huxleyi | 0.010 | 0.123 | 0.043 | 0.049 | 0.047 | 0.078 | 0.053 | 0.046 | 0.045 | 0.055 | 0.056 | X |  |  |  |  |  |
| 12 | A. may | 0.010 | 0.123 | 0.065 | 0.063 | 0.066 | 0.076 | 0.067 | 0.057 | 0.057 | 0.051 | 0.045 | 0.063 | X |  |  |  |  |
| 13 | A. nazariani | 0.001 | 0.131 | 0.071 | 0.066 | 0.054 | 0.075 | 0.065 | 0.062 | 0.062 | 0.059 | 0.054 | 0.063 | 0.059 | X |  |  |  |
| 14 | A. sallee | 0.001 | 0.123 | 0.062 | 0.057 | 0.056 | 0.079 | 0.057 | 0.055 | 0.051 | 0.060 | 0.058 | 0.051 | 0.060 | 0.064 | X |  |  |
| 15 | A. salut | 0.001 | 0.154 | 0.082 | 0.080 | 0.075 | 0.052 | 0.082 | 0.080 | 0.075 | 0.091 | 0.072 | 0.083 | 0.092 | 0.088 | 0.089 | X |  |
| 16 | A. vondrona | 0.000 | 0.103 | 0.022 | 0.033 | 0.056 | 0.075 | 0.041 | 0.035 | 0.035 | 0.062 | 0.058 | 0.051 | 0.068 | 0.064 | 0.059 | 0.083 | X |
|  | average | 0.003 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

## Results and discussion

Our total dataset contained specimens from $>400$ colonies and sequence data from 357 Madagascan individuals, plus global representatives. COI barcodes, totaling 1011 bp , were obtained from most sequenced specimens and 336 individuals were included in the barcode analysis (Supplementary Material). Three additional loci were obtained from a small subset for a phylogenetic matrix of 3409 bp for 114 global terminals (Figure 1). Morphological examination and phylogenetic analyses reveal ten new Anelosimus species from four forest fragments in Madagascar. The only intensely surveyed forest, Périnet Special Reserve in the Andasibe-Mantadia National Park contained a remarkable assemblage of 10 species, adding four new species to the six recently described ones. Some of the species are fully allopatric with respect to their closest relative in the tree, consistent with allopatric speciation in this clade. However, others occur in sympatry with their closest relatives. Anelosimus is globally distributed, but this local diversity far exceeds that of any other locality in the World. Other forests in Madagascar contained fewer species, but in two of them we still uncovered impressive diversity given the limited sampling. For example, we were able to collect for only a few hours in Ambohitantely, finding approximately 15 colonies, and these contained a total of 5 species, thereof four new to science. Further systematic sampling of these and other montane forest fragments in Madagascar thus promises to continue to discover diversity in this clade. Anelosimus has been most thoroughly studied in the Americas (Agnarsson et al. 2007; 2010c; Albo et al. 2007; Aviles 1986; Aviles and Bukowski 2006; Aviles and Gelsey 1998; Aviles et al. 2006) where the genus was thought to be most species rich. However, the island of Madagascar now seems to harbor a comparable radiation.

Curiously, our phylogenetic results establish that the subsocial montane forest 'Madagascar group' is sister to the most diverse American group, the 'eximius lineage', and the solitary group from Madagascar is sister to solitary species also from the Americas (Fig. 1). These results do not support prior hypothesis of the sister relationship of Madagascar and African Anelosimus (Agnarsson et al. 2010b). Given that the genus is likely much too young to reflect a Gondwanan distribution, somewhere between 15-35 my (Agnarsson et al. unpublished data), this close relatedness of Madagascar to American species is difficult to explain. The genus is likely diverse-though poorly studied-in Africa, however, species studied to date seem unrelated to either of the two Madagascar clades. These are the South African A. nelsoni Bryant, 1945 included here, and a diverse clade of mostly undescribed east African species that have been phylogenetically placed in a diverse African plus Asian clade, based on morphological data (Agnarsson 2006). The majority of the biota of Madagascar traces back its history to colonization events from Africa with only about 5\% of examined groups colonizing the island from the Americas (Agnarsson and Kuntner 2012). Seeing such a rare pattern replicated twice within a single genus is thus a significant finding. Further systematic sampling of African Anelosimus is necessary, however, to adequately address the biogeography of the lineage.

0.2

Figure I. A Bayesian phylogeny of Madagascar Anelosimus and worldwide relatives. Terminal taxa are replaced by species names, full results including all taxon details are found in Suppl. material 1. Black stars indicate 100 posterior probability support, gray stars $85-99 \%$. The replicated sister relationship between Madagascan and American clades is highlighted in blue and red, with the total distribution of each lineage indicated on the maps in lower left. Dots on right map indicate collection localities for the current study. Spider photograph is of the European Anelosimus vittatus, adapted from a photo by Glenn Halvor Morka.

The intense survey of Périnet secured fresh samples of all known Madagascan Anelosimus species and led to the discovery of males of several species. These species are all re-illustrated and males described. Several new species were also discovered in Périnet and in pilot surveys of Ambohitantely, Ranamofana, and Montagne d'Ambre. Many of these new species are only known from single or a few female specimens, which is far from ideal for taxonomic purposes. These are nevertheless described here to highlight the diversity of this group in Madagascar and especially to call attention to the uniqueness of the tiny and isolated forest fragment of Ambohitantely. Our findings underline the need to thoroughly survey eastern Madagascan montane forests, and to preserve their extraordinary biodiversity.

DNA delimitation and diagnosis. Given that it can be difficult to delimit and distinguish species of Madagascan Anelosimus based on morphology alone, especially in species where males are unknown, we offer DNA diagnosis in addition to standard morphological diagnosis. In total we obtained 334 COI barcodes for specimens of the 16 species. Average distances among species barcodes ranged from $16.2 \%$ to $1.9 \%$ (Table 1). One clade of four species (A. buffoni sp. n., A. wallacei sp. n., A. lamarcki sp. n. and $A$. andasibe) has the lowest average interspecific distances ranging from 1.9$2.2 \%$. However, there are several lines of evidence suggesting these are heterospecific. All are represented by multiple individuals and show intraspecific distances ranging from $0.1-0.2 \%$ - roughly 10 times less than that among species, often referred to as a 'barcoding gap' (Table 1). Given that three of these coexist, not only regionally but can be found at trivial distances in colonies sharing the same trees and branches we do not expect these genetic distances to reflect any dispersal biases, such as male-mediated dispersal. Furthermore, even the most densely sampled species within Périnet with nearly 100 COI sequences and those found in more than one of the forests sampled, have low to very low intraspecific sequence divergences suggesting panmixia at local and even regional scales. Finally, distances of around 2\% separate some closely related Anelosimus species pairs elsewhere, and even lower distances are known to separate sympatric species of other organisms, such as skippers in Guanacaste (Hebert et al. 2004).

## Taxonomy

Species diagnoses are based on the standard DNA COI barcodes with a reference alignment starting at base 1 of the routinely used 658 bp fragment and in the matrix submitted as supplementary material (and available from the authors) but extending beyond the standard barcode to cover a 1000 bp fragment. We list 1 ) unique mtDNA nucleotide substitutions at alignment positions for each species as distinct diagnostic features, e.g. $G(100)$ indicating this species uniquely has a $G$ in position 100, following e.g. Bond and Stockman (2008); 2) and also unique combination of nucleotide substitutions that may be shared with one or maximally two additional species, e.g. G (184, except $A$. buffoni sp. n. and $A$. andasibe) indicating this species can be diagnosed from all species except those named in parenthesis by this substitution, and from all
species by the combination of multiple such partially shared substitutions. This approach does not exhaust the diagnosability of species based on DNA barcodes. For example, four species ( $A$. may, A. torfi, $A$. darwini, and $A$. wallacei), none of which are sister species, can each be diagnosed from their respective closest relatives by A (241). In fact $\mathrm{A}(241)$ is one of relatively few features that readily diagnoses $A$. wallacei from the related and very similar $A$. buffoni. However, listing every DNA substitution-based diagnosis is not feasible and we set the arbitrary limit to substitutions shared with no more than two species in our diagnosis sections. The matrix of barcodes is made available as supplementary material for complete diagnoses. Four of the 16 species were represented only by a single sequence such that no variation is known and the diagnoses involving these species are thus preliminary - a common enough predicament with morphological diagnoses in rare species. However, given that the average within species distances among densely sampled species were $0.3 \%$ and $0.1 \%$ for those species limited to a single forest, there is no a priory reason to expect these rarely collected species to be outliers with abundant intraspecific variation.

## Family Theridiidae Sundevall, 1833

Genus Anelosimus Simon, 1891

Type species. Anelosimus socialis Simon, 1891 = Anelosimus eximius (Keyserling, 1884).

## The "Madagascar group" sensu Agnarsson and Kuntner (2005) and Agnarsson (2006)

Brief diagnosis (see Agnarsson and Kuntner (2005) for a full diagnosis): Males uniquely with a large and distally rugose embolic division b (Figs 2D-E, 3H-I) and a hooked proximal embolic sclerite (PES, Fig. 2E) in between the tegulum and the embolus. Sperm duct pathway of male palps highly complex with up to ten switchbacks. Females uniquely possess a pendulum-like epigynal septum (Figs 2H, 3C).

## Anelosimus may Agnarsson, 2005 in Agnarsson and Kuntner (2005)

Notes. The species is here redescribed to clarify earlier taxonomic confusion; the female previously described (see Agnarsson and Kuntner 2005), was in fact not conspecific with the male holotype, and is below described as $A$. ata sp. n. Here we therefore redescribe the female of $A$. may based on specimens collected at the type locality of the male. An additional male specimen also allowed a more detailed documentation of the male palp (Fig. 2D-G).

Type material. Holotype male from Ambohitantely Special Reserve, Analamanga region, Ankazobe district, Madagascar, ( $18.161^{\circ} \mathrm{S}, 47.302^{\circ} \mathrm{E}$ ), 17-22.iv.2001, mon-


Figure 2. Anelosimus may: A-B female dorsal and lateral views $\mathbf{C}$ male lateral view $\mathbf{D}-\mathbf{G}$ male palp ventral, subectal, mesal, dorsal $\mathbf{H}$ epigynum ventral $\mathbf{I}$ epigynum dorsal.
tane forest, 1500 m alt, col. J. J. Rafanomezantsoa et al., in CAS, examined (see Agnarsson and Kuntner 2005).

Other material. Additional three females from same locality, 28.iv.2008, col. Agnarsson and Kuntner, a male and multiple females from Périnet Special Reserve, Andasibe Mantadia National Park, Madagascar ( $18.933^{\circ} \mathrm{S}, 48.417^{\circ} \mathrm{E}$ ), montane forest, $900-1000 \mathrm{~m}$ alt, $3-20 . \mathrm{iv} .2008$ and 12-28.xi.2008, col. Agnarsson, Kuntner, and Hanitriniaina and eight females from Ranamofana National Park ( $21.25^{\circ} \mathrm{S}, 47.43^{\circ} \mathrm{E}$ ), montane rainforest, 980-1050 m alt, 27.iv.-2.v.2013, col. Pruitt.

Diagnosis. Males are diagnosed from other species by the shape of the theridiid tegular apophysis, bifurcated with the lower branch longer than the upper (Fig. 2G) and the voluminous Eb (Fig. 2E). Females differ from others of the Madagascar group, except $A$. ata sp. n. by the anchor-shaped septum (Fig. 2H) and from A. ata sp. n.
by the more acute curving of the copulatory duct (Fig. 2I). Anelosimus may can be diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: A (31), A (223), A (274), G (517), G (529). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: T (58, except $A$. huxleyi sp. n.), G (100, except $A$. hookeri sp. n., and some $A$. darwini sp. n.), T (181, except $A$. ata sp. n.), G (244, except $A$. darwini sp. n.), T (352, except $A$. sallee and A. darwini sp. n.), T (355, except $A$. ata sp. n.), T (484, except $A$. torfi sp. n. and $A$. nazariani), T (781, except $A$. huxleyi sp. n. and $A$. salut sp. n.), G (805, except rarely $A$. nazariani), A (871, except $A$. nazariani), G (973, except $A$. sallee).

Description. Female: Total length 6.02 Cephalothorax 2.77 long, 1.94 wide, 1.58 high, brown. Sternum 1.49 long, 1.23 wide, extending half way between coxae IV, brown. Abdomen 3.85 long, 2.74 wide, 2.8 high. Brown base with white line and dot patterns with red near the spinnerets. Eyes subequal in size about 0.14 in diameter. Clypeus height about 2.9 times one AME diameter. Chelicerae with one large tooth, three denticles prolaterally. Leg I femur 3.4, patella 1.06, tibia 3.22, metatarsus 2.92, tarsus 1.18. Leg formula 2314, with leg 4 slightly longer than leg 1 . Legs 1 and 2 brown, legs 3 and 4 light brown-yellow with dark brown at junctions between tibia and metatarsus, and metatarsus and tarsus. 4 small trichobothria dorsally on tibia I, 4 on tibia II. Trichobothria on all metatarsi (1-2), 4-5 dorsal trichobothria on female palpal tibia.

Variation: Total length 5.70-6.20, Cephalothorax 2.60-2.80, femur 13.00-3.50.
Male (from Ranamofana, see Agnarsson and Kuntner (2005) for description of holotype male): Total length 4.01. Cephalothorax 2.05 long, 1.61 wide, 0.91 high, dark brown. Abdomen 2.21 long, 1.54 wide, 1.41 high. Light brown base with black/ brown spots, two jagged white longitudinal stripes and a central red longitudinal band. Eyes subequal in size about 0.13 in diameter. Leg I femur 3.15, patella 0.76 , tibia 2.98, metatarsus 2.65 , tarsus 1.05 . Leg formula 1243. Leg yellow, with alternating light and dark reddish shaded bands.

Variation: Total length 3.25-4.01, Cephalothorax 1.63-2.05, femur I 2.67-3.15.
Distribution. Eastern Madagascan montane forest. This is the most widespread species of the Madagascar group, documented from Périnet, Ambohitantely and Ranamofana, and can be expected to be found in additional montane forest reserves in eastern Madagascar.

Natural history. In Ranamofana, eight complete $A$. may colonies along trails in the forest interior were found. We found two colonies containing one adult female and her egg case and eight colonies containing a female with a group of small juveniles, likely instars I-III post egg sac. Females actively guarded their egg cases by seizing them in their chelicerae. We also noted one instance of a female feeding her young via regurgitation. Our observations indicate that $A$. may primarily exhibits subsocial behavior, as do other members of the Madagascar group. An unidentified salticid inhabited six of the eight colonies sampled.

## Anelosimus vondrona Agnarsson \& Kuntner, 2005

Fig. 3

Notes. We here describe the male of $A$. vondrona for the first time and illustrate both sexes to facilitate identification.

Type material. Holotype female from Périnet Special Reserve (P.N. Andasibe Mantadia), Toamasina Province, Madagascar, (18.935 ${ }^{\circ}$ S, $48.418^{\circ}$ E), 7-8.v.2001, montane forest, $900-1000 \mathrm{~m}$, (I. Agnarsson and M. Kuntner), in NMNH, examined.

Other material. Multiple additional specimens from same locality, 3-20.iv. 2008 and 12-28.xi.2008, col. Agnarsson, Kuntner, and Hanitriniaina, and from Ranamofana National Park ( $21.25^{\circ} \mathrm{S}, 47.43^{\circ} \mathrm{E}$ ), montane rainforest, $980-1050 \mathrm{~m}$ alt, $27 . \mathrm{iv} .-$ 2.v.2013, col. Pruitt.

Diagnosis. Anelosimus vondrona females can be diagnosed from all other species except $A$. huxleyi by the relatively broad septum that extends the entire width of the epigynum (Fig. 3C) and from $A$. huxleyi by the less heavily sclerotized lower margin of the epigynal plate. Males can be diagnosed by the shape of the TTA with curved and elongate upper branch (Fig. 3G), and the shape of the Eb (Fig. 3H-I). Anelosimus vondrona can be diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: $G$ (802), $T$ (820). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: $\mathrm{A}(163$, rarely also in $A$. nazariani), G (466, except $A$. hookeri sp. n.), G (493, except some $A$. may), G (521, except $A$. salut), G (619, except $A$. huxleyi sp. n.), G (628, except some $A$. may), G (655, except some A. huxleyi sp. n.), G (760, except most $A$. nazariani), G (799, except $A$. buffoni sp. n.)

Description. Male (same locality as holotype): Total length 4.47 Cephalothorax 2.03 long, 1.53 wide, 0.49 high. Sternum 1.11 long, 0.94 wide, extending halfway between coxae IV, dark brown. Abdomen 2.40 long, 1.87 wide, 1.89 high, color (Fig. 3A). Eyes subequal in size about 0.13 in diameter. Clypeus height about 2 times one AME diameter Chelicerae with one large tooth, and 3-4 denticles retrolaterally Leg 1 femur 3.16, patella 0.88 , tibia 3.15, metatarsus 2.60, tarsus 1.11. Leg formula 1243 Legs are light brownyellow. 7 small trichobothria dorsally on tibia I and II, and two dorsally on metatarsi.

Distribution. Eastern Madagascan montane forest, documented from Périnet and Ranamofana.

Natural history. In Ranamofana, we sampled ten colonies of $A$. vondrona. Six colonies contained a singleton female and four colonies contained a singleton female with a discolored, collapsed egg sac. All of these colonies were found along roadsides and ornamental shrubbery. In Périnet a large number of colonies were collected, almost exclusively in open forest, including many with female and up to 53 spiderlings coexisting. An adult female was more commonly present in webs with small juveniles but also found in some nests containing antepenultimate and subadult ( $5^{5^{\mathrm{h}}-6^{\text {th }} \text { instar) juveniles, suggesting }}$ prolonged cohabitation of mother and young. Foreign spiders were abundant in $A$. vondrona colonies, including several saliticids, a sparassid, a thomisid, and many theridiids.


Figure 3. Anelosimus vondrona: A-B female dorsal, lateral $\mathbf{C}$ epigynum ventral view $\mathbf{D}$ epigynum, dorsal E-F male dorsal, lateral G-I male palp mesal, ectal, ventral.

## Anelosimus salut Agnarsson \& Kuntner, 2005

Fig. 4
Notes. New material of males of this species allowed a more detailed study of the palpal organ and we provide new illustrations and diagnosis of the male; the original description included a single drawing (see Agnarsson and Kuntner 2005, fig. 5D).


Figure 4. Anelosimus salut: A-B male lateral and dorsal views C-E male palp mesal, ectal, ventral F epigynum, ventral.

Type material. Holotype male and paratype female, Périnet Special Reserve (P.N. Andasibe Mantadia), Toamasina Province, Madagascar, ( $18.935^{\circ} \mathrm{S}, 48.418^{\circ} \mathrm{E}$ ), 24.xii.2001, montane forest, 1000 m , col. M. E. Irwin, E. I. Schlinger, H.H. Rasolondalao, in CAS, examined.

Other material. Additional specimens from same locality, 3-20.iv. 2008 and 1228.xi.2008, col. Agnarsson, Kuntner, and Hanitriniaina.

Diagnosis. Anelosimus salut females can be diagnosed by having a broad 'inverted T-shape' septum that differs from $A$. vondrona in not extending the entire
length of the epigynum (Fig. 5J). Males can be diagnosed from all other Anelosimus by the relatively short bifurcated TTA (Fig. 4C) and the bilobed embolic division b that is longer and narrower than in other species (Fig. 4D). Anelosimus salut can be diagnosed from other Madagascan Anelosimus on the basis of the following unique $m t D N A$ nucleotide substitutions at the following standard DNA barcode alignment positions: A (38), T(43), T(97), T (369), A (371), T (415), G (460), A (470), A (494), A (568), T (796). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: A (256, except $A$. torfi sp. n. and $A$. hookeri sp. n.), T (370, except $A$. torfi sp. n.), T (412, except $A$. torfi sp. n.), A (469, except A. torfi sp. n.). A (474, except $A$. nazariani), G (521, except $A$. vondrona), G (541, except $A$. sallee and some $A$. wallacei sp. n.), A (622, except $A$. torfi sp. n.), T (631, except $A$. darwini sp. n.), A (754, except $A$. torfi sp. n.), T (781, except $A$. may and $A$. huxleyi sp. n.), T (940, except $A$. torfi sp. n.), A (961, except $A$. torfi sp. n.), G (994, except most $A$. huxleyi sp. n.).

Distribution. Only known from type locality.
Natural history. As other species in this group $A$. salut appears to be subsocial with colonies consisting of single females and up to 39 spiderlings.

## Anelosimus nazariani Agnarsson \& Kuntner, 2005

Fig. 5
Notes. The male of $A$. nazariani is here described and diagnosed for the first time, the female epigynum is re-illustrated.

Type material. Holotype and paratype females from Périnet Special Reserve (P.N. Andasibe Mantadia), Toamasina Province, Madagascar, (18.935º $\left.{ }^{\circ} 48.418^{\circ} \mathrm{E}\right)$, 7-8.v.2001, montane forest, $900-1000 \mathrm{~m}$, (I. Agnarsson and M. Kuntner), in NMNH, examined.

Other material. Additional specimens from same locality, 3-20.iv. 2008 and 1228.xi.2008, col. Agnarsson, Kuntner, and Hanitriniaina.

Diagnosis. Anelosimus nazariani differs from other species in being distinctly the largest Anelosimus species recorded to date with female total length exceeding 7 mm , with other species ranging from $1.9-5.5 \mathrm{~mm}$. The males are easily diagnosed by the dark, bulky, and comparatively smooth Eb (Fig. 5E-F), and all but A. sallee by the very elongated upper branch of the TTA (Fig. 5D-E). The TTA differs in shape from that of $A$. sallee, being less curved. The epigynum differs from all but $A$. andasibe, $A$. buffoni sp. n., and $A$. wallacei sp. n. by the W pattern on the septum, and from these three by the larger distance between the septum and the epigynal margin (Fig. 5A). Anelosimus nazariani can be diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: $\mathrm{T}(24), \mathrm{T}(45), \mathrm{T}(100), \mathrm{T}$ (202), G (322), T (424), G (583), T (814), T (859). It can also be readily diagnosed from most other Anelosimus based the


Figure 5. Anelosimus nazariani: A epigynum, ventral B epigynum, dorsal; C, female abdomen, ventral D-F male palp dorsal, mesal, ventral.
following partially shared nucleotide substitutions, and all other species by their unique combination: A (46, except $A$. torfi sp. n.), T (121, except $A$. tita sp. n.), T (127, except A. darwini sp. n. and $A$. ata sp. n.), T (130, except $A$. darwini sp. n.), G (262, except $A$. tita sp. n.), G (307, except some $A$. salut), G (313, except $A$. sallee and some $A$. huxleyi sp. n.), A (474, except $A$. salut), T (479, except $A$. andasibe), T (484, except $A$. may and A. torfi sp. n.), G (556, except A. darwini sp. n.), G (736, except some A. may), G (745, expect $A$. wallacei sp. n.and some $A$. ata sp. n.), G (841, except $A$. torfi sp. n.), A (871, except $A$. may).

Description. Male: Total length 5.89 Cephalothorax 2.70 long, 1.99 wide, 0.27 high. Sternum 1.40 long, 1.17 wide, extending halfway between coxae IV, light brown. Abdomen 3.19 long, 2.31 wide, 2.08 high (add color). Eyes subequal in size about 0.15 in diameter. Clypeus height about times one AME diameter Chelicerae with one large tooth, $4-5$ denticles retrolaterally Leg 1 femur 3.71, patella 1.18, tibia 3.94, metatarsus 3.45, tarsus 1.23 Leg formula 1243 Legs light brown-yellow with
brown at junctions between tibia and metatarsus, and metatarsus and tarsus. 7 small trichobothria dorsally on tibia I and II, 3 dorsally on metatarsi.

Distribution. Only known from type locality.
Natural history. As in other species of this group a female can be found in its web with close to 50 juveniles and juveniles appear to cohabit in the web until close to adulthood.

## Anelosimus sallee Agnarsson \& Kuntner, 2005

Fig. 6

Notes. The species is rediagnosed and genitalia re-illustrated.
Type material. Holotype male, paratype female from Périnet Special Reserve (P.N. Andasibe Mantadia), Toamasina Province, Madagascar, (18.935º,$~ 48.418^{\circ} \mathrm{E}$ ), 24.xii. 1999 (M.E. Irwin et al.), in CAS, examined.

Other material. Additional specimens from same locality, 3-20.iv. 2008 and 1228.xi.2008, col. Agnarsson, Kuntner, and Hanitriniaina.

Diagnosis. Males are readily diagnosed from all species other than $A$. nazariani by the elongate upper branch of the TTA (Fig. 6B) and from $A$. nazariani by the greater curvature of this branch. Females can be diagnosed by the shape of the septum being almost as high as wide (Fig. 5C). Anelosimus sallee can be diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: $G$ (190), C (284), C (401), G (403), A (421), G (433), A (482), A (718). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: G (211, except some $A$. may), G (313, except $A$. nazariani and some $A$. huxleyi sp. n.), T (139, except $A$. huxleyi sp. n.), T (352, except $A$. may and $A$. darwini sp. n.), G (541, except $A$. salut and some $A$. wallacei sp. n.), G (550, except rarely $A$. nazariani), T (838, except $A$. huxleyi sp. n.), G (934, except some $A$. nazariani), G (973, except $A$. may).

Distribution. Only known from type locality.
Natural history. This species is rare at the type locality, and too few colonies have been sampled to comment on its natural history, though it is expected to be subsocial like related species.

## Anelosimus andasibe Agnarsson \& Kuntner, 2005

Fig. 6E-F
Notes. The species, known only from females, is rediagnosed and genitalia re-illustrated.
Type material. Holotype female from Périnet Special Reserve (P.N. Andasibe Mantadia), Toamasina Province, Madagascar, (18.9355, 48.418 ${ }^{\circ}$ E), 7-8.v.2001, montane forest, $900-1000$ m, (I. Agnarsson and M. Kuntner), in NMNH, examined.


Figure 6. Anelosimus sallee: A-B palp, ventral, mesal C epigynum, ventral. Anelosimus andasibe: D-E epigynum, ventral, dorsal.

Other material. Additional specimens from same locality, 3-20.iv. 2008 and 1228.xi.2008, col. Agnarsson, Kuntner, and Hanitriniaina.

Diagnosis. Anelosimus andasibe differs from all but $A$. nazariani, A. buffoni sp. n., and $A$. wallacei sp. n. by the W-shaped septum (Fig. 6E), and from $A$. nazariani by the small distance between the septum and the epigynal margin and by being smaller. Clear diagnostic features separating females of the very similar $A$. andasibe, A. buffonisp. n., and $A$. wallacei sp. n. have not been established, however, we predict they will be readily diagnosable based on palpal organs once males are discovered. Anelosimus andasibe can be diagnosed from $A$. wallacei sp. n. by lacking substitution A (241), and from $A$. wallacei sp. n. and $A$. buffoni sp. n. by lacking substitution G (249). It can be diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: $\mathrm{C}(124), \mathrm{G}$ (415), G (496), G (769). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: $G$ (79, except some $A$. lamarcki sp. n.), G (184, except A. buffoni sp. n. and A.wallacei sp. n.), G (202, except most $A$. ata sp. n.), T (479, except $A$. nazariani), G (511, except $A$. buffoni sp. n.and $A$.wallacei sp. n.), T (553, except $A$. tita), T (709, except
A. ata), G (772, except A. lamarcki), (796, except A. buffoni and some A. may), G (838, except A. darwini).

Distribution. Only known from type locality.
Natural history. As in other species of this group a female can be found in its web with close to 50 juveniles and juveniles appear to cohabit in the web until close to adulthood.

## Anelosimus torfi Agnarsson, sp. n.

http://zoobank.org/77C3E795-7A2A-4163-87E9-408C92BB5D19
Fig. 7
Type material. Holotype female from Ambohitantely Special Reserve ( $18.161^{\circ} \mathrm{S}$, $47.302^{\circ} \mathrm{E}$ ), 1500 m alt, Analamanga region, Ankazobe district, Madagascar, 28.iv.2008, montane forest, col. Agnarsson and Kuntner, in NMNH.

Other material. Only known from holotype.
Etymology. The species epithet is a noun in apposition and honors Torf Agnarsson, the senior author's brother.

Diagnosis. Anelosimus torfi can be diagnosed from all other Anelosimus based on the distinctly dark coloration and from all but $A$. vondrona based on its pendulum-like septum. Anelosimus torfi can be diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: G (43), C (620), A (764), G (952), T (953), G (955). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: A (46, except $A$. nazariani), A (256, except $A$. salut and $A$. hookeri), T (364, except A. darwini), T (370, except A. salut), T (412, except A. salut), A (469, except $A$. salut), T (484, except $A$. may and $A$. nazariani), A ( 622 , except $A$. salut), G ( 625 , except $A$. ata and $A$. huxleyi), A (754, except $A$. salut), $\mathrm{G}(817$, except $A$. huxleyi), G (841, except A. nazariani), T ( 940 , except $A$. salut), A ( 943 , except $A$. moramora), A (961, except $A$. salut).

Description. Female: Total length 4.1. Cephalothorax 1.95 long, 1.4 wide, 1.06 high, dark black-brown. Sternum 1.13 long, .99 wide, extending half way between coxae IV, brown. Abdomen 2.67 long, 1.76 wide, 1.67 high.black base with yellow patterns. Eyes subequal in size about 0.12 in diameter. Clypeus height about 2.1 times one AME diameter. Chelicerae with one large tooth, three denticles not visible on specimen. Leg I femur 2.21, patella 0.76 , tibia 2.81, metatarsus 2.47 , tarsus 1.08 . Leg formula 3214, with leg 4 significantly longer than leg 1 . Legs primarily black-brown with yellow bands, dark at junction between each leg segment. 4 small trichobothria dorsally on all tibia. Trichobothria on all metatarsi (2), single tricobothria on tarsi. Four dorsal trichobothria on female palpal tibia.

Variation: Total length 4.1-4.32. Abdomen 2.67-2.84 long, 1.76-1.9 wide, 1.67-2.04 high. Femur 2.21-2.47.


Figure 7. Anelosimus torf: A-C female lateral, dorsal, ventral D epigynum ventral view E-F cleared epigynum, ventral, dorsal.

Distribution. Only known from type locality.
Natural history. Unknown, predicted to be subsocial.

## Anelosimus hookeri Agnarsson, Kuntner \& Jencik, sp. n. <br> http://zoobank.org/1A2C51AA-F24E-4E60-8167-E948A507F3AF <br> Fig. 8

Type material. Holotype female from Ambohitantely Special Reserve ( $18.161^{\circ} \mathrm{S}$, $47.302^{\circ} \mathrm{E}$ ), 1500 m alt, Analamanga region, Ankazobe district, Madagascar, 28.iv.2008, montane forest, col. Agnarsson and Kuntner, in NMNH.

Other material. Only known from holotype.
Etymology. The species epithet is a noun in genitive case that honors the evolutionary biologist Joseph Dalton Hooker, who was among the first scientists to publish work announcing support for Darwin's theory of evolution by natural selection.

Diagnosis. Anelosimus hookeri differs from all other Anelosimus by the combination of pale coloring (Fig. 8A-C), and a pendulum-like septum that is widest at its extremes (Fig. 8E). Anelosimus hookeri can be diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: G (85), G (479). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: T (22, except $A$. tita and $A$. huxleyi), G (100, except $A$. may, and some A. darwini), A (256, except $A$. torfi and $A$. salut), G (379, except $A$. wallacei), G (466, except $A$. vondrona), G (487, except $A$. ata), G (514, except $A$. lamarcki and most $A$. vondrona).

Description. Female: Total length 4.76. Cephalothorax 2.17 long, 1.43 wide, 1.26 high, dark brown. Sternum 1.26 long, 0.99 wide, extending half way between coxae IV, brown. Abdomen 2.99 long, 2.05 wide, 2.17 high. White base with black/ brown spots, red marks near spinnerets, dark brown around genitalia. Eyes subequal in size about 0.12 in diameter. Clypeus height about 2.1 times one AME diameter. Chelicerae with one large tooth, three denticles prolaterally. Leg I femur 1.76, patella 0.63 , tibia 1.99, metatarsus 1.89 , tarsus 0.88 . Leg formula 1243 , with leg 2 slightly longer than leg 1 and leg 3 slightly longer than leg 4 . Leg light orange-brown, with alternating light and dark shaded bands, and very dark at metatarsus/tarsus junction and distal tip of tarsus. Numerous (4-5) small trichobothria dorsally on all tibia, 4 on tibia II, 5 on tibia I. Trichobothria on all metatarsi (2-3). Four dorsal trichobothria on female palpal tibia.

Variation: only known from holotype.
Distribution. Only known from type locality.
Natural history. Unknown, predicted to be subsocial.


Figure 8. Anelosimus hookeri: A-C female lateral, dorsal and ventral views $\mathbf{D}$ epigynum ventral view E-F cleared epigynum ventral, dorsal.

## Anelosimus lamarcki Agnarsson \& Goh, sp. n.

http://zoobank.org/1A46E0A6-99BE-4AFE-BDE1-5FD9C96E36C1
Fig. 9
Type material. Holotype female from Ranamofana National Park ( $21.25^{\circ} \mathrm{S}, 47.43^{\circ} \mathrm{E}$ ), montane rainforest, 9801050 m alt, 27.iv.-2.v.2013, col. Pruitt, in NMNH.

Other material. Same locality and collection, several adult females.


Figure 9. Anelosimus lamarcki: A-B female dorsal and lateral views $\mathbf{C}$ epigynum ventral view $\mathbf{D}$ epigynum cleared dorsal.

Etymology. The species epithet is a noun in genitive case that honors the early evolutionary biologist Jean-Babtiste Lamarck, the first scientists to develop a thorough and coherent evolutionary theory, though it was later shown by Darwin to be flawed in major ways.

Diagnosis. Anelosimus lamarcki can be diagnosed from other Madagascan Anelosimus by the heavily sclerotized copulatory ducts and small spermathecae that barely exceed the diameter of the copulatory ducts. Anelosimus lamarcki can be diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: G (280), C (562). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: $G$ (502, except rarely in $A$. may), G (514, except $A$. hookeri and most $A$. vondrona), G (553, except some $A$. huxleyi), G (766, except some $A$. may), G (772, except $A$. andasibe), G (814, except most $A$. vondrona).

Description. Female (holotype): Total length 5.16. Cephalothorax 2.32 long, 1.70 wide, 0.98 high, dark brown. Abdomen 2.88 long, 2.04 wide, 1.90 high. Light brown base with black/white spots, black and white longitudinal band extending just beyond half of abdomen, red marks near spinnerets. Eyes subequal in size about 0.14 in diameter. Leg I femur 2.77, patella 0.84 , tibia 2.34 , metatarsus 2.28 , tarsus 0.91 . Leg formula 1423. Leg light orange-brown, with alternating light and dark shaded bands, and very dark at distal tips of femur, patella, tibia and metatarsus. Numerous ( $6-7$ ) small trichobothria dorsally on all tibia, 7 on tibia I, 6 on tibia II, 7 on tibia III, 6 on tibia IV.

Variation: Total length 5.00-6.80. Prosoma 2.30-2.90 long. Abdomen 2.70-3.20 long. Femur I 2.70-3.20.

Distribution. Only known from type locality.
Natural history. We sampled twelve colonies of $A$. lamarcki. Colonies were found both along trails in the forest interior and along roadsides and ornamental shrubbery. The ten colonies in the forest interior contained females with groups of small juveniles, likely instars I-II, and colonies along road sides contained one penultimate or mature female. Like A. vondrona, A. lamarcki webs contained an impressive diversity of foreign spiders including multiple theridiids, saliticids, sparassids, a thomisid, and several linyphiids. We observed multiple co-feedings events between $A$. lamarcki and its web associates during staged prey capture events. Whether $A$. lamarcki or its web associate was the first to subdue the prey differed across trials.

## Anelosimus tita Agnarsson, Kuntner \& Jencik, sp. n. http://zoobank.org/85C991FF-0021-4D6E-80A0-65813CCD3F5E Fig. 10

Type material. Holotype female from Ambohitantely Special Reserve $\left(18.161^{\circ} \mathrm{S}\right.$, $47.302^{\circ} \mathrm{E}$ ), 1500 m alt, Analamanga region, Ankazobe district, Madagascar, 28.iv.2008, montane forest, col. Agnarsson and Kuntner, in NMNH.

Other material. Only known from holotype.
Etymology. The species is a noun in apposition named in the honor of the first author's mother-in-law Yadira Collado Ulloa, affectionately known to her grandchildren as 'Tita'.

Diagnosis. Anelosimus tita can be diagnosed from other Madagascan Anelosimus by the triangular shape of the septum (Fig 10E) and on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: $\mathrm{T}(30), \mathrm{G}(37), \mathrm{T}(80), \mathrm{T}(81), \mathrm{T}(82), \mathrm{A}(83), \mathrm{G}(109), \mathrm{G}(214)$, $T(220), G(319), T(328), T(586), T(625), G(873), G(883), A(903), G(919)$. It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: T (22, except $A$. hookeri and $A$. huxleyi), T (121, except $A$. nazariani), G (190, except some $A$. huxleyi), G (262, except $A$. nazariani), T (532, except some $A$. huxleyi), T (553, except $A$. andasibe).

Description. Female: Total length 3.87 Cephalothorax 1.9 long, 1.34 wide, 1.09 high, brown. Sternum 1.02 long, 0.87 wide, extending half way between coxae IV, orange. Abdomen 2.44 long, 1.68 wide, 1.33 high. Mixed pattern of white, grey, and black. Eyes subequal in size about 0.11 in diameter. Clypeus height about 2 times one AME diameter. Chelicerae with one large tooth, three denticles prolaterally. Leg I femur 2.01, patella 0.66 , tibia 2.02 , metatarsus 1.84 , tarsus 0.91 . Leg formula 3421, with leg 1 significantly longer than leg 2 . Legs alternating between light orange and


Figure 10. Anelosimus tita: A-D female lateral, dorsal, ventral, and ventral abdomen E epigynum ventral F epigynum cleared, dorsal.
dark brown bands. 3-4 small trichobothria dorsally on tibia, 3 on tibia 1.3 or 4 dorsal trichobothria on female palpal tibia.

Variation: only known from holotype.
Distribution. Only known from type locality.
Natural history. Unknown, predicted to be subsocial.

## Anelosimus ata Agnarsson, Kuntner \& Jencik, sp. n. <br> http://zoobank.org/E5EE186A-D324-413B-AD04-8F395EFB6EA6

Fig. 11

Notes. In 2005 we described Anelosimus may Agnarsson, based on a holotype male and females both from Ambohitantely and Périnet (Agnarsson and Kuntner 2005). Here we establish based on DNA analyses that $A$. may as currently circumscribed contains two species. Anelosimus may is indeed as originally thought found both in Ambohitantely and Périnet, while the very similar species described here, $A$. ata is so far restricted to Périnet.

Type material. Holotype female from Périnet Special Reserve (P.N. Andasibe Mantadia), Toamasina Province, Madagascar, (18.9355, 48.418E), 7-8.v.2001, montane forest, 900-1000 m, (I. Agnarsson and M. Kuntner) (NMNH), based on the paratype originally attributed to $A$. may, see Agnarsson and Kuntner (2005) p. 580.

Other material. Several female specimens from same locality.
Etymology. The species epithet is a noun in apposition named in the honor of the first author's father-in-law Jorge May-Barquero, affectionately known to his grandchildren as 'Ata'.

Diagnosis. Anelosimus ata can be diagnosed from all other Anelosimus, expect $A$. may, by the anchor-shaped septum (Fig. 11D) and from A. may by the juxtaposed spermathecae and the pathway of the copulatory ducts following the septum edge (Fig. 11E). Anelosimus ata can be diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: A (88), G (166), A(169), G (253), G (358), T (835), G (910). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: T (127, except $A$. nazariani and $A$. darwini), T (181, except $A$. may), T (355, except $A$. may), G (487, except $A$. hookeri), G ( 625 , except $A$. torfi and A. huxleyi), T (709, except $A$. andasibe), G (751, except some $A$. may).

Description. Female: Total length 5.01. Cephalothorax 2.28 long, 1.82 wide, 1.45 high, brown. Sternum 1.35 long, 1.16 wide, extending halfway between coxae IV, brown. Abdomen 2.93 long, 2.44 wide, 2.52 high. Pattern as in Fig 3A. Eyes subequal in size about 0.12 in diameter. Clypeus height about 2.4 one AME diameter. Chelicerae with one large and two small prolateral teeth, three denticles retrolaterally. Leg I femur 2.89, patella 0.98 , tibia 2.70, metatarsus 2.57 , tarsus 0.94 . Femur about


Figure II. Anelosimus ata: A-B female and her web $\mathbf{C}$ female abdominal venter $\mathbf{D}$ epigynum, ventral $\mathbf{E}$ epigynum, dorsal. Images are schematic and not to scale.

5 longer than wide, metatarsus I about 16longer than wide. Leg formula 1243, with leg II very slightly longer than leg IV. Leg base colour as carapace, light orange-brown, with distal tip of tibia darkened, and metatarsus/tarsus junction dark. Tarsal organs slightly distal ( $0.55-0.60$ ) on tarsi I and II, central ( 0.5 ) on III, slightly proximal ( 0.45 ) on IV, distal ( 0.85 ) on female palp, positions vary slightly between specimens. Numerous (seven to eight) small trichobothria dorsally on all tibia, seven on tibia III, eight on tibia I. Trichobothria on metatarsi I-III central or slightly proximal (about $0.45-0.50$ ), absent on metatarsus IV. Four to five dorsal trichobothria on female palpal tibia.

Variation: female total length 4.90-5.15.
Distribution. Only known from type locality.
Natural history. This species is common at its type locality and webs have been found with females and up to 80 spiderlings. Juveniles cohabit in the web with the mother until she dies and appear to disperse close to adulthood. Webs without adult females generally contained instar 4-6 juveniles.


Figure 12. Anelosimus darwini: A female dorsal $\mathbf{B}$ epigynum, ventral $\mathbf{C}$ epigynum cleared, dorsal.

Anelosimus darwini Agnarsson, Kuntner \& Jencik, sp. n. http://zoobank.org/DAA2F5AE-0AF1-49A4-82B5-72F79AB99333
Fig. 12

Type material. Holotype female holotype from Ambohitantely Special Reserve $\left(18.197^{\circ} \mathrm{S}, 47.285^{\circ} \mathrm{E}\right), 1600 \mathrm{~m}$ alt, Analamanga region, Ankazobe district, Madagascar, 28.iv.2008, montane forest, col. Agnarsson and Kuntner, in NMNH.

Other material. Juveniles from same locality.
Etymology. The species epithet is a noun in the genitive case and honors Charles Darwin, the father of evolutionary biology.

Diagnosis. Anelosimus darwini can be diagnosed from all other Anelosimus, expect A. may, and $A$. ata, by the anchor-shaped septum (Fig. 12B) and from $A$. may and $A$. ata by the pathway of the copulatory duct with a near $90^{\circ}$ bend (Fig. 12C). Anelosimus darwini can be diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: T (84), T (190), T (526), A (848). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: T (127, except $A$. nazariani and $A$. ata), T (130, except $A$. nazariani), A (133, except $A$. huxleyi), G (229, except some $A$. may), G (244, except $A$. may), T (352, except $A$. may and $A$. sallee), T (364, except $A$. torfi), G (556, except A. nazariani), T (631, except $A$. salut), G (838, except $A$. andasibe).

Description. Female (holotype): Total length 3.70. Cephalothorax 1.88 long, 1.27 wide, 0.93 high. Red-brown. Sternum 1.04 long, 0.82 wide, extending between coxae IV, dark brown. Abdomen 1.79 long, 1.63 wide, 2.45 high. Brown pattern with 2 white streaks. Eyes subequal in size about 0.11 in diameter. Chelicerae each with 1 large tooth, 3 denticles located prolaterally. Clypeus height about 2.3 times one AME diameter. Leg I femur 2.10, patella 0.62, tibia 1.86, metatarsus 1.49 , tarsus 0.93 . Legs roughly same color as cephalothorax. Leg formula 1432. Numerous (3-4) small trichobothria dorsally on all tibiae. 2-3 trichobothria on metatarsus, absent on tarsus.

Variation: only known from holotype.
Distribution. Only known from type locality.
Natural history. Unknown, predicted to be subsocial.

## Anelosimus wallacei Agnarsson, Veve \& Kuntner, sp. n.

http://zoobank.org/46C8F33C-EE8C-4844-9F07-4A66D0F9110C
Fig. 13A-E

Type material. Holotype female from Périnet Special Reserve (P.N. Andasibe Mantadia), Toamasina Province, Madagascar, (18.935 ${ }^{\circ}$ S, $48.418^{\circ} \mathrm{E}$ ), 12-28.xi.2008, montane forest, $900-1000 \mathrm{~m}$, col Hanitriniaina, in NMNH.

Other material. Additional specimens from same locality, 3-20.iv. 2008 and 1228.xi.2008, col. Agnarsson, Kuntner, and Hanitriniaina.

Etymology. The species epithet is a noun in the genitive case and honors the evolutionary biologist Alfred Russel Wallace, a contemporary of Darwin and co-author of the first paper on natural selection.

Diagnosis. Anelosimus wallacei can be diagnosed from all other Anelosimus, expect $A$. andasibe and $A$. buffoni by the W pattern of the septum (Fig. 13C) and from A. andasibe and $A$. buffoni by substitutions A (241), G (379) and G (745). Anelosimus wallacei can be diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: C (283), G (679). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: $G(184$, except $A$. buffoni and $A$. andasibe), G (379, except A. hookeri), G (511, except $A$. buffoni and A.andasibe), G (745, expect $A$. nazariani and some $A$. ata).

Description. Female: Total length 4.72 Cephalothorax 2.14 long, 1.57 wide, 0.44 high. Sternum 1.26 long, 1.05 wide, extending halfway between coxae IV, dark brown. Abdomen 2.58 long, 2.01 wide, 1.78 high, color and pattern as in Fig. 13A. Eyes subequal in size about 0.13 in diameter. Clypeus height about 2 times one AME diameter. Chelicerae with one large tooth, three denticles retrolaterally. Leg 1 femur 2.35 , patella 0.88 , tibia 3.13 , metatarsus 1.49 , tarsus 0.76 . Leg formula 1243. Legs light brown-yellow with brown at junctions between tibia and metatarsus, and metatarsus and tarsus. 5 small trichobrothia dorsally on tibiae, two dorsally on metatarsi.


Figure 13. Anelosimus wallacei: A-B female dorsal and lateral views $\mathbf{C}$ abdomen ventral $\mathbf{D}$ epigynum ventral view $\mathbf{E}$ epigynum cleared ventral $\mathbf{F}$ epigynum cleared, dorsal.

Variation: Total length 4.72-4.8. Cephalothorax 2.14-2.25 long. Femur I 1.76-2.35.
Distribution. Only known from type locality.
Natural history. This species occurs almost exclusively in closed forest at its type locality. Like other species of the group it makes webs with females and spiderlings cohabiting, with up to 83 spiderlings found in a single web. Webs without adult females generally contained instar 4-6 juveniles.

## Anelosimus huxleyi Agnarsson, Veve \& Kuntner, sp. n.

 http://zoobank.org/CB0DEF79-3C74-4755-AB91-4A7EAD86CEE6 Fig. 14Type material. Holotype female from Périnet Special Reserve (P.N. Andasibe Mantadia), Toamasina Province, Madagascar, (18.935 $\left.{ }^{\circ} \mathrm{S}, 48.418^{\circ} \mathrm{E}\right), 12-28 . x i .2008$, montane forest, $900-1000 \mathrm{~m}$, col Hanitriniaina, in NMNH.


Figure 14. Anelosimus huxleyi: A-C female dorsal, ventral and lateral views $\mathbf{D}$ epigynum ventral view E-F cleared epigynum ventral, dorsal.

Other material. Additional specimens from same locality, 3-20.iv. 2008 and 1228.xi.2008, col. Agnarsson, Kuntner, and Hanitriniaina.

Etymology. The species epithet is a noun in the genitive case and honors the evolutionary biologist Thomas Henry Huxley; 'Darwin's bulldog'.

Diagnosis. Anelosimus huxleyi females can be diagnosed from all other species except $A$. vondrona, by the relatively broad septum that extends the entire width of the epigynum (Fig. 14D-E) and from $A$. vondrona by the more heavily sclerotized lower margin of the epigynal plate (Fig. 14D-F). Anelosimus huxleyi can be diagnosed from
other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: A (283), A (418), T (760), G (784). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: T (22, except $A$. tita and $A$. hookeri), T (58, except $A$. may), A (133, except A. darwini), G (181), T (139, except $A$. sallee), G (619, except $A$. vondrona), G ( 625 , except $A$. torfi and $A$. ata), T (781, except $A$. may and $A$. salut), G (817, except $A$. torfi), T (838, except $A$. sallee).

Description. Female: Total length 5.64 Cephalothorax 2.54 long, 1.79 wide, 0.63 high. Sternum 1.30 long, 1.11 wide, extending halfway between coxae IV, dark brown. Abdomen 3.10 long, 2.45 wide, 2.63 high, color and pattern as in Fig.14A. Eyes subequal in size about 0.16 in diameter. Clypeus height about 2 times one AME diameter. Chelicerae with one large tooth, 3-4 denticles retrolaterally. Leg 1 femur 3.17, patella 0.85 , tibia 2.75 , metatarsus 2.38 , tarsus 1.07 . Leg formula 1243. Legs are light brown-yellow with dark brown at junctions between tibia and metatarsus, and metatarsus and tarsus. 5 small trichobothria dorsally on tibiae, 4 dorsally on metatarsi.

Variation: Total length 5.50-5.70. Cephalothorax 2.50-2.55 long. Femur I 3.103.20 .

Distribution. Only known from type locality.
Natural history. As in other species of this group a female can be found in its web with close to 50 juveniles and juveniles appear to cohabit in the web until close to adulthood.

## Anelosimus buffoni Agnarsson, Kuntner \& Jencik, sp. n. http://zoobank.org/F0FFD4BF-E1F6-446B-9996-184EA7D846B7 <br> Fig. 15

Notes. Unfortunately most adult specimens of this species, almost all of which were collected in April 2008, were lost during the transport of the Agnarsson lab from UPR to UVM. Of the only two remaining adult females the carapace and legs had been consumed for DNA extraction, though total length was measured before specimens were processed for DNA extraction. The species description is therefore abbreviated and limited to the abdomen, genitalia, and DNA barcode.

Type material. Holotype and paratype female from Périnet Special Reserve (P.N. Andasibe Mantadia), Toamasina Province, Madagascar, (18.935 ${ }^{\circ}$ S, $\left.48.418^{\circ} \mathrm{E}\right), 12-28$. xi.2008, montane forest, $900-1000 \mathrm{~m}$, col Hanitriniaina, in NMNH.

Etymology. The species epithet is a noun in the genitive case and honors the great naturalist Georges-Louis Leclerc, Comte de Buffon.

Diagnosis. Anelosimus buffoni females can be diagnosed from all other species expect $A$. andasibe and $A$. wallacei by the W pattern of the septum (Fig. 15B-C) and from $A$. wallacei by lacking substitution $\mathrm{A}(241)$, from $A$. andasibe by having substitution G (349), and by both by having substitution A (559). Anelosimus buffoni can be


Figure 15. Anelosimus buffoni: A female abdomen dorsal B epigynum ventral view $\mathbf{C}$ epigynum cleared ventral $\mathbf{D}$ epigynum cleared dorsal.
diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions: $G$ (742), $T$ (769). It can also be readily diagnosed from most other Anelosimus based the following partially shared nucleotide substitutions, and all other species by their unique combination: $G(364$, except some $A$. salut), $G(184$, except $A$. wallacei and $A$. andasibe), G (511, except $A$. andasibe and $A$.wallacei), G (796, except $A$. andasibe and some $A$. may), G (799, except $A$. vondrona).

Description. Total length 4.1, Abdomen 2.59 long, 1.96 wide, color and pattern as in Fig. 15A.

Distribution. Only known from type locality.
Natural history. As in other species of this group a female can be found in its web with close to 50 juveniles and juveniles appear to cohabit in the web until close to adulthood.

## Anelosimus moramora Agnarsson, Kuntner \& Jencik, sp. n.

 http://zoobank.org/4404333D-C40F-458E-AC01-7FFF94EC9D4DFig. 16
Type material. Holotype female from Montagne d'Ambre National Park (12.516972 ${ }^{\circ}$, $49.178778^{\circ} \mathrm{E}$ ), 1005 m alt, Antsiranana district, Madagascar, 4.iv.2008, montane forest, col. Agnarsson and Kuntner, in NMNH.

Etymology. The species epithet is a Madagascan aphorism or motto meaning 'no rush' or 'take it easy'.

Diagnosis. Anelosimus moramora females can be diagnosed from all other species except $A$. ata by the relatively small and pointy shape of the septum and from $A$. ata by its smaller size. Anelosimus moramora can be diagnosed from other Madagascan Anelosimus on the basis of the following unique mtDNA nucleotide substitutions at the following standard DNA barcode alignment positions (note that only a short fragment


Figure 16. Anelosimus moramora: A-C female ventral, lateral and abdomen ventral $\mathbf{D}$ epigynum ventral view $\mathbf{E}$ epigynum cleared, dorsal.
of the divergent $A$. moramora barcode is available starting at position 824): C (843), C (888), T (897), A (901), C (906), A (914), C (924), C (939), C (967), A (979). It can also be diagnosed from all Anelosimus except $A$. torfi based on A (943).

Description. Female: Total length 3.31. Cephalothorax 1.4 long, 1.06 wide, 0.87 high, brown. Sternum 0.78 long, 0.67 wide, extending half way between coxae IV, brown. Abdomen 2.02 long, 1.6 wide, 2.26 high. Red-brown with 2 white streaks. Eyes subequal in size about 0.09 in diameter. Clypeus height about 2.5 times one AME diameter. Chelicerae with one large tooth, three denticles prolaterally. Leg I femur 1.67, patella 0.5 , tibia 1.41 , metatarsus 1.1 , tarsus 0.65 . Leg formula 1243. Legs light brown-yellow with dark brown at junctions between tibia and metatarsus, and metatarsus and tarus. 4 small trichobothria dorsally on tibiae, 3 on all metatarsi. 3-4 dorsal trichobothria on female palpal tibia.

Variation: only known from holotype.
Distribution. Only known from type locality.
Natural history. Unknown, predicted to be subsocial.

## Anelosimus decaryi (Fage, 1930)

Fig. 17

Notes. Anelosimus decaryi does not belong to the 'Madagascar group' of subsocial montane species but rather to the 'solitary clade' of Anelosimus (see Agnarsson et al. 2010). It was recently redescribed (see Agnarsson et al. 2010) and is here only reillustrated for completeness so that all Anelosimus species currently known to occur in Madagascar can be identified from a single source.


Figure 17. Anelosimus decaryi: A-B female dorsal, lateral C-D male dorsal, lateral E-F epigynum ventral, dorsal G-H male palp mesal, ventral.

Distribution. Orangea Peninsula (N Madagascar), Ranamofana (Central Madagascar), Comoros Islands, and Aldabra atoll.

Natural history. Anelosimus decaryi is solitary with very brief cohabitation between mother and offspring, which disperse at early instars (Agnarsson et al. 2010). The species was previously thought to be restricted to coastal habitats in the north, however, it also occurs in Ranamofana. Seven colonies of $A$. decaryi were sampled along the riparian zones directly flanking the Namorona River. A. decaryi has previously only been known from coastal habitats at low elevations. At Ranomafana (600-800m elevation) we found six colonies containing singleton females and one colony containing a female with an egg case. In contrast to the other species of Anelosimus at Ranomafana, $A$. decaryi colonies did not contain any foreign spiders.

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## Supplementary material I

## Data matrix

Authors: Ingi Agnarsson, Brian B. Jencik1, Giselle M. Veve, Sahondra Hanitriniaina, Diego Agostini, Seok Ping Goh, Jonathan Pruitt, Matjaž Kuntner
Data type: NEXUS file
Explanation note: Data matrix set of five aligned and concatenated mtDNA and nuDNA loci.
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# Redescription of two subterranean amphipods Niphargus molnari Méhely, 1927 and Niphargus gebhardti Schellenberg, 1934 (Amphipoda, Niphargidae) and their phylogenetic position 

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#### Abstract

A detailed redescription of two endemic, cave-dwelling niphargid species of the Hungarian Mecsek Mts., Niphargus molnari Méhely, 1927 and Niphargus gebhardti Schellenberg, 1934 is given based on newly collected material. Morphology was studied under light microscopy and with scanning electon microscopy. Morphological descriptions are complemented with mitochondrial cytochrome c oxidase subunit I (COI) sequences as barcodes for both species and with notes on their ecology. Using three independent molecular markers we showed that $N$. gebhardti belongs to the clade distributed between Central and Eastern Europe, whereas phylogenetic relationship of $N$. molnari to the rest of Niphargus species is not clear. The two species from the Mecsek Mts. are phylogenetically not closely related. Both species need to be treated as vulnerable according to IUCN Red List of Threatened Species.


## Keywords

Hungary, Mecsek Mts., Niphargus, redescription, morphology, phylogeny, endemism, SEM

## Introduction

Fragmented mountain areas in East-Central Europe had been suggested to be centres of endemisms that evolved through a complex geological history including Eocene marine regression-transgression cycles and Pleistocene glacial cycles (Hou et al. 2013, Meleg et al. 2013, Mamos et al. 2014). The Mecsek is one of these isolated mountain ranges, that is situated in Southern Hungary and surrounded by Pannonian plains. The closest mountain ranges are the Croatian Papuk Mts. ( 80 km ) and the Hungarian Transdanubian Mts. ( 150 km ) (Fig. 1). The area is small of approximately $545 \mathrm{~km}^{2}$. In biological sense, it is populated by numerous endemic species the origin of which may date back to Tertiary and which therefore apparently have survived mass extinctions in glacial periods. The upper geological layers comprise of Triassic and Jurassic limestones and dolomites, where extensive karstification has created over 200 caves. The subterranean environment of the area harbours numerous terrestrial and aquatic highly endemic invertebrates, known only from one or a few caves. Although the region apparently harbours an important piece of European and Hungarian natural heritage, until now only one species, the Hungarian blind snail (Bythiospeum hungaricum (Soós, 1927)) has been protected by law. A serious impediment for conservation biology is that our knowledge of species is only limited, beginning with poor taxonomic descriptions. The aim of this study is to bridge this gap at the most basic level. We morphologically redescribe and present phylogenetic relationships of two amphipod species from the genus Niphargus, both endemic to this area.

Niphargus molnari Méhely, 1927 was described from the stream of the Mánfai-kőlyuk Cave (Méhely 1927). The description is not detailed, as it contains only a few information about the body lenght, the pereonits, the pleon segments, the first antenna, the uropods and the telson, and two drawings about the epimeral plates and the pereion segments. Further drawing of the right lacinia mobilis can be found in Méhely's summarizing work (Méhely 1941). At approximately the same period the species was also studied by Schellenberg, who analysed samples fom Abaligeti Cave. In his early study he first treated it as N. leopoliensis molnari (Schellenberg, 1933), but later he acknowledged its species status and supplemented description with data about the seta number of the palpus of the first maxilla (Schellenberg 1935). The species was found in the Mánfai-kőlyuk Cave (Gebhardt 1933, 1934, 1963, 1967) and in the stream of the Abaligeti Cave too (Gebhardt 1934, 1963, 1967). Recently, the species was found in other two localities, the Spirál Sinkhole and the Vadetetős Sinkhole (Angyal and Balázs 2013). During our research in the caves of the Western Mecsek between 2010 and 2013, the species could not have been re-collected on the type locality, which is supposedly related to the artificial utilization of the Mánfai-kőlyuk Cave. The intrusive introduction of waterworks in the 1960-s and 1970-s has caused irreversible changes in the cave's character, hidrology and ecosystem (Angyal 2012).

Niphargus gebhardti Schellenberg, 1934 was described from the pools formed by dripping water of the Abaligeti Cave, originally as Niphargus foreli gebhardti (Schellenberg 1934). Brief description reports on only few characters, like the pereopods, the


Figure I. Location of the Mecsek Mts. and the nearby isolated mountain ranges within Europe.
antennae and the mouth parts, and two drawings about the second gnathopod's propodus and the telson. Later the author gave additional data on the body length and the telson (Schellenberg 1935). Gebhardt mentioned the species'distribution from pools of the Abaligeti Cave's main passage in various papers (Gebhardt 1934, 1963, 1967). The species rank was proposed for the first time in Méhely's synthetic work (Méhely 1941), wherein a drawing of the pleopod's retinacles and some data about the lacinia mobilis are also presented. Dudich (1941) discussed 'Niphargus foreli gebhardti' from the Abaligeti Cave as a fauna element of the historical Hungary. More recent sampling revealed new records of the species from Vadetetős Sinkhole, Szajha-felső Sinkhole, Spirál Sinkhole, Gilisztás Cave and Trió Cave (all Mecsek Mts.; see Angyal and Balázs 2013).

The holotypes of both species are either in an unknown place or had been destroyed. Although we identified the distinguishing characters of $N$. gebhardti and $N$. molnari, and presented comparative drawings of them (Angyal and Balázs 2013), the morphology of both species is unsuficiently known and cannot be used in a broader comparative research of Niphargus. In order to follow modern trends in taxonomy, we revised all possible sources of data that might increase the robustness of taxonomic conclusions (Padial et al. 2010). We provide a detailed and richly illustrated redesription of $N$. molnari and $N$. gebhardti with cytochrome c oxidase subunit I (COI) sequences as barcodes. We also present comparative scanning electron micrographies which
are - to our knowledge - the first comparative micrographies of Niphargus. Moreover, we present phylogenetic relationships of both species within the genus Niphargus using three independent molecular markers and summarize field observations that may indicate species' ecology.

## Material and methods

## Sampling sites and sampling

Samples for the redescription were collected in the Abaligeti Cave (N46ㅇ́'11.89", E18 $6^{\prime} 59.40^{\prime \prime}$ ), which is located in Southern Hungary, Western Mecsek in Abaliget village, near Pécs city. The altitude of the cave entrance is 219 m above sea level. With its three collaterals and the main passage, the total length of the cave is 2000 m . Its lowest point below the entrance is 10 m , while its highest point is 38 m . Shallow pools of water in the cave are of two types: some are formated by dripping water of the dripstones whereas others are filled during floods and contain residual water. The cave was regulary visited between 2010 and 2013 to characterize its fauna. For the morphological and molecular taxonomic analysis in total 18 and 20 specimens of $N$. molnari and N. gebhardti respectively were collected on 23 March 2013. Niphargus molnari was found in the stream of the Western 2. collateral and N. gebhardti was collected from a permanent pool in a lateral chamber of 'Karthago romjai' hall in the main passage and from a pool at the end of Western 2. collateral, near Akácos Sinkhole's entrance (Fig. 2). An additional specimen of $N$. gebhardti for molecular studies was collected from a pool of the Szajha-felső Sinkhole ( $46^{\circ} 8^{\prime} 5.4^{\prime \prime N}$, $18^{\circ} 7^{\prime} 8.22$ E) 30 m vertical distance and 100 m horizontal distance from the entrance. The cave is situated in the area of a platform right above the Abaligeti Cave, 283 m above sea level. The two caves are supposedly connected, their entrances are approximately 1 km from each other (Dezső 2011). Specimens were collected using entomological (soft) forceps and were fixed and stored in $96 \%$ ethanol.

## Morphological studies

Cleared and stained exoskeletons of 10 ( $N$. molnari) and 11 ( $N$. gebhardti) specimens were dissected under a Leica MZ75 and a Leica M125 stereomicroscope. Slides were examined using a Leica DM 1000 light microscope. Drawings were made using a drawing tube mounted on the light microscope. Measurements were made using the AnalySIS Program Package, the computer was connected with a Zeiss Axioscope II light microscope. In total 230 morphological characters on each speciemens were examined according to the characters of the DELTA program package (Fišer et al. 2009) which were recorded in an Excel data matrix. Scanning micrographs of two individuals of each species about the main characters were made with a HITACHI S-2600 N scan-


Figure 2. Distribution of the two species within the Abaligeti Cave. I N . molnari along the stream of the Western 2. collateral $\mathbf{2} \mathrm{N}$. gebhardti in a permanent pool of 'Karthago romjai' $\mathbf{3} \mathrm{N}$. gebhardti in a permanent pool near the Akácos Sinkhole's entrance.
ning electron microscope. Specimens were placed in absolute alcohol for one day, then cleaned in an EMAG Emmi-16 Ultrasonic Cleaner and dried out on air. Dry samples were sticked onto holders and were sputter-coated by gold-palladium. Micrographs were digitally edited.

## Molecular studies

DNA extraction was performed using QIAamp DNA Microcit ${ }^{\circ}$ (Qiagen) or Sigma Aldrich GenElute Mammalian Genomic DNA Miniprep Kit ${ }^{\ominus}$ following the manufacturer's instructions. Only a few pereopods were used for DNA isolation of each animal. The following primer pairs were used for PCR amplifications of COI, 28 S rDNA fragment and histone (H3). For COI: LCO 1490 - HCO 2198 (Folmer et al. 1994), for 28 S rDNA: 28 S lev2 - 28 S des 2 or 28 S rtest2 (Verovnik et al. 2005, Zakšek et al. 2007) and H3aF2-H3aR2 (Colgan et al. 2000) for histone (H3). Details on PCR conditions are listed in Suppl. material 1. PCR products were cleaned using Roche High Pure Purification Kit ${ }^{\ominus}$ or Exonuclease I and Alkaline Phosphatase (Fermentas,

Germany) according to manufacturer's instruction. The fragments were sequenced in both directions using PCR amplification primers using ABI 3130 sequencer in the Laboratory of Molecular Taxonomy in Budapest or Macrogen Europe (Amsterdam, The Netherlands). Contigs were assembled and sequences were edited using Geneious Pro 5.5.6. (Biomatters, New Zeland).

## Phylogenetic analysis

In order to recover phylogenetic relationships of $N$. molnari and $N$. gebhardti within the genus Niphargus, a dataset of three molecular markers were complied, using available Niphargus sequences from previous studies (see Suppl. material 2 for references) and Synurella ambulans as outgroup taxon (Švara et al. submitted, Meleg et al. 2013). Altogether 104 taxa were included in the final dataset. List of taxa and sequences with GenBank accession numbers used in the analyses are listed in Suppl. material 2. The sequences were aligned using MAFFT 7 (Katoh and Standley 2013). Each sequence alignment was concatenated to the joint dataset and analysed as a single dataset in phylogenetic analysis. The length of combined dataset, including sequences of COI, 28S rDNA and H3 was 2068 bp . A general time-reversible model with a proportion of invariant sites and a gamma distribution of rate heterogeneity $(\mathrm{GTR}+\mathrm{I}+\Gamma)$ assuming six discrete gamma categories was chosen as the most appropriate model according to AIC and BIC criteria, using ModelGenerator (Keane et al. 2006). Phylogenetic relationships were reconstructed with Bayesian inference (BA) using MrBayes v3.2 (Ronquist and Huelsenbeck 2003). Two parallel searches with four chains each were run for 20 million generations, sampled every $1000^{\text {th }}$ generation. After discarding the first $25 \%$ of the sampled trees, the final tree was constructed according to the $50 \%$ majority rule. MrBayes phylogenetic analysis was run on the CIPRES Science Gateway, www.phylo.org (Miller et al. 2012).

## Results

Redescription of Niphargus molnari Méhely, 1927
Order Amphipoda Latreille, 1816
Suborder Gammaridea Latreille, 1802
Family Niphargidae G. Karaman, 1962
Genus Niphargus Schiödte, 1849

Niphargus molnari Méhely, 1927
Niphargus molnari sp. n.: Méhely 1927 type locality: Mánfai-kőlyuk Cave; Data from the original description is available in Suppl. material 3.
N. leopoliensis molnari: Schellenberg 1933, samples from the Abaligeti Cave, morphological data.
N. molnari: Schellenberg 1935, morphological data.
N. leopoliensis molnari, N. molnari: Gebhardt 1933, 1934, 1963, 1967 distributional data N. molnari: Méhely 1941 additional morphological data.
N. molnari: Angyal and Balázs 2013 morphological and distributional data.

Material examined for redescription. 7 females and 3 males from the stream of the Western 2. collateral of the Abaligeti Cave (Cadastre number: 4120-1, Hungarian Cave Cadastre), collected in 23 March 2013 (leg. D. Angyal and A. Illés), dissected and mounted on slides; additional 4 specimens not dissected. Slides were deposited in the Collection of Crustaceans of the Hungarian Natural History Museum with the following codes: N.MOL-02, N.MOL-03, N.MOL-04, N.MOL-06, N.MOL-07, N.MOL-08, N.MOL-09, N.MOL-10, N.MOL-11, N.MOL-12. Diagnostic voucher number of specimen used for molecular studies: NB555 (N. molnari, coll. data: Abaligeti Cave, Western 2. collateral, stream, 23 March 2013, leg. D. Angyal \& A. Illés).

COI Gen Bank Accession Number: KP967552
Diagnosis. Small to medium-sized niphargid; epimeral plate III postero-ventral corner sharply inclined. Telson with 3-4 apical spines, 1-3 lateral spines, $0-2$ lateral plumose setae, $0-2$ spines in cleft, dorsal surface with $1-3$ spines in mediobasal position. Maxilla I outer lobe with 7 spines, 1.-3. pluri-toothed, 4.-7. variable (uni-, bi-, pluri-toothed). Gnathopod I and gnathopod II dactyli with single seta on outer margin. Gills II-VI ovoid, approximately same size as pereopod VI coxa, posterior margin slightly concave. Pleopods I-III with 2 retinacles on each. Uropod I lenght of endopodite: length of exopodite ratio as 1.00: $(1.00-1.20)$ on males and $1.00:(1.15-1.18)$ on females. Uropod III sexually dimorphic, exopodite rod-shaped, distal article of exopodite on males $83-115 \%$ of proximal article length and $18-73 \%$ on females.

Description. Body and telson. Small to medium-sized species, females are 6.4 mm to 9.0 mm , males are 7.8 mm to 10.6 mm . Head length up to $13 \%$ of body length; rostrum absent. Pereonites I-VI without setae; pereonite V, VI, VII with 1 postero-ventral seta each. Pleonites I-III with 3-6 setae along dorso-posterior margin (Fig. 3). Epimeral plate II ventral and posterior margins straight or sinusoid, ventro-postero-distal corner approximately perpendicular and pointed; along ventral margin 1-3 spiniform setae; along posterior margin 4-6 thin setae (Figs 3, 4). Epimeral plate III ventral margin convex and posterior margin straight, ventropostero-distal corner sharply inclined, strongly produced; along ventral margin $2-3$ spiniform setae; along posterior margin 4-6 thin setae (Figs 3, 4). Urosomite I postero-dorso-laterally with 1-2 spiniform seta; urosomite II postero-dorso-laterally with 2-3 spiniform setae; urosomite III without setae. Near insertion of uropod I 1 spiniform seta (Fig. 3).

Telson length: width as 1.0: $0.6-0.8$; cleft $71-87 \%$ of length; lobes apically rounded. Telson spines (per lobe): 3-4 apical spines; lateral margins with $1-3$ spine, $0-2$ plumose setae; 0-2 in cleft spines, dorsal surface with 1-3 basal spines in mediobasal position (Figs 4, 9). Length of apical spines $20-25 \%$ of telson length.


Figure 3. N. molnari, male from the Abaligeti Cave, lateral view. Telson, mouthparts and pleopods II-III are not drawn.

Antennae and mouthparts. Antenna I 35-48\% of body length. Flagellum with up to 19 articles; each article with 1 long aesthetasc. Peduncle article 1:2:3 proportions 1.0: 0,78 ( $0.72-0.88$ ): $0,4(0.36-0.46)$. Proximal article of peduncle dorso-distally slightly produced. Accessory flagellum biarticulated; distal article shorter than one-half of the proximal article. Lengths of antennae I: II as 1.0: 0.50. Flagellum of antenna II with $6-8$ articles. Lengths of peduncle articles $4: 5$ as 1.0: ( $0.84-0.95$ ); flagellum $54-70 \%$ of peduncle length (articles $4+5$ ) (Fig. 5).

Inner lobes of labium longer than half of outer lobes (Fig. 5).
Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth; between lacinia and molar 6-9 thick, serrated setae, long seta at base of molar absent (Fig. 5).

Right mandible: incisor processus with 4-5 teeth, lacinia mobilis with several small denticles (more then 12), between lacinia and molar 6-7 thick, serrated setae, long seta at base of molar present. Proportions of mandibular palp articles 2: 3 (distal) as 1.0: 1,20 (1.17-1.32). Proximal palp article without setae; second article with 9-11 seta in 5-6 groups; distal article with 1 group of 3-5 'A setae'; 3 groups of 'B setae'; 16-24 'D setae'; 3-5 'E setae' (Fig. 5).

Maxilla I distal palp article with 2-3 apical and subapical setae. Outer lobe of maxilla I with 7 spines, 1-3 spines are always pluri-toothed with 3-6 lateral tooth while 4-6 spines are uni-, or bitoothed. Inner lobe with 1-2 setae (Fig. 5).

Maxilla II inner lobe slightly smaller than outer lobe; both of them setose apically and subapically, number of setae is approximately 13-23 per lobe (Fig. 5).

Maxilliped palp article 2 with 11-17 rows of setae along inner margin; distal article with dorsal seta and group of small setae at base of nail. Maxilliped outer lobe with 6-12 flattened, thick setae and 3-8 serrated setae; inner lobe with 2-3 flattened, thick setae apically and 5-9 serrated setae (Fig. 5).


Figure 4. N. molnari, scanning electron micrographs. A epimeral plates (Ep1-3 = epimeral plates 1-3) B honeybee-cell pattern on the exosceleton (tipical feature of amphipods) $\mathbf{C}$ pleopod with two retinacles (pl-r = pleopod ramus, ret = retinaculum) $\mathbf{D}$ retinaculi on the pleopod (ret = retinaculum) $\mathbf{E}$ gnathopod II propodus (prop $=$ propodus, sup-spine $=$ supporting spine, dact $=$ dactylus) $\mathbf{F}$ palmar region of gnathopod II propodus (dent-spine $=$ denticulated spine, sup-spine $=$ supporting spine, $n=$ nail, palm-spine = palmar spine).

Coxal plates. Coxal plate I width: depth as 1.00: 1.03 (0.89-1.16), of flattened rhomboid shape, antero-ventral corner subrounded; anterior and ventral margin of coxa I with 3-6 setae (Fig. 6). Coxal plate II width: depth as 1.00: 0.84 (0.76-0.95); anterior and ventral margin with 5-8 setae. Coxal plate III width: depth as 1.00: 0.82 ( $0.71-1.00$ ); along antero-ventral margin $4-7$ setae (Fig. 7). Coxal plate IV width:


Figure 5. $N$. molnari, $\mathrm{aI}=$ antenna $\mathrm{I}, \mathrm{aII}=$ antenna $\mathrm{II}, \mathrm{mxI}=$ maxillaI, $\mathrm{mxII}=$ maxilla $\mathrm{II}, \mathrm{md}-\mathrm{R}=$ right mandibula, $\mathrm{lm}=$ lacinia mobilis, $\mathrm{inc}=$ incisor, $\mathrm{md}-\mathrm{L}=$ left mandibula, $\mathrm{lb}=$ labium, mxpe : maxilliped.
depth as 1.00: 1.03 (1.26-0.88); posteriorly concave; along antero-ventral margin 5-7 setae (Fig. 7). Coxal plates V-VI: anterior lobe well developed; along posterior margin 1 seta (Fig. 7). Coxal plate VII half-egg shaped, along posterior margin 1 seta (Fig. 7). Gills II-VI ovoid, with approximately same size as coxa VI (Fig. 7).

Gnathopods. Basis width is $38(33-45) \%$ of basis length. Gnathopod I ischium with $4-8$ posterodistal setae in 1 row. Carpus length $62(57-75) \%$ of basis length and $87(80-100) \%$ of propodus length. Anterior margin of carpus only with distal group of setae; carpus posteriorly with transverse rows of setae proximally and a row of lateral setae, posterior enlargment small. Propodus subquadrate, palm convex. Along posterior margin 6-8 rows of denticulated setae. Anterior margin with $10-17$ setae in $2-3$ groups, antero-distal group with 6-12 setae. Group of 2-4 facial setae below (proximal of) palmar spine; 2-4 single surface setae present. Palmar corner with palmar spine, single supporting spine on inner surface, and 3 (rarely 4) denticulated, thick spiniform setae on outer side. Nail length 36 (34-37)\% of total dactylus length; along anterior margin single seta; along inner margin $4-5$ setae (Fig. 6).


Figure 6. $N$. molnari, $\mathrm{gpI}=$ gnathopod $\mathrm{I}, \mathrm{gpII}=$ gnathopod II .

Gnathopod II basis width: length as 1.0: 0.26 ( $0.21-0.29$ ). Ischium with 2-6 postero-distal setae. Carpus length $56(50-61) \%$ of basis length and $86(71-94) \%$ of propodus lenght. Anterior margin of carpus only with distal row of setae; carpus posteriorly with transverse rows of setae proximally, a row of lateral setae; posteroproximal bulge small, positioned proximally. Propodus medium-sized (sum of length, diagonal and palm length measures up to $19(15-21) \%$ of body length) and larger than propodus of gnathopod I (1.0: 0.57 ( $0.65-0.85$ )). Propodus rectangular, palm convex. Posterior margin convex with 6-9 rows of denticulated setae. Anterior margin with $10-20$ setae in 3-5 groups; antero-distal group with 7-9 setae. 1 group of $2-3$ facial setae below (distal of) palmar spine; 1-4 individual surface setae present. Palmar corner with strong palmar spine, single supporting spine on inner surface, and 1 denticulated, thick spiniform seta on outer side. Nail length $31(22-36) \%$ of total dactylus length. Along anterior margin single seta; along inner margin 4-6 short setae (Figs 4, 6).


Figure 7. $N$. molnari, $\mathrm{ppIII}=$ pereopod III, $\mathrm{ppIV}=$ pereopod $\mathrm{IV}, \mathrm{ppV}=$ pereopod $\mathrm{V}, \mathrm{g}=$ gill, oost $=$ oostegit.

Pereopods III-IV. Proportions of pereopods III: IV as 1: 0.95 (0.93-0.97). Dactylus IV $45(39-51) \%$ of propodus IV; nail length 47 (39-52)\% of total dactylus length. Dactyli III-IV with one dorsal plumose seta, one spine-like seta at the base of the nail, and tiny seta near the spine-like seta (sometimes not visible or absent). Additional spiniform setae on posterior margin are absent (Fig. 7).

Pereopods V-VII. Proportions of pereopods V: VI: VII as 1.00: 1.4 (1.37-1.54): 1.5 (1.42-1.61). Pereopod VII length $47(42-52) \%$ of body length. Basis V-VII narrow with convex posterior margins. Basis V width is 70 ( $60-78$ )\% of length, basis VI is $67(59-76) \%$ of length and basis VII is $66(56-76) \%$ of length. Basis V with small posterodistal lobe, posterior margin with 8-13 setae, anterior margin with 6-8 groups of setae. Dactylus $V$ with one dorsal plumose seta, one spine-like seta at the base of the nail, and tiny seta near the spine-like seta (sometimes not visible or


Figure 8. $N$. molnari, $\mathrm{ppVI}=$ pereopod $\mathrm{VI}, \mathrm{ppVII}=$ pereopod $\mathrm{VII}, \mathrm{plpII}=$ pleopod II.
absent). Additional spiniform setae on posterior margin are absent (Fig. 7). Basis VI with small posteriodistal lobe, posterior margin with 9-14 setae, anterior margin with $6-10$ setae. Dactylus VI with one dorsal plumose seta (sometimes not visible or absent), one spine-like seta at the base of the nail, and tiny seta near the spinelike seta (sometimes not visible or absent). Additional spiniform setae on posterior margin are absent (Fig. 8).

Basis VII posterior margin with 6-13 setae, anterior margin with $6-11$ groups of setae. Total number of basis setae is 15-21. Dactylus VII length 26 (24-29)\% of propodus VII length; nail length 26 (16-33)\% of total dactylus length. Dactylus VII with one spine-like seta at the base of the nail. Additional spiniform setae on posterior margin are absent (Fig. 8).


Figure 9. N. molnari, $\mathrm{t}=$ telson, upI $=\operatorname{uropod} \mathrm{I}$, upII = uropod II, upIII-f = female's uropod III, upIII-m = male's uropod III.

Pleopods. Pleopods I-III with 2-hooked retinacles. Pleopod II rami of 16-20 articles each (Figs 4, 8).

Uropods. Uropod I basipodite with 6 dorso-lateral and 6 dorsomedial spinifom setae. Length ratio endopodite: exopodite as 1.00: 0.89 (0.83-1.0); rami slightly curved. Endopodite total setae number $2-4$ in $2-3$ groups, apically 5 spinifom setae. Exopodite with $2-7$ spines; apically 5 spinifom setae (Fig. 9).

Uropod II endopodite: exopodite length as 1.00: 0.81 (0.77-0.9) (Fig. 9).
Uropod III up to $38-46 \%$ (males) and $12-42 \%$ (females) of body length. Basipodite with no lateral seta and 3-6 apical spiniform and thin setae. Endopodite 58-61\% (males) and 48-70\% (females) of basipodite length, endopodite apically with 1-2 thin-flexible and spiniform setae; laterally $0-1$ seta. Exopodite of uropod III rod-shaped, distal article of exopodite $83-115 \%$ (males) and 18-73\% (females) of proximal exopodite article length. Proximal article with $4-5$ groups of plumose, thin-flexible and spiniform setae along inner margin and 4 groups of thin-flexible and spiniform setae along outer margin. Distal article with 3-6 apical setae; lateral setae only in males (Fig. 9).

# Redescription of Niphargus gebhardti Schellenberg, 1934 

Order Amphipoda Latreille, 1816
Suborder Gammaridea Latreille, 1802
Family Niphargidae G. Karaman, 1962
Genus Niphargus Schiödte, 1849

## Niphargus gebhardti Schellenberg, 1934

Niphargus foreli gebhardti n. subsp.: Schellenberg 1934; Type locality: Abaligeti Cave.
Data from the original description is available in Suppl. material 3.
N. foreli gebhardti: Schellenberg 1935, additional morphological data
N. foreli gebhardti, N. gebhardti: Gebhardt 1934, 1963, 1967, distributional data
N. gebhardti: Méhely 1941, morphological data
N. foreli gebhardti: Dudich 1941, distributional data
N. gebhardti: Angyal and Balázs 2013, morphological and distributional data

Material examined for redescription. 7 females and 4 males from a permanent pool in the main passage near 'Karthago romjai' hall of the Abaligeti Cave (Cadastre number: 4120-1, Hungarian Cave Cadastre), collected on 23 March 2013 (leg. D. Angyal \& A. Illés), dissected and mounted on slides; additional 4 specimens not dissected. Slides were deposited in the Collection of Crustaceans of the Hungarian Natural History Museum with the following codes: N.GEB-02, N.GEB-03, N.GEB-04, N.GEB-05, N.GEB-08, N.GEB-10, N.GEB-14, N.GEB-15, N.GEB-17, N:GEB-18, N.GEB-20. Diagnostic voucher numbers of specimens used for molecular studies: NB 550 ( N. gebgardti, coll. data: Abaligeti Cave, main passage, pool, 23 March 2013, leg. D. Angyal \& A. Illés), NB 551 (N. gebgardti, coll. data: Szajha-felső Sinkhole (Cadastre number: 4120-16), small pool, 2 April 2013, leg. D. Angyal \& Z. Tegzes).

COI Gen Bank Accession Numbers: KP967553 (Abaligeti Cave), KP967554 (Szajha-felső Sinkhole)

Diagnosis. Small-sized niphargid; epimeral plate III postero-ventral corner subrounded. Telson with 3-6 apical spines, $0-2$ lateral spines, $0-1$ lateral plumose setae, $0-1$ spines in cleft and $0-1$ dorsal surface spines. Maxilla I outer lobe with 7 spines, pluri-, uni-, bi-toothed spines alternating. Gnathopod I and gnathopod II dactyli with single seta on outer margin. Gills II-VI ovoid. Pleopods I-III with 3, rarely 4 retinacles on each. Uropod I lenght of endopodite: length of exopodite ratio as 1.00: (1.09-1.11) on males and 1.00: (1.03-1.17) on females. Uropod II sexually dimorphic, exopodite rod-shaped, distal article of exopodite on males $95-155 \%$ of proximal article length and $52-72 \%$ on females.

Description. Body and telson. Small-sized niphargid species, females $4.9-5.9 \mathrm{~mm}$, males 5.9-7.0 mm. Head length up to $9 \%$ of body length; rostrum absent. Pereonites


Figure IO. N. gebhardti, female from the Abaligeti Cave, lateral view. Mouthparts, rami of pleopods and telson are not drawn.

I-VI without setae; pereonite V, VI, VII with 1 postero-ventral seta each. Pleonites I-III with 1-2 setae along dorso-posterior margin. Epimeral plate II posterior and ventral margins convex, ventro-postero-distal corner rounded. Along ventral margin 1-3 spiniform setae; along posterior margin 3-4 thin setae. Epimeral plate III ventral and posterior margins convex, ventro-postero-distal corner rounded; along ventral margin 2-3 spiniform setae; along posterior margin 4 thin setae. Urosomite I postero-dorso-laterally with 1 seta; urosomite II postero-dorso-laterally with 1 spiniform seta; urosomite III without setae. Near insertion of uropod I 1 spiniform seta (Figs 10, 11).

Telson length: width as 1.0 : 0.88 ; cleft $74(70-79) \%$ of length; lobes apically widely rounded. Telson spines (per lobe): 2-4 apical spines, 33.5 (28-39)\% of telson length; lateral margins with $0-2$ spine and $0-1$ plumose setae; $0-1$ in cleft spines, 0 or 1 dorsal surface spines, 1 basal spine (Figs 11, 16).

Antennae and mouthparts. Antenna I 37 (34-41)\% of body length. Flagellum with up to 13-16 articles; each article with 1 long aesthetasc (Fig. 11). Peduncle article 1: 2: 3 as 1.0: 0.69 ( $0.60-0.76$ ): 0.37 ( $0.30-0.4$ ). Proximal article of peduncle dorsodistally slightly produced. Accessory flagellum biarticulated; distal article 52 (38-67)\% of proximal article. Lengths of antennae I: II as 1.0: 0.48 ( $0.42-0.52$ ). Flagellum of antenna II with 6-8 articles. Lengths of peduncle articles 4: 5 as 1.0: 0.85 (0.81-0.91); flagellum 73 (57-81)\% of peduncle length (articles 4+5) (Fig. 12).

Inner lobes of labium longer than half of outer lobes (Fig. 12).
Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth; between lacinia and molar 5-7 thick, serrated setae, long seta at base of molar absent (Fig. 12).


Figure II. N. gebhardti, scanning electron micrographs. A epimeral plates with uropods (Ep1-3 = epimeral plates $1-3$, upI $=$ uropod I , upII $=$ uropod II, upIII-f $=$ female's uropod III) B epimeral plates (Ep1-3 = epimeral plates 1-3) C telson ( $\mathrm{t}=$ telson, pl-seta $=$ plumose seta) $\mathbf{D}$ pleopods (plp = pleopod) $\mathbf{E}$ aesthetasc on antenna I (aest = aesthetasc) $\mathbf{F}$ pereopod VI dactylus (sl-seta $=$ spine-like seta at the base of the nail, pl-seta = plumose seta).

Right mandible: incisor processus with 4 teeth, lacinia mobilis with 5-6 denticles, between lacinia and molar 6-8 thick, serrated setae, 1 long seta at base of molar present. Proportions of mandibular palp articles 2: 3 (middle: distal) as 1.0: 1.1 (1.001.21). Proximal palp article without setae; second article with 4-6 seta in 3-4 groups; distal article with 1 group of 3-4 'A setae'; 2-4 of 'B setae' (single or in groups); 9-13 'D setae' and 3-5 'E setae' (Fig. 12).


Figure 12. N. gebhardti, aI = antenna I , aII = antenna $\mathrm{II}, \mathrm{mxI}=$ maxilla $\mathrm{I}, \mathrm{mxII}=$ maxilla $\mathrm{II}, \mathrm{md}-\mathrm{R}=$ right mandibula, $\mathrm{inc}=$ incisor, $\mathrm{lm}=$ lacinia mobilis, $\mathrm{md}-\mathrm{L}=$ left mandibula, $\mathrm{lb}=$ labium, $\mathrm{mxpe}=$ maxilliped.

Maxilla I distal palp article with 3-6 apical and subapical setae. Outer lobe of maxilla I with 7 spines, pluri-, uni-, bi-toothed spines alternating. Inner lobe with 1 seta (Fig. 12).

Maxilla II inner lobe slightly smaller than outer lobe; both of them setose apically and subapically, number of setae is approximately $6-11$ on inner lobe and $8-12$ on outer lobe (Fig. 12).

Maxilliped palp article 2 with 8-11 rows of setae along inner margin; distal article with dorsal seta and group of small setae at base of nail. Maxilliped outer lobe with 6-8 flattened, thick setae and 3-5 serrated setae; inner lobe with 2-3 flattened, thick setae apically and 2-4 serrated setae (Fig. 12).


Figure I3. N. gebhardti, gpI = gnathopod I, gpII = gnathopod II.

Coxal plates. Coxal plate I width: depth as 1.00: 0.76 (0.6-0.9) of flattened rhomboid shape, antero-ventral corner subrounded; anterior and ventral margin of coxa I with 4-6 setae (Fig. 13). Coxal plate II width: depth as 1.00: 0.97 (0.83-1.21); anterior and ventral margin with 3-6 setae (Fig. 13). Coxal plate III width: depth as 1.00: 1.12 (1.05-1.2); along antero-ventral margin 4-6 setae. Coxal plate IV width: depth as 1.00: 1.04 (0.97-1.12); posteriorly concave; along antero-ventral margin $4-5$ setae (Fig. 14). Coxal plates V-VI with well developed anterior lobe, and smaller posterior lobe with usually 2 setae (occasionally with 1 or 3) in postero-ventral corner. Coxal plate VII half-egg shaped, along posterior margin 2 setae. Gills II-VI ovoid, of approximately similar size as coxa VI (Fig. 15).

Gnathopods. Gnathopod I basis width 42 (38-47) \% of basis length. Ischium with 3-4 posterodistal setae in 1 row. Carpus length 61 (52-82)\% of basis length and 98
(87-110)\% of propodus length. Anterior margin of carpus only with distal group of setae; carpus posteriorly with transverse rows of setae proximally and a row of lateral setae, posterior enlargment small. Propodus subquadrate, palm and posterior margin convex. Along posterior margin 3-4 rows of denticulated setae. Anterior margin with $6-11$ setae in $2-3$ groups, antero-distal group with $4-8$ setae. Group of 2-3 facial setae below (proximal of) palmar spine; 1-4 surface setae in $1-2$ groups present. Palmar corner with palmar spine, single supporting spine on inner surface, and 2-3 denticulated, thick spiniform setae on outer side. Nail length 33 (30-39)\% of total dactylus length; along anterior margin single seta; along inner margin 3-4 setae (Fig. 13).

Gnathopod II basis width: length as 1.0: 0.34 (0.27-0.45). Ischium with 3-4 postero-distal setae in 1 row. Carpus length 59 (48-69)\% of basis length and 106 (96111)\% of propodus length. Anterior margin of carpus only with distal row of setae; carpus posteriorly with transverse rows of setae, proximally a row of lateral setae; poste-ro-proximal bulge small and positioned proximally. Propodus small to medium-sized (sum of length, diagonal and palm length measures up to $12-15 \%$ of body length) and larger than propodus of gnathopod I (1.0: $0.87(0.78-0.96)$ ). Propodus rectangular, palm convex. Posterior margin straight or convex with $4-5$ rows of denticulated setae. Anterior margin with 3-9 setae in 1-2 groups; antero-distal group with 4-8 setae. Group of 2-4 facial setae below (proximal of) palmar spine; 2-3 surface setae in 1-2 groups present. Palmar corner with strong palmar spine, single supporting spine on inner surface, and 2-3 denticulated, thick spiniform setae on outer side. Nail length $34(29-42) \%$ of total dactylus length. Along anterior margin single seta; along inner margin 3 short setae (Fig. 13).

Pereopods III-IV. Proportions of pereopods III: IV as 1: 0.96 (0.89-1). Dactylus IV 51 (46-57)\% of propodus IV lenght; nail length 53 (44-61)\% of total dactylus length. Dactyli III-IV with dorsal plumose seta (sometimes not visible or absent), one spine-like seta at the base of the nail, and tiny seta near the spine-like seta (sometimes not visible or absent). Additional spiniform setae on posterior margin are absent (Fig. 14).

Pereopods V-VII. Proportions of pereopods V: VI: VII as 1.00: 1.3 (1.27-1.49): 1.5 (1.46-1.58). Pereopod VII length $42-45 \%$ of body length. Basis V-VII with convex posterior margins. Basis V width is $71(66-80) \%$ of length, basis VI is 68 (64-73)\% of length, and basis VII is 66 (63-69)\% of length. Basis V with small posterodistal lobe, posterior margin with 4-6 setae, anterior margin with 4-9 setae in $3+1$ groups (Fig. 14). Pereopod dactylus V with one dorsal plumose seta (sometimes not visible or absent), and one spine-like seta at the base of the nail (Fig. 14). Basis VI with small posterodistal lobe, posterior margin with 6-7 setae, anterior margin with 5-8 setae in 3-4 groups. Dactylus VI with one spine-like seta at the base of the nail, and tiny seta near the spine-like seta (sometimes not visible or absent). Additional spiniform setae on posterior margin are absent (Fig. 15). Basis VII posterior margin with 5-8 setae, anterior margin with 3-5 groups of setae. Total number of basis setae is $11-15$. Dactylus VII length 26 (23-35)\% of propodus VII length; nail length 28.5 (25-38)\% of total dactylus length. Dactyli VI with one dorsal plumose seta (sometimes not visible or absent), one spine-like seta at the base of the nail, and tiny seta


Figure 14. N. gebhardti, ppIII = pereopod III, $\mathrm{ppIV}=$ pereopod $\mathrm{IV}, \mathrm{ppV}=$ pereopod V .


Figure I5. $N$. gebhardti, ppVI = pereopod VI, ppVII = pereopod VII, plpII = pleopod II.


Figure 16. N. gebhardti, t: telson, upI: uropod I, upII: uropod II, upIII-m: male's uropod III, upIII-f: female's uropod III.
near the spine-like seta (sometimes not visible or absent). Additional spiniform setae on posterior margin are absent (Fig. 15).

Pleopods. Pleopods I-III with 3, rarely 4 hooked retinacles. Pleopod II rami of 11-13 articles each (Figs 11, 15).

Uropods. Uropod I basipododite with 4-5 dorso-lateral and 1-3 dorsomedial spiniform setae including spiniform setae in distal position. Length ratio endopodite: exopodite as 1.00: 0.91 ( $0.87-0.97$ ); rami slightly curved. Endopodite with $1-2$ setae, apically 5 spinifom setae. Exopodite with $1-4$ setae or spines in $1-2$ groups; apically 5 spinifom setae (Figs 11, 16).

Uropod II endopodite: exopodite length as 1.00: 0.84 (0.77-0.95) (Figs 11, 16).
Uropod III 38 (37-39)\% (males) and 26 (24-30)\% (females) of body length. Basipodite with 0-1 lateral setae and 5-6 apical spiniform and thin setae. Endopodite $41(39-44) \%$ (males) and $48(41-54) \%$ (females) of basipodite length; endopodite apically with $0-2$ thin-flexible and spiniform setae; laterally with $0-1$ seta. Exopodite of uropod III rod-shaped, distal article of exopodite 100 (95-105)\% (males) or $60(52-78) \%$ (females) of proximal article length. Proximal article with $3-4$ groups of plumose, thin-flexible and spiniform setae along inner margin and 2-4 groups of thin-flexible and spiniform setae along outer margin. Distal article without lateral seta (males) or with 3 setae in 1 group (females); apically $4-7$ setae (Figs 11, 16).

## Comparison with phylogenetically related and geographically close species

N. molnari and N. gebhardti share few main traits (the same body size class, slender body, sexually dimorphic uropod III but not uropod I), but differ from each other in the shape of epimeral plates, the size of gnathopod propodi, in denticulation of spines on outer lobe of maxilla I and in the number of retinacles (Angyal and Balázs 2013). Keeping these differences in mind we compare both species to the species that are either closely related according to molecular phylogeny, or to the species that live in the same geographic area.

Niphargus vadimi Birstein, 1961 is known from Crimea. Despite its close position suggested by the presented molecular tree, this species differs from phylogenetically related $N$. gebhardti and non-related $N$. molnari in considerably larger body size and much larger gnathopods.

High morphological similarity to the focal pair of species reveal another four species phylogenetically related to $N$. gebhardti, namely Niphargus bihorensis Schellenberg, 1940, Niphargus fongi Fišer \& Zagmajster, 2009, Niphargus carniolicus Sket, 1960, and Niphargus dobati Sket, 1999. Epikarstic N. bihorensis is known from Romania and Italy, whereas the latter three are known from epikarst and karst river beds from Slovenian caves. All four species share with focal species main traits (body size, slender body, sexually dimorphic uropod III but not uropod I).
$N$. bihorensis and $N$. fongi differ from the focal species in the shape of gills (being narrow instead of ovoid as in focal species) and in higher number of retinacles on pleopods. In addition, $N$. fong $i$ differs from $N$. molnari and $N$. gebhardti by (i) the elevated number of setae along posterior margin of epimeral plate III, (ii) the longer apical telson spines, (iii) and the reduced number of denticulated spines in palmar corners of both gnathopods. $N$. bihorensis, which is a complex of at least two morphologically indistinguishable species (Meleg et al. 2013), differs from the focal species by (i) reduced number of spines on maxilla I outer lobe (only 6), (ii) more numerous setae on maxilla I palpus (7-8), (iii) and by more numerous retinacles.
$N$. carniolicus and $N$. dobati differ from the focal pair of species in the length of rami of uropod I (expopodite equal to or slightly longer than endopodite versus exopodite consistently shorter to endopodite in focal species). In addition, $N$. carniolicus differs from $N$. molnari and $N$. gebhardti by (i) shorter apical spines on telson, and (ii) fewer denticulated spines on palmar corner of gnathopods. N. dobati differs from the two focal species by (i) the elevated number of spines on uropod I basipodite, (ii) the length of pereopod V and VI (which are longer comparing with pereopod VII), and the (iii) elevated number of mandibular palp 'D seta'.

Phylogenetic relationship of $N$. molnari to the rest of Niphargus species is not clear, however a few morphologically similar species, like Niphargus schellenbergi S. Karaman, 1932 are known. It differs from $N$. molnari and $N$. gebhardti by (i) the differently ornamented telson ( $5-7$ long apical spines and $2-5$ lateral spines in $N$. schellenbergi, respectively), (ii) more numerous apical setae on uropod III endopodit, (iii) elevated number of pleopod retinaculi, (iv) by the length of uropod I exopodite, which
is slightly longer than endopodit, (v) by several setae along outer margin of gnathopod dactyli, and (vi) by bigger body size ( $>10 \mathrm{~mm}$ ).

The following species are compared with $N$. molnari and $N$. gebhardti due to their geographical vicinity. Niphargus forroi G. Karaman, 1986 was described from Northeast Hungary, and is known from only a couple of caves from the Bükk Mts. Beside the close body size, $N$. forroi agree with $N$. molnari by the similar seta numbers and arrangement on the gnathopods, by the telson spine-pattern, as well as by the number of different spine and seta types on pereopod dactyli. $N$. forroi differs from $N$. molnari by (i) the subrounded posteroventral corner of the epimeral plates, (ii) the lower number of mandibular palp 'D setae' and by (iii) the reduced number of maxilla distal article apical seta. $N$. forroi differs from both $N$. molnari and $N$. gebhardti by the number of posterior margin setae on pereopods V-VII. The description of Niphargus hungaricus Méhely, 1937 (endemic species of the Kőszegi Mts.) contains no drawings and not enough characters that would be needed for proper comparison. A later work of Méhely (1941) is only partially filling this gap by containing a drawing on the first gnathopod and some additional data on its seta arrangement. According to the available information, $N$. hungaricus differs from $N$. molnari and $N$. gebhardti by (i) the setae number of gnathopods dactyli outer margin (always more than 1 seta of $N$. hungaricus) and by (ii) the length of male's uropod I endopodite (inner ramus is elongated and two times long as outer ramus in $N$. hungaricus). There are different Niphargus populations in the Bükk Mts. and in the Aggtelek Karst belonging to the Niphargus tatrensis Wrzesniowsky, 1888 species group including Niphargus aggtelekiensis Dudich, 1932. Although the taxonomic status of these populations is not clear, the complex shares several distinct morphological characters that can be compared with the focal species. Populations of $N$. tatrensis - N. aggtelekiensis complex differ from $N$. molnari and $N$. gebhardti by (i) larger body size ( $>15 \mathrm{~mm}$ ), (ii) the elevated number of setae along outer margin of gnathopods dactyli (there are more than one), (iii) the lower mandibular 'A' and 'D seta' number and (iv) the elongated distal article of uropod III of both gender. Main diagnostic characters are presented in Table 1.

## Molecular taxonomy

Phylogenetic relationships within the genus Niphargus (Fig. 17) showed that the two redescribed species of Niphargus from Hungary are not phylogenetically closely related. Phylogenetic relationship of $N$. molnari to the rest of Niphargus species is unclear; species is nested within basal polytomy. N. gebhardti belongs to the clade of Central to Eastern European species. The focal species is in sister relationship with a pair of morphologically cryptic species endemic to Western Carpathian (N. bihorensis, see Meleg et al. 2013). Other closely related species include N. vadimi from Crimea, Pontoniphargus racovitzai from Eastern Romania and a clade of epikarstic and interstitial species from Southern Slovenia ( $N$. fongi, $N$. carniolicus, $N$. wolfi and $N$. dobati).
Table I. Comparison of the main diagnostic characters of N. molnari, N. gebhardti and the phylogenetically related and geographically close species.

| Species | No. apical telson spines | No. lateral telson spines | Pleopod I. no. hooks in retinacle | Pleopod II. no. hooks in retinacle | Pleopod III. no. hooks in retinacle | Uropod I endopodite/ exopodite length | Gnathopod dactylus anterior margin seta no. | Shape of gills II-IV | Epimeral plates postero-ventral corner shape | Source of data |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| N. molnari Méhely, 1927 | 3-4 | 1-3 | 2 | 2 | 2 | endopodite slightly longer | single | ovoid | sharply inclined | own slides |
| N. gebhardti <br> Schellenberg, 1934 | 3-6 | 0-2 | 3 (rarely 4) | 3 (rarely 4) | 3 (rarely 4) | endopodite slightly longer | single | ovoid | subrounded | own slides |
| N. carniolicus Sket, 1960 | 4-5 | 1-2 | 4-5 | 4-5 | 4-5 | exopodite slightly longer | single | ? | subrounded | Sket 1960, G. <br> Karaman 1989 |
| $\begin{gathered} \text { N. dobati Sket, } \\ 1999 \end{gathered}$ | $3+1$ | 2 | 3-4 | 3-4 | 3-4 | nearly equal | single | narrow | subrounded | Sket 1999 |
| N. vadimi Birstein, 1960 | ? | 3 | ? | ? | ? | ? | ? | ? | sharply inclined | Birstein 1961 |
| N. fongi Fišer \& Zagmajster, 2009 | 3-5 | 1-2 | 4-7 | 3-5 | 4-5 | equal | single | narrow | subrounded | Fišer and Zagmajster 2009 |
| N. bihorensis Schellenberg, 1940 | 5-7 | 1 pair, plumose | 4-6 | 4-6 | 4-6 | exopodite slightly longer | single | long and recurved | I., II. subrounded, III. angular | $\begin{aligned} & \text { G. Karaman } \\ & 1980 \\ & \hline \end{aligned}$ |
| N. schellenbergi S. Karaman, 1932 | 5-7 | 2-5 | 4-6 | 3-5 | 3-6 | exopodite slightly longer | more than 1 | ? | subrounded | $\begin{gathered} \text { S. Karaman } \\ 1932 \\ \hline \end{gathered}$ |
| N. forroi G . <br> Karaman, 1986 | 2 | 2 | 2 | 2 | 2 | endopodite longer | single | narrow | subrounded | G. Karaman and Ruffo 1986 |
| $N$. hungaricus Méhely, 1937 | 3-5 | 1-2 | ? | ? | ? | endopodite 2 x longer | more than 1 | ? | subrounded | Méhely 1937, 1941 |
| N. tatrensis Wrzesniowsky, 1888 | 3-4 | 0-3 | 2 | 2 | 2 | nearly equal | more than 1 | large, irregularly ovoid | III. sharply inclined | Fišer et al. 2010 |



Figure 17. Bayesian phylogenetic tree of 104 amphipod taxa (including N. molnari and N. gebhardti) based on COI, 28S and histone (H3) sequences. Map represents distribution of the clade with N. gebharti. Squeres represent epikarstic species and circles species from other subterranean habitats.


Figure 18. Distribution of $N$. molnari and $N$. gebhardti within the Western Mecsek.

## Remarks on ecology and distribution

Among the studied two species, $N$. gebhardti was collected more frequently, as it was found in five other caves of the Western Mecsek in addition to the type locality, namely Trió Cave, Gilisztás Sinkhole, Szajha-felső Sinkhole, Vadetetős Sinkhole and Spirál Sinkhole (Fig. 18). In most of these, two types of water bodies exist: i) small pools of residual- or percolated/dripping water and ii) streams or minor streaming water. Amount of water in the caves is dependent on the rainfall in the surface. In all six caves, $N$. gebhardti specimens were found in isolated, shallow pools in limestone, sinter or clay, most likely formed by dripping water (Fig. 19). Specimens were never observed in streams or any other streaming waters. During our repeated visits between 2010 and 2013 (altogether 24 visits in the 6 caves), the same pools were checked every time and some specimens were always found in them (except when the pools dried out). Once it was observed that a group of $N$. gebhardti (approximately 20 specimens) were fed upon a dead Oxychilus snail in a pool.
N. molnari was observed in the Abaligeti Cave and in two sinkholes that the other species ( $N$. gebhardti) was also inhabited, Spirál Sinkhole and Vadetetős Sinkhole (Fig. 18). Density of $N$. molnari was high in the stream of the Western 2 collateral of the Abaligeti Cave, however in the other two caves only a few specimens were found in streaming water, always in deeper parts of the caves. The two species were always spatially well segregated. In the Abaligeti Cave N. molnari coexisted with Protelsonia bungarica Méhely, 1924 (endemic aquatic troglobiont isopod of the cave) and with the troglomorph specimens of Gammarus fossarum Koch, 1836.


Figure 19. Small pool formed by dripping water, one of the occupied microhabitats of N. gebhardti in the Trió Cave.

## Discussion

Due to its protected geographical situation, since the Tertiary, the area of Mecsek may have played a refugial role during the alternating warmer and colder eras, preserving old lineages of Crustaceans. They presumably ensconced into subterranean aquatic habitats from searing creaks of the Paratethys Sea, that encompassed the islands of the Mecsek. Then, by degress, they had been adapted to the subterranean conditions in both physiological and morphological features (Méhely 1925). According to results of our phylogenetic analysis, N. molnari and N. gebhardti represent completely distinct lineages, which colonized the Mecsek area independently. The two species are spatially segregated within the same caves. N. gebhardti inhabits isolated pools of stagnant water, which fed by percolating water from the limestone fissures, so called epikarst. Interestingly N. gebhardti is apparently phylogenetically related to epikarstic species from Slovenia. On the contrary, $N$. molnari was always found in streaming waters.

The distribution range of the two endemic species is small, the most distant caves are seven kilometers far. These caves belong to three different catchment areas (Fig. 18). Despite of our repeated visits and careful searching, Niphargus specimens were not found in the Mánfai-kőlyuk Cave. N. molnari supposedly has gone extinct in its type locality as it is ruined due to the industrial utilization of the cave (Angyal 2012). Moreover, the type locality of N. gebhardti - which is a touristic cave with 80.000 annual visitors - may be also endangered. Considering the extremely narrow distributional range of the two species and the vulnerability of their populations, $N$. molnari and N. gebhardti are suggested to be placed into the 'Vulnerable (VU)'
category according to the following criteria of IUCN Red List of Threatened Species (IUCN 2012): i) number of locations is $\leq 10$ ('B2') and ii) area of occupancy is less than $20 \mathrm{~km}^{2}$ ('D2').'

Hidrologically connected caves are in quadrats.

## Conclusions

Some highly endemic, troglobiont invertebrate taxa are known from the Southern Hungarian Mecsek Mts. Two of them, the blind amphipod Niphargus molnari Méhely, 1927 and Niphargus gebhardti Schellenberg, 1934 have been rediscribed, applying the modern approach of integrative taxonomy. Comparative scanning electron microscopy used for first time on niphargids, and it proved to be a rather useful method in analysing and illustrating of barely visible diagnostic characters. As contributions to the future molecular genetic studies on niphargids, cytochrome c oxidase subunit I (COI) sequences as barcodes of $N$. molnari and $N$. gebhardti are now available for the public. The phylogenetic analyses have shown that the two species - which are spatially segregated in caves where they coexist - represent completely distinct lineages and may have colonized the Mecsek area independently. Phylogenetic relationship of N. molnari to the rest of Niphargus species is for the present not clear. $N$. gebhardti is closely related to a clade of epikarstic species from Southern Slovenia and to cryptic species endemic to Western Carpathians. New localities of both species have been found. The two species are suggested for legal protection, they should be listed into 'Vulnerable' category of the IUCN Red List of Threatened Species.

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## Supplementary material I

## Protocols and thermo profiles used in molecular studies

Authors: Dorottya Angyal, Gergely Balázs, Valerija Zakšek, Virág Krízsik, Cene Fišer Data type: Primers, molecular protocols
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

## Supplementary material 2

## List of taxa and sequence data used in phylogenetic analysis

Authors: Dorottya Angyal, Gergely Balázs, Valerija Zakšek, Virág Krízsik, Cene Fišer Data type: Sequence data
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

## Supplementary material 3

## Original descriptions of $N$. molnari and N. gebhardti

Authors: Dorottya Angyal, Gergely Balázs, Valerija Zakšek, Virág Krízsik, Cene Fišer
Data type: Descriptions
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

# A taxonomic review of the Gyrinidae (Coleoptera) in Korea 

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#### Abstract

A taxonomic review of Korean Gyrinidae is presented. Seven species [Dineutus orientalis (Modeer, 1776), Gyrinus gestroi Régimbart, 1883, G. japonicus Sharp, 1873, G. pullatus Zaitzev, 1908, Orectochilus punctipennis Sharp, 1884, O. Regimbarti Sharp, 1884 and O. villosus (Müller, 1776)] in three genera are recognized, one of which ( $O$. punctipennis Sharp, 1884) is reported for the first time in Korea. We also found that G. curtus Motschulsky, 1866 previously recorded in Korea was an incorrect identification of $G$. pullatus Zaitzev, 1908. Habitus and SEM photographs, distribution maps, keys, and diagnoses of genera and species are provided.


## Keywords

Taxonomy, Gyrinidae, Coleoptera, Korea

## Introduction

The Gyrinidae are water beetles with unique swimming behavior where adults rapidly gyrate and whirl on the surface of water. They inhabit stagnant or slowly running water and prefer clean oxygen-rich habitats (Brinck 1955). Members of the Gyrinidae
are characterized by the combination of the following characters: compound eyes divided completely with one pair on the dorsal surface of the head (above the water line) and another on the ventral surface of the head (below the water line); antenna short with a broad, cup-shaped scape, subtriangular pedicel, elongate but compact flagellum; middle and hind legs broadly expanded and fringed with setae for swimming (Miller and Bergsten 2012).

The whirligig beetles contain about 1000 species in 25 genera worldwide (Slipinski et al. 2011) and 118 species in seven genera in the Palaearctic region (Mazzoldi 2003). In Korea, seven species in three genera have been recorded (Jung et al. 2011), 50 species in six genera from China, 16 species in three genera from Japan and 11 species in three genera from the Far East of Russia (Mazzoldi 2003).

It was Kolbe (1886) who recorded the first gyrinid species, Gyrinus japonicus Sharp, 1873 in Korea. About 50 years later, Japanese entomologists, Takizawa (1931) and Kamiya (1936) reported two species (Dineutus orientalis Modeer, 1776 and G. japonicus Sharp, 1873) with descriptions and illustrations. Cho (1957) was the first Korean beetle taxonomist who studied Korean gyrinid fauna in detail. Since then, a few entomologists have studied Korean gyrinid beetles, mainly in the local fauna and no taxonomic review has been performed since Yoon (1988). Accordingly, this review is an updated contribution worth undertaking for Korean Gyrinidae.

In this paper we recognized seven species [D. orientalis (Modeer, 1776), G. gestroi Régimbart, 1883, G. japonicus Sharp, 1873, G. pullatus Zaitzev, 1908, Orectochilus punctipennis Sharp, 1884, O. Regimbarti Sharp, 1884 and O. villosus (Müller, 1776)]: O. punctipennis Sharp is identified for the first time in Korea and G. curtus Motschulsky previously recorded in Korea was an incorrect identification of G. pullatus Zaitzev. We provided habitus and SEM photographs, distribution maps, keys, and diagnoses of genera and species.

## Materials and methods

To identify Korean Gyrinidae more reliably, we compared them with type and voucher specimens in the National History Museum (NHM, London, United Kingdom). The specimens used in this study are deposited in Chungnam National University Insect Collection (CNUIC), Daejeon, Korea and Ehime University Museum (EUMJ), Matsuyama, Japan. Habitus photographs were prepared from single or multi-layered shots taken with Olympus DP71 camera with several images amalgamated using Helicon Focus 5.3 (Helicon Soft Ltd, Kharkov, Ukraine) and edited by Adobe Photoshop CS4 (Adobe Systems, San Jose, CA, USA). Dry specimens for scanning electron microscope (SEM) photographs were sputter coated with platinum (Cressington 208 auto sputter coater, Hertfordshire, UK) and examined under SEM (S-4800, Hitachi, Tokyo, Japan). The terminology of taxonomic characters and measurements of specimens mainly follow Holmen (1987) and Miller and Bergsten (2012). The subdivision of China and Russia follows the standards of Löbl and Smetana (2003).

## Results

Gyrinidae Latreille, 1810

## Key to the genera of Korean Gyrinidae

1 Pronotum with transverse depression on median part; elytra with rows of punctures

Gyrinus

- Pronotum without transverse depression; elytra without rows of punctures... 2

2 Labrum short and broad; elytra without compact setae. $\qquad$ Dineutus

- Labrum long and subtriangular; elytra with compact setae. $\qquad$ Orectochilus


## Genus Dineutus MacLeay, 1825

Figs 7-8, 10-14
Dineutus MacLeay, 1825: 133. Type species: Dineutus politus MacLeay, 1825.
Diagnosis. Head about 2.0 times as wide as long, with polygonal micro-reticulation. Clypealium with long setae. Antenna with 6 antennomeres. Galea absent; cardo and stipes with few setae on apico-lateral part. Pronotum (Fig. 7) convex without transverse groove; anterior margin bisinuate, posterior margin rounded, anterior angle acute, posterior angle nearly rectangular. Scutellum (Fig. 8) concealed when elytra closed. Elytra (Fig. 8) widest at middle, without punctato-striae and compact setae; subelytral suture absent. Prosternum (Fig. 10) transverse; anterior margin rounded; prosternal process (Fig. 10) linear-form and posterior margin rounded. Anterior margin of mesoventrite (Fig. 11) acute, posterior part bifid. Metaventrite (Fig. 12) broad, diamond-shaped, flattened; anterior part acute, lateral parts transverse. Sternite II (Fig. 13) without pit and groove. Sternite VIII (Fig. 14) with compact and short setae on lateral margins.

## Subgenus Spinosodineutes Hatch, 1925: 447

Spinosodineutes Hatch, 1925: 447. Type species: Gyrinus spinosus Fabricius, 1781.
Gyrinodineutus Ochs, 1926: 66. Type species: Dineutus unidentatus Aubé, 1838. Synonymized by Brinck (1955: 104).

Dineutus (Spinosodineutes) orientalis (Modeer, 1776)
Figs 1, 7-14, 41-43, 59
Gyrinus orientalis Modeer, 1776: 160.
Dineutus marginatus Sharp, 1873: 56. Synonymized by Ochs (1926: 136).
Dineutus quadrispina Fairmaire, 1878: 88. Synonymized by Ahlwarth (1910: 6).
Dineutus (Spinosodineutes) orientalis: Ochs (1930: 9).


Figures I-6. Habitus. I Dineutus orientalis, 9.0 mm 2 Gyrinus gestroi, 4.8 mm 3 G. japonicus, 7.0 mm 4 G. pullatus, 6.0 mm 5 Orectochilus punctipennis, 6.5 mm 6 O. villosus, 5.7 mm .

Specimens examined. NORTH KOREA: Gangwon Prov.: $1 \overparen{\sigma}^{\Uparrow} 1 q$, Uonsan-city, Anby-on-gun, Pisan-ri, 23.VII.2008, Changdo Han. SOUTH KOREA: Gangwon Prov.: 2 ふ̋ ふ̋ 1 , Cheorwon-gun, Dongsong-eub, Odeok-ri, Hakji-reservoir, 15.IX.1990; Gyeongbuk Prov.: 3 đ̋ 3 q $q$, Euiseong-gun, Geumseo-myeon, Sujeong-ri, 27.VII.2010, SW Jung, DH Lee, valley ( $1 \delta^{\lambda}$, on slide); $1 \circlearrowleft^{\lambda} 1$ q, Gumi-si, Okgye-dong, 18.VI.1990, SH Lee; 1 Q, Gyeongju-si, Geoncheon-eub, Sinpyeong-ri, 28.V.1993, SH Lee; 1 §̃, Gyeongju-si, Wolseong-dong, 5.VI.1987, HM Lee; Gyeonggi Prov.: $1 \diamond^{\top} 2$ 우, Hwaseong-si, Songsanmyeon, Dokji-ri, N37º $15^{\prime} 25.08^{\prime \prime}$, E126 $40^{\prime} 49.68^{\prime \prime}, 5 \mathrm{~m}, 4 . \mathrm{VII} .2013$, DH Lee, SG Lee,
pond near brackish zone; 1 P, Incheon-si, Ganghwa-gun, Gyodong-myeon, 6.X.2009, HM Lim; $1 \AA^{\lambda}$, same data as former except for, Naega-myeon, 16.IX.1990, SH Lee, pond; 1 Q, Suwon-si, 24.VI.1969; Jeju Prov.: 1 ठ, Bukjeju-gun, Jocheon-eub, Seonheul-ri, 11.VI.2005, DH Lee, pond; $1 \widehat{ }^{\pi}$, Seoguipo-si, Daejeong-eub, Boseong-ri, 9.VII.1985; Jeonnam Prov.: 1 §, Haenam-gun, Hwangsan-myeon, Namri-ri, 13.VI.2010, SH Lee; 1 §, Hawsun-gun, Dong-myeon, Jangdong-ri, 3.VIII.2009, SH Lee.

Published Korean records. Dineutus (Spinosodineutes) orientalis: Mazzoldi (1995: 160); Mazzoldi (2003: 26). Dineutus orientalis: Kamiya (1936: 16); Mochizuki and Tsunekawa (1937: 78); Mochizuki and Matsui (1939: 56); Kamiya (1940: 130); Cho (1957: 201); Cho (1963: 46); Cho (1969: 191); Kim and Nam (1982: 25); Lee et al. (1985: 402); Kwon and Suh (1986: 98); Yoon (1988: 617); Lee et al. (1992: 55); Kim et al. (1994: 14); Lee (1995: 15); Hua (2002: 41); Han et al. (2007: 271); Han et al. (2008: 261); Park et al. (2008a: 248); Cho and Park (2010: 95). Dineutus marinatus: Okamoto (1924: 167); Takizawa (1931: 15); Yoshino (1935: 16); Kusanagi (1936: 323).

Diagnosis. Length $9.0-10.0 \mathrm{~mm}$. Dorsal surface mostly dark grey; clypeus, labrum, interorbital area metallic green; lateral parts of pronotum, margin of elytra yellow. Maxillary palpi shorter than labial palpi, palpomere 4 as long as $1-3$ combined. Anterior margin of ligula slightly bisinuate; labial palpomere 3 as long as 1-2 combined. Gula with few setae on lateral margin. Postero-lateral and apical parts of elytron sharply pointed (arrows in Fig. 9). Antero-medial margin of prosternum (Fig. 10) strongly rounded. Prosternal process nearly parallel-sided (arrow in Fig. 10). Median lobe of aedeagus (Figs 41-43) shorter than paramere; apical part acute; sperm-groove as in Fig. 42. Paramere (Figs 41, 43) slightly curved at anterior fourth; apical margin rounded.

Distribution. Korea, China (Fujian, Guangdong, Guizhou, Guangxi, Hebei, Jiangsu, Liaoning), Japan, Russia (Far East) (Mazzoldi 2003).

Habitat. Most Korean specimens are found in ponds or mountain streams with plentiful vegetation and low water temperature. Some specimens were collected in ponds near brackish water.

## Genus Gyrinus Geoffroy, 1762

Figs 15-22
Gyrinus Geoffroy, 1762: 194. Type species: Dytiscus natator Linné, 1758.

Diagnosis. Head (Fig. 15) about 3.0 times wider than long, with micro-reticulation. Frons with two rounded depression (arrows in Fig. 15). Antenna with 9 antennomeres, antennomere 2 rugous with sparse punctures, 9 with compact short setae on apico-lateral part. Clypealium with long setae on antero-lateral part. Galea 1-articled. Pronotum with transverse depression on median parts (arrow in Fig. 16), lateral parts rugous, anterior margin slightly sinuate, posterior margin slightly rounded, anterior angle acute, posterior angle acute. Elytron (Fig. 16) with 11 punctato-striae and with-


Figures 7-14. Dineutus orientalis. $\mathbf{7}$ pronotum (dorsal aspect) $\mathbf{8}$ basal part of elytron (dorsal aspect) 9 apical part of elytron (dorsal aspect) $\mathbf{I 0}$ prosternum (ventral aspect) II mesoventrite (ventral aspect) $\mathbf{1 2}$ metaventrite (ventral aspect) $\mathbf{1 3}$ sternite II (ventral aspect) $\mathbf{1 4}$ sternite VIII (ventral aspect).
out compact setae, apical margin rounded; subelytral suture present (arrow in Fig. 17); epipleura reached on lateral part of sternite VII, anterior part of epipleura rounded. Prosternum transverse; anterior margin slightly rounded (Fig. 18). Prosternal process (Fig. 18) linear-form, gradually broad at posterior part. Metaventrite (Fig. 19) elongated, diamond-shaped, flattened; anterior part very acute; lateral parts curved upwardly. Tergite VIII (Fig. 20) with compact long setae and posterior margin rounded. Sternites III-VII (Fig. 21) with weak depression on lateral part. Sternite VII (Fig. 21) with long setae on lateral margin. Sternite VIII (Fig. 22) with long setae on postero-lateral parts and posterior margin rounded.

## Subgenus Gyrinus Geoffroy, 1762

Gyrinus Geoffroy, 1762: 194. Type species: Dytiscus natator Linné, 1758.

## Key to the species of Korean Gyrinus

1 Hypomera and epipleura dark brown; apical part of median lobe less than 3.0 times as narrow as basal part2

- Hypomera and epipleura yellowish brown to brown; apical part of median lobe (Figs 50-52) more than 3.0 times as narrow as basal part.... G. pullatus 2 Median part of mesoventrite (Fig. 23) with deep pit and large groove; median lobe (Figs 44-46) slightly shorter than paramere, apical margin rounded......
G. gestroi
- Median part of mesoventrite (Fig. 26) with shallow pit and vertical plica; median lobe (Figs 47-49) distinctly shorter than paramere, apical margin broadly rounded
G. japonicus

Gyrinus (s. str.) gestroi Régimbart, 1883
Figs 2, 18, 23-25, 44-46, 58
Gyrinus gestroi Régimbart, 1883: 165.

Specimens examined. SOUTH KOREA: Jeju Prov.: $1 \circlearrowleft^{\lambda}$, Bukjeju-gun, Jocheon-eub, Gyorae-ri, 10.V.1974; $1 \delta^{\lambda} 1$, same data as former except for, 27.VII.2005, DH Lee,
 slide); $1 \AA^{\lambda}$, same data as former except for, 28.VII.2005, SH Lee, ( 1 § , on slide); 2 $\circlearrowleft^{\lambda} \delta^{\lambda} 1 q$, same data as former except for, 22.V.2006; $2 \widehat{\delta} 2 q Q$, same data as former except for, 25.IX.2008; 1 §, same data as former except for, 15.VI. 2011 (1 § , on slide); 2 §§ 2 q $q$, Jeju-si, Ara-dong, Gwaneum-temple, 22.VII.1990, SH Lee; 1 , same data as former except for, Jeju National University, 11.VII.1985; 1 §, Seoguiposi, Pyoseon-eub, 10.VII.1985; 1 Q, Seoguipo-si, Seoho-dong, 23.VII.1990, SH Lee, pond; $1 \circlearrowleft^{\lambda}$, Seoguipo-si, Seongsan-eub, Ojo-ri, 24.VII.1990, SH Lee.


Figures I5-22. I5-I7, I9-22 Gyrinus japonicus 18 G. gestori $1 \mathbf{5}$ head (dorsal aspect) $\mathbf{1 6}$ pronotum (dorsal aspect) $\mathbf{I 7}$ apical part of elytron (dorsal aspect) $\mathbf{I} \mathbf{8}$ prosternum (ventral aspect) $\mathbf{1 9}$ metaventrite (ventral aspect) $\mathbf{2 0}$ tergite VIII (dorsal aspect) $\mathbf{2 I}$ sternites IV-VII (ventral aspect) $\mathbf{2 2}$ sternite VIII (ventral aspect).

Published Korean records. Gyrinus gestroi: Kwon and Suh (1986: 98); Lee et al. (1992: 54); Kim et al. (1994: 134); Lee (1995: 13); Park et al. (2008: 247); Cho and Park (2010: 95).

Diagnosis. Length $4.5-5.5 \mathrm{~mm}$. Ventral surface mostly black; ventral part of antennomere 2, mouthparts, prosternum, legs, posterior part of sternite VII, sternite VIII yellowish brown to brown; hypomera, epipleura dark brown. Ligula with a row of sparse spines on antero-medial part. Anterior margin of mesoventrite rounded, posterior margin bifid with very deep pit (arrow in Fig. 23), large groove present on postero-medial and antero-lateral parts. Metepisternum with a deep pit (arrow in Fig. 24), oval groove present on anterior part. Sternite II with deep pit (arrow in Fig. 25), transverse groove on anterior part. Median lobe of aedeagus (Figs 44-46) nearly parallel-sided at middle to apex, slightly shorter than paramere; apical margin rounded; sperm-groove as in Fig. 45. Paramere (Figs 44, 46) broader than median lobe; apical margin broadly rounded.

Distribution. Korea, Japan (Mazzoldi 2003).
Habitat. All specimens were collected in ponds with plentiful vegetation in Jejudo Island. They are frequently found together with $D$. orientalis or $G$. japonicus.

## Gyrinus (s. str.) japonicus Sharp, 1873

Figs 3, 15-17, 19-22, 26-28, 47-49, 58
Gyrinus japonicus Sharp, 1873: 55.
Gyrinus (Gyrinus) japonicus: Cheo (1934: 210).
Gyrinus japonicus francki Zaitzev, 1953: 355. Synonymized by Mazzoldi (2003: 27).

Type specimens examined. Syntypes: 1 đ 1 (NHM), with labels as follows: "Gyrinus japonicus Types D. S. Yokohama. Lewis, Type, Sharp Coll. 1905-313., Japan. G. Lewis.".

Additional material examined. NORTH KOREA: $6 \Uparrow$ む 9 q $q$, Pyongyangcity, Around Pyongyang-Hotel, Near Daedong-River, 15 vii 2008, Changdo Han; 16 ơ $^{\top} 13$ q + , Pyongyang-city, Mt. Daesong-San, 24.VI.2009, Changdo Han. SOUTH KOREA: Chungbuk Prov.: $1 \widehat{J}^{\lambda}$, Yeongdong-gun, Yongsan-myeon, Sin-hang-ri, 9.V.2009, SH Lee, pond; Chungnam Prov.: 1 §, Daejeon-si, Yusong-gu, Gyesan-dong, Sutonggol, 11.VII.1999, KJ Ahn, valley; 8 đ̄̃ 12 q ㅇ, Gongjusi, Banpo-myeon, Hakbong-ri, Mt. Gyeoryongsan, 25.VII.1992, SH Lee; 2 q $q$, Yesan-gun, Deoksan-myeon, Sacheon-ri, Surak-temple, 26.VII.1995, SH Lee; Gangwon Prov.: 7 đ̋ 6 O $q$, Samcheok-si, Geundeok-myeon, Hamaengbang-ri, Chodang-reservoir, 7.IX.1990; Gyeongbuk Prov.: 1 q, Daegu-si, Buk-gu, Baekandong, 4.VI.1985; 13 đ̋ 12 q $\uparrow$, Gumi-si, Haepyeong-myeon, Songgok-ri, Doritemple, 5.VIII.1994, SH Lee; 1 Q, Gumi-si, Okgye-dong, 18.VI.1990, SH Lee; 1 む, Gunwi-gun, Hyoryeong-myeon, Gogok-ri, 6.VI.2010, SH Lee, pond; 4 đ̋ 6 ㅇ $\uparrow$, Gyeongju-si, Geoncheon-eub, Sinpyeong-ri, 28.V.1993, SH Lee; 1 , , Gyeo-


Figures 23－3I．23－25 Gyrinus gestroi 26－28 G．japonicus 29－3I G．pullatus $\mathbf{2 3}$ mesoventrite（ventral aspect） $\mathbf{2 4}$ metepisternum（ventral aspect） $\mathbf{2 5}$ sternite II（ventral aspect） $\mathbf{2 6}$ mesoventrite（ventral aspect） $\mathbf{2 7}$ metepisternum（ventral aspect） $\mathbf{2 8}$ sternite II（ventral aspect） $\mathbf{2 9}$ mesoventrite（ventral aspect） $\mathbf{3 0}$ me－ tepisternum（ventral aspect） $\mathbf{3 I}$ sternite II（ventral aspect）．
ngju－si，Jinhyeon－dong，Bulguk－temple，23．VI．1991，SH Lee； 6 §§ 6 q $q$ ，Gyeo－ ngju－si，Naenam－myeon，Yongjang－ri，Hawgok－pond，25．IV．1994，SH Lee，pond； 1 O，Kimcheon－si，Buhang－myeon，Daeya－ri，29．VIII．2011，DH Lee，SW Jung， mountain stream； 36 § 38 q ㅇ，Pohang－si，Buk－gu，Jukjang－myeon，Duma－ri， 1．X．1989，SH Lee； 1 o 2 q $q$ ，Pohang－si，Gigye－myeon，Hwabong－ri，5．IV．1993， SH Lee，pond； $1 \AA^{\Uparrow} 1$ ，Sangju－si，Jungdong－myeon，Osang－ri，18．VI．1990，SH Lee， pond； $1 \circlearrowleft^{\top} 3$ q $q$ ，Uljin－gun，Seo－myeon，Wangpi－ri，23．IV．1994，SH Lee，stream； $1{ }^{\top}$ ，Ulsan－si，Ulju－gun，Samnam－myeon，Gacheon－ri 30．VI．2003，YB Cho，MJ Jeon，DH Lee，mountain stream（1 ぶ，on slide）； 8 ふた 8 q $q$ ，Yeongdeok－gun， Yeonghae－myeon，Myogok－ri，6．VI．1994，SH Lee，pond；Gyeonggi Prov．： 1 § 1 ， Anseong－si，10．IX．1977，DW Oh； 1 Q，Pocheon－si，Byeolnae－myeon，Yongam－ri， Mt．Yongamsan［＝Sori－bong］，16．VII．1992，SH Lee； 1 Q，Seoul－si，Gangbuk－ gu，Ui－dong，Sogui－stream，23．VI．2007，JG Lee，valley； 1 ठ̃，Yongin－si，Suji－gu， Sinbong－dong，19．VII．1988，JH Lee；Gyeongnam Prov．： 1 ô，Busan－si，Seo－ gu，Ulmang－dong，26．IV．2009，SH Lee； 1 §̃，Goseong－gun，Gaecheon－myeon，

Bukpyeong－ri，13．VIII．1995，SH Lee； 2 q $q$ ，Euiryeong－gun，Yongdeok－myeon， Imok－ri，Deokam－pond，18．V．2009，SH Lee，pond； 2 ô 5 q $q$ ，Geoje－si，Geo－ je－myeon，Seosang－ri，Geoje－reservoir，28．VII．2009，SH Lee，reservoir； 2 ふ̋， Habcheon－gun，Samga－myeon，Eojeon－ri，8．V．2009，SH Lee，pond； 1 q，Hamy－ ang－gun，Aneui－myeon，Sangwon－ri，Yongchu－temple，16．VII．1985； 5 đふ 2 q $q$ ， Sacheon－si，Gonmyeong－myeon，Yongsan－ri，Dasol－temple，14．VIII．1995，SH Lee； 1 ő 2 q $q$ ，Sacheon－si，Sanan－myeon，13．VIII．1995，SH Lee； 1 §̃，Sancheong－ gun，Sancheong－eub，Jeonggok－ri，Jipum－church，8．V．2009，SH Lee；Jeju Prov．： 1 ठ，Bukjeju－gun，Jocheon－eub，Gyorae－ri，18．VIII．1992； 4 ő 3 우，Jeju－si， Ara－dong，Gwaneum－temple，22．VII．1990，SH Lee（1 §，on slide）； 1 §＇，Jeju－si， Hangyeong－myeon，Yongsu－ri，17．VII．1992，SH Lee；Jeonbuk Prov．： 20 す̃ 27 ¢ $\uparrow$ ，Jeongeub－si，Naejang－dong，Mt．Naejangsan，4．VIII．1990，SH Lee； 2 ふ̋ 2 우，Namwon－si，Sandong－myeon，Daesang－ri，Guijeong－temple，28．VII．2008， DH Lee，pond； 6 ふ̋ 6 q $q$ ，Namwon－si，Sannnae－myeon，Ibseok－ri，Silsang－tem－ ple，12．VI．2008，DH Lee，pond（ $1 \sigma^{\lambda}$ ，on slide）； 1 Q，Namwon－si，Unbong－eub， Maeyo－ri，9．V．2009，SH Lee；Jeonnam Prov．： 2 §̃ 1 ，Haenam－gun，Songji－ myeon，Geumgang－ri，27．VII．2010，SH Lee，pond； 1 ठ，Gurye－gun，Gurye－eub， Sinseong－ri，Si－dong，18．VI．2003，CH Park； 1 §̉，Janseong－gun，Bukha－myeon， Sinseong－ri，Mt．Naejangsan，2．IV．2010，JC Jeong（ $\delta^{\lambda}$ ，on slide）．

Published Korean records．Gyrinus japonicus：Kolbe（1886：179）；Takizawa （1931：18）；Kamiya（1936：20）；Mochizuki and Tsunekawa（1937：78）Mochizuki and Matsui（1939：55）；Ishii（1940：43）；Cho（1957：201）；Cho（1969：191）；Kim and Nam（1982：25）；Lee et al．（1985：402）；Yoon（1988：616）；Kim and Lee（1991：65）； Lee et al．（1992；52）；Lee（1994：15）；Lee（1995：13）；Mazzoldi（1995：158）；Nilsson et al．（2001：29）；Hua（2002：41）；Mazzoldi（2003：27）．Gyrinus japonicus franki（syno－ nym）：Kwon and Suh（1986：98）；Kim et al．（1994：134）；Kim（1995：132）；Han et al． （2007：271）；Han et al．（2008：259）；Cho and Park（2010：95）．

Diagnosis．Length $6.5-8.5 \mathrm{~mm}$ ．Ventral surface mostly black；mouthparts，pros－ ternum，legs，sternite VIII brown to reddish brown；hypomera，epipleura dark brown． Ligula with a row of compact spines on antero－medial part．Anterior margin of mes－ oventrite acute and posterior margin bifid；shallow pit（arrow in Fig．26）and vertical plica present on postero－medial part；shallow plica present on antero－lateral parts．Me－ tepisternum with a pit（arrow in Fig．27）and shallow groove on anterior part．Sternite II with small pit and transverse plica on anterior part（arrow in Fig．28）．Median lobe of aedeagus（Figs 47－49）parallel－sided at middle to apex，shorter than paramere，nar－ rowest at middle；apical margin nearly straight；sperm－groove as in Fig．48．Paramere as in Figs 47 and 49.

Distribution．Korea，China（Northeast Territory），Japan，Russia（Far East） （Mazzoldi 2003）．

Habitat．Specimens were collected in ponds with plentiful vegetation and low water temperature．In summer，we often found that a large number of individuals gathered whirling on surface of water．

## Gyrinus（s．str．）pullatus Zaitzev， 1908

Figs 4，29－31，44， 46
Gyrinus pullatus Zaitzev，1908： 244.

Specimens examined．SOUTH KOREA：Gyeongbuk Prov．： 1 §，Gyeongju－si，Jin－ hyeon－dong，Bulguk－temple，23．VI．1991，SH Lee； 1 §，Gyeongju－si，Naenam－myeon， Hwagok－ri，25．IV．1993，SH Lee，pond； $1 \Uparrow 1$ ， ，Pohang－si，Nam－gu，Yeongil－eub， 22．VI．1992，SH Lee； 1 Q，Uljin－gun，Wonnam－myeon，Maehwa－ri，Maehwa－stream， 10．VI．1995，SH Lee； 1 Q，Yeongdeok－gun，Changsu－myeon，Changsu－ri，5．VI．1994， SH Lee，pond； 2 ふた $\begin{gathered}\text { ，Yeongdeok－gun，Yeonghae－myeon，17．VI．1985，SH Lee；} 15\end{gathered}$ ふす 15 q Y ，Yeongdeok－gun，Yeonghae－myeon，Myogok－ri，6．VI．1994，SH Lee，pond （ $1{ }^{\text {た }}$ ，on slide）．

Published Korean records．Gyrinus pullatus：Holmen（1987：49）；Mazzoldi （1995：159）；Nilsson et al．（2001：31）；Hua（2002：41）；Mazzoldi（2003：28）；Gyri－ nus curtus（misidentification）：Mochizuki and Tsunekawa（1937：78）；Mochizuki and Matsui（1939：56）；Kim and Nam（1982：25）；Kwon and Suh（1986：98）；Kim et al． （1994：134）；Lee（1994：15）；Cho and Park（2010：95）．

Diagnosis．Length $6.0-7.0 \mathrm{~mm}$ ．Ventral surface mostly reddish brown；ven－ tral part of antennomere 2，mouthparts，prosternum，legs，posterior part of sternite VII，sternite VIII yellowish brown to brown；hypomera，epipleura yellowish brown． Ligula with a row of sparse spines on antero－medial part．Anterior margin of mes－ oventrite rounded and posterior margin bifid；deep pit（arrow in Fig．29）and verti－ cal groove present on postero－medial part；deep groove present on antero－lateral parts．Metepisternum with a deep pit（arrow in Fig．30）on anterior part．Sternite II with pit，transverse and thick plica on anterior part（arrow in Fig．31）．Median lobe of aedeagus（Figs 50－52）narrowed apically，shorter than paramere；narrowest at anterior fifth；apical margin nearly straight；sperm－groove as in Fig．51．Paramere as in Figs 50 and 52.

Distribution．Asia：Korea，China（Liaoning，Jilin），Russia（East Siberia，Far East）， Europe：Estonia，Finland，Russia（North European Territory），Sweden（Mazzoldi 2003）．

Remarks．Gyrinus curtus was first recorded in Korea by Mochizuki and Tsunekawa （1937）．After that，many entomologists［Mochizuki and Matsui（1939：56）；Kim and Nam（1982：25）；Kwon and Suh（1986：98）；Kim et al．（1994：134）；Lee（1994： 15）；Cho and Park（2010：95）］reported this species in Korea，only in the local fauna without any taxonomic comments．After examining specimens（ $2 \sigma^{\top} \sigma^{\top}$ ，Yeongdeok－ gun，Yeonghae－myeon，17．VI．1985，SH Lee）previously studied by Kwon and Suh （1986），Kim et al．（1994），Lee（1994），and Cho and Park（2010），we found that they had been incorrectly identified and actually represent $G$ ．pullatus．This species can be distinguished from G．curtus by the hypomera being yellowish brown to brown and the apical margin of the median lobe being nearly straight．

## Genus Orectochilus Dejean, 1833

Figs 32-40
Orectochilus Dejean, 1833: 59. Type species: Gyrinus villosus Müller, 1776.
Diagnosis. Body long oval, with micro-reticulation, compact setae present on most dorsal part. Labrum semicircular, slightly wider than long, long setae present on anterior margin. Antenna with 9 antennomeres. Pronotum without transverse depression. Scutellum (Fig. 32) transverse and visible when elytra closed. Elytron (Figs 33, 34) with compact setae. Prosternum (Fig. 35) with few setae on anterior part. Prosternal process (arrow in Fig. 35) sagittiform, widest anterior three forth (Fig. 35). Mesoventrite (Fig. 36) with setae on anterior margin, vertical plica on postero-medial part; anterior part acute. Metaventrite cruciform, vertical process on median part (arrow in Fig. 37). Metepisternum (Fig. 36) without pit and groove. Metatibia with two spines of equal length. Sternite II (Fig. 38) without pit, transverse groove present on anterior part. Sternites IV-VI (Fig. 39) with short setae on median parts. Sternites VII-VIII (Figs 39, 40) with compact long setae on medial parts. Sternite VII (Fig. 39) with long setae on posterior margin. Sternite VIII (Fig. 40) longer than wide, long setae present on medial and lateral parts; apex bifid.

## Key to the species of Korean Orectochilus

$1 \quad$ Body less than 7.0 mm ; apical part of elytron not protruded; median lobe of aedeagus shorter than paramere2

- $\quad$ Body more than 7.0 mm ; apical part of elytron protruded; median lobe of aedeagus longer than paramere O. regimbarti

2 Body black; apical part of elytra acute in dorsal view; paramere curved at middle, apical margin of gonocoxa (Fig. 56) rounded ............. O. punctipennis

- Body brown; apical part of elytra broadly round in dorsal view; paramere nearly straight, apical margin of gonocoxa (Fig. 57) broadly rounded. O. villosus


## Orectochilus punctipennis Sharp, 1884

Figs 5, 56, 59
Orectochilus punctipennis Sharp, 1884: 449.
Type material examined. Syntype: 1 (NHM), with labels as follows: "Orectochilus punctipennis. Types D. S. Yokio. Japan. Lewis, Type, Japan. G. Lewis., Sharp Coll. 1905-313."

Additional material examined. SOUTH KOREA: Gangwon Prov.: 1 , Gang-neung-si, Okgyeo-myeon, Jusu-ri, 17.VIII.2011, SW Jung.


Figures 32-40. Orectochilus villosus. $\mathbf{3 2}$ scutellum (dorsal aspect) $\mathbf{3 3}$ basal part of elytron (dorsal aspect) 34 apical part of elytron (dorsal aspect) $\mathbf{3 5}$ prosternum (ventral aspect) $\mathbf{3 6}$ mesoventrite (ventral aspect) $\mathbf{3 7}$ metaventrite (ventral aspect) $\mathbf{3 8}$ sternite II (ventral aspect) $\mathbf{3 9}$ sternites III-VII (ventral aspect) $\mathbf{4 0}$ sternite VIII (ventral aspect).

Diagnosis. Length 6.5 mm . Dorsal and ventral surface mostly black; antenna, mouthparts, hypomera, epipleura, front leg brown; middle and hind legs, sternites V-VII yellowish brown. Head without pubescence on vertex and postero-lateral part. Pronotum widest at posterior margin; anterior angle rectangular; posterior angle rounded. Elytra (Fig. 5) widessst at middle; posterior margin acute in dorsal view. Protarsal claw as long as protarsomere 1. Posterior part of mesoventrite bifid. Median lobe of aedeagus shorter than paramere. Apical margin of gonocoxa (Fig. 56) rounded.

Distribution. Korea, Japan, Russia (Far East) (Mazzoldi 2003).
Remarks. Orectochilus punctipennis is recorded for first time in Korea. This species can be distinguished from $O$. regimbarti by the small size (less than 7.0 mm ) and median lobe of aedeagus shorter than paramere. It also differs from $O$. villosus by the black body, posterior margin of the elytron acute, paramere curved at middle and apical margin of gonocoxa (Fig. 56) rounded.

Habitat. A single female specimen was collected near the margin of a stream with plentiful vegetation and slow flow velocity.


Figures 4I-55. Aedeagus 4I-43 Dineutus orientalis 44-46 Gyrinus gestroi 47-49 G. japonicus 50-52 G. pullatus 53-55 Orectochilus villosus 41 dorsal aspect $\mathbf{4 2}$ median lobe (dorsal aspect) $\mathbf{4 3}$ ventral aspect $\mathbf{4 4}$ dorsal aspect $\mathbf{4 5}$ median lobe (dorsal aspect) $\mathbf{4 6}$ ventral aspect $\mathbf{4 7}$ dorsal aspect $\mathbf{4 8}$ median lobe (dorsal aspect) $\mathbf{4 9}$ ventral aspect $\mathbf{5 0}$ dorsal aspect $\mathbf{5 I}$ median lobe (dorsal aspect) $\mathbf{5 2}$ ventral aspect $\mathbf{5 3}$ dorsal aspect $\mathbf{5 4}$ median lobe (dorsal aspect) $\mathbf{5 5}$ ventral aspect.


Figures 56-57. Gonocoxa. 56 Orectochilus punctipennis (dorsal aspect) 57 O. villosus (dorsal aspect). Scales $=0.1 \mathrm{~mm}$.

## Orectochilus regimbarti Sharp, 1884

Orectochilus regimbarti Sharp, 1884: 448.
Published Korean records. Orectochilus regimbarti: Kwon and Suh (1986: 99); Kim et al. (1994: 134); Cho and Park (2010: 95).

Distribution. Korea, Japan, Russia (Far East) (Mazzoldi 2003).
Remarks. This species has been recorded in Korea by Kwon and Suh (1986), Kim et al. (1994) and Cho and Park (2010), only in their checklists without any taxonomic comments and specimens. We could not find any Korean specimens and the occurrence of this species in Korea is suspicious. However, its occurrence in Korea is possible because it is known from Japan and Russia (Far East).


Figures 58-59. Distribution maps. 58 Gyrinus japonicus (circle), G. gestroi (triangle), G. pullatus (square) 59 Dineutus orientalis (circle), Orectochilus punctipennis (triangle), O. villosus (square).

## Orectochilus villosus (Müller, 1776)

Figs 6, 32-40, 53-55, 57, 59
Gyrinus villosus Müller, 1776: 68.
Gyrinus modeeri Marsham, 1802: 100. Synonymized by Illiger (1807: 299).
Orectochilus villosus: Dejean 1883: 59.
Specimens examined. SOUTH KOREA: Gyeonggi Prov.: 2 đ ${ }^{\top} 3 q$, Namyangjusi, Wabu-eub, Paldang-ri, Paldang-lake, 10.VI.1962, JS Lee (1 ठ 1 q, on slide).

Published Korean records. Orectochilus villosus: Yoon (1988: 615); Kim et al. (1994: 134); Cho and Park (2010: 96).

Diagnosis. Length $5.5-6.5 \mathrm{~mm}$. Dorsal surface dark brown; ventral surface mostly brown; ventral part of antennomere 2, mouthparts, hypomera, epipleura; legs yellowish brown. Head without pubescence on postero-lateral margins. Posterior angle of pronotum rectangular. Elytra (Fig. 34) widest at middle, posterior margin broadly rounded. Posterior part of mesoventrite (Fig. 36) slightly acute. Median lobe of aedeagus (Figs 53-55) slender, shorter than paramere; apical part acute; sperm-groove as in Fig. 54. Paramere (Figs 53,55) nearly straight, long setae present on lateral and apical parts; apical part rounded. Apical margin of gonocoxa (Fig. 57) broadly rounded.

Distribution. Europe, Asia; Korea, China (Liaoning), Cyprus, Iran, Iraq, Israel, Japan, Kazakhstan, Russia (East Siberia, Far East, West Siberia), Syria, Turkey, Uzbekistan (Mazzoldi 2003).

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# Records of Coendou ichillus (Rodentia, Erethizontidae) from the Lower Urubamba Region of Peru 

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#### Abstract

Coendou ichillus was first described in 2001 by Voss and da Silva, with a range from Amazonian Ecuador to Iquitos, Peru. Here, we describe an adult female Coendou ichillus specimen collected in a Tomahawk trap in the forest canopy of the Lower Urubamba Region of Peru in October 2013. We also describe pathologies and behaviors observed through 379 camera trapping photo events ( 2,196 photos) gathered in natural canopy bridges over the course of a year ( 7,198 trap nights), including information on activity period over the course of the day and over the course of the lunar cycle. We conservatively estimate that 17 individuals were photographed, including one juvenile. Being 900 km away from Iquitos, Peru (the site of the closest record), discovery of this species in the Lower Urubamba constitutes a significant range extension.


## Keywords

Neotropical porcupines, Coendou ichillus, Peru, biodiversity, distribution, Urubamba, Rodentia, camera trap

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## Introduction

Our understanding of the diversity and distributions of Neotropical porcupines of the vestitus-group has been very much thwarted by the lack of specimens and locality records. Voss and da Silva (2001) discussed two hypotheses for why these diminutive species might be so rarely detected: 1) they might have very restricted distributions in western Amazonia, and 2) their apparent rarity might be an artifact of inadequate collection. In a recent methods paper, Gregory et al. (2014) demonstrated how specialized camera trapping methods can result in the documentation of a greater abundance of vestitus-group porcupines than might have been detected using conventional survey methods (sensu Voss and Emmons 1996) alone. Testing either of these hypotheses would begin with efforts to first map the known distribution of the species through the steady accumulation of point locality data. In this paper we report one such new locality record-and a significant range extension-for the recently described species Coendou ichillus, which is here reported from the Lower Urubamba Region (LUR) of Peru.

Coendou ichillus was previously known from six sight records and only five speci-mens-three collected from the Amazonian lowlands of eastern Ecuador, one from Iquitos, Peru, and the fifth of unverified natural origins that was purchased in a market in Iquitos (Voss 2011; Voss and da Silva 2001; Voss et al. 2013). The skulls of the specimens analyzed by Voss and da Silva (2001) exhibit damage that presumably occurred during the process of collecting and preparing these specimens, and none include postcranial elements beyond those left in the hands and feet of their respective study skins. Morphometric information was not provided for the specimen from Iquitos mentioned by Voss (2011) and Voss et al. (2013) (TTU 115491). The specimen that forms the basis of our new record consists of a study skin, a skeleton, and an intact skull. To maximize the utility of the specimen, we cleaned the skeletal elements from one side of the study skin, while leaving the other side intact as a traditional study skin. We provide measurements and descriptions of these and compare them to the previously known specimens reported in Voss and da Silva (2001).

In 2011, Gregory and colleagues began a study in the Lower Urubamba Region (LUR) of Peru assessing the effectiveness of natural canopy bridges (connections between the branches of large trees) over a $\sim 15$-meter-wide natural gas pipeline clearing in mitigating the ensuing canopy fragmentation. The study began before the natural bridges were exposed and continued through pipeline right-of-way (RoW) construction, revegetation, and eventual closure of the RoW to motorized vehicles. In September 2012, soon after the 13 natural bridges were exposed in July, camera traps were placed in the canopy at all potential crossing points. The 24 cameras were left for a year, and the photos revealed extensive small and medium-size mammal activity in the canopy, including thousands of photos of a dwarf porcupine species. With no dwarf porcupine species known to the area, the research team opted to attempt trapping a specimen for identification as the study concluded. One female specimen was collected.

In this paper we report the range extension for this species, describe its morphology, comment on its behavior as revealed in the camera trap photos, and discuss the significance of the range extension. This is the second in a series of four papers that address the methods used and results from the three-year-long study (Gregory et al. 2014).

## Methods

The study site is in the Lower Urubamba Region, adjacent to the confluence of the Camisea and Urubamba Rivers ( $11^{\circ} 43.28^{\prime} \mathrm{S}, 72^{\circ} 56.52^{\prime} \mathrm{W}(\mathrm{DDM})$, Figure 1). The study area is in predominantly primary forest between $450-500 \mathrm{~m}$ with three major habitat types: terra firme, riverine terrace, and mixed upland (Comiskey et al. 2001). It is near the Pluspetrol-controlled Pagoreni A natural gas well and the pipeline that connects the well to the Malvinas processing plant. The site is also near the Matsigenka communities of Camisea and Shivankoreni and is not part of the national system of protected areas, for which reason subsistence hunting of wildlife is legal.

This study is part of a larger study aimed at understanding the utility of natural canopy bridges in reducing the forest fragmentation impact caused by a natural gas pipeline right-of-way (RoW) clearing. In order to evaluate natural bridge use by arboreal mammals, the 13 bridges were monitored with 24 Reconyx PC800 Hyperfire ${ }^{\mathrm{TM}}$ Professional (Reconyx Inc., Holmen, WI, USA) camera traps placed at all potential crossing points over the course of a year. An additional 28 camera traps were placed on the ground below the bridges and 26 more in a control area along the RoW with no canopy bridges. The branches comprising the bridges were left in July 2012, and monitoring occurred from September 2012, the middle of the pipeline construction period, to September/October 2013, approximately six months post construction. All behavioral data presented here were gathered from camera trap photos. Camera trap photographs were processed by a team of three people who separated the photos into trigger events and identified the vertebrates in each event. The information recorded for each event included the following: date and time, bridge, species present, number of individuals, behaviors exhibited, and moon phase.

To evaluate activity hours of Coendou ichillus, data from all cameras over the course of the year were pooled by hour of the day. For the moon phase analysis, data from all cameras were pooled by moon phase for each lunar cycle over the course of 13 cycles. Waning and waxing moon phases with similar amounts of moonlight were pooled for the analysis for a total of 5 moon phase categories: $0=$ new moon, $1=$ waxing and waning crescent, 2 = first and last quarters, 3 = waxing and waning gibbous, and 4 = full moon. The number of events per hour of the day and per moon phase category per lunar cycle were divided by the corresponding number of camera trap nights and multiplied by 100 , for analysis of event rates. A Spearman's correlation was performed on the moon phase data for the 13 lunar cycles through which the study occurred to explore the relationship between activity and phase.


Figure I. Map of trapping locations and canopy bridge distribution. Yellow circles indicate canopy bridges in which Coendou ichillus was photographed with a camera trap. The blue star indicates the bridge in which the specimen was trapped.

Regarding other local fauna besides Coendou ichillus, the camera trap photos have recorded 20 other mammal species in the canopy, with an additional two species of monkeys registered through visual surveys, and cameras on the ground recorded 22 mammal species ( 5 species overlapped between the canopy and the ground) (Gregory et al. 2012; Gregory et al. 2014).

Porcupine specimen collection took place in October 2013. Six Tomahawk $(20 \times 60 \times 20 \mathrm{~cm})$ traps were placed in the mid-canopy over 12 days (trapping methods will be described in detail in Gregory, in prep.). Each Tomahawk trap was monitored with a camera trap.


Figure 2. Adult female Coendou ichillus (CORBIDI-MA-00973) specimen in life.

The specimen described here was captured on the first day of trapping at $2: 26 \mathrm{AM}$ (determined by camera trap photo) in a trap at 17.5 m in height (Figure 2). The trap had been baited with a section of wood soaked in salt water and a mixture of canned tuna, oatmeal, tomato paste, and fresh tomato. There was no sign of any remaining bait when the individual was captured. The camera trap documented the individual consuming the bait.

The morning after the specimen was trapped, it was immediately transported in the trap back to the research camp. The entire trap was placed in a large plastic bag with isoflurane-soaked cotton in one corner, out of contact with the animal. After the animal was subdued, it was injected with sodium pentobarbital ( 6 mL injected into the chest and 2 mL injected into the cranium). The specimen was identified as a female with developed, turgid nipples and a closed vagina. The skin was then prepared and the body was placed in alcohol. Tissue samples, quills, fleas, feces, and stomach contents were also collected. The specimen was deposited at the Centro de Biología y Biodiversidad (CORBIDI, MA-00973) in Lima, Peru, and then borrowed and transported to the United States. Maceration and cleaning of the carcass and cranium and morphometric analyses were performed at the National Museum of Natural History (NMNH). A portion of each tissue sample was deposited at NMNH (USNM 599500).

All reported measurements are in millimeters (mm). External measurements of the specimen that constitutes our new record are those that were recorded in the field by the collector. Cranio-dental measurements are those defined by Voss and da Silva (2001); long-bone measurements are greatest total length. All osteological measurements were recorded to the nearest 0.1 mm using digital calipers at NMNH.

## Results and discussion

Coendou ichillus was recorded by the camera traps in nine of the 13 natural canopy bridges. Behavioral inferences drawn from the photos (i.e. animals searching for the best branch by which to cross) and data recorded on the characteristics of the bridges suggest that the only bridges not used were those without direct contact between the branches that composed the bridge. Lack of saltatory ability is therefore likely to have prevented use of these bridges.

There were 379 total camera trapping events ( 2,196 photos) of Coendou ichillus over 7,198 trap nights for a rate of 5.3 events/ 100 trap nights. To identify the number of individuals captured during these events, we carefully analyzed the photos, using identifying characteristics to distinguish between individuals. Our count of the total number of individuals was conservative, accounting for the possibility that close proximity between bridges and favorable topography would allow individuals to be photographed in more than one bridge. In total, we counted 17 individuals, including one juvenile. While adult males and females were not distinguishable, during 11 of the trapping events, two individuals were recorded together. During five of these events (all in the same bridge), one individual was significantly smaller than the other, suggesting it was a mother and juvenile. During the other six events (in two bridges), both animals seemed to be adults and may have been a male and female (Figures 3b and 7c).

Pathologies recorded in the photographs include botfly wounds or lumps on the right and left rear paws and a broken tail. In addition, two individuals showed an enlarged abdomen and may have been pregnant ( 1 photo event in May, 3 events in July, and 6 events in August). Behaviors recorded include body shaking ( $\mathrm{N}=4$ events, Figure 3a), auto-grooming ( $\mathrm{N}=3$ ), pilo-erection ( $\mathrm{N}=42$ ), biting the tree trunk $(\mathrm{N}=2)$, and sniffing the tree trunk $(\mathrm{N}=8)$, and social behaviors recorded include one individual following the other ( $\mathrm{N}=11$ ) and body contact between individuals ( $\mathrm{N}=2$, Figure 3 b ). Four of the camera traps were heavily gnawed on by Coendou ichillus (evidenced by photos); the latches were also opened causing the cameras to flood with rain water.


Figure 3. Behaviors demonstrated by Coendou ichillus: body shaking (a) and body contact between two individuals (b).


Figure 4. Daily activity pattern of Coendou ichillus evaluated through photo events per 100 trap nights over the course of one year.


Figure 5. Activity pattern in relation to moon phase evaluated through photo events per 100 trap nights during each moon phase over the course of 13 lunar cycles. Moon phase categories are labeled in order of increasing moonlight ( $0=$ new moon and $4=$ full moon $)$. Moon phase categories $1-3$ represent data pooled for waning and waxing periods with similar moonlight (e.g. $1=$ waxing and waning crescent events pooled). Bars represent the standard error of the mean.

Events of Coendou ichillus occurred between 18:00 and 5:00 hours, with an activity peak at 20:00 (Figure 4). With regards to moon phase, there was a significant relationship between activity and phase, and there was more activity during the lower light phases (Figure 5, $\varrho(4)=-0.33, \mathrm{p}=0.007$ ).

Four interspecific interactions were recorded in three bridges, and they were all with Aotus nigriceps. In one of the bridges ( $\mathrm{N}=2$ events), an adult $A$. nigriceps chased


Figure 6. Adult Aotus nigriceps chasing adult Coendou ichillus.
an adult Coendou ichillus (Figure 6). Coendou bicolor individuals (2 total) were also photographed in two of the same bridges used by C. ichillus during 20 events ( 1 event in one bridge and 19 in the other). For five of these events, the two species used the same bridge on the same night. Their extreme difference in body size makes them easily distinguishable (Figure 7).

Our specimen from the LUR (CORBIDI-MA-00973) fits the description of the holotype of Coendou ichillus (Figure 8), as described in Voss and da Silva (2001), and differs only in some details of the pelage as follows: 1) the dorsal view of the skin of the holotype, as photographed in Figure 9 of Voss and Da Silva (2001), shows a distinct, apparently spineless patch over the lower back and on the dorsal surface of the base of the tail, whereas our specimen from Peru is well-quilled over much of this area, there being only a small patch of quill-less skin over the base of the tail that we believe was the result of de-quilling during the skinning of the specimen; 2) Voss and da Silva (2001) describe the dorsal part of the tail of C. ichillus as having tricolored bristles extending along the lateral caudal surfaces of the tail converging to form an indistinct whitish or yellowish "chevron" near the middle of the tail, but our specimen from the LUR did not exhibit this trait and had a rather sparse covering of tricolored bristles along the sides of the base of the tail; 3) the


Figure 7. Coendou ichillus ( $\mathbf{a}$ and $\mathbf{c}$ ) and C. bicolor ( $\mathbf{b}$ and $\mathbf{d}$ ) in the same two locations. The species are primarily distinguishable by size differences.
small tufts of bristles on the outer canthus of the pinnae appear to be set higher on the pinnae compared to those in the animal photographed in figure 12 of Voss and da Silva (2001) such that the ear bristle tufts on our specimen are in a line directly anterior to the eye, whereas in the live animal photographed, these bristles are slightly below an imaginary line drawn posteriorly through the midline of the eye (Figures 2 and 8 ).

In addition to providing measurements of long bones in Table 1, some additional comments on the overall condition of the bony elements are warranted. The animal appears to have suffered injuries in life that, although healed, are readily apparent on the specimen. There is a healed fracture on the left zygoma, which is slightly indented compared to the normal right side. There are also apparently related distortions to the rostrum, which is somewhat asymmetrical in dorsal view and twisted to the animals' right side. Finally, the animal appeared to have lost, during its lifetime, the part of the last phalange bearing the claw on the third digit of its left foot. This toe was observed in the field as being clawless but healed over at the time of capture.

The vertebral column of our specimen (CORBIDI-MA-00973) presents seven cervical vertebrae, of which C 2-4 were fused. There were 15 thoracic vertebrae with 15


Figure 8. Images of skull (a dorsal b ventral, and $\mathbf{c}$ lateral views), mandible (d), skin (e dorsal and $\mathbf{f}$ ventral views), and skeleton (g) of our Coendou ichillus specimen (CORBIDI-MA-00973).
pairs of ribs, six lumbar vertebrae as defined by a conspicuous transverse process, three fused sacral vertebrae, and 29 caudal vertebrae. Greatest lengths of long bones from the left side of the animal are provided in Table 1.

Table I. Comparisons of morphometrics of the present specimen of Coendou ichillus (CORBIDI-MA-00973) in millimeters with those described by Voss and da Silva (2001, AMNH, EPN, FMNH).

|  | AMNH | EPN | FMNH | CORBIDI-MA-00973 |
| :---: | :---: | :---: | :---: | :---: |
| Sex | unk | female | male | female |
| HBL | ca. 290 | - | ca. 260 | 307 |
| LT | ca. 250 | - | ca. 210 | 251 |
| HF | - | 59 | 58 | 56.7 |
| LA | - | - | - | 22.9 |
| LA2 | - | - | - | 17.6 |
| CIL | 58.8 | 64.2 | 64.4 | 64.4 |
| LD | 14 | 15.8 | 16.9 | 17.2 |
| LIF | 3.7 | - | 3.7 | 3.7 |
| BIF | 2.7 | - | 3.1 | 3.5 |
| MTR | 14.1 | 15.2 | 14 | 14 |
| LM | 10.3 | 11.4 | 10.5 | 10.4 |
| BP4 | 4.1 | 4.5 | 3.9 | 4.1 |
| BM1 | 3.8 | 4.2 | 3.8 | 3.6 |
| APB | 4.4 | 4.5 | 4.1 | 4.7 |
| PPB | 5.9 | 6.2 | 6.5 | 6.5 |
| PZB | 39.2 | 39.1 | 39 | 38.5 |
| HIF | 8.6 | 8.3 | 9.8 | 8.2 |
| ZL | 24 | 25.7 | 26.2 | 24 |
| LN | - | 18.8 | - | 19.6 |
| BNA | - | 9.9 | - | 9.4 |
| BB | 29.3 | 31.3 | 28.9 | 29.5 |
| DI | 2.9 | 2.9 | - | 2.9 |
| BIT | 4.7 | 4 | - | 4.5 |
| Weight | - | - | - | 770 g |

Our specimen represents the sixth known Coendou ichillus voucher, and it substantially extends the known range of the species. All previous unambiguous records of the species were from the Amazonian lowlands of eastern Ecuador and Iquitos, Peru (Figure 9), our new record extends the range of the species approximately 900 km further south into the Peruvian Amazon. This unambiguous record of C. ichillus from the Lower Urubamba Region of Peru confirms that this species has a much wider distribution outside of eastern Ecuador and northeastern Peru.

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Figure 9. Map of Coendou ichillus collection sites and observation sites, including that of the present specimen (blue star) and those of the specimens analyzed by Voss and da Silva (2001), Voss (2011) and Voss et al. (2013) (black circles). Coordinates for all sites, except La Selva (live animal observations) and CORBIDI-MA-00973 are estimated.

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# The genus Omphreus in Bosnia and Herzegovina and Montenegro, with two new subspecies of O . morio (Coleoptera, Carabidae, Omphreini) 

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#### Abstract

Two new ground beetle subspecies, Omphreus (Omphreus) morio sandeli ssp. n. (from Mts. Zelengora and Maglić, eastern Bosnia and Herzegovina) and $O$. (O.) morio durmitorensis ssp. n. (from Mt. Durmitor, northwestern Montenegro) are here described and diagnosed. The male and female genitalia and other taxonomically important characters are illustrated. The new taxa are distinctly different from the nearest relatives and represent both endemics and relicts inhabiting limited high-altitude Dinaric areas in Bosnia and Herzegovina and Montenegro. A key to Omphreus taxa from Montenegro and a key to subspecies of Omphreus (Omphreus) morio Dejean, 1828 are presented.


## Keywords

Ground beetles, soil-dwelling fauna, Balkan Peninsula

## Introduction

The genus Omphreus Dejean, 1828 is divided into three subgenera: Omphreus s. str., Neomphreus Winkler, 1933 and Paromphreus Ganglbauer, 1887. The genus is the only one constituting the tribe Omphreini (Bousquet 2003, Lorenz 2005). No phylogenetic analysis exists which makes possible to assess the true systematic position of Omphreus within Har-
palinae. It currently contains 18 species and 13 subspecies inhabiting the Balkan Peninsula and Asia Minor (Trautner and Geigenmüller 1987, Bousquet 2003, Guéorguiev 2007, Ćurčić et al. 2008a, 2008b). All Omphreus taxa are distributed very locally (endemics), mostly representing montane to alpine forms living in forests (under stones and tree bark), but can be found beyond the timberline under stones as well. These ground beetles are large in size (R 16-28 mm), black colored and recognizable by the habitus and the long shaftlike antennomere 1. It is possible that the present scarcity of specimens of this genus in the collections does not mean that they are really rare, but reflects our still insufficient knowledge regarding their life history (Trautner and Geigenmüller 1987, Arndt et al. 2011).

The following Omphreus taxa live in Bosnia and Herzegovina (Apfelbeck 1902, 1904, Winkler 1933, Drovenik and Peks 1999, Bousquet 2003):

- Omphreus (Neomphreus) apfelbecki apfelbecki Reitter, 1893 (Loc. typ.: Bukovi peak near Bileća, Herzegovina, southern Bosnia and Herzegovina; also known from Gornje Hrasno, Hutovo Blato near Gabela, Ljubinje, Mt. Velež and Doljani, Herzegovina, southern Bosnia and Herzegovina),
- O. (N.) apfelbecki cabuljensis Winkler, 1933 (Loc. typ.: Mt. Čabulja, southwestern Bosnia and Herzegovina),
- O. (N.) apfelbecki dinaricus Apfelbeck, 1904 (Loc. typ.: Mt. Kamešnica, western Bosnia and Herzegovina),
- O. (N.) apfelbecki plasensis Apfelbeck, 1904 (Loc. typ.: Mt. Plasa, central Bosnia and Herzegovina),
- O. (Omphreus) morio morio Dejean, 1828 (Loc. typ.: Montenegro, without precise locality; also known from Mt. Orjen, southern Bosnia and Herzegovina),
- O. (O.) morio beckianus Ganglbauer, 1888 (Loc. typ.: Mt. Visočica, central Bosnia and Herzegovina; from central and southern Bosnia to border with Montenegro and northern Herzegovina),
- O. (O.) morio strupii Winkler, 1933 (Loc. typ.: Čajniče surroundings, eastern Bosnia and Herzegovina),
- O. (O.) weiratheri Winkler, 1933 (Loc. typ.: Mt. Prenj, southern Bosnia and Herzegovina).

The territory of Montenegro is inhabited by the following Omphreus taxa (Dejean 1828, Winkler 1933, Meschnigg 1934, Drovenik 1984, Drovenik and Peks 1999, Bousquet 2003, Ćurčić et al. 2008a):

- Omphreus (Neomphreus) apfelbecki meridionalis Winkler, 1933 (Loc. typ.: Kremeni Do near the village of Gornje Stravče, Mt. Žijovo, southeastern Montenegro; also known from the village of Lijeva Rijeka, Mt. Žijovo, southeastern Montenegro, and the Island of Mljet, southern Croatia),
- O. (Omphreus) morio morio Dejean, 1828 [Loc. typ.: Montenegro, without precise locality; also known from the regions of Njeguši (Mt. Lovćen), southwestern Montenegro, and Krivošije (Mt. Radostak), western Montenegro],
- O. (O.) morio beckianus Ganglbauer, 1888 (Loc. typ.: Mt. Visočica, central Bosnia and Herzegovina; also known from northwestern Montenegro),
- O. (O.) morio malissorum Winkler, 1933 (Loc. typ.: Mt. Prokletije, eastern Montenegro),
- O. (O.) wohlberedti Winkler, 1933 (Loc. typ.: Virpazar near the Skadar Lake, southern Montenegro),
- O. (O.) bischoffi Meschnigg, 1934 (Loc. typ.: Mt. Mokra Gora, eastern Montenegro),
- O. (O.) prekornicensis Ćurčić, 2008 (Loc. typ.: Međeđe peak, village of Jugovići, Mt. Prekornica, near Nikšić, central Montenegro),
- O. (O.) bjelasicensis Ćurčić \& Ilić, 2008 (Loc. typ.: Biogradska Gora National Park, Mt. Bjelasica, near Mojkovac, eastern Montenegro).

A few field trips organized by two of the authors of this paper (S.Ć. and D.A.) and F. Sandel and P. Zanandrea in eastern Bosnia and Herzegovina and northwestern Montenegro resulted in the discovery of the two new Omphreus subspecies: O. (O.) morio sandeli ssp. n. and $O$. (O.) morio durmitorensis ssp. n. Both the descriptions and diagnoses are presented in the current paper.

## Materials and methods

The diagnosis of $O$. (O.) morio sandeli ssp. n . is based on the study of the type series of 12 males and four females collected during 2011 and 2012 on Mt. Zelengora and Mt. Maglić (eastern Bosnia and Herzegovina), while the diagnosis of O. (O.) morio durmitorensis ssp. n . is based on the study of the type series of three males and two females collected during 2006, 2007 and 2014 on Mt. Durmitor (northwestern Montenegro). All specimens were collected by pitfall trapping on Mt. Zelengora, Mt. Maglić (eastern Bosnia and Herzegovina), and Mt. Durmitor (northwestern Montenegro) (Figures 1-3).

These were studied in the laboratory of the Institute of Zoology, University of Belgrade - Faculty of Biology, Belgrade, Serbia. The beetle specimens were dissected, studied, and imaged. Dry specimens and the genitalia were glued onto separate rectangular paper labels situated on the same pin.

Carl Zeiss - Stemi 2000 and Carl Zeiss Discovery V8 binocular stereomicroscopes with a Canon G10 digital camera, as well as Nikon Eclipse E100 microscope with a Moticam 2000 digital camera attached were used in the study.

## Measurements

M mean value for certain measurements
$\mathbf{R} \quad$ range of the total measurements performed
TL total body length (measured from the anterior margin of clypeus to the apex of elytra)


Figures I-3. Some of the type localities of Omphreus (Omphreus) morio sandeli ssp. n. (I, 2) and O. (O.) morio durmitorensis ssp. n. (3). I eastern Bosnia and Herzegovina, southern slope of Mt. Zelengora, Čemerno, $1,450 \mathrm{~m}$ a.s.l., near Gacko (photo Franco Sandel) 2 eastern Bosnia and Herzegovina, Mt. Maglić, Tjentište, $1,450 \mathrm{~m}$ a.s.l., near Foča (photo Franco Sandel) $\mathbf{3}$ northwestern Montenegro, Mt. Durmitor, Sedlo pass, 2,100-2,200 m a.s.l., near Žabljak.

HW/HL ratio maximum width of head/length of head
HW/PW ratio maximum width of head/maximum width of pronotum, as greatest transverse distance
AL total antennal length including the scape
AL/TL ratio total antennal length including the scape/total body length (measured from the anterior margin of clypeus to the apex of elytra)
PW/PL ratio maximum width of pronotum, as greatest transverse distance/length of pronotum (measured along the median line)
EW/EL ratio maximum width of elytra/length of elytra (as linear distance measured along the suture from the elytral base to the apex)
EL/EW ratio length of elytra (as linear distance measured along the suture from the elytral base to the apex)/maximum width of elytra
EW maximum width of elytra

## Collections

IZFB collection of the Institute of Zoology, University of Belgrade - Faculty of Biology, Belgrade, Serbia
CRS private collection of Riccardo Sciaky, Milan, Italy
CNI private collection of Nastas Ilić, Belgrade, Serbia

## Other examined taxa

Omphreus (Omphreus) prekornicensis Ćurčić, 2008: holotype male, Montenegro, Mt. Prekornica, village of Jugovićí, Međeđe peak, 900 m a.s.l., near Nikšić, 28.VIII09.IX.2002, leg. Z. Zlatić (IZFB); eight paratype males and one paratype female, same locality as for holotype, 10-28.VII.2001, leg. N. Ilić (IZFB, CNI).
O. (O.) bjelasicensis Ćurčić \& Ilić, 2008: holotype male, six paratype males and two paratype females, Montenegro, Mt. Bjelasica (Biogradska Gora National Park), near Mojkovac, VIII.2002, leg. N. Ilić (IZFB, CNI).
O. (O.) ovcarensis Ćurčić \& Ilić, 2008: holotype male, four paratype males and two paratype females, Republic of Serbia, Čačak, Mt. Ovčar, village of Ovčar Banja, vicinity of the Preobraženje Monastery, 24.V.1996, leg. N. Ilić (IZFB, CNI).
O. (O.) serbooccidentalis Ćurčić, 2008: holotype male, two paratype males and one paratype female, Republic of Serbia, Valjevo, Mt. Maljen, village of Mrčići, Bukovi peak, 900 m a.s.l., 01.VI.1997, leg. N. Ilić (IZFB, CNI).
O. (O.) morio serbicus Winkler, 1933: topotype male, Republic of Serbia, Mt. Murtenica, village of Draglica, $1,100 \mathrm{~m}$ a.s.l., 31.VII.1996, leg. N. Ilić (IZFB).

## Taxonomy

## Omphreus Dejean, 1828

## Omphreus (Omphreus) morio sandeli Ćurčić \& Sciaky, ssp. n.

 http://zoobank.org/D7777A72-0E69-4CAC-84BC-1EAE851BD480Figures 4-8

Material examined. Holotype male labeled as follows: "eastern Bosnia and Herzegovina, southern slope of Mt. Zelengora, Čemerno, 1,450 m a.s.l., near Gacko, 22.VI-14. VII.2012, from pitfall traps, leg. F. Sandel" (white label, printed) / Holotypus Omphreus (Omphreus) morio sandeli ssp. n. S. Ćurčić \& R. Sciaky det. 2014" (red label, printed) (IZFB). Paratypes: four males and one female, same data as for holotype (CRS); one male labeled as follows: "eastern Bosnia and Herzegovina, southern slope of Mt. Zelengora, Čemerno, 1,600 m a.s.l., near Foča, 07-24.VIII.2011, from pitfall traps, leg. F. Sandel" (CRS); four males and two females labeled as follows: "eastern Bosnia and Herzegovina, Mt. Maglić, Tjentište, $1,450 \mathrm{~m}$ a.s.l., near Foča, 23.VI-13.VII.2012, from pitfall traps, leg. F. Sandel" (CRS); two males and one female labeled as follows: "eastern Bosnia and Herzegovina, northern slope of Mt. Zelengora, Tjentište, $1,450 \mathrm{~m}$ a.s.l., near Foča, 23.VI-13.VII.2012, from pitfall traps, leg. F. Sandel" (CRS). All paratypes are labeled with white, printed locality labels and with red printed labels "Paratypus Omphreus (Omphreus) morio sandeli ssp. n. S. Ćurčić \& R. Sciaky det. 2014".

Description. Size large: TL: R 16.88-19.73 mm (M 18.49 mm ). Body elongate; elytra ovoid (Figure 4). Body color black, mouthparts, apical antennomeres, and tarsi black-brownish. Tegument shiny, except slightly matt elytra.

Head rounded, somewhat elongated [HW/HL: M 0.96 (R 0.91-1.05)], narrower (HW/PW: M 0.69) than pronotum (Figure 4). Head below eye level somewhat constricted. Labrum broad, medially rounded, carrying four setae. Epistome large, concave anteriorly, with two setae. Both vertex and occiput wrinkled. Frontal foveae deepened and long. Gula bisetose. Mandibles elongated, sickle-shaped, broadened basally. Labial palpomere 1 short, without setae. The labial palpomeres 2 and 3 longer. Both labial and maxillar palpomere 3 broadened distally and densely pubescent. AL: M 10.41 mm (R 9.25-11.16mm). Antennae pubescent from antennomere 4. AL/TL: M 0.56 (R $0.51-0.58)$. Antennomere 1 club-like, sharply widened distally, with a few long setae distally, somewhat shorter than the following three antennomeres combined. Antennomere 2 slightly shorter than antennomere 3 .

Pronotum sub-campaniform, elongate, PW/PL: M 0.91 (R 0.885-0.94). Fore angles somewhat prominent, rounded, hind angles obtuse, well rounded (Figure 4). Lateral margins well developed, thickened, arcuate anteriorly, then sinuate and narrowing posteriorly. Anterior pronotal margin somewhat concave, base strongly concave. Pronotum widest between its fore fourth and third. Lateral furrows narrow and shallow, with four anterior setae, one median seta, and one posterior seta each. Median furrow


Figures 4-8. Omphreus (Omphreus) morio sandeli ssp. n. from eastern Bosnia and Herzegovina, southern slope of Mt. Zelengora, Čemerno, 1,450 m a.s.l., near Gacko. 4 holotype male, habitus (dorsal view) 5 holotype male, aedeagus (dorsal view) $\mathbf{6}$ holotype male, aedeagus (lateral view) $\mathbf{7}$ holotype male, abdominal sternite IX (urite) 8 paratype female, genitalia. Scale bars $5 \mathrm{~mm}(\mathbf{4})$ and $1 \mathrm{~mm}(\mathbf{5 - 8})$.
long and deep. Basal foveae deep and long, slightly shorter than one half of pronotum length. Pronotal disc somewhat convex proximally.

Elytra ovoid, relatively wide, rounded laterally, EW/EL: M 0.58 (R 0.54-0.61) (Figure 4). EL/EW: M 1.73 . EW: M 6.26 mm in males, while 6.36 mm in females. Elytral striae shallow, weakly punctate, points somewhat deeper basally. Elytral intervals flattened. Scutellum large, triangular. Scutellar stria present, but without scutellar puncture. Elytra widest around at the middle. A setiferous puncture in interval 7 basally (close to stria 6), 4-6 setiferous punctures in interval 7 medially (close to stria 7), and two setiferous punctures situated on stria 7 apically on each elytron. The punctures deep and large. Shoulders rounded. Umbilicate series regular, with the setae densely distributed. Elytral disc somewhat convex distally.

Protarsomeres 1 and 2 widened in males. Metacoxae long and rounded. Tarsal claws elongate, glabrous, smooth (Figure 4).

Aedeagus long, median lobe somewhat widened sub-apically in dorsal view (Figure 5), while curved and strongly widened sub-apically in lateral view (Figure 6), with a straight long acute triangular apex (Figures 5 and 6). Parameres wide, the right being much broader (Figure 5). Basal bulb wide and short (Figures 5 and 6).

Male abdominal sternite IX (urite) large, sub-triangular (Figure 7).
Both gonocoxites and gonosubcoxites IX as presented in Figure 8. Gonocoxites IX wide and elongated, somewhat curved, gradually narrowing distally, each with a rounded apex, basally joined with massive rounded gonosubcoxites IX.

Variability. It was noticed that the specimens from the population from the southern slope of Mt. Zelengora are of somewhat larger size and more elongate elytra (EW/EL: M 0.56) compared with the specimens belonging to the populations both from Mt. Maglić and the northern slope of Mt. Zelengora (EW/EL: M 0.59). Other characteristics, including the structure of aedeagus, do not show any significant differences among the three analyzed populations of the taxon.

Differential diagnosis and remarks. The new subspecies is compared here with the morphologically nearest subspecies of Omphreus (Omphreus) morio Dejean, 1828. These are $O$. (O.) morio beckianus Ganglbauer, 1888, O. (O.) morio serbicus Winkler, 1933, and $O$. (O.) morio durmitorensis ssp. n. (the first antennomere being club-like distally, somewhat shorter than the following three antennomeres combined in all the subspecies mentioned) (Ganglbauer 1888, Winkler 1933).

Omphreus ( $O$.) morio sandeli ssp. n. differs from $O$. ( $O$.) morio beckianus in the shape of the elytra (distinctly rounded laterally vs. slightly rounded laterally), the elytra length/width ratio (M 1.73 vs. around 1.66), and the body length ( $\mathrm{R} 16.88-19.73 \mathrm{~mm}$ vs. $16-18 \mathrm{~mm}$ ) (Ganglbauer 1888, Winkler 1933). The former taxon is distributed both on Mt. Zelengora and Mt. Maglić, while the latter one inhabits Mt. Visočica (the type locality) (Ganglbauer 1888). In the literature Omphreus (O.) morio beckianus is reported to have a wide distribution in Bosnia and Herzegovina, Montenegro and Serbia (from central and southern Bosnia to border with Montenegro, southwestern Serbia and northern Herzegovina, including the high-altitude area of the surroundings of Sarajevo, Mts. Ivan, Bjelašnica, Volujak and Durmitor and the Lim River Valley)
(Apfelbeck 1894, 1904, Wohlberedt-Triebes 1909, Drovenik 1984). This appears to us very doubtful since Omphreus taxa (species or subspecies) mostly inhabit single mountain ranges (Winkler 1933). The specimens from Mt. Durmitor belong to a new subspecies described herein - Omphreus (O.) morio durmitorensis ssp. n.; Mt. Volujak is relatively close to Mt. Zelengora and Mt. Maglić and there is a possibility that the population from this mountain might actually belong to Omphreus ( $O$.) morio sandeli ssp. n., but this has to be proven. The specimens from all known sites should be compared in the future (including the structure of the male genitalia) in order to conclude whether they belong to a single taxon (subspecies) or to different taxa. Additionally, a detailed study of all Omphreus (O.) morio subspecies (with comparisons of numerous morphological characteristics) would be needed in order to define their real taxonomic status (subspecies or species).

Omphreus ( $O$.) morio sandeli ssp. n. differs from $O$. (O.) morio serbicus in the shape of the elytra (elongate vs. short), the elytra length/width ratio (M 1.73 vs. around 1.50 ), and the body length ( $\mathrm{R} 16.88-19.73 \mathrm{~mm}$ vs. $15-16 \mathrm{~mm}$ ) (Winkler 1933). Omphreus ( $O$.) morio sandeli ssp. n . is found in eastern Bosnia and Herzegovina (Mt. Zelengora and Mt. Maglić), while $O$. (O.) morio serbicus lives in southwestern Serbia (Mt. Murtenica and Mt. Zlatibor) (Winkler 1933).

Eventually, Omphreus (O.) morio sandeli ssp. n. differs from O. (O.) morio durmitorensis ssp. n . in the antennal length ( $\mathrm{M} 10.41 \mathrm{~mm} v .10 .38 \mathrm{~mm}$ ), the shape of the hind pronotal angles (more rounded $v$ s. less rounded), maximum width of the pronotum (between its fore fourth and third $v s$. in front of the fore third), the shape of the elytra (somewhat widened $v s$. more elongate), the elytral width/length ratio (M 0.58 vs. 0.56), the elytra length/width ratio (M 1.73 vs. 1.79), the elytral width (M 6.26 mm in males, 6.36 mm in females vs. 5.94 mm in males, 6.12 mm in females), maximum width of the elytra (around at the middle $v s$. slightly below the middle), the shape of the shoulders (rounded vs. obtusely rounded), the form of the median lobe (somewhat widened sub-apically in dorsal view, while curved and strongly widened sub-apically in lateral view, with a straight long acute triangular apex $v s$. strongly widened sub-apically in dorsal view, while arcuate, moderately widened and with a shallow concavity in the sub-apical part in lateral view, with a straight short rounded triangular apex), the shape of the basal bulb (wide and short $v s$. narrow and elongated), the shape of the male abdominal sternite IX (urite) (less elongate $v s$. more elongate), the form of the apex of the gonocoxites IX (rounded $v s$. pointed), and the total body length (M 18.49 mm vs. 18.13 mm ).

Etymology. This new subspecies is named after Franco Sandel, friend of the second author and excellent collector, who collected the whole type series of this new subspecies allowing us to freely study the material.

Distribution. So far known only from the type locality (Mt. Zelengora) and the nearby Mt. Maglić, eastern Bosnia and Herzegovina.

Habitat. The subspecies prefers high-altitude habitats (1,450-1,600 m a.s.l.) on Mt. Zelengora (at both the northern and southern slopes) and Mt. Maglić in eastern Bosnia and Herzegovina. Type series of the new subspecies was collected by pitfall
traps filled with alcoholic vinegar, placed at different sites in beech forests by the alpine meadows on both mountains, and on a pass and near the first hairpin bend after the pass on the southern slope of Mt. Zelengora.

## Omphreus (Omphreus) morio durmitorensis Ćurčić \& Sciaky, ssp. n. http://zoobank.org/BA83B21E-917D-4674-ACC1-B40D65FE121E

Figures 9-13

Material examined. Holotype male labeled as follows: "northwestern Montenegro, Mt. Durmitor, Sedlo pass, 2,100-2,200 m a.s.l., near Žabljak, 28.VI-17.VII.2014, from pitfall traps, leg. S. Ćurčić" (white label, printed) / Holotypus Omphreus (Omphreus) morio durmitorensis ssp. n. S. Ćurčić \& R. Sciaky det. 2014" (red label, printed) (IZFB). Paratypes: one female, same data as for holotype (IZFB); one male labeled as follows: "northwestern Montenegro, Mt. Durmitor, Sedlo pass, 2,100 m a.s.l., 28-29.VI.2014, from pitfall traps, leg. S. Ćurčić \& D. Antić" (IZFB); one male labeled as follows: "northwestern Montenegro, Mt. Durmitor, Žabljak, 1,950 m a.s.l., VIII.2006, leg. P. Zanandrea" (CRS); one female labeled as follows: "northwestern Montenegro, Mt. Durmitor, Sedlo pass, 2,200 m a.s.l., 07.VIII.2007, leg. F. Sandel" (CRS). All paratypes are labeled with white, printed locality labels and with red printed labels "Paratypus Omphreus (Omphreus) morio durmitorensis ssp. n. S. Ćurčić \& R. Sciaky det. 2014".

Description. Size large: TL: R $17.73-18.91 \mathrm{~mm}$ (M 18.13 mm ). Body elongate; elytra ovate (Figure 9). Body color black, mouthparts, apical antennomeres, and tarsi black-brownish. Tegument shiny, except slightly matt elytra.

Head rounded, somewhat elongated [HW/HL: M 0.95 (R 0.93-0.99)], shorter and narrower (HW/PW: M 0.695) than pronotum (Figure 9). Head beyond eye level somewhat constricted. Labrum broad, medially rounded, carrying four setae. Epistome huge, concave anteriorly, with two setae. Both vertex and occiput wrinkled. Frontal foveae deepened and long. Gula bisetose. Mandibles elongated, sickle-formed, broadened basally. Labial palpomere 1 short, without setae. Labial palpomeres 2 and 3 longer. Both the labial palpomere 3 and maxillar palpomere 3 broadened distally and densely pubescent. AL: M 10.38 mm (R $10.07-10.64 \mathrm{~mm}$ ). Antennae pubescent from antennomere 4. AL/TL: M 0.57 (R 0.54-0.60). Antennomere 1 club-like, sharply widened distally, with a few long setae distally, somewhat shorter than the following three antennomeres combined. Antennomere 2 slightly shorter than antennomere 3.

Pronotum sub-campaniform, elongate, PW/PL: M 0.91 (R 0.90-0.92). Fore angles somewhat prominent, rounded, hind angles obtuse, somewhat rounded (Figure 9). Lateral margins well developed, thickened, arcuate anteriorly, then sinuate and narrowing posteriorly. Anterior pronotal margin somewhat concave, while the base strongly concave. Pronotum widest in front of the fore third. Lateral furrows narrow and shallow, with four anterior setae, one median seta, and one posterior seta each. Median furrow long and deep. Basal foveae deep and long, slightly shorter than half of pronotum length. Pronotal disc somewhat convex proximally.


Figures 9-13. Omphreus (Omphreus) morio durmitorensis ssp. n. from northwestern Montenegro, Mt. Durmitor, Sedlo pass, 2,100-2,200 m a.s.l., near Žabljak. $\mathbf{9}$ holotype male, habitus (dorsal view) $\mathbf{I O}$ holotype male, aedeagus (dorsal view) I I holotype male, aedeagus (lateral view) $\mathbf{I} \mathbf{2}$ holotype male, abdominal sternite IX (urite) $\mathbf{1 3}$ paratype female, genitalia. Scale bars $5 \mathrm{~mm}(\mathbf{9})$ and $1 \mathrm{~mm}(\mathbf{1 0}-1 \mathbf{3})$.

Elytra ovate, relatively long, arcuate laterally, EW/EL: M 0.56 (R 0.53-0.58) (Figure 9). EL/EW: M 1.79 . EW: M 5.94 mm in males, 6.12 mm in females. Elytral striae shallow, weakly punctate, points somewhat deeper basally; elytral intervals flattened. Scutellum large, triangular; scutellar stria present, but without scutellar punctures. Elytra widest slightly below the middle; one setiferous puncture in interval 7 basally (close to stria 6), four or five setiferous punctures in interval 7 medially (close to stria 7), and two setiferous punctures situated on stria 7 apically on each elytron; the punctures deep and large. Shoulders obtusely rounded. Umbilicate series regular, with the setae densely distributed. Elytral disc somewhat convex distally.

Protarsomeres 1 and 2 widened in males. Metacoxae long and rounded. Tarsal claws elongated, glabrous, without teeth (Figure 9).

Aedeagus long, median lobe strongly widened sub-apically in dorsal view (Figure 10), while arcuate, moderately widened and with a shallow concavity in the sub-apical part in lateral view (Figure 11), with a straight short rounded triangular apex (Figures 10 and 11). Parameres wide, the right being much huger (Figure 10). Basal bulb narrow and elongated (Figures 10 and 11).

Male abdominal sternite IX (urite) large, sub-triangular (Figure 12).
Both gonocoxites and gonosubcoxites IX as presented in Figure 13. Gonocoxites IX wide and elongated, somewhat curved, gradually narrowing distally, each with a pointed apex, basally joined with massive rounded gonosubcoxites IX.

Differential diagnosis and remarks. The new subspecies is compared here with the morphologically nearest subspecies of Omphreus (Omphreus) morio. These are $O$. (O.) morio beckianus, $O$. (O.) morio serbicus, and $O$. (O.) morio sandeli ssp. n. In all these subspecies the first antennomere is club-like distally, somewhat shorter than the following three antennomeres combined (Ganglbauer 1888, Winkler 1933).

Omphreus (O.) morio durmitorensis ssp. n. differs from $O$. (O.) morio beckianus in the shape of the elytra (arcuate laterally $v s$. somewhat rounded laterally), the elytra length/width ratio (M 1.79 vs. around 1.66 ), and the body length ( $\mathrm{R} 17.73-18.91 \mathrm{~mm}$ vs. $16-18 \mathrm{~mm}$ ) (Ganglbauer 1888, Winkler 1933). The former taxon is distributed on Mt. Durmitor (northwestern Montenegro), while the latter inhabits Mt. Visočica (central Bosnia and Herzegovina) (the type locality) (Ganglbauer 1888). Apfelbeck (1904) and Drovenik (1984) recorded the presence of an Omphreus on Mt. Durmitor reported as $O$. (O.) morio beckianus. While the former author recorded it for the mountain without precise localities, the latter author found it in several different sites (Crno Jezero Lake surroundings, forests below Mali Štulac, Zminje Jezero Lake surroundings, Veliki Štulac, Ališnica, Međed, Velika Karlica, Savin Kuk, Lokvice, Bobotov Kuk, Dobri Do, Sedlo, Todorov Do, Prutaš, Škrčka Jezera Lakes surroundings, Bolj, forest below Ćurevac, Nagorje, and Šljeme) (Apfelbeck 1904, Drovenik 1984). On the basis of the material collected from Mt. Durmitor (at Sedlo and Žabljak sites) loaned by a few colleagues, as well as the one collected by the first and third author (S.Ć. and D.A.), we have concluded that the taxon from Mt. Durmitor actually represents a new subspecies, $O$.(O.) morio durmitorensis ssp. n.

Omphreus (O.) morio durmitorensis ssp. n. differs from $O$. (O.) morio serbicus in the shape of the elytra (elongate, arcuate laterally $v$ s. short, rounded laterally), the elytra length/width ratio (M 1.79 vs. around 1.50), and the body length ( $\mathrm{R} 17.73-18.91$ mm vs. 15-16 mm) (Winkler 1933). Furthermore, they inhabit quite distant areas: Omphreus (O.) morio durmitorensis ssp. n . is found in northwestern Montenegro (Mt. Durmitor), while O. (O.) morio serbicus lives in southwestern Serbia (Mt. Murtenica and Mt. Zlatibor) (Winkler 1933).

The diagnostic differences between Omphreus (O.) morio durmitorensis ssp. n. and $O$. (O.) morio sandeli ssp. n . are presented in the differential diagnosis of the latter.

Etymology. The new subspecies is named after Mt. Durmitor, its terra typica.
Distribution. So far known only from the type locality, Mt. Durmitor, northwestern Montenegro.

Habitat. The subspecies prefers high-altitude habitats (1,950-2,200 m a.s.l.) on Mt. Durmitor in northwestern Montenegro. The type series of the new subspecies was collected by pitfall traps filled with alcoholic vinegar, placed at different sites on the border between alpine meadows and rocks, up to a timberline on Mt. Durmitor.

## Key to Omphreus taxa from Montenegro (modified after Winkler 1933 and Ćurčić et al. 2008b)

1 The first two male protarsomeres broadened. A triangular field on the top of the underside of the first male protarsomere, while the second male protarsomere on the underside almost entirely brush-like setose. The second male protarsomere square-formed (subgenus Omphreus s. str.) 2

- The first two male protarsomeres neither broadened, nor brush-like setose on the underside. The second male protarsomere elongate. Seventh intervals of the matt elytra with 5-7 setiferous punctures each. Head behind eyes clearly narrowed. The first antennomere equally broadened towards the top, as long as the following three antennomeres combined (subgenus Neomphreus Winkler, 1933). Elytra elongately oval, laterally less rounded, twice as long as broad, with strongly impressed striae. Pronotum weakly heart-shaped, laterally very slightly rounded, for one fourth longer than broad. Size R 26 mm (southeastern Montenegro and southern Croatia)
............ Omphreus (Neomphreus) apfelbecki meridionalis Winkler, 1933
2 Elytra without rows of punctures in the second intervals ............................ 3
- Elytra in the middle of the second intervals with a fine and dense row of punctures each, which is solely in the exterior basal and apical part absent. Head proportionally small, the first antennomere as long as the following three antennomeres combined, equally broadened towards the top. Pronotum somewhat longer than broad, in the fore third considerably strongly widened and equally rounded, the hind angles equally rounded. Elytra considerably
equally oval, moderately rounded laterally, slightly more than twice as long as broad. Broadened male protarsomeres brush-like setose in a small extent. Size R 26 mm (southern Montenegro)
O. (Omphreus) wohlberedti Winkler, 1933

3 Head well constricted behind eyes, pronotum as long as broad O. (O.) bischoffi Meschnigg, 1934

- Head moderately constricted behind eyes, pronotum longer than broad..... 4

4 Lateral pronotal margins narrowed or almost parallel basally, never divergent 5

- Lateral pronotal margins divergent basally
O. (O.) bjelasicensis Ćurčić \& Ilić, 2008

5 Lateral pronotal margins sub-parallel basally, size larger (more than 20 mm ), elytral striae less impressed, elytra broader
O. (O.) prekornicensis Ćurčić, 2008

- Lateral pronotal margins somewhat narrowed or almost parallel basally, size smaller (R 15-20 mm), elytral striae more impressed, elytra narrower [subspecies of $O$. (O.) morio Dejean, 1828]6

6 First antennomere club-like thickened distally, mostly somewhat shorter than the following three antennomeres combined (northwestern Montenegro) ....
O. (O.) morio durmitorensis ssp. n.

- First antennomere gradually thickened distally, as long as the following three antennomeres combined 7

7 Size larger (R 20 mm ), pronotum anteriorly stronger widened, head proportionally smaller, with longer antennae (western and southwestern Montenegro and southern Bosnia and Herzegovina)
O. (O.) morio morio Dejean, 1828

Size smaller (R 17-18 mm), narrower, pronotum less widened, head proportionally larger, with shorter antennae (northern Albania and eastern Montenegro)
O. (O.) morio malissorum Winkler, 1933

## Key to the subspecies of Omphreus (Omphreus) morio (modified after Winkler 1933) (Figure 14)

1 First antennomere club-like thickened distally, mostly somewhat shorter than the following three antennomeres combined. 2

- First antennomere gradually thickened distally, as long as the following three antennomeres combined5

2 Smaller subspecies (R 15-16 mm), with elytra short (length/width ratio: M around 1.5 ) and well rounded laterally (southwestern Serbia)
O. (O.) morio serbicus Winkler, 1933

- Larger subspecies (size larger than 16 mm ), with elytra more elongate and less rounded laterally


Figure 14. The distribution of Omphreus (Omphreus) morio subspecies. Scale bar 50 km .

3
Subspecies of somewhat smaller size (R 16-18 mm), with less elongate elytra (length/width ratio: $M$ around 1.66) (central Bosnia and Herzegovina). $\qquad$ O. (O.) morio beckianus Ganglbauer, 1888

- Subspecies of somewhat larger size (M more than 18 mm ), with more elongate elytra (the length/width ratio: $M>1.66$ ). 4
4 Antennae longer, hind pronotal angles more rounded, maximum width of pronotum between its fore fourth and third, elytra somewhat less elongate (length/width ratio: M 1.73) and wider, maximum width of elytra around the middle, shoulders rounded. Median lobe of aedeagus somewhat widened sub-apically in dorsal view, while curved and strongly widened sub-apically in lateral view, with a straight long acute triangular apex, basal bulb wide and short, male abdominal sternite IX (urite) less elongate, apex of the gonocoxites IX rounded, somewhat larger body size (M 18.49 mm ) (eastern Bosnia and Herzegovina) $\qquad$ O. (O.) morio sandeli ssp. n.
- Antennae shorter, hind pronotal angles less rounded, maximum width of pronotum in front of the fore third, elytra more elongate (length/width ratio: M 1.79) and narrower, maximum width of elytra slightly below the middle, shoulders obtusely rounded. Median lobe of aedeagus strongly widened subapically in dorsal view, while arcuate, moderately widened and with a shallow concavity in the sub-apical part in lateral view, with a straight short rounded triangular apex, basal bulb narrow and elongated, male abdominal sternite IX
(urite) more elongate, apex of the gonocoxites IX pointed, somewhat smaller body length (M 18.13 mm ) (northwestern Montenegro)
O. (O.) morio durmitorensis ssp. n.

5 Elytra short, around 1 and $1 / 2$ times longer than broad, evidently widened laterally, length R 18-19 mm (eastern Bosnia and Herzegovina) $\qquad$
O. (O.) morio strupii Winkler, 1933

- Elytra slender, at least for 1 and 2/3 times longer than broad, less rounded and widened laterally6

6 Pronotum markedly longer than broad, somewhat narrowed towards the hind angles, elytra slender, almost twice as long as broad, with weakly expressed shoulders 7

- Pronotum slightly longer than broad, with sides basally parallel, the hind angles less rounded, elytra around 1 and $2 / 3$ times longer than broad, hardly rounded laterally, with strongly expressed shoulders, length R 18-19 mm (northern, northeastern and central Albania and southern Serbia)
O. (O.) morio albanicus Apfelbeck, 1906

7 Size larger ( R 20 mm ), pronotum anteriorly stronger widened, head proportionally smaller, with longer antennae (western and southwestern Montenegro and southern Bosnia and Herzegovina) ... O. (O.) morio morio Dejean, 1828

- $\quad$ Size smaller (R $17-18 \mathrm{~mm}$ ), narrower, pronotum less widened, head proportionally larger, with shorter antennae (northern Albania and eastern Montenegro).
O. (O.) morio malissorum Winkler, 1933


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# Description of the male of Leptotyphlus kovaci Šustek, 2000, the only Central European species of the Mediterranean genus Leptotyphlus Fauvel, 1874 (Coleoptera, Staphylinidae, Leptotyphlinae) 

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#### Abstract

The previously unknown male of Leptotyphlus kovaci Šustek, 2000 is described and illustrated. The relationship of the species is discussed. The species is also reported from Gemerskoteplická jaskyňa near Jelšavská Teplica (Slovakia).


## Keywords

Leptotyphlus kovaci, male characters, Silicka planina, Jelšavský kras

## Introduction

The genus Leptotyphlus Fauvel, 1874 with more than 200 named species (Herman 2001 and subsequent descriptions) is the most speciose genus of the subfamily Leptotyphlinae (worldwide distribution, 43 genera, 515 species). Members of the subfamily are adapted to subterranean life and soil-dwelling; they are frequently found near caves. The known distribution of Leptotyphlus is from the Pyrenees and Southern France to Italy,
with a few species in Spain and Tunisia. With its predominantly Western and Central Mediterranean distribution, it was a great surprise when a new species of this genus was described from Ardovská jaskyňa at Ardovo (Pelsőcardó), Slovakia, not far from the Hungarian border (Šustek 2000). While there are no real gaps in the known distribution of this genus (only about 150 km ), L. kovaci Šustek, 2000 was found about 630 km away from the nearest other member of this genus (L. foroiuliensis Pace, 1976). The description was based on two females (in microscopic preparation). The specimens came from soil at a forested cave entrance of Ardovská jaskyňa near the village Ardovo, a few kilometers SE of Plešivec. Attempts to gather more specimens remained unsuccessful until in 2009 the present writer collected 4 specimens, including one male, at the type locality. In 2010 the species was also found at Gemerskoteplická jaskyňa near Jelšavská Teplica. In contrast to the type locality, this is a watery cave, with a stream exiting and providing humid microclimate, not allowing the soil to dry out. The description of the male is very important, since this species is the only Central European member of this very speciose genus, and also because only based on characters of the aedeagus can its phylogenetic affiliations be assessed.

## Material and methods

In the framework of the Gemer-ATBI+M program (EDIT WP7), the author had an opportunity to collect at the type locality on two occasions. The cave is situated at the edge of Silicka planina (Szilicei-fennsík). The new record is from Jelšavský kras, an adjacent large karstic area. For collecting, the soil-washing method was used: soil together with roots and stones is immersed in water, repeatedly stirred, and the substance gathering on the water surface is collected with a fine tea-filtering net. The organic matter was gathered in a fabric sack which was then drained from excess water and the material was eventually placed in Berlese funnels for further drying and was heated with small (20W) lamps from above. Sampling was done at 6-7 different spots per site to increase the chance of capture. The specimens are dry mounted. Genitalia drawings were made by embedding into Euparal mounting medium on small plastic slides pinned with the specimens. Drawing was done with a Jenalab (Carl Zeiss, Jena) compound microscope and drawing tube (camera lucida), SEM imaging of uncoated specimens with a Hitachi S-2600 N scanning electron microscope. The terminology of the description follows Orousset $(1987,2013)$.

The following codens indicate collections in which the listed specimens are deposited: The Natural History Museum (formerly British Museum of Natural History), London, United Kingdom (BMNH), Hungarian Natural History Museum, Budapest, Hungary (HNHM). Numbers in brackets "\{\}" stand for collecting events.

## Taxonomy

## Leptotyphlus (Leptotyphlus) kovaci Šustek, 2000

Figs 1-10
Leptotyphlus (Leptotyphlus) kovaci Šustek, 2000: 151.

Material examined. SLOVAKIA, Silická planina, 1 km SSE Ardovo, Ardovská jaskyňa, dark oak-maple forest at cave entrance, 310 m , top 15 cm of soil (roots, stones, humus), soil-washing $\{016\}, 48^{\circ} 31^{\prime} 18^{\prime \prime} \mathrm{N}, 20^{\circ} 25^{\prime} 16^{\prime \prime} \mathrm{E}, 30 . \mathrm{V} .2009$, leg. Gy. Makranczy ( 1 male, 2 females, HNHM, 1 female, BMNH); SLOVAKIA, Jelšavský kras, Gemerskoteplická jaskyňa, 1.5 km E Jelšavská Teplica, forest at cave entrance, 230 m , soilwashing $1-4 \mathrm{~m}$ from outcoming stream, 20 cm deep $\{115\}, 48^{\circ} 36^{\prime} 18^{\prime \prime} \mathrm{N}, 20^{\circ} 17^{\prime} 42^{\prime \prime} \mathrm{E}$, 25.IV.2010, leg. Gy. Makranczy (1 male, HNHM).

Partial redescription (male). Measurements in mm (separately for male from Ardovská jaskyňa / male from Gemerskoteplická jaskyňa): head width $=0.14 / 0.155$; maximum width of pronotum $=0.135 / 0.15$; maximum width of elytra $=0.12 / 0.14$; maximum width of abdomen $=0.14 / 0.15$; head length from front margin of clypeus to the beginning of neck $=0.14 / 0.14$; length of pronotum in the middle-line $=0.14$ / 0.16; length of elytra from hind apex of scutellum $=0.09 / 0.10$; length of abdomen $=0.71 / 0.75$ (all measured from dorsal view). Forebody as in Fig. 1 (Ardovská jaskyňa) and Fig. 4 (Gemerskoteplická jaskyňa). Head subrectangular, length about 4/5 of width, parallel-sided, neck separated by transversal groove. Pronotum very slightly narrower than head, both anterior and posterior pronotal margins truncate, sides narrowing behind, gently arched anteriorly, more strongly on posterior portion; anterior angles narrowly rounded, posterior angles more broadly. Elytra together less broad than pronotum, trapezoid, shoulders not developed, posterior margin almost truncate, very slightly concave at suture. Abdomen (Gemerskoteplická jaskyňa) as in Fig. 5, very elongate and parallel-sided; segment VII with much shortened inner laterosclerites. Antenna (Gemerskoteplická jaskyňa) as in Fig. 6, antennomeres 3-11 strongly transverse, articles 9-11 with modified setae. Dissected genital segments of male from Ardovská jaskyňa: sternite VIII (dorsal view) as in Fig. 2, segments IX-X (ventral view) as in Fig. 3.

Aedeagus (Figs 7-10). Greatly asymmetrical. Sternal lamina (l) strongly developed, elongate, with a broadened, hammer-like apex. Sternal lobe (ls) well-developed, in lateral view on the side of sternal lamina opposite to basal orifice, exceeding half length of sternal lamina; in parameral view sternal lobe situated at right side of sternal lamina. Both left and right parameres well-developed, each with 4 strong setae on apex; apices approaching but not reaching half of length of sternal lamina from basal capsule. Copulatory pieces: p1 not developed (in most other related species a large, broad blade-like structure, often approaching apex of sternal lamina), p2 present but weakly developed, without any peculiar formation, p3 (often helicoid in related species) present but inconspicuous (a little sticking out in the Gemerskoteplická jaskyňa specimen, but can be explained as variability). Proximal callus (cp) on basal capsule (cb) very strongly developed, apically with a deflexed edge.


Figures I-6. Leptotyphlus kovaci Šustek, 2000 male from Ardovská jaskyňa (I-3) and male from Gemerskoteplická jaskyňa (4-6). I, $\mathbf{4}$ forebody $\mathbf{2}$ sternite VIII, dorsal view $\mathbf{3}$ abdominal segments IX-X, ventral view $\mathbf{5}$ abdomen, dorsal view $\mathbf{6}$ antenna. Scale bar: $0.06 \mathrm{~mm}(\mathbf{6}), 0.08 \mathrm{~mm}(\mathbf{2}), 0.1 \mathrm{~mm}(\mathbf{I}, \mathbf{4})$, $0.11 \mathrm{~mm}(\mathbf{3}), 0.15 \mathrm{~mm}(\mathbf{5})$.


Figures 7-I0. Leptotyphlus kovaci Šustek, 2000 male from Ardovská jaskyňa (7-8) and male from Gemerskoteplická jaskyňa (9-I0). 7, 9 lateral view 8, 10 "parameral" view. Scale bar: 0.1 mm .

Sexual dimorphism: none besides the usual differences in terminalia. The much wider genital segments mentioned in the original description are an artefact resulting from squeezing the specimens by mounting them between glass surfaces.

Remarks. Based on the structures of the male genitalia, the species belongs to the subgenus Leptotyphlus as correctly stated in the original description and to the $L$. (L.) tyrrhenius species group (sensu Pace (1996)). Similar species are L. (L.) uccellinensis Pace, 1978 and Leptotyphlus (Leptotyphlus) aithaliensis Orousset, 1983, but in L. (L.) kovaci the sternal lobe is on the opposite side of the sternal lamina, compared to most other known species, and the p1 lobe of copulatory pieces is not developed. The sole male specimen from Gemerskoteplická jaskyňa is slightly larger, with somewhat broader head. However, the comparison of the aedeagi reveals only minor differences that may be due to infraspecific variation or distortions. There is no evidence suggesting that the two male specimens may represent different species. On the other hand, the aedeagus of $L$. (L.) kovaci differs quite remarkably from the most similar congeners in Tuscany and Corsica. The knowledge of the male genitalia may gain greater significance when more species are discovered, especially from more southern mountain ranges of the Carpathians or from the northern Balkans.

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