RESEARCH ARTICLE



New Bythinella (Gastropoda, Bythinellidae) species from western Turkey

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Abstract

Bythinella anatolica **sp. n.**, *Bythinella istanbulensis* **sp. n.**, *Bythinella magdalenae* **sp. n.**, and *Bythinella wilkei* **sp. n.** from western Turkey are described herein. Illustrations of the shell and genitalia of the newly described taxa, together with comparisons with previously known *Bythinella* taxa and a key to the species from western Turkey, are also provided.

Keywords

Bythinella, new species, freshwater, springs, Turkey

Introduction

Bythinella Moquin-Tandon, 1856, the sole genus of the caenogastropod family Bythinellidae (Szarowska 2006; Wilke et al. 2013), is composed of small sized (1–3 mm) species occurring almost exclusively in springs (rarely in upper courses of nutrient poor montane streams or caves) having relatively cold waters below 10 °C (Boeters 1998). Although typically characterized by cylindrical (sometimes ovate-conic) shells with rounded apertures, congeners are difficult to discriminate owing to intraspecific variation and the morphostatic mode of divergence observed in the genus (Falniowski et al. 2012). The genitalia of these snails is characterized by a penial appendix with a flagellum in the male (Glöer and Pesic 2010), and a J-shaped cylindrical bursa copulatrix in the female (Falniowski et al. 2009b).

Bythinella contains 132 species and subspecies (Yıldırım et al. 2006; Georgiev 2009; Glöer and Georgiev 2009; Bank 2013; Georgiev and Glöer 2013; Glöer 2013; Odabaşı and Georgiev 2014; Georgiev and Glöer 2014; Glöer and Pešić 2014) and is among the most species-rich genera in the Truncatelloidea. The geographic range of the genus extends from northern Africa and the Iberian Peninsula through central Europe to the Balkan countries, Ukraine and Turkey (Kristensen 1985; Haase et al. 2007). Until recently, only a few species had been recorded in the eastern half of this range. All 21 of the species in Bulgaria and 10 of 12 species in Romania were described within the last decade (Falniowski et al. 2009 a,b; Georgiev and Glöer 2013, 2014); although only three species are known from continental Greece (Bank 2013), the actual number is estimated to be 10 based on molecular data (Falniowski and Szarowska 2011). Together with difficulties in conchological identification mentioned above, the small number of local researchers and scant material from the area have contributed to the recognition of only a few species of Bythinella in its eastern range until recently. The case in Turkey, forming the eastern limit for the genus, does not differ much in this regard. The first mention of the genus from this country was by Schütt (1965), who reported Bythinella opaca (Frauenfeld, 1857) from Belgrad Forest in Prov. Istanbul. The assignment of the species from Istanbul to B. opaca (Frauenfeld, 1857) was later refuted by Schütt (1980) and Yıldırım et al. (2006), however the taxonomic status of this snail remains uncertain. Three additional species from the Asian part of Turkey have also been described (Kebapçı and Yıldırım 2010; Odabası and Georgiev 2014): Bythinella turca Radoman, 1976 from Cire spring (Prov. Isparta), B. occasiuncula Boeters & Falkner, 2001 from Kırkoluk springs (Prov. İzmir), and recently described B. kazdaghensis Odabaşı & Georgiev, 2014 from Ayazma Stream (Prov. Canakkale).

In the present study, four new *Bythinella* species are described from western Turkey based on field surveys between 2006 and 2013. These species are discriminated based on details of shell morphometry, head and cephalic tentacle pigmentation, penial appendix, tubular gland, female genitalia, central tooth of radula and operculum. A comparison with other species and an identification key to other congeners described from Turkey are also presented.

Material and methods

Specimens were collected by hand-netting and preserved in 75% ethanol. Dissections and measurements of the genital organs and the shells were carried out using a Olympus SZ12 stereo microscope; photographs were taken with a digital camera system.

Morphological terminology largely follows Radoman (1973, 1976) and Hershler and Ponder (1998). The scale bars in the figures are 1 mm.

Abbreviations: SW: shell width, SH: shell height, rs: reseptaculum seminis, ah: aperture height, aw: aperture width, Coll. Yıldırım: Collection of M. Zeki Yıldırım in Zoological Museum of the Mehmet Akif University (Burdur, Turkey).

Systematics

Genus Bythinella Moquin-Tandon, 1856

Type species. Bulimus viridis Poiret, 1801.

Bythinella anatolica Yıldırım, Kebapçı & Bahadır Koca, sp. n. http://zoobank.org/F4B75E78-F447-40C7-BCF0-5B2FA8D00A57 Figs 1, 2, 6a

Holotype (Coll. Yıldırım): SH 2.78 mm, SW 1.67 mm, ah 1.22 mm, aw 1.11 mm. Coll. Yıldırım; TURKEY, Manisa, Çırpıcıdede hill on Spil Mountain, N 38°44.66', E 27°24.30', 17. 07. 2006. Leg. M. Z. Yıldırım. Paratypes: 18 ex. (5 dissected), same data and locality as holotype in Coll. Yıldırım.

Type locality. A spring below Çırpıcıdede hill on Spil Mountain and its small outflow stream down the hill, Manisa.

Etymology. Named after Anatolia.

Description. Shell thin, usually cylindrical-ovoid (SW/SH 57.19%), appearing blackish (owing to darkly pigmented animal), with slightly rough surface; apex blunt, depressed on the left side; having $3 \frac{1}{2}$ -4 very turid whorls (more convex the left side), last whorl more inflated, sutures deep. Aperture ovoid or pear shaped, height of the aperture usually greater than penultimate whorl, umbilicus relatively broad and deep, sometimes covered by the outer lip, palatal lip margin not reflected, columellar and parietal margins broadly reflected.

Head black; tentacles unpigmented. Operculum oval and with distinct growth lines.

Penis unpigmented and variable in natural position among individuals (straight to bent or folded), though not in shape. Tubular gland thickened, penial appendix usually very short (varying according to the shell size of the individuals). Bursa copulatrix narrow and elongated, rs₁ large and globular in shape. Central tooth of radula with 9 pointed cusps, 1 median and 4 each on sides; lateral margin without any cusps, but undulated; basal lip roundish (Figures 1, 2).

Measurements (n=19): SH: 2.79 mm (min 2.37/max 3.07), SW: 1.65 mm (min 1.37/max 1.92), SH/SW: 1.7 (min 1.59/max 1. 91), SW/SH : 0.58 (min 0.52/max 0.62), ah/SH: 0.44 (min 0.41/max 0.46).

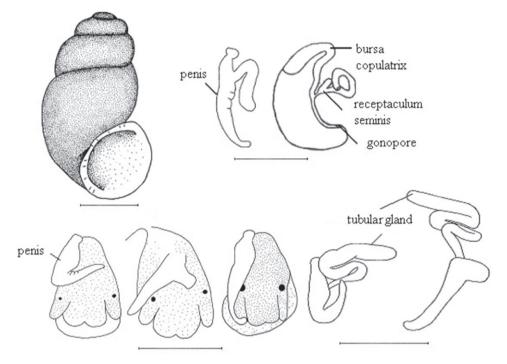


Figure 1. Shell and anatomy of a *Bythinella* species (*B. anatolica* sp. n.). Scale bars = 1 mm.

Differential diagnosis: Identified by its larger shell (except *B. wilkei* sp. n.) and earlike aperture having reflected margins, a character state not observed in other Turkish species.

Habitat. Specimens were collected from under the stones in the spring outflow down the hill.

Remarks. The new species is distinguished from other Turkish species by its larger shell dimensions and ear like shell aperture. *Bythinella turca* Radoman 1976 is distinguished from *B. anatolica* sp. n. by its blunter and shorter shell (SH $2,42 \pm 0,13$; max. 2.81 mm). SW/SH is close to *B. istanbulensis* sp. n., from which it can be distinguished by its oval shell with convex whorls. *Bythinella occasiuncula* and *B. magdalenae*, the geographically most proximate congeners, have smaller shell dimensions, while *B. occasiuncula* can be identified by the shorter and blunter shell shape and *B. magdalenae* sp. n. by smoother periphery and shallow sutures.

According to our current knowledge of Peri-Aegean *Bythinella* species, the area of the Aegean coast of Turkey is a center of diversity for the genus. The Eastern Aegean Islands Ikaria, Kos, Lesbos, and Chios are inhabited by *B. kosensis* (Schütt 1980; Bank 1988), while within the provinces of Aydın, İzmir, and Manisa on adjacent Anatolian mainland there are three species (*B. anatolica* sp. n., *B. magdalenae* sp. n. and *B. occasiuncula*) geographically isolated from the remainder of the species recorded in Turkey (Figure 7).

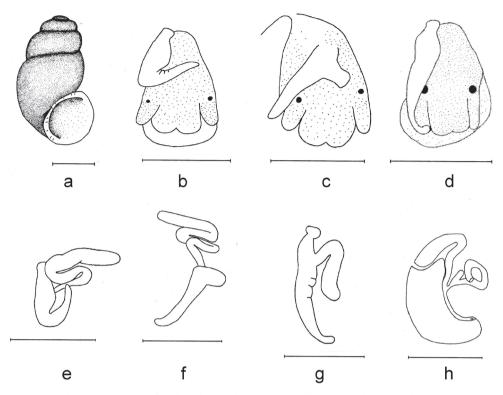


Figure 2. Shell and anatomy of *Bythinella anatolica* sp. n.: **a** shell **b**, **c**, **d** male head and various positions of penis **e**, **f**, **g** penes **h** female genitalia. Scale bars = 1 mm.

Bythinella istanbulensis Yıldırım, Kebapçı & Yüce, sp. n.

http://zoobank.org/8958B919-4F97-4EAA-A6F8-EB7059A8FEB7 Figs 3, 6b

- *Bythinella opaca*, Schütt, H., 1965 Zur Systematik und Ökologie Türkischer Süsswasserprosobranchier. Zoologische Mededelingen, 41: 43–71. (misidentification).
- *Bythinella "opaca*", Schütt, H., 1980 Zur Kenntnis griechischer Hydrobiiden, Arch. Moll.110 (4/6):115.
- *Bythinella* sp. A, Yıldırım et al. 2006 Supplement to the Prosobranchia (Mollusca: Gastropoda) Fauna of Fresh and Brackish Waters of Turkey, Tr. J. Zool. 30: 197–204.

Holotype (Coll. Yıldırım): Shell height: 2.74 mm, width 1.43 mm (Coll. M.Z. Yıldırım); TURKEY, Istanbul, Bahçeköy, a small spring at the entrance of Bahçeköy in Belgrad Forest, N41°11.09', E28°59.5', 23.02.2013, leg. A. Yüce. Paratypes: 27 ex., same data and locality as holotype; 5 ex. Zoologisches Museum Hamburg (ZMH 79661), 5 ex. Naturhistorisches Museum in Wien (NHMW 109174), rest in the Coll. Yıldırım. Additional material: 5 ex. in the University of Giessen (Coll. Prof. Wilke), rest in the Coll. Yıldırım (5 dissected).

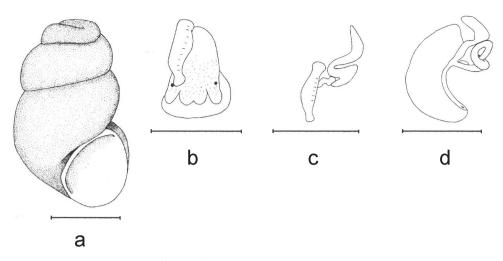


Figure 3. Shell and anatomy of *B. istanbulensis* sp. n.: **a** shell **b** male head and penis in natural position **c** penis **d** female genitalia. Scale bars = 1 mm.

Type locality. A small spring at the entrance of Bahçeköy in Belgrad Forest, Bahçeköy, Istanbul.

Etymology. Named after the type locality.

Description. Shell broad cylindrical oval, yellowish horn-colored, surface smooth and glossy covered by a thick, solid and rough encrustation due to chemical conditions of the spring water; apex blunt; $3\frac{1}{2}$ -4 convex whorls, rapidly and regularly increasing to form a cylindrical shape, last whorl about $\frac{2}{3}$ of the shell height, sutures relatively deep; aperture pear-shaped, height nearly the same as or slightly less than the penultimate whorl, columellar margin with an distinct inner lip reaching basal and apical corners of the aperture; operculum orange, umbilicus narrow and slit-shaped (Figs. 2a, 6a).

Tubular gland relatively short and thick. Pallial roof unpigmented, head having little pigmentation (Fig. 2b), rs₁ small and attached to oviduct (Fig. 2c), thus not easily discernible.

Measurements (n=28): SH: 2.67 mm (min 2.18/max 2.87), SW: 1.66 mm (min 1.37/max 1.94), SH/SW: 1.61 (min 1.39/max 1.84), SW/SH: 0.62 (min 0.54/max 0.72), ah/SH: 0.41 (min 0.37/max 0.50).

Differential diagnosis. This new species is distinguished from other Turkish *Byth-inella* species by its nearly cylindrical shell with broad and blunt apex. Small and attached rs₁, unpigmented and light coloured pallial roof are the other key characters.

Habitat. Specimens were collected from the stones in a small spring and its pond.

Remarks. Based on shell characters only, Schütt (1965) misidentified *Bythinella* specimens from Belgrad Forest as *Bythinella opaca* (Frauenfeld, 1857), a species originally described from Carniola (Slovenia) and Italy. *Bythinella angelitae* was introduced as a replacement name for the preoccupied name *Paludinella opaca* Frauenfeld, 1857, a

homonym of *Paludinella opaca* M. von Gallenstein, 1848, by Haase et al. (2007). The two closely related species *B. angelitae* and *B. opaca* can be morphologically separated only by radular dentition, and the geographic range of *Bythinella angelitae* is restricted to the border areas of Austria and Slovenia (Haase et al. 2007). Although they have been recorded in previous studies (Wagner 1941; Grossu 1956; Angelov 1960, 2000), *Bythinella austriaca* (Frauenfeld, 1857) and *Bythinella opaca* (M. von Gallenstein, 1848) have not been confirmed from the eastern Balkan countries in recent studies (Falniowski et al. 2009a, b). Therefore, there is a large distribution gap between the ranges of Central European taxa and that of *B. istanbulensis* sp. n.

Despite the conchological similarities, the new species is anatomically distinct from the Central European species *B. austriaca* and *B. opaca*. While the lighter pallial roof colouration is also observed in *Bythinella opaca* (Glöer & Pešic, 2006), *B. austriaca* has a dark pallial roof colouration. The new species differs from both of these taxa in having a very short and broad penial appendix (cf. long trumpet-shaped penial appendix in these species).

Bythinella magdalenae Yıldırım, Kebapçı & Bahadır Koca, sp. n.

http://zoobank.org/669E7842-7985-473B-837B-C06917BF0962 Figs 4, 6c

Holotype (Coll. Yıldırım): SH 2,5 mm, SW 1,62 mm, ah 1,12 mm, aw 1,06 mm; TURKEY, Aydın, İncirliova, Karagözler Village, Karapınar spring above the village, 37°57,796'N, 27°49,375'E, leg. S. Bahadır Koca. Paratypes: 17 ex in Coll. Yıldırım, same data and locality as holotype; 18 ex in Coll. Yıldırım, TURKEY, Aydın, İncirliova, Karagözler Village, Çaycuk spring above the village, 37°57,829'N, 27°49,230'E, leg. S. Bahadır Koca.

Type locality. Karapınar Spring, Karagözler Village, İncirliova, Aydın.

Etymology. Named after the late Polish malacologist Magdalena Szarowska (1952–2013), who contributed greatly especially to the knowledge of the Balkan Truncatelloidea.

Description. Shell ovate-conical and with 3–3,5 whorls. Apex truncated. Last whorl slowly increasing and broader than previous whorls, sutures not deep and periphery nearly flat. Aperture roundish oval. Umbilicus small, hollow shaped and covered by the lip. Operculum nucleus along left margin, oval shaped and with distinct growth lines.

Head having little amount of pigmentation, rather concentrated on the tips of the tentacles and around the mouth. Tentacles slightly longer than snout. Penis unpigmented, tapering towards the tip, tip not very pointed at its distal end but club-shaped. Tubular gland externally visible, thin, elongated and convoluted. Bursa copulatrix narrow and elongated, rs₁ globular in shape. Central tooth of radula with 9 pointed cusps, 1 median and 4 each on sides; lateral margin without any cusps, but undulated.

Measurements: Karapınar Spring (n=18): SH: 2.55 mm (min 2.19/max 2.91), SW: 1.64 mm (min 1.37/max 1.78), SH/SW: 1.55 (min 1.42/max 1.72), SW/SH :

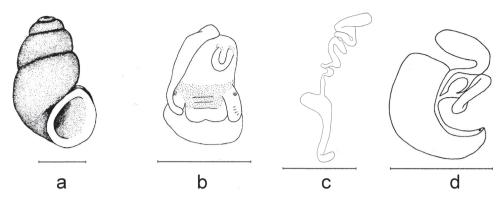


Figure 4. Shell and anatomy of *B. magdalenae* sp. n.: **a** shell **b** male head and penis in natural position **c** penis **d** female genitalia. Scale bars = 1 mm.

0.65 (min 0.58/max 0.70), ah/SH: 0.45 (min 0.44/max 0.46). Çaycuk Spring (n=18): SH: 2.41 mm (min 2.06/max 2.78), SW: 1.59 mm (min 1.31/max 1.87), SH/SW: 1.52 (min 1.37/max 1.66), SW/SH : 0.66 (min 0.64/max 0.67), ah/SH: 0.46 (min 0.46/max 0.48).

Differential diagnosis. *Bythinella magdalenae* sp. n. is distinguished from *B. co-sensis* Schütt 1980 (from Kos island) by the smaller number of shell whorls and the differing number of cusps on the central tooth of radula. It can be distinguished from other Turkish congeners by the flat periphery of the whorls and shallow sutures.

Habitat. Specimens were collected from two small, closely proximal seepage springs.

Remarks. Çaycuk Spring is very close to the type locality, thus indeed *B. magdalenae* sp. n. can be considered a single spot endemic as in the case of other species known from Turkey.

Bythinella wilkei Yıldırım, Kebapçı & Bahadır Koca, sp. n. http://zoobank.org/3C7814DB-62E3-458B-9FB9-E4A026F339DD

Figs 5, 6d

Holotype (Coll. Yıldırım): SH 2.75 mm, SW 1.84 mm, ah 1.28 mm, aw 1.12 mm; TURKEY, Kocaeli, Maşukiye, spring along the road to Kartepe, 40°40.603'N, 30°08.605'E, leg. S. Bahadır Koca. Paratypes: 29 ex in Coll. Yıldırım, same data and locality as holotype.

Type locality. Spring along the road to Kartepe, Maşukiye, Kocaeli.

Etymology. Named after the malacologist Thomas Wilke (Justus Liebig University, Germany).

Description. Shell oval conical, light brown, but appearing blackish due to encrustation; having 3–3.5 tumid whorls; last whorl slowly increasing and broader than previous whorls, sutures deep. Apex blunt. Aperture roundish oval, last whorl strongly descending towards aperture and aperture projected forward, lip in some individuals

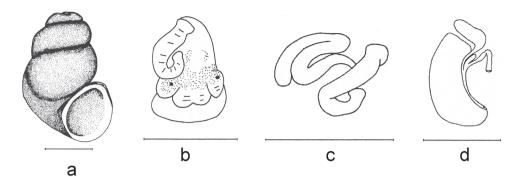


Figure 5. Shell and anatomy of *Bythinella wilkei* sp. n.: **a** shell **b** male head and penis in natural position **c** penis **d** female genitalia. Scale bars = 1 mm.

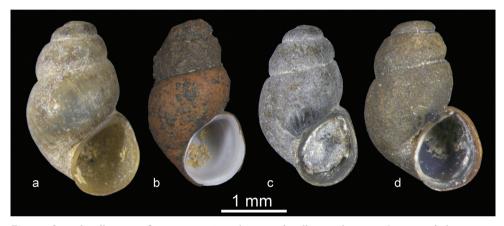


Figure 6. *Bythinella* species from western Anatolia: **a** *Bythinella anatolica* sp. n. **b** *B. istanbulensis* sp. n. **c** *B. magdalenae* sp. n. **d** *B. wilkei* sp. n.

having a small protuberance in upper palatal margin. Umbilicus small, hollow or completely covered by the lip. Operculum ovate, translucent and with distinct growth lines.

Head having little pigmentation, pigment rather concentrated on bases of the tentacles and around mouth. Tentacles short and broad. Unpigmented penis tapering towards the tip, tip not pointed at its distal end; tubular gland thick and with two convolutions. Bursa copulatrix narrow and elongated, rs₁ elongate. Central tooth of radula with 9 pointed cusps, 1 median and 4 each on sides; lateral margins lacking cusps, but undulated.

Measurements (n=22): SH: 2.80 mm (min 2.56/max 3.03), SW: 1.88 mm (min 1.56/max 2.19), SH/SW: 1.50 (min 1.31/max 1.68), SW/SH: 0.66 (min 0.60/max 0.76), ah/SH: 0.47 (min 0.41/max 0.51).

Differential diagnosis. Excluding *B. anatolica* sp. n. described from Manisa Province, *Bythinella wilkei* sp. n. has a larger shell than other Turkish congeners. The nearly circular aperture, which is strongly descending and projected forward, is also characteristic of the new species.

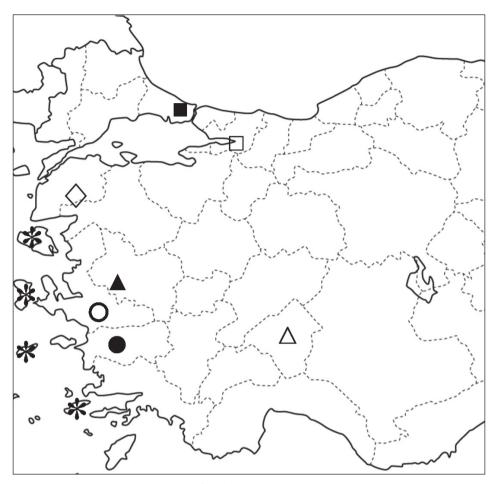


Figure 7. Map showing the locations of *Bythinella* species in Turkey and the Eastern Aegean Islands: *Bythinella anatolica* sp. n. (black triangle), *B. istanbulensis* sp. n. (closed square), *B. kazdaghensis* (diamond), *B. kosensis* (asterix), *B. magdalenae* sp. n. (closed circle), *B. occasiuncula* (open circle), *B. turca* (open triangle), *B. wilkei* sp. n. (open square).

Habitat. The specimens were collected from the outflow of a small spring having little vegetation and a gravel substrate.

Remarks. The species is one of the two northernmost distributed congeners in Turkey. Unlike *B. istanbulensis* sp. n. the new species is found in higher altitudes.

Identification key to the Bythinella species from Turkey

1	Shell having 3.5–4 whorls	.2
_	Shell having 3–3.5 whorls	.4
2	Shell elongated oval, aperture margins reflected	

_	Shell nearly cylindrical, aperture margins not reflected
3	Pallial roof black, penis shorter than penial appendix
_	Pallial roof light coloured, penis longer than penial appendix B. istanbulensis
4	Tubular gland thin, long and convoluted (more than 2 loops)5
_	Tubular gland thick, short and less convoluted
5	Shell oval, SH<2.3 mm
_	Shell ovate-conic and usually SH>2.3 mm
6	Periphery almost straight, sutures shallow, tentacles longer than proboscis
	B. magdalenae
_	Periphery convex, sutures deep, tentacles shorter than proboscisB. turca

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SHORT COMMUNICATION



A pioneer survey and DNA barcoding of some commonly found gastropod molluscs on Robben Island

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Abstract

Nineteen species of abundant gastropods were collected at Robben Island, including introduced dune snails and European brown garden snails. They were identified using morphology and DNA barcoding. It was expected that the species recorded would be similar to those from the Cape peninsula, South Africa, but we were surprised to find some exceptions: the very abundant invasive mussel species in South Africa, the South American bisexual mussel (*Semimytilus algosus*), and the beaded topshells (*Oxystele impervia*) were not found on Robben Island. Possible explanations are presented for these differences.

Keywords

Mollusca, Gastropoda, mitochondrial gene COI, species identification

Introduction

Robben Island is most renowned for its maximum security prison, which housed President Nelson Mandela of SA. Robben Island is 7 km from Bloubergstrand (SA), the length of Robben Island is 5.4 km, the width is 2.5 km, and has an area of 574 hectares (approximately 5.74 km²) with the highest point at 30 m above sea level and an annual rain fall of between 300 mm and 400 mm. It is less known for being the island to which lepers were banned to become miserable island outcasts. The leper colony was initiated in 1846 with special water police patrols to ensure no escapees. Robben Island has recently become a tourist attraction, possibly contributing towards the decline of African penguin numbers to the extent that they became an endangered species (Weller et al. 2014).

Unfortunately, little research on molluscs has been carried out on the island. The only available reference is to one mussel species at Robben Island by Pollock (1979). The aim of this study is to report results from a survey of the biodiversity, confirmed by voucher identifications and DNA barcoding of the common molluscs of Robben Island as part of the international Barcoding of Life Data Systems (BOLD; http://www.barcodinglife.org) project. We were very interested to determine the extent to which alien species have invaded the island, as alien invasive species are becoming a big problem in SA (Picker and Griffiths 2011). The South American bisexual mussel is especially worrisome because it proliferates extremely rapidly and is replacing endemic mussel species in South Africa.

Materials and methods

Samples were collected from random sampling sites during July 2011. Standard DNA barcoding protocols were used to ensure correct identification of individuals (e.g. Van der Bank et al. 2013).

DNA extraction, polymerase chain reactions (PCR) and sequencing of the COI region (animal DNA barcode) were done at the Canadian Centre for DNA Barcoding (CCDB). Standard CCDB protocols for PCR reactions were followed as described by Hajibabaei et al. (2005). Multiple Sequence Comparison by Log-Expectation (MUS-CLE vs. 3.8.31, Edgar 2004 was performed) for sequence alignment. GenBank accession numbers, BOLD process identification numbers and voucher information are all available online (www.boldsystems.org). The Kimura 2-parameter (K2P) model (Kimura 1980) was used to measure genetic distances.

We reconstructed Bayesian phylogenetic trees using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). jModelTest v0.1.1 (Posada 2008) under the Akaike information criterion (Posada and Buckley 2004) was used as the best-fit model of DNA sequence evolution. The Bayesian tree was generated by selecting the TrN + I model and nine million generations, with sampling one tree every 100 generations, was used in the analysis.

PAUP* v4.10b10 (Swofford 2002) was used for maximum parsimony (MP) analyses and the data and tree searches were done using heuristic searches with 1 000 random sequence additions keeping only 10 trees. All character transformations were treated as equally likely i.e. Fitch parsimony (Fitch 1971) for the tree bisection-reconnection as performed with MP searches and bootstrap resampling (Felsenstein 1985) done using PAUP* v4.10b10 (Swofford 2002). The American bisexual mussel (*Semimytilus algosus* (Gould, 1850); BOLD process Id: HvdB-12-2010-116) was used as outgroup.

Depending on availability, up to 10 individuals per species were collected (Table 1) from random sampling sites (Figure 1) generated with the aid of computer software

BOLD Process	Sample identity	Species	Family		
HVDBM476-11	HVDBM-ROB-96	Af_{i} litter in a horizontal (DL ilingi 1947)	Littorinidae		
HVDBM495-11	HVDBM-ROB-97	Afrolittorina knysnaensis (Philippi, 1847)	Littorinidae		
HVDBM569-11	HVDBM-ROB-94	Burnupena catarrhacta (Gmelin, 1791)	Buccinidae		
HVDBM528-11	HVDBM-ROB-53				
HVDBM529-11	HVDBM-ROB-54				
HVDBM530-11	HVDBM-ROB-55	Burnupena cincta (Röding, 1798)	Buccinidae		
HVDBM531-11	HVDBM-ROB-56				
HVDBM532-11	HVDBM-ROB-57				
HVDBM533-11	HVDBM-ROB-58				
HVDBM534-11	HVDBM-ROB-59				
HVDBM535-11	HVDBM-ROB-60	Cornu aspersum (Müller, 1774)	Helicidae		
HVDBM536-11	HVDBM-ROB-61	_			
HVDBM537-11	HVDBM-ROB-62				
HVDBM525-11	HVDBM-ROB-50				
HVDBM526-11	HVDBM-ROB-51				
HVDBM527-11	HVDBM-ROB-52				
HVDBM553-11	HVDBM-ROB-78	Crepidula complanata (Krauss, 1848)	Calyptraeidae		
HVDBM554-11	HVDBM-ROB-79	Crepiunui compuniui (Klauss, 1040)	Caryptiacidae		
HVDBM555-11	HVDBM-ROB-80				
HVDBM556-11	HVDBM-ROB-81				
HVDBM568-11	HVDBM-ROB-93				
HVDBM496-11	HVDBM-ROB-21				
HVDBM497-11	HVDBM-ROB-22	Cymbula compressa (Linnaeus, 1758)	Patellidae		
HVDBM552-11	HVDBM-ROB-77				
HVDBM481-11	HVDBM-ROB-06				
HVDBM482-11	HVDBM-ROB-07				
HVDBM483-11	HVDBM-ROB-08				
HVDBM484-11	HVDBM-ROB-09				
HVDBM485-11	HVDBM-ROB-10	<i>Cymbula granatina</i> (Linnaeus, 1758)	Patellidae		
HVDBM486-11	HVDBM-ROB-11	Cyntowa granavna (Linnaeds, 1790)	1 atcilitat		
HVDBM500-11	HVDBM-ROB-25				
HVDBM540-11	HVDBM-ROB-65				
HVDBM541-11	HVDBM-ROB-66				
HVDBM542-11	HVDBM-ROB-67				
HVDBM487-11	HVDBM-ROB-12				
HVDBM488-11	HVDBM-ROB-13	Cymbula oculus (Born, 1778)	Patellidae		
HVDBM489-11	HVDBM-ROB-14				
HVDBM539-11	HVDBM-ROB-64				
HVDBM499-11	HVDBM-ROB-24		E: 11: 1		
HVDBM570-11	HVDBM-ROB-95	Fissurella mutabilis (Sowerby, 1835)	Fissurellidae		
HVDBM561-11	HVDBM-ROB-86	Helcion pectunculus (Gmelin, 1791)	Patellidae		
HVDBM506-11	HVDBM-ROB-31				
HVDBM507-11	HVDBM-ROB-32				
HVDBM508-11	HVDBM-ROB-33				
HVDBM509-11	HVDBM-ROB-34				
HVDBM510-11	HVDBM-ROB-35				

Table 1. BOLD process and sample identity numbers, species collected, authorities and families.
 Bold font indicates samples for which no DNA barcodes were obtained from BOLD.
 Image: Collected of the sampl

BOLD Process	Sample identity	Species	Family			
HVDBM511-11	HVDBM-ROB-36					
HVDBM512-11	HVDBM-ROB-37		Trochidae			
HVDBM513-11	HVDBM-ROB-38	Oxystele variegata (Anton, 1839)				
HVDBM514-11	HVDBM-ROB-39					
HVDBM515-11	HVDBM-ROB-40					
HVDBM498-11	HVDBM-ROB-23	Scutellastra barbara (Linnaeus, 1758)	Patellidae			
HVDBM477-11	HVDBM-ROB-02					
HVDBM478-11	HVDBM-ROB-03	Scutellastra cochlear (Born, 1778)	Patellidae			
HVDBM479-11	HVDBM-ROB-04	Scuteuastra cochiear (Born, 1/78)	Patellidae			
HVDBM480-11	HVDBM-ROB-05					
HVDBM490-11	HVDBM-ROB-15					
HVDBM491-11	HVDBM-ROB-16					
HVDBM492-11	HVDBM-ROB-17	Soutollastus manularis (Linnoous, 1759)	Patellidae			
HVDBM493-11	HVDBM-ROB-18	Scutellastra granularis (Linnaeus, 1758)	Patellidae			
HVDBM494-11	HVDBM-ROB-19					
HVDBM562-11	HVDBM-ROB-87					
HVDBM563-11	HVDBM-ROB-88					
HVDBM564-11	HVDBM-ROB-89	Siphonaria serrata (Fischer von	Siphonariidae			
HVDBM565-11	HVDBM-ROB-90	Waldheim, 1807)				
HVDBM566-11	HVDBM-ROB-91					
HVDBM501-11	HVDBM-ROB-26					
HVDBM502-11	HVDBM-ROB-27					
HVDBM503-11	HVDBM-ROB-28	Siphonaria oculus (Krauss, 1848)	Siphonariidae			
HVDBM504-11	HVDBM-ROB-29		-			
HVDBM505-11	HVDBM-ROB-30					
HVDBM538-11	HVDBM-ROB-63					
HVDBM1129-12	HVDBM_ROB_1010					
HVDBM1130-12	HVDBM_ROB_1011	Theba pisana (Müller, 1774)	Helicidae			
HVDBM1131-12	HVDBM_ROB_1012	<i>Theor pisuna</i> (Wuner, 1774)				
HVDBM1132-12	HVDBM_ROB_1013					
HVDBM1133-12	HVDBM_ROB_1014					
HVDBM1134-12	HVDBM_ROB_1015					
HVDBM1135-12	HVDBM_ROB_1016					
HVDBM1136-12	HVDBM_ROB_1017	Trigonephrus globulus (Müller, 1774)	Dorcasiidae			
HVDBM1137-12	HVDBM_ROB_1018	.8				
HVDBM1138-12	HVDBM_ROB_1019					
HVDBM551-11	HVDBM-ROB-76	Turbo cidaris (Gmelin, 1791)	Turbinidae			

(http://www.random.org/). Some terrestrial, but mostly marine snails were collected from the *Littorina* to *cochlear* zones. Data capture as prescribed by BOLD (including GPS, altitude, temperature, photographs of the localities and species, and voucher information) was done. Voucher specimens (shells) were also collected and deposited at the KwaZulu-Natal Museum (SA) in an attempt to limit future bio-prospecting.

An unedited BOLD identification tree of barcoded southern African individuals is available from the corresponding author. This includes 815 sequenced individuals from 184 species, 118 genera, and 76 families in southern Africa (excluding the ones that were published already in other journals such as in Van der Bank et al. 2013).



Figure 1. Random sampling sites on Robben Island from which species were collected.

Results and discussion

BOLD process and sample identity numbers, species collected, authorities and families are listed in Table 1. We did not receive DNA barcodes from BOLD for Burnupena catarrhacta (Röding, 1798) and Scutellastra cochlear (Born, 1778), and for a few individuals from other species (sample identity numbers in bold, Table 1).

The most abundant terrestrial snail we encountered was *Theba pisana* (Müller, 1774), an introduced dune snail. As expected, more Cape endemic terrestrial snails were found (*Trigonephrus globulus*, Müller, 1774) and fewer introduced European brown garden snails (*Cornu aspersum*, Müller, 1774, formerly *Helix aspersa*) were recorded. Figure 2 indicates that the land snail species are monophyletic and were well-supported (bootstrap values 74-100%; average 93.5% using the South American bisexual mussel as outgroup, also in Figure 3 for the marine molluscs). The aligned COI matrix is 654 base pairs long, the numbers of constant characters are 335; 319

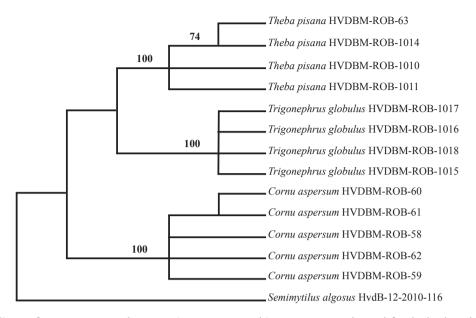


Figure 2. A parsimonious bootstrap (50% majority-rule) consensus tree obtained for the land snails, using *Semimytilus algosus* as the outgroup.

characters are variable (of which 146 are parsimony uninformative and 173 are parsimony informative). The tree length is 414 steps with a consistency index of 0.942 and a retention index of 0.963. Mean intra-specific divergence is 0.15 and 24.5 for the mean distance to nearest neighbour. This indicates that there is a barcode gap (Meyer and Paulay 2005) in the dataset, thus confirming COI as an appropriate DNA region for taxon identification within these studied molluscs.

Approximately 85–90% of the habitat at the coast consists of rocky shores, with low biodiversity of sparsely populated barnacles and molluscs (bivalves, limpets, and mussels): it consisted mostly of individuals of the same species. According to the island conservationists, this can be attributed to the rough seas.

We were pleasantly surprised not to have found any South American bisexual mussel on Robben Island, as they are extremely common on the SA coastline (only approximately 6.9 km away) and Robben Island was joined with SA approximately 10 000 years ago (Thackeray 2001). They are extremely prolific breeders and are a threat to SA and Namibian mussels because they outcompete endemic mussel species. They only grow to 5 cm (Branch et al. 2010) and are therefore not suitable as a human food source as are the endemic species. It is conceivable that the Benguela Upwelling System might be responsible for their offspring to be transported away from the island in a more northerly direction. The aligned COI matrix in Figure 3 is 654 base pairs long, the numbers of constant characters are 258, and 396 characters are variable (of which 51 are parsimony uninformative and 345 are parsimony informative). The tree length is 1599 steps with a consistency index of 0.439 and a retention index of 0.862. Mean

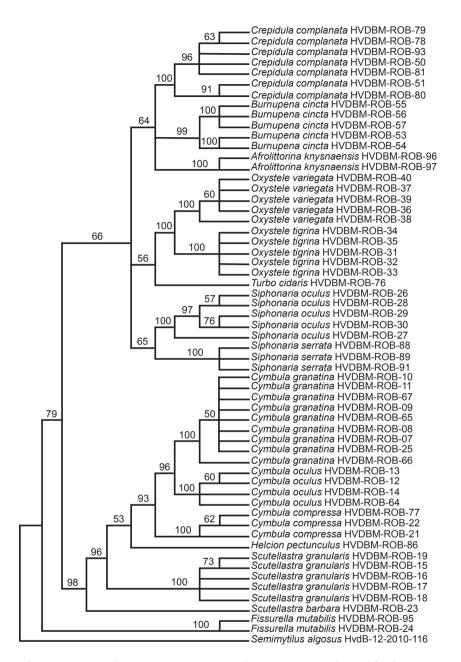


Figure 3. A parsimonious bootstrap (50% majority-rule) consensus tree obtained for the marine snails, using *Semimytilus algosus* as the outgroup.

intra-specific divergence is 0.79 and 20.04 for the mean distance to nearest neighbour. This, again, indicates that there is a barcode gap in the dataset; thus confirming COI as an appropriate DNA region for taxon identification for the marine molluscs.

At the generic level, all of the marine species are monophyletic and were well supported (bootstrap values 98–100%; Figure 3), except for the single representatives of *Helcion pectunculus* (Gmelin, 1791) and *Turbo cidaris* (Gmelin, 1791) (bootstrap values 53% and 56% respectively). *Helcion* clusters as a sister group to Cymbula, as also reported by Nakano and Ozawa (2007).

We were also surprised not to find any *Oxystele impervia* (Menke, 1843) individuals on the island, which could be due to differences in habitat preferences: they are found more abundantly higher up the shore than, for example, *O. variegata* (Heller and Dempster 1991), and possibly avoided the lower shore when SA and Robben Island were linked. It is also possible that the Benguela Upwelling System might prevent the free-swimming larvae of *O. impervia* to reach the island, but it is more likely that thorough sampling is required before this can be confirmed.

The slipper-limpet (*Crepidula complanata* Krauss, 1848) was found with smaller males on top of the larger females; both live on other shells but are not parasites. Two clades with 91% and 97% bootstrap support were obtained (Figure 3). One specimen of *Cymbula granatina* (Linnaeus, 1758; HVDBM-ROB-66) was found that differed only by one sequence from the other *C. granatina* individuals.

Almost no molluscs occurred on the man-made structures surrounding the harbour, most probably because these were installed recently, in 2004. This area was studied because introductions from ships (which transport tourists from SA as well as island staff and children) are most likely to occur at the harbour.

Surveys that are much more detailed are required to report on the biodiversity of the island; however, this is the first study to link DNA sequence results with the morphology of the commonly found molluscs on Robben Island and thereby to contribute to the global biodiversity fauna data that could enhance future efforts in conservation and management.

Acknowledgements

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RESEARCH ARTICLE



A new species of *Hyalella* (Crustacea, Amphipoda, Dogielinotidae) from the Atlantic Forest of Misiones, Argentina

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Abstract

The freshwater genus *Hyalella* Smith, 1874 has a distribution restricted to the Western Hemisphere with most species being found in South America. In this report we describe a new species of *Hyalella* from the Atlantic Forest of the Misiones province, Argentina.

Keywords

Hyalella, taxonomy, freshwater amphipods, novel-species description, Argentina

Introduction

The genus *Hyalella* includes approximately 70 valid species distributed in only the Americas (Baldinger 2004, WoRMS 2014). The hyalellids inhabit different freshwater environments, associated with either the bottom sediments (benthic fauna) or the aquatic vegetation (Poi de Neiff 1992), where these amphipods constitute a fundamental link in the transfer of matter and energy in those ecosystems (Casset et al. 2001, González et al. 2006, Castiglioni and Bond-Buckup 2008).

Currently, nine species of *Hyalella* have been recorded for Argentina: *H. curvispina* Shoemaker, 1942, *H. fossamancinii* Cavalieri, 1959, *H. pampeana* Cavalieri, 1968, *H. neonoma* Stock & Platvoet, 1991, *H. falklandensis* Bousfield, 1996, *H. rionegrina* Grosso & Peralta, 1999, *H. araucana* Grosso & Peralta, 1999, *H. kochi* González & Watling, 2001, and *H. bonariensis* Bond-Buckup, Araujo & Santos, 2008 (Santos et al. 2008, De los Ríos-Escalante et al. 2012). Although studies on the genus have increased in recent years, essential aspects of the taxonomy and ecology of *Hyalella* in Argentina still remain poorly known.

The Atlantic Forest of South America – a species-rich and ecologically highly complex system – is considered one of the biodiversity "hot spots" of the world (Myers et al. 2000). In Argentina, the Atlantic Forest includes the province of Misiones, where part of the remaining forest biome is partially protected by the Yabotí Biosphere Reserve.

The aim of this work was to describe a new species of freshwater amphipod of the genus *Hyalella* from the Atlantic Forest in Misiones, Argentina.

Materials and methods

The Yabotí Biosphere Reserve is located in the eastern central region of the Misiones province (Fig. 1). The climate is hot and humid without dry season, with an annual mean precipitation of 2000 mm and an annual mean temperature of 21 °C (Cabrera 1971).

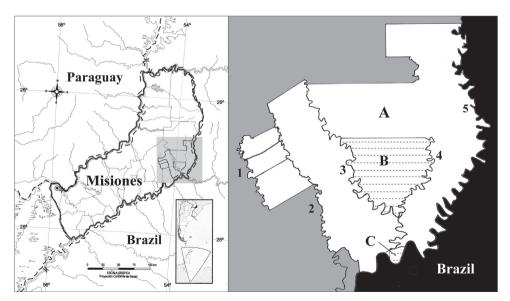


Figure 1. Map of the study area. Left panel: Location of the Province of Misiones, Argentina and the Yabotí Biosphere Reserve in the eastern-central portion. Right panel: **A** Yabotí Biosphere Reserve (entire white area) **B** Esmeralda Provincial Park (textured area) **C** Moconá Provincial Park (textured area). **I** Soberbio stream. **2** Paraíso stream. **3** Yabotí stream. **4** Yabotí Miní stream. **5** Pepirí Guazú River.

Amphipods were collected by hand from the epilithic vegetation (bryophytes) growing on the rocks of the waterfall Salto Isipós, near Paraíso stream (27°13.19'S; 54°02.73'W).

In the laboratory, the cephalothorax length (CL) and total length (TL) of 30 specimens (15 males and 15 females) was measured under a stereoscopic microscope with a milimetric scale (LEICA EZ4). The anatomical pieces were placed in semipermanent slides and the drawings realized by means of a drawing tube mounted on a microscope ocular (LEICA DMLS). The terminology used for the setae of the appendices follows Zimmer et al. (2009). Morphological description is generalized from 10 individuals dissected (5 males and 5 females). We have mentioned the variations when appropiate.

Type material is deposited on Colección de Carcinología, División Zoología Invertebrados (DZI), Facultad de Ciencias Naturales y Museo (FCNyM), Universidad Nacional de La Plata (UNLP), Argentina.

Taxonomy

Family Dogielinotidae Gurjanova, 1953 Subfamily Hyalellinae Bulycheva, 1957 Genus *Hyalella* Smith, 1874

Hyalella misionensis sp. n. http://zoobank.org/02941807-666D-4A05-8A3E-80DACD6F0FBA

Type material. Holotype male, Argentina, Province of Misiones, Yabotí Biosphere Reserve, San Pedro and Guaraní Departments, Salto Isipós (27°13.19'S; 54°02.73'W) (MLP 26978), October, 19, 2011. César, I. I. and Martín, S. M., collectors.

Paratypes. 15 males, 15 females, 7 ovigerous females, and 70 juveniles (MLP 26979), same data as holotype.

Type locality. Argentina, Province of Misiones, Yabotí Biosphere Reserve, San Pedro and Guaraní Departments, Salto Isipós (27°13.19'S; 54°02.73'W).

Diagnosis. Body surface smooth. Coxa 4 excavated posteriorly. Eyes pigmented. Antenna 1 shorter than antenna 2. Antenna 2 less than half the body length. Maxilla 1 palp short, reaching to less than half the distance between base of palp and tip of setae on outer plate; inner plate slender, with two strong, pappose apical setae. Maxilla 2 with two strong pappose setae on inner margin. Gnathopod 1 propodus length less than twice maximum width, hammer-shaped, inner face with six to nine serrate setae, comb scales on distoposterior border. Gnathopod 2 propodus ovate, palm shorter than posterior margin, distal margin of palm irregular. Pereiopods 3 and 4 merus and carpus posterior margin with three hind marginal clusters of short setae; propodus posterior margin with five groups of setae. Uropod 3, peduncle slender (rectangular), wider than ramus, with five strong distal setae, basal width more than twice apex of ramus. Telson as long as wide, entire, apically

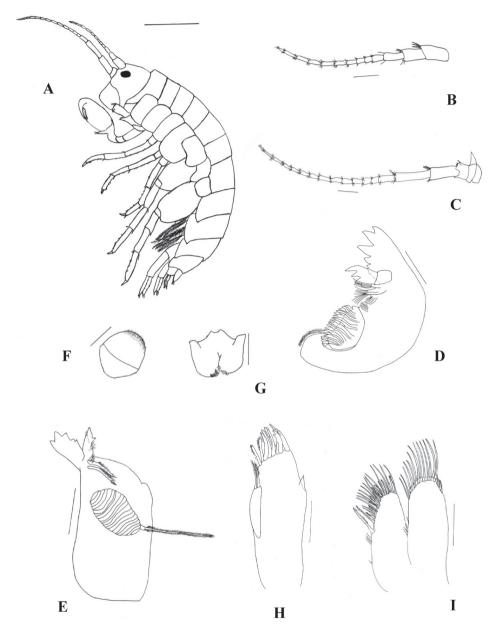


Figure 2. *Hyalella misionensis* sp. n., male. **A** holotype and habitus **B** antenna 1. **C** antenna 2 **D** left mandible **E** right mandible. **F** upper lip **G** lower lip **H** maxilla 1. I maxilla 2. Scale bar equals 1 mm for **A**; 200 μm for **B**, **C**, **F**, and **G**; 100 μm for **D**, **E**, **H**, and I.

rounded, bearing two long simple setae symmetrically distributed on distal margin, and three small submarginal setae close to each main setae. Sternal gills present on segments 2 to 7.

Description of male (Figs 2 to 5). Mean body length: 5.9 ± 1.09 mm; mean cephalothorax length: 0.61 ± 0.08 mm (n = 15). Body surface smooth. Epimeral plate 1, 2, and 3 acuminate. Coxae 1 to 4 subequal in size and shape, slightly overlapping. Acumination in coxae absent. Coxa 1 similar to 2 and 3. Coxa 3 narrower than 4. Coxa 4 as wide as deep, excavated posteriorly. Coxa 5 posterior lobe deeper than anterior lobe. Coxa 6 posterior lobe deeper than anterior lobe, anterior lobe small.

Head typically gammaridean, as long as the first two thoracic segments, rostrum absent. Eyes pigmented, medium, rounded, located between insertion of antenna 1 and antenna 2 (Fig. 2A).

Antenna 1 (Fig. 2B) less than half the body length, shorter than antenna 2 but longer than peduncle of antenna 2; peduncle as long as head; article 3 shorter than article 1 and article 2 in length; flagellum of 10–11 articles, longer than peduncle; aesthetascs on flagellum, present on articles 4 (2 aesthetascs), 5 (2), 6 (3), 7 (1), and 8 (1).

Antenna 2 (Fig. 2C) less than half of the body length, peduncle longer than head, article 4 shorter than article 5, setal groups on articles 4 and 5 scarce, flagellum with 13–14 articles and longer than peduncle.

Mandible without palp; incisor toothed; left lacinia mobilis with five teeth; setal row on left mandible with three main pappose setae plus accessory setae (Fig. 2D), right mandible with two main pappose setae plus accessory setae; molar large, cylindrical, and triturative; accessory seta present (Fig. 2E).

Upper-lip ventral margin round (Fig. 2F).

Lower-lip outer lobes rounded without notches or excavations, mandibular projection of outer lobes truncated (Fig. 2G).

Maxilla 1 (Fig. 2H) palp short, uniarticulate, reaching to less than half the distance between base of palp and tip of setae on outer plate, distally pointed; inner plate slender, smaller than outer plate, with two strong, pappose apical setae; outer plate with nine stout and serrate setae.

Maxilla 2 (Fig. 2I) inner plate subequal in length and width to outer plate, with two strong pappose setae on mid-inner margin; outer and inner plates with abundant setules.

Maxilliped (Fig. 3A) inner plates apically truncated, with three connate setae and pappose setae apically and medially; outer plates larger than inner plates, apically truncated, apical, medial, and facial setae simple. Palp of four articles: article 2 wider than long, medial margin with long simple setae; article 3 outer distal face (at the base of article 4) with several long simple setae, inner distal face with long plumose setae, inner distal margin with long setae, outer margin with one or two strong and long plumose setae; dactylus unguiform, shorter than article 3, distal setae simple and shorter than nail, inner margin with setae, distal nail present.

Gnathopod 1 (Fig. 3B and C) subchelate; carpus longer than wide, longer and wider than propodus, with strong and wide posterior lobe, and forming a scoop-like structure, open to the inside, inner face with five serrate setae; propodus (Fig. 3D) length less than two times maximum width, hammer-shaped, with no setae on anterior border, with three simple setae on posterior border; inner face (Fig. 3E) with six to nine serrate setae, several small triangular setae, comb scales on distoposterior border,

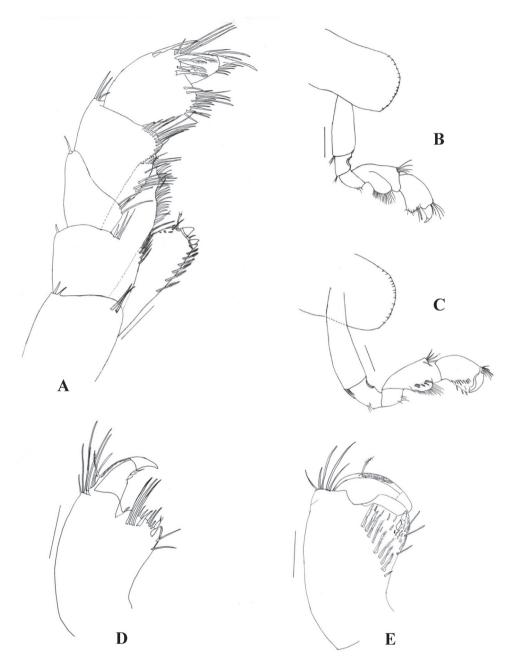


Figure 3. *Hyalella misionensis* sp. n., male. **A** maxilliped **B** dorsal view of gnathopod 1 **C** ventral view of gnathopod 1 **D** dorsal view of propodus of gnathopod 1 **E** ventral view of propodus of gnathopod 1. Scale bar equals 100 μ m for **A**, **D**, and **E**; 200 μ m for **B** and **C**.

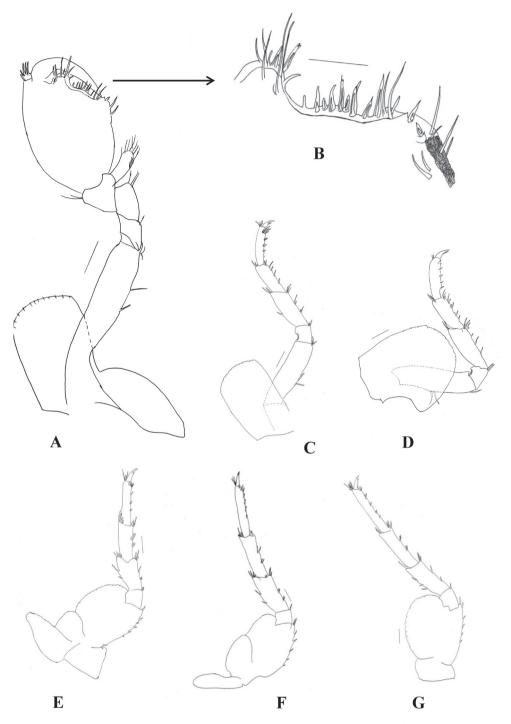


Figure 4. *Hyalella misionensis* sp. n., male. **A** gnathopod 2 **B** detail of the palm of gnathopod 2 **C** pereiopod 3 **D** pereiopod 4 **E** pereiopod 5 **F** pereiopod 6 **G** pereiopod 7. Scale bar equals 200 μ m for **A**, **C**, **D**, **E**, **F**, and **G**; 100 μ m for **B**.

palm slope transverse, margin convex, posterior distal corner with robust setae, dactylus claw-like with comb scales.

Gnathopod 2 (Fig. 4A) subchelate; basis hind margin with two long setae; merus with less than seven setae on posterior margin, posterodistal margin straight, distal corner rounded; carpus posterior lobe elongated, produced between merus and propodus, distal end of carpal lobe with cuticular denticles and with several serrate setae; propodus ovate, distoposterior border with comb scales, palm (Fig. 4B) shorter than posterior margin, slope oblique, margin irregular, bearing several strong short setae, anterior edge with a wide truncated or rounded process, posterior distal corner with strong setae and with cup for dactylus; dactylus claw-like, as long as palm, with seven short simple setae symmetrically distributed on inner border. Triangular space between propodus and dactylus when dactylus is closed.

Pereiopods 3 to 7 simple. Pereiopods 3 (Fig. 4C) and 4 (Fig. 4D) with merus and carpus posterior margin having three hind marginal clusters of short setae; propodus posterior margin with five groups of setae; dactylus less than half the propodus length. Pereiopods 5 to 7, all similar in structure and successively slightly longer; dactylus less than half the propodus length. Pereiopod 5 (Fig. 4E) longer than pereiopod 4, basis posterior lobe longer than wide, smaller than posterior lobe longer than wide, larger than posterior lobe of pereiopod 7. Pereiopod 7 (Fig. 4G) slightly longer than pereiopod 6, basis posterior lobe longer than wide.

Pleopods (Fig. 5A) not modified; peduncle slender; longest ramus longer than peduncle.

Uropod 1 (Fig. 5B) longer than uropod 2; peduncle longer than rami, with 4-5 dorsal setae; rami subequal; inner ramus with 2–3 dorsal setae and 5 distal setae; male without curved setae on inner side of the ramus; outer ramus with 3–4 dorsal setae and 5 distal setae.

Uropod 2 (Fig. 5C) peduncle as long as rami, with 3 dorsal setae; rami subequal; inner ramus with 3 dorsal setae and 6 distal setae, outer ramus with 4 dorsal and 4 distal setae.

Uropod 3 (Fig. 5D) as long as peduncle of uropod 2; peduncle slender (rectangular), wider than ramus, with 5 strong distal setae of variable length, inner ramus absent; outer ramus uniarticulate, as long as peduncle, basal width more than twice apex of ramus, with 4–5 simple slender apical setae and one connate seta.

Telson (Fig. 5E) as long as wide, entire, apically rounded, bearing two long simple setae symmetrically distributed on distal margin, and three small setae close to each main seta.

Coxal gills sac-like, present on segments 2 to 6. Sternal gills tubular, present on segments 2 to 7.

Female (Fig. 6). Mean total length: 4.52 ± 0.71 mm; mean cephalothorax length: 0.51 ± 0.07 mm (n = 15). Antenna 1 (Fig. 6A) flagellum of 9–10 articles. Antenna 2 (Fig. 6B) similar in length and shape to male, flagellum of 15 articles.

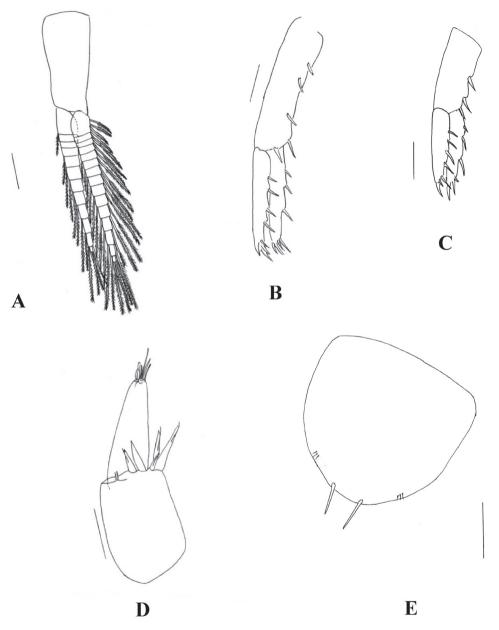


Figure 5. *Hyalella misionensis* sp. n., male. **A** pleopod **B** uropod 1 **C** uropod 2 **D** uropod 3 **E** telson. Not all setae are represented in the figures. Scale bar equals 200 µm for **A**, **B**, and **C**; 100 µm for **D** and **E**.

Gnathopod 1 (Fig. 6C and D) similar in size and shape to gnathopod 2. Propodus inner face with six serrate setae (Fig. 6E). Gnathopod 2 (Figs 6F and G) propodus of length less than two times maximum width, normally subchelate, palm transverse. Propodus inner face with two serrate setae.

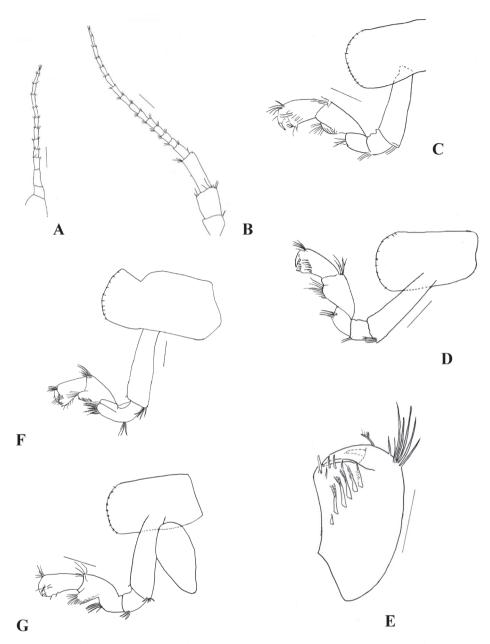


Figure 6. *Hyalella misionensis* sp. n., female. **A** antenna 1 **B** antenna 2 **C** dorsal view of gnathopod 1 **D** ventral view of gnathopod 1 **E** ventral view of propodus of gnathopod 1 **F** dorsal view of gnathopod 2 **G** ventral view of gnathopod 2. Scale bar equals 200 µm for **A**, **B**, **C**, **D**, **F**, and **G**; 100 µm for **E**.

Habitat. Freshwater, epigean.

Distribution. Argentina, Province of Misiones, Yabotí Biosphere Reserve, San Pedro and Guaraní Departments, Salto Isipós (27°13.19'S; 54°02.73'W).

Species	Curved setae in inner ramus of uropod 1	Articles of flagellum of antenna 1	Articles of flagellum of antenna 2	Sternal gills	Inner face of propodus of gnathopod 1	Telson
H. curvispina	Present	11	13	3–7	5–7 setae	Wider than long, with 3 simple spines.
H. pampeana	Present	11–12	Up to 18	2–7	5–6 setae	As wide as long, apically rounded, with 2–5 spines.
H. falklandensis	Present	10	12	2–6	5 setae	Wider than long, broadly rounded apex with 4–5 short fine spines.
H. bonariensis	Present	9–12	12–15	2–7	5 setae	Quadrangular, with 2–3 cuspidate setae apically.
H. kochi	Present	9	11	3–7	7 setae	As wide as long, apically truncated, with two long simple setae.
H. fossamancinii	Absent	9–10	9–14	3–7	More than 10 setae	Wider than long, with more than two small or minute setae.
H. neonoma	Absent	12	25	3–7	8–10 setae	Wider than long, apex rounded, with 3–4 very small slender spines.
H. araucana	Absent	9	12	3–7	10–11 setae	As wide as long, apically rounded, with more than two small or minute setae.
H. misionensis	Absent	10–11	13–14	2–7	6–9 setae	As long as wide, apically rounded, with two long simple setae on distal margin.
<i>H. rionegrina</i> (female)	?	4	5	2–7	?	Semieliptic, with 4 simple spines.

Table 1. Morphological characters of *Hyalella* species of Argentina and Falkland Islands. All characters are referred to males, with exception of *H. rionegrina*.

Etymology. The species name *misionensis* refers to the location where this new member was found, the Province of Misiones in Argentina.

Remarks. *H. misionensis* has some morphological similarities to *H. pampeana* Cavalieri, 1968, a common freshwater amphipod of the Province of Buenos Aires. The principal similarity is the shape of gnathopod 2 in the males, with a triangular space between the propodus and the dactylus in both species; the number of antennal segments (*H. misionensis*, antenna 1: 10–11 articles and antenna 2: 13–14 articles; *H. pampeana*, antenna 1: 11–12 articles and antenna 2: up to 18); and the total length (*H. misionensis*: 5.9 mm, *H. pampeana*: 5 mm). Although, the two species differ in the presence of a curved seta in the inner ramus of uropod 1 in the males of *H. pampeana*; this seta is absent in *H. misionensis*. The width of the propodus of gnathopod 1 is about 3/4 of its length in *H. pampeana*, but about 2/3 of its length in *H. misionensis*. In addition, the inner face of propodus in gnathopod 1 of *H. pampeana* has 5 to 6 pappose setae, but in *H. misionensis* has 6 to 9 serrate setae. The setation of the telson is also

Species	Body surface	Length of antenna 2	Inner margin of maxila 2	Inner face of propodus of gnathopod 1	Telson	Type locality
H. misionensis	Smooth	Less than half the body length	Two pappose setae	6–9 serrate setae	As long as wide, with 2 simple setae	Salto Isipós, Province of Misiones, Argentina
H. castroi	Smooth	More than half the body length	One pappose setae	More than 10 serrate setae	Wider than long, with 8 setae	Vale das Trutas, Rio Grande du Sul, Brazil
H. pleoacuta	With dorsal flanges on some segments	More than half the body length	Two pappose setae	9 serrate setae	As long as wide, with 2 simple setae	Vale das Trutas, Rio Grande du Sul, Brazil
H. gracilicornis	Smooth	More than half the body length	One pappose setae	4 pappose setae	As long as wide with 2 simple setae	Campos, Rio de Janeiro, Brazil
H. longistila	Smooth	More than half the body length	One pappose setae	5 pappose setae	Longer than wide, with 2 simple setae	Swamp 3 miles from Campos, Rio de Janeiro, Brazil
H. warmingi	Smooth	More than half the body length	One pappose setae	10 pappose setae	Longer than wide, with 2 simple setae	Lagoa Santa, Minas Gerais State, Brazil

Table 2. Main morphological differences between *Hyalella misionensis* and five *Hyalella* species from nearby areas in Brazil.

different with 2 to 5 setae of variable length in *H. pampeana* and only two simple setae in *H. misionensis*. Comparisons between morphological characters of *Hyalella* species of Argentina and Falkland Islands are presented in Table 1.

The study area where *Hyalella misionensis* was found is geographically close to Brazil, where fourteen species of the genus have been reported (Bueno et al. 2013). In Table 2 we compared the main morphological characters of *H. misionensis* with those of the Brazilian *Hyalella* species that were geographically close to where the newly described species was found: *H. castroi* Gonzalez, Bond Buckup & Araujo, 2006, *H. pleoacuta* Gonzalez, Bond Buckup & Araujo, 2006, *H. gracilicornis* Faxon, 1876, *H. longistila* Faxon, 1876 and *H. warmingi* Stebbing, 1899 (Gonzalez and Watling 2003, Gonzalez et al. 2006).

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RESEARCH ARTICLE



A review of Solenysa spiders from Japan (Araneae, Linyphiidae), with a comment on the type species S. mellotteei Simon, 1894

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Abstract

The present paper gives a review of *Solenysa* species from Japan and provides a solution for the species bearing the generotype name *S. mellotteei* Simon, 1894. A total of six species are recorded, including two new species *S. macrodonta* **sp. n.** and *S. trunciformis* **sp. n.** The species collected from Kawasaki (NSMT-Ar 11154) and Hachioji should be the generotype *S. mellotteei*, with *S. akihisai* Tu, 2011, **syn. n.** as its junior synonym. To distinguish these congeneric species from each other, their genital characters are provided in detail based on images collected by scanning electron microscopy and light microscopy.

Keywords

Genitalia, morphology, new species, taxonomy

Introduction

The spider genus *Solenysa* was erected by Simon (1894) to accommodate the linyphiid species, *S. mellotteei* Simon, 1894, which was collected from Japan by a French diplomat, A. Mellottée. Other *Solenysa* species were described successively from other places in Japan, the Chinese mainland, Taiwan, and the Korean Peninsula (see review by Tu and Li 2006). In recent studies, several new species were sorted from the

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Solenysa collections deposited in the Department of Zoology, National Museum of Nature and Science (ex National Science Museum, Tokyo), Japan (Tu et al. 2007, Tu and Hormiga 2011, Ono 2011). Prior to this study, there were five *Solenysa* species reported from Japan: *S. mellotteei* Simon, 1894 (type locality: Yokohama, Kanagawa Prefecture), *S. akihisai* Tu, 2011 (type locality: Hachioji, Tokyo), *S. ogatai* Ono, 2011 (type locality: Okazaki-shi, Aichi Prefecture), *S. partibilis* Tu, Ono & Li, 2007 (type locality: Itsuki-mura, Kumamoto Prefecture). According to results of a phylogenetic analysis based on morphological data, the twelve known *Solenysa* species were divided into four groups, and the four species from Japan share a complex of genital characters, forming the *S. mellotteei* group (Tu and Hormiga 2011).

As more species were recognized, a problem regarding the type species of Solenysa emerged. Generally, the Solenysa species occurring in Japan are endemic, have a restricted distribution with little overlap (Fig. 7). Small in body size, similar in somatic features and genital morphology, it is difficult to distinguish them from each other without examining their genitalia in detail (Tu and Hormiga 2011). Consequently, all Solenysa spiders collected from the islands of Japan have long been identified as S. mellotteei (Oi 1960, Yaginuma 1986, Irie and Saito 1987, Chikuni 1989, Lee et al. 2004, Tu and Li 2006). Redescriptions for the species currently bearing the generotype name, S. mellotteei, in reviews of Solenysa were not based on the type material (Tu and Li 2006, Tu and Hormiga 2011) and the species are different from those collected from the places more adjacent to the inferred type locality (Ono 2011). It is necessary to make a review to distinguish the species of the S. mellotteei group and to establish the identity of the generotype S. mellotteei. From the materials collected throughout the islands of Japan, we identified six species in total, including two new species and one new synonymy. In the present study, all these Solenysa spiders were studied by using scanning electric microscopy (SEM) and light microscopy to show genital characters in detail. Descriptions for the new species and redescriptions for the known species are presented.

Materials and methods

Specimens were examined and measured by using a Leica MZ16A stereo microscope. Further details, such as epigynes, were studied with a Leica DM5500B compound microscope. Digital images were taken with a Leica DFC 500 camera and as a composite of multiple focus images assembled using the software package Leica Application Suite. Epigynes were cleared in methyl salicylate (Holm 1979) for examination under the microscope and temporarily mounted as described by Grandjean (1949) and Coddington (1983). SEM images were taken by using a Hitachi S-3400N scanning electron microscope at China Agriculture University. For SEM examination, the

specimens were prepared as described by Álvarez-Padilla and Hormiga (2008). The non-chitinous abdominal tissue was digested with Sigma Pancreatin LP 1750 enzyme complex to expose the internal structures for examination. Due to the unavailability of specimen, no SEM image provided for the male palp of *S. reflexlis*.

All measurements are given in millimeters. The leg measurements are given in the following sequence: Total (femur, patella+tibia, metatarsus, tarsus). Terminology for the genital characters follows Tu and Hormiga (2011). The specimens examined here have been deposited in the Department of Zoology, National Science Museum, Tokyo, Japan (NSMT) and in College of Life Sciences, Capital Normal University, Beijing (China).

Anatomical abbreviations used in the text and figures

Male palp

ATA	anterior terminal apophysis
DSA	distal suprategular apophysis
Ε	embolus
LC	lamella characteristica
LC ₁	anterior branch of LC
LC ₂	median branch of LC
LC_{3}	posterior branch of LC
MŤA	median terminal apophysis
Р	paracymbium
PBP	cymbial probasal process
PTA	posterior terminal apophysis
R	radix
STT	<i>solenysa</i> tegular triangle
Т	tegulum

Epigyne

- CG copulatory groove
- **CO** copulatory opening
- **DP** dorsal plate
- EC epigynal collar
- FG fertilization groove
- **S** spermatheca
- SL solenoid
- VP ventral plate

Taxonomy

Linyphiidae Blackwall, 1859

Solenysa Simon, 1894

Type species. Solenysa mellotteei Simon, 1894.

Composition. Fourteen species including two new species: Solenysa geumoensis Seo, 1996, S. lanyuensis Tu, 2011, S. longqiensis Li & Song, 1992, S. macrodonta sp. n., S. mellotteei Simon, 1894, S. ogatai Ono, 2011, S. partibilis Tu, Ono & Li, 2007, S. protrudens Gao, Zhu & Sha, 1993, S. reflexilis Tu, Ono & Li, 2007, S. retractilis Tu, 2011, S. tianmushana Tu, 2011, S. trunciformis sp. n., S. wulingensis Li & Song, 1992 and S. yangmingshana Tu, 2011.

Diagnosis. *Solenysa* species can be distinguished from all other linyphilds by the four lobes at the sides of carapace, the rounded pits scattered on the carapace and the tubular-shaped petiole (Fig. 1A–B). Females are also diagnosed by the presence of a long membranous solenoid, connecting between the epigyne and the abdomen (Fig. 1D), males by the presence of *Solenysa* tegular triangle in male palp (Fig. 2A).

Description. See Tu and Li (2006) and Tu and Hormiga (2011).

Distribution. Japan, Chinese mainland, Taiwan, Korea.

Comments. The subfamily placement of *Solenysa* remains controversial as its complex type of male palp with well developed lamella characteristica and terminal apophysis is like those in Micronetinae Hull, 1920, but the simple type of epigyne is like those in Erigoninae Emerton, 1882. Based on the movable epigyne, Saaristo (2007) included it in his new subfamily Ipainae Saaristo, 2007. However, the results of a phylogenetic analysis of Linyphiidae queried the monophyly of "ipaines", and suggested that "micronetines" and erigonines form a monophyletic group (Arnedo et al. 2009). Furthermore, the results of a phylogenetic analysis of erigonines based on morphological data showed that all *Solenysa* species form a monophyly robustly supported by a long list of synapomorphies, and other synapomorphies suggested its close relationship with erigonines although its sister group remained unresolved (Tu and Hormiga 2011). Accordingly, the well-developed lamella characteristica and terminal apophysis in Solenysa should be regarded as homologous to those of "micronetines" and secondarily lost in erigonines; their simple type epigyne also derived from the complex type of "micronetines". The morphology of solenoid in Solenysa is different from the extensive basal parts in Acanoides beijingensis Sun, Marusik & Tu, 2014 and A. hengshanensis (Chen & Yin, 2000) (Sun et al. 2014: figs 4G, 5G), and in Wubanoides uralensis (Pakhorukov, 1981), Epibellowia enormita (Tanasevitch, 1988) and E. septentrionalis (Oi, 1960) (Tanasevitch 1996: figs 7–9). Whether the movable epigyne has a single origin or independently evolved multiple times in linyphiids needs to be tested in future studies.

A phylogenetic analysis based on morphological data (Tu and Hormiga 2011) suggested that the twelve known *Solenysa* species are divided into four clades. Among

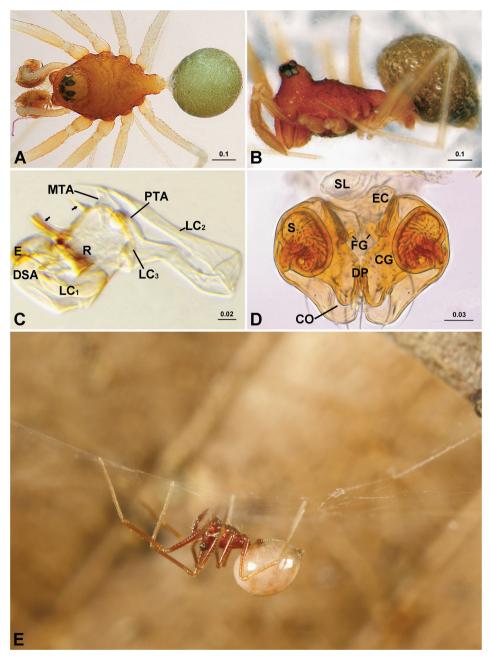


Figure 1. *Solenysa trunciformis* sp. n. (**A–D**) and *S. partibilis* (**E**). **A** male, dorsal **B** female, lateral **C** male palpal embolic division, ventral, arrows indicate two anterior protrusions of MTA **D** epigyne, dorsal **E** female, lateral in living state, showing non-functional state of epigyne. **CO** copulatory opening; **CG** copulatory groove; **DP** dorsal plate; **DSA** distal suprategular apophysis; **E** embolus; **EC** epigynal collar; **FG** fertilization groove; **LC** lamella characteristica; **LC**₁ anterior LC branch; **LC**₂ median LC branch; **LC**₃ posterior LC branch; **MTA** median terminal apophysis; **PTA** posterior terminal apophysis; **R** radix; **S** spermatheca; **SL** solenoid. Photo of *S. partibilis* provided by Akihisa Andoh. [Scale bars: mm]

them, the four species occurring in Japan formed a monophyletic clade, unambiguously supported by the following synapomorphies: the presences of hook shaped cymbial probasal process, half rounded *Solenysa* tegular triangle and copulatory grooves enter the spermathecae from the outer sides.

Solenysa mellotteei group Tu & Hormiga, 2011

Composition. Six species: *Solenysa mellotteei* Simon, 1894, *S. macrodonta* sp. n., *S. ogatai* Ono, 2011, *S. partibilis* Tu, Ono & Li, 2007, *S. reflexilis* Tu, Ono & Li, 2007 and *S. trunciformis* sp. n.

Diagnosis. Males of *S. mellotteei* group are distinguished from all other three groups by the spiral plate-shaped embolus (Fig. 3E), the hook-shaped cymbial probasal process and by the half rounded *Solenysa* tegular triangle (Fig. 2A). Females are characterized by the dorsoventrally folded solenoid (Figs 4C, 5C), the spherical spermathecae and the pocket shaped copulatory grooves entering the spermathecae from the outer sides (Fig. 1D).

Description. All *Solenysa* species have quite uniform somatic morphology. Somatic characters as in the genus description (see also Tu and Li 2006, Tu and Hormiga 2011).

Male palp (Fig. 2A–B). Tibia twice as long as patella, with proximal process furnished by two long bristles. Cymbium with hook-like proximal process and small retrolateral process, forming articulation with proximal arm of U-shaped paracymbium. Tegulum with half rounded *Solenysa* tegular triangle and stout distal suprategular apophysis. Embolic division (Fig. 6): embolus spiral plate shaped with two apophyses, one at outer margin, and one distally (Fig. 3E). Radix embedded within membranous area connecting terminal apophysis and lamella characteristica (Figs 1C, 2B). Terminal apophysis divided into three parts, with median one as enlarged sclerite. Lamella characteristica with three well-developed branches, anterior branch (LC₁) stout and extending forward, following embolus trajectory; median one (LC₂) long and slender, dragging backwards and pointing forward, bifid in some species (Fig. 3A); posterior one (LC₃) sharp and strongly sclerotized, bifid in some species (Fig. 3B).

Epigyne (Figs 1D, 4A–B). Strongly sclerotized box-shaped, having a well-developed epigynal collar at anterior part connecting with solenoid. Solenoid flexible, dorsoventrally folded (Fig. 5C) in non-functional stage, holding epigyne up (Fig. 1E). Spermathecae large spherical. Copulatory openings as a pair of crescent shaped slits hidden on dorsal surface. Copulatory grooves enlarged into half round pocket-shaped, matching to spiral plate-shaped embolus, entering spermathecae outboard. Fertilization grooves convergent, extending forward.

Distribution. Japan (Honshu, Shikoku, Kyushu, Fig. 7).

Solenysa mellotteei Simon, 1894

Figs 2, 4A-B, 6A

S. mellottei Simon, 1894: 677; Lee et al. 2004: 100; Ono et al. 2009: 330, figs 1087–1091.

S. mellotteei: Yaginuma 1986: 78, fig. 42.2; Irie and Saito 1987: 23, fig. 21; Chikuni 1989: 56, fig. 48.

S. akihisai: Tu and Hormiga 2011: 499, fig. 8A-I.

Material examined. 1 and 1 (NSMT-Ar 11154), Japan, Honshu, Kanagawa Prefecture, Kawasaki-shi, Asao-ku, Kurokawa, 35°32'N, 139°43'E, 15 Nov. 1997, coll. Mitsuru Ban; 1 and 2 \bigcirc , Japan, Honshu, Tokyo, Hachioji, 35°42'N, 139°18'E, 20 Dec. 2003, coll. Akihisa Andoh; 3 and 3 \bigcirc (CNU-J02), Japan, Honshu, Ibaraki Prefecture, Mito-shi, Tara, 36°24.35'N, 140°24.55'E, 27 Nov. 2000, coll. Akihisa Andoh; 3 and 7 \bigcirc , Japan, Honshu, Tokyo, Hachioji, Kamikawa, 35°42.55'N, 139°15.23'E, alt. 230 m, 9 Nov. 2008, coll. Akihisa Andoh; 5 and 2 \bigcirc (CNU-J22), Japan, Honshu, Kanagawa Prefecture, Miura, Ko-ajiro, 35°09.88'N, 139°37.65'E, alt. 20 m, 1 Mar. 2008, coll. Akihisa Andoh; 2 \land (CNU-J32), Japan, Honshu, Ibaraki Prefecture, Mito, Tano, 36°24.55'N, 140°24.38'E, alt. 45 m, 13 Jun. 2009, coll. Akihisa Andoh.

Diagnosis. Solenysa mellotteei is similar to S. partibilis and S. ogatai in male palps having the posterior branch of lamella characteristica (LC_3) divided into two parts (Fig. 6A, C, D), and in females having an apple-shaped epigyne. Males can be distinguished by: the anterior part of LC_3 is flag-shaped in S. mellotteei (Fig. 2B), long spike-shaped in S. ogatai (Fig. 3C) and S. partibilis (Fig. 3D); the posterior part of LC_3 S-curved in S. ogatai (Fig. 3C), L-curved in S. partibilis (Fig. 3D). Females can be distinguished by the inverse triangular epigynal collar and the dorsal plate as wide as long in S. mellotteei (Fig. 4A, Tu and Hormiga 2011: fig. 8I), the dorsal plate wider than long in S. partibilis and S. ogatai (Fig. 5B, D), and the epigynal collar more than four times wider than long in S. ogatai (Fig. 11I).

Description. *S. mellotteei* has somatic morphology typical of *Solenysa* (Fig. 1A, B, E) and a genital pattern of the *S. mellotteei* group (Fig. 2A–B). For somatic and genital characters, see the description provided by Tu and Hormiga (2011) for *S. akihisai*, the junior synonym of *S. mellotteei*.

Distribution. Japan (Honshu, Fig. 7).

Comments. The problem with the identification of the generotype *Solenysa mellotteei* arose because *Solenysa* species occurring in Japan, previously all identified as *S. mellotteei*, are now distinguished as six species. Since most of them have restricted distributions without any overlap (Fig. 7), it has long remained ambiguous which species is the original *S. mellotteei* described by Simon (1894). The type material of *S. mellotteei* was not located (Tu and Li 2006), and the original description by Simon (1894) did not provide detailed information about the type locality. According to Ono

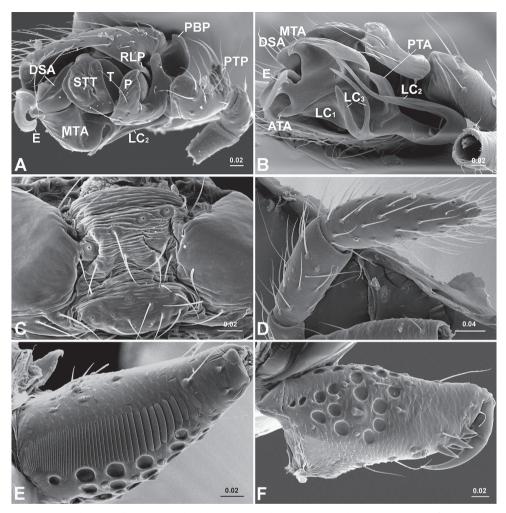


Figure 2. *Solemysa mellotteei.* **A** male palp, retrolateral **B** ditto, ventral **C** anterior part of male abdomen, ventral, shows epiandrous fusules absent and smooth book lung cover **D** female palp, shows tarsus claw absent **E** male chelicera, ectal, shows stridulatory striae **F** female chelicerae. **ATA** anterior terminal apophysis; **DSA** distal suprategular apophysis; **E** embolus; **LC** lamella characteristica; **LC**₁ anterior LC branch; **LC**₂ median LC branch; **LC**₃ posterior LC branch; **MTA** median terminal apophysis; **P** paracymbium; **PBP** probasal cymbial process; **PTA** posterior terminal apophysis; **PTP** proximal tibial process; **RLP** cymbial retrolateral process; **STT** *Solenysa* tegular triangle; **T** tegulum. [Scale bars: mm]

(2011), the French diplomat A. Mellottée, who had spent only two years in Japan, stayed in the foreign settlement at Yokohama and collected spiders in the surrounding area. All his collections were contributed to the National Museum of Natural History, Paris (Ono 1987, Takahashi 2000) and studied by Simon (1886a, 1886b, 1889, 1893, 1894, 1895). For that reason, Ono (2011) inferred the type locality of *S. mellotteei* should be Yokohama, Kanagawa Prefecture. In the first review of the genus by Tu and

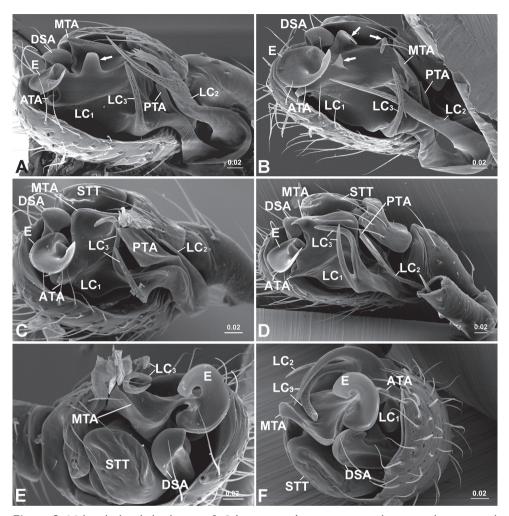


Figure 3. Male palpal embolic division. **A** *Solenysa macrodonta* sp. n., ventral, arrow indicates central tooth **B** *S. trunciformis* sp. n., ventral, arrows indicate central tooth and two anterior protrusions of MTA **C** *S. ogatai*, ventral **D** *S. partibilis*, ventral **E** *S. ogatai*, anterior **F** *S. partibilis*, anterior. **ATA** anterior terminal apophysis; **DSA** distal suprategular apophysis; **E** embolus; **LC** lamella characteristica; **LC**₁ anterior LC branch; **LC**₂ median LC branch; **LC**₃ posterior LC branch; **MTA** median terminal apophysis; **PTA** posterior terminal apophysis; **STT** *Solenysa* tegular triangle. [Scale bars: mm]

Li (2006), the redescription of *S. mellotteei* was based on a pair of specimens sent by a Japanese scholar and did not include any collecting data. In the phylogenetic revision of *Solenysa* (Tu and Hormiga 2011), the supplementary material of the same species did not come from the type locality, but from Esuzaki, Susami-cho, Wakayama Prefecture. However, specimens collected from Hachioji, Tokyo, which is much closer to the type locality (Fig. 7), were proposed as a new species *S. akihisai*. In the present study we examined material collected from three localities adjacent to Yokohama: Hachioji,

Kawasaki (NSMT-Ar 11154) and Miura (Fig. 7), as well as specimens from Mito, and found that they are the same species, which should bear the generotype name *S. mellotteei*, and *S. akihisai* is a junior synonym of it. The materials collected from Wakayama Prefecture, and those from Shikoku Island are proposed here as a new species *S. trunciformis* sp. n.

Solenysa macrodonta sp. n.

http://zoobank.org/E937495C-A852-4FB6-8739-F40F5AA5C1E8 Figs 3A, 4C–D

Types. Male holotype (CNU-J21), Japan, Honshu, Shimane Prefecture, Yunotsu, Nishida, 35°05.06'N, 132°24.10'E, 27 Jul. 2006, coll. Akihisa Andoh. Paratype, 1, same data as holotype.

Diagnosis. The male palp of *Solenysa macrodonta* sp. n. is similar to those of *S. trunciformis* sp. n. and *S. refrexilis* in the presence of a central tooth at the membranous area embedded the radix (Figs 1C, 3A, 6B), the forked apex of the median branch of lamella characteristica and the long spike-shaped posterior branch (Fig. 3A–B). They can be distinguished from each other by the median part of terminal apophysis, which has a serrate margin in *S. macrodonta* sp. n. (Fig. 3A), but with two anterior protrusions in *S. trunciformis* sp. n. (Fig. 3B) and *S. refrexilis* (Tu et al. 2007: fig. 1D), which is truncate in the former species and pointed in the latter species. The short epigyne of *S. macrodonta* sp. n. is similar to those of *S. partibilis* and *S. reflexilis*, having the dorsal plate wider than long (Fig. 5D). They can be distinguished from each other by the anterior part in *S. partibilis* (Fig. 5C), in the middle in *S. macrodonta* sp. n. (Fig. 4C), and posterior in *S. reflexilis* (Fig. 4E), which also has a straight posterior margin.

Description. Male holotype. Total length 1.33. Carapace, 0.8 long, 0.48 wide. Abdomen, 0.53 long, 0.38 wide. Chelicera with four promarginal and two retromarginal teeth. Length of legs: I 2.53 (0.68 + 0.80 + 0.58 + 0.47); II 2.25 (0.60 + 0.66 + 0.50 + 0.49); III 1.69 (0.47 + 0.50 + 0.39 + 0.33); IV 1.98 (0.61 + 0.64 + 0.43 + 0.30). Tm I: 0.23, Tm IV absent. Measurements for the female were not possible since the single specimen was prepared for SEM examination. Other somatic characters are as in the genus description (Fig. 1A, B, E; see also Tu and Li 2006, Tu and Hormiga 2011).

Male palp (Fig. 3B). General male palpal characters are as in the description for the *S. mellotteei* group. Embolic division (Fig. 6B): radix embedded in the central membranous area connecting with terminal apophysis and lamella characteristica, from where a central tooth protrudes. Median part of terminal apophysis as large sclerite with serrated margin. Anterior branch of lamella characteristica reduced, stout and extending forward following embolus; the median branch ribbon-like,

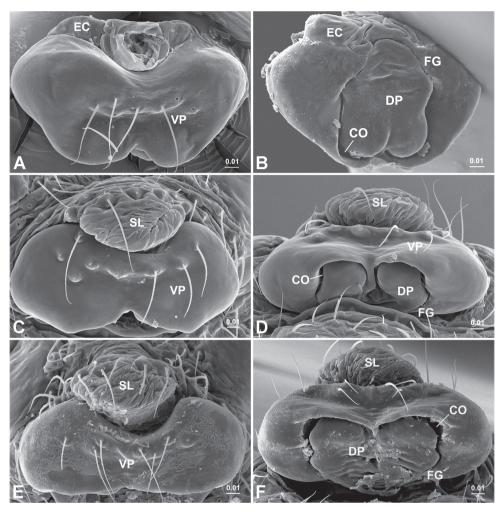


Figure 4. Epigyne. *Solenysa mellotteei* (**A**–**B**), *S. macrodonta* sp. n. (**C**–**D**), *S. reflexilis* (**E–F**). **A, C, E** ventral, **A** with epigynal collar removed **B, D, F** dorsal. **CO** copulatory opening; **DP** dorsal plate; **EC** epigynal collar; **FG** fertilization groove; **VP** ventral plate; **SL** solenoid. [Scale bars: mm]

long and slender, dragging backwards, then folding forward, with forked apex, one sharp, one with threaded margin; the posterior long spike-shaped and strongly sclerotized.

Epigyne (Fig. 4C–D). Twice as wide as long in ventral view, with maximum width in the middle. Posterior margin centrally incised. Dorsal plate wider than long.

Etymology. The species name is based on the Latin '*macrodontus*' in reference to the large central tooth protruding from the membranous area connecting with terminal apophysis and lamella characteristica (Fig. 3A).

Distribution. Japan (Honshu, Fig. 7).

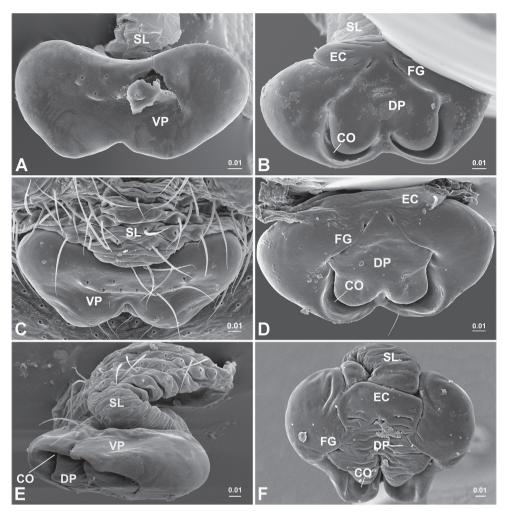


Figure 5. Epigyne. *Solenysa ogatai* (**A–B**), *S. partibilis* (**C–D**), *S. trunciformis* sp. n. (**E–F**). **A, C** ventral **B, D, F,** dorsal **E** lateral, with solenoid artificially loosened. **CO** copulatory opening; **DP** dorsal plate; **EC** epigynal collar; **FG** fertilization groove; **VP** ventral plate; **SL** solenoid. [Scale bars: mm]

Solenysa ogatai Ono, 2011 Figs 3C, E, 5A–B

Solenysa ogatai Ono, 2011: 126, figs 11-17.

Types. Male holotype (NSMT-Ar 9741), Japan, Honshu, Aichi Prefecture, Okazakishi, Okuyamada-cho, Mt. Murazumi-yama, alt. 200–250 m, 5 May 2011, coll. Kiyoto Ogata. Paratypes, 1 \bigcirc (NSMT-Ar 9742), same data as holotype; 2 \bigcirc \bigcirc and 2 \bigcirc \bigcirc (NSMT-Ar 9743), same data as holotype.

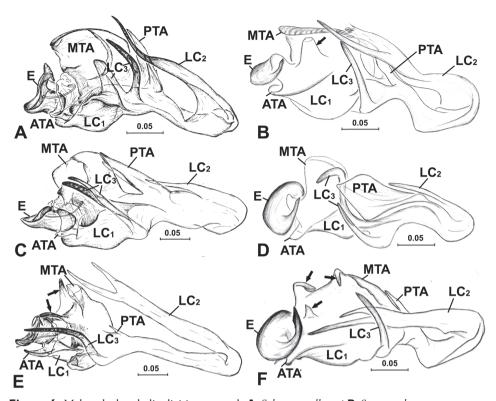


Figure 6. Male palpal embolic division, ventral. **A** *Solenysa mellotteei* **B** *S. macrodonta* sp. n., arrows indicate central tooth **C** *S. partibilis* **D** *S. ogatai* **E** *S. reflexilis*, arrows indicate two anterior protrusions **F** *S. trunciformis* sp. n., arrows indicate central tooth and two anterior protrusions of MTA. **ATA** anterior terminal apophysis; **E** embolus; **LC** lamella characteristica; **LC**₁ anterior LC branch; **LC**₂ median LC branch; **LC**₃ posterior LC branch; **MTA** median terminal apophysis; **PTA** posterior terminal apophysis; **STT** *Solenysa* tegular triangle. [Scale bars: mm]

Diagnosis. The genital characters of *S. ogatai* are very similar to those of *S. partibilis* (Figs 3C–F, 6C–D). The male palp is diagnosed by the posterior branch of the lamella characteristica with two long free ends, the longer one in *S. ogatai* is sigmoid curved in ventral view (Fig. 3C), almost a circle in anterior view (Fig. 3E), while in *S. partibilis* L-curved in ventral view (Fig. 3D), half circle in anterior view (Fig. 3F). The epigyne can be distinguished by the epigynal collar, which is more than four times wider than long in *S. ogatai* (Fig. 5B), but less than twice as wide than long in *S. partibilis* (Tu and Hormiga 2011: fig. 11I).

Description. Somatic characters as in the genus description and for genital characters see Ono (2011).

Distribution. Japan (Honshu, Fig. 7).

Solenysa partibilis Tu, Ono & Li, 2007

Figs 1E, 3D, F, 5C-D

S. melloteei: Oi 1960: 153, figs 52-54 (misidentification).

Solenysa partibilis Tu, Ono & Li, 2007: 60, fig. 2A–D; Ono et al. 2009: 332, figs 1092–099; Tu and Hormiga 2011: figs 11I, 12A–H, 13A–H.

Type. Male holotype (NSMT-Ar 2776), Japan, Honshu, Shiga Prefecture, Mt. Ibuki, 35°12'N, 136°12'E, 11 Nov. 1957, coll. Ryoji Oi.

Additional Material examined. 333 and 399, Japan, Honshu, Tokyo, Omeshi, Mitake, 35°48'N, E139°10.80'E, 17 Oct. 2004, coll. Akihisa Andoh; 3 and 3 \bigcirc (CNU-J01), Japan, Houshu, Tokyo, Ome-shi, Mitake, 35°48.08'N, E139°11.15′E, 17 Oct. 2004, coll. Akihisa Andoh; $3 \bigcirc \bigcirc$ (CNU-J25), Japan, Honshu, Fukushima Prefecture, Fukushima-shi, Kanayagawa, 37°41.42'N, 140°27.18'E, alt. 190 m, 28 Feb. 2009, coll. Akihisa Andoh; $2 \eth \eth$ and $3 \clubsuit \clubsuit$ (CNU-J31), Japan, Honshu, Shiga Prefecture, Maibara (base of Mt. Ibuki), Ohshimizu, 35°22.37'N, 136°24.08′E, alt. 190 m, 2 Jun. 2009, coll. Akihisa Andoh; 1♀ (CNU-J33), Japan, Honshu, Niigata Prefecture, Niitsu, Akihayama, 37°47.02'N, 139° 08.32'E, alt. 50 m, 20 Jun. 2009, coll. Akihisa Andoh; 1º (CNU-J34), Japan, Honshu, Niigata Prefecture, Niitsu, 37°46'N, 139°08.20'E, alt. 50 m, 20 Jun. 2009, coll. Akihisa Andoh; $2\Im$ (CNU-J35), Japan, Honshu, Akita Prefecture, Akita-shi, Katsurane, 39°39.32'N, 140°05.10'E, alt. 60 m, 2 Jul. 2009, coll. Akihisa Andoh; 4 \eth \eth and 7 \Im \Im (CNU-J36), Japan, Honshu, Miyagi Prefecture, Sendai, Mt.Takamori, 38°19.03'N, 140°56.17'E, 23 Aug. 2009, coll. Akihisa Andoh; $2\Im \Im$ and $1\Im$ (CNU-J39), Japan, Honshu, Tokyo, Ome, Yugi, 35°48.18'N, 139°11.98'E, alt. 240 m, 12 Sept. 2009, coll. Akihisa Andoh.

Diagnosis. See diagnosis for S. ogatai.

Description. Somatic characters as in the genus description (Fig. 1E, see also Tu and Li 2006, Tu and Hormiga 2011), and genital characters see the descriptions by Tu et al. (2007) and Tu and Hormiga (2011).

Distribution. Japan (Honshu, Fig. 7).

Solenysa reflexilis Tu, Ono & Li, 2007

Fig. 4E-F

Solenysa reflexilis Tu, Ono & Li, 2007: 58, fig. 1A–H; Ono et al. 2009: 332, figs 1100–1104.

Types. Male holotype (NSMT-Ar 3905), Japan, Kyushu, Kumamato Prefecture, Kumagun, Itsukimura, Shimo-kajiwara, 32°12'N, 130°30'E, 27 Oct. 1981, coll. Teruo Irie. Paratype, 1 $^{\circ}_{\circ}$ and 2 $^{\circ}_{\circ}_{\circ}$, same data as holotype.

Additional material examined. $2 \stackrel{\bigcirc}{\downarrow} \stackrel{\bigcirc}{\downarrow}$ (CNU-J28), Japan, Kyushu, Kumamoto Prefecture, Itsuki, Touji, 32°23.63'N, 130°49.67'E, alt. 310 m, 27 Apr. 2009, coll.

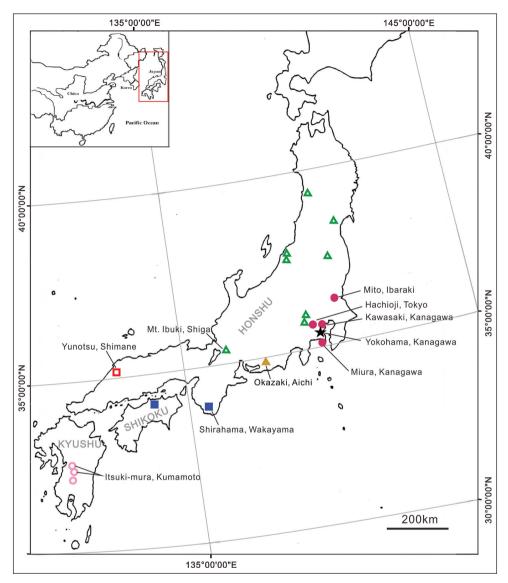


Figure 7. Collecting locations of *Solenysa* species from Japan. ● *S. mellotteei* □ *S. macrodonta* sp. n. ▲ *S. ogatai* ▲ *S. partibilis* ◎ *S. reflexilis* ■ *S. trunciformis* sp. n. ★ type locality of *S. mellotteei*.

Akihisa Andoh; 1 \bigcirc (CNU-J29), Japan, Kyushu, Kumamoto Prefecture, Sagara, 32°8.67'N, 130°51.53'E, alt. 590 m, 28 Apr. 2009, coll. Akihisa Andoh; 1 \bigcirc (CNU-J30), Japan, Kyushu, Kumamoto Prefecture, Sagara, Nagae, 32°18.67'N, 130°51.53'E, alt. 170 m, 22 Jul. 2006, coll. Akihisa Andoh.

Diagnosis. See the diagnosis for S. macrodonta sp. n.

Description. Somatic characters as in the genus description (see also Tu and Li 2006, Tu and Hormiga 2011), and genital characters as in the description by Tu et al. (2007).

Distribution. Japan (Kyushu, Fig. 7).

Solenysa trunciformis sp. n.

http://zoobank.org/AD5A06F1-6832-4FFB-B5E0-652F483A491A Figs 1A–D, 3B, 5E–F

Solenysa melloteei: Tu and Li 2006: 91, figs 21–30; Tu and Hormiga 2011: 499, fig. 8A–I (misidentification).

Types. Male holotype (CNU-J26), Japan, Honshu, Wakayama Prefecture, Shirahama, Tondazaka, 33°37.53'N, 135°25.35'E, alt. 310 m, 31 Mar. 2009, coll. Akihisa Andoh. Paratypes, $2\sqrt[3]{3}$ and 922, same data as holotype; $1\sqrt[3]{3}$ and 12 (CNU-J23), Japan, Shikoku, Kagawa Prefecture, Takamatsu, Nishi-ueda, 34°13.22'N, 134°04.62'E, alt. 130 m, 19 Jul. 2008, coll. Akihisa Andoh; $1\sqrt[3]{3}$ and 322 (CNU-J05), Japan, Honshu, Wakayama Prefecture, Susami-cho, Esuzaki, 33°30'N, 135°34.20'E, 24 Aug. 1981, coll. Yoshito Ishii.

Diagnosis. The male palpal characters of *S. trunciformis* sp. n. (Figs 1C, 3B, 6F) are similar to those of *S. macrodonta* sp. n. and *S. refrexilis*; to distinguish them see the diagnosis for *S. macrodonta* sp. n. The female is distinguished by the apple-shaped epigyne with a rectangular epigynal collar (Fig. 5F).

Description. Somatic characters as in the genus description and genital characters as in the descriptions for *S. mellotteei* by Tu and Li (2006) and Tu and Hormiga (2011).

Etymology. The species name comes from the Latin '*trunciformis*' in reference to truncate apex of anterior protrusion in front of median terminal apophysis (Fig. 1C).

Distribution. Japan (Honshu, Shikoku, Fig. 7).

Key to Solenysa species from the islands of Japan

1	Male
_	Female7
2	LC, with a forked apex, LC ₃ unbranched (Fig. 3A) 3
_	LC, with a sharp apex, LC ₃ includes two parts (Fig. 3C)
3	MTA with serrated margin (Fig. 3A)S. macrodonta sp. n.
_	MTA with smooth margin and two anterior protrusions (Fig. 3B)
4	First protrusion truncate (Fig. 3B)
_	First protrusion pointed (Tu et al. 2007, fig. 1D)
5	Anterior part of LC ₃ flag-shaped (Fig. 2B)
_	Anterior part of LC ₃ spike-shaped (Fig. 3C–D)6
6	Posterior part of LC ₃ S-curved (Fig. 3C)
_	Posterior part of LC ₃ L-curved (Fig. 3D)
7	Dorsal plate almost as wide as long (Fig. 4B)8
_	Dorsal plate wider than long (Fig. 5B)
8	Epigynal collar inversed triangular (Fig. 4A) S. mellotteei
_	Epigynal collar rectangular (Fig. 5F)
9	Posterior margin of epigyne centrally incised (Fig. 4A–C)10

_	Posterior margin of epigyne straight (Fig. 4E)	<i>lexilis</i>
10	Maximum width at anterior part (Fig. 5A)	11
_	Maximum width in middle (Fig. 4C)S. macrodonta	sp. n.
11	Epigynal collar more than four times wider than long (Fig. 5B)S.	ogatai
_	Epigynal collar less twice wider than long (Tu and Hormiga 2011: fig. 1	1I)
		rtibilis

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RESEARCH ARTICLE



Trophic relations of *Opatrum sabulosum* (Coleoptera, Tenebrionidae) with leaves of cultivated and uncultivated species of herbaceous plants under laboratory conditions

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Abstract

We carried out a quantitative assessment of the consumption of herbaceous plants by *Opatrum sabulosum* (Linnaeus, 1761) – a highly significant agricultural pest species. We researched the feeding preferences of this pest species with respect to 33 uncultivated and 22 cultivated plant species. This species of darkling beetle feeds on many uncultivated plant species, including those with hairy leaves and bitter milky sap, such as *Scabiosa ucrainca* (5.21 mg/specimen/24 hours), *Euphorbia virgata* (3.45), *Solanum nigrum* (3.32), *Centauria scabiosa* (2.47), *Lamium album* (2.41), *Aristolochia clematitis* (1.76), *Chenopodium album* (1.73), *Arctium lappa* (1.51), *Asperula odorata* (1.20). A high rate of leaf consumption is also characteristic for cultivated species, for example, *Perilla nankinensis* (5.05 mg/specimen/24 hours), *Lycopersicon esculentum* (3.75), *Tropaeolum majus* (3.29), *Nicotiana tabacum* (2.66), *Rumex acetosa* (1.96), *Beta vulgaris* (1.27). *O. sabulosum* is capable of feeding on plants which are poisonous to cattle. This species of darkling beetle consumes 95.5% of the cultivated and 48.5% of the uncultivated herbaceous plants researched.

Keywords

Opatrum sabulosum, Tenebrionidae, Food Preferences, Laboratory Experiments, Plant-eating Insects

Introduction

For many species of phytophages and saprophages the consumption of leaves of herbaceous plants is the main aspect of their negative influence on natural ecosystems. If a particular species of insect feeds on one particular species of grass, it is quite easy to control its numbers in agricultural conditions (Fattorini 2011; Jia et al. 2013). The situation is much more difficult with polyphages potentially able to feed on many species of fodder plants (Whicker and Tracey 1987; Crawford 1988; Rogers et al. 1988; De Los Santos et al. 2002). *Opatrum sabulosum* (Linnaeus, 1761), a member of the Tenebrionidae family, is a pest species with a wide range of consumption preferences. This species has a wide distribution (Chernej 2005; Abdurahmanov and Nabozhenko 2011). It is numerous in the majority of steppe and meadow ecosystems, in pine forests and, most significantly, in agricultural ecosystems (Parmenter and Macmahon 1984; Minoranskij and Kuzina 1987). Its abilty to eat herbaceous plants from different families enables populations of this species to thrive in high and stable numbers over a long period of years in spite of all agro-technical measures directed against them (Kabanov and Sedin 1981; Leo et al. 2011).

The imagines of *O. sabulosum* are most active in the first half of spring (Rejnhardt 1936). During this period it is usual to observe a few dozen to hundreds of this darkling beetle species in a single square meter plot. According to modern data the imagines of *O. sabulosum* cause extensive damage to a large number of agricultural plant species, both on ploughed fields with wide furrows and densely sown fields with narrow gaps between the rows (Medvedev 1968). Newly planted pines suffer similar damage (Chernej 2005). According to Medvedev (1968) the imagines of *O. sabulosum* prefer to feed on roots, the lower parts of stems and also root crops, making long, narrow passages in them. It is worth noting that these beetles readily eat the decaying parts of plants (Kabanov 1977; De Los Santos et al. 1988).

In natural conditions the imago of *O. sabulosum* feeds on the leaves of steppe plants, and in fields it begins to damage both weeds and agricultural crops (Rejnhardt 1936). Cases of consumption of dry horse manure and dry remains of vegetation have been recorded (Chen et al. 2004). The peak of the feeding activity of *O. sabulosum*, when it causes serious damage to sown crops, is observed at the end of April and in May (Dolin 1975).

The new generation emerges at the end of August (Knor 1975; Allsopp 1980; Carpaneto and Fattorini 2001). The number of actively feeding imagines declines considerably in mid summer (Naidu and Hattingh 1986; Gehrken and Somme 1994).

According to the information in the literature, the imagines of *O. sabulosum* feed on wild and weedy species of plants in natural environments, and in agricultural ecosystems they transfer their consumption to cultivated plants and weeds. It is also widely assumed that this species of darkling beetle does not feed on species with bitter milky juice, such as *Euphorbia stepposa* Zoz ex Prokh. and *Cichorium intybus* L., and on hairy leaf species, such as *Agrimonia eupatoria* L. and *Asclepias syriaca* L., though statements on this point are fragmentary and require support. Controlling the numbers of *O. sabulosum* in its capacity as a highly significant pest on agricultural crops is impossible without a quantitative assessment of its consumption spectrum with respect to herbaceous plants. There is only fragmentary information in the scientific literature on the damage caused by this species to specific species of agricultural crops and this lacks a quantitative assessment of the amount of food consumed by an individual beetle. As a result of our preliminary research we have established that, though this species of darkling beetle has traditionally been considered a saprophage, its consumption of six types of soil and four types of steppe litter have not been observed in laboratory conditions (Nazimov and Brygadyrenko 2013). For this reason we consider *O. sabulosum* to be a phytophage, feeding predominantly on herbaceous vegetation.

So, the following questions are of considerable interest: (1) whether *O. sabulosum* eats the leaves of plants poisonous to cattle, (2) whether it eats green leaves of hairy plants, (3) whether the beetles prefer species from the natural flora or cultivated plants. In connection with these questions, the aim of this study is to establish in laboratory conditions the potential trophic relations of *O. sabulosum* with the leaves of herbaceous plants belonging to different taxonomic groups.

Materials and methods

The research was carried out on the outskirts of Dnipropetrovsk, Ukraine, at the end of July beginning of August 2013. A total of 1,920 *O. sabulosum* individuals were collected on plots in steppe habitat and kept for 10 days on an optimal diet consisting of lettuce, cabbage and vine leaves. Sprinkling devices were placed in the containers so the beetles did not experience lack of water.

Each food item was offered in eight transparent plastic containers ($8 \times 12 \times 10$ cm) without any substrate provided, each with four beetles (two male, two female), a total of 32 imago specimens being involved in each experiment with a particular plant species. The temperature in the laboratory was maintained at 25–28 °C and the humidity at 60–80%. Each experiment lasted for five days. A control group was kept in 32 containers identical to those used in the main experiment, also without any substrate, but without any food, each holding four specimens.

The leaves of naturally occurring herbaceous plant species were collected from natural ecosystems which were not affected by anthropogenic pollution. The leaves of cultivated plants were collected from a private plot where the plants had been cultivated without the use of growth stimulators, herbicides, organic or mineral fertilisers. The green leaves were dried out over a period of 12 to 20 days in the open air on shelves in an open sided roofed structure. After this procedure the drying of the leaves was completed with a 24 hour period in a drying chamber.

To determine the mass of food consumed we took into account the degree of decomposition of the leaves under the influence of microorganisms. For this purpose, simultaneously with the main experiment we placed leaves of each plant species in eight identical containers (making a total of 440 containers) without *O. sabulosum*. The consumption of food by the beetles was calculated using the optimised formula proposed by David (1998). All experiments were carried out in identical light, temperature and humidity conditions.

The weight of the food and the beetles was determined on analytical scales JD-100 (precision 1 mg). In the statistical analysis of the data we calculated $x \pm S_x$, the median and range of fluctuation for each characteristic. The most significant characteristic is the median (the normal distribution of the characteristics was not observed as the beetles do not eat daily in equal portions, but at an uneven rate, each "meal" varying considerably in the weight of food consumed).

Results

Consumption of leaves of wild herbaceous plant species

From the wild growing herbaceous plants imagines of *O. sabulosum* consume predominantly the following species (Table 1): *Scabiosa ucranica* (5.21 mg/specimen/24 hours), *Euphorbia virgata* (3.45), *Solanum nigrum* (3.32), *Centaurea scabiosa* (2.47), *Lamium album* (2.41), *Aristolochia clematitis* (1.76), *Chenopodium album* (1.73), *Arctium lappa* (1.51), *Asperula odorata* (1.20). For the other plant species the intensity of food consumption did not exceed 1 mg per specimen over 24 hours. Among the above-mentioned species are both plants edible for cattle (*Chenopodium album* and *Centaurea scabiosa*) and species of plants which are not edible for the majority of phytophages (*Aristolochia clematitis, Euphorbia virgata* and *Solanum nigrum*).

In various containers the maximum speeds of food consumption significantly exceeded the average figures for each plant species, an effect most likely connected with the prolonged reproductive period of individual beetles and the intensive consumption of food for the development of eggs.

The following species were practically not consumed by *O. sabulosum: Cirsium vulgare* (0.88), *Euphorbia stepposa* (0.74), *Hypercium perforatum* (0.60), *Salvia nemorosa* (0.60), *Astragalus borysthenicus* (0.55), *Chelidonium majus* (0.55), *Convallaria majalis* (0.54), *Artemisia absinthium* (0.40), *Vinca minor* (0.40), *Viola tricolor* (0.36), *Potentilla argentea* (0.35), *Arctium tomentosum* (0.21), *Aegopodium podagraria* (0.20), *Asclepias syriaca* (0.18), *Senecio vernalis* (0.16), *Fragaria vesca* (0.15) and *Agrimonia eupatoria* (0.08). For this group of plants the maximum rate of food consumption for any container did not exceed 1.2 mg per specimen per 24 hours.

The decrease in beetles' body weight during the experiment (Table 2) is connected, first of all, with loss of moisture (during the entire five day period of the experiment the *O. sabulosum* imagines did not have access to water). The control group of *O. sabulosum*, denied access to food, decreased in body weight by 1.02 ± 0.27 mg/specimen/24 hours (2.05% over 24 hours). A larger decrease in body weight relative to the control group can be connected with the purgative effect on the intenstines of beetles from

Family	Species	Median	$x \pm S_x$	Min-Max
Apiaceae	Aegopodium podagraria L.	0.20	0.21 ± 0.11	0.04-0.35
Apocynaceae	Vinca minor L.	0.40	0.39 ± 0.27	0.04-0.77
Aristolochiaceae	Aristolochia clematitis L.	1.76	1.76 ± 0.75	0.65-3.00
Asclepiadaceae	Asclepias syriaca L.	0.18	0.18 ± 0.10	0.05-0.35
Asteraceae	Arctium lappa L.	1.51	1.42 ± 0.59	0.00-2.13
"	A. tomentosum Mill.	0.21	0.25 ± 0.20	0.00-0.71
"	Artemisia absinthium L.	0.40	0.36 ± 0.22	0.00-0.65
"	Cirsium vulgare (Savi) Ten.	0.88	0.79 ± 0.31	0.00-1.03
"	Cichorium intybus L.	0.60	0.64 ± 0.77	0.10-2.55
"	Centaurea scabiosa L.	2.47	2.47 ± 0.84	1.50-4.30
	Hieracium pilosella L.	0.85	0.86 ± 0.58	0.15-2.15
"	Senecio vernalis Waldst. & Kit.	0.16	0.16 ± 0.09	0.00-0.25
Cannabaceae	Humulus lupulus L.	0.58	0.72 ± 0.56	0.00-1.63
Chenopodiaceae	Chenopodium album L.	1.73	2.03 ± 1.74	0.00-5.52
Convallariaceae	Convallaria majalis L.	0.54	0.44 ± 0.39	0.00-1.08
Dipsacaceae	Scabiosa ucranica L.	5.21	5.21 ± 1.31	3.10-7.45
Euphorbiaceae	Euphorbia stepposa Zoz ex Prokh.	0.74	0.74 ± 0.21	0.40-1.15
"	E. virgata W.K.	3.45	3.86 ± 1.76	2.50-8.30
Fabaceae	Astragalus borysthenicus Klokov.	0.55	0.55 ± 0.27	0.00-0.94
"	Medicago romanica Prodan.	0.95	1.11 ± 0.56	0.40-2.05
Hypericaceae	Hypericum perforatum L.	0.60	0.60 ± 0.30	0.00-1.15
Lamiaceae	Ajuga genevensis L.	0.25	0.62 ± 0.73	0.15-2.40
_"	Lamium album L.	2.41	2.49 ± 1.36	0.00-5.31
_"	Salvia nemorosa L.	0.60	0.58 ± 0.34	0.00-1.05
"	Thymus marschallianus Willd.	0.65	1.02 ± 1.25	0.15-4.25
Papaveraceae	Chelidonium majus L.	0.55	0.55 ± 0.30	0.16-1.04
Polygonaceae	Polygonum aviculare L.	0.70	0.87 ± 0.78	0.00-2.31
Rosaceae	Agrimonia eupatoria L.	0.08	0.08 ± 0.05	0.00-0.15
_"	Fragaria vesca L.	0.15	0.17 ± 0.10	0.00-0.35
"	Potentilla argentea L.	0.35	0.35 ± 0.20	0.10-0.60
Rubiaceae	Asperula odorata L.	1.20	0.89 ± 0.65	0.00-1.60
Solanaceae	Solanum nigrum L.	3.32	3.15 ± 1.67	0.00-5.14
Violaceae	Viola tricolor L.	0.36	0.38 ± 0.31	0.00-0.97

Table 1. Consumption of leaves (mg/specimen/24 hours) of different species of uncultivated herbaceous plants by *O. sabulosum* in laboratory conditions (n = 32).

compounds contained in the food plants, intoxication of their organs or damage to their intestinal walls. This effect was observed for the following species; *Vinca minor* (-1.85 mg/24 hours), *Cichorium intybus* (-1.60), *Asperula odorata* (-1.44), *Solanum nigrum* (-1.44), *Salvia nemorosa* (-1.35), *Cirsium vulgare* (-1.20), *Potentilla argentea* (-1.20), *Euphorbia stepposa* (-1.19), *E. virgata* (-1.14), *Artemisia absinthium* (-1.14).

The minimum loss in body weight of *O. sabulosum* compared to the start of the experiment was observed for the following species: *Astragalus borysthenicus* (-0.25 mg/

Family	Species	Median	x ± S _x	Min–Max
Apiaceae	Aegopodium podagraria L.	-0.33	$-0.33 \pm 0.31^*$	-1.00-+0.15
Apocynaceae	Vinca minor L.	-1.85	$-2.41 \pm 2.09^{*}$	-6.25-0.50
Aristolochiaceae	Aristolochia clematitis L.	-0.65	$-0.69 \pm 0.25^{*}$	-1.20-0.35
Asclepiadaceae	Asclepias syriaca L.	-0.95	-0.89 ± 0.12	-1.05-0.65
Asteraceae	Arctium lappa L.	-0.80	$-0.73 \pm 0.18^{*}$	-1.05-0.40
"	A. tomentosum Mill.	-0.67	$-0.67 \pm 0.19^*$	-0.95-0.40
"	Artemisia absinthium L.	-1.14	-1.14 ± 0.30	-1.45-0.55
"	Cirsium vulgare (Savi) Ten.	-1.20	$-1.14 \pm 0.14^*$	-1.35-0.85
"	Cichorium intybus L.	-1.60	$-1.66 \pm 1.19^*$	-4.15-+0.25
" _"_	Centaurea scabiosa L.	-0.55	$-0.60 \pm 0.14^*$	-0.80-0.45
"	Hieracium pilosella L.	-0.95	-0.95 ± 0.24	-1.35-0.65
"	Senecio vernalis Waldst. & Kit.	-0.90	-0.94 ± 0.29	-1.60-0.65
Cannabaceae	Humulus lupulus L.	-1.05	-0.94 ± 0.29	-1.20-0.40
Chenopodiaceae	Chenopodium album L.	-0.65	$-0.71 \pm 0.27^*$	-1.25-0.40
Convallariaceae	Convallaria majalis L.	-0.80	$-0.72 \pm 0.36^{*}$	-1.20-0.15
Dipsacaceae	Scabiosa ucranica L.	-0.53	$-0.53 \pm 0.24^{*}$	-0.95-0.25
Euphorbiaceae	Euphorbia stepposa Zoz ex Prokh.	-1.19	$-1.19 \pm 0.40^{*}$	-2.00-0.75
"	E. virgata W.K.	-1.14	-1.14 ± 0.40	-1.75-0.65
Fabaceae	Astragalus borysthenicus Klokov.	-0.25	$-0.21 \pm 0.25^{*}$	-0.55-+0.25
"	Medicago romanica Prodan.	-0.80	$-0.77 \pm 0.19^{*}$	-0.95-0.40
Hypericaceae	Hypericum perforatum L.	-0.65	$-0.48 \pm 0.55^{*}$	-0.80-+0.95
Lamiaceae	Ajuga genevensis L.	-0.95	-0.88 ± 0.44	-1.45-+0.15
"	Lamium album L.	-0.79	$-0.79 \pm 0.19^{*}$	-1.20-0.55
"	Salvia nemorosa L.	-1.35	$-1.47 \pm 0.47^{*}$	-2.15-0.95
"	Thymus marschallianus Willd.	-1.05	-1.05 ± 0.42	-1.70-0.40
Papaveraceae	Chelidonium majus L.	-0.90	-1.06 ± 0.49	-2.20-0.55
Polygonaceae	Polygonum aviculare L.	-0.86	$-0.86 \pm 0.27^{*}$	-1.20-0.40
Rosaceae	Agrimonia eupatoria L.	-1.06	-1.06 ± 0.35	-1.55-0.55
"	Fragaria vesca L.	-0.68	$-0.68 \pm 0.16^{*}$	-0.95-0.40
"	Potentilla argentea L.	-1.20	-1.14 ± 0.34	-1.60-0.55
Rubiaceae	Asperula odorata L.	-1.44	$-1.44 \pm 0.41^*$	-2.15-0.75
Solanaceae	Solanum nigrum L.	-1.44	$-1.44 \pm 0.41^{*}$	-2.15-0.75
Violaceae	Viola tricolor L.	-0.66	$-0.66 \pm 0.22^*$	-1.05-0.40

Table 2. Changes in body weight (mg/specimen/24 hours) of *O. sabulosum* on diet of different uncultivated herbaceous plant species in laboratory conditions (n = 32).

Note: *–loss of body weight, observed at the end of the experiment, considered reliable at P < 0.05 differs from the body weight of the control group of beetles without access to food (1.02 ± 0.27 mg/specimen/24 hours); average body weight of beetles at start of experiment –49.61 ± 7.86 mg).

specimen/24 hours), Aegopodium podagraria (-0.33), Scabiosa ucrainca (-0.53), Centaurea scabiosa (-0.55), Aristolochia clematitis (-0.65), Chenopodium album (-0.65), Hypericum perforatum (-0.65), Viola tricolor (-0.66), Arctium tomentosum (-0.67), Fragaria vesca (-0.68), Lamium album (-0.79), Medicago romanica (-0.80), Convallaria majalis (-0.80) and Arctium lappa (-0.80). Maximum faecal formation by the beetles was observed following diets of *Polygonum aviculare* (0.73) and *Solanum nigrum* (0.70), and the minimum rate (equal to 0 mg/specimen/24 hours in all eight experimental containers) was observed after feeding on *Convallaria majalis* and *Vinca minor*. The intensity of faecal formation was at an intermediate level with the other plant species tested.

It is interesting that for *Aegopodium podagraria* one of the minimum rates of food consumption was observed (0.20 mg/specimen/24 hours), one of the minimum losses of body weight compared to the control group of beetles (-0.33 compared to -1.02 mg/specimen/24 hours for the group without access to food) and also the minimum rates of excrement formation (0.10 mg/specimen/24 hours). Thus, from 0.20 mg of food consumed per day 0.10 mg of excrement was formed, the remainder being expended on anabolism and respiration.

Consumption of leaves of cultivated herbaceous plant species

The leaves of cultivated herbaceous plant species were consumed on average with the same intensity as the leaves of wild plant species (Table 3). The rates of leaf consumption were highest for the following species: *Perilla nankinensis* (5.05 mg/specimen/24 hours), *Lycopersicon esculentum* (3.75), *Tropaeolum majus* (3.29), *Nicotiana tabacum* (2.66), *Rumex acetosa* (1.96), *Beta vulgaris* (1.27).

Leaves of the following species were those consumed least intensively by *O. sabulo-sum*: *Oenothera biennis* (0.73), *Aquilegia vulgaris* (0.68), *Citrullus lanatus* (0.61), *Matricaria recutita* (0.54), *Phytolacca americana* (0.49), *Phlox paniculata* (0.44), *Cucurbita pepo* (0.37), *Helianthus tuberosus* (0.32), *Echinacea purpurea* (0.29), *Fragaria moschata* (0.24) and *Zea mays* (0.17 mg/specimen/24 hours). It is interesting that of all the cultivated grasses researched, the minimum quantity of dried leaves was consumed for maize despite the fact that this is the main crop damaged by *O. sabulosum*. It is worth emphasising once again that phytophages eat the fresh or decaying leaves of this species but hardly ever dry leaves.

Compared to the control group without access to food, for which we observed a decrease in body weight of 1.02 ± 0.27 mg/specimen/24 hours, the consumption of many species of cultivated plants minimises the loss of the original body weight. This can be seen with *Daucus carota* (-80 mg/specimen/24 hours), *Nicotiana tabacum* (-0.80), *Phlox paniculata* (-0.80), *Capsicum annuum* (-0.76), *Phytolacca americana* (-0.75), *Helianthus tuberosus* (-0.74), *Malva erecta* (-0.74), *Oenothera biennis* (-0.70), *Rumex acetosa* (-0.67), *Lycopersicon esculentum* (-0.65), *Fragaria moschata* (-0.64), *Helianthus annuus* (-0.58), *Matricaria recutita* (-0.55), *Zea mays* (-0.55), *Citrullus lanatus* (-0.53), *Aquilegia vulgaris* (-0.49), *Tropaeolum majus* (-0.40), *Cucurbita pepo* (-0.25) and *Borago officinalis* (-0.20). In our experiment the consumption of dry leaves of *Beta vulgaris* did not lead to a reliable tendency towards preservation in the beetles' body weight compared to the control group (-1.08 and -1.02 mg/ specimen/24 hours respectively).

Family	Species	Median	$x \pm S_{x}$	Min–Max
Apiaceae	Daucus carota L.	0.80	0.80 ± 0.47	0.00-1.74
Asteraceae	Echinacea purpurea (L.) Moench.	0.29	0.49 ± 0.61	0.00-2.04
" _"_ _"_	Matricaria recutita L.	0.54	0.65 ± 0.50	0.00-1.54
_"	Helianthus annuus L.	1.05	1.05 ± 0.26	0.77-1.55
"	H. tuberosus L.	0.32	0.36 ± 0.26	0.00-0.77
Boraginaceae	Borago officinalis L.	0.86	1.05 ± 1.13	0.00-2.97
Chenopodiaceae	Beta vulgaris L.	1.27	1.49 ± 0.73	0.58-2.82
Cucurbitaceae	Citrullus lanatus (Thunb.) Matsum. & Nakai.	0.61	0.76 ± 0.68	0.00-1.89
"	Cucurbita pepo L.	0.37	0.37 ± 0.25	0.00-0.76
Lamiaceae	Perilla nankinensis (Lour.) Decne.	5.05	4.60 ± 1.75	0.00-5.66
Malvaceae	Malva erecta J. Presl & C. Presl	1.13	1.13 ± 0.51	0.32-1.74
Onagraceae	Oenothera biennis L.	0.73	0.96 ± 0.77	0.23-2.82
Phytolaccaceae	<i>Phytolacca americana</i> L.	0.49	0.57 ± 0.47	0.03-1.23
Poaceae	Zea mays L.	0.17	0.17 ± 0.08	0.02-0.31
Polemoniaceae	Phlox paniculata L.	0.44	0.44 ± 0.13	0.26-0.56
Polygonaceae	Rumex acetosa L.	1.96	2.00 ± 1.52	0.00-4.36
Ranunculaceae	Aquilegia vulgaris L.	0.68	0.63 ± 0.38	0.05-1.03
Rosaceae	Fragaria moschata (Duchesne) Weston.	0.24	0.87 ± 1.71	0.00-5.38
Solanaceae	Capsicum annuum L.	1.10	1.25 ± 0.40	0.82-2.04
"	Lycopersicon esculentum Mill.	3.75	3.74 ± 2.66	0.00-8.20
"	Nicotiana tabacum L.	2.66	3.05 ± 2.72	0.00-9.08
Tropaeolaceae	Tropaeolum majus L.	3.29	3.10 ± 2.23	0.00-6.54

Table 3. Consumption of leaves (mg/specimen/24 hours) of different cultivated herbaceous plant species by *O. sabulosum* in laboratory conditions (n = 32).

The maximum intensity of faecal formation for *O. sabulosum* was characteristic for diets of dry leaves of the following species: *Daucus carota* (1.03 mg/specimen/24 hours), *Lycopersicon esculentum* (0.65), *Fragaria moschata* (0.55), *Perilla nankinensis* (0.55), *Citrullus lanatus* (0.53), *Rumex acetosa* (0.45), *Nicotiana tabacum* (0.41), *Zea mays* (0.41), *Capsicum annuum* (0.30), *Cucurbita pepo* (0.30) and *Helianthus annuus* (0.30).

Discussion

The research showed that with 16 of the 33 wild and 21 of the 22 cultivated herbaceous plant species investigated the species of leaf consumed led to a reliable loss in the beetles' body weight (see Tables 2 and 4). Overall, *O. sabulosum* consumed 95.5% of the cultivated and 48.5% of the wild herbaceous plant species researched. Of the 11 species in the Asteraceae family 8 were consumed, of the 5 Lamiaceae species 4 were consumed, of the 4 Rosaceae species 1 was consumed and of the 4 Solanaceae species 1 was consumed. Representatives of the following families included in our research were not consumed at all: Apocynaceae, Asclepiadaceae, Convallariaceae, Hypericaceae, Papaveraceae, Rosaceae and Violaceae.

Family	Species	Median	$x \pm S_x$	Min–Max
Apiaceae	Daucus carota L.	-0.80	$-0.87 \pm 0.40^{*}$	-1.60-0.40
Asteraceae	<i>Echinacea purpurea</i> (L.) Moench.	-0.95	-0.95 ± 0.39	-1.85-0.40
" _"_ _"_	Matricaria recutita L.	-0.55	$-0.66 \pm 0.23^{*}$	-1.20-0.40
"	Helianthus annuus L.	-0.58	$-0.58 \pm 0.21^{*}$	-0.80-0.15
"	H. tuberosus L.	-0.74	$-0.74 \pm 0.36^{*}$	-1.35-0.25
Boraginaceae	Borago officinalis L.	-0.20	$-0.22 \pm 0.28^{*}$	-0.80-+0.15
Chenopodiaceae	Beta vulgaris L.	-1.08	-1.08 ± 0.30	-1.65-0.55
Cucurbitaceae	Citrullus lanatus (Thunb.) Matsum. & Nakai.	-0.53	$-0.53 \pm 0.15^{*}$	-0.80-0.40
"	Cucurbita pepo L.	-0.25	$-0.34 \pm 0.19^{*}$	-0.65-0.15
Lamiaceae	Perilla nankinensis (Lour.) Decne.	-0.95	-0.96 ± 0.32	-1.45-0.55
Malvaceae	Malva erecta J. Presl & C. Presl.	-0.74	$-0.74 \pm 0.20^{*}$	-1.05-0.40
Onagraceae	Oenothera biennis L.	-0.70	$-0.70 \pm 0.40^{*}$	-1.45-0.15
Phytolaccaceae	Phytolacca americana L	-0.75	$-0.74 \pm 0.20^{*}$	-1.05-0.40
Poaceae	Zea mays L.	-0.55	$-0.54 \pm 0.18^{*}$	-0.80-0.20
Polemoniaceae	Phlox paniculata L.	-0.80	$-0.96 \pm 0.43^{*}$	-2.00-0.40
Polygonaceae	Rumex acetosa L.	-0.67	$-0.67 \pm 0.27^{*}$	-1.05-0.20
Ranunculaceae	Aquilegia vulgaris L.	-0.49	$-0.49 \pm 0.46^{*}$	-1.05-+0.55
Rosaceae	Fragaria moschata (Duchesne) Weston.	-0.64	$-0.64 \pm 0.33^{*}$	-1.35-0.25
Solanaceae	Capsicum annuum L.	-0.76	$-0.76 \pm 0.22^{*}$	-1.20-0.55
_"	Lycopersicon esculentum Mill.	-0.65	$-0.65 \pm 0.14^{*}$	-0.80-0.40
"	Nicotiana tabacum L.	-0.80	$-0.72 \pm 0.16^{*}$	-0.95-0.40
Tropaeolaceae	Tropaeolum majus L.	-0.40	$-0.43 \pm 0.31^{*}$	-0.80-+0.25

Table 4. Changes in body weight (mg/specimen/24 hours) of *O. sabulosum* on diet of leaves of different cultivated herbaceous plant species in laboratory conditions (n = 32).

Note: *-see Table 2.

According to the work of Rejnhardt (1936) *O. sabulosum* eats the roots and leaves of wild steppe weeds such as *Atriplex hortensis* L., *Chenopodium album* L., *Convolvulus arvensis* L. and *Polygonum aviculare* L. Our experiments have confirmed that imagines of *O. sabulosum* do feed on weed species. However, they do not show a clear preference for this type of food compared to cultivated plants. Indeed, the lowest decreases in original body weight were observed when the beetles fed on cultivated plants such as *Z. mays*, *D. carota*, *C. pepo*, *H. annuus*, *H. tuberosus*, *B. officinalis* and *N. tabacum*. At the same time consumption of weeds also helps to reduce the loss of original body weight. Based on the results of our research, we can state that *O. sabulosum* damages in almost equal ratios both cultivated plants and weeds.

Opatrum sabulosum has shown an ability to feed on species of plants with hairy leaves and a bitter milky sap. The beetles lost hardly any weight when feeding on the bitter leaves of *A. borysthenicus* and *A. clematitis*, and also experienced insignificant weight loss in variants of the experiments with hairy plant species such as *S. ucrainca* and *C. scabiosa*. It follows that this species of darkling beetle consumes a fairly wide range of bitter species not eaten by livestock.

According to modern data (Kabanov 1977; Kabanov and Sedin 1981), the imagines of *O. sabulosum* damage quite a large number of agricultural plants, including *Hordeum sativum* L., *Avena sativa* L., *Panicum virgatum* L., *Triticum durum* L., *Cicer arietnum* L., *Lens culinaris* Medikus., *Phaseolus vulgaris* L., *Sorghum saccharatum* (L.) Moench, *Sorghum bicolor* (L.) Moench, *Z. mays*, *Allium cepa* L., *N. tabacum*, *Solanum tuberosum* L., *L. esculentum*, *B. vulgaris*, *H. annuus*, *Cannabis sativa* L., *P. nankinensis*, *Brassica napus* L., *Papaver somniferum* L., *C. lanatus* and *Cucumis sativus* L. (Bryzova and Kelejnikova 1964; Medvedev 1968). They also damage the leaves of *Vitis vinifera* L., and eat the cotyledons of shoots of fruit trees.

It is clear that outside the reproductive period *O. sabulosum* is able to feed intensively on both wild and cultivated species of herbaceous plants. According to information from the literature (Parmenter et al. 1989a, 1989b; Semida et al. 2001), there is considerable seasonal change in the diet of this species of darkling beetle (Cloudsley-Thompson 1975): this is connected both with the passage of definite phenological phases in the development of herbaceous plants (shoots, the formation of leaf rosettes near the roots), and with the spring reproductive period of the beetles themselves. Nevertheless, during the period of decreased trophic activity in the second half of summer (our experiment was carried out in late July – early August) the trophic activity of *O. sabulosum* imagines continued at a pretty high level.

The seasonal dynamic of the trophic activity of this species of darkling beetle requires further research, especially the characteristics of its trophic activity (the quantitative and qualitative differences in its diet) during the period of intensive spring feeding and during the egg laying period. The peculiarities of the larval consumption of the root parts of wild and cultivated plants requires detailed research. The sex and age differences in the diet of the beetles in their first and second years of life remain unstudied. Besides this, the differences in the consumption of dry, fresh and decaying leaves of the beetle's main species of food plants are of considerable interest. The results of studies of the chemical content of the plants consumed by *O. sabulosum* will form the basis for the construction of models of the trophic relations of this species of polyphage, which is one of the most intensively studied and economically significant species of insect.

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RESEARCH ARTICLE



The Bostrichidae of the Maltese Islands (Coleoptera)

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Abstract

The Bostrichidae of the Maltese Islands are reviewed. Ten species are recorded with certainty from this Archipelago, of which 6 namely, *Trogoxylon impressum* (Comolli, 1837), *Amphicerus bimaculatus* (A.G. Olivier, 1790), *Heterobostrychus aequalis* (Waterhouse, 1884), *Sinoxylon unidentatum* (Fabricius, 1801), *Xyloperthella picea* (A.G. Olivier, 1790) and *Apate monachus* Fabricius, 1775 are recorded for the first time. Two of the mentioned species (*H. aequalis* and *S. unidentatum*) are alien and recorded only on the basis of single captures and the possible establishment of these species is discussed. Earlier records of *Scobicia pustulata* (Fabricius, 1801) from Malta are incorrect and should be attributed to *S. chevrieri* (A. Villa & J.B. Villa, 1835). A zoogeographical analysis and an updated checklist of the 12 species of Bostrichidae recorded from the Maltese Islands and neigbouring Sicilian islands (Pantelleria, Linosa and Lampedusa) are also provided.

Rhizopertha dominica (Fabricius, 1792) form *granulipennis* Lesne in Beeson & Bhatia, 1937 from Uttarakhand (northern India) was overlooked by almost all subsequent authors. Its history is summarized and the following new synonymy is established: *Rhizopertha dominica* (Fabricius, 1792) form *granulipennis* Lesne in Beeson & Bhatia, 1937 = *Rhyzopertha dominica* (Fabricius, 1792), **syn. n.**

Finally, records of *Amphicerus bimaculatus* from Azerbaijan, of *Bostrichus capucinus* (Linnaeus, 1758) from Jordan and Syria, of *Scobicia chevrieri* from Jordan and Italy, of *Xyloperthella picea* from Italy, and of *Apate monachus* from Corsica (France) and Italy, are also provided.

Keywords

Bostrichidae, new records, new synonym, alien species, Malta, Italy

Introduction

The larvae of most species of this family are wood borers, and as other saproxylic organisms they play an important role in the decomposition processes. They are thus significant for nutrient cycling in natural and semi-natural ecosystems, particularly forests (cf. Stokland et al. 2012). Several species of this family are of great economic importance since they can cause extensive damage to dry and dead wood, to seasoned sapwood timber, to bamboo, and to wooden or bamboo artifacts through the boring behavior of both adults and larvae. Moreover, several other species are pests of living trees and vines, a few species are store product pests attacking commodities such as grain and tubers. These beetles are frequently transported between countries, especially in wood packing materials such as crating and dunnage, and are often intercepted at ports and cargo distribution centres (cf. Haack and Slansky 1987, Geis 2002, Ivie 2002, Haack 2006, Liu et al. 2006, Bahillo de la Puebla et al. 2007, Liu et al. 2008, Lawrence 2010).

The earliest record of the family comes from the mid Cretaceous (Peris et al. 2014). This family is now mainly represented in subtropical and tropical regions, and currently about 600 described species accommodated in 90 genera are known to occur worldwide (cf. Lawrence and Slipinski 2013, Zahradník and Háva 2014).

The knowledge on Maltese Bostrichidae was very limited, with only five previously recorded species. New data on Maltese Bostrichidae emerged from recent studies carried out on collections made from the Maltese Islands, the results of which are included in the present work.

Material and methods

Study area

The study area comprises all of the Maltese islands (c. 316 km²). Chetcuti et al. (1992), Schembri (1993, 1997) and Giusti et al. (1995) can be consulted for general environmental information on this Archipelago situated in the centre of the Mediterranean basin.

Nomenclature and classification

The suprageneric classification of the family and the nomenclature (family-group names and genus-group names) adopted in the present work follow Zahradník and Háva (2014), but the subfamilies follow Liu and Schönitzer (2011). The species are listed alphabetically as in Borowski and Węgrzynowicz (2007) and Ivie (2010).

Faunistic list

For each species, the following information is provided: nomenclatural combinations (listed chronologically-alphabetically) of the Maltese records found in the literature, literature records on the Maltese Islands, material examined, chorotype, data on ecology, and notes.

When possible, the following data was also provided for each record: island, municipality, locality of collection, altitude, date of collection, collector/s, collecting method, number of specimens, and, in parenthesis, literature reference or abbreviation of the depository. The records are listed in alphabetic order with respect to localities of collection. A semicolon separates different records; if these are from the same site, the name of the site is listed only at the beginning with the older record. When deemed useful for the discussion of some species, material examined from other countries ("Other material examined") is also provided.

Possible interpolations are given in square brackets; collecting data of Italian specimens that is originally written in Italian, is hereunder provided in English. Information on very old specimens is written in double quotation marks.

Regarding literature records, Luigioni (1929) listed the two previously recorded bostrichids from Malta (cf. Cameron and Caruana Gatto 1907), but both Porta (1929, 1934, 1949, 1959) and Borowski (2007) overlooked these records. Records from "Malta" of Nardi (2004a, 2004b) are based only on previous literature. Finally, Schembri and Lanfranco (1996: 6) recorded an undentified bostrichid collected in a consignment of tropical logs imported from Africa, but the material was presumably lost (D. Mifsud, unpublished data).

The identifications of the material examined were made by one of us (G. Nardi), and were based on Lesne (1899, 1901b, 1904, 1906, 1909, 1924), Español (1955, 1956a), Cymorek (1961), Liu et al. (2006), Bahillo de la Puebla et al. (2007), Sitticaya et al. (2009) and Beiriger (2010). References, including illustrations of the genitalia are listed when available. This was done since so far little attention was given to the study of the genitalia of these beetles.

Zoogeography

Chorotypes, which were also used in the zoogeographical analysis, were assigned according to Vigna Taglianti et al. (1993, 1999) and are based on the distributions provided by the authors cited for each species. Moreover, the chorotypes of many species show extensions or more confined distributions when compared to the standard ones; these differences were mentioned only when the change is significant. For further information on global distributions, references cited in the text may be consulted.

Acronyms

Specimen depositories

BMNH CCI	The Natural History Museum, London, UK; private collection P. Cornacchia, Porto Mantovano (Mantua), Italy;
CMM	private collection D. Mifsud, Malta;
CNBFVR	Centro Nazionale per lo Studio e la Conservazione della Biodiversità Fo-
	restale "Bosco Fontana" di Verona. Sede di Bosco Fontana. Marmirolo
	(Mantua), Italy;
CNI	private collection G. Nardi, Cisterna di Latina (Latina), Italy;
MCSV	Museo civico di Storia Naturale, Verona, Italy;
MCSVA	F. Angelini collection c/o Museo civico di Storia Naturale, Verona, Italy;
MCSVD	G. Dellabeffa collection c/o Museo civico di Storia Naturale, Verona, Italy;
MCZRD	E. De Maggi collection c/o Museo civico di Zoologia, Rome, Italy;
MCZRL	P. Luigioni collection c/o Museo civico di Zoologia, Rome, Italy;
MZUF	Museo di Storia Naturale dell'Università degli Studi di Firenze, Sezione di
	Zoologia "La Specola", Florence, Italy;
MZUR	Museo di Zoologia, Università degli Studi di Roma "Sapienza", Rome,
	Italy.

Main collectors

AF	A. Falzon;
DD	D. Dandria;
D.P.I.T.	Dana Project Invertebrate Team = [S. De Felici, T. Di Micco De Santo,
	M. Shubat, F. Al-Eman Al-Husseini & A. Abu Hantash];
DM	D. Mifsud;
GN	G. Nardi;
HB	H. Borg Barthet;
LC	L.F. Cassar;
LF	L. Fancello;
PS	P. Sammut.

Other abbreviations

ex = specimen/s; Fraz. = Frazione = Hamlet; prov. = province; pt = pitfall trap.

Results

Subfamily Lyctinae Billberg, 1820, Tribe Lyctini Billberg, 1820

Lyctus brunneus (Stephens, 1830)

Lyctus brunneus Steph.: Cameron and Caruana Gatto 1907: 398. *Lyctus (Xylotrogus) brunneus* Steph.: Luigioni 1929: 642. *Lyctus brunneus* (Stephens, 1830): Nardi 2004a, Denux and Zagatti 2010: 366.

Literature records. Malta: Valletta (Cameron and Caruana Gatto 1907); "Mal." [= Malta] (Luigioni 1929); Malta (Nardi 2004a, Denux and Zagatti 2010).

Material examined. Malta: Naxxar, 7.IX.1997, DD, [in human abitation], $2 \stackrel{\diamond}{\circ} \stackrel{\diamond}{\circ}$ (CMM); Rabat, Wied ta l-Isqof, 28.VI.2002, PS, [in an agricultural area], $1 \stackrel{\circ}{\circ}$ (CNI).

Chorotype. Cosmopolitan (Borowski 2007, as L. (X.) brunneus). This species is widespread in the Palaeartic region (cf. Nardi 2004a, Borowski 2007), but the followings countries were overlooked in the species distribution summarized by Nardi (2004a) and/or Borowski (2007): Algeria (Peyerimhoff 1919, as L. (X.) brunneus, Lesne 1924), Austria (Horion 1961, Lucht 1987, Adlbauer 1998, Nardi 2004a, Denux and Zagatti 2010, Querner et al. 2011), Azores (cf. Borges 1990, Nardi 2004a, Oromí et al. 2010), Belarus, Bulgaria (Denux and Zagatti 2010), Canary Islands (cf. Machado and Oromí 2000, Nardi 2004a, Oromí et al. 2009), Corsica (cf. Becker 1969, Geis 2002), Croatia (Damoiseau 1966, Nardi 2004a), Cyprus (Baudi di Selve 1873, 1874, Georghiou 1977, Nardi 2004a), Egypt (Kaszab 1959, Attia and Kamel 1965, in both cases as L. (X.) brunneus, Hanna 1970, Alfieri 1976, Hamad and Aly 1985), Fujian (southeastern China) (Vrydagh 1960), Greece (Damoiseau 1966, Nardi 2004a, Denux and Zagatti 2010), Iran (Adeli 1972, Niloufari 1985), Israel (cf. Halperin and Geis 1999, Chikatunov et al. 2004b, 2006), Latvia (Denux and Zagatti 2010), Poland (cf. Burakowsi et al. 1986, Nardi 2004a, Jabłoński et al. 2007, Krajewski and Mazurek 2009), Serbia and Montenegro (Glavendekic et al. 2005, Denux and Zagatti 2010) and Turkey (cf. Gerberg 1957, Akbulut et al. 2008).

Ecology. In tropical areas this species develops in the wood of a large number of unrelated plants, whereas in temperate regions (where the species is considered as an established alien) it develops mainly in hardwood timber (e.g. *Castanea sativa, Fraxinus exelsior, Junglans regia, Quercus* spp., *Ulmus* spp., etc.) primarily in synanthropical environments (workshops, plywood industries, private houses). In the West Palaearctic region, the species was also found in local trees (*Alnus, Eucalyptus, Ulmus*, etc.) which were in poor health conditions. The passive transport of this beetle has been documented with both wooden products (timbers, tables, furniture, ethnographic collection, briers, etc.) and manioc (cf. Lesne 1924, Lepesme 1944, Español 1956a, Gerberg 1957, Cymorek 1961, Burlini 1972, Aitken 1975, Cavalloro and Ratti 1978,

Gambetta and Orlandi 1982a, Gambetta 1983, Wang et al. 1996, Halperin and Geis 1999, as *L.* (*X.*) *brunneus*, Geis 2002, Peters et al. 2002, Chikatunov et al. 2004, Bahillo de la Puebla et al. 2007, as *L.* (*X.*) *brunneus*, Mattson et al. 2007, Krajewski and Mazurek 2009, Denux and Zagatti 2010, Geis 2012). According to Gambetta and Orlandi (1982b: 55), *L. brunneus* and *Trogoxylon impressum* (Comolli, 1837) are the two species of Lyctinae which are the most widespread in woods in Italian storage situations.

Notes. A species native to Asia which has been established throughout Europe for more than 150 years (Denux and Zagatti 2010). The recently collected material confirm its presence in Malta. In the nearby countries, the species is recorded from Tunisia (Lesne 1924, Normand 1936, Borowski 2007) but not from Sicily (cf. Audisio et al. 1995, Sparacio 1997, Chiappini et al. 2001, Nardi 2004a, Denux and Zagatti 2010). Accurate information and illustrations on the morphology of all stages of this species were provided by Iwata and Nishimoto (1981, 1982) and Kucerová and Stesjkal (2008). Moreover the genitalia of both sexes was illustrated by different authors (e.g. Altson 1924: pls. 31–34, Gerberg 1957: pl. II, figs 15–16, Cymorek 1961: 81, fig. 3c, Iwata and Nishimoto 1982: 19, figs 42–44).

Subfamily Lyctinae Billberg, 1820, Tribe Trogoxylini Lesne, 1921

Trogoxylon impressum (Comolli, 1837)

Material examined. Malta: Busket, 15.VI.2003, DM, 1 ex (CMM); Mistra Valley, 21.IV.1994, DM, under leves of *Ficus carica*, 1 ex (CMM); Qormi, Hal-Farrug, 5.V.2003, DM, [on] *Ceratonia siliqua*, 1 ex (CNI); 5.V.2003, DM, 2 ex (CMM; CNI); Rabat, Ta Koronja, 14.VI.2002, PS, 1 ex (CMM); Wied Badu, 3.VII.2002, DM, 1 ex (CMM).

Chorotype. Turanic-European-Mediterranean. This species is widespread from the Canary Islands to Turkmenistan; to the north it reaches Austria, Czech Republic, Germany, Hungary, Slovakia and Switzerland, moreover it has been introduced in some northern European states (Denmark, Finland, Norway and Sweden), into Argentina, Chile and USA (cf. Borowski 2007, Borowski and Węgrzynowicz 2007, Barriga and Cepeda 2009), and it is considered as an established alien in the latter two mentioned territories (Gerberg 1957). The species is possibly also establishment in China (cf. Peters et al. 2002), South Africa and Australia (Geis 2002), but for these territories this was not confirmed (Borowski and Węgrzynowicz 2007). This species was also recorded from Iraq (Knopt 1972) and was introduced to Benelux (Lucht 1987, Drumont and Grootaert 2011) and both countries must be added to its distribution which was summarized by Borowski (2007). Hopkins (1911: 137, as *Lyctus impressus*) in an Appendix to Kraus (1911), recorded this species also for "Cordova, Mex. [= Mexico]"; this record was overlooked since Lesne (1938) probably because this Appendix, which also included data on additional specimens, was added after the submission

of the paper of Kraus (1911) (cf. Hopkins 1911: 130, footnote) and Mexico was not included in the mentioned work.

Ecology. *Trogoxylon impressum* is a polyphagous species; in the Mediterranean area it has been reared from many local and exotic trees and shrubs (e.g. *Ceratonia siliqua, Cercis siliquastrum, Eucalyptus* spp., *Ficus carica, Pistacia lentiscus, P. vera, Punica granatum, Quercus* spp., *Tamarix* sp., *Vitis vinifera*), and also from timber (cf. Nardi and Ratti 1995, Halperin and Geis 1999, Geis 2002, Liberto and Audisio 2005, Bahillo et al. 2007, Baena and Zuzarte 2013).

Notes. This species is a new record for the Maltese Islands; it is recorded also from the nearby Island of Pantelleria (Nardi and Ratti 1995) and from the Balearic Islands (cf. Schaufuss 1882, as *L. impressus capitalis* n. var., Bahillo de la Puebla and López-Colón 2001, Nardi 2004a).

Its male genitalia was figured by Gerberg (1957: pl. XI, figs 15–16) and Iablokoff-Khnzorian (1980: 284, fig. 12.9).

Subfamily Dinoderinae C.G. Thomson, 1863

Rhyzopertha dominica (Fabricius, 1792)

Rhyzopertha dominica: Hyde and Daubney 1960: 120. *Rhyzopertha dominica* (Fabricius, 1792): Denux and Zagatti 2010: 348.

Literature records. Malta: Floriana, St. Publius site, 1958, on grain, 1 ex (Hyde and Daubney 1960); "MT" [= Malta] (Denux and Zagatti 2010).

Material examined. Malta: St. Thomas Bay, 27.VI.1990, DM, 1 ex (CMM); Zejtun, 20.VII.1989, DM, attracted to light, 1 ex (CMM); 15.IX.1989, DM, 1 ex (CNI); 21.IX.1989, DM, 1 ex (CMM); 28.IX.1989, DM, 1 ex (CMM); 10.IX.2001, DM, 1 ex (CMM); 24.XI.2001, DM, 2 ex (CMM; CNI); 3.VII.2002 DM, 1 ex (CMM).

Chorotype. Cosmopolitan (Potter 1935, as *Rhizopertha dominica*, Borowski 2007). This species is widespread in the Palaeartic region (cf. Nardi 2004b, Borowski 2007). The followings territories were not included in its distribution provided by Borowski (2007): Bhutan (Taylor and Halliday 1986), Bulgaria (Zidan and Obretenchev 2009), China (Anhui, Chongqing, Guangdong, Guizhou, Hebei, Henan, Hubei, Hunan, Jiangxi, Shandong, Sichuan) (Lesne 1904, as *Rhi. dominica*, Song et al. 2012), Iran (cf. Abivari 2001, as *Rhi. dominica*, Ziaee et al. 2006, Ashouri and Shayesteh 2009), Israel (Halperin and Damoiseau 1980, as *Rhi. dominica*, Chikatunov et al. 2004, as *Rhyzoperta* [sic!] *dominica*, Chikatunov et al. 2006, as *Rhi. dominica*), Jordan (Sharaf et al. 1983), Libya (cf. Zanon 1922, Gridelli 1930, Zavattari 1934, in all cases as *Rhi. dominica*, Champ 1986), Lithuania (Ivinskis et al. 2009, as *Rhi. dominica*), Morocco (Martínez de la Escalera 1914, Rungs 1946, Español 1956b, Kocher 1956, in all cases as *Rhi. dominica*, Bartali et al. 1990, López-Colon 2000, as *Rhi. dominica*, Benhalima et al. 2004), Nepal (Taylor and Halliday 1986), Pakistan (cf. Quddus and Qayyum

1982, Ishaque et al. 1982, in both cases as *Rhi. dominica*, Taylor and Halliday 1986, Sardar Alam et al. 1999, Anwar et al. 2005, in both cases as *Rhi. dominica*, Wakil et al. 2006, Ahmed et al. 2008, as *Rhi. dominica*, Wakil et al. 2013), Portugal (Seabra 1943, as *Rhi. dominica*, Nardi 2004b, Bahillo de la Puebla et al. 2007, Baena and Zuzarte 2013), Romania (Ghizdavu and Deac 1995, Nardi 2004b), Saudi Arabia (Damoiseau 1979, Amin et al. 1986, in both cases as *Rhi. dominica*, Mostafa et al. 1981, Rostom 1993, Ahmed 1996), Switzerland (Hoppe 1981, Buchi 1993, Kenis 2005, as *Rhyzoperta* [sic!] *dominica*, Denux and Zagatti 2010), Turkey (Gerini 1971, Aydin and Soran 1987, Yucel 1988, Emekçi and Ferizli 2000), Ukraine (Podobivskiĭ 1991) and Uzbekistan (Asanov 1980).

Ecology. Originally, this species was only associated with wood but is now considered as a primary pest of all kinds of stored grains. Both larvae and adults are able to attack whole grain, causing considerable damage. The species is thought to have originated from the Indian subcontinent, and was introduced worldwide by commerce. It is an economically important pest since it can cause significant economic losses in terms of grain mass and nutrient depletion, and pose a public health risk from contamination by allergens, such as uric acid (cf. Potter 1935, Beeson and Bhatia 1937, Lepesme 1944, in all cases as *Rhi. dominica*, Fisher 1950, Aitken 1975, Maes 1995, Pollini 1998, in both cases as *Rhi. dominica*, Geis 2002, Gelosi and Süss 2001, Nguyen 2006, Borowski 2007, Liu et al. 2008, Denux and Zagatti 2010, Kenis and Branco 2010, Arthur et al. 2012, Edde 2012). This species can also damage books (Hoffman 1933, as *Rhi. dominica*).

Notes. The record from Malta by Hyde and Daubney (1960) was later overlooked by all authors (cf. Saliba 1963, Nardi 2004b, Borowski 2007). Thus this beetle must have been introduced in Malta at least since 1958 where it is now a well established species.

In Italy, *Rhyzopertha dominica* was first collected in Sicily during the nineteenth century (cf. Bertolini 1874, as *Rhi. pusilla* Fabr. [= (Fabricius, 1798)]; Ragusa 1896, as *Rhi. pusilla*), and since the 1950's it was widespread in all regions (cf. Norato 1957, Dal Monte 1958, as *Rhi. dominica*, Genduso 1963). According to Denux and Zagatti (2010: 348) the first European record of this species was from Czech Republic and is dated 1900, but the above Sicilian records, as well as those from other countries (cf. Reitter 1883, as *Rhi. pusilla*, Lesne 1901b), are older. Moreover, this species has been present in Europe at least since 3500–2551 BP, as testified by its archelogical presence in Spain and Greece (cf. King 2009). So the species was introduced and established before 1492 A.D. at least in Spain and Greece and must be considered as parautoc-thonous (cf. Zapparoli 2008: 98). Illustrations of its male and female genitalia were provided by Potter (1935: 474–475, figs 21–25), Lesne (1945: 149, figs 9–13, as *Rhi. dominica*) and Surtees (1961: 149, fig. 11, as *Rhi. dominica*).

Rhizopertha dominica granulipennis Lesne in Beeson & Bhatia, 1937 from northern India (Uttarakhand, Chandi Randge) (Beeson and Bhatia 1937: 283, Lesne 1945: 146, as *Rhi. dominica granulipennis*) was overlooked in recent catalogues (cf. Borowski 2007, Borowski and Węgrzynowicz 2007, Ivie 2010). In its original description – "A large form with exceptionally strongly developed granulation of the elytral declivity was bred from Shorea robusta [(Dipterocarpaceae)] and labelled R. dominica granulipennis by Lesne" (Beeson and Bhatia 1937: 283) - its rank is not unambiguously given, so, according to the Code (ICZN 1999, art. 45.6.4), it is an available subspecific name. Its authorship is here attributed to Lesne in Beeson and Bhatia (1937: 283), since Lesne (1945: 146) has reaffirmed the authorship of this name: "J'ai donné le nome de *R. dominica granulipennis* (1 [= footnote: Cf. Beeson et Bhatia, mémoire cité, p. 283.]) à cette forme remarquable" [= I named R. dominica granulipennis (1 [= footnote: Cf. Beeson et Bhatia, memory cited, p. 283.]) this remarkable form]. Unfortunately, it was not possible to study the type material of this taxon, that, according to Lesne (1945: 146), is housed in the Muséum National d'Histoire Naturelle of Paris (France) and in the Forest Research Institute of Derha Dun (India). However, on the basis of numerous specimens of this species from different territories (Nardi, unpublished data) which show a significant range of variability in the granulation of the elytral declivity, the following new synonymy, is here established: Rhizopertha dominica form granulipennis Lesne, in Beeson and Bhatia 1937: 283 = Rhyzopertha dominica (Fabricius, 1792: 359), syn. n.

Subfamily Bostrichinae Latreille, 1802, Tribe Bostrichini Latreille, 1802

Amphicerus bimaculatus (A.G. Olivier, 1790)

Material examined. Malta: Girgenti, 9.XII.2002, DM, $3 \stackrel{\diamond}{\circ} 4 \stackrel{\diamond}{\circ} 4 \stackrel{\diamond}{\circ}$ (CMM; CNI); Zabbar, 29.IX.1995, DM, $1 \stackrel{\diamond}{\circ}$ (CMM); Zejtun, 29.V.1991, DM, $1 \stackrel{\diamond}{\circ}$ (CMM).

Other material examined. [Azerbaijan:] Caucase, Elisabetpol [= Ganja], [no date], Babadjanides [leg.], $1 \triangleleft 1 \subsetneq (MCSVD)$.

Chorotype. Turanic-Mediterranean except for Libya and Egypt, with extension into Portugal, Hungary, Tajikistan and Kyrgyzstan (cf. Borowski 2007, as A. (Caenophrada) bimaculata [sic!]). The occurrence of this species in Azerbaijan (Lesne 1899, 1905, Clermont 1909, Khalilov 1972, in all cases as Schistoceros bimaculatus, Ciampolini et al. 1989) is here confirmed. In fact, the distributional record of this species by Borowski (2007: 321), as "E [= Europe]: AF [= Afghanistan]") is incorrect and should be "E: AB [=Azerbaijan]" (cf. Borowski and Węgrzynowicz 2007, as A. (C.) bimaculatus). A record from Iraq (Derwesh 1965, as S. bimaculatus) was later overlooked, while those from Germany (cf. Soro 1964, Zocchi 1971, in both cases as S. bimaculatus, Ciampolini et al. 1989, Pollini 1998) were never confirmed (cf. Lucht 1987, Köhler and Klausnitzer 1998, Geis 2002, Nardi 2004b, as S. bimaculatus, Borowski 2007, Borowski and Wegrzynowicz 2007). Amphicerus bimaculatus was intercepted at US ports, but it is not an established species in North America (Fisher 1950, as A. (S.) bimaculatus, Ivie 2002, Borowski and Wegrzynowicz 2007), while its establishment in Uruguay (cf. Soro 1964, Zocchi 1971, Pollini 1998), was not reported in recent publications (Borowski 2007, Borowski and Wegrzynowicz 2007, Barriga and Cepeda 2009).

Ecology. Larval development of this species takes place mainly in dead wood of *Vitis* spp. and *Tamarix* spp., but other host plants are also recorded including: *Acacia* sp., *Annona cherimola, Cerasus* sp., *Citrus* sp., *Delonix regia, Ficus carica, Lycium* sp., *Malus communis, Olea europea, Prunus amygdalus, Punica granatum, Pyrus malus* and *Tamarindus indica* (cf. Lesne 1901b, as *S. bimaculatus*, Fisher 1950, Novak 1952, Caillol 1954, in both cases as *S. bimaculatus*, Español 1955, Kocher 1956, as *S. bimaculatus*, Soro 1964, Zocchi 1971, Halperin and Damoiseau 1980, Lundberg et al. 1987, in both cases as *S. bimaculatus*, Moleas 1988, Ciampolini et al. 1989, Ragusa and Russo 1989, fig. 8, as [sic!] *Apate monachus*, Pollini 1998, Akşit et al. 2005, Liberto and Audisio 2005, in both cases as *S. bimaculatus*, Bahillo de la Puebla et al. 2007, as *A. (C.) bimaculata* [sic!], Tezcan 2008, as *S. bimaculatus*).

Notes. First record for Malta. *Amphicerus bimaculatus* is not recorded from the neigbouring Sicilian islands (Tab. 1), but is known from mainland Sicily (cf. Audisio et al. 1995, Sparacio 1997, Nardi 2004b, in all cases as *S. bimaculatus*) and Tunisia (Borowski 2007). It is recorded also from two circumsardinian islands (Piras and Pisano 1972, as *S. bimaculatus*). Its aedeagus was figured by Iablokoff-Khnzorian (1976: 232, fig. 5, as *S. bimaculatus*).

The above specimens collected during 2002 are almost entirely black, probably for a *post mortem* colouration.

The correct grammatical gender for the specific name of this taxon is *bimaculatus*, since it was described as *Bostrichus bimaculatus* from "Provence" (southern France) (Olivier 1790: 109) and the above mentioned usage of *bimaculata* (Bahillo de la Puebla et al. 2007, Borowski 2007) is a subsequent incorrect spelling of this taxon (cf. Borowski 2013: 3).

The nomenclatorial problems for Schistoceros Lesne, 1899 were discussed by Ivie (2010).

Bostrichus capucinus (Linnaeus, 1758)

Bostrychus capucinus (L.): Aitken 1975: 8. Bostrichus capucinus (Linnaeus, 1758): Nardi 2004b.

Literature records. Malta: "Malta: wooden ornament" (Aitken 1975); "Malta" (Nardi 2004b).

Other material examined. Jordan: Dana Reserve, El-Barrah, 1150 m, NE slope, 36R YU 517 926, 23.IV.–8.V.1995, D.P.I.T., Mediterranean environment, pt, 1 ex (MZUR); ditto, Wadi Araba Camp Site, 15–20.IV.1995, D.P.I.T., pt, 1 ex (MZUR). **Syria:** Palmyra, 15.VII.2003, G. Serra leg., 1 ex (MZUF).

Chorotype. Centralasiatic-Mediterranean, including parts of Northern Europe (cf. Nardi and Ratti 1995, as *Bostry. capucinus*, Borowski 2007), the Algerian Sahara (Lesne 1899, 1901b, both as *Bostry. capucinus*) and coastal Sudan (Cloudsley-Thompson 1962, as *Bostry. capucinus*). This species reaches Altai mountains (Lesne 1901b,

Borowski and Węgrzynowicz 2007), Asian Kazakhstan (Borowski 2007), Kyrgyzstan (Vrydagh 1956, Ovtchinnikov 1996, in both cases as *Bostry. capucinus*), Tajikistan (Lesne 1901b), Northwest China (Lesne 1904, Horion 1961, in both cases as *Bostry. capucinus*, Borowski 2007), China (without further details) (Borowski 2007, Yan et al. 2010, as *Bostry. capucinus* and *Bostry. capucinus* var. *rubrirenttis* [sic!] Zouf [= *rubrive-ntris* Zoufal, 1894]) and eastern Siberia (Borowski 2007). It was intercepted numerous times at US ports, but is not yet established in North America (cf. Fisher 1950, Ivie 2002). The above record from Jordan is the first for this country (cf. Sharaf et al. 1983, Borowski 2007), even though the presence of this species in Jordan was expected because of its occurence in neibouring countries (e.g. Israel, Syria, etc.); in Israel the species was probably introduced with timber from Europe (Bytinsky-Salz 1966, Bytinski-Salz and Sternlicht 1967, Halperin and Damoiseau 1980, in all cases as *Bostry. capucinus*, Borowski 2007).

Ecology. This species develops chiefly in the wood of oaks (*Quercus ilex, Q. robur, Q. toza*, etc.), but is also recorded from many other broadleaves trees and scrubs such as *Pinus*, and timber (cf. Nardi and Ratti 1995, Sparacio 1997, Liberto and Audisio 2005, Bahillo de La Puebla et al. 2007, Baena and Zuzarte 2013). Occasionally it can produce house infestations (Saccà 1940, as *Bostry. capucinus* Geoffroy [sic!], Cavalloro and Ratti 1978, Lodos 1985, Hellrigl 2006, in all cases as *Bostry. capucinus*).

Notes. The above record of Aitken (1975) was based on material collected in UK from cargo originating from Malta. The presence of this species in Malta needs to be confirmed. Although *Quercus ilex* is present in Malta, its abundance is very scarce on the archipelago (cf. Haslam et al. 1977, Schembri 1993, 1997). *Bostrichus capucinus* is known from other similarly small Mediterranean islands, such as the nearby Pantelleria Island, three circumsardinian islands (Piras and Pisano 1972, as *Bostry. capucinus*, Nardi and Ratti 1995), two northern Adriatic islands (Müller 1923: 28, Schatzmayr and Müller 1925: 74, Luigioni 1929: 611, in all cases as *Bostry. capucinus*), and the Balearic Islands (cf. Español 1955, as *Bostry. capucinus*).

Heterobostrychus aequalis (Waterhouse, 1884)

Material examined. Malta: Rabat, 21.IX.2001, PS, 1 👌 (CMM).

Chorotype. A cosmopolitan species of Indo-Malaysian origins. It is mainly distributed in tropical and sub-tropical regions and restricted to 40° north and south of the equator (cf. Borowski 2007, Borowski and Węgrzynowicz 2007, Azmi et al. 2011: 500, fig. 2). In the Mediterranean, it was intercepted in Israel, Italy and Spain (cf. Gambetta 1983, Geis 2002, Ratti 2002, 2004b, Bahillo de la Puebla et al. 2007, Ratti 2007, Azmi et al. 2011). Ireland (O'Mahony 1949, as *Heterobostrichus* [sic!] *aequalis*), France (cf. Brustel and Aberlenc 2014) and Oregon (Westcott et al. 2006) must be added to the countries in which this species was intercepted (cf. Azmi et al. 2011), eventhough the former record was based on collection of death specimens (larvae and adults), which was later ignored (cf. Anderson et al. 1997, Geis 2002, Nardi 2004b, Borowski 2007, Denux and Zagatti 2010, Alexander and Anderson 2012).

Ecology. Polyphagous species attacking some 36 unrelated host-plant genera; this species breeds not only in logs, but also in planks, furniture, plywood and roots of manioc (cf. Fisher 1950, Kalshoven 1963a, 1963b, Horion 1972, Gambetta 1983, Wang et al. 1996, as *Heterobostrachus* [sic!] *aequalis*, Geis 2002, Maes 2005, Aguilera 2006, Bahillo de la Puebla et al. 2007, Sitticaya et al. 2009, Robinson 2013).

Notes. First record for Malta. This species become established in some countries where it was accidentally introduced. Temperatures of 17 °C and below are said to be unsuitable for the species to breed (cf. Ivie 2002, Azmi et al. 2011). Thus, considering the warm climate of the Maltese Islands, (Chetcuti et al. 1992), it is highly likely that the species is already an established one. The above specimen was collected with a light trap on a terrace, mainly surrounded by agricultural land (P. Sammut, pers. comm., 2002).

Subfamily Bostrichinae Latreille, 1802, Tribe Sinoxylini Marseul, 1857

Sinoxylon unidentatum (Fabricius, 1801)

Material examined. Malta: Malta: Marsa, Ghammieri, 24.I.2007, DM, taken from wood packaging material originating from India, 1 ex (CMM).

Chorotype. A cosmopolitan species native to tropical eastern Asia, and widespread in the intertropical regions of the world. In Europe, it was intercepted in France, Germany, Great Britain, Italy, Poland, Russia, Spain and Ukraine, but its establishment was never confirmed. Moreover, the species is not reported from North Africa and the Middle East (except from Yemen) (cf. Vrydagh 1955, Poggi et al. 1994, Peck et al. 1998, Geis 2002, Ratti 2004, Maes 2005, Iwata and Nakano 2006, Liu et al. 2006, Peres Filho et al. 2006, Westcott et al. 2006, in all cases as *S. conigerum* Gerstäcker 1855, Bahillo de la Puebla et al. 2007, Borowski 2007, Borowski and Węgrzynowicz 2007, Barriga and Cepeda 2009, as *S. conigerum*, Savoldelli and Regalin 2009, Price et al. 2011, Brustel and Aberlenc 2014).

Ecology. In its place of origin, *S. unidentatum* develops in the wood of many unrelated plant families mainly: Anacardiaceae, Combretaceae, Dipterocarpaceae, Euphorbiaceae, Lamiaceae, Lauraceae, Leguminosae, Mimosaceae, Myrtaceae, Rubiaceae, Tiliaceae, Ulmaceae, etc. (cf. Poggi et al. 1994, Peres Filho et al. 2006, as *S. conigerum*, Bahillo de la Puebla et al. 2007, Savoldelli and Regalin 2009).

Notes. This alien species is a new record for the central Mediterranean area. Due to its polyphagy, it is frequently exported with wooden packing material (cf. Bahillo de la Puebla et al. 2007). The Maltese climate (Chetcuti et al. 1992, Schembri 1997) is unsuitable for its establishment (J. Borowski, pers. comm. 2012), but in an indoor site of northern Italy, this species was able to complete its development and to spread the infestation (Savoldelli and Regalin 2009).

Subfamily Bostrichinae Latreille, 1802, Tribe Xyloperthini Lesne, 1921

Scobicia chevrieri (A. Villa & J.B. Villa, 1835)

Xyloperta pustulata F.: Cameron and Caruana Gatto 1907: 398. *Scobicia pustulata* Fabr.: Luigioni 1929: 641. *Scobicia pustulata* (Fabricius, 1801): Nardi 2004b. *Scobicia chevrieri* (A. Villa & J.B. Villa, 1835): Mifsud et al. 2012: 9.

Literature records. Malta: [Malta,] "Coll. Gatto" (Cameron and Caruana Gatto 1907); "Mal." [= Malta] (Luigioni 1929); "Malta" (Nardi 2004b); Buskett, adults emerged between 5–25.X.2011 from dead twigs of *Ficus carica* collected on 8.VII.2011, AF & DM leg., 56 ex (Mifsud et al. 2012).

Material examined. Gozo: Marsalforn Valley, 6.VI.1990, DM, 1 ex (CMM). Malta: Bahrija, 5.VIII.1992, LC, 1 ex (CMM); Bingemma, 10.IX.2001, DM, attracted to light, 8 ex (CMM); Buskett, 24.VI.2003, DM, attracted to light, mixed woodland *Pinus/Cupressus*, 5 ex (CMM); Marsa, Ghammieri, 24.III.2002, DM, 1 ex (CMM); "Malta, 9/[= IX.]1901", "Xylopertha", "pustulata", "M. Cameron Coll. / B.M. 1936-555", "5777" [= *Xylopertha pustulata* F. Marsa Scirocco [= Marsaxlokk]/ id. EAN [= ?; maybe identified by E. A. Newbery (cf. Cameron and Caruana Gatto 1907: 383)]], 10 ex (BMNH); Rabat, 14.VI.2002, PS, 1 ex (CMM); 3.VIII.2002, PS, 3 ex (CMM); Rabat, Dwejra, 21.VI.2002, PS, 1 ex (CMM); Rabat, Ta Koronja, 6.VI.2002, PS, 2 ex (CMM); Wied Badu, 3.VIII.2002, DM, 5 ex (CMM); Wied tallsqof, 16.VII.2002, DM, 1 ex (CMM); 2.VIII.2002, DM, 2 ex (CMM); Zejtun, 10.XI.1989, DM, 1 ex (CMM); 29.IX.1990, DM, development of larvae took place in dead branches of vines, 2 ex (CMM); 27.V.2002, DM, 1 ex (CMM).

Other material examined. Italy: Marche region, Ancona prov., Gole di Frasassi, 20.VI.2001, A. B. Biscaccianti leg., ex [larvae from] *Corylus avellana*, 2 ex (CNI); ditto, Pesaro e Urbino prov., Foce Fiume Metauro, area golenale [= Mouth of Metauro River, floodplain area], 2.VI.1999, A. B. Biscaccianti leg., ex larvae from *Salix* sp., 4 ex (CNBFVR; CNI). Latium region, Rome prov., Tenuta Presidenziale di Castelporziano, Ponte della Focetta, 10.IX.1997, A. B. Di Giulio leg., hygrophilous wood, light trap 15 W, 1 ex (CNI); ditto, ditto, ditto, Villa di Capocotta, 21.VI.2000, P. Maltzeff leg., mixed light trap 160 W, 2 ex (CNI); ditto, Latina prov., Cisterna di Latina, [33T 319824.15 E 4606546.61 N], 29.IX.1987, GN, night, in a garden, at light, 2 ex (CNI); ditto, ditto, Fraz. Cerciabella, [33T 319479.96 E 4605030.39 N], 17.VIII.1998, GN, in a garden, at light, 20–21 hours, 2 ex (CNI). **Jordan:** Dana Reserve, Acacia Area, 17.IV.1995, D.P.I.T., night catch, 1 ex (MZUR); ditto, El-Barrah, 1150 m, NE slope, 36R YU 517 926, 23.IV.–8.V.1995, D.P.I.T., Mediterranean environment, pt, 1 ex (MZUR); ditto, Irano Turanian Area 1, 18.IV.1995, D.P.I.T., 1 ex (MZUR).

Chorotype. Mediterranean (northward upto Austria, French Alps, Hungary and Switzerland), with extension westward upto Portugal, and eastward upto Azerbaijan,

Georgia, Iran and southern Russia; this species was intercepted in the USA and Canada, but so far it has not established itself (cf. Fisher 1950, as *S. chevrieri* (Villa) [sic!], Vrydagh 1952, as *S. Chevrieri* (Villa) [sic!], Ivie 2002, Borowski 2007, Borowski and Węgrzynowicz 2007, McCaffrey 2011, as *S. chevrieri* Villa & Villa, 1835 [sic!]). It is known also from Romania (Lesne 1904, Vrydagh 1956, in both cases as *S. Chevrieri* Villa [sic!], Nardi 2004b) and Sinai (cf. Alfieri 1976, as *S. chevrieri* Villa [sic!]), but these two regions were overlooked by Borowski (2007). The above record from Jordan is the first for this country (cf. Sharaf et al. 1983, Borowski 2007).

Ecology. Polyphagous species, with development taking place in death or debilitated branches of several woody plants. The following are plants known to be infested by this species and are present in Malta (cf. Haslam et al. 1977): *Acacia* sp., *Amygdalus communis, Arundo* sp., *Bambusa* sp., *Ceratonia siliqua, Cercis* sp., *Citrus* sp., *Eucalyptus* sp., *Ficus carica, Hibiscus sabdariffa, Laurus nobilis, Morus alba, Olea* sp., *Pinus halepensis, Pistacia lentiscus, P. vera, Prunus avium, P. dulcis, Punica granatum, Quercus* spp., *Rhamnus alaternus, Ulmus* sp. and *Vitis* spp. (cf. Lesne 1901b, as *S. Chevrieri* Villa [sic!], Peyerimhoff 1919, Novak 1952, both as *S. Chevrieri* (Villa) [sic!], Caillol 1954, Español 1955, as *S. chevrieri* Vill. [sic!], Bytinski-Salz and Sternlicht 1967, as *S. chevrieri* Villa [sic!], Halperin and Damoiseau 1980, as *S. chevrieri* (Villa) [sic!], Lundberg et al. 1987, as *S. chevrieri* Villa [sic!], Nardi and Ratti 1995, Borowski and Mazur 2001, Nardi and Zahradník 2004, Akşit et al. 2005, as *S. chevrieri* Villa [sic!], Baena and Zuzarte 2013).

In central Italy (Marche region), this species (see above) was reared also from wood of *Salix* sp. and *Corylus avellana*, that represent new host-plant records for this Bostrichid (see above listed literature).

This species is often collected at light (Angelini 1996a, 1998, Chikatunov et al. 2006, Baena and Zuzarte 2013) and by window flight traps. Using these traps, large number of specimens were collected in forests of *Quercus calliprinos, Pinus halepensis* and *P. brutia* from northern Israel (Buse et al. 2010), in *Q. suber* forests from southern France (Brin et al. 2005, Brin and Brustel 2006), in an oak-hornbeam forest (*Querco-Carpineto boreoitalicum*) from northern Italy (Nardi and Zahradník 2004), in *Quercus ilex* forests of Sardinia, in a floodplain remnant of northern Italy (Nardi, unpublished data) and in mixed beechwoods of central Italy (Redolfi De Zan et al. 2014).

This species was also recorded from urban areas (Nardi 1997, Inglebert 2004).

Notes. *Scobicia pustulata* (Fabricius, 1801), a closely related Mediterranean species (cf. Borowski 2007), is here excluded from the Maltese fauna, since the record by Cameron and Caruana Gatto (1907) should refer to *S. chevrieri* as established by the examination of the above mentioned historical material. This is not a case of misidentification by Cameron and Caruana Gatto (1907), since they (Cameron and Caruana Gatto 1907: 383) based the nomenclature of this species on Heyden et al. (1891: 467) who listed "*Xyloperta pustulata* F.Kiesw.", and "*Xyloperta pustulata* Kiesenw. (non F.)" is *S. chevrieri* (Lesne 1938: 57, as *S. Chevrieri* Villa [sic!], 1835). Only on the basis of this old litterature record, *S. pustulata* was erroneously listed from Malta by the above subsequent authors (Luigioni 1929, Nardi 2004b).

Scobicia chevrieri is a good colonizer of Mediterranean islands, since it is recorded also from other islands such as Montecristo (Tuscan Archipelago), Pantelleria, Lampedusa (cf. Nardi and Ratti 1995), Balearic and Columbretes Islands (cf. Lesne 1901b, as *S. Chevrieri* Villa [sic!], 1835, Español 1955, Vrydagh 1960b, as *S. chevrieri* Villa [sic!], 1835, Compte 1970, Nardi 2004b).

Xyloperthella picea (A.G. Olivier, 1790)

Material examined. Malta: Qormi, Hal-Farrug, 5.V.2003, DM [under bark of *Ceratonia siliqua* in an agricultural environment], 1 \bigcirc (CMM); Rabat, 4.VI.1989, PS, attracted to light [on the roof of private residence], 1 ex (CMM); 18.VI.1992, PS, [attracted to light, in an agricultural environment], 2 ex (CMM); 4.VI.1999, PS, attracted to light [in an agricultural environment], 1 \Diamond , (CMM); 28.VI.2001, PS, [attracted to light, in an agricultural environment], 1 \Diamond , (CMM); 21.VI.2002, PS, [ditto], 2 $\Diamond \Diamond$ (CNI); 23.VI.2002, PS, [ditto], 2 $\bigcirc \bigcirc$ (CMM; CNI); 1.VII.2002, PS, [ditto], 1 \Diamond 1 \bigcirc (CMM); 3.VI.2003, PS, [ditto] 1 \bigcirc (CNI); Rabat, Ta Koronja, 21.VI.2002, PS, [attracted to light], 1 \bigcirc (CMM).

Other material examined. Italy: Apulia region, Lecce prov., S. Cataldo, Ris. [= Riserva = Reserve] WWF Le Cesine, 11–21.VI.1995, F. Angelini, 2 ex (MCSVA). Sardinia region, Sassari prov., Berehidda, 15.VII.1985, M. Daccordi leg., 1 ex (MCSV). Sicily region, Siracusa prov., Noto, Oasi di Vendicari, Cala Mosche, 10 m, N°36 49,066' E15°5,834', 3.VII.2011, D. Birtele & P. Birtele leg., 1 ♀ (CCI).

Chorotype. Afrotropical-Mediterranean species which was also intercepted in Germany, Great Britain and The Netherlands (cf. Lesne 1924, 1938, in both cases as *Xylopertha picea*, Aitken 1975, Akşit et al. 2005, Borowski 2007, in both cases as *Xyloperthella p. picea*, Bahillo de la Puebla et al. 2007, Borowski and Węgrzynowicz 2007, Baena and Zuzarte 2013). According to several authors (cf. Lesne 1901a, 1924, 1938, Gridelli 1939, 1940, Blackwelder 1945, Da Costa Lima 1953, in all cases as *Xylopertha picea*, Vrydagh 1958, 1960a, 1960b, 1961, 1962, Geis 2002, Ivie 2002, Borowski 2007, Peres Filho et al. 2007, Barriga and Cepeda 2009, Baena and Zuzarte 2013), it became established in parts of the Neotropical region (Argentina, Brazil, Colombia, French Guyana, Jamaica, Paraguay, Perù) since a long time but its presence in this region was recently reported as doubtful (Borowski and Węgrzynowicz 2007).

Notes. First record for Malta and the Apulia region; the species is not known from neighbouring Sicilian Islands (Tab. 1) but is widespread in Africa. Whether this species is autochthonous or has been introduced into Malta might be therefore open to debate. It is known from Tunisia and from all other mainland countries of North Africa (Lesne 1901b, Normand 1936, in both cases as *Xylopertha picea*, Vrydagh 1956, Borowski 2007), while from southern Europe it is recorded only from Portugal (cf. Serrano 1981, as *X. picea*, Baena and Zuzarte 2013), Greece (Samos Island) (Vrydagh 1962, Nardi 2004b, as *Xyloperthella p. picea*), southern mainland Italy (Basilicata region), Sardinia,

Sicily, Spain and the Balearic Islands (cf. Lesne 1901a, 1901b, 1905, Winkler 1927, Luigioni 1929, Porta 1929, in all cases as *Xylopertha picea*, Cobos 1950, as *Xyloperthe* [sic!] *picea*, Español 1955, 1974, Angelini and Montemurro 1986, Audisio et al. 1995, Sparacio 1997, Angelini 1998, Nardi 2004b, Bahillo de la Puebla et al. 2007, Borowski 2007).

According to some authors (cf. Lesne 1924, Español 1955), the Mediterranean populations may belong to a distinct subspecies, *Xyloperthella picea heydeni* (Schilsky, 1899), nevertheless the species is currently monotypic since this subspecies and also *X. picea plumbeipennis* Lesne, 1924 from Gabon and Democratic Republic of Congo (Lesne 1924), were recently listed as a synonym of *X. picea* (Borowski and Węgrzynowicz 2007). The species was described from Cape Verde Islands (Olivier 1790, as *Bostrichus piceus*) and not from Senegal as indicated by some authors (López-Colón 2000, López-Colón et al. 2001, Bahillo de la Puebla et al. 2007); in this African archipelago it is probably autochthonous (Oromí et al. 2005).

Subfamily Apatinae Billberg, 1820, Tribe Apatini Billberg, 1820

Apate monachus Fabricius, 1775

Material examined. Malta: Gharghur, 18.VII.2013, DM, on living branchs of *Ficus carica*,1 \bigcirc (CMM); Manikata, VII.2012, DM, in healthy branch (3–5 cm in diameter) of *Ceratonia siliqua* tunneled by this beetle, 1 \bigcirc death (CMM). Mellieha, Kortin, 24.VII.2004, HB, U.V. light trap, 1 \bigcirc (CNI); 28.VII.2004, HB, U.V. light trap, 2 \bigcirc \bigcirc (CMM); 29.VII.2004, HB, U.V. light trap, 1 \bigcirc (CMM); 15.VIII.2004, HB, U.V. light trap, 1 \bigcirc (CMM); 15.VIII.2004, HB, U.V. light trap, 1 \bigcirc (CMM); 3.VII.2005, HB, U.V. light trap, 1 \bigcirc (CMM); 28.VI.2005, HB, UV lights, 1 \bigcirc (CMI); 16.VII.2005, HB, U.V. light trap, 2 \bigcirc \bigcirc (CMM; CNI); 5.VII.2006, HB, UV lights, 1 \bigcirc (CNI); 16.VII.2006, HB, U.V. light trap, 2 \bigcirc \bigcirc (CMM); 20.VI.2006, HB, UV lights, 1 \bigcirc (CMI); 20.VI.2006, HB, UV lights, 1 \bigcirc (CMI); 19.VI.2009, HB, UV lights, 1 \bigcirc (CMM); 5.VII.2009, HB, UV lights, 3 \bigcirc \bigcirc 1 \bigcirc (CMM); 11.VIII.2009, HB, UV lights, 2 \bigcirc \bigcirc (CMM); 13.VIII.2009, HB, UV lights, 1 \bigcirc (CMM); Mellieha, Santa Maria Estate, 24.VII.2004, HB, 1 \bigcirc (CMM).

Other material examined. Italy: Calabria region, [Cosenza prov.,] Sibari, VII.1924, G. Leoni leg., 1 ex (MCZRL) (cf. Luigioni 1929: 642). Sardinia region, Cagliari prov., Geremeas, 18.VIII.2001, LF, 1 \bigcirc (CNI); ditto, ditto, [Island of Sant'Antioco,] Sant'Antioco, Torre Canai, 25.VIII.[19]79, Ferrara leg., 1 \bigcirc (MZUR); ditto, Oristano prov., Arborea, S. Anna, 10.VI.2004, LF, 6 \bigcirc 1 \bigcirc (CNI); ditto, Nuoro prov., Orosei, VI.1956, E. De Maggi leg., 1 ex (MCZRD); ditto, [Nuoro prov., Siniscola,] Capo Comino, 31.VIII.1973, L.G. Donadini leg., 1 \bigcirc (MCSV); ditto, [Sassari prov.,] Stintino, Punta Negra, 15.VII.1998, G. Mambrini leg., 1 \bigcirc (CCI). **France:** Corsica, Bastia, Pineto, 3.VIII.[19]80, A. Sette leg., 1 \bigcirc (MCSV).

Chorotype. Afrotropical-Mediterranean (northward upto Corsica and northern Spain). This species is established in the Neotropical region (Greater Antilles, Brazil,

etc.), and was intercepted in southern France, in central European countries and USA (cf. Vrydagh 1960b, Reichardt 1964, Horion 1972, Aitken 1975, Geis 2002, fig. 31, Ivie 2002, Nardi 2004b, Bahillo de la Puebla et al. 2007, Borowski 2007, Barriga and Cepeda 2009, Ciesla 2011, Brustel and Aberlenc 2014).

Ecology. The genus Apate Fabricius, 1775 is one of the most notorious and troublesome forest pests in Africa (cf. Schabel 2006). Apate monachus is a polyphagous beetle, with over 80 host-plants used for larval development (cf. Lesne 1924, Rungs 1946, Boselli 1959, as A. monachus var. rufiventris P.H. Lucas, 1843, Peretz and Cohen 1961, Boselli 1962, as Apathe [sic!] monachus, Chararas and Balakowski 1962, Prota 1963, Zanardi et al. 1969, Zocchi 1971, Halperin and Damoiseau 1980, Luciano 1982, Benfatto and Longo 1985, Sadok and Gerini 1988, Borowski and Mazur 2001, Gobbi 2003, Pisano et al. 2003, Schabel 2006, Bahillo de la Puebla at al. 2007, Di Franco and Benfatto 2008, Bonsignore et al. 2011, Ciesla 2011, Bonsignore 2012, Cillo and Bazzato 2012) of which, the following occur also in Malta (E. Lanfranco, pers. comm., 2014): Acacia spp., Ailanthus glandulosa, Amigdalis comunis, Arbutus unedo, Armeniaca vulgaris, Ceratonia siliqua, Citrus bigaradia, C. limon, C. limonia, C. nobilis, C. sinensis, Cupressus sp., Erica sp., Erythrina sp., Eucalyptus spp., Grevillea sp., Malus communis, M. domestica, Melia azedarach, Myrtus comunis, Olea europaea, O. europaea var. oleaster, Persica vulgaris, Phoenix dactylifera, Pinus pinea, Pisidium guajava, Pistacia lentiscus, Pyrus amigdaliformis, P. dulcis, P. communis, P. communis var. piraster, Prunus amygdalus, P. armeniaca, P. domestica, P. persica, P. spinosa, Punica granatum, Quercus ilex, Schinus sp., Tamarix sp., and Vitis spp. Adults are nocturnal and as observed also in Malta, they are frequently collected at light (cf. Lesne 1924, Sparacio 1997, Angelini 1998, Ragusa and Russo 1989, Chikatunov et al. 2006, Bahillo de la Puebla et al. 2007). In the Afrotropical region, Lyctoderma africanum (Grouvelle, 1900) and L. testaceum Lesne, 1913 (Bostrichidae, Lyctinae) are associated with adults of A. monachus, usually living under the abdomen and between the legs of these beetles (Lesne 1932, Paulian 1988: 501).

Notes. First record for Malta and it is not known from neighbouring Sicilian Islands (Tab. 1). Most of the Maltese specimens were collected at Mellieha, Kortin using U.V. lights, with sex ratio of 1:1. This habitat can be best described as garigue but with pockets of low lying *Ceratonia siliqua* and *Pistacia lentiscus*. At Manikata, several healthy branches (3–5 cm in diameter) of *Ceratonia siliqua* were found tunneled by this beetle; the wood was drilled in the late summer, and the above mentioned specimen was found death in one of these holes. This area was recently converted into an agritouristic area and the owners were very concerned when they found the healthy branches of *Ceratonia siliqua* damaged by this beetle. According to the above records, the earliest capturs of this species from Malta was 2004. This beetle is very conspicuous (10–19 mm of lenght: Bahillo de la Puebla et al. 2007) and it may be hypothesised that it is a recently established species in Malta. Having said this however, it is also worth mentioning that very few people in Malta were interested in collecting and studying these beetles in recent years. *Apate monachus* is also recorded from Tunisia (Lesne 1924, Borowski 2007) and Italy. In Italy it is known from three southern mainland

regions (Apulia, Basilicata and Calabria), Sicily and Sardinia (including some small circumsardinian islands) (cf. Dodero 1908, as *A. monachus* ab. *rufiventris*, Luigioni 1929, Porta 1929, as *A. monachus* a. *rufiventris*, Boselli 1959, 1962, Chararas and Balachowsky 1962, Prota 1963, Tassi 1967, Zanardi et al. 1969, Zocchi 1971, Luciano 1982, Benfatto and Longo 1985, Ragusa and Russo 1989, Audisio et al. 1995, Angelini 1996b, Sparacio 1997, Angelini 1998, Gobbi 2003, Pisano et al. 2003, Bonsignore et al. 2011, Bonsignore 2012, Cillo and Bazzato 2012). Its doubtfull presence "N?", in northern Italy (Audisio et al. 1995) was later confirmed by records from South Tyrol (Kahlen and Hellrigl 1996) that are probably based only on interceptions, since its establisment in the mentioned Alpine region is climatically improbable (Nardi, unpublished data).

The aedeagus of this species was figured by Jeannel and Paulian (1944: 91, figs 75, 87) and by Jeannel (1955: 57, fig. 32c).

The nomenclatorial problems for *Apate* Fabricius, 1775 were discussed by Borowski and Węgrzynowicz (2009).

Discussion

Table 1 summarized the species of Bostrichidae recorded from the Maltese Archipelago and neigbouring Sicilian Islands with each assigned to a chorotype. Altogether 12 species are known: 4 from Pantelleria, 1 from Linosa, 1 from Gozo, 9 from Malta and 1 from Lampedusa; and only *Scobica chevrieri* is recorded from four islands (except from Linosa).

For Sicily and Tunisia, 22 (Catara and Barbagallo 1980, Benfatto and Longo 1985, Audisio et al. 1995, Nardi 2004b, 2004b, Suma and Russo 2005, Muscarella et al. 2013) and 30 species respectively (Borowski 2007) of Bostrichidae are recorded. The relatively small number of species recorded from the Sicilian Channel Islands (Tab. 1) is probably due to the limited surface area, by the absence of many host plants, and the absence of certain habitats such as moutain and sub-mountain ranges.

Excluding Sinoxylon sexdentatum (A.G. Olivier, 1790) (= muricatum (Linnaeus, 1767) nomen oblitum) and Xylopertha praeusta (Germar, 1817), Malta hosts all species recorded from the Sicilian Channel Islands (Tab. 1). Some of the host plants (e.g. Vitis spp., Pistacia spp., Ficus carica, Malus domestica, Olea europaea, Prunus persica, Pyrus communis, Quercus ilex, Ceratonia siliqua, Cercis siliquastrum, Citrus spp.) of the former species (cf. Lesne 1901b, 1906, Peyerimhoff 1919, Halperin and Damoiseau 1980, Gobbi 1984, Benfatti and Longo 1985, Moleas 1988, Mart et al. 1995, Ratti and Nardi 1995, Mourikis et al. 1998, Pollini 1998, Akşit et al. 2005) are present also in Malta (cf. Borg 1962, Saliba 1963, Haslam et al. 1977), so this polyphagous beetle could be eventually found in Malta or may establish itself. The occurrence of X. praeusta, which develops chiefly in the wood of oak trees (Quercus ilex, Q. suber, Q. robur, Q. mirbecki) (Lesne 1901b, as Xylonites praeustus Germar, Rungs 1946, as X. praeustus Germ., Español 1955, Bahillo de la Puebla et al. 2007), is less probable since oak trees are rare in the Maltese Islands.

Table I. Bostrichidae recorded from Maltese Archipelago and neigbouring islands with respective chorotype codes. Abbreviations: A = Aitken 1975; AFM = Afrotropical-Mediterranean; C = Cameron and Caruana Gatto 1907; CEM = Centralasiatic-European-Mediterranean; COS = Cosmopolitan; D = Denux and Zagatti 2010; EUR = European; F = Falzon et al. 2012; G = Goggi 2004; H = Hyde and Daubney 1960; L = Luigioni 1929; LAMP. = Lampedusa; LINO. = Linosa; MED = Mediterranean; Na = Nardi 2004a; Nb = Nardi 2004b; NR = Nardi and Ratti 1995; PANTEL. = Pantelleria; SEU = S-European; TEM = Turanic-European-Mediterranean; TUM = Turanic-Mediterranean; WME = W-Mediterranean; ZGG = Chorotype; ! = this paper; () = misinterpretation.

Species	PANTEL.	LINO.	GOZO	MALTA	LAMP.	ZGG
Lyctus brunneus				C L Na D !		COS
Trogoxylon impressum	NR			!		TEM
Rhyzopertha dominica				HD!		COS
Amphicerus bimaculatus				!		TUM
Bostrichus capucinus	NR			A Nb		CEM
Heterobostrychus aequalis				!		COS
Scobicia chevrieri	NR		!	(C L Nb) F !	NR	MED
Sinoxylon sexdentatum	NR					MED
Sinoxylon unidentatum				!		COS
Xylopertha praeusta		G				WME
Xyloperthella picea				!		AFM
Apate monachus				!		AFM
TOTAL/ISLANDS	4	1	1	10	1	

With the current available data, no conclusions can be drawn on how the bostrichid fauna colonised the Maltese Islands, but we can say that almost all species are also known from Sicily and North Africa, and are normally widely spread in the Mediterranean area. In fact, from a zoogeographical point of view (cf. Tab. 2) wide chorotypes prevails: 40% Cosmopolitan, 30% Palaearctic, 20% Afrotropical-Mediterranean and 10% Mediterranean. European chorotypes are absent; this situation was observed in other families of Coleoptera (Mifsud and Bílý 2002, Nardi and Mifsud 2003, Háva and Mifsud 2006), whereas they are rappresented in two other families of saproxylic beetles (Mifsud 2002, Mifsud and Knížek 2009).

Species of bostrichids are easly transported with infested woods and cereal, and are frequently observed in harbour areas (cf. Aitken 1975, Contessi 1991, Contessi and Mucciolini 1993, 1998, Peck et al. 1998, Haack and Cavey 2000, Geis 2002, Nicoli Aldini 2003, 2004, Ratti 2004, Borges et al. 2006, Haack 2006, Liu et al. 2006, Majka 2007, Ratti 2007, Beiriger 2010, Humble 2010, Leal et al. 2010, Price et al. 2011). The interception in Malta of other alien species from warm climatic regions is highly likely, and their possible establishment could also be promoted by global warming (cf. Parmesan 2006, Franceschini et al. 2009, Walther et al. 2009, Fronzek et al. 2010, Robinet and Roques 2010, Roques 2010, Thuiller et al. 2011).

Chorotypes	Number of species (%)	Pantelleria	Linosa	Maltese Islands	Lampedusa
COS	4 (33.33)			4 (40)	
AFM	2 (16.66)			2 (20)	
CEM	1 (8.33)	1 (25)		1 (10)	
TEM	1 (8.33)	1 (25)		1 (10)	
TUM	1 (8.33)			1 (10)	
MED	2 (16.66)	2 (50)		1 (10)	1 (100)
WME	1 (8.33)		1 (100)		
Total	12	4	1	10	1

Table 2. Chorotypes of the Bostrichidae from Malta Archipelago and neigbouring islands, their distribution and percents (abbreviations as in Table 1).

From a faunistic point of view, six species are here recorded for the first time, namely: *Trogoxylon impressum*, *Amphicerus bimaculatus*, *Heterobostrychus aequalis*, *Sinoxylon unidentatum*, *Xyloperthella picea* and *Apate monachus*.

The Mediterranean area is one of the most significantly altered biodiversity hotspots on Earth, since it has been intensively affected by human activity for millennia. As a result, only 4.7% of its primary vegetation remained unaltered and the landscape has been repeatedly transformed (cf. Myers et al. 2000, Cuttelod et al. 2008, Geri et al. 2010, Carroll et al. 2012). This transformation has negatively influenced xylophagous insect populations, especially in small and isolated environments, like those of islands (cf. Becker 1975, Howart and Ramsay 1991, Becker 1992, Crisp et al. 1998, Mantisi 2001, Pasta and La Mantia 2002). In this framework, half of bostrichid species recorded from the Maltese Islands (*Amphicerus bimaculatus, Bostrichus capucinus, Scobicia chevrieri, Xyloperthella picea, Apate monachus*), are included in the European Red list of saproxylic beetles (Nieto and Alexander 2010: 29), but luckily all belong only to the IUCN category "LC" (Least Concern).

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RESEARCH ARTICLE



Rhinolekos capetinga: a new cascudinho species (Loricariidae, Otothyrinae) from the rio Tocantins basin and comments on its ancestral dispersal route

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Abstract

The present study deals with the description of a new species of *Rhinolekos*. It can be distinguished from its congeners by having 31 vertebrae, the anterior portion of the compound supraneural-first dorsal-fin proximal radial contacting the neural spine of the 9th vertebra, the absence of transverse dark bands in the pectoral, pelvic and anal-fin rays, 24–28 plates in the dorsal series, the lack of odontodes on the ventral tip of the snout, the absence of accessory teeth, a greater prenasal length, a smaller head length, and by a greater snout length. *Rhinolekos capetinga* is restricted to the headwaters of the rio Tocantins and it is the first species of this genus in the Amazon basin. Additionally, we present a brief discussion of a biogeographic scenario that may explain the dispersal of the new species from the rio Paranaíba to the rio Tocantins basin. We suggest that the ancestral lineage of *R. capetinga* reached the rio Tocantins from portions of the rio Paranaíba at the end of the Miocene, about 6.3 Mya (4.1–13.9 Mya 95% HPD), probably as a result of headwater capture processes among adjacent drainages.

Keywords

Biodiversity, Freshwater, Neotropical fish, South America, Taxonomy

Introduction

Otothyrinae sensu Chiachio et al. (2008) is one of the most diverse and widespread members of Loricariidae, and is composed of about 97 species (Eschmeyer and Fong 2014). Fishes of this subfamily are characterized by morphological specializations such as the presence of metapterygoid channel, the ventral margin of preopercle medially reflected, the rostral plate with posterior notch articulated with mesethmoid, the fourth infraorbital expanded ventrally, and the almost complete fusion of pectoral dermal bony plates forming a strong pectoral armor (Schaefer 1998; Chiachio et al. 2008). In the last fifteen years, new genera and species have been described and assigned (Roxo et al. 2014a) to the subfamily Otothyrinae (e.g. *Gymnotocinclus* Carvalho, Lehmann & Reis, 2008; *Rhinolekos* Martins & Langeani, 2011a), indicating that the great diversity of this group still remains fairly known (Martins and Langeani 2011a).

Rhinolekos is the most recently described genus of Otothyrinae and differs from its members, mainly by having the anterior portion of the compound supraneural-first dorsal-fin proximal radial contacting the neural spine of the 9th or 10th vertebrae and by the presence of the lateronasal plate. Currently, *Rhinolekos* includes three valid species: *R. britskii* Martins & Langeani, 2011a, *R. schaeferi* Martins & Langeani, 2011a and *R. garavelloi* Martins & Langeani, 2011a, all of which were described from drainages of the rio Paranaíba (upper rio Paraná basin).

Furthermore, several authors discussed the historical dispersal of the ancient fauna among adjacent drainages of South America Platform (e.g. Ribeiro 2006; Albert and Reis 2011) and specifically species of the families Hypoptopomatinae, Neoplecostominae, and Otothyrinae (e.g. Roxo et al. 2012; Roxo et al. 2014a). The geological process responsible for this distribution pattern is head water captures (also known as stream capture or stream piracy). This is a geomorphological process by which the flow of part of a stream or river drainage basin is diverted into neighboring basin. River capture may facilitate the dispersal of fish species among adjacent drainage and can have profound consequences in isolated local fauna, which watershed boundaries strongly limit their dispersal (Grant et al. 2007; Muneepeerakul et al. 2008; Bertuzzo et al. 2009).

Recently, during collecting expeditions in small tributaries of the rio Tocantins, the major drainage of the Brazilian Shield (Carvalho and Albert 2011), an undescribed species of Otothyrinae, which meets the diagnosis of *Rhinolekos*, was collected and is formally described in the present study. Moreover, we used a time calibrated phylogenetic analysis and parametric biogeographic methods based on available data of Roxo et al. (2014a) to estimate ancestral geographic ranges and hypothesize when the new species reached the rio Tocantins from sections of the rio Paranaíba, probably as a result of head water capture events between these two hydrographic systems in Late Miocene.

Material and methods

Morphological analysis

After collection, fish were anesthetized using 1% benzocaine in water, fixed in 10% formaldehyde, and preserved in 70% ethanol for morphological study. Institutional acronyms follow Fricke and Eschmeyer (2014). Vouchers of the morphological study were deposited in the collection of the Laboratório de Biologia e Genética de Peixes (LBP) and Museu de Zoologia da Universidade de São Paulo (MZUSP), Brazil. Measurements and counts were taken on the left side of specimens. Measurements followed Boeseman (1968) with modifications of Armbruster and Page (1996), Schaefer and Provenzano (1993), and Ribeiro et al. (2005) and were taken point to point to the nearest 0.1 mm with digital calipers. Meristic data include numbers of premaxillary and dentary teeth, dorsal, middorsal, median, mid-ventral and ventral plates following Schaefer (1997). Abbreviations used in the text followed Carvalho and Reis (2009). Specimens were cleared and stained (c&s) according to the method of Taylor and Van Dyke (1985). Head plate and osteology nomenclature followed Schaefer (1997). Dorsal-fin ray counts include spinelet as the first unbranched ray. Vertebral counts also include the five vertebrae that comprise the Weberian apparatus. The compound caudal centrum (PU1 + U1) was counted as one element. Zoological nomenclature follows the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999).

Molecular analysis

We used *Diplomystes mesembrinus* to root our phylogeny. Additionally, samples of *Corydoras imitator, Corydoras oiapoquensis, Hoplosternum littorale, Callichthys callichthys, Astroblepus* sp. 1 and *Astroblepus* sp. 2, *Hemipsilichthys gobio, H. papillatus, Delturus parahybae, Rineloricaria lanceolata, Spatuloricaria* sp. 1, *Hypostomus ancistroides, H. nigromaculatus* and *H. microstomus* were used as additional outgroups. We included in the analysis 155 specimens representing 115 loricariid species (see Suppl. material 3 – Table S1 to all species names, localities, deposits in museums and GenBank accession numbers).

Vouchers of the molecular study were deposited at the collection of the Laboratório de Biologia e Genética de Peixes (LBP); the Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul (MCP); the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (NUP); and the Museum of Natural History of the City of Geneva (MHNG).

Sequencing

Total DNA was extracted from ethanol preserved muscle samples with the DNeasy Tissue Kit (Qiagen), following manufacturer's instructions. Partial sequences of the genes 16S rRNA (Kocher et al. 1989), cytochrome b (Cytb) (Oliveira et al. 2011), cytochrome c oxidase subunit I (COI) (Ward et al. 2005) and F-reticulon 4 (Chiachio et al. 2008) were amplified using polymerase chain reaction (PCR) with the primers described in Suppl. material 4 - Table S2. Amplifications were performed in a total volume of 12.5 µl with 1.25 µl of 10X buffer (10 mM Tris-HCl+15 mM MgCl2), 0.5 µl dNTPs (200 nM of each), 0.5 µl each 5 mM primer, 0.05 µl Platinum[®] Taq Polymerase (Invitrogen), 1 µl template DNA (12 ng), and 8.7 µl ddH2O. The PCR reactions consisted of 30-40 cycles, 30 s at 95 °C, 15-30 s at 48-58 °C, and 45-90 s at 72 °C. Nested-PCRs were used to amplify the nuclear marker; the first amplification was performed using the primers Freticul4-D and Freticul4-R with a total volume of 12.5 µl for 30–40 cycles (30 s at 95 °C, 30 s at 48 °C, and 135 s at 72 °C); the second amplification was performed using the primers Freticul4 D2 and Freticul4 R2 with a total volume of 12.5 μ l for 30–40 cycles (30 s at 95 °C, 30 s at 53–54 °C, and 135 s at 72 °C). All PCR products were first visually identified on a 1% agarose gel and then purified using ExoSap-IT[®] (USB Corporation) following instructions of the manufacturer. The purified PCR products were sequenced using the "Big DyeTM Terminator v3.1 Cycle Sequencing Ready Reaction Kit" (Applied Biosystems), purified again by ethanol precipitation and loaded on an automatic sequencer 3130-Genetic Analyzer (Applied Biosystems) in the Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo.

Phylogenetic analysis

The phylogenetic analysis was performed according to Roxo et al. (2014a) (Suppl. material 1 – Fig. S1). All individual sequences for each species were initially analyzed using the software program BioEdit 5.0.9 (Hall 1999) and consensus sequences were obtained. All sequences for each gene were independently aligned using MUSCLE (Edgar 2004) under default parameters and the alignments inspected by eye for any obvious misalignments. After that, sequences of all genes were concatenated to perform all phylogenetic and biogeography analysis.

Maximum likelihood analyses were performed using RAxML Web-Servers (Stamatakis et al. 2008). RAxML implements a faster algorithm of heuristic searches with bootstrap pseudoreplicates (RBS). Bootstrap (BS) resampling (Felsenstein 1985) was applied to assess support for individual nodes using 1,000 replicates. Random starting trees were used for each independent ML tree search and all other parameters were set on default values.

Bayesian inference (BI) (Huelsenbeck and Ronquist 2001) was performed evaluating alternative tree topologies through the estimation of posterior probabilities (P) using MrBayes v.3.0 (Ronquist and Huelsenbeck 2003). The ML tree obtained from ML analysis was used as a starting three for the Markov chain Monte Carlo searches. Eight chains were run simultaneously for 100,000,000 generations and every 1000th generation a tree was sampled. The above analysis was performed twice. The distribution of log-likelihood scores was examined to determine stationary phase for each search and to decide if extra runs were required to achieve convergence, using the program Tracer 1.5 (Rambaut and Drummond 2007a). All sampled topologies beneath the asymptote (25,000,000 generations) were discarded as part of a burn-in procedure, and the remaining trees were used to construct a 50% majority-rule consensus tree in TreeAnnotator v1.7.5 (Rambaut and Drummond 2007b).

Time calibrated phylogeny and hypothesis on the ancestor

The time calibrated phylogeny was performed according to Roxo et al. (2014a) (Suppl. material 2 – Fig. S2). The uncorrelated relaxed molecular clock was calibrated using BEAST (Bayesian Evolutionary Analysis Sampling Trees) v1.6.2 (Drummond and Rambaut 2007). Two fossil calibration points were used to constrain divergence times for all clades of the phylogenetic tree. The first calibration point was implemented as a normally distributed prior, with an offset of 125 million years ago (Ma), and a standard deviation of 15 million years. These date-estimate parameters were selected to match current knowledge of the timing of siluriform origins. Information from the stratigraphic record and geographic distributions of living taxa indicate an origin for Siluriformes during the Lower Cretaceous (145–100 Ma; Lundberg 1993; Sullivan et al. 2006; Lundberg et al. 2007). We used a birth–death model for speciation likelihood and a starting tree obtained from ML. The analysis was run for 100 million generations and sampled every 1000th generation. Stationarity and sufficient mixing of parameters (ESS>200) was checked using Tracer v1.5 (Rambaut and Drummond 2007a). A consensus tree was built using TreeAnnotator v1.6.2 (Rambaut and Drummond 2007b).

Data on the geographic distributions of species were taken from the original species descriptions and information available at the Catalog of Eschmeyer (2014). We assigned taxa to geographic areas using the ecoregion classifications of Vari and Malabarba (1998) and Chiachio et al. (2008), within the following five biogeographic regions: A, Atlantic Coastal Drainages of Southeastern Brazil; B, Upper Paraná Basin; C, Paraguay, Lower Paraná and Uruguay basins; D, Amazon and Orinoco basins; E, São Francisco basin and Coastal Drainages of Northeastern of Brazil (see Roxo et al. 2014a for more details about biogeographic area classifications). The new species *R. capetinga* is assigned to the D area (Amazon and Orinoco basins) in the present paper.

The maximum-likelihood analysis of biogeographic history was performed in Lagrange v2.0 (Ree et al. 2005; Ree and Smith 2008) using all available data and parameters of Roxo et al. (2014a). Four DEC models were tested to estimate distribution ranges inherited by the descending lineages at each node of the tree. The differences between the models are in the rate of dispersal among adjacent and no adjacent areas (see Suppl. material 5 – Table S3 for the likelihood values and dispersal rate among adjacent and no adjacent areas for each model). The model that obtained the highest ML values was model 3 (M3) that constrained the dispersal rates between adjacent areas at 0.5 and areas separated by one or more intercalated areas at 0.0001.

Results

Rhinolekos capetinga sp. n.

http://zoobank.org/53CB690E-E969-4C06-8C1E-4991C103F19F Figs 1, 3; Table 1

Rhinolekos sp. 1 – Roxo et al. 2014a: 9(8) e105564 (phylogenetic relationships).

Holotype. MZUSP 116102, (male, 37.5 mm SL), Brazil, Goiás State, municipality of Água Fria de Goiás, córrego da Branca, drainage of the rio Tocantizinho, rio Tocantins basin, 14°53'47.2"S, 47°34'58.4"W, 30 June 2014, FF Roxo, GSC Silva, LE Ochoa.

Paratypes. Brazil, Goiás State, rio Tocantins basin (56 specimens). LBP 17089 (1 male, 39.1 mm SL), municipality of Agua Fria de Goiás, córrego da Branca, drainage of the rio Tocantizinho, 14°57'01.6"S, 47°35'57.0"W, 21 November 2012, R Devidé, BF Melo, JMH Martinez, GSC Silva; LBP 18996, (1 female, 24.1 mm SL), municipality of São João D'Aliança, córrego Roncador, drainage of the rio Tocantizinho, 14°43'51.3"S, 47°32'34.0"W, 30 June 2014, FF Roxo, GSC Silva, LE Ochoa; LBP 19001 (15 females, 26.8–36.2 mm SL, 20 males, 39.5–30.2 mm SL, 3 c&s, 37.2–32.6 mm SL, 9 unsexed juveniles not measured), collected with holotype. LBP 19466 (2 females, 36.5–37.1 mm SL) municipality of Água Fria de Goiás, córrego da Branca, drainage of the rio Tocantizinho, 14°53'47.2"S, 47°34'58.4"W, 09 November 2014, FF Roxo, LH Roxo, GSC Silva, LE Ochoa; MZUSP 113920 (2 females, 29.3–37.3 mm SL, 3 males, 30.4–39.0 mm SL), municipality of Água Fria de Goiás, córrego da Branca, drainage of the rio Tocantizinho, 14°53'47.2"S, 47°34'58.4"W, 09 November 2014, FF Roxo, LH Roxo, GSC Silva, LE Ochoa; MZUSP 113920 (2 females, 29.3–37.3 mm SL, 3 males, 30.4–39.0 mm SL), municipality of Água Fria de Goiás, córrego da Branca, drainage of the rio Tocantizinho, 14°53'47.2"S, 47°34'58.4"W, 09 November 2014, FF Roxo, LH Roxo, GSC Silva, LE Ochoa; MZUSP 113920 (2 females, 29.3–37.3 mm SL, 3 males, 30.4–39.0 mm SL), municipality of Água Fria de Goiás, córrego da Branca, drainage of the rio Tocantizinho, 14°53'47.2"S, 47°34'58.4"W, 27 November 2012, OT Oyakawa, AM Zanata, P Camelier, M Melo.

Diagnosis. Rhinolekos capetinga differs from R. garavelloi and R. schaeferi in that it has a lower number of vertebrae, 31 (vs. 32) and the anterior portion of the compound supraneural-first dorsal-fin proximal radial contacts the neural spine at the 9th vertebra (vs. 10th, Fig. 2a). The new species can be distinguished from *R. britskii* by the absence of transverse dark bands in the pectoral, pelvic and anal-fin rays (vs. present), lower number of plates in the dorsal series 24-28 (vs. 30-35), lack of odontodes on the ventral tip of the snout (vs. tip of snout completely covered by odontodes), and by having a greater prenasal length, 41-60% of HL (vs. 32-40% of HL). Moreover, the new species differs from *R. schaeferi* by the absence of accessory teeth (vs. present) and from all congeners by the smaller head length, 20-27% of SL (vs. 28-32% of SL in R. britskii; 29-35% of SL in R. garavelloi; 29-32% of SL in R. schaeferi), and by the greater snout length, 61-85% of HL (vs. 52-57% of SL in R. britskii; 49-60% of SL in R. garavelloi; 53–59% of SL in R. schaeferi). It differs from R. britskii and R. garavelloi by the smaller caudal-peduncle depth, 6–9% of SL (vs. 9–11% of SL in R. britskii and 10-13% of SL in R. garavelloi); it differs from R. garavelloi by the smaller thoracic length 10–15% of SL (vs. 18–21% of SL), and by the smaller folded dorsal-fin length, 14-21% of SL (vs. 22-26% of SL).



Figure 1. *Rhinolekos capetinga* MZUSP 116102, holotype, male, 37.5 mm SL, Goiás State, rio Tocantins basin, Brazil.

Description. Morphometric and meristic data presented in Table 1. Maximum body length 39.1 mm SL; dorsal profile of head in lateral view convex to straight from upper part of rostrum to anterior margin of eyes, slightly curved from eyes to posterior margin of parieto supraoccipital, almost straight to dorsal-fin origin; dorsal profile of trunk almost straight, descending from base of dorsal-fin origin to caudal peduncle; ventral profile slightly concave from snout tip to pelvic-fin origin, slightly convex to caudal peduncle; greatest body depth at dorsal-fin origin; greatest body width at cleithral region, gradually decreasing towards snout and caudal-fin. Cross-section of caudal peduncle almost ellipsoid; rounded laterally and almost flat dorsally and ventrally.

Head rounded in dorsal view. Snout slightly pointed, its tip rounded, elongated (61–85% of HL) and depressed in front of each nostril on dorsal surface. Anterior margin of snout covered with odontodes, except ventral tip of snout; odontodes of margin of snout similar in size to remaining ones found on head. Odontodes on head

	Rhinolekos capetinga, holotype and paratypes (n=30)				
	Holotype	Range	Mean	SD	
SL	37.5	22.9-39.1	34.3	3.6	
Percents of SL					
Predorsal length	42.2	38.9-49.9	44.7	2.0	
Preanal length	53.4	48.2-60.3	54.0	2.7	
Prepectoral length	26.2	19.1-26.2	23.2	1.4	
Prepelvic length	33.3	31.5-39.5	35.2	2.0	
Postanal length	34.4	28.0-38.7	34.8	2.5	
Thoracic length	13.1	9.6-15.2	12.9	1.5	
Abdominal Length	19.7	11.8-23.5	19.2	2.2	
Caudal peduncle depth	6.6	5.8-8.6	6.9	0.6	
Head length	21.6	19.6-26.6	22.4	1.5	
Head width	22.1	17.6-26.6	21.8	1.8	
Head depth	11.9	10.8-15.7	12.8	1.0	
Base of dorsal-fin length	10.9	9.3-13.0	10.5	1.0	
Folded dorsal-fin length	20.7	13.9-21.3	19.1	1.4	
Pectoral-fin unbranched ray length	20.1	13.6-22.9	19.9	1.9	
Pelvic-fin unbranched ray length	15.3	13.3-17.5	15.6	1.2	
Snout-opercle length	21.8	18.8-26.3	21.9	1.5	
Percents of HL					
Snout length	60.9	60.7-85.2	72.4	5.8	
Orbital diameter	19.6	12.2-23.2	17.1	2.3	
Interorbital length	45.4	40.4-55.8	46.6	3.8	
Prenasal length	48.8	41.3-60.2	51.4	4.4	
Suborbital depth	26.3	19.0-39.7	25.6	4.5	
Meristics	Holotype	Range	Mode	SD	
Left premaxillary teeth	26	15-34	22	_	
Left dentary teeth	24	14-31	26	_	
Dorsal plates	27	24-28	27	_	
Mid-dorsal plates	17	16-20	18	_	
Median plates	25	23-27	25	_	
Mid-ventral plates	25	20-24	22		
Ventral plates	18	15-18	17	_	

Table 1. Morphometrics and meristic data for *R. capetinga*. SD, standard deviation.

and trunk well defined and not forming longitudinal rows; eye small (12–23% of HL), dorsolaterally positioned; iris operculum not present; lips roundish and papillose; papillae uniformly distributed on base of dentary and premaxillary and slightly decreasing distally. Lower lip larger than upper lip; its border fringed; maxillary barbel present; Teeth slender and bicuspid; mesial cusp larger than lateral cusp; premaxillary teeth 15–34. Dentary teeth 14–30.

Dorsal fin ii,6-7; dorsal-fin spinelet short, roughly triangular shaped, locking mechanism non-functional; dorsal-fin origin slightly posterior of vertical through

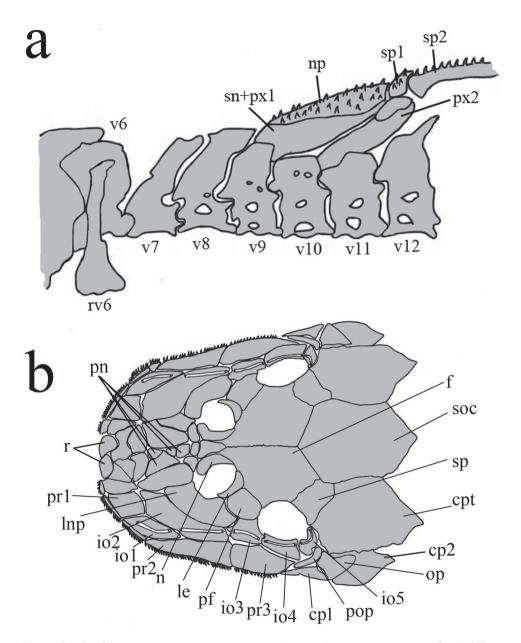


Figure 2. *Rhinolekos capetinga*, LBP 19001, paratype, 34.5 mm SL. **a** Anterior portion of axial skeleton and dorsal-fin supports (left side, lateral view). Vertebrae counts included five vertebrae of the Weberian apparatus. **np** nucal plate; **rv6** rib of sixth vertebrae; **px2** compound proximal and medial radial 2; **sn+px1** compound supraneural first dorsal-fin proximal radial; **sp1** first dorsal-fin spinelet; **sp2** second dorsal-fin spine; **v6-12** vertebrae 6-12 **b** Skull of *R. capetinga*; **f** frontal; **soc** supraoccipital; **cpt** parietosupraoccipital; **op** opercle; **io1-5** infraorbitals; **pop** preopercle; **cp 1-2** cheek plates; **pr 1-3** postrostral plates; **pf** prefrontal plates; **le** lateral ethmoid; **n** nasal; **lpn** lateronasal plate; **r** rostral plate; **pn** prenasal; **sp** sphenotic.



Figure 3. *Rhinolekos capetinga*, live specimen, MZUSP 116102, holotype, male, 37.5 mm SL, rio Tocantins basin, Goiás State, Brazil. Photo: FF Roxo.

pelvic-fin origin. Anterior portion of compound supraneural-first dorsal-fin proximal radial contacting neural spine of 9th vertebrae (Fig. 2a). Tip of adpressed dorsal-fin rays slightly surpassing end of anal-fin base. Pectoral fin i,5-6; tip of longest pectoral-fin ray almost reaching to middle of adpressed pelvic-fin, when depressed. Pectoral axillary slit not present even in juveniles. Pectoral spine supporting odontodes anteroventrally; pelvic fin i,5; its tip not exceeding anal-fin origin when depressed in both sexes. Pelvic-fin unbranched ray with dermal flap along its dorsal surface in males; anal fin i,5; its tip reaching 7th and 8th plate from its origin; Caudal fin i,14,i; distal margin forked; Adipose-fin absent. Total vertebrae 31 (3 c&s).

Body covered with bony plates except on ventral part of head, around pectoral and pelvic-fin origin and on dorsal-fin base. Cleithrum and coracoid totally exposed; Arrector fossae partially enclosed by ventral lamina of coracoids. Abdomen entirely covered by plates in adults (about 25.0 mm SL); lateral plate series with elongate and large plates, formed by two lateral plate series, similar in size; median plates formed by four to five irregular plate series reaching anal shield. Lateral side of body entirely covered by plates; mid-dorsal and mid-ventral plates well developed, reaching typical adipose-fin region.

Parts of head osteology presented in Fig. 2b. Tip of snout formed by two square rostral plates. Nasal almost rectangular forming anterior medial nostril margin in contact posteriorly with frontals, and anterior and lateral margins contacting pre-nasals. Lateral surface of head formed by three posterior rostrum plates, second one large and triangular shaped. Complete infraorbital plate series, present mesial to posterior rostrum series, composed of five plates; fourth infraorbital expanded ventrally, all associated with latero-sensory canal system; first and second infraorbitals largest and fifth smallest. Large lateronasal plate mesial to second infraorbital, forming anterior distal nostril margin in contact anteriorly with prenasals and posteriorly with prefrontal. Preopercle present just ventral to fifth infraorbital; an elongated bone covered by latero-sensory canal. Subocular cheek plates present ventral to preopercle plate. Top of head composed of compound pterotic-supracleithrum, supraoccipital, prefrontal, frontal, and sphenotic; parieto-supraoccipital bearing fenestrae irregularly distributed and of different sizes and shapes.

Color in life. Pale yellowish ground color. Dorsal surface of head dark brown, except for pale yellowish areas on snout tip. Four dark-brown saddles crossing dorsum, reaching longitudinal dark strip on side of trunk: first at dorsal-fin origin, second below dorsal-fin base, third typically at adipose-fin region, and fourth at end of caudal peduncle. Caudal-fin black, with small hyaline circular area on each lobe, tip of lobes hyaline; some specimens with caudal-fin lobe entirely dark (Fig. 3).

Color in alcohol. Similar pattern described for living specimens, but with ground color dark brown (Fig. 1).

Sexual dimorphism. Adult males are distinguished by having a papilla at the urogenital opening (vs. papilla absent in females), and by an unbranched pectoral- and pelvic-fin ray supporting a dermal flap on their proximal dorsal surface in males.

Etymology. The specific name *capetinga* from the Tupi-guarani dialect is in reference to the old and unused name of São João D'Aliança municipality. The name «capetinga» means white, or clear water. A noun in apposition.

Distribution. *Rhinolekos capetinga* is known from two localities at the córrego da Branca and one locality at the córrego Roncador, all drainages of the rio Tocantizinho, rio Tocantins basin (Fig. 4a).

Habitat. *Rhinolekos capetinga* was collected on flat areas of the córrego da Branca and córrego Roncador, rio Tocantins basin, in places of shallow clear waters, about 1 m depth and median to fast current flow. The fishes captured were associated with the vegetation that covers the bottom and the border of the headwaters (Fig. 4b).

Phylogenetic and time calibrated tree

Partial sequences of the three mitochondrial genes (16S rRNA, COI, Cytb) and one nuclear gene (F-reticulon 4) were obtained from GenBank (Suppl. material 3 – Table S1) (same data available in Roxo et al. 2014a). The combined sequence data resulted in a matrix of 4,500 base pairs. This matrix was used to perform all phylogenetic and biogeographic analyses. Bayesian and ML phylogenetic analyses resulted in very similar topologies (Suppl. material 1 – Fig. S1). Our results illustrate the same phylogenetic relationship of Roxo et al. (2014) that the clades Hypoptopomatinae, Neoplecostominae and Otothyrinae are monophyletic with strong statistical support (BS = 96, P = 0.99 for Hypoptopomatinae; BS = 99, P = 1.00 for Neoplecostominae; BS = 96, P = 0.99 with BI for Otothyrinae). The new species *R. capetinga* formed sister group to the species *R. garavelloi*, and both species formed sister group to the species *R. britskii*.

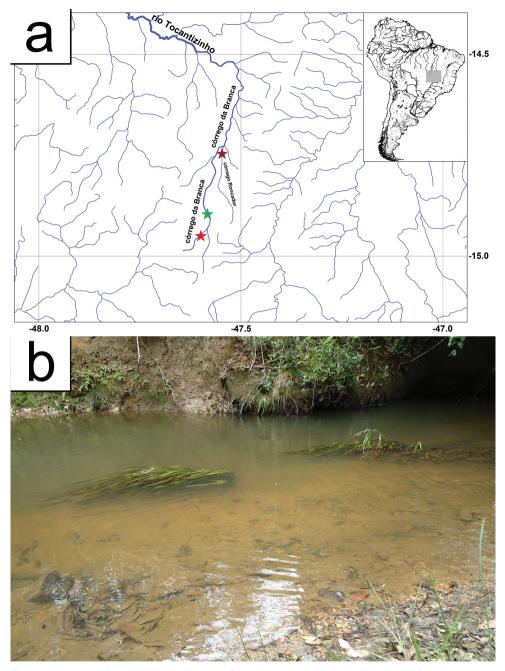


Figure 4. a Map showing the distribution of *R. capetinga*. Type locality at córrego da Branca, green star – 14°53'47.2"S, 47°34'58.4"W. Paratype localities at córrego da Branca, red star – 14°57'01.6"S, 47°35'57.0"W, and at córrego Roncador, pink star – 14°43'51.3"S, 47°32'34.0"W **b** Habitat and submerged vegetation where the specimens were found in type locality of córrego da Branca, 14°53'47.2"S, 47°34'58.4"W. Photo: LH Roxo.

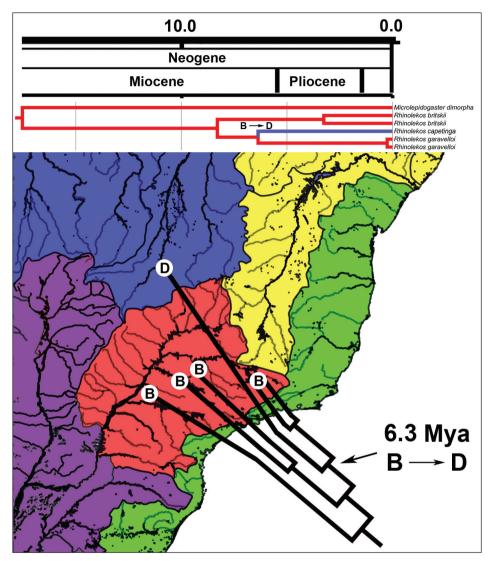


Figure 5. Biogeographic distribution and time-calibrated phylogenetic tree of *Microlepidogaster* and *Rhi-nolekos* species, based on three mitochondrial (16SrRNA, COI, Cytb) and one nuclear marker (F-reticulon 4), modified from figure 7 of Roxo et al. (2014a). The map colorations indicate distinct biogeographic regions according to classification available in Roxo et al. (2014a): Green – Coastal drainages (**A**); Red – upper rio Paraná basin (**B**); Purple – Paraguay, Lower Paraná and Uruguay basins (**C**); Blue – Amazon basin (**D**); Yellow – São Francisco basin (**E**).

Our time calibrated phylogeny and the ancestral area reconstruction (Suppl. material 2 – Fig. S2; Fig. 5) suggested that the genus *Rhinolekos* originated in the upper rio Paraná basin about 17.5 Mya (9.6–27.9 Mya 95% HPD) and the new species *R. capetinga* reached the area D (Amazon and Orinoco basins) from drainages of the rio Paranaíba about 6.3 Mya (4.1–13.9 Mya 95% HPD) at the end of Miocene.

Discussion

The new species *Rhinolekos capetinga* is a typical species of the genus, given that it presents the main characters used by Martins and Langeani (2011a): the lateronasal plate just above second infraorbital, forming anterior distal nostril margin in contact anteriorly with prenasals, and the anterior portion of the compound supraneural-first dorsal-fin proximal radial contacting the neural spine of the 9th vertebrae, a character that, according to Schaefer (1998), is a homoplastic condition evolved independently many times among the Otothyrinae.

Martins and Langeani (2011a) suggested that the presence of the lateronasal plate is a character state that is present not only in *Rhinolekos* but also shared among species of *Acestridium* Haseman, 1911 and *Gymnotocinclus*. The works of Cramer et al. (2011) and Roxo et al. (2014a) suggested that species of these two genera are not closely related to *Rhinolekos. Gymnotocinclus* was found to be the sister group to the genus *Corumbataia* Britski, 1997, and *Acestridium* was included in a polytomy with other Otothyrinae species (Cramer et al. 2011). In contrast, *Acestridium* was found to be the sister group to *Hypoptopoma* Günther, 1868 by Roxo et al. (2014a). Considering these hypotheses, the lateronasal plate, the main character used to distinguish *Rhinolekos* from *Microlepidogaster* Eigenmann & Eigenmann, 1889, as proposed by Martins et al. (2011a), is a homoplasy.

The anterior portion of the compound supraneural-first dorsal fin proximal radial contacting the neural spine of the 9th vertebrae (Fig. 2a) is present in the new species *R. capetinga* and that character state is shared with *R. britskii*, the most similar species externally. However, in a phylogenetic and biogeographic study of Hypoptopomatinae, Neoplecostominae, and Otothyrinae, Roxo et al. (2014a) suggested that *R. capetinga* (identified in that study as *Rhinolekos* sp. 1) form a sister group with *R. garavelloi*, and both species form a sister group with *R. britskii*. This result suggests that the compound supraneural-first dorsal fin proximal radial contacting the neural spine of the 9th vertebrae is a homoplasy, since that in *R. garavelloi* and *R. schaeferi* it contacting the neural spine of the 10th vertebrae. Furthermore, in all other Hypoptopomatinae and Otothyrinae species the compound supraneural-first dorsal fin-proximal radial contacting the neural spine of the 7th vertebrae and in *Epactionotus* contacting the neural spine of the 8th vertebrae (Martins and Langeani 2011a).

Martins et al. (2014a) reported that the pectoral-fin axillary slit is present in many species of Otothyrinae. Within *Rhinolekos*, the slit was reported to be absent in adults, but present in juveniles (Martins and Langeani 2011a). However, it was not observed in the new species, even in very young specimens. The complete absence of the pectoral-fin axillary slit is a condition shared with *Otothyris travassosi* Garavello, Britski & Schaefer, 1998, *O. rostrata* Garavello, Britski & Schaefer, 1998, *O. rostrata* Garavello, Britski & Schaefer, 1998, *O. lophophanes* (Eigenmann & Eigenmann, 1889), *O. juquiae* Garavello, Britski & Schaefer, 1998, and *Schizolecis guntheri* (Miranda Ribeiro, 1918) (Martins et al. 2014a), and, according to Reis and Schaefer (1998), its presence is a derived condition in Otothyrinae and its absence is a secondarily derived condition.

Biogeography and geodispersal route

Rhinolekos capetinga is the first species of *Rhinolekos* described in the rio Tocantins basin. Results of Roxo et al. (2014a) suggested that this genus originated in the upper rio Paraná basin at 17.5 Mya (9.6–27.9 Mya 95% HPD) (also see Suppl. material 2 – Fig. S2 of the present study). However, in last paper the species *R. capetinga* (*Rhinolekos* sp. 1 in Roxo et al. 2014a) was erroneously assigned to the B area (upper Paraná River basin). In our study we corrected this misunderstanding and assigned the species *R. capetinga* to the D area (Amazon and Orinoco basins) during the ancestral area estimation performed in Lagrange and found that dispersal events in the end of the Miocene, about 6.3 Mya (4.1–13.9 Mya 95% HPD), let the ancestor of *R. capetinga* reach the rio Tocantins basin (Suppl. material 2 – Fig. S2; Fig. 5) from drainages of rio Paranaíba basin.

Several authors (e.g. Eigenmann and Eigenmann 1891; Jordan 1896; Pearson 1937; Carvalho and Albert 2011; Ribeiro et al. 2013) suggested that headwater captures could explain the movement of fish lineages among the rio Paraguay, the Amazon River tributaries (e.g. Madeira, Tocantins and Xingu) and the drainages of the Brazilian Shield (e.g. rio Paranaíba). By definition, headwater captures change the spatial location of a watershed acting simultaneously as a vicariant process and occasioning biological dispersal. This geological process has been widely reported as responsible for fish movement among adjacent drainages (Ribeiro 2006; Albert et al. 2011; Carvalho and Albert 2011; Roxo et al. 2012; Roxo et al. 2014b), and thus for fish movements of the subfamily Otothyrinae (Roxo et al. 2014a).

Lima and Ribeiro (2011) substantiated that the ichthyofauna of the rio Tocantins shares species with the trans-boundary river basins, namely the Paraguay, Paraná, São Francisco, and Xingu drainages. Additionally, Montoya-Burgos (2003) also associated cladogenetic events within species of *Hypostomus* Lacepède, 1803 with divisions among the rio Amazon tributaries and the Paraguay-Paraná system. Therefore, considering the previous hypothesis, we believe that headwater captures may have influenced the movement of ancestral species of *R. capetinga* from the drainages of the rio Paranaíba to the rio Tocantins at the end of the Miocene (Fig. 5).

Comparative material

Microlepidogaster arachas Martins, Calegari & Langeani, 2013: LBP 10882, 3, 22.3–36.3 mm SL, rio Araguari, rio Paranaíba basin; LBP 11724, 9, 38.0–41.2 mm SL, 1 c&s, 39.1 mm SL, córrego sem nome, rio Paranaíba basin; *Microlepidogaster discus* Martins, Rosa & Langeani, 2014b: MZUSP 115384, 2, 38.8–40.4 mm SL, rio Itacambiruçu, rio Jequitinhonha basin; *Microlepidogaster dimorpha* Martins & Langeani, 2011b: LBP 10683, 2, 28.8–35.6 mm SL, rio Uberaba, upper rio Paraná basin; *Microlepidogaster longicolla* Calegari & Reis, 2010: LBP 17077, 4, 39.7–46.4 mm SL, rio Pipiripari, upper rio Paranaíba basin; *Microlepidogaster perforatus* Eigenmann

& Eigenmann, 1889: LBP 19498, 1, 28.9 mm SL, rio Carandaí, rio São Francisco basin; *Rhinolekos britskii* Martins & Langeani, 2011a: LBP 7245, 3, 28.9–30.5 mm SL, rio Arapuca, rio Paranaíba basin; LBP 7253, 15, 21.7–35.2 mm SL, córrego sem nome, rio Paranaíba basin; *RLUSP* 103698, 6 paratypes, 27.1–36.1 mm SL, córrego sem nome, rio Paranaíba basin; *Rhinolekos garavelloi* Martins & Langeani, 2011a: LBP 7246, 24, 24.1–34.8 mm SL, córrego Fazenda Lageado, rio Paranaíba basin; *RLUSP* 103697, 5 paratypes, 21.4–31.9 mm SL, córrego da Fazenda Lageado, rio Paranaíba basin; *Rhinolekos schaeferi* Martins & Langeani, 2011a: LBP 19460, 1, 28.5 mm SL, córrego Fazenda Garaíbas, rio Paranaíba basin; *Rhinolekos* sp.: LBP 7247, 26, 24.1–33.1 mm SL, córrego Fazenda Balsamo, rio Paranaíba basin.

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Supplementary material I

Fig. S1

Authors: Fábio F. Roxo, Luz E. Ochoa, Gabriel S. C. Silva, Claudio Oliveira Data type: Adobe PDF file

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Supplementary material 2

Fig. S2

Authors: Fábio F. Roxo, Luz E. Ochoa, Gabriel S. C. Silva, Claudio Oliveira

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Supplementary material 3

Table S1

Authors: Fábio F. Roxo, Luz E. Ochoa, Gabriel S. C. Silva, Claudio Oliveira Data type: Microsoft Word document

Explanation note: Species included in the present study.

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Supplementary material 4

Table S2

Authors: Fábio F. Roxo, Luz E. Ochoa, Gabriel S. C. Silva, Claudio Oliveira

Data type: Microsoft Word document

- Explanation note: Primers used in the present study to amplify partial sequences of F-reticulon 4, 16S rRNA, cytochrome oxidase subunit I (COI) and cytochrome B (CytB).
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Supplementary material 5

Table S3

Authors: Fábio F. Roxo, Luz E. Ochoa, Gabriel S. C. Silva, Claudio Oliveira

Data type: Microsoft Word document

- Explanation note: DEC models tested to estimate distribution ranges inherited by the descending lineages at each node of the tree. The differences between the models are in the rate of dispersal among adjacent and no adjacent areas. * Represent the model used in the analysis.
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RESEARCH ARTICLE



IMp:The customizable LEGO[®] Pinned Insect Manipulator

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Abstract

We present a pinned insect manipulator (IMp) constructed of LEGO[®] building bricks with two axes of movement and two axes of rotation. In addition we present three variants of the IMp to emphasise the modular design, which facilitates resizing to meet the full range of pinned insect specimens, is fully customizable, collapsible, affordable and does not require specialist tools or knowledge to assemble.

Keywords

Specimen Manipulator, Entomology, Stage, Digitization, Imaging

Introduction

Natural history collections are one of the most fundamentally important institutions in science, where voucher specimens are housed in perpetuity, embodying the research of generations of scientists. Within entomology collections insects were historically preserved by drying on an appropriately sized pin. Today insect groups are preserved in ethanol, mounted on microscope slides or in paper or plastic envelopes, but the dry pinned method has not changed for the majority of insect orders since its development in the early 18th century. This has resulted in millions of pinned insect specimens housed in natural history collections globally, for example the pinned insect collection of the Natural History Museum (NHM) in London comprises 136500 drawers and is estimated to contain 27 million pinned specimens.

Although pinned specimens preserve well they become fragile with age and are prone to damage when handled. This has become especially apparent in the digital age where there is an increased focus on mobilizing the vast amounts of biodiversity data stored in the collections through digitization activities. Institutions are now able to provide images of specimens on request (termed a "digital loan" at the NHM), when researchers cannot view the specimens directly and they are too fragile to ship. In addition some institutions do not loan primary type material, compounding the need to image specimens in order to examine them remotely. The net result of improving access to collections through digitization efforts is that the specimens are made available without the need of excessive handling. This goal however can only be achieved when there are images of multiple taxonomically meaningful views available, as required for accurate remote examination.

Most commonly, pinned insect specimens are observed through stereo microscopes at the $10-80 \times$ range of magnification. Under magnification specimens can become difficult to handle as minute movements are amplified. In order to provide stabilization specimens are often pinned to a flexible material such as cork, plastazote foam or putty when the desired angle is found or alternatively the specimen is mounted into a specimen manipulator that enables repositioning during observation.

With the rapid increase in collections digitization, museum specimens are handled to a much larger extent than ever before. Positioning and repositioning of specimens during digitization is often required for the majority of specimen handling in a collection. As handling of pinned specimens carries the most immediate risk of damage, especially to the fragile extremities (e.g. legs, antenna and wings) specimen manipulators are of great value to the overall preservation of a functioning pinned natural history collection.

A good insect specimen manipulator requires the following properties: (i) Foremost the manipulator should allow for easy positioning and repositioning of specimens especially if used for imaging at multiple views or comparing structures at different angles; (ii) Stability to prevent the specimen moving once in the correct position; (iii) Capability of fine scale adjustment to enable positioning the specimen under magnification and (iv) Open design to allow for both specimen placement/removal and adequate illumination of the specimen to be examined.

There are several good designs available from commercial and amateur DIY websites such as the Universal Stage (http://extreme-macro.co.uk/universal-stage/), Rose Entomology (http://www.roseentomology.com/Pinned_Specimen_Manipulator.htm), BioQuip Microscope Stages 6186 and 6188 (http://www.bioquip.com/search/Disp-Product.asp?pid=6186; http://www.bioquip.com/search/DispProduct.asp?pid=6188) and the Watkins & Doncaster Insect Examination Stage (http://www.watdon.co.uk/ acatalog/Microscope_Accessories.html). In addition there are variants of the steel / brass ball & ring stage combination (Ento / Ergo Ball: details available on request).

Previous authors have provided custom designs for insect specimen manipulators (Köppen 1966, Oliver 1969, Lobanov and Kotjurgin 1975, Boyadzhiev and Bozhinova

2006, Boyadzhiev et al. 2012), however most commercial examples are of a fixed standard size while DIY manipulators are custom-made from materials and tools that are not readily available to everyone. Furthermore most DIY setups are specifically designed for a particular group of insects and may not be of an appropriate size for other insect groups. We believe that the design presented here is a solution to an insect specimen manipulator that is (a) universally applicable, (b) readily available, (c) cost effective, (d) portable and (e) fully customizable.

Material and methods

The idea of a holding mechanism for pinned specimens is as old as the pinned specimen itself. The design of these particular models were inspired by the daily grind of comparative morphology and the association to mass digitization and digital loans that the first author has had at the natural history museums of Denmark and London. Although the LEGO[®] brick has always been a working tool it has served more as a means of prototyping ideas, but in this case the authors found the plastic bricks to have the right properties for the product presented here. It is in fact the simple nature of the LEGO[®] bricks, their availability and ease of use that we feel make these models so customizable, user friendly, affordable and hassle free.

The Insect specimen manipulator (IMp) and subsequent size/design variants were built and designed using both the LEGO[®] building blocks and the LEGO[®] Digital designer software version 4.3.8 (http://ldd.lego.com/en-gb/) using beams, beam connectors, connecting pins, an 8 tooth spur gear and a worm gear. For a complete parts list and assembly manual for all IMp models see the supplementary information (http:// dx.doi.org/10.5519/0036449).

Data resources

The data underpinning the analysis reported in this paper are deposited in the NHM Data Portal at http://dx.doi.org/10.5519/0036449.

Results and discussion

Etymology: IMp is an abbreviation of Insect Manipulator and references the attendant imp of folklore that is usually cast as the small, mischievous helper, associated with witches and warlocks, the academics of mythology.

Initially a single enclosed IMp design was conceived (Figures 1b, 2), capable of accommodating insect specimens up to 50 mm in length, with 5 mm clearance on either side of the specimen. Three subsequent models were then designed to facilitate the examination of insects of varying sizes, and to display the customizable nature of

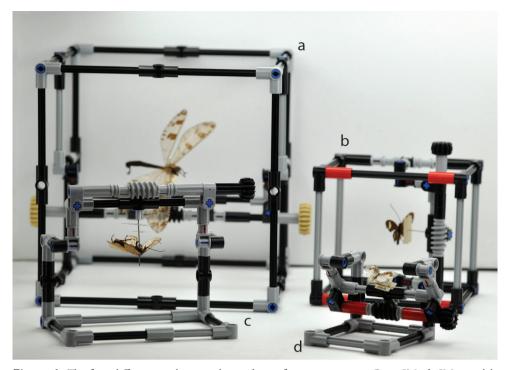


Figure 1. The four different sized manipulators shown for comparison: **a** Giant-IMp **b** IMp models with encasing support beams **c** Open-IMp **d** Micro-IMp models that are not encased. The specimens in the manipulators are: **a** *Nosa tristis* (Hagen, 1853) – Neuroptera: Myrmeleontidae **b** *Perissoneura paradoxa* McLachlan, 1871 – Trichoptera: Odontoceridae **c** *Pteronarcys californica* Newport, 1848 – Plecoptera: Pteronarcyidae and **d** *Psychopsis coelivaga* (Walker, 1853) – Neuroptera: Psychopsidae.

the IMp base design, the relative size of each can be seen in Figure 1. The design of the IMp model on which all subsequent variants are based, and the axes of movement and rotation, are shown in more detail in Figure 2 and the Suppl. material 2. The original IMp and the Giant-IMp models are encased with support beams, adding stability to the design and physical protection for the specimens, whereas the Micro-IMp and Open-IMp models are of an open design that allows for a smaller working distance between the specimen and the stereo microscope. The size and cost (excluding shipping) of each model is summarized in Table 1.

The bricks for all models can be bought directly from the LEGO[®] websites' Pick a Brick (http://shop.lego.com/en-GB/Pick-A-Brick-ByTheme) and the Bricks and Pieces selection (https://service.lego.com/en-gb/replacementparts#BasicInfo). Besides the LEGO[®] bricks the models each require a small plastazote foam / cork plug or other material that will allow for the pin to be held in place (Figure 3). The models presented here all use a 3 mm × 10 mm plastic tube with nylon toothbrush bristles in the center. This allows for any size pin to be held firmly in place while allowing repeated use without the degradation that is common when repeatedly pinning into foam or cork

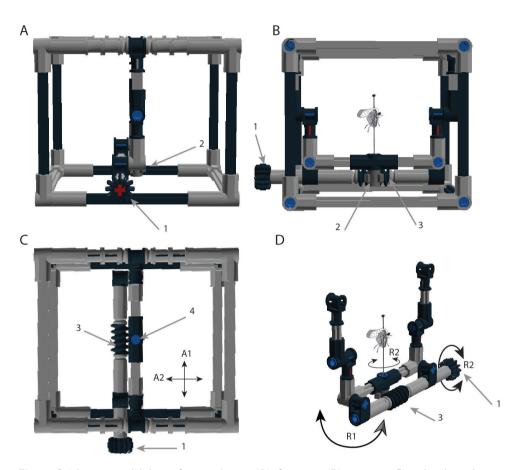


Figure 2. The IMp model shown from a side view (**A**), front view (**B**), top view (**C**) and without the stabilizing case exposing the pivot arm wherein the specimen is placed (**D**). Figure labels and abbreviations: pivot handle (**I**), 8 tooth gear (**2**), worm gear (**3**), connector peg that holds and rotates the specimen (**4**), axis of movement 1 (**AI**), axis of movement 2 (**A2**), axis of rotation 1 (**RI**) and axis of rotation 2 (**R2**).

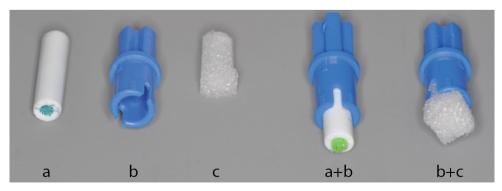


Figure 3. Two options for the modification to the connector pegs (**b**) to allow for insertion of the specimen pin: a 3 mm diameter tube with 0.1 mm nylon fibers (**a+b**); or a small plastazote plug (**b+c**).

Model name	Maximum specimen size ¹ (mm)	Cost ² (£)	Design ³
IMp	50	9	closed ⁴
Micro-	30	7	open
Open-	60	8	open
Giant-	110	15	closed ⁴

Table 1. Summary of the features of each of the IMp models.

¹ allowing 5 mm clearance on either side; ² cost (rounded to nearest pound) at time of publication and excluding shipping; ³ open design facilitates a closer working distance, while the closed design includes the supporting cube structure for stability and additional specimen protection; ⁴ supports can be removed if a closer working distance is required.

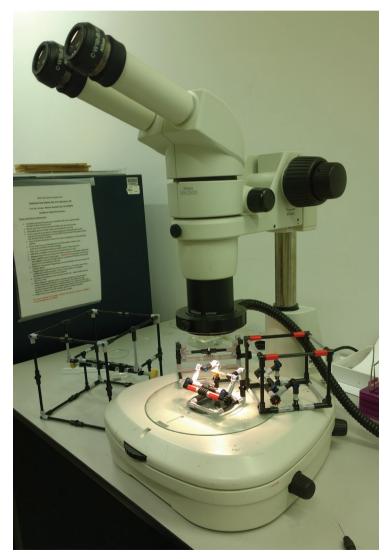


Figure 4. The IMp models being used with the micro-IMp holding a specimen positioned for viewing.

inserts over time. Except for the Giant-IMp the smaller versions are small enough to fit under a standard stereo microscope (Figure 4). The Giant-IMp however was designed for use with large specimens that are usually imaged using a standard DSLR setup with a much larger working distance.

We believe the insect specimen manipulators presented here are a valuable addition to any entomologist's toolbox and that the use of any insect manipulator is in the interest of anyone dealing with valuable specimens as the actual handling of the specimen is reduced to a minimum during examination. In case of the original IMp and Giant-IMp models the specimens are further protected from accidental contact during examination by the supporting cube structure. These LEGO[®] based manipulators benefit from their modular design as they are inexpensive and made from readily available components. Furthermore, even the largest of the models can be disassembled for travel. The open design further allows for the addition of portable lighting solutions (such as LEDs) and an endless amount of customization which makes them ideal for specimen imaging. Future modifications of the IMp models may include the addition of motorized control, using Arduino controllers or native LEGO[®] motors and software from the LEGO[®] mindstorms range.

The authors welcome correspondence on ideas for the next generation of IMps, and although the current models are easy to assemble the authors are happy to assist if no children can be sourced locally.

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Supplementary material I

Annotated building instructions

Authors: Steen Dupont, Benjamin Price, Vladimir Blagoderov

Data type: Adobe PDF file.

Explanation note: Assembly manual for all IMp models.

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Supplementary material 2

Video demontration

Authors: Steen Dupont, Benjamin Price, Vladimir Blagoderov

Data type: MOV file.

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