

Discovery of an alien species of mayfly in South America (Ephemeroptera)

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Abstract

Despite its wide, almost worldwide distribution, the mayfly genus *Cloeon* Leach, 1815 (Ephemeroptera: Baetidae) is restricted in the Western hemisphere to North America, where a single species is reported. In the Neotropics, except for some species wrongly attributed to the genus in the past, there are no records of *Cloeon*. Recently, however, specimens of true *Cloeon* were collected along the coast of Espírito Santo, Southeastern Brazil. In order to verify the hypothesis that this species was recently introduced to Brazil, our aim was to identify the species based on morphological and molecular characters and to confirm the presence of true representatives of the genus in the Neotropics. Our results revealed that the specimens found in Brazil belong to the Afrotropical species *C. smaeleni* Lestage, 1924. The identity of the species, its distribution, along with its previous absence in regularly sampled sites, is a clear sign that the specimens of *C. smaeleni* found in Espírito Santo are introduced, well established, and that the colonization took place very recently.

Keywords

Aquatic insects, Ephemeroptera, invasive species, Baetidae, *Cloeon*, Neotropics

Introduction

Aquatic insects, despite their dominance in terms of diversity in most freshwater ecosystems, show a disproportional low number of invasive species when compared to other freshwater macroinvertebrates (Karatayev et al. 2009). Among entirely aquatic insect orders, there are no Plecoptera or Megaloptera and very few Trichoptera that are invasive (de Moor 1992). In mayflies (Ephemeroptera), a single case is documented, a Southeast Asia lentic species of the family Caenidae introduced accidentally in Hawaii during WWII (Zimmermann 1957) and now well established (Smith 2000). Among the necessary characteristics for invasive species, Kleunen et al. (2010) mentioned phenotypic plasticity, ability for uniparental reproduction, and fast growth in disturbed habitats. Besides that, de Moor (1992) mentioned some important characteristics in the case of invasive aquatic insects, such as: the generalist feeding, e.g. detritivores, year-round breeding capacities, the ability to colonize peri-urban environment and artificial waterbodies, and the climatic similarity of invaded and source environments. The ability to colonize water bodies with low level of oxygen, such as polluted streams and rivers or lentic habitats, can also be added to the characteristics mentioned by de Moor (1992). Tolerant species are much more likely to find a suitable site, and pass through physiological filters, than those with high requirements (see Karatayev et al. 2009).

Due to their diversity, abundance and role in nutrient cycling, mayflies are a critical component in freshwater ecosystems throughout the world, and most species are good bioindicators of the water quality (Menetrey et al. 2008; Brittain and Sartori 2009). Around 3500 species are known over the world, the vast majority colonizing running waters (Barber-James et al. 2008). Some species, however, are capable of colonizing lentic habitats, eutrophic water bodies, and present most of the aforementioned requirements for potential invaders.

The genus *Cloeon* Leach, 1815 (Ephemeroptera) is one of the most common and most diversified genera of mayflies. It encompasses 75 species with 24 of them in the Palearctic realm, 23 in the Afrotropical and 20 in the Oriental. It is also present, though less diversified, in the other realms, except the Neotropics where true *Cloeon* have never been reported. Because of its long imaginal stage in females, ca 14 days for mated (Degrange 1960) to 28 days for virgin ones (Oehme 1972), *Cloeon* presents an unusual potential for dispersion in mayflies; it is reported even from remote islands such as the Azores in the Northern Atlantic Ocean (Brinck and Scherer 1961), La Réunion in the Indian Ocean (Gattolliat 2004), or Vanuatu in the Pacific Ocean (Gattolliat and Staniczek 2011).

Cloeon colonizes all kind of still and standing waters. It can be collected in the riparian vegetation of streams, in ponds and lakes as well as artificial habitats. Larvae feed on detritus and small algae. They swim rather rapidly and actively move their gills. They support water of low quality, i.e. α - β mesosaprobic (Zelinka and Marvan 1961) and even temporary anoxia (Nagell 1977). At least some of the species are recognized

as being ovoviviparous [e.g. *C. dipterum* Linnaeus, 1761, *C. smaeleni* Lestage, 1924, *C. gambiae* Gillies, 1980, *C. perkinsi* Barnard, 1932, and *C. cylindroculum* (Kimmins, 1956) (Degrange 1959, Gillies 1985)] implying a long imaginal stage necessary for the maturation of the eggs.

The genus *Cloeon* is characterized at the larval stage by double rounded gills on segments I to VI and gills VII simple; sclerotized spines on the lateral margins of the abdomen; segment III of labial palp apically tapered or falcate; legs long and slender, tarsal claws elongated with two rows of numerous denticles; median caudal filament equal to the cerci. In the imaginal stage: forewing with single intercalary veins; hindwings absent; female forewing with costal and subcostal fields coloured in some species; male with 3-segmented gonopods without lateral extensions, segment III reduced, genital plate rounded or conical.

Historically, several species of *Cloeon* were described from the Western Hemisphere, but all of them have been transferred to other genera. While the species from the Nearctic realm were transferred to *Centroptilum* Eaton, 1869 or *Procloeon* Bengtsson, 1915 (McCafferty and Waltz 1990), those from the Neotropics were all transferred to other genera mainly *Pseudocloeon* Klapalek, 1905 - and subsequently to *Americabaetis* Kluge, 1992 (Ulmer 1920, Gillies 1990, Lugo-Ortiz and McCafferty 1999), or to *Callibaetis* Eaton, 1883 (Gillies 1990).

The case of *Cloeon dipterum* (mentioned as *C. cognatum* Stephens, 1835 in some papers) is of great interest; this widespread European species was first reported from the U.S.A. based on a single female from Illinois (Burks 1953), but in the succeeding years additional records were provided from several areas in U.S.A. and Canada (Traver 1962, Flowers 1978, Burian and Gibbs 1991, Randolph et al. 2002, McCafferty et al. 2008). Its presence in North America has long been controversial, with some authors regarding the species as a non-native mayfly (Traver 1962, McCafferty 1996), while others sustaining an old Holarctic distribution (Randolph et al. 2002). Therefore, since 1990, *C. dipterum* is considered the only representative of the genus in the Western Hemisphere where it is restricted to temperate North America.

Recently, unexpected adults and larvae of *Cloeon* were found in the State of Espírito Santo, Southeastern Brazil. During the last seven years this state was one of the most sampled and studied areas in Brazil regarding mayflies (e.g., Salles et al. 2010), including some of the sites where *Cloeon* has been found now. This fact, along with the previous absence of report of true *Cloeon* in South America (Domínguez et al. 2006) and the ecology of the genus (i.e., tolerance to low water quality, ovoviviparity), indicates a recent introduction of a non-native species. In order to verify this hypothesis, our aim is to identify the species based on morphological and molecular characters and to confirm the presence of true representatives of the genus in the Neotropics. A brief discussion on the significance of this finding to the origin of *Cloeon dipterum* in North America is also provided, as well as the problems that may arise from the colonization of this taxon in Brazil.

Material and methods

Larvae were collected with the usual techniques for aquatic insects, such as surber samples and other net sampling methods. *Cloeon* female adults were gathered mostly inside houses, while male adults were obtained by rearing larvae in the laboratory. The examined material is housed in the Coleção Zoológica Norte Capixaba, Universidade Federal do Espírito Santo, São Mateus, Brazil; Coleção Entomológica Prof. José Alfredo Dutra, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; and Musée cantonal de zoologie, Lausanne, Switzerland. The geographic records of the species were mapped with DIVA-GIS (version 7.17.2, <http://www.diva-gis.org/>) and then edited with Adobe Illustrator and Adobe Photoshop CS6.

For the specific identification, the most important morphological characters of the genus were examined (e.g. Gillies 1980, Gattolliat and Rabeantoandro 2002, Gattolliat et al. 2008): for the immature stage, the shape of the labial palp, the number of segments of the maxillary palp, the number of abdominal segments with lateral spines and the number of lateral spines, and the shape and denticulation of the tarsal claws; for the imagos, the abdominal color pattern, the shape of the genital plate, the coloration of the costal and subcostal fields of female fore wings. As no identification key for all the species of *Cloeon* are available, several papers were consulted, such as: Gillies (1980, 1985); Gattolliat and Rabeantoandro (2002) for Afrotropical species; Sowa (1975), Bauernfeind and Humpesch (2001), Bauernfeind and Soldán (2012) for Palaearctic species.

For the molecular analyses DNA was extracted from specimens stored in pure alcohol using a Qiagen Extraction Kit. The 658 pb of a mitochondrial protein-coding gene fragment (cytochrome oxidase subunit I, or CO1) were amplified using primers LCO 1490 (GGTCAACAAATCATAAAGATATTGG) and HCO 2198 (TAAACTTCAGGGTGACCA A AAAATCA) (Folmer et al. 1994). All laboratory procedures, edition and alignment of sequences were conducted as described in Vuataz et al (2011). The sequence divergence between haplogroups was calculated using the Kimura 2-parameter as implemented in Mega 5.05 (Tamura et al. 2011). We considered 3% sequence divergence (K2P=0.03) as the maximal value for intraspecific divergence (Hebert et al. 2003). GenBank accession numbers are provided for the material in which DNA has been analysed.

Material examined

Cloeon smaeleni

Brazil

Conceição da Barra, Parque Estadual de Itaúnas, Alagado, 17/v/2012, S18°24'34.66", W39°41'59.84", 54 larvae, 1 female subimago, 1 female imago, 3 male subimagos and 2 male imagos. Same data, 18/v/2012, 9 larvae (GenBank ac-

cession numbers: HG935106 and HG935107). **São Mateus:** Meleira, 17/vi/2011, S18°43'14.8692", W39°46'9.1302", 2 female imagos; Bairro Colina, 09/x/2012, 1 male imago; Chácara do Cricaré, Rua Traíra, 17 female imagos, 07/vi/2013, S18°42'57.9162", W39°50'29.5902", 4 female imagos; Rio Preto, 31/x/2012, S18°44'8.16", W39°47'47.0394", 4 larvae; Rio Preto, 25/ix/2012, S18°44'8.16", W39°47'47.03", 3 female imagos. **Jaguare:** Córrego Água Limpa, 04/viii/2012, S18°55'40.3962", W39°59'9.8988", 7 larvae; Santa Maria, Cachoeira do Bereco, 22/ix/2011, S18°53'4.45", W40°12'23.14", 2 larvae. **Vitória,** Port Complex of Tubarão: impoundment on Carapina stream, 20°15'23.09"S / 40°14'57.95"W, 15.x.2009, 108 larvae. Same data, 16–17.xii.2009, 5 larvae. Impoundment on Carapina Stream, 20°15'45.89"S / 40°15'1.93"W, 15.x.2009, 7 larvae. Same data, 16–17.xii.2009, 9 larvae. Impoundment on Carapina Stream, S20°15'32.71" W40°15'34.29", 14.x.2009, 4 larvae. Same data, 16–17.xii.2009, 54 larvae. **Guarapari,** Parque Estadual Paulo César Vinha: Lagoa Feia, 03/v/2012, S19°26'33.71", W40°24'7.2", 8 larvae; Lagoa Manilha, 02/v/2012, S19°23'40.92", W40°25'20.27", 3 larvae. **Bom Jesus do Norte,** Ilha do Vicente, Rio Itabapoana, 31/vii/2012, S21°6'53.59", W41°41'30.90", 2 larvae.

Madagascar

Antananarivo, Atanandrano, 23/v/2003, 25 larvae. (GenBank accession numbers: HG935104 and HG935105).

South Africa

S2125, Limpopo Prov., Louis Trichardt, Bass. Limpopo, Riv. Luvuvhu, Alt. 700m, 24/v/2003, 23°05'11"S, 30°10'29"E, 15 larvae.

Cloeon cf. smaeleni

Saudi Arabia

AR47, Al-Itnayn, dam, Alt. 2300m, 14/xi/2012, 18°01'21"N, 42°45'50"E, 10 larvae, 5 female imagos and subimagos. (GenBank accession numbers: HG935108 and HG935109).

Cloeon dipterum

Switzerland

Zurich, Kleinandelfingen, Räubrichsee, 15/v/2012, N47°36'46", E8°40'35", 2 larvae. (Unpublished sequences from Sereina Rutschmann, IGB, Berlin).

Korea

(GenBank accession number: KC135930).

Cloeon cf. dipterum**Canari Islands**

GC01: Gran Canaria, Telde, Barranco de los Cernicalos, 25/i/2009, N27°57'54", W15°29'46", 6 larvae. (GenBank accession numbers: KF438141 and KF438144).

TF03: Tenerife, Igueste de St Andrés, Alt. 100m, 18/iii/2007, N28°32'21", W16°09'26", 20 larvae. (GenBank accession numbers: KF438163 and KF438120).

Cloeon peregrinator**Madeira**

Funchal, Alt. 270m., 17/iv/2006, 32°39'43"N, 16°53'44"O, 27 larvae

Funchal, Alt. 70m., 02/xii/2005. 32°38'30"N , 16°55'36"O, 2 female imagos.

Cloeon praetextum* (gr. simile)*Norway**

Finnmark (GenBank accession number: PRJNA37833).

Cloeon* sp1*Saudi Arabia**

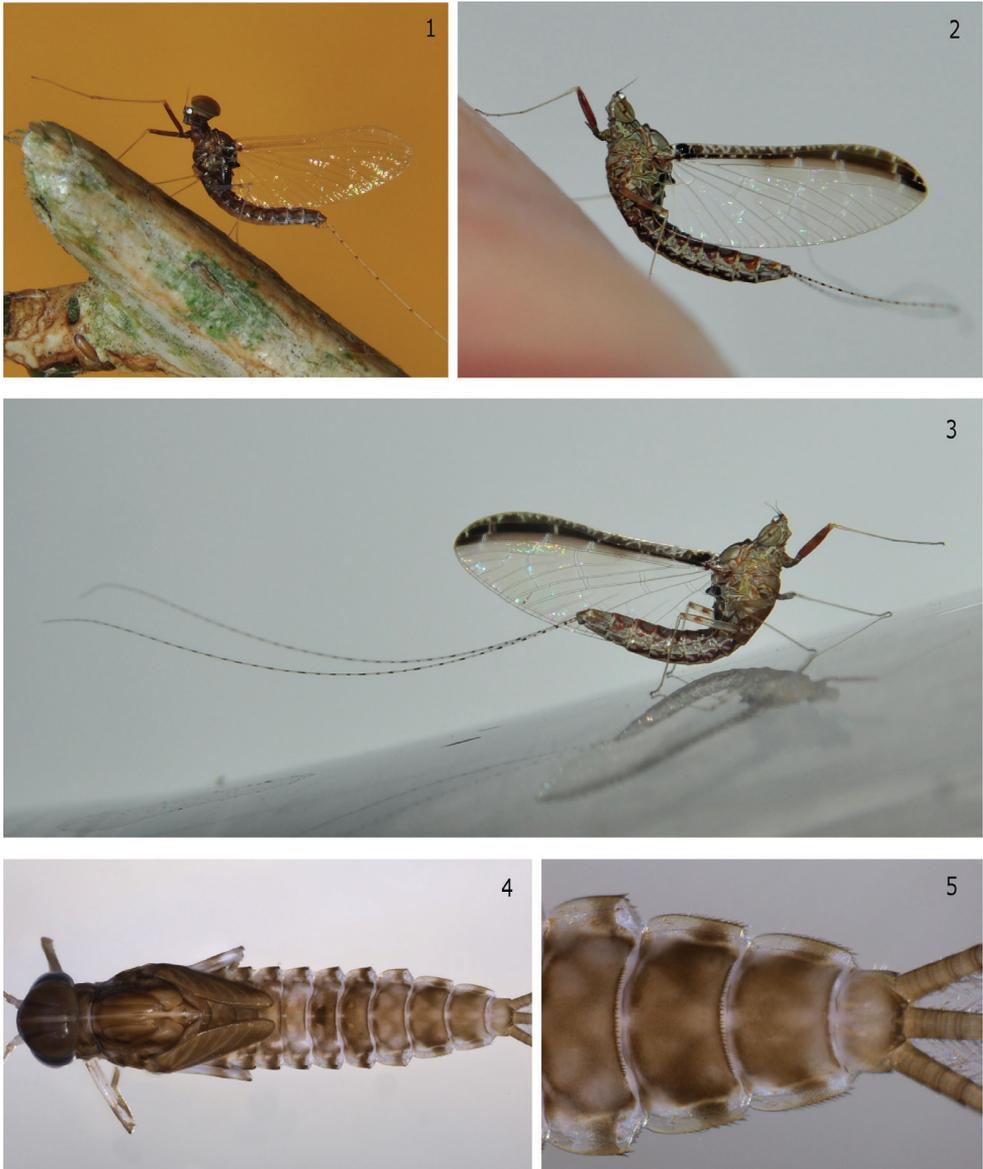
AR44, Wadi Shahadan, Alt. 190m, 13/ii/2012, 17°27'7"N, 42°42'49"E, 20 larvae. (GenBank accession number: KF438120).

Cheleocloeon soldani**Saudi Arabia**

AR39, Wadi Damad, Alt. 260m, 11/ii/2012, 17°27'7"N, 42°42'49"E, 35 larvae. (GenBank accession number: HG935111).

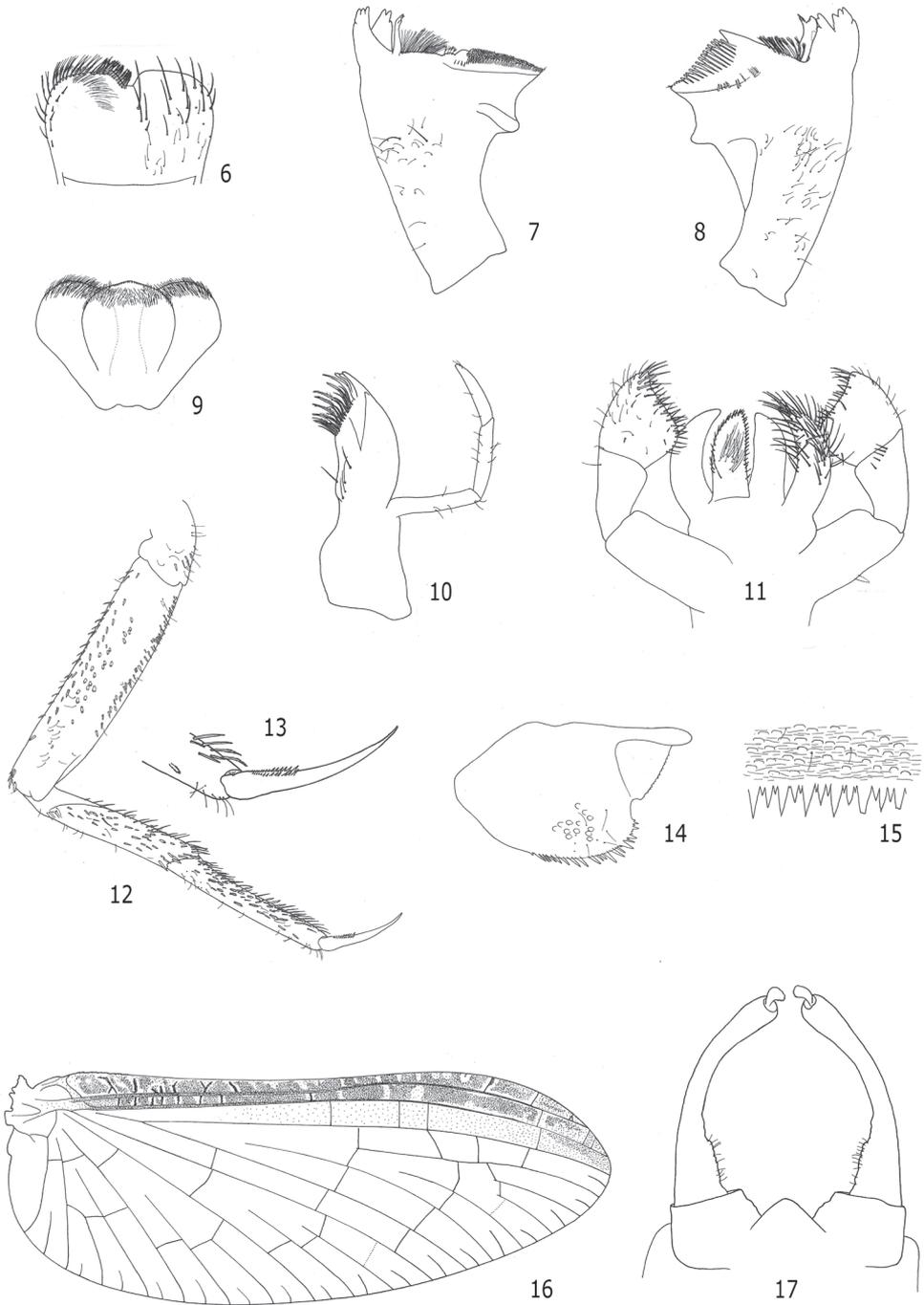
Results

Morphological, as well as molecular analyses revealed that the specimens found in Brazil belong to the Afrotropical species *C. smaeleni*. The main diagnostic characteristics were, as usual among *Cloeon* species, the color pattern of adults, especially the fore wing (Figs 1, 2, 3 and 16) and abdominal sterna (Figs 1, 2 and 3), along with the color of the fore legs (Figs 1, 2 and 3). In addition, the maxillary palp three-segmented

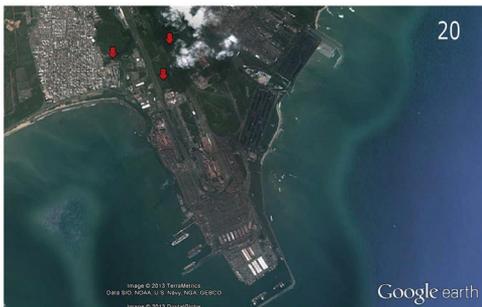
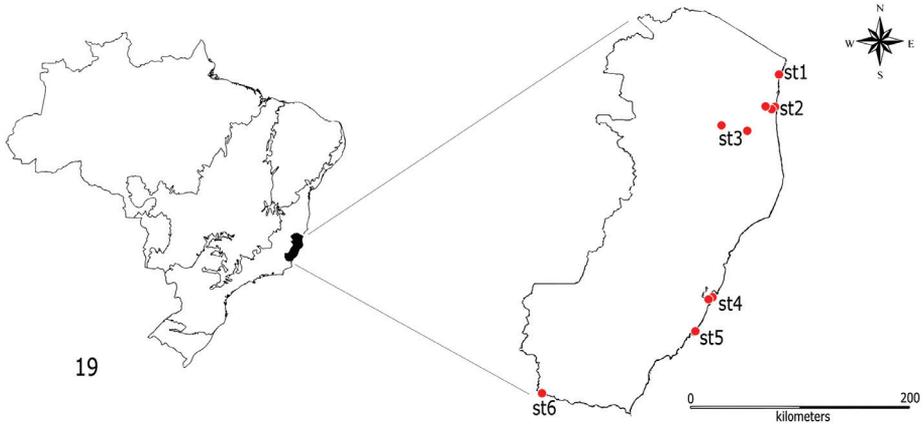
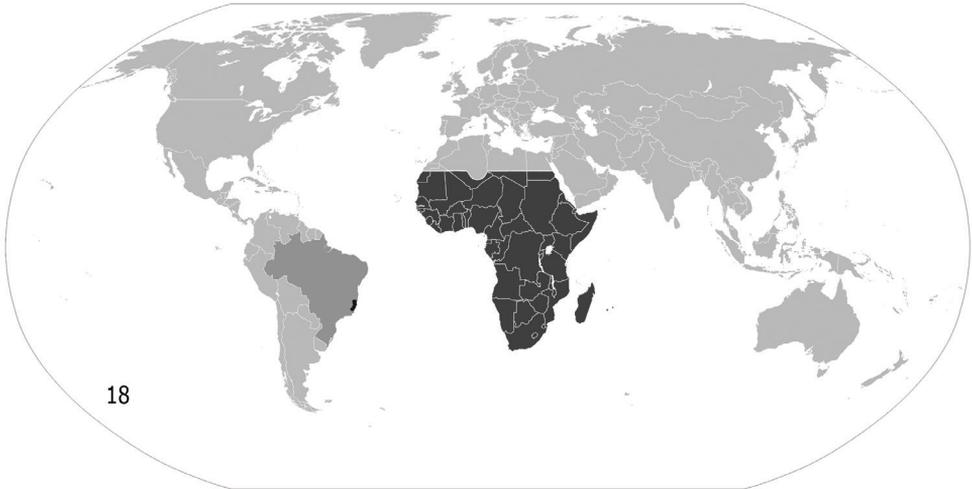


Figures 1–5. *Cloeon smaeleni*. **1** Male imago (lateral view of living specimen) **2** Female imago (lateral view of living specimen) **3** Female imago (lateral view of living specimen) **4** Male larva (dorsal view) **5** Detail of male larval tergites VII to X.

(Fig. 10), the labial palp segment III clavate (Fig. 11), the lateral spines restricted to segments VIII and IX (Figs 4 and 5), the teeth and shape of the tarsal claws (Figs 12 and 13), the spines on posterior margin of abdominal terga alternating one long one short (Fig. 15), and the male genitalia with a conical genital plate (Fig. 17) were crucial for the specific identification. Other morphological features such as labrum



Figures 6–17. *Cloeon smaeleni*: **6–15** larva morphology **6** Labrum **7** Right mandible **8** Left mandible **9** Hypopharynx **10** Maxilla **11** Labium **12** Fore leg **13** Fore tarsal claw **14** Paraproct **15** Posterior margin of abdominal tergum **16** and **17** adult morphology **16** Female fore wing **17** Male genitalia.



Figures 18–21. Distribution of *Cloeon sraeleni*. **18** World map (gray, Brazil; dark gray, species distribution) **19** Map of Brazil subdivided in biomes, with detail of the State of Espírito Santo and collection stations (red circles) (st1, Conceição da Barra; st2, São Mateus; st3, Jaguaré; st4, Vitória; st5, Guarapari; st6, Bom Jesus do Norte) **20** Satellite picture from the Tubarão Complex Port in Vitória (red arrows indicate collection stations) **21** General view of one of the stations at Parque Estadual de Itaúnas.

Table 1. Sequences using the Kimura 2-parameter: **Taxa:** CS = *Cloeon smaeleni* or *Cloeon cf. smaeleni*; CD = *Cloeon dipterum* or *Cloeon cf. dipterum*; CL: *Cloeon* sp1; CP = *Cloeon praetextum*; CHS = *Cheleocloeon soldani*. **Countries:** MA = Madagascar; BR = Brazil; SA = Saudi Arabia; CH = Switzerland; KO = South Korea; TF = Tenerife (Canari Islands); GC = Gran Canaria (Canari Islands); NO = Norway.

	CS-MA	CS-BR	CS-SA	CD-CH	CD-KO	CD-TF	CD-GC	CL-SA	CP-NO
CS-BR	0.02								
CS-SA	0.12	0.11							
CD-CH	0.19	0.20	0.18						
CD-KO	0.21	0.21	0.19	0.08					
CD-TF	0.21	0.22	0.20	0.10	0.09				
CD-GC	0.22	0.23	0.21	0.11	0.10	0.10			
CL-SA	0.19	0.19	0.20	0.17	0.17	0.18	0.20		
CP-NO	0.23	0.23	0.21	0.21	0.20	0.21	0.20	0.19	
CHS-SA	0.24	0.25	0.23	0.20	0.20	0.22	0.23	0.21	0.23

(Fig. 6), mandibles (Figs 7 and 8), hypopharynx (Fig. 9), and paraproct (Fig. 14) are also illustrated as they may be useful to separate *Cloeon* from other Neotropical genera especially *Callibaetis* Eaton, 1881 and *Callibaetoides* Cruz, Salles & Hamada, 2013.

There is no genetic distance between the Brazilian specimens and they are highly supported as sister group to Afrotropical haplotypes of *Cloeon smaeleni* (Table 1). The genetic distance between Brazilian and Afrotropical haplotypes clearly corresponds to intraspecific variation (K2P distance = 0.02). We also sequenced material from Saudi Arabia morphologically similar to *C. smaeleni*. These haplotypes appear as sister group to the Afrotropical + Brazilian clade, but present interspecific distance with this clade (K2P distance > 0.11). Brazilian haplotypes are not genetically related to any Palaearctic species including *Cloeon dipterum* s.l. (K2P distance > 0.20).

The specimens examined were found exclusively in the State of Espírito Santo, Southeastern Brazil. They have been reported from at least six localities, most of them along the coast of the state (Figs 18 and 19) and always in low altitude areas (from the sea level to 65 meters above it). In Vitória (st4), Guarapari (st5) and Conceição da Barra (st1) (Fig. 19), larvae were collected in ponds very close to the shoreline. In Vitoria (st4), larvae were gathered in artificial impoundments colonized by *Pistia* spp. or *Typha* spp. macrophytes on the final section of the Carapina stream. All impoundments are located inside the Port Complex of Tubarão (Fig. 20), the biggest iron ore export port in the world. In São Mateus (st2), larvae were found in Rio Preto, a small black water tributary (Fig. 19) of the main river of the region, the Rio São Mateus or Cricaré. Female adults were caught inside a house very close to a large tributary of the Cricaré, the Rio Mariricu, suggesting that the species might also be present there. Attempts to collect material at the Rio Cricaré, however, were unsuccessful. Jaguaré (st3) and Bom Jesus do Norte (st6) are located more distant from the Ocean coast (around 100 km); in these localities, specimens were collected in approximately five meter wide streams. In the streams or rivers, larvae were found exclusively in areas with slow or no current, among organic substrates, such as roots, macrophytes or leaf litter.

Discussion

Cloeon smaeleni is a widespread species (Fig. 18) originally described from a female imago from Katanga, Congo. Subsequently, all ontogenetic stages of *C. smaeleni* were described (Gillies 1980, Gattolliat and Rabeantoandro 2002) and this species was reported from the whole Afrotropical Region: Central Africa (Lestage 1924), Southern Africa (Demoulin 1970), West Africa (Gillies 1980), East Africa (Gillies 1985), Madagascar (Gattolliat and Rabeantoandro 2002), and La Réunion (Gattolliat 2004). *Cloeon smaeleni* was also reported from the Arabian Peninsula (Gillies 1985). Our molecular (Table 1) and morphological analyses (unpublished results) established that in fact Arabian populations constitute a new undescribed species related to *C. smaeleni*.

Larvae of *C. smaeleni* are found in many types of slow waters: temporary ponds, rice fields, reservoirs, slow moving streams and the margins of lakes (Gillies 1980), sometimes associated with hydrophytes (Petr 1968). Imagos, as well as other species of the genus, are ovoviviparous (Gillies 1985).

The identity of the species, its distribution, along with its previous absence in regularly sampled sites, is a clear sign that the specimens of *Cloeon smaeleni* found in Espírito Santo are introduced, well established, and that the colonization took place very recently. It is not possible to ascertain where or how this event has occurred, or even if there was a single or multiple entrances. The presence of larvae of *Cloeon smaeleni* in a port, though highly speculative, suggests that they may have arrived in by ship traveling from Africa.

It is also difficult to predict the impact caused by the presence of *Cloeon smaeleni* in Brazil. In its original habitats, this species feeds on detritus contributing to the recycling of organic matter. It is often the eudominant species but generally co-occurs with other *Cloeon* species. As this species is not a predator and has no significant economic importance, the impact is probably more related to its competition with other native species at the same trophic level (or controphic species, according to Davis 2003). In the case of the mayfly community, species of the genus *Callibaetis* occupy a very similar ecological niche when compare to those of *Cloeon* (Domínguez et al. 2006). *Callibaetis guttatus* Navás, 1915, for example, is found along the Brazilian tropical coast (Da Silva 1991, Salles et al. 2010, Lima et al. 2012) and is a common species in the sites where *Cloeon smaeleni* was collected. Based on our observations in the north of Espírito Santo, at least for now, larvae of *Callibaetis* are more abundant than those of *Cloeon*. Following Davis (2003), competition from introduced species is not likely to be a common cause of extinctions of long-term resident species. However, a study on the population dynamics of both species would be interesting in order to monitor the potential impact of *Cloeon smaeleni* on a resident species, especially because the sites where the species was found in Conceição da Barra (st1) and Guarapari (st5) are located in nature reserves, the Parque Estadual Paulo Cesar Vinha and the Parque Estadual de Itaúnas (Fig. 21).

Our finding raises again the idea, put forward by earlier authors (e.g. Traver 1962, McCafferty 1996, McCafferty and Mauremootoo 2001), but contested by Randolph et al. (2002), that *Cloeon dipterum* is also an exotic species in the Western Hemisphere. Whereas *C. smaeleni* is a tropical species that encountered a suitable area for reproduction along the

coast of Brazil, similar to its native habitat, the same is also plausible for *C. dipterum* in North America. This result also has important implication on the insular populations of *Cloeon* that are often considered as endemics but may be in fact recent natural colonizations or introductions related to human activities (McCafferty and Mauremootoo 2001, Gattolliat and Staniczek 2011, Gattolliat 2013). On the other hand, *Cloeon dipterum* is not the only species of mayfly, or even Baetidae, with a Holarctic distribution. Kjaerstad et al. (2012), for example, listed 10 species found in Norway and North America. Besides these species, the CO1 sequences of *Cloeon praetextum* Bengtsson, 1914 studied by these authors are highly similar to those of *Procloeon mendax* (Walsh, 1862) from northern Canada (less than 1%) and, therefore, they maybe conspecific. The historical processes that lead to the presence of unlikely invasive species in both realms, such as those listed by Kjaerstad et al. (2012), could be the same that lead to the Holarctic distribution of *C. dipterum*.

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References

- Barber-James HM, Gattolliat JL, Sartori M, Hubbard MD (2008) Global diversity of mayflies (Ephemeroptera, Insecta) in freshwater. *Hydrobiologia* 595: 339–350. doi: 10.1007/s10750-007-9028-y
- Bauernfeind E, Humpesch UH (2001) Die Eintagsfliegen Zentraleuropas (Insecta: Ephemeroptera): Bestimmung und Ökologie. Naturhistorisches Museum, Wien, 239 pp.
- Bauernfeind E, Soldán T (2012) The Mayflies of Europe. Apollo Books, Ollerup, 781 pp.
- Brinck P, Scherer E (1961) On the Ephemeroptera of the Azores and Madeira. *Boletim do Museu Municipal do Funchal* 14: 55–66. http://www.ephemeroptera-galactica.com/pubs/pub_b/pubbrinckp1961p55.pdf
- Brittain JE, Sartori M (2009) Ephemeroptera. In: Resh VH, Cardé R (Eds) *Encyclopedia of insects*, 2nd edition. Academic Press, New York, 328–333.
- Burian SK, Gibbs KE (1991) Mayflies of Maine: an annotated faunal list, Orono, 109 pp. http://www.ephemeroptera-galactica.com/pubs/pub_b/pubburians1991p1.pdf

- Burks BD (1953) The mayflies, or Ephemeroptera, of Illinois. Bulletin of the Illinois Natural History Survey 26: 1–216. http://www.ephemeroptera-galactica.com/pubs/pub_b/pub-burksb1953p1.pdf
- Da Silva ER (1991) Descriçao da ninfa de *Callibaetis guttatus* Navás, 1915, com notas biológicas e comentarios sobre a imago (Ephemeroptera: Baetidae). Anais da Sociedade Entomológica do Brasil 20: 345–352.
- Davis MA (2003) Biotic globalization: Does competition from introduced species threaten biodiversity? Bioscience 53: 481–489. <http://cedarcreek.umn.edu/biblio/fulltext/t1904.pdf>, doi: 10.1641/0006-3568(2003)053[0481:BGDCFI]2.0.CO;2
- de Moor FC (1992) Factors influencing the establishment of aquatic insect invaders. Transactions of the Royal Society of South Africa 48: 141–158. <http://www.tandfonline.com/doi/abs/10.1080/00359199209520259#.UpIF78Ssim4>, doi: 10.1080/00359199209520259
- Degrange C (1959) L'ovolarviparité de *Cloëon dipterum* (L.) (Ephemeroptera-Baetidae). Bulletin de la Société Entomologique Francaise 64: 94–100.
- Degrange C (1960) Recherches sur la reproduction des Ephéméroptères. Travaux du Laboratoire de Pisciculture de l'Université de Grenoble 50–51: 7–193. http://www.ephemeroptera-galactica.com/pubs/pub_d/pubdegrangec1960p7.pdf
- Demoulin G (1970) Ephemeroptera des faunes éthiopienne et malgache. South African Animal Life 14: 24–170. http://www.ephemeroptera-galactica.com/pubs/pub_d/pubdemouling1970p24.pdf
- Domínguez E, Molineri C, Pescador ML, Hubbard MD, Nieto C (2006) Ephemeroptera of South America. Pensoft Press, Sofia and Moscow, 650 pp.
- Flowers RW (1978) Occurrence of *Cloëon cognatum* Stephens in the United States (Ephemeroptera, Baetidae). Entomological News 89: 79–80. http://www.ephemeroptera-galactica.com/pubs/pub_f/pubflowersr1978p79.pdf
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–9. http://www.mbari.org/staff/vrijen/PDFS/Folmer_94MMBB.pdf
- Gattolliat J-L (2004) First reports of the genus *Nigrobaetis* Novikova & Kluge (Ephemeroptera: Baetidae) from Madagascar and La Réunion with observations on Afrotropical biogeography. Revue Suisse de Zoologie 111: 657–669. http://www.ephemeroptera-galactica.com/pubs/pub_g/pubgattolliatj2004p657.pdf
- Gattolliat J-L, Hughes SJ, Monaghan MT, Sartori M (2008) Revision of Madeiran mayflies (Insecta, Ephemeroptera). Zootaxa: 52–68. http://www.ephemeroptera-galactica.com/pubs/pub_g/pubgattolliatj2008p52.pdf
- Gattolliat J-L, Rabeantoandro SZ (2002) The genus *Cloëon* (Ephemeroptera, Baetidae) in Madagascar. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 74: 195–209. http://www.ephemeroptera-galactica.com/pubs/pub_g/pubgattolliatj2002p195.pdf
- Gattolliat J-L, Staniczek A (2011) New larvae of Baetidae (Insecta: Ephemeroptera) from Espiritu Santo, Vanuatu. Stuttgarter Beiträge zur Naturkunde A, Neue Serie 4: 75–82. http://www.ephemeroptera-galactica.com/pubs/pub_g/pubgattolliatj2011p75.pdf

- Gattolliat JL (2013) Subclass PTERYGOTA Lang, 1888; Order EPHEMEROPTERA Hyatt & Arms, 1891. In: Gerlach J (Ed) Hemiptera, Hymenoptera and other insects of the Seychelles islands. Siri Scientific Press, Manchester, 41–49.
- Gillies MT (1980) An Introduction to the study of *Cloeon* Leach (Baetidae, Ephemeroptera) in West Africa. Bulletin de l'I.F.A.N. serie A 42: 135–156. http://www.ephemeroptera-galactica.com/pubs/pub_g/pubgilliesm1980p135.pdf
- Gillies MT (1985) A preliminary account of the East African species of *Cloeon* Leach and *Rhithrocloeon* gen. n. (Ephemeroptera). Aquatic Insects 7: 1–17. http://www.ephemeroptera-galactica.com/pubs/pub_g/pubgilliesm1985p1.pdf
- Gillies MT (1990) A revision of the Argentine species of *Callibaetis* Eaton (Baetidae; Ephemeroptera). Revista de la Sociedad Entomológica Argentina 48: 15–39. http://www.ephemeroptera-galactica.com/pubs/pub_g/pubgilliesm1990p15.pdf
- Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of The Royal Society B-Biological Sciences 270: 313–321. doi: 10.1098/rspb.2002.2218, <http://www.urbanbarcodeproject.org/images/pdf/Biological-identifications-through-DNA-barcodes.pdf>
- Jobb G, von Haeseler A, Strimmer K (2004) TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. BMC Evolutionary Biology 4: 1–9. doi: 10.1186/1471-2148-4-18, <http://link.springer.com/article/10.1186%2F1471-2148-4-18#page-1>
- Karatayev AY, Burlakova LE, Padilla DK, Mastitsky SE, Olenin S (2009) Invaders are not a random selection of species. Biological Invasions 11: 2009–2019. doi: 10.1007/s10530-009-9498-0, <http://link.springer.com/article/10.1007/s10530-009-9498-0#page-1>
- Kjaerstad G, Webb JM, Ekrem T (2012) A review of Ephemeroptera of Finnmark – DNA barcodes identify Holarctic relations. Norwegian Journal of Entomology 59: 182–195.
- Kleunen M, Dawson W, Schlaepfer D, Jeschke JM, Fischer M (2010) Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. Ecology Letters: 947–958.
- Lestage J-A (1924) Etudes sur les Ephémères du Congo Belge. II. Un nouveau Cloëon du Katanga (*Cloeon smaeleni* nov. sp.). Revue zoologique africaine 12: 426–428. http://www.ephemeroptera-galactica.com/pubs/pub_l/publestagej1924p426.pdf
- Lima LRC, Salles FF, Pinheiro U (2012) Ephemeroptera (Insecta) from Pernambuco State, northeastern Brazil. Revista Brasileira De Entomologia 56: 304–314. doi: 10.1590/S0085-56262012005000043
- Lugo-Ortiz CR, McCafferty WP (1999) Revision of South American species of Baetidae (Ephemeroptera) previously placed in *Baetis* Leach and *Pseudocloeon* Klapalek. Annales de Limnologie 35: 257–262. doi: 10.1051/limn/1999034
- McCafferty WP (1996) The Ephemeroptera species of North America and index to their complete nomenclature. Transactions of the American Entomological Society 122: 1–54. http://www.ephemeroptera-galactica.com/pubs/pub_m/pubmccaffertyw1996p1.pdf
- McCafferty WP, Jacobus LM, Webb JM, Meyer MD (2008) Insecta, Ephemeroptera: range extensions and new records for Ontario and Canada. Check List 4(4): 445–448. <http://www.checklist.org.br/getpdf?NGD125-08>

- McCafferty WP, Mauremootoo JR (2001) The first mayfly taken from the island of Mauritius (Ephemeroptera, Baetidae). *Ephemera* 2: 105–111. http://www.ephemeroptera-galactica.com/pubs/pub_m/pubmccaffertyw2001p105.pdf
- McCafferty WP, Waltz RD (1990) Revisionary Synopsis of the Baetidae (Ephemeroptera) of North and Middle America. *Transactions of the American Entomological Society* 116: 769–799. http://www.ephemeroptera-galactica.com/pubs/pub_m/pubmccaffertyw1990p769.pdf
- Menetrey N, Oertli B, Sartori M, Wagner A, Lachavanne JB (2008) Eutrophication: are mayflies (Ephemeroptera) good bioindicators for ponds? *Hydrobiologia* 597: 125–135. doi: 10.1007/s10750-007-9223-x
- Nagell B (1977) Survival of *Cloeon dipterum* (Ephemeroptera) larvae under anoxic conditions in winter. *Oikos* 29: 161–165. doi: 10.2307/3543308, http://www.ephemeroptera-galactica.com/pubs/pub_n/pubnagellb1977p161.pdf
- Oehme G (1972) Zur maximalen Lebensdauer von *Cloeon dipterum* L. (Eph. Baetidae). *Entomologische Nachrichten* 16: 131–133. http://www.ephemeroptera-galactica.com/pubs/pub_o/puboehmeg1972p131.pdf
- Petr T (1968) Population changes in aquatic invertebrates living on two water plants in a tropical man-made lake. *Hydrobiologia* 32: 449–484. doi: 10.1007/BF00155401, http://www.ephemeroptera-galactica.com/pubs/pub_p/pubpetrt1968p449.pdf
- Randolph RP, McCafferty WP, Zaranko D, Jacobus LM, Webb JM (2002) New Canadian records of Baetidae (Ephemeroptera) and adjustments to North American *Cloeon*. *Entomological News* 113: 306–309. http://www.ephemeroptera-galactica.com/pubs/pub_r/pubrandolphr2003p306.pdf
- Salles FF, do Nascimento JMC, Massariol FC, Angeli KB, Silva PBE, Rudio JA, Boldrini R (2010) First survey of mayflies (Ephemeroptera, Insecta) from Espírito Santo State, South-eastern Brazil. *Biota Neotropica* 10: 293–307. doi: 10.1590/S1676-06032010000100025, <http://www.biotaneotropica.org.br/v10n1/en/abstract?inventory+bn02610012010>
- Smith GC (2000) Rediscovery of the introduced mayfly *Caenis nigropunctata* (Ephemeroptera: Caenidae) in Waimānalo stream, O’ahu. *Bishop Museum Occasional Papers* 64: 30–31. http://www.ephemeroptera-galactica.com/pubs/pub_s/pubsmithg2000p30.pdf
- Sowa R (1975) What is *Cloeon dipterum* (Linnaeus, 1761)? - The nomenclatural and morphological analysis of a group of the European species of *Cloeon* Leach (Ephemera: Baetidae). *Entomologica Scandinavia* 6: 215–223. http://www.ephemeroptera-galactica.com/pubs/pub_s/pubsowar1975p215.pdf
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731–2739. http://kumarlab.net/pdf_new/TamuraKumar11.pdf
- Traver JR (1962) *Cloeon dipterum* (L.) in Ohio (Ephemeroptera: Baetidae). *Bulletin of the Brooklyn Entomological Society* 57: 47–50. http://www.ephemeroptera-galactica.com/pubs/pub_t/pubtraverj1962p47.pdf
- Ulmer G (1920) Übersicht über die Gattungen der Ephemeropteren, nebst Bemerkungen über einzelne Arten. *Stettiner Entomologische Zeitung* 81: 97–144. http://www.ephemeroptera-galactica.com/pubs/pub_u/pubulmerg1920p97.pdf

- Vuataz L, Sartori M, Wagner A, Monaghan MT (2011) Toward a DNA taxonomy of Alpine *Rhithrogena* (Ephemeroptera: Heptageniidae) using a mixed Yule-coalescent analysis of mitochondrial and nuclear DNA. PLoS ONE 6: e19728. doi: 10.1371/journal.pone.0019728
- Zelinka M, Marvan P (1961) Zur Präzisierung der biologische Klassifikation der Reinheit fließender Gewässer. Archiv für Hydrobiologie 57: 389–407
- Zimmermann EC (1957) Volume 6 Ephemeroptera-Neuroptera-Trichoptera and supplement to volumes 1 to 5. Insects of Hawaii. University of Hawaii Press, Honolulu, 212 pp.

Ultrastructure of antennal sensilla of four skipper butterflies in *Parnara* sp. and *Pelopidas* sp. (Lepidoptera, Hesperiiidae)

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Abstract

Most species of *Parnara* and *Pelopidas* (Hesperiiidae) are important pests of rice. In this study, the antennal morphology, types of sensilla, and their distribution of four skipper butterflies, including *Parnara guttata* (Bremer & Grey), *Pa. bada* (Moore), *Pelopidas mathias* (Fabricius) and *Pe. agna* (Moore), were observed using a scanning electron microscope. Six distinct morphological types of sensilla were found on the antennae of all of these species: sensilla squamiformia, sensilla trichodea, sensilla chaetica, sensilla auricillica, sensilla coeloconica, and Böhm sensilla. The sensilla trichodea are the most abundant sensilla among the four skipper butterflies, and the sensilla auricillica are confirmed on the antennae of butterflies for the second time. In addition, the possible functions of these sensilla are discussed in the light of previously reported lepidopteran insects, which may provide useful information for further study of the function of these antennal sensilla and for related pests control by applying sex pheromones.

Keywords

Lepidoptera, Hesperiiidae, morphology, fine structure

Introduction

The antennae of insects have various types of sensilla that play important roles in insect behaviors, including host location, feeding, mate attraction and oviposition (Zacharuk 1980; Skiri et al. 2005). Antennal sensilla have been extensively recorded in many insect groups (Amer and Mehlhorn 2006; Sukontason et al. 2004; Bleeker et al. 2004). Although the structure and function of antennal sensillae in Lepidoptera have been well known for decades (Anderson et al. 2000), little research has involved butterflies, especially some important pest species.

Parnara guttata (Bremer & Grey), *Parnara bada* (Moore), *Pelopidas mathias* (Fabricius) and *Pelopidas agna* (Moore) are among the most important pests of rice in China. The larvae of these four species feed on the leaves of rice, causing considerable damage and great loss of rice production. So far, the control of rice plant skippers chiefly relies on the use of chemical insecticides, which in turn causes many negative consequences. Biological controls, including the application of sex pheromones, have become increasingly important. Consequently, research of pest antennae has immediate application to the suppression of pests (Smith and Wall 1998). In order to better understand their olfactory system related to the biological control of these four skippers, we researched the type, size, and distribution of antennal sensilla of *Pa. guttata* (Bremer & Grey), *Pa. bada* (Moore), *Pe. mathias* (Fabricius) and *Pe. agna* (Moore).

Materials and methods

Insects

All insects studied are specimens in the entomological museum of Northwest A&F University. More specific information is provided in Table 1.

Table 1. Material localities and collection dates.

Species	Collection location	Collection date
<i>Pa. guttata</i> (Bremer & Grey)	Huxian County, Shaanxi Province	2009.08.15
	Lantian County, Shaanxi Province	2012.08.15
	Fuzhou City, Fujian Province	2006.07.01
	Zhenkang County, Yunnan Province	2007.07.09
<i>Pa. bada</i> (Moore)	Ding'an County, Hainan Province	2002.08.08
	Fuzhou City, Fujian Province	2005.11.19
	Jinghong City, Yunnan Province	2007.07.21
<i>Pe. mathias</i> (Fabricius)	Hanzhong City, Shaanxi Province	1993.07.23
	Fuzhou City, Fujian Province	2003.12.28
	Minqing County, Fujian Province	2005.10.21
<i>Pe. agna</i> (Moore)	Wuzhi Mountain, Hainan Province	2007.05.20
	Luxi County, Yunnan Province	2005.08.19

Scanning electron microscope

The antennae of 10 adults of each of the four species were removed under a microscope (Nikon SMZ1500) by using sharp blades. The antennae were washed for 20 s (four times, each for 5 s) in 70% ethanol solution in an ultrasonic cleaner (KH-250DB; 15°C, 50HZ). After critical point drying, the specimens were attached to a holder using electric adhesive tape, sputter-coated with gold, examined and photographed with a S-4800 SEM (at 10 kV-15 kV).

Results

Antennal morphology

The antennae of the four studied species of skipper butterflies are located between the compound eyes, and each consists of three components: a basal scape, pedicel, and an elongated flagellum. The first two components consist of a single short segment each one

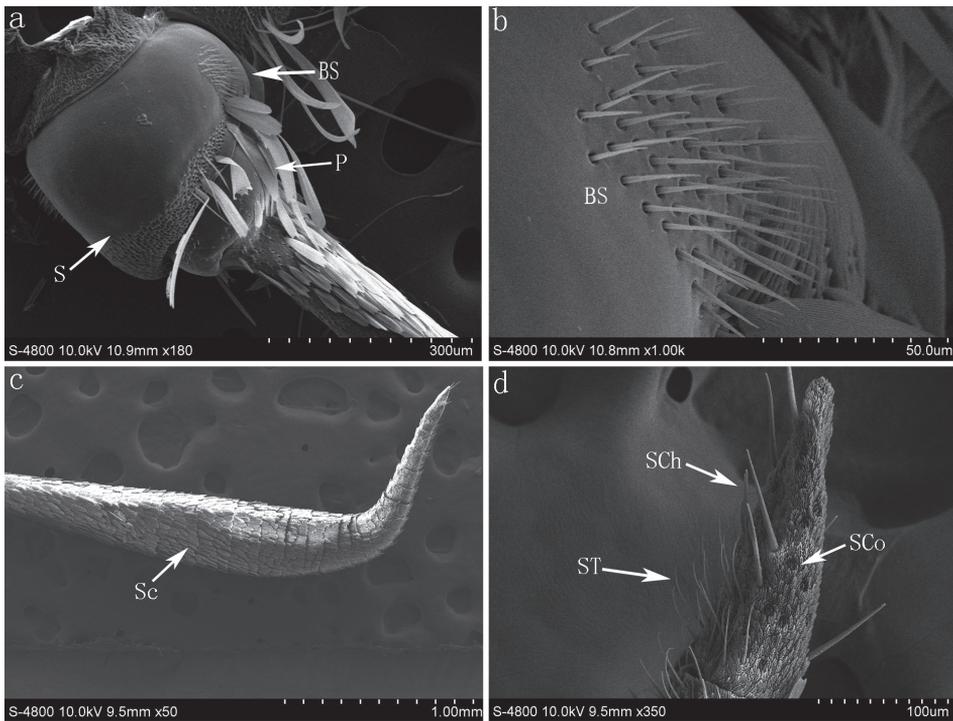


Figure 1. SEM photomicrographs of *Pa. guttata*. (a) the scape and pedicel of *Pa. guttata* and location of the Böhm sensilla (b) the Böhm sensilla on the scape of antenna (c) profile of the flagellum with scales (d) profile of last flagellar subsegment of the antenna and the sensilla chaetica, sensilla trichodea and sensilla coeloconica. S Scape; P Pedicel; BS Böhm sensilla; Sc Scales; SCh sensilla chaetica; ST sensilla trichodea; SCo sensilla coeloconica.

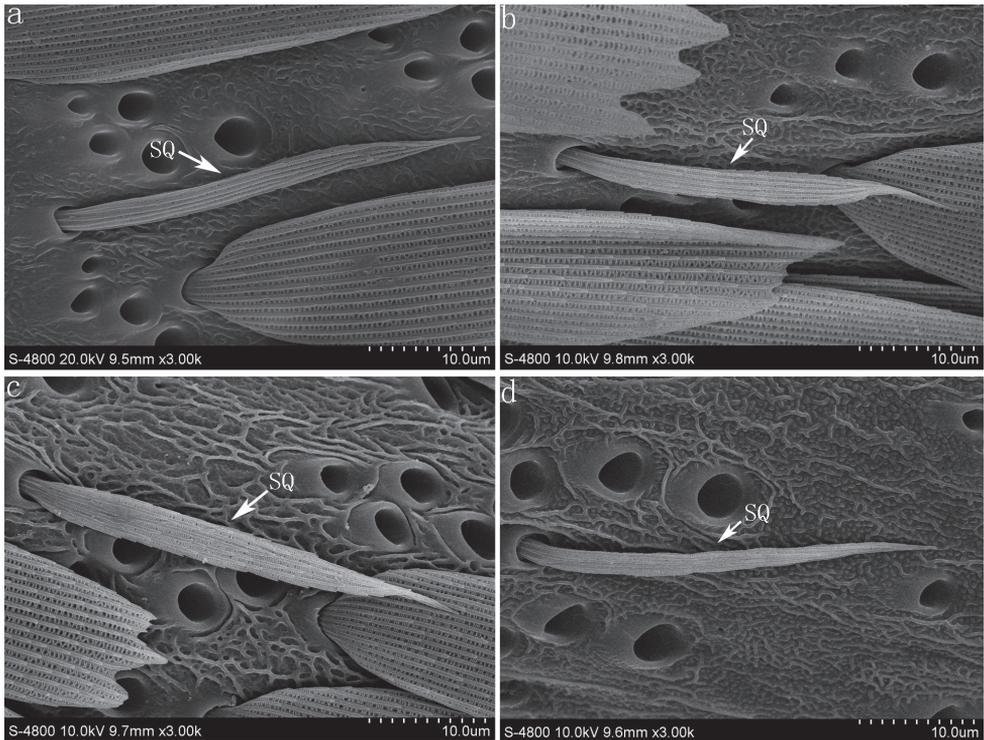


Figure 2. (a) sensilla squamiformia on the flagellum of *Pa. guttata* (b) sensilla squamiformia of *Pa. bada* (c) sensilla squamiformia of *Pe. mathias* (d) sensilla squamiformia of *Pe. agna*. SQ sensilla squamiformia.

of them (Fig. 1a). The third component, the flagellum, consists of many subsegments. The typical flagellum is thin basally and becomes gradually thicker and curved, covered with scales (Fig. 1c). More types of sensilla are observed on the curved hook (Fig. 1d).

Types of antennal sensilla

In total, six types of sensilla were observed on the antennae of these four skippers: sensilla squamiformia, sensilla trichodea, sensilla chaetica, sensilla auricillica, sensilla coeloconica, and Böhm sensilla.

Sensilla squamiformia (SQ)

This type of sensillum is scale-like and elongated with a distal end tapering, found along the base or center flagellum among the scales (Fig. 2a–d). The length of the sensilla squamiformia is $43.5 \pm 4.0 \mu\text{m}$ (*Pa. guttata*), $48.5 \pm 6.7 \mu\text{m}$ (*Pa. bada*), $47.5 \pm 5.8 \mu\text{m}$ (*Pe. mathias*), $46.3 \pm 3.8 \mu\text{m}$ (*Pe. agna*). The number of sensilla is 1–4 per flagellomere, with the terminal flagellomeres without any among the four skipper butterflies.

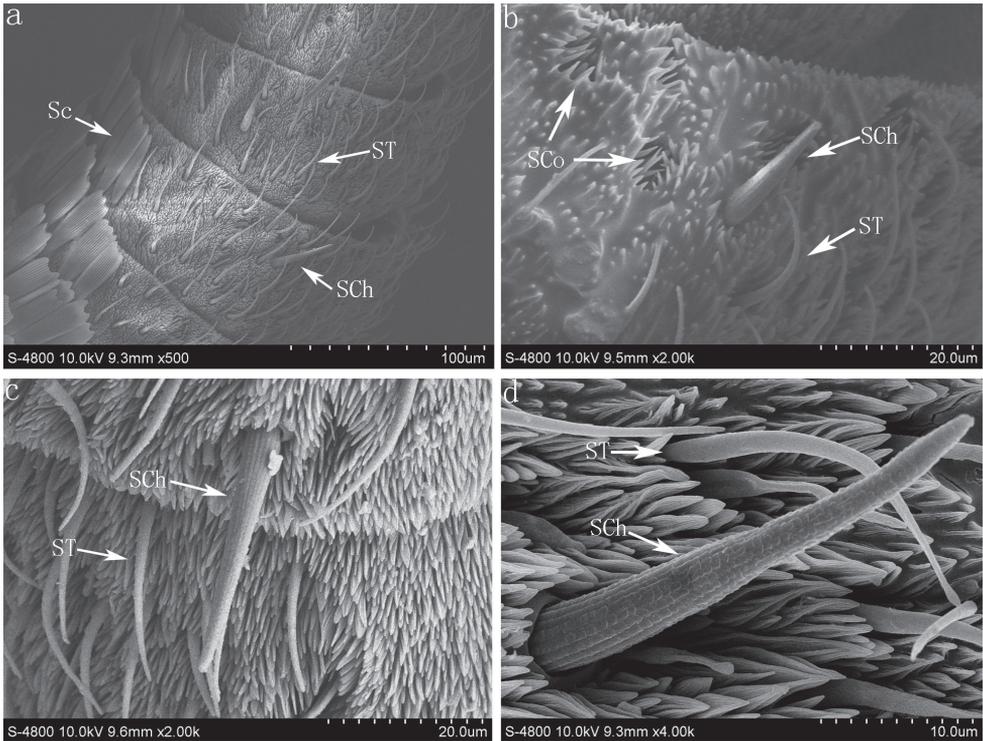


Figure 3. (a) The sensilla chaetica, sensilla trichode and scales on the flagellum of *Pa. guttata* (b) the sensilla chaetica, sensilla trichode and sensilla coeloconica on the flagellum of *Pa. bada* (c) the sensilla chaetica and sensilla trichode on the flagellum of *Pe. mathias* (d) the sensilla chaetica and sensilla trichode on the flagellum of *Pe. agna*. **Sc** Scales; **SCh** sensilla chaetica; **ST** sensilla trichodea; **SCo** sensilla coeloconica.

Sensilla trichodea (ST)

The sensilla trichodea are hair-like, tapering apically. They occur along the distal segments on the ventral surface (Figs 1d and 3a and b). The surface of the cuticular wall of sensilla trichodea is smooth and the wall pores are not seen with scanning electron microscope (Fig. 3c and d). These sensilla (range $27.1 \pm 3.2 \mu\text{m}$ – $28 \pm 1.5 \mu\text{m}$) are the most abundant with about 32–69 per flagellomere in the four species.

Sensilla chaetica (SCh)

The sensilla chaetica have a straight needle-like appearance with a grooved surface (Figs 1d and 3a–c). Each sensilla arise from a round socket, is wide at the base and sharp at the distal end (Fig. 3d). These sensilla (range from $29.5 \pm 4.1 \mu\text{m}$ to $39.5 \pm 7.5 \mu\text{m}$) are distributed evenly (1–3 per flagellomere) among the scales at the base and center of the flagellomere and among the sensilla trichodea along the flagellum. 4–7 larger sensilla chaetica ($80.3 \pm 5.8 \mu\text{m}$) are distinct and can be found on the terminal segment of flagellum.

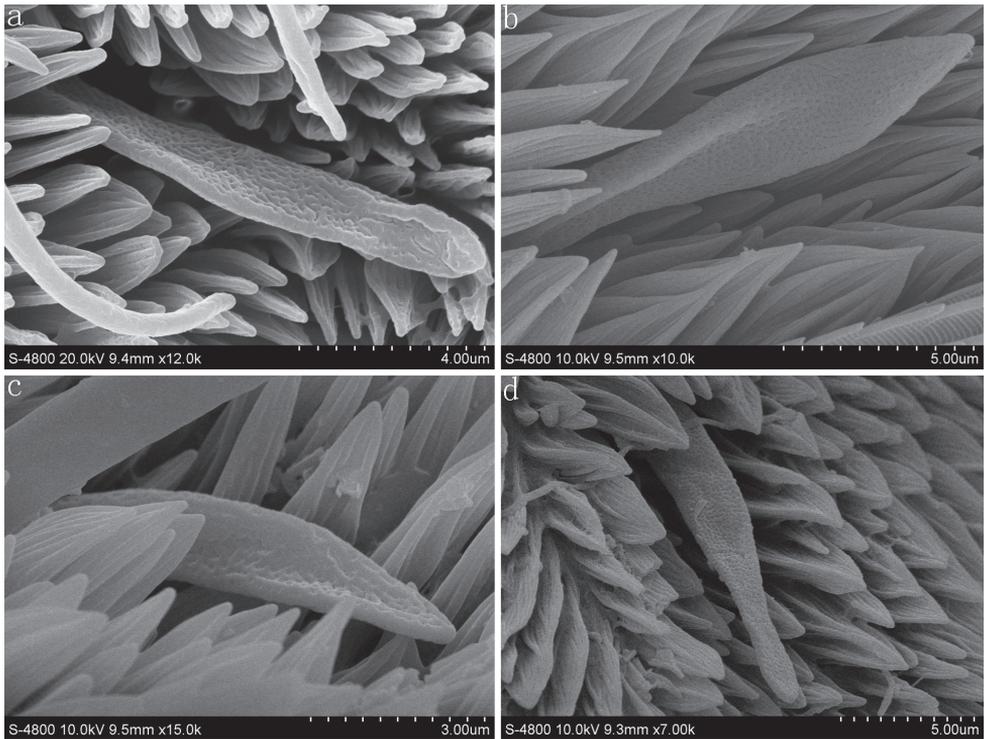


Figure 4. The sensilla auricillica of *Pa. guttata* (a) *Pa. bada* (b) *Pe. mathias* (c) and *Pe. agna* (d).

Sensilla auricillica (SAu)

The sensilla auricillica are short and ear-shaped with a blunt and rounded tip. The surface of the cuticular wall of ear-shaped sensilla is covered with small pores (Fig. 4a–d). These sensilla are only scattered along the distal end of the flagellum. These sensilla (about 6–14 per flagellomere) are very similar and the length varies from $12.8 \pm 3.4 \mu\text{m}$ to $15.5 \pm 0.3 \mu\text{m}$ among all four skipper butterflies.

Sensilla coeloconica (SCo)

The sensilla coeloconica consist of a submerged central peg with a grooved surface and blunt tip surrounded by a ring of cuticular spines (Figs 3b, 5a–d). They are found on the distal end of the flagellum (about 6–12 per flagellomere) in the four species (Fig. 1d). In *Pe. mathias* and *Pe. agna*, these sensilla are also found occasionally on the base or center of the flagellomere as they are difficult to discern since the scales will conceal them (Fig. 5c and d).

Böhm sensilla (BS)

Böhm sensilla are spine-like structures with smooth cuticles. Böhm sensilla, in clusters, are inserted to the base of scape and pedicel segments only (Fig. 1a and b). Each cluster has approximately 56, 59, 34 and 32 sensilla respectively among *Pa. guttata*, *Pa. bada*, *Pe. mathias* and *Pe. agna*.

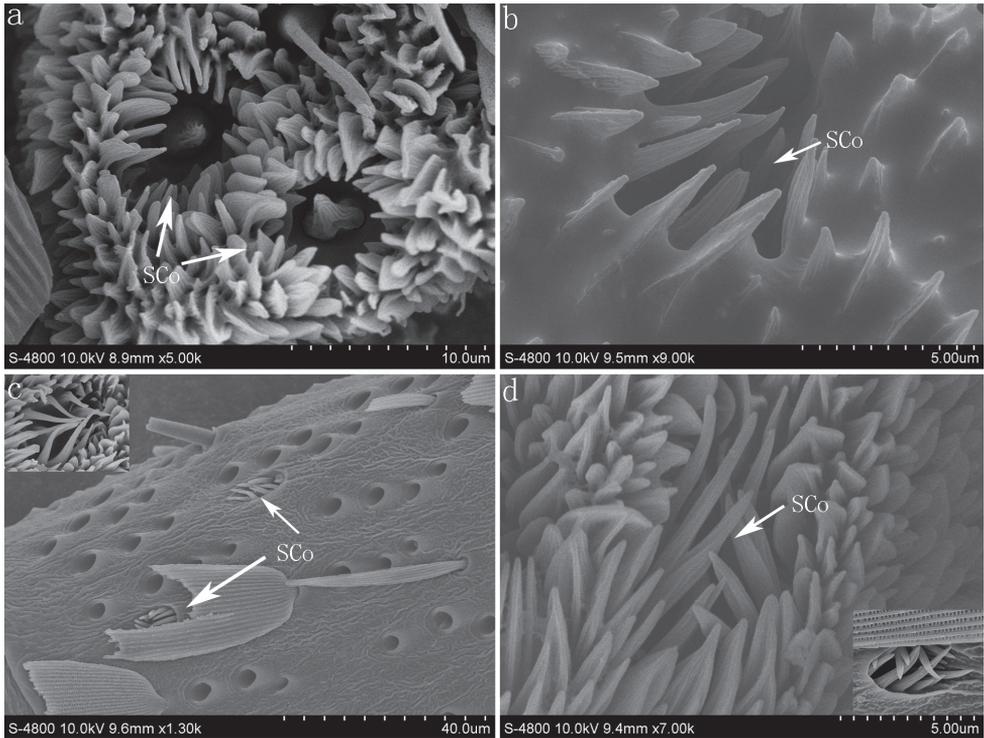


Figure 5. The sensilla coeloconica of *Pa. guttata* (a) *Pa. bada* (b) *Pe. mathias* (c) and *Pe. agna* (d). SCo sensilla coeloconica

Discussion

Sensilla squamiformia are commonly present in lepidopteran insects (Faucheux 1999). The sensilla squamiformia found in this study are similar in shape and distribution to those reported in two other butterfly species, *Teinopalpus aureus* Mell and *Heliophorus phoenicoparyphus* (Holland) (Jiang et al. 2000; Xu and Wang 2013); but the shape and distribution of these sensilla are different from several reported moth species, *Synanthedon scitula* (Harris), *Coleophora* sp. and *Zamagiria dixolophella* Dyar (Frank et al. 2010; Faucheux 2011; Gómez et al. 2003) and also different from Coleoptera (Hix et al. 2003; Gao et al. 2013). These aporous sensilla are inferred to have a mechanoreceptive function (Schneider 1964).

We identified only one type of sensilla trichodea among the four skipper butterflies. However, studies of other moth species have shown those sensilla can be divided into more subtypes according to their size and pore density (Faucheux 1999). For example, three subtypes of these sensilla are found on *Synanthedon scitula* and *Ostrinia nubilalis* (Hübner) (Frank et al. 2010; Hallberg et al. 1994). Furthermore, the number of these sensilla in Bombycidae, Tortricidae, Tineidae and Pyralidae is significantly different between male and female antennae (Steinbrecht 1973; George and Nagy 1984; Faucheux 1987; Wang et al. 2008). The accumulated studies have shown that multiporous sensilla trichodea are associated with olfactory reception of sex pheromones (Hansson et al. 1995; Ebbinghaus et al. 1997; Ma and Du 2000).

Sensilla chaetica found in this study are similar in structure to those reported for the Lycaenidae: *Chilades pandava* (Horsfield) and *Heliophorus phoenicoparyphus* (Jian et al. 2011; Xu and Wang 2013). These sensilla have also been observed in many other moth species, viz, *Cydia nigricana* (Fabricius), *Bactra furfurana* (Haworth), and *Zamagiria dixolophella* (Wall 1978; Razowski and Wojtusiak 2004; Gómez et al. 2003). Several studies noted that these uniporous sensilla to be contact chemoreceptors (Altner and Prillinger 1980; Hallberg et al. 1994).

Although the sensilla auricillica have been easily observed in the moths, these sensilla on the antenna of butterfly was described for the first time in *Pieris rapae* L. (Faucheux 1996, 1999). Our observations on the HesperIIDae confirm their presence in the butterflies. Several studies on moth species considered multiporous sensilla auricillica as olfactory receptors for plant volatiles (Boekh et al. 1965; Kaissling 1971). Others suggest they respond to sex pheromone compounds (Ebbinghaus et al. 1997; Anderson et al. 2000; Faucheux 2006).

In this study, the multiporous sensilla coeloconica closely resemble those observed in many other Lepidoptera. This type of sensilla is considered to have a humidity and temperature sensitive function (Altner et al. 1977). Pophof (1997) reported that in *Bombyx mori* L., they are sensitive to plant volatiles and are possibly involved in the selection of oviposition sites. Sensilla coeloconica were found under the scales on the antennae of *Pe. mathias* and *Pe. agna*, as has not been reported in other insects.

Böhm sensilla observed here are morphologically similar to those presented in other families of Lepidoptera, e.g., Pyralidae, Tortricidae, Sesiidae (Gómez et al. 2003; Gómez and Carrasco 2008; Frank et al. 2010). The absence of dendrite in the sensillum lumen and the presence of a tubular body at the base of the hair, observed in the Böhm sensilla of *Tineola bisselliella* Humm. (Faucheux 1987) are characteristic of the mechanoreceptors with a proprioceptive function (Schneider 1964; Faucheux 1999).

In summary, we identified six different types of sensilla on the antennae of *Pa. guttata*, *Pa. bada*, *Pe. mathias* and *Pe. agna*. The external morphology and distribution of these sensilla among *Parnara* and *Pelopidas*, is very similar and also somewhat similar to other reported Lepidoptera. However, documents on morphology of antennal sensilla in butterfly species are still very limited yet. Further exploration on antennal sensilla of these group need merits to be conducted, which may provide useful information for taxonomy and phylogeny of Lepidoptera, and for further studies on the function of antennal sensilla and related pests control by application of sex pheromones.

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References

- Amer A, Mehlhorn H (2006) The sensilla of *Aedes* and *Anopheles* mosquitoes and their importance in repellency. *Parasitology Research* 99: 491–499. doi: 10.1007/s00436-006-0185-0
- Anderson P, Hallberg E, Subchev M (2000) Morphology of antennal sensilla auricillica and their detection of plant volatiles in the Herald moth, *Scoliopteryx libatrix* L. (Lepidoptera: Noctuidae). *Arthropod Structure and Development* 29: 33–41. doi: 10.1016/S1467-8039(00)00011-6
- Altner H, Prillinger L (1980) Ultrastructure of invertebrate chemo-, thermo-, and hygroreceptors and its functional significance. *International Review Cytology* 67: 69–139. doi: 10.1016/S0074-7696(08)62427-4
- Altner H, Sass H, Altner I (1977) Relationship between structures and function of antennal chemo-hygro-, and thermo-receptive sensilla in *Periplaneta americana*. *Cell and Tissue Research* 176: 389–405. doi: 10.1007/BF00221796
- Bleeker MAK, Smid HM, Van Aelst AC, Van Loon JJA, Vet LEM (2004) Antennal sensilla of two parasitoid wasps: a comparative scanning electron microscopy study. *Microscopy Research and Technique* 65(5): 266–273. doi: 10.1002/jemt.20038
- Boeck J, Kaissling KE, Schneider D (1965) Insect olfactory receptors. *Cold Spring Harbor Symposia on Quantitative Biology* 30: 263–280. doi: 10.1101/SQB.1965.030.01.028
- Ebbinghaus D, Losel PM, Lindemann M, Scherkenbeck J, Zebitz CPW (1997) Detection of major and minor sex pheromone components by the male codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). *Journal of Insect Physiology* 44(1): 49–58. doi: 10.1016/S0022-1910(97)00101-7
- Faucheux MJ (1987) Recherches sur les organes sensoriels impliqués dans le comportement de ponte chez deux Lépidoptères à larves kératinophages, *Tineola bisselliella* Humm. et *Monopis crocicapitella* Clem. (Tineidae). Thèse doct. état es sciences, Université de Nantes, 511 pp.
- Faucheux MJ (1996) Sensilles auricilliformes sur l'antenne des Rhopalocères: étude de la Piéride de la rave, *Pieris rapae* L. (Lepidoptera: Pieridae). *Bulletin de la Société des Sciences naturelles de l'Ouest de la France* (n.s.) 18: 93–97.
- Faucheux MJ (1999) Biodiversity and unity of sensory organs in lepidopteran insects. *Société des Sciences naturelles de l'Ouest de la France, Nantes*, 296 pp.
- Faucheux MJ (2006) Antennal sensilla of male *Lophocorona pediasia* Common 1973 and their phylogenetic implications (Lepidoptera: Lophocoronidae). *Annales de la Société entomologique de France* 42(1): 113–118. doi: 10.1080/00379271.2006.10697456
- Faucheux MJ (2011) Antennal sensilla in adult males of five species of *Coleophora* sp. (Lepidoptera: Coleophoridae). Considerations on their structure and function. *Nota lepidopterologica* 34(1): 61–69.
- Frank DL, Leskey TC, Bergh JC (2010) Morphological characterization of antennal sensilla of the Dogwood Borer (Lepidoptera: Sesiidae). *Annals of the Entomological Society of America* 103(6): 993–1002. doi: 10.1603/AN09182
- Gao Y, Chen ZM, Sun XL (2013) Antennal sensilla of the tea weevil *Mylocerinus aurolineatus*. *Plant Protection* 39(3): 45–50.
- George JA, Nagy BAL (1984) Morphology, distribution, and ultrastructural differences of sensilla trichodea and basiconica on the antennae of the oriental fruit moth, *Grapholitha molesta*

- (Busck) (Lepidoptera: Tortricidae). International Journal of Insect Morphology and Embryology 13(2): 157–170. doi: 10.1016/0020-7322(84)90023-0
- Gómez VRC, Nieto G, Valdes J, Castrejón F, Rojas JC (2003) The antennal sensilla of *Zama-giria dixolophella* Dyar (Lepidoptera: Pyralidae). Annals of the Entomological Society of America 96(5): 672–678. doi: 10.1603/0013-8746(2003)096[0672:TASOZD]2.0.CO;2
- Gómez VRC, Carrasco JV (2008) Morphological characteristics of antennal sensilla in *Talponia batesi* (Lepidoptera: Tortricidae). Annals of the Entomological Society of America 101(1): 181–188. doi: 10.1603/0013-8746(2008)101[181:MCOASI]2.0.CO;2
- Hix RL, Johnson DT, Bernhardt JL (2003) Antennal sensory structures of *Lissorhoptrus oryzo-philus* (Coleoptera: Curculionidae) with notes on aquatic adaptations. The Coleopterists Bulletin 57(1): 85–94. doi: 10.1649/0010-065X(2003)057[0085:ASSOLO]2.0.CO;2
- Hallberg E, Hansson BS, Steinbrecht RA (1994) Morphological characteristics of antennal sensilla in the European cornborer *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Tissue and Cell 26(4): 489–502. doi: 10.1016/0040-8166(94)90002-7
- Hansson BS, Blackwell A, Hallberg E, Lofqvist J (1995) Physiological and morphological characteristics of the sex pheromone detecting system in male corn stemborers, *Chilo partellus* (Lepidoptera: Pyralidae). Journal of Insect Physiology 41(2): 171–178. doi: 10.1016/0022-1910(94)00086-V
- Jiang GF, He DC, Yan ZG (2000) Scanning electron microscopy observations of antennal sensilla of male *Teinopalpus aureus* Mell. Guangxi Science 7(2): 144–146, 149.
- Jan ML, Zhang LL, Mao RQ (2011) Studies on the antennal sensilla of *Chilades pandava* by scanning electron microscopy. Journal of South China Agricultural University 32(2): 52–54.
- Kaissling KE (1971) Insect olfaction. In: Beidler LM (Ed) Handbook of sensory physiology, vol IV: Chemical senses: olfaction. Springer-Verlag, Berlin, 351–431.
- Ma RY, Du JW (2000) Insect antennal sensilla. Entomological Knowledge 37(3): 179–183.
- Pophof B (1997) Olfactory responses recorded from sensilla coeloconica of the silkworm *Bombyx mori*. Physiological Entomology 22: 239–248. doi: 10.1111/j.1365-3032.1997.tb01164.x
- Razowski J, Wojtusiak J (2004) Tortricidae from Venezuela (Lepidoptera: Tortricidae). Genus 15(2): 257–266.
- Skiri HT, Stranden M, Sandoz JC, Menzel R, Mustaparta H (2005) Associative learning of plant odorants activating the same or different receptor neurones in the moth *Heliothis virescens*. The Journal of Experimental Biology 208: 787–796. doi: 10.1242/jeb.01431
- Smith KE, Wall R (1998) Suppression of the blowfly *Lucilia sericata* using odour-baited tri-flumuron-impregnated targets. Medical Veterinary Entomology 12(40): 430–437. doi: 10.1046/j.1365-2915.1998.00134.x
- Schneider D (1964) Insect antennae. Annual Review of Entomology 9: 103–122. doi: 10.1146/annurev.en.09.010164.000535
- Steinbrecht RA (1973) Der Feinbau olfaktorischer sensillen der Seidenspinners (Insecta, Lepidoptera). Rezeptorforsätze Apparat. Zeitschrift für Zellforschung und mikroskopische Anatomie 139: 533–565. doi: 10.1007/BF02028392
- Sukontason K, Sukontason KL, Piangjai S, Boonchu N, Chaiwong T, Nger-nklun R, Sripak-dee D, Vogtsberger RC, Olson JK (2004) Antennal sensilla of some forensically important

- flies in families Calliphoridae, Sarcophagidae and Muscidae. *Micron* 35(8): 671–679. doi: 10.1016/j.micron.2004.05.005
- Wang X, Xu J, Liu FY, Chen HB, Wu JX, Du YJ (2008) Ultrastructure of antennal sensilla of *Maruca testulalis* (Lepidoptera: Pyralidae) adult and its sensory responses to sex pheromone and plant volatiles. *Acta Entomologica Sinica* 51(12): 1225–1234.
- Wall C (1978) Morphology and histology of the antenna of *Cydia nigricana* (F.) (Lepidoptera: Tortricidae). *International Journal of Insect Morphology and Embryology* 7(3): 237–250. doi: 10.1016/0020-7322(78)90006-5
- Xu M, Wang M (2013) Scanning electron microscopy observation of antennal sensilla of *Heliophorus phoenicopyrphus* (Holland). <http://www.paper.edu.cn/releasepaper/content/201303-415>
- Zacharuk RY (1980) Ultrastructure and function of insect chemosensilla. *Annual Review of Entomology* 25: 27–47. doi: 10.1146/annurev.en.25.010180.000331

First record of *Eubroncus* (Hymenoptera, Mymaridae) from China, with description of three new species

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Abstract

The genus *Eubroncus* Yoshimoto, Kozlov & Trjapitzin is first recorded from China, and three species, *E. hani* **sp. n.**, *E. tibetanus* **sp. n.** and *E. vertexus* **sp. n.** are described as new. A key to the six described species is given, with photomicrographs to illustrate morphological characters.

Keywords

Chalcidoidea, Mymaridae, *Eubroncus*, taxonomy, new species, China

Introduction

Yoshimoto et al. (1972) established the new subfamily Eubroncinae, including two genera, *Eubroncus* and *Stomarostrum*, based on prominent mandibles that were almost as long as the head height. *Eubroncus* was erected for the type species *E. orientalis* Yoshimoto, Kozlov & Trjapitzin (male) from Malaysia, and *Stomarostrum* was erected for the type species *S. prodigiosum* Yoshimoto, Kozlov & Trjapitzin (female) from the Russian Far East. Triapitsyn and Huber (2000) synonymized *Stomarostrum* with

Eubroncus. Triapitsyn and Berezovskiy (2002) redescribed *E. prodigiosus* (Yoshimoto, Kozlov & Trjapitzin) and keyed males of the two known species based on color of mesosoma and whether the pronotum is divided or not. Viggiani (2003) described the male genitalial structure of *E. prodigiosus* from Japan. Lin et al. (2007) recorded an unidentified species from Australia. Hayat and Khan (2009) described the third species, *E. indicus* Hayat & Khan, from a single female from India. Here we report the genus from China and describe three new species.

Materials and methods

Specimens were collected from Xizang Province (Tibet) and Yunnan Province (Southwest China) using yellow pan traps.

Specimens were dissected and mounted dorsally or laterally in Canada balsam on slides following the method described by Noyes (1982) and modified for the Mymaridae by Huber (1988).

Photographs were taken with a digital CCD camera attached to an Olympus BX51 compound microscope, and most measurements were made from slide-mounted specimens using an eye-piece reticle. Total body length excluding ovipositor was measured mostly with an eye-piece reticle from alcohol-preserved specimens before being dissected, but sometimes from slide-mounted specimens (meso- and metasoma, without head). All measurements are given in micrometers (μm).

Morphological terminology and abbreviations are those of Gibson (1997) and Huber (2012), as follows (with some additions):

YPT	Yellow pan trap
OD	Mid ocellar diameter
POD	Post ocellar diameter
OOL	Ocular-ocellar length
OCL	Least post ocellus-occipital margin length
LOL	Least ocellar length
POL	Postocellar length
MOL	Least mid ocellus-occipital margin length
Fl_n	Flagellar segment
Mps	Multiporous plate sensilla
Gt_n	Gastral tergum
Gs_n	Gastral sternum

Specimens studied are deposited in the following institution:

NEFU Northeast Forestry University, Harbin, China.

Taxonomy

Genus *Eubroncus* Yoshimoto, Kozlov & Trjapitzin, 1972

<http://species-id.net/wiki/Eubroncus>

Eubroncus Yoshimoto, Kozlov & Trjapitzin, 1972: 879. Type species: *Eubroncus orientalis* Yoshimoto, Kozlov & Trjapitzin, 1972, by original designation.

Stomarostrum Yoshimoto, Kozlov & Trjapitzin, 1972: 879. Type species: *Stomarostrum prodigiosum* Yoshimoto, Kozlov & Trjapitzin, 1972, by original designation; synonymy by Triapitsyn and Huber 2000: 603.

Diagnosis. Head strongly angular (or subtriangular) in lateral view. Vertex (Figs 1, 10, 20, 28) with a pair of placoid sensilla in front of post ocelli. Mandibles (Figs 2, 11, 21) not crossing medially, extremely long and narrow, with strong apical teeth and rows of denticles on ventral margin. Female antenna with funicle 6-segmented and clava 1-segmented. Pedicel distinctly longer than fl₁, fl₁ without mps, fl₂–fl₆ and clava each with numerous mps. Hind wing (Figs 6, 15, 25, 30) relatively wide with broadly rounded apex, disc begins at wing's base, submarginal vein striped by alternating hyaline and infusate areas. Tarsi 4-segmented. Protibial spur (Figs 7, 16, 26) comb-like. Male antennal flagellum (Fig. 29) 11-segmented.

(Note: this diagnosis applies to examined species from China because authors have not examined any other material of this genus. Hosts of all *Eubroncus* are unknown)

Key to species of *Eubroncus* of the world

(Note: females are not known for *orientalis*; males are not known for *indicus*, *hani*, and *tibetanus*)

- 1 ♀: flagellum clavate, funicle 6-segmented and clava 1-segmented (Figs 3, 12, 22) **2**
- ♂: flagellum filiform, 11-segmented (Fig. 29) **6**
- 2 Pronotum entire, without longitudinal carina medially (Yoshimoto et al. 1972, Fig. 7) **3**
- Pronotum with a faint longitudinal carina medially (Figs 4, 13, 20) **4**
- 3 Scape about 3.0× as long as wide; Gt₁ with prominent carinae; eye circular ..
..... ***E. prodigiosus***
- Scape about 5.5× as long as wide; Gt₁ without prominent carinae; eye oval ..
..... ***E. indicus***
- 4 Hind wing without a group of long setae on disc behind the distal part of marginal vein (Fig. 25); petiole with short and blunt spine-like projections anterolaterally (Fig. 27); vertex entirely covered with conspicuous scale-like sculpture (Fig. 20); ovipositor 0.87–0.90× as long as mesotibia ***E. vertexus* sp. n.**
- Hind wing with a group of notably long setae on disc behind the distal part of marginal vein (Figs 6, 15); petiole with relatively long spine-like projec-

- tions anterolaterally (Figs 8, 19); vertex only partly covered with conspicuous sculpture or the sculpture inconspicuous; ovipositor 0.66–0.68× as long as mesotibia5
- 5 Vertex with conspicuous scale-like sculpture in anterior half, smooth in posterior half or nearly so (Fig. 10); propodeum with distinct reticulate sculpture medially (Fig. 13); scutellum with transverse row of fovea extending to lateral margins (Fig. 13) *E. tibetanus* sp. n.
- Both vertex and propodeum with fine reticulate sculpture (Fig. 1); scutellum with short transverse row of fovea medially (Fig. 4) *E. hani* sp. n.
- 6 Pronotum entire, without longitudinal carina medially *E. prodigiosus*
- Pronotum with a faint longitudinal carina medially 7
- 7 Forewing with a notch on basal third of posterior margin (Yoshimoto et al. 1972, Fig. 4) *E. orientalis*
- Forewing without a notch on basal third of posterior margin (Fig. 30)
..... *E. vertexus* sp. n.

***Eubroncus hani* Jin & Li, sp. n.**

<http://zoobank.org/0EE9C19F-A59C-4D9A-A984-F2A09F13A2FA>

http://species-id.net/wiki/Eubroncus_hani

Figs 1–9

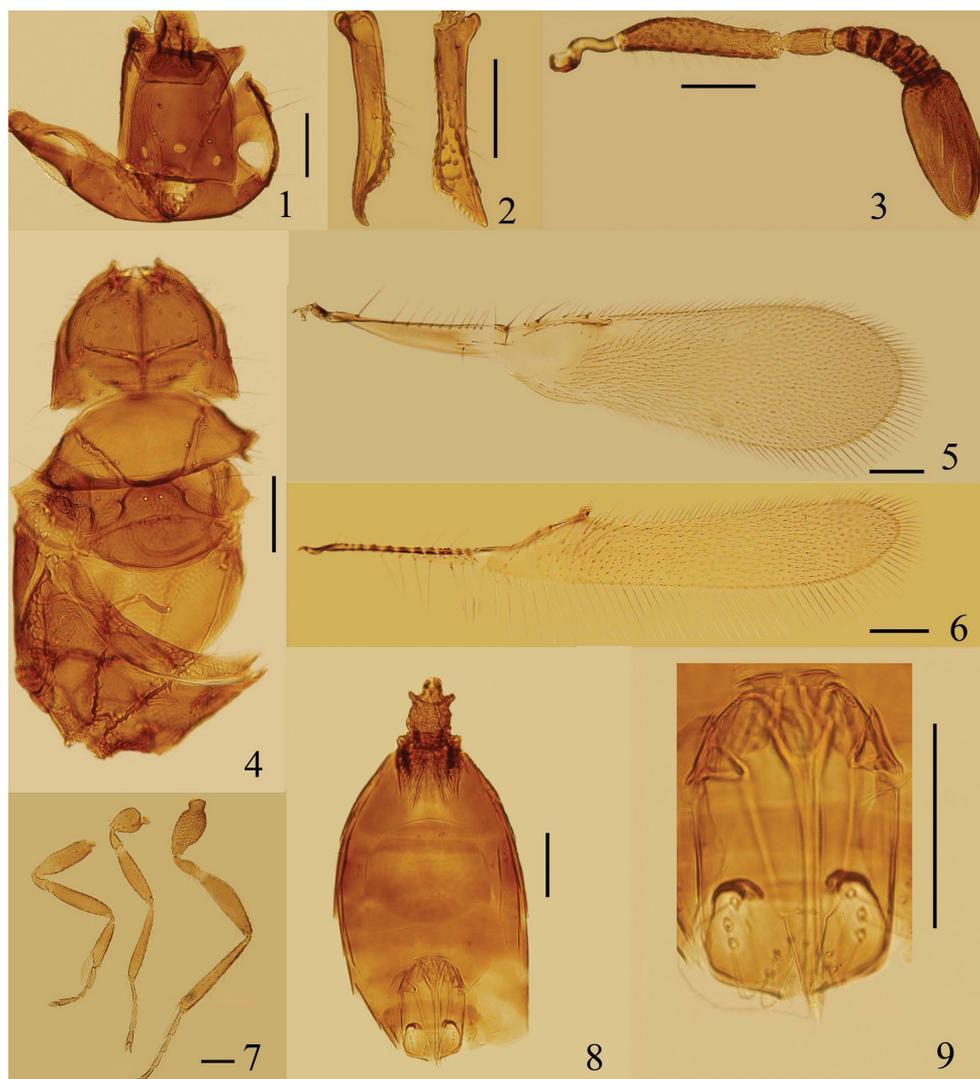
Holotype. ♀ China, Yunnan Province, Lijiang City, Mt. Yulongxueshan, 3000m, 8–9. VII. 2012, Xiang-Xiang Jin, Hui-Lin Han, Hui Geng, Chao Zhang (NEFU), YPT.

Paratypes. With same data as holotype (3♀, NEFU).

Diagnosis. Vertex with light reticulation; pronotum with a faint longitudinal carina medially; scutellum with short transverse row of fovea medially; hind wing with a group of notably long setae on disc behind the distal part of marginal vein; petiole with relatively long spine-like projections anterolaterally.

Description. Female. Body length 1.08–1.15 mm. Head black with ocelli and mandibles brown and eyes pinkish. Antenna dark brown with apical part of radicle brown and scape yellowish-brown. Mesosoma blackish with pronotum dark brown. Wings infuscate with base of forewing dark brown, largely less infuscate behind the marginal vein and apical part of submarginal vein. Legs brown except protibial spur, trochanters, basal three tarsal segments light brown. Metasoma brown with ovipositor light brown.

Head (Fig. 1) 1.11–1.15× as long as wide. Eye subtriangular, with relatively long setae, each seta longer than the diameter of a facet. Vertex 1.1–1.3× as long as wide, with light reticulate sculpture. Ocelli in an obtuse triangle; mid ocellus oval, MOL 1.2–1.8× as long as OD; post ocellus oval, OCL approximately as long as POD; POL about 4.7–5.1× as long as OOL. Mandibles (Fig. 2) 0.68–0.78× as long as head and 1.0–1.1× as long as vertex in dorsal view. Antenna (Fig. 3) with radicle 0.2–0.4× as long as scape; scape with light reticulate sculpture, 4.0–5.4× as long as wide; pedicel



Figures 1–9. *Eubroncus hani* sp. n., holotype female: **1** head, dorsal **2** mandibles **3** antenna **4** mesosoma, dorsal **5** forewing **6** hind wing **7** legs **8** metasoma, dorsal **9** ovipositor. Scale bars = 100 μ m.

1.6–2.0 \times as long as wide, 2.5–2.8 \times as long as fl_1 ; fl_2 – fl_6 each with 2 mps; clava a little shorter than scape, 2.2–2.5 \times as long as wide, with 6–8 mps.

Mesosoma (Fig. 4) 1.80–2.07 \times as long as wide. Pronotum 0.61–0.76 \times as long as wide, with a faint longitudinal carina medially, each lobe with about 24–33 setae dorsally. Mesoscutum 0.41–0.47 \times as long as wide, and 0.72–0.83 \times as long as pronotum. Scutellum 0.83–0.96 \times as long as mesoscutum, with short transverse row of fovea medially; distance between placoid sensilla 1.4–1.6 \times as long as their own diameter. Propodeum 0.85–1.00 \times as long as mesoscutum, 1.0–1.1 \times as long as scutellum, with light reticulate sculpture, with one pair of tooth-like projections posterolaterally and 2–3 fine setae.

Forewing (Fig. 5) 3.86–4.10× as long as wide, longest marginal setae about 0.25–0.34× as long as greatest wing width. Beneath or on the submarginal vein with 9–12 setae. Marginal vein with 8–11 setae along anterior margin. Hind wing (Fig. 6) 7.6–8.0× as long as wide, longest marginal setae about 0.86–0.93× as long as greatest wing width, with 1 long seta and 1 short seta on marginal vein, and a group of notably long setae on disc behind the distal part of marginal vein.

Petiole (Fig. 8) 1.22–1.45× as long as wide, with relatively long spine-like projections anteriolaterally. Gaster oblong, 0.90–1.05× as long as mesosoma; Gt_1 and Gs_1 with numerous prominent and sclerotized carinae; ovipositor (Fig. 9) not or only slightly exerted; about 0.66× as long as mesotibia (Fig. 7).

Measurements (length/width, mm): head 0.30–0.35/0.26–0.30, scape 0.180–0.220/0.048–0.054, pedicel 0.067–0.072/0.036–0.038, fl_1 0.024–0.031/0.031–0.036, fl_2 0.024–0.034/0.038–0.043, fl_3 0.024–0.031/0.036–0.043, fl_4 0.024–0.029/0.038–0.048, fl_5 0.024–0.034/0.036–0.048, fl_6 0.024–0.036/0.045–0.055, clava 0.192–0.211/0.084–0.091, forewing 1.00–1.20/0.24–0.29, longest marginal setae 0.063–0.082, hind wing 0.94–1.10/0.12–0.14, longest marginal setae 0.102–0.125, ovipositor 0.16–0.17.

Relative measurements. OD 16–18, OCL 16–18, OOL 16–18, POL 82–84, LOL 36, POD 14–16.

Male. Unknown.

Etymology. The species is named for Dr. Hui-Lin Han, Northeast Forestry University, China.

***Eubroncus tibetanus* Jin & Li, sp. n.**

<http://zoobank.org/CF35CCD0-1196-4672-A46B-7B805D804554>

http://species-id.net/wiki/Eubroncus_tibetanus

Figs 10–19

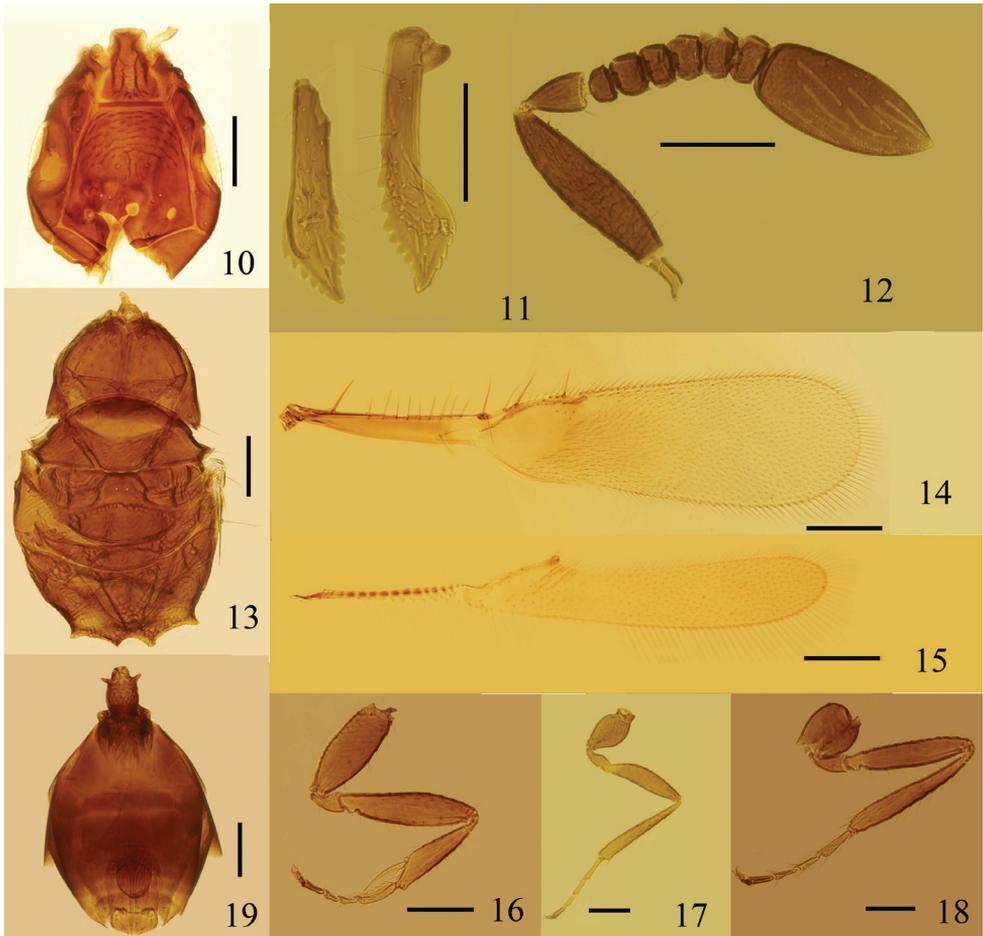
Holotype. ♀ China, Xizang (=Tibet), Linzhi County, Pailong Village, 2000m, 22–23. IX.2011, Hui-Lin Han (NEFU), YPT.

Paratypes. With same data as holotype (4♀♀, NEFU).

Diagnosis. Vertex with distinct scale-like sculpture in anterior half, smooth or almost smooth in posterior half; pronotum with a faint longitudinal carina medially; hind wing with a group of notably long setae on disc behind the distal part of marginal vein; petiole with relatively long spine-like projections anterolaterally.

Description. Female. Body length 1.00–1.32 mm. Head black with ocelli and mandibles brown and eyes pinkish. Antenna dark brown with radicle yellowish-brown. Mesosoma black. Wings infusate with base of forewing dark brown. Legs brown except protibial spur, trochanters, basal three tarsal segments light brown. Metasoma brown with apex fading to brown to yellow brown.

Head (Fig. 10) 1.1–1.2× as long as wide. Eye subtriangular, with relatively long setae, each seta distinctly longer than the diameter of a facet. Vertex 1.10–1.25× as



Figures 10–19. *Eubroncus tibetanus* sp. n., holotype female: **10** head, dorsal **11** mandibles **12** antenna **13** mesosoma, dorsal **14** forewing **15** hind wing **16** fore leg **17** middle leg **18** hind leg **19** metasoma, dorsal. Scale bars = 100 μ m.

long as wide, its posterior margin 1.6–2.0 \times as long as anterior margin, with conspicuous scale-like sculpture in anterior half, almost smooth in posterior half. Ocelli in an obtuse triangle; mid ocellus round, MOL approximately twice as long as OD; post ocellus oval, OCL about as long as POD; POL about 3.8–4.0 \times as long as OOL. Mandibles (Fig. 11) 0.7 \times as long as head and 1.0–1.1 \times as long as vertex in dorsal view. Antenna (Fig. 12) with radicle 0.25–0.30 \times as long as scape; scape 3.8–4.7 \times as long as wide; pedicel 1.6–1.8 \times as long as wide, 2.5–3.0 \times as long as fl_1 ; fl_2 – fl_6 each with 2 mps; clava slightly shorter than scape, 2.00–2.35 \times as long as wide, with 7 mps.

Mesosoma (Fig. 13) in dorsal view 1.6–1.8 \times as long as wide. Pronotum 0.6–0.8 \times as long as wide, with a faint longitudinal carina medially, each lobe with about 29–33 setae dorsally. Mesoscutum 0.7–0.8 \times as long as pronotum. Scutellum about as long as

mesoscutum, with transverse row of fovea extending to lateral margins; distance between placoid sensilla about 1.6–2.2× as long as their own diameter. Propodeum with strong reticulate sculpture medially, less conspicuous laterally, about as long as mesoscutum, with one pair of tooth-like projections posterolaterally and 2–3 fine setae.

Forewing (Fig. 14) 4.0–4.2× as long as wide, longest marginal setae about 0.20–0.25× as long as greatest wing width. Beneath or on the submarginal vein with 8–13 setae. Marginal vein with 8–10 setae along anterior margin. Hind wing (Fig. 15) 7.2–7.6× as long as wide, longest marginal setae about as long as greatest wing width, with 1 long seta and 1 short seta on marginal vein, and a group of notably long setae on disc behind the distal part of marginal vein.

Petiole (Fig. 19) about 1.5× as long as wide, with relatively long spine-like projections anterolaterally. Gaster oblong, 0.86–1.05× as long as mesosoma; Gt_1 and Gs_1 with numerous prominent and sclerotized carinae. Ovipositor not or only slightly exerted, about 0.68× as long as mesotibia (Fig. 17).

Measurements (length/width, mm): head 0.35–0.39/0.30–0.32, scape 0.192–0.214/0.043–0.058, pedicel 0.060–0.070/0.036–0.043, fl_1 0.024–0.026/0.034–0.043, fl_2 0.024–0.034/0.036–0.048, fl_3 0.024–0.034/0.041–0.053, fl_4 0.022–0.031/0.041–0.050, fl_5 0.022–0.036/0.041–0.050, fl_6 0.024–0.036/0.048–0.055, clava 0.192–0.197/0.079–0.094, forewing 0.98–1.00/0.23–0.25, longest marginal setae 0.049–0.061, hind wing 0.90–0.95/0.12–0.14, longest marginal setae 0.122–0.129, ovipositor 0.15.

Relative measurements. OD 16, OCL 15–18, OOL 20, POL 80, LOL 32–36, POD 16.

Male. Unknown.

Etymology. The specific name is derived from the name of the collection locality of the type species.

***Eubroncus vertexus* Jin & Li, sp. n.**

<http://zoobank.org/795F2E2F-1D12-4B09-BBD4-4E4697E0235F>

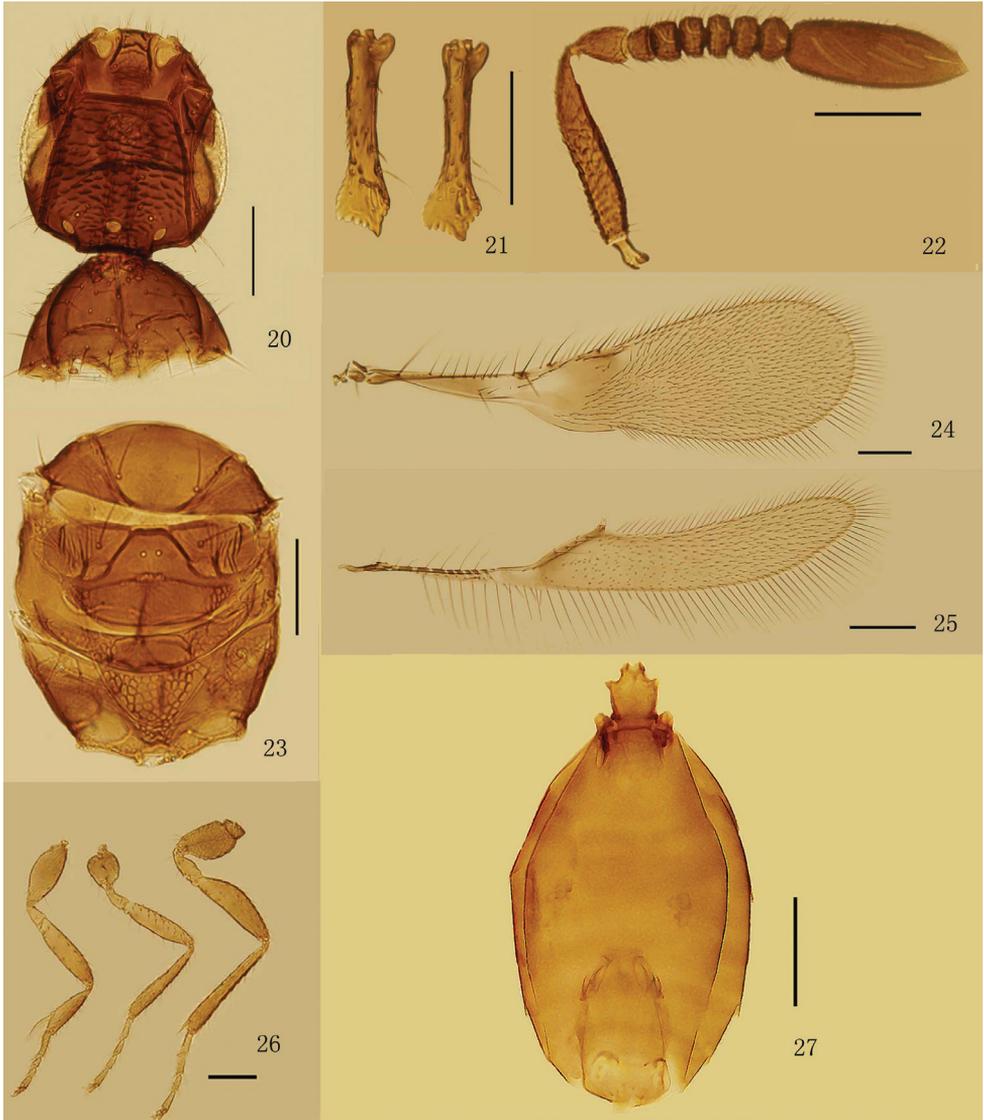
http://species-id.net/wiki/Eubroncus_vertexus

Figs 20–31

Holotype. ♀ China, Yunnan Province, Baoshan City, Tengchong County, Laifengshan National Forest Park, 16–19.VII. 2012, Xiang-Xiang Jin, Hui-Lin Han, Hui Geng, Chao Zhang (NEFU), YPT.

Paratypes. CHINA. Yunnan Province. Longchuan County, Zhangfeng Town, 26–27.IV.2013, Xiang-Xiang Jin, Hui-Lin Han, Guo-Hao Zu, Chao Zhang (3♀♀, NEFU), YPT; Lincang City, Yongde County, Yongkang Town, 23–24. IV.2013, Xiang-Xiang Jin, Hui-Lin Han, Guo-Hao Zu, Chao Zhang (1♀, 2♂♂, NEFU), YPT.

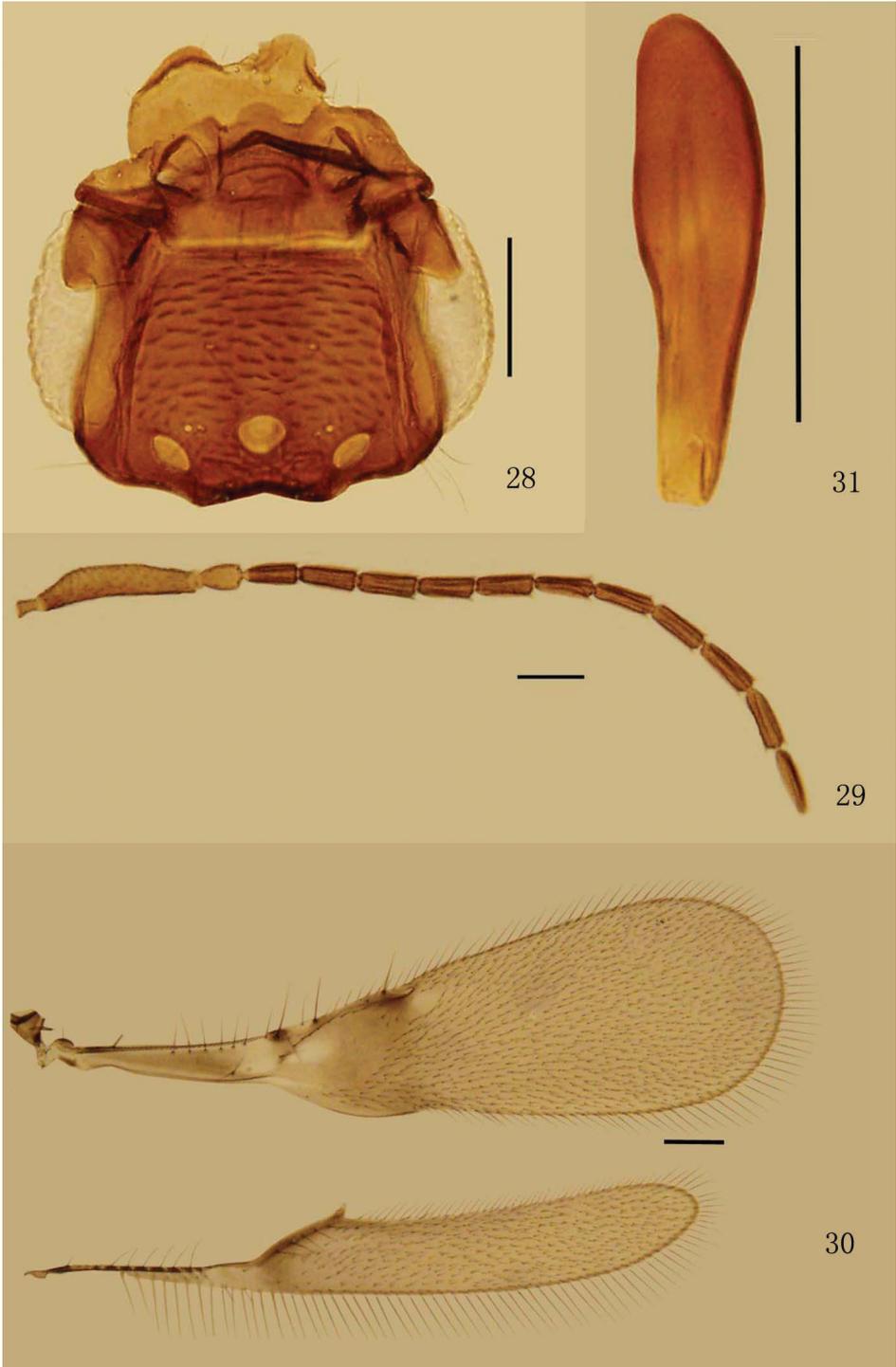
Diagnosis. Vertex entirely covered with conspicuous scale-like sculpture; pronotum with a faint longitudinal carina medially; propodeum with strong reticulate sculpture medially, less conspicuous laterally; hind wing with 3–6 long setae and 1 short



Figures 20–27. *Eubroncus vertexus* sp. n., holotype female: **20** head and pronotum, dorsal **21** mandibles **22** antenna **23** mesosoma (except pronotum) **24** forewing **25** hind wing **26** legs Paratype female: **27** metasoma. Scale bars = 100 μ m.

seta on marginal vein, disc uniformly setose; petiole with relatively short spine-like projections anterolaterally; ovipositor 0.87–0.90 \times as long as mesotibia.

Description. Female. Body length 0.9–1.1 mm. Head black with ocelli and mandibles brown and eyes pinkish. Antenna dark brown with radicle yellowish-brown. Mesosoma black with pronotum dark brown. Wings infuscate, with base of forewing under the venation dark brown, and two transparent spots, one on the behind the api-



Figures 28–31. *Eubroncus vertexus* sp. n., paratype male: **28** head, dorsal **29** antenna **30** wings **31** genitalia. Scale bars = 100 μ m.

cal part of submarginal vein and the other on base of marginal vein. Legs brown except protibial spur, trochanters, basal three tarsal segments light brown. Metasoma brown with ovipositor light brown.

Head (Fig. 20) 1.1× as long as wide. Eye subtriangular, 1.8–2.0× as long as wide, finely setose, each seta about as long as the diameter of a facet. Vertex about as long as wide, with conspicuous scale-like sculpture entirely, its posterior margin 1.6–1.7× as long as anterior margin. Ocelli in an obtuse triangle; mid ocellus oval, MOL shorter than OD; post ocellus oval, OCL approximately a little shorter than POD; POL about 3.4–4.0× as long as OOL. Mandible (Fig. 21) 0.6× as long as head and 0.8× as long as vertex in dorsal view. Antenna (Fig. 22) with radicle 0.15–0.25× as long as scape; scape 4.5–5.3× as long as wide; pedicel 1.4–1.7× as long as wide, and 2.4–2.7× as long as fl_1 ; fl_2 – fl_6 each with 2 mps; clava shorter than scape, 2.4–2.7× as long as wide, with 8 mps.

Mesosoma (Fig. 23) 1.5–2.0× as long as wide. Pronotum (Fig. 20) 0.5× as long as wide, with a faint longitudinal carina medially, each lobe with about 18–22 setae dorsally. Propleuron with reticulate sculpture. Prepectus with strong reticulate sculpture. Mesoscutum 0.8× as long as pronotum. Scutellum about 1.0–1.1× as long as mesoscutum, with short transverse row of fovea medially, distance between placoid sensilla 1.6× as long as their own diameter. Propodeum 0.83–0.97× as long as mesoscutum, and 0.86–0.93× as long as scutellum, with strong reticulate sculpture medially, less conspicuous laterally, with one pair of tooth-like projections posterolaterally and 2–4 fine setae.

Forewing (Fig. 24) 3.55–3.85× as long as wide, longest marginal setae about 0.25× as long as greatest wing width. Beneath or on the submarginal vein with 6–8 setae. Marginal vein with 6–8 setae along anterior margin. Hind wing (Fig. 25) 7.5–8.4× as long as wide, longest marginal setae about 0.8–1.2× as long as greatest wing width, with 3–6 long setae and 1 short seta on marginal vein; disc uniformly setose, without a group of long setae behind the distal part of marginal vein.

Petiole (Fig. 27) 1.25–1.45× as long as wide, with relatively short spine-like projections anterolaterally. Gaster oblong, 0.86–1.05× as long as mesosoma; Gt_1 and Gs_1 with numerous prominent and sclerotized carinae. Ovipositor not or only slightly exerted; 0.87–0.90× as long as mesotibia (Fig. 26).

Measurements (length/width, mm): head 0.20–0.30/0.20–0.26, scape 0.173–0.228/0.038–0.055, pedicel 0.046–0.060/0.034–0.036, fl_1 0.022–0.034/0.024–0.036, fl_2 0.022–0.034/0.034–0.043, fl_3 0.019–0.034/0.036–0.043, fl_4 0.019–0.024/0.038–0.043, fl_5 0.019–0.024/0.038–0.046, fl_6 0.019–0.026/0.038–0.048, clava 0.156–0.206/0.050–0.091, forewing 0.98–1.00/0.19–0.26, longest marginal setae 0.055–0.063, hind wing 0.67–0.93/0.12–0.14, longest marginal setae 0.087–0.098, ovipositor 0.14–0.19.

Relative measurements. OD 12–18, OCL 6–10, OOL 16–20, POL 64–74, LOL 28–30, POD 14–18.

Male. Body length 1.1 mm. Similar to female except for normal sexually dimorphic characters and the following. Head (Fig. 28) about 0.91× as long as wide. POL about 5.4× as long as OOL. Antenna (Fig. 29) with all the flagellar segments longer than wide, each with several mps. Distance between placoid sensilla 2.0–2.4× as long

as their own diameter. Forewing (Fig. 30) relatively wider than in female, 3.29–3.31× as long as wide. Hind wing (Fig. 30) 7.60–7.68× as long as wide. Genitalia (Fig. 31) simple, phallobase without parameres.

Measurements (length/width, mm): head 0.30/0.33, scape 0.228–0.235/0.048–0.058, pedicel 0.046–0.060/0.034–0.036, fl₁ 0.070–0.084/0.036–0.041, fl₂ 0.077–0.084/0.036–0.041, fl₃ 0.082–0.084/0.036–0.041, fl₄ 0.079–0.082/0.036–0.041, fl₅ 0.079–0.084/0.036–0.041, fl₆ 0.082–0.084/0.036–0.041, fl₇ 0.084–0.086/0.036–0.041, fl₈ 0.084/0.036–0.041, fl₉ 0.086/0.036–0.041, fl₁₀ 0.089–0.094/0.036–0.041, fl₁₁ 0.089–0.096/0.036–0.041, forewing 1.22–1.24/0.37–0.38, hind wing 1.10–1.17/0.14–0.15, genitalia 0.12–0.14.

Relative measurements. OD 24, OCL 6, OOL 14, POL 76, LOL 30, POD 26.

Etymology. The specific name refers to the vertex entirely covered with conspicuous scale-like sculpture.

Acknowledgements

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References

- Gibson GAP (1997) Morphology and terminology. In: Gibson GAP, Huber JT, Woolley JB (Eds) Annotated keys to the Genera of Nearctic Chalcidoidea (Hymenoptera). NRC Research Press, Ottawa, 16–44.
- Hayat M, Khan FR (2009) First record of *Eubroncus* from India (Hymenoptera: Chalcidoidea: Mymaridae), with description of a new species. *Journal of Threatened Taxa* 1(8): 439–440. doi: 10.11609/JOTT.o2079.439-40
- Huber JT (1988) The species groups of *Gonatocerus* Nees in North America with a revision of the *sulphuripes* and *ater* groups (Hymenoptera: Mymaridae). *Memoirs of the Entomological Society of Canada* 141: 1–109. doi: 10.4039/entm120141fv
- Huber JT (2012) Revision of *Ooctonus* (Hymenoptera: Mymaridae) in the Nearctic region. *Journal of the Entomological Society of Ontario* 143: 15–105.
- Lin NQ, Huber JT, La Salle J (2007) The Australian genera of Mymaridae (Hymenoptera: Chalcidoidea). *Zootaxa* 1596: 1–111.
- Noyes JS (1982) Collecting and preserving chalcid wasps (Hymenoptera: Chalcidoidea). *Journal of Natural History* 16: 315–334. doi: 10.1080/00222938200770261

- Triapitsyn SV, Berezovskiy VV (2002) Review of the Mymaridae (Hymenoptera, Chalcidoidea) of Primorskii Krai: genera *Anagroidea* Girault and *Eubroncus* Yoshimoto, Kozlov et Trjapitzin. Far Eastern Entomologist 114: 1–17.
- Triapitsyn SV, Huber JT (2000) Fam. Mymaridae-mymarids. In: Ler PA (Ed) Key to the insects of Russian Far East 4(4), Dal'nauka, Vladivostok, 603–614. [In Russian]
- Viggiani G (2003) A further contribution to the knowledge of the male genitalia in the Mymaridae (Hymenoptera: Chalcidoidea). Bollettino del Laboratorio di Entomologia Agraria Filippo Silvestri, Portici 59: 59–74.
- Yoshimoto CM, Kozlov MA, Trjapitzin VA (1972) A new subfamily of Mymaridae (Hymenoptera, Chalcidoidea, Mymaridae). Entomologicheskoe Obozrenie 51(4): 878–885. [In Russian] [English translation: Entomological Review 51: 521–525]

Species composition, richness, and distribution of marine bivalve molluscs in Bahía de Mazatlán, México

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Abstract

We describe the composition and distribution of bivalve molluscs from the sandy and rocky intertidal and the shallow subtidal environments of Bahía de Mazatlán, México. The bivalve fauna of the bay is represented by 89 living species in 28 families, including 37 new records and four range extensions: *Lithophaga hastasia*, *Adula soleniformis*, *Mactrellona subalata*, and *Strigilla ervilia*. The number of species increases from the upper (44) and lower intertidal (53) to the shallow subtidal (76), but only 11 (17%) have a wide distribution in the bay (i.e., found in all sampling sites and environments). The bivalve assemblages are composed of four main life forms: 27 epifaunal species, 26 infaunal, 16 semi-infaunal, and 20 endolithic. A taxonomic distinctness analysis identified the sampling sites and environments that contribute the most to the taxonomic diversity (species to suborder categories) of the bay. The present work increased significantly (31%) to 132 species previous inventories of bivalves of Bahía de Mazatlán. These species represent 34% of the bivalve diversity of the southern Golfo de California and approximately 15% of the Eastern Tropical Pacific region.

Keywords

Mollusca, Bivalves, Taxonomic distinctness, Bahía de Mazatlán, Mexican Pacific

Introduction

Studies on molluscs from Bahía de Mazatlán, located in the Mexican Pacific, have focused mainly on the conspicuous species of gastropods and bivalves from the rocky intertidal (Arreguín-Romero 1982, Sánchez-Vargas 1984, Olabarria et al. 2001, Camacho-Montoya et al. 2007, Vega et al. 2008, Rendón-Díaz 2010) and rocky-sandy subtidal environments (Parker 1963, Orozco-Romo 1980, Sánchez-Vargas 1984). Altogether these studies have reported 83 species of bivalves. However there has never been a complete inventory, as there are many inconspicuous infaunal, semi-infaunal, and endolithic forms which have been recorded elsewhere in the Mexican Pacific region (Keen 1971, Keen and Coan 1974, Hendrickx and Toledano-Granados 1994, Hendrickx and Brusca 2002, Ríos-Jara et al. 2008), but not collected yet in Bahía de Mazatlán. For example, Parker (1963) recorded 380 bivalve species only in the Golfo de California; Coan (1968) recorded 75 species in Bahía de los Ángeles, located in the northern portion of this gulf; Hendrickx et al. (2007) recorded up to 565 species; Keen (1971) listed 567 species for the Panamic Province.

According to Bouchet et al. (2002), mollusc species richness has been frequently underestimated in ecological studies mainly because of an inadequate coverage of the spatial heterogeneity and sampling effort. This is particularly important in the case of bivalves because they possess a wide variety of life forms and exploit a large number of habitats, which require specialized sampling techniques. Thus, special consideration should be given to the complexity of the environment and to sampling techniques in order to obtain a better understanding of the structure of the assemblages.

Many studies on marine biodiversity have used species accumulation curves (i.e., sample-based rarefactions) to evaluate the sampling effort; this technique indicates when a sufficiently large percentage of species has been observed with a definite number of samples with respect to a theoretical expected total number of species of a given community (Magurran 2004). The evaluation of sampling effort is particularly important in the case of molluscan assemblages, which often contain a large number of rare species, including unique (recorded in only one sample) (Bouchet et al. 2002) and duplicate species (recorded only in two samples). Therefore, the use of different estimators has been recommended when many unique and duplicate species are found in a large set of samples since they have complementary characteristics (Escalante-Espinoza 2003, Magurran 2004).

Marine biodiversity has been evaluated with the taxonomic distinctness approach (Warwick and Light 2002, Clarke and Gorley 2006), which integrates the species richness and all taxonomic categories of an assemblage of species. The average taxonomic distinctness measures the extent to which the species in a sample are taxonomically related. This is the average taxonomic distance between all pairs of species across a taxonomic tree. This analysis determines the extent to which certain taxa contribute to the total diversity of a certain environment or site using only the species presence-absence data, and it is insensitive to differences in sampling effort and sampling techniques used across different scales (Clarke and Warwick 1999). The assessment of

biodiversity at a regional scale is occasionally difficult, but the taxonomic distinctness parameter facilitates this measurement (Warwick and Light 2002). Furthermore, the average taxonomic distinctness indexes (Δ^+ and Λ^+) are an easy-to-use tool to measure biodiversity in the time and space scales (Warwick and Clarke 1998), as confirmed in studies on fish communities (Roger et al. 1999), macrobenthic communities (Mistri et al. 2000), marine nematodes (Clarke and Warwick 2001), assemblages of empty molluscan shells (Warwick and Light 2002, Smith 2008), freshwater organisms (Heino et al. 2005), and aquatic insects (Heino et al. 2008).

Bahía de Mazatlán is located in the southern portion of Golfo de California. The alternating warm and temperate seasons of this region create conditions that favor the development of a very diverse marine biota composed by species from both Golfo de California and the Mexican Tropical Pacific biogeographic subprovinces (Brown and Lomolino 1998). In this work we describe the taxonomic composition of the bivalve communities inhabiting the intertidal and shallow subtidal (depths 3–10 m) environments from four rocky and two sandy shores of Bahía de Mazatlán. Bivalve specimens were collected using various sampling techniques and during different seasons of the year to obtain a good representation of the epifaunal, infaunal, semi-infaunal, and endolithic species of the bay. Species accumulation curves were used to evaluate the sampling effort performed during the study period, and to predict the theoretical expected total number of bivalve species of these environments. We also provide a comparative analysis of previous inventories performed in the bay, the new records and the geographical range extensions. Finally, the taxonomic composition is analyzed using the average taxonomic distinctness index and its variation to evaluate the variability in the composition and distribution of the different taxonomic categories from the species to the subordinal level.

Material and methods

Study Area. Bahía de Mazatlán is located at the mouth of the Golfo de California (23°15'–23°11'N, 106°29'–106°25'W) (Figure 1). The bay has a total extent of approx. 3,500 hectares and a coastline of 13.5 km. There are three major islands (Venados, Pájaros, and Lobos), located approximately 1.5 km off the coast. These islands are protected as ecological reserves for migratory birds and marine animals and plants, and part of the “Islands of the Golfo de California Protection Area” (CONABIO 2012).

The bay belongs to the Cortesian Eco-Region included in the Warm-Temperate Northeast Pacific Province (Spalding et al. 2007). It is seasonally influenced by the California Current with cold water from the north, the North-Equatorial Counter-current with flow of warm tropical waters, and the temperate waters from Golfo de California itself (Wyrтки 1966, Zamudio et al. 2001, Alonso-Rodríguez 2004). The climate is tropical-subtropical with two very distinct seasons (Bell and Carballo 2008). The wet season occurs from July to October, and the dry season, with little

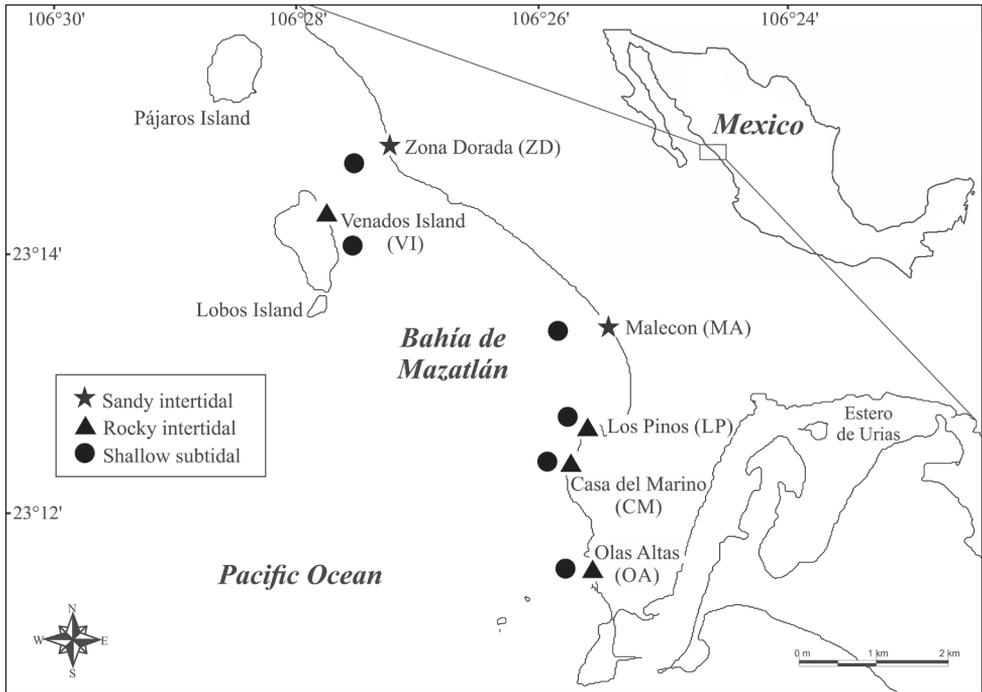


Figure 1. Study area and sampling sites at Bahía de Mazatlán, México.

or no rainfall, occurs from November to June (Montaño-Ley 1985). The sea surface temperature ranges from 13–21 °C in the dry season and from 28–31 °C in the wet season (Wilkinson et al. 2009).

Fieldwork. Six sampling sites protected or exposed to wave action conditions were established along Bahía de Mazatlán: four rocky beaches and two sandy beaches. Three environments were considered in each site: upper intertidal (UI), lower intertidal (LI), and shallow subtidal (SS) (3–10 m depth) adjacent to each beach. The intertidal zones were defined according to the natural zonation of benthic invertebrates (Peres 1982, Sánchez-Vargas 1984, Esqueda et al. 2000).

Some of the general characteristics of these beaches are: 1) **Olas Altas** (OA), a partially-protected beach with a well-developed rocky area of approx. 150 m long and smaller areas of medium-to-coarse sand; 2) **Los Pinos** (LP), a protected rocky beach approx. 100 m long, with some areas of medium-to-coarse sand; 3) **Casa del Marino** (CM), a semi-protected to exposed rocky beach approx. 250 m long mixed with small sandy areas of fine-to-medium size grains; 4) **Venados Island** (VI), the east side of the island has an extensive protected sandy beach approx. 850 m long and 30–60 m wide with medium-to-coarse sand; towards the northern part of the beach there is a rocky beach approx. 200 m long with many tidal pools and boulders; 5) **Malecon** (MA), an exposed and very dynamic sandy beach approx. 400 m long of medium-to-coarse

sand and pebbles. The beach runs along the urban sector of the city of Mazatlán and it is frequently visited by locals and tourists; and 6) **Zona Dorada** (ZD), a protected beach of medium-to-fine sand approx. 300 m long located in the tourist hub of the city just in front of hotels and extensively visited by tourists. The adjacent shallow subtidal environments of all these beaches include mixed substrates composed by coarse and fine sand, rocky reef areas, and many shell fragments. In Venados Island and Los Pinos small patches of live coral are also frequent.

Different sampling techniques (transect-quadrats, dredges, and direct searches) were applied during four expeditions in December 2008, and March, June, and August 2009. The transects (15 m long) were set parallel to the coastline, two in each environment (UI, LI, SS) of each beach. Two to four ($\bar{x} = 3$) quadrats (0.5 m²) were placed equidistant along each transect and all bivalves found in each quadrat identified *in situ* or collected for taxonomic identification in the laboratory. In the shallow subtidal, sampling was performed during SCUBA diving. The total sampling effort was 126 quadrats (63 m²) in the rocky intertidal and 52 quadrats (26 m²) in the sandy intertidal. A total of 90 quadrats (45 m²) were sampled in the shallow subtidal environments of all beaches. Additionally, in order to increase the inventory of bivalves, the specimens found in the areas immediately adjacent to the quadrats were also identified *in situ* or collected during direct searches in the intertidal and shallow subtidal environments. Dredges (24) were carried out in the shallow subtidal zone (8–15 m depth) of the six beaches, using a naturalist's dredge (mesh size = 2.5 cm, cod-end mesh size = 1.3 cm) (English et al. 1997) during 15 min. at an approximate speed of 2 knots.

Laboratory methods. A detailed examination of each sample was conducted to search for bivalves. Only living specimens were considered. Endolithic specimens (i.e., those growing within rocks or other hard substrates) were obtained by breaking rocks and shells, coral fragments, polychaete tubes, and rodoliths. Epifaunal specimens (i.e., species attached to a hard substrate) were obtained by scraping the surface of rocks. Semi-infaunal specimens (i.e., partially buried in the sediment but protruding above it) and infaunal specimens (< 4 mm) (i.e., those living buried in soft substrate) were obtained by screening the sandy sediment (Levinton 2001). A stereo microscope was used for examining soft and hard substrates in search of specimens < 10 mm and for taxonomic determination. The following references were used for the taxonomic identification of bivalves: Abbott (1974), Keen (1971), Morris (1980), and Coan and Valentich-Scott (2012). Previous inventories, additions, and taxonomic changes which include records from Bahía de Mazatlán were also reviewed (Parker 1963, Keen 1971, Orozco-Romo 1980, Arreguín-Romero 1982, Sánchez-Vargas 1984, Skoglund 2001, Olabarria et al. 2001, Hendrickx et al. 2005, Camacho-Montoya et al. 2007, Rendón-Díaz 2010, Coan and Valentich-Scott 2012).

The absolute frequency of every species in each environment and site was estimated by calculating the ratio between the number of sites where that species was recorded and the total number of sites. A reference collection was set up with all the

locality information in the Laboratory of Marine Ecosystems and Aquaculture at the Department of Ecology, University of Guadalajara, México. Voucher specimens were also deposited in this laboratory.

Analysis of the data. Only specimens recorded with the transect-quadrat method in the rocky intertidal and the adjacent shallow subtidal zones were used to evaluate sampling effort. Species accumulation curves were based on the cumulative number of species per quadrat. The expected richness was calculated using the nonparametric estimators Chao 2, Jackknife 1, and Jackknife 2. Plots were constructed with 10,000 non-replacement iterations based on samples for each site and environment, using the software EstimateS v8 (Colwell 2006).

A species presence-absence matrix was constructed using information from the records obtained from the transect-quadrats, dredges, and direct search techniques on the rocky beaches (Olas Altas, Los Pinos, Casa del Marino, and Venados Island). Six taxonomic levels (species, genus, family, superfamily, order, and superorder) were considered based on the classification schemes of Coan and Valentich-Scott (2012) (species to family) and Bouchet and Rocroi (2010) (superfamily to superorder). These taxa were weighted according to Warwick and Clarke (1995), as follows: w1, species within the same genus; w2, species within the same family but in different genera; w3, species within the same superfamily but in different family; w4, species within the same order but in a different superfamily; and so on. The average taxonomic distinctness Δ^+ and its variation Λ^+ were estimated for each site, environment and site-by-environment combination. Models were made with 95% confidence intervals, and the statistical significance of Δ^+ and Λ^+ were tested using 1,000 permutations in the program PRIMER v6 + PERMANOVA (Clarke and Gorley 2006).

Results

Species accumulation curves

The species accumulation curves show a trend towards an asymptote in all environments (Figure 2). The observed species representativeness with respect to the estimators Chao 2, Jackknife 1, and Jackknife 2 ranged between 64 and 80%, with Jackknife 1 always estimating the lowest expected richness, while Jackknife 2 always estimating the highest values. The species accumulation curves revealed a similar and more evident trend in the four sampling sites (Figure 3). The observed species representativeness ranged between 64 and 85% with respect to the estimators, with Jackknife 2 being the estimator that yielded the highest expected richness. Los Pinos and Casa del Marino possessed the highest species representativeness ($\geq 79\%$), while Olas Altas had the lowest value (68–75%). A large number of unique (12–17) and duplicate (6–9) species were obtained in the three environments and the four sites (Table 1).

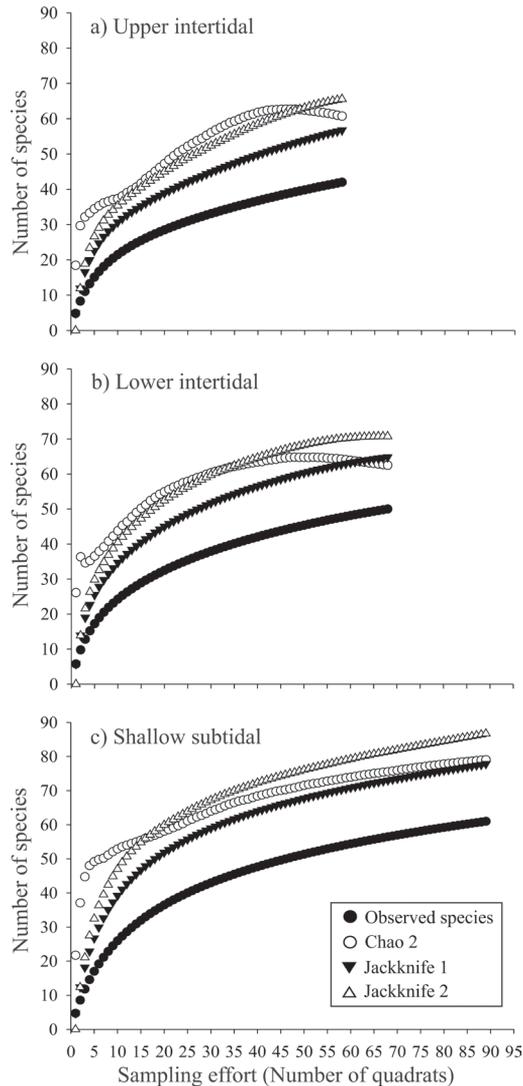


Figure 2. Observed and expected bivalves species accumulation curves, with nonparametric indices Chao 2, Jackknife 1, and Jackknife 2, in the three environments of Bahía de Mazatlán (**a-c**). Plots were constructed with 10,000 non-replacement iterations.

Richness, composition, and distribution of species

A total of 21,694 live bivalve specimens was recorded, representing 28 families, 55 genera, and 89 species (Table 2). The most diverse families were Mytilidae (14 species), Veneridae (10), and Arcidae (8). Ten families (35%) included only one species. The number of species increased from the upper (44) and lower intertidal (53) to the shallow subtidal (76). In addition, the numbers of unique species were 7 (upper), 4

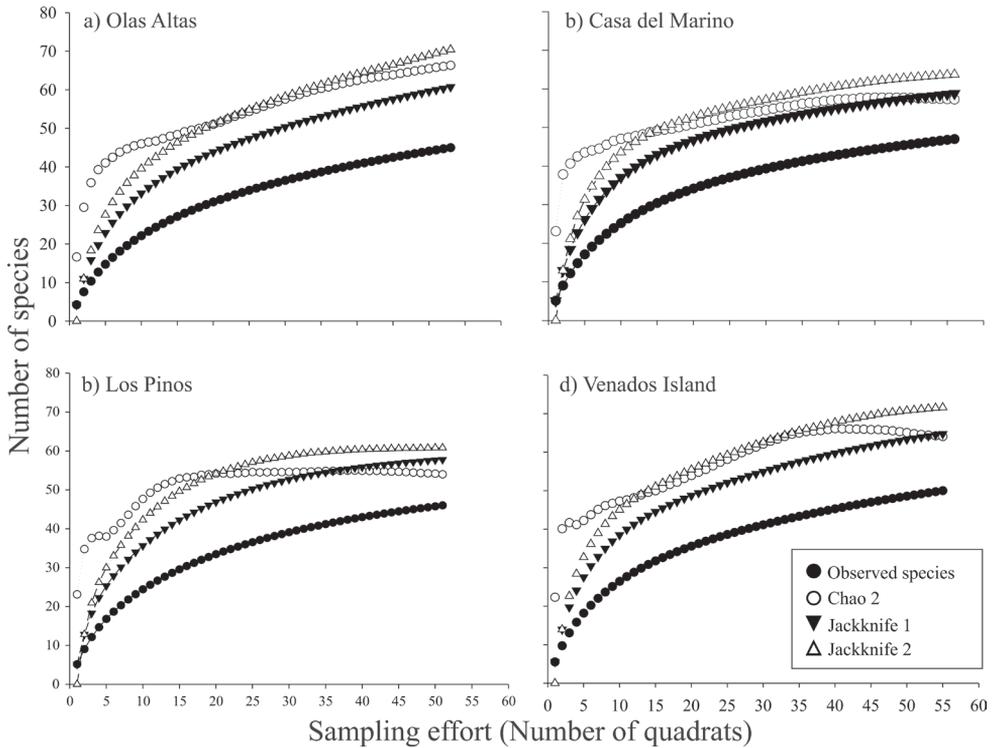


Figure 3. Observed and expected bivalves species accumulation curves, with nonparametric indices Chao 2, Jackknife 1, and Jackknife 2, of four sites of Bahía de Mazatlán (a–d). Plots were constructed with 10,000 non-replacement iterations.

Table 1. Rarity of species in four sites and three environments in Bahía de Mazatlán, México.

Rarity of species	Sites				Environments		
	Olas Altas	Los Pinos	Casa Marino	Venados Island	Upper intertidal	Lower intertidal	Shallow subtidal
Uniques	16	12	12	15	15	15	17
Duplicates	6	9	7	8	6	9	8

(lower), and 18 (subtidal). The species richness was similar in the adjacent shallow subtidal zone of all beaches (28–36 species) except for Venados Island (55 species), which had the highest number of species restricted to this island (7) (Table 3).

Several species of small size (5–10 mm) were recorded in rocky and sandy substrates: *Crassinella coxa*, *Crassinella nuculiformis*, *Liralucina approximata*, *Ctena mexicana*, *Kellia suborbicularis*, *Neolepton subtrigonum*, *Nutricola* cf. *humilis*, *Pitar* cf. *omissa*, *Sphenia fragilis*, *Chioneryx squamosa*, and *Transennella* cf. *puella*. Only three species were collected in the sandy intertidal environment: *Strigilla cicercula*, *Strigilla dichotoma*, and *Donax punctatostriatu*s.

Table 2. Systematic list of species and sampling method used in the different environments of Bahía de Mazatlán, México. Q = quadrat & transect, D = dredge, DS = direct search, I = infaunal, E = epifaunal, S = semi-infaunal, En = endolithic; * = geographical range extensions; ** = species in only one environment; + = new record for the bay.

Species	Environments			Life forms
	Upper intertidal	Lower intertidal	Shallow subtidal	
Mytilidae				
1. <i>Brachidontes adamsianus</i> (Dunker, 1857)	Q	Q	Q, DS	E
2. <i>Brachidontes semilaevis</i> (Menke, 1849)	Q	Q	Q	E
3. <i>Gregariella coarctata</i> (P. P. Carpenter, 1857)	Q	Q	Q, D, DS	En
4. <i>Lioberus salvadoricus</i> (Hertlein & Strong, 1946)	-	-	Q, DS	E
5. <i>Lithophaga (Diberus) plumula</i> (Hanley, 1843)+	Q	Q	Q, D, DS	En
6. <i>Lithophaga (Labis) attenuata</i> (Deshayes, 1836)	Q	Q	Q, DS	En
7. <i>Lithophaga (Myoforceps) aristata</i> (Dillwyn, 1817)	Q	Q	Q, D, DS	En
8. <i>Lithophaga (Rupiphaga) hastasia</i> Olsson, 1961 *, +	-	-	Q, DS	En
9. <i>Adula soleniformis</i> (Olsson, 1961)*, +	-	-	Q, D, DS	En
10. <i>Botula cylista</i> S. S. Berry, 1959	-	Q	Q, D, DS	En
11. <i>Leiosolenus spatiosus</i> P. P. Carpenter, 1857	-	Q	Q, D, DS	En
12. <i>Modiolus americanus</i> (Leach, 1815)	-	-	Q, D	E
13. <i>Modiolus capax</i> Conrad, 1837	-	Q	Q	E
14. <i>Septifer zeteki</i> Hertlein & Strong, 1946+	-	-	Q	E
Arcidae				
15. <i>Arca mutabilis</i> (G. B. Sowerby I, 1833)	Q	Q	D	E
16. <i>Arca pacifica</i> (G. B. Sowerby I, 1833)+	-	-	Q	E
17. <i>Acar bailyi</i> Bartsch, 1931	Q	-	-	E
18. <i>Acar gradata</i> Broderip & G. B. Sowerby I, 1829	Q	Q	Q, D, DS	E
19. <i>Acar rostrae</i> (S. S. Berry, 1954)	Q	Q	Q, D, DS	E
20. <i>Barbatia reeveana</i> (d'Orbigny, 1846)	-	Q	-	E
21. <i>Barbatia illota</i> (G. B. Sowerby I, 1833)+	-	-	D	E
22. <i>Anadara formosa</i> (G. B. Sowerby I, 1833)	-	-	D, DS	S
Noetiidae				
23. <i>Arcopsis solida</i> (G. B. Sowerby I, 1833)	Q	Q	Q, D, DS	E
Pteriidae				
24. <i>Pinctada mazatlanica</i> (Hanley, 1856)	Q	Q	Q, DS	E
Isognomonidae				
25. <i>Isognomon (Melina) janus</i> P. P. Carpenter, 1857	Q	Q	Q, D, DS	E
26. <i>Isognomon (Melina) recognitus</i> (Mabille, 1895)+	-	Q	-	E
Ostreidae				
27. <i>Ostrea conchaphila</i> P. P. Carpenter, 1857	Q	Q	Q, DS	E
28. <i>Saccostrea palmula</i> (P. P. Carpenter, 1857)	Q	Q	Q, D, DS	E
29. <i>Striostrea prismatica</i> (J. E. Gray, 1825)	Q	Q	Q, D, DS	E
Plicatulidae				
30. <i>Plicatula penicillata</i> P. P. Carpenter, 1857	Q	-	Q, D, DS	E
31. <i>Plicatulostrea anomioides</i> (Keen, 1958)	Q	Q	Q, DS	E
Limidae				
32. <i>Limaria pacifica</i> (d'Orbigny, 1846)	-	Q	Q, DS	E

Species	Environments			Life forms
	Upper intertidal	Lower intertidal	Shallow subtidal	
Lucinidae				
33. <i>Liralucina approximata</i> (Dall, 1901)	-	Q	-	S
34. <i>Ctena mexicana</i> (Dall, 1901)+	Q	Q	Q, D	S
Carditidae				
35. <i>Carditamera affinis</i> (G. B. Sowerby I, 1833)	Q	Q	Q, D, DS	I
36. <i>Cardites laticostatus</i> (G. B. Sowerby I, 1833)	Q	Q	Q, DS	I
Crassatellidae				
37. <i>Crassinella coxa</i> Olsson, 1964+	-	-	Q	S
38. <i>Crassinella ecuadoriana</i> Olsson, 1961	-	Q	Q	S
39. <i>Crassinella nuculiformis</i> S. S. Berry, 1940+	-	Q	Q	S
40. <i>Crassinella</i> aff. <i>pacifica</i> (C. B. Adams, 1852)+	Q	Q	Q	S
Cardiidae				
41. <i>Laevicardium substriatum</i> (Conrad, 1837)+	Q	-	-	I
Chamidae				
42. <i>Chama buddiana</i> C. B. Adams, 1852	Q	Q	Q, D, DS	E
43. <i>Chama coralloides</i> Reeve, 1846+	Q	Q	Q, D, DS	E
44. <i>Chama sordida</i> Broderip, 1835	Q	Q	Q, DS	E
45. <i>Chama</i> cf. <i>frondosa</i> Broderip, 1835	-	-	D	E
Lasaeidae				
46. <i>Kellia suborbicularis</i> (Montagu, 1803)+	-	Q	Q, D, DS	En
Mactridae				
47. <i>Mactrellona subalata</i> (Mörch, 1860) *, +	-	-	D	I
48. <i>Mulinia pallida</i> (Broderip & G. B. Sowerby I, 1829)+	-	-	D	I
Tellinidae				
49. <i>Strigilla</i> (<i>Strigilla</i>) <i>cicercula</i> (R. A. Philippi, 1846)	-	Q	Q, D, DS	I
50. <i>Strigilla</i> (<i>Strigilla</i>) <i>dichotoma</i> (R. A. Philippi, 1846)	Q	Q	Q	I
51. <i>Strigilla</i> (<i>Strigilla</i>) <i>ervilia</i> (R. A. Philippi, 1846) *, +	Q	-	-	I
52. <i>Tellina</i> (<i>Laciolina</i>) <i>ochracea</i> P. P. Carpenter, 1864+	-	-	Q	I
53. <i>Tellina</i> (<i>Moerella</i>) <i>coani</i> Keen, 1971+	-	Q	Q, D	I
54. <i>Tellina</i> (<i>Moerella</i>) <i>felix</i> Hanley, 1844	-	Q	D	I
Donacidae				
55. <i>Donax</i> (<i>Chion</i>) <i>punctatostriatus</i> Hanley, 1843+	Q	Q	-	I
56. <i>Donax</i> (<i>Paradonax</i>) <i>gracilis</i> Hanley, 1845	-	Q	D	I
Semelidae				
57. <i>Cumingia lamellosa</i> G. B. Sowerby I, 1833	Q	Q	-	I
58. <i>Semele</i> (<i>Semele</i>) cf. <i>bicolor</i> (C. B. Adams, 1852)	Q	-	-	I
59. <i>Semele</i> (<i>Semele</i>) <i>californica</i> (Reeve, 1853)+	-	-	Q	I
60. <i>Semele</i> (<i>Semele</i>) <i>flavescens</i> (A. A. Gould 1851)+	-	Q	-	I
61. <i>Semele jovis</i> (Reeve 1853)+	Q	-	-	I
62. <i>Semele hanleyi</i> Angas, 1879+	Q	Q	Q	I
Ungulinidae				
63. <i>Diplodonta orbella</i> (A. A. Gould, 1851)+	Q	-	Q, D, DS	En
64. <i>Diplodonta</i> (<i>Pegmapex</i>) <i>caelata</i> (Reeve, 1850)	-	Q	Q, D, DS	En
65. <i>Diplodonta</i> (<i>Timothyus</i>) <i>inezensis</i> (Hertlein & Strong, 1947)+	-	-	Q	En

Species	Environments			Life forms
	Upper intertidal	Lower intertidal	Shallow subtidal	
Veneridae				
66. <i>Chione subimbricata</i> (G. B. Sowerby I, 1835)	Q	Q	Q, D	S
67. <i>Chione undatella</i> (G. B. Sowerby I, 1835)+	-	-	Q	S
68. <i>Chioneryx squamosa</i> (P. P. Carpenter, 1857)+	Q	Q	Q	S
69. <i>Paphonotia elliptica</i> (G. B. Sowerby, 1834)	Q	-	-	S
70. <i>Periglypta multicostata</i> (G. B. Sowerby, 1835)+	Q	-	-	S
71. <i>Megapitaria squalida</i> (G. B. Sowerby, 1835)	-	-	Q	I
72. <i>Nutricula</i> cf. <i>humilis</i> (P. P. Carpenter, 1857)	-	-	D	S
73. <i>Pitar</i> cf. <i>omissa</i> (Pilsbry & Lowe, 1932)	-	-	Q	I
74. <i>Transennella modesta</i> (G. B. Sowerby, 1835)+	-	-	Q	S
75. <i>Transennella</i> cf. <i>puella</i> (P. P. Carpenter, 1864)	-	-	Q, D	S
Neoleptonidae				
76. <i>Neolepton</i> (<i>Neolepton</i>) <i>subtrigonum</i> (P. P. Carpenter, 1857)	-	Q	Q, D, DS	S
Myidae				
77. <i>Sphenia fragilis</i> (H. & A. Adams 1854)+	Q	Q	Q, D, DS	En
Corbulidae				
78. <i>Caryocorbula binadiata</i> (G. B. Sowerby I, 1833)	Q	Q	Q	I
79. <i>Caryocorbula marmorata</i> (Hinds, 1843)+	Q	Q	Q, DS	I
80. <i>Caryocorbula nasuta</i> G. B. Sowerby I, 1833	Q	-	Q, D	I
81. <i>Juliacorbula bicarinata</i> G. B. Sowerby I, 1833	Q	Q	Q	I
Petricolidae				
82. <i>Choristodon robustus</i> (G. B. Sowerby I, 1834)+	-	-	D	En
83. <i>Petricola</i> (<i>Petricola</i>) <i>linguafelis</i> (P. P. Carpenter, 1857)+	-	Q	Q, D, DS	En
84. <i>Petricola</i> (<i>Petricolirus</i>) <i>californiensis</i> Pilsbry & Lowe, 1932+	-	-	DS	En
Phadidae				
85. <i>Parapholus calva</i> (G. B. Sowerby I, 1834)+	-	-	Q	En
86. <i>Pholadidea</i> (<i>Hatasia</i>) <i>melanura</i> (G. B. Sowerby I, 1834)	-	-	D	En
Hiatellidae				
87. <i>Hiatella arctica</i> (Linnaeus, 1767)	Q	Q	Q, D, DS	En
Gastrochaenidae				
88. <i>Lamychaena truncata</i> (G. B. Sowerby I, 1834)	-	-	Q, D	En
Lyonsidae				
89. <i>Entodesma brevifrons</i> (G. B. Sowerby I, 1834)+	-	Q	Q, D, DS	I
Total sampling species richness (**)	44 (7)	53 (4)	76 Q = 64 (10) D = 42 (7) DS = 38 (1)	-
Total infaunal species richness		-		26
Total semi-infaunal species richness		-		16
Total endolithic species richness		-		20
Total epifaunal species richness		-		27

More species were recorded with the quadrat-transect technique in the shallow subtidal (64 species) than with either dredges (42 species) or direct searches (38 species). However, the species composition was different since some were collected only with quadrat-transects (10 species), dredges (7) or direct searches (1). The life-forms recorded included epifaunal (27), infaunal (26 species), endolithic (20), and semi-infaunal (16). Endolithic species were found in various hard substrates such as sedimentary rocks, corals, polychaete tubes, bivalve shells, and rodoliths. The rock-drilling bivalve *Parapholas calva* was found only in sedimentary rocks; all other species were found in two or more types of hard substrates.

Compared to previous studies in the region, this study includes 37 new records for Bahía de Mazatlán (Table 1), and geographic range extensions for four species: *Lithophaga hastasia*, *Adula soleniformis*, *Mactrellona subalata*, and *Strigilla ervilia*.

Twelve (13.5%) of the 89 species recorded were widely distributed in the bay (e.g., found in six sites), and 11 were recorded at three of the environments (UI, LI, and SS): *Acar rostrae*, *Carditamera affinis*, *Gregariella coarctata*, *Hiatella arctica*, *Lithophaga aristata*, *Lithophaga plumula*, *Leiosolenus spatiosus*, *Ostrea conchaphila*, *Saccostrea palmula*, *Striostrea prismatica*, and *Sphenia fragilis*. However, a large number of species (27) were unique to one environment and sampling site. Only nine species were recorded in the three environments and in five or six sampling sites: *Acar rostrae*, *Arcopsis solida*, *Brachidontes adamsianus*, *Carditamera affinis*, *Isognomon janus*, *Ostrea conchaphila*, *Plicatulostrea anomioides*, *Saccostrea palmula*, and *Striostrea prismatica* (Table 3).

There are several distribution patterns revealed by the life forms recorded in the different environments of Bahía de Mazatlán. Epifaunal bivalves were more frequent in the upper and lower rocky intertidal (12–17 species), followed by the endolithic species (1–8), whereas the number of infaunal species was very similar in all six sites (4–5), except for Venados Island. Similarly, epifaunal species dominated the shallow subtidal and intertidal zones (10–22), followed by the endolithic (7–14), infaunal (3–8) and semi-infaunal species (2–10) (Table 3).

Taxonomic distinctness

The average taxonomic distinctness analysis revealed complementary information on the bivalve assemblages recorded in the sampling sites and in the environments. The values of Δ^+ for Los Pinos and Olas Altas fell within the probability funnel (e.g. within the confidence intervals of 95%, $p > 0.05$), indicating a greater contribution to the mean taxonomic diversity of Bahía Mazatlan. However, values of Δ^+ fell within the probability funnel for the four sites, suggesting that these are significantly representative of the bay's bivalve assemblage (Figures 4a–b). On the other hand, the values of Δ^+ for the SS zone and the values of Δ^+ of the UI and SS fell within the confidence funnel, close to the bay's mean taxonomic inventory (Figures 4c–d). Finally, in the case of the site-by-environment analysis, most sites fell within the Δ^+ probability funnel ($p > 0.05$), except for Olas Altas upper intertidal zone and Los Pinos and Venados Island lower

Table 3. Distribution of bivalve species at six sites in the Bahía de Mazatlán, México. Sites: OA = Olas Altas, LP = Los Pinos, CM = Casa del Marino, VI = Venados Island, MA = Malecon, ZD = Zona Dorada. Environments: UI = upper intertidal, LI = lower intertidal, SS = shallow subtidal, * = species recorded in only one site or environment. AF = absolute frequency, by sites and environment (for each environment, the number of sites where there was a species / total sites).

Species	Sites and Environments																																									
	OA						LP						CM						VI						ZD						MA						AF					
	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS									
<i>Acar bailyi</i>	X			X						X			X																		0.5	-	-									
<i>Acar gradata</i>	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.5	0.7	0.7									
<i>Acar rostrae</i>	X	X	X	X	X		X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	0.7	0.7	1.0									
<i>Adula soleniformis</i>																									X						-	-	0.3									
<i>Anadara formosa</i>																									X						-	-	0.3									
<i>Arca mutabilis</i>				X	X		X	X					X	X											X	X	X	X	X	X	0.3	0.5	0.2									
<i>Arca pacifica*</i>						X																									-	-	0.2									
<i>Arcopsis solida</i>	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X							0.7	0.7	0.7									
<i>Barbatia illota*</i>																									X						-	-	0.2									
<i>Barbatia reeveana*</i>																			X												-	0.2	-									
<i>Botula cylindrica</i>		X				X						X						X													-	0.2	0.8									
<i>Brachidontes adamsianus</i>	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.7	0.7	0.7									
<i>Brachidontes semilaevis</i>	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X							0.7	0.7	0.5									
<i>Cardiumera affinis</i>	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.7	0.7	1.0									
<i>Cardites laticostatus</i>	X	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X	X	X	X	X	0.3	0.5	0.5									
<i>Caryocorbula binadiata</i>	X	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X								0.5	0.5	0.2									
<i>Caryocorbula marmorata</i>																															0.2	0.3	0.5									
<i>Caryocorbula nasuta</i>			X																						X	X	X	X	X	X	0.2	-	0.5									
<i>Chama buddiana</i>		X	X	X	X		X	X	X	X	X		X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	0.3	0.7	0.8									
<i>Chama cf. frondosa*</i>																															-	-	0.2									
<i>Chama comilloides</i>	X	X	X	X	X		X	X	X	X	X		X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	0.5	0.5	0.8									
<i>Chama sordida</i>	X	X		X	X		X	X		X	X		X	X		X	X		X	X	X	X	X		X	X	X	X	X	X	0.5	0.3	0.5									
<i>Chione subimbricata</i>	X	X		X	X		X	X		X	X		X	X		X	X		X	X	X	X	X		X	X	X	X	X	X	0.5	0.3	0.5									
<i>Chione undatella*</i>																															-	-	0.2									
<i>Chioneryx squamosa</i>	X					X						X						X													0.2	0.2	0.3									

Species	Sites and Environments																																									
	OA						LP						CM						VI						ZD						MA						AF					
	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS						
<i>Choristodon robustus*</i>																																										
<i>Crassinella</i> aff. <i>pacifica</i>																			X																							
<i>Crassinella coxa*</i>															X																											
<i>Crassinella ecuadoriana</i>															X																											
<i>Crassinella nuculiformis</i>																																										
<i>Ctena mexicana</i>													X																		X	0.2	0.2	0.5								
<i>Cunningia lamellosa</i>							X	X																							0.2	0.2	-									
<i>Diplodonta caelata</i>							X	X					X	X											X						X	-	0.3	0.8								
<i>Diplodonta inezensis</i>													X	X																												
<i>Diplodonta orbella</i>	X												X																													
<i>Donax gracilis</i>																															X	-	0.2	0.2								
<i>Donax punctatostriatus</i>																									X	X																
<i>Entodesma brevifrons</i>																															X	-	0.2	0.5								
<i>Gregariella coarctata</i>	X	X													X										X	X					X	0.2	0.3	0.8								
<i>Hiatella arcica</i>	X	X	X				X	X							X	X															X	0.2	0.5	1.0								
<i>Isoptomon janus</i>	X	X	X				X	X					X	X											X	X					X	0.7	0.7	0.8								
<i>Isoptomon recognitus*</i>															X																											
<i>Juliacorbula bicarinata</i>	X	X											X	X																	X	0.5	0.3	0.2								
<i>Kellia suborbicularis</i>															X																											
<i>Laevicardium substriatum*</i>	X																																									
<i>Lamycheuena truncata</i>															X																X	-	-	0.7								
<i>Leiosolenus spatiosus</i>							X	X							X																X	-	0.5	1.0								
<i>Limaria pacifica</i>																																										
<i>Lioberus salvadoricus</i>																															X	-	-	0.3								
<i>Liraducina approximata*</i>																															X	-	-	0.2								
<i>Lithophaga aristata</i>	X	X	X												X	X															X	0.5	0.5	1.0								
<i>Lithophaga attenuata</i>	X	X	X												X																X	0.5	0.3	0.8								
<i>Lithophaga hastasia*</i>																																										
<i>Lithophaga plumula</i>							X	X																							X	0.2	0.3	1.0								

Species	Sites and Environments																																									
	OA						LP						CM						VI						ZD						MA						AF					
	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS	UI	LI	SS						
<i>Striostrea prismatica</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
<i>Tellina coani</i>							X	X	X																																	
<i>Tellina felix</i>																																										
<i>Tellina ochracea*</i>																																										
<i>Transennella cf. puella*</i>						X																																				
<i>Transennella modesta*</i>																																										
Exclusive species	3	-	4	2	-	1	1	1	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1							
Total (environment by site)	30	28	28	25	32	34	22	22	22	36	17	33	53	2	3	30	1	1	33																							
Total epifaunal species richness	17	15	10	16	17	18	15	16	15	15	12	17	22	-	-	10	-	-	11																							
Total endolithic species richness	6	8	7	3	8	10	1	1	1	13	2	7	14	-	-	14	-	-	13																							
Total semi-infaunal species richness	3	1	3	2	2	3	1	1	1	4	2	6	10	-	-	3	-	-	2																							
Total infaunal species richness	4	4	8	4	5	3	5	4	4	4	1	3	7	2	3	3	1	1	7																							
Total species richness by sites	48			47			49				55		32				34																									

intertidal zone (Figure 4e). Also the Λ^+ values of all sites and environments fell within the funnel, i.e., all sites contribute significantly to the bay's total taxonomic diversity (Figure 4f; Table 4).

Discussion

The present work increased significantly (31%) the inventory of bivalve species of Bahía de Mazatlán to an updated total number of 132 species, including 37 new records (Table 5). According to these figures, the bay contributes 34% to the bivalve diversity of the southern Golfo de California (390 species) (Hendrickx et al. 2007), and approximately 15% to the Eastern Tropical Pacific region (890 species) (Coan and Valentich-Scott 2012). The intensive sampling strategy applied during this survey contributed considerably to the thoroughness of the inventory. In the subtidal zone, the Van Veen dredge and trawl net that had been used in previous studies failed to collect many epifaunal and endolithic life forms that we obtained during SCUBA diving in all six sites of the bay. Also, the number of species previously recorded in the intertidal zone (9–19) by using a single sampling technique, either quadrats, quadrat-transects, transects, or direct searches (Arreguín-Romero 1982, Sanchez-Vargas 1984, Camacho-Montoya et al. 2007, Vega et al. 2008, Rendón-Díaz 2010), was increased to 63 by using a combination of techniques during the four sampling expeditions throughout the year. The distribution patterns of bivalves in the two main environments of the bay, the intertidal and shallow intertidal, are also extended with the records of eight species previously known only from the subtidal zone and recorded for the first time in the intertidal zone of the bay: *Caryocorbula biradiata*, *C. nasuta*, *Donax gracilis*, *Gregariella coarctata*, *Strigilla cicercula*, *S. dichotoma*, and *Parvilucina aproximata* (Orozco-Romo 1980, Sánchez-Vargas 1984); and the records of 16 bivalve species in the shallow subtidal, previously reported only in the intertidal zone (Arreguín-Romero 1982, Sánchez-Vargas 1984, Olabarría et al. 2001, Camacho-Montoya et al. 2007, Rendón-Díaz 2010).

Our surveys yielded a substantial increase in the number of infaunal (29%) and endolithic (23%) species of bivalves; most of them (67%) not recorded previously in the bay. This is particularly important since frequently the species richness of molluscs has been underestimated in ecological investigations due to two main factors that, alone or combined, contribute to incomplete inventories (Bouchet et al. 2002). The first factor is inadequate coverage of the spatial heterogeneity, due to inappropriate sampling techniques. These limitations result in missing specialized species that live in a limited or specific area or habitat. The second factor is the overvaluation of macromolluscs (i.e., collection only of conspicuous species): studies that include species inventories tend to focus on large species (≥ 10 mm) and ignore the small ones. One reason for excluding molluscs less than 10 mm in size is the difficulty for taxonomic determination. Another factor that contributes to the exclusion of small molluscs from ecological studies is the failure of a detailed review of the sediments where bivalves in

this size range are common. Therefore, it was important to address these factors for a more complete inventory of bivalves.

We also report range extension of four species previously known in other regions of the Eastern Pacific coast: *Lithophaga hastasia* (from Bahía de Banderas, Jalisco, to Perú); *Strigilla ervilia* (from Bahía de Tenacatita, Jalisco, to Salinas, Ecuador); *Mactrel-lona subalata* (from La Peñita, Nayarit to Tumbes, Perú); and *Adula soleniformis* (El Lagartillo, Los Santos, Panamá to Paita, Perú) (Coan and Valentich-Scott 2012).

A total of 83 additional species were collected during field work; these are not reported here because they were not living specimens however they were identified from complete and well preserved shells. Interestingly, most of these species (64) have not been recorded previously in the bay, thus raising the total inventory (living specimens plus empty shells) to 196 species. Many ecological investigations include the species recorded from empty mollusc shells assuming that they are components of the regional community (i.e., Warwick and Light 2002, Smith 2008). However, at the local scale (i.e., sites, environments), most authors exclude them, arguing that empty shells may be transported by both currents and invertebrates (i.e. hermit crabs) so their presence may be incidental and there is no guarantee that these empty shells are part of the community at the time of collecting (Bouchet et al. 2002). Thus, on a regional scale (i.e., Bahía del Mazatlán) this complete inventory (196 species) including empty shells and live specimens may be taken into consideration. However, since the composition of the assemblage is described here in a more detailed way and it was associated with sampling sites and specific environments, we decided to exclude the empty shells and define the assemblage of bivalves in a conservative way using only live specimen associated with a narrow vertical distribution range which includes two adjacent interconnected environments of the bay: the intertidal and shallow subtidal (< 10 m depth).

Some implications that emerge from the taxonomic identification of five bivalve taxa classified here as “cf.” (from the Latin *confer* which means “compare with”, that is, similar to and probably the same as, the parent taxon) are worth mentioning. These specimens corresponded to juvenile stages (*Chama* cf. *frondosa*, *Semele* cf. *bicolor*, *Nutricola* cf. *humilis*, *Transennella* cf. *puella*, and *Pitar* cf. *omissa*), which restricts their taxonomic determination because the keys and photographs in the literature consistently refer to adult specimens (i.e., Keen 1971, Coan and Valentich-Scott 2012). However, the juvenile specimens collected displayed certain distinctive features that prove their resemblance to that species. The specimens of *Crassinella* aff. *pacifica*, collected in Venados Island, have all the characteristics of the species, although some of them display a slight variation in the beak that does not correspond to the taxon. Therefore, for practical purposes all individuals collected in this study in all sites were determined as *C.* aff. *pacifica*. Nonetheless, these specimens warrant a detailed examination to rule out a potential new species (Figure 5A–E).

Some specimens of the rock oyster *Striostrea prismatica* did not show the thick lamellae on the outer shell surface which characterize this species. Instead, they exhibited tubular spines as *Ostrea tubulifera*. The spines are located on the outer edge of the shell, and the features of the inner surface match those of *S. prismatica*. If only the

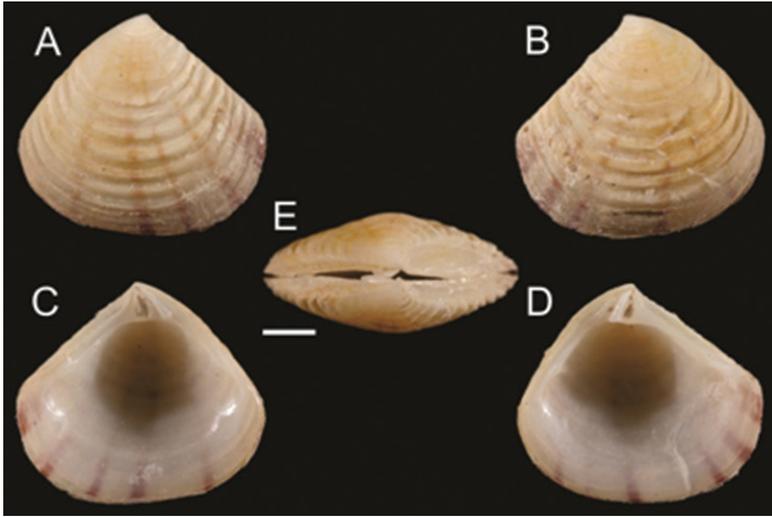


Figure 5. *Crassinella* aff. *pacifica*. Length = 4.92 mm **A** Exterior of right valve **B** Exterior of left valve **C** Interior of right valve **D** Interior of left valve **E** Dorsal view of both valves joined. Scale = 1 mm. Venados Island, Bahía de Mazatlán, México. LEMA-BI-14. Photography credit: Paul Valentich-Scott.

analysis of morphological traits is considered, the problem could be explained as either hybridization between the two species—as this phenomenon is very common among oysters (Leitão et al. 2007), or an atypical species trait, or ontogenetic variation. Only a genetic analysis could resolve the true identity.

According to the different projections obtained with species accumulation curves, the expected total numbers of species is 32% (for the intertidal zone) and 57% (for the subtidal zone) higher than the number of species we actually collected. This difference relates to the large number of rare species recorded and it is a good estimator of the potential number of species expected in these environments at Bahía de Mazatlán. Even so, the species accumulation curves confirmed that our sampling effort was sufficient to calculate the theoretical total number of bivalve species in the bay.

Although different sampling techniques were used in the bay's different environments, the sampling effort was estimated only for the quadrat-transect technique. Thus, whether all the bivalve species that inhabit the bay were collected in this study was not satisfactorily demonstrated. Some bivalves may be present either only in some seasons or impermanently, so these will not be recorded irrespective of the sampling intensity, which in turn is reflected in the sampling effort outcome (Figures 2, 3).

The high marine biodiversity of Golfo de California has been related to its irregular coastal geomorphology (i.e., open and protected bays and inlets, rocky and sandy beaches, estuaries, and numerous islands), the local dynamics of the surface currents and the seabed heterogeneity (Hendrickx and Brusca 2002, Hendrickx et al. 2005). According to Roy et al. (2000), there is a remarkable increase in infaunal and epifaunal bivalve species in the northeastern Pacific coast, between latitudes 5°N (i.e., Punta Paita, Perú) and 23°N (i.e., Bahía de Mazatlán, México). The bivalve species

Table 5. Previous studies in Bahía de Mazatlán, México. * = Species list not provided.

Environments	Sampling method	Total species	Shared species	Reference
Subtidal (10–15m)	Grab (Van Veen)	3	2	Parker (1963)
Subtidal (3.5–27m)	Grab (Van Veen)	42	11	Orozco-Romo (1980)
Subtidal	Trawls	2	1	
Rocky intertidal	Quadrats & transects	15	14	Arreguín-Romero (1982)
Middle rocky intertidal	Direct search	4	4	Sánchez-Vargas (1984)
Lower rocky intertidal	Direct search	12	11	
Shallow subtidal (1–5m)	Grab (Van Veen) & trawls	22	12	
Rocky– sandy intertidal	Quadrats	7	5	Olabarria et al. (2001)
Rocky– sandy intertidal	Quadrats	13	5	Camacho-Montoya (2007)
Intertidal	Quadrats & transects	9	-	Vega et al. (2008)*
Rocky– sandy intertidal	Transect band	19	13	Rendón-Díaz (2010)
Total species previous studies		83		
Total shared species			40	
Rocky– sandy intertidal	Quadrats & transects	60		This study
Sandy intertidal	Quadrats & transects	3		
Subtidal (4–10 m)	Quadrats & transects	64		
Subtidal (8–15 m)	Naturalist's dredge	42		
Subtidal (4–10 m)	Direct search	38		
Total species present study		89		
Total species in Bahía de Mazatlán		132		

richness in Golfo de California has been documented by Parker (1963) who reported 380 species; Hendrickx et al. (2007) who reported 565 species; and Zamorano and Hendrickx (2007) who reported 137 species. Therefore, Bahía de Mazatlán has approximately 23% of the bivalve species reported for this region. Coan (1968) recorded 75 bivalve species in Bahía de los Ángeles, which has a similar size to Bahía de Mazatlán and it is located in the northern portion of the Golfo de California. Many of the bivalves from Bahía de los Ángeles are infaunal forms mostly associated with sandy-silt substrates which prevail in this bay. A detailed review of the malacological fauna of these bays indicates that they are quite different with only 12 bivalve species shared. According to Hendrickx et al. (2007), invertebrate diversity generally tends to decrease from south to north in the Golfo de California. Actually, these authors document a reduction in the number of bivalve species along a south-to-north latitudinal gradient of the gulf. The region is generally considered to be warm temperate with a combination of elements from two adjacent provinces and ecoregions: the Cortezian Ecoregion, in the southern end of the Warm Temperate Northeast Pacific province and the Mexican Tropical Pacific Ecoregion, in the northern part of the Tropical Eastern Pacific province (Spalding et al. 2007).

The characteristics of the Bahía de Mazatlán coastline provide a variety of benthic habitats to support a large number of bivalve species. A number of studies on the Mexican Pacific coast have shown that the high species richness and diversity of bivalve life forms

are related to substrate heterogeneity, wave exposure and particle size of the sediments in the intertidal and shallow subtidal environments (Parker 1963, Coan 1968, Esqueda et al. 2000, González-Medina et al. 2006, Hendrickx et al. 2007, Ríos-Jara et al. 2008, Vega et al. 2008, López-Uriarte et al. 2009, Ríos-Jara et al. 2009). Bahía de Mazatlán comprises islands, rocky reefs, small aggregations of coral, rocky and sandy shores, all of which increase the heterogeneity and availability of marine habitats. In addition, there are two well-defined seasons throughout the annual cycle – the dry season and the wet season – with major changes in primary productivity, nutrients, and phytoplankton (Alonso-Rodríguez 2004). This environmental heterogeneity contributes to the presence of numerous bivalve species and life forms, and contrasts with the low richness observed by Parker (1963) in deeper bays (> 10 m) with more homogeneous soft bottoms (Orozco-Romo 1980).

Our analysis combined data from three different sampling techniques, which was a major advantage, as the average taxonomic distinctness analysis is not affected by the various techniques and sampling effort used (Warwick and Clarke 2001, Leonard et al. 2006). With this method we identified the sites and environments that, according to their species taxonomic composition, are within the 95% probability funnel of average taxonomic distinctness (Δ^+) and its variation (Λ^+). Their inclusion within the probability funnel indicates that they involve a good representativeness of the bay's taxonomic diversity.

In this study, the combination of sites and environments provided better values of Δ^+ and Λ^+ when rocky shores and shallow subtidal adjacent zones were taken into consideration. This is because, although the three environments are clearly different from each other, all the sites contribute towards the taxonomic diversity of the bay. For example, Venados Island and Casa del Marino had the highest number of taxa in the shallow subtidal zone; Los Pinos and Venados Island, in the lower intertidal zone; and Olas Altas, in the upper intertidal zone. Theoretically, populations with a high genetic diversity have a high evolutionary potential or ability to adapt to changing environmental conditions (Price 2002). Further comparative studies among regions, clades, and functional groups are needed to understand the bivalve assemblage of Bahía de Mazatlán.

Conclusion

The present work demonstrates that the bivalve fauna in Bahía de Mazatlán is well represented by various life forms (epifaunal, infaunal, semi-infaunal, and endolithic) in all the sites studied. Venados Island is an area protected by two government agencies; this is significant because it displayed high species richness and a large number of unique species. Since the bay is now a popular destination for tourists, efforts to preserve its ecosystems and species are essential, including those bivalves of economic importance such as the rock oyster *Striostrea prismatica* and the pearl oyster *Pinctada mazatlanica*. The latter species is on the Mexican Official List of Protected Species (NOM-059-SEMARNAT-2010).

The information on bivalve assemblages in Bahía de Mazatlán should be supplemented with analysis including an assessment of α , γ , and β diversity in order to de-

termine their relative distribution at different spatial scales. A quantitative analysis investigating the relationship between bivalve assemblage structure and local and seasonal environmental parameters is also required. Such an analysis, would contribute to a comprehensive framework on the ecology of these bivalves, which is essential for further studies on the conservation of the bay.

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References

- Abbott RT (1974) American seashells. Van Nostrand Reinhold Co., New York, N.Y. U. S. A. 391 pp.
- Alonso-Rodríguez R (2004) Hidrología y condiciones ambientales que determinan la proliferación de dinoflagelados causantes de marea roja en la bahía de Mazatlán, Sin. México. Ph.D. thesis. Centro de Investigaciones Biológicas del Noroeste, 117 pp.
- Arreguín-Romero JL (1982) Estudio de los moluscos de la zona rocosa litoral de la Bahía de Mazatlán, con referencia especial a su zonación. Tesis de Licenciatura. Escuela de Biología, Universidad Autónoma de Guadalajara, 100 pp.
- Bell JJ, Carballo JL (2008) Patterns of sponge biodiversity and abundance across different biogeographic regions. *Marine Biology* 155: 563–570. doi: 10.1007/s00227-008-1036-6 [accessed 15 June 2013]
- Bouchet P, Lozouet P, Maestrati P, Heros V (2002) Assessing the magnitude of species richness in tropical marine environments: exceptionally high numbers of molluscs at a New Caledonia site. *Biological Journal of the Linnean Society* 75: 421–436. doi: 10.1046/j.1095-8312.2002.00052.x
- Bouchet P, Rocroi JP (2010) Nomenclator of Bivalve Families. *Malacologia* 52(2): 1–184. doi: 10.4002/040.052.0201

- Brown JH, Lomolino MV (1998) Biogeography. Sinauer, Sunderland, Massachusetts, 691 pp.
- Camacho-Montoya C, Valdez-Pineda MC, Pérez-González R, Rodríguez-Negrete RE (2007) Moluscos en un área de crianza de las langostas *Panulirus inflatus* y *P. gracilis* (Decapada: Palinuridae) en la Bahía de Mazatlán, Sinaloa. In: Ríos-Jara E, Esqueda-González MC, Galván-Villa CM (Eds) Estudios sobre la Malacología y Conquiliología en México. Universidad de Guadalajara, México, 65–67.
- Clarke KR, Gorley RN (2006) Primer v6: User manual and tutorial. Primer-E, Plymouth, UK.
- Clarke KR, Warwick RM (1999) The taxonomic distinctness measure of biodiversity: weighting of step lengths between hierarchical levels. Marine Ecology Progress Series 184: 21–29. doi: 10.3354/meps184021
- Clarke KR, Warwick RM (2001) A further biodiversity index applicable to species lists: variation in taxonomic distinctness. Marine Ecology Progress Series 216: 265–278. doi: 10.3354/meps216265
- Coan VE (1968) A biological survey of Bahía de los Angeles, Gulf of California, Mexico. III. Benthic Mollusca. Transactions of the San Diego Society of Natural History 15(8): 107–132.
- Coan EV, Valentich-Scott P (2012) Bivalve seashells of tropical west America. Marine Bivalve Mollusks from Baja California to Northern Perú. Santa Barbara Museum of Natural History, 1258 pp.
- Colwell RK (2006) EstimateS: statistical estimation of species richness and shared species from samples. <http://viceroy.eeb.uconn.edu/estimates> [accessed 5 November 2011]
- CONABIO (2012) http://www.conabio.gob.mx/conocimiento/regionalización/doctos/rmp_020.html [accessed 2 February 2012]
- English S, Wilkinson C, Baker V (1997) Survey Manual for Tropical Marine Resources. 2nd Edition. Australian Institute of Marine Sciences. Townsville, Australia, 390 pp.
- Escalante-Espinoza T (2003) Cuántas especies hay? Los estimadores no paramétricos de Chao. Elementos 52: 53–56.
- Esqueda MC, Ríos-Jara E, Landa-Jaime V, Michel-Morfin E (2000) The vertical distribution and abundance of gastropods and bivalves from rocky beaches of Cuatecomate Bay, Jalisco. International Journal of Tropical Biology and Conservation 48(4): 765–775.
- González-Medina FJ, Holguín-Quiñones OE, de la Cruz-Agüero G (2006) Spatiotemporal variability of some shallow-bottom macroinvertebrates (Gastropoda, Bivalvia and Echinodermata) from Espíritu Santo Archipelago, Baja California Sur, México. Ciencias Marinas 32(1A): 33–44.
- Heino J, Soinen J, Lappalainen J, Virtanen R (2005) The relationship between species richness and taxonomic distinctness in freshwater organisms. Limnology and Oceanography 50(3): 978–986.
- Heino J, Mykrä H, Kotanen J (2008) Weak relationships between landscape characteristics and multiple facets of stream macroinvertebrate biodiversity in a boreal drainage basin. Landscape Ecology 23: 417–426. doi: 10.1007/s10980-008-9199-6 [accessed 20 July 2013]
- Hendrickx ME, Toledano-Granados A (1994) Catálogo de moluscos. Colección de referencia de la estación Mazatlán, ICMYL, UNAM. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad e Instituto de Ciencias del Mar y Limnología. UNAM, México, D.F., 71 pp.

- Hendrickx ME, Brusca RC (2002) Mollusca. In: Hendrickx ME, Brusca RC (Eds) Distributional checklist of the macrofauna of the Gulf of California, México. Arizona-Sonora Desert Museum, Tucson, Arizona, 159–273.
- Hendrickx ME, Brusca RC, Findley LT (Eds) (2005) A Distributional Checklist of the Macrofauna of the Gulf of California, Mexico. Part 1. Invertebrates. [Listado y Distribución de la macrofauna del Golfo de California, México, Parte 1. Invertebrados]. Arizona-Sonora Desert Museum, 429 pp.
- Hendrickx ME, Brusca RC, Cordero M, Ramírez G (2007) Marine and brackish-water molluscan biodiversity in the Gulf of California, Mexico. *Scientia Marina* 71: 637–647.
- Keen AM (1971) Seashells of Tropical West America. Marine mollusks from Baja California to Peru. Stanford University Press, Stanford, California, 1065 pp.
- Keen MA, Coan E (1974) Marine molluscan genera of western North America (from Baja California to Peru). Stanford University Press. Stanford, California, 208 pp.
- Leitão A, Chaves R, Santos S, Guedes-Pinto H, Boudry P (2007) Interspecific hybridization in oysters: Restriction enzyme digestion chromosome banding confirms *Crassostrea angulata* x *Crassostrea gigas* F1 hybrids. *Journal of Experimental Marine Biology and Ecology* 343(2): 253–260. doi: 10.1016/j.jembe.2006.12.007 [accessed 27 December 2013]
- Leonard DPR, Clarke KR, Somerfield P, Warwick RM (2006) The application of an indicator based on taxonomic distinctness for UK marine biodiversity assessment. *Journal of Environmental Management* 78: 52–62. doi: 10.1016/j.jenvman.2005.04.008 [accessed 20 July 2013]
- Levinton JS (2001) Marine Ecology. Function, Biodiversity, Ecology. 2nd ed. Oxford University Press, Inc., 515 pp.
- López-Uriarte E, Ríos-Jara E, Galván-Villa CM, Juárez-Carrillo E, Enciso-Padilla I, Robles-Jarero EG, Pérez-Peña M (2009) Macroinvertebrados bénticos del litoral somero de Punta La Rosada, Bahía Chamela, Jalisco. *Scientia-CUCBA* 1-2: 57–68.
- Magurran EA (2004) Measuring Biological Diversity. Blackwell Publishing, 248 pp.
- Mistri M, Ceccherelli VU, Rossi R (2000) Taxonomic distinctness and diversity measures: responses in lagoonal macrobenthic communities. *Italian Journal Zoology* 67: 297–301. doi: 10.1080/11250000009356327 [accessed 22 July 2013]
- Montaño-Ley Y (1985) Estudio del transporte litoral de arenas en la Isla de la Piedra, Mazatlán, Sin., usando trazadores fluorescentes. *Anales Instituto Ciencias del Mar y Limnología Universidad Nacional Autónoma de México* 12: 15–32.
- Morris PA (1980) A field guide to Pacific coast shells, including shells of Hawaii and the Gulf of California. Houghton Mifflin Company Boston, USA, 297 pp.
- Olabarria C, Carballo JL, Vega C (2001) Spatio-Temporal changes in the tropics structure of rocky intertidal mollusc assemblages on a tropical shore. *Ciencias Marinas* 27(2): 235–254.
- Orozco-Romo ML (1980) Estudio del macrobentos de la Bahía de Mazatlán. Tesis de licenciatura. Universidad Autónoma de Guadalajara, 131 pp.
- Parker RH (1963) Zoogeography and ecology of some macro-invertebrates, particularly mollusks, in the Gulf of California and the continental slope off Mexico. *Dansk Naturhistorisk Forening (Copenhagen), Videnskabelige Meddelelser* 126: 1–178.
- Peres JM (1982) Zonations. In: Kinne O (Ed) *Marine Ecology*. Wiley, New York, 9–45.

- Price ARG (2002) Simultaneous 'hotspots' and 'coldspots' of marine biodiversity and implications for global conservation. *Marine Ecology Progress Series* 241: 23–27. doi: 10.3354/meps241023
- Rendón-Díaz LY (2010) Diversidad ecológica de moluscos del estero de Urías y costas de la Bahía de Mazatlán, Sinaloa, México. Tesis de licenciatura. Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, 81 pp.
- Ríos-Jara E, López-Uriarte E, Galván-Villa CM (2008) Bivalve molluscs from the continental shelf of Jalisco and Colima, Mexican Central Pacific. *American Malacology Bulletin* 26: 119–131. doi: 10.4003/006.026.0212
- Ríos-Jara E, Navarro-Caravantes CM, Galván-Villa CM, López-Uriarte E (2009) Bivalves and gastropods of the Gulf of Tehuantepec, Mexico: A checklist of species with notes on their habitat and local distribution. *Journal of Marine Biology* 2009: 1–12. doi: 10.1155/2009/176801 [accessed 20 January 2012]
- Roger SI, Clarke KR, Reynolds JD (1999) The taxonomic distinctness of coastal bottom-dwelling fish communities of the North-east Atlantic. *Journal of Animal Ecology* 68: 769–782. doi: 10.1046/j.1365-2656.1999.00327.x
- Roy K, Jablonsky D, Valentine JW (2000) Dissecting latitudinal diversity gradients: functional groups and clades of marine bivalves. *Proceedings of the Royal Society London* 267: 293–299. doi: 10.1098/rspb.2000.0999
- Sánchez-Vargas DP (1984) Ecología y estructura de las comunidades de moluscos y crustáceos decápodos en la ensenada de Puerto Viejo, Mazatlán, Sinaloa. Tesis de licenciatura. Escuela de Biología, Universidad Nacional Autónoma de México, 186 pp.
- Skoglund C (2001) Panamic Province Molluscan Literature: Additions and Changes from 1971 through 2000. I. Bivalvia; II. Polyplacophora. *The Festivus (Supplement)*, 140 pp.
- Smith SDA (2008) Interpreting molluscan death assemblages on rocky shores: Are they representative of the regional fauna? *Journal of Experimental Marine Biology and Ecology* 366: 151–159. doi: 10.1016/j.jembe.2008.07.019 [accessed 22 July 2013]
- Spalding MD, Fox HE, Allen GR, Davidson N, Ferdaña ZA, Finlayson M, Halpern BS, Jorge MA, Lombana A, Lourie SA, Martin KD, McManus E, Molnar J, Recchia CA, Robertson J (2007) Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. *BioScience* 57(7): 573–583. doi: 10.1641/B570707 [accessed 12 January 2012]
- Vega C, Olabarria C, Carballo JL (2008) Variación espacio temporal de moluscos y macroalgas en sustratos rocosos intermareales en la Bahía de Mazatlán. *Ciencia y Mar* XII (34): 3–16.
- Warwick RM, Clarke KR (1995) New "biodiversity" measures reveal a decrease in taxonomic distinctness with increasing stress. *Marine Ecology Progress Series* 129: 301–305. doi: 10.3354/meps129301
- Warwick RM, Clarke KR (1998) Taxonomic distinctness and environmental assessment. *Journal of Applied Ecology* 35: 532–543. doi: 10.1046/j.1365-2664.1998.3540532.x
- Warwick RM, Clarke KR (2001) Practical measures of marine biodiversity based on relatedness of species. *Oceanography Marine Biology Annual Review* 39: 207–231.
- Warwick RM, Light J (2002) Death assemblages of mollusks on St Martin's Flats, Isles of Scilly: a surrogate for regional biodiversity? *Biodiversity and conservation* 11: 99–112. doi: 10.1023/A:1014094829984

- Wilkinson T, Wiken E, Bezaury-Creel J, Hourigan T, Agardy T, Herrmann H, Janishevski L, Madden C, Morgan L, Padilla M (2009) Ecoregiones marinas de América del Norte. Comisión para la Cooperación Ambiental, Montreal, 200 pp.
- Wyrski L (1966) Oceanography of the Eastern Equatorial Pacific Ocean. *Oceanography Marine Biology Annual Review* 4: 33–68
- Zamorano P, Hendrickx ME (2007) Biocenosis y distribución de los moluscos de aguas profundas en el Pacífico mexicano: una evaluación de los avances. In: Ríos-Jara E, Esqueda-González MC, Galván-Villa CM (Eds) *Estudios sobre la Malacología y Conquiliología en México*. Universidad de Guadalajara, México, 48–49.
- Zamudio L, Leonardi AP, Meyers SD, O'Brien JJ (2001) ENSO and eddies on the Southwest coast of Mexico. *Geophysical Research Letters* 28: 13–16. doi: 10.1029/2000GL011814

A new earthworm species within a controversial genus: *Eiseniona gerardo* sp. n. (Annelida, Lumbricidae) - description based on morphological and molecular data

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Abstract

The morphological and anatomical simplicity of soil dwelling animals, such as earthworms, has limited the establishment of a robust taxonomy making it sometimes subjective to authors' criteria. Within this context, integrative approaches including molecular information are becoming more popular to solve the phylogenetic positioning of conflictive taxa. Here we present the description of a new lumbricid species from the region of Extremadura (Spain), *Eiseniona gerardo* sp. n. The assignment to this genus is based on both a morphological and a phylogenetic study. The validity of the genus *Eiseniona*, one of the most controversial within Lumbricidae, is discussed. A synopsis of the differences between the type species and the west-European members of the genus is provided.

Keywords

Earthworms, lumbricids, *Eiseniona*, species description

Introduction

Earthworm fauna is still poorly known within vast areas of the Iberian Peninsula. The available data indicate the common presence of cosmopolitan species such as *Aporrectodea trapezoides* (Dugès, 1828) or *A. rosea* (Savigny, 1826). In contrast, other species show more restricted distributions but are locally abundant (Díaz Cosín et al. 1992, Rodríguez et al. 1997). The region of Extremadura is one of the best documented, thanks to the work by Sánchez et al. (1998, 1999). These authors found that *A. trapezoides* and *A. rosea* are the dominant species, while other species can be locally important in river sides and flooded areas, such as the species of the complex *Allolobophora molleri* Rosa, 1889 sensu Barros et al. (1992) that was placed in *Eophila* by Blakemore (2008).

An intensive earthworm sampling campaign was accomplished between 2009 and 2012 in the surroundings of Plasencia (North of Cáceres, Extremadura, Spain) within the European Project “BioBio, Biodiversity Indicators for European Farming Systems, Indicators for Biodiversity in Organic and Low Input Farming Systems”. The Spanish team within this project studied the potential use of soil fauna as bioindicators in *dehesas* (i.e., Mediterranean grazed open woodlands of *Quercus ilex* Linné and olive groves under different types of land management. Among the several thousands of earthworm specimens collected during this sampling campaign, nineteen individuals sampled close to El Bronco (Cáceres, Spain) are of special taxonomical interest as they represent a new species as described in the present study.

The taxonomical assignment to a genus level in earthworm lumbricid taxonomy is confusing and varies regarding the criteria used by the different authors. In addition, it lacks robustness because it is not necessarily based on phylogenetic relationships. The number of genera proposed for the family Lumbricidae varies from five when reviewed by Michaelsen (1900) (*Eiseniella*, *Eisenia*, *Helodrilus* –with four subgenera – *Octolasion* and *Lumbricus*) to 44 proposed by Blakemore (2008) or 45 considered by Qiu and Bouché (1998a), including 29 subgenera. Some of these genera are well-defined and characterized by consistent and stable characters. A good example is the genus *Lumbricus*, with a tanylobic prostomium, paired chaetae and reddish body colour. Unfortunately, this is not the case in the great majority of the other genera, as often overlapping or slightly variable characters are used to define them. Therefore, the proper assignment to the level of genus is challenging and sometimes even subjective, but should nevertheless comply with ICZN requirements to be consistent with its type-species.

Soil dwelling animals are subject to a series of limitations in their corporal design. This is reflected in earthworms that present a very simple body externally without many differential morphological characters. The position of clitellum and tubercula pubertatis, type of prostomium, pigmentation, chaetal arrangement, number and position of spermathecae, seminal vesicles, Morren’s glands, nephridia or typhosole are

some of the most widely-used morphological characters in earthworm systematics. Nevertheless, these characters may probably have evolved as adaptations to a particular soil environment or independently in several phylogenetic lineages, therefore hindering establishment of a robust taxonomical system based on morphology. The solution to this taxonomical chaos would be the phylogenetic resolution of earthworms based on molecular and morphological studies. This would allow the generation of stable and robust phylogenies in which systematic classifications are properly defined. Unlike earthworms from the family Hormogastridae (e.g., Novo et al. 2011), only some attempts have been done to study the phylogenetic relationships of lumbricids (e.g., Briones et al. 2009). Nonetheless, these studies are limited because of incomplete sampling or limited number of genes sequenced. Fortunately, a multigene phylogeny of lumbricid earthworms represented by a high number of species and genera is about to be published (Pérez Losada et al., *pers. comm.*) and hopefully it will help to convert lumbricid systematics into a more stable system.

In the context of this controversial classification of genera in lumbricid earthworms, one of the most conflictive ones is *Eiseniona* (Omodeo, 1956). This genus was established by Omodeo (1956) and was accepted by some authors (Álvarez 1970; Qiu and Bouché 1998a, d; Omodeo and Rota 2004; Rota 2013) but rejected by others (Bouché 1972; Zicsi 1982; Easton 1983; Mrcsic 1991 and Blakemore 2008). Most of the species included in *Eiseniona* are distributed in Italy, Greece and other countries of Central or Eastern Europe. Some examples of species described in Western Europe are *E. paradoxa* (Cognetti, 1904) and *E. gavarnica* (Cognetti, 1904) in France [both retained in genus *Orodriilus* Bouche, 1972 by Blakemore (2008)] and in the Iberian Peninsula *Eiseniona oliveirae* (Rosa, 1894), *Eiseniona carpetana* (Álvarez, 1970) and *Eiseniona albolineata* Díaz Cosín et al., 1989 [the former retained in *Allolobophora* Eisen, 1874 and the latter two retained in genus *Iberoscolex* Qiu & Bouche, 1998 by Blakemore (2008)].

Despite the extended use of morphological and anatomical characters in earthworm taxonomy, during the last years the concept of integrative taxonomy as a tool to describe and delimit species has become more popular. This concept, consisting of a multidisciplinary approach including the morphological, molecular, ecological and geographical available data, has been applied to earthworms (e.g., Novo et al. 2012 for hormogastrids, Blakemore and Kupriyanova 2010, Blakemore 2010, Blakemore et al. 2010, Blakemore and Grygier 2011 and Blakemore 2012a for lumbricids) The implementation of molecular techniques has allowed presumption of a high cryptic diversity in earthworms completely unknown when based on traditional systematic methods (e.g. King et al. 2008, Novo et al. 2009, 2010, Dupont et al. 2011, Fernández et al. 2011), but see critique in Blakemore et al. (2010). In addition, molecular barcoding has become a widely used technique for taxonomical evaluation, allowing interesting discoveries such as the proposed separation of *L. terrestris* and *L. herculeus* (James et al., 2010), but see correction by Blakemore (2013).

In this context, this manuscript aims to describe a new lumbricid species (*E. gerardoii* sp. n.) based on morphological, molecular and ecological data.

Material and methods

Earthworm specimens and sampling points

Nineteen individuals were collected at four different but geographically-close sampling points nearby El Bronco (Cáceres, Extremadura, Spain). Soil was a sandy-loam on underlying slate (Figure 1); collectors G. Moreno, E. Juárez.

D4 Le1: **2 ex.** (1 adult, 1 subadult) (40°12'42.76"N, 6°19'0.68"W). Altitude 430 m. Grazed *dehesa* with *Quercus ilex*. Mean precipitation 876 mm. Present plant species: *Eleocharis palustris*, *Pulicaria paludosa*. Other earthworm species: *A. molleri* 1 ex. (0.75 g).

D4 R2: **2 ex.** (2 adults) (40°12'45.22"N, 6°18'39.22"W). Altitude 414 m. Grazed *dehesa* with *Quercus ilex*. Mean precipitation 876 mm. Present plant species: *Anthoxanthum aristatum*, *Isoetes hystrix*. Other earthworm species: *A. molleri* 8 ex. (6.72 g), *A. trapezoides* 16 ex. (4.96 g).

D4 S1: **2 ex.** (2 subadults) (40°12'41.51"N, 6°19'1.20"W). Altitude 430 m. Grazed *dehesa* with *Quercus ilex*. Mean precipitation 879 mm. Present plant species: *Festuca ampla*, *Trifolium dubium*. Other earthworm species: *A. molleri* 2 ex. (2.02 g), *A. trapezoides* 3 ex. (2.01 g).

D5 P2: **13 ex.** (5 adults, 8 subadults) (40°13'38.80"N, 6°18'36.04"W). Altitude 428 m. Grazed *dehesa* with *Quercus ilex*. Mean precipitation 923 mm. Present plant species: *Juncus bufonius*, *Conyza sp.* Other earthworm species: *A. molleri* 6 ex. (5,32 g), *A. rosea* 4 ex (1.05 g), *A. trapezoides* 32 ex (18,91 g).

Molecular sequencing and analyses

The following molecular regions were amplified by the methods described in Novo et al. (2011): mitochondrial subunit I of cytochrome c oxidase (COI), 16S rRNA and tRNA Leu, Ala, and Ser (16S-tRNAs), two nuclear ribosomal genes (complete 18S and a portion of 28S) and two nuclear protein-encoding genes (histones H3 and H4).

In order to have an evaluation of the selection of species to include in the molecular analyses, M. Pérez-Losada and J. Domínguez (Universidad de Vigo) kindly compared the sequences of 16S and 28S rRNA from the specimens included in this study with an unpublished database that includes most lumbricid genera. This comparison provided the first evidence indicating that the new species was closely related to *E. albolineata* and *E. oliveirae*. As a second method, we collected some individuals belonging to *E. albolineata* and sequenced the mitochondrial gene COI. In addition, we retrieved from GenBank all available COI sequences from as many different lumbricid species as possible to date (Table 2), although many of these have their identities unconfirmed. We excluded from the analyses the sequenced

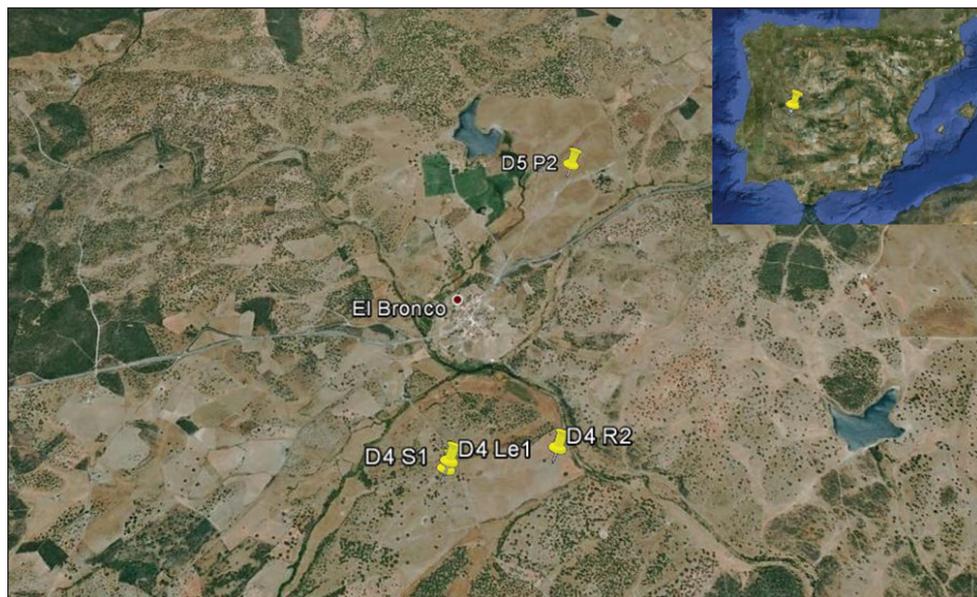


Figure 1. Map showing the position of sampling points.

Table 1. Paragenotypes (sensu Chakrabarty 2010) of *Eiseniona gerardoi* sp. n. paratypes, with GenBank accession numbers. As a consequence of the UCM scientific collections restructuring, the earthworms are now deposited within UCMLT (Universidad Complutense de Madrid Lombrices de Tierra).

Voucher	COI	16S-tRNAs	18S rRNA	28S rRNA	H3	H4
UCMLT 60001	KF737142	KF737134	KF737140	KF737148	KF737150	HG780373
UCMLT 60002	KF737143	KF737135	KF737141	KF737149	KF737151	HG780374
UCMLT 60007	KF737144	KF737136			KF737152	HG780375
UCMLT 60013	KF737145	KF737137				
UCMLT 60015	KF737146	KF737138				
UCMLT 60017	KF737147	KF737139				

genes in the public databases for which information is scarce and biased. Bayesian phylogenetic inference was then explored with the COI sequences as described in Fernández et al. (2012).

Uncorrected pairwise differences were calculated between these species with Arlequin 3.5 (Excoffier et al. 2005).

Data resources

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at doi: 10.5061/dryad.5k76c

Table 2. Taxa and specimens included in the molecular analysis. GenBank accession numbers are indicated. Species names were literally taken from GenBank. The correct name [after Blakemore (2008)], of the species marked with asterisk is, *Bimastos parvus*, *Allolobophoridella eiseni* and *Iberoscolex albolineatus*.

Species	COI GeneBank accession number
<i>Allolobophora chlorotica</i>	GU013806
<i>Aporrectodea longa</i>	JN850544
<i>Aporrectodea rosea</i>	FJ214232
<i>Aporrectodea trapezoides</i>	JF313567
<i>Aporrectodea tuberculata</i>	JN869877
* <i>Bimastos parvus</i>	EF077605
<i>Dendrobaena attemsi</i>	FJ214224
<i>Dendrobaena octaedra</i>	GU013836
<i>Dendrobaena veneta</i>	FJ214233
<i>Dendrodrilus rubidus</i>	GU013839
<i>Eisenia andrei</i>	DQ914619
* <i>Eisenia eiseni</i>	AY874488
<i>Eisenia fetida</i>	EF077596
* <i>Eiseniona albolineata</i>	KF746384
<i>Helodrilus ocellatus</i>	FJ374775
<i>Hormogaster elisae</i>	EF653905
<i>Lumbricus festivus</i>	FJ937290
<i>Lumbricus rubellus</i>	GU206189
<i>Lumbricus terrestris</i>	JN869936
<i>Octodrilus juvyi</i>	HE611693
<i>Octolasion cyaneum</i>	JQ909144
<i>Octolasion lacteum</i>	DQ092909

Results

The specimen with voucher number UCMLT 60000 is the designated holotype. The paratypes bear the numbers UCMLT 60001 to 60018.

Morphological description

The specimens were sketched using an Olympus binocular microscope with digital camera, dissected, and described.

Taxonomic results

Phylum Annelida Lamarck, 1802

Subphylum Clitellata Michaelsen, 1919

Class Oligochaeta Grube, 1850

Order Haplotaxida Michaelsen, 1900

Family Lumbricidae Rafinesque-Schmaltz, 1815

Genus *Eiseniona* Omodeo, 1956

Type-species. *Allolobophora handlirschi* Rosa, 1897 by original designation.

***Eiseniona gerardoi* Díaz Cosín, sp. n.**

<http://zoobank.org/E14BF86D-EFF1-47E7-BE5B-6F59ACCD4B>

http://species-id.net/wiki/Eiseniona_gerardoi

Material examined. *Holotype.* Adult (Catalog # UCMLT 60000), 40°13'38.80"N, 6°18'36.04"W ("spanish dehesa" mediterranean grazed open woodlands of *Quercus ilex*), near El Bronco (Cáceres, Spain), leg. G. Moreno, E. Juárez, April 2010.

Paratypes. 18 specimens (Catalog # UCMLT 60001 to 60018), leg G. Moreno, E. Juárez, April 2010.

Morphological description. *External morphology* (Figures 2, 3). Length of mature specimens: 21–40 mm, \bar{x} : 28mm, SD: 5.6 mm, holotype: 31 mm. Diameter: clitellar \bar{x} : 2.5 mm, SD: 0.4 mm, holotype: 2.5 mm, postclitellar \bar{x} : 1.8 mm, SD: 0.2 mm, holotype: 1.7 mm. Body cylindrical in the anterior part, wider at clitellum and trapezoidal or rectangular in postclitellar region, with chaetae in the corners. Number of segments: 89 to 124, \bar{x} : 109.5, SD: 10.7, holotype: 117. Weight (fixed specimens): 38 to 64 mg, \bar{x} : 52 mg, SD: 13 mg, holotype 62 mg.

Colour: When alive, the anterior part is red-brownish showing noticeable antero-posterior and dorso-ventral gradients. Cream-coloured or whitish clitellum. After a long period within alcohol the red pigment is gradually lost and transformed into brown of different intensities (Figure 2).

Prostomium epilobic $\pm 1/3$. No longitudinal lines are noticeable in segments 1 and 2. First dorsal pore in (3/4) 4/5. Nephridial pores inconspicuous in a row slightly above *b*. Spermathecal pores at intersegments 9/10 and 10/11, at the level of chaetae *cd* (Figure 3).

Male pores as vertical grooves in the segment 15 between chaetae *b* and *c* showing small porophores with whitish areolae shape. Female pores in 14 slightly above *b*. Chaetae paired, interchaetal ratio at segment 40, *aa*: 16, *ab*: 1.4, *bc*: 7, *cd*: 1, *dd*: 24. Chaetae are simple with a wider base and a sharp and bent distal end. (Figure 4).

Clitellum white or cream-coloured, saddle-shaped extending over 22,23–29,30, in the holotype 1/n 22, 23–29. When well developed it invades the ventral area and the intersegmental lines are hard to distinguish. Tubercula pubertatis extended as a belt in 23-(27)28,29, in the holotype in 23–29. Occasionally they appear folded or wrinkled. No noticeable papillae are present in any of the specimens.

Internal anatomy. Slightly thickened anterior septa. Last pair of oesophageal hearts in 11. Morren's glands with small diverticula in 10 and little lamellae in 11 and 12. Crop in 15,16, gizzard in (17)18,19. First section of the intestine is not dilated. Simple typhlosole pleated, which begins in 20, 21 and ends near the anus leaving only 10–15 atyphlosolate segments.



Figure 2. External view of the anterior part of the body of *E. gerardoii*.

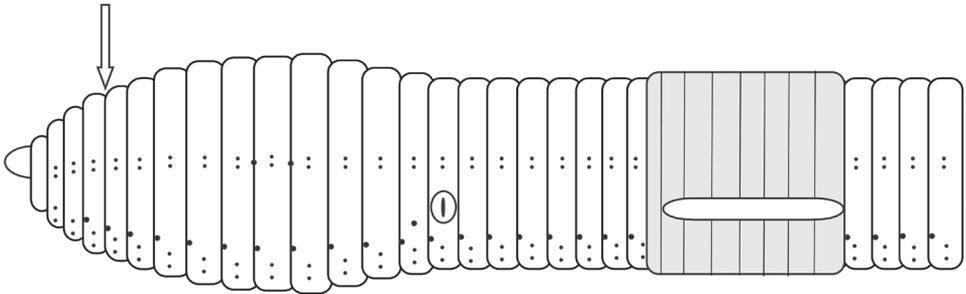


Figure 3. Schematic view of the external morphology of *E. gerardoii*.

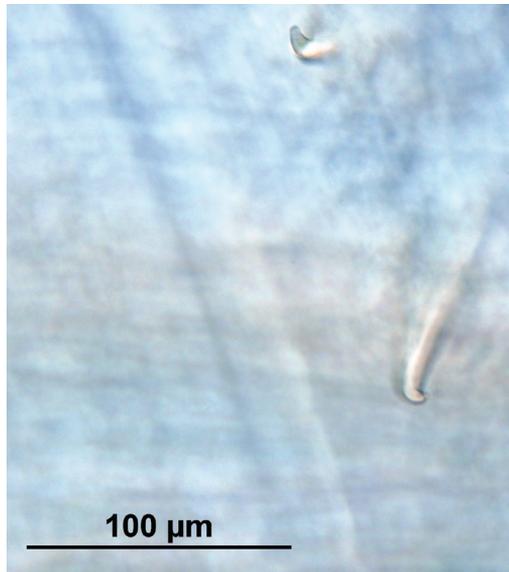


Figure 4. Chaetae *ab* from segment 40–41 (DIC Nomarski).

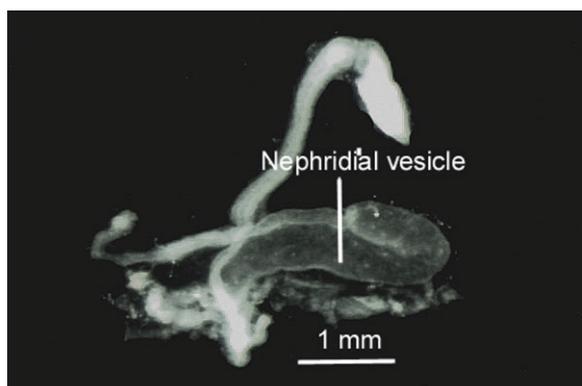


Figure 5. Posterior nephridium isolated by dissection, showing the nephridial curved bladder.

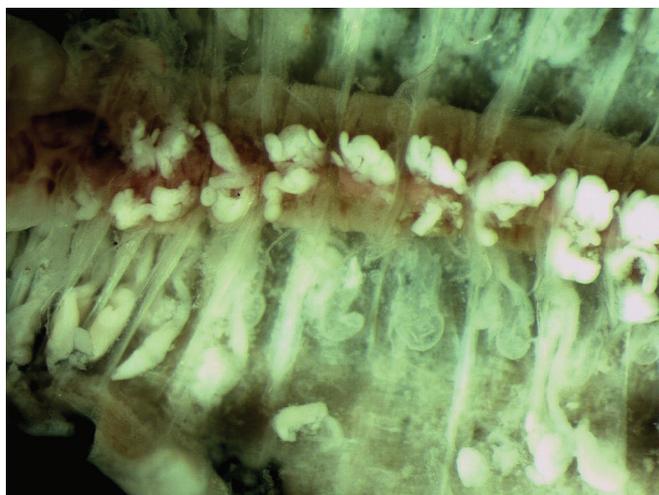


Figure 6. White tissue associated with the dorsal vessel.

Fraying testes and iridescent and very large seminal funnels in 10 and 11. Three pairs of seminal vesicles in 9, 11 and 12. The last pair is very large pushing back the septum 12/13. Large ovaries and female funnels in 13, ovarian receptacles (ovisacs) in 14. Two pairs of very large and iridescent spermathecae in segments 10 and 11.

In the posterior region of the body the nephridia are much enlarged, the nephridial bladders are curved and J-shaped with curved section 1/3 of total length. (Figure 5).

An important characteristic is the presence of dense white glands on top of the dorsal vessel initially around segment 20 and externally visible as a whitish line extending to the end of the body. (Figure 6).

Distribution. Known only from its type locality.

Etymology. The species is dedicated to Prof. Gerardo Moreno from Centro Universitario de Plasencia, Universidad de Extremadura, Spain. He is the PI for the Bio-Bio program in Spain and collected the specimens described in this paper.

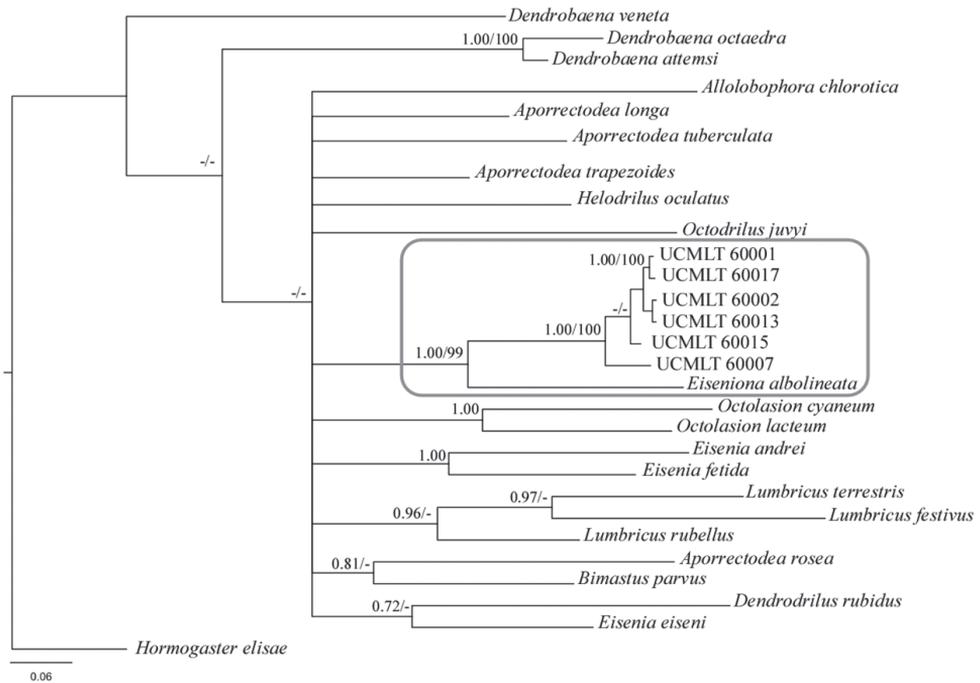


Figure 7. Bayesian inference tree based on COI sequences of *E. gerardoi* and other lumbricids represented in GeneBank. *E. gerardoi* (see UCMLT codes in Table 1) clusters with *E. albolineata*.

Molecular characters. Sequences of the used genes have been deposited in GenBank (see Table 1). According to Drs. Pérez Losada and Domínguez (*pers. comm.*), the 16S and 28S sequences of *E. gerardoi* clustered with the two species classified as *Eiseniona*, *E. albolineata* and *E. oliveirae*.

The phylogenetic tree presented here, based on the COI gene and including some of the available species in GenBank (Figure 7), shows that *E. gerardoi* specimens form a highly supported group (1.00 posterior probability, 0.99 bootstrap) with *E. albolineata*. The two species share the presence of whitish glands on top of the dorsal vessel. COI genetic divergence (uncorrected p-distances) between *E. albolineata* and *E. gerardoi* is 14.09%, and the intraspecific variability of the latter is 2.81% showing a very close relationship.

Habitat and ecological characters. All the soils from sampling sites have been developed on slates and are sandy-loams. Precipitation corresponds to the typical values of intermediate semi-humid Spain. The associated species *A. molleri* is always present and this species is bound to terrains that are flooded during several months per year. Additionally, the presence of plants typical from wetlands, such as *Eleocharis palustris*, *Pulicaria paludosa* or *Juncus bufonius* indicates that in these sites there is enough humidity during most of the year, which supports hygrophile communities. Nevertheless they could be desiccated in the summer, which would force the earthworms to

undergo aestivation in order to survive to these dry periods, resuming activity when humidity is restored. All these details are compatible with the diagnosis of the genus by Omodeo (1956) as he highlighted the semiaquatic characteristics of *Eiseniona*.

Discussion

Eiseniona genus was created by Omodeo (1956) for the inclusion of five earthworm species presenting small to medium sizes, number of segments inferior to 170, closely paired chaetae, Morren's glands with lateral bags in segment 10 and red or light pink subepidermic pigment (absent in some instances). Their clitella start between segments 23 and 27 and tubercula pubertatis appear as continuous bands. Male pores lack the glandular atrium (but show a small atrium in some instances) and papillae are present in segments near the spermathecae and male pores. They show three or four pairs of seminal vesicles, the last reaching to segments 13 or 14. Their habitat is semiaquatic. Omodeo and Rota (2004) subsequently added or specified other characters such as: "body central and posterior parts with trapezoidal cross section, with the pairs of chaetae at the four corners, nephridial bladders as an inverted J with the ental limb oriented backward, typhlosole cylindrical "en accordéon" spermathecae large, intracoelomic with openings in 9/10 and 10/11, three pairs of seminal vesicles in IX, X, XI the latter being very large, protruding in XIII".

The species originally included in this genus were *E. handlirschi* (Rosa, 1897) [the designated type, now placed in *Aporrectodea* according to Blakemore (2008) and Csuzdi (2012)], *E. oliveirae* (Rosa, 1893), *E. intermedia* (Michaelsen, 1901), *E. paradoxa* (Cognetti, 1904) and *E. sineporis* (Omodeo, 1952). Two new species from Spain were included afterwards, *E. carpetana* Álvarez, 1970 and *E. albolineata* Díaz Cosín et al. 1989, Qiu and Bouché (1998a, d) accept the genus *Eiseniona* in which they include 17 species or subspecies, most of them distributed in the Balkans. However, they place *albolineata* and *carpetana* within the genus *Iberoscolex*; *gavarnica* and *paradoxa* within *Orodrilus* and *oliveirae* within *Koinodrilus* Qiu and Bouché (1998b, c). The diagnosis of *Eiseniona* by these authors is slightly different from the one by Omodeo and Rota (2004), mainly regarding details such as pigment absence, pinnate typhlosole or the presence of four pairs of seminal vesicles in 9, 10, 11 and 12.

Blakemore (2008) did not accept the genus *Eiseniona* and considered it as a synonym of *Aporrectodea*. This author highlighted that it was neither accepted by Bouché (1972), who included hemiandric forms such as *paradoxa* and *gavarnica* within the genus *Orodrilus* and the remainder within *Allolobophora*. Neither was it accepted by Zicsi (1981, 1982b) nor Mrcsic (1991), who note that "the diagnosis of this genus is deficient (the species are hard to distinguish from those of the genus *Aporrectodea*), so I ignore it." It is evident that the validity of *Eiseniona* is controversial and in this sense Blakemore (2008) stresses that "These issues need to be thoroughly resolved, with reference to types, before we can consider restoration of either *Eiseniona* or *Koinodrilus*".

Phylogenies recovered by molecular methods can aid to solve this problem by providing key information to support systematics and therefore approaching a natural system (Novo et al. 2011). On this topic Blakemore (2012a) states the need of basing the molecular analyses on the types of the genera. A molecular comparison with the type species *E. handlirschi* could not be carried out in this study due to lack of material. However, in the phylogenetic trees we present here, *E. gerardoii* clustered together with *E. albolineata* and it is clearly differentiated from the other genera. The assignment of this new species to the genus *Eiseniona* is further supported by the fact that analyses with 16S and 28S regions place it near *E. albolineata* and *E. oliveirae* within an unpublished phylogeny of lumbricids that includes most of their genera (Pérez Losada and Domínguez pers. comm.). Hence, the new species can be located within an explicit phylogenetic context, near *albolineata* and *oliveirae* regardless of their generic assignment.

Some of the features of our specimens, such as the lack of papillae or the presence of porophores in segment 15, are different from the ones described for most *Eiseniona*. However male porophores of *E. gerardoii* are relatively small and Omodeo's (1956) indicates in its diagnosis that in some instances small porophores might be present in the genus. Apart from that, most of the traits of *E. gerardoii* are compatible with those originally diagnosed as the generic features of *Eiseniona*. Moreover *E. gerardoii* shares with *E. albolineata* the white tissue developed on the dorsal vessel.

Considering all this data, we opt to include this new species, at least provisionally, within *Eiseniona* because it is the less troublesome position within the current genera system for Lumbricidae. This is suggested not only by morphological and ecological considerations but also by the molecular data placing it near *E. albolineata* and *E. oliveirae*.

The phylogeny of species historically included within *Iberoscolex*, *Koinodrilus* and *Eiseniona* will need to be thoroughly revised in the future, in order to clarify whether they represent good genera and to find a robust grouping of the species within genera, which does not seem possible exclusively with morphological tools. It is also noteworthy that within *Eiseniona* there is a group of species from Southern France and Iberian Peninsula and another one from Italy, Greece and Central and Eastern Europe. Future studies will unravel whether these two groups constitute independent phylogenetic units susceptible to be taxonomically divided.

A considerable effort is still necessary to establish a robust genera system based on phylogeny within lumbricids. This system should integrate the study of mitochondrial and nuclear markers with morphological characters and include representatives from all the proposed genera and type species. Until the moment when such big picture is available controversy on lumbricids' genera system will continue and different authors will apply subjective criteria.

Differences with other species of the genus

The most similar species to *E. gerardoii* regarding clitellum position and tubercula pubertatis is *E. intermedia*, but the last has a much greater size, its tubercula pubertatis start in

Table 3. Comparison of species living in the western part of the geographic range of *Eiseniona*. The type species *E. handlirschi* is included and the hemiandric *E. paradoxa* and *E. gavarnica* are excluded.

	<i>E. albolineata</i>	<i>E. carpetana</i>	<i>E. oliveirae</i>	<i>E. gerardoi</i>	<i>E. handlirschi</i>
Length (mm)	78–122 matures	52–74	85–110 * 30–86** 45***	21–40	50–60* 50–170** 50–95***
Segments	138–172	129–150	167 * (77) 100–131** 125***	89–124	120–130* 115–163** 78–119***
Colour	Grey, posterior white line	Rose violet	Light flesh tone* Brown or violet “in vivo”, greyish when fixed** Brown, red***	Red-brownish “in vivo”, posterior white line	Colourless* Colourless** Pale reddish***
Chetae	Separate 2.5 - 1.2 - 2.2 - 1 - 5	Separate	Closely paired* 6.7 – 1.3 – 6.2 – 1 – 11.8** Closely paired 9 – 1.5 – 7.5 – 1 - 18***	Paired 16 - 1.4 - 7 - 1 - 24	Closely paired 8 – 1.15 – 6 – 1 – 20***
First dorsal pore	(4/5) 5/6	4/5	4/5* (4/5) 5/6** 5/6***	(3/4) 4/5	From 4/5, usually 19/20** 17/18 to 23/24***
Spermathecae	10,11, pores 9/10, 10/11 near <i>d</i>	10, 11, pores 9/10, 10/11 <i>c</i>	10, 11, pores 9/10 10/11 near <i>c</i>	10, 11, large, iridescent, pores 9/10, 10/11 <i>cd</i>	9, 10, pores in 9/10 10/11
Clitellum	(24)25 – 30(31)	Annular in (1/2) 24)25 -1/2 31(31)	24–30* (23)24–29(30)** 24–29***	22,23–29,30	26–33* (25,26)27–32(33)** 25,26(27)- (32)33***
T. pubertatis	1/n 26 – 28(1/2 29)	25–30	24–30* 24–29,30** 1/2 25–28***	23-(27)28,29	29–32* (1/2 27,28)29–30 (31,32)** 1/n 28–31,32***
Gld. Morren	10–12, diverticula in 10	11- 12, no diverticula	10–13 diverticula in 10** 11–14, no diverticula***	10,11,12 small diverticula in 10	Diverticula in 10 10–13***
Nephridial vesicle	S - shaped	?	Curved, reclined***	J - shaped	Inverted J***
Typhlosole	Bifid initially, later simple	?	Simple	Simple, pleated	Circular, transversally pleated***
Seminal vesicles	9,10,11,12	9,10,11,12	9,11,12* 9, 10,11,12** 9,11,12***	9,11,12	9,11,12* 9,(10),11,12** 9,11,12***
Others	White tissue on top of the dorsal vessel.		*Rosa (1894) ** Díaz Cosín et al. (1985) *** Qiu and Bouché (1998b)	White tissue on top of the dorsal vessel	*Rosa (1897) **Bouché (1972) ***Omodeo and Rota (2004)

a more posterior segment and presents four pairs of seminal vesicles. In addition, it was only found in Bashkiria (Bashkortostan, Russia) (data from Omodeo 1956). The differences of *E. gerardoi* with the remaining species included within *Eiseniona* by Omodeo

(1956) and Qiu and Bouché (1998a, d) are clear in terms of the beginning of clitellum in segments 22,23 and the tubercula pubertatis in segment 23. A comparison of some characters of the species living in the western part of the geographic range of *Eiseniona* is shown in Table 3, excluding the hemiandric *E. paradoxa* and *E. gavarnica*.

Genetic divergence between *E. gerardoii* and *E. albolineata* (COI, uncorrected distances) is 14.09%, which is within the interval of uncertainty proposed by Chang and James (2011), but still near the 15% that these authors consider as indicative for different species in earthworms. Nevertheless there are enough morphological characters that permit the separation of the two species.

References

- Álvarez J (1970) Nuevas formas de oligoquetos terrícolas para la fauna de la Península Ibérica. Boletín de la Real Sociedad española de Historia Natural (Sección Biológica) 68: 17–24.
- Barros F, Carracedo F, Díaz Cosín DJ (1992) Karyological and allozymic study of *Allolobophora molleri* and several related taxa. Soil Biology & Biochemistry 24, 12: 1242–1255. doi: 10.1016/0038-0717(92)90100-C
- “BioBio, Biodiversity Indicators for European Farming Systems, Indicators for Biodiversity in Organic and Low Input Farming Systems” <http://www.biobio-indicator.org/index.php?l=1>
- Blakemore RJ (2008) An updated list of valid, invalid and synonymous names of Criodrilioidea and Lumbricoidea (Annelida: Oligochaeta: Criodrilidae, Sparganophilidae, Ailoscolecida, Hor-mogastridae, Lumbricidae, Lutodrilidae). A Series of Searchable Texts on Earthworm Biodiversity, Ecology and Systematics from Various Regions of the World - Supplemental. CD publication under ICZN (1999: Art. 8). <http://www.annelida.net/earthworm/> [accessed July, 2013]
- Blakemore RJ (2010) Unraveling some Kinki worms (Annelida: Oligochaeta: Megadrili: Megascolecidae) Part II. Opuscula Zoologica 42(2): 191–206.
- Blakemore RJ (2012a) On Schmarda’s lost worm and some newly found New Zealand species. Journal of Species Research 1(2): 105–132. doi: 10.12651/JSR.2012.1.2.105
- Blakemore RJ (2012b) Cosmopolitan Earthworms – an Eco-Taxonomic Guide to the Peregrine Species of the World. Vth Edition. VermEcology, Yokohama, 900 pp + 300 figs.
- Blakemore RJ (2013) Restoration of London type of first earthworm – *Lumbricus terrestris* Linnaeus, 1758 (Annelida: Oligochaeta: Lumbricidae), and setting aside of a ‘neo-neotype’. Opuscula Zoologica Budapest 44(2): 211–212. http://opuscula.elte.hu/opuscula44_2.htm
- Blakemore RJ, Grygier MJ (2011) Unraveling some Kinki worms (Annelida: Oligochaeta: Megadrili: Lumbricidae) Part III. Journal of Soil Organisms 83(2): 231–244.
- Blakemore RJ, Kupriyanova EK (2010) Unraveling some Kinki worms (Annelida: Oligochaeta: Megadrili: Moniligastridae) Part I. Opuscula Zoologica 40: 3–18.
- Blakemore RJ, Kupriyanova E, Grygier MJ (2010) Neotypification of *Drawida hattamimizu* Hatai, 1930 (Oligochaeta: Megadrili: Moniligastridae) and the first COI sequence from an earthworm type. ZooKeys 41: 1–29. doi: 10.3897/zookeys.41.374

- Bouché MB (1972) Lombriciens de France Écologie et Systématique. Institut National de la Recherche Agronomique. Annales de Zoologie Ecologie animale. Numéro hors-série. Paris, 671 pp.
- Briones MJI, Moran P, Posada D (2009) Are the sexual, somatic and genetic characters enough to solve nomenclatural problems in lumbricid taxonomy? *Soil Biology & Biochemistry* 41: 2257–2271. doi: 10.1016/j.soilbio.2009.07.008
- Chang CH, James S (2011) A critique of earthworm molecular phylogenetics. *Pedobiologia* 54 (supplement S3–S9).
- Chakrabarty P (2010) Genetypes: a concept to help integrate molecular phylogenetics and taxonomy. *Zootaxa* 2632: 67–68.
- Csuzdi Cs (2012) Earthworm species, a searchable database. *Opuscula Zoologica, Budapest*, 43(1): 97–99.
- Cognetti L (1904) Lombricidi dei Pirinei. *Bolletino dei Musei di Zoologia ed Anatomia comparata della R. Univesità di Torino* 19, 476: 1–14.
- Díaz Cosín DJ, Trigo D, Mato S (1989) Contribución al conocimiento de los lumbrícidos de la Península Ibérica. III. *Eiseniona albolineata* n. sp. *Boletín de la Real Sociedad española de Historia Natural (Sección Biológica)* 84, 3–4: 363–370.
- Díaz Cosín DJ, Trigo D, Mascato R (1992) Earthworms of Iberian Peninsula. Species list and some biogeographical considerations. *Soil Biology & Biochemistry* 24: 1351–1356. doi: 10.1016/0038-0717(92)90117-G
- Dupont L, Lazrek F, Porco D, King RA, Rougerie R, Symondson WOC, Livet A, Richard B, Decaëns T, Butt KR, Mathieu J (2011) New insight into the genetic structure of the *Allolobophora chlorotica* aggregate in Europe using microsatellite and mitochondrial data. *Pedobiologia* 54: 217–224. doi: 10.1016/j.pedobi.2011.03.004
- Easton EG (1983) A guide to the valid names of Lumbricidae (Oligochaeta). In: Satchell JE (Ed) *Earthworm Ecology from Darwin to vermiculture*. Chapman et Hall, London, 475–485. doi: 10.1007/978-94-009-5965-1_41
- Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47–50.
- Fernández R, Almodóvar A, Novo M, Gutiérrez M, Díaz Cosín DJ (2011) A vagrant clone in a peregrine species: phylogeography, high clonal diversity and geographical distribution in the earthworm *Aporrectodea trapezoides* (Dugès, 1828). *Soil Biology & Biochemistry* 43, 10: 2085–2093. doi: 10.1016/j.soilbio.2011.06.007
- Fernández R, Almodóvar A, Novo M, Simancas B, Díaz Cosín DJ (2012) Adding complexity to the complex: New insights into the phylogeny, diversification and origin of parthenogenesis in the *Aporrectodea caliginosa* species complex (Oligochaeta, Lumbricidae). *Molecular Phylogenetics and Evolution* 64: 368–379. doi: 10.1016/j.ympev.2012.04.011
- James SW, Porco D, Decaëns T, Richard B, Rougerie R, Erseus C (2010) DNA Barcoding Reveals Cryptic Diversity in *Lumbricus terrestris* L., 1758 (Clitellata): Resurrection of *L. herculeus* (Savigny, 1826). *PLoS ONE* 5(12): e15629. doi: 10.1371/journal.pone.0015629
- King RA, Tibble AL, Symondson WOC (2008) Opening a can of worms: unprecedented sympatric speciation within British lumbricid earthworms. *Molecular Ecology* 17: 4684–4698. doi: 10.1111/j.1365-294X.2008.03931.x

- Michaelsen W (1900) Oligochaeta. Das Tierreich 10. P. Friedlander and Sohn, Berlin, 575 pp.
- Mrsic N (1991) Monograph on earthworms (Lumbricidae) of the Balkans I, II. Slovenska akademija znanosti in umetnosti, Ljubljana 1881, 757 pp.
- Novo M, Almodóvar A, Díaz Cosín DJ (2009) High genetic divergence of hormogastrid earthworms (Annelida, Oligochaeta) in the central Iberian Peninsula: evolutionary and demographic implications. *Zoologica Scripta* 38: 537–552. doi: 10.1111/j.1463-6409.2009.00389.x
- Novo M, Almodóvar A, Fernández R, Trigo D, Díaz Cosín DJ (2010) Cryptic speciation of hormogastrid earthworms revealed by mitochondrial and nuclear data. *Molecular Phylogenetics and Evolution* 56: 507–512. doi: 10.1016/j.ympev.2010.04.010
- Novo N, Almodóvar A, Fernández R, Giribet G, Díaz Cosín DJ (2011) Understanding the biogeography of a group of earthworms in the Mediterranean basin—The phylogenetic puzzle of Hormogastridae (Clitellata: Oligochaeta). *Molecular Phylogenetics and Evolution* 61: 125–135. doi: 10.1016/j.ympev.2011.05.018
- Novo M, Fernández R, Fernández Marchán D, Gutiérrez M, Díaz Cosín DJ (2012) Compilation of morphological and molecular data, a necessity for taxonomy: The case of *Hormogaster abbatissae* sp. n. (Annelida, Clitellata, Hormogastridae). *Zookeys* 242: 1–16. doi: 10.3897/zookeys.242.3996
- Omodeo P (1956) Contributo alla revisione dei Lumbricidae. *Archivio Zoologico Italiano* 1956, 41: 129–212.
- Omodeo P, Rota E (2004) Taxonomic Remarks on the earthworms inhabiting the Western Alps. In: Moreno AG, Borges S (Eds) *Advances in Earthworm Taxonomy*. Editorial Complutense, Madrid, Spain, 220–259.
- Qiu JP, Bouché MB (1998a) Révision des taxons supraspécifiques de Lumbricoidea. *Documents Pédozoologiques et Intégrologiques* 3: 179–216.
- Qiu JP, Bouché MB (1998b) Contribution à la taxonomie des Avelonini trib. nov. (Oligochaeta: Lumbricidae). *Avelona* gen. nov., *Koinodrilus* gen. nov. et *Nicodrilus cuendeti* sp. nov. *Documents Pédozoologiques et Intégrologiques* 4: 109–116
- Qiu JP, Bouché MB (1998c) Contribution à la taxonomie des Dendrobaenini trib. Nov. (Oligochaeta: Lumbricidae). *Iberoscolex* gen. nov. et nouveaux taxons de *Dendrobaena* Eisen, 1874 et *Satchellius* Gates, 1975. *Documents Pédozoologiques et Intégrologiques* 4: 153–163.
- Qiu JP, Bouché MB (1998d) Liste classe des taxons valides de lombriciens (Oligochaeta, Lumbricoidea), après l'étude des trois cinquièmes d'entre eux. *Documents Pédozoologiques et Intégrologiques* 4: 181–200.
- Rodríguez T, Trigo D, Díaz Cosín DJ (1997) Biogeographical zonation of the western Iberian peninsula on the basis of the distribution of earthworm species. *Journal of Biogeography* 24: 893–901. doi: 10.1046/j.1365-2699.1997.00112.x
- Rosa D (1894) *Allolobophora ganglbaueri* ed *A. oliveirae* nuove specie di lumbricidi europei. *Bollettino dei Musei di Zoologia ed Anatomia comparata della R. Università di Torino* 9, 170: 1–3.
- Rosa D (1897) Nuovi lombrichi dell'Europa orientale. *Bollettino dei Musei di Zoologia ed Anatomia comparata della R. Università di Torino* 12, 269: 1–5.

- Rota E (2013) *Eiseniona*. In: Fauna Europaea version 2.6.2. <http://www.faunaeur.org>, http://www.faunaeur.org/full_results.php?id=178037 [accessed january 2014]
- Sánchez EG, Jesús JB, Muñoz B, Parejo C (1998) Lombrices de tierra de Cáceres, Badajoz y Huelva. I. Géneros *Allolobophora*, *Eisenia*, *Eiseniella* y *Lumbricus* (Annelida, Oligochaeta). Boletín de la Real Sociedad española de Historia Natural (Sección Biológica) 91(3–4): 31–40.
- Sánchez EG, Jesús JB, Muñoz B, Parejo C (1999) Lombrices de tierra de Cáceres, Badajoz y Huelva. II. Familias *Acanthodrilidae* (género *Microscolex*), *Megascolecidae* (género *Amyntas*), *Ocnerodrilidae* (género *Eukerria*) y otros Lumbricidae (géneros *Dendrobaena*, *Octodrilus* y *Octolasion*). Boletín de la Real Sociedad española de Historia Natural (Sección Biológica) 95(1–2): 57–62.
- Zicsi A (1982) Verzeichnis der bis 1971 beschriebenen und revidierten Taxa der Familie Lumbricidae (Oligochaeta). Acta Zoologica Academiae Scientiarum Hungaricae 28: 421–454.

