RESEARCH ARTICLE



DNA barcoding and morphological analyses revealed validity of *Diadema clarki* Ikeda, 1939 (Echinodermata, Echinoidea, Diadematidae)

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Abstract

A long-spined sea urchin *Diadema*-sp reported from Japanese waters was genetically distinct from all known *Diadema* species, but it remained undescribed. Extensive field surveys in Japan with molecular identification performed in the present study determined five phenotypes (I to V) in *Diadema*-sp according to the presence and/or shape of a white streak and blue iridophore lines in the naked space of the interambulacral area. All phenotypes were distinct from *Diadema setosum* (Leske, 1778) and *Diadema savignyi* (Audouin, 1829), of which a major type (I) corresponded to *Diadema clarki* Ikeda, 1939 that was questioned and synonymized with *D. setosum* by Mortensen (1940). The holotype of *D. clarki* has not

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been found, but three unlabeled dried tests of *Diadema* were found among Ikeda's original collection held in the Kitakyushu Museum of Natural History and Human History, Fukuoka, Japan. A short mtDNA *COI* fragment (ca. 350bp) was amplified from one of the tests, and the nucleotide sequence determined (275bp) was nearly identical with that of *Diadema*-sp. Arrangements of the primary tubercles on the coronal plates in *Diadema*-sp and the museum specimen also conformed with *D. clarki*, indicating that *Diadema*-sp is identical to *D. clarki* and a valid species. Narrow latitudinal distribution (31°N to 35°N) of *D. clarki* in Japan was observed, where it co-existed with abundant *D. setosum* and rare *D. savignyi*. No *D. clarki* was found in the southern islands in Japan, such as Satsunan Islands to Ryukyu Islands and Ogasawara Island, where *D. setosum* and *D. savignyi* were commonly observed.

Keywords

Diadema clarki, Diadematidae, DNA barcoding, Echinoidea

Introduction

Long-spined sea urchins of the genus Diadema Gray, 1825 are abundant, widespread and ecologically important species in tropical to temperate areas (Muthiga and Mc-Clanahan 2007). Morphological similarity among Diadema species has made systematics a difficult task (Clark 1925, Mortensen 1940, Lessios et al. 2001, Muthiga and McClanahan 2007). Although Mortensen (1940) recognized six extant species in this genus, Diadema antillarum Philippi, 1845, Diadema ascensionis Mortensen, 1909, Diadema mexicanum A. Agassiz, 1863, Diadema paucispinum A. Agassiz, 1863, Diadema savignyi (Audouin, 1829), and Diadema setosum (Leske, 1778), considerable room for systematic revision has remained. Ikeda (1939) described a new species of Diadema from Japan under the name Diadema clarki, but Mortensen (1940) synonymized this new species with D. setosum. Baker (1967) added a new species Diadema palmeri Baker, 1967 from the north coast of New Zealand, and Pawson (1978) demoted D. ascensionis to a subspecies of D. antillarum. Advancements in molecular genetic analyses have shed further light on Diadema systematics, in which Lessios et al. (2001) using mitochondrial DNA (mtDNA) sequence analysis reported that D. ascensionis was nested within D. antillarum. Lessios et al. (2001) also detected substantially divergent sub-clades within D. antillarum, D. paucispinum and D. setosum, which strongly suggest the presence of cryptic species within the nominal species. Rodríguez et al. (2013) using mtDNA and morphological analyses raised eastern Atlantic population of D. antillarum to a new species Diadema africanum Rodríguez et al. 2013, which corresponds to the *D. antillarum*-b sub-clade reported by Lessios et al. (2001). Lessios et al. (2001) further found a genetically distinct species among specimens originally identified as D. savignyi or D. setosum in Japan and Marshal Islands, and tentatively designated them as Diadema-sp.

Recently, Chow et al. (2014) analyzed mtDNA of *D. savignyi*-like individuals from Sagami Bay (Kanagawa Prefecture, Pacific side) and Iki Island (Nagasaki Prefecture, Japan Sea side) in Japan and found these had the same mtDNA sequence as those that Lessios et al. (2001) called *Diadema*-sp. Considering the similar geographic

origin, Lessios et al. (2001) suspected that *Diadema*-sp might be *D. clarki* Ikeda, 1939. Ikeda (1939) proposed the conspicuous white streaks running along the interambulacral zones and the arrangement of interambulacral tubercles to be diagnostic characteristics of *D. clarki*, which corresponded to those of *Diadema*-sp observed by Chow et al. (2014). Ikeda (1939) mentioned that "The type specimen is kept in the Zoological Laboratory, Kyushu Imperial University", but he gave no further deposition information on the type specimen of *D. clarki*. All of Ikeda's collections were not maintained at the laboratory, and we found meanwhile that the collection was moved to the Kitakyushu Museum of Natural History and Human History, Fukuoka, Japan. It was unfortunate that the labels of large number of specimens seemed to have been lost upon transfer, and three dried tests of *Diadema* found in the Ikeda's original collection were not the exception. However, a short DNA fragment was amplified from one of these tests, and hence this dried test was utilized as a reference specimen.

In this study, molecular and phenotypic evidence are provided that *D. clarki* is *Diadema*-sp and hence a valid species, and we report the geographic distribution of *D. clarki* based on extensive field surveys.

Materials and methods

The twenty localities where field observations and/or collecting of Diadema specimens were carried out in Japanese waters are shown in Figure 1A-T. Based on the phenotypes to discriminate among D. setosum, D. savignyi and Diadema-sp as described in Chow et al. (2014), we selected *Diadema* individuals possessing characteristics neither of D. setosum nor D. savignyi. Although orange ring on the anal cone and white spots in naked space of the interambulacral areas are known to be characteristics of D. setosum, we found some individuals having the orange ring but no white spot during present survey. These "unusual" individuals were also determined to be Diadema-sp. Detailed locality information are presented in Table 1. Since many Diadema-sp might have been miss-identified as D. savingnyi in Japan mainland (see Chow et al. 2014), we recorded the number of Diadema-sp and D. savignyi encountered during the field survey. A quantitative survey of the phenotype variants of Diadema-sp was attempted in samples from Kanagawa (Figure 1A), Mie (Figure 1E), Nagasaki (Figure 1I-K), and Kagoshima (Figure 1M) Prefectures. A monthly scuba diving survey has been performed in order to investigate abundance and fecundity of *Diadema* spp. in Kanagawa Prefecture. The Diadema-sp individuals collected were transferred to aquaria, in which phenotype variation was studied. In Mie, Nagasaki, and Kagoshima Prefectures, Diadema-sp individuals encountered during scuba or skin diving surveys were photographed, and phenotype variation was examined based on photograph images. Ad hoc photographing in situ or in aquarium was performed in other areas, using which species identification was attempted. Of four D. savignyi individuals found and photographed at Motobu in Okinawa Island (Figure 1R), three (designated as OK2 to OK4) were transferred to the laboratory for subsequent analysis. Data of D. savignyi from Sesoko in Okinawa

Locality	Prefecture	Figure 1	Lat (N)	Long (E)	Date	\mathbf{n}^{\dagger}
Arasaki	Kanagawa	A	35°11'50"	139°35'59"	Dec. 2011 [‡] March-Sep. 2014	>400:0
Tateyama	Chiba	В	34°59'26"	139°49'28"	March to June, 2014	3:0
Mera	Shizuoka	С	34°39'39"	138°47'10"	May 2 and 23, 2015	22:0
Shikine-jima	Tokyo	D	34°19'13"	139°13'11"	Aug. 6, 2015	21:0
Haida-ura	Mie	E	33°59'48"	136°15'39"	March 10, 2012; April 15, 2015	70:0
Kushimoto	Wakayama	F	33°28'33"	135°44'29"	Sep. 29, 2014	1:3
Uachiia iima	Talma	C	33°05'53",	139°46'30",	Feb. 26, 2007	0:1
	Токуо	G	33°07'20"	139°49'00"	July 30-31, 2015	4:22
Uchidomari	Ehime	Н	32°56'31"	132°29'14"	Oct. 26, 2014	9:1
Iki-no-shima	Nagasaki	Ι	33°44'58"	129°38'56"	Sep. 2, 2014	37:0
Ojika	Nagasaki	J	33°11'05"	129°04'21"	July 19, 2014	102:0
Mie	Nagasaki	K	32°48'	129°45'	May 30, 2014	36:0
Shibushi	Kagoshima	L	31°27'55"	131°08'13"	May 17, 2014	2:0
Kaimon	Kagoshima	М	31°10'39"	130°33'14"	Oct. 22, 2014	19:0
Tanega-shima	Kagoshima	N	30°49'	131°02'	April 26, 2015	0:2
Yaku-shima	Kagoshima	0	30°27'	130°30'	Feb. 10, 2004	0:2
Amami Ohshima	Kagoshima	Р	28°24'12"	129°27'15"	July 29, 2008	0:4
Ogasawara	Tokyo	Q	27°05'44"	142°11'58"	June 21, 2015	0:26
Motobu	Okinawa	R	26°39'16"	127°52'44"	July 16, 2014	0:4
Sesoko	Okinawa	S	26°38'09"	127°51'55"	May, 2013 [‡]	0:4
Ishigaki-jima	Okinawa	Т	24°27'	124°12'	Oct., 2013 [‡]	0:7
Tulamben, Bari	(Indonesia)	not shown	8°16'29" [§]	115°35'40"	March 8, 2015	0:2

Table I. Locality information for field survey and number of *Diadema*-sp and *D. savignyi* observed.

[†] Number of individuals (*Diadema*-sp: *Diadema savignyi*) observed. [‡] Data from Chow et al. (2014). § Southern hemisphere.

Island and Ishigaki-jima (Figure 1S and T) were obtained from previous study (Chow et al. 2014).

Six individuals of *Diadema*-sp (designated as AT1 to AT3, AR54, AR59, AR70) showing phenotypic variation were chosen among the specimens collected at Arasaki during March to August 2014 (Table 2) and photographed in an aquarium. Tube feet of these six specimens collected in Arasaki along with those of three *D. savignyi* collected at Motobu (Table 2) were preserved in 70 % ethanol. Remaining bodies were fixed in neutralized 10% formaldehyde-sea water solution for two days, rinsed in running tap water overnight and transferred to 80 % ethanol. These samples were transferred to 70 % ethanol several months later and deposited to the Kanagawa Prefectural Museum of Natural History, Odawara, Kanagawa, Japan (Table 2). Three dried tests of *Diadema* were found among Ikeda's original collection held in the Kitakyushu Museum of Natural History and Human History, but these tests were accompanied with no label and we numbered them as IK1 to IK3 (Table 2). Considering research field of Prof. Ikeda, these specimens were likely from Kumamoto, Nagasaki or Fukuoka Prefectures, all in Kyushu, Japan. Pieces of dried tissue remains from the base of spines or from the corona were collected and preserved in 70% ethanol.



Figure 1. Localities where field observation and sampling of *Diadema* were performed. See Table 1 for detailed information. A Arasaki (Kanagawa Prefecture) B Tateyama (Chiba) C Mera (Shizuoka) D Shi-kine-jima (Tokyo) E Haida-ura (Mie) F Kushimoto (Wakayama) G Hachijo-jima (Tokyo) H Uchidomari (Ehime) I Iki-no-shima (Nagasaki) J Ojika (Nagasaki) K Mie (Nagasaki) L Shibushi (Kagoshima) M Kaimon (Kagoshima) N Tanega-shima (Kagoshima) O Yaku-shima (Kagoshima) P Amami Ohshima (Kagoshima) Q Ogasawara (Tokyo) R Motobu (Okinawa) S Sesoko (Okinawa) T Ishigaki-jima (Okinawa). *Diadema setosum* was observed in all areas surveyed. No *Diadema savignyi* but *Diadema*-sp were observed at localities with yellow circle, Both *D. savignyi* and *Diadema*-sp were observed at localities with white circle, No *Diadema*-sp but *D. savignyi* were observed at localities with red circle.

All tissues fixed in ethanol and extracted DNA are kept in the National Research Institute of Fisheries Science, Kanagawa, Japan.

Molecular analysis

Crude DNAs were extracted from the ethanol tissues preserved in ethanol and used for PCR amplification. In addition to the primers (COI120F and COI1300R) previously described (Chow et al. 2014), three internal primers (COI330F: 5'-TGATCAGTYTT-TATCACCGC-3'; COI531F: 5'-ATGATTTCTCATGTAATTGC-3'; COI874R: 5'-AGTACAACGTCTATAGATGA-3') were designed and used in this study. PCR amplification, nucleotide sequencing and phylogenetic analysis were performed as described in Chow et al. (2014).

Sample			Test size	(mm)		Collection		GenBank
No.	Voucher	Phenotype	diameter	height	locality	date	depth (m)	Accession No.
AT1	KPM-NJL000035	Ι	42.0	22.4	Arasaki	Mar. 24, 2014	2	LC037355
AT2	KPM-NJL000036	II	46.5	19.6	Arasaki	Mar. 24, 2014	3	LC037356
AT3	KPM-NJL000037	III	54.0	22.2	Arasaki	Mar. 24, 2014	2	LC037357
AR59	KPM-NJL000039	IV	44.0	19.2	Arasaki	July 28, 2014	2	LC037358
AR54	KPM-NJL000038	V	67.0	32.6	Arasaki	June 25, 2014	3	LC037359
AR70	KPM-NJL000040	V	53.8	20.8	Arasaki	Aug. 29, 2014	2	LC037360
IK1	KMNH IvR 500879	-	88.5	38.6	Kyushu	1933 or 1934	-	-
IK2	KMNH IvR 500880	-	65.4	36.9	Kyushu	1933 or 1934	-	-
IK3	KMNH IvR 500788	-	64.2	31.6	Kyushu	1933 or 1934	-	LC037361
OK2	KPM-NJL000041	-	91.7	38.6	Motobu	July 16, 2014	2	LC037362
OK3	KPM-NJL000042	-	64.0	31.5	Motobu	July 16, 2014	2	LC037363
OK4	KPM-NJL000043	-	40.0	19.5	Motobu	July 16, 2014	2	LC037364

Table 2. Information of five phenotypes (I to V) of six *Diadema*-sp individuals collected in Arasaki area, three museum specimens.

Results

Phenotype analysis

Diadema-sp

Underwater images of several phenotypes in D. setosum, D. savignyi and Diadema-sp were presented in Chow et al. (2014). Phenotype variants in *Diadema*-sp observed in the present study and reference images of D. setosum and D. savignyi obtained in the previous study (Chow et al. 2014) are shown in Figures 2 and 3. D. setosum had characteristics of five white spots in naked space of the interambulacral areas and orange ring on the anal cone (Figure 3G). Many individuals of this species had small blue iridophore dots aligned along the naked space of the interambulacral areas. D. savignyi had characteristics of Y-shaped blue iridophore lines (YBIL) running along the naked space of the interambulacral areas and no orange ring on the anal cone (Figure 3H). Some D. savignyi had small white crescent at the fork region of YBIL but never resembled white spot of *D. setosum*. Three phenotypes in *Diadema*-sp were reported in Chow et al. (2014), corresponding to those presented in Figure 2 and Figure 3A, B. YBIL shape of Diadema-sp was substantially different from that of D. savignyi (see also Chow et al. 2014). We have never observed D. savignyi in monthly survey performed at Arasaki area since 2011, but encountered a few Diadema individuals having orange ring on the anal cone with orange spot at the fork region of YBIL but no white spot. We here determined five phenotype variants (I to V) (Figure 2 and Figure 3A-F) in *Diadema*sp. Phenotype I was the most common, having conspicuous white streak at the fork region of the intact YBIL (Figure 2), corresponding to the description of *D. clarki* by Ikeda (1939). The other phenotypes had no white streak. YBIL of phenotype II was intact (Figure 3A), while that in the other phenotypes was broken (Figure 3B-E). Phe-



Figure 2. Underwater aboral view of phenotype I of *Diadema*-sp, KPM-NJL000035, original specimen number is AT1.

notype III had broken YBIL (Figure 3B). Phenotype IV had orange ring on the anal cone (Figure 3C). Phenotype V was similar to phenotype IV but had small orange dot at the fork region of broken YBIL (Figure 3D, E). White streaks in some individuals were small (not shown) and some individuals had red streaks (Figure 3F), but all these variants were classified as phenotype I.

These characteristics in living specimen conspicuous underwater were not well preserved after fixation (Figure 4). In the preserved specimens, YBILs of all phenotypes were completely disappeared, while white streak in phenotype I (Figure 4A) and orange ring and orange dot in phenotype V (Figure 4D) were barely seen.

Museum specimens

Aboral views of the dried test of IK3 are presented in Figure 5. No PCR amplification was observed in the other museum specimens (IK1 and 2). A streak-like white line was observed on the naked space of the interambulacral areas, and the outer and inner series of primary tubercles initiated on the 3rd and 5th coronal plates, respectively (Figure 5B). These correspond to Ikeda's (1939) description on *D. clarki* and to observations by Chow et al. (2014) on *Diadema*-sp.



Figure 3. Underwater aboral views of four phenotypes (**A–E**) and color variants of phenotype I (**F**) of *Diadema-sp*, *Diadema setosum* (**G**), and *Diadema savignyi* (**H**). **A** KPM-NJL000036, original specimen number is AT2 designated as phenotype II **B** KPM-NJL000037, original specimen number is AT3 designated as phenotype III **C** KPM-NJL000039, original specimen number is AR59 designated as phenotype IV **D** KPM-NJL000038, original specimen No. is AR54 designated as phenotype V **E** KPM-NJL000040, original specimen No. is AR70 designated as phenotype V **F** color variant of phenotype I observed at Haida-ura in Mie Prefecture (Figure 1E) photographed by T. Ishikawa **G** *Diadema setosum* (DST2 in Chow et al. (2014)) **H** *Diadema savignyi* (DSV23 in Chow et al. (2014)).



Figure 4. Preserved specimen of phenotypes I (**A**), II (**B**), III (**C**) and V (**D**) of *Diadema*-sp, corresponding to AT1 (KPM-NJL000035) in Figure 2, and AT2, AT3 and AR54 (KPM-NJL000036–KPM-NJL000038) in Figures 3A, B and D, respectively. All scale bars = 10 mm.

Genetic analysis of museum and field specimens of Diadema

Approximately 1,100 bp fragments could be amplified in three *D. savignyi* (OK) and six *Diadema*-sp (AT and AR) individuals using a primer pair (COI120FxCOI1300R). All possible primer combinations were tested in three museum specimens (IK1 to IK3), but successful amplification (c.a. 350 bp fragment) was obtained only in IK3 by one primer pair (COI531FxCOI874R). Nested PCR was also attempted for the other museum specimens, but no amplification was observed. IK3 was therefore designated as a reference specimen of the Ikeda's collection and deposited to the Kitakyushu Museum of Natural History and Human History (voucher: KMNH IvR 500788). Nucleotide sequences determined were 275 bp for IK3, 411 bp for AR samples, 440 bp for OK samples, and 944–950 bp for AT samples. These sequences are available in DDBJ/EMBL/GenBank database (LC037355 to LC037364). Using MEGA v6 (Tamura et al. 2013), these sequences were aligned with several sequences of *D. setosum*, *D. savignyi* and *Diadema*-sp previously published by Lessios et al. (2001) and Chow et al. (2014), in which the gamma-corrected Kimura's two parameter (K2P) distance was selected as the best-fit model for nucleotide substitution. The phylogenetic analysis



Figure 5. Aboral view (**A**) and enlarged view of a naked space of the interambulacral area (**B**) in a reference dried specimen of *Diadema* found in Ikeda's collection. KMNH IvR 500788, original specimen No. is IK3. White streak-like remnant can be seen (**A**, **B**), and the outer and inner series of primary tubercles initiated on the 3^{rd} and 5^{th} coronal plates, respectively (**B**). Scales bars = 20 mm (**A**) and 10 mm (**B**).

	IK3	Diadema-sp	D. savignyi	D. setosum-a	D. setosum-b
IK3 (1)	-				
Diadema-sp (14)	0.16±0.09	0.26±0.09			
D. savignyi (9)	12.06±2.39	12.14±1.56	1.11±0.25		
D. setosum-a (7)	13.13±2.81	16.96±1.93	18.50±2.21	0.84±0.19	
D. setosum-b (2)	12.72±2.79	13.89±2.07	19.30±2.61	7.22±1.24	0.00±0.00

Table 3. Mean percent nucleotide sequence divergence (K2P±SE) within (on the diagonal) and between (below diagonal) species. Number of individuals within brackets.

See Figure 6 for nucleotide sequences obtained from database.

clearly indicates that specimen IK3 and the six *Diadema*-sp individuals collected in this study (AT and AR specimens) are clustered together within a unique clade, distinct from other clades (Figure 6). Mean nucleotide sequence divergences (K2P: pairwise deletion option) within and between species are presented in Table 3. Average K2P between IK3 and *Diadema*-sp was 0.16±0.09%, which was well within the intraspecific divergence values of *Diadema* (see Lessios et al. 2001, Chow et al. 2014). This estimate is much smaller than those between IK3 and *D. savignyi* (12.06±2.35%), IK3 and *D. setosum*-a (13.13±2.84%), and IK3 and *D. setosum*-b (12.72±2.80%). These indicate that all *Diadema*-sp phenotypes and IK3 are conspecific.

Ecology

Diadema-sp was seen in the subtidal zone, ranging to depths of 8 m but no further attempt was performed to investigate their distribution in deeper zones. Both *D. setosum* and *Diadema*-sp were observed in the same habitats and depths, but the former had tendency to aggregate and the later was usually seen as solitary specimens; in consequence both usually did not occur side by side. Although relative abundances of *D. setosum* and *Diadema*-sp were not quantitatively investigated, the former species was relatively abundant and ubiquitously observed throughout all the areas examined. However, after the severe winters of 2014 and 2015 (January to February), *Diadema*sp were observed to be predominated at Arasaki area, suggesting that it may be more tolerant to cold water than *D. setosum*. In addition, *D. savignyi* may be less tolerant to lower temperatures than *D. setosum*, since *D. savignyi* was never found at Arasaki area and is not common around Japan mainland.

Distribution and phenotype frequency

In contrast to the ubiquitous distribution of *D. setosum* throughout the survey areas, *Diadema*-sp was only observed in a narrow latitudinal range around Japan mainland, from Kanagawa (35°11' N) to Kagoshima (31°10' N) (see Figure 1 and Table 1). No



Figure 6. Neighbor-joining phylogenetic tree drawn using from *COI* sequence data. Bootstrap support (>50%) after 1,000 replications is shown at each node. Italic accession numbers with dagger (AY012732, AY012733, AY012742–AY012747) are from Lessios et al. (2001) and red accession numbers with asterisk (AB900024, AB909927, AB909929–AB909931, AB909933–AB909935, AB909942, AB909945, AB909947, AB909949, AB909955, AB909957) are from Chow et al. (2014).

Diadema-sp was found in the Satsunan Islands (Tanega-shima, Yaku-shima, and Amami Oshima) to the Ryukyu Archipelago (Figure 1N–P, R–T), nor in Ogasawara Island (Figure 1Q), where *D. setosum* and *D. savignyi* were commonly observed. *D. savignyi*

Kanagawa		Mie		Kagoshima				
Phenotype		Arasaki		Haida-ura	Iki Isl.	Ojika	Mie	Kaimon
	June 2014	July 2014	Sep. 2014	April 2015	Sep. 2014	July 2014	May 2014	Oct. 2014
Ι	64.1	58.8	71.4	73.1	64.6	52.6	38.9	52.6
II	14.8	4.1	7.7	20.9	17.7	21.1	30.5	21.1
III	12.0	27.8	15.4	3.0	17.7	10.5	16.7	15.8
IV	6.3	6.2	3.3	3.0	0.0	5.3	11.1	10.5
V	2.8	3.1	2.2	0.0	0.0	10.5	2.8	0.0
total (n)	142	97	91	67	34	57	36	19

Table 4. Relative abundance (percentage) of the five phenotypes of *Diadema*-sp observed in Kanagawa, Mie, Nagasaki and Kagoshima Prefectures.

was rare around Japan mainland and observed with *Diadema*-sp and *D. setosum* at Kushimoto in Wakayama (Figure 1F) and Uchidomari in Ehime (Figure 1H) (see also Table 1). The three species also co-existed in Hachijo-jima (Figure 1G), where *Diadema*-sp became a minority (Table 1).

Frequency of the five phenotypes observed in Kanagawa, Mie, Nagasaki and Kagoshima Prefectures is presented in Table 4. Phenotype I was most commonly observed at all localities and during all sampling days, followed by phenotypes II and III. Phenotypes IV and V were much less frequently observed. Frequency distribution of these phenotypes was found significantly different among sampling days at Arasaki area (χ^2 test, P = 0.024) but not among three localities in Nagasaki (P = 0.089). Significant heterogeneity in the phenotype frequency was observed among pooled samples of the four Prefectures (P < 0.001).

Discussion

The present investigation together with previous studies (Lessios et al. 2001, Chow et al. 2014) revealed phenotypic and genetic characteristics of *Diadema*-sp distinct from congeneric species (*D. setosum* and *D. savignyi*) occurring in Japan. The conspicuous white streak of phenotype I and arrangements of the outer and inner series of primary tubercles observed in *Diadema*-sp correspond to the description on *D. clarki* by Ikeda (1939). One of Ikeda's specimen (IK3) was genetically identified to be *Diadema*-sp and the arrangements of the outer and inner series of primary tubercles were similar to *D. clarki*. Furthermore, the present distributions of *Diadema*-sp correspond to that of *D. clarki*. These indicate that *Diadema*-sp appears to be *D. clarki* and a valid species. Ikeda (1939) did not provide specific size data and museum repository numbers for his type specimens of *D. clarki*, but he only stated that the largest specimen was 65 mm in diameter among 22 individuals collected in 1933 and 1934. IK1 (88.5 mm in diameter) is too large to be in his type series, while IK2 and 3 (65.4 and 64.2 mm, respectively) could be. Aboral view photograph of a dried

D. clarki test presented in Ikeda (1939) could not be compared with IK2 and 3, since it was of "smaller individual" (Ikeda 1939).

Among the several characters of D. clarki described by Ikeda (1939), the white or red streaks running along the interambulacrals may be the most distinguishing character from other species in living specimen. Ikeda (1939), however, did not mention any variation in the white streak appearance, and he might have considered only the phenotype I to be D. clarki. Size and shape of the white (or red) streak appear to vary (see also Chow et al. 2014), of which a smaller one might be miss-identified as white spot of D. setosum and individuals having no white streak might be miss-identified as D. savignyi. In fact, photographs shown as D. savignyi in previous reports (Shigei 1986, Kohtsuka 2005) are obviously of D. clarki. Although the tridentate pedicellariae may be a diagnostic characteristic for species identification in the genus *Diadema* (Coppard and Campbel 2006a), Mortensen (1940) and Ikeda (1939) both did not consider the tridentate pedicellariae of D. clarki to be specific characteristic for discriminating it from D. setosum. As Mortensen (1940) examined preserved specimens of D. clarki, the white streak might have been obscured and hence regarded to be a variant of diagnostic white spot of *D. setosum*. Although YBIL of D. clarki and D. savignyi and small blue iridophore dots of D. setosum may be better diagnostic keys for discriminating all these three species as already demonstrated by Coppard and Campbell (2006b), Ikeda (1939) did not mention this character at all. Characters not suitable for preservation might be neglected or unnoticed. Thus, underwater coloration images of living specimens are necessary for properly identifying D. clarki, although the white streak may occasionally remain observable even after preservation. These characteristics of *D. clarki*, distinct from *D. setosum* and *D. savignyi*, were noted for some of the samples collected from the Seto Marine Biological Laboratory, Shirahama, Japan, and used in Lessios et al. (2001), but specimens were assumed to be hybrids of *D. setosum* and *D. savignyi* (J.S. Pearse, pers. comm.).

Ikeda (1939) stated that all 22 *D. clarki* individuals observed had an orange ring at the end of the anal cone as in *D. setosum*, and it was observed that *D. clarki* individuals (phenotypes IV and V) have this same orange ring (Figure 3C–E) but much less frequently. There is another discrepancy between Ikeda (1939) and our observations: Ikeda (1939) described his *D. clarki* individuals to have the white streak and the orange ring together, but such a combination has never been observed by the authors. Assuming the phenotypic characters to be heritable, genetic drift may explain the change of a phenotype frequency. However, fixation of a phenotype combination in Ikeda's time and the separation of these phenotypes at present time are unexplainable by genetic drift alone. The size or color variation in the white streak, orange ring and orange dot described in the present study might be partially an environment-associated character, which may be responsible for type frequency difference between localities or among sampling dates (Table 4).

Since experimental hybridization between *D. setosum* and *D. savignyi* produced viable hybrids (Uehara et al. 1990) and occurrence of natural hybrids between *D. setosum*, *D. savignyi* and *D. paucispinum* was reported by Lessios and Pearse (1996), hybridization between *D. clarki* and other species may not be ruled out. All phenotype variants of *D. clarki* had nearly identical *COI* sequences one another, but asymmetrical fertilization success may be the case for *D. clarki* as observed in strongylocentroid sea urchins (Addison and Pogson 2009). Since *D. clarki* was not recognized as a valid species and the phenotype variants were similar to some of the suspected hybrids reported by Lessios and Pearse (1996), it is highly probable that the suspected hybrids specifically from Shirahama, Japan, examined by them may be *D. clarki*. Nevertheless, genetic analysis on nuclear genome may be necessary for investigating occurrence of natural hybrids.

So far as published data, *D. clarki* is not observed in the Ryukyu Archipelago (Lessios et al. 2001, Chow et al. 2014), and no *D. clarki* were observed in Indonesia. On the other hand, the distribution was genetically confirmed in remote tropical islands such as Marshal Island (Lessios et al. 2001). Given that some of the suspected hybrids observed by Lessios and Pearse (1996) were *D. clarki*, the distribution could be much wider extending to Papua New Guinea and Indonesia.

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RESEARCH ARTICLE



Redescription and designation of a neotype for Caecum floridanum (Littorinimorpha, Truncatelloidea, Caecidae) with a characterization of the protoconch and growth stages

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Abstract

After an extensive search for the type specimens of *Caecum floridanum* Stimpson, 1851, we believe that these specimens may have been either lost or destroyed in the Chicago fire (1871). This paper presents a redescription of the species and a neotype is designated based on material from the type locality (Florida). Protoconch and growth stages of *C. floridanum* are described and illustrated herein. The teleoconch IV of *C. floridanum* is characterized by strong, wide, low, rounded, closely arranged axial ribs, except last three to four preceding the aperture, which are larger and more widely separated. *Caecum compactum* Dall, 1892 is here synonymized under *C. floridanum*. *Caecum annulatum* Emmons, 1858 and *C. dux* Folin, 1871 are not considered synonyms of *C. floridanum* in this report.

Keywords

Micromollusks, Caenogastropoda, Caecinae, Western Atlantic, South America, continental shelf, shallow waters

Introduction

Stimpson (1851: 112) described the marine gastropod *Caecum floridanum* from specimens collected on the coast of Florida (USA). Stimpson's description for this species is brief, with no illustration and no information on the type material, depository institution(s) or habitat.

According to Dance (1966: 302), shells studied by Stimpson were deposited in the Chicago Natural History Museum (CNHM), currently called the Field Museum of Natural History (FMNH), Illinois, Chicago, USA, and destroyed in the Chicago fire (1871). However, the institution destroyed was the Chicago Academy of Sciences (CAS), where Stimpson had stored the malacological material studied (Hendrickson and Beecher 1972). According to Bartsch et al. (1946: 10) and Warén (1980), types described by Stimpson were deposited in the "J.G. Jeffreys" collection and Zoological Museum of the University of Copenhagen (ZMUC), respectively. However, Cernohorsky (1974) and Dr Ole S. Tendal (Curator of Mollusca – personal communication, June 2008) found no specimens of C. floridanum in the ZMUC collection. Moreover, a number of years after Jeffreys death, his conchological collection was given by Dall to the U.S. National Museum of Natural History (USNM, Smithsonian Institution) (Dance 1966: 289-290, Warén 1980: 3). Some years later, a part of the material collected during the Lightning, Porcupine and Triton expeditions was given to BMNH (actually NHMUK) (Warén 1980: 4). However, based on information from the respective curators, no type material for C. floridanum was found in either institution. Thus, we conclude that all types of this species were deposited in the CAS and lost or destroyed in the Chicago fire.

Caecum floridanum is a shallow water species widespread throughout the Western Atlantic and associated with a variety of ecosystems and biotopes (Abbott 1974, Vokes and Vokes 1983, Leal 1991, Lightfoot 1992, Diaz and Puyana 1994, Bandel 1996, Rios 2009, Tunnell et al. 2010, Redfern 2013, Lima et al. 2015).

The present study provides a detailed re-description for *Caecum floridanum* based on a large number of specimens studied from the Western Atlantic and the designation of a neotype for the species based on a specimen from the type locality (Florida). In addition, the protoconch and all growth stages of this species are described and figured here based on scanning electron microscopy.

Materials and methods

Identification of the material was performed under a stereomicroscope. Specimens were also studied based on photographs taken with scanning electron microscopy (SEM), at the Electron Microscope Laboratory of the "Museu Nacional do Rio de Janeiro (MNRJ)".

Growth stages in shells were recognized based on truncation regions characterized herein as strangulation (Fig. 2C), suture (Fig. 2G), pronounced increase in diameter

(Fig. 3A), or with an interface of sculpture patterns (Figs 2A–B to 2C–E, 3–4). Roman numerals discriminate and arrows delimit each growth stage. Some growth stages were characterized together (e.g., Fig. 2D: II–III) due to the lack of a distinct truncation region [see approach originally proposed in Lima et al. (2013)].

The following standard measures are based on Lima et al. (2013) and were taken using a stereomicroscope with an eyepiece micrometer: total length (Tol), length from the aperture to the point of maximum arc (Larc), maximum arc (Arc), diameter of aperture (Da), diameter of posterior extremity (Dpe), length of mucro (Lm) and width of mucro (Wm). Only undamaged shells were measured. Simple descriptive statistics were performed to determine the range of meristic and morphometric variables. Other abbreviations used: number (N), mean (M), range (R), standard deviation (SD). The number inside brackets indicates the number of specimens in each lot.

Part of the material examined was obtained from the following projects organized by Brazilian Government: Estudo Multidisciplinar da Plataforma Continental da Amazônia (AMASSEDS/Brazil: 1970/1979); "Geologia Marinha da Plataforma Continental do Brasil" (GEOMAR: 1989-1990/1997, Brazil); "Programa de Avaliação do Potencial Sustentável dos Recursos Vivos da Zona Econômica Exclusiva" (REVIZEE/Brazil).

Most of specimens analyzed was obtained on loan and are deposited in the following scientific collections: **ANSP** – Academy of Natural Sciences of Philadelphia, Philadelphia, USA; **IBUFRJ** – Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; **LMUFRPE** – Laboratório de Malacologia, Departamento de Pesca e Aquicultura, Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil; **MNHN** – Muséum national d'Histoire naturelle, Paris, France; **MNRJ** – Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; **MORG** – Museu Oceanográfico Prof. Eliezer de Carvalho Rios, Fundação Universidade Federal do Rio Grande, Rio Grande, Rio Grande do Sul, Brazil; **MZSP** – Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil; **PRI** – Paleontological Research Institution, New York, USA; **UF** – Florida Museum of Natural History, University of Florida, Gainesville, Florida, USA; **UFPB MOLL** – Laboratório de Invertebrados Paulo Young, Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba, João Pessoa, Paraíba, Brazil.

Systematics

Caecidae Gray, 1850 Caecinae Gray, 1850

Caecum Fleming, 1813

Type species. *Dentalium trachea* Montagu, 1803 (by subsequent designation, Gray 1847: 203) from the Atlantic coasts of Europe, the Mediterranean Sea and northwestern Africa (Vannozzi et al. 2015).

Caecum floridanum Stimpson, 1851

Figs 1-4

Caecum floridanum Stimpson 1851: 112 (Recent, Florida).

- *Caecum irregulare* Folin 1867: 47, pl. 3, fig. 6 (Bahia state, northeastern Brazil; syntype MNHN 25729; Recent) – Dall (1892: 298), Abbott (1974: 92, fig. 874), Rios (1975: 40, pl. 11, fig. 142, 1985: 44, pl. 17, fig. 199, 1994: 57, pl. 18, fig. 211, 2009: 98, fig. 237), Diaz and Puyana (1994: 141, pl. XLV, fig. 489), Redfern (2001: 41, pl. 20, fig. 174a–b) [Fig. 4D, type material].
- Caecum phronimum Folin 1867: 44, pl. 3, fig. 4 (Port au Prince, Haiti; syntype MNHN 25728; Recent) Abbott (1974: 92, fig. 874), Rios (1985: 44, pl. 17, fig. 199, 1994: 57), Diaz and Puyana (1994: 141, pl. XLV, fig. 489), Absalão and Pizzini (2002: pl. 1, fig. 2, pl. 2, Figs 15–16) [Fig. 4E, type material].
- Caecum floridanum var. compactum Dall 1892: 298, pl. 20, fig. 9b (Caloosahatchie River, near Fort Thompson, Florida; type USNM 83590; fossil, Pliocene) – new synonym [Fig. 4F, type material].
- *Caecum cayoense* Rehder 1943: 190, pl. 20, fig. 9 (Bonefish Key, Florida Keys; holotype USNM 536045; Recent) Abbott (1974: 92, fig. 874), Tunnell et al. (2010: 144).
- *Caecum puntagordanum* Weisbord 1962: 165, pl. 14, Figs 13–14 (south flank of Punta Gorda anticline, Venezuela; holotype PRI 26107, paratype, PRI 26108; fossil, Pliocene] Abbott (1974: 92, fig. 874), Tunnell et al. (2010: 144) [Fig. 4G–H, type material].

Type material. NEOTYPE ANSP 407671 (herein designated – Fig. 1), USA, Florida: Venice – collected by Donald R. Moore, June 1963.

Additional material examined. United States OF America: -- off Florida State: [8] ANSP 100196, Bahia Honda Key; [1] ANSP 141044, Dry Tortugas, 1925, 29 m; [13] ANSP 306229, John's Pass, 1965, 62 m; [1] UF 350743, Palm Beach, 01 April 1979; [1] UF 359106, Crawl Key, 01 August 1978; [1] UF 359111, Cayo Costa, beach drift, 01 April 1992; [11] UF 359112, Palm Beach, beach drift, 01 February 1988; [1] IBUFRJ 1920, collector Tarrasconi, subtidal, 04 February 1999; [5] MZSP 42358, [6] MZSP 91154, collector P.J. Souza, Deerfield Beach, 3 to 5 m, January 1999; Bahamas: [3] UF 359107, Cat Key, beach drift, 01 March 1981; [3] UF 359108, Sampson Cay, beach drift, 01 June 1992; West Indies: -- off Virgin Islands: [1] UF 359109, St. Croix, 19.81 m, 01 January 1993; -- off ABC Islands: -- Aruba Island: [4] IBUFRJ 6500, [11] IBUFRJ 6906, collector F. Verberne; -- off Venezuela: [1] PRI 26107, holotype and [1] PRI 26108, paratype (Caecum puntagordanum), Tertiary, Lower Pliocene, Mare Formation, Punta Gorda Anticline; -- off Trinidad and Tobago: [6] UF 359105, Tobago Island, 1.82 to 8.53 m, 01 January 1992; [14] UF 359113, Tobago Island, beach drift, 01 April 1991; Brazil: -- off Amapá State: AMASSEDS, collector R/V 'Columbus Iselin' – [5] MORG 39.824, April 1997; [8] MORG 43.297, station 4134, 45 to 50 m, March 1997; -- off Pará State: GEOMAR, collector R/V 'Almirante Saldanha' - [2] MORG 15.815, Cânion do Amazonas,



Figure 1. Photos of *Caecum floridanum*, teleoconch IV (neotype, ANSP 407671): **A–B** lateral view **C** Apical region showing mucro **D** Detail of longitudinal lines and axial interspaces/ribs **E** Anterior region view **F** Operculum (outer surface view) **G** Operculum (internal surface view). Scale bars: 500 μ m (**A–B**), 200 μ m (**C, E**), 100 μ m (**D, F–G**).

station 2438, 40 m, 1970; [5] MORG 15.902, Rio Pará, 25 m, 1970; [2] MORG 16.517, Foz do Amazonas, station 2438, 40 m, 08 November 1970; AMASSEDS, collector R/V 'Columbus Iselin', cruise III – [3] IBUFRJ 18306, station 3209, 01°21'N, 48°00'W, 53 m, May 1990; [2] IBUFRJ 18308, station 3210, 01°52'N, 48°16'W, 47 m, 12 May 1990; [1] IBUFRJ 18309, station 3228, 03°25'N, 49°55'W, 74 m, 17 May 1990; [3] IBUFRJ 18310, station 3201, 00°29'N, 48°11'W, 12 m, May 1990; [1] IBUFRJ 18377, station 3210, 01°52.45'N; 48°16'W, 47 m, 05 December 1990; -- off Maranhão State: REVIZEE/Score NORTE, collector Márcia - [15] IBUFRJ 18316, Banco do Tarol, 20 July 1997; -- off Rio Grande do Norte State: collector MORG research group - [dozens] MORG 19.119, [7] MORG 26.453/28.186, Atol das Rocas, February 1977; -- off Paraíba State: [2] MZSP 77776, Formosa beach, Cabedelo, January 1979, collector L.R.L. Simone; [12] UFPB MOLL 3545, [02] MZSP 114729, Cabo Branco Beach, in rhodolith beds, 22 December 2011, collector André, Emerson, Jéssica, Lívia, Rafael and Silvio; -- off Pernambuco State: [4] IBUFRJ 11179, Rata Island, Fernando de Noronha Archipelago, 08 August 1999, collector IBUFRJ research group; [3] MORG 32.949, Fernando de Noronha Archipelago, 40 m, 05 December 1986, collector M. Cabeda; [3] MZSP 32004, Fernando de Noronha Archipelago, 0-6 m, 20 July 1999, collector P.J. Souza and L.R.L. Simone; collector LMUFRPE research group - [3] LMUFRPE, Porto de Galinhas beach, 05 October 1982; [3] LMUFRPE, Suape, 24 May 1982; -- off Alagoas State: [2] MORG 12.494, Rec. da Marinha, 1964, collector Sá Cardoso; -- off Bahia State: [9] IBUFRJ 7408,

[2] IBUFRJ 7287, Ribeira, Salvador, 1994, collector L. Trinchão; [3] MORG 41.867, Recôncavo Baiano, 29 April 1997, collector 'fishing-boat'; [1] MORG 45.602, Boipeba, 45 m, December 2002, collector R/V 'Astro Garoupa'; [3] MORG 45.639, Camamu Bay, 52 m, 11 December 2002, collector R/V 'Astro Garoupa'; [5] MZSP 44883, Coroa Vermelha Reef, Salvador, 13 January 2000, collector E.P. Gonçalves and P.J. Souza; [7] MORG 18.052, Abrolhos Archipelago, 5 m, July 1972, collector L.C. Araújo; collector MORG research group - [23] MORG 20.113, Abrolhos Archipelago, February 1978; [27] MORG 23.836, Abrolhos Archipelago, January 1985; [29] MORG 26.418, I. Guarita, Abrolhos Archipelago, 5 m, February 1987, collector A.S.J.L. Laurino; [1] MZSP 36863, Alcobaça, Parcel Paredes, 2-3 m, 16 January 2000, collector E.P. Gonçalves and P.J.S. Souza; REVIZEE/Score Central, collector R/V 'Antares' - [132] IBUFRJ 10134, [4] IBUFRJ 12679, [10] IBUFRJ 12750, station C76, 15°54'22"S, 38°31'09"W, 66 m, 30 April 1996; [9] IBUFRJ 14688, station 2R, 13°38'S, 38°44'W, 55 m, 02 July 2001; [3] IBUFRJ 18307, [7] IBUFRJ 18315, [4] IBUFRJ 18376, [2] IBUFRJ 18378, [1] IBUFRJ 18379, station R4#1, 13°45'S, 38°23'W, 91 m, 23 June 2002; [5] IBUFRJ 18313, station R3#1, 15°49'S; 38°36'W, 83 m, 21 June 2002; local project - [6] MNRJ 14061, 13°29'22"S, 38°48'43"W, vi.2007; [5] MNRJ 14062, 13°28'17"S, 38°48'44"W, August 2007; [2] MNRJ 14069, 13°29'20"S, 38°47'37"W, August 2007; [3] MNRJ 14071, 13°28'17"S, 38°48'44"W, August 2007; [1] MNRJ 14073, 13°29'20"S, 38°47'37"W, August 2007; [1] MNRJ 14076, 13°16'00"S, 38°55'07"W, 12 January 2007; [6] MNRJ 14081, 13°19'51"S, 38°52'51"W, 12 January 2007; [1] MNRJ 14090, 13°28'58"S, 38°49'06"W, August 2007; [3] MNRJ 14092, 13°28'58"S, 38°48'21"W, August 2007; -- off Espírito Santo State: [1] IBUFRJ 8629, Piúma, 1993, collector IBUFRJ research group; GEOMAR XII, collector R/V 'Almirante Câmara' - [7] IBUFRJ 7289, 20°53'S, 40°12'W, 26 August 1979; REVIZEE/Score Central, collector R/V 'Antares' - [2] IBUFRJ 9280, station C63, 19°40'42"S, 38°08'15"W, 61 m, 25 April 1996; [30] IBUFRJ 9421, [2] IBUFRJ 12752, station C65, 18°53'37"S, 39°06'23"W, 50 m, 25 April 1996; [30] IBUFRJ 9817, [31] IBUFRJ 12689, station C62, 20°30'02"S, 37°28'51"W, 96 m, 25 April 1996; [8] IBUFRJ 10841, station VV31, 18°52' S, 39°35'W, 23 m, 28 February 1996; [4] IBUFRJ 11360, station VV22, 20°20'S, 40°15'W, 33 m, 27 February 1996; [4] IBUFRJ 12681, station VV21, 20°38'S, 40°00'W, 56 m, 27 February 1996; [2] IBUFRJ 12687, station VV16; 21°10'S, 40°27'W, 28 m, 26 February 1996; [2] IBUFRJ 14574, station 42R, 20°44'S, 31°49'W, 85 m, 11 July 2001; [10] IBUFRJ 18311, station Y7, 20°50'S, 40°10'W, 75 m, 28 June 2002; [4] IBUFRJ 18314, station VV22, 20°20'S; 40°59'W, 33 m, 27 February 1996; [6] MORG 40.457, station VV31, 18°52'S, 39°35'W, 23 m, 28 February 1996); [5] MORG 41.084, station VV21, 20°38'S, 40°00'W, 56 m, 27 February 1996; [17] MORG 33.637, Trindade and Martim Vaz Archipelago, 75 m, 25 April 1996; [3] MORG 39.124, 18°53'S, 39°06'W, 50 m, 25 April 1996; -- off Rio de Janeiro State: [2] MZSP 63394, Rio das Ostras, September 1971, collector MZSP research group; GEOMAR XII, collector R/V 'Almirante Câmara' - [2] IBUFRJ 7288, 22°05'S, 40°17'W, 29 August 1979.



Figure 2. Scanning electron micrographs of Caecum floridanum shells at different growth stages: **A–B** Protoconch and teleoconch I (Bandel 1996: pl. 7, fig. 8, 0.3 mm, fig. 7, 0.7 mm, respectively) **C** Teleoconch II to IV (IBUFRJ 12687) **D** Teleoconch II to IV (IBUFRJ 12689) **E** Teleoconch II to IV (MORG 41.867) **F** Truncation region between teleoconch II and III **G** Truncation region between teleoconch II and III. Scale bars: 500 μm (**C–E**), 100 μm (**F**), 50 μm (**G**).

Original description. "Shell much arcuated, somewhat thick, white, shining; with about thirty-two sharp, elevated ribs, much narrower than their interspaces. Aperture slightly oblique, not contracted. In some specimens there is a broad rib just above the aperture. Long. .075; lat. .02. poll. *Hab.* Florida." (Stimpson 1851: 112).

Diagnosis. Teleoconch with strong, wide, low, rounded, closely arranged axial ribs, except last three to four preceding the aperture, which are larger and more widely separated.

Redescription (shell – neotype). Teleoconch IV (last growth stage) small (Tol 3.85 mm), tubular, rather thick, moderately and regularly arched (Larc 1 mm; Arc 0.30 mm), with slight increase in caliber from apical region to aperture, opaque-white to cream-white with brownish markings (Fig. 1A-B). Surface sculptured with longitudinal striae, faint to well-defined longitudinal threads (Fig. 1D) and 26 prominent, wide, low, rounded, closely arranged and regularly spaced axial ribs (Fig. 1A-B), except last three preceding aperture, which are larger and more widely separated (Fig. 1E).. Striae and threads cross ribs and interspaces (Fig. 1D); threads producing a very slightly beaded effect on ribs (Fig. 1D). Axial interspaces very narrow and shallow, except last two to three preceding aperture, which become wider and deeper (Fig. 1E). Apical region circular (Dpe: 0.57 mm) (Fig. 1C). Septum slightly convex, deeply recessive (Fig. 1C). Mucro finger-shaped, conical, moderately slender (Lm: 0.12 mm; Wm: 0.15 mm), positioned on dorsal margin, straight (Fig. 1C). Aperture circular (Da 0.75 mm), prominent varix around (Fig. 1A–B, E). Operculum yellowish-brown, horny; outer surface concave, with nucleus subcentral, about eight slight coil (Fig. 1F); inner surface convex, smooth (Fig. 1G).

Characterization. Protoconch to teleoconch IV. Protoconch paucispiral (about 1.5 whorls), planispiral, smooth; suture deep, grooved; transition to teleoconch I abrupt, marked by slight axial edge (Figs 2A-B, 4A). Teleoconch I short, sculptured with 9 to 15 wide, very weak, slightly wavy, closely arranged axial riblets and very fine, slight longitudinal striae (Figs 2A-B, 4A); transition to teleoconch II not observed. Teleoconch II sculptured with 9 to 15 faintly demarcated, well-spaced axial riblets and very weak longitudinal threads and striae (Figs 2C-E, 4A); transition to teleoconch III not clear or marked by very slight axial strangulation/suture (Fig. 2F-G). Teleoconch III to IV sculptured with wide, rounded, low, closely arranged axial ribs, longitudinal striae and threads that increase in prominence with the progression of stages (Figs 2C-E, 3A-C). Teleoconch III with about 18 axial ribs (Fig. 2C-E); transition to teleoconch IV not clear (Figs 2E, C) or marked by very slight axial strangulation to pronounced increase in diameter (Figs 2C-D, 3A-B). Teleoconch IV small (Tol 2.90-4.25 mm, M 3.53 mm, N 50), arched (Larc 0.85–1.50 mm, M 1.11 mm, N 50; Arc 0.20–0.40 mm, M 0.28 mm, N 50), apical region circular (Dpe 0.37-0.57 mm, M 0.45 mm, N 50), mucro finger-shaped to triangular, conical (Lm 0.07-0.25 mm, M 0.15 mm, N 49; Wm 0.07-0.20 mm, M 0.13 mm, N 50), aperture circular (Da 0.50-0.75 mm, M 0.58 mm, N 50), sculptured with 22 to 33 axial ribs, wider in comparison to previous stages (Figs 2C-E, 3A-F, 4A), last three to four usually larger and more separated (Figs 1E, 3D-F, 4A, D, F). Figure 4A shows the reconstruction of the growth stages.



Figure 3. Scanning electron micrographs of *Caecum floridanum* shells at different growth stages and operculum: **A** Teleoconch II to III (MORG 41.867) **B** Truncation region between teleoconch II and III (**A**) **C** Teleoconch II to III (IBUFRJ 18376) **D–F** Teleoconch IV (**E–F** Arrows pointing to last three to four axial ribs at anterior end) (**D–F** IBUFRJ 18376) **G** Operculum, outer surface (IBUFRJ 7408) **H** Operculum, inner surface (IBUFRJ 7408). Scale bars: 500 μm (**A**, **C**, **E–H**), 100 μm (**B**, **I**), 200 μm (**D**).

Type locality. Florida (Venice), United States (here established).

Geographic distribution. North Carolina to Florida (Dall 1892, Rehder 1943, Olsson and Harbison 1953, Abbott 1974, Gomes and Absalão 1996); Texas (Tunnell et al. 2010); Mexico (Vokes and Vokes 1983, Lightfoot 1992); Bahamas (Kisch 1959, Redfern 2001); Cuba (Espinosa et al. 1995); Puerto Rico (Rosenberg 2009); Haiti (Fo-



Figure 4. Hypothesis in the reconstruction of growth stages and synonyms of *Caecum floridanum*: **A** Protoconch to teleoconch IV **B** Sculpture of teleoconch I **C** Sculpture of teleoconch II to IV **D** *C. irregular*, teleoconch IV (syntype – MNHN 25729) **E** *C. phronimum* at different growth stages, teleoconch II to III (syntype – MNHN 25728) **F** *Caecum compactum*, teleoconch IV (USNM 83590) **G–H** *C. puntagordanum* (holotype – PRI 26107 and paratype – PRI 26108, respectively), teleoconch IV. Measures and scale bars: **A** protoconch and first half of teleoconch I (Bandel, 1996: pl. 7, fig. 7, 0.7 mm), second half of teleoconch II to III (200 µm), teleoconch IV (500 µm), 100 µm (**B–C**), 500 µm (**D–E**), 1 mm (**F–H**)

lin 1867); Virgin Islands and Saint Martin (Kisch 1959); Trinidad and Tobago Archipelago (Lightfoot 1992); ABC Islands (Jong and Coomans 1988, Gomes and Absalão 1996); Costa Rica (Sevilla et al. 2003); Panama (Olsson and McGinty 1958, Sevilla et al. 2003); Colombia (Diaz and Puyana 1994, Bandel 1996); Venezuela (Weisbord 1962, Rios 2009); Guiana (Princz 1977); Surinam (Rosenberg 2009); Brazil: Amapá, Pará, Maranhão, Ceará, Rio Grande do Norte, Paraíba (presente study), Pernambuco, Alagoas, Bahia, Espírito Santo (Folin 1867, Dall 1892, Kisch 1959, Leal 1991, Gomes and Absalão 1996, Rios 2009), Rio de Janeiro (present study).

Discussion

The brief original description (without illustration) and the loss of the types does not permit recognition of the morphotype originally proposed for *Caecum floridanum*. These issues are more than sufficient to make the taxon a nomen dubium. However, since 1892 a typical morphotype, which is not in agreement with the conchological characters described by Stimpson (1851) (see also Jong and Coomans 1988: 35, C. irregulare) has been universally accepted for C. floridanum in the vast majority of taxonomic and ecological papers. Although the original description is brief, we can recognize that there are considerable discrepancies between the morphotype of the original description and that universally accepted for C. floridanum. Stimpson described this species as having "thirty-two sharp elevated ribs much narrower than the interspaces", while the most papers recognize that the taxon has 22 to 33 low, rounded, closely arranged axial ribs and very narrow and shallow axial interspaces, except the last one preceding the aperture. Dall (1892: 298) was the first to characterize this species in disagreement with the original proposition based on C. irregulare Folin, 1867 (Fig. 4D), which was included as a synonym in the author's study, without, however, giving any reasons for such an action. Thereafter, a new concept of C. floridanum sensu Dall was established and followed by practically all authors addressing the taxon (Rehder 1943, Olsson and Harbison 1953, Olsson and McGinty 1958, Moore 1970: fig. 2, Abbott 1974, Vokes and Vokes 1983, Leal 1991, Lightfoot 1992, Diaz and Puyana 1994, Bandel 1996, Gomes and Absalão 1996, Lee 2009, Rios 2009, Tunnell et al. 2010, Redfern 2013, Lima et al. 2015). Caecum floridanum cannot be identified accurately based on Stimpson's description, which is too vague and might be applied to various Caecum taxa from the Western Atlantic. Therefore, any nomenclature decision regarding this taxon (e.g., description of the taxon as a new species or validating its synonym C. irregulare, making C. floridanum a nomen dubium) will cause instability, inconsistency and taxonomic confusion (unless some type material is found).

Thus, we believe that the best course is to designate a neotype for *Caecum floridanum* based on a specimen deposited at the ANSP (International Commission on Zoological Nomenclature, 1999: art. 75.3.7.) and collected from the type locality (ICZN 1999: art. 76.3.) due to the rather vague original description (in our view, an exceptional need before the ICZN 1999: art. 75.3.). This neotype replaces the lost or destroyed original type material (ICZN 1999: art. 75.3.4, see Introduction to review the steps taken to trace the type material) and clarifies inconsistencies between the concepts put forth by Stimpson (1851) and subsequent authors (ICZN 1999: art. 75.3.1.), conserving the current usage of the name and the universally accepted morphological concept of the species (as have been used in most of the literature) beyond doubt (ICZN 1999: art. 75.3.5.). Vokes and Vokes (1983: 120, fig. 12) recognized a hypotype for *Caecum floridanum*, but this nomenclatural type does not appear in the ICZN (1999) and has no scientific value.

The characterization of teleoconch II presented herein for *Caecum floridanum* is consistent with that of Lightfoot (1992: 179). Bandel (1996) recognized four to five growth stages in the ontogeny of this species, but did not describe each stage separately. Thus, reconstruction of the stages presented by him is an assumption not supported with clear data. Still according to Bandel (1996), a varix is seen on the penultimate and last growth phases, but it is characterized here only at the end of the last stage.

Caecum floridanum has been mistakenly figured as C. imbricatum Carpenter, 1858 by Rios (1994: pl. 19, fig. 212, 2009: 99, fig. 238) and Bandel (1996: fig. 13, pl. 7, Figs 5-8). Caecum annulatum Emmons, 1858 and C. dux Folin, 1871 have usually been considered synonyms of C. floridanum (Dall 1892, Pilsbry and Aguayo 1933, Rosenberg 2009). A reassessment of the shell morphology of C. annulatum based on Emmons (1858: 183, fig. 190) and of C. dux from photos of type material (MNHN), allow us to conclude that both species have somewhat different conchological characters, when compared to C. floridanum. Caecum annulatum has an inflated, domeshaped septum and rounded, raised, axial ribs, which are not slightly larger and more widely separated preceding aperture (Emmons 1858: 183, fig. 190), while C. dux has a broad, blunt mucro, raised, widely separated axial ribs and no evidence of longitudinal sculpture on the teleoconch. Two type specimens of Caecum floridanum var. compactum were recognized by Dall (1892), but at least five shells are deposited in USNM (83590). Only two of these specimens represent C. floridanum (Fig. 4F). The most distinguishing features of C. floridanum are the recessive septum, rather triangular mucro, longitudinal striae and threads cross axial ribs and interspaces, aperture with prominent varix and low, rounded, closely arranged axial ribs, except last preceding aperture, which become larger and wider (ICZN 1999: art. 75.3.2.).

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RESEARCH ARTICLE



Papiliocoelotes gen. n., a new genus of Coelotinae (Araneae, Agelenidae) spiders from the Wuling Mountains, China

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Abstract

One new genus of the spider subfamily Coelotinae, *Papiliocoelotes* gen. n., with five new species is described for both sexes: *P. guanyinensis* sp. n., *P. guitangensis* sp. n., *P. jiepingensis* sp. n., *P. meiyuensis* sp. n., *P. yezhouensis* sp. n. All new species were collected from caves in the Wuling Mountains of Hubei and Hunan Provinces, China. DNA barcodes were obtained for future use.

Keywords

Taxonomy, coelotine, description, diagnosis, Asia

Introduction

Coelotine spiders are only distributed in the temperate and tropical areas of the Northern Hemisphere. So far, a total of 657 valid species belonging to 24 genera (Wang 2012, Chen et al. 2015b, Chen et al. 2016) are known, and 18 genera are distributed in China. Wang (2002) erected 10 new genera, and more than half of them are primarily distributed in southern China. This distribution shows that the potential generic-level diversity of coelotine spiders is yet to be discovered (although three of those genera are considered synonyms and no longer used) (World Spider Catalog 2016). In the last three years, a new genus from southern China (Chen et al. 2015b) and many new coelotine species were described successively from China (Chen et al. 2015a, Jiang and Chen 2015) and adjacent regions: Caucasus (Kovblyuk et al. 2013), Japan (Okumura 2013) and Korea (Kim and Ye 2013, Kim and Ye 2014, Seo 2014, Ye and Kim 2014), suggesting that there are still many poorly known species and genera in those areas.

In this study, a new genus of coelotine spider, *Papiliocoelotes* gen. n. and five new species from Hubei and Hunan Provinces in southern China are reported.

Material and methods

Specimens were examined with a LEICA M205C stereomicroscope. Images were captured with an Olympus C7070 wide zoom digital camera (7.1 megapixels) mounted on an Olympus SZX12 dissecting microscope. Epigynes and male palps were examined after dissection from the spiders' bodies.

All measurements were obtained using a LEICA M205C stereomicroscope and are given in millimeters. Leg measurements are shown as: total length (femur, patella + tibia, metatarsus, tarsus). Only structures (palp and legs) on the left side of the body were described and measured. The abbreviations and terminology used in the text follows Wang (2002). The pattern was not described for each species because it is shown in the figures and is nearly the same in all species. Abbreviations used in this paper and in the figure legends: ALE = anterior lateral eye; AME = anterior median eye; AME-ALE = distance between AME and ALE; AME-AME = distance between AME and AME; ALE-PLE = distance between ALE and PLE; BH = basal hematodocha; C = conductor; CD = copulatory duct; CO = copulatory opening; CF = cymbial furrow; E = embolus; EB = embolic base; FD = fertilization duct; H = epigynal hood; PA = patellar apophysis; PLE = posterior lateral eye; PME = posterior median eye; PME-PLE = distance between PME and PLE; PME-PME = distance between PME and PME; RTA = retroventral tibial apophysis; S = spermatheca; ST = subtegulum; T = tegulum; TA = tegular apophysis; PC = patellar condyle.

DNA barcodes were obtained for future use. A partial fragment of the mitochondrial gene cytochrome oxidase subunit I (COI) was amplified and sequenced for *P. guanyinensis* sp. n., *P. guitangensis* sp. n., *P. jiepingensis* sp. n., *P. meiyuensis* sp. n. and *P. yezhouensis* sp. n. using Primers LCO1490-oono (5'-CWACAAAYCA-TARRGATATTGG-3') (Folmer et al. 1994; Miller et al. 2010) and HCO2198-zz (5'-TAAACTTCCAGGTGACCAAAAAATCA-3') (Folmer et al. 1994; Chen et al. 2015a). For additional information on extraction, amplification and sequencing procedures, see Zhao et al. (2013). All sequences were analyzed using BLAST and are deposited in GenBank. The accession numbers are provided in Table 1.

All species were collected from caves in the Wuling Mountains. All specimens (including molecular vouchers) are deposited in the Institute of Zoology, Chinese Academy of Sciences in Beijing (IZCAS).

Species	GenBank accession number	Sequence length	Collection localities
P. guanyinensis sp. n.	KU991801	630 bp	Hefeng County, Enshi Prefecture, Hubei, China
P. guitangensis sp. n.	KU991804	630 bp	Longshan County, Hunan, China
P. jiepingensis sp. n.	KU991803	630 bp	Xianfeng County, Enshi Prefecture, Hubei, China
P. meiyuensis sp. n.	KU991802	630 bp	Hefeng County, Enshi Prefecture, Hubei, China
P. yezhouensis sp. n.	KU991800	627 bp	Jianshi County, Enshi Prefecture, Hubei, China

Table 1. Voucher specimen information.

Systematics

Family Agelenidae C.L. Koch, 1837 Subfamily Coelotinae F.O.P.-Cambridge, 1893

Genus Papiliocoelotes gen. n.

http://zoobank.org/223E0874-B0AF-413C-879D-9BB08DC8CF4E

Type species. Papiliocoelotes yezhouensis sp. n.

Etymology. The generic name is derived from the Latin word "Papilio", meaning "butterfly, moth", referring to the shape of the endogyne, and "Coelotes" referring to the similarity with the nominal genus of the subfamily. The gender is masculine.

Diagnosis. Males can be easily distinguished from other coelotines, except *Platocoelotes* Wang, 2002, by the absence of a median apophysis and the presence of an elongated tegular apophysis. They can be distinguished from *Platocoelotes* by the broad conductor without the embolus inside and the relatively short embolus that terminates at the base of conductor (Fig. 1A–C; Wang 2002: figs 338, 339; Xu and Li 2008: figs 11–16; Chen et al. 2015a: fig. 1A–C). Females can be easily distinguished from other coelotines, except *Platocoelotes* and *Spiricoelotes* Wang, 2002, by having no epigynal teeth and the presence of epigynal hoods. They can be distinguished from *Platocoelotes* by the shape of the copulatory ducts, which are weakly sclerotized and spiraled, whereas the copulatory ducts are usually broad in *Platocoelotes* (Fig. 2A–B; Chen et al. 2015a). They can distinguished from *Spiricoelotes* by the positions of the epigynal hoods that are located mediolaterally or posterolaterally on the epigynal plate, whereas the epigynal hoods are usually located anterolaterally in *Spiricoelotes*, and by the sclerotized and spiral copulatory ducts (Fig. 2A–B; Wang 2002: figs 360, 361; Chen et al. 2016: fig. 2A–B).

Description. Small to medium-sized, with a total length of 4–7 mm; body color is shallow, with black stripes; carapace nearly pear-shaped, with radial pattern; sternum yellowish; abdomen nearly oval-shaped, with herringbone pattern in dorsal view; chelicerae usually with 1 to 3 promarginal and 2 retromarginal teeth in both sexes; leg formula ($4 > 1 > 2 \ge 3$). Male palp with 1 patellar apophysis and 1 patellar condyle; retroventral tibial apophysis extending beyond the distal margin of tibia; conductor broad;

tegulum with tegular apophysis; embolus filiform, relatively short and terminates at the base of conductor. Epigynal teeth absent; atrium usually small or indistinct; epigynal hoods located mediolaterally or posterolaterally; copulatory openings usually located centrally or posterior centrally on the epigyne plate; the shape of spermathecae and copulatory ducts butterfly-like; spermathecae located in posterior of epigyne; spermathecal head indistinct; copulatory ducts sclerotized and spiral.

Comments. In addition to morphological study, we reconstructed the phylogeny of coelotine spiders based on molecular data from 18 genera and 286 coelotine species (the phylogenetic analysis results will be published in a subsequent paper). The molecular phylogenetic analyses support *Papiliocoelotes* as monophyletic and closely related to *Platocoelotes* and *Spiricoelotes*.

Distribution. China (Hubei, Hunan) (Fig. 11).

Papiliocoelotes yezhouensis sp. n.

http://zoobank.org/5466FCB3-9F26-4FE7-A40E-5B77283191E6 Figs 1–2, 11

Type material. Holotype \mathcal{E} : China: Hubei: Enshi Prefecture: Jianshi County: Yezhou Town: near gas station, a unnamed cave (near a sandpit), N30.63685°, E109.72212°, 588 m, 21.I.2014, Y. Li and J. Liu leg. **Paratypes:** $4\mathcal{Q}2\mathcal{E}$, same data as holotype.

Etymology. The specific name is derived from the type locality; adjective.

Diagnosis. The male can be distinguished from *P. meiyuensis* sp. n. by the short and wide tegular apophysis with a bifurcated tip, the broad conductor with a slightly bifurcated distal process and a long patellar condyle (Fig. 1A–C). The female can be distinguished from *P. meiyuensis* sp. n. by the thick copulatory ducts that roll into a circle (Fig. 2A–B).

Description. Male (holotype): Total length 4.85. Carapace 2.50 long, 1.75 wide. Abdomen 2.40 long, 1.60 wide. Eye sizes and interdistances: AME 0.07, ALE 0.11, PME 0.13, PLE 0.13; AME-AME 0.05, AME-ALE 0.03, ALE-PLE 0.05, PME-PME 0.05, PME-PLE 0.07. Leg measurements: I: 9.45 (2.50, 3.15, 2.30, 1.50); II: 8.20 (2.25, 2.65, 1.95, 1.35); III: 7.85 (2.10, 2.45, 2.10, 1.20); IV: 10.60 (2.75, 3.25, 3.10, 1.50). Chelicerae with 3 promarginal teeth. Palp: patellar apophysis long, scarcely curved, with pointed tip, extending anteriorly; patellar condyle long; retroventral tibial apophysis almost rectangular apically; cymbial furrow broad and about 2/5 length of cymbium; conductor broad, blunt apically; conductor with slightly bifurcated distal process; tegular apophysis short with bifurcated tip, shorter than the length of the cymbial furrow (Fig. 1A–C).

Female (one of paratypes): Total length 5.05. Carapace 2.45 long, 1.60 wide. Abdomen 2.45 long, 1.70 wide. Eye sizes and interdistances: AME 0.07, ALE 0.11, PME 0.12, PLE 0.12; AME-AME 0.05, AME-ALE 0.02, ALE-PLE 0.03, PME-PME 0.05, PME-PLE 0.07. Leg measurements: I: 8.15 (2.25, 2.80, 1.85, 1.25); II: 7.00 (2.00, 2.25, 1.60, 1.15); III: 6.65 (1.85, 2.00, 1.75, 0.95); IV: 9.00 (2.40, 2.85, 2.50, 1.85, 1.25); IV: 9.00 (2.40, 2.85, 2.50, 1.85, 2.50); IV: 9.00 (2.40, 2.85, 2.50, 1.85, 2.50); IV: 9.00 (2.40, 2.85, 2.50); IV: 9.00 (2.40, 2.50); IV: 9.00 (2.40, 2.50); IV: 9.00 (2.40, 2.50); IV: 9


Figure 1. Left male palp of *Papiliocoelotes yezhouensis* sp. n., holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A**, **B**, **C**.

1.25). Chelicerae as in male. Epigyne: copulatory openings located centrally; epigynal hoods located mediolaterally, sulci small and shallow; copulatory ducts roll into a circle; width of spermathecae subequal to width of copulatory ducts (Fig. 2A–B).

Distribution. Known only from the type locality (Fig. 11).

Papiliocoelotes guanyinensis sp. n.

http://zoobank.org/50C51224-FA28-48AA-A419-646C7075C840 Figs 3–4, 11

Type material. Holotype ♂: China: Hubei: Enshi Prefecture: Hefeng County: Guanyinping, Guanyin Cave, N29.93238°, E110.05344°, 758 m, 11.I.2014, Y. Li and J. Liu leg. **Paratypes:** 3♀2♂, same data as holotype.

Etymology. The specific name is derived from the type locality; adjective.

Diagnosis. The male can be distinguished from *P. yezhouensis* sp. n. by the large tegular apophysis that is longer than the length of the cymbial furrow, the lack of a patel-



Figure 2. Epigyne and habitus of *Papiliocoelotes yezhouensis* sp. n. **A** Epigyne, ventral **B** Vulva, dorsal **C** Male habitus, dorsal **D** Female habitus, dorsal **E** Female habitus, ventral. Scale bars: equal for **A** and **B**, equal for **D** and **E**.



Figure 3. Left male palp of *Papiliocoelotes guanyinensis* sp. n., holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A**, **B**, **C**.

lar condyle, the fin-shaped conductor and the dorsally extending patellar apophysis (Fig. 3A–C). The female can be distinguished from *P. yezhouensis* sp. n. by the distinct copulatory openings and the epigynal hoods which are located posterolaterally (Fig. 4A–B).

Description. Male (holotype): Total length 4.55. Carapace 2.25 long, 1.65 wide. Abdomen 2.35 long, 1.55 wide. Eye sizes and interdistances: AME 0.06, ALE 0.12, PME 0.13, PLE 0.13; AME-AME 0.05, AME-ALE 0.01, ALE-PLE 0.01, PME-PME 0.04, PME-PLE 0.03. Leg measurements: I: 8.60 (2.25, 2.85, 2.00, 1.50); II: 7.40 (2.00, 2.40, 1.85, 1.15); III: 7.10 (1.95, 2.25, 1.75, 1.15); IV: 10.00 (2.60, 3.05, 2.90, 1.45). Chelicerae with 1 promarginal tooth. Palp: patellar apophysis long, scarcely curved, with pointed tip, extending dorsally; patellar condyle absent, only dark distally; retroventral tibial apophysis sharply pointed; cymbial furrow short and indistinct, about 1/3 length of cymbium; conductor fin-shaped; tegular apophysis elongate, slightly blunt at subdistal part and longer than the length of the cymbial furrow (Fig. 3A–C).



Figure 4. Epigyne and habitus of *Papiliocoelotes guanyinensis* sp. n. **A** Epigyne, ventral **B** Vulva, dorsal **C** Male habitus, dorsal **D** Female habitus, dorsal **E** Female habitus, ventral. Scale bars: equal for **A** and **B**, equal for **D** and **E**.

Female (one of paratypes): Total length 4.75. Carapace 2.15 long, 1.50 wide. Abdomen 2.65 long, 1.95 wide. Eye sizes and interdistances: AME 0.07, ALE 0.10, PME 0.12, PLE 0.13; AME-AME 0.04, AME-ALE 0.02, ALE-PLE 0.03, PME-PME 0.05, PME-PLE 0.04. Leg measurements: I: 6.90 (2.00, 2.25, 1.45, 1.20); II: 6.26 (1.76, 2.00, 1.50, 1.00); III: 6.15 (1.75, 1.95, 1.45, 1.00); IV: 8.75 (2.00, 2.35, 2.25, 1.15). Chelicerae with 3 promarginal teeth. Epigyne: copulatory openings located centrally; epigynal hoods located posterolaterally, sulci round and deep; copulatory ducts thick and curled; the width of spermathecae subequal to the width of the copulatory ducts (Fig. 4A–B).

Distribution. Known only from the type locality (Fig. 11).

Papiliocoelotes guitangensis sp. n.

http://zoobank.org/951855FC-F477-4DA1-8AAB-043D7726349E Figs 5–6, 11

Type material. Holotype \Diamond : China: Hunan: Longshan County: Guitangba Town: Wulongshan Park, Feihu Cave, N29.21000°, E109.30569°, 436 m, 13.I.2014, Y. Li and J. Liu leg. **Paratypes:** $2 \bigcirc 2 \circlearrowright$, same data as holotype.

Etymology. The specific name is derived from the type locality; adjective.



Figure 5. Left male palp of *Papiliocoelotes guitangensis* sp. n., holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A**, **B**, **C**.



Figure 6. Epigyne and habitus of *Papiliocoelotes guitangensis* sp. n. **A** Epigyne, ventral **B** Vulva, dorsal **C** Male habitus, dorsal **D** Female habitus, dorsal **E** Female habitus, ventral. Scale bars: equal for **A** and **B**, equal for **D** and **E**.

Diagnosis. The male can be distinguished from *P. yezhouensis* sp. n. by the dorsally curved patellar apophysis, the apically rounded retroventral tibial apophysis, the large tegular apophysis with pointed tip and the conductor with 2 pointed distal processes (Fig. 5A–C). The female can be distinguished from *P. yezhouensis* sp. n. by the small and shallow epigynal hoods, and the width of the copulatory ducts is slightly wider than the spermathecae (Fig. 6A–B).

Description. Male (holotype): Total length 6.05. Carapace 3.25 long, 2.20 wide. Abdomen 2.85 long, 1.75 wide. Eye sizes and interdistances: AME 0.11, ALE 0.14, PME 0.14, PLE 0.15; AME-AME 0.05, AME-ALE 0.02, ALE-PLE 0.03, PME-PME 0.06, PME-PLE 0.08. Leg measurements: I: 11.55 (2.85, 4.00, 2.70, 2.00); II: 10.30 (2.80, 3.25, 2.50, 1.75); III: 8.95 (2.60, 3.00, 2.25, 1.10); IV: 13.05 (3.25, 4.00, 3.80, 2.00). Chelicerae with 1 promarginal tooth. Palp: patellar apophysis long, curved dorsally, with pointed tip; patellar condyle short; retroventral tibial apophysis rounded apically; cymbial furrow broad, about 2/5 length of the cymbium; conductor wide, with 2 pointed distal processes; tegular apophysis elongate with pointed tip, subequal to the length of the cymbial furrow (Fig. 5A–C).

Female (one of paratypes): Total length 5.85. Carapace 2.75 long, 1.80 wide. Abdomen 3.15 long, 2.25 wide. Eye sizes and interdistances: AME 0.11, ALE 0.13, PME 0.13, PLE 0.14; AME-AME 0.04, AME-ALE 0.03, ALE-PLE 0.03, PME-PME 0.05, PME-PLE 0.08. Leg measurements: I: 9.15 (2.50, 3.05, 2.15, 1.45); II: 7.95 (2.20, 2.50, 1.95, 1.30); III: 7.75 (2.05, 2.45, 1.95, 1.30); IV: 10.30 (2.70, 3.25, 2.90, 1.45). Chelicerae with 3 promarginal teeth. Epigyne: copulatory openings located centrally; epigynal hoods small, located mediolaterally, sulci small and shallow; copulatory ducts roll into a ball; the width of copulatory ducts slightly wider than the spermathecae (Fig. 6A–B).

Distribution. Known only from the type locality (Fig. 11).

Papiliocoelotes jiepingensis sp. n.

http://zoobank.org/B4D3118D-E2CA-4711-BF8F-52BC4DE810B5 Figs 7–8, 11

Type material. Holotype \mathcal{S} : China: Hubei: Enshi Prefecture: Xianfeng County: Zhongtangpu Town: Jieping Village, Shangjieping, Xiangjie, a cave without name, N29.61330°, E109.17803°, 1004 m, 17.I.2014, Y. Li and J. Liu leg. **Paratypes:** $4 \mathfrak{Q} 1 \mathcal{S}$, same data as holotype.

Etymology. The specific name is derived from the type locality; adjective.

Diagnosis. The male can be distinguished from *P. yezhouensis* sp. n. by the slender, needle-like tegular apophysis, the short patellar condyle and the flat conductor with 1 pointed retrolateral process (Fig. 7A–C). The female can be distinguished from *P. yezhouensis* sp. n. by the thin and coiled copulatory ducts, the width of copulatory ducts obviously wider than the spermathecae (Fig. 8A–B).



Figure 7. Left male palp of *Papiliocoelotes jiepingensis* sp. n. (expanded), holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A**, **B**, **C**.

Description. Male (holotype): Total length 5.45. Carapace 2.55 long, 2.00 wide. Abdomen 2.85 long, 1.80 wide. Eye sizes and interdistances: AME 0.10, ALE 0.13, PME 0.13, PLE 0.14; AME-AME 0.03, AME-ALE 0.02, ALE-PLE 0.03, PME-PME 0.07, PME-PLE 0.08. Leg measurements: I: 9.75 (2.55, 3.20, 2.40, 1.60); II: 8.85 (2.50, 2.85, 2.00, 1.50); III: 8.20 (2.25, 2.45, 2.10, 1.40); IV: 11.40 (3.00, 3.50, 3.15, 1.75). Chelicerae with 2 promarginal teeth. Palp: patellar apophysis short, extending anteriorly, with curved, pointed tip; patellar condyle short; retroventral tibial apophysis small; cymbial furrow short but obvious, about 1/3 length of cymbium; conductor flat with 1 pointed retrolateral process; tegular apophysis slender, needle-like and longer than the length of cymbial furrow (Fig. 7A–C).

Female (one of paratypes): Total length 5.50. Carapace 2.65 long, 1.80 wide. Abdomen 2.90 long, 2.10 wide. Eye sizes and interdistances: AME 0.09, ALE 0.11, PME 0.12, PLE 0.13; AME-AME 0.04, AME-ALE 0.03, ALE-PLE 0.04, PME-PME 0.07, PME-PLE 0.08. Leg measurements: I: 7.90 (2.10, 2.75, 1.80, 1.25); II: 7.20 (2.00, 2.40, 1.65, 1.15); III: 6.90 (1.95, 2.10, 1.85, 1.00); IV: 9.20 (2.45, 3.00, 2.50, 1.25). Chelicerae like in male with 2 promarginal teeth. Epigyne: copulatory openings located posteromedially; epigynal hoods located mediolaterally on epigynal plate, sulci rounded and deep; copulatory ducts thin and coiled with more than 2 loops; the width of copulatory ducts obviously wider than the spermathecae (Fig. 8A–B).

Distribution. Known only from the type locality (Fig. 11).



Figure 8. Epigyne and habitus of *Papiliocoelotes jiepingensis* sp. n. **A** Epigyne, ventral **B** Vulva, dorsal **C** Male habitus, dorsal **D** Female habitus, dorsal **E** Female habitus, ventral. Scale bars: equal for **A** and **B**, equal for **D** and **E**.

Papiliocoelotes meiyuensis sp. n.

http://zoobank.org/10118C37-ACDA-4D50-9B4C-14E499048F90 Figs 9–10, 11

Type material. Holotype ♂: China: Hubei: Enshi Prefecture: Hefeng County: Zouma Town: Meiyuping, Xini Village, Xianren Cave, N29.73239°, E110.31914°, 853 m, 10.I.2014, Y. Li and J. Liu leg. **Paratypes:** 2♀1♂, same data as holotype.

Etymology. The specific name is derived from the type locality; adjective.

Diagnosis. The male can be distinguished from *P. yezhouensis* sp. n. by the sharply pointed retroventral tibial apophysis, the shorter patellar condyle, the dorsally extending patellar apophysis, the tegular apophysis with pointed tip and the large conductor with 1 distal process and 1 small spine-like retrolateral process (Fig. 9A–C). The female can be distinguished from *P. yezhouensis* sp. n. by the distinct copulatory openings, the epigynal hoods located posterolaterally and by the width of the copulatory ducts, which are narrower than the spermathecae (Fig. 10A–B).



Figure 9. Left male palp of *Papiliocoelotes meiyuensis* sp. n., holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A**, **B**, **C**.



Figure 10. Epigyne and habitus of *Papiliocoelotes meiyuensis* sp. n. **A** Epigyne, ventral **B** Vulva, dorsal **C** Male habitus, dorsal **D** Female habitus, dorsal **E** Female habitus, ventral. Scale bars: equal for **A** and **B**, equal for **D** and **E**.



Figure 11. Localities of new Papiliocoelotes species from China. 1 P. guanyinensis sp. n. 2 P. guitangensis sp. n. 3 P. jiepingensis sp. n. 4 P. meiyuensis sp. n. 5 P. yezhouensis sp. n.

Description. Male (holotype): Total length 4.98. Carapace 2.55 long, 1.80 wide. Abdomen 2.40 long, 1.45 wide. Eye sizes and interdistances: AME 0.08, ALE 0.12, PME 0.13, PLE 0.13; AME-AME 0.03, AME-ALE 0.02, ALE-PLE 0.03, PME-PME 0.05, PME-PLE 0.05. Leg measurements: I: 10.20 (2.75, 3.25, 2.35, 1.85); II: 9.00 (2.40, 2.90, 2.10, 1.60); III: 8.65 (2.35, 2.50, 2.35, 1.45); IV: 11.75 (3.00, 3.50, 3.40, 1.85). Chelicerae with 3 promarginal teeth. Palp: patellar apophysis long, slightly curved, with pointed tip, extending dorsally; patellar condyle short; retroventral tibial apophysis sharp pointed, extending beyond the tibia anteriorly; cymbial furrow small, about 1/3 length of cymbium; conductor large, with 1 distal process and 1 small spine-like retrolateral process; tegular apophysis relatively short, with pointed tip and subequal to the length of cymbial furrow (Fig. 9A–C).

Female (one of paratypes): Total length 4.13. Carapace 2.10 long, 1.50 wide. Abdomen 2.10 long, 1.40 wide. Eye sizes and interdistances: AME 0.05, ALE 0.11, PME 0.11, PLE 0.12; AME-AME 0.02, AME-ALE 0.02, ALE-PLE 0.03, PME-PME 0.05, PME-PLE 0.04. Leg measurements: I: 6.60 (1.95, 2.25, 1.50, 0.90); II: 6.05 (1.75, 1.95, 1.30, 1.05); III: 5.90 (1.55, 1.85, 1.55, 0.95); IV: 7.65 (2.25, 2.45, 2.10, 0.85). Chelicerae with 2 promarginal teeth. Epigyne: copulatory openings located centrally; epigynal hoods located posterolaterally, sulci round; copulatory ducts small and spiraled; the width of the copulatory ducts narrower than the spermathecae (Fig. 10A–B).

Distribution. Known only from the type locality (Fig. 11).

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RESEARCH ARTICLE



Megastigmus seed chalcids (Hymenoptera, Torymidae) radiated much more on Angiosperms than previously considered. I- Description of 8 new species from Kenya, with a key to the females of Eastern and Southern Africa

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Abstract

A survey of seed chalcids from woody plants in Kenya revealed 12 species belonging to the genus *Megastigmus* Dalman, 1820, and has increased to 16 the number of *Megastigmus* species presently recorded from the Afrotropical Region, of which at least 13 are seed feeders. A key to female *Megastigmus* of the Afrotropical Region is provided. Eight new species are described from morphological evidence: *M. lanneae* Roques & Copeland, *M. laventhali* Roques & Copeland, *M. ozoroae* Roques & Copeland, and *M. smithi* Roques & Copeland in seeds of species of the family Anacardiaceae, *M. copelandi* Roques & Copeland and *M. grewianae* Roques & Copeland in seeds of Malvaceae, *M. helinae* Roques & Copeland in seeds of the family Anacardiaceae, *M. helinae* Roques & Copeland in seeds of harmaceae as hosts of *Megastigmus* seed chalcids, which appear to have radiated in Angiosperms much more than previously considered. Analyses of the mitochondrial (cytochrome oxidase subunit one – COI) and nuclear DNA (28S ribosomal region) could be carried out on 8

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of the 16 African species of which 5 were newly described ones. The species associated with Anacardiaceae always clustered together in phylogenies, confirming the existence of a strong and ancestral monophyletic clade, unlike the ones associated with Malvaceae and Rhamnaceae, whose position remains unclear. All holotypes are deposited in the National Museums of Kenya.

Keywords

Chalcidoidea, Megastigmus, seed, Anacardiaceae, Malvaceae, Rhamnaceae, new species, Africa, plant hosts, fruit

Introduction

Globalization, the accelerating worldwide movement of goods and people, has led to an exponential increase in the transport of alien organisms across geographical barriers (DAISIE 2009; Essl et al. 2011), and the international trade in plant propagative material is widely recognized as a major mode of introduction of alien arthropod pests and microbial diseases (Roques et al. 2010; Liebhold et al. 2012). The seed trade, in particular, has been identified as highly prone to pest invasion as a result of the limited measures in place to regulate seed traffic and the specific biology of the associated alien organisms (Auger-Rozenberg and Roques 2012). Indeed, phytosanitary regulations regarding the trade in tree seeds are non-existent in a number of countries, including those of the European Union, whilst the exploding development of trade through the internet allows seeds to move quite freely all over the world. In addition, the endophytic way of life of most insects associated with seeds makes it difficult to detect their presence during the transport and importation processes. In most cases, examining the outside of the seeds does not provide any evidence of insect larvae which can be detected only when seeds are X-rayed (Auger-Rozenberg and Roques 2012). As a result, 11 of the 21 seed wasp species in the genus Megastigmus (Hymenoptera, Torymidae) known to date in Europe are of exotic origin (Roques and Skrzypczyńska 2003). This level of invasion has already had negative consequences for both natural regeneration of native trees and seed niche exploitation by native seed feeders (Auger-Rozenberg and Roques 2012). Therefore, it is important to increase our knowledge about the species that may be introduced with tree seeds.

Seeds of woody plants can be infested by larvae of a number of arthropod groups, including, among others, dipteran seed midges in the family Cecidomyiidae (e.g., *Pleme-liella, Mayetiola*), coleopteran seed beetles in the family Bruchidae, and mites in the genus *Trisetacus* (Nalepellidae) (Křístek et al. 1992). However, the most important group consists of hymenopteran seed chalcids in the families Torymidae (e.g., *Megastigmus, Boo-tanellus, Bootania, Torymus*), Eurytomidae (e.g. *Bephratelloides, Bruchophagus, Eurytoma, Risbecoma, Striateurytoma*), Pteromalidae (*Homodecatoma*), Eulophidae (*Anselmella*), and Tanaostigmatidae (*Tanaoneura*) (Roques et al. 2003). Among these seed chalcids, the genus *Megastigmus* appears dominant. In his checklist of the world Megastigminae, Grissell (1999) listed 126 *Megastigmus* species of which about one-third were phytophagous in tree seeds, about one-third were parasitoids or inquilines of gall-forming insects in the

fruits, leaves and stems of plants, and about one-third had no known hosts. Since 2000, a number of new *Megastigmus* species have been described (Roques and Skrzypczyńska 2003; Auger-Rozenberg et al. 2006; Ghramh and Shati 2011; Grissel 2006; Sureshan 2009; Narendran et al. 2010; Doğanlar and Hassan 2010; Galindo-González et al. 2012; Doğanlar et al. 2013; Doğanlar 2015). Doğanlar (2011) also transferred the metallic-coloured, parasitic species into the genus *Bootanomyia*.

Data from these recent studies indicate that, worldwide, the genus *Megastigmus* currently includes 135 species, 70 of which are seed feeders, 45 develop in galls, and 20 for which behavioral data is lacking (Noyes 2015). Among the seed feeders, species associated with conifers are most abundant with 49 species (24 spp. in the family Pinaceae; 22 spp. in Cupressaceae; one sp. in Taxodiaceae, and two spp. in unidentified conifers), whereas 21 species have been shown to develop within seeds of five families of Angiosperms (Rosaceae: 13 spp.; Anacardiaceae: four spp.; Fabaceae: two spp.; Aquifoliaceae: one sp.; Hamamelidaceae: one sp.). Although found in all biogeographic regions, most species of *Megastigmus* have been described from the Palearctic, Australian, and Nearctic Regions.

To date, relatively few species have been reported from subtropical and tropical areas. In the Afrotropical region, only seven species have been recognized. Four species develop as phytophages in angiosperm seeds. Three of these, Megastigmus hypogeus (Hussey), M. thomseni (Hussey), and M. transvaalensis (Hussey) (= M. rhusi [Hussey]) attack seeds of Anacardiaceae (Hussey 1956a; Grissell and Prinsloo 2001). A fourth species, Megastigmus aculeatus (Swederus), is specific to seeds of Rosa spp. (Rosaceae) and, in the Afrotropical region, was previously known only from a single record from a US quarantine inspection in French Somaliland (Roques and Skrzypczynska 2003). It has since been collected in the wild in Eastern Cape, South Africa (Simon Van Noort, personal communication, IZIKO catalogue SAM-HYM-P005791). Another species, M. somaliensis Hussey, develops in seeds of Juniperus procera Endl. (Cupressaceae) (Hussey 1956b). Two final Afrotropical species, M. pretorianensis Doğanlar and M. zebrinus Grissell, develop in Eucalyptus galls, the former as an inquiline or parasitoid in leaf, stem and petiole galls induced by the eulophid Leptocybe invasa Fisher & LaSalle (Doğanlar 2015) and the latter as a gall maker in seeds of Eucalyptus camaldulensis Dehnhardt and Syzygium cordatum Hochst. ex Krauss, both Myrtaceae (Grissell 2006). Of these seven species, at least two, M. aculeatus and *M. zebrinus*, are not native to Africa but were introduced with their host from Eurasia and Australia, respectively. Thus far, M. pretorianensis is known only from South Africa (Doğanlar 2015). The native status of *M. transvaalensis* which develops in seeds of the exotic pink pepper trees, Schinus spp., introduced from South America, but also in Rhus species native to Africa (Grissell and Prinsloo 2001), is still debatable, although molecular studies by Scheffer and Grissell (2003) suggested a South African origin.

The lower diversity of *Megastigmus* seed chalcids observed in angiosperms and in subtropical/ tropical areas may be due to a more limited radiation process in these hosts and regions or simply reflect more limited investigations in the tropics compared to those carried out in conifers of the Holarctic region in relation to afforestation programs and establishment of seed orchards (Hedlin et al. 1980; Roques and Skrzypczyńska 2003). The

present paper aims at contributing to the knowledge of African *Megastigmus*. It presents the results of a large collection campaign carried out by the International Centre of Insect Physiology and Ecology (ICIPE) that included, primarily, fruits of Angiosperm trees and shrubs from different regions of Kenya. Between 1999 and 2012 two projects were conducted in this country, both of which focused on the rearing of insects, especially Tephritidae, from wild fruits. The first of these (1999–2004) was a country-wide survey of fruits from diverse habitat types. The second (2011–2012) focused on the northernmost representatives of the Eastern Arc mountains, the Taita Hills and the outlying Kasigau Mountain, both located in southeastern Kenya. Most of the collections from this second project were from highland (ca.1600–2200 m elevation) wet-forest remnants, with a smaller number of samples from mid-altitude dry savanna and woodland.

Over 700 insect species were reared from fruits and much of the data from the two projects has been published previously (e.g. Copeland et al. 2002, White et al. 2003, Copeland et al. 2004, Copeland et al. 2006, Copeland et al. 2009, Adamski et al. 2012, Razowski and Brown 2012, Brown et al. 2014). Surprisingly, *Megastigmus* were reared from several species of wild fruits. *Megastigmus* specimens were also recovered, though rarely, from Malaise trap samples from an unrelated project, also conducted by the second author. In this paper, combining the use of morphological and molecular tools, we report range expansion of some previously described species, add two plant families of Angiosperms to the list of known hosts of seed-feeding *Megastigmus*, describe eight new species from Kenya, and provide a key to females of all known Afrotropical species.

Material and methods

Fruit and insect collection and rearing

During the 1999–2004 wild-fruit survey, 3839 fruit collections were made throughout much of Kenya. An additional 347 wild-fruit collections were made during the 2011–2012 survey in the Taita Hills, bringing the total number of fruit samples to 4186, the great majority from woody plants. Over the course of the two surveys approximately 930 species of plants were sampled, representing 122 families. Details of fruit collection and insect rearing methods are available in Copeland et al. (2002) and Copeland (2006). Briefly, fruits were collected from plants or on the ground below them. An effort was made to collect ripe, but not rotting fruit. Binoculars were used to associate fallen fruit under tall trees with fruit still remaining on the tree. Leaf and stem specimens, including flowers when present, were pressed in the field. Photographic vouchers of fruits and leaf and stem specimens were made at the time of collection. A ball point pen was included in each photograph to provide scale. The approximate diameter of the barrel of the pen was 10mm.

During transport, damage to fruits was minimised by placing each fruit sample in a separate polythene bag and suspending the bag above the bottom of a two-litre plastic storage container. A tight-fitting plastic lid fixed the bag and its contents in place. A large rectangular piece had been cut out of the lid and replaced with fine mesh cloth to allow for ventilation of the fruits. In the laboratory, fruits were removed from transport bags and placed within one-litre, rectangular plastic containers that had small elliptical holes cut out of the bottom. Each one-litre container (also provided with a mesh-covered lid) was nested within a fresh two-litre container, the bottom of which had a layer of heat-treated sand. Fruits were usually held for up to two months. Emerged adult insects were held for 1–3 days before being killed.

Beentje (1994) and Agnew and Agnew (1994) were used for preliminary plant identifications. Identifications made by the second author were confirmed or corrected by Quentin Luke of the East African Herbarium. Plant names and authors correspond to those found in The African Plant Database (2015) and the Missouri Botanical Garden (Tropicos 2015). Voucher specimens of plants are deposited in the collection of the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi.

Independent of the wild-fruit surveys, at various times between 2005 and 2014, the second author surveyed Kenyan insects using Townes-style Malaise traps (Townes 1972). Traps were run in a total of 76 locations representing diverse habitats, mostly throughout the southern half of the country. Some of the traps were run for a full year. Collecting bottles with 85–90% EtOH were changed every two weeks. Together, these collections represent ca. 458 sampling months (=ca. 13,740 sampling days). Moreover, after completion of the two fruit surveys, occasional collections of wild fruits were made during field trips to collect Malaise trap samples.

Morphological study

Adult morphology was examined using a MZ12 Leica stereomicroscope equipped with a Leica IC A camera. When more than three specimens were obtained per species and sex, one individual of each sex was dissected and wings of both sexes and male genitalia were mounted on glass slides using Dimethyl hydantoin formaldehyde (DMHF; Mendel 1982). Length of body, head, thorax, gaster and ovipositor sheaths was measured for each specimen using Leica QWIN V2.3° image processing and analysis software. Measurements of forewing stigma characteristics (stigma length, stigma maximal width, length of uncus, upper part of stigmal vein), marginal and post-marginal veins, and of antennal parts were made as described above. To increase depth of field, photographs of entire specimens and body parts were made by combining images taken at multiple focal planes using a Leica Z16 APO A microscope and JVC digital camera KY-F75U. Microvision Cartograph[®] software was used to combine pictures.

In a few specimens, head, antenna and thoracic sculpture were examined using a Cambridge StereoscanB 90 scanning electron microscope equipped with OrionB[®] image processing software. When less than three specimens were available per species and sex, these specimens were kept intact and mounted on triangular cardboards. Photographs and measurements of body and body parts (wings, antenna, head, thorax, gaster and ovipositor) were made as above without dissecting the mounted specimens. Morphological terminology follows Roques and Skrzypczyńska (2003). The following abbreviations are used in the text: F1, F2, ... Fn: Segment number of antennal funicle; OOL: Ocellocular line: the minimum distance between the eye margin and the adjacent posterior ocellus; POL: Posterior ocellar line: the minimum distance between the posterior ocelli; T3: first apparent tergite of gaster; T4: second apparent tergite; T5, T6, T7, T8, T9: subsequent gaster tergites.

DNA sequence analysis

• DNA extraction, molecular markers, amplification and sequencing

In order to include the Kenyan species in an existing phylogeny of *Megastigmus*, we used mtDNA sequences previously obtained by Boivin et al. (2014) for 25 species of seed chalcids associated with different host families (Pinaceae, Cupressaceae, Rosaceae, Anacardiaceae; GenBank accession numbers KF531833 to KF531858. Total genomic DNA was isolated by crushing, individually, whole adult females. A segment of the cytochrome oxidase I (COI) gene was amplified by PCR using the primers "Clyde and Bonnie" following Boivin et al. (2014). Whenever possible, the amplification was tested for one to five individuals per species, but for several species, only one specimen had amplifiable DNA although other specimens of the same species were collected. When these two primers did not allow sequencing, we used Bonnie and another primer C1-J-2183 ("Jerry", 5'-CAACATTTATTTTGATTTTTGG-3'; Simon et al. 1994), which supplied a shorter dataset but on a larger number of species. PCR products were purified using Nucleospin gel and PCR clean-up kit (Macherey-Nagel, Düren, Germany). Sequencing was performed using the big-dye terminator sequencing kit (PE Applied Biosystems) and carried out with an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Specimens were sequenced in both directions.

In addition to the COI gene, a nuclear fragment, the D2 region of the 28S ribosomal subunit (rDNA), was used to build a phylogenetic tree of the studied *Megastigmus* species. Nuclear primers, D1F (5'-ACCCGCTGAATTTAAGCATAT-3') and D3R (5'-TAGTTCACCATCTTTCGGGTC-3'), previously used for reconstructing a molecular phylogeny of *Megastigmus* spp. on conifers (Auger-Rozenberg et al. 2006), were chosen due to their utility for molecular identification at the intrageneric level. Some sequences were previously obtained by Auger-Rozenberg et al (2006) for 13 species of seed chalcids associated with conifer host families (AY900454, AY90048, AY900460, AY900463, AY900470 to AY900472, AY900474, AY900479, AY900481, AY900486, AY900487, AY900490), and the others were obtained for this study. The species studied molecularly, their collection localities and host plants are summarized in Suppl. material 1

Phylogenetic analyses

For all datasets, sequences were aligned using Clustal W (Thompson et al. 1994) as implemented in BioEdit 7.05 (Hall 1999). COI was aligned unambiguously and all

sequences were truncated to the same length (810 bp for the longest fragment and 417 bp for the shortest). All sequences were translated into amino acids using MEGA 6 (Tamura et al, 2013) to check that no stop codons occurred (Tamura et al. 2013). Genetic distances were calculated on the two COI datasets with MEGA 6 using Kimura-2-parameter (K2P) distance model. Interspecific sequence divergences based on K2P distances were calculated for all species, and mean interspecific K2P divergences were calculated from the pairwise comparisons within and between the clades identified according to host families. For 28S sequences, final alignment was obtained manually and gaps were treated as missing data. The alignment was 924 pb long including gaps.

Phylogenetic analyses were performed using maximum likelihood (ML) inference with PhyML v3.0 (Guindon et al. 2010). The appropriate model of evolution was evaluated with jModeltest v2.1.4 (Darriba et al. 2012). The models selected were GTR+I+G for the different genes. The robustness of the nodes was assessed with 500 bootstrap replicates. Additionally, Bayesian inferences (BI) were also used to reconstruct phylogenies with MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) using settings leading to convergence between two independent runs (200000 MCMC generations and sampling every 100 generations). Finally, trees were edited with Figtree v1.4.0 (A. Rambaut, http://tree.bio.ed.ac.uk/software/figtree).

Specimen depositories

Abbreviations used for insect specimen depositories or private collections cited in the study are as follows:

NMKE	National Museums of Kenya
RSC	Robert Copeland personal collection, ICIPE, Nairobi, Kenya
ARC	Alain Roques personal collection, INRA Zoologie Forestière Orléans, France
ICIPE	International Centre of Insect Physiology and Ecology, Nairobi, Kenya
SAMC	IZIKO South African Museum, Capetown, South Africa
BNHM	British National History Museum, London, UK

Results and discussion

Megastigmus diversity in Kenya

Megastigmus wasps were obtained from 31 of the fruit samples (0.74%- Table 1). Additionally, 10 *Megastigmus* specimens were recovered from four Malaise trap samples. The distribution in Kenya of previously described *Megastigmus* species that we collected from fruit samples and Malaise traps is shown in Figure 1, while sampling locations that yielded new *Megastigmus* species are indicated in Figure 2. Specimens were obtained across the length and breadth of the country, from the Indian Ocean to Lake

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Family / Host plant species	Megastigmus species	Region	Location	Latitude N	Longitude E	Elevation ¹ (m)
Anacardiaceae						
Lannea of schimperi (A.Rich.) Engl.	<i>M. smithi</i> Roques & Copeland, sp. n.	Eastern Arc Mountains	Sagalla Mountain	-3,4786	38,5746	1090
Lannea rivae (Chiov.) Sacl.	<i>M. lanneae</i> Roques & Copeland, sp. n.	Central highlands	Machakos	-1,5609	37,2338	1586
Lannea schweinfurthii (Engl.) Engl.	<i>M. laventhali</i> Roques & Copeland, sp. n.	Eastern Arc Mountains	Base of Kasigau Mountain	-3,8130	38,6405	619
Ozoroa insignis Del. subsp. reticulata (Bak.f.) Gillett	<i>M. ozorode</i> Roques & Copeland, sp. n.	Western mid-altitude	Gembe Hills	-0,4894	34,2433	1362
O. insignis subsp. reticulata	M. ozoroae	Western mid-altitude	Sindo-Mbita Road	-0,4849	34,1765	1205
O. insignis subsp. reticulata	M. hypogeus (Hussey)	Western mid-altitude	Sindo-Mbita Road	-0,4849	34,1765	1205
Ozoroa obovata (Oliv.) R. & A. Fernandes	M. hypogeus	Coastal forests	Arabuko-Sokoke Forest	-3,3103	39,9978	55
O. obovata	M. hypogeus	Coastal forests	Arabuko-Sokoke Forest	-3,3716	39,8949	55
O. obovata	M. hypogeus	Coastal forests	Arabuko-Sokoke Forest	-3,2030	39,9271	98
O. obovata	M. hypogeus	Coastal forests	Shimba Hills	-4,1349	39,4680	285
<i>Pistacia lentiscus</i> L. subsp. <i>emarginata</i> (Engl.) AL-Saghir	<i>M. pistaciae</i> Walker	Western highlands	nr. Narok on main road	-1,1023	36,0192	1919
<i>P. lentiscus</i> subsp. <i>emarginata</i>	M. pistaciae	Western highlands	nr. Narok on main road	-1,1243	35,9868	1830
P. lentiscus subsp. emarginata	M. pistaciae	Northern highlands	Mt Kulal	2,6290	36,9278	1639
P. lentiscus subsp. emarginata	M. pistaciae	Western highlands	Mai Mahiu-Narok Road	-1,1121	35,9765	1900
Rhus natalensis Krauss	<i>M. transvaalensis</i> (Hussey)	Central Highlands	Kikuyu Escarpment	-1,0290	36,6025	2100
R. natalensis	M. transvaalensis	Central Highlands	Kikuyu Escarpment	-1,0717	36,6033	2100
R. natalensis	M. transvaalensis	Central Highlands	Burguret	-0,1128	37,0375	1960
R. natalensis	M. transvaalensis	Northern mid-altitude	south of Marsabi town	2,2659	37,8976	1200
R. natalensis	M. transvaalensis	Western mid-altitude	Gembe Hills	-0,4894	34,2433	1370
R. natalensis	M. transvaalensis	Eastern Arc Mountains	Ronge-Nyika, mixed shrub- and grassland	-3,3963	38,4309	1050
R. natalensis	M. transvaalensis	Eastern Arc Mountains	Kasigau forest	-3,8194	38,6619	1280

Family / Host plant species	Megastigmus species	Region	Location	Latitude N	Longitude E	Elevation ¹ (m)
Rhus vulgaris Meikle	M. transvaalensis	Central Highlands	Kikuyu escarpment	-1,0780	36,6020	2200
Schinus molle L.	M. transvaalensis	Central Highlands	Nairobi	-1,2211	36,8963	1610
Schinus terebinthifolius Raddi	M. transvaalensis	Central highlands	Nairobi	-1,2212	36,8963	1610
S. terebinthifolius	M. transvaalensis	Central highlands	Nairobi	-1,2501	36,7835	1750
Cupressaceae						
Juniperus procera Endl.	M. somaliensis (Hussey)	Central highlands	Mt. Kenya Forest	-0,0308	37,1230	2130
J. procera	M. somaliensis	Northern highlands	Mt. Kulal	2,6319	36,9282	1650
Rhamnaceae						
Helinus integrifolius (Lam.) Kuntze	<i>M. helinae</i> Roques & Copeland, sp. n.	Northern highlands	Mathews Range	1,1777	37,3141	1340
Malvaceae						
Grewia stubhnannii K. Schum.	<i>M. grewianae</i> Roques & Copeland, sp. n.	Coastal forests	Arabuko-Sokoke Forest	-3,2997	39,9869	60
Grewia tephrodermis K. Schum.	M. grewianae	Eastern savanna	Tsavo	-2,6760	38,3325	640
Grewia tephrodermis	<i>M. copelandi</i> Roques & Copeland, sp. n.	Eastern Arc Mountains	Mwatate area, on small farm	-3,4894	38,3330	1050
n.a., Malaise trap	M. grewianae	Eastern savanna	Kasaala	-2,0749	38,2250	740
n.a., Malaise trap	M. grewianae	Eastern Arc Mountains	Kasigau Mountain Forest	-3,8270	38,6488	1070
n.a., Malaise trap	<i>M. icipeensis</i> Roques & Copeland, sp. n.	Central Highlands	Nairobi, ICIPE	-1,2232	36,8965	1600
n.a., Malaise trap	M. laventhali	Coastal island	Funzi Island	-4,5778	39,4413	0



Figure 1. Distribution in Kenya of the previously described African Megastigmus species.

Victoria in the west and from near Tanzania in the south to Mt. Kulal and Marsabit in the arid north (Figures 1 and 2). They were collected at elevations between near sealevel (Funzi Island) and 2200 m (Kikuyu Escarpment).

A total of 12 *Megastigmus* species were found during the survey, of which eight were new to science (Table 1). Among the four previously described species that we reared, *M. hypogeus* was described from specimens collected in Kenya (Hussey 1956a), and before our surveys it was the only *Megastigmus* species known from the country. *Megastigmus transvaalensis* was reared from Anacardiaceae fruits during the early stages of our project in Kenya and these data were reported in Grissell and Prinsloo (2001) and Scheffer and Grissell (2003). Numerous records of this species also exist for other regions of tropical Africa such as South Africa (Iponga et al. 2008) and the La Réunion island (Roques and Skrzypczynska 2003). Our rearing of *M. pistaciae* represents the



Figure 2. Distribution in Kenya of the new Megastigmus species identified during our study.

first record in the Afrotropical region of this widespread Palaearctic species (Roques and Skrzypczynska 2003). In addition, two samples of *Juniperus procera* produced *M. somaliensis*, a species previously described from specimens collected in the former British Somaliland (Hussey 1956b).

Megastigmus chalcids were reared from fruits of seven genera of shrubs and trees native to tropical Africa (Table 2), belonging to the families Anacardiaceae (*Lannea, Ozoroa, Pistacia* and *Rhus*; Figures 3–9), Rhamnaceae (*Helinus*; Figure 10), Malvaceae (*Grewia*; Figure 11–12) and Cupressaceae (*Juniperus*; Figure 13).

Of the host families, Anacardiaceae was most important. Including the collections from two exotic *Schinus* species, Anacardiaceae contributed 10 (67%) of the host fruit species (n=15) from which *Megastigmus* were reared. Among these plant hosts, *Lannea* was the most important genus with three of five species sampled producing *Megastigmus*.



Figures 3–9. Anacardiaceae host fruits of *Megastigmus* spp. in Kenya. 3 *Lannea cf. schimperi* 4 *L. rivae* 5 *Lannea schweinfurthii* 6 *Ozoroa insignis* subsp. *reticulata* 7 *O. obovata* 8 *Pistacia lentiscus* subsp. *emarginata* 9 *Rhus natalensis* (note the chalcid hole in a fruit).

Family	Genus	Total no. of Kenyan species	No. of Kenyan species sampled	No. of sampled Kenyan species yielding Megastigmus (%)	Total number of Afrotropical species
Anacardiaceae	Lannea ¹	10	5	3 (60)	29
	Ozoroa ¹	2	2	2 (100)	44
	Pistacia ^{1,2}	1	1	1 (100)	2
	Rhus ¹	6	4	2 (50)	106
	Schinus ^{1,5}	25	25	2 (100)	25
Cupressaceae	Juniperus ¹	1	1	1 (100)	4
Rhamnaceae ³	Helinus ^{1,2,4}	2	2	1 (50)	3
Malvaceae ³	Grewia ^{1,2,4}	28	14	2 (14)	122

Table 2. Host-plant genera of Megastigmus species in Kenya.

¹First record of genus as a host of *Megastigmus* in Kenya; ²First record of genus as a host of *Megastigmus* in Afrotropical region; ³New host-plant family; ⁴New host-plant genus; ⁵Introduced in Afrotropical region

Each Lannea species was attacked by a different, previously undescribed chalcid: Lannea cf. schimperi (A.Rich.) Engl. (Figure 3) by Megastigmus smithi Roques & Copeland, sp. n., L. rivae (Chiov.) Sacl. (Figure 4) by Megastigmus lanneae Roques & Copeland, sp. n., and L. schweinfurthii (Engl.) Engl. (Figure 5) by Megastigmus laventhali Roques & Copeland, sp. n., (Table 1). Both species of Kenyan Ozoroa were hosts of Megastigmus. Ozoroa insignis Del. subsp. reticulata (Bak.f.) Gillett (Figure 6) and Ozoroa obovata (Oliv.) R. & A. Fernandes (Figure 7) represent the first confirmed hosts of M. hypogeus (see next paragraph). The former plant is also reported for the first time as a host of Megastigmus. Ozoroa insignis var. reticulata also yielded an undescribed Megastigmus species, M. ozoroae Roques & Copeland, sp. n. Other Anacardiaceae hosts were Pistacia lentiscus L. subsp. emarginata (Engl.) Al-Saghir (Figure 8), a new host record for Megastigmus pistaciae Walker, and Rhus natalensis Krauss (Figure 9), Rhus vulgaris Meikle and the two exotic Anacardiaceae, Schinus terebinthifolius Raddi and Schinus molle L., all hosts of M. transvaalensis. The two Rhus species represent new host records for M. transvaalensis.

Before our study the host of *M. hypogeus* was an unresolved issue. When transcribing label information in his description of *M. hypogeus* from specimens then available to him, Hussey (1956a) reported the host as "Oil seed, probably *Helianthus* (Compositae)". This seemed unlikely as no Asteraceae has been reported as a host of phytophagous *Megastigmus*. We were able to examine Hussey's holotype and paratypes of *M. hypogeus* as well as additional material deposited at BNHM and it appeared that our Kenyan specimens undoubtedly belong to the same species. Thus, our study appears to have resolved this host question as we reared *M. hypogeus* from several collections of *O. insignis* subsp. *reticulata* and *O. obovota*. Another *Megastigmus* species, *M. thomseni* (Hussey), is known to develop in seeds of *O. obovota* as well as in those of *Ozoroa paniculosa* (Sond.) and *Lannea discolor* (Sond.) Engl. in South Africa (Grissell and Prinsloo 2001). The examination of Hussey's type material kept at BNHM confirmed that *M. thomseni* was not present in the chalcid specimens collected during our survey.



Figures 10–13. Other host fruits of *Megastigmus* spp. in Kenya. 10 *Helinus integrifolius* (Rhamnaceae) 11 *Grewia tephrodermis* (Malvaceae) 12 *G. stuhlmannii* 13 *Juniperus procera* (Cupressaceae).

Among non-Anarcardiaceae host families, two new *Megastigmus* species were obtained from fruits of two species of Malvaceae in the genus *Grewia. Megastigmus grewianae* Roques & Copeland, sp. n. emerged from both *Grewia tephrodermis* K. Schum. (Figure 11) and *G. stuhlmannii* K. Schum. (Figure 12), while *Megastigmus copelandi* Roques & Copeland, sp. n. was reared from *G. tephrodermis. Megastigmus helinae* Roques & Copeland, sp. n. was reared from seeds of the Rhamnaceae *Helinus integrifolius* (Lam.) Kuntze (Figure 10), collected in the northern Kenya mountains of the Mathews Range. These are the first records of *Megastigmus* seed chalcids from Malvaceae and Rhamnaceae. Finally, we reared *M. somaliensis* from two collections of *Juniperus procera* (Figure 13). The examination of Hussey's type material kept at BNHM allowed us to confirm the species identity. The remaining species, *M. icipeensis* Roques & Copeland, sp. n., has no known host, having been collected only in Malaise trap samples.

Table 3 presents the infestation indices of the host fruits. The percentage of fruits infested by chalcids ranged from 0.1 to 14.8% depending on both the *Megastigmus* species and the host plant. However, the infestation only exceeded 10% of the fruits in two species of Anacardiaceae, *Rhus vulgaris* and the introduced pinkpepper tree *Schinus molle*, both attacked by *M. transvaalensis*, and in the Malvaceae *Grewia tephrodermis*, attacked by *M. grewianae*.

The four Malaise trap samples containing *Megastigmus* yielded a total of 10 specimens. Given our estimate of 13,740 Malaise trap sampling days, *Megastigmus* were captured at the very low rate of approximately one specimen per 1374 sampling days.

Plant family/ Plant species	No. of fruits	Megastigmus species	No. of reared <i>Megastigmus</i>	% of infestation by <i>Megastigmus</i>
Anacardiaceae				
Lannea cf. schimperi	786	M. smithi	3	0,4
Lannea rivae	338	M. lanneae	11	3,3
Lannea schweinfurthii	168	M. laventhali	1	0,6
Ozoroa insignis subsp. reticulata	2806	M. ozoroae	226	8,1
Ozoroa obovata	1505	M. hypogeus	42	2,8
Ozoroa obovata	698	M. hypogeus	1	0,1
Ozoroa obovata	2442	M. hypogeus	22	0,9
Pistacia lentiscus subsp. emarginata	2603	M. pistaciae	12	0,5
Pistacia lentiscus subsp. emarginata	1019	M. pistaciae	1	0,1
Rhus natalensis	674	M. transvaalensis	41	6,1
Rhus natalensis	524	M. transvaalensis	6	1,1
Rhus natalensis	2394	M. transvaalensis	14	0,6
Rhus natalensis	894	M. transvaalensis	36	4,0
Rhus natalensis	412	M. transvaalensis	6	1,5
Rhus vulgaris	769	M. transvaalensis	113	14,7
Schinus molle	535	M. transvaalensis	79	14,8
Rhamnaceae				
Helinus integrifolius	82	M. helinae	8	9,8
Malvaceae				
Grewia tephrodermis	448	M. copelandi	3	0,7
Grewia tephrodermis	100	M. grewianae	13	13,0
Grewia stuhlmannii	1056	M. grewianae	29	2,7

Table 3. Host fruit infestation indices for *Megastigmus* species. Only samples whose fruits were counted are included; no samples of *Juniperus procera* fruits were counted.

Despite this, a total of three species of *Megastigmus* were collected, all of them representing previously undescribed species. Single specimens of *Megastigmus grewianae* Roques & Copeland, sp. n. were collected in Malaise traps set in dry savanna in Eastern Kenya (Kasaala) and in a small campsite clearing in Kasigau Forest (Table 1, Figure 2). In addition to being reared from *Lannea schweinfurthii*, a single specimen of *Megastigmus laventhali* Roques & Copeland, sp. n. was captured in a Malaise trap set on Funzi Island, just off the coast of Kenya. Finally, a Malaise trap set alongside a small stream in a sprawling semi-urban part of Nairobi yielded five female specimens of *Megastigmus icipeensis* Roques & Copeland, sp. n. and three males, which probably belong to the same species. In contrast with other groups of Microhymenoptera (e.g. Dryinidae; Olmi et al. 2015) Malaise traps produced very few specimens of *Megastigmus*. Although each of the four Malaise samples with *Megastigmus* contained an undescribed species, only one of the species (*M. icipeensis*), was not represented in the fruit samples. Fruit collection is presently the best hope for discovering new host plant taxa and new *Megastigmus* species.

Our fruit collections bring to six the number of Afrotropical plant families that are hosts of *Megastigmus*, including Rosaceae which are attacked by the introduced species, *M. aculeatus*. The listing, in a summary table, by Grissell (1999) of the presence in the Afrotropical region of *M. rosae* Bouček, another palearctic species associated with seeds of wild roses, was probably in error as it is not mentioned in the species accounts (Grissell 1999). Moreover, no such record is found in Noyes (2015).

Table 2 summarizes data on the number of species of known host-plant genera found in Kenya and in the Afrotropical region. The Anacardiaceae genera *Rhus* and, to a lesser extent, *Ozoroa* and *Lannea* are speciose, as is the Malvaceae genus *Grewia*. Considering that the great majority of species of these plant genera remain to be sampled it is reasonable to expect that targeted fruit collections will yield considerably more species of Afrotropical *Megastigmus*. Without exception, fruits used by *Megastigmus* species were small and relatively dry, with little pulp surrounding the seed (Figures 3–13). Of plant families known to be hosts, species with larger and wetter fruits were never found to harbor *Megastigmus*. For example, among the Anacardiaceae with large, fleshy fruits that we sampled, the genera *Anacardium* (1 sample), *Harpephyllum* (6 samples), *Mangifera* (3 samples), *Pseudospondias* (2 samples), *Sclerocarya* (15 samples), and *Sorindeia* (10 samples) all failed to produce *Megastigmus* (Copeland et al. 2009).

Molecular confirmation of specific identity of the Megastigmus species and phylogeny

MtDNA was sequenced for nine of the 12 African species defined morphologically, including five of the eight new species and the four previously described ones. Using the longest mitochondrial marker ("Clyde-Bonnie"), 810 bp- long COI sequences were obtained for *M. helinae* (1 specimen), *M. grewianae* (4 specimens), *M. lanneae* (1 specimen), M. pistaciae (2 specimens), M. somaliensis (1 specimen) and M. zebrinus (1 specimen). In the case of *M. hypogeus* and *M. ozoroae*, six specimens per species were extracted but we obtained only a short fragment of 417 bp with the internal primer "Jerry" for one individual of *M. ozoroae*, and for two of *M. hypogeus*. Similarly, we obtained this short fragment for the sole specimen of *M. icipeensis*. DNA from the other species was amplified with the different sets of primers and, although we obtained data from more species with the shorter fragment than with the longer, we studied and compared the two datasets because some short sequences showed the presence of overlapping fragments (for less than 2% of the short sequence) which could be due to heteroplasmy or numt's (nuclear copies of mitochondrial DNA), even if they were of the correct length and reading frame and contained no stop codons. These trends were observed in *M. lanneae* and *M. ozoroae* and their occurrence seemed to be species specific. These problems (no amplification and/or overlapping fragments) could be due to the preservation quality of the specimens. For *M. transvaalensis*, we used sequences from specimens previously obtained from Morocco, because we failed to amplify any Kenyan specimens. Megastigmus copelandi, M. laventhali and M. smithi were not analyzed molecularly because of the limited number of available specimens necessary to

define holotype and paratypes. For the nuclear marker 28S, one specimen per species was successfully amplified for all the species analyzed in the COI dataset except *M. icipeensis*, and an alignment of 924 bp including gaps was obtained.

The inclusion of the nine Kenyan species in an already existing molecular phylogeny (Boivin et al. 2014) and their resulting phylogenetic position confirmed without ambiguity that none of the Kenyan species was synonymous with previously examined species. After grouping *Megastigmus* species according to their host plant families (Pinaceae / Cupressaceae / Taxodiaceae / Rosaceae / Anacardiaceae / Malvaceae / Rhamnaceae), we calculated within- and between-group mean distances for the two COI datasets (Table 4). In both cases lower values were observed within groups than between groups, with values ranged from 6% to 7.9% for COI-long fragment and from 4.6% to 6.8% for COI-short fragment (with no values for Malvaceae, Rhamnaceae or gall-former because there was only one species per group). Values within the Anacardiaceae group were quite similar for both datasets (6.8% and 6.3%), and are consistent with the values observed within the Holarctic groups (Pinaceae / Cupressaceae / Taxodiaceae / Rosaceae).

Between groups, similar values were observed between Holarctic groups, as well as between the species related to Malvaceae and the Holarctic groups, whereas pairwise K2P distances showed higher values for the other host families. In the COI-long fragment dataset, the values between *M. somaliensis* and the two other species related to the genus *Juniperus* (Cupressaceae) were 3.5%. The two specimens identified as *M. pistaciae* (one from Greece and one from Kenya) diverged by 4.5%, suggesting that the two are probably sibling species.

The use of maximum likelihood and Bayesian inference methods resulted in similar information about phylogenetic relationships within the genus Megastigmus. Therefore, we present only the phylogenetic trees built with the Bayesian inference method (Figure 14). The phylogenetic reconstruction was carried out by adding, where possible, one sequence (selected at random when there was more than one specimen) per Kenvan species to the sequences of the 25 Megastigmus species considered in Boivin et al. (2014). The current dataset thus included 32 taxa for the COI-long fragment, 35 for the COI-short one and 34 for the 28S marker. To obtain phylogenetic trees including as many taxa as possible, after alignment we compared the sequenced regions common to all taxa for analysis; i.e. the 28S and COI-short fragment. For the mtDNA analysis, the trees obtained with COI-long fragment were considered first because of the greater reliability and safety of long fragments vs short ones. Our phylogenetic reconstruction based on evidence from two markers confirmed previous studies (Auger-Rozenberg et al. 2006; Boivin et al. 2014) that linked host families specialization with genetic similarity. The phylogenetic trees were analysed with a focus on the Kenyan species, and some of the new species are associated with host families already present in the phylogeny (Anacardiaceae and Cupressaceae). In those cases, they always clustered in the corresponding clade regardless of the marker. For the other host families (i.e. associated with Rhamnaceae and Malvaceae or described as gall-former) for which no species were already described, the phylogenetic position remains unclear.

Table 4. Mean genetic distances within and between groups of Megastignus sequences. For each cell: COI long fragment before backslash (/), and COI short fragment after backslash (in this case, the group "unknown" is corresponding to the only specimen of M. icipeensis, caught in malaise trap and only sequenced in short fragment).

quorgroop										-/-
umouyun										-\0.113
Gall-former									-\0.083	0.154\0.121
Апасагдіасеае							0.068\0.063	0.105\0.092	-\0.082	0.146\0.115
Кһатпасеае						-/-	0.107\0.078	0.094\0.086	-\0.083	0.140\0.113
Tiliaceae					-1-	0.111\0.097	0.111\0.095	0.106\0.080	-\0.096	0.161\0.132
экээкгоЯ				0.079\0.068	0.102\0.087	0.108\0.088	0.108\0.100	0.100\0.086	-\0.094	0.157\0.134
эвээвірохвТ			-/-	0.086\0.077	0.094\0.083	0.107\0.096	0.099\0.104	0.094\0.096	-\0.096	0.158\0.135
Cupressaceae		0.060\0.046	0.084\0.074	0.093\0.080	0.089\0.080	0.112\0.105	0.112\0.099	0.100\0.085	-\0.089	0.151\0.123
Ріпасеае	0.061\0.057	0.088\0.073	0.081\0.074	0.085\0.074	0.098\0.086	0.113\0.095	0.111\0.096	0.102\0.092	-\0.094	0.157\0.127
	Pinaceae	Cupressaceae	Taxodiaceae	Rosaceae	Tiliaceae	Rhamnaceae	Anacardiaceae	Gall-former	Unknown	Outgroup

In the different trees, robust clades (Pinaceae, Cupressaceae, Rosaceae, Anacardiaceae) included several species, whereas the positions of single species varies depending of the marker. Whatever the marker and method, the Megastigmus species associated with Anacardiaceae clustered together in a highly supported monophyletic clade (Figure 14). Within this clade, the two specimens collected on *Pistacia* always clustered indicating a phylogenetic proximity. The basal position of this clade in the COI dataset and its large branch length in the nuclear dataset indicated an ancestral split between the species associated with Anacardiaceae and those reared from other plant families. However, the phylogenetic position of *M. helinae* (Rhamnaceae), *M. grewianae* (Malvaceae) and *M.* zebrinus (gall-former) was unstable and unresolved. Similarly, the position of M. icipeensis (caught in malaise trap) remained unclear. For this last species, as for *M. hypogeus* and M. ozoroae (both associated with Anacardiaceae), mtDNA amplification produced only the shortest COI fragment. The study of this gene fragment was very useful because some species were only available in this dataset, although it is recognized that longer fragments are usually more reliable in identification of insects (Aly 2014) and that the presence of numts can skew the phylogenetic relationships, leading to mtDNA divergence that is often lineage specific (Song et al. 2008). Details for each species studied both morphologically and molecularly are provided in the key below.

Despite amplifying for all markers used, the phylogenetic position of *M. zebrinus* remains uncertain. According to Grissell (2006), *M. zebrinus*, a gall-forming species introduced with its *Eucalyptus* host, has modified its feeding behaviour by adapting to the seeds of a new host, *Syzygium cordatum*, endemic to the Afrotropical region. In our phylogenetic trees (in addition to the genetic distance calculated from the COI fragments) it clusters with the strictly seminiphagous species, and this brings it close to the Kenyan species. It would be very useful to confirm its degree of molecular similarity with Australian gall-formers in order to disentangle the evolutionary strategies that allow a species to shift to new host-plant taxa and adapt to new host-plant reproductive structures.

The mtDNA sequences and nuclear sequences determined in the course of this study are registered under the following GenBank accession numbers: KU984677 to KU984706.

Key to the species of Megastigmus of Eastern and Southern Africa

In some *Megastigmus* species, males, unlike females, are highly variable in size and colour. This is observed especially in species associated with Anacardiaceae (e.g., *M. transvaalensis*; Grissell and Prinsloo 2001; *M. pistaciae*, Roques and Skrzypczyńska 2003) and Cupressaceae (*M. amicorum* Bouček, *M. wachtli* Seitner; Roques and Skrzypczyńska 2003), but also in species associated with galls such as *M. zebrinus* (Grissell, 2006). In *M. transvaalensis*, Grissell and Prinsloo (2001) observed two extreme morphs, small yellow specimens vs. large ones predominantly colored in black and with a large, infuscate stigma, and pointed out that these forms are bridged by intermediates along a gradual cline. Many pale and dark male morphs were seen by us in rearings of *M. lanneae* (Figures 103 and 110), *M. ozoroae* (Figures 49 and 56) and *M. hypogeus* (Figures 123 and





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130) These males were easily assigned to their respective species because they emerged together with many females of only one species. In the absence of DNA confirmation, when few specimens are reared, variations in size and colour make it difficult to assign males and females to the same species with certainty, particularly when adults emerge at different times or at different sites, or when they were caught in Malaise traps.

Therefore, we provide a key to females only, including the species associated with rose hips, conifers, and galls that were not collected during the surveys (i.e. *M. aculeatus, M. thomseni, M. pretorianensis* and *M. zebrinus*). We also include *M. asir* Ghramh & Shati recorded from seeds of *Juniperus procera* in a region of Saudi Arabia facing Eritrea and Ethiopia (Ghramh and Shati 2011). The key relies strongly on the relative length of the exserted part of the ovipositor which we consider to be a stable character, related to the species' adaptation to host exploitation (Roques and Skrzypczyńska 2003).

Key to Megastigmus females of Eastern and Southern Africa

1	Ovipositor sheaths shorter $(0.4-0.9\times)$ than gaster length (Figures 16, 31, 37, 43)
-	Ovipositor sheaths longer (1.1×–1.8×) than gaster length (Figures 63, 76, 97, 117, 137, 151, 163) 5
2	Ovipositor sheaths much shorter $(0.4-0.6\times)$ than gaster length (Figures 16, 31)3
	Ovipositor sheaths 0.8–0.9× as long as gaster length (Figures 37, 43)4
3	Body colour nearly entirely orange-yellow; only a narrow black band along
	anterior suture of pronotum (Figures 15, 16)
	<i>M. helinae</i> Roques & Copeland, sp. n.
-	Body colour with a large longitudinal, median rufous band extending from
	pronotum to scutellum (Figure 30, 32) and parts of gaster dark brown (Fig-
	ure 30) M. smithi Roques & Copeland, sp. n.
4	Pilosity on head entirely pale (Figure 39); pilosity on propodeum, coxa and
	legs entirely pale; body extensively orange except a few black patterns (Figure
	37)
_	Pilosity pale on face but dark on other parts of head (Figure 45); pilosity on
	propodeum, coxa and legs dark; body colour yellow and black (Figures 42,
	43, 44)M. ozoroae Roques & Copeland, sp. n.
5	Minute species, body length less than 1.5 mm; Thorax pilosity pale; 2 pairs of
	hairs on scutellum (Figure 64)6
_	Larger species, body length more than 2.5 mm; Thorax pilosity dark; from 3
	to 8 pairs of hairs on scutellum (Figures 77, 83, 98, 118, 131, 138, 152)7
6	Body colour mostly black
_	Body colour nearly completely orange-yellow (Figure 64)
	<i>M. icipeensis</i> Roques & Copeland, sp. n.
7	Thorax colour orange or pale yellow without extensive dark markings (Figures
	77, 138, 152) 8

-	Thorax with extensive dark markings (Figures 83, 98, 118, 163)12
8	Ovipositor sheaths less than 1.2× as long as gaster length (Figure 137)9
_	Ovipositor sheaths at least 1.4× as long as gaster length (Figures 76, 151) 10
9	Forewing with an elongate stigma, 1.7× as long as wide
_	Forewing with an oval- rounded stigma, $1.2-1.3 \times as$ long as wide (Figure
	141)
10	Ovipositor sheaths at least 1.8 × as long as gaster length and 0.8× as long as
	body length (Figure 76); forewing stigma oval, $1.5 \times as$ long as wide (Figure
	80)
_	Ovipositor sheaths at most 1.6× as long as gaster length and 0.6× as long as
	body length (Figure 151); forewing stigma more elongate, at least $1.6 \times as$
	long as wide (Figure 155)11
11	Forewing stigma oblong, $1.7 \times \text{as}$ long as wide, with a very short stigmal vein,
	at most $0.2 \times$ as long as stigma length; gaster tergites pale yellow with translu-
	cent median longitudinal stripes, transversely infumateM. zebrinus Grissell
-	For ewing stigma elongate-oval, 2.0 \times as long as wide, with an elongate stigmal
	vein, $0.4 \times$ as long as stigma length (Figure 155); gaster tergites light orange-
	brown with transverse dark brown bands
12	Ovipositor sheaths never longer than 1.5× gaster length (Figures 117, 163)13
-	Ovipositor sheaths at least 1.8× longer than gaster length (Figures 82, 97) 15
13	Pronotum with a large median dark brown band progressively decreasing in
	size from the anterior to posterior suture (Figure 118); scutellum with 3-4
	pairs of hairs; in seeds of Ozoroa
-	Pronotum yellow or brownish, without median dark band; scutellum with
	7-8 pairs of hairs; in seeds of <i>Juniperus procera</i> 14
14	Propodeum smooth; forewing stigma elongate, more than $2 \times$ as long as
	broad <i>M. asir</i> Ghramh & Shati
-	Propodeum rugose, with reticulate striae (Figure 166); forewing stigma oval,
	1.5 × as long as broad (Figure 167) <i>M. somaliensis</i> Hussey
15	Ovipositor sheaths at least 1.2× longer than body length. In Rosa seeds
-	Ovipositor sheaths never longer than body length. In Malvaceae or Anacardi-
	aceae seed
16	Pronotum orange with a conspicuous longitudinal black stripe on each side
	(Figures 81, 82, 83); in Malvaceae seeds
	<i>M. grewianae</i> Roques & Copeland, sp. n.
-	Pronotum without lateral black stripes but with a large, nearly rectangular dark
	brown band medially extending from anterior to posterior suture (Figures 96,
	98); in Anacardiaceae seeds
Species description

Megastigmus helinae Roques & Copeland, sp. n. http://zoobank.org/A2A398C5-E7D8-4B8E-B858-2136275BC907

Type material. Holotype ♀, Kenya, Scandent climber, Rift Valley Prov. Matthews Range, 1.1777°N, 37.3141°E, 1342m, 16 Jan 2004, ex. *Helinus integrifolius* fruits, A&M coll. N°2692, R. Copeland leg. (NMKE).

Paratypes. $2 \bigcirc \bigcirc$, same collection data as holotype (ARC, 1 destroyed); $2 \bigcirc \bigcirc$, same collection data as holotype (NMKE, ICIPE).

Description. Holotype \bigcirc . A small species with body length 3.2 mm (without ovipositor); length of ovipositor sheaths 0.8 mm. Body colour nearly entirely orange-yellow (Figures 15, 16). Head orange with a black ring around each ocellus. Pilosity on lower face pale, but black hairs on malar space, gena, and on dorsum of head, with a row of long hairs on vertex behind ocelli. Antenna entirely yellow. Pronotum orange; remainder of thorax orange except a bisinuate black band along pronotum suture at the anterior part of mid-lobe of mesoscutum (Figure 16), and two small black spots at wing insertions. Pilosity black on thorax dorsum; sparse long hairs on pronotum and mid-lobe of mesoscutum along notauli; scutellum with 3 pairs of black hairs on the sides. Legs entirely pale yellow. Forewing stigma brown without infuscation; basal cell well defined, with disc including 8 long setae, closed by a basal setal line with 8 long hairs and a costal setal line with 6 long hairs; coastal cell with several rows of small setae (Figure 21). Propodeum orange with a narrow, curved black stripe on the center of its anterior part; propodeum hairs black. Gaster entirely orange, with black hairs on sutures. Ovipositor sheaths black, very short, only 0.6 × as long as gaster, 0.2 × as long as body (Figure 16).

Head transverse (Figure 18), width: height ratio: 1.3 in dorsal view; POL: OOL: 0.8; torulus $1.2 \times as$ long as wide; inter-antennal area as broad as torulus width; scrobe elongate, ca. $2.3 \times as$ long as wide; Scape $1.2 \times as$ long as combined length of pedicel, anellus and F1 (Figure 19); scape $0.8 \times as$ long as combined length of pedicel, anellus, F1 and F2; anellus subquadrate; F1 $1.2 \times as$ long as pedicel, $1.9 \times as$ long as wide; F2 $1.8 \times as$ long as wide; following funicular segments progressively tending to subquadrate from F4 on, with F7 $1.3 \times as$ long as wide. Pronotum, mid- and lateral lobes of mesoscutum, with coarse transverse carinae (Figure 20); axilla with longitudinal striae; mid-lobe of mesoscutum $1.1 \times as$ long as scutellum. Scutellum $1.3 \times as$ long as wide, with irregular transverse striae; frenum $0.3 \times as$ long as scutellum length, mostly smooth with a few longitudinal carinae on sides. Forewing stigma oval, about $1.5 \times as$ long as wide (Figure 21); upper part of stigmal vein elongate, $0.4 \times as$ long as stigma length; uncus $0.6 \times as$ long as upper part of stigmal vein; marginal vein $0.8 \times as$ long as postmarginal vein. Propodeum with coarse longitudinal carinae, and a median carina interrupted in the middle (Figure 20).

Male. Body colour similar to that of female (Figures 22, 23), orange-yellow with a few black markings: a ring around each ocellus, a transverse fuzzy band on the center of the anterior part of propodeum, a large spot on T3 at gaster insertion, a comma- like spot



Figures 15–21. *Megastigmus helinae* Roques & Copeland, sp. n. female. 15 dorsal view of the body 16 lateral view of body 17 dorsal view of thorax 18 front view of head 19 electroscan of antenna 20 electroscan of dorsal view of thorax 21 forewing.

on the lateral sides of T4, and a smaller, rounded, brownish spot on the lateral sides of the three following tergites. Pilosity as in female. Forewing stigma brown without infuscation (Figure 28); basal cell well defined, the disc with 11 long setae, closed by a basal setal line



Figures 22–29. *Megastigmus helinae* Roques & Copeland, sp. n. male. 22 dorsal view of the body 23 lateral view of body 24 dorsal view of thorax 25 front view of head 26 electroscan of antenna 27 electroscan of dorsal view of thorax 28 forewing 29 genitalia.

with 7 long setae and a costal setal line with 6 long setae; coastal cell with several rows of small setae.

Head rectangular (Figure 25), width: height ratio: 1.5 in dorsal view; POL: OOL 0.9; torulus $1.2 \times as$ long as wide; inter-antennal area $0.9 \times as$ wide as torulus width;

scrobe 2.5 × as long as wide. Scape $1.1 \times$ as long as combined length of pedicel, anellus and F1 (Figure 26); scape $0.8 \times$ as long as combined length of pedicel, anellus, F1 and F2; F1 $1.3 \times$ as long as pedicel, $2.1 \times$ as long as wide; F2 $2.0 \times$ as long as wide; following funicular segments also elongate, with F7 $1.5 \times$ as long as wide. Pronotum, mid- and lateral lobes of mesoscutum, with coarse transverse carinae (Figure 27); axilla with longitudinal striae; mid-lobe of mesoscutum short, $0.9 \times$ as long as scutellum. Scutellum $1.2 \times$ as long as wide, with irregular, transverse striae; frenum $0.3 \times$ as long as scutellum length, mostly smooth with a few longitudinal carinae on sides; Forewing stigma more rounded than in female, about $1.4 \times$ as long as wide (Figure 28); upper part of stigmal vein elongate, $0.5 \times$ as long as stigma length; uncus $0.7 \times$ as long as upper part of stigmal vein; marginal vein $0.8 \times$ as long as postmarginal vein. Propodeum with coarse longitudinal carinae, and short median carina in its upper part (Figure 27). Gaster conspicuously petiolate (Figure 23). Genitalia with rather small aedeagus, its part above digitus only $1.2 \times$ as long as digitus length; digitus with only 2 teeth (Figure 29).

Variation. The transverse brown band at the anterior part of mid-lobe of mesoscutum is less marked in female paratypes. In males, the lateral black-brown spots could be absent on T5–T7.

Host plants. *Helinus integrifolius* (Rhamnaceae). Probably a seed feeder, based on its place in the molecular phylogeny of *Megastigmus* spp. (Figure 14). Nevertheless we only sequenced one specimen and it would be necessary to analyze other samples in order to clarify the phylogenetic position, which appeared to vary depending on the marker.

Distribution. Known only from the Matthews Range, Kenya where it was observed to emerge from 9.8 % of the collected fruits (Table 3).

Etymology. Named after its host plant.

Diagnosis and comments. This is the only species of *Megastigmus* found to date to emerge from fruits of the Rhamnaceae. Females are easily distinguished from those of other species present in the region by the very small ovipositor associated with an orange body colour. Genitalic characters differentiate males *M. helinae* by the presence of only two teeth on the digitus whereas the other species bear three teeth except *M. icipeensis* but the phallobase of the latter species is less elongate (Figure 74).

Megastigmus smithi Roques & Copeland, sp. n.

http://zoobank.org/3A22FAA9-278F-4741-8288-123D074A276B

Type material. Holotype \bigcirc , Kenya, Coast Province, Sagalla Mountain, 3.47864°S, 38.57463°E, 1090m, 22 Sep 2014, ex. *Lannea* cf. *schimperi* fruits, CHIESA coll. N°343, R. Copeland leg. (NMKE).

Paratypes $2 \stackrel{\bigcirc}{\downarrow} \stackrel{\bigcirc}{\downarrow}$, same collection data as holotype ($1 \stackrel{\bigcirc}{\downarrow}$ ICIPE; $1 \stackrel{\bigcirc}{\downarrow}$ ARC).

Description. Holotype \bigcirc . Medium-sized species, body length (without ovipositor) 4.6 mm; length of ovipositor sheaths 0.8 mm. Body colour orange-yellow and brown

(Figures 30, 31, 32). Head with face yellow except a blackish infuscation extending from base of eye along malar line, a large brown spot covering most of head dorsum and extending to the top of the scrobe on the face and to the temple on the back; parascrobal area and gena yellow. Pilosity on lower face pale; upper face and dorsum of head with conspicuous black hairs (Figure 33). Antenna brownish except scape entirely yellow and pedicel yellow underneath. Pronotum yellow with a brown, irregular median band extending from anterior to posterior suture (Figure 32); mid-lobe of mesoscutum yellow with a large triangular brownish band in the middle, narrowing from anterior to posterior suture; lateral lobe of mesoscutum with a large orange rectangular spot at apex; axilla with a large longitudinal orange band on its lateral half (Figure 32). Scutellum with a large brownish band, covering about half of the surface until frenum; frenal area quite completely brownish, with a narrow yellow band on borders; pleura and ventrum yellow except a large dark orange spot along mesopleural line. Pilosity on thorax entirely black; 3 pairs of black hairs on scutellum. Legs entirely pale yellow except claws brown.

Forewing stigma brown without infuscation; basal cell indistinct, lacking setae, no setae on basal setal line and costal setal line (Figure 35). Propodeum with a large, oval longitudinal black band in its middle (Figure 30, 32); callus and metapleuron yellow with the suture dark orange. Propodeum hairs entirely pale; Gaster brown, T3 orange with a black spot at insertion, following tergites with a dark brown band covering the middle part (Figure 30), lateral parts orange with a whitish rectangular spot on the side. Pilosity on gaster black. Ovipositor sheaths black, very short, only $0.4 \times as$ long as gaster, $0.2 \times as$ long as body (Figure 31).

Head transverse (Figure 33), width: height ratio: 1.5; POL: OOL: 1.4. Scape $1.2 \times$ as long as combined length of pedicel, anellus and F1 (Figure 34); scape $0.9 \times$ as long as combined length of pedicel, anellus, F1 and F2; anellus subquadrate; F1 $1.1 \times$ as long as pedicel, $1.5 \times$ as long as wide; F2 $1.5 \times$ as long as wide; following funicular segments longer than wide, F7 $1.3 \times$ longer than wide. Pronotum, mid- and lateral lobes of mesoscutum with transverse carinae; axilla striated. Mid-lobe of mesoscutum $1.1 \times$ as long as scutellum. Scutellum $1.3 \times$ as long as wide, with irregular transverse striae; frenum $0.4 \times$ as long as scutellum length; completely covered with irregular, concentric wrinkles. Forewing stigma oval elongate, $1.6 \times$ as long as wide (Figure 35); upper part of stigmal vein relatively elongate, $0.4 \times$ as long as stigma length; uncus short, $0.4 \times$ as long as upper part of stigmal vein; marginal vein $0.9 \times$ as long as postmarginal vein. Propodeum with coarse longitudinal carinae, and conspicuous spiracles.

Variation: Body length from 4.5–4.9 mm, ovipositor sheaths from 0.8–0.9 mm. No variation was observed in colour or pilosity.

No males reared.

Host plants. Lannea cf. schimperi (Anacardiaceae). Like other species also reared from Anacardiaceae fruits, e.g., *M. pistaciae* and *M. transvaalensis*, it is probably a seed chalcid.

Distribution. Known only from Sagalla Mountain, Taita Hills, Kenya. Specimens emerged from only 0.4% of sampled fruits (Table 3).



Figures 30–35. *Megastigmus smithi* Roques & Copeland, sp. n. female. 30 dorsal view of the body 31 lateral view of body 32 dorsal view of thorax 33 front view of head 34 antenna 35 forewing.

Etymology. Named in honour of Robert J. Smith, noted musicologist and exceptional left fielder.

Diagnosis and comments. Its comparatively very short ovipositor, nearly $2 \times$ smaller than gaster length, allows to separate easily this species from the other ones developing in Anacardiacae fruits and showing resembling body colour patterns but a comparatively much longer ovipositor, quite as long as gaster length (*M. ozoroae*) or longer (*M. lanneae*; *M. hypogeus*).

Megastigmus copelandi Roques & Copeland, sp. n. http://zoobank.org/7FD3FBF4-C166-4172-B1F0-98CEFA571BC8

Type material. Holotype ♀: Kenya, Coast Province, Taita hills, Mwatate area, 3.48940°S, 38.33298°E, 1050m, 4 Feb 2012, ex. *Grewia tephrodermis* seeds, CHIESA coll. N°98, R. Copeland leg. (NMKE)

Paratypes: 2 \bigcirc \bigcirc , same collection data as holotype (1 \bigcirc RSC; 1 \bigcirc ARC)

Description. Holotype \bigcirc . Small species with body length (without ovipositor) 2.6 mm; length of ovipositor sheaths 1.1 mm. Body colour entirely orange to dark orange except a few black patterns including contours of each ocellus, a spot at wing insertions, contour of propodeal spiracle, rounded spots on the lateral parts of T3, a lunule on the middle of T4, and 2 elongated blackspots on the lateral sides of T5 (Figures 36, 37). Head and gaster dark orange contrasting with the paler orange of the thorax (Figure 37). Pilosity on head entirely pale (Figure 39). Antenna brownish. Thoracic dorsum with long black hairs but lateral parts with pale hairs; scutellum with 3 lateral pairs of long black hairs (Figure 38). Propodeum with pale hairs, coxa and legs with pale hairs. Forewing stigma black without infuscation; basal cell with two small setae, closed by a basal setal line with five long setae and a costal setal line with seven long setae (Figure 41). Ovipositor sheaths black, 0.9 × as long as gaster, 0.4 × as long as body (Figure 37).

Head rounded, width: height ratio: $1.3\times$; POL: OOL 1.0; inter-antennal area as broad as torulus width; scrobe short, ca. $2.6 \times$ as long as wide. Scape $1.2 \times$ as long as combined length of pedicel, anellus, and F1 (Figure 40); scape $0.8 \times$ as long as combined length of pedicel, anellus, F1 and F2; anellus subquadrate; F1 1.1 × as long as pedicel, $1.6 \times$ as long as wide; F2 1.9 × as long as wide; following funicular segments becoming increasingly elongate, with F7 2.0 × as long as wide. Pronotum, mid- and lateral lobes of mesoscutum, and axilla with very coarse transverse carinae; mid-lobe of mesoscutum $1.1 \times$ as long as scutellum. Scutellum $1.2 \times$ as long as wide, with irregular transverse striae; frenum $0.4 \times$ as long as scutellum length, densely wrinkled. Stigma elongate, oval-oblique, about $1.4 \times$ as long as wide (Figure 41); upper part of stigmal vein short, $0.3 \times$ as long as stigma length; uncus $0.9 \times$ as long as upper part of stigmal vein; marginal vein $0.9 \times$ as long as postmarginal vein. Propodeum with cross- striae tending to reticulate in the anterior part but without median carina.

Variation. Females range in length from 2.6–2.7 mm. In one paratype, gaster is dark brown. Wing chaetotaxy is variable with basal setal line with 5–6 long setae and costal setal line with 3–6 setae.

No males reared.

Host plants. Grewia tephrodermis (Malvaceae).

Distribution. Known only from the Mwatate area, Taita Hills, Kenya. *Megastigmus copelandi* emerged from 0.7% of collected fruits (Table 3). Probably a seed feeder.

Etymology. Named after Dr. Robert Copeland, who collected the specimen.



Figures 36–41. *Megastigmus copelandi* Roques & Copeland, sp. n. female. 36 dorsal view of the body 37 lateral view of body 38 dorsal view of thorax; 39 front view of head 40 antenna 41 forewing.

Diagnosis. The combination of an almost entirely orange body, pale pilosity on the head, and an ovipositor a bit shorter than gaster length readily distinguishes this species from other Afrotropical *Megastigmus*, and from *M. grewianae*, in particular, which also attacks *Grewia* fruits. The latter species is much larger (4.1 mm vs. 2.6–2.7) and has an ovipositor ca. $1.8 \times$ longer than gaster length (Figure 82) vs. $0.9 \times$ in *M. copelandi*.

Megastigmus ozoroae Roques & Copeland, sp. n. http://zoobank.org/DB27B108-C725-4654-938C-2C1ED26FAA59

Type material. Holotype. ♀, Kenya, Nyanza Province, Sindo-Mbita Road, 0.4849°S, 34.1765°E, 1205m, 11 Dec 2004, A&M coll. N°3064, ex. *Ozoroa insignis ssp reticulata*, R. Copeland leg. (NMKE)

Paratypes. 8 \bigcirc , same collection data as holotype; 6 \bigcirc , same collection data as holotype, except 19 Dec 2004, A&M coll. N°3077 (2 \bigcirc \bigcirc , 1 \bigcirc , NMKE; 2 \bigcirc \bigcirc , 1 \bigcirc RSC; 2 \bigcirc \bigcirc , 1 \bigcirc ARC, 1 \bigcirc , 1 \bigcirc ICIPE, 1 \bigcirc , 1 \bigcirc SAMC)

Additional material examined. $23 \bigcirc \bigcirc$, $11 \circlearrowright \circlearrowright$ same collection data as holotype, except 19 Dec 2004, A&M coll. N°3077 ($11 \circlearrowright \bigcirc$, $5 \circlearrowright \circlearrowright RSC$; $12 \circlearrowright \bigcirc$, $6 \circlearrowright \circlearrowright ARC$)

Description. Holotype \bigcirc . Large species, body length (without ovipositor) 4.8 mm; length of ovipositor sheaths 1.5 mm. Body colour yellow and black (Figures 42-44). Head pale yellow except a conspicuous black ring around occipital carina extending on vertex in a distinct median band reaching the inter-antennal area, malar sulcus blackish, and a black dot on each side of clypeus (Figure 45). Pilosity pale on lower face; upper face with a row of long black hairs along parascrobal area and on dorsum of head (Figure 45). Antenna brown with scape and pedicel yellow beneath. Pronotum pale yellow- grey with a large, nearly rectangular, longitudinal black spot in the middle, its colour turning to brownish at the posterior end (Figure 44). Remainder of thorax mostly pale yellow with conspicuous darker patterns: a large brownish spot covering most of the mid-lobe of mesoscutum (except the lateral parts) including a large black triangle at the anterior suture extending in a median, distinct black line to the posterior suture, a large triangular brownish spot covering most of the scutellum, a rectangular orange spot on the upper part of the lateral lobe of mesoscutum followed by a grey spot of same size, a large orange spot on the axilla, all sutures brownish- black along prepectus and lateral panel of pronotum, mid-lobe of mesoscutum, lateral lobe of mesoscutum and scutellum, and a brownish oval spot including a small triangular black spot in the middle of the mesepimeron. Metanotum yellow with a black transverse line interrupted in the middle. Pilosity on thorax dark, with 5 pairs of conspicuous setae on the lateral yellow sides of the posterior part of mid-lobe of mesoscutum, 4 pairs on lateral sides of scutellum, and 1 pair of large setae at the anterior corner of axilla.

Legs pale yellow except claws brownish; coxae with numerous pale hairs extending from small black dots, especially on hind coxa; femora with small black hairs; tibiae with 3 rows of long black hairs. Forewing stigma brown without infuscation; basal cell only partly closed, the disc with 5 very small setae; basal line with 2 setae; no setae on costal line; 4 setae in coastal cell (Figure 47). Propodeum pale yellow with black markings including a large central band extending in a lateral line along the suture with metanotum (Figure 44), a longitudinal spot along suture between callus and propodeum, and the sutures of metapleuron and callus. Pilosity pale on propodeum. Gaster brown- black with yellowish sides; T3 black on dorsum; the 4 following tergites with large transverse black bands progressively narrowing and becoming brownish on sides; a row of long black hairs along lateral parts of all tergite sutures; sterna pale yel-



Figures 42–47. *Megastigmus ozoroae* Roques & Copeland, sp. n. female. 42 dorsal view of the body 43 lateral view of body 44 dorsal view of thorax 45 front view of head 46 antenna 47 forewing.

low; Ovipositor sheaths black, short, only $0.8 \times as$ long as gaster, $0.3 \times as$ long as body (Figure 43).

Head about $1.1 \times$ as wide as long in front view (Figure 45). POL: OOL: 1.5; inter-antennal area as broad as torulus width; scrobe elongate, ca. $3 \times$ as long as wide.

Scape 1.2 × as long as combined length of pedicel, anellus, and F1 (Figure 46); scape 0.8 × as long as combined length of pedicel, anellus, F1 and F2; F1 1.3 × as long as pedicel, 1.7 × as long as wide; F2 1.6 × as long as wide; following funicular segments tending to subquadrate from F3, with F7 as long as wide. Pronotum with coarse transverse carinae; mid-lobe of mesoscutum and axilla with finer cross-striae; mid-lobe of mesoscutum 1.3 × as long as scutellum; scutellum 1.1× as long as wide, noticeably covered with longitudinal, irregular wrinkles; frenum quite indistinct from scutellum, roughly 0.4 × as long as scutellum length, completely covered with irregular wrinkles. Forewing stigma oval- oblique, about 1.6 × as long as wide (Figure 47); upper part of stigmal vein elongate, 0.4 × as long as stigma length; uncus short, 0.4 × as long as upper part of stigmal vein; marginal vein 0.8 × as long as postmarginal vein. Propodeum with a short median carina in its anterior part and cross- striae on its upper part, the lower part smooth and shining.

Variation. Females range in length from 3.5 to 4.8 mm. Five of the examined specimens out of 14 have lateral lobes of mesoscutum and scutellum pale yellow. The black spots on pronotum and mid-lobe of mesoscutum as well as the other black markings are absent in one specimen, except at base of eye, on the suture of the lateral lobe of mesoscutum and on the central part of propodeum. The general colour of this specimen is orange, with the gaster brownish and not blackish in its middle part, the brownish colour beginning only at T5. Another specimen has the central part of propodeum completely shining black. The relative length of the ovipositor sheaths varies between 0.7 to $0.8 \times$ the gaster length, and $0.3-0.4 \times$ the body length.

Males. They are highly variable in colour, and two extreme forms can be distinguished with intermediates.

Pale form. Body length from 4.3 to 5.7 mm. Body colour pale yellow and black (Figures 48-50). Head with a large black spot extending from lower face to frons and vertex, and laterally to the lower part of eye which is entirely surrounded by a black line, and malar space (Figure 51); yellow only on gena, temples, base of clypeus and lower part of malar space and parascrobal area; ocellar area black, with anterior ocellus covered with black but not the lateral part of posterior ocelli; black extending in a conspicuous black ring around occiput. Pilosity on face pale, but conspicuous black hairs on parascrobal area, temple, gena and dorsum of head (Figure 51). Antenna yellow. Pronotum yellow with a longitudinal black band in the center, narrowing towards mid-lobe of mesoscutum. Remainder of thorax yellow, except mid-lobe of mesoscutum with a large triangular black-brown spot covering the anterior part, prolongated in its center by an irregular longitudinal black line, narrowing in the middle, and expanding to the posterior part of mid-lobe of mesoscutum (Figure 50). Anterior of scutellum with a brownish; a conspicuous squareshaped grey spot in the posterior part of lateral lobe of mesoscutum; axilla yellow; lateral panel of pronotum black; prepectus mostly black with a few yellow infuscations near the suture of lower mesepisternum and prothoracic spiracle; upper and lower mesepisternum mostly black except in the center a yellow band extending



Figures 48–54. *Megastigmus ozoroae* Roques & Copeland, sp. n. male pale form. 48 dorsal view of the body 49 lateral view of body 50 dorsal view of thorax; 51 front view of head 52 antenna 53 genitalia 54 forewing.

to median coxa; lateral part of upper and lower mesepimeron yellow; all sutures black along mid-lobe of mesoscutum, lateral lobe of mesoscutum, and scutellum. Thoracic ventrum mostly black. Pilosity on thorax consisting of long black hairs, including 5 pairs on the postero- lateral parts of mid-lobe of mesoscutum, 1 pair on axilla, and 3 pairs on scutellum.

Legs yellow expect anterior part of coxae with large black spots (extending to the posterior part in fore coxa), and small brownish spots at femur insertion, 3 rows of large hairs on tibia. Forewing stigma brown without infuscation (Figure 54); basal cell partly closed, with 8 setae on disc; basal setal line with 2 long setae, but costal setal line quite indistinct with 3 small setae; coastal cell with more than 20 long setae in several rows in the apical 1/2 to 2/3. Propodeum yellow- grey with a black band along the anterior suture black and a large oval black spot medially (Figure 50); callus and metapleuron yellow but the sutures black. Propodeum hairs pale. Gaster colour mostly black-brown; T3 petiolate and black; T4 with anterior part brown, followed by a large yellow band; T5 with the anterior part brown, followed by a small yellow band; T6 with a large black spot in form of shield; T7 brown; T8- T9 yellow with a brown lateral band. Long black hairs on tergite sutures. Genitalia with elongate aedeagus, its part above digitus about 1.8 × as long as digitus length; digitus compressed, about 2.2 × longer than its maximum width, with 3 teeth (Figure 53).

Head about 1.2 × as wide as long in front view. POL: OOL: 2.0; inter-antennal area as broad as torulus width; scrobe short, ca. 1.5 × as long as wide. Scape 1.3 × as long as combined length of pedicel, anellus, and F1 (Figure 52); scape 0.9 × as long as combined length of pedicel, anellus, F1 and F2; F1 1.5 × as long as pedicel, $1.7 \times$ as long as wide; F2 1.6 × as long as wide; following funicular segments elongate, only tending to subquadrate from F7. Pronotum with coarse transverse carinae, mid-lobe of mesoscutum and axilla with transverse striae finer than on pronotum. Mid-lobe of mesoscutum 1.3 × as long as scutellum length; Scutellum 1.2 × as long as wide, noticeably covered with longitudinal, irregular wrinkles; frenum nearly indistinct from scutellum, roughly 0.4 × as long as scutellum length, completely covered with irregular, arching, concentric wrinkles. Forewing stigma oval- oblique, about 1.4 × as long as wide (Figure 54); upper part of stigmal vein very short, only 0.3 × as long as stigma length; uncus short, 0.6 × as long as upper part of stigmal vein; marginal vein 0.9 × as long as postmarginal vein. Propodeum with strong, irregular striae medially

Dark form. Larger than pale form, with body length 5.0 to 6.5 mm. Body colour mostly black (Figures 55–57) with a conspicuous, large triangular infuscation around the forewing stigma (Figure 61). Head entirely black except a triangular dark grey spot along parascrobal area (Figure 58). Thorax black except a triangular grey spot on sides of pronotum posteriorly, a narrow yellowish line on the sides of mid-lobe of mesoscutum and on lateral sutures of lateral lobes of mesoscutum, axillae yellowish (Figure 57). Antero-lateral part of scutellum yellowish but frenum black. Metanotum with a transverse yellow line. Propodeum entirely black. Legs mostly black (Figure 56); coxae black except the posterior part yellowish; mid- and hind femora black except the basal and apical part brownish; fore femur with the apical part yellow; tibiae and tarsi yellow. Forewing with basal cell closed, disc with 10 strong setae; basal setal line with 2 long setae, costal setal line with 8 small setae; coastal cell with more than 100 small setae in several rows in the apical 1/2 to 2/3 (Figure 61). Thoracic sculpture, pilosity and genitalia (Figure 60) same as in pale forms.



Figures 55–61. *Megastigmus ozoroae* Roques & Copeland, sp. n. male dark form. 55 dorsal view of the body 56 lateral view of body 57 dorsal view of thorax; 58 front view of head 59 antenna 60 genitalia 61 forewing.

Intermediates. In some specimens, black patterns on thorax are limited to a sharp median line on pronotum and mid-lobe of mesoscutum, and to the sutures of lateral panel of pronotum, prepectus, and tegula. These specimens also show a grey spot on

lateral lobes of mesoscutum and a brownish median line on scutellum. Gaster is mostly black with a few yellow patterns as follows: an annelation at the base of T3, the sides of T4, a small lateral spot on T5 and the last two segments. Forewing stigma not infuscated in these individuals. In some others, the lateral parts of thorax are black (panel of pronotum, prepectus, mesepisternum, and upper mesepimeron) except lower mesepimeron and metapleuron and callus yellow (with black spots on callus), and scutellum is black at the base with the remainder dark brown. In these individuals, the forewing stigma is infuscated. Pilosity may include 5–6 pairs of setae on mid-lobe of mesoscutum and 3–4 pairs on scutellum.

Host plants. Ozoroa insignis (Anacardiaceae). Its position in the molecular phylogeny is close to those *Megastigmus* species known to be seed feeders in Anacardiaceae, strongly suggesting it is also a seed feeder. Although the presence of numts was noticed in the analyzed specimen of *M. ozoroae* it belonged whatever the marker (short mitochondrial or nuclear fragment) to the "Anacardiaceae clade" confirming the existence of a strong monophyletic clade, the most divergent from the others (Figure 14).

Distribution. Known only from western Kenya, near Lake Victoria. In one collection, 8.1% of fruits were infested (Table 3).

Etymology. Named after the genus of the host plant.

Diagnosis. Females are easily distinguished from those of other species observed to emerge from fruits of Anacardiaceae by the relatively small ovipositor, nearly as long as gaster length whereas it is at least $1.2 \times$ the gaster length in *M. hypogeus* (Figure 117), *M. lanneae* (Figure 97), *M. laventhali* (Figure 76), *M. pistaciae* (Figure 137), *M. thomseni* (see Hussey 1956a) and *M. transvaalensis* (Figure 151). In contrast, the ovipositor of *M. smithi* is significantly shorter ($0.4 \times$ the gaster length; Figure 31). Genitalic characters differentiate male *M. ozoroae* from those of males described for other species that feed in Anacardiaceae; the aedeagus part above digitus being significantly longer in *M. ozoroae* ($1.8 \times$ as long as digitus length) than in *M. hypogeus* ($1.2-1.3 \times$; Figure 127, 134), *M. pistaciae* ($0.8 \times$; Figure 148) and *M. transvaalensis* ($0.5 \times$; Figure 162). The aedaegus dimension compared to digitus is similar in *M. lanneae* but the phallobase is more elongate in the latter species (Figures 107, 115), being $2.8 \times$ longer than wide vs. $2.6 \times$ in *M. pistaciae*.

Megastigmus icipeensis Roques & Copeland, sp. n.

http://zoobank.org/4EE8D716-63AB-48F4-8B9E-E6277136C6D8

Type material. Holotype \bigcirc , Kenya, Nairobi Province, ICIPE Campus, Kasarani, 1.2232°S,36.8965°E, 1600m elevation, Malaise trap, meadow in degraded shrub-/ grassland, 24 Feb to 3 Mar 2014, R. Copeland leg. (NMKE)

Paratypes $4 \bigcirc \bigcirc$, the same as holotype ($1 \bigcirc NMKE$; $1 \bigcirc RSC$; $1 \bigcirc ARC$; $1 \bigcirc ICIPE$). **Additional material.** $3 \bigcirc \bigcirc$, collected together with the four females ($2 \bigcirc \bigcirc RSC$; $1 \bigcirc ARC$).

Description. Holotype \bigcirc . Very small species with body length (without ovipositor) 1.2 mm; length of ovipositor sheaths 0.9mm. Body colour nearly entirely orangeyellow (Figures 62-64). Head colour orange, except mouthparts brown (Figure 65). Pilosity on lower face pale; dorsum of head with conspicuous black hairs: a row along the internal side of each eye from parascrobal area to temple, a row along the occiput, and three isolated hairs around each of the lateral ocelli; two interocellar bristles. Antenna dark- brown except scape and pedicel yellow underneath (Figure 66). Thorax entirely orange except a small black spot on axilla at wing insertions and on lateral panel of metanotum (Figure 64). Pilosity on thorax mostly pale; sparse long hairs on pronotum, especially along the suture with mid-lobe of mesoscutum; 3 pairs of pale hairs on the lateral side of mid-lobe of mesoscutum, very close to suture with the lateral lobe; 2 pairs of pale hairs on scutellum. Legs entirely pale yellow except claws brown; Forewing stigma brown without infuscation; basal cell without setae, closed by a basal setal line with 5 setae and a costal setal line with 3 setae (Figure 67). Propodeum orange with a transverse black stripe along the suture with metanotum (Figure 64). Propodeum hairs pale; Gaster orange, with four large transverse orange-brown bands along the anterior sutures of the tergites 4-8; pilosity on gaster black. Ovipositor sheaths black, $1.6 \times \text{longer than gaster}$, $0.7 \times \text{as long as body}$ (Figure 63).

Head about $1.2 \times as$ wide as long in front view (Figure 65). POL large, POL: OOL: 2.7. Scape $1.1 \times as$ long as combined length of pedicel, anellus, and F1 (Figure 66); scape $0.9 \times as$ long as combined length of pedicel, anellus, F1 and F2; pedicel elongate, $1.5 \times longer$ than F1; F2 subquadrate, small, $0.8 \times as$ long as F1; following funicular segments subquadrate from F2. Pronotum, mid-lobe of mesoscutum, axilla and scutellum with fine transverse striae. Mid-lobe of mesoscutum conspicuously short, only $0.9 \times as$ long as scutellum length; scutellum $1.1 \times as$ long as wide; frenum roughly $0.4 \times as$ long as scutellum length, with the same sculpture as the remainder of scutellum. Propodeum with a zig-zag median carina. Forewing stigma oval, without infuscation, about $1.4 \times as$ long as wide (Figure 67); upper part of stigmal vein short, $0.2 \times as$ long as postmarginal vein.

Variation. Body ranges 1.2–1.3 mm with ovipositor up to 1.2 mm. Ovipositor length varies from 0.7 to $0.9 \times$ the body length, and from 1.6 to $2.0 \times$ the gaster length. No variation in colour and pilosity.

Male. In the absence of molecular analyses, the males caught in Malaise traps along with the four female *M. icipeensis* could not be attributed with certainty to this species but noticeable convergences in morphology make it probable. Body length 1mm. Same body colour as in female of *M. icipeensis* (Figures 68–70) except a paler yellow pronotum and some additional black patterns on head and thorax: an irregular spot surrounding the three ocelli, an annelation at insertion of pronotum, a darkening of the lateral sutures of mid-lobe of mesoscutum, a narrow transverse band on lateral panel of metanotum, and a transverse band covering the base of propodeum and extending in its middle into a large longitudinal spot in shape of inverted Y (Figures 62, 64). Gaster petiolate, mostly brown- black on dorsum: T3 black, T4 brown black with



Figures 62–67. *Megastigmus icipeensis* Roques & Copeland, sp. n. female. 62 dorsal view of the body 63 lateral view of body 64 dorsal view of thorax 65 front view of head 66 antenna 67 forewing.



Figures 68–74. *Megastigmus icipeensis* Roques & Copeland, sp. n. male. 68 dorsal view of the body 69 lateral view of body 70 dorsal view of thorax 71 front view of head 72 antenna 73 forewing 74 genitalia.

a large transverse yellow band distally, T5 similar but the yellow band narrower, T6 brown-black, T7 yellow.

Legs yellow with the two last tarsal segments and claws black. Pilosity pale on head and thorax, showing the same setal pattern as in female *M. icipeensis*, including the interocellar brisles and 2 pairs of pale hairs on scutellum (Figure 70). Forewing stigma without infuscation (Figure 73); basal cell closed, but without setae on disc; basal setal line with 6 setae, costal setal line with 3 long setae; costal cell with only 3 small setae in the apical 1/2 to 2/3.

Head transverse, about $1.3 \times as$ wide as long in front view (Figure 71) . OOL short, POL: OOL: 2.8. Scape $1.2 \times as$ long as combined length of pedicel, anellus, and F1 (Figure 72); scape as long as combined length of pedicel, anellus, F1 and F2; pedicel elongate, $1.8 \times longer$ than F1; F2 subquadrate, small, $0.8 \times as$ long as F1; following funicular segments also subquadrate. Pronotum, mid-lobe of mesoscutum, axilla and scutellum finely transversely striated. Mid-lobe of mesoscutum conspicuously short, only as long as scutellum length; scutellum $1.1 \times as$ long as wide; frenum roughly $0.4 \times as$ long as scutellum length, with the same sculpture as the rest of scutellum. Propodeum with an irregular median carina. Forewing stigma nearly rounded, only $1.3 \times as$ long as wide (Figure 74); upper part of stigmal vein very short, only $0.2 \times as$ long as stigma length; uncus $1.5 \times longer$ than upper part of stigmal vein; marginal vein $1.1 \times longer$ than postmarginal vein. Genital digitus with only 2 teeth (Figure 73).

Host plants. Unknown. In the molecular study, *M. icipeensis* did not group with other taxa, but the phylogenetic position (Figure 14) and the genetic distances between this species and the other groups led to suggest that it could be a seed-feeder associated with another plant family than those identified in this paper.

Distribution. Only known from the collection site in a degraded meadow near a small stream.

Etymology. Named after the institution on whose grounds the species was collected, the campus of the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi.

Diagnosis. The species can be easily distinguished from other tiny *Megastigmus* spp. known from the Afrotropical region by the pale bristles on the thoracic dorsum whereas they are mostly black in *M. zebrinus* (Grissell, 2006), and by its orange body whereas *M. pretorianensis* is mostly black with brown areas (Doğanlar 2015). In contrast to most other species, the male genital digitus bears only two teeth such as in *M. helinae* but the phallobase of the latter species is more elongate (Figure 29).

Megastigmus laventhali Roques & Copeland, sp. n. http://zoobank.org/81764A75-CF49-4AB8-977F-497F56F9A6DA

Type material. Holotype \bigcirc , Kenya, Coast Province, Base of Kasigau Mountain, 3.81301°S, 38.64050°E, 619m, 5 Mar 2012, ex. *Lannea schweinfurthii* fruits, CHIE-SA coll. N°141, R. Copeland leg. (NMKE);

Paratype 1 \bigcirc Kenya, Coast Province, Funzi island, 4.57776°S, 39.44127°E, near sea level, Malaise trap in mixed grass and woodland, 24 to 28 Jul 2012, R. Copeland leg. (ICIPE).

Description. Holotype \bigcirc . Medium-sized species with body length (without ovipositor) 4.0 mm; length of ovipositor sheaths 3.4 mm. Body colour predominantly orange (Figures 75–77). Head entirely orange (Figure 78); pilosity on lower face pale; upper face and dorsum of head with conspicuous black hairs. Antenna light brown except scape entirely orange (Figure 79). Thorax entirely light orange except a small black spot at wing insertion (Figure 77). Pilosity on thorax black; a row of 6 black hairs along each lateral suture of mid lobe of mesoscutum; 3 pairs of black hairs on scutellum (Figure 77). Legs entirely pale yellow except claws brown. Forewing stigma brown without infuscation; basal cell indistinct, with two small setae on disc; basal setal line with 1 seta; costal setal line without setae; costal cell without setae (Figure 80). Propodeum light orange with a darker longitudinal line medially (Figure 75); propodeal spiracle very conspicuous; propodeum hairs entirely pale. Gaster dark orange, darker than propodeum; pilosity on gaster black. Ovipositor sheaths black, long, 1.9 × as long as gaster, 0.8 × as long as body, 0.9 × as long as thorax plus gaster (Figure 76).

Head rounded, width: height ratio: 1.0 (Figure 78); POL:OOL: 1.1. Scape 1.1 × as long as combined length of pedicel, anellus and F1 (Figure 79); scape 0.8 × as long as combined length of pedicel, anellus, F1 and F2; anellus subquadrate; F1 1.1 × as long as pedicel, twice as long as wide; F2 1.1 × as long as 1st funicular segment; following funicular segments longer than wide, with F7 1.4 × longer than wide. Pronotum, mid- and lateral lobes of mesoscutum with coarse transverse carinae; axilla with finer striae; mid-lobe of mesoscutum elongate, $1.3 \times$ as long as scutellum; scutellum 1.2 × as long as wide, reticulate; frenum 0.4 × as long as wide (Figure 80); upper part of stigmal vein 0.4 × as long as stigma length; uncus relatively elongate, $0.8 \times$ as long as upper part of stigmal vein; marginal vein 0.9 × as long as postmarginal vein. Propodeum with coarse longitudinal carinae and conspicuous spiracles.

Variation. In the paratype, the basal cell presents two long setae on disc, and the basal setal line has 1 seta.

No males reared.

Host plants. *Lannea schweinfurthii* (Anacardiaceae). Probably a seed feeder but it could not be integrated in the molecular analysis because too few specimens were available.

Distribution. Known from the base of Kasigau Mountain, a Taita Hills outlier. Only 1 of 168 fruits (0.6%) was infested (Table 3). Also collected in a Malaise trap sample from Funzi Island, just off the southeast coast of Kenya (Figure 2).

Etymology. Named in honour of Leo Laventhal, historian, Yiddish scholar and union man.



Figures 75–80. *Megastigmus laventhali* Roques & Copeland, sp. n. female. 75 dorsal view of the body 76 lateral view of body 77 dorsal view of thorax 78 front view of head 79 antenna 80 forewing.

Diagnosis. Females can be separated easily from those of other species associated with fruits of Anacardiaceae by the combination of a predominantly orange body and a relatively long ovipositor, at least $1.8 \times as$ long as the gaster. In most of the other species, body colour combines dark and yellow patterns (*M. hypogeus, M. ozoroae, M. lanneae, M. smithi*). For the remaining species that have a predominantly orange body colour, the ovipositor length is at most $1.4 \times gaster$ length (*M. pistaciae, M. thomseni* and *M. transvaalensis*).

Megastigmus grewianae Roques & Copeland, sp. n. http://zoobank.org/5AA95119-9BC7-4DF3-AE4E-3CB3409147EB

Type material. Holotype \bigcirc , Kenya, Coast Province, Arabuko-Sokoke forest, 3.2997°S, 39.9869°E, 55 m, 17 Feb 2000, ex. *Grewia stuhlmannii* fruits, Coll. N° Kip-356, R Copeland leg. (NMKE)

Paratypes: Kenya, 1, 1, 1, 3, same collection data as holotype (RSC); 2, 2, 1, same collection data as holotype (ARC); 1, 7, 7, 7, Eastern Province, Tsavo, 2.6760°S, 38.3325°E, 638 m, 20 Feb 2000, ex. fruits of *Grewia tephrodermis*, A&M Coll. N°514, R. Copeland leg. (1, 3, 3, NMKE, 4, 7, ICIPE); 1, Coast Province, Kasigau Mountain, 3.82700°S, 38.64875°E, 1065 m, Malaise trap in campsite clearance, 19 May to 2 Jun 2011, R. Copeland leg. (SAMC); 1, Eastern Province, Kasaala area, 2.07486°S, 38.22530°E, 741 m, Malaise trap, 28 Nov. to 4 Dec 2013, leg. J. Bukhebi & R. Copeland (ICIPE).

Additional material in alcohol. Kenya, $7 \bigcirc \bigcirc$, $1 \circlearrowright$, same as holotype (ARC); $1 \bigcirc$, Eastern Province, Tsavo, 2.6760°S; 38.3325°E, 638 m, 20 Feb 2000, ex. fruits of *Grewia tephrodermis*, Coll. #514, R. Copeland leg. (ARC)

Description. Holotype \bigcirc . Body length (without ovipositor) 4.1mm; length of ovipositor sheaths 3.0 mm. Body colour orange with some darker patterns (Figures 81–83). Head colour orange with a very narrow occipital black line and outer part of ocelli black. Pilosity on face pale, dark on dorsum of head but hairs not strong (Figure 85). Antenna brownish except scape and pedicel yellowish (Figure 85). Pronotum orange with a conspicuous longitudinal black stripe running for 7/8 of the lateral part of the pronotum, interrupted just before the suture with mid-lobe of mesoscutum (Figures 82–83). Remainder of thorax orange except black spots on the posterior part of axilla at wing insertion. Pilosity on thorax black but hairs not strong; a few on pronotum; 5 pairs on latero-posterior part of mid-lobe of mesoscutum; 4 pairs on lateral lobe of mesoscutum along suture with mid-lobe of mesoscutum; 4 pairs on axilla. Scutellum orange with 5 pairs of lateral hairs with conspicuous insertion dots (Figures 83–84).

Legs entirely pale yellow except claws brown, coxae with conspicuous hair dots. Forewing stigma brown without infuscation; basal cell closed, with 13 setae on disc; basal setal line with 6 long setae; costal setal line with 16 small setae, costal cell with 11 setae in 2 rows (Figure 87). Propodeum dark orange, with a small oblique brown-



Figures 81–87. *Megastigmus grewianae* Roques & Copeland, sp. n. female. 81 dorsal view of the body 82 lateral view of body 83 dorsal view of thorax 84 electroscan of dorsal view of thorax 85 front view of head 86 electroscan of antenna 87 forewing.

ish spot around the spiracle; not wrinkled transversally; propodeum hairs dark. Gaster orange with two lateral rows composed of 5 brown elongated spots, one on each of the first five segments, placed latero-medially, the third spot shaped like a comma, larger than the others, followed in size by the 4th one. Ovipositor sheaths black, 1.8 × as long as gaster, $0.7 \times as$ long as body (Figure 82).

Head rounded, width: height ratio: 1.1 × (Figure 85); POL: OOL: 1.3; torulus ca. $1.2 \times as$ long as wide; inter-antennal area much smaller (0.6×) than torulus width; scrobe elongate, ca. $3.3 \times$ as long as wide. Scape elongate, $1.2 \times$ as long as combined length of pedicel, anellus and F1 (Figure 86); scape $0.8 \times as$ long as combined length of pedicel, anellus, F1 and F2; Pedicel elongate, 1.3× longer than F1; anellus subquadrate; F1 1.8 \times as long as wide, shorter than the other segments, 0.8 \times shorter than F2; F2 1.9 × as long as wide; following funicular segments same as F2, except F7 and F8 tending to subquadrate, with F7 $1.4 \times$ as long as wide. Pronotum with 8 very strong transverse carinae (Figure 84), mid-lobe of mesoscutum with 7 coarse transverse carinae extending onto lateral lobes; axillae with 3 weaker, longitudinal, carinae. Mid-lobe of mesoscutum $1.2 \times$ as long as scutellum length. Scutellum $1.2 \times$ as long as wide, with transverse, irregular striae anteriorly; frenum smooth, $0.3 \times as$ long as scutellum length. Stigma oval, about $1.2 \times as$ long as wide (Figure 87); upper part of stigmal vein very short, $0.3 \times as$ long as stigma length; uncus comparatively elongate, $1.2 \times longer$ than upper part of stigmal vein; marginal vein 0.8 × as long as postmarginal vein. Propodeum reticulate with 2 oblique carinae diverging from the center of the anterior suture (Figure 84).

Male. Body length 4.2 mm; Body colour, pilosity and black patterns similar to female (Figures 88–91) except gaster with 6 rows of brown spots laterally on dorsum; those on the first two segments elongate with the second longer, the 3 following more rounded, comma-like, and the last a simple spot (Figure 88). Forewing stigma brown without infuscation; basal cell closed, with 21 setae on disc; basal setal line with 7 setae; costal setal line with 21 setae; costal setal line with 21 setae.

Head subquadrate, width: height ratio: 1.1 × (Figure 91); POL: OOL 1.3; torulus ca. $1.2 \times$ as long as wide; inter-antennal area short, $0.6 \times$ as broad as torulus width; scrobe elongate, ca. 3.9× as long as wide; eyes protruding. Scape as long as combined length of pedicel, anellus and F1 (Figure 92); scape 0.7× as long as combined length of pedicel, anellus, F1 and F2; pedicel 1.2× longer than F1; anellus subquadrate; F1 2.1× as long as wide; F2 1.9× as long as wide; following funicular segments similar to F2, only F7 tending to suquadrate $(1.2 \times \text{as long as wide})$. Pronotum with coarse transverse carinae as in female but irregular; some carinae interrupted in their middle on mid-lobe of mesoscutum (Figure 93); axilla with curved longitudinal striae; mid-lobe of mesoscutum $1.1 \times$ as long as scutellum length. Scutellum $1.2 \times$ as long as wide, with transverse striae in the anterior part only, then smooth; frenum entirely smooth, 0.2 × as long as scutellum length (Figure 93). Forewing stigma subquadrate, 1.1 × as long as wide (Figure 94); upper part of stigmal vein short, $0.3 \times as$ long as stigma length; uncus as long as upper part of stigmal vein; marginal vein 0.8 × as long as postmarginal vein. Propodeum with several oblique, irregular carinae (Figure 93). Genitalia with aedeagus relatively elongate, its part above digitus about 1.6 × as long as digitus length; digitus enlarged at its extremity, only twice as long than its maximum width, with 3 teeth (Figure 95).



Figures 88–95. *Megastigmus grewianae* Roques & Copeland, sp. n. male. 88 dorsal view of the body 89 lateral view of body 90 dorsal view of thorax 91 front view of head 92 electroscan of antenna 93 electroscan of dorsal view of thorax 94 forewing 95 genitalia.

Variation. Females range in length from 3.5 to 4.0mm. The number of lateral spots on gaster varies from 6 (with two on T3) to 2 (only the large ones remaining), the 3 last lateral spots sometimes fused into a line. Pilosity on thorax paler in one female

paratype. Males range in length from 4.1 to 4.5 mm. The spots on gaster sometimes larger, the pairs of lateral hairs on scutellum ranging up to 6 in both sexes.

Host plants. Grewia stuhlmannii, Grewia tephrodermis (Malvaceae). Probably a seed feeder, based on its place in the molecular phylogeny of *Megastigmus* spp. (Figure 14). Sequencing of four specimens from Arabuko-Sokoke forest showed they differed by less than 0.3% in COI, confirming the validity of the single specimen that we used in the analyses, even if the position remains unresolved. A large number of species of *Grewia* coexist in East Africa (Brink and Achigan-Dako 2012) and it would be interesting to find if other *Megastigmus* species or sub-species are able to attack their seeds.

Distribution. Known from dry *Acacial Commiphora* savanna, moderately wet mid-altitude mountain forest, and bush associated with costal forest. Adults emerged from 2.7% of *G. stuhlmannii* fruits and 13% of *G. tephrodermis* fruits (Table 3).

Etymology. Named after the genus of its host plant.

Diagnosis. Females are easily separated from those of *M. copelandi*, the other species reared from *Grewia* fruits, by their significantly larger size (4.1 mm vs. 2.6–2.7) and the relatively longer ovipositor (ca. $1.8 \times$ longer than gaster length vs. $0.9 \times$ in *M. copelandi*). Females and males are also easily distinguished from the other species of Afrotropical *Megastigmus* by the unique pattern of a longitudinal black stripe extending 7/8 of the length of the side of the pronotum (Figures 82–83, 89–90).

Megastigmus lanneae Roques & Copeland, sp. n.

http://zoobank.org/A19A4C74-A315-46B7-90B9-828196351FAF

Type material. Holotype \bigcirc : Kenya, Eastern Province, Machakos, 1.5609°S, 37.2338°E, 1586m elevation, 3 May 2005, ex. *Lannea rivae* fruits, Coll. N°3109, R. Copeland leg. (NMKE)

Additional material in alcohol. Kenya; $7 \ \bigcirc \ \bigcirc \ \bigcirc$ same collection data as holotype; $2 \ \bigcirc \ \bigcirc \ \bigcirc$, same collection data as holotype except 25 Nov. 2004, ex. *Lannea rivae* seeds, Coll. N°3042, R. Copeland leg. (ARC).

Description. Holotype \bigcirc . Large species, body length (without ovipositor) 5.4 mm; length of ovipositor sheaths 4.2 mm. Body colour yellow and brownish (Figures 96–98). Head yellow with the following dark patterns (Figure 99): a brownish band from frons to occiput, covering the interocellar area, base of eye at malar line black, malar line with a brownish infuscation, temple with a brownish infuscation. Pilosity on face and gena pale, dark hairs on temple, vertex and parascrobal area (Figure 99). Antenna light brownish, scape and pedicel yellow underneath (Figure 100). Pronotum yellow with a large, nearly rectangular, dark median band extending from anterior to posterior suture (Figure 98); mid-lobe of mesoscutum with a large triangular band covering most of the surface, narrowing from anterior to posterior, its colour turning



Figures 96–101. *Megastigmus lanneae* Roques & Copeland, sp. n. female. 96 dorsal view of the body 97 lateral view of body 98 dorsal view of thorax 99 front view of head 100 antenna 101 forewing.

progressively from brown-red to dark brownish; lateral lobe of mesoscutum with a large orange rectangular band on the antero-lateral corner continuing as a smaller grey band; axilla with an orange band at apex; lateral and ventral sides entirely yellow except two blackish spots at wing insertion; scutellum with a large triangular dark-brownish band, covering about half of the surface until reaching the frenum; frenal area nearly completely dark brownish, with a narrow yellow band on sides; lateral panel of metanotum with a narrow brownish band. Pilosity on thorax dark with long hairs on the yellow parts of pronotum, mid-lobe of mesoscutum (eight pairs of long hairs),

lateral lobe of mesoscutum (three rows of hairs of different sizes), and scutellum (four lateral pairs, one on them on the frenum). Legs yellow except claws brown; fore femora with several long black hairs at apex, hind femora with numerous pale hairs; tibiae with 3 rows of long black hairs. Forewing stigma brown, without infuscation; basal cell closure poorly defined, with 9 small setae on disc; only 1 seta on basal setal line; 4 small setae on costal line; costal cell with 4 large setae (Figure 101). Propodeum with a large black rectangular band in its middle, and a brownish spot around spiracle. Gaster with a narrow, yellow longitudinal median band (Figure 96); T3 dark orange, T4 with a dark brown triangular spot extending from the median yellow line to the side of the tergite, continuing as an orange- brown band, thus delimiting a triangular yellow cell closed laterally by a comma- shaped blackish spot; lateral parts of gaster whitish. Ovipositor sheaths black, 1.8 × longer than gaster, 0.7 × as long as body (Figure 97).

Face quadrate, width: height ratio: 1.0 (Figure 99); POL: OOL 1.7; scrobe elongate, ca. 4.2× as long as wide. Scape 1.1 × as long as combined length of pedicel, anellus and F1 (Figure 100); scape 0.8× as long as combined length of pedicel, anellus, F1 and F2; anellus subquadrate; F1 1.2 × longer than pedicel, $1.7 \times$ as long as wide; F2 $1.9 \times$ as long as wide; following funicular segments progressively tending to subquadrate, with F7 1.2 × as long as wide. Pronotum, mid- and lateral lobes of mesoscutum, and axilla with coarse transverse cross- striae. Mid-lobe of mesoscutum 1.5 × as long as scutellum length. Scutellum 0.9 × as long as wide, with reticulate striation; frenum 0.4 × as long as scutellum, with reticulate striation in the middle, and carinae laterally. Stigma oval- elongate, about 1.5 × as long as wide (Figure 101); upper part of stigmal vein elongate, $0.5 \times$ as long as stigma length; uncus $0.7 \times$ as long as upper part of stigmal vein; marginal vein 0.9× as long as postmarginal vein. Propodeum with coarse transverse striae.

Variation. Length of female body varies from 4.8 to 5.4 mm. The relative length of the ovipositor sheaths varies between 1.6 and $1.8 \times$ the gaster length, and between 0.6 and 0.7 × the body length. In three out of the eleven specimens examined, the brownish bands on thorax and abdomen are closer to to dark orange, becoming less distinct. One specimen, with two setae on the basal setal line, a second specimen with four setae.

Males. Like in most other *Megastigmus* species related to Anacardiaceae, males are highly variable in colour, and two extreme forms can be distinguished with intermediates.

Pale form. Body length from 4.2 to 6.2 mm. Body colour mostly orange-yellow (Figures 102–104). Head orange with a blackish ring around occiput. Pilosity on lower face pale but conspicuous black hairs on parascrobal area, temple, gena and dorsum of head. Antenna yellow. Thorax orange-yellow except a small black spot at wing insertion and a more or less distinct median longitudinal band of darker orange colour extending such as in female from anterior suture of pronotum to scutellum. Pilosity on thorax black with 4 pairs of long setae on scutellum (Figure 104). Legs entirely yellow; pilosity pale on femurs, black on tibiae. Forewing stigma brown without infuscation (Figure 108); basal cell partly closed, with 2 long setae on disc; basal setal line with 2 long setae, costal setal line quite indistinct with 1 setae. Propodeum orange with a

median black band; hairs on callus pale. Gaster colour mostly brown- yellow; T3 petiolate, black at insertion and then turning to brownish; T4 yellow; T5 with a transverse brown band on anterior suture; T6 with a small triangular brown spot on the middle; T7 with a larger triangular brown spot on the middle, and a small rounded brown spot on each side; T8 with a small rounded brown spot on each side. Long black hairs on tergite sutures. Genitalia with elongate aedeagus, its part above digitus about 1.8 × as long as digitus length; digitus with 3 teeth; phallobase elongate, about 2.8× as long as wide (Figure 107).

Head about $1.1 \times as$ wide as long in front view (Figure 105). POL: OOL: 1.2. Scape $1.3 \times as$ long as combined length of pedicel, anellus, and F1 (Figure 106); scape $0.9 \times as$ long as combined length of pedicel, anellus, F1 and F2; F1 $1.4 \times as$ long as pedicel, $2.1 \times as$ long as wide; F1 $1.2 \times as$ long as F2; following funicular segments elongate, not tending to subquadrate; F7 $2.0 \times as$ long as wide. Pronotum with coarse transverse carinae, mid-lobe of mesoscutum and axilla with transverse striae finer than on pronotum (Figure 104). Mid-lobe of mesoscutum $1.2 \times as$ long as scutellum length; Scutellum $1.3 \times as$ long as wide, noticeably covered with irregular, arching, concentric wrinkles; frenum nearly indistinct from scutellum. Forewing stigma oblique, rounded, about $1.2 \times as$ long as wide (Figure 108); upper part of stigmal vein $0.4 \times as$ long as stigma length; uncus very short, $0.4 \times as$ long as upper part of stigmal vein.

Dark form. Larger than pale form, with body length 5.7 to 6.4 mm. Body colour substantially black (Figures 109–112) with a conspicuous, large triangular infuscation around the forewing stigma (Figure 114). Head mostly black except a transverse dirty yellow band on the lower face (restricted to gena in some specimens) and a dirty yellow spot between eye and posterior ocellus (Figure 112). Thoracic dorsum dirty yellow with a large median black band extending from the anterior suture of pronotum to frenum (Figure 111). Sides of thorax entirely black (Figure 110). Legs mostly dark; coxae black; fore femora brownish in its basal part, mid- and hind femora quite entirely brownish; tibiae and tarsi yellow. Propodeum entirely black. Antenna (Figure 113), thoracic sculpture (Figure 111), pilosity and genitalia (Figure 115) similar to pale form.

Intermediates. Some specimens show a slightly infuscated stigma along with head and thorax mostly orange but with more blackish patterns than in the pale form: a black-brown band between eye margin and torulus, a brownish median band on thorax from anterior suture of pronotum to posterior suture of mid lobe of mesoscutum which prolongates in a fuzzy median band on scutellum, and a brown longitudinal band on upper and lower part of mesepimeron. Legs yellow with a large black spot on fore coxa, a smaller spot on mid-coxa, and a brownish infuscation on hind coxa. Propodeum with a large median black band, black spots around spiracles, and a brownish infuscation at suture with callus. Pilosity is similar to the one of pale from but some individuals have 3 pairs of hairs on scutellum.

Host plants. Lannea rivae (Anacardiaceae). Probably a seed feeder. Although the presence of numts was noted in the COI sequences of the only specimen of *M. lanneae*



Figures 102–108. *Megastigmus lanneae* Roques & Copeland, sp. n. male pale form. 102 dorsal view of the body 103 lateral view of body 104 dorsal view of thorax 105 front view of head; 106 antenna 107 genitalia 108 forewing.

which amplified for DNA analysis, this species clearly clustered within the "Anacardiaceae clade" (Figure 14) as already noticed for *M. ozoroae*.

Distribution. Known only from farmland tree in Machakos area. Adults emerged from 3.3% of the collected fruits (Table 3).

Etymology. Named after the genus of its host plant.



Figures 109–115. *Megastigmus lanneae* Roques & Copeland, sp. n. dark form. 109 dorsal view of the body 110 lateral view of body 111 dorsal view of thorax 112 front view of head; 113 antenna 114 forewing 115 genitalia.

Diagnosis. Females can be separated easily from those of other species associated with fruits of Anacardiaceae by the combination of an ovipositor at least $1.8 \times$ as long as gaster length and a yellow and brownish body. In other species having dark and yellow patterns, the ovipositor length is either shorter than gaster length (*M. ozoroae*- Figure 43, *M. smithi*- Figure 31) or at most $1.5 \times$ longer (*M. hypogeus*-

Figure 117). Body colour is predominantly orange in *M. laventhali, M. pistaciae, M. thomseni* and *M. transvaalensis*. Genitalic characters allow separation of *M. lanneae* males from those of other species developing in seeds of Anacardiaceae. The aedeagus part above digitus is significantly longer ($1.8 \times as$ long as digitus length) than in *M. hypogeus* ($1.2-1.3\times$; Figures 127, 134), *M. pistaciae* ($0.8\times$; Figure 148) and *M. transvaalensis* ($0.5\times$; Figure 162). The aedaegus dimension compared to digitus is similar in *M. pistaciae* but the phallobase of *M. lanneae* is more elongate ($2.8\times vs. 2.6\times longer$ than wide; Figures 53, 60).

M. hypogeus (Hussey, 1956)

Type material examined. Holotype \bigcirc "Bred ex. oil seed, Nairobi; 5–37; Kenya, Corydon Museum; Pres. by Com. Inst. Ent., B.M. 1957–41; B.M. TYPE HYM. 5.1653; NHMUK010263947" (BNHM); **Paratypes**: $3 \bigcirc \bigcirc$, $4 \oslash \oslash$, same collection data as holotype (BNHM);

Additional material. 1199, 1433, Kenya, Coast Province, Shimba Hills, 4°08.096'S, 39°28.082'E, 285m elevation, 25 Nov. 2001, ex. *Ozoroa obovata* seeds A&M coll. N°1609, R. Copeland leg.; 19, Nyanza Province, Sindo-Mbita Road, 0°29.091'S, 34°10.592'E alt 1205m, 11 Dec 2004, ex. *Ozoroa insignis* ssp *reticulata* seeds, A&M coll. N°3064, R. Copeland leg.; 1199, 833, Arabuko-Sokoke forest, 3.3716°S, 39.8949°E, 55m elevation, 18 Feb 2000., coll. N°Kip-372, ex. *Ozoroa obovata* seeds, R. Copeland leg; 299, 333, Arabuko-Sokoke forest, 3.3716°S, 39.8949°E, 55m elevation, 18 Feb 2000, coll. N°Kip-372, ex. *Ozoroa obovata* seeds, R. Copeland leg; 299, 333, Arabuko-Sokoke forest, 3.3716°S, 39.8949°E, 55m elevation, 18 Feb 2000, ex. *Ozoroa obovata* seeds, A&M coll. N°509, R. Copeland leg.; 19, Arabuko-Sokoke forest, 3.2030°S, 39.9271°E, 98m elevation, 17 Mar 2001, ex. *Ozoroa obovata* seeds, A&M coll. N°1103, R. Copeland leg. (all material in RSC except 599, 533 from Arabuko-Sokoke forest in ARC)

This species has been first described by Hussey (1956a) under the name of *Eumegastigmus hypogea*, and erroneously reported to emerge from "Oil seed, probably *Helianthus* sp." in Nairobi, Kenya. No subsequent records have been published since this original description. In his paper, Hussey mentioned that it is a very variable species. Our collection of a large number of specimens from different hosts, all belonging to the Anacardiaceae genus *Ozoroa*, allowed us to supplement Hussey's primary description which was rather limited.

Female. Females range in length from 3.9 to 5.1 mm (without ovipositor), length of ovipositor sheaths from 2.1 to 2.5 mm. Body colour usually yellow and black (Figures 116–118) but some individuals present a dominant orange colour, all the blackish patterns becoming orange-brown. Head yellow with upper part of scrobe black (but interocellar area yellow), and vertex with a brownish infuscation. Pilosity on face, gena and temples pale but with a few long black hairs near clypeus and parascrobal area (Figure 119); vertex with long black hairs. Antenna brownish.

Thorax yellowish-brown with blackish patterns (Figures 116, 118). Pronotum with a large, median dark brown band progressively decreasing in width from anterior



Figures 116–121. *Megastigmus hypogeus* Hussey female. 116 dorsal view of the body 117 lateral view of body 118 dorsal view of thorax 119 front view of head 120 antenna 121 forewing.

to posterior suture (Figure 118) but in a few specimens, the median pronotum line is very light and interrupted at its distal end; mid-lobe of mesoscutum completely black at the anterior part, continuing as a large triangular brown- black band progressively narrowing from anterior to posterior, the sides yellow; lateral lobe of mesoscutum yellow with an orange rectangular band becoming grey posteriorly, continuing on axilla as a similar orange band; a brownish longitudinal line on lower mesepimeron; scutellum with a triangular blackish band increasing in size from the anterior suture to frenal area (but sometimes reduced to a narrow line with an orange-brown infuscation); conspicuous black sutures between the following parts: lateral panel of pronotum and collar, mid-lobe of mesoscutum and lateral lobe of mesoscutum, lateral lobe of mesoscutum and prepectus, axilla and scutellum, callus and metapleuron. In the individuals with a dominant orange colour, the sutures between lateral lobe of mesoscutum and prepectus, wing insertion and middle part of propodeum remain black. Pilosity on thorax with long black hairs; mid-lobe of mesoscutum with five lateral pairs of hairs on the yellow parts; scutellum with 3-4 lateral pairs of hairs on the yellow sides (4 pairs on holotype; 3 pairs on most other specimens). Legs yellow except claws brown; pale hairs on coxa, with conspicuous blackish insertion dots; femora with both pale and black hairs; hairs black at apex of femora; 3 rows of long black hairs on tibia. Forewing stigma brown without infuscation; basal cell indistinct, basal setal line with only 1 seta; costal cell with 3 setae (Figure 121). Propodeum light brown with a large, rectangular longitudinal black band medially. Gaster mostly brown with a narrow yellow line in the middle; T3 black; subsequent terga with transverse brown bands on the dorsum, narrowing progressively towards the lateral sides which are whitish; gaster completely brown on some specimens with only a few whitish dots on sides; in a few others, gaster has an orange T3, with the subsequent tergites having an orange longitudinal band anteriorly, the band becoming yellow posteriorly. The brownish lines on the lateral sides of gaster segments are sometimes absent. Ovipositor sheaths black, Ovipositor sheaths $1.2 \times \text{longer than gaster}, 0.6 \times \text{as long as body (Figure 117)}.$

Face subquadrate, width: height ratio in front view: $1.2\times$; POL: OOL 1.6×; interantennal area as broad as torulus width; scrobe rather short, ca. $2.9 \times$ as long as wide. Scape $1.3 \times$ as long as combined length of pedicel, anellus, and F1 (Figure 120); scape $0.8 \times$ as long as combined length of pedicel, anellus, F1 and F2; anellus subquadrate; F1 $1.3 \times$ as long as pedicel, $2.1 \times$ as long as wide; F2 $2.1 \times$ as long as wide; following funicular segments elongate tending to subquadrate only from F6, with F7 $1.3 \times$ as long as wide. Pronotum with coarse cross- striae, mid- and lateral lobes of mesoscutum, and axilla with finer strong cross- striae (Figure 118). Mid-lobe of mesoscutum $1.4 \times$ as long as scutellum length. Scutellum $1.4 \times$ as long as wide, reticulate; frenum indistinct, $0.4 \times$ as long as scutellum, reticulate in the middle, and with smooth striae on sides. Stigma rectangular, elongate, about $1.9 \times$ as long as wide (Figure 121); upper part of stigmal vein elongate, $0.4 \times$ as long as stigma length; uncus $0.8 \times$ as long as upper part of stigmal vein; marginal vein $0.9 \times$ as long as postmarginal vein. Propodeum with cross- striae becoming reticulate anteriorly, a very weak median carina posteriorly.

Males. They are highly variable in colour, and two extreme forms can be distinguished with intermediates. Paratypes $\stackrel{\circ}{\supset}$ include three specimens with pale patterns and one with dark patterns whereas our collection included nine specimens of pale form, 13 of dark form, and three of intermediate color.

Pale form. Body length from 4.3 to 5.4 mm. Body colour mostly orange-yellow (Figures 122–124). Head yellow with a black spot covering the interantennal area and the scrobes (Figure 125), extending in a semi-circle to the anterior ocellus, then lengthening in a median irregular line until pronotum insertion. Pilosity on lower face pale, black on the remainder of head; parascrobal area with a line of small setae along the scrobes, and a line of long black bristles along the eye contour; two small setae in the interocellar area; two rows of bristles around pronotum insertion. Antenna yellow.

Thorax mostly yellow- orange with a few black patterns (Figure 124). Pronotum yellow with a triangular black spot (very light in a few specimens) in the middle of pronotum, enlarging apically but not reaching the mid lobe of mesoscutum, and a fuzzy blackish band along the posterior suture; mid lobe of mesoscutum orange with a narrow, median black band extending from anterior to posterior suture (very light in one of the δ paratypes); lateral lobes of mesoscutum yellow; axilla yellow with a transparent, rectangular band on the antero-lateral part; scutellum yellow with the anterior suture black prolongating in its middle by a small fuzzy blackish spot surrounded by an orange spot. Posterior sutures of metanotum black. Pilosity on thoracic dorsum black but pale on mesepisternum, mesepimeron and callus; 4 pairs of long black bristles on scutellum (Figure 124). Legs yellow. Forewing stigma of paratype brown with a slight yellowish contour but the specimens we collected did not show such a contour (Figure 128); basal cell only partly closed, the disc with 6 setae; basal line and costal line each with 2 setae; costal cell more than 20 small hairs in 4-5 rows in the apical 1/2 to 2/3. Propodeum yellow with a black, median patch of rombhoid shape, extending from anterior to posterior part; sutures with callus blackish. Gaster petiolate, mostly black with few yellow patterns (Figure 122); T3 elongate, entirely black on dorsum; T4 with a large transvers blackish band on the anterior part and a narrower yellow band on the posterior part, extending on sides; T5 similar; T6 and T7 with the vellow band limited to the sides; last segments yellow. Gaster entirely black on dorsum in a few specimens. A row of long black bristles on the lateral parts of tergites 5, 6, 7 and numerous bristles on T8.

Face rounded, width: height ratio in front view: $1.0\times$ (Figure 125); POL: OOL 1.4×. Scape $1.2 \times$ as long as combined length of pedicel, anellus, and F1 (Figure 126); scape $0.9 \times$ as long as combined length of pedicel, anellus, F1 and F2; anellus subquadrate; F1 elongate, $1.5 \times$ as long as pedicel, $2.1 \times$ as long as wide; F2 and following funicular segments similarly elongate, with F7 $1.4 \times$ as long as wide.. Pronotum with strong transverse cross-striae; mid lobe of mesoscutum with finer, very irregular cross-striae (Figure 124); mid-lobe of mesoscutum $1.1 \times$ as long as scutellum length; scutellum $1.2 \times$ as long as wide, irregularly reticulate; frenum $0.4 \times$ as long as scutellum length, reticulate as scutellum in its middle but with longitudinal carinae on the sides. Forewing stigma nearly rectangular, $1.4 \times$ as long as wide (Figure 128); upper part of stigmal vein; marginal vein $0.8 \times$ as long as postmarginal vein. Propodeum with coarse, irregular carinae extending laterally but no median carina. Genitalia with short aedeagus, its part above digitus about $1.2 \times$ as long as digitus



Figures 122–128. *Megastigmus hypogeus* Hussey male pale form. 122 dorsal view of the body 123 lateral view of body 124 dorsal view of thorax 125 front view of head; 126 antenna 127 genitalia 128 forewing.

length; digitus compressed, about $2.3 \times \text{longer}$ than its maximum width, with three teeth (Figure 127).

Dark form. Body length slightly longer than in pale form, from 5.0 to 5.5 mm. Body substantially black (Figures 129–132). Head entirely black (Figure 132) but antenna yellow with scape brownish (Figure 133). Pronotum dark-yellowish with a large
median black extending from head insertion to mid lobe of mesososcutum, a large black rectangular patch on the sides, and a larger fuzzy blackish band along the posterior suture (Figure 131); mid lobe of mesoscutum with a large median, dark orange brown band covering most of the surface; lateral lobes of mesoscutum and axilla dark orange; scutellum with a large brown- black median band from anterior to posterior suture. Lateral parts of the thorax (mesepisternum, mesepimeron and callus) entirely black except acropleuron dark orange. Legs mostly black; coxae black; femora black on the apical 2/3 in fore- and mid- femur, and quite completely in hind femur; tibiae and tarsi yellow. Forewing stigma brown with a large, triangular infuscation (Figure 135); basal cell with 16 strong setae on disc; basal line with 3 strong setae; costal line with 26 small setae; costal cell with more than 100 small hairs in a number of rows. Pilosity of thorax similar to that in pale form. Propodeum entirely black (Figure 131). Gaster conspicuously petiolate, entirely black (Figure 129–130).

Relative dimensions of head and antenna similar as in pale form; sculpture of thorax and propodeum same as in pale form. Forewing stigma nearly rectangular, $1.3 \times as$ long as wide (Figure 135); upper part of stigmal vein very short, only $0.2 \times as$ long as stigma length; uncus as long as upper part of stigmal vein; marginal vein $0.8 \times as$ long as postmarginal vein. Aedeagus similar to pale form but one digitus with four teeth, the other one with three teeth (Figure 134).

Intermediates. Body length as in pale form, from 4.6 to 5.4 mm. Some specimens with infuscate stigma show lighter coloration patterns than in the extreme dark form, with the median band on pronotum smaller, the lateral lobes of mesoscutum and axilla yellow, the scutellum with the median band limited to the anterior part, and the gaster with narrow yellow bands from T4 to T6.

Host plants. Ozoroa obovata, Ozoroa insignis ssp reticulata (Anacardiaceae). Probably a seed feeder, based on its place in the molecular phylogeny of *Megastigmus* spp. where it clusters with the other species developing in seeds of Anacardiaceae (Figure 14), such as *M. ozoroae* and *M. lanneae*, and despite the presence of numts in the short COI fragment.

Distribution. Mixed bush-/grassland in western Kenya near Lake Victoria and in similar habitat in coastal areas. Adults emerged from up to 2.8% of the fruits in some collections (Table 3).

Diagnosis. The key from Hussey (1956a) distinguished the females of *M. hypogeus* by their large body (>5 mm), the ocelli surrounded with black, and the thoracic dorsum with longitudinal black stripe. Actually, it does not allow one to separate females of this species from those of some other species associated with fruits of Anacardiaceae, which are also large and present a thoracic dorsum with a longitudinal dark band; i.e., *M. lanneae*, *M. ozoroae*, and *M. smithi*. *M. hypogeus* differs from these species by the relative length of its ovipositor being 1.2–1.3 × longer than gaster length whereas it is shorter than gaster length in *M. ozoroae* (Figure 43), and *M. smithi* (Figure 31), and much longer in *M. lanneae* (1.8×; Figure 97).

Genitalic characters also allow separation of *M. hypogeus* males from those of other species developing in seeds of Anacardiaceae, the aedeagus part above digitus being



Figures 129–135. *Megastigmus hypogeus* Hussey male dark form. 129 dorsal view of the body 130 lateral view of body 131 dorsal view of thorax 132 front view of head; 133 antenna 134 genitalia 135 forewing.

significantly less elongated (1.2 × as long as digitus length) than in *M. ozoroae* (1.8×; Figures 53, 60) and *M. lanneae* (1.8×; Figures 107, 115) but much longer than in *M. pistaciae* (0.8×; Figure 148) and *M. transvaalensis* (0. 5×; Figure 162).

M. pistaciae Walker, 1871

Material examined. $3 \bigcirc \bigcirc$, $3 \bigcirc \bigcirc$, Kenya, Mount Kulal, 2.6290°S, 36.9278°E, 1640m elevation, 11 Dec 2008, ex. *Pistacia lentiscus* subsp. *emarginata* seeds, R. Copeland leg. $(2 \bigcirc \bigcirc, 1 \oslash RSC; 1 \bigcirc, 1 \oslash ARC; 1 \oslash destroyed)$

Male and female specimens fit the detailed description of the species by Roques and Skrzypczyńska (2003) as shown by figures 136-141 presenting the most important characteristics of female specimens from Kenya. Similarly as in most Megastigmus species developing in Anacardiaceae seeds in Kenya, males are highly variable ranging from pale forms, mostly yellow with forewing stigma without infuscation (Figures 142-144 and 146-149,) to dark forms, mostly black with infuscated stigma (Figure 145). However, the forewing description of female M. pistaciae used by Grissell and Prinsloo (2001) as a diagnostic character in the key to separate the species of Megastigmus reared from seeds of Anacardiaceae appears largely different from our observations. To separate it from female M. thomseni, Grissell and Prinsloo (2001) stated that the costal cell of the forewing of M. pistaciae presents 3 or 4 rows of setae in the apical 1/2 to 2/3 and the basal cell more than 5 setae whereas there is at most a single row of setae in the costal cell and at most 3-4 setae in the basal cell of *M. thomseni*. Actually, all the specimens of *M*. pistaciae we examined from Kenya, but also from other parts of the world (Europe, North Africa, California) did not show the characters mentioned by Grissell & Prinsloo but were closer to those attributed to the forewing of *M. thomseni*. Since these authors did not mention the origin of the specimens they examined, it is difficult to evaluate their observations.

In the molecular phylogeny of *Megastigmus*, the Kenyan specimen clusters with the other specimen of *M. pistaciae* from Southern Europe (Figure 14), confirming their status of sister species belonging to the same "Anacardiaceae clade". However, there was a genetic distance of 4.5% between these specimens and according to Auger-Rozenberg et al (2006) and Scheffer and Grissell (2003), clearly differentiated species of *Megastigmus* diverge by more than 4.0%. This suggests an opportunity to develop further studies to evaluate the possibility that the Kenyan populations represent a sibling species of *M. pistaciae*.

Host plants. Pistacia lentiscus subsp. emarginata seeds.

Distribution. Reared from samples collected in highland areas (1640–1920 m above sea level) of the Rift Valley, in northern and southwestern Kenya. Few fruits (0.6%) were infested (Table 3).

Diagnosis. Females can be separated from those of other species with a predominantly orange colour associated with fruits of Anacardiaceae by the shape of the forewing stigma and the relative length of ovipositor. The stigma is oval in *M. pistaciae* (1.2–1.3× as long as wide; Figure 141) whereas it is much more elongate in both *M. transvaalensis* (2.0×; Figure 155) and *M. thomseni* (1.7×, according to the drawing by Hussey 1956a). The ovipositor is only a bit longer than gaster length (Figure 137)



Figures 136–141. *Megastigmus pistaciae* Walker female. 136 dorsal view of the body 137 lateral view of body 138 dorsal view of thorax 139 front view of head 140 antenna 141 forewing.

whereas it is nearly twice as long as gaster in *M. laventhali* (1.8×; Figure 76) and $1.4 \times$ as long as gaster length in *M. transvaalensis* (Figure 151).

The genitalia allow one to separate males from those of other species identified in seeds of Anacardiaceae. The aedeagus part above digitus is comparatively longer than in *M. transvaalensis* ($0.8 \times vs. 0.5 \times as$ long as digitus length; Figures 148 and 162, respectively) whereas this part is much more elongated in *M. hypogeus* ($1.2 \times as$ long



Figures 142–149. *Megastigmus pistaciae* Walker male. 142 dorsal view of the body (pale form) 143 lateral view of body (pale form) 144 dorsal view of thorax(pale form) 145 lateral view of body (dark form) 146 front view of head (pale form); 147 antenna (pale form) 148 genitalia (pale form) 149 forewing (pale form).



Figures 150–155. *Megastigmus transvaalensis* Hussey female. 150 dorsal view of the body 151 lateral view of body 152 dorsal view of thorax 153 front view of head 154 antenna 155 forewing.



Figures 156–162. *Megastigmus transvaalensis* Hussey male. 156 dorsal view of the body 157 lateral view of body 158 dorsal view of thorax 159 front view of head; 160 antenna 161 forewing 162 genitalia.

as digitus length; Figures 127, 134), *M. ozoroae* (1.8×; Figures 53, 60) and *M. lanneae* (1.8×; Figures 107, 115).

M. transvaalensis (Hussey, 1956)

Material examined. $3 \bigcirc \bigcirc$, $7 \circlearrowright \circlearrowright$, Kenya, Kikuyu Escarpment, Central Province, 1.0290°S, 36.6025°E, 2100 m, coll. 85, ex. *Rhus vulgaris* seeds, 29 Apr 1999, R. Copeland leg.; $2 \bigcirc \bigcirc$, $3 \circlearrowright \circlearrowright$, Kenya, Burguret, Central Province, 0.1128°S, 37.0375°E, coll. 2162, ex. *Rhus natalensis* seeds, 16 Aug 2002, R. Copeland leg.; $3 \bigcirc \bigcirc$, Kenya, Nairobi Province, 1.2212°S, 36.8963°E, 1610m, coll. 2787, ex. *Schinus terebinthifolius* seeds, 28 Apr 2004, R. Copeland leg.; $4 \bigcirc \bigcirc$, $10 \circlearrowright \circlearrowright$ RSC; $4 \bigcirc \bigcirc$, $7 \circlearrowright \circlearrowright$ ARC)

Male and female specimens fit the description of the species by Hussey (1956a) and Grissell and Prinsloo (2001), then detailed by Roques and Skrzypczyńska (2003). Figures 150–155 present the most important characteristics of female specimens from Kenya whereas Figures 156–162 show those of males.

Host plants. Schinus molle, Schinus terebinthifolius, Rhus natalensis, R. vulgaris (Anacardiaceae). A seed feeder. Schefer and Grissell (2003) presented a molecular analysis of the populations of this species, suggesting its origin in Austral Africa but we were unable to amplify any specimens of the Kenyan populations we obtained.

Distribution. Adults emerged from 14.8% of the fruits of *S. molle*, 14.7% of those of *R. vulgaris* and up to 6.1% of *R. natalensis* fruits (Table 3).

Diagnosis. Females can be distinguished from these of other species associated with fruits of Anacardiaceae and showing a predominantly orange body by the relative size of the ovipositor and the shape of the forewing stigma. In *M. transvaalensis*, the ovipositor is $1.4 \times as$ long as gaster length (Figure 151) whereas it is nearly twice as long as the gaster in *M. laventhali* ($1.8\times$; Figure 76) and only a bit longer than gaster in *M. thomseni* and *M. pistaciae* ($1.1-1.2\times$; Figure 137). The stigma is conspicuously elongate, and quite rectangular in *M. transvaalensis* ($2.0 \times as$ long as wide; Figure 155), less elongate in *M. thomseni* ($1.7\times$, according to the drawing by Hussey 1956a) whereas it is oval and rounded in *M. pistaciae* ($1.2-1.3\times$; Figure 141).

Genitalia allows the separation of males from those of other species reared from seeds of Anacardiaceae. The aedeagus part above digitus is conspicuously shorter than digitus length (0. 5×; Figure 162) whereas this part is more elongated in *M. pistaciae* (0.8×; Figure 148), *M. hypogeus* (1.2 × as long as digitus length; Figures 127, 134), *M. ozoroae* (1.8×; Figures 53, 60) and *M. lanneae* (1.8×; Figures 107, 115).

M. somaliensis Hussey, 1955

Type material examined. Holotype Q "Bristish Somaliland; ex. seeds of *Juniperus procera*; coll. i.1954, em. 25.ii.1954, J.T. Lawrie; Brit. Mus. 1956–294; Com. Inst. Ent.







Figures 163-167. Megastigmus somaliensis Hussey. 163 lateral view of female body 164 electroscan of front view of male head 165 electroscan of male antenna 166 electroscan of dorsal view of male thorax **167** male forewing.

coll. 13661; B.M. TYPE HYM. 5.1623a; NHMUK010263946 5.1623a"(BNHM). Paratypes $4 \bigcirc \bigcirc , 2 & \bigcirc$, same collection data as holotype (BNHM);

Additional material. 19 "Abyssinia, Mulu, above Muger Valley; circa 8000 ft., 18–23. 12. 1926, Dr. H. Scott" (BNHM); 19 "Abyssinia, Mt. Chillálo Digula; circa 9500 ft., 27.11.1926, from foliage giant juniper, Dr. H. Scott" (BNHM); 399, 233, Kenya, Central Prov., Mt. Kenya Forest, 0.0308°S, 37.1230°E, 2125m, coll. 3034, ex. *Juniperus procera* seeds, 2 Nov. 2004, R. Copeland leg. (299, 13, RSC; 19 ARC; 13 destroyed).

Male and female specimens obtained from Kenya fit the general description of the species by Hussey (1956b). Figure 163 presents a view of female body whereas Figures 164–167 show the major morphological characteristics of male specimens from Kenya. Body colour is dark orange in the Kenyan specimens instead of pale orange in the type material from former British Somaliland, at present Somalia. However, at least one dark orange female was noticed in the BNHM collection, which had been collected in Digula, Abyssinia, at present Ethiopia.

Host plants. Juniperus procera. A seed feeder clustering in the molecular phylogeny of *Megastigmus* with the other species developing in seeds of Juniperus in Europe (Figure 14). This species clearly belongs with strong support to the "Cupressaceae clade" whatever the marker and the analysis. It is close to *M. amicorum*, a seed chalcid which is widely distributed on Juniperus from the Mediterranean basin. The host of *M. somaliensis*, *J. procera*, is the only juniper out of more than 60 species growing in the Arabian Peninsula and in Africa (Mao et al. 2010). It suggests that the host and the associated chalcid originated from regions more northern than the ones where they are presently observed, which could explain the phylogenetic proximity with other juniper seed chalcids.

Distribution. Sampled in highland forest and woodland in central and northwestern Kenya (Figure 1). Also present in Somalia and Ethiopia.

Diagnosis. This species, as well as the related *M. asir* which also attacks *Juniperus procera* (Ghram and Shati 2011), are easily differentiated from the other Afrotropical *Megastigmus* by the 7–8 pairs of hairs on the lateral parts of scutellum (Figure 166) whereas at most 5 pairs are present in the other species. Females of *M. somaliensis* are distinguished from those of *M. asir* by the shape of the forewing stigma and the sculpture of the propodeum. In the first species, forewing stigma is oval (1.6 × as long as wide measured from the drawing of Hussey 1956b) whereas it is much more elongate *M. asir* (2.2 × as long as wide when measured on the corresponding wing photo in Ghram and Shati (2011), although the authors mentioned in their text this value to be $1.5\times$). Propodeum is rugose and irregularly striated in *M. somaliensis* whereas it is smooth in *M. asir* (Ghram and Shati 2011). The propodeum of males shows the same differences, the one of *M. somaliensis* additionally presenting a very short median carina in its anterior part (Figure 166). Forewing stigma is quite similar in the two species ($1.4 \times as long$ as wide in M. somaliensis- Figure 167- vs. $1.35 \times in M. asir$; Ghram and Shati 2011).



Figure 168. Updated synthesis of biological habits of the world *Megastigmus* species recorded to date with a detail of the species present in Eastern and Southern Africa. The four bars on the top detail the chalcid feeding regimes. The following ones present the host plant families colonized by the phytophagous seed chalcids, splitted into angiosperms and conifers.

Conclusion

Is the radiation on Angiosperms more important than previously considered?

Our results increase to 16 the number of *Megastigmus* species presently known from the Afrotropical region, of which at least 13 are seed feeders. The results also increase to 28 the number of species shown to be associated worldwide with angiosperm seeds vs. 48 with conifers, and to 7 the number of angiosperm families hosting *Megastigmus* seed chalcids (Figure 168). These angiosperm families are rather distant taxonomically, belonging to different clades and orders within the new, molecular-based classification system of angiosperms (APG III; The Angiosperm Phylogeny Group 2009): Rosaceae and Rhamnaceae in order Rosales and Fabaceae in order Fabales of the clade Fabids; Anacardiaceae in order Sapindales and Malvaceae in order Malvales of the clade Malvids; Hamamelidaceae in order Saxifragales of the clade Core eudicots; and Aquifoliaceae in order Aquifoliales of the clade Campanulids. Rosaceae still host the largest number of chalcid species but there is increasing evidence of a large radiation in Anacardiaceae. Thus, Megastigmus seed chalcids appear to have radiated in Angiosperms much more than previously considered, and it is likely that new host genera, and possibly new host families are to be discovered. The combination of morphological with molecular evidence provides a more robust method for analyzing relationships between chalcid species and their host plants. In the future, such a combined analysis could also help to reliably link the different male colour forms with conspecific females.

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Supplementary material I

Sampling details on specimens used in the genetic study

Authors: Alain Roques, Robert S. Copeland, Laurent Soldati, Olivier Denux, Marie-Anne Auger-Rozenberg

Data type: Collection data

Explanation note: Collection data for the specimens used in the genetic study:

- 25 species already sequenced and used in a previous phylogenetic study (Boivin et al, 2014). (Note that *Torymus azureus* was used as an outgroup for building the phylogenetic trees of the *Megastigmus* genus).
- 4 species already described in the literature but sequenced and analysed for this paper.
- 5 new species described in this paper.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

REVIEW ARTICLE



Records of Limoniidae and Pediciidae (Diptera) from Armenia, with the first Armenian checklist of these families

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Abstract

Records of species of the families Limoniidae and Pediciidae are presented from Armenia. A total of 38 species of Limoniidae and four species of Pediciidae are listed. Of these, 27 species of Limoniidae and one species of Pediciidae represent the first records from Armenia. The first checklist of these families from Armenia is appended, containing 77 species of Limoniidae and six species of Pediciidae.

Keywords

Diptera, Limoniidae, Pediciidae, distributions, first records, checklist, Armenia

Introduction

Compared to some European countries, Transcaucasia (Georgia, Armenia, Azerbaijan) is among less-investigated territories, as far as the families Limoniidae and Pediciidae are concerned. The territory occupies the southern macro-slopes of the Great Caucasus and the mountains and plateaux as far south as the Turkish and Iranian borders.

Savchenko (e.g. 1971, 1972a,b, 1973, 1974, 1976a,b, 1978a,b, 1979, 1981, 1983, and others) contributed considerably to the knowledge of the local fauna and summarized his results in a comprehensive study (Savchenko 1989) dealing with the fauna of the former USSR. Faunal records from all relevant publications are registered in the Catalogue of the Craneflies of the World (Oosterbroek 2015). A total of 50 species of Limoniidae and five species of Pediciidae have been previously listed from Armenia.

The material we present in this paper was mostly collected in the north-western part of Armenia during a recent sampling campaign from August 26 to September 4, 2015. Thirty-eight species of Limoniidae and four species of Pediciidae were identified from this material, of which 27 species of Limoniidae and one species of Pediciidae represent the first records from Armenia. Seven species of Limoniidae and one species of Pediciidae are new to the whole Transcaucasia. In addition, we append the first checklist of Limoniidae and Pediciidae from Armenia, containing 77 species of Limoniidae and six species of Pediciidae.

Material and methods

Samples were collected by sweep-netting from vegetation along streams and lakes by the first, third, and fourth authors (JO, PM, LH) and preserved in 75% ethanol. A list of 33 sampling sites, with coordinates and altitudes, is given in Table 1, and the locations of the sites are shown in Map 1. The material is deposited in the collection of the second author (JS) who also identified the species. Some specimens were dried and mounted on points in the course of the study. The male terminalia, if necessary, were prepared by boiling in a solution of 10% KOH and preserved in glycerine in a sealed plastic tube pinned with the appropriate specimen after examination. Classification, nomenclature, and distribution for individual species are given as summarized by Oosterbroek (2015).

Results

Faunistic records

Family Limoniidae Subfamily Limnophilinae

Paradelphomyia (Oxyrhiza) brevifurca Savchenko, 1976

Material examined. Tavush: below Jukhtakvank Monastery, (site 26), 29.viii.2015, 1 3.

Distribution. So far only known from North Caucasus and Georgia. First record from Armenia.

Site No.	site name (province, short description of locality)	latitude/ N	longitude/ E	altitude/ m a.s.l.
1	Ararat Province: Garni, below Garni Temple, Azat River	40°06'39.4"	44°43'45.3"	1273
2	Ararat Province: nr. Lanjazat, Azat River	40°03'27.0"	44°34'38.3"	976
3	Gegharkunik Province: Tsovagyugh, nr. Sevan Lake	40°37'01.8"	44°57'44.2"	1930
4	Kotayk Province: between Marmarik and Aghavnadzor, tributary of Marmarik River	40°33'52.0"	44°40'09.1"	1872
5	Kotayk Province: E of Hankavan, Marmarik River	40°38'04.7"	44°29'19.4"	1974
6	Kotayk Province: E of Hankavan, Marmarik River	40°38'09.2"	44°32'23.2"	1913
7	Kotayk Province: Hrazdan Sewage Treatment Plant, Hrazdan River	40°29'12.8"	44°43'55.9"	1705
8	Kotayk Province: Meghradzor, behind railway, tributary of Marmarik River	40°37'12.7"	44°40'18.3"	1825
9	Kotayk Province: Meghradzor, below "Gold Mine", tributary of Marmarik River	40°37'53.0"	44°40'17.5"	1870
10	Kotayk Province: near Artavaz, Marmarik River	40°36'49.9"	44°34'18.2"	1849
11	Kotayk Province: N of Solak, Hrazdan River	40°28'19.7"	44°42'42.2"	1567
12	Kotayk Province: SW of Hrazdan Reservoir, tributary of Hrazdan River	40°30'10.2"	44°44'22.4"	1718
13	Kotayk Province: between Marmarik and Aghavnadzor, Marmarik River	40°34'29.3"	44°41'02.2"	1760
14	Lori Province: W of Vahagnadzor, Zamanlu River	40°53'07.0"	44°34'39.0"	1092
15	Lori Province: Lermontov, tributary of Aghstev River (Fig. 2)	40°45'24.6"	44°38'42.0"	1853
16	Lori Province: Meghvahovit, road H31, small steppe brook	41°03'59.9"	44°05'44.2"	1949
17	Lori Province: N of Dzoraget, tributary of Pambak River	40°56'52.7"	44°37'37.2"	1030
18	Lori Province: N of Pushkin, tributary of Dzoraget River	40°58'04.8"	44°24'49.7"	1485
19	Lori Province: NE of Geghasar, Pambak River	40°51'17.4"	44°11'40.8"	1627
20	Lori Province: road H23 to Pushkin Pass, small brook	40°54'22.9"	44°25'33.3"	1839
21	Shirak Province: between Aghvoik and Ardenis, tributary of Akhurian River	41°04'21.2"	43°44'44.8"	2052
22	Shirak Province: E of Torosgyugh, tributary of Akhurian River	40°55'55.0"	43°52'45.3"	1885
23	Shirak Province: NE of Musayelyan, tributary of Akhurian River	41°00'13.2"	43°57'24.9"	2195
24	Shirak Province: NW of Amasia, tributary of Akhurian River (Fig. 1)	40°58'20.5"	43°46'06.9"	1987
25	Shirak Province: Zuygaghbyur, meanders of tributary of Akhurien River	41°00'56.1"	43°54'18.8"	2034
26	Tavush Province: below Jukhtakvank Monastery	40°45'11.8"	44°48'25.7"	1411
27	Tavush Province: car park on road M4, tributary of Aghstev River	40°50'32.0"	45°06'56.8"	760
28	Tavush Province: W of Dilijan, Bldan River (Fig. 3)	40°44'49.1"	44°49'03.5"	1354
29	Tavush Province: E of Haghartsin, tributary of Aghstev River	40°48'09.3"	44°53'43.7"	1382
30	Tavush Province: E of Matosavank Monastery	40°44'59.6"	44°48'29.2"	1392
31	Tavush Province: N of Gosh, Getik River	40°45'16.5"	45°01'18.4"	940
32	Tavush Province: NW of Teghut, tributary of Aghstev River	40°47'15.2"	44°54'58.0"	1197
33	Tavush Province: vicinity of Parz Lake	40°44'57.7"	44°57'33.3"	1376

Table 1. List of sampling sites.



Map 1. Map showing all sampling sites in Armenia.

Paradelphomyia (Oxyrhiza) fuscula (Loew, 1873)

Material examined. Kotayk: between Marmarik and Aghavnadzor, tributary of Marmarik R. (site 4), 27.viii.2015, 1 3.

Distribution. Europe; Iran. First record from Armenia and Transcaucasia.

Paradelphomyia (Oxyrhiza) senilis (Haliday, 1833)

Material examined. Lori: N of Dzoraget, tributary of Pambak R. (site 17), 1.ix.2015, 1 Å. Distribution. Europe; Azerbaijan, Turkey; Kirghizia. First record from Armenia.

Phylidorea (Phylidorea) ferruginea (Meigen, 1818)

Material examined. Kotayk: Hrazdan Sewage Treatment Plant, Hrazdan R. (site 7), 27.viii.2015, $1 \stackrel{\circ}{\circ} 3 \stackrel{\circ}{\ominus}$.

Distribution. Europe; Azerbaijan, Turkey, Israel; Central Asia, Mongolia; West Siberia. First record from Armenia.



Figures 1–3. Sampling sites with highest species diversity. **I** Site No. 24 - NW of Amasia, tributary of Akhurian River (9 species, including *Erioconopa symplectoides, Molophilus (M.) pleuralis, Ormosia (O.) hederae*, and *Limonia macrostigma*) **2** Site No. 15 - Lermontov, tributary of Aghstev River (8 species, including *Hoplolabis (P.) iranica, O. (O.) hederae, Rhabdomastix (R.) filata, Dicranomyia (D.) melanantha, L. macrostigma*, and *L. stigma*) **3** Site No. 28 - W of Dilijan, Bldan River (8 species, including *Pseudolimnophila (P.) melanura, Antocha (A.) vitripennis, Dicranomyia (D.) pontica*, and *Limonia hercegovinae*).

Pseudolimnophila (Pseudolimnophila) melanura Savchenko, 1984

Material examined. Tavush: W of Dilijan, Bldan R. (site 28), 28.viii.2015, 6 ♂ 3 ♀.

Distribution. So far only known from Tajikistan. First record from Armenia and Transcaucasia; first record since original description.

Remark. Due to syntopic occurrence in Armenia of P. (P.) *melanura* and P. (P.) *sepium* the former cannot be considered a subspecies of the latter, as suggested by Savchenko et al. (1992).

Pseudolimnophila (Pseudolimnophila) sepium (Verrall, 1886)

Material examined. Tavush: W of Dilijan, Bldan R. (site 28), 28.viii.2015, 1 3.

Distribution. Europe; Morocco; Georgia, Azerbaijan, Turkey; Central Asia. First record from Armenia.

Subfamily Chioneinae

Erioconopa symplectoides (Kuntze, 1914)

Material examined. Kotayk: Hrazdan Sewage Treatment Plant, Hrazdan R. (site 7), 27.viii.2015, 1 $\stackrel{\circ}{\circ}$; Shirak: NE of Musayelyan, tributary of Akhurian R. (site 23), 2.ix.2015, 2 $\stackrel{\circ}{\circ}$; Shirak: Zuygaghbyur, meanders of tributary of Akhurian R. (site 25), 2.ix.2015, 3 $\stackrel{\circ}{\circ}$ 1 $\stackrel{\circ}{\circ}$; Shirak: NW of Amasia, tributary of Akhurian R. (site 24), 2.ix.2015, 5 $\stackrel{\circ}{\circ}$; Shirak: between Aghvoik and Ardenis, tributary of Akhurian R. (site 21), 3.ix.2015, 1 $\stackrel{\circ}{\circ}$ 1 $\stackrel{\circ}{\circ}$; Shirak: E of Torosgyugh, tributary of Akhurian R. (site 22), 3.ix.2015, 4 $\stackrel{\circ}{\circ}$ 1 $\stackrel{\circ}{\circ}$; Lori: NE of Geghasar, Pambak R. (site 19), 3.ix.2015, 2 $\stackrel{\circ}{\circ}$ 1 $\stackrel{\circ}{\circ}$.

Distribution. Europe, except for northern countries; Morocco; Georgia, Azerbaijan, Turkey. First records from Armenia.

Erioptera (Erioptera) fusculenta Edwards, 1938

Material examined. Kotayk: NW of Artavaz, Marmarik R. (site 6), 26.viii.2015, 3 \bigcirc 1 \bigcirc ; Kotayk: Hrazdan Sewage Treatment Plant, Hrazdan R. (site 7), 27.viii.2015, 5 \bigcirc ; Kotayk: SW of Hrazdan Reservoir, tributary of Hrazdan R. (site 12), 27.viii.2015, 1 \bigcirc ; Kotayk: near Artavaz, Marmarik R. (site 10), 27.viii.2015, 1 \bigcirc ; Kotayk: Meghradzor, behind railway, tributary of Marmarik R. (site 8), 27.viii.2015, 1 \bigcirc ; Tavush: W of Dilijan, Bldan R. (site 28), 28.viii.2015, 3 \bigcirc ; Ararat: Garni, below Garni Temple, Azat R. (site 1), 31.viii.2015, 5 \bigcirc ; Lori: Meghvahovit, road H31, small steppe brook (site 16), 2.ix.2015, 1 \bigcirc ; Lori: N of Gosh, Getik R. (site 31), 4.ix.2015, 6 \bigcirc 5 \bigcirc ; Shirak: NE of Musayelyan, tributary of Akhurian R. (site 23), 2.ix.2015, 5 \bigcirc 2 \bigcirc ; Shirak: NW of Amasia, tributary of Akhurian R. (site 24), 2.ix.2015, 8 \Diamond 2 \heartsuit ; Shirak: between Aghvoik and Ardenis, tributary of Akhurian R. (site 21), 3.ix.2015, 1 \heartsuit ; Shirak: E of Torosgyugh, tributary of Akhurian R. (site 22), 3.ix.2015, 2 \Diamond .

Distribution. Europe; Georgia, Armenia, Azerbaijan, Turkey, Israel; Turkmenia.

Erioptera (Erioptera) lutea Meigen, 1804

Material examined. Kotayk: NW of Artavaz, Marmarik R. (site 6), 26.viii.2015, $1 \stackrel{\circ}{\circ}$; Kotayk: between Marmarik and Aghavnadzor, Marmarik R. (site 13), 26.viii.2015, $1 \stackrel{\circ}{\circ}$; Kotayk: Hrazdan Sewage Treatment Plant, Hrazdan R. (site 7), 27.viii.2015, $1 \stackrel{\circ}{\circ}$; Kotayk: between Marmarik and Aghavnadzor, tributary of Marmarik R. (site 4), 27.viii.2015, $1 \stackrel{\circ}{\circ}$; Tavush: W of Dilijan, Bldan R. (site 28), 28.viii.2015, $1 \stackrel{\circ}{\circ}$; Tavush: below Jukhtakvank Monastery, (site 26), 29.viii.2015, $1 \stackrel{\circ}{\circ}$; Gegharkunik: Tsovagyugh, nr. Sevan L. (site 3), 29.viii.2015, $1 \stackrel{\circ}{\circ}$; Lori: N of Dzoraget, tributary of Pambak R. (site 17), 1.ix.2015, $1 \stackrel{\circ}{\circ}$; Lori: Meghvahovit, road H31, small steppe brook (site 16), 2.ix.2015, $3 \stackrel{\circ}{\circ}$; Shirak: NE of Musayelyan, tributary of Akhurian R. (site 23), 2.ix.2015, $1 \stackrel{\circ}{\circ} 1 \stackrel{\circ}{\circ}$; Shirak: NW of Amasia, tributary of Akhurian R. (site 24), 2.ix.2015, $1 \stackrel{\circ}{\circ} 1 \stackrel{\circ}{\circ}$

Distribution. Europe; Georgia, Armenia, Azerbaijan, Turkey, Israel, ?Iran; Central Asia; West Siberia.

Erioptera (Mesocyphona) bivittata (Loew, 1873)

Material examined. Lori: N of Pushkin, tributary of Dzoraget R. (site 18), 3.ix.2015, 1 Å. Distribution. Europe; Azerbaijan, Turkey, Israel, Iran; Central Asia, Mongolia; West Siberia, East Siberia. First record from Armenia.

Hoplolabis (Parilisia) iranica (Alexander, 1973)

Material examined. Lori: Lermontov, tributary of Aghstev R. (site 15), 1.ix.2015, 4 $\stackrel{\circ}{\supset}$ 2 $\stackrel{\circ}{\bigcirc}$.

Distribution. Russia (North Caucasus); Georgia, Azerbaijan, Iran. First record from Armenia.

Ilisia maculata (Meigen, 1804)

Material examined. Gegharkunik: Tsovagyugh, nr. Sevan L. (site 3), 29.viii.2015, 2 \Diamond ; Lori: Lermontov, tributary of Aghstev R. (site 15), 1.ix.2015, 1 \Diamond ; Shirak: E of Torosgyugh, tributary of Akhurian R. (site 22), 3.ix.2015, 3 \Diamond 1 \Diamond .

Distribution. Europe; Georgia, Armenia, Azerbaijan, Turkey, Iran.

Molophilus (Molophilus) lackschewitzianus habetatus Savchenko, 1976

Material examined. Tavush: E of Haghartsin, tributary of Aghstev R. (site 29), 29.viii.2015, 1 ♂.

Distribution. Russia (North Caucasus); Georgia, Armenia. **Remark.** Possibly a valid species.

Molophilus (Molophilus) obscurus (Meigen, 1818)

Material examined. Shirak: NE of Musayelyan, tributary of Akhurian R. (site 23), 2.ix.2015, 2 ♂.

Distribution. Europe; Morocco; Georgia, Armenia, Turkey, Cyprus, Lebanon, Israel.

Molophilus (Molophilus) ochraceus (Meigen, 1818)

Material examined. Lori: N of Dzoraget, tributary of Pambak R. (site 17), 1.ix.2015, 2 Å. Distribution. Europe; Georgia, Azerbaijan, Turkey. First record from Armenia.

Molophilus (Molophilus) propinquus (Egger, 1863)

Material examined. Tavush: N of Gosh, Getik R. (site 31), 4.ix.2015, 10 ♂ 3 ♀.
 Distribution. Europe; Morocco; Georgia, Turkey; West Siberia, East Siberia, Far East of Russia. First record from Armenia.

Molophilus (Molophilus) pleuralis de Meijere, 1920

Material examined. Kotayk: NW of Artavaz, Marmarik R. (site 6), 26.viii.2015, 3 ♂; Shirak: NW of Amasia, tributary of Akhurian R. (site 24), 2.ix.2015, 2 ♂.

Distribution. Europe; Georgia, Azerbaijan, Turkey, Cyprus, Israel, Iran; Central Asia; as far east as Far East of Russia. First records from Armenia.

Molophilus (Molophilus) stroblianus decoloratus Savchenko, 1978

Material examined. Kotayk: Meghradzor, below "Gold Mine", tributary of Marmarik R. (site 9), 27.viii.2015, 2 ♂ 2 ♀; Tavush: W of Dilijan, Bldan R. (site 28), 28.viii.2015, $3 \stackrel{\circ}{\circ} 1 \stackrel{\circ}{\circ}$; Tavush: below Jukhtakvank Monastery, (site 26), 29.viii.2015, $1 \stackrel{\circ}{\circ}$; Lori: Lermontov, tributary of Aghstev R. (site 15), 1.ix.2015, $4 \stackrel{\circ}{\circ} 4 \stackrel{\circ}{\circ}$.

Distribution. Ukraine, Russia (North Caucasus); Georgia, Armenia, Azerbaijan. First records since original description.

Remark. Possibly a valid species.

Ormosia (Ormosia) cuspidata Savchenko, 1973

Material examined. Lori: N of Dzoraget, tributary of Pambak R. (site 17), 1.ix.2015, 1 Å. Distribution. ?European Russia (southeast); Georgia. First record from Armenia.

Ormosia (Ormosia) hederae (Curtis, 1835)

Material examined. Gegharkunik: Tsovagyugh, nr. Sevan L. (site 3), 29.viii.2015, 1 \bigcirc ; Lori: Lermontov, tributary of Aghstev R. (site 15), 1.ix.2015, 2 \bigcirc ; Shirak: NW of Amasia, tributary of Akhurian R. (site 24), 2.ix.2015, 1 \bigcirc ; Shirak: E of Torosgyugh, tributary of Akhurian R. (site 22), 3.ix.2015, 1 \bigcirc .

Distribution. Europe; Georgia, Azerbaijan, Turkey; Tajikistan. First records from Armenia.

Rhabdomastix (Rhabdomastix) filata Starý, 2004

Material examined. Lori: Lermontov, tributary of Aghstev R. (site 15), 1.ix.2015, 1 \bigcirc 1 \bigcirc .

Distribution. Bulgaria, Greece, Russia (North Caucasus); Georgia, Armenia, Turkey, Lebanon. First record since original description.

Symplecta (Symplecta) hybrida (Meigen, 1804)

Material examined. Shirak: NW of Amasia, tributary of Akhurian R. (site 24), 2.ix.2015, 1 \bigcirc .

Distribution. Nearctic (Canada, USA, Greenland); widespread in Palaearctic, including Europe; North Africa; Georgia, Armenia, Azerbaijan, Turkey, Lebanon, Israel, Iran; Central Asia, Mongolia; as far east as North Korea, Japan and China; Oriental (India, Nepal, Pakistan).

Subfamily Limoniinae

Achyrolimonia decemmaculata (Loew, 1873)

Material examined. Tavush: W of Dilijan, Bldan R. (site 28), 28.viii.2015, 1 \bigcirc ; Tavush: NW of Teghut, tributary of Aghstev R. (site 32), 29.viii.2015, 1 \bigcirc .

Distribution. Europe; Georgia, Armenia, Azerbaijan, Iran.

Antocha (Antocha) vitripennis (Meigen, 1830)

Material examined. Kotayk: between Marmarik and Aghavnadzor, tributary of Marmarik R. (site 4), 27.viii.2015, 1 3; Tavush: W of Dilijan, Bldan R. (site 28), 28.viii.2015, 1 9 1 3.

Distribution. Europe; Turkey, Israel; Afghanistan. First records from Armenia and Transcaucasia

Dicranomyia (Dicranomyia) circassica Lackschewitz, 1941

Material examined. Tavush: car park on road M4, tributary of Aghstev R. (site 27), 4.ix.2015, 1 ♂.

Distribution. So far only known from North Caucasus and Georgia. First record from Armenia.

Dicranomyia (Dicranomyia) didyma (Meigen, 1804)

Material examined. Kotayk: E of Hankavan, Marmarik R. (site 5), 26.viii.2015, 1 \bigcirc ; Kotayk: N of Solak, Hrazdan R. (site 11), 27.viii.2015, 1 \bigcirc ; Ararat: nr. Lanjazat, Azat R. (site 2), 31.viii.2015, 1 \bigcirc ; Shirak: NE of Musayelyan, tributary of Akhurian R. (site 23), 2.ix.2015, 1 \bigcirc ; Shirak: NW of Amasia, tributary of Akhurian R. (site 24), 2.ix.2015, 1 \bigcirc .

Distribution. Europe; Morocco, Algeria; Georgia, Armenia, Azerbaijan, Turkey, Iran; Afghanistan, Mongolia, ?China.

Dicranomyia (Dicranomyia) longipennis (Schummel, 1829)

Material examined. Kotayk: Meghradzor, behind railway, tributary of Marmarik R. (site 8), 27.viii.2015, $4 \stackrel{>}{\circ}$, $1 \stackrel{\bigcirc}{\circ}$.

Distribution. Nearctic (Canada, USA); widespread in Palaearctic, including Europe; Georgia, Azerbaijan, ?Syria, Iran; Central Asia, Mongolia; as far east as Far East of Russia and Japan; Oriental (India). First record from Armenia.

Dicranomyia (Dicranomyia) melanantha Savchenko, 1984

Material examined. Kotayk: between Marmarik and Aghavnadzor, tributary of Marmarik R. (site 4), 27.viii.2015, 1 \bigcirc ; Lori: Lermontov, tributary of Aghstev R. (site 15), 1.ix.2015, 2 \bigcirc 1 \bigcirc ; Lori: Meghvahovit, road H31, small steppe brook (site 16), 2.ix.2015, 6 \bigcirc 1 \bigcirc ; Shirak: NE of Musayelyan, tributary of Akhurian R. (site 23), 2.ix.2015, 1 \bigcirc ; Tavush: car park on road M4, tributary of Aghstev R. (site 27), 4.ix.2015, 1 \bigcirc .

Distribution. France (Corsica), Russia (North Caucasus); Georgia, Azerbaijan, ?Lebanon. First records from Armenia.

Dicranomyia (Dicranomyia) modesta (Meigen, 1818)

Material examined. Gegharkunik: Tsovagyugh, nr. Sevan L. (site 3), 29.viii.2015, 1 \Diamond ; Lori: NE of Geghasar, Pambak R. (site 19), 3.ix.2015, 1 \Diamond ; Tavush: N of Gosh, Getik R. (site 31), 4.ix.2015, 1 \Diamond .

Distribution. Nearctic (Canada, USA, Greenland); widespread in Palaearctic, including Europe; Georgia, Armenia, Azerbaijan, Turkey, Iran; Central Asia, Mongolia; as far east as Far East of Russia and Japan.

Dicranomyia (Dicranomyia) pallidinota Starý, 2009

Material examined. Gegharkunik: Tsovagyugh, nr. Sevan L. (site 3), 29.viii.2015, 1 Å. Distribution. Bulgaria, Greece, France (Corsica); Lebanon, Syria. First record from Armenia and Transcaucasia; first record since original description.

Dicranomyia (Dicranomyia) pontica Lackschewitz, 1941

Material examined. Tavush: W of Dilijan, Bldan R. (site 28), 28.viii.2015, 1 3; Lori: W of Vahagnadzor, Zamanlu R. (site 14), 1.ix.2015, 2 3 1 \bigcirc .

Distribution. So far only known from North Caucasus and Georgia. First records from Armenia.

Dicranomyia (Numantia) fusca (Meigen, 1804)

Material examined. Kotayk: near Artavaz, Marmarik R. (site 10), 27.viii.2015, 1 ♂; Kotayk: between Marmarik and Aghavnadzor, tributary of Marmarik R. (site 4), 27.viii.2015, 2 ♂; Lori: road H23 to Pushkin Pass, small brook (site 20), 3.ix.2015, 1 ♂.

Distribution. Nearctic (Canada, USA); Europe; Georgia, Azerbaijan, Turkey, Iran; Far East of Russia, Japan. First records from Armenia.

Dicranoptycha livescens Loew, 1871

Material examined. Tavush: E of Matosavank Monastery (site 30), 29.viii.2015, 1 \bigcirc ; Tavush: E of Haghartsin, tributary of Aghstev R. (site 29), 29.viii.2015, 1 \bigcirc .

Distribution. Europe, except for northern countries. First records from Armenia and Transcaucasia.

Limonia hercegovinae (Strobl, 1898)

Material examined. Tavush: W of Dilijan, Bldan R. (site 28), 28.viii.2015, 1 \bigcirc ; Tavush: below Jukhtakvank Monastery, (site 26), 29.viii.2015, 1 \bigcirc 1 \bigcirc ; Tavush: NW of Teghut, tributary of Aghstev R. (site 32), 29.viii.2015, 1 \bigcirc ; Tavush: E of Haghartsin, tributary of Aghstev R. (site 29), 29.viii.2015, 1 \bigcirc ; Lori: W of Vahagnadzor, Zamanlu R. (site 14), 1.ix.2015, 2 \bigcirc ; Lori: road H23 to Pushkin Pass, small brook (site 20), 3.ix.2015, 1 \bigcirc .

Distribution. Europe, except for northern countries; Morocco; Azerbaijan, Turkey, Iran. First records from Armenia.

Limonia macrostigma (Schummel, 1829)

Material examined. Lori: Lermontov, tributary of Aghstev R. (site 15), 1.ix.2015, 1 ♂; Shirak: NW of Amasia, tributary of Akhurian R. (site 24), 2.ix.2015, 1 ♂.

Distribution. Europe; Morocco; Georgia, Azerbaijan, Turkey, Cyprus; Central Asia; as far east as Far East of Russia, and ?North Korea; Oriental (Pakistan). First records from Armenia.

Limonia stigma (Meigen, 1818)

Material examined. Gegharkunik: Tsovagyugh, nr. Sevan L. (site 3), 29.viii.2015, 1 3; Lori: Lermontov, tributary of Aghstev R. (site 15), 1.ix.2015, 1 3.

Distribution. Europe. First records from Armenia and Transcaucasia.

Metalimnobia (Metalimnobia) quadrinotata (Meigen, 1818)

Material examined. Kotayk: E of Hankavan, Marmarik R. (site 5), 26.viii.2015, 1 ♂. Distribution. Europe; Kirghizia, Mongolia; West Siberia, East Siberia, Far East of Russia. First record from Armenia and Transcaucasia.

Rhipidia (Rhipidia) maculata Meigen, 1818

Material examined. Gegharkunik: Tsovagyugh, nr. Sevan L. (site 3), 29.viii.2015, 1 ♂. Distribution. Nearctic (Canada, USA); widespread in Palaearctic, including Europe; Georgia; Mongolia; as far east as Far East of Russia, China, and Japan; Oriental (China). First record from Armenia.

Family Pediciidae

Dicranota (Dicranota) crassicauda Tjeder, 1972

Material examined. Shirak: Zuygaghbyur, meanders of tributary of Akhurien R. (site 25), 2.ix.2015, 1 ♂; Shirak: E of Torosgyugh, tributary of Akhurian R. (site 22), 3.ix.2015, 1 ♂.

Distribution. Finland, Norway, Sweden; Kazakhstan, Tajikistan. First records from Armenia and Transcaucasia.

Dicranota (Paradicranota) landrocki Czižek, 1931

Material examined. Shirak: NW of Amasia, tributary of Akhurian R. (site 24), 2.ix.2015, 1 ♂.

Distribution. Europe, except for northern countries; Morocco; Georgia, Armenia, Azerbaijan, Lebanon; Tajikistan.

Dicranota (Paradicranota) subtilis Loew, 1871

Material examined. Tavush: vicinity of Parz L. (site 33), 28.viii.2015, 1 ♂; Tavush: below Jukhtakvank Monastery, (site 26), 29.viii.2015, 1 ♂; Lori: Meghvahovit, road H31, small steppe brook (site 16), 2.ix.2015, 1 ♂.

Distribution. Europe; Georgia, Armenia, Azerbaijan.

Pedicia (Amalopis) occulta (Meigen, 1830)

Material examined. Lori: road H23 to Pushkin Pass, small brook (site 20), 3.ix.2015, 1 Å. Distribution. Europe; Georgia, Armenia, Azerbaijan, Turkey, Cyprus, Lebanon.

Discussion

A total of 38 species of Limoniidae and four species of Pediciidae are recorded from Armenia. Of these, 27 species of Limoniidae and one species of Pediciidae represent the first records for Armenia. These are the following: Paradelphomyia (O.) brevifurca, P. (O.) fuscula, P. (O.) senilis, Phylidorea (P.) ferruginea, Pseudolimnophila (P.) melanura, P. (P.) sepium, Erioconopa symplectoides, Erioptera (M.) bivittata, Hoplolabis (P.) iranica, Molophilus (M.) ochraceus, M. (M.) pleuralis, M. (M.) propinguus, Ormosia (O.) cuspidata, O. (O.) hederae, Antocha (A.) vitripennis, Dicranomyia (D.) circassica, D. (D.) longipennis, D. (D.) melanantha, D. (D.) pallidinota, D. (D.) pontica, D. (N.) fusca, Dicranoptycha livescens, Limonia hercegovinae, L. macrostigma, L. stigma, Metalimnobia (M.) quadrinotata, Rhipidia (R.) maculata, and Dicranota (D.) crassicauda. Seven species of Limoniidae and one species of Pediciidae are new to the whole Transcaucasia, viz. Paradelphomyia (O.) fuscula, Pseudolimnophila (P.) melanura, Antocha (A.) vitripennis, Dicranomyia (D.) pallidinota, Dicranoptycha livescens, Limonia stigma, Metalimnobia (M.) quadrinotata, and Dicranota (D.) crassicauda. Four species/subspecies are here recorded for the first time since their original descriptions, viz. Pseudolimnophila (P) melanura, Molophilus (M.) stroblianus decoloratus, Rhabdomastix (R.) filata, and Dicranomyia (D.) pallidinota.

Altogether 50 species of Limoniidae and five species of Pediciidae were previously known to occur in Armenia (Oosterbroek 2015). Our records increase the number of Armenian species to 83, 77 species of Limoniidae and six species of Pediciidae.

Checklist of Limoniidae and Pediciidae of Armenia

species new to Armenia are marked with an asterisk (*)

Limoniidae: Limnophilinae

- 1. Afrolimnophila minima (Savchenko, 1971)
- 2. Dicranophragma (Brachylimnophila) nemorale (Meigen, 1818)
- 3. Dicranophragma (Mixolimnomyia) rufulum (Savchenko, 1979)
- 4. Eloeophila maculata (Meigen, 1804)
- 5. Hexatoma (Cladolipes) haiasana Savchenko, 1972
- 6. Hexatoma (Hexatoma) gaedii (Meigen, 1830)
- 7. Limnophila (Limnophila) pictipennis (Meigen, 1818)

- 8. *Paradelphomyia (Oxyrhiza) brevifurca Savchenko, 1976
- 9. *Paradelphomyia (Oxyrhiza) fuscula (Loew, 1873)
- 10. *Paradelphomyia (Oxyrhiza) senilis (Haliday, 1833)
- 11. **Phylidorea (Phylidorea) ferruginea* (Meigen, 1818)
- 12. Pseudolimnophila (Pseudolimnophila) lucorum (Meigen, 1818)
- 13. *Pseudolimnophila (Pseudolimnophila) melanura Savchenko, 1984
- 14. *Pseudolimnophila (Pseudolimnophila) sepium (Verrall, 1886)

Limoniidae Chioneinae

- 15. Ellipteroides (Ptilostenodes) omissus (Lackschewitz, 1940)
- 16. *Erioconopa symplectoides (Kuntze, 1914)
- 17. Erioconopa trivialis (Meigen, 1818)
- 18. Erioptera (Erioptera) fusculenta Edwards, 1938
- 19. Erioptera (Erioptera) lutea Meigen, 1804
- 20. *Erioptera (Mesocyphona) bivittata (Loew, 1873)
- 21. Gonomyia (Gonomyia) basilobata Alexander, 1975
- 22. Gonomyia (Gonomyia) conoviensis Barnes, 1924
- 23. Gonomyia (Gonomyia) lucidula de Meijere, 1920
- 24. Gonomyia (Gonomyia) papposa Savchenko, 1983
- 25. Hoplolabis (Eurasicesa) amseliana (Nielsen, 1961)
- 26. *Hoplolabis (Parilisia) iranica (Alexander, 1973)
- 27. Hoplolabis (Parilisia) yezoana (Alexander, 1924)
- 28. Idiocera (Idiocera) laterospina (Alexander, 1975)
- 29. Idiocera (Idiocera) pulchripennis (Loew, 1856)
- 30. Ilisia maculata (Meigen, 1804)
- 31. Molophilus (Molophilus) lackschewitzianus hebetatus Savchenko, 1976
- 32. Molophilus (Molophilus) obscurus (Meigen, 1818)
- 33. *Molophilus (Molophilus) ochraceus (Meigen, 1818)
- 34. *Molophilus (Molophilus) pleuralis de Meijere, 1920
- 35. Molophilus (Molophilus) politonigrus Savchenko, 1983
- 36. *Molophilus (Molophilus) propinquus (Egger, 1863)
- 37. Molophilus (Molophilus) stroblianus decoloratus Savchenko, 1978
- 38. Molophilus (Molophilus) urodontus Savchenko, 1978
- 39. *Ormosia (Ormosia) cuspidata Savchenko, 1973
- 40. Ormosia (Ormosia) fascipennis (Zetterstedt, 1838)
- 41. *Ormosia (Ormosia) hederae (Curtis, 1835)
- 42. Ormosia (Ormosia) longispina Savchenko, 1983
- 43. Phyllolabis ghilarovi Savchenko, 1983
- 44. Rhabdomastix (Rhabdomastix) eugeni Stary, 2004
- 45. Rhabdomastix (Rhabdomastix) filata Stary, 2004
- 46. Symplecta (Psiloconopa) stictica (Meigen, 1818)
- 47. Symplecta (Symplecta) hybrida (Meigen, 1804)

Limoniidae: Limoniinae

- 48. Achyrolimonia decemmaculata (Loew, 1873)
- 49. Antocha (Antocha) libanotica Lackschewitz, 1940
- 50. *Antocha (Antocha) vitripennis (Meigen, 1830)
- 51. *Dicranomyia (Dicranomyia) circassica Lackschewitz, 1941
- 52. Dicranomyia (Dicranomyia) didyma (Meigen, 1804)
- 53. Dicranomyia (Dicranomyia) chorea (Meigen, 1818)
- 54. *Dicranomyia (Dicranomyia) longipennis (Schummel, 1829)
- 55. Dicranomyia (Dicranomyia) lucida de Meijere, 1918
- 56. *Dicranomyia (Dicranomyia) melanantha Savchenko, 1984
- 57. Dicranomyia (Dicranomyia) modesta (Meigen, 1818)
- 58. *Dicranomyia (Dicranomyia) pallidinota Starý, 2009
- 59. *Dicranomyia (Dicranomyia) pontica Lackschewitz, 1941
- 60. Dicranomyia (Glochina) transsilvanica Lackschewitz, 1928
- 61. Dicranomyia (Melanolimonia) caledonica Edwards, 1926
- 62. Dicranomyia (Melanolimonia) morio (Fabricius, 1787)
- 63. *Dicranomyia (Numantia) fusca (Meigen, 1804)
- 64. Dicranoptycha fuscescens (Schummel, 1829)
- 65. *Dicranoptycha livescens Loew, 1871
- 66. Dicranoptycha recurvispina Savchenko, 1974
- 67. Limonia caucasica Lackschewitz, 1940
- 68. Limonia eos Stary & Savchenko, 1976
- 69. Limonia flavipes (Fabricius, 1787)
- 70. *Limonia hercegovinae (Strobl, 1898)
- 71. *Limonia macrostigma (Schummel, 1829)
- 72. Limonia nubeculosa Meigen, 1804
- 73. *Limonia stigma (Meigen, 1818)
- 74. Limonia subaequalis Savchenko, 1979
- 75. Metalimnobia (Metalimnobia) quadrimaculata (Linnaeus, 1760)
- 76. *Metalimnobia (Metalimnobia) quadrinotata (Meigen, 1818)
- 77. *Rhipidia (Rhipidia) maculata Meigen, 1818

Pediciidae

- 78. *Dicranota (Dicranota) crassicauda Tjeder, 1972
- 79. Dicranota (Ludicia) iranensis (Alexander, 1975)
- 80. Dicranota (Paradicranota) landrocki Czižek, 1931
- 81. Dicranota (Paradicranota) subtilis Loew, 1871
- 82. Pedicia (Amalopis) occulta (Meigen, 1830)
- 83. Tricyphona (Tricyphona) immaculata (Meigen, 1804)

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DATA PAPER



A database on the distribution of butterflies (Lepidoptera) in northern Belgium (Flanders and the Brussels Capital Region)

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This paper describes version 1.3 of this resource: http://dataset.inbo.be/dagvlinders-inbo-occurrences&v=1.3

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Dataset/Occurrence. http://doi.org/10.15468/ezfbee Data paper: http://dx.doi.org/10.3897/zookeys.585.8019 This paper describes version 1.2 of this resource: http://dataset.inbo.be/dagvlinders-natuurpunt-occurrences&v=1.2

Abstract

In this data paper, we describe two datasets derived from two sources, which collectively represent the most complete overview of butterflies in Flanders and the Brussels Capital Region (northern Belgium). The first dataset (further referred to as the *INBO dataset* – http://doi.org/10.15468/njgbmh) contains

761,660 records of 70 species and is compiled by the Research Institute for Nature and Forest (INBO) in cooperation with the Butterfly working group of Natuurpunt (Vlinderwerkgroep). It is derived from the database Vlinderdatabank at the INBO, which consists of (historical) collection and literature data (1830-2001), for which all butterfly specimens in institutional and available personal collections were digitized and all entomological and other relevant publications were checked for butterfly distribution data. It also contains observations and monitoring data for the period 1991-2014. The latter type were collected by a (small) butterfly monitoring network where butterflies were recorded using a standardized protocol. The second dataset (further referred to as the *Natuurpunt dataset* – http://doi.org/10.15468/ ezfbee) contains 612,934 records of 63 species and is derived from the database http://waarnemingen.be, hosted at the nature conservations by volunteers (citizen scientists), mainly since 2008. Together, these datasets currently contain a total of 1,374,594 records, which are georeferenced using the centroid of their respective 5×5 km² Universal Transverse Mercator (UTM) grid cell. Both datasets are published as open data and are available through the Global Biodiversity Information Facility (GBIF).

Keywords

Butterflies, distribution, collection, literature, citizen science, observations, monitoring

Data published through

INBO dataset: http://doi.org/10.15468/njgbmh (http://www.gbif.org/dataset/7888f666-f59e-4534-8478-3a10a3bfee45) Natuurpunt dataset: http://doi.org/10.15468/ezfbee (http://www.gbif.org/dataset/1f968e89-ca96-4065-91a5-4858e736b5aa)

Rationale

Butterflies are among the best studied insects in the world and have always attracted the attention of both professional researchers, amateur naturalists, butterfly collectors, and the wider public (Kühn et al. 2008). Butterflies are widely considered as interesting study systems for ecology, evolution, behaviour, and conservation biology (e.g., Watt and Boggs 2003). Many butterflies have been collected and subsequently stored in museum or private collections. Furthermore, entomologists have often published lists of observed species during excursions to special habitats or have made overviews of regional or national butterfly faunas. In Belgium, entomology in general and lepidopterology in particular, have a long tradition with the first faunas already published only seven years after its independence in 1830 (De Selys-Longchamps 1837). Since then, several authors have updated the Belgian butterfly fauna based on collections or observations (e.g., Hackray et al. 1969; De Prins 1998). In 1991, the youth and nature organization Jeugdbond voor Natuur en Milieu (JNM) launched a butterfly project with the aim to publish a distribution atlas of the butterflies of Flanders, northern Belgium (Daniëls 1991). To do so, a first step consisted of collecting all historical collection and
literature data. Secondly, a working group was organised in cooperation between JNM, De Wielewaal (which later became Natuurpunt) and the INBO that set up a citizen science project to obtain as many butterfly observations with a good spatial coverage over Flanders. The data gathered during this project (period 1991-1998) were used to compile a first Red List (Maes and Van Dyck 2001) and a distribution atlas of butterflies in Flanders, including the Brussels Capital Region (Maes and Van Dyck 1999). Recently, both the Red List (Maes et al. 2012) and the distribution atlas (Maes et al. 2013) were updated using recent distribution data recorded through www.waarnemingen.be, a data portal launched by Natuurpunt, the largest nature conservation NGO in Belgium, where citizen-scientists can store and keep track of their recordings. Here, we publish both the historical and the more recent data used for the Red List and the distribution atlases as a data paper on a UTM grid cell resolution of 5×5 km².

Taxonomic coverage

The datasets cover all 67 indigenous and 3 regular migrant butterfly species (*Colias croceus, Colias hyale, Vanessa cardui*). In the INBO dataset vagrant or doubtful species (*Apatura ilia, Arethusana arethusa, Boloria dia, Brenthis ino, Coenonympha arcania, Colias alfacariensis, Colias palaeno, Cupido argiades, Danaus plexippus, Erebia aethiops, Erebia ligea, Erebia medusa, Hamearis lucina, Iphiclides podalirius, Lampides boeticus, Lasiommata maera, Limenitis populi, L. reducta, Lycaena dispar, Lycaena helle, Lycaena hippothoe, Lycaena virgaureae, Melitaea aurelia, Pontia daplidice)* and introduced species (*Cacyreus marshalli* and *Polyommatus damon*) were excluded because no evidence of the observation was available. In the Natuurpunt dataset, however, eight vagrant species with photographic evidence, that most likely spontaneously reached Flanders were included (*Apatura ilia, Brenthis ino, Cupido argiades, Iphiclides podalirius, Lampides boeticus, Nymphalis xanthomelas, Polyommatus coridon* and *Pontia daplidice*). Three additional species (*Aporia crataegi, Argynnis adippe* and *A. aglaja*) are considered as indigenous species, but recent observations are all vagrant individuals. Nomenclature is according to Fauna Europaea (http://www.faunaeur.org/full_results.php?id=7).

Taxonomic ranks

Kingdom: Animalia

Phylum: Arthropoda, Subphylum: Hexapoda, Class: Insecta, Order: Lepidoptera, Superfamilies: Hesperoidea, Papilioidea, Families: Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae, Pieridae, Subfamilies: Apaturinae, Coliadinae, Dismorphiinae, Heliconiinae, Heteropterinae, Hesperiinae, Limenitidinae, Lycaeninae, Melitaeinae, Nymphalinae, Papilioninae, Pierini, Polyommatinae, Pyrginae, Satyrinae, Theclinae, Genera: Aglais, Anthocharis, Apatura, Aphantopus, Aporia, Araschnia, Argynnis, Aricia, Boloria, Callophrys, Carcharodus, Carterocephalus, Celastrina, Coenonympha, Cupido, Cyaniris, Erynnis, Euphydryas, Favonius, Gonepteryx, Hesperia, Heteropterus, Hipparchia, Issoria, Lasiommata, Leptidea, Limenitis, Lycaena, Maniola, Melitaea, Melanargia, Nymphalis, Ochlodes, Papilio, Pararge, Phengaris, Pieris, Plebejus, Polygonia, Polyommatus, Pyrgus, Pyronia, Satyrium, Spialia, Thecla, Thymelicus, Vanessa

Species: Table 1 gives an overview of the species, together with the number of records present in the respective datasets.

Common names: Butterflies

Table 1. The number of records per species in the two datasets and the sum of the records in both datasets. v = observations with photographic evidence, but the species most probably do not have populations in Flanders. [†]indicates that a species is considered as extinct in Flanders; the year of extinction is also given. Observations after the year of extinction are considered as vagrant individuals. ^M: regular migrant species, ^(M): the species is indigenous, but the regional population is supplemented by migrant individuals.

Species name	INBO	Natuurpunt	Total	
Aglais io	54,329	52,471	106,800	
Aglais urticae	35,237	25,047	60,284	
Anthocharis cardamines	15,689	17,393	33,082	
Apatura ilia	-	4 ^v	4	
Apatura iris	141	304	445	
Aphantopus hyperantus	8,156	7,636	15,792	
Aporia crataegi ^{†1960}	120	2 ^v	122	
Araschnia levana	24,772	18,531	43,303	
Argynnis adippe ^{†1947}	22	3 ^v	25	
Argynnis aglaja ^{†1971}	54	1 ^v	55	
Argynnis niobe ^{†1977}	21	-	21	
Argynnis paphia	272	697	969	
Aricia agestis	6,867	5,251	12,118	
Boloria euphrosyne ^{†1949}	37	-	37	
Boloria selene ^{†1994}	181	-	181	
Brenthis ino	-	7°	7	
Callophrys rubi	2,008	1,552	3,560	
Carcharodus alceae	16	402	418	
Carterocephalus palaemon	1,159	2,478	3,637	
Celastrina argiolus	21,857	20,579	42,436	
Coenonympha hero ^{†1912}	16	-	16	
Coenonympha pamphilus	9,886	10,429	20,315	
Coenonympha tullia ^{†1994}	70	-	70	
Colias croceus ^M	3,380	12,762	16,142	
Colias hyale ^M	617	277	894	
Cupido argiades	-	1 ^v	1	
Cupido minimus	82	43	125	
Cyaniris semiargus	222	76	298	
Erynnis tages	102	130	232	
Euphydryas aurinia ^{†1959}	65	-	65	

Species name	INBO	Natuurpunt	Total
Favonius quercus	2,217	3,051	5,268
Gonepteryx rhamni	20,011	22,357	42,368
Hesperia comma	145	471	616
Heteropterus morpheus ^{†1995}	29	-	29
	4,157	5,160	9,317
Hipparchia statilinus ^{†1930}	11	-	11
Iphiclides podalirius	-	5 ^v	5
Issoria lathonia	2,794	3,216	6,010
Lampides boeticus	-	44 ^v	44
Lasiommata megera	4,089	1,882	5,971
Leptidea sinapis	144	585	729
Limenitis camilla	1,154	2,323	3,477
Limenitis populi ^{†1957}	14	-	14
Lycaena phlaeas	16,393	15,246	31,639
Lycaena tityrus	303	263	566
Maniola jurtina	35,117	31,782	66,899
Melanargia galathea	53	23	76
Melitaea athalia ^{†1968}	80	-	80
Melitaea cinxia	300	466	766
Melitaea diamina ^{†1954}	28	-	28
Nymphalis antiopa	240	63	303
Nymphalis polychloros	323	362	685
Nymphalis xanthomelas	-	5 ^v	5
Ochlodes sylvanus	11,484	15,660	27,144
Papilio machaon	10,322	8,927	19,249
Pararge aegeria	65,290	56,129	121,419
Phengaris alcon	441	342	783
Phengaris teleius ^{†1980}	136	-	136
Pieris brassicae	45,713	22,030	67,743
Pieris napi	54,313	28,294	82,607
Pieris rapae	94,957	52,188	147,145
Plebejus argus	1,436	1,711	3,147
Plebejus idas ^{†1984}	15	-	15
Polygonia c-album	33,660	36,058	69,718
Polyommatus coridon	-	12 ^v	12
Polyommatus icarus	20,269	21,186	41,455
Pontia daplidice	-	3 ^v	3
Pyrgus armoricanus ^{†1952}	18	-	18
Pyrgus malvae	589	527	1116
Pyronia tithonus	31,771	21,184	52,955
Satyrium ilicis	397	617	1,014
Satyrium w-album	97	504	601
Spialia sertorius ^{†1937}	8	-	8
Thecla betulae	835	2,191	3,026
Thymelicus lineola	17,087	5,029	22,116

Species name	INBO	Natuurpunt	Total
Thymelicus sylvestris	1,012	387	1,399
Vanessa atalanta ^(M)	69,965	55,306	125,271
Vanessa cardui ^M	28,865	21,269	50,134
Total	761,660	612,934	1,374,594
N species	70	63	78
Number of grid cells surveyed	631	634	637
Number of different observers	1,697	3,856	



Figure 1. The location of Belgium in Europe (left) and the three administrative regions of Belgium (right): Flanders (yellow), the Brussels Capital Region (black) and Wallonia (red).

Geographic coverage

Flanders and the Brussels Capital Region

Flanders and the Brussels Capital Region cover an area of 13,522 km² and 162 km² respectively (13,684 km² in total – Figure 1). This area is situated in the northern of Belgium and represents 45% of the Belgian territory. Flanders is largely covered by agricultural land and urban areas while the Brussels Capital Region is mainly urban (Table 2). Both regions have a very high population density (Table 2).

Bounding box

50°40'48"N to 51°30'36"N latitude, 2°32'24"E to 5°55'12"E longitude

Georeferencing method

All distribution data of butterflies in Flanders and the Brussels Capital Region were attributed to grid cells of 5×5 km² of the Universal Transverse Mercator (UTM) projection (Figure 2). The centroids of the 5×5 km² grid cells were calculated using the

Land use type	Flanders	Land use type	Brussels Capital Region
Agricultural land	702 276 (51%)	Urban areas	11 917 (73%)
Urban areas	411 144 (30%)	Woodlands	1988 (12%)
Woodlands	138 595 (10%)	Other green areas	1568 (10%)
Other green areas	39 516 (3%)	Agricultural land	544 (3%)
Water	32 008 (2%)	Water	185 (1%)
Semi-natural grasslands	15 315 (1%)	Semi-natural grasslands	27 (<1%)
Heathlands	8140 (<1%)	Marshes	17 (<1%)
Coastal dunes	1818 (<1%)	Heathlands	3 (<1%)
Marshes	1742 (<1%)		
Mud flats and salt marshes	1497 (<1%)		
Population density	474/km ²		7210/km ²

Table 2. Area of the main land use types in Flanders and the Brussels Capital Region in ha (ranked in descending order of percentage in both regions). Source: Biological Valuation Map Flanders and the Brussels Capital Region (Vriens et al. 2011).



Figure 2. $10 \times 10 \text{ km}^2$ UTM grid cells in Flanders and in the Brussels Capital Region. The partitioning of $10 \times 10 \text{ km}^2$ UTM grid cells (left) into $5 \times 5 \text{ km}^2$ UTM grid cells is shown on the right. The $5 \times 5 \text{ km}^2$ UTM grid cells were used to georeference the distribution data in Flanders and the Brussels Capital Region.

WGS84 projection with a *coordinateUncertaintyInMeters* of 3,769 meters (Wieczorek et al. 2004).

In total, Flanders and the Brussels Capital Region cover 638 (622 with records) and 9 (all nine with records) grid cells, respectively. The grid cells without records only cover a very small area within Flanders.

Temporal coverage

The INBO dataset mainly covers the historical museum and literature records (since 1830), butterfly monitoring records (since 1991) and observations (until 2008) while the Natuurpunt dataset covers the recent observations (mostly since 2008). Between



Figure 3. Number of collected records between 1830 and 1985 (left) and between 1986 and 2014 (right) in the two datasets (INBO and Natuurpunt). Each number on the x-axis stands for a period of 5 years (e.g., 1905 = 1901–1905, 1910 = 1906–1910, etc.). Note the different scales on the y-axis for both figures.

2000 and 2006, a butterfly survey project was organised in the province of West-Flanders (Cuvelier et al. 2007) and in the period 2006-2008, a similar project was undertaken in the Brussels Capital Region by the INBO on demand of Leefmilieu Brussel – BIM (Beckers et al. 2009). Both datasources were integrated in the INBO dataset. Since the introduction of the data portal www.waarnemingen.be for storing observations by the NGO Natuurpunt in 2008, the number of records has strongly increased and now reaches almost 150,000 records per year (Figure 3). The datasets will be updated on a yearly basis.

Methodology

Sampling methods

Butterfly distribution data were collected in four different ways: i) collection data, ii) literature data, iii) monitoring transect data and iv) observations.

Collection data were digitized from the following museum collections: Bosmuseum Groenendaal, Royal Institute for Natural Sciences (Brussels), Agricultural Faculty of Gembloux, Ghent university and the Antwerp Zoo. Furthermore, the private butterfly collections of the following people were also incorporated into the INBO dataset: A. Artoisenet, R. Bracke, A. Caljon, S. Cuvelier, A. De Boer, K. Desender, P. Halflants, D. Hilven, J. & T. Jaeken, M. Keirens, H. Kinders, P. & W. Pardon, W. Tips, W. Troukens, F. Turelinckx, O. Van De Kerckhove, R. Van Heuverswijn, B. Vandepitte, J. Vervaeke & R. Winnen. The source collection is indicated in the field *associatedReferences*.

Published observations were searched for in different literature sources (see section "References to literature checked for occurrence data" in the Suppl. material 1) and indicated in the field *associatedReferences*. Since most of the records in collections and in the literature were only reported at the municipality level, the UTM 5×5 km² grid cell of the centre of the municipality was attributed to the record.

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Figure 4. Frequency distribution of the observers per number of records in the datasets of INBO and Natuurpunt.

Butterfly monitoring counts were conducted along fixed transects of maximum 1 km, consisting of smaller sections, each with a homogeneous habitat (e.g., woodland, hay meadow, dry heathland – see van Swaay et al. 2008; van Swaay et al. 2011 for a detailed description of the monitoring method).

Observations (species, date, location, observer) were recorded by volunteers/citizen scientists and stored in the INBO dataset (mainly for the period 1991-2007, usually with a resolution of $1 \times 1 \text{ km}^2$ or $5 \times 5 \text{ km}^2$) or in the Natuurpunt dataset. Since 2011, 69% of the records had a precision of 25m or less. Because of the increasing popularity of mobile apps using GPS readings in the field, this proportion increased with 5% per year to reach 77% in 2015. The number of observers in the INBO and the Natuurpunt datasets is given in Table 1. The frequency distribution of the recorders per number of records is given in Figure 4.

A list of references that used data described in this paper can be found in the section "Publications based on this dataset" in the Suppl. material 1.

Quality control

The data in both datasets were carefully verified by butterfly experts (including professional entomologists) taking collection specimens, the observer's species knowledge, added photographs and known species list of locations into account. The validation procedure from www.waarnemingen.be consists of an interactive procedure in which observers can be asked for additional information by a team of validators, after which the validator manually adds a validation status. Records that are not manually validated are additionally checked by an automated validation procedure that takes into account the number of manually validated observations within a specified date and distance range. 11% of the butterfly records submitted to the data portal www.waarnemingen.be are supported by photographs. The validation status is indicated in the field *identificationVerificationStatus*.

Information withheld

In the original databases, the observer's name, the exact XY-coordinates and the toponym are known.

Datasets

Dataset description

The butterfly occurrence data are published as two separate Darwin Core Archives: 1) collection and literature data, observations and butterfly monitoring in Flanders and in the Brussels Capital Region (1830-2014) hosted at the Research Institute for Nature and Forest (INBO) and 2) recent observations (1974-2014) from the Natuurpunt data portal (www.waarnemingen.be). The data models used for both datasets are identical and can be merged easily. The INBO dataset contains 761,660 records and the Natuurpunt dataset 612,934 records totalling to almost 1.4 million records. The data compiled for the butterfly atlas of the Brussels Capital Region are marked as INBO/LB-BIM in the *ownerInstitutionCode* field in the INBO dataset.

The distribution of the number of records and species per grid cell for both datasets is given in Figure 5.

The data are standardized to Darwin Core (Wieczorek et al. 2012) with a custom SQL view on the original INBO and Natuurpunt butterfly database respectively. They were published using the GBIF Integrated Publishing Toolkit (Robertson et al. 2014) instance at the INBO (http://data.inbo.be/ipt). The Darwin Core terms (http:// rs.tdwg.org/dwc/terms/) in the dataset at the time of publication are:

occurrenceID, type, language, license, rightsHolder, accessRights, references, datasetID, institutionCode, datasetName, ownerInstitutionCode, basisOfRecord, informationWithheld, dataGeneralizations, recordedBy, individualCount, sex, lifestage, associatedReferences, samplingProtocol, samplingEffort, eventDate, verbatimEventDate, continent, countryCode, stateProvince, municipality, verbatimLocality, verbatimCoordinates, verbatim-CoordinateSystem, verbatimSRS, decimalLatitude, decimalLongitude, geodeticDatum, coordinateUncertaintyInMeters, georeferenceRemarks, identificationVerificationStatus, scientificName, kingdom, phylum, class, order, taxonRank, scientificNameAuthorship, vernacularName, nomenclaturalCode.



Figure 5. Number of records (left, increasing dot sizes represent 100, 1000, 2500, 5000 and >5000 records per grid cell) and species (right, increasing dot sizes represent 10, 20, 30, 40 and >40 species per grid cell) in the INBO dataset (1830–2014, top row) and in the NP dataset (1981–2014, bottom row). Squares indicate grid cells without records.

INBO dataset

- Object name: Vlinderdatabank Butterflies in Flanders and the Brussels Capital Region, Belgium
- Format name: Darwin Core Archive format
- Format version: 1.0
- Character encoding: UTF-8
- Language: English
- License: http://creativecommons.org/publicdomain/zero/1.0/
- Usage norms: http://www.inbo.be/en/norms-for-data-use
- Publication date: 2016-01-13
- Distribution: http://dataset.inbo.be/dagvlinders-inbo-occurrences
- DOI: http://doi.org/10.15468/njgbmh

Natuurpunt dataset

- **Object name:** Waarnemingen.be Butterfly observations in Flanders and the Brussels Capital Region, Belgium
- Format name: Darwin Core Archive format
- Format version: 1.0
- Character encoding: UTF-8
- Language: English
- License: http://creativecommons.org/publicdomain/zero/1.0/
- Usage norms: http://www.natuurpunt.be/normen-voor-datagebruik

- Publication date: 2016-02-02
- Distribution: http://dataset.inbo.be/dagvlinders-natuurpunt-occurrences
- DOI: http://doi.org/10.15468/ezfbee

Usage norms

To allow anyone to use the datasets described here, we released the data to the public domain under a Creative Commons Zero waiver (http://creativecommons.org/public-domain/zero/1.0/). Users of published datasets are encouraged to follow the respective norms for data use (http://www.inbo.be/en/norms-for-data-use and http://www.natu-urpunt.be/normen-voor-datagebruik [in Dutch]) and to provide a link to the original dataset (http://doi.org/10.15468/njgbmh and http://doi.org/10.15468/ezfbee), whenever appropriate. If used for a scientific paper, it is recommended to cite the dataset following the applicable citation norms (e.g. GBIF 2012) and/or to contact the authors for additional information (dirk.maes@inbo.be, marc.herremans@natuurpunt.be or dimitri.brosens@inbo.be). Dataset issues can also be reported via opendata@inbo.be.

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Supplementary material I

A database on the distribution of butterflies (Lepidoptera) in northern Belgium (Flanders and the Brussels Capital Region)

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Data type: Additional references

- Explanation note: The supplementery material provides 1) a list of published entomological references that were checked for occurrence data and 2) a list of references that used data described in this paper.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.