

# ***Olindias deigo* sp. nov., a new species (Hydrozoa, Trachylinae, Limnomedusae) from the Ryukyu Archipelago, southern Japan**

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## **Abstract**

A new hydromedusa belonging to the order Limnomedusae is reported from the Ryukyu Archipelago, southern Japan. *Olindias deigo* sp. nov. can be distinguished from other Olindiidae species by the number and color of tentacles. Mature medusae of *O. deigo* sp. nov. were collected to observe the life history, including polyp (hydroid) and medusa formation. A comparative table of the primary diagnostic characters of the genus is provided.

## **Keywords**

Development, flower hat jellyfish, hydroid, medusa, Okinawa, polyp

## **Introduction**

The order Limnomedusae comprises about 60 species in five families: Armorhadridae Swedmark & Teissier, 1958; Geryoniidae Eschscholtz, 1829; Microhydrulidae Bouillon & Deroux, 1967; Monobrachiidae Mereschowsky, 1877; and Olindiidae Haeckel, 1879 (Bouillon et al. 2006; Bentlage et al. 2018). Olindiidae is the largest family which includes 16 genera and 49 species (Bentlage et al. 2018). The species of Olindiidae have

been reported from the Pacific and the Atlantic in tropical, subtropical, mild, and cold localities (Mayer 1910; Kramp 1961). Most species inhabit salt waters; however, some species have been found in fresh and brackish waters (Oka and Hara 1922; Jankowski 2001; Toyokawa and Fujii 2015). Olindiidae species have a planktonic sexual medusa and a benthic asexual polyp in their life cycles (Kakinuma 1971; Nagao 1973; Toshino 2017; Kayashima et al. 2019).

Species of the genus *Olindias* Müller, 1861 are large hydrozoans with umbrella diameters reaching 10 cm (Kramp 1961). *Olindias formosus* (Goto, 1903) is a very beautiful jellyfish called the “flower hat jellyfish” and is popular for exhibition in public aquaria worldwide (Yasuda 2003; Patry et al. 2014). Venomous stings by another species, *Olindias sambaquiensis* Müller, 1861, have been reported around South American seashores, and it is regarded as a venomous jellyfish (Mianzan and Ramírez 1996; Resgalla et al. 2011). To date, a single incidence of lethal envenomation has been documented for *O. formosus* in Japan (Yasuda 1988; Purcell et al. 2007), which occurs near seashore.

Recently, ten specimens of *Olindias* were collected from Okinawa Island, southern Japan. In this study, morphology and molecular phylogenetic analyses revealed that the specimens represent a new species of *Olindias*. Furthermore, we observed and documented the life history of this new species of *Olindias*.

## Material and methods

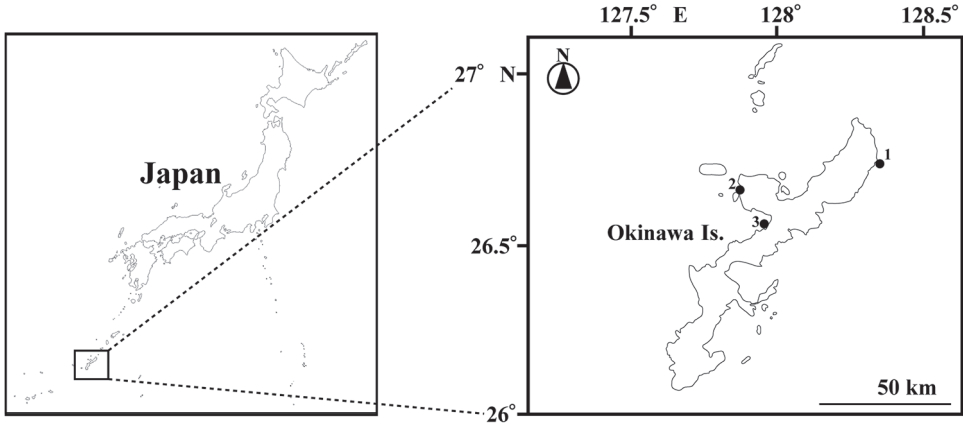
### Collection and fixing

Ten medusae were collected from Kunigami, Motobu, and Nago, Okinawa Prefecture, Ryukyu Archipelago, southern Japan between March 29, 2015 and April 8, 2018 (Fig. 1). The medusae were collected using a dipper net (diameter 20 cm) and plastic bags while scuba diving, or a set net. Additionally, specimens of *O. formosus* collected from Iwate, Oita and Miyazaki prefectures were used for comparison of morphology and for molecular phylogenetic analyses (Table 1). After preserving a subsample in ethanol (for molecular analysis), collected medusae were fixed in 5% formalin seawater and deposited in the National Museum of Nature and Science, Tsukuba, Japan (NSMT). Part of the tentacles were preserved in 99.5% ethanol for DNA extraction.

### Morphological investigations

Morphological observations and measurements were made on living or preserved specimens. Measurements were made with digital calipers (CD-20CPX, Mitutoyo Corporation, Japan) to the nearest 0.01 mm. For nematocyst identification in the medusae, squashes prepared from fresh tissues were examined under a compound microscope (BX53, OLYMPUS, Japan). In this study, the following morphological characters were recorded (Fig. 2): umbrella height, umbrella diameter, number of centripetal canals,

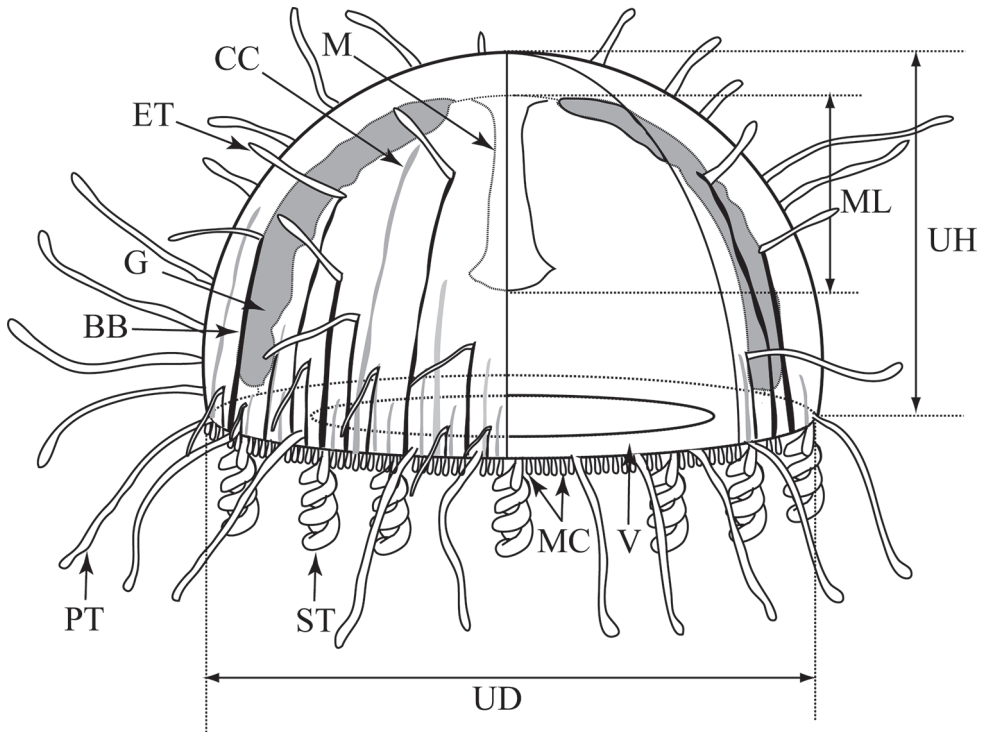




**Figure 1.** Map of the sampling sites **1** Off Ada, Kunigami **2** Off Motobu, Motobu **3** Off Kyoda, Nago.

**Table 1.** Taxa included in the phylogenetic analyses and accession numbers for sequences. Sequences obtained in this study are in bold. a Collins et al. (2008); b Collins et al. (2005); c He et al. unpublished; d Goto et al. unpublished; e Patry et al. unpublished; f Bentlage et al. (2018).

Species	Accession No.	Locality (Origin)	Reference
<i>Aglauroopsis aeora</i>	EU293973	Unknown	a
<i>Astrohydra japonica</i>	EU293975	Universität Hamburg, Germany	a
<i>Craspedacusta sinensis</i>	AY512507	China	b
<i>Craspedacusta sowerbyi</i>	EU293971	Unknown	a
<i>Craspedacusta ziguiensis</i>	EU293974	Unknown	a
<i>Gonionemus</i> sp.	KF962480	Unknown	c
<i>Gonionemus vertens</i>	EU293976	Friday Harbor, WA, USA	a
<i>Limnognathia tanganyicae</i>	EU293972	Unknown	a
<i>Maeotias marginata</i>	AY512508	Suisun Bay, CA, USA	a
<i>Monobrachium parasiticum</i>	EU293970	Unknown	a
<i>Scolionema suvaense</i>	AB720909	Unknown	d
<b><i>Olindias deigo</i></b>	<b>LC508961</b>	Ada, Kunigami, Okinawa, Japan	This study
<b><i>Olindias deigo</i></b>	<b>LC508962</b>	Ada, Kunigami, Okinawa, Japan	This study
<b><i>Olindias deigo</i></b>	<b>LC508963</b>	Motobu, Okinawa, Japan	This study
<b><i>Olindias deigo</i></b>	<b>LC508964</b>	Kyoda, Nago, Okinawa, Japan	This study
<b><i>Olindias formosus</i></b>	<b>LC508965</b>	Nagoya, Saiki, Oita, Japan	This study
<b><i>Olindias formosus</i></b>	<b>LC508966</b>	Nobeoka, Miyazaki, Japan	This study
<b><i>Olindias formosus</i></b>	<b>LC508967</b>	Nobeoka, Miyazaki, Japan	This study
<b><i>Olindias formosus</i></b>	<b>LC508968</b>	Nobeoka, Miyazaki, Japan	This study
<b><i>Olindias formosus</i></b>	<b>LC508969</b>	Nobeoka, Miyazaki, Japan	This study
<b><i>Olindias formosus</i></b>	<b>LC508970</b>	Ryori Bay, Ofunato, Iwate, Japan	This study
<i>Olindias formosus</i>	KF184031	Unknown	e
<i>Olindias mulleri</i> (identified as <i>O. phosphorica</i> )	AY512509	Mallorca	b
<i>Olindias sambauquiensis</i>	EU293978	Unknown	a
<i>Olindias sambaquiensis</i>	EU293977	Brazil	a
<i>Olindias tenuis</i>	MG979369	Atrantic	f



**Figure 2.** Key characters for identification and measurement of parts of the *Olindias*. BB = Black band; CC = centripetal canal; ET = exumbrella tentacle; G = gonad; M = manubrium; MC = marginal club; ML = manubrium length; PT = primary tentacle; ST = secondary tentacle; UH = umbrella height; UD = umbrella diameter; V = velum.

primary tentacles, secondary tentacles, marginal clubs, and exumbrella tentacles. Goto (1903) distinguished exumbrella tentacles, those arising from the exumbrella at any level, from those occurring proximal to the apex – just a short distance from the velum; however, he did not distinguish exumbrella from primary tentacles. In this study, the exumbrella tentacles are defined as tentacles that arise from the black band on the exumbrella, rather than those arising from the margin of the umbrella.

Nematocysts were identified according to Östman (2000) from wild and cultured specimens. Measurements were made using ImageJ (NIH, USA) to the nearest 0.1  $\mu\text{m}$ .

### Molecular phylogenetic analyses

Near complete sequences of the nuclear 16S rDNA genes (approximately 600 bp) were used for molecular phylogenetic analyses. Genomic DNA was extracted from the 99.5% ethanol preserved tissue of specimens using the DNeasy Blood and Tissue Kit (QIAGEN, Germany) following the manufacturer's protocol. 16S rDNA was PCR amplified and sequenced using primers and protocols outlined in Collins et al. (2008). Unidentified and deposited olindiid sequences in GenBank (Table 1) were used for

molecular comparison and to clarify the unidentified *Olindias* species. The generated sequences were aligned using MEGA 6.06 with built in ClustalW (Tamura et al. 2013). Phylogenetic analysis and pairwise distance measurements were determined using the maximum likelihood method with 1000 bootstrap replications in MEGA 6.06 (Tamura et al. 2013). All sequences have been deposited in DNA Data Bank of Japan (DDBJ) under accession numbers LC508961–LC508970 for the new species (Table 1).

### Observation of life cycle

Collected male and female medusae were transferred to an aquarium tank (18 × 32 × 22 cm, volume 13 L) to obtain fertilized eggs. Spawning was induced by alternation of light and dark conditions using an LED lamp (8 W) with a blue filter. The medusae were incubated in light between 20:30 and 7:00 and in dark between 7:00 and 20:30. Obtained fertilized eggs were kept in Petri-dishes (diameter 8 cm, height 4 cm) with filtered seawater (5 µm) at about 20 °C in the laboratory at Okinawa Churaumi Aquarium. *Artemia* nauplii were fed to primary and secondary polyps twice to thrice a week. Full water changes were conducted with filtered seawater (5 µm) about three hours after feeding. Newly detached medusae were kept in Petri-dishes (diameter 8 cm, height 4 cm) with filtered seawater (5 µm) at about 20 °C. *Artemia* nauplii were fed to the young medusae daily. The medusae that grew to about 4 cm of umbrella diameter were transferred into a tank (38 × 48 × 58 cm, volume 96 L). Juvenile anchovies and krill were fed to the medusae daily. Culture water was replaced with filtered seawater (5 µm) about three hours after feeding.

### Results

**Phylum Cnidaria** Verrill, 1865

**Subphylum Medusozoa** Peterson, 1979

**Class Hydrozoa** Owen, 1843

**Subclass Trachylinae** Haeckel, 1879

**Order Limnomedusae** Kramp, 1938

**Family Olindiidae** Haeckel, 1879

**Genus *Olindias*** Müller, 1861

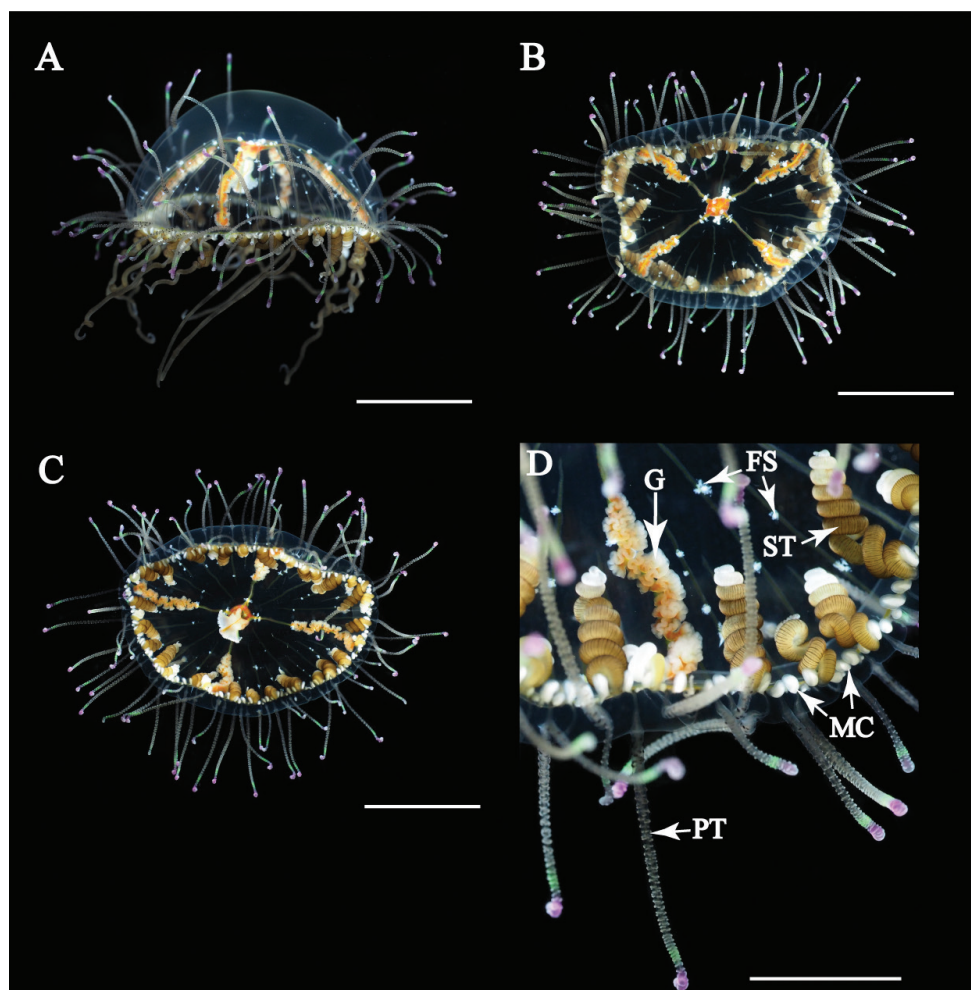
***Olindias deigo* sp. nov.**

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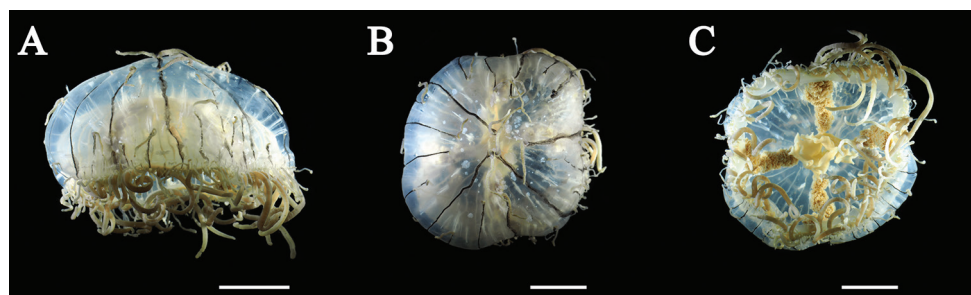
Figs 3–10

**New Japanese name.** Deigo-hanagasa-kurage.

**Material examined. Holotype:** NSMT-Co1690. Ada, Kunigami, Okinawa Prefecture, Ryukyu Archipelago, southern Japan; 26°43'29.0"N, 128°19'7.0"E; March 11, 2018; collector: Shuhei Odoriba. **Paratypes:** NSMT-Co1691. Same locality as holotype, March 16, 2018, collector: Shuhei Odoriba. NSMT-Co1692. Motobu, Okinawa

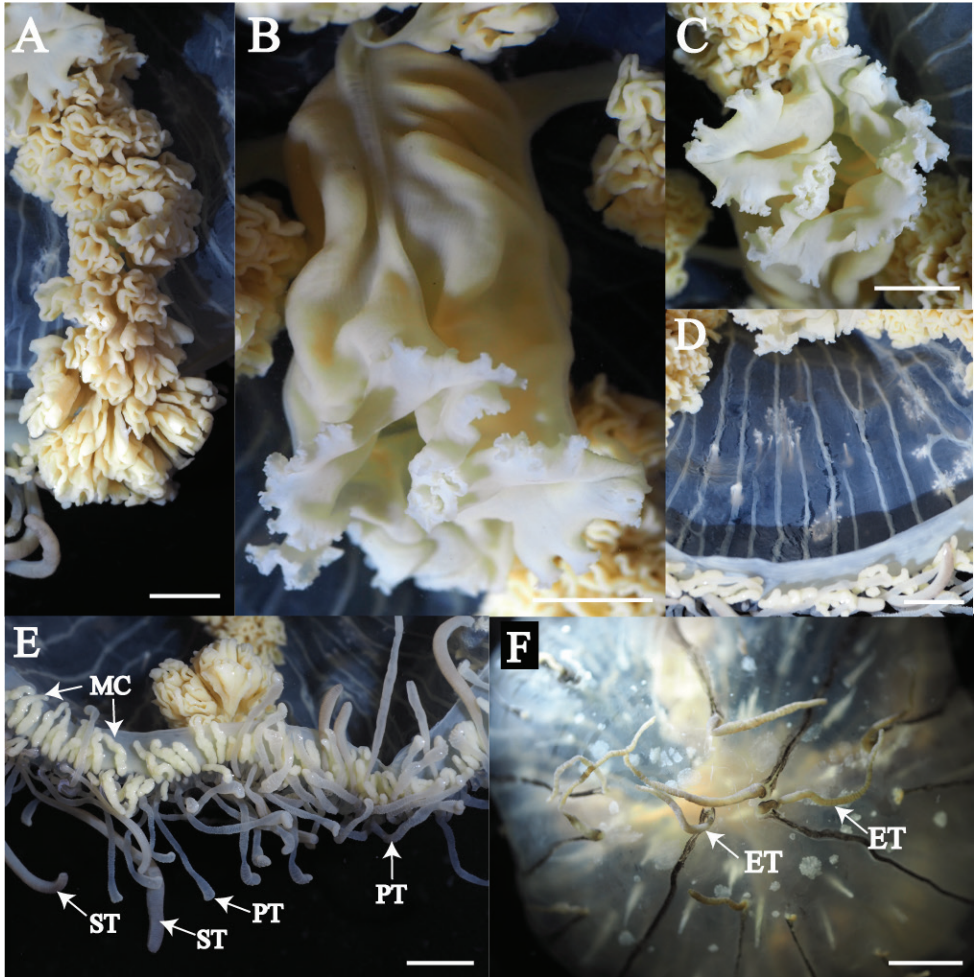


**Figure 3.** *Olindias deigo* sp. nov., live **A** lateral view **B** apical view **C** oral view **D** umbrella margin. FS = fibrous structure; G = gonad; MC = marginal club; PT = primary tentacle; ST = secondary tentacle. Scale bars: 2 cm (**A–C**), 1 cm (**D**).



**Figure 4.** *Olindias deigo* sp. nov., holotype **A** lateral view **B** apical view **C** oral view. All scale bars represent 2 cm.





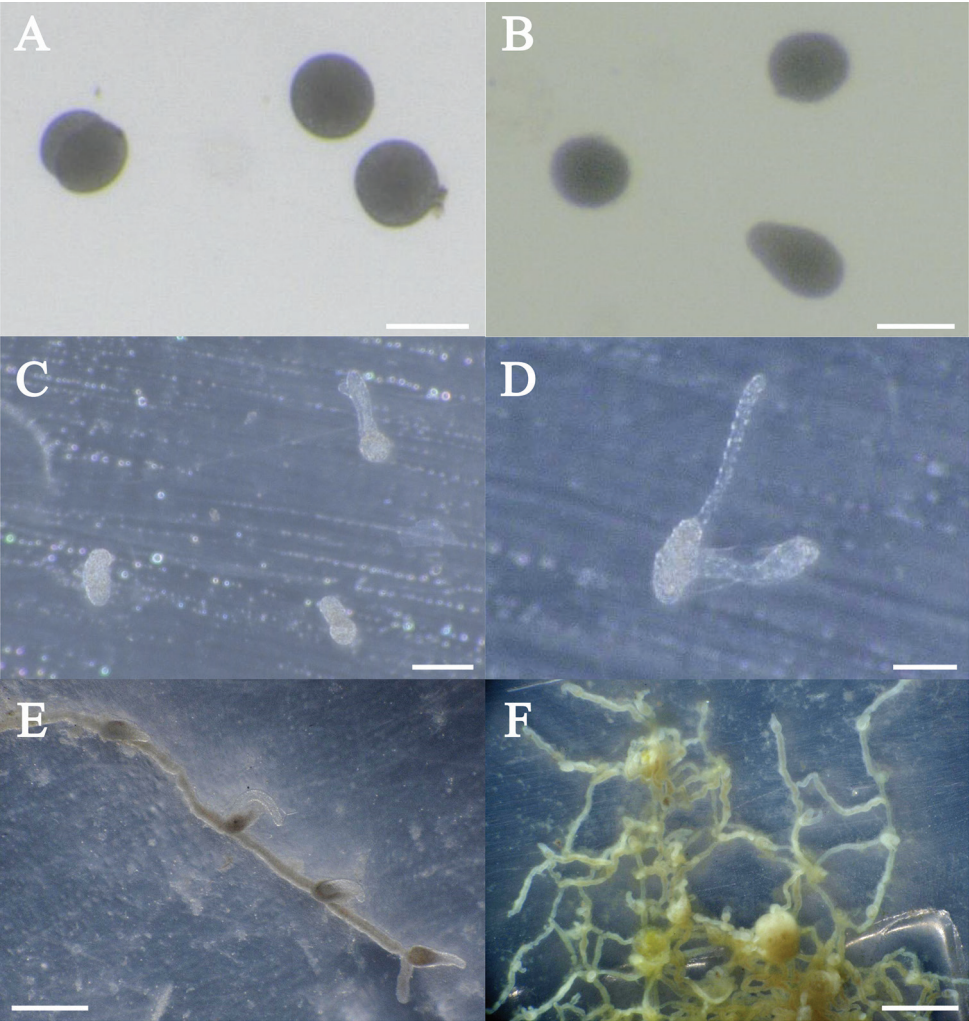
**Figure 5.** *Olindias deigo* sp. nov., holotype **A** gonad **B** manubrium **C** mouth rips **D** centripetal canals **E** umbrella margin **F** exumbrella. ET = exumbrella tentacle; MC = marginal club; PT = primary tentacle; ST = secondary tentacle. Scale bars: 0.5 cm (**A–E**), 1 cm (**F**).

Prefecture, Ryukyu Archipelago, southern Japan; 26°40'18.0"N, 127°52'49.0"E; April 19, 2015; collector: Shinichi Arakawa.

**Description.** Mature medusae with transparent, dome-like exumbrella (Figs 3A, 4A). Umbrella height about 40 mm and umbrella diameter about 80 mm (Table 2). Exumbrella smooth, lacking nematocyst warts (Fig. 3B). Four radial canals elongate from four corners of stomach (Figs 3B, C, 4B). Folded gonads attached along entire length of four radial canals (Fig. 5A). Immature gonads light red to orange (Figs 3D, 4C) while mature gonads are milky-white in color. Manubrium long, length about 50% of umbrella height, with quadrate base, light red to orange in color, folded (Fig. 5B, C). Mouth quadrate to rhomboid (Fig. 5C). Oral rips complexly folded (Fig. 5C). White

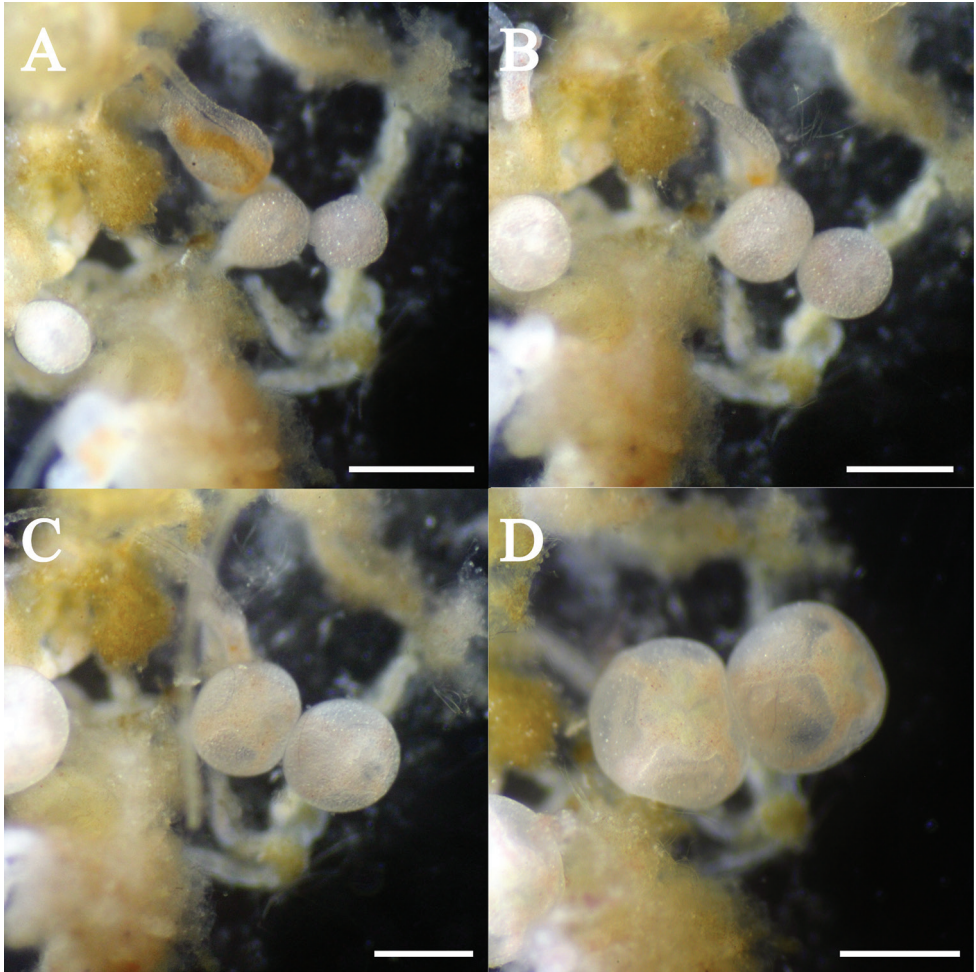
**Table 2.** Size (mm) of *Olindias deigo* sp. nov. \*: the holotype. Nos. Co1691-1692 are paratypes. \*\*: damaged. CC = centripetal canal; ET = exumbrella tentacle; PT = primary tentacle; MC = Marginal club; ST = secondary tentacle; UD = umbrella width; UH = umbrella height.

Specimen No.	UH (mm)	UD (mm)	No. of ET	No. of CC	No. of PT	No. of ST	No. of MC	Sampling site	Date	Lat./ long.
NSMT-Co1690*	39.5	67.1	33	83	112	51	238	Ada, Kunigami, Okinawa	11/03/2018	26°43'29.0"N, 128°19'7.0"E
NSMT-Co1691	44.7	83.7	66	104	141	(29)**	242	Ada, Kunigami, Okinawa	16/03/2018	26°43'29.0"N, 128°19'7.0"E
NSMT-Co1692	29.9	61.8	30	86	78	49	168	Motobu, Okinawa	19/04/2015	26°40'18.0"N, 127°52'49.0"E



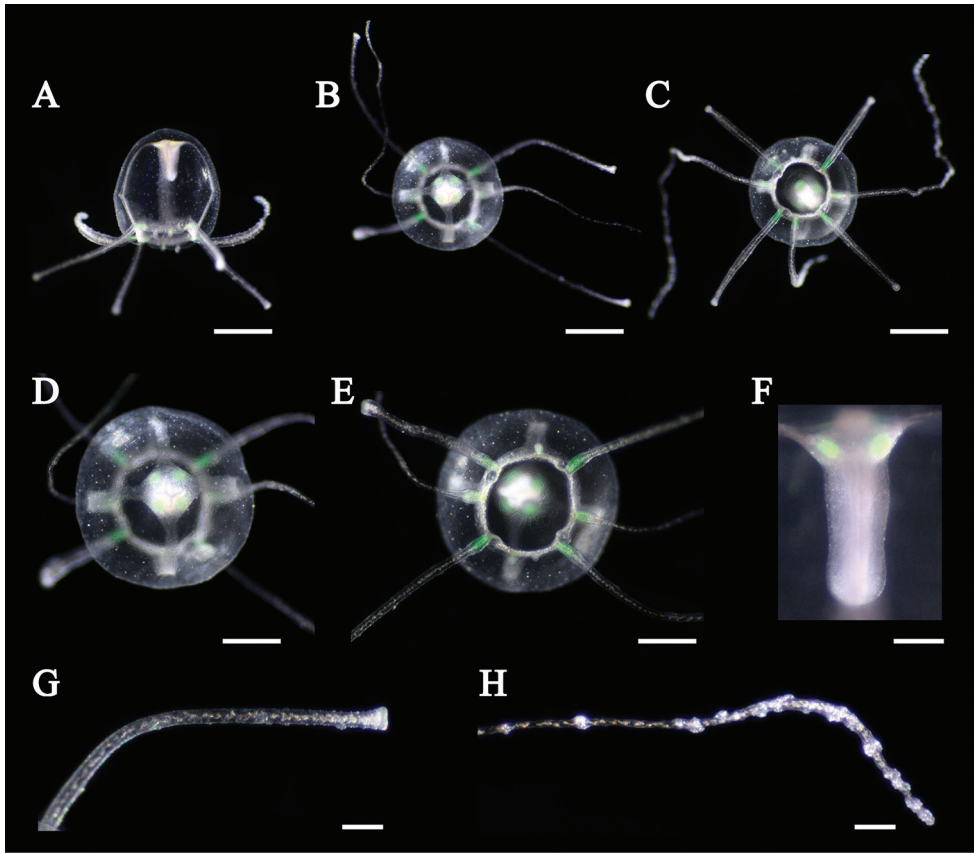
**Figure 6.** Early embryogenesis and polyps of *Olindias deigo* sp. nov. **A** fertilized eggs **B** planulae **C–D** primary polyps **E–F** mature polyps. Scale bars: 0.1 mm (**A–B**), 0.2 mm (**C–D**), 1 mm (**E–F**).





**Figure 7.** The process of medusa budding in the hydroid of *Olindias deigo* sp. nov. All scale bars represent 1 mm.

fibrous structures scattered in mesoglea of exumbrella (Fig. 5D). Different length of black bands elongated from umbrella margin to the apex of exumbrella (Fig. 5F). Centripetal canals about 80 to 100, long and short alternately aligned (Fig. 5D). Long canals reached apex of the umbrella while short ones were half or quarter length that of long canals terminating in tentacles. Some canals connected or branched (Fig. 5D). Tentacles and marginal clubs aligned on the umbrella margin (Figs 3D, 5E). Primary tentacles about 80 to 140, thin, short with distal adhesive pads and cnidocysts in transverse clasps. Color of exumbrella tentacles and primary tentacles pale black with purple and glowing green tips and with black base (Fig. 3D). Number of secondary tentacles about 50, thick, no adhesive pads, cnidocysts in rings, deep-brown in color (Fig. 3D). Contracted secondary tentacle short, coil-like while elongate ones reaching 2 m in length. Exumbrella tentacles about 30 to 60, developing on tip of black bands (Fig. 5F). Shape and color similar to those of primary tentacles (Fig. 3D). Number of



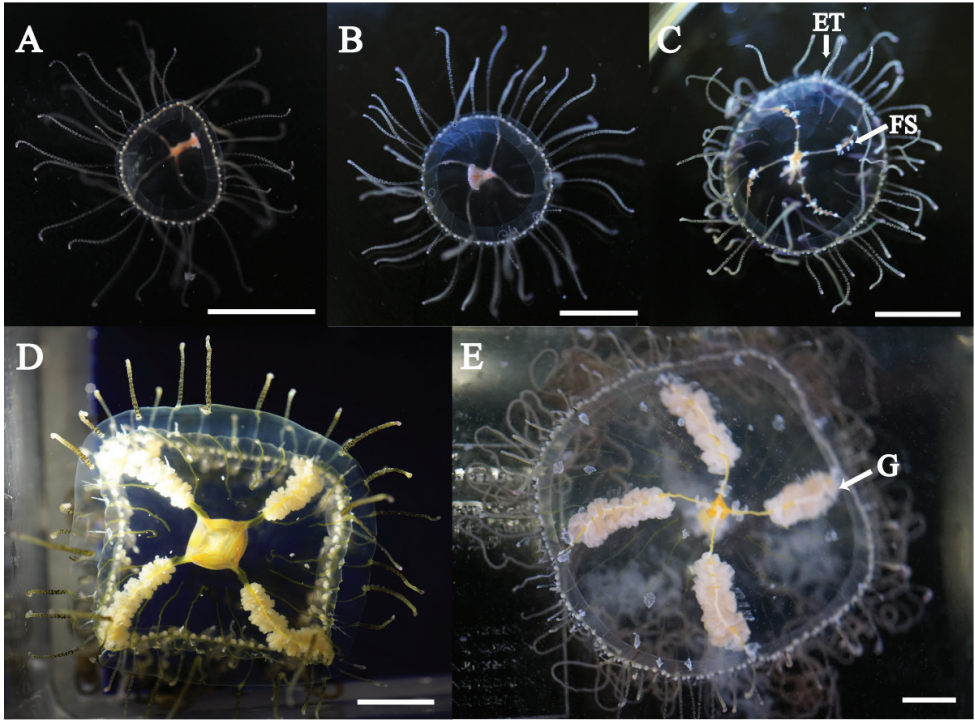
**Figure 8.** Newly detached medusa of *Olindias deigo* sp. nov. **A** lateral view **B, D** apical view **C, E** oral view **F** manubrium **G** primary tentacle **H** secondary tentacle. Scale bars: 1 mm (**A–C**), 0.5 mm (**D, E**), 0.1 mm (**F–H**).

marginal clubs about 170 to 240, rounded, short, whitish in color (Fig. 3D). Statocysts were not found in fixed mature medusae.

**Life cycle. Fertilization and polyp formation.** Spawning occurred in dark conditions. Thousands of fertilized eggs were collected from the bottom of the tank in the early morning (from 8 to 9 am); diameter of blastocysts ~100  $\mu$ m (Fig. 6A). Blastocysts developed into planulae within two days. Planulae had a pear-shaped body, 70  $\mu$ m in diameter and 130  $\mu$ m in length (Fig. 6B); they developed into polyps within 20 days.

The polyps form small colonies by elongation of the stolon, developing into a network (Fig. 6C–F). The hydrorhizae were cylindrical with small egg-shaped or cylindrical hydranths developing on the stolon. The hydranths had an ovoid body, 0.7 mm in length (Fig. 6E). The body was divided in two parts, gastric region (0.3 mm in diameter and 0.5 mm in length) and hypostome (0.2 mm in diameter and 0.2 mm in length). Tentacle single, filiform, 1.7 mm in length (Fig. 6E, F). The hydroid, usually brownish or yellowish, became orange or pink owing to the consumption of *Artemia* nauplii. Tentacle and hypostome transparent.





**Figure 9.** The process of young medusa development in *Olindias deigo* sp. nov. ET = exumbrella tentacle; FS = fibrous structure; G = gonad. All scale bars represent 1 cm.

**Budding and development of young medusa.** Budding of young medusae was observed after 8 months of polyp formation. Medusa bud formation occurred on stolon (Fig. 7A) at temperatures below 20 °C. The shape of the buds was ovoid and 0.3 mm in diameter (Fig. 7A). Two days after onset of budding, four radial canals and a circular canal appeared, but were obscure (Fig. 7B). Eight days after onset of budding, rudiments of tentacles developed from the bud (Fig. 7C). Fourteen days after onset of budding, the buds enlarged (0.8 mm in diameter) and green fluorescence was observed on the tentacles (Fig. 7D). Fifteen days after onset of budding, the medusa buds detached.

Newly detached medusae had a spherical umbrella translucent in color (Fig. 8A–C). Umbrella height about 1.6 mm and diameter about 1.5 mm. Exumbrella with tiny nematocysts along entire exumbrella (Fig. 8D). Four simple radial canals from four corners of the stomach (Fig. 8B, D). Statocysts four, enclosed in mesoglea, adjacent to primary tentacles (Fig. 8E). Manubrium long, about 50% that of umbrella height (Fig. 8F). Marginal tentacles of two types (Fig. 8C, G, H). Primary tentacles four, short (about 1 to 2 times that of umbrella diameter) bearing nematocyst clusters on the tips (Fig. 8G). Secondary tentacles two, long (about 5 times that of umbrella diameter) bearing 10 to 20 nematocyst batteries (Fig. 8H). The medusae attached using the tip of the primary tentacles, but adhesive pad was not observed (Fig. 8G). Green fluorescence was observed at the base of tentacles and four corners of the stomach (Fig. 8D–F).



**Figure 10.** Nematocysts of *Olindias deigo* sp. nov. **A, B** macrobasic b-mastigophore (small and large), adult medusae. Intact (**A**), discharged (**B**) **C, D** eurytele, adult medusae. Intact (**C**), discharged (**D**) **E, F** macrobasic b-mastigophore, young medusae. Intact (**E**), discharged (**F**) **G, H** eurytele (Large), young medusae. Intact (**G**), discharged (**H**) **I, J** eurytele (Small), young medusae. Intact (**I**), discharged (**J**) **K, L** microbasic eurytele, mature polyp. Intact (**K**), discharged (**L**). Scale bars: 10 µm (**A–F**), 5 µm (**G–L**).

Ninety-day-old medusae were about 10 mm in diameter (Fig. 9A). Umbrella bowl-shaped. Primary and secondary tentacles about 40 and 20, respectively. About 20 centripetal canals were observed. Medusae aged 120-day-old were about 15 mm in diameter (Fig. 9B). White fibrous structures appeared around radial canals. Manubrium elongated and mouth lips developed. Number of primary and secondary tentacles and radial canals not increased much. Medusae aged 150-day-old were about 20 mm in diameter (Fig. 9C). Primary and secondary tentacles about 60 and 20, respectively. About 20 centripetal canals observed. Exumbrella tentacles developed near umbrella margin, but were not observed on the apex of exumbrella. Medusae aged 200-day-old were about 40 mm in diameter (Fig. 9D). Primary and secondary tentacles about 80 and 40, respectively. About 60 centripetal canals were observed. Gonad developed. Exumbrella tentacles developed near the margin of umbrella and the middle part of exumbrella. Medusae aged 240-day-old were about 60 mm in diameter (Fig. 9E). Primary and secondary tentacles about 120 and 40, respectively. About 60 centripetal canals observed. Gonad developed and matured. Spawning observed (Fig. 9E).

**Cnidome.** Two different nematocyst types were identified and measured in the adult medusae, young medusae, and mature polyps (Table 3). Adult medusae had two nematocyst types. Two sizes of macrobasic b-mastigophores (Fig. 10A, B) and microbasic euryteles (Fig. 10C, D) were found on primary, secondary, and exumbrella tentacles. Young medusae had two nematocyst types. Macrobasic b-mastigophores (Fig. 10E, F) were found only on tentacles while two sizes of microbasic euryteles

**Table 3.** Cnidomes of *Olindias deigo* sp. nov. D, L represent capsule diameter and length, respectively, in  $\mu\text{m}$ .

Stage	Part	Type		Min	Max	Mean	SD	N
Adult medusae	Primary tentacle	Macrobasic p-mastigophore (Large)	D	5.69	8.75	7.37	0.63	50
			L	34.19	42.44	38.95	1.99	50
		Macrobasic p-mastigophore (Small)	D	3.24	5.15	4.02	0.45	50
			L	13.01	18.58	16.48	1.18	50
		Microbasic eurytele	D	8.01	10.91	9.84	0.77	50
			L	20.56	28.48	24.61	1.94	50
Young medusae	Exumbrella	Microbasic eurytele (Large)	D	5.66	8.32	7.10	0.72	14
			L	13.70	20.02	17.62	1.78	14
		Microbasic eurytele (Small)	D	2.09	4.68	3.40	0.49	28
			L	6.39	10.47	8.64	1.07	28
	Tentacle	Macrobasic p-mastigophore	D	6.04	7.85	6.77	0.46	50
			L	26.29	34.62	30.42	2.25	50
		Microbasic eurytele (Large)	D	6.33	9.49	7.70	0.68	44
			L	15.70	23.62	20.04	2.35	44
		Microbasic eurytele (Small)	D	2.62	4.33	3.53	0.43	50
			L	6.82	11.97	9.37	1.03	50
Hydroids	Body	Microbasic eurytele	D	4.01	8.31	5.59	0.72	100
			L	9.29	16.95	12.62	1.50	100
	Tentacle	Microbasic eurytele	D	3.79	7.35	5.93	0.72	94
			L	10.75	16.61	13.05	1.20	94

(Fig. 10G–J) were found on primary, secondary, and exumbrella tentacles. The mature polyps had one nematocyst type, microbasic euryteles (Fig. 10K, L).

**Molecular phylogenetics.** In the resulting maximum likelihood tree (Fig. 11), four major monophyletic clades were formed in the genus *Olindias*: 1) *O. formosus*; 2) *Olindias muelleri* Haeckel, 1879; 3) *O. sambaquiensis*; 4) *Olindias tenuis* (Fewkes, 1882); and 5) a fifth group (*O. deigo*). The monophyly of *O. deigo* was evident in the 16S phylogenetic tree with high bootstrap values (99%), strongly supporting the validity of the new species. The Kimura 2-parameter distance between *O. deigo* and *O. formosus* was 0.03, below the distance 0.06–0.11 between olindiids (Table 4). Interspecific distance 0.000–0.002 between *O. formosus* from Iwate Prefecture, eastern Japan and *O. formosus* from Oita and Miyazaki prefectures, western Japan. Therefore, K2P divergence factor between 0.03–0.11 could be a threshold for discriminating olindiid species.

**Habitat and ecology.** Medusae of *O. deigo* appeared in shallow waters (from 3 to 10 m) during winter and spring in a range of subtropical temperature localities in the Ryukyu Archipelago, southern Japan. The medusae rested on the sandy bottom or in areas with a good slope and movement of water during the daytime while they drifted and swam by extending their tentacles during the night. Thus, the species seems to be nocturnal in behavior. Stinging events attributable to *O. deigo* have not been reported thus far.

**Etymology.** The species name comes from the beautiful appearance of the jellyfish. The Japanese name *deigo* (noun in apposition) means *Erythrina variegata* which is popular as the “prefectural flower” of Okinawa.

**Differential diagnosis.** A comparison of key features of the species in the genus *Olindias* is presented in Table 5. All species of *Olindias* have four radial canals and numerous centripetal canals; numerous tentacles of two kinds: primary ones issuing above the umbrella



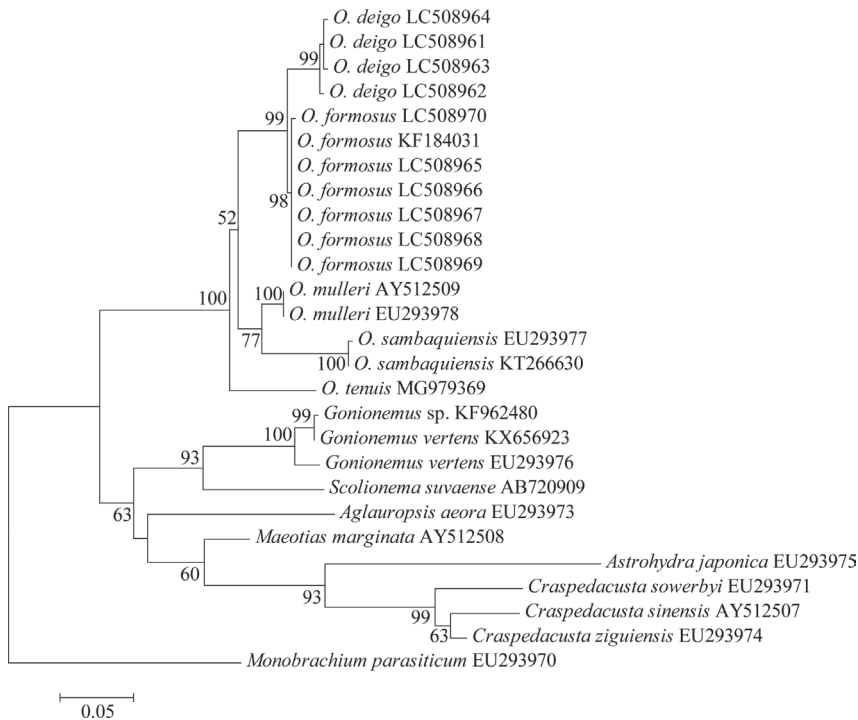
No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
18	<i>Olindias formosus</i>																									
	LC508968	0.189	0.254	0.230	0.237	0.204	0.201	0.188	0.197	0.169	0.182	0.028	0.028	0.025	0.030	0.000	0.000	0.000								
19	<i>Olindias formosus</i>																									
	LC508969	0.189	0.254	0.230	0.237	0.204	0.201	0.188	0.197	0.169	0.182	0.028	0.028	0.025	0.030	0.000	0.000	0.000								
20	<i>Olindias formosus</i>																									
	LC508970	0.188	0.253	0.233	0.240	0.207	0.200	0.187	0.197	0.169	0.181	0.030	0.030	0.027	0.033	0.002	0.002	0.002	0.002							
21	<i>Olindias formosus</i>																									
	KF184031	0.188	0.253	0.230	0.237	0.204	0.200	0.187	0.197	0.169	0.181	0.027	0.027	0.025	0.030	0.000	0.000	0.000	0.000	0.002						
22	<i>Olindias mulleri</i>																									
	AY512509	0.217	0.274	0.244	0.254	0.213	0.210	0.197	0.206	0.175	0.191	0.072	0.072	0.069	0.074	0.061	0.061	0.061	0.061	0.064	0.061					
23	<i>Olindias mulleri</i>																									
	EU293978	0.217	0.274	0.244	0.254	0.213	0.210	0.197	0.206	0.175	0.191	0.072	0.072	0.069	0.074	0.061	0.061	0.061	0.061	0.064	0.061	0.000				
24	<i>Olindias sambaquensis</i>																									
	EU293977	0.214	0.260	0.253	0.257	0.226	0.220	0.216	0.220	0.197	0.204	0.094	0.096	0.094	0.099	0.088	0.088	0.088	0.088	0.088	0.088	0.066	0.066			
25	<i>Olindias sambaquensis</i>																									
	KT266630	0.218	0.263	0.257	0.260	0.229	0.216	0.213	0.216	0.194	0.200	0.096	0.099	0.096	0.102	0.091	0.091	0.091	0.091	0.091	0.091	0.064	0.064	0.002		
26	<i>Olindias tenuis</i>																									
	MG979369	0.217	0.253	0.233	0.230	0.213	0.207	0.207	0.207	0.157	0.200	0.096	0.096	0.093	0.099	0.088	0.088	0.088	0.088	0.091	0.088	0.077	0.077	0.108	0.105	
27	<i>Monobrachium paraiticum</i>																									
	EU293970	0.234	0.344	0.314	0.351	0.299	0.265	0.258	0.261	0.218	0.276	0.241	0.241	0.241	0.245	0.224	0.224	0.225	0.225	0.224	0.224	0.231	0.231	0.259	0.262	0.262

**Table 5.** Morphology of adult medusae in previous and the present study. Bars represent a lack of data.

	<i>O. deigo</i> sp. nov.		<i>O. formosus</i>		<i>O. malayensis</i>	<i>O. mulleri</i>		<i>O. sambaquiensis</i>	<i>O. singularis</i>	<i>O. tenuis</i>	<i>Olindias</i> sp. (young medusa)
UD (mm)	62–84	83.2	75	25–35	40–60	22–44	50–100	13–36	35	7	
UH (mm)	30–45	42.6	about 1/2 of UD	over 1/2 of UD	–	–	–	half of UD	–	5.5	
No. of ET	30–60	84	present	absent	absent	absent	absent	absent	absent	absent	
No. of PT	78–141	168	264	20–30	50–60	48–60	80–100	28–86	32–54	12	
No. of ST	49–51	57	325	30–40	100–120	96–120	200–300	16–50	38–70	–	
No. of MC	168–242	283	–	120	100–170	–	100–200	32 to more than 100	64–69	–	
No. of CC (per quadrant)	20–26	19–23	18–23	7–9	11–19	7–11	21–27	4–12	7–10	1	
No. of gonads	4	4–6	4–6	4	4	4	4	4	4	4	
Gonads	Folded/ along nearly whole length of radial canals	Folded/ along nearly whole length of radial canals	–	Papilliform/ along nearly whole length of radial canals	Linear, swollen, with surfaces covered with branched processes/ over nearly entire length of radial canals	Papilliform/ along the radial canals	Folded/ along nearly whole length of radial canals	Papilliform/ outer half of radial canals	Papilliform/ outer half of radial canals	Folded/ upper half of the radial canals	
Statocysts	Not examined	Not examined	Twice as many as primary tentacles	Twice as many as primary tentacles	Twice as many as primary tentacles	–	Twice as many as primary tentacles	Single orolith at base of each primary tentacle	Single orolith at base of each tentacle	Two at the base of two centripetal canals	



	<i>O. deigo</i> sp. nov.	<i>O. formosus</i>		<i>O. malayensis</i>	<i>O. mulleri</i>		<i>O. sambaquiensis</i>	<i>O. singularis</i>	<i>O. tenuis</i>	<i>Olindias</i> sp. (young medusa)
Color	Manubrium light red to orange. Gonads milky-white. Primary tentacles pale black with purple and glowing green tips and black base. Secondary tentacles deep-brown.	Manubrium lilac to red orange. Each corner of base of manubrium smaragdine-green. Gonads egg-yellow. Tips of primary and exumbrellar tentacles transparent, lilac and smaragdine-green. Marginal clubs and base of primary and exumbrella tentacles ivory-black. Radial canals and circular canals deep scarlet. Centripetal canals lighter scarlet.	–	Similar to <i>O. mulleri</i>	Similar to <i>O. tenuis</i> but apparently browner and duller.	Gonads orange	Bright and variable, with mingled yellow, red, brown, and black. Colors similar to <i>O. tenuis</i> .	Entoderm of stomach, gonads, and ring-canal opaque (cream color?).	Entoderm of manubrium, tentacle-bulbs, and gonads opaque yellowish-green, streaked with purple. Exumbrella tentacles white or dark-purple. Marginal tentacles red and yellow.	–
Distribution (Sampling locality)	Ryukyu archipelagos, Okinawa, southern Japan	Oita, Japan	Japan; Korea	Malay Archipelago	Bahamas; Bermudas; Mediterranean Sea; West Africa	Aegean Sea	Brazil; Argentina	Maldivive Is.; Low Archipelago; Chagos Archipelago; Philippines; India Australia; Iranian Gulf; Pakistan	Florida; Bahamas; Barnudas; Cuba	Sunda Strait
References	This study	This study	Goto (1903) Kramp (1961) Park (2006)	Maas (1905) Mayer (1910) Kramp (1961)	Mayer (1910) Kramp (1961)	Ayran et al. (2019)	Müller (1861) Mayer (1910) Kramp (1961) Chiaverano et al. (2004)	Browne (1905) Mayer (1910) Kramp (1961)	Fewkes (1883) Maas (1905) Mayer (1910) Kramp (1961)	Uchida (1947)



**Figure 11.** Maximum likelihood tree for 15 limnomedusan taxa based on the nuclear 16S rDNA data set. Scale bars indicate branch length in substitutions per site. Nodal support values are presented as the ML bootstrap value; only values >50% are shown.

margin, with distal adhesive pads and cnidocysts in transverse clasps and secondary ones on the umbrella margin, no adhesive pads, cnidocyst in rings; gonads with papilliform processes, on radial canals; numerous marginal clubs, statocyst usually in pairs at base of primary tentacles (Bouillon et al. 2006). *Olindias deigo* can be distinguished from other Olindiidae species by the number and color of tentacles in adult medusae. Many more primary tentacles than secondary tentacles in *O. deigo*, *O. formosus*, and *O. singularis*, while fewer primary tentacles than secondary tentacles in *O. malayensis*, *O. muelleri*, *O. sambaquiensis*, and *O. tenuis* (Table 5). Several exumbrella tentacles present in *O. deigo* and *O. formosus* while lacking in others. Exumbrella tentacles of *O. deigo* many more than those of *O. formosus* (84 vs 30–60, respectively). The primary tentacles were colorful (black, purple, and glow green) in *O. deigo* and *O. formosus*, while they were red and yellow in *O. malayensis*, *O. muelleri*, *O. sambaquiensis*, and *O. tenuis* (no data for *O. singularis* and *Olindias* sp.) (Table 5).

## Discussion and conclusions

Prior to our study, only one olindiid, *O. formosus*, had been recorded from Japan (Goto 1903). This species was described by Goto (1903) based on specimens collected from Misaki, Kanagawa Prefecture, eastern Japan. The medusae of the species have been



reported from warm and cold localities in the Sea of Japan and the Pacific coast of Honshu (Uchida and Uchida 1965), and Jejudo Island, Korea (Park 2006). Distribution of the two species, *O. formosus* and *O. deigo*, do not overlap.

Development of olindiids is known in only two species *O. formosus* (Patry et al. 2014) and *O. muelleri* (identified as *Olindias phosphorica* (Delle Chiaje, 1841)) (Weill 1936). Polyps form colonies which are stolonial, and hydroids bear a single tentacled hydranth, but lack hydrotheca in *O. deigo* and *O. formosus* (Patry et al., 2014) (Table 5). However, polyps of *O. muelleri* are solitary, and hydranth lacks tentacle but enclosed by hydrotheca. Young medusae of *O. deigo* resemble those of *O. formosus* in umbrella sizes and number of tentacles (Patry et al. 2014).

Asexual reproduction and medusa budding of *O. deigo* were observed at 20 °C. The temperature corresponds with that of winter waters around Okinawa Island (Japan Meteorological Agency 2019). Mature medusae appear between winter and spring in Okinawa. Polyps of *O. deigo* may produce medusae during fall and winter.

Morphological and molecular phylogenetic analyses in this study provide evidence that *Olindias* from the Ryukyu Archipelago is a new species. Olindiids are very beautiful and popular but harmful because of their venomous stings (Mianzan and Ramírez 1996; Resgalla et al. 2011). Additional investigations are needed to understand the ecology and distribution of *O. deigo*.

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# A new species of *Onitis* Fabricius, 1798 from south-eastern Africa (Coleoptera, Scarabaeidae, Scarabaeinae, Onitini)

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## Abstract

*Onitis albertcollarti* **sp. nov.** is described and illustrated. The new species is closely related to and occurs sympatrically with *O. lycophron* Klug, 1855.

## Keywords

Democratic Republic of the Congo, Dung beetle, Mozambique, new species, *Onitis albertcollarti*, Tanzania, Zambia

## Introduction

While surveying dung beetles in Zambia and Mozambique, we found a series of specimens that key out to *Onitis lycophron* Klug, 1855 in the Ferreira (1978) key to the Afrotropical species of Onitini. These specimens were clearly distinct from *O. lycophron* and all other *Onitis* based on a unique protibial protrusion in males and the distinct fine rugae on the apical surface of the parameres and belonged to a new species. This new species belongs to the *O. lycophron* species complex (defined by Krikken 1977), which is part of the larger *Onitis* 19<sup>th</sup> species group as defined by Janssens (1937).

The main purpose of describing this new species of *Onitis* is to make the name available for a field guide of the dung beetle species of Parque Nacional das Quirimbas (FG, in preparation). Although the taxonomy of the species belonging to group 19 needs to be reviewed, the uniquely shaped parameres unambiguously separate this undescribed taxon from all others in the genus.

## Materials and methods

Type specimens were deposited in the following collections:

- CMNC** Canadian Museum of Nature, Ottawa, Ontario, Canada;  
**FGIC** François Génier personal collection, Gatineau, Québec, Canada;  
**IRSNB** Royal Belgian Institute of Natural Sciences, Brussel, Belgium;  
**JFJC** Jean-François Josso personal collection, Muzillac, France;  
**PMOC** Philippe Moretto personal collection, Toulon, France;  
**RMIC** Collection Robert Minetti, La Ciotat, France.

The methods are the same as described in Génier and Moretto (2017). All specimens are types and have a World Scarab. Database number.

## Taxonomy

### *Onitis albertcollarti* sp. nov.

<http://zoobank.org/560344F2-06A1-4BD2-B5BD-ABF1C2748DC1>

Figures 1–9

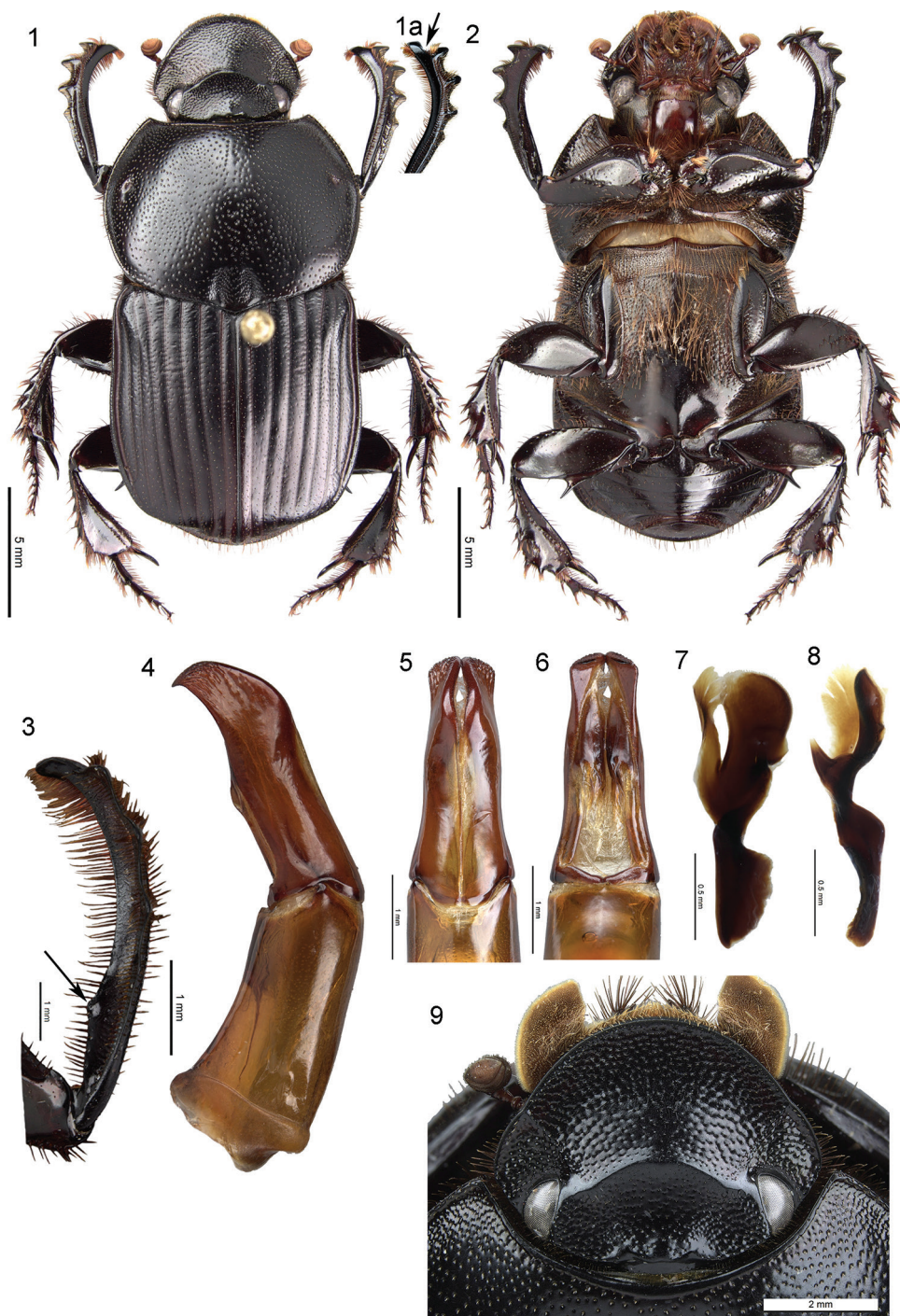
**Type locality.** 1 km SE Kacheleko Outpost (15°01'35"S 26°25'23"E, 1250 m), Central Province, Zambia.

**Holotype** ♂ (CMNC, WSD00038914): [ ZAMBIA: CENTRAL PR. | 1 km SE Kacheleko Outpost, | 1250 m, 15°01'35"S 26°25'23"E | 10.XII.2010, Central Zambesian | & Miombo woodland, prairie edge | light trap, F. Génier, 2010-56 ]; [ WORLD | SCARAB. | DATABASE | WSD00038914 ] barcode label; [ HOLOTYPE ♂ | *Onitis albertcollarti* | des. Génier & Moretto, 2019 ] red card.

**Allotype** ♀ (CMNC, WSD00038918): same data as holotype.

**Paratypes** 68 ♂♂, 55 ♀♀, 402 unsexed specimens. DEMOCRATIC REPUBLIC OF THE CONGO: KATANGA, [unspecified locality], [no date], [anonymous], – 1 ♂ (IRSNB); MOZAMBIQUE: CABO DELGADO, Ravia (site 1), P.N. Quirimbas, 380 m (12°39'41"S, 39°25'22"E), 1.i.2013, F. & S. Génier & M. Denja, eastern Miombo woodlands, light trap (2013-24) – 1 ♀, 1 ♂ (FGIC); same locality, 2.i.2013, F. & S. Génier & M. Denja, eastern Miombo woodlands, light trap (2013-26) – 1 ♂ (FGIC); Ravia (site 6), P.N. Quirimbas, 380 m (12°31'2"S, 39°18'38"E), 3.i.2013, F. & S. Génier & M. Denja, eastern Miombo woodlands, light trap (2013-35) – 2 ♂♂ (FGIC);





**Figures 1–9.** *Onitis albertcollarti* sp. nov. **1–8** male holotype **9** female allotype **1** habitus, dorsal view **1a** protibia, slightly oblique dorsal view to show apical tooth shape **2** habitus, ventral view **3** protibia, oblique view **4** aedeagus, lateral view **5** parameres, dorsal view **6** parameres, ventral view **7** frontolateral peripheral endophallite (FLP), flat view **8** frontolateral peripheral endophallite (FLP), side view **9** head, dorsal view.

Taratibu (site 4), P.N. Quirimbas, 340 m (12°47'32"S, 39°41'50"E), 7.i.2013, F. & S. Génier & M. Denja, eastern Miombo woodlands, elephant dung (2013-39) – 3 ♂♂ (FGIC); Taratibu (site 5), P.N. Quirimbas, 340 m (12°47'3"S, 39°42'10"E), 10.i.2013, F. & S. Génier, eastern Miombo woodlands, elephant dung (2013-67) – 1 ♀, 3 ♂♂ (FGIC); TANZANIA: DODOMA, Dodoma, (6°11'S, 35°46'E), xii.2006, [anonymous], – 43 specimens (RMIC); IRINGA, Tandala, Ruaha National Park, 912 m (7°47.412'S, 35°30.219'E), 6.xii.2006, R. Minetti, – 4 ♀♀, 4 ♂♂, 96 specimens (FGIC, PMOC); MOROGORO, savane de Mikesse Hills, 378 m (6°14.457'S, 37°58.312'E), 12–14.xii.2006, R. Minetti, – 1 ♂ (PMOC); Uluguru Mountains, (7°6'S, 37°39'E), xii.2006, Local collectors, – 103 specimens (PMOC); Vuma Hills, (7°25'S, 37°8'E), 3–5.i.2009, D.C. Moore, – 3 ♀♀, 1 ♂ (PMOC); RUKWA, 10 km N Namanyere, Luafi Game reserve, 510 m (7°27.289'S, 30°54.498'E), 19.xi.2006, R. Minetti, – 1 ♂ (PMOC); ZAMBIA: CENTRAL PROVINCE, 1 km SE Kacheleko Outpost, 1250 m (15°1'35"S, 26°25'23"E), 10.xii.2010, F. Génier, central Zambebian & Miombo woodland, prairie edge, light trap (2010-56) – 1 ♀, 3 ♂♂ (CMNC, FGIC); 25 km NE Lilemone, 1250 m (15°13'14"S, 26°19'41"E), 5.xii.2010, F. Génier, central Zambebian & Miombo woodland, light trap (2010-46) – 6 ♀♀, 8 ♂♂ (FGIC); same locality, 6.xii.2010, F. Génier, central Zambebian & Miombo woodland, dung trap (2010-47) – 1 ♂ (FGIC); 5.6 km SW Kacheleko Outpost, 1250 m (15°3'28"S, 26°23'55"E), 7.xii.2010, F. Génier, central Zambebian & Miombo woodland, prairie edge, dung trap (2010-48) – 7 ♀♀, 7 ♂♂ (FGIC); 6.2 km W Mukambi Lodge Jct. on M9, 1100 m (14°57'2"S, 25°56'21"E), 18.xi.2010, F. Génier, open central Zambebian & Miombo woodland, elephant dung (2010-02) – 4 ♀♀, 3 ♂♂ (FGIC); 6.5 km N Chunga, 1100 m (14°59'40"S, 26°1'11"E), 4.xii.2010, F. Génier, open central Zambebian & Miombo woodland, light trap (2010-43) – 15 ♀♀, 18 ♂♂ (FGIC); Chunga, Kafue National Park [site 1], (15°2.362'S, 25°59.437'E), 11–12.xii.2009, Josso, Juhel & Minetti, piège lumineux – 20 specimens, 1 ♀, 1 ♂ (JFJC); Kacheleko Wildlife Outpost, Kafue National Park, (15°1'S, 26°25'E), 2–3.xii.2007, Josso, Juhel & Monfort, piège lumineux – 6 ♀♀, 4 ♂♂ (JFJC); same locality, 6–7.xii.2008, J.-F. Josso & R. Minetti, piège lumineux – 2 ♀♀ (JFJC); same locality, 10–18.xii.2009, Josso, Juhel & Minetti, piège lumineux – 91 specimens (JFJC); Kafue river east, (14°57'S, 25°55'E), 4.xii.2007, Josso, Juhel & Monfort, – 49 specimens (JFJC); Mukambi Safari Lodge, 1250 m (14°58'32"S, 25°59'29"E), 8.xii.2010, F. Génier, open central Zambebian & Miombo woodland, light trap (2010-50) – 2 ♀♀, 1 ♂ (FGIC); same locality, 9.xii.2010, F. Génier, open central Zambebian & Miombo woodland, light trap (2010-52) – 1 ♀, 1 ♂ (FGIC); COPPERBELT, Kasompe, (12°36'S, 27°53'30"E), ii.1982, [anonymous], – 1 ♀, 3 ♂♂ (PMOC).

**Diagnosis.** Male *Onitis albertcollarti* sp. nov. will key to couplet 4 on page 159 in the Krikken (1977) key to species of the *O. lycophron* species complex. The presence of a single tooth on the profemur ventral surface on basal half (Fig. 3, arrow) will separate it from *O. mendax* Gillet, 1918 (interrupted carina) and *O. pseudojanssenii* Krikken,



1977 and *O. janssenii* Gomes Alves, 1854 (both species with two separate protibial protrusions on the ventral surface basally in moderate to large males).

*Onitis albertcollarti* will key to *O. lycophron* (couplet 12, page 323) in the Ferreira (1978) key to Onitini species. Males differ from *O. lycophrons* by the distinct projecting apical tooth of the protibia (Fig. 1a, arrow) and the external edges of the apical tooth forming a distinct angle with the anterior edge of the apical lateral tooth. Females differ from *O. lycophron* by the smaller subtriangular pygidium.

**Description.** Holotype ♂ (Figs 1–8). **Overall aspect.** Length 20.0 mm, maximum width 9.5 mm. Colour dark brown to black, lacking metallic sheen. Dorsal surface slightly sericeous on head and pronotum, elytra less glossy. Setae minute on head, pronotum, and dorsal surface of elytra, with longer setae on elytral apical declivity. Venter with long dense pubescence on mesosternum and median lobe of metasternum. **Head.** Anterior clypeal edge rounded, margin abruptly upturned. Clypeogenial sutures not carinate, weakly defined. Clypeal surface with short, transversal scabrous punctures. Genal surface finely granulate. Clypeofrontal carina arcuate medially, slightly sinuous and tuberculate near the clypeogenial suture. Frontal surface finely punctate medially, with denser granulate punctures laterally. Frontal tubercle obtuse, in line with cephalic posterior edge. **Pronotum.** Lateral edges broadly arcuate in dorsal view, maximum width midway between anterior angle and posterior angle. Surface moderately convex. Pronotal punctures simple and coarse, becoming scabrous on anterior angles; punctures irregularly distributed, separated by one to six diameters. Posteromedian fossae fused, surface with coarse microsculpture and with fine, irregular, scabrous punctures. Posterior edge finely crenulate on each side of posteromedian fossae. **Scutellum.** Small and triangular. **Elytra.** Moderately convex. Striae 1–7 moderately wide, slightly wider at basal third. Strial punctures weakly defined on apical declivity. Interstriae 1–8 feebly convex, with fine, irregularly-spaced punctures. Elytral striae 8 straight, wide, and deeply impressed from humeral callus to junction with striae 7. Elytral interstria 9 narrowly bulging dorsally. Elytral striae 8 and 9 wide and deeply impressed at apical third. **Pygidium.** Subtriangular, distinctly smaller than in *O. lycophron*. Pygidial surface convex, sericeous, with scattered, minute, setigerous granules. **Antennal club.** Fulvous, mostly covered with dense, minute, yellow setae and some scattered long, brown setae. **Ventrites.** Metasternal surface with two more-or-less triangular depressions on each side of midline posteriorly. Abdominal sternite 3 with few minute, scattered setae medially. **Legs.** Profemur anterior surface flat with minute punctures. Apicoventral edge produced into a small, inwardly bent denticle. Protibia long and slender, anterior half evenly bent inward. External edge with four teeth. Apex produced into a semi-trapezoidal and downwardly bent tooth flanked internally by a short but thick setal brush (Fig. 1a, arrow). Distal edge of apical tooth forming a distinct angle with external edge of apical projection. Longitudinal carina of ventral surface obtusely toothed at basal fourth (Fig. 3, arrow). Mesofemur posterior edge crenulate in ventral view, lacking a denticle or projection on apicoventral angle. Mesotibial internal edge straight. Metafe-

mur lacking ventrally bent tooth on anterior edge at middle, posterior edge produced into an obliquely oriented, acute tooth medially, basal portion of tooth never fused with ventroposterior edge of metafemur. **Aedeagus.** Phallobase as long as parameres (Fig. 4). Parameres with apical surface covered with coarse, fused granulation forming fine longitudinal rugae (Figs 4, 5). Frontolateral peripheral endophallite (FLP) bilobate, with a spiniform process at distal third (Figs 7, 8).

**Variation.** Measurements (59 ♂♂, 46 ♀♀). Length: male 15.0–21.0 mm ( $18.0 \pm 1.5$  mm), female 15.5–21.0 mm ( $18.1 \pm 1.3$  mm). Female as male except clypeal edges ogival in dorsal view (Fig. 9); pygidium smaller and triangular with surface flat and with denser, minute, setigerous granules; legs unmodified, except for mesofemoral posterior edge as in male. Some variation in the coarseness of dorsal microsculpture with some individuals appearing entirely dull.

**Etymology.** While visiting the Royal Belgian Institute of Natural Sciences in Brussels, we found a specimen of this species bearing the “*Onitis collarti*” paratype label of André Janssens. Since Janssens’ name was never formally described we decided to honour this homage with the modification to “*O. albertcollarti*”. Albert DCH Collart was a colleague and friend of André Janssens a well-known scarab worker. Collart first worked as a sanitary agent in the former Belgian Congo from 1923 to 1930. For health reason he had to come back to Belgium and started to work as a scientific collaborator for the entomology department of the former Royal Museum of Natural History in 1932. He was promoted several times and concluded his career as the Laboratory Director of the Institution in 1964. He retired in January 1965 and remained associated as a scientific collaborator of the Royal Belgian Institute of Natural Sciences until his death in 1993.

**Distribution.** From southern Democratic Republic of the Congo (Katanga) to Tanzania in the north and eastern Zambia through northern Mozambique in the south. *Onitis albertcollarti* occurs sympatrically with *O. lycophron* on most of its northern distribution.

**Natural history.** Specimens with data were collected using pitfall traps baited with human faeces and elephant dung and were attracted to light traps. Some individuals were collected in eastern Miombo woodlands and central Zambezian woodlands.

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# Altitudinal and seasonal distribution of butterflies (Lepidoptera, Papilionoidea) in Cerro Bufo El Diente, Tamaulipas, Mexico

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## Abstract

Butterflies are one of the most recognized and useful groups for the monitoring and establishment of important conservation areas and management policies. In the present study, we estimate the richness and diversity, as well as the association value of submontane scrub, oak forest, and cloud forest species at Cerro Bufo El Diente, within the Sierra de San Carlos priority land region, located in the Central-western region of Tamaulipas, Mexico. Three sampling sites were established based on criteria of vegetation distribution per altitudinal floor. One site for each altitudinal floor and vegetation type. Sampling was carried out in permanent transects on a monthly basis at each site, using an aerial entomological net and ten Van Someren-Rydon traps, during four sampling periods: early dry season, late dry season, early wet season and late wet season. In total, 7,611 specimens belonging to six families, 20 subfamilies, 32 tribes, 148 genera and 243 species of the study area were collected. Nymphalidae was the most abundant family with 3,454 specimens, representing 45.38% of total abundance in the study area. Lower abundance was recorded in Hesperidae (19.17%), Pieridae (16.41%), Lycaenidae (10.17%), Papilionidae (5.12%), and finally Riodinidae (3.74%). The highest species richness was presented in the family Hesperidae with 34.57% of the total obtained species followed by Nymphalidae (30.45%), Lycaenidae (15.23%), Pieridae (9.88%), Papilionidae (5.76%), and Riodinidae (4.12%). Twenty-seven species were categorized

as abundant, these species, *Anaea aidea* (Guérin-Ménéville, 1844), *Libytheana carinenta larvata* (Strecker, 1878), *Pyrgus oileus* (Linnaeus, 1767), *Mestra amymone* (Ménétriés, 1857) and *Phoebis agarithe agarithe* (Boisduval, 1836) presented the highest number of specimens. Sixty-five species were considered common, constituting 41.73% of the total number of butterflies, 63 frequent (9.76% of the total abundance), 55 limited (2.54%) and 33 rare (0.43%). The greatest number of specimens and species, as well as alpha diversity, were presented on the lowest altitudinal floor, made up of submontane scrub, and decreased significantly with increasing altitude. According to the cluster analysis, low and intermediate altitude sites constitute an area of distribution of species that prefer tropical conditions, while the third-floor site forms an independent group of high mountain species. The greatest abundance and richness of species, as well as alpha diversity, was obtained during the last wet season, decreasing significantly towards the early dry season. Moreover, through the use of the association value, 19 species were designated as indicators, three for the last altitudinal floor, three for the intermediate and 13 for the first. The present work represents the first report of the altitudinal variation in richness, abundance and diversity of butterflies in the northeast of Mexico. These results highlight the importance of the conservation of this heterogeneous habitat and establish reference data for the diurnal Lepidoptera fauna of the region.

### Keywords

Diurnal Lepidoptera, diversity, elevation, indicator species, priority land region, seasonality

## Introduction

More than 155,000 species of Lepidoptera have been described to date (Nieukerken et al. 2011), as such the order comprise 10% of the known animal diversity (Kristensen et al. 2007). The Butterflies (Papilionoidea) comprise six families: Papilionidae, Pieridae, Lycaenidae, Riodinidae, Nymphalidae and Hesperidae, and together represent 13% of total species in Lepidoptera worldwide (Kawahara and Breinholt 2014; Llorente et al. 2014). In Mexico, according to Warren (2000), Llorente et al. (2006) and Llorente et al. (2014), it is estimated that there are 2,049 species, corresponding to 9.4% of the Papilionoidea described worldwide. Butterflies are among the best environment quality indicator insects, because they are highly diverse and abundant (Prince-Chacón et al. 2011), easy to identify at field and due to their rapid biological cycles, they are easy to sample in any time of the year (Freitas et al. 2006). In addition, they are affected by constant landscape changes, because they are closely related to the vegetation (Marín et al. 2014), and most of their life cycle is associated with specific plants (Orozco et al. 2009). Furthermore, they respond to the stratification of the vegetation in terms of light, wind, humidity and temperature gradients (Montero-Muñoz et al. 2013). Therefore, they are very sensitive to climatic and ecological variations occurring in natural gradients, such as elevation (Camero et al. 2007).

Numerous studies show the close association between altitude and changes in composition and diversity of species (Muñoz and Amarillo-Suárez 2010). Several hypotheses have been proposed to explain among which the Rapoport effect states that the richness and distribution ranges of species are inversely related to altitude, with higher richness at low elevations (Sanders 2002), while the hypothesis of average domain in-

dicates that the greatest number of species occurs at intermediate altitudes (Brown and Lomolino 1998). Besides, McCoy (1990) determined that, if the distribution differs between elevations, then the time scale used would strongly influence the evaluation of species richness. Thus, seasonal variations are strongly linked to elevational patterns of communities (Castro and Espinosa 2016).

In Mexico, several checklists of butterflies from altitudinal transects ranging from 600 to 3,100 m asl, including different vegetation types, have been published (Llорente et al. 1986; Luis and Llorente 1990; Luis et al. 1991; Vargas et al. 1994, 1999; Díaz-Batres et al. 2001; Luna and Llorente 2004; Luna et al. 2008; Luna et al. 2010; Álvarez et al. 2016). In addition, at a temporal level, the climatic factors influencing butterfly species turnover have been addressed in previous studies (Luis and Llorente 1990, 1991; Vargas et al. 1994; Hernández-Mejía et al. 2008; Luna et al. 2008; Pozo et al. 2008; Luna et al. 2010). However, little is known about the entomofauna and especially about the butterfly ecology of the extreme, humid and dry environments of northeastern Mexico, which is inhabited by a very special group that represents about 15% of national entomofauna, and harbors elements of the Atlantic District of the United States (Luz and Madero 2011). Knowing the distribution of the species richness and abundance of butterflies in altitudinal gradients, allows to elucidate patterns and processes of biological diversification, occupying an important role to demonstrate the conservation value of a particular habitat (DeVries and Walla 2001). Likewise, the study of communities and populations of butterflies over time, can offer important information to implement urgent measures before the effects of environmental disturbance become irreversible (Núñez-Bustos et al. 2011).

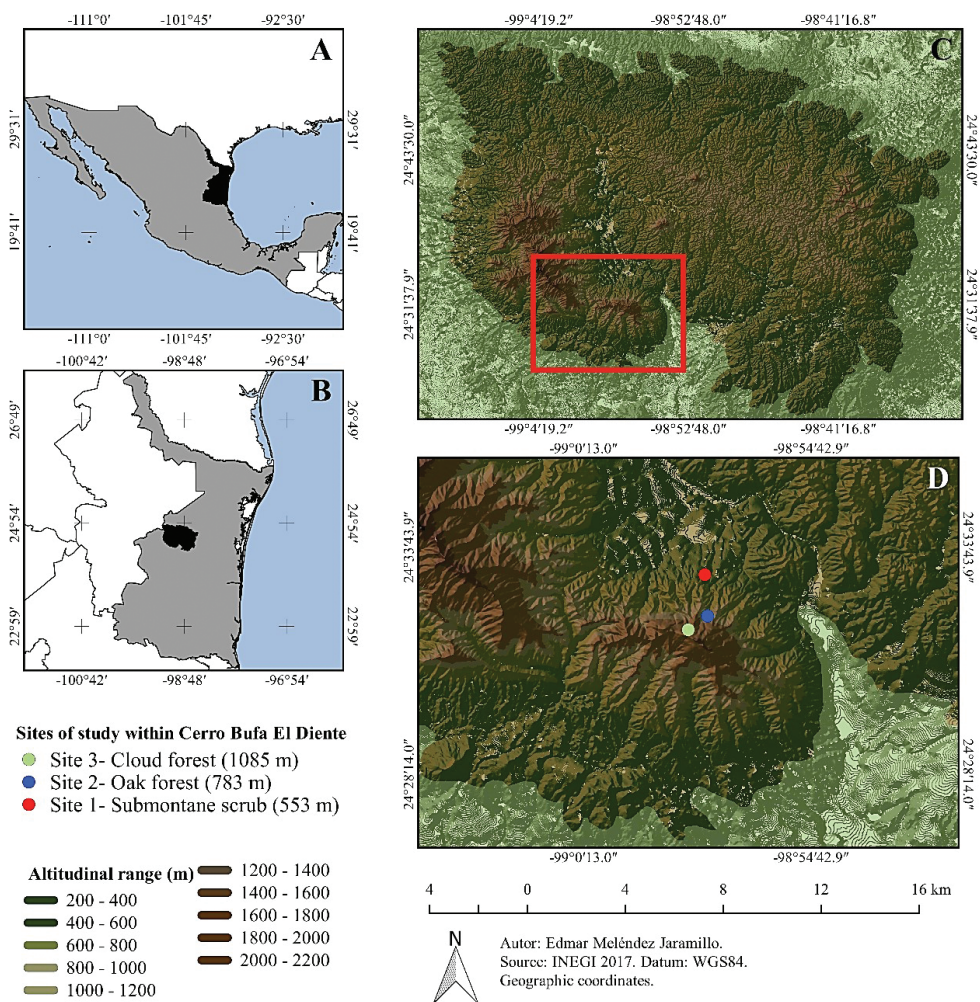
In this context, the Bufo El Diente mountain constitutes one of the highest elevation gradients (up to 1,460 m asl) in the Sierra de San Carlos, which is an isolated orographic unit within the coastal plain of the North Gulf of Mexico (Treviño et al. 2002). The region is considered an area of special interest for conservation and requires an evaluation of its natural resources (Arriaga et al. 2000). The objectives of the present study were: 1) to determine the butterflies species richness in Cerro Bufo El Diente, Tamaulipas, Mexico; 2) analyze the variation of *Rhopalocera* species richness, abundance and diversity along an altitudinal gradient, and during different seasons of the year; 3) analyze the influence of climatological variables (temperature, precipitation, relative humidity and solar radiation) on the abundance and richness of butterfly species; and 4) quantify the indicator value of species by each altitudinal site.

## **Methods**

### **Study area**

The Cerro Bufo El Diente mountain is located in the Sierra de San Carlos, located in the central-western portion of the State of Tamaulipas, between 24°23.03' and 24°51.60'N, and 98°32.40' and 99°12.04'W (Figure 1). Sierra de San Carlos (also





**Figure 1.** Study area and location of sampling sites **A** location of Tamaulipas in Mexico **B** location of Sierra de San Carlos within Tamaulipas **C** study area (red square) within Sierra de San Carlos **D** elevation sites in Cerro Bufo El Diente.

known as Sierra Chiquita or Sierra de Cruillas) is a physiographic discontinuity in the Coastal Plain of the Gulf of Mexico. Due to its relative geographical isolation in relation to the Sierra Madre Oriental, it can be conceived as an ecological island, where relatively particular populations and communities have been originated or conserved (Briones-Villarreal 1991). The area is considered as a Mexican Priority Region for Conservation (RTP) of biodiversity by the National Commission for the Knowledge and Use of Biodiversity (CONABIO). The vegetation types of this RTP mainly comprise temperate ecosystems in the mountain part and submontane scrub in the piedmont (Arriaga et al. 2000). A main characteristic of the region is that it represents the boreal limit of the cloud forest in northeastern Mexico (Valdez-Tamez et al. 2003).



Climate of the region is semi-warm sub-humid with summer rains; average annual temperature is 18 to 22 °C, and the annual precipitation ranges between 500 and 2,500 mm (Treviño et al. 2002).

### Site locations

Three sites were established based on Llorente (1984) and Briones (1991) criteria for the altitudinal gradient and vegetation types. Site 1 has the lowest elevation at 553 m asl and corresponds to submontane scrub (SS) (24°33.04'N, 98°57.16'W). Site 2 is located at an intermediate altitude of 783 m asl where the plant community consists of oak forest (OF) (24°32.04'N, 98°57.13'W). Site 3 is the highest elevation with 1085 m asl and a community of cloud forest (CF) (24°31.44'N, 98°57.41'W; Table 1).

### Collection and processing of specimens

The collection of individuals was conducted using aerial entomological nets. At each site, routes were made along a 1 km permanent transect, following the techniques recommended by Villarreal et al. (2006). Also, along with the use of the aerial entomological nets, the sampling was carried out using Van Someren-Rydon traps (Rydon 1964). Ten traps were placed along a permanent transect 500 m long, at a distance of 50 m from one another, and between 1 to 2.5 m high from the ground. Bait used for the traps consisted of a fermented mixture of seasonal fruits: plantain (*Musa paradisiaca*), pineapple (*Ananas comosus*), mango (*Mangifera indica*), and guava (*Psidium guajava*).

Monthly samplings were made for each of the sites, during the period from September 2012 to August 2013, resulting in a total of three samples-months per season: Early dry season (EDS: December, January, February), Late dry season (LDS: March, April, May), Early rainy season (ERS: June, July, August), and Late rainy sea-

**Table 1.** Synthesis of the collection sites.

Site	Vegetation	Frequent species	General description
1	Submontane scrub (SS)	The dominant shrubs are <i>Helietta parvifolia</i> , <i>Leucophyllum frutescens</i> and <i>Acacia rigidula</i> , or <i>Havardia pallens</i> , <i>Cordia boissieri</i> and <i>Acacia berlandieri</i> .	It grows in the piedmont and hillsides with south exposure, at altitudes of 500 to 800 m asl.
2	Oak forest (OF)	Along with <i>Quercus canbyi</i> , it is common to find <i>Arbutus xalapensis</i> , <i>Quercus clivicola</i> and <i>Quercus virginiana</i> , or, in addition to <i>Quercus rysophylla</i> , there are other oak species: <i>Q. sartorii</i> , <i>Q. laceyi</i> , <i>Q. clivicola</i> , as well as <i>Arbutus xalapensis</i> , <i>Pinus pseudostrobus</i> , <i>Persea podadenia</i> , <i>Carya ovata</i> , <i>Prunus serotina</i> and <i>Platanus occidentalis</i> .	It is possible to recognize two variants of this type of vegetation. The first one is the <i>Quercus canbyi</i> forest. It is found around 700 m asl on slopes with north exposure, bordering the submontane scrub. From there it extends up to 1,000 m asl, where it comes into contact with the <i>Quercus rysophylla</i> forest, which is the second variant.
3	Cloud forest (CF)	<i>Abies guatemalensis</i> , is the most abundant species, followed by <i>Carya ovata</i> . Oaks as a whole are also important, followed by <i>Carpinus caroliniana</i> , <i>Ostrya virginiana</i> , <i>Gleditsia triacanthos</i> , <i>Persea podadenia</i> , <i>Ilex rubra</i> , <i>Acer saccharum</i> , <i>Ungnadia speciosa</i> and <i>Crataegus rosei</i> .	Restricted to the upper parts of Cerro Bufo El Diente, with north exposure, between 1,300 and 1,400 m asl.

son (LRS: September, October, November). Seasons were defined on basis of historical data of total monthly values of temperature and precipitation (average of 1990 to 2010), which were obtained from a meteorological station located within the study area in the municipality of San Carlos. Therefore, a total of 36 sampling units (three samplings per four seasons per three sites) were considered. Additionally, for each site and date of collection, the temperature and relative humidity variables were recorded using a Kestrel 3500 portable weather station, while values of precipitation and solar radiation were extracted with QGIS 2.18 software (Quantum GIS 2017) from the WorldClim database available in <http://worldclim.org/> and described by Fick and Hijmans (2017).

The collected entomological specimens were mounted according to the procedure described by Andrade et al. (2013). All specimens were labeled and deposited in the entomological collection of the Instituto Tecnológico de Cd. Victoria, Ciudad Victoria, Tamaulipas, Mexico, and in the collection of the Department of Conservation of the Faculty of Forestry Sciences at the Universidad Autónoma de Nuevo León, Linares, Nuevo León, Mexico. For taxonomic identification of specimens, the works of Scott (1986), Llorente et al. (1997), Luis et al. (2003), Garwood and Lehman (2005), Glassberg (2007), Vargas et al. (2008) and Luis et al. (2010), were consulted. Phylogenetic arrangement of species followed Warren et al. (2012).

## **Data analysis**

The abundance was quantified based on the total number of individuals per species collected at each site, season and for the entire study area. Five categories of species were considered according to the total abundance recorded: rare (species with one specimen), scarce (from 2 to 5), frequent (from 6 to 21), common (from 22 to 81), and abundant (with 82 or more specimens) (Luna et al. 2010). To corroborate significant differences between the abundance associated to each site, as well as to each season of the year, nonparametric tests of Kruskal-Wallis and Mann-Whitney were carried out. As a measure of specific richness, the total number of species obtained was used for each site, season and for the entire study area. A permutation test was conducted to determine significant variations in the number of species. Both tests (for abundance and species richness) were carried out using the Rcommander package (Fox 2005) in the program R 3.2.3 (R Development Core Team 2015). To calculate the potential number of species, the nonparametric estimators of Chao 1 and Jackknife 1 were used. These indices were chosen according to: 1) a distribution model of abundance is not previously assumed, 2) they are robust in calculating the minimum estimate of specific richness, 3) they are necessary as a complementary measure in biodiversity analyzes, and 4) Chao 1 considers the association between the number of species represented by an individual (singletons) and those represented by two individuals (doubletons) in the sample, while Jackknife 1 is a conservative index based on incidence data (presence or absence) of those species found only in one sample (uniques) (Magurran 2004; Hortal et al. 2006; Villarreal et al. 2006; Gotelli and Colwell 2011). The estimators were cal-

culated with 100 randomizations without replacement using the software EstimateS 9.1 (Colwell 2013), based on the abundance of the species recorded by each sampling unit, and were obtained for each site, station of the year and for the entire study area. To complement the estimation of richness, and as a measure for the analysis of sampling efficiency, the linear dependence model was used. It assumes that as the list of species increases, the probability of adding new taxa decreases exponentially, and is an ideal model for studying small areas and known taxa (Gómez-Anaya et al. 2014). The value obtained from the coefficient of determination ( $R^2$ ) was used, as well as the slope value, which allows to measure the quality of the faunistic inventory. The calculation was based on the number of samples for each site, as well as for each season of the year and for the entire study area; the procedure was performed in the program Statistica 13.3 (TIBCO Software Inc. 2017).

In this study, alpha diversity was considered a measure of association or relation between abundance and number of species. Therefore, Simpson's dominance index and Shannon's entropy or uncertainty index were used for its measurement; these indices were calculated for the entire study area, as well as for each site and season using the vegan package (Oksanen et al. 2012) of the platform R 3.2.3. The SHE analysis S (species richness), H (Shannon-Wiener diversity index) and E (evenness as measured using the Shannon-Wiener evenness index) is a method that consists of analyzing the behavior of three components: diversity, the natural logarithm of evenness and the proportion of the previous two as a function of abundance (Buzas and Hayek 1996). To discriminate between the types of distribution, the component with the least variation was identified in relation to different values of number of species and abundance. If the diversity parameter remains more stable, then the distribution corresponds to a logarithmic series; if the most stable is the proportion between natural logarithm of evenness and diversity, a normal log distribution is attributed; and if evenness is the most stable, then the distribution will be of a broken stick type (Carreño-Rocabado 2006). The SHE test was carried out for the entire study area, as well as for each site using the forams package (Aluizio 2015) in R 3.2.3. Beta diversity was measured as the faunal similarity between sites and seasons, using the Bray-Curtis similarity index. In addition, a cluster analysis was carried out to define groups of sites and seasons according to their species composition, using the adjusted Euclidean units as distance measure and the Ward method as an amalgamation algorithm. Calculations were made in the Rcommander package (Fox 2005) in the R 3.2.3 program. A Spearman correlation test was applied between the monthly averages of microclimate variables (temperature, precipitation, relative humidity and solar radiation) and ecological parameters (number of species and abundance) using the Rcommander package (Fox 2005) in R 3.2.3.

Finally, to calculate the association value of each butterfly species to the habitat type, the indicator value index (IndVal) was used (Dufrene and Legendre 1997). This is based on the degree of specificity (exclusivity of the species to a particular site based on its abundance), and the degree of fidelity (frequency of occurrence within the same habitat) (Tejeda-Cruz et al. 2008), expressed in a percentage value. The analyzes were carried out in the abds package in platform R 3.2.3, using 1,000 random permutations to define the level of significance. Indicator species with an index equal to or

greater than 75% were categorized as “characteristics”, which are defined by their high specificity to a given habitat, while species with a value less than 75% but equal to or greater than 50% considered as “detectors”, which present different degrees of preference for diverse habitats (McGeoch et al. 2002).

## Results

### Abundance, richness, and diversity of butterflies in Cerro Bufa El Diente

A total of 7,611 specimens of Papilionoidea was collected from 36 samples, between September 2012 to August 2013. These belong to six families, 20 subfamilies, 32 tribes, 148 genera, and 243 species (Appendix 1). Nymphalidae was the most abundant family with 3,454 specimens, representing 45.38% of total abundance in the study area. Lower abundance was recorded in Hesperidae (19.17%), Pieridae (16.41%), Lycaenidae (10.17%), Papilionidae (5.12%), and finally Riodinidae (3.74%). The highest species richness was also presented found in the family Hesperidae with 34.57% of the total obtained species followed by Nymphalidae (30.45%), Lycaenidae (15.23%), Pieridae (9.88%), Papilionidae (5.76%), and Riodinidae (4.12%). Twenty-seven species were categorized as abundant (with more than 82 specimens) and accounted for 45.54% of the total abundance. These abundant species, *Anaea aidea* (Guérin-Ménéville, 1844) (442 individuals), *Libytheana carinenta larvata* (Strecker, 1878) (213), *Pyrgus oileus* (Linnaeus, 1767) (176), *Mestra amymone* (Ménétriés, 1857) (172) and *Phoebis agarithe* (Boisduval, 1836) (167), among others presented the highest number of specimens. Sixty-five species were considered common, constituting 41.73% of the total number of butterflies. Sixty-three species were considered frequent (743 specimens) by occupying 9.76% of the total abundance. Fifty-five species were scarce (2.54% of total abundance) and 33 were rare (0.43%) (Appendix 1).

The richness estimators indicated that the total number of butterfly species in the study area was 278 species using Chao 1 and 283 through Jackknife 1 (Table 2, Figure 2), suggesting that the observed total of 243 species represents 87.35% (Chao 1) or 85.91% (Jackknife 1) of the actual richness. The data showed a good fit to the linear dependence model ( $R^2 = 0.93$ ), with a registered proportion of species of 92.40% and a slope less to 0.1. Total diversity values of Papilionoidea in Cerro Bufa El Diente were 0.98 for the Simpson index and 4.16 for the Shannon index (Table 2). The SHE analysis shows an assemblage with less variation in the natural logarithm of evenness, suggesting a broken stick type distribution (Table 3, Figure 3).

### Altitudinal variation of butterflies

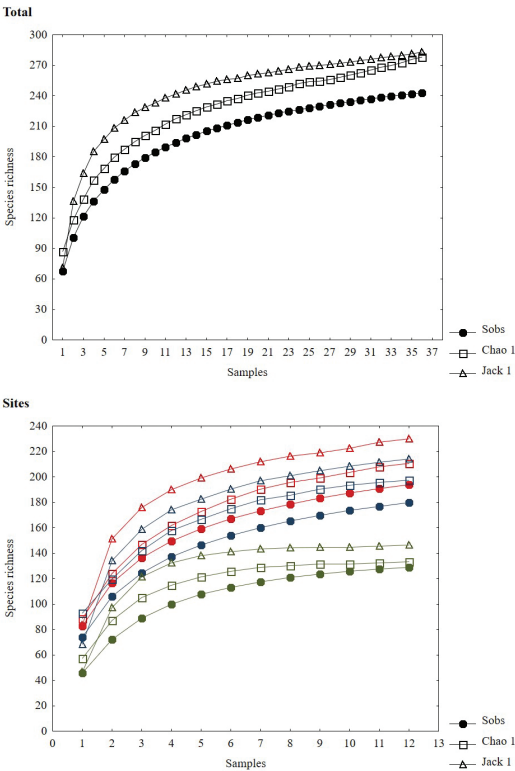
Abundance and number of species of butterflies was significantly different ( $p < 0.05$ ) only between the highest site compared to the intermediate and low sites (Site 3 compared to the Site 2 and 1; Table 2). Both abundance and species richness decreased

**Table 2.** Richness, abundance and diversity parameters of Papilionoidea in Cerro Bufo El Diente, Tamaulipas, Mexico. Key: S obs = Observed richness; N = Abundance; S est = Estimated richness; LDM = Linear dependence model; R<sup>2</sup> = LDM determination coefficient; 1-D = Simpson diversity index; H' = Shannon diversity index.

Ecological parameter	Site			Season				Total
	Submontane scrub (553 m asl)	Oak forest (783 m asl)	Cloud forest (1085 m asl)	Dry		Rainy		
				Early (Dec–Feb)	Late (Mar–May)	Early (Jun–Aug)	Late (Sep–Nov)	
S obs *	194 a	180 a	129 b	65 a	165 b	187 b	207 b	243
N *	3726 a	2641 a	1244 b	297 a	1970 b	2637 b	2707 b	7611
S est								
Chao 1	210.67	197.55	133.11	69.33	198.07	208.00	233.46	278.20
Jackknife 1	229.75	213.92	146.42	84.56	204.11	232.33	266.56	282.86
LDM								
R <sup>2</sup>	0.96	0.96	0.99	1.00	0.95	0.95	0.94	0.93
S est	219.51	205.61	154.23	95.75	195.55	217.60	243.83	262.99
Slope	0.34	0.39	0.41	1.58	1.04	0.93	1.35	0.04
Diversity								
1-D **	0.97 a	0.97 a	0.89 b	0.84 a	0.98 b	0.98 b	0.98 b	0.98
H' **	4.06 a	3.93 a	3.19 b	2.37 a	4.11 b	4.17 b	4.25 b	4.16

\* Values with different letters between columns are significantly different using Kruskal-Wallis and Mann-Whitney Tests: abundance between sites, K= 10.16, DF= 2, *p*= 0.006; richness between sites, K= 7.93, DF= 2, *p*= 0.019; abundance between seasons, K= 21.09, DF= 3, *p*= 0.000, richness between seasons, K= 21.31, DF= 3, *p*= 0.000.

\*\* Diversity values with different letters between columns are significantly different at *p*< 0.05, using permutation tests in R 3.2.3 program.



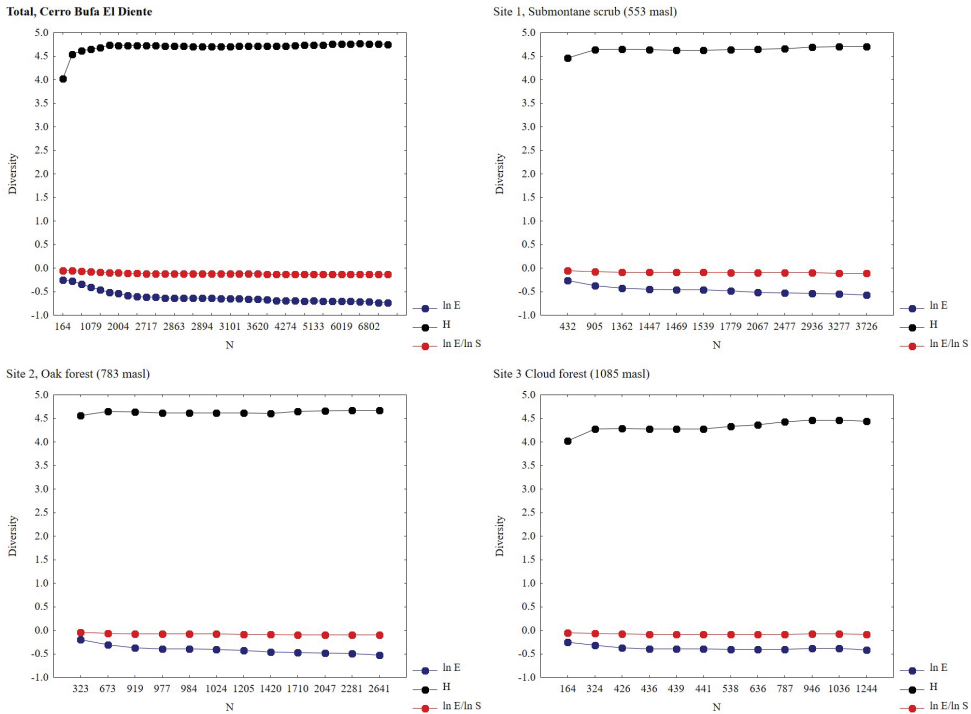
**Figure 2.** Species accumulation and estimator curves in the Cerro Bufo El Diente, Tamaulipas, Mexico. Upper graphic: accumulation curves for all study area. Lower graphic: Site 1 (red color), Site 2 (blue color) and Site 3 (green color).

with increasing altitude (Table 2). In the lowest altitude site, 194 species were recorded which represented between 84.44 andto 92.09% of the estimated richness with the models used. In the second site, the number decreased to 180 species (84.14–91.12% of the estimated) and at the highest site, 129 species were recorded (83.64–96.91% of the estimated) (Figure 2). Determination coefficient in all sites was greater than 0.95, suggesting a good fit of the linear dependence model to the data obtained at each site; contrarily, the slope values was greater than 0.1 in all sites (Table 2).

Alpha diversity decreased progressively with increasing altitude and was significantly different between the highest altitude and the other two sites ( $p < 0.05$ ) (Table 2). The result of the SHE analysis for three sites showed a lower variation in natural logarithm of evenness, indicating a broken stick type distribution (Table 3, Figure 3).

**Table 3.** SHE analysis to identify the type of abundance distribution of butterflies in Cerro Bufo El Diente, Tamaulipas, Mexico. Marked cells (\*) highlight the component with the lowest percentage variation.

Sites	Abundance range	ln E	H	ln E/ln S	Distribution
Site 1, Submontane scrub (553 m asl)	432 to 3726	46.57 *	94.96	51.90	Broken stick
Site 2, Oak forest (783 m asl)	323 to 2641	36.45 *	97.76	39.82	Broken stick
Site 3 Cloud forest (1085 m asl)	164 to 1244	59.83 *	90.65	67.99	Broken stick
Total, Cerro Bufo El Diente	164 to 7611	33.83 *	84.71	43.45	Broken stick



**Figure 3.** SHE analysis of diversity for the Cerro Bufo El Diente and for each one of altitudinal sites. H diversity (Shannon index); ln E natural logarithm of evenness; ln E/ ln S quotient of two previous.



Of the 243 species recorded in the Cerro Bufo El Diente, 98 were distributed along the entire altitudinal gradient, 64 were recorded only in two sites, and 81 were unique to one of the three sites. Of these 81 unique species, 50 were exclusively from Site 1, 19 for Site 2, and 12 for Site 3 (Appendix 1). The similarity values were greater than 50% between the nearest sites (Site 1 and 2, Site 2 and 3), and less than 50% between the more distant sites (Site 1 and 3). According to the cluster analysis, sites 1 and 2 composed an area of distribution for species that prefer warm climatic conditions, while Site 3 form an independent group of high mountain species (Figure 4).

### Seasonal variation of butterflies

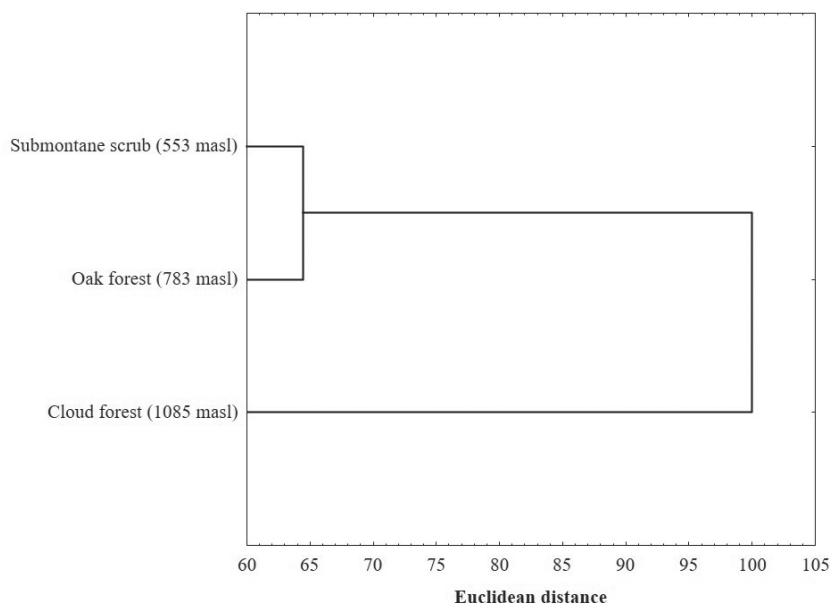
Differences in abundance and richness of Papilionoidea were found between early dry season and the other three seasons (Table 2, Figure 7). The highest number of specimens was obtained during the rainy season, with 2,707 individuals in the late period and 2,637 in the early one. Lower abundance was found during the late and early dry seasons (1,970 and 297 specimens, respectively) (Table 2). Species richness was higher in the late rainy season, with 207 species representing between 77.66 and 88.67% of estimated richness. Such value decreased to the early dry season (65 species, 67.89 to 93.75%), but increased at the end of dry season (165 species, 80.84 to 84.38%) (Table 2, Figure 5). Determination coefficients of the linear dependence model were higher than 0.90 for all seasons, while the slope values were greater than 0.1 (Table 2).

Highest values of temperature, precipitation, relative humidity and solar radiation were found during both periods of the rainy season (Figure 6). Relative humidity was highly correlated with abundance, while precipitation was better correlated with species richness. Interaction between climate variables compared with the abundance and species richness was positive; however, the correlation between abundance and solar radiation was not significant (Table 4).

According to diversity indices, early dry season was statistically different to the other three seasons ( $p < 0.05$ ) (Table 2). Shannon and Simpson indices indicated the highest diversity during the end of dry season and both periods of the rainy season. Lower diversity was found in early dry season (Table 2). Only 49 species from the total observed, were present during all seasons, 84 were recorded in three seasons, 66 in only two and 44 were exclusive of one season. Of these exclusive species, 19 were recorded

**Table 4.** Spearman correlations of abundance and richness of butterfly species with climatic factors in Cerro Bufo El Diente, Tamaulipas, Mexico. Marked (\*) correlations are significant at  $p < 0.05$ .

	Abundance	Species richness
Mean temperature (°C)	0.720 *	0.706 *
Total precipitation (mm)	0.734 *	0.713 *
Solar radiation (kJ)	0.580	0.608 *
Relative humidity (%)	0.748 *	0.664 *



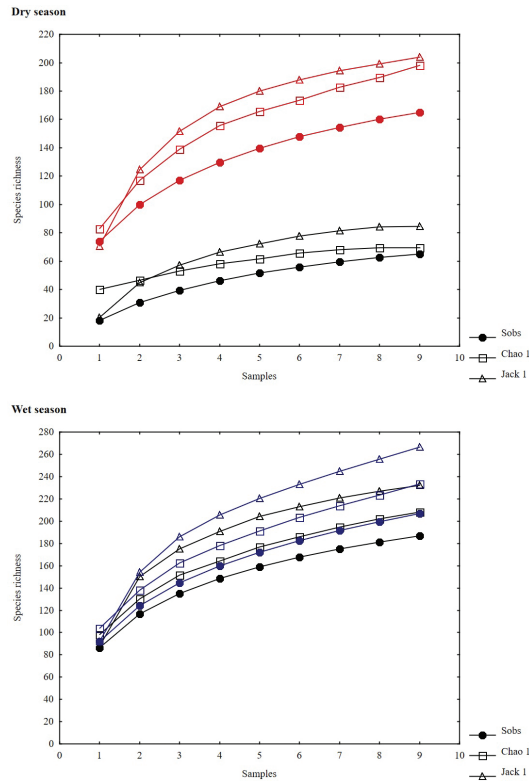
**Figure 4.** Cluster analysis from sites in the Cerro Bufo El Diente, Tamaulipas, Mexico.

at the end of the rainy season, 14 in the early rainy season, 10 at the beginning of the dry season, and only one in the late dry season (Table 2).

According to the Bray-Curtis index, the early and late rainy season had the greatest similarity (80.50%). Rest of the comparisons are above 50%, in the case of the end of the rainy season and the late dry season (72.57%), and from the early rainy season with the late dry season (68.20%), and below 50%, between the beginning and end of the dry season (24.26%), the beginning of the dry and rainy season (19.15%), and the end of the rainy season and the early dry season (19.11%). Cluster analysis shows the formation of two groups, according to the species composition in each season. The first group is composed only of species of the early dry season, and the second group includes species in the late dry season and the beginning and end of the rainy season (Figure 8).

### Indicator species

The IndVal allowed to quantify the percentage of association for the 243 species in the study area, of which 168 had a higher probability ( $p < 1$ ) of being considered as indicators (Appendix 1). Of these, 66 presented values equal to or greater than 50%, categorizing themselves as detectors or characteristics, while only 19 presented a significant indicator value ( $p < 0.05$ , Appendix 1). The remaining 75 species had association values equal to or less than 33.33%, with null probabilities ( $p = 1$ ) of being considered as characteristics of a habitat (Appendix 1). The detector species with the highest values of the index were: *Ministrymon azia* (Hewitson, 1873) (70.37%), *Urbanus procne* (Plötz, 1881) (63.89%) and *Chioides zilpa* (Butler, 1872) (57.14%)



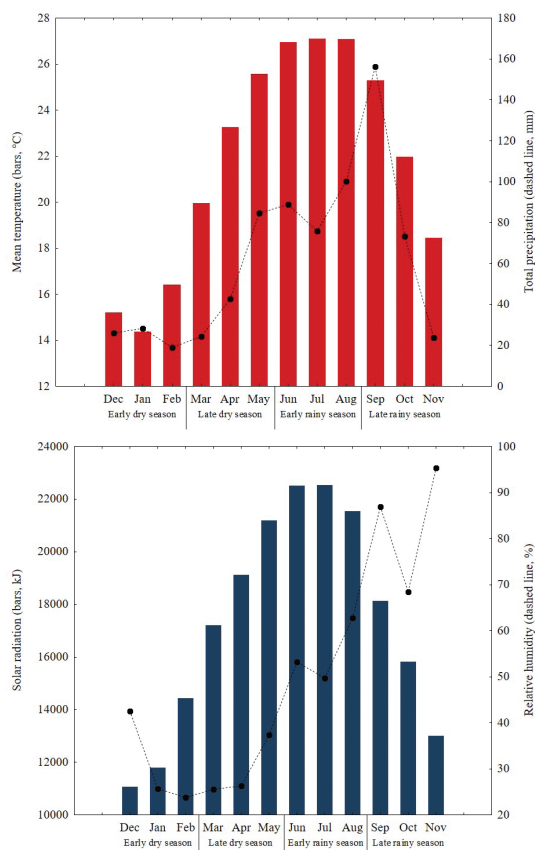
**Figure 5.** Species accumulation and estimator curves by season in the Cerro Bufo El Diente, Tamaulipas, Mexico. Upper graphic: Early dry season (black color) and late dry season (dark red color). Lower graphic: Early rainy season (black color) and late rainy season (dark blue color).

for the Site 1; *Achlyodes pallida* (R. Felder, 1869) (69.70%), *Smyrna blomfieldia datis* Fruhstorfer, 1908 (62.96%) and *Rekoa marius* (Lucas, 1857) (62.32%) for the Site 2; as well as *Amblyscirtes celia* Skinner, 1895 (60.61%) for the Site 3. With respect to the characteristic species, *Microtia elva elva* H. Bates, 1864 (94.23%), *Chlosyne theona bollii* (WH Edwards, 1877) (90.91%) *Heraclides anchisiades idaeus* Fabricius, 1793 (86.67%) presented the highest values for the Site 1; *Oarisma edwardsii* (W. Barnes, 1897) (83.33%) and *Quinta cannae* (Herrich-Schäffer, 1869) (80.00%) were characteristic species of the Site 3 (Appendix 1).

## Discussion

### Faunistic inventory and biodiversity of butterflies in Cerro Bufo El Diente

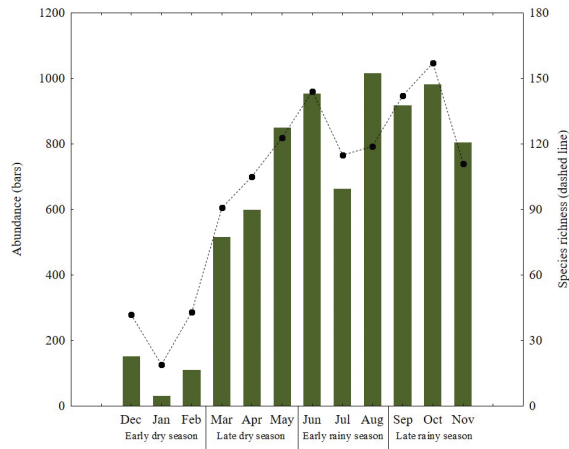
In Cerro Bufo El Diente, the superfamily Papilionoidea consists of 243 species that represent 69.43% of the richness recorded for Tamaulipas (García 2005; García et al. 2009), and 11.86% in relation Mexico (Warren 2000; Llorente et al. 2006). Hesperii-



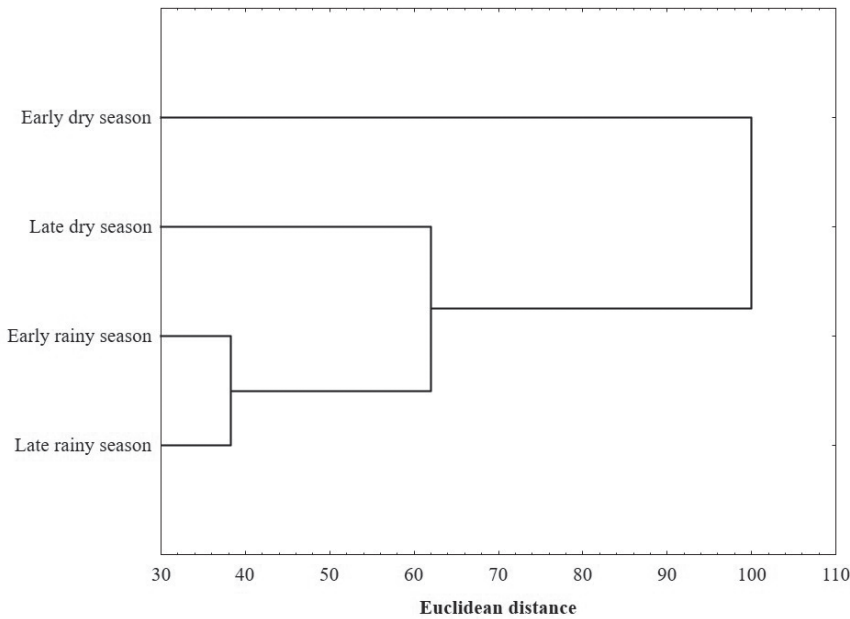
**Figure 6.** Monthly climate variation in Cerro Bufo El Diente, Tamaulipas, Mexico. Upper graphic: Variation of temperature and precipitation. Lower graphic: Variation of solar radiation and relative humidity.

dae are the family with greatest richness, which represents 75.68% of the diversity of the family for the State, and 9.78% in comparison with that of the country. The abundance and species richness by families found in this study is very different when compared to research conducted in other parts of the country. This is due to the specific biotic and abiotic characteristics of each ecoregion, which allow the development of a particular type of fauna (Espinosa and Ocegueda 2008), in this case of the butterflies. This also may be occurring at the species level, where the characteristics of the area, as well as the presence and abundance of its host plants will determine the dominant species (Luis and Llorente 1990; Vargas et al. 1994).

When comparing results found in this research with the few systematic and rigorously sampled inventories of Papilionoidea in Mexico, it can be observed that the species richness in the present study area is high. De la Luz and Madero (2011) in collaboration with the North American Butterfly Association listed 266 species for the state of Nuevo Leon. Luna-Reyes et al. (2010) recorded 145 species for the Lobos



**Figure 7.** Monthly variation of abundance and richness of butterfly species in Cerro Bufo El Diente, Tamaulipas, Mexico.



**Figure 8.** Cluster analysis from seasons in the Cerro Bufo El Diente, Tamaulipas, Mexico.

Canyon, Yauatepec, Morelos state. In the same way, Peña-Morales (2009) listed 120 for two fragments of tropical deciduous forest from the state of Tamaulipas, one in Gómez Farías and the other in Victoria. Luna-Reyes et al. (2008) recorded 142 species for the Huautla mountain range, in the states of Morelos and Puebla. Hernández-Mejía et al. (2008) listed 213 species for Malinalco, State of Mexico. Luna-Reyes and Llorente

(2004) listed 85 species for four entities that comprise the Sierra Nevada. Díaz-Batres et al. (2001) recorded 55 species in La Michilía, Durango state. Bizuet-Flores et al. (2001) obtained 69 species for El Chico National Park, Hidalgo state. Luis and Llorente (1993) listed 161 species for Omiltemi Park, Guerrero. Balcázar (1993) presented 205 species for Pedernales, Michoacán. Luis and Llorente (1990) recorded 65 species for the Dinamos, Magdalena Contreras, D.F. Beutelspacher (1982) listed 141 species for El Chorreadero, Chiapas. Considering that these authors used sampling methodologies similar to our study, it can be suggested that Cerro Bufa El Diente is a very important area for distribution and diversity of *Rhopalocera* in Tamaulipas and Mexico.

Richness estimators suggested that the diurnal butterfly fauna was obtained almost entirely in the Cerro Bufa El Diente, although it is possible that there are still some species to be recorded. In this regard, several authors point out that the increase in number of samples and time of study, or selection of other sampling methods, can aid in complementing faunistic inventories (Sparrow et al. 1994; Daily and Ehrlich 1995; DeVries et al. 1997, 1999; Hughes et al. 1998; Caldas and Robbins 2003; Jiménez et al. 2004; Romo and García 2005; Sackmann 2006; Hernández-Mejía et al. 2008; Bonebrake and Sorto 2009; Pedraza et al. 2010; Álvarez-García et al. 2016; González-Valdivia et al. 2016). However, the critical value in which a faunal inventory can be considered as reliable or complete is from 70% representativeness (observed richness in relation to estimated richness), since above that limit, the number of samples required to register all of the species increases remarkably and disproportionately (Jiménez and Hortal 2003). Taking into account the high percentage of representativeness obtained in this study, it would be necessary to conduct a large number of additional samples only to record a minimum number of possible missing species, since these are considered as accidental species that come from adjacent sites (Thomas 1994; Pozo et al. 2005; Hortal et al. 2006).

Comparing the number of species between different habitats is often enough to give a rapid assessment of a biodiversity measure. However, it is necessary to resort to the use of other statistical measures in order to make comparisons with other studies (Magurran 2004). In this investigation, quantification of diversity was done mainly by the values obtained from Shannon (4.16) and Simpson (0.98). The diversity index of Simpson gives a greater weight to the abundant species and underestimates rare ones, returning values between 0 (low diversity) to a maximum of  $1 - 1/S$  (Moreno 2001). Values of the Shannon index are usually between 1.5 and 3.5, rarely surpassing a value of 4 in very diverse communities (Margalef 1972). This suggests that diversity of butterflies in the study area is actually very high. Moreover, observed values were higher than the diversity present in some tropical communities, where the existing conditions favors a high number of species and individuals, as observed in Montero and Ortiz (2013) for Tablazo Paramo, Cundinamarca, and Camero et al. (2007) in Combeima River, department of Tolima, both in Colombia, and who obtained a total Shannon value of 3.9 for each zone. Accordingly, the broken stick distribution proposed by MacArthur (1960), corroborates ecology heterogeneity of Cerro Bufa El Diente according to the SHE analysis, corresponds



biologically to communities of species that colonize and distribute resources at random. In this type of distribution, the most common species are more susceptible to being invaded by the colonizing species than the rare species, resulting in a more equitable community (Gotelli and Graves 1996). The opposite of this distribution is the geometric series, since it reflects the lowest equity among the species of a community (Tokeshi 1990; Fattorini 2006).

On the other hand, the community structure of butterflies may represent evidence of the ecological characteristics of the study area, as a semi-preserved habitat. Community was formed by a moderate group of frequent species and few rare species, which is a characteristic pattern of areas with intermediate ecological quality. On the contrary, and according to Pedraza et al. (2010), a locality with excellent ecological quality is characterized by an assemblage with few frequent species, and a large number of scarce species. All this evidence agrees with previous values of ecological integrity obtained for Sierra de San Carlos (Arriaga et al. 2000). In addition, species that according to Pozo et al. (2005) and Raguso and Llorente (1991) are considered as indicators of disturbed habitats, were present in the study area.

### **Elevational effects on diversity patterns of butterflies**

Altitude is a variable frequently related to changes in species richness and abundance (Janzen 1993), producing changes in distribution patterns along altitudinal gradients (Llorente 1984; Andrade-Correa 2002), which was demonstrated in this study. In general, a negative correlation of altitude was observed with abundance or species richness; that is, a reduction in the number of specimens and species as the altitudinal gradient increases. According to Andrade-Correa (2002), it is observed that diversity and percentage of exclusive species decrease towards higher altitude areas. Moreover, Hernández-Mejía et al. (2008) states that the overall tendency of richness and abundance is to decrease with the altitudinal gradient. Although each family shows a different rate of decline, Nymphalidae decreases faster, which may be because of their higher number of species accentuates the altitudinal effect. Contrarily the Pieridae family comprises many eurytopic species, and therefore the change in richness is almost imperceptible as the altitude increases. In relation to the general abundance of each family, it can be observed how this decreased notably with the increase in altitude. This pattern in the number of individuals has been observed in other studies with butterflies (Luis and Llorente 1990; Vargas et al. 1994, 1999; Andrade-Correa 2002; Luna-Reyes and Llorente 2004; Palacios and Constantino 2006; Camero et al. 2007; Hernández et al. 2008; Ospina et al. 2010; De León 2012; Carrero et al. 2013), as well as in different groups of insects, such as the necrophilous entomofauna (Sánchez et al. 1993) and Scarabaeoidea beetles (Morón 1994).

The variation found in the community patterns could be originate in the abiotic factors that are modified along the altitudinal gradient, such as the air pressure (which decreases with the increase in altitude), solar radiation and precipitation (both increase

with the increase in elevation) (McCain and Grytnes 2010), as well as the increase of unfavorable environments and the reduction in availability of resources (Camero 2003; Camero et al. 2007). This can favor some species in particular, allowing them to increase their abundance at a certain altitudinal level, as was observed in the case of the species categorized as indicators, three for the last altitudinal site, three at the intermediate site and 13 for the first. In addition, the available area that species can occupy decreases with altitude (McCain and Grytnes 2010), which may cause a reduction in the number of individuals per species in higher sites (Camero 2003; Camero et al. 2007). Besides, the linear decrease in temperature, which decreases on average 0.68 °C per 100 meters of increase in elevation, is maybe one of the most important abiotic factors in the altitudinal distribution of species (McCain and Grytnes 2010). Therefore, the lower abundance in the higher altitude site could be related to its lower temperature, which represent an unfavorable factor for these insects (Kremen et al. 1993; Fagua 1999). The importance of this variable has also been observed in other studies of Lepidoptera (Luis and Llorente 1990; Vargas et al. 1994, 1999; Luna-Reyes and Llorente 2004; Hernández et al. 2008; De León 2012).

Vegetation is another factor of great influence for butterfly composition along altitudinal gradients (Llorente 1984; Luis et al. 2000). In the study area, the first altitudinal site corresponds to submontane scrub, which shows a high density of plants in the herbaceous and shrub layers (Briones 1991; Martínez 1998). Such condition represents a greater availability of food resources, allowing the increase in abundance of diurnal Lepidoptera in this area. On the other hand, the intermediate altitudinal site represents a transitional zone between the Papilionoidea fauna of the submontane scrub and the fauna of the cloud forest, which was corroborated with the Bray-Curtis index. In relation to this, the lower similarity between the extreme sites of the altitudinal gradient would be determined by the distance between both zones. In addition, the fact that the percentages of similarity were mostly greater than 50%, establishes that the compositions of the communities in the study area are similar in each site, this maybe because the Sierra de San Carlos and especially the rocky massif Bufo El Diente represent ideal sites for research on biodiversity over a period of a year due to its small area (Martínez 1998).

According to the behavior of both variables, abundance and diversity in the different sites, it can be suggested that vegetation and perhaps temperature and humidity are the determining factors in the abundance and richness of species of butterflies in the study area, parameters that decrease with altitude. Protecting populations of Papilionoidea in mountain areas, often depends on the conservation of lower adjacent areas, where the greatest abundance may occur (Andrade-Correa 2002). Another issue directly associated with the conservation of the populations, is that middle and high mountain areas are frequently used as natural corridors in the migration of butterfly species (Monteagudo et al. 2001). It is also necessary to take into account the displacements that occur from the lower parts towards the high elevation areas, because species search for foraging sites and better climatic conditions (Bonebrake et al. 2010). Therefore, biodiversity inventories along an altitudinal gradient, such as the one carried out

in this research, serve as monitoring studies of habitat quality, which allows identifying important areas in conservation and management policies (Dewenter and Teja 2000; Hoyle and Harbone 2005; Fattorini 2006).

### **Seasonal effects on diversity patterns of butterflies**

In general, the pattern of monthly variation of abundance and species richness was similar to the results reported by Luis and Llorente (1990), Luis et al. (1991), Vargas et al. (1994), Hernández-Mejía et al. (2008), Luna et al. (2008), Pozo et al. (2008) and Luna et al. (2010). From March to November, the greatest number of species and specimens was recorded, with lower values between December and February, the first months of the dry season. As for the Shannon index, both the end of the dry season as early and late wet season had the highest values, which were above 4.0, and so they represent a high diversity (Margalef 1972). In addition, the Bray-Curtis index and Cluster analysis indicated that late dry season and two periods of the wet season had a very different faunal composition compared to the early dry season. Therefore, the most favorable flight period for butterflies in the study area occurs during the last months of the dry season and the months corresponding to the wet season.

Seasonality is a very important factor in species distribution, being of great relevance for insects, since they cannot regulate their body temperature and therefore require favorable environmental conditions for metabolic activities and development of their life cycles (Brown 1984; Morón and Terrón 1984; Wolda 1988). Among the microclimatic factors that influence the seasonal distribution of butterflies in Cerro Bufa El Diente are the temperature and relative humidity. This temporal association is commonly recorded in tropical areas (Arteaga 1991; Luis et al. 1991; Vargas et al. 1992, 1999; Balcázar 1993), in which the imagos are most active during the early and late wet seasons, that is, when the availability of resources is greater, wintering in diapause (Scott 1979; Courtney 1986).

Additionally, butterflies are closely associated to plants, and their presence depends on the flora and structure of the vegetation (Shapiro 1974). Thus, it is possible that the wetter conditions in June to November favored the increase of diversity and biomass of the plant community, which can lead to the establishment of more species and larger populations of butterflies (Rhoades 1983). Temperature is more stable in this period, but humidity conditions are contrasting and remarkably superior with respect to the dry season, in which the total precipitation is 225 mm, while that in the rainy season is 518.7 mm. Although the first rains take place towards the end of May, the greater precipitation occurs from September to November, and as a consequence there is greater cloudiness that reduces evaporation. During this season, vegetation diversity and density increases, thus providing a greater amount of resources that are used by butterflies for their feeding, oviposition and protection, which favor the presence of more species with larger populations. Besides, the presence of rainfall correlates directly with abundance and richness of insects (Wolda 1988), since it affects the physiology of the re-

production, the ontogenetic development and the behavior of the imagoes; indirectly, it can also affect populations because of its effects on plant phenology (Vargas et al. 1999). As in other studies, the late wet season would represent the period where the greatest number of Lepidoptera species complete their diapause stage and begin their feeding, reproduction and oviposition stage (Owen 1971; Wolda 1988).

On the contrary, the highest variation in temperature as well as the highest number of clear days occur during the months of November to April, leading to high evaporation rates. Under these conditions, most of the vegetation is dry, especially some herbaceous plants that, when flowering, provide food for imagoes. During the drought period, water reserves of tree and shrub species are also reduced, modifying their growth, nectar production, nutritional content, or even texture and turgor of leaves, which constitute food resources for most lepidoptera species. Therefore, although trees and shrubs are present in the habitat, many of them cannot be used by butterflies during this period due to their deciduous phenology, affecting in this way the community composition and populations of butterflies in these months. In addition, some compounds present in plants can vary in each season and not be palatable in certain months, so they are not nutritious for the immature stages of many species. Nevertheless, it is possible that the species are in diapause during the cold months (Scott 1979).

The results obtained in this work may have implications for the conservation of biodiversity, mainly butterflies, as they provide information to build a research line focused on detecting the effects of climatic variations on the composition of species and providing an approximation of the behavior of its diversity. In the particular case of diurnal Lepidoptera, the impact of climate change on populations can be measured by monitoring the temporary replacement of the composition of species in the community and the environmental gradients of temperature and relative humidity. This information can be used in the evaluation and use of environmental services by pollination of a large variety of plants, which is carried out by Lepidoptera (Grøtan et al. 2012, 2014; Checa et al. 2014; Forrest 2016).

## Conclusions

For the first time in northeastern Mexico, the Papilionoidea group was systematically sampled during an annual sampling period. A total of 7,611 specimens belonging to six families, 20 subfamilies, 32 tribes, 148 genera, and 243 species of butterfly was collected from the study area. The highest abundance and richness of species, as well as alpha diversity was recorded in the lowest elevation site, and decreases significantly with increasing altitude, the tendency of altitudinal distribution of the Papilionoidea butterflies in Cerro Bufo El Diente is well defined to the environmental characteristics of the lower zone, agreeing with the Rapoport rule. The sites of low and intermediate altitude constitute an area of distribution of tropical species, while the site of the third floor forms an independent group of high mountain species, according to the conglomerate analysis carried out.

The greatest abundance and richness of species, as well as alpha diversity, was obtained during the late wet season, decreasing towards the beginning of the dry season. The geographical location of the study area plus the different plant compositions of the three sampled sites could be the main reason for the variation found here in the butterfly communities with altitude and season. In addition, relative humidity and temperature can influence the community of Rhopalocera in the study area; however, both abiotic factors directly affect plant composition, which is assumed to be the main factor in determining the composition and abundance of butterfly species.

This work is one of the first studies of diurnal butterflies in a specific area of north-eastern Mexico, in which altitude and season are analyzed. The information presented here provides reference data that allow the comparison of the diversity and richness of Papilionoidea species at a regional and national scale. This information could be used as an initial step to analyze the possible use of butterflies as a biodiversity indicator group in Mexico.

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## Appendix I

**Table 10.** Taxonomic list of Papilionoidea by season and site in Cerro Bufo El Diente, Tamaulipas, Mexico. Abundance (upper row) and indicator values (lower row) are indicated for each species. N = Total abundance; 1 = Submontane scrub, 553 m asl; 2 = Oak forest, 783 m asl; 3 = Cloud forest, 1085 m asl; marked species (\*) had a significant indicator value at  $p < 0.05$ .

Taxon	Dry Season						Rainy Season						N
	Early (Dec-Feb)			Late (Mar-May)			Early (Jun-Aug)			Late (Sep-Nov)			
	1	2	3	1	2	3	1	2	3	1	2	3	
Papilionidae Latreille, 1802													
Papilioninae Latreille, 1802													
Troidini Talbot, 1939													
<i>Battus philenor philenor</i> (Linnaeus, 1771)	0	0	0	9	11	6	2	2	3	5	2	3	43
	0.0	0.0	0.0	20.9	25.6	14.0	1.6	1.6	2.3	3.9	1.6	2.3	73.6
<i>Battus polydamas polydamas</i> (Linnaeus, 1758)	1	1	0	2	4	3	9	10	6	7	6	6	55
	0.6	0.6	0.0	1.2	2.4	1.8	16.4	18.2	10.9	12.7	10.9	10.9	86.7
Leptocircini W. F. Kirby, 1896													
<i>Protographium epidaus epidaus</i> (Doubleday, 1846)	0	0	0	1	0	0	0	0	0	2	0	0	3
	0.0	0.0	0.0	11.1	0.0	0.0	0.0	0.0	0.0	22.2	0.0	0.0	33.3
<i>Protographium philolaus philolaus</i> (Boisduval, 1836)	0	0	0	3	1	2	5	2	3	2	1	1	20
	0.0	0.0	0.0	5.0	1.7	3.3	16.7	6.7	10.0	3.3	1.7	1.7	50.0
Papilionini Latreille, 1802													
<i>Papilio polyxenes asterius</i> (Stoll, 1782)	2	1	0	8	5	3	6	5	5	8	9	5	57
	1.2	0.6	0.0	14.0	8.8	3.5	10.5	8.8	5.8	14.0	15.8	8.8	91.8
<i>Pterourus alexiarses garcia</i> Rothschild & Jordan, 1906	0	0	0	0	0	6	0	0	0	0	1	4	11
	0.0	0.0	0.0	0.0	0.0	36.4	0.0	0.0	0.0	0.0	3.0	12.1	51.5
<i>Pterourus pilumnus</i> Boisduval, 1836	0	0	0	4	4	2	4	6	4	6	3	3	36
	0.0	0.0	0.0	7.4	7.4	3.7	7.4	16.7	11.1	11.1	5.6	5.6	75.9
<i>Pterourus palamedes leontis</i> Rothschild & Jordan, 1906	0	0	0	0	0	0	0	3	0	2	1	0	6
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	11.1	5.6	0.0	50.0
<i>Pterourus garamas abderus</i> Höpffer, 1856	0	0	0	0	1	4	0	0	6	0	0	7	18
	0.0	0.0	0.0	0.0	1.9	14.8	0.0	0.0	33.3	0.0	0.0	38.9	88.9
<i>Pterourus victorinus victorinus</i> E. Doubleday, 1844	0	0	0	0	0	3	0	0	0	0	0	2	5
	0.0	0.0	0.0	0.0	0.0	40.0	0.0	0.0	0.0	0.0	0.0	13.3	53.3

Taxon	Dry Season						Rainy Season						N
	Early (Dec-Feb)			Late (Mar-May)			Early (Jun-Aug)			Late (Sep-Nov)			
	1	2	3	1	2	3	1	2	3	1	2	3	
<i>Heraclides crespontes</i> Cramer, 1777	1	1	0	0	0	0	19	14	11	25	12	6	89
	0.4	0.4	0.0	0.0	0.0	0.0	21.3	15.7	12.4	28.1	13.5	6.7	98.5
<i>Heraclides astyalus pallas</i> G. Gray, 1853	0	0	0	1	0	0	3	1	0	1	0	0	6
	0.0	0.0	0.0	5.6	0.0	0.0	16.7	5.6	0.0	5.6	0.0	0.0	33.3
<i>Heraclides ornythion</i> Boisduval, 1836	0	0	0	0	0	0	13	5	0	5	3	0	26
	0.0	0.0	0.0	0.0	0.0	0.0	33.3	12.8	0.0	12.8	7.7	0.0	66.7
<i>*Heraclides anchisiades idaeus</i> Fabricius, 1793	0	0	0	8	1	0	0	0	0	5	1	0	15
	0.0	0.0	0.0	53.3	2.2	0.0	0.0	0.0	0.0	33.3	2.2	0.0	91.1
Pieridae Swainson, 1820													
Coliadinae Swainson, 1821													
<i>Kricogonia lyside</i> (Godart, 1819)	4	2	0	14	8	5	28	24	20	19	21	14	159
	1.7	0.8	0.0	8.8	5.0	3.1	17.6	15.1	12.6	11.9	13.2	8.8	98.7
<i>Nathalis iole iole</i> Boisduval, 1836	2	1	0	5	3	8	1	3	3	6	6	10	48
	1.4	0.7	0.0	10.4	6.3	16.7	0.7	2.1	2.1	12.5	12.5	20.8	86.1
<i>Eurema daira eugenia</i> (Wallengren, 1860)	0	0	0	0	0	0	2	0	0	1	0	0	3
	0.0	0.0	0.0	0.0	0.0	0.0	22.2	0.0	0.0	11.1	0.0	0.0	33.3
<i>Eurema boisduvaliana</i> (C. Felder & R. Felder, 1865)	0	0	0	0	0	0	1	0	0	3	0	0	4
	0.0	0.0	0.0	0.0	0.0	0.0	8.3	0.0	0.0	50.0	0.0	0.0	58.3
<i>Eurema mexicana mexicana</i> (Boisduval, 1836)	1	1	0	8	6	2	4	5	2	10	5	2	46
	0.7	0.7	0.0	17.4	13.0	2.9	5.8	7.2	1.4	21.7	10.9	1.4	83.3
<i>Eurema salome jamapa</i> (Reakirt, 1866)	0	0	0	0	0	0	1	2	0	2	0	0	5
	0.0	0.0	0.0	0.0	0.0	0.0	6.7	13.3	0.0	13.3	0.0	0.0	33.3
<i>Abaeis nicippe</i> (Cramer, 1779)	0	0	0	6	8	3	13	5	7	4	5	1	52
	0.0	0.0	0.0	7.7	10.3	1.9	25.0	9.6	13.5	5.1	6.4	0.6	80.1
<i>Pyrisitia proterpia</i> (Fabricius, 1775)	4	4	0	6	7	3	9	6	4	8	3	0	54
	7.4	4.9	0.0	11.1	13.0	3.7	16.7	11.1	4.9	14.8	3.7	0.0	91.4
<i>Pyrisitia lisa centralis</i> (Herrich-Schäffer, 1865)	5	4	0	12	10	3	12	8	9	12	10	6	91
	5.5	2.9	0.0	13.2	11.0	2.2	13.2	8.8	9.9	13.2	11.0	4.4	95.2
<i>Pyrisitia nise nelphe</i> (R. Felder, 1869)	5	1	0	11	10	7	9	9	2	10	9	4	77
	6.5	0.4	0.0	14.3	13.0	9.1	11.7	7.8	0.9	13.0	11.7	5.2	93.5
<i>Pyrisitia dina westwoodii</i> (Boisduval, 1836)	0	0	0	0	0	0	0	0	0	6	3	0	9
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	44.4	22.2	0.0	66.7
<i>Colias eurytheme</i> Boisduval, 1832	0	0	0	0	0	0	0	0	0	1	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	33.3
<i>Zerene cesonia cesonia</i> (Stoll, 1790)	1	2	0	2	3	5	20	10	11	31	15	7	107
	0.3	0.6	0.0	0.6	0.9	1.6	18.7	9.3	10.3	29.0	14.0	6.5	91.9
<i>Anteos clorinde</i> (Godart, 1824)	0	0	0	0	0	0	1	1	2	1	5	5	15
	0.0	0.0	0.0	0.0	0.0	0.0	2.2	2.2	4.4	2.2	33.3	22.2	66.7
<i>*Anteos maerula</i> (Fabricius, 1775)	2	0	0	0	0	0	10	6	3	8	5	4	38
	3.5	0.0	0.0	0.0	0.0	0.0	26.3	15.8	5.3	21.1	13.2	7.0	92.1
<i>Phoebis sennae marcellina</i> (Cramer, 1777)	4	3	1	19	16	9	15	6	10	19	14	7	123
	2.2	2.4	0.3	15.4	13.0	7.3	12.2	4.9	8.1	15.4	11.4	5.7	98.4
<i>Phoebis philea philea</i> (Linnaeus, 1763)	1	0	0	0	0	0	5	7	7	11	4	3	38
	0.9	0.0	0.0	0.0	0.0	0.0	13.2	18.4	12.3	28.9	10.5	5.3	89.5
<i>Phoebis argante argante</i> (Fabricius, 1775)	0	0	0	1	2	0	2	3	1	4	3	1	17
	0.0	0.0	0.0	2.0	3.9	0.0	3.9	5.9	2.0	15.7	5.9	2.0	41.2
<i>Phoebis agarithe agarithe</i> (Boisduval, 1836)	2	1	3	25	15	12	24	23	14	22	17	9	167
	0.4	0.2	0.6	15.0	9.0	7.2	14.4	13.8	8.4	13.2	10.2	5.4	97.6
Pierinae Swainson, 1820													
Pierini Swainson, 1820													
<i>Glutophrissa drusilla tenuis</i> (Lamas, 1981)	0	0	0	6	7	5	9	5	5	7	5	7	56
	0.0	0.0	0.0	7.1	8.3	6.0	10.7	6.0	6.0	8.3	6.0	8.3	66.7
<i>Pieriballia viardi viardi</i> (Boisduval, 1836)	0	0	0	0	0	0	0	0	0	1	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	33.3
<i>Pontia protodice</i> (Boisduval & Le Conte, 1830)	0	0	0	0	0	0	15	16	20	3	3	4	61
	0.0	0.0	0.0	0.0	0.0	0.0	24.6	26.2	32.8	1.6	1.6	2.2	89.1
<i>Ascia monuste monuste</i> (Linnaeus, 1764)	0	0	0	0	0	0	6	5	2	5	7	3	28
	0.0	0.0	0.0	0.0	0.0	0.0	14.3	11.9	2.4	11.9	16.7	3.6	60.7

Taxon	Dry Season						Rainy Season						N
	Early (Dec-Feb)			Late (Mar-May)			Early (Jun-Aug)			Late (Sep-Nov)			
	1	2	3	1	2	3	1	2	3	1	2	3	
<i>Ganyra josephina josepha</i> (Salvin & Godman, 1868)	0	0	0	3	1	1	8	6	7	12	8	3	49
	0.0	0.0	0.0	2.0	0.7	0.7	16.3	8.2	9.5	24.5	16.3	2.0	80.3
Lycaenidae Leach, 1815													
Theclinae Swainson, 1831													
Eumaeini E. Doubleday, 1847													
<i>Eumaeus childrenae</i> (G. Gray, 1832)	0	3	0	3	3	2	17	13	18	8	13	7	87
	0.0	1.1	0.0	1.1	1.1	0.8	19.5	14.9	20.7	9.2	14.9	5.4	88.9
<i>Atlides halesus corcorani</i> Clench, 1942	0	0	0	1	0	0	0	0	0	0	0	0	1
	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Rekoa palegon</i> (Cramer, 1780)	0	0	0	0	1	0	0	1	0	1	0	0	3
	0.0	0.0	0.0	0.0	11.1	0.0	0.0	11.1	0.0	11.1	0.0	0.0	33.3
<i>*Rekoa marius</i> (Lucas, 1857)	1	2	0	0	0	0	3	6	0	3	7	1	23
	1.4	5.8	0.0	0.0	0.0	0.0	8.7	26.1	0.0	13.0	30.4	1.4	87.0
<i>Anawacus jada</i> (Hewitson, 1867)	0	0	0	0	1	0	0	1	0	1	0	0	3
	0.0	0.0	0.0	0.0	11.1	0.0	0.0	11.1	0.0	11.1	0.0	0.0	33.3
<i>Ocaria ocrisia</i> (Hewitson, 1868)	0	0	0	0	0	0	0	0	0	0	1	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	33.3
<i>*Chlorostrymon simaethis sarita</i> (Skinner, 1895)	0	0	0	0	0	0	3	0	0	14	1	0	18
	0.0	0.0	0.0	0.0	0.0	0.0	5.6	0.0	0.0	77.8	1.9	0.0	85.2
<i>Cyanophrys herodotus</i> (Fabricius, 1793)	0	0	0	3	1	1	2	0	0	3	1	0	11
	0.0	0.0	0.0	9.1	3.0	3.0	6.1	0.0	0.0	9.1	3.0	0.0	33.3
<i>Cyanophrys miserabilis</i> (Clench, 1946)	0	0	0	1	0	0	3	0	0	1	0	0	5
	0.0	0.0	0.0	6.7	0.0	0.0	40.0	0.0	0.0	6.7	0.0	0.0	53.3
<i>Cyanophrys longula</i> (Hewitson, 1868)	0	0	0	0	1	1	0	0	1	0	0	0	3
	0.0	0.0	0.0	0.0	11.1	11.1	0.0	0.0	11.1	0.0	0.0	0.0	33.3
<i>Callophrys xami texami</i> Clench, 1981	0	0	0	0	0	1	0	0	0	0	0	0	1
	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Allosmaitia strophius</i> (Godart, 1824)	0	0	0	0	0	0	0	0	0	1	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	33.3
<i>Electrostrymon hugon</i> (Godart, 1824)	0	0	0	1	2	0	3	3	0	2	1	0	12
	0.0	0.0	0.0	2.8	11.1	0.0	8.3	16.7	0.0	5.6	2.8	0.0	47.2
<i>Electrostrymon guzanta</i> (Schaus, 1902)	0	0	0	0	0	0	2	0	0	2	1	0	5
	0.0	0.0	0.0	0.0	0.0	0.0	13.3	0.0	0.0	26.7	6.7	0.0	46.7
<i>Calycopis isobeeon</i> (Butler & H. Druce, 1872)	1	2	0	7	11	1	3	11	3	5	13	1	58
	0.6	2.3	0.0	12.1	19.0	0.6	3.4	12.6	1.7	5.7	14.9	0.6	73.6
<i>Strymon melinus melinus</i> Hübner, 1818	2	0	0	5	5	3	22	15	5	8	5	1	71
	0.9	0.0	0.0	2.3	2.3	1.4	31.0	21.1	7.0	7.5	4.7	0.5	78.9
<i>Strymon rufofusca</i> (Hewitson, 1877)	2	0	0	0	0	0	1	0	0	4	0	0	7
	9.5	0.0	0.0	0.0	0.0	0.0	4.8	0.0	0.0	38.1	0.0	0.0	52.4
<i>Strymon albata</i> (C. Felder & R. Felder, 1865)	0	0	0	0	0	0	1	0	0	0	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	33.3
<i>Strymon alea</i> (Godman & Salvin, 1887)	0	0	0	1	0	0	4	0	0	8	0	0	13
	0.0	0.0	0.0	2.6	0.0	0.0	20.5	0.0	0.0	41.0	0.0	0.0	64.1
<i>Strymon bebrycia</i> (Hewitson, 1868)	0	0	0	1	0	0	0	0	0	2	0	0	3
	0.0	0.0	0.0	11.1	0.0	0.0	0.0	0.0	0.0	44.4	0.0	0.0	55.6
<i>Strymon yojoa</i> (Reakirt, 1867)	1	0	0	0	0	0	0	0	0	0	0	0	1
	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Strymon bazochii bazochii</i> (Godart, 1824)	0	0	0	0	0	0	4	2	0	5	1	0	12
	0.0	0.0	0.0	0.0	0.0	0.0	22.2	11.1	0.0	41.7	2.8	0.0	77.8
<i>Strymon istapa istapa</i> (Reakirt, 1867)	1	1	0	10	6	3	6	10	1	14	7	1	60
	0.6	0.6	0.0	16.7	10.0	1.7	10.0	16.7	0.6	23.3	11.7	0.6	92.2
<i>Strymon senapio</i> (Godman & Salvin, 1887)	0	0	0	0	0	0	0	1	0	0	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	33.3
<i>Ministrymon clytie</i> (W. H. Edwards, 1877)	3	0	0	4	5	1	9	10	3	9	7	1	52
	3.8	0.0	0.0	5.1	6.4	0.6	17.3	19.2	3.8	17.3	13.5	0.6	87.8
<i>*Ministrymon azia</i> (Hewitson, 1873)	0	0	0	5	0	0	2	0	0	2	0	0	9
	0.0	0.0	0.0	55.6	0.0	0.0	7.4	0.0	0.0	7.4	0.0	0.0	70.4
<i>Strephonota tephraeus</i> (Geyer, 1837)	0	0	0	0	0	0	0	0	0	0	1	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	33.3

Taxon	Dry Season						Rainy Season						N
	Early (Dec-Feb)			Late (Mar-May)			Early (Jun-Aug)			Late (Sep-Nov)			
	1	2	3	1	2	3	1	2	3	1	2	3	
<i>Panthiades bathildis</i> (C. Felder & R. Felder, 1865)	0	0	0	0	0	0	0	0	0	1	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	33.3
<i>Parrhasius moctezuma</i> (Clench, 1971)	0	0	0	0	0	0	0	1	0	1	1	0	3
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.1	0.0	11.1	11.1	0.0	33.3
Polyommatinae Swainson, 1827													
<i>Leptotes cassius cassidula</i> (Boisduval, 1870)	6	2	0	18	12	6	15	7	2	13	12	5	98
	6.1	1.4	0.0	18.4	12.2	6.1	15.3	7.1	0.7	13.3	12.2	3.4	96.3
<i>Leptotes marina</i> (Reakirt, 1868)	0	0	0	9	3	0	11	5	0	11	5	0	44
	0.0	0.0	0.0	13.6	4.5	0.0	25.0	7.6	0.0	16.7	7.6	0.0	75.0
<i>Brephidium exilis exilis</i> (Boisduval, 1852)	0	0	0	0	0	0	1	0	0	0	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	33.3
<i>Zizula cyna</i> (W. H. Edwards, 1881)	0	0	0	2	0	0	2	0	0	1	0	0	5
	0.0	0.0	0.0	26.7	0.0	0.0	13.3	0.0	0.0	6.7	0.0	0.0	46.7
<i>Cupido comyntas comyntas</i> (Godart, 1824)	0	0	0	1	0	0	0	0	0	0	0	0	1
	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Celastrina ladon</i> (Cramer, 1780)	0	0	1	1	0	1	0	0	0	0	0	2	5
	0.0	0.0	6.7	6.7	0.0	6.7	0.0	0.0	0.0	0.0	0.0	13.3	33.3
<i>Echinargus isola</i> (Reakirt, 1867)	0	0	0	15	10	7	16	9	4	16	10	5	92
	0.0	0.0	0.0	16.3	10.9	7.6	17.4	9.8	2.9	17.4	10.9	3.6	96.7
<i>*Hemiargus ceraunus astenidas</i> (Lucas, 1857)	2	0	0	15	7	1	9	4	1	10	9	3	61
	2.2	0.0	0.0	24.6	11.5	0.5	9.8	4.4	0.5	16.4	14.8	3.3	88.0
Riodinidae Grote, 1895													
Euselasiinae Kirby, 1871													
<i>Euselasia eubule</i> (R. Felder, 1869)	0	0	0	0	0	0	0	1	0	0	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	33.3
Riodininae Grote, 1895													
<i>*Calephelis nemesis australis</i> (W. H. Edwards, 1877)	0	0	0	3	1	1	16	9	3	11	9	2	55
	0.0	0.0	0.0	1.8	0.6	0.6	29.1	16.4	3.6	20.0	16.4	1.2	89.7
<i>Calephelis perditalis perditalis</i> W. Barnes & McDunnough, 1918	0	0	0	16	12	5	14	7	2	15	12	3	86
	0.0	0.0	0.0	18.6	14.0	5.8	16.3	8.1	0.8	17.4	14.0	2.3	97.3
<i>Calephelis rawsoni</i> McAlpine, 1939	0	0	0	0	0	0	1	0	0	0	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	33.3
<i>Caria ino melicerta</i> Schaus, 1890	0	0	0	10	6	1	10	6	1	9	5	1	49
	0.0	0.0	0.0	20.4	12.2	0.7	20.4	8.2	0.7	18.4	10.2	0.7	91.8
<i>Lasaia sula peninsularis</i> Clench, 1972	0	0	0	0	0	0	0	0	0	3	0	0	3
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	33.3
<i>Emesis tenedia</i> C. Felder & R. Felder, 1861	2	0	0	0	0	0	3	7	4	4	9	7	36
	1.9	0.0	0.0	0.0	0.0	0.0	2.8	13.0	11.1	7.4	25.0	19.4	80.6
<i>Emesis emesia</i> (Hewitson, 1867)	2	1	0	0	0	0	9	5	1	14	12	6	50
	1.3	0.7	0.0	0.0	0.0	0.0	18.0	6.7	0.7	28.0	24.0	12.0	91.3
<i>Apodemia hypoglaucia hypoglaucia</i> (Godman & Salvin, 1878)	0	0	0	0	0	1	0	0	1	0	1	0	3
	0.0	0.0	0.0	0.0	0.0	11.1	0.0	0.0	11.1	0.0	11.1	0.0	33.3
<i>Apodemia walkeri</i> Godman & Salvin, 1886	0	0	0	0	0	0	0	0	0	0	1	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	33.3
Nymphalidae Rafinesque, 1815													
Libytheinae Boisduval, 1833													
<i>Libytheana carinenta larvata</i> (Strecker, 1878)	0	0	0	27	13	5	56	33	18	37	16	8	213
	0.0	0.0	0.0	8.5	4.1	0.8	26.3	15.5	8.5	17.4	7.5	2.5	90.9
Danainae Boisduval, 1833													
Danaini Boisduval, 1833													
<i>Danaus plexippus plexippus</i> (Linnaeus, 1758)	0	0	0	2	3	3	0	0	0	3	3	9	23
	0.0	0.0	0.0	2.9	4.3	4.3	0.0	0.0	0.0	8.7	8.7	26.1	55.1
<i>Danaus gilippus thersippus</i> (H. Bates, 1863)	5	2	0	12	7	3	14	10	5	12	10	6	86
	5.8	1.6	0.0	14.0	8.1	2.3	16.3	11.6	3.9	14.0	11.6	7.0	96.1
<i>Danaus eresimus montezuma</i> Talbot, 1943	2	0	0	0	0	0	5	2	1	14	9	6	39
	1.7	0.0	0.0	0.0	0.0	0.0	8.5	1.7	0.9	35.9	23.1	15.4	87.2

Taxon	Dry Season						Rainy Season						N
	Early (Dec-Feb)			Late (Mar-May)			Early (Jun-Aug)			Late (Sep-Nov)			
	1	2	3	1	2	3	1	2	3	1	2	3	
<b>Heliconiinae Swainson, 1822</b>													
<b>Heliconiini Swainson, 1822</b>													
<i>Agraulis vanillae incarnata</i> (N. Riley, 1926)	5	2	1	14	10	6	13	9	2	12	7	3	84
	4.0	1.6	0.4	16.7	11.9	7.1	15.5	10.7	1.6	14.3	8.3	2.4	94.4
<i>Dione moneta poeyii</i> Butler, 1873	0	0	0	4	1	0	2	1	0	5	4	1	18
	0.0	0.0	0.0	14.8	1.9	0.0	3.7	1.9	0.0	18.5	14.8	1.9	57.4
<i>Dryadula phaetusa</i> (Linnaeus, 1758)	0	0	0	0	0	0	0	0	0	1	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	33.3
<i>Dryas iulia moderata</i> (N. Riley, 1926)	0	0	0	13	11	3	12	9	4	12	7	1	72
	0.0	0.0	0.0	18.1	15.3	2.8	16.7	12.5	3.7	16.7	9.7	0.5	95.8
<i>Eueides isabella eva</i> (Fabricius, 1793)	0	0	0	0	0	0	0	0	0	1	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	33.3
<i>Heliconius charithonia vazquezae</i> W. Comstock & F. Brown, 1950	7	6	1	14	11	3	14	12	5	20	10	6	109
	4.3	3.7	0.3	8.6	6.7	0.9	8.6	7.3	3.1	18.3	9.2	3.7	74.6
<b>Argynniini Swainson, 1833</b>													
<i>Euptoieta claudia</i> (Cramer, 1775)	0	0	0	7	3	0	11	8	1	5	3	1	39
	0.0	0.0	0.0	12.0	2.6	0.0	28.2	20.5	0.9	8.5	2.6	0.9	76.1
<i>Euptoieta hegesia meridiania</i> Stichel, 1938	0	2	0	4	3	1	14	14	4	14	10	2	68
	0.0	1.0	0.0	2.0	1.5	0.5	20.6	20.6	3.9	20.6	14.7	1.0	86.3
<b>Limenitidinae Behr, 1864</b>													
<b>Limenitidini Behr, 1864</b>													
<i>Limnitis arthemis astyanax</i> (Fabricius, 1775)	0	0	0	0	2	1	0	5	1	0	5	2	16
	0.0	0.0	0.0	0.0	4.2	2.1	0.0	20.8	2.1	0.0	20.8	4.2	54.2
<i>Adelpha eulalia</i> (E. Doubleday, 1848)	0	0	0	0	0	2	0	0	0	0	0	2	4
	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	16.7	50.0
<i>Adelpha paraena massilia</i> (C. Felder & R. Felder, 1867)	0	0	0	0	0	0	1	0	0	0	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	33.3
<i>Adelpha fessonia fessonia</i> (Hewitson, 1847)	1	0	0	12	9	6	11	9	3	13	12	3	79
	0.4	0.0	0.0	15.2	11.4	7.6	13.9	11.4	1.3	16.5	15.2	2.5	95.4
<i>Adelpha basiloides</i> (H. Bates, 1865)	0	0	0	8	8	1	7	7	2	7	3	1	44
	0.0	0.0	0.0	18.2	18.2	0.8	10.6	15.9	1.5	10.6	2.3	0.8	78.8
<b>Apaturinae Boisduval, 1840</b>													
<i>Asterocampa celtis antonia</i> (W. H. Edwards, 1878)	0	0	0	4	0	0	9	7	0	6	8	1	35
	0.0	0.0	0.0	3.8	0.0	0.0	17.1	13.3	0.0	11.4	15.2	1.0	61.9
<i>Asterocampa leilia</i> (W. H. Edwards, 1874)	0	0	0	7	1	0	7	0	0	7	2	0	24
	0.0	0.0	0.0	19.4	1.4	0.0	19.4	0.0	0.0	19.4	2.8	0.0	62.5
<i>Asterocampa clyton louisa</i> D. Stallings & Turner, 1947	0	0	0	0	0	0	7	4	0	0	0	0	11
	0.0	0.0	0.0	0.0	0.0	0.0	42.4	24.2	0.0	0.0	0.0	0.0	66.7
<i>Asterocampa idyia argus</i> (H. Bates, 1864)	0	0	0	0	0	0	0	1	0	2	2	0	5
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7	0.0	13.3	26.7	0.0	46.7
<i>Doxocopa pavon theodora</i> (Lucas, 1857)	0	0	0	0	1	0	0	0	0	0	0	0	1
	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Doxocopa laure laure</i> (Drury, 1773)	0	0	0	0	0	0	6	1	1	4	1	0	13
	0.0	0.0	0.0	0.0	0.0	0.0	30.8	2.6	2.6	20.5	2.6	0.0	59.0
<b>Biblidinae Boisduval, 1833</b>													
<b>Biblidini Boisduval, 1833</b>													
<i>Biblis hyperia aganisa</i> Boisduval, 1836	1	1	0	13	13	6	12	10	2	9	9	1	77
	0.4	0.4	0.0	16.9	16.9	7.8	15.6	13.0	1.7	11.7	11.7	0.4	96.5
<i>Mestra amymone</i> (Ménétriés, 1857)	10	3	0	23	16	7	30	21	8	29	16	9	172
	5.8	1.2	0.0	13.4	9.3	4.1	17.4	12.2	3.1	16.9	9.3	5.2	97.9
<b>Catonephelini Orfila, 1952</b>													
<i>Eunica tatila tatila</i> (Herrich-Schäffer, 1855)	5	3	0	13	13	8	21	16	7	27	20	9	142
	1.2	0.7	0.0	9.2	9.2	5.6	14.8	11.3	3.3	19.0	14.1	6.3	94.6
<i>Eunica monima</i> (Stoll, 1782)	0	0	0	0	0	0	10	6	3	7	8	2	36
	0.0	0.0	0.0	0.0	0.0	0.0	27.8	16.7	5.6	19.4	22.2	3.7	95.4
<i>Myscelia ethusa ethusa</i> (Doyère, 1840)	0	0	0	7	5	4	12	12	6	7	9	3	65
	0.0	0.0	0.0	7.2	5.1	4.1	18.5	18.5	9.2	7.2	9.2	3.1	82.1



Taxon	Dry Season						Rainy Season						N
	Early (Dec-Feb)			Late (Mar-May)			Early (Jun-Aug)			Late (Sep-Nov)			
	1	2	3	1	2	3	1	2	3	1	2	3	
Ageroniini E. Doubleday, 1847													
<i>Hamadryas februa ferentina</i> (Godart, 1824)	4	2	0	12	8	3	8	7	5	12	13	7	81
	3.3	0.8	0.0	14.8	9.9	2.5	9.9	8.6	4.1	14.8	16.0	8.6	93.4
<i>Hamadryas glauconome glauconome</i> (H. Bates, 1864)	0	0	0	9	5	2	7	4	1	7	6	3	44
	0.0	0.0	0.0	20.5	11.4	3.0	15.9	6.1	0.8	15.9	9.1	4.5	87.1
<i>Hamadryas guatemalena marmarice</i> (Fruhstorfer, 1916)	0	0	0	0	0	0	0	0	0	2	0	0	2
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	33.3
Epiphelini Jenkins, 1987													
<i>Epiphile adrasta adrasta</i> Hewitson, 1861	0	0	0	0	3	3	1	7	3	1	8	6	32
	0.0	0.0	0.0	0.0	3.1	3.1	1.0	14.6	6.3	1.0	25.0	18.8	72.9
Eubagini Burmeister, 1878													
<i>Dynamine dyonis</i> Geyer, 1837	0	0	0	0	0	0	3	1	0	3	1	0	8
	0.0	0.0	0.0	0.0	0.0	0.0	25.0	4.2	0.0	12.5	4.2	0.0	45.8
Cyrestinae Guenée, 1865													
Cyrestini Guenée, 1865													
<i>Marpesia chiron</i> (Fabricius, 1775)	0	0	0	0	0	0	0	0	0	1	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	33.3
<i>*Marpesia petreus</i> (Cramer, 1776)	0	0	0	0	0	0	1	0	0	4	0	0	5
	0.0	0.0	0.0	0.0	0.0	0.0	6.7	0.0	0.0	80.0	0.0	0.0	86.7
Nymphalinae Rafinesque, 1815													
Coeini Scudder, 1893													
<i>Historis acheronta acheronta</i> (Fabricius, 1775)	0	0	0	0	0	0	0	0	0	0	1	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	33.3
Nymphalini Rafinesque, 1815													
<i>*Smyrna blomfieldia datis</i> Fruhstorfer, 1908	0	0	0	0	3	1	3	1	0	0	9	1	18
	0.0	0.0	0.0	0.0	11.1	1.9	5.6	1.9	0.0	0.0	50.0	1.9	72.2
<i>Vanessa virginiensis</i> (Drury, 1773)	0	0	0	2	1	1	6	5	2	3	1	0	21
	0.0	0.0	0.0	3.2	1.6	1.6	19.0	15.9	6.3	4.8	1.6	0.0	54.0
<i>Vanessa cardui</i> (Linnaeus, 1758)	0	0	0	0	4	0	2	4	0	6	3	0	19
	0.0	0.0	0.0	0.0	14.0	0.0	3.5	14.0	0.0	21.1	10.5	0.0	63.2
<i>Vanessa atalanta rubria</i> (Fruhstorfer, 1909)	0	0	0	14	10	8	12	16	10	22	10	8	110
	0.0	0.0	0.0	12.7	9.1	7.3	10.9	14.5	9.1	20.0	9.1	7.3	100
<i>Nymphalis antiopa antiopa</i> (Linnaeus, 1758)	0	0	0	0	0	0	0	0	0	0	1	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	33.3
<i>Polygonia interrogationis</i> (Fabricius, 1798)	0	0	0	0	3	0	2	4	0	0	2	0	11
	0.0	0.0	0.0	0.0	9.1	0.0	6.1	12.1	0.0	0.0	6.1	0.0	33.3
Victorinini Scudder, 1893													
<i>Anartia jatrophae luteipicta</i> (Fruhstorfer, 1907)	3	2	0	5	2	2	14	13	6	22	11	5	85
	1.2	0.8	0.0	2.0	0.8	0.8	16.5	15.3	7.1	25.9	12.9	3.9	87.1
<i>*Anartia fatima fatima</i> (Fabricius, 1793)	0	0	0	21	3	0	8	7	0	18	1	0	58
	0.0	0.0	0.0	36.2	1.7	0.0	9.2	8.0	0.0	31.0	0.6	0.0	86.8
<i>Siproeta stelenes biplagiata</i> (Fruhstorfer, 1907)	0	0	0	3	3	1	8	6	3	6	5	0	35
	0.0	0.0	0.0	2.9	2.9	1.0	15.2	11.4	5.7	11.4	9.5	0.0	60.0
Junoniini Reuter, 1896													
<i>Junonia coenia coenia</i> Hübner, 1822	0	0	0	2	1	0	0	0	0	4	1	0	8
	0.0	0.0	0.0	8.3	4.2	0.0	0.0	0.0	0.0	33.3	4.2	0.0	50.0
<i>Junonia evarete</i> (Cramer, 1779)	0	0	0	0	0	0	0	0	0	0	1	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	33.3
Melitaeini Newman, 1870													
<i>Chlosyne janais janais</i> (Drury, 1782)	5	0	0	13	9	4	16	6	1	11	12	2	79
	4.2	0.0	0.0	16.5	11.4	3.4	20.3	7.6	0.4	13.9	15.2	1.7	94.5
<i>Chlosyne definita definita</i> (E. Aaron, 1885)	0	0	0	3	0	0	2	0	0	1	0	0	6
	0.0	0.0	0.0	33.3	0.0	0.0	11.1	0.0	0.0	5.6	0.0	0.0	50.0
<i>Chlosyne melitaeoides</i> (C. Felder & R. Felder, 1867)	0	0	0	0	0	0	3	1	0	9	0	0	13
	0.0	0.0	0.0	0.0	0.0	0.0	7.7	2.6	0.0	46.2	0.0	0.0	56.4
<i>Chlosyne endeis pardelina</i> Scott, 1986	0	0	0	0	0	0	2	0	0	5	1	0	8
	0.0	0.0	0.0	0.0	0.0	0.0	8.3	0.0	0.0	41.7	4.2	0.0	54.2

Taxon	Dry Season						Rainy Season						N
	Early (Dec-Feb)			Late (Mar-May)			Early (Jun-Aug)			Late (Sep-Nov)			
	1	2	3	1	2	3	1	2	3	1	2	3	
<i>Chlosyne rosita browni</i> Bauer, 1961	0	0	0	0	0	0	12	8	1	14	8	5	48
	0.0	0.0	0.0	0.0	0.0	0.0	16.7	11.1	0.7	19.4	11.1	6.9	66.0
* <i>Chlosyne theona bollii</i> (W. H. Edwards, 1877)	0	0	0	0	0	0	8	0	0	3	0	0	11
	0.0	0.0	0.0	0.0	0.0	0.0	72.7	0.0	0.0	18.2	0.0	0.0	90.9
<i>Chlosyne lacinia adjutrix</i> Scudder, 1875	2	0	0	7	4	1	11	8	0	19	7	0	59
	1.1	0.0	0.0	7.9	4.5	0.6	12.4	9.0	0.0	32.2	7.9	0.0	75.7
* <i>Microtia elva elva</i> H. Bates, 1864	0	0	0	0	0	0	14	1	0	35	2	0	52
	0.0	0.0	0.0	0.0	0.0	0.0	26.9	0.6	0.0	67.3	1.3	0.0	96.2
<i>Texola elada ulrica</i> (W. H. Edwards, 1877)	0	0	0	0	0	0	1	0	0	2	0	0	3
	0.0	0.0	0.0	0.0	0.0	0.0	11.1	0.0	0.0	22.2	0.0	0.0	33.3
<i>Anthanassa texana texana</i> (W. H. Edwards, 1863)	2	3	0	18	14	6	10	15	7	15	11	1	102
	0.7	1.0	0.0	17.6	13.7	5.9	9.8	14.7	4.6	14.7	10.8	0.3	93.8
<i>Anthanassa ardyi</i> (Hewitson, 1864)	0	0	0	0	3	1	0	0	0	0	1	0	5
	0.0	0.0	0.0	0.0	40.0	6.7	0.0	0.0	0.0	0.0	6.7	0.0	53.3
<i>Anthanassa ptylca</i> (H. Bates, 1864)	0	0	0	0	0	0	0	1	0	0	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	33.3
<i>Anthanassa argentea</i> (Godman & Salvin, 1882)	1	1	0	10	13	3	10	9	2	12	14	1	76
	0.4	0.4	0.0	13.2	17.1	2.6	13.2	11.8	0.9	15.8	18.4	0.4	94.3
<i>Anthanassa tulcis</i> (H. Bates, 1864)	0	0	0	0	0	1	0	0	3	0	2	2	8
	0.0	0.0	0.0	0.0	0.0	4.2	0.0	0.0	25.0	0.0	8.3	8.3	45.8
<i>Phyciodes graphica</i> (R. Felder, 1869)	3	0	0	8	5	0	8	7	0	8	7	0	46
	2.2	0.0	0.0	17.4	10.9	0.0	17.4	10.1	0.0	17.4	10.1	0.0	85.5
<i>Phyciodes mylitta mexicana</i> A. Hall, 1928	0	0	0	1	0	0	4	0	0	3	0	0	8
	0.0	0.0	0.0	4.2	0.0	0.0	33.3	0.0	0.0	12.5	0.0	0.0	50.0
<i>Phyciodes phaon phaon</i> (W. H. Edwards, 1864)	0	0	0	4	0	0	0	0	0	1	0	0	5
	0.0	0.0	0.0	53.3	0.0	0.0	0.0	0.0	0.0	6.7	0.0	0.0	60.0
<i>Phyciodes tharos tharos</i> (Drury, 1773)	6	2	0	8	14	4	7	14	1	14	10	6	86
	7.0	0.8	0.0	9.3	16.3	4.7	8.1	16.3	0.4	16.3	11.6	7.0	97.7
Charaxinae Guenée, 1865													
Anaeni Reuter, 1896													
<i>Anaea aidea</i> (Guérin-Ménéville, 1844)	16	6	4	40	28	17	79	58	28	77	50	39	442
	3.6	0.9	0.6	9.0	6.3	3.8	17.9	13.1	6.3	17.4	11.3	8.8	99.2
<i>Anaea andria</i> Scudder, 1875	0	0	0	9	5	5	10	8	3	17	10	6	73
	0.0	0.0	0.0	12.3	4.6	4.6	13.7	11.0	1.4	23.3	13.7	8.2	92.7
<i>Fountainea glycerium glycerium</i> (E. Doubleday, 1849)	0	0	0	6	5	2	5	3	0	0	2	0	23
	0.0	0.0	0.0	17.4	21.7	2.9	21.7	8.7	0.0	0.0	2.9	0.0	75.4
<i>Memphis pithyusa pithyusa</i> (R. Felder, 1869)	0	0	0	1	0	0	2	1	0	4	3	0	11
	0.0	0.0	0.0	3.0	0.0	0.0	6.1	3.0	0.0	24.2	9.1	0.0	45.5
Satyrinae Boisduval, 1833													
Satyrini Boisduval, 1833													
<i>Cyllopsis</i> sp. R. Felder, 1869	0	0	0	0	0	4	0	3	3	0	0	3	13
	0.0	0.0	0.0	0.0	0.0	20.5	0.0	7.7	15.4	0.0	0.0	7.7	51.3
<i>Cyllopsis dospassosi</i> L. Miller, 1974	1	1	0	9	3	0	11	8	1	8	5	1	48
	0.7	0.7	0.0	18.8	4.2	0.0	22.9	16.7	0.7	16.7	6.9	0.7	88.9
<i>Cyllopsis gemma freemani</i> (D. Stallings & Turner, 1947)	6	2	1	23	15	7	19	14	7	20	14	7	135
	4.4	1.0	0.2	17.0	11.1	5.2	14.1	10.4	5.2	14.8	10.4	3.5	97.3
<i>Megisto rubricata rubricata</i> (W. H. Edwards, 1871)	0	0	0	4	1	0	6	0	0	2	0	0	13
	0.0	0.0	0.0	20.5	2.6	0.0	30.8	0.0	0.0	5.1	0.0	0.0	59.0
<i>Hermeuptychia hermes</i> (Fabricius, 1775)	0	0	0	14	7	2	12	6	1	12	6	1	61
	0.0	0.0	0.0	23.0	7.7	1.1	19.7	6.6	0.5	19.7	6.6	0.5	85.2
Hesperiidae Latreille, 1809													
Eudaminae Mabille, 1877													
<i>Phocides polybius lilea</i> (Reakirt, 1867)	0	0	0	0	0	0	2	0	0	0	0	0	2
	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	33.3
<i>Phocides urania urania</i> (Westwood, 1852)	0	0	0	0	0	0	3	0	0	0	2	0	5
	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	13.3	0.0	33.3
<i>Epargyreus socus orizaba</i> Scudder, 1872	0	0	0	3	1	0	2	0	0	2	0	0	8
	0.0	0.0	0.0	12.5	4.2	0.0	8.3	0.0	0.0	8.3	0.0	0.0	33.3

Taxon	Dry Season						Rainy Season						N
	Early (Dec-Feb)			Late (Mar-May)			Early (Jun-Aug)			Late (Sep-Nov)			
	1	2	3	1	2	3	1	2	3	1	2	3	
<i>Polygonus leo arizonensis</i> (Skinner, 1911)	0	0	0	0	2	0	0	0	0	0	0	0	2
	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Chioides albofasciatus</i> (Hewitson, 1867)	0	2	0	8	12	3	7	3	0	10	6	1	52
	0.0	1.3	0.0	15.4	23.1	3.8	9.0	3.8	0.0	19.2	11.5	0.6	87.8
* <i>Chioides zilpa</i> (Butler, 1872)	0	0	0	11	7	2	6	0	0	9	6	1	42
	0.0	0.0	0.0	26.2	16.7	1.6	9.5	0.0	0.0	21.4	14.3	0.8	90.5
<i>Aguna asander asander</i> (Hewitson, 1867)	0	0	0	1	0	0	4	0	0	0	0	0	5
	0.0	0.0	0.0	6.7	0.0	0.0	53.3	0.0	0.0	0.0	0.0	0.0	60.0
<i>Aguna metopis</i> (Latreille, 1824)	0	0	0	0	0	0	1	0	0	1	0	0	2
	0.0	0.0	0.0	0.0	0.0	0.0	16.7	0.0	0.0	16.7	0.0	0.0	33.3
<i>Typhedanus undulatus</i> (Hewitson, 1867)	0	0	0	0	0	0	3	0	0	5	0	0	8
	0.0	0.0	0.0	0.0	0.0	0.0	12.5	0.0	0.0	41.7	0.0	0.0	54.2
<i>Codatractus bryaxis</i> (Hewitson, 1867)	0	0	0	1	0	0	0	0	0	0	0	0	1
	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Urbanus proteus proteus</i> (Linnaeus, 1758)	0	0	0	2	0	0	0	0	0	0	0	0	2
	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Urbanus dorantes dorantes</i> (Stoll, 1790)	0	0	0	1	0	0	1	0	0	0	1	0	3
	0.0	0.0	0.0	11.1	0.0	0.0	11.1	0.0	0.0	0.0	11.1	0.0	33.3
* <i>Urbanus procne</i> (Plötz, 1881)	0	0	0	5	0	0	2	1	0	3	1	0	12
	0.0	0.0	0.0	41.7	0.0	0.0	5.6	2.8	0.0	16.7	2.8	0.0	69.4
<i>Urbanus teleus</i> (Hübner, 1821)	0	0	0	4	0	0	1	0	0	3	0	0	8
	0.0	0.0	0.0	33.3	0.0	0.0	4.2	0.0	0.0	12.5	0.0	0.0	50.0
<i>Urbanus doryssus</i> (Swainson, 1831)	0	0	0	5	1	0	4	1	1	5	2	0	19
	0.0	0.0	0.0	17.5	1.8	0.0	7.0	1.8	1.8	26.3	7.0	0.0	63.2
<i>Astraptes fulgerator azul</i> (Reakirt, 1867)	1	0	0	7	3	2	7	2	0	7	2	0	31
	1.1	0.0	0.0	15.1	6.5	2.2	15.1	2.2	0.0	15.1	2.2	0.0	59.1
<i>Astraptes alector hopfferi</i> (Plötz, 1881)	0	0	0	0	0	0	0	0	2	0	0	0	2
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	33.3
<i>Autochton cellus</i> (Boisduval & Le Conte, 1837)	0	0	0	0	1	4	0	1	5	0	2	0	13
	0.0	0.0	0.0	0.0	2.6	10.3	0.0	2.6	25.6	0.0	5.1	0.0	46.2
<i>Autochton cincta</i> (Plötz, 1882)	0	1	0	0	0	6	0	5	4	0	3	2	21
	0.0	1.6	0.0	0.0	0.0	19.0	0.0	15.9	6.3	0.0	9.5	3.2	55.6
<i>Achalarus toxeus</i> (Plötz, 1882)	0	0	0	8	3	0	6	1	0	6	1	0	25
	0.0	0.0	0.0	32.0	8.0	0.0	16.0	1.3	0.0	16.0	1.3	0.0	74.7
<i>Thorybes pylades albosuffusa</i> H. Freeman, 1943	0	0	0	5	3	2	0	0	0	0	1	0	11
	0.0	0.0	0.0	30.3	18.2	6.1	0.0	0.0	0.0	0.0	3.0	0.0	57.6
<i>Cabares potrillo potrillo</i> (Lucas, 1857)	0	1	0	7	9	1	0	4	0	9	6	1	38
	0.0	0.9	0.0	18.4	23.7	0.9	0.0	3.5	0.0	15.8	10.5	0.9	74.6
<i>Spathilepia clonius</i> (Cramer, 1775)	0	0	0	0	2	0	0	0	0	2	0	0	4
	0.0	0.0	0.0	0.0	16.7	0.0	0.0	0.0	0.0	33.3	0.0	0.0	50.0
<i>Cogia hippalus hiska</i> Evans, 1953	0	0	0	0	0	0	0	0	0	0	0	2	2
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	33.3
Pyrginae Burmeister, 1878													
Carcharodini Verity, 1940													
<i>Arteurotia tractipennis tractipennis</i> Butler & H. Druce, 1872	0	0	0	0	2	0	1	0	0	0	1	0	4
	0.0	0.0	0.0	0.0	16.7	0.0	8.3	0.0	0.0	0.0	8.3	0.0	33.3
<i>Polycctor enops</i> (Godman & Salvin, 1894)	0	0	0	0	0	0	0	4	0	0	2	0	6
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	44.4	0.0	0.0	11.1	0.0	55.6
<i>Noctuana lactifera bipuncta</i> (Plötz, 1884)	0	0	0	0	0	0	3	3	0	0	3	0	9
	0.0	0.0	0.0	0.0	0.0	0.0	11.1	22.2	0.0	0.0	22.2	0.0	55.6
<i>Bolla brennus brennus</i> (Godman & Salvin, 1896)	0	0	0	1	0	0	0	0	0	0	0	0	1
	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Bolla chytius</i> (Godman & Salvin, 1897)	0	0	0	0	0	0	0	0	1	0	0	1	2
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.7	0.0	0.0	16.7	33.3
<i>Staphylus mazans</i> (Reakirt, 1867)	0	1	0	9	6	2	4	3	1	6	3	0	35
	0.0	1.0	0.0	25.7	17.1	3.8	7.6	5.7	1.0	11.4	2.9	0.0	76.2
<i>Staphylus azteca</i> (Scudder, 1872)	0	0	0	0	1	0	0	3	0	0	0	0	4
	0.0	0.0	0.0	0.0	8.3	0.0	0.0	50.0	0.0	0.0	0.0	0.0	58.3

Taxon	Dry Season						Rainy Season						N
	Early (Dec-Feb)			Late (Mar-May)			Early (Jun-Aug)			Late (Sep-Nov)			
	1	2	3	1	2	3	1	2	3	1	2	3	
<i>Pholisora catullus</i> (Fabricius, 1793)	0	0	0	1	0	0	3	0	0	2	0	0	6
	0.0	0.0	0.0	5.6	0.0	0.0	16.7	0.0	0.0	11.1	0.0	0.0	33.3
Erynnini Brues & F. Carpenter, 1932													
<i>Gorgythion begga pyralina</i> (Möschler, 1877)	2	4	1	2	3	0	0	0	0	0	0	0	12
	5.6	11.1	2.8	5.6	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Grais stigmaticus stigmaticus</i> (Mabille, 1883)	0	0	0	0	3	0	0	3	0	0	5	0	11
	0.0	0.0	0.0	0.0	9.1	0.0	0.0	18.2	0.0	0.0	30.3	0.0	57.6
<i>Timochares ruptifasciata</i> (Plötz, 1884)	0	0	0	1	0	0	4	1	0	4	1	0	11
	0.0	0.0	0.0	3.0	0.0	0.0	24.2	3.0	0.0	24.2	3.0	0.0	57.6
<i>Chiomara georgina georgina</i> (Reakirt, 1868)	5	4	1	22	14	5	12	13	5	19	10	3	113
	2.9	2.4	0.3	19.5	12.4	2.9	10.6	11.5	2.9	16.8	8.8	1.8	92.9
<i>Gesta invisus</i> (Butler & H. Druce, 1872)	0	0	0	9	4	3	6	4	0	9	11	3	49
	0.0	0.0	0.0	12.2	5.4	4.1	4.1	2.7	0.0	18.4	22.4	4.1	73.5
<i>Erynnis tristis tatus</i> (W. H. Edwards, 1883)	0	1	0	7	7	2	8	7	2	12	11	5	62
	0.0	0.5	0.0	7.5	7.5	2.2	8.6	7.5	2.2	19.4	17.7	5.4	78.5
<i>*Erynnis funeralis</i> (Scudder & Burgess, 1870)	1	0	0	5	0	0	0	0	0	6	0	0	12
	2.8	0.0	0.0	41.7	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	77.8
Achlyodidini Burmeister, 1878													
<i>*Achlyodes pallida</i> (R. Felder, 1869)	0	0	0	0	3	0	0	2	0	0	6	0	11
	0.0	0.0	0.0	0.0	9.1	0.0	0.0	6.1	0.0	0.0	54.5	0.0	69.7
<i>Eantis tamenund</i> (W. H. Edwards, 1871)	6	4	0	16	11	5	14	10	3	19	14	6	108
	5.6	2.5	0.0	14.8	10.2	4.6	13.0	9.3	1.9	17.6	13.0	5.6	97.8
<i>Zera hyacinthinus hyacinthinus</i> (Mabille, 1877)	0	0	0	0	2	0	0	2	0	1	0	0	5
	0.0	0.0	0.0	0.0	13.3	0.0	0.0	26.7	0.0	6.7	0.0	0.0	46.7
Pyrgini Burmeister, 1878													
<i>Carrhenes canescens canescens</i> (R. Felder, 1869)	1	0	0	0	0	0	0	0	0	1	0	0	2
	16.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.7	0.0	0.0	33.3
<i>Systasea pulverulenta</i> (R. Felder, 1869)	0	0	0	2	2	0	1	1	0	2	0	0	8
	0.0	0.0	0.0	8.3	16.7	0.0	4.2	4.2	0.0	16.7	0.0	0.0	50.0
<i>Celotes nessus</i> (W. H. Edwards, 1877)	0	0	0	0	2	0	0	0	0	2	1	0	5
	0.0	0.0	0.0	0.0	26.7	0.0	0.0	0.0	0.0	13.3	6.7	0.0	46.7
<i>Pyrgus communis communis</i> (Grote, 1872)	0	0	0	2	1	1	14	12	7	3	4	1	45
	0.0	0.0	0.0	1.5	0.7	0.7	31.1	26.7	15.6	2.2	3.0	0.7	82.2
<i>Pyrgus albescens</i> Plötz, 1884	2	4	0	13	13	7	12	10	4	21	10	7	103
	0.6	2.6	0.0	12.6	12.6	6.8	11.7	9.7	2.6	20.4	9.7	6.8	96.1
<i>Pyrgus oilens</i> (Linnaeus, 1767)	6	7	1	23	18	9	22	24	12	26	17	11	176
	2.3	4.0	0.2	13.1	10.2	5.1	12.5	13.6	6.8	14.8	9.7	6.3	98.5
<i>Pyrgus philetas</i> W. H. Edwards, 1881	0	2	0	0	3	1	0	0	0	0	5	1	12
	0.0	11.1	0.0	0.0	8.3	2.8	0.0	0.0	0.0	0.0	27.8	2.8	52.8
<i>Heliopyrgus domicella domicella</i> (Erichson, 1849)	1	0	0	1	0	0	0	0	0	0	0	0	2
	16.7	0.0	0.0	16.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Heliopyrgus sublinea</i> (Schaus, 1902)	0	0	0	0	0	0	3	2	0	0	1	0	6
	0.0	0.0	0.0	0.0	0.0	0.0	16.7	22.2	0.0	0.0	5.6	0.0	44.4
<i>Heliopetes laviana laviana</i> (Hewitson, 1868)	5	2	0	11	10	4	10	12	1	23	17	6	101
	3.3	0.7	0.0	10.9	9.9	4.0	9.9	11.9	0.3	22.8	16.8	5.9	96.4
<i>Heliopetes macaira macaira</i> (Reakirt, 1867)	0	0	0	1	0	0	0	0	0	0	0	0	1
	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3
Hesperiinae Latreille, 1809													
Megathymini J. H. Comstock & A. Comstock, 1895													
<i>Agathymus remingtoni</i> (D. Stallings & Turner, 1958)	0	0	0	0	0	0	1	0	0	0	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	33.3
Thymelicini Tutt, 1905													
<i>Ancyloxypha arene</i> (W. H. Edwards, 1871)	0	0	0	0	0	4	0	0	1	0	0	3	8
	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	4.2	0.0	0.0	12.5	50.0
<i>*Oarisma edwardsii</i> (W. Barnes, 1897)	0	0	0	0	0	1	0	0	9	0	0	2	12
	0.0	0.0	0.0	0.0	0.0	2.8	0.0	0.0	75.0	0.0	0.0	5.6	83.3
<i>Copaodes aurantiaca</i> (Hewitson, 1868)	0	0	0	0	0	2	0	0	0	0	0	1	3
	0.0	0.0	0.0	0.0	0.0	44.4	0.0	0.0	0.0	0.0	0.0	11.1	55.6

Taxon	Dry Season						Rainy Season						N
	Early (Dec-Feb)			Late (Mar-May)			Early (Jun-Aug)			Late (Sep-Nov)			
	1	2	3	1	2	3	1	2	3	1	2	3	
<i>Copaodes minima</i> (W. H. Edwards, 1870)	0	0	0	0	0	0	0	0	1	0	0	1	2
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.7	0.0	0.0	16.7	33.3
<b>Calpodini A. Clark, 1948</b>													
<i>Panoquina lucas</i> (Fabricius, 1793)	0	0	0	0	0	0	0	1	0	0	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	33.3
<b>Anthoptini A. Warren, 2009</b>													
<i>Synapte pecta</i> Evans, 1955	0	0	0	0	0	0	0	1	0	0	2	0	3
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.1	0.0	0.0	22.2	0.0	33.3
<b>Moncini A. Warren, 2008</b>													
<i>Mnasicles geta</i> Godman, 1901	0	0	0	0	0	0	0	2	0	0	0	0	2
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	33.3
<i>Remella rita</i> (Evans, 1955)	0	0	0	0	0	0	0	1	3	0	2	2	8
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.2	25.0	0.0	8.3	16.7	54.2
<i>Amblyscirtes aenus erna</i> H. Freeman, 1943	0	0	0	0	1	0	0	0	2	0	0	1	4
	0.0	0.0	0.0	0.0	8.3	0.0	0.0	0.0	16.7	0.0	0.0	8.3	33.3
<i>*Amblyscirtes celia</i> Skinner, 1895	0	0	0	0	0	2	0	1	6	0	2	0	11
	0.0	0.0	0.0	0.0	0.0	6.1	0.0	3.0	54.5	0.0	6.1	0.0	69.7
<i>Amblyscirtes fimbriata fimbriata</i> (Plötz, 1882)	0	0	0	0	0	0	2	1	0	0	0	0	3
	0.0	0.0	0.0	0.0	0.0	0.0	22.2	11.1	0.0	0.0	0.0	0.0	33.3
<i>Amblyscirtes anubis</i> (Godman, 1900)	0	0	0	1	0	0	2	2	0	0	0	0	5
	0.0	0.0	0.0	6.7	0.0	0.0	13.3	26.7	0.0	0.0	0.0	0.0	46.7
<i>Repens florus</i> (Godman, 1900)	0	0	0	5	5	1	0	0	0	5	2	0	18
	0.0	0.0	0.0	9.3	18.5	1.9	0.0	0.0	0.0	18.5	3.7	0.0	51.9
<i>Monca crispinus</i> (Plötz, 1882)	1	0	0	0	0	0	0	0	0	2	0	0	3
	11.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.2	0.0	0.0	33.3
<i>Nastra julia</i> (H. Freeman, 1945)	0	0	0	0	0	1	0	0	0	0	0	0	1
	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Cymaenes trebius</i> (Mabille, 1891)	0	0	0	0	3	1	0	0	0	0	0	1	5
	0.0	0.0	0.0	0.0	40.0	6.7	0.0	0.0	0.0	0.0	0.0	6.7	53.3
<i>Lerodea eufala eufala</i> (W. H. Edwards, 1869)	0	0	0	0	4	0	2	0	0	0	0	0	6
	0.0	0.0	0.0	0.0	44.4	0.0	11.1	0.0	0.0	0.0	0.0	0.0	55.6
<i>Lerodea arabus</i> (W. H. Edwards, 1882)	0	0	0	1	0	0	1	0	0	0	0	0	2
	0.0	0.0	0.0	16.7	0.0	0.0	16.7	0.0	0.0	0.0	0.0	0.0	33.3
<i>Lerema accius</i> (J. E. Smith, 1797)	3	1	0	11	7	2	5	0	0	1	2	0	32
	3.1	1.0	0.0	22.9	14.6	2.1	5.2	0.0	0.0	1.0	2.1	0.0	52.1
<i>Lerema liris</i> Evans, 1955	0	0	0	0	0	0	8	8	3	2	3	2	26
	0.0	0.0	0.0	0.0	0.0	0.0	10.3	20.5	7.7	2.6	3.8	2.6	47.4
<i>Vettius fantasos</i> (Cramer, 1780)	0	0	0	1	0	0	2	0	0	0	0	0	3
	0.0	0.0	0.0	11.1	0.0	0.0	22.2	0.0	0.0	0.0	0.0	0.0	33.3
<b>Hesperiini Latreille, 1809</b>													
<i>Hylephila phyleus phyleus</i> (Drury, 1773)	0	0	0	5	2	0	5	0	0	0	0	0	12
	0.0	0.0	0.0	13.9	5.6	0.0	27.8	0.0	0.0	0.0	0.0	0.0	47.2
<i>Polites vibex praeceps</i> (Scudder, 1872)	0	0	0	2	0	0	3	0	0	0	0	0	5
	0.0	0.0	0.0	13.3	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	33.3
<i>Wallengrenia otho otho</i> (J. E. Smith, 1797)	0	0	0	0	0	0	0	0	0	1	0	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	33.3
<i>Atalopedes campestris buron</i> (W. H. Edwards, 1863)	0	0	0	0	3	2	0	0	5	0	2	6	18
	0.0	0.0	0.0	0.0	5.6	3.7	0.0	0.0	9.3	0.0	3.7	22.2	44.4
<i>Poanes zabulon</i> (Boisduval & Le Conte, 1837)	0	0	0	0	0	1	0	0	0	0	2	0	3
	0.0	0.0	0.0	0.0	0.0	11.1	0.0	0.0	0.0	0.0	22.2	0.0	33.3
<i>Poanes melane vitellina</i> (Herrich-Schäffer, 1869)	0	0	0	0	0	1	0	0	0	0	0	1	2
	0.0	0.0	0.0	0.0	0.0	16.7	0.0	0.0	0.0	0.0	0.0	16.7	33.3
<i>Quasimellana eulogius</i> (Plötz, 1882)	0	0	0	0	0	0	0	0	0	0	1	0	1
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	33.3
<i>*Quinta cannae</i> (Herrich-Schäffer, 1869)	0	0	0	0	0	4	0	0	0	0	1	0	5
	0.0	0.0	0.0	0.0	0.0	80.0	0.0	0.0	0.0	0.0	6.7	0.0	86.7
<i>Nyctelius nyctelius nyctelius</i> (Latreille, 1824)	0	0	0	2	0	0	3	5	1	0	0	2	13
	0.0	0.0	0.0	5.1	0.0	0.0	7.7	12.8	2.6	0.0	0.0	5.1	33.3

# Four new species and seven new records of *Promalactis* Meyrick, 1908 (Lepidoptera, Oecophoridae) from Laos

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## Abstract

The genus *Promalactis* Meyrick, 1908 is recorded for the first time from Laos in mainland Southeast Asia and four new species are described: *P. crassa* **sp. nov.**, *P. retusa* **sp. nov.**, *P. senispina* **sp. nov.**, and *P. uniclavata* **sp. nov.** Additionally, seven species are newly reported from the country: *P. albisquama* Kim & Park, *P. apicisetifera* Du & Wang, *P. bitrigona* Kim & Park, *P. zolotuhini* Lvovsky, *P. parasuzukiella* Wang, *P. suzukiella* (Matsumura), and *P. spiraliola* Kim. Distributional data and diagnoses and/or descriptions for all species in Laos are provided, along with illustrations of adults and genitalia.

## Keywords

First record of genus, fungivores and scavengers, PKK National Park, systematics

## Introduction

*Promalactis* Meyrick, 1908 is one of the largest genera of the family Oecophoridae (Lepidoptera, Gelechioidea), comprising 341 species (Kim et al. 2018; Wang and Liu 2019). They are highly diverse in China and the Indomalayan realm (Kim et al. 2017b). *Promalactis*, which includes fungivores and scavengers, is close to the genera *Harpella* Schrank, *Oecophora* Latreille, and *Schiffermuelleria* Hübner in the recent



phylogeny of Kim et al. (2016), but it can be distinguished from them in having the forewing ground color brownish yellow to yellowish brown with distinct markings, such as white bands, patches, or fuscous suffusions (Kim et al. 2017c), and in having  $R_4$  and  $R_5$  stalked (Kim et al. 2014).

In Mainland Southeast Asia (Fig. 1A), approximately 54 species of *Promalactis* have been reported: two species in Myanmar (Meyrick 1908b; Wang et al. 2013); six species in Thailand (Wang et al. 2013); 36 species in Vietnam (Lvovsky 1988, 1997, 2007, 2013; Kim et al. 2010, 2012, 2014) and 11 species in Cambodia (Kim et al. 2017c, 2018). However, no species has been recorded from Laos to date.

Laos is a landlocked and mountainous country located in Mainland Southeast Asia, bordered by Myanmar and China to the northwest, Vietnam to the east, Cambodia to the southwest, and Thailand to the west and southwest. It lies in the monsoon belt and experiences a rainy season between May and November, and a dry season from December to April.

The aim of this study is to report the first distributional data of *Promalactis* in Laos, with four new species described and seven other species newly recorded. Illustrations of all known species in Laos have been provided along with diagnosis and distributional information.

## Material and methods

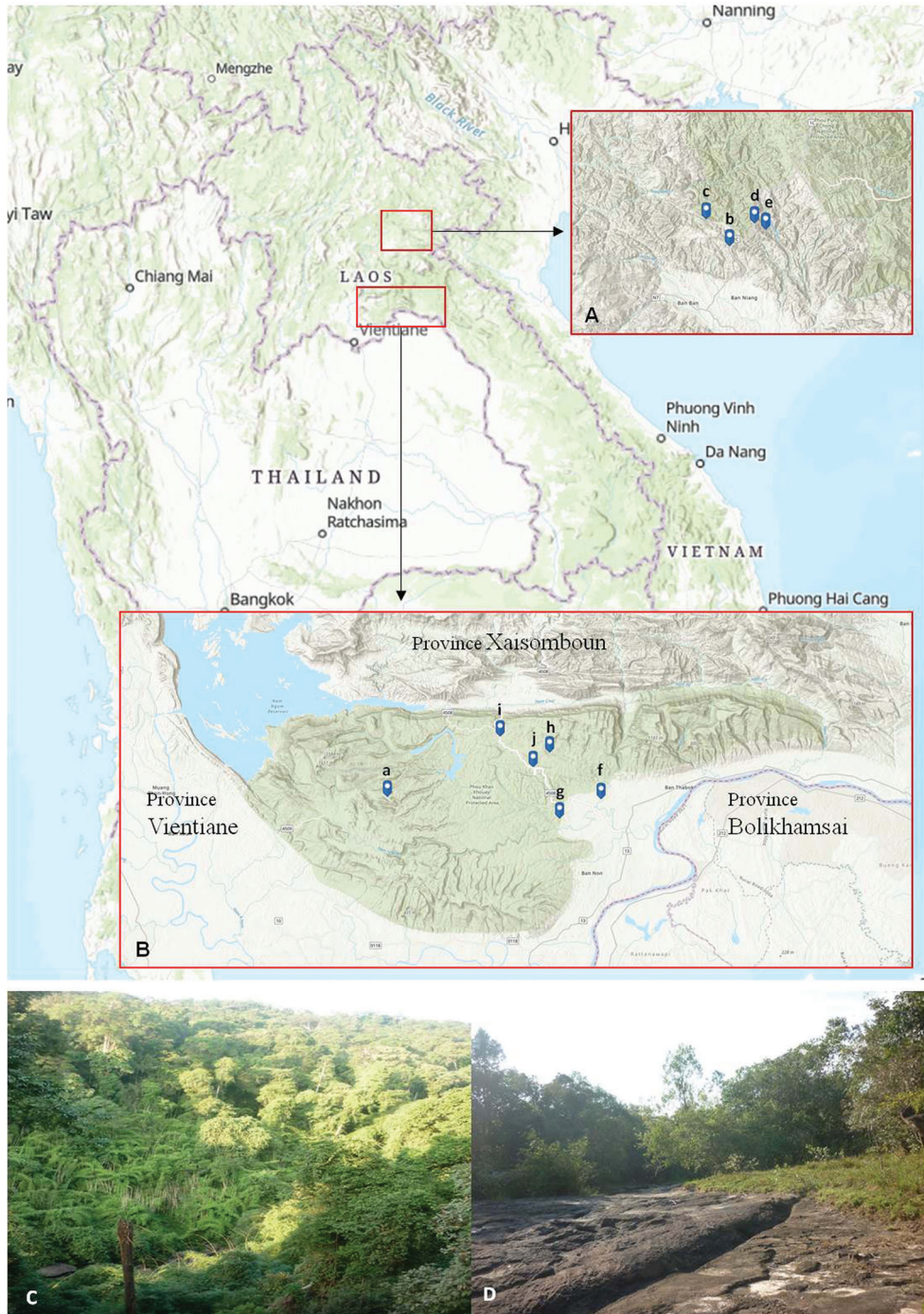
Materials in this study were collected from 2012–2017 at several sites of three provinces of Laos, Vientiane (Northwest), Xiangkhaung (Northeast), and Bolikhamsai (Central) (Fig. 1; Table 1). The collections were mainly from Phou Khao Khouay National Biodiversity Conservation Area (PKK National Park). It is considered as one of the most stunning preserves, with mixed deciduous forest dominating the lighter which less humid and shallow soils.

The forest consists of *Dipterocarpus* and *Shorea*, with a dry, evergreen forest centrally. Using either a mercury vapor lamp (220V/400W) or a black light lamp (20W), all individuals were taken alive, put in vials with cork lids, and killed with ammonia. Genitalia preparations for voucher specimens followed Kim et al. (2017a). All specimens were examined under a Leica 400B microscope (Leica Microsystems, Germany) and digital images were taken using Image Lab software, version 2.2.4.0 (MCM Design, Hillerød, Denmark). All specimens including type specimens will be deposited in two institutions: SNU, Seoul National University; INU, Incheon National University in Republic of Korea.

## Taxonomy

### Genus *Promalactis* Meyrick, 1908

*Promalactis* Meyrick, 1908a: 806. Type species. *Promalactis holozona* Meyrick, 1908a.



**Figure 1.** Locality of collection areas of *Promalactis* in Laos **A** Ban Tha area in Xiang khaung province **B** PKK National Park area **C** Forest of PKK National Park **D** collection sites near waterfall in PKK National Park.

**Table 1.** Collection sites and seasons of *Promalactis* in Laos.

Province of Laos	Locality	Geographic position	Date, season
Vientiane (Northwest)	PKK National Park Fig. 1B(a)	18°24'43.67"N, 102°50'57.92"E	7 December 2012, dry season
Xiang khaung (Northeast)	Ban Tha, 1204 m Fig. 1A(b)	19°43'0.70"N, 103°35'24.20"E	30 June 2017, rainy season
	Ban Tha, 1298 m Fig. 1A(c)	19°45'07.35"N, 103°33'25.34"E	7 August 2016, rainy season
			12 November 2015, rainy season
			5–6 April 2016, dry season
			7 August 2017, rainy season
	Ban Tha, 1513 m Fig. 1A(d)	19°44'50.2"N, 103°37'28.1"E	20 February 2017, dry season
	Ban Tha, 1524 m Fig. 1A(e)	19°44'17.99"N, 103°38'25.06"E	01 January 2017, dry season
Bolikhamsai (Central)	Hat Khay Fig. 1B(f)	18°24'33.03"N, 103°9'37.08"E	22 December 2012, dry season
	Thaphabath, Phaset Fig. 1B(g)	18°25'37.53"N, 103°16'19.64"E	21 February 2013, dry season
	PKK National Park, 322 m Fig. 1B(h)	18°28'25.28"N, 103°5'6.11"E	01 April 2016, dry season
	PKK National Park, 452 m Fig. 1B(i)	18°27'11.9"N, 103°03'40.5"E	03 July 2017, rainy season
	PKK National Park, 561 m Fig. 1B(j)	18°29'43.79"N, 103°00'48.02"E	02 July 2017, rainy season

***Promalactis parasuzukiella* Wang, 2006**

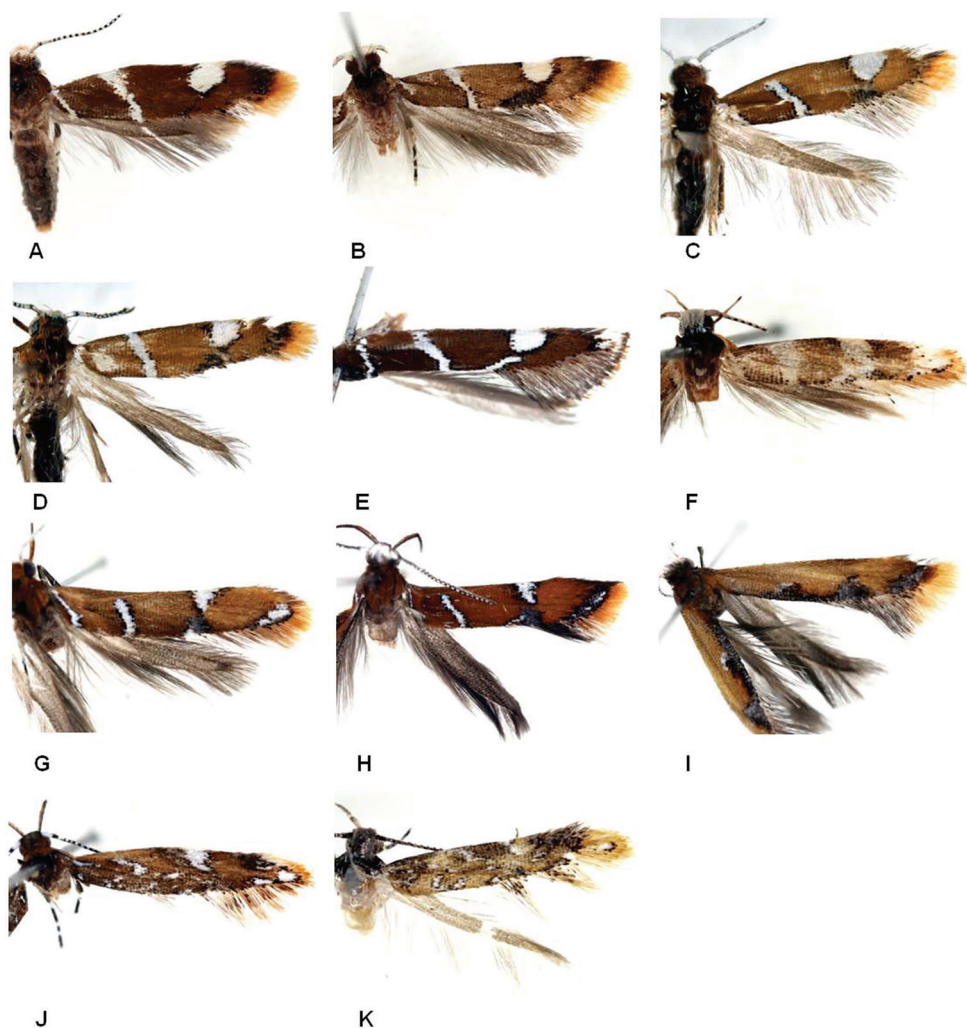
Figures 2A, 3A–D, 6A–C

*Promalactis parasuzukiella* Wang, 2006: 44. Type locality: China.

**Material examined.** 1♀, Laos, Bolikhamsai Prov., Hat Khay, 22 December 2012, Kim, gen. slide no. 9513/S. Kim; 1♀, Laos, Xiang khaung Prov., Ban Tha, 1298 m, 12 November 2015, Bae et al.; 1♂, Laos, Bolikhamsai prov., Phou Khao Khouay National Protected Area National Park, 322 m, 1 April 2016, Bae et al., gen. slide no. 9846/S. Kim; 1 extra (missing abdomen), Laos, Xiang khaung Prov., Ban Tha, 1298 m, 6 April 2016, Bae et al.; 1♀, same locality, 7 August 2017, Bae et al., gen. slide no. 9848/S. Kim.

**Diagnosis.** This species (Fig. 2A) is externally most similar to *P. suzukiella* (Matsumura) in the forewing pattern, but it can be easily distinguishable from that species by the subbasal band which traverses from the costa to the posterior margin and the fuscous suffusion rarely developed under the circular costal patch at 2/3 of the forewing. The male genitalia (Fig. 3A–D) are differentiated from those of *P. suzukiella* in having the gnathos with a pointed apex and the aedeagus bent at 3/5 of its length. The female genitalia (Fig. 6A–C) are distinguished from those of *P. suzukiella* in having the ductus bursae bearing a long, serrated spine and another tiny spine.

**Distribution.** Laos (northeast, central; new record), China (south) and Thailand (central).



**Figure 2.** Wing pattern of Laos *Promalactis* **A** *Promalactis parasuzukiella* **B** *P. suzukiella* **C** *P. uniclavata* sp. nov. **D** *P. albisquama* **E** *P. spiraliola* **F** *P. senispina* sp. nov. **G** *P. apicisetifera* **H** *P. zolotuhini* **I** *P. bitrigona* **J** *P. crassa* sp. nov. **K** *P. retusa* sp. nov.

***Promalactis suzukiella* (Matsumura, 1931)**

Figures 2B, 3E–H

*Borkhausenia suzukiella* Matsumura, 1931: 1089. Type locality: Japan.

**Material examined.** 1♂, Laos, Xiang khaung Prov., Ban Ta, 1524 m, 1 January 2017, Bae et al., gen. slide no. 9829/S. Kim.

**Diagnosis.** This species (Fig. 2B) is similar to *P. uniclavata* sp. nov. in the wing pattern but can be easily distinguished from the latter in having the forewing without



a subbasal band which traverses from the costa to the posterior margin, and in having the male genitalia (Fig. 3E–H) with the uncus having a thumb-like apex and a tongue-like gnathos.

**Distribution.** Laos (northeast; new record), China (south), Vietnam; Palearctic: Korea (South), China (northwest, northeast, central), Japan, Russia (Far East); Nearctic: USA.

***Promalactis uniclavata* Kim, sp. nov.**

<http://zoobank.org/32E673C6-160C-496B-A589-B2FD72AE5B95>

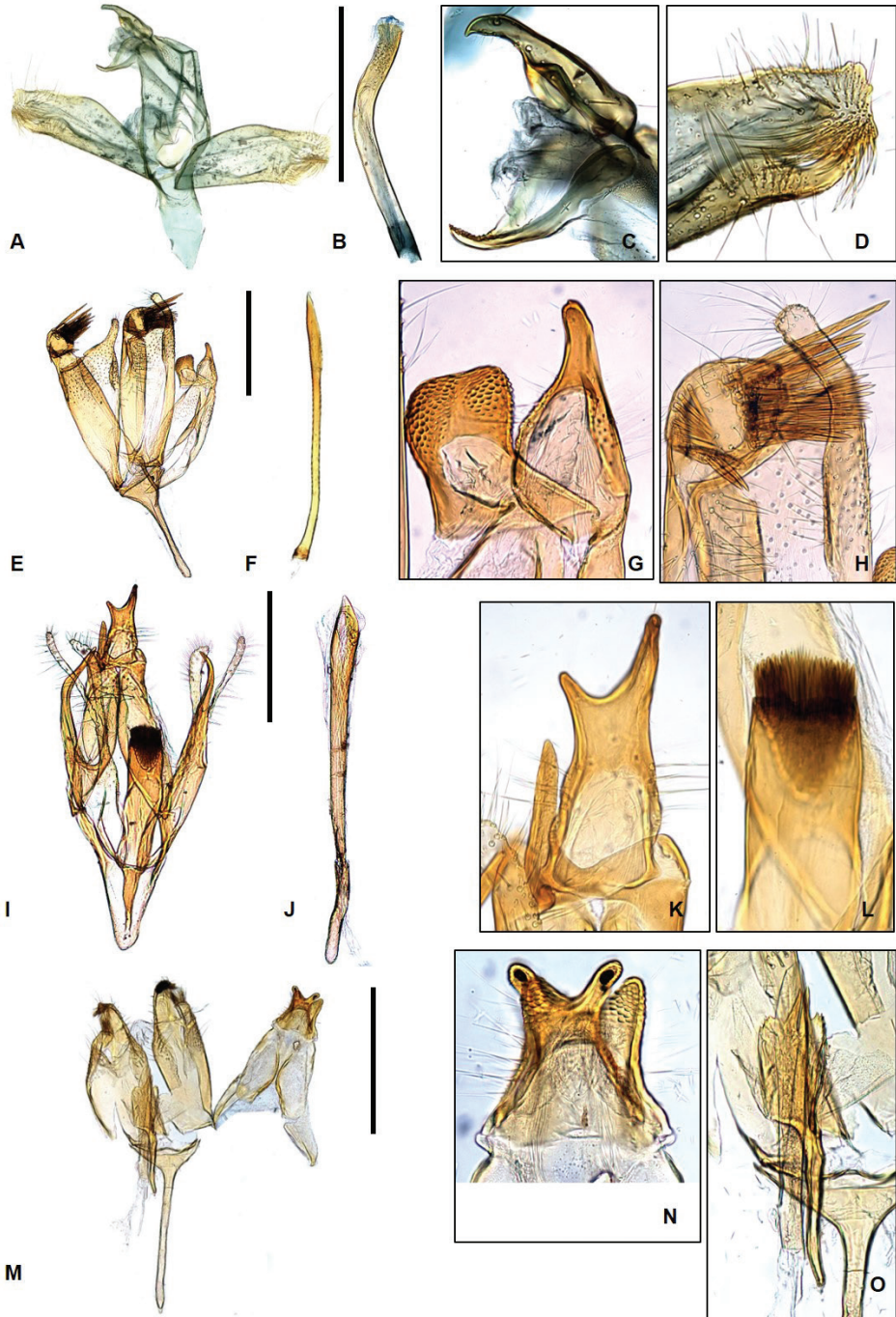
Figures 2C, 3I–L, 6D–F

**Type material. Holotype:** ♂, Laos, Xiang khaung Prov., Ban Tha, 1513 m, 20 February 2017, Bae et al., gen. slide no. 9834/S. Kim. Paratype: 1♂, 1♀, Laos, Vientiane Prov., Phou Khao Khouay National Protected Area, 7 December 2012, Lee et al., gen. slide no. 9512(M), 9509(F)/S. Kim; 1 ex., Laos, Bolikhamsai prov., Phou Khao Khouay National Protected Area, 322 m, 1 April 2016, Bae et al.; 1♂, Laos, Xiang khaung Prov., Ban Tha, 1298 m, 6 April 2016, Bae et al. [Holotype is deposited in INU, paratypes are separately deposited into SNU and INU.]

**Diagnosis.** The species is superficially most similar to *P. suzukiella* in the similar forewing pattern but can be easily distinguished from the latter by the subbasal band which traverses from the costa to the posterior margin and with a fuscous suffusion below the costal patch at 2/3 of the posterior margin. The male genitalia are characterized in having the uncus bifurcate, the gnathos digitate, and the juxta large and club-shaped.

**Description. Adult** (Fig. 2C). **Head:** frons grayish brown tinged with white; vertex white, tinged with dark brown; occiput yellowish brown. **Antenna:** scape white entirely, shorter than diameter of eye; flagellum white from base to 2/3 of its length, dorsally dark brown and white alternately from 2/3 to apex. **Labial palpus:** 2<sup>nd</sup> palpomere pale-yellow tinged with dark brown, as long as 3<sup>rd</sup> palpomere; 3<sup>rd</sup> palpomere dark brown, except pale yellowish white at apex dorsally. **Thorax:** thorax and tegula dark brown. Wingspan 7.5–8.0 mm. Forewing ground color brownish yellow, darker near base; two bands and one costal patch all white, edged by fuscous scales: one subbasal band traversed from costal margin to posterior margin just near wing base; one antemedial band, oblique, not reaching costa; one costal patch semicircular at 3/4 of forewing, connected to fuscous suffusion before tornus; fuscous scales somewhat dense near apex; fringes grayish brown near tornus, yellow near apex. Hindwing ground color grayish brown; fringes grayish brown.

**Male genitalia** (Fig. 3I–L). Uncus wide at base, bifurcate distally, setose from base to middle; lateral lobes asymmetrical. Gnathos digitate, wide at base, shorter than uncus. Valva short; costal margin elongated, bifurcate, with different length of lobes, setose entirely; sacculus heavily sclerotized with pointed apex, shorter than costal margin. Juxta large, club-shaped, bearing U-shaped apical margin with dense hairs, as long as sacculus. Saccus large, triangular, longer than uncus. Aedeagus straight, gradually broader to apex, slightly longer than valva. Cornutus absent.



**Figure 3.** Genitalia of Laos *Promalactis* **A–D** *P. parasuzukiella*: **A** male genitalia **B** aedeagus **C** uncus and gnathos **D** apical part of valva **E–H** *P. suzukiella*: **E** male genitalia **F** aedeagus **G** uncus and gnathos **H** apical part of valva **I–L** *P. uniclavata* sp. nov.: **I** male genitalia **J** aedeagus **K** uncus and gnathos **L** dense hairs on apical part of juxta **M–O** *P. albisquama*: **M** male genitalia **N** uncus and gnathos **O** juxta and aedeagus. Scale bars: 0.5 mm.



**Female genitalia** (Fig. 6D–F). Papillae anales setose. Apophyses posteriores almost twice longer than apophyses anteriores. Lamella postvaginalis large, incised medially, setose on caudal margin. Lamella antevaginalis wide, surrounded by dense hairs, concave on caudal margin. Antrum cylindrical, somewhat sclerotized posteriorly, gradually membranous anteriorly, as long as ductus bursae vertically. Ductus bursae membranous, sclerotized projection at 3/4 posteriorly. Corpus bursae small, circular; signum bearing one tiny spine and one sclerotized plate consisting of 3 or 4 tiny spines.

**Distribution.** Laos (northeast, northwest, central; new).

**Etymology.** The name is derived from the Latin, *uni* (= one) plus the Latin *clava* (= club), referring from the large, club-shaped juxta in the male genitalia.

### *Promalactis albisquama* Kim & Park, 2010

Figures 2D, 3M–O

*Promalactis albisquama* Kim & Park, 2010: 548. Type locality. Vietnam, Tamdao.

**Material examined.** 1♂, Laos, Vientiane Prov., Phou Khao Khouay National Protected Area, 7 December 2012, Lee et al., gen. slide no. 9511/S. Kim.

**Diagnosis.** The species (Fig. 2D) is externally similar to *P. suzukiella* in the wing pattern but can be easily differentiated from the latter in having the forewing without a subbasal band and in having the fuscous suffusion denser near apex. The male genitalia (Fig. 3M–O) are also distinguished from those of *P. suzukiella* by the bifurcate uncus, the distinct juxta, and the extremely longer saccus.

**Distribution.** Laos (northwest; new record), Cambodia (northwest), China (south), Vietnam (north).

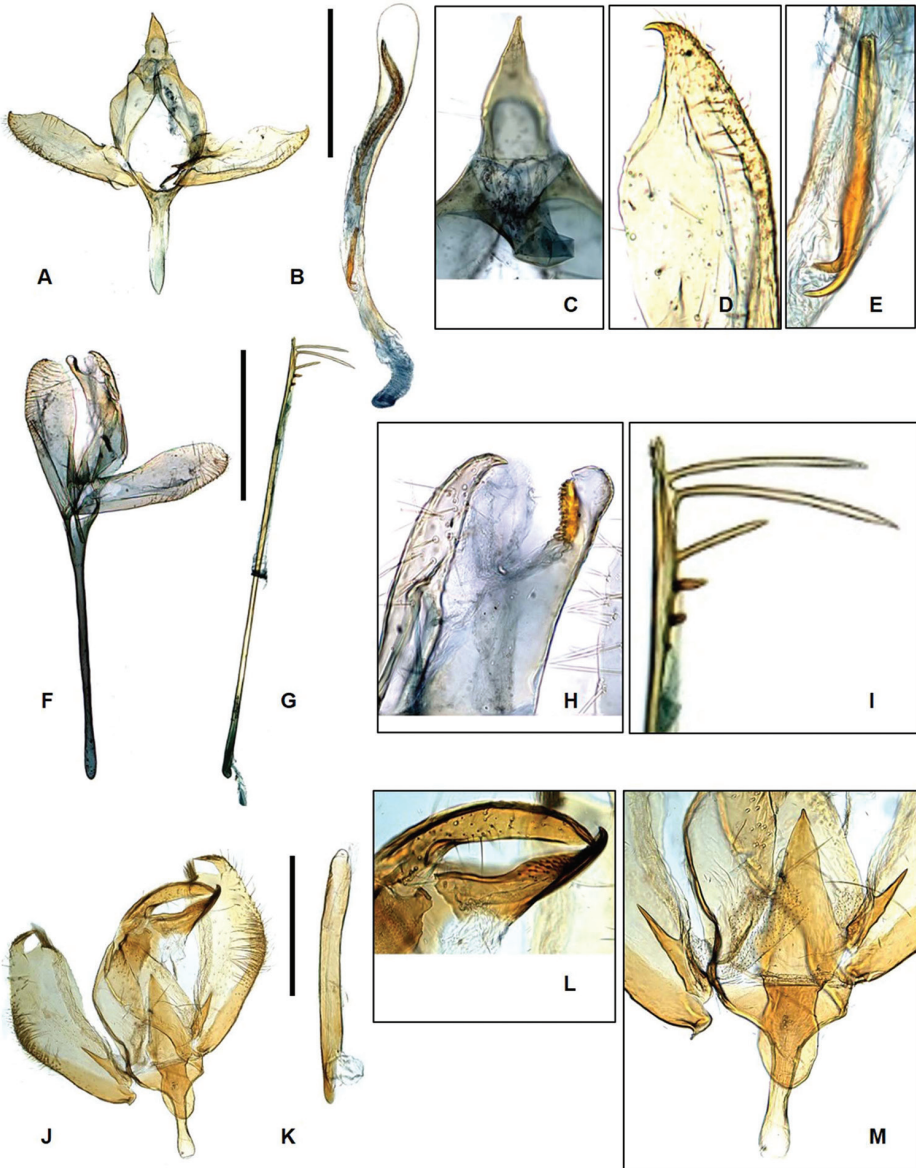
### *Promalactis spiraliola* Kim, 2017

Figures 2E, 4A–E, 6G, H

*Promalactis spiraliola* Kim, 2017: 1710. Type locality: Cambodia, Koh Kong.

**Material examined.** 2♂, 1♀ and 1 ex., Bolikhamsai Prov., Phou Khao Khouay National Protected Area National Park, 561 m, 2 July 2017, gen. slide no. 9862(♂), 9858(♂) and 9863 (♀)/S. Kim. 1♂, Laos, Bolikhamsai prov., Phou Khao Khouay National Protected Area National Park, 561 m, 2 July 2017, Bae et al., gen. slide no. 9858/S. Kim.

**Diagnosis.** The species (Fig. 2E) is externally characterized by the narrow, medial band not connected to the white costal patch on the forewing. The male genitalia (Fig. 4A–E) are also distinguished by the weakly developed gnathos and large aedeagus. The female genitalia (Fig. 6G, H) are characterized in having the ductus bursae coiled



**Figure 4.** Genitalia of Laos *Promalactis* **A–E** *P. spiraliola*: **A** male genitalia **B** aedeagus **C** uncus and gnathos **D** apical part of valva **E** bifurcate part of cornutus **F–I** *P. senispina* sp. nov.: **F** male genitalia **G** aedeagus **H** sclerotized projection of gnathos **I** apical projections of aedeagus **J–M** *P. apicisetifera*: **J** male genitalia **K** aedeagus **L** uncus and gnathos **M** juxta. Scale bars: 0.5mm.

several times and bearing numerous tiny spines, and in having the corpus bursae small, membranous, and without signum.

**Distribution.** Laos (central; new record), Cambodia (southwest).

***Promalactis senispina* Kim, sp. nov.**

<http://zoobank.org/9468C7C7-C403-4D0A-BA93-E5D8D98DB353>

Figures 2F, 4F–I

**Type material. Holotype:** ♂, Laos, Bolikhamsai prov., Phou Khao Khouay National Protected Area National Park, 322 m, 1 April 2016, Bae et al., gen. slide no. 9860/S. Kim. [Holotype is deposited in INU.]

**Diagnosis.** This species is externally similar to *P. lophacantha* Wang, Du & Li by having irregular bands of forewing, but it can be easily differentiated by the extended saccus and the prolonged aedeagus, which bears six projections in male genitalia.

**Description. Adult** (Fig. 2F). **Head:** frons pale yellowish white; vertex white; occiput white. Antenna: scape white entirely, longer than diameter of eye; flagellum dark brown and white alternately from base to apex dorsally. Labial palpus: 2<sup>nd</sup> palpomere pale yellowish brown, slightly longer than 3<sup>rd</sup> palpomere; 3<sup>rd</sup> palpomere pale yellow tinged with white, except dark brown apically. **Thorax:** thorax and tegula dark brown. Wingspan 7.0–7.5 mm. Forewing ground color yellowish brown; three bands, one costal patch, and one apical patch all white: one subbasal band traversed from costal margin to posterior margin near wing base; one antemedial band broad, slightly oblique toward wing base, traversed from costa to posterior margin; one medial band connected to postmedial short band on lower margin of cell; one apical patch occupied after 3/4 of forewing to apex; fuscous scales scattered followed by posterior margin of forewing; fringes yellow, except pale yellowish white near medial and postmedial bands. Hindwing ground color grayish dark brown; fringes dark grayish brown.

**Male genitalia** (Fig. 4F–I). Uncus wide at base, gradually narrowed to pointed apex, setose apically, as long as gnathos. Gnathos wide at base, gradually narrowed to apex, thumb-shaped apex, bearing sclerotized and serrated projection inner margin, as long as uncus. Valva symmetrical; costal margin convex after middle; saccular margin moderate, setose from 1/2 of its length to apex. Saccus extremely long, 1.5 times longer than valva. Aedeagus straight, elongated, as long as total length of genitalia, with six spines, differently sized apically; cornutus absent.

**Female** unknown.

**Distribution.** Laos (central; new).

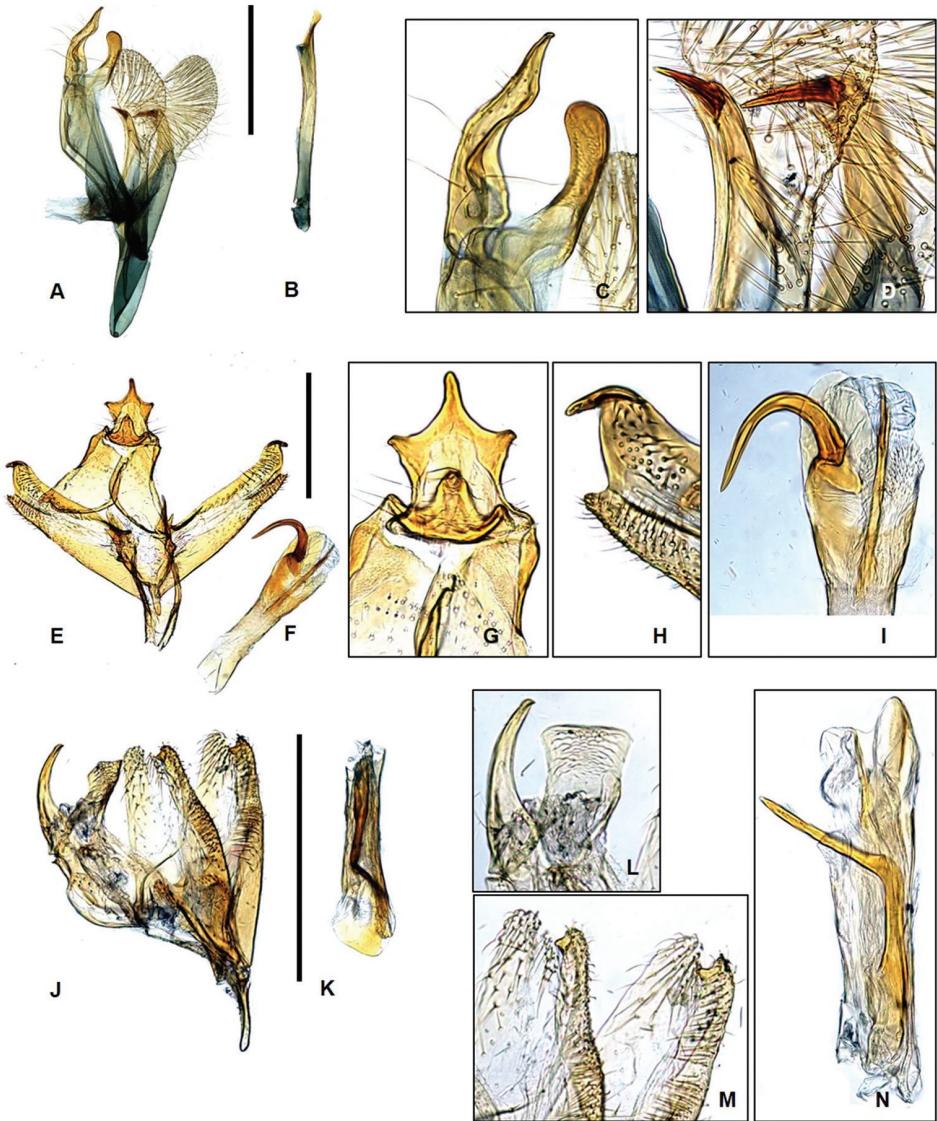
**Etymology.** The specific name is derived from the Latin, *seni* (= six) and *-spina* (= spine), referring from the aedeagus bearing six spines in males.

***Promalactis apicisetifera* Du, Li & Wang, 2011**

Figures 2G, 4J–M

*Promalactis apicisetifera* Du, Li & Wang, 2011: 52. Type locality: China

**Material examined.** 1♂, Laos, Xiang khaung Prov., Ban Tha, 1298 m, 7 August 2017, Bae et al., gen. slide no. 9842/S. Kim.



**Figure 5.** Genitalia of Laos *Promalactis* **A–D** *P. zolotuhini*: **A** male genitalia **B** aedeagus **C** uncus and gnathos **D** cucullus **E–I** *P. crassa* sp. nov.: **E** male genitalia **F** aedeagus **G** uncus and gnathos **H** apical part of valva **I** cornutus and spine of aedeagus **J–N** *P. retusa* sp. nov.: **J** male genitalia **K** aedeagus **L** uncus and gnathos **M** apical part of valva **N** cornutus. Scale bar: 0.5 mm.

**Diagnosis.** The species (Fig. 2G) is similar to *P. zolotuhini* Lvovsky, 2013 in the wing pattern but can be easily recognized from the latter species by the yellowish-brown ground color and relatively broad subbasal band and the absence of medial band of the forewing. The male genitalia (Fig. 4J–M) are characterized in having the triangular juxta bearing lateral cylindrical lobes.

**Distribution.** Laos (northeast; new record), China (south).

***Promalactis zolotuhini* Lvovsky, 2013**

Figures 2H, 5A–D

*Promalactis zolotuhoni* Lvovsky, 2013: 133. Type locality: Vietnam.

**Material examined.** 1♂, Laos, Bolikhamsai Prov., Phou Khao Khouay National Protected, 452 m, 3 July 2017, Bae et al., gen. slide no. 9840/ S. Kim.

**Diagnosis.** This species (Fig. 2H) is close to *P. apicisetifera* Du & Wang in its wing pattern, but it can be easily distinguished in having the forewing ground color tinged with reddish dark brown. The male genitalia (Fig. 5A–D) are differentiated from the those of *P. apicisetifera* in having the gnathos bearing round apical tips, the cucullus sclerotized, and the valva rounded apically.

**Distribution.** Laos (northeast; new record), Vietnam (north).

***Promalactis bitrigona* Kim & Park, 2012**

Figures 2I, 7A–C

*Promalactis bitrigona* Kim & Park, 2012: 900. Type locality: Vietnam.

**Material examined.** One female, Laos, Xiang khaung Prov., Ban Tha, 1204 m, 30 June 2017, Bae et al., gen. slide no. 9847/ S. Kim.

**Diagnosis.** The species (Fig. 2I) is distinguished from congeners by the three suffusions on the antemedial, postmedial, and apical areas of posterior margin of forewing. The male genitalia are characterized in having the valva with dense hairs on the costal margin (Kim et al. 2012; Fig. 3D–F), and by the female genitalia (Fig. 7A–C), which is characterized in having the antrum with a thumb-shaped caudal margin and the signum somewhat triangular-pyramid-shaped.

**Distribution.** Laos (northeast; new record), Vietnam (north).

***Promalactis crassa* Kim, sp. nov.**<http://zoobank.org/A61E6ECE-9D3B-483E-8CB6-C4D83A1294D3>

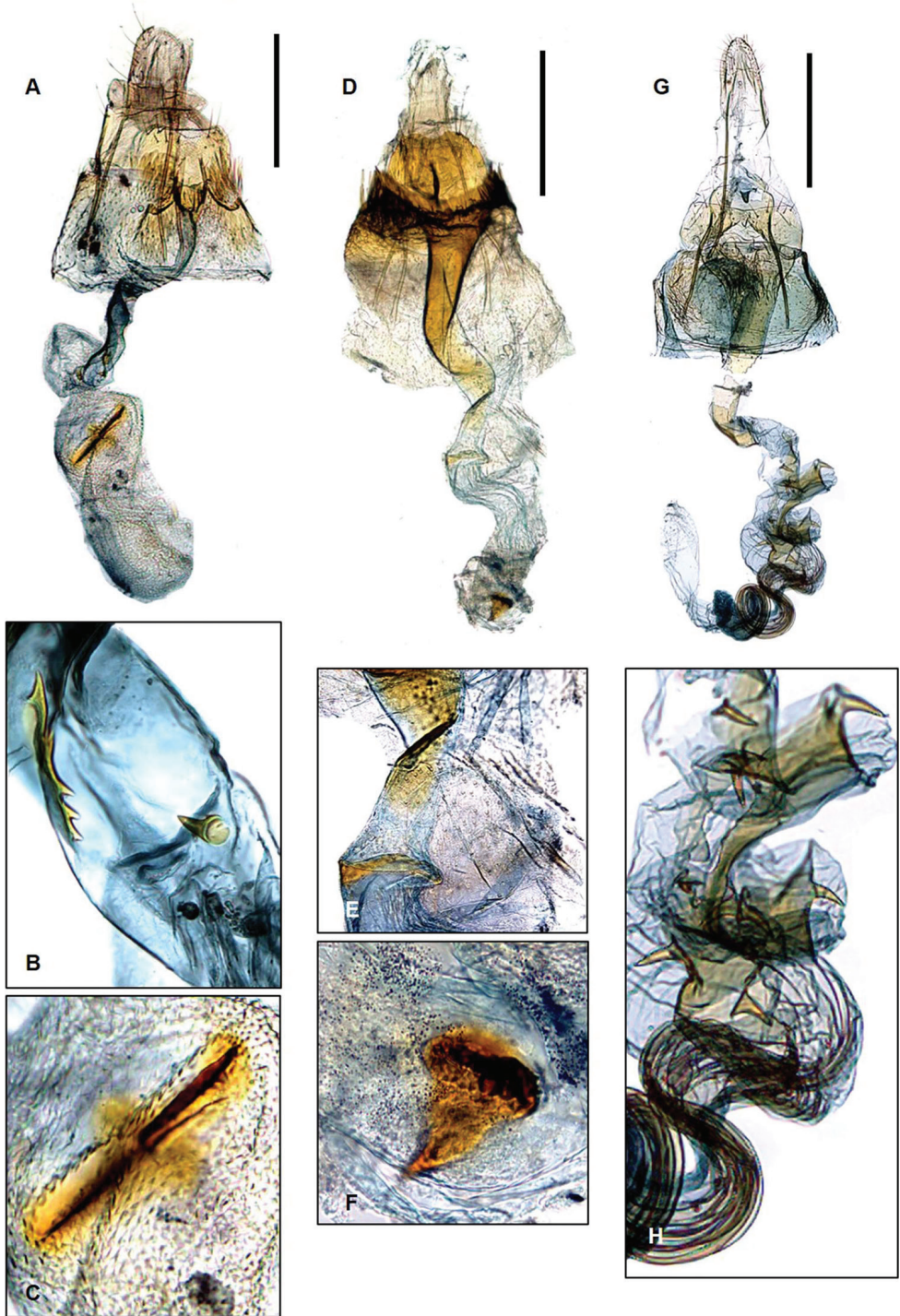
Figures 2J, 5E–I, 7D–F

**Type material. Holotype:** ♂, Laos, Xiang khaung Prov., Ban Tha, 1298 m, 7 August 2017, Bae et al., gen. slide no. 9592/ S. Kim. **Paratype:** 1♀, same locality, date and collector, gen. slide no. 9595. [Holotype and paratype are deposited in INU].

**Diagnosis.** This species is superficially similar to *P. diorbis* Kim & Park, 2012, but it can be differentiated in having the large costal patch at 4/5 of the length of the forewing and in having the small antrum and the thick ductus bursae bearing several tiny spines in the female genitalia.

**Description. Adult** (Fig. 2J). **Head:** frons pale grayish dark brown, tinged with dark brown; vertex dark brown; occiput yellowish dark brown. Antenna: scape entirely white,





**Figure 6.** Genitalia of Laos *Promalactis* **A–C** *P. parasuzukiella*: **A** female genitalia **B** spines in ductus bursae **C** signum **D–F** *P. uniclavata* sp. nov.: **D** female genitalia **E** projection in ductus bursae **F** signum **G–H** *P. spiraliola*: **G** female genitalia **H** numerous spines in ductus bursae. Scale bars: 0.5 mm.



except dark brown apically, shorter than diameter of eye; fragellum dark brown and white alternately from base to apex dorsally. **Labial palpus:** 2<sup>nd</sup> palpomere pale yellowish dark brown, 1.5 times longer than 3<sup>rd</sup> palpomere; 2<sup>nd</sup> palpomere dark brown, except white at apex. **Thorax:** thorax blackish dark brown; tegula dark brown. Wing expanse 11.0–11.5 mm. Forewing ground color yellowish brown; five bands, one spot, two costal patches, one posterior patch, and one apical patch, all white edged with fuscous scales: one sub-basal band short and one tiny spot near base, not connected each other; two antemedial bands: one just below subcostal (Sc) vein and the other at 1/6 posterior margin, both irregularly shaped, connected to each other at antemedial part of medial cell; the other antemedial band just before middle narrowed, arched connected to postmedial band; two costal patches: large one at 3/5 and somewhat semi-ovate, after pale grayish suffusion; small one at 4/5 and irregularly shaped, after fuscous suffusion; one small posterior patch after tornus; one apical patch larger than near costal and posterior patches; fringes yellowish brown near apex, mixed with fuscous scales near tornus. Hindwing more or less lanceolate; Hindwing ground color grayish brown; fringes dark grayish dark brown.

**Male genitalia** (Fig. 5E–I). Uncus large, thumb-shaped, with sclerotized, trifurcate projection, laterally setose near base. Gnathos wide at base, gradually narrowing to apex, inverted bell-shaped, shorter than uncus. Tegumen simple. Valva symmetrical; costal margin slightly concave basally, slightly convex medially, upward at sub-apex, bearing sclerotized, tiny spine at apex, setose after 2/3 to apex; saccular margin moderate, gradually narrowed to blunt apex, setose after 3/4 to apex, shorter than costal margin of valve. Juxta small bearing lateral spinous lobes, length of 2/3 of valve. Saccus wide at base, triangular, longer than uncus. Aedeagus gradually narrowed from base to 1/5, straight from 1/5 to 1/2, gradually broader from 1/2 to apex, bearing spine with bifurcate apexes at middle, 1/2 length of aedeagus; cornutus large, heavily sclerotized, hook-shaped at sub-apex.

**Female genitalia** (Fig. 7D–F). Apophyses posteriors almost 1.5 times longer than apophyses anteriores. Apophyses anteriores as long as papillae anales. Lamella post-vegenalis small, bearing lateral circular lobes on caudal margin. Lamella antevaginalis wide at base, incised centrally. Antrum small, tiny cup-shaped. Ductus bursae thickly developed, wide at base, gradually narrowed from 4/5 to 3/5, somewhat straight from 3/5 to corpus bursae, wrinkled longitudinally after middle, with numerous scattered spines. Corpus bursae membranous, semi-ovate. Signum absent.

**Distribution.** Laos (northeast; new).

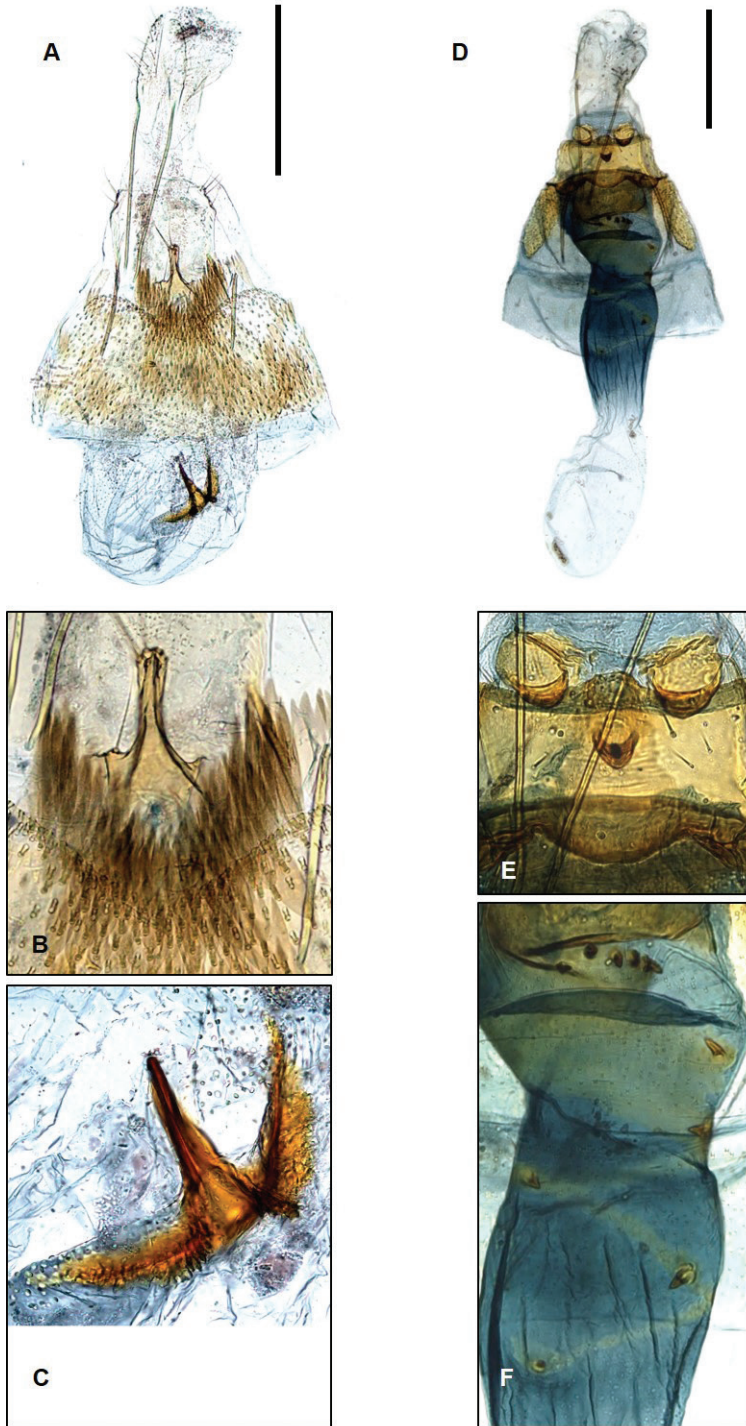
**Etymology.** The name of this species is derived from the Latin, *crass* (= thick) and the suffix *-a*, referring from the ductus bursae thickly developed in the female genitalia.

***Promalactis retusa* Kim, sp. nov.**

<http://zoobank.org/9D115097-1D7D-4C8F-B91E-51487818BB8A>

Figures 2K, 5J–N

**Type material. Holotype:** ♂, Laos, Bolikhamsai prov., Thaphabath, Phaset, 21 February 2013, Kim et al., gen. slide no. 9508/ S. Kim. Paratype: 1 ♂, same locality, data as holotype and collector. [Holotype and paratype are deposited in SNU.]



**Figure 7.** Genitalia of Laos *Promalactis* **A–C** *P. bitrigona*: **A** female genitalia **B** antrum **C** signum **D–F** *P. crassa* sp. nov.: **D** female genitalia **E** lamella post-and ante-vaginalis **F** tiny spines in ductus bursae. Scale bars: 0.5 mm.

**Diagnosis.** This species is externally similar to *P. crassa* sp. nov. in its wing pattern, but it can be easily recognized in having the costal and apical patches not large in the forewing. The male genitalia are differentiated by the inverted funnel-shaped uncus and the rectangular gnathos with blunt apex.

**Description. Adult** (Fig. 2K). **Head:** frons and vertex grayish dark brown; occiput grayish dark brown. Antenna: scape dark brown dorsally, white ventrally, shorter than diameter of eye; fragellum dark brown dorsally. **Labial palpus:** 2<sup>nd</sup> palpomere pale yellowish brown, except dark brown at apex dorsally, 1.5 times longer than 3<sup>rd</sup> palpomere; 3<sup>rd</sup> palpomere dark brown, except white at base dorsally. **Thorax:** thorax grayish dark brown partly mixed with whitish scales; tegula dark grayish brown. Wing expanse 8.0–8.5mm. Forewing ground color yellowish brown tinged with grayish dark brown near wing base, middle, and sub-apex on costa; five bands, one costal patch, four spots, and one apical patch, all white edged with fuscous scales: two subbasal bands, one from Sc vein, oblique toward apex, the other from anal vein, oblique toward costa, both connected to each other; two antemedial bands at 1/5, 2/7 of posterior margin, short, oblique toward costa; one antemedial band at 1/3 of costa, oblique toward before tornus; two spots medially, one on posterior vein of medial cell, the other at 1/2 of posterior margin; one costal patch, not connected to under tiny spot on posterior vein of medial cell; one spot at tornus; one apical patch occupied after 6/7 to apex with fuscous scales; fringes yellow near apex, mixed with fuscous scales middle and near tornus. Hindwing more lanceolate; ground color pale grayish dark brown; fringes grayish brown.

**Male genitalia** (Fig. 5J–N) Uncus inverted funnel-shaped, wide at base, gradually narrowing to apex, slightly bent inward, longer than gnathos. Gnathos rectangular, concave laterally, blunt apically, gradually narrowed from base to 1/2, gradually broadened from 1/2 to apex. Tegumen simple, as long as uncus. Valva symmetrical; costal margin straight to apex, roundly edged, setose after middle; saccular margin moderate, gradually narrowed to apex, apex bearing tiny spine, setose after middle. Saccus short, finger-shaped, shorter than uncus. Aedegus gradually broader to apex, as long as valve; cornutus sclerotized, bifurcate after middle.

**Female** unknown.

**Distribution.** Laos (northeast; new).

**Etymology.** The name of species is derived from the Latin, *retus* (= blunt) and the suffix –a, referring from the gnathos with blunt apex of male genitalia.

## Acknowledgements

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# A new leafmining pest of guava: *Hesperolyra guajavifoliae* sp. nov., with comments on the diagnostics of the endemic Neotropical genus *Hesperolyra* van Nieukerken (Lepidoptera, Nepticulidae)

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## Abstract

We describe a new pest of guava (*Psidium guajava* L.), *Hesperolyra guajavifoliae* Stonis & Vargas, **sp. nov.**, that was recently discovered in western Colombia. *Hesperolyra* van Nieukerken is a small, Neotropical genus of pygmy moths (Nepticulidae). We re-examine and document the complex morphology of the male genitalia of the generic type species, *H. diskusi* (Puplesis & Robinson). We discuss the diagnostics and composition of the genus and provide a simple pictorial differentiation scheme for all currently known representatives of the genus. The new species is illustrated with photographs of the adults, some of the immature stages, male and female genitalia, and leaf mines. A link to the COI barcodes of *H. guajavifoliae* **sp. nov.** is provided and the relationship of *Hesperolyra* to other genera is discussed.

## Keywords

New species, pygmy moths, guayaba, *Psidium guajava*, leaf mines, taxonomy, Colombia

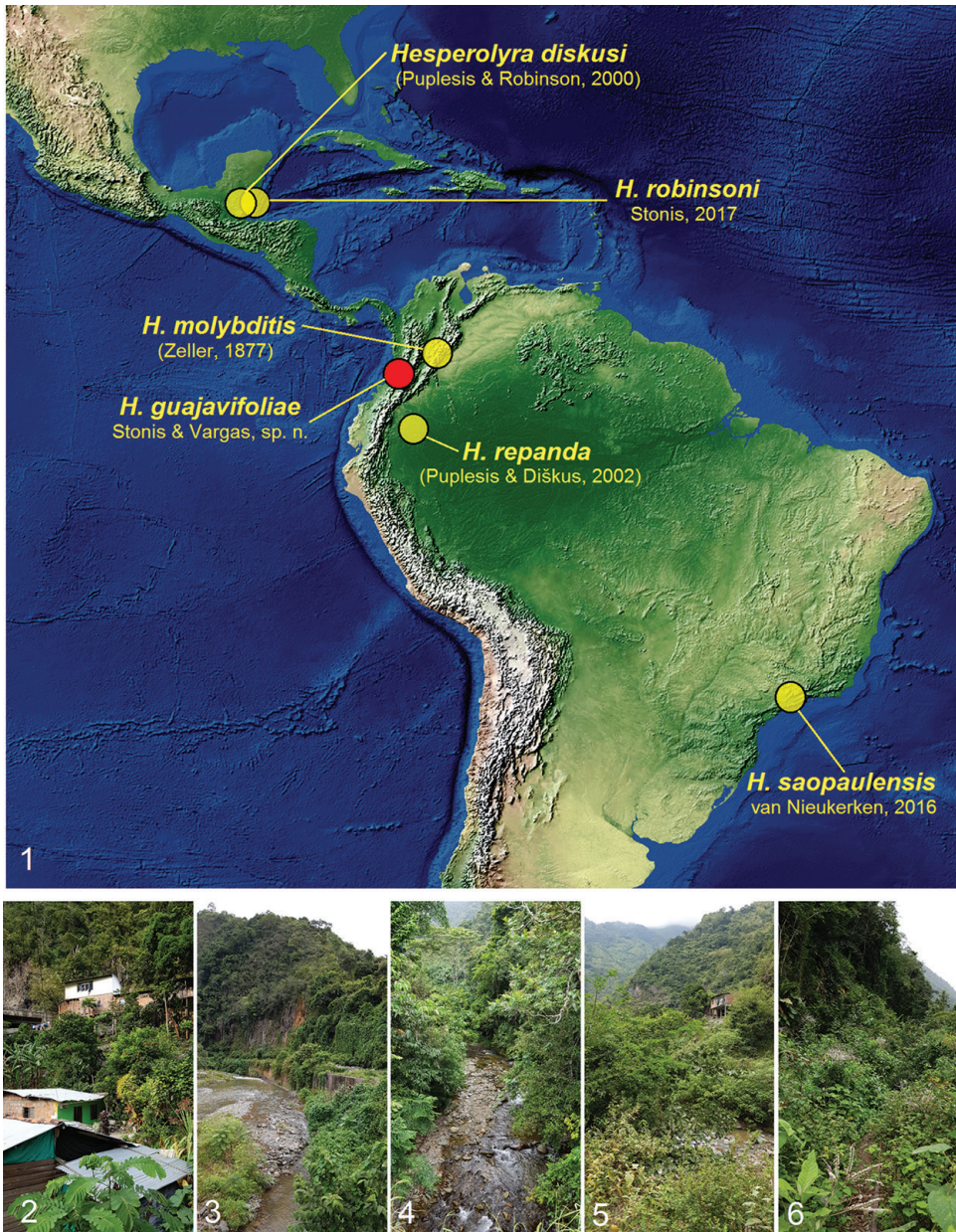


## Introduction

Guava or guayaba (*Psidium guajava* L.) is an important shrub or small tree cultivated for its fruit in many tropical countries in Asia, Africa, South America and the Caribbean. The fruit can be eaten raw or cooked, but, it is mostly known for its processed fruit products, and can be an integral part of local and international cuisine. The leaves and fruits are also fed to livestock (Heuzé et al. 2017). In some countries, this plant is also used in local traditional medicine (ethnopharmacology) to deal with numerous medical issues such as inflammation, diabetes, hypertension, tooth decay, wounds, ulcers, fever, diarrhea, lung ailments, rheumatism, and as a pain-relief remedy (Gutiérrez et al. 2008). Guava usually grows in areas below 1000 m, annual mean temperatures ranging from 23 to 28 °C, and 1500–2000 mm rainfall (Heuzé et al. 2017). In some tropical areas the plant can occur at altitudes up to 2000 m, in temperatures of 15–45 °C, and quiescent trees can even survive light frosts (Heuzé et al. 2017). Although the origin of this widespread species is not clear, it is believed to be native to countries in tropical America (Germplasm Resources Information Network 2019).

In late January to early March 2019, we conducted fieldwork in the Departamento de Valle del Cauca, northwest of Dagua in southwestern Colombia (Figs 2–6), where *Psidium guajava* is a common plant cultivated in orchards and widespread in the wild in various anthropogenic or natural habitats. We expected to find *Ozadelpha guajavae* (Puplesis & Diškus) (Lepidoptera, Nepticulidae), a guava-feeding nepticulid species described a few degrees south from the same western tropical slopes of the Andes in equatorial Ecuador (Puplesis et al. 2002a). It was later recorded in large numbers in the Andes of southern Ecuador near the Peruvian border (Remeikis et al. 2014). However, during our fieldwork in western Colombia, we discovered another species producing leaf mines in mass quantities on *P. guajava*. It appeared to be a new and distinctive species belonging to the recently erected, Neotropical genus *Hesperolyra* van Nieukerken. Including the newly discovered species described below, *Hesperolyra* now comprises six species that occur from Central America to the Atlantic coast of Brazil. Prior to our study, *H. molybdis* (Zeller, 1877), of which the host plant is unknown, was known to occur in central Colombia (Fig. 1). Previously, only one species of *Hesperolyra* had host-plant family data; *H. saopaulensis* van Nieukerken 2016 was reared from an unidentified Myrtaceae plant (van Nieukerken et al. 2016b).

Nepticulidae, or pygmy moths, are miners (occasionally gall inducers, e.g., van Nieukerken et al. 2016b) of assimilative tissues of plants. Some species have been included on lists of cultivated plant pests (e.g., Kuznetzov and Puplesis 1994). A general characterization of this family was provided by several authors, notably Scoble (1983), van Nieukerken (1986), Johansson et al. (1990), Puplesis (1994), Puplesis and Robinson (2000), Puplesis and Diškus (2003), Diškus and Stonis (2012), and recently van Nieukerken et al. (2016b). Nepticulidae are distributed worldwide and occur in almost all terrestrial habitats. Because of species endemism and a high degree of stenophagy, these tiny lepidopterans may serve as tools for monitoring the biodiversity richness of habitats and provide data on the evolutionary processes



**Figures 1–6.** Distribution map of currently known *Hesperolyra* species and habitat of *H. guajavifoliae* Stonis & Vargas sp. nov. **1** Distribution map (the map base, courtesy of Tom Patterson, USA) **2, 5, 6** El Naranjo, 3°46'46"N, 76°43'63"W, 550 m **3, 4** Cisneros, 3°46'27"N, 76°44'40"W, 450 m.

(Diškus and Stonis 2012; Remeikis 2017). Being some of the smallest moths, adults remain rare in many scientific collections, especially in the tropical countries of Latin America. However, in the field, leaf mines produced by nepticulid larvae are usually

distinctive and easy to spot. Sometimes these leaf mines may appear in very large numbers, dramatically reducing the assimilative tissue of mined host plants. Sometimes hundreds or thousands of larvae may feed upon a single tree at the same time, as in the case of the Holarctic *Ectoedemia occultella* (Linnaeus) and East-Asian *E. picturata* Puplesis (Puplesis 1994).

Below, we provide a description of the new species, documentation of leaf mines, adults, and their male and female genitalia. We also provide comments on the diagnostics of *Hesperolyra*, with a simple pictorial tool for identification of the currently known *Hesperolyra* species.

## Material and methods

### Material

The material was collected in 2019 in the Valle del Cauca in Colombia by Jonas R. Stonis and Sergio A. Vargas. We were assisted by Franklin J. Galindo (Collecting Permit No. 2019007511-1-000 by *Autoridad Nacional de Licencias Ambientales*, Bogotá, Colombia). The material is deposited at the collection of the Laboratorio de Entomología, UNESIS, Departamento de Biología, Pontificia Universidad Javeriana, Bogotá, Colombia (**MPUJ**). Additional material of the type species *Hesperolyra diskusi* (Puplesis & Robinson), used for comparison and re-study of the complicated morphology of the male genitalia, is currently at the Lithuanian University of Educational Sciences, Vilnius, Lithuania (**LEU**) and will be transferred for permanent deposition to the collection of the Zoological Museum, Natural History Museum of Denmark, Copenhagen (**ZMUC**).

### Methods

We followed collecting methods and protocols for species description outlined in Johansson et al. (1990), Puplesis and Diškus (2003), and Stonis et al. (2016). After maceration of the abdomen in 10% KOH and subsequent cleaning, male genital capsules were removed from the abdomen and mounted ventral side up. Both male and female genitalia were mounted in Euparal. In most cases the phallus was severed from the genital capsule. Abdominal pelts and female genitalia were stained with Chlorazol Black (*Direct Black 38/Azo Black*), male genitalia were left unstained (for a detailed description of the used method see Stonis et al. 2014).

Permanent preparations on microscope slides were photographed and studied using a Leica DM2500 microscope and a Leica DFC420 digital camera. Adults were photographed using a Leica S6D stereoscopic microscope with attached Leica DFC290 digital camera, except for Figs 13–20, 23–27, which were photographed using a Lomo MBS10 stereoscopic microscope and temporary attached cellular telephone Samsung



Galaxy S7 with a camera. The specimens were subjected to high intensity, daylight illumination and rotated to ascertain ground colour and reflection of the adult scaling.

The descriptive terminology of morphological structures follows Puplesis and Robinson (2000), except for the term “aedeagus”, which is here referred to as “phallus” and the term “cilia”, which is here referred to as “fringe”.

**Molecular analysis.** The fragment of the mitochondrial COI gene that includes the standard barcode region for the animal kingdom (Hebert et al. 2003) was analysed to evaluate the molecular distinctness of the newly described *Hesperolyra guajavifoliae* sp. nov. from closely related Nepticulidae species. To achieve this goal, eight specimens of *H. guajavifoliae* sp. nov., two specimens of *Acalyptris* Meyrick (Lepidoptera, Nepticulidae), and one specimen of *Pseudopostega* Kozlov (Lepidoptera, Opostegidae) were barcoded (Table 1). The total genomic DNA was extracted from legs or the whole specimens stored in 96% ethanol, using the GeneJet Genomic DNA Purification kit (Thermo Fisher Scientific Baltics) according to the manufacturer's specifications. A 674 bp fragment of the mitochondrial COI gene was amplified with the primers T3Lep-f (5'-ATTAACCCCTCACTAAAGTCWACHAATCATAAARATATTGG-3'; modified Lep-f1 (Hebert et al. 2004)) and T7Nancy-r (5'-AATACGACTCACTATAGGDA-RAATTARAATRTAAACYTCWG-3'; modified Nancy (Simon et al. 1994)). All PCR reactions were carried out in a volume of 25 µL containing 12.5 µL of 2× PCR buffer (Thermo Fisher Scientific Baltics), 2.5 µL of 10 pmol of each primer (Macrobio), 6.5 µL of deionised water, and 1 µL of genomic DNA. All amplification reactions were performed with the MasterCycler personal thermocycler (Eppendorf) with the following conditions: initial denaturation at 95 °C for 1 min; 45 cycles of denaturation at 94 °C for 40 s, annealing at 45 °C for 40 s, extension at 72 °C for 1 min; with a final extension at 72 °C for 5 min. PCR product quality was checked by electrophoresis on 1.5 % agarose gel (Thermo Fisher Scientific Baltics) stained with 10 000× GelRed (Biotium) and visualized under 305 nm UV light. The excess of primers and dNTPs was removed with exonuclease I and alkaline phosphatase (Thermo Fisher Scientific Baltics) prior to automatic sequencing by the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) in Macrogen Inc. (Seoul, South Korea). The sequences were manually aligned using BioEdit 7.2.5 (Hall 1999). The final aligned length of the dataset was 657 bp. The shorter than expected (609 bp-long) sequence of *Acalyptris* sp. involved in the analysis was due to unsuccessful sequencing. All sequences obtained in this study have been deposited in the GenBank database ([www.ncbi.nlm.nih.gov/Genbank](http://www.ncbi.nlm.nih.gov/Genbank)) under the accession numbers provided in Table 1. In addition, previously published Nepticulidae sequences downloaded from the BOLD platform (Ratnasingham and Hebert 2007) (<https://www.boldsystems.org>) were involved in further analysis. The nucleotide-sequence divergence was calculated using the Kimura 2-parameter distance (Kimura 1980) model and graphically displayed in the Neighbour-Joining (NJ) tree by the MEGA 6 software (Tamura et al. 2013). Robustness of the inferred tree was evaluated by bootstrapping with 10,000 replicates; the distantly related *Pseudopostega* sp. was used as an outgroup. MEGA 6 was also used for the calculation of pairwise distances, the mean distances within and between species.

**Table 1.** Data of studied Lepidoptera specimens and their DNA barcodes.

Species	Sex	Locality	Coordinates	Date	Collector	Sample ID	Genbank accession
<b>Nepticulidae:</b>							
<i>Acalyptis bifidus</i> Puplesis & Robinson	♂	COLOMBIA, Valle del Cauca, El Naranjo	3°47'2"N, 76°43'14"W	21–23. ii.2019	J. R. Stonis & S. Vargas	AB2517	MN732881
<i>Acalyptis</i> Meyrick sp.	♀	COLOMBIA, Valle del Cauca, Lobo Guerrero	3°45'42"N, 76°39'46"W	8.ii–3. iii.2019	J. R. Stonis & S. Vargas	AC2521	MN732881
<i>Hesperolyra guajavifoliae</i> Stonis & Vargas, sp. nov.	♂	COLOMBIA, Valle del Cauca, Cisneros	3°46'27"N, 76°44'40"W	11.ii–3. iii.2019	J. R. Stonis & S. Vargas	HG2527	MN732873
	♀	COLOMBIA, Valle del Cauca, Cisneros	3°46'27"N, 76°44'40"W	11.ii–3. iii.2019	J. R. Stonis & S. Vargas	HG2528	MN732874
	♂	COLOMBIA, Valle del Cauca, Cisneros	3°46'27"N, 76°44'40"W	11.ii–3. iii.2019	J. R. Stonis & S. Vargas	HG2529	MN732875
	♂	COLOMBIA, Valle del Cauca, Cisneros	3°46'27"N, 76°44'40"W	11.ii–3. iii.2019	J. R. Stonis & S. Vargas	HG2530	MN732876
	♀	COLOMBIA, Valle del Cauca, Cisneros	3°46'27"N, 76°44'40"W	11.ii–3. iii.2019	J. R. Stonis & S. Vargas	HG2532	MN732877
	♀	COLOMBIA, Valle del Cauca, Cisneros	3°46'27"N, 76°44'40"W	11.ii–3. iii.2019	J. R. Stonis & S. Vargas	HG2534	MN732878
	♂	COLOMBIA, Valle del Cauca, Cisneros	3°46'27"N, 76°44'40"W	11.ii–3. iii.2019	J. R. Stonis & S. Vargas	HG2535	MN732879
	♀	COLOMBIA, Valle del Cauca, Cisneros	3°46'27"N, 76°44'40"W	11.ii–3. iii.2019	J. R. Stonis & S. Vargas	HG2536	MN732872
<b>Opostegidae:</b>							
<i>Pseudopostega</i> Kozlov sp.	♂	COLOMBIA, Valle del Cauca, SW of Cali, Via Villa Carmelo, Desarrollo Biodiverso	none	29–30.i.2019	J. R. Stonis & S. Hill	PC2516	MN732882

## New species description

### *Hesperolyra guajavifoliae* Stonis & Vargas, sp. nov.

<http://zoobank.org/C4224ABF-1778-4651-BC9F-E5E3A48A100D>

**Type-specimen. Holotype:** male, pinned, with genitalia slide no. RA1033. Original label: Colombia, Departamento de Valle del Cauca, Municipio de Dagua, Cisneros, 3°46'27"N, 76°44'40"W, 450 m, larva on *Psidium guajava*, fieldcard no. SV003, 11 Feb – 3 Mar 2019, J. R. Stonis and S. A. Vargas. (MPUJ).

**Diagnosis.** Externally, adults of the new species are distinguishable from all other Neotropical Nepticulidae, including congeneric *Hesperolyra*, by a dark, oblique fascia and two small, dark, basal and apical spots on the forewing. However, in some specimens, including worn ones, the spots may be inconspicuous or absent. In the male genitalia, a large apical process of the valva, two large, horn-like processes fused with the transtilla and weakly developed cornuti in the phallus distinguish *H. guajavifoliae* sp. nov. from all other *Hesperolyra* species. In the female genitalia, the unique, large vaginal sclerite and distally wide vesicles of ductus spermathecae are hypothesized to be unique to this species, but this character may not remain valid for species differentiation because females of many nepticulid species are unknown and remain to be discovered. *Hesperolyra guajavifoliae* sp. nov. is distinguishable from another guava feeder, *Ozadelpha guajavae* Puplesis & Robinson, by a dark, oblique fascia and two small spots

on the forewing of the adults, and by blotch-like leaf mines (leaf mines of *O. guajavae* are slender and sinuous, see Remeikis et al. 2015: figs 1, 7).

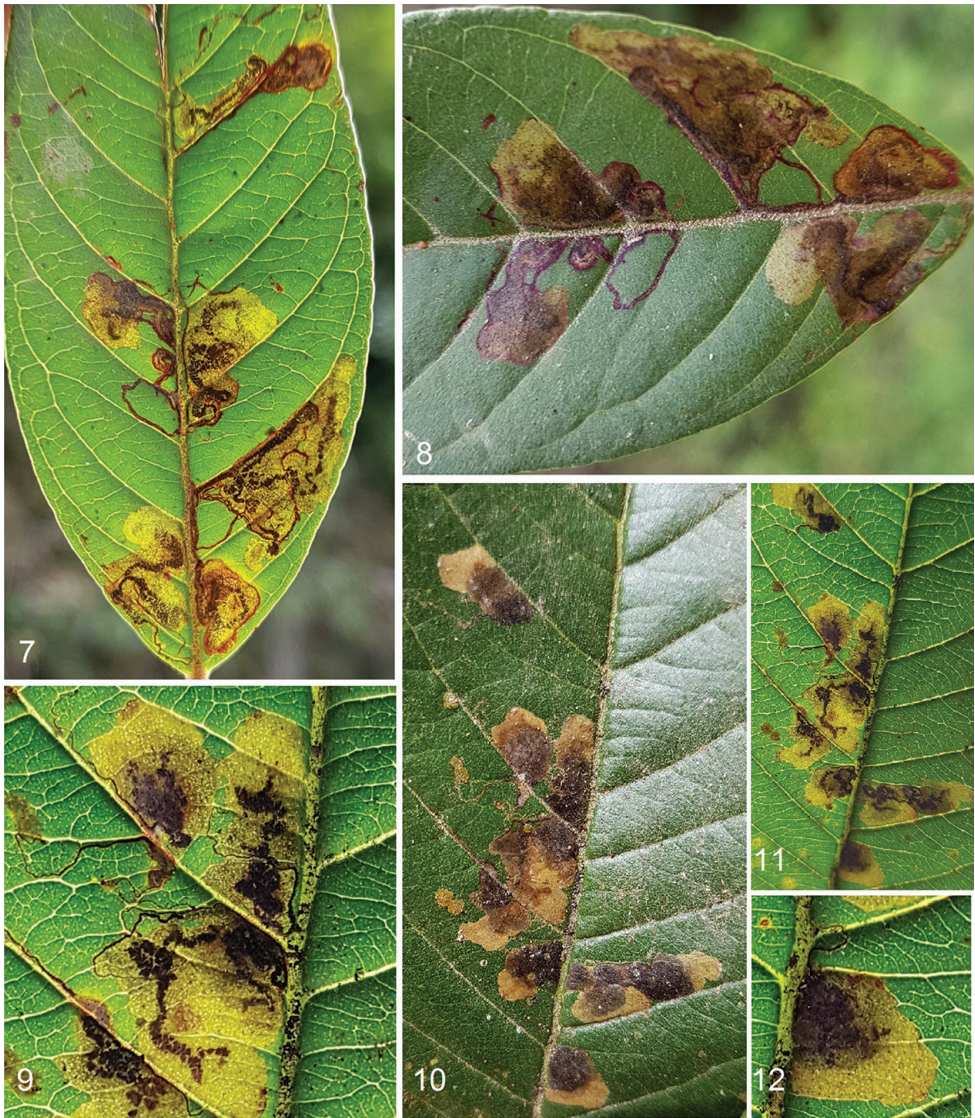
**Description. Male** (Figs 21, 30, 31, 34, 35). Forewing length 1.8–2.0 mm; wingspan 4.0–4.5 mm ( $n = 7$ ). **Head:** frontal tuft orangish ochre to ochre-brown; collar inconspicuous, comprised of piliform, cream scales; scape yellow cream to pale ochre, with some scattered brown scales; sometimes scape entirely cream, without brown scales, glossy; antenna slightly shorter than length of forewing; flagellum with 27–28 segments, pale grey to dark grey, with little purple iridescence. **Thorax,** tegula and forewing ochreous cream, sparsely speckled with dark brown scales; forewing with an oblique, postmedian fascia formed by black-brown scales, and with two small, black-brown apical and basal spots (the latter may be absent or inconspicuous in some specimens); fringe cream, fringe line irregular, inconspicuous; on underside, forewing pale grey or cream grey in basal half of wing, pale grey in rest; under fold with a distinct row of special scales, only visible in descaled wings (Figs 34, 35); venation with four distal veins:  $Rs_3$ ,  $Rs_4$ , M, and A (Figs 28–31). Hindwing glossy, cream to pale grey; on underside, basal third to half usually cream grey, pale grey in rest, or entire hindwing pale grey; fringe pale grey; venation with two distal veins: Rs and M (Figs 32, 33). **Legs** cream to ochre cream; on upper side, foreleg and midleg usually densely covered with dark grey or black-grey scales. Abdomen grey-brown on upper side, cream to pale ochre with some brown scales on underside; anal tufts cream, short, inconspicuous.

**Female** (Figs 22–24, 28, 29, 32, 33). Very similar to male but tends to be slightly darker and larger: forewing length 2.0–2.5 mm; wingspan 4.4–5.4 mm ( $n = 8$ ). Flagellum with about 25–26 segments. Forewing and hindwing undersides pale grey. Abdominal apex wide, truncated, and without anal tufts (Fig. 24). Otherwise as male.

**Male genitalia** (Figs 36–57). Capsule much longer (ca 325  $\mu\text{m}$ ) than wide (ca 185  $\mu\text{m}$ ). Vinculum large; ventral plate of vinculum widely rounded, truncated, without lateral lobes. Tegumen almost truncated or forming an inconspicuous, short, widely bilobed pseuduncus, with many setae on each lobe. Uncus thickened, inverted Y-shaped (Figs 41, 42). Gnathos with short but wide central process and slender lateral arms (Figs 36, 37). Valva (Figs 45, 46) 170–200  $\mu\text{m}$  long, 70–90  $\mu\text{m}$  wide, with long apical process (Fig. 45); transtilla without or with short sublateral processes (Figs 50, 54, 55), and with two large, horn-like processes (Figs 47, 48, 50, 55). Anellus thickened laterally (Figs 44, 49, 51, 55) and ventrally (Figs 55–57), membranous dorsally. Phallus (Figs 38, 40) 70–75  $\mu\text{m}$  long; minimal width 35–50  $\mu\text{m}$ , maximal width at base 70–85  $\mu\text{m}$ , without carina; vesica with an inconspicuous cathrema and plate-like cornutus, and thickened folds which in slides resemble cornuti (Fig. 40).

**Female genitalia** (Figs 58–64). Total length about 560  $\mu\text{m}$ . Anterior apophyses distally bent inwardly, slightly longer or equal to posterior ones (Figs 58, 64). Vestibulum with a wide, complex sclerite (Fig. 63). Corpus bursae rather small (reduced), without pectinations or signa, oval-shaped (Figs 58, 64). Accessory sac enlarged, equal or longer than corpus bursae; ductus spermathecae wide to slender proximally

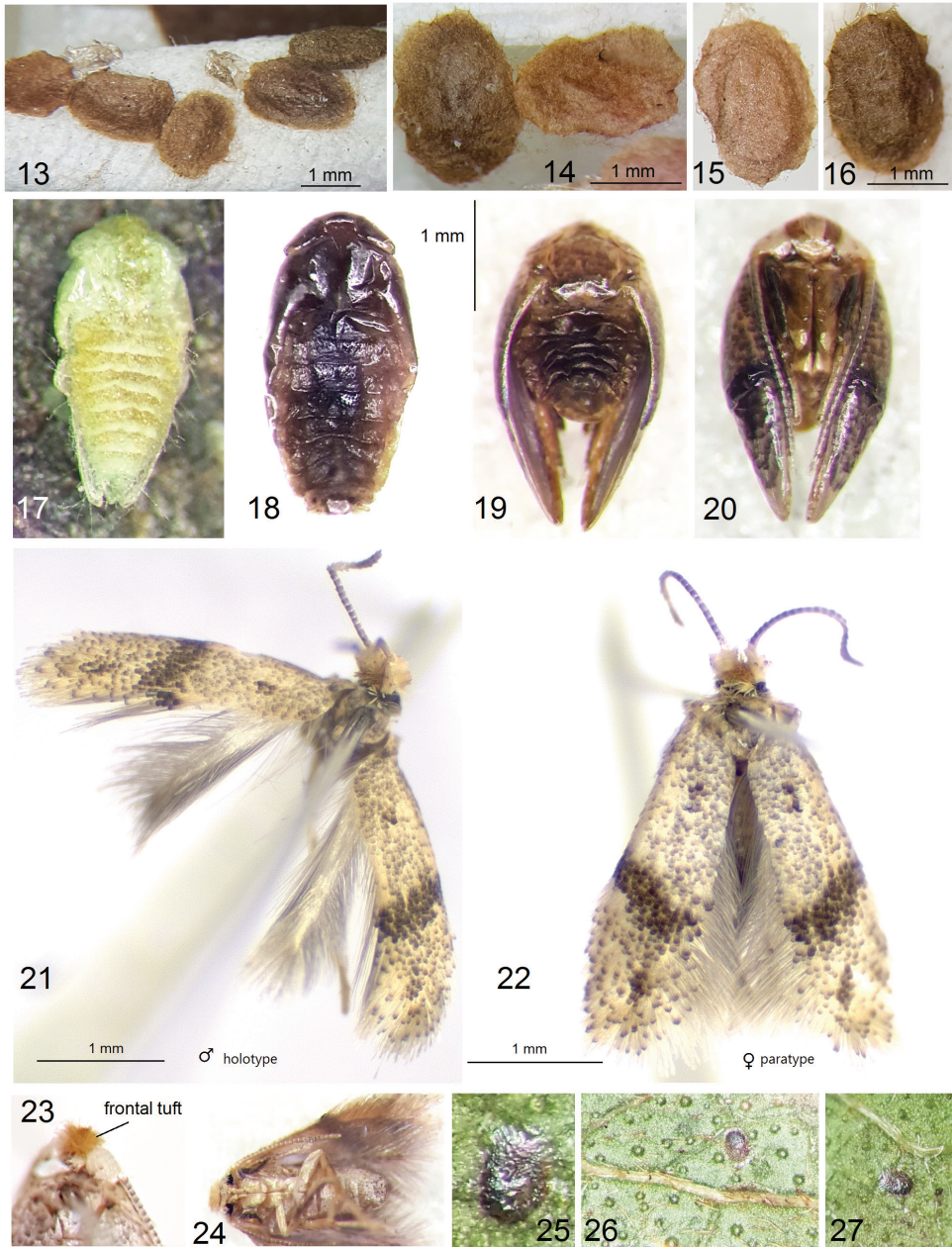




**Figures 7–12.** Leaf mines of *Hesperolyra guajavifoliae* Stonis & Vargas sp. nov. on *Psidium guajava* (Myrtaceae), Colombia, Valle del Cauca, Cisneros, 3°46'27"N, 76°44'40"W, 450 m.

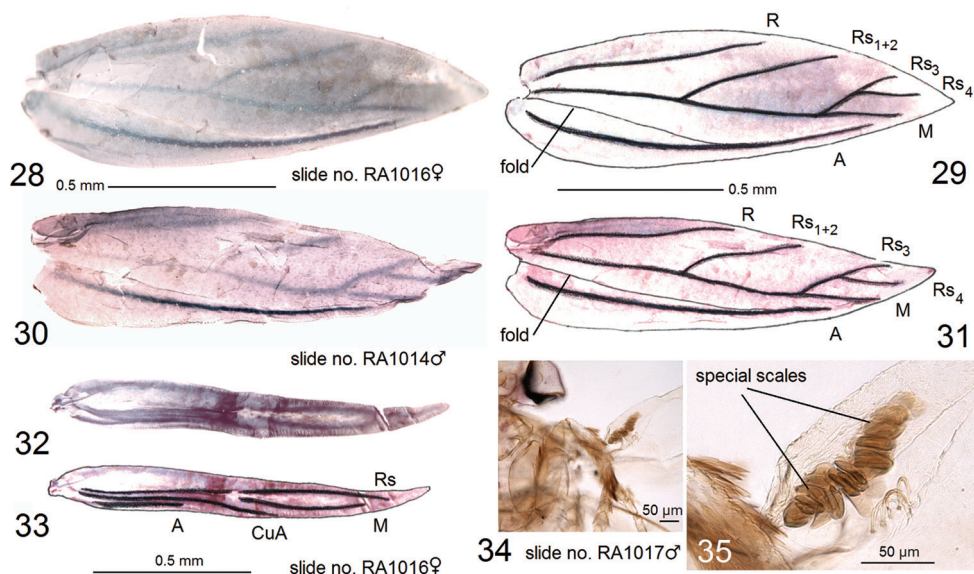
(see Figs 60, 62), with about three shallow convolutions (Fig. 64) and 2–2.5 large, rounded, plate-like vesicles distally (Figs 59, 61). Abdominal apex wide, truncated.

**Biology** (Figs 7–20, 25–27). Host plant: *Psidium guajava* (Myrtaceae). Egg (Figs 25, 27) laid singly on underside of leaf; egg case flat, 0.25 mm long ( $n = 6$ ), shiny, black-grey when filled with frass. Larvae mine leaves in February to early March; based on numerous older, vacant leaf mines, the mining may start as early as late December and be particularly active in January, i.e., during the drier season from late



**Figures 13–27.** *Hesperolyra guajavifoliae* Stonis & Vargas sp. nov. **13–16** cocoons **17–20** pupae (found dead in cocoons at different stages of development and with various levels of dehydration) **21** male holotype (MPUJ) **22** female paratype **23** frontal tuft, female paratype **24** ventral view, female paratype **25–27** AnEgg on a leaf underside of the host plant *Psidium guajava*.



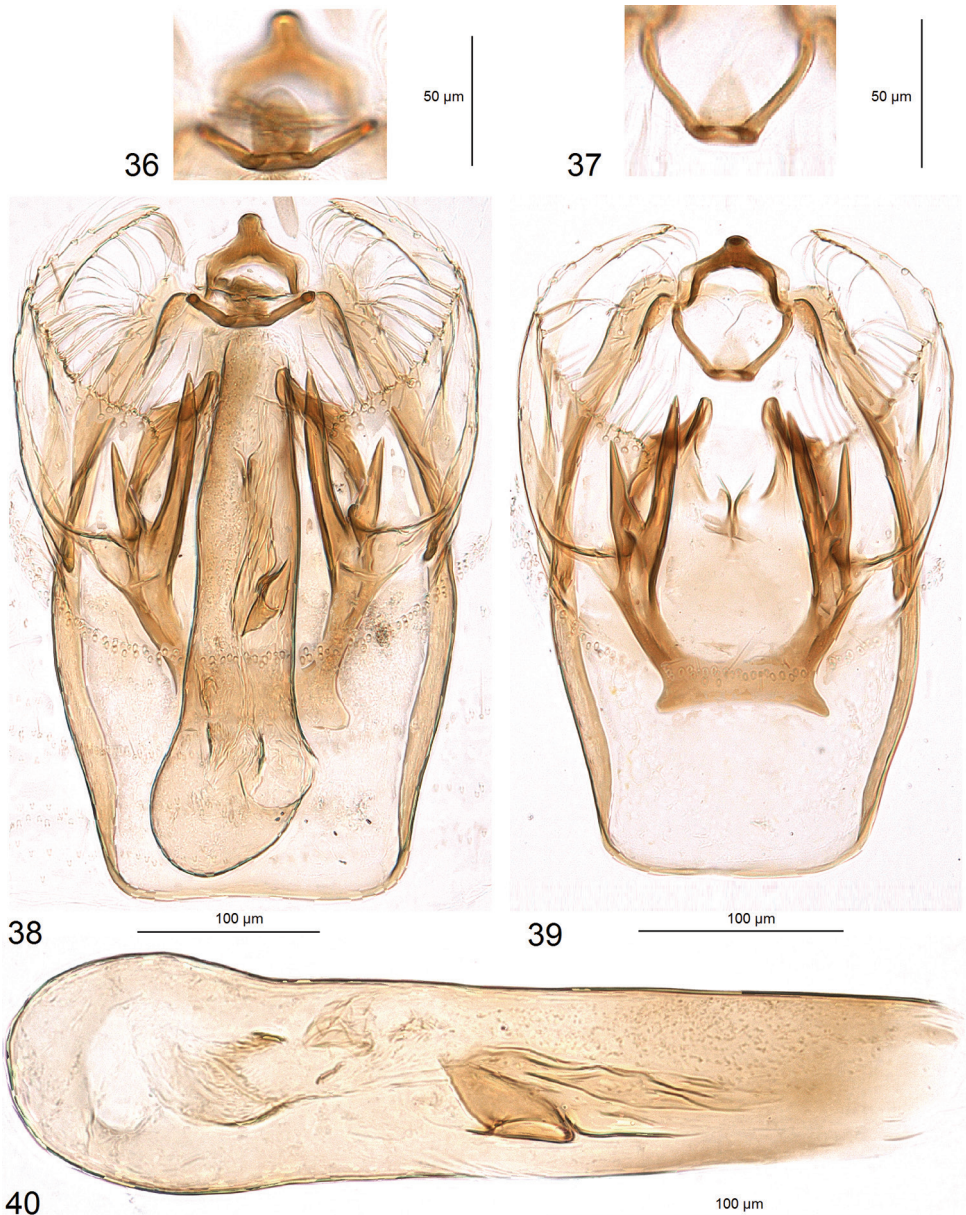


**Figures 28–35.** Morphology of *Hesperolyra guajavifoliae* Stonis & Vargas sp. nov. **28** forewing venation, female paratype, slide RA1016 **29** same, enhanced and labelled, with veins reinforced **30** forewing venation, male paratype, slide RA1014 **31** same, enhanced and labelled, with veins reinforced **32** hindwing venation, female paratype, slide RA1016 **33** same, enhanced and labelled, with veins reinforced **34, 35** special scales on descaled male paratype, slide no. RA1017 (MPUJ).

December to February in the exceptionally humid region of western Colombia (see Distribution); voltinism unknown. Larva pale green with a pale brown head and dark green intestine. Leaf mine (Figs 7–12) starts as a slender gallery filled with black frass; later the gallery almost abruptly widens to a blotch with irregularly scattered brown-black or black frass. Pupation occurs outside the leaf mine, possibly in debris or litter, because no cocoons were observed on the host plants. Exit slit on upperside of leaf. Pupation (Figs 17–20) inside cocoon; immature stages will be described elsewhere (Sergio A. Vargas, personal communication). Cocoon (Figs 13–16) 1.9–2.2 mm long, 1.2–1.5 mm wide ( $n = 9$ ), brown to blackish brown or dark green-brown (slightly paler when vacant and dried), usually with a rather distinct flat rim around the main body (Figs 15, 16). Adults emerged late February to March; moths were not collected at a light trap in localities where the species occurred, therefore, we do not know how readily moths fly to light. Otherwise, biology is unknown.

**Distribution** (Figs 1–6). So far, this species is known to occur at altitudes from 450 to 850 m on the western slopes of the Andes (Valle del Cauca, western Colombia), bordering with the lowland Choco province. The latter is possibly the most humid area on Earth, where annual rainfall reaches 11,770 mm (Wettest places on Earth 2019) and is equally distributed except for only slight dry season(s) (Figs 2–6).

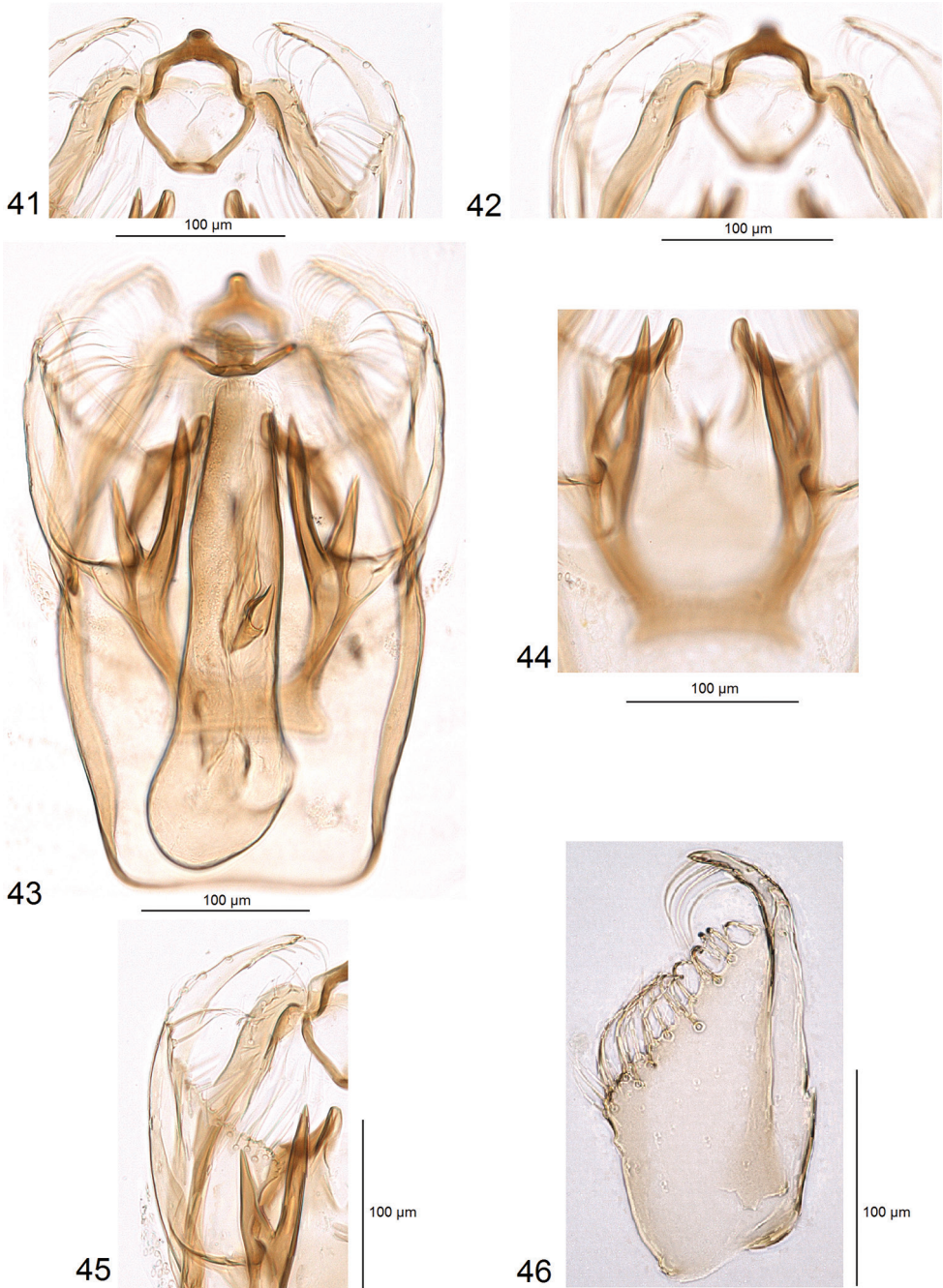
**DNA barcode.** We barcoded eight specimens of the type series, but not the holotype; sequences are available in GenBank under voucher/sample IDs MN732873, MN732874, MN732875, MN732876, MN732877, MN732878, MN732879, MN732872.



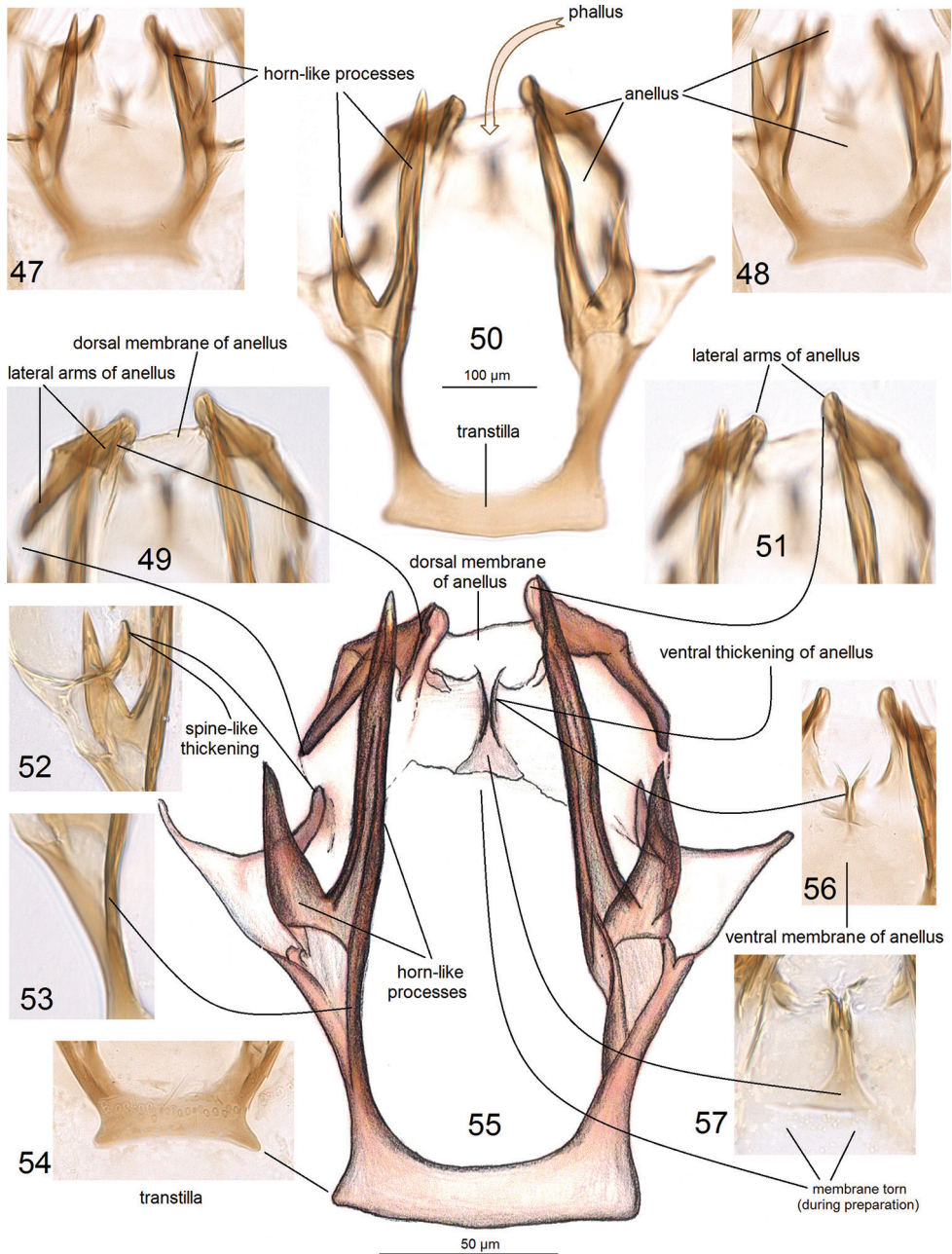
**Figures 36–40.** Male genitalia of *Hesperolyra guajavifoliae* Stonis & Vargas sp. nov. **36** gnathos, paratype, genitalia slide no. RA1017 **37** same, holotype, genitalia slide no. RA1033 **38** complete genitalia, paratype, slide no. RA1017 **39** capsule with phallus removed, holotype, genitalia slide no. RA1033 **40** phallus, holotype, genitalia slide no. RA1033 (MPUJ).

**Etymology.** The species name derives from the Latin name of the host plant *guajava*, in combination with the Latin *folium* (a leaf), in reference to the feeding habit of the new species; although the ending -ae here is not correct Latin (van Nieukerken, personal comm.), we preferred to name the species as *guajavifoliae* and not otherwise.



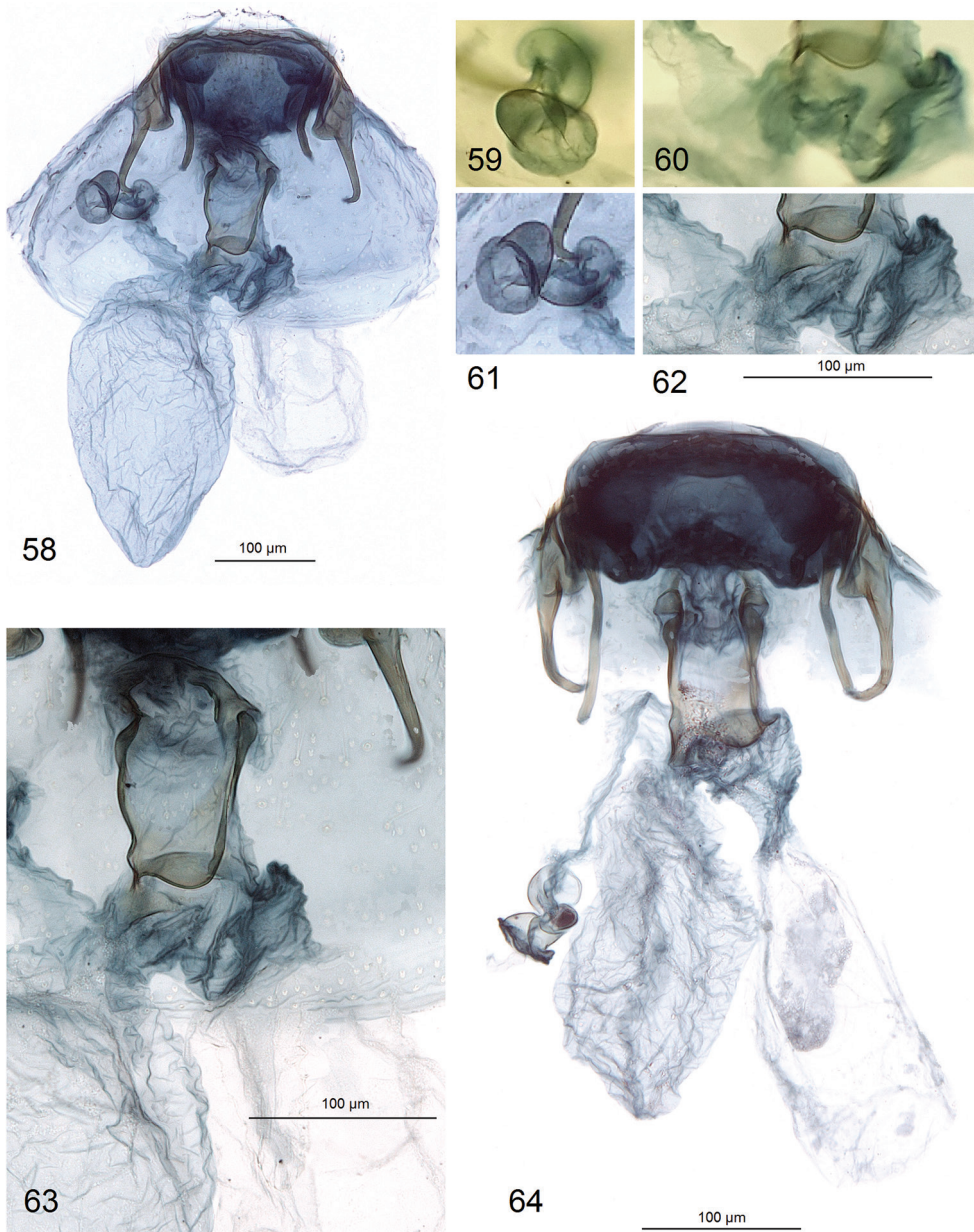


**Figures 41–46.** Male genitalia of *Hesperolyra guajavifoliae* Stonis & Vargas sp. nov. **41** tegumen, uncus, and gnathos, holotype, genitalia slide RA1033 **42** same, at different focus **43** complete genitalia, paratype, genitalia slide no. RA1017 **44** anellus and horn-like processes, holotype, genitalia slide RA1033 **45** valva, holotype, genitalia slide no. RA1033 **46** same, paratype, genitalia slide no. RA1018 (MPUJ).



**Figures 47–57.** Male genitalia of *Hesperolyra guajavifoliae* Stonis & Vargas sp. nov. Details of morphology **47, 48, 54, 56** holotype, genitalia slide no. RA1033 **49–53, 55, 57** paratype, genitalia slide no. RA1018 (MPUJ).





**Figures 58–64.** Female genitalia of *Hesperolyra guajavifoliae* Stonis & Vargas sp. nov. **58–63** paratype, genitalia slide no. RA1015 **64** same, genitalia slide no. RA1034 (MPUJ).

**Other material examined.** 13 ♂, 13 ♀, paratypes: Colombia, Departamento de Valle del Cauca, Municipio de Dagua, Cisneros, 3°46'27"N, 76°44'40"W, 450 m, larva on *Psidium guajava* (Myrtaceae), fieldcard no. SV003, 11 Feb. – 3 Mar. 2019, Jonas R. Stonis and Sergio A. Vargas leg., genitalia slide nos RA1014♂, RA1015♀, RA1016♀, RA1034♀ (MPUJ).

## Discussion

In the first review of the Neotropical Nepticulidae (Puplesis and Robinson 2000) we noticed that some nepticulid species from Belize and Colombia looked different, mostly because of the long transtilla and horn-like processes in the male genitalia. We hypothesized that these species could belong to *Fomoria* Beirne, but the observed difference in the forewing venation in comparison to *Fomoria* (Puplesis and Robinson 2000: fig. 64) was incorrectly hypothesized to be a case of partial venational reduction. Later, a formal species group within *Fomoria* was erected for species with these male genitalic and forewing venational characters (Puplesis et al. 2002b). In the most recent review and global catalogue (van Nieukerken et al. 2016a, 2016b), the *molybdis* group was excluded from *Fomoria* and erected as a separate genus.

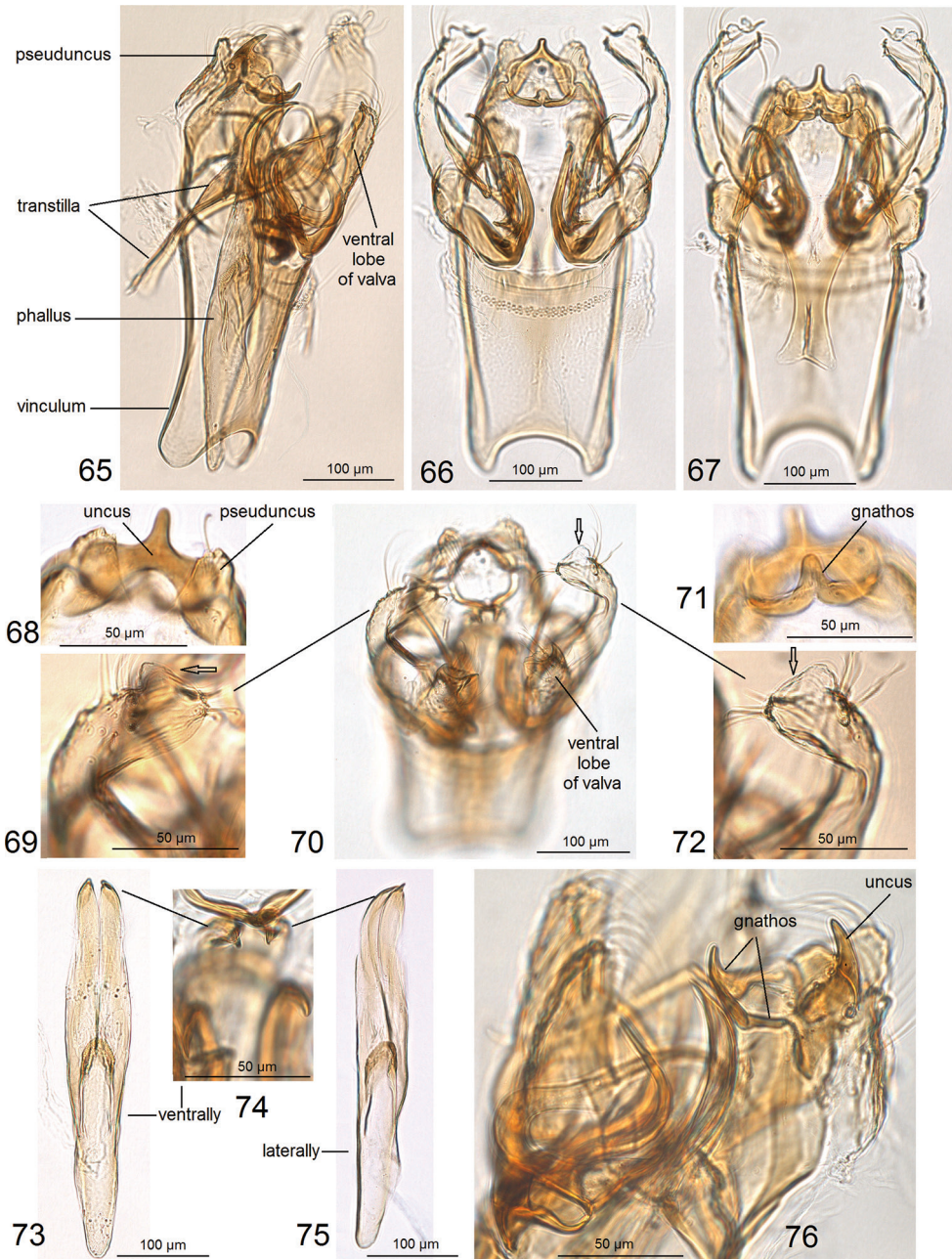
For this study, we re-examined the type species of *Hesperolyra*, *H. diskusi* (Puplesis & Robinson, 2000), that is characterized by a complex morphology of the male genitalia (Figs 65–86). The horn-like processes are the most remarkable genitalic features of this species. Characterization of *H. diskusi* was provided in the original description (Puplesis and Robinson 2000) and later in the redescription by van Nieukerken et al. (2016b). In the current study, we found two, not three, large processes (Figs 83–86), and observed that they are attached to the valva (Figs 78–83), not the anellus as was supposed earlier (van Nieukerken et al. 2016b); we did not observe the presence of an anellus. We also found that the transtilla in *H. diskusi* is more flexible and movable in comparison to most Nepticulidae that possess a transtilla; it can be lifted slightly dorsally at an angle even if the valvae are fixed (Figs 65, 77), and the connections of the transtilla to the valva are unusually weak, easy to break since they are supported by slender, little chitinized arms (Fig. 67).

We provide photographic documentation of the genital structures at different angles (Figs 65–86); they, including the spine-like thickening on the ventral lobe of the valva (Fig. 84) or the ventrally-bent spines of the phallus (Fig. 74), are usually not available for observation or appear different in permanent mounts. After our examination, we became convinced that none of the large, horn-like processes are carinal processes of the phallus.

Upon comparison of the male genitalia, we found that *Hesperolyra guajavifoliae* sp. nov. fundamentally differs in morphology from *H. diskusi*. We discovered that in *H. guajavifoliae* the horn-like processes are connected not with the valva itself, but are fused with the transtilla (Figs 50, 55), and, in contrast to *H. diskusi*, the anellus is present in the male genitalia. We found that the anellus is comprised of a weakly chitinized dorsal membrane (Figs 49, 55), strongly thickened lateral arms (Figs 49, 51, 55), and a ventral membrane with an elaborate thickening (Figs 55–57) surrounding the phallus from all sides (Fig. 50, best seen in Figs 38, 43).

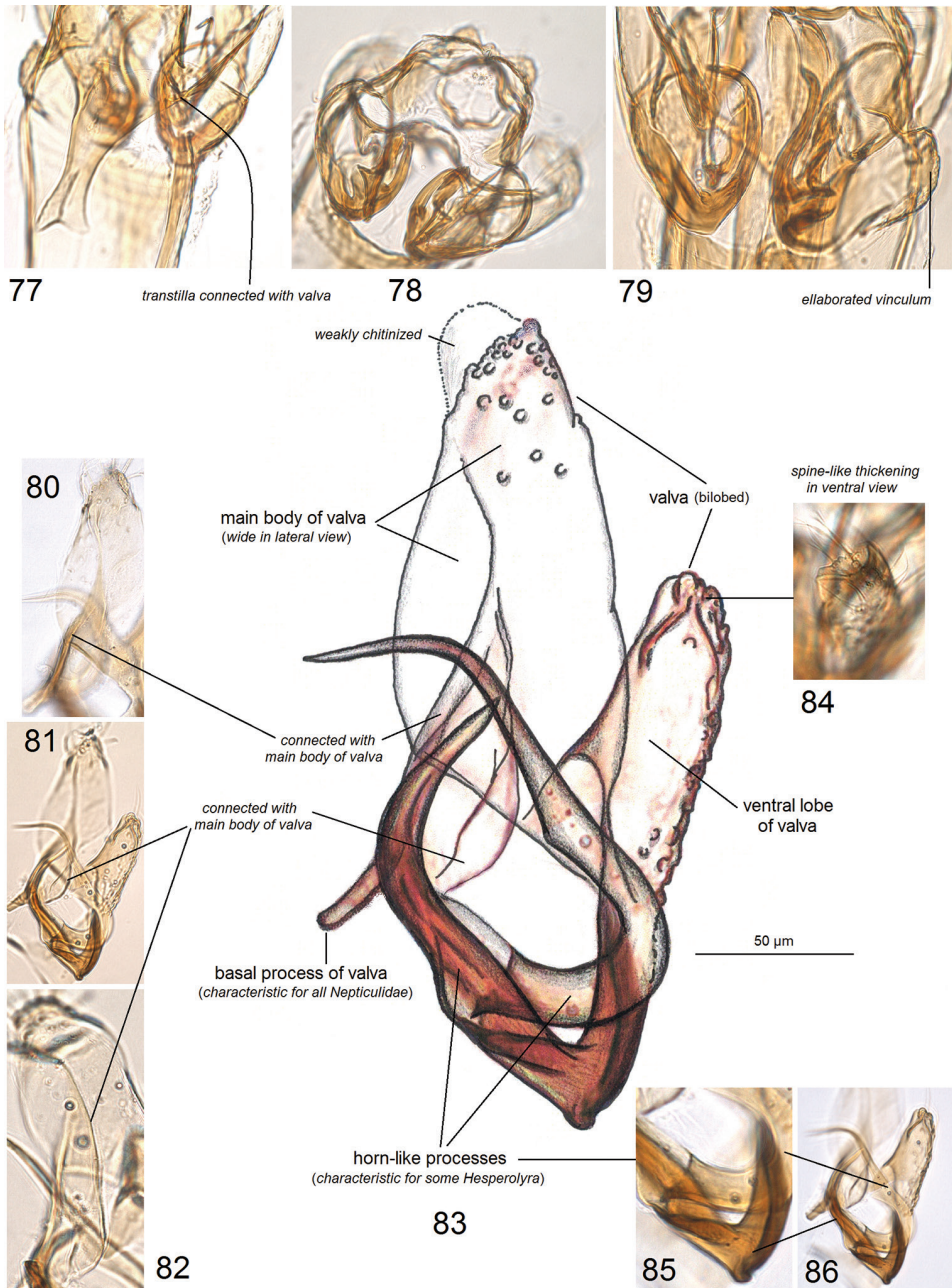
Currently, there are six species of *Hesperolyra* distributed from Central America (Belize) to the Atlantic coast of Brazil (Fig. 1); the species from Brazil was described from a female only (van Nieukerken et al. 2016b). All species are distinctive; therefore, diagnostics of *Hesperolyra* species, including the Brazilian *H. saopaulensis*, is clear (see Fig. 87). However, it also raises some questions: do all the species really belong to the same genus, and what is their relationship to other genera?





**Figures 65–76.** Details of male genitalia of *Hesperolyra diskusi* Puplesis & Robinson, paratype, genitalia slide no. AD989 (ZMUC).

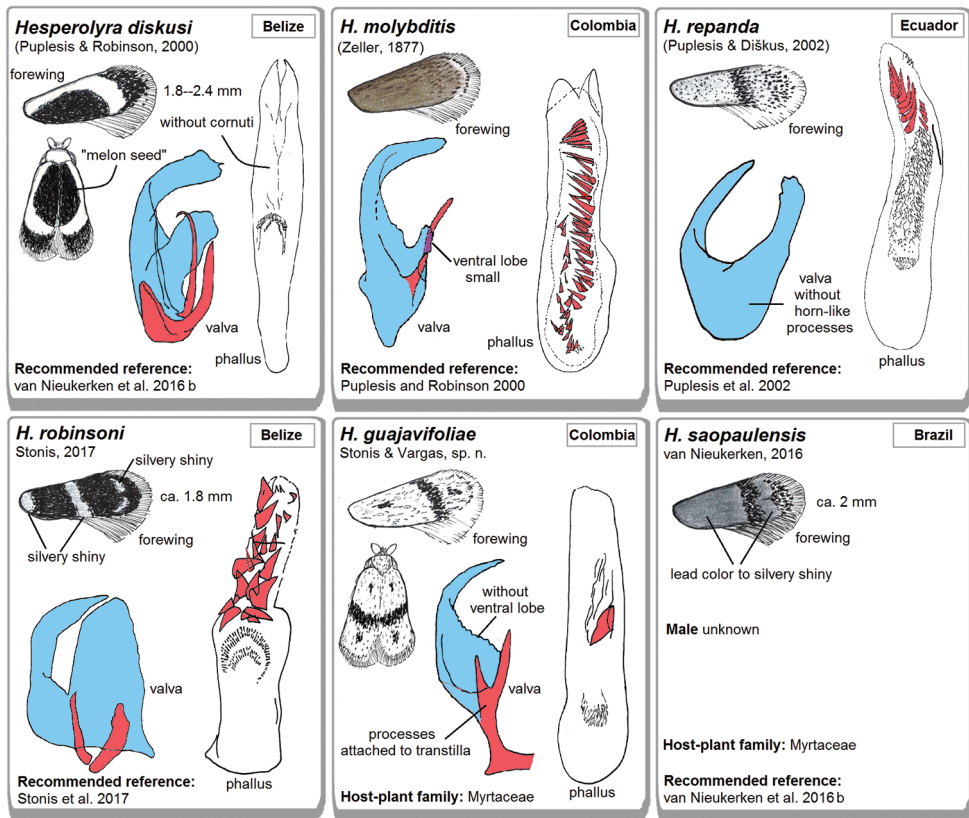
The wing venation of *Hesperolyra guajavifoliae* sp. nov. (Figs 28–33) is almost identical to *H. saopaulensis* (see van Nieuwerkerken et al. 2016b: fig. 116) and similar to *H. diskusi* (see Puplesis and Robinson 2000: fig. 64); the venation of remaining species is unstudied. It is important to note that unique, special scales hidden under



**Figures 77–86.** Details of male genitalia of *Hesperolyra diskusi* Puplesis & Robinson **77–79, 84** paratype, genitalia slide no. AD989 (NHMUK) **80–83, 85, 86** paratype, genitalia slide no. AD962 (ZMUC).

the forewing fold are characteristic of both *H. diskusi* and *H. guajavifoliae* sp. nov. We have concluded that *Hesperolyra* most likely represents a separate, monophyletic taxon, characterized by a wide forewing, with more or less uniform, but unique, simplified venation, extended, lyre-shaped transtilla, elaborate valva, and the presence of horn-

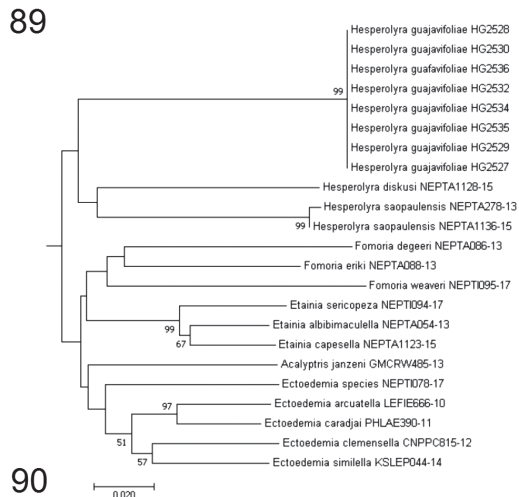
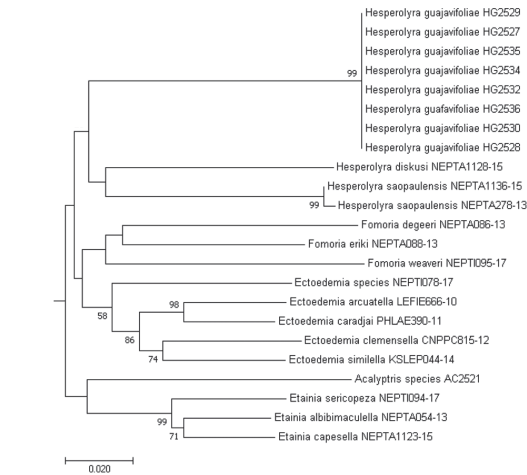
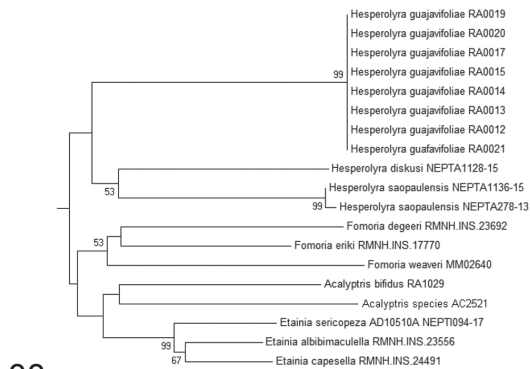




**Figure 87.** A pictorial tool for quick diagnostics of the currently known *Hesperolyra* species. Note: the morphological structures are drawn at different scales.

like processes in the male genitalia, and possibly the feeding on Myrtaceae (host plant known for only two species). *Hesperolyra* was also supported by a multi-gene molecular analysis by Doorendeerd et al. (2016), that grouped it with *Neotrifurcula* van Nieukerken and *Bohemannia* Stainton. *Neotrifurcula* was subsequently synonymized with *Glaucolepis* Braun (Stonis et al. 2017).

During our study, ten sequences of 657 bp and 1 sequence of 609 bp of the mtDNA COI gene belonging to three Nepticulidae and one Opostegidae species were successfully obtained (Table 1). These data were supplemented by the sequences of other species downloaded from the BOLD website (Table 2). In these sequences, 184 parsimony-informative sites were detected. The overall mean distance between analysed species estimated using the same mtDNA sequence was  $14.8 \pm 1.0$ . The interspecific pairwise distances between the pairs of the studied species varied from  $5.6 \pm 1.0\%$  (between *Etainia albibimaculella* (Larsen) and *E. capesella* (Puplesis)), and  $22.1 \pm 2.5\%$  (between *H. guajavifoliae* sp. nov. and *Pseudopostega* sp.). The smallest interspecific distance from *H. guajavifoliae* sp. nov. to any other species was  $15.3 \pm 1.9\%$  (i.e., between *H. guajavifoliae* sp. nov. and *Fomoria eriki*) (Table 2). This indicates that COI can be used as a useful diagnostic tool for the identification of this new species. On the other



**Figures 88–90.** Fragments of different versions of Neighbour-Joining tree of *Hesperolyra* and other Nepticulidae genera (the full molecular phylogeny will be published elsewhere; a general phylogeny of Nepticulidae is not presented or discussed here). The divergence was calculated using the Kimura 2-parameter model based on 657 bp mtDNA COI sequences. Bootstrap values below 50 are not shown. *Pseudopostega* sp. (Opotegeidae) was used as outgroup.



**Table 2.** Pairwise distances between sequences. The number of base substitutions per site between sequences (%) are shown; standard error estimates (%) are shown above the diagonal and were obtained by a bootstrap procedure (10,000 replicates). Analyses were conducted using the Kimura 2-parameter model.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26		
<i>Hesperolyna guajayifoliae</i> HG2536		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.020	0.020	0.023	0.019	0.023	0.022	0.021	0.023	0.019	0.020	0.019	0.020	0.021	0.019	0.019	0.020	0.025	
2 <i>H. guajayifoliae</i> HG2527		0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.020	0.020	0.023	0.019	0.023	0.022	0.021	0.023	0.019	0.020	0.019	0.020	0.021	0.019	0.019	0.020	0.025	
3 <i>H. guajayifoliae</i> HG2528		0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.021	0.020	0.020	0.023	0.019	0.023	0.022	0.021	0.023	0.019	0.020	0.019	0.020	0.021	0.019	0.019	0.020	0.025	
4 <i>H. guajayifoliae</i> HG2529		0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.021	0.020	0.020	0.023	0.019	0.023	0.022	0.021	0.023	0.019	0.020	0.019	0.020	0.021	0.019	0.019	0.020	0.025	
5 <i>H. guajayifoliae</i> HG2530		0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.021	0.020	0.020	0.023	0.019	0.023	0.022	0.021	0.023	0.019	0.020	0.019	0.020	0.021	0.019	0.019	0.020	0.025	
6 <i>H. guajayifoliae</i> HG2532		0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.021	0.020	0.020	0.023	0.019	0.023	0.022	0.021	0.023	0.019	0.020	0.019	0.020	0.021	0.019	0.019	0.020	0.025	
7 <i>H. guajayifoliae</i> HG2534		0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.021	0.020	0.020	0.023	0.019	0.023	0.022	0.021	0.023	0.019	0.020	0.019	0.020	0.021	0.019	0.019	0.020	0.025	
8 <i>H. guajayifoliae</i> HG2535		0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.021	0.020	0.020	0.023	0.019	0.023	0.022	0.021	0.023	0.019	0.020	0.019	0.020	0.021	0.019	0.019	0.020	0.025	
9 <i>H. diskus</i> NEPTA1128-15		0.174	0.174	0.174	0.174	0.174	0.174	0.174	0.174		0.018	0.018	0.022	0.019	0.023	0.022	0.019	0.023	0.019	0.020	0.021	0.018	0.018	0.019	0.018	0.019	0.023	
10 <i>H. saopaulensis</i> NEPTA1136-15		0.159	0.159	0.159	0.159	0.159	0.159	0.159	0.159	0.142		0.002	0.021	0.020	0.021	0.022	0.019	0.022	0.020	0.021	0.021	0.020	0.019	0.017	0.019	0.018	0.022	
11 <i>H. saopaulensis</i> NEPTA278-13		0.164	0.164	0.164	0.164	0.164	0.164	0.164	0.164	0.146	0.003		0.021	0.020	0.021	0.022	0.019	0.022	0.020	0.021	0.021	0.020	0.019	0.017	0.019	0.018	0.022	
12 <i>Fomoria degeeri</i> NEPTA086-13		0.203	0.203	0.203	0.203	0.203	0.203	0.203	0.203	0.190	0.172	0.172		0.017	0.022	0.020	0.019	0.024	0.018	0.018	0.019	0.018	0.017	0.019	0.018	0.018	0.023	
13 <i>E. eriki</i> NEPTA088-13		0.153	0.153	0.153	0.153	0.153	0.153	0.153	0.153	0.155	0.165	0.169	0.134	0.019	0.021	0.019	0.021	0.017	0.018	0.017	0.017	0.017	0.017	0.019	0.018	0.017	0.024	
14 <i>E. weaveri</i> NEPT1095-17		0.194	0.194	0.194	0.194	0.194	0.194	0.194	0.194	0.192	0.172	0.175	0.143		0.023	0.021	0.024	0.020	0.021	0.020	0.021	0.022	0.021	0.023	0.023	0.020	0.024	
15 <i>Aatlypris bifidus</i> AB2517		0.184	0.184	0.184	0.184	0.184	0.184	0.184	0.184	0.177	0.187	0.192	0.164	0.171	0.188		0.019	0.020	0.016	0.016	0.018	0.020	0.019	0.019	0.020	0.020	0.021	
16 <i>A. janzeni</i> GMCRW485-13		0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.154	0.157	0.162	0.155	0.151	0.167	0.141		0.020	0.016	0.017	0.017	0.016	0.016	0.014	0.015	0.018	0.022	
17 <i>Aatlypris</i> sp. AC2521		0.192	0.192	0.192	0.192	0.192	0.192	0.192	0.192	0.187	0.179	0.185	0.199	0.168	0.194	0.150	0.164		0.019	0.019	0.019	0.020	0.020	0.021	0.019	0.023	0.025	
18 <i>Ectasia albithinacutella</i> NEPTA054-13		0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.173	0.178	0.148	0.127	0.165	0.121	0.121	0.148		0.010	0.011	0.015	0.014	0.016	0.014	0.018	0.023	
19 <i>E. capella</i> NEPTA1123-15		0.165	0.165	0.165	0.165	0.165	0.165	0.165	0.165	0.163	0.175	0.180	0.144	0.142	0.168	0.121	0.128	0.147	0.056		0.011	0.014	0.014	0.016	0.015	0.018	0.023	
20 <i>E. serripes</i> NEPT1094-17		0.156	0.156	0.156	0.156	0.156	0.156	0.156	0.172	0.178	0.183	0.155	0.135	0.154	0.135	0.130	0.148	0.067	0.066		0.015	0.016	0.017	0.016	0.018	0.022		
21 <i>Ectodermia arcuella</i> LEFTE666-10		0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.150	0.168	0.173	0.148	0.127	0.169	0.154	0.120	0.166	0.111	0.112	0.123		0.010	0.015	0.013	0.015	0.022	
22 <i>E. canadai</i> PHILAE390-11		0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.150	0.159	0.163	0.140	0.125	0.177	0.148	0.126	0.162	0.107	0.112	0.128	0.059		0.014	0.012	0.014	0.021	
23 <i>E. demersella</i> CNPPC815-12		0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.157	0.143	0.143	0.158	0.148	0.173	0.150	0.100	0.174	0.125	0.126	0.141	0.106	0.096		0.012	0.016	0.022
24 <i>E. similis</i> KSLEP044-14		0.163	0.163	0.163	0.163	0.163	0.163	0.163	0.163	0.143	0.162	0.166	0.153	0.138	0.181	0.150	0.112	0.159	0.113	0.112	0.123	0.085	0.081	0.081		0.017	0.023	
25 <i>Ectodermia</i> sp. NEPT1078-17		0.165	0.165	0.165	0.165	0.165	0.165	0.165	0.165	0.154	0.139	0.144	0.149	0.149	0.131	0.159	0.159	0.135	0.189	0.138	0.137	0.143	0.112	0.100	0.116	0.120	0.022	
26 <i>Pseudopostega</i> sp. PC2516		0.221	0.221	0.221	0.221	0.221	0.221	0.221	0.204	0.187	0.190	0.215	0.220	0.215	0.173	0.185	0.204	0.197	0.197	0.190	0.189	0.175	0.194	0.199	0.182			

hand, intraspecific divergence in *H. guajavifoliae* sp. nov. has not been observed yet; however, all studied specimens were from the same locality, and additional specimens from different localities would certainly enrich our knowledge about divergence within the species.

Depending on the combination of species set, several versions of the Neighbour-Joining tree with different topology were obtained; some of them are presented in Figs 88–90. In our preliminary analysis using only the COI barcode fragment, *Hesperolyra* always appeared as a separate clade. *Hesperolyra guajavifoliae* sp. nov. always clustered at a distance from *H. diskusi* + *H. saopaulensis* (Fig. 88). In most of our numerous, different attempts, the *Hesperolyra* clade consistently grouped either with *Fomoria* + *Ectoedemia* (Figs 89), or *Fomoria* + [*Acalyptis* + *Etainia*] (Fig. 88), or [*Fomoria* + *Etainia*] + [*Acalyptis* + *Ectoedemia*] (Fig. 90), or even only with *Fomoria*, but never with *Glaucolepis* or *Bohemannia*, or other genera, as presented in Doorenweerd et al. (2016). We found that the relationships between approximately half of the clades remain unsupported according to bootstrapping results; however, according to Nieukerken et al. (2012), bootstrap support values for the Neighbour-Joining similarity tree are not necessary. NJ trees are never robust due to the nature of the method; therefore, adding bootstrap supports to indicate the robustness does not add much. NJ trees are useful for indicating pairwise differences between clusters (of species) and estimating whether COI can be used as a diagnostic marker, what has been shown in the case of *H. guajavifoliae* sp. nov. Although our data are far from complete, the tendency of *Hesperolyra* to group with *Fomoria* or other genera causes us to re-evaluate their relationships; i.e., it may be possible that *Hesperolyra* is related to these taxa. It is interesting to note that, unexpectedly, *Acalyptis* most often clustered with *Etainia* (e.g., Figs 88, 90); this was also found by other workers (Doorenweerd et al. 2016).

Our molecular analysis did not show a close relationship between the guava-feeding *Hesperolyra guajavifoliae* sp. nov. and other Myrtaceae-feeding Nepticulidae, including the South American guava-feeding nepticulid species, *Ozadelpha guajavae*, which was recently barcoded by us; the sequence is available in the BOLD database: ADH4024.

So far, *Hesperolyra guajavifoliae* sp. nov. is the only Nepticulidae pest discovered in western Colombia. However, during our fieldwork we observed a couple of old leaf mines on guava with a wider gallery that did not extend into an obvious blotch at the final stage of development. Although these differently looking leaf mines may belong to *Ozadelpha guajavae*, there is no confirmed evidence that both species *H. guajavifoliae* sp. nov. and *O. guajavae* occur together in western Colombia.

## Contributions the research

Contributions to this research are as follows: JRS: concept and design of the research and fieldwork, discovery and rearing of the adults from the mining larvae, photographic documentation of leaf mines and habitats; writing the manuscript and technical preparation of all plates of illustrations, and discussion on results of the molecular

research and diagnostics of *Hesperolyra*; AR: preparation of the material collected in Colombia in 2019, dissection and photographic documentation of *H. guajavifoliae* sp. nov., molecular research, discussion on diagnostics of *Hesperolyra* and general Nepticulidae phylogeny, and writing of comments on the results of molecular research; AD: dissection and photographic documentation of type species (*H. diskusi*), discussion on morphology and diagnostics of *Hesperolyra*, compiling of list of cited references; SO: molecular research, discussion on molecular Nepticulidae phylogeny, writing of comments on the results of DNA research, and deposition of sequences in GenBank; SA: fieldwork in Colombia, assistance in rearing some *H. guajavifoliae* sp. nov., obtaining of research permits (together with Igor Dimitri Forero Fuentes, see Acknowledgements), various contributions to the new species description, specimen deposition at MPUJ; MAS: manuscript writing, scientific expertise of the data, elaboration of the concept, and discussion on the results.

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# Notes on the genus *Psychostrophia* Butler, 1877 (Lepidoptera, Epicopeiidae), with description of a new species

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## Abstract

New information on the genus *Psychostrophia* Butler, 1877 is provided. A new species, *Psychostrophia micronymphidiaria* Huang & Wang, **sp. nov.**, is described from western, northern and northwestern Yunnan Province, southwestern China; it is similar to *P. nymphidiaria* (Oberthür, 1893) which is widely distributed in eastern, southern, western and central China. A new synonym is established: *Psychostrophia nymphidiaria* (Oberthür, 1893) (= *Stiboges lushanica* Chou & Yuan, 2001, **syn. nov.**). Some other taxonomic and nomenclatural notes on the genus are presented. A key to the species of the genus *Psychostrophia* is provided.

## Keywords

cryptic species, East Asia, Geometroidea, oriental swallowtail moth, taxonomy

## Introduction

*Psychostrophia* Butler, 1877 is a small genus belonging to the family Epicopeiidae, which is widely distributed across Japan, China, and Indochina (Inoue 1992; Zhu et al. 2004; Owada 2011). This genus is characterized by the following characters: 1) hindwing with cilia mostly black, except for a white area between veins M1 and M3;

2) uncus long, thin, and tubular for most of its length; and 3) aedeagus with a cluster of slender cornuti, and coecum well developed and long (Minet 2003; Huang et al. 2019). Adults are diurnal, delicate moths, usually found flying along the forest edge or near water, visiting flowers or sucking nutrients from the damp ground. The immature stage is unknown for most of the members, and only the Japanese *P. melanargia* is found to feed on *Clethra barbinervis* of the family Clethraceae (Inoue 1982; Owada 2011). Until now only four species are known in this genus, viz. *P. melanargia* Butler, 1877, *P. nymphidiaria* (Oberthür, 1893), *P. picaria* Leech, 1897, and *P. endoi* Inoue, 1992, all of which have been previously recorded from China (Zhu et al. 2004; Huang et al. 2019).

Thus far, this genus has only been represented by *P. endoi* in Deqin County in Yunnan Province (Huang et al. 2019). The first observation of another member was of a worn-out male of a *Psychostrophia* species with a *P. nymphidiaria*-like appearance, flying together with another epicopeiid, *Burmeia leesi* Minet, 2003, at the edge of an evergreen broad-leaved forest near water at an altitude around 2550 m. This unexpected recording by the authors took place during a field survey conducted in Yaojiaping, Lushui County, on the western slope of the Gaoligong Mountains in western Yunnan province in the summer of 2018. Subsequently, the first author discovered in the Lepidoptera collection of the South China Agricultural University several males of this *P. nymphidiaria*-like species, which were collected from a vast area in Yunnan. Although at first glance they showed a striking similarity to *P. nymphidiaria* from other parts of China, some small but distinctive morphological differences were noticed. After examining the genitalia, these individuals were found to have distinguishing genital features, confirming them to represent a species distinct from the true *P. nymphidiaria*, making it the fifth species of the genus *Psychostrophia* Butler, 1877. It is described herein.

During the course of studying the genus *Psychostrophia*, the taxon *Stiboges lushmanica* Chou & Yuan, 2001, originally described as a new butterfly, was found to be synonymous with *P. nymphidiaria* (Oberthür, 1893). Two names, *P. melanargia* ab. *hemimelaena* Seitz, 1912 and *P. melanargia* ab. *catenifer* Seitz, 1912 are unavailable as infrasubspecific, even after Zhu et al. (2004) provided a description of *P. melanargia* ab. *hemimelaena*. All the synonymic relationships and unavailable names mentioned above are discussed in detail below.

## Material and methods

Specimens examined in this study were all collected in daytime using an insect net and subsequently deposited in the collection of South China Agricultural University (SCAU), Guangzhou. The photographs of the holotype of *S. lushmanica* in the collection of Northwest Agriculture and Forestry University (NWFU) were provided courtesy of Dr Guo-xi Xue and used here under permission of Dr Xiangqun Yuan. Photographs of all adult specimens examined were taken using a Nikon CoolPix S7000 camera and the habitat photographs with a Sony DSC-RX100 v1.00 camera. Abdomens were

removed and macerated in 10% NaOH for examination of genitalia. Photographs of genitalia of *Psychostrophia* spp. were taken under a Keyence VHX-5000 digital microscope. Adult and genitalia photographs were all processed using Adobe Photoshop CS5 software. Terminology for adults and genitalia follows Klots (1970) and Minet (2003). The specimen code for linking adult and genitalia together is numbered from PSY001 to PSY017.

## Taxonomy

### Genus *Psychostrophia* Butler, 1877

*Psychostrophia* Butler 1877: 401.

**Type species.** *Psychostrophia melanargia* Butler, 1877 (Yokohama, Japan).

### *Psychostrophia micronymphidiaria* Huang & Wang, sp. nov.

<http://zoobank.org/AF2ECA37-9EE3-4EDD-A074-5BA0766976E6>

Figs 1–4, 9, 10

*Psychostrophia nymphidiaria*: Huang et al. 2019: 40 [misidentification].

**Type material.** *Holotype*: male, altitude 2779–2927 m, 27.V.2016, near Shajiamia Bridge, Tacheng Town, Weixi Lisu Autonomous County, Diqing Tibetan Autonomous Prefecture, Yunnan Province, leg. Zhen-fu Huang, Qi-tong Huang and Jing Tang, PSY001. *Paratypes*: 1 male, same label as holotype, PSY002; 1 male, altitude 2550 m, 15.VII.2018, Yaojiaping, Lushui County, Nujiang Lisu Autonomous Prefecture, Yunnan Province, PR China, leg. Si-yao Huang, PSY003; 1 male, 6.VII.2014, altitude 2900–3000 m, Mt Diancang, Dali Bai Autonomous Prefecture, Yunnan Province, leg. Hao Huang, PSY004; 1 male, altitude 2850 m, 7.VII.2013, Tacheng Town, Weixi Lisu Autonomous County, Diqing Tibetan Autonomous Prefecture, Yunnan Province, leg. Zhen-fu Huang, Hai-ling Zhuang and Min Wang, PSY005. The type series is deposited in the Insect Collection of Department of Entomology, South China Agricultural University (SCAU), Guangzhou, P. R. China.

**Diagnosis.** Externally, *P. micronymphidiaria* sp. nov. is characterized and distinguished from its closest relative, *P. nymphidiaria* by a smaller size (length of forewing 16–17 mm vs 18–22 mm in *P. nymphidiaria*), more slender discal cell bar with the tip pointing to the tornus (in *P. nymphidiaria* the discal cell bar is robust and short, the tip shifting basally and pointing to the dorsum), and a narrower costal black border on the dorsal forewing. The male genitalia of *P. micronymphidiaria* sp. nov. can be distinguished from those of *P. nymphidiaria* by the following points: 1) the juxta is much narrower and more strongly sclerotized, while it is much broader and more membra-



**Figures 1–8.** Males of *Psychostrophia* spp. **1** *Psychostrophia micronymphidiaria* sp. nov., holotype, Weixi, Yunnan, PSY001 **2** ditto, paratype, Weixi, Yunnan, PSY002 **3** ditto, paratype, Lushui, Yunnan, PSY003 **4** ditto, paratype, Dali, Yunnan, PSY004 **5** *Psychostrophia nymphidiaria*, Jiangshan, Zhejiang, PSY006 **6** ditto, Qingyuan, Zhejiang, PSY008 **7** ditto, Nanling, Guangdong, PSY009 **8** ditto, Yingjing, Sichuan, PSY007. Scale bar: 1 cm.

nous in *P. nymphidiaria*; 2) the valva has a narrower praesacculus, while it is broader in *P. nymphidiaria*; 3) the aedeagus is longer than the coecum, while it is shorter than the coecum or equal to it in *P. nymphidiaria*; 4) coecum and aedeagus are more sclerotized, while they are more membranous in *P. nymphidiaria*.

**Description. Male** (Figs 1–4). Forewing length 16–17 mm ( $n = 5$ ). Head black; antenna black, filiform. Thorax and abdomen black dorsally. Forewing ground color black with well-developed white patterns. White triangular zone extending from wing base to postmedial area, ending in wavy edge; cell bar at end of discal cell slender; subapical area with oval white patch, center sometimes extending outwards. Submarginal series comprising four white spots extending from vein  $M_2$  to anal angle. Cilia black from apex to vein  $R_5$ , white from  $R_5$  to middle portion of cell  $M_1$ , becoming black again from medial portion of cell  $M_1$  to tornus; sometimes cilia white only between vein  $R_5$  and vein  $M_1$ . Dorsally, hindwing ground color white at inner two-thirds and black at outer one-third, junction line between white and black area wavy; submarginal series consisting of four to six white spots of different sizes, extending from apex to tornus; cilia black from apex to vein  $M_1$ , white from  $M_1$  to medial portion of cell  $M_2$ , becoming black again from medial portion of cell  $M_2$  to tornus.

**Male genitalia** (Figs 9, 10). Uncus tubular, relatively long, and slender. Tegumen broadly U-shaped in ventral view, rather short and broad. Subscaphium moderately sclerotized, bearing setae in ventral and distal areas. Costula at base of costa, consisting of two sclerotized, crescent-shaped processes connected by a membrane. Juxta small and shield-like, strongly sclerotized. Saccus sclerotized, short and diamond-shaped. Valva shape varies from broad and stout to relatively slender, inner surface densely setose. Costa strongly sclerotized. Sacculus strongly sclerotized, broadened basally, narrowing distally. Praesacculus strongly sclerotized and bending upwards, ending with long and sharp tip. Aedeagus long and slender, sclerotized, cluster of long and thin cornuti present distally. Coecum strongly sclerotized, slightly shorter than aedeagus.

**Female.** Unknown at present.

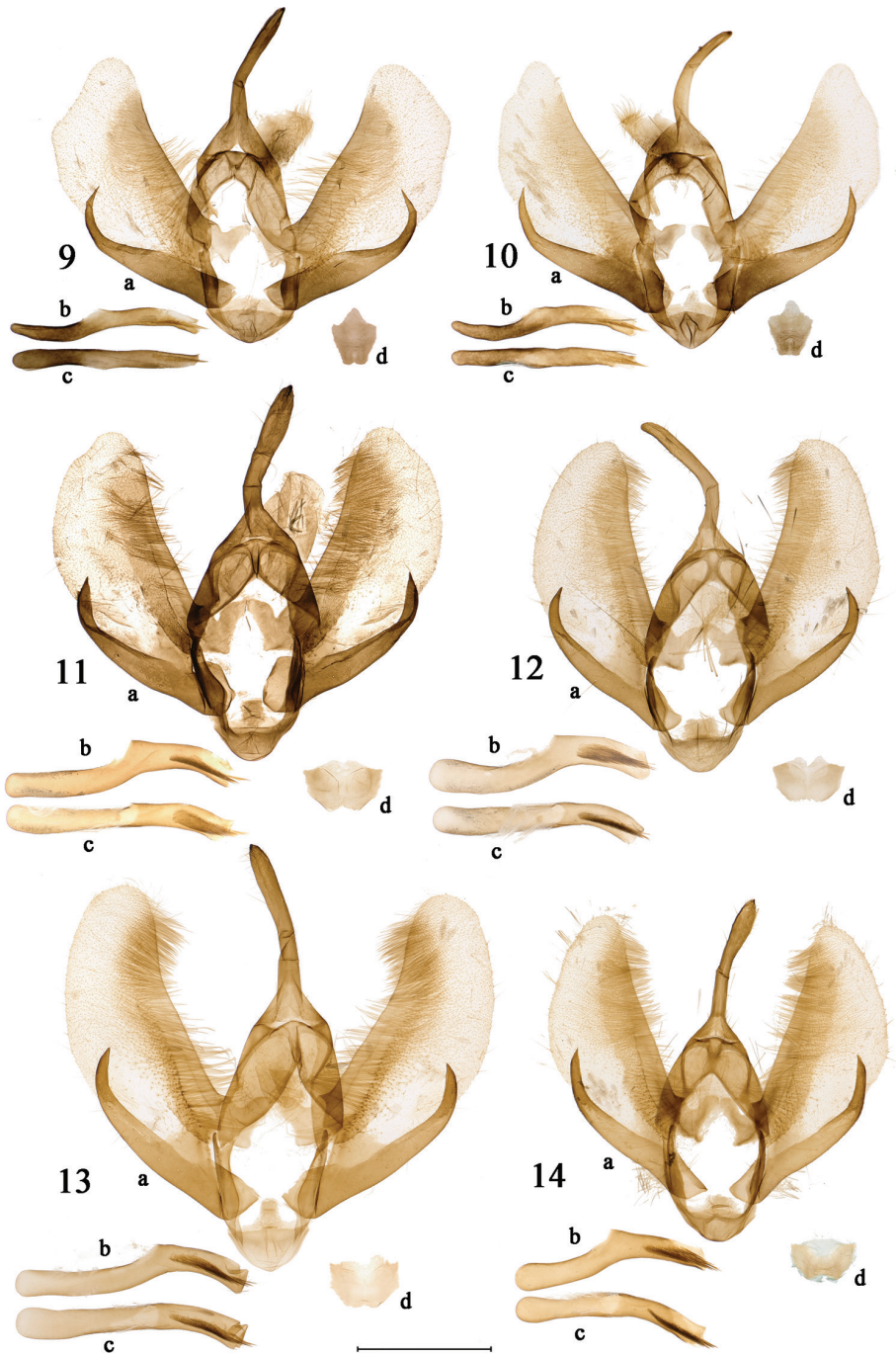
**Distribution.** This species is currently known to occur in western, northern and northwestern Yunnan province of China (Fig. 25).

**Etymology.** The specific name *micronymphidiaria* is the combination of prefix *micro-* and *nymphidiaria*, referring to the size of the new species, which is smaller than *P. nymphidiaria*.

**Bionomics.** This species has been found to fly at the periphery of evergreen broad-leaf forests or conifer-broadleaf forests near water, at altitudes above 2500 m (Figs 21, 22) from late May to mid July. Adults are diurnal and commonly found flying at a slow pace above bushes.

**Remarks.** At present this species is restricted to habitats at altitudes above 2500 m in the Yunnan Province of southwestern China. Conversely, *P. nymphidiaria* is distributed across a vast area ranging from Sichuan Province to Zhejiang Province and extending southwards to northern Guangdong Province, typically preferring habitats where the altitude does not exceed 2200 m (usually from 300 to 2000 m).





**Figures 9–14.** Male genitalia of *Psychostrophia* spp. **9** *Psychostrophia micronymphidiaria* sp. nov., holotype, Weixi, Yunnan, PSY001 **10** ditto, paratype, Weixi, Yunnan, PSY002 **11** *Psychostrophia nymphidiaria*, Jiangshan, Zhejiang, PSY006 **12** ditto, Qingyuan, Zhejiang, PSY008 **13** ditto, Nanling, Guangdong, PSY009 **14** ditto, Yingjing, Sichuan, PSY007. **a** = male genitalia capsule with juxta removed; **b** = aedeagus lateral view; **c** = aedeagus dorsal view; **d** = juxta. Scale bar: 1 mm.

***Psychostrophia nymphidiaria* (Oberthür, 1893)**

Figs 5–8, 11–15

*Abraxas nymphidiaria* Oberthür 1893: 34, pl. 2, fig. 28. [Type locality: “Rencontrée pendant le voyage de Ta-Tsien-Lou à Mou-Pin” (Road from Kangding to Baoxing)].

*Psychostrophia nymphidiaria* (Oberthür): Leech 1897: 189; Seitz 1912: 278; Minet 2003: 473, 479, fig. 4.

*Stiboges lushanica* Chou and Yuan 2001: 142, fig. 15, 16. syn. nov. (Riodinidae).

**Material examined.** Photos of holotype of *Stiboges lushanica*, male, printed label in Chinese “[Sichuan, Lushan, leg. Bing-hong Wang]”/ printed red label “Holotype” / red label “*Stiboges lushanica* Chou et etc., IDENT. IO CHOU” (NWAUFU); 1 male, 22.VI.2003, Nanling Mts, Guangdong Province, leg. Min Wang, PSY009 (SCAU); 1 female, 2.VIII.2003, Huanjiang Maonan Autonomous County, Guangxi Zhuang Autonomous Prefecture, leg. Min Wang (SCAU); 1 male, 10.V.2018, Luding County, Sichuan Province, leg. Min Wang (SCAU); 1 male, altitude 1700–1900 m, 13.VI.2012, Mt Niba, Yingjing County, Ya’an City, Sichuan Province, leg. Xiao-hua Deng & Hou-shuai Wang, PSY007 (SCAU); 1 male, 11.VIII.2016, Shuangxikou Town, Jin-yun County, Jiangshan City, Zhejiang Province, leg. Shu-qin Ji & Hou-shuai Wang, PSY006 (SCAU); 1 male, 25.VII–15.VIII.2018, Qingyuan County, Lishui City, Zhejiang Province, leg. Qing-song Wu, PSY008 (SCAU); 1 male, altitude 1500 m, 3. VI. 2019, Guanmenshan, Shennongjia, Yichang City, Hubei Province (SCAU); 2 males, 21.V.2011, Huanggangshan, Mt Wuyi, Fujian Province, leg. Zhen-fu Huang & Qi-tong Huang (SCAU); 2 males, altitude 1300 m, 13.VIII.2014, Mt Tianping, Zhangjiajie City, Hunan Province, leg. Lan-lan Huang, Wan Lu, Qi-tong Huang & Min Wang (SCAU).

**Remarks.** Taxon *Stiboges lushanica* Chou & Yuan, 2001 was described based on two specimens taken in Lushan County, Ya’an City in western Sichuan Province. Kishida (2006) was first to point out that this “butterfly” taxon is conspecific with *P. nymphidiaria*, but did not synonymize it formally. With the help of Dr Guo-xi Xue and the permission from Dr Xiangqun Yuan, the photographs of the holotype could be examined. The holotype is a male of the oriental swallowtail moth species *P. nymphidiaria*. Although the male genitalia were not illustrated, the description by Chou and Yuan (2001) as well as the photographs suggest that these individuals are undoubtedly conspecific with this epicopeiid moth species commonly found in that area, rather than a bona species of the riodinid butterfly genus *Stiboges* Butler, 1876. Although their mimicry relationship makes them morphologically similar, one can easily recognize this moth species simply by the filiform antenna. Thus, *S. lushanica* is considered a junior synonym of *P. nymphidiaria* (syn. nov.).

Nevertheless, according to Dr Guo-xi Xue and Dr Xiangqun Yuan, there is another specimen also bearing the holotype red label of *S. lushanica* in the collection of NWAUFU, and this specimen is illustrated here for the first time (Fig. 16). The information on the labels is interpreted as follows: “[Sichuan, Lushan, leg. Jing-hua Wang]”/ printed red label “Holotype” / red label “芦山白蛱蝶, *Stiboges lushanica* Chou et Yuan, IDENT. IO



**Figures 15–18.** Adults of *Psychostrophia* spp. and *Stiboges* sp. **15, 17–18** male **16** female: **15** *Psychostrophia nymphidiaria*, holotype of *Stiboges lushanica*, Lushan, Sichuan **16** *Stiboges elodinia*, mislabeled specimen of "holotype" of *Stiboges lushanica*, Lushan, Sichuan **17** *Psychostrophia picaria*, Shennongjia, Hubei, PSY017 **18** holotype of *Psychostrophia endoi*, Xam Neua, Laos, from Inoue (1992). Scale bars: 1 cm.



CHOU". This specimen is a female of *Stiboges elodinia* Fruhstorfer, 1914 in the opinion of Callaghan (2009). Chou and Yuan (2001) stated in English that the sex of the holotype of *S. lushanica* was female, which would suggest that this taxon is a true butterfly. However, the descriptions in both Chinese and English have all clearly expressed that the holotype is conspecific with the male of *P. nymphidiaria* and definitely not a female riordinid butterfly. According to Article 73.1.1 of the Code (ICZN 1999), if an author, when establishing a new nominal species-group taxon, states in the original publication that one specimen, and only one, is the holotype, or "the type", or uses some equivalent expression, that specimen is the holotype fixed by original designation. The specimen of *P. nymphidiaria* pictured in figure 16 of Chou and Yuan (2001) was fixed as the holotype of *S. lushanica* since the word "holotype" was plainly used in Chinese in the legend under this figure. The holotype status of that female riordinid butterfly, which was subsequently labeled as the holotype of *S. lushanica*, is therefore invalid, making the true holotype of this taxon the specimen of *P. nymphidiaria* shown in figure 16 of Chou and Yuan (2001).

During the study of the populations of *P. nymphidiaria* from various localities, it was found that males vary externally and in their genitalia. The median white zone on the dorsal forewing varies in shape and size, and the genitalia vary in the shape of the valva, length and width of the praesacculus, and the ratio of the aedeagus to the coecum (Figs 5–8, 11–14). It is possible that cryptic species still exist within *P. nymphidiaria* sensu lato, a possibility that deserves a more careful investigation including the examination of more adults and genitalia of both sexes, as well as conducting DNA barcoding in the future.

**Distribution.** China (Sichuan, Hubei, Hunan, Zhejiang, Fujian, Guangdong, Guangxi Zhuang Autonomous Region) (Fig. 25)

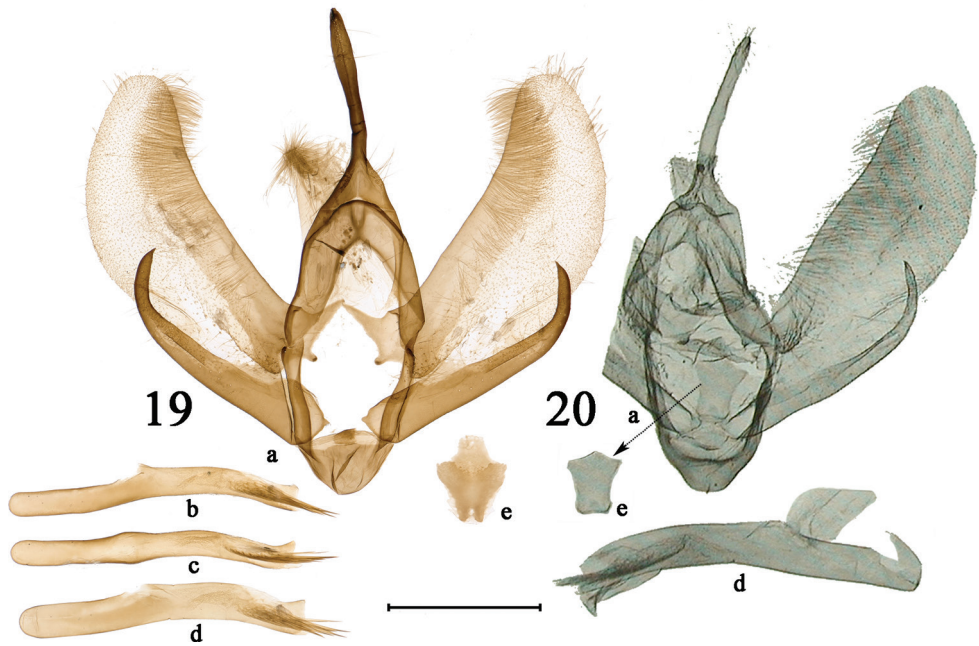
### *Psychostrophia picaria* Leech, 1897

Figs 17, 19

*Psychostrophia picaria* Leech 1897: 189, pl. VI, fig. 11. [Type locality: Changyang, Ichang (now Yichang), Central China]; Seitz 1912: 278; Minet 2003: 473, 478, fig. 5, 24.

**Material examined.** 1 male, altitude 1000–1400 m, 13.V.2015, Muyu Town, Shennongjia, Yichang City, Hubei Province, leg. Yu-fei Li, PSY017 (SCAU); 1 female, 11–14.V.2007, Mt Tianping, Zhangjiajie City, Hunan Province, leg. Liu-sheng Chen, Zhen Li & Yang Long (SCAU); 3 males, 9.VII.2015, Mt Simian, Chongqing City, leg. Si-yao Huang (SCAU); 1 male, 1 female, 1.VII.2003, Mt Maoer, Guangxi Zhuang Autonomous Region, leg. Min Wang (SCAU).

**Remarks.** Huang et al. (2019) stated that the difference in male genitalia between *P. endoi* and *P. picaria* lies in the shape of the valva, which protrudes more at the apex in *P. endoi*. However, the more protruding valva apex is actually found in *P. picaria*. The photographs of adult and male genitalia of a *P. picaria* collected in Shennongjia, Yichang, Hubei Province have been illustrated here for comparison. Judging from the structures of the male genitalia, both the sacculus and praesacculus of *P. picaria* are



**Figures 19, 20.** Male genitalia of *Psychostrophia* spp. **19** *Psychostrophia picaria*, Shennongjia, Hubei, PSY017 **20** holotype of *Psychostrophia endoi*, Xam Neua, Laos, from Inoue (1992). **a** = male genitalia capsule with juxta removed; **b** = aedeagus lateral view; **c** = aedeagus dorsal view; **d** = flattened aedeagus in lateral view; **e** = juxta. Scale bar: 1 mm (**19**).

thicker than those of *P. endoi*, and the upper lobe of the juxta is much broader and longer than that of *P. endoi*. However, given that the valva structure is variable in *P. nymphidiaria*, it is possible that the differences in valva structure mentioned above between these two species are still not constant. Thus, only the shape of the juxta can currently be regarded as a true distinguishing characteristic. More material of male *P. endoi* should be examined to confirm such differences.

**Distribution.** China (Hubei, Hunan, Chongqing, Guangxi Zhuang Autonomous Region) (Fig. 25).

### *Psychostrophia endoi* Inoue, 1992

Figs 18, 20

*Psychostrophia endoi* Inoue 1992: 149, figs 1, 2. [Type locality: Sam Neua (Xam Neua), Laos]; Huang et al. 2019: 44, figs 25–29.

**Remarks.** The adult and genitalia figures of the holotype from Inoue (1992) have been reproduced for comparison.

**Distribution.** China (Yunnan, Guizhou, Guangxi Zhuang Autonomous Region), Laos (Xam Neua) (Fig. 25).



***Psychostrophia melanargia* Butler, 1877**

*Psychostrophia melanargia* Butler 1877: 401. [Type locality: Yokohama, Japan]; Leech 1897: 189; Seitz 1912: 278, pl. 48, line f; Minet 2003: 473, fig. 3; Zhu et al. 2004: 224, fig. 156, pl. VI, fig. 3.

*Psychostrophia hemimelaena* Seitz, 1913 [sic]: Zhu et al. 2004: 225, fig. 157, pl. VI, fig. 4.

**Remarks.** Zhu et al. (2004) recorded *P. melanargia* Butler, 1877 and *P. hemimelaena* Seitz, 1913 (sic), which was originally described as an aberration of *P. melanargia*, viz. *P. melanargia* ab. *hemimelaena* Seitz, 1912, from Dailing, Heilongjiang Province and Mt Changbai, Jilin Province, respectively. According to Article 45.6.2 of the Code (ICZN 1999), the name *hemimelaena* as well as the name *catenifer* Seitz, 1912, which was also published as a new aberration, are invalid because they are infrasubspecific due to the use of the term “ab.” when described. Although these two names were subsequently regarded as *P. melanargia* var. *hemimelaena* and *P. melanargia* var. *catenifera* (sic) in the catalogue by Dalla Torre (1924), this action is at most an “elevation in rank” because no description and definition of these taxa can be traced throughout the catalogue. According to Article 45.5.1 of the Code (ICZN 1999), an infrasubspecific name cannot be made available from its original publication by any subsequent action (such as “elevation in rank”) except by a ruling of the Commission. Thus, the name *P. melanargia* ab. *catenifer* Seitz, 1912 is still unavailable. It should also be clarified that the correct spelling of this aberration is *catenifer*, not *catenifera*, as indicated in Dalla Torre (1924) and Beccaloni et al. (2003).

The matter of the name *P. melanargia* ab. *hemimelaena* is more complicated. As already mentioned above, according to Article 45.5.1 of the Code (ICZN 1999), a name that has infrasubspecific rank under the provisions of this Article cannot be made available from its original publication by any subsequent action (such as “elevation in rank”) except by a ruling of the Commission. Article 45.5.1 also states that when a subsequent author applies the same word to a species or subspecies in a manner that makes it an available name (Articles 11–18), even if he or she attributes authorship of the name to the author of its publication as an infrasubspecific name, that subsequent author thereby establishes a new name with its own authorship and date. The name *hemimelaena* seemed to have been made available under Article 45.5.1 by Zhu et al. (2004) as *Psychostrophia hemimelaena* Zhu, Wang & Han, 2004 because they gave a description in Chinese. This would mean that Zhu, Wang and Han 2004 published a new name with its own authorship and date. However, according to Dr Gerardo Lamas (pers. comm.), the actions of Zhu et al. (2004) did not actually comply with Articles 13.1.1, 16.4.1 and 16.4.2 (ICZN 1999). They require every new specific and subspecific name published after 1999, except a new replacement name (a nomen novum), for which the name-bearing type of the nominal taxon it denotes to is fixed automatically (Art. 72.7), must also be accompanied in the original publication by a description or definition that states in words characters that are purported to differentiate the taxon (Article 13.1.1), by the explicit fixation of a holotype, or syntypes, for the



**Figures 21, 22.** Habitats of *Psychostrophia micronymphidiaria* sp.nov. **21** near Shajiamia Bridge, Weixi County **22** Yaojiaping, Lushui County.

nominal taxon (Article 16.4.1) and where the holotype or syntypes are extant specimens, must be a statement of intent that they will be (or are) deposited in a collection and a statement indicating the name and location of that collection (Article 16.4.2). Zhu et al. (2004) did not differentiate *hemimelaena* from any other taxon in the genus *Psychostrophia* in their description, fix a holotype for the name *hemimelaena*, nor state where the “holotype” was because their actions were not deliberate, nor did they intend to make the name *hemimelaena* available. Thus, the name *Psychostrophia melanargia* ab. *hemimelaena* Seitz, 1912 is still unavailable, bearing the original authorship and date.





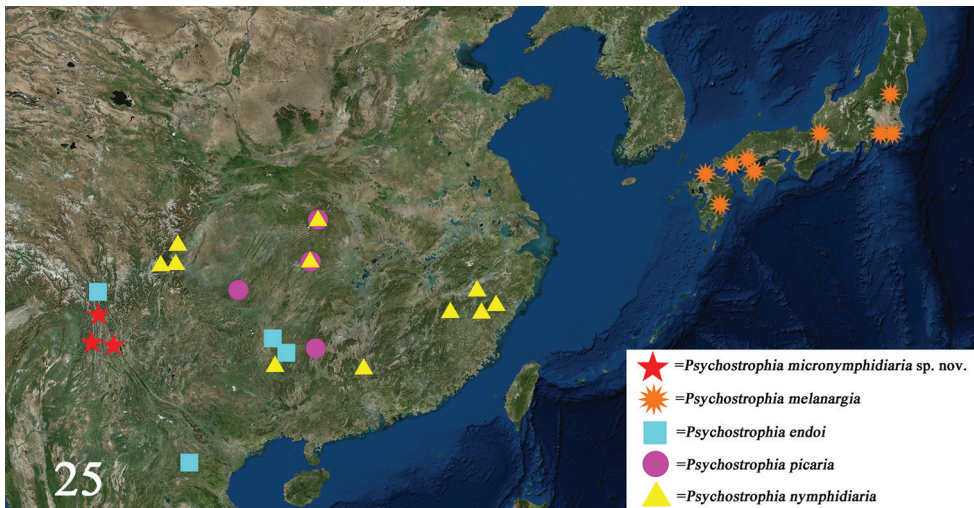
**Figures 23, 24.** Habitats of *Psychostrophia nymphidiaria* **23** Mt Niba, Yingjing County **22** Shennongjia, Yichang City.

It is worth noting that the year of publication of *P. melanargia* ab. *hemimelaena* is 1912, not 1913 as indicated by Dalla Torre (1924), Beccaloni et al. (2003) and Zhu et al. (2004). This name had been published on page 278 in the section on the Uraniidae written by A. Seitz. Although no information on date can be traced throughout the whole section, according to Griffin (1936), the text of the Uraniidae section in the German version of Seitz (1912) was published in Lieferung 99 and received at the British Museum of Natural History on 25.VI.1912. Part 99 encompasses pages 265–344

and plates 49 and 53. The figure of *P. melanargia* ab. *hemimelaena* first appeared on plate 48 published in Lieferung 100, which was received at the British Museum of Natural History on 13.VIII.1912. Part 100 encompasses pages 345–392 and plates 48 and 50. Thus, the publication date of this name must be earlier than 25.VI.1912 and definitely not 1913.

The presence of *P. melanargia* in northeast China is still debatable. Its only known host plant, *Clethra barbinervis*, the Japanese sweet shrub, is distributed across Japan, Korea, and South and East China. The northernmost distribution record in China is from Mt Lao in the Shandong Province, and this plant is not currently recorded in the flora of northeast China (Qin and Fritsch 2005). Moreover, the specimen figured in Zhu et al. (2004) is not significantly different from the individuals commonly found in Japan, which suggests that the individuals examined by Zhu et al. (2004) were collected from somewhere within the geographic range of *P. melanargia* in Japan and mislabeled as being collected in northeast China. Thus, it is unlikely that the geographic range of *P. melanargia* extends to northeast China, and this species should therefore be excluded from the Chinese fauna. There is a similar case in Lepidoptera regarding the distribution of *Neope niphonica* Butler, 1881. Takahashi (1996) concluded that an individual of *N. niphonica* labeled as “Kirin, Manchoukuo, 1941-VII-17 (now Jilin Province, PR China)” had been mislabeled, because the host plant genus of this butterfly was not found in northeast China and the wing pattern did not differ from populations found in central Japan.

**Distribution.** Japan (Honshu, Shikoku, Kyushu) (Fig.25).



**Figure 25.** Distribution map of the genus *Psychostrophia*. Records of distribution are taken from Inoue (1992), Chou and Yuan (2001), Owada (2011), Huang et al. (2019), An Identification Guide of Japanese Moths Compiled by Everyone <http://www.jpmoth.org>, Shiiba Research Forest, Kyushu University <http://www.forest.kyushu-u.ac.jp/miyazaki/index.php>, and the present study.



**Key to the genus *Psychostrophia* Butler, 1877**

- 1 Forewing from base to medial zone with two yellow or whitish areas, the basal one situated along discal cell, the outer one extending from costal region to postmedial region..... *Psychostrophia melanargia*
- Forewing from base to medial zone with only one pale white or whitish area .... **2**
- 2 Pale area forming a slender band ..... **3**
- Pale area forming a trapezoidal or triangular zone ..... **4**
- 3 Hindwing postmedial series comprised of a single transverse band ..... *Psychostrophia picaria*
- Hindwing postmedial series comprised of several separated dots ..... *Psychostrophia endoi*
- 4 Forewing discal cell bar slender ..... *Psychostrophia micronymphidiaria* sp. nov.
- Forewing discal cell bar short and robust..... *Psychostrophia nymphidiaria*

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We express our sincere thanks to Dr Xiangqun Yuan for permission for using the photos of the true holotype of *Stiboges lushanica* and another "holotype" of this taxon, to Dr Mamoru Owada (Tsukuba, Japan) for providing valuable literature on *Psychostrophia endoi*, to Mr Hao Huang (Qingdao, Shandong, PR China), Dr Liu-sheng Chen (Guangdong Academy of Forestry, Guangzhou, PR China), Dr Guo-xi Xue (Zhengzhou, Henan, PR China), Dr Hou-shuai Wang (SCAU, Guangzhou, PR China), Mr Qi-tong Huang (Northwest Agricultural and Forestry University, Yangling, PR China), Dr Hai-ling Zhuang (Shenzhen, Guangdong, PR China), Ms Jing Tang (Nanjing Normal University, Nanjing, PR China), Mr Zhi-peng Miao, Ms Lan-lan Huang, Mr Yang Long, Mr Zhen Li, Ms Wan Lu (SCAU, Guangzhou, PR China), and Mr Yu-fei Li (Xi'an, PR China) for collecting valuable materials and providing useful information and photographs. We are also grateful to Mr Sun-bin Huang, Ms Meng-zhen Chen and Ms Zi-jun Ma (SCAU, Guangzhou, PR China) for helping us with genitalia photographs. Special thanks are due to Dr Gerardo Lamas (Universidad Nacional Mayor de San Marcos, Lima, Peru) and Dr Hao Xu (Mianyang Teachers' College, Mianyang, PR China) for critical advises on nomenclature problem on *Psychostrophia melanargia* ab. *hemimelaena*. We are also grateful to Editage (www.editage.cn) for English language editing.

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# Description of the male of *Craugastor yucatanensis* (Lynch, 1965) (Anura, Craugastoridae), its advertisement call, and additional data on females

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## Abstract

The male of *Craugastor yucatanensis* (Lynch, 1965) is described for the first time, as the original description was based on four females. The advertisement call is described and additional morphological data on females are presented. Also, information is provided on the sexual dimorphism and natural history of the species.

## Keywords

Natural history, sexual dimorphism, vocalization, Yucatán Rainfrog

## Introduction

Lynch (1965) described *Craugastor yucatanensis* from a cave near Nuevo Xcán, Quintana Roo, Mexico, attributing the specific name to the Yucatán Peninsula. However, the description was based exclusively on four females. *Craugastor yucatanensis* is a

member of the *Craugastor* (*Hylactophryne*) *bocourti* species series (sensu Hedges et al. 2008), which includes 19 species. Subsequently, Padial et al. (2014) rejected species groups within the subgenus *Hylactophryne*. *Craugastor yucatanensis* inhabits the central and northeastern parts of the Yucatán Peninsula and is the only member of the genus found in the Mexican portion of this peninsula (Lee 1996; Köhler 2011; González-Sánchez et al. 2017). In recent years we have observed and collected specimens of both sexes of *C. yucatanensis* near the type locality, and reviewed museum specimens from additional localities. We describe herein the previously unknown male and advertisement call, and present morphological data on females. Also, we provide information on sexual dimorphism and natural history.

## Materials and methods

We conducted field trips in 2015–2018 to locate individuals of *C. yucatanensis* in the vicinity of the type locality at Nuevo Xcán, Quintana Roo, Mexico. Specimens used in the description are deposited in the herpetological collection of El Colegio de la Frontera Sur (ECOSUR) at Chetumal, Quintana Roo, Mexico (ECO-CH-H). We followed Lynch's (1965) measurements from the species description. Abbreviations used are: SVL (snout-vent length), TM (tympanum length), EL (eye length), IOD (interorbital distance), SL (tibia segment), F3 (width of pad of 3<sup>rd</sup> finger), EN (eye to nostril distance), HW (head width), and HL (head length). The measurements follow Duellman (1970), expressed in millimeters, to the nearest 0.1 mm, and were obtained by a digital caliper (Mitutoyo). Adult specimens included in the measurement series were maintained in 70% ethyl alcohol. Male specimens were identified at the time of collection based on advertisement call. The sex of preserved specimens was determined by the presence of white prepollical nuptial excrescences in adult males and their absence in females. Color descriptions of live specimens were based on Köhler (2012). Color descriptions in the diagnoses refer to live specimens. We calculated the mean, standard deviation, and range for each morphometric variable. We tested differences between males and females in SVL with the Student *t*-test, after testing variables for normality with the Kolmogorov-Smirnov test. We tested differences in TM, EL, ID, SL, F3, EN, HL and HW with analyses of covariance, with SVL as the covariate, and sex as a dependent variable. All variables were log transformed, and all statistical analyses were performed in Statistica (StatSoft Inc., Tulsa, Oklahoma, USA), with a statistical significance threshold of  $P \leq 0.05$ .

We recorded advertisement calls of male frogs while they were actively calling in the field, using the WavePad free recording software (NCH Software 2015) on a Samsung Galaxy J7 smartphone with an internal directional microphone. We recorded the calls at distances of 50–150 cm. Digital sonograms were executed to identify the frequencies emitted, as well as determine the other sound sources that also formed part of the landscape. We selected the frequencies of the species to later filter unwanted frequencies through multiple parametric equalizers using the Ableton Live 10 program



(Ableton 2017). Finally, the recordings were edited to emphasize the time cycles of the species and create a one-minute sample with the following sampling rate: 48,000 hertz, 2,880,000 samples, and 24 bits of resolution. We obtained a frequency spectrogram using the “seewave” version 1.6.4 package (Sueur et al. 2008) of R version 3.5.0, 64-bit version (R Core Team 2018). The “seewave” settings were as follows: window name (Fast Fourier Transform window) = Hanning; window length = 512 samples; sampling rate = 48,000 hertz; number of samples = 2,880,000; and overlap = 80%.

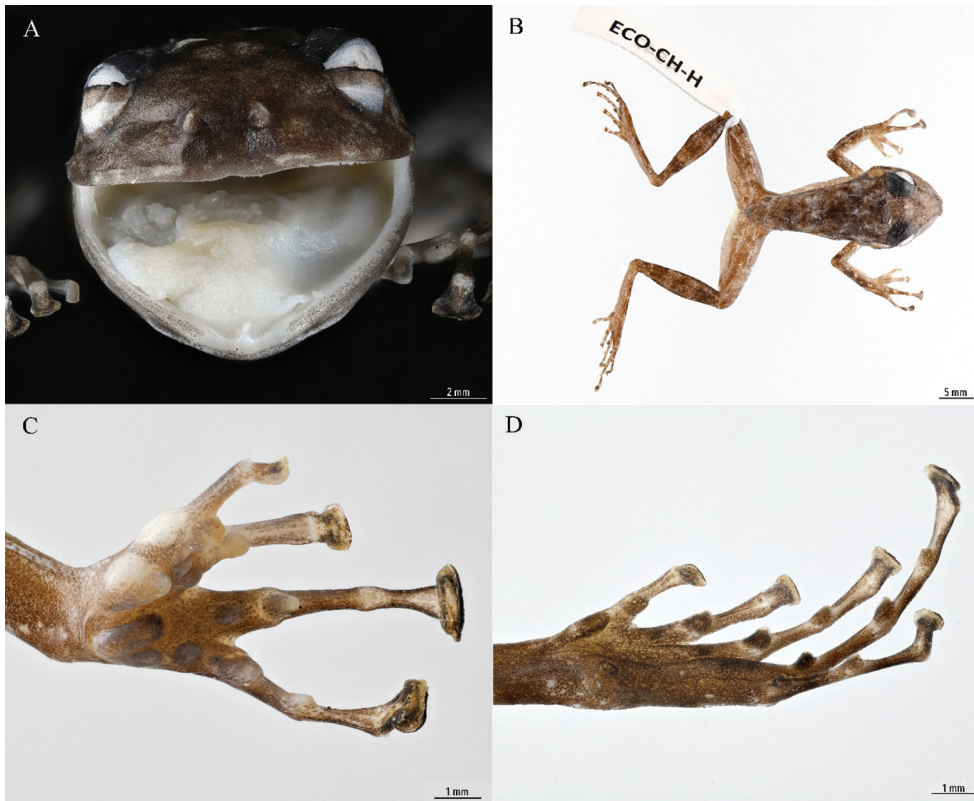
## Results

### *Craugastor yucatanensis* (Lynch, 1965)

**Material examined.** *Craugastor yucatanensis* (23). MEXICO – **Quintana Roo State:** Benito Juárez Mun. ECO-CH-H-1655; Felipe Carrillo Puerto Mun. ECO-CH-H-1878, 1904, 1932, 1949, 2014, 2015, 2016, 2042, 2105, 2393, 3538 –**Yucatán State:** Chemax Mun. ECO-CH-H-3790, 3791, 3792, 3793, 4537, 4538, 4539, 4540, 4541, 4542; Tinum Mun. ECO-CH-H-3539.

**Diagnosis.** A member of the *Craugastor* (*Hylactophryne*) *bocourti* species series, most closely related to *C. alfredi* (Boulenger, 1898) (Lynch, 1965), characterized by having greatly expanded and truncate digital pads on the outer two fingers, possessing no vocal sac or slits (Fig. 1A), a relative large tympanum in adult males (74.7% [65.7–85.5%] of eye diameter) and females (52.4% [42–65.5%] of eye diameter), canthus rostralis rounded; supernumerary tubercles on palm and sole; venter semi-transparent or pinkish; finger pads large, slightly emarginate, and having a fine tarsal ridge. Differs from the closest related species within the series lacking vocal sacs (e.g., *C. alfredi*, *C. campbelli* (Smith, 2005), *C. cyanochthebius* McCranie & Smith, 2006, *C. galacticorhinus* (Canseco-Márquez & Smith, 2004), *C. glaucus* (Lynch, 1967), *C. megalotympanum* (Shannon & Werler, 1955), *C. nefrens* (Smith, 2005), *C. stuarti* (Lynch, 1967), *C. taylori* (Lynch, 1966), and *C. xucanebi* (Stuart, 1941)) by a relatively larger tympanum in males – 74.7% of eye diameter (versus about two-thirds of eye diameter), supratympanic fold absent (versus poorly developed), and numerous small tubercles on the sole (versus few or absent).

**Description and variation of males.** Adult males ( $N = 19$ ) averaged SVL =  $27.1 \pm 1.7$  mm (range 24.2–30.5 mm). Head somewhat broader (HW =  $11.2 \pm 0.9$  mm [9.6 – 13.2]) than long (HL =  $10.5 \pm 0.6$  mm [9.7–12.1]). Tympanum distinct, more than two-thirds the diameter of the eye (TM =  $2.8 \pm 0.2$  mm [2.4–3.2]). Eye slightly longer (EL =  $3.7 \pm 0.3$  mm [3.1–4.4]) than distance from EN =  $3.6 \pm 0.3$  mm [3.1 – 4.0]). Average IOD =  $3.4 \pm 0.3$  mm (2.8–4.0). Canthus rostralis rounded; loreal region slightly concave; tympanum transparent with a dark spot in the center; no dorsolateral, paravertebral, or occipital folds; supra and post-tympanic folds not distinct. Dorsum smooth; skin on venter smooth except for ventral surface of thigh which is areolate; scattered melanophores on chin, chest, and limbs; ventral disc present although obscure; posterior border of thighs slightly granular (Fig. 1B).



**Figure 1.** Male of *Craugastor yucatanensis* (ECO-CH-H-4542) view inside of mouth (**A**) in dorsal aspect (**B**) ventral view of hand (**C**) and ventral view of foot (**D**). Photos by Humberto Bahena Basave.

Tips of fingers expanded, slightly emarginate; the width of pad of 3<sup>rd</sup> finger (F3) averages  $1.6 \pm 0.2$  mm (1.2–2.1); thumb with an enlarged thenar tubercle, almost as large as semi-divided cordiform palmar tubercle; subarticular tubercles round to slightly obtuse and projecting in lateral profile, rounded to ovoid in basal outline, Fingers I and II with one, Fingers III and IV with two; accessory palmar tubercles globular to slightly conical in lateral profile, rounded in basal outline; first finger as long as second; with two white glandular nuptial pads, one on thenar tubercle and the other, also on pollex, opposite and slightly lateral to distal subarticular tubercle (Fig. 1C). Toes expanded slightly. Supernumerary tubercles on metatarsus; tarsal fold present for one-third length of tarsus; inner metatarsal tubercle elongate, not compressed; outer metatarsal tubercle present, round, diameter one-fifth length of inner metatarsal tubercle; subarticular tubercles globular (Fig. 1D). Tibia length averages  $14.9 \pm 0.7$  mm (14.1–16.3). Heel reaching anterior edge of eye; legs held at a little less than a right angle to the body.

**Description and variation of females.** Adult females ( $N = 8$ ; including four from the original description) had an average SVL =  $35.0 \pm 1.9$  mm (range 31.2–37.1 mm). Head is broader (HW =  $14.1 \pm 1.1$  mm [12.5–15.1]) than long (HL =  $13.1 \pm 1.7$  mm [11.8–17.1]). Tympanum distinct, half the diameter of the eye (TM =  $2.5 \pm 0.4$  mm [2.0–3.1]).



**Figure 2.** An adult male *Craugastor yucatanensis* found sitting on vegetation at Xcán, Yucatán, Mexico. Photo by Pedro E. Nahuat Cervera.

Eye slightly shorter ( $EL = 4.7 \pm 0.4$  mm [4.1–5.1]) than distance from  $EN = 4.9 \pm 0.3$  mm [4.4–5.3]. Average  $IOD = 4.0 \pm 0.3$  mm (3.4–4.5). Canthus rostralis rounded; loreal region slightly concave; no dorsolateral, paravertebral, or occipital folds; supra and post-tympanic folds not distinct. Tips of fingers expanded, slightly emarginate; the width  $F3 = 2.3 \pm 0.4$  mm (1.6–2.7). Tibia length averages  $18.2 \pm 1.0$  mm (17.0–20.4).

**Color in life.** The coloration depends on the substrate and the time when the specimens are found. When males are active at night over vegetation or leaf litter, they have an Olive Yellow (117) to Smoke Gray (267) dorsal coloration, with Glaucous (289) to Sepia (279) blotches on dorsum and bars on limbs; sometimes a thin clear vertebral stripe is distinguishable (Fig. 2). When found during the day in caves, they exhibit a coloration similar to that observed at night, but with a paler tone. When found during the day on leaf litter they have a Cinnamon-Rufous (31) dorsal coloration, where the blotches and bars are less evident. The dorsal coloration of females is Olive Yellow (117) to Smoke Gray (267) with scattered Glaucous (289) to Sepia (279) blotches when active at night, whether in caves or leaf litter. When found during the day inside caves, their coloration is paler (Fig. 3), sometimes becoming completely Pale Pinkish Buff (3), similar to some karstic limestone inside the caves. When they are found in leaf litter, their coloration is Cinnamon-Rufous (31), and in both cases the blotches and bars are little evident. The arms and legs are banded, the iris Pearl Gray (262) with metallic bronze tones, and the lateral and ventral surfaces are semi-transparent or Pinkish White (216) in both sexes.



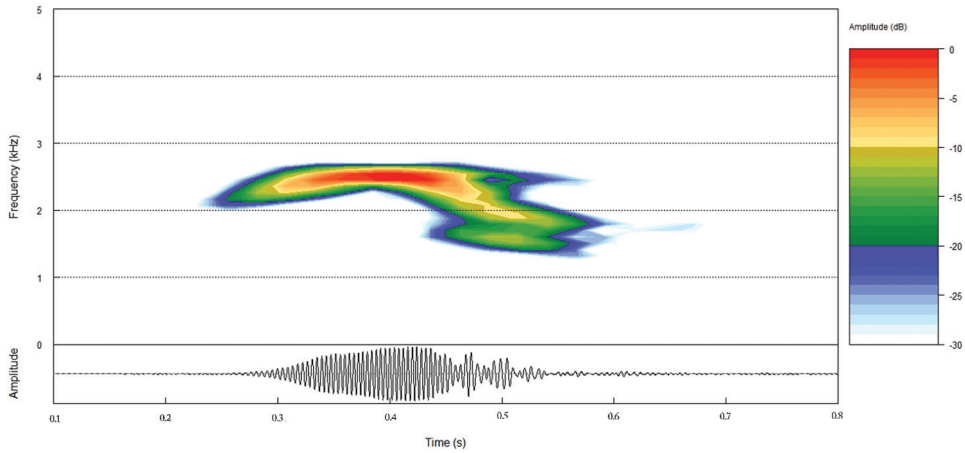
**Figure 3.** An adult female *Craugastor yucatanensis* found inside a cave at Opichén, Yucatán, Mexico. Photo by Pedro E. Nahuat Cervera.

**Color in preservative.** Dorsum Pale Neutral Gray (296), with Glaucus (289) to Brownish Olive (292) blotches on dorsum and bars on limbs; pupil Smoky White (261), iris and upper eyelid Grayish Olive (273); tympanum Pale Cinnamon (55); venter semi-transparent or Pale Buff (1) to Pale Pinkish Buff (3) (Fig. 1B).

**Advertisement call.** The advertisement call of *Craugastor yucatanensis* is part of a communication system that consists of repetitive notes emitted every 10 seconds (6 times per minute). Every note has a duration of approximately 460 MS at a dominant frequency around 2600 kHz. These notes sound like a very short “peep” that resembles the weak chirping of a bird chick (Fig. 4). The digital audio file can be accessed online at Díaz-Gamboa et al. (2019) at Soundcloud.

**Distribution and natural history.** *Craugastor yucatanensis* is known from near sea level to 60 m elevation throughout its range on the central and northeastern portion of the Yucatán Peninsula (Lee 1996; Ortiz-Medina et al. 2016). The vegetation in this area is classified as tropical deciduous forest, low and medium semideciduous forest; high, medium and low semi-evergreen forest; tall evergreen forest, with karstic limestone outcroppings (Torrescano-Valle and Folan 2015). The males were located calling on 18–19 October 2016 and 22 July 2018, on vegetation 1.5–6.0 m above ground. Calling occurred at night (20:00–03:00 h) following afternoon or early evening rainfall, and during light rain later in the night. Many males could be heard calling from





**Figure 4.** Spectrogram (top), and oscillogram (bottom) from a single note of the *Craugastor yucatanensis* advertisement call, recorded 11 km south of Nuevo Xcán, Yucatán, Mexico.

the vegetation. When we attempted to capture vocalizing males, they jumped to the ground where they jumped erratically, then stopped suddenly and became immobile in the leaf litter; crypsis was enhanced by rapid color change to darker tones (metachrosis). Additional males were found during the day and appeared to have been dislodged from a resting place in lower vegetation or leaf litter. Most females were found inside caves or at their entrance during day or night, and some females were found during the day in the leaf litter around rocky outcrops or caves. We did not observe egg laying, and juveniles were not detected. Predators of this species remain undocumented.

**Sexual dimorphism.** Only males have prepollical nuptial excrescences. There was a significant difference between the SVL of adult males and females of *C. yucatanensis* ( $t = -9.72$ ,  $df = 25$ ,  $P < 0.05$ ). When the effect of body length (SVL) was removed, there was a significant difference between sexes in TM ( $F_{1,24} = 11.21$ ,  $P < 0.05$ ), where males possess larger TM average, SL ( $F_{1,24} = 4.87$ ,  $P < 0.05$ ) where females are larger in average, and EN ( $F_{1,24} = 12.61$ ,  $P < 0.05$ ) where females possess larger EN. There was no significant difference in EL ( $F_{1,24} = 1.26$ ,  $P > 0.05$ ), IOD ( $F_{1,24} = 0.01$ ,  $P > 0.05$ ), F3 ( $F_{1,24} = 0.11$ ,  $P > 0.05$ ), HL ( $F_{1,24} = 3.14$ ,  $P > 0.05$ ), and HW ( $F_{1,24} = 0.48$ ,  $P > 0.05$ ) between sexes.

## Discussion

*Craugastor yucatanensis* is the only member of the genus occurring in the central and northeastern portion of the Yucatán Peninsula (Quintana Roo and Yucatán), while *C. alfredi* is known from the base of the Peninsula (Chiapas, Tabasco, and Guatemala). Increased sampling efforts in current range gaps are necessary to improve our understanding of the distribution of both species. Here we describe the previously unknown male, and document the arboreal behavior and advertisement call of *C. yucatanensis*,



previously mentioned but with little detail by Calderón-Mandujano et al. (2008). We heard multiple males calling from the bushes and canopy, but the challenge of climbing trees during rain or high humidity made it difficult to capture many individuals. Similarly, Taylor (1942) mentioned the difficulty in obtaining specimens of *C. decoratus* (Taylor, 1942). Campbell (1998) noted that *C. alfredi* is usually encountered after dark, especially after rains, sitting in low vegetation in the vicinity of rocky outcroppings. Campbell et al. (1989) found the holotype of *C. polymniae* (Campbell, Lamar & Hillis, 1989) (a species with vocal sac and slits) calling from vegetation (1–3 m) at night and described its voluminous and varied vocalizations, composed of four different calls. Within the species of *Craugastor* lacking vocal slits and sac, the holotype of *C. galacticorhinus* was found calling at 18:25 h from within a hole at the base of a dirt bank, on the side of a trail; the call was described as an extremely soft single “peep” repeated about every minute (Canseco-Márquez and Smith 2004). The holotype of *C. campbelli* was found sitting on a leaf at 0.75 m above the ground on a foggy night, additional females were observed on vegetation 1 m above the ground and males 2 m above, but not calling (Smith 2005). The holotype of *C. nefrens* was found at night on a *Cecropia* leaf at 0.75 m above the ground, and additional specimens during rainy and clear nights on low vegetation or the forest floor (0.3–2.0 m), without calling (Smith 2005). Specimens of *C. cyanochthebius* were found at night on vegetation (0.25–1.0 m above the ground) in an area of outcropping limestone; the collectors heard a soft frog-like call, but the call could not be confidently associated with this species (McCranie and Smith 2006).

Here we demonstrate that *C. yucatanensis* possesses an advertisement call, despite the absence of vocal slits and sac in both sexes. Lynch (1965) mentioned that *C. yucatanensis* lacks vocal slits and sac, but curiously his sample was based only on females. Lee (1996) stated that the call of *C. yucatanensis* is unknown, and the species might be mute. The quantification of the advertisement call of *C. yucatanensis* will allow new studies of the behavior and ecology of this species, as well as comparisons with related species, and suggests that other congeners lacking vocal sacs might also vocalize. The vocal repertoire of *C. yucatanensis* that we describe in this work was recorded in situ from a population near the type locality; however, it will be essential to extend this research to different populations to identify possible intraspecific variation. *Craugastor yucatanensis* is sexually dimorphic in many characters, notably snout-vent length and tympanum diameter. Males in most of the species in the *bocourti* species series have a larger tympanum than females, with the exception of *C. spatulatus* (Smith, 1939), but we must consider that there are species where data for males are not available (e.g., *C. batrachylus* (Taylor, 1940), *C. bocourti* (Brocchi, 1877), *C. megalotympanum*, and *C. silvicola* (Lynch, 1967); Martin 1958; Campbell et al. 1989). The tympanum-to-eye ratio of all species in the series for which males are known is  $\geq 50\%$ , except for *C. galacticorhinus* (40%), *C. polymniae* (32%), and *C. spatulatus* (30%). In females, the tympanum-to-eye ratio is 43–72% (Campbell et al. 1989; Canseco-Márquez and Smith 2004). *Craugastor yucatanensis* is endemic to the Yucatán Peninsula in Mexico. Its conservation status has been evaluated as Near Threatened by the IUCN (2016), and as a species of special protection (Pr) by SEMARNAT (2010). Wilson et al. (2013) determined its Environmen-

tal Vulnerability Score as 17, placing it in the middle portion of the high vulnerability category. The description of males and advertisement call presented here should help in locating additional populations in the Yucatán Peninsula, encourage further research, and eventually generate strategies for the protection of frogs and their habitat.

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