Revision of the genus *Epiparbattia* Caradja, 1925 (Lepidoptera, Crambidae, Pyraustinae), based on morphology and molecular data

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**Abstract**

The genus *Epiparbattia* Caradja, 1925 is revised based on general appearance, including genitalia. A new species, *Epiparbattia multispinalis* Zhang & Chen, \textit{sp. nov.} is described. The external characters and genitalia morphology of all species are figured. The phylogeny of *Epiparbattia* species is investigated using molecular data. Monophyly of the genus is well supported by phylogenetic analysis based on sequence data of COI, 16S rRNA, EF-1\(\alpha\) and 28S rRNA gene regions.

**Keywords**

China, *Epiparbattia*, molecular phylogeny, new species, *Sclerocona*, taxonomy

**Introduction**

The genus *Epiparbattia* Caradja, 1925 comprises only two species, *E. gloriosalis* Caradja, 1925 and *E. oligotricha* Zhang & Li, 2005, distributed in southern China, India and Bhutan (Munroe and Mutuura 1971; Nuss et al. 2003–2020; Irungbam et al. 2016). These two species are easily recognized by the yellow body and the creamy white wings bearing dark brown markings. Three specimens, having a different colour but similar genital morphology, represent a new species of *Epiparbattia*.
which is described below. In this paper, we also redefine the genus and summarize the
diagnoses of all species based on the external morphological and genitalic characters.
The phylogeny of *Epiparbattia* based on sequence data of *COI*, 16S rRNA, EF-1α and
28S rRNA gene regions is reconstructed.

**Material and methods**

Morphological studies

The material studied, including the types of the newly described species, are all deposited
at the Museum of Biology, Sun Yat-sen University, China (SYSBM) except those specified
as being in the Insect Collection of the College of Life Sciences, Nankai University, China
(NKU), the Institute of Zoology, Chinese Academy of Sciences (IZCAS) and the National
History Museum, London, United Kingdom (NHMUK). Slides of genitalic dissections
were prepared according to Robinson (1976) and Li and Zheng (1996), with some modific-
ations. Genitalia terms follow Klots (1970), Munroe (1976), Maes (1995) and Kristensen
(2003). Images of the specimens at different focal levels were made using a Canon EOS
1DX camera in combination with the Helicon Remote image stacking program; the geni-
talia pictures were taken using Zeiss Axio Scope.A1 in combination with a Zeiss AxioCam
camera and the Axio Vision SE64 programme on a Windows PC; source images were then
aligned and stacked on Helicon Focus to obtain a fully sharpened composite image.

Molecular analyses

In total seven species of four genera were included for molecular phylogenetic
analyses (Table 1). *Euclasta stoetzneri* (Caradja, 1927) was chosen as the outgroup
because the genus *Euclasta* Lederer, 1855 is considered as the basal lineage of the
Pyraustinae (Mally et al. 2019). One species of *Sclerocona* and two species of *Croci-
dophora* were included as closely related groups based on the fovea of the forewing,
the minute basal and apical outer spurs of the hindleg in males and the similar
genitalic characters. Total DNA was extracted from two legs and, sometimes in
addition, from the abdomen of the dry specimens using the TIANGEN DNA ex-
traction kit following the manufacturer’s instructions. The nucleotide sequences of
two mitochondrial genes, cytochrome c oxidase subunit I (*COI*) and 16S ribosomal
RNA (*16S rRNA*), and two nuclear genes, Elongation factor-1 alpha (*EF-1α*) and
28S ribosomal RNA (*28S rRNA*) were selected for study. Primers used in this study
are as follow: LCO/Nancy for *COI* and LR-J-12888/ LR-N-13398 for *16S rRNA*
(Simon et al. 2006), Oscar-6143/Bosie-6144 for *EF-1α* (Hundsdoerfer et al. 2009)
and 28S-f1/28S-r1 for *28S rRNA* (Lee and Brown 2008). All PCRs were performed
in 25 µl of solution, containing 2 µl 10×PCR Buffer, 2 µl dNTP (2.5 mM each),
0.6 µl MgCl₂ (25 mM), 1 µl of each primer (10 pmol/µl), 0.2 µl Takara Taq DNA
Polymerase (Takara Bio Inc., 5 u/µl), 4 µl of template DNA and 14.2 µl ddH₂O
for *COI*, *16S rRNA* and *28S rRNA*, and 10 µl 2×PCR Buffer (8 mM MgCl₂, 2 µl
dNTP (10 mM each), 1 µl of each primer (10 pmol/µl), 0.4 µl KOD FX DNA Polymerase (TOYOBO CO., LTD., 1 u/µl), 4 µl of template DNA and 6.6 µl ddH₂O for EF-1α. PCR cycle conditions were set to an initial denaturation of 5 min at 95 °C, 35 cycles of 30 seconds at 94 °C, 30 seconds at 48 °C (COI and 16S rRNA) or 52 °C (EF-1α and 28S rRNA) and 1 min at 72 °C for amplification, and a final extension at 72° C for 10 min. PCR products were confirmed with 1.5% agarose gel electrophoresis in TAE buffer, then were purified and direct-sequenced at Majorbio Bio-pharm Technology Co., Ltd (Guangzhou), utilizing the same primers used for PCR amplification.

The sequences were aligned using Clustal W (Thompson et al. 1994) in MEGA 6 (Tamura et al. 2013) with default settings. The aligned matrix was corrected by eye. Gaps were treated as missing data. Phylogenetic analyses were inferred using Bayesian inference (BI) method in MrBayes 3.2.6 (Ronquist et al. 2012) and maximum likelihood (ML) in RAxML 8.2.10 (Stamatakis 2014). BI analysis was run with independent parameters for the COI and the 16S rRNA gene partitions under the GTR + G model, the EF-1α gene partition under the GTR + I model, and 28S rRNA gene partition under the GTR + G model, as suggested by jModelTest 0.1.1 (Posada 2008). Two independent runs, each with four Markov Chain Monte Carlo (MCMC) simulations, were performed for 20 million generations sampled every 1000th generation. The first 25% trees were discarded as burn-in, and posterior probabilities (PP) were determined from remaining trees. ML analysis was executed under the GTR + G model for all gene partitions and with 1000 iterations for the bootstrap test. The pairwise Kimura 2-Parameter (K2P) distances between species were calculated from the COI gene using MEGA 6 (Tamura et al. 2013).

Results

Epiparbattia Caradja, 1925

Epiparbattia Caradja, 1925: 358.

Type species. Epiparbattia gloriosalis Caradja, 1925, by monotypy.
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**Diagnosis.** This genus is related to *Sclerocona* Meyrick, 1890 in the forewings bearing a fovea basally beyond the cell and another fovea between R$_{3+4}$ and R$_{5}$, as well as the minute basal and apical outer spurs of the hindlegs in the male, the developed and sclerotized lamella postvaginalis, the coiled and partly sclerotized posterior part of the ductus bursae and the second signum with two almost parallel ridges in the female genitalia, but can be distinguished by the larger body size, the relatively short labial palpi and the prominent markings on the wings. In the genitalia, *Epiparbattia* differs from *Sclerocona* by the relatively broad uncus, the uninflated sacculus, the stout, ventrodistally sclerotized phallus and the broad, nearly elliptical signum.

**Redescription.** Frons rounded, sometimes weakly flat. Labial palpi obliquely porrect, third segment porrect; exceeding frons by less than the diameter of eyes. Maxillary palpi slightly broadened terminally. Hindlegs of male with basal and apical outer spurs minute. Forewings elongated triangular, costa slightly curved near apex, apex obtuse, termen slightly curved and oblique, tornus rounded; reniform stigma developed and connected with postmedial band, postmedial band comprising of a series of patches and with a broad patch on posterior margin, subterminal band comprising of a series of broad, elliptical patches; length of cell less than half of forewing, male with posterior margin of cell, Cu$_{A2}$ and 1A basally curved, and forming a fovea, with a scale-tuft on the underside surface at position of fovea, R$_3$ and R$_4$ stalked about 1/2–2/3 length of R$_4$, R$_5$ basally curved, a fovea present between R$_{3+4}$ and R$_5$ but without scale-tuft on the underside surface of fovea. Hindwings fan-shaped; length of cell less than half length of hindwing; Sc+$R_4$ and Rs anastomosed for 1/3–1/2 length of Rs; subterminal band narrow. **Male genitalia.** Uncus nearly triangular, distally narrowly rounded, with distal half densely setose laterally and dorsally, basal half sparsely setose laterally. Tegumen with dorsal 1/3–1/2 narrow and basal 2/3–1/2 broad. Transtilla connected, arms nearly triangular or trapezoidal, sparsely bearing slender setae, with ventral process extending to juxta. Valvae tongue-shaped, costa nearly straight, ventral margin convex, apex rounded; sella weakly sclerotized and setose, dorsally bearing a large cluster of curved and thick setae or several slender setae forming an edium, ventrally with strongly sclerotized processes; sacculus not inflated; saccus nearly triangular. Juxta with dorsal part divided into two arms. Phallus stoutly cylindrical, distally with ventral part sclerotized. **Female genitalia.** Ovipositor lobes flat, densely setose. Apophyses stout, anterior apophyses about same length of posterior apophyses, 8$^{th}$ tergite with base of anterior apophysis strongly extended forward and connected with lamella postvaginalis. Lamella postvaginalis developed, sclerotized. Antrum reduced. Ductus bursae usually longitudinally wrinkled, about 2–3 times length of corpus bursae; with most of posterior part coiled and partially sclerotized, usually inflated; colliculum elongated hourglass-shaped; ductus seminalis arising closely from anterior end of colliculum. Corpus bursae globular or oval, wrinkled; appendix bursae originating from posterior part; signum broadly rhomboid, nearly elliptical; second signum located between base of appendix bursae and entrance of ductus bursae, plate-shaped and curved, usually with two almost parallel ridges.

**Distribution.** China, India, Bhutan.
Key to species of *Epiparbattia*

1. Forewings ground colour pale yellow and the covering dark brown scales forming markings in male (Fig. 3), wings pale yellow in female (Fig. 4); ventral processes of sella with rows of densely set spines ventrally, dorsalmost process long and straight (Fig. 7).............. *E. multispinalis* Zhang & Chen, sp. nov.

   - Wings creamy white bearing dark brown markings; ventral processes of sella with the dorsalmost one curved and with sparse spines ventrally..............2

2. Forewings with postmedial band interrupted (Fig. 1); ventral margin and costa of valva approximately parallel, dorsal part of sella densely covered with thick setae forming editum, the setae with apex curved and divided into several filaments, ventral part of sella with dorsalmost process slightly curved and extending inward (Fig. 5).................. *E. gloriosalis* Caradja, 1925

   - Postmedial band of forewing not interrupted (Fig. 2); valvae gradually widened from base to apex, dorsal part of sella sparsely covered with slender and simple setae forming editum, ventral processes of sella with the dorsalmost one extending dorsad (Fig. 6)..................*E. oligotricha* Zhang & Li, 2005

*Epiparbattia gloriosalis* Caradja, 1925

Figs 1, 5, 8

*Epiparbattia gloriosalis* Caradja, 1925: 359.

*Epiparbattia gloriosalis whalleyi* Munroe & Mutuura, 1971: 506.

*Type material examined.* Paratype of *Epiparbattia gloriosalis* Caradja, 1925: 1♀, [China: Guangdong]: Lienping [Lianping], 26. April (NHMUK). Types of *Epiparbattia gloriosalis whalleyi* Munroe & Mutuura, 1971: holotype ♂, [India]: Assam, 5000 ft, Shillong, 19.May.1924, Fletcher coll., Pyralidae Brit. Mus. Slide no. 8708 (NHMUK); allotype ♀, [India]: Assam, 5000 ft, Shillong, at light, 18.V.[19]28, T. Bainbridge Fletcher, Pyralidae Brit. Mus. Slide no. 8709 (NHMUK); paratypes: 2♀♀, [India]: Assam, Shillong, at light, H. M. Parish., Pyralidae Brit. Mus. Slide no. 5384 (NHMUK).

*Other material examined.* China: Fujian: 1♂, Sangang, 15.VIII.1979 (IZCAS); Guangdong: 2♂♂, Mt. Danxiashan, Renhua, alt. 408 m, 15. IV.2008, leg. Wang Fengwei; 1♂, 1♀, Mt. Dinghushan, Zhaoqing, 23.17°N, 112.55°E, alt. 56 m, 8.IV.2013, leg. Li Jinwei, genitalia slide no. CXH12039 (♂), SYSU1036 (♀, molecular voucher no. SYSU-LEP0244); Guangxi: 1♀, Mt. Shiwandashan, 21.91°N, 107.91°E, alt. 352 m, 18.IV.2012, leg. Li Jinwei; Yunnan: 1♂, Lufeng, 22.VI.1982, leg. Song Shimei (IZCAS); 1♀, Kunming, 10.V.1980, leg. Zhong Tiesen (IZCAS); 1♀, Muding, V.1975 (IZCAS); Xizang: 1♀, Pailong, Linzhi, 30.01°N, 95.00°E, alt. 2010 m, 5.VII.2013, leg. Li Jinwei.
Diagnosis. Wingspan 32.0–41.0 mm. This species is similar to *Epiparbattia oligotricha* in appearance, but can be distinguished from it by the tegulae with only one black spot at base, the interrupted postmedial band and the narrower patch on the posterior margin; in male genitalia by the dorsal side of the sella densely covered with thick setae forming editum, and the setae subapically curved and divided into several filaments, the ventral processes of the sella with the dorsalmost curved inward, apically bifurcated, the ventrally curved ventrad, and by the longer arms of the juxta; in female genitalia by the absence of a U-shaped concave unsclerotized window of the lamella postvaginalis anteriorly and the uninflated posterior part of the ductus bursae.

Distribution. China (Fujian, Hubei, Guangdong, Guangxi, Sichuan, Yunnan, Xizang), India, Bhutan.

Biology. Larvae bore in the stems of bamboo shoots of *Sinocalamus affinis* (Rendle) McClure (Wang 1980).

*Epiparbattia oligotricha* Zhang & Li, 2005

Figs 2, 6, 9

*Epiparbattia oligotricha* Zhang & Li, 2005: 40.

Type material examined. **Holotype **♂, CHINA: Guizhou: Mt. Fanjingshan, 27°33′N, 108°24′E, alt. 1700 m, 1.VI.2002, leg. Wang Xinpu (NKU); **Paratypes**: Yunnan:
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**Other material examined.** China: Sichuan: 5♀, Labahe, Tianquan, 30.09N, 102.52E, alt. 1860 m, 8.VII.2012, leg. Li Jinwei, genitalia slide no. SYSU1035, molecular voucher no. SYSU-LEP0243, SYSU-LEP0335; Yunnan: 1♂, Qinlangdang Reserve Station, Gaoligongshan Reserve, Nujiang, 27.69°N, 98.27°E, alt. 380 m, 30.V.2017, leg. Teng Kajian et al., genitalia slide no. ZDD12109, molecular voucher no. SYSU-LEP0359 (NKU).

**Diagnosis.** Wingspan 32.0–47.0 mm. This species is superficially similar to *Epi-parbattia gloriosalis*, but can be distinguished from it by the tegulae bearing a second large black spot in the center, the large orbicular stigma, the uninterrupted postmedial band and the wider patch of the postmedial band at the posterior margin; in male genitalia by the dorsal side of the sella sparsely covered with slender simple setae forming editum, the ventral processes of the sella with the dorsalmost curved dorsad and with

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*Figures 5–7.* Male genitalia of *Epiparbattia* spp. 5 *E. gloriosalis*, Guangdong (genitalia slide no. CXH12039) 6 *E. oligotricha*, Yunnan (genitalia slide no. ZDD12109) 7 *E. multispinalis* sp. nov., Hubei (genitalia slide no. ZDD12101). Scale bars: 1.0 mm.
ventral margin sparsely bearing spines, the ventralmost slightly curved inward, as well as the shorter arms of the juxta; in female genitalia by the presence of a deep, U-shaped concave unsclerotized window of the lamella postvaginalis anteriorly and the inflated posterior part of the ductus bursae.

**Distribution.** China (Sichuan, Guizhou, Yunnan).

*Epiparbattia multispinalis* Zhang & Chen, sp. nov.
http://zoobank.org/CC9D9746-060F-43D4-A173-50F6AA904464
Figs 3, 4, 7, 10

**Material examined.** **Holotype** ♂, **China:** Hubei: Shuangping, Zhuxi, 31.57N, 109.87E, alt. 1201 m, 5.VII.2017, leg. Qi Wanding, genitalia slide no. ZDD12101, molecular voucher no. SYSU-LEP0378 (NKU). **Paratypes:** 2♀♀, same data as holotype, genitalia slide no. ZDD12074, ZDD12095, molecular voucher no. SYSU-LEP0351 (NKU, SYSBM).

**Diagnosis.** The new species differs from the other two species by the pale yellow ground colour of forewings with dark brown markings in the male and the pale-yellow wings in the female. In the male genitalia, *Epiparbattia multispinalis* is similar to *E. oligotricha*, but differs from the latter in the concave lateral margin of the uncus, the more convex ventral margin of the valva, the ventral processes of the sella with the dorsalmost straight and long, narrowly triangular, with rows of spines ventrally, transversely extending inward, and by the large drop-shaped cornutus. In the female genitalia, this species is different from *E. gloriosalis* and *E. oligotricha* by the prominently inflated posterior part of the ductus bursae, approximately 2–3 times the width of the remainder of the ductus bursae.

**Description.** Wingspan 29.0–33.0 mm. **Male** (Fig. 3). **Head.** Frons flat or round, brown. Vertex brown. Labial palpi brown, pale yellow at base beneath. Maxillary palpi brown, paler at apex. Basal scales of proboscis pale brown. Antennae yellowish brown. **Thorax.** Pale brown dorsally, tegulae bearing scales pale yellow with pale brown apex; greyish white ventrally. Legs yellowish brown, hindlegs of male with basal and apical outer spurs minute, about 1/5 length of inner spurs. Forewings ground colour pale yellow, with area from base to postmedial band densely covered with dark brown scales, only with a diffuse pale yellow medial area between wing base and postmedial band; reniform stigma dark brown, nearly triangular; postmedial band dark brown, from costal 2/3 to middle of posterior margin; subterminal band broad, brown, with veins pale yellow; termen with brown spots at veins end; fringe pale yellow, scattered with dark browns scales. Forewings with a fovea beyond posterior margin of cell and another outside of cell. Hindwings pale yellow, with basal half sparsely scattered with dark brown scales and a narrow pale brown postmedial line as outer demarcation; subterminal band dark brown, with inner margin suffusing and irregular; termen and fringe same as forewing. **Abdomen.** Brown, dorsally with posterior margin of
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**Male genitalia** (Fig. 7). Uncus laterally strongly concave and with distal end narrowly rounded. Valvae slightly curved, costal margin slightly concave and ventral margin convex; width of basal half relatively even and slightly tapering from middle towards bluntly rounded apex; sella nearly rhombic and weakly sclerotized, dorsal part sparsely bearing slim setae and several thick setae forming editum, ventral processes of sella with the dorsalmost sclerotized, straight and long, narrowly triangular, transversely extending inward and densely bearing rows of spines ventrally, another process of sella spine-shaped and small. Juxta small, nearly inversely trapezoidal, dorsal arms weak and widely separated. Phallus slightly narrowed in middle, with a large drop-shaped, weakly sclerotized cornutus. **Female** (Fig. 4). Head and thorax yellow, antennae pale brown. Forewings yellow, with a pale yellow band indistinct, wider than that in male; termen with pale brown spots at veins end; fringe pale yellow. Hindwings pale yellow; subterminal band indistinct; termen and fringe as in forewing. Abdomen brown. **Female genitalia** (Fig. 10). Lamella postvaginalis densely covered with minute spines, strongly extended dorsad and connected dorsally, with dorsal part forming a pair of closely associated rounded sclerites. Ductus bursae about three times diameter of corpus bursae, with posteriormost part prominently inflated, about 2–3 times width of the remainder; colliculum hourglass-shaped. Corpus bursae globular, length of signum about 2/3 of diameter of corpus bursae, ends of the long

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**Figures 8–10.** Female genitalia of *Epiparbattia* spp. 8 *E. gloriosalis*, Guangdong (genitalia slide no. SYSU1036) 9 *E. oligotricha*, Sichuan (genitalia slide no. SYSU1035) 10 *E. multispinalis* sp. nov., Hubei (genitalia slide no. ZDD12074). Scale bars: 1.0 mm.
axis approximately right-angled, ends of the short axis (perpendicular to long axis) produced into short acute-angled tips.

**Etymology.** The specific name is derived from the Latin multi- (= many) and spinalis (= spine) corresponding to the ventral processes of the sella bearing many spines.

**Distribution.** China (Hubei).

**Phylogenetic relationships**

The concatenated dataset of four genes consisted of 2510 nucleotide positions (658 for COI, 468 for 16S rRNA, 619 for 28S rRNA, and 765 for EF-1α). Both BI and ML analyses of the concatenated dataset inferred congruent topologies with only subtle differences in posterior probability and bootstrap values (Fig. 11). The monophyly of Epiparbattia is robustly supported (PP = 1.00, BS = 99), the genus Sclerocona and Epiparbattia are sister groups with moderate support. The results of the current phylogenetic analyses support the placement of E. multispinalis sp. nov. in Epiparbattia, with E. oligotricha as its sister species, with strong support in the BI analysis (PP = 0.98, BS = 75).

Pairwise distances of the barcoding region (COI) are given in Table 2. The genetic distances between Epiparbattia and other genera range from 8.5% (Crocidophora) to 15.5% (Euclasta). Interspecific genetic distances within Epiparbattia range from 5.6% (E. oligotricha to E. multispinalis) to 7.9% (E. oligotricha to E. gloriosalis), while intraspecific genetic distances range from 0.2% (E. multispinalis) to 1.9% (E. oligotricha).

**Figure 11.** Phylogenetic hypothesis inferred from Bayesian inference. Numbers on branches indicate Bayesian posterior probabilities and ML bootstrap values respectively.
Table 2. Pairwise distances of the COI barcode region based on Kimura-2-parameter model (intraspecific distances are highlighted in bold).

|   | 1          | 2          | 3          | 4          | 5          | 6          | 7          | 8          |
|---|------------|------------|------------|------------|------------|------------|------------|------------|
| 1 | SYSU-LEP0334 Euclasta stoetzneri |            |            |            |            |            |            |            |
| 2 | SYSU-LEP0088 Crocidophora lutusalis | 0.133      |            |            |            |            |            |            |
| 3 | SYSU-LEP0090 Crocidophora pallidulalis | 0.136      | 0.077      |            |            |            |            |            |
| 4 | SYSU-LEP0243 Epiparbattia oligotricha | 0.138      | 0.098      | 0.085      |            |            |            |            |
| 5 | SYSU-LEP0359 Epiparbattia oligotricha | 0.133      | 0.101      | 0.089      | **0.019**  |            |            |            |
| 6 | SYSU-LEP0351 Epiparbattia multispinalis | 0.153      | 0.103      | 0.098      | 0.056      | 0.070      |            |            |
| 7 | SYSU-LEP0378 Epiparbattia multispinalis | 0.155      | 0.105      | 0.099      | 0.058      | 0.071      | **0.002**  |            |
| 8 | SYSU-LEP0244 Epiparbattia gloriosalis | 0.135      | 0.098      | 0.089      | 0.072      | 0.079      | 0.067      | 0.069      |
| 9 | SYSU-LEP0152 Sclerocona acutella  | 0.118      | 0.113      | 0.092      | 0.092      | 0.103      | 0.087      | 0.089      | 0.096      |

Discussion

The monophyly of *Epiparbattia* is robustly supported by the results of the molecular analysis. Three species can be recognized as members of *Epiparbattia* by a series of external and genital characters provided above in the diagnosis of the genus. As is apparent from the tree topology (Fig. 11), *E. multispinalis* is more closely related to *E. oligotricha* than to *E. gloriosalis* which makes good sense with respect to the similar hair-like editum in male genitalia (Figs 6, 7). According to the tree topology (Fig. 11), *Epiparbattia* is more closely related to *Sclerocona*. Species of *Epiparbattia* and *Sclerocona* have two foveae on the forewing in males, a developed and sclerotized lamella postvaginalis, and a weakly developed, almost reduced antrum in females.

Additionally, several pyraustine genera, *Anamalaia* Munroe & Mutuura, 1969, *Lepidoplaga* Warren, 1895, *Limbobotys* Munroe & Mutuura, 1970 and *Torulisquama* Zhang & Li, 2010, are similar to *Epiparbattia* and *Sclerocona* by bearing fovea (at least one fovea) on the forewing, minute basal and apical outer spurs of hindleg in males, as well as a developed and sclerotized lamella postvaginalis in females, but can still be distinguished from each other by the number and position of the fovea and other genital characters. The relationships among all these genera need to be further studied.

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