Identification and Redescription of Eumorphobotys eumorphalis, with Remarks on Its Relationship with Prodasycnemis inornata Referring to Abdominal Terminalia and DNA Barcode Information (Lepidoptera: Crambidae)

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Identification and Redescription of *Eumorphobotys eumorphalis*, with Remarks on Its Relationship with *Prodasycnemis inornata* Referring to Abdominal Terminalia and DNA Barcode Information (Lepidoptera: Crambidae)

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Abstract. A pyraustine *Eumorphobotys eumorphalis* (Caradja, 1925) is estimated to be an invader into Japan from China. Redescription of this species is given with the additional morphological features including tympanic organ based on the specimens collected in Gifu and Aichi Prefectures, Central Honshu, Japan. The adult female is peculiar to have a cavity on the posterior portion of pleural membrane of 7th abdominal segment laterally. This feature is also recognized in a related pyraustine *Prodasycnemis inornata* of Japan. DNA barcode analyses are made to refer the identification of *E. eumorphalis* of Japanese form and to estimate the relationship with *P. inornata*. It is suggested that *E. eumorphalis* is more closely related to *Prodasycnemis inornata* than to Chinese *Loxoneptera* species.

Key words: Bambuseae, China, DNA barcode, *Eumorphobotys*, genitalia, *Loxoneptera*, *Prodasycnemis*, Pyraustinae, tympanal organ.

Introduction

The second author discovered an unrecorded pyraustine species during the survey of Lepidoptera in Gifu Prefecture, Central Honshu, Japan, performed in 2021. The first author determined this species as *Eumorphobotys eumorphalis* (Caradja, 1925), after the morphological examination of this species. The species was originally described from Mokanshan, Chekiang Province, China in 1925 (Caradja 1925). In the course of this determination on this species, the first discovery of this species was recorded from Aichi Prefecture, adjacent to Gifu Prefecture, Japan (Iwashita and Matsui 2022; Mano 2022). In their report, adults of both sexes and photos of the male genitalia were shown with remarks on its biological notes as a bamboo pest and a possible invasion into Japan from South China. However, they did not offer detail morphological description of this newly invasive pest species.

In this paper, we will redescribe this species with the genitalia of both sexes based on specimens collected in Japan to compare with those of a related *Prodasycnemis inornata* of Japan, referring to Munroe & Mutuuta (1969) and Chen *et al*. (2018). A second objective is to discuss on the relationship of *Eumorphobotys* with the related genera such as *Prodasycnemis* Warren, 1892 and *Loxoneptera* Hampson, 1896 by the comparison of the morphological characters, together with the DNA barcode analysis.

Materials and methods

(1) Material examined for *Eumorphobotys eumorphalis*; 2♀, Nishiazai-cho, Nishio City, Aichi Pref., 5.viii.2019 (T. Mano leg.); 1♀, Kise-cho, Toyota City, Aichi Pref., 23.vi.2020 (T. Mano leg.), 1♂, 2♀, Nishiazai-cho, Nishio City, Aichi Pref .,10.viii.2020 (T. Mano leg.), 2♂, 1♀, Dachibokubora, Gifu City, Gifu Pref. (30 m als), 30.viii.2021(S. Funakoshi leg.)(1♂, 1♀ for DNA extraction); 1♂, Nishiazai-cho, Nishio

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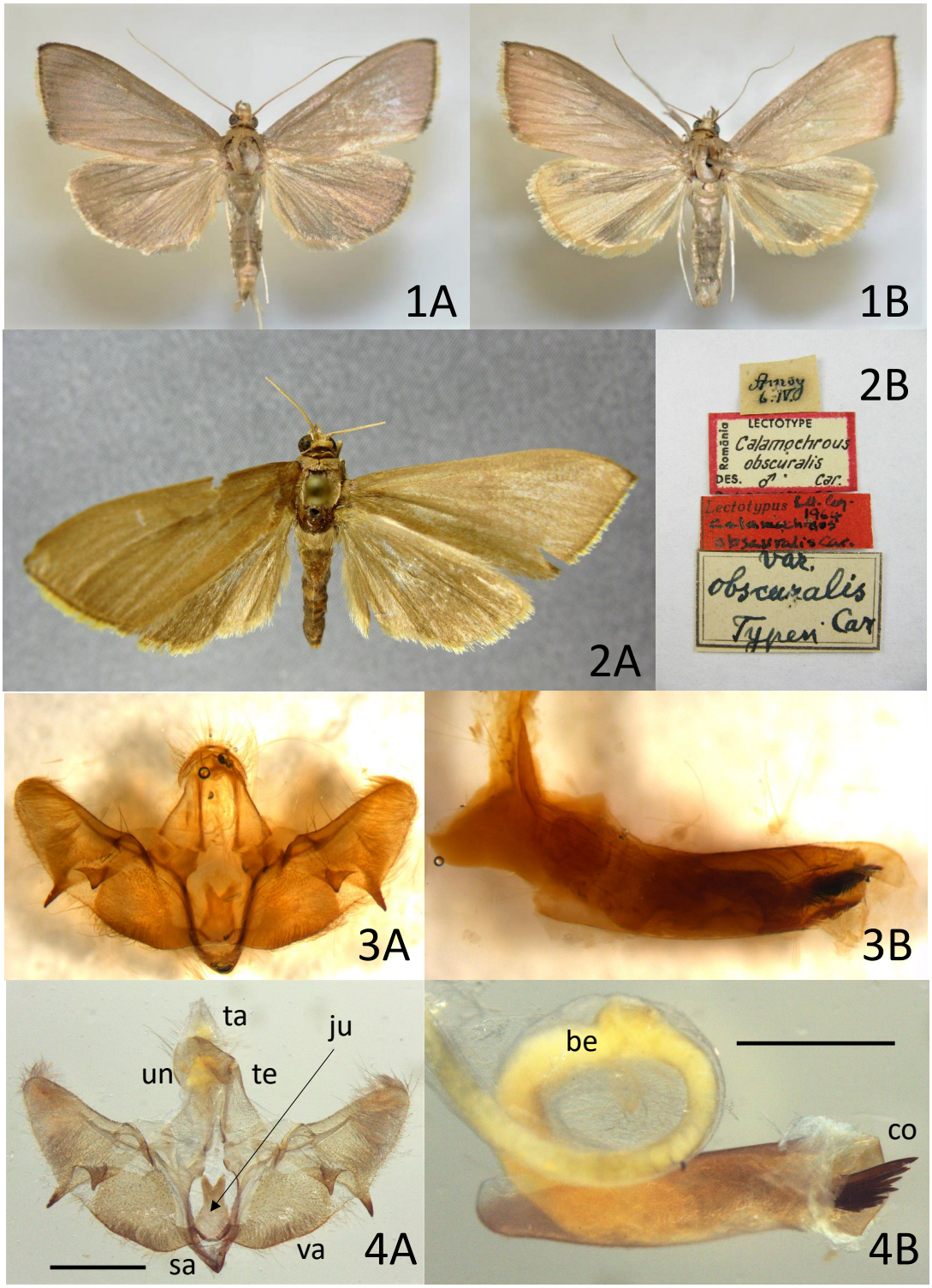


FIGURES 1–4. 1: Adult habitus of *Eumorphobotys eumorphalis*; A, Male (forewing length: 14.5 mm); B, female (forewing length: 15.1 mm). 2: Lectotype of *Calamochrous obscuralis* Caradja, 1925 (male), (Amoy, 6.IV) (redesignated by Eugene Munroe, 1964), in NMNHB. 3: Male genitalia (slide no. 621, NMNHB) of paralectotype of *Calamochrous obscuralis* Caradja, 1925 (coll. 28.IV, Amoy, China); 4: Male genitalia of *E. eumorphalis* (1); A, Ventral view, phallus removed; B, phallus, lateral view (be=burbus ejaculatorius; co=cornuti; ju=juxta; sa=saccus; ta=tuba analis; te=tegumen; un=uncus; va=valva). Scale: 1.0 mm.

City, Aichi Pref., 6.ix.2021(T. Mano leg.) (preserved in Osaka Metropolitan University(=OMU)). Reference specimens (picture) for identification: Lectotype (♂) and allolectotype(♀) of *Calamochrous eumorphalis* Caradja, 1925; Lectotype (♂) of *Calamochrous obscuralis* Caradja, 1925 ; male genitalia of paralectotype of *Calamochrous obscuralis* (slide No. 621, preserved in "Grigore Antipa" National Mueum of Natural History, Bucharest, Romania (=NMNHB)).

(2) Material examined for *Prodasycnemis inornata*: 1♀, Ino-cho, Kochi Pref., ix.1972 (Y. Suzuki leg.);1♂, Minami-yamashiro-mura, Kyoto Pref., 24.vi.1989 (Y. Yoshiyasu leg.);1♀, 25.v.2020, 1♀, 27.viii.2020, 1♀, 10.ix.2021, 1♂, 28.ix.2021, 1♂, 20.iv.2021, Kisarazu City, Chiba Pref. (O. Saito leg.) (1♂, 1♀ from Chiba Pref. for DNA extraction) (OMU).

(3) Observation method and terminology: The abdomen of the adult was removed, and macerated in 10% KOH solution for 10 minutes in hot water. Then it was transferred to the petri dish with 75 % ethanol solution, and dissected for observing the genital organ under the microscope (Leica M205C). The photos except for adult habitus were taken pictures by IC80 HD, attached to the microscope. The terminology of the genitalia followed Chen *et al*. (2018) and Xiang *et al*. (2021) and that of the tympanal organ followed Maes (1985).

(4) DNA extraction and barcode analysis: Two specimens of each species mentioned above were selected for DNA extraction. Total DNA was extracted from the right hindleg of the sample using the DNeasy Tissue Kit (Qiagen, Hilden, Germany). The mitochondrial COI gene, which encodes cytochrome oxidase subunit I, was amplified by using the primers LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al*. 1994). Other procedures were basically done following the methods described in Kobayashi *et al*. (2011). The aligned sequence in this study was compared with the samples of *E. eumorphalis* and *P. inornata* registered in the database.

Results

Redescription of species

***Eumorphobotys eumorphalis* (Caradja, 1925)**

Calamochrous eumorphalis Caradja, 1925: 362 (type-locality: Mokanshan, China).

Calamochrous obscuralis Caradja, 1925: 363 (type-locality: Amoy, China).

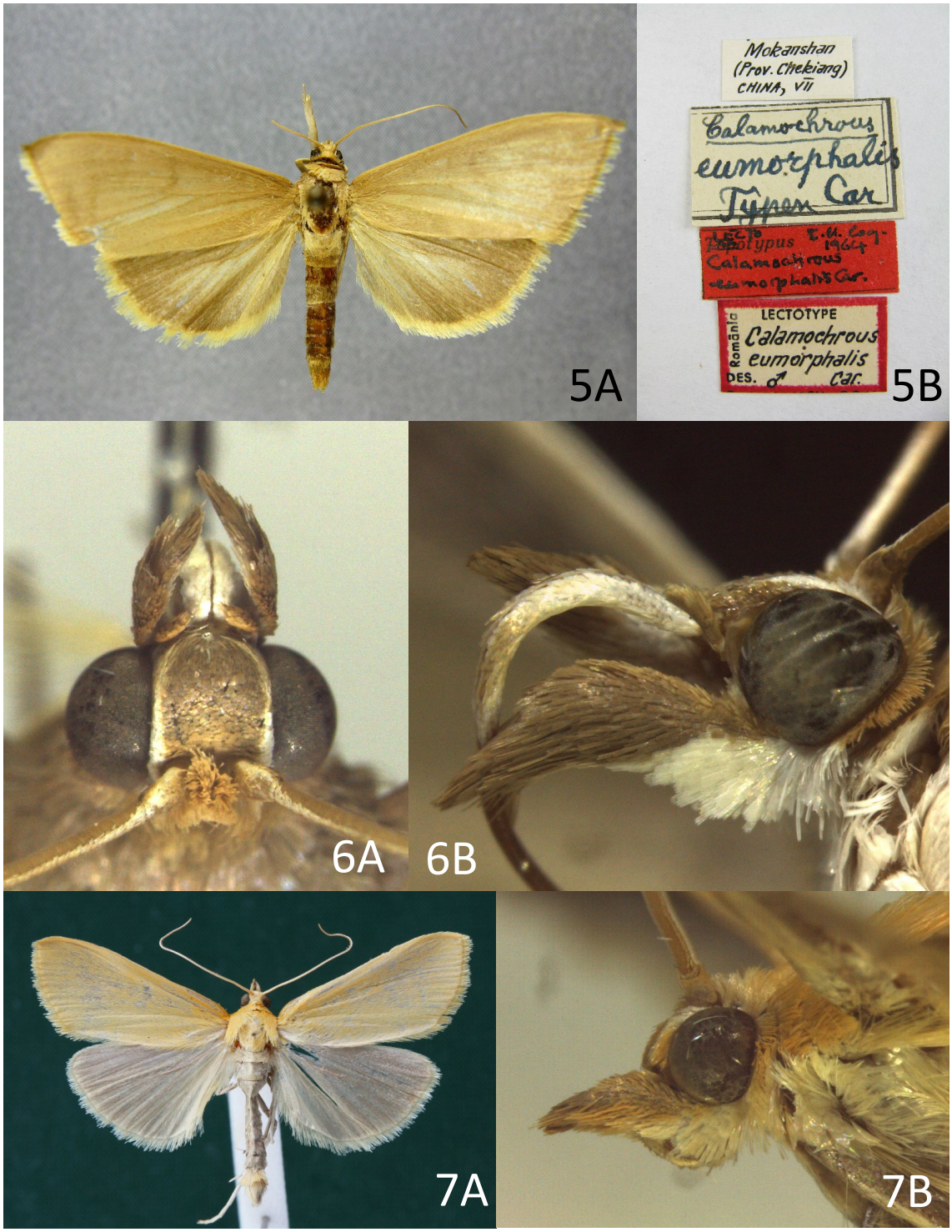
Eumorphobotys eumorphalis: Munroe & Mutuura, 1969: 303 (Figs 3 & 7); Wang, 1980: 173 (pl. 30, fig. 200); Chen *et al*., 2018: 495 (Figs 3–7, 11 & 14).

Male and female (Fig. 1). Forewing length: ♂13.5–14.5 mm (n=4), ♀14.3–14.7 mm (n=6) (wing span ♂♀ : 30-33 mm)

Head with vertex narrow, with erect long and brown scales. Ocellus clearly present. Frons rather flat, covered with brownish scales except for a whitish band along compound eye (Fig. 6A). Antenna about 2/3 as long as forewing length, ciliate in male and filiform in female; dorsal surface with yellowish white scales in both sexes. Maxillary palpus developed, with chocolate brown scales. Proboscis long, whitish scales at base. Labial palpus (Fig. 6B) porrect, slightly declining downwards apically; the lst segment whitish and terminal 2 segments chocolate brown, in male almost 1.5 times and in female about twice as long as eye diameter. Thorax above pale ochreous brown, beneath glossy white. Abdomen above pale brown, beneath glossy white tinged with pale yellow. Legs whitish; foreleg with a tuft of scales from proximal portion of coxa (Fig. 8A); midleg with inner spur of tibia twice as long as outer one; hindleg longer than midleg, with both inner spurs twice as long as outer ones.

Wing shape and coloration: Forewing broad, with apex acute, especially in female; termen almost stratight, but rather slightly curved to tornus comparing with linear termen in the other 2 *Eumorphobotys* species (*E. horacae* and *E. concavuncus*). Wing marking above in male uniformly pale chocolate brown tinged with purplish in both wings; with cilia dark brown in proximal 1/2 and fulvous in distal 1/2; in female a little lighter than in male, hindwing being fulvous behind discoidal cell and behind vein 1+2A, cilia fulvous. Wing marking beneath in male with forewing pale brown except for discoidal cell and its outer portion, with hindwing evenly pale fuscous brown; cilia as in above; hindwing beneath evenly slightly smoky fulvous in both sexes.

Tympanal organ (Fig. 8B): Praecinctorium



FIGURES 5–7. 5: Lectotype (male) of *Calamochrous eumorphalis* Caradja, 1925 (Mokanshan, Chekiang, China, vii) (redesignated by Eugene Munroe, 1964), in NMNHB. 6: Head of adult of *E. eumorphalis*; A, Male, dorsal view; B, female, left side. 7: Adult of *Prodasycnemis inornata*; A, Male (forewing length: 13.5 mm); B, *do*, head, left side.

rather small and narrow, long triangular in shape extending ventrally. Tympanum and pons tympani rather deeply emerged inside. Ramus tympani distinctly marked, with both sides making a straight line transversely. Puteolus tympani broad. Venula secunda well developed, extending to posterior margin of 2nd sternite.

♂**genitalia and related organs** (Figs 9 &10): Tegumen wide, deeply incurvate in dorsal view; lateral side with several sockets of long scales; fenestrula developed laterally. Vinculum wide at dorsal side and narrowing to ventral. Saccus rather flat extending anteriorly, with rather narrow tip in ventral view. Uncus wide with rounded distal margin, with several setae along subapical margin. Valva wide at base, gently narrowing to apex; sacculus very broad at base extending at basal 2/5; sella distinct, with a finger like process (editum *sensu* Xiang *et al*. 2021) dorsally and with a triangular ventral hook from sella extending ventrally, somewhat distinct setae marginally. Phallus (Figs 4B & 9) very long (almost as long as the length from anterior end of saccus to valval margin) and stout, slightly curved at middle portion in dorsal view (Fig. 9B); vesica with some distinct needle-shaped cornuti; burbus ejaculatorius thick, coiled twice in the left side of phallus before ductus ejaculatorius emitting from it. Juxta long, narrowing to its dorsal emarginate apex.

♀**genitalia and related organs** (Figs 12 & 13A–C): Ostium bursae very wide, with a broad and asymmeric subquadrate plate (lamella postvaginalis in this study) (Fig. 12C, D) dorsally which is supposed to be an antrum of the other pyraustine species. Lamella antevaginalis narrowly present along ventral margin of ostium bursae. Ductus bursae wide near ostium bursae, long and narrow, weakly curved and coiled twice before reaching to corpus bursae. Ductus seminalis emitting from right side of ductus bursae near ostium bursae. Spermathecal gland long, with a pouch of lagena. Corpus bursae almost spherical; signum well developed, present at anterior portion of corpus bursae, narrowly oblong with slightly produced lateral sides at middle and having a pair of distinct carinae transversely together with some rows of minute spinules directing inside (Fig. 13A). Appendix bursae developed around posterior margin of corpus bursae, spherical in shape, with its proximal portion concealed by coiled ductus bursae (Fig. 12B) Apophysis anterioris about 2/3 as long as 7th abdominal tergite, with anterior 1/2 curved downward. Eighth tergite narrow, without setae. Apophysis posterioris short, almost 1/2 as long as its anterioris. Papilla analis broad, having dense setae spherically, with ventral portion widened and slightly curved posteriorly.

Posterior portion of 7th abdominal tergite extending laterally and invading into a cavity in posterior pleural membrane where a sac or pocket-like hollow is formed (Figs 12 &13B). Seventh abdominal sternite shorter than the tergite, with posterior portion a little widened laterally.

Distribution: Japan (Central Honshu, temporary occurrence?); China, Taiwan.

DNA barcode: DNA sequences of the COI barcode region were successfully obtained for two specimens (1♂, 1♀) of *P. inornata*. They are completely identical, then one of them was submitted to GenBank (Accession number: LC724048).

***Prodasycnemis inornata* (Butler, 1879)**

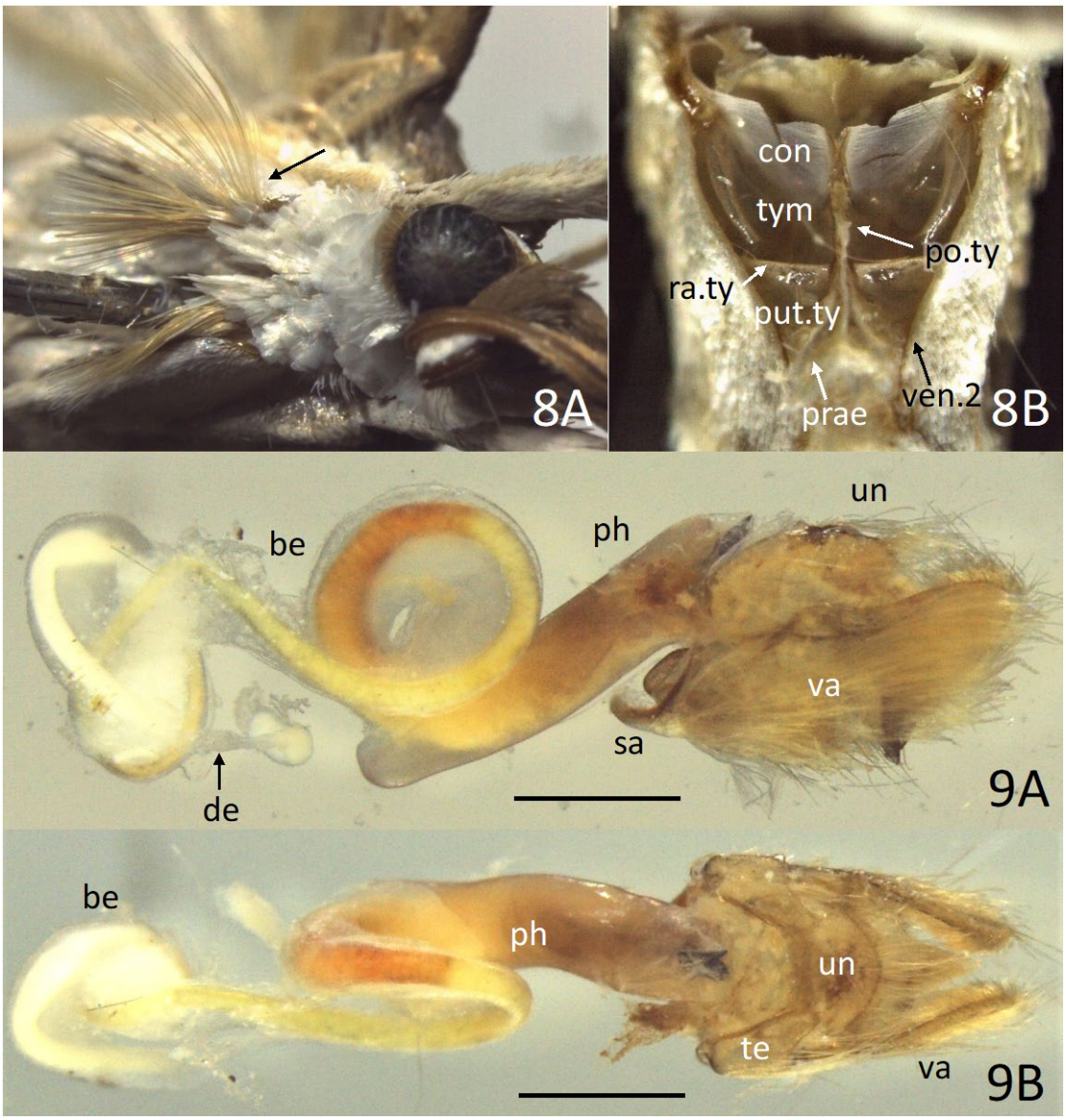
Botys inornata Butler, 1879: 76, pl. 59, fig. 11 (type-locality: Yokohama, Japan)

Prodasycnemis inornata: Warren, 1892: 301; Mutuura, 1954: 20; Inoue, 1982: 359; Yamanaka, 2013: 419.

This pyraustine is the only species of the genus *Prodasycnemis* and widely distributed in Japan. In this section we give the description of the terminalia of both sexes and the DNA barcode analysis for the comparison with those of *E. eumorphalis*.

Diagnosis. The external appearance of this species (Fig. 7) and tympanal organ (Fig. 8B) are almost same to *E. eumorphalis*, but different from the latter as follows: Forewing length a little shorter and paler (especially in female), with termen more curved; labial palpus shorter, in male ca 1.2 times and in female ca 1.5 times as long as eye diameter; midleg of male with a characteristic tuft of scales on its tibia (no tuft of scales in *E. eumorphalis*).

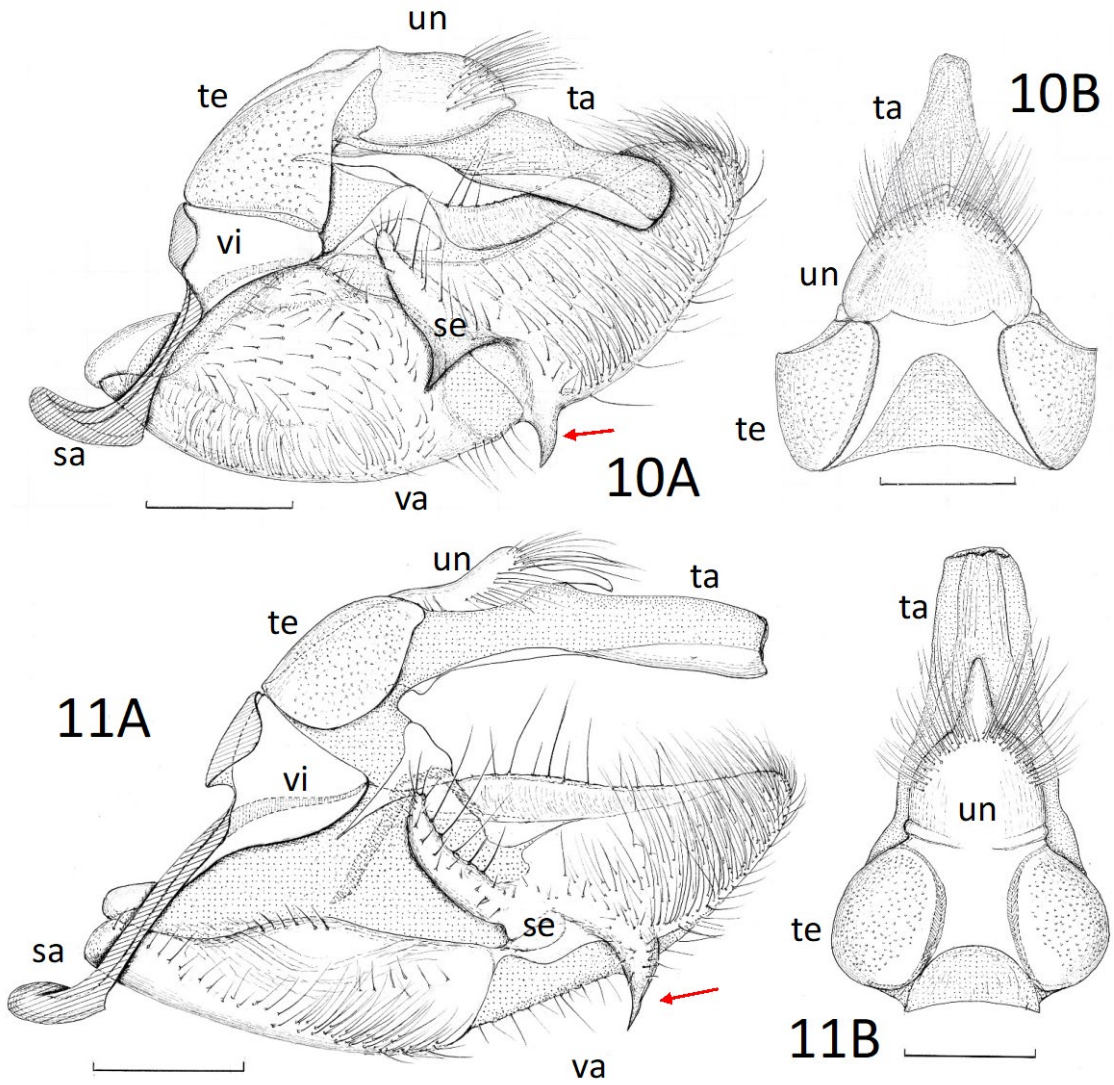
♂ **genitalia and related organs** (Fig. 11): Fundamentally similar to those of *E. eumorphalis*, but different in the following points: Tegumen with lateral side evenly rounded, with anterior excurvation weaker in dorsal view; vinculum relatively longer; saccus smaller and rather flattened; uncus narrower at base, with a series of setae along posterior margin, and with a corniculate production



FIGURES 8–9. 8: Adult male of *E. eumorphalis*; A, A tuft of specialized scales (arrow) on base of right foreleg, ventral view; B, tympanal organ, ventral view. con: conjunctivum; prae: praecinctorium; po.ty: pons tympani; put.ty: puteolus tympani; ra.ty: ramus tympani; tym: tympanum; ven.2: venula secunda. 9: Male genitalia of *E. eumorphalis*; A, Lateral view; B, dorsal view. be=bulbus ejaculatorius; ph=phallus; sa=saccus; te=tegumen; un=uncus; va=valva. Scale: 1.0 mm.

apically in dorsal view; valva almost as in *E. eumorphalis* in shape, but sacculus wider with a series of setae ventrally; sella with developed editum dorsally, without a central process seen in *E. eumorphalis*; phallus with very long and stout, with vesica having a group of some needle-like spines of cornuti as in *E. eumorphalis*; juxta much different from *E. eumorphalis* by having numerous setae along apex.

♀**genitalia and related organs** (Figs 13D, E, F & 14): Ostium bursae wide, distinctly marked. Eighth tergite narrow. Lamella postvaginalis representing a pair of weakly sclerotized plates. Antrum broad and wide, strongly sclerotized. Ductus bursae long and narrow, coiled twice near corpus bursae. Ductus seminalis emitting from right side of proximal portion of ductus bursae. Corpus bursae almost spherical to long ovoid; signum well developed as in *E.*



FIGURES 10–11. 10: Male genitalia of *E. eumorphalis*; A, Lateral view, phallus and left valva removed. An arrow indicates a ventral hook of sella; B, tegumen and uncus, dorsal view. 11: Male genitalia of *Prodasycnemis inornata*; A, Lateral view, phallus and left valva removed. An arrow indicates a ventral hook of sella; B, tegumen and uncus, dorsal view. ju=juxta; sa=saccus; se=sella; ta=tuba analis; te=tegumen; un=uncus; va=valva; vi=vinculum. Scale: 0.5 mm.

eumorphalis. Appendix bursae from posterior portion of corpus bursae, spherical but wholly concealed by ductus bursae coiled twice before reaching to corpus bursae. Apophyses and papilla analis short, as in *E. eumorphalis*.

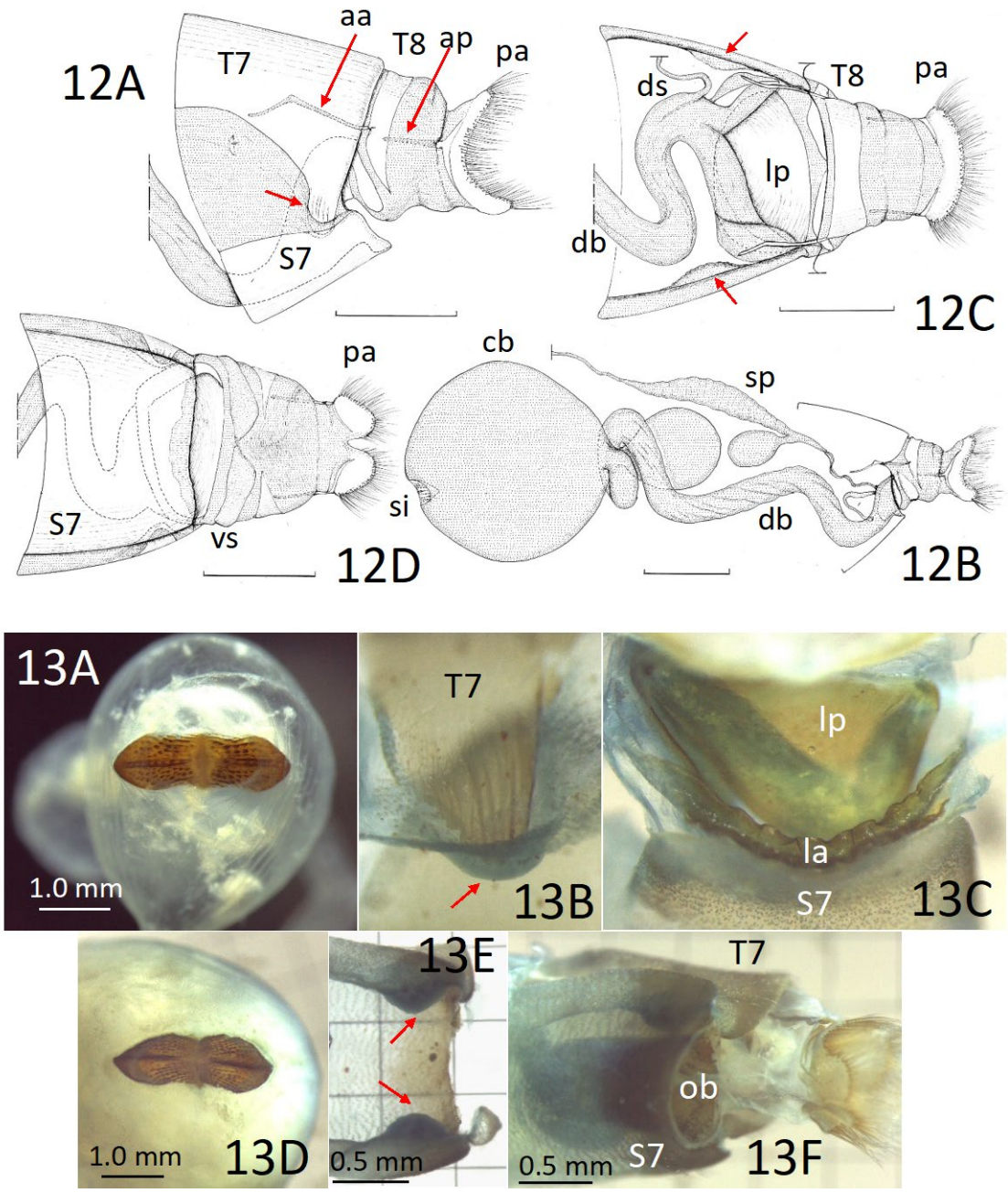
Seventh abdominal tergite gradually broader to posterior margin, which is narrowly converging ventrally. Postero-ventral portion of pleural membrane strongly concaved, forming a distinct cavity (Figs 13 & 14).

Distribution. Japan (Hokkaido, Honshu, Shikoku, Kyushu, Tsushima Is.); China, South Korea, Russia (Primorsky).

DNA barcode: DNA sequences of the COI barcode region were successfully obtained for four specimens (2♂, 2♀) of *P. inornata*. They are completely identical, then one of them was submitted to GenBank (Accession number: LC724047). Using this information, a neighbor-joining tree including related genera is shown in Fig. 15.

Discussion

1. Identification of "*Eumorphobotys eumorphalis*"



FIGURES 12–13. 12: Female terminalia of *E. eumorphalis*; A, 7th to 10th segments, lateral view. (an arrow indicates ventral expansion of T7 to a vicinity of lateral membrane); B, whole genitalia, lateral view; C, 7th to 10th segments, dorsal view, 7th tergite removed (arrows indicate cavities in pleural membrane); D, *do*, ventral view. aa=apophysis anterioris, ap=apophysis posterioris, cb=corpus bursae, db=ductus bursae, ds=ductus seminalis, lp=lamella postvaginalis, pa=papilla analis, si= signum, sp=spermathecal gland, vs=ventral sclerite; T7=7th tergite, S7=7th sternite, T8=8th tergite. Scale: A, C, D: 1.0 mm; B: 2.0 mm. 13: Female terminalia of *E. eumorphalis* and *P. inornata*; A, *E. eumorphalis*, signum on corpus bursae, anterior view; B, *do*, lateral cavity of 7th tergite, lateral view (an arrow indicates a cavity of pleural membrane invaginating inside); C, *do*, lamella antevaginalis, postero-ventral view; D, *P. inornata*, signum, ventral view; E. *do*, lateral cavity of pleural membrane of 7th segment, dorsal view; F. *do*, posterior end of 7th tergite and ostium bursae, ventral view. la=lamella antevaginalis; lp=lamella postvaginalis; os=ostium bursae.

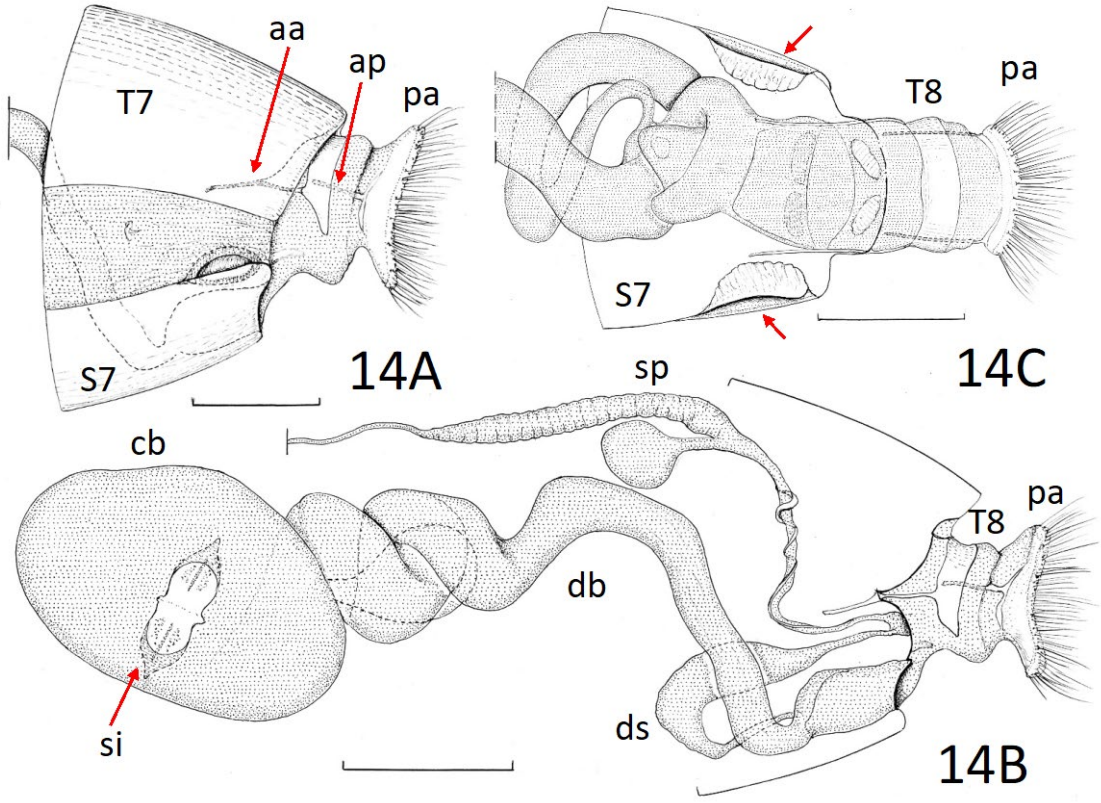


FIGURE 14. Female terminalia of *P. inornata*. A, 7th to 10th abdominal segments, left side; B, whole genitalia, lateral view; C, dorsal view, 7th tergite removed (arrows indicate cavities in pleural membrane). aa=apophysis anterioris, ap=apophysis posterioris, cb=corpus bursae, db=ductus bursae, ds=ductus seminalis, pa=papilla analis, si= signum, sp=spermathecal gland, T7=7th tergite, S7=7th sternite, T8=8th tergite. Scale: A, C: 1.0 mm; B: 2.0 mm.

The adult (Fig. 1) and the male genitalia (Figs 4 & 10) of the Japanese form are coincident with the male specimen (Fig. 2) of the lectotype and the male genitalia ((Fig. 3 (slide No. 621)) of paralectotype of *E. obscuralis*. Munroe and Mutuura (1969) treated *E. obscuralis* (Caradja) as a distinct species from *E. eumorphalis* (Caradja) . The forewing of lectotype of *E. eumorphalis* (Fig. 5) is yellowish brown, lighter than in *E. obscuralis* (Fig. 2). In addition, the costa of forewing of *E. eumorphalis* is fringed with yellowish fulvous, especially remarkable in the female. Moreover, there are some slight differences in the genitalia between these two species. However, Chen *et al*. (2018) treated *E. obscuralis* as a junior synonym of *E. eumorphalis* after examination on many specimens belonging to these two species preserved in NMNHB and in Chinese Institutes and Universities. The DNA barcode of Japanese specimen (Accession number: LC724048) examined in this study is almost coincident with those of *E. eumorphalis* (Accession numbers: MW085610 and MG739574), resulting 99 % similarity in mean. And the presence of rather lighter wing color in male specimens collected in the same place in Aichi Prefecture, also in Japan. By these facts, we followed the taxonomic treatment proposed by Chen *et al*. (2018) in this report. As both Munroe and Mutuura (1969) and Chen *et al*. (2018) did not observe the genitalia of the lectotype or specimen of type locality (Mokanshan (Caradja, 1925)) of *E. eumorphalis*, there is a possibility to be revised this taxonomic treatment in the future.

2. A peculiar modification of tergite and pleural membrane on 7th abdominal segment in the females in *E. eumorphalis* and *P. inornata*

In *E. eumorphalis* the ventral margin of 7th

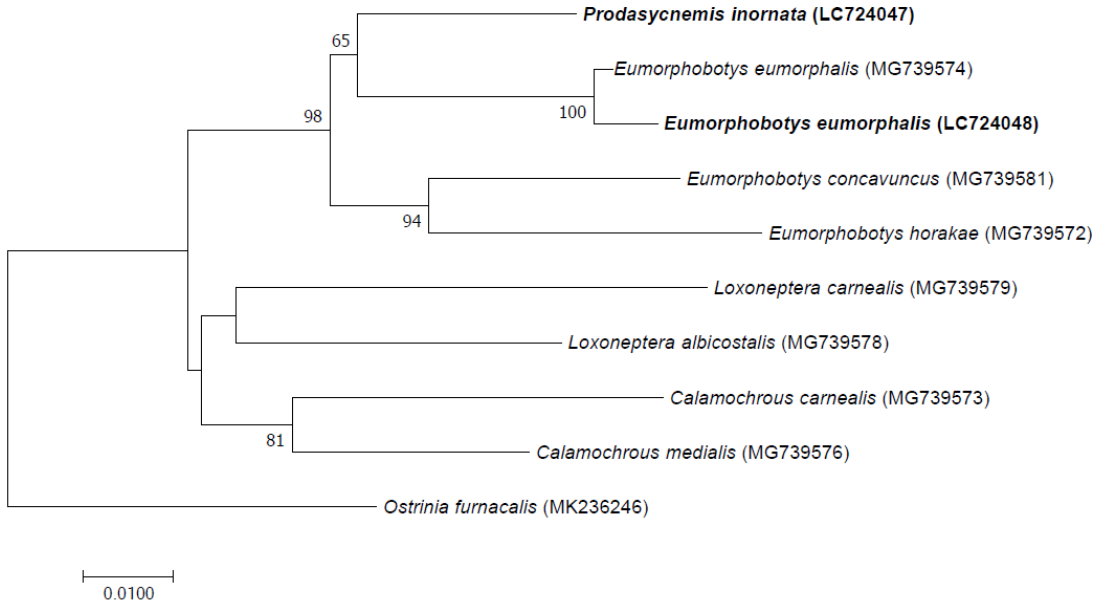


FIGURE 15. Neighbor-joining tree based on the mitochondrial COI gene partial sequences (658 bp) of *E. eumorphalis*, *Prodasycnemis inornata* and *Loxoneptera* species. *Ostrinia furnacalis* was used as an outgroup. Numbers beside the branches indicate bootstrap values greater than 50% in 1,000 replicates. Branch lengths represent nucleotide substitutions. The alphabets and numbers in parentheses were shown accession numbers.

abdominal tergite gradually expanding laterally and the posterior portion extending ventrally almost to reach the sternite. As far as we know, there is no report on this peculiar modification in the female terminalia in the Pyraustinae. In addition to the modification, the lateral membrane on this segment having the characteristic cavity near the postero-lateral corner. The resembling feature is also seen in *P. inornata*, which is also unique in the Pyraustinae. It is suggested that this cavity will become a pocket to receive something by the shape. In both *E. eumorphalis* and *P. inornata*, there is a strong ventral hook of sella on valva in their male genitalia (Figs10 &11). By the position of the hook, it is possible to estimate that this hook is relating to the cavity of the female 7th abdominal segment during copulation; i.e. the cavity is considered to be a "pocket" receiving a ventral hook of sella of the male genitalia.

There is no information on the 7th abdominal segment in the other *Eumorphobotys*, *E. concuvuncus* Chen & Zhang, 2018 and *E. horakae* Chen & Zhang, 2018. The latter species was described based on 8 male specimens from China and has a different valval morphology without a ventral hook of sella (Chen *et al*. 2018). More detail observations of these two species will reveal the function of the male valval hook of sella and female modification of 7th abdominal segment in two genera.

3. Relationship of *Eumorphoboty* and *Prodasycnemis*

Munroe and Mutuura (1969) reported that *Eumorphobotys* is distinct from a related genus *Prodasycnemis* Warren, 1892 in having "the angulate forewing, the dorsally emarginate juxta, the rounded uncus, the presence of a triangular ventral process on the clasper (=sella), in the basally expanded sacculus" in the male genitalia. Chen *et al*. (2018) also discussed the morphological similarity of *E. eumorphalis* with *Prodasycnemis inornata*, but no taxonomic treatment was made. Recently Xiang *et al*. (2021) reviewed *Loxoneptera* Hampson in China, resulting 10 species including 3 species using morphological traits, and gave molecular analysis for 5 species among them and 3 *Eumorphobotys* species. They also gave a phylogentic relationships with in *Loxoneptera* species, and noted the genus is a sister group position to *Eumorphobotys* (PP=1.00, BS=100) based on molecular analysis. In this study,

Eumorphobotys species is more related to *Prodasycnemis inornata* not only in morphological characters but also in the DNA barcode information (Fig. 15).

On the other hand, the larva of *E. eumorphalis* is known as a pest of Bambuseae such as *Phyllostachys* sp., *Bambusa* sp., *Dendrocalamus* sp. feeding on the leaves and branches（Wang 1980; Chen *et al*. 2018). While, the host plant of *Prodasycnemis inornata* is *Sasa kurilensis* (Rupr.) Makino et Shibata (Bambuseae) (Inoue 1982). Both species use Bambuseae plants as the hosts. The similarity of their host range could reflect close phylogenetic relationship of these two taxa.

Acknowledgments

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