



A new record family Neopseustidae (Insecta: Lepidoptera) from Chongqing of China, with the first description of the *Neopseustis archiphenax* female adult

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Abstract

In 2022, the insect inventories organized by Prof. Zhi-Sheng Zhang of Southwest University were constructed in Yintiaoling Nature Reserve, Chongqing Municipality, China. The neopseustid moth from Chongqing based on three specimens of *Neopseustis archiphenax* by light trapping were reported in this paper. The photos of the male and female adults, genitalia, and abdominal special structures are presented with the female described firstly. Also, the DNA barcoding sequence data is provided, and the key to the *Neopseustis* species is given.

Key words: New record, Female genitalia, Neopseustidae, Yintiaoling Nature Reserve

Introduction

The family Neopseustidae is an archaic lepidopteran group which consists of 4 genera and 14 species, and distributed in Southeast Asia and South America (Davis 1975; Davis & Nielsen 1980, 1985; Chen *et al.* 2009; Liao *et al.* 2021; Huang *et al.* 2021). Two genera and eight species have been recorded from mainland China (Chen *et al.* 2009; Liao *et al.* 2021; Huang *et al.* 2021), but no species has been reported from Chongqing Municipality. The insect inventories organized by Prof. Zhi-Sheng Zhang of Southwest University were constructed in Yintiaoling Nature Reserve, Chongqing of China. On June 25, 2022, three specimens of *Neopseustis archiphenax* Meyrick, 1928 were collected by Mr. Wei-Wei Zhang at 23:30 pm to 6:00 am.

Neopseustis archiphenax was described by Meyrick, 1928 from Myanmar, and later discovered in Sichuan and Henan Provinces of China (Chen *et al.* 2009; Liao *et al.* 2021). In this paper, we describe the female adult of *N. archiphenax* for the first time and re-describe the male adult in detail. The DNA barcoding sequence data of the specimen is provided, and the key to the *Neopseustis* species is also given.

Material and methods

Taxon sampling. Adult specimens were collected by light trapping in Yintiaoling National Natural Reserve, Chongqing, China. Male and female genitalia were dissected following the methods of Liao *et al.* (2021). The photos of adults and genitalia were taken by using a Canon EOS 50D camera and a LEICA S8APO stereo microscope, respectively. The terminology in this study follows Davis (1975) and Kristensen (2003). All examined specimens are preserved in Hunan Agricultural University, Changsha City, Hunan Province, China (HUNAU).

DNA sequencing and Phylogenetic analysis. The genomic DNA was extracted from legs and thorax muscles of the dry adult specimen HAUHL077900, and the protocols for total DNA extraction was followed the kit operating

instructions. The 585 bp fragments of the cytochrome oxidase I gene (*COI*) barcoding fragment was sequenced following the methods of Liao *et al.* (2019). In this study, two *N. archiphenax* sequences from BOLD systems (www.boldsystems.org), and ten *Neopseustis* sequences and one outgroup taxa sequence from NCBI (www.ncbi.nlm.nih.gov) (Table 1) were used in the phylogenetic analysis with the maximum likelihood (ML) tree constructed (Kumar *et al.*, 2018).

TABLE 1. *Neopseustis* species and outgroup information used for molecular analysis.

Sample ID	Species	Locality	Date
LNAUT030-14	<i>N. archiphenax</i>	Henan, China	24.VII.2002
LNAUT031-14	<i>N. archiphenax</i>	Henan, China	24.VII.2002
HAUHL077900	<i>N. archiphenax</i>	Chongqing, China	25.VI.2022
HAUHL039474	<i>N. rectagnatha</i>	Hunan, China	15.VIII.2020
HAUHL039473	<i>N. rectagnatha</i>	Hunan, China	15.VIII.2020
LS-06-0068	<i>N. meyricki</i>	Taiwan, China	N/A
BX1	<i>N. sinensis</i>	Sichuan, China	VII.2009
YJ1	<i>N. sinensis</i>	Sichuan, China	VII.2009
MX1	<i>N. moxiensis</i>	Sichuan, China	2.VIII.2004
YJ2	<i>N. bicornuta</i>	Sichuan, China	VII.2009
HAUHL041880	<i>N. fanjingshana</i>	Hunan, China	10.VIII.2019
SZ1	<i>N. fanjingshana</i>	Hunan, China	VIII.2008
CT1	<i>N. chentangensis</i>	Xizang, China	23.V.2021
HN20170409020	<i>Endoclita davidi</i>	Hunan, China	1.XI.2015

Results

Taxonomy

Key to all nine species of the genus *Neopseustis*

- 1 Parameres well developed and narrow 2
- Parameres poorly developed. 5
- 2 Gnathos curved, sickle-shaped 3
- Gnathos distinctly straight *N. rectagnatha*
- 3 Lateroposterior process of anellus forked, forming two equally thick branches 4
- Lateroposterior process of anellus forked, one branch slender and another is coarser and S-shaped with serrations on one side *N. archiphenax*
- 4 Branches of lateroposterior process of anellus all slender *N. meyricki*
- One branch of lateroposterior process of anellus sharp and another irregularly serrate *N. sinensis*
- 5 Latero-posterior process of anellus apex deeply bifurcate, bending anteriorly *N. chentangensis*
- Latero-posterior process of anellus apex not bifurcate and pointed posteriorly 6
- 6 Valvae with a long, curved, hook-tipped process *N. moxiensis*
- Valvae without a ventral process 7
- 7 Lateroposterior process of anellus angular, curved inward with sharp tip 8
- Lateroposterior process of anellus paddle-shaped, not sharp with rounded tip *N. calliglauca*
- 8 Lateroposterior process of anellus apical beyond uncus *N. fanjingshana*
- Lateroposterior process of anellus not reach uncus *N. bicornuta*

Neopseustis archiphenax Meyrick, 1928 (Figures 1–4)

Neopseustis archiphenax Meyrick, 1928: 404; Davis 1975: 20–21, figs 1, 5, 7–10, 15, 21–22, 35, 53, 55; Davis 1997: 5; Chen *et al.* 2009: 17; Huang *et al.* 2021: 45; Liao *et al.* 2021: 340.

Diagnosis. *Neopseustis archiphenax* is very similar to *N. rectagnatha* (Liao *et al.* 2021) in the external morphology, but it can be distinguished by the genital structure of male adult: i) the gnathos is curved, while straight in *N. rectagnatha*; ii) the lateroposterior process of anellus is S-like bifurcated, while h-like in *N. rectagnatha*. In addition, the female genitalia of this species can be distinguished from other *Neopseustis* species by the shorter, more rounded corpus bursae.

Description. Male. Forewing length 10.0 mm; wingspan 22.0 mm.

Head. Vertex and frons pale brown. Antennae with about 80 nodes; brown to dark brown, darkening near base (Fig. 1). Eyes large, purplish black, with irregular reticulate pattern. Maxillary palpi well developed, yellow brown; fourth segments longer than fifth. Labial palpi of the same color and thickness as the maxillary palpi, but shorter than the latter.

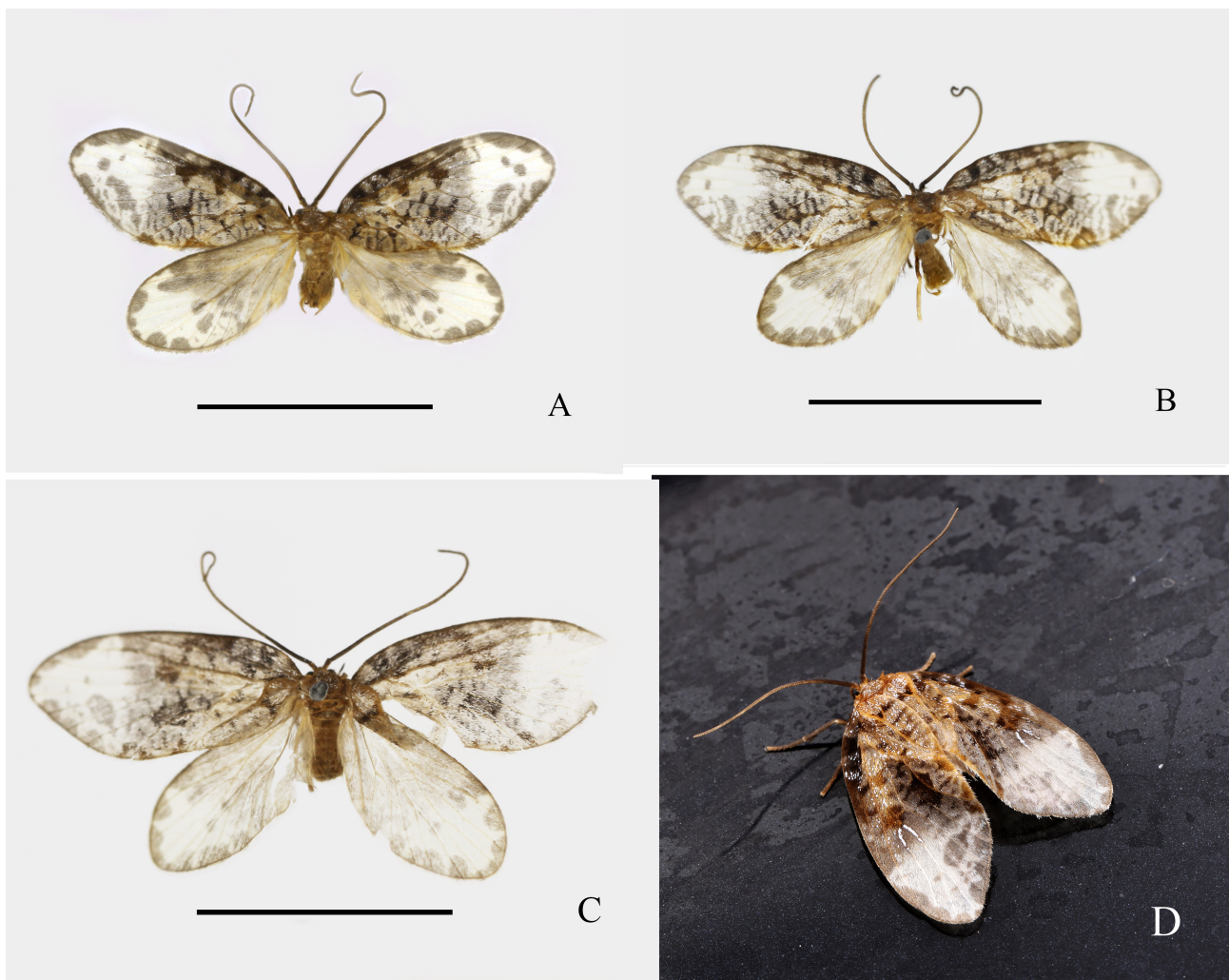


FIGURE 1. *Neopseustis archiphenax* from Yintiaoling. HAUHL077900-077902. **A–B.** male; **C.** female; **D.** male at rest. Scales bar: 5 mm

Thorax. Dorsum brown, central bare, margin covered with brown scales. Forewings chiefly of three colors and can be divided into two parts of different shades. Basal two-thirds of wing grayish brown, distributed with blackish brown patterns and spots; out third of wing white, slightly transparent, streaked with grayish brown patterns and spots. Between M2 and CuA1, usually with a large dark patch in the middle of the wing; an almost continuous line of semi-circular grayish brown spots along edge of the wing. The patterns and spots on the wings of different individuals may be quite different.

Abdomen. Slender brown scales covering the abdomen. Seventh sternite with a small spinous conical projection at the top of the center; eighth sternite with a low median ridge-like lobe with a dozen rod-like spines.

Male genitalia. Gnathos slender, curved process that gradually becomes sharp from the bottom to the top. Surface of socii covered with thick bristles, which are separated to both sides. Tegumenal lobes extended to form

a pair of slender, slightly curved projecting arms with rounded tips. Valvae wide, with a pair of small, rod-shaped projections on both sides, and the top close to each other. Lateroposterior process of anellus developed, symmetrical on both sides, and bifurcate near the base, producing two branches of different shapes: inner branch stouter, S-like, roughly serrated along entire length of outer edge; the other one shorter, slender, straight, with small spines at the top. Paired processes of anellus hardened, curved, with serrated spinules at the ends, and close to each other at the middle trailing edge of anellus. Juxta with a broad flat central lobe extending forward, slightly beyond lateral arms of vinculum. Parameres symmetrical, very slender, transparent near the base, and two-thirds near the top are densely covered with short bristles, with sharp ends (Fig. 2).

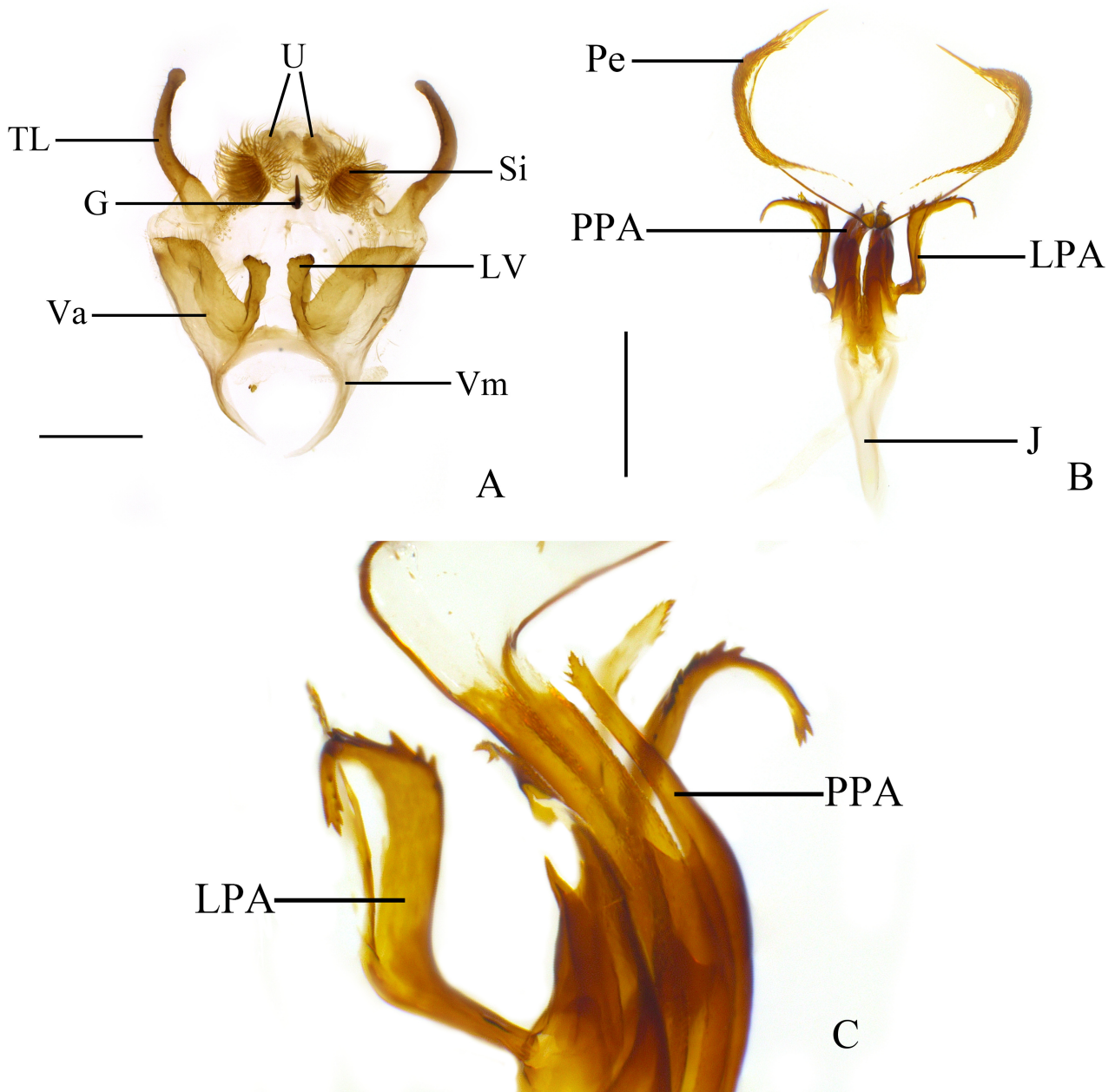


FIGURE 2. Male genitalia of *Neopseustis archiphenax*. **A.** Ventral view except anellus; **B.** Anellus-juxta-parameres; **C.** Detail of anellus. Scales: 0.5 mm. Abbreviations: G, gnathos; J, Juxta; LPA, lateroposterior process of anellus; LV, lobe of valva; Pe, paramere; PPA, paired process of anellus; Si, socii; TL, tegumenal lobes; U, uncus; V a, valva; Vm, vinculum.

Female. Forewing length 11.2 mm; wingspan 24.5 mm.

Head and thorax. Similar to male.

Abdomen. Surface covered with brown scales like male. A pair of oval glands can be seen at the back of the fourth sternite forming a pair of membranous windows on the integument (Fig. 3). Seventh sternite strongly hard-

ened, with a small backward conical bulge in the middle. The rear edge of seventh sternite with some bristles and bend inward at the middle, many small tumor-like bulges on it. (Figs 4A-B).

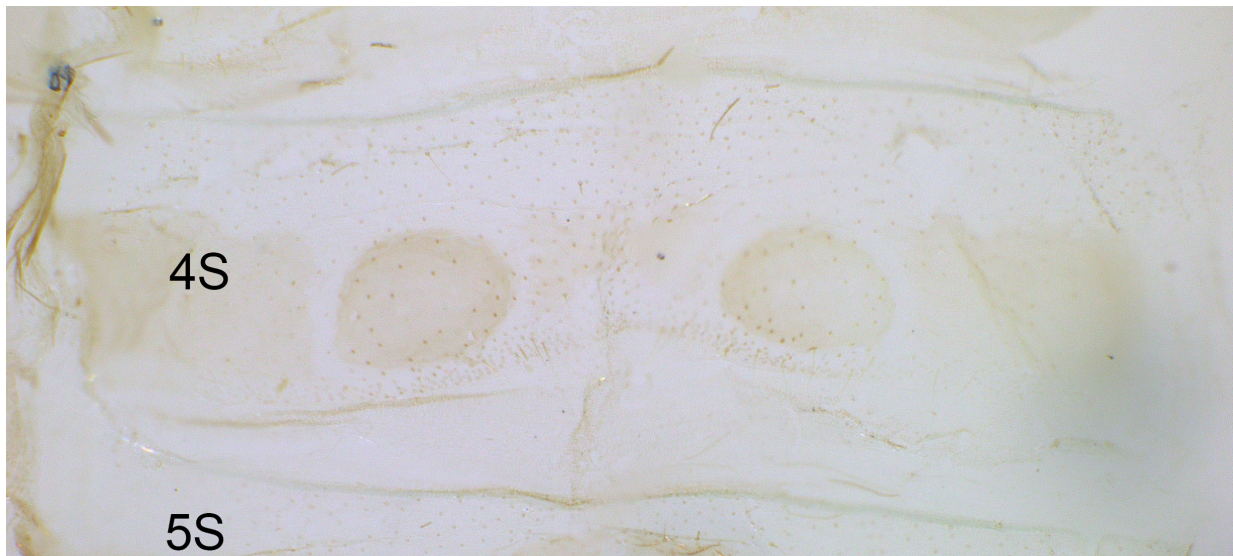


FIGURE 3. Glands of the fourth abdominal sternite of female.

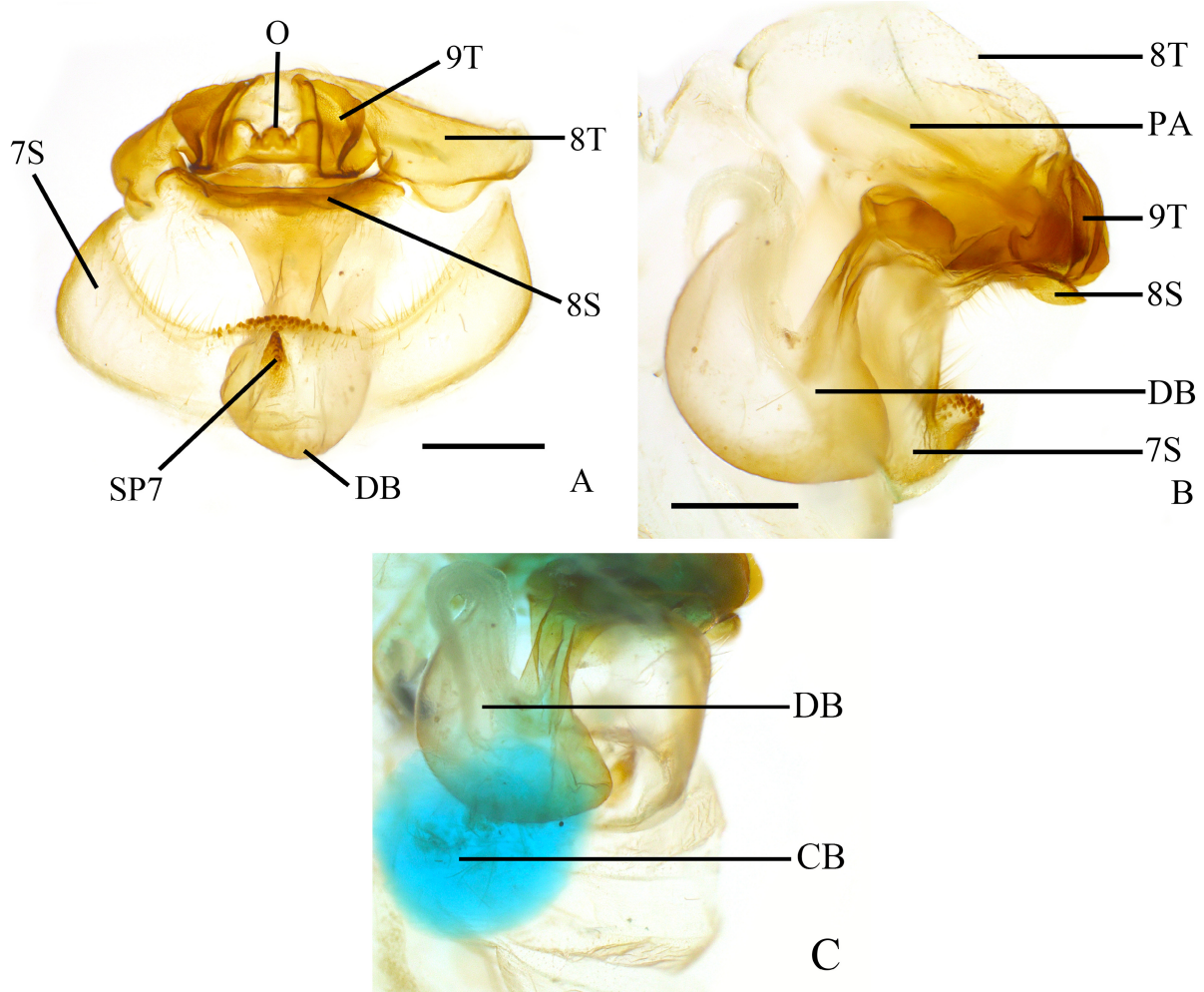


FIGURE 4. Female genitalia of *Neopseustis archiphenax*: A. Ventral view; B. Lateral view, excluding seventh sternite; C. Corpus bursae and ductus bursae. Scales: 0.5 mm. Abbreviations: CB, corpus bursae; DB, ductus bursae; O, ovipositor; PA, posterior apophysis; 7S, seventh abdominal sternite; SP7, sternal process of seventh abdominal segment; 8T, eighth abdominal tergite.

Female genitalia. Eighth tergite extends downward at the rear end to form a hood-like structure. Eighth sternite concave inward, forming two wide triangular lateral lobes at the tail end. Ninth sternite reduced to a pair of hardened plates, and ninth tergite with a pair of semicircular plates on both sides of ovipositor, with the inner edge bent and raised. Posterior apophysis hardened and extends to more than half of eighth tergite (Figs 4A–B). Ductus bursae thick, and the middle part obviously expanded, D-shaped. Corpus bursae suborbicular, membranous. (Fig. 4C). Spermatheca small and transparent, ovoid, with slender ducts.

The females of *Neopseustis* are rarer than males, had been reported only in two species, *N. meyricki* and *N. rectagnatha*. The female genital structure of the latter is very similar to that of *N. archiphenax*, indicating that they are closely related, which is consistent with the results of COI sequences analysis.

Examined specimens: 2 males, China: Chongqing, Wuxi County, Yintiaoling National Nature Reserve, Hongqi Management and Protection Station, June 25, 2022, light trapping, leg. W.-W. Zhang; voucher number: HAUHL077900-077901 (HUNAU); 1 female, same locality as the male with the voucher number: HAUHL077902 (HUNAU).

Host. Unknown.

Molecular analysis. According to the *COI* barcoding sequences analyses, the Kimura-2-parameter distances of the species population individuals in the genus *Neopseustis* are given in Table S1. The minimum interspecific divergence occurred between *N. fanjingshana* and *N. bicornuta* was 1.6%. The maximum intraspecific genetic divergence among individuals of *N. archiphenax* is 0.5% (N = 3) (Table. S1). In the ML tree based on *COI* sequences, *N. archiphenax* was closest to *N. rectagnatha*, *N. meyricki* and *N. sinensis*. Compared with other species, these four species were clearly clustered (bootstrap value: 98%) (Fig 5.).

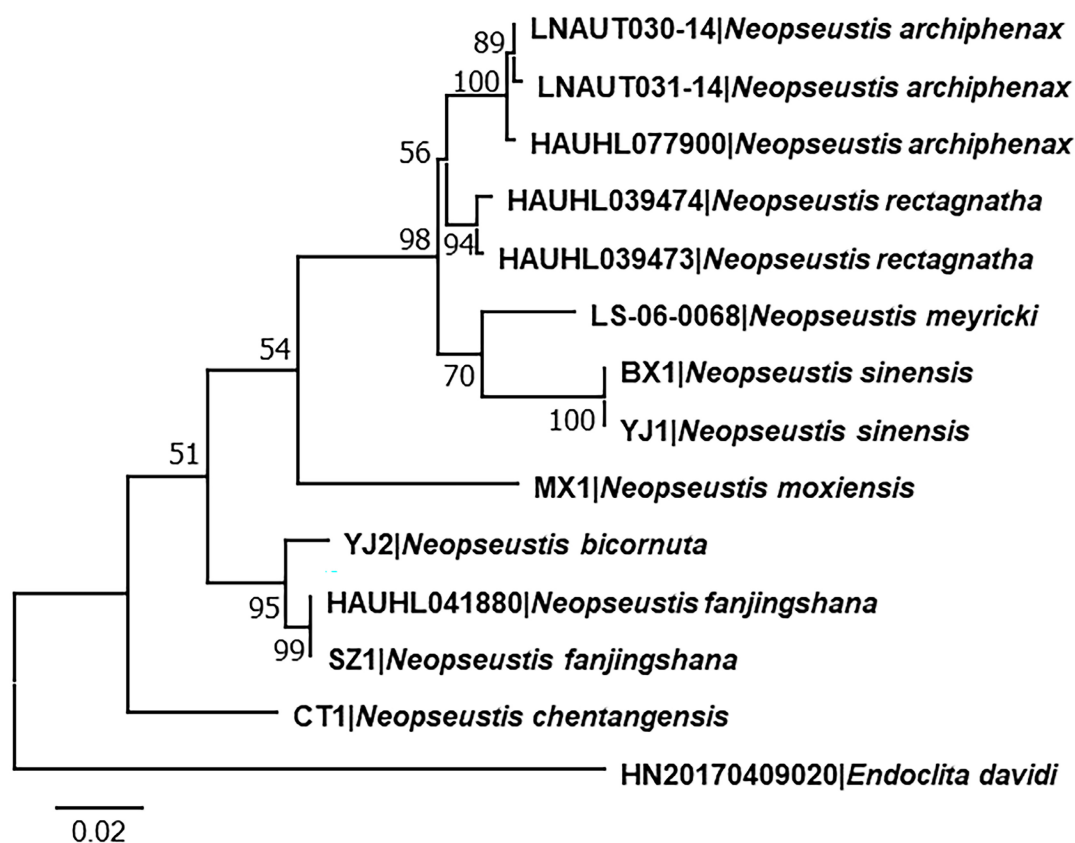


FIGURE 5. Phylogenetic tree of *Neopseustis* species based on *COI* sequences (585 bp) analysis by maximum likelihood method.

Discussion

The female adult of *N. archiphenax* was firstly described, and was recorded from Chongqing for the first time. The female adult morphology and genitalia of *N. archiphenax* were improved in this paper. Davis (1975) considered *Neopseustis* is divided into two groups: one is characterized principally by the well-developed, densely pubescent parameres, and the other one is characterized by the poorly developed and naked parameres. Huang *et al.* (2021) consider that this genus may actually comprise of at least four groups after utilizing more data from previously unsampled taxa. However, in the present study, *N. archiphenax*, *N. rectagnatha*, *N. meyricki* and *N. sinensis* belong to one group is suggested. These species share the following characteristics: i) the valvae have a slender, bilateral pair of clavate lobes ventrally; ii) the parameres are greatly lengthened, weakly sclerotized, densely pubescent over ventral half; iii) the lateroposterior process of anellus is forked with two branches, and iv) the gnathos is slender and sharp, thinner than other species of *Neopseustis*. In addition, according to the female genital structure of *N. archiphenax*, *N. rectagnatha* and *N. meyricki*, it is speculated that the semicircular dilated ductus bursae may also be a feature common to these species, but confirmation of this will require the discovery of more female individuals of *Neopseustis*.

In previous studies, *N. archiphenax* has been described as wing expanse 26–27 mm or exceeding 25 mm, which was also used to the key to species of *Neopseustis*. However, the three specimens collected this time suggest that this description may be problematic, at least in Chongqing's population of *N. archiphenax*, wing expanse exceeding 25 mm may not be a common feature. The three adults from Yintiaoling, two males and one female are much smaller, and no individual has a wingspan of more than 25 mm. So a new key to males of all known species of *Neopseustis* Meyrick, 1909 independent of wing expanse is constructed. Because of the scarcity of specimens, it is uncertain whether there are significant differences in body size between the *N. archiphenax* populations in central China and those in eastern Myanmar.

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TABLE S1. The Kimura-2-parameter distance on *COI* sequences between different taxon of the genus *Neopseustis* sampled for the current study.