A new species in the genus *Cirrhochrista* (Lepidoptera: Crambidae: Spilomelinae) from China

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Abstract: A new species, *Cirrhochrista tridentalis* **sp. nov.** from Hainan, China is described. This new species resembles *Cirrhochrista spinuella* Chen, Song & Wu, 2006 in morphology, yet there are differences in the characteristics of the hindwing and male genitalia. Neighbour-joining analysis based on *COI* gene sequences also validates *Cirrhochrista tridentalis* **sp. nov.** as a monophyletic lineage. The diagnostic features and illustrations of adults and male genitalia are provided.

Key words: Margaroniini; *COI* gene; taxonomy

中国黄缘野螟属 *Cirrhochrista* 一新种记述 (鳞翅目：草螟科：斑野螟亚科)

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摘要: 记述采自中国海南的黄缘野螟属 *Cirrhochrista* 1 新种：三棘黄缘野螟 *Cirrhochrista tridentalis* **sp. nov.**。该新种的外形特征与单棘黄缘野螟 *Cirrhochrista spinuella* 相似，但在后翅和雄性外生殖器特征上存在差异。基于 *COI* 基因序列采用邻接法建树，其结果也证实该新种为一个单系分支。文中还提供了新种的形态描述和特征插图。

关键词: 珍翅斑野螟族；*COI* 基因；分类

Introduction

The genus *Cirrhochrista* was established by Lederer with *Cirrhochrista aetherialis* Lederer, 1863 as its type species, but Ragonot (1891) subsequently designated *Margaronia brizoalis* Walker, 1859 as the type species and recognized *aetherialis* as a junior synonym of *brizoalis*. Warren (1892) reconsidered *C. aetherialis* as the type species (Warren 1892; Chen *et al.* 2006; Nuss *et al.* 2003−2023). Over the past decades, this genus has been placed into diverse subfamilies. It was assigned to the subfamily Schoenobiinae in many previous studies (Hampson 1895; Shibuya 1928; Marumo 1934; Chen *et al.* 2006). But Viette (1961) and Inoue (1982) later classified it to the subfamily Pyraustinae. Shaffer & Munroe (2007) placed it in the subfamily Spilomelinae. The affiliation of the genus *Cirrhochrista* to the tribe Margaroniini Swinhoe & Cotes, 1889 in Spilomelinae was confirmed based on the

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morphological investigation (Mally *et al*. 2019).

The genus *Cirrhochrista* is an enigmatic group of Crambidae and distributed in tropical and subtropical areas. The larvae are oligophagous and significant pests in forestry. They only feed on *Ficus* spp. (Moraceae) (Sugiura & Yamazaki 2004; Novotny *et al*. 2005). There are currently 38 described species. The vast majority occur in the Old-World tropics (Shaffer *et al*. 1996; Mathew 2006; Chen *et al*. 2006; Irungbam *et al*. 2016; Ko *et al*. 2020; Nuss *et al*. 2003–2023). *Cirrhochrista* spp. have a discontinuous distributional pattern with two centers in the world: the Oriental Region and circumjacent areas and Africa and adjoining islands. The Oriental Region and its surrounding areas (Southeast Asia; the Malay Islands; Oceania; Australia) contain the most diverse species (Nuss *et al*. 2003–2023). Chen *et al*. (2006) reported nine species in China and classified these species into three species-groups based on the morphology of female external genitalia. In this study, the classification of this genus from Hainan Island was investigated based on a morphological and DNA barcoding analysis, revealing a new species to science. Diagnostic features and illustrations of this new species are supplied.

Material and methods

Light traps were utilized to collect the specimens of the genus *Cirrhochrista* from Hainan, China. The length of forewing and hindwing are defined as the distance from the base to its apex through the cell. Orange G in lactic acid was used to stain genital preparations. The slide-mounted genitalia were photographed using a Nikon AZ100 Multi-Zoom microscope (Yang *et al*. 2012). The adult dorsal view and the lateral view of the head were obtained with a Leica M205A stereomicroscope, and the lateral view of the head was created by stacking software using Combine Z. The Adobe Photoshop CC 2021 software was used to process all of the images. The terminology follows Hampson (1895). The genital terminology follows Lewvanich (1981). The type specimen is deposited in the Entomological Museum, Northwest A&F University (NWAFU) in Yangling, Shaanxi, China.

Before removing the genitalia for dissection, DNA was extracted from the entire abdomen (Knölke *et al*. 2005). The 658 bp barcode region of the mitochondrial *COI* gene (Hebert *et al*. 2003) was amplified using the LepF1/LepR1 primers (Hebert *et al.* 2004). In general, 2.5 mm $MgCl_2$, 50 μm dNTPs, 1.25 pm of each primer, 50 mm KCl, 10 mm Tris-HCl (pH 8.3), 10–20 ng (1–2 μL) of genomic DNA and 0.3 U of Taq DNA polymerase (Platinum Taq DNA polymerase; Invitrogen, Burlington, Ontario, Canada) were included in the 12.5-μL PCR reaction mixtures. The reaction cycle was as follows: 94°C for 1 min of initial degeneration, followed by 5 cycles of 40 s at 94℃, 40 s at 45℃ and 1 min at 72℃, followed by 35 cycles of 40 s at 94℃, 40 s at 51℃ and 1 min at 72℃, with a final extension of 5 min at 72℃. The PCR products were examined using a 2% agarose E-Gel 96-well system (Invitrogen). The sequences were generated on an ABI 3730xl DNA Analyzer (Applied Biosystems) (Hajibabaei *et al.* 2005).

A total of 19 *COI* sequences were utilized in the molecular analysis. The sequences used in this work are available in BOLD (Table 1). CLUSTAL W was used to align the sequences, and the Kimura 2-parameter (K2P) algorithm in MEGA v.11.0 was used to determine genetic distances within and between lineages. Bootstrap values were calculated with 1000 replicates

(Kimura 1980; Tamura *et al*. 2011), and a distance-based neighbour-joining (NJ) tree was constructed in MEGA v.11.0. We selected 2 species of the genus *Obtusipalpis*, viz. *O. pardalis* and *O. brunneata*, as the outgroups (Shaffer & Munroe 2007).

Table 1. Specimen information for 19 DNA barcoding sequences in this study

Species	Country	Sample ID	Length of sequence (bp)	Barcode Index Number (BIN)
C. brizoalis	Australia	BIOUG01407-G01	658	BOLD: AAD0191
C. kosemponialis	China	Pyr001731	658	BOLD: ACE8369
C. oxylalis	Seychelles	USNM ENT 01067589	658	BOLD: ABV9107
C. convoluta	Kenya	USNM ENT 00719747	658	BOLD: ABU6504
C. xanthographis	Papua New Guinea	USNM ENT 00733427	658	BOLD: ACF2296
C. metisalis	Madagascar	USNM ENT 01068308	658	BOLD: ACY3107
C. annulifera	Pakistan	NIBGE MOT-03363	658	BOLD: AAG0612
C. caconalis	Australia	10ANIC-10765	658	BOLD: ACE8368
C. arcusalis	Australia	10ANIC-10764	658	BOLD:AAC8421
C. aetherialis	Australia	10ANIC-10758	658	BOLD: AAI4720
C. punctulata	Australia	10ANIC-10751	658	BOLD: AAJ1073
C. fumipalpis	China	ARB00029232	658	BOLD: ABV8863
C. fuscusa	China	ARB00023959	658	BOLD: ABU9386
C. tridentalis **sp. nov.**	China	Pyr001730	658	BOLD: AAL8540
C. bracteolalis	China	IOZ_LEP_M_2808	658	BOLD: AAQ2048
C. spissalis	Philippines	CCDB-32975-F10	658	BOLD: ADF0673
C. grabczewskyi	South Africa	BIOUG02073-G09	658	BOLD: ABV9107
Obtusipalpis pardalis	Angola	MK459723	658	BOLD: AAZ8289
Obtusipalpis brunneata	Madagascar	KLM Lep 02259	658	BOLD: ACS5664

Taxonomy

***Cirrhochrista tridentalis* Li & Yang sp. nov.** (Figs 1, 2)

Description (Fig. 1). Head covered with white scales, antenna filiform. Frons white, no anteriad-projection; chaetosema and ocellus absent, compound eye round; labial palpus fuscous on the inside and ocherous on the outside, with white tufts at the base and length approximately twice the diameter of the compound eye; the 3rd labial palpomere porrect; proboscis obsolete. Thorax is covered with white tufts. Forewing white, the length is about 13.0 mm, with a cuspidate apex; the costal margin is brown with a fuscous basal patch; the antemedial fascia is a yellow, triangular patch, and the median region has an orange-yellow, triangular patch at costa, all with a brown edge. A fuscous triangular patch across apex area. Outer margin oblique, edged with brown lines; terminal fascia brown, irregularly wavy, with yellow stripe between them; fringe ocherous; inner margin straight. Hindwing white, about 10 mm long, with a rounded,

nearly vertical apex; outer margin oblique, with four continuous yellow but fuscous-edged triangular patches, and the tornus is broadly rounded. Thoracic leg is white, with four apical spurs on the hind tibia: a proximal and a distal pair.

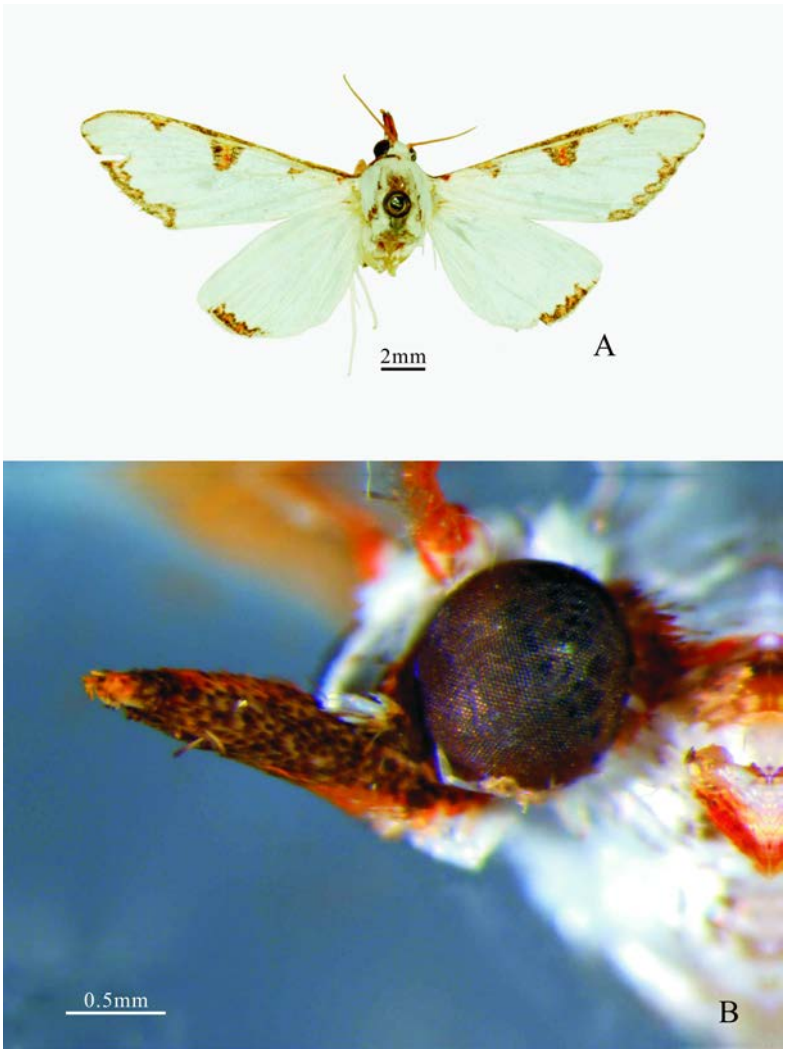

Figure 1. *Cirrhochrista tridentalis* **sp. nov.**, adult. A. ♂, Holotype, Hainan, Jianfengling; B. Head, lateral view.

Male genitalia (Fig. 2). Uncus slender, rhabdoid, swollen and rounded at apex, with bushy and elongated setae; tegumen triangular, the region between subscaphium and dorsal tegumen membranous, gnathos absent; valva oval, weakly sclerotized; sacculus and costa sclerotized, with spare setae; two elongate spine-like fibulas originate from the middle region of costa, directed towards the distal costa; juxta almost rectangular, vinculum and saccus U-shaped. Phallus is cylindrical and equal in length to the valva; vesica with a tridental cornutus possessing three serrated spines at the apex.

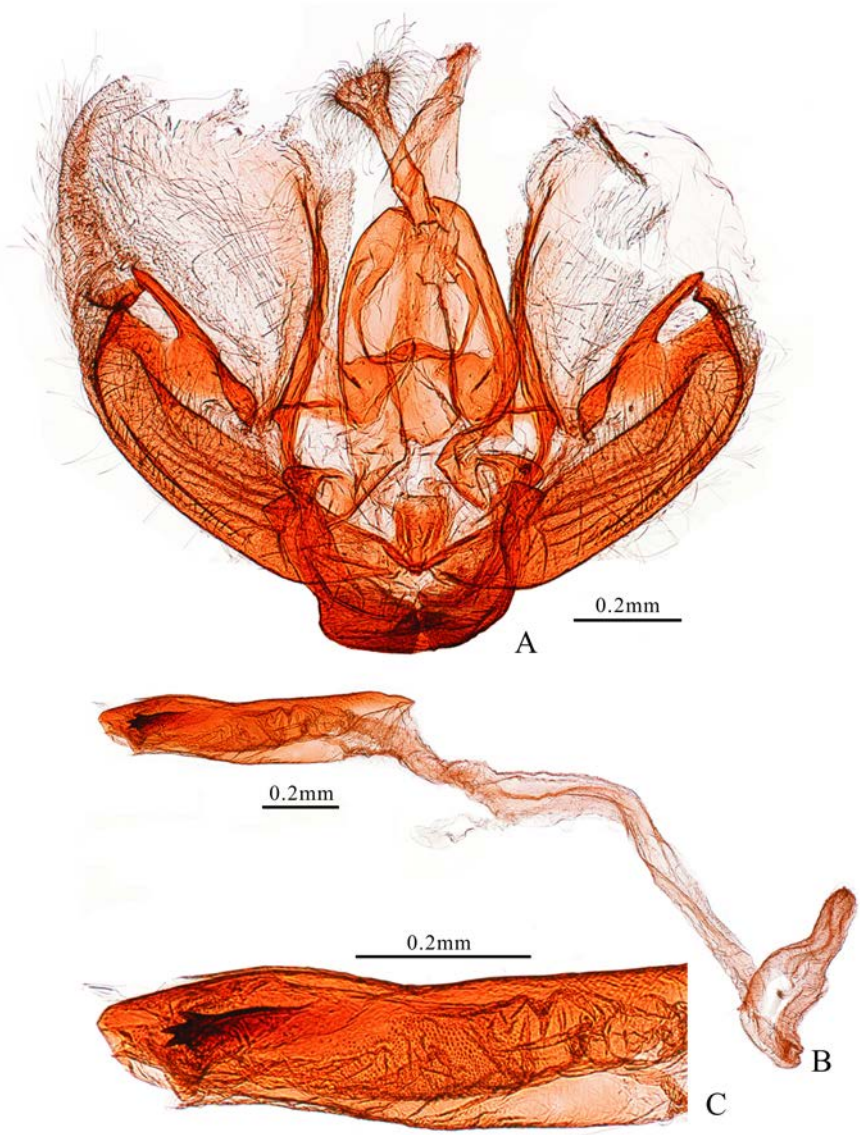

Figure 2. *Cirrhochrista tridentalis* **sp. nov.** A. Male genitalia; B. Phallus; C. Distal portion of phallus (genitalia slide: LEPyr001730).

Holotype. ♂, **China**, Hainan, Jianfengling, 18°42'25″N, 108°49'48″E, 980 m, 06-V-2008, Qiulei MEN [white, printed]; BOLD Sample ID and Process ID: Pyr001730, CNPYB069-10 [yellow, printed]; Genitalia slide number: LEPyr001730. 'HOLOTYPE| *Cirrhochrista tridentalis*| Yang 2010| by Yang 2010' [red, printed]. Institution storing specimen: Entomological Museum, Northwest A&F University, Yangling, Shaanxi, China.

Etymology. The specific epithet indicates the trifurcate cornutus of male genitalia.

Diagnosis. This new species resembles *C. spinuella* Chen, Song & Wu, 2006 in

morphology. But there are several differences in the characteristics of the hindwing and male genitalia: the outer margin of hindwing of *C. tridentalis* **sp. nov.** has four continuous yellow and fuscous-edged triangular patches, whereas *C. spinuella* merely has a fuscous stripe. In addition, the cornutus of this new species has three serrated spines at the apex, while there is a long spine-shaped cornutus in *C. spinuella*.

Sequence analysis. For molecular analysis, 19 *COI* gene sequences were assembled, including two *Obtusipalpis* spp. and 17 *Cirrhochrista* species. In NJ analysis, the new species formed a well-supported monophyletic lineage and was significantly divergent from other congeneric species (Fig. 3). The interspecific genetic distance between *C. tridentalis* **sp. nov.** and remaining *Cirrhochrista* spp. ranged from 5.56% to 12.02%, with a high support value. These results support the classification of *C. tridentalis* as a new species.

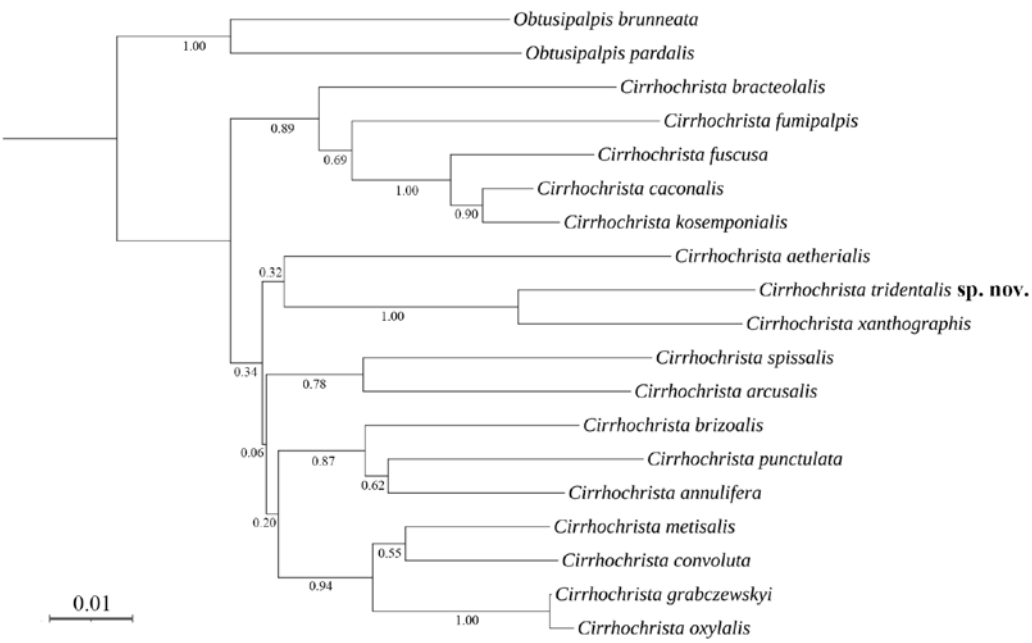

Figure 3. Neighbour-joining (NJ) tree of 19 species based on 19 *COI* sequences.

Discussion

In this study, we describe a new species of *Cirrhochrista* based on morphological and molecular analyses. The main characteristics of *C. tridentalis* **sp. nov.** are the outer margin of the hindwing with four continuous yellow fuscous-edged triangular patches, and the cornutus of the male genitalia with three serrated spines at the apex. These morphological features indicate that this new species is considerably different from other congeneric species. Similarly, the molecular analyses support *C. tridentalis* **sp. nov.** as separate from other species with *COI* divergence, which is consistent with Hebert *et al*. (2003) that found interspecific *COI* sequence differences are generally greater than 3.0% in Lepidoptera.

Since the 19th century, there have been numerous studies of this genus, but the majority

are described primarily based on their external characteristics. However, taxonomic revisions and molecular phylogenetic analysis of this genus are still insufficient (Yoshiyasu 1975; Novotny *et al*. 2005; Chen *et al*. 2006; Shaffer & Munroe 2007; Ko *et al*. 2020). Species identification still remains difficult and the status of many taxa remains uncertain. Therefore, more investigation of this genus worldwide is needed, including their biology and their host plant associations.

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