A new species in the genus *Synanthedon* (Lepidoptera: Sesiidae) from China

Yufeng ZHENG^{1, 2*}, Jinyu ZHAN^{1, 2*}, Zhaofu YANG^{1, 2}^①

- 1. Key Laboratory of Plant Protection Resources and Pest Management, Ministry of Education, Northwest A&F University, Yangling, Shaanxi 712100, China
- 2. Entomological Museum, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China

*These authors contributed equally to this work.

Abstract: A new species, *Synanthedon elaeagnus* **sp. nov.** feeding on *Elaeagnus mollis*, is described from Shanxi, China. This new species is similar to *Synanthedon ulmicola* Yang & Wang, 1989 in general morphology, but it differs from this latter species by features of the forewing, female and male genitalia. Neighbor-joining and maximum likelihood analysis both recover *Synanthedon elaeagnus* **sp. nov.** as a well-supported monophyletic lineage with a high support value based on *COI* gene sequences. The key features of this new species are illustrated.

Key words: Synanthedonini; COI gene; taxonomy; Elaeagnus mollis

中国兴透翅蛾属 Synanthedon 一新种记述 (鳞翅目: 透翅蛾科)

郑御丰^{1,2*}, 詹金钰^{1,2*}, 杨兆富^{1,20}

1. 西北农林科技大学植物保护资源利用与害虫防治重点实验室,陕西 杨凌 712100; 2. 西北农林科技 大学植物保护学院昆虫博物馆,陕西 杨凌 712100

* 同等贡献作者

摘要:记述危害翅果油树的中国兴透翅蛾属 Synanthedon 1 新种: 翅果兴透翅蛾 Synanthedon elaeagnus sp. nov.。其外形与榆兴透翅蛾 Synanthedon ulmicola Yang & Wang, 1989 相似,但前翅、雌性和雄性生殖器区别于后者。此外,基于 COI 基因序列及采用邻接树和最大似然法建树分析结果均强烈支持该新种是一个区别于其他种的单系分支。提供了新种的鉴别特征图。

关键词:兴透翅蛾族; COI 基因;分类;翅果油树

Introduction

Synanthedon Hübner, 1819 is the largest genus in the tribe Synanthedonini of Sesiidae, with 298 species described worldwide and 27 species recorded in China (Pühringer & Kallies 2017; Jin *et al.* 2008).

The most diverse fauna of this group is found in the Palearctic, Oriental, Ethiopian and Neotropical Regions (Agassiz & Kallies 2018; Liang & Hsu 2015). However, the worldwide

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① Corresponding author, E-mail: yangzhaofu@nwsuaf.edu.cn

fauna of *Synanthedon* remains still unstudied with many more species in need of verification and likely new species awaiting discovery (Heppner & Duckworth 1981). The larvae have been less studied. Known species are typically wood-boring pests which create destructive tunnels in the stem and branches of fruit and forestry trees (Xu *et al.* 2019).

During research into the lepidopteran species diversity of *Elaeagnus mollis* (Elaeagnaceae) plantations in Shanxi, a number of specimens in the genus *Synanthedon* were identified as *Synanthedon ulmicola* Yang & Wang, 1989 (Zhan *et al.* 2021). However, several specimens sampled in these same localities along with *S. ulmicola* were undetermined due to characteristics in general appearance. We combined *COI* gene DNA barcoding analyses with a detailed morphological study of wing pattern and genital features of these specimens, revealing a new species to science. Diagnostic characters and the biology of *S. elaeagnus* **sp. nov.** are provided.

Material and methods

Adult specimens were collected from the plantations of *Elaeagnus mollis* in Xiangning, Shanxi, China.

The forewing span is defined as the distance between the left and right forewing apices (Hook *et al.* 2012). Genitalia slides were prepared following procedures of Yang *et al.* (2012). Terminology of genitalia follows Kristensen (2003). The wing pattern and venation are described following Špatenka *et al.* (1999). Type specimens are deposited in the Entomological Museum, Northwest A&F University (NWAFU), Yangling, Shaanxi, China.

We generated the 658 bp barcode region of mitochondrial *COI* sequences from a single leg using the DNAeasy DNA Extraction kit following the manufacturer's protocols (Zhou *et al.* 2020; Volponi Skowron & Volponi 2018). The fragment of the *COI* gene was amplified using the primers LepF1 (forward) 5'- ATTCAACCAATCATAAAGATATTGG-3' and LepR1 (reverse) 5'-TAAACTTCTGGATGTCCAAAAAATCA-3' (Hebert *et al.* 2004). PCR reactions were performed in a total volume of 25 μ l using 2 μ l of DNA extract, 1 μ l each of forward and reverse primer, 12.5 μ l Green-Mix, and 8.5 μ l ddH₂O. The reaction cycle consisted of pre-degeneration at 94°C for 5 min, denaturation at 94°C for 0.5 min, annealing at 51°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 7 min, with 40 cycles. PCR products were separated by electrophoresis in 1.0% agarose gel, and samples showing a correct-sized band were sequenced in both directions by AuGCT Biotech (Beijing, China).

A dataset of 29 *COI* sequences was used to perform the molecular analyses. We included four sequences of *S. elaeagnus* **sp. nov.**, four sequences generated for *S. ulmicola*, and 20 sequences for 20 *Synanthedon* spp. downloaded from GenBank as ingroups. *Bombyx mori* (Linnaeus, 1758) was selected as the primary out-group to build the tree (Table 1). Phylogenetic analyses were conducted through maximum likelihood (ML) and neighbor-joining (NJ) methods using PhyloSuite and Mega X. ML analysis was created with 1000 ultrafast bootstrap replicates in PhyloSuite (Minh *et al.* 2013). NJ analysis was carried out using the Kimura 2-parameter (K2P) molecular evolutionary model with 1000 bootstrap replications in MEGA X (Kumar *et al.* 2018).

Species	Country	Sample ID	Length of sequence (bp)	GenBank Accession Number
S. elaeagnus sp. nov.	China	XA18051500086(A1)	658	OM316815
S. elaeagnus sp. nov.	China	XA18051500086(A2)	658	OM316816
S. elaeagnus sp. nov.	China	XA18051500086(A3)	658	OM316817
S. elaeagnus sp. nov.	China	XA18051500086(A4)	658	OM316818
S. ulmicola	China	C1803210053/1	658	OM316811
S. ulmicola	China	C1803210053/2	658	OM316812
S. ulmicola	China	C1803210053/3	658	OM316813
S. ulmicola	China	C1803210053/4	658	OM316814
S. acerni	USA	TAMUICEGR-0226	658	HQ977575
S. acerrubri	USA	CCDB-04613 F09	658	JF848555
S. bibionipennis	Canada	UASM99827	658	HM902929
S. culiciformis	Finland	MM00513	658	HM871045
S. decipiens	USA	TAMUICEGR-0230	658	HQ977579
S. exitiosa	USA	TAMUICEGR-0233	658	HQ977582
S. flaviventris	Finland	MM18837	658	JF854645
S. formicaeformis	Finland	MM08402	658	HM873903
S. fulvipes	USA	CCDB-04613 G06	658	JF848560
S. kathyae	USA	TAMUICEGR-0235	658	HQ977584
S. myopaeformis	Canada	jflandry2571	658	MH592898
S. novaroensis	Canada	BOX-2219 A04	658	GU661886
S. polaris	Finland	MM22834	658	MZ610629
S. rubrofascia	USA	TAMUICEGR-0240	658	HQ977587
S. sapygaeformis	USA	TAMUICEGR-0241	658	HQ977588
S. scitula	USA	TAMUICEGR-0242	658	HQ977589
S. scoliaeformis	Finland	MM08397	658	HM873899
S. soffneri	Finland	MM22770	658	KT782535
S. spheciformis	Finland	MM18790	658	JF854620
S. tipuliformis	Finland	MM24101	658	MZ609921
Bombyx mori	China	AS43MT04	658	JF700138

Table 1. Specimen information for 29 DNA barcoded sequences in this study

Taxonomy

Synanthedon elaeagnus Zheng & Yang sp. nov. (Figs 1, 2)

Description (Fig. 1). Antenna length about 7.5 mm (n = 2); wing expanse about 22.0 mm (n = 2). Head. Frons purple black; labial palpus black, somewhat greyish black ventrally; vertex black with purplish sheen; pericephalic scales black with a few black-purple scales

dorsally; antenna filiform, black with blue-violet sheen, a tuft of black setae at tip of club. Thorax. Patagium black with violet sheen; tegula black, bronzed-blue sheen with a yellow dorsal line; mesonotum black with blue-violet sheen; metathorax black; thorax laterally yellow with a few brown scales. Forewing: well-developed transparent area covered with dark brown scales, basally black; costal margin dark brown to black; discal spot and veins within exterior transparent area dark brown to black; apical angle dark brown with admixture of black scales; discal spot broad; anterior transparent area divided into three pellucidum areas and exterior transparent area large, divided into seven pellucidum areas; forewing media vein present; R_3 and R_4 segregated basally, R_4+R_5 bifurcated in fenestrated plaques, but inter-forked with dark-brown scales; M_1 extended to middle opaque area, Cu_1 and Cu_2 originate at common point; posterior transparent area reaching discal spot; cilia black. Hindwing: transparent basally; outer margin, inner margin, and veins are dark brown to black; base of the wing with long gray hairs; A1 well-developed; vein M2 originates from the middle and upper segment of cross-vein; M_3 and Cu_1 bifurcated in the middle, distance less than M_2 ; cilia dark brown. Legs. Fore coxa, femur, tibia and tarsus black, with violet sheen; mid coxa and femur black, with violet sheen; mid tibia black with two spurs, spurs black brown and varying in length; mid-tarsus dark black, with admixture of short scales distally; hind leg similar to mid leg, hind tibia and tarsus much longer than abdomen; all legs with black-purple sheen in sunlight. Abdomen. Smooth-scaled; dark brown to black with bluish-purple sheen; seven abdominal segments, narrow yellow bands on distal margin of the 2nd, 4th and 6th tergite and the yellow stripe at the posterior margin of tergite; the length of the 4th is the sum of tergites 2nd and 6th; abdomen ends with a black tuft of scaly hair, the tuft of the female is a "V"-shaped oblique vertical on the posterior and the male's tuft is flat on both sides; anal tuft small, black.

Male genitalia (Figs 2A–D). Tegumen-uncus complex broad; socii well-developed with scopula androconialis; valva elongated, somewhat trapeziform, slightly concave and slightly tapered; crista sacculi well-developed, oblique arising from the middle saccular margin to the inner part of the valva, densely dark-scaled; saccus rounded basally, long, moderately sclerotized at the tip; phallus stout at the base, shorter than valva; vesica with two cornuti, one is curved and fork-shaped, the other is spiculate.

Female genitalia (Fig. 2E). 8th tergite large and broad, with a few setae at distal margin; posterior apophysis about $1.3 \times$ length of anterior apophysis; ostium bursae opening near anterior margin of the 8th sternite; antrum broad, well-sclerotized; ductus bursae as long as posterior apophysis, membranous; corpus bursae ovoid, with many microfolds over the surface, without signum.

Holotype. \Im , China, Shanxi, Xiangning County, $35^{\circ}41'30''N-36^{\circ}09'06''N$, $110^{\circ}30'20''E-111^{\circ}17'13''E$, 04-V-2019, Fangyuan ZHANG. **Paratypes**. $1 \text{ } 21 \Im$, China, same data as holotype.

Etymology. The specific epithet is named after its host plant Elaeagnus mollis.

Remarks. *S. elaeagnus* is similar to *S. ulmicola* Yang & Wang, 1989 in markings of body, but it can be distinguished by the following genital and wing characters: the base of saccus is broad in *S. ulmicola*, but evenly thin, long and moderately sclerotized at the tip in *S. elaeagnus*; phallus is slightly shorter than valva in *S. ulmicola* and lacking cornuti, but in *S. elaeagnus* phallus is much shorter than valva with two cornuti (fork-shaped and speculate-like,

respectively); the length of anterior apophysis is equal to the posterior apophysis in *S. ulmicola*, but posterior apophysis is about $1.3 \times$ length of anterior apophysis in *S. elaeagnus*; corpus bursae long ovate and relatively small in *S. ulmicola*, but ovoid and large correspondingly in *S. elaeagnus*; forewing media vein vestigial in *S. ulmicola*, but present in *S. elaeagnus* (Fig. 1E).



Figure 1. Synanthedon elaeagnus **sp. nov.**, adults. A. \mathcal{O} , Holotype, Shanxi: Xiangning; B. \mathcal{Q} , Paratype, Shanxi: Xiangning; C. Head, ventral view; D. Head, lateral view; E. Wing venation.

Biology. The larva bores into burls within 20.0 cm above the ground in the phloem layer on the trunk of *Elaeagnus mollis* (Elaeagnaceae) (Fig. 3) that is an endemic woody plant and oil species in China. It feeds on damaged tissue around the trunk, which is then covered with mucus and frass.

Sequence analysis. A total of 29 *COI* gene sequences including *Bombyx mori* and 22 species within *Synanthedon* were assembled for molecular analyses. The average length is 658 bp. In both ML and NJ analysis, the four sequences generated for new species formed a well-supported monophyletic lineage and were significantly divergent from those for other congeneric species (Fig. 4). The interspecific genetic distance among *Synanthedon* spp. ranged from 9.92% to13.11%. The phylogenetic tree shows *S. elaeagnus* **sp. nov.** is close to *S. tipuliformis* and the interspecific genetic distance between them is 10.63%. These results confirm the validity of *S. elaeagnus* as a new species.



Figure 2. Genitalia of *Synanthedon elaeagnus* **sp. nov.** A. Male genitalia; B. Distal portion of saccus in detail (enlarged area marked with a rectangle in a); C. Phallus; D. Distal portion of phallus (enlarged area marked with a rectangle in C); E. Female genitalia.



Figure 3. A, B. Habitat of larvae of Synanthedon elaeagnus sp. nov. and damage to trunk of Elaeagnus mollis.



Figure 4. Maximum-likelihood (ML) tree and neighbor-joining (NJ) tree of 23 species constructed based on 29 *COI* sequences. Support values are ML/NJ.

Discussion

Based on both morphological and molecular evidence, we describe a new species within the genus *Synanthedon*, *S. elaeagnus* **sp. nov.**, occurring with *S. ulmicola* in sympatry in Shanxi, China. The key diagnostic characteristics of this new species are in the external genitalia and wing venation: the base of saccus is thin, long and moderately sclerotized at the tip, phallus with two cornuti; the length of posterior apophysis is longer than anterior apophysis; and the forewing media vein is present. These morphological features clarify that *S. elaeagnus* **sp. nov.** is significantly different from all other congeneric species. Similarity, our molecular analyses support *S. elaeagnus* **sp. nov.** as separate from other species with significant *COI* gene sequence divergence. In addition, this study reveals that *S. elaeagnus* **sp. nov.** and *S. ulmicola* are two major lepidopteran pests damaging *Elaeagnus mollis* (Elaeagnaceae) in Shanxi, China. *Elaeagnus* has rarely been reported as a larval host plant for *Synanthedon* spp. in China aside from *S. unocingulata* Bartel, 1912 (Špatenka *et al.* 1993, 1999; Arita *et al.* 2004). However, more taxa sampling and nuclear gene markers are necessary to illuminate the relationship between these two species and further detail the phylogeny of *Synanthedon*.

The members of the family Sesiidae are well-known for their striking mimicry of various Hymenoptera. However, field collection of clearwing moths is very difficult due to their enigmatic behavior both in larval and adult stages. Over the past few decades, some species of sesiids have been investigated and recorded by using sex pheromones (Duckworth & Eichlin 1977). However, the mimicry and behavior of *Synanthedon* species remain largely unexplored. More investigation of the range of host plants and the biology of *S. elaeagnus* **sp. nov.** and its sibling species is needed in future field work.

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