

# Two new species of *Oobius* (Hymenoptera: Encyrtidae) and their phylogenetic relationship with other congeners from northeastern Asia

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**Abstract**—Two new species of egg parasitoids, *Oobius saimaensis* Yao and Mottern **new species** and *Oobius fleischeri* Yao and Duan **new species** (Hymenoptera: Encyrtidae), are described from eggs of *Agrilus fleischeri* Obenberger, 1925 (Coleoptera: Buprestidae). *Agrilus fleischeri* is a phloem-feeding woodborer of poplar (*Populus* Linnaeus; Salicaceae) in northeastern China. These two species can be distinguished morphologically as *O. fleischeri* has five tarsomeres and *O. saimaensis* has four tarsomeres. Although *O. saimaensis* is morphologically similar to its sympatric congener *O. agrili* Zhang and Hang, 2005, an important natural enemy of the invasive emerald ash borer, *Agrilus planipennis* Fairmaire, 1888, molecular phylogenetics and morphological data indicate that they are distinct species. Phylogenetic relationships among the new species and other closely related species are also inferred by using DNA sequence data from several ribosomal and mitochondrial genes. In addition, we expand the known distribution of *Oobius primorskyensis* Yao and Duan, 2016 to include South Korea.

## Introduction

The genus *Oobius* Trjapitzin, 1963 (Hymenoptera: Encyrtidae) was not well studied until recently. Before 2010, only nine species were described (Trjapitzin and Volkovitsh 2011), with six species from the Palearctic Region (Nowicki 1928; Trjapitzin 1963; Myartseva 1979; Guerrieri *et al.* 1989; Zhang *et al.* 2005), and three species from the Afrotropical Region (Annecke 1967). Subsequently, Noyes (2010) synonymised *Avetianella* Trjapitzin, 1968, *Szelenyiola* Trjapitzin, 1977, and *Oophagus* Liao, 1987 under *Oobius* and described 20 new species of *Oobius* from the Neotropical Region

(Costa Rica). More recently, two new *Oobius* species were described from the Nearctic Region (United States of America) (Triapitsyn *et al.* 2015) and one from the Palearctic Region (Russia) (Yao *et al.* 2016).

Most species of *Oobius* are solitary egg parasitoids of wood-boring Coleoptera in the families Buprestidae and Cerambycidae (Zhang *et al.* 2005; Triapitsyn *et al.* 2015). Among those, *O. longoi* (Siscaro, 1992) was introduced from Australia to the United States of America (California) in the late 1990s, where it successfully established as a biocontrol agent of the invasive eucalyptus longhorned beetle, *Phoracantha semipunctata*

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(Fabricius, 1775) (Coleoptera: Cerambycidae), which feeds on ornamental *Eucalyptus* L'Héritier (Myrtaceae) trees (Hanks *et al.* 1996; Paine and Millar 2002). Also, *O. agrili* Zhang and Huang, 2005 was introduced from China to the United States of America (Michigan) in 2007 for biocontrol of the invasive emerald ash borer, *Agrilus planipennis* Fairmaire, 1888 (Coleoptera: Buprestidae), which feeds on ash trees (*Fraxinus* Linnaeus; Oleaceae) and has become a devastating ash tree pest in North America (Federal Register 2007; Liu *et al.* 2007; Duan *et al.* 2012, 2014; Abell *et al.* 2014; Bauer *et al.* 2015). Most recently, *O. primorskyensis* Yao and Duan, 2016 from the Russian Far East, is being evaluated for possible use in biocontrol of the emerald ash borer in North America (Larson and Duan 2016; Yao *et al.* 2016).

Research in northeast China on *Agrilus fleischeri* Obenberger, 1925 (Coleoptera: Buprestidae), an emerging wood-boring pest of *Populus* Linnaeus (Salicaceae), led to the discovery of several species of insect natural enemies. These included two new species of *Oobius* that parasitise eggs of *A. fleischeri*, with combined egg parasitism rates on poplar trees ranging from 7% to 48% (Zang *et al.* 2016). These two species are described here as *O. saimaensis* Yao and Mottern, **new species** and *O. fleischeri* Yao and Duan, **new species**. Herein, we describe the morphological, biological, and molecular differences between these two species, and contrast them to the morphologically similar congeners *O. agrili* (sympatric) and *O. primorskyensis* (allopatric), both of which parasitise emerald ash borer eggs on ash trees. In addition, we sequenced and analysed several ribosomal and mitochondrial genes to infer their phylogenetic relationships.

## Materials and methods

### Sample collection and rearing

Parasitised *A. fleischeri* eggs, which are darker in colour than healthy eggs, were first collected from European black poplar trees (*Populus nigra* Linnaeus; Salicaceae) infested with *A. fleischeri* on 18 September 2013 in Saima Village, Liaoning Province, China (40°57.473'N, 124°15.788'E). Additional parasitised *A. fleischeri* eggs were collected from other infested exotic poplar trees *Populus nigra* var. *italica* Münchhausen

(Salicaceae) and native poplar trees *P. davidiana* Dode in Saima Village and nearby Nanmiao Village, Liaoning Province, China (40°54.658'N, 124°16.305'E) from May to September 2014, and 2–5 May 2016. The parasitised host eggs were placed in 1.5 × 7.5 cm glass vials and reared at the room temperature in the Key Laboratory of Forest Protection, China State Forestry Administration, at the Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry (CAF), Beijing, China. Newly emerged adult egg parasitoids were either killed directly in 100% ethanol for molecular studies or killed with carbon dioxide and critical point dried for card or slide-mounting. Parasitoid wasps that emerged from the *A. fleischeri* eggs collected in 2013 were also reared individually on *A. planipennis* eggs freshly laid on coffee filter paper (Larson and Duan 2016) for one generation in the quarantine laboratory at the United States Department of Agriculture, Agricultural Research Service, Beneficial Insects Introduction Research Unit, Newark, Delaware, United States of America. While some laboratory-reared (F<sub>1</sub>) parasitoids were designated as paratypes, most were used for DNA sequencing for molecular phylogenetic analysis at the Laboratories of Analytical Biology (Washington, District of Columbia, United States of America), Smithsonian Institution, National Museum of Natural History (NMNH).

### Taxonomic studies

Selected parasitoid specimens were slide-mounted in Canada balsam (Platner *et al.* 1999) for detailed examination. All specimens were examined under a ZEISS Stemi 508 stereomicroscope (Bio-Research Scientific Cooperation, Agent Company, Beijing, China). Slide-mounted specimens were imaged using a Leica DM6000B microscope with a Leica DFC 300FX high sensitivity digital camera (Leica Microsystems, Wetzlar, Germany) according to the methods described in Yao *et al.* (2016). Some specimens were also examined in ethanol to determine the number of multiporous plate sensillae on the antennae. Images of card-mounted specimens were generated using Helicon Focus software (Helicon Soft, Kharkiv, Ukraine) after generating an image stack with an Olympus CX31 microscope (Olympus, Tokyo, Japan) fitted with a

Canon EOS 700D digital camera. Scanning electron microscope images were produced with a Hitachi/S4800 desktop unit (Hitachi, Tokyo, Japan). Some specimens were manually cleaned of external debris with forceps or brushes and affixed to 12.7 × 3.2 mm Leica/Cambridge aluminum scanning electron microscope stubs with carbon adhesive tabs (Electron Microscopy Sciences, #77825-12). Stub-mounted specimens were imaged after sputter coating using a Cressington Scientific 108 Auto with gold from all angles to ensure complete coverage (~10–20 nm coating). Morphological characters and measurements were obtained by examination of card-mounted specimens, slide-mounted specimens and scanning electron microscope photos. Morphological terms used in the species description follow Bouček (1988) and Gibson (1997). The holotypes and a portion of the paratypes are deposited in the Insect Collection Unit at the Chinese Academy of Forestry, Beijing, China. Additional paratypes are deposited in the Smithsonian Institution, Washington, District of Columbia, United States of America.

### DNA extraction, amplification, and sequencing

DNA extraction, amplification, and sequencing were performed independently on different sets of specimens at two institutions: the Key Laboratory of Forest Protection at China State Forestry Administration, and the Laboratories of Analytical Biology at NMNH. At the China State Forestry Administration, a 610 base pair fragment of the mitochondrial cytochrome oxidase subunit I (*COI*) gene was amplified and sequenced from one female ( $F_0$ ) and one male ( $F_0$ ) specimen of *O. saimaensis* and one female ( $F_0$ ) *O. fleischeri* (GenBank, [www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank): MF568022–MF568026). In addition, we extracted DNA from two female ( $F_0$ ) *O. agrili*. Genomic DNA was extracted from the whole body of adult wasps using the Tiangen Blood and Animal Tissue Kit (Tiangen Biotech, Beijing, China). A region of COI of mtDNA was amplified using the following primer pair: New Par: 5'-TAAGWTTAATTATTCGRTTAGAATTARG-3' (forward), 5'-TAAACTTCWGGATGACCAAAAAATCA-3' (reverse) (Santos *et al.* 2011). The amplification reactions were done in 0.5 µL of each primer (10 µmol), 12.5 µL 2 × Taq PCR Master Mix

(Tiangen Biotech), 10 µL ddH<sub>2</sub>O and 1.5 µL DNA template for a final volume of 25 µL. The amplification was performed in 0.2 mL tubes in Eppendorf B Mastercycler (Eppendorf, Hamburg, Germany) with the following profile: incubation for five minutes at 94 °C (initial denaturation step), then for 35 cycles consisting of 94 °C for 45 seconds, 46 °C for 45 seconds and 72 °C for one minute and a final extension for five minutes at 72 °C. The sequencing reaction was carried out using ABI BigDye Terminator version 3.1 cycle sequencing kit on an ABI 3730XL (SinoGenoMax, Beijing, China). Sequences were edited and aligned with Staden Package (Staden *et al.* 1999) and DNAMAN version 6 (Lynnon Biosoft, San Ramon, California, United States of America).

At the National Museum of Natural History, Laboratories of Analytical Biology, DNA was nondestructively extracted from one female ( $F_0$ ) and four male ( $F_1$ ) *O. saimaensis* (USNMENT01231522, USNMENT01231524–26) and two male ( $F_1$ ) *O. fleischeri* (USNMENT01231521, USNMENT01231523). In addition to the two new species, we extracted DNA from one female *O. agrili* (USNMENT01119386) one female *O. primorskyensis* from Russia (USNMENT01119387), and one female *O. primorskyensis* from South Korea (new locality record, USNMENT01119503). To serve as outgroups for our molecular analysis, we included one female *O. longoi* (USNMENT01119464) and one female *O. buprestidis* (USNMENT01119332). These outgroups were chosen based upon a larger and more comprehensive molecular phylogeny of *Oobius* currently in developed by the co-authors J.L.M. and M.W.G. (unpublished data). Extraction, polymerase chain reaction amplification, purification, sequencing, and sequence verification/assembly followed the protocols outlined in Liu and Mottern (2017). Amplified gene regions included the following, though not all genes were amplified for all specimens: a 766 base pair fragment of the small subunit 18S rDNA (GenBank: MF573302–MF573309), large subunit 28S rDNA expansion regions *D2–5* (GenBank: MF573310–MF573322), a 1041 base pair fragment of *COI* (GenBank: MF568009–MF568025), and a 254 base pair fragment of cytochrome oxidase subunit II (*COII*) (GenBank MF567996–MF568008). All primer sequences are reported in Liu and Mottern (2017) with the exception of the pair used for *COII*, which were as

follows: 5'-ATTGGACATCAATGATATTGA-3' (forward) (Simon *et al.* 1994) and 5'-CCACAAATTTCTGAACATTGACCA-3' (reverse) (Dowton and Austin 1997).

### Sequence alignment and phylogenetic analysis

Ribosomal DNA sequences were aligned using the E-INS-i algorithm in MAFFT v6 (Kato *et al.* 2009) with default settings. Ribosomal and mitochondrial genes were then concatenated using SequenceMatrix v100.0 (Vaidya *et al.* 2010) for a final alignment length of 3196 base pairs. Maximum likelihood analysis of the concatenated alignment was conducted using RAxML v8.2.10 (Stamatakis 2014) under a GTR+ $\Gamma$  substitution model as implemented through the CIPRES Web Portal (Miller *et al.* 2010) accessed at [www.phylo.org](http://www.phylo.org). The data were analysed with 1000 rapid bootstraps using five gene partitions: *18S*, *28S-D2*, *28S D3-5*, *COI + COII* codon positions 1 and 2, and *COI + COII* codon position 3. The resulting best tree (highest likelihood) was visualised and drawn using FigTree v1.3.1 (Rambaut 2009).

## Results

### Molecular analyses

As the molecular data obtained at the National Museum of Natural History are more comprehensive in terms of gene and taxon sampling, the National Museum of Natural History data were used for phylogenetic reconstruction, and the *COI* sequences from Beijing were used for the barcoding gap analysis. Both laboratories found the same result regarding *COI*: *O. saimaensis* and *O. fleischeri* share identical *COI* sequences across the regions of the gene that we examined, with the exception of a single synonymous transition (C/T) in one *O. saimaensis* specimen. After trimming the low-quality bases from the edge, the data matrix consisted of 610 base pairs for the *COI* genes, and no insertions or deletions were found in the trimmed sequence. The average compositions of the nucleotides A, G, C, and T were 30.9%, 13.0%, 12.5%, and 43.6%, respectively. As expected, the sequences were rich in A + T, which accounted for 74.5% of the nucleotides.

The genetic distance between *O. saimaensis/fleischeri* and *O. agrili* was computed using *COI* haplotype data based on the Kimura two-parameter and p-distance model. A barcode gap was discovered between the maximum intraspecific variation (0.17%) and the minimum interspecific divergence (K2P distance: 7.74%; p-distance: 7.32%). The application of character-based methods provides a combination of diagnostic nucleotides that can be used to distinguish the two new species from *O. agrili*.

The species-specific nucleotide positions of *COI* (pure diagnostic barcode characters) for the two closely related species are listed in Table 1. There are differences in 44 selected nucleotide positions between the two distinct *COI* haplotypes (*O. saimaensis* versus *O. agrili*), and there is a switch between T and C at position 69 for *O. agrili* (Table 1).

A phylogram (Fig. 1) demonstrates the close evolutionary relationship among *O. agrili*, *O. primorskyensis*, *O. saimaensis*, and *O. fleischeri*. The ribosomal genes are invariant among these four species, though there are likely multiple copies of *28S-D2* as evidenced by the presence of two double peaks (one C/G and the other C/T) in most sequence reads. Only *COI* and *COII* provide signal among the ingroup taxa, and none of our sampled genes could distinguish between *O. saimaensis* and *O. fleischeri*.

During a visit to the Canadian National Collection of Insects, Nematodes, and Arachnids in Ottawa, Ontario, Canada, we found a single *Oobius* specimen in an ethanol lot collected in South Korea (USNMENT01119503: Gangwon-do: Chuncheon, Nam-myeon Hudong-li, 6-31.vii.2003, malaise trap in pastured area on trails close to forest edge). This specimen was both a morphological and molecular match with *O. primorskyensis* specimens collected in the Russian Far East.

### *Oobius saimaensis* Yao and Mottern, new species

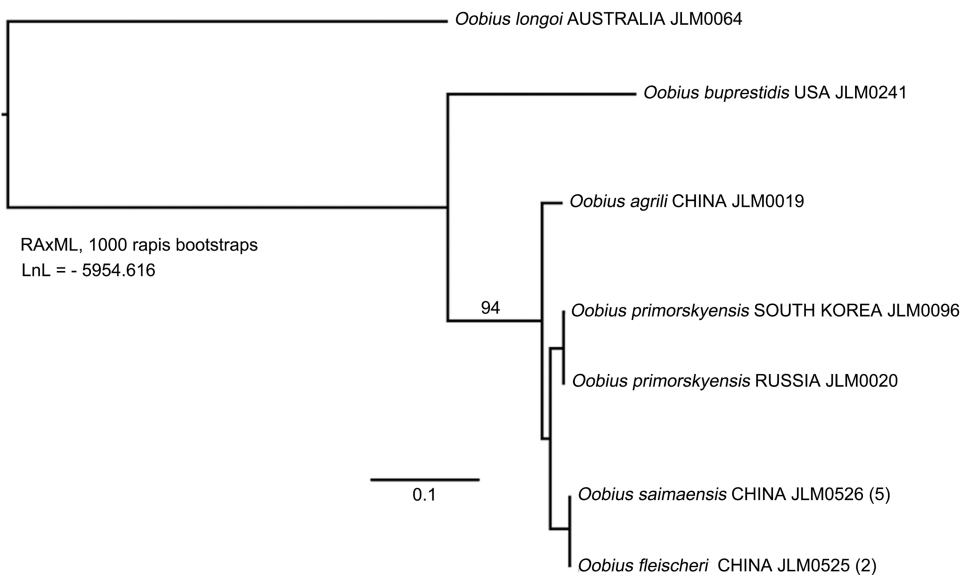
Figures 2–3.

**Type material.** Holotype: ♀, China: Liaoning Province: Saima Village [40°57.473'N, 124°15.788'E], v-ix, 2014, Kai Zang, ex *Agrilus fleischeri* eggs, deposited in CAF. Paratypes: ♀: China: Liaoning Province, Saima Village

**Table 1.** Character-based DNA barcodes (*COI*) for *Oobius saimaensis*/*Oobius fleischeri* (new species) and *Oobius agrili*.

| Position | <i>O. agrili</i> | New species | Position | <i>O. agrili</i> | New species | Position | <i>O. agrili</i> | New species |
|----------|------------------|-------------|----------|------------------|-------------|----------|------------------|-------------|
| 28       | A                | G           | 49       | G                | A           | 58       | G                | A           |
| 69       | T/C              | C           | 76       | A                | T           | 88       | T                | A           |
| 112      | A                | T           | 127      | G                | A           | 139      | A                | G           |
| 163      | G                | A           | 178      | A                | G           | 212      | T                | C           |
| 214      | A                | T           | 215      | T                | C           | 247      | G                | A           |
| 259      | A                | G           | 262      | G                | T           | 268      | A                | T           |
| 271      | G                | A           | 277      | T                | A           | 289      | T                | C           |
| 313      | T                | A           | 322      | C                | T           | 325      | A                | T           |
| 349      | T                | A           | 352      | G                | A           | 361      | A                | G           |
| 365      | A                | G           | 385      | G                | A           | 400      | T                | A           |
| 419      | A                | T           | 430      | A                | G           | 484      | A                | G           |
| 493      | A                | G           | 499      | A                | T           | 505      | A                | T           |
| 508      | A                | T           | 517      | T                | A           | 523      | A                | G           |
| 532      | C                | T           | 574      | T                | G           | 577      | C                | T           |
| 580      | T                | A           | 589      | T                | C           |          |                  |             |

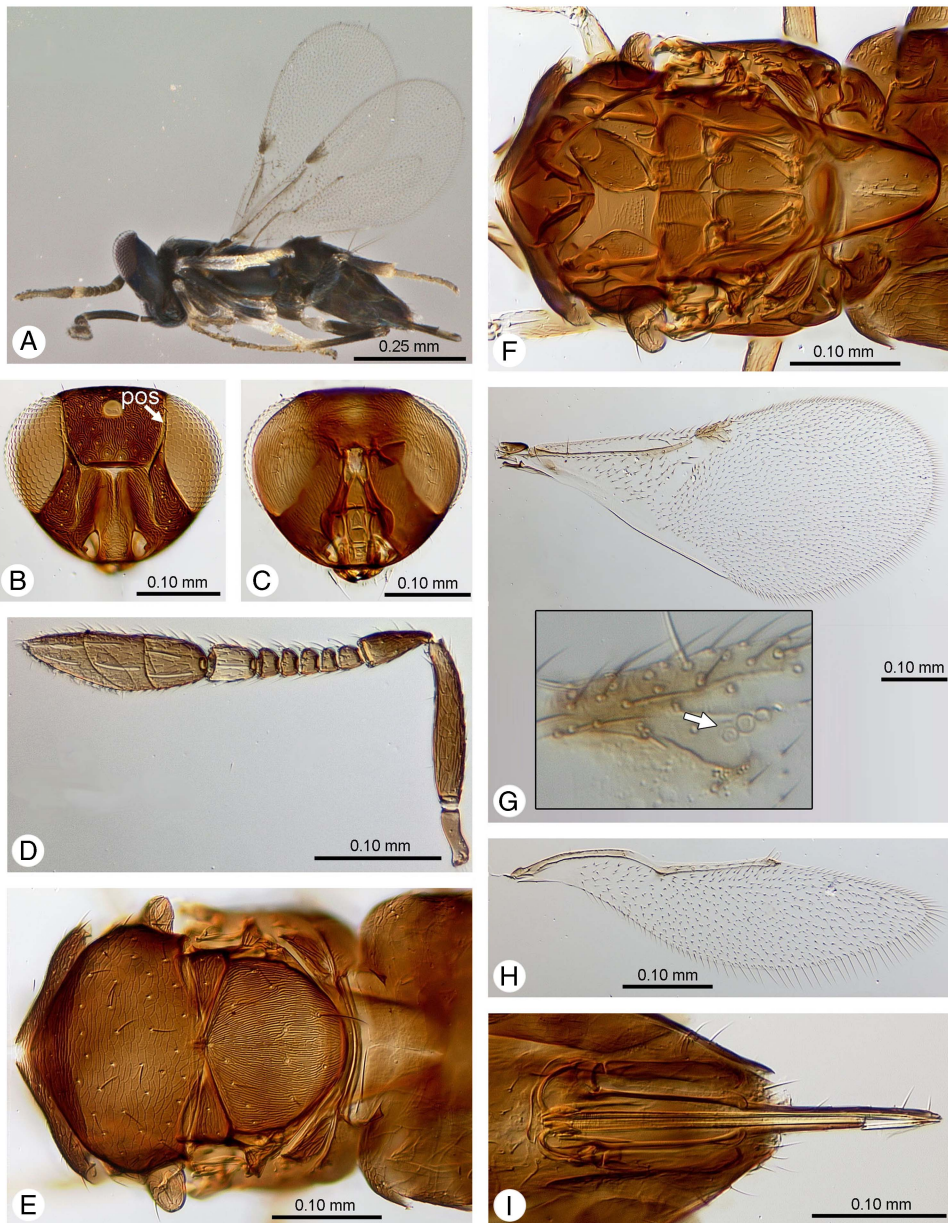
**Fig. 1.** Maximum likelihood analysis of far eastern species of *Oobius*. Outgroups include *Oobius longoi* (Australia) and *Oobius buprestidis* (North America) base on *COI*, *COII* and *28S D2–5* ribosomal DNA. Support values are maximum likelihood bootstraps; values below 90% not shown.



(40°57.473'N, 124°15.788'E), 18.ix.2010, J.J. Duan, ex *Agrilus fleischeri* eggs, deposited in CAF; 3♀ and 2♂, China: Liaoning Province: Saima Village [40°57.473'N, 124°15.788'E], v–ix, 2014, Kai Zang, ex *Agrilus fleischeri* eggs, deposited in CAF; ♀: China: Liaoning Province: Saima Village (40°57.473'N, 124°15.788'E), 16.ix.2013, ex

*Agrilus fleischeri* eggs collected from poplar trees, emerged 25–27.iv.2014 [USNM01231527, deposited USNM]. 4♂: progeny produced from parental generation collected from holotype locality, ex *Agrilus planipennis* eggs, emerged 24.xi–1.xii.2014 in quarantine at the Beneficial Insects Introduction Laboratory, Newark, Delaware

**Fig. 2.** *Oobius saimaensis* Yao and Mottern, new species, female. **A**, Lateral habitus; **B**, anterior head, arrow indicating preorbital sensillum; **C**, posterior head; **D**, antenna; **E**, dorsal mesosoma; **F**, interior and ventral mesosoma; **G**, forewing, inset showing venation and annulated first stigmal sensillum; **H**, hind wing; **I**, ovipositor.

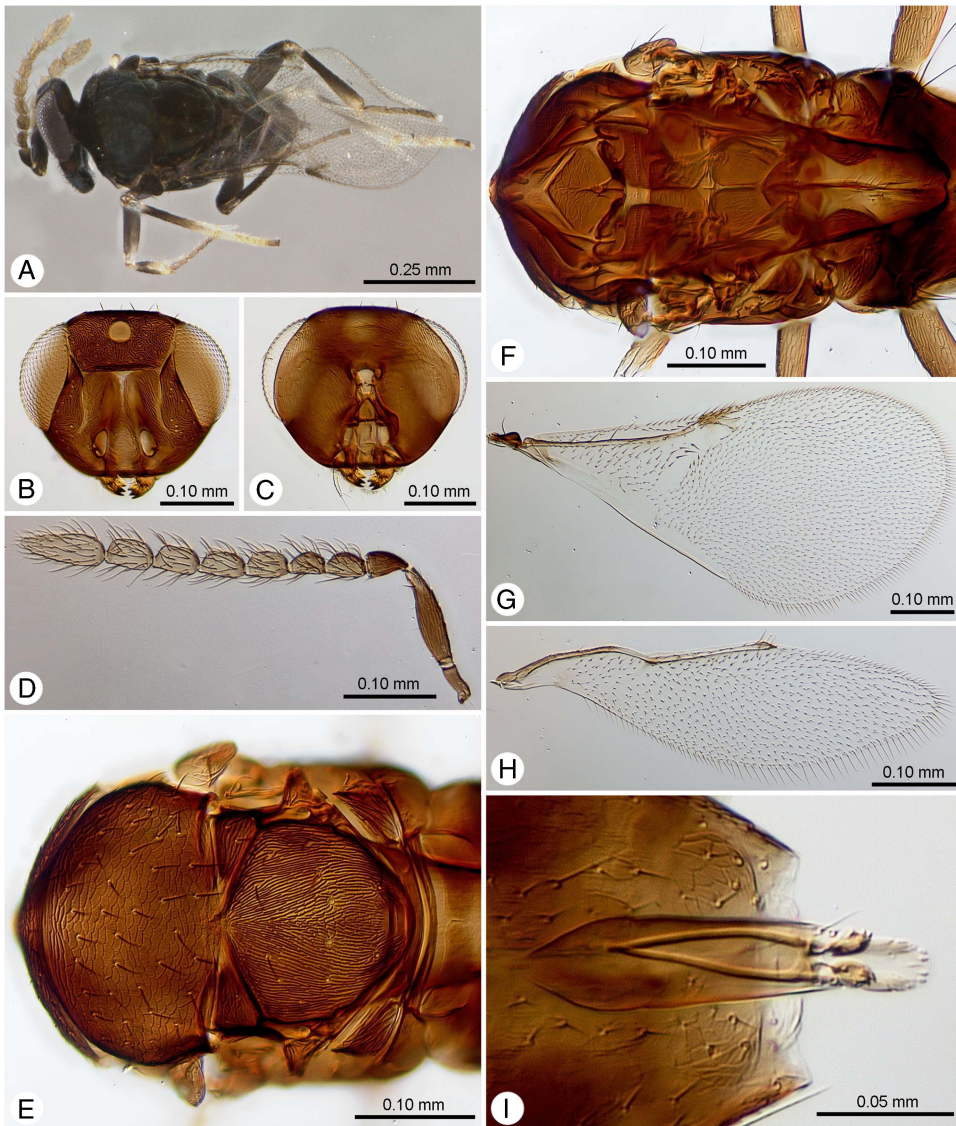


[USNMENT01231522, USNMENT01231524-26, deposited in USNM].

**Diagnosis.** *Oobius saimaensis* is morphologically similar to *O. agrili*, *O. primorskyensis*, and *O. fleischeri*. Therefore, the following combination of characters is required to distinguish females from all other *Oobius* species: body

length 0.63–0.74 mm; all tarsi with four tarsomeres; pedicel longer than the combined lengths of the first three funicular antennomeres; clava 2.81–3.25× as long as broad; exerted portion of ovipositor 0.33–0.42× as long as metasoma; ovipositor at least 1.3× the length of the mesotibia. Males can be distinguished from all other *Oobius*

**Fig. 3.** *Oobius saimaensis* Yao and Mottern, new species, male. **A**, Dorsal habitus; **B**, anterior head; **C**, posterior head; **D**, antenna; **E**, dorsal mesosoma; **F**, interior and ventral mesosoma; **G**, forewing; **H**, hind wing; **I**, aedeagus.



species by the combination of the tarsi with four tarsomeres and clava rounded apically, lacking transverse truncation.

**Description.** Female (Fig. 2). Body length 0.63–0.74 mm (including exerted ovipositor) (Fig. 2A).

**Colour.** Body dark brown to black with blue-green metallic sheen. Mandible yellowish brown with distal two teeth darker apically. Antenna dark brown except sixth funicular antennomere

brownish yellow (in card-mounted specimens) or yellow (in slide-mounted specimens); multiporous plate sensilla pale yellow; clava brown. Scutellum dark brown with coppery sheen. Coxae brown, femora dark brown, lighter at apices; metatibia dark brown except distal third yellow; mesotibia mostly pale yellow with dark brown band in proximal quarter; protarsi and metatarsi pale brown, tarsomeres becoming darker distally; mesotarsus mostly pale yellow with fourth

tarsomere dark brown. Wings hyaline except venation mostly brown, stigmal vein and middle third of submarginal vein yellowish brown. Metasoma dark brown; metasomal tergite 1 lighter than remaining terga. Visible portion of third valvula dark brown with coppery sheen.

*Head* (Fig. 2B, 2C). Frontovertex with coarse irregular reticulate sculpture and large setiferous punctures, its maximum width measured in anterior view about half that of the head. Ocelli form obtuse triangle, posterior ocellus separated by less than half its diameter from inner eye margin. Eye sparsely setose with each seta shorter than the width of an ommatidium; eye height 1.6–2.3× malar space. Frons with irregular rugulose sculpture; upper face with two lines of setae each along preorbital carina, interantennal area having two lines of setae each along inner edge of torulus; malar space with scattered setae. Torulus longer than broad, separated by greater than largest torular diameter; scrobal depression shaped like an inverted V, smooth, not reaching anterior edge of frontovertex. Preorbital sensillum (arrow, Fig. 2B, pos) present, directly adjacent to the compound eye, positioned on the frontovertex lower than the ventral margin of the anterior ocellus. Mandible tridentate, with basal tooth shorter than apical two tenths. Maxillary with three palpomeres, and labial with one palpomere. Antenna with scape subcylindrical, not reaching anterior ocellus, 4.32–5.00× as long as maximum width; pedicel 1.33–2.07× as long as the first three funicle antennomeres together, 1.60–2.67× as long as broad; funicular antennomeres 1–2 distinctly transverse with the following length width ratios: funicular antennomere 1, 0.70–0.85×, funicular antennomere 2, 0.52–0.64×, funicular antennomere 3, 0.45–0.50×, funicular antennomere 4, 0.50–0.57×, funicular antennomere 5, 0.60–0.64×, funicular antennomere 6, 1.18–1.53× as long as broad and with 1 or 2 multiporous plate sensilla. Clava with three antennomeres, 2.81–3.25× as long as broad, strongly obliquely truncate, truncation slightly more than a half the length of clava. Clava multiporous plate sensilla: claval antennomere 1: claval antennomere 2: claval antennomere 3 = 3:3:4.

*Mesosoma* (Fig. 2E–F). Mesosomal dorsum, 0.94–1.05× as long as broad, 0.68–0.89× as long as metasoma (including ovipositor). Pronotum short, with imbricate reticulate sculpture,

posterior margin with a line of the longer bristles. Mesoscutum 0.39–0.53× as long as broad, with imbricate reticulate sculpture similar to pronotum but more superficial, with about 40 scattered setae, and posterior margin slightly produced medially. Axillae with superficial reticulation, not advanced and nearly meeting medially, but often covered by posterior margin of mesoscutum in dried specimens, making them appear well separated in dorsal view; usually with three setae each, forming a triangle with two setae laterally and one in about the center of the axilla. Mesoscutellum 0.74–1.14× as long as broad, with fine longitudinally striate sculpture, and with nine or 10 more-or-less paired setae, posterior-most pair the longest; mesoscutellar campaniform sensilla separated from each other by about 5× the diameter of a sensillum, and located ~0.33× length of the mesoscutellum from posterior margin. Forewing 1.94–2.51× as long as broad; marginal vein 0.87–0.90× as long as postmarginal vein, 0.81–0.90× as long as stigmal vein; stigmal vein with three sensilla, basal most sensillum with distinct annulation (arrow, Fig. 2G inset); lineae calva closed posteriorly by several setae. Hind wing 3.76–4.40× as long as broad. All legs with four tarsomeres; mesotibial spur about 0.71–0.81× length of basitarsus, and basitarsus of mesothoracic leg with four stout lateral pegs and five ventral pegs.

*Metasoma*. Oval acuminate, 0.94× as long as broad excluding ovipositor. Cerci arise just posteriorly to the middle of the metasoma. Hypopygium nearly reaching apex of metasoma. Ovipositor about 2.04× as long as third valvula and 1.31–1.76× as long as mesotibia, ovipositor sheath exerted 0.41–0.50× metasomal length beyond apex of metasoma, apical half symmetrically lined with pairs of setae.

Male (Fig. 3). Body length 0.51–0.59 mm. Male similar to female except genitalia and the following: legs with coxae, femur, and tibiae dark brown with metallic tint, tarsi brown; antenna with scape 3.41–3.65× as long as broad, pedicel 1.58× as long as first funicular antennomere, 1.25× as long as broad; funicular antennomeres 1–2 distinctly smaller (both length and width) and darker in colour than the following antennomeres; all funicular antennomeres with long setae, the longest setae about as long as the width of the antennomere; length width ratios for funicular



antennomeres as follows: funicular antennomere 1, 1.41–1.70 $\times$ , funicular antennomere 2, 1.44–1.48 $\times$ , funicular antennomere 3, 1.48–1.67 $\times$ , funicular antennomere 4, 1.69–1.70 $\times$ , funicular antennomere 5, 1.63–1.69 $\times$ , funicular antennomere 6, 1.51–1.68 $\times$ . Clava solid (segmented), without oblique truncation, 2.39–2.61 $\times$  as long as broad; multiporous plate sensilla absent from funicular antennomeres 1–2; funicular antennomeres 3–6 each with two multiporous plate sensillae (one dorsal and one lateral) and clava with three multiporous plate sensillae. Forewing with marginal vein 0.70–0.86 $\times$  the length of postmarginal vein. External male genitalia as in Figure 3I.

**Etymology.** The specific epithet is derived from the name of the region (Saima, Liaoning province, China) where the species was collected.

**Distribution.** China, Liaoning Province.

**Natural history.** *Oobius saimaensis* is a bisexual solitary egg parasitoid of *A. fleischeri* infesting *Populus* trees. Rearing experiments conducted at the United States Department of Agriculture, Agricultural Research Service, Beneficial Insects Introduction Research Unit quarantine laboratory confirmed that this species could successfully parasitise and develop in *A. planipennis* eggs, at least under laboratory-rearing conditions.

### ***Oobius fleischeri* Yao and Duan, new species**

Figures 4–5.

**Type material.** Holotype: ♀: China: Liaoning Province: Saima Village (40°57.473'N, 124°15.788'E), v–ix.2014, Kai Zang, ex *Agrilus fleischeri* eggs, deposited in CAF. Paratypes: 3♀2♂: China: Liaoning Province: Saima Village (40°57.473'N, 124°15.788'E), v–ix.2014, Kai Zang, ex *Agrilus fleischeri* eggs, deposited in CAF; 2♂: progeny produced from parental generation collected from holotype locality, ex *Agrilus planipennis* eggs, emerged 31.x–14.xi.2014 in quarantine at the Beneficial Insects Introduction Laboratory, Newark, Delaware [USNMENT012 31521, USNMENT1231523, deposited in USNM].

**Diagnosis.** *Oobius fleischeri* is morphologically similar to *O. agrili*, *O. primorskyensis*, and *O. saimaensis*. Therefore, the following combination of characters is required to distinguish

females from all other *Oobius* species: body length 0.69–0.76 mm; all tarsi with five tarsomeres; pedicel longer than the combined lengths of the first three funicular antennomeres; clava 2.97–3.33 $\times$  as long as broad; exerted portion of ovipositor 0.23–0.38 $\times$  as long as metasoma; ovipositor at least 1.2 $\times$  the length of the mesotibia. Males can be distinguished from all other *Oobius* species by the combination of the tarsi with five tarsomeres and clava rounded apically, lacking transverse truncation.

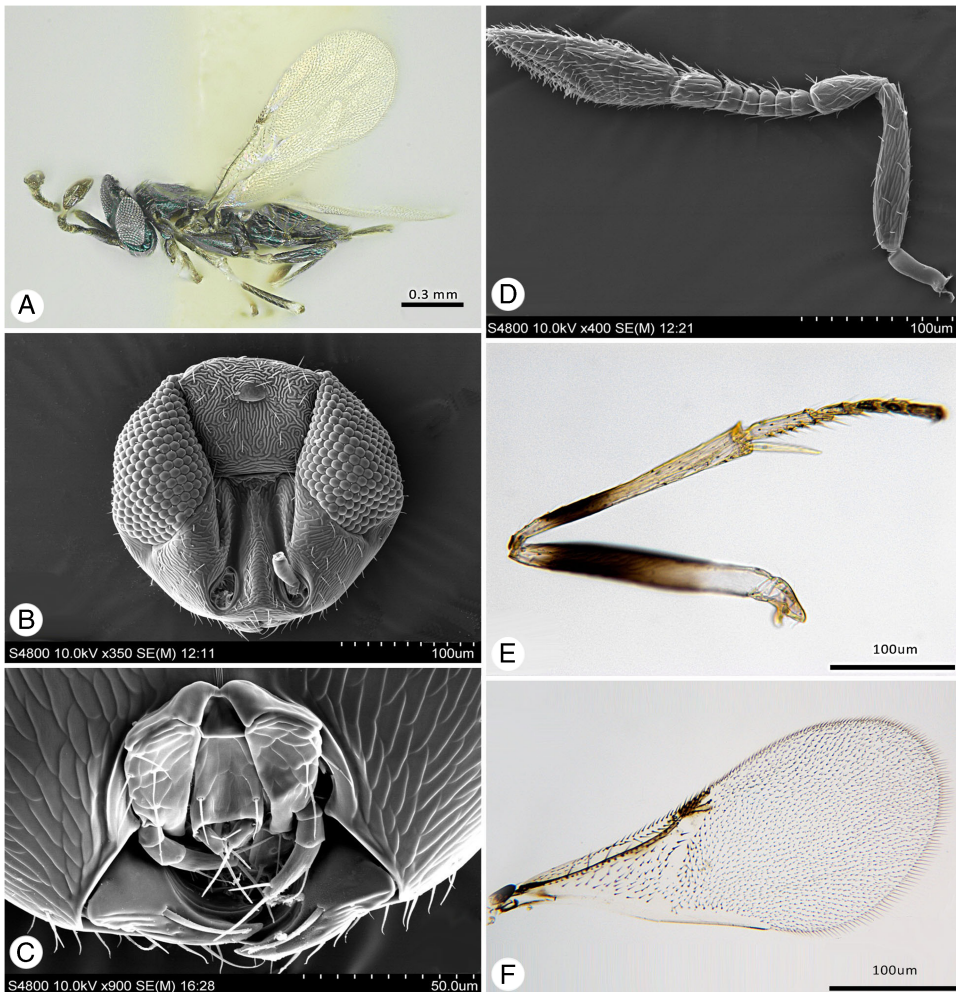
**Description.** Note: because *O. saimaensis* and *O. fleischeri* can only be reliably distinguished based on tarsomere count, we include only measurements and some counts within the *O. fleischeri* description, as some of these may eventually prove to differ statistically between the two species.

Female: Body length 0.69–0.76 mm (including exerted ovipositor) (Fig. 4A) **Colour.** As in *O. saimaensis*.

**Head** (Fig. 4B). Eye height about 2.20–2.45 $\times$  malar space. Scape 4.16–4.86 $\times$  as long as maximum breadth; pedicel 1.11–1.47 $\times$  as long as the first three funicular antennomeres together, 1.78–2.20 $\times$  as long as broad; funicular antennomere 1 1.13–1.31 $\times$  as broad as long, funicular antennomere 2–5 distinctly transverse with the following length: width ratios: funicular antennomere 2, 0.59–0.65 $\times$ , funicular antennomere 3, 0.56–0.61 $\times$ , funicular antennomere 4, 0.47–0.57 $\times$ , funicular antennomere 5, 0.48–0.58 $\times$ , funicular antennomere 6, 1.04–1.09 $\times$  as long as broad and with one or two multiporous plate sensillae. Clava 2.97–3.33 $\times$  as long as broad, strongly obliquely truncate, truncation about a half of the length of clava, multiporous plate sensilla: claval antennomere 1: claval antennomere 2: claval antennomere 3 = 3:3:4, but the number of multiporous plate sensillae in claval antennomere 2 and claval antennomere 3 variable.

**Mesosoma.** Mesosoma 0.79–0.85 $\times$  as long as broad, 0.63–0.76 $\times$  as long as metasoma (including ovipositor). Mesoscutum 0.50–0.59 $\times$  as long as broad, scattered with about 40 setae. Scutellum about as long as or slightly longer than mesoscutum, 0.69–0.75 $\times$  as long as broad, with 8–10 setae (four or five pairs). Forewing (Fig. 4G) 1.95–2.17 $\times$  as long as broad, marginal vein 0.75–1.15 $\times$  as long as postmarginal vein, 1.00–1.15 $\times$  as long as stigmal vein. Hind wing 3.64–3.93 $\times$  as long as broad (Fig. 4H). All legs with five

**Fig. 4.** *Oobius fleischeri* Yao and Duan, new species, female. **A**, Lateral habitus; **B**, anterior head; **C**, posterior mouthparts; **D**, antenna; **E**, mesothoracic leg; **F**, forewing.



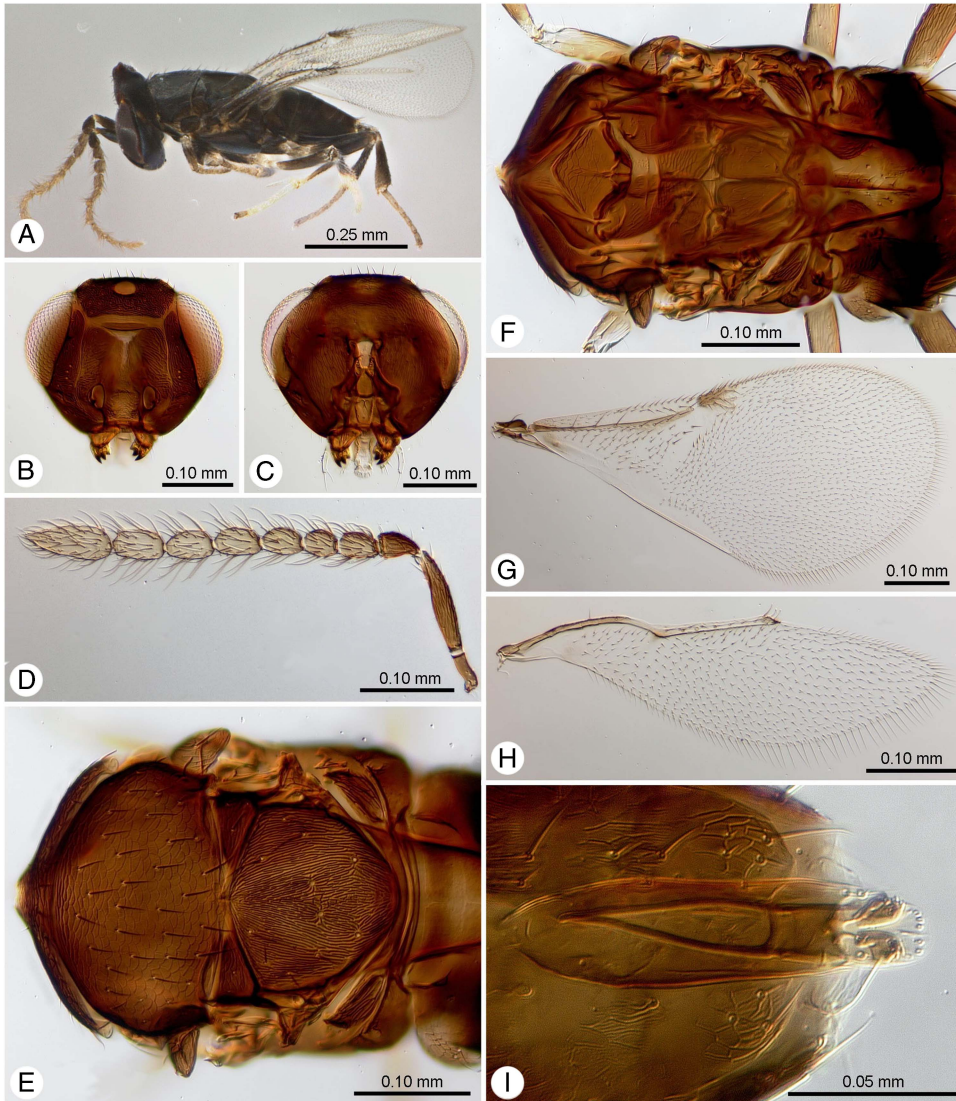
tarsomeres; mesotibial spur about 0.67–0.83× length of basitarsus.

*Metasoma.* 1.29–1.46× as long as broad excluding ovipositor. Ovipositor 2.19–2.65× as long as outer plate of the ovipositor, 1.17–1.50× length of mesotibia, ovipositor sheath exerted 0.23–0.38× metasomal length beyond apex of metasoma.

Male (Fig. 5): Body length 0.53–0.57 mm. Similar to female except genitalia and the following: antenna with scape 2.65× as long as broad, pedicel just about or slightly shorter than first funicular antennomere, 1.38–1.48× as long as broad; all funicular antennomere longer than broad and with long setae, length: width ratios for

funicular antennomeres as follows: funicular antennomere 1, 1.48–1.63×, funicular antennomere 2, 1.17–1.46×, funicular antennomere 3, 1.42–1.52×, funicular antennomere 4, 1.50–1.58×, funicular antennomere 5, 1.44–1.56×, funicular antennomere 6, 1.38–1.62×. Clava solid, without oblique truncation, 2.27–2.68× as long as broad; multiporous plate sensilla absent from funicular antennomeres 1–2; funicular antennomeres 3–6 each with two multiporous plate sensillae (one dorsal and one lateral) and clava with three multiporous plate sensillae. Forewing with marginal vein 0.55–0.60× as long as postmarginal vein, 0.86–1.05× as long as stigmal vein. External male genitalia as in Figure 5I.

**Fig. 5.** *Oobius fleischeri* Yao and Duan, new species, male. **A**, Lateral habitus; **B**, anterior head; **C**, posterior head; **D**, antenna; **E**, dorsal mesosoma; **F**, interior and ventral mesosoma; **G**, forewing; **H**, hind wing; **I**, aedeagus.



**Etymology.** The specific epithet is derived from the scientific name of its host *A. fleischeri*.

**Distribution.** China, Liaoning Province.

**Natural history.** *Oobius fleischeri* is a bisexual solitary egg parasitoid of *A. fleischeri* infesting *Populus* trees. Rearing experiments conducted at the United States Department of Agriculture, Agricultural Research Service, Beneficial Insects Introduction Research Unit quarantine laboratory (Newark, Delaware) confirmed that this species could successfully parasitise and develop in

*A. planipennis* eggs, at least under laboratory-rearing conditions.

## Discussion

Most species of *Oobius* are primary egg parasitoids of buprestid and cerambycid beetles, and a total of 44 species were recorded worldwide before the present study (Noyes 2015; Triapitsyn *et al.* 2015; Yao *et al.* 2016). The two new species of *Oobius* described here are the primary and

solitary egg parasitoids of *A. fleischeri*, a newly emerging pest of poplar trees in northeastern China. Both species appears to have co-existed on *A. fleischeri* hosts infesting poplar trees in the same geographic region (northeastern China). Our field collection records showed that both species appeared to be equally abundant in northeastern China (Y.X.Y., X.Y.W. unpublished data) and inflicted a combined parasitism of *A. fleischeri* as high as 48% (Zang *et al.* 2016). More recently, a laboratory study at the quarantine facility of the United States Department of Agriculture, Agricultural Research Service, Beneficial Insects Introduction Research Unit showed that both species successfully parasitised eggs of the emerald ash borer *A. planipennis*. As *A. planipennis* also occurs in Liaoning Province (part of its native range), where both *O. fleischeri* and *O. saimaensis* were collected from *A. fleischeri* eggs, we recommend future studies to determine if these two parasitoids species also attack *A. planipennis* in this region. If so, *O. fleischeri* and *O. saimaensis* may also be studied for biological control introduction against *A. planipennis* in North America.

We acknowledge that this may be an unusual polymorphism within a single species, especially in light of our inability to separate the two species using molecular data. However, as these wasps are part of a cryptic species complex that is being used for classical biological control, giving different names to these wasps that are morphologically diagnosable will allow biological control researchers to clearly communicate the identity of the agent to regulatory agencies during non-target risk assessment and host specificity testing. More importantly, we did not observe any variation in tarsomere numbers of the F<sub>1</sub> males produced on *A. planipennis* eggs by the unmated female wasps collected in the field in each of the species. Although it is true that tarsomere counts vary within Chalcidoidea at various taxonomic levels, to our knowledge there are no cases where tarsomere counts vary intraspecifically.

The distribution of *O. fleischeri* and *O. saimaensis* overlaps with that of *O. agrili*, the egg parasitoid of *A. planipennis* in northeastern China. While *O. fleischeri* may be easily distinguished from *O. agrili* and *O. saimaensis* based on its five tarsomeres versus four tarsomeres in the latter two species, *O. agrili* and *O. saimaensis* are morphologically extremely similar. However,

morphological results show that *O. saimaensis* is smaller than *O. agrili* and has a much longer ovipositor (at least 1.3× the length of the mesotibia) relative to overall body size than *O. agrili*. In addition, our molecular data show clear genetic divergence between these two species (Table 1).

As the three genera *Avetianella*, *Szelenyiola*, and *Oophagus*, were incorporated to *Oobius* (Noyes 2010), a few studies have examined the intra-specific and interspecific variation in morphological characteristics as well as the phylogenetic relationships among different species within *Oobius* as currently defined. In addition, morphological similarities among some species combined with the lack of molecular data and morphological detail in many of the early species descriptions has made the taxonomy more challenging. Further taxonomic and phylogenetic studies are clearly needed, particularly with the addition of more molecular markers through the use of next generation sequencing and biological crossing studies to further clarify species boundaries.

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