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First Record of the Oak Gall Wasp (Hymenoptera, Cynipidae) in Myanmar

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Abstract This is the first record of *Andricus mukaigawae* (Mukaigawa, 1913) (Hymenoptera: Cynipidae) in Myanmar. This is also the first record of the tribe Cynipini (which induces galls on oaks and their relatives) in the country. Galls of *A. mukaigawae* were collected from *Quercus griffithii* Hook.f. & Thomson ex Miq. in Tedim Township, Falam District, during a field expedition to the Chin Hills in northwestern Myanmar. Since the species diversity of potential host plants, *Quercus* and allies, is high in the mountainous areas of the north to northwestern Myanmar where galls of *A. mukaigawae* were found, further field surveys in these areas will help uncover the species diversity of Cynipidae in Myanmar.

Key words: *Andricus mukaigawae*, Burma, Chin Hills, DNA barcoding, gall inducer, new record, *Quercus griffithii*.

Introduction

The family Cynipidae is a group of tiny, herbivorous wasps whose larvae inhabit plant galls induced by themselves or others. The taxon contains approximately 1,400 species worldwide but remains poorly known in Southeast Asia, including Myanmar (Abe *et al.*, 2007; Pénzes *et al.*, 2018). *Lithosaphonecrus mindatus* Ide, Aung and Tanaka, 2020, is the only cynipid species reported in Myanmar (Ide *et al.*, 2020).

Andricus mukaigawae (Mukaigawa, 1913) was originally described in Japan (Mukaigawa, 1913). The species is also known in the Russian Far East, Korea, mainland China, and northeastern India (Abe, 1986; Abe *et al.*, 2012; Pujade-Villar *et al.*, 2014, 2016, 2020). They inhabit burr-shaped galls that exceed 40 mm in diameter (Yukawa and Masuda, 1996). On 21 November 2019 we found three similar galls on *Quercus griffithii* Hook.f. & Thomson ex Miq. in Tedim Township, Falam District, during a field expedition to the Chin Hills in northwestern Myanmar (Fig. 1), and successfully collected three female cynipid wasps from these galls. Here, we summarise the identification of the wasp based on morphological examination and DNA barcoding.

Materials and Methods

Three unidentified cynipid female wasps collected from Tedim Township were killed by immersion in 99.5% (v/v) ethanol. The voucher specimen was dried following the t-butyl-alcohol freeze-drying method (originally developed by Inoué and Osatake, 1988) using a freeze-drying device (JFD-320; JEOL, Tokyo, Japan) following the manufacturer's protocol. The external structures of dry-mounted wasps were examined under binocular stereomicroscopes (S8APO and

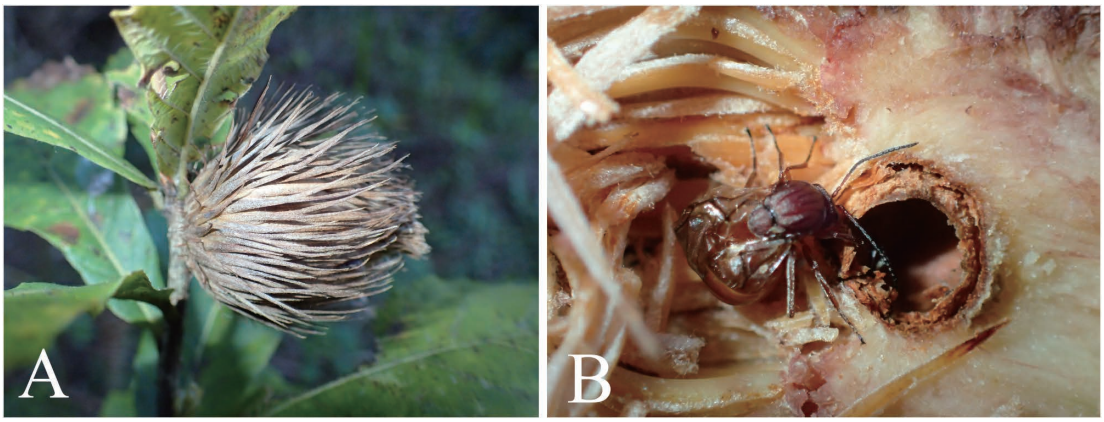

Fig. 1. Asexual generation gall of *Andricus mukaigawae* collected in Myanmar. A, Gall appearance; B, Longitudinal section of gall with an adult wasp collected from inside.

MZ APO; Leica Microsystems KK, Tokyo, Japan) fitted with digital single-lens reflex cameras (E-30; Olympus, Tokyo, Japan), and under a scanning electron microscope (JSM-6380LV; JEOL, Tokyo, Japan) operating at 1.5 kV. The length of each body part was measured using an ocular micrometre. Focus stacking was conducted using the CombineZP software (https://combinezp.software.informer.com/). All images were processed using the GNU Image Manipulation Program (GIMP 2.10.20; https://www.gimp.org/).

DNA was extracted from one of the three wasps using the DNeasy Blood and Tissue Kit (Qiagen K.K., Tokyo, Japan). The mitochondrial cytochrome *c* oxidase subunit I (COI) region was amplified by polymerase chain reaction (PCR) using primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTCAGGGTGACCAAAAAATCA-3′) (Folmer *et al.*, 1994). The PCR thermal conditions followed Hebert *et al.* (2003a): 94°C for 60 s; five cycles at 94°C for 60 s, 45°C for 90 s, and 72°C for 90 s; 35 cycles at 94°C for 60 s, 50°C for 90 s, and 72°C for 60 s; and a final extension at 72°C for 5 min. The PCR product was purified using a QIAquick PCR Purification Kit (Qiagen K.K., Tokyo, Japan). The sequencing was carried out by Eurofins Genomics K.K., Tokyo, Japan, using a 3730xl DNA Analyser (Thermo Fisher Scientific K.K., Tokyo, Japan). The complementary forward and reverse sequences were assembled in Mega 7.0.26 (Kumar *et al.*, 2016) using ClustalW and analysed visually.

The morphological characteristics and determined partial sequence (658 bp) of the COI region (OL873579; GenBank: https://www.ncbi.nlm.nih.gov/genbank/) were compared with those of two *A. mukaigawae* specimens collected in Japan (OL873580, OL873581). The voucher cynipid specimens will be deposited at the Department of Zoology, National Museum of Nature and Science, Tsukuba, Ibaraki, Japan (NSMT) and the Biodiversity Research Center, Yezin, Myanmar (BRC). Voucher herbarium specimens of the host plant (Collection number of the herbarium specimen: Tanaka *et al.* MY5833) will be deposited in the herbaria TNS and RAF (acronyms according to Thiers, 2016).

The following morphological abbreviations were used: POL, postocellar line (the distance between the inner edges of the two lateral ocelli); OOL, ocular-ocellar line (the distance from the outer edge of a lateral ocellus to the compound eye); LOL, lateral-ocellar line (the distance between the median and lateral ocelli); and F1–F13, the first to thirteenth flagellomeres. The morphological terminologies follow Richards (1977), Ronquist and Nordlander (1989), and

Liljeblad *et al.* (2008); the description of the surface sculptures follow Harris (1979).

Results and Discussion

The COI sequence of the unidentified cynipid specimen from Myanmar differed by 2.0–2.1% (13–14 bp) from that of the two *A. mukaigawae* specimens from Japan. Although this may be considered as an interspecific variation in animals (Hebert *et al.*, 2003b), large intraspecific genetic variation is commonly observed in Cynipidae; e.g., the divergence of COI sequences within *Andricus qurcuslanigera* (Ashmead, 1881) was 0–10.85% in Hood *et al.* (2018). In addition, no significant morphological differences were observed between the Myanmar and Japanese specimens (Figs. 2–3). Therefore, we identified the unidentified cynipid specimens collected in Myanmar as *A. mukaigawae*.

This species has sexual and asexual generations with different morphologies of galls and wasps between the two generations (Abe, 1986). The asexual generation gall of *A. mukaigawae* is burr-shaped, sometimes exceeding 40 mm in diameter (Yukawa and Masuda 1996). This is consistent with the characteristics of the galls found in Myanmar, indicating that the *A. mukaigawae* recorded here is the asexual generation. Although the sexual generation gall of *A. mukaigawae* has not yet been found in Myanmar, it will be found on young leaves some weeks after bud burst according to the collection data in Japan (Abe, 1986; Yukawa and Masuda, 1996).

Andricus mukaigawae (Mukaigawa, 1913)

Specimens examined. Myanmar (new record): 3 ♀♀, asexual generation, Tedim Township, Falam District, Chin State, gall collection and dissection: 21. XI. 2019, N. Tanaka *et al.* leg., host: *Quercus griffithii*.

Summary of taxonomic characteristics based only on a Myanmar specimen used for DNA extraction. Head brown, except for black areas around antennal rim, ventral margin of face, including clypeus, and occipital foramen; mandible brown, with black teeth; antenna dark brown, except for light brown scape, pedicel, and F1; palpi light brown. Mesosoma brown, except for black areas around anterolateral margin of mesoscutum, anteroadmedian and parapsidal signa, mesoscutal fovea, metascutellum, lateral propodeal carina, central propodeal area, subalar area,

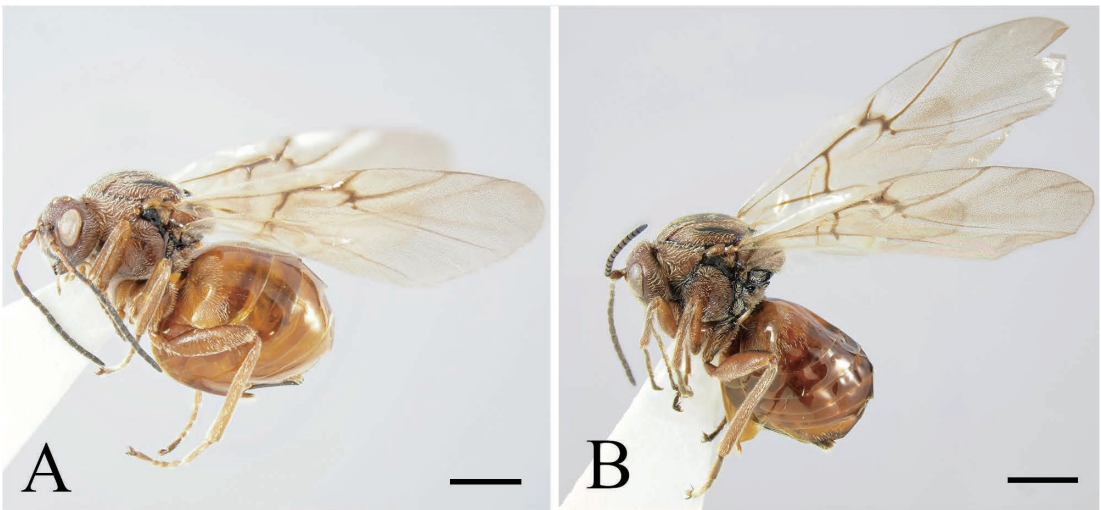

Fig. 2. Habitus of asexual generation wasps of *Andricus mukaigawae* used for DNA extraction (scale bar = 1 mm). A, Specimen from Chin, Myanmar; B, Specimen from Nagano, Japan.

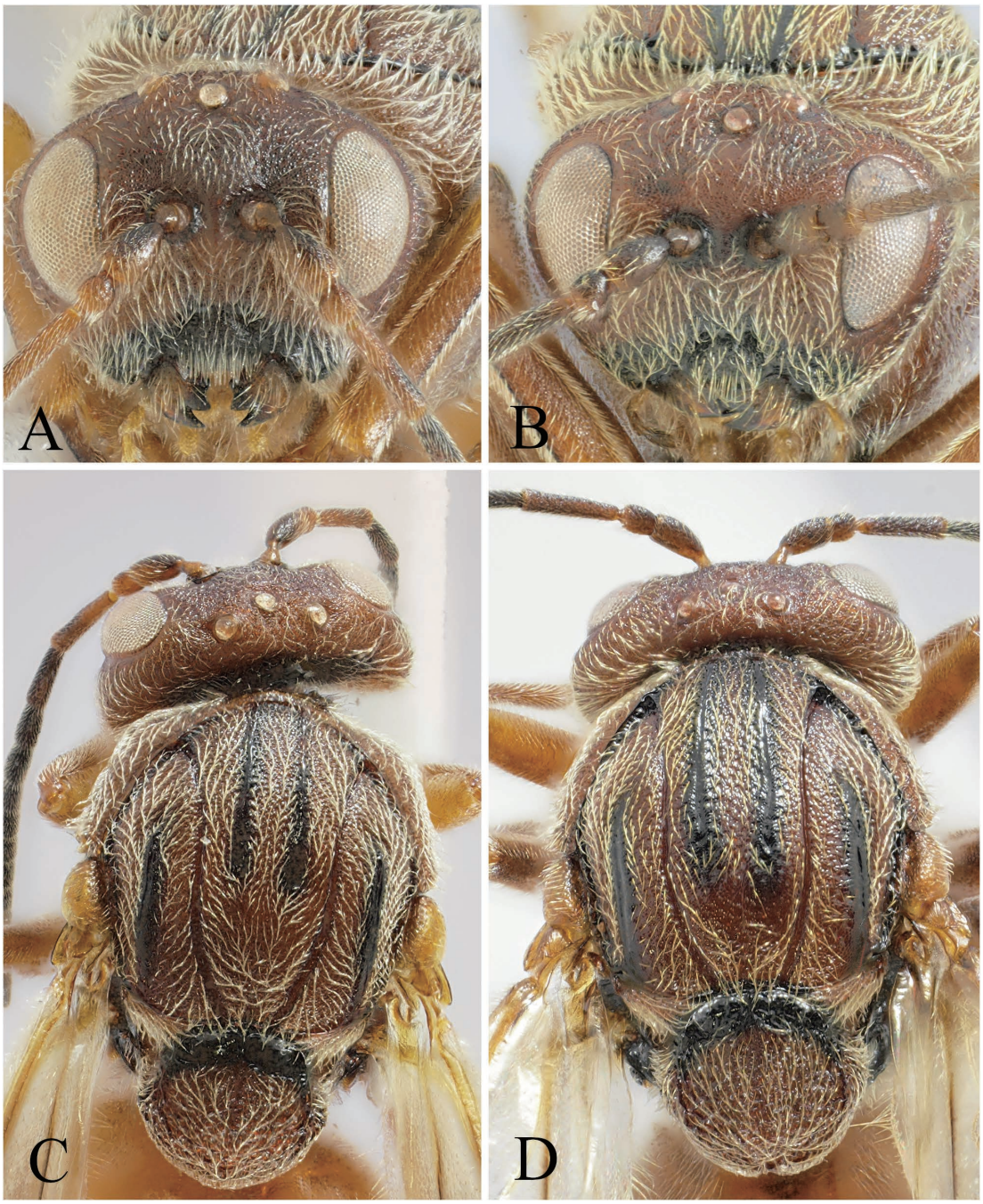

Fig. 3. Head and mesosoma of asexual generation wasps of *Andricus mukaigawae*, collected in Chin, Myanmar (A and C) and Nagano, Japan (B and D). A–B, Head, frontal view; C–D, Head and mesosoma, dorsal view.

and carinae on metapleural area; legs brown, except for darker tarsal claws. Metasoma brown, except for darker dorsal surface, light brown hypopygium and black third valvula.

Head slightly narrower than mesosoma in dorsal view, rudely coriarious to finely colliculate,

with dense setae. Ventral clypeal margin slightly incised medially. Facial strigae radiating from lateral clypeus obscure, reaching about half ways to eye margin. Subocular impression absent. Diameter of antennal rim 1.5 times as broad as distance between inner margins of rims, 1.3 times as broad as distance between lateral margin of antennal rim and inner margin of compound eye. Gena broadened behind eye. POL : OOL : LOL = 16 : 11 : 7. Antenna with 15 antennomeres; relative lengths of scape, pedicel, and F1–F13: 38, 20, 61, 50, 35, 32, 32, 25, 22, 22, 22, 20, 20, 20, 20; F12 and F13 fused, separated by obscure transverse furrow.

Mesosoma almost as long as high in lateral view, with dense setae, except for bared surface of anteroadmedian and parapsidal signa, scutellar fovea, subalar area, and central propodeal area. Pronotum rugose. Mesoscutum rudely coriarious to finely colliculate; base of each seta with or without small round depression; median mesoscutal line present only as slight impression at posterior margin of mesoscutum; notaulus percurrent. Scutellum rugose; bottom of scutellar fovea smooth. Mesopleuron coriarious; setae relatively sparse, except at mesopleural triangle. Propodeum with distinct lateral propodeal carina; central propodeal area smooth. Metatarsal claw strongly bent at apex; base expanded to pronounced and pointed tooth. Marginal cell of forewing open, 3.2 times as long as broad. R1 not reaching wing margin. Length of forewing 5.2 mm, of hind tibia 1.7 mm.

Metasoma smooth; tergum II with dense setae anterolaterally. Projecting part of hypopygial spine 4.5 times as long as high in lateral view, 2.5 times as long as broad in ventral view; subapical setae long, reaching beyond apex of spine.

Variations within Myanmar material (n = 3). Length of forewing 5.1–5.3 mm. Length of hind tibia 1.6–1.7 mm. Marginal cell of forewing 3.2–3.6 mm.

Remarks. In the present study, we recorded *A. mukaigawae* for the first time in Myanmar. Abe *et al.* (2012) recorded *A. mukaigawae* in northeastern India and regarded it as the first record of a gall wasp with a distribution from East Asia to the Indian subcontinent. Our finding of *A. mukaigawae* in Myanmar supports and expands on this contention. In both instances, *A. mukaigawae* was recorded from *Q. griffithii*, which is widely distributed in the Oriental region, including Myanmar, Thailand, Laos, and Vietnam (Govaerts & Frodin 1998). This suggests that *A. mukaigawae* may have a wide distribution across East Asia, the Indian subcontinent, and the Indochinese Peninsula. Myanmar lies at the northwestern edge of the Indochinese Peninsula, most of which is in the Oriental region, but northern Myanmar is adjacent to the Palearctic region (Udvardy, 1975; Morrone, 2015). Therefore, Myanmar is important in terms of the biogeographic connection between the Oriental and Palearctic regions inhabited by Asian Cynipidae.

Lithosaphonecrus mindatus, the only known cynipid species in Myanmar before this study, belongs to the tribe Synergini. *Andricus mukaigawae* belongs to the tribe Cynipini, which is known for inducing galls on oaks and their relatives, and is thus known as the oak gall wasp. Therefore, this is the first record of the oak gall wasp and the second record of the family Cynipidae in Myanmar. Both Cynipini and Synergini consist of herbivorous insects associated with oaks and their relatives of the family Fagaceae (Ronquist *et al.*, 2015; Buffington *et al.*, 2020), and host plant diversity is important when estimating the species richness of herbivorous insects (Lin *et al.*, 2015). As approximately 80% of all known cynipid species belong to the two tribes (Ronquist *et al.*, 2015), the diversity of Fagaceae is important for cynipid diversity. Two species of *Castanea*, 15 of *Castanopsis*, 31 of *Lithocarpus*, and 33 of *Quercus*, which belong to Fagaceae, have been recorded in Myanmar (Kress *et al.*, 2003). In particular, the mountainous areas of the northern to northwestern ranges of Myanmar (Kachin, Sagaing, and Chin) are species-rich in oaks and their relatives; at least one species of *Castanea*, seven of *Castanopsis*, 17 of *Lithocarpus*, and 21 of *Quercus* have been recorded (Kress *et al.*, 2003). This suggests that

further field surveys focusing on these areas will help uncover the species diversity of the Cynipidae in Myanmar.

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