NOTE

Deposition of phasmid eggs (Phasmatodea) in the nests of Acanthomyrmex glabfemoralis Zhou & Zheng, 1997 (Hymenoptera: Formicidae: Myrmicinae)

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ABSTRACT: Ant-mediated secondary dispersal (myrmecochory) of phasmid eggs using a fleshy nutrient-rich appendage called capitulum remains poorly studied, albeit the egg capitula are present in a large number of phasmid species worldwide. We here report our unexpected collection of 42 multi-species phasmid eggs deposited together with seeds in the four nests of the myrmicine ant species, Acanthomyrmex glabfemoralis Zhou & Zheng, 1997, in southern China and northern Vietnam. The collected phasmid eggs were composed of five egg morphospecies that most likely belong to some related genera in the two lonchodid subfamilies, Necrosciinae and Lonchodinae. None of the collected phasmid eggs had an intact capitular structure, indicating a strong likelihood of their consumption by the ants. To our knowledge, these records are the first published field evidence of myrmecochory in the Oriental phasmid species and these two subfamilies, supporting the broad occurrence of the myrmecochory in phasmids. Notes on the seeds deposited in these nests are also provided herewith.

KEY WORDS: Ants, Asia, dispersal, myrmecochory, myrmecophily, stick insects, symbiosis.

INTRODUCTION

Ant-mediated secondary dispersal (myrmecochory) of plant seeds is a well-known and widespread phenomenon. Most myrmecochorous plants produce seeds with a fleshy nutrient-rich appendage called elaiosome as a food reward that induces ants to pick up and transport the seeds to their nests (Ness et al., 2010). However, it is lesser-known that the myrmecochory has also evolved in phasmid insects (Phasmatodea), known as stick and leaf insects (Stanton et al., 2015).

The eggs of phasmids are generally hard-shelled and often closely resemble seeds in their external appearance, and exhibit great morphological diversity among species. Their egg morphology is highly species-specific across the entire order, with specific egg forms that correspond to oviposition mode (Robertson et al., 2018; O’Hanlon et al., 2020). The most common (and ancestral) oviposition mode in phasmids is dropping or flicking single eggs to the ground (Goldberg et al., 2015; Robertson et al., 2018). The eggs of many species with this oviposition mode have a fleshy appendage called capitulum attached on the operculum (detachable lid of the egg capsule, which the nymph pushes off when it hatches). The capitula have been shown to function analogously to the seed elaiosomes of myrmecochorous plants, that is, to induce ants to pick up and transport the egg to their nests (Compton and Ware, 1991; Hughes and Westoby, 1992; Windsor et al., 1996; Stanton et al., 2015).

Biological information on the myrmecochory in phasmids is, however, scarce, albeit the egg capitula are present in a large number of phasmid species worldwide. To our knowledge, the dispersal of phasmid eggs by ants has so far been documented in only four phasmid species from limited geographic regions (South Africa, Australia, and Costa Rica). These species include Phalces brevis (Burmeister, 1838) (Bacillinae; formerly Bacillus coecyz, see Compton and Ware, 1991), Ctenomorpha marginipennis Gray, 1833 (Phasmatinae; formerly C. chroma, see Hughes and Westoby, 1992), Calynda bicuspis Stål, 1875 (Diapheromerinae; see Windsor et al., 1996), and Eurycyna goliath (Gray, 1834) (Phasmatinae; see Stanton et al., 2015). Few observations have been made so far under natural conditions without experimental manipulation. There is also no published study on the myrmecochory in the Oriental phasmid species.

Acanthomyrmex Emery, 1893 is a small genus of myrmicine ants consisting of 19 valid species and nearly endemic to the Oriental realm (Bolton, 2020). Acanthomyrmex species generally form small colonies
(usually with less than 100 adult individuals) in cavities in dead twigs and wood fragments, under or between stones on the forest floor. Their nests often contain deposition of small seeds that are thought to be their important food source, although they are not exclusively granivore (Moffett, 1985; Eguchi et al., 2004, 2008; Ito et al., 2017; Yamada et al., 2018). The natural history of this genus is still poorly understood, including the details of seed-foraging behavior (but see Ito et al., 2017).

In the present paper, we report our unexpected collection of 42 multi-species phasmid eggs deposited together with seeds in the four nests of Acanthomyrmex glabfemoralis Zhou & Zheng, 1997 in southern China and northern Vietnam. The most likely identifications of the collected phasmid eggs are provided based on egg morphology, with COI DNA barcodes of each egg morphospecies. Notes on the seeds deposited in these nests are also provided herewith.

MATERIALS AND METHODS

In the course of the recent field survey in Huaping National Nature Reserve, Guilin, Guangxi of China (N25.57°, E109.94°, 1000–1500 m alt.) on 6 July 2017, three colonies of A. glabfemoralis (Eg06vii17-31, AKY06vii17-03, AKY06vii17-04), whose nests contained a deposition of many seeds and phasmid eggs, were collected. The seeds and phasmid eggs in these nests were collected as many as possible and then preserved in ethanol. Additional phasmid egg specimens were collected from an additional A. glabfemoralis colony (Eg13ix17-136) collected from Mau Son, Lang Son, Vietnam (N21.846°, E106.922°, ca. 900 m alt.) on 13 September 2017. The phasmid eggs and seeds were examined in laboratory under a Nikon SMZ1270 stereomicroscope and sorted into morphospecies. The phasmid egg morphospecies were then morphologically studied and compared with historical records (Clark-Sellick, 1997, 1998) and with a large series of egg specimens of different north Vietnamese species (collection in Royal Belgian Institute of Natural Sciences (RBINS)), leading to an identification to the most possible extent.

A 658 bp DNA barcoding region of the mitochondrial COI gene was determined from representative specimens of each phasmid egg morphospecies to allow future molecular identification. Protocols for DNA barcoding were the same as those used in Yamada et al. (2018): the egg opercula were detached to extract DNA from the inside embryos. The determined sequences were searched against Barcode of Life Data Systems (BOLD: https://www.boldsystems.org) and GenBank (https://www.ncbi.nlm.nih.gov/genbank) to check if the sequences are derived from their egg parasitoids, and submitted to International Nucleotide Sequence Database (INSD: http://www.insdc.org) via DNA Data Bank of Japan (DDBJ): accession number LC601950–LC601955. The voucher specimens are deposited in the following collections: Institute of Ecology and Biological Resources, Hanoi, Vietnam; Systematic Zoology Laboratory, Tokyo Metropolitan University, Tokyo, Japan (curated by the first author); Guangxi Key Laboratory of Rare and Endangered Animal Ecology, Guangxi Normal University, Guilin, China.

RESULTS

Nesting habitat and composition of deposited phasmid eggs and seeds: The three colonies of A. glabfemoralis (Eg06vii17-31, AKY06vii17-03, AKY06vii17-04) from Huaping (Guangxi of China) were found nesting under or between stones and located nearby (with distances of several meters) on the forest floor (Fig. 1A–B). Phasmid eggs and plant seeds were found aggregated in a corner of the simple nest chamber of each nest (as indicated by a dashed circle in Fig. 1A). A total of 39 phasmid eggs of four morphospecies (“sp. 1–4” in Figs. 1–2) and 114 seeds of seven morphospecies (“sp. a–g” in Fig. 1) were collected from the three nests (Table 1). Besides, three phasmid eggs of another distinct morphospecies (“sp. 5”) were collected from another A. glabfemoralis nest (Eg13ix17-136) from Mau Son (Lang Son, Vietnam): several seeds collected from the same nest were lost.

No apparently fatal damage to the phasmid eggs, such as exposure of the inside embryo, was observed when collected. The embryos appeared at least not to be decayed or deformed in some eggs whose opercula were detached during preservation in ethanol or by dissection for DNA extraction. None of the collected eggs had an intact capitular structure, and its partial remnant was clearly recognized in some eggs of each egg morphospecies except “sp. 4” (indicated by arrows in Fig. 2) (indicated by arrows in Fig. 2, see also the morphological remarks below).

Table 1. Composition of phasmid eggs and seeds collected from the nests of Acanthomyrmex glabfemoralis colonies. Abbreviation for localities: HP, Huaping National Nature Reserve, Guilin, Guangxi of China; MS, Mau Son, Lang Son, Vietnam. n/a: data not available.

<table>
<thead>
<tr>
<th>Colony code</th>
<th>Locality</th>
<th>No. of phasmid eggs</th>
<th>No. of seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sp 1 Sp 2 Sp 3 Sp 4 Sp 5</td>
<td></td>
</tr>
<tr>
<td>Eg06vii17-31</td>
<td>HP</td>
<td>17 4 6 2 0</td>
<td>54 (4 spp.)</td>
</tr>
<tr>
<td>AKY06vii17-03</td>
<td>HP</td>
<td>0 0 3 0 0</td>
<td>41 (5 spp.)</td>
</tr>
<tr>
<td>AKY06vii17-04</td>
<td>HP</td>
<td>1 1 1 3 0</td>
<td>17 (4 spp.)</td>
</tr>
<tr>
<td>Eg13ix17-136</td>
<td>MS</td>
<td>0 0 0 0 3</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Morphological remarks and identification of the phasmid eggs: The collected phasmid egg morphospecies showed morphological affinities with the following related genera of the two lonchodid subfamilies, Necrosciinae and Lonchodinae. A summary of the identifications is shown in Table 2. Morphological remarks...
Table 2. Morphological identification of phasmid egg morphospecies collected from the nests of *Acanthomyrmex glabfemoralis* colonies.

<table>
<thead>
<tr>
<th>Phasmid egg morphospecies</th>
<th>Morphological identification</th>
<th>COI accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>sp. 1</td>
<td>Lonchodes or Phraortes</td>
<td>Lonchodinae LC601951</td>
</tr>
<tr>
<td>sp. 2</td>
<td>Lopaphus</td>
<td>Necrosciinae LC601955</td>
</tr>
<tr>
<td>sp. 3</td>
<td>Micadina</td>
<td>Necrosciinae LC601954</td>
</tr>
<tr>
<td>sp. 4</td>
<td>Pachyscia</td>
<td>Necrosciinae LC601953</td>
</tr>
<tr>
<td>sp. 5</td>
<td>Lopaphus cf. sphalerus (Redtenbacher, 1908)</td>
<td>Necrosciinae LC601950</td>
</tr>
</tbody>
</table>

Fig. 1. Nesting habitat of *Acanthomyrmex glabfemoralis* colonies in Huaping (Guangxi of China) and phasmid eggs and seeds collected from their nests. A: nest of the colony Eg06vii17-31, whose roof stone was removed; location of deposition of seeds and phasmid eggs are shown by a dashed circle. B: worker (minor, left) and soldier (major, right) of the ant species. C–E: all contents of deposited seeds and phasmid eggs in the three colonies from Huaping; C, Eg06vii17-31; D, AKY06vii17-03; E, AKY06vii17-03; phasmid eggs were indicated by black circles. F: adult male phasmid of *Micadina* sp. (Necrosciinae) photographed in Huaping by the first author, which can perhaps be conspecific with sp. 3 eggs.
Fig. 2. Phasmid egg morphospecies collected from the nests of *Acanthomyrmex glabfemoralis* colonies. A–F: eggs in dorsal, lateral, and anterior views respectively (from left); A, “sp. 1” (*Lonchodes* or *Phraortes*); B, “sp. 2” (*Lopaphus*); C, “sp. 3” (*Micadina*); D, “sp. 4” (*Pachyscia*), less-damaged egg; E, “sp. 4” (*Pachyscia*), severely damaged egg; F, “sp. 5” (*Lopaphus* cf. *sphalerus*). Arrows indicate remnants of the capitula. In the three eggs shown in D–F, their opercula were once artificially opened to extract DNA from embryos and glued after extraction. Scale bars: 1.0 mm.

Fig. 3. *Oecophylla smaragdina* workers carrying a capitulum-bearing egg of *Tirachoidea* sp. (Cilumninae), photographed in Cambodia (near Angkor temples) by Jérôme Constant (Royal Belgian Institute of Natural Sciences, Belgium) and used with permission.

of each egg morphospecies are provided below (based on comparison with historical records and reference specimens in RBINS).

The morphospecies “sp. 1” has eggs bearing a stalked capitulum. Stalked capitula are not present in the Necrosciinae, and the general morphology of the egg places the morphospecies into the Lonchodinae (Clark-Sellick, 1997). The egg closely resembles that of species in the genus *Lonchodes* Gray, 1835 or *Phraortes* Stål, 1875.

Eggs of the morphospecies “sp. 2” and “sp. 5” are believed to represent those of the genus *Lopaphus* Westwood, 1859, and the latter most resembles the egg of *L. sphalerus* (Redtenbacher, 1908). The *Lopaphus* is widespread over the Oriental realm (Brock *et al.*, 2021) and has a wide variety in egg morphology, with all species bearing a capitulum (except for *L. lampethusa* (Westwood, 1859)) (Clark-Sellick, 1997; Bragg, 1995), an exception considering that very few necrosciines possess capitular structures (Bradler *et al.*, 2014). In the case of *L. sphalerus*, the capitulum is large and discoid, and was nearly completely consumed in the conspecific or closely related “sp. 5” eggs. The other representatives of species currently attributed to the genus have a smaller, raised, and round to cylindrical capitulum placed centrally
on the operculum, as can be observed in the “sp. 2”.

Eggs of the morphospecies “sp. 3” and “sp. 4” are believed to represent those of the closely related genera Micadina Redtenbacher, 1908 and Pachyscia Redtenbacher, 1908, respectively. Some representatives of the Micadina have a capitulum-like circular structure centrally on the egg operculum. Figure 2F is the photograph of a male Micadina sp. taken by the first author in Huaping, which can perhaps be conspecific with sympatric “sp. 3” eggs. No distinctive capitulum has been observed in the Pachyscia, instead it has a central hump on the operculum. In “sp. 4” eggs, the external coating structures of the egg capsule and operculum, were damaged to different degrees: three of the four collected eggs were especially severely damaged, making their appearance somewhat distinct from the remaining (see Figs. 1, 2D–E); the COI sequences from the less-damaged egg (specimen in Fig. 2D, accession no. LC601952) and one of the severely damaged eggs (in Fig. 2E, LC601953) were nearly identical (only one site difference), supporting their conspecificity.

The inter-morphospecies genetic distances (p-distance) among the determined COI sequences from six representative specimens of the five morphospecies were 10.3–14.9% (two eggs of “sp. 4” were sequenced as mentioned above). The highest matches of these sequences against BOLD and GenBank databases were indeed phasmids. However, it did not provide reliable implications for their genus- or species-level identity due to the lack of a homologous COI barcode reference library for the Oriental phasmids. The highest matches in BOLD were as follows (searched on 9 December 2020): Phraortes illepidus (Lonchodinae) for “sp. 1” (89% similarity), Candovia annulate (Necrosciinae) for “sp. 2” (91%), Damasippus batesianus (Prisopodinae) for “sp. 3” (89%), Sipyloidea sipylus (Necrosciinae) for “sp. 4” (87%) and “sp. 5” (89%).

**DISCUSSION**

We recorded five phasmid egg morphospecies that most likely belong to some related genera of lonchodid subfamilies Necrosciinae and Lonchodinae being deposited in the nests of Acanthomyrmex glabfemoralis. The absence of an intact capitular structure in the collected phasmid eggs indicated a strong likelihood of their consumption by the ants. To our knowledge, these records are the first published field evidence for the commensality of the myrmecochory across the order. It also suggests that various ant species, including those having arboreal nests (the weaver ants are obligately arboreal and construct nests by weaving together leaves using larval silk; Hölldobler and Wilson, 1990), can be involved in the dispersal of phasmid eggs.

The seeds deposited in the nests of Acanthomyrmex ants have been reported to be fig seeds in a few studied species, and their soldiers (majors) equipped with a huge head and robust edentate mandibles (see Fig. 1B) are considered to be important for crushing fig seeds (Moffett, 1985; Ito et al., 2017; Yamada et al., 2018). Ito et al. (2017) reported that A. ferox Emery, 1893 workers (minors) harvest fig seeds exclusively from bird feces dropped on the leaves of lower vegetation. However, we found that the nests of A. glabfemoralis contained various kinds of seeds and phasmid eggs. The collected seeds appeared non-figs, although we could not identify them. Moreover, many of the seeds and phasmid eggs collected from their nests seem to be too large to be crashed by the soldier’s mandibles (soldiers of A. glabfemoralis have head width of around 2.5 mm, but see scales in Fig. 1C–E, and Fig. 2): the condition of deposited “sp. 4” (Pachyscia) eggs are particularly intriguing, because the severe damage on their coating structures of egg capsule was observed in three of the four eggs, implying a possibility that the ants might have grazed these structures.

The above circumstances suggest that workers of A. glabfemoralis may often collect seeds with an easily edible part such as elaiosome, as well as capitulum-bearing phasmid eggs, and then keep them inside their nests even after the edible parts are consumed. Considering that both egg capitula and seed elaiosomes were shown to have similar fatty acid compositions (Stanton et al., 2015), workers of A. glabfemoralis are most likely attracted to not only the former but also the latter. The workers of A. glabfemoralis are also attracted to cheese bait (Eguchi et al., 2004), and their colony can be reared for more than one year in laboratory by feeding them by cut pieces of mealworms and cockroaches (personal observation by the first author). Therefore, A. glabfemoralis seems to be a generalist that utilizes various food sources and contributes secondary dispersal of different kinds of seeds and phasmid eggs. However, the long-term fates of the seeds and phasmid eggs deposited in their nests remain unknown.

Eguchi et al. (2004) investigated foraging distances and nesting sites of various ant species in northern Vietnam and reported that the maximum foraging distance of A. glabfemoralis is 0.58 m. Thus, phasmid eggs (and seeds) found in A. glabfemoralis nests were unlikely dispersed much longer distances than this from...
the place initially dropped. The major potential advantage of myrmecochory in phasmids has been thought about survival against their predators and parasitoids rather than dispersal itself (Hughes and Westoby, 1992; Windsor et al., 1996; Goldberg et al., 2015). Two cuckoo wasp subfamilies Amiseginae and Loboscelidinae are considered to be obligatory parasitoids to phasmid eggs, and their geographic distribution overlaps worldwide with those of phasmids (Goldberg et al., 2015; Baker, 2016). Being deposited in ant nests may protect phasmid eggs from such parasitoids, but much remains to be studied. It is also noteworthy that members of the rare loboscelidinae wasps are suspected to be myrmecophilous (Riek, 1970; Kimsey, 2012; Hisasue and Mita, 2020), although their detailed biology is yet to be known.

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