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*Amanita ibotengutake* (Agaricales: Amanitaceae)

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SHORT COMMUNICATION

New host record for three scuttle flies, *Megaselia flava*, *M. kanekoi* and *M. gotoi* (Diptera: Phoridae), on the poisonous fungus *Amanita ibotengutake* (Agaricales: Amanitaceae)

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**Abstract**

We identified three species of fungivorous scuttle fly – *Megaselia flava*, *M. kanekoi* and *M. gotoi* – from eight fruit bodies of a fungus, *Amanita ibotengutake*, which has not previously been recorded as the host of these flies.

**Key words:** food habit, fungivor, fungus–insect relationship, molecular identification.

Many large, evolutionarily successful groups of insect owe much of their proliferation to fungal food sources, and fungi frequently profit from insects: insects aid fungi in spore dispersal and habitat provision, and can even supply nutrient resources (Wheeler & Blackwell 1984). Many of the secondary metabolites present in fungi are harmful or toxic to insects in general (e.g. Martin 1979; Hanski 1989), and may be strongly involved in insect–fungus interaction. Interestingly, poisonous mushrooms, such as the family Amanitaceae, which includes species toxic to mammals, have the highest proportion of polyphagous Diptera, while many edible mushrooms have the smallest proportion of polyphagous species (e.g. Kukor & Martin 1987; Hanski 1989). Hanski (1989) suggested that spatial and temporal unpredictability of resources for fungivorous insects is the force that primarily selects for polyphagy and exposes populations to a wide range of chemicals. However, little information is available on insect–fungus interaction, particularly at the species level (Disney 1994), mainly due to the difficulty and confusion of fungi taxonomy (e.g. Oda *et al.* 2002). Therefore, host information for

a fungivorous insect based on the morphologically and molecularly identified fungus at species level is much needed to promote further study of insect–fungus relationships.

*Amanita ibotengutake* is poisonous and is one of the causal agents of Pantherina syndrome in humans (Bresinsky & Besl 1990; Oda *et al.* 2002). The fruit body of this mushroom contains the neurotoxic metabolites ibotenic acid and muscimol, which are non-fatal to mammals but are highly insecticidal (Takemoto *et al.* 1964; Seeger & Stijve 1980; Oda *et al.* 2002). We collected eight fruit bodies of *A. ibotengutake* on 5 October 2002 in the Kamigamo Experimental Forest of Kyoto University (35°04'N, 135°45'E), Kyoto, Japan. In Japan, *A. ibotengutake* has long been confused with *Amanita pantherina* due to similarities in their habitat and the macromorphology of the fruit body. The species can be distinguished microscopically and through molecular phylogenetics (Oda *et al.* 2002). Subhymenial tissues are usually clamped in *A. ibotengutake*, whereas they are rarely clamped in *A. pantherina* (Jenkins 1977; Oda *et al.* 2002).

Differential interference contrast microscopy observations of tissues of voucher specimens were made in 30 mg/mL KOH. For some voucher specimens of which subhymenial tissues were difficult to observe microscopically, we analyzed the DNA sequence of the internal

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transcribed spacer (ITS) region using polymerase chain reaction (PCR) primers ITS4 and ITS5 (White *et al.* 1990). DNA preparation, PCR amplification and DNA sequencing were conducted following the methods of Oda *et al.* (1999). The resulting sequences (accession numbers AB211054–AB211057) from the voucher specimens were compared to those of *A. ibotengutake* deposited in the DDBJ/EMBL/GenBank nucleotide sequence database, and were identical to or showed high homology (more than 99.6%) with those of *A. ibotengutake*. In the laboratory, the fruit bodies of individual mushrooms were kept on moistened vermiculite in a clear polystyrene container (160 mm high, 90 mm in diameter) with a pierced cap (cap: 90 mm in diameter, hole: 30 mm in diameter). The hole was covered with two pieces of Tetron gauze (50 mm in diameter; T-No. 90T; NBC, Tokyo, Japan). Each container was kept at 20°C, 16 h light : 8 h dark, and was moistened if necessary. Within 2 weeks, 2295 adult scuttle flies (Phoridae) emerged from the four fruiting bodies. We chose the following 31 undamaged specimens for morphological identification: *Megaselia flava* (Fallén, 1823), 1♂; *M. kanekoi* Disney, 1989, 11♂ and 13♀; and *M. gotoi* Disney, 1989, 3♂ and 3♀. Many species of scuttle fly are parasitoids or specialist predators, while some species, such as those in the genus *Megaselia*, are a significant component of the dipteran fauna of fungus sporophores (Hackman & Meinander 1979; Disney 1994). *Megaselia flava* (referred to as *Ophiochaeta matsutakei* by Sasaki (1935) and *Aphiochaeta matsutakei* by Kiyoku (1958)) is a pest on the mushroom *Tricholoma matsutake* (referred to as *Armillaria matsudake* by Sasaki (1935) as cited by Disney (1994), and as the Japanese Pine mushroom by Kiyoku (1958), cited as *T. edodes* by Disney (1994)). In addition, *M. flava* has previously been reared from a wide range of fungal taxa, including *Gymnopilus hybridus*, *Leccinum scabrum*, *Pluteus cervinus*, *Russula aeruginea*, *R. heterophylla*, *Peziza micropus*, *P. vesiculosa*, *P. varia* and *P. repanda* (Disney 1994). The only previous record of this species reared from a species of Amanitaceae was from *A. muscaria* (Yakovlev 1994). *Megaselia gotoi* has been reared from *A. farinosa* and *A. spissacea*, and *M. kanekoi* has been reared from *A. spissacea* (Disney 1989). *Amanita farinosa* is phylogenetically related to *A. pantherina* and allies, including *A. ibotengutake* (Oda *et al.* 1999, 2002), and is suspected to be poisonous to humans (Seeger & Stijve 1980), but ibotenic acid and muscimol were not detected (Chilton & Ott 1976). *Amanita spissacea* is also suspected to be poisonous, but the toxic element of this fungus has not been elucidated.

The toxins ibotenic acid and muscimol were only found in *A. muscaria*, *A. pantherina* and their allies, including *A. ibotengutake* (Seeger & Stijve 1980; Oda *et al.* 2002). *Amanita spissacea* is distantly related to those species (Oda *et al.* 1999), and it is unlikely that this fungus contains those toxins. Disney (1989) summarized four *Megaselia* species that were fungivores found with *Amanita* species containing ibotenic acid and muscimol (*M. lata* and *M. rubella* from *A. muscaria*, and *M. lata*, *M. lutea* and *M. rubella* from *A. pantherina*). *Amanita ibotengutake*, which contains ibotenic acid and muscimol, thus represents a previously undescribed host of *M. flava*, *M. kanekoi* and *M. gotoi*.

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