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Thread-like appendix on *Arisaema urashima* (Araceae) attracts fungus gnat pollinators

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Plant floral traits have evolved convergently with pollinator traits (Faegri & van der Pijl, 1979), giving rise to tremendous diversity in floral forms. Floral traits associated with particular pollinators are recognized as pollination syndromes, and pollination syndromes associated with well-studied groups, such as bees, have been developed over the past 150 years (Dellinger, 2020). Nonetheless, there is a strong historical bias toward diurnal pollinators, flower color, and temperate-zone model systems in pollination syndrome studies. Given that Diptera is a morphologically and ecologically diverse lineage and many dipteran families have recently been recognized as vital pollinators, a deeper understanding of fly diversity is essential for a better understanding of niche dimensions associated with fly pollination (Raguso, 2020).

Although fly-pollinated plants are morphologically diverse, many fly-pollinated flowers exhibit similar floral traits (Mochizuki & Kawakita, 2018). For instance, long filiform appendices are common among fly-pollinated plant species, such as some species of *Arisaema* (Araceae), *Aristolochia* (Aristolochiaceae), and *Tacca* (Taccaceae) (Vogel, 1978; Zhang et al., 2005). Consequently, filiform appendices are considered an adaptation attracting fly pollinators (Vogel, 1978; Vogel & Martens, 2000; Zhang et al., 2005). In particular, filiform appendices have often been hypothesized as "the guiding paths" in plants pollinated by fungus gnats because fungus gnats usually remain on the ground and seldom fly, and they move through leaf litter by the jerky movement of their spiny legs (Vogel, 1978; Vogel & Martens, 2000). However, it remains largely unknown whether the distinctive morphological traits attract pollinators and subsequently increase reproductive success (e.g., Zhang et al., 2005). Troll (1928) even considered that a long thread-like extension of the sterile appendix in some *Arisaema* species might represent evolutionary luxuriance without ecological significance because many related species do not possess such an unusual reproductive morphology. Consequently, despite more than 100 years of speculation (Knuth, 1904; Troll, 1928), little progress has been made in determining the function of the thread-like appendix in fly-pollinated plants .

In this study, we focused on a Japanese endemic plant, *Arisaema urashima* (=*A. thunbergii* ssp. *urashima*),

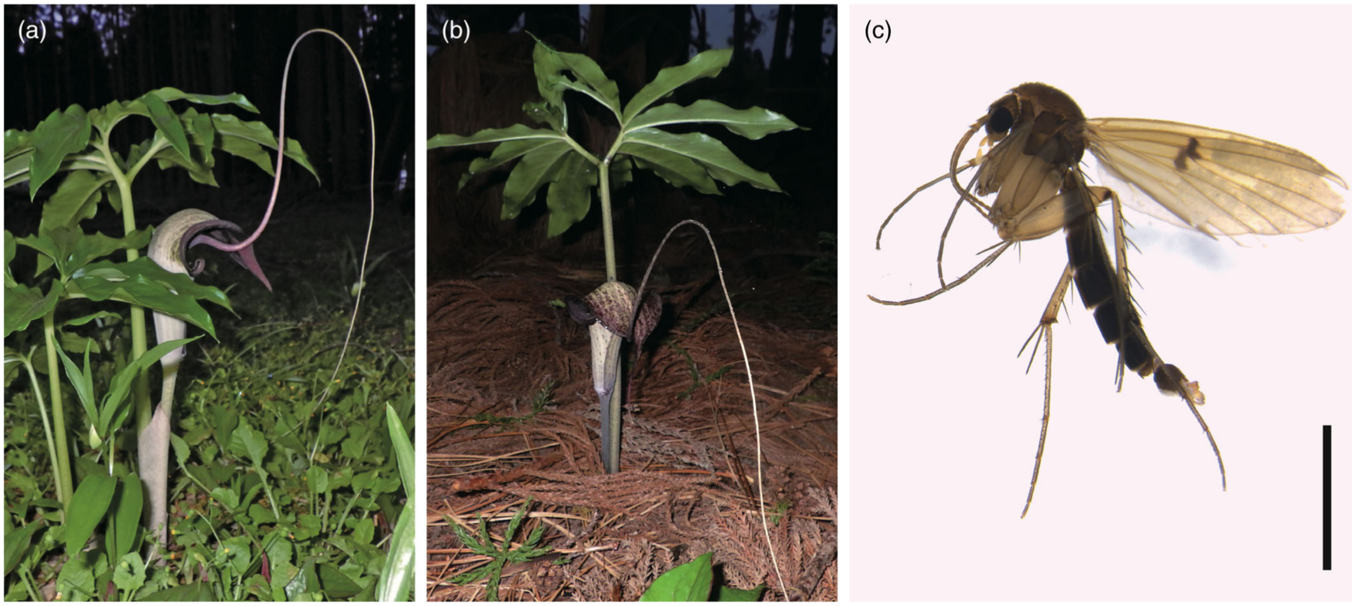

FIGURE 1 *Arisaema urashima* and its pollinators. (a, b) An *Arisaema urashima* inflorescence bearing a thread-like sterile appendix. (c) *Mycetophila ruficollis* trapped in an *A. urashima* spathe. Scale bar = 2 mm.

supposedly pollinated by fungus gnats of *Mycetophila* spp. (Mycetophilidae) (Figure 1; Tanaka et al., 2013). As with several other Araceae species, the *Arisaema* inflorescence is characterized by a spadix with fertile flowers at its base, with the upper part modified into a sterile appendix. The genus *Arisaema* possesses a lethal pollination mechanism, in which they permanently trap pollinators (mainly Mycetophilidae and Sciaridae) in the female spathes (Appendix S1: Figure S1). The appendix often plays a vital role in pollinator attraction, even when it is inconspicuous and hidden by the spathe, because the olfactory cues emitted by the appendix are the basis for the attraction (Suetsugu, 2022; Suetsugu et al., 2021). In *A. urashima*, the appendix is thread-like, 40–60 cm long, and protruding, with the upper half bent abruptly downward; it gradually thickens toward the base, transitioning into the floriferous part of the plant (Appendix S1: Figure S1). Here we conducted a removal experiment to test the impact of the thread-like appendix on pollinator attraction and reproductive success in *A. urashima* in its natural habitats. Specifically, we applied three different treatments, including (i) an unmanipulated control, (ii) thread removal, leaving an appendix the same size as that found in typical *Arisaema* species, and (iii) complete removal of the appendix (Appendix S1: Section S1).

Our experiments revealed that the most frequent insect visitors were mycetophilid fungus gnats (48.8% of all visitors), followed by the families Sciaridae (29.4%) and Drosophilidae (10.6%). These dipteran visitors (ca. 1 mm in width) are small enough to leave the inflorescences via exit holes (ca. 2 mm internal diameter) in the male spathes. Among the Mycetophilidae, *Mycetophila* spp. are the most common mycetophilid visitors (70.5% of all mycetophilids) (Appendix S1: Figure S2). Given that *Mycetophila* is known as a potential pollinator of *A. urashima* in other populations (Tanaka et al., 2013), *Mycetophila* spp. may be the primary pollinators throughout the distribution. Additionally, many other dipteran species, such as the obligately mycophagous drosophilid, *Mycodrosophila*, were trapped with the spathes. Because some fungus gnats other than *Mycetophila* and *Mycodrosophila* trapped in the female spathes carried pollen grains, they also acted as pollinators. Notably, some *Arisaema* species, such as *A. angustatum* and *A. peninsulae*, are pollinated exclusively by the males of one or two mycetophilid species, possibly through sexual deception (Suetsugu et al., 2021). However, *Mycetophila* pollinators were not skewed toward males in *A. urashima* (44.7%; 21/47). Considering the lack of male bias and visitation by diverse mycophagous insects (*Mycodrosophila* and other fungus gnats), mushroom mimicry rather than sexual deception is likely the luring mechanism in *A. urashima*. In this regard, it is interesting to note that the swollen basal part of *A. urashima* appendix visually resembles a fungal fruiting body and emits a faint musty odor that can be perceived by the human nose.

Importantly, visits by the most abundant fungus gnat, *Mycetophila*, were significantly less frequent in not only complete removal ($p < 0.001$) but also thread removal ($p < 0.05$; Figure 2) treatments. The visitation rate by other insects (both insects except fungus gnats [$p = 0.84$] and fungus gnats except *Mycetophila* [$p = 0.54$]) was not significantly reduced by thread removal, although their visitation rate significantly decreased by complete removal ($p < 0.01$; Appendix S1: Table S1 and Appendix S1: Table S2). These results suggest that the most abundant fungus gnat, *Mycetophila*, is dramatically impacted by the experimental removal of the thread-like appendix, whereas other fly visitors are affected only by the further removal of the swollen basal part of the appendix, which probably ceases the emission of the scent that attracts them to the inflorescence (Miyake & Yafuso, 2003; Suetsugu et al., 2021). An alternative explanation for our results is that the damage done by appendix removal can cause plants to emit herbivore-repellant volatiles (Howe & Jander, 2008); however, such volatiles must render flowers less attractive to both primary pollinators and other floral visitors (Suetsugu et al., 2021). Therefore, the specific decline of *Mycetophila* visits by thread removal could not have resulted solely from these volatiles. Thus, we can conclude that the appendix appeals specifically to *Mycetophila* spp. in *A. urashima*.

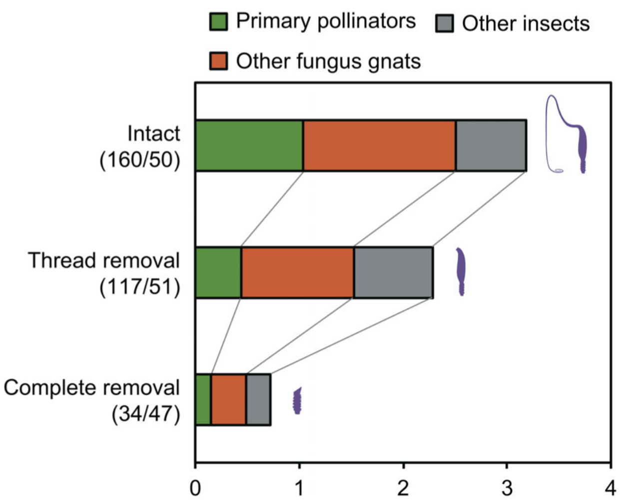

FIGURE 2 Visitation frequencies of floral visitors following thread or complete appendix removal of *Arisaema urashima*. *X*-axis: Mean number of insects trapped per spathe. The number of insect individuals and spathes examined is indicated in parentheses.

Additionally, we have found that there is a significant reduction in the fruit set (42.2 ± 24.0% [$n = 5$] vs. 78.7 ± 12.3% [$n = 7$]; average ± SD %, $p < 0.01$) and seed set (11.4 ± 19.4% [$n = 1076$] vs. 36.5 ± 29.0% [$n = 1005$]; average ± SD %, $p < 0.001$; Figure 3) when the thread-like appendix is removed. Therefore, our results demonstrate that the thread is essential for the reproductive fitness of *A. urashima*. Given that its removal did not alter the frequency of visits by other fly species, the reproductive success must be primarily affected by *Mycetophila* visitation, further supporting the idea that *Mycetophila* fungus gnats are likely the most important pollinators. Although fungal gnats were historically considered ineffective pollinators, owing to their small size and poor flight ability (but also see Mochizuki & Kawakita, 2018), fungus gnat pollination will be advantageous in the moist and shady forest understory where *A. urashima* grows. Most other plant species pollinated by fungus gnats are also associated with moist and shady environments where fungus gnats are abundant throughout the year and where other common pollinators, such as bees, are less abundant (Mochizuki & Kawakita, 2018; Suetsugu & Sueyoshi, 2018; Vogel, 1978).

In conclusion, our study indicates that the thread-like appendix of *A. urashima* is critical in attracting *Mycetophila* fungus gnat pollinators and, thus, in increasing fruit and seed set (Appendix S1: Figure S3). It is noteworthy that many fly-pollinated plants, such as *Aristolochia* and *Tacca*,

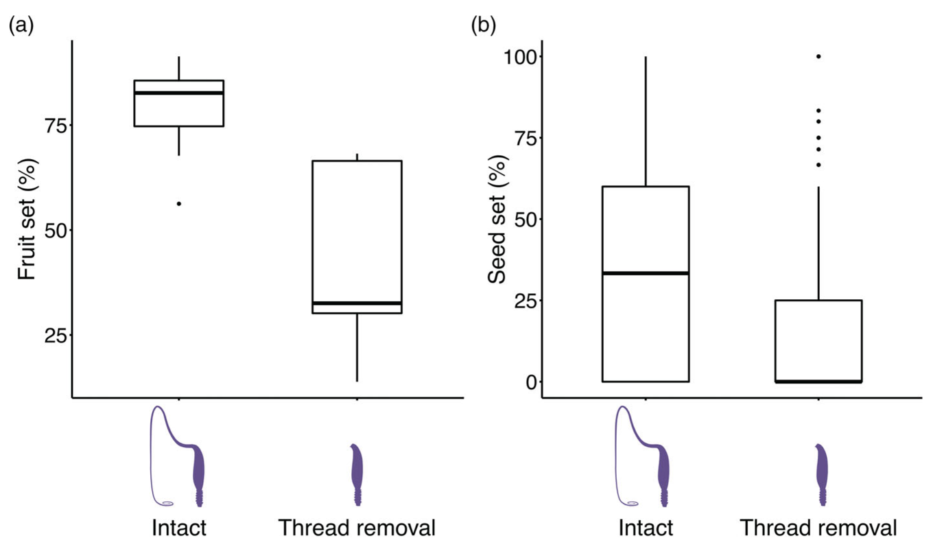

FIGURE 3 Effect of thread-like appendix removal on (a) fruit set and (b) seed set of *Arisaema urashima*.

have evolved a thread-like appendix independently and globally (Vogel, 1978; Zhang et al., 2005). The convergent evolution of a thread-like appendix and our demonstration of its functional significance strongly suggest that the filiform appendix is a widespread adaptive trait that impacts fly pollinator attraction. Nonetheless, the mechanism by which the filiform appendix attracts pollinators remains unclear. Given that some fungus gnat–pollinated flowers are thought to attract pollinators visually (Gaskett, 2011), it may act as a visual attraction. Alternatively, the filiform appendix may improve scent dissipation, considering that floral scent has a vital role in attracting fungus gnats in some plants (Okamoto et al., 2015). Therefore, further investigation is needed to determine how the thread-like appendix attracts pollinators. For example, after removing an actual thread-like appendix, attaching a nonliving thread-like object would verify whether it helps fungus gnats crawl on the thread even without scent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Suetsugu et al., 2022) are available in Figshare: https://doi.org/10.6084/m9.figshare.19386980.v1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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