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研究課題名（和文）花に隠された生物多様性：蕾利用タマバエ類の多様性と生態系機能の解明

研究課題名（英文）Biodiversity hidden in flowers: Elucidation of the diversity of gall midges inhabiting flower buds and their ecosystem functions

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：本研究では、2022年および2023年に多数の植物の花や花芽に生息するタマバエを調査しました。収集されたタマバエ幼虫の分子解析により、数十種のタマバエの存在が確認されました。収集されたタマバエの大多数は、単一の植物種に関連する花芽を利用していましたが、中には2種類以上の植物種の花芽に見られる寡食性または多食性の種も存在しました。分類学的には、花芽に生息するタマバエはCecidomyiinae亜科の少なくとも5つの族に属しています。これらの結果は、種子植物の花芽を利用する特性がCecidomyiidae内で複数回独立して進化したことを強く示唆しています。

研究成果の学術的意義や社会的意義
Gall midges (Diptera: Cecidomyiidae) are estimated to be the most diverse insect taxon, with up to 1.8 million species estimated to exist worldwide. However, little is known about gall midge diversity and ecological functions. This research sheds light on their diversity in flower buds.

研究成果の概要（英文）：In Japan, about 600 sorts of galls formed by gall midges (Diptera: Cecidomyiidae) are known so far after decades of intensive research, including those unidentified species. In this study, gall midges were investigated in the flowers and flower buds of numerous plants in 2022 and 2023.The molecular analyses of the collected gall midge larvae indicated the presence of dozens of gall midge species. Of the collected gall midges, the majority of species utilize flower buds associated with a single plant species, while some are oligo or polyphagous, found on flower buds of two or more plant species. Taxonomically, the gall midges inhabiting flower buds belong to at least five tribes within the Cecidomyiinae subfamily. These results strongly suggest that the trait of utilizing the flower buds of seed plants has evolved independently multiple times within Cecidomyiidae.

研究分野：生産環境農学およびその関連分野

キーワード：Cecidomyiidae　Biodiversity　Diptera　タマバエ　ハエ目　多様性

１．研究開始当初の背景

Insects are the most adaptively radiated group in terrestrial ecosystems, having evolved various characteristics in response to their environment and resources. A recent DNA barcoding study revealed that the biodiversity of the fly family Cecidomyiidae (gall midges) is extraordinary, with up to 1.8 million species worldwide (Hebert et al. 2016). Previously, the highest estimated number of insect species was in the order Coleoptera (beetles), with 1.5 million species across 142 families. Therefore, Cecidomyiidae alone surpasses this estimate, suggesting that Cecidomyiidae is the most speciose animal taxon on Earth.

Most known species of Cecidomyiidae are responsible for the induction of conspicuous abnormal structures, termed “galls”. The species-specific shape of galls attracted the attention of many researchers over decades, and they have been extensively studied (Yukawa & Tokuda 2021). However, only about 7,000 species are currently known, representing a mere 0.4% of the estimated total number of species (Gagné & Jaschhof 2021). Such a small number of known gall-forming species cannot explain the extraordinary diversity of Cecidomyiidae, suggesting that the family harbors significant unexplored hidden diversity.

Several examples of hidden gall midges whose larvae develop on/in their hosts without showing remarkable signs of infestation were reported recently (Fig. 2; Dorchin & Freidberg 2008; Dorchin et al. 2022; Elsayed 2023; Elsayed et al. 2015, 2018, 2021, 2023; Elsayed & Kawakita 2022a,b), but no studies focused on their real diversity and evolutionary scenarios were done so far. Through the applicant’s recent investigations on pollination by gall midges (e.g. Elsayed & Kawakita 2022b), he noticed many undescribed gall midge species develop in flowers and flower buds, and some of them have pollination importance to their hosts. Since gall midges were established in the Upper Cretaceous concordant with the major radiation of angiosperms (Dorchin et al. 2019), flowers must have played an important role in the diversification of gall midges. Thus, inventories on gall midges in flowers and flower buds is a necessary step toward understanding the reasons behind their extraordinary diversification and potential critical roles in ecosystems.

２．研究の目的

A. Clarification of diversity of gall midges (Diptera: Cecidomyiidae) that develop in the flower buds of various and unravel their phylogenetic relationship based on molecular

analyses.

B. Description of new species of gall midges obtained through laboratory rearing experiments and morphological comparisons.

３．研究の方法

Flowers and flower buds were collected randomly from numerous plants of in various localities in Japan in 2022 and 2023. Samples were kept in plastic bags in the laboratory until the emergence of larvae. Most larvae of each gall midge species emerged were preserved in 99.55 ethanol for morphological examination and molecular analyses. Some larvae were transferred to rearing cups to rear adults following the technique outlined in Elsayed et al. (2018).

Total DNA was extracted from 504 gall midge larval specimens using the DNeasy Blood and Tissue kit (Qiagen, Tokyo, Japan) following the manufacturer's protocol, with a few modifications following Elsayed et al. (2023). PCR amplification was performed using the KOD FX Neo kit (Toyobo Co., LTD, Osaka, Japan), TaKaRa Ex Taq (Takara Bio Inc., Shiga, Japan), and using the following primer set: Forward: J-1718 (5'–GGA GGA TTT GGA AAT TGA TTA GTT CC–3') (Simon et al. 1994) and reverse: COIA (5'–CCC GGT AAA ATT AAA ATA TAA ACT TC–3') (Funk et al. 1995). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and submitted as a premix with water and primers to the FASMAC Corporation (Kanagawa, Japan) for Sanger sequencing.

The sequence data obtained were aligned with FFT-NS-1 algorithm in MAFFT ver. 7 online servers (https://mafft.cbrc.jp/alignment/server/). Genetic distances were calculated in MEGA11 using Kimura 2-parameter model (Tamura et al., 2021). Gall midge species were separated into operational taxonomic unites (OTUs) based on variations of 3% in the COI sequences.

４．研究成果

Among the collected flower and flower bud samples, over 50% contained at least one gall midge species without showing any signs of infestation. This indicates that collecting random samples of healthy-like flower buds and flowers guarantees discovering gall midges with over 50% success rate (Elsayed et al. in prep.).

The molecular analyses of the collected gall midge larvae indicated the presences of dozens of gall midge species. Of the collected gall midges, the majority of species utilize flower buds associated with a single plant species, while some are oligo or polyphagous, found on flower buds of two or more plant species. Taxonomically, the gall midges

inhabiting flower buds belong to at least five tribes within the Cecidomyiinae subfamily. These results strongly suggest that the trait of utilizing the flower buds of seed plants has evolved independently multiple times within Cecidomyiidae (Elsayed et al. in prep.).

Among the collected species, two new species and a new genus were described and published (Elsayed et al. 2023; Elsayed 2023). However, the species identification through morphological comparisons and molecular phylogenetic analyses, as well as reporting the distribution information, host ranges, annual generations, and the seasonal emergence patterns of dozens of species is ongoing (Elsayed in prep.).

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　0件／うち国際共著　0件／うちオープンアクセス　0件）

１．著者名 Elsayed Ayman Khamis、Nemoto Shuichi、Tokuda Makoto	４．巻 26
２．論文標題 Pseudasphondylia ishigakiensis, a new gall midge species (Diptera: Cecidomyiidae) from Japan developing in flower buds of Melicope triphylla (Rutaceae), with notes on its life history	５．発行年 2023年
３．雑誌名 Journal of Asia-Pacific Entomology	６．最初と最後の頁 102107～102107
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.aspen.2023.102107	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Elsayed Ayman Khamis	４．巻 zlad152
２．論文標題 Integrative taxonomy reveals a new gall midge genus and species (Diptera: Cecidomyiidae) developing in the flower buds of Pongamia pinnata (Fabaceae) in Japan	５．発行年 2023年
３．雑誌名 Zoological Journal of the Linnean Society	６．最初と最後の頁 1～13
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/zoolinnean/zlad152	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Elsayed Ayman Khamis、Kobayashi Yasuko、Gagne Raymond J.、Tokuda Makoto	４．巻 37
２．論文標題 New hypothesis of the tribal placement puzzle of Enigmadiplosis (Diptera: Cecidomyiidae), with description of a new pest species on Rhododendron (Ericaceae) in Japan	５．発行年 2023年
３．雑誌名 Invertebrate Systematics	６．最初と最後の頁 782～796
掲載論文のDOI（デジタルオブジェクト識別子） 10.1071/IS23033	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計4件（うち招待講演　3件／うち国際学会　0件）

１．発表者名 Ayman Khamis Essayed, Ko Mochizuki, Atsushi Kawakita
２．発表標題 Gall midges associated with flowers of Dioscorea tokoro
３．学会等名 The 79th Annual Meeting of the Entomological Society of Japan（招待講演）
４．発表年 2022年

１．発表者名
Ayman Khamis Essayed, Ko Mochizuki, Atsushi Kawakita

２．発表標題
Gall midges associated with flowers of Dioscorea tokoro

３．学会等名
佐賀昆虫同好会（招待講演）

４．発表年
2023年

１．発表者名
南　瑶・古川　晶啓・Ayman Khamis Elsayed・徳田　誠

２．発表標題
マレーズトラップによるタマバエ類の捕獲消長佐賀県の森林と開放地での比較（予報）

３．学会等名
2022年度九州・沖縄昆虫研究会大会（招待講演）

４．発表年
2022年

１．発表者名
Elsayed A.K.

２．発表標題
Untold secret of gall midges in angiosperm flowers

３．学会等名
Joint Seminar between China Agricultural University and Saga University in Entomology

４．発表年
2024年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関