



# Unveiled species diversity of moss-feeding mites (Stigmaeidae: *Eustigmaeus*): a research on their distribution, habitat, and host plant use in Japan

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## Abstract

The genus *Eustigmaeus* Berlese, 1910 represents the unique phytophagous group within the superfamily Raphignathoidea. Four species within this genus have been known to inhabit mosses and feed on them as larvae, nymphs, and adults. However, the interactions with mosses have remained poorly understood. In order to reveal the diversity and host-plant use of the moss-feeding species, we conducted an extensive field study in Japan. This study revealed an array of moss-feeding species inhabiting various moss species, with 10 morphologically distinctive species newly documented in Japan. Through DNA barcoding based on cytochrome *c* oxidase subunit I (COI) sequences, these morphospecies were recovered as distinct entities. Notably, the host-plant use of four species was elucidated. Among these, *Eustigmaeus* sp. 9 exhibited polyphagy, while three species (*Eustigmaeus* spp. 1–3) demonstrated varying degrees of host specificity, each using moss species from the Hypnales, *Philonotis*, and Dicranidae, respectively. While a few moss-feeding species were frequently found in the same geographic area, more than one species rarely co-occurred within the same moss colonies. *Eustigmaeus* offers a unique study system, with its diverse moss-feeding species and indications of specific host plant use. Consequently, the moss-feeding *Eustigmaeus* serves as a valuable model for exploring the macroevolutionary patterns underlying diversification in moss-feeding arthropods.

**Keywords** Phytophagy · Raphignathoidea · Behavior · Bryophyte · Host specificity · DNA barcoding

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## Introduction

Obligate phytophagy has independently evolved several times within the Acariformes (Lindquist 1998). The obligate phytophagous mites typically exhibit a suite of adaptations to their host plants, including specialized mouthparts (De Lillo et al. 2001). Particularly, two superfamilies, Tetranychoidae and Eriophyoidea, have achieved enormous diversity in their interactions with vascular plants (Zhang et al. 2011). These groups have been relatively well-studied, primarily owing to their economic impact as agricultural pests (Lindquist and Oldfield 1996; Bolland et al. 1998). In contrast, the biology of other obligate phytophagous mite groups within Heterostigmata, Raphignathoidea, and Eupodoidea, remains poorly understood (Krantz and Lindquist 1979; Lindquist 1998). We herein focus on an obligate phytophagous group in Raphignathoidea that inhabits mosses (Bryophyta).

The genus *Eustigmaeus* Berlese, 1910 (Prostigmata: Stigmaeidae) is the unique phytophagous group within the family Stigmaeidae under the superfamily Raphignathoidea, which primarily comprises predatory species (Fan and Zhang 2005). *Eustigmaeus* mites are characterized by their reddish coloration and sluggish movement, with a reproduction strategy involving arrhenotokous parthenogenesis (Gerson 1972). Their ranges encompass all ecozones except the Antarctic (Fan et al. 2016). Among the more than 120 known species, at least 70 species have been collected from substrates including mosses (Fan et al. 2016; Beron 2020; Bizarro et al. 2020; Khaustov 2021a, b; Khaustov et al. 2023). Notably, Gerson (1972) collected 11 species inhabiting mosses, and the rearing experiments clarified that four of these species, *E. frigidus* (Habeeb, 1958), *E. rhodomelus* (Koch, 1841), *E. clavatus* (Canestrini and Fanzago, 1876), *E. schusteri* (Summers and Price 1961), were moss-feeders. These mites complete their entire life cycle on mosses (Gerson 1972), utilizing moss patches as sources of food for their larvae, nymphs, and adults and even as mating sites. Their feeding strategy involves piercing the cell walls of moss leaves with their chelicerae and extracting the cell contents (Gerson 1972). After Gerson's (1972) work, some *Eustigmaeus* species were inferred to be moss-feeding based on their habitats and the presence of green gut contents (Flechtmann 1985; Swift 1994). However, the diversity of moss-feeding species and their host plant use remains veiled. Moss gametophytes, including leaves and stems, exhibit diverse morphology, habitat preferences, and chemistry across different lineages (Ueno et al. 2009). This diversity may create opportunities for specialized host-plant use, akin to some bryophyte-feeding insects (Imada et al. 2011; Imada and Kato 2016; Imada 2021; Kato et al. 2022). In fact, Gerson (1972) noted variations in the performance of *E. frigidus* on different moss species. The moss-feeding species can thus be more diverse than previously thought and potentially be host specific.

Revealing moss-mite interactions is inherently challenging, primarily due to the minuscule size of mites and the intricate structure of moss colonies. Moss colonies often form complex life forms, including cushions, mats, and tufts, where various moss species coexist. However, our extensive fieldwork across Japan, together with the meticulous host-plant identification through the observation of mite habit, has revealed a diversity of moss-feeding species of *Eustigmaeus* and their varying degrees of host specificity.

## Materials and methods

### Sampling

To reveal the diversity of moss-feeding *Eustigmaeus* species, extensive fieldwork was conducted mainly in Western Japan, with additional sampling in some locations in Central and Eastern Japan, from March 2021 to May 2022. This approach was informed by preliminary assessments conducted between April and December 2020, which revealed that *Eustigmaeus* mites were typically found within moss colonies of varying sizes situated in consistently moist microhabitats, e.g., areas along riverbanks, streams, pavement roads, and mountainous pathways. Consequently, our focus was on moss colonies thriving in such moisture-rich environments. Furthermore, moss patches where the leaves of the moss were fully spread out and in a wet condition were targeted, as these were the preferred habitats of the mites.

Mosses were sampled by hand and carefully placed in plastic containers (NK200, Risu-pack Inc.). Each container was sealed in a plastic bag to prevent the potential mixing of mites originating from different moss patches. To ensure the purity of the samples, great care was taken to collect moss colonies composed of a single moss species from the same patch. Even in such diligent collection, occasionally, a moss colony that appeared to consist of a single moss species might contain a few other species. A colony size of 300–1000 cm<sup>3</sup> (in field conditions) was sampled for each moss species. At a locality, a patch was defined as a spatially isolated moss colony composed of one or more moss species, assigning each patch a unique “patch code” (Table 1). Occasionally, a moss patch was ill-defined in case the specific type of substrate or microhabitat where the moss colonies overlain was continuous at a moderately large spatial scale; patches were then distinguished when each cluster was several meters apart and a patch code was assigned to each of them. In case the cooccurrence of multiple moss species within a patch was evident (e.g., patch code AH shown in Fig. 1e), each species was segregated by allocating them into different plastic containers (Table 1). When the same moss species was encountered in different patches within a locality, the colonies were placed in separate plastic containers. After completing the sampling, the characteristics of the habitats and substrates at each patch were documented. Subsequently, the sealed samples were transported to our laboratory, where they were stored in darkness at 4 °C until they were subjected to microscopic analysis.

### Confirmation of moss-feeding habit and determination of host plant

In addition to four moss-feeding species, *Eustigmaeus* contains six insect parasite species (Chaudhri 1965; Abonnenc 1970; Zhang and Gerson 1995; Reeves et al. 2008; Majidi et al. 2019), and the rest of the known species in the genus have unknown feeding habits. Additionally, some *Eustigmaeus* species have been collected from habitats other than moss colonies (Fan et al. 2016). Thus, not all moss-inhabiting species of *Eustigmaeus* are moss-feeders. The following method ensures not only the confirmation of the moss-feeding habit of the species, but also the determination of their precise host plants within moss colonies, which can sometimes consist of multiple moss species.

For the collected living mites, the moss-feeding habit was determined and their host plants were identified by closely observing them on moss shoots. Initially, a moss colony enclosed in a plastic container was placed under a stereomicroscope (Leica M125C), and

**Table 1** Information on sampling details and host plant records

| Eusigmæus species | Locality code | Prefecture | Elevation (m) | Sampling date | Patch code | Habitat   |              | Host plant    |                   | Genus                  | Species            |                    |
|-------------------|---------------|------------|---------------|---------------|------------|-----------|--------------|---------------|-------------------|------------------------|--------------------|--------------------|
|                   |               |            |               |               |            | Substrate | Environment  | Order         | Family            |                        |                    |                    |
| sp. 1             | 8             | Tokushima  | 1360          | 2021/5/22     | A          | a         | a            | Hymenales     | Brachytheciaceae  | <i>Rhynchosstegium</i> | <i>riparioides</i> |                    |
|                   | 9             | Tokushima  | 1100          | 2021/5/22     | B          | a         | a            | Hymenales     | Brachytheciaceae  | <i>Rhynchosstegium</i> | <i>riparioides</i> |                    |
|                   | 18            | Tottori    | 540           | 2021/3/29     | C          | a         | a            | Hymenales     | Brachytheciaceae  | <i>Sciuro-hypnum</i>   | <i>plumosum?</i>   |                    |
|                   | 18            | Tottori    | 540           | 2021/3/29     | C          | a         | a            | Hymenales     | Brachytheciaceae  | <i>Rhynchosstegium</i> | <i>riparioides</i> |                    |
|                   | 21            | Wakayama   | 60            | 2021/10/12    | D          | a         | b            | Hymenales     | Brachytheciaceae  | <i>Rhynchosstegium</i> | <i>riparioides</i> |                    |
|                   | 21            | Wakayama   | 60            | 2021/10/12    | E          | a         | b            | Hymenales     | Entodontaceae     | <i>Enodon</i>          | <i>luridas</i>     |                    |
|                   | 22            | Wakayama   | 90            | 2021/10/11    | F          | a         | a            | Hymenales     | Brachytheciaceae  | <i>Rhynchosstegium</i> | <i>riparioides</i> |                    |
|                   | 31            | Gifu       | 590           | 2022/4/20     | G          | a         | a            | Hymenales     | Brachytheciaceae  | <i>Rhynchosstegium</i> | <i>riparioides</i> |                    |
|                   | 31            | Gifu       | 590           | 2022/4/20     | H          | a         | a            | Hymenales     | Brachytheciaceae  | <i>Sciuro-hypnum</i>   | <i>plumosum</i>    |                    |
|                   | 36            | Saitama    | 1140          | 2021/10/27    | I          | a         | a            | Hymenales     | Brachytheciaceae  | <i>Rhynchosstegium</i> | <i>riparioides</i> |                    |
|                   | sp. 2         | 2          | Kochi         | 860           | 2021/9/27  | J         | b            | c             | Bryales           | Mniaceae               | <i>Pohlia</i>      | sp.                |
|                   |               | 5          | Ehime         | 630           | 2021/3/1   | K         | b            | c             | Bartramiales      | Bartramiaceae          | <i>Philonotis</i>  | <i>falcata</i>     |
|                   |               | 6          | Tokushima     | 470           | 2021/7/31  | L         | d            | d             | Bartramiales      | Bartramiaceae          | <i>Philonotis</i>  | <i>turneriana</i>  |
|                   |               | 7          | Tokushima     | 270           | 2021/7/31  | M         | d            | d             | Bartramiales      | Bartramiaceae          | <i>Philonotis</i>  | <i>turneriana</i>  |
|                   |               | 10         | Tokushima     | 600           | 2021/9/11  | N         | e            | c             | Bartramiales      | Bartramiaceae          | <i>Philonotis</i>  | <i>turneriana?</i> |
|                   |               | 11         | Tokushima     | 420           | 2022/5/7   | O         | a            | b             | Bartramiales      | Bartramiaceae          | <i>Philonotis</i>  | <i>falcata</i>     |
|                   |               | 13         | Tokushima     | 50            | 2021/9/11  | P         | a            | b             | Bartramiales      | Bartramiaceae          | <i>Philonotis</i>  | <i>turneriana</i>  |
| 13                |               | Tokushima  | 50            | 2021/9/11     | Q          | d         | b            | Bartramiales  | Bartramiaceae     | <i>Philonotis</i>      | <i>falcata</i>     |                    |
| 14                |               | Hiroshima  | 10            | 2021/3/26     | R          | a         | b            | Bartramiales  | Bartramiaceae     | <i>Philonotis</i>      | <i>hustata</i>     |                    |
| 23                |               | Wakayama   | 80            | 2021/10/11    | S          | d         | c            | Bartramiales  | Bartramiaceae     | <i>Philonotis</i>      | <i>thwaitesii</i>  |                    |
| 24                |               | Wakayama   | 270           | 2021/10/10    | T          | b         | d            | Bartramiales  | Bartramiaceae     | <i>Philonotis</i>      | <i>falcata</i>     |                    |
| 27                | Shizuoka      | 180        | 2022/4/22     | U             | b          | c         | Bartramiales | Bartramiaceae | <i>Philonotis</i> | <i>falcata</i>         |                    |                    |
| 28                | Shizuoka      | 250        | 2022/4/21     | V             | d          | c         | Bartramiales | Bartramiaceae | <i>Philonotis</i> | <i>falcata</i>         |                    |                    |

Table 1 (continued)

| Eusigmaeus species | Locality code | Prefecture | Elevation (m) | Sampling date | Patch code | Habitat   |             |           | Host plant    |                  |                   | Species          |
|--------------------|---------------|------------|---------------|---------------|------------|-----------|-------------|-----------|---------------|------------------|-------------------|------------------|
|                    |               |            |               |               |            | Substrate | Environment | No. Indiv | Order         | Family           | Genus             |                  |
|                    |               |            |               |               |            |           |             |           |               |                  |                   |                  |
| sp. 3              | 1             | Kochi      | 100           | 2021/4/18     | X          | a         | a           | 2         | Pottiales     | Pottiaceae       | Chionoloma        | subarcticusculum |
|                    | 12            | Tokushima  | 280           | 2021/5/22     | Y          | d         | c           | 5         | Pottiales     | Pottiaceae       | Trichostomum      | brachydontium    |
|                    | 16            | Hiroshima  | 370           | 2021/3/30     | Z          | d         | d           | 3         | Dicranales    | Amphidiaceae     | Amphidium         | mougoieiti       |
|                    | 16            | Hiroshima  | 370           | 2021/3/30     | AA         | d         | d           | 6         | Pottiales     | Pottiaceae       | Chionoloma        | orthodontium     |
|                    | 17            | Hyogo      | 550           | 2021/3/29     | AB         | d         | c           | 2         | Dicranales    | Amphidiaceae     | Amphidium         | mougoieiti       |
|                    | 17            | Hyogo      | 550           | 2021/3/29     | AB         | d         | c           | 1         | Pottiales     | Pottiaceae       | Chionoloma        | tenuirostre      |
|                    | 17            | Hyogo      | 550           | 2021/3/29     | AB         | d         | c           | 1         | Bartramiales? | Bartramiales?    | Philonis?         | sp.              |
|                    | 17            | Hyogo      | 550           | 2021/3/29     | AB         | d         | c           | 1         | Pottiales     | Pottiaceae       | Chionoloma        | subarcticusculum |
|                    | 19            | Hyogo      | 430           | 2021/3/29     | AC         | d         | d           | 6         | Dicranales    | Amphidiaceae     | Amphidium         | mougoieiti       |
|                    | 19            | Hyogo      | 430           | 2021/3/29     | AD         | d         | d           | 10        | Pottiales     | Pottiaceae       | Anoetangium       | thomsonii        |
|                    | 26            | Nara       | 350           | 2021/10/13    | AE         | e         | c           | 1         | Dicranales    | Dicranaceae      | Ceratodon         | purpureus        |
|                    | 26            | Nara       | 350           | 2021/10/13    | AE         | e         | c           | 1         | Hymenales     | Brachytheciaceae | Brachythecium     | helminthocladium |
|                    | 30            | Shizuoka   | 1000          | 2022/4/21     | AF         | d         | c           | 2         | Pottiales     | Pottiaceae       | Anoetangium       | thomsonii        |
|                    | 33            | Saitama    | 900           | 2021/10/27    | AG         | d         | c           | 1         | Pottiales     | Pottiaceae       | Anoetangium       | thomsonii        |
| sp. 4              | 4             | Ehime      | 1020          | 2021/3/16     | AH         | b         | c           | 7         | Hymenales     | Hymenaceae       | Gollania          | ruginosa         |
|                    | 29            | Shizuoka   | 1460          | 2022/4/19     | AI         | f         | d           | 1         | Hymenales     | Brachytheciaceae | Okamuraea         | hakoniensis      |
| sp. 5              | 4             | Ehime      | 1020          | 2021/3/16     | AH         | b         | c           | 7         | Bryales       | Bryaceae         | Rosulabryum       | capillare        |
|                    | 4             | Ehime      | 1020          | 2021/3/16     | AH         | b         | c           | 1         | Bryales       | Bryaceae         | Bryum             | paradoxum        |
|                    | 4             | Ehime      | 1020          | 2021/3/16     | AH         | b         | c           | 3         | Hymenales     | Hymenaceae       | sp.               | sp.              |
| sp. 6              | 8             | Tokushima  | 1360          | 2021/5/22     | AJ         | b         | c           | 8         | Hymenales     | Brachytheciaceae | Brachythecium     | rivulare         |
|                    | 8             | Tokushima  | 1360          | 2021/5/22     | AK         | c         | c           | 2         | Hymenales     | Brachytheciaceae | Brachythecium     | rivulare         |
| sp. 7              | 32            | Nagano     | 1350          | 2022/4/20     | AL         | a         | a           | 2         | Hymenales     | Pyralisaceae     | Pseudohygrohypnum | engyrium         |
|                    | 35            | Saitama    | 1030          | 2021/10/27    | AM         | a         | b           | 2         | Hymenales     | Pyralisaceae     | Pseudohygrohypnum | engyrium         |
|                    | 35            | Saitama    | 1030          | 2021/10/29    | AN         | a         | a           | 1         | Hymenales     | Enodontaceae     | Enodon            | luridus          |

**Table 1** (continued)

| <i>Eusigmæus</i> species | Locality code | Prefecture | Elevation (m) | Sampling date | Patch code | Habitat   |             | Host plant    |                  | Species                               |
|--------------------------|---------------|------------|---------------|---------------|------------|-----------|-------------|---------------|------------------|---------------------------------------|
|                          |               |            |               |               |            | Substrate | Environment | Order         | Family           |                                       |
| sp. 8                    | 20            | Tottori    | 760           | 2021/3/27     | AO         | a         | a           | Hymenales     | Brachytheciaceae | <i>Brachythecium rivulare</i>         |
| sp. 9                    | 2             | Kochi      | 860           | 2021/9/27     | J          | b         | c           | Bryales       | Mniaceae         | <i>Pohlia</i> sp.                     |
| 3                        | Ehime         | 1520       | 2021/10/2     | AP            | c          | c         | b           | Bryales       | Bryaceae         | <i>Bryum paradoxum</i>                |
| 11                       | Tokushima     | 420        | 2022/5/7      | O             | a          | b         | b           | Bryales       | Bryaceae         | <i>Bryum cyclophyllum</i>             |
| 13                       | Tokushima     | 50         | 2021/9/11     | P             | a          | a         | b           | Bartramiiales | Bartramiaceae    | <i>Philonotis falcata</i>             |
| 13                       | Tokushima     | 50         | 2021/9/11     | P             | a          | a         | b           | Bryales       | Bryaceae         | <i>Bryum cyclophyllum</i>             |
| 15                       | Hiroshima     | 40         | 2021/3/26     | AQ            | a          | a         | a           | Bartramiiales | Bartramiaceae    | <i>Philonotis falcata</i>             |
| 15                       | Hiroshima     | 40         | 2021/3/26     | AQ            | a          | a         | a           | Bryales       | Bryaceae         | <i>Bryum cyclophyllum</i>             |
| 26                       | Nara          | 350        | 2021/10/13    | AE            | e          | e         | c           | Hymenales     | Brachytheciaceae | <i>Brachythecium helminthocladium</i> |
| 34                       | Saitama       | 700        | 2021/10/27    | AR            | a          | a         | a           | Hymenales     | Brachytheciaceae | <i>Rhynchostegium riparioides</i>     |
| 35                       | Saitama       | 1030       | 2021/10/27    | AS            | c          | c         | c           | Bryales       | Bryaceae         | <i>Bryum recurvulum</i>               |
| sp. 10                   | 25            | Mie        | 100           | 2021/10/14    | AT         | e         | d           | Pottiales     | Pottiaceae       | <i>Weisia</i> sp.                     |
| 25                       | Mie           | 100        | 2021/10/14    | AT            | e          | e         | d           | Hymenales     | Leskeaceae       | <i>Haplocladium microphyllum</i>      |

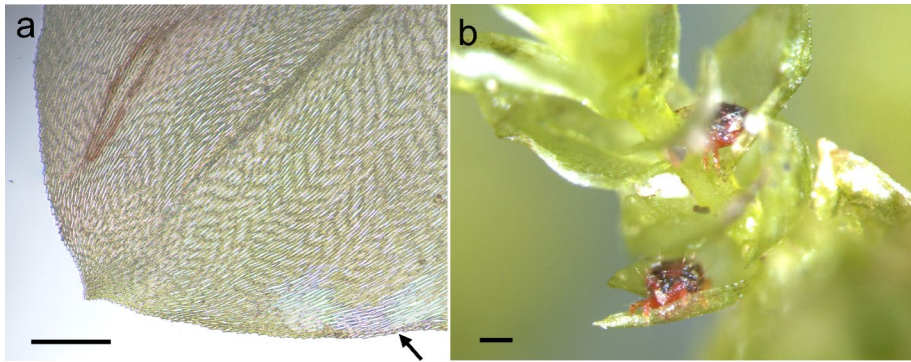
Substrates are categorized as follows: wet stone/rock/boulder (a); wet/dripping concrete wall (b); wet/dripping concrete face (c); wet/dripping rock face (d); wet soil (e); wet tree root (f). Surrounding environments are categorized as follows: the substrates in the stream/river (a), by the stream/river (b), along the pavement road (c), and the mountain path (d). Locality and patch codes correspond to those in the Supplementary Material Table S1



**Fig. 1** Various habitats of moss-feeding *Eustigmaeus* mites. **a** Patch code A (arrow). **b** Patch code K. **c** Patch code AG. **d** Patch code AI. **e** Patch code AH. **f** Patch code AM. **g** Patch code AP (arrow). **h** Patch code AE (arrow). **i** Patch code AT

a mite individual within the colony was searched with tweezers. Before observation, we ensured that the mites were active by incubating the moss colonies at a temperature of  $20 \pm 5$  °C for at least 12 h, with the light condition as “Light 2” of the incubator (SANYO MLR-350). If an immobile mite was found on a moss shoot, whether there were characteristic feeding traces on the moss leaves was verified. Specifically, we observed if the mite was actively feeding on the leaf cells on a moss shoot. We identified transparent cell patterns, which were typically a small part of a whole leaf but continuous to some extent, as feeding marks caused by *Eustigmaeus* mites. This identification was based on Gerson’s (1972) description and figures and our observations of their feeding behavior (Fig. 2). We determined whether the mite species exhibited moss-feeding habit, either through the presence of feeding marks on moss leaves or their active feeding behavior. The damaged moss shoots were then recorded as the host plant taxa. To precisely determine the moss-mite associations to the species level, each individual mite and its associated host-plant shoot(s) were recorded and preserved as paired voucher specimens. For selected mite specimens, genomic DNA was extracted for DNA barcoding and subsequent phylogenetic analysis before they were mounted in Hoyer’s medium for morphological identification.

The mounted mite specimen was identified at the species level. Only adult females of the mites were captured for identification, although some were later found to be nymphs (Table S1). On sampling, we obtained one to around 20 pairs of mites per colony with the shoots of host mosses. The number of collected mite specimens per colony is indicated in Table 1. Any remaining mite individuals in the moss colony were extracted using



**Fig. 2** Behavioral observation. **a** Leaf of *Rhynchosstegium riparioides* with characteristic feeding mark caused by *Eustigmaeus* mite. Cells in the infested area (arrow) are transparent due to the suction feeding of cell contents. **b** Two individuals of *Eustigmaeus* sp. 1 on *Rhynchosstegium riparioides* shoot. Scale bar = 200  $\mu\text{m}$

the Berlese-Tullgren funnel. Most of these mite individuals were stored in 70% ethanol for future morphological identification, while a few were preserved in 99% ethanol for DNA extraction.

As identification of the host plant taxa based on a few moss shoots was technically difficult, a handful of moss shoots that occurred near the host shoot were used as a reference specimen. Several shoots were randomly chosen from the reference specimen and identified based on the morphology. The identified moss species was designated as the host plant. All mite specimens used in this study were deposited in the Zoological Collection of Kyoto University (KUZ) (Tables S1–S3). Reference specimens of mosses were also deposited at the herbarium of National Museum of Nature and Science (TNS) (Table S1).

### Morphological identification

To distinguish species among the collected mites, we assessed 52 morphological characters in adult females that are commonly used for the species-level taxonomy of *Eustigmaeus* (Figs. S1 and S2; Table S4). The examined characters classified into idiosomal size, dorsal shield ornamentation, presence or absence of eye, dorsal seta length and shape, the number of seta pairs on dorsal shields, the distance between dorsal setae, endopodal shield state, the number of endopodal seta pairs, the number of aggenital and pseudanal seta pairs, were primarily extracted from the keys in Fan and Zhang (2005), Stathakis et al. (2016), and Khaustov et al. (2023). Humeral, endopodal, and aggenital shield ornamentations were also examined as their variations among congeners have been reported (Stathakis et al. 2016; Khaustov 2019; Khaustov et al. 2023). For each species, we examined one to four individuals from every collection site where that species was found (Table S4). Specimens were mounted in Hoyer's medium.

Each species was assigned into four species groups according to the definitions by Summers and Price (1961), Chaudhri (1965), Wood (1972), and Kaźmierski (2000). *Eustigmaeus* species with flat, arcuate or sabre-shaped dorsal setae and one pair of aggenital seta were classified into “*segnis*” group; species with bushy dorsal setae and three pairs of aggenital setae were classified into “*pectinatus*” group; species with flat, arcuate or sabre-shaped dorsal setae and three pairs of aggenital setae, three or four



pairs of pseudoanal setae, and genu II with setae *k* were classified into “*pinnatus*” group; species with varied shapes of dorsal setae (but not flat, arcuate, sabre-shaped or bushy) and two or three pairs of aggenital setae were classified into “*maculatus*” group. The species group assignment is indicated in Tables S1 and S2.

## DNA extraction

Mite samples for DNA extraction were preserved in 99% ethanol and stored at -20 °C. Genomic DNA was extracted from a whole body of a mite specimen using a NucleoSpin® Tissue (Takara) following the manufacturer’s protocol with some modifications to retrieve the mite body. The specimen was placed at the bottom of the 1.5 ml microcentrifuge tube and left it for 30 min for dehydration; 180 µl of buffer T1 and 25 µl of Proteinase K (100 µg/ml or 20 mg/ml) were then added. Particularly for a specimen with hard sclerotization, the body was crushed with a pestle. The solution was incubated at 56 °C for 24 h, and briefly vortexed, 200 µl of buffer B3 was then added, vortexed again for 5 s, and incubated at 70 °C for 10 min. The mixture was then centrifuged for 5 min, 11,000 g at room temperature. The supernatant was then transferred to a new microcentrifuge tube. The mite body that remained in the tube was retrieved and stored in 70% ethanol, which was subsequently mounted on a microscope slide with Hoyer’s medium as a voucher specimen. The parts of the body of the voucher specimen were sometimes lacking but still preserved the most of morphological details, even in the case the body was crushed, allowing us to use it for identification. For the rest of the protocol for DNA extraction, we followed the manufacturer’s protocol.

## PCR amplification and sequencing

The mitochondrial cytochrome *c* oxidase subunit I (COI) region was selected for DNA barcoding and validation of the morphological species. The COI region was amplified by a nested PCR, using primer sets for the initial PCR were COX1\_16F and COX1\_1324R; those for the second PCR were COX1\_25Fshort\_T and COX1\_1282R\_T (Klimov et al. 2018). For the initial PCR, the total reaction volume was 20 µl. This contained 8.05 µl of distilled water, 10 µl of KOD One PCR Master Mix -Blue- (TOYOBO), 0.8 µl of each primer of COX1\_16F and COX1\_1324R, 0.35 µl of DNA template. For the second PCR, the total PCR reaction volume was 20 µl: it contained 7.5 µl of distilled water, 10 µl of KOD One PCR Master Mix -Blue- (TOYOBO), 0.8 µl of each primer of COX1\_25Fshort\_T and COX1\_1282R\_T, 0.9 µl of the initial PCR product. The thermal cycler conditions for the first and second PCRs followed Klimov et al. (2018). The second PCR products were purified either with ExoSAP-IT or with NucleoSpin® Gel and PCR Clean-up (Takara). Sequencing was done in both directions. Sequencing reactions were conducted by Eurofins Genomics Inc. (Tokyo, Japan). After trimming the primer and low-quality regions in the obtained sequence data, 883–1163 bp of 33 OTUs were obtained. Neither indels nor stop codons were found. The sequence data were deposited on International Nucleotide Sequence Databases (INSD) through the DNA Data Bank of Japan under the accession numbers from LC789942 to LC789974.

## Molecular phylogenetic analysis

A phylogenetic analysis focusing on the species within the genus *Eustigmaeus* was conducted to infer the phylogenetic relationships of the moss-feeding species. To determine the outgroup of *Eustigmaeus*, there was no available inference on the phylogenetic position of each genus in Stigmaeidae with molecular data, so we preliminary reconstructed the phylogenetic tree, including 77 sequences of 10 genera in Stigmaeidae, which based on 700–1300 bp length of COI sequences from INSD (Table S2). Two sequences of unidentified species of *Homocaligus* (Homocaligidae) were used as the outgroup (Table S2). The sequences were aligned with ClustalW in MEGA11 (Tamura et al. 2021). A maximum-likelihood (ML) phylogeny was reconstructed with IQ-TREE 1.6.12 (Nguyen et al. 2015). As a result, *Stigmaeus akimovi* (OP960167) and *Stigmaeus dignus* (MW367928) were selected as the outgroup due to their highest log-likelihood value.

In addition to the 33 newly obtained sequences of *Eustigmaeus* spp., sequences of *Eustigmaeus* spp., *Villersia* sp., *Ledermuelleriopsis* spp., *Cheylostigmaeus* spp., and *Postumius* sp., were downloaded from INSD and included in the ingroup (Table S2). The analyzed *Eustigmaeus* species were assigned into the four species groups as described in the “**Morphological identification**” section (Table S2). Their morphological traits were based on the original descriptions or redescriptions. The alignment of sequences for in- and outgroups followed the same method as described above. ML phylogeny was reconstructed with IQ-tree 1.6.12 (Nguyen et al. 2015). The best-fitting substitution model was estimated by Modelfinder (Kalyaanamoorthy et al. 2017), and K3Pu + F + I + G4, TN + F + R2, GTR + F + I + G4 were then selected for 1st, 2nd, 3rd positions, respectively. UF-bootstrap (Hoang et al. 2018) and SH-like approximate likelihood ratio test (SH-aLRT; Guindon et al. 2010) with 1000 replicates were performed. Clades with support values of both SH-aLRT  $\geq 80\%$  and UFboot  $\geq 95\%$  were considered reliable (Minh et al. 2022). The resultant tree was visualized with Figtree v1.4.4 (Rambaut 2018). The genetic distances were calculated by Kimura 2-parameter model (Kimura 1980) with MEGA 11, with pairwise deletion of missing data (Tamura et al. 2021).

## Species delimitation analysis

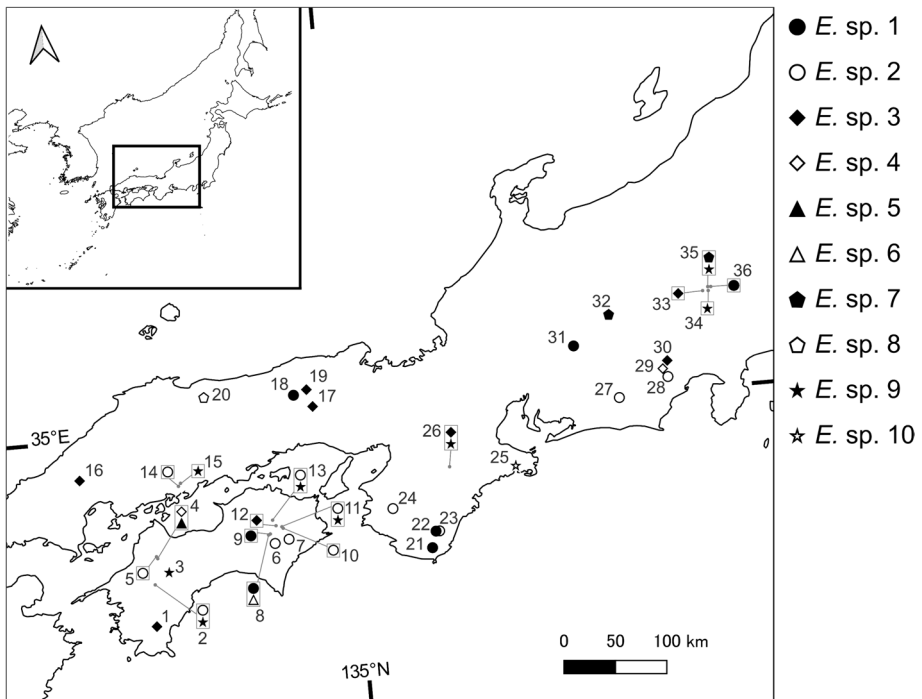
To examine the species delimitation based on genetic distance and to determine if it aligns with the boundary of newly recognized morphospecies, the species delimitation tests, Automatic Barcoding Gap Discovery (ABGD; Puillandre et al. 2012) and Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2021), were applied to the COI dataset of OTUs comprising Clade A (Fig. 4). The ABGD analysis was performed in a webserver (<https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>) using the K2P model, with default prior value of maximum intraspecific distance ( $P_{min}=0.001$ ,  $P_{max}=0.1$ ), relative gap width (X) was specified to 1.0, with 1000 steps. The ASAP analysis is a hierarchical clustering algorithm based on the pairwise genetic distance. This method does not require a user to define any prior value and provides a score for each partition which helps the user identify the best one. The ASAP analysis was carried out online (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>) using a default setting with the K2P model. As the great sequence length variation was present in the datasets (ranging from 792 to 1216 bp), the input sequences were trimmed to 740 bp to remove the effects of missing data.

## Results

### Species identification and phylogenetic relationship

The moss-feeding species of *Eustigmaeus* were collected from 36 localities in Japan (Fig. 3; Table 1), which were all identified as the member of the “*maculatus*” group and distinguished into 10 species based on a combination of morphological characters such as dorsal shield ornamentation, dorsal seta length and shape, and the number of aggenital setae pairs (shown in Figs. S1 and S2; Table S4). These species were all likely to be new to Japan because their morphological features were clearly distinct from those of the four *Eustigmaeus* species—*E. anauniensis* (Canestrini, 1889), *E. lirellus* (Summers and Price 1961), *E. segnis* (Koch, 1836), *E. arcuatus* (Chaudhri 1965)—recorded from Japan (Beron 2020). In the following, they are tentatively referred to as ‘*Eustigmaeus* sp. 1’, although this treatment does not necessarily indicate that they are undescribed species. The COI sequence of all morphospecies except *Eustigmaeus* sp. 5 was obtained. For the widely distributed species, *Eustigmaeus* sp. 1, *Eustigmaeus* sp. 2, *Eustigmaeus* sp. 3, and *Eustigmaeus* sp. 9, the sequences were based on individuals from localities that largely cover their distribution (Table S2). However, the sequence of the *Eustigmaeus* sp. 4 sampled from LC 29 was not obtained.

The resultant phylogeny recovered four clades (labeled Clade A–D). Of these, Clade D was robustly supported, but the other clades were less supported, and the phylogenetic



**Fig. 3** Sampling localities of moss-feeding *Eustigmaeus* species. Locality codes correspond to those in Table 1

relationships between these clades could not be fully resolved. Clade A (SH-aLRT=87.2%, UFBoot=80%) included all examined “*maculatus*” species and one *Villiersia* species. Clade B contained a part of “*pectinatus*” species. Clade C was composed of the other part of “*pectinatus*” species, “*pinnatus*” species, and *Ledermuelleriopsis* species. Clade D consisted of “*segnis*” species. A clade consisting of *Cheylostigmaeus* and *Postumius* species was resolved as a sister of the four clades.

Within Clade A, the Japanese moss-feeding species did not form a monophyletic group. The respective monophyly was well-supported for four morphological species from Japan (*Eustigmaeus* sp. 1, *Eustigmaeus* sp. 2, *Eustigmaeus* sp. 3, and *Eustigmaeus* sp. 7), but weakly supported for *Eustigmaeus* sp. 9 (SH-aLRT=80.8%, UFBoot=70%). Three of the four singletons of morphospecies respectively formed a clade with a different named species from abroad. Pairwise genetic distances were varied among closely related species, e.g., 1.6% between *Eustigmaeus* sp. 8 and *Eustigmaeus pseudolacunus* and 6.1% between *Eustigmaeus* sp. 6 and *Eustigmaeus grandis* (Table S5). *Eustigmaeus* sp. 9 formed a well-supported clade (SH-aLRT=97.3%, UFBoot=98%) with *Eustigmaeus rhodomelus* (OL863247 and OP960172). The non-monophyly of *Eustigmaeus rhodomelus* may be related to its genetic differences among the four sequences because they were taken from a geographically wide range (Table S2).

The pairwise genetic distance analysis between 80 OTUs showed that intraspecific divergence was below 3.7%, while interspecific divergence exceeded 7.8% in the morphological species (Table S5). The intra- and inter-specific distances between Japanese moss-feeding species are summarized in Table 2. The partitioning hypotheses inferred by ABGD and ASAP are presented in Fig. 4. The ABGD detected 20 ( $P=0.006805-0.024626$ ), 24 ( $P=0.002707-0.006774$ ), and 27 ( $P=0.001000-0.002719$ ) groups. The ASAP assigned the best score (asap-score=2.50) to the 20 groups hypothesis. In the 20-grouping hypothesis, nine morphospecies were classified into nine discrete groups. *Eustigmaeus rhodomelus* from Russia (OL863247 and OP960172) and *Eustigmaeus pseudolacunus* were assigned to the same group as *Eustigmaeus* sp. 9 and *Eustigmaeus* sp. 8, respectively. In the 24-grouping hypothesis, a group composed of *Eustigmaeus* sp. 9 and *Eustigmaeus rhodomelus* (OL863247 and OP960172) was split into four, and *Eustigmaeus* sp. 2 group was separated into two. In the 27-grouping hypothesis, in addition to the subdivision in the 24-grouping hypothesis, a group of *Eustigmaeus* sp. 1 was classified into four groups.

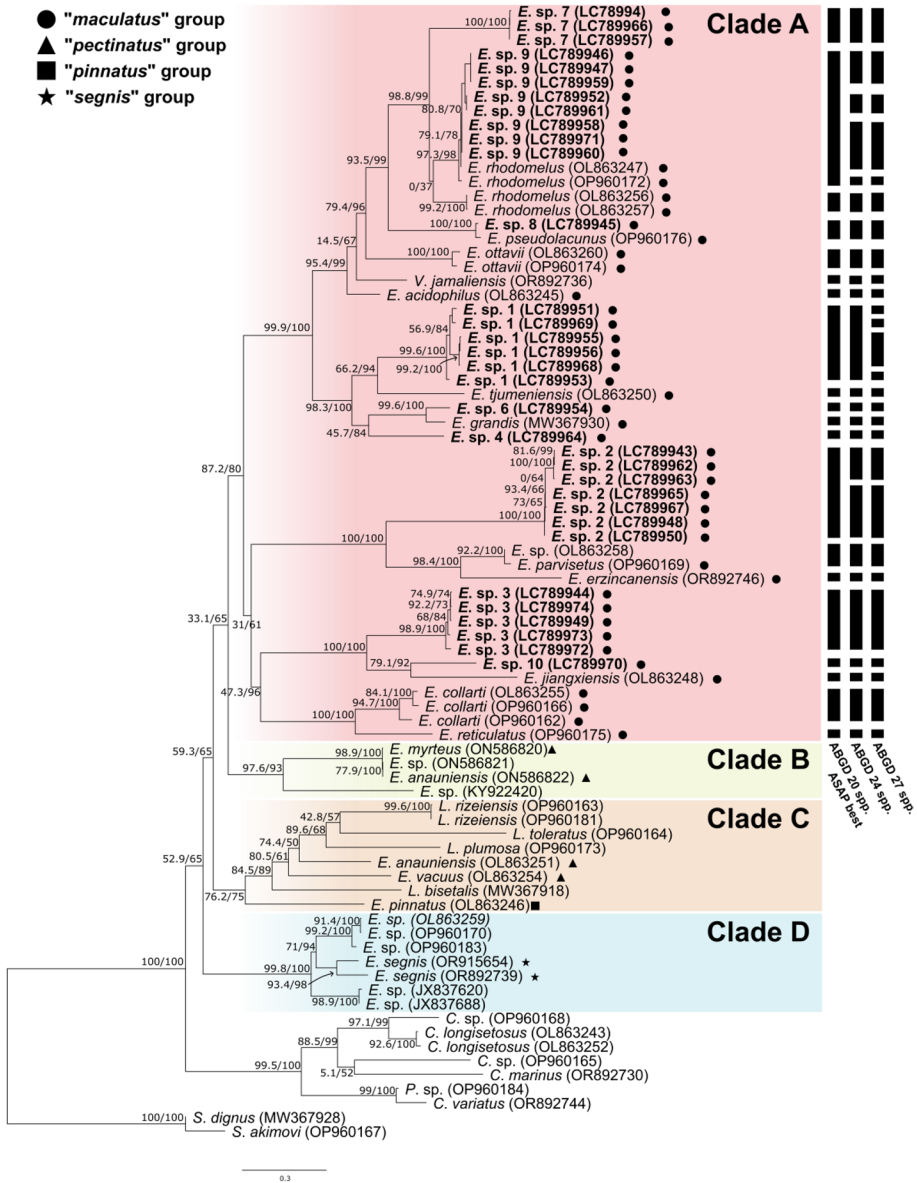
## Distribution, habitat, and host plant use

Although the species sampled from more than seven localities, *Eustigmaeus* sp. 1, *Eustigmaeus* sp. 2, *Eustigmaeus* sp. 3, and *Eustigmaeus* sp. 9, were widely distributed in Japan, the geographic ranges of the other species (i.e., *Eustigmaeus* sp. 4, *Eustigmaeus* sp. 5, *Eustigmaeus* sp. 6, *Eustigmaeus* sp. 7, *Eustigmaeus* sp. 8, and *Eustigmaeus* sp. 10) were not clarified in this study because they were collected only from one or two localities (Fig. 3; Table 1). The distributions of *Eustigmaeus* sp. 1, *Eustigmaeus* sp. 2, *Eustigmaeus* sp. 3, and *Eustigmaeus* sp. 9 were overlapped with each other. Notably, multiple species were frequently sampled from the same locality or closely located locality (Fig. 3; Table 1). For example, *Eustigmaeus* sp. 1, *Eustigmaeus* sp. 3, *Eustigmaeus* sp. 7, *Eustigmaeus* sp. 9 were sampled from closely located localities (LC 33–36), and distances between them were less than 10 km.

The moss-feeding species were found in a moss colony that was constantly wet retaining high humidity and water supply. Such moss patches were on various substrates in a diverse

**Table 2** The intraspecific and interspecific genetic distance (%) of nine *Eusigmaeus* species based on the K2P model in MEGA 11 (mean ±SD)

|                  | sp. 1        | sp. 2        | sp. 3        | sp. 4        | sp. 6        | sp. 7        | sp. 8       | sp. 9        | sp. 10 |
|------------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------|--------------|--------|
| <i>E. sp. 1</i>  | 2.21 ± 0.97  |              |              |              |              |              |             |              |        |
| <i>E. sp. 2</i>  | 25.01 ± 0.34 | 2.22 ± 1.48  |              |              |              |              |             |              |        |
| <i>E. sp. 3</i>  | 17.83 ± 0.38 | 26.41 ± 0.45 | 1.48 ± 0.72  |              |              |              |             |              |        |
| <i>E. sp. 4</i>  | 12.13 ± 0.35 | 25.84 ± 0.08 | 18.73 ± 0.48 | –            |              |              |             |              |        |
| <i>E. sp. 6</i>  | 13.83 ± 0.31 | 26.24 ± 0.26 | 18.07 ± 0.28 | 12.26        | –            |              |             |              |        |
| <i>E. sp. 7</i>  | 15.72 ± 0.32 | 24.99 ± 0.39 | 17.53 ± 0.33 | 16.68 ± 0.12 | 17.32 ± 0.13 | 0.29 ± 0.15  |             |              |        |
| <i>E. sp. 8</i>  | 13.62 ± 0.17 | 24.84 ± 0.15 | 16.12 ± 0.28 | 14.06        | 15.16        | 11.29 ± 0.05 | –           |              |        |
| <i>E. sp. 9</i>  | 14.15 ± 0.33 | 24.12 ± 0.83 | 16.31 ± 0.31 | 14.55 ± 0.19 | 15.69 ± 0.27 | 8.59 ± 0.32  | 10.83 ± 0.1 | 1.19 ± 0.71  |        |
| <i>E. sp. 10</i> | 19.1 ± 0.28  | 27.42 ± 0.24 | 14.33 ± 0.22 | 19.55        | 18.53        | 20.4 ± 0.03  | 19.02       | 19.08 ± 0.33 | –      |



**Fig. 4** Phylogenetic relationships for moss-feeding *Eustigmaeus* species reconstructed by maximum-likelihood analysis based on mitochondrial COI gene sequences. Sequences newly obtained in this study are shown in bold. Nodal value (%) consists of SH-aLRT value and UF-boot value; the values are not shown for some terminal nodes. Symbol near the tip indicates species group assignment. Bars on the right represent the results of species delimitation analyses

ecological setting (Fig. 1; Table 1). Some mite species tended to be sampled from a specific type of habitat. In the case of frequently-sampled *Eustigmaeus* sp. 1, *Eustigmaeus* sp. 2, *Eustigmaeus* sp. 3, and *Eustigmaeus* sp. 9, we observed the following patterns. *Eustigmaeus* sp. 1

was consistently sampled from mosses on wet rocks and boulders around the stream and river across all nine patches. *Eustigmaeus* sp. 2 was predominantly collected from rock faces and the concrete walls in wet environments, observed in nine out of 13 patches. *Eustigmaeus* sp. 3 was found on rock faces along pavement roads or mountain paths in eight out of 10 patches. *Eustigmaeus* sp. 9 exhibited a wider range of habitat variability, including open areas with relatively low surrounding humidity, at patch code AP and AS (Table 1).

Table 1 shows a list of 10 moss-feeding species and their respective host plants, encompassing 26 species in total. These host plants belong to the Bryopsida, comprising 18 genera, 11 families, and five orders. Based on the field observations, different host plant use among the frequently-sampled species was suggested: *Eustigmaeus* sp. 1, *Eustigmaeus* sp. 2, *Eustigmaeus* sp. 3, and *Eustigmaeus* sp. 9. *Eustigmaeus* sp. 1 exclusively inhabited Hypnales mosses at all nine patches, with a majority of samples found on *Rhynchostegium riparioides*. *Eustigmaeus* sp. 2 predominantly used *Philonotis* species across 12 out of 13 patches. While *Eustigmaeus* sp. 3 was exclusively discovered from the dicranid moss lineages, Pottiales and Dicranales, we found two examples (patch codes AB, AE) that an individual was on other lineages of mosses shoots with feeding mark that coexisted in a Dicranidae moss colony. *Eustigmaeus* sp. 3 may feed on them as well. *Eustigmaeus* sp. 9 was found in several distantly related moss species, suggesting its polyphagous nature.

Notably, at certain localities, different mite species within a pair specifically occurred on different mosses growing in immediate proximity. At LC 4, although both *Eustigmaeus* sp. 4 and *Eustigmaeus* sp. 5 were present in the same patch on a concrete wall (Fig. 1e; Table 1), *Eustigmaeus* sp. 4 was only collected from *Gollania ruginosa* and *Eustigmaeus* sp. 5 was mainly found from *Rosulabryum capillare*. Similarly, the recorded host-plant species were distinctive between a pair of *Eustigmaeus* sp. 2 and *Eustigmaeus* sp. 9 at patch code P in LC 13, *Eustigmaeus* sp. 9 occurred on *Bryum cyclophyllum* and *Philonotis falcata* but *Eustigmaeus* sp. 2 used *Philonotis turneriana*. In other instances, a pair of mite species used different moss species from different patches within the same locality. At LC 8, *Eustigmaeus* sp. 6 was sampled from *Brachythecium rivulare* on the wet concrete wall and nearby along a pavement road, and *Eustigmaeus* sp. 1 inhabited *Rhynchostegium riparioides* on a wet rock in a stream (Fig. 1a; Table 1). Additionally, *Eustigmaeus* sp. 7 was collected from *Pseudohygrohypnum eugyrium* on a wet boulder by a river (Fig. 1f) but *Eustigmaeus* sp. 9 used *Bryum recurvulum* on a wet concrete beside a pavement road at LC 35 (Table 1). We noted only two cases where multiple *Eustigmaeus* species coexisted within the same colony of a particular moss species (i.e., *Eustigmaeus* sp. 2 and *Eustigmaeus* sp. 9 from *Pohlia* sp. at LC 2, *Eustigmaeus* sp. 3 and *Eustigmaeus* sp. 9 from *Brachythecium helminthocladum* at LC 26). *Eustigmaeus* sp. 2 and *Eustigmaeus* sp. 9 both used *Philonotis falcata* at LC 13 but they were derived from different patches. With these two exceptions, *Eustigmaeus* species sharing the same host plant did not coexist within the same colony in the field (e.g., *Eustigmaeus* sp. 2 and *Eustigmaeus* sp. 9 shared *Philonotis falcata*, *Eustigmaeus* sp. 1 and *Eustigmaeus* sp. 7 shared *Entodon luridus*, *Eustigmaeus* sp. 5 and *Eustigmaeus* sp. 9 shared *Bryum paradoxum*, *Eustigmaeus* sp. 1 and *Eustigmaeus* sp. 9 shared *Rhynchostegium riparioides*, and *Eustigmaeus* sp. 6 and *Eustigmaeus* sp. 8 shared *Brachythecium rivulare*) (Table 1).

## Discussion

### Diversity of moss-feeding species

The field sampling and microscopic observation among 10 species have provided a glimpse into the previously overlooked diversity of moss-feeding species in *Eustigmaeus*. These 10 species have been identified as distinct entities based on the morphological identification, phylogenetic analyses, and genetic distance-based species delimitation methods (Fig. 4; Table S4), except for *Eustigmaeus* sp. 5, for which DNA sequence data was not obtained. Although ABGD provided three partitioning hypotheses to the species within Clade A (20, 24, and 27 groups), the 20-grouping hypothesis that ASAP gave the best score (threshold value was 5%) was thus consistent with the morphological identification. However, the taxonomic position of these species warrants further taxonomic studies. It is important to highlight that six of 10 species have only been collected from one or two localities, suggesting that the true extent of diversity within moss-feeding *Eustigmaeus* mites remains largely unexplored.

The 10 species discovered in this study, as well as the four species previously reported as moss-feeders (Gerson 1972), all fall within the “*maculatus*” group (Summers and Price 1961; Fan and Zhang 2004). The phylogeny represented the “*maculatus*” species formed a clade with a *Villersia* species. Although the species in *Villersia* are characterized by the presence of setae *sce* and *d2* on independent platelets [seta *d2* of *Villersia jamaliensis* is not on independent platelet but included in this genus by Khaustov (2019)], other characteristics are generally in common with “*maculatus*” species in *Eustigmaeus* (Summers 1966; Wood 1972). Given the morphological similarities and the result of phylogenetic analysis, it is inferred that the autapomorphy of *Villersia* is merely a variation within the “*maculatus*” group and this genus is reasonably considered as a member of this group. Currently, the species that matches the definition of the “*maculatus*” group counts more than 50 species. Revealing the prevalence of moss-feeding habit within this group is crucial for understanding the evolution of this habit. Additionally, to trace the origin of the moss-feeding habit found in *Eustigmaeus*, it is necessary to solve the phylogenetic relationships among different species groups and related taxa. It is also crucial to accumulate knowledge regarding their feeding habits, an aspect that is largely lacking in the existing body of information.

### Ecology of moss-feeding species

Extensive field sampling of *Eustigmaeus* species and accurate host determination accumulated knowledge on their host plant records and geographic ranges. For frequently-sampled species, *Eustigmaeus* sp. 1, *Eustigmaeus* sp. 2, *Eustigmaeus* sp. 3, the geographic ranges were overlapped, as did the period of occurrence for adult females, while they differ in their utilization of host plants (Fig. 3; Table 1). The recorded host plant lineages, Hypnales, *Philonotis*, Dicranidae, are phylogenetically and morphologically distant from each other (Liu et al. 2019). Furthermore, these species exhibited different preferences for environmental conditions in the current study (Table 1). These findings suggest that the distinct niches for each mite species are shaped not only by the inherent characteristics of the moss plant body, such as chemistry and gametophyte morphology, but also by various factors influenced by the environment, including abiotic factors and



predator fauna. These factors possibly play a role in determining the host plant use of the three *Eustigmaeus* species.

We rarely observed multiple *Eustigmaeus* species coexisting within the same colony of a given moss species in the field, even though some mite species shared the same host plant (Table 1). Phytophagous mites are known to undergo long-distance dispersal mainly through aerial dispersal (Li and Margolies 1993; Lawson et al. 1996; Osakabe et al. 2008; Michalska et al. 2010; Galvão et al. 2012; Majer et al. 2021), which may also be the case for *Eustigmaeus*. Consequently, if the distributions of *Eustigmaeus* species overlap, it is possible for them to disperse to host plants already occupied by other mite species. This scenario could lead to frequent interactions between the established mite species and newly arrived species on mosses. Therefore, interspecific interactions such as resource competition (López-Olmos and Ferragut 2023) and reproductive interference (Takafuji et al. 1997), which prevent the coexistence of species on a host plant, may also play a key role in determining the host plant use among *Eustigmaeus* species.

Moss-feeding *Eustigmaeus* species demonstrate a preference for constantly moist moss colonies (Table 1). This preference may be linked to the poikilohydry of moss. Mosses are poikilohydric plants, meaning that their bodies dehydrate when exposed to dry conditions and rehydrate when water becomes available (Proctor 2008). However, as *Eustigmaeus* mites rely on fluid feeding, it is likely that they cannot suck out the cell contents from dehydrated moss cells. Consequently, mosses growing in periodically drying environments may not be suitable as host plants, and the mites may instead favor mosses in constantly wet environments. Such mosses would provide a stable source of food for the mites, as they would remain hydrated and available at all times. Therefore, drying environment may strikingly restrict the host plant use of *Eustigmaeus* over the natures of moss gametophyte.

The current study highlights the genus *Eustigmaeus* as a rare group that contains diverse moss-feeding species, with some species exhibiting specific host plant use. Mosses are seldom consumed by phytophagous arthropods (Gerson 1969, 1982) and some studies have shown that mosses are typically unsuitable food resources for generalist consumers in arthropods, likely due to their chemical defense mechanisms (Markham et al. 2006; Parker et al. 2007; Haines and Renwick 2009; Duhin et al. 2022). Moreover, known moss-feeding insects are either host-specific but less diverse (Imada 2021), diverse but not host-specific (Pyszko et al. 2020; Ruan et al. 2020), or for which information about host plants is scarce (Gerson 1982). The association between *Eustigmaeus* mites and mosses thus presents a promising model for studying the macroevolutionary patterns of diversification in moss-feeding arthropods. However, the complex associations between *Eustigmaeus* species and mosses are only partially understood. Thus, further information on the diversity of moss-feeding species and their host plant use around the world is necessary to fully elucidate these relationships.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10493-024-00954-z>.

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**Author contributions** Satsuki Ikeda and Yume Imada designed this study, conducted field sampling, and acquired funds. Satsuki Ikeda prepared the specimens, recorded the specimen data, conducted molecular analysis, and wrote the main manuscripts. Yuya Inoue identified moss specimens and edited the manuscript. Yume Imada supervised the manuscript. All authors read and approved the final manuscript.

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**Data availability** The sequence data were deposited on International Nucleotide Sequence Databases (INSD) through the DNA Data Bank of Japan under the accession numbers from LC789942 to LC789974.

## Declarations

**Competing interest** The authors declare no competing interests.

**Ethical approval** No approval of research ethics committees was required to accomplish this study.

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