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Floral Visitors of *Eriocaulon heleocharioides* (Eriocaulaceae), an Extinct Aquatic Species in the Wild

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Abstract Insects were observed visiting the inflorescences of *Eriocaulon heleocharioides* (Eriocaulaceae), an extinct aquatic species in the wild, at Sanuma Lake, Japan. Twenty-three floral visitors to *E. heleocharioides* belonging to three orders, four suborders, and ten families were collected. Of them, members of the order Diptera-suborder Brachycera accounted for 74% and those of the suborder Nemato accounted for 17%. The *E. heleocharioides* visitors were hygrophytes and terrestrial species.

Key words: aquatic plant, *Eriocaulon heleocharioides*, extinct species in the wild, pollination.

Introduction

Eriocaulon heleocharioides Satake (Eriocaulaceae) is an annual aquatic herb endemic to Japan and is an extinct species in the wild (Ministry of the Environment, 2012). This species disappeared from its last habitat in the Sanuma Lake, Shimotsuma City, Ibaraki Prefecture, Japan, immediately after a change in water management in 1994 and has only been preserved *ex situ* (Tanaka *et al.*, 2014). Since 2008, a project to return this species at the Sanuma Lake has been conducted by the Tsukuba Botanical Garden, National Museum of Nature and Science, Japan. From 2009 onward, many *E. hereocharioides* individuals survived and bore seeds in autumn (ca. 70 individuals in 2009, ca. 800 in 2010, ca. 13,600 in 2011).

Although the knowledge of the pollination system is essential for the conservation of the annual species, few reports have been available for the family Eriocaulaceae. Indirect studies based on floral morphology have reported inconsistencies. Some authors presumed that the pollination system of the Eriocaulaceae members is anemophily based on their floral morphological characteristics (Kral, 1966; Cronquist, 1981; Judd *et al.*, 2002), whereas others have speculated that it is entomophily (Dahlgren *et al.*, 1985; Hensold, 1988; Rosa and Scatena, 2003). Only two studies have revealed that entomophily is the pollination system of some *Syngonanthus* species (Eriocaulaceae) using direct observations and experiments, including *Syngonanthus mucugensis* and *S. curralensis* (Ramos *et al.*, 2005) and *S. elegans* (Oriani *et al.,* 2009). Sawyer *et al.* (2005) showed that *E. parkerii* relies heavily on geitonogamy for seed production, but the pollinators of this genus remain unclear.

The male *E. heleocharioides* flower has a nectary and its pollen grains are sticky. These floral morphological characteristics argue regarding

entomophily. In this study, we identified insects that visited the inflorescences of this species in a wild habitat.

Materials and Methods

The study site was Yanagi Wando, Sanuma Lake, Shimotsuma City, Ibaraki Prefecture, Japan. Seeds of *E. heleocharioides* have been reintroduced to this site since 2009, and seven small communities (ca. 22 m^2; ca. 13,600 individuals) have been regenerated as of 2011. The observations were conducted within two of these communities. One was a 3 m^2 area with 1,920 individuals and the other was 9 m^2 area with 6,354 individuals. The two sites were located at 3 m from each other.

We observed the insects that visited the inflorescences of *E. heleocharioides* from 9 a.m. to 5 p.m. between 26 September and 30 September, 2011. We observed through the night only on 30 September. Almost all *E. heleocharioides* in the Sanuma Lake were flowering during this period. Insects that visited the inflorescences were collected after they moved among the flowers and were preserved with naphthalene in silica gel. The number of pollen grains attached to the insects that were collected was measured using a stereoscopic microscope.

Results and Discussion

Twenty-three floral visitors to *E. heleocharioides* belonging to three orders, four suborders, and ten families were collected (Table 1, Fig. 1A). Of them, members of the order Diptera-suborder Brachycera accounted for 74% and those of the suborder Nemato accounted for 17%. The number of pollen grains attached to the insects varied from 0 to 50 for each individual. Most of the pollen grains were found around the body or legs, but for Hymenoptera they were found around the mouth (No. 22 in Table 1).

Dipterans were the main visitors to *E. heleocharioides* in the Sanuma Lake. No reports are available on the genus or species of the pollinators. Previous studies have reported that the orders Coleoptera and Hymenoptera are pollina-

Table 1. Floral visitors of *Eriocaulon hereocharioides* and the number of pollen grains attached to the visitors

Individual No.	Order	Suborder	Family	Genus	Species	The number of attached pollen grains
1	Diptera	Brachycera	Ephydridae		*sp.*-A	3
2	Diptera	Brachycera	Ephydridae		*sp.*-A	10
3	Diptera	Brachycera	Ephydridae		*sp.*-A	0
4	Diptera	Brachycera	Ephydridae		*sp.*-B	50–
5	Diptera	Brachycera	Ephydridae		*sp.*-B	2
6	Diptera	Brachycera	Ephydridae		*sp.*-B	4
7	Diptera	Brachycera	Ephydridae	*Ochthera*	*sp.*-C	4
8	Diptera	Brachycera	Ephydridae	*Ochthera*	*sp.*-C	0
9	Diptera	Brachycera	Ephydridae	*Ochthera*	*sp.*-C	1
10	Diptera	Brachycera	Ephydridae	*Ochthera*	*sp.*-C	0
11	Diptera	Brachycera	Tachinidae		*sp.*-D	10–20
12	Diptera	Brachycera	Sciomyzidae	*Sepedon*	*sauteri*	2
13	Diptera	Brachycera	Sarcophagidae		*sp.*-F	5
14	Diptera	Brachycera	Sarcophagidae		*sp.*-G	5
15	Diptera	Brachycera	Calliphoridae	*Lucilia*	*caesar*	12
16	Diptera	Brachycera	Calliphoridae	*Lucilia*	*caesar*	1
17	Diptera	Brachycera	Syrphidae		*sp.*-H	1
18	Diptera	Nemato	Ceratopogonidae		*sp.*-I	1
19	Diptera	Nemato	Ceratopogonidae		*sp.*-I	0
20	Diptera	Nemato	Ceratopogonidae		*sp.*-I	2
21	Diptera	Nemato	Culicidae		*sp.*-J	0
22	Hymenoptera	Symphyta	Tenthredinidae		*sp.*-K	3
23	Hemiptera	Homoptera	Cicadellidae		*sp.*-L	0

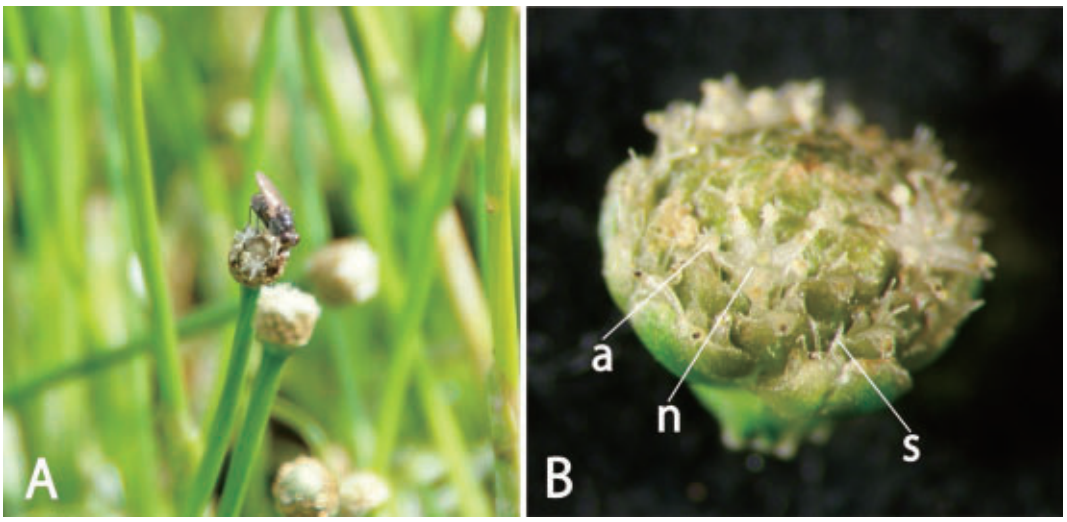

Fig. 1. A. Ephydridae sp. visiting *Eriocaulon heleocharioides* at Sanuma Lake. B. Inflorescences of *Eriocaulon heleocharioides*. a: anther of male flower, n: nectary of male flower, s: stigma of female flower.

tors of some *Syngonanthus* species (Oriani *et al.,* 2009; Ramos *et al.,* 2005).

Inflorescences of the Eriocaulaceae usually consists of many male and female flowers borne in dense heads. Only the number of stamens (4–6 in *Eriocaulon* and 2–3 in *Syngonanthus*) and appendices of female flowers (absent in *Eriocaulon* and present in *Syngonanthus*) (Stutzel, 1998) differ between *Eriocaulon* and *Syngonanthus*. It is uncertain whether the difference in visitors is because of the basic floral morphology of these genera. Future studies on Eriocaulaceae pollinators may reveal whether the pollinators are associated with floral morphology, the faunal habitat, or other factors.

Eriocaulon and *Syngonanthus* species are hygrophytes or submerged. *E. heleocharioides* inhabits underwater areas in spring and summer and marshes during autumn. Of the visitors identified in this study, *Sepedon sauteri* (Sciomyzidae) lives under water around paddy fields (Nagatomi and Kushigemachi, 1965). Most *Ochthera* (Ephydridae) adults are found near water (streams, rivers, and lake shores) and most larvae are aquatic or semi-aquatic (McAlpine *et al.*, 1987). In contrast, *Lucilia caesar* (Calliphoridae) widely inhabits urban areas and forests. Although previous studies about *Syngonanthus* did not refer to the habitats of the pollinators, the visitors to *E. heleocharioides* were not limited to hygrophytous insects. If terrestrial visitors, such as *L. caesar,* are effective pollinators, they will pollinate *E. heleocharioides* even if the environment surrounding the Sanuma Lake is urbanized in the future. *E. heleocharioides* has nectary at the basal part of sterile pistil in a male flower. Most of visitors except for the Ochthera of Diptera, Hymenoptera and Hemiptera, suck nectar (McAlpine *et al.*, 1987). Because these visitors did not accidentally perch on the inflorescences, they would be expected to visit there constantly.

We showed that most of the insects that visited *E. heleocharioides* probably transported some of the pollen grains among its flowers and/or inflorescences. However, because the number of pollen grains attached to the insects varied within each species and the number of samples was small, the effectiveness and contribution of each species could not be assessed. A more detailed study in the future is necessary to resolve these points.

Seed propagation of *E. heleocharioides* after its reintroduction into the wild is essential for *in situ* conservation. Our results provide basic information to determine the pollination system and reproductive ecological traits of *E.*

heleocharioides.

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