

バラ科キジムシロ属の1 新雑種, エチゼンキジムシロ

メタデータ	言語: eng 出版者: 公開日: 2019-03-07 キーワード (Ja): キーワード (En): 作成者: メールアドレス: 所属:
URL	https://doi.org/10.24517/00053417

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Naohiro Naruhashi¹, Takashi Sato² and Yoshikane Iwatsubo³ : *Potentilla* × *echizenensis* (Rosaceae), a new natural hybrid from Japan

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Abstract

A new plant was found in two localities of Oono-shi, Fukui Prefecture; Ikegahara, Shimouchinami and Mt. Koarashima-dake. In these sites we found *Potentilla freyniana* and *P. toyamensis* growing together with the plant or growing in the place that is not far. The new plant was morphologically intermediate between the two plants above mentioned, and is believed to be a hybrid between them, from the results of pollen fertility of the plant in addition to ecological points of view, i.e., same phenology, same habitat and same flowering season. The present new plant, *P. ×echizenensis*, is described as a new natural hybrid between *P. freyniana* and *P. toyamensis*.

Key words : chromosome, description, natural hybrid, pollen fertility, *Potentilla ×echizenensis*.

Introduction

In literature on Japanese *Potentilla* 23 species have been reported (Naruhashi 2001). Makino (1956) described *P. ×musashinoana* Makino as a hybrid between *P. sprengeliana* Lehm. and *P. freyniana* Bornm. Afterward Naruhashi (1970, 1981, 2001) discovered 3 natural hybrids of Japanese *Potentilla*.

One of the authors, Sato found the present plant (Fig. 1) at Ikegahara, Shimouchinami, Oono-shi, Fukui Prefecture in 1978, when he visited to observe the cultivation of *Coptis japonica* (Thunb.) Makino with Messrs. I. Sasaki and T. Hashimoto. After this, the plant has been cultivated at the botanical garden of University of Toyama, Toyama Prefecture.

On the other hand, Naruhashi also found the specimen of the similar plant on Mt. Koarashima-dake in Oono-shi, Fukui Prefecture that was collected by S. Watanabe in 1968 and deposited in the herbarium of Kanazawa University (KANA) in 1988. By guidance of Mr. S. Watanabe in 1989 Naruhashi visited the locality of the specimen collected by him (Fig. 2).

At Ikegahara the present plant is growing with *P. freyniana* and *P. toyamensis* Naruh. et Tak. Sato. At Koarashima-dake the plant that is

growing with *P. toyamensis* and *P. freyniana* is found at a slightly remote place of the same mountain.

The authors of this paper considered that the new plant might be a hybrid between *P. freyniana* and *P. toyamensis*, and therefore examined the morphology and cytology of the three taxa.

Materials and methods

Plants from Ikegahara have been cultivated at the botanical garden of University of Toyama from 1989 and were morphologically compared with *P. freyniana* and *P. toyamensis* cultivated in the same garden. In addition to the observation in the field, many dried specimens from each locality of Koarashima-dake and Ikegahara were used. For karyotype study, excised root tips from the potted plants were pretreated with 2 mM 8-hydroxyquinoline for one hour at room temperature, and continuously kept for 15 hours at 5°C. The root tips were fixed in a 1 : 3 acetic acid and ethyl alcohol mixture for 40 minutes at room temperature, soaked in 1 N HCl for one hour, macerated with 1 N HCl at 60°C for 11.5 minutes, and then immersed in distilled water. The meristematic cells were stained in 1.5% lacto-propionic orcein, and observed by the ordi-



Fig. 1. *Potentilla* × *echizenensis* collected at Ikegahara. Bar represents 5 cm.

nary squash method.

To study the meiotic chromosome behavior, young flower buds were fixed in Newcomer's fluid for 3 hours at room temperature and treated similarly to the root tips. After stained with Schiff's reagent, anthers of the flower buds were squashed in 1.5% lacto-propionic orcein. Pollen fertility was estimated by the grain size and stainability in lacto-propionic orcein in more than two thousand grains of respective plants.

Results and discussion

Morphology

The three present taxa belong to *P. fragarioides* group (Naruhashi and Sato 1978 ; Sato and Naruhashi 1978), are very similar to each other,

i.e., perennial plants, radical leaves consisting of 3-7 leaflets, 1-6 erect flowering stems. Flowers are 10-15 mm across. Flowering season is in April to June. Inflorescences are cyme. Petals are 5, obcordate-orbicular in shape, emerginate at apex, yellow in color. Stamens are 20. Pistils are numerous. Plants have a few runners developing from flowering season. The differences among them are not qualitative differences, they are quantitative ones (Table 1). The new hybrid is an intermediate between *P. freyniana* and *P. toyamensis* at a glance. *Potentilla freyniana*, however, is distinguishable from the other two plants clearly, because of the leaves that are without accessory leaflets and only large leaves at flowering. On the other hand, *P. toyamensis*



Fig. 2. Habitat and plants of *Potentilla* × *echizenensis*. A : Habitat of the type locality in Mt. Koarashima-dake, 26 Apr. 1989. B : Flowering plants at the type locality, 26 Apr. 1989. C : Plants cultivated at University of Toyama, Toyama City, 20 Apr. 1989 (from Ikegahara). D : Flowers at the type locality, 26 Apr. 1989.

and the new plants have rosette leaves in winter, and then they consist of two types of leaves, i.e., rosette leaves and large leaves developing at flowering season.

Characters of flower are variable in the three taxa as a matter of course, because of the flowers which are in flowering order on a flowering stem, different individuals and different populations. The new plant is intermediate between *P. freyniana* and *P. toyamensis*, especially in flower size, petal and the shape of calyx segment and epicalyx segment (Fig. 3).

Karyotype

Both of the plants from the two sites of Koarashima-dake and Ikegahara were all diploid with $2n=14$ chromosomes (Fig. 4 D, E, F, G).

The mitotic metaphase chromosomes of the plant collected at Koarashima-dake ranged in length from $0.9\ \mu\text{m}$ to $1.4\ \mu\text{m}$ and in arm ratio from 1.0 to 3.5 (Table 2). These in the plant collected at Ikegahara ranged in length from $0.9\ \mu\text{m}$ to $1.5\ \mu\text{m}$ and in arm ratio from 1.0 to 3.5 (Table 2). In either plants, the 14 chromosomes were divided into three groups constituting 4 metacentric pairs, 2 submetacentric pairs, and one satellite subtelocentric pair (Fig. 4 F, G). Their karyotypes were formulated as $2n=14=12(m+sm)+2^{st}$, as the same as those of their putative two parent species: *P. freyniana* and *P. toyamensis* (Iwatsubo and Naruhashi 1991).

Chromosome pairing and pollen fertility

In the plant collected at Koarashima-dake, a

Table 1. Diagnostic characters in *Potentilla freyniana*, *P. × echizenensis* and *P. toyamensis*

	<i>P. freyniana</i>	<i>P. × echizenensis</i>	<i>P. toyamensis</i>
Rhizome	tuberous	not tuberous	not tuberous
Root	not stout	stout	stout
Number of leaflets	3	(3-) 4-5*	5-7
Shape of terminal leaflet	elliptic to obovate	elliptic to rhombic oblong	rhombic oblong to rhombic obovate
Apex of terminal leaflet	obtuse to rounded	obtuse to acute	acute
Lower surface of leaf	often somewhat purplish	often somewhat purplish	not purplish
Stipule of radical leaf	linear to lanceolate	lanceolate to triangular obovate	lanceolate to triangular obovate
Stipule of leaf on flowering stem	entire or 1-6 deeply incised	entire or 1-3 slightly dentate	entire or 1-3 slightly dentate
Stipule of leaf on runner	entire or 1-3 incised	entire or 1-3 slightly dentate	entire or 1-3 slightly dentate
Calyx segment	elliptic	triangular-ovate	triangular

* There is not the individual consisting of trifoliolate leaves.

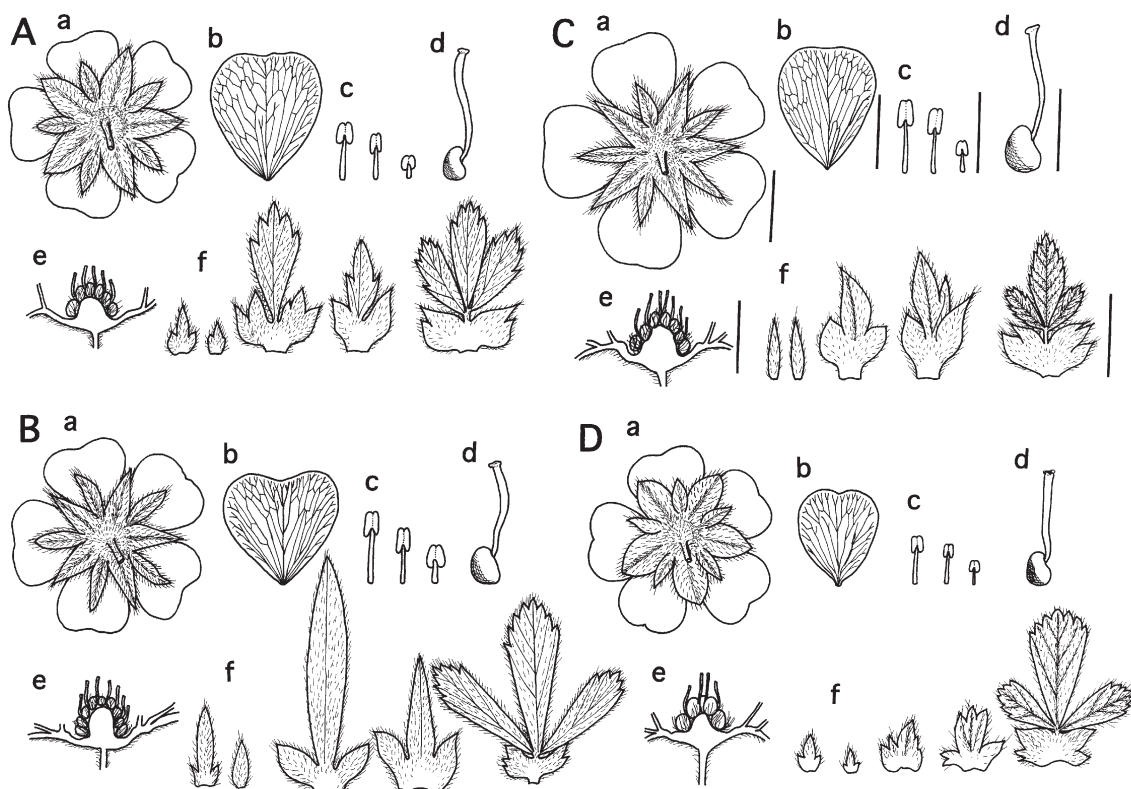


Fig. 3. *Potentilla × echizenensis* and putative parents, *P. toyamensis* and *P. freyniana*. A: *P. × echizenensis* from Koarashima-dake. B: *P. × echizenensis* from Ikegahara. C: *P. toyamensis* from Ikegahara. D: *P. freyniana* from Ikegahara. Scale in A, B and D as the same as C. Bars of a, b, c, e, f: 5 mm. Bar of d: 1 mm. a: Back view of flower. b: Petal. c: Stamens. d: Pistil. e: Longitudinal section of flower. f: A series of cauline leaves from apex to basal part on stem (from left to right).

Table 2. Measurements of somatic chromosomes of *Potentilla* × *echizenensis* collected at Koarashima-dake and Ikegahara, Fukui Prefecture

Koarashima-dake

No.	Length	Total (μm)	A. R.	Form	No.	Length	Total (μm)	A. R.	Form
1	0.7 + 0.7	1.4	1.0	M	8	0.4 + 0.7	1.1	1.8	sm
2	0.6 + 0.7	1.3	1.2	m	9	0.4 + 0.7	1.1	1.8	sm
3	0.6 + 0.6	1.2	1.0	M	10	0.4 + 0.7	1.1	1.8	sm
4	0.6 + 0.6	1.2	1.0	M	11	0.4 + 0.6	1.0	1.5	m
5	0.6 + 0.6	1.2	1.0	M	12	0.4 + 0.6	1.0	1.5	m
6	0.5 + 0.7	1.2	1.4	m	13	t-0.2 + 0.7	0.9	3.5	st
7	0.4 + 0.7	1.1	1.8	sm	14	t-0.2 + 0.7	0.9	3.5	st

Ikegahara

No.	Length	Total (μm)	A. R.	Form	No.	Length	Total (μm)	A. R.	Form
1	0.7 + 0.8	1.5	1.1	m	8	0.4 + 0.7	1.1	1.8	sm
2	0.7 + 0.7	1.4	1.0	M	9	0.4 + 0.7	1.1	1.8	sm
3	0.7 + 0.7	1.4	1.0	M	10	0.4 + 0.7	1.1	1.8	sm
4	0.6 + 0.8	1.4	1.3	m	11	0.4 + 0.6	1.0	1.5	m
5	0.6 + 0.6	1.2	1.0	M	12	0.4 + 0.6	1.0	1.5	m
6	0.5 + 0.7	1.2	1.4	m	13	t-0.2 + 0.7	0.9	3.5	st
7	0.4 + 0.7	1.1	1.8	sm	14	t-0.2 + 0.7	0.9	3.5	st

A. R. : Arm ratio. t : satellite.

total of 213 pollen mother cells (PMCs) at the first metaphase were analyzed (Fig. 4 A, B, C, Table 3). Of these, 207 cells (97.2%) had 7 bivalents, and the rest had 6 bivalents and 2 univalents (2.8%). Pollen fertility was 64.0% (3,659 pollen grains were examined in total). Similarly, in the plant collected at Ikegahara, the PMCs at same stage had 7 bivalents (93.9%), except for some few cells showing 6 bivalents and 2 univalents (6.1%) (Table 3). Pollen fertility of the plant was 52.6% (2,568 pollen grains).

In contrast to this results, meiotic chromosomes of the parental species, *P. freyniana* and *P. toyamensis*, show normal bivalent formation at the first metaphase in PMCs: seven bivalents in 212 cells (100%) of *P. freyniana* and in 211 cells (100%) of *P. toyamensis* (Iwatsubo and Naruhashi 1991). Pollen fertilities of both species were 96.7% (2,322 pollen grains examined) in *P. freyniana* and 77.9% (2,632 pollen grains) in *P. toyamensis*.

The karyotypes of the two plants (Koarashima-dake and Ikegahara) were identical to those of *P. freyniana* and *P. toyamensis* and had showed somewhat lower bivalent formation than the two

putative parent species of *P. freyniana* and *P. toyamensis*.

In our previous report for the *P. fragarioides* group including the putative parents of *P. × echizenensis*, every species examined showed the same karyotype (Iwatsubo and Naruhashi 1991). Moreover, our cytogenetical studies on both the artificial F₁ hybrids (Iwatsubo and Naruhashi unpublished) and natural hybrids (Iwatsubo and Naruhashi 1992) among the species belonging to this group showed that all the hybrids had low pollen fertility and somewhat lower bivalent formation than every species in the group. As found in the observation, the two plants of *P. × echizenensis* also had low pollen fertility and somewhat lower bivalent formation in common with those of the artificial and natural hybrids.

The new plants are growing with the putative parents, *P. freyniana* and *P. toyamensis*, and furthermore their flowering season is the same. They are alike in morphology and karyotype (Figs. 3, 4). Therefore the new plant may be understood as the hybrid of the above mentioned two taxa.

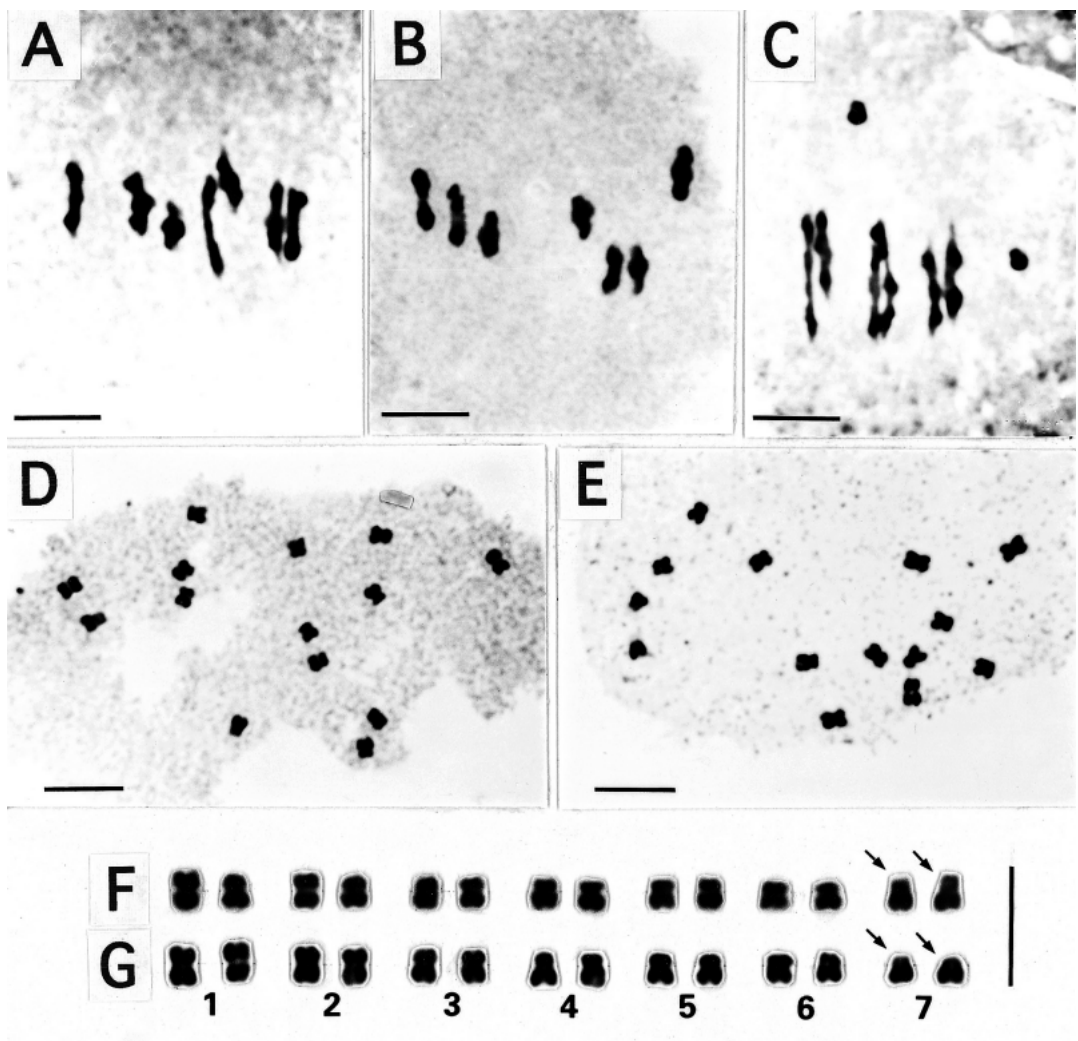


Fig. 4. Meiotic chromosomes at metaphase, mitotic metaphase and karyotype in *Potentilla* × *echizenensis*. A–C : Showing 7 II (A, B), 6 II + 2 I (C) of PMCs, Koarashima-dake. D : 2n=14, Koarashima-dake. E : 2n=14, Ikegahara. F : Koarashima-dake. G : Ikegahara. Arrows indicate chromosome arms with satellites. Bars represent 5 μm.

Table 3. Chromosome pairing at M I in *Potentilla* × *echizenensis* collected at Koarashima-dake and Ikegahara, Fukui Prefecture

	Configuration	No. of PMCs observed	Frequency (%)
Koarashima-dake	7 II	207	97.2
	6 II + 2 I	6	2.8
		(213)	
Ikegahara	7 II	232	93.9
	6 II + 2 I	15	6.1
		(247)	

() : Counted numbers in total.

Taxonomic description

Potentilla × *echizenensis* Naruh. et Tak. Sato, hybr. nov. (Figs. 1, 2, 3 A, B)

P. × *musashinoana* non Makino; Watanabe, Flora of Fukui Prefecture p.150. (1989).

P. × *echizensis* Naruhashi et Sato in Watanabe, Flora of Fukui Prefecture p.176. (2003), nom. nud.

P. freyniana Bornm. × *P. toyamensis* Naruh. et Tak. Sato

Haec hybrida *Potentillae toyamense* affinis est, sed foliis nullis vel 1 accessoris foliolatis et foliis inferioris saepe pallide purpuratis diversa. Ab *P. freynianae* rhizomate non-tubuliformi et foliis 1–2 accessoris foliolatis et parvis rhizophylli foliatis sub anthesi distinguitur.

Perennial herbs, pilose, stoloniferous. Stolons 1–4, loosely few leaved, 15–30 cm long. Roots somewhat stout. Rhizomes elongate, thickened, not tuberous, sublignose. Flowering stems ascendent, pilose, 10–20 cm tall. Leaves on rhizome trifoliate or quartefoliate- or quinquefoliate-pinnate, larger terminal leaflets 3, extremely smaller leaflets 1–2, petiolate, stipulate, 6–25 cm long; leaflets sessile or nearly so, elliptic to rhombic-oblong, acute to obtuse, attenuate to obtuse, 1–5.5 cm long, 1–3 cm wide, serrate, pilose on both surfaces. Lower surface of leaves often somewhat purplish. Petioles pilose. Stipules membranaceous, greenish brown, segments lanceolate to triangular-ovate, acuminate, pilose outside and on margin. Leaves on flowering stem trifoliate or unifoliate, with or without petiole, stipulate; stipules ovate to oblong, entire or slightly 1–3 dentate, pilose. Leaves on runner trifoliate, petiolate, stipulate; stipules ovate to lanceolate, entire or slightly 1–3 dentate, pilose. Inflorescences cymes, 3–10-flowered. Flowers April to May, 5-merous, ca. 1.5 cm across. Pedicels slender, densely pilose, 5–20 mm long. Petals yellow, widely obovate, 6–8 mm long, 5–8 mm wide, emarginate. Calyx segments triangular-ovate, acute, entire, ca. 4mm long, ca. 2mm wide, pilose outside. Epicalyx segments narrowly oblong, acute, pilose outside, smaller and narrower than calyx segments. Stamens 20; anthers ovate-elliptic. Pistils numerous; styles sublateral, glabrous, nearly filiform. Receptacles white pilose. Achenes glabrous, slightly rugose.

Nom. Jap. Echizen-kijimushiro (Naruhashi 1979

unpublished)

Type : Mt. Koarashima in Oono-shi, Fukui Prefecture, 960 m alt., Naruhashi no. 89042604, 26 Apr. 1989 (**Holotype** OSA, **isotype** KYO, MAK, MBK, OSA, TI, TNS)

Hab. Japan. Honshu. Fukui Prefecture : Ikegahara, Shimouchinami, Oono-shi, ca. 920 m alt., N. Naruhashi and T. Sato no. 89052101 and no. 89052102, 21 May 1989 (HYO, KANA, KYO, MAK, MBK, NAC, OSA, TI, TNS, TOYA) ; cult. in Univ. of Toyama, Naruhashi no. 89042001, 20 Apr. 1989 (HYO, KANA, KYO, MAK, MBK, NAC, OSA, TI, TNS, TOYA) Mt. Koarashima, ca. 1,200 m alt., S. Watanabe s. n., 2 Jun. 1968 (KANA) ; ibidem, 940 m alt., Naruhashi no. 89042603, 26 Apr. 1989 (KYO, OSA).

Acknowledgments

We wish to give our hearty thanks to Messrs. Ichiro Sasaki and Takejiro Hashimoto who kindly guided Sato to the growing sites at Ikegahara, and to Mr. Sadamichi Watanabe who kindly guided Naruhashi to the growing sites at Koarashima-dake. We wish also to give our sincere gratitude to Mr. Mamoru Sugimoto for proofreading this article and to Dr. Madjit Hakki who kindly revised our manuscript. We also thank the curator, the late Prof. Nobuo Satomi of Kanazawa University (KANA) for permission to examine specimens.

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- (Received August 10, 2009; accepted December 10, 2009)

鳴橋直弘¹・佐藤 卓²・岩坪美兼³：バラ科キジムシロ属の 1 新雑種，エチゼンキジムシロ

福井県大野市下打波池ヶ原と大野市小荒島岳で見つかったミツバツチグリとエチゴツルキジムシロとの中間的形態を所有する植物 (Fig. 1) を調べたところ、両種の自然雑種と推測されたので、エチゼンキジムシロ *Potentilla* × *echizenensis* Naruh. et Tak. Sato としてここに記載発表する。

この植物は、佐々木一郎氏、橋本竹二郎氏、佐藤によって、1978 年 7 月 19 日に池ヶ原で発見されたもので、その後、1989 年 5 月 21 日に、佐藤と鳴橋が現地を訪れている。一方鳴橋は、金沢大学理学部所蔵の 1968 年 6 月 2 日の渡辺定路氏採集の小荒島岳産の標本を見て、渡辺氏に採集地への案内をお願いし、彼の案内で 1989 年 4 月 26 日に現地を調査している。

本雑種は渡辺定路氏の『福井県植物誌』(1989)

のオオミツバツチグリと同定されたものと同じであり、同氏の『改訂増補 福井県植物誌』(2003)には裸名で“エチゼンキジムシロ *P. × echizenensis* Naruhashi et Sato”と掲載されている。

この新雑種、エチゼンキジムシロはミツバツチグリとエチゴツルキジムシロの中間的形質を示すが、前者とは根茎が塊茎状にならないこと、および植物体が 1-2 個の側小葉を持つ葉があることで区別できる。また、後者とは、植物体が側小葉を持たない葉や 1 個の側小葉を持つ葉があることで区別できる。ミツバツチグリとエチゼンキジムシロは、葉の下面が時に紫色を帯びる。エチゴツルキジムシロとエチゼンキジムシロは、冬期にロゼット葉を持ち、花期にはこの葉が残る。これら 2 分類群の根出葉の托葉は、披針形～三角状卵形で大きく目立つ。また、花期か花期後には大きな葉を展開する。そのため、これら 2 分類群は、花期には大小の葉 (冬期のロゼット葉と春に展開した大きな葉) をもっている (Fig. 1)。それに対して、ミツバツチグリは冬芽がしっかりしており、春の葉の展開は遅く、花期には春に展開した大きな葉のみからなる。また、根出葉の托葉は線形～披針形で目立たない。

エチゼンキジムシロは推定両親種と同じ染色体数と核型を持ち (Fig. 4)、花粉母細胞の減数分裂第 1 分裂中期の対合では、ミツバツチグリとエチゴツルキジムシロが全て 7 個のⅡ価を示したのに対し、エチゼンキジムシロは 6 個のⅡ価と 2 個のⅠ価を示す細胞が観察された (Fig. 4, Table 3)。また、花粉稔性も小荒島岳 64.0%、池ヶ原 52.6% と低い。これらのことは、新植物が雑種であることを示唆している。

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