ヨモギ属近縁2種北海道産 Artemisia rubripes およびヨーロッパ産 A. vulgaris の染色体数と核形態

Junko NOGUCHI*, Shuichi NAKAYAMA** and Shoichi KAWANO***: Karyotype Analysis of *Artemisia rubripes*, an Introduced Species from the Asiatic Continent into Hokkaido, Japan, and a European Species, *A. vulgaris*.

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ヨモギ属近縁2種北海道産 *Artemisia rubripes* および
ヨーロッパ産 *A. vulgaris* の染色体数と核形態

In the previous paper, it was shown by NAKAYAMA (1983 in press) that a taxonomically unknown species of *Artemisia*, which at present occurs widely in eastern to central Hokkaido, was identified as *A. rubripes* NAKAI (Sect. Abrotanum Subsect. Polycarpicae Series Vulgares-Mongolicae) (KITAMURA, 1940) based upon its morphological characters, although it had been once tentatively referred to *A. vulgaris* L., a European species (cf. NAKAYAMA, 1983 in press). It was also suggested that this species was possibly introduced from north-eastern part of China into Hokkaido just after the World War II, and had expanded its distribution range over eastern to central Hokkaido. To supplement a previous taxonomic study, the present karyological analysis was conducted on both *Artemisia rubripes* collected from Tokachi, Hokkaido, by one of the authors (S. NAKAYAMA), and *Artemisia vulgaris*, authentic seeds of which were kindly sent from Prof. H. MERXMÜLLER of the University of München Botanical Garden, München, Federal Republic of Germany.

Chromosome counts have so far been made on *A. vulgaris* by various authors, i. e., 2n = 16 (TISCHLER, 1934; CLAUSEN *et al.*, 1940; KECK, 1946; PÓLYA, 1949; WULFF, 1950; LÖVE and LÖVE, 1956; MULLIGAN, 1957; SORSA, 1962; KAWATANI and OHNO, 1964; KOUL, 1964 a, b; KHOSHOO and SOBTI, 1958), 2n = 18 (KOUL, 1964 a, b; KHOSHOO and SOBTI, 1958; WEINEDEL-LIEBAU, 1928; MEHRA *et al.*, 1965), 2n = 36 (KHOSHOO and SOBTI, 1958; KOUL, 1964 b; CLAUSEN *et al.*, 1938), and 2n = 54 (KOSHOO and SOBTI, 1958; KOUL, 1964 b). It appears clear that the European *A. vulgaris* is a diploid species with the basic number of X = 8 chromosomes; whereas North American plants of *A. vulgaris* form a polyploid series, 2n = 36 (4X) and 2n = 54 (6X), possessing a different basic number of X = 9 (KECK, 1946). From *Artemisia rubripes* two different chromosome numbers, i. e., 2n = 16 and 2n = 18, have previously been reported by KUROSAWA (1979) on the materials collected from Lake Furen, Nemuro in eastern Hokkaido, and also from Takamori in Kumamoto Prefecture, Kyushu, respectively. All the previous papers, however, have not reported detailed karyotypes of these *Artemisia* plants. The purpose of this paper is first to report on the chromosome number of *A. vulgaris* and *A. rubripes*, and second, to describe critically their karyotypes.

Material and Methods

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Artemisia rubripes was collected in 1982 from the grassy roadside in the campus of Obihiro Chikusan University, Hokkaido, Japan. The collected rhizomes were planted in pots and cultivated in the experimental garden of Toyama University in 1983. The seeds of *A. vulgaris* were kindly supplied by Prof. H. MERXMÜLLER and were sowed in vermiculite in a pot. For the chromosome counts and karyotype analysis, somatic chromosomes were observed in root tip meristematic cells. Fifteen individuals of *A. rubripes*, and twenty-two of *A. vulgaris* were examined in this study.

The root tips were pretreated with 0.002 M 8-hydroxyquinoline for 2-2.5 hours at 18 °C, and fixed in 45 % acetic acid solution for 10-15 minutes at 4-8 °C. Subsequently, root tips macerated in a 1 : 2 mixture of 45 % acetic acid and 1 N HCl were stained with 1 % aceto-orcein for 15 minutes, and squashed.

Results

A. rubripes NAKAI

The morphology of interphase nuclei of *Artemisia rubripes* NAKAI belongs to the complex chromocenter type, which contains 10-20 small round heteropycnotic bodies in lightly staining chromatin (Fig. 1 A) (TSCHERMAK-WOESS, 1963 ; TANAKA, 1971 a). The condensing pattern at prophase to prometaphase corresponds to interstitial type which initiates to condense from the interstitial or proximal parts of chromosome arms (TANAKA, 1977). In particular, the terminal interstitial part of the ninth chromosome, the proximal interstitial part of the fifteenth, and the proximal part of the sixteenth begin to strongly condense earlier at prophase to prometaphase (Fig. 2 A). In this study *A. rubripes* examined (collected from Obihiro, Hokkaido) possess 2n = 16 chromosomes. The longest chromosome pair in the complement is about 6 μm in size, and the shortest about 4 μm in size. The second to the seventh pairs, intermediate of the two extremes in size, gradually vary in size. The metaphase

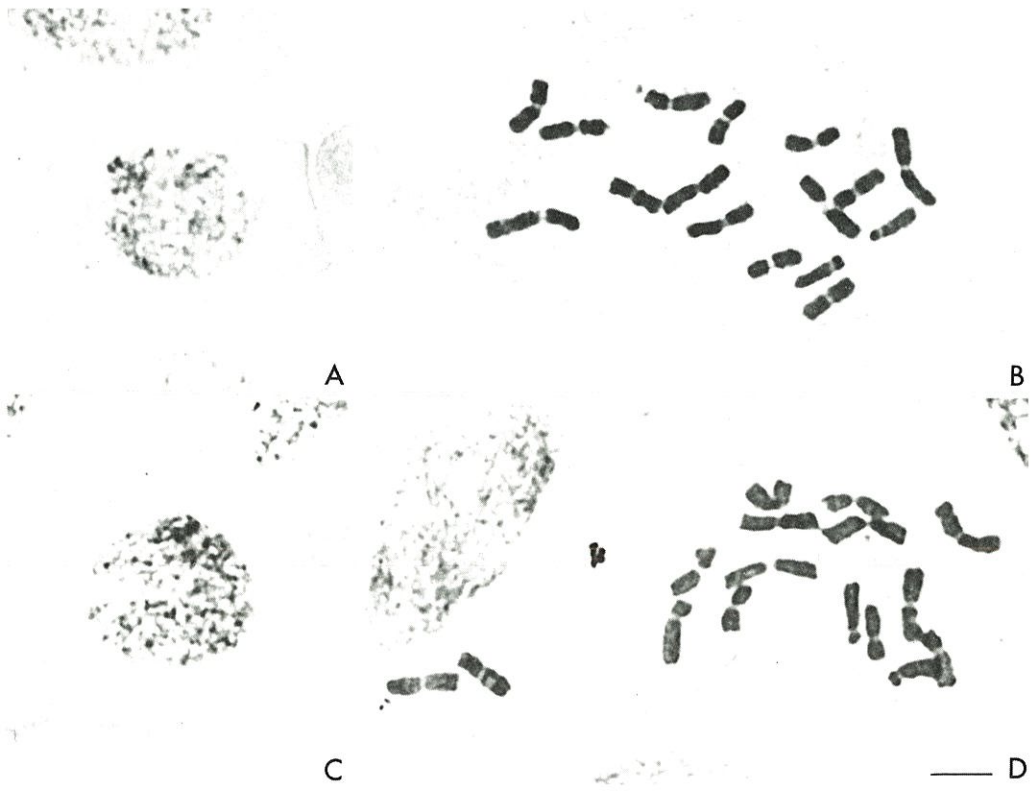

Fig. 1. Photomicrographs of interphase nuclei and mitotic metaphase chromosomes. A and B, *A. rubripes* ; C and D, *A. vulgaris*. The bars in the photos specify 5 μm.

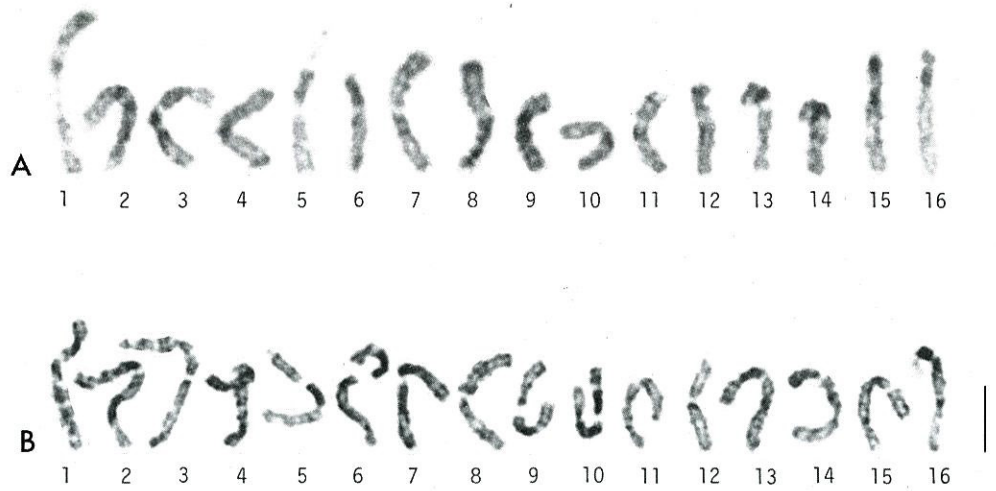

Fig. 2. Photomicrographs of prometaphase chromosomes in two species of *Artemisia*. A, *A. rubripes* and B, *A. vulgaris*.

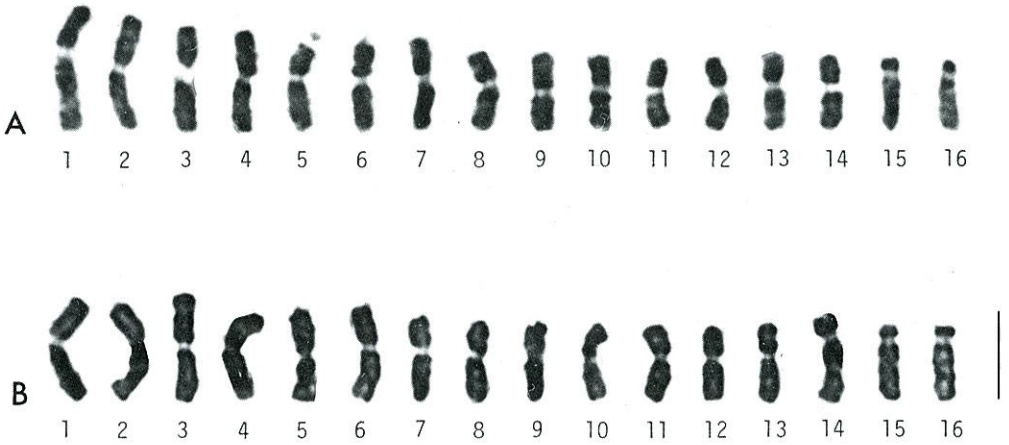

Fig. 3. Karyotypes at mitotic metaphase of two *Artemisia* species. A, *A. rubripes* (2n=16) and B, *A. vulgaris* (2n=16).

chromosome complement, consists of 14 median and 2 subterminal chromosomes (Table 1). The fifth, sixth, and ninth chromosomes are all satellited which have a satellite at the terminal part of the short arm. The satellites are 0.4-0.5 μm in size in the fifth chromosome, and 0.3-0.4 μm in sixth and ninth chromosomes. Regarding the size and occurrence of satellites, the third (chromosome no. 5 and 6) and the fifth (chromosome no. 9 and 10) pairs investigated always consisted of heterogeneous pairs of chromosomes, respectively (Fig. 3 A, 4 A).

A. vulgaris L.

Interphase nuclei of *A. vulgaris* showed the characteristics of the complex chromocenter type nuclei, just as was observed on *A. rubripes*, having 25-40 small round heteropycnotic bodies in lightly staining chromatin (Fig. 1 C). The condensation pattern of prophase to prometaphase is the same interstitial type as *A. rubripes*. However, the initiating parts which begin to condense in *A. vulgaris* are different from those in *A. rubripes*. In *A. vulgaris*, the interstitial part of the long arm and the whole short arm in the sixteenth chromosome are very strongly condensed (Fig. 2 B). The chromosome number of *A. vulgaris* was also

Table 1. Chromosome length, arm ratio, and chromosome morphology of *A. rubripes*

Complement	Chromosome length (μm)	Arm ratio	Chromosome morphology
1	2.8 + 3.6 = 6.4	1.3	m
2	2.4 + 3.8 = 6.2	1.6	m
3	2.5 + 2.8 = 5.3	1.1	m
4	2.3 + 2.8 = 5.1	1.2	m
5	0.5 + 1.9 + 2.8 = 5.2	1.5	m
6	0.4 + 2.0 + 2.8 = 5.2	1.4	m
7	2.0 + 2.6 = 4.6	1.3	m
8	1.8 + 2.6 = 4.4	1.4	m
9	0.4 + 2.0 + 2.0 = 4.4	1.0	m
10	1.8 + 2.0 = 3.8	1.1	m
11	1.6 + 2.0 = 3.6	1.2	m
12	1.6 + 2.0 = 3.6	1.2	m
13	1.5 + 2.2 = 3.7	1.5	m
14	1.5 + 2.0 = 3.5	1.3	m
15	0.7 + 2.8 = 3.5	3.7	st
16	0.7 + 2.8 = 3.5	3.8	st

Table 2. Chromosome length, arm ratio, and chromosome morphology of *A. vulgaris*.

Complement	Chromosome length (μm)	Arm ratio	Chromosome morphology
1	2.3 + 3.3 = 5.6	1.4	m
2	2.4 + 3.4 = 5.8	1.4	m
3	2.8 + 2.9 = 5.7	1.1	m
4	2.6 + 2.8 = 5.4	1.1	m
5	0.3 + 2.2 + 2.7 = 5.2	1.2	m
6	2.4 + 2.6 = 5.0	1.1	m
7	2.0 + 2.3 = 4.3	1.2	m
8	1.9 + 2.4 = 4.3	1.3	m
9	1.8 + 2.4 = 4.2	1.3	m
10	1.8 + 2.4 = 4.2	1.3	m
11	1.6 + 2.1 = 3.7	1.3	m
12	1.6 + 2.3 = 3.9	1.4	m
13	1.6 + 2.6 = 4.2	1.6	m
14	1.1 + 3.6 = 4.7	3.1	st
15	0.6 + 3.2 = 3.8	5.5	st
16	0.8 + 3.2 = 4.0	4.0	st

turned out to be 2n = 16. The size of metaphase chromosome complement is 4 μm to 6 μm, ranging continuously in size in different pairs. The metaphase chromosomes are composed of 13 median and 3 subterminal chromosomes. A chromosome in the third chromosome pair (chromosome no. 5) has a very small satellite at the terminal part of the short arm. At metaphase, it was not possible to discriminate a satellite at the terminal part of the short arm of a chromosome (no. 15) in the eighth chromosome pair. At prometaphase, however, a diffusing satellite was often observed in that part. Both heterogeneous pairs (consisting of median and subterminal chromosomes) and homogeneous pairs of two median chromosomes were observed in the seventh chromosome pair (Fig. 3 B).

Discussion

Both *Artemisia rubripes* NAKAI and *A. vulgaris* L. examined in this study proved to possess the same somatic chromosome number, i. e., 2n = 16, agreeing with the previous counts made by KUROSAWA (1979) for *A. rubripes* from Nemuro, Hokkaido and also for European *A. vulgaris* (TISCHLER, 1934; CLAUSEN *et al.*, 1940; KECK, 1946; PÖLYA 1949; WULFF, 1950; LÖVE and LÖVE, 1956; MULLIGAN, 1957; SORSA, 1962; KAWATANI and OHNO, 1964; KOUL, 1964 a, b; KHOSHOO and SOBTI, 1958), although 2n = 18 chromosome numbers are also known from *A. rubripes* (KUROSAWA, 1979) and 2n = 18, 36, and 54 from *A. vulgaris* (KOUL, 1964 a, b; KHOSHOO and SOBTI, 1958; WEINEDEL-LIEBAU, 1928; MEHRA *et al.*, 1965). The morphology of inter-

phase nuclei, the condensation pattern of chromosomes at prophase to prometaphase, and the chromosome length at metaphase of these two species were similar to each other. However, differences were observed in the following characteristics, i. e., (1) the number of heteropycnotic bodies at interphase, (2) the portions of early condensation in chromosome complements at prophase to prometaphase, and also (3) the number and morphology of satellite chromosomes, as was described in the results.

Although both species possess very similar somatic karyotypes at metaphase, the third pair (no. 5 and 6) in the chromosome complement of *A. rubripes* is "submedian" (more strictly) with a satellite at the distal end of the short arm; whereas in *A. vulgaris* somatic chromosomes referred to the third pair are "median", often heteromorphic in the presence of a satellite, although the third chromosomes can be classified exclusively as "median" according to LEVAN (1964).

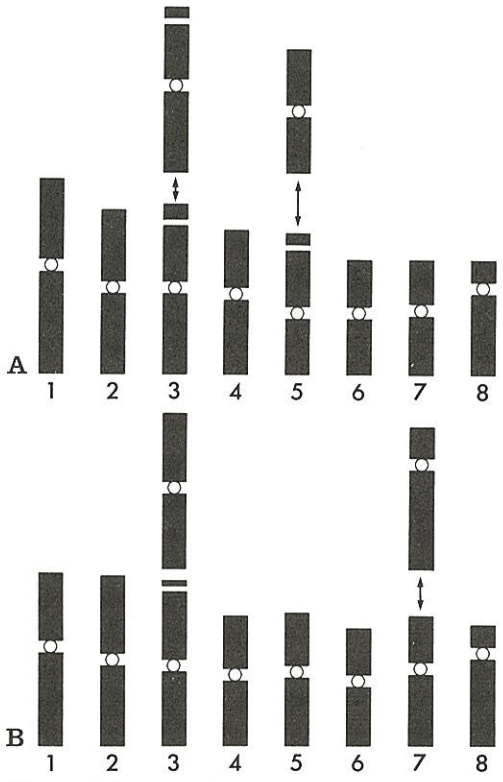

Fig. 4. Idiogram of haploid chromosomes at mitotic metaphase. The upper chromosome pair with the same number represents heteromorphic chromosomes observed. A, *A. rubripes* and B, *A. vulgaris*.

Further difference in size and morphology of chromosomes, and also presence of satellite can be found in the fifth pair and seventh pair of the complement between these two species (cf. Fig. 4).

In addition to gross morphological characters, the karyological data gathered in this study provided us with additional evidence for further consideration on the taxonomic status of *A. rubripes*. However, since two different chromosome numbers are presently known from this species, i. e., 2n = 16 from the plants in Hokkaido (KUROSAWA, 1979 and the present observations) and 2n = 18 from those in Kyushu (KUROSAWA l. c.), the origin of *A. rubripes* in Hokkaido may be different from that in Kyushu. Future karyological as well as ecological studies on the plants in China, Korea, and also in Kyushu must be considered in a thorough review of this problem.

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References

CLAUSEN, J., KECK, D. D. and HIESEY, W. M. 1940. Experimental studies on the nature of species. The genus *Artemisia*. Carnegie. Inst. Washington Publ., **520**: 325-350.

KAWATANI, T. and OHNO, T. 1964. Chromosome numbers in *Artemisia*. Bull. Natl. Inst. Hygienic Sci. **82**: 183-193.

KECK, D. D. 1946. A revision of the *Artemisia vulgaris* complex in North America. Proc. California Acad. Sci. Ser. 4, **25** (17): 421-468.

KITAMURA, S. 1940. Compositae Japonicae II. Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, **15** (3): 285-446.

KHOSHOO, T. N. and SOBTI S. N. 1958. Cytology of Indian species of *Artemisia*. Nature **181**: 853-854.

KOUL, M. L. H. 1964 a. Chromosome numbers in some medicinal Composites. Proc. Ind. Acad. Sci., Sect. B. **59** (2): 72-77.

————. 1964 b. Cytomorphological survey of Indian *Artemisia* L. J. Sci. Res. Banaras Hindu Univ., **14** (2): 103-110.

KUROSAWA, S. 1979. Notes on chromosome numbers of Spermaphytes (2). J. Jap. Bot. **54**: 155-160.

LEVAN, A. K., FREDGA, K. and SANDBERG, A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas. **52**: 201-220.

LÖVE, A. and LÖVE, D. 1956. Cytotaxonomical conspectus of the Icelandic flora. Act. Horti Gotob. **20** (4): 65-291.

MULLIGAN, G. A. 1957. Chromosome numbers of Canadian weeds. I. Can. J. Bot. **35** (5): 779-789.

NAKAYAMA, S. 1983. On the identity of a naturalized *Artemisia* species in Hokkaido, Japan. J. Phytogeogr. & Taxon. (in press).

PÓLYA, L. 1949. Chromosome numbers of some Hungarian plants. Act. Geobot. Hung. **6** (2): 124-137.

SORSA, V. 1962. Chromosomenzahlen Finnischer Kormophten I. Ann. Acad. Sci. Fenn. Ser. A, IV. Biol. **58**: 1-14.

TANAKA, R. 1971. Types of resting nuclei in *Orchidaceae*. Bot. Mag. Tokyo. **84**: 118-122.

————. 1977. A new approach in Karyology (in Japanese). In: ZOKUSAIBOUGAKUTAIKEI, Vol. 3. SYOKUBUTSUSAIBOUGAKU, p. 293-326. Asakura, Tokyo.

TISCHLER, G. 1934. Die Bedeutungen der Ployploidie für die Verbreitung der Angiospermen, erläutert an den Arten Schleswig-Holsteins, mit Ausblicken auf andere Florengebiete. Bot. Jahrb. **67**: 1-36.

TSCHERMAK-WOESS, E. 1963. Strukturtypen der Ruhederne von Pflanzen und Tieren. Protoplasmatologia. **5**: 158.

WULFF, H. D. 1950. Chromosomenstudien an der schleswigholsteinischen Angiospermen-Flora. V. Ber. Deutsch. Bot. Ges. **63** (3): 64-70.

摘　要

中山ら（植物地理・分類研究 31：71-77 参照）は，北海道に生育する 10 種余のヨモギ属 *Artemisia* の種に関して詳細な種生態学的研究を行なってきたが，その内の 1 種（仮に *A. vulgaris* に当てられていた）については，その正確な分類学的帰属が不明であった。前報の中山（1983）の詳しい外部形態の比較から明らかにされたように，この taxon は中国大陸，朝鮮半島と九州の一部に広く分布するヤブヨモギ *A. rubripes* であることが確認された。

この報告では，北海道帯広市で採集した *A. rubripes* と，西ドイツミュンヘン植物園から入手したヨーロッパ産 *A. vulgaris* について，その染色体数，体細胞分裂環の間期，前中期および中期の核型の比較を行なった。その結果，染色体数は 2 種ともに 2n = 16 であり，さらに 2 種の間期核の形態および前期での凝縮パターンも類似していた。また，体細胞中期の染色体の核型は，14 個の中部型染色体と 2 個の次端部型染色体からなっていた。しかし，詳細にみると，2 種間にはつぎのような差異が認められた。付随体染色体数は，*A. rubripes* では 3 本であるのに対し，*A. vulgaris* では 1 本であった。付随体を有する第 3 対目の染色体組についてみると，*A. rubripes* では動原体の位置は次中部に近い部位にあるのに対し，*A. vulgaris* では正中部に近い部位に認められる。また，*A. vulgaris* では第 7 対目の染色体は，非相同な染色体組（中部型と次端部型）からなる個体がみられた。間期の凝縮塊数と前中期で早くかつ強く凝縮する部位にも違いがみられた。

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ホウライシダの新産地（河野和博）Kazuhiro KOHNO: *Adiantum capillus-veneris* Found in Hokuriku District.

昭和 58 年 5 月中旬，金沢市内で *Adiantum* の一種が自生しているのを見つけ，採集した。調べてみると，形態的にはホウライシダ（*Adiantum capillus-veneris* L.）のようであるが，従来，この種の日本海側の分布は北九州以北には報告されていない。〔東京大学出版会：日本のシダ植物図鑑 Vol. 1, p.p. 82～83 (1979)〕

金沢大学理学部の里見信生先生の御意見を求めたところ，ホウライシダであると同定されたが，念のためその標本は国立科学博物館の中池敏之先生にも送られたところ，やはりホウライシダであると断定された。

石川県では先にマツバラン，オニクラマゴケの発見があり，この種の発見も同様に極めて興味がある。生育地は日当りの良い石垣のすき間で，個体数は必ずしも多くないので，絶滅をおそれ，詳しい産地の記載は省略する。なお証拠標本は，東京国立科学博物館（TNS 365962）と，金沢大学理学部（KAN 107903）に収めてある。最後に，本種の同定をして下さった国立科学博物館中池敏之・金沢大学理学部里見信生先生に，厚く御礼申し上げる。