

Coryphopteris castanea (Thelypteridaceae), a Polymorphic Diploid Species Distributed in Taiwan and Japan

Atsushi Ebihara^{1,*}, Narumi Nakato², Yi-Hang Chang³ and Li-Yaung Kuo⁴

¹ Department of Botany, National Museum of Nature and Science,
4–1–1 Amakubo, Tsukuba, Ibaraki 305–0005, Japan

² 1–363 Narahashi, Higashiyamato 207–0031, Japan

³ Hengchun Research Center, Taiwan Forestry Research Institute, Hengchun,
Pingtung Co. 946005, Taiwan

⁴ Institute of Molecular & Cellular Biology, National Tsing Hua University, Hsinchu City, Taiwan

*E-mail: ebihara@kahaku.go.jp

(Received 9 February 2021; accepted 24 March 2021)

Abstract Diploid chromosome number $2n = 62$ observed in the Taiwanese populations of *Coryphopteris castanea* supported the distinctness of this species from tetraploid *Thelypteris* (*Coryphopteris*) *japonica*. Although much variation in frond size was observed across its populations of Taiwan and Iriomote Island in southern Ryukyu of Japan, their alleles of nuclear *gapCp* “short” region formed a monophyletic clade, suggesting that those populations are highly likely to be populations of a single species. Spores of *C. castanea* with membranous ornamentation are apparently similar to those of *T. japonica*, but they are slightly smaller and more folds and small pits were observed under the light and scanning electronic microscopes.

Keywords: chromosome number, fern, spore.

Thelypteris castanea (Tagawa) Ching [= *Parathelypteris castanea* (Tagawa) Ching] is a terrestrial or epilithic fern species originally described from northern Taiwan (Tagawa, 1935), and later discovered in Iriomote Island in the southern Ryukyu, Japan (Hatusima, 1971; Serizawa, 1975) (Fig. 1). This species, morphologically closest to *Thelypteris japonica* (Baker) Ching [= *Parathelypteris japonica* (Baker) Ching] distributed in temperate East Asia (China, Japan and Korea), is usually distinguished from *T. japonica* by its castaneous (vs. reddish purple or stramineous) stipes of the fronds and denser (vs. sparse) hairs on the frond axis. There is a conflicting opinion that *T. castanea* should not be distinguished from *T. japonica* (e.g. DeVol and Kuo, 1975; Kuo, 1985; Yang and Liu, 2002;

Knapp, 2011).

Nakato and Ebihara (2016) observed the chromosome number of *T. castanea* collected in Iriomote Island, and the result ($2n = 62$) suggested that the Ryukyu population of the species is diploid—cytologically distinct from tetraploid *T. japonica* (Nakato *et al.*, 2004). The results of molecular phylogeny (Ebihara, 2011; Ebihara *et al.*, 2020) also supported the identity of the Ryukyu population of *T. castanea* as being independent from *T. japonica*. However, we cannot draw a conclusion about the relationship of the two species until Taiwanese populations of *T. castanea* are sampled for cytological and molecular phylogenetic studies. In fact, frond sizes of the Ryukyu populations tend to be smaller than those of Taiwanese populations (Serizawa, 1975) (Figs. 1b, 1d). Thus, a careful study on infraspecific variation of *T. castanea* is necessary.

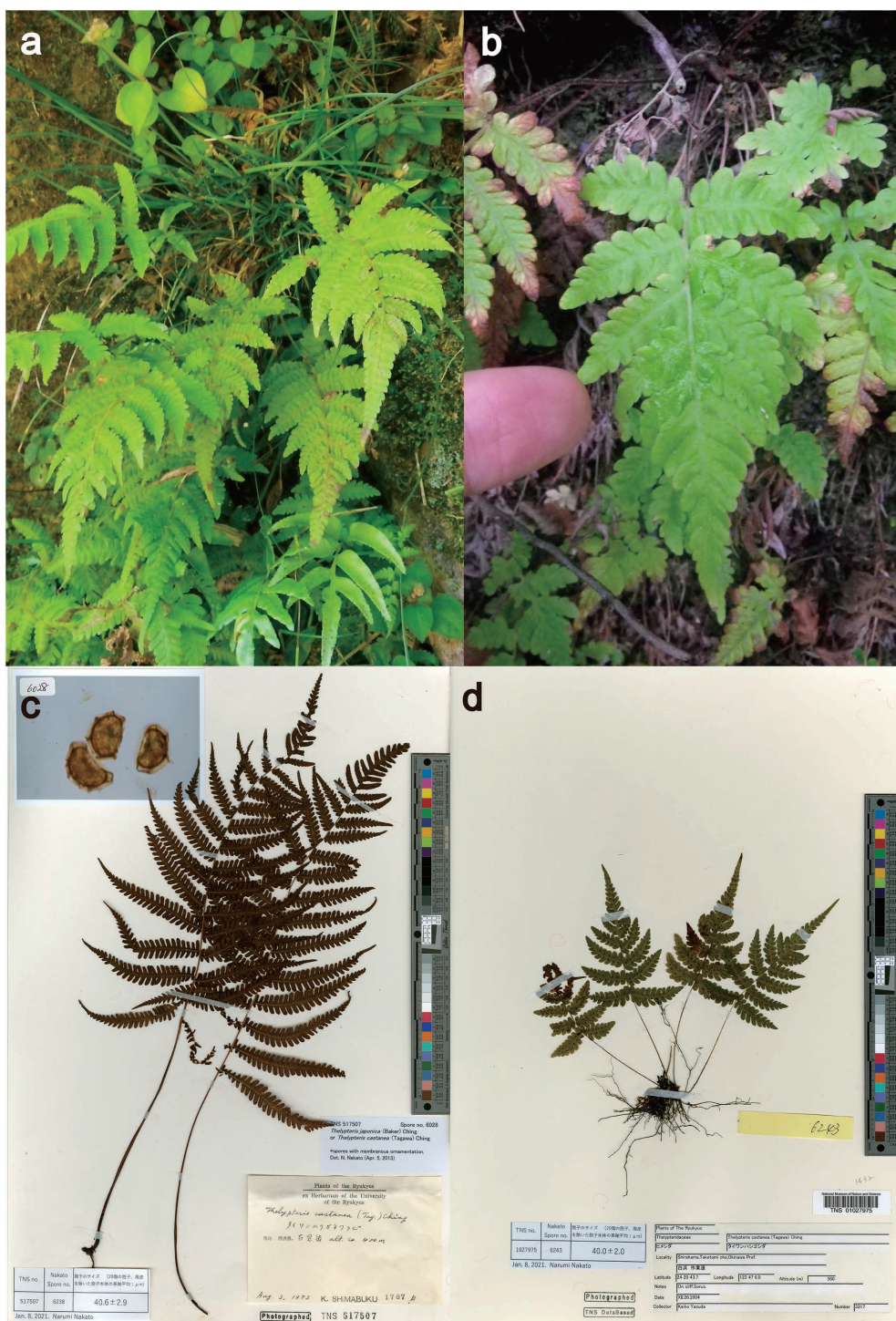


Fig. 1. *Coryphopteris castanea*. a. Sihyuan Yakou, Taichung City, Taiwan; b. Iriomote Island, Japan; c. a specimen with normal-sized fronds collected from Iriomote Island (TNS VS-517507); d. a specimen with smaller fronds collected from Iriomote Island (TNS VS-1027975).

Materials and Methods

A total of ten living stocks from two locations of Taiwan (the northern north-central parts) were collected and used for chromosome count and molecular analysis (Table 1). Chromosome number was counted in the metaphase of root tip cells using the same methods as in Ebihara *et al.* (2014). Plastid *rbcL* sequences and nuclear *gapCp* “short” sequences (Schuettelpelz *et al.* 2008) were obtained, following the methods used in Ebihara *et al.* (2010, 2020). For the *gapCp* “short” sequences, molecular phylogenetic analysis was performed under the same methods as in Ebihara *et al.* (2020) combining newly generated

sequences (GenBank accession numbers are listed in Table 2) and the dataset used by that study. Frond and spore sizes of *T. castanea* were measured in a total of 21 specimens (8 from Japan and 13 from Taiwan) and 18 specimens (8 from Japan and 10 from Taiwan), respectively. All of the specimens are deposited in the herbarium, National Museum of Nature and Science (TNS). For measuring frond sizes, one mature frond was selected from each herbarium sheet. For spore sizes, 20 spores derived from multiple sporangia were selected and the length along the long axis excluding perispore was measured. Spores of a Taiwanese individual (*Chang* 20120707-011 [TAIF], Mt. Chitan, Dist. Xindian,

Table 1. The material of *Coryphopteris castanea* used in the present study

Locality & Coll. No.	Voucher specimen [TNS VS-]	Chromosome number	<i>gapCp</i> genotype	Spore size (μm)
Taiwan, Taipei city, Beitou district, Zhuzihu				
<i>Ebihara & Kuo</i> 3136	1170755	$2n = 62$ (2x)	FK	—
<i>Ebihara & Kuo</i> 3137	1170754	$2n = 62$ (2x)	II	—
<i>Ebihara & Kuo</i> 3138	1170753	$2n = 62$ (2x)	IJ	35.3 ± 2.6
<i>Ebihara & Kuo</i> 3139	1170752	—	IL	—
Taiwan, Taichung city, Sihyuan Yakou				
<i>Ebihara & Kuo</i> 3113	1170785	$2n = 62$ (2x)	FF	36.4 ± 2.1
<i>Ebihara & Kuo</i> 3114	1170784	$2n = 62$ (2x)	HH	36.1 ± 2.1
<i>Ebihara & Kuo</i> 3115	1170783	$2n = 62$ (2x)	FH	36.6 ± 2.0
<i>Ebihara & Kuo</i> 3116	1170782	$2n = 62$ (2x)	FF* ¹	38.5 ± 1.9
<i>Ebihara & Kuo</i> 3117	1170781	$2n = 62$ (2x)	II	35.8 ± 2.0
<i>Ebihara & Kuo</i> 3118	1170778	—	FF* ¹	36.9 ± 2.3
Japan, Okinawa Pref., Iriomote Island				
<i>Ebihara</i> 060930-01	759279	—	GG* ²	—
The following specimens were used only for morphological observation and measurements.				
Taiwan, Datunshan				
<i>Sasaki s.n.</i>	387481	—	—	38.1 ± 2.4
Taiwan, Yangmingshan				
<i>Masuda</i> 8354	9539122	—	—	38.6 ± 2.5
Taiwan, Chihshingshan				
<i>Liew</i> 1190	446647	—	—	38.6 ± 1.7
Japan, Okinawa Pref., Iriomote Island				
<i>Shimabuku</i> 999	1327691	—	—	36.9 ± 2.0
<i>Takara & Takara s.n.</i>	533125	—	—	39.6 ± 2.3
<i>Yasuda</i> 3317	1027975	—	—	40.0 ± 2.0
<i>Kokubugata & al.</i> 5441	9518896	—	—	40.5 ± 2.5
<i>Sugasawa s.n.</i>	601255	—	—	40.6 ± 2.5
<i>Ebihara</i> 3177	1176780	$2n = 62$ (2x)* ³	—	41.6 ± 3.0
<i>Shimabuku</i> 1787	517507	—	—	42.0 ± 3.0
<i>Shimabuku</i> 1790	533124	—	—	39.3 ± 2.4

*¹ Alleles identified by a comparison of banding patterns of a SSCP gel.

*² Ebihara *et al.* (2020).

*³ Nakato & Ebihara (2016).

Table 2. GenBank accession numbers of *gapCp* “Short” sequences

Collection No.	Allele	GenBank accession No.
<i>Ebihara & Kuo 3136</i>	F	LC605144
<i>Ebihara & Kuo 3136</i>	K	LC605145
<i>Ebihara & Kuo 3137</i>	I	LC605146
<i>Ebihara & Kuo 3138</i>	I	LC605147
<i>Ebihara & Kuo 3138</i>	J	LC605148
<i>Ebihara & Kuo 3139</i>	I	LC605149
<i>Ebihara & Kuo 3139</i>	L	LC605150
<i>Ebihara & Kuo 3113</i>	F	LC605151
<i>Ebihara & Kuo 3114</i>	H	LC605152
<i>Ebihara & Kuo 3115</i>	F	LC605153
<i>Ebihara & Kuo 3115</i>	H	LC605154
<i>Ebihara & Kuo 3117</i>	I	LC605155

New Taipei City, 7 July, 2012) were also observed by Miniscope TM3000 scanning electronic microscope (SEM) (Hitachi High-Tech, Tokyo).

Results

The diploid chromosome number, $2n = 62$ was successfully observed in eight of the ten living samples (Fig. 2 shows one example; others were similar). Plastid *rbcL* sequences of the ten samples from Taiwan completely match that of Japan (GenBank accession AB575014). For nuclear *gapCp* “short” sequences, a total of seven alleles (Tables 1, 2) were identified, and all the alleles of the samples from *T. castanea* of Taiwan and Japan formed a robustly supported monophyletic clade in the tree (Fig. 3). Frond sizes (lamina length and width) were plotted in Fig. 5. Approximately 64 or more than 32 spores were observed per sporangium. The spores are bilateral, and the size (long axis) is $38.8 \pm 3.0 \mu\text{m}$. Under a light microscope, the perispore looks membranous (Fig. 4a–c), and numerous folds and pits can be seen on the membranous wings when observed using an electron microscope (Fig. 4d).

Discussion

Our results of *gapCp* phylogeny and chromosome counts supported that both Taiwanese and Japanese populations of *T. castanea* are closely

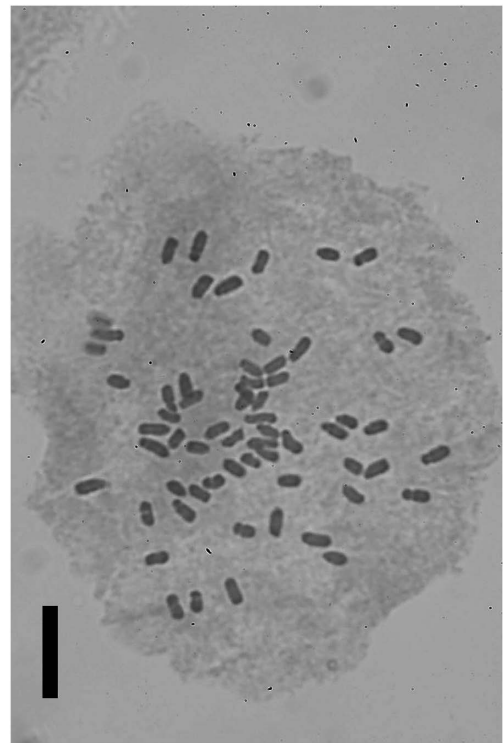


Fig. 2. Microphotographs of mitotic metaphase chromosomes of *Coryphopteris castanea* (Ebihara & Kuo 3117, Taiwan). $2n = 62$. Scale bar = $10 \mu\text{m}$.

related to each other and that they most likely belong to a single species. The results also suggested that *T. castanea* is a diploid species, and that it is not a progenitor of *T. japonica*, which is probably allotetraploid. Therefore, *T. japonica* should be excluded from the flora of Taiwan as was done by Taiwan Pteridophyte Group checklist (TPG) (2019, 2020).

It is interesting that relatively high allelic variation was detected in the *gapCp* “short” region of *T. castanea* collected only in three locations in contrast to poor or almost no infraspecific variation in *T. japonica*, *T. musashiensis*, *T. nipponica* and *T. sylvia-nipponica*. (Ebihara *et al.*, 2020). The species is also polymorphic in frond size—populations in Japan tend to have smaller fronds than those in Taiwan (Fig. 5). It is still unclear whether or not the frond size variation depends on environmental conditions of the habitat. We

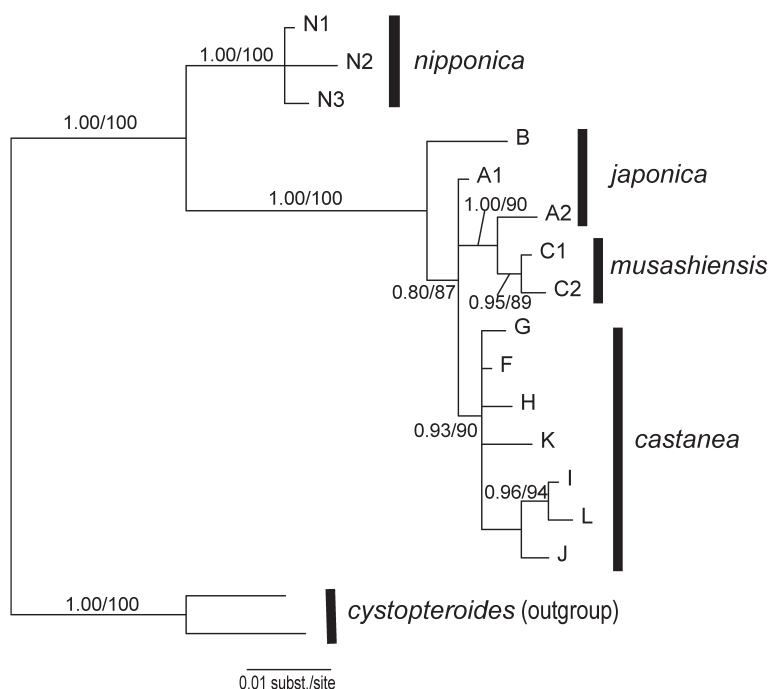


Fig. 3. A majority-rule consensus tree of nuclear *gapCp* "Short" region of *Coryphopteris castanea* and related species inferred by the Bayesian method rooted with *Parathelypteris cystopteroides*. Support values of nodes: posterior probabilities of Bayesian inference (>0.80 are shown)/bootstrap values (%) in maximum likelihood analysis (>50% are shown).

noticed a specimen, *Shimabuku 1787 TNS* (VS-517507) collected from Mt. Komidake, Iriomote Island, which bears exceptionally large (>20 cm long) lamina among specimens collected in Japan. Although we have not yet succeeded rediscovering any similar "large" individuals there, it would be worth examining both morphological and genetic variation of the species in Iriomote Island more carefully.

Spore morphology is often an important character for identifying species belonging to *Coryphopteris sensu* Fawcett *et al.* (in revision, see Taxonomic Treatment). In the case of *T. japonica* and its closely related species, the regularity of spore shape is useful for recognizing interspecific hybrids, which are frequently produced, and spore ornamentation pattern is the most reliable character for distinguishing diploid *T. musashinesis* from tetraploid *T. japonica* (Nakato *et al.*, 2004). According to our present observation, the spore size of *T. castanea*

($38.8 \pm 3.0 \mu\text{m}$) tends to be slightly smaller than *T. japonica* ($44.7 \pm 3.2 \mu\text{m}$, Nakato *et al.*, 2004) and *T. musashiensis* ($44.4 \pm 3.8 \mu\text{m}$, Nakato *et al.*, 2004). The spore ornamentation of *T. castanea* is apparently similar to that of *T. japonica* in that it has a membranous or alate appearance under a light microscope, though folds and small pits are more frequently observed than in *T. japonica*. The pits are more distinct in the spore image by SEM (Fig. 4d). However, there is a certain degree of infraspecific variation in the spore morphology of *T. castanea*. The spores on a specimen collected in Taiwan (*Liew 1190*, TNS 446647, Fig. 4c) showed more or less echinate perispore, an intermediate feature between *T. japonica* and *T. musashiensis*.

In conclusion, *T. castanea* is a phylogenetically distinct diploid species distributed in Taiwan and Iriomote Island in Japan. In spite of its restricted distribution range, the species possesses abundant morphological and genetic variation.

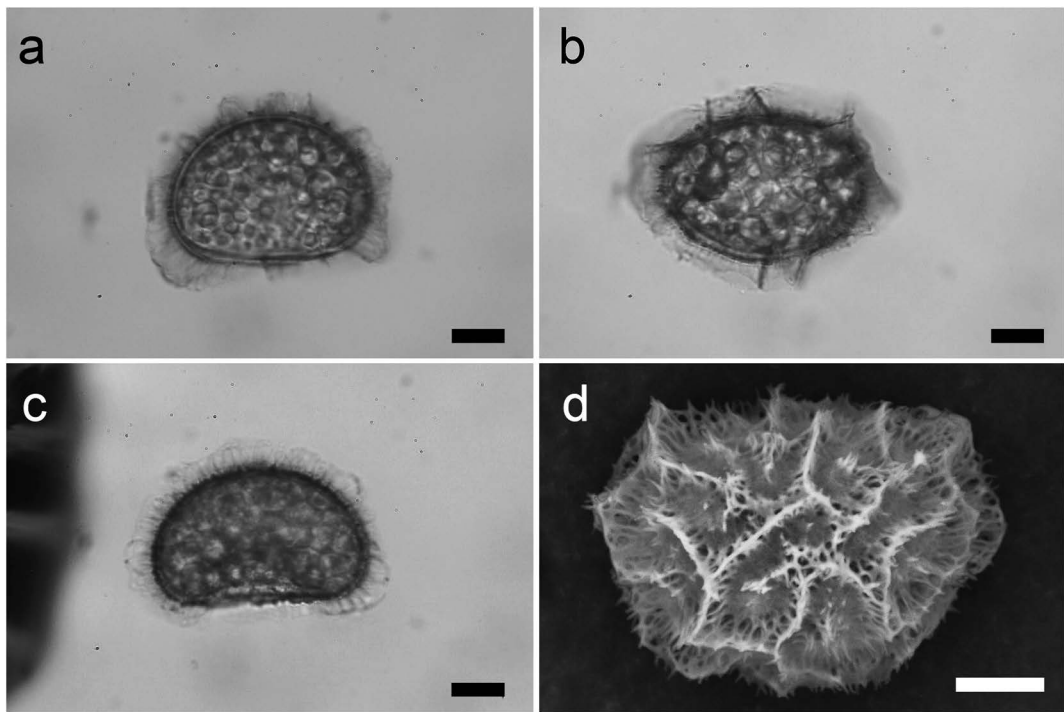


Fig. 4. Spores of *Coryphopteris castanea*. a. Ebihara & Kuo 3116, Taiwan; b. Ebihara 3179, Japan; c. TNS VS-446647, Taiwan; d. Chang 20120707-011, Taiwan. SEM image. Scale bar: 10 μm.

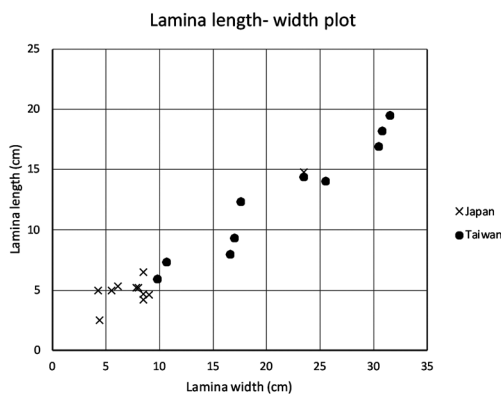


Fig. 5. A plot diagram of lamina sizes of *Coryphopteris castanea*.

Taxonomic treatment

The generic classification of Thelypteridaceae accepted in PPG1 (Pteridophyte Phylogeny Group, 2016) was premature, particularly for several polyphyletic genera including *Parathelypteris*, and more recently Fawcett *et al.* (in revision)

sion) proposed a generic revision based on an updated global phylogeny of the family. They subdivided *Parathelypteris* sensu PPG1 into two genera *Parathelypteris* sensu stricto and *Coryphopteris*, classifying *T. castanea*, *T. japonica*, *T. nipponica* and related species into the latter genus. Taiwan Pteridophyte Group (TPG) (2019) proposed a new generic combination "*Coryphopteris castanea* (Tagawa) Y.H.Chang", but the publication without citing the basionym did not meet ICN Art. 41.1 (Turland, 2018), hence this name has not been validly published.

Coryphopteris castanea (Tagawa) Y.H.Chang, A.Ebihara & L.Y.Kuo, **comb. nov.** —*Dryopteris castanea* Tagawa, Acta Phytotax. Geobot. 4(3): 132, 1935 —*Thelypteris castanea* (Tagawa) Ching, Bull. Fan Mem. Inst. Biol., Bot. 6(5): 315, 1936 —*Parathelypteris castanea* (Tagawa) Ching, Acta Phytotax. Sin. 8(4): 302, 1963.

Type: Taiwan, Taipei city, Beitou District, Zhuzihu 'Formosa, prov. Taihoku, Sitiseigun,

Tikusiko', *Tagawa 616* (holo KYO; iso BM [2 sheets], MICH)

Acknowledgments

The authors thank K. Uno and C. Takaboshi for their assistance in DNA sequencing. This study was partly supported by JSPS KAKENHI (Grant Nos. 15K07204 and 19H03288 to AE).

References

- DeVol, C. E. and Kuo, C. M. 1975. Flora of Taiwan. Volume 1. Epoch Publishing, Taipei.
- Ebihara, A. 2011. *RbcL* phylogeny of Japanese pteridophyte flora and implications on infrafamilial systematics. Bulletin of National Museum of Nature and Science, Series B 37: 63–74.
- Ebihara, A., Nitta, J. H. and Ito, M. 2010. Molecular species identification with rich floristic sampling: DNA barcoding the pteridophyte flora of Japan. PLoS ONE 5: e15136.
- Ebihara, A., Nakato, N., Ohi-Toma, T. and Iwatsuki, K. 2020. *Thelypteris sylvanipponica*, a new allotetraploid species in the *Parathelypteris* group (Thelypteridaceae). Phytotaxa 477: 229–242.
- Ebihara, A., Nakato, N., Saito, Y., Oka, T. and Minamitani, T. 2014. New records of *Asplenium varians* (Aspleniaceae) and two new hybrids in Japan. Acta Phytotaxonomica et Geobotanica 65: 53–65.
- Fawcett S., Smith, A. R., Sundue, M., Burleigh, J. G., Sessa, E. B., Kuo, L. Y., Chen, C. W., Testo, W. L., Kessler, M., Goflag Consortium and Barrington, D. S. A global phylogenomic study of the Thelypteridaceae. Systematic Botany (in revision).
- Hatusima, S. 1971. Flora of the Ryukyus. Okinawa Association of Biology Education, Okinawa (in Japanese).
- Knapp, R. 2011. Ferns and Fern Allies of Taiwan. KBCC Press & Yuan-Liou. Publishing Co., Ltd., Taipei.
- Kuo, C.-M. 1985. Taxonomy and phytogeography of Taiwanese pteridophytes. Taiwania 30: 5–99.
- Nakato, N. and Ebihara, A. 2016. Chromosome numbers of 18 ferns in Japan: Toward completion of chromosome information in Japanese ferns. Bulletin of National Museum of Nature and Science, Series B 42: 25–40.
- Nakato, N., Sahashi, N. and Kato, M. 2004. Cytotaxonomy of the *Thelypteris japonica* complex (Thelypteridaceae). Acta Phytotaxonomica et Geobotanica 55: 89–105.
- Pteridophyte Phylogeny Group. 2016. A community-derived classification for extant lycophytes and ferns. Journal of Systematics and Evolution 54: 563–603.
- Schuettpelz, E., Grusz, A. L., Windham, M. D. and Pryer, K. M. 2008. The utility of nuclear *gapCp* in resolving polyploid fern origins. Systematic Botany 33: 621–629.
- Serizawa, S. 1975. Pteridophytes of the Ryukyu Islands (1). Science Reports of the Takao Museum of Natural History 7: 1–53.
- Tagawa, M. 1935. *Spicilegium pteridographiae Asiae Orientalis* 8. Acta Phytotaxonomica et Geobotanica 4: 132–148.
- Taiwan Pteridophyte Group (TPG). 2019. Updating Taiwanese pteridophyte checklist: a new phylogenetic classification. Taiwania 64: 367–395.
- Taiwan Pteridophyte Group (TPG). 2020. Taiwan Pteridophyte Checklist v2020.2. <https://groups.google.com/forum/#!forum/taiwan-pteridophyte-group> [Accessed 31 January 2021].
- Turland, N. J., Wiersema, J. H., Barrie, F. R., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T. W., McNeill, J., Monro, A. M., Prado, J., Price, M. J. and Smith, G. F. 2018. International Code of Nomenclature for Algae, Fungi, and Plants (Shenzhen Code), adopted by the Nineteenth International Botanical Congress, July 2017, Shenzhen, PR China. Regnum Vegetabile 159. Koeltz Botanical Books, Glashütten.
- Yang, Y. P. and Liu, H. Y. 2002. Manual of Taiwan Vascular Plants, vol. 6. Taipei: The Council of Agriculture, The Executive Yuan.