A taxonomic revision of cleistocarpous species of *Weissia* (Pottiaceae, Bryophyta) in Japan

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Abstract

Four species, including one newly described, of Japanese cleistocarpous species of *Weissia* (Pottiaceae): *W. exserta*, *W. japonica*, *W. kiiensis* and *W. parajaponica* sp. nov. are recognized based on molecular phylogenetic inference and morphological reassessment. For each species, typification, description, distribution, illustrations and photographs are presented, and a key to the species is included. Rapid sporophyte modifications in *Weissia* and monophyletic positions of these four species are supported by the analysis using concatenated chloroplast *rbcL* and *rps4* gene sequences. A lectotype is designated for *W. controversa* which is the type species of the genus, and a new combination, *W. loncophylla* is proposed for *Trachycarpidium lonchophyllum*.

Key words: Gametophyte, Sporophyte, Stegocarpous

Introduction

Among bryophytes, mosses show the most complex and diverse sporophytes. Sporophyte diversification can be explained in relation to their habitat preferences, and an understanding of sporophyte modification will help to clarify ideas of evolutionary parallelisms and adaptive specialization in mosses (Vitt 1981). The Pottiaceae Hampe (1853: 329) is the most generic and species rich family of mosses, with around 1,400 species in 83 genera, comprising more than 10 % of the known extant moss species (Frey & Stech 2009), and exhibit a great variety of apparent morphological, physiological and geneecological adaptations to their particular environments (Zander 1993). Geometric morphometric analyses together with evolutionary hypothesis testing have revealed that Pottiaceae is one of the lineages in which most shifts in sporangium shape have occurred, and the genus *Weissia* Hedwig (1801: 64) one of the most notable where a shift in both sporangium shape and also habitat is seen (Rose et al. 2016). These results indicate the potential for the genus to be used as a model organism for investigating morphological diversification in moss sporophytes.

The genus *Weissia* s.l. grows mainly on arable land which is a transient habitat subject to regular disturbance such as by cultivation (Porley 2008). Sporophytes of the genus show a great range of variability, including having exerted stegocarpous capsules, immersed cleistocarpous capsules, and various combination of sporophyte characters, while the gametophytes are essentially identical and distinguishing species when sterile is difficult (Stoneburner 1985). These characteristics have caused incongruence between gametophyte based and sporophyte based classifications, and there has been no consensus on the species or even generic circumscriptions of this group (see review by Stoneburner 1985). *Weissia* s.l. is often divided into four genera: *Astromum* Hampe (1837: 285), *Hymenostomum* Brown (1819: 572), *Phasconica* Müller (1882: 438) and *Weissia* s. str. *Astromum* is characterized by immersed cleistocarpous capsules, *Hymenostomum* by exerted stegocarpous, eperistomate capsules with hymenium, *Phasconica* is characterized by immersed stegocarpous (macrostomous), eperistomate capsules, and *Weissia* s. str. is characterized by exerted stegocarpous, peristomate capsules. Morphological, cytological and molecular phylogenetic studies have shown close relationships among these genera and resulted in the subdivision of *Weissia* into several genera, and also lent support to the congeneric treatment of *Weissia* (*Weissia* s.l.). There have been many reports of morphologically intermediate...
or malformed sporophytes presumably caused by hybridization between the species of *Astromum* and *Weissia s. str.* or of *Astromum* and *Hymenostomum* in nature (Nicholson 1905, 1906; Smith 1964, Reese & Lemmon 1965, Crundwell & Nyholm 1972, Khanna 1960, Anderson & Lemmon 1972, Williams 1966), and cytodetical analysis has also provided circumstantial evidence of hybrid sporophytes (Khanna 1960, Anderson & Lemmon 1972). Superficial characters of spores in *Astromum*, *Hymenostomum* and *Weissia s. str.*, are very nearly the same, favoring a congeneric concept (Saito & Hirohama 1974). The phylogenetic tree based on nuclear ribosomal internal transcribed spacer (nr ITS) sequences has shown the independent origin (parallelism) of sporophyte structures and rapid diversification and radiation in this group (Werner et al. 2005). Based on this morphological and molecular evidence, we follow the congeneric concept of *Weissia* and include *Astromum, Hymenostomum* and *Phasconica* within the broader concept of the genus in the present study.

In the Far East region, many species with different types of sporophytes have been described (e.g. Chen 1941, Saito 1975, Eddy 1990, Akiyama 1996). However, there are few DNA sequence data for species in this region, and a revisional study using integrated morphological and molecular data is necessary to clarify the evolutionary history and systematics of *Weissia* on a global scale. In the present study we have focused on cleistocarpous species of the genus (traditionally treated as *Astromum*) which include many heterogeneous capsule taxa. In Japan, five cleistocarpous species of *Weissia* had been reported as *Astromum: A. acuminatum* Dixon & Thériot in Dixon (1942: 11), *A. crispum* (Hedwig 1801: 21) Hampe (1837: 285), *A. exsertum* Brotherus (1899: 212), *A. japonicum* Roth (1911: 32) and *A. kiense* Okamura (1911: 140). In a monograph of Japanese Pottiaceae, Saito (1975) recognized two cleistocarpous species under *Weissia* subg. *Astromum* (Hampe) Kindberg (1897: 283): *W. longifolia* Mitten (1851: 317) [as *W. crispa* (Hedw.) Mitten (1851: 316)] and *W. exserta* (Broth.) Chen (1941: 158), with *A. acuminatum* and *A. kiense* synonymized in *W. longifolia*. The taxonomic status of *A. japonicum* was not discussed since the type material was not available. Based on a morphological study of the type specimens, Inoue & Tsubota (2017) recognized *A. japonicum* as a well-established species and proposed a new combination, *W. japonica* (G.Roth) Y.Inoue & H.Tsubota (2017: 86) for the species. In the present study, phylogenetic relationships and species circumscriptions of the cleistocarpous species of *Weissia* in Japan were reassessed based on molecular phylogenetic inference and detailed morphological investigation.

**Material & Methods**

**Species delimitation**

In the present study we recognized species as the population which is morphologically homogeneous and phylogenetically monophyletic or paraphyletic on the DNA tree except for *W. controversa* Hedwig (1801: 67) and *Trichostomum* Bruch in Müller (1829: 396) which have been shown to be polyphyletic (Werner et al. 2005), but by accepting the current broad concept of these taxa we have avoided making any premature taxonomic changes.

**Molecular phylogenetic analyses**

Sampling for DNA was based mainly on material collected by field research on *Weissia* growing in Japan (Honshu, Shikoku, Kyushu, and Ogasawara and Ryukyu Islands) during 2011–2016. Two phylogenetic markers were selected for the present analyses: chloroplast ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) and ribosomal protein S4 (*rps4*) genes. 33 *rbcL* and *rps4* gene sequences were newly obtained respectively. The supposed ingroup species represent species of Trichostomoideae *sensu* Werner et al. (2005). Outgroup species [*Barbula unguiculata* Hedwig (1801: 118) and *Didymodon japonicus* (Brotherus 1921: 6) Saito (1975: 508)] were selected based on Werner et al. (2005) and Inoue & Tsubota (2016). A total of 53 concatenated *rbcL* and *rps4* gene sequences were examined in the present analysis, as shown in Table 1.

Genomic DNA was extracted from leaves of plants bearing sporophytes. The protocol for extraction of total DNA followed Suzuki et al. (2013). Conditions of PCR amplification for both *rbcL* and *rps4* genes followed Inoue & Tsubota (2014). Direct sequence analyses of the PCR products were performed following Inoue et al. (2012). The list of primers used for PCR amplification and DNA sequencing is shown in Appendix 1. Sequences obtained in the present study have been submitted to DDBJ/EMBL/GenBank International Nucleotide Sequence Database Collaboration (INSDC).

Sequences of two genes were aligned separately by using the program MAFFT ver. 7.027 (Katoh & Standley 2013) with some manual adjustment on the sequence editor of MEGA ver. 5.2 (Tamura et al. 2011). Start and stop codons were removed, and the resulting total length was 2,025 bp. Duplicated sequences were eliminated using Phylogears2 (ver. 2.0.2013.10.22, Tanabe 2008).
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<th>Taxon</th>
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<th>Accession No.</th>
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**TABLE 1. (Continued)**

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<td>Japan: Nagano Pref., Y. Inoue 4040 (HIRO)*</td>
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*After reexamination of the voucher specimen (*Y. Inoue 4040* in HIRO) used by Inoue & Tsubota (2016) as *Didymodon constrictus* var. flexicuspis, we reidentified it as *D. japonicus*.

Phylogenetic analysis using the concatenated sequences of *rbcL* and *rps4* genes was performed based on a maximum likelihood (ML) method (Felsenstein 1981) with a codon substitution model, and the approximate unbiased (AU) test (Shimodaira 2002, 2004) in the final stage of the analysis scheme.

Prior to the phylogenetic reconstruction, Kakusan4 (ver. 4.0.2015.01.23, Tanabe 2011) was used to determine the appropriate substitution model and partitioning scheme for our data based on corrected Akaike Information Criterion (AICc: Sugiyama 1978). Since the codon substitution model is inappropriate for a heuristic search due to the huge computational burden, phylogenetic trees were constructed using the following three program packages to obtain the candidate topologies: (1) RAxML ver. 8.2.8 (Stamatakis 2014) with ML method using the equal mean rate model among codon positions (GTR + Γ for all codon positions of *rbcL* and *rps4*) with 1,000 heuristic searches; (2) PAUPRat (Sikes & Lewis 2001) over PAUP* ver. 4.0b10 (Swofford 2002) with the maximum parsimony (MP) method (Fitch 1971) to implement Parsimony Ratchet searches (Nixon 1999) using the Parsimony Ratchet search strategy with random weighting of each character in fifty 200 iteration runs; (3) MrBayes ver. 3.2.5 (Ronquist et al. 2012) with Bayesian inference (BI) method using the proportional model among codon positions (GTR + Γ + Invariant for first and second codon positions of *rbcL*, GTR + Γ for third codon position of *rbcL*, HKY85 + Γ for first and second codon positions of *rps4*, GTR + Homogeneous for third codon position of *rps4*) with 10,000,000 generations, sampling trees every 1,000 generations. A 50 % majority-rule consensus tree was calculated after the convergence of the chains and discarding 25 % of the sampled trees as burn-in.

Based on the ML criteria, re-calculation of likelihood values for each tree topology was performed with the codon substitution model which was more or less equivalent to the Goldman-Yang 1994 model implemented in Garli var. 2.01 (Zwickl 2006). The set of candidate topologies was evaluated by the AU test and Bayesian posterior probability (PP) calculated by the BIC approximation (Schwarz 1978, Hasegawa & Kishino 1989) using CONSEL ver. 0.20 (Shimodaira & Hasegawa 2001). A strict condensed tree for the topologies with high ranking log-likelihood values that passed both AU and PP tests was also computed by MEGA. Supporting values more than 50 % obtained by CONSEL were overlaid to assess the robustness of each branch of the highest likelihood topology: AU test (AU), bootstrap probabilities (NP), and Bayesian posterior probabilities (PP) are shown on or near each branch (AU/NP/PP).

**Morphological investigation**

The morphological investigation was made based on specimens included in the molecular phylogenetic analysis and additional specimens to assess whether each molecular grouping corresponds to species that could be recognized morphologically. Approximately 400 herbarium specimens of cleistocarpous species of *Weissia*, including several type specimens, were borrowed from BM, CBFS, H, HIRO, KOCH, MUB, NICH, NUM, NY, PC, S, SP, TNS and W were examined in the present study. This study also includes new material collected by field research during 2011–2016 on *Weissia* growing in Japan and which have been deposited at HIRO. Our morphological identification was made based only on the plants bearing sporophytes. Morphological characters were examined with a light microscope and scanning electron microscope (SEM). Preparation for SEM observation followed Inoue et al. (2011). To avoid developmental deviations, the descriptions and measurements were made only from plants with mature sporophytes.
We defined mature sporophyte as the sporophyte which possesses mature spores that are a brownish color and are densely papillate.

Results

Molecular phylogenetic analysis

The concatenated data matrix had a total length of 2,025 bp, of which 269 (13.3 %) were variable, and 135 (50.2 % of the variable sites) were parsimony-informative.

A total of 291 topologies were obtained from the three analyses: 233 ML topologies by RAxML; 57 MP by PAUPRat over PAUP*; and one BI by MrBayes. More detailed topologies were searched through the obtained trees using a log-likelihood measure. Fig. 1 shows the best-supported tree with the highest likelihood value (In $L = -5770.556$). The strict condensed tree was also obtained for the two topologies with high-ranking log-likelihood values that passed both AU and PP tests (not shown). These best-supported and strict condensed trees had identical topologies. Values for the percentage of supported topologies for each branch were superimposed in Fig. 1.

*Weissia* was resolved as monophyly with inclusion of *Trachycarpidium lonchophyllum* (Roth 1911: 182) Zander (1993: 213) with high supporting values (100/100/1.00). The *Weissia* clade was sister to *Trichostomum brachydontium* Bruch in Müller (1829: 393). Accessions of the exserted stegocarpous, peristomate species *W. controversa* are dispersed throughout the clade. Four cleistocarpous clades were confirmed in Japanese *Weissia*, corresponding to *W. kiiensis*, *W. japonica*, *W. exserta* and a new species *W. parajaponica*. *Weissia exserta* was sister to *W. parajaponica*, and *W. kiiensis* was sister to *W. japonica* with high supporting values (both 100/100/1.00). The relationships among these cleistocarpous species and stegocarpous species (*W. controversa* 1–3) were ambiguous in the present analysis.

Morphology and ecology

Our morphological investigations supported the molecular groupings of cleistocarpous species of *Weissia* in Japan, each circumscribed by a combination of sporophytic and perichaetial leaves characters. These characters are illustrated and described in the Taxonomy section. The most outstanding sporophytic feature shared by *W. exserta*, *W. japonica* and *W. parajaponica* is the presence of annulus (Fig. 2 A–C), which had been overooked in Japanese cleistocarpous species until it was pointed out by Inoue & Tsubota (2017). The annulus consists of several rows of much smaller cells than adjacent exothecial cells of the urn and operculum. The deoperculation found in these species is nonfunctional, that is, spores are not released from the dehiscent part of the capsule. We also observed that capsules of all four species have a fragile, capsule-abscission tissue region located at the junction of the capsule and seta, where the mature capsules are easily detached from the seta.

All four species grow on ground in sunny places such as arable land, gardens, parks, temples, schools, shrines, and roadside cliffs covered with thin soil at low elevation as described by Saito (1975). In the Japanese archipelago, *W. exserta*, *W. japonica* and *W. kiiensis* are all distributed in Honshu, Shikoku and Kyushu. In Hokkaido, only *W. kiiensis* is known and in Ogasawara and Ryukyu Islands, only *W. parajaponica* is known.

Discussion

Implications for the evolutionary history of *Weissia*

Our study has provided the first DNA sequences and phylogenetic relationships of the cleistocarpous *Weissia* species in Japan, and suggested monophyly of each species. The inferred length of the branches subtending nodes in Japanese *Weissia* is relatively short (< 0.0031), suggesting rapid and parallel sporophyte modifications (cleistocarpy) in this clade, as also shown in European and North American *Weissia* by Werner et al. (2005). Gametophytes often display a high degree of polymorphism while sporophytes remain less variable at intra- and inter-specific levels in bryophytes (Stanton & Reeb 2016). In the case of *Weissia*, however, our results suggest that sporophytes in *Weissia* species are more plastic than gametophytes between species, as also found in Funariaceae Schwägrichen (1830: 43) (Fife 1985). These groups usually occur in highly seasonal habitats, characterized by an alternation of moist and dry conditions over short periods and with bare soil not covered by larger plants, such as arable land. Vitt (1981) suggested that mosses occurring in highly seasonal habitat can be characterized by cleistocarpous, gymnostomous capsules that are...
FIGURE 1. Phylogenetic tree based on analysis with the concatenated sequences of chloroplast \textit{rbcL} and \textit{rps4} genes, depicted by the best-supported tree with highest likelihood value (In \( L = -5770.556 \) by Garli). Supporting values more than 50 \% obtained by the program CONSEL were overlaid: AU test (AU), bootstrap probabilities (NP), and Bayesian posterior probabilities (PP) are shown on or near each branch (AU/NP/PP). Thickened branches indicate that all three supporting values are 100 \%. 
often ovate and immersed. It appears as if selective pressures or relaxation in highly seasonal habitats are driving
the diversification rather than the conservation of sporophytic architecture (Liu et al. 2012). The relatively short
branch length in the Japanese Weissia clade also suggests reticulate evolution within the genus, as recently shown
in the Physcomitrium–Physcomitrella species complex (McDaniel et al. 2010, Bike et al. 2014). The hybrids are
usually found among weedy and semi-weedy species, that is species with potential life spans of a few years, and their
highly seasonal habitats promote the growth of colonies of different species in close proximity, increasing the chance
of intermixing and cross fertilization (Anderson 1980, Natcheva & Cronberg 2004). Rapidly rampant sporophyte
diversification within Weissia might result in adaptation to highly seasonal habitats and the formation of a syngameon,
which is the most inclusive unit of interbreeding in a hybridizing species group (Grant 1981).

In the Japanese cleistocarpous species, our DNA data showed that all four species are resolved in the monophyletic
clade (Fig. 1). Morphologically, however, W. japonica and W. parajaponica are nearly the same and they cannot always
be distinguished without DNA data. These two species partially share the sporophytic characters with W. exserta
(annulate capsules) and W. kiiensis (immersed capsules), and their urns show an intermediate shape between W. exserta
and W. kiiensis. These results imply the following two hypotheses, as suggested in vascular plants (Kato et al. 1996).
The first, that W. japonica and W. parajaponica originate from the hybrid-derived population: hybridization once
occurred between W. exserta and W. kiiensis, and subsequent back-crosses repeatedly occurred with one of mother
species. The second, that according to the morphological reduction series of the Pottiaceae (e.g. Saito 1975, Zander
1993), W. japonica and W. parajaponica (immersed capsules with annulus) originate from the ancient populations of
W. exserta (exserted capsules with annulus), and rapidly diverged in Honshu, Shikoku and Kyushu (W. japonica), and
Ogasawara and Ryuku Islands (W. parajaponica). Weissia kiiensis (immersed capsules without annulus) originates
from the ancient population of W. japonica and rapidly diverged in Hokkaido, Honshu, Shikoku and Kyushu. Formation
of a hybrid sporophyte and the production of viable spores support the former hypothesis (Reese & Lemmon 1965).
However, more solid evidence is necessary to untangle the evolutionary history among these species, provided by the
comparison of chloroplast and nuclear DNA sequences, microsatellite analysis, or the comparison of genome size by
flow cytometry based on broad geographical sampling.

**Systematic position of Trachycarpidium lonchophyllum**
The inferred tree supported monophyly of the genus Weissia with inclusion of a species of Trachycarpidium Brotherus
(1901). Trachycarpidium is characterized by long-lanceolate, plane-margined, entire leaves with a stout costa ending in a short awn, basal cells differentiated in a vee up the margins, and bulging, strongly protuberant cells of the body (not the apiculus) of the immersed, cleistocarpous capsule (Zander 1993). Its gametophytic similarity to Weissia and the possibility of it being included in the genus was suggested by Stone (1975). Trachycarpidium lonchophyllum was originally described as a species of Astomum from South America (Roth 1911). Later, Zander (1993) placed the species in Trachycarpidium due to its protuberant cells of the capsule (Fig. 2 E). The present study supports the recognition of Trachycarpidium as a member of Weissia. According to our phylogenetic tree we concluded that T. lonchophyllum should be transferred to Weissia. However we retained Trachycarpidium as a genus because the phylogenetic position of the type species T. verrucosum (Bescherelle 1873: 187) Brotherus (1901: 383) remains unclear.

Circumscriptions of Weissia controversa and the genus Trichostomum
The inferred tree suggested the current concept of W. controversa being polyphyletic, as shown by Werner et al. (2005) using nuclear ITS sequence data. The exserted stegocarpous, peristomate capsules of the species are reverted in the Weissia clade. A taxonomic revision of this species based on a broad geographical sampling is required for a comprehensive molecular phylogenetic analysis and reassessment of its defining morphological characters.

The type species of Trichostomum (T. brachydontium) was sister to the Weissia clade. Based on the analysis using ITS sequence data, this species was also resolved nested in Weissia and formed a subclade together with T. brittonianum R.H.Zander (1993: 92), T. crispulum Brach in Müller (1829: 295), and T. jamaiense (Mitten 1889: 147) Jaeger (1873: 397) [as W. jamaiicensis (Mitt.) Grout (1938: 157)] (Werner et al. 2005). These results supports a broad circumscription of the genus Weissia including Trichostomum (e.g. Dixon 1913, Andrews 1945). However, other species belonging to Trichostomum are polyphyletic (Werner et al. 2005, and present study). The current taxon sampling of Trichostomum and other genera in the subfamily Trichostomoideae appears to be insufficient to make a final conclusion whether Trichosomum should be transferred to Weissia.

Taxonomy

Based on the present investigation, the following taxonomic treatment on the genus Weissia in Japan is presented. We follow the Melbourne Code of Nomenclature (McNeill et al. 2012) for nomenclatural elements.

Description of the genus
= Cavanillea Borkh., Tent. Disp. Pl. German., op. posth. 251. 1809 (Borechhausen 1809), nom. illeg. [ICN Art. 53.1; later homonym (non Medik., non Desr.).]
= Simophyllum Lindb., Acta Soc. Sci. Finn. 10: 74. 1871 (Lindberg 1871), nom. illeg. (ICN Art. 52.1; type of earlier name included).

Description:—Plants small, forming low cushions, turfs or loosely caespitose. Stems simple or branched, erect, smooth, rounded in cross section; central strand present; scleroderms weakly differentiated; hyalodermis undifferentiated to well differentiated; axillary hairs hyaline throughout. Rhizoids sparse at base; rhizoidal tubers occasionally developed. Leaves strongly crisped when dry, spreading when moist, lanceolate to linear-lanceolate, tapering to an acute to acuminate apex from a broad to narrow oblong base; lamina unistratose; margins entire, incurved above the leaf base or plane throughout; costa single, stout, ending below apex to excurrent, papillose on adaxial surface, smooth or papillose on abaxial surface; cross section at midleaf ovate, occasionally circular or semicircular; adaxial epidermis present; adaxial stereid band present; guide cells in a single row or seldom scattered bistrate pairs; hydroid strand absent or present, abaxial stereid band present, abaxial epidermis present or occasionally absent; upper laminal cells subquadrate to hexagonal, papillose on both surfaces; basal laminal cells irregularly oblong, smooth. Laminal KOH color reaction...
yellow. *Sexual condition* monoicous or dioicous. *Perichaetium* terminal; perichaetial leaves little different from vegetative leaves or somewhat larger. *Perigonium* appearing as stalked lateral buds on perichaetiate plants (but variably present) or terminal on usually smaller perigoniate plants; perigonal leaves much smaller than vegetative leaves, ovate. *Seta* dextrosely twisted throughout or straight. *Capsules* stegocarpous or cleistocarpous, spherical to cylindrical; exothecial cells irregularly quadrate to oblong, smooth or mammillose (except the apiculus); stomata phaneroporous at base of capsules; annulus absent or present, when present consisting of much smaller cells than adjacent exothecial cells of urn and operculum, or persistent thick-walled larger cells; peristome teeth absent or present, when present erect or weakly dextrosely twisted. *Operculum* undifferentiated or differentiated, when differentiated conic to rostrate; cells straight to weakly dextrosely arranged. *Calyptra* cucullate. *Spores* brown to yellowish brown, papillose.

**Lectotypification of Weissia controversa**

Towards a better circumscription of the genus *Weissia*, we designate here a lectotype for *W. controversa*, the type species of the genus.


Type:—Lipsiae ad rivulum post collem Bienitz. Humo theca loca, nec non sabulosa, uda, praeprimis regionum montosarum amat (lectotype designated here, Tab. 5. B. in Hedwig 1791–1792).

Typification notes:—The genus *Weissia* Hedw. was typified on *W. controversa* Hedw. by Mitten (1856). When *W. controversa* was proposed by Hedwig (1801), he used the validating descriptions and illustrations which he had previously given to the same species (Hedwig 1791–1792). Although there was no designation of the holotype in either publication (Hedwig 1791–1792, 1801), in the photologue (Hedwig 1801) he referred to a specimen from Leipzig. One specimen from Leipzig, named *W. controversa* in Hedwig’s herbarium (G; Supplemental Information Fig. S.1), is the best candidate for a lectotype. In his taxonomic revision of *Weissia* for the Iberian Peninsula, Guerra (2002) selected this specimen as a lectotype for *W. controversa*. However, he did not include the phrase “designated here” or an equivalent, thus making this an ineffective typification (ICN Art. 7.10). This specimen has unfortunately been lost while on loan (Price 2005, p. 378). Hedwig’s illustration (Hedwig 1971–1792, Tab. 5. B.) is from the original material and is considered to be the only element that certainly fits Hedwig’s concept of the species, being the safest choice as lectotype. Hedwig (1791–1792, 1801) cited Vaillant’s and Dillenius’ pre-Linnean phrase-names with reference to their illustrations under *W. controversa* as synonyms. Vaillant (1727) and Dillenius (1742) did not refer to any particular specimen and made only general comment on habitat information. These two illustrations given by Vaillant and Dillenius can be considered parts of original material, but we believe that the Hedwig’s illustration provides much more morphological information and is therefore better to select this as the lectotype.

To ensure nomenclatural stability a specimen from the type locality, Bienitz in Leipzig, with DNA information should be selected as the epitype supporting the lectotype illustration rather than selecting an old specimen without DNA information, because the modern concept of *W. controversa* is thought to be polyphyletic (Werner et al. 2005, and present study) and a morpho-molecular revision is necessary to provide a better circumscription of the species.

**Key to the cleistocarpous species of Weissia in Japan**

| 1. Perichaetial leaves little differentiated from vegetative leaves; capsules with functionally dehiscent operculum (spore release with opening of capsule mouth) | Stegocarpous species |
| - Perichaetial leaves well differentiated and much larger than vegetative leaves; capsules without functionally dehiscent operculum (spore release with irregular dehiscence of capsule) | |
| 2. Annulus absent | *W. kiensis* |
| - Annulus present | |
| 3. Seta 0.5–1.2 mm long; capsules exserted from perichaetial leaves; urn ellipsoidal | *W. exserta* |
| - Seta less than 0.4 mm long; capsules deeply immersed among perichaetial leaves; urn ovoid to subovoid | |
| 4. Urn (550–)625–750(–840) × (450–)505–600(–720) μm; costa excurrent in a point reaching (80–)90–130(–160) μm...*W. japonica* |
| - Urn (400–)500–660(–760) × (360–)415–515(–620) μm; costa excurrent in a point reaching (80–)100–250(–280) μm... | *W. parajaponica* |

Notes:—Although *W. parajaponica* tends to have a smaller urn and longer excurrent costa than *W. japonica*, their dimensions sometimes show considerable overlap, and these two species cannot always be distinguished without phylogenetic analysis based on chloroplast DNA data.

*WEISSIA* (POTTIACEAE) Phytotaxa 306 (1) © 2017 Magnolia Press • 9
Description of the species

1. Weissia exserta (Broth.) P.C. Chen, Hedwigia 80: 158. 1941 (Chen 1941).

Basionym:—Astomum exsertum Broth., Hedwigia 38: 212. 1899 (Brotherus 1899). Type:—JAPAN. Nagasaki Pref.: 20 January 1861, Hichiro 1379a (lectotype designated here, H 190018).

≡ Systegium exsertum (Broth.) Paris, Index Bryol. Suppl. 317. 1900 (Paris 1900).

≡ Hymenostomum exsertum (Broth.) Broth., Nat. Pflanzenfam. 1 (3): 386. 1902 (Brotherus 1902).

Description:—(Figs. 2 A, & 3 A–J). Plants when moist ca. 5–10 mm high, including capsules. Stems simple or branched, erect; central strand present; sclerodermis weakly differentiated; hyalodermis undifferentiated. Leaves of when dry, spreading when moist, gradually becoming larger toward shoot apex. Autoicous. Perichaetial leaves much larger than vegetative leaves, lanceolate to linear lanceolate, (2.3–)2.9–4.3(–4.7) mm long and 0.4–0.6(–0.75) mm wide at base, tapering to an acuminate apex from a broad oblong base; margins incurved in distal 1/2–2/3, plane in basal portion, smooth or nearly smooth with faint projections at shoulder part of leaf base; costa stout, excurrent in a point reaching (70–)80–115(–130) µm, papillose on adaxial surface and smooth on abaxial surface; guide cells 4 in a single row at midleaf; adaxial and abaxial sestrogones 2–4 atrostrate at midleaf; upper laminal cells subquadrate, 6–9(–10) × 6–9 µm, papillose on both surfaces with bifid papillae; basal laminal cells enlarged, rectangular, (50–)65–85 × 8–10(–12) µm, smooth. Perigonial leaves much smaller than vegetative leaves, oval, acuminate, concave. Asexual reproduction unknown. Setae (450–)670–920(–1140) µm long; epidermal cells elongated, thick walled. Capsules cleistocarpous, exserted from perichaetial leaves; urn ellipsoidal, (690–)760–970(–1180) × (460–)570–625(–760) µm; exothecial cells irregularly quadrate, smooth; stoma phaneroporous, 4–6 at base of capsule; annulus present at the base of the apiculus, consisting of much smaller cells than adjacent exothecial cells of urn and operculum. Operculum differentiated as a slightly oblique finger-like beak, (280–)285–340(–385) µm long. Calyptra cucullate, (700–)905–1110(–1280) µm long. Spores (16–)18–20(–22) µm in diam., densely papillose.

Typification notes:—When Brotherus (1899) described A. exsertum, he cited two specimens: Wiechura 1379a and 1379b. However, he did not specify the holotype, so each of these specimens is a syntype (ICN Art. 9.5). Thus it is necessary to select the lectotype from these two specimens. Saito (1975) cited the specimen Wiechura 1396a (H) as the holotype of A. exsertum. However, the specimen Wiechura 1396a is the type for Hyophila propagulifera Brotherus (1899). In a taxonomic account of Indian Pottiaceae, Aziz & Vohra (2008) cited the specimen Wiechura 1379a (H) as the type of A. exsertum. However, they did not validly designate a lectotype for A. exsertum, because they did not include the phrase “designated here” (ICN Art. 7.10). In the present study we could confirm that the specimen Wiechura 1379a agrees well with the original description of Brotherus (1899) and we designate it as the lectotype of A. exsertum. (The specimen Wiechura 1379b was not found in H: Curator, pers. comm., March 2015).

Distribution:—Japan (Honshu, Shikoku and Kyushu).

Representative specimens examined:—JAPAN. Honshu, Ibaraki Pref.: Nishi-ibaraki District, Iwase-cho, Ohta, 14 December 1981, Z. lwatsuki 9546 (NICH-M 185373); Kanagawa Pref.: Kamakura City, Imaizumidai, ca. 100 m elev., 35°20′05″N, 139°32′55″E, 9 March 2013, Y. Inoue 1794 (Hiro); Aichi Pref.: Toyokawa City, Solar-Terrestrial Environment Laboratory of Nagoya University, Toyokawa Branch, 7 January 1953, N. Takaki s.n. (NUM-Bt 13762); Nara Pref.: Ikoma District, Tomio-mura, Hirano, ca. 100 m elev., 25 March 1949, M. Mizutani 1487 (NICH-M 31106); Hiroshima Pref.: Higashihiroshima City, Hiroshima University, ca. 220 m elev., 34°24′08″N, 132°42′42″E, 23 February 2012, Y. Inoue 912 (Hiro, DNA voucher); Hatakaihi City, Miyajima Isl., 10 m elev., 23 January 1969, coll. T. Seki. in lb. Miyajima Natural Botanical Garden no. 798 (Hiro); Shikoku, Ehime Pref.: Imabari City, Ohshima Isl., ca. 100 m elev., 34°10′39″N, 133°03′59″E, 14 May 2011, H. Tsubota 7699 (Hiro); Kyushu, Nagasaki Pref.: 20 January 1861, Wiechura 1379a (lectotype of Astomum exsertum, H 190018); Kumamoto Pref.: Hitoyoshi, Isshochi, ca. 100 m elev., 26 February 1971, K. Saito 8546 (TNS 70370); Oita Pref.: Tsukumi City, Chiu, ca. 20 m elev., 33°04′29″N, 132°52′53″E, 2 March 2013, Y. Inoue 1788a (Hiro, DNA voucher).


Basionym:—Astromum japonicum G.Roth, Aussereur. Laubm. 187. 1911 (Roth 1911). Type:—JAPAN, s.loc. & s.d, Siebold s.n. [lectotype—(designated by Inoue & Tsubota 2017), PC 657676; isolectotypes, BM 867124!, S B3524!).
FIGURE 3. *Weissia exserta* (A–J) and *W. japonica* (K–T). A, Habit (dry); B, Cross section of stem; C, Perichaetal leaf; D & E, Vegetative leaves; F & G, Cross sections of perichaetal leaf; H, Sporophyte; I, Cross section of seta; J, Calyptra; K, Habit (dry); L, Cross section of stem; M, Perichaetal leaf; N & O, Vegetative leaves; P & Q, Cross sections of perichaetal leaf; R, Sporophyte; S, Cross section of seta; T, Calyptra. A–J drawn from Wichura 1379a (H 190018, lectotype of *Astomum exsertum*); K–T from Y. Inoue 914 (HIRO).
Description:—(Figs. 2 B, & 3 K–T). Plants when moist ca. 5–10 mm high including capsules. Stems simple or branched, erect; central strand present; scleroderms weakly differentiated; hyalodermis undifferentiated. Leaves strongly crisped when dry, spreading when moist, gradually becoming larger toward shoot apex. Autoicous. Perichaetal leaves much larger than vegetative leaves, lanceolate to linear lanceolate, (2.0–)2.6–4.2(–4.7) mm long and (0.4–)0.5–0.7(–0.9) mm wide at base, tapering to an acumenate apex from a broad oblong base; margins incurved in distal 1/3–1/2, plane in basal portion, smooth or nearly smooth with faint projections at shoulder part of leaf base; costa stout, excurrent in a point reaching (70–)85–120(–125) μm, papillose on adaxial surface and smooth on abaxial surface; guide cells 4 in a single row at midleaf; adaxial and abaxial stereids 2–3 stratose at midleaf; upper laminal cells subquadrate, 6–9(–10) × 6–8 μm, papillose on both surfaces with bifid papillae; basal laminal cells enlarged, rectangular, (55–)60–90(–100) × 8–12(–15) μm, smooth. Perigonial leaves much smaller than vegetative leaves, oval, acumenate, concave. Asexual reproduction unknown. Setae (35–)120–190(–260) μm long; epidermal cells quadrate to subquadrulate, thin walled. Capsules cleistocarpous, deeply immersed among perichaetal leaves; urn ovoid to subovoid, (550–)620–750(–840) × (445–)505–600 (–720) μm; exothecial cells irregularly quadrate, smooth; stomata phaneroporous, (3–)4–5 at base of capsule; annulus present at the base of the apiculus, consisting of much smaller cells than adjacent exothecial cells of urn and operculum. Operculum differentiated as a slightly oblique finger-like beak, (130–)185–240(–315) μm long. Calyptra cucullate, (520–)550–675(–715) μm long. Spores (17.5–)20.2–22.5(–26) μm in diam., densely papillose.

Taxonomic notes:—This species is very similar to W. parajaponica, and sometimes difficult to identify based only on morphological characters. However, W. japonica tends to have larger urns and shorter excurrent costae of perichaetal leaves. Separation of this species is also supported by their geographical distribution: W. japonica is distributed in Honshu, Shikoku and Kyushu, while W. parajaponica is distributed in Ryukyu and Ogasawara Islands. Saito (1975) synonymized A. acuminatum with W. longifolia Mitt. [as W. crispa (Hedw.) Mitt.] due to gametophytic identity with W. longifolia. After detailed examination of the holotype, we concluded that A. acuminatum should instead be considered a synonym of W. japonica since the plants of holotype have immersed capsules with an annulus.

Distribution:—Japan (Honshu, Shikoku and Kyushu).

Representative specimens examined:—JAPAN. Honshu, Miyagi Pref.: Sendai City, Osaki-hachiman, 7 April 1907, S. Okamura s.n. (NICH-M 35935); Ibaragi Pref.: Mt. Mayumi, ca. 300 m elev., 17 February 1972, S. Okamura, Bot. Mag. (Tokyo) 25: 140. 1911 (Okamura 1911). Type:—JAPAN. Hyogo Pref.: Awaji Island, Toshi-mura, 24 November 1917, G. Takata s.n. in hb. H. Sasaoka 293 (holotype, BM 867097!).

3. Weissia kiensis (S.Okamura) Y.Inoue & H.Tsubota, comb. nov.

Basionym:—Astonum kiense S.Okamura, Bot. Mag. (Tokyo) 25: 140. 1911 (Okamura 1911). Type:—JAPAN. Wakayama Pref.: Wakanoura, the foot of Mt. Goboyama, 9 December 1900, K. Minakata s.n. (holotype, NICH-M 37518!).

Description:—(Figs. 2 D, & 4 A–I). Plants when moist ca. 2–10 mm high including capsules. Stems simple or branched, erect; central strand present; scleroderms weakly differentiated; hyalodermis undifferentiated. Leaves strongly crisped when dry, spreading when moist, gradually becoming larger toward shoot apex. Autoicous. Perichaetal leaves much larger than vegetative leaves, lanceolate to linear lanceolate, (1.7–)2.3–3.1(–3.6) mm long and (0.45–)0.5–0.7(–0.8) mm wide at base, tapering to an acumenate apex from a broad oblong base; margins incurved in distal 1/2–2/3, plane in basal portion, smooth or nearly smooth with faint projections at shoulder part of leaf base; costa stout, excurrent in a point reaching (40–)70–115(–160) μm, papillose on adaxial surface and smooth on abaxial surface; guide cells 4 in a single row at midleaf; adaxial and abaxial stereids 2–3 stratose at midleaf; upper laminal cells subquadrulate, 6–10 × 6–10
FIGURE 4. Weissia kiiensis (A–I) and W. praepponica (J–W). A, Habit (dry); B, Cross section of stem; C, Perichaetal leaf; D, Vegetative leaf; E & F, Cross sections of perichaetal leaf; G, Sporophyte; H, Cross section of seta; I, Calyptra; J, Habit (dry); K, Cross section of stem; L–N, Perichaetal leaves; O–R, Vegetative leaves; S & T, Cross sections of perichaetal leaf; U, Sporophyte; V, Cross section of seta; W, Calyptra. A–I drawn from K. Minakata s.n. (NICH-M 37518, holotype of Astomum kiiense); J, V from T. Yamaguchi 36925 (HIRO, paratype); K, L, O, P, S, T, W from Y. Inoue 3864 (HIRO, holotype); M, Q from Y. Inoue 3925 (HIRO, paratype); N, R from T. Yamaguchi 30497 (HIRO, paratype); U from T. Yamaguchi 18666 (HIRO, paratype).

**Taxonomic notes:**—Saito (1975) synonymized *A. kiense* with *W. longifolia* Mitt. [as *W. crispa* (Hedw.) Mitt.]. However, in their taxonomic revision of European *Weissia* subg. *Astomum*, Crucnell & Nyholm (1972) suggested that Japanese plants that had been named *W. crispa* belonged to a non-European species. After examination of the holotypes of *A. kiense* (NICH-M 37518) and *W. longifolia* (NY 1408141), we conclude that *A. kiense* should be resurrected and transferred to *Weissia*. *W. kiensis* has a similar appearance to *W. longifolia* in having the deeply immersed capsules without an annulus, but the capsule shape of the former is spherical while that of the latter is ellipsoidal. *W. kiensis* is also quite similar to the North American species *W. muhlenbergiana* (Swartz 1829: 74) Reese & Lemmon (1965: 282) as suggested by Andrews (1922). Crum & Anderson (1981) shared Crundwell’s opinion (in litt.) that Japanese plants referred to *A. crispum* were identical with the North American species *W. muhlenbergiana* [as *A. muhlenbergianum* (Sw.) Grout (1938: 152)]. In the present study we examined some specimens identified as *W. muhlenbergiana* (Appendix 2) and confirmed two morphological groups: (1) capsules without an annulus and (2) capsules with an annulus. No distinct morphological differences are apparent between *W. kiensis* and the former group identified as *W. muhlenbergiana*. In the plotologue of *Phascum muhlenbergianum* Swartz (1829) did not refer to whether the capsules have or lack an annulus. We have not been able to locate the type specimen of *P. muhlenbergianum*. Until additional morpho-molecular data are obtained to clarify the taxonimic identities of Japanese and North American plants, we consider these species best regarded as distinct.

**Distribution:**—Japan (Hokkaido, Honshu, Shikoku and Kyushu)

Representative specimens examined:—JAPAN. Hokkaido, Hokkaido Pref.: Obihiro City, Midorigaoka Park, ca. 50 m elev., 42°54′17″N, 143°11′17″E, 12 September 2012, Y. Inoue 1493 (HIRO, DNA voucher); Honshu, Fukushima Pref.: Fukushima City, Mt. Shinobu, ca. 95 m elev., 37°46′18″N, 140°28′42″E, 9 March 2015, Y. Inoue 3169 (HIRO, DNA voucher); Tokyo Pref.: Nishitokyo City, The University of Tokyo Tanashi Forest, ca. 90 m elev., 35°44′05″N, 139°32′28″E, 10 March 2015, Y. Inoue 3183 (HIRO, DNA voucher); Niigata Pref.: Tsubame City, Shinocho, ca. 10 m elev., 37°38′07″N, 138°49′48″E, 26 October 2015, T. Sato 1430 (HIRO, DNA voucher); Shizuoka Pref.: Kakegawa City, Nagaya, ca. 55 m elev., 34°45′30″N, 137°59′40″E, 19 December 2015, Y. Inoue 3816 (HIRO, DNA voucher); Aichi Pref.: Shinshiro City, Yanai, ca. 30 m elev., 34°51′52″N, 137°27′34″E, 18 March 2013, Y. Inoue 1816 (HIRO, DNA voucher); Nara Pref.: Ikoma District, Ikaruga-cho, Horyuji Temple, ca. 60 m elev., 5 March 2010, K. Une 10243 (TNS 211531); Wakayama Pref.: Wakanoura, the foot of Mt. Goboyama, 9 December 1900, K. Minakata s.n. (holotype of *Astomum kiense*, NICH-M 37518); Hiroshima Pref.: Kure City, Kamikamagarijima Island, ca. 25 m elev., 34°11′23″N, 132°43′09″E, 20 December 2015, Y. Inoue 3826 (HIRO, DNA voucher); Shikoku, Kochi Pref.: Kochi City, Mononobe-cho, Odachi, ca. 200 m elev., 33°41′52″N, 133°52′25″E, 8 March 2014, Y. Inoue 2606 (HIRO, DNA voucher); Kyushu, Oita Pref.: Tsukumi City, Chinu, ca. 20 m elev., 33°04′29″N, 132°52′53″E, 2 March 2013, Y. Inoue 1788b (HIRO, DNA voucher); Miyazaki Pref.: Nichinan City, Hoshikura, ca. 20 m elev., 31°37′29″N, 131°21′33″E, 28 November 2015, Y. Inoue 3813 (HIRO, DNA voucher).


**Holotype:**—JAPAN. Ryukyu Islands: Ishigakijima Isl., ca. 30 m elev., 24°29′25″N, 124°16′41″E, 18 January 2016, Y. Inoue 3864 (HIRO, DNA voucher [rbcL/psbA]: LC183870/LC183813)).

**Paratypes:**—JAPAN. Ogasawara Islands: Mokojima Isl., ca. 15 m elev., 27°40′53″N, 142°07′47″E, 14 July 2008, S. Uchida 10069 (HIRO, DNA voucher); Nakoudojima Isl., 12 July 2008, T. Katagiri 409 (HIRO); Yomejima Isl., ca. 80 m elev., 27°29′47″N, 142°12′36″E, 11 July 2008, S. Uchida 10008 (HIRO); Chichijima Isl., ca. 120 m elev., 27°05′39″N, 142°11′11″E, 12 June 2009, T. Yamaguchi 10497 (HIRO); Hahajima Isl., ca. 30 m elev., 26°37′06″N, 142°10′47″E, 17 September 2008, S. Uchida 10685 (HIRO, DNA voucher); Ryukyu Islands: Yakushima Isl., ca. 2 m elev., 30°27′02″N, 130°29′06″E, 3 January 2015, coll. S. Uchida in hb. *Y. Inoue 3143* (HIRO, DNA voucher); Amamioshima Isl., ca. 5 m elev., 28°22′53″N, 129°29′55″E, 25 February 2016, coll. A. Ohno in hb. *Y. Inoue 3951* (HIRO, DNA voucher); Okinawarubu Isl., 200–250 m elev., 30 March 1967, N. Takaki & H. Katsurayama s.n. (NUM-Bt 38114); Yoron Isl., ca. 70 m elev., 28 March 1967, N. Takaki & H. Katsurayama s.n. (NUM-Bt 38053); Izena Isl., ca. 80 m elev., 14 April 2004, H. Sato 464 (HIRO); Okinawa Isl., ca. 70 m elev., 26°13′39″N, 127°42′58″E, 24 February 2016, Y. Inoue 3912 (HIRO, DNA voucher); Kitadaishojima Isl., ca. 50 m elev., 25 March 2000, T. Yamaguchi 18666 (HIRO); Minamidaitojima Isl., ca. 20 m elev., 37°38′07″N, 138°49′48″E, 26 October 2015, T. Sato 1430 (HIRO, DNA voucher).
Description:—(Figs. 2 C, & 4 J–W). Plants when moist ca. 5 mm high including capsules. Stems simple or branched, erect; central strand present; sclerodermis weakly differentiated; hyalodermis undifferentiated. Leaves strongly crisped when dry, spreading when moist, gradually becoming larger towards shoot apex. Autoicous. Perichaetial leaves much larger than vegetative leaves, lanceolate to linear lanceolate, (2.1–)2.4–3.3(--4.2) mm long and (0.3–)0.4–0.55(--0.7) mm wide at base, tapering to an acuminate apex from a broad oblong base; margins incurved in distal 1/3–1/2, plane in basal portion, smooth; costa stout, excurrent in a point reaching (72–)105–160(--225) μm, papillose on adaxial surface and smooth on abaxial surface; guide cells 4 in a single row at midleaf; adaxial and abaxial stereids 2–3 stratose at midleaf; upper laminal cells subquadrate, 6–8 × 6–8 μm, papillose on both surfaces with bifid papillae; basal laminal cells enlarged, rectangular, (45–)60–100 × 10–15 μm, smooth. Perigonial leaves much smaller than vegetative leaves, oval, acuminate, concave. Asexual reproduction unknown. Setae (55–)125–185(--280) μm long; epidermal cells quadrate to subquadrate, thin walled. Capsules cleistocarpous, deeply immersed among perichaetial leaves; urn ovoid to subovoid, (400–)500–660(--760) × (360–)415–515(--620) μm; exothecial cells irregularly quadrate, smooth; stomata phaneroporous, (3–)4–5(--6) at base of capsule; annulus present at the base of the apiculus, consisting of much smaller cells than adjacent exothecial cells of urn and operculum. Operculum differentiated as a slightly oblique flange, (125–)165–225(--300) μm long. Calyptra cucullate, (390–)520–645(--680) μm long. Spores (15–)19–22(--25) μm in diam., densely papillose.

Taxonomic notes:—This species is very similar to W. japonica, and sometimes difficult to identify based only on morphological characters. However, W. parajaponica tends to have smaller urns and longer excurrent costae of the perichaetial leaves.

Distribution:—Japan (Ogasawara and Ryukyu Islands).

Taxonomic status of Trachycarpidium lonchophyllum
When Roth (1911) described Trachycarpidium lonchophyllum he did not specify the herbarium where the holotype was deposited. A number of duplicates were distributed. Costa (2016) cited the original materials of L. lonchophyllum as isotypes. However, each of these duplicates constitutes a syntype (ICN Art. 9.5).

Based on our molecular phylogenetic analysis, we consider that T. lonchophyllum is better placed in Weissia and we here propose the transfer of Trachycarpidium lonchophyllum to the genus Weissia as follows:

Weissia lonchophylla (G.Roth) Y.Inoue & H.Tsubota, comb. nov.
Basionym:—Astomum lonchophyllum G.Roth, Aussereur. Laubm. 182. 1911 (Roth 1911). Type:—BRASIL. Santa Catarina: Tubarão, July 1889, E. Ule 7 [holotype: herbarium not cited in the protologue; syntypes: G, GOET, JE, LE, MICH, PC, R, fide Costa (2016); non vid].


Acknowledgements

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in techniques for SEM observation and helpful suggestions; Prof. Rod Seppelt for checking the English text and valuable suggestions on the manuscript. This study was partly supported by JSPS KAKENHI for HT (Grant Number 16K07481). Sequencing for this study was carried out at the Analysis Center of Life Science, Natural Science Center for Basic Research and Development, Hiroshima University.

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Appendix 1. List of primer sequences used for PCR amplification and DNA sequencing of the rbcL and rps4 genes.

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<tr>
<th>Primer name</th>
<th>Sequence (5’-3’)</th>
<th>Target region</th>
<th>Reference</th>
<th>Note</th>
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<tr>
<td>rbcL-53h</td>
<td>TCGAGTAGAC CTATCCTTG C</td>
<td>rbcL</td>
<td>Inoue &amp; Tsubota (2014)</td>
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<td>HrL1</td>
<td>ATGTCAACAC AAGAGGAGAC</td>
<td>rbcL</td>
<td>Masuzaki et al. (2010)</td>
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<td></td>
<td>TAAAGCAGG</td>
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<tr>
<td>rbcL7</td>
<td>TGGATTTAAA GCTGGTTA AAG</td>
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<td>Tsubota et al. (1999)</td>
<td>Sequencing</td>
</tr>
<tr>
<td>rbcL862</td>
<td>CAATGCGATGC AGTATTGAC</td>
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<td>rbcL919G</td>
<td>CATGGTATGC ATTTCCGTA A</td>
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<td>tmT36R</td>
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<td>TCGACTCCGA TA</td>
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<td>rps4_1R</td>
<td>ATGTCCCGGT ATGAGGGACC TCGGT</td>
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<tr>
<td>rps4_19Fi</td>
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<td><strong>Reverse</strong></td>
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<td>trnR24R</td>
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<td>rbcL1301RL</td>
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<td>rbcL650Rmas</td>
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<td>rbcL600R</td>
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<td>rbcL</td>
<td>Tsubota et al. (1999)</td>
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<td>rbcL270R</td>
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<td>Sequencing</td>
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<tr>
<td>tmS</td>
<td>TACCAGGAGGT TGCAATTC</td>
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<td>Souza-Chies et al.</td>
<td>PCR</td>
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<td>(1997)</td>
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<td>rps4_602Fn</td>
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<td>rps4</td>
<td>Inoue &amp; Tsubota (2014)</td>
<td>Sequencing</td>
</tr>
<tr>
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<td>rps4</td>
<td>Inoue &amp; Tsubota (2014)</td>
<td>Sequencing</td>
</tr>
</tbody>
</table>

Appendix 2. Exotic specimens examined for comparison.

**Weissia longifolia** Mitt. var. longifolia

ENGLAND. Goldstone Barn, near Brighton, 1836, *Borrer s.n.* (holotype, NY 1408141)

**Weissia muhlenbergiana** (Sw.) W.D.Reese & B.A.E.Lemmon


Morphological group 2 (Capsules with annulus):—USA. Kansas: 12.8 km E of Wilmore, 1 June 1978, *S.P. Churchhill 9980* (HIRO); Louisiana: bluffs along the Ouachita River, 4 3/4 mi. se of Columbia, 13 May 1966, *W.D. Reese 9229* (NICH 290052); vic. of Pont Brule, 4 mi. due sw of Arnaudville, 18 March 1965, *W.D. Reese 1919* (NICH 242842).