



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 loncophyllum*.

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 genecological 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 exserted 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: *Astomum* Hampe (1837: 285), *Hymenostomum* Brown (1819: 572), *Phasconica* Müller (1882: 438) and *Weissia* s. str. *Astomum* is characterized by immersed cleistocarpous capsules, *Hymenostomum* by exserted stegocarpous, eperistomate capsules with hymenium, *Phasconica* is characterized by immersed stegocarpous (macrostomous), eperistomate capsules, and *Weissia* s. str. is characterized by exserted 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 *Astomum* and *Weissia s. str.* or of *Astomum* and *Hymenostomum* in nature (Nicholson 1905, 1906; Smith 1964, Reese & Lemmon 1965, Crundwell & Nyholm 1972, Khanna 1960, Anderson & Lemmon 1972, Williams 1966), and cytological analysis has also provided circumstantial evidence of hybrid sporophytes (Khanna 1960, Anderson & Lemmon 1972). Superficial characters of spores in *Astomum*, *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 *Astomum*, *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 *Astomum*) which include many heterogeneous capsule taxa. In Japan, five cleistocarpous species of *Weissia* had been reported as *Astomum*: *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. kiiense* Okamura (1911: 140). In a monograph of Japanese Pottiaceae, Saito (1975) recognized two cleistocarpous species under *Weissia* subg. *Astomum* (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. kiiense* 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 (Hokkaido, 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).

TABLE 1. List of species investigated for *rbcL* and *rps4* gene sequences with the voucher information and the accession number. Bold accession numbers indicate newly obtained sequences for the present study.

Taxon	Voucher information	Accession No.	
		<i>rbcL</i>	<i>rps4</i>
Ingroup species			
<i>Ardeuma aurantiacum</i> (Mitt.) R.H.Zander & Hedd.	Japan: Yamaguchi Pref., <i>Y. Inoue 4007</i> (HIRO)	LC176249	LC176270
<i>Ardeuma recurvirostrum</i> (Hedw.) R.H.Zander & Hedd.	Japan: Nagano Pref., <i>Y. Inoue 1323</i> (HIRO)	LC176251	LC176272
<i>Chionoloma angustata</i> (Mitt.) M.Menzel	Japan: Miyazaki Pref., <i>Y. Inoue 3238</i> (HIRO)	LC176254	LC176276
<i>Chionoloma tenuirostre</i> (Hook. & Taylor) M.Alonso, M.J.Cano & J.A.Jiménez	Japan: Hiroshima Pref., <i>Y. Inoue 3218</i> (HIRO)	LC176252	LC176274
<i>Ephemerum crassinervium</i> (Schwägr.) Hampe	Japan: Tochigi Pref., <i>T. Kamiyama 8980</i> (HIRO)	LC176246	LC176267
<i>Eucladium verticillatum</i> (With.) Bruch & Schimp.	Japan: Kanagawa Pref., <i>Y. Inoue 1803</i> (HIRO)	LC176247	LC176268
<i>Gymnostomiella longinervis</i> Broth.	Japan: Okinawa Pref., <i>Y. Inoue 3902</i> (HIRO)	LC176248	LC176269
<i>Hydrogonium hiroshii</i> (K.Saito) Jan Kučera	Japan: Shizuoka Pref., <i>T. Suzuki 61397</i> (HIRO)	LC176250	LC176271
<i>Hyophila propagulifera</i> Broth.	Japan: Hiroshima Pref., <i>Y. Inoue 1745</i> (HIRO)	AB853074	AB853084
<i>Pachyneuropsis miyagii</i> T.Yamag.	Japan: Okinawa Pref., <i>T. Yamaguchi 34243</i> (HIRO)	AB853078	AB759969
<i>Pottiopsis caespitosa</i> (Bruch ex Brid.) Blockeel & A.J.E.Sm.	Czech Republic: S Moravia, CBFS14602 (duplicate in HIRO)	LC176253	LC176275
<i>Tortella tortuosa</i> (Schrader ex Hedw.) Limpr.	Japan: Nagano Pref., <i>Y. Inoue 1297</i> (HIRO)	AB853080	AB853089
<i>Trichostomum brachydontium</i> Bruch	Spain: Murcia, CBFS 13652 (duplicate in HIRO)	LC176256	LC176278
<i>Trichostomum crispulum</i> Bruch	Spain: Asturias, MUB 45068 (duplicate in HIRO)	LC176257	LC176279
<i>Trichostomum platyphyllum</i> (Broth. ex Iisiba) P.C.Chen	Japan: Okinawa Pref., <i>Y. Inoue 3869</i> (HIRO)	LC176258	LC176280
<i>Tuerckheimia svihlae</i> (E.B.Bartram) R.H.Zander	Japan: Fukuoka Pref., <i>T. Suzuki 61444</i> (HIRO)	LC176259	LC176281
<i>Uleobryum naganoi</i> Kiguchi, I.G.Stone & Z.Iwats.	Japan: Kagawa Pref., <i>H. Sato 377</i> (HIRO)	AB194717	LC176282
<i>Weissia condensata</i> (Voit) Lindb.	Spain: Málaga, MUB 41163 (duplicate in HIRO)	LC183764	LC183797
<i>Weissia controversa</i> Hedw. 1	Japan: Ehime Pref., <i>Y. Inoue 2580</i> (HIRO)	LC183769	LC183802
<i>Weissia controversa</i> Hedw. 1	Japan: Oita Pref., <i>Y. Inoue 1783</i> (HIRO)	LC183766	LC183799
<i>Weissia controversa</i> Hedw. 2	Japan: Fukushima Pref., <i>Y. Inoue 2524</i> (HIRO)	LC183767	LC183800
<i>Weissia controversa</i> Hedw. 2	Japan: Hiroshima Pref., <i>Y. Inoue 2568</i> (HIRO)	LC176261	LC176284
<i>Weissia controversa</i> Hedw. 2	Japan: Kagawa Pref., <i>H. Tsubota 7704</i> (HIRO)	LC183765	LC183798
<i>Weissia controversa</i> Hedw. 3	Japan: Hiroshima Pref., <i>Y. Inoue 2564</i> (HIRO)	LC183768	LC183801
<i>Weissia controversa</i> Hedw. 4	Spain: Burgos, MUB 34121 (duplicate in HIRO)	LC183770	LC183803
<i>Weissia exserta</i> (Broth.) P.C.Chen	Japan: Hiroshima Pref., <i>Y. Inoue 912</i> (HIRO)	LC183792	LC183825
<i>Weissia exserta</i> (Broth.) P.C.Chen	Japan: Oita Pref., <i>Y. Inoue 1788a</i> (HIRO)	LC183793	LC183826
<i>Weissia japonica</i> (G.Roth) Y.Inoue & H.Tsubota	Japan: Hiroshima Pref., <i>Y. Inoue 3830</i> (HIRO)	LC183782	LC183815
<i>Weissia japonica</i> (G.Roth) Y.Inoue & H.Tsubota	Japan: Fukuoka Pref., <i>Y. Inoue 3947</i> (HIRO)	LC183785	LC183818
<i>Weissia kiiensis</i> (S.Okamura) Y.Inoue & H.Tsubota 1	Japan: Hokkaido Pref., <i>Y. Inoue 1493</i> (HIRO)	LC183772	LC183805
<i>Weissia kiiensis</i> (S.Okamura) Y.Inoue & H.Tsubota 1	Japan: Niigata Pref., <i>T. Sato 1430</i> (HIRO)	LC183790	LC183823
<i>Weissia kiiensis</i> (S.Okamura) Y.Inoue & H.Tsubota 1	Japan: Shizuoka Pref., <i>Y. Inoue 3816</i> (HIRO)	LC183788	LC183821
<i>Weissia kiiensis</i> (S.Okamura) Y.Inoue & H.Tsubota 1	Japan: Aichi Pref., <i>Y. Inoue 1816</i> (HIRO)	LC183774	LC183807
<i>Weissia kiiensis</i> (S.Okamura) Y.Inoue & H.Tsubota 1	Japan: Hiroshima Pref., <i>Y. Inoue 3826</i> (HIRO)	LC183789	LC183822
<i>Weissia kiiensis</i> (S.Okamura) Y.Inoue & H.Tsubota 1	Japan: Miyazaki Pref., <i>Y. Inoue 3813</i> (HIRO)	LC183787	LC183820
<i>Weissia kiiensis</i> (S.Okamura) Y.Inoue & H.Tsubota 1	Japan: Oita Pref., <i>Y. Inoue 1788b</i> (HIRO)	LC183773	LC183806
<i>Weissia kiiensis</i> (S.Okamura) Y.Inoue & H.Tsubota 2	Japan: Kochi Pref., <i>Y. Inoue 2606</i> (HIRO)	LC183775	LC183808
<i>Weissia kiiensis</i> (S.Okamura) Y.Inoue & H.Tsubota 3	Japan: Fukushima Pref., <i>Y. Inoue 3169</i> (HIRO)	LC183777	LC183810
<i>Weissia kiiensis</i> (S.Okamura) Y.Inoue & H.Tsubota 4	Japan: Tokyo Pref., <i>Y. Inoue 3183</i> (HIRO)	LC183778	LC183811
<i>Weissia lonchophylla</i> (G.Roth) Y.Inoue & H.Tsubota	Brazil: São Paulo, <i>O. Yano & B.L. Morretes 28820</i> (SP 382923)	LC183763	LC183796
<i>Weissia longidens</i> Cardot	Japan: Hiroshima Pref., <i>Y. Inoue 918</i> (HIRO)	LC183794	LC183827
<i>Weissia newcomeri</i> (E.B.Bartram) K.Saito	Japan: Hiroshima Pref., <i>Y. Inoue 2781</i> (HIRO)	LC183795	LC183828
<i>Weissia parajaponica</i> Y.Inoue & H.Tsubota 1	Japan: Ryukyu, Miyakojima Isl., <i>Y. Inoue 3910</i> (HIRO)	LC183781	LC183814
<i>Weissia parajaponica</i> Y.Inoue & H.Tsubota 1	Japan: Ryukyu, Ishigakijima Isl., <i>Y. Inoue 3864</i> (HIRO)	LC183780	LC183813
<i>Weissia parajaponica</i> Y.Inoue & H.Tsubota 2	Japan: Kagoshima Pref., Yakushima Isl., <i>Y. Inoue 3143</i> (HIRO)	LC183776	LC183809
<i>Weissia parajaponica</i> Y.Inoue & H.Tsubota 2	Japan: Ryukyu, Iriomotejima Isl., <i>Y. Inoue 3849</i> (HIRO)	LC183779	LC183812

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TABLE 1. (Continued)

Taxon	Voucher information	Accession No.	
		<i>rbcL</i>	<i>rps4</i>
<i>Weissia parajaponica</i> Y.Inoue & H.Tsubota 3	Japan: Ryukyu, Amamioshima Isl., <i>Y. Inoue</i> 3951 (HIRO)	LC183791	LC183824
<i>Weissia parajaponica</i> Y.Inoue & H.Tsubota 3	Japan: Ryukyu, Okinawa Isl., <i>Y. Inoue</i> 3912 (HIRO)	LC183783	LC183816
<i>Weissia parajaponica</i> Y.Inoue & H.Tsubota 3	Japan: Ryukyu, Minamidaitojima Isl., <i>Y. Inoue</i> 3925 (HIRO)	LC183784	LC183817
<i>Weissia parajaponica</i> Y.Inoue & H.Tsubota 4	Japan: Ogasawara, Mukojima Isl., <i>S. Uchida</i> 10069 (HIRO)	LC183771	LC183804
<i>Weissia parajaponica</i> Y.Inoue & H.Tsubota 5	Japan: Ogasawara, Hahajima Isl., <i>S. Uchida</i> 10685 (HIRO)	LC183786	LC183819
Outgroup species			
<i>Barbula unguiculata</i> Hedw.	Japan: Hiroshima Pref., <i>Y. Inoue</i> 113 (HIRO)	AB670696	LC176265
<i>Didymodon japonicus</i> (Broth.) K.Saito	Japan: Nagano Pref., <i>Y. Inoue</i> 4040 (HIRO)*	LC176245	LC176266

*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: Sugiura 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 ver. 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 ($\ln L = -5770.556$). 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 overlooked 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

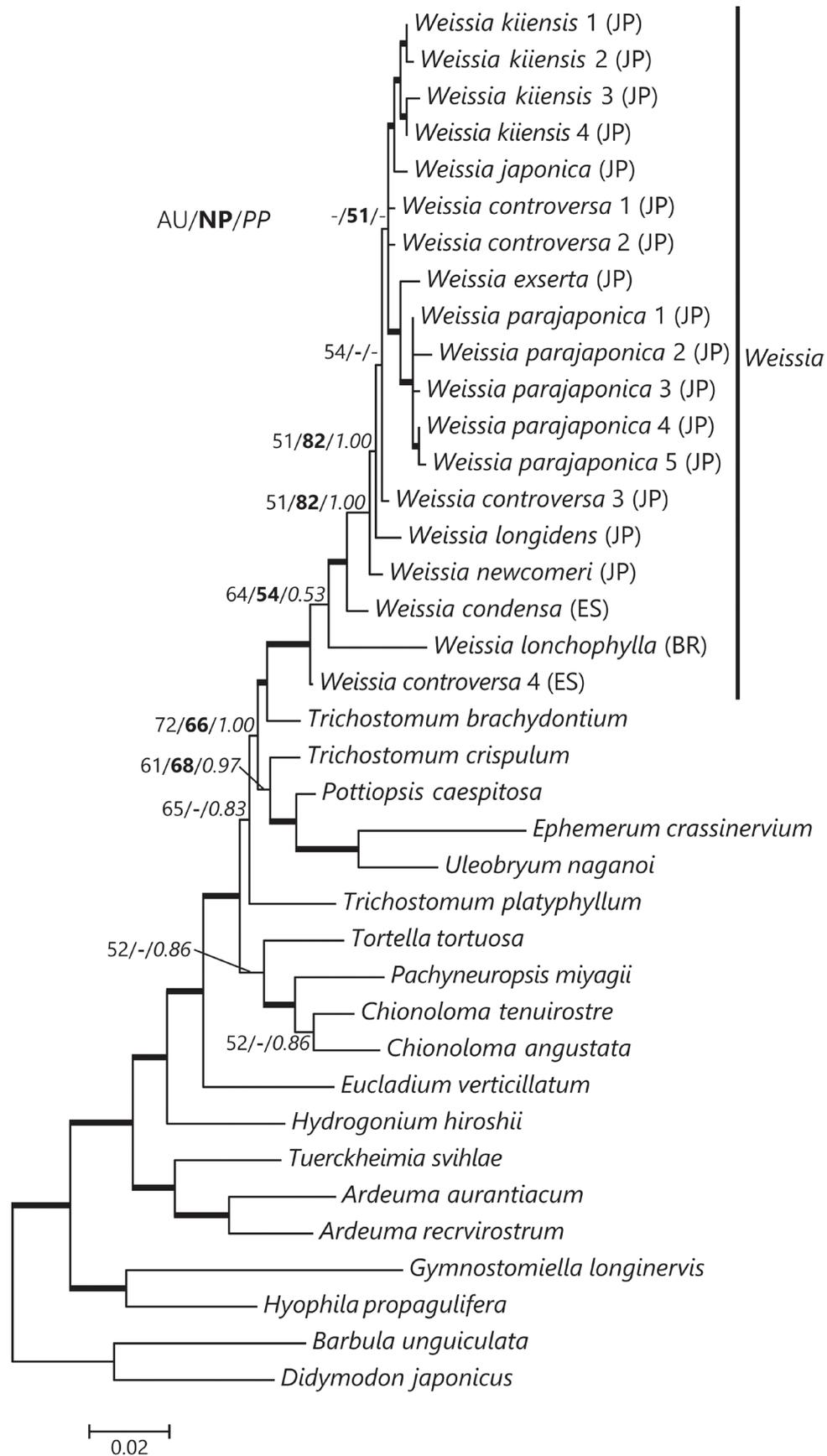


FIGURE 1. Phylogenetic tree based on analysis with the concatenated sequences of chloroplast *rbcL* and *rps4* genes, depicted by the best-supported tree with highest likelihood value ($\ln L = -5770.556$ by Garli). Supporting values more than 50 % obtained by the program CONSEL were overlaid: AU test (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 %.

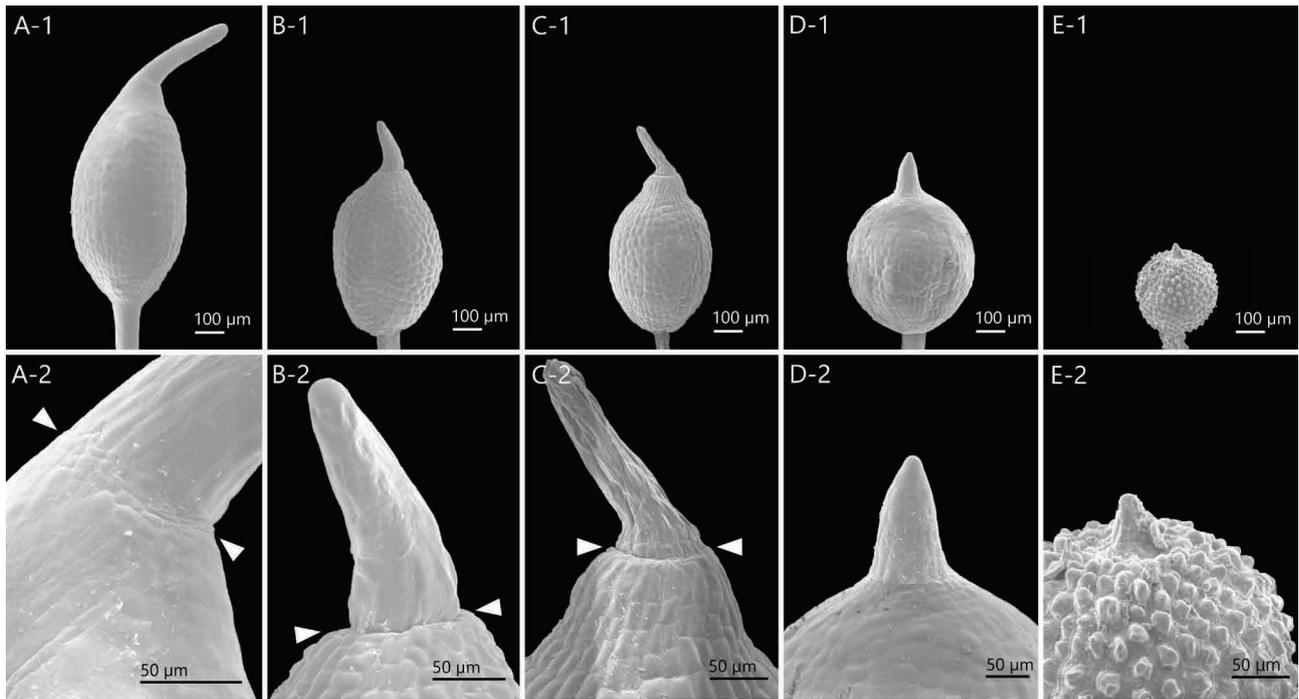


FIGURE 2. Scanning electron microscope (SEM) images of cleistocarpous capsules of *Weissia* in Japan and of *Trachycarpidium lonchophyllum*. A, *W. exserta* (Y. Inoue 3828 in HIRO); B, *W. japonica* (Y. Inoue 3830 in HIRO); C, *W. parajaponica* (T. Yamaguchi 36925 in HIRO); D, *W. kiiensis* (Y. Inoue 3813 in HIRO); E, *T. lonchophyllum* (O. Yano & B.L. Morretes 28820 in SP). 1, Capsule overviews; 2, Close up of upper portion of capsules (Arrowheads indicate dehiscence line).

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 Ryukyu 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 exerted 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* Bruch in Müller (1829: 395), and *T. jamaicense* (Mitten 1869: 147) Jaeger (1873: 397) [as *W. jamaicensis* (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 *Trichostomum* 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

- Weissia*** Hedw., Sp. Musc. Frond. 64. 1801 (Hedwig 1801). Lectotype:—*W. controversa* Hedw. *vide* Mitten (1856). = *Cavanillea* Borkh., Tent. Disp. Pl. German., op. posth. 251. 1809 (Borckhausen 1809), *nom. illeg.* [ICN Art. 53.1; later homonym (non Medik., non Desr.)].
- = *Hymenostomum* R.Br., Trans. Linn. Soc. London 12: 572. 1819 (Brown 1819). Type:—*H. microstomum* (Hedwig 1801: 33) Nees von Esenbeck & Hornschuch (1823: 199).
- = *Astomum* Hampe, Flora 20: 285, 1837 (Hampe 1837). Lectotype:—*A. crispum* (Hedw.) Hampe *vide* Margadant (1959).
- = *Systegium* Schimp., Syn. Musc. Eur. 30. 1860 (Schimper 1860), *nom. illeg.* (ICN Art. 52.1; type of earlier name included).
- = *Simophyllum* Lindb., Acta Soc. Sci. Fenn. 10: 74. 1871 (Lindberg 1871), *nom. illeg.* (ICN Art. 52.1; type of earlier name included).
- = *Phasconica* Müll.Hal., Linnaea 43: 438. 1882 (Müller 1882). Lectotype:—*P. lorentzii* Müll.Hal. *vide* Zander (1993).
- = *Rechingerella* J.Froehl., Ann. Naturhist. Mus. Wien 66: 36. 1963 (Froehlich 1963), *nom. illeg.* [ICN Art. 53.1; later homonym (non Petr.)]. Type:—*R. macedonica* J.Froehl.

Description:—*Plants* small, forming low cushions, turfs or loosely caespitose. *Stems* simple or branched, erect, smooth, rounded in cross section; central strand present; sclerodermis 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 bistratose 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. *Perichaetia* terminal; perichaetial leaves little different from vegetative leaves or somewhat larger. *Perigonia* appearing as stalked lateral buds on perichaetiate plants (but variably present) or terminal on usually smaller perigoniolate plants; perigonial leaves much smaller than vegetative leaves, ovate. *Setae* 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 phaneroporously 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.

Weissia controversa Hedw. Sp. Musc. Frond. 67. 1801 (Hedwig 1801).

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 plotologue (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 1791–1792, 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
2. Annulus absent *W. kiiensis*
- Annulus present3
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 subovoid4
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.

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, *Wichura 1379a* (lectotype designated here, H 190018!).

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

≡ *Hymenostomum exsertum* (Broth.) Broth., Nat. Pflanzenfam. I (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* strongly crisped 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 stereids 2–4 stratose at midleaf; upper laminal cells subquadrate, 6–9(–10) \times 6–9 μm , papillose on both surfaces with bifid papillae; basal laminal cells enlarged, rectangular, (50–)65–85 \times 8–10(–12) μm , smooth. *Perigonal 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) \times (460–)570–625(–760) μm ; exothecial cells irregularly quadrate, smooth; stomata 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: *Wichura 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 *Wichura 1396a* (H) as the holotype of *A. exsertum*. However, the specimen *Wichura 1396a* is the type for *Hyophila propagulifera* Brotherus (1899). In a taxonomic account of Indian Pottiaceae, Aziz & Vohra (2008) cited the specimen *Wichura 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 *Wichura 1379a* agrees well with the original description of Brotherus (1899) and we designate it as the lectotype of *A. exsertum* (The specimen *Wichura 1379b* was not found in H: Curator, pers. comm., March 2015).

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

Representative specimens examined:—JAPAN. Honshu, Ibaraki Pref.: Nishi-ibaraki District, Iwase-cho, Ohta, 14 December 1981, *Z. Iwatsuki 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.: Higashi-hiroshima City, Hiroshima University, ca. 220 m elev., 34°24'08"N, 132°42'42"E, 23 February 2012, *Y. Inoue 912* (HIRO, DNA voucher); Hatsukaichi City, Miyajima Isl., 10 m elev., 23 January 1969, coll. *T. Seki*. in hb. *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, *Wichura 1379a* (lectotype of *Astomum exsertum*, H 190018); Kumamoto Pref.: Hitoyoshi, Isshouchi, ca. 100 m elev., 26 February 1971, *K. Saito 8546* (TNS 70370); Oita Pref.: Tsukumi City, Chinu, ca. 20 m elev., 33°04'29"N, 132°52'53"E, 2 March 2013, *Y. Inoue 1788a* (HIRO, DNA voucher).

2. *Weissia japonica* (G.Roth) Y.Inoue & H.Tsubota, Cryptog. Bryol. 38: 86. 2017 (Inoue & Tsubota 2017).

Basionym:—*Astomum 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!].

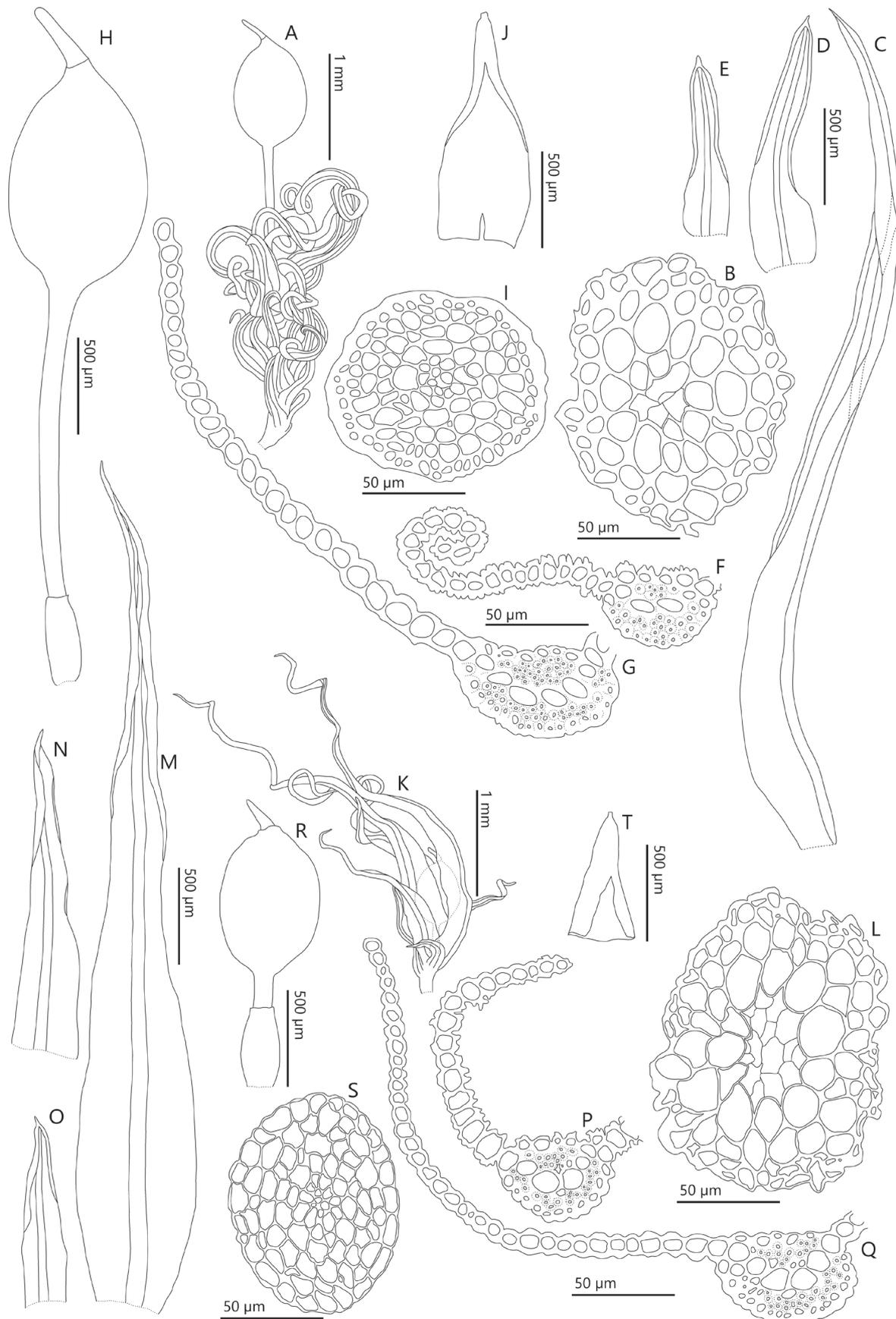


FIGURE 3. *Weissia exserta* (A–J) and *W. japonica* (K–T). A, Habit (dry); B, Cross section of stem; C, Perichaetial leaf; D & E, Vegetative leaves; F & G, Cross sections of perichaetial leaf; H, Sporophyte; I, Cross section of seta; J, Calyptra; K, Habit (dry); L, Cross section of stem; M, Perichaetial leaf; N & O, Vegetative leaves; P & Q, Cross sections of perichaetial 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).

- = *Systegium crispum* auct. non (Hedw.) Schimp.: Sande Lacoste, Ann. Mus. Bot. Lugduno-Batavum 2: 292. 1866 (Sande Lacoste 1866).
 = *Astomum crispum* auct. non (Hedw.) Hampe: Sande Lacoste, Ann. Mus. Bot. Lugduno-Batavum 2: 292. 1866 (Sande Lacoste 1866).
 = *Systegium japonicum* Besch. in Paris, Index Bryol. ed. 2. 352. 1905 (Paris 1905), *nom. inval.* [ICN Art. 38.1; no description].
 = *Astomum acuminatum* Dixon & Thér., Trav. Bryol. 1: 11. 1942 (Dixon 1942). Type:—JAPAN. Hyogo Pref.: Awaji Island, Toshi-mura, 24 November 1917, *G. Takata s.n.* in hb. *H. Sasaoka 293* (holotype, BM 867097!), *syn. nov.*

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; sclerodermis weakly differentiated; hyalodermis undifferentiated. *Leaves* strongly crisped when dry, spreading when moist, gradually becoming larger toward shoot apex. *Autoicous*. *Perichaetial 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 acuminate 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) \times 6–8 μm , papillose on both surfaces with bifid papillae; basal laminal cells enlarged, rectangular, (55–)60–90(–100) \times 8–12(–15) μm , smooth. *Perigonal leaves* much smaller than vegetative leaves, oval, acuminate, concave. *Asexual reproduction* unknown. *Setae* (35–)120–190(–260) μm long; epidermal cells quadrate to subquadrate, thin walled. *Capsules* cleistocarpous, deeply immersed among perichaetial leaves; urn ovoid to subovoid, (550–)620–750(–840) \times (445–)505–600 (–720) μm ; exothelial 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 exothelial 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–)20–22.5(–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 perichaetial leaves. Separation of these 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, *K. Saito 10596* (TNS 72161); Shizuoka Pref.: Mikkabi, 21 February 1973, *K. Saito 13966* (TNS 72163); Hyogo Pref.: Awaji Island, Toshi-mura, 24 November 1917, *G. Takata s.n.* in hb. *H. Sasaoka 293* (holotype of *Astomum acuminatum*, BM 867097); Wakayama Pref.: Tanabe City, 25 March 1972, *H. Deguchi 9432* (KOCH); Hiroshima Pref.: Hiroshima City, Asakita-ku, Miiriminami, ca. 60 m elev., 33°32'18"N, 132°31'40"E, 18 March 2012, *Y. Inoue 914* (HIRO); ditto, 18 March 2012, *Y. Inoue 3830* (HIRO, DNA voucher); Shikoku, Ehime Pref.: Matsuyama City, Gogoshima Isl., ca. 50 m elev., 33°53'01"N, 132°40'24"E, 8 February 2012, coll. *T. Seki* in hb. *Y. Inoue 4034* (HIRO); Kochi Pref.: Kochi City, Kochi University, ca. 5 m elev., 23 March 1986, *H. Hidaka 271* (KOCH, voucher specimen used for phenological study by Deguchi & Hidaka 1987, as *A. crispum*); Kyushu, Fukuoka Pref.: Fukuoka City, Fukuoka Castle, ca. 10 m elev., 33°35'01"N, 130°22'51"E, 13 March 2016, coll. *T. Katagiri* in hb. *Y. Inoue 3947* (HIRO, DNA voucher).

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

Basionym:—*Astomum kiiense* 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; sclerodermis weakly differentiated; hyalodermis undifferentiated. *Leaves* strongly crisped when dry, spreading when moist, gradually becoming larger toward shoot apex. *Autoicous*. *Perichaetial 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 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 (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 subquadrate, 6–10 \times 6–10

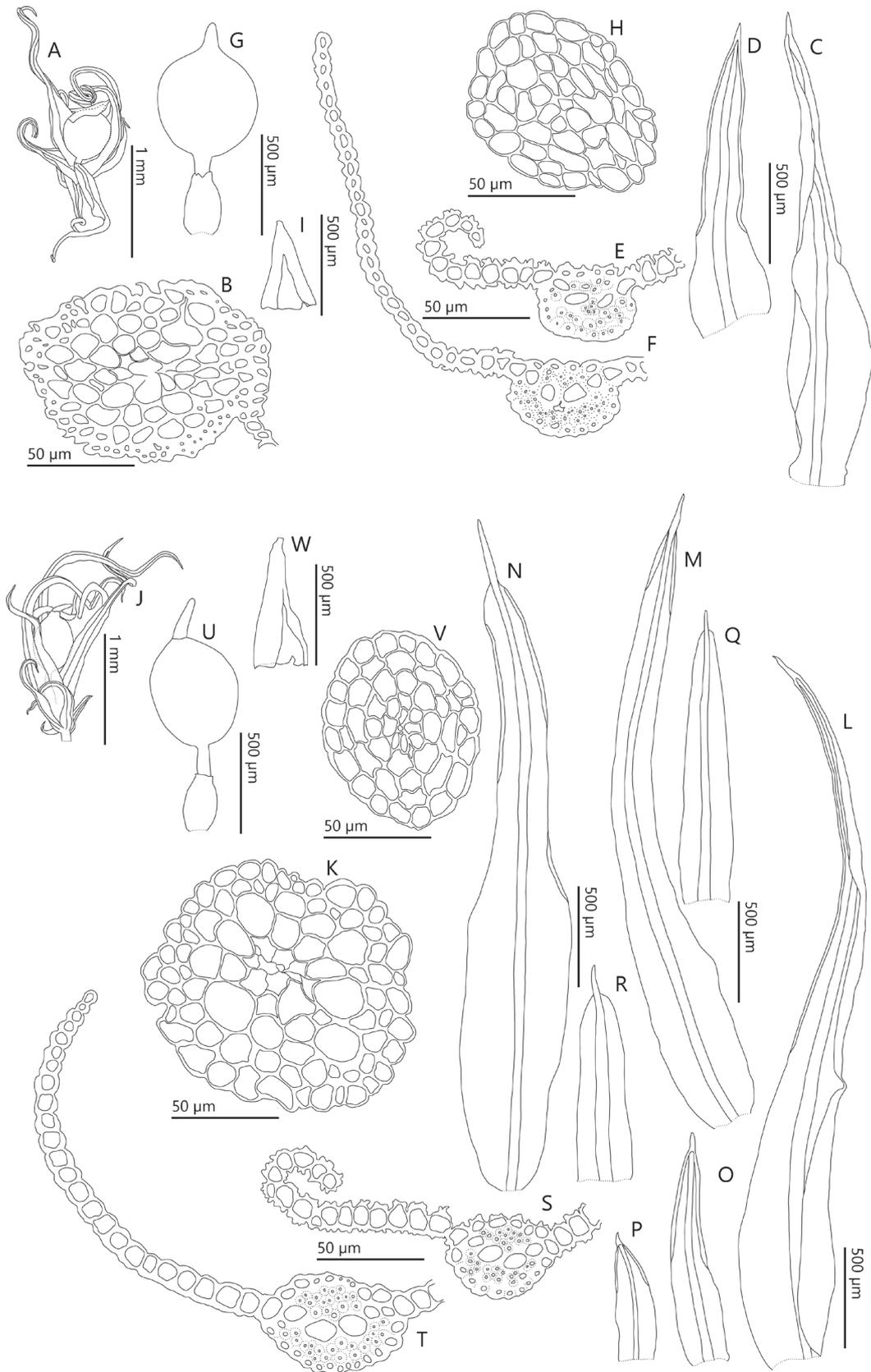


FIGURE 4. *Weissia kiiensis* (A–I) and *W. prajaponica* (J–W). A, Habit (dry); B, Cross section of stem; C, Perichaetial leaf; D, Vegetative leaf; E & F, Cross sections of perichaetial leaf; G, Sporophyte; H, Cross section of seta; I, Calyptra; J, Habit (dry); K, Cross section of stem; L–N, Perichaetial leaves; O–R, Vegetative leaves; S & T, Cross sections of perichaetial 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).

µm, papillose on both surfaces with bifid papillae; basal laminal cells enlarged, rectangular, 50–120 × 8–14 µm, smooth. *Perigonal leaves* much smaller than vegetative leaves, oval, acuminate, concave. *Asexual reproduction* unknown. *Setae* (70–)130–240(–350) µm long; epidermal cells quadrate to subquadrate, thin walled. *Capsules* cleistocarpous, deeply immersed among perichaetial leaves; urn spherical, (490–)645–770(–840) × (460–)580–700 (–800) µm, with a slightly oblique finger-like apiculus reaching (110–)150–205(–230) µm long; exothecial cells irregularly quadrate, smooth; stomata phaneroporous, (3–)4–6(–9) at base of capsule; annulus absent. *Operculum* undifferentiated. *Calyptra* cucullate, (510–)575–680(–775) µm long. *Spores* (16.25–)20–24(–30) µm in diam., densely papillose.

Taxonomic notes:—Saito (1975) synonymized *A. kiiense* with *W. longifolia* Mitt. [as *W. crispa* (Hedw.) Mitt.]. However, in their taxonomic revision of European *Weissia* subg. *Astomum*, Crundwell & 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. kiiense* (NICH-M 37518) and *W. longifolia* (NY 1408141), we conclude that *A. kiiense* should be resurrected and transferred to *Weissia*. *W. kiiensis* 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. kiiensis* 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. kiiensis* 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 taxonomic 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, Shincho, 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 kiiense*, NICH-M 37518); Hiroshima Pref.: Kure City, Kamikamagarijima Isl., 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).

4. *Weissia parajaponica* Y. Inoue & H. Tsubota, *sp. nov.*

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/rps4*: LC183780/LC183813)].

Paratypes:—JAPAN. Ogasawara Islands: Mukojima 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 30497* (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); Okinoerabu 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., 11 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); Kitadaitoshima Isl., 20–50 m elev., 25 March 2000, *T. Yamaguchi 18666* (HIRO); Minamidaitojima Isl., ca. 20 m

elev., 25°49'38"N, 131°13'00"E, 25 February 2016, *Y. Inoue 3925* (HIRO, DNA voucher); Irabu Isl., ca. 80 m elev., 24°48'59"N, 125°12'58"E, 22 January 2016, *T. Yamaguchi 36877* (HIRO); Miyakojima Isl., ca. 10 m elev., 24°48'51"N, 125°16'58"E, 21 January 2016, *Y. Inoue 3910* (HIRO, DNA voucher); ditto, ca. 15 m elev., 24°48'51"N, 125°16'58"E, 25 March 2016, *T. Yamaguchi 36925* (HIRO); ditto, ca. 20 m elev., 24°48'56"N, 125°17'04"E, 25 March 2016, *T. Yamaguchi 36926* (HIRO); Hatomajima Isl., ca. 5 m elev., 17 March 1982, *T. Yamaguchi 2146* (HIRO); Ishigakijima Isl., ca. 30 m elev., 18 January 2016, *Y. Inoue 3884* (HIRO); Iriomotejima Isl., ca. 30 m elev., 24°26'01"N, 123°46'55"E, 16 January 2016, *Y. Inoue 3849* (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 \times 6–8 μm , papillose on both surfaces with bifid papillae; basal laminal cells enlarged, rectangular, (45–)60–100 \times 10–15 μm , smooth. *Perigonal 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) \times (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 finger-like beak, (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 *A. 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, *vide* Costa (2016); *non vidi*].

≡ *Trachycarpidium lonchophyllum* (G.Roth) R.H.Zander, *Bull. Buffalo Soc. Nat. Sci.* 32: 213. 1993 (Zander 1993).

Specimen examined:—BRAZIL. São Paulo: Pirassununga, Cerrado de Emas, 27 March 2006, *O. Yano & B.L. Morretes 28820* (SP 382923, DNA voucher).

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

Primer name	Sequence (5'-3')	Target region	Reference	Note
Forward				
rbcL-53h	TCGAGTAGAC CTTATCCTTG C	<i>rbcL</i>	Inoue & Tsubota (2014)	PCR
HrL1	ATGTCACCAC AAACGGAGAC TAAAGCAGG	<i>rbcL</i>	Masuzaki <i>et al.</i> (2010)	PCR
rbcL7	TGGATTTAAA GCTGGTGTTA AAG	<i>rbcL</i>	Tsubota <i>et al.</i> (1999)	Sequencing
rbcL862	CAATGCATGC AGTTATTGAC	<i>rbcL</i>	Tsubota <i>et al.</i> (1999)	Sequencing
rbcL919G	CATGGTATGC ATTTCCGTGT A	<i>rbcL</i>	Tsubota <i>et al.</i> (2001)	Sequencing
rbcL921G	GGTATGCATT TCCGTGTATT AGC	<i>rbcL</i>	Tsubota <i>et al.</i> (2001)	Sequencing
trnT36R	GTAATGCGAT GGTCATCGGT TCGACTCCGA TA	<i>rps4</i>	Inoue <i>et al.</i> (2012)	PCR
rps4_1R	ATGTCCTGTT ATCGAGGACC TCGTGTA	<i>rps4</i>	Inoue <i>et al.</i> (2012)	Sequencing
rps4_19Fi	CCTCGTGTA GAATAATACG TC	<i>rps4</i>	Inoue & Tsubota (2014)	Sequencing
Reverse				
trnR24R	CTCTAATCCA CTGAGCTACA	<i>rbcL</i>	Tsubota <i>et al.</i> (1999)	PCR
rbcL1346hR	GCAGCTAATT CAGGACTCC	<i>rbcL</i>	Tsubota <i>et al.</i> (1999)	Sequencing
rbcL1301RL	CTTCATTACG TGCTTGTA CAAGCTTCTA	<i>rbcL</i>	Inoue <i>et al.</i> (2011)	PCR
rbcL1145R	TTAATGCTGG CATATGCCAA AC	<i>rbcL</i>	Tsubota <i>et al.</i> (1999)	Sequencing
rbcL1098R	AACACCTGGT AAAGAAACC	<i>rbcL</i>	Tsubota <i>et al.</i> (1999)	Sequencing
rbcL804hR	TGCAGTAAAA CCACCTG	<i>rbcL</i>	Tsubota <i>et al.</i> (1999)	Sequencing
rbcL650Rmas	CGATCTCTCC AACGCA	<i>rbcL</i>	Masuzaki <i>et al.</i> (2010)	Sequencing
rbcL600R	GTGAAATCAA GTCCACCACG	<i>rbcL</i>	Tsubota <i>et al.</i> (1999)	Sequencing
rbcL270R	GCAATATATT GATTTTCTTC TCCAG	<i>rbcL</i>	Tsubota <i>et al.</i> (1999)	Sequencing
trnS	TACCGAGGGT TCGAATC	<i>rps4</i>	Souza-Chies <i>et al.</i> (1997)	PCR
rps4_602Fn	TGACGAGAAT AATATTCTAC AACTA	<i>rps4</i>	Inoue & Tsubota (2014)	Sequencing
rps4_578Ri	CGAGAATAAT ATTCTACAAC TA	<i>rps4</i>	Inoue & Tsubota (2014)	Sequencing

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 1 (Capsules without annulus):—USA. Indiana: Dearborn, Tanner's Creek near outlet with Ohio River just below Lawrenceburg, 21 April 1984, *B.H. Allen 3924* (HIRO); Dearborn, 19 March 1983, *B.H. Allen 3038* (HIRO); Texas: North side of Ft. Worth–Oakhurst Div., 18 December 1967, *D. Griffin, III s.n.* (NICH 299840); Maryland: Prince Georges Country, Hillenest Heights, Marlborough House, 31 January 1968, *F.S. Hermann 22294* (HIRO); Beltsville, USDA Plant Industry Station, 1 mile S., 13 April 1958, *F.J. Hermann 14210* (NICH 202584); Tennessee: Nashville, 3 May 1958, *R.F. Cain s.n.* (NICH 202585); Garden, near Lake City, ca. 335 m elev., 13 January 1957, *Z. Iwatsuki s.n.* (NICH 202588); Texas: Pasture and alluvial soil in vicinity of junction Piney River & US 63 ca. 1 mile north of Cabool, 16 April 1960, *P.L. Redfearn, Jr. 5478* (HIRO).

Morphological group 2 (Capsules with annulus):—USA. Kansas: 12.8 km E of Wilmore, 1 June 1978, *S.P. Churchill 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).