

## A note on *Pottia intermedia* (Turner) Fürnr. (Pottiaceae, Bryopsida) with special reference to its phylogeny and new localities in SW Japan

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Eight new localities of *Pottia intermedia* (Turner) Fürnr. are recorded from Honshu and Shikoku (Hiroshima, Kagawa and Ehime Prefs.), SW Japan. Although it is widely distributed in the world, this species is a rare moss in Japan and is previously known from Honshu, Shikoku and Kyushu for Japan. Japanese populations of *P. intermedia* grow on soil in open sites, especially in citrus orchards. Analytical illustrations with SEM images of peristome, spores and leaf papillae and a discussion on the phylogenetic position of this species are provided based on the materials collected.

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### Introduction

*Pottia intermedia* (Turner) Fürnr (Pottiaceae) is an acrocarpous moss, and despite its wide distribution range in the world, it is a rare species in Japan. It was reported from Nagasaki Pref., Kyushu, SW Japan by Brotherus (1899) and later from Kyoto, Osaka, Hyogo, Tottori, Shimane, Hiroshima, Yamaguchi and Ehime Prefs. (Tatebe & Nakajima 1953; Saito 1973; Deguchi 1998; Tsubota *et al.* 2003; Hayashi & Shiomi 2004; Kimura & Sakuma 2008). We report here its new localities in Hiroshima, Kagawa and Ehime Prefs., Honshu and Shikoku, SW Japan, and provide a morphological description based on Japanese populations. The phylogenetic position of this species is also discussed on the basis of the plastid marker, ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene sequences with maxi-

mum likelihood analysis.

### Materials and methods

For SEM observation and DNA data, fresh materials and a dried specimen were used. Collection details are: JAPAN, HONSHU, Hiroshima-ken, Onomichi-shi, Setoda-cho, Ikuchijima Isl., 34°16'47"N, 133°04'41"E, ca. 30 m alt., 17 iv 2011, *Y. Inoue 135* (HIRO); ibid, 7 xi 2011, *Y. Inoue 315* (HIRO); Kure-shi, Toyohama-cho, Toyoshima Isl., 34°10'56"N, 132°46'42"E, 0–20 m alt., 26 iii 2009, *H. Tsubota 6400* (HIRO); SHIKOKU, Kagawa-ken, Shozu-gun, Shodoshima-cho, Shodoshima Isl., 34°29'45"N, 134°17'45"E, ca. 130 m alt., 15 v 2011, *Y. Inoue 194* (HIRO); Ehime-ken, Imabari-shi, Ohmishima-cho, Ohmishima Isl., 34°14'51"N, 133°00'27"E, ca. 20 m alt., 20 ii 2011, *Y. Inoue 56* (HIRO).

Table 1. Primers used for PCR amplification and sequencing of the *rbcL* regions. The primers number from the 5' end correspond to approximate positions in the sequence of *Marchantia polymorpha* (Ohyama *et al.* 1986).

Primers	Sequence (5'-3')	Note
Forward		
rbcL-130 <sup>1</sup>	ACAATGATACTGTTGTTAT AG	1st PCR
HrL1 <sup>2</sup>	ATGTCACCACAAACGGAGAC TAAAGCAGG	Sequencing
rbcL7 <sup>1</sup>	TGGATTAAA GCTGGTGTAAAG	Sequencing
rbcL549 <sup>1</sup>	TGTCTCGTG GTGGAC	Sequencing
rbcL919G <sup>3</sup>	CATGGTATGCAATTCCGTGT A	Sequencing
Reverse		
HrL260R <sup>2</sup>	ATATCATAGCATCGACCTTT GTAACGATC	Sequencing
rbcL600R <sup>1</sup>	GTGAAATCAA GTCCACCACG	Sequencing
rbcL650Rmas <sup>4</sup>	CGATCTCTCC AACGCA	Sequencing
rbcL804hR <sup>1</sup>	TGCAGTAAAA CCACCTG	Sequencing
rbcL1301RL <sup>5</sup>	CTTCATTACGTGCTTGTACA CAAGCTTCTA	Sequencing
rbcL1346hR <sup>1</sup>	GCAGCTAATT CAGGACTCC	Sequencing
trnRn <sup>6</sup>	GGGTTAGAAG GGATTGAAAC CCTTGAC	1st PCR

<sup>1</sup>Indicates primers designed by Tsubota *et al.* (1999); <sup>2</sup>indicates primers designed by Tsubota and Matsuda and published by Masuzaki *et al.* (2010); <sup>3</sup>indicates primers designed by Tsubota and Ageno in Tsubota *et al.* (2001); <sup>4</sup>Masuzaki *et al.* (2010); <sup>5</sup>indicates primers designed by Tsubota (published here); <sup>6</sup>indicates primers designed by Manhart (1994) and shortened by Tsubota *et al.* (1999).

### SEM observation

Fresh samples were fixed with 4% glutaraldehyde in 0.05 M phosphate buffer (pH 7.0) for 2 hrs at room temperature, then rinsed in the same buffer and postfixed in 1% buffered OsO<sub>4</sub> at 4°C for 2 hrs followed by dehydration in a graded EtOH series and immersed in *t*-butyl alcohol. The samples immersed in *t*-butyl alcohol were then freeze-dried in an evacuator (VFD-21; Vacuum Device, Ibaraki, Japan) and mounted on aluminum stubs with double-sided carbon tape. The samples were coated with a thin layer of gold using a sputter coater (JFC-1500; JEOL, Tokyo, Japan) and examined with a SEM (JSM-T220A; JEOL, Tokyo, Japan). Some air-dried specimens were also examined with a low-vacuum SEM (TM-1000; Hitachi, Tokyo, Japan) without gold coating.

### DNA extraction, PCR amplification and DNA sequencing

Total DNA was extracted using the simplified

chloroform method (Tsubota *et al.* 2009) or the glass wool binding method (Tsubota *et al.* 2005). Stems were placed under a stereo microscope and the green part of the shoot dissected. Single shoots were used for the DNA extraction procedure. Several segments of chloroplast ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene were amplified by standard polymerase chain reaction (PCR) or nested PCR with Ex Taq polymerase PCR amplification kit following the manufacturer's protocols (Takara Bio, Inc., Otsu) using a DNA thermal cycler (Mastercycler gradient, Eppendorf Co., Ltd., Tokyo) with synthetic primers (Table 1). Direct DNA sequence analyses of the PCR products were performed by the dideoxy chain termination method using the ABI kits with the additional internal primers (Table 1) using an automated sequencer (ABI 3130 Genetic Analyzers, Life Technologies Japan, Tokyo). Conditions of PCR and sequencing were based on Tsubota *et al.* (1999, 2000) and Handa *et al.* (2003). The sequences obtained in the present study have been submitted

Table 2. List of species and families investigated for *rbcL* gene sequences with the accession number and voucher or reference of the sequences\*.

Class/Order/Family/Species	Accession No.	References/Origin
<b>Bryopsida</b>		
<b>Bryoxiphiales</b>		
Bryoxiphiacae		
<i>Bryoxiphium norvegicum</i> (Brid.) Mitt. subsp. <i>japonicum</i> (Berggr.) Å.Löve & D.Löve	AB194720	Sato <i>et al.</i> (2004)
<b>Archidiales</b>		
Archidiaceae		
<i>Archidium stellatum</i> I.G.Stone	AF231066	DNA Database
<b>Dicraeales</b>		
Fissidentaceae		
<i>Fissidens adianthoides</i> Hedw.	DQ645988	Qiu <i>et al.</i> (2006)
<i>F. dubius</i> P.Beauv	AF231303	La Fage <i>et al.</i> (2000)
<i>F. mooreae</i> H.Whittier & H.A.Mill.	AF226810	DNA Database
Ditrichaceae		
<i>Ditrichum pallidum</i> (Hedw.) Hampe	AF231302	La Fage <i>et al.</i> (2000)
Rhabdoweisiaceae		
<i>Arctoa fulvella</i> (Dicks.) Bruch & Schimp.	AF231293	La Fage <i>et al.</i> (2000)
<i>Cynodontium jenneri</i> (Schimp.) Stirt.	AF231318	La Fage <i>et al.</i> (2000)
<i>Rhabdoweisia crenulata</i> (Mitt.) H.Jameson	AF005544	Goffinet <i>et al.</i> (1998)
Eriodiaceae		
<i>Aulacopilum hodgkinsoniae</i> (Hampe & Hüll.Hal.) Broth.	AF005545	Goffinet <i>et al.</i> (1998)
<i>Venturiella sinensis</i> (Venturi) Müll.Hal.	AB125591	Tsubota <i>et al.</i> (2003)
Dicranaceae		
<i>Brothera leana</i> (Sull.) Müll.Hal.	AB122033	Oguri <i>et al.</i> (2003)
<i>Dicranodontium pulchroatare</i> Broth.	AF226831	DNA Database
Leucobryaceae		
<i>Campylopus umbellatus</i> (Schwägr. & Gaudich. ex Arn.) Paris	AF226814	DNA Database
<i>Leucobryum humillimum</i> Cardot	AB124789	Oguri <i>et al.</i> (2003)
<i>L. sanctum</i> (Nees ex Schwägr.) Hampe	AB124787	Oguri <i>et al.</i> (2003)
<i>L. scabrum</i> Sande Lac.	AB029388	Tsubota <i>et al.</i> (1999)
<b>Pottiales</b>		
Pottiaceae		
<i>Acaulon muticum</i> (Schreb. ex Hedw.) Müll.Hal.	AF231078	DNA Database
<i>Barbula convoluta</i> Hedw.	AF478225	Magombo (2003)
<i>B. unguiculata</i> Hedw.	AB670696	This study
<i>Cinclidotus mucronatus</i> (Brid.) Guim.	AF231079	DNA Database
<i>Ephemerum spinulosum</i> Bruch & Schimp.	AB194719	Sato <i>et al.</i> (2004)
<i>Hymenostylium recurvirostrum</i> (Hedw.) Dixon	DQ645992	Qiu <i>et al.</i> (2006)
<i>Hypodontium pomiforme</i> (Hook.) Müll.Hal.	AF226803	DNA Database
<i>Leptophascum leptophyllum</i> (Müll.Hal.) J.Guerra & Cano		
[= <i>Chenia rhizophylla</i> (Sakurai) R.H.Zander]	AB670695	This study
<i>Pottia intermedia</i> (Turner) Fürnr [= <i>Tortula modica</i> R.H.Zander]	AB125592	Tsubota <i>et al.</i> (2003)
	AB670694	This study
<i>P. truncata</i> (Hedw.) Bruch & Schimp. [= <i>T. truncata</i> (Hedw.) Mitt.]	DQ463105	Newton <i>et al.</i> (2006)
<i>Pseudosymblepharis schimperiana</i> (Paris) H.A.Crum	AF226805	DNA Database
<i>Stegonia latifolia</i> (Schwägr.) Venturi ex Broth.	AF231314	La Fage <i>et al.</i> (2000)
<i>Streptopogon calympères</i> Müll.Hal.	AF478231	Magombo (2003)
<i>Syntrichia ruralis</i> (Hedw.) F.Weber & D.Mohr [= <i>Tortula ruralis</i> (Hedw.) Gaertn.]	AJ275169	Cox <i>et al.</i> (2000)
	FJ546412	Oliver <i>et al.</i> (2010)
<i>Tortula obtusissima</i> (Müll.Hal.) Mitt.	AF226823	DNA Database
<i>Uleobryum naganoi</i> Kiguchi	AB194717	Sato <i>et al.</i> (2004)
<i>Weissia controversa</i> Hedw.	AB194718	Sato <i>et al.</i> (2004)
<b>Orthotrichales</b>		
Orthotrichaceae		
<i>Uleastrum palmicola</i> (Müll.Hal.) R.H.Zander	AF005547	Goffinet <i>et al.</i> (1998)
<b>Hypnales</b>		
Hypnaceae		
<i>Campylophyllum halleri</i> (Sw. ex Hedw.) M.Fleisch	AB332270	Arikawa <i>et al.</i> (2008)
Outgroup taxa		
<b>Grimmiales</b>		
Grimmiaceae		
<i>Grimmia apiculata</i> Hornsch.	AB125576	Tsubota <i>et al.</i> (2003)
<i>G. pulvinata</i> (Hedw.) Sm.	AB125580	Tsubota <i>et al.</i> (2003)
Ptychomitriaceae		
<i>Ptychomitrium dentatum</i> (Mitt.) A.Jaeger	AB125587	Tsubota <i>et al.</i> (2003)
<i>P. wilsonii</i> Sull. & Lesq.	AB125588	Tsubota <i>et al.</i> (2003)
<i>Racomitrium fasciculare</i> (Hedw.) Brid. var. <i>atroviride</i> Cardot	AB125581	Tsubota <i>et al.</i> (2003)

\*Treatment of families follows Hill *et al.* (2006). Scientific name of *Pottia* and *Leptophascum* follow Guerra *et al.* (2006), see also Werner *et al.* (2002). Further information for the sequences obtained in the present study is shown in Appendix 2.

to DDBJ/EMBL/GenBank International Nucleotide Sequence Database Collaboration (INSDC; Appendix 1).

#### *Taxon sampling, sequence alignment, and phylogenetic analysis*

BLAST searches were carried out based on the sequences obtained in the present study, and additional sequences registered in the DNA database (Tsubota *et al.* 2003, 2004; Sato *et al.* 2004). The *rbcL* gene sequences were aligned using the program MUSCLE (Edgar 2004) on MEGA5 (Tamura *et al.* 2011) with some manual adjustment. The data set includes 18 OTUs of Pottiales, 20 OTUs of other mosses and five OTUs for outgroups (Table 2, with DDBJ/EMBL/GenBank accession nos.), and used for the following phylogenetic analyses.

Phylogenetic analysis using the *rbcL* gene sequences was performed based on maximum likelihood criteria as previously described (Tsubota *et al.* 2004; Ozeki *et al.* 2007; Masuzaki *et al.* 2010), with some differences as follows: Prior to the phylogenetic reconstruction, Kakusan4 (Tanabe 2011) was implemented in AICc (Sugiura 1978) to make a rational decision regarding the nucleotide-based substitution model that best fitted our data, and the approximate unbiased test (AU; Shimodaira 2002, 2004) in the final stage of the analysis scheme. Phylogenetic trees were constructed using the following five program packages to obtain the candidate topologies: (1) RAxML ver. 7.2.6 (Stamatakis 2006) with maximum likelihood (ML) method (Felsenstein 1981) using GTR + gamma and GTR + CAT models; (2) PhyML ver. 3.0 (Guindon & Gascuel 2003) with ML method using GTR + gamma model; (3) PAUPRat (Sikes & Lewis 2001) over PAUP\* ver. 4.0b10 (Swofford 2002) with 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 50 iteration runs; (4) TNT ver. 1.1 (Goloboff *et al.* 2008) with MP method; and (5) MrBayes ver. 3.1.2 (Ronquist & Huerlenbeck 2003) with Bayesian inference (BI) method using GTR + gamma model with 1,000,000 generations. Based on the ML criteria, re-calculation of likelihood values for each tree topology was performed with the GTR + gamma model by PAUP, with the

set of candidate topologies being evaluated by 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 50% majority-rule condensed tree for the topologies with best ranking log-likelihood values that passed the AU test was also computed by MEGA5. Supporting values more than 50% were overlaid to assess the robustness of each branch of the condensed topology: AU test (AU), bootstrap probabilities (NP), and Bayesian posterior probabilities (PP) are shown on or near each branch (AU/NP/PP; in %).

## Results

### *Description (Figs. 1 & 2)*

Plants forming dense tufts, green above, brown below. Stems to 1 cm long, sparingly branched; central strand weakly differentiated. Leaves appressed, weakly twisted when dry, spreading and weakly reflexed when moist; oblong to narrowly ovate, 1.5–2.3 mm long and 0.5–0.8 mm wide, with acute apex, somewhat keeled above, concave below; margins entire throughout, upper marginal cells occasionally with 1–4 faint papillae, narrowly recurved in middle; costa stout, percurrent, smooth on both adaxial and abaxial sides in cross-section; guide cells 2 or 3 in a single layer, adaxial stereids absent, abaxial stereids present, 1–4 stratose; upper laminal cells quadrate, 13–23 × 13–23 µm thin-walled, smooth, occasionally with 1–2 faint papillae; basal laminal cells enlarged, rectangular, 22–30 × 10–12 µm, thin-walled, smooth. Autoicous. Perichaetal leaves scarcely differentiated, slightly shorter and narrower than upper leaves. Perigonial leaves smaller than vegetative leaves, ca. 0.2 mm long. Rhizoidal tubers present, irregularly globose to ellipsoidal, reddish brown. Capsule short-cylindric, 0.5–1.4 × 0.2–0.7 mm, urn with slightly narrowed mouth, exothelial cells rectangular to hexagonal, 21–34 × 13–26 µm, thin-walled, stomata at base of capsule; peristome absent or rudimentary; rudimentary teeth with weakly developed minute papillae on both surfaces, disappearing after spore shedding; operculum with a short oblique rostrum. Seta 4–7 mm long, reddish brown, twisted to the right above and the left below. Calyptra cuillate,

smooth, *ca.* 2 mm long. Spores 25–35  $\mu\text{m}$  in diam, brown, finely papillose. Capsules mature in December to June in Japan. KOH laminal color reaction none.

Note: leaf papillae would not be stable character, because some showed papillae on lamina or only on margin, and others lack papillae among collections examined.

Specimen examined from Hiroshima, Kagawa and Ehime Prefs. (also Appendix 2): Japan, HON-SHU, Hiroshima-ken, Onomichi-shi, Setodacho, Ikuchijima Isl., 34°16'47"N, 133°04'41"E,

*ca.* 30 m alt., 17 iv 2011, *Y. Inoue* 135 (HIRO); ibid, 7 xi 2011, *Y. Inoue* 315 (HIRO); Mihara-shi, Sagi-cho, Sunami, Sagishima Isl., 34°20'34"N, 133°06'21"E, *ca.* 40 m alt., 19 vi 2011, *Y. Inoue* 258 (HIRO); Higashi-hiroshima-shi, Akitsu-cho, Ooshibajima Isl., 34°16'20"N, 132°48'01"E, *ca.* 20 m alt., 19 v 2011, *Y. Inoue* 195 (HIRO); Kure-shi, Toyohama-cho, Toyoshima Isl., 34°10'56"N, 132°46'42"E, 0–20 m alt., 26 iii 2009, *H. Tsubota* 6397, 6400, 6402 (HIRO); SHIKOKU, Kagawa-ken, Shozu-gun, Shodoshima-cho, Shodoshima Isl., 34°29'45"N, 134°17'45"E, *ca.* 130 m alt., 15

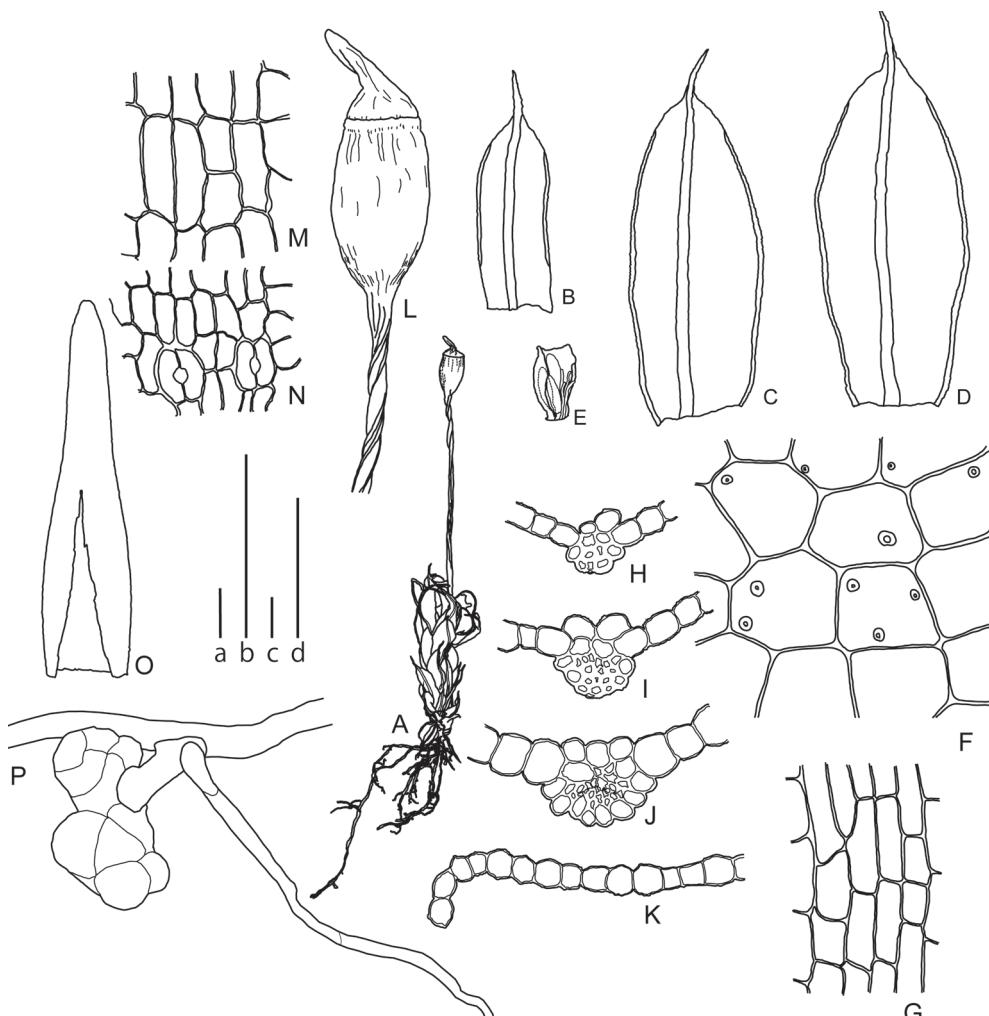


Fig. 1. *Pottia intermedia* (Turner) Fürnr. A. Plant. B–D. Leaves. E. Perigonal leaf. F. Cells from middle of leaf. G. Cells from base of leaf. H–J. Cross section of costa. K. Cross section of leaf-margin. L. Capsule. M. Exothelial cells. N. Stomata. O. Calyptra. P. Rhizoidal tuber. Scale bars: a = 1 mm (A); b = 1 mm (B–E, L); c = 10  $\mu\text{m}$  (F); d = 100  $\mu\text{m}$  (G–K, M–P). (A, L and P drawn from *Y. Inoue* 194 in HIRO; B–D, F–K and M–P from *Y. Inoue* 56 in HIRO; E, from *Y. Inoue* 315 in HIRO).

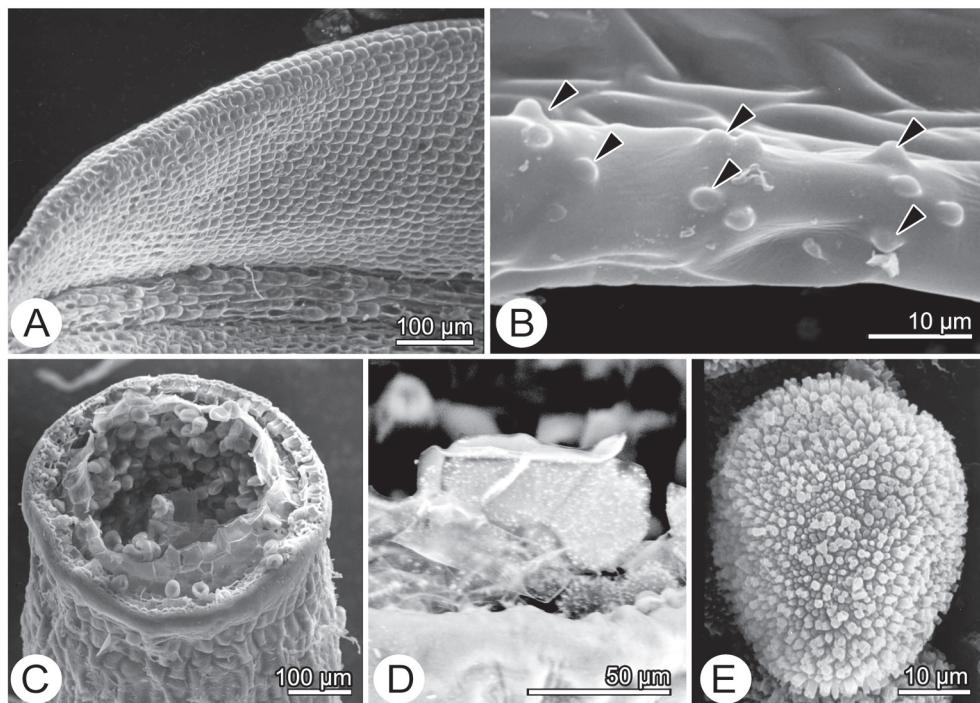


Fig. 2. *Pottia intermedia* (Turner) Fürnr. A. Leaf with smooth laminal cells. B. Upper marginal cells with faint papillae (arrowheads). C. Capsule with peristome. D. Peristome teeth with faint papillae. E. Spore. (A–C and E from *Y. Inoue* 315 in HIRO; D from *Y. Inoue* 194 in HIRO).

v 2011, *Y. Inoue* 194 (HIRO); Ehime-ken, Imabari-shi, Ohmishima-cho, Ohmishima Isl., 34°14'51"N, 133°00'27"E, ca. 20 m alt., 20 ii 2011, *Y. Inoue* 56 (HIRO); Hakata-cho, Hakatajima Isl., 34°12'40"N, 133°06'49"E, ca. 10 m alt., 2 v 2011, *H. Kubo* 696

(HIRO); Miyakubo-cho, Hayakawa, Ohshima Isl., 34°11'37"N, 133°02'26"E, ca. 10 m alt., 14 v 2011, *H. Tsubota* 7702 (HIRO).

*P. intermedia* differs from its close relative *P. truncata* (Hedw.) Bruch & Schimp. by (1) the leaf margins narrowly recurved, (2) urn with slightly narrowed mouth and (3) rudimentary peristome teeth.

Distribution in Japan: W. Honshu, Shikoku, Kyushu (Fig. 3).

Icones: Sakurai, J. Jap. Bot. 28: 61, f. 7 [as

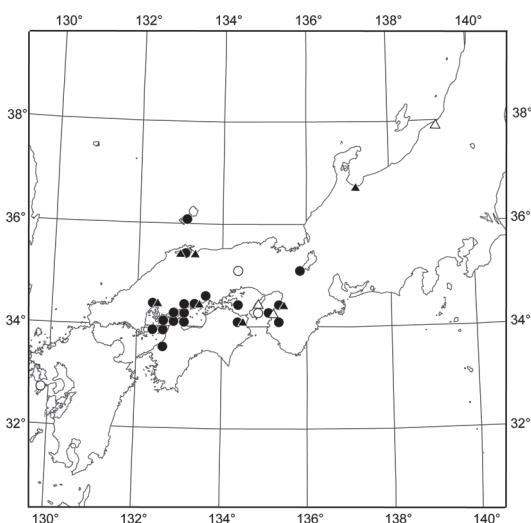


Fig. 3. Distribution range in Japan of *Pottia intermedia* (Turner) Fürnr (closed circles or open circles) and *P. truncata* (Hedw.) Bruch & Schimp. (closed triangles or open triangles). Occurrence of species within the area of each unit-geoquadrat (latitude 10'×longitude 15') is represented by one dot, whether it is known from a single or more localities, based on the method of Horikawa (1963). Closed circles or triangles are based on specimens examined; open circles or triangles are based on literature reports (Brotherus 1899; Sakurai 1953; Tatebe & Nakajima 1953; Saito 1973).

*Tortula tutigae* Sakurai] (1953); Sakurai, Muscol. Jap.: pl. 19 [as *Tortula tutigae* Sakurai] (1954); Noguchi, Misc. Bryol. Lichenol. 3: 123, f. 19 (1964); Saito, Bull. Nat. Sci. Mus. Tokyo 16: 66, f. 2 (1973); Noguchi *et al.*, Ill. Moss Fl. Jap. 3: 319, f. 132 (1988); Deguchi, Bryophytes: 112, f. 56 (1998);

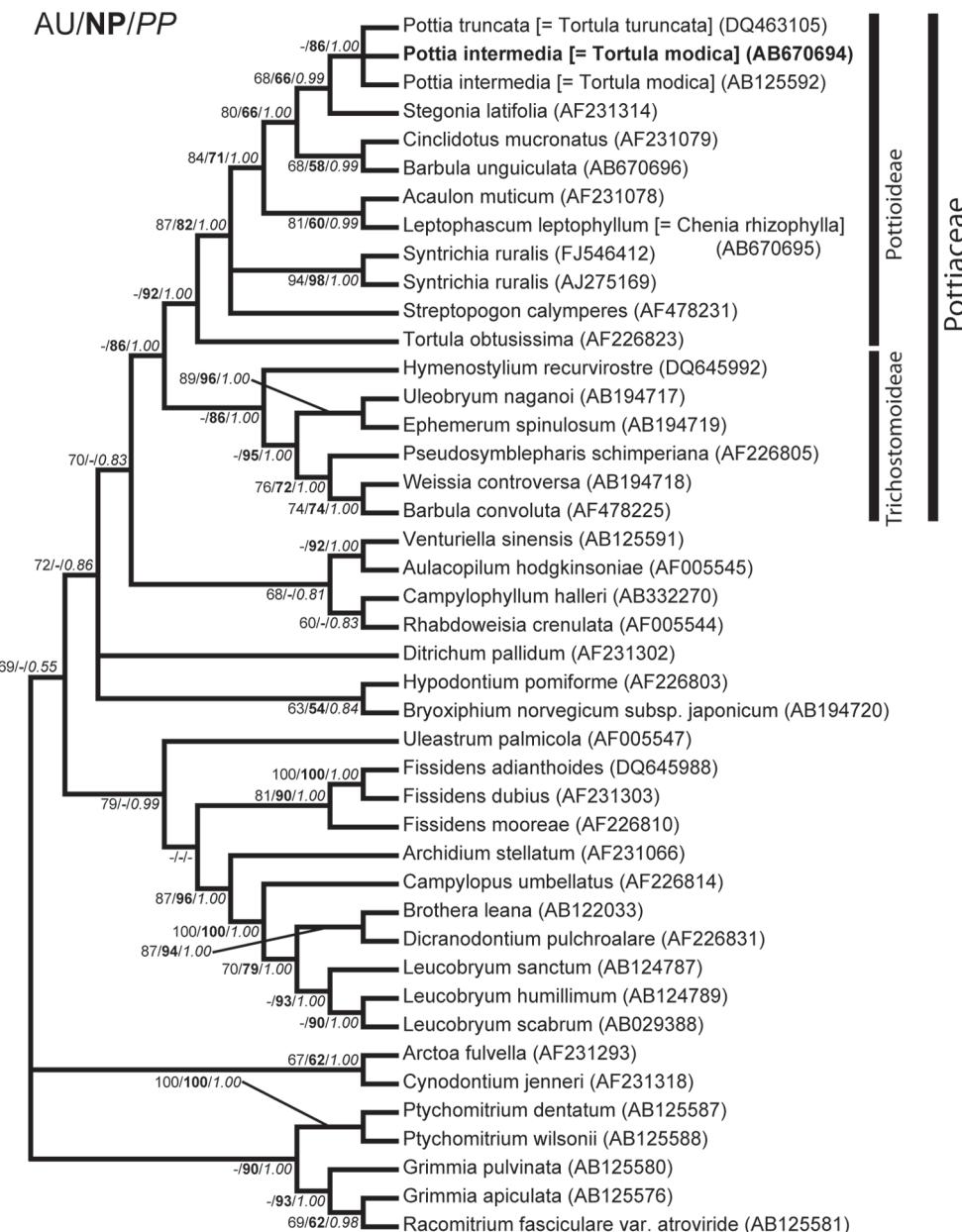


Fig. 4. Phylogenetic position of *Pottia intermedia* (Turner) Fürnr based on cp *rbcL* gene sequences depicted by a 50% majority-rule condensed tree for the 27 topologies with the best ranking log-likelihood value. Supporting values more than 50% obtained by the program CONSEL were overlaid: the values by the AU test (AU), bootstrap probabilities (NP), and Bayesian posterior probabilities (PP) are shown on or near each branch (AU/NP/PP, in %). The root is arbitrarily placed on the branch leading to the clade which includes members of the Grimmiaceae following Tsubota *et al.* (2003, 2004) and Sato *et al.* (2004).

Iwatsuki *et al.*, Mosses Liverworts Jap.: 89, f. 22, pl. 39 (2001).

**Habitat:** Fertilized clayey soil, especially in citrus orchards, occasionally associated with *Bryum* spp., *Physcomitrium* spp. and/or *Leptophascum leptophyllum* (Müll.Hal.) J.Guerra & M.J.Cano.

#### *Phylogenetic analysis*

The GTR + gamma model provided the best fit model for the dataset. A total of 10,271 distinct topologies were obtained in the ML, MP and BI analyses, of which 7,760 topologies passed the AU test. Fig. 4 shows the condensed topology of the 27 topologies with best ranking log-likelihood values. The best 27 topologies supported the monophyly of Pottiaceae with high supporting values (AU/NP/PP = -/86/1.00; Fig. 4). Within the Pottiaceae clade, two subclades were resolved: the *Pottia*–*Stegonia*–*Cinclidotus*–*Barbula*–*Acalon*–*Leptophascum*–*Syntrichia*–*Tortula*–*Streptopogon* clade (-/92/1.00), and the *Hymenostylium*–*Uleobryum*–*Ephemerium*–*Pseudosymbelpharis*–*Weissia*–*Barbula* clade (-/86/1.00). *P. intermedia* appeared in the former clade together with *P. truncata*. *P. intermedia* is closely related to *P. truncata* (-/86/1.00). Homology values of the partial *rbcL* sequences (= 1,322 bp) of *P. intermedia* and *P. truncata* proved to be identical with the value 100%.

## Discussion

#### *Morphological characters*

SEM observations of recent Japanese collections confirm the slightly papillose laminal cells and rudimentary peristome reported for the species. These characters have been repeatedly described from specimens from Europe, China and North America in previous works (Nyholm 1956; Chamberlain 1978; Pedrotti & Puntillo 2001; Xing-jian *et al.* 2001; Ros & Werner 2006; Zander & Eckel 2007). Saito (1973) noted that laminal cells of Japanese specimens were smooth and that the peristome was usually absent or rarely rudimentary. Noguchi *et al.* (1988) indicated that the upper laminal cells rarely have low papillae, and Deguchi (1998) showed the presence of laminal cells with faint papillae and rudimentary peristome teeth for Japanese populations.

Based on our careful examination of fresh

materials, rhizoidal tubers are also confirmed in Japanese populations. Although there are several reports of rhizoidal tubers from European and Russian specimens (Roth 1904; Savicz-Ljubitkaja & Smirnova 1970; Risso 1985; Arts 1987), there have been no previous reports of tubers in Japanese populations. Their occurrence appears to be infrequent and the number of tubers is small.

#### *Habitat and distribution*

In the present field investigations, *P. intermedia* was found usually on fertilized clayey soil in open sites, especially citrus orchards. Deguchi (1998) also reported this species from similar habitats. However, little attention has been paid to habitat preference by Japanese Bryologists.

A new locality for *P. intermedia* is first reported here from Kagawa Pref., Shikoku, SW Japan, together with additional localities from Hiroshima and Ehime Prefs., Honshu and Shikoku, SW Japan. Although it is widely distributed in the world, *P. intermedia* can be regarded as a rare moss in Japan, and was previously known only from a limited number of localities on Honshu, Shikoku and Kyushu. *P. truncata* also has a similar habitat to that of *P. intermedia*, though this species has a wider distribution range in Japan, extending northward to Toyama and Niigata Prefs. (Fig. 3). In North America, Zander & Eckel (2007) reported the distributions of *P. intermedia* [as *T. modica*] from eastern part of North America and that of *P. truncata* [as *T. truncata*] from a much wider range across North America and Canada. Deguchi & Iwatsuki (1984), when discussing geographical relationships of the moss flora of Japan, mentioned a close affinity between the floras of Japan and North America. They mentioned that many eastern North American elements have a major disjunctive distribution pattern and are confined to southern areas and the Pacific side of Honshu, suggesting the explanation lay in the climatic differences, such as the amount of snow fall in winter or rainfall in summer. The distribution of Japanese populations of *Pottia* may also reflect these climatic parameters.

#### *Phylogeny*

The present results, based on cp *rbcL* gene sequences, suggests monophyly of Pottiaceae with the inclusion of *Ephemerum spinulosum* Bruch & Schimp. and *Uleobryum naganoi* Kiguchi *et*

*al.* and the exclusion of *Hypodontium pomiforme* (Hook.) Müll.Hal. with high supporting values, as previously reported by Sato *et al.* (2004). Two subclades corresponding to the subfamilies Pottioideae and Trichostomoideae (*sensu* Werner *et al.* 2004) were also resolved. *P. intermedia* is confirmed in the Pottioideae clade, as previously shown on the basis of the data on *rbcL* gene sequences (Tsubota *et al.* 2003, 2004; Sato *et al.* 2004).

*P. intermedia* has a close relationship with *P. truncata*. Chamberlain (1978) discussed *P. intermedia* in Europe, indicating its possible origin through hybridization between *P. truncata* and *P. lanceolata* (Hedw.) Müll.Hal., since it frequently grew with one or both species. However, in Japan we have not found *P. intermedia* associated with *P. lanceolata* and/or *P. truncata*. In addition, these species do not appear on the same branch of the phylogenetic tree, based on *rps4* gene sequences (Werner *et al.* 2002). Therefore, the possibility of a hybrid origin is unlikely.

Zander (1993) considered that the genus *Hypodontium* might be placed in a family of its own because of its unusual characters, while retaining it in Pottiaceae. Werner *et al.* (2004) mentioned that the taxonomic position of *Hypodontium* might be placed near *Fissidens* based on cp *rps4* sequence data of Pottiaceae. Recently, Stech and Frey (2008) made a re-evaluation of *Hypodontium* and elevated the genus to the new family Hypodontiaceae in their morpho-molecular classification of the mosses. Our phylogenetic tree based on cp *rbcL* gene sequences also supports its placement as a monotypic family.

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#### Appendix 1. Additional specimen examined of *Pottia intermedia*

Japan, HONSHU, Kyoto-fu, Kyoto-shi, Maruyama Park, 7 xi 1918, *H. Sasaoka* 1495, 40550 (TNS); Osaka-fu, Sennan-gun, Kinyuji (Sennan-shi, Shindachikinyuji), ca. 80 m alt., 26 vi 1960, *T. Nakajima* 10731 (TNS); Wakayama-ken, Naga-gun, Momoyama-cho, Tsukatuki (Kinokawa-shi, Momoyama-cho, Tsukatuki), ca. 90 m alt., 3 xii 1982, *T. Nakajima* 41863 (NICH); Okayama-ken, Asakuchi-gun, Satoso-machi (Asakuchi-gun, Satosho-cho), 6 xii 1930, *H. Sasaoka* 5969 (TNS); Funaho-mura (Kurashiki-shi, Funao-cho), 8 i 1931, *H. Sasaoka* 5979 (TNS); Tsurajima-machi (Kurashiki-shi, Tsurajima-cho), 6 iv 1930, *H. Sasaoka* 5769 (TNS); Hiroshima-ken, Eiju Hospital (Fukuyama-shi, National Hospital Organization Fukuyama Medical Center), 24 xii 1931, *A. Noguchi* 2756 (NICH); Ogiji (Mihara-shi, Nagatani-cho), 29 xii 1934, *H. Sasaoka* 18798 (TNS); Toyota-gun (Higashi-hiroshima-shi), Akitsu-cho, Oshiba Isl., 17 ii 2002, *H. Tsubota* 4461 (HIRO); Aki-gun, Kamagari-cho (Kure-shi, Kamagari-cho), 14 xii 1995, *H. Tsubota* 49 (HIRO); ibid., 6 iii 1996, *H. Tsubota* 371 (HIRO); ibid., 4 iv 1999, *M. Izawa* (NICH 24972); Aki-gun, Shimokamagari-cho, 23 iv 2000, *H. Tsubota* 3850 (HIRO); ibid., 23 iv 2000, *T. Yamaguchi* 18701 (HIRO); Hiroshima-shi, 5 v 1961, *A. Noguchi* 61237 (NICH); Shimane-ken, Matsue-shi, Sotonakabara-cho, 3 xii 1957, *A. Noguchi* 42170 (NICH); Oki-gun, Ama-cho, Hishi-ura, 11 i 1973, *H. Omae* (HIRO); Yamaguchi-ken, Ohshima-gun, Kuka-cho, Nakaseta (Suo-ohshihama-cho, Kuka-nakaseta), ca. 60 m alt., 25 xi 2001, *M. Hayashi* 10222, 10237, 10238

(Yamaguchi Pref. Museum); SHIKOKU, Ehime-ken, Matsuyama-shi, i 1930, *S. Yagi* 6 (NICH); Matsuyama-minami High School, 1 ii 1950, *A. Noguchi* 65330 (NICH & TNS); Ohguki (Kita-gun, Uchiko-cho, Ohguki), 3 v 1930, *K. Oti* 12232 (HIRO); Tokushima-ken, 27 xii 1931, *S. Murai* 55A (NICH).

Note: Saito (1973) reported *Pottia intermedia* from Tottori Pref. (NICH 37167), but the specimen is *P. truncata*.

#### Appendix 2. Taxa whose *rbcL* sequences were utilized in this study

The list includes accession numbers, their source and voucher specimen information. Voucher specimens are kept in HIRO.

The sequence of information is: the name of taxon, accession number, locality and specimen number. Pottiaceae: *Barbula unguiculata* Hedw., AB670696, Japan, Honshu, Hiroshima-ken, Hatsukaichi-shi, Miyajima-cho, Miyajima Isl., 34°16'36"N, 132°18'56"E, ca. 300 m alt., 8 iii 2011, *Y. Inoue* 113 (HIRO); *Leptophascum leptophyllum* (Müll.Hal.) J.Guerra & M.J.Cano [as *Chenia rhizophylla* (Sakurai) R.H.Zander], AB670695, Shikoku, Ehime-ken, Imabari-shi, Ohmishima-cho, Omishima Isl., 34°14'51"N, 133°00'27"E, ca. 20 m alt., 20 ii 2011, *Y. Inoue* 57 (HIRO); *Pottia intermedia* (Turner) Fürnr. [= *Tortula modica* R.H.Zander], AB670694, Ehime-ken, Imabari-shi, Ohmishima-cho, Ohmishima Isl., 34°14'51"N, 133°00'27"E, ca. 20 m alt., 20 ii 2011, *Y. Inoue* 56 (HIRO).

井上侑哉・坪田博美・久保晴盛・内田慎治・向井誠二・嶋村正樹・出口博則：センボンゴケ *Pottia intermedia* (Turner) Fürnr. (センボンゴケ科) の新産地とその分子系統学的位置

センボンゴケ *Pottia intermedia* (Turner) Fürnr. の新たな産地を報告する。本種は世界的に広く分布し、国内では近畿以西の本州および四国、九州で報告されているが比較的稀な頂蘚類であり、今回本州（広島）および四国（香川・愛媛）の8か所で新たに生育を確認した。本種は日当たりの良い畑の土上に生育している。今回得られた標本にもとづいて形態解析図およびこれまで正確に記載されていない日本産のものについての蒴歯や胞子、葉細胞の電子顕微鏡写真を与えると共に、本種の系統的位置についても言及した。