



On the systematic position of the genus *Timmiella* (Dicranidae, Bryopsida) and its allied genera, with the description of a new family Timmiellaceae

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Abstract

Based on our molecular phylogenetic analysis of haplolepidous mosses with concatenated sequences of chloroplast *rps4* and *rbcL* genes, a new family Timmiellaceae is erected to accommodate the genera *Timmiella* and *Luisierella*, both of which have been formerly included in the family Pottiaceae. The family Timmiellaceae is resolved as a second-branching clade together with *Distichium* (Distichiaceae) within the Dicranidae (haplolepidous moss) lineages and phylogenetically distinct from the Pottiaceae. Reassessment of morphological characters suggests that a combination of the characters: 1) adaxially bulging and abaxially flat leaf surfaces, 2) sinistrorse or straight peristomes, when present, and 3) sinistrorsely arranged operculum cells is unique to Timmiellaceae and discriminates it from other haplolepidous moss families.

Introduction

The genus *Timmiella* (De Notaris 1865: 100) Limpricht (1888: 590) is a haplolepidous moss which has been placed in the family Pottiaceae Schimper (1856: 24) since it was first described as a section of the genus *Trichostomum* Bruch in Müller (1829: 396) by De Notaris (1865). Although many authors have placed the genus in the subfamily Trichostomoideae (Schimper 1860: 141) Brotherus (1902: 381) of the Pottiaceae (Limpricht 1888, Brotherus 1902, 1924a, Hilpert 1933, Chen 1941, Podpěra 1954, Saito 1975, Corley *et al.* 1981, Walther 1983), the systematic position of the genus has been questioned because of its unique morphological characters [e.g. denticulate to dentate leaf margins, bistratose lamina, adaxially bulging and abaxially flat lamina, and sinistrorse peristomes (twisted to the left when viewed from the side)]. These characters indicate that it has a different evolutionary line from the other genera of Trichostomoideae as noted by Saito (1975). Based on cladistic analysis using morphological characters, Zander (1993) established the subfamily Timmielloideae Zander (1993: 68) with its sole genus *Timmiella*.

Recent molecular phylogenetic studies have suggested the exclusion of *T. anomala* (Bruch & Schimper 1842: 196) Limpricht (1888: 592) or *T. crassinervis* (Hampe 1860: 456) Koch (1950: 11) from the Pottiaceae and their repositioning as an early-diversing clade within the Dicranidae Ochyra (2003: 104) (haplolepidous mosses) (La Farge *et al.* 2000, 2002, Werner *et al.* 2004, Hedderson *et al.* 2004, Tsubota *et al.* 2004, Wahrmond *et al.* 2009, 2010, Cox *et al.* 2010). However, *Timmiella* was retained as a member of the Pottiaceae because of its morphological affinity to the family, especially the distinctive twisted peristome (Zander 2006, 2007). No taxonomic changes had been made based on the monophyletic groupings because the phylogenetic position of the genus in the early-diversing haplolepidous mosses remained to be fully resolved.

In the present study, the phylogenetic position and taxonomic treatment of *Timmiella* and its allied genera are reassessed based on phylogenetic analysis with concatenated sequences of chloroplast ribosomal protein S4 (*rps4*) and ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) genes. We also discuss morphological characters that support the monophyly inferred from our analysis.

Materials and Methods

Taxon sampling

16 *rps4* and 17 *rbcL* gene sequences of the Dicranidae, including the type species of *Timmiella*, *T. anomala*, were newly obtained for the present study. A total of 85 concatenated *rps4* and *rbcL* gene sequences were examined in the present analysis, as shown in Appendix 1. Taxa were selected to represent the haplolepidaceous moss families recognized by Frey & Stech (2009), as well as taxa placed in or near the Dicranidae by Cox *et al.* (2010). We also included representatives of peristomate moss orders as outgroup taxa and used *Buxbaumia aphylla* Hedwig (1801: 166) and *Diphyscium fulvifolium* Mitten (1891: 193) as root of the tree following Tsubota *et al.* (2003, 2004) and Cox *et al.* (2010).

DNA extraction, PCR amplification and DNA sequencing

The protocol of the DNA extraction of total DNA followed Tsubota *et al.* (2009) and Suzuki *et al.* (2013). Condition of PCR amplification for both *rps4* and *rbcL* genes followed Tsubota *et al.* (1999, 2000) and Tsubota *et al.* (2013) with modifications: denaturation at 98°C for 10 sec., annealing at 58°C for 35 sec., and extension at 65°C for 1–1.5 min. for total 45 cycles. Direct sequence analyses of the PCR products were performed following Inoue *et al.* (2012). The design of the PCR and DNA sequencing primers followed Nadot *et al.* (1994), Tsubota *et al.* (1999, 2001), Masuzaki *et al.* (2010) and Inoue *et al.* (2011, 2012). The list of the primers newly designed in the present study is shown in Appendix 2. Sequences obtained in the present study have been submitted to DDBJ/EMBL/GenBank International Nucleotide Sequence Database Collaboration (INSDC).

Sequence alignment and phylogenetic analysis

The sequences were aligned using the program MAFFT ver. 7.027 (Katoh & Standley 2013) with some manual adjustment on the sequence editor of MEGA5.2 (Tamura *et al.* 2011). The indel confirmed in the *rps4* sequence of *Catoscopium nigrutum* (Hedwig 1801: 72) Bridel (1826: 368) was treated as missing data.

Phylogenetic analysis using concatenated *rps4* and *rbcL* gene sequences was performed based on maximum likelihood (ML) criteria (Felsenstein 1981) as previously described (Tsubota *et al.* 2003, Ozeki *et al.* 2007, Masuzaki *et al.* 2010) with some differences as follows: Prior to the phylogenetic reconstruction, model testing was performed based on AICc (Sugiura 1978) using Kakusan4 (ver. 4.0.2012.12.14; Tanabe 2011) to make a rational decision regarding the partitioning scheme and nucleotide substitution model that best fitted our data, and AU test in the final stage of the analysis scheme. Phylogenetic trees were constructed using the following four program packages to obtain the candidate topologies: (1) RAxML ver. 8.0.0 (Stamatakis 2014) with ML method using codon-partitioned model (GTR + G for all codon positions); (2) Garli ver. 2.01 (Zwickl 2006) with ML method using codon partitioned model (GTR + G + I for all codon positions); (3) 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; (4) BEAST v1.8.0 (Drummond *et al.* 2012) with Bayesian inference (BI) method using codon-partitioned model (GTR + G for all codon positions) with 100,000,000 generations. Re-calculation of likelihood values for each tree topology was performed with the GTR + G + I model which is the best fitted model for our data by PAUP. Alternative topology test and edge analysis were performed using the *p*-value of the approximate unbiased test (AU; Shimodaira 2002, 2004), bootstrap probability calculated through the same theory as AU (NP), and Bayesian posterior probability calculated by the BIC approximation (PP; Schwarz 1978, Hasegawa & Kishino 1989) as implemented in CONSEL ver. 0.20 (Shimodaira & Hasegawa 2001). A 50% majority-rule consensus tree for the topologies passing both AU and PP tests was also computed by MEGA. Supporting values more than 50% were overlaid to assess the robustness of each branch of the consensus topology: AU, NP and PP are shown on or near each branch.

Morphological investigation

Both fresh materials and dried specimens were used for light microscopic and scanning electron microscopic (SEM) observations. Preparation for SEM observation followed Inoue *et al.* (2011). Voucher specimen information is listed in Appendix 3.

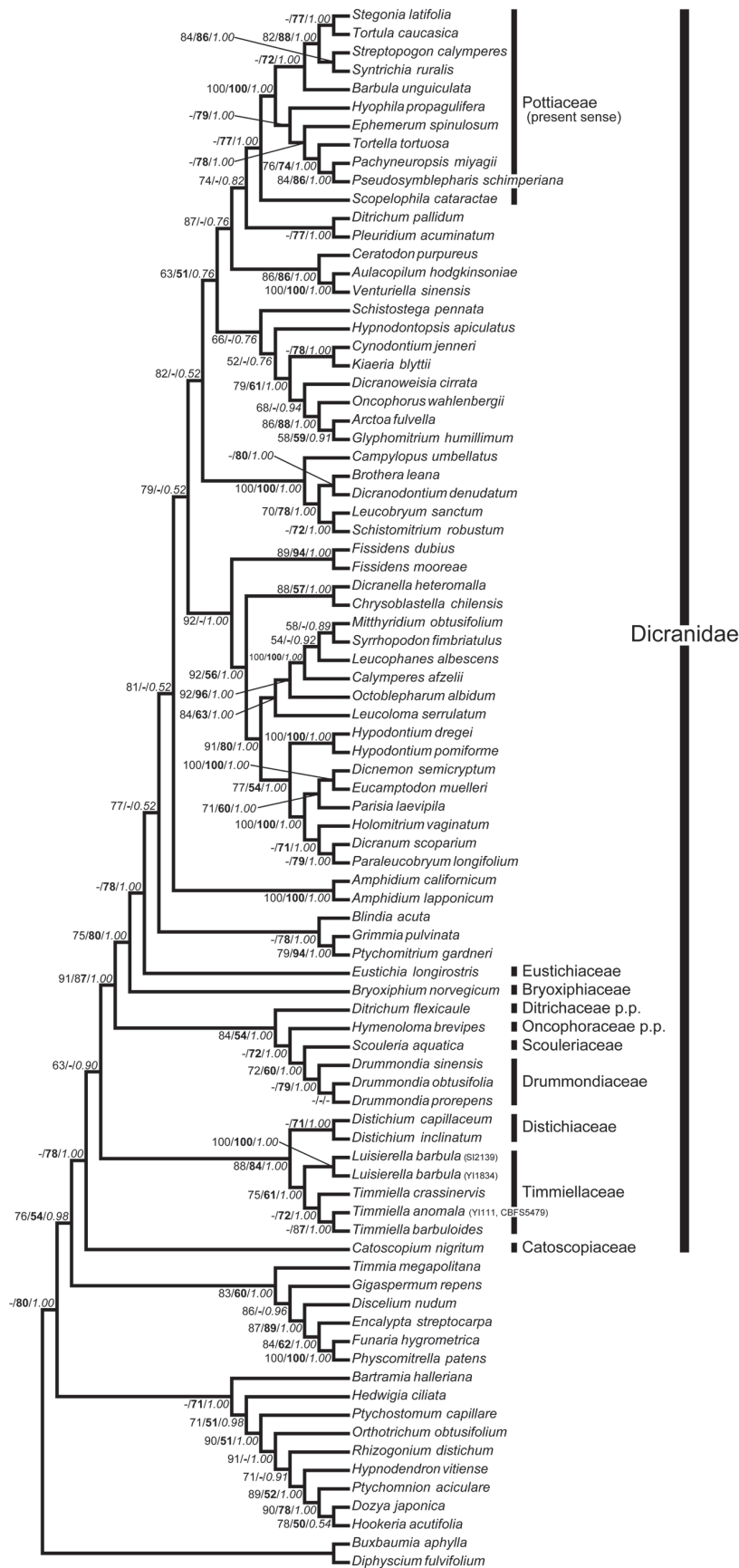


FIGURE 1. Phylogenetic tree based on analysis with the concatenated sequences of chloroplast *rps4* and *rbcL* genes, depicted by a 50% majority-rule consensus tree for the 9 topologies passing both AU and PP tests. Supporting values more than 50% obtained by the program CONSEL were overlaid: the values by the AU test (AU), bootstrap probabilities calculated through the same theory as AU (NP), and Bayesian posterior probabilities (PP) are shown on or near each branch (AU/NP/PP). The root is arbitrarily placed on the branch leading to the clade which includes members of the genera *Buxbaumia* and *Diphyscium* following Tsubota *et al.* (2003, 2004) and Cox *et al.* (2010).

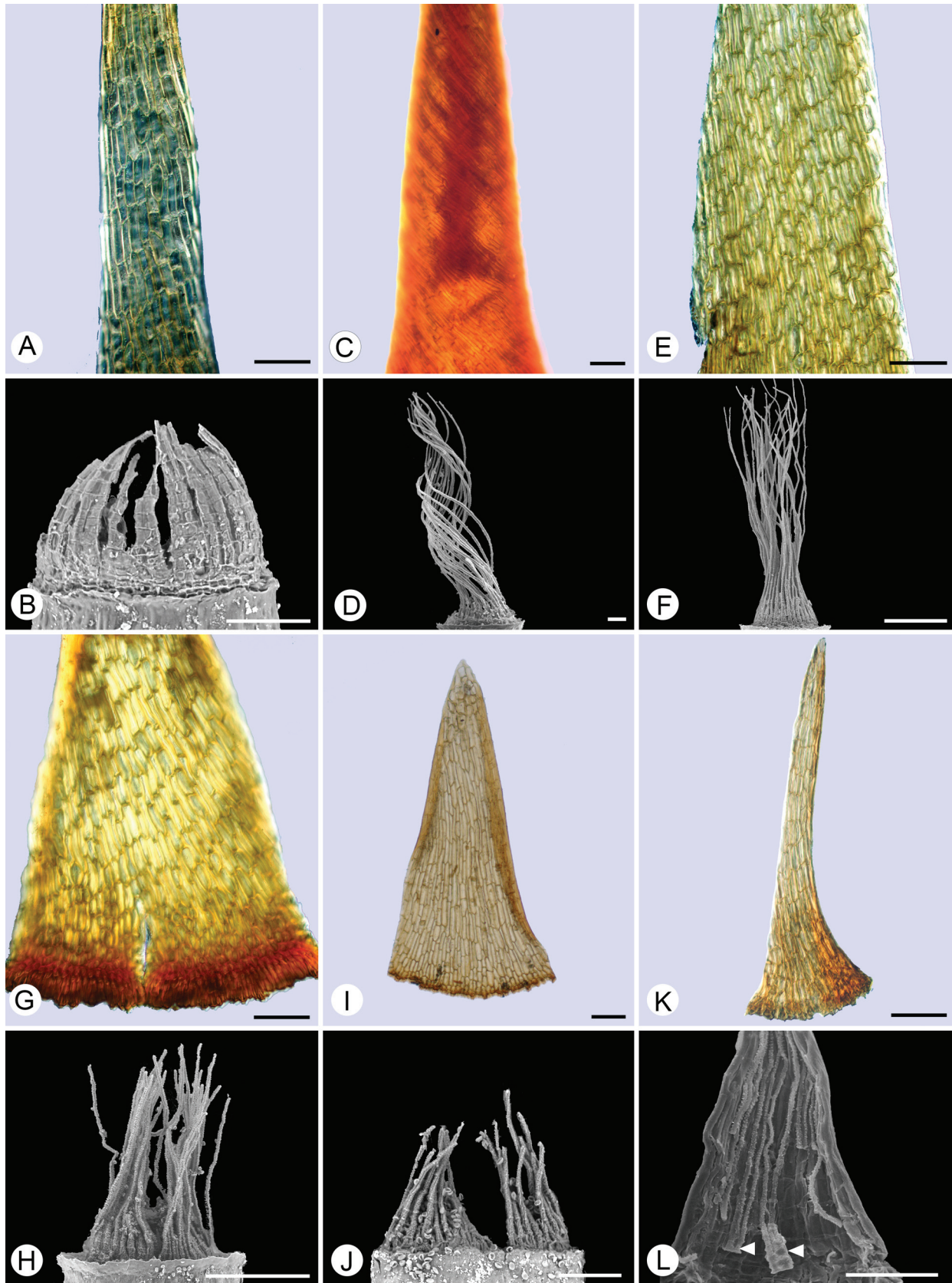


FIGURE 2. Opercula (A, C, E, G, I, K) and peristomes (B, D, F, H, J, L) of *Timmia* and *Luisierella*. A, B. *Timmia acaulon*. C, D. *T. anomala*. E, F. *T. barbulooides*. G, H. *T. crassinervis*. I, J. *T. diminuta*. K, L. *Luisierella barbula* (Peristome teeth indicated by arrowheads). A, B from C. C. Hosseus 396 (HIRO). C, D from Y. Inoue 1910 (HIRO). E, F from C. C. Townsend s.n. (HIRO). G, H from W. B. Schofield 14404 (HIRO). I, J from C. Y. Chang s.n. (TNS). K from R. A. Pursell 632 (HIRO). L from R. L. Redfearn Jr. 73–55 (HIRO). Scale bars = 100 μ m.

Results

A total of 1595 distinct topologies were obtained in the ML, MP and BI analyses, of which 978 topologies passed the AU test and 9 topologies passed the PP test. Fig. 1 shows the 50% majority-rule consensus tree for the topologies passing both AU and PP tests. Five main clades are confirmed in the early-diversing haplolepidous moss lineages: Catoscopiaceae Boulay ex Brotherus (1904: 629), *Timmiella*—*Luisierella* Thér. & P.de la Varde in Potier de la Varde (1936: 73)—Distichiaceae Schimper (1860: 135), Drummondiaaceae Goffinet in Buck & Goffinet (2000: 99)—Scouleriaceae Churchill (1981: 143)—*Hymenoloma brevipes* (Müller 1890: 300) Ochyra (2003: 114)—*Ditrichum flexicaule* (Schwägrichen 1811: 113) Hampe (1867: 182), Bryoxiphiaceae Bescherelle (1892: 183), and Eustichiaceae Brotherus (1924b: 420). The clade consisting of *Timmiella*, *Luisierella* and *Distichium* Bruch & Schimper (1846a: 153) is resolved as the second-branching clade in the haplolepidous moss lineages. In this clade, *Timmiella* is sister to *Luisierella* with moderate supporting values (AU/NP/PP = 75/61/1.00; Fig. 1).

Based on the phylogenetic tree, we reassessed the morphological characters shared with *Timmiella* and *Luisierella* which discriminate them from other haplolepidous moss families. In addition to gametophytic similarity: adaxially bulging and abaxially flat leaf surfaces, the sinistrorsely arranged operculum cells are unique to them. The operculum cells of *T. anomala* (type species) are sinistrorsely arranged and correlate with their sinistrorse peristome (Fig. 2, C and D). *T. acaulon* (Müller 1879: 320) R.H.Zander (1993: 70), *T. barbulooides* (Bridel 1806: 233) Mönkemeyer (1927: 273), *T. crassinervis* and *T. diminuta* (Müller 1898: 177) Chen (1941: 176), whose peristomes are apparently straight, have sinistrorsely arranged operculum cells (Fig. 2, A, B and E–J). *Luisierella barbula* (Schwägrichen 1826: 77) Steere (1945: 84), also has sinistrorsely arranged operculum cells, although its peristome is delicate and sometimes absent (Fig. 2, K and L).

Discussion

In the present study, we have shown the precise phylogenetic position of *Timmiella* by using all basal haplolepidous taxa suggested in previous studies (La Farge *et al.* 2000, 2002, Werner *et al.* 2004, 2013, Hedderson *et al.* 2004, Tsubota *et al.* 2004, Wahrmund *et al.* 2009, 2010, Cox *et al.* 2010, Stech *et al.* 2012). The genus is distinct from the Pottiaceae-clade and resolved as the second-branching clade together with *Luisierella* and *Distichium* among the Dicranidae lineages.

Zander (1993) distinguished *Timmiella* from the other members of the Pottiaceae and established a monogeneric subfamily Timmielloideae based on a combination of characters: very wide costa with multiple hydroid strands, epapillose leaf cells, adaxially bulging and abaxially nearly flat laminal cells, weakly sinistrorse (clockwise) or straight peristome. Our study suggests that the direction of twist of the operculum cells, as well as the peristome, is a significant character that discriminates the genus from Pottiaceae and the other haplolepidous moss families. In *Timmiella* spp. with peristomes that are apparently straight, the operculum cells are sinistrorsely arranged. This suggests that the genus has a fundamentally sinistrorse amphithecium.

Although its peristome is delicate and sometimes absent, *Luisierella*, which is a monotypic genus of Pottiaceae and phylogenetically sister to *Timmiella*, also has sinistrorsely arranged operculum cells, adaxially bulging and abaxially flat leaf cell surfaces. The close relationship between *Timmiella* and *Luisierella* is thus both morphologically and phylogenetically supported. *Luisierella* is much smaller than *Timmiella* in plant size, and often grows in association with cyanobacteria (blue-green algae) (Reese 1984, Deguchi 1987, Zander 1993). The genus *Seligeria* Bruch & Schimper (1846b: 7) which is a very small moss and phylogenetically sister to Grimmiaceae Arnott (1825: 19) (e.g. Tsubota *et al.* 2003) also grows in association with cyanobacteria (Longton 1988). In the course of evolution, the association with cyanobacteria might have led these genera to reduced plant size.

The combination of characters: 1) adaxially bulging and abaxially flat leaf cell surfaces, 2) when present, sinistrorse or straight peristomes, and 3) sinistrorsely arranged operculum cells, supports the molecular groupings inferred from our analysis, and discriminates *Timmiella* and *Luisierella* from the other haplolepidous moss families.

No significant characters that link *Distichium* and *Timmiella* + *Luisierella* are confirmed, although the two groups are phylogenetically sister to each other and both have the saxicolous habitat especially in limestone area (cf. Tanaka 2012, Inoue *et al.* 2014). The genus *Distichium* has distinct sporophytic and gametophytic characters: peristome teeth with dextrorse spiral thickenings in the basal portion, the distichous leaf arrangement and the mammillose subula. Although the two groups share mammillose leaf surfaces, our observation proved that the mammillae are present

in both adaxial and abaxial surfaces in *Distichium*, whereas in *Timmiella* + *Luisierella* they are restricted to adaxial surface.

The family Distichiaceae was originally proposed by Schimper (1860) to include *Distichium* and *Eustichium* Bruch & Schimper (1849: 159) [= *Bryoxiphium* Mitten (1869: 24)], and later Limpricht (1887) placed *Distichium* in Ditrichaceae Limpricht (1887). Due to its universal acceptance, Magill (1977) proposed Ditrichaceae as a conserved name against Distichiaceae and Ceratodontaceae Schimper (1860), and this proposal was adopted in the Berlin Code (Greuter *et al.* 1988). From our study, the resultant tree suggests that *Distichium* should be treated as a distinct family from the other genera of Ditrichaceae. The family name Distichiaceae can be used to accommodate *Distichium*, because Distichiaceae and Ditrichaceae are heterotypic synonyms and either can be adopted as correct names when they are considered distinct from each other (Art 14.6, in ICN, Melbourne, McNeill *et al.* 2012).

Taxonomy

Based on phylogenetic and morphological distinctions from the other haplolepidous moss families, we concluded that *Timmiella* and *Luisierella* are excluded from Pottiaceae and warrant accommodation within a new family. However, from the results no final decision regarding the order within which these families are accommodated can be made. Further analyses based on increased taxa, especially polyphyletic families such as Dicranaceae Schimper (1856: 11), Ditrichaceae and Oncophoraceae Stech (2008: 14), are necessary for further resolution.

Timmiellaceae Y.Inoue & H.Tsubota, *stat. nov.*

Timmielloideae R.H.Zander, Bull. Buffalo Soc. Nat. Sci. 32: 68. 1993.

Type: *Timmiella* (De Not.) Limpr., Laubm. Deutch. 1: 590. 1888. [based on *Trichostomum* sect. *Timmiella* De Not., Comment. Soc. Crittog. Ital. 2: 100. 1865.]

Included genera: *Timmiella* (De Not.) Limpr. and *Luisierella* Thér. & P.de la Varde

Diagnosis: Plants acrocarpous; leaves incurved and tubulose when dry, spreading when moist, leaf cell surfaces adaxially bulging and abaxially flat; peristomes straight to sinistrorse or absent, operculum cells sinistrorsely arranged.

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Appendix 1. Alphabetical list of taxa investigated for *rps4* and *rbcL* gene sequences with accession numbers. Newly obtained sequences (in bold) are shown with voucher information (in order of scientific name, accession number, locality, and specimen number with abbreviation).

Amphidium californicum (Hampe ex Müll. Hal.) Broth., AF226762/AF226812; *A. lapponicum* (Hedw.) Schimp., AF222896/AF005543; *Arctoa fulvella* (Dicks.) Bruch & Schimp., AF231266/AF231293; *Aulacopilum hodgkinsoniae* (Hampe & Müll. Hal.) Broth., AF222897/AF005545; *Barbula unguiculata* Hedw., AF480952/AB670696; *Bartramia halleriana* Hedw., AF265358/AF231090; *Blindia acuta* (Hedw.) Bruch & Schimp., AF023781/AF226817; *Brothera leana* (Sull.) Müll. Hal., AY908129/AB122033; *Bryoxiphium norvegicum* (Brid.) Mitt., AF231267/AF231294; *Buxbaumia aphylla* Hedw., AF306959/AF231062; *Calymperes afzelii* Sw., AF226744/AF226788; *Campylopus umbellatus* (Schwägr. & Gaudich. ex Arn.) Paris, AF226764/AF226814; ***Catoscopium nigratum* (Hedw.) Brid., AB914711/AB914712, Austria, Styria, Hochschwab Mts., J. Kučera s.n., CBFS 15674 in HIRO**; *Ceratodon purpureus* (Hedw.) Brid., AY908122/DQ463103; ***Chrysoblastella chilensis* (Mont.) Reimers, AB914713/AB914714, Australia, Tasmania, Mt. Wellington, R. D. Seppelt 26697 in HIRO**; *Cynodontium jenneri* (Schimp.) Stirt., AF231271/AF231318; *Dicnemon semicryptum* Müll. Hal., AF478274/AF478228; *Dicranella heteromalla* (Hedw.) Schimp., AF231272/AF231296; *Dicranodontium denudatum* (Brid.) E. Britton, AF231273/AF231317; *Dicranoweisia cirrata* (Hedw.) S.O. Lindberg, AF478279/AF478227; *Dicranum scoparium* Hedw., AF231277/AF231300; *Diphyscium fulvifolium* Mitt., AF478266/AF478222; *Discelium nudum* (Dicks.) Brid., AF223063/EU095320; ***Distichium capillaceum* (Hedw.) Bruch & Schimp., AB853082/AB853072, Japan, Ngano-ken, Mt. Shiomi, Y. Inoue 1236 in HIRO**; ***D. inclinatum* (Hedw.) Bruch & Schimp., AB914715/AB914716, Czech Republic, NE Bohemia, Krkonoše Mts., J. Kučera s.n., CBFS 8013 in HIRO**; ***Ditrichum flexicaule* (Schwägr.) Hampe, AB914717/AB914718, Austria, Styria, Totes Gebirge Mts., J. Kučera s.n., CBFS 12464 in HIRO**; *D. pallidum* (Hedw.) Hampe, AF231279/AF231302; *Dozya japonica* Sande Lac., AY908262/AB125593; *Drummondia obtusifolia* Müll. Hal., AF223038/AF232697; *D. prorepens* (Hedw.) E. Britton, AF306977/AF005542; ***D. sinensis* Müll. Hal., AB853081/AB853071, Japan, Hiroshima-ken, Miyajima Isl., H. Tsubota 7707 in HIRO**; *Encalypta streptocarpa* Hedw., AF478282/AF478239; *Ephemerum spinulosum* Bruch & Schimp., AF223055/AB194719; *Eucampodon muelleri* Hampe & Müll. Hal., AF231280/AF231319; *Eustichia longirostris* (Brid.) Brid., AY908091/GQ497665; *Fissidens dubius* P. Beauv., AF231281/AF231303; *F. mooreae* H. Whittier & H.A. Mill., AF226760/AF226810; *Funaria hygrometrica* Hedw., AJ845203/AF005513; *Gigaspermum repens* (Hook.) Lindb., JN088984/AF231064; *Glyphomitrium humillimum* (Mitt.) Cardot, EU246851/AB125585; *Grimmia pulvinata* (Hedw.) Sm., AF222900/AF231305; *Hedwigia ciliata* (Hedw.) P. Beauv., AF478289/AF478234; *Holomitrium vaginatum* (Hook.) Brid., AF226761/AF226811; *Hookeria acutifolia* Hook. & Grev., AF143071/AF158170; ***Hymenoloma brevipes* (Müll. Hal.) Ochyra, AB914719/AB914720, Chile, Prov. de Tierra del Fuego, J. Larrain s.n., CBFS 14901 in HIRO**; ***Hyophila propagurifela* Broth., AB853084/AB853074, Japan, Hiroshima-ken, Kami-kamagari-jima Isl., Y. Inoue 1745 in HIRO**; *Hypnodendron vitiense* Mitt., AY524471/AY524443; ***Hypnodontopsis apiculatus* Z. Iwats. & Nog., AB853083/AB853073, Japan, Aichi-ken, Yanai Shrine, Y. Inoue 1815 in HIRO**; *Hypodontium dregei* (Hornsch.) Müll. Hal., AF226755/AF226804; *H. pomiforme* (Hook.) Müll. Hal., AJ554020/AF226803; *Kiaeria blyttii* (Bruch & Schimp.) Broth., AF231283/AF231306; *Leucobryum sanctum* (Nees ex Schwägr.) Hampe, AF226769/AF226826; *Leucoloma serrulatum* Brid., AF231286/AF231309; *Leucophanes albescens* Müll. Hal., AF226751/AF226798; ***Luisierella barbula* (Schwägr.) Steere, AB853085/AB853077, Japan, Hiroshima-ken, Taishaku-kyo Gorge, S. Ideshita 2139 in HIRO**; ***L. barbula* (Schwägr.) Steere, AB853086/AB853076, Japan, Shizuoka-ken, Mt. Okami-yama, Y. Inoue 1834 in HIRO**; *Mitthyridium obtusifolium* (Lindb.) H. Rob., AF226733/AF226777; *Octoblepharum albidum* Hedw., AF226747/AF226794; *Oncophorus wahlenbergii* Brid., AF231287/AF231310; *Orthotrichum obtusifolium* Schrad. ex Brid., AF306969/AF005537; ***Pachyneuroopsis miyagii* T. Yamag., AB759969/AB853078, Japan, Okinawa-ken, Mt. Boujimui, T. Yamaguchi 34243 in HIRO**; *Paraleucobryum longifolium* (Ehrh. ex Hedw.) Loeske, AF226772/AF226829; *Parisia laevipila* (Cardot & Thér.) Tixier, HM236404/HM236405; *Physcomitrella patens* (Hedw.) Bruch & Schimp. subsp. *patens*, AP005672/AP005672; *Pleuridium acuminatum* Lindb., AF231289/AF231312; *Pseudosymblepharis schimperiana* (Paris) H.A. Crum, AF226756/AF226805; *Ptychomitrium gardneri* Lesq., AF231290/AF231313; *Ptychomnion aciculare* (Brid.) Mitt., DQ186845/DQ196094; *Ptychostomum capillare* (Hedw.) Holyoak & N. Pede, AF521682/AY163027; *Rhizogonium distichum* (Sw.) Brid., AY524461/AY524433; *Schistomitrium robustum* Dozy & Molk., AF226768/AF226825; *Schistostega pennata* (Hedw.) F. Weber & D. Mohr, AY631171/AY631206; ***Scopelophila cataractae* (Mitt.) Broth., AB853087/AB853075, Japan, Kochi-ken, Mt. Yokogugra-yama, Y. Inoue 318 in HIRO**; *Scouleria aquatica* Hook., AF023780/AF226822; *Stegonia latifolia* (Schwägr.) Venturi ex Broth., AF222901/AF231314; *Streptopogon calymperes* Müll. Hal., AF478285/AF478231; *Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr, FJ546412/FJ546412; *Syrrhopodon fimbriatulus* Müll. Hal.,

AF226742/AF226786; *Timmia megapolitana* Hedw., AY908619/AY312938; *Timmiella anomala* (Bruch & Schimp.) Limpr., AB853088/AB853079, Japan, Hiroshima-ken, Miyajima Isl., *Y. Inoue 111* in HIRO; *T. anomala* (Bruch & Schimp.) Limpr., AB914721/AB914722, Spain, Andalucia, Prov. de Sierra Nevada, *J. Kučera s.n.*, CBFS 5479 in HIRO; *T. barbulooides* (Brid.) Mönk., AB914723/AB914724, Spain, Andalucia, Prov. de Málaga, *J.-C. Vadam s.n.*, CBFS 10739 in HIRO; *T. crassinervis* (Hampe) L.F.Koch, AF478275/AF478236; *Tortella tortuosa* (Hedw.) Limpr., AB853089/AB853080, Japan, Ngano-ken, Mt. Shiomi, *Y. Inoue 1297* in HIRO; *Tortula caucasica* S.O.Lindberg, AB759970/AB670694; *Venturiella sinensis* (Venturi) Müll.Hal., AY908117/AB125591.

Appendix 2. Newly designed primer sequences used for PCR amplification and sequencing of the *rps4* and *rbcL* genes.

Primers	Sequence (5'-3')	Target region	Note
Forward			
¹ rps4_19Fi	CCTCGTGTA GAATAATACG TC	<i>rps4</i>	Sequencing
² rbcL-53h	TCGAGTAGAC CTTATCCTTG C	<i>rbcL</i>	PCR
Reverse			
¹ rps4_578Ri	CGAGAATAAT ATTCTACAAC TA	<i>rps4</i>	Sequencing
³ rps4_602Fn	TGACGAGAAT AATATTCTAC AACTA	<i>rps4</i>	Sequencing

1: primers designed by A. Sadamitsu & Y. Inoue. 2: primer designed by H. Tsubota. 3: the PCR primer “rps4_602F” of Table 2 in Inoue *et al.* (2012) was published with the sequences based on mistranscription of the sequence data, which should be rectified here. In avoiding future confusion we gave a new name to the sequence (rps4_602Fn).

Appendix 3. Alphabetical list of taxa used for morphological observations. The list includes the name of taxon, locality and specimen number with abbreviation.

Distichium capillaceum (Hedw.) Bruch & Schimp., Japan, Nagano-ken, Mt. Shiomi, *Y. Inoue 1236* in HIRO; *Luisierella barbula* (Schwägr.) Steere, America, Florida, Wakulla, *R. L. Redfearn Jr. 73-55* in HIRO; ditto, *R. A. Pursell 632* in HIRO; ditto, Japan, Shizuoka-ken, Mt. Okami-yama, *Y. Inoue 1834* in HIRO; *Timmiella acaulon* (Müll.Hal.) R.H.Zander, Argentina, Prov. Córdoba, Villa Allende, *C. C. Hosseus 396* in HIRO; *T. anomala* (Bruch & Schimp.) Limpr., Japan, Hiroshima-ken, Kurahashi-jima Isl., *Y. Inoue 1910* in HIRO; *T. barbulooides* (Brid.) Mönk., Greece, Peloponnese, Mistra, ex herb. *C. C. Townsend s.n.* in HIRO; *T. crassinervis* (Hampe) L.F.Koch, America, Georgia, Saturna Isl., *W. B. Schofield 14404* in HIRO; *T. diminuta* (Müll.Hal.) P.C.Chen, China, Peking, Hai-daian, *C. Y. Chang s.n.* in TNS 037219.