

The phylogenetic position of *Vandiemenia ratkowskiana* (Metzgeriales, Marchantiophyta)

HIROAKI MASUZAKI, TATSUWO FURUKI, PATRICK J. DALTON, HIROMI TSUBOTA, RODNEY D. SEPPELT AND HIRONORI DEGUCHI

MASUZAKI, H., FURUKI, T., DALTON, P. J., TSUBOTA, H., SEPPELT, R. D. & DEGUCHI, H. 2011. The phylogenetic position of *Vandiemenia ratkowskiana* (Metzgeriales, Marchantiophyta). *Hikobia* 16: 51–57.

A molecular phylogenetic analysis was carried out on a range of simple thalloid liverworts, particularly focusing on the genus *Vandiemenia*. Two major clades were indicated: the Metzgeriales clade comprising Metzgeriaceae, Vandiemeniaceae, and Aneuraceae and a clade comprising the other orders of sampled thalloid liverworts: Pelliales, Fossombroniales, and Pallaviciniales. In the Metzgerialean clade, the Aneuraceae appeared at the most basal position, and *Vandiemenia* was sister to *Metzgeria* with high supporting values. The results support the taxonomic conclusion that *Vandiemenia* should be placed in the Metzgeriaceae.

Hiroaki Masuzaki, Department of Biofunctional Science and Technology Graduate School of Biosphere Science, 1–4–4, Kagamiyama, Higashi-hiroshima-shi, Hiroshima-ken 739–8528, Japan.

Tatsuwo Furuki, Natural History Museum and Institute, 955–2 Aoba-cho, Chuo-ku, Chiba-shi 260–8682, Japan.

Patrick J. Dalton, School of Plant Science, University of Tasmania, Private Bag 55, Hobart, Tasmania 7001, Australia.

Hiromi Tsubota, Miyajima Natural Botanical Garden, Graduate School of Science, Hiroshima University, Mitsumaruko-yama 1156–2, Miyajima-cho, Hatsukaichi-shi, Hiroshima-ken 739–0543, Japan.

Dodney D. Seppelt, Australian Antarctic Division, Department of Science and Technology, Channel Highway, Kingston Tasmania 7050, Australia.

Hironori Deguchi, Department of Biological Science, Graduate School of Science, Hiroshima University, 1–3–1 Kagamiyama, Higashi-hiroshima-shi, Hiroshima-ken 739–8526, Japan.

Introduction

Vandiemenia ratkowskiana Hewson was described on the basis of a single specimen from Tasmania (Hewson 1982). It is presently registered as a threatened species in the world red list of bryophytes by the IUCN (International Union for the Conservation of Nature and Natural Resources, <http://www.redlist.org>). *Vandiemenia* grows on decaying logs and has a similar habit to those of the genus *Riccardia* Gray (Aneuraceae). The thallus structure resembles that of *Riccardia*, but the male branches show characteristics

of Metzgeriaceae. According to the descriptions (Hewson 1982; Furuki & Dalton 2008), the species is characterized by (1) thalli prostrate, pale green to green, to 15 mm long, 0.5–2.0 mm wide, flat to incurved at margin, with undefined costal region; (2) thallus convex–concave in cross section, to 300 µm thick and 4–7 cells high, the cells thin walled, lacking trigones; (3) rhizoids unicellular, mostly sparse on ventral surface of costal region, rarely on wings and marginal cells; (3) oil bodies absent; (4) dioicous, sexual branches reduced, ventral endogenous, arising from ventral inner cells of thallus, arranged in multiple rows

from central part of thallus; (5) capsules subspherical, irregularly divided into 4–valves, rarely 3–valves, with elaterophores at apex of each valve; (6) valves bistratose; (7) cells of outer layer with nodular thickenings on adaxial radial walls, very rarely on abaxial radial wall adjacent to median wall; and (8) cells of inner layer with nodular thickenings on adaxial and abaxial radial walls extending to outer and inner tangential walls.

Hewson (1982) considered *Vandiemenia* to have ancestral features suggestive of a relationship to the families Aneuraceae and Metzgeriaceae, and established the new monotypic family Vandiemeniaceae in the Metzgeriales to accommodate the genus. Schuster (1992) reduced Vandiemeniaceae to the rank of subfamily within Aneuraceae, recognizing only three families: Aneuraceae, Metzgeriaceae, and Mizutaniaceae. He noted that the multistratose thallus of *Vandiemenia* shows a close resemblance to that of *Riccardia*. In 2004, fresh specimens with female plants bearing sporophytes were discovered in southern Tasmania. Furuki and Dalton (2008) described for the first time, the rhizoids, female branches, calyptrae and sporophytes. They placed *Vandiemenia* in the Metzgeriaceae on the basis of morphological similarities.

A large number of species have been described within the Metzgeriales based on the diversity of the gametophyte, while important characters of the sporophyte and molecular data are limited. For example, *Mizutania* Furuki & Z.Iwats. is an enigmatic species and was considered to be a simple thalloid liverwort (Furuki & Iwatsuki 1989). However, *Mizutania* has unistratose thalloid features and the sexual organs and branches resemble those of leafy liverworts. Masuzaki *et al.* (2010a) reduced Mizutaniaceae to synonymy of the Calypogeiaceae Arnell of the Jungermanniales H.Klinggr., based on the phylogenetic relationship and morphological affinities between *Mizutania* and the other genera of Calypogeiaceae. There remain several enigmatic taxa necessary for a complete molecular phylogenetic analysis of the order Metzgeriales.

In the present study, a phylogenetic analysis was undertaken to resolve the phylogenetic position of *Vandiemenia* within the Metzgeriales and to confirm the determination from previous morphological studies.

Materials and methods

DNA extraction, PCR amplification and sequencing

Extraction protocols follow Tsubota *et al.* (2004, 2005). The primers for PCR and DNA sequencing that were used in the present study are shown in Table 1. Three exemplars with full length were newly obtained. The sequences obtained in the present study have been submitted to the DNA database (DDBJ/EMBL/GenBank International Nucleotide Sequence Database Collaboration).

Taxon sampling

The phylogenetic tree was constructed based on the ribulose biphosphate carboxylase large subunit (*rbcL*, 1428 bps). The data set comprised 21 exemplars including *Vandiemenia ratkowskiana* Hewson (Dalton *et al.* 19704). The three newly obtained exemplars: *Vandiemenia ratkowskiana*, *Lobatiriccardia yakusimensis* (S.Hatt.) Furuki, and *Riccardia marginata* (Colenso) Pearson var. *pacifica* Furuki and 15 exemplars downloaded from the DNA database were used for the analysis. The *rbcL* sequences for *Cavicularia densa* Steph. (AB476559), *Conocephalum* sp. (AB476563), and *Marchantia polymorpha* L. (X04465) were downloaded from the DNA database and added to the data set as outgroups. The *rbcL* gene sequences were aligned manually.

Voucher specimen of *Vandiemenia ratkowskiana* is deposited in the herbarium of the Tasmanian Museum and Art Gallery (HO) and duplicates in herbaria of the Natural History Museum and Institute, Chiba (CBM), Hiroshima University, Hiroshima, Japan (HIRO), and the Field museum, Chicago, USA (F), that of *Lobatiriccardia yakusimensis* is in HIRO, and that of *Riccardia marginata* var. *pacifica* is in CBM and duplicates in HIRO (See Appendix). Liverwort taxonomy and nomenclature followed that of Crandall-Stotler *et al.* (2008).

Phylogenetic analysis

Phylogenetic analysis using the *rbcL* gene sequences was performed based on maximum likelihood criteria (Cavalli-Sforza & Edwards 1967; Felsenstein 1981) as previously described (Tsubota *et al.* 2004; Ozeki *et al.* 2007; Masuzaki *et al.*

Table 1. The designed primer sequences used in the present study for PCR amplification and sequencing of the *rbcL* (5' to 3') gene.

<i>rbcL</i> gene (5' to 3')	Sequences	Reference
Forward primers		
rbcL-26Fh	TATTAATTGA TTTGTAGGGA GG	in this study*
HrL1	ATGTCACCAC AAACGGAGAC TAAAGCAGG	Masuzaki <i>et al.</i> (2010a)
rbcL39Fmas	AAAGCTGGTG TTAAAGATTA	Masuzaki <i>et al.</i> (2010a)
rbcL111Fmas	GCAGCATTTT GTATGAC	Masuzaki <i>et al.</i> (2010a)
rbcL256	GCTATGATCT TGAAGCAGTT CCTGGAGAAG	Tsubota <i>et al.</i> (2000)
rbcL333mas	AATCAATATA TTGCTTATGT	in this study
rbcL634Fmas	ATGCGTTGGA GAGA	Masuzaki <i>et al.</i> (2010a)
rbcL920Fmas	CATGGTATGC ATTTCCGTGT	Masuzaki <i>et al.</i> (2010a)
rbcL1117	GGTATTCATG TTTGGC	Tsubota <i>et al.</i> (1999)
Reverse primers		
rbcL128Rmas	GTCATACGAA ATGCTGC	Masuzaki <i>et al.</i> (2010a)
rbcL302Rmas	ACATAAGCAA TATATTGATT	Masuzaki <i>et al.</i> (2010a)
rbcL600R	CGTGGTGGAC TTGATTTTAC	Tsubota <i>et al.</i> (1999)
rbcL650Rmas	CGATCTCTCC AACGCA	Masuzaki <i>et al.</i> (2010a)
HrL911R	CGTGCAATGC ATGCAGTTAT TGATAGACA	in this study
trnRn	GGGTAGAAG GGATTCTGAAC CCTTGAC	Manhart (1994), Tsubota <i>et al.</i> (1999)

*The PCR primer “rbcL-26Fmas” of Table 1 in Masuzaki *et al.* (2010a) was published with the sequences based on mistranscription of the sequences data, which should be rectified as “5'-TATTAATTGA TTTGTAGGGA GG-3'”. In avoiding future confusion we gave a new name to the sequences (rbcL-26Fh: 5'-TATTAATTGA TTTGTAGGGA GG-3').

2010b), with some differences as follows:

Prior to the phylogenetic reconstruction, Kaksan4 (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 AU test in the final stage of the analysis scheme. Phylogenetic trees were constructed using the following seven 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 model; (2) PhyML ver. 3.0 (Guindon & Gascuel 2003) with ML method using GTR + gamma model; (3) nucml in MOLPHY ver. 2.3b3 package (Adachi & Hasegawa 1996) with ML method using TN model; (4) baseml in PAML ver. 4.4d package (Yang 2007) with ML method using GTR + gamma model; (5) 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 Parsi-

Ratchet search strategy with random weighting of each character in 50 iteration runs; (6) MEGA5 (Tamura *et al.* 2011) with NJ and ML methods using GTR + gamma model and MP method; and (7) MrBayes ver. 3.1.2 (Ronquist & Huelsenbeck 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 the approximate unbiased test using the multi-scale bootstrap technique (AU: Shimodaira & Hasegawa 2001; Shimodaira 2002, 2004) and Bayesian posterior probability (PP) calculated by the BIC approximation (Schwarz 1978; Hasegawa & Kishino 1989) using CONSEL 0.20 (Shimodaira & Hasegawa 2001). ML topologies that passed the AU test was also reconstructed to calculate branch lengths by baseml. Supporting values more than 50% were overlaid to assess the robustness of each branch of the condensed topology:

the p -values based on the AU test (AU) by CONSEL, the bootstrap probabilities with the scaled bootstrap replicates (NP) by CONSEL, the standard bootstrap probabilities (BP: Efron 1979; Felsenstein 1985) by MEGA5, the local bootstrap probabilities (LBP) by nucml, and Bayesian posterior probabilities (PP) calculated by the Bayesian information criterion (BIC) approximation (Schwarz 1978, Hasegawa & Kishino 1989) by CONEL are shown on or near each branch (AU/NP/BP/LBP/PP; in%).

Results

A total of 1,461 distinct topologies were obtained from the ML, MP, NJ and BI analyses, and

1,277 topologies passed the AU test. The ML tree with supporting values (AU/NP/BP/LBP/PP) is shown in Fig. 1. The best fit model for the *rbcL* sequence is GTR + gamma.

Two major clades were recognized, corresponding to a Metzgeriales clade, comprising Metzgeriaceae, Vandiemeniaceae, and Aneuraceae, and a clade including the other orders of sample thalloid liverworts: Pelliales, Fossombroniales, and Pallaviciniales (AU/NP/BP/LBP/PP = 64/91/94/99/1.00, 77/58/65/95/0.60), respectively. In the former clade, the Aneuraceae appeared at the most basal position, and *Vandiemenia* was sister to *Metzgeria* with high supporting values (52/92/99/99/1.00). The relationships within the Fossombroniales, Pallaviciniales, Pelliales, and

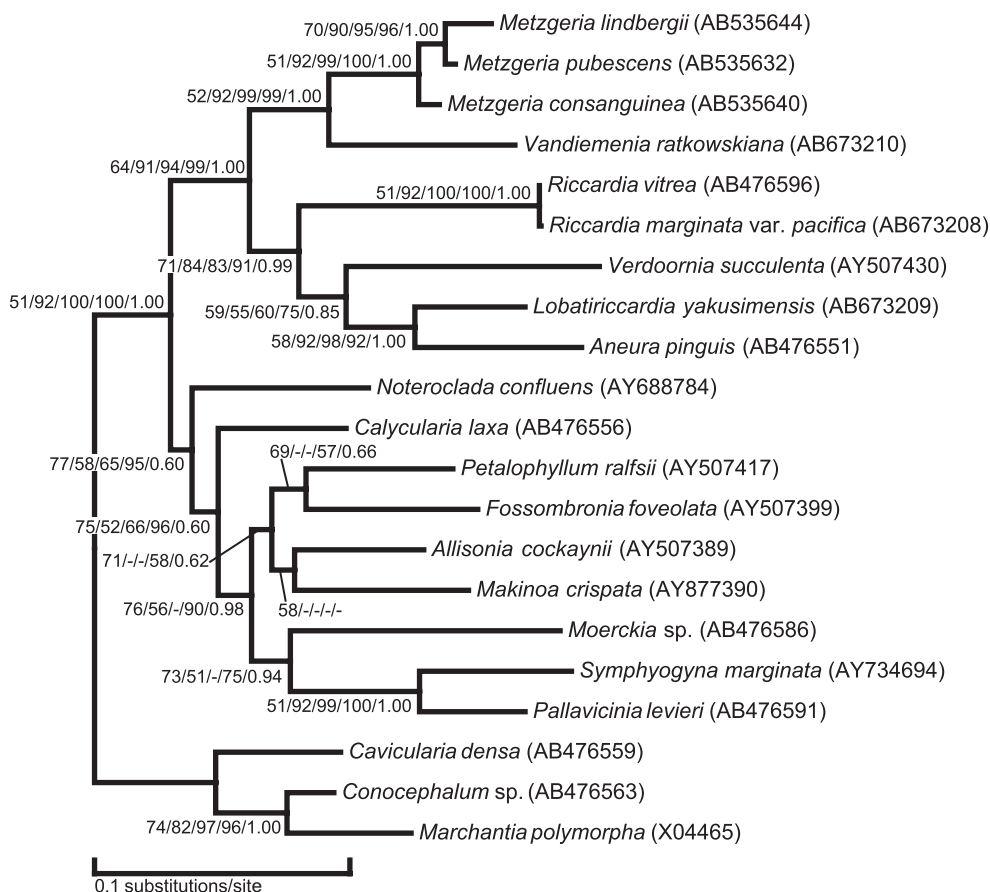


Fig. 1. Maximum Likelihood tree based on analysis of the *rbcL* sequence. Supporting values more than 50% were overlaid: values obtained by the AU test (AU), bootstrap probabilities (NP), standard bootstrap probabilities (BP), local bootstrap probabilities (LBP) and Bayesian posterior probabilities (PP) are shown on or near each branch (AU/NP/BP/LBP/PP; in%). The root is arbitrarily placed on the branch leading to the clade which includes members of the Marchantiales.

Aneuraceae essentially agreed with Crandall-Stotler *et al.* (2005), Forrest and Crandall-Stotler (2005), Forrest *et al.* (2006), and Masuzaki *et al.* (2010a).

Discussion

On the basis of the revised description of *Vandiemenia* (Furuki & Dalton 2008), it shares with the Metzgeriales several key characters: (1) sexual branches reduced; (2) capsule with elaterophores at the apex of each valve; (3) capsule walls 2-stratose; and (4) cells of the capsule elongate and linear to oblong. *Mizutania* has reduced sexual branches which resemble those of the Metzgeriales, although no sporophytes of *Mizutania* have been discovered. *Mizutania* belongs to the Calypogeiaceae of the Jungermanniales (Masuzaki *et al.* 2010a). The sharing of these important criteria amongst the three taxa and the derived phylogenetic tree (Fig. 1) support the inclusion of *Vandiemenia* in the Metzgeriales. In addition, *Vandiemenia* shares with the other genera of Metzgeriaceae several morphological characters: (1) thalli with terminal-lateral or ventral endogenous branching, (2) rhizoid-like hairs on the successive junctions or corners of epidermal cells of the thallus, (3) the absence of oil bodies, (4) sexual branches arising endogenously from ventral inner cells of the costal region, (5) antheridia not enclosed within the thallus chamber, and (6) sexual organs located on the dorsal surface of sexual branches. *Vandiemenia* differs from the Metzgeriaceae by: (1) mucilage papillae are two-celled and arise from ventral epidermal cells, and (2) the thickening patterns on the layers of the capsule wall. Two-celled mucilage papillae are exceptionally found on stalk cells in the Aneuraceae (Furuki 1991), and are unknown in the Metzgeriaceae. Although the thickening patterns of the capsule wall layers are an important taxonomic criterion in Metzgeriaceae, the character corresponds to differences at the subgeneric level (Masuzaki *et al.* 2010b). Furuki and Dalton (2008) placed *Vandiemenia* in the Metzgeriaceae. The phylogenetic relationships would be supported that their taxonomic conclusion was appropriate.

The result (Fig. 1) suggests that *Vandiemenia* is a primitive form of Metzgeriaceae. The

highly diverse features seen in the genera *Metzgeria*, *Steereella*, *Austrometzgeria*, and subgen. *Apometzgeria* may be evolutionarily derived as consequences of environmental adaptation. The evolution of the Metzgeriaceae from an ancestral form having a rhizomatous system, as proposed by Kuwahara (1966), seems unlikely, but needs further evaluation.

Acknowledgments

This work was supported by research grants from the Ministry of Education, Science, Sports and Culture (MESSC) to H. Deguchi (No. 19570088 and 23370045), T. Furuki (No. 13640712), and H. Tsubota (23770089).

Literature cited

- Adachi, J. & Hasegawa, M. 1996. MOLPHY version 2.3: Programs for molecular phylogenetics based on maximum likelihood. Computer science monographs 28: 1–150. Institute of Statistical Mathematics, Tokyo.
- Crandall-Stotler, B. J., Forrest, L. L. & Stotler, R. E. 2005. Evolutionary trends in the simple thalloid liverworts (Marchantiophyta, Jungermanniopsida subclass Metzgeriidae). *Taxon* 54: 299–316.
- , Stotler, R. E. & Long, D. G. 2008. Morphology and classification of the Marchantiophyta. In Shaw, A. J. & Goffinet, B. (eds.), *Bryophyte Biology*. Second edition. Pp. 1–54. Cambridge Univ. Press, Cambridge.
- Cavalli-Sforza, L. L. & Edwards, A. W. F. 1967. Phylogenetic analysis: models and estimation procedures. *Am. J. Hum. Genet.* 19: 233–257.
- Efron, B. 1979. Bootstrap methods: another look at the jackknife. *Ann. Stat.* 7: 1–26.
- Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* 17: 368–376.
- . 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.
- Fitch, W. M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* 20: 406–416.
- Forrest, L. L. & Crandall-Stotler, B. J. 2005. Progress towards a robust phylogeny for the liverworts, with particular focus on the simple thalloids. *J. Hattori Bot. Lab.* 97: 127–159.
- , Davis, E. C., Long, D. G., Crandall-Stotler, B. J., Clark, A. & Hollingsworth, H. 2006. Unraveling the

- evolutionary history of the liverworts (Marchantiophyta): multiple taxa, genomes and analyses. *Bryologist*. 109: 303–334.
- Furuki, T. 1991. A taxonomical revision of the Aneuraceae (Hepaticae) of Japan. *J. Hattori Bot. Lab.* 70: 293–397.
- & Dalton, P. J. 2008. *Vandiemenia ratkowski-ana* Hewson (Marchantiophyta): A revised description and reassessment of its taxonomic status. *J. Bryol.* 30: 48–54.
- & Iwatsuki, Z. 1989. *Mizutania riccardioides*, Gen. et sp. nov. (Mizutaniaceae, fam. nov.), a unique liverwort from tropical Asia. *J. Hattori Bot. Lab.* 67: 291–296.
- Guindon, S. & Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52: 696–704.
- Hasegawa, M. & Kishino, H. 1989. Confidence limits on the maximum likelihood estimate of the hominoid tree from mitochondrial DNA sequences. *Evolution* 43: 672–677.
- Hewson, H. J. 1982. Vandiemeniaceae: A new family in the Metzgeriales. *J. Hattori Bot. Lab.* 52: 163–165.
- Kuwahara, Y. 1966. The family Metzgeriaceae in North and South East Asia, Pacific Oceania, Australia and New Zealand. *Rev. Bryol. Lichénol.* 34: 191–239.
- Masuzaki, H., Shimamura, M., Furuki, T., Tsubota, H., Yamaguchi, T., Haji, M. A. M. & Deguchi, H. 2010a. Systematic position of an enigmatic liverwort *Mizutania* (Mizutaniaceae, Bryophyta) inferred from molecular phylogenetic analyses. *Taxon* 59: 448–458.
- , Tsubota, H., Shimamura, M., Yamaguchi, T. & Deguchi, H. 2010b. A taxonomic revision of the genus *Apometzgeria* (Metzgeriaceae, Marchantiophyta). *Hikobia* 15: 427–452.
- Manhart, J. R. 1994. Phylogenetic analysis of green plant *rbcl* sequences. *Mol. Phylogenet. Evol.* 3: 114–127.
- Nixon, K. C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407–414.
- Ozeki, M., Isagi, Y., Tsubota, H., Jacklyn, P. & Bowman, D. M. J. S. 2007. Phylogeography of an Australian termite, *Amitermes laurensis* (Isoptera, Termitidae), with special reference to the variety of mound shapes. *Mol. Phyl. Evol.* 42: 236–247.
- Ronquist, F. & Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinform.* 19: 1572–1574.
- Schuster, R. M. 1992. The Hepaticae and Anthocerotae of North America, East of the Hundredth Meridian, Vol. V. Filed Mus. Nat. Hist., Chicago.
- Schwartz, G. 1978. Estimating the dimension of a model. *Ann. Stat.* 6: 461–464.
- Shimodaira, H. 2002. An approximately unbiased test of phylogenetic tree selection. *Syst. Biol.* 51: 492–508.
- . 2004. Approximately unbiased tests of regions using multistep-multiscale bootstrap resampling. *Ann. Stat.* 32: 2616–2641.
- & Hasegawa, M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 17: 1246–1247.
- Sikes, D. S. & Lewis, P. O. 2001. Software manual for PAUPRat: a tool to implement Parsimony Ratchet searches using PAUP*. 12 pp. University of Connecticut, Storrs.
- Stamatakis, A. 2006. RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinform.* 22: 2688–2690.
- Sugiura, N. 1978. Further analysis of the data by Akaike's information criterion and the finite corrections. *Commun. Stat. Theory Meth.* 7: 13–26.
- Swofford, D. L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0 beta version. 142 + ii pp. Sinauer Associates, Massachusetts.
- Tanabe, A. S. 2011. Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Mol. Ecol. Res.* 11: 914–921.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28: 2731–2739.
- Tsubota, H., De, Luna E., Gonzalez, D., Ignatov, M. S. & Deguchi, H. 2004. Molecular phylogenetic and ordinal relationships based on analyses of a large-scale data set of 600 *rbcl* sequences of mosses. *Hikobia* 14: 149–169.
- , Nakao, N., Arikawa, T., Yamaguchi, T., Higuchi, M., Deguchi, H. & Seki, T. 1999. A preliminary phylogeny of Hypnales (Musci) as inferred from chloroplast *rbcl* sequence data. *Bryol. Res.* 7: 233–248.
- , ———, Yamaguchi, T., Seki, T. & Deguchi, H. 2000. Preliminary phylogenetic relationships of the genus *Brotharella* and its allied genera (Hypnales, Musci) based on chloroplast *rbcl* sequence data. *J. Hattori Bot. Lab.* 88: 79–99.
- , Takahashi, K., Nakahara, M., Mohamed, H. & Deguchi, H. 2005. A simple procedure for DNA isolation using small quantities of lichen thallus. *Lichenology* 4: 25–28.

Yang, Z. 2007. PAML 4: phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* 24: 1586–1591.

Accepted 10. XI. 2011

Appendix. Information on specimens from which DNA extracted in this study.

Here is a list of taxa whose *rbcL* sequences were utilized in this study with their accession numbers, showing their sources and voucher specimen information. The sequence of information is the name of taxon, accession number, locality and specimen number.

Lobatiriccardia yakusimensis (Schiffner) Furuki, AB673209, Japan, Kyushu, Yakushima Isl., Kagoshima-ken, Kumage-gun, Yaku-cho, Mt. Mocchomu, 3 Aug. 2005, T. Yamaguchi in hb. H. Masuzaki No. 2200 (HIRO); *Riccardia marginata* (Colenso) Pearson var. *pacifica* Furuki, AB673208, Japan, Fukui Pref., Tsuruga-shi, 28 Aug. 2005, T. Furuki 20339 (CBM & HIRO); *Vandiemena ratkowskiana* Hewson, AB673210, Australia, Tasmania Is., 2004, Dalton et al. 19704 (HO, CBM, HIRO & F).

榊崎浩亮・古木達郎・Patrick J. Dalton・坪田博美・Rodney D. Seppelt・出口博則： *Vandiemena ratkowskiana* の系統学的位置づけ

Vandiemena ratkowskiana は、タスマニア島から記載された単純な葉状体体制をもつ苔類である。 *Vandiemena* の葉状体体制や生育環境は、スジゴケ科 Aneuraceae (特にスジゴケ属 *Riccardia*) のものと酷似するが、生殖器官や胞子体などの特徴は、フタマタゴケ科 Metzgeriaceae のものと類似している。 *Vandiemena* を記載した Hewson (1982) は、 *Vandiemena* を Aneuraceae や Metzgeriaceae とは異なる科 Vandiemeniaceae に置いた。しかし、植物体と雄枝の形態情報しか記載されていなかったため、 *Vandiemena* を Aneuraceae の亜科とする見解もあった (Schuster 1992)。 Furuki and Dalton (2008) は、新たに *Vandiemena* の生殖器官、胞子体などの形態情報を記載し、 *Vandiemena* を Metzgeriaceae に置いた。本研究では、これらの分類学的取り扱いについて検討を行うために、分子系統学的解析を行った。その結果、 *Vandiemena* は Metzgeriaceae に属することが示唆された。