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

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


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, Vandiemiaceae, 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; (4) 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 sub-spherical, 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 Calypogeaceae. 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 bisphosphate 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. *pacific* 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. *pacific* 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. 2010a).
Prior to the phylogenetic reconstruction, Kaku-san4 (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 Parsimony 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 porobabilities (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 = 64/91/99/99/1.00, 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, Pallaviniciales, Pelliales, and...
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 Jungermaniales (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**


Schuster, R. M. 1992. The Hepaticae and Anthocerotae of

Masuzaki, H., Shimamura, M., Furuki, T., Tsubota, H.,

Ozeki, M., Isagi, Y., Tsubota, H., Jacklyn, P. & Bowman,

Kuwahara, Y. 1966. The family Metzgeriaceae in North


Guindon, S. & Gascuel, O. 2003. A simple, fast, and

Hasegawa, M. & Kishino, H. 1989. Con

Deguchi, H. 2010b. A taxonomic revision of the ge-

Hikobia 15: 427–452.


Ozeki, M., Isagi, Y., Tsubota, H., Jacklyn, P. & Bowman,

D. 2007. Phylogeography of an Australian ter-

mite, Amitermes laurensis (Isoptera, Termitidae), with

special reference to the variety of mound shapes. Mol.


Ronquist, F. & Huelsenbeck, J. P. 2003. MrBayes 3:

Bayesian phylogenetic inference under mixed models.


Schuster, R.M.1992. The Hepaticae and Anthocerotae of


Stamatakis, A. 2006. RAXML-VI-HPC: maximum like-


Tanabe, A. S. 2011. Kakusan4 and Aminosan: two pro-


Tsubota, H., De, Luna E., Gonzalez, D., Ignatov, M. S. & Deguchi, H. 2004. Molecular phylogenetic and or-


———, Nakao, N., Arikawa, T., Yamaguchi, T., Higuchi, M., Deguchi, H. & Seki, T. 1999. A preliminary phy-

logeny of Hypnales (Musci) as inferred from chloro-


———, ———, Yamaguchi, T., Seki, T. & Deguchi, H. 2000. Preliminary phylogenetic relationships of the genus Broth-


———, Takahashi, K., Nakahara, M., Mohamed, H. & Deguchi, H. 2005. A simple procedure for DNA iso-

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.


Vandiemenia 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。Vandiemenia 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）のもと似似するが、生殖器官や胞子体などの特徴は、フタタゴケ科 Metzgeriaceae のものと類似している。Vandiemenia を記載したHewson (1982) は、Vandiemenia を Aneuraceae や Metzgeriaceae とは異なる科 Vandiemiaceae に置いた。しかし、植物体と枝葉の形態情報しか記載されていなかったため、Vandiemenia を Aneuraceae の亜科とする見解もあった (Schuster 1992)。Furuki and Dalton (2008) は、新たな Vandiemenia の生殖器官、胞子体などの形態情報を記載し、Vandiemenia 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、Vandiemenia は Metzgeriaceae に属することが示唆された。