Hattoria 4: 107–118, 2013

Notes on *Aptychella* (Sematophyllaceae, Bryopsida): *Yakushimabryum longissimum*, syn. nov.

Tadashi Suzuki[1], Yuya Inoue[2], Hiromi Tsubota[2] and Zennoske Iwatsuki[3]

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Abstract. *Yakushimabryum longissimum* H.Akiyama, Y.Chang, T.Yamag. & B.C.Tan is proposed as a new synonym of *Aptychella tonkinensis* (Broth. & Paris) Broth. Morphological comparisons and phylogenetic analysis based on chloroplast *rbcL* gene sequences supported our taxonomic treatment.

Introduction

Yakushimabryum longissimum was described from Yakushima Island, Japan by Akiyama et al. (2011). The type specimens of *Y. longissimum* and *Gammiella touwii* B.C.Tan, the latter a synonym of *Gammiella tonkinensis* (Broth. & Paris) B.C.Tan (Tan & Jia 1999), were compared. No morphological differences between these two species were found. To evaluate the morphological conclusions we undertook a phylogenetic analysis based on sequences of the chloroplast ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene.

Materials and Methods

Source of plants studied

Fresh material of *Aptychella tonkinensis* was gathered from Miyazaki Pref., Japan (see Appendix A). For comparison, herbarium specimens of *Yakushimabryum longissimum*, *Gammiella touwii* and *Clastobryum glomerato-propaguliferum* were obtained from HIRO and NICH (see Specimens examined).

DNA extraction, PCR amplification and DNA sequencing

Total DNA was extracted using modified the method of Tsubota et al. (2009). Stems were placed under a stereo microscope and the green part of the shoots were dissected. The shoots were grinded with 30 μl extraction buffer (TE) using a polypropylene pestle in a 1.5

ml microcentrifuge tube. Ca. 200 μl of extraction buffer (TE) is added and mixed gently. Several segments of chloroplast *rbcL* gene were amplified by standard polymerase chain reaction (PCR) or nested PCR with Ex Taq polymerase PCR amplification kit (Takara Bio, Inc., Otsu). Reactions were performed using a DNA thermal cycler (Mastercycler gradient, Eppendorf Co., Ltd., Tokyo) with synthetic primers (see Tsubota et al. 1999; Masuzaki et al. 2010; Inoue et al. 2011). Condition of PCR was based on Tsubota et al. (1999, 2000). Direct DNA sequence analyses of the PCR products were performed by the dideoxy chain termination method using the ABI kits according to the manufacturer's instructions with the additional internal primers (see Tsubota et al. 1999, 2001a; Masuzaki et al. 2010). The sequences were electrophoresed on automated sequencer (ABI 3130 Genetic Analyzers, Life Technologies Japan, Tokyo).

Sequence alignment and phylogenetic analysis

The sequences were aligned using the program MAFFT var. 7.027 (Katoh & Standley 2013) with some manual adjustment on sequence editor of MEGA5 (Tamura et al. 2011). Phylogenetic analysis using the *rbcL* gene sequences was performed based on maximum likelihood (ML) criteria as previously described (Inoue et al. 2012) using PAUP 4.0b10 (Swofford 2003) instead of baseml in PAML 4 software package (Yang 2007).

Morphological investigation and taxonomy

As a result of the present detailed morphological and molecular phylogentic study, *Yakushimabryum longissimum* is considered a synonym of *Aptychella tonkinensis*. The genera *Aptychella* and *Gammiella* are doubtfully distinct and supporting evidence for this assertion will be the subject of a separate paper.

Aptychella tonkinensis (Broth. & Paris) Broth. in Nat. Pfl., ed 2, 11: 406 (1925). Figs. 1 & 2.

Basionym. *Clastobryum tonkinense* Broth. & Paris, Rev. Bryol. 35: 47 (1908).

Gammiella tonkinensis (Broth. & Paris) B.C.Tan, Bryologist 93(4): 433 (1990).

Gammiella touwii B.C.Tan, Bryologist 93(4): 432, f. 13–17 (1990).

Clastobryum glomerato-propaguliferum Toyama, Acta Phytotax. Geobot. 4: 214, f. 2 (1935).

Clastobryella glomerato-propagulifera (Toyama) Sakurai, Muscologia Japonica: 152 (1954).

Aptychella glomerato-propagulifera (Toyama) Seki, J. Sci. Hiroshima Univ., sér. B, div. 2, 12: 72 (1969).

Yakushimabryum longissimum H.Akiyama, Y.Chang, T.Yamag. & B.C.Tan, J. Bryol. 33 (1): 42–49 (2011), ***syn. nov.***

Plants densely branched. Stem and primary branch leaves similar, polymorphic, *1.0–1.5* mm long, 0.25–0.5 mm wide, ovate, gradually attenuate to an acuminate point, becoming lanceolate, acuminate on secondary branches; margins serrulate above; costa short and

double; lamina cells linear, 85–110 μm long, alar regions swollen or concave, consisting of groups of quadrate and short-rectangular, thick-walled, coloured cells. Asexual propagulae tangled, frequently with numerous flagellate branches, 10–20 mm long, with leaves shorter and narrower than vegetative leaves, gemmae few in number, scattered on branches or clustered on the apical part of branch, uniseriate, smooth or verrucose. Dioicous?; perigonial plants and sporophytes not found.

Additional descriptions: Toyama 1935, 214, f. 2 (as *Clastobryum glomerato-propaguliferum*); Noguchi 1994, 1079, f. 475, A (as *Clastobryella glomerato-propagulifera*); Tan 1990, 430, f. 13–17 (as *Gammiella touwii*); Akiyama et al. 2011, 42, f. 1 (as *Yakushimabryum longissimum*).

Specimens examined. **Philippines**, Mindanao, Bukidnon Prov., Mt. Kitanglad, on log, ca. 1,800 m alt., *Tan, Navarez & Amoroso 84-307* (isotype of *Gammiella touwii*, NICH); **Japan**, Tokyo-to, Hachijo Island, Mt. Hachijo-fuji, old crater, 790 m alt., on branch, *Kiguchi 16973*, NICH; Kyushu, Kagoshima-ken, Mt. Takakuma, *R. Toyama 1111* (isotype of *Clastobryum glomerato-propaguliferum*, NICH); Yakushima Island, Yakusugi-land, *Iwatsuki & Smith 10889* (paratype of *Yakushimabryum longissimum*, NICH); 800 m alt., on twig, *H. Deguchi 34995* (paratype of *Y. longissimum*, HIRO); between upper reach of Ambo River and Hananoego moor, *Iwatsuki, Smith & T. Suzuki 11131* (paratype of *Y. longissimum*, NICH); Kosugi-dani, 660–700 m alt., on tree trunk, *Iwatsuki & Sharp 14172*, NICH; 1000–1200 m alt, on tree trunk, *Iwatsuki & Sharp 15128*, NICH; Miyazaki-ken, Nichinan-shi, Sakatani, Kobuse, 150 m alt., on tree trunk, *Matsumoto 512*; Inohae, 80 m alt., on branch, *Matsumoto 659, 1832*, NICH; Miyazaki-shi, Kagamisu, along Kaeda river, 200 m alt., on fallen tree trunk, *Matsumoto 1756 & 2001*, NICH.

Distribution: Kampuchea, Laos, Vietnam, Indonesia, Philippines, China and Japan.

Buck (1998) recognized *Aptychella* by its lanceolate leaves with quadrate, thick-walled, mostly reddish alar cells and numerous propagular clusters along the stems or sometimes with flagellate branches.

The distinguishing characters of *Aptychella tonkinensis* (including *Yakushimabryum longissimum*) are: alar regions swollen or concave, consisting of groups of quadrate and short-rectangular, thick-walled, coloured cells; flagellate branches with leaves shorter and narrower than vegetative leaves; gemmae few in number, scattered on branches or clustered on the apical part of branches, uniseriate, smooth or verrucose.

The species is better recognized as *Aptychella* rather than *Gammiella*. In the materials studied we found gemmae scattered on branches or clustered on the apical part of branches, and smooth or verrucose gemmae (sometimes on the same plant). Plants of *A. tonkinensis* with gemmae can be quite variable in morphology.

Molecular phylogenetic analysis

A total of 110 *rbcL* gene sequences were examined, as shown in Appendix A. The *rbcL* gene sequences of *Aptychella tonkinensis* and *Dichelyma japonicum* Cardot were newly obtained. Based on our molecular studies, three sequences registered in

DDBJ/EMBL/GenBank International Nucleotide Sequence Database Collaboration (INSDC) as *Aptychella glomerato-propagulifera* (AB051217; as *Yakushimabryum longissimum* in Akiyama et al. 2011), *Y. longissimum* (GU327375) and *Gammiella tonkinensis* (AY346097) all relate to the same taxon - *A. tonkinensis*.

In the sequences AB796435 (as *Aptychella tonkinensis*), AB051217 (as *Aptychella glomerato-propagulifera*), GU327375 (as *Yakushimabryum longissimum*) and AY346097 (as *Gammiella tonkinensis*), there were 4–8 nucleotide differences (0 or 1 amino acid differences) between the materials from different localities (Table 1). We concluded that these materials represented the same taxon considering also the morphological range of gemmae found in *A. tonkinensis*. Further, there were 44–47 nucleotide differences (5 or 6 amino acid differences) between materials of AB796435 (as *A. tonkinensis*), AB051217 (as *A. glomerato-propagulifera*), GU327375 (as *Y. longissimum*) and AY346097 (as *G. tonkinensis*) and that of GU327380 (as *G. tonkinensis*). The sequence data from GU327380 was also phylogenetically distinct from those of AB796435, AB051217, GU327375 and AY346097 (Fig. 3). Although the voucher specimen of GU327380 was not available for the present study, examination of morphological features will be needed for the resolution of taxonomic treatment of this material. Arikawa & Higuchi (1999, 2003) have also demonstrated that in some species of *Plagiothecium*, *Pylaisia* and *Rhytidiadelphus*, similar *rbcL* nucleotide differences are found.

The GTR + gamma model provided the best fitted model for the dataset. A total of 4,087 distinct topologies were obtained in the ML, maximum parsimony (MP), Baysian inference (BI) and neighbor-joining (NJ) analyses, of which 3,363 satisfied the approximately unbiased (AU) test (Shimodaira 2002) criteria and one topology satisfying the Bayesian posterior probability (PP) test criteria respectively. Fig. 3 shows the ML tree with the best ranking log-likelihood and PP values. The tree strongly supports the monophyly of the Sematophyllaceae (*sensu lato*) including a small number of species so far included in the Hypnaceae and Hylocomiaceae together with the Entodontaceae (AU/BP/PP = 100/100/1.00). *A. tonkinensis* formed a monophyletic clade being sister to the *Isopterygium minutirameum* – *I. propagulifera* clade with high supporting values (73/69/1.00). The result supports our taxonomic treatment based on morphological investigations that *A. tonkinensis* and *Y. longissimum* are the same taxon.

Acknowledgements

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Appendix A. Taxon sampling and DDBJ/EMBL/GenBank accession number in the present study. Newly obtained sequences (in bold) are shown with voucher information (in order of scientific name, accession number, locality, and specimen number with abbreviation).

Acanthorrhynchium papillatum (Harv.) M.Fleisch., AB051224; AY346095; *A. scabrifolium* (Broth.) B.C.Tan & C.Ying, AY346098 (as *Mastopoma scabrifolium* in database); *Acroporium aciphyllum* Dixon, AY320236; *A. brevipes* (Broth.) Broth., AY320237; *A. joannis-winkleri* Broth., AY320238; *A. lamprophyllum* Mitt., AY320239; *A. procerum* (Müll.Hal.) M.Fleisch., AY320240; *A. pungens* (Hedw.) Broth., AF233572; *A. rigens* (Broth. ex Dixon) Dixon, AY320241; *A. rufum* (Reinw. & Hornsch.) M.Fleisch., AY320242; *A. secundum* (Reinw. & Hornsch.) M.Fleisch., GQ254037; *A. stramineum* (Reinw. & Hornsch.) M.Fleisch., AB051225; AY320243; *A. strepsiphyllum* (Mont.) B.C.Tan, AY320244; ***Aptychella tonkinensis* (Broth. & Paris) Broth., AB796435 (Japan, Miyazaki-ken, Miyazaki-shi, Kagamisu, along Kaeda river, 200 m alt., Matsumoto 2001, NICH)**; AB051217 (as *Aptychella glomerato-propagulifera* in database and as *Yakushimabryum longissimum* in Akiyama et al. 2011); AY346097 (as *Gammiella tonkinensis* in database); GU327375 (as *Y. longissimum* in database); *Boulaya mittenii* (Broth.) Cardot, AB024963; *Brotherella complanata* Reimers & Sakurai, AB039785; *B. fauriei* (Besch. ex Cardot) Broth., AB039786; *B. henonii* (Duby) M.Fleisch., AB029167; *B. herbacea* Sakurai, AB039787; *B. nakanishikii* (Broth.) Nog., AB051222; *B. recurvans* (Michx.) M.Fleisch., L13475; *Callicladium haldanianum* (Grev.) H.A.Crum, AB332268; *Clastobryopsis brevinervis* M.Fleisch., GU327378; *C. imbricata* H.Akiyama, Y.Chang & B.C.Tan, GU560188 (as *Clastobryopsis* sp. in database); *C. planula* (Mitt.) M.Fleisch., GU327377; *C. robusta* (Broth.) M.Fleisch., GU327376; *Clastobryum cuculligerum* (Sande Lac.) Tixier, AY346096; *C. glabrescens* (Z.Iwats.) B.C.Tan, Z.Iwats. & D.H.Norris, GU327381; ***Dichelyma japonicum* Cardot, AB796436 (Japan, Shiga-ken, Takashima-shi, Makino-cho, Dairaike, 488 m alt., coll. T. Seki & H. Taoda in herb. H. Tsubota 7721, HIRO)**; *Entodon challengeri* (Paris) Cardot, AB050993; *E. hampeanus* Müll.Hal., DQ467877; *E. luridus* (Griff.) A.Jaeger, AB050994; *E. myurus* (Hook.) Hampe, AB024640; *E. rubicundus* (Mitt.) A.Jaeger, AB029386; *E. scabridens* Lindb., AB050995; *Fontinalis antipyretica* Hedw., AB050949; *Foreauella orthothecia* (Schwägr.) Dixon & P. de la Varde, AB194962; *Gammiella pterogonioides* (Griff.) Broth., GU327379; *G. tonkinensis* ? (Broth. & Paris) B.C.Tan, GU327380; *Glossadelphus ogatae* Broth. & Yasuda, AB050950; *Heterophyllium affine* (Hook.) M.Fleisch., AB051218; *H. nematosum* Broth., AB029391; *Hypnum cupressiforme* Hedw., AB032077; *Isocladiella surcularis* B.C.Tan & Mohamed, AY320245; *Isopterygium albescens* (Hook.) A.Jaeger, AY320234; *I. limatum* (Hook. f. & Wilson) Broth., AB332308; AB332307; *I. minutirameum* (Müll.Hal.) A.Jaeger, AB332306; *I. propaguliferum* Toyama, AB332286; *I. tenerum* (Sw.) Mitt., AF233569; *I. vineale* E.B.Bartram, AB024650; *Leptocladiella delicatula* (Broth.) J.R.Rohrer, GQ254039; *L. psilura* (Mitt.) M.Fleisch., AB491802; AB332250; *Macrohymenium muelleri* Dozy & Molk., AY320246;

Macrothamnium macrocarpum (Reinw. & Hornsch.) M.Fleisch., AB491803; *Mastopoma subfiliferum* Horik. & Ando, AB071411; *M. uncinifolium* (Broth.) Broth., AB071412; *Meiothecium microcarpum* (Harv.) Mitt., AB051223; *Neacroporium flagelliferum* (Sakurai) Z.Iwats. & Nog., AB039784; *Papillidiopsis bruchii* (Dozy & Molk.) W.R.Buck & B.C.Tan, AY320248; *Piloecium pseudorufescens* (Hampe) Müll.Hal., AY320247; *Plagiomnium japonicum* (Lindb.) T.J.Kop., AB050992; *Plagiothecium nemorale* (Mitt.) A.Jaeger, AB029387; *Platygyrium repens* (Brid.) Schimp., AB091271; *Pseudotrismegistia undulata* (Broth. & Yasuda) H.Akiyama & H.Tsubota, AB071415 (as *Trismegistia perundulata* in database); AB051229 (as *Trismegistia undulata* in database); *Pterogonidium pulchellum* (Hook.) Müll.Hal., AY320249; *Pylaisia speciosa* (Mitt.) Wilson ex Paris, AB246749; *Pylaisiadelpha tenuirostris* (Bruch & Schimp. ex Sull.) W.R.Buck, AB051219; *P. tristoviridis* (Broth.) O.M.Afonina, Ignatova & H.Tsubota, AB050991 (as *Hypnum tristoviride* in database); *Radulina hamata* (Dozy & Molk.) W.R.Buck & B.C.Tan, AY320256; *Rhaphidostichum macromonostictum* J.Froehl., AB051220; *Rhynchostegium pallidifolium* (Mitt.) A.Jaeger, AB024944; *Rhytidiadelphus japonicus* (Reimers) T.J.Kop., AB039788; *Sematophyllum pulchellum* (Cardot) Broth., AB071413; *S. subhumile* subsp. *japonicum* (Broth.) Seki, AB039675; *S. subpinnatum* (Brid.) E.Britton, GQ254045; *Stereodontopsis pseudorevoluta* (Reimers) Ando, AB332245; *Symphyodon asper* (Mitt.) A.Jaeger, AB491807; *S. erinaceus* (Mitt.) A.Jaeger, AB491808; *S. leiocarpus* H.Akiyama & H.Tsubota, AB491804; *S. scaber* (Tixier) S.He & Snider, AB491805; *Taxithelium nepalense* (Schwägr.) Broth., AY320250; *T. planum* (Brid.) Mitt., AF233573; *Thuidium delicatulum* (Hedw.) Schimp., AF158177; *T. pristocalyx* (Müll.Hal.) A.Jaeger, AB071416; *T. recognitum* (Hedw.) S.O.Lindberg, AB019476; DQ645996; *T. tamariscinum* (Hedw.) Schimp., DQ463113; *Trichosteleum cuspidatum* B.C.Tan & Ho B.C., nom. sched., AY320235; *T. papillosum* (Hornsch.) A.Jaeger, AF233574; *T. singapurense* M.Fleisch., AY320251; *T. stissophyllum* (Hampe & Müll.Hal.) A.Jaeger, AB051226; *Trismegistia calderensis* (Sull.) Broth., AB071414; *T. korthalsii* (Dozy & Molk.) Broth., AB051227; *T. plicata* H.Akiyama, AB051228; *T. pulchella* Herzog, AB071410; *T. rigida* (Mitt.) Broth., AY320252; *Vesicularia reticulata* (Dozy & Molk.) Broth., AY320253; *Warburgiella leptocarpa* (Schwägr.) M.Fleisch., AY320254; *Wijkia concavifolia* (Cardot) H.A.Crum, AB095271; *W. deflexifolia* (Mitt. ex Renauld & Cardot) H.A.Crum, AB051221; *W. hornschuchii* (M.Fleisch.) H.A.Crum, AB029383; *W. tanytricha* (Mont.) H.A.Crum, AY320255.

Appendix B. Supplementary information is shown on the website of the Digital Natural History Museum of Hiroshima University at "https://www.digital-museum.hiroshima-u.ac.jp/~museum/data/SuzukiEtAl2013HattoriaAppendixB.pdf".

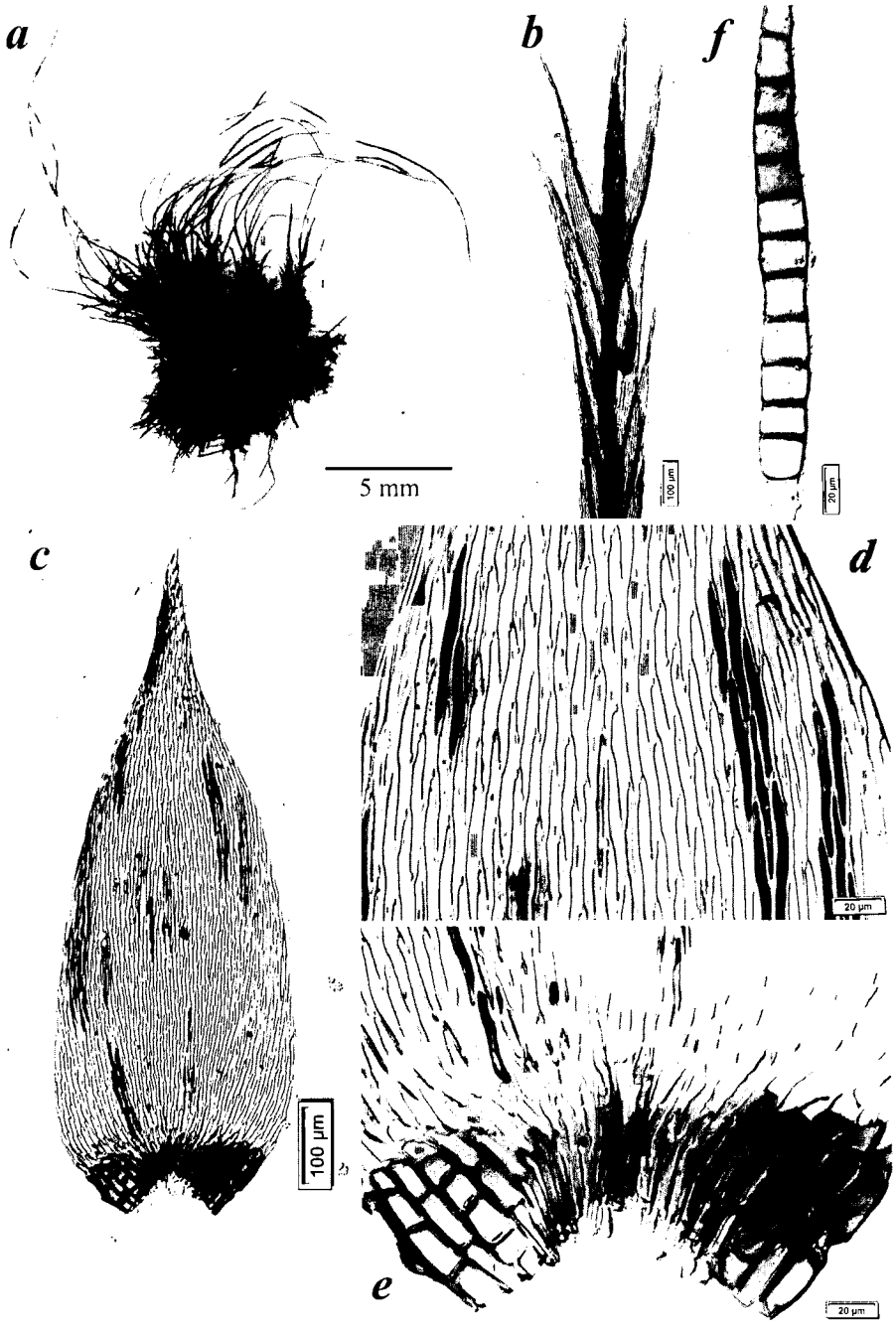

Fig. 1. *Aptychella tonkinensis* (Broth. & Paris) Broth.: a, habit. b, flagellate branch. c, leaf. d, upper median cells of leaf. e, alar region. f, gemma. All figures were taken from the isotype of *Gammiella touwii* in NICH.

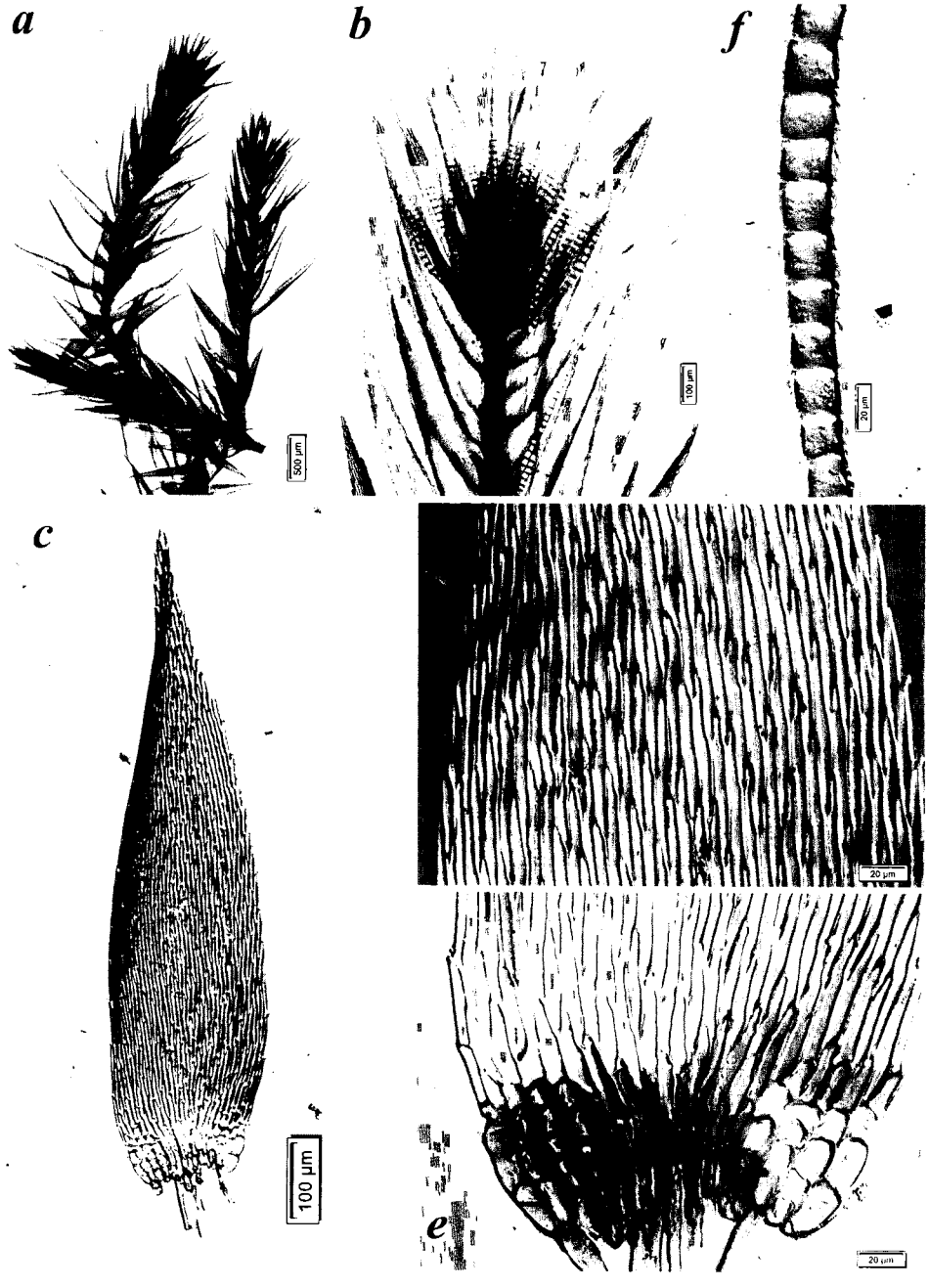

Fig. 2. *Aptychella tonkinensis* (Broth. & Paris) Broth.: a, habit. b, stem apex with gemmae. c, leaf. d, upper median cells of leaf. e, alar region. f, gemma. All figures were taken from the isotype of *Clastbryum glomerato-propaguliferum* in NICH.

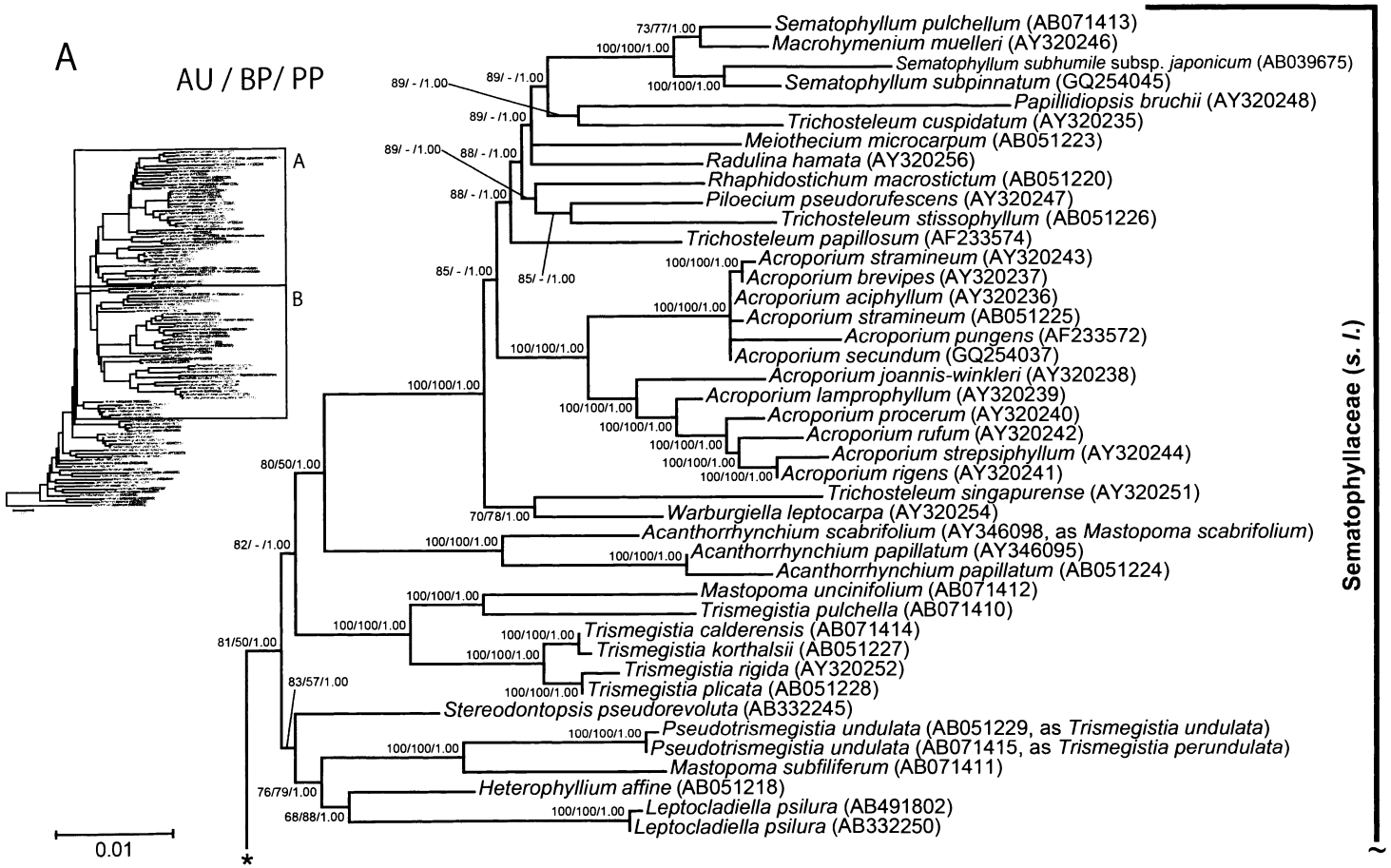

Fig. 3. Phylogenetic tree based on analysis of the chloroplast *rbcL* gene sequences, depicted by the ML tree with the best ranking log-likelihood value (see also Appendix B). Supporting values more than 50% obtained by the program CONSEL were overlaid: the values by the AU test (AU), bootstrap probabilities (BP), and Bayesian posterior probabilities (PP) are shown on or near each branch (AU/BP/PP; in %). The root is arbitrarily placed on the branch leading to *Plagiomnium japonicum* following Tsubota et al. (2001b) and Akiyama & Tsubota (2009).

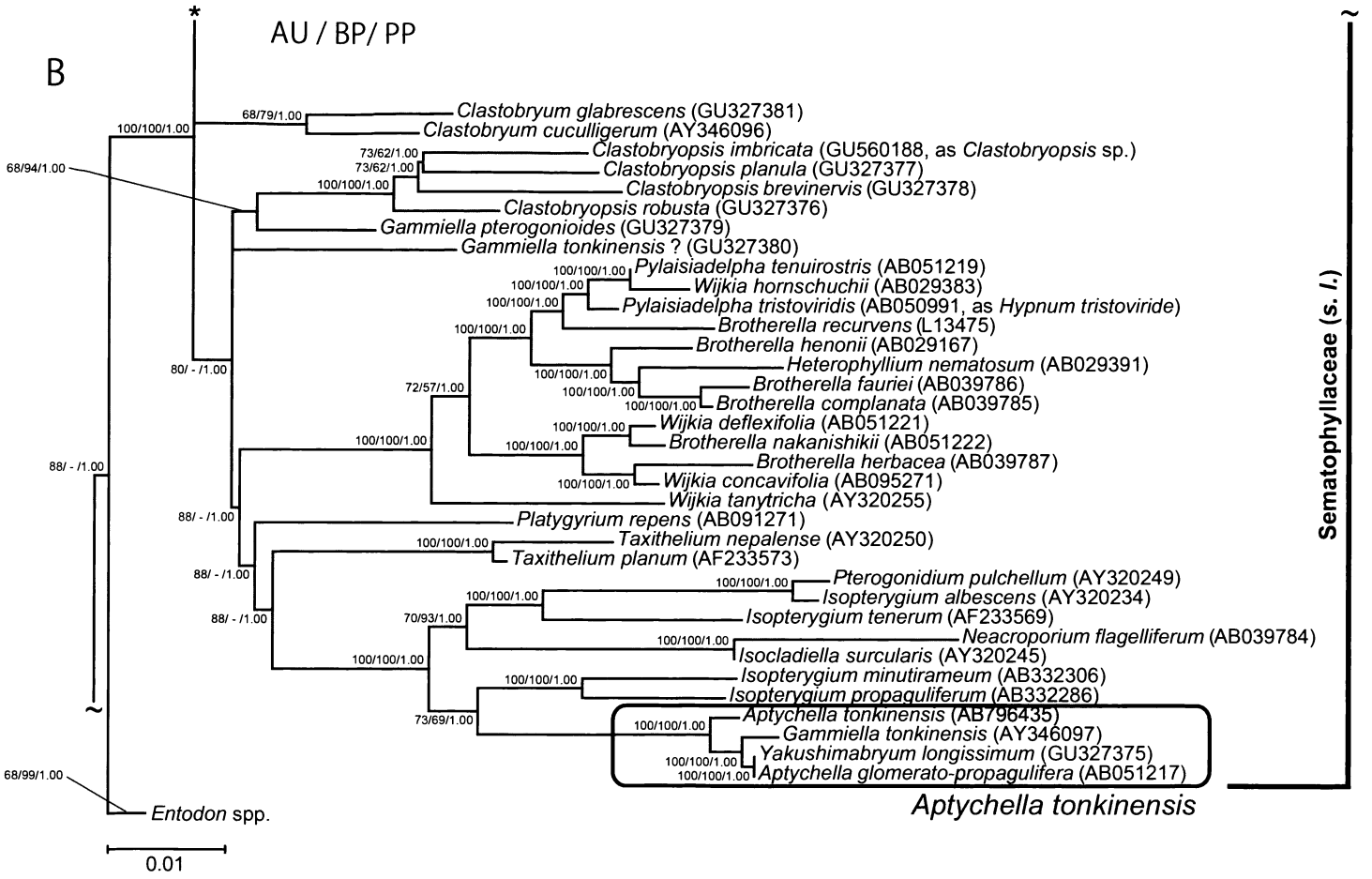

Fig. 3. Continued.

Table 1. Uncorrected pairwise differences among chloroplast *rbcL* gene sequences. Above diagonal: total numbers of differences of the nucleotide sequences within 1,292 bp. Below diagonal: differences of estimated amino acid sequences within 430 aa. Each Material is shown with voucher information (in order of accession number, scientific name in database, locality).

Material	1	2	3	4	5
1 AB796435, *Aptychella tonkinensis*, Japan, Miyazaki, Kagamisu	-	6	6	8	47
2 AB051217, *A. glomerato-propagulifera*, Japan, Kagoshima, Yakushima Isl.	0	-	0	4	46
3 GU327375, *Yakushimabryum longissimum*, Japan: Kagoshima, Yakushima Isl.	0	0	-	4	46
4 AY346097, *Gammiella tonkinensis*, Malaysia, Pahang, Fraser Hill	1	1	1	-	44
5 GU327380, *G. tonkinensis*, Japan, Kagoshima, Yakushima Isl.	6	6	6	5	-