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Aptychella chilensis belongs to the Ptychomniaceae and not Pylaisiadelphaceae based on DNA and morphological analyses

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Abstract – Inferences from molecular data (*rps*4 and *trn*L-F) and morphological features of the Chilean endemic *Aptychella chilensis* reveal that the species should be excluded from *Aptychella* and the Pylaisiadelphaceae, and transferred to *Ombronesus* in the Ptychomniaceae. The new combination *Ombronesus chilensis* (Herzog) Frank Müll. & H. Akiyama is proposed with the new synonym *O. stuvensis* N.E. Bell, N. Pedersen & A.E. Newton. A lectotype for *A. chilensis* is selected.

Chile / *Ombronesus* / pleurocarpous mosses / Ptychomniaceae / Pylaisiadelphaceae / taxonomy / Valdivian rainforest

Résumé – Des inférences de données moléculaires (*rps*4 and *trn*L-F) et de caractères morphologiques de l'espèce endémique du Chili, *Aptychella chilensis* révèlent que cette espèce doit être exclue d'*Aptychella* et des Pylaisiadelphaceae, et transférée dans *Ombronesus* au sein des Ptychomniaceae. La nouvelle combinaison *Ombronesus chilensis* (Herzog) Frank Müll. & H. Akiyama est proposée, avec le nouveau synonyme *O. stuvensis* N.E. Bell, N. Pedersen & A.E. Newton. Un lectotype pour *A. chilensis* est choisi.

Chili / *Ombronesus* / mousses pleurocarpes / Ptychomniaceae / Pylaisiadelphaceae / taxonomie / forêt pluvieuse Valdivienne

INTRODUCTION

The genus *Aptychella* (Broth.) Herzog (Pylaisiadelphaceae) comprises *ca* 10 species and occurs predominantly in East and Southeast Asia (Akiyama *et al.*, 2015). Beside *A. proligera* (Broth.) Herzog, widely distributed in tropical Central and South America, *A. chilensis* Herzog is the only member of the genus reported from the New World. In the course of a phylogenetic study of the genus, we have studied the morphology and sequenced two loci of recent collections of *A. chilensis*. Surprisingly blast searches revealed that the species has close affinities not to

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Aptychella, but to the monospecific genus *Ombronesus* N.E. Bell, N. Pedersen & A.E. Newton of the family Ptychomniaceae. Here we report our molecular analyses as well as a morphological comparison with type material of *O. stuvensis* N.E. Bell, N. Pedersen & A.E. Newton with a new taxonomic proposal.

MATERIALS AND METHODS

We studied two recent collections of *Aptychella chilensis* deposited in DR (*Frank Müller C925* from Chile Región X, Aisén province, and *Frank Müller C2410* from Chile Región XI, Ranco province) as well as type specimens for this species (*G. H. Schwabe, no 8/a* and *8/d* JE [04004133 & 04004134]), respectively, and one syntype of *Ombronesus stuvensis* (*N.E. Bell 1247* BM [000710897]).

We extracted total DNA from the two specimens of *Aptychella chilensis*. We followed Akiyama *et al.* (2015) for DNA extraction, amplification and sequencing of the *rps*4 and *trn*L-F loci. We also tried to amplify *rbc*L and *nad*5, which Bell *et al.* (2007) included in their analyses, but our attempts failed. The sequences were complemented with those of taxa from Bell *et al.* (2007). *Lepyrodon pseudolagurus* B.H. Allen (Lepyrodontaceae) and *Rhytidiadelphus triquetrus* (Hedw.) Warnst. (Hylocomiaceae) were selected as outgroups as in Bell *et al.* (2007).

We performed Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses using MEGA5.2.2 (Tamura *et al.*, 2011), and also Bayesian (BI) analyses using MrBayes ver. 3.2.2 (Ronquist & Huelsenbeck, 2003). For MP analyses, we used default settings with TBR branch-swapping with 1000 random addition replicates. We applied the T92 + G model in the ML analysis based on model selection according to the Akaike's Information Criterion (AIC: Akaike, 1973) as implemented in MEGA5.2.2. Non-parametric bootstrapping analyses for ML and MP analyses were performed with 200 pseudo-replicates with simple taxon addition, with bootstrap frequencies, respectively labelled MLBS and MPBS, inferred from the consensus trees of 200 optimal pseudoreplicate trees. We used T92 + G model for BI analysis and four chains (tem parameter 2.0) were run simultaneously for 1,000,000 generations; trees were sampled every 1,000 generations. The first 12,500 trees were removed for the burn-in phase. The consensus of the remaining trees was constructed with resulting nodes frequencies treated as Bayesian posterior probabilities (PP).

RESULTS

We were only successful in obtaining *rps*4 and *trn*L-F sequences, for one (i.e., *Frank Müller C2410*) or both samples of *Aptychella chilensis*, respectively. We tried to amplify *rbc*L and *nad*5 but our attempts failed. This is probably due to the poor condition of the specimens available because *Frank Müller C925* was collected in 1999 and *Frank Müller C2410* in 2001. Newly obtained sequences of *A. chilensis* are 580 nucleotides (nts) long for *rps*4 (LC052331 for *Frank Müller C2410*) and 441 nts for *trn*L-F (LC052330 for *Frank Müller C925* and LC052329 for *Frank*

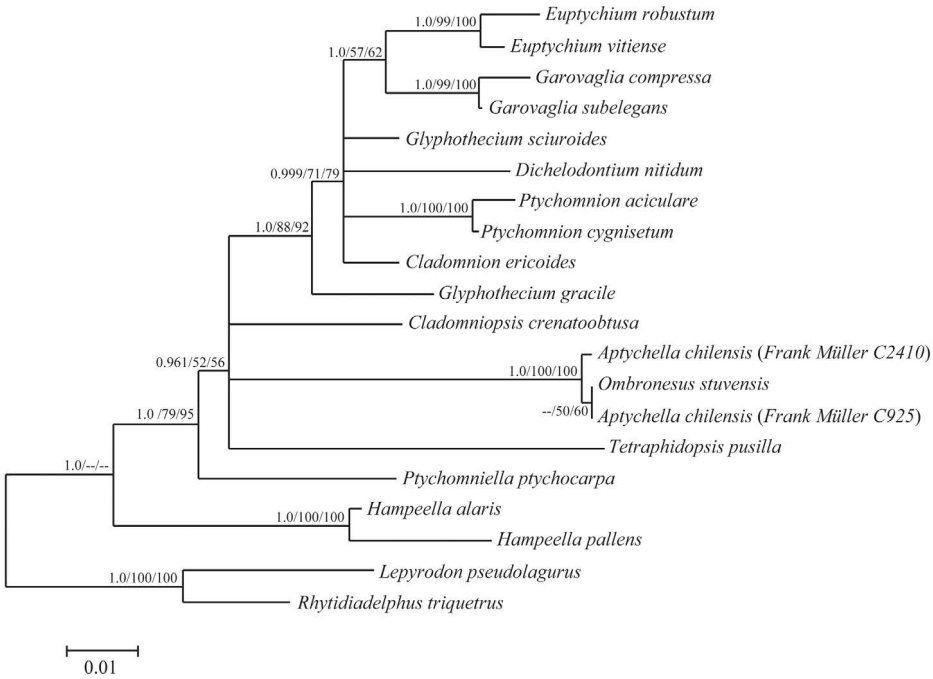


Fig 1. Phylogenetic relationships of 18 samples (13 genera and 17 species) of Ptychomniaceae based on combined *rps*4 and *trn*L-F sequences. The tree represents the 50% consensus Maximum Likelihood inference. Branches below 50% reliabilities are collapsed. Bootstrap values (> 50 %) of Maximum Likelihood inference and Maximum Parsimony inference, and PP values (> 0.94) from the Bayesian inference are indicated. Scale bar for branch lengths shows the number of substitutions per site. Note that scientific names of *Aptychella chilensis* and *Ombronesus stuvensis* (both are treated as *O. chilensis* in the text) are used here.

Müller C2410). *Trn*L-F sequences of the two samples of *A. chilensis* were identical to one another and to the available sequence for *Ombronesus stuvensis*. The *rps*4 sequence of *A. chilensis* differed from that of *O. stuvensis* by two substitutions. Matrices of aligned sequences for each locus from 20 Ptychomniaceae and outgroup lineages comprised 692 nts for *rps*4 and 476 nts for *trn*L-F, and hence a total of 1168 nts when combined. The sequences could be unambiguously aligned. Gaps were treated as missing data.

Inferences from each locus yielded no conflicting branches with high bootstrap or posterior probabilities. MP analysis yielded four most parsimonious trees each 307 steps long (CI = 0.625551, RI = 0.731861). These trees did not differ from those obtained under ML and BI analyses, and hence the 50% majority consensus tree of optimal ML trees (Fig. 1) is used for the following discussion. The general topology is congruent with the presented in Bell *et al.* (2007). Two samples of *Aptychella chilensis* form a clade with *Ombronesus stuvensis* well supported in BI, ML and MP analyses (1.0, 100, 100, respectively).

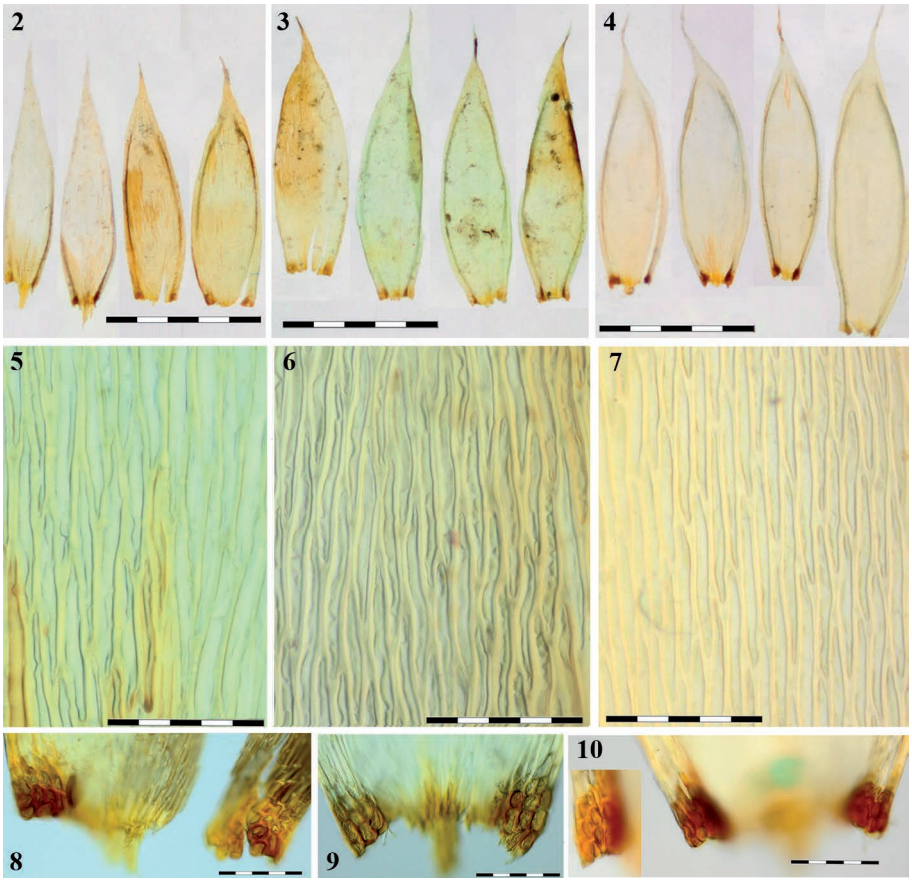
DISCUSSION

Aptychella chilensis was described on the basis of material without sporophytes by Herzog (1954) from the Aisén region of southwestern Chile. After its description the species was only found a few times again. Seki (1974) reported it from Aisén province of Chile, M. R. Crosby collected it in Chiloé in the Región Los Lagos (He, 1998) and Müller (2009) reported it from Ranco province. During excursions in 1999 and 2001 the senior author had the opportunity to collect materials of *A. chilensis* in Aisén and Ranco province of Chile. Comparison of these collections against the type material of *A. chilensis* deposited in JE confirmed their identity. Blasts of the *rps*4 and *trn*L-F sequences suggest that this species may, however, be a member of the Ptychomniaceae. Phylogenetic inferences (ML, MP, and BI) suggest indeed a close affinity of these two samples to *Ombronesus stuvensis* (Fig. 1).

On the basis of this result, we have contrasted morphological traits of the isotype material of *Ombronesus stuvensis* (BM) and two syntypes of *A. chilensis* in JE and our two recent collections. These specimens share (1) strongly concave leaves with abruptly acuminate-piliferous, often twisted acumen, (2) the elongate, somewhat incrassate leaf areolation, (3) a strongly differentiated alar region with enlarged, and distinctly incrassate, orange, irregularly quadrate or isodiametric to oblong alar cells, suggesting that they belong to a single taxon (Figs 2-10; for *O. stuvensis* see also Bell *et al.*, 2007).

Herzog (1954) described and illustrated leaf costae of *A. chilensis* as reaching half the leaf length. According to our observations of the syntypes, they are in reality much shorter and many leaves lack a costa entirely. Therefore, this feature of *A. chilensis* corresponds well with those of our recent collections as well as of the isotype of *O. stuvensis*. Herzog (1954) also mentioned the presence of filiform gemmae in clusters at branch apices in *A. chilensis*. We confirmed such gemmae in the syntypes as well as in our own collections (Figs 11, 12, 14, 15); they are restricted to branch apices of flagellate branches. Though Bell *et al.* (2007) did not mention any asexual reproductive organs in the description of *O. stuvensis*, we recognized similar gemmae in the isotype specimen of the species, especially at the apices of a few flagellate branches (Figs 13, 16). Such gemmae are also found in the monospecific *Tetraphidopsis* Broth. & Dixon (Ptychomniaceae), although Bell *et al.* (2007) treated it as one of the features distinguishing it from *Ombronesus*. Comparison with material of *T. pusilla* (Hook. f. & Wilson) Dixon (New Zealand, North Island, Wairata region, Waioeka Gorge Scenic Area, *J.R. Shevock 39438*, DR) as well as the report presented by Bell *et al.* (2007), however, reveal more differences between these two genera, e. g., *Tetraphidopsis* has shorter propagules, not suddenly narrowed leaf apex, thinner alar cells, the possession of a rhomboid, very lax leaf areolation, a very slender habit, a less reduced endostome, a fairly short seta and (unique in the Ptychomniales) a single costa extending to the middle part of the leaf. Therefore our view is to treat both genera as separate.

Aptychella chilensis was described by Herzog (1954) on the basis of material without sporophytes. First reports of sporophytes were made by Seki (1974), but he provided no description. We requested a loan of the material of Seki, now deposited in Hiroshima University, Miyajima Branch (HIRO), but we received no response or access to specimens. One of the newly collected specimens (*Frank Müller C2410*) bears sporophytes and while examining the sporophytes the senior author already had some doubts of the placement of the species in *Aptychella*, because the 8-ribbed capsules are not typical for *Aptychella* as well as for any

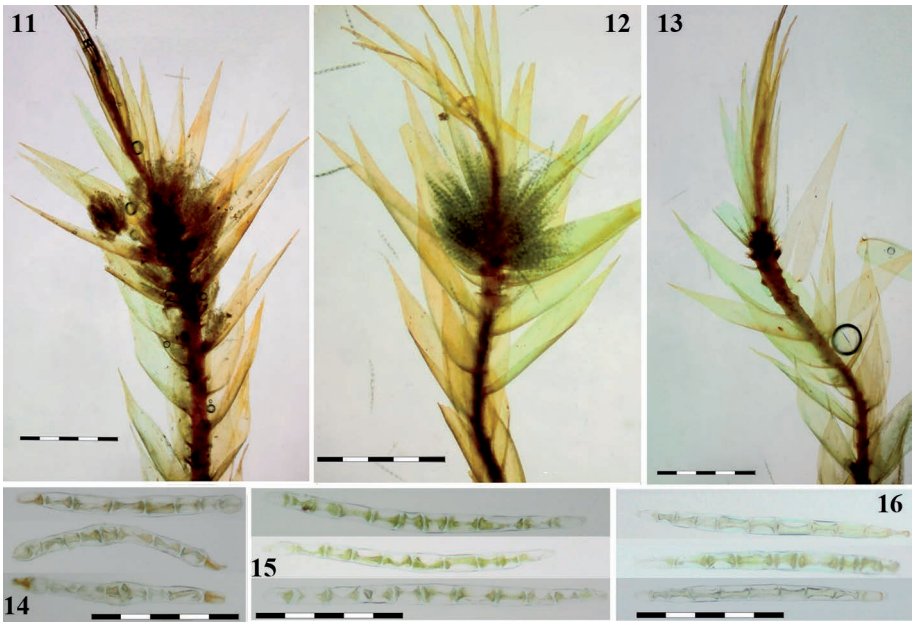


Figs 2-10. Leaf characteristics of *Ombronesus chilensis*. **2-4.** Leaves; **5-7.** Leaf cells in mid-leaf; **8-10.** Basal part of leaf with alar region. Left line (2, 5, 8): Type material of *Aptychella chilensis* (*G.H. Schwabe no. 8/d* JE); Middle line (3, 6, 9): Recent collections of *A. chilensis* (*Frank Müller C925* DR); Right line (4, 7, 10): Type material of *O. stuvensis* (*N.E. Bell 1247* isotype BM). Scale bars: 2, 3, 4 = 1 mm; 5, 6, 7 = 50 µm; 8, 9, 10 = 100 µm.

Hypnalean or Hookeralian family. Beside the 8-ribbed capsules, the development of the endostome consisting of narrow and fragmentary segments fits well with the description of *O. stuvensis*. Finally, the type material of *A. chilensis* (*G.H. Schwabe 8/a*) contains perichaetia with perichaetial leaves with a broad ovate base and a long-flexuose acumen, which also fits well the description of *O. stuvensis*.

TAXONOMIC TREATMENT

Morphological and molecular surveys show no significant differences between *A. chilensis* and *O. stuvensis*. Since *A. chilensis* has been described earlier than *O. stuvensis*, we propose the following taxonomic change:



Figs 11-16. Branches with clusters of gemmae and gemma morphology of *Ombronesus chilensis*. **11-13.** Branches with clusters of gemmae; **14-16.** Gemmae. 11, 14: Type material of *A. chilensis* (*G.H. Schwabe no. 8/d* JE); 12, 15: Recent collections of *A. chilensis* (*Frank Müller C925* DR); 13, 16: Type material of *Ombronesus stuvensis* (*N.E. Bell 1247* isotype BM). Scale bars: 11, 12, 13 = 1 mm; 14, 15, 16 = 200 µm.

Ombronesus chilensis (Herzog) Frank Müll. & H. Akiyama, ***comb. nov.*** **Figs 2-16**

Basionym: *Aptychella chilensis* Herzog, *Revue Bryologique et Lichénologique* 23: 89. 24. 1954. **Type**: Westpatagonien: Pto. Puyuhuapi, beim Muggelbach an Baumrinde, 15.10.39, *G. H. Schwabe, no 8/d* [Lectotype JE (*04004133*)!, selected here]; Westpatagonien: Pto. Puyuhuapi, am Muggelbach, 100-150 m, 15.10.39, *G. H. Schwabe, no. 8/a* [Syntype JE (*04004134*)].

Ombronesus stuvensis N.E. Bell, N. Pedersen & A.E. Newton, *Taxon* 56: 893. 2007. Type: Chile, Region XI (Aysén). Reserva Nacional Katalalixar. Juan Stuven Island. Mature *Nothofagus* forest behind sheltered bay, 15/02/2003, *N.E. Bell 1247* [Holotype SGO, Isotypes BM (*000710897*)!, NY (*00756696*)], **syn. nov.**

Description: The complete description given by Bell *et al.* (2007) is complemented by the following detail. Gemmae often present, in clusters at branch apices of flagellate branches, 8-11-celled, uniseriate, smooth, 290-450 µm long, 24-31 µm wide (Figs 11-16).

Additional specimens examined: **CHILE.** Monumento Natural "Alerce Costera" c. 40 km SW of Valdivia, Chile Región X, 40°10' S, 73°33' W, forest dominated by *Fitzroya cupressoides*, epiphytic on thin twigs of *Nothofagus*, c. 600 m, 30.03.1999, *Frank Müller C925* (DR). Puerto Puyuguapi NNE, northern part of the National Park Queulat, at the NW shore of Lago Risopatron, Chile Región XI, c. 80 m, 44°13' S, 72°30' W, temperate rainforest, epiphytic on thin twigs, 05.03.2001, *Frank Müller C2410* (DR).

Distribution: *Ombronesus chilensis* is endemic to southwestern Chile and known from coastal areas in the political regions of Los Ríos (región XIV, province

Ranco), Aisén (región XI, provinces Capitán Prat, Aisén [from this province the type of *A. chilensis* are from]) and Los Lagos (región X, province Chiloé). The northernmost record is situated in the Monumento Natural "Alerce Costera" about c. 40 km SW of Valdivia (40°10' S). The southernmost record is on Juan Stuven Island in the Reserva Nacional Katalalixar (c. 47°49' S). This distribution spans 900 km along the west coast of Chile, and is characterised by a temperate, humid and foggy climate with annual precipitation of about 2000 mm or more. The altitudes of the known records varied from near sea level at the southernmost site in province Aisén to about 600 m at the northernmost site in the Región de Los Ríos.

Habitat: All the known localities lie in areas within the Valdivian temperate rain forest as the predominant vegetation type, characterized by *Nothofagus* sp., *Fitzroya cupressoides* (Molina) I.M. Johnst., *Drimys winteri* J.R. Forst. & G. Forst., *Pilgerodendron uviferum* (D. Don) Florin, *Amomyrtus luma* (Molina) D. Legrand & Kausel, and *Podocarpus nubgenus* Lindl. *Ombronesus chilensis* is normally growing pendent on small twigs of rainforest trees, especially of *N. betuloides* (Mirb.) Oerst., and *N. nitida* (Phil.) Krasser.

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APPENDIX

20 samples (13 genera and 19 species) and their associated GenBank accession numbers for *rps*4 and *trn*L-F, respectively. Dashes (—) indicate lack of corresponding data. New accessions are indicated with an asterisk (*) before species names and with voucher information in parentheses.

Hylocomiaceae: *Rhytidiadelphus triquetrus* (Hedw.) Warnst., DQ186851, AF397811. **Lepyrodontaceae:** *Lepyrodon pseudolagurus* B.H. Allen, AF143014, AF161107. **Ptychomniaceae:** *Cladomnion ericoides* (Hook.) Wilson, AY306884, AY306718. *Cladomniopsis crenato-obtusa* M. Fleisch., AY306883, AY306717. *Dichelodontium nitidum* (Hook. f. & Wilson) Broth., AY449664, AY449670. *Euptychium robustum* Hampe, AY306907, AY306741. *E. vitiense* Dixon, AY306909, AY306743. *Garovaglia compressa* Mitt., AY306914, AY306748. *G. subelegans* Broth., DQ186838, DQ194218. *Glyphothecium gracile* (Hampe) Broth., DQ186840, DQ194220. *G. sciuroides* (Hook.) Hampe, DQ186841, DQ194221. *Hampeella alaris* (Dixon & Sainsbury) Sainsbury, DQ186842, DQ194222. *H. pallens* (Sande Lac.) M. Fleisch., DQ186844, DQ194224. **Ombronesus* (*Aptychella*) *chilensis* Frank Müll. & H. Akiyama (*Frank Müller C925*); —, LC052330. **O. chilensis* (*Frank Müller C2410*), LC052331, LC052329. *O. stuvensis* N.E. Bell, N. Pedersen & A.E. Newton, DQ219413, DQ219414. *Ptychomniella ptychocarpon* (Broth.) W.R. Buck, C.J. Cox, A.J. Shaw & Goffinet, DQ186849, DQ194229. *Ptychomnion aciculare* (Brid.) Mitt., DQ186845, DQ194225. *Ptychomnion cygnisetum* (Müll. Hal.) Kindb., DQ186846, DQ194226. *Tetraphidopsis pusilla* (Hook. f. & Wilson) Dixon, AY307001, AY306835.