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ORIGINAL ARTICLE

Phylogenetic relationships in the genus *Hennediella* (Pottiaceae, Bryophyta) inferred from nrITS sequence data

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Abstract The first phylogenetic study of the genus *Hennediella* using nuclear ITS rDNA for 11 of the 15 species recognized is presented. Maximum parsimony, maximum likelihood, and Bayesian analyses were performed to evaluate the monophyly of the genus and to investigate phylogenetic relationships between the species. The molecular data suggest that the core *Hennediella* is monophyletic only including *Tortula platyphylla*, although the affinities of two species (*Hennediella heteroloma* and *H. longipedunculata*) are ambiguous. The ITS sequences did not fully resolve the internal relationships of *Hennediella* while its affinities with *Dolotortula*, *Leptophascum*, *Sagenotortula*, or *Tortula* remain ambiguous. The present circumscription of *Hennediella* is provisional until a morphological revision of genus *Tortula* is completed.

Keywords *Hennediella* · Pottiaceae · nrITS sequence · Phylogeny · Taxonomy

Introduction

Hennediella Paris is a small genus of the Pottiaceae distributed throughout much of the world from 81°N in the Arctic to 78°S in the Antarctic, although its highest diversity is found in the Southern Hemisphere. The species of this genus occur from near sea level to 5,000 m in puna and paramo formations, growing mainly on soil or on ledges, taluses, walls, and rocks with accumulated soils (Cano 2008).

The genus as presently defined includes small plants characterized mainly by leaves edged with cells that are different in shape, usually rectangular to linear, in 1–4 strata, less papillose and thicker-walled than the inner cells, leaf margins plane, occasionally slightly recurved, mostly denticulate to dentate at least close to the apex, costa usually with dorsal and ventral surface cells differentiated, one band of dorsal stereids usually semicircular, surface of the laminal cells frequently plane, and KOH color reaction orange to reddish. The sporophyte varies from long seta with emergent capsules and peristomes of 32 spirally teeth with a long basal membrane to short seta with capsules immersed and gymnostomous (Zander 1993; Cano 2008).

The genus was erected by Brown (1893) and later synonymyzed with *Pottia* Ehrh. ex Fürnr (Sainsbury 1952; Wijk et al. 1962). Zander (1989) resurrected the genus *Hennediella* and revised its circumscription (Zander 1993), on the basis of contributions by Blockeel (1991). The genus, as defined by Blockeel (1991) and Zander (1989, 1993), comprises 20 species that had previously been included in *Desmatodon* Brid., *Pottia*, or *Tortula* Hedw.

Cano (2008) monographed the genus, reducing the number of species to 15. Although she established the differences between *Hennediella* and *Syntrichia* Brid., the boundaries between *Tortula* and *Hennediella* remained ambiguous. *Tortula* is distinguished by broader leaves, more convex surface of the laminal cells, absence of elongate marginal cells border, and yellow KOH reaction; however, some species with markedly recurved margins, for example *T. platyphylla* Mitt., have differentiated marginal cells disposed in 1–2 strata and orange to reddish KOH leaf reaction. Species such as *T. solmsii* (Schimp.)

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Limpr. have similar lax areolation and differentiated border, but in this case the leaf KOH reaction is yellow. Cano (2008) noted that most of the species treated in her study fulfil the requirements for belonging to *Hennediella* (*H. antarctica* (Ångstr.) Ochyra & Matteri, *H. arenae* (Besch.) R.H. Zander, *H. densifolia* (Hook. f. & Wilson) R.H. Zander, *H. denticulata* (Wilson) R.H. Zander, *H. heimii* (Hedw.) R.H. Zander, *H. kunzeana* (Müll. Hal.) R.H. Zander, *H. longirostris* (Hampe ex Müll. Hal.) R.H. Zander, *H. macrophylla* R. Br. bis) Paris, *H. marginata* (Hook. f. & Wilson) R.H. Zander, *H. polyseta* (Müll. Hal.) R.H. Zander, *H. stanfordensis* (Steere) Blockeel and *H. steereana* (R.H. Zander & H.A. Crum) R.H. Zander). However, *H. bellii* (E.B. Bartram) R.H. Zander, *H. heteroloma* (Cardot) R.H. Zander, and *H. longipedunculata* (Müll. Hal.) R.H. Zander, which lack marginal teeth on their leaves, have a combination of characteristics that suggest a close relationship with *Tortula*. According to Zander and Eckel (2007), the two genera intergraded somewhat and a less arbitrary distinction than simple emphasis on KOH color reaction awaits more study.

The genus *Tortula* is one of the most complex and diverse genera in terms of morphological variation in the Pottiaceae. With the exception of the treatment of Brotherus (1924), the genus *Tortula* has never been monographed. Only the taxonomic revision of the genus in South America (Cano and Gallego 2008) and floristic treatments such as Zander and Eckel (2007) for North America, Mishler (1994) for Mexico, Smith (2004) for Great Britain and Ireland, and Cano (2006) for the Iberian Peninsula are outstanding. Zander (1989) recognized genera such as *Chenia* R.H. Zander, *Dolotortula* R.H. Zander, *Hennediella*, *Hilpertia* R.H. Zander, *Sagenotortula* R.H. Zander, *Stonea* R.H. Zander or *Syntrichia*, as segregates of *Tortula*, but included taxa that traditionally were placed in other genera such as some species of *Pottia*, *Phascum* Hedw. (e.g. *Phascum cuspidatum* Hedw.), and the genus *Desmatodon*.

In his classification of genera of Pottiaceae, Zander (1993) placed *Hennediella* close to *Dolotortula* in the Pottieae. *Dolotortula* has a reddish orange KOH reaction of the leaf, broad and smooth laminal cells, border of elongated cells, and a costa with dorsal and ventral surface cells differentiated and one semicircular dorsal band of stereids like *Hennediella*. The genera only differ in the multistratose border of stereid cells, undifferentiated dentate leaf margins and smooth laminal cells of *Dolotortula* instead of the usually unistratose to tristratose border, dentate leaf margins, and, usually, papillose laminal cells of *Hennediella*. Later, Zander (2006) included *Tortula* and related genera (*Aloinella* Cardot, *Crossidium* Jur., *Globulinella* Steere, *Pterygoneurum* Jur., and *Stegonia* Vent.) in the Pottieae and the rest of the genera of Pottioideae (*Acaulon* Müll. Hal., *Aloina* Kindb., *Chenia* (including *Leptophascum* (Müll. Hal.) J. Guerra & M.J. Cano), *Crumia* W.B. Schofield, *Dolotortula*, *Hennediella*, *Hilpertia*, *Microbryum* Schimp., *Phascopsis* I.G. Stone, *Sagenotortula*, *Stonea*, *Streptopogon* (Taylor) Wilson ex Mitt., *Syntrichia* and *Willia* Müll. Hal.) in Syntrichieae.

To date, there has been no molecular phylogenetic work on *Hennediella*. DNA-based analyses of *Tortula* and related genera have been also very limited (Werner et al. 2002; Cano et al. 2005). Molecular data using *rps*4 sequence (Werner et al. 2002) suggest that some similar genera, for example *Crossidium*, *Stegonia*, or species of *Pterygoneurum*, could also be included in the *Tortula* concept. In addition, *Syntrichia* formed a monophyletic clade clearly separated from *Tortula*.

In this paper, we present a preliminary phylogenetic analysis based on ITS1, 5.8S, and ITS2 ribosomal DNA sequence data. The ITS region has been widely used to resolve phylogenetic relationships in different bryophytes groups such as *Sphagnum* L. (Shaw 2000), *Amblystegium* Schimp. (Vanderpoorten et al. 2001), *Campylopus* Brid. (Stech 2004), *Didymodon* Hedw. (Werner et al. 2005), or *Tortula* (Cano et al. 2005). A review of the applications of the ITS region in bryophyte systematics is provided in Vanderpoorten et al. (2006). The objectives of this study are to test the monophyly of the species of *Hennediella* and the internal relationship between them based on ITS sequences; and to explore the phylogenetic relationship of *Hennediella* with other members of Pottiaceae to which it is believed to be closely related, for example *Dolotortula*, *Leptophascum*, *Sagenotortula*, and *Tortula*, on the basis of ITS sequences.

Materials and methods

Plant material

A total of 22 accessions were used from material collected in the field and herbarium material from the following herbaria: AAS, ALTA, CAS, CBG, MUB, NY, and personal herbarium of T.L. Blockeel. Eighteen accessions belong to species in *Hennediella*, representing 11 species of the 15 presently recognized in the genus (Cano 2008). The specimens included were selected from approximately 1,100 collections used for a taxonomic revision of the genus (Cano 2008). Material from some species such as *H. heimii* is relatively abundant in the herbaria, however, most of the studied species of this genus are very scarce and the collections in the herbaria available are often old. When possible, several accessions of the same species were extracted and amplified because of the taxonomic problems of the group. However, in many cases it was not possible.

Hennediella marginata, and *H. steereana* were not included in the study because material suitable for sequencing was not available. Unfortunately, the amplification of *H. macrophylla* and *H. antarctica* extracts was unsuccessful. The amplifications of some species from the genus *Syntrichia*, which were used in the study, for example *S. serrulata* (Hook. & Grev.) M.J. Cano, *S. robusta* Dusén, and *S. pseudorobusta* (Dusén) R.H. Zander, also failed. *Tortula platyphylla* was also sampled. Ten sequences were downloaded from GenBank. These represent various outgroups and one population of *H. heimii*. The outgroup species were selected from previous results in Pottiaceae (Cano et al. 2005, Werner et al. 2002, 2003, 2004). These include *Barbula unguiculata*, *Dolotortula mniifolia*, *Leptophascum leptophyllum*, *Pseudocrossidium hornschuchianum*, *Sagenotortula quitoensis*, *Tortula canescens*, *T. cuneifolia*, *T. mucronifolia*, *T. muralis*, and *T. schimperi*. Reference or voucher information, GenBank accession numbers, and the geographical origin of the specimens are summarized in Table 1.

DNA extraction, PCR amplification, and sequencing

Total DNA of gametophore tips from dried herbarium specimens or recent collections was isolated using the CTAB method described by Doyle and Doyle (1987), and stored frozen at −20°C until the PCR reaction was carried out. Amplification reactions were conducted in 50 µl volumes containing approximately 20 ng genomic DNA 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 2 U Taq Polymerase (Biotools), the buffer provided by the manufacturer, and the primers 18S (5′-GGAGAAGTCGTAACAAGGTTTCCG-3′), designed by Spagnuolo et al. (1999) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′; White et al. 1990) at a final concentration of 0.4 mM. PCR reactions were performed in an Eppendorf Mastercycler using the following program: an initial cycle at 94°C for 3 min; 35 cycles of 30 s at 94°C, 30 s at 50°C, and 1 min at 72°C. A final cycle at 72°C for 8 min was included to terminate amplification products. Finally, 2 µl of the amplification products were visualized on 1.5% agarose gel and successful amplifications were cleaned with the GenElute PCR clean-up kit (Sigma–Aldrich). For sequencing, purified PCR products were reacted with BigDye terminator cycle sequencing ready reaction (Perkin–Elmer, Applied Biosystems) using amplification primers. For each DNA, both strands were sequenced. Sequences have been submitted to GenBank (Table 1).

Sequences were aligned using CLUSTALX (Thompson et al. 1997). Bioedit (Hall 1999) was used for minor manual adjustments of the alignment. The aligned matrix is available on request. Maximum parsimony (MP) and maximum likelihood (ML) analyses were conducted using PAUP 4.0b10 (Swofford 2002). Bayesian analyses were performed using MrBayes v 3.1 (Huelsenbeck and Ronquist 2001). Gaps were excluded from all analyses. Both MP and ML analyses were conducted with heuristic searches as described in Harrison and Langdale (2006). For ML analysis, the choice of the model of sequence evolution was performed using the program Modeltest 3.7 (Posada and Crandall 1998) and Modeltest WebServer (http://darwin.uvigo.es) with all options set to default. Modeltest returned TIM + I + G as the optimal model of evolution. Bootstrap analyses (Felsenstein 1985) were carried out with 1,000 replicates for both MP and ML analyses. For the Bayesian analysis, 500,000 generations were run sampling every 100th generation using the default settings: Nst = 6, rates = invgamma (general model of DNA substitution with gamma distributed rate variation across sites and invariant sites). On the basis of empirical evaluation, burn-in (the number of starting generations discarded from further analysis) was set at 100,000 generations. A 50% majority rule tree was constructed using the "sumt" command of MrBayes. The tree was edited using TreeView version 1.6.6 (Page 1996).

Results

The resulting alignment had a total length of 973 bp. The individual sequences vary between 605 bp for one sample of *Tortula mucronifolia* and 788 bp for *Hennediella heteroloma*. Lengths of the ITS1 sequences lay in the range of 214 bp for *T. mucronifolia* and 350 bp for *Dolotortula mniifolia*. The 5.8S rRNA gene has a length of 159 bp in all cases. The final region of ITS2 was excluded from phylogenetic analyses because of missing data in several accessions (approximately 50–60 bp.). Of the aligned sequences 517 sites were constant, 192 variable but parsimony uninformative, and 265 parsimony informative.

MP analyses resulted in 133 most parsimonious trees of 1,224 steps, CI = 0.535, RI = 0.509. The strict consensus tree with bootstrap values for supported nodes is present in the Fig. 1. The ML analysis and the Bayesian inference resulted in trees with a very similar topology (Figs. 1, 2). No incongruence was found between Bayesian, MP, and ML trees. The core *Hennediella* (Figs. 1, 2) was in all the analysis well supported as monophyletic with bootstrap values of 82% (MP) and 86% (ML), and Bayesian inference 100%. All the analysis reveal a *Tortula* clade, which receives low bootstrap support (MP 65%, ML 63%) and maximal Bayesian inference (100% clade credibility).

The *Hennediella* core comprises two clearly separated groups: a clade (group I) formed by *H. bellii* and *T. platyphylla* recognized with a bootstrap support of 99% (MP)

Table 1 List of the species included in the analysis with the voucher's reference and GenBank accession number, and the geographic origin of the specimens

Species	Country	Voucher specimen or reference	GenBank accession no.
Barbula unguiculata Hedw.	Germany	Werner et al. (2005)	AY437129
Dolotortula mniifolia (Sull.) R.H. Zander	Ecuador	*Cano & Gallego 3054* (MUB)	**GQ339748**
Leptophascum leptophyllum J. Guerra & M.J. Cano	Spain	Cano et al. (2005)	AY934544
Hennediella arenae (Besch.) R.H. Zander	Crozet	*Bell 2246* (AAS)	**GQ339744**
Hennediella bellii (E.B. Bartram) R.H. Zander	Ecuador	*Cano 3136a* (MUB)	**GQ339745**
Hennediella densifolia (Hook. f. & Wilson) R.H. Zander	Chile	*Cano 721* (MUB)	**GQ339746**
Hennediella denticulata (Wilson) R.H. Zander	Bolivia	*Cano* et al. *3408a* (MUB)	**GQ339747**
Hennediella heimii (Hedw.) R.H. Zander	Chile	*Buck 41273* (NY)	**GQ339743**
Hennediella heimii (Hedw.) R.H. Zander	Bolivia	*Cano and Jiménez 3745* (MUB)	**GQ339750**
Hennediella heimii (Hedw.) R.H. Zander	Estonia	*Kannukene s.n.* (CBG)	**GQ339749**
Hennediella heimii (Hedw.) R.H. Zander	Antarctica	_	AY13331
Hennediella heteroloma (Cardot) R.H. Zander	Mexico	*Delgadillo 6827* (MUB)	**GQ339751**
Hennediella kunzeana (Müll. Hal.) R.H. Zander	Chile	*Ireland & Bellolio 32390* (MUB)	**GQ339752**
Hennediella longipedunculata (Müll. Hal.) R.H. Zander	South Africa	*Magill & Schelpe 3894* (ALTA)	**GQ339753**
Hennediella longirostris (Hampe ex Müll. Hal.) R.H. Zander	Bolivia	*Cano & Jiménez 3824* (MUB)	**GQ339755**
Hennediella longirostris (Hampe ex Müll. Hal.) R.H. Zander	Ecuador	*Cano 3197* (MUB)	**GQ339754**
Hennediella polyseta (Müll. Hal.) R.H. Zander	Peru	*Cano 2325* (MUB 20612)	**GQ339758**
Hennediella polyseta (Müll. Hal.) R.H. Zander	Ecuador	*Cano 3093* (MUB 21704)	**GQ339759**
Hennediella polyseta (Müll. Hal.) R.H. Zander	Bolivia	*Cano & Jiménez 3877* (MUB)	**GQ339760**
Hennediella stanfordensis (Steere) Blockeel	Australia	*Streimann 48847* (CBG)	**GQ339762**
Hennediella stanfordensis (Steere) Blockeel	Greece	*Blockeel 17/71* (herb. T.L. Blockeel)	**GQ339764**
Hennediella stanfordensis (Steere) Blockeel	USA	*Kellman 3416* (CAS)	**GQ339763**
Sagenotortula quitoensis (Taylor) R.H. Zander	Bolivia	*Cano* et al. *3416* (MUB)	**GQ339761**
Pseudocrossidium hornschuchianum (Schultz) R.H. Zander	Spain	Werner et al. (2005)	AY437134
Tortula canescens Mont.	Spain	Cano et al. (2005)	AY934542
Tortula cuneifolia (Dicks.) Turner	Spain	Cano et al. (2005)	AY934543
Tortula mucronifolia Schwägr.	Denmark	Cano et al. (2005)	AY934590
Tortula muralis Hedw.	Serbia	Cano et al. (2005)	AY437132
Tortula platyphylla Mitt. (1)	Chile	*Cano 111* (MUB 20840)	**GQ339756**
Tortula platyphylla Mitt. (2)	Chile	*Cano 22* (MUB 20841)	**GQ339757**
Tortula schimperi M.J. Cano, O. Werner & J. Guerra	USA	Cano et al. (2005)	AY934575
Tortula subulata Hedw.	France	Cano et al. (2005)	AY934572

Sequences in bold were obtained in this study

The collector and collection number used in this study are in italics

and 100% (ML) and Bayesian inference 100%, and another clade (group II) formed by *H. heimii*, *H. longirostris*, *H. polyseta*, *H. stanfordensis*, *H. kunzeana*, *H. arenae*, and *H. densifolia* moderately supported by bootstrap values of 82% (MP) and 70% (ML), and a clade credibility value of 100% under Bayesian inference (Figs. 1, 2). The affinities of *H. denticulata* are unresolved under MP and ML analysis, although it is included in the group II in the Bayesian inference with 86% clade credibility. In group II, a subclade is formed by *H. heimii*, *H. longirostris*, and *H. polyseta* unsupported by MP analysis, weakly supported by ML analysis (76% bootstrap value), and a clade credibility value of 100% under Bayesian inference. The relative position of the remaining species of *Hennediella* is less clear but a clade comprising *H. kunzeana* and *H. stanfordensis* is supported by bootstrap values of 82% (MP) and 86% (ML), and 98% of Bayesian inference.

Hennediella heteroloma and *H. longipedunculata* are included in the clade that comprises *Leptophascum leptophyllum*, *Sagenotortula quitoensis*, *Dolotortula mniifolia*, *Hennediella* core, and *Tortula* clade supported by bootstrap values of 96% (MP) and 93% (ML), and Bayesian inference 100% clade credibility. However, the internal relationships of these groups remain ambiguous.

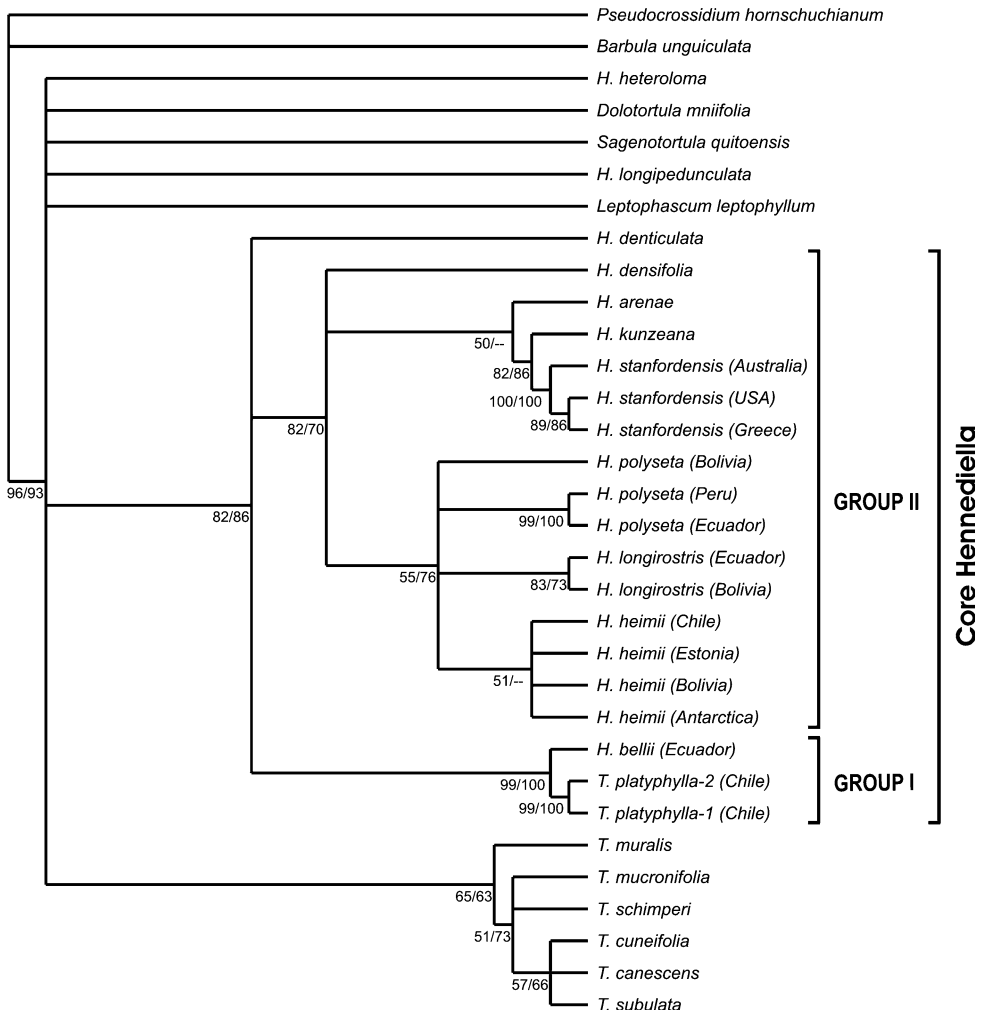

Fig. 1 Strict consensus of 133 most parsimonious trees with tree length of 1,224 steps (RI = 0.509, CI = 0.53, considering only parsimony-informative sites), based on ITS sequences. Bootstrap values above 50% are given below the clades using maximum parsimony/maximum likelihood. The tree was rooted with *Pseudocrossidium hornschuchianum* as outgroup

Discussion

The ITS sequences support the recognition of the core *Hennediella* (including *Tortula platyphylla*), as monophyletic involving two clades, marked as group I and II in Figs. 1 and 2. Group I includes *H. bellii* and *T. platyphylla*. Group II is formed by *H. heimii*, *H. longirostris*, *H. polyseta*, *H. stanfordensis*, *H. kunzeana*, *H. arenae*, and *H. densifolia*. All these species share a red KOH reaction, a differentiated leaf border, and, usually, dentate margins.

Group I, which includes the South American *T. platyphylla* and *H. bellii* is supported by a number of morphological characteristics, for example intramarginal leaf border and absence of teeth in the leaf margins. Its morphological similarity has already been observed in previous taxonomic revisions of the team (Cano 2008; Cano and Gallego 2008); the generic affinities of both species were uncertain, however. Our molecular data suggest that *T. platyphylla* is included in *Hennediella*.

All the species included in group II exhibit marginal border and dentate leaf margins, although their relative affinities are less clear. The affinities of *H. denticulata* remain ambiguous in the core *Hennediella*. The diagnostic characteristics of *H. denticulata* are its smooth (rarely with low papillae) and broad upper and middle laminal cells. The rest of *Hennediella* species has papillose laminal cells, except, generally, *H. heimii*. The position of *H. arenae* and *H. densifolia* within group II are not resolved in our analysis. *Hennediella arenae* is sister of the *H. kunzeana*–*H. stanfordensis* clade with MP and sister of the remaining species of the group II with Bayesian inference. That *Hennediella polyseta*, *H. longirostris*, and *H. heimii* form a group is well supported. A characteristic that defines this subclade is the absence of peristome teeth. *Hennediella kunzeana* and *H. stanfordensis* are resolved into a subclade with good support. According to Cano (2008) both species exhibit the same gametophytic morphologic characteristics and only they can be separated by sporophytic characteristics that in *H. stanfordensis* are based on immature capsules, which resemble those of *H. macrophylla*. The inference based on ITS data is congruent with the hypothesis that these taxa could be conspecific.

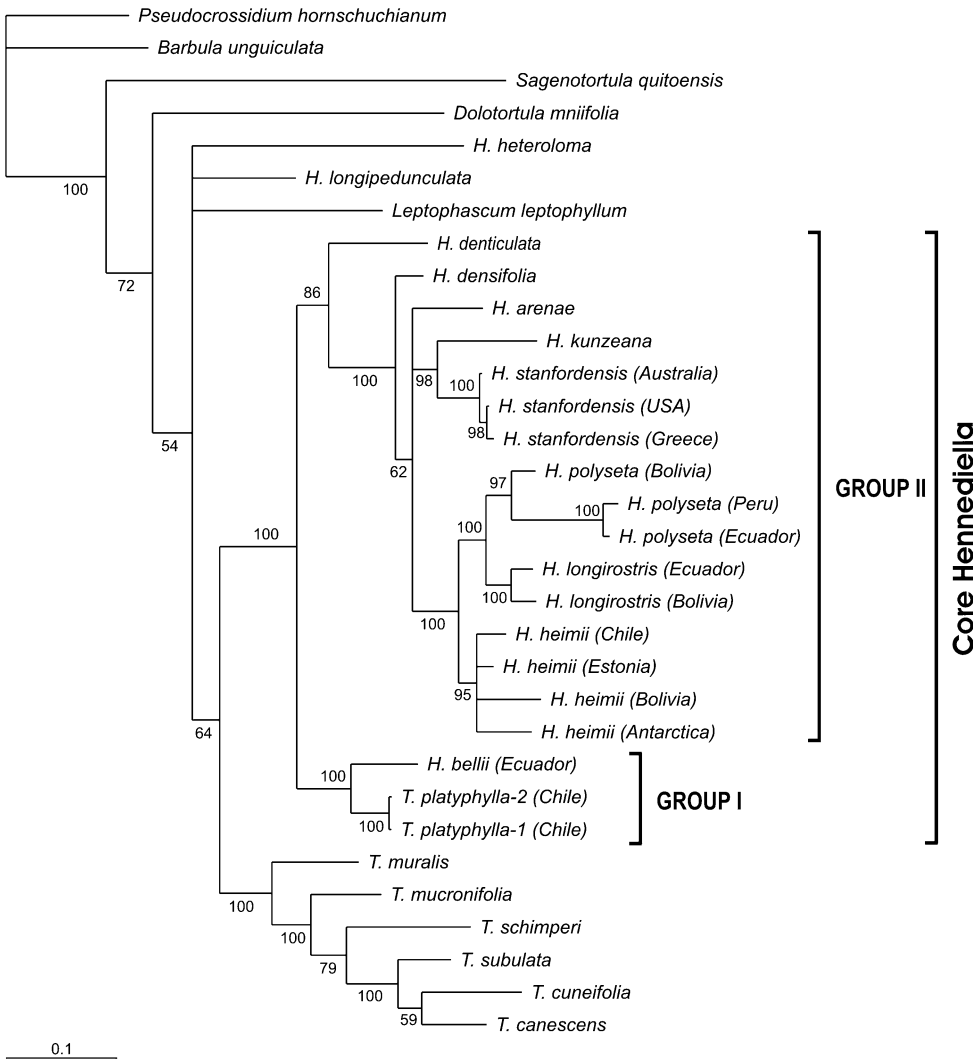

Fig. 2 Phylogram based on the Bayesian approach with ITS sequence data. *Numbers* indicate the clade credibility values of the nodes. Values above 50% are shown. The tree was rooted with *Pseudocrossidium hornschuchianum* as outgroup

The affinities among *Hennediella heteroloma*, and *H. longipedunculata* and *Hennediella* core and the *Tortula* clade are ambiguous. *Hennediella heteroloma* is only known from some localities of Mexico. It is characterized by its intramarginal unistratose leaf border of elongate smooth cells in several rows edged by a usually single row of shorter cells with many minute papillae, obovate to lingulate leaves, with the costa generally ending before the apex and upper and middle laminal cells with (7)10–24(30) papillae. The intramarginal border and the absence of dentate margins relate this species with *H. bellii* or *Tortula platyphylla*, but these species have bistratose leaf margins and smaller leaves and middle laminal cells.

Hennediella longipedunculata is endemic in Namibia and South Africa. It is characterized by lingulate to ovate leaves, not dentate leaf margins, a circular and prominent cross-section of the costa, small upper and middle laminal cells, outer middle marginal cells usually oblate (ratio length/width (0.3)0.4–1), and the inner marginal cells quadrate or short-rectangular (ratio length/width (0.4)1–2(2.6)), and by a peristome of 32 slightly spirally twisted teeth, with a short basal membrane (22.5–62.5 μm long). This species has plane leaf margins, orange leaf KOH reaction, and marginal border of differentiated cells (in some cases not very conspicuous), which supports its inclusion in the genus *Hennediella*. However, the circular and prominent cross-section of the costa was not found in any other species of *Hennediella*. In addition, it lacks dentate leaf margins, the cells of the outer rows of the leaf margins are oblate and the inner cells quadrate or short-rectangular (instead of long-rectangular or linear as most of the species of the genus), which supports its inclusion in *Tortula*. Further sampling of *Hennediella* or *Tortula* or the inclusion of sequences of other members of Pottiaceae related with *Tortula* could clarify the phylogenetic position of both species.

Hennediella marginata could not be included in the study. According to Cano (2008), it has deviant characteristics of the genus, for example the prorate and mitrate

calyptra. These characteristics are reminiscent of the recently described species *Ludorugbya springbokorum* Hedd. and R.H. Zander (Hedderson and Zander 2007). Both species share the short-cylindrical capsules with a very long-conic operculum, and the mitrate calyptra. They can be separated by the strongly differentiated pluristratose leaf border of (7)8–13 rows of cells in *H. marginata*, weakly differentiated border of 1–3 narrower thicker-walled and less papillose cells in *L. springbokorum* and the strongly differentiated perichaetial leaves in *L. springbokorum*, which are weakly differentiated in *H. marginata* and, in general, in all the species of *Hennediella*.

The topology here obtained suggests a close relationships among *Tortula* and *Hennediella*. However, the results are preliminary because of the small number of *Tortula* species included. In addition, the rest of the closely related genera (*Dolotortula*, *Sagenotortula*, *Leptophascum*) included in our analysis and the correct placement of *H. heteroloma* and *H. longipedunculata* are not resolved in this study. Additional sampling to assess the relationship to other species of *Tortula* and genera of Pottiaceae such as *Crumia* or *Ludorugbya* Hedd. and R.H. Zander are required.

Therefore, the present circumscription of *Hennediella* is provisional until a complete morphologic revision of genus *Tortula* can be performed. In addition, an extension of the taxon sampling with the addition of more species and samples of *Hennediella* and *Tortula* and the use of other phylogenetic markers are required to better understand of the internal phylogenetic relationships of *Hennediella* and its relationship to close genera.

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