CSIRO PUBLISHING
www.publish.csiro.au/journals/asb *Australian Systematic Botany* **17**, 407–421

Inter- and infraspecific relationships in the Gondwanan liverwort genus *Hymenophyton* (Hymenophytaceae, Hepaticophytina). Studies in austral temperate rain forest bryophytes 23

Tanja Pfeiffer[A,C], *Friederike Schaumann*[A], *Gabriela G. Hässel de Menéndez*[B] *and Wolfgang Frey*[A]

[A]Freie Universität Berlin, Institut für Biologie, Systematische Botanik und Pflanzengeographie, Altensteinstraße 6, D–14195 Berlin, Germany.
[B]Consejo Nacional de Investigaciones Científicas y Técnicas, Museo Argentino de Ciencias Naturales B. Rivadavia, Av. Angel Gallardo 470, DJR 1405 Buenos Aires, Argentina.
[C]Corresponding author: tanja_pfeiffer@gmx.de

Abstract. Inter- and infraspecific relationships in *Hymenophyton* Dumort. were studied by sequencing of the cpDNA *trn*T_{UGU}-*trn*L_{UAA} 5′exon intergenic spacer, *trn*L_{UAA} 5′exon and *trn*L_{UAA} intron and nrDNA internal transcribed spacer 2 (ITS2), and by morphological examination of representative specimens. Based on the molecular data, four taxa were recognised, comprising (i) Australasian *H. flabellatum* (Labill.) Dumort. ex Trevis., (ii) Chilean specimens, (iii) New Zealand *H. leptopodum* (Hook.f. & Taylor) A.Evans samples and (iv) a Tasmanian *H. leptopodum* specimen, respectively. The former three clades are supported by high bootstrap values, while the affinities of the latter specimen remain ambiguous in the calculated *trn*T-*trn*L and ITS2 trees. The observed sequence divergence supports the existence of a distinct *Hymenophyton* taxon in southern South America, delimitated from *H. flabellatum* and *H. leptopodum* on species level: *H. pedicellatum* Steph. This reinstated species is described in detail; differentiating morphological and molecular characters are presented. *Hymenophyton* taxa have a basically palaeoaustral distribution pattern. Biogeography, dispersal biology and phylogeography of the taxa are discussed.

Introduction

Hymenophyton Dumort. is a small genus of dendroid thalloid liverworts belonging to the Hymenophytaceae R.M.Schust. in the Metzgeriidae. Habitually, *Hymenophyton* species resemble the dendroid (to fan-shaped) Metzgerialean taxa of the Pallaviciniaceae, e.g. *Jensenia* Lindb. spp., *Symphyogyna hymenophyllum* Mont. & Nees and *S. podophylla* (Thunb.) Mont. & Nees. The taxa are characterised by plagiotropic, often branched rhizome-like axes with aerial shoots divided into erect stalks and flabellate or palmate fronds with the forked thallus wings spreading out more or less horizontally. However, in *Hymenophyton*, the antheridia and archegonia are in ventral position, developing on very reduced branches postical on the midrib (cf. Fig. 7).

The genus *Hymenophyton* shows a strictly antipodal distribution pattern (Schuster 1982) with a main centre in Australasia. It occurs in temperate rain forests of New Zealand and adjacent islands, Tasmania, eastern and south-eastern Australia (Qld, NSW, Vic.), New Caledonia and Fiji Is, and is known from only seven localities in southern South America [e.g. Evans 1925; Pfeiffer 2000*a*; Frey and Schaumann 2002; coll. Frey 2003, cf. Table 1; Fig. 1]. [Specimens reported from the Neotropics are either probably erroneous (Colombian specimen collected by Weis, see Uribe and Gradstein 1998) or, more commonly, misidentifications of *Symphyogyna* Nees & Mont. or *Jensenia* Lindb. (e.g. Costa Rican specimens, Gradstein *et al*. 1994). The latter is also true for the Tristan da Cunha record (Grolle 1987).]

A similar distribution pattern with palaeoaustral disjunctions can be found in other bryophyte taxa with dendroid habit, e.g. species of *Jensenia, Symphyogyna* Nees & Mont. and *Hypopterygium* Brid. (e.g. Kruijer 2002; Schaumann *et al*. 2003). These taxa, concentrated in the Southern hemispheric temperate rain forests (mainly *Nothofagus* forests) of New Zealand, Tasmania, south-eastern Australia and southern Chile and Argentina, have been interpreted as remnants of a palaeoaustral flora with Gondwanan origin (Schuster 1982; Frey 1990; Frey and Beever 1995).

The number of species included in *Hymenophyton* (excluding the prostrate and rather simple, ± ligulate

 26 August 2004 10.1071/SB03022 1030-1887/04/040407

Table 1. List of studied *Hymenophyton* (ingroup-) and *Symphyogyna* (outgroup-) specimens with abbreviations, sampling and voucher data and GenBank database accession numbers for sequences of the cpDNA *trn*T_{UGU}-*trn*L_{UAA} 5′exon intergenic spacer and *trn*L_{UAA} intron and nrDNA ITS2 (in italics)

#, partial sequence, ##, no reliable PCR product(s)

Taxon abbreviation	Location of origin	Voucher; herbarium	GenBank accession no. (spacer/intron *ITS2*)
		Hymenophyton leptopodum	
HL1	New Zealand, Hutt Valley	L.J. Porter, 20 Aug. 1976; MPN 17030	AY368636 / AF143525
HL2	New Zealand, Karamea	H. & W. Frey 94–155; CHR	AY368637 / AF143526 *AY640219*
HL3	New Zealand, Karamea	W. Frey & T. Pfeiffer 98-Mo37; CHR	AY368638 / AF143527
HL4	New Zealand, Haast	W. Frey & T. Pfeiffer 98-Mo27; CHR	AY368639 / AF143528
HL5	New Zealand, South Otago	E.O. Campbell 98-Mo67	AY368640 / AF143529 *AY640220*
HL Tas	Australia, Tasmania	J. Jarman, 12 Jan. 1998; HO 52508	AY368641 / AF143524 *AY640218*
		Hymenophyton flabellatum	
HF1	New Zealand, Waikato	E.O. Campbell, 30 Oct. 1995; MPN 18962	AY368630 / AF143519
HF2	New Zealand, Urewera National Park	H. & W. Frey 94–83; CHR	AY368631 / AF071840
HF3	New Zealand, Urewera National Park	T. Pfeiffer 98-Mo59; CHR	AY368632 / AF143520 *AY640216*
HF4	New Zealand, Hutt Valley	L.J. Porter, 20 Aug 1976; MPN 17029	AY368629 / AF143518
HF5	New Zealand, Karamea	W. Frey & T. Pfeiffer 98-Mo38; CHR	AY368633 / AF143521 *AY640217*
HF6	New Zealand, Franz-Josef Glacier	W. Frey & T. Pfeiffer 98-Mo3; CHR	AY368634 / AF143523
HF Tas	Australia, Tasmania	J. Jarman, 9 Feb 1999; HO 51654	AY368635 / AF143522
HF Aus1	Australia, Victoria	J.A. Curnow 1494; B 30 0274476	AY368646
HF Aus2	Australia, Victoria	R.D. Seppelt; B 30 0239329	AY368647
		Southern South American *Hymenophyton* specimens	
CHILE 1	Chile, Puyehue, frente Lago Toro	G. Hässel de Menéndez 10849, BA, herb. GHM	AY368648 / (#)
CHILE 2	Chile, X. Región, Hornopirén	W. Frey & F. Schaumann 01–145a; VALD	AY368649 *AY640221*
CHILE 3	Chile, X. Región, S Hornopirén	W. Frey 03–01; BA, herb. Frey	AY368650 *AY640222*
CHILE 4	Chile, X. Región, Galeta Gonzalo	W. Frey 03–03; BA, herb. Frey	AY368651
CHILE 5	Chile, XII. Región Magallanes, Pto. Toro	M.M. Schiavone 2274b; TBPA-B	# / ##
JF	Juan Fernández Is Mas a Fuera	R. Hatcher & J. Engel, 5-XII-1965 (no. 553 Bryophyta of the Juan Fernández Islands); MSC, BA	##
ARG	Argentina, Río Negro Puerto Blest	C.C. de Donterberg & G. Hässel de Menéndez 1859*a*; BA 12955	##
		Outgroup: *Symphyogyna* spp.	
S. podophylla 1 (S pod 1)	La Réunion Cirque de Mafate	T. Pfeiffer 2002–21; herb. Pfeiffer	AY368642 / AY289140
S. podophylla 2 (S pod 2)	Chile, XII. Región Prov. Magallanes, Punta Arenas	J.-P. Frahm 1–20; BONN	AY368643 / AY289141
S. podophylla 3 (S pod 3)	Chile, X. Región Parque Nacional Puyehue	W. Frey & F. Schaumann 01–301; VALD	AY368644 / AY289145 *AY289169*
S. hymenophyllum (S hym)	New Zealand, Urewera National Park	T. Pfeiffer 98-T206; CHR	AY368645 / AY289153 *AY289177*

Podomitrium taxa) varied. In 1892, Evans recognised three species, *H. flabellatum* (Labill.) Dumort. ex Trevis. and *H. leptopodum* (Hook.f. & Taylor) A. Evans of New Zealand and *H. mülleri* (Gottsche) A. Evans of Australia. Stephani (1900) reduced the latter to synonymy of *H. flabellatum*, extending the species' range to include New Zealand, Australia and Tasmania, and reported *H. leptopodum* from New Zealand and Tasmania. Later, *H. flabellatum* was also recorded from the Fiji Is based on a collection of Seemann (cf. Evans 1925). In southern South America, *Hymenophyton* specimens were described as *H. pedicellatum* Steph., a rather delicate species with few dichotomies (≤ 3) from Isla Huafo, and as *Symphyogyna integerrima* Steph. from the Juan Fernández Is (Mas a Fuera; Stephani 1911). For New Caledonia, Pearson (1922) described *H. furcatum* Pearson as a new species larger than *H. flabellatum*.

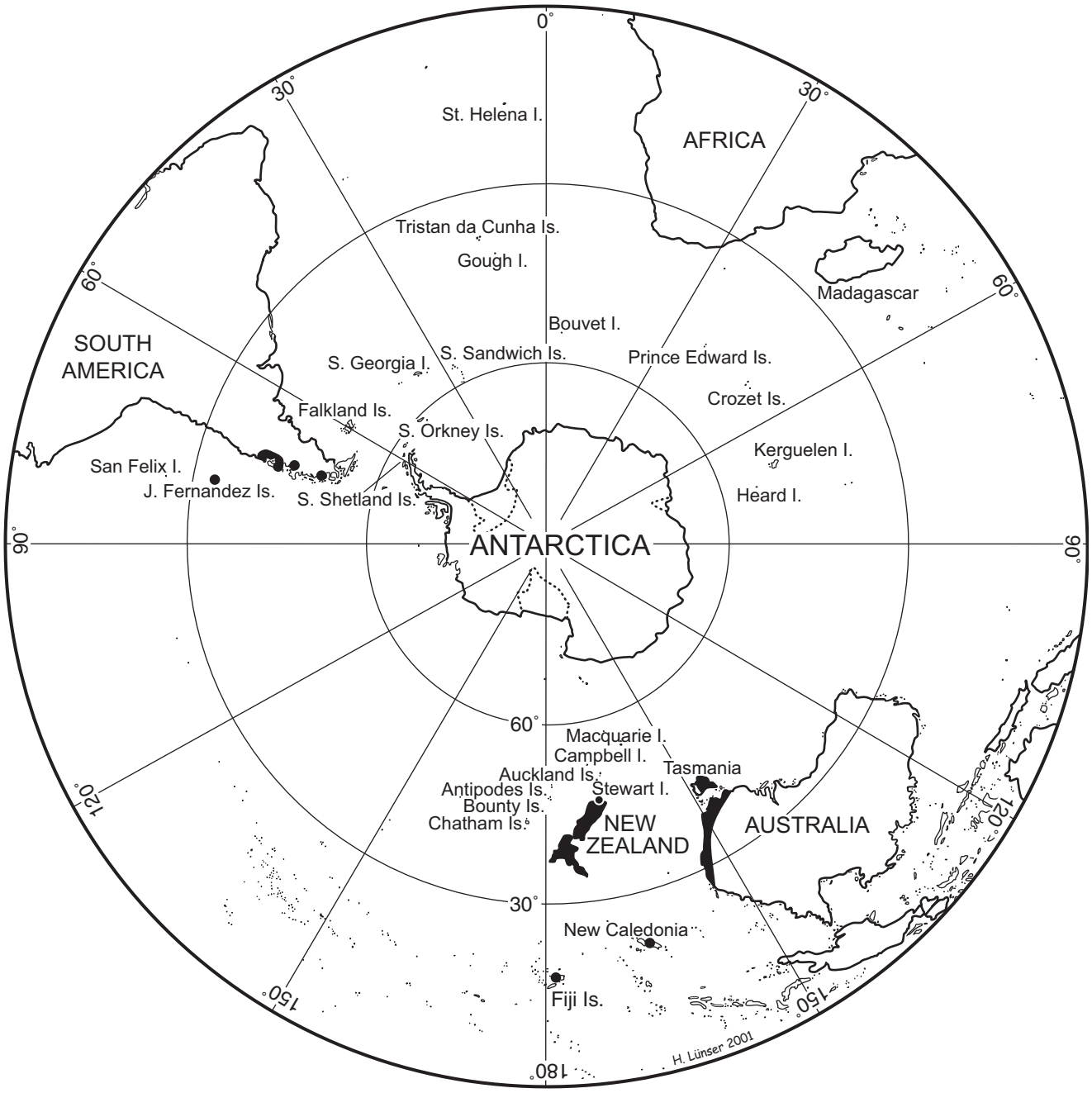

Fig. 1. World-wide distribution of *Hymenophyton* (black areas and dots).

After an investigation of specimens from all these regions, Evans (1925) concluded that there was only one species, *H. flabellatum*. He reduced all described previously species of the genus to its synonymy. This monotypic circumscription of the genus was accepted by many later authors (e.g. Schuster 1963; Miller *et al*. 1983). More recent studies, however, including phytochemical and molecular analyses, have shown the existence of at least two distinct species in New Zealand and Tasmania, *H. flabellatum* and *H. leptopodum* (Campbell *et al*. 1975; Markham *et al*. 1976; Grolle 1987; Pfeiffer 2000*a*). Specimens from the other regions are usually still recognised as *H. flabellatum* (e.g. in southern South America, Hässel de Menéndez and Solari 1985; Frey and Schaumann 2002).

A first insight in the molecular phylogenetic relationships of New Zealand and Tasmanian *Hymenophyton* taxa was presented by Pfeiffer (2000*a*) based on a sequencing of the cpDNA *trn*L_{UAA} intron. In the present study, the inter- and

infraspecific relationships in *Hymenophyton* are further examined. *Hymenophyton* specimens from nearly the whole distribution range of the genus are studied morphologically and by a sequencing of the cpDNA *trn*T_{UGU}-*trn*L_{UAA} 5′exon intergenic spacer/*trn*L_{UAA} 5′exon/*trn*L_{UAA} intron of the *trn*T-F region and the nrDNA internal transcribed spacer 2 (ITS2) in representative specimens. Based on the analysis of these data, the main aims of the study were to clarify the situation regarding the number of species and resolve the inter- and infraspecific relationships of these taxa, with a focus on biogeography and phylogeography of the palaeoaustral Gondwanan taxa.

Material and methods

Plant material

A total of 19 *Hymenophyton* specimens from New Zealand, Tasmania, Australia and southern Chile were studied by DNA-sequencing of the cpDNA *trn*T-*trn*L spacer, *trn*L 5′exon and *trn*L intron, including the New Zealand and Tasmanian *H. flabellatum* (Labill.) Dumort. ex Trevis. and *H. leptopodum* (Hook.f. & Taylor) A. Evans specimens investigated by Pfeiffer (2000*a*; *trn*L intron). For some further specimens from southern Chile, southern Argentina and Juan Fernández Is unfortunately no or no reliable PCR-products could be obtained from the rather old material (see Table 1). Sequences of nrDNA ITS2 were obtained for seven *Hymenophyton* specimens. The voucher data, abbreviations and respective GenBank accession numbers for the specimens are summarised in Table 1.

Specimens were also investigated morphologically, along with older samples from these regions (not suitable for sequencing), including material from Fiji (deposited in NY) and the type specimens of *H. pedicellatum* Steph. and *Symphyogyna integerrima* Steph. (UPS). Only from New Caledonia (cf. *H. furcatum* Pearson) was no material available.

Molecular analysis

Deoxyribonucleic acid preparation, PCR and sequencing

Deoxyribonucleic acid was extracted from fresh plant material or herbarium tissue (partly Silica-dried) following the method of Doyle and Doyle (1990). Amplifications were performed with primer pairs A_M/D_x (*trn*T_{UGU}-*trn*L_{UAA} 5′exon intergenic spacer, *trn*L_{UAA} 5′exon), C_M/D_M (*trn*L_{UAA} intron) or A_M/D_M (complete cpDNA region; A_M, C_M, D_M slightly modified after Taberlet *et al*. 1991; Meißner *et al*. 1998, for D_x see Schaumann *et al*. 2004), and 5.8F/25R for nrDNA internal transcribed spacer 2 (ITS2; after Baldwin 1992). Amplifications, purification of PCR products and sequencing follow the method described by Quandt *et al*. (2001).

Phylogenetic analyses

The sequences were aligned manually in the alignment editor Align32 (Hepperle 1997, 2003). In addition to substitution data, indels differentiating between the *Hymenophyton* specimens in the non-coding cpDNA segments were coded in a binary matrix and included in some analyses as additional characters.

In several specimens it was not possible to determine a few (*c*. 10) of the first bases of the *trn*T-*trn*L spacer. These bases were excluded from the analyses in all specimens. The lengths of the *trn*T-*trn*L spacer given in the Results are based on this dataset, and values are hence smaller than the ‘real’ lengths. Similarly, the calculated sequence divergences may be underestimations with the real values being slightly higher because of the possibility of undetected substitutions within the excluded first part of the spacer sequences.

In a second alignment of the *trn*T-*trn*L region, four specimens of *Symphyogyna hymenophyllum* and *S. podophylla* were used as outgroup representatives (Table 1). Some parts of the *trn*T-*trn*L spacer (3′ end) and *trn*L intron were difficult to align between *Hymenophyton* and the *Symphyogyna* specimens. Therefore, analyses were conducted with different datasets, including or excluding these sequence parts, respectively.

ITS2 sequences were obtained for seven *Hymenophyton* specimens and aligned with two outgroup *Symphyogyna* spp.

Calculations of molecular trees from the *trn*T-*trn*L dataset (*trn*T-*trn*L spacer, *trn*L 5′exon, *trn*L intron) and nrDNA ITS2, respectively, were performed with PAUP 4.0b10 (Swofford 2002). Maximum parsimony trees were evaluated separately for the cpDNA and ITS datasets (performing a heuristic search or branch-and-bound search, respectively) with the following options: all characters unweighted and unordered, multistate characters interpreted as uncertainties, gaps coded as missing data, performing TBR branch swapping, collapse zero length branches, MulTrees option in effect. Heuristic bootstrap searches were performed with 1000 replicates, 1000 random addition replicates per bootstrap replicate, and the same options in effect.

For the *trn*T-*trn*L dataset, maximum likelihood analyses were executed assuming a general time reversible model (GTR + G) and a rate variation among sites following gamma distribution (three categories represented by mean), with the shape being estimated. Likelihood bootstrap analyses were performed with 100 replicates.

Results

Molecular analysis

The aligned sequences of the cpDNA *trn*T-*trn*L region of 19 *Hymenophyton* specimens from New Zealand, Tasmania, Australia and southern Chile (Table 1) are given in Fig. 2. The Tasmanian sample HL Tas has the longest spacer (243 bp) and shortest (332 bp) intron sequences.

All studied *Hymenophyton* specimens have an identical *trn*L 5′exon sequence (35 bp); in comparison with the outgroup *Symphyogyna* specimens (alignment not shown) two substitutions can be observed. Differences between ingroup specimens are obvious in the *trn*T-*trn*L spacer and the *trn*L intron. The studied specimens form four distinct groups, which comprise specimens of the different taxa or regions: the *H. flabellatum* (HF) samples from (i) Australasia [i.e. New Zealand HF1–5, Tasmanian HF Tas and Australian HF Aus1, 2], from (ii) Chile [CHILE 1–4], the (iii) New Zealand [HL1–5] and (iv) Tasmanian *H. leptopodum* [HL Tas] specimens. These groups can also be detected in the nrDNA ITS2 dataset.

Divergence within the groups

The Australasian HF samples have *trn*T-*trn*L spacer lengths of 250–251 bp and intron lengths of 344 or 352 bp. Apart from a length polymorphism in HF Aus2 caused by an indel at the variable 3′end, the *trn*T-*trn*L spacer sequences are identical. In the *trn*L intron, an indel is observed in HF4 (cf. Pfeiffer 2000*a*); the sequence divergence amounts to 0–0.9% because of up to three substitutions.

The Chilean *Hymenophyton* samples are even more uniform, with identical *trn*T-*trn*L spacer and *trn*L intron

sequences of 241 and 335 bp in length, respectively. Another specimen (CHILE 5) also showed no divergence in a partial *trn*T-*trn*L spacer sequence (not shown).

The same spacer and intron lengths (241, 335 bp) are recorded for most New Zealand HL specimens, solely in HL1 the *trn*T-*trn*L spacer sequence is 1 bp shorter due to an indel in a poly-T stretch (Fig. 2). This latter specimen is further differentiated by one substitution in the *trn*T-*trn*L spacer (sequence divergence 0.4%). The spacer sequences of the other and the *trn*L intron sequences of all New Zealand HL samples are identical.

The ITS2 has a length of 248–252 bp in *Hymenophyton*. The two analysed specimens of both New Zealand HL (HL2, 5) and Australasian HF (HF3, 5) have identical sequences of 251 and 248 bp in length, respectively. In the Chilean specimens, eight substitutions differentiate between CHILE 2 and CHILE 3 (sequence divergence 3.2%; length 252 bp).

Divergence between the groups (interregional infraspecific and interspecific variation)

Comparing the Australasian and Chilean *H. flabellatum* specimens, the sequence divergence amounts to 7.5–7.9% (18 or 19 substitutions) in the *trn*T-*trn*L spacer and 3.3–3.9% (11–13 substitutions) in the *trn*L intron sequences. Additionally, at least three indels differentiate between specimens from the two regions.

In *H. leptopodum*, 7 or 8 substitutions are recorded in both sequenced regions between the Tasmanian and New Zealand specimens, corresponding to a sequence divergence of 2.9–3.4% (*trn*T-*trn*L spacer) and 2.4% (*trn*L intron), respectively. Furthermore, at least three differentiating indels are observed, including an indel unique to HL Tas.

On the interspecific scale, between Australasian HF and HL specimens, 15 (6.2%, HL Tas) to 18 or 19 substitutions (7.5–8.0%, New Zealand HL1–5) are detected in the *trn*T-*trn*L spacer, and a further 11–14 (3.3–4.2%, HL Tas) and 9–11 substitutions (2.7–3.3%, HL1–5) in the *trn*L intron. Additionally, 4 or 5 indels of 1–12 bp in length cause differences in sequence lengths (Fig. 2).

The Chilean *Hymenophyton* specimens are differentiated from *H. leptopodum* by a sequence divergence of 5.4–5.9% (13 or 14 substitutions) in the *trn*T-*trn*L spacer. In the *trn*L intron, sequence divergence is lower with 2.4% (New Zealand HL) and 3.6% (HL Tas), which is due to 8 and 12 substitutions, respectively. In the *trn*T-*trn*L spacer, two or three indels differentiate between Chilean HF and New Zealand HL despite of the uniform length; in the *trn*L intron no indels are observed in these specimens. Length differences between HL Tas and Chilean samples are related to four indels in spacer and intron.

In the nrDNA ITS2, 18 substitutions are observed between Australasian HF and New Zealand HL (7.3%). HL Tas is differentiated by 11 substitutions (4.4%) and 15 substitutions (6.1%) from Australasian HF and New Zealand HL, respectively. The highest sequence divergence is recorded between the Chilean specimens and the other samples (18–24 substitutions, 7.3–9.6%), especially with New Zealand HL (20 and 24 substitutions, 8.0 and 9.6%). The length differences in the ITS2 are caused by 1–3 indels of 1–2 bp in length.

Molecular phylogenetic trees

The alignment of the *trn*T-*trn*L sequences of *Hymenophyton* and *Symphyogyna* spp. (available on request) contains 687 characters, with 167 variable (24.3%) including 155 parsimony-informative characters (92.8%, 22.6% of total characters). Different analyses were performed using either the complete alignment or datasets excluding parts that were difficult to align between the in- and outgroup taxa.

The *Hymenophyton* alignment (Fig. 2) has a length of 642 bp, out of 49 variable characters (7.6%) 40 are parsimony-informative (81.6%, 6.2% of total characters). In some analyses, seven indels observed between taxa were included as additional parsimony-informative characters.

The calculated molecular phylogenetic trees from the maximum parsimony (MP; Fig. 3) and maximum likelihood (ML; Fig. 4) analyses show monophyly of the *Hymenophyton* specimens. Within *Hymenophyton*, the analyses found the four groups detected by comparison of the sequences. With the exception of HL Tas (see below), the clades are clearly separated, with very high to maximal bootstrap support (98–100%) of the Australasian HF, New Zealand HL and Chilean clades, respectively, in the MP trees. Within these groups, specimens are placed on polytomic branches with only HF6 and HL1 (62–63%) slightly differentiated from the remaining Australasian HF and New Zealand HL samples, respectively (Fig. 3). This topology is robust, the same clades are obtained in the trees of the ML calculations (Fig. 4), even though partly with lower bootstrap support.

In MP analyses of the *Hymenophyton* alignment (without outgroup representatives, Fig. 5), the generated unrooted trees also show a clear differentiation of Australasian HF, New Zealand HL and the Chilean samples. The main topology of these trees (apart from the position of HL Tas, see below) is unaffected by the dataset used, i.e. whether 4 bp at the 3′ end of the *trn*T-*trn*L spacer (Fig. 2) are in- or excluded and whether indels are coded as additional characters or not. Differences are only obvious in the number of calculated trees, their lengths and the bootstrap support for the respective branches.

This infrageneric differentiation within *Hymenophyton* is also obvious in the trees calculated from the ITS2 alignment of 272 positions with 70 variable (25.7%) including 51 parsimony-informative characters (71.4%, 18.4% of total characters),

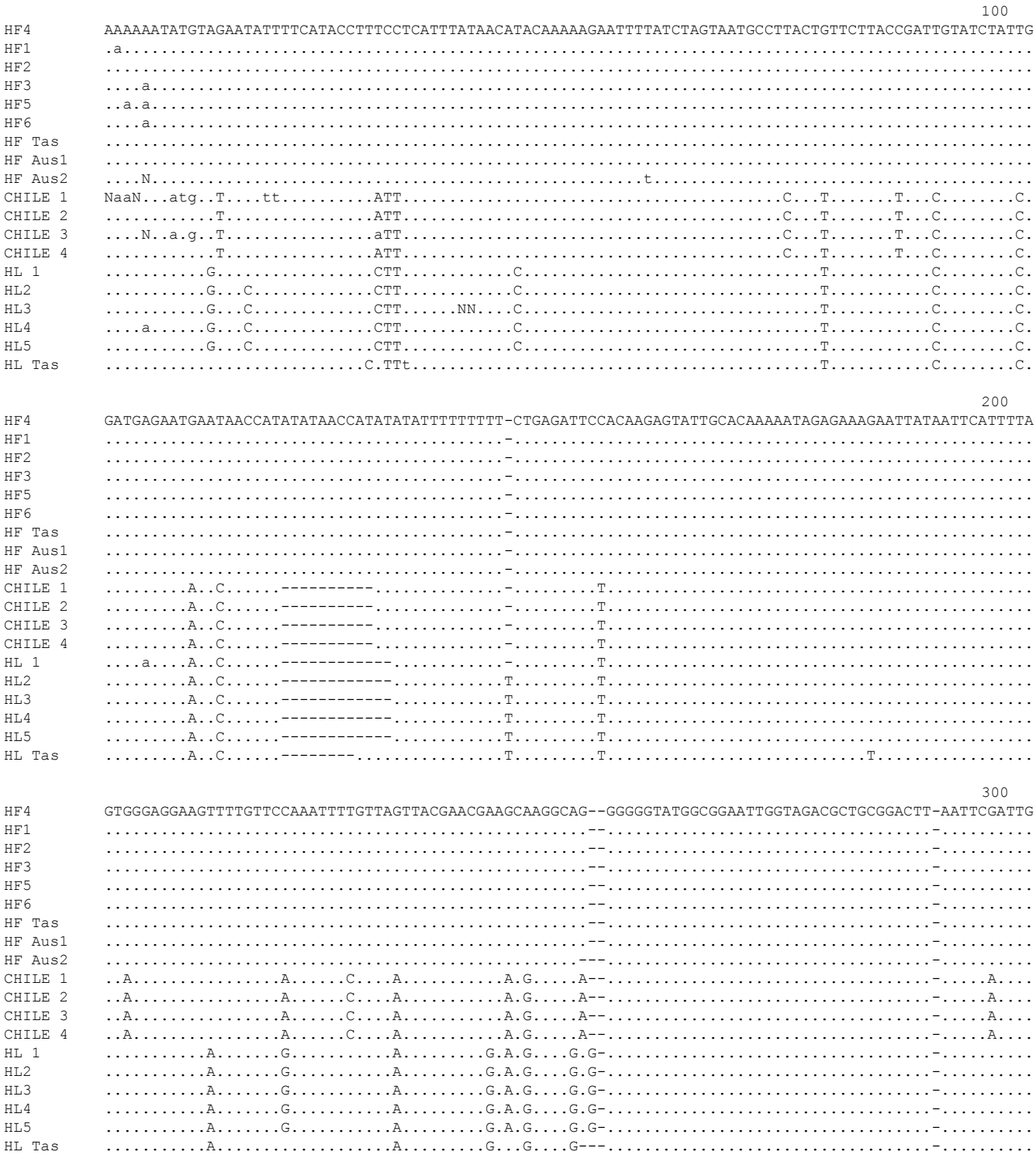

Fig. 2. Aligned cpDNA *trn*T-*trn*L 5′exon spacer, *trn*L 5′exon and *trn*L intron sequences of *Hymenophyton flabellatum* [HF], *H. leptopodum* [HL] and Chilean *Hymenophyton* specimens [CHILE] (for abbreviations see Table 1). Positions equal with first sequence are given as dots. Positions 1–253 - *trn*T-*trn*L spacer, 255–289 - *trn*L 5′exon, 291–642 - *trn*L intron.

```
                                                                                                              400
HF4        AGCCTTGGTGGAGAAATCTACTAAGTGATTGTTTCCATATTCAGGGAAACCTAGGTTGAAAAAGAAGAACCTACTAGGTAATCCTGAGCCAAATTTCTAT
HF1        ....................................................................................................
HF2        ....................................................................................................
HF3        ................................................................................................A...
HF5        ....................................................................................................
HF6        ....................................................................................................
HF Tas     ....................................................................................................
HF Aus1    ....................................................................................................
HF Aus2    ....................................................................................................
CHILE 1    .............................................................N..a-----------------------------------
CHILE 2    ....................................................................................................
CHILE 3    ....................................................................................................
CHILE 4    ....................................................................................................
HL 1       ....................................................................................................
HL2        ....................................................................................................
HL3        ....................................................................................................
HL4        ....................................................................................................
HL5        ....................................................................................................
HL Tas     ....................................................................................................

                                                                                                              500
HF4        TGCGGAATAGGTGCAGAGACTCGAAGGAAACTATCCCAAAAATTTTTCGATGTTCTGCGATTTGTTTCATGCACCAGAGGATGAGTGAGAATAACAATAG
HF1        ....................................................................................................
HF2        ....................................................................................................
HF3        ....................................................................................................
HF5        ....................................................................................................
HF6        ....................................................................................................
HF Tas     ....................................................................................................
HF Aus1    ....................................................................................................
HF Aus2    ....................................................................................................
CHILE 1    ----------------------------------------------------------------------------------------------------
CHILE 2    ..............................................................C......C............A......C..........
CHILE 3    ..............................................................C......C............A......C..........
CHILE 4    ..............................................................C......C............A......C..........
HL 1       .....................................................................C.........A..A...C.............
HL2        .....................................................................C.........A..A...C.............
HL3        .....................................................................C.........A..A...C.............
HL4        .....................................................................C.........A..A...C.............
HL5        .....................................................................C.........A..A...C.............
HL Tas     ...............................................T.....................C.....G......A.................

                                                                                                              600
HF4        TCAATGCTTATATGGTTATATGGTTATATGGTTATTGCTACCACTATTTCACGTTCCATGAATCTCAATTCATACACGAGGATAAAGAGAGAGTCCGTTT
HF1        .............--------...............................................................................
HF2        .............--------...............................................................................
HF3        .............--------............................A..................................................
HF5        .............--------...............................................................................
HF6        .............--------...........................C...................................................
HF Tas     .............--------...............................................................................
HF Aus1    .............--------...............................................................................
HF Aus2    .............--------...............................................................................
CHILE 1    ----------------------------------------------------------------------------------------------------
CHILE 2    ...........G.----------------...................CT........C.......-.............................A...
CHILE 3    ...........G.----------------...................CT........C.......-.............................A...
CHILE 4    ...........G.----------------...................CT........C.......-.............................A...
HL 1       .............----------------...................CT........C.......-.G...........................A...
HL2        .............----------------...................CT........C.......-.G...........................A...
HL3        .............----------------...................CT........C.......-.G...........................A...
HL4        .............----------------...................CT........C.......-.G...........................A...
HL5        .............----------------...................CT........C.......-.G...........................A...
HL Tas     ....---......----------------...................C.....C...C.......-.G...........................A...

                                          642
HF4        TTACGAGCTATGATTAGCAACGATGCGAATCGTAGTGAAAAG
HF1        ..........................................
HF2        ..........................................
HF3        ..........................................
HF5        ..........................................
HF6        ..........................................
HF Tas     ..........................................
HF Aus1    ..........................A...............
HF Aus2    ..........................................
CHILE 1    ------------------------------------------
CHILE 2    .A...........C............................
CHILE 3    .A...........C............................
CHILE 4    .A...........C............................
HL 1       .............C............................
HL2        .............C............................
HL3        .............C............................
HL4        .............C............................
HL5        .............C............................
HL Tas     ...........A.C......................A.....
```

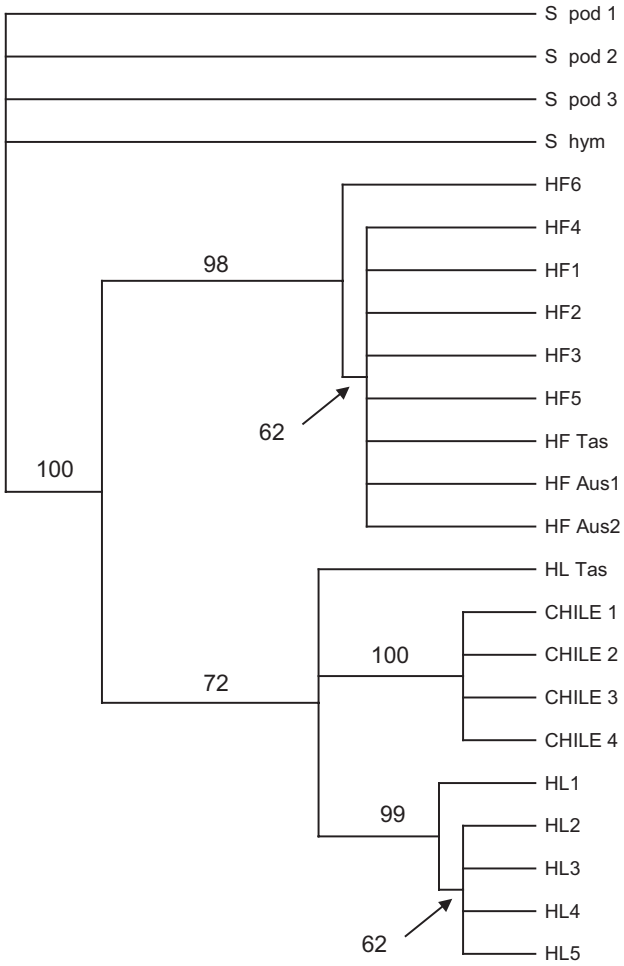

Fig. 3. Strict consensus of 20 most parsimonious trees (length 194, CI = 0.9227, RI = 0.9750) inferred from *trn*T-*trn*L sequences of 19 *Hymenophyton* [*H. flabellatum* HF; *H. leptopodum* HL, HL Tas; and CHILE (*H. pedicellatum*)] and 4 *Symphyogyna* (outgroup) specimens by a heuristic search with PAUP 4.0b10 (for specimen abbreviations see Table 1). Numbers above branches indicate bootstrap values from 1000 replicates with 1000 random addition replicates per bootstrap-replicate.

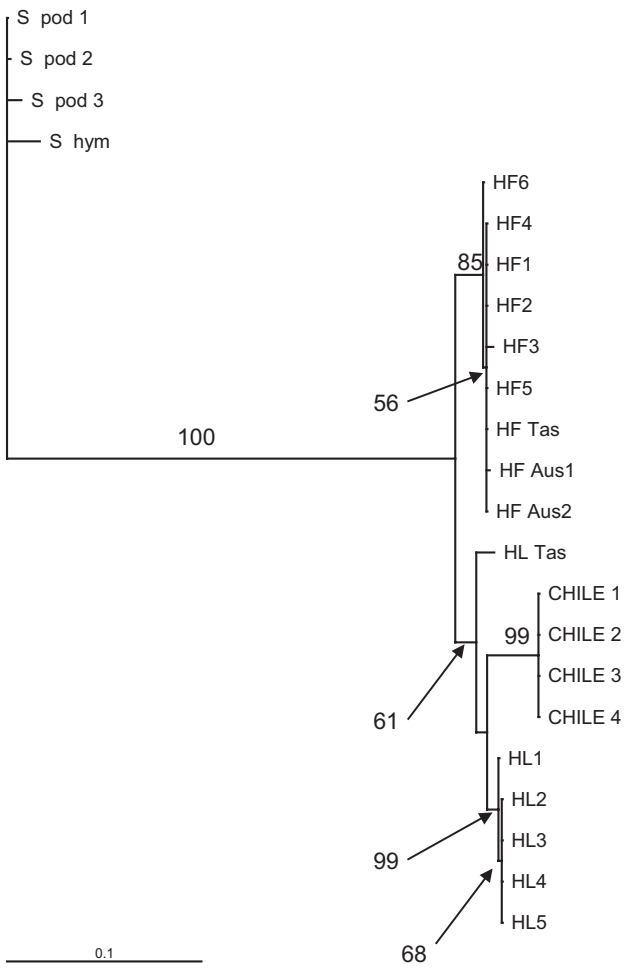

Fig. 4. Maximum likelihood phylogram based on *trn*T-*trn*L sequences of 19 *Hymenophyton* [*H. flabellatum* HF; *H. leptopodum* HL, HL Tas; and CHILE (*H. pedicellatum*)] and 4 *Symphyogyna* (outgroup) specimens calculated with PAUP 4.0b10. Identical topologies are obtained using either the complete *trn*T-*trn*L alignment (score = 1790.07219; 4384 rearrangements) or partial sequences (of 613 characters; score = 1571.65592, 4414 rearrangements) (for specimen abbreviations see Table 1). Numbers above branches indicate bootstrap values from 100 replicates per bootstrap replicate for the partial alignment.

which were completely used for tree construction. Compared to the trees based on cpDNA sequences, the topology differs slightly: the ITS2 trees (strict consensus tree of two most parsimonious trees shown in Fig. 6) suggest a closer relationship of the Australasian specimens (HF, HL, HL Tas) compared to the Chilean taxon, although with low bootstrap support for the former clade (62%).

In all analyses, the relationships of HL Tas remain ambiguous, its position differs depending on the dataset used to generate the tree(s). In the *trn*T-*trn*L analyses, the specimen is either resolved as sister clade to New Zealand HL [with low to moderate bootstrap support of up to 80% (analyses without outgroup specimens, Fig. 5)], as sister to both New Zealand HL and the Chilean samples [e.g. in MP-analyses of the complete (bootstrap support 72%; Fig. 3) or only slightly shortened alignment with outgroup taxa (57%), tree not shown], or the specimen forms a polytomous clade with all other three *Hymenophyton* taxa [when larger sequence parts are excluded in MP and ML analyses with *Symphyogyna*, trees not shown]. In the ITS2 phylogram, HL Tas forms a polytomous clade with the other Australasian taxa (HF and HL).

Morphological data

On the basis of morphological-anatomical characters, the examined specimens can be split into three groups with consistent character sets, i.e. the southern South American *Hymenophyton* specimens, Australasian *H. flabellatum* and

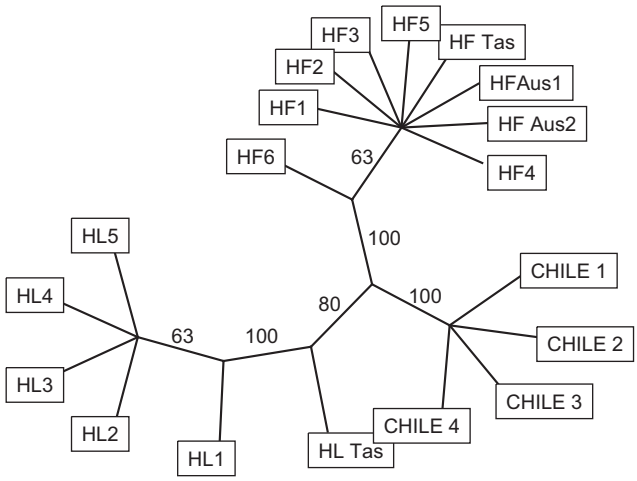

Fig. 5. Strict consensus of two most parsimonious trees (length 54, CI = 0.9630, RI = 0.9911) inferred from *trn*T-*trn*L sequences of 19 *Hymenophyton* [*H. flabellatum* HF; *H. leptopodum* HL, HL Tas; and CHILE (*H. pedicellatum*)] specimens by a heuristic search with PAUP 4.0b10 (for specimen abbreviations see Table 1). Numbers above branches indicate bootstrap values from 1000 replicates with 1000 random addition replicates per bootstrap-replicate.

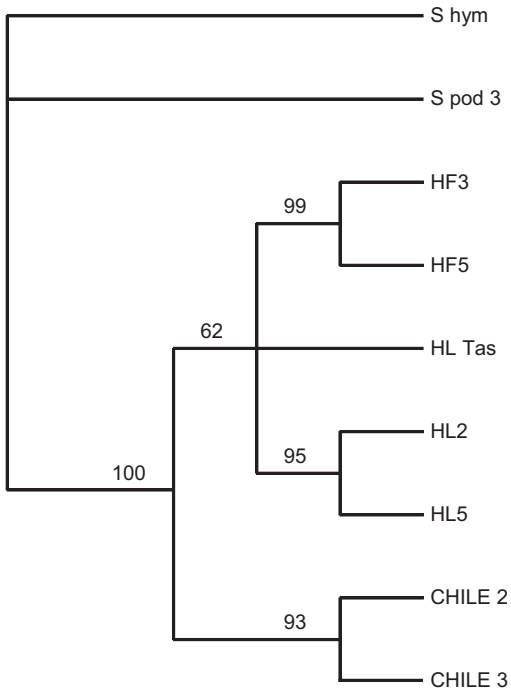

Fig. 6. Strict consensus of 2 most parsimonious trees (length 91, CI = 0.8681, RI = 0.8310) inferred from nrDNA ITS2 sequences of seven *Hymenophyton* [*H. flabellatum* HF; *H. leptopodum* HL, HL Tas; and CHILE (*H. pedicellatum*)] and two *Symphyogyna* (outgroup) specimens by a heuristic search with PAUP 4.0b10 (for specimen abbreviations see Table 1). Numbers above branches indicate bootstrap values from 1000 replicates with 1000 random addition replicates per bootstrap-replicate.

New Zealand *H. leptopodum*. Although the observed differences are sometimes slight, they allow for unambiguous assignment of specimens to one of the groups. The main differentiating characters are summarised in Table 2.

Discussion

Marker characteristics

In the molecular analysis, inter- and infraspecific divergence was detected between the studied *Hymenophyton* specimens. These results corroborate the usefulness of the studied cp- and nrDNA markers for investigations on these taxonomic levels in liverworts.

The amount of observed sequence divergence varies between the two sequenced non-coding cpDNA regions. In most cases, the differences between the specimens are greater in the *trn*T-*trn*L 5′exon intergenic spacer than in the adjacent *trn*L intron. Exceptions are the Australasian HF specimens with sequence identity in the spacer but slight variability in the intron (sequence divergence 0–0.9%). Generally, the sequence divergence recorded in the *trn*T-*trn*L spacer is 1.2-fold (New Zealand HL/HL Tas) to 3-fold (Australasian HF/New Zealand HL) higher than between the *trn*L intron sequences of the same groups. The obtained values are in accordance with other bryological studies reporting higher variability in the *trn*T-*trn*L spacer compared with the *trn*L intron (e.g. Meißner *et al*. 1998; Quandt *et al*. 2000). The divergence in the intron is also mainly restricted to the most variable P8 region, which is consistent with the assumption of lower evolutionary constraints in this region as well as in the *trn*T-*trn*L spacer (Quandt and Stech 2004).

In the nrDNA ITS2, the observed sequence divergence between the *Hymenophyton* specimens of the four groups (Australasian HF, New Zealand HL, HL Tas, Chilean specimens; 4.4–9.6%) is in the same range as the interspecific divergence in *Symphyogyna* spp. (6.8–10.6%; Schaumann *et al*. 2003); the ‘infrataxonomic’ divergence between the two studied Chilean specimens (3.2%) is considerably smaller.

Molecular and morphological differentiation

Despite of the different scales of variability, the sequenced regions reveal a consistent regional as well as a taxonomic differentiation within *Hymenophyton*, which is further supported by morphological data. The studied specimens form four regional or taxonomic groups, i.e. Australasian HF, Chilean samples, New Zealand HL and Tasmanian HL with little to no divergence between specimens from the same but pronounced differentiation between specimens from different groups.

For New Zealand and Tasmania, the existence of at least two *Hymenophyton* species, *H. flabellatum* and *H. leptopodum*, has already been stated based on

Table 2. Distribution, morphological, cp- and nrDNA characteristics of *Hymenophyton flabellatum, H. leptopodum* and southern South American *Hymenophyton* specimens (*H. pedicellatum*)

Characteristic	*H. flabellatum*, Australasia	*H. leptopodum*	Southern South American *Hymenophyton* specimens (*H. pedicellatum*)
Distribution	New Zealand, Tasmania, south-eastern Australia; probably Fiji Is and New Caledonia (see text for details)	New Zealand, Tasmania (but see comments on HL Tas)	Chile IX.-XII. Región, adjacent Argentina, Juan Fernández Is
Fronds	Olive green	Translucent green	Emerald green
Forking	5–6 times	(3–)4–5 times	(3–) 4 times
Segments	Flat, only borders slightly undulate 0.8–1 (–1.2) mm wide	Flat 1–1.5 (–2) mm wide	Flat, borders slightly recurved 1.5–2 mm wide
Apex	Obtuse, segments with limited grow	Emarginate, segments with limited growth	Obtuse or attenuate obtuse, with limited growth
Lamina cells	35–49 µm long, 23–30 µm wide, 23–35 µm thick	54–70 µm long, 28–35 µm wide, 44–47 µm thick	54–70 µm long, 30–42 µm wide, 28–35 µm thick
Marginal cells	Shorter than wide, 18–23 µm long (parallel to border), 33–35 µm wide	Elongated, 35–47 µm long, 23–35 µm wide	Elongated, 37–51 µm long, 28–35 µm wide
Growing apex	With 1-celled marginal slime papillae	No apical slime papillae seen	With 1-celled marginal slime papillae
Papillae	Persistent papillae 3 cells long (70–94 µm); lateral, and dorsally and ventrally on nerve	Evanescent slime papillae 2 cells long (117 µm); lateral to nerve	Evanescent slime papillae 2 cells long (46 µm); lateral to nerve
Male branches	(3–4) 5–6 per frond, lateral on stalk or nerve bifurcation	5–14 per frond on nerve bifurcation	12–13 per frond, lateral on stalk and some on nerve bifurcation
Margin of female scale	Often toothed and (when young) fringed with 12 or more cilia (EO Campbell unpubl. data.; Pfeiffer 2000*a*)	Entire or slightly lobed (EO Campbell unpubl. data; Pfeiffer 2000*a*)	Partially entire with some triangular teeth
Pseudoperianth	n.d.	n.d.	3.5 mm long
Infraspecific molecular variability			
*trn*T-*trn*L spacer: length	250 bp (HF Aus2) or251 bp (all other specimens); no substitutions	240 bp (HL1), 241 bp (all other specimens); no substitutions	241 bp; no substitutions
*trn*L intron: length	352 bp (HF4) or 344 bp (all other specimens); sequence divergence 0–0.9% due to 0–3 substitutions	335 bp; identical sequences in all specimens	335 bp; no substitutions
ITS2: length	248 bp; no substitutions	251 bp; no substitutions In HL Tas length 243 bp (*trn*T-*trn*L spacer), 332 bp (intron), and 249 (ITS2), differentiated from New Zealand specimens by 7–8 (2.9–3.4%) / 8 substitutions (2.4%) and 3–4 indels (*trn* T-*trn*L region); 15 substitutions (6.1%) and 3 indesl (ITS2)	252 bp; 8 substitutions (3.2%, CHILE2–3)
Interspecific molecular divergence	Compared with: New Zealand, *H. leptopodum* 18–19 substitutions (7.5–8.0%, spacer) and 9–11 substitutions (2.7–3.3%, intron), and 4–5 indels; and 18 substitutions (7.3%) and 2 indels (ITS2); HL Tas 15 substitutions (6.2%, spacer) and 11–14 substitutions (3.3–4.2%, intron), and at least 5 indels; 11 substitutions (4.4%) and 1 indel (ITS2); Chilean *H. pedicellatum* 18–19 substitutions (7.5–7.9%, spacer) and 11–13 substitutions (3.3–3.9%, intron), and at least 3 indels; 18 substitutions (7.3%) and 3 indels (ITS2)	Compared with: Chilean *H. pedicellatum* 13–14 substitutions (5.4–5.9%, spacer) and 8 (New Zealand HL, 2.4%) or 12 substitutions (HL Tas, 3.6%) in the intron, and 2–3 (New Zealand HL) or 4 indels (HL Tas), respectively; ITS2: 20/24 substitutions (8.0/9.6%, New Zealand HL) or 19 substitutions (7.6%; HL Tas) and 3 and 2 indels, respectively	

phytochemical, molecular (*trn*L intron) and morphological evidence (Campbell *et al*. 1975; Markham *et al*. 1976; Pfeiffer 2000*a*). The *trn*T-*trn*L spacer data support this interspecific differentiation. Furthermore, the analysis clearly shows that *H. flabellatum* also occurs in continental Australia (Victoria, specimens HF Aus1, 2).

The Chilean *Hymenophyton* specimens, since Evans' study (1925) traditionally recognised as *H. flabellatum*, form a separate clade in all molecular analyses (Figs 3–6). In the *trn*T-*trn*L region, they exhibit a higher sequence similarity with New Zealand *H. leptopodum* (spacer 94.1–94.6%, intron 97.6%) than with Australasian *H. flabellatum* (92.1–92.5%, 96.1–96.7%); indicating a closer molecular relationship with the former taxon. This has a morphological equivalent: Stephani (1911), in his description of Patagonian *H. pedicellatum*, already remarked that the taxon is related with and resembles *H. leptopodum* ('Die Pflanze steht dem *H. leptopodum* nahe', p. 11). Morphological similarities between the taxa have been shown in the present study, e.g. cell sizes in the thallus wings and two-celled evanescent papillae. However, the Chilean specimens are also clearly differentiated from New Zealand HL; in the phylogenetic trees both groups are sister to Australasian HF (cpDNA, Figs 3, 4), or the Chilean specimens form the sister clade to all Australasian *Hymenophyton* taxa (ITS2 data, Fig. 6).

Like for Australasia, these findings for southern South America contradict Evans' (1925) monotypic circumscription of the genus, instead they support Stephani (1911) and confirm the existence of a separate taxon in South America, delimitated from its Australasian relatives on species level.

Taxonomic consequences

Originally, two *Hymenophyton* species were described for southern South America by Stephani (1911), *H. pedicellatum* from Isla Huafo, Patagonia and *Symphyogyna integerrima* from Juan Fernández Is. Both type specimens could not be analysed by DNA sequencing. The sequenced specimens from Chile, however, originate from localities and close to the type locality of *H. pedicellatum*; from Juan Fernández Is unfortunately no fresh material was available. Morphologically, all examined southern South American specimens, including samples from Juan Fernández Is, form one entity and can be unambiguously grouped with the holotype of *H. pedicellatum*. For these southern South American *Hymenophyton* specimens, we propose to reinstate *H. pedicellatum* as a distinct species.

Hymenophyton pedicellatum Steph., *Kungl. Svenska Vetenskapsakad. Handl.* 46 (9); p. 11, fig. 2*a* (1911). Type: Chile, Westpatagonien, Isla Huafo im Hochwalde, *Halle & Skottsberg 123* (UPS ! holotypus). [On label: Chile Australis: Insula Huafo. In silva primaeva. *T. Halle & C. Skottsberg no 123*, 27.vii.1908]

= *Symphyogyna integerrima* Steph., *Kungl. Svenska Vetenskapsakad. Handl.* 46 (9); p. 13, fig. 2*e* (1911). Type: Chile, Juan Fernández Is, Masafuera in der Farnsteppe, 1200 m, *Skottsberg 43* (UPS ! holotypus). [On label: Juan Fernandez: Masafuera. *C. Skottsberg no 43*, 28.vii.1908]

Description

Plants with emerald green, palmate, furcate thallus segments, brown stalks and stolons. Stalk 1.5–2.0 cm high, cylindrical, simple or distally furcate, 0.34–0.48 mm (18–22 cells + central strand) in diameter; cells 11–35 μm in diameter with hyaline cell walls, excepted those subepidermal ones slightly thickened; the strand formed by 22 cells of brown thickened walls, each 3 μm in diameter; epidermal cells, 62–117 μm long, 14–23 μm wide and 12–16 μm thick, cuticle thin, brown. Rhizoids at bottom of stalk, ± 16 μm in diameter with thickened walls. Thallus palmate, firm, to 1.3 cm wide, bent horizontally towards substrate, (3)–4 furcate; each segment flat, the borders slightly recurved, 1.5–2 mm wide; by limited growth apex obtuse, sometimes acute; growing apex with apical notch and marginal evanescent slime papillae, 28–30 μm long. Lamina cells oriented oblique to border, 54–70 μm long, 28–35 μm wide, 30–42 μm thick; with thin walls; marginal cells, elongated parallel to the border, 37–51 μm long, 28–35 μm wide. Oil bodies spherical yellowish refringent 2.5–5 μm in diameter, (13)–15–16 (–26) per lamina cell. Nerve in adult segment not reaching the apex, 0.24–0.35 mm (on surface ± 8 cells) wide, 4–6 stratose + central strand of small cells with thickened walls; dorsal cells 51–100 μm long, 26–30 μm wide; ventral cells 59–70 μm long, 23–26 μm wide; laterally and close to nerve, on both surfaces, 2 cell long (± 70 μm) evanescent slime papillae. Occasionally stolons born ventrally from nerves. Dioicous. Male plants with (3)–9 androecia branches, some of them laterally on the stalk, others close to bifurcations of nerves on the expanded thallus. Female plants with ventral gynoecia, the scales ± 5.1 mm wide and ± 4.4 mm high; cells similar to lamina cells, margin entire or with few triangular broad teeth, some acute with 1-seriate tip and 3 cells at base; slime papillae present on the inner surfaces. Archegonia (?-12-?). Pseudoperianth 3.5 mm long. (cf. Fig. 7).

Specimens examined.

CHILE. Región IX, Puyehue, frente Lago Toro, (fem., masc.), *GHM 10849*, 30 Apr. 1994 (BA). Región IX, Seno Reloncavi, arroyo entre Lenca y La Arena, *GHM 11439*, 13 Jan. 1997 (BA). Región X, Hornopirén, Río Blanco, *Frey & Schaumann 01–145a*, 2 Mar. 2001 (VALD). id., Punta Río Santa Juana bei Pichanco, *Frey 03–01*, Feb. 2003 (BA). Caleta Gonzalo, Parque Pumatin, *Frey 03–03*, 17 Feb. 2003 (BA). Región XI, camino de Coihaique a Aysen, (masc.), *GHM & M.Rubies* 12276, 21 Mar. 1999 (BA). Región XII, Ultima Esperanza, Seno Ultima Esperanza, Puerto Toro, *Schiavone 2274b*, 22 Jan. 1977 (TBPA-B). Isla Juan Fernández, Masafuera, (fem.), *Skottsberg* 27-8-1908 [con nº 38 *Plagiochila riparia* (= *Nothostrepta bifida*)], 27-8-1908 (UPS). ARGENTINA. Prov. Río Negro, Puerto Blest,

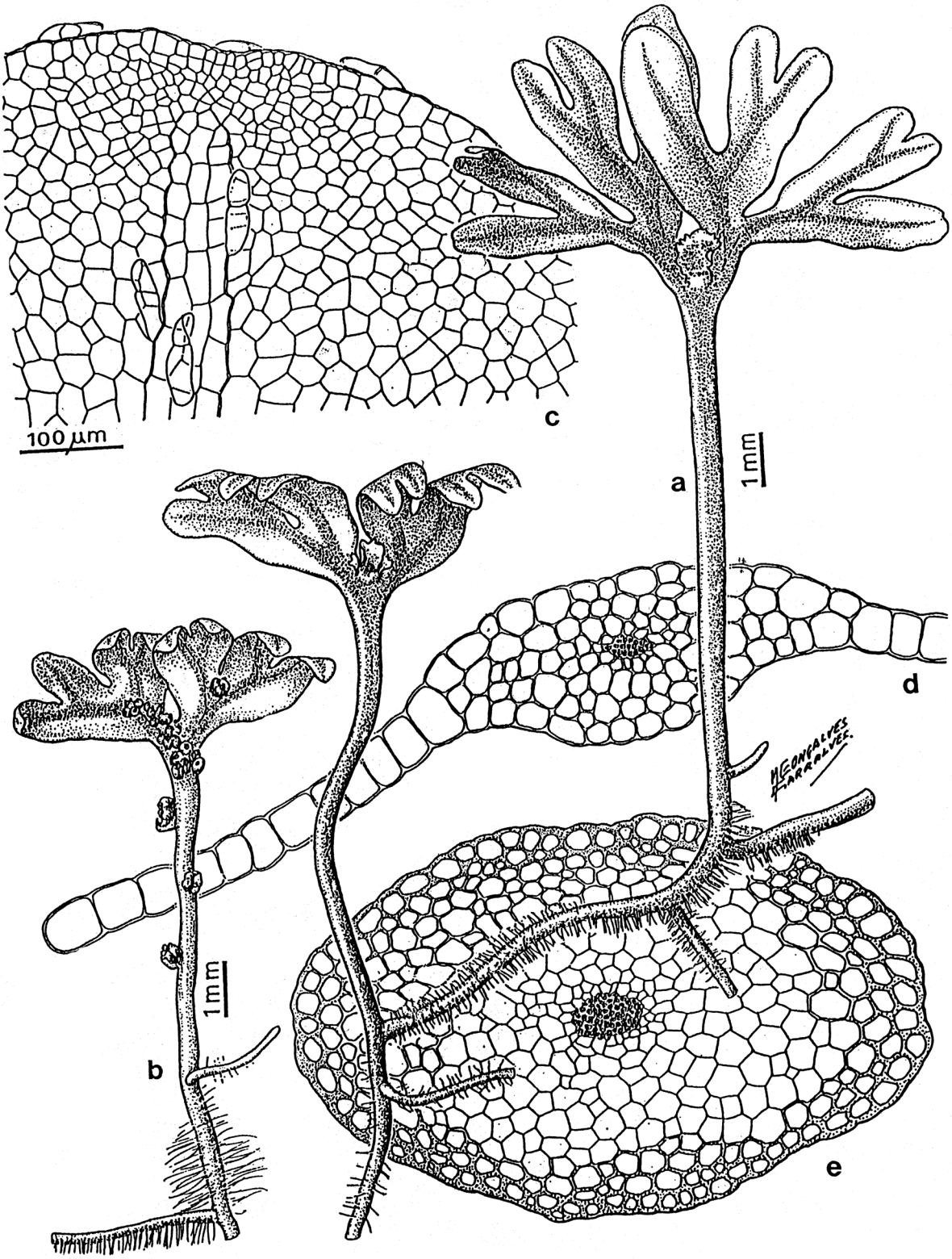

Fig. 7. *Hymenophyton pedicellatum* Steph. (*a*) Female plant, ventral view, with scales. (*b*) Male plant, ventral view with androecia. (*c*) Detail of apical portion of thallus segment. (*d*) Transversal section of segment. (*e*) Transversal section of stem. (*a*, *c–e*) Chile, X Región, Hornopirén, Río Blanco. *Frey & Schaumann 01–145a* (VALD). (*b*) Chile, Puyehue, frente a Lago Toro, *G.H.M. 10849* (BA).

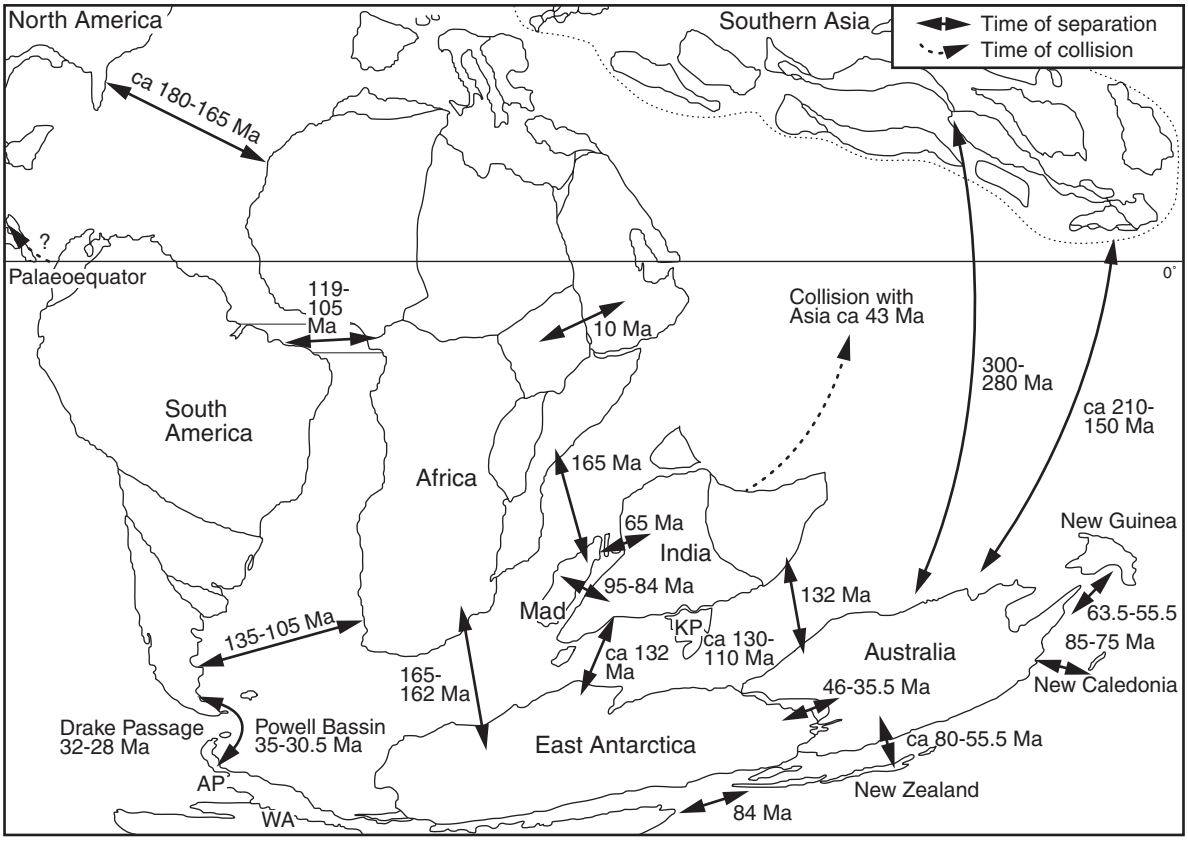

Fig. 8. Gondwanan breakup episodes. Reconstruction of the South Atlantic-Indian Ocean-Neotethys Ocean regions during the late Early Cretaceous (110 million years ago) showing the timing of separation and amalgamation or collision of Gondwanan and Asian terranes (compiled by McLoughlin 2001). AP, Antarctic Peninsula; KP, Kerguelen Plateau; Mad, Madagascar; WA, West Antarctica.

camino a Lago Frias (masc.), *C.C. Donterberg & GHM 1859a*, 27 Apr. 1965 (BA 12955)

Ecology and Distribution

Plants are solitary or gregarious in moist, shaded bryophyte carpets, on soil and on stream banks, on granite blocks near streams, at the base of trees and on rotten logs. Present in Chile from IX Región to XII Región and on Juan Fernández Island. In Argentina the species was only found once, in Río Negro, close to Puerto Blest at Lago Nahuel Huapi.

Most of the known Chilean *H. pedicellatum* collections originate from populations concentrated in the Valdivian and northern Patagonian regions. The sequences of specimens from this region are, however, completely identical with the partial *trn*T-*trn*L spacer sequence of a specimen from the single known Magallanian collection from south of the Patagonian ice-shield (CHILE 5, cf. Hässel de Menéndez and Solari 1985). This indicates a continuous distribution in Chile from region IX to XII throughout the *Nothofagus betuloides* forests situated to the west of the ice-shield.

The main differences (distributional range, morphological–anatomical characters, cpDNA *trn*T-*trn*L and nrDNA ITS2 data) differentiating between the three *Hymenophyton* species are summarised in Table 2.

Biogeographic, phylogeographic and dispersal implications

The extant distribution of the genus *Hymenophyton* is basically palaeoaustral Gondwanan. The taxa primarily occur in temperate rain forests of southern South America, New Zealand, Tasmania, Australia and New Caledonia, with few range extensions into geologically probably younger regions such as Juan Fernández and Fiji Is. This pattern suggests that the genus is of Gondwanan origin and had a continuous range until the Cretaceous-Tertiary Gondwana break-up started to affect these regions (approximately 84 million years ago with respect to New Zealand, cf. McLoughlin 2001; Fig. 8). Following the disruption, the long isolation led to the differentiation of separate species in either region, i.e. of *H. pedicellatum* in southern South America, *H. flabellatum* in Australasia and *H. leptopodum* in New Zealand (and probably Tasmania).

In the analysed cp- and nrDNA sequences, distinct divergence is obvious between the species, accompanied by smaller morphological differences. This is partly in

accordance with a study in *Pyrrhobryum mnioides* (Hook.) Manuel showing rather strong genetic divergence despite of relative morphological stasis; the moss is interpreted as a Gondwanan species-vicariant between South America and Australasia (McDaniel and Shaw 2003).

Today, *Hymenophyton* taxa of the two main distribution centres (southern South America, Australasia) are completely isolated, and no species is common to both regions. This separation, in conjunction with the lack of *Hymenophyton* on oceanic and subantarctic islands lying between the two centres, indicates that no long-range intercontinental dispersal occurs. This is surprising, because rather small spores (diameter 13–16 μm, Schuster 1964; Allison and Child 1975 for *H. flabellatum*) are ± regularly produced (at least in some New Zealand populations, cf. Pfeiffer 2003), which would facilitate transoceanic dispersal. Thus, the spores are either not able to leave their sheltered and humid microsites within the rain forests, could not withstand conditions during transport at jetstream altitudes (cf. van Zanten and Gradstein 1988) or are unable to establish after successful dispersal events.

Over shorter distances, dispersal and hence genetic exchange seems to happen at least infrequently. The (near) total sequence identity between *H. leptopodum* specimens in New Zealand or in Chilean *H. pedicellatum* (*trn*T-*trn*L data), respectively, as well as the strong uniformity of *trn*T-*trn*L-sequences of HF samples from New Zealand, Tasmania and Australia (nowadays separated by ≥ 1500 km of Tasman Sea), suggest recent or ongoing migration and gene flow within these regions; especially as simultaneously some variability can be observed on local to regional scales, e.g. between *H. flabellatum* populations in the Urewera Nationalpark (HF2, HF3) or in Victoria (HF Aus1, HF Aus2).

Furthermore, the occurrence of *H. pedicellatum* populations on the geologically probably younger volcanic Juan Fernández Is indicates rather recent colonisation event(s) from 'Gondwanan' populations across the intervening seas.

Taxa/specimens with unresolved affinities

The taxonomic affinities of the Tasmanian *H. leptopodum* sample remain ambiguous (cf. Pfeiffer 2000*a*). The studied specimen is, both morphologically and molecularly, related to New Zealand *H. leptopodum*, but it is nevertheless also divergent from these rather uniform samples (Figs 3–5). A similar molecular differentiation of New Zealand and Tasmanian specimens was observed in the palaeoaustral moss *Hypopterygium didictyon*, but could not be substantiated by morphological characters (Pfeiffer 2000*b*; Kruijer 2002). To reveal the affinities of HL Tas, additional *H. leptopodum* specimens from Tasmania (and, if possible, also from Australia) should be examined.

Similarly, the affinities of the New Caledonian *Hymenophyton* taxon (cf. *H. furcatum*) cannot be clarified, because no fresh material was available for comparison. Based on the original description (Pearson 1922, type not seen), the taxon is larger than *H. flabellatum*, but the larger, elongated cells resemble *H. leptopodum*. The taxon could be either of Gondwanan origin, separated from Australian and New Zealand relatives 85–75 million years ago (McLoughlin 2001; Fig. 8), or extant populations are the result of a recent migration event (compare comments on Fijian sample).

For the Fijian specimen, a mislabelling cannot be wholly excluded (as is the case with the Colombian specimen, see Introduction): the taxon is known from a single collection accessioned 1906 in the herbarium of W. Mitten (now deposited in NY), bearing only the information 'Fiji Seemann.' in handwriting, but no additional data. [Most probably, the specimen was collected by B. C. Seemann during a British expedition in 1860–1861; compare Frahm and Eggers (2001)]. To our knowledge, the taxon has not been recorded from the Fiji Is ever since.

Acknowledgments

We thank the Department of Conservation, New Zealand (Taupo/Tongariro, Nelson Marlborough and West Coast Conservancies) and the Corporación Nacional Forestal, Chile, for the research permits and logistic support. We are indebted to colleagues providing fresh or herbarium specimens of *Hymenophyton* for DNA-sequencing and/or comparison: Dr J. Jarman (Hobart, Tasmania) and Dame Dr E.O. Campbell (Palmerston North, New Zealand), as well as Drs H. Nowak-Krawietz and H. Sipman (B), Dr P. Heenan and D. Glenny (CHR), Dr G. Kantvilas (HO), Dr S. Edwards (MANCH), L. van Essen (MPN), Dr W. Buck (NY) and Dr R. Moberg (UPS) for loan of herbarium specimens. We further express our thanks to D. Glenny, the late Dame Dr E.O. Campbell and Professor Dr J.-P. Frahm for useful comments, Dr M. Stech for help with the phylogenetic analyses and critical reading of draft versions of the paper, Ms B. Giesicke for technical assistance, Mrs M. Gonçalves-Carralves for drawing Fig. 7, Mr. H. Lünser for drawing Figs. 1 and 8, and Drs M. Stech and K. Meißner for determining sequences.

The study was supported by a grant from the Deutsche Forschungsgemeinschaft for the project 'BRYO AUSTRAL' (Fr404/3–2) and a travel grant from the Freie Universität Berlin to W. Frey in 2003.

References

Allison KW, Child J (1975) 'The liverworts of New Zealand.' (University of Otago Press: Dunedin)

Baldwin BG (1992) Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* **1**, 3–16.

Campbell EO, Markham KR, Porter LJ (1975) Dendroid liverworts of the order Metzgeriales in New Zealand. *New Zealand Journal of Botany* **13**, 593–600.

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* **12**, 13–15.

Evans AW (1892) An arrangement of the genera of Hepaticae. *Transactions of the Connecticut Academy of Arts and Sciences* **8**, 2–20.

Evans AW (1925) A taxonomic study of *Hymenophytum. Bulletin of the Torrey Botanical Club* **52**, 491–506.

Frahm J-P, Eggers J (2001) 'Lexikon deutschsprachiger Bryologen.' (Selbstverlag/Books on Demand GmbH: Norderstedt)

Frey W (1990) Genoelemente prä-angiospermen Ursprungs bei Bryophyten. *Botanische Jahrbücher für Systematik* **111**, 433–456.

Frey W, Beever JE (1995) Dendroid bryophytes communities of New Zealand. *Nova Hedwigia* **61**, 323–354.

Frey W, Schaumann F (2002) Records of rare southern South American bryophytes. Studies in austral temperate rain forest bryophytes 18. *Nova Hedwigia* **74**, 533–543. doi:10.1127/0029-5035/2002/0074-0533

Gradstein SR, Lücking A, Morales ZMI, Dauphin G (1994) Additions to the hepatic flora of Costa Rica. *Lindbergia* **19**, 73–86.

Grolle R (1987) Miscellanea Hepaticologica 251–260. *Journal of the Hattori Botanical Laboratory* **63**, 437–443.

Hässel de Menéndez GG, Solari SS (1985) Catalogo de las Hepaticas. In 'Transecta Botánica de la Patagonia Austral'. (Eds O Boelcke, DM Moore, FA Roig) pp. 299–342. (Conicet/Argentina, Royal Society/Gran Bretaña, Instituto de la Patagonia/Chile: Buenos Aires)

Hepperle D (1997) 'Alignment-Editor Align32.' (Available on line at http://wwwuser.gwdg.de/~dhepper/)

Hepperle D (2003) 'Align v01/2003: manual sequence alignment for PCs.' (Available on line at http://wwwuser.gwdg.de/~dhepper/)

Kruijer JD (2002) Hypopterygiaceae of the World. *Blumea Supplement* **13**, 1–388.

Markham KR, Porter LJ, Campbell EO, Chopin J, Bouillant M-L (1976) Phytochemical support for the existence of two species in the genus *Hymenophyton. Phytochemistry* **15**, 1517–1521. doi:10.1016/S0031-9422(00)88928-1

McDaniel SF, Shaw AJ (2003) Phylogeographic structure and cryptic speciation in the trans-antarctic moss *Pyrrhobryum mnioides. Evolution* **57**, 205–215.

McLoughlin S (2001) The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany* **49**, 271–300. doi:10.1071/BT00023

Meißner K, Frahm J-P, Stech M, Frey W (1998) Molecular divergence patterns and infrageneric relationship of *Monoclea* (Monocleales, Hepaticae). Studies in austral temperate rain forest bryophytes 1. *Nova Hedwigia* **67**, 289–302.

Miller HA, Whittier HO, Whittier BA (1983) Prodomus Florae Hepaticarum Polynesiae with a key to genera. *Bryophytorum Bibliotheca* **25**, 1–423.

Pearson H (1922) Hepaticae. In 'A systematic account of the plants collected in New Caledonia and the Isle of Pines by Mr RH Compton, MA, in 1914, Part III, Cryptogams'. *Journal of the Linnean Society of London/Botany* **46**, 13–44.

Pfeiffer T (2000*a*) Molecular relationship of *Hymenophyton* species (Metzgeriidae, Hepaticophytina) in New Zealand and Tasmania. Studies in austral temperate rain forest bryophytes 5. *New Zealand Journal of Botany* **38**, 415–423.

Pfeiffer T (2000*b*) Relationship and divergence patterns in *Hypopterygium* '*rotulatum*' s.l. (Hypopterygiaceae, Bryopsida) inferred from *trn*L intron sequences. Studies in austral temperate rain forest bryophytes 7. *Edinburgh Journal of Botany* **57**, 291–307. doi:10.1017/S0960428600000226

Pfeiffer T (2003) Terricolous bryophyte vegetation of New Zealand temperate rain forests. Communities, adaptive strategies and divergence patterns. Studies in austral temperate rain forest bryophytes 14. *Bryophytorum Bibliotheca* **59**, 1–147.

Quandt D, Tangney RS, Frahm J-P, Frey W (2000) A molecular contribution for the understanding of the Lembophyllaceae (Bryopsida) based on noncoding chloroplast regions (cpDNA) and ITS2 (nrDNA) sequence data. Studies in austral temperate rain forest bryophytes 8. *Journal of the Hattori Botanical Laboratory* **89**, 71–92.

Quandt D, Frahm J-P, Frey W (2001) Patterns of molecular divergence within the palaeoaustral genus *Weymouthia* Broth. (Lembophyllaceae, Bryopsida). Studies in austral temperate rain forest bryophytes 11. *Journal of Bryology* **23**, 305–311.

Quandt D, Stech M (2004) Molecular evolution of the *trn*T_{UGU}-*trn*F_{GAA} region in bryophytes. *Plant Biology*, (In press)

Schaumann F, Frey W, Hässel de Menéndez G, Pfeiffer T (2003) Geomolecular divergence in the Gondwanan dendroid *Symphyogyna* complex (Pallaviciniaceae, Hepaticophytina, Bryophyta). Studies in austral temperate rain forest bryophytes 22. *Flora* **198**, 404–412.

Schaumann F, Pfeiffer T, Frey W (2004) Molecular divergence patterns within the Gondwanan liverwort genus *Jensenia* (Pallaviciniaceae, Hepaticophytina, Bryophyta). Studies in austral temperate rain forest bryophytes 25. *Journal of the Hattori Botanical Laboratory* **96**, (In press)

Schuster RM (1963) Studies on Antipodal Hepaticae. I. Annotated keys to the genera of Antipodal Hepaticae with special reference to New Zealand and Tasmania. *Journal of the Hattori Botanical Laboratory* **26**, 185–309.

Schuster RM (1964) Studies on Antipodal Hepaticae, IV. Metzgeriales. *Journal of the Hattori Botanical Laboratory* **27**, 183–216.

Schuster RM (1982) Generic and familial endemism in the hepatic flora of Gondwanaland: origin and causes. *Journal of the Hattori Botanical Laboratory* **52**, 3–35.

Stephani F (1900) Species Hepaticarum. *Hymenophytum* Dum. 1835. *Mémoires de L'Herbier Boissier* **11**, 307–309.

Stephani F (1911) Botanische Ergebnisse der schwedischen Expedition nach Patagonien und dem Feuerlande 1907–1909. II. Die Lebermoose. *Bihang til Kungl. Svenska Vetenskapsakademiens Handligar* **46**(9), 1–92.

Swofford DL (2002) 'PAUP*: Phylogenetic analysis using parsimony (* and other methods), version 4.0b10.' (Sinauer: Sunderland)

Taberlet P, Gielly L, Patou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**, 1105–1109.

Uribe JM, Gradstein SR (1998) Catalogue of the Hepaticae and Anthocerotae of Colombia. *Bryophytorum Bibliotheca* **53**, 1–99.

van Zanten BO, Gradstein SR (1988) Experimental dispersal geography of neotropical liverworts. *Nova Hedwigia Beiheft* **90**, 41–94.

Manuscript received 24 August 2003, accepted 13 May 2004