RELATIONSHIPS AND DIVERGENCE PATTERNS IN *HYPOPTERYGIUM 'ROTULATUM'* S.L. (*HYPOPTERYGIACEAE*, BRYOPSIDA) INFERRED FROM *trn*L INTRON SEQUENCES. STUDIES IN AUSTRAL TEMPERATE RAIN FOREST BRYOPHYTES 7

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The phylogenetic relationship of species within the *Hypopterygium 'rotulatum'* (Hedw.) Brid. s.l. complex is analysed by sequencing the *trn*L intron of cp DNA. Furthermore, the divergence in variable New Zealand *H. 'rotulatum'* samples is investigated along a latitudinal gradient. The presented data strongly support the delimitation of at least two distinct species within *Hypopterygium 'rotulatum'* s.l. in New Zealand, Australia and Tasmania, which correspond with the morphological species *H. muelleri* Hampe (possibly conspecific with *H. debile* Reichardt) and *H. didictyon* Müll. Hal., a species known from Chile. The distribution patterns of these species are discussed on a regional (New Zealand) and global scale; indicating an australasian distribution pattern of *H. muelleri* and a palaeoaustral pattern of *H. didictyon*.

Keywords. Australasian distribution pattern, cp DNA, palaeoaustral, *trn*L_{UAA} intron.

INTRODUCTION

Hypopterygium Brid. is the largest genus within the small pleurocarpous moss family *Hypopterygiaceae* Mitt. It contains c.10–14 accepted species (Kruijer, 1995, 1997). Most of the species occur in humid temperate to tropical forests from Australasia to continental southern and south-eastern Asia and in south-western South America, showing a mainly Gondwanan distribution pattern (Kruijer, 1995; Stech *et al.*, 1999).

In New Zealand, which is one of the centres of diversity of the *Hypopterygiaceae*, the genus *Hypopterygium* s.str. is represented by at least two species. *Hypopterygium filiculaeforme* (Hedw.) Brid. is a stenoecious endemic taxon, predominantly confined to wet sites in lowland to montane conifer forests (up to 900m), especially *Dacrydium cupressinum–Dacrycarpus dacrydioides* forests (Frey & Beever, 1995). *Hypopterygium rotulatum* (Hedw.) Brid. s.l. (sensu Dalton *et al.*, 1991; Beever *et al.*, 1992; Fife, 1995) is more widespread and quite common, occurring not only in primary but also in secondary forests and more open country. The taxon is found on the main islands of New Zealand and some of its outlying archipelagos (e.g. the Kermadec,

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Chatham and Auckland Islands), in southern and south-eastern Australia, Tasmania, and on Norfolk and Lord Howe Islands.

Initially, in the *Hypopterygium 'rotulatum'* s.l. complex, two species were described and distinguished: *H. rotulatum* s.str. and *H. novae-seelandiae* Müll. Hal. (e.g. Dixon, 1927; Sainsbury, 1955). In 1973, Matteri placed *H. novae-seelandiae* under synonymy of the Chilean *H. didictyon* Müll. Hal., a view accepted (or at least mentioned) in most recent treatments and supported by *trn*L intron data (Stech *et al.*, 1999). Some authors additionally point out that *H. didictyon* is not satisfactorily distinguished from *H. rotulatum* (Scott & Stone, 1976; Beever *et al.*, 1992; see also Dalton *et al.*, 1991). Other authors solely list *H. rotulatum*, reducing *H. novae-seelandiae* to its synonymy (for New Zealand see Fife, 1995).

Kruijer (1995 and pers. comm.) on the other hand, regards *H. rotulatum* as a doubtful species. He considers that New Zealand *H. 'rotulatum'* specimens are often misidentified and belong to austral *H. didictyon* (sensu Matteri, 1973) or to australasian *H. muelleri* Hampe, a species known previously only from Australia (Scott & Stone, 1976; Streimann & Curnow, 1989). It is, according to Scott & Stone (1976: 398), 'very like a large *H. rotulatum*, vegetatively'. Kruijer (1995, 1997) places *H. muelleri* within the *H. tenellum* complex [*H. tenellum* Müll. Hal. from Indo-Malaysian and Sino-Japanese Asia, *H. laricinum* (Hook.) Brid. from Africa, *H. tamarisci* (Sw.) Müll. Hal. from South and Central America and the Caribbean, *H. debile* Reichardt from Melanesia and Polynesia], a 'group of five very similar and hence probably closely related species' (Kruijer, 1997: 11).

All these *Hypopterygium* taxa are characterized by a dendroid habit and the typical Hypopterygiacean foliation with two rows of asymmetrical lateral leaves and a third row of smaller symmetrical underleaves ('amphigastria') on the branches. The species are variable in morphological characters such as colour, tomentum, strength of leaf border, and branching. Problems in delimiting species within the *H. 'rotulatum'* complex are mainly caused by the high vegetative plasticity.

Based on *trn*L intron sequences Stech *et al.* (1999) have already shown a strong relationship of one New Zealand sample of *H. 'novae-seelandiae'* with the Chilean *H. didictyon*, supporting the opinion that these two taxa are conspecific.

The present study aims to resolve the phylogenetic relationships between taxa in the *H. 'rotulatum'* complex; furthermore the divergence within this complex along a geographical (latitudinal) gradient within New Zealand is analysed. *Hypopterygium 'rotulatum'* s.l. specimens from different sites (Fig. 1) in temperate rain forests located on a transect through the North and South Islands of New Zealand, together with specimens from Tasmania and mainland Australia, and several specimens of *H. muelleri* are analysed by sequencing the *trn*L_{UAA} intron of chloroplast (cp) DNA. This intron, located in the large single copy region, has been used for examining relationships of closely related taxa in phanerogams (e.g. Gielly & Taberlet, 1994; Böhle *et al.*, 1996; Mast, 1998) as well as in bryophytes (e.g. Meißner *et al.*, 1998; Frey *et al.*, 1999; Stech & Frahm, 1999; Stech *et al.*, 1999). For comparison, sequences of the possibly conspecific *H. didictyon* (from Chile) and *H. debile* (from Samoa) (drawn from Stech *et al.*, 1999) are included in the study.

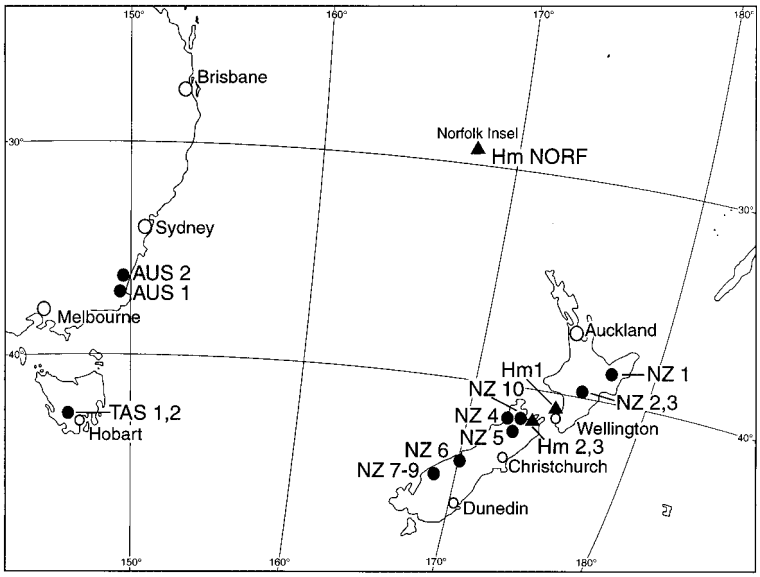

FIG. 1. Origin of the specimens of *H. 'rotulatum'* s.l. (NZ1–10, AUS1, 2, TAS1, 2; marked as dots) and *H. muelleri* (Hm 1–3, Hm NORF; triangles) investigated in this study.

MATERIAL AND METHODS

Plant material

The investigated specimens, their abbreviations in the following analyses and additional information (collecting data, origin, deposit of voucher etc.) are listed in Table 1 (see also Fig. 1). The sequences of NZ2, *H. didictyon* from Chile and *H. debile* from Samoa were analysed by Stech *et al.* (1999). Additionally, sequences of New Zealand *Lopidium concinnum* (Hook.) Wilson and African *L. struthiopteris* (Brid.) M. Fleisch. (Frey *et al.*, 1999) are included; *Achrophyllum quadrifarium* (Sm.) Vitt & Crosby is used as outgroup (Table 1).

DNA preparation, PCR and sequencing reactions

DNA preparations from herbarium material (NZ1, 3, 4, 7, 8 dried in Silicagel [Roth], all other specimens air-dried) were carried out using the DNA extraction method described by Doyle & Doyle (1990), with the exception of using smaller amounts of material and only 76% ethanol (v/v) to wash the pellet after the precipitation with cold isopropanol. PCR reactions were performed using 0.2mM dNTPs (Roth), 1–2U Taq polymerase, 1 × buffer, magnesium chloride (all GibCo or Qiagen), and 12.5 pmol primers C, D or F that were slightly modified compared with the original method of Taberlet *et al.* (1991): C_M: 5′-CGA AAT TGG TAG ACG CTG CG-3′ and D_M: 5′-GGG GGT AGA GGG ACT TGA AC-3′ or F_M: 5′-ATT TGA ACT

TABLE 1. List of investigated specimens with abbreviations, sampling data, origin (locality, habitat), herbarium in which a voucher is held, and GenBank database accession numbers. Specimens in bold were identified as *H. muelleri* by *trn*L intron data. Abbreviations: Acc. no. = GenBank database accession numbers; NZ = New Zealand; W. Frey = voucher specimen in the herbarium of W. Frey, Berlin

	Sampling data	Origin (locality, habitat)	Herbarium	Acc. no.
Hypopterygium 'rotulatum'				
NZ1	98-Mo 62* leg. T. Pfeiffer, 21 iii1998	NZ, North Is., Urewera National Park, track to Lake Waikareiti; 680m; vertical clay bank at roadside	CHR, W. Frey	AF170585
NZ3	98-Mo 54* leg. T. Pfeiffer, 14 iii 1998	NZ, North Is., Tongariro National Park, Mt Ruapehu, Ohakune Mt Rd; 1020m; on clay	CHR, W. Frey	AF170586
NZ4	98-Mo 40* leg. W. Frey & T. Pfeiffer, 1 iii 1998	NZ, South Is., near Karamea, Mt Stormy; 795m; on soil/ rotting wood	CHR, W. Frey	AF170587
NZ5	94–138 leg. H. & W. Frey, 6 iii 1994	NZ, South Is., Nelson Lakes National Park, Lake Rotoiti, lake track; 810m; on soil – analysed by K. Meißner	CHR, W. Frey	AF170588
NZ6	98-Mo 68 leg. W. Frey, 20 ii 1998	NZ, South Is., Bruce Bay; 5m; *Dacrycarpus dacrydioides* forest	CHR, W. Frey	AF170589
NZ7	98-Mo 18* leg. W. Frey & T. Pfeiffer, 21 ii 1998	NZ, South Is., Haast Pass Rd, 200m N of Robinson creek; 470m	CHR, W. Frey	AF170590
NZ8	98-Mo 19* leg. W. Frey & T. Pfeiffer, 22 ii 1998	NZ, South Is., Haast Pass Rd, Roaring Billy Bush Walk; 70m; on soil in *Nothofagus menziesii*-conifer forest	CHR, W. Frey	AF170591
NZ9	9–7 leg. J.-P. Frahm, 22 ii 1998	NZ, South Is., Haast Pass, forest walk along rd from Haast to Haast Pass, c.25km S Haast; 40m; *Dacrydium–Nothofagus* forest	CHR, BONN, W. Frey	AF170592
NZ10	98-Mo 52 leg. W. Frey & T. Pfeiffer, 11 iii 1998	NZ, South Is., Waikoropupu Springs; 20m; on soil	CHR, W. Frey	AF170595

TABLE 1. (*Cont'd*)

	Sampling data	Origin (locality, habitat)	Herbarium	Acc. no.
TAS1	HO 52617 leg. J. Jarman, 9 ii 1999	Tasmania; Bermuda Rd, 43°04″ 146°54′E; 490m; in mixed forest (eucalypts over rainforest)	HO, W. Frey	AF170593
TAS2	HO 64727 leg. J. Jarman, 19 xiii 1998	Tasmania; Manuka Rd, 43°06″ 146°41′E; 125m; in *Eucalyptus obliqua* forest	HO, W. Frey	AF170594
AUS1	CANB 604569 leg. H. Streimann, 8 i 1999	Australia; Maxwells Rd, Nadgee State Forest, 41km SWW of Eden, 37°25″ 149°49′E; 230m; *Acmena smithii*, *Eucryphia morrei*, *Cyathea*-dominated valley, on dead fallen tree fern trunk	CANB, W. Frey	AF170601
AUS2	CANB 604570 leg. H. Streimann, 11 i 1999	Australia; Marble Arch, 18km SSW of Major's Creek, 35°43″ 149°41′E; 580m; deep limestone valley surrounded by dry sclerophyll forest, on semi-exposed limestone-boulds	CANB, W. Frey	AF170599
Hypopterygium* cf. *muelleri (originally determined as *H. rotulatum*)				
Hm 1	WELT M029155 leg. P.J. Brownsey, 3 iv 1993	NZ, North Is., Wellington, Kapiti Island, Trig Track, 40°51′S 174°55′E; 100m; *Corynocarpus laevigatus*, *Dysoxylum spectabile*, *Vitex lucens*, *Melicytus ramiflorus*, *Macropiper excelsum* forest, on boulder in creek bed	WELT, W. Frey	AF170596
Hm 2	WELT M028292 leg. P.J. Brownsey, 19 x 1991	NZ, South Is., Nelson, Hira State Forest, Sharland Creek Track, 41°15′S 173°20′E; 180m; *Nothofagus solandri* forest with *Coprosma grandifolia*, *Beilschmiedia tawa*, *Melicytus ramiflorus* and tree ferns, on rock at edge of creek	WELT, W. Frey	AF170597

TABLE 1. (*Cont'd*)

	Sampling data	Origin (locality, habitat)	Herbarium	Acc. no.
Hypopterygium muelleri (sic!)				
Hm 3	ex H. Streimann 51393 pro parte leg. H. Streimann, 5 ii 1993	NZ, South Is., Sharland Creek, Hira Forest, 4km E of Nelson, 41°16′S 173°20′E; 70m; *Nothofagus* (beech) forest on moderately steep slopes surrounding creek, on wet shaded rock at waterfall *picked out of a collection of *Canalohypopterygium tamariscinum**	L etc., W. Frey	AF170598
Hm NORF	ex H. Streimann 49667 leg. H. Streimann, 15 vi 1992	Australia/Norfolk Island, track E of Mt Bates, Norfolk Island National Park, 29°00′40″S 167°56′20″E; 280m; sub-tropical forest on ridge, on shaded rock outcrop on side of ridge	L etc., W. Frey	AF170600
Achrophyllum quadrifarium (as outgroup)				
	98-T2 leg. W. Frey & T. Pfeiffer, 15 ii 1998	NZ, South Is., Franz-Josef-Glacier, Alex Knob; 190m; on soil in lowland forest	CHR, W. Frey	AF170584
Specimens investigated in previous studies				
NZ2	as *Hypopterygium 'novae-seelandiae'*	*See Stech et al.*, 1999	CHR, W. Frey	AF134636
CHILE	*Hypopterygium didictyon*	See Stech *et al.*, 1999	W. Frey	AF134639
SAMOA	*Hypopterygium debile*	see Stech *et al.*, 1999	B	AF134635
	Lopidium concinnum	see Frey *et al.*, 1999	CHR, W. Frey	AF033233
	Lopidium struthiopteris	see Frey *et al.*, 1999	W. Frey	AF034835

GGT GAC ACG AG-3′ (each 5′biotin-modified, MWG-Biotech, Roth). They were processed in a Biometra thermocycler with the following protocols: for primers C_M–D_M 3min 94°C, 34 cycles (1min 94°C, 1min 50°C, 1min 72°C) 3min 72°C, for primers C_M–F_M 5min 94°C, 35 cycles (1min 94°C, 1min 55°C, 1min 72°C) 2min 72°C. PCR products were purified using the Qiaquick PCR Purification Kit (Qiagen). Sequencing reactions (2min 94°C, 35 cycles [30s 94°C, 30s 60°C, 30s 72°C] 1min 72°C) were carried out in a Perkin Elmer thermocycler using the primers C_M and D_M mentioned above and the SequiTherm EXCEL™II DNA sequencing Kit (Epicentre Technologies). Sequencing reactions were separated in the GATC-1500-System, transferred to Nylon-membranes (Pall) and became visible after applying a standard protocol based on treatment with Streptavidin-Alkaline Phosphatase (Promega) and BCIP/NBT (Roth). To verify results, sequencing reactions were performed on two independent PCR products generated from each sample.

Alignment and tree construction

All sequences of the *trn*L intron were determined and aligned manually in the alignment editor Align32 (Hepperle, 1997). Molecular systematic trees were generated according to the maximum parsimony principle with PAUP 4.0b (Swofford, 1997). A heuristic search was performed with the specimens mentioned above; *Achrophyllum quadrifarium* (*Hookeriaceae*) was used as outgroup. In addition to substitution data, indels (insertions and/or deletions of nucleotides) which distinguished between the ingroup-specimens were included in the data matrix as a second set of characters. The search was performed with the following options: multistate characters interpreted as uncertainties, gaps coded as missing data, TBR branch swapping, collapse zero length branches, MulTrees option in effect, addition sequence 'random'. A bootstrap analysis was performed with 1000 bootstrap replicates of the heuristic search and the same options applying. For all generated trees, consistency index (CI) and retention index (RI) were calculated.

The *trn*L intron sequences of the specimens are deposited in the GenBank data base under the accession numbers given in Table 1.

RESULTS

In the *Hypopterygium* samples investigated, the *trn*L intron has a length of 298 (NZ10, AUS1) or 296bp (all other samples). In the *Lopidium* species it is 304bp long, which is mainly a result of an indel of 8bp length (indel 2). The alignment for all specimens is given in Fig. 2.

The sequences of all New Zealand *H. 'rotulatum'* specimens from primary forests (NZ1–9) are absolutely identical. Furthermore, neither indels nor substitutions are observed in comparing these samples with the Chilean *H. didictyon* (CHILE). The *H. 'rotulatum'* specimen NZ10, originating from a secondary (regenerating) open

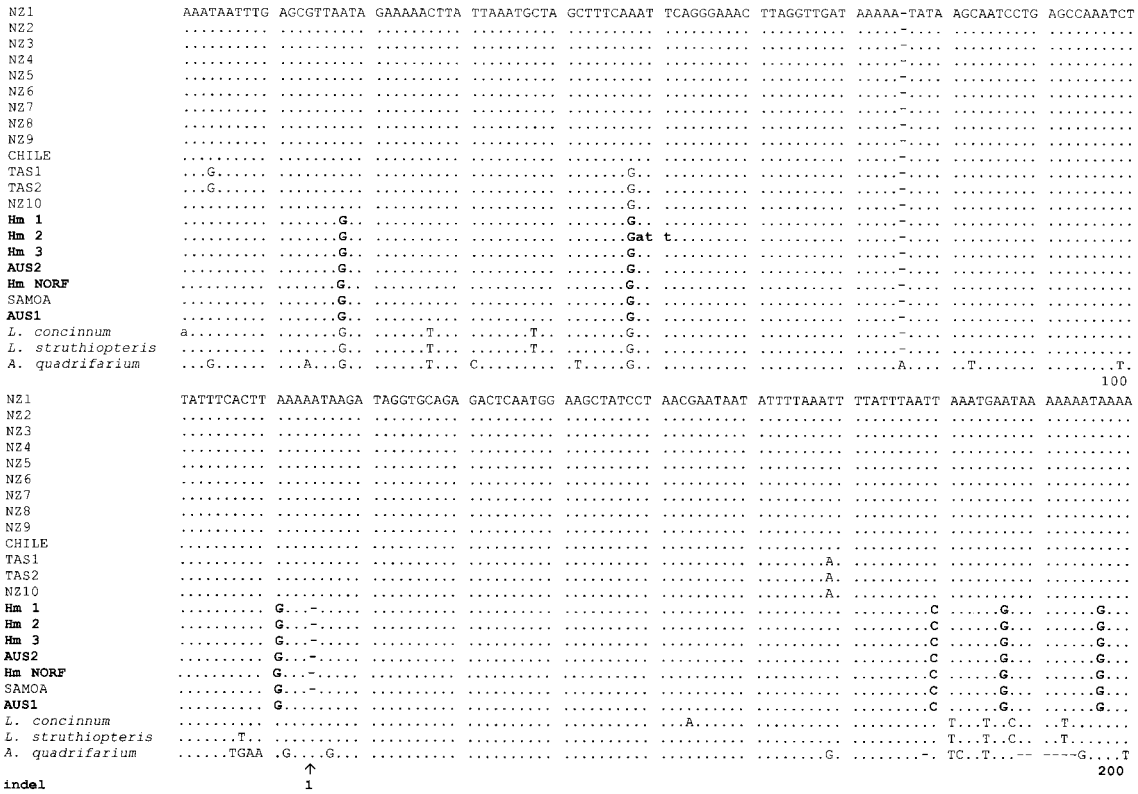

FIG. 2. Aligned sequences of *trn*L intron from cp genomes of *Hypopterygium 'rotulatum'* s.l. specimens from New Zealand (NZ1–10), Tasmania (TAS1, 2) and Australia (AUS1, 2) along with sequences of *H. muelleri* (Hm 1–3, Hm NORF), Chilean H. *didictyon* (CHILE), Samoan *H. debile* (SAMOA), *Lopidium concinnum, Lopidium struthiopteris* and *Achrophyllum quadrifarium* as outgroup. Positions equal with first sequence (NZ1) are given as dots. Specimens identified molecularly as *H. muelleri* are in bold letters. Indels differentiating between the ingroup-specimens are marked and numbered serially.

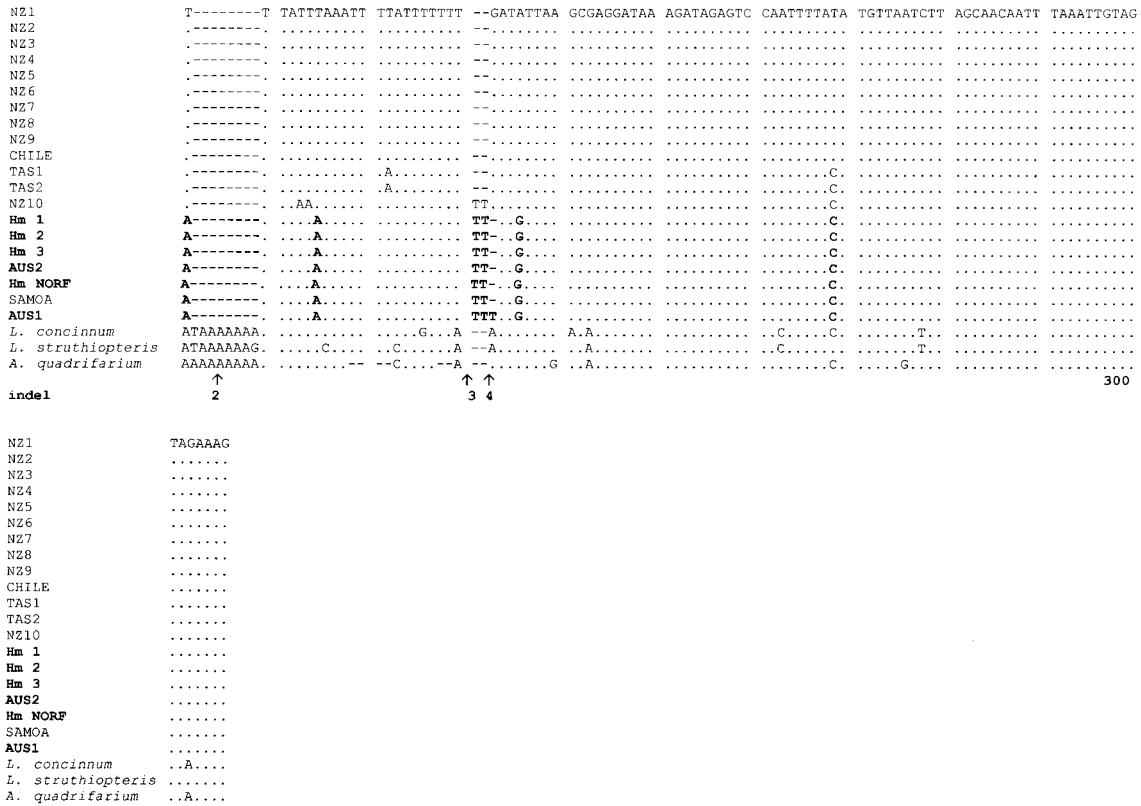
NZ1 T--------T TATTTAAATT TTATTTTTTT --GATATTAA GCGAGGATAA AGATAGAGTC CAATTTTATA TGTTAATCTT AGCAACAATT TAAATTGTAG
NZ2 .--------. .... --........ ....
NZ3 .--------. .... --........ ....
NZ4 .--------. .... --........ ....
NZ5 .--------. .... --........ ....
NZ6 .--------. .... --........ ....
NZ7 .--------. .... --........ ....
NZ8 .--------. .... --........ ....
NZ9 .--------. .... --........ ....
CHILE .--------. .... --........ ....
TAS1 .--------. .... .A........ --........ ....C. ....
TAS2 .--------. .... .A........ --........ ....C. ....
NZ10 .--------. ..AA...... .... TT........ ....C. ....
Hm 1 A--------. ....A..... .... TT-..G.... ....C. ....
Hm 2 A--------. ....A..... .... TT-..G.... ....C. ....
Hm 3 A--------. ....A..... .... TT-..G.... ....C. ....
AUS2 A--------. ....A..... .... TT-..G.... ....C. ....
Hm NORF A--------. ....A..... .... TT-..G.... ....C. ....
SAMOA A--------. ....A..... .... TT-..G.... ....C. ....
AUS1 A--------. ....A..... .... TTT..G.... ....C. ....
L. concinnum ATAAAAAAA. ....G...A --A..... A.A..... ....C.....C. ....T.. ....
L. struthiopteris ATAAAAAAG. .....C.... ..C......A --A..... ..A..... ....C..... ......T.. ....
A. quadrifarium AAAAAAAAA. ....-- --C.....-A --.....G ..A..... ....C. .....G.... ....
300
indel 2 3 4
NZ1 TAGAAAG
NZ2 ....
NZ3 ....
NZ4 ....
NZ5 ....
NZ6 ....
NZ7 ....
NZ8 ....
NZ9 ....
CHILE ....
TAS1 ....
TAS2 ....
NZ10 ....
Hm 1 ....
Hm 2 ....
Hm 3 ....
AUS2 ....
Hm NORF ....
SAMOA ....
AUS1 ....
L. concinnum ..A....
L. struthiopteris ....
A. quadrifarium ..A....

FIG. 2. (*Cont'd*).

forest is 2bp longer (298bp), a result of one 2bp insertion (indel 3 in Fig. 2). Identical *trn*L intron sequences are also obtained for both Tasmanian *H. 'rotulatum'* samples and when comparing *H.* (cf.) *muelleri* from New Zealand (Hm 1–3) and Norfolk Island (Hm NORF) with AUS2 and *H. debile* (SAMOA). In AUS1, the intron has the same length as in NZ10, but both specimens are readily differentiated by 12 substitutions. Distinct differences are observed when comparing the identical specimens NZ1–9/CHILE and the Tasmanian samples TAS1, 2 with *H.* (cf.) *muelleri*/*H. debile* and the Australian samples (10–12 substitutions; proportions of substitutions 3.7–4.1%). The number of substitutions (transversions and transitions), the proportions of substitutions and the indels differentiating between all *Hypopterygium* specimens are compiled in Table 2 (compare with Fig. 2).

The alignment for the maximum parsimony analysis (Fig. 2) has a length of 307bp, the indels code for four additional characters. Out of these, 257 are constant (82.6%), and 30 (9.7%) of the 54 (17.4%) variable characters are parsimony-informative. The heuristic search resulted in a single most parsimonious tree with a length of 62 steps, CI = 0.919 and RI = 0.957. The phylogram of the heuristic search (Fig. 3) and a 50% majority rule consensus tree from a bootstrap analysis (Fig. 4, length 62 steps, CI = 0.919, RI = 0.957) show the same topology. The investigated *Hypopterygium* taxa appear monophyletic. They form one well supported clade (bootstrap support, BS = 99%) which is separated from the branch of both *Lopidium* species. Within *Hypopterygium*, the investigated specimens are split into two distinct groups. One clade, consisting of New Zealand and Norfolk Island *H.* (cf.) *muelleri* (Hm 1–3, Hm NORF), *H. debile* (SAMOA) and the Australian *H. 'rotulatum'* samples, is supported by the maximum bootstrap value of 100%. Within this clade, the Australian sample AUS1 is separated from the other specimens (BS = 85%), which is a result of indels 1 and 4. The second group is also divided into two clades, one comprising the New Zealand *H. 'rotulatum'* specimens NZ1–9 and *H. didictyon* (CHILE) and a second with the Tasmanian specimens (BS = 84%) and next to NZ10. But the bootstrap support of 56% for this clade leaves this position ambiguous.

The same tree topology was generated when using an alignment with *Canalohypopterygium tamariscinum* (Hedw.) Kruijer (AF134632) and *Catharomnion ciliatum* (Hedw.) Wilson (AF134633; see Stech *et al.*, 1999) instead of the *Lopidium* species (data not shown).

DISCUSSION

The sequencing of the *trn*L intron of cp DNA provides new insights into the relationship of the *Hypopterygium 'rotulatum'* complex. From the *trn*L intron data, two taxa, which also show differences in their global distribution patterns, can be separated at species level.

The New Zealand specimen NZ2 has already been shown to have *trn*L intron sequence identity with the Chilean *H. didictyon* (CHILE), and the formerly two species are regarded as conspecific (Stech *et al.*, 1999, as *H. 'novae-seelandiae'*). The

TABLE 2. Compilation of *trn*L intron sequence differences between the investigated *Hypopterygium* samples. Number of transversions (tv), transitions (ts) and proportions of substitutions are given in upper right, in the lower left the indels differentiating between the samples are listed. For abbreviations of specimens see Table 1

	NZ1–NZ9, CHILE	NZ10	TAS1, TAS2	AUS2, *H.* (cf.) *muelleri*, *H. debile*	AUS1
NZ1-NZ9, CHILE	—	3 tv + 2 ts, 1.7%	3 tv + 2 ts, 1.7%	2 tv + 8 ts, 3.4%	3 tv + 8 ts, 3.7%
NZ10	indel 3	—	4 tv + 0 ts, 1.4%	5 tv + 6 ts, 3.7%	6 tv + 6 ts, 4.0%
TAS1, TAS2	—	indel 3	—	5 tv + 6 ts, 3.7%	6 tv + 6 ts, 4.1%
AUS2, *H.* (cf.) *muelleri, H. debile*	indels 1, 3, 4	indels 1, 4	indels 1, 3, 4	—	0
AUS1	indel 3	indel 4	indel 3	indels 1, 4	—

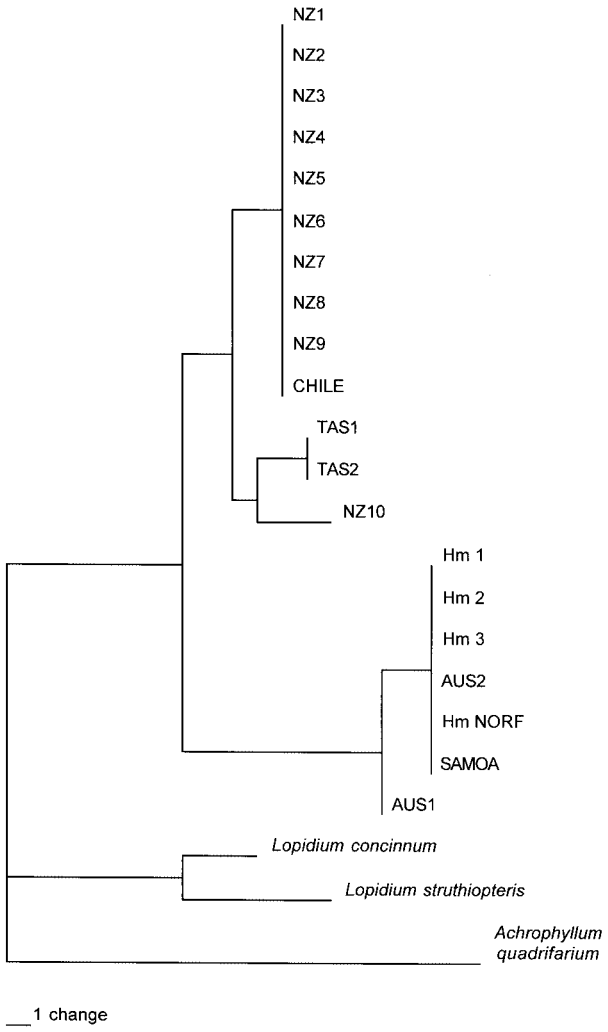

FIG. 3. Single most parsimonious tree obtained from 1,000 random replicates of a heuristic search with specimens of the *Hypopterygium 'rotulatum'* complex, *H. muelleri, H. debile, Lopidium concinnum, L. struthiopteris* and, as outgroup, *Achrophyllum quadrifarium*, performed with PAUP 4.0b (length 62 steps, CI = 0.919, RI = 0.957), displayed as a phylogram. For abbreviations see Table 1.

eight *H. 'rotulatum'* specimens from primary rain forests in New Zealand (NZ1, NZ3–9) additionally investigated in this study also show 100% sequence identity with the Chilean *H. didictyon*. These results provide further support for the opinion of Matteri (1973) that the two taxa are conspecific. Hence, the samples NZ1–9 are interpreted as belonging to *H. didictyon* (sensu Matteri, 1973; Kruijer, 1995). For samples NZ2 and NZ9 this was confirmed by a morphological determination (Kruijer, pers. comm.).

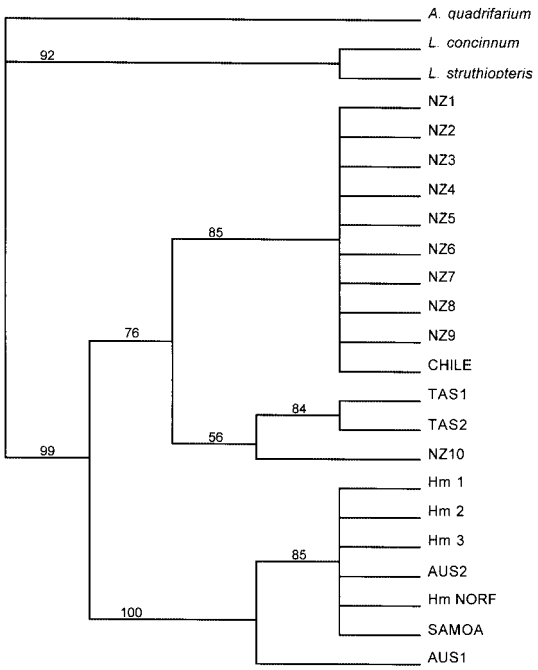

FIG. 4. 50% majority rule consensus tree of a bootstrap analysis with 1,000 resamplings of a heuristic search with specimens of the *Hypopterygium 'rotulatum'* complex, *H. muelleri, H. debile, Lopidium concinnum, L. struthiopteris* and *Achrophyllum quadrifarium* as outgroup, performed with PAUP 4.0b (length 62 steps, CI = 0.919, RI = 0.957). For abbreviations see Table 1.

The extant disjunct distribution pattern of *H. didictyon* (New Zealand–Australia/Tasmania–south-western South America; Matteri, 1973) can be explained by a Gondwanan origin. After the break-up of the palaeoaustral floristic region (palaeoaustral *Nothofagus* region) and the opening of the Tasman Sea approximately 82 million years ago (White, 1990; Hill, 1994), the former continuous range of the species was disrupted and the populations isolated. The other possible explanation of the present-day range as the result of a more recent long-distance dispersal of the species by transport of spores via the prevailing strong westerly winds of the Southern hemisphere is less likely for the following reasons: although the relatively small spores of *H. didictyon* (diameter about 10–17μm, Matteri, 1973) may conceivably traverse the 8000km separating New Zealand and Australia from southern South America, a successful establishment of the species is unlikely. As *H. didictyon* is strictly dioicous (Matteri, 1973; Kruijer, pers. comm.) and, in addition, has no specialized asexual diaspores to rely on for vegetative reproduction s.str., the regular fruiting of the species and hence the existence of bisexual stands ('breeding centres') in both Australasia and southern South America strongly supports a Gondwanan origin rather than long-distance dispersal (for monoicism/dioicism and long-range dispersal see also van Zanten & Pócs, 1981; Schuster, 1983: 471f.).

For *Lopidium concinnum* (with a distribution pattern similar to *H. didictyon*) Frey *et al.* (1999) have shown the great molecular similarity of New Zealand and South American specimens and interpreted the species as a remnant of the palaeoaustral flora and a 'stenoevolutionary' taxon (Frey *et al.*, 1999). For *H. didictyon* also, we consider this interpretation of the extant disjunction to be most likely.

Although separated by 'only' c.2000km of the Tasman Sea from the New Zealand populations, the Tasmanian *H. 'rotulatum'* samples differ in 1.7% of the *trn*L intron sequence. This proportion of base substitution suggests a separation of the Tasmanian specimens from *H. didictyon* at infraspecific level, as the substitution proportions in taxa differentiated at interspecific level are generally higher (c.3% in *Lopidium* and *Hymenophyton*, Frey *et al.*, 1999; Pfeiffer, 2000). This is reflected in Figs 3 and 4, in which the specimens from Tasmania (TAS1, 2) form a separate branch within *H. didictyon*. Morphologically, the Tasmanian specimens are characterized by dark-brown, non-tomentose stipes. However, the morphological characteristics/features are not consistent within Tasmanian material, thus not justifying a delimitation of taxa (Kruijer, pers. comm.).

The sequence differences between the New Zealand/Chilean/Tasmanian *H. didictyon* and the continental Australian *H. 'rotulatum'* samples AUS1, 2 are much greater. They indicate a separation and treatment of these specimens as a distinct species. The proportions of substitutions of 3.4–3.7% (compared with NZ1–9 and CHILE) and 3.7–4.1% (compared with TAS1, 2), respectively, are more significant than the proportions separating New Zealand and South American *Lopidium concinnum* and African *L. struthiopteris* (3.0–3.3%, Frey *et al.*, 1999) or the 2.7–3.3% observed between New Zealand *Hymenophyton flabellatum* and *H. leptopodum* (Pfeiffer, 2000). The strong relationship of the continental Australian specimens with *H. muelleri* (Hm 3, Hm NORF and the tentatively named samples Hm 1, 2) is evident from Figs 3 and 4, indicating that both Australian *H. 'rotulatum'* samples in fact belong to *H. muelleri*.

This 'molecular identification' is in accordance with a morphological determination (Kruijer, pers. comm.). These conclusions are further verified by the absolute identity of the *trn*L intron sequences with the Samoan *H. debile*. Thus, Kruijer's (1997) assumption that *H. tenellum, H. debile* and *H. muelleri* are possibly conspecific is supported for *H. debile* and *H. muelleri* by *trn*L intron data. The slightly separated position of AUS1 (Figs 3, 4) is a result of indels 1 and 4, but no substitutions differentiate it further from the other specimens within this clade (see also Fig. 2, Table 2).

The molecular-systematic position of the *H. 'rotulatum'* sample NZ10 is ambiguous. According to Kruijer (pers. comm.) the specimen belongs to *H. didictyon*, although in habit closely resembling *H. muelleri*; the superficial similarity may be caused by the more open, secondary forest habitat it was growing in. Kruijer's morphological determination is in accordance with the sequence similarity being greatest with *H. didictyon* from Tasmania (98.6%), followed by New Zealand/Chile (98.3%). From *H. muelleri* and the – possibly conspecific – *H. debile* (SAMOA) it is clearly differentiated by the much lesser degree of sequence identity (96–96.3%);

on the other hand, NZ10 shares indel 3 with *H. muelleri*/SAMOA. Consequently, the sample is placed in the *H. didictyon* clade (BS = 76%), but its position within this group is not fully resolved (Fig. 4).

The two species delimited within the *H. 'rotulatum'* s.l. complex, *H. didictyon* and *H. muelleri*, show different distribution patterns. In New Zealand, where both species occur, *H. didictyon* seems to be far more abundant in the terrestrial bryophyte communities of the primary temperate rain forests than *H. muelleri*. The c.15 *H. 'rotulatum'* samples collected during the BRYO AUSTRAL project trip in various forest sites along an approximately NE–SW-oriented transect in New Zealand belong exclusively to *H. didictyon*. In contrast, the three New Zealand *H. muelleri* specimens included in this study originate from slightly different habitats: they did not grow on soil but on boulders/rocks in or adjacent to streams or near waterfalls. Thus, there may be an additional ecological differentiation of the two species (at least in New Zealand), but this conclusion remains speculative until further material is investigated.

On a global scale, the two species formerly comprising *H. 'rotulatum'* s.l. exhibit different distribution patterns. *H. muelleri* is an Australasian and Western Pacific species (and more common in the North than the South Island of New Zealand), whereas *H. didictyon* has a palaeoaustral distribution pattern. If *H. didictyon* is a Gondwanan taxon and a species of the palaeoaustral floristic region, the dioicous or monoicous and gemmiferous *H. muelleri* (Kruijer, 1997) and the possibly conspecific *H. debile* could have originated from this 'stock' and drifted to the more northern-north-eastern (temperate to subtropical) regions they are now mainly distributed in.

In New Zealand *H. didictyon*, the high vegetative plasticity observed is not evident in the sequences of the *trn*L intron. By means of this marker, no effects of the latitudinal gradient (approximately NE–SW-oriented transect) or the altitudinal differences between samples from the same region (Tongariro: NZ2, 3; Haast: NZ7–9, Fig. 1) can be detected. The conformity of the samples allows no statements on a possible geomolecular divergence of *H. didictyon* specimens within New Zealand.

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