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Molecular data support *Ramalina ovalis* as a distinct lineage (*Ramalinaceae*, Ascomycota)

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Abstract: *Ramalina celastri* is a highly variable, widely distributed pan-subtropical lichen species. In Australasia the species had been separated into two subspecies; *R. celastri* subsp. *celastri* and *R. celastri* subsp. *ovalis*. This study compares morphological variation, substratum preference and sequences of the internal transcribed spacer (ITS) and intergenic spacer (IGS) regions of ribosomal DNA from a range of specimens from New Zealand and one from Australia. Bayesian and ML trees generated using the sequence data form two well-supported clades corresponding to the two previously recognized subspecies. Molecular, morphological and geographical differences support the recognition of *R. ovalis* at the species rank.

Key words: Australasia, IGS, ITS, lichens, *Ramalinaceae*, ribosomal DNA, species delimitation

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Introduction

Species delimitation in lichen-forming fungi has changed recently (Lumbsch & Leavitt 2011) with readily available DNA sequence data and the discovery of distinct lineages that were either morphologically indistinguishable or shown to differ in subtle, previously overlooked characters and hence are referred to as cryptic or sometimes semi-cryptic species (e.g. Crespo & Pérez-Ortega 2009; Vondrak *et al.* 2009; Crespo & Lumbsch 2010; Otálora *et al.* 2010; Hodkinson & Lendemer 2011; Leavitt *et al.* 2011, 2012, 2013; Lumbsch & Leavitt 2011; Molina *et al.* 2011; Nuñez-Zapata *et al.* 2011; Parnmen *et al.* 2012; Wirtz *et al.* 2012). Environmental factors have been shown to influence phenotypic characters in various groups of lichens (Culberson *et al.* 1983; Fernandez-Mendoza *et al.* 2011; Pérez-Ortega *et al.* 2012). Also, a remarkable amount of morphological disparity within clades of lichenized fungi has been shown (Wedin & Döring 1999; Blanco *et al.* 2004; Högnabba 2006; Tehler & Irestedt 2007; Lumbsch *et al.* 2010; Parnmen *et al.* 2010). This suggests that morphological characters have limited taxonomic value, at least in some groups of lichenized fungi. However, there are also cases in which similar species that were only recently distinguished based on morphological characters, which were previously interpreted as intraspecific variation, have been confirmed by molecular data. Examples include *Cladia moniliformis* Kantvilas & Elix as distinct from *C. aggregata* (Sw.) Nyl. (Parnmen *et al.* 2012, 2013), *Punctelia jeckeri* (Roum.) Kalb as being distinct from *P. subrudecta* (Nyl.) Krog (Crespo *et al.* 2004, 2010; Lendemer & Hodkinson 2010) or *Usnea lambii* (Imshaug) Wirtz & Lumbsch and *U. sphacelata* R. Br. (Wirtz *et al.* 2008, 2012; Lumbsch & Wirtz 2011) .

Here we focus on the interpretation of morphotypes within *Ramalina celastri* (Spreng.) Krog & Swinscow, which is a pan-subtropical lichen species with flattened yellow-green

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strap-like branches and numerous laminal and marginal apothecia. It is found in Australia (Stevens 1987), New Zealand (Blanchon *et al.* 1996), Africa (Krog & Swinscow 1976), India (Pant & Awasthi 2003) and South America (Kashiwadani & Kalb 1993). Stevens (1987) and Blanchon *et al.* (1996) also recognized a subspecies, *R. celastri* subsp. *ovalis* (J. D. Hook. & Taylor) N. Stevens, for Australasia, although later treatments of the New Zealand species (Bannister & Blanchon 2003; Bannister *et al.* 2004; Galloway 2007) did not recognize the subspecies for New Zealand. *Ramalina celastri* s. str. appears to be a very variable taxon, with Stevens (1987) reporting two morphotypes for Australia and Blanchon *et al.* (1996) noting a wide range of thallus forms, particularly an inland, fine, narrow thallus form and a coastal rock form with robust and broad thalli. In New Zealand, the species is widespread and common on a range of native and introduced trees and shrubs in urban, rural and natural areas. This paper addresses two objectives: 1) to compare morphological variation, substratum preference and molecular data for a range of specimens of *Ramalina celastri* collected from selected areas of New Zealand; and 2) to evaluate the taxonomic status of *Ramalina ovalis* J. D. Hook. & Taylor in Australasia.

Materials and Methods

Taxon sampling

A total of 105 specimens from the *Ramalina celastri* complex were collected from sites in the North and South Islands of New Zealand, including a range of land use types (urban, agricultural, native forest, coastal) and substrata (native and introduced trees and shrubs, and rocks). A specimen from Victoria, Australia was included in this study and samples of *R. inflexa* and *R. glaucescens* were collected from New Zealand as outgroups. Voucher specimens are deposited in the herbarium, Unitec Institute of Technology (UNITEC).

Chemical examination

Standard methods were used for thin-layer chromatography (TLC) using the solvent system A and C (Culberson & Ammann 1979).

DNA extraction and PCR amplification

DNA was extracted from medullary hyphae from whole thalli of recently collected specimens (less than one year old). Hyphae were dissected from washed thalli, soaked in acetone, dried and DNA was extracted using a Qiagen DNeasy Plant Mini Kit, following the manufacturer's instructions. Two loci were amplified: nuclear ITS rDNA using primer pairs ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990), and a fragment of the 3′-end of the nuclear IGS rDNA using primer pairs IGSf and IGSr (Wirtz *et al.* 2008). PCR was carried out in an Eppendorf Mastercycler Personal. The amplification programs were: initial denaturation for 2 min at 95°C, followed by 30 cycles of 30 s at 95°C, 30 s at 48°C, 1 min at 72°C; with a final elongation of 10 min at 72°C for both ITS and IGS. PCR products were purified using the Qiagen MinElute PCR purification kit. Sequencing was performed by the Massey Genome Service (Massey University, Palmerston North, New Zealand) using an Applied Biosystems model 3730 automated capillary DNA sequencer, using the same primers as for PCR.

Sequence editing and alignment

Sequences were assembled, edited and aligned using Geneious Pro 5.5.6 (Drummond *et al.* 2011). Sequences of 33 specimens (Table 1) were aligned separately for the two loci.

Phylogenetic analyses

The data sets were analyzed separately and combined using maximum likelihood (ML) and a Bayesian approach. ML analyses were performed using the single-locus and concatenated data sets as implemented in RAxML (Stamatakis 2006) with 2000 bootstrap replicates. Tree searches were performed with 1000 replicates, using the GTR+G model. Bayesian tree inference was carried out for the concatenated data set using MrBayes version 3.2.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Two parallel MCMCMC runs were performed, each using four chains and 5 000 000 generations, sampling trees every 1000th generation. A 50% majority-rule consensus tree was generated from the combined sampled trees of both runs after discarding the first 25% as burn-in. Conflicts were assumed to be significant if two different relationships for the same set of taxa were supported with ML bootstrap values above 75%.

Results

We generated 33 new nuITS rDNA, and 33 nuIGS rDNA sequences for this study (Table 1). The matrix of the nuITS data set had 546 unambiguously aligned nucleotide position characters, while the nuIGS data set had 416 unambiguously aligned nucleotide position characters. A total of 739 positions were constant in the combined data set.

The two single locus ML trees did not show any incongruence and hence combined analyses were performed. The Bayesian and

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TABLE 1. *Material used in this study. Vouchers, their geographical origin, substratum, herbarium accession numbers and GenBank accession numbers for ITS and IGS sequences are listed.*

Species	Locality, substratum, collector number and herbarium accession number	GenBank Acc. No.	
		ITS	IGS
Ramalina celastri	New Zealand, Auckland, Whatipu, rock, *G. C. Hayward* 120 (UNITEC 4616)	KF583526	KF594436
R. celastri	New Zealand, Auckland, Huia, *Plagianthus divaricatus G. C. Hayward* 121 (UNITEC 4618)	KF583527	KF594437
R. celastri	New Zealand, Auckland, Little Huia, *Prunus*, *G. C. Hayward* 128 (UNITEC 4617)	KF583528	KF594438
R. celastri	New Zealand, Auckland, Mt Albert, *Metrosideros excelsa*, *G. C. Hayward* 133 (UNITEC 4620)	KF583529	KF594439
R. celastri	New Zealand, Auckland, Mt Albert, *Sophora*, *G. C. Hayward* 134 (UNITEC 4637).	KF583530	KF594440
R. celastri	New Zealand, Tawharanui, rock, *G. C. Hayward* 135 (UNITEC 4478)	KF583531	KF594441
R. celastri	New Zealand, Auckland, Otuataua, basalt, *G. C. Hayward* 139 (UNITEC 4685)	KF583532	KF594442
R. celastri	New Zealand, Auckland, Wenderholm, *Avicennia marina* subsp. *australasica*, *G. C. Hayward* 144 (UNITEC 4646)	KF583533	KF594443
R. celastri	New Zealand, Whangarei, *Avicennia marina* subsp. *australasica*, *G. C. Hayward* 149 (UNITEC 4639)	KF583534	KF594444
R. celastri	New Zealand, Whangarei, *Plagianthus divaricatus*, *G. C. Hayward* 150 (UNITEC 4641)	KF583535	KF594445
R. celastri	New Zealand, Auckland, Remuera, *Avicennia marina* subsp. *australasica*, *G. C. Hayward* 151 (UNITEC 4482)	KF583536	KF594446
R. celastri	New Zealand, Auckland, Meadowbank, *Prunus*, *G. C. Hayward* 152 (UNITEC 4483)	KF583537	KF594447
R. celastri	New Zealand, Auckland, Browns Island, *Cupressus macrocarpa*, *G. C. Hayward* 153 (UNITEC 4650)	KF583538	KF594448
R. celastri	New Zealand, Auckland, Browns Island, rock, *G. C. Hayward* 154 (UNITEC 4649)	KF583539	KF594449
R. celastri	New Zealand, Canterbury, Christchurch, *Discaria toumatou*, *G. C. Hayward* 161 (UNITEC 4744)	KF583540	KF594450
R. celastri	New Zealand, Wellington, Ngaio, *Prunus*, *D. J. Blanchon* (GCH 188) (UNITEC 4683)	KF583541	KF594451
R. celastri	New Zealand, Waikato, Meremere, *Callistemon*, *H. R. Nessia* (GCH 191) (UNITEC 4752)	KF583542	KF594452
R. celastri	New Zealand, Northland, North Cape, iron nodule, *P. J. de Lange* & *M. A. M. Renner* 10227 (GCH 192) (AK 328039)	KF583543	KF594453
R. celastri	New Zealand, Hauraki Gulf, Kaikoura Island, volcanic conglomerate, *D. J. Blanchon* s. n. (UNITEC 5417)	KF583544	KF594454
R. glaucescens	New Zealand, Canterbury, West Melton, *Sophora microphylla*, *P. J. de Lange* 11097 (UNITEC 5429)	KF583545	KF594455
R. inflexa	New Zealand, Canterbury, Okains Bay, deciduous tree, *G. C. Hayward* 190 (UNITEC 4656)	KF583546	KF594456
R. inflexa	New Zealand, Otago, Dunedin, deciduous tree, *A. Knight* (UNITEC 5331)	KF583547	KF594457
R. ovalis	New Zealand, Canterbury, Governor's Bay, *Prunus*, *G. C. Hayward* 159 (UNITEC 4659)	KF583548	KF594458
R. ovalis	New Zealand, Canterbury, Lake Ellesmere, *Prunus*, *G. C. Hayward* 160 (UNITEC 4663)	KF583549	KF594459
R. ovalis	New Zealand, Canterbury, Little River, deciduous tree, *G. C. Hayward* 163 (UNITEC 4660)	KF583550	KF594460
R. ovalis	New Zealand, Canterbury, Taylor's Mistake, *Banksia*, *G. C. Hayward* 166 (UNITEC 4652)	KF583551	KF594461
R. ovalis	New Zealand, Canterbury, Teddington, deciduous tree, *G. C. Hayward* 167 (UNITEC 4657)	KF583552	KF594462
R. ovalis	New Zealand, Canterbury, Barry's Bay, *Cordyline australis*, *G. C. Hayward* 173 (UNITEC 4665)	KF583553	KF594463
R. ovalis	New Zealand, Canterbury, Kaiapoi, *Prunus*, *G. C. Hayward* 177 (UNITEC 4672)	KF583554	KF594464
R. ovalis	New Zealand, Canterbury, Ilam, deciduous tree, *G. C. Hayward* 179 (UNITEC 4670)	KF583555	KF594465
R. ovalis	New Zealand, Canterbury, Spreydon, deciduous tree, *G. C. Hayward* 186 (UNITEC 4681)	KF583556	KF594466
R. ovalis	New Zealand, Otago, Dunedin, fence post, *A. Knight* (UNITEC 5329)	KF583557	KF594467
R. ovalis	Australia, Victoria, Melbourne, corticolous, *V. Stajsic* (UNITEC 5607/MEL 2363318)	KF583558	KF594468

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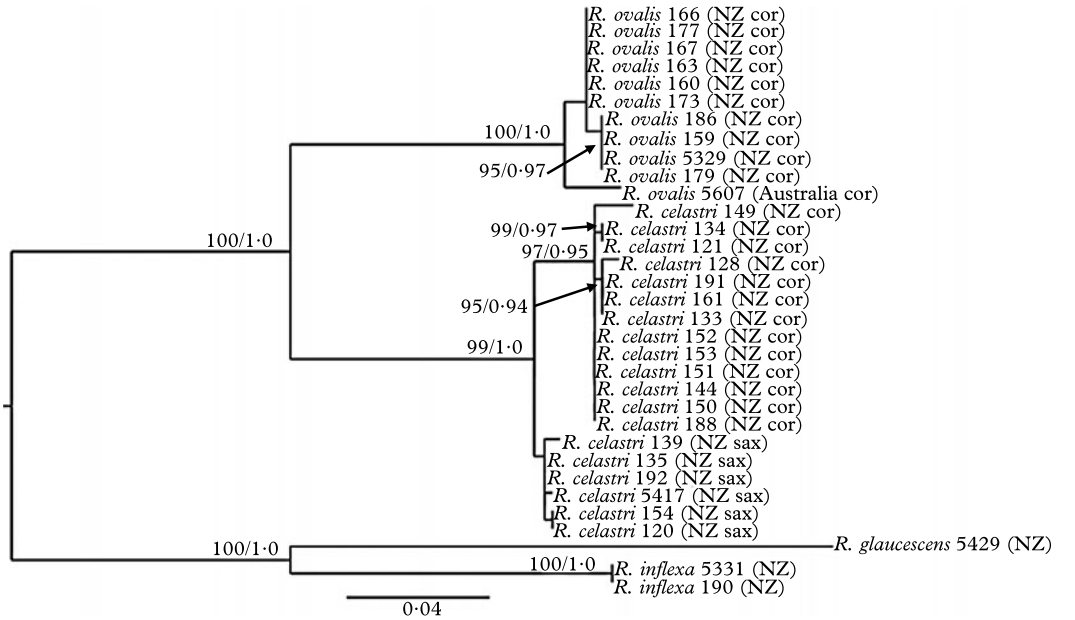


FIG. 1. Phylogenetic relationships of *Ramalina celastri* s. lat. samples based on a maximum-likelihood analysis using two ribosomal loci (ITS, IGS). Most likely tree obtained with RAxML; ML-bootstrap support equal or above 70% are indicated first, and posterior probabilities equal or above 0·95 as the second number at branches. The country in which the sample was collected and the substratum (sax = saxicolous, cor = corticolous) are indicated in brackets.

ML trees generated from the combined data set had an identical topology and so only the ML tree is shown here (Fig. 1). The *Ramalina celastri* samples in the tree form two well-supported clades. One of the clades includes specimens which morphologically fit into the concept of *Ramalina celastri* subsp. *ovalis*. The other clade represents *Ramalina celastri* s. str. These two clades are separated with high bootstrap support (100) and high posterior probability in a Bayesian framework (1·0). The latter clade is further separated into corticolous and saxicolous individuals but this is not well supported in our analysis. Within the corticolous *R. celastri* subclade there was no apparent correlation with geography or substratum tree species, with samples from seven native and six non-native tree substrata from rural, urban and natural areas interspersed.

Discussion

The separation of *R. ovalis* from *R. celastri* s. str. is strongly supported by the molecular data in our study. There are also morphological and geographical differences between these two groups, previously reported by Stevens (1987) and Blanchon *et al.* (1996). *Ramalina ovalis* has blunt or rounded apices, laminal apothecia and one or a few broad branches (Fig. 2A & B). In contrast, *Ramalina celastri* s. str. is morphologically variable, but tends to have narrower branches which are sometimes canaliculate, marginal and laminal apothecia and densely branched thalli (Fig. 2C). In combination, we regard these differences as significant enough to recognize *Ramalina ovalis* at the species rank.

Ramalina ovalis appears to be a strictly corticolous species from the drier, cooler parts of southern and south-eastern Australia and south-eastern New Zealand. Stevens (1987) recorded this species (as *R. celastri* subsp. *ovalis*) from East Africa (Uganda and Tanzania) and a specimen from Kenya (AK 310393) examined by us shows the morphology of *R. ovalis*, as does an image published by Krog & Swinscow (1976; fig. 2, p. 160). Additional studies on fresh material from

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FIG. 2. A & B, *Ramalina ovalis*, habit; A, New Zealand; *G. C. Hayward* 185 (UNITEC); B, Australia; *J. A. Elix* 21335 (MEL). C & D, *R. celastri*, habit; C, corticolous specimen, New Zealand; *G. C. Hayward* 121 (UNITEC); D, saxicolous specimen, New Zealand; *G. C. Hayward* 154 (UNITEC). Scales: A–D = 10 mm. In colour online.

East Africa are needed to confirm the presence of that species on the African continent, but a disjunction of species between East Africa and Australasia has been reported for other species of *Ramalina*, including *R. exiguella* Stirt., *R. fimbriata* Krog & Swinscow (Stevens 1987) and *R. luciae* Molho *et al.* (Bannister & Blanchon 2003), and other groups of lichens (Kantvilas 1991). *Ramalina celastri* s. str. is either corticolous or saxicolous and is more common in warmer, wetter parts of Australia (New South Wales and Queensland) and northern New Zealand.

Saxicolous specimens of *Ramalina celastri* s. str. were found in the North Island in strictly coastal habitats and differed morphologically from corticolous specimens by being more robust with broader, longer thalli (Fig. 2D). In cross-section, the medulla in saxicolous specimens was thicker. The samples from the two different substrata were not randomly distributed (Fig. 1) and further studies are needed to understand the relationships between populations on different substrata. There are several published examples of lichen species displaying a correlation between molecular differences and substratum preferences. This includes corticolous populations of *Parmelia saxatilis* (L.) Ach. s. lat. in the Mediterranean that were found to be genetically distinct and accommodated in a separate species, *P. serrana*, despite displaying only subtle morphological differences (Molina *et al.* 2004). Similarly, molecular data supported the delimitation of *Bacidina arnoldiana* (saxicolous) and *B. sulphurella* (corticolous), despite these species being very similar morphologically (Czarnota

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& Guzow-Krzeminska 2012). More research is required before assigning any taxonomic significance to our results of the potential of substratum specificity.

Taxonomy

Ramalina ovalis Hook. f. & Taylor

Lond. J. Bot. **3:** 655 (1844).—*Ramalina calicaris* var. *ovalis* (Hook. f. & Taylor) Church. Bab., *Flora Tasmaniae* **3:** 344 (1860).—*Ramalina ecklonii* var *ovalis* (Hook. f. & Taylor) F. Wilson, *Pap. Proc. R. Soc. Tasm.* **1892:** 159 (1893).—*Ramalina yemensis* var *ovalis* (Hook. f. & Taylor) Zahlbr., *Cat. Lich. Univ.* **6:** 529 (1930).—*Ramalina celastri* subsp. *ovalis* (Hook. f. & Taylor) G. N. Stevens, *Bull. Br. Mus. Nat. Hist. (Bot.)* **16:** 184 (1987); type: Australia, *Gunn* s.n. (BM—holotype).

(Fig. 2A & B)

Thallus corticolous, green-grey, erect, 1·0–5·0(–8·5) cm long; branching sparse with usually only one or a few branches making up the whole thallus; branch width 4–25 mm, flat, apices blunt or rounded, small branchlets often occurring at the apices; surface matt, occasionally having longitudinal ridges; pseudocyphellae usually present, short linear or labriform; holdfast present. *Soredia* absent.

Apothecia laminal only, numerous; *disc* 0·4–2·5 mm diam., plane to convex; margin entire. *Ascospores* ellipsoid, mostly curved, occasionally straight, 10–16(–18) × 4–6 µm.

Chemistry. Usnic acid. Medulla K–.

Notes. The type material of *R. ovalis* from Australia (Van Dieman's Land) has the oval branch form seen in most Australasian specimens. Material that is correctly placed within *R. ovalis* has blunt or rounded tips, laminal apothecia only, one or a few branches and is corticolous. Coastal, saxicolous specimens of *R. celastri* from northern New Zealand can be differentiated from *R. ovalis* by the pointed tips, marginal and laminal apothecia, multiple branches, branching along the entire thallus, and canaliculate branches. *Ramalina inflexa* can be distinguished from *R. ovalis* by its dichotomous branching, canaliculate branches and marginal and subterminal spurred apothecia. *Ramalina glaucescens* often co-occurs with *R. ovalis* in New Zealand and Australia, but can be distinguished by its chemistry (usually sekikaic and/or divaricatic acids), narrower branches, the presence of side branchlets, and generally marginal and subterminal apothecia (some thalli have some laminal apothecia, but not to the extent seen in specimens of *R. ovalis*).

Distribution and habitat. Ramalina ovalis is common in the drier areas of the eastern South Island of New Zealand, including inland Canterbury, Otago and Southland, and also occurs, but is less commonly found, in Marlborough, Nelson, Wellington, Palmerston North and New Plymouth (Blanchon *et al.* 1996; Bannister *et al.* 2004). Stevens (1987) also recorded this species (as *R. celastri* subsp. *ovalis*) from southern Australia (New South Wales, Victoria, South Australia, Western Australia and Tasmania) and the higher altitude areas of Africa (Tanzania and Uganda), although Swinscow & Krog (1988) did not recognize the taxon for Africa. This species is corticolous, and grows on a wide range of native and introduced trees and shrubs. The most common substrata in New Zealand are *Carmichaelia* spp. (broom), *Coprosma crassifolia*, *Crataegus monogyna* (hawthorn), *Malus* x *domestica* (apple), *Prunus* x *domestica* (plum), *Salix* spp. (willow), and *Sophora microphylla* (kowhai), and in Australia species of *Acacia*. In Australia it may be found growing with *R. glaucescens* and in New Zealand with *R. glaucescens* and *R. inflexa*.

Selected additional specimens examined. **New Zealand:** *Otago*: Dunedin, Sawyer's Bay, *A. Knight* (UNITEC). *Canterbury*: Banks Peninsula, Little Akaloa, *G. Hayward* 162 (UNITEC); Christchurch, Mona Vale, *G. Hayward* 178 (UNITEC); Christchurch, Bexley Reserve, *G. Hayward* 181 (UNITEC).—**Australia:** *South Australia*: Mount Lofty Ranges, Springton, *J. A. Elix* 21335 (MEL). *Victoria*: Otway Ranges, Yeodene, *A. C. Beauglehole* (MEL). *Tasmania*: Stanley, The Nut, *J. H. Willis* (MEL).

Ramalina celastri (Spreng.) Krog & Swinscow

Norw. J. Bot. **23:** 159 (1976).— *Parmelia celastri* Spreng., *Syst. Veget.* **4** (2)**:** 328 (1827); type: South Africa, Cape of Good Hope, *Eklon* (S—holotype; FH—isotype; usnic acid).

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(Fig. 2C & D)

Thallus corticolous or saxicolous, pale green to grey-green, occasionally blue-grey, erect or pendulous, 1–12(–30) cm long; branching trichotomous, subdichotomous or irregular, small branches at right angles to the main branch usually present; branch width (1–)3–10 mm, occasionally to 20 mm, branches ranging from narrow and canaliculate with fine apices to broad and flat with blunt or tattered tips; surface matt, smooth to ridged or rugose, thin to coarse and thick, torn or fenestrate in older thalli, sometimes producing small lobules from torn areas; pseudocyphellae common, short and linear or punctiform; holdfast present. *Soredia* absent.

Apothecia numerous, marginal on narrow branches, marginal and laminal on broader branches; *disc* 0·3–2·0(–4·0) mm diam., plane to convex; margin narrow, entire, smooth or incised, often occluded with age. *Ascospores* oval, ellipsoid, straight, 9·5–15·5(–17·5) × 4–6 μm.

Chemistry. Usnic acid. Medulla K–.

Notes. The holotype of *R. celastri* is from South Africa and consists of three small thalli, and these resemble New Zealand material. The isotype from FH is in better condition, and this also closely resembles typical New Zealand specimens.

A wide range of thallus forms can be seen in nature, ranging from the very robust and broad thalli found on coastal rocks, to fine narrow thalli which appear to be almost geniculate and are found at more inland locations and in mangrove swamps. New Zealand material is very similar to the Australian thalli, and both possess the same chemistry, usnic acid and occasionally one or more faint unknowns. A wide range of names has been applied to herbarium material of *R. celastri*, and these are covered elsewhere (Krog & Swinscow 1976; Stevens 1983, 1987; Blanchon *et al.* 1996).

Other species which could be mistaken for *R. celastri* include *R. inflexa*, a species which differs from *R. celastri* by having subterminal or terminal spurred apothecia; *R. glaucescens*, which can be distinguished from *R. celastri* by its chemistry and (usually) subterminal or terminal apothecia; and *R. exiguella*, which can be mistaken for morphotypes of *R. celastri*, but the former species is truly terete and has subterminal or terminal apothecia.

Distribution and habitat. Ramalina celastri is found throughout New Zealand and its offshore islands, including the Chatham Islands and Stewart Island. This species is gradually replaced by *R. ovalis* from Canterbury southwards, but can still be found on the coast as far south as Otago and Stewart Island. It is usually the only species of *Ramalina* present on the west coast of the South Island. *Ramalina celastri* is pan-subtropical in its distribution, and is found in Australia (Stevens 1987), Africa (Swinscow & Krog 1988), India (Pant & Awasthi 2003) and South America (Kashiwadani & Kalb 1993). It is the most common of the New Zealand *Ramalina* taxa, and is a very effective colonizer. In New Zealand it is often found on introduced trees such as fruit trees, *Salix* spp. (willow), *Crataegus monogyna* (hawthorn), ornamental trees, as well as fence posts. It is also common on *Avicennia marina* subsp. *australasica* (mangroves), *Metrosideros excelsa* (pohutukawa), *Sophora microphylla* (kowhai), *Leptospermum scoparium* (manuka), *Agathis australis* (kauri), *Plagianthus divaricatus*, and rocks of various types (andesite, argillite, basalt, greywacke, iron nodules and rhyolite). In a number of northern New Zealand sites, *R. celastri* is found growing with *R. pacifica, R. australiensis* and *R. geniculata* (on mangroves), or *R. meridionalis* (rocky coastal areas). Further south it may be found with *R. inflexa* or *R. glaucescens*. In Australia, Stevens (1987) recorded *R. celastri* commonly growing on species of *Acacia*, often in association with *R. inflata* subsp. *inflata*, *R. inflata* subsp. *perpusilla* and *R. reducta*.

Additional specimens examined. **New Zealand:** *Northland*: Waipu, *G. C. Hayward* 148 (UNITEC). *Auckland*: Kaipara Harbour, Mt Rex, *D. J. Blanchon* & *N. W. Blanchon* (UNITEC); Kawau Island, Vivian Bay, *G. C. Hayward* (UNITEC). *East Cape*: Pokara, Lottin Point, *P. J. de Lange* 11593 (UNITEC). *Wairarapa*: Cape Palliser, *D. J. Blanchon* (UNITEC).—**Australia:** *Queensland*: Moreton, Lamington National Park, *T.*

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Hand (MEL). *New South Wales*: 20 mi S of Batemans Bay, *G. C. Bratt* 1020 (MEL).

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