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Phylogenetic relationships within *Plantago* (Plantaginaceae): evidence from nuclear ribosomal ITS and plastid *trnL-F* sequence data

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A molecular phylogenetic study of *Plantago* L. (Plantaginaceae) analysed nucleotide variation in the internal transcribed spacers (ITS) of nuclear ribosomal and plastid *trnL-F* regions. Included are 57 *Plantago* species, with two *Aragoa* species as the ingroup and three *Veronica* species as the outgroup. Phylogenetic analysis using maximum parsimony identified five major clades, corresponding to the taxonomic groups *Plantago* subgenera *Plantago, Coronopus, Psyllium, Littorella* and *Bougueria*. *Aragoa* is sister to genus *Plantago*. *Plantago* subgenus *Littorella* is sister to the other subgenera of *Plantago*. The results are in general correlated with a morphological phylogenetic study and iridoid glucoside patterns, but *Plantago* subgenus *Albicans* is paraphyletic and should be included in *Plantago* subgenus *Psyllium sensu lato* to obtain a monophyletic clade with six sections. *Plantago* section *Hymenopsyllium* is more closely related to section *Gnaphaloides* than to section *Albicans*. *Plantago* subgenus *Bougueria* is sister to subgenus *Psyllium s.l.* section *Coronopus* in *Plantago* subgenus *Coronopus* is subdivided in two series. Only some of the sections can be resolved into series. DNA variation within genus *Plantago* is high, a result that would not have been predicted on the basis of morphology, which is relatively stereotyped. If we calibrate a molecular clock based on the divergence of *P. stauntoni*, endemic to New Amsterdam in the southern Indian Ocean, we calculate the time of the split between *Plantago* and *Aragoa* to be 7.1 million years ago, which is congruent with the fossil record. *Botanical Journal of the Linnean Society,* 2002, **139,** 323–338.

ADDITIONAL KEYWORDS: *Aragoa* – *Bougueria* – iridoid glucosides – *Littorella* – molecular systematics – Veronicaceae.

INTRODUCTION

Plantago (Plantaginaceae) consists of roughly 200 annual and perennial herbs and subshrubs with a world-wide distribution. In Rahn (1996), *Plantago* was considered monophyletic and supported by a number of synapomorphies: phyllodial and parallel-veined leaves with hairs in the axils, scarious corolla, absence of a disc, long, filiform, dry stigmas with two lateral bands of papilla, protogynous flowers, anthers with an extension of the connective and forate pollen grains with 4–15 apertures. The relationship of the genus within Dahlgren's (1989) superorder *Lamianae* was considered unclear. However, in two studies on Scrophulariaceae, based on plastid DNA data (Olmstead & Reeves, 1995; Olmstead *et al*., 2001), a number of genera previously considered to belong to Scrophulariaceae were found to fall in a separate clade with *Plantago*. Olmstead *et al*. (2001) suggested that the family name Veronicaceae would be appropriate if this 'scroph II' clade were recognized as a separate family, but they also mentioned that the valid name would be

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Plantaginaceae (which name is used here). Rahn (1996) considered that Plantaginaceae should contain only one genus, *Plantago*, including the four species sometimes placed in the small genera *Bougueria (*monotypic) and *Littorella*.

Olmstead & Reeves (1995) and Olmstead *et al*. (2001) found *Veronica* to be the sister group of *Plantago*. *Plantago* and *Veronica* were also sister groups in an analysis of the asterids using 18S nrDNA and plastid *rbcL*, *ndhF* and *atpB* DNA sequence data (Albach *et al*., 2001). In another phylogenetic study of *Veronica* using just nuclear ribosomal ITS sequences, but increased generic sampling, Albach & Chase (2001) found that several other genera of Veroniceae were closer to *Plantago* than *Veronica*. Recently, Bello *et al*. (in press) discovered that *Aragoa*, an endemic to the páramos of Columbia and Venezuela, was sister to *Plantago*, and both fell in a clade also including *Veronica, Hemiphragma* and *Hebe* (Veroniceae of Scrophulariaceae *sensu auct. mult.*).

The most recent phylogenetic study of *Plantago* by Rahn (1996) relied upon 91 mainly morphological and embryological characters. Hair and seed characters were the most informative for his estimation of the infrageneric relationships. In all, 213 species, grouped in six subgenera and a number of sections and series, were recognized (Table 1). A number of taxonomic studies have evaluated the use of iridoid glucosides as chemical markers in *Plantago* (Andrzejewska-Golec, 1997; Rønsted *et al*., 2000; and references therein). Iridoid glucosides seem to be valuable taxonomic characters at the subgenus and section level but cannot resolve species relationships, partly due to the restricted number of compounds. An early attempt to use molecular data to study the relationships of five *Plantago* species employed restriction endonuclease site variability (Wolff & Schaal, 1992), which produced a pattern in agreement with Rahn's taxonomy (1996). In a study of angiosperm mitochondrial genome structure, Palmer *et al*. (2000) found that *Plantago* was one of two lineages with highly accelerated substitution rates. The increased rates appeared to be restricted to the mitochondrial genomes, but these were not uniform throughout the species of *Plantago*.

The aim of this paper is to examine the phylogenetic relationships within *Plantago* and compare these with the groupings found in Rahn's (1996) morphological and embryological analysis (Table 1). Secondly, we will focus on the age of *Plantago* and its sister genus *Aragoa* by using a calibration point within *Plantago* based on divergence of *P. stauntoni* Reichardt, native to New Amsterdam. To evaluate phylogenetic rela-

Table 1. Subgenera of *Plantago* after Rahn (1996). *Series not included in this study due to lack of material

Species No.	Subgenera, section and series	Distribution
1–42	*Plantago* sect. *Plantago*	Africa, Asia, Europe, N. America, Oceania
43–48	*Plantago* sect. *Micropsyllium*	America, Asia, Europe
49–80	*Plantago* sect. *Mesembrynia*	Australia, New Zealand, Amsterdam and St. Paul's Isl.
81–108	*Plantago* sect. *Virginica*	N. and S America
109–116	*Plantago* sect. *Oliganthos* ser. *Oliganthos*	S. America, Auckland, Tasmania
117–118	*Plantago* sect. *Oliganthos* ser. *Carphophorae*	The Andes and Mexico
119–131	**Plantago* sect. *Oliganthos* ser. *Microcalyx*	New Guinea, Australia, New Zealand
132–135	*Coronopus* sect. *Maritima*	Mediterranean
136–142	*Coronopus* sect. *Coronopus*	Mediterranean
143–145	*Littorella*	N. and S. America, Europe
146–161	*Psyllium*	Mediterranean, Macaronesia
162	*Bougueria*	Andes
163–168	*Albicans* sect. *Montana*	N. and S. Africa, Spain, C. Europe, W. Asia
169–174	*Albicans* sect. *Lanceifolia*	Mediterranean
175	*Albicans* sect. *Bauphula*	N. Africa, W. Asia
176–178	*Albicans* sect. *Hymenopsyllium*	Mediterranean
179	*Albicans* sect. *Albicans* ser. *Ovatae*	Mediterranean, NW America
180–182	*Albicans* sect. *Albicans* ser. *Minutae*	C. and W. Asia
183–185	*Albicans* sect. *Albicans* ser. *Albicantes*	Mediterranean
186–190	*Albicans* sect. *Albicans* ser. *Ciliatae*	N. Africa, W. Asia
191–195	*Albicans* sect. *Gnaphaloides* ser. *Hispidulae*	Chile, Peru
196–203	*Albicans* sect. *Gnaphaloides* ser. *Sericeae*	Mexico, Guatemala, Andes, E. Argentina
204–206	*Albicans* sect. *Gnaphaloides* ser. *Brazilienses*	Chile, Argentina, Uruguay, S. Brazil
207–213	*Albicans* sect. *Gnaphaloides* ser. *Gnaphaloides*	N. America, Argentina

tionships, we employ both the nuclear ribosomal ITS region (Baldwin, 1992) and plastid *trnL* intron and *trnL-F* intergenic spacer (hereafter, the *trnL-F* region; Taberlet *et al*., 1991). Both regions were known to show suitable variation in the close relatives, *Veronica* (Albach & Chase, 2001; Albach, Martínez-Ortega & Chase, in press).

MATERIAL AND METHODS

Material

Most of the material was obtained as seeds from botanical gardens and propagated at the Copenhagen Botanical Garden. Some taxa were obtained as fresh material, most of which was silica-gel dried prior to DNA extraction. Some taxa were obtained as frozen material from stocks propagated for phytochemical investigations (Jensen *et al*., 1996; Rønsted *et al*., 2000). This material was freeze dried. Three taxa representing minor groups were only available as herbarium material. *Veronica* and *Aragoa* sequences were from other studies (Albach & Chase, 2001; Albach *et al*., in press; Bello *et al*., in press). Author names and details of material examined are presented in Table 2.

DNA extraction, amplification and sequencing

DNA extractions used 0.2–0.3 g dried or 1.0–1.5 g fresh leaves in the 2 × CTAB method of Doyle & Doyle (1987). Before precipitation, an aliquot was purified using QIAquick mini columns (Qiagen, Inc.) following the manufacturer's protocols for PCR products. The remainder of the DNA was purified on caesium chloride/ethidium bromide gradients (1.55 g mL^{-1} density) and dialysed before inclusion in the DNA Bank at the Royal Botanic Gardens, Kew, http://www.rbgkew.org.uk/data/dnaBank/homepage.html (aliquots are available upon request and payment of a small handling charge). Amplification of the ITS region was carried out using the 17SE and 26SE primers designed by Sun *et al*. (1994). DMSO (2%) was added to reduce secondary structure problems common to ITS (Winship, 1989; Baldwin *et al*., 1995; Chase *et al*. in press). The *trnL-F* region was amplified with the 'c' and 'f' primers of Taberlet *et al*. (1991). PCR mastermixes 1.5 and 2.5 mM MgCl (Invitrogen Advanced Biotechnologies, Ltd) were used for ITS and *trnL-F,* respectively. Amplified products were purified with QIAquick mini columns following the manufacturer's protocol. Old herbarium specimens often have highly degraded DNA, and nine such species were extracted. Only *P. stocksii, P. ciliata* and *P. nubicola* were successfully amplified, and none of them for both ITS and *trnL-F*.

Amplification of the ITS region consisted of 2 min at 94°C followed by 28 cycles of: 1 min denaturation (94°C), 1 min annealing (50°C) and 3 min extension (72°C). After the last cycle, the temperature was kept at 72°C for a final 7 min extension. Amplification of the *trnL-F* region consisted of 2 min at 94°C followed by 28 cycles of: 1 min denaturation (94°C), 0.5 min annealing (50°C) and 1 min extension (72°C). After the last cycle, the temperature was kept at 72°C for a 7-min extension. Cycle sequencing reactions were carried out using BigDye Terminator Mix (Applied Biosystems, Inc.). This protocol consisted of 26 cycles of: 10 s denaturation (96°C), 5 s annealing (50°C) and 4 min elongation (60°C). All PCR and sequencing reactions were run using Perkin-Elmer GenAMP. model 9600 or 9700 PCR system, and sequencing products were run on an ABI 377 automated sequencer according to the manufacturer's protocols (Applied Biosystems, Inc.). Each region was sequenced for both strands for all but four taxa.

Alignment and phylogenetic analyses

Approximately 690 base pairs (bp) of the ITS region (ITS1 ranged from 212 to 230 bp, 5.8S from 161 to 163 and ITS2 from 191 to 202 bp) and about 840 bp of the *trnL-F* region (475–530 bp for the *trnL* intron, 52–53 bp for the *trnL* 3′ exon, and 319–369 bp for the *trnL-F* intergenic spacer, the species in section *Gnaphaloides* only having 164 bp after the exon) were used in the analysis for each taxon. Sequences were edited and assembled using Sequencher 3.1.1 software (Gene Codes Corp.). Each base position was checked for agreement of the two strands. ITS sequences were aligned easily by eye because of their conserved length (Baldwin *et al*., 1995). In the final matrix, 72 insertion/deletions (indels) were observed: 1 of 22 bp, 2 of 11 bp and the rest 1–4 bp. The *trnL-F* sequences could likewise be aligned by eye even though they had more and often longer indels. In the final matrix, 233 indels of 1–7 bp were observed, 6 of 12–16 bp and 1 of 22 bp. The seven species from *Plantago* section *Gnaphaloides* shared a large deletion of 221 bp in the *trnL-F* spacer. The matrices of ITS and *trnL-F* can be obtained as NEXUS files from the first and second authors (ninaroensted@hotmail.com, m.chase@rbgkew.org.uk). All sequences were submitted to GenBank (Table 2).

Cladistic analyses were undertaken using PAUP v. 4.0b5 for Macintosh (Swofford, 2001). Data were analysed as separate ITS and *trnL-F* matrices, and then because no bootstrap supported difference were present, as a combined data set. *Veronica chamaedrys* L., *V. fruticulosa* L., and *V. glandulosa* Hochst ex Benth. were collectively designated as the outgroup based on Albach & Chase (2001). All changes were

Table 2. Details of material included in the present study. [1]Voucher cannot be substantiated unambiguously because of lack of flowers or other crucial characters. [2]Obtained as total DNA from the DNA bank at (K). Herbaria and botanical gardens (origin of seeds): (B) = BGBM Berlin-Dahlem (BONN) = Bonn University (BPU) = Eötvös Loránd University, Budapest (C) = University of Copenhagen (COI) = University of Coimbra (DUSS) = Dusseldorf (GOET) = Göttingen University (HAL) = Martin-Luther-University, Halle (K) = Royal Botanic Gardens, Kew (PAL) = Palermo University (RSA) = Rancho Santa Ana Botanic Garden, Claremont (SAAR) = Saarlandes University, Saarbrücken (TELA) = Tel Aviv University (UDM) = Museo Friulano di Storia Naturale, Udine (WU) = University of Wienna. Coll. = Collected in, Cult. = Cultivated in, Acc. = Accession year

Rahn No.	Species	Distribution	Origin and type	Voucher	GenBank Acc. No ITS/*trnL-F*
155	*P. afra* L.	Mediterranean	Origin unknown, cult. (C)	*Rønsted 7* (C)	AY101892/AY101945
184	*P. albicans* L.	Mediterranean	Cult. (C), acc. 1955	*Rønsted 25* (C)	AY101905/AY101958
132	*P. alpina* L.	Europe	Origin unknown, cult. (C)	Jensen *et al*. 1996	AY101877/AY101932
175	*P. amplexicaulis* Cav.	Mediterranean	Origin unknown, cult. (C)	*Rahn 663* (C)	AY101900/AY101954
146	*P. arborescens* Poir.	Macronesia	Madeira, Pinacula, cult. (C), acc. 1968	Rønsted *et al*. 2000	AY101886/
154	*P.* arenaria Waldst. & Kit.	Mediterranean	Origin unknown, cult. (C)	*Rønsted 3* (C)	AY101891/AY101944
210	*P. aristata* Michx.	E USA	Cult. (C), acc. 1855	*Rahn 665* (C)	AY101911/AY101963
29	*P. asiatica* L.	S & E Asia	China, cult. (C)	*Rønsted 42* (C)	AY101862/AY101918
166	*P. atrata* Hoppe.	Europe, W Asia	Kaukasus, cult. (C), acc. 1989	*Rønsted 36* (C)	AY101895/AY101949
108	*P. australis* Lam.	America	Origin unknown, cult. (C)	Rønsted *et al*. 2000	AY101874/AY101929
178	*P. bellardii* All.	Mediterranean	Cult. (C), acc. 1912.	Rønsted *et al*. 2000	AY101902/AY101956
190	*P. ciliata* Desf.	Mediterranean	Pakistan, Herbarium material	*Rodin 5285* (K)	AY101906/
23	*P. cornuti* Gouan.	S Europe	No data available, cult. (UDM)	*Rønsted 31* (C)	AY101859/AY101915
140	*P. coronopus* L.	Mediter., Europe	Helnæs, Denmark, cult. (C)	*Rønsted 8* (C)	AY101882/AY101937
137	*P. crassifolia* Forssk.	Mediter., S Africa	Valencia. Cult. (C), acc. 1995	*Rønsted 17* (C)	AY101881/AY101936
176	*P. cretica* L.	E Mediterranean	Cyprus, Mimosa Beach, coll. 1999	*Albach 126* (WU)	AY101901/AY101955
60	*P. debilis* R.Br.	Australia, Tasmania	Origin unknown, cult. (C), acc. 1993	*Rønsted 45* (C)	AY101868/AY101922
207	*P. erecta* Morris.	W USA	California, Los Angeles (RSA)	*Boyd 8553* (RSA)	AY101909/AY101962
148	*P. famarae* Svent.	Lanzarote	No data available (DUSS)	*Chase 11183* (K)	AY101888/
212	*P. hookeriana* Fisch. & Mey.	S USA	Cult. (C), acc. 1959	*Rønsted 24* (C)	AY101913/AY101965
169	*P. lagopus* L.	Mediterranean	Origin unknown, cult. (C)	*Rønsted 6* (C)	AY101897/AY101951
170	*P. lanceolata* L.	Cosmopolite	Origin unknown, cult. (C)	*Rønsted 33* (C)	AY101898/AY101952
171	*P. leiopetela* Lowe.	Madeira	Madeira. Seeds from (COI)	*Rønsted 26* (C)	AY101899/AY101953
194	*P. lundborgii* Sparre.	San Ambrosio Isl.	San Ambrosio, cult. (C), acc. 1965	*Rønsted 52* (C)	AY101907/AY101959

141	*P. macrorhiza* Poir.	Meditteranean	No data available (PAL)	*Rønsted 20* (C)	AY101883/AY101938
26	*P. major* L.	Cosmopolite	Sachsen-Anhalt (HAL)	*Rønsted 41* (C)	AY101861/AY101917
135	*P. maritima* L.	Cosmopolite	Origin unknown, cult. (C)	*Rønsted 23* (C)	AY101879/AY101934
151	*P. mauritanica* Boiss. & Reut.	NW Africa	Origin unknown, cult. (BONN)	*Albach* 141(BONN)	AY101890/AY101943
40	*P. maxima* Jacq.	E Europe, C Asia	No data available, cult. (SAAR)	*Rønsted 28*[1] (C)	AY101864/
41	*P. media* L.	Europe, C Asia	Origin unknown, cult. (C)	*Rønsted 50* (C)	AY101865/AY101920
91	*P. myosuros* Lam.	S America	Origin unknown, cult. (C), acc. 1929	*Rønsted 47*[1] (C)	AY101873/AY101928
168	*P. nivalis* Boiss.	S Spain	Sierra Nevada. (TROM), coll. 1998	Rønsted *et al.* 2000	AY101896/AY101950
162	*P. nubicola* (Decne.) Rahn.	Andes	Peru, Andes, 4175 m. Herbarium material	*HHCH 5079* (C)	/AY101948
179	*P. ovata* Forssk.	Mediter., W USA	California, Riverside (RSA)	*Rønsted 40* (C)	AY101903/AY101957
24	*P. palmata* Hook.f.	Tropical Africa	Rwanda, Cyangu, cult. (C), acc. 1975	*Rønsted 9* (C)	AY101860/AY101916
211	*P. patagonica* Jacq.	W USA, Argentina	Origin unknown, cult. (C)	Rønsted *et al.* 2000	AY101912/AY101964
68	*P. raoulii* Decne.	N Zealand	Origin unknown, cult. (C)	Rønsted *et al.* 2000	AY101867/AY101923
22	*P. reniformis* Beck.	SE Europe	No data available (GOET)	*Rønsted 32* (C)	AY101858/AY101914
118	*P. rigida* Kunth.	Andes	Cult. (K), 1984–3185	*Chase 2767* (K)	AY101876/AY101931
35	*P. rugelii* Decne.	E & N America	Ontario, Halton county, cult. (C), acc. 1987	*Rønsted 37* (C)	AY101863/AY101919
160	*P. sarcophylla* Zohary.	E Mediterranean	Israel, Sharon (TELA)	*Rønsted 39* (C)	AY101893/AY101946
149	*P. sempervirens* Crantz.	SW Europe	France, Haute Savoi, cult. (C)	*Rønsted 27* (C)	AY101889/AY101942
200	*P. sericea* Ruiz. & Pav.	Andes	Venezuela, Merida, páramo	*Chase 2768* (K)	AY101910/AY101960
136	*P. serraria* L.	Mediterranean	No data available (PAL)	*Chase 9613* (K)	AY101880/AY101935
76	*P. spathulata* Hook.f.	N Zealand	France, Reamur, cult. (C), acc. 1993	*Rønsted 43* (C)	AY101869/AY101924
161	*P. squarrosa* Murray.	E Mediterranean	Israel, Jerusalem, cult. (C), acc. 1992	Voucher lacking	AY101894/AY101947
78	*P. stauntonii* Reichardt.	Amst. & St. Paul I.	Amsterd. & St. Paul Isl., cult. (C)	Rønsted *et al.*2000	AY101870/AY101925
182	*P. stocksii* Boiss.	W Asia	Baluchistan, Herbarium material	*Lamond 523* (C)	AY101904/
142	*P. subspathulata* Pilg.	Madeira	Madeira, Porto Moniz, cult. (C)	*Rahn 714* (C)	AY101884/AY101939
133	*P. subulata* L.	Mediterranean	Origin unknown, cult. (C)	Rønsted *et al.* 2000	AY101878/AY101933
205	*P. tandilensis* Pilg.	S America	Argentina, Prov. Buenos Aires (B)	*Rønsted 51* (C)	AY101908/AY101961
43	*P. tenuiflora* Waldst. & Kit.	E Europe, C Asia	Hortobágy, Hungary (BPU)	*Rønsted 30* (C)	AY101866/AY101921
84	*P. tomentosa* Lam.	S America	Argentina: Prov. Buenos Aires (B)	*Rønsted 29* (C)	AY101872/AY101927
81	*P. trinitatis* Rahn.	Ilha Trinidade	Ilha Trinidade, cult. (C)	Living plant (C)	AY101871/AY101926
143	*P. uniflora* L.	Europe	England, Cumberland, Lake District	*Chase 2798* (K)	AY101885/AY101940
114	*P. uniglumis* Walp.	S America	Argentina, Pr. Sta., cult. (C), acc. 1989	*Rahn 4393* (C)	AY101875/AY101930
147	*P. webbii* Barnéoud.	Macronesia	Origin unknown, cult. (C)	Rønsted *et al.* 2000	AY101887/AY101941

assessed as unordered and equally weighed (Fitch parsimony; Fitch, 1971). Indels were coded as missing and not included in the analyses because in all cases they coincided with groups that were well supported on the basis of the sequence analysis alone.

Heuristic searches of 1000 replicates of random taxon addition with tree bisection-reconnection swapping (TBR) and no tree limit (saving all shortest trees) were conducted. The analysis of the *trnL-F* matrix had to be limited due to the number of trees produced. Initially, 1000 replicates of random taxon addition with TBR and a limit of 10 trees per replicate were run. The trees from this analysis were then used as starting trees in a second heuristic search with no tree limit. All trees collected were then swapped to completion. The combined matrix was analysed in two phases. The first search included taxa with either ITS or *trnL-F* missing (Table 2), whereas a second search excluded all taxa with one or the other region missing. Missing data can lead to spurious cladograms and reduce resolution (Cameron *et al*., 2001; Richardson *et al*., 2001a) so we felt it was important to investigate the effects of missing data on our results. In this study, excluding the taxa with either region missing reduced the number of most parsimonious trees from 72 to four but did not influence the bootstrap percentages or topology. Thus, only the results of the full matrix (i.e. including the taxa with missing data) are shown.

Robustness was assessed by the bootstrap (Felsenstein, 1985) with 500 replicates of simple addition, TBR swapping, equal weights and a limit of 10 trees for each replicate. We report only scores of greater than 50% for clades present in the strict consensus tree. For the separate analyses, we show only the bootstrap consensus tree to establish that there were no cases of incongruent groups present in the separate analyses so that it was appropriate to directly combine all data. We did not perform tests for data incongruence because these methods have been shown to be unreliable for establishing data combinability (Reeves *et al*., 2001; Yoder, Irwin & Payseur, 2001).

Molecular clock

If it can be safely assumed that timing of dispersal to a datable oceanic island occurred soon after emergence of an island from the ocean, then the number of nucleotide substitutions per million years can be estimated. We have thus used an approach here similar to that of Richardson *et al*. (2001a,b). In our case, we have used the endemicity of *P. stauntoni* on the southern oceanic island of New Amsterdam, which is known to be approximately 0.5–0.7 Myr old. *Plantago stauntoni* has two ITS autapomorphies, which then leaves us with a rate for ITS of 4.27 ($\pm$0.6) $\times$ 10^{-9} per site per year. This is similar although somewhat faster than previously calculated rates of substitution, such as 2.44 $\times$ 10^{-9}–3.94 ($\pm$1.0) $\times$ 10^{-9} per site per year calculated for *Phylica* and *Dendroseris* (Rhamnaceae, Richardson *et al*., 2001a; Asteraceae, Sang *et al*., 1995), respectively. To apply this rate, it is first necessary to determine if rate heterogeneity is present by using a maximum likelihood (ML) relative rate test. To do this, ITS branch lengths were mapped onto one of the four most parsimonious trees from combined analysis using a three-parameter ML model of transition-transversion ratio and gamma distribution of rate variation amongst sites, which was then examined using a likelihood ratio test (Felsenstein, 1981). This method involves a comparison of twice the difference in log likelihood of branch lengths between a rate-constrained tree (enforcing a molecular clock in PAUP*) and a result in which there are no such constraints. If log likelihoods are significantly different, then rate constancy is rejected, which was the case here. We therefore produced an ultrametric tree from the unconstrained ML branch lengths employing the NPRS method of Sanderson (1997) in TreeEdit (v. 1.0 alpha 4–61; Rambaut & Charleston, 2000). We used this tree to calibrate the timing of other nodes in the *Plantago* tree based on fixing the rates with the timing of dispersal of *P. stauntoni* to New Amsterdam. We assessed dates only for nodes that received high Bootstrap percentages, thus avoiding difficulties associated with nodes that were either not consistent in all shortest trees or not well supported.

RESULTS

Analysis of ITS

The ITS matrix included 56 taxa of *Plantago*, two of *Aragoa* and three of *Veronica*. The aligned matrix included 761 positions, of which 333 (44%) were variable and 262 (34%) were potentially parsimony informative. Analysis produced 10 most parsimonious trees that were 919 steps long with a consistency index (CI) = 0.55 and a retention index (RI) = 0.81. The 50% bootstrap tree is shown in Figure 1. The ingroup is monophyletic (100 bootstrap percentage, BP), and *Aragoa* is sister to *Plantago*, which is supported by 96 BP. *Plantago* is divided into four clades that correspond to subgenera: *Plantago* (99 BP), *Coronopus* (100 BP), *Psyllium* s.l. (including *Plantago* subgenus *Albicans;* 74 BP) and *Littorella* (*Bougueria* was not sequenced for ITS). *Plantago* subgenus *Albicans* is paraphyletic to subgenus *Psyllium*; this clade (74 BP) contains six subclades that correspond to the six component sections of these subgenera, most of them with high bootstrap support: *Plantago* sects. *Psyllium* (94 BP), *Albicans* (75 BP), *Lanceifolia* (100 BP),

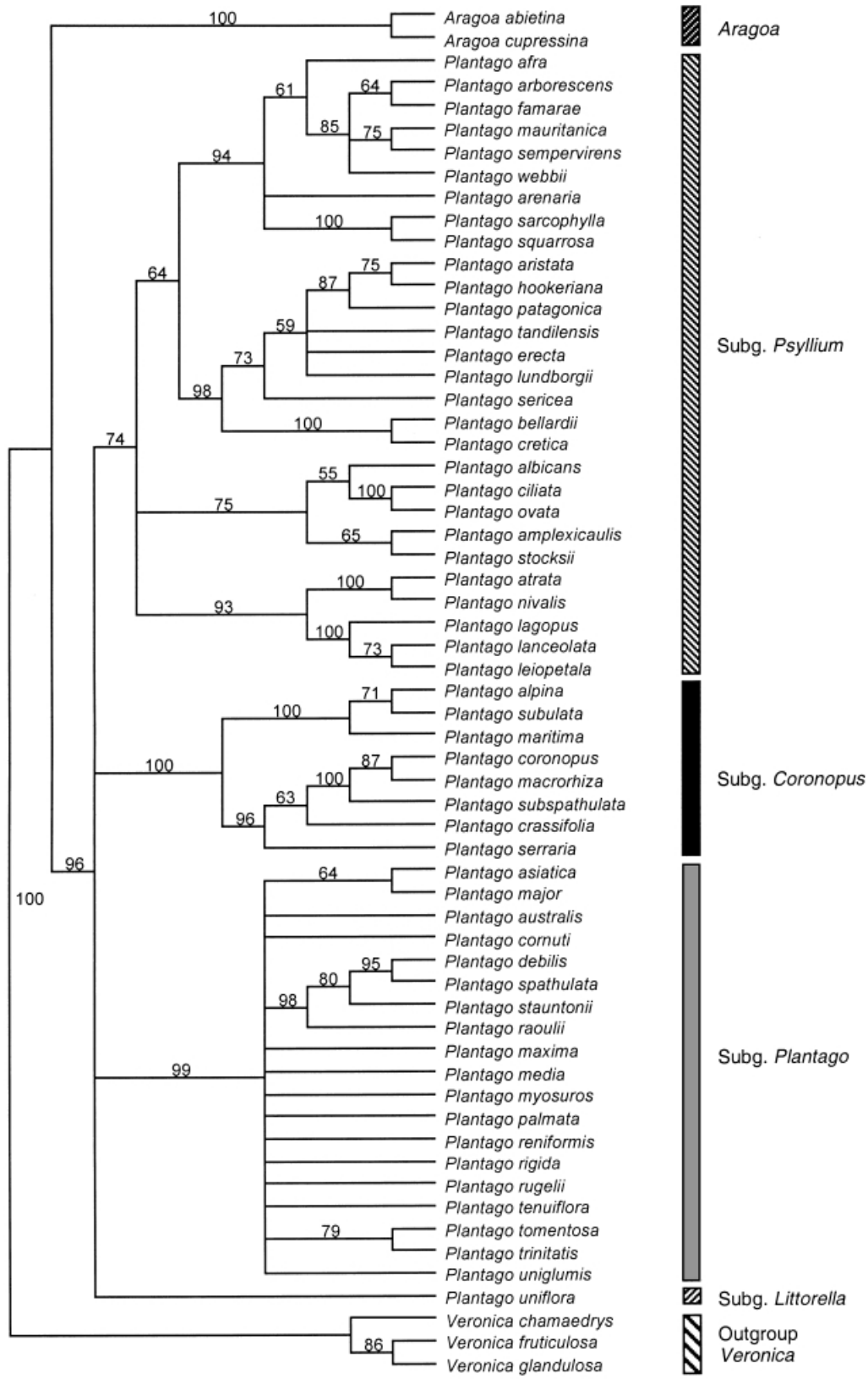

Figure 1. Bootstrap tree (50% consensus) from analysis of ITS nrDNA sequences. The three *Veronica* taxa were designated as the outgroup. The subgenera supported by the ITS nrDNA sequence data are indicated.

Montana (100 BP), *Hymenopsyllium* (100 BP) and *Gnaphaloides* (73 BP). *Plantago s*ubgenus *Coronopus* is subdivided into sects. *Maritima (*100 BP) and *Coronopus* (96 BP). *Plantago s*ubgenus *Plantago (*99 BP) is monophyletic but not well resolved internally; *Plantago* sect. *Mesembrynia* (*P. debilis, P. spathulata, P. stauntoni* and *P. raoulii;* 98 BP) constitutes a monophyletic subclade.

Analysis of *trnL-F*

The *trnL-F* matrix included 52 species of *Plantago* and three of *Veronica*. The aligned *trnL-F* region included 1069 positions, of which 281 were variable (27%) and 199 (19%) were potentially parsimony informative. Analysis produced 1368 most parsimonious trees that were 426 steps long with CI = 0.79 and RI = 0.91 (Fig. 2). The ingroup is monophyletic (100 BP), and the tree generally has the same topology as the tree obtained from analysis of the ITS matrix (Fig. 1). The same four subgenera of *Plantago* are present along with a fifth, subgenus *Bougueria*, which is sister to subgenus *Psyllium s.l.* (92 BP), the latter supported by 70 BP. *Plantago* sect. *Coronopus* (98 BP) is subdivided into two series, one containing *P. crassifolia* and *P. serraria* (98 BP) and the other containing the three remaining sampled species (99 BP). *Plantago* subgenus *Plantago* is not well resolved in this analysis either, and *Plantago* sect. *Mesembrynia* does not constitute a separate clade; another subclade (74 BP) is formed by species belonging to *Plantago* sect. *Virginica* (*P. australis, P. trinitatis, P. myosuros* and *P. tomentosa*). *Plantago uniglumis* (*Plantago* sect. *Oliganthos*) is associated with this subclade, but this is not well supported (59 BP). *Plantago tenuiflora* from *Plantago* sect. *Micropsyllium* is sister to the other species of *Plantago* subgenus *Plantago (*96 BP), the latter supported by 86 BP.

Combined matrix

Results from the two separate matrices produced highly similar patterns; in no case did differing results obtain high bootstrap support (BP greater than 80%), so we directly combined the two matrices in one analysis. The combined matrix included all taxa with at least ITS or *trnL-F* and thus contained 57 species from *Plantago*, two from *Aragoa* and three from *Veronica*. The matrix consisted of 1830 positions, of which 614 were variable (34%) and 461 (25%) were potentially parsimony informative. Analysis produced 72 most parsimonious trees that were 1365 steps long with CI = 0.61 and RI = 0.84. One of the most parsimonious trees is shown with bootstrap percentages (>50%) below the branches and branch lengths above (ACCTRAN optimization; Fig. 3). Nodes not present in the strict consensus of all 72 shortest trees are marked with an arrowhead.

Analysis of the combined matrix excluding the taxa with either ITS (*P. nubicola*) or *trnL-F* (*P. arborescens, P. ciliata, P. famarae, P. maxima, P. stocksii*) missing produced four most parsimonious trees that were 1332 steps long with a CI = 0.63 and RI = 0.84 (results not shown). In general, there are no contradictions between the topology of the tree obtained from the combined analysis (Fig. 3) and those from analyses of each of the separate matrices (Figs 1, 2).

As in the separate analyses, the ingroup is monophyletic (100 BP), and *Aragoa* is sister to *Plantago* (100 BP). The clade including subgenera *Psyllium s.s* and *Albicans* (subgenus *Psyllium s.l*) is now supported by 83 BP instead of 70–74 BP in the separate analysis. Support of the other four clades, corresponding to *Plantago* subgenera *Bougueria, Coronopus*, *Plantago* and *Littorella* is again close to 100 BP. The same six subclades are found in *Plantago* subgenus *Psyllium s.l.*, and *Plantago* sect. *Montana* is sister to sect. *Lanceifolia* as in the ITS analysis with even stronger support (99 BP instead of 75 BP). In the *trnL-F* analysis, *P. arenaria* is sister to *P. afra* with 74 BP, but there is no support in the combined analysis for this relationship. The support of *P. aristata* and *P. hookeriana* being sisters dropped from 75 BP in the ITS analysis down to 68 BP in the combined analysis, whereas the support of addition of *P. patagonica* to this clade, went up from 87 to 93 BP, addition of *P. lundborgii, P. erecta* and *P. tandilensis* from 59 BP to 72 BP and addition of *P. sericea* went up from 73 BP to 99 BP. A clade with *P. ciliata, P. ovata* and *P. albicans* also got stronger support in the combined analysis than in the ITS analysis (73 BP instead of 55 BP) as well as the addition of *P. amplexicaulis* and *P. stocksii* to this clade (98 BP instead of 75 BP). The sister relationship of *P. lanceolata* and *P. leiopetala* found in both of the separate analysis is now well supported (90 BP instead of 64–73 BP). The subdivision of *Plantago* sect. *Coronopus* in two clades in the *trnL-F* analysis is also found in the combined analysis, but the support of the one clade with *P. serraria* and *P. crassifolia* has dropped from 98 BP to 88 BP.

In *Plantago* subgenus *Plantago* (100 BP), the clades with sects. *Mesembrynia* and *Virginica* found in the ITS and the *trnL-F* analysis, respectively, are both present and sect. *Virginica* has stronger support (90 BP excluding *P. uniglumis* and 71 BP including it as opposed to 74 and 59 BP, respectively, in the *trnL-F* analysis). *Plantago tenuiflora* from sect. *Micropsyllium* is still sister to the other species of subgenus *Plantago*, but the support of the latter has dropped from 86 BP to 69 BP. In the ITS analysis *P. cornuti* was sister to *P. major* (88 BP), whereas *P. asiatica* was the sister to *P. major* in the *trnL-F* analysis (64 BP). In the

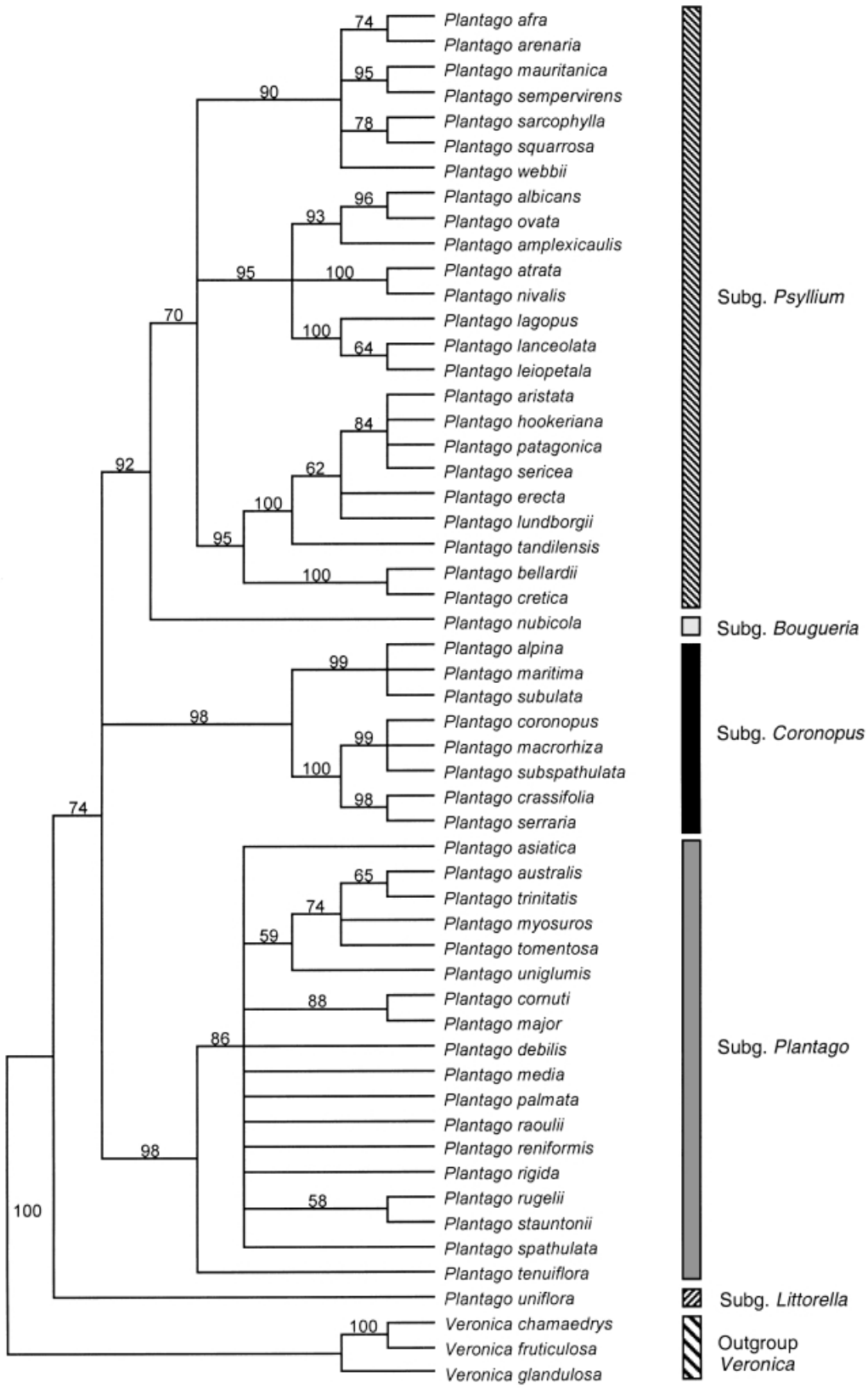

Figure 2. Bootstrap tree (50% consensus) from analysis of *trnL-F* plastid DNA sequences. The three *Veronica* taxa were designated as the outgroup. The subgenera supported by the *trnL-F* plastid DNA sequence data are indicated.

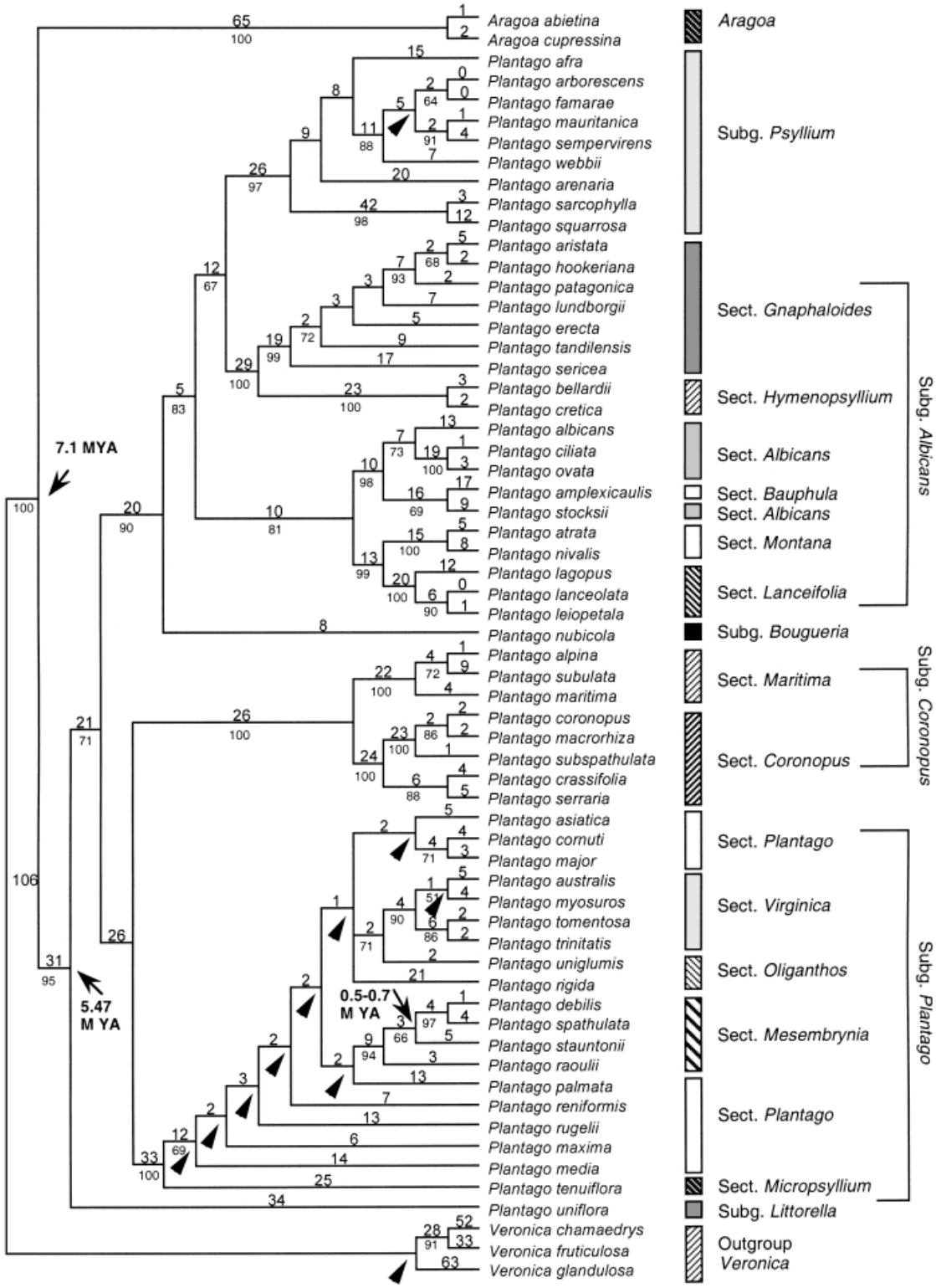

Figure 3. One of the 72 most parsimonious trees obtained from the combined analysis of ITS nrDNA and *trnL-F* plastid DNA sequences. Tree length 1365, CI = 0.61, RI = 0.84. Branch length and bootstrap percentages (50% consensus) are shown above and below the branches, respectively. Branches not in the strict consensus tree are marked with an arrowhead. Dates of nodes mentioned in the text are marked with an arrow and the age indicated. The three *Veronica* taxa was designated as the outgroup. The subgenera and sections recognized by Rahn (1996) are indicated.

combined analysis, only the former relationship is retained (71 BP).

Molecular clock and timing of the *Plantago* radiation

We produced an ultrametric tree with the NPRS method with the corrected estimates for ITS branch lengths (we used one of the four trees found in the combined analysis, which is more reliable than either of the separate analyses, but we have used only the ITS data; we could not use the combined data because this would involve too much complexity due to different parameters having to be used for each of the two regions involved, one nuclear and the other plastid). This gives a date for the divergence of *Aragoa* and *Plantago* of 7.1 Myr ago (Mya). The radiation of *Plantago* dates to 5.47 Mya, and most of the subgenera of *Plantago* were in existence 2–3.5 Mya. The ultrametric tree is not shown, but divergence times are indicated in Figure 3.

DISCUSSION

Molecular evolution

Palmer *et al*. (2000) found highly accelerated substitution rates of *Plantago*, 50–100 times higher than typical, in portions of several protein-coding and rRNA genes in the mitochondrial genome but not for plastid or nuclear sequences. As Palmer *et al*. (2000) mentioned, accelerated rates are known from other groups, such as the grasses (Eyre-Walker & Gaut, 1997), in which all three genomes show higher substitution rates, but only several-fold in relation to palms. The nuclear (ITS) and plastid (*trnL-F*) regions we sequenced exhibited relatively high DNA sequence variation, but not at levels comparable to the sequence divergence that Palmer *et al*. (2000) reported for the mitochondrial regions. The high levels of DNA variation in *Plantago* are not paralleled in *Aragoa*, which showed no sequence variation among the eight species sequenced for ITS in the study of Bello *et al*. (in press). Rate heterogeneity similar to that shown by *Plantago* and *Aragoa* was also reported among sister genera in subtribe Oncidiinae of Orchidaceae (Williams, Chase & Whitten, 2001), so it is not an uncommon phenomenon.

Taxonomic implications

Plantago subgenus *Plantago*

Plantago subgenus *Plantago*, most members of which are perennial, is characterized mainly by plesiomorphic characters. The primary root is usually a more or less fleshy taproot, and an elongated stem is usually absent. Rahn (1996) included 131 species here, which are distributed on all continents and found on many oceanic islands, often in mesic or moist habitats. Morphological variation among species in subgenus *Plantago* is low, and how the species are grouped is unclear (Rahn, 1996). No chemical markers for the subgenus have been found so far, apart from lack of certain iridoid glucosides characteristic of other subgenera (Rønsted *et al*., 2000). Rahn (1996) recognized five sections: *Plantago*, *Mesembrynia, Micropsyllium, Virginica* and *Oliganthos*. In his analysis, sect. *Plantago* was paraphyletic to the other four sections, and sect. *Mesembrynia* was paraphyletic to section *Virginica*. *Plantago* sect. *Plantago* is absent from New Guinea, Australia, New Zealand and South America, where it is replaced by three others: sects. *Oliganthos*, *Mesembrynia* and *Virginica*. This pattern is probably due to long distance dispersal followed by local speciation, a conclusion that is supported by their derivative position in the phylogenetic tree and low levels of sequence divergence. A tectonic (vicariance) explanation is clearly not viable. In our combined analysis (Fig. 3), this subgenus is monophyletic but not well resolved due to low levels of sequence divergence. The combined data indicate at least three of the sections are monophyletic; more of them may turn out to be so when sufficient data to resolve relationships are produced. Our results do not strongly refute their monophyly.

Plantago sect. *Micropsyllium,* represented by *P. tenuiflora,* is sister to the other species of subgenus *Plantago* (Fig. 3). The six species in this section grow in temperate zones in North and South America, Europe and Asia. They are small annuals with minute floral parts, narrow leaves and antrorse non-glandular hairs with a spur-like elongation (Rahn, 1996).

Plantago sect. *Mesembrynia* is found in Australia, New Zealand and New Guinea, with a few outliers, such as *P. stauntoni* from New Amsterdam and St. Paul Islands, part of the French Southern and Antarctic Territory in the southern Indian Ocean. Rahn (1996) also included six species from Asia and eastern Europe in this section, but this may be incorrect (Rahn, pers. comm.). In his analysis, *Plantago* sect. *Mesembrynia* included sect. *Virginica*, which is not supported by our data. Both sections have an ovary with a third compartment at the top on the adaxial side of the placenta.

The clade of *Plantago* section *Virginica* in our study included the Patagonean species *P. uniglumis* (but only with 71 BP) from Rahn, 1996) sect. *Oliganthos*, which is ecologically and morphologically distinct from the American section *Virginica*. The species in our sect. *Virginica* clade are from South America, except the peculiar endemic *P. trinitatis,* found on Ilha

Trinidade, 1500 km east of Rio de Janeiro in the Atlantic Ocean (Rahn, 1996). The other species sampled from *Plantago* sect. *Oliganthos*, *P. rigida,* is not unambiguously placed but might also belong to this clade.

The remaining species of *Plantago* subgenus *Plantago* included in this study, *P. palmata*, *P. reniformis*, *P. cornuti*, *P. major*, *P. asiatica*, *P. maxima*, *P. rugelii* and *P. media,* all belong to Rahn's (1996) paraphyletic section *Plantago*, which is also paraphyletic to sects. *Oliganthos, Virginica* and *Mesembrynia* in our analyses (Fig. 3). As noted above, BP for this result is low, so we consider the question of the monophyly of this section to require further study. *Plantago major* and *P. cornuti* also form a separate clade, probably including *P. asiatica*, but there is no BP-supported resolution sufficient to show how the remaining species in sect. *Plantago* relate to this clade.

Plantago subgenus *Coronopus* (Lam. & DC.) Rahn

Rahn (1996) included 11 species, distributed around the Mediterranean, in his *Plantago* subgenus *Coronopus*. *Plantago maritima* is also found in other parts of Europe, central Asia, North America and southern South America (Moore, Williams & Yates, 1972). The members of this subgenus always have short hairs covering the corolla tube, whereas all other species of the genus have a glabrous tube, except *P. lachnantha* Bunge, which has a lanate tube (Rahn, 1996). Our results (Fig. 3) confirm the monophyly of subgenus *Coronopus* and its division into two sections as in Rahn's results (1996): *Plantago* sect. *Coronopus* Lam. & DC and sect. *Maritima* H. Dietr. with seven species and four species, respectively. Our analysis indicates that these sections are sister groups, whereas sect. *Maritima* was paraphyletic to sect. *Coronopus* in Rahn's 1996 study. *Plantago* sect. *Coronopus* is subdivided into two well-supported groups or series, which Rahn (1996) described but did not recognize as series. The first series has 1–4 ovules and a glabrous ovary (a reversal), as is also found in *P. serraria* and *P. crassifolia*. The other series has an upper, abaxial compartment in the ovary and lacks the carbohydrate ribose in the seeds, as in *P. coronopus*, *P. macrorhiza* and *P. subspathulata*. None of the species of the first series have been investigated for iridoid glucosides, but in the latter there seems to be a characteristic lack of iridoid glucosides. *Plantago* sect. *Maritima* contains 5-substituted iridoids such as monomellitoside (Rønsted *et al*., 2000).

Plantago subgenus *Psyllium* (Juss.) Harms & Reiche

Our results (Fig. 3) indicate that *Plantago* subgenus *Albicans* is paraphyletic to subgenus *Psyllium sensu stricto* and should be included in a broader subgenus *Psyllium* (Juss.) Harms & Reiche. Rahn's (1996) monophyletic subgenus *Albicans* contains 51 species adapted to dry habitats in Asia, Europe, Africa, North and South America (they are absent from New Guinea, Australia and New Zealand). The group has hairs with highly refracting walls, making the narrow lumen invisible, and swollen joints (Rahn, 1996). The iridoid glucosides catalpol and asperuloside are characteristic for this subgenus, but catalpol was also found in subgenus *Littorella* (Andrzejewska-Golec, 1997, 1999; Rønsted *et al*., 2000). Rahn (1996) recognized six sections and a number of series: sects. *Montana, Lanceifolia*, *Bauphula*, *Hymenopsyllium*, *Albicans*, with series *Ovatae, Minutae, Albicans* and *Ciliatae* and sect. *Gnaphaloides*, with series *Hispidulae, Sericeae, Braziliense* and *Gnaphaloides*; all of these were monophyletic except series *Sericeae,* which was paraphyletic to series *Gnaphaloides.*

In our analysis, sect. *Hymenopsyllium* Pilg., is sister to sect. *Gnaphaloides* Barnéoud. It was expected that sect. *Hymenopsyllium* would be closer to sect. *Albicans* Barnéoud, which is also Mediterranean. The former group also has distinctive chemistry; both investigated species lack the iridoid glucoside, catalpol, which is otherwise characteristic for subgenus *Albicans,* and they both contain chlorogenic acid, a caffeic acid derivative not found in other species of *Plantago* (Rønsted *et al*., 2000). *Plantago* sect. *Gnaphaloides* is not well resolved in our analysis. Only series *Gnaphaloides (*excluding *P. erecta*) constitutes a subclade. *Plantago erecta* was also placed in this series of annuals with a characteristic corolla by Rahn (1996), but this may be inappropriate. Rahn (1996) also recognized another distinct series of annuals, series *Hispidula* from the coast of Chile and Peru, which also have a characteristic corolla. The placement of *P. lundborgii* from this series is ambiguous here.

Rahn's (1996) monophyletic subgenus *Psyllium sensu stricto* contained only one section with 16 species indigenous to dry habitats in the Mediterranean area and Macaronesia. Easily recognized synapomorphies are a stem with elongated internodes and leaves either opposite or in whorls of three. This subgenus is also supported by the iridoid glucosides bartsioside and plantarenaloside (Andrzejewska-Golec *et al*., 1993; Rønsted *et al*., 2000). Rahn (1996) did not subdivide subgenus *Psyllium*.

The combined analysis (Fig. 3) shows a monophyletic clade with a well-supported subclade of perennials. Rahn (1996) placed the annual species into three unnamed groups. His group (1) is here represented by *P. arenaria* (2) by *P. afra* and (3) by *P. sarcophylla* and *P. squarrosa*. The two latter species form a separate group here. There is no bootstrap support (>50%) to indicate how *P. arenaria* and *P. afra* are related to the

other two, leaving their placements ambiguous. *Plantago arenaria* is distinctive in not having long glandular hairs as in the other annual members of the group. Andrzejewska-Golec (1992) divided sect. *Psyllium* into four series: (1) *P. squarrosa* alone in series *Squarrosae* (*P. sarcophylla* was not mentioned, but it should probably belong to this series as well); (2) *P. arenaria* and *P. afra* in series *Arenariae*; (3) *P. sempervirens* in its own series *Sempervirens,* and (4) the remaining species included in our study, all belonging to her series *Arborescens* (*P. mauritanica* was not mentioned either). As previously stated, our results (Fig. 3) indicate that subgenus *Albicans*, in the sense of Rahn (1996), is paraphyletic and should be included in subgenus *Psyllium* (Juss.) Harms & Reiche to obtain a monophyletic clade containing six sections, each with high BP: *Psyllium* (Juss.) Lam & DC*, Albicans* Barnéoud*, Lanceifolia* Barnéoud*, Montana* Barnéoud, *Hymenopsyllium* Pilg. and *Gnaphaloides* Barnéoud. *Plantago* subgenus *Psyllium sensu* Rahn (1996) thus becomes *Plantago* sect. *Psyllium* (Juss.) Lam & DC., as previously suggested by Rahn (1978). Such a broader concept of subgenus *Psyllium* has been repeatedly criticized by Andrzejewska-Golec and coworkers. Rahn's (1996) two subgenera *Psyllium* and *Albicans* do share a number of characters, such as an ovary with two (one) ovules and no rudiment of an upper compartment. The inner side of the seeds is deeply concave (Rahn, 1996), but Andrzejewska-Golec and coworkers have argued that the species from subgenus *Albicans* that they investigated have no hairs with multicellular stalks and unicellular heads as in the species of *Psyllium s.s.* (Andrzejewska-Golec & Swiatek, 1989a,b; Andrzejewska-Golec & Swietoslawski, 1993). The iridoid glucosides, plantarenaloside and bartsioside, that are characteristic for species of subgenus *Psyllium s.s.* are also not found in species of subgenus *Albicans* (Andrzejewska-Golec & Swiatek, 1984; Andrzejewska-Golec *et al*., 1993; Rønsted *et al*., 2000).

Plantago subgenus *Bougueria* (Decne) Rahn

Plantago nubicola was only successfully sequenced for *trnL-F*. In both the *trnL-F* tree (Fig. 2) and the combined analysis tree (Fig. 3), *P. nubicola* is sister to the clade of subgenus *Psyllium sensu lato*. *Plantago nubicola* grows at high altitudes in the Andes, and the only available material was a few tiny leaves of herbarium material collected in 1971 in Peru. The flowers are different from those of other *Plantago* species, and it is difficult to find synapomorphies uniting it with other subgenera. In Rahn's phylogenetic analysis (1996), *Bougueria* was sister to subgenus *Albicans,* a result not drastically different from what we obtained here.

Plantago subgenus *Littorella* (P.J. Bergius) Rahn

According to Rahn (1996), the three species in *Plantago* subgenus *Littorella* are united by several synapomorphies such as unisexual, monoecious flowers, an ovary with only one fertile compartment and one anatropous ovule attached to a basal placenta (Rahn, 1996). The species of *Littorella* are stoloniferous and their habitat is oligotrophic lakes, in which they are submerged to more or less inundated. Our analyses show *Littorella* as the sister group to all the other species of *Plantago* (Fig. 3). Pilger (1937) considered the species of *Littorella* to be a separate genus, whereas Rahn (1996) included *Littorella* as a subgenus of *Plantago*. Chemical investigations may be interpreted to support the inclusion of *Littorella* as a subgenus in *Plantago*. The one species investigated here, the Eurasian *P. uniflora,* contains the iridoid aucubin, which is characteristic for *Plantago* as well as catalpol, which is also present in *Plantago* subgenus *Albicans* (Andrzejewska-Golec, 1999; Rønsted *et al*., 2000). However, catalpol and aucubin are not restricted to *Plantago* and are widespread in Scrophulariaceae s.*l.* (Hegnauer, 1973; Andrzejewska-Golec, 1997). The iridoid pattern in the closely related genus, *Aragoa,* has not yet been studied for comparison. *Littorella* has few obvious differences from *Plantago*, apart from the stoloniferous aquatic habit and the monoecious flowers, but *Littorella* is a widely used name for a group of widespread species. Our results do not preclude keeping *Littorella* as a distinct genus, but we agree with Rahn's treatment of it as a member of *Plantago*.

Biogeography and timing of the *Plantago* radiation

Pollen referred to *Plantago* extends to the upper Miocene (5–11 Myr; Muller, 1981). In consulting the original references reviewed by Muller (1981) to obtain a more precise dating of the early *Plantago* pollen, we found that the reference to the work of Krutzsch (1966c in Muller, 1981) was incorrectly stated, and Gray (1964) only specified upper Miocene. However, Van Campo (1976) identified pollen of *Littorella* and *Plantago* from about 6 Myr old deposits of Venta del Moro (Valencia, Spain) and Naud & Suc (1975) reported on *Plantago* pollen from Mirabel (Ardèche, France) and dated the deposits there to 6.4 ± 0.2 Myr, which is concordant with the date we assigned to the genus using a molecular clock approach, 5.47 Myr.

We looked for more calibration points associated with other species of *Plantago* occurring on datable oceanic islands to confirm our rate estimate. In this case, there are several, but thus far we have been unable to find literature providing dates for species

such as *P. lundborgii* on San Ambrosio and *P. trinitatis* on Ilha Trinidade. Two species, *P. leiopetala* and *P. subspathulata*, occur on Madeira, for which a date is known, but both appear to have arrived at different times, the former relatively recently; it exhibits such low levels of divergence from its closest relatives that if we used it as a calibration point it would indicate that ITS was evolving far more slowly than any other published estimates.

It is our assumption that all of the autapomorphies found in *P. stauntoni* occurred after it dispersed to New Amsterdam. If some of this change took place before it reached New Amsterdam, the inferred rate would be too fast, but this hypothesis would assume that *P. stauntoni* subsequently became extinct in Australia and New Zealand, which is where its closest relatives now occur.

Our rate estimate is similar, although somewhat faster, than others hypothesized for ITS rDNA sequences (Sang *et al*., 1995; Richardson *et al*., 2001a). *Plantago* is well adapted to dispersal; there are few places in the World without at least one species, including small and remote oceanic islands such as New Amsterdam. We think it is therefore safe to conclude that none of the patterns of relationships indicate that plate tectonics has anything to do with where *Plantago* species occur today. Many of the clades recognized taxonomically by Rahn (1996) and the system proposed here, which is similar to that of Rahn, are focused on particular geographical areas, but many also contain at least one species that does not fit the general pattern. This could be explained by the fact that the testa of many *Plantago* seeds become sticky when wet, due to swelling of the polysaccharides present in the testa, which make the seeds easy to disperse even over long distances on the feet of birds (Pilger, 1937; Moore *et al*., 1972). The swelling property of some *Plantago* seeds make them useful as laxatives, and the testa of *P. ovata* are described under the name 'isaphagula husk' in the *European Pharmacopoeia* (European Pharmacopoeia Secretariat, 1999). If our molecular clock date is correct, then *Plantago* is a modern genus that has been as incredibly successful at dispersal to new land masses as it has been in colonizing open sites within the current distribution of its species. There are some narrow endemics in the genus, but we agree with the assessment of Carlquist (1970) in his paper on the woody insular species that these could not be considered palaeoendemics.

With such a complicated distribution driven by long-distance dispersal, it is difficult to develop a strong hypothesis about where *Plantago* originated. Adding the páramo endemic *Aragoa* to the picture appears to clarify little. *Plantago* subgenus *Littorella* is mostly European, and the next major split is among groups that are collectively cosmopolitan and without any clear geographical patterns that would shed any light on origins. We therefore conclude that the question of the origin of *Plantago* is at present highly speculative and unlikely to be robustly addressed in the context of these phylogenetic patterns. Perhaps with a great deal more sampling at the species level, a more consistent pattern will emerge. *Plantago* and related genera such as *Veronica* should be the focus of future broadly based phylogenetic studies because they represent excellent examples of life history strategy adaptability.

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APPENDIX

In summary, this study supports the following taxonomic conclusions:

Plantaginaceae Juss**.**, Gen. Pl. 89 (1789). Nom. cons ("Plantagines"). Type: *Plantago* L.

Plantago L., Sp. Plant. 112 (1753). Lectotype (Britton & Brown, 1913): *Plantago major* L.

Plantago subgen. *Littorella* (P.J. Bergius) Rahn, Bot. J. Linn. Soc. 120: 197 (1996). Basionym: *Littorella* P. J. Bergius, Vet. Acad. Handl. 29: 341 (1768). Type: *Plantago uniflora* L. Sp. Plant. 115 (1753).

Plantago subgen. *Plantago*

Plantago subgen. *Coronopus* (Lam. & DC.) Rahn, Bot. Tidsskr. 73: 107 (1978).

Basionym: *Plantago* sect. *Coronopus* Lam. & DC., Fl. Fr. éd. 3: 417 (1805).

Plantago sect. *Coronopus* Lam. & DC., Fl. Fr. éd. 3: 417 (1805). Type: *Plantago coronopus* L.

Plantago sect. *Maritima* H. Dietr., Wiss. Z. Friedrich-Schiller-University Jena, Math.-Naturwiss. Reihe 24, 4: 455 (1975). Type: *Plantago maritima* L.

Plantago subgen. *Bougueria* (Decne) Rahn, Bot. J. Linn. Soc. 120: 197 (1996).

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Plantago subgen. *Psyllium* (Juss.) Harms & Reiche in Engler, Die natürlichen Pflanzenfam. IV 3b: 373 (1895). Basionym: *Psyllium* Juss., General Pl. 89 (1789). Lectotype: *Plantago sempervirens* Crantz.

*Plantago*ect. *Psyllium* (Juss.) Lam & DC., Fl. Fr. éd. 3,3 (1805).

Plantago sect. *Gnaphaloides* Barnéoud, Mem. Bot. Acad. Paris, Fac. Sc. 19 (1844) (Thesis), and Monogr. Plantag.: 42 (1845). Type: *Plantago gnaphaloides* Nutt. (= *P. patagonica* Jacq.).

Plantago sect. *Hymenopsyllium* Pilg., Bot Jb. 57 : 320 (1921). Lectotype (Rahn, 1978): *Plantago cretica* L**.**

Plantago sect. *Albicans* Barnéoud**,** Mem. Bot. Acad. Paris, Fac. Sc. 18 (1844) (Thesis), and Monogr. Plantag. 36 (1845). Type: *Plantago albicans* L.

Plantago sect. *Montana* Barnéoud, loc. cit. (1844) (Thesis), and loc. cit. (1845). Type: *Plantago montana* Lam. (= *P. atrata* Hoppe).

Plantago sect. *Lanceifolia* Barnéoud, loc. cit. (1844) (Thesis), and loc. cit. (1845). Type (Rahn, 1978): *Plantago lanceolata* L.