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PHYLOGENETIC RELATIONSHIPS WITHIN *KORTHALSELLA* (VISCACEAE) BASED ON NUCLEAR ITS AND PLASTID *TRNL-F* SEQUENCE DATA[1]

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The nuclear encoded internal transcribed spacer (ITS) region and the plastid encoded *trnL-F* region were sequenced for 25 populations of *Korthalsella*, a genus of reduced, monoecious, Old World misletoes. The molecular study confirms the hypothesis that branch shape and cladotaxy (the arrangement of branches with respect to their parent axis) are unreliable indicators of relationship in the genus and demonstrates that many of the taxa previously recognized are not monophyletic. Both gene regions identify three major subgroups within the genus and find lower level relationships within these subgroups highly correlated with geographic distance. An analysis based upon 18S and *rbcL* sequences identifies *Ginalloa* as the sister group to *Korthalsella*, which together with the branching order within the genus, indicates that *Korthalsella* originated in Papuasia and aids in elucidating evolution of the peculiar inflorescence structure. There are problems associated with species delimitation when evolutionary units are more restricted than morphological lineages, and justification is offered for recognizing only morphologically diagnosable monophyletic lineages as species. Varying substitution rates and differing modes of inheritance in ITS and *trnL-F* result in complementary utility of the two regions for elucidating infrageneric relationships in *Korthalsella*.

Key words: character evolution; internal transcribed spacer (ITS); *Korthalsella*; molecular phylogeny; species delimitation; *trnL-F*; Viscaceae.

Korthalsella Van Tieghem (Viscaceae) is a small genus of squamate, hemiparasitic mistletoes found on a wide range of hosts but favoring Myrtaceae. Other families parasitized include Apocynaceae, Araliaceae, Campanulaceae, Ebenaceae, Ericaceae, Fabaceae, Myrsinaceae, Oleaceae, Podocarpaceae, Rubiaceae, Rutaceae, and Sapotaceae. *Korthalsella* has an unusual and patchy distribution (Fig. 1) within the area bounded by Hawaii and the Marquesas in the east, Japan in the north, Australia in the south, and Ethiopia and Madagascar to the west.

Current estimates of the size of the genus vary depending upon the monographer and the choice of characters used to delimit taxa. Danser (1940), for instance, recognized 23 species and Barlow (1983) estimated 30, whereas Molvray (1997) recognized eight. The disagreement between treatments is due to an absence of taxonomically informative characters resulting from the reduction of many organ systems, thus focusing attention on relatively few features, most of which are of dubious taxonomic utility.

Reduction is most extreme within the vegetative portion of the plant. The vestigial leaves are represented by minute crests of tissue at the nodes (Fig. 2A, "br") and display no taxonomically significant variation among species. *Korthalsella* lacks roots, and its haustorium has not been examined for phylogenetically informative characters. Morphological variation is found in color and size of plants, cladotaxy, and shape of the branches and their internodes.

The inflorescences are axillary and either spike-like (with short internodes between successive whorls of flowers) or abbreviated, cushion-like masses of flowers (without discernible internodes). In some taxa inflorescences are borne only on specific branches, usually of different shape (referred to here as differentiated inflorescence branches), whereas in others inflorescences may develop on any of the branches (termed undifferentiated inflorescences branches). There is variation in the number of flowers per inflorescence–three in *K. geminata* vs. many in all other species. Developmentally, the cushion-like inflorescences in *Korthalsella* start as a typical viscoid triad of flowers with one staminate and two flanking pistillate flowers, but subsequent flowers grow below these without a discernible pattern. Multicellular, uniseriate trichomes (Fig. 2C) are thickly interspersed in the inflorescence and can vary somewhat in color. They contain a brownish substance extraordinarily resistant to chemical attack, possibly a resin.

Individual flowers are reduced, unisexual, generally less than 1 mm in length, with the perianth reduced to three, somewhat fleshy subdeltoid sepals. Staminate flowers (Fig. 2D–E) have three confluent anthers forming a six-locular synandrium enclosed by the sepals. The synandrium is one of the diagnostic features of *Korthalsella* and a structure unique in the angiosperms, but it is invariant within the genus. The highly reduced pistillate flowers (Fig. 2C) have an umbonate stigma partially enclosed within the sepals and are similar among species of *Korthalsella*. Mature fruits are subclavate and may attain a size of 3.5 mm in larger plants, but they have no taxonomically useful characters associated with them.

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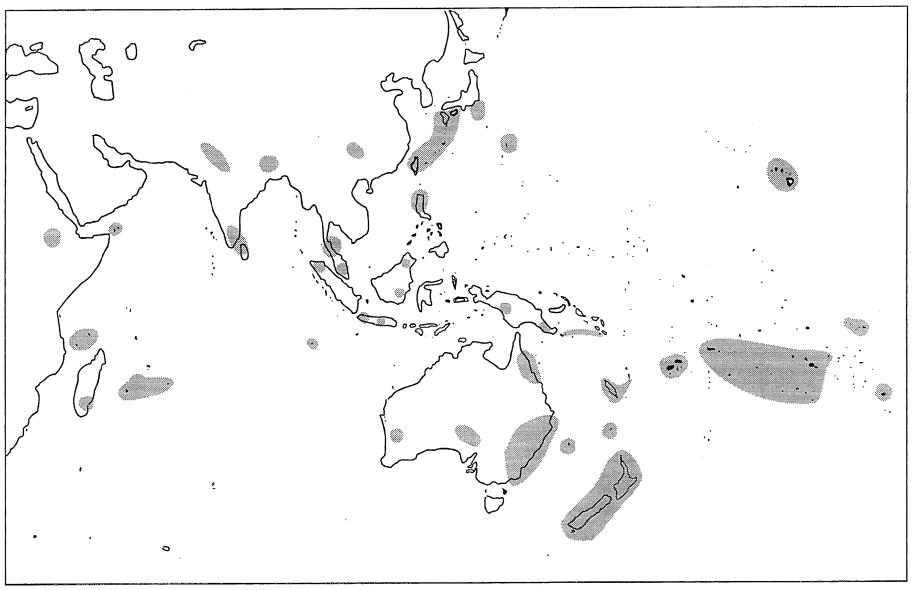

Fig. 1. Distribution of *Korthalsella*.

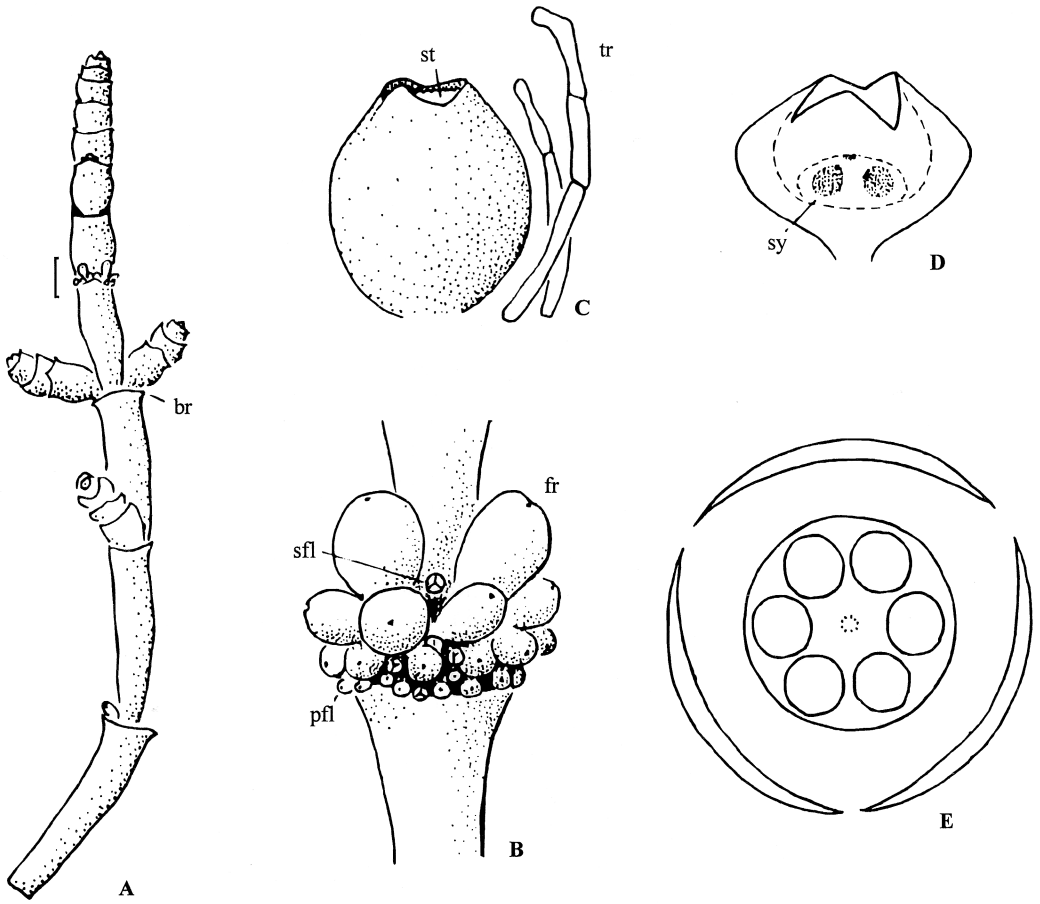

Fig. 2. (A) *Korthalsella salicornioides*, a species with undifferentiated inflorescence branches. (B) Enlargement of nodal area bracketed in (A). (C) Pistillate flower and detail of trichomes. (D) Staminate flower. Dotted outlines: hidden synandrium (sy) and thecae. (E) Floral diagram of staminate flower, showing single pore of synandrium. Abbreviations: br—bract or vestigial leaf, fr—fruit, pfl—pistillate flower, sfl—staminate flower, st—stigmatic surface, sy—synandrium, tr—trichomes.

All the variable characters mentioned above, plant size and color, branch shape and orientation, and location of inflorescences and number of flowers per cushion, have been used in previous treatments of *Korthalsella* (Van Tieghem, 1896; Engler, 1897; Danser, 1937, 1940), but their validity as taxonomically informative characters was never assessed. Van Tieghem (1896) recognized *Korthalsella* and two other segregate genera, *Bifaria* and *Heterixia*, as distinct from *Viscum* on the basis of their inflorescence cushions, which are unique among Viscaceae. Van Tieghem was aware of the synandrium but did not explicitly discuss this structure. His three genera were characterized as follows: *Korthalsella* had "unspecialized" inflorescence branches and decussate stems; *Bifaria* also had "unspecialized" inflorescence branches, but distichous stems; and *Heterixia* had "specialized" inflorescence branches. Working largely from specimens at the Paris Herbarium, Van Tieghem described over 60 species in these three genera based in many cases on minute variations in color or internode shape.

The following year Engler (1897) reduced *Bifaria* and *Heterixia* to sections of *Korthalsella*. He also published a number of new combinations within *Korthalsella* necessitated by these changes in rank but did not formally reevaluate any of Van Tieghem's species. However, he suggested that some of Van Tieghem's species might better be given infraspecific rank. Lecomte (1916) also dealt with *Korthalsella*, although he did not propose a formal revision of the genus. His work has a number of taxonomic errors, but his lasting contribution was explicitly recognizing the significance of the synandrium.

The most recent revision of *Korthalsella* is that of Danser (1937, 1940). He recognized that many of the fine distinctions drawn between species by previous workers could not be supported. Accordingly, he reduced the number of taxa to 23 species and ten varieties. Danser maintained the infrageneric classification established by Engler (1897) and continued to use cladotaxy and the presence of "specialized" inflorescence branches to distinguish the three sections of the genus. An amended synopsis of Danser's treatment of *Korthalsella* appears in Table 1. By accepting many of the taxonomic criteria used by Van Tieghem, but broadening the circumscription of some species to accommodate the apparent plasticity of these characters, Danser effectively inherited the problems that plagued Van Tieghem's treatment. Danser's solution appears to have been to assume that sympatry meant conspecificity, hence specimens from one land mass would tend to be placed in the same species. Continents therefore had highly variable species found in numerous populations, whereas species found on islands often had lower variability and were found in only one or a few populations.

Danser's treatments of *Korthalsella* did much to clarify our understanding of this taxonomically difficult genus, but his work stopped short of critically reevaluating taxonomically informative characters in the genus. In an attempt to rectify this problem, Molvray (1990) undertook a morphometric study of shape and size characters previously used to delimit taxa in *Korthalsella*. Multivariate statistical analysis of quantitative characters demonstrated that variations in plant size and internode length and shape were continuous and could not be partitioned into discrete character states. Stevens (1991) argued that continuous characters with no discontinuities in their states were best avoided in phylogenetic analyses. Of all the morphological characters used by previous workers, only the presence of differentiated inflorescence branches proved taxonomically reliable at the specific rank. Comparative anatomical studies also shed light on taxonomic relationships. Differences in bundle number could consistently differentiate among taxa: some had four or fewer bundles, others had eight or more (Touw, 1984). Using the characters that morphological and anatomical studies indicated were reliable, Molvray (1997) recognized eight species and proposed a new infrageneric classification based on presence or absence of differentiated inflorescence branches. Cladotaxy, the second character formerly used to delimit sections, was proven unreliable at any rank. Field work demonstrated that this character sometimes varies within populations.

Since the classification proposed for *Korthalsella* by Molvray (1997) differs significantly from previous treatments and there are few reliable morphological or anatomical characters, DNA sequence data were used to evaluate species delimitations and examine infrageneric relationships. Portions of two genomes were used, the *trnL-F* region from the plastid and the internal transcribed spacer (ITS) regions of the nuclear ribosomal DNA (nrDNA). Both of these contain noncoding regions that are characterized by sometimes higher nucleotide substitution rates (Taberlet et al., 1991; Baldwin, 1993; Baldwin et al., 1995) and have proven useful for elucidating phylogenetic relationships at the infrageneric level (Baldwin, 1993; Hahn and Sytsma, 1993; Bogler and Simpson, 1996; Freemann and Ybarra, 1996). In addition, ITS has already been used infragenerically within Viscaceae to clarify relationships in *Arceuthobium* (Nickrent, Schuette, and Starr, 1994) and *Phoradendron* (Ashworth, 1996).

MATERIALS AND METHODS

Plant samples—Details of the plant material sampled for sequence analysis, voucher information, and their collection localities are listed in Table 2. DNA was obtained from individuals from 25 populations of *Korthalsella* representing six of the eight species currently recognized within the genus (Molvray, 1997), as well as three other genera of Viscaceae: *Notothixos, Viscum*, and *Phoradendron*. Unfortunately, two species of *Korthalsella, K. dacrydii* and *K. geminata*, are exceedingly rare. Repeated attempts over a 10-yr period to obtain additional collections for DNA extraction, as well as attempts to use existing herbarium material for this purpose have failed. As a result these taxa are not included here.

DNA extraction, amplification, and sequencing—DNA samples were obtained from fresh, silica-dried, or cryogenically preserved material stored at 80°C. In addition, shoots from some recent herbarium collections were also used, but the success rate for this material was poor with only ~5% of the herbarium samples yielding high molecular weight DNA. Genomic DNA was extracted following the CTAB method described by Doyle and Doyle (1987) and purified by CsCl-ethidium bromide centrifugation. After centrifugation the portion of the sample containing the DNA was extracted with water-saturated n-butanol to remove the ethidium bromide and dialyzed to remove excess salts. The *trnL-F* and ITS regions were amplified from the purified genomic DNA using the polymerase chain reaction (PCR; Mullis and Faloona, 1987). Primers used were as follows: *trnL-F* "c" and "f" (Taberlet et al.,

TABLE 1. Species, infraspecific taxa, and sections of *Korthalsella* recognized by Danser (1937, 1940) and additional taxa described by Barlow (1983) contrasted with those accepted by Molvray (1997).

Danser and Barlow	Molvray
Section *Heterixia*	Section *Heterixia*
K. amentacea (Tieghem) Engler	*K. geminata* (Korthals) Engler
K. clavata (T. Kirk) Cheeseman	*K. lindsayi* (Oliver ex J. D. Hooker) Engler var. *lindsayi*
K. geminata (Korthals) Engler	var. *clavata* (T. Kirk) Danser
K. lindsayi (Oliver ex J. D. Hooker) Engler	*K. papuana* Danser
K. papuana Danser	*K. salicomioides* (A. Cunningham) Tieghem
Section *Korthalsella*	Section *Korthalsella*
K. dacrydii (Ridley) Danser	*K. cylindrica* (Tieghem) Engler
K. grayi Barlow	*K. dacrydii* (Ridley) Danser
K. horneana Tieghem	*K. japonica* (Thunberg) Engler f. *japonica*
K. madagascarica Danser	f. *grayi* (Barlow) Molvray
K. remyana Tieghem var. *remyana*	*K. taenioides* (Commerson ex DC) Engler f. *taenioides*
var. *wawrae* (Tieghem) Danser	f. *disticha* (Endlicher) Molvray
K. salicornioides (A. Cunningham) Tieghem	f. *emersa* (Barlow) Molvray
K. striata Danser	f. *horneana* (Tieghem) Molvray
Section *Bifaria*	f. *pendula* (Wawra) Molvray
K. aoraiensis (Nadeaud) Engler	f. *remyana* (Tieghem) Molvray
K. breviarticulata (Tieghem) Danser	
K. commersoni (Tieghem) Danser	
K. complanata (Tieghem) Engler	
K. cylindrica (Tieghem) Engler var. *cylindrica*	
var. *planiuscula* Danser	
K. degeneri Danser	
K. dichotoma (Tieghem) Engler var. *dichotoma*	
var. *balansae* (Tieghem) Danser	
K. disticha (Endlicher) Engler	
K. emersa Barlow	
K. japonica (Thunberg) Engler subsp. *japonica*	
subsp. *brassiana* (Blakely) Barlow	
K. latissima (Tieghem) Danser var. *latissima*	
var. *crassa* Danser	
K. leucothrix Barlow	
[a][*K. opuntia* (Thunberg) Merrill var. *opuntia*]	
= *K. japonica* sensu Barlow	
var. *boieri* (Tieghem) Danser	
var. *fasciculata* (Tieghem) Danser	
var. *gaudichaudii* (Tieghem) Danser	
var. *richardii* (Tieghem) Danser	
K. platycaula (Tieghem) Engler var. *platycaula*	
var. *rapensis* (F. Brown) Danser	
var. *vitiensis* (Tieghem) Danser	
K. rubra (Tieghem) Engler subsp. *rubra*	
subsp. *geijericola* Barlow	
K. rubescens Tieghem	

[a] *Korthalsella opuntia* is an illegitimate name (see Barlow, 1983, or Molvray, 1997, for details). The earliest available epithet is *K. japonica* (Thunberg) Engler, however the varieties listed here have never been formally transferred to that epithet.

1991), which amplified the intron, 3′ exon, and intergenic spacer, and "ITS5" and "ITS4" (White et al., 1990), which amplified ITS1, the 5.8S gene, and ITS2. For both *trnL-F* and ITS, all segments were included in separate as well as combined analyses. The double-stranded PCR products were purified using Promega Wizard PCR™ minicolumns (Promega Corp., Madison, Wisconsin) in accordance with the manufacturer's protocols, and 10 μL sequencing reactions were carried out in a Perkin-Elmer GenAmp model 9600 thermocycler using the Applied Biosystems Inc. (ABI) Taq DyeDeoxy Terminator Cycle Sequencing Kit™ (Perkin-Elmer Corp., Norwalk, Connecticut). Sequencing was done on an ABI model 373 automated sequencer, and each region was sequenced for both strands. Sequences were assembled and edited electronically using Sequencher™ software from Gene Codes Corporation (Ann Arbor, Michigan). The completed sequences were manually aligned prior to analysis.

Data analysis—Posted sequences of nrDNA for other genera of Viscaceae obtained from GenBank were used to determine the boundaries of the ITS1 and ITS2 regions. The boundaries of the *trnL* intron and *trnL-F* intergenic spacer were identified by comparing sequences with posted sequences from Genbank of the highly conserved *trnL* 5′ and 3′ exon and the *trnF* gene.

Phylogenetic analysis was performed using PAUP Version 3.1.1 (Swofford, 1993) and Macintosh computers. Data were analyzed as separate ITS and *trnL-F* sets, and then as a combined data set. All transformations were unordered and weighted equally in both data sets (Fitch parsimony; Fitch, 1971); gaps were coded as missing values, but also separately coded as binary characters using PaupGap (Cox, 1997) and appended to the respective data sets. Heuristic analyses were performed on all data sets (ITS, *trnL-F*, and combined) with tree-bisection-reconnection (TBR) branch-swapping and the MULPARS option in effect. Tree space was searched by using 100 random taxon addition sequence replicates with a limit of five trees saved per replicate. All trees saved were then swapped to completion. This method is applied to increase

TABLE 2. *Korthalsella* voucher information. Collection numbers refer to Molvray collections except where otherwise indicated. Both Molvray (boldface) and Danser (regular type) identifications provided.

	Coll. no. or ID		Locality	Host	Herbarium
K. taenioides					
	302	*K. platycaula*	Hawaii Kauai Waininiua trail	*Nestegis*	MO
	304	*K. latissima*	Hawaii Kauai Pihea—Alakai trail	*Clermontia, Pelea*	MO
	307	*K. platycaula*	Hawaii Oahu Waianae Mts., Kaala, midlevel Puu Kaua	*Planchonella*	MO
	308	*K. complanata*	Hawaii Oahu Waialae ridge	*Eugenia*	MO
	309	*K. latissima*	Hawaii Oahu Waianae Mts., Kaala summit	*Myrsine*	MO
	312	*K. remyana*	Hawaii Oahu Koolau Mts., Niu ridge	*Diospyros*	MO
	313	*K. complanata*	Hawaii Oahu Koolau Mts., Niu ridge	*Eugenia*	MO
	361	*K. rubra* subsp. *geijericola*	Australia, New South Wales, Croppa Creek	multiple hosts	MO
	362	*K. rubra* subsp. *geijericola*	Australia, New South Wales, Robertson	*Doryphora*	MO
Lorence	R19	["*K. opuntia*" var. *bojeri*] = *K. japonica*	Réunion, Cilaos near les Thermes	*Eugenia*	K
Lorence	1967	["*K. opuntia*" var. *richardii*] = *K. japonica*	Mauritius, Piton de la Rivière Noire	*Nuxia*	K
Strasberg	s.n.	["*K. opuntia*" var. *richardii*] = *K. japonica*	Réunion, without specific locality	unknown	K
K. cylindrica					
	303	*K. cylindrica*	Hawaii Kauai Pihea ridge trail	*Metrosideros*	MO
	310	*K. cylindrica*	Hawaii Oahu Waianae Mts., Kaala summit	*Metrosideros*	MO
K. japonica					
	358a	*K. japonica* subsp. *brassiana*	Australia, Queensland, Mt. Lewis	*Rapanea, Uromyrtus*	MO
Klackenberg & Lundin	228	*K. japonica* subsp. *japonica*	India Western Ghats, Tamil Nadu	*Rhododendron*	K
Inouye	s.n.	*K. japonica* subsp. *japonica*	Japan, Oshina Island	unknown	K
K. salicornioides					
	350	*K. salicornioides*	New Zealand, North Isl., Whakarewarewa	*Kunzea*	MO
	355	*K. salicornioides*	New Zealand, South Isl., Price's Valley	*Kunzea*	MO
K. lindsayi					
	315	*K. lindsayi*	New Zealand, South Isl., DSIR parking lot[a]	*Melicope*	MO
	351	*K. lindsayi*	New Zealand, North Isl., A'Dean's Reserve	*Melicope*	MO
	352	*K. lindsayi*	New Zealand, South Isl., Carluke	*Melicope*	MO
	354	*K. lindsayi*	New Zealand, South Isl., Price's Valley	*Lophomyrtus, Coprosma*	MO
	356	*K. lindsayi* var. *clavata*	New Zealand, South Isl., View Creek	*Coprosma*	MO
	357	*K. lindsayi*	New Zealand, South Isl., Lake Coleridge	*Coprosma, Discaria*	MO
K. papuana					
	359	*K. papuana*	Australia, Queensland, Mt. Lewis	*Acmena, Syzygium*	MO
Notothixos					
Forster	9555	*N. subaureus*	Australia, Queensland		K
Phoradendron					
Kores	s.n.	*P. serotinum*	USA, New Orleans, Audubon Park	*Quercus*	K
Viscum					
Sheahan	s.n.	*V. album*	United Kingdom, vicinity of London	*Crataegus*	K

[a] Host, with attached parasite, transplanted from Price's Valley.

the chances of finding the shortest tree(s) (Olmstead et al., 1993). In *trnL-F* for which more than one most parsimonious tree was obtained, trees resulting from the heuristic search were successively weighted to reduce the influence of homoplasious characters (Farris, 1969). Examination of downweighted characters showed these to be among the most variable in *trnL-F* with three to eight changes per site. The combined and ITS data sets were also successively weighted to facilitate comparison among tree scores. Successive weighting did not alter tree topology, but rather produced one of the trees found with Fitch weights. Relative support for the clades identified by the parsimony analysis was assessed by bootstrapping (Felsenstein, 1985), with 1000 replicates for each matrix.

Potential rooting within *Korthalsella* was evaluated by comparing ITS and *trnL-F* sequences from *Viscum album, Notothixos subaureus*, and *Phoradendron serotinum* with those of *Korthalsella*. These ITS sequences were too divergent to be aligned with sequences from *Korthalsella*, thereby precluding the use of these genera as outgroups. However, the *trnL-F* region from these genera could be aligned with sequences from *Korthalsella*, and an analysis based upon these aligned sequences roots *Korthalsella* at the midpoint of the longest branch. (Midpoint rooting of the ITS tree provides the same tree topology.) Thus, the *K. papuana* clade was sister to the rest of the genus in the *trnL-F* analysis and was used as the outgroup in subsequent analyses of both *trnL-F* and ITS matrices. Since not all genera within Viscaceae were available for the *trnL-F* analysis, the sister group to *Korthalsella* was identified by a combined analysis of ten 18S rDNA and plastid *rbcL* sequences obtained from GenBank. These sequences represent all seven genera of Viscaceae. Outgroups were two genera of Santalaceae and one genus of Loranthaceae. The taxa used, their Genbank accession numbers and individuals who posted these sequences are listed in Table 3.

TABLE 3. GenBank numbers of taxa used to determine the sister group to *Korthalsella* and of *Korthalsella* specimens examined.

Taxon	GenBank accession number	
	18S	***rbcL***
Loranthaceae		
Gaiadendron punctatum (Ruiz and Pav.) G. Don	GBANL24143[1]	GBANL26072[1]
Santalaceae		
Osyris lanceolata Hochst. & Steud. ex A. DC	GBANU42803[2]	GBANL11196[5]
Santalum album L.	GBANL24416[1]	GBANL26077[1]
Viscaceae		
Arceuthobium verticilliflorum Engler	GBANL24042[4]	GBANL26067[1]
Dendrophthora clavata (Benth.) Urban	GBANL24086[1]	GBANL26069[1]
Ginalloa arnottiana Korthals	GBANL24144[1]	GBANL26070[1]
Korthalsella lindsayi (Oliver) Engler	GBANL24150[1]	GBANL26073[3]
Notothixos subaureus Oliver	GBANL24403[1]	GBANL26075[1]
Phoradendron serotinum (Raf.) M. Johnston	GBANX16607[3]	GBANL11199[5]
Viscum album L.	GBANL24426[1]	GBANL26078[1]
	ITS	***trnL-F***
Korthalsella		
Korthalsella papuana (359)	GBANAF051951	GBANAF055673
Korthalsella salicornioides (355)	GBANAF051952	GBANAF055674
Korthalsella salicornioides (350)	GBANAF051953	GBANAF055675
Korthalsella lindsayi (315)	GBANAF051954	GBANAF055676
Korthalsella lindsayi (351)	GBANAF051955	GBANAF055677
Korthalsella lindsayi (352)	GBANAF051956	GBANAF055678
Korthalsella lindsayi (354)	GBANAF051957	GBANAF055679
Korthalsella lindsayi var. *clavata* (356)	GBANAF051958	GBANAF055680
Korthalsella cylindrica (310)	GBANAF051959	GBANAF055681
Korthalsella cylindrica (303)	GBANAF051960	GBANAF055682
Korthalsella remyana (312)	GBANAF051961	GBANAF055683
Korthalsella latissima (309)	GBANAF051962	GBANAF055684
Korthalsella platycaula (302)	GBANAF051963	GBANAF055685
Korthalsella platycaula (307)	GBANAF051964	GBANAF055686
Korthalsella latissima (304)	GBANAF051965	GBANAF055687
Korthalsella complanata (308)	GBANAF051966	GBANAF055688
Korthalsella complanata (313)	GBANAF051967	GBANAF055689
Korthalsella japonica ssp. *brassiana* (358a)	GBANAF051968	GBANAF055690
Korthalsella rubra ssp. *geijericola* (362)	GBANAF051969	GBANAF055691
Korthalsella rubra ssp. *geijericola* (361)	GBANAF051970	GBANAF055692
Korthalsella japonica (L1967)	GBANAF051971	GBANAF055693
Korthalsella japonica (LR19)	GBANAF051972	GBANAF055694
Korthalsella japonica (STR)	GBANAF051973	GBANAF055695
Korthalsella japonica (K&L228)	GBANAF051974	GBANAF055696
Korthalsella japonica (INO)	GBANAF051975	GBANAF055697

Note: The prefix GBAN has been added for linking the on-line version of *American Journal of Botany* to GenBank and is not part of the actual GenBank accession number. Superscripts after the GenBank accession numbers refer to the author(s) of the sequence: [1]D. L. Nickrent and D. E. Soltis, [2]D. E. Soltis, [3]D. L. Nickrent, [4]D. L. Nickrent and E. M. Starr, [5]D. R. Morgan and D. E. Soltis. Numbers in parentheses after specific names in *Korthalsella* refer to collection or ID numbers.

Sequence data for the *trnL-F* and ITS analyses used in this paper have been submitted to GenBank. PAUP matrices and tree files are available on request from the first author.

RESULTS

The sister group to Korthalsella—The analysis of the Viscaceae based on 18S and *rbcL* sequences yielded one most parsimonious tree, 723 steps long, with a consistency index of 0.72 and a retention index of 0.55 (Fig. 3). *Ginalloa* is the sister to *Korthalsella* with 100% bootstrap support. *Phoradendron* and *Dendrophthora* are sister taxa, also with bootstrap support of 100%, and this clade is sister to the *Ginalloa-Korthalsella* clade with 75% bootstrap support. Although this tree is fully resolved, all other nodes within the Viscaceae have bootstrap support below 50%.

Size, variability, and sequence alignments—The aligned *trnL-F* region in *Korthalsella* consists of 1091 bp, including the intron, *trnL* 3′ exon, and *trnL-F* intergenic spacer. When only *Korthalsella* species are used in the alignment, the region is 978 bp long. The intron consists of 597 bp, of which 66 (11.1%) are potentially phylogenetically informative, and contributes 164 steps when mapped onto the tree obtained in combined analysis. The *trnL* 3′ exon has 52 bp, five of which (9.6%) are informative, and contributes 14 steps to the combined tree. The intergenic spacer extends for 329 bp, with 45 (13.7%) informative characters, and contributes 174 steps to the combined tree. This study indicates that the level of variability within the *trnL-F* intergenic spacer is approximately twice that of the *trnL* intron; however, the former is only about half the length of the latter so the

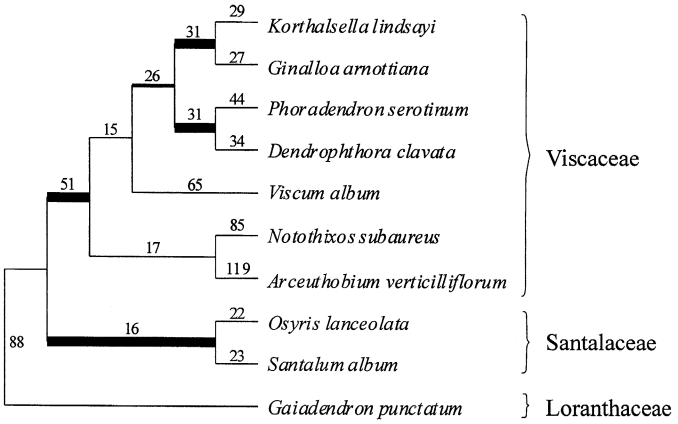

Fig. 3. Phylogenetic relationships in the Viscaceae based on 18S nrDNA and plastid *rbcL*. All sequences were previously posted on GenBank. One most parsimonious tree, length 723, CI = 0.72, RI = 0.55. Branch lengths are above. Bootstrap support indicated by line thickness: thickest, 100–90%; medium thickness, 89–80% (not present in this figure); less thickness, 79–50%; thinnest, below 50%. *Ginalloa* is the sister group to *Korthalsella*. *Phoradendron* and *Dendrophthora* are sister taxa, and this clade is sister to the *Ginalloa–Korthalsella* clade. All other nodes within the Viscaceae have bootstrap support of <50%.

number of characters provided by each segment is similar. The coding region has a 1:1 ratio of transitions to transversions; the two noncoding regions have ~1:2 ratios, i.e., an excess of transversions. The mean G + C content of the three regions within the *trnL-F* sequences is as follows: 32.7% for the intron, 48.3% for the 3′ exon, and 26.5% for the intergenic spacer. Details of measures of variability can be seen in Table 4.

After alignment there are 175 indels in the *trnL-F* data matrix. These indels range in size from 1 to 182 bp; 31% are only 1 bp long, 26% are either 2 or 3 bp, 16% are 4 or 5 bp, 12% are 6–9 bp, while the remaining 13% are >9 bp. Fifty-nine (33.7%) of these indels are phylogenetically informative, and nine are of particular interest from the standpoint of this study. These indels have been mapped onto the cladogram in Fig. 4. One of these, a 3-bp insertion at positions 91 to 93 (I-1) is shared by all sequences from New Zealand (clade "B" in Fig. 4). Two others, a 2-bp deletion at positions 529 to 530 (I-5) and a 9-bp deletion spanning positions 707 to 715 (I-8), are shared by all sequences in clade "C" (Fig. 4). Two additional indels, a 2-bp deletion at positions 148 to 149 (I-2) and a 6-bp deletion from positions 407 to 412 (I-4), are common to those sequences from the Hawaiian Islands. In addition, three other indels are congruent with some of the minor subclades within Fig. 4: a 6-bp insertion from position 570 to 575 (I-6) common to the Indian and Japanese collections of *K. japonica*; a 4-bp insertion at positions 586 to 589 (I-7) found in all collections of *K. japonica* from the Mascarene Islands; and a 5-bp deletion from position 754 to 758 (I-9) found in the two collections of *K. rubra* subsp. *geijericola* from Australia. Also of interest is a 4-bp deletion at positions 262 to 265 (I-3) that is shared by the Asian and Mascarene collections of *K. japonica*, suggesting an affinity between these two subclades. There are no unique indels shared by all the Australian taxa sampled in this study.

We evaluated the feasibility of aligning ITS sequences from *Korthalsella* with three other genera of Viscaceae by attempting multiple alignment using the CLUSTAL W computer software package (Thompson, Higgins, and Gibson, 1995) under a number of different assumptions. Depending on the gap penalty the number of potentially informative characters varies from 259 to 300, whereas the branch length between *Korthalsella* and the other genera ranges from 235 to 237 steps. Regardless of which gap penalty is chosen, the number of changes in ITS between *Korthalsella* and the other genera included in this study is greater than the number of variable sites within *Korthalsella* and is too great to allow any valid conclusions to be drawn about intergeneric relationships. These findings agree with previous reports that the Viscaceae have highly divergent ITS sequences at the generic level (Nickrent, Schuette, and Starr, 1994; Baldwin et al., 1995). Furthermore, the topology within *Korthalsella* is unstable depending on the gap penalty. Since outgroup genera could not be aligned, *Korthalsella papuana* was used as the outgroup in ITS analyses based on results from *trnL-F* analysis.

The aligned region comprising ITS1, 5.8S, and ITS2

TABLE 4. Summary of descriptive measures, indexes of variation, and comparison of sequence variation between ITS and *trnL-F* in 24 populations of *Korthalsella* (excluding binary coded gap data).

	ITS				*trnL-F*			
Comparison	ITS1	5.8S	ITS2	Total	*trnL* intron	*trnL* 3′ exon	*trnL-F* spacer	Total
Number of characters in aligned matrix	278	168	271	717	597	52	329	978
Percentage of characters variable	41.7	10.1	44.6	35.4	18.6	17.3	32.8	23.3
Percentage informative characters	27.3	5.4	26.2	21.8	11.1	9.6	13.7	13.6
Number of most parsimonious trees	12	640	36	2	1095	21	2340	93
Tree length	182	24	182	392	159	11	167	345
Consistency index (CI)	0.74	0.83	0.78	0.76	0.79	0.82	0.73	0.74
CI excluding autapomorphies	0.66	0.75	0.70	0.67	0.69	0.67	0.63	0.64
Retention index (RI)	0.86	0.90	0.89	0.87	0.87	0.75	0.82	0.83
Base composition (%)								
Adenine	18.2	23.8	19.8	20.3	39.2	24.8	34.7	36.9
Cytosine	22.6	24.2	22.5	23.0	14.1	28.1	14.8	15.2
Guanine	32.6	27.9	28.7	29.9	18.6	20.2	11.7	16.5
Thymine	26.5	24.1	29.0	26.9	28.1	26.9	38.8	31.4
Transition/transversion ratio	1.23:1	2.17:1	1.01:1	1.10:1	0.55:1	1:1	0.53:1	0.56:1

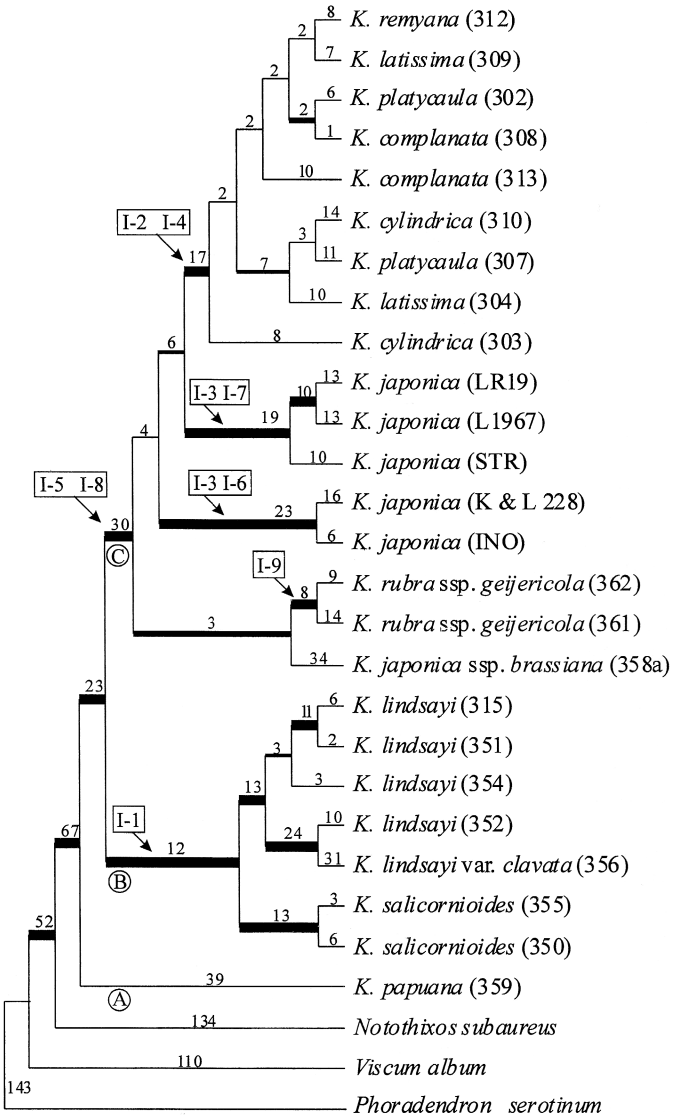

Fig. 4. Tree of *Korthalsella* and outgroups based on plastid *trnL-F* data. Taxa are labeled according to names in Danser (1937, 1940) and Barlow (1983), and population identifiers are in parentheses. One tree obtained after successive weighting, length 1035, CI = 0.94, RI = 0.92. Indels discussed in the text are mapped onto the tree as I-1 through I-9. Clades A, B, and C are discussed in the text. Branch lengths are above. Bootstrap support is indicated as in Fig. 3.

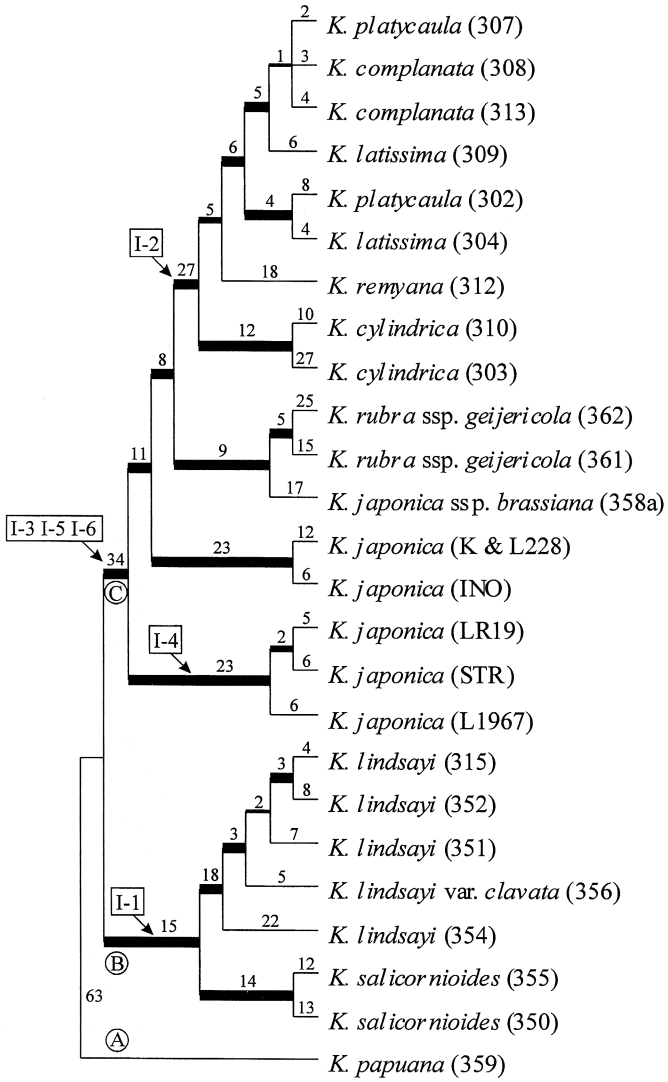

Fig. 5. Tree of *Korthalsella* based on nuclear ITS. One tree was obtained before and after successive weighting, length 538, CI = 0.70, RI = 0.83. Indels discussed in the text are mapped onto the tree as I-1 through I-6. Clades A, B, and C are discussed in the text. Branch lengths are above. Taxa labeled as in Fig. 4. Bootstrap support indicated as in Fig. 3.

in *Korthalsella* consists of 717 bp. The shortest sequence (650 bp) is found in *K. japonica* from Mauritius. The longest sequence (679 bp) is the outgroup, *K. papuana*. This is the longest ITS region reported for angiosperms (Baldwin et al., 1995). ITS1 extends for 278 bp, of which 76 (27.3%) are potentially phylogenetically informative, and contributes 183 steps in the combined analysis. The 5.8S gene is 168 bp long with nine (5.4%) informative characters, and provides 24 steps. The ITS2 region extends for 271 bp, yields 71 (26.2%) informative characters, and provides 188 steps. As the step lengths and numbers of informative sites indicate, the substitution rate for ITS is ~2.5× that of the *trnL-F* region in *Korthalsella* when their size difference is taken into account. ITS1 and ITS2 have an ~1:1 ratio of transitions to transversions, while the ratio in the 5.8S gene is ~2:1, i.e., an excess of transitions. The mean G + C content of the ITS region is as follows: 55.2% for ITS1, 52.1% for the 5.8S gene, and 51.2% for ITS2. (Data are summarized in Table 4.)

After alignment there are 81 indels in the ITS data matrix. These indels range in size from 1 to 26 bp; 67% are only 1 bp long, 15% are 2 or 3 bp, while the remaining 18% are >4 bp. Fifty-nine of these indels (72%) are phylogenetically informative. Six indels of particular interest to this study are mapped onto the cladogram in Fig. 5. One of these, a 15-bp deletion at positions 82 to 96 (I-1) is shared by the New Zealand sequences (clade "B" in Fig. 5). A second, a 3-bp deletion from position 99 to 101 (I-2), is found only in the Hawaiian sequences. A third, a 26-bp deletion from position 223 to 248 (I-4) is common to the Mascarene collections. The remaining three indels, a 5-bp insertion at positions 195 to 199 (I-

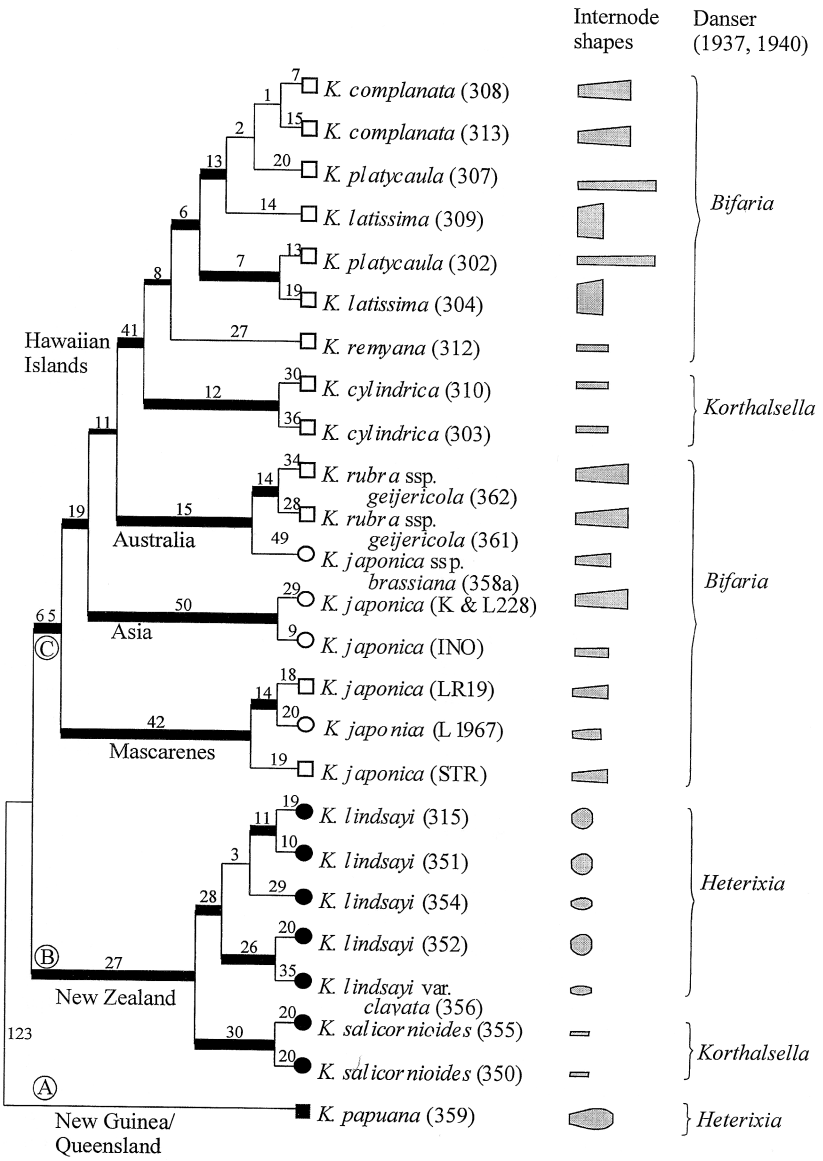

Fig. 6. Tree of *Korthalsella* based on combined ITS and *trnL-F* data sets, including binary coded gap data. One tree, length 1108, CI = 0.91, RI = 0.95. Clades A, B, and C are discussed in the text and correspond to sections sensu Molvray: (A) *Korthalsella* sect. *Papuana*, (B) *Korthalsella* sect. *Heterixia*, (C) *Korthalsella* sect. *Korthalsella*. Geographic regions corresponding to clades are indicated. Circles at terminal branches indicate taxa with four or fewer internodal vascular bundles, whereas squares indicate those with eight or more. Filled circles or squares indicate taxa with differentiated inflorescence branches, and white ones indicate taxa with undifferentiated branches. Internode shapes and previous sectional classification are shown to the right of the tree. Branch lengths are above. Taxa are labeled as in Fig. 4. Bootstrap support is indicated as in Fig. 3.

3), a 2-bp insertion at positions 528 to 529 (I-5) and a 1-bp deletion at position 688 (I-6), are all shared by the sequences grouped as clade "C" in Fig. 5. As in the *trnL-F* study, there is no unique indel that is found only among all of the Australian samples.

Phylogenetic analyses—Our analysis of the plastid *trnL-F* region yielded eight equally parsimonious trees 1035 steps long, which were reduced to one after successive weighting. The tree is 1035 steps (645 654 weighted steps) and has a consistency index (CI) of 0.94 and a retention index (RI) of 0.92. Bootstrap analysis (Fig. 4) shows 100% support for the monophyly of the ingroup. This analysis indicates that *Korthalsella papuana* is sister to all other species in the genus (92% bootstrap support). The remaining taxa sampled form two strongly supported sister subclades. These correspond to the taxa with differentiated inflorescence branches (clade B in Fig. 4, with 100% bootstrap support) and those taxa that lack differentiated inflorescence branches (clade C in Fig. 4, with 100% bootstrap support). Branch lengths within clade C are short, but a number of smaller clades are well supported, especially those in which relationships are correlated with geography. Three such subclades are the collections of *K. japonica* from the Mascarenes with bootstrap support of 100%; the collections of *K. complanata, K. cylindrica, K. latissima, K. platycaula,* and *K. remyana* from the Hawaiian Islands with 100%

bootstrap support; and the three collections from Australia: *K. rubra* ssp. *geijericola* from New South Wales, Australia, and *K. japonica* ssp. *brassiana* from Queensland with 82% bootstrap support. However, the levels of variability found in the *trnL-F* region proved insufficient for resolution of several clades.

Our analysis of the ITS data yielded one most parsimonious tree, 531 steps long, with a consistency index of 0.71 and a retention index of 0.84 (Fig. 5). Successive weighting results in the same tree (CI = 0.90, RI = 0.95, weighted length 311 618). If gaps are excluded, two minimal trees are obtained (not shown; CI = 0.76, RI = 0.87, length 392), which are maintained after successive weighting (CI = 0.90, RI = 0.95, weighted step length 260 453). The topology of the ITS tree is similar to those based on *trnL. Korthalsella papuana* is sister to all other species within the genus, and these species are further resolved into two well-supported subclades corresponding to the taxa with differentiated inflorescences branches and those lacking them (clades B and C in Fig. 5). The composition of all three clades, A, B, and C, is identical in the two data sets.

An analysis based upon both data sets echoes the results found in the separate analyses, but with stronger levels of bootstrap support for many of the clades. This analysis yielded one most parsimonious tree, 1108 steps long, with a consistency index of 0.91 and a retention index of 0.95 (Fig. 6). All of the clades are fully resolved, and it is evident in Fig. 6 that many of the species formerly recognized by Danser (1937, 1940) and Barlow (1983) are polyphyletic.

In brief, the results obtained indicate that *Korthalsella* is monophyletic and is resolved into three clades comprising a monotypic clade, *K. papuana*, which has differentiated inflorescence branches and eight or more vascular bundles, a second clade, *K. salicornioides* and *K. lindsayi*, with differentiated inflorescence branches and four or fewer vascular bundles, and a third clade, characterized by plants with undifferentiated inflorescence branches and four or more vascular bundles (Figs. 4–6). Based upon the results presented here, many of the species formerly recognized within this clade are not monophyletic.

DISCUSSION

The polyphyly of *Korthalsella* species and sections (Fig. 6) based on branch characters is evident, necessitating a complete revision of the genus. However, how to effect the revision is equivocal from molecular data because they do not resolve the difficult question of species delimitation. Isozyme studies (Molvray, 1990), using 25–30 individuals from each population, showed that almost every population is resolved as its own evolutionary unit. Because *Korthalsella* populations probably seldom interbreed since neither seeds nor pollen move far from the parent plant, such a pattern among populations is expected. Seeds are deposited near or even on the parent plant except in rare cases of animal-mediated dispersal, and the sticky pollen is carried on the feet of minute thrips, mites, and similar arthropods with limited mobility. Thus, the evolutionary units in the genus are considerably more restricted than the morphological lineages, a situation also found in cave spiders, which have similar problems bridging gaps between populations (Hedin, 1997). The molecular data could be used to hypothesize the existence of microspecies, morphologically undiagnosable and recognizable only by their DNA sequences. Such a proliferation of unrecognizable species is both philosophically and practically unattractive (Olmstead, 1995).

If species delimitations are to be drawn more broadly, however, there needs to be some consistency in circumscription. Without reliance on branch shape or other labile characters Molvray (1997) found only eight diagnosable species in *Korthalsella*: *K. geminata, K. papuana, K. lindsayi, K. salicornioides, K. dacrydii, K. taenioides, K. japonica*, and *K. cylindrica*. However, two of these, *K. cylindrica* and *K. japonica,* need further molecular study to determine whether they can be supported. The existing molecular evidence indicates that both taxa may be polyphyletic. In our study *K. cylindrica* appears embedded within the larger clade comprising *K. taenioides* (clade C in Fig. 6), and a partial ITS sequence from a Tahitian specimen referable to *K. cylindrica* is not placed on the same clade as the Hawaiian material. Similarly, Molvray (1997) distinguished *K. japonica* from *K. taenioides* on the basis of a difference in the number of internodal vascular bundles (two or four in the former as opposed to eight or more in the latter), but the molecular phylogeny indicates that a reduction in bundle number has occurred at least three times within the clade with undifferentiated inflorescence branches (indicated in Fig. 6).

It is worth stressing that species concepts have a predictive component (O'Hara, 1993) in that they seek to discover lineages that will remain discrete in the future, not just those that have been so in the past. Hence, although it appears counterintuitive initially to group very different morphs, such groupings are consistently diagnosable, they are monophyletic, and they are unlikely to be mere accidents of geographic distance or the chance visits of birds. The high level of variability in some aspects of these species can be understood in terms of the considerable isolation of most populations.

Using broad species delimitations, data from the two gene trees yield the same species tree composed of a few morphologically plastic taxa. Supporting broad delimitation is some evidence of gene flow within species. Gene flow can result in differences in tree topology between the nuclear, biparentally inherited ITS and plastid, probably uniparentally inherited *trnL-F* that is more likely to show the effects of lineage sorting. There is at least one instance of a well- to moderately supported incongruence in terminal branches between the ITS and *trnL-F* trees: for instance, *K. lindsayi* (315) and *K. lindsayi* (352) are sister clades on a well-supported branch in the ITS tree but separated on other well-supported branches in the *trnL-F* tree (Figs. 4, 5). Incongruences such as these may be evidence of chloroplast capture or gene flow (Wolfe and Elisens, 1995), which in *Korthalsella* is likely to be seed mediated.

Korthalsella geminata, an Indonesian-Malaysian taxon missing from our molecular data, is almost certain either to form a clade sister to the rest of the genus or to group with *K. papuana* on clade A. This conclusion is based on morphological analysis by Molvray (1990), the results of which have been largely confirmed by our molecular studies. The other unsampled taxon, *K. dacrydii* from

Malaysia and Indonesia, will most likely be sister to other Asian populations and thus represent a highly derived terminal clade on a lineage generally showing reduced size and number of vascular bundles. The accuracy of the overall tree topology is likely to be unaffected due to the large amount of data supporting the tree, which should compensate for the missing elements. Thus, biogeographic and other implications discussed below are likely to remain valid.

It is possible that ITS sequences appear congruent due to paralogy (Buckler and Holtsford, 1995). However, all sequences studied using the computer program RNAdraw (Matzura, 1995) have similar secondary structure stability, which indicates that all are similarly constrained and therefore comparable. It is possible that skewed nucleotide composition in *trnL-F* might result in differential effects among lineages, but the generally high levels of support for the same topology in trees based on different regions indicate to us that such biases are not a factor.

The great variation in branch shape among closely related plants on different hosts raises the question whether there may be host-mediated changes in morphology of the parasite. Especially striking examples can be found in the Hawaiian terminal clades, in which plants with long thin internodes (e.g., "*K. platycaula*") are most closely related to plants with short and broad internodes (e.g., "*K. latissima*"; Fig. 6). An interesting area for further research would be to look at the degree to which the host itself may be influencing the shape of the parasite. If there is a host effect, the use of morphological characters subject to that effect may be suspect not only in *Korthalsella* but in any parasite similarly affected.

Another potentially significant trend is the degree of differentiation that may develop due to adaptation to a given host. Some *K. taenioides* found in Hawaii growing on *Metrosideros* (e.g., "*K. cylindrica*" 303, 310 in Figs. 4 and 5) have unusually high levels of both molecular and isozyme divergence from their conspecifics, and cluster together despite their geographic separation on different islands of the Hawaiian archipelago. Possibly, the physiological adaptations required to grow on *Metrosideros* are such that a host race is forming.

The *Korthalsella taenioides* clade (C in Figs. 4–6) illustrates the misconceptions that can arise when characters are not analyzed for their phylogenetic significance. When faced with a high level of variation in one character in an otherwise difficult group, the first tendency is to try to recognize that variation. If this is a phylogenetically uninformative character, it will lead only to incorrect assessments as with branch shape in *Korthalsella*. Another implication of general relevance is that continuous characters, such as shape and size, need quantitative evaluation (Stevens, 1991). Without it, spurious groupings may be recognized, which either contain at best no information or at worst misleading patterns.

Morphological character analysis is also necessary at the sectional level. Danser's section *Bifaria*, composed of distichously branching plants, is polyphyletic, as are his sections *Korthalsella*, with decussate branching, and *Heterixia*, with differentiated inflorescence branches. The molecular tree indicates that, in addition to clade C (Figs. 3–6) with undifferentiated branches, there are two sections with differentiated branches, one containing *K. papuana* (clade A, Figs. 4–6), and one that could continue to be called *Heterixia* (clade B, Figs. 4–6), containing *K. salicornioides* and *K. lindsayi*. Morphologically, *Heterixia* in the molecular sense appears paradoxical since *K. salicornioides* does not obviously have differentiated branches. However, the branch morphology of *K. salicornioides* has been misinterpreted. *Korthalsella salicornioides* bears inflorescences only on distal nodes, as does its sister species, *K. lindsayi*. *Korthalsella salicornioides* must, in effect, be viewed as a plant with differentiated inflorescence branches in which vegetative branches have secondarily acquired a terete shape. In contrast, plants with truly undifferentiated branches have flowers on almost every node. The terete vegetative branches of *K. salicornioides*, seemingly different from the flattened obovate segments found in *K. lindsayi*, have led to the mistaken assumption of a fundamental difference in inflorescence development between these two species. Instead, branch shape is highly plastic in the genus, and this is simply another instance in which different branch morphologies have no bearing on phylogenetic relationship.

The character evolution of differentiated and undifferentiated inflorescence branches Molvray postulated (1990) is also confirmed by the molecular data. Danser considered the differentiated state to be advanced and called it "specialized branches," whereas Molvray postulated that differentiation is a symplesiomorphy because of its association with the primitive condition of floral triads found in the extremely rare *K. geminata*, a species known only from three collections in Sabah and one collection from Sumatra. *Ginalloa*, found in Malesia and sister to *Korthalsella* (Fig. 1), has triads of flowers at the nodes of branch segments, with flowering tending to occur on the more distal segments. Although the mature plants are different in appearance, the position and arrangement of flowers in *Ginalloa* are similar to that found in *Korthalsella*, and *Korthalsella*-type inflorescence cushions could have evolved from such an ancestor by reduction of inflorescence-bearing branches. *Korthalsella geminata* has the longest inflorescence branches in the genus, with several tens of nodes on each branch. A compression of nodes during development would result in the formation of inflorescences with multiple flowers per node as is found in *K. papuana, K. lindsayi, and K. salicornioides*, which also have noticeably fewer nodes per flowering branch. Complete compression of all flowering nodes results in plants with flowers confined to inflorescence cushions at the nodes. Nodal anatomy is complex (Touw, 1984; Molvray, 1990), supporting the concept that the nodes are derived from larger structures.

The molecular and the morphological data both have implications for the biogeography of *Korthalsella*. The most closely related genus, *Ginalloa*, is found in Malesia, the species that is sister to the rest of the genus comes from the same area, and the levels of both sectional and species diversity are highest there. It is logical to hypothesize that dispersal proceeded from this core area outward. We speculate that the dispersal agents responsible for the patchy, disjunct distribution found in the genus are birds and possibly butterflies in some restricted locations. The southern hemisphere distribution has also been explained by vicariance events following the breakup of Gondwana (Barlow, 1981; Barlow, personal com-

munication, CSIRO), but the hypothesis appears superfluous given that *Korthalsella* has clearly dispersed across oceanic distances to islands no more than five million years old. Previous taxonomy of *Korthalsella* (Danser, 1937, 1940) had the highest diversity, both of species and sections, in Hawaii. High diversity at the sectional level suggests the genus had a longer history in Hawaii than elsewhere, which is biogeographically improbable. The previous alignment of *Korthalsella* caused species found on different continents to be included in the same section, whereas some species on one island were put in different sections. Although not impossible, such a situation also seems improbable.

Korthalsella is a good model system to explore several important topics in evolutionary biology. It provides an example of the phylogenetic resolving power of combining independent data sets and especially of a comparative molecular/morphological approach. The interaction among ecology, physiology, and systematics is exceptionally clear in the genus, especially in potentially host-influenced branch shape and size variation. Most importantly, *Korthalsella* exemplifies the interdependence between critical character analysis and species delimitation, and ultimately phylogeny itself, which rests upon the foundation provided by our understanding of characters and species.

LITERATURE CITED

ASHWORTH, V. E. 1996. Phylogenetic relationships within the mistletoe genus *Phoradendron* (Viscaeae). *American Journal of Botany* 83: (Supplement Abstract) 387.

BALDWIN, B. G. 1993. Molecular phylogenetics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. *American Journal of Botany*. 80: 222–238.

———. M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, AND M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.

BARLOW, B. A. 1983. A revision of the Viscaceae of Australia. *Brunonia* 6: 25–57.

———. 1981. The Australian flora: its origin and evolution. *Flora of Australia* 1: 25–75.

BOGLER, D. J., AND B. B. SIMPSON. 1996. Phylogeny of Agavaceae based on ITS DNA sequence variation. *American Journal of Botany* 83: 1225–1235.

BUCKLER, E., AND T. P. HOLTSFORD. 1995. *Zea* ribosomal repeat evolution and substitution patterns. *Molecular Biology and Evolution* 13: 623–632.

COX, A. 1997. PaupGap. Computer program available from A. Cox, Jodrell Laboratory, Royal Botanic Gardens Kew, London.

DANSER, B. H. 1937. A revision of the genus *Korthalsella. Bulletin du Jardin Botanique Buitenzorg* 14(2): 115–159.

———. 1940. A Supplement to the revision of the genus *Korthalsella. Bulletin du Jardin Botanique Buitenzorg* 16: 329–342.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* 19: 11–15.

ENGLER, A. 1897. *In* Engler and Prantl, Die Natürliche Pflanzenfamilien, Nachträge II-IV. p. 138.

FARRIS, J. S. 1969. A successive approximations approach to character weighting. *Systematic Zoology* 18: 374–385.

———, V. A. ALBERT, M. KÄLLERSJÖ, D. LIPSCOMB, AND A. G. KLUGE. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FITCH, W. M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* 20: 406–416.

FREEMANN, C. E. AND G. R. YBARRA. 1996. The utility of chloroplast *trnL* intron DNA sequences in inferring phylogeny within *Keckiella* (Scorphulariaceae). *American Journal of Botany* (supplement) 83: 590 (Abstract).

GIELLY, L., AND P. TABERLET. 1994. The use of chloroplast DNA to resolve plant phylogenies: noncoding versus *rbcL* sequences. *Molecular Biology and Evolution* 11: 769–777.

———. 1996. A phylogeny of the European gentians inferred from chloroplast *trnL* (UAA) intron sequences. *Botanical Journal of the Linnaean Society* 120: 57–75.

———. 1996. Chloroplast DNA sequencing to resolve plant phylogenies between closely related taxa. *In* T. B. Smith and R. K. Wayne [eds.], Molecular genetic approaches in conservation, 143–153. Oxford University Press, Oxford.

HAHN, W. J., AND K. J. SYTSMA. 1993. Nuclear ribosomal internal transcribed spacer phylogenetics of the genus *Clarkia* (Onagraceae). *American Journal of Botany* (Supplement) 80: 152 (Abstract).

HEDIN, M. C. 1997. Molecular phylogenetics at the population/species interface in cave spiders of the southern Appalachians (Araneae: Nesticidae: *Nesticus*). *Molecular Biology and Evolution* 14: 309–324.

LECOMTE, P. H. 1916. Le genre *Korthalsella. Bulletin du Museum d'Histoire Naturelle de Paris* 22: 260–267.

MATZURA, O. 1995. RNAdraw v. 1.1. Computer program available from the author, ole@mango.mef.ki.se, Karolinska Institut, Stockholm, Sweden.

MERRILL, E. D. 1916. *Korthalsella, Bifaria* and *Pseudixus. Botanical Magazine Tokyo*, 30: 66–68.

MOLVRAY, M. 1990. A revision of *Korthalsella* (Viscaceae). Ph.D. dissertation, Tulane University, New Orleans, LA.

———. 1997. A synopsis of *Korthalsella* (Viscaceae). *Novon* 7: 268–273.

MULLIS, K. B., AND F. A. FALOONA. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymology* 155: 335–350.

NICKRENT, D. L., K. P. SCHUETTE, AND E. M. STARR. 1994. A molecular phylogeny of *Arceuthobium* (Viscaceae) based on nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany*. 81: 1149–1160.

O'HARA, R. J. 1993. Systematic generalization, historical fate, and the species problem. *Systematic Biology* 42: 231–246.

OLMSTEAD, R. G. 1995. Species concepts and plesiomorphic species. *Systematic Botany* 20: 623–630.

———, B. BREMER, K. M. SCOTT, AND J. D. PALMER. 1993. A molecular systematic analysis of the Asteridae *sensu lato* based on *rbcL* sequences. *Annals of the Missouri Botanical Garden* 80: 700–722.

SMITH, A. C. 1985. *Flora Vitiensis Nova* Vol. 3, p. 747. Pacific Tropical Botanical Garden: Lawai, Kauai, HI.

STEVENS, P. F. 1991. Character states, morphological variation, and phylogenetic analysis: a review. *Systematic Botany* 16: 553–583.

SWOFFORD, D. L. 1993. PAUP: phylogenetic analysis using parsimony. Version 3.1.1. Illinois Natural History Survey, Champaign.

TABERLET, P. T., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

THOMPSON, J. D., D. G. HIGGINS, AND T. J. GIBSON. 1995. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position, specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680.

TOUW, M. (NOW MOLVRAY). 1984. Preliminary observations on *Korthalsella* (Viscaceae) with special reference to vascular patterns. *Blumea* 29: 525–545.

VAN TIEGHEM, P. 1896. Sur le groupement des éspèces en genres dans les Ginalloées, Bifariées, Phoradendrées et Viscées, quatre tribus de la famille des Loranthacées. *Bulletin du Societé Botanique de France* 43: 161–194.

WHITE, T. L., T. BRUNS, S. LEE, AND J. TAYLOR. 1991. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols, 315–322. Academic Press, San Diego, CA.

WOLFE, A. D., AND W. J. ELISENS. 1995. Evidence of chloroplast capture and pollen-mediated gene flow in *Penstemon* sect. *Peltanthera* (Scrophulariaceae). *Systematic Botany* 20: 395–412.