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A Phylogenetic Analysis of *Doronicum* (Asteraceae, Senecioneae) Based on Morphological, Nuclear Ribosomal (ITS), and Chloroplast (*trnL-F*) Evidence

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A phylogenetic analysis of the Old World genus *Doronicum* (26 species, 4 subspecies) based on sequence data of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA, the chloroplast spacer *trnL-F*, and morphology is presented. Congruence among the three data sets was explored by the computing of several indices, all of which suggest homogeneity between only the two molecular matrices. We argue that the morphological data set contains poor phylogenetic signal and advocate simultaneous analysis of the three data sets (total evidence approach) so that morphological characters are tested for homology by congruence with molecular data. The resulting phylogenetic hypothesis allows several well-supported conclusions including the placement of a Corsican endemic (*D. corsicum*), sister to the remainder of the genus, and the inference that an early southern European or Mediterranean diversification took place in the genus. Shifts in morphological characters (e.g., homocarpy to heterocarpy) are confirmed to have evolved several times. Results from comparative studies of sequence data of the chloroplast gene *ndhF* support inclusion of *Doronicum* in tribe Senecioneae.
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INTRODUCTION

The genus *Doronicum* comprises 26 species and 4 subspecies distributed in Asia, Europe, and north Africa (I. Álvarez, unpublished PhD dissertation). All the members of this genus are perennial, rhizomatous herbs bearing one to several radiate, yellow-flowered capitula. The involucre is composed of two or three rows of similar bracts. The plants occur in open or forested habitats from sea level up to 5000 m in elevation.

Despite old taxonomic misconceptions that allied *Doronicum* to genera as diverse as *Arnica* or *Nannoglottis*, the genus is now unanimously accepted as a member of tribe Senecioneae based on shared morphological, karyological, and chemical characters (Nordenstam, 1977; Jeffrey, 1987; Bremer, 1994).

Although several taxonomic studies circumscribing species from particular regions are available (Widder, 1925; Edmondson, 1973, 1975, 1978; Avetisyan, 1980; Chacón, 1987; Pérez Morales and Penas, 1990; Duvigneaud, 1992; Pérez Morales *et al.*, 1994), there is only one previous comprehensive revision of *Doronicum* (Cavillier, 1907, 1911). This author included a hypothetical phylogenetic scheme for what he considered the core of the genus (his Sect. *Doronicastrum*), to the exclusion of his two monotypic sections, *Soulieastrum* (*D. stenoglossum*) and *Hookerastrum*. The latter section included a species that is currently placed in another genus, in a different tribe (*Nannoglottis hookeri*, tribe Astereae). This classification was based almost exclusively on the shape of the basal leaves. This character is highly variable and thus a substantial portion of the species groupings based on it is likely to be the result of incorrect homology assessments. Cavillier (1911) recognized seven subsections. The species in subsection *Plantaginea* were suggested to be ancestral, having given rise to the rest of the subsections, some of them directly and some through subsection *Grandiflorae*. Based on data presented here, none of Cavillier's subsections are monophyletic.

The main objective of this paper is to present a phylogenetic analysis of the genus based on three different data sets: morphology, the internal transcribed spacers (ITS) of the nuclear ribosomal DNA, and the chloroplast spacer region *trnL-F*. In addition, we present a preliminary assessment of the placement of *Doronicum* within the family based on the chloroplast gene *ndhF*. A few studies investigating relationships among genera of Asteraceae have sampled members of the Senecioneae but none of them have included *Doronicum* (Jansen *et al.*, 1990, 1991; Kim *et al.*, 1992; Kim and Jansen, 1995; Kadereit and Jeffrey, 1996).

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Integrating data from different sources into a single reliable estimate of phylogenetic relationships within a group of organisms is one the most intensively debated issues in phylogeny reconstruction (Bull *et al.,* 1993; Eernisse and Kluge, 1993; Patterson *et al.,* 1993; Olmstead and Sweere, 1994; De Queiroz *et al.,* 1995; Farris *et al.,* 1995; Huelsenbeck *et al.,* 1996; Wiens, 1998; Slowinski and Page, 1999). However, there is no general agreement as to how such integration should be done. Some authors, representing the "total evidence" (character congruence, simultaneous analysis) approach, argue that all available data should be combined and analyzed simultaneously (Kluge and Wolf, 1993; Nixon and Carpenter, 1996). Other authors maintain that data from different sources should not be combined but should be independently analyzed (Miyamoto and Fitch, 1995), the latter representing the consensus approach (taxonomic congruence, partitioned analysis). A third, intermediate approach advocates merging the data into a single matrix only if there is evidence that the various matrices do not contain serious incongruence, i.e., that they do not represent different branching histories or that they have not been affected by different evolutionary mechanisms (Bull *et al.,* 1993; Johnson and Soltis, 1998).

In this paper, incongruence among the three different data sets is examined by use of several indices. Despite the fact that the two molecular data sets are incompatible with the morphological data set, we argue that the best solution for handling the three data sets is to combine them all into a single matrix. We discuss the reasons that justify our approach, including the apparently low phylogenetic signal contained in the morphological data and the homogeneity between two molecular markers with different modes of inheritance.

MATERIAL AND METHODS

Plant Material and Data Sets

Forty-five specimens representing the 26 species and 4 subspecies accepted in *Doronicum* and two outgroups (*Ligularia sibirica* and *Tussilago farfara*) were sampled (Table 1). Sequences from these specimens were included in the separate matrices of the ITS1 and ITS2, each containing more than one sample for each of several species, and are hereafter called the ITS extended matrices. To allow comparison and combination of the ITS data with both the *trnL-F* and the morphological data, the original extended ITS matrices were trimmed to include only 1 ITS sequence per taxon and combined to cover the whole ITS region (ITS1 + ITS2, the 5.8S excluded). This matrix is hereafter referred to as the ITS reduced matrix (Table 1). In those instances in which more than 1 specimen from the same species was sampled for the three markers, 1 of the specimens was arbitrarily chosen (Table 1). This was the case for both *D. grandiflorum* and *D. austriacum*. Based on the same criterion of use of the same samples in the three data sets (ITS, *trnL-F*, morphology) to allow combination, 3 species represented in the ITS extended matrices were removed from the ITS reduced matrix. These are *D. carpaticum, D. cataractarum*, and *D. macrolepis* (synonym of *D. macrophyllum* in our taxonomic treatment), for which we repeatedly failed to amplify the *trnL-F*. In contrast, a representative sample of *D. turkestanicum*, from Xinjiang (China), was included in the reduced matrix despite our taxonomic treatment which considers this taxon a synonym of the central Asian species *D. falconeri* (Table 1). With these adjustments, the resulting reduced matrix contains 30 sequences of *Doronicum* plus the two outgroup species.

In addition to *trnL-F*, another chloroplast region was assayed for variation within *Doronicum*. The *trnT-L* spacer (Taberlet *et al.,* 1991) was sequenced in samples of 14 species of *Doronicum*. Although the sequences ranged in length from 529 to 545 bp, only five positions were variable, and they have no phylogenetic value as they represent autapomorphies. In addition, there are two regions that contain poly(T) and poly(A) stretches and require gaps of different length for alignment. This marker was eventually abandoned because of lack of informative variation.

The morphological data set has been elaborated on the basis of a comprehensive taxonomic revision of the genus and thus is based on the study of ca. 4300 herbarium specimens (I. Álvarez, unpublished PhD dissertation). Features that are autapomorphic, quantitative, continuous, plastic, intraspecifically polymorphic, and seemingly nonhomologous were trimmed from a raw matrix containing 51 characters. As a result, the morphological data set contains only 12 characters (Table 2).

Ligularia sibirica and *Tussilago farfara*, both members of the Old World Senecioneae–Tussilagininae (Bremer, 1994), were chosen as outgroups. Several attempts were made to align the ITS sequences of *Doronicum* with those of other genera of Senecioneae suggested in the literature as closely related to the genus. Sequences from other tribes were also examined. These include *Psacalium, Roldana*, and *Tephroseris* in the Tussilagininae, *Gynura, Packera*, and *Pericallis* in the Senecioninae, and *Arnica* (Helenieae), *Calendula* (Calenduleae), and *Myriactis* (Astereae). These sequences were provided by J. Panero and J. Francisco-Ortega (*Psacalium, Tephroseris, Gynura, Packera, Roldana, Pericallis*) and retrieved from GenBank (*Arnica, Calendula, Myiriactis*). Unambiguous alignment of any of those sequences with *Doronicum* by visual inspection or with CLUSTALW was not possible, thus suggesting distant relationships to our study genus.

TABLE 1

Specimens Sampled in This Study

Taxon	Geographic origin[a]	Marker	ACRONYM[b]	GenBank Accession No.
D. altaicum	Siberia, Altai, river Surijza	ITS1, ITS2, *trnL-F*	**ALTA**	AJ400002, AJ400048, AJ400104
D. atlanticum	Algeria, Djurdjura, Tala Guilef	ITS1, ITS2, *trnL-F*	**ATLA**	AJ400003, AJ400049, AJ400105
D. austriacum	Greece, Makedonien, Nomos Florinis	ITS1, ITS2, *trnL-F*	**AUS1**	AJ400004, AJ400050, AJ400106
D. austriacum	Poland, Bieszczady	ITS1, ITS2	AUS2	AJ400005, AJ400051
D. briquetii	China, Tibet–Qinghai, Tha Chu valley	ITS1, ITS2, *trnL-F*	**BRIQ**	AJ400006, AJ400052, AJ400107
D. cacaliifolium	Turkey, Isauria, Vil Antalya	ITS1, ITS2, *trnL-F*	**CACA**	AJ400007, AJ400053, AJ400108
D. carpaticum	Romania, Comit, Fogaras	ITS1, ITS2	CRTC	AJ400008, AJ400054
D. carpetanum subsp. *carpetanum*	Spain, Madrid, Sierra de Guadarrama	ITS1, ITS2, *trnL-F*	**CARP**	AJ400009, AJ400055, AJ400109
D. carpetanum subsp. *diazii*	Spain, Soria, Picos de Urbión	ITS1, ITS2, *trnL-F*	**DIAZ**	AJ400014, AJ400060, AJ400113
D. carpetanum subsp. *kuepferi*	Spain, Ávila, Sierra de Béjar	ITS1, ITS2, *trnL-F*	**KUE1**	AJ400024, AJ400070, AJ400120
D. carpetanum subsp. *kuepferi*	Spain, Ávila, Sierra de Béjar	ITS1, ITS2	KUE2	AJ400025, AJ400071
D. carpetanum subsp. *kuepferi*	Spain, Ávila, Sierra de Gredos	ITS1, ITS2	KUE3	AJ400026, AJ400072
D. carpetanum subsp. *pubescens*	Spain, León, Cordillera Cantábrica	ITS1, ITS2, *trnL-F*	**PUBE**	AJ400039, AJ400085, AJ400095
D. cataractarum	Austria, Koralpe	ITS1, ITS2	CATA	AJ400010, AJ400056
D. cavillieri	China North-central, Lianhuashan	ITS1, ITS2, *trnL-F*	**CAVI**	AJ400044, AJ400090, AJ400100
D. clusii	Italy, Bormio, Passo dello Stelvio	ITS1, ITS2, *trnL-F*	**CLUS**	AJ400011, AJ400057, AJ400110
D. columnae	Italy, Trento, Passo Gardena	ITS1, ITS2, *trnL-F*, *ndhF*	**COLU**	AJ400012, AJ400058, AJ400111, AJ276493
D. corsicum	Corsica, mount Renoso	ITS1, ITS2, *trnL-F*	**CORS**	AJ400013, AJ400059, AJ400112
D. dolichotricum	Caucasus, Transcaucasus, Gruziya	ITS1, ITS2, *trnL-F*	**DOLI**	AJ400015, AJ400061, AJ400114
D. falconeri	Pakistan, Nanga Parbat	ITS1, ITS2, *trnL-F*	**FALC**	AJ400016, AJ400062, AJ400115
D. falconeri (sub. *D. turkestanicum*[c])	China, Xinjiang, Uygur Zizhiqu, Tien Shan	ITS1, ITS2, *trnL-F*	**TURK**	AJ400045, AJ400091, AJ400101
D. glaciale	Italy, Trento, Col Rodella,	ITS1, ITS2, *trnL-F*	**GLAC**	AJ400017, AJ400063, AJ400116
D. grandiflorum	Spain, Asturias, Picos de Europa	*trnL-F*	GRA1	AJ400117
D. grandiflorum	France, Pyrenees, Vallée d'Ossoue	ITS1, ITS2	**GRA2**	AJ400018, AJ400064
D. grandiflorum	Andorra, Pyrenees, circo de Pessons	ITS1, ITS2	GRA3	AJ400019, AJ400065
D. grandiflorum	Spain, Huesca, Pyrenees, peak Aneto	ITS1, ITS2	GRA4	AJ400020, AJ400066
D. grandiflorum	Spain, Girona, Pyrenees, peak Noufonts	ITS1, ITS2	GRA5	AJ400021, AJ400067
D. haussknechtii	Turkey, Giresun, Karagöl	ITS1, ITS2, *trnL-F*	**HAUS**	AJ400022, AJ400068, AJ400118
D. hungaricum	Romania, Oltenia, Craiova	ITS1, ITS2, *trnL-F*	**HUNG**	AJ400023, AJ400069, AJ400119
D. kamaonense	Jammu-Kashmir, Sind Valley	ITS1, ITS2, *trnL-F*	**KAMA**	AJ400041, AJ400087, AJ400097
D. macrophyllum subsp. *macrophyllum* (sub *D. macrolepis*[c])	Turkey, Boejuk dere supra Artabir	ITS1, ITS2	MCRL	AJ400027, AJ400073
D. macrophyllum subsp. *macrophyllum*	Caucasus, Transcaucasus, Azerbaydzhan	ITS1, ITS2, *trnL-F*	**MACR**	AJ400028, AJ400074, AJ400121
D. macrophyllum subsp. *sparsipilosum*	Turkey, Çankiri, Ilgaz Kastamonu	ITS1, ITS2, *trnL-F*	**SPAR**	AJ400042, AJ400088, AJ400098
D. maximum	Turkey, Erzurum	ITS1, ITS2, *trnL-F*	**MAXI**	AJ400029, AJ400075, AJ400122
D. oblongifolium	Caucasus, Transcaucasus, Armeniya	ITS1, ITS2, *trnL-F*	**OBLO**	AJ400030, AJ400076, AJ400123
D. orientale	Greece, Arcadia, mount Melanon	ITS1, ITS2, *trnL-F*	**ORIE**	AJ400031, AJ400077, AJ400124
D. pardalianches	Spain, Huesca, sierra de Guara	ITS1, ITS2, *trnL-F*	**PAR1**	AJ400032, AJ400078, AJ400125
D. pardalianches	Spain, Lleida, Sierra del Cadi	ITS1, ITS2	PAR2	AJ400033, AJ400079
D. plantagineum	Spain, Almería, sierra de Gádor	ITS1, ITS2, *trnL-F*	**PLA1**	AJ400034, AJ400080, AJ400094
D. plantagineum	Spain, Murcia, Moratalla	ITS1, ITS2	PLA2	AJ400035, AJ400081
D. plantagineum	Spain, Asturias, Cordillera Cantábrica	ITS1, ITS2	PLA3	AJ400036, AJ400082
D. plantagineum	Spain, Guadalajara, Cantalojas	ITS1, ITS2	PLA4	AJ400037, AJ400083
D. plantagineum	Spain, Montes de Toledo	ITS1, ITS2	PLA5	AJ400038, AJ400084
D. reticulatum	Turkey, Bursa, Belvedere	ITS1, ITS2, *trnL-F*	**RETI**	AJ400040, AJ400086, AJ400096
D. stenoglossum	China, Tibet-Qinghai, Dari Xian	ITS1, ITS2, *trnL-F*	**STEN**	AJ400043, AJ400089, AJ400099
Ligularia sibirica	France, Pyrenees, Capcir	ITS1, ITS2, *trnL-F*	**LIGU**	AJ400046, AJ400092, AJ400102
Tussilago farfara	Spain, Cuenca, El Cubillo	ITS1, ITS2, *trnL-F*	**TUSS**	AJ400047, AJ400093, AJ400103

[a] Main geographic subdivisions follow Hollis and Brummitt (1992).
[b] Samples included in the ITS reduced matrix (see text) are in boldface.
[c] In the trees and appendixes.

DNA Extraction, Amplification, and Sequencing

Total DNA was extracted both from living plants and from herbarium material following the CTAB method of Doyle and Doyle (1987). A sample of 30–50 mg of leaf material from each of the specimens was used.

Amplification of the whole ITS region (ITS1 + 5.8S + ITS2) was performed with primers ITS7A (White *et al.*, 1990) slightly modified by J. L. Panero and A. Plovanovich-Jones (pers. comm.) and ITS4 (White *et al.*, 1990). However, in most cases ITS1 and ITS2 spacers were amplified separately with primers ITS7A and ITS2 for ITS1 and with ITS3 and ITS4 for ITS2. The *trnT-L* spacer region was amplified with primers a and b, and *trnL-F* was amplified with primers e and f (Taberlet *et al.*, 1991). For ITS, the PCR cocktail followed Kim and Jansen (1994). For *trnT-L* and *trnL-F*, we followed Taberlet *et al.* (1991). When a first amplification of ITS was not adequate, 5–10% of dimethyl sulfoxide was added to the cocktail in a new reaction. The standard PCR program for ITS amplification began with 1 min

TABLE 2

Characters Included in the Morphological Data Set

1. Rhizome hairy on the nodes (1); rhizome glabrous on the nodes (0).
2. Rhizome with unequal internodes, thin and long internodes alternating with short and wide internodes (1); rhizome with internodes uniform in length and width (0).
3. Rhizome with buds (1); rhizome without buds (0).
4. Basal leaves with three main nerves converging toward the apex (triplinerved) (1); basal leaves with other venation pattern (0).
5. Basal leaves with pinnate–reticulate venation (1); basal leaves with other venation pattern (0).
6. Scapiform stems (most leaves basal) (1); stems leafy (0).
7. Tall herbs with one to four very large reticulately veined caulinar leaves and several to many capitula (1); other architecture (0).
8. Involucral bracts ciliate (1); involucral bracts not ciliate (0).
9. Homocarpic capitula (1); heterocarpic capitula (marginal cypselae thicker and epappose) (0).
10. Cypselae with glands (1); cypselae without glands (0).
11. Involucre with shorter supplementary bracts at base (0); involucre without shorter supplementary bracts (1).
12. Involucral bracts in two to three rows (1); involucral bracts in one row (0).

at 94°C, 40 s at 92°C, 40 s at 50°C, 90 s at 75°C, followed by 29 cycles of 40 s at 92°C, 40 s at 50°C, and 90 s at 75°C. The program was completed with 5 min at 75°C and then held at 4°C. In many cases this protocol was modified to make it suitable for specific samples. For *trnL-F* and *trnT-L* amplification, we followed the PCR cycle profile proposed by Taberlet *et al.* (1991). The PCR products were purified either by centrifugation with Millipore Ultrafree-MC 30,000 NMWL cellulose filters or with a Boehringer Mannheim PCR Clean Up Kit. In both cases we followed manufacturer's recommendations.

ITS amplification of recalcitrant samples or those for which DNA was isolated from herbarium specimens required additional manipulation and purification via cloning to obtain readable sequences. Approximately 48% of the ITS sequences used in this study were cloned. Ligation and transformation reactions were performed with the Invitrogen TOPO TA Cloning Kit Version D. Transformed bacteria were spread on LB plates containing ampicillin (100 mg/ml), and colonies with inserts were raised on new plates. Approximately 10 of these colonies were screened via PCR for the presence of the ITS insert with primers ITS5 and ITS4. The PCR protocol followed with the selected colonies was the same as that above except for the thermocycler program. After a 10-min hot start at 94°C, *Taq* polymerase was added. An initial step of 5 min at 57°C was followed by 30 cycles at 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min. Colonies with desired inserts were grown in LB liquid medium with ampicillin for approximately 12 h at 250 rpm and 37°C. Plasmids were harvested with the Promega Wizard Plus SV Minipreps kit under the specified protocol. To amplify cloned fragments, a PCR was prepared with 1 μl of plasmid and universal primers M13F and M13R for the plasmid. PCR products were digested with restriction enzyme *Spe*I (New England BioLabs) to discriminate for desired ITS sequence as all *Doronicum* species have a conserved *Spe*I site in their ITS1 region.

For directly sequenced samples, nucleotide sequences of both strands were determined from PCR fragments with the dideoxy chain termination method (Sanger *et al.,* 1977). The sequencing primers used for amplification were the same as those above. Standard protocols of the manufacturer for *Taq* DNA polymerase-initiated cycle sequencing reactions with fluorescently labeled dideoxynucleotide terminators (Applied Biosystems) were followed. For cloned sequences, purified PCR products were sequenced with a dye terminator cycle-sequencing ready-reaction kit (Perkin–Elmer, Applied Biosystems Division). Sequencing products were separated and analyzed on an ABI 377 Automated DNA sequencer (Perkin–Elmer, Applied Biosystems Division) at the Institute of Cellular and Molecular Biology of the University of Texas at Austin. Chromatograms were examined and edited with Edit-View (Applied Biosystems) and Sequencher (Gene Codes Corp., Ann Arbor, MI). All the sequences were recorded in both strands with a 100% overlap. The 125 sequences have been submitted to the EMBL database (Table 1).

Data Analysis

Alignment of the sequences was performed manually (Appendixes 1 and 2). It was relatively easy for *Doronicum* but somewhat more challenging for outgroups in variable domains of the ITS. Pairwise distance values between sequences were calculated with the Kimura two-parameter model as implemented by PAUP 4.0b2 for Power PC (Swofford, 2000) for all data sets.

Phylogenies were reconstructed by the application of the Fitch parsimony criterion (unordered character-states, equal weights) as implemented by PAUP. Searches for the most parsimonious trees (m.p.t.) in the analysis of the different data sets were performed with the heuristic algorithm and the TBR and MULPARS options in effect. ACCTRAN option was used for character optimization. To attempt to locate trees on multiple islands of most parsimonious trees (Maddison, 1991), 10 replicate searches with random taxon addition were performed. Gaps were treated as

missing data. Bootstrap analysis was performed to assess relative branch support from the data. In half of the analyses, bootstrap values were obtained from PAUP with 100 replicates, each with 10 random taxon addition and the TBR and MULPARS options in effect. However, we compared these bootstrap values with those obtained by the fast bootstrap option in PAUP (10,000 replicates) and found that they were very similar. This is consistent with an empirical study by Mort *et al.* (2000) that compared fast bootstrap approaches with those obtained by branch-swapping. Thus, the remaining bootstrap analyses of the morphological matrix, the *trnL-F*, and the ITS1 and ITS2 extended matrices were calculated with the fast bootstrap method. A successive weighting procedure (Farris, 1969) was followed in PAUP to try to reduce the effects of the most homoplastic characters in the morphological data set. We reweighted characters with the rescaled consistency index until the weights assigned to each character were identical in two successive iterations, thus resulting in the same number of m.p.t. and length. To check for consistency in the topology with other methods of phylogeny reconstruction, a neighbor-joining tree was constructed based on pairwise sequence distances within the ITS reduced matrix.

Congruence among Data Sets

To examine the feasibility of integrating the three data sets into a single matrix for estimating phylogenetic relationships within *Doronicum*, all possible combinations of the three data sets were analyzed with parsimony and the results compared. In these independent and combined analyses, the data set used for the ITS marker was the reduced matrix (32 taxa).

Several indices that assess the degree of congruence between data sets for the same organisms were also calculated (Johnson and Soltis, 1998). We computed two topological congruence indices. The partition metric (PM) measures the rearrangements needed to transform one of the two trees that we compared into the other (Robinson and Foulds, 1981). The other topological index used is the greatest agreement subtree metric, D_1 (Kubicka *et al.,* 1995). It measures the number of taxa that we have to prune in two trees to arrive at a minimum topology in which the two trees agree. Both topological indices were computed in PAUP under the "tree to tree distances" menu, with the "symmetric-difference distance" and "agreement metric D1" commands, respectively. To maintain a reasonable number of pairwise comparisons with the molecular trees, only the 52 m.p.t. obtained after three rounds of the successive weighting procedure on the morphological matrix were considered in these topological indices.

To address the question of how much conflicting phylogenetic information exists between two data sets rather than how different are two phylogenetic trees derived from them, we calculated other indices that are more directly based on the data sets. The incongruence metric of Miyamoto, I_M (Miyamoto in Swofford, 1991), calculates the extra homoplasy needed to explain each data set on the topology recovered from the alternative data set. For the ITS/*trnL-F* comparison, this means the number of homoplastic steps required to explain the ITS data set on the most parsimonious tree(s) recovered from the *trnL-F* data set, plus the number of homoplastic steps required to map the *trnL-F* data on the most parsimonious tree(s) recovered from the ITS data. The incongruence metric of Mickevich and Farris (I_{MF}) (Mickevich and Farris, 1981) is similar but instead of mapping each data set on the other data set, it uses the number of homoplastic steps required by each individual data set to explain the shortest tree recovered from the combined matrix. Both indices were computed with the length and fit measures given by PAUP and following the indications by Johnson and Soltis (1998).

We also applied a significance test for heterogeneity that addresses the problem of how large a character congruence index needs to be to indicate a serious conflict between data sets. We used the test proposed by Farris *et al.* (1995) based on the incongruence length difference of Mickevich and Farris (I_{MF}). This test is implemented in PAUP as the "partition homogeneity test" and referred to as HT_F by Johnson and Soltis (1998). The I_{MF} metric is computed for a number of random partitions of the combined data set, each partition consisting of two subsets of the same size as the two data sets. When 95% or more of those random partitions show an I_{MF} smaller than the original, we reject the null hypothesis and conclude that the data sets are significantly heterogeneous. This test was computed by execution of the "partition homogeneity test" command in PAUP on each of the three combined matrices (ITS + *trnL-F*, ITS + morphology, *trnL-F* + morphology).

Placement of Doronicum

For a preliminary assessment of the placement of *Doronicum* within the Senecioneae, the chloroplast gene *ndhF* was used. Because the last 800 bp of the gene are the most variable (Kim and Jansen, 1995), we initially sequenced this portion in two morphologically distinct species (*D. columnae* and *D. stenoglossum*), placed in different clades in the ITS trees. For amplification, we used primers 1201 and 1417 kindly provided by R. K. Jansen and based on the coding sequence of tobacco (Shinonzaki *et al.,* 1986). The sequences in both species are identical, and we therefore sequenced the entire gene only for *D. columnae* using primers 52 and 590. The 2.2-kb sequence was aligned with a representative selection of the Asteraceae matrix generated by Kim and Jansen (1995). The matrix that we analyzed included 2238 characters and 59 terminals, 5 of them outside Asteraceae (*Boopis,*

Campanula, Dampiera, Menyanthes, and *Scaevola*) which were used as outgroups. A parsimony heuristic search, with the same options as for the analysis within *Doronicum*, was conducted with PAUP.

RESULTS

ITS Sequences

The total aligned length of the ITS sequence, excluding the 5.8S gene, is 496 positions, corresponding to 265 bp in ITS1 and 231 bp in ITS2. Within *Doronicum*, ITS1 is 255–260 bp (*D. atlanticum* and *D. oblongifolium*), and ITS2 ranges from 210 bp (*D. columnae, D. macrophyllum*) to 214 bp (*D. briquetii* and several others). In all, 12 indels were required for the alignment of the ITS region. In ITS1, 3 are 2 bp in length, and 1 is 3 bp. In ITS2, 4 are 1 bp, 3 are 2 bp, and 1 is 3 bp. Eighty-eight variable positions (33.2%) within *Doronicum* are found in the ITS1 extended matrix, 53 of which are autapomorphic. Sixty-two variable positions (26.8%) within *Doronicum* are found in the ITS2 extended matrix, 33 of which are autapomorphic. When the reduced matrix (ITS1 + ITS2) is considered, the following features are obtained: 144 variable positions (29%) within *Doronicum*, 89 of which are autapomorphic and 55 of which are potentially informative within the ingroup in a parsimony analysis. Four polymorphisms were detected that follow an additive pattern with respect to extant taxa. This feature increases to eight when the ITS1 and ITS2 extended matrices are considered.

ITS pairwise distance values within *Doronicum*, calculated separately for the two spacers, range from 0 to 0.115 in ITS1 and from 0 to 0.13 in ITS2. The greatest distance was found between *D. corsicum* and *D. macrophyllum* subsp. *sparsipilosum* in ITS2. Distances between sequences from the same taxon range from 0 to 0.023 in ITS1 and from 0 to 0.024 in ITS2. In particular, within *D. austriacum* divergence was 0.023 in ITS1 and 0.024 in ITS2, within *D. grandiflorum* it was 0 in ITS1 and ranged from 0 to 0.009 in ITS2, within *D. pardalianches* it was 0 in ITS1 and 0.004 in ITS2, within *D. plantagineum* it was 0 to 0.003 in ITS1 and 0 to 0.014 in ITS2, and within *D. carpetanum* subsp. *kuepferi* it was 0 in both ITS1 and ITS2. When the ITS1 + ITS2 reduced matrix is considered, the distances range from 0.002 to 0.114. The shortest distance between a species of *Doronicum* and the outgroup was displayed by *D. corsicum* (0.26 with respect to *Ligularia sibirica* in the reduced matrix).

Parsimony analysis of ITS1, including all sequences, i.e., 46 terminals from 35 taxa, resulted in 1492 optimal trees (length = 143; CI, excluding autapomorphic characters = 0.68; RI = 0.83). Unambiguous resolution is modest (tree not shown). However, the most strongly supported phylogenetic conclusions reported in this paper are already apparent or suggested in this analysis. These are the monophyly of *Doronicum* relative to the outgroups included, the basal position of *D. corsicum*, the *D. plantagineum* clade, and the relationship between some of the central and eastern Asian taxa.

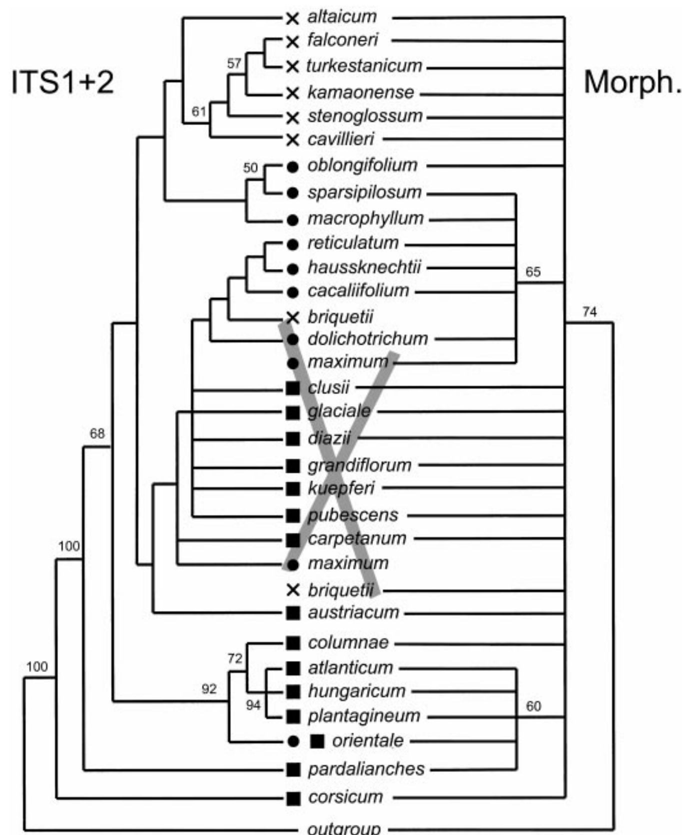

FIG. 1. Congruence between data sets in *Doronicum*: Comparison of the strict consensus trees from the independent analyses of the internal transcribed spacers (ITS) of the nrDNA and a morphological data set. Taxa represented twice to allow comparison of the two trees are linked by gray lines. Bootstrap values above 50% are shown along the branches. Geographical distribution of the taxa analyzed: central and eastern Asia (crosses), southwest Asia (circles), and Europe and northern Africa (squares).

The ITS2 extended matrix (46 sequences from 35 taxa) analyzed under parsimony resulted in 1118 optimal trees (length = 95; CI, excluding autapomorphic characters = 0.71; RI = 0.86). As with ITS1, the strict consensus shows moderate resolution, and the first two of the phylogenetic conclusions mentioned hold (tree not shown).

When the reduced matrix (32 sequences) was analyzed under parsimony, the number of most parsimonious trees dropped to 12, and the resolution increased compared to the independent analyses of the two spacers (Fig. 1) while homoplasy and length increased (length = 373; CI, excluding autapomorphic characters = 0.61; RI = 0.72). Bootstrap support above 90% is limited to the *Doronicum* clade (the ingroup), the same

clade excluding *D. corsicum*, and the clade including *D. plantagineum, D. hungaricum, D. atlanticum, D. columnae*, and *D. orientale* (hereafter called the *D. plantagineum* clade).

The topology of the neighbor-joining tree from the analysis of the ITS reduced matrix is largely consistent with the strict consensus tree from the parsimony analysis of the same matrix (tree not shown). The only difference is the placement of *D. austriacum*, which appears on a deep node in both analyses. In the parsimony tree, *D. austriacum* is sister to a large clade comprising seven European taxa, five southwestern Asian taxa, and a single central Asian taxon, *D. briquetii* (Fig. 1). In the neighbor-joining tree, *D. austriacum* appears as sister to the clade including the six remaining central and eastern Asian taxa (*D. altaicum, D. falconeri, D. turkestanicum, D. kamaonense, D. stenoglossum*, and *D. cavillieri*) and three southwestern Asian taxa (*D. oblongifolium*, and *D. macrophyllum* subsp. *macrophyllum* and subsp. *sparsipilosum*). However, such a difference in topology between the neighbor-joining and the parsimony trees is negligible given that the clades that include *D. austriacum* in both analyses receive bootstrap support <5%.

TrnL-F Sequences

Length of the spacer ranges from 348 bp (*D. pardalianches*) to 381 bp (*D. altaicum*). Total aligned length of the *trnL-F* is 397 bp. It includes six gaps, of which two are 1 bp, and the remaining four are 8, 9, 10, and 25 bp. Twelve positions (3%) are variable within *Doronicum*, of which 9 are autapomorphic and 3 are potentially informative in a parsimony analysis. Pairwise distance values are considerably lower than those in ITS, ranging from 0 to 0.019. The greatest distance is displayed between *D. corsicum* and *D. cacaliifolium*. The parsimony analysis of the *trnL-F* sequences resulted in 18 m.p.t. (length = 28; CI, excluding autapomorphic characters = 0.90; RI = 0.94). Resolution was very poor. In the strict consensus tree, only three clades were recovered: the *Doronicum* clade (100% bootstrap), the *Doronicum* clade excluding *D. corsicum* (91% bootstrap), and a small clade composed of *D. hungaricum* and *D. plantagineum* (63% bootstrap) (see majority rule consensus in Fig. 2).

Morphological Data

Given that 2 of the characters in the morphological data set are synapomorphic for the entire genus, only 10 characters are potentially informative within *Doronicum*. The parsimony analysis resulted in 675 m.p.t. (length = 20; CI = 0.60; RI = 0.88). Resolution was very poor, with the strict consensus recovering only two clades within the ingroup, both of them with bootstrap support of 60% (Fig. 1). The semistrict consensus recovers a few small clades with bootstrap values below 50%. The successive weighting procedure improved

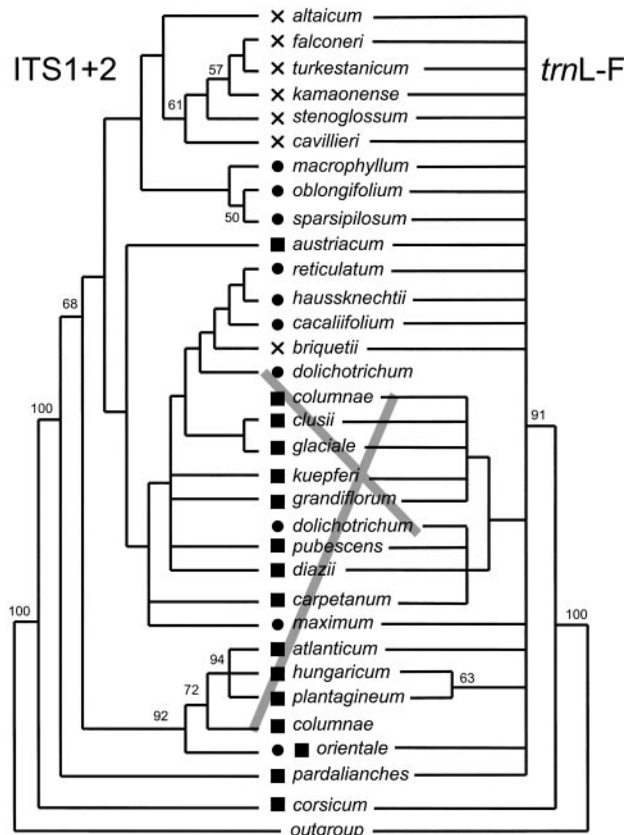

FIG. 2. Congruence between data sets in *Doronicum*: Comparison of the 50% majority rule trees from the independent analyses of the internal transcribed spacers (ITS) of the nrDNA and the chloroplast spacer *trnL-F*. Taxa represented twice to allow comparison of the two trees are linked by gray lines. Bootstrap values above 50% are shown along the branches. Geographical distribution of the taxa analyzed: central and eastern Asia (crosses), southwest Asia (circles), and Europe and northern Africa (squares).

resolution relative to the original matrix after three rounds, particularly at deeper nodes, and CI increased to 0.78. The clades recovered in the strict consensus of the 52 m.p.t. obtained after those three rounds are incompatible with the topology obtained from the ITS reduced matrix. The only exceptions are two three-taxon clades (*D. plantagineum* + *D. hungaricum* + *D. atlanticum* and *D. reticulatum* + *D. haussknechtii* + *D. cacaliifolium*) appearing in the ITS tree (Fig. 1) compatible with polytomies involving five and seven taxa, respectively, in the morphological successively weighted tree. The increase in CI and resolution after successive weighting reveals the importance of homoplasy in the morphological data set (see Discussion).

Combined Data Sets: Congruence Indices and Phylogenetic Analyses

The values obtained in the independent and combined analyses of the three data sets can be seen in Table 3. Consensus from the topologies of the m.p.t.

TABLE 3

Summary Statistics from the Parsimony Analyses of the Independent and Combined Data Sets for *Doronicum*

	Independent			Combined			
	ITS 1+2	*trnL-F*	Morph.	ITS + *trnL-F*	ITS + Morph.	Morph. + *trnL-F*	ITS + *trnL-F* + Morph.
No. of informative characters	117	19	12	136	129	31	148
No. of most parsimonious trees	12	18	675	21	24	172	228
Length	373	28	20	405	409	52	441
C.I.	0.61	0.9	0.6	0.62	0.57	0.69	0.58
R.I.	0.72	0.94	0.88	0.72	0.7	0.86	0.71

from these analyses can be seen in Fig. 1 to 5 with the exception of the *trnL-F* + morphology and the ITS + morphology combined data sets.

Comparisons of the topologies of the cladograms resulting from the independent analyses of the three data sets do not provide clear agreement (Figs. 1–3). The partition metric is low among trees resulting from the same data set (ITS, *trnL-F*, or morphology) and rises considerably when trees from different data sets are compared (Table 4). The normalized values between data sets range from 0.31 to 0.75. Normalized

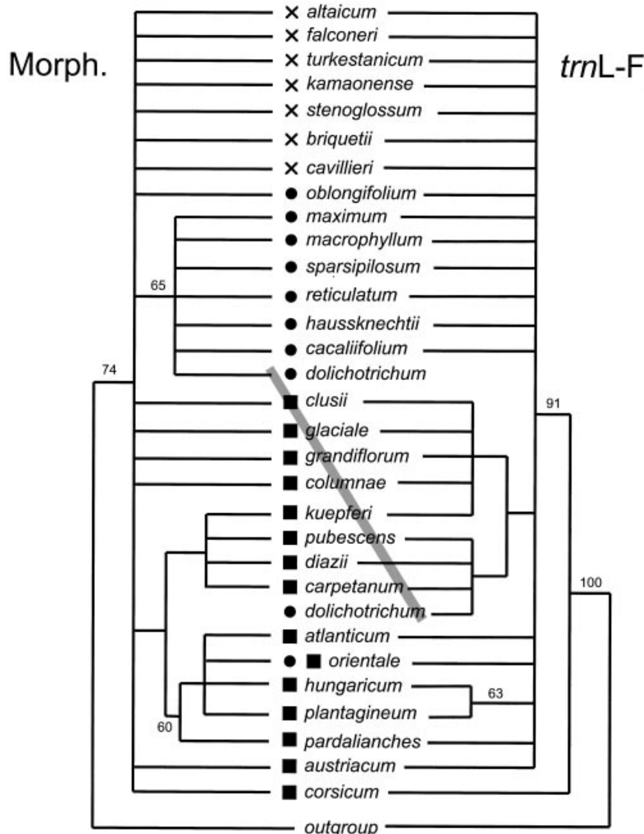

FIG. 3. Congruence between data sets in *Doronicum*: Comparison of the 50% majority rule trees from the independent analyses of the chloroplast spacer *trnL-F* and a morphological data set. A gray line links the only taxon represented twice to allow comparison of the two trees. Bootstrap values above 50% are shown along the branches. Geographical distribution of the taxa analyzed: central and eastern Asia (crosses), southwest Asia (circles), and Europe and northern Africa (squares).

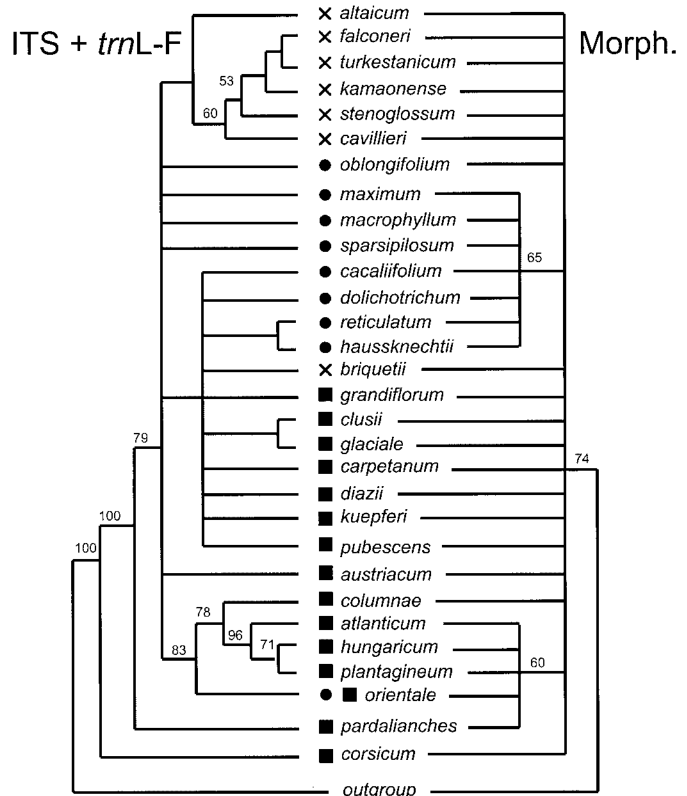

FIG. 4. Congruence between data sets in *Doronicum*: Comparison of the strict consensus tree from the combined analysis of two molecular data sets (nuclear ribosomal ITS and the chloroplast spacer *trnL-F*) and the strict consensus from the independent analysis of a morphological data set. Bootstrap values above 50% are shown along the branches. Geographical distribution of the taxa analyzed: central and eastern Asia (crosses), southwest Asia (circles), and Europe and northern Africa (squares).

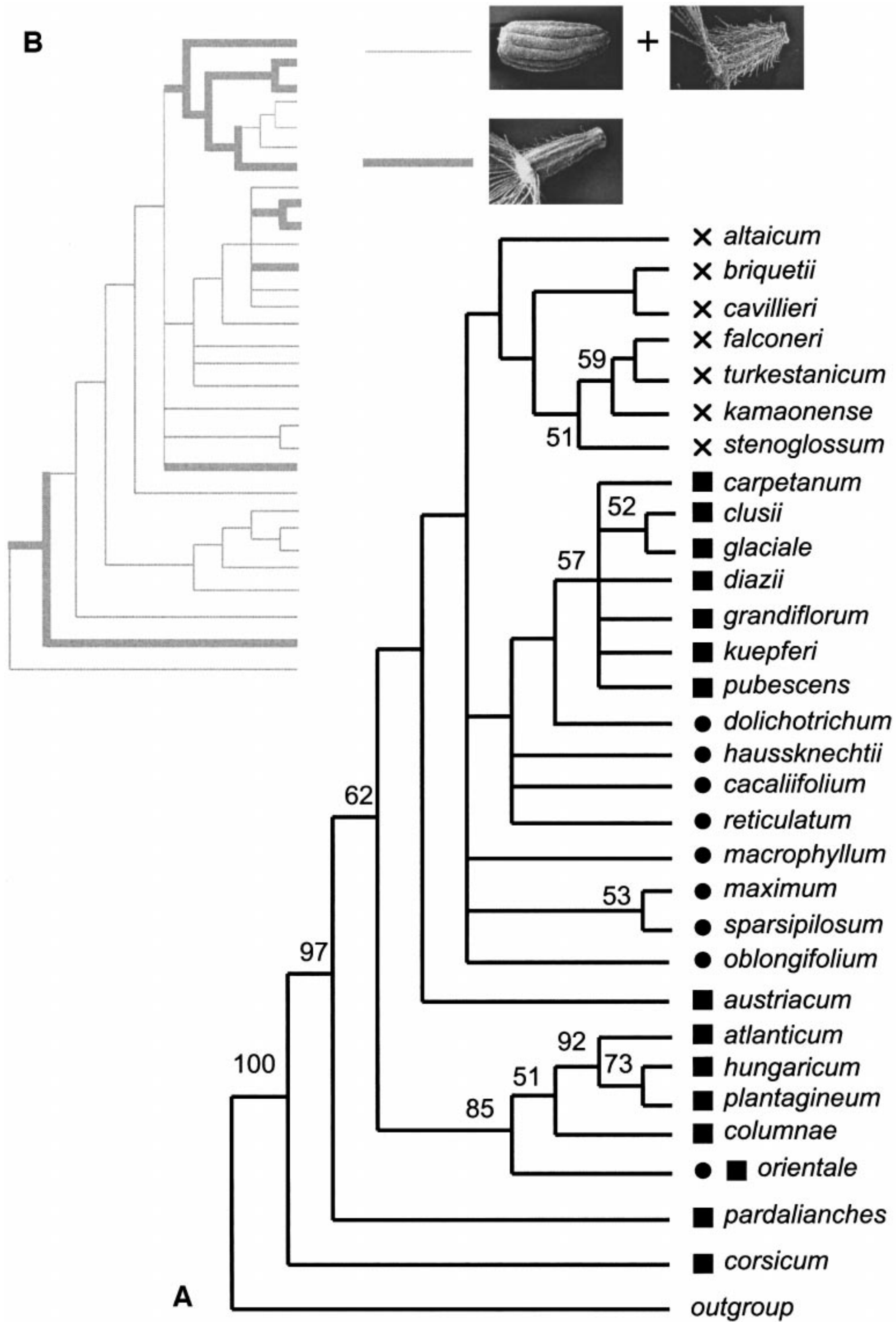

FIG. 5. (A) Strict consensus from 228 most parsimonious trees (length = 441; CI, excluding uninformative characters = 0.58; RI = 0.71) resulting from the combined analysis of three data sets in *Doronicum* (morphology, ITS1 + 2, *trnL-F*). Bootstrap values above 50% are shown along the branches. Geographical distribution of the taxa analyzed: central and eastern Asia (crosses), southwest Asia (circles), and Europe and northern Africa (squares). (B) Mapping of homocarpy (thick branches) onto the same tree.

values of the greatest agreement subtree metric range from 0.17 to 0.31, thereby suggesting more incongruence than does the PM.

Of the two character congruence indices, I_{MF} and I_M, the former is lower than or equal to the latter (Table 4). Both indicate that the most incongruent pair of data

TABLE 4

Measures of Incongruence between Data Sets for *Doronicum*

		Within data sets			Between data sets		
		ITS	*trnL-F*	Morph.	ITS–*trnL-F*	ITS–morph.	*trnL-F*–morph.
PM	Mean	4 (0.93)	4 (0.93)	4 (0.93)	29 (0.50)	37 (0.36)	18 (0.69)
	Range	1–10 (0.98–0.82)	1–8 (0.98–0.86)	1–10 (0.98–0.82)	26–32 (0.55–0.44)	34–40 (0.41–0.31)	14–22 (0.75–0.62)
D_1	Mean	3 (0.89)	6 (0.79)	5 (0.82)	23 (0.2)	22 (0.24)	22 (0.24)
	Range	1–7 (0.96–0.75)	1–9 (0.96–0.69)	1–9 (0.96–0.69)	21–24 (0.27–0.17)	21–24 (0.27–0.17)	20–24 (0.31–0.17)
I_{MF}					4.9%	14.1%	28.5%
I_M					51.9%	50.2%	84.8%
HT_F					0.93	0.01*	0.02*

Note. Topological congruence indices within and between data sets (partition metric, PM; greatest agreement subtree metric, D_1), character congruence indices (index of Mickevich and Farris, I_{MF}; index of Miyamoto, I_M), and a significance test for heterogeneity of the data sets (HT_F) by Farris *et al.* (1995). For PM and D_1, normalized values are indicated in parentheses (0 less similar, 1 most similar topologies). For I_{MF} and I_M, 0% indicates least incongruence and 100% indicates most incongruence.

sets is morphology/*trnL-F* and the most congruent is ITS/*trnL-F*. This result implies that the morphological matrix, containing a number of informative characters more than nine times smaller than that of the ITS matrix, is the most heterogeneous data set of the three.

The significance test for heterogeneity, based on the incongruence length difference of Mickevich and Farris (1981), rejects the null hypothesis of homogeneity between data sets in the two pairs in which the morphological data are involved (morphology/ITS and morphology/*trnL-F*). This test thus confirms that the morphological data are discordant with the two molecular data sets.

Placement of Doronicum within Senecioneae

The parsimony analysis of *ndhF* in a sample of Asteraceae resulted in 4600 optimal trees (length = 2447; CI, excluding autapomorphic characters = 0.44; RI = 0.56). The topology of the strict consensus is the same as that obtained by Kim and Jansen (1995) (Fig. 6). The five genera belonging in the Senecioneae (*Senecio, Lopholaena, Blennosperma, Syneilesis*, and *Doronicum*) form a monophyletic group, although the bootstrap support is not strong. Topological relationships within the clade are identical in all the optimal trees. *Doronicum* is sister to the other four genera.

DISCUSSION

ITS Variability and Intraspecific Sampling

The ITS region is considerably variable within *Doronicum* (55 parsimony-informative characters), implying favorable signal for reconstruction of interspecific relationships. The size of ITS1 in *Doronicum* is within the limits reported by Baldwin *et al.* (1995) for Asteraceae. ITS2 is 6 bp shorter but clearly within the ranges determined by more recent work in Asteraceae (Susanna *et al.,* 1995; Kim *et al.,* 1996, 1999; Francisco-Ortega *et al.,* 1997, 1999; Kornkoven *et al.,* 1998; Panero *et al.,* 1999; Torrell *et al.,* 1999).

Although our sampling does not allow comprehen-

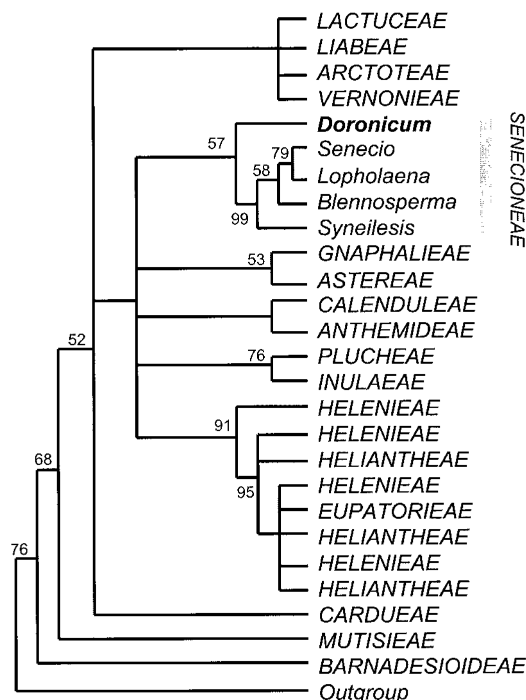

FIG. 6. Abbreviated strict consensus from 4600 most parsimonious trees resulting from the analysis of the chloroplast gene *ndhF* in a sample of the Asteraceae (59 terminal taxa, length = 2447; CI, excluding uninformative characters = 0.44; RI = 0.56). Bootstrap values above 50% are shown along the branches. The data matrix is a subset of that in Kim and Jansen (1995) to which a sequence from *Doronicum* has been added.

sive assessment of the intraspecific level of ITS variation, some conclusions can be drawn. The main question is whether or not intraspecific variation is sufficient, given limited sampling (the rule in systematically and phylogenetically oriented studies), to cause errors in reconstructing the species phylogeny. We cannot exclude this possibility for certain taxa, mostly Asian, that are included in unresolved clades and for which only one sequence is available. Nevertheless, such a possibility is minimized in those species for which we have more than one molecular sample, once the intraspecific variation detected in them and its effects are examined.

In our study, more than 1 sequence per species or subspecies was obtained for six different taxa. This sampling resulted in 12 additional sequences, mostly from Europe, included in only the separate analyses of ITS1 and ITS2. The intrataxon variability detected in those sequences does not have serious effects on the analysis. When the results of the analysis of the ITS1 + ITS2 reduced matrix are compared to the independent analyses of the ITS1 and ITS2 extended matrices, the number of m.p.t. drops dramatically (12 vs 1492 and 1118, respectively). However, the reason for improvement in resolution and reduction in number of optimal trees is not the removal of conflicting variation at the intraspecific level. When ITS1 and ITS2 are separately analyzed under parsimony with only one sample per species, i.e., by removal of the additional sequences for each taxon (trees not shown), the number of trees dropped to half for ITS2 (516 vs 1118) but increased more than sixfold for ITS1 (9522 vs 1492). Further, the C.I. not only did not increase as one might expect from reduction of the number of terminals in a parsimony analysis (Sanderson and Donoghue, 1996), but it dropped slightly for ITS1 (0.65 vs 0.68) and did not change for ITS2 (0.71). Unambiguous resolution remained as poor as that obtained from the analysis of intact extended ITS1 and ITS2 matrices.

In two of the six taxa for which we have more than one sequence, polyploid populations are known: *D. pardalianches* and *D. plantagineum*. Therefore, these two species would be candidates to contain intraspecific variability for ITS sequences. However, sequence distances within each of these two species are not noteworthy. More importantly, the two sequences from *D. pardalianches* and the five from *D. plantagineum* cluster in their own respective clades. This suggests that intraspecific variability does not interfere with variation that is informative (phylogenetic signal) at the species level. Neither the four sequences of *D. grandiflorum* nor the three sequences of *D. carpetanum* subsp. *kuepferi* form exclusive clades in the strict consensus tree from the analyses of the ITS1 and ITS2 extended matrices. However, this apparent lack of monophyly is not due to differences among the sequences (e.g., those from *kuepferi* are identical), but is due to lack of characters that are exclusive to either taxa, i.e., synapomorphies for *D. grandiflorum* or for *D. carpetanum* subsp. *kuepferi*. A similar situation is found in *D. falconeri*. The sample included from Xinjiang (China) under the synonym *D. turkestanicum* in the ITS reduced matrix is almost identical to the other sample from Pakistan (distances: 0 in ITS1, 0.009 in ITS2, 0 in *trnL-F*). The two ITS sequences cluster in a clade in most of the analyses, a result that supports our taxonomy and provides another example of very low intraspecific ITS polymorphism within *Doronicum*.

The greatest divergence found in this study between sequences of the same species occurs in *D. austriacum*. The two sequences, from Greece and Poland, differ by 11 nucleotide substitutions (6 in ITS1 and 5 in ITS2), of which only 2 are autapomorphic. However, 4 of the remaining 9 differences correspond to nucleotides (sites 6, 50, 308, and 423) that do not show a character-state distribution consistent either with geography or with taxonomically recognizable groups and are thus likely to be highly variable positions. Not evidently noisy positions include 130, for which the Greek sample of *D. austriacum* (but not the Polish sample) shares an "A" with the *D. plantagineum* group, *D. macrophyllum* and its allies, *D. carpetanum, D. carpaticum*, and two additional southwestern Asian taxa (*D. macrolepis* and *D. maximum*) (Appendix 1). As a whole, there is not a clear pattern that could suggest differentiation or hybridization as the cause behind those differences between the samples of *D. austriacum*. Moreover, both sequences form a clade when the Polish sample is added to the ITS1 + ITS2 reduced matrix (tree not shown). We conclude that the intraspecific variability detected in our study that is not autapomorphic seems to be homoplastic. Therefore intraspecific differences do not question the potential informativeness of ITS variation in reconstructing the species phylogeny because sequences from the same species either form exclusive clades or, if lacking synapomorphies, cluster in the same clade with other terminals.

The four subspecies recognized within *D. carpetanum* (subsp. *carpetanum, diazii, kuepferi*, and *pubescens*) are not monophyletic but they nest in a clade containing other species in the molecular, morphological, and total evidence trees (Figs. 1, 4, and 5). *D. macrophyllum* subsp. *sparsipilosum* exhibits slight morphological differences (fewer cauline leaves and capitula) from subsp. *macrophyllum*. Results from our ITS analysis show, however, that subsp. *sparsipilosum* is not sister to subsp. *macrophyllum* but is instead sister to *D. oblongifolium* (Fig. 1), and if all evidence is used, subsp. *sparsipilosum* is sister to *D. maximum* (Fig. 5). The latter result has weak support (Fig. 5). Divergence between the ITS sequences of the two subspecies is 0.032 in ITS1 and 0.024 in ITS2. Another potential case of conflict is represented by the northern African populations described under *D. atlanticum*, a

taxon that we have kept in the analyses despite the lack of consistent morphological features to differentiate it from *D. plantagineum*. The divergences between *D. atlanticum* and the five sequences of *D. plantagineum* range from 0.027 to 0.035 in ITS1 and from 0.019 to 0.034 in ITS2. Whereas the samples belonging to *D. plantagineum* are nested together and form a monophyletic group in both the separate ITS1 and ITS2 trees (trees not shown), the ITS1 and ITS2 sequences of *D. atlanticum* fell out of the five-sequence *plantagineum* clade as did *D. hungaricum*. The possibility exists that the apparent contradictory placement of these species as not sister to each other is the result of sampling error, introgression, or the possibility that the northern African populations of *D. atlanticum* should be regarded as a different species.

The latter two cases are the only cases revealing intraspecific ITS variability that might suggest problems when trying to infer organismic phylogeny from gene trees. However, in both cases underlying taxonomic issues may be responsible for the apparent contradictory placements of different samples from the same species. That is, the differences in ITS sequences between *D. atlanticum* and *D. plantagineum* may be relevant at the species-level phylogeny even though morphological differentiation is poor. Taxonomic treatment that would turn this "intraspecific" ITS variability into interspecific variation might be justified. Alternatively, sequence differences might be due to introgression from *D. orientale*, which would have implications for the species phylogeny but might not demand taxonomic adjustments. Further sampling is therefore required to determine the most likely reason for those two discordant cases. In all, it seems that informativeness of ITS data for reconstructing the phylogeny of the genus is not precluded by intraspecific variability coupled with sampling error.

Combined and Independent Analyses

Theoretical issues aside, a combined (total evidence) analysis improves the opportunity to detect phylogenetic signal amid background noise by increasing the number of characters (Soltis *et al.,* 1998). On the other hand, analysis of different data sets separately allows the identification of serious heterogeneity that would otherwise contribute additional noise and thus might override the true phylogenetic signal. A justification for the third (conditional combination) approach is that it attempts to reduce the risk of mixing different signals, which is another source of noise in addition to homoplasy. However, arriving at the conclusion that two data partitions are incongruent does not mean that they are both equally reliable. The following argument is in the spirit of Wendel and Doyle's (1998) contention that incongruence between data sets need not be perceived as negative results, since they may be indicators of previously unsuspected biological processes. A serious incongruence between two data sets allows more than the mere questioning of their merging into a single data matrix for analysis. In *Doronicum*, we claim that even such questioning may be reconsidered in favor of a total evidence approach when all the evidence about characters, analyses, etc. is taken into account.

Given the results of the significance test for heterogeneity, an application of the conditional combination approach (Bull *et al.,* 1993) in *Doronicum* would suggest that we combine only the ITS and *trnL-F* data into a single matrix. The morphology should be analyzed independently and the results of the two analyses compared for common clades. However, when we look for common clades in the strict consensus of the analyses of molecular and morphological data (Fig. 4), we find that there are none within the ingroup, although there are some minor clades that are compatible with the alternative topology. A strict application of this procedure would leave us without a single component of information supported by the three data sets. This result appears to be too radical, particularly given that the discrepancies between data sets do not seem to be strongly supported by each data set and thus suggest an insightful biological alternative explanation (Wendel and Doyle, 1998). We believe that following the conditional combination approach leads, in these specific data sets, to some kind of reductio ad absurdum.

Reasons for disagreement between molecular trees and species trees have been described elsewhere (Doyle, 1992, 1997; Maddison, 1997). Because some of them involve molecular mechanisms (gene duplication, lineage sorting), we might suspect that the molecular tree (based on the ITS + *trnL-F* data matrices) could be a worse representation of the species phylogeny than the morphological tree. However, it seems that such is not the case. Despite the fact that ITS is inherited biparentally and *trnL-F* is inherited maternally, these two data sets are homogeneous. The most likely explanation for the branching histories of the two molecular markers agreeing despite their differences in inheritance and relevant mechanisms (recombination, concerted evolution, etc.) is that they are a good reflection of the species branching pattern. In addition, the summary statistics resulting from the parsimony analysis of the morphological data set are clearly worse than those from the combined molecular data set. The C.I. is similar (0.62) even though the number of informative characters is 11 times greater in the molecular data, and the bootstrap support for most groups is lower in the morphological trees, as is the resolution. In contrast, the number of m.p.t. is much higher in the morphological analyses (675 vs 21). Therefore, although morphological characters are few, they contain a considerable amount of incongruence among themselves, probably the result of errors in homology assessment (primary homology sensu de Pinna, 1991). These mor-

phological characters seem to contain poor recoverable signal for the reconstruction of species phylogeny despite the fact that a detailed morphological study has been conducted on the basis of more than 4000 herbarium specimens. The fact that after three rounds of successive weighting, the clades obtained are incompatible with those from the ITS tree seems to provide support for our interpretation that the morphological data set contains an excess of incorrect homology assessments.

Based on the above argument, either we could rely exclusively on the phylogenetic hypothesis based on the molecular matrix and disregard the morphological matrix or we could merge all the data together into a single (total evidence) matrix (Fig. 5). The first solution produces slightly better parameters in the number of m.p.t. (Table 3) but discarding a set of empirical data seems difficult to justify epistemologically, even if we map the morphological characters a posteriori on the molecular trees. In contrast, the combined analysis of the three data sets constitutes a homology test for the morphological characters against the molecular characters (Patterson, 1988). In fact, the most stringent homology test for a set of characters is to analyze it together with other characters to see which patterns are reinforced and which are questioned by congruence. The results of such a test can be read on the resulting cladograms generated from the parsimony analysis of the combined matrix. There is an additional justification for letting other sets of (in this case molecular) characters decide which of the morphological characters contain more noise in reconstructing the species phylogeny: the low number of morphological characters. Provided that the molecular data set appears to convey more phylogenetic signal for reconstructing the species phylogeny, if we were to merge the molecular data together with a high number of morphological characters containing much homoplasy, the true phylogenetic signal might be partially overridden. Thus, the low number of morphological characters is, in this case, appropriate for preferring the total evidence approach and performing the congruence test.

The results of the combined analysis show that 7 of the 12 morphological characters require high amounts of homoplasy to be optimized in the m.p.t. Therefore, their coding as the same character is not confirmed by the parsimony analysis and instead seems to result from incorrect homology assessment (primary homology). In particular, the occurrence of two types of fruit within a single inflorescence (heterocarpy) is not uncommon within groups of Asteraceae (Zohary, 1950). Although it has ecological significance for dispersal and germination and the shift from heterocarpy to homocarpy is frequent (Venable and Levin, 1985; Imbert *et al.,* 1996), in *Doronicum* this character has been considered of taxonomic importance even at the generic level (Candolle, 1838; Cavillier, 1907, 1911). The fact that homocarpy requires five independent gains (seven steps) to be optimized on the m.p.t. indicates that it fails to pass a test of homology by congruence with the rest of the characters (Fig. 5). A similar case is that of the presence of hairs (six steps) and buds (seven steps) in the rhizome, as well as the shape of the internodes in the rhizome (four steps), and the pinnate–reticulate leaf venation (five steps), the architecture of the *D. macrophyllum* group (four steps), and the occurrence of glands in the cypselae (five steps). The 5 remaining morphological characters require one step and thus contain no homoplasy.

Systematic and Biogeographic Implications

We consider that the best estimate of phylogenetic relationships within *Doronicum* is that which is obtained from the combined analysis of the three data sets (Fig. 5). This analysis allows a number of well-supported conclusions and suggestions.

Doronicum is monophyletic in all the analyses that we have conducted. This is consistent irrespective of which data set or combination of them is used. In all analyses, except the morphological, bootstrap support for the *Doronicum* clade is 100%, and our sampling of the genus in terms of species is comprehensive (100% of the species in the ITS extended matrices, 92% in the ITS reduced matrix and in the *trnL-F* matrix). It can be argued that the outgroup taxa are too far removed to test the monophyly of the genus. However, we lack information as to which other genus could be the immediate sister taxon of *Doronicum*. *Doronicum* has not been included in any of the most comprehensive molecular studies for the tribe to date (Kadereit and Jeffrey, 1996; Knox, 1996; Kim and Jansen, 1995). Historically, the Chinese endemic genus *Nannoglottis* has been allied to *Doronicum*. In fact, *N. hookeri* was included in *Doronicum* by Cavillier (1907, 1911) but placed in its own section. The placement of *Nannoglottis* within Senecioneae is not supported based on the morphology of reproductive features such as the stylar branches (Nordenstam, 1977). We follow Bremer (1994) who includes the genus within tribe Astereae. Attempts to isolate and amplify DNA of *N. hookeri* have been unsuccessful. *D. stenoglossum* was placed in a monotypic section created by Cavillier (1911). Morphological features separating *D. stenoglossum* from the rest of the species are all autapomorphic (e.g., the linear greenish ligules or the arrangement and structure of the pappus) and thus we believe that it should be classified with its sister species. In all our analyses *D. stenoglossum* is always deeply nested within *Doronicum*.

The most outstanding result of this study is the basal position of *D. corsicum*, sister to the remaining species, a result that has strong biogeographic implications (Fig. 5). Based on our study, the evolution of *Doronicum* appears to have followed successive splitting lead-

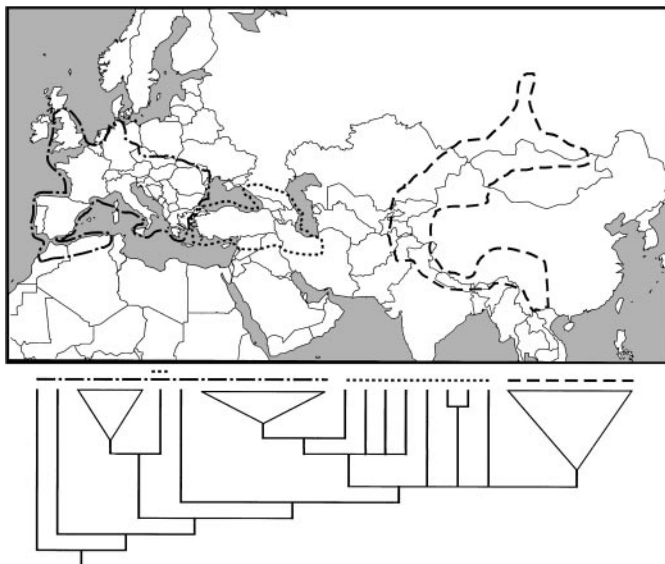

FIG. 7. Correspondence between the geographic distribution of the species of *Doronicum* and the strict consensus tree from the combined analysis of the three data sets shown in Fig. 5.

ing to the European species *D. pardalianches* and the *D. plantagineum* clade. These species are mostly Mediterranean in distribution, except for *D. hungaricum* (eastern European). Therefore, it appears that an early diversification took place on the European continent, or more specifically, in the Mediterranean basin (Fig. 7).

The core species of the *D. plantagineum* group (*D. plantagineum, D. atlanticum, D. hungaricum, D. orientale*) share a number of morphological characters that are the most congruent with the molecular data. These characters include the scapiform habit (few reduced cauline leaves), triplinerved leaf venation, and ciliate involucral bracts. The species belonging to this group occur in areas dominated by Mediterranean forests. However, the inclusion of *D. columnae*, which usually occurs at higher elevations, is uncertain despite the high support for the clade. Morphologically, the scapiform habit in *D. columnae* is clearly consistent with that of the other species, the leaf venation is slightly different, and the involucral cilia are present, although not in every population. Furthermore, the position of *D. columnae* in the ITS trees, nested within the *D. plantagineum* group, differs from that in the *trnL-F* trees (Fig. 2). The different placement of *D. columnae* in the two gene trees and its partial morphological affinity with the *D. plantagineum* group suggest a possible hybrid origin.

Another group that tends to appear in the cladograms of the various analyses, although with low bootstrap support in the total evidence analysis (47%), includes central and eastern Asian taxa. Of these taxa, the placement of *D. briquetii* is equivocal, nested in a clade with taxa from southwestern Asia in the ITS tree (Fig. 5).

The clade containing *D. grandiflorum*, although weakly supported (57% bootstrap), includes a group of species that share similar ecological requirements and parapatric distributions. The *D. grandiflorum* clade contains *D. grandiflorum* (from the eastern Alps to the Cantabrian mountains in northern Spain), its close relatives *D. glaciale* (Alps) and *D. clusii* (Alps and Carpathians), and four closely related Iberian taxa (the four subspecies of *D. carpetanum*, subsp. *carpetanum, kuepferi, pubescens*, and *diazii*). All of them occur on mountain habitats and exhibit a fleshy rhizome with minute hairs on the nodes.

Cavillier (1911) recognized subsection *Macrophylla* which includes species from Turkey, the Caucasus, and the Caspian area (*D. cacaliifolium, D. dolichotrichum, D. haussknechtii, D. macrophyllum, D. maximum, D. reticulatum*). Morphologically, all species share a growth habit typical of alpine European plants growing on rich, humid soils receiving large amounts of snow, namely, tall herbs with a few very large reticulately veined leaves with several to many capitula. The molecular data, however, do not support the recognition of this group. The above-mentioned European diversification is supported by four subsequent basal nodes ending in European terminals in the strict consensus tree (Fig. 5). The rest of the inferred cladogenetic events may roughly conform to a west–east sequence (Fig. 7). However, any conclusion beyond those four basal nodes is tentative because either bootstrap support is low or resolution is poor. In fact, in the remaining portion of the tree, a European clade (*D. grandiflorum, D. glaciale, D. clusii, D. carpetanum*) occurs distally to several southwestern Asian species. In all, the geographic structure of the tree seems noticeable because the only species occurring on two of the three large areas marked in Fig. 7 is *D. orientale*. Such a structure does not answer the question of whether the geographic structure has been shaped mainly by vicariance or by dispersal events.

Placement of Doronicum

The Senecioneae is the largest tribe in the Asteraceae, with 3200 species and approximately 120 genera (Bremer, 1994). The placement of *Doronicum* within the Senecioneae was proposed by Cassini (1819) and subsequently accepted by Bentham and Hooker (1873–1876). In addition, *Doronicum* shares with other Senecioneae a basic chromosome number (probably secondary) of $x = 30$ and pyrrolizidine alkaloids (Nordenstam, 1977; Jeffrey, 1987; Bremer, 1994). The few molecular studies that have sampled Senecioneae species did not include *Doronicum* (Jansen *et al.,* 1990, 1991; Kim *et al.,* 1992; Kim and Jansen, 1995; Knox 1996; Kadereit and Jeffrey, 1996), and thus, the present study addresses for the first time the position of this genus on the basis of molecular evidence.

Phylogenetic analyses of *ndhF* sequence data reveal

that *Doronicum* is a member of tribe Senecioneae (Fig. 6). The position of *Doronicum* as sister to a clade containing the genera *Blennosperma, Lopholaena, Senecio*, and *Syneilesis* contradicts the widely held assumption that members of the Blennospermatinae (here represented by *Blennosperma*) are the basal group of the Senecioneae (Bremer, 1994 and other references therein). Despite the weak sampling, it is important to note that the four other genera form a strongly supported clade (bootstrap 99%), with the sister position of *Doronicum* being weakly supported (bootstrap 57%). These results lead to speculation that the widely held phylogenetic assumptions about the relationships of the Senecioneae are at best controversial and may prove incorrect once more extensive studies are completed. In sum, our analysis using *ndhF* data provides support, albeit not conclusive, for the placement of *Doronicum* within tribe Senecioneae.

APPENDIX 1

Aligned Sequences of the ITS1 and IST2 Regions in a Sample of Doronicum (Refer to Table 1 for Acronym Identification)

ALTA	TCGAAGCCTG	CAAAGCAGAA	CGACCCGTGA	ACTTGTACTA	ACAACCACCC	GCTGT---GA	GGATCGGACA	CGTGTTTGAG	ACTCATGGCG	TTTC-GTCGA	TGTGTGCTCA	A-GGTCGCCC	GATAAGGGCG
ATLA	....	....	....	....	....	....	..T.......	.T........	....	....	....	....A.A..T	.T........
AUS1	.....A....	....	....	....	.........T	....	.....A....	.T........	........T.	.A........	....	......A...	.T.......A
AUS2	....	....	....	....	....	....	.....A....	.T........	........T.	.A........	....	......A...	.TC.....T.
BRIQ	....	....	....	........C.	....	....	....	..--......	........T.	...T...T..	....A.....	......A...	.T........
CACA	....	....	....	....	.T.C..G...	....	........T.	.C...CC...	....	....	......A...	......A...	.T........
CARP	....	....	....	....	....	....	......A...	.T........	....	....	....	......A...	.T.......A
CATA	....	....	....	....	....	....	....	.C........	....	....	....	...T..A...	.T........
CAVI	....	....	....	........C.	....	....	....	.--.......	........T.	....	..C.A.....	......A...	.T........
CLUS	.....T....	....	....	....	....	....	....	.T........	....	....	....	......A...	.T.......A
COLU	....	....	....	....	....	..C.......	..T.T.....	.T........	....	....	....	....A.A...	.T........
CORS	....	....	....	..A.......	....	..C.......	....T..G.T	TT........	C.........	....	....	T.....A.T.	.T........
CRTC	....	....	....	....	....	.T........	......A...	.T........	....	...T......	....	......A...	.T.......A
DIAZ	....	....	....	....	....	....	......A...	.T........	....	....	....	......A...	.T.......A
DOLI	....	....	....	....	....	.T........	....	.C........	....G.....	....	....	......A...	.T........
FALC	....	....	....	....	....	....	....	....	....	....	....A.....	......A..T	.T........
GLAC	....	....	....	....	.........T	...A......	........T.	.T........	....	..C.......	....	......A...	.T.......A
GRA2	....	....	....	....	....	....	......A...	.T........	....	....	....	......A...	.T.......A
GRA3	....	....	....	....	....	....	......A...	.T........	....	....	....	......A..Y	.T.......A
GRA4	....	....	....	....	....	....	......A...	.T........	....	....	....	......A...	.T.......A
GRA5	....	....	....	....	....	....	......A...	.T........	....	....	....	......A...	.T.......A
HAUS	....	....	....	....	........T.	.T........	....	.C........	....	....	....	......A...	.T........
HUNG	....	....	T.........	....	....	..C.......	..T.......	.T........	....	....	....	....A.A..T	.T........
KAMA	.....A....	....	....	....	....	...A......	....	....	.........A	....	....	......A..T	.T........
KUE1	....	....	....	....	....	....	......A...	.T........	....	....	....	......A...	.T.......A
KUE2	....	....	....	....	....	....	......A...	.T........	....	....	....	......A...	.T.......A
KUE3	....	....	....	....	....	....	......A...	.T........	....	....	....	......A...	.T.......A
MACR	.....R....	....	....	....	........YT	A.........	....	.C........	........TA	....	....	......A...	.T........
MAXI	....	....	....	....	....	.T........	......A...	.T........	....	...T......	....	......A...	.T.......A
MCRL	....	....	....	....	....	....	......A...	.T........	....	....	.....A....	......A...	.T........
OBLO	....	....	....	....	....	....	....	.C........	....	....	....	...T..A...	.T........
ORIE	....	....	....	....	....	..C.......	..T.......	.T........	....	....	....	..A.A.A...	.T........
PAR1	....	....	....	....	...G....T.	.TC.......	....	.C...C....	....	....	....	......A...	.T......T.
PAR2	....	....	....	....	...G....T.	.TC.......	....	.C...C....	....	....	....	......A...	.T......T.
PLA1	....	....	T.........	....	....	..C.......	..T.......	.T........	....	....	....	....A.A...	.T...T....
PLA2	....	....	T.........	....	....	..C.......	..T.......	.T........	....	....	....	....A.A...	.T...K....
PLA3	....	....	T.........	....	....	..C.......	..T.......	.T........	....	....	....	....A.A...	.T...T....
PLA4	....	....	T.........	....	....	..C.......	..T.......	.T........	....	....	....	....A.A...	.T...T....
PLA5	..C.......	....	T.........	....	....	..C.......	..T.......	.T........	....	....	....	....A.A...	.T...T....
PUBE	....	....	....	....	....	....	......A...	.T........	.....A....	....	....	......A...	.T.......A
RETI	....	....	....	........A.	....	.7........	....	.C........	.........T	....	....	......A...	.T......T.
SPAR	....	....	....	....	........T.	.7........	....	.C........	....	....	....	......A...	.T........
STEN	....	.G........	....	....	.........T	....	....	.--.......	....	....	....	......A..T	.T........
TURK	....	....	....	....	....	....	....	....	....	....	....A.....	......A..T	.T........
LIGU	----------	----------	----------	-.A....AC.	....---T.G	.G...CCTTG	.T.....G.T	.T....C..T	TAATTG.AT.	CC.T...T..	....C.TCTC	T.....AG..	CT.TU..T.T
TUSS	.....A....	..T....A..	.....T....	..A...TAC.	....---T.T	TG.C.CCTT.	.AG.T...GT	.T....C..T	T..TTG.ATT	CC.T......	....CATCTT	T....TAG..	T..CC..TTC

APPENDIX 1—Continued

ALTA	TCATGGACTT	CACATCGGCA	CAACAACAAA	CCCC-GGCAC	GGAATGTGCC	AAGGAAAACA	AAACTTGAAA	AGGGCTCGTG	CCATGCATGC	CCCGTTTGCG	GTGCGC--TC	ATGGCACGAA	GCTTCTTTGT
ATLA	......T...	....	...A......	.........T	....	....	....	...A......	..T....A..	....	........C.	......T.C.	....
AUS1	....	....	....	....	....	....	....	....	.T........	....	....	........C.	....
AUS2	....	....	....	....	....	....	....	..A.......	.T........	....	....	........C.	....
BRIQ	....	....	....	....	....	....	....	.A....T...	....	....	....	G....T....	....T.....
CACA	....	....	.........T	....	....	....	....A.....	....	.T........	....	....	....	....
CARP	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
CATA	....	....	....	....	....	....	....	....	....	....	....	....	....
CAVI	....	....	....	....	....	....	....	......T...	....	....	....	G......A..	....
CLUS	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
COLU	....	.......T..	....	....	....	....	....	...A......	.......A..	....	....	......T.C.	.T........
CORS	....	....	....	....	....C.....	C	........G.	.........A	....	....	....	...A....TG	.T........
CRTC	....	....	....	....	....	.G........	....	G.....T...	....	....	....	......T.T.	....
DIAZ	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
DOLI	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
FALC	....	....	....	....	....	........T.	....	......T...	....	....	....	G.........	....
GLAC	....	....	....	....	....	....	....	......T...	....	....	....	........T.	....
GRA2	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
GRA3	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
GRA4	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
GRA5	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
HAUS	....	....	....	....	....	....	....	....	....	..T.......	....	....	....
HUNG	......T...	....	....	....	....	....	....	...A......	.......A..	....	....	......T.C.	T.........
KAMA	....	....	....	....	....	....	........T.	......T...	....	....	....	G.........	....
KUE1	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
KUE2	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
KUE3	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
MACR	....	....	....	....	....	....	....	....	....	....	...T......	....	....
MAXI	....	....	....	....	....	....	.G........	G.....T...	....	....	....	......T.T.	....
MCRL	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
OBLO	....	....	....	....	....	....	....	....	....	....	....	....	....
ORIE	.....C....	....	....	....	....	....	....	...A......	....	....	....	........C.	....
PAR1	....	....	....	....	....	....	....	....	....	....	....	........CG	...C......
PAR2	....	....	....	....	....	....	....	....	....	....	....	........CG	...C......
PLA1	......T...	....	....	....	....	....	....	...A......	.......A..	....	....	......T.C.	..A.......
PLA2	......T...	....	....	....	....	....	....	...A......	.......A..	....	....	......T.C.	..A.......
PLA3	......T...	....	....	....	....	....	....	...A......	.......A..	....	....	......T.C.	..A.......
PLA4	......T...	....	....	....	....	....	....	...A......	.......A..	....	....	......T.C.	..A.......
PLA5	......T...	....	....	....	....	....	....	...A......	.......A..	....	....	......T.C.	..A.......
PUBE	....	....	....	....	....	....	....	......T...	....	....	....	......T.T.	....
RETI	....C.....	....	....	....	....	....	....	....	....	....	....	.......A..	....
SPAR	.......T..	....	....	....	....	....	....	....	....	..T.......	....	....	....
STEN	....	....	....	....	....	....	....	.....CT...	....	....	....	G.....T...	....
TURK	....	....	....	....	....	........T.	....	......T...	....	....	....	G.........	....
LIGU	C..-....G.	.....T....	....	....C.....	..C.......	........TY	......A.G.	......T..A	......-.T.	....	.G.T.T..G.	....G...TG	........A.
TUSS	.A.A.ATG..	-.TG.T...C	T..T.....C	....	..C.......	........TC	......A.G.	.........A	T..A..-.C.	....	.G.TTTT.G.	....G...TG	....

APPENDIX 1—Continued

```
           ┌──────→ ITS2
ALTA  AATTAG---C ACG----TTC ACCCCATCA CGAATC---- ------ATGT GTGAAATGGG GGCGGAGAAT GGTCTCCCGT TCCCTCGGTG CGGCTGGCCA AAATTAGAGT CCCCTTCGGC GGACGCACGA
ATLA  .......T.. .......... ......... .......... .......CAC .......... .......... .......... ....AT.... .......... .......... ...T....AT ..........
AUS1  .......... .......... ......... .......... .......CAC .......... .......... .......... .......... .......... .......... .......... ..........
AUS2  .......... .......... ......... .......... .........C .......... .......... .......... .......... .......... .......... .........T ..........
BRIQ  .......T.. .T........ ......... .......... .......CTC .......... .......... .......... ....A..... .......... .......... .......... ..........
CACA  .......T.. .T........ ......... .......... .......CTC .......... .......... .......... ....A..... .......... .......... .......... ..........
CARP  .......T.. .......... ......... .......... .........C .......... .......... .......... .......... .......... .......... .........T ..........
CATA  .......T.. .T........ ......... .......... .......CTC .......... .......... .......... ....A..... .......... .......... .......... ..........
CAVI  .......T.. .......... ......... .......... .........C .......... .......... .......... .......... .......... .......... .......... ..........
CLUS  .......T.. .T........ ......... .......... .......CCC .......... .......... .......... ....A..... .......... .......... .A........ ..........
COLU  .......... .......... ......... .......... .......CAC .......... .......... .......... ....A..... .......... ........A. .......... ..........
CORS  .....TAT.. ..A....... ......... T.C....... .........C .GTG...... ..A....... .......... ....A..... ...T...... .......... .........T ..........
CRTC  .......T.. .......... ......... .......... .......CAC .......... .......... .......... ....A..... .......... .......... .......... ..........
DIAZ  .......T.. .T........ ......... .......... .......CTC .......... .......... .......... ....A..... .......... .......... .......... ..........
DOLI  .......T.. .T........ ......... .......... .......CTC .......... .......... .......... ....A..... .......... .......... .......... ..........
FALC  .......T.. .......... ......... .......... .........C .......... .......... .......... .......... .......... .......... .......... ..........
GLAC  ......AT.. .T........ ......... .......... .......CCC .......... .......... .......... ....A..... .......... .......... .......... ..........
GRA2  .......T.. .T........ ......... .......... .......CTC .......... .......... .......... ....A..... .......... .......... .......... ..........
GRA3  .......T.. .T........ ......... .......... .......CTC .......... .......... .......... ....A..... ...T...... .......... .......... ..........
GRA4  .......T.. .T........ ......... .......... .......CTC .......... .......... .......... ....A..... ...Y...... .......... .......... ..........
GRA5  .......T.. .T........ ......... .......... .......CTC .......... .......... .......... ....A..... .......... .......... .......... ..........
HAUS  .......T.. .T........ ......... .......... .......CTC ......A... ..T....... .......... C...A..... .......... .......... .......... ..........
HUNG  .......T.. .......... ......... .......... .......CAC .......... .......... .......... ....A..... .......... .......... ........AT ..........
KAMA  .......T.. .......... ......... .......... .........C .......... .......... .......... .......... .......... .......... .......... ..........
KUE1  .......... .T........ ......... .......... .......CTC .......... .......... .......... ....A..... .......... .......... .......... ..........
KUE2  .......T.. .T........ ......... .......... .......CTC .......... .......... .......... ....A..... .......... .......... .......... ..........
KUE3  .......T.. .T........ ......... .......... .......CTC .......... .......... .......... ....A..... .......... .......... .......... ..........
MACR  .......T.. .......... ......... .......... .......C.C .......... .......... .......... .......... .......... .......... .......... A..T......
MAXI  .......T.. .......... ......... .......... .......C.C .......... .......... .......... .......... .......T.. .......... T......... A.........
MCRL  .......T.. .T........ ......... .......... .......CTC ......A... .......... .......... C...A..... .......... .......... .......... ..........
OBLO  .......T.. .......... ......... .......... .......C.C .......... .......... .......... .......... .......... .......... .......... A.........
ORIE  .......T.. .......... ......... .......... .......CAC .......... .......... .......... ....AT.... .......... ........A. .......... .........G
PAR1  .....TAT.. .......... ......... .......... .......... .......... .......... .......... ....A..... ...A....T. .......... .......... ..........
PAR2  .....TAT.. .......... ......... .......... .......... .......... .......... .......... ....A..... ...A....T. .......... .......... ..........
PLA1  .......... .......... ......... .......... .......CAC .......... ..Y....... .......... ....A..... ........A. .......... ........AT ..........
PLA2  .......T.. .......... ......... .......... .......CAC .......... ..Y....... .......... ....A..... ........A. .......... ........AT ..........
PLA3  .......T.. .......... ......... .......... .......CAC .......... .......... .......... ....A..... ........A. .......... ........AT ..........
PLA4  .......T.. .......... ......... .......... .......CAC .......... .......... .......... ....A..... ........A. .......... ........AT ..........
PLA5  .......T.. .......... ......... .......... .......CAC .......... .......... .......... ....A..... ........A. .......... ........AT ..........
PUBE  .......T.. .T........ ......... .......... .......CAC .......... .......... .......... ....A..... .......... .......... .......... ..........
RETI  .......T.. .T........ ......... .......... .......CTC ......R... .......... .......... ....A..... .......... .......... .......... ..........
SPAR  .......T.. .......... ......... .......... .......C.C .......... .......... .......... .....T.... .......... .......... .......... A.........
STEN  .......T.. .......... ......... .......... .........C .......... ..T....... .......... .......... .......... .......... .......... ..........
TURK  .......T.. .......... ......... .......... .........C .......... .......... .......... .......... .......... .......... .......... ..........
LIGU  ...C..TCA. ..ATCGTG.. G.T....C. ..CC..CTCG ATGGGG...C T..G.-..T. .......T.. .......... ..TAT..... ...T....T. ...CAG.... .......... ..........
TUSS  ...A.-...- ---....--T G....T.C. T.CC.TCTTG ATAGGG...C T..G.-.T.. .......T.. .......... ..TAT..... ...T....T. ...C.G.... ..T.....AA ........T.
```

APPENDIX 1—Continued

Taxon	Sequence
ALTA	CTAGTGGTGG TTGACAAAAC CCTCGTATCG AGCCGTGCGT TGTGAGTCGT AAGGGAAGAC CT--ACATAG ACCCTAACGT GTTGTCTTGT GAT-GACGCT TCGATT
ATLA	....T.. ....A. ...T...... ......
AUS1	....A.... ....
AUS2	....T....... .....A...C ....
BRIQ	....T....... ....T.. ......G... ..CT...... ....A. ....
CACA	....T....... ....CT...C.. ....A. ....A. ....
CARP	....T....... .....A...C ....-.T...... ....
CATA	....G...... ....T....... ....CT...... ....A. ....
CAVI	....TT..... ....T... ......
CLUS	....CT...... ....A. ....
COLU	....Y.. ....A. ....
CORS	....T....... ....TT...T.. ......C... ....T..A.. ....C.A. ....A.
CRTC	....T.. ....-.T...... ....A. ....
DIAZ	....T....... ....CT...... ....A. ....A. ....
DOLI	....T....... ....CT...... ....A. ....A. ....
FALC	....T... ......
GLAC	....T....... ....CT...... ....A. ......TT.G ......
GRA2	....Y...... ....CT...... ....A. ....
GRA3	....T. ....CT...... ....A. ....
GRA4	....CT...... ....A. ....
GRA5	....CT...... ....A. ....
HAUS	....T....... ....CT...... ....A. ....
HUNG	....A.-..... .-.T...... ....A. ...T...... ......
KAMA	....-.T...... ....T... ......
KUE1	....T....... ....CT...... ....A. ....
KUE2	....T....... ....CT...... ....A. ....
KUE3	....T....... ....CT...... ....A. ....
MACR	....T....... ....
MAXI	....TG.... ....
MCRL	....T....... ....CT...... ....A. ....
OBLO	....TG.... ....-.T...... ....
ORIE	....T.. .....T.... ....-..... ....G.
PAR1	....T...T.. ....-...T. ....G...T. ....
PAR2	....T...T.. ....-...T. ....T. ....
PLA1	....-..... ....A. ...T...... ......
PLA2	....-..... ....A. ...T...... ......
PLA3	....-..... ....A. ...T...... ......
PLA4	....-..... ....C.A. ....TT.... ......
PLA5	....-..... ....A. ...T...... ......
PUBE	....T.....A. ....T.. ....CT...... ....A. ....
RETI	....T....... ....T.. ....CT...... ....A. ....
SPAR	....TG.... ....
STEN	..G....... ....C ....C...T... ......
TURK	....AA..... ....T... ......
LIGU	T......... ....G.. ....T.T..A ..TT...... .C.A..--.. ....-..T.T ..CTT..AT. ....T.. ..C...---- ---.------ ------
TUSS	T......... ....G..G.. ....T....A ..TT...T.. .CCA..GA.. C......T.T ..CTTT.AT. ....----T. ..C..T..TA ..C...T.A. .T..CC

APPENDIX 2

Aligned Sequences of the trnL-F Chloroplastic Spacer in a Sample of Doronicum (Refer to Fig. 1 for Acronym Identification)

```
ALTA  CGTATCCTT- TTGATTTATC CTTTTTTCGT TATTTTTCGT TAGCGGTTCA AAA------- --TTTCTCAT TCACTACTCT TTATACAAAT GGATCTGAGC GTAAATGCTG TTCTCTTATC ACAT----GT GATATATATG
ATLA  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
AUS1  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
BRIQ  .........T G......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
CACA  .......... .......... .......... ..-------- --........ .......... .....A.... .......... .......... .......... .......... .......... .......... ..........
CARP  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
CAVI  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
CLUS  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
COLU  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
CORS  .......... .......... .......... ..-------- --........ ...CTCCTTA TC........ .......... .......... .......... .g........ .......... .......... ..........
DIAZ  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
DOLI  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
FALC  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
GLAC  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
GRA1  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
HAUS  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
HUNG  .......... ..T....... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
KAMA  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
KUE1  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
MACR  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
MAXI  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
OBLO  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
ORIE  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
PAR1  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
PLA1  .......... ..T....... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
PUBE  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
RETI  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
SPAR  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
STEN  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
TURK  .......... .......... .......... ..-------- --........ .......... .......... .......... .......... .......... .......... .......... .......... ..........
LIGU  .........T .-........ .......... ..-------- --........ ...CTCCTTA TC........ .......... .......... ........A. .G....A... .......... ....CACATG ..---.....
TUSS  .........T .-........ .......... ..-------- --........ ...CTCCTTA TC........ .......... .......... ........A. .G....A... .......... ........TG ..---.....
```

APPENDIX 2—Continued

ALTA	ATACATGTAA	AAATGAACAT	CTTTGAGCAA	GGAATCCCCA	TTTGAATGAT	TCACGATCGA	GATTTTT--A	TTCATACTGA	AACTTACAAA	GTTGTTCTTT	TAACAAGTTA	TAGGACCTGG	ATGAGGCTTT	GTAATACCCC
ATLA	....	....	....	....	....	....	....	....	....	....	....	....	....	....
AUS1	....	....	....	....	....	....	....	....	....	....	....	....	....	....
BRIQ	....	....	....	....	....	....	....	....	....	....	....	....	....	....
CACA	....	....	....	....	....	....	....	....	....	....	....	....	....	.G........
CARP	....	....	....	....	....	....	T.........	....	....	....	....	.....T....	....	....
CAVI	....	....	....	....	....	....	....	....	....	....	....	....	....	....
CLUS	....	....	....	....	....	....	....	....	....	....	....	.....T....	....	....
COLU	....	....	....	....	....	....	....	....	....	....	....	.....T....	....	....
CORS	....	....	....	....	....	....	T.........	....	....	....	.G........	....	....	....
DIAZ	....	....	....	....	....	....	T.........	....	....	....	....	.....T....	....	....
DOLI	....	....	....	....	....	....	T.........	....	....	....	....	.....T....	....	....
FALC	....	....	....	....	....	....	....	....	....	....	....	....	....	....
GLAC	....	....	....	....	....	....	....	....	....	....	....	.....T....	....	....
GRA1	....	....	....	....	....	....	....	....	....	....	....	.....T....	....	....
HAUS	....	....	....	....	....	....	T.........	....	....	....	....	....	....	....
HUNG	....	....	....	....	....	....	....	....	....	....	....	....	....	....
KAMA	....	....	....	....	....	....	....	....	....	....	....	....	....	....
KUE1	....	....	....	....	....	....	....	....	....	....	....	.....T....	....	....
MACR	....	....	....	....	....	....	....	....	....	....	....	....	....	....
MAXI	....	....	....	....	....	....	....	....	....	....	....	....	....	....
OBLO	....	....	....	....	....	....	....	....	....	....	....	....	....	....
ORIE	....	....	....	....	....	....	....	....	....	....	....	....	....	....
PAR1	....	....	....	....	....	....	....	....	....	....	....	....	....	....
PLA1	....	....	....	....	....	....	....	....	....	....	....	....	....	....
PUBE	....	....	....	....	....	....	T.........	....	....	....	....	.....T....	....	....
RETI	....	....	....	....	....	....	....	....	....	C.........	....	....	....	....
SPAR	....	....	....	....	....	....	T.........	....	....	....	....	....	....	....
STEN	....	....	....	....	....	....	....	....	....	....	....	....	....	....
TURX	....	....	....	....	....	....	....	....	....	....	....	....	....	....
LIGU	C	....	....	....	....	....	.......TT.	....G.....	....	....	.G.....A..	C	.....A....	.........T
TUSS	C	....	.......A..	........T.	....	....	.......TT.	....G.....	....	....	.G.....A..	C	.....A....	.........T

APPENDIX 2—Continued

ALTA	TTCAATTGAC ATAGACCAC GTTCTCTAGT AAAATGAAAA TGAGGAGGAG ACATCAGGAA TAGTCGGGAT AGCTCAGTCG GTAGAGGAGA GGACTGAAAA TCCTCGTGTC ACCAGTT
ATLA	....
AUS1	....
BRIQ	....
CACA	....
CARP	....
CAVI	....
CLUS	....
COLU	....
CORS	....T... ....T. ....
DIAZ	....
DOLI	....
FALC	....
GLAC	....
GRA1	....
HAUS	....
HUNG	....
KAMA	....
KUE1	....
MACR	....
MAXI	....
OBLO	....
ORIE	....
PAR1	....
PLA1	....
PUBE	....
RETI	....
SPAR	....
STEN	....T...... ....
TURK	....
LIGU	....G... ....T... ....T..... ....T. ....
TUSS	....G... ....T... ....T..... ....T. ....

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