



Major clades within Apiaceae subfamily Apioideae as inferred by phylogenetic analysis of nrDNA ITS sequences

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With 1 figure and 2 tables

Abstract

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The results of phylogenetic analyses of 1240 nrDNA ITS sequences of Apiaceae subfamily Apioideae, representing 292 genera and 959 species from all major clades of the subfamily except those most basally branching, were compared to a preexisting phylogenetic classification for the group based on several molecular markers and a smaller sampling of taxa. This was done to confirm previously defined tribal and generic limits and to identify additional major clades and genera whose monophyly and relationships can be tested in future studies. All species for which ITS data are available in GenBank plus newly obtained sequences for 53 additional taxa were considered for inclusion in this study and their simultaneous analysis permitted misidentifications and other problematic sequences to be revealed. Analyses of these ITS data, in conjunction with results of recently published molecular studies, identified 41 major clades in Apioideae, of which 21 have already been recognized at the tribal or subtribal rank. No major changes to the preexisting phylogenetic classification of Apioideae are proposed, other than the recognition of additional major clades and the expansion of others to accommodate increased sampling. As noted in previous studies, many genera are not monophyletic; indeed, 18 genera are highly polyphyletic, with their members occurring in 2 to 7 major clades. Each of the 1240 accessions examined can be unambiguously assigned to a particular major clade. Resolution of relationships, as well as the formal recognition of several major clades, must await supporting data, such as that from the cpDNA *trnQ*^(UUG)–5′ *trnK*^(UUU) intergenic spacer region. An ITS-based classification of the subfamily is proposed that enables direct and precise references to specific groups and facilitates sampling and hypothesis testing in future systematic studies.

Keywords: Apiaceae subfamily Apioideae, classification, nrDNA ITS, phylogeny.

Introduction

Apiaceae subfamily Apioideae is, for the most part, a readily identifiable group of flowering plants, with 404 genera and 2827–2935 species recognized (Pimenov & Leonov

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1993). Many of its members are distinguished from those of the other subfamilies by the shared possession of compound umbels, characteristic fruits consisting of two one-seeded mericarps suspended from a free carpophore, and well-developed vittae. However, while the group is undoubtedly monophyletic, the many tribes and subtribes traditionally recognized within the subfamily are not (reviewed in Downie et al. 2001).

In the Proceedings of the 3rd International Symposium on the Apiales, Downie et al. (2001) summarized the results of previously published molecular systematic studies of Apioideae and presented a revised classification of the group that reflects its phylogeny. This classification was based on taxonomic congruence among the results of phylogenetic analyses of different molecular data sets, including chloroplast DNA (cpDNA) gene (*rbcL*, *matK*) and intron (*rpl16*, *rps16*, *rpoC1*) sequences, cpDNA restriction sites, and nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences. Ten tribes (Aciphyllaeae, Bupleureae, Careae, Echinophoreae, Heteromorphaeae, Oenantheae, Pleurospermeae, Pyramidoptereae, Smyrnieae, and Scandiceae, the latter comprising subtribes Daucinae, Scandicinae, and Torilidinae) were erected or confirmed as monophyletic (taxonomic authorities for all tribes and subtribes are presented in Table 1). Some of these tribes maintain long-standing names, but are radically different in generic composition from those groups traditionally recognized. Seven other major clades were also identified but were not treated formally, pending confirmation from additional studies or the sampling of nomenclatural types. These groups were the *Angelica* Clade, *Apium* Clade, *Arracacia* Clade, *Conioselinum chinense* Clade, *Heracleum* Clade, *Komarovia* Clade, and *Pimpinella* Clade. In addition, representatives from 23 genera were of dubious clade placement. Subsequent studies based primarily upon ITS sequences have treated the *Angelica* and *Arracacia* clades as tribe Selineae (Spalik et al. 2004) and have referred the *Apium*, *Heracleum*, and *Pimpinella* clades to tribes Apieae, Tordylieae, and Pimpinelleae, respectively (Spalik & Downie 2007, Ajani et al. 2008, Kurzyna-Młynik et al. 2008, Winter et al. 2008, Zhou et al. 2008, 2009).

At present, and likely in the foreseeable future, ITS sequences comprise the most comprehensive database for Apioideae phylogenetic study. These sequences are readily obtainable, even from old herbarium specimens, the phylogenies at low taxonomic levels are generally congruent with those inferred from chloroplast markers, and the few intra-individual polymorphisms revealed to date do not interfere with the phylogeny estimation (Spalik & Downie 2007). As of early September 2008, there were 2762 nucleotide sequence records for the ITS region in GenBank (ascertained using “Apioideae and internal transcribed spacer” as the search string). While many of these records represent separate ITS1 and ITS2 regions, there are significantly more apioid taxa for which ITS sequences are available for phylogenetic study than there are for all commonly used loci from the chloroplast genome combined (specifically, *rbcL*, *rpl16*, *rpoC1*, *rps16*, and *matK*, totaling 966 sequence records). An analysis of all available ITS sequences in which to sort taxa into smaller natural units is the first step towards a global phylogeny of Apioideae. The most widely used region from the chloroplast genome for Apioideae phylogenetic study is the gene *rps16* (specifically, its intron) and its flanking intergenic spacer regions, with 378 sequence records currently available in GenBank.

Table 1. Composition of major clades within Apiaceae subfamily Apioideae supported on the basis of phylogenetic analyses of cpDNA and/or nrDNA ITS sequence data. Names in brackets are allied closely to the indicated clade and may even be included within that clade pending confirmation from further study. Asterisks indicate genera that are confirmed as not monophyletic within their respective clades; the absence of an asterisk, however, does not mean that the genus is monophyletic if it has not been subject to comprehensive study. Genera assigned to two or more major clades are indicated and are also summarized in Table 2. Cited references provide further information, such as discussions of taxonomic problems and phylogenetic relationships.

Aciphyllae M.F. Watson & S.R. Downie^{1,2,3,14,16}

Aciphylla J.R. Forst. & G. Forst.*
Anisotome Hook.f.*
Gingidia J.W. Dawson*
Lignocarpa J.W. Dawson
Scandia J.W. Dawson*

Acronema Clade^{4,10}

Acronema Falc. ex Edgew.
Angelica anomala Avé-Lall.
Halosciastrum Koidz.
Harrysmithia H. Wolff
Kitagawia Pimenov
Ligusticum L.*
Meeboldia H. Wolff
Oreocomopsis Pimenov & Kljuykov
Ostericum grosseserratum (Maxim.) Kitag., *Ostericum scaberulum*
 (Franch.) C.Q. Yuan & R.H. Shan
Pachypleurum Ledeb.
Pimpinella brachycarpa Nakai
Pleurospermum hookeri C.B. Clarke, *P. yunnanense* Franch.
Pternopetalum Franch.
Pterygopleurum Kitag.
Rupiphila Pimenov & Lavrova
Sinocarum H. Wolff ex R.H. Shan & F.T. Pu
 (includes *Apium ventricosum* H. Boissieu¹⁰)
Spuriopimpinella (H. Boissieu) Kitag.
Tilingia Regel & Tiling*

Annesorhiza Clade^{5,6,15}

Annesorhiza Cham. & Schldl.*
Chamarea Eckl. & Zeyh.*
Itasina Raf.
 [*Astydamia* DC., *Ezosciadium* B.L. Burtt, *Molopospermum* W.D.J. Koch]

Apiace Takht. ex V.M. Vinogr.^{7,8,9}

Ammi L.
Anethum L.
Apium L.
Billburttia Magee & B.-E. van Wyk
Deverra DC.
Foeniculum Mill.
Naufraga Constance & Cannon
Petroselinum Hill
Pseudoridolfia J.-P. Reduron, J. Mathee & S.R. Downie
Ridolfia Moris

Sclerosciadium W.D.J. Koch ex DC.
Seseli webbii Coss.
Stoibrax Raf.

Arcuatopterus Clade^{4,10}

Arcuatopterus M.L. Sheh & R.H. Shan

Bupleureae Spreng.^{2,10,11,12}

Bupleurum L. (includes *Hohenackeria* Fisch. & C.A. Mey.)

Cachrys Clade^{13,41}

Alococarpum Riedl & Kuber
Azilia Hedge & Lamond
Bilacunaria Pimenov & V.N. Tikhom.
Cachrys L.
Diplotaenia Boiss.
Eriocycla Lindl.
Ferulago W.D.J. Koch
Prangos Lindl.*

Careae Baill.^{7,13,14,16,48}

Aegokeras Raf.
Aegopodium L.*
Carum L.*
Chamaele Miq.
Chamaesciadium C.A. Mey.
Falcaria Fabr.
Fuernrohria K. Koch
Gongylosciadium Rech.f.
Grammosciadium DC.*
Hladnikia Rechb.
Pimpinella anthriscoides Boiss.
Rhabdosciadium Boiss.

Chamaesium Clade⁴ [Tribe Chamaesieae J. Zhou & F.D. Pu⁵⁰]

Chamaesium H. Wolff

Conioselinum chinense Clade^{2,7}

Conioselinum chinense (L.) Britton, Sterns & Poggenb.,
C. scopulorum (A. Gray) J.M. Coult. & Rose
Ligusticum canadense (L.) Britton, *L. porteri* J.M. Coult. & Rose
Meum Mill.
Mutellina Wolf
Trochiscanthes W.D.J. Koch

Conium Clade

Conium L.

Coriandreae W.D.J. Koch

Bifora Hoffm.
Coriandrum L.

Diplolophium Clade¹⁵

Diplolophium Turcz.

Echinophoreae Benth. & Hook.f.^{7,16}

Anisosciadium DC.
Dicyclophora Boiss.
Echinophora L.*

Pycnocycla Lindl.

[*Mediasia* Pimenov, *Nirarathamnos* Balf.f., *Pimpinella heyneana* Wall ex Kurz,
“*Rughidia*” ined., *Trachyspermum aethusifolium* Chiov.]

Erigenieae Rydb.^{5,16}

Erigenia Nutt.

Heteromorpheae M.F. Watson & S.R. Downie^{2,5,15,16}

Heteromorpha Clade⁵

Anginon Raf. (includes *Glia* Sond.⁵)

Heteromorpha Cham. & Schldl.

Polemanna Eckl. & Zeyh.

Malagasy Clade⁵

Andriana B.-E. van Wyk

Anisopoda Baker

Cannaboides B.-E. van Wyk

Dracosciadium Hilliard & B.L. Burtt

“*Oreofraga*” ined.

Pseudocannaboides B.-E. van Wyk

Pseudocarum C. Norman*

Tana B.-E. van Wyk

Komarovia Clade^{2,5,17,46} [Tribe Komarovieae J. Zhou & S.R. Downie⁵⁰]

Calyptrosiadium Rech.f. & Kuber

Changium H. Wolff

Chuanminshen M.L. Sheh & R.H. Shan

Cyclorhiza M.L. Sheh & R.H. Shan

Komarovia Korovin

Parasilaus Leute

Sphaerosciadium Pimenov & Kljuykov

Lichtensteinia Clade^{5,18}

Lichtensteinia Cham. & Schldl.

Oenantheae Dumort.^{2,8,14,19,20,21,22}

Atrema DC.

Berula W.D.J. Koch (includes *Afrocarum* Rauschert)

Cicuta L.

Cryptotaenia DC. (includes *Lereschia* Boiss.)

Cynosciadium DC.

Daucosma Engelm. & A. Gray ex A. Gray

Harperella Rose (*Ptilimnium* pro parte)

Helosciadium W.D.J. Koch

Lilaeopsis Greene

Limnosciadium Mathias & Constance

Neogoezia Hemsl.

Oenanthe L.

Oxypolis Raf.

Perideridia Rehb.

Ptilimnium Raf.

Sium L.

Tiedemannia DC. (*Oxypolis* pro parte)

Trepocarpus Nutt. ex DC.

Opopanax Clade¹³

Opopanax W.D.J. Koch

Smyrniopsis Boiss.

[*Crenosciadium* Boiss. & Heldr. ex Boiss., *Kruberia* Hoffm., *Magydaris* W.D.J. Koch ex DC., *Petroedmondia* Tamamsch., *Stefanoffia* H. Wolff, *Visnaga* Mill.]

Physospermopsis Clade (East Asia Clade)^{4,5,10}

Hansenia Turcz.

Haplosphaera Hand.-Mazz.

Heptaptera Margot & Reut.

Hymenolaena DC.

Keraymonia Farille

Notopterygium H. Boissieu*

Physospermopsis H. Wolff*

Pimpinella acuminata (Edgew.) C.B. Clarke, *P. henryi* Diels,

P. purpurea (Franch.) H. Boissieu

Sinocarum cruciatum (Franch.) H. Wolff ex F.T. Pu

S. dolichopodum (Diels) H. Wolff ex F.T. Pu

Sinolimprichtia H. Wolff

Spuriopimpinella brachycarpa (Nakai) Kitag.

Tongoloa H. Wolff*

Trachydium simplicifolium W.W. Sm.

Vicatia bipinnata R.H. Shan & F.T. Pu

Pimpinelleae Spreng.^{4,7,8,13,16,26,41}

Aphanopleura Boiss.*

Arafoe Pimenov & Lavrova

Bubon L.

Cryptotaenia africana (Hook.f.) Drude, *C. calycina* C.C. Towns.

Demavendia Pimenov

Frommia H. Wolff

Hausknechtia Boiss.

Nothosmyrnum Miq.

Opsicarpium Mozaff.

Phellolophium Baker

Physospermopsis cuneata H. Wolff

Pimpinella L.*

Psammogeton Edgew.*

Seseli diffusum (Roxb. ex Sm.) Santapau & Wagh.

Trachyspermum scaberulum (Franch.) H. Wolff, *T. triradiatum* H. Wolff

Zeravschania Korovin*

Pleurospermeae M.F. Watson & S.R. Downie^{2,4,5}

Aulacospermum Ledeb.

Eleutherospermum K. Koch

Eremodaucus Bunge

Hymenidium Lindl.*

Korshinskya Lipsky

Physospermopsis muliensis R.H. Shan & S.L. Liou,

P. shaniana C.Y. Wu & F.T. Pu

Physospermum Cusson

Pleurospermum Hoffm.*

Pseudotrachydium (Kljuykov, Pimenov & V.N. Tikhom.) Pimenov &

Kljuykov

Trachydium Lindl.*

Pleurospermopsis Clade

Pleurospermopsis C. Norman

Pyramidoptereae Boiss.^{7,8,14,16,23,38}

- Ammooides* Adans.
Astomaea Rchb.
Bunium L.*
Carum apuanum (Viv.) Grande, *C. buriaticum* Turcz., *C. heldreichii* Boiss.,
C. piovanii Chiov., *C. rigidulum* (Viv.) W.D.J. Koch ex DC.
Crithmum L.
Cyclospermum Lag.
Elaeosticta Fenzl
Galagania Lipsky
Gongylotaxis Pimenov & Kljuykov
Hellenocarum H. Wolff
Hyalolaena Bunge*
Indoschulzia Pimenov & Kljuykov
Kosopoljanskia Korovin
Lagoecia L.
Ligusticum rhizomaticum Hartvig
Lipskya (Koso-Pol.) Nevski
Mogoltavia Korovin
Muretia Boiss.
Notiosciadium Speg.
Oedibasis Koso-Pol.*
Oreoschimperella Rauschert
Ormopterum Schischk.
Pimpinella siifolia Leresche
Postiella Kljuykov
Pyramidoptera Boiss.
Scaligeria DC.*
Schrenkia Fisch. & C.A. Mey.
Schtschurowskia Regel & Schmalh.
Schulzia Spreng.
Sison L.* (includes *Petroselinum segetum* (L.) W.D.J. Koch)
Tamamschjanella Pimenov & Kljuykov*
Trachyspermum ammi (L.) Sprague ex Turrill

Scandiceae Spreng.^{2,14,26,27}

- Artemisia* Clade²⁷
Artemisia L.

Daucinae Dumort.^{8,26,27,28}
Agrocharis Hochst.
Ammodaucus Coss. & Durieu ex Coss.
Athamanta della-cellae Asch. & Barbey ex E.A. Durand & Barratte
Cryptotaenia elegans Webb ex Bolle
Cuminum L.
Daucus L.*
Laser Borkh. ex P. Gaertn., B. Mey. & Schreb.
Laserpitium L.*
Melanoselinum Hoffm.
Monizia Lowe
Orlaya Hoffm.
Pachyctenium Maire & Pampan. ex Pampan.
Polylophium Boiss.
Pseudorlaya (Murb.) Murb.*
Thapsia L.

Tornabenea Parl. ex Webb

Ferulinae Engl.^{13,25,27}

Ferula L. (includes *Dorema* D. Don, *Leutea* Pimenov, *Soranthus* Ledeb.,
Schumannia Kuntze, and *Talassia* Korovin)

[*Autumnalia* Pimenov, *Fergania* Pimenov, *Kafirnigania* Kamelin & Kinzik.⁴⁶]

Glaucosciadium Clade^{16,25}

Glaucosciadium B.L. Burt & P.H. Davis (includes *Mozaffariania* Pimenov &
Maassoumi)

Scandicinae Tausch^{24,26,29,42,43,44}

Anthriscus Pers.

Athamanta L. (includes *Tinguarra* Parl.)

Chaerophyllum H. Boissieu⁵⁰

Chaerophyllum L. (includes *Myrrhoides* Fabr. and *Oreomyrrhis* Endl.)

Conopodium W.D.J. Koch (includes *Balansaea* Boiss. & Reut.)

Geocaryum Coss.

Kozlovina Lipsky (includes *Krasnovia* Popov ex Schischk. and *Neoconopodium*
(Koso-Pol.) Pimenov & Kljuykov)

Myrrhis Mill.

Osmorhiza Raf.

Scandix L.

Sphallerocarpus DC.

Todaroa Parl.

Torilidinae Dumort.^{27,28}

Astrodaucus Drude

Caucalis L.

Glochidotheca Fenzl

Lisaea Boiss.

Szovitsia Fisch. & C.A. Mey.

Torilis Adans. (includes *Chaetosciadium* Boiss.²⁷)

Turgenia Hoffm.

Yabea Koso-Pol.

Selineae Spreng.^{4,7,13,30,39,40,46,47,49}

Aethusa L.

Ammoselinum Torr. & A. Gray

Angelica L.* (includes *Coelopleurum* Ledeb., *Czernaevia* Turcz. ex Ledeb.,

Ostericum Hoffm. pro parte, *Sphenosciadium* A. Gray³⁰,

Melanosciadium H. Boissieu, and *Pimpinella smithii* H. Wolff⁵⁰)

Apiastrum Nutt. ex Torr. & A. Gray

Carlesia Dunn.

Cervaria Wolf*

Chymydia Albov

Cnidiocarpa Pimenov

Cnidium Cusson

Cortia DC.*

Cortiella C. Norman

Dichoropetalum Fenzl (includes *Holandrea* Reduron, Charpin & Pimenov
and *Johreniopsis* Pimenov⁴⁵)

Dimorphosciadium Pimenov

Dystaenia Kitag.*

Endressia J. Gay

Exoacantha Labill.

Ferulopsis Kitag.
Glehnia F. Schmidt ex Miq.*
Imperatoria L.
Johrenia DC.
Kadenia Lavrova & V.N. Tikhom.
Kailashia Pimenov & Kljuykov
Karatavia Pimenov & Lavrova
Katapsuxis Raf.
Kedarnatha P.K. Mukh. & Constance
Kitagawia Pimenov
Ledebouriella H. Wolff
Libanotis Haller ex Zinn*
Ligusticopsis Leute*
Ligusticum daucoides Franch., *L. involucratum* Franch.,
L. mucronatum (Schrenk) Leute, *L. oliverianum* (H. Boissieu) Shan,
L. physospermifolium Albov, *L. pteridophyllum* Franch. ex Oliv.,
L. scapiforme H. Wolff
Lomatocarpa Pimenov
Macroscladium V.N. Tikhom. & Lavrova
Magadania Pimenov & Lavrova
Oligocladus Chodat & Wilczek
Oreocome Edgew.*
Oreoselinum Mill.
Ormosolenia Tausch
Pachypleurum mutellinoides (Crantz) J. Holub
Paraligusticum V.N. Tikhom.
Peucedanum L.*
Phlojodicarpus Turcz. ex Ledeb.
Pilopleura Schischk.
Pteroselinum (Rchb.) Rchb.
Rumia Hoffm.
Sajanella Soják
Saposhnikovia Schischk.
Selinum L.*
Seseli L.*
Spermolepis Raf.
Stenocoelium Ledeb.
Thecocarpus Boiss.
Thysselinum Adans.
Tommasinia Bertol.
Trinia Hoffm.
Vicatia DC.
Xanthogalum Lallem.
Xanthoselinum Schur

Arracacia Clade^{2,16,20,30,31,37}

Arracacia Bancr.*
Coaxana J.M. Coult. & Rose*
Coulterophytum B.L. Rob.*
Dahliaphyllum Constance & Breedlove
Donnellsmithia J.M. Coult. & Rose
Enantiophylla J.M. Coult. & Rose
Mathiasella Constance & C. Hitch.
Neonelsonia J.M. Coult. & Rose

Myrrhidendron J.M. Coult. & Rose
Ottoa Kunth
Prionosciadium S. Watson*
Rhodosciadium S. Watson*
 [*Cotopaxia* Mathias & Constance, *Niphogeton* Schltdl.,
Perissocoeleum Mathias & Constance]

Perennial Endemic North American Clade^{31,32,33,34}

Aletes J.M. Coult. & Rose*
Cymopterus Raf.*
Eurytaenia Torr. & A. Gray
Harbouria J.M. Coult. & Rose
Lomatium Raf.*
Musineon Raf.*
Neoparrya Mathias
Oreonana Jeps.
Oreoxis Raf.*
Orogenia S. Watson
Podistera S. Watson*
Polytaenia DC.*
Pseudocymopterus J.M. Coult. & Rose*
Pteryxia (Nutt. ex Torr. & A. Gray) J.M. Coult. & Rose*
Shoshonea Evert & Constance
Taenidia (Torr. & A. Gray) Drude
Tauschia Schltdl.*
Thaspium Nutt.
Zizia W.D.J. Koch

Sinodielsia Clade⁴

Angelica sinensis (Oliv.) Diels, *A. paeoniifolia* C.Q. Yuan & R.H. Shan,
A. tianmuensis Z.H. Pan & T.D. Zhuang
Cenolophium W.D.J. Koch
Cnidium dahuricum (Jacq.) Turcz. ex Fisch. & C.A. Mey.,
C. officinale Makino
Conioselinum Hoffm.
 [*Conioselinum chinense* (L.) Britton, Sterns & Poggenb.⁴⁰]
Levisticum Hill
Ligusticum acuminatum Franch.,
L. sinense 'Chuanxiong' S.H. Qiu, Y.Q. Zeng, K.Y. Pan, Y.C. Tang &
 J.M. Xu, *L. jeholense* (Nakai & Kitag.) Nakai & Kitag.,
L. tenuissimum (Nakai) Kitag.
Lithosciadium Turcz.
Paulita Soják
Pleurospermum prattii H. Wolff
Prangos haussknechtii Boiss.
Pterocyclus Klotzsch
Seselopsis Schischk.
Silaum Mill.
Sinodielsia H. Wolff
Sphaenolobium Pimenov
Vicatia tibetica H. Boissieu

Smyrnieae Spreng.^{2,13,16}

Lecokia DC.
Smyrnum L.

Tordylieae W.D.J. Koch^{7,9,13,36}

Tordyliinae Engl.

Angelica likiangensis H. Wolff, *A. oncosepala* Hand.-Mazz.
Heracleum L.*
Kandaharia Alava
Lalldhwojia Farille
Leiotulus Ehrenb.*
Malabaila Hoffm.*
Mandenovia Alava
Pastinaca L.*
Semenovia Regel & Herder*
Symphyoloma C.A. Mey.
Tetrataenium (DC.) Manden.*
Tordyliopsis DC.
Tordylium L. (includes *Ainsworthia* Boiss. and *Synelcosciadium* Boiss.)
Trigonosciadium Boiss.*
Vanasushava P.K. Mukh. & Constance
Zosima Hoffm.

Cymbocarpum Clade^{9,13}

Cymbocarpum DC. ex. C.A. Mey. (includes *Kalakia* Alava)
Ducrosia Boiss.

Lefebvrea Clade^{9,35}

Afroligusticum C. Norman
Afrosciadium P.J.D. Winter
Capnophyllum Gaertn.
Cynorrhiza Eckl. & Zeyh.
Dasispermum Raf. (includes *Sonderina* H. Wolff)
Lefebvrea A. Rich
Nanobubon Magee
Notobubon B.-E. van Wyk
Scaraboides Magee & B.-E. van Wyk
Stenosemis E. Mey. ex Harv. & Sond.

¹ Mitchell et al. (1998); ² Downie et al. (2000b); ³ Radford et al. (2001); ⁴ Zhou et al. (2008); ⁵ Calvino et al. (2006); ⁶ Magee et al. (2008); ⁷ Downie et al. (2000c); ⁸ Spalik & Downie (2007); ⁹ Winter et al. (2008); ¹⁰ Valiejo-Roman et al. (2002b); ¹¹ Valiejo-Roman et al. (2002a); ¹² Neves & Watson (2004); ¹³ Ajani et al. (2008); ¹⁴ Downie et al. (1998); ¹⁵ Downie & Katz-Downie (1999); ¹⁶ Downie et al. (2001); ¹⁷ Katz-Downie et al. (1999); ¹⁸ Tilney et al. (2008); ¹⁹ Downie et al. (2008); ²⁰ Plunkett et al. (1996); ²¹ Spalik et al. (2009); ²² Hardway et al. (2004); ²³ Degtjareva et al. (2008); ²⁴ Spalik et al. (2001a); ²⁵ Kurzyna-Młynik et al. (2008); ²⁶ Downie et al. (2000a); ²⁷ Lee & Downie (2000); ²⁸ Lee & Downie (1999); ²⁹ Chung et al. (2005); ³⁰ Spalik et al. (2004); ³¹ Downie et al. (2002); ³² Sun & Downie (2004); ³³ Sun et al. (2004); ³⁴ Sun & Downie (2010); ³⁵ Magee et al. (2009); ³⁶ Logacheva et al. (2008); ³⁷ Downie & Katz-Downie (1996); ³⁸ Papini et al. (2007); ³⁹ Xue et al. (2007); ⁴⁰ Valiejo-Roman et al. (2006a); ⁴¹ Valiejo-Roman et al. (2006b); ⁴² Spalik & Downie (2001); ⁴³ Spalik et al. (2001b); ⁴⁴ Chung (2007); ⁴⁵ Pimenov et al. (2007); ⁴⁶ Terentjeva et al. (2008); ⁴⁷ Valiejo-Roman et al. (2008); ⁴⁸ Papini (2006); ⁴⁹ Reduron et al. (1997); ⁵⁰ Zhou et al. (2009).

In this paper, we review the results of phylogenetic studies of Apiaceae subfamily Apioideae published since the 3rd International Symposium on the Apiales (Watson et al. 2001), the vast majority of which have been based exclusively on ITS sequences. We use these results, as well as our own analyses of currently available and newly generated ITS sequence data, to test previously defined tribal and generic limits within

the subfamily, ascertain the phylogenetic placements of taxa not included in the aforementioned studies, and identify additional major clades. Our ultimate goal, which by necessity will be a multinational, interdisciplinary, and collaborative effort, is to produce an explicit, species-level phylogenetic hypothesis for this large and taxonomically complex subfamily. As a major step towards reaching this goal, we update the phylogenetic classification proposed by Downie et al. (2001) by recognizing additional major clades inferred through examination of ITS sequences whose monophyly and relationships can be tested using evidence from cpDNA and other molecular markers. If future studies corroborate these clades, additional tribes and subtribes in Apioideae may be realized. As ancillary objectives, we identify polyphyletic genera for future collaborative study and discuss problematic ITS sequences currently available in GenBank.

ITS Phylogenetic Analysis

ITS sequences from 1240 taxa of Apiaceae subfamily Apioideae, representing most higher apioid species in GenBank for which ITS data are currently available plus 53 newly obtained sequences, were subjected to a phylogenetic analysis. Only those taxa that included complete and high quality data from both intergenic spacer regions were considered. Approximately half of these taxa lacked data from the intervening 5.8S gene region. These 1240 accessions represent 292 genera and 959 species from throughout the geographic range of the subfamily and include all tribes and major clades inferred previously on the basis of molecular systematic studies, except its most early diverging branches (Heteromorphaeae, *Annesorhiza* Clade, *Lichtensteinia* Clade), other basal apioid genera of uncertain phylogenetic position (*Astydamia* DC., *Choritaenia* Benth., *Ezosciadium* B.L. Burt, *Marlothiella* H. Wolff, *Molopospermum* W.D.J. Koch), and a lineage of tribe Oenantheae characterized by rapid ITS sequence evolution (North American Endemics Clade in Hardway et al. 2004). All taxa excluded from the phylogenetic analysis have ITS sequences that cannot be unambiguously aligned with those of most members of the crown clades. Similarly, we did not include several other taxa where previous studies had identified ITS sequences with numerous length mutations or high sequence divergence estimates that also led to ambiguities in alignment. Alignment of these sequence data resulted in a matrix of 735 characters, with none excluded because of alignment ambiguities. Initially, the ITS sequences were aligned using ClustalX (Jeanmougin et al. 1998), but missing data in the 5.8S region for about half of the taxa caused problems, especially when altering the gap-opening and gap-extension parameters. Manual adjustments of the ensuing alignments were very time consuming because of the large number of accessions involved. Subsequently, the data matrices representing the greatest diversity of apioid taxa from our previous studies (which included data for the 5.8S region) were aligned to each other (Spalik & Downie 2007, Ajani et al. 2008, Zhou et al. 2008) and all additional sequences appended and aligned to this large matrix manually. The DNA sequences in these previously published data matrices were aligned using default parameters for gap opening and gap extension, followed by manual adjustment. These data were analyzed using

maximum parsimony (MP) and neighbor-joining methods in PAUP* vers. 4.0b10 (Swofford 2002), using the methods described in our previous studies for large data sets (e.g., Downie et al. 2000a, Spalik & Downie 2007). Bootstrap values were calculated from 100,000 replicate analyses using fast stepwise addition of taxa and only those values compatible with a majority-rule consensus tree were recorded. The trees were rooted with three species of *Bupleurum* (tribe Bupleureae), as previous studies of several plastid markers have supported its sister group relationship to all other apioid genera, excluding those of its most early diverging branches (Downie et al. 2001, Calviño et al. 2006).

We reiterate that our major objectives are to test previously defined tribal and generic limits within the subfamily Apioideae, as already inferred using ITS sequences and several cpDNA markers (Downie et al. 2001), and to identify additional major clades using the wealth of currently available ITS sequences in GenBank and those generated specifically for this study. The monophyly of these newly recognized clades and their interrelationships can be tested subsequently using additional molecular evidence, such as that from cpDNA. Past studies have reported that ITS data are often inadequate to estimate intertribal and, especially, deep level relationships within Apioideae (Downie et al. 1998, Katz-Downie et al. 1999). While similar major groupings of taxa are resolved in different phylogenetic studies, the relationships among them may be ambiguous, weakly supported and/or conflicting depending upon the sampling and method of analysis. In general, the ITS region is simply too small and the data too homoplastic for confident resolution at these levels. Even among distally branching clades, such as those major clades comprising the apioid superclade (Plunkett & Downie 1999), resolution of relationships is ambiguous. As such, in this study, we refrain from discussing relationships among the tribes and major clades revealed exclusively through phylogenetic analysis of ITS data and, therefore, do not present phylogenetic trees containing all 1240 taxa. Moreover, differences were apparent between the results of the MP and neighbor-joining analyses with regard to the relative placements of some of the major clades. We reveal only the best-supported, major clades inferred through these analyses and present a very conservative estimate of their interrelationships. Resolution of relationships among these major clades, especially those within the apioid superclade, must await consideration of multiple loci from both chloroplast and nuclear genomes and a broad sampling of taxa from all major clades.

An ITS-based Phylogenetic Classification of Apioideae

A previous classification of Apiaceae subfamily Apioideae, presented in the Proceedings of the 3rd International Symposium on the Apiales (Downie et al. 2001), was based on results of phylogenetic analyses of diverse molecular data sets, including chloroplast gene and intron sequences, cpDNA restriction site variation, and ITS sequences. Ten tribes and three subtribes were erected or confirmed as monophyletic and seven additional major clades were identified. In constructing this classification, a conservative approach was taken so that only well-supported clades revealed by multiple analyses of both chloroplast and nuclear markers were recognized. Clades were recognized

at the tribal or subtribal level based on priority of validly published names, as indicated by Pimenov & Constance (1985) and regulated by the *International Code of Botanical Nomenclature*. If material from the type of a genus that had priority was not examined, we refrained from formally recognizing that clade. Representatives of 23 genera were not assigned to any tribe or major clade because of uncertainties in their clade assignment. This prior phylogenetic classification of Apioideae was based on results from 195 genera and 450 species, representing about one-half of the 404 genera and just over one-sixth of the 2827–2935 species recognized in the subfamily by Pimenov & Leonov (1993).

Herein, we amend the classification presented by Downie et al. (2001) upon consideration of the many published molecular phylogenetic studies since that time and the results of our analyses of ITS data. In this study, 1240 ITS sequences from 292 genera and 959 species were analyzed, representing 100 more genera and over double the number of species as examined previously. Forty-one major clades within Apioideae are identified (Table 1), of which 21 have already been recognized at the tribal or subtribal rank, and a conservative estimate of their relationships, as inferred through this study and, for the basally branching lineages, prior phylogenetic studies using cpDNA, is presented in Fig. 1. We exclude from this number the *Arracacia* Clade and the Perennial Endemic North American Clade, as they occur within a paraphyletic tribe Selineae (see discussion below). The composition of each of these major clades is also presented, based primarily on the results of the phylogenetic analyses presented herein, but also on the results of other studies cited in Table 1. Changes to the tribal classification of Apiaceae subfamily Apioideae outlined by Downie et al. (2001) are minimal, and are as follows: (1) The transfer of *Cyclospermum* Lag. from Careae to Pyramidoptereae (as a result of an identification error). (2) The transfer of *Molopospermum* from Pleurospermeae to near or within the *Annesorhiza* Clade (Calviño et al. 2006). *Molopospermum* was provisionally included in Pleurospermeae by Downie et al. (2001) based on results of the serological studies of Shneyer et al. (1992) who reported that *Molopospermum* and the “*Physospermum-Pleurospermum* group” are serologically similar. (3) The transfer of *Chamaele* Miq. from the *Angelica* Clade (tribe Selineae) to tribe Careae. Its placement in tribe Selineae by Downie et al. (2001) was influenced by the *rbcl* results of Kondo et al. (1996), where *Chamaele decumbens* (Thunb. ex Murr.) Makino was sister group to a clade comprising several genera of tribe Selineae. (4) The transfer of *Notopterygium* H. Boissieu from Selineae to the *Physospermopsis* Clade. The initial placement of *Notopterygium* in Selineae (Downie et al. 1998) was based on an identification error (Valiejo-Roman et al. 2002b, Spalik et al. 2004). (5) The transfer of *Hansenia* Turcz. and *Physospermopsis* H. Wolff from the *Komarovia* Clade to the *Physospermopsis* Clade based on additional sampling of the taxonomically complex taxa comprising each of these clades (Calviño et al. 2006). (6) The transfer of *Meum* Mill. from Selineae to the *Conioselinum chinense* Clade, as a result of an identification error. Representatives of the 23 genera that could not be unambiguously assigned to a tribe or major clade previously are now each assigned to a particular group. Overall, the tribes and major clades of Apioideae outlined by Downie et al. (2001) are still valid today, although the increased sampling since that time has expanded the circumscriptions of many of these lineages.

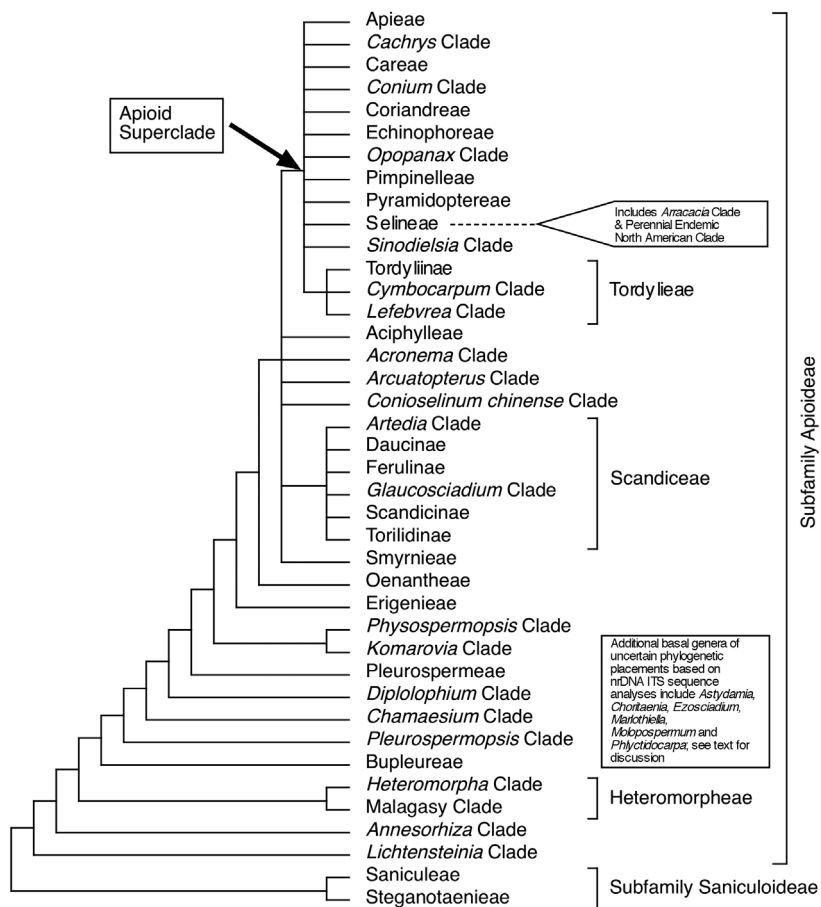


Fig. 1. Summary of relationships among the major clades of Apiaceae subfamily Apioideae as revealed by phylogenetic analysis of molecular data. Forty-one major clades are identified (not including the *Arracacia* and Perennial Endemic North American clades in tribe Selineae, see text for discussion), of which 21 are recognized at the tribal or subtribal rank. This tree is based, in part, on previous studies using both ITS and cpDNA markers (Downie et al. 2001, Calviño et al. 2006) and, in part, on the results of recently published studies and the phylogenetic analyses presented herein based exclusively on ITS sequences. The apioid superclade is a large and morphologically variable group of umbellifers containing a minimum of 14 major clades of largely uncertain relationship. The phylogenetic placements of basal genera *Astydamia*, *Choritaenia*, *Ezosciadium*, *Marlothiella*, *Molopospermum* and *Phlyctidocarpa* have yet to be confirmed using both ITS and cpDNA markers; those genera that have putative affinities with Apioideae are listed in Table 1. Apiaceae subfamily Saniculoideae comprises two tribes: Saniculeae and Steganotaenieae (Calviño & Downie 2007).

Since the classification of Apioideae presented by Downie et al. (2001), additional clades and tribes have been identified. Some of these new groups have now received support from both ITS and cpDNA studies (Apieae, Coriandreae, Erigenieae, Scandiceae subtribe Ferulinae, *Annesorhiza* Clade, *Arracacia* Clade, *Glaucosciadium* Clade, *Komarovia* Clade, *Lefebvrea* Clade, *Lichtensteinia* Clade, Perennial Endemic

North American Clade, *Physospermopsis* Clade; Calviño et al. 2006, Kurzyna-Młynik et al. 2008, Magee et al. 2008, Sun & Downie, 2010, C.A. Danderson, University of Illinois at Urbana-Champaign, USA, unpublished data), whereas other groups are recognized primarily on the basis of ITS sequence data (*Acronema* Clade, *Cachrys* Clade, *Chamaesium* Clade, *Conioselinum chinense* Clade, *Cymbocarpum* Clade, *Opopanax* Clade, Pimpinelleae, Selineae, *Sinodielsia* Clade, Tordylieae subtribe Tordyliinae; Spalik et al. 2004, Spalik & Downie 2007, Ajani et al. 2008, Winter et al. 2008, Zhou et al. 2008, 2009). Further molecular systematic studies are necessary to corroborate the monophyly of these primarily ITS-derived clades, if they are to be recognized formally at the tribal or subtribal level or, as with the case of tribes Pimpinelleae, Selineae, and Tordylieae, if they continue to be recognized as currently circumscribed.

Several additional changes to the classification outlined by Downie et al. (2001) have been made (or will have to be made pending further study). The *Conioselinum chinense* Clade (Downie et al. 2000b) was recognized originally as a lineage comprising only two species, *Conioselinum chinense* (L.) Britton, Sterns & Poggenb. and *Ligusticum porteri* J.M. Coult. & Rose (Downie et al. 1998). Subsequent studies included additional species of *Conioselinum* Hoffm. and *Ligusticum* L. (Katz-Downie et al. 1999) and, in the current study, the clade is expanded to include *Mutellina* Wolf and *Trochiscanthes* W.D.J. Koch. The type species for *Conioselinum*, *C. tataricum* Hoffm., occurs in the *Sinodielsia* Clade, adjacent to *Ligusticum acuminatum* Franch. and another accession of *C. chinense*, and the type species of *Ligusticum*, *L. scoticum* L., occurs in the *Acronema* Clade. The placement of *C. chinense* in two separate clades indicates that one of these specimens is misidentified. *Conioselinum* and *Ligusticum* are both in need of revision. Pending further study, however, we continue to recognize this group as the *Conioselinum chinense* Clade.

Three major lineages are recognized in tribe Tordylieae: (1) The *Heracleum* Clade of previous molecular phylogenetic studies (Downie et al. 2000b, 2000c, 2001), coinciding partly with Peucedaneae subtribe Tordyliinae Engl. sensu Drude (1898) or tribe Tordylieae W.D.J. Koch sensu Pimenov & Leonov (1993) and containing such genera as *Heracleum* L., *Malabaila* Hoffm., *Pastinaca* L., *Semenovia* Regel & Herder, and *Tordylium* L. (2) The *Cymbocarpum* Clade, comprising the genera *Cymbocarpum* DC. ex C.A. Mey. (incl. *Kalakia* Alava) and *Ducrosia* Boiss. (Ajani et al. 2008). *Ducrosia* and *Kalakia* have also been treated previously in tribe Tordylieae. (3) The “African peucedanoid group” or the *Lefebvrea* Clade (Spalik & Downie 2007, Winter et al. 2008, Magee et al. 2009). These three clades have already been considered in a broad tribe Tordylieae (Ajani et al. 2008, Winter et al. 2008, Magee et al. 2009), but the relationships among them are equivocal. Most analyses reveal a sister group relationship between the *Cymbocarpum* and *Lefebvrea* clades, whereas a MP strict consensus tree reveals a trichotomy (Ajani et al. 2008). Therefore, we recognize tribe Tordylieae as constituting three distinct clades of equal rank and recognize the aforementioned *Heracleum* clade as Tordylieae subtribe Tordyliinae Engl. Recognition of the *Cymbocarpum* and *Lefebvrea* clades at subtribal ranks is forthcoming.

With representatives from at least 94 genera, tribe Selineae is the largest clade within the subfamily and thus, would also benefit by the recognition of subtribes. Previously, two major groups have been recognized within the tribe, the Central and South

American *Arracacia* Clade and the Perennial Endemic North American Clade of primarily western North American distribution, but each has been subsumed without formal recognition within tribe Selineae (Spalik et al. 2004). Recognizing these groups as subtribes would render the Eurasian members of the tribe paraphyletic, as the type species of the tribe, *Selinum carvifolia* (L.) L., is placed in one of its basal branches and these two New World clades are placed in the crown branches. Hence, based on our current ITS phylogeny, we would have to recognize numerous subtribes, many of which would be monotypic. However, we do acknowledge that a comprehensive cpDNA-based phylogeny for Selineae is not currently available and, until that time we maintain recognition of these two major clades in our provisional ITS-based classification as they are subjects of ongoing studies (Sun 2003 and unpublished data; C.A. Danderson, unpublished data).

Tribe Heteromorphae constitutes two major clades, one of which we have recognized as the Malagasy Clade based on the work of Calviño et al. (2006). This clade includes five genera endemic to Madagascar and other genera from Socotra or more broadly distributed in Africa. The recognition of the *Heteromorpha* and Malagasy clades at subtribal ranks will be considered in an upcoming paper (C.I. Calviño et al., INIBIOMA, CONICET - Universidad Nacional del Comahue, Argentina, manuscript in preparation).

Ten clades are recognized as monogeneric (*Arcuatopterus* Clade, *Artemisia* Clade, Bupleureae, *Chamaesium* Clade, *Conium* Clade, *Diplolophium* Clade, Erigenieae, *Glaucosciadium* Clade, *Lichtensteinia* Clade, and the *Pleurospermopsis* Clade), three of which are monotypic (*Artemisia squamata* L., *Erigenia bulbosa* (Michx.) Nutt., and *Pleurospermopsis sikkimense* (C.B. Clarke) C. Norman). These clades represent either isolated lineages with no known close relatives or, as in the case of the *Artemisia* and *Glaucosciadium* clades, genera whose sister group relationships within Scandiceae are neither suggested by morphology nor unambiguously inferred from molecular data and, thus, are treated as distinct lineages of equal rank within the tribe. Two clades (Bupleureae and Erigenieae) have already been treated at the tribal level because their isolated positions have been corroborated through multiple studies using both ITS and cpDNA data. Future studies incorporating cpDNA evidence and further sampling are necessary to confirm the isolated status of the remaining clades and their recognition at tribal ranks.

The phylogenetic placements of several basal genera excluded from the ITS analysis presented herein have been or are currently being considered elsewhere. *Astydamia*, *Ezoscadiium* and *Molopospermum* are allied closely with the *Annesorhiza* clade and in some trees comprise a clade that is sister group to it (Calviño et al. 2006, Magee et al. 2008, C.I. Calviño, unpublished data). The relationships among these taxa, however, are generally poorly supported and additional studies are necessary to confirm their phylogenetic positions among basal Apioideae. Similarly, a basal position of *Choritaenia* within Apioideae has also been proposed, with preliminary analyses suggesting an allegiance with the *Lichtensteinia* Clade (Calviño et al. 2006, and unpublished data).

To date, the only major lineage of Apioideae that has been comprehensively sampled and extensively studied is that of tribe Oenantheae (Downie et al. 2004, 2008, Hardway et al. 2004, Lee & Downie 2006, Spalik & Downie 2006, 2007, Bone 2007,

Feist & Downie 2008, Spalik et al. 2008, M.A. Feist, University of Illinois at Urbana-Champaign, unpublished data). Of the 117 species pertaining to the tribe, 109 have been included in molecular systematic investigations. Nearly all genera within the tribe have been or are currently being revised, with 18 monophyletic genera now recognized (Table 1). Oenantheae are well defined morphologically and ecologically, so it was relatively straightforward to identify potential members and include them in molecular studies. However, this is not the case for the vast majority of other major clades of Apioideae. It has not been possible to delimit many of these newly recognized tribes, subtribes or major clades using any obvious morphological or anatomical synapomorphies. It is hoped that future studies will provide this information, but if not, we would have to accept that the task of reclassifying Apioideae at the suprageneric level is to be accomplished primarily on the basis of molecular data rather than morphology. Given that many of the clades recognized represent no more than a morphologically heterogeneous assemblage of taxa that bear no resemblance to any traditional system of classification, confirmation of the composition of these clades will only be accomplished once all apioid taxa have been included in molecular studies.

Polyphyletic Genera

Our results revealed that numerous genera are not monophyletic within their respective major clades; these genera are indicated by asterisks in Table 1. These genera are often species-rich and poorly delimited, and are exemplified by such taxa as *Aletes* J.M. Coult. & Rose, *Angelica* L., *Arracacia* Bancr., *Bunium* L., *Carum* L., *Cymopterus* Raf., *Heracleum* L., *Ligusticum* L., *Lomatium* Raf., *Peucedanum* L., *Pimpinella* L., *Prangos* Lindl., *Seseli* L. and *Tauschia* Schltdl. Previous molecular systematic studies have already indicated the nonmonophyly of most of these genera (Downie et al. 1998, 2000b, 2000c, Katz-Downie et al. 1999, Zhou et al. 2008). Furthermore, 18 genera are assigned to two or more major clades (Table 2). The genus *Angelica* is assigned to a minimum of four major clades, *Ligusticum* is assigned to a minimum of five major clades, and *Pimpinella* is assigned to a minimum of seven major clades. No doubt, with additional sampling of these large and polymorphic genera, their placements in additional major clades will occur. Like the “*Peucedanum* problem” of previous worldwide interest, any one of these highly polyphyletic genera can therefore be considered for further collaborative study.

Problematic Sequences in GenBank

Given that Apiaceae subfamily Apioideae is such a large and taxonomically difficult group with many taxa being notoriously difficult to identify, and given the large number of Apioideae ITS sequences currently available in GenBank, it was surprising to find that there are relatively few ITS sequences obviously misidentified. Some of these misidentified sequences have already been reported (e.g., Valiejo-Roman et al. 2002b, Spalik et al. 2004, Ajani et al. 2008, Kurzyna-Młynik et al. 2008). During this study,

Table 2. Genera assigned to two or more major clades. The nomenclatural type of the boldfaced clade has been included in molecular studies.

Genus	Number of major clades	Clade name
<i>Angelica</i>	4	<i>Acronema</i> Clade, Selineae , <i>Sinodielsia</i> Clade, Tordyliinae
<i>Athamanta</i>	2	Daucinae, Scandicinae
<i>Carum</i>	2	Careae , Pyramidoptereae
<i>Cnidium</i>	2	Selineae , <i>Sinodielsia</i> Clade
<i>Conioselinum</i>	2	<i>Conioselinum chinense</i> Clade, <i>Sinodielsia</i> Clade
<i>Cryptotaenia</i>	3	Oenantheae , Pimpinelleae, Daucinae
<i>Ligusticum</i>	5	<i>Acronema</i> Clade , <i>Conioselinum chinense</i> Clade, Pyramidoptereae, Selineae, <i>Sinodielsia</i> Clade
<i>Ostericum</i>	2	<i>Acronema</i> Clade, Selineae
<i>Pachypleurum</i>	2	<i>Acronema</i> Clade , Selineae
<i>Physospermopsis</i>	2	<i>Physospermopsis</i> Clade, Pimpinelleae
<i>Pimpinella</i>	7	<i>Acronema</i> Clade, Careae, Echinophoreae, <i>Physospermopsis</i> Clade, Pimpinelleae , Pyramidoptereae, Selineae
<i>Pleurospermum</i>	3	<i>Acronema</i> Clade, Pleurospermeae , <i>Sinodielsia</i> Clade
<i>Prangos</i>	2	<i>Cachrys</i> Clade , <i>Sinodielsia</i> Clade
<i>Seseli</i>	3	Apiaceae, Pimpinelleae, Selineae
<i>Sinocarum</i>	2	<i>Acronema</i> Clade , <i>Physospermopsis</i> Clade
<i>Trachydium</i>	2	<i>Physospermopsis</i> Clade, Pleurospermeae
<i>Trachyspermum</i>	3	Echinophoreae, Pimpinelleae, Pyramidoptereae
<i>Vicatia</i>	2	Selineae , <i>Sinodielsia</i> Clade

misidentifications or other sources of errors, such as mix-ups of DNA samples or sequences, were identified through the simultaneous analysis of multiple ITS sequences from the same taxa. Extreme cases of error include an ITS sequence assigned to Asteraceae (*Atractylodes macrocephala* Koidz.) that is almost identical to two ITS sequences of *Ligusticum tenuissimum* (Nakai) Kitag., an ITS sequence of *Ligusticum sinense* Oliv. that is 99% identical to that of *Lycium barbarum* L. (Solanaceae), and an ITS sequence of *Peucedanum praeruptorum* Dunn that is identical to an ITS sequence from an endophytic fungi. Similarly, the ITS sequence of *Dickinsia hydrocotyloides* Franch. (Apiaceae subfamily Azorelloideae; Chandler & Plunkett 2004) is also likely misidentified, as it allies with *Apium ventricosum* H. Boissieu (*Acronema* Clade) in subfamily Apioideae. These problematic sequences and others, their GenBank accession numbers (for ITS1 and ITS2 regions, if submitted separately), and sources are as follows: *Atractylodes macrocephala* (AY548206 in H.Y. Choi et al., Kyunghee University, South Korea, unpublished); *Cyclospermum leptophyllum* (Pers.) Sprague ex Britton & E.H. Wilson (AF358471 and AF358538 in Downie et al. 2002); *Dickinsia hydrocotyloides* (AF337180 and AF337188 in Valiejo-Roman et al. 2002a); *Dorema aucheri* Boiss. (AY911858 and AY911864 in Valiejo-Roman et al. 2006b); *Ferula assa-foetida* L. (U78391 and U78451 in Downie et al. 1998); *Ferula communis* L. (U79615 and U79616 in Downie et al. 1998); *Ferula tingitana* L. (AF164826 and AF164851 in Downie et al. 2000c); *Foeniculum vulgare* Mill. (AY551289 in H.Y. Choi et al., Kyunghee University, South Korea, unpublished); *Glehnia littoralis* F. Schmidt ex Miq. subsp. *littoralis* (AY146849 and AY146915 in Sun et al. 2004);

Glehnia littoralis (DQ270202 in H. Xue et al., Nanjing Normal University, China, unpublished); *Heracleum stenopterum* Diels (DQ270203, H. Xue et al., Nanjing Normal University, China, unpublished); *Heteromorpha arborescens* (Thunb.) Cham. & Schlecht. (U27578 and U30314 in Downie and Katz-Downie 1996); *Ligusticum sinense* (AF467943, H.Y. Choi and Y.H. Chung, Kyunghee University, South Korea, unpublished); *Notopterygium incisum* Ting ex H.-T. Chang (U78412 and U78472 in Downie et al. 1998); *Peucedanum delavayi* Franch. (EU418386 in Feng et al., Sichuan University, China, unpublished); *Peucedanum elegans* Komarov (EU418385 in Feng et al., Sichuan University, China, unpublished); *Peucedanum japonicum* Thunb. (AJ131343 in Lee and Rasmussen 2000); *Peucedanum praeruptorum* Dunn (AY925159 in Mak et al., The Chinese University of Hong Kong, unpublished); *Thysselum palustre* (L.) Hoffm. (AF008621 and AF009100 in Katz-Downie et al. 1999). In addition, there are other instances of two or more accessions of the same species not occurring as monophyletic in our preliminary phylogenetic analyses, suggestive that some of these may also be misidentifications. These species include, but are not limited to, *Aletes macdougalii* J.M. Coult. & Rose, *Conioselinum chinense*, *Ligusticum sinense* 'Chuanxiong' S.H. Qiu et al., *Peucedanum terebinthaceum* (Fisch. ex Spreng.) Ledeb. (*Kitagawia terebinthacea* (Fisch. ex Spreng.) Pimenov), *Pimpinella peregrina* L., *Pimpinella rhodantha* Boiss., *Pteryxia terebinthina* (Hook.) J.M. Coult. & Rose, and *Sinocarum coloratum* H. Wolff ex R.H. Shan & F.T. Pu. No doubt, further studies with additional sampling will confirm the phylogenetic placements of these taxa and reveal that many are of questionable taxonomic assignment. Alternatively, further studies may show that differences in sequences among these accessions may actually be due to one of the many molecular genetic processes that impact ITS repeat sequence variation, such as those described by Álvarez & Wendel (2003), although such phenomena have been rarely observed in Apiaceae to date and, for the most part, do not mislead phylogenetic inferences (Chung et al. 2005, Spalik & Downie 2006, but see Calviño et al. 2006 and Zhou et al. 2009 for exceptions).

Additional problematic sequences in GenBank include ITS data that are of overall poor quality, have incomplete ITS1 or ITS2 regions, contain large insertions that may not be homologous with the ITS region (*Pimpinella brachycarpa* (Komarov) Nakai AF169259, AF169261), or have 48-bp (*Changium smyrnioides* EU515300) or 29-bp (*Peucedanum japonicum* AF169273, AF169275, AF169277) transpositions in ITS2 that are probably attributable to human error. These sequences often differed considerably from other sequences of the same species or their presumed congeners and it is suggested that these sequences should be withdrawn from GenBank. These problematic sequences include *Heracleum antasiaticum* Manden. (AM168440, AM168441, AM167913, AM167914), *H. crenatifolium* Boiss. (AM167919, AM167920, AM168446, AM168447), *H. pastinacifolium* K. Koch (AM168434, AM168435, AM167908), *H. persicum* Desf. (AM167917, AM167918, AM168444, AM168445), *H. sosnowskyi* Manden. (AM167915, AM167916, AM168442, AM168443), *H. sphondylium* L. (AM167911, AM167912, AM168438, AM168439), *H. trachyloma* Fisch. & C.A. Mey. (AM167909, AM167910, AM168436, AM168437), *Pimpinella brachycarpa* (AF169258, AF169259, AF169260, AF169261), and *Angelica dahurica* Maxim. (AY925155, AY925162).

A Standard System of Apioideae Phylogenetic Classification

Family-level molecular systematic analyses and studies of specific lineages within Apiaceae subfamily Apioideae reveal that traditional classifications of the subfamily based largely on fruit morphology and anatomy, such as the widely used system of Drude (1898) and modifications thereof (e.g., Pimenov & Leonov 1993), are only partially predictive with respect to phylogenetic relationships. Of Drude's 8 tribes, only the small morphologically distinct Echinophoreae is retained as monophyletic in light of results of phylogenetic analyses of molecular data. Of Drude's 10 subtribes and of those where sampling has been extensive, only Scandiceae subtribe Scandicinae and Peucedaneae subtribe Tordyliinae show some resemblance to groups inferred by molecular study. The present investigation represents the most comprehensive, single-locus-based phylogenetic analysis of Apioideae published to date and, along with the results of recent molecular systematic investigations based on chloroplast markers (e.g., Calviño et al. 2006), has helped to elucidate the natural subdivisions within the entire subfamily. We identify 41 major clades within Apioideae, of which 21 have already been recognized at the tribal or subtribal rank.

While it is stated that the NCBI taxonomy database is not an authoritative source for nomenclature or classification, it does provide a highly visible classificatory reference system for the family. However, the listing of many genera under such categories as "apioid superclade *incertae sedis*," "Apioideae *incertae sedis*," or even "Apiaceae *incertae sedis*" does not serve a useful purpose other than to simply befuddle individuals who are submitting their sequences to GenBank, especially if they are not familiar with the most recent scientific literature. Often, newly submitted sequences are placed in incorrect tribes or major clades, such as *Demavendia* Pimenov, *Haussknechtia* Boiss. and *Zeravschania* Korovin (Pimpinelleae not Selineae), *Changium* H. Wolff and *Chuanminshen* R.H. Shan & M.L. Sheh (*Komarovia* Clade not Scandiceae subtribe Scandicinae), *Stoibrax* Raf. (Apieae not Tordylieae), *Sonderina* H. Wolff and *Stenosemis* E. Mey. ex Harv. & Sond. (*Lefebvrea* Clade not Selineae), and *Dorema* D. Don and *Schumannia* Kuntze (Scandiceae subtribe Ferulinae not subtribe Scandicinae). Moreover, the names of clades and other taxonomic groups are not consistent in the molecular systematic literature or the clades are simply assigned ad hoc numbers that do not coincide from one paper to the next (e.g., Valiejo-Roman et al. 2006a, b). To facilitate communication and to avoid taxonomic confusion, a standardized system of phylogenetic classification of subfamily Apioideae is necessary. We propose that the system outlined herein be used, both as a reference system for GenBank and as a modern classification system for the subfamily, because (1) for many clades it reflects the results of multiple molecular phylogenetic studies using both ITS and chloroplast markers from several different laboratories, (2) there is no reasonable competing system available, other than those traditionally based systems such as that of Drude (1898) and Pimenov & Leonov (1993), and (3) so far, it has stood the test of time, for no major changes to the system of Downie et al. (2001) resulted upon consideration of an additional 100 genera and 500 species. We provide a workable framework for subfamily Apioideae that enables direct and precise references to specific groups. It is possible that future studies would necessitate revision of particular clades, especially those in-

ferred recently on the basis of only ITS data, but the groups proposed herein are much needed to encapsulate the progress made to date and to provide a general framework for sampling in future systematic studies.

Lastly, we make an appeal that researchers use the same molecular loci to resolve Apioideae phylogenetic relationships. The goal of achieving a global, species-level phylogeny for the entire subfamily can only be achieved through multinational, collaborative study, and the consideration of common plastid, nuclear, and mitochondrial loci will be necessary to facilitate this objective. However, the lack of a comprehensive, cpDNA-based estimate of phylogenetic relationships is a major hurdle towards the realization of this goal. To date, the chloroplast *trnQ*^(UUG)-5'*trnK*^(UUU) locus, which contains the *rps16* intron and two intergenic spacer regions (*trnQ*-5'*rps16* and 3'*rps16*-5'*trnK*), has been sequenced widely in Apioideae and Saniculoideae (with at least 20 studies using these data to date) and holds great promise for resolving many remaining issues of Apioideae phylogeny. Shaw et al. (2007) considered the utility of 34 noncoding chloroplast regions for molecular studies at low taxonomic levels and identified the *trnQ*-5'*rps16* and 3'*rps16*-5'*trnK* intergenic spacer regions as being among the most variable. The *rps16* intron was previously considered highest among their "Tier II" group of loci, meaning that it may also provide some useful information for low level phylogenetic studies (Shaw et al. 2005). Until we have evidence to the contrary, it appears that these three noncoding regions are likely to be among the best choices from the chloroplast genome for continued molecular systematic studies of Apioideae. No doubt, much more sequence data than that from the cpDNA *trnQ*^(UUG)-5'*trnK*^(UUU) locus will be required to produce a fully-resolved and well-supported phylogenetic tree for Apioideae. Until we have these data, the continued acquisition of ITS sequences from all higher apioid species heretofore not included in molecular studies and the proposed multinational development of a database of cpDNA *trnQ*^(UUG)-5'*trnK*^(UUU) sequences for representatives of each of the 41 major clades of Apioideae identified herein are an excellent start towards producing a "New Drude," a modern, phylogeny based classification of Apioideae for the 21st century.

Note Added in Proof

The following clades or basal genera have been recently recognized at the tribal level (Magee & al. 2010): *Annesorhiza* Clade (Annesorhizeae Magee, C.I. Calviño, M. Liu, S.R. Downie, P.M. Tilney & B.-E. van Wyk, and includes the genera *Annesorhiza*, *Astydamia*, *Chamarea*, *Ezoscadium*, *Itasina*, and *Molopospermum*); Choritaenieae Magee, C.I. Calviño, M. Liu, S.R. Downie, P.M. Tilney & B.-E. van Wyk, and includes only the genus *Choritaenia*; *Lichtensteinia* Clade (Lichtensteinieae Magee, C.I. Calviño, M. Liu, S.R. Downie, P.M. Tilney & B.-E. van Wyk, and includes only the genus *Lichtensteinia*); Marlothielleae Magee, C.I. Calviño, M. Liu, S.R. Downie, P.M. Tilney & B.-E. van Wyk, and includes only the genus *Marlothiella*; and Phlyctidocarpeae Magee, C.I. Calviño, M. Liu, S.R. Downie, P.M. Tilney & B.-E. van Wyk, and includes only the genus *Phlyctidocarpa*.

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