

Generic limits of Pyrinae: Insights from nuclear ribosomal DNA sequences

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ABSTRACT. The subtribe Pyrinae, formerly the Maloideae, is a monophyletic group of about 1,000 species that includes well known fruit crops such as apple (*Malus*), pear (*Pyrus*), quince (*Cydonia*), loquat (*Eriobotrya*), chokeberry (*Aronia*), and serviceberry (*Amelanchier*). Generic limits have been fluid in Pyrinae, especially in *Malus*, *Sorbus* and *Photinia*. This study evaluated the generic limits of 180 samples of multiple species or accessions from each of the traditional genera using sequences of the nrDNA ITS region. The ITS data recognized 24 genera, including *Amelanchier*, *Aria* (including *Micromeles*), *Aronia*, *Chaenomeles*, *Chamaemespilus*, *Chamaemeles*, *Cormus*, *Cotoneaster*, *Crataegus*, *Cydonia*, *Dichotomanthes*, *Eriobotrya*, *Hesperomeles*, *Malacomeles*, *Malus* (including *Chloromeles*, *Docynia*, *Docyniopsis*, and *Eriolobus*), *Mespilus*, *Osteomeles*, *Peraphyllum*, *Pourthiaea*, *Pseudocydonia*, *Pyrus*, *Rhaphiolepis*, *Sorbus*, and *Torminalis*. However, both *Photinia* and *Pyracantha* are polyphyletic. *Photinia* is separated into different clades, one of which contains species of *Heteromeles* and *Stranvaesia*. Asian species of *Pyracantha* do not form a clade with *P. coccinea* of southern Europe and Iran. Our results support the close relationship of *Amelanchier*, *Malacomeles*, and *Peraphyllum*, and of *Crataegus* and *Mespilus*, and for the first time recognize the sister relationship of the South American genus *Hesperomeles* with the *Crataegus-Mespilus* clade.

Keywords: Generic limits; *Hesperomeles*; Maloideae; nrDNA ITS Pyrinae.

INTRODUCTION

The Rosaceae subtribe Pyrinae, formerly subfamily Maloideae (Potter et al., 2007), contains about 1000 species (Phipps et al., 1990), many of which are economically important, such as apple (*Malus domestica* Borkh.), pear (*Pyrus pyrifolia* Nakai), loquat (*Eriobotrya* Lindl.), and chokeberry (*Aronia* Mitchell). The Pyrinae is defined by several synapomorphic characters: the pome fruit, base number of chromosomes $x=17$ (Phipps et al., 1991), rust parasites (Savile, 1979), and gametophytic apomixis (Campbell et al., 1991). Menz (1964) divided Pyrinae into two tribes: Crataegeae, with fruit called polypyrenous drupes (Kalkman, 1988; Baird and Thieret, 1989), in which most or the entire ovary wall becomes hard, and each carpel forms a separate nutlet or pyrene (Rohrer et al., 1991), as with *Crataegus* and *Pyracantha*; and Sorbeae (Maleae), with connate endocarps, a membranous to cartilaginous inner ovary wall, and connate carpels form-

ing a single multilocular core (Rohrer et al., 1991), as with *Malus* and *Pyrus*. Although Rohrer et al. (1991) studied Pyrinae fruit structure and could not substantiate its division based on core textures, the circumscription of Pyrinae has never been seriously challenged. Minor changes have included the removal of *Dichotomanthes* S. Kurz by Gladkova (1969), the inclusion of *Vauquelinia* Corrêa ex Bonpl in the subfamily by Goldblatt (1976), the inclusion of *Vauquelinia*, *Lindleya* and *Kageneckia* in two tribes of Pyroideae, and the division of Pyrinae into Maleae and Crataegeae by Takhtajan (1997). Recent phylogenetic studies, however, support the placement of *Dichotomanthes* in the Pyrinae and the sister relationship Pyrinae has with *Vauquelinia*, *Lindleya* and *Kageneckia* (Evans et al., 2000).

Generic limits within Pyrinae, however, have been controversial (Linnaeus, 1753; Lindley, 1822; de Candolle, 1825; Lindley, 1837; Decaisne, 1874; Focke, 1888; Koehne, 1891; Fritsch, 1898; Fritsch, 1899; Lindley, 1845; Roemer, 1847; Wenzig, 1883; Rehder, 1940; Rehder, 1949; Robertson et al., 1991). The center of the controversy lies in the circumscriptions of *Sorbus* L., *Malus* Mill., and *Photinia* Lindl. There are two concepts of *Sorbus*. Wenzig (1883) used *Sorbus* broadly and included *Chamaemespi-*

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lus, *Aria*, *Torminaria* M. Roem., *Aronia*, *Eriolobus*, *Cornus*, and *Sorbus* species. Roemer (1847) and Robertson et al. (1991), however, gave them all generic status. *Chloromeles* (Decne.) Decne. was placed with *Malus* Wenzig (1883), and Robertson et al. (1991). *Stanvaesia* Lindl. is morphologically very similar to *Photinia* Lindl. and the two genera have sometimes been merged under *Photinia* Lindl. (Vidal, 1965; Kalkman, 1973); *Aronia* Medik. has been considered a close relative of *Photinia*, and is sometimes listed with that genus (Robertson et al., 1991), *Pourthiaea* Decaisne is sometimes considered morphologically or anatomically distinct from *Photinia* (Iketani and Ohashi, 1991; Zhang, 1992; Lu et al., 2003).

A cladistic analysis of morphological characters by Phipps et al. (1991) concluded that while the genera formed clusters, the consistency was low, such that adding a few OTUs changed the placement of the genera. The low consistency reflects the fact that Pyrinae genera hybridize easily (Robertson et al., 1991), as with *Crataegus* × *Sorbus*, *Cotoneaster* × *Sorbus*, *Crataegus* × *Pyrus*, *Pyracantha* × *Osteomeles*, *Pyracantha* × *Cotoneaster*, *Cydonia* × *Pyrus*, *Cydonia* × *Malus*, *Malus* × *Sorbus*. Only a few of these hybrids, such as *Sorbus* × *Aria*, *Sorbus* × *Torminalis* and *Sorbus* × *Chamaemespilus*, however, regularly occur in nature, and this has been taken to indicate close relationships among these genera.

Although several molecular phylogenetic analyses of the Pyrinae have been conducted in recent years (Morgan et al., 1994; Campbell et al., 1995; Evans et al., 2000; Evans and Campbell, 2002; Campbell et al., 2007; Potter et al., 2007), intergeneric relationships remain unresolved. The lack of phylogenetic information may be partially due to possible rapid radiation of the Pyrinae generic lineages (Campbell et al., 2007). To date, no molecular phylogenetic analyses have focused on the generic limits of the Pyrinae. Our objective was thus to evaluate these generic limits by sampling multiple species or accessions from each of the possible genera. We used sequences from the internal transcribed spacer regions of nuclear ribosomal DNA (nrDNA ITS), as is common in phylogenetic reconstructions of flowering plants, including Rosaceae (Campbell et al., 1995; Campbell et al., 1997; Oh and Potter, 2003; Lo et al., 2007).

MATERIALS AND METHODS

This study included 180 samples representing the geographic distribution and morphological diversity of all genera of the Pyrinae (81 samples, representing 73 species, are studied here for the first time) (Table 1). *Lindleya* Kunth, *Kageneckia* Ruiz & Pav., and *Vauquelinia* were used for rooting purposes since they are most closely related to the Pyrinae (Campbell et al., 1995; Evans and Campbell, 2002; Morgan et al., 1994).

Genomic DNAs were extracted from fresh or silica gel-dried leaf material using a DNeasy Plant Mini Kit following manufacturer's instructions (Qiagen, Valencia, CA).

Polymerase chain reactions (PCR) were conducted using a MJ Research Thermocycler or an Eppendorf Mastercycler in a 25 µl reaction system. The PCR protocols and thermocycler programs followed Li (2008). PCR products of the expected size were cut from 1% agarose gels and purified using a Qiagen Gel Purification Kit. Direct sequencing of the purified PCR products was done using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit with *AmpliTaq* DNA polymerase, FS. Sequences were obtained using an ABI 3730 Automated Genetic Analyzer and edited in Sequencher (version 4.0, Ann Arbor Gene Code, Inc.). Sequences were aligned using the MUSCLE program (Edgar, 2004), available freely at <http://www.drive5.com/muscle/download3.6.html>, with a slight manual adjustment. Ambiguously aligned regions, where indels could be inserted in more than one site, were excluded from phylogenetic analyses.

Both maximum parsimony (MP) and Bayesian inference (BI) analyses were used to reconstruct phylogenetic trees of the Pyrinae. Characters were equally weighted and their states were unordered. MP analyses were done in PAUP* (version 4.0b) (Swofford, 2002) using the heuristic tree search algorithm with the following options: random sequence addition of 5000 replicates with one tree held per replicate, MAXTREES set to 20,000, TBR branch swapping, MULTTREES on, and STEEPEST DESCENT off. Bootstrap analyses of 10,000 replicates were performed to evaluate support for individual clades (Felsenstein, 1985) using the FAST STEPWISE ADDITION search in PAUP* due to the large data set size. Bayesian analyses were conducted for two runs using the MRBAYES computer program (Ronquist and Huelsenbeck, 2003) and the best-fit model selected by the hierarchical likelihood ratio test in the MODELTEST (Posada and Crandall, 1998). Four MCMC chains were run for 2,000,000 generations with trees sampled every 1,000 generations. The likelihood scores were plotted against the number of generations; all generations prior to likelihood stationarity were discarded as burn-in. The 50% majority rule consensus of the remaining 4001 trees was used to calculate posterior probabilities (pp) for individual clades.

RESULTS

The ITS data set had a total of 180 sequences, including 81 sequences reported here for the first time. The sequence alignment included 710 sites, 385 of which were variable and 247 of which were parsimony informative. A 63-bp fragment in the ITS-1 was excluded from analyses due to alignment ambiguity. The MP analyses produced 20,000 trees (the limit set on Maxtrees) with a consistency index of 0.39 and retention index of 0.76. The best-fit evolutionary model of the nrDNA ITS data was the TVM+I+G model as selected by the MODELTEST. In the BI analyses, the maximum likelihood scores reached plateau in 148,000 generations; thus the first 148 trees were discarded as burn-in, and the remaining trees were used to obtain the pp for individual clades.

Table 1. Species, their source, and vouchers used in Pyrinae phylogenetic analyses.

Species	Source	Voucher	GenBank accessions
<i>Amelanchier</i> Medikus			
<i>Amelanchier arborea</i>			EF127041
<i>Amelanchier bartramiana</i>			U15191
<i>Aria</i> (Persoon) Host			
<i>Aria coronata</i>	Yunnan, China	Qingyan Li YN-003	FJ810012
<i>Aria hemsleyi</i>	Arnold Arboretum, U.S.A.	Qingyan Li 1771-80C	FJ810010
<i>Aria yuana</i>	Arnold Arboretum, U.S.A.	Qingyan Li 1539-80C	FJ810007
<i>Aronia</i> Mitchell			
<i>Aronia pyrifolia</i> 1			U16199
<i>Aronia pyrifolia</i> 2			U16199
<i>Aronia</i> sp.			EF127043
<i>Aronia arbutifolia</i>	Arnold Arboretum, U.S.A.	Qingyan Li1905-81	FJ796911
<i>Aronia melanocarpa</i>	Arnold Arboretum, U.S.A.	Qingyan Li 1906-81MASS	FJ810003
<i>Aronia prunifolia</i>	Arnold Arboretum, U.S.A.	Qingyan Li 1389-83C	FJ810001
<i>Chaenomeles</i> Lindley			
<i>Chaenomeles cathayensis</i>			U16186
<i>Chamaemeles</i> Lindley			
<i>Chamaemeles speciosa</i>			AF186530
<i>Chamaemeles coriacea</i>			DQ811768
<i>Chamaemespilus</i> Medikus			
<i>Chamaemespilus alpina</i>			DQ811769
<i>Chamaemespilus alpina</i>	Arnold Arboretum, U.S.A.	Qingyan Li 1110-65A	FJ810045
<i>Chloromeles</i> (Decaisne) Decaisne			
<i>Chloromeles coronaria</i>			AF186524
<i>Chloromeles coronaria</i>			AF186525
<i>Chloromeles ioensis</i>			AF186526
<i>Chloromeles angustifolia</i>			AF186523
<i>Cormus</i> Spach			
<i>Cormus domestica</i> 1			U16187
<i>Cormus domestica</i> 2	Arnold Arboretum, U.S.A.	Qingyan Li 1043-64A	FJ810017
<i>Cotoneaster</i> Medikus			
<i>Cotoneaster acutifidius</i>	Arnold Arboretum, U.S.A.	Qingyan Li 00165718	FJ796931
<i>Cotoneaster acutinatus</i>	Arnold Arboretum, U.S.A.	Qingyan Li 00191805;00191728	FJ796921
<i>Cotoneaster atropurpureus</i>	Arnold Arboretum, U.S.A.	Qingyan Li 00166599	FJ796922
<i>Cotoneaster przewalskii</i>	Arnold Arboretum, U.S.A.	Qingyan Li 00223832	FJ796903
<i>Cotoneaster adpressus</i>	Arnold Arboretum, U.S.A.	Qingyan Li 00191505	FJ796933
<i>Cotoneaster conspicuus</i>	Arnold Arboretum, U.S.A.	Qingyan Li 00191716	FJ796937
<i>Cotoneaster dielelanus</i>	Arnold Arboretum, U.S.A.	Qingyan Li 00166620	FJ796919
<i>Cotoneaster apiculatus</i>	Arnold Arboretum, U.S.A.	Qingyan Li 7275A	FJ796933
<i>Cotoneaster dielsianus</i>	Arnold Arboretum, U.S.A.	Qingyan Li 13428B	FJ796920
<i>Cotoneaster integerrimus</i>	Xinjiang, China	Qingyan Li 780074	FJ796948
<i>Cotoneaster melanocarpus</i>	Xinjiang, China	Qingyan Li 780006	FJ796949
<i>Cotoneaster melanocarpus</i>	Arnold Arboretum, U.S.A.	Qingyan Li 00223183;00191532	FJ796946
<i>Cotoneaster morrisonensis</i>	Arnold Arboretum, U.S.A.	Qingyan Li 271-98A	FJ796941

Table 1. (Continued)

Species	Source	Voucher	GenBank accessions
<i>Cotoneaster perpusillus</i>	Arnold Arboretum, U.S.A.	Qingyan Li 7157C	FJ796928
<i>Cotoneaster procumbens</i>	Arnold Arboretum, U.S.A.	Qingyan Li 1979-0164 A	FJ796938
<i>Cotoneaster rotundifolius</i>	Nanjing, China	Qingyan Li 0706-014	FJ796950
<i>Cotoneaster soongoricus</i>	Xi'an, China	Qingyan Li 780177	FJ796936
<i>Cotoneaster verruculosus</i>	Yunnan, China	Lihua Zhou GLGS 22004	FJ796935
<i>Crataegus</i> L.			
<i>Crataegus aestivalis</i>			EF127023
<i>Crataegus brachyacantha</i>			EF127032
<i>Crataegus calpodendron</i>			EF127018
<i>Crataegus chlorosarca</i>			EF127009
<i>Crataegus crusgalli</i>			EF127010
<i>Crataegus dahurica</i>			EF127028
<i>Crataegus heldreichii</i>			EF127016
<i>Crataegus hupehensis</i>			EF127038
<i>Crataegus kansuensis</i>			EF127029
<i>Crataegus laevigata</i>			EF127015
<i>Crataegus marshallii</i>			EF127037
<i>Crataegus maximowiczii</i>			EF127030
<i>Crataegus mollis</i> 1			U16190
<i>Crataegus mollis</i> 2			EF127012
<i>Crataegus monogyna</i>			EF127014
<i>Crataegus nigra</i>			EF127007
<i>Crataegus opaca</i>			EF127022
<i>Crataegus pentagyna</i>			EF127035
<i>Crataegus phaenopyrum</i>			EF127034
<i>Crataegus pubescens</i>			EF127021
<i>Crataegus punctata</i>			EF127011
<i>Crataegus saligna</i>			EF127031
<i>Crataegus sanguinea</i>			EF127027
<i>Crataegus songarica</i>			EF127036
<i>Crataegus spathulata</i>			EF127033
<i>Crataegus suksdorfii</i> 1			EF127025
<i>Crataegus suksdorfii</i> 2			EF127026
<i>Crataegus triflora</i>			EF127019
<i>Crataegus uniflora</i>			EF127020
<i>Crataegus viridis</i>			EF127013
<i>Crataegus wilsonii</i>			EF127008
<i>Crataegus lasa</i>			EF127024
<i>Cydonia</i> Miller			
<i>Cydonia oblonga</i> 1			U16189
<i>Cydonia oblonga</i> 2			AF186531
<i>Dichotomanthes</i> Kurz			
<i>Dichotomanthes tristanicarpa</i> 1			DQ811770
<i>Dichotomanthes tristanicarpa</i>	Yunnan, China	Wei Guo 8305	FJ796909

Table 1. (Continued)

Species	Source	Voucher	GenBank accessions
<i>Docynia</i> Decaisne			
<i>Docynia delavayi</i>	Yunnan, China	Lihua Zhou GLGS19031	FJ796912
<i>Docyniopsis</i> (C. K. Schneider) Koidzumi			
<i>Docyniopsis prattii</i>			AF186511
<i>Docyniopsis tschonokii</i> 1			AF186527
<i>Docyniopsis tschonokii</i> 2			DQ811771
<i>Docyniopsis yunnanensis</i>			AF186508
<i>Eriobotrya</i> Lindley			
<i>Eriobotrya cavaleriei</i>	Guangxi, China	Xiaomin Fu, 1060435	FJ810022
<i>Eriobotrya</i> sp.	Yunnan, China	Qiang Fan Q6002	FJ810023
<i>Eriobotrya fragrans</i>	Guangdong, China	Xiaomin, Fu, 6050113	FJ810024
<i>Eriobotrya fragrans</i>	Guangxi, China	Wei Guo7236	FJ810025
<i>Eriobotrya japonica</i>			U16192
<i>Eriobotrya tengyuehensis</i>	Yunnan, China	Lihua Zhou GLGS 24171	FJ796915
<i>Eriolobus</i> (A. P. de Candolle) M. J. Roemer			
<i>Eriolobus trilobatus</i>			AF186521
<i>Hesperomeles</i> Lindley			
<i>Hesperomeles palcensis</i>		Paniagua 5770 (MOBOT)	FJ796914
<i>Hesperomeles latifolia</i>		Paniagua 5764 (MOBOT)	FJ810044
<i>Heteromeles</i> M. J. Roemer			
<i>Heteromeles arbutifolia</i>			U16193
<i>Malacomeles</i> (Decaisne) Engler			
<i>Malacomeles denticulata</i>			U16194
<i>Malus</i> Miller			
<i>Malus asiatica</i>			EF442030
<i>Malus asiatica</i>			AF186494
<i>Malus baccata</i>			AF186501
<i>Malus domestica</i>			U16195
<i>Malus doumeri</i>			AF186529
<i>Malus florentina</i>			AF186520
<i>Malus floribunda</i>			EF493836
<i>Malus fusca</i>			AF186514
<i>Malus halliana</i>			AF186502
<i>Malus honanensis</i>			AF186510
<i>Malus hupehensis</i>			AF186503
<i>Malus kansuensis</i>			AF186512
<i>Malus mandshurica</i>			AF186504
<i>Malus neidzwetzkyana</i>			AF186495
<i>Malus ombrophila</i>			AF186513
<i>Malus orientalis</i>			AF186498
<i>Malus orientalis</i>			AF186499
<i>Malus prunifolia</i>			AF186500

Table 1. (Continued)

Species	Source	Voucher	GenBank accessions
<i>Malus sargentii</i>			AF186507
<i>Malus sieboldii</i>			AF186505
<i>Malus toringoides</i>			AF186517
<i>Malus transitoria</i>			AF186518
<i>Mespilus</i> L.			
<i>Mespilus canescens</i>			EF127039
<i>Mespilus germanica</i> 1			U16196
<i>Mespilus germanica</i> 2			EF127040
<i>Micromeles</i> Decaisne			
<i>Micromeles alnifolia</i> 1			U16185
<i>Micromeles alnifolia</i> 2	Arnold Arboretum, U.S.A.	Qingyan Li 00160096--00160099	FJ796908
<i>Micromeles alnifolia</i> 3	Arnold Arboretum, U.S.A.	Qingyan Li 413-94-B	FJ810006
<i>Micromeles caloneura</i>	Yunnan, China	Wei Guo YN-019	FJ810008
<i>Micromeles thomsonii</i>	Jiangxi, China	Wei Guo SQ0809502	FJ810009
<i>Micromeles tsinglingensis</i>	Arnold Arboretum, U.S.A.	Qingyan Li 544-88E	FJ810011
<i>Osteomeles</i> Lindley			
<i>Osteomeles anthyllidifolia</i>			AY864895
<i>Osteomeles schwerinae</i> 1			U16197
<i>Osteomeles schwerinae</i> 2	Yunnan, China	Wei Guo YN-30	FJ796910
<i>Peraphyllum</i> Nuttall ex Torrey & A. Gray			
<i>Peraphyllum ramosissimum</i>			U16198
<i>Photinia</i> Lindley			
<i>Photinia davidsoniae</i>	Nanjing, China	Qingyan Li, 0706019	FJ810005
<i>Photinia glabra</i>	Jiangxi, China	Wei Guo 10218	FJ796905
<i>Photinia nussia</i>	Arnold Arboretum, U.S.A.	Jianhua Li 1974-5668	FJ810004
<i>Photinia prinophylla</i>	Yunnan, China	Wei Guo YN-35	FJ810018
<i>Photinia prunifolia</i>	Zhejiang, China	Qingyan Li 8230	FJ810019
<i>Photinia serralata</i>	Jiangxi, China	Wei Guo 8564	FJ810021
<i>Photinia tushanensis</i>	Guangxi, China	Wei Guo 70722003	FJ810020
<i>Pourthiaea</i> Decaisne			
<i>Pourthiaea beauverdiana</i>	Zhejiang, China	Qingyan Li 0706003	FJ796907
<i>Purthiaea benthamiana</i>			
<i>Pourthiaea benthamiana</i>	Guangdong, China	Wei Guo 0013	FJ810014
<i>Pourthiaea parvifolia</i>	Jiangxi, China	Wei Guo 20120	FJ810015
<i>Pourthiaea villosa</i>	Guizhou, China	Wei Guo 283-82B	FJ810016
<i>Pseudocydonia</i> (C. K. Schneider) C. K. Schneider			
<i>Pseudocydonia sinensis</i>			U16201
<i>Pyracantha</i> Roemer			
<i>Pyracantha angustifolia</i>	Zhejiang, China	Qingyan Li H0706-006	FJ796916
<i>Pyracantha coccinea</i> 1			DQ811772
<i>Pyracantha coccinea</i>	Brooklyn Botanic Garden, U.S.A.	Jinshuang Ma BBG67068	FJ821024
<i>Pyracantha fortuneana</i>	Zhejiang, China	Qingyan Li 706003	FJ810049

Table 1. (Continued)

Species	Source	Voucher	GenBank accessions
<i>Pyrus</i> L.			
<i>Pyrus calleryana</i>			U16202
<i>Pyrus caucasica</i>	Arnold Arboretum, U.S.A.	Qingyan Li 1335-80B	FJ796917
<i>Pyrus elaeagnifolia</i>	Arnold Arboretum, U.S.A.	Qingyan Li 00186151	FJ810046
<i>Pyrus pyrifolia</i> 1	Arnold Arboretum, U.S.A.	Qingyan Li 00223812;00190718	FJ810047
<i>Pyrus pyrifolia</i> 2			AF287246
<i>Pyrus salicifolia</i>			AF186532
<i>Pyrus ussuriensis</i>	Arnold Arboretum, U.S.A.	Qingyan Li 00223291	FJ810050
<i>Rhaphiolepis</i> Lindley			
<i>Rhaphiolepis indica</i>			GU947645
<i>Rhaphiolepis indica</i>			U16203
<i>Sorbus</i> L.			
<i>Sorbus acuparia</i>	Arnold Arboretum, U.S.A.	Qingyan Li 1257-84A	FJ796913
<i>Sorbus amabilis</i>	Jiangxi, China	Wei Guo SQ0809501	FJ810033
<i>Sorbus americana</i>	Arnold Arboretum, U.S.A.	Qingyan Li 1845-66A	FJ810037
<i>Sorbus aronioides</i>	Yunnan, China	Wei Guo YN-013	FJ810031
<i>Sorbus aucuparia</i>			U16204
<i>Sorbus discolor</i>	Arnold Arboretum, U.S.A.	Qingyan Li 136-79A	FJ810026
<i>Sorbus dumisa</i>	Arnold Arboretum, U.S.A.	Qingyan Li 423-88A	FJ810041
<i>Sorbus forrestii</i>	Arnold Arboretum, U.S.A.	Qingyan Li 814-77-D	FJ810028
<i>Sorbus huphensis</i>	Arnold Arboretum, U.S.A.	Qingyan Li 1675-80C	FJ810027
<i>Sorbus intermedia</i>	Arnold Arboretum, U.S.A.	Qingyan Li 136-56A	FJ810036
<i>Sorbus koehneana</i>	Arnold Arboretum, U.S.A.	Qingyan Li 1693-80B	FJ810029
<i>Sorbus pohuashanensis</i>	Arnold Arboretum, U.S.A.	Qingyan Li 477-80B	FJ810034
<i>Sorbus prattii</i>	Yunnan, China	Lihua Zhou GLGS20390	FJ810032
<i>Sorbus pteridophylla</i>	Yunnan, China	Lihua Zhou GLGS20376	FJ810030
<i>Sorbus rufo-ferruginea</i>	Arnold Arboretum, U.S.A.	Qingyan Li 367-80A	FJ810038
<i>Sorbus sambucifolia</i> 1	Arnold Arboretum, U.S.A.	Qingyan Li 1730-77A	FJ810042
<i>Sorbus sambucifolia</i> 2	Arnold Arboretum, U.S.A.	Qingyan Li 1730-77A	FJ810048
<i>Sorbus scopulina</i>	Arnold Arboretum, U.S.A.	Qingyan Li 310-75A	FJ810039
<i>Sorbus tianschanica</i>	Xinjiang, China	Qingyan Li 0780061	FJ810043
<i>Sorbus vilmorinii</i>	Arnold Arboretum, U.S.A.	Qingyan Li 151-87B	FJ810040
<i>Sorbus wilfordii</i>	Arnold Arboretum, U.S.A.	Qingyan Li 326-86A	FJ810035
<i>Stranvaesia</i>			
<i>Stranvaesia davidiana</i>	Yunnan, China	Lihua Zhou GLGS22604	FJ796906
<i>Torminalis</i> Medikus			
<i>Torminalis clusii</i> 1	Arnold Arboretum, U.S.A.	Qingyan Li 246-98C	FJ796918
<i>Torminalis clusii</i> 2			DQ811773
<i>Torminalis clusii</i> 3			AF186533
<i>Vauquelinia</i>			
<i>Vauquelinia californica</i>			DQ811766
<i>Vauquelinia corymbosa</i>			DQ811767
<i>Kageneckia angustifolia</i>			DQ811764
<i>Lindleya mespiloides</i>			DQ811765

The MP (Figure 1) and BI (Figure 2) trees were largely congruent, with a few minor differences. In the MP tree (Figure 1), *Amelanchier* Medik., *Malacomeles* (Decne.) Engl. and *Peraphyllum* Nutt. formed a clade (bootstrap, bs=63%) that was sister to the remainder of the tribe (bs=89%). In the BI tree (Figure 2), however, *Amelanchier*, *Malacomeles*, *Peraphyllum*, *Crataegus* L., *Mespilus* L., and *Hesperomeles* Lindl. formed a robust clade (posterior probability, pp=100%). *Crataegus* and *Mespilus* each formed their own clades, and were sister to each other in both the MP and BI trees (Figures 1-2). *Hesperomeles* formed a clade (bs=pp=100%) but its sister relationship to the *Mespilus-Crataegus* clade was also poorly supported (pp=51%). *Sorbus* was moderately supported in the MP tree (bs=75%). In the BI tree, however, *Sorbus* had strong support (pp=99%).

The ITS data provided moderate to strong support in both MP and BI trees (Figures 1-2) for monophyly of several genera, including *Aronia* (bs=98%, pp=100%), *Chaenomeles* Lindl. (bs=68%, pp=70%), *Eriobotrya* (bs=87%, pp=100%), *Osteomeles* Lindl. (bs=71%, pp=98%), *Pourthiaea* Decne (bs=93%, pp=100%), and *Pyrus* (bs=95%, pp=100%). Monophyly of *Cotoneaster* Rupp. was weakly supported by the MP analyses (bs=50%), but had strong support from the BI analyses (pp=95%). *Pyracantha* M. Roem. did not form a clade in either MP or BI tree (Figures 1-2). Neither *Aria* nor *Micromeles* Decne. formed its own clade. Nevertheless, together they formed a robust clade in both MP and BI trees (pp=100%, Figures 1-2). *Malus* was paraphyletic to *Chloromeles*, *Eriolobus*, *Docynia* Decne., and *Docyniopsis* (C.K. Schneid.) Koidz. (Figures 1-2). *Rhaphiolepis* Lindl. was sister to *Eriobotrya* (bs=83%, pp=100%), while *Photinia* species appeared in different clades: some with *Heteromeles* M. Roem. and *Stranvaesia* Lindl., and others with unclear affinities. Nevertheless, the support for the relationships among *Photinia*, *Heteromeles*, *Stranvaesia*, and other genera was weak (Figures 1-2).

DISCUSSION

Generic limits have been controversial in the Pyrinae. Since Linnaeus's (1753, 1754) recognition of only four genera, many new Pyrinae genera have been proposed. The number of currently recognized genera is 28. Many genera (e.g., *Cotoneaster*, *Crataegus*, *Osteomeles*, *Rhaphiolepis*, *Eriobotrya*, *Pyrus*) can be explicitly circumscribed by morphological characters, but the limits of some genera, (e.g., *Amelanchier*, *Sorbus*, *Photinia*, *Malus*, and *Stranvaesia*) have remained unclear. Relationships at the genus and species levels have been successfully resolved in Rosaceae using sequences of nrDNA ITS (Campbell et al., 1995; Lo et al., 2007). Incomplete concerted evolution may lead to the existence of paralogous copies within a single species and the failure to sample all copies may result in erroneous relationships. The paralogy of the ITS region is probably only a minor issue in our analysis because

multiple individuals of the same species formed clades and our focus was on testing generic limits. To our knowledge, this study provides the first molecular evaluation of the generic limits of the Pyrinae with a comprehensive taxon sampling of each genus.

Amelanchier is a disjunct genus between Eurasia and North America with most species in North America and only a few in Asia (Campbell et al., 1997). Apomixis, polyploidy and hybridization have caused the number of recognized species in the genus to range from six to thirty-three (Landery, 1975, Phipps et al., 1991). *Amelanchier* is easily distinguished from other Pyrinae genera by a combination of characters including racemose inflorescence, narrow petals, false locular septa in each locule, and pseudoberries (Robertson et al., 1991). *Peraphyllum* and *Malacomeles* share fruit characters with *Amelanchier*. However, *Peraphyllum*, a monotypic genus, has narrow, fascicled leaves, reduced inflorescences, and orange-colored fruits, while *Malacomeles*, with three species, has a xeromorphic habit and barely connate carpels. In the ITS trees, *Amelanchier* is monophyletic and closely related to *Peraphyllum* and *Malacomeles*, as reported in previous studies (Campbell et al., 1995; Campbell et al., 2007).

Crataegus is a shrub or small tree genus of 186-256 species with a distribution in Eurasia, as well as North America (Phipps et al., 1990). The genus usually possesses lobed leaves, small fruits containing from one to five hard pyrenes, and most species have thorns, which do not occur in any other Pyrinae genera. Our broad analysis of the ITS data supports the monophyly of *Crataegus*. *Mespilus* was separated from *Crataegus* by Medikus in 1793 in light of the fact that its pyrenes are covered while *Crataegus*' are exposed. It is a small genus comprised of two species, one in southern Europe (*M. germanica* L.) and the other in Arkansas (*M. canescens* J.B. Phipps). They form a clade with weak support (Figures 1-2). *Mespilus* differs from *Crataegus* in having entire or sub-entire leaves, large flowers with flattish hypanthia, and pomes with wide-spreading persistent sepals giving a "hollow" appearance to the fruit. Nonetheless, both genera have thorns and two superposed seeds per locule. Their sister relationship is well supported (Figures 1-2). However, a recent study, based on two nuclear (ribosomal ITS and *LEAFY* intron2) and four plastid intergenic regions (*trnS-trnG*, *psbA-trnH*, *trnH-rpl12*, and *rpl20-rps12*) has pointed out that *M. canescens* might be a hybrid species between *Mespilus* and *Crataegus* (Lo et al., 2007).

Eriobotrya has a confined distribution in the subtropical and tropical regions of southern and eastern Asia, and is an evergreen taxon with 26 species (Phipps et al., 1990; Robertson et al., 1991). Our ITS data support the monophyly of *Eriobotrya*. *Rhaphiolepis indica*, distributed in eastern and southern Asia, is sister to the *Eriobotrya* clade. Their close relationship has been suggested based on various shared morphological traits including the coreless fruit with a large seed and thin endocarp (Robertson et al., 1991).

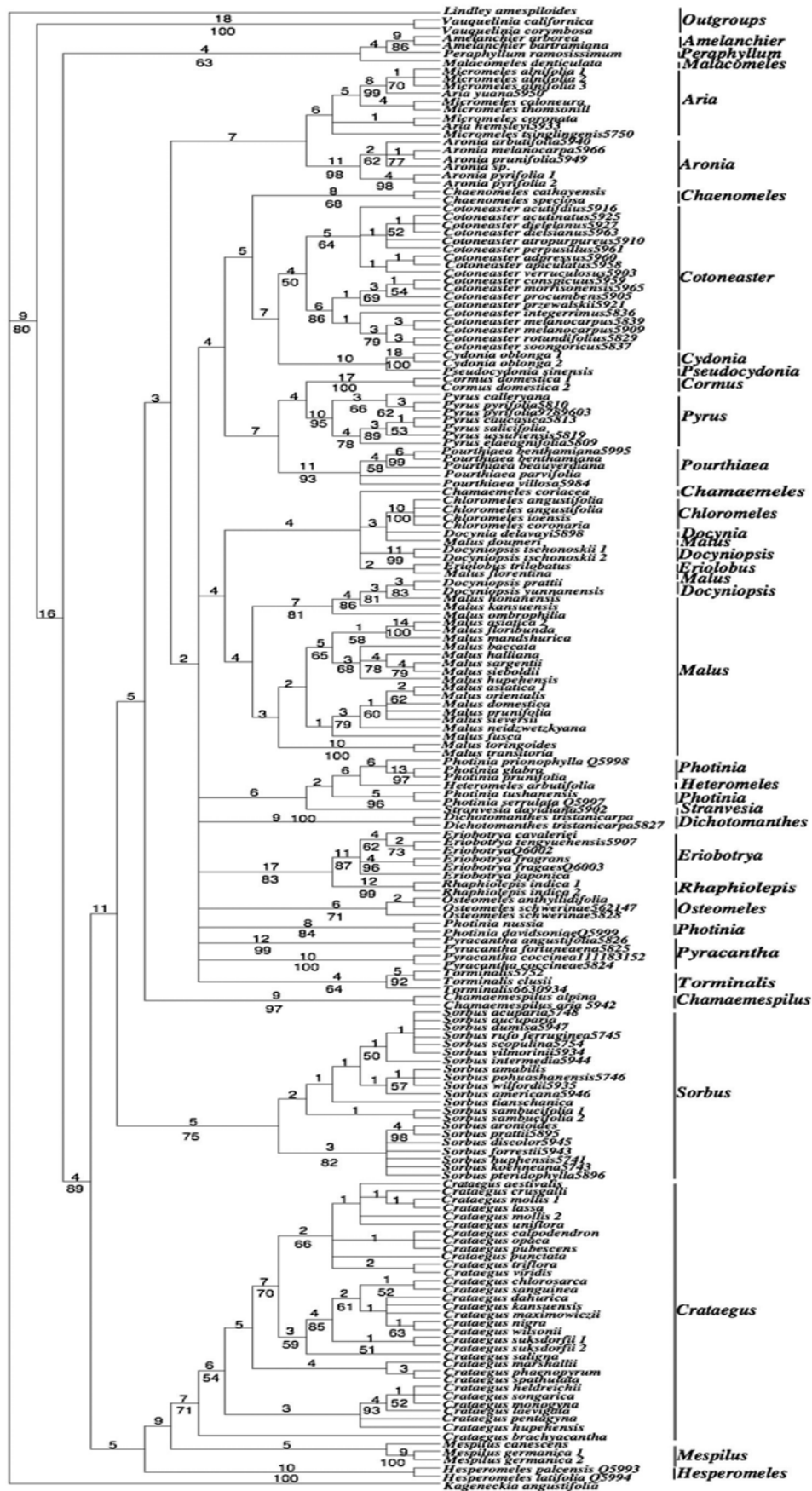


Figure 1. Strict consensus of 20,000 trees based on parsimony analyses of nrDNA ITS sequences (CI=0.39, RI=0.76). Numbers above and below branches are branch lengths and bootstrap percentages, respectively.

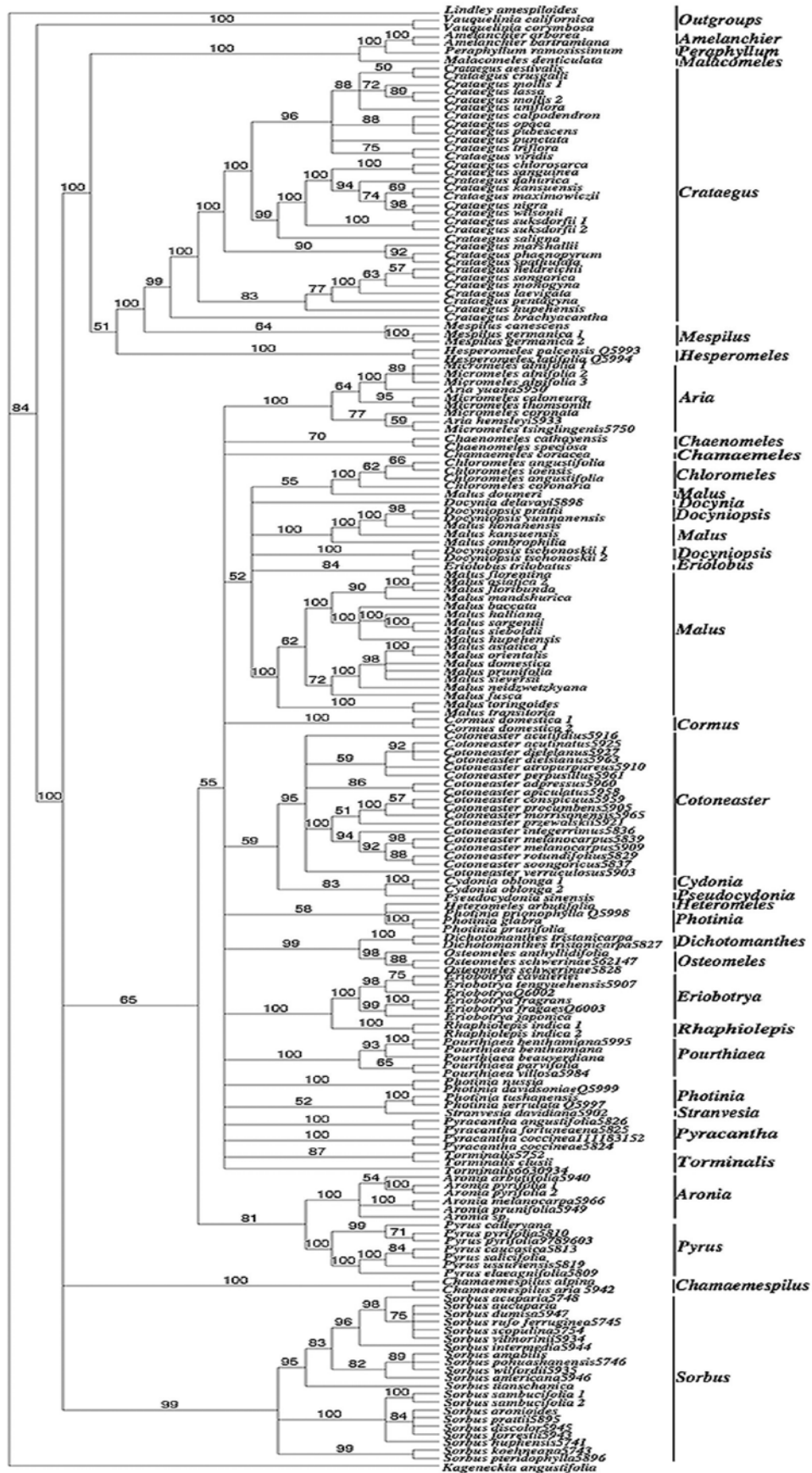


Figure 2. Majority-rule consensus of 3852 trees based on the Bayesian inference with 4,000,000 generations. Numbers at branches indicate posterior probability percentages.

Hesperomeles is an evergreen genus, with or without thorns, having simple leaves, generally 1-4 flowered inflorescences, and small, pinkish flowers. It has five carpels, is fully adnate, has a free style, one ovule per carpel, red to black fruits, and very hard pyrenes separated by a fleshy layer (Robertson et al. 1991). *Hesperomeles* is comprised of 11 species, endemic to the Andes Mountains of South America. It shares solitary ovules and hard pyrenes with *Osteomeles* (Rohrer et al., 1991). But Robertson et al. (1991) considered *Hesperomeles* to be a distinct genus based on the simple leaves and reduced inflorescences. As the first molecular evaluation of *Hesperomeles*, our ITS data suggest that *Hesperomeles* is distantly related to *Osteomeles* and may be sister to the clade of *Crataegus* and *Mespilus* (Figures 1-2). The three genera share morphologically similar simple leaves, possible thorns, and a hard core. Most species of *Crataegus* are distributed in North America, but the distribution of *Crataegus mexicana* DC. extends to Guatemala; and in the *Hesperomeles* genus, *Hesperomeles obovata* (Pittier) Standl. and *Hesperomeles heterophylla* (Ruiz. & Pav.) Hook can be found in Costa Rica. Based on the distribution of the two genera, Phipps (1983) suggested that *Hesperomeles* may have originated from *Crataegus mexicana* or an extinct relative. Our data provide weak support for the close relationship of *Amelanchier-Malacomeles-Peraphyllum* and *Crataegus-Mespilus-Hesperomeles*, as shown in Campbell et al., 2007, but do not support the derivation of *Hesperomeles* from within *Crataegus*.

Osteomeles has three species in eastern Asia and Hawaii, is the only evergreen genus, and has compound leaves and hard pyrenes (Robertson et al., 1991). It is supported as a clade in the MP tree (71%, Figure 1). In the BI tree, however, *Osteomeles* forms a clade with *Dichotomanthes* with strong support (99%) in the BI tree. *Dichotomanthes* is a monotypic genus found in limited areas of Yunnan and Sichuan provinces of China, and is unique with its single carpel and oblique style that is not adnate to the hypanthium. Nevertheless, evidence from cytology, flavonoid chemistry and wood anatomy indicates a strong affinity between *Dichotomanthes* and the remaining genera of Pyrinae (Rohrer et al. 1994). Albeit without morphological synapomorphy, *Dichotomanthes* seems to be most closely related to *Osteomeles* (pp=99% in the BI tree).

Chamaemeles is a monotypic genus endemic to Madeira. Although with a single carpel as in *Dichotomanthes*, *Chamaemeles* has carpels almost fully inferior. In the ITS trees (Figures 1-2) and Campbell et al.'s study (2007), the relationship of *Chamaemeles* is unresolved.

Cydonia Mill. is a monotypic genus in southwestern and central Asia, and shares multiple ovules per locule with *Pseudocydonia* C.K. Schneid., another monotypic genus in Asia. *Chaenomeles* Lindley is distributed in eastern Asia. In both our MP and BI trees (Figures 1-2), *Cydonia* and *Pseudocydonia* form a weak clade that is sister to *Cotoneaster* (pp=59%). *Cotoneaster* is a species-rich

genus with over 250 species. Two subgenera have been recognized based on petal characters: subg. *Chaenopetalum* with white, spreading petals; subg. *Cotoneaster* with pinkish flowers and erect petals (Robertson et al., 1991). In the ITS trees, although neither of the two subgenera is monophyletic, together they form a robust clade (pp=95%, Figures 1-2). Morphologically, *Cotoneaster* is distinguished from other genera by a combination of characters including lack of thorns, simple and entire leaves with camptodromous venation, 2-3 carpels, 2/3 adnation, no connation, free styles, fruits with hard pyrenes, and calyx lobe flesh, incurved, and persistent.

Pyracantha consists of nine species in Eurasia and has occasionally been included in *Cotoneaster* (Focke, 1888; Wenzig, 1883). *Pyracantha* differs from *Cotoneaster* in having thorns, toothed leaves, and five carpels. Albeit with poor resolution, our ITS data and Campbell et al.'s (2007) results do not support the close relationship of *Cotoneaster* and *Pyracantha* (Figures 1-2). Asian species of *Pyracantha* form a clade, but they do not show a close relationship with *P. coccinea* of southern Europe and Iran (Figures 1-2). Therefore, *Pyracantha* may not be monophyletic.

Photinia differs from other genera of the Pyrinae in having the combination of simple leaves, calyx lobes that are persistent, incurved, and fleshy and a soft to leathery core. However, other characters are diverse: unarmed or armed with thorns, toothed or entire leaves, red, black, or purple fruits. Therefore, several segregate genera have been recognized from *Photinia*: *Stranvaesia*, *Pourthiaea*, and *Aronia*. *Stranvaesia* is separated from *Photinia* due to its dehiscent carpels (Lindley, 1837). However, this diagnostic character may have resulted from the artificial pressing of herbarium specimens (Kalman, 1973) and is confidently rejected today. In the ITS tree (Figures 1-2), *Stranvaesia* is sister to the clade containing *Heteromeles*, *Photinia glabra* (Thunb.) Franch. & Sav., *P. prunifolia* Lindl., and *P. tushanensis* T.T. Yu. The support, however, is weak. *Stranvaesia* and *Photinia* do not form a clade in either cpDNA or nuclear DNA trees (Campbell et al., 2007). *Pourthiaea* forms a well-supported clade in our ITS trees, indicating that it may be recognized as a separate genus. The potential synapomorphy of *Pourthiaea* is the deciduous habit. *Aronia* is different from *Photinia* in having glands along the upper midribs of the leaves, a feature also present in other Pyrinae genera (Robertson, 1992). Nevertheless, the monophyly of *Aronia* is supported by our molecular data (Figures 1-2) and by Guo et al. (2010).

Pyrus consists of 73 species with corymbose-racemose inflorescences, 2 ovules per locule, free styles, a cartilaginous endocarp and dense sclereids in the fruits (Robertson et al., 1991). In our ITS trees (Figures 1-2), *Pyrus* forms a well supported clade.

Malus and *Sorbus* are the most controversial genera in the Pyrinae. The disagreement on the generic limits of *Malus* rests on whether or not to recognize several monotypic or small genera: *Chloromeles*, *Eriolobus*, *Docynia*, and *Docyniopsis*. *Chloromeles* differs from other species

of *Malus* in having greenish, fragrant, often waxy fruits with a dense layer of sclereids around the core and just under the skin. *Eriolobus*, with a single species in the eastern Mediterranean, is unique in having deeply lobed simple leaves, incomplete adnation of carpels, and abundant sclereids in fruits. *Docynia* has two species, one in the Himalayas and from Assam to Vietnam and the other in southwestern China. *Docyniopsis* consists of four species, all in eastern Asia, and differs from *Docynia* in having only two ovules per locule (vs. 3-10 in *Docynia*). Nevertheless, the two genera share similar flavonoids chemistry (Williams, 1982). In the phylogenies, *Docynia delavayi* C.K. Schneid. is closely related to *Malus doumeri* A. Chev., *M. florentina* C.K. Schneid. and *Eriolobus*. *Docyniopsis tschonokii* (Maxim.) Koidz., *D. prattii* (C.K. Schneid.) Koidz., and *D. yunnanensis* (C.K. Schneid.) Koidz. do not form a clade, and the latter two species are closely allied with *Malus honanensis* Rehder, *M. kansuensis* (Batalin) C.K. Scheid., and *M. ombrophila* Hand.-Mazz. (bs=86%, pp=100%). *Chloromeles* forms a clade, but its relationship with other clades within *Malus* remains unresolved. Similarly, *Docyniopsis*, *Eriolobus* and *Malus* form a robust clade (95%) in Campbell et al.'s (2007) GBSSI-2B tree, but their relationships are unresolved. Therefore, it is appropriate to circumscribe *Malus* broadly, containing *Chloromeles*, *Docynia*, *Docyniopsis*, *Eriolobus*, and *Malus*.

Some authors recognize *Sorbus* in the broad sense, while others divide it into five genera (Robertson et al., 1991): *Sorbus*, *Aria*, *Cormus*, *Torminalis*, and *Chamaemespilus*. A major reason that taxonomists in Europe and western Asia include these other genera in *Sorbus* is the large number of apomictic microspecies intermediate between them in those regions (McAllister H. 2005). Robertson et al. (1991) cited several examples of intergeneric hybrids involving *Sorbus* and other genera of the Pyrinae, such as \times *Sorbocotoneaster*, \times *Sorbaronia*, \times *Amelosorbus* and \times *Crataegosorbus*, and concluded that "the extensive hybridization between genera and subgeneric groups seems to reflect weak overall barriers to hybridization rather than indicating evolutionary relationships", and "it seems best to discount intergeneric hybridization when setting generic limits."

Cormus and *Sorbus* have pinnately compound leaves, *Torminalis* leaves are pinnately lobed, and those of *Chamaemespilus* are simple and toothed with camptodromous venation. However, *Aria* is diverse in leaf morphology; some species have coarsely toothed leaves with craspedodromous venation, while others have simple leaves and camptodromous venation (Robertson, 1992). Kovanda and Challice (1981) segregated species with deciduous calyx lobes into *Micromeles*. However, the calyx feature is inconsistent in the Pyrinae, and thus *Micromeles* should not be recognized (Robertson, 1992; Rohrer et al., 1991). In the ITS trees, *Micromeles* species are intermixed with those of *Aria* (Figures 1-2), while *Cormus*, *Torminalis*, *Chamaemespilus* each form their own clades. Our ITS

data thus support their generic status in the Pyrinae.

CONCLUSIONS

Our ITS data, from multiple species representing the diversity of traditionally recognized genera, support recognition of 24 genera that are resolved as monophyletic: *Amelanchier*, *Aria* (including *Micromeles*), *Aronia*, *Chaenomeles*, *Chamaemespilus*, *Chamaemeles*, *Cormus*, *Cotoneaster*, *Crataegus*, *Cydonia*, *Dichotomanthes*, *Eriobotrya*, *Hesperomeles*, *Malacomeles*, *Malus* (including *Chloromeles*, *Docynia*, *Docyniopsis*, and *Eriolobus*), *Mespilus*, *Osteomeles*, *Peraphyllum*, *Pourthiaea*, *Pseudo-cydonia*, *Pyrus*, *Rhaphiolepis*, *Sorbus*, and *Torminalis*.

Most of these genera are essentially in agreement with recent works (Robertson et al., 1991). Among those genera, *Aronia* and *Pourthiaea* are separated from *Photinia* as independent genera, and *Pourthiaea* is for the first time supported by molecular data as a genus; *Hesperomeles* is also examined for the first time using molecular data and may have a close relationship to *Crataegus-mespilus* instead of *Osteomeles*. Our data support the inclusion in *Malus* of *Chloromeles*, *Docynia*, and *Docyniopsis* and suggest that *Pyracantha* may be polyphyletic. *Photinia* is found to be polyphyletic and possibly closely related to *Heteromeles* and *Stranvaesia*. However, more extensive sampling is needed to determine the generic limits of *Pyracantha*, *Photinia*, and *Stranvaesia*.

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LITERATURE CITED

- Campbell, C.S., C.W. Greene, and T.A. Dickinson. 1991. Reproductive biology in subfam. Maloideae, Rosaceae. *Syst. Bot.* **16**: 333-349.
- Campbell, C.S., M.J. Donoghue, B.G. Baldwin, and M.F. Wojciechowski. 1995. Phylogenetic-Relationships in Maloideae (Rosaceae) - Evidence from Sequences of the Internal Transcribed Spacers of Nuclear Ribosomal DNA and Its Congruence with Morphology. *Amer. J. Bot.* **82**: 903-918.
- Campbell, C.S., M.F. Wojciechowski, B.G. Baldwin, L.A. Alice, and M.J. Donoghue. 1997. Persistent nuclear ribosomal DNA sequence polymorphism in the *Amelanchier* agamic complex (Rosaceae). *Mol. Biol. Evol.* **14**: 81-90.

- Campbell, C.S., R.C. Evans, D.R. Morgan, T.A. Dickinson, and M.P. Arsenault. 2007. Phylogeny of subtribe Pyrinae (formerly the Maloideae, Rosaceae): Limited resolution of a complex evolutionary history. *Plant Syst. Evol.* **266**: 119-145.
- de Candolle, A.P. 1825. Rosaceae. *Prodromus* **2**: 525-639.
- Decaisne, M.J. 1874. Memoirs sur le famille des Pomacees. *Nouv. Arch. Mus. Hist. Nat.* **10**: 113-192.
- Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Res.* **32**: 1792-1797.
- Evans, R.C. and C. Campbell. 2002. The origin of the apple subfamily (Maloideae; Rosaceae) is clarified by DNA sequence data from duplicated GBSSI genes. *Am. J. Bot.* **89**: 1478-1484.
- Evans, R.C., L.A. Alice, C.S. Campbell, E.A. Kellogg, and T.A. Dickinson. 2000. The granule-bound starch synthase (GBSSI) gene in the Rosaceae: Multiple loci and phylogenetic utility. *Molec. Phylog. Evol.* **17**: 388-400.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.
- Focke, W.O. 1888. Rosaceae Nat. Pflanzenfam. III., 1-61, Lepig. pp. 1-61.
- Fritsch, K. 1898. Zur Systematik der Gattung Sorbus. *Osterr. Bot. Z.* **48**: 1-4, 47-49, 167-171.
- Fritsch, K. 1899. Zur Systematik der Gattung Sorbus. *Osterr. Bot. Z.* **49**: 381-385, 426-429.
- Gladkova, V.N. 1969. On the systematic position of the genus *Dichotomanthes*. *Bot. Zhur.* **54**: 421-436.
- Goldblatt, P. 1976. Cytotaxonomic studies in the tribe Quillajeae (Rosaceae). *Ann. Missouri Bot. Gard.* **63**: 200-206.
- Kalman, C. 1973. The Malesian species of the subfamily Maloideae (Rosaceae). *Blumea* **21**: 413-442.
- Koehne, E. 1891. Die Gattungen der Pomaceen. *Gartenflora* **40**: 4-7, 35-38, 59-61.
- Kovanda, M. and J.S. Challice. 1981. The genus *Micromeles* revisted. *Folia Geobotanica Phytotaxonomica* **16**: 181-193.
- Li, J. 2008. Phylogeny of *Catalpa* (Bignoniaceae) inferred from sequences of chloroplast *ndhF* and nuclear ribosomal DNA. *J. Syst. Evol.* **46**: 341-348.
- Lindley, J. 1822. Observations on the natural group of plants called Pomaceae. *Trans. Linn. Soc. London* **13**: 88-106.
- Lindley, J. 1837. *Stranvaesia glaucescens*. *Bot. Reg.* **23**: t.1956.
- Lindley, J. 1845. *Nagelia denticulata*. *Bot. Reg.* **31 (misc.)**: 40.
- Linnaeus, C. 1753. *Sp. Pl.* pp.
- Lo, E.Y.Y., S. Stefanovic, and T.A. Dickinson. 2007. Molecular reappraisal of relationships between *Crataegus* and *Mespilus* (Rosaceae, Pyreae) - two genera or one? *Syst. Bot.* **32**: 596-616.
- Morgan, D.R., D.E. Soltis, and K.R. Robertson. 1994. Systematic and Evolutionary Implications of *RbcL* Sequence Variation in Rosaceae. *Amer. J. Bot.* **81**: 890-903.
- Oh, S.H. and D. Potter. 2003. Phylogenetic utility of the second intron of *LEAFY* in *Neillia* and *Stephanandra* (Rosaceae) and implications for the origin of *Stephanandra*. *Mol. Phylogenetics Evol.* **29**: 203-215.
- Phipps, J.B., K.R. Robertson, P.G. Smith, and J.R. Rohrer. 1990. A checklist of the subfamily Maloideae (Rosaceae). *Canad. J. Bot.* **68**: 2209-2269.
- Phipps, J.B., K.R. Robertson, J.R. Rohrer, and P.G. Smith. 1991. Origin and evolution of subfam. Maloideae (Rosaceae). *Syst. Bot.* **16**: 303-332.
- Posada, D. and K.A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics Application Note* **14**: 817-818.
- Potter, D., T. Eriksson, R.C. Evans, S. Oh, J.E.E. Smedmark, D.R. Morgan, M. Kerr, K.R. Robertson, M. Arsenault, T.A. Dickinson, and C.S. Campbell. 2007. Phylogeny and classification of Rosaceae. *Plant Syst. Evol.* **266**: 5-43.
- Rehder, A. 1940. Manual of cultivated trees and shrubs hardy in North America exclusive of the subtropical and warmer temperature regions. 2nd edition ed. Macmillan, New York, pp.
- Rehder, A. 1949. bibliography of cultivated trees and shrubs hardy in the cooler temperature regions of the northern hemisphere Arnold Arboretum of Harvard University, Jamaica Plain, MA.
- Robertson, K.R. 1992. Summary of leaves in the genera of Maloideae (Rosaceae). *Ann. Miss. Bot. Gard.* **79**: 81-94.
- Robertson, K.R., J.B. Phipps, J.R. Rohrer, and P.G. Smith. 1991. A synopsis of genera in Maloideae (Rosaceae). *Syst. Bot.* **16**: 376-394.
- Roemer, M.J. 1847. *Familiarum naturalium regni vegetabilis synopsis monographicae. III. Rosiflorae. Amygdalacearum et Pomacearum Landes-Industrie-Comptoir*, Weimar.
- Rohrer, J.R., K.R. Robertson, and J.B. Phipps. 1991. Variation in structure among fruits of Maloideae (Rosaceae). *Amer. J. Bot.* **78**: 1617-1635.
- Ronquist, F. and J. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574.
- Savile, D.B.O. 1979. Fungi as aids in higher plant classification. *Bot. Rev. (Lancaster)* **45**: 377-503.
- Swofford, D.L., 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods), 4.0b10 ed. Sinauer Associates, Sunderland.
- Wenzig, T. 1883. Die Pomaceen. Charaktere der Gattungen und Arten. *Jahrb. Konigl. Bot. Gart. Berlin* **2**: 287-307.
- Williams, A.H. 1982. Chemical evidence from the flavonoids relevant to the classification of *Malus* species. *J. Linn. Soc. Bot.* **84**: 31-39.

基於核基因序列探討蘋果亞科的屬間界限

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Pyrinae 亞族（原蘋果亞科）為單系類群，約具 1,000 個種。本亞族包括了許多著名的水果：如蘋果、梨、榲桲、枇杷、野櫻莓、唐棣。本亞族的分類一直很混亂，特別是對蘋果屬、花楸屬和石楠屬的分類一直存在爭議。本次研究共包括 180 個 ITS 序列，代表了本亞族內所有的屬，本文即利用 ITS 分子序列分析來研究 Pyrinae 亞族的屬間關係。ITS 序列分析結果顯示，以下 24 屬得到確認，即：*Amelanchier*、*Aria*（包括 *Micromeles*）、*Aronia*、*Chaenomeles*、*Chamaemespilus*、*Chamaemeles*、*Cormus*、*Cotoneaster*、*Crataegus*、*Cydonia*、*Dichotomanthes*、*Eriobotrya*、*Hesperomeles*、*Malacomeles*、*Malus*（包括 *Chloromeles*、*Docynia*、*Docyniopsis* 和 *Eriolobus*）、*Mespilus*、*Osteomeles*、*Peraphyllum*、*Pourthiaea*、*Pseudocydonia*、*Pyrus*、*Raphiolepis*、*Sorbus* 和 *Torminalis*。但石楠屬和火棘屬則顯示為多起源，包括了 *Heteromeles* 和 *Stranvaesia*，所以它們與亞族內其它屬的關係尚未解決。研究結果支持唐棣屬與 *Malacomeles*、*Peraphyllum* 具有較近的親緣關係，山楂屬和歐楂屬具有較近的親緣關係，並第一次確定了南美的 *Hesperomeles* 與 *Crataegus-Mespilus* 具較近的親緣關係。

關鍵詞：蘋果亞科；Pyrinae；nrDNA ITS；屬間關係；*Hesperomeles*。