Araştırma Makalesi **BAUN Fen Bil. Enst. Dergisi, 23(2), 716-731, (2021)**

DOI:10.25092/baunfbed.930696 **J. BAUN Inst. Sci. Technol., 23(2), 716-731, (2021)**

Defining phylogenetic relationship between the genera *Opopanax* and *Crenosciadium* using nuclear and chloroplast DNA sequence data

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Geliş Tarihi (Received Date): 16.10.2020
Kabul Tarihi (Accepted Date): 03.04.2021

Abstract

A phylogenetic study of the Opopanax and Crenosciadium was conducted to elucidate their circumscription and infrageneric relationships. Sequence data from nrDNA ITS, cpDNA matK and trnL-F were used to determine their phylogenetic relationship. Polymerase Chain Reaction (PCR) amplifications of all regions were performed using universal primers. Maximum Likelihood (ML) trees were constructed using PAUP software, another phylogenetic tree of ITS sequences showing node ages was obtained from MrBayes programme and Neighbour Net Diagrams were constructed using Splits Tree programme. Our results clearly showed that Smyrniopsis and Opopanax are allied strongly with Petroedmondia syriaca, Magydaris panacifolia, and Crenosciadium siifolium.*

Keywords: *Opopanax, Crenosciadium, Apiaceae, Phylogeny*

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Opopanax ve *Crenosciadium* cinsleri arasındaki filogenetik ilişkinin çekirdek ve kloroplast DNA dizisi verilerinin kullanılarak belirlenmesi

Öz

Opopanax ve Crenosciadium üzerine filogenetik bir çalışma, cinslerin sınırlarını ve cinsler arası ilişkilerini aydınlatmak için yapıldı. Çekirdek DNA'sından ITS, kloroplast DNA'sından matK ve trnL-F''den elde edilen dizi verileri, filogenetik ilişkilerini belirlemek için kullanıldı. Tüm bölgelerin Polimeraz Zincir Reaksiyonu (PCR) amplifikasyonları evrensel primerler kullanılarak yapıldı. Maksimum Olabilirlik (ML) ağaçları, PAUP yazılımı kullanılarak oluşturuldu, ITS dizilerinin nod yaşlarını gösteren bir diğer filogenetik ağacı MrBayes programından elde edildi ve Komşu Ağ Diyagramları, Splits Tree Programı kullanılarak oluşturuldu. Sonuçlarımız, Smyrniopsis ve Opopanax'ın Petroedmondia syriaca, Magydaris panacifolia ve Crenosciadium siifolium ile güçlü bir şekilde yakın ilişkili olduğunu açıkça gösterdi.*

Anahtar kelimeler: *Opopanax, Crenosciadium, Apiaceae, Filogeni.*

1. Introduction

The flowering plant family Apiaceae (Umbelliferae) comprises approximately 450 genera and 3700 species [1-2]. It is widely distributed in the temperate zones of both the northern and southern hemispheres and is highly diverse in Central Asia [3]. Apiaceae comprises 105 genera and approximately 493 species in Turkey [4].

The genus *Opopanax* W.D.J.Koch is distributed in southern Europe, Mediterranean region and Western Asia. *Crenosciadium* Boiss. & Heldr. is a monotypic endemic genus, which is distributed in Central Anatolia in Turkey. *Opopanax* is represented by two species, *O. hispidus* Griseb. and *O. persicus* Boiss. & Heldr., in Flora Iranica Rechinger [5], by only one species, *Opopanax hispidus*, in Flora of Cyprus [6], by only one species, *O. armeniacum* Bordz., in Flora of USSR [7], by two species, *O. chironium* W.D.J. Koch and *O. hispidus* in Flora Europaea [8]. According to Flora of Turkey and the latest study, *Opopanax* represented three species in Turkey [9-10]. Turkey is the only area where all *Opopanax* species can proliferate together. *O. persicus* has limited geographical extension in the world and is distributed in Iran, Armenia, and Eastern part of Turkey. The eastern part of Turkey is the western boundary of distribution of this species. *O. hispidus* has wider geographic distribution than the other *Opopanax* species and it is distributed from Europe to the western parts of Asia. *O. chironium* is distributed from the western part of Europe to Balkans.

The earlier molecular studies on Apiaceae family showed that there were problems in most tribal and intergeneric classifications. The classification of the family was reconstructed based on molecular data. The first comprehensive molecular phylogenetic analysis of *Opopanax*, based ITS sequence [11] show that the genus very closely related to *Smyrniopsis* Boiss. and these positions were confirmed by Ajani et al. [12].

The aim of this study was to determine the genetic relationships between *Opopanax* and its allies and to resolve their unclear and controversial status by using a DNA-based molecular marker system.

2. Material and methods

1.1. Plant specimens

Specimens were collected from different localities in Turkey (Appendix 1) and plant samples were identified according to Tutin [8], Chamberlain [9], Meikle [6], Shishkin [7] and Rechinger [5].

1.2. DNA extraction and amplification

DNA extractions (both of gDNA and cpDNA) were performed using the DNeasy Plant Mini Kit (QIAGEN, Germany), following the manufacturer's instructions with some modifications (incubation at 65 ^{0}C and waiting on the ice have been set to 30 minutes). Powdered plant tissues were obtained from dried green leaves using liquid nitrogen in a mortar with a pestle 100 µL of elution buffer was used for each the first and the second elutions. PCR procedures were performed with the second elutions. ITS region of studied taxa was amplified using ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') and ITS5a (5' GGA AGG AGA AGT CGT AAC AAG 3') primers [13-14]. For the chloroplast DNA, two different regions were used: trnL-c as forward (5'-CGA AAT CGG TAG ACG CTA CG-3') and trnL-f as reverse (5'-ATT TGA ACT GGT GAC ACG AG-3') primers [15] for *trn*L-F region, and matK- 3F_KIM (forward) (5'-CGT ACA GTA CTT TTG TGT TTA CGA G-3') and matK-1R_KIM (reverse) (5'-ACC CAG TCC ATC TGG AAA TCT TGG TTC-3') primers [16] were used to amplify the desired matK region. PCR conditions set to 95°C for 5 min initial denaturation, 35 cycles of 94 °C for 1 m denaturation, 50 °C for 1 m annealing, and 72 °C for 1 min extension, 72 °C for 10 min final extension. PCR products were visualized by agarose jel.

1.3. DNA sequencing and analysis

The amplified fragments were sequenced using the same PCR primers by BGI Europe. ITS1+5.8S rDNA+ITS2 nrDNA, matK and *trn*L-F cpDNA sequences of the studied taxa were edited Sequencher 5.4 and aligned via Bioedit version 7.2.5. PAUP* 4.0.164 [17] were used to construct phylogenetic trees. Phylogenetic tree criterion was set to likelihood. Bootstrap resampled method was chosen, and search type were set full heuristic. Swapping algorithm was TBR. Also, MrBayes programme [18] was used to obtain another phylogenetic tree to show the node ages of different clades. In addition to these phylogenetic tree, Neighbour Net diagrams were done using Splits Tree 5.0.12.

3. Results and discussion

ITS data—The results were obtained from 56 specimens, *Opopanax* group and 13 outgroups genera (*Echinophora* L., *Ammi* L., *Anethum* L., *Apium* L., *Grammosciadium* DC., *Caropodium* Stapf & Wettst. ex Stapf.) *Pimpinella* L., *Lagoecia* L., *Seseli* L., *Cymbocarpum* DC., *Tordylium* Tourn. ex L., *Heracleum* L., *Tetrataenium* (DC.) Manden., *Pastinaca* L.) (Figure 1-3). The aligned ITS sequences yielded a total of 576 characters and contained 267 conserved sites, 68 variable sites, of which 241 were parsimony informative. ML tree generating from ITS dataset is shown in Figure 1, another phylogenetic tree conducted by MrBayes and a Neighbour-Net diagram are given in Figure 2 and 3, respectively. Maximum Likelihood and MrBayes trees constitute seven major clades.

The member of the first clade is belonging to tribus Echinophoreae. *Echinophora* was first included in Smyrneae by de Candolle [19], and then tribus Echinophoreae was recognized as comprising the genera *Echinophora* and *Pycnocycla* Lindl. [20]. This circumscription of the tribe was expanded to include *Anisosciadium* DC. and *Dicyclophora* Boiss. by Downie et al. [21].

The members of the third clade are belonging to genera *Grammosciadium* in tribus Careae. The members of fourth and fifth clades are *Pimpinella tragium* Vill. and *Lagoecia cuminoides* L. *Smyrneae* was recognized as comprising the genera *Lagoecia* and *Magydaris*, and the others by de Candolle [19]. Then *Lagoecia* was transferred to Saniculeae by Bentham & Hooker [20]. According to phylogenetic analysis, *Lagoecia* is closely related to members of Pyramidoptereae.

The members of the sixth clade are represented by only two taxa, *Ammi* and *Anethum*, in this study. Historically, *Anethum* was placed in tribe Peucedaneae with *Opopanax* and other genera, but phylogenetic studies have revealed that *Anethum* is closed to tribe Apieae.

The members of seventh clade are represented by *Opopanax*, *Smyrniopsis*, *Petroedmondia*, *Magydaris* in this study. The "*Opopanax*" clade is recognized as comprising the genera *Opopanax*, *Smyrniopsis*, *Crenosciadium*, *Petroedmondia* Tamamsch., *Magydaris* W.D.J.Koch ex DC. *Smyrniopsis* and *Opopanax* are allied strongly with *Petroedmondia syriaca*, *Magydaris panacifolia*, and *Crenosciadium siifolium* within the Apioid super clade in study of Ajani et al. [12] and our phylogenetic analyses strongly supported this alliance. In addition to this phylogenetic tree, the other phylogenetic tree based on Bayesian analysis presented in Figure 2 supported ML analysis. And also, clustering of the mentioned genera members is shown the phylogenetic relationships of *Opopanax* and its allies.

The genus *Smyrniopsis* which placed in Smyrnieae of classification of Drude [22] is nested *Opopanax* clade and is more closely related to *Opopanax* than the other genera placed in this group. The tribal name Smyrnieae has been used by virtually all authors of Apiaceae suprageneric classifications. First described by Sprengel [22] and later modified by Koch [24], de Candolle [19], Bentham [20], and Drude [22], the size and composition of the tribe have varied considerably [25]. The artificiality of the tribe, however, has been demonstrated by different researcher [26-30]. The genus *Smyrniopsis* was placed in tribe Ammineae of classifications of Bentham & Hooker [20] and then transferred in the tribe Smyrnieae by Drude [21]. The immunochemical study of Shneyer et al. [26] showed that *Smyrnium* occupies an isolated position away from all other Smyrnieae and other Apiaceae investigated [25]. Recent phylogenetic studies confirm distinct position of *Smyrniopsis* from this clade.

Smyrniopsis was revised by Stevens in Flora of Turkey and he noted that *Smyrniopsis* is not a natural genus. He pointed out the two sections recognized by Boissier have been in the past treated as separate genera. However, he hesitated to change Boissier's

classification, without more extensive study of these genera. Then *Smyrniopsis cachroides* was recognized as new species by S.M.Tamamschian, and this species was treated as a synonym of *Petroedmondia syriaca* in Flora Iranica. The mericarps are concave in transverse section in *Opopanax* while mericarps are nearly semicircular in transverse section in *Petroedmondia*. Moreover, all ribs are equally projecting or narrowly winged in *Petroedmondia*, while lateral and median ribs are slightly projecting and filiform, marginal ribs are strongly projecting and winged in *Opopanax*. The fruits of *Petroedmondia* are multi vittate and vittae ring around the endosperm, while there are 6-8 commissural vittae as well as 1−3 vallecular vittae in each furrow in *Opopanax*.
There are some obvious differences between *Opopanax* and *Smyrniopsis* which is readily distinguished from *Opopanax* by its unwinged and laterally compressed fruit. Moreover, all ribs are filiform in *Smyrniopsis*, although the median and lateral ribs are filiform and marginal one isdistincly winged in *Opopanax*. *Smyrniopsis* is monotypic and endemic to the Caucasus according to Tamamschian [31]. But this genus also includes *Smyrniopsis syriaca* Boiss. and *S. behboudiana* Rech.f. & Esfand. which are currently remain unresolved.

Magydaris which was placed in tribe Smyrnieae of classification of Drude [22] is nested in *Opopanax* clade like as *Smyrniopsis*. *Magydaris* was placed in tribe Seselineae by Bentham & Hooker [20]. The genera *Magydaris, Cachrys* L., and *Prangos* Lindl., together with *Phellopterus* (Nutt. ex Torr. & A.Gray) J.M.Coult. & Rose were referred to subtribe Cachrydeae of the tribe Seselineae, far from Ammineae-Smyrnieae [26] but phylogenetic studies have revealed that *Magydaris* is closed to *Opopanax* clade.

Bentham & Hooker [20] considered that *Crenosciadium* was closely allied to and possibly congeneric with *Opopanax*. Menemen [32] rearranged *Crenosciadium siifolium* as a synonym of *Opopanax siifolium* but the latter study showed their distinct position. *Crenosciadium* apparently differs from the *Opopanax* which has dorsally flattaned fruit, a character which would clearly differentiate the two genera. Mericarp ribs are also significant characteristics to differentiate these two genera. Mericarps of *Crenosciadium* show five distinctly projecting ribs, whereas those of *Opopanax* have 3 slender, filiform dorsal ridges.

According to Chamberlain [9], *O. hispidus* and *O. persicus* are closely related and may prove to be conspecific. The two accessions of *O. hispidus* do not unite as monophyletic in study of Ajani et al. [12]. Leaf lamina indumentum is the distinguishing character of the genus *Opopanax*. They are stellate hairy on both sides in *O. hispidus*, and glabrous beneath in *O. persicus*.

The members of the eighth clade are belonging *Cymbocarpum*, *Tordylium*, *Tetrataenium*, *Heracleum*, *Pastinaca*. Tribe Tordylieae was circumscribed by Pimenov & Leonov [1] to include the genera *Ainsworthia* Boiss., *Ducrosia* Boiss., *Heracleum*, *Kalakia* Alava, *Malabaila*, *Pastinaca*, *Semenovia* Regel & Herder, *Symphyoloma* C.A.Mey., *Tetrataenium, Tordylium, Vanasushava* P.K.Mukh. & Constance, and *Zosima* [12]. The circumscription of Tordylieae was expanded the to include *Cymbocarpum* by Ajani et al. [11].

The classification of Bentham & Hooker [20], *Opopanax* was placed into tribe Peucedaneae with *Johrenia* DC., *Tordylium, Zosimia, Ducrosia* Boiss., *Malabaila, Polytaenia* DC., *Leptotaenia* Nutt. ex Torr. & A.Gray, *Heracleum*, *Lefeburia* Lindl., *Peucedanum*, *Dorema* D.Don, *Perula* Schreb. Some genera placed in this tribus was transferred to *Tordylieae.* Recent phylogenetic studies confirm distinct position of *Opopanax* from this clade.

matK data.— The analysis comprises 27 accessions including 21 outgroups (*Crenosciadium siifolium, Apium nodiflorum* (L.) Lag., *A. graveolens* L., *Grammosciadium daucoides* DC., *Heracleum maximum* W.Bartram, *H. candicans* var. *obtusifolium* (Wall. ex DC.) F.T.Pu & M.F.Watson, *Tetrataenium canescens* (Lindl.) Manden., *Seseli yunnanense* Franch., *S. mairei* H.Wolff., *Anethum graveolens* L., *Ammi trifoliatum* Trel., *Pimpinella tragium* Vill., *Lagoecia cuminoides, Ammi visnaga* (L.) Lam., *Ammi majus* L., *Petroedmondia syriaca*) (Figure 3). Of the aligned matK sites, yielded a total of 770 characters, 79 were variable and 42 parsimony informative. 649 of the total characters were constant. RI and CI indices were 0.853 and 0.868, respectively, and homoplasy index was 0.132.

trnL-F data.— The analysis comprises 21 accessions including 8 outgroups (*Crenosciadium siifolium, Apium nodiflorum, Lagoecia cuminoides, Ammi visnaga, Ammi majus, Pimpinella tragium* Vill., *Grammosciadium daucoides, Petroedmondia syriaca, Seseli mairei, S. squarrulosum* R.H.Shan & M.L.Sheh, *Heracleum candicans* var. *obtusifolium, H. dissectifolium* K.T.Fu, *Pastinaca sativa* L.) of the aligned trnL-F sites (Figure 4), yielded a total of 812 characters, 92 were variable and 30 parsimony informative. RI and CI indices were 0.732 and 0.873, respectively, and homoplasy index was 0.127.

4. Conclusion

Fruit morphology and anatomy were traditionally viewed as the most promising sources of taxonomic characters, exhibiting some variation in features such as fruit shape, the degree and direction of mericarp compression, modifications of the pericarp ribs (e.g., wings or spines), and the shape of mericarp commissural faces. Thus, most traditional classifications of Apiaceae have relied almost exclusively on fruit characters [35-37].
Bentham & Hooker [20] emphasized the importance of the number of mericarp ribs and proposed three series, Heterosciadiae, Haplozygiae and Diplazygiae, and 9 tribus. *Opopanax* was placed into tribe Peucedaneae with *Johrenia*, *Tordylium*, *Zosimia*, *Ducrosia*, *Malabaila*, *Polytaenia*, *Leptotaenia*, *Heracleum*, *Lefeburia*, *Peucedanum*, *Dorema*, *Perula*.

According to Boissier [38], *Opopanax* was placed in tribe Peucedaneae with *Crenosciadium*, *Ferulago* W.D.J.Koch, *Johrenia*, *Peucedanum*, *Anethum*, *Krubera* Hoffm., *Cymbocarpum*, *Ormosciadium* Boiss., *Tordylium*, *Heracleum*, *Zosima*, *Ainswortia* and 14 other genera.

Drude [22] recognized three subfamilies (Apioideae, Saniculoideae, and Hydrocotyloideae) of Apiaceae and dividing each into a series of tribes and subtribes. The genus Opopanax was placed in Peucedaneae in subfamily Apioideae with *Pastinaca*, *Peucedanum*, *Ferulago*, *Ferula*, *Johrenia*, *Diplotaenia*, *Capnophyllum* Gaertn., and 13 other genera.

The tribal classification of *Opopanax* and *Crenosciadium* has not changed for many years and these genera was evaluated in tribe Peucedanee. A revised tribal classification of *Opopanax* and *Crenosciadium* was proposed by Ajani et al. [12] and Downie et al. [21]. The first comprehensive molecular phylogenetic analysis of *Opopanax*, based ITS sequence [10] show that the genus very closely related to *Smyrniopsis aucheri* and these positions were confirmed by Ajani et al. [12] and Downie et. al [21]. According to phylogenetic study of Ajani et al. [12], *Opopanax* and *Smyrniopsis* are allied strongly with *Petroedmondia syriaca* (Boiss.) Tamamsch., *Magydaris panacifolia* (Vahl) Lange, and *Crenosciadium siifolium* Boiss. & Heldr. within the Apioid superclade.

According to phylogenetic analysis performed in this study, ITS data gave more information about the relationship between *Opopanax* and *Crenosciadium* genera. And, because of there are more specimens in NCBI, we presented a better solution according to ITS data. In ITS phylogenetic tree (Figure 1), we can see that *Petroedmondia syriaca* shares the same clade with *Opopanax*, *Smyrniopsis*, *Magydaris* and *Creneosciadium* members. *Smyrniopsis aucheri* (Ajani854 which a NCBI data) appears to be the closest specimen to *Opopanax* members examined in this study. Six different *Opopanax* specimens show that this species is probably not monophyletic. But on the other hand, the genus *Opopanax* seems be monophyletic. But on the other hand, the genus *Opopanax* seems be monophyletic. matK and *trn*L-F data did not solve the phylogenetic relationship as enough as nrITS data. According to matK phylogenetic tree (Figure 4), *Petroedmondia syriaca* and *Crenosciadium siifolium* differs *Opopanax* species, and according to *trn*L-F data (Figure 5) *C. siifolium* is the sister group with *Opopanax* species and *Petroedmondia syriaca* is the closest out group member of this two genera. In conclusion, our previously published rearrangements [10] have been confirmed by phylogenetic analysis in this new study. *Opopanax* species show a correlation between morphologic diagnostic characters and molecular taxonomic classification in the dendogram. We believe that this study will play a role in resolving the problems of taxonomy in *Opopanax* group.

Acknowledgment

We express our thanks to the Scientific Investigation Projects Coordinate Office of the Selçuk University (Project number: 17401082) for financial support.

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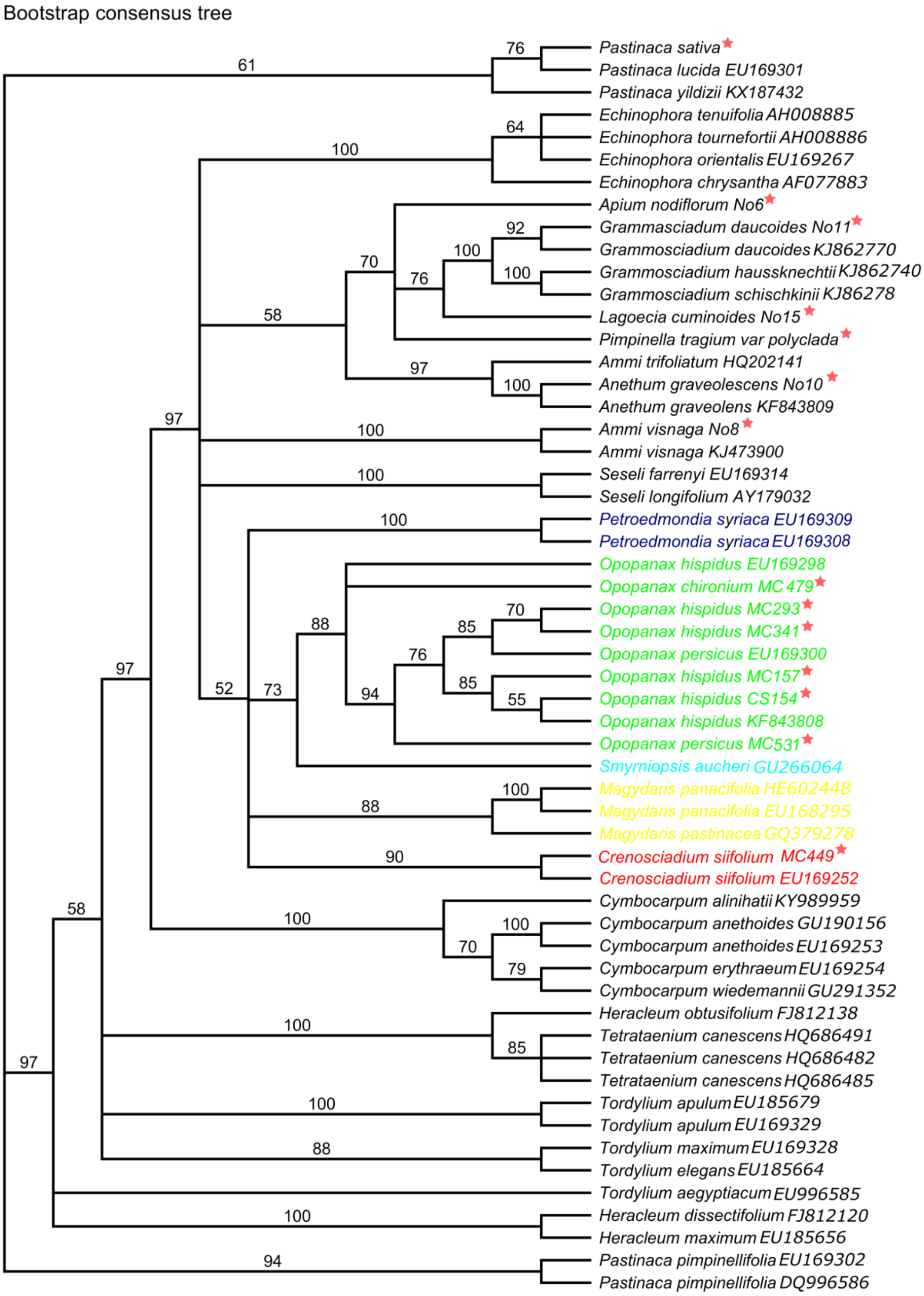


*: taxa examined in this study.

Figure 1. Maximum Likelihood phylogenetic tree based on nrITS DNA data.

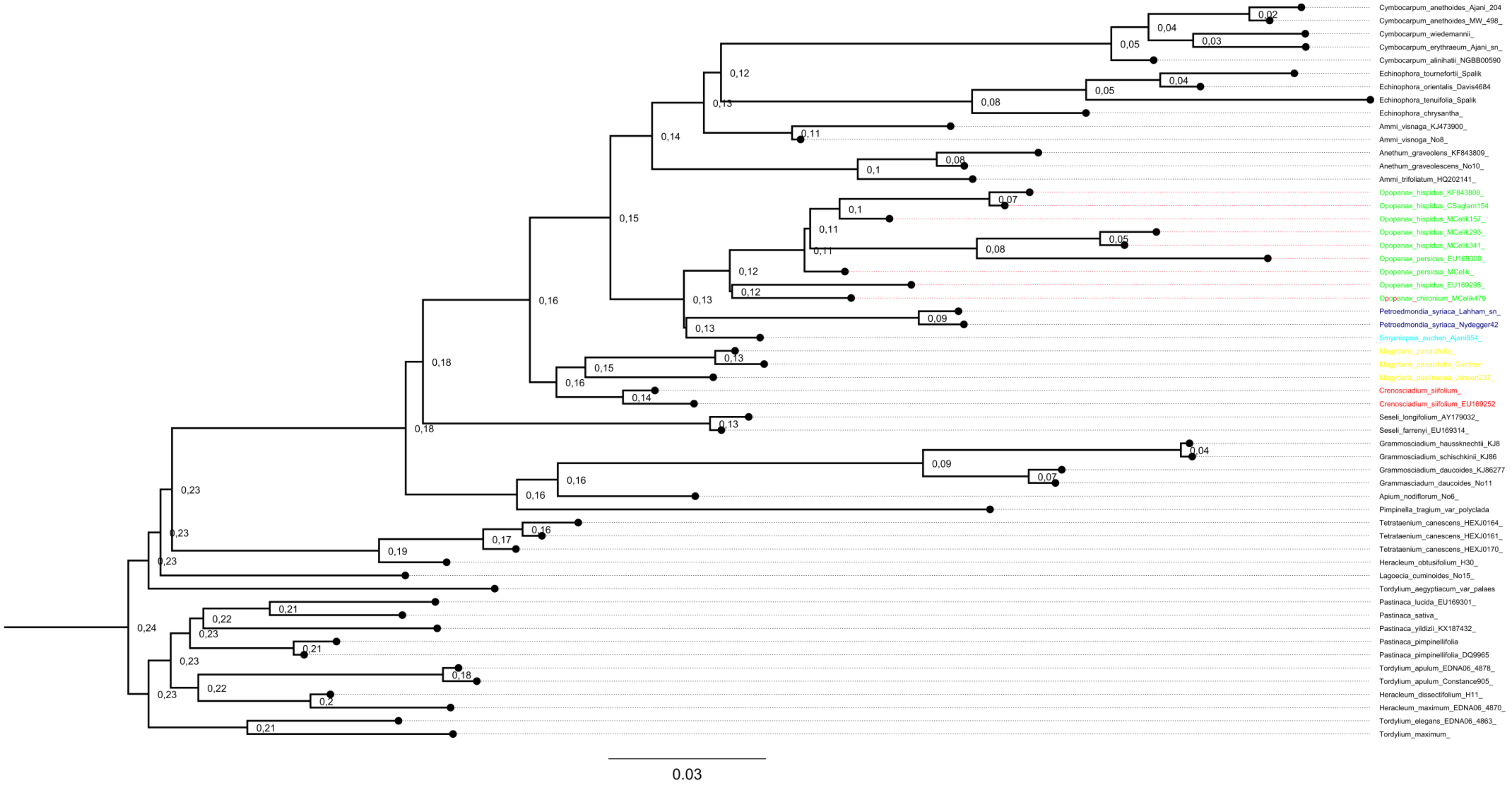


Figure 2. MrBayes phylogenetic tree based on nrITS DNA data.

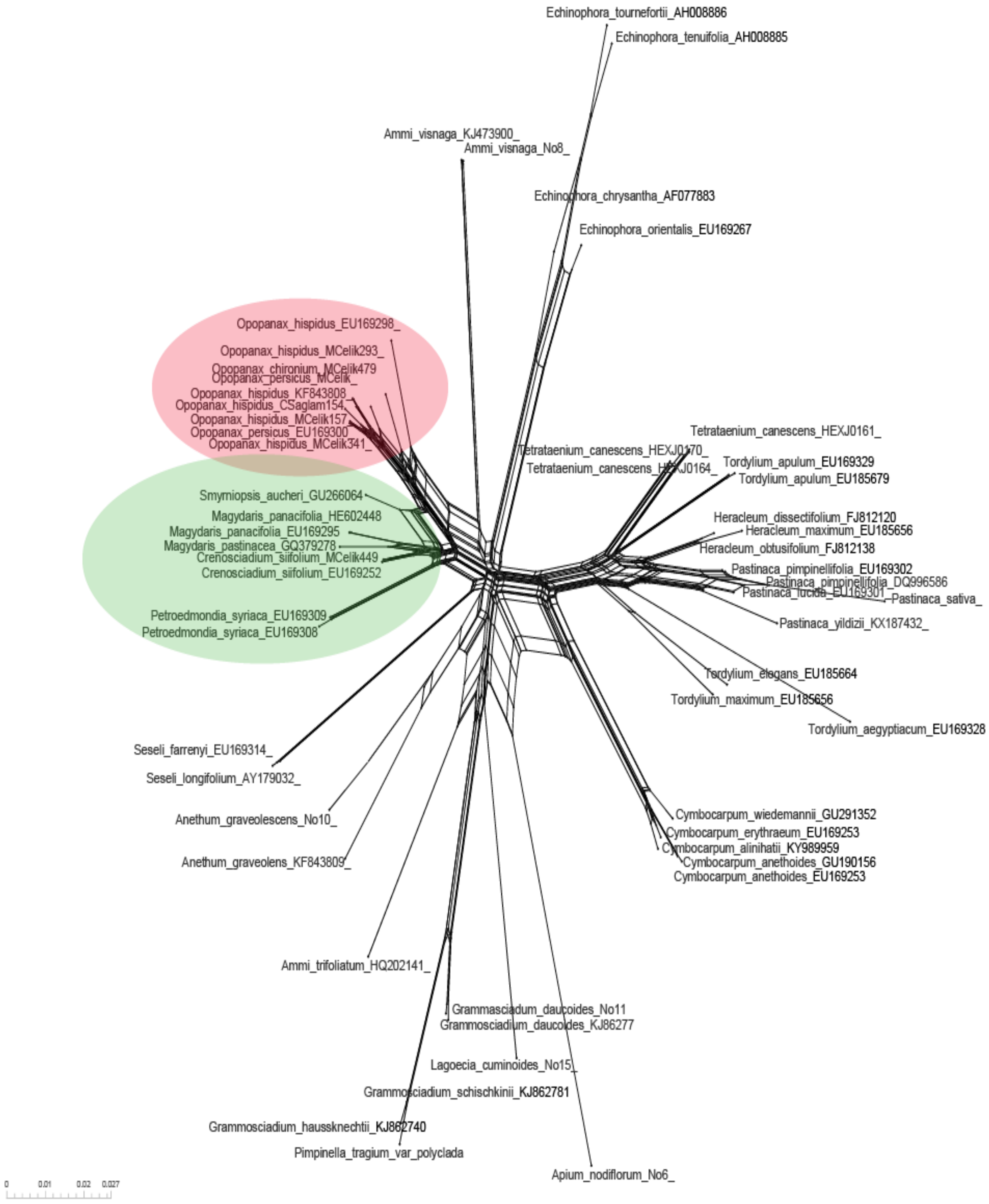
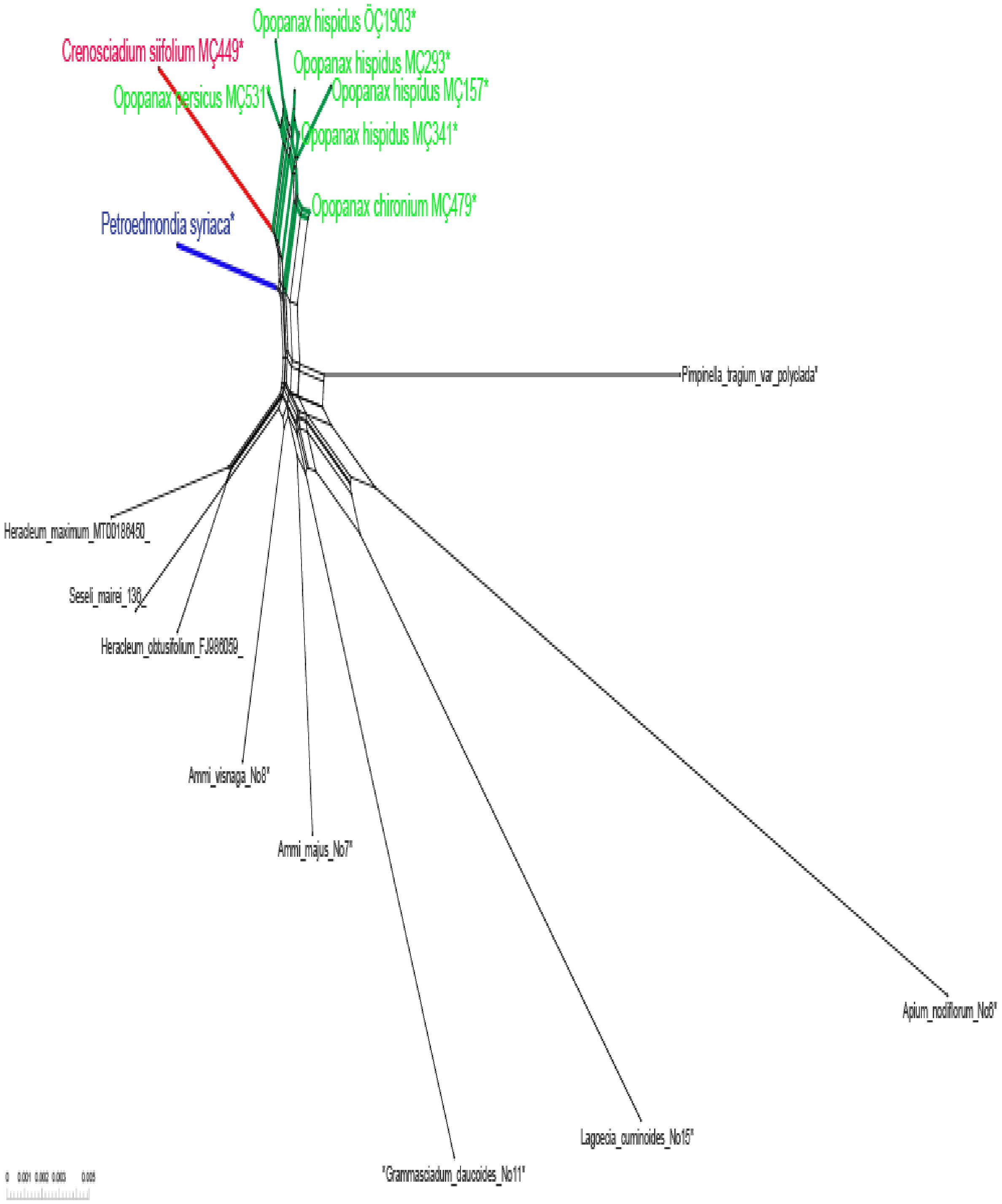
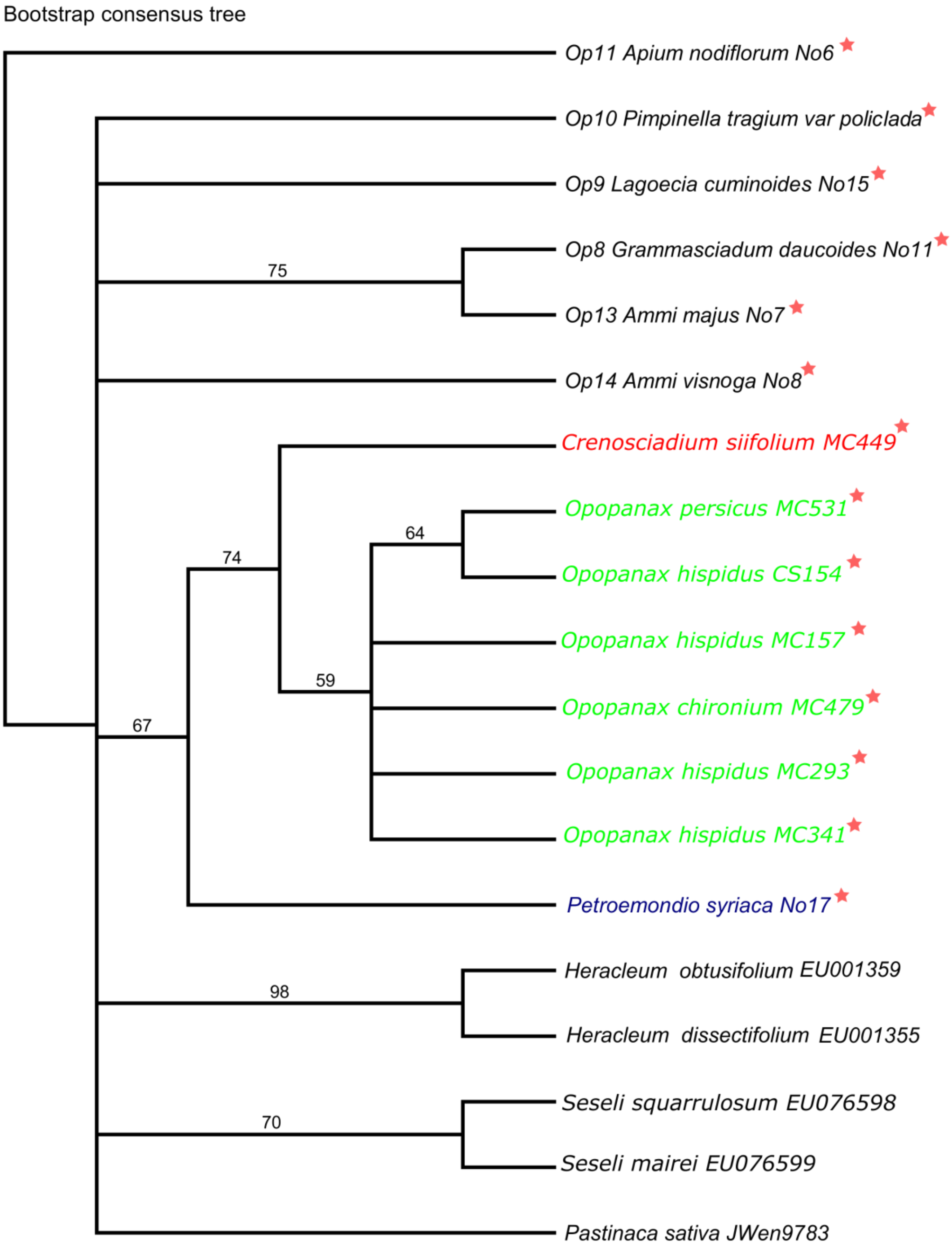


Figure 3. Neighbour Net Diagram based on nrITS DNA data.



*****: taxa examined in this study.

Figure 4. Neighbour Net Diagram based on combined matK-*trn*L-F cpDNA data.



*****: taxa examined in this study.
Figure 5. ML phylogenetic tree based on *trn*L-F cpDNA data.

Appendix

List of NCBI accessions of Apiaceae included in the phylogenetic analysis of nuclear rDNA ITS, matK, trnL-F sequences and voucher information is presented for those sequences obtained in this investigation.

Echinophora tenuifolia, Downie et al. (2000), AH008885.2; *Echinophora tournefortii*, Downie et al. (2000), AH008886.2; *Echinophora orientalis*, Ajani et al. (2008), EU169267.1; Echinophora chrysantha, Valiejo-Roman et al. (1998) AF077883.1; *Ammi trifoliatum*, Schaefer et. al. (unpubl.), HQ202141.1; Schaefer et al. (unpubl.), HM850718; *Anethum graveolens*, Menemen (unpubl.), KF843809.1; *Smyrniopsis aucheri*, Logacheva et al. (2010), GU266064.1; *Magydaris panacifolia*, Kool et al. (2012), HE602448.1; Spalik et. al. (2010), GQ379278.1; *Grammosciadium haussknechtii*, Koch and Augsten (unpubl.), KJ862740.1; *Grammosciadium schischkinii*, Koch and Augsten (unpubl.), KJ862781.1; *Seseli farrenyi*, Ajani et. al. (2008), EU169314.1; *Seseli longifolium*, Spalik et. al. (unpubl.), AY179032.1; Seseli yunnanense, Zhang and Yang (unpubl.), KU853710.1; Seseli mairei Zhang and Yang (unpubl.), KU853709.1; Zhang and Yang (unpubl.), KU853708.1; He et al. (unpubl.), EU076599; Seseli squarrulosum He et al. (unpubl.), EU076598; *Cymbocarpum alinihatii*, Menemen et al. (2017), KY989959.1; Cymbocarpum anethoides, Logacheva et al. (2010), GU190156.1; Ajani et. al. (2008), EU169253.1; *Cymbocarpum erythraeum* EU169254.1; *Cymbocarpum wiedemannii*, Logacheva et al. (2010), GU291352.1; *Tordylium maximum*, Logacheva et al. (2010), DQ996585.1; *Tordylium elegans*, Paik and Watson, (unpubl.), EU185664.1; *Heracleum obtusifolium*, Yu et al. (unpubl.), FJ812138.1; Deng et al. (unpubl.), FJ986059; *Tetrataenium canescens*, Li et al. (2011), HQ686491.1; Deng et al. (unpubl.), FJ986062; Li et al. (2011), HQ686482.1; Li et al. (2011), HQ686485.1; *Tordylium apulum*, Paik and Watson (unpubl.), EU185679.1; Ajani et. al. (2008), EU169329.1; *Tordylium aegyptiacum*, Ajani et. al. (2008), EU169328.1; *Heracleum dissectifolium*, Yu et al. (unpubl.), FJ812120.1; *Heracleum maximum*, Paik and Watson (unpubl.), EU185656.1; Kuzmina et. al. (2017), MG224911.1; *Pastinaca yildizii*, Menemen et. al. (unpub.), KX187432.1; *Pastinaca pimpinellifolia*, Ajani et. al. (2008), EU169301.1; Logacheva et al. (2010), DQ996586.1; *Pastinaca lucida*, Ajani et. al. (2008), EU169301.1; *Pastinaca sativa*, Ajani et. al. (2008), EU169303.1; KJ157767.1; ***Opopanax chironium****:* A1 Tekirdağ: Malkara, between Malkara-Karaiğdemir dam, 200 m, 14 July 2017, *Ö.Çetin 1930 & M.Çelik*; ***Opopanax hispidus***, Ajani et. al. (2008), EU169298.1; Menemen (unpubl.), KF843808.1; B7 Malatya: between Malatya- Pütürge, 8 km away from Kubbe pass 8 km, 1632 m, 25 June 2015, rocky slopes, *M.Çelik 293*, C2 Muğla: Datça, Kocadağ, Mesudiye, road side, 35S 0551420 N, 4063578, 544 m, 24 April 2015, *M.Çelik 157 & F.Altinordu*, C2 Burdur: Gölhisar, Uylupınar, Gölhisar Lake, 02 July 2017, *Ö.Çetin 1903*; ***Opopanax persicus***, EU169300.1; C9 Van: Gürpınar, steppe, 2484 m, 08 June 2017, *M.Çelik 531*; ***Crenosciadium siifolium****, Ajani et. al. (2008), EU169252.1;* C4 Karaman: Ermenek, Balkusan, Aktepe, Boğazçayır around, 36°44'N, 32°55'E, 1687 m, 28 June 2017, *M.Çelik 449; Ammi visnaga, Jimenez-Mejias and Vargas (unpubl.),KJ473900.1; Schaefer et al. (unpubl.) HM850725;* Kahramanmaraş, Kıyıkçı village, 12 June 2015, *Ö.Çetin 1569;* ***Ammi majus*****:** C4 Mersin: Mut, Between Evren and Kırıkkavak, Kırıkkavak entrance, 143 m, 31.05.2015, 36 S 527919, 4047529, *M.Çelik 204, F.Altınordu;* ***Anethum graveolens*****:** Erzincan: Tatlısu village, 1500 m; Petroedmondia syriaca, Ajani et. al. (2008), EU169309.1, EU169308.1; C7 Şanlıurfa: Birecik, Arat Village, Arat mountain, 839 m, 10 June 2014, *M.Çelik 123*; Apium graveolens KP900771.1; C7 Şanlıurfa: Birecik dam, 340 m, 02 June 2012, *Ö.Çetin 1076;* ***Pimpinella tragium*:—** Isparta: Garip Forest, 1500 m, 04 August 2013, *Ö.Çetin 1362 & M.Çelik*; ***Lagoecia cuminoides*:—** C2 Muğla: between Datça-Marmaris, 255 m, 24 April 2015, *M.Çelik 175 & F.Altınordu;* ***Grammosciadium daucoides****, Koch and Augsten (unpubl.), KJ862772.1;* C4 Antalya: Alanya, Karapınar Avenues, Dikmetaş plateu, 1380 m, 11.06.2015, 36 S 448613, 4052049, *M.Çelik 272* & F.*Altınordu*.