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Original article

The proximity of *Hydrocotyle umbellata* L. with araliaceae as evident from plastome and phylotranscriptomic analyses

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ABSTRACT

The recent massive development in the next-generation sequencing (NGS) platforms and bioinformatics tools including cloud-based NGS data analyses have proven extremely useful in understanding the deeper-level phylogenetic relationships of angiosperms. The family 'Apiaceae Lindley / Umbelliferae Jussieu' and Araliaceae Jussieu resemble each other in the structure of their gynoecia, were placed in the order Apiales Nakai, and are closely related. The family Araliaceae with the subfamily 1. *Hydrocotyloideae* Link, 2. *Harmsiopanax* Harms and 3. *Aralioideae* Eaton accepted as a monophyletic branch within the Apiales, an order within the Asterids. Of these, *Hydrocotyle* L. from *Hydrocotyloideae* Link of Apiaceae transferred to Araliaceae based on molecular phylogenetic studies. The present study evaluates the proximity of *H. umbellata* with Araliaceae based on plastome and phylotranscriptomic analyses using the minimum evolution method. The analyses revealed the nesting of the *H. umbellata* under the family Araliaceae in the MPT (Maximum Parsimony Tree), and the proximity of *H. umbellata* under the family Araliaceae further supported by the evolutionary divergence between the sequences and plastome alignment.

1. Introduction

The order Apiales [(Nakai, Hisi-Shokubutsu 58 (1930)] which includes seven families {[i.e. 1. Apiaceae [(Lindl., Intr. Nat. Syst. Bot. (ed. 2) 21, (1836)], 2. Araliaceae [(Juss., Gen. Pl. 217, (1789)], 3. Griseliniaceae [(Akht., Sist. Magnol. 209 (1987)], 4. Myodocarpaceae [(Doweld, Prosyllab. Tracheophyt. Lii (2001)], 5. Pennantiaceae [(J. Agardh, Theoria Syst. Pl. 301 (1858)], 6. Pittosporaceae [(R. Br., Voy. Terra Austral. 2: 542 (1814)], and 7. Torricelliaceae [(H.H. Hu, Bull. Fan Mem. Inst. Biol., Bot. 5: 311, 1934)]} have been placed within the Asterid group of Eudicots as circumscribed by group Campanulids (APG IV, 2016). The family 'Apiaceae Lindley / Umbelliferae Jussieu' (with approx. c. 434 genera and c. 3700 species) and Araliaceae Jussieu (with approx. c. 43 genera, c.1450 species; APG IV, 2016; https://www.mobot.org/mobot/research/apweb/) resemble each other in the structure of their gynoecia, and were placed in the order Ariales, superorder Araliiflorae (Dahlgren, 1980), and are closely related (Plunkett et al., 2004). The family Araliaceae with the subfamily 1. *Hydrocotyloideae* Link, 2. *Harmsiopanax* Harms and 3. *Aralioideae* Eaton accepted as a monophyletic branch within the Apiales (Kim et al., 2017), an order within the Asterids (APG IV, 2016). Of these, the genus *Hydrocotyle* L. which comprises c.130 species (Hiroe, 1979; Pimenov and Leonov, 1993; Du and Ren, 2010; APG IV, 2016, https://www.mobot.org/mobot/research/apweb/) from *Hydrocotyloideae* Link of the family Apiaceae transferred to the family Araliaceae based on molecular phylogenetic studies (Lowry et al., 2004; Plunkett et al., 2004). The recent massive development in the next-generation sequencing platforms and bioinformatics tools including cloud-based bioinformatic analyses has proven extremely useful in understanding the deeper-level phylogenetic relationships of angiosperms (Ali, 2021). The present study evaluates the proximity of *Hydrocotyle umbellata* with Araliaceae based on plastome and phylotranscriptomic analyses.

2. Materials and methods

2.1. Phylotranscriptomic analyses of the selected taxon

The RNA transcriptome SRA data of *Hydrocotyle umbellata* L. [(Sp. Pl. 1: 234 (1753)] available (https://zenodo.org/record/

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3255100#.X9QFGtgza70) from the previous study (Leebens-Mack et al., 2019) was retrieved, and analyzed together with (1) ingroup taxon: *Angelica archangelica* L. [(Sp. Pl. 1: 250–251 (1753)], *Centella asiatica* (L.) Urb. [(Fl. Bras. 11(1): 287 (1879)], *Hedera helix* L. [(Sp. Pl. 1: 202 (1753)], *Griselinia littoralis* (Raoul) Raoul [(Choix Pl. Nouv.-Zél. 22 (1846)], *Griselinia racemosa* (Phil.) Taub. [(Bot. Jahrb. Syst. 16: 390 (1892)], *Pennantia corymbosa* J.R. Forst. & G. Forst. [(Char. Gen. Pl. 67 (1775)], *Pittosporum sahnianum* Gowda [(J. Arnold Arbor. 32(4): 305–307 (1951)], *Pittosporum resiniferum* Hemsl. [(Bull. Misc. Inform. Kew 1894: 344 (1894)], *Dipsacus asper* Wall. ex DC. [(Prodr. 4: 646 (1830)], and (2) outgroup: *Dipsacus asper* [(Prodr. 4: 646 (1830)] (Table 1). The selected aligned data set were imported to MEGA X (Kumar et al., 2018) and converted into .mega format, and the evolutionary analyses were performed using Maximum Parsimony (MP) bootstrap methods (Felsenstein, 1985).

2.2. Analyses of plastome data

The plastome (chloroplast genome) of the family Apiaceae and Araliaceae e.g. 1. *Angelica dahurica* (Fisch.) Benth. & Hook. f. {[Enum. Pl. Jap. 1(1): 187 (1873)]}, 2. *Eleutherococcus senticosus* {(Rupr. ex Maxim.) Maxim. [Mém. Acad. Imp. Sci. St.-Pétersbourg Divers Savans 9: 132 (1859)], 3. *Hydrocotyle sibthorpioides* Lam. {[Encycl. 3(1): 153 (1789)]}, 4. *Kalopanax septemlobus* (Thunb.) Koidz. {[Bot. Mag. (Tokyo) 39(468): 306 (1925)]}, and 5. *Petroselinum crispum* (Mill.) Mansf. {[Repert. Spec. Nov. Regni Veg. 46 (1168–1170): 307 (1939)]} were retrieved from NCBI GenBank (Table 2), and the genomic rearrangements and the relationships among the selected taxon were detected using MAUVE (Darling et al., 2004).

3. Results

3.1. Plastome and phylotranscriptome dataset characteristics

The present phylotranscriptomic analyses is from 11,495 parsimony informative sites out of a total of 1,42,796 positions in the aligned (the aligned data set contains 85,153 conserved, 48,037 variable and 35,240 singleton sites, Fig. 1) transcriptome dataset of *A. archangelica* (Apiaceae), *C. asiatica* (Apiaceae), *H. umbellate* (Araliaceae), *H. helix* (Araliaceae), *G. littoralis* (Griseliniaceae), *G. racemose* (Griseliniaceae), *P. corymbosa* (Pennantiaceae), *P. sahnianum* (Pittosporaceae), *P. resiniferum* (Pittosporaceae) and *D. asper* (Caprifoliaceae).

Table 1
The list of ingroup and outgroup taxon with their family and SRA accession used in the phylotranscriptomic analyses.

S. No.	Taxon	Family	SRA accession
Ingroup			
1.	*Angelica archangelica* L.	Apiaceae	ERS1829701
2.	*Centella asiatica* (L.) Urb.	Apiaceae	ERS1829708
3.	*Hydrocotyle umbellata* L.	Araliaceae	ERS1829705
4.	*Hedera helix* L.	Araliaceae	ERS1829702
5.	*Griselinia littoralis* (Raoul) Raoul	Griseliniaceae	ERS1829706
6.	*Griselinia racemose* (Phil.) Taub.	Griseliniaceae	ERS1829707
7.	*Pennantia corymbosa* J.R. Forst. & G. Forst.	Pennantiaceae	ERS1829709
8.	*Pittosporum sahnianum* Gowda	Pittosporaceae	ERS3670337
9.	*Pittosporum resiniferum* Hemsl.	Pittosporaceae	ERS1829710
Outgroup			
10.	*Dipsacus asper* Wall. ex DC.	Caprifoliaceae	ERS1829762

Table 2
The list of the taxon with their family and NCBI GenBank accession number used to detect genomic rearrangements and the relationships among taxon.

S. No.	Taxon	Family	NCBI GenBank accession number
1.	*Angelica dahurica* (Fisch.) Benth. & Hook. f.	Apiaceae	NC_029392.1/ KT963037.1
2.	*Petroselinum crispum* (Mill.) Mansf.	Apiaceae	NC_015821.1/ HM596073.1
3.	*Eleutherococcus senticosus* (Rupr. ex Maxim.) Maxim.	Araliaceae	NC_016430.1/ JN637765.1
4.	*Hydrocotyle sibthorpioides* Lam.	Araliaceae	NC_035502.1/ KT589392.1
5.	*Kalopanax septemlobus* (Thunb.) Koidz.	Araliaceae	NC_022814.1/ KC456167.1

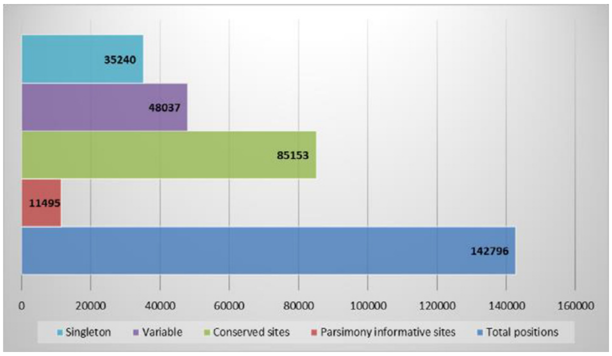

Fig. 1. The aligned transcriptome data set showing several different sites. A total number of 11,495 parsimony informative sites out of a total number of 14,27,95 sites were used in phylogenetic analysis.

3.2. Phylotranscriptomic analyses

The phylotranscriptomic analyses recovered the MPT with tree length of 0.628 [consistency index 0.689, retention index 0.404, composite index 0.362]. The MPT revealed the proximity (bootstrap support 99%) of *H. umbellata* (ex Apiaceae, Araliaceae) with *H. helix* (Araliaceae), while *A. archangelica* (Apiaceae), *C. asiatica* (Apiaceae) clade (supported by 99% bootstrap support)- *P. sahnianum* (Pittosporaceae)- *G. littoralis* (Griseliniaceae)- *P. corymbosa* (Pennantiaceae) shows proximity of Araliaceae clade (70% bootstrap support) (Fig. 2), which is also evident from the estimates of evolutionary divergence between the sequences (Table 3) and the alignment of plastome of *A. dahurica* (Apiaceae), *P. crispum* (Apiaceae), *E. senticosus* (Araliaceae), *H. sibthorpioides* (Araliaceae) and *K. septemlobus* (Araliaceae). The Araliaceae clade [constitute with *E. senticosus* (Araliaceae) - *H. sibthorpioides* (Araliaceae) with branch length 0.08] clade with *K. septemlobus* (Araliaceae) (branch length 0.15), while *A. dahurica* (Apiaceae) (branch length 0.15) clade together with *P. crispum* (Apiaceae) (branch length 0.17)

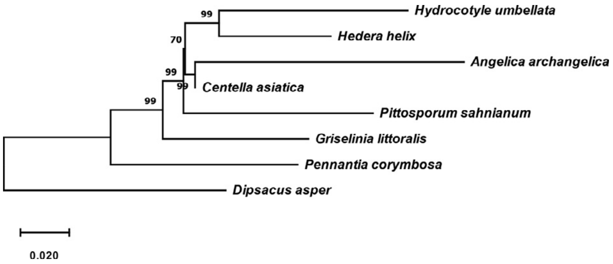

Fig. 2. The evolutionary tree to evaluates the proximity of the genus *Hydrocotyle* with Araliaceae based on phylotranscriptomic analyses.

Table 3
The estimates of evolutionary divergence between the sequences (Zuckerkandl and Pauling, 1965) used to infer the phylogeny using MEGA X (Kumar et al., 2018).

	G. littoralis	*P. sahnianum*	*H. umbellata*	*P. corymbosa*	*H. helix*	*A. archangelica*	*C. asiatica*	*D. asper*
G. littoralis								
P. sahnianum	0.145							
H. umbellata	0.164	0.171						
P. corymbosa	0.150	0.182	0.202					
H. helix	0.118	0.134	0.123	0.168				
A. archangelica	0.187	0.193	0.201	0.220	0.180			
C. asiatica	0.158	0.167	0.179	0.198	0.146	0.195		
D. asper	0.223	0.243	0.248	0.211	0.231	0.269	0.252	

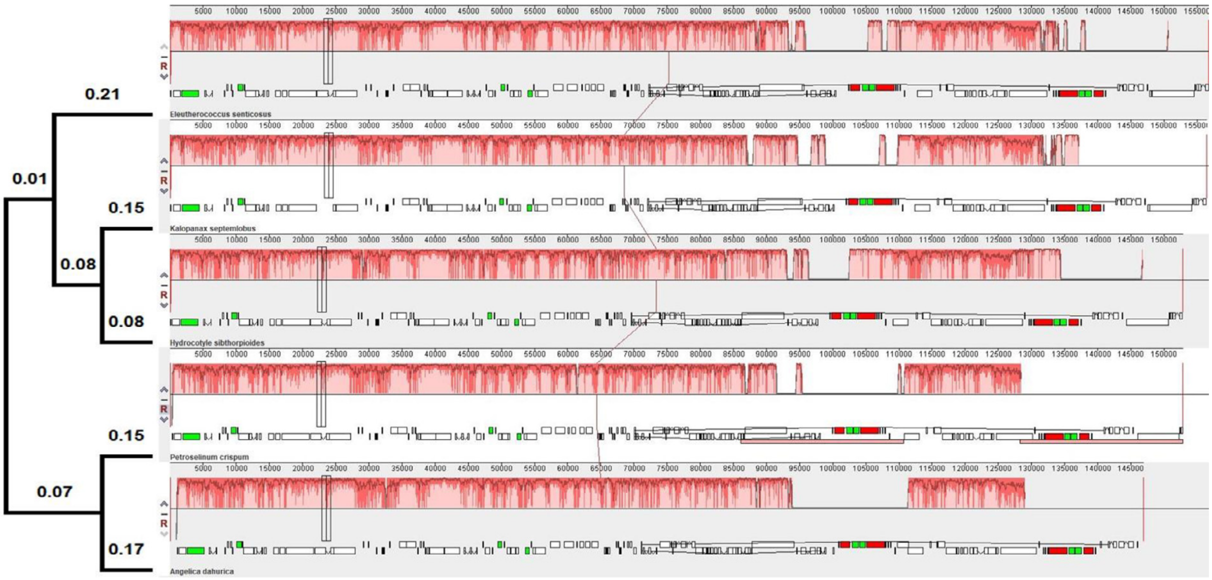

Fig. 3. The plastome alignment of the representative of the family Apiaceae and Araliaceae using MAUVE (Darling et al., 2004). Alignment Lane1: *Eleutherococcus senticosus, 2. Kalopanax septemlobus, 3. Hydrocotyle sibthorpioides, 4. Petroselinum crispum, 5. Angelica dahurica.*

(Fig. 3). In the maximum likelihood analyses, the tree topology and the proximity of *H. umbellata* with the taxon included in the analyses found similar to MPT.

3.3. Plastome genome size and CDS

The comparison of the plastome size and coding sequence (CDS) of 29 species of the family Apiaceae (e.g. *Anethum graveolens* L. (KR011055.1), *Angelica acutiloba* (Siebold & Zucc.) Kitag. (KT963036.1), *Angelica dahurica* (Fisch.) Benth. & Hook. f. (KT963037.1), *Angelica gigas* Nakai (KT963038.1), *Anthriscus cerefolium* (L.) Hoffm. (GU456628.1), *Arracacia xanthorrhiza* Bancr. (KY117235.1), *Bupleurum boissieuanum* H. Wolff (MF663725.1), *Bupleurum falcatum* L. (KM207676.1), *Bupleurum latissimum* Nakai (KT983258.1), *Carum carvi* L. (KR048286.1), *Cicuta virosa* L. (KX352466.1), *Coriandrum sativum* L. (KR002656.1), *Crithmum maritimum* L. (HM596072.1), *Daucus carota* L. (DQ898156.1), *Foeniculum vulgare* Mill. (KR011054.1), *Glehnia littoralis* F. Schmidt ex Miq. (KU866532.1), *Hansenia forbesii* (H.Boissieu) Pimenov & Kljuykov (KX808492.1), *Hansenia forrestii* (H.Wolff) Pimenov & Kljuykov (KX808494.1), *Hansenia oviformis* (R.H.Shan) Pimenov & Kljuykov (KX808493.1), *Hansenia weberbaueriana* (Fedde ex H.Wolff) Pimenov & Kljuykov (KX808491.1), *Ledebouriella seseloides* (Hoffm.) H. Wolff (KU866529.1), *Ligusticum tenuissimum* (Nakai) Kitag. (KT963039.1), *Ostericum grosseserratum* (Maxim.) Kitag. (KT852844.1), *Petroselinum crispum* (Mill.) Fuss (HM596073.1), *Peucedanum insolens* Kitag. (KU041143.1), *Peucedanum japonicum* Thunb. (KU866530.1), *Pleurospermum camtschaticum* Hoffm. (KU041142.1), *Prangos trifida* (Mill.) Herrnst. & Heyn (MG386251.1), *Pterygopleurum neurophyllum* (Maxim.) Kitag. (KT983257.1), and 19 species of the family Araliaeae (e.g. *Aralia undulata* Hand.-Mazz. (KC456163.1), *Brassaiopsis hainla* (Buch.-Ham.) Seem. (KC456164.1), *Dendropanax dentiger* (Harms) Merr. (KP271241.1), *Dendropanax morbiferus* H.Lév. (KR136270.1), *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (JN637765.1), *Fatsia japonica* (Thunb.) Decne. & Planch. (KR021045.1), *Hydrocotyle sibthorpioides* Lam. (KT589392.1), *Hydrocotyle verticillata* Thunb. (HM596070.1), *Kalopanax septemlobus* (Thunb.) Koidz. (KC456167.1), *Metapanax delavayi* (Franch.) J.Wen & Frodin (KC456165.1), *Panax ginseng* C.A.Mey. (AY582139.1), *Panax japonicus* (T.Nees) C.A.Mey. (KP036469.1), *Panax notoginseng* (Burkill) F. H.Chen (KJ566590.1), *Panax quinquefolius* L. (KM088018.1), *Panax stipuleanatus* H.T.Tsai & K.M.Feng (KX247147.1), *Panax trifolius* L. (MF100782.1), *Panax vietnamensis* Ha & Grushv. (KP036470.1), *Schefflera delavayi* (Franch.) Harms (KC456166.1), *Schefflera heptaphylla* (L.) Frodin (KT748629.1), revealed that the plastome size ranges between 140,000 to 160,000 nucleotides base-pairs in both Apiaceae and Araliaceae, but the CDS varies e.g. from 71 to 99 CDS in Apiaceae, and while narrowly varies (CDS 85 to 87) in Araliaceae, indicating similar content and pattern of CDS in the genus *Hydrocotyle* with Araliaceae.

4. Discussion

The phylogenetic reconstruction of evolutionary histories in plants based on molecular data has been based largely on nuclear sequences or chloroplast/plastid DNA markers (Ali et al., 2014). The recent massive development in the next-generation sequencing platforms has brought cost-effective sequencing of genome or organelle genome e.g. chloroplast and mitochondria, which have proven extremely useful in understanding the deeper-level phylogenetic relationships of angiosperms (Ali, 2021). The transcriptome/ RNA-Seq (Wang et al., 2009) have attracted the attention for the reconstruction of evolutionary histories in plants because it allows massively parallel sequencing of expressed genes within a single genome which offers a powerful means of investigating the signals for evolutionary implications in higher taxonomic level such as Charophytes, Caryophyllales, Vitaceae, Brassicaceae, Betulaceae, Asteraceae, Hydnoraceae, Asclepias, Ferns; at a lower taxonomic level such as *Camelina sativa*, *Artemisia tridentata*, *Ranunculus*, *Flaveria*, or at angiosperms level; and have also been proven very useful in comparative transcriptomics, character evolution, domestication of crops and genome evolution (Wen et al., 2014).

The present phylotranscriptomic analyses revealed the proximity of *H. umbellata* (ex Apiaceae, Araliaceae) with *H. helix* (Araliaceae). The family Araliaceae (-Ginseng family) have distributed mostly in tropical regions, consists of c. 41 plus genera under Subfamily *Aralioideae* (1. *Anakasia* Philipson, 2. *Aralia* L., 3. *Astrotricha* DC., 4. *Brassaiopsis* Decne. & Planch., 5. *Cephalaralia* Harms, 7. *Cheirodendron* Nutt. ex Seem., 8. *Cussonia* Thunb., 9. *Dendropanax* Decne. & Planch., 10. *Eleutherococcus* Maxim., 11. *Fatsia* Decne. & Planch., 12. *Gamblea* C.B. Clarke, 13. *Harmsiopanax* Warb., 14. *Hedera* L., 15. *Heteropanax* Seem., 16. *Hunaniopanax* C.J. Qi & T.R. Cao, 17. *Kalopanax* Miq., 18. *Macropanax* Miq., 19. *Megalopanax* Ekman ex Harms, 20. *Merrilliopanax* H.L. Li, 21. *Meryta* J.R. Forst. & G. Forst., 22. *Metapanax* J. Wen & Frodin, 23. *Motherwellia* F. Muell., 24. *Oplopanax* (Torr. & A. Gray) Miq., 25. *Oreopanax* Decne. & Planch., 26. *Osmoxylon* Miq., 27. *Panax* L., 28. *Plerandra* A. Gray, 29. *Polyscias* J. R. Forst. & G. Forst., 30. *Pseudopanax* C. Koch, 31. *Raukaua* Seem., 32. *Schefflera* J.R. Forst. & G. Forst., 33. *Sciadodendron* Griseb., 34. *Seemannaralia* R. Vig., 35. *Sinopanax* H.L. Li, 36. *Stilbocarpa* (Hook. f.) Decne. & Planch., 37. *Tetrapanax* (K. Koch) K. Koch, 38. *Trevesia* Vis., 39. *Woodburnia* Prain) and Subfamily *Hydrocotyloideae*: 40. *Hydrocotyle*, 41. *Trachymene* (APG, 2016); out of these, some possess immense medicinal impotence such as *Hedera* sp., *Panax* sp. (Ginseng), and *Eleutherococcus senticosus* (Wen et al., 2000; Plunkett et al., 2004), are distinctive in being palmate or pinnate leaves, heads, reduced calyx, apopetalous to sympetalous corolla, and a 1–∞-carpellate inferior ovary, apical-axile placentation, fruit a berry, drupe, or schizocarp (Plunkett et al. 2004). The family Araliaceae show considerable floral variation. The family Apiaceae and Araliaceae resemble each other in the structure of their gynoecia, and are closely related (Plunkett et al., 2004), the family Araliaceae with the subfamily *Hydrocotyloideae*, *Harmsiopanax* and *Aralioideae* accepted as a monophyletic branch within the Apiales, an order within the Asterids; of these, *Hydrocotyle* from *Hydrocotyloideae* of Apiaceae transferred to Araliaceae based on molecular phylogenetic studies (Lowry et al., 2004; Plunkett et al., 2004). The Hydrocotyloideae are sister to the rest of the family (Chandler and Plunkett, 2004; Plunkett et al., 2004; Nicolas and Plunkett, 2009), the species of the genus *Hydrocotyle* are highly polyploidy (Yi et al., 2004), show early corolla tube initiation (Leins and Erbar, 1997; Erbar and Leins, 2004), calyx remains absent (Tseng, 1967), and possess has trilacunar nodes, laterally flattened fruits with a sclerified endocarp and stipules are cauline or borne on the leaf base (Sinnott and Bailey 1914).

In conclusion, the present study evaluates the proximity of *Hydrocotyle* with Araliaceae based on plastome and phylotranscriptomic analyses using the minimum evolution method. The family 'Apiaceae/Umbelliferae and Araliaceae resemble each other in the structure of their gynoecia, and were placed in the order Apiales, and are closely related. The family Araliaceae with the subfamily 1. *Hydrocotyloideae* Link, 2. *Harmsiopanax* Harms and 3. *Aralioideae* Eaton accepted as a monophyletic branch within the Apiales, an order within the Asterids. Of these, *Hydrocotyle* L. from *Hydrocotyloideae* Link of Apiaceae transferred to Araliaceae based on molecular phylogenetic studies. The present analyses revealed the nesting of the *Hydrocotyle* under Araliaceae in the MPT, and the proximity is further supported by the evolutionary divergence between the sequences and plastome alignment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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