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DISSECTING MOLECULAR EVOLUTIONARY RELATIONSHIP OF KRAMERIACEAE INFERRED FROM PHYLOTRANSCRIPTOMIC ANALYSIS

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Abstract

The systematic relationships of Krameriaceae have changed considerably. The phylotranscriptomic data sets provide highly informative data for resolving deeper-level phylogenetic relationships. The phylotranscriptomic analyses to infer evolutionary relationships of Krameriaceae in the order Zygophyllales using the Minimum Evolution, Maximum Parsimony and Maximum Likelihood methods recovered similar topology and taxon proximity. Under the Zygophyllales clade, *Krameria lanceolata* Torr. of the family Krameriaceae nested with *Tribulus eichlerianus* K.L. Wilson and *Larrea tridentata* (Sessé & Moc. *ex* DC.) Coville belonging to the family Zygophyllaceae with strong nodal support. The phylotranscriptomic analyses suggest that the family Krameriaceae is sister to Zygophyllaceae.

Introduction

The order Zygophyllales Link in the Fabids comprises two families *viz*. Zygophyllaceae R. Br. and Krameriaceae Dumort. (APG IV, 2016). The Zygophyllaceae commonly known as the ‘Caltrop Family’, possesses mostly opposite, compound leaves, pinnate or 2-foliolate with paired persistent stipules, flowers with disc, distinct stamens bearing basal scales, polycarpellary syncarpous with 5 carpals and 4-5 loculed ovary. The Zygophyllaceae consists of c. 25 genera [e.g. *Augea* Thunberg, *Balanites* Delile, *Bulnesia* C. Gay, *Fagonia* L., *Gonopterodendron* (Grisebach) Godoy-Bürki, *Guaiacum* L., *Kallstroemia* Scopoli, *Kelleronia* Schinz, *Larrea* Cavanilles, *Melocarpum* (Engl.) Beier & Thulin, *Metharme* Engler, *Morkillia* Rose & Painter, *Neoluederitzia* Schinz, *Pintoa* C. Gay, *Plectrocarpa* Gillies, *Porlieria* Ruíz & Pavón, *Roepera* A. de Jussieu, *Seetzenia* R. Brown, *Sericodes* A. Gray, *Sisyndite* Sonder, *Tetraena* Maximowicz, *Tribulopis* R. Br., *Tribulus* L., *Viscainoa* Greene, *Zygophyllum* L.] and c. 325 species under 5 subfamilies (e.g. Larreoideae, Morkillioideae, Seetzenioideae, Tribuloideae and Zygophylloideae), distributed in dry and warm or cool temperate and tropical regions (Beier *et al*., 2004; Brummitt, 2007; APG IV, 2016; Godoy-Bürki *et al*., 2018). The monogeneric family Krameriaceae (e.g. *Krameria* Loefl.) commonly known as ‘Rhatany’ is characterized by small-leaved, moderate-sized shrubs to subshrubs with somewhat woody underground stems and roots with the prostrate

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herbaceous stems and comprises c. 18 species distributed in South and North America, and the West Indies (Simpson, 1989; Simpson *et al*., 2004) possessing astringent properties (Simpson, 1991).

The systematic relationships of the Zygophyllaceae and Krameriaceae have often been debatable (APG IV, 2016). The Krameriaceae was considered as a subfamily of Fabaceae, or near to Polygalaceae (Simpson, 1989; Simpson *et al*., 2004); however, aligned as sister to Zygophyllaceae based on evidences from the anatomical (Gregory, 1994; Carlquist, 2005), DNA (Savolainen *et al*., 2000; Wang *et al*., 2009) and pollen data (Tao *et al.*, 2018). While the wood anatomy (Carlquist, 2005) and plastosome analysis (Ali *et al*., 2019) revealed the separation of Krameriaceae from the Zygophyllaceae, Granot and Grafi (2014) emphasized the phylogenetic significance of the epigenetic information with reference to Zygophyllaceae, and argued that the placement of Krameriaceae under the Zygophyllales needs to be re-examined.

The advances in the next generation sequencing and data analysis during the last decade have made transcriptomics a cost-efficient means for investigating systematic and evolutionary questions at species to larger clades (Wickett *et al*., 2014). Transcriptomics refers to the study of the transcriptome - the complete set of RNA transcripts which are produced by the genome under specific circumstances or in a specific cell by using high-throughput methods. The use of transcriptome data sets provides novel insights into evolutionary history (Cannon *et al*., 2015; Smith *et al.*, 2015; Yang *et al*., 2018) for resolving deeper-level phylogenetic relationships which are not obtainable from a handful of loci and with limited taxon sampling (Wickett *et al*., 2014; Smith *et al*., 2018). A handful of loci and limited taxon sampling result in artificially inflated support (Seo, 2008). Therefore, the present analyses aimed to explore the utility of transcriptome data to infer evolutionary relationships in Zygophyllales.

Materials and Methods

Selection of taxon

Transcriptome data of *Tribulus eichlerianus* K.L. Wilson and *Larrea tridentata* (Sessé & Moc. *ex* DC.) Coville belonging to the family Zygophyllaceae, and *Krameria lanceolata* Torr. of the family Krameriaceae available in the SRA database from the study of 'One Thousand Plant Transcriptomes Initiative' (Leebens-Mack, 2019) were retrieved, and analyzed together with *Rhus radicans* L., *Gleditsia triacanthos* L. and *Polygala lutea* L. as representatives from Anacardiaceae, Fabaceae and Polygalaceae, respectively. Transcriptome data of *Tetrastigma obtectum* (Wall. *ex* M.A. Lawson) Planch. *ex* Franch. (Vitaceae) was used as the outgroup in the phylotranscriptomic analysis. The representative in group taxon e.g. *R. radicans, G. triacanthos, P. lutea* and the outgroup taxon were also retrieved from the SRA database available from the study of One Thousand Plant Transcriptomes Initiative (Leebens-Mack, 2019) (Table 1). The retrieved aligned data were then subjected to phylogenetic analyses using MEGA X (Kumar *et al.*, 2018).

Phylogenetic analyses

All ambiguous positions were removed for each sequence pair (pairwise deletion option). The evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018). The evolutionary history was inferred using the Minimum Evolution method (Rzhetsky and Nei, 1992), Maximum Likelihood method based on the JTT matrix-based model (Jones *et al*., 1992) and Maximum parsimony bootstrap method (Felsenstein, 1985) using the Subtree-Pruning-Regrafting algorithm (Nei and Kumar, 2000). A timetree inferred using the RelTime method (Tamura *et al.*, 2012, 2018) and estimates of branch lengths were calculated using the Neighbor-Joining method (Saitou and Nei, 1987).

Table 1. List of species used in the phylotranscriptomic analyses to infer relationship between Zygophyllaceae and Krameriaceae.

Sl.	Species	Clade	Order	Family	GenBank
Ingroup					
1.	*Tribulus eichlerianus* K.L. Wilson	Core Eudicots/ Rosids /Fabids	Zygophyllales	Zygophyllaceae	ERS3670314
2.	*Larrea tridentata* (Sessé & Moc. *ex* DC.) Coville	Core Eudicots/ Rosids/Fabids	Zygophyllales	Zygophyllaceae	ERS368254
3.	*Krameria lanceolata* Torr.	Core Eudicots/ Rosids /Fabids	Zygophyllales	Krameriaceae	ERS1829389
4.	*Rhus radicans* L.	Core Eudicots/ Rosids/ Malvids	Sapindales	Anacardiaceae	ERS1829525
5.	*Gleditsia triacanthos* L.	Core Eudicots/ Rosids /Fabids	Fabales	Fabaceae	ERS631106
6.	*Polygala lutea* L.	Core Eudicots/ Rosids/Fabids	Fabales	Polygalaceae	ERS631118
Outgroup					
7.	*Tetrastigma obtectum* (Wall. *ex* M.A. Lawson) Planch. *ex* Franch.	Core Eudicots/ Rosids	Vitales	Vitaceae	ERS1829368

Results and Discussion

The present study revealed the molecular phylogenetic analyses of 10376 parsimony informative sites (out of a total of 142796 positions in the final transcriptome dataset) of *Tribulus eichlerianus, Larrea tridentata, Krameria lanceolata, Rhus radicans, Gleditsia triacanthos, Polygala lutea* and *Tetrastigma obtectum* (Fig. 1). The evolutionary tree topology and taxon proximity recovered in all the three method i.e. ME, ML and MP were found similar (Fig. 2).

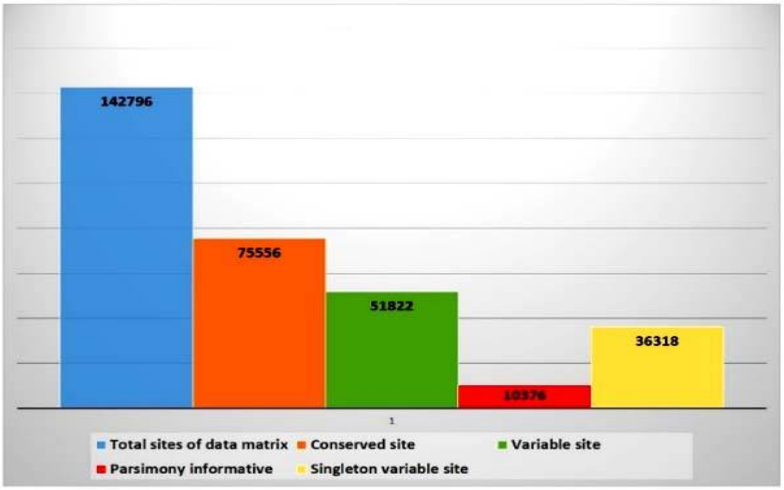

Fig. 1. The final transcriptome data set of *T. eichlerianus, L. tridentata, K. lanceolata, R. radicans, G. triacanthos, P. lutea* and *T. obtectum.*

Under the Zygophyllales clade, *Krameria lanceolata* (Krameriaceae) nested with *Tribulus eichlerianus* and *Larrea tridentata* (Zygophyllaceae) with strong nodal support having ME/ML/MP: 99/100/100 (Fig. 2). The equality of evolutionary rate between sequences of *T. eichlerianus* and *K. lanceolata*, with sequence of *Tetrastigma obtectum* used as an outgroup in Tajima's relative rate test (Tajima, 1993) performed using MEGA X (Kumar *et al*., 2018) revealed 23009 identical sites in all three sequences, 1948 divergent sites in all three sequences, 2098 unique differences in *T. eichlerianus* (Zygophyllaceae), 2005 unique differences in *K. lanceolata* (Krameriaceae) and 3056 unique differences in *T. obtectum*.

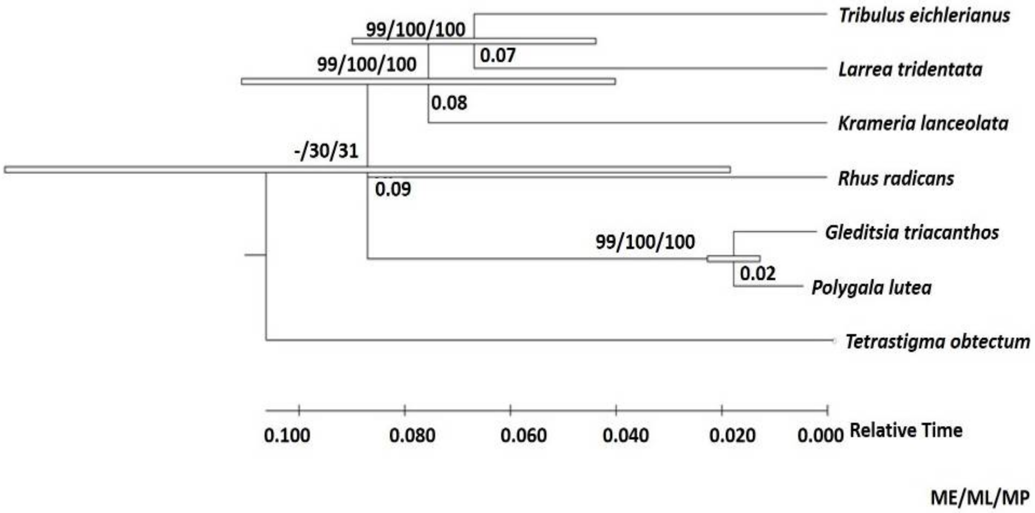

Fig. 2. The evolutionary history inferred using the Minimum Evolution method (the optimal tree with the sum of branch length 0.72511142) and Maximum Likelihood method (the tree with the highest log likelihood -818278.73). Tree #1 out of 2 most parsimonious trees having length: 83949, consistency index: 0.704273, retention index: 0.435550, composite index: 0.400719. A timetree inferred using the RelTime method and estimates of branch lengths inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches (ME/ML/MP).

The order Zygophyllales is distinct by the presence of harman alkaloids (Kubitzki, 2007), diversity of lignans and neolignans (Sheahan, 2006; Simpson *et al.*, 2006), and lack of mycorrhizae; however, arbuscular mycorrhizae have been reported from roots of *L. tridentata* in the Mojave desert (Apple *et al*., 2005); deep cortical or pericyclic (superficial) cork cambium; vessel elements with simple perforation plates; rays (predominantly) uniseriate; transverse stomatal orientation (Carlquist, 2005); pollen colpate (Tao *et al*., 2018); micropyle endostomal; seeds more or less exotestal; and lack of endosperm (APG IV, 2016).

The systematic relationships of Krameriaceae have often been changed. Earlier works placed Krameriaceae as a subfamily of Fabaceae or within Fabaceae, or near to Polygalaceae (Simpson, 1989; Simpson *et al.*, 2004). Wood anatomy comparison between Krameriaceae and Zygophyllaceae species showed that they share several wood characters that might reflect ancestral relationships, but significant differences have been noticed in their wood anatomy *viz.* vessels with non-vestured pits in Zygophyllaceae vs vestured pits in Krameriaceae; imperforate tracheary elements in all tracheids in Zygophyllaceae vs tracheids and fibre-tracheids in

Krameriaceae; axial parenchyma usually with single cell per strand in Zygophyllaceae vs 2–4 cells per strand in Krameriaceae; rays paedomorphic type III in Zygophyllaceae vs heterogeneous types II and III as well as homogeneous type III in Krameriaceae; storying absent or nearly so in Zygophyllaceae vs present in axial parenchyma, sometimes in rays in Krameriaceae; and crystals many per cell, of varied sizes, rare in wood but common in axial parenchyma of secondary phloem in Zygophyllaceae vs crystals one per cell or septate portion of cell in wood or secondary phloem in Krameriaceae (Carlquist, 2005). These anatomical variations support the separation of Krameriaceae from the Zygophyllaceae. In the present phylotranscriptomic analyses, the Krameriaceae did not show proximity with Fabaceae or Polygalaceae, rather proximity of Krameriaceae with Zygophyllaceae was found consistent with previous reports based on DNA data (Chase *et al*., 1993; Savolainen *et al*., 2000; Soltis *et al*., 2000; Wang *et al*., 2009).

Further, many *Krameria* species are shrubs, inhabiting similar habitats to species of Zygophyllaceae, and have the ability to tolerate similarly low water potentials, but they differ from Zygophyllaceae species in other respects; *Krameria* species are root parasites and have zygomorphic flowers, and an obligate relationship with oil-collecting centridine bees (Simpson, 1989). Previous study has shown that *K. cistoidea*, in contrast to Zygophyllaceae species, possesses H3K9me2 binding protein indicating that this unique epigenetic trait might have been developed exclusively in Zygophyllaceae or have been lost in Krameriaceae during evolution (Granot and Grafi, 2014). Finally, our study based on phylotranscriptomic analyses suggests that the family Krameriaceae is sister to Zygophyllaceae.

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