Introduction

The order Zygophyllales Link in the Fabids comprises two families viz. Zygophyllaceae R. Br. and Krameriacaeae Dumort. (APG IV, 2016). The Zygophyllaceae commonly known as the ‘Caltrop Family’, possess mostly opposite, compound leaves, pinnate or 2-foliate 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, Metharne Engler, Morkillia Rose & Painter, Neoluederitzia Schinz, Pintoa C. Gay, Plectrocarpa Gillies, Porlieria Ruiz & Pavón, Roepera A. de Jussieu, Setexenia R. Brown, Sericodes A. Gray, Sisyndite Sonder, Tetraena Maximowicz, Tribulopsis R. Br., Tribulus L., Viscainoa Greene, Zygophyllum L.] and c. 325 species under 5 subfamilies (e.g. Larreoideae, Morkillioideae, Setexenioideae, Tribuloideae and Zygophyllioideae), 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 Krameriacaeae (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
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.

<table>
<thead>
<tr>
<th>Sl.</th>
<th>Species</th>
<th>Clade</th>
<th>Order</th>
<th>Family</th>
<th>GenBank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ingroup</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td><em>Tribulus eichlerianus</em></td>
<td>Core Eudicots/ Rosids/Fabids</td>
<td>Zygophyllales</td>
<td>Zygophyllaceae</td>
<td>ERS3670314</td>
</tr>
<tr>
<td></td>
<td>K.L. Wilson</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><em>Larrea tridentata</em></td>
<td>Core Eudicots/ Rosids/Fabids</td>
<td>Zygophyllales</td>
<td>Zygophyllaceae</td>
<td>ERS368254</td>
</tr>
<tr>
<td></td>
<td>(Sessé &amp; Moc. ex DC.) Coville</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td><em>Rhus radicans</em> L.</td>
<td>Core Eudicots/ Rosids/Malvids</td>
<td>Sapindales</td>
<td>Anacardiaceae</td>
<td>ERS1829525</td>
</tr>
<tr>
<td>5.</td>
<td><em>Gleditsia triacanthos</em> L.</td>
<td>Core Eudicots/ Rosids/Fabids</td>
<td>Fabales</td>
<td>Fabaceae</td>
<td>ERS631106</td>
</tr>
<tr>
<td>6.</td>
<td><em>Polygala lutea</em> L.</td>
<td>Core Eudicots/ Rosids/Fabids</td>
<td>Fabales</td>
<td>Polygalaceae</td>
<td>ERS631118</td>
</tr>
<tr>
<td></td>
<td>Outgroup</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td><em>Tetrastigma obtectum</em></td>
<td>Core Eudicots/ Rosids</td>
<td>Vitales</td>
<td>Vitaceae</td>
<td>ERS1829368</td>
</tr>
<tr>
<td></td>
<td>(Wall. ex M.A. Lawson) Planch. ex Franch.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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](image_url)

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 vested 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 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.

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project (RG-1439-84). This study was also supported by the Chungnam National University, Daejeon, Republic of Korea.

References


MOLECULAR EVOLUTIONARY RELATIONSHIP OF KRAMERIACEAE


(Manuscript received on 13 July 2020; revised on 22 November 2020)