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Structural rearrangements in plastid genomes of Apiaceae as phylogenetic markers

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Abstract. Apiaceae belong to angiosperm families with frequent plastome structural rearrangements, some of which are generally regarded as synapomorphic for large clades, although typically with limited taxon sampling. Our study aims to improve understanding of the structural rearrangements in plastome within the Tordylieae tribe (Apiaceae-Apioideae) with a dense sampling scheme of its species. We showed that presence of *psb*A pseudogene in inverted repeats near the border with a large single-copy region, which is found in the Tordylieae tribe, may be a clade-specific synapomorphy.

1 Introduction

Apiaceae are one of the largest and most economically important families of angiosperms. Many species are highly valued as essential oil (coriander, cumin), vegetable (carrot, parsley, celery, dill) and medicinal (dill, ferula) plants. They are also a family with a long history of taxonomic uncertainty. Challenges in accurate species identification persist and phylogenetic relationships among genera in the family continue to be unresolved. Herewith, it is not possible to delimit many molecular clades using morphological traits [1].

Recently, it has been repeatedly stipulated that structural reorganizations in plastid genomes such as the change of gene order, small deletions and duplications in non-coding regions, losses and additions of whole genes or their separate parts, can serve as phylogenetic markers in addition to the results obtained from the analysis of nucleotide substitutions [2-4]. Instability in the plastid genome is often associated with the junctions between inverted repeats (IR) and single-copy regions.

Apiaceae plastomes appear to be unusual in angiosperm families due to the frequency and large size of IR junction shifts [5-6]. To date, all major IR expansions and contractions uncovered in the Apiales are restricted to the “apioid superclade,” a large, distally branching group within Apiaceae subfamily Apioideae [7]. Specific large expansions of the IR have shown to demarcate large monophyletic groups, whereas small expansions or contractions tend to occur in closely related species.

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The aim of our study was to conduct molecular analysis with a dense sampling to elucidate whether IR fluxes could be served as a synapomorphy within a certain group. As a group for our study, Tordylieae tribe was selected. Within this group, occurrence of specific *psb*A pseudogene was detected in *Pastinaca* and *Heracleum* plastomes between *ycf*2 and *trn*L-CAA genes (Fig. 1) [8-9].

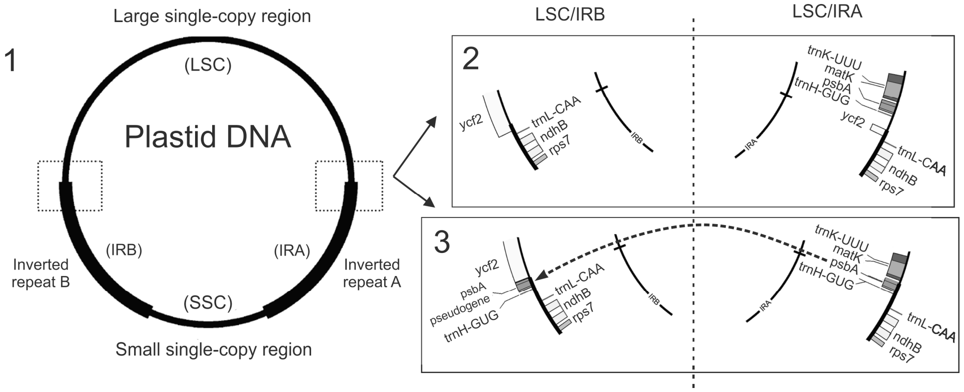

Fig. 1. LSC / IR junctions with gene content changes at the adjacent regions in plastid genomes.

2 Experimental

For study of polymorphism of junction position, 17 Tordylieae taxa were selected. The material was kindly provided by the Botanical Garden of Moscow State University or MW.

Total DNA was isolated from fruits or leaf tissue using the NucleoSpin Plant isolation kit (Macherey-Nagel, Düren, Germany) following manufacturer instructions. To determine the structure of IRb-LSC borders we designed primers Pas3 27248U22: 5’-TTATTTGCAGATGAAGAGATGT-3’; Pas3 27868L20: 5’-GAACTCCCAATTCTCTAGCAAAT-3’) based on the sequence of whole plastome of *Pastinaca pimpinellifolia* M.Bieb. (GenBank accession number KM035850). When the IRb/LSC junctions were typical for *Daucus carota* subsp. *sativus* (GenBank accession number DQ898156), PCR amplification products were about 700 bp length. When the pseudogene *psb*A was located in the IRb as it happened in plastids of *Pastinaca*, PCR product was about 1,0-1,2kb. PCR products were purified with the Gel Extraction & PCR Cleanup Kit (Evrogen, Russia). Sequencing was performed with ABI Prism BigDye Terminator v. 3.1 reagents with subsequent analysis on an ABI Prism 3100-Avant (Applied Biosystems) automated sequencer. The precise localization of JIRb/LSC in Tordylieae species was defined after the sequencing of PCR-products.

3 Results and discussion

Tordylieae W.D.J.Koch is a tribe containing 23 genera [10] with a wide distribution in Middle Asia, Siberia, Europe and South Africa. This group includes a number of important medicinal and vegetable plants (*Heracleum*, *Pastinaca*) while others are noxious invasive plants (*Heracleum sosnowskyi* Manden. and *H. mantegazzianum* Sommier & Levier). The tribe is morphologically well recognizable due to its fruits, which are strongly flattened dorsally and characterized by inner mesocarp composed of thick-walled lignified fibres. However, delimitation of the genera presents considerable difficulties. The largest genera within the group are *Pastinaca* (14-143 species), *Heracleum* (60-200 species), *Tordylium*

(20-70 species) and *Semenovia* (18-39 species), which are characterized by great morphological diversity and a complicated taxonomic history.

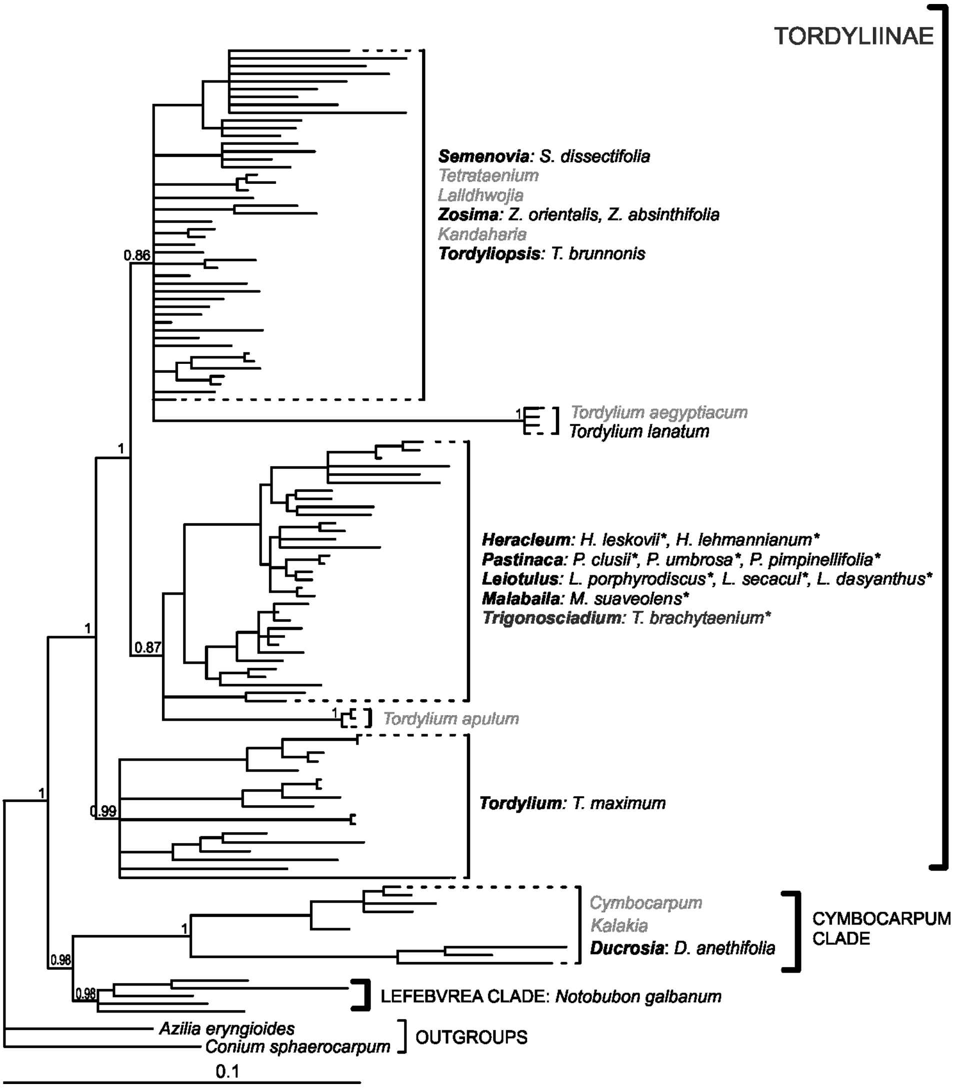

Fig. 2. Summary of the current ITS+ETS based view of phylogenetic relationships in the Tordylieae. Species marked by asterisks possess *psb*A pseudogene in their plastid genome.

Phylogenetic studies based on sequence data from nuclear ribosomal DNA [e.g., 11] and chloroplast DNA [12-13] have provided important insights into the evolution and diversification of Tordylieae. Molecular phylogenetic analyses have resolved five major lineages within the tribe (Fig. 2). Due to molecular data, the Tordylieae was expanded to include African taxa previously residing in *Peucedanum* (*Notobubon*, *Dasispermum*, and others). Majority of genera traditionally recognized within Tordylieae fall into three clades: (1) *Tordylium*; (2) *Heracleum*+*Pastinaca*; (3) *Semenovia*+*Tetrataenium*.

PCR-based analyses of close relatives of *Pastinaca* (*Heracleum*, *Leiotulus*, *Malabaila*, *Trigonosciadium*) detected presence of a *psb*A pseudogene (Fig. 2) and its absence in *Zosima*, *Ducrosia*, *Tordylium* and *Tordyliopsis*, making this rearrangement a likely clade-specific synapomorphy. Our screening for presence of the *trn*H+*psb*A insertion between *ycf*2 and *trn*L-CAA genes showed that this structural feature is characteristic only for *Heracleum*+*Pastinaca* clade. The insertion arises as a consequence of a shift of the junction between the IRA and LSC: the inverted repeat A expands and includes the gene *trn*H-GUG with the 3' part of the *psb*A, and, eventually, their copies emerge in inverted repeat B, between *ycf*2 and *trn*L-CAA genes.

Thus, using representatives of the tribe Tordylieae as an example, we have showed that some changes in the plastid DNA structure (in this case, caused by the shift of IR junctions from the LSC), can be used for testing the hypotheses about taxonomic position at the tribal level.

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