

Comparative Genomics, Codon Usage Bias and Phylogenetic Relationships of Species from Biebersteiniaceae and Nitrariaceae Based on Complete Chloroplast Genomes

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Supplement Materials

Table S1. Primer used for Illumina library amplification.

| Primer | Sequence |
|------------------|---|
| Universal Primer | 5'-AATGATAACGGCGACCACCGAGATCTACACTTTCCCTACACGACGCTTCCGATCT-3' |
| Index Primer | 5'-CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTTCCGATC-3' |

Table S2. Protein-coding genes used for nucleotide substitution rate, codon usage bias and phylogenetic analysis.

| Category | Name |
|------------------------|--|
| Rubisco | <i>rbcL</i> |
| Photosystem I | <i>psaA, psaB, psaC, psaI, psaJ</i> |
| Photosystem II | <i>psbA, psbB, psbC*, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ</i> |
| ATP synthase | <i>atpA, atpB, atpE, atpF, atpH, atpI</i> |
| Cytochrome b/f complex | <i>petA, petB, petD, petG, petL, petN</i> |
| Cytochrome c synthesis | <i>ccsA</i> |
| NADPH dehydrogenase | <i>ndhA*, ndhB, ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK</i> |
| Transcription | <i>rpoA, rpoB, rpoC1, rpoC2</i> |
| Ribosomal proteins | <i>rps2*, rps3, rps4, rps7, rps8, rps11*, rps12, rps14, rps15, rps16, rps18, rps19*, rpl2, rp114, rp116, rp120, rp122*, rp123, rp132, rp133, rp136</i> |
| RNA processing | <i>matK</i> |
| Carbon metabolism | <i>cemA</i> |
| Fatty acid synthesis | <i>accD*</i> |
| Proteolysis | <i>c1pP</i> |

* genes that not used for phylogenetic analysis

Table S3. Information of the species used for the phylogenetic analysis

| NO. | Species | Genebank Accession | Family |
|-----|------------------------------------|--------------------|-------------------|
| 1 | <i>Anacardium occidentale</i> | KY635877.1 | Anacardiaceae |
| 2 | <i>Mangifera indica</i> | KY635882.1 | Anacardiaceae |
| 3 | <i>Pistacia weinmannifolia</i> | MF630953.1 | Anacardiaceae |
| 4 | <i>Rhus chinensis</i> | KX447140.1 | Anacardiaceae |
| 5 | <i>Sclerocarya birrea</i> | MK002721.1 | Anacardiaceae |
| 6 | <i>Spondias bahiensis</i> | KU756561.1 | Anacardiaceae |
| 7 | <i>Spondias mombin</i> | KY828469.1 | Anacardiaceae |
| 8 | <i>Spondias tuberosa</i> | KU756562.1 | Anacardiaceae |
| 9 | <i>Biebersteinia heterostemon</i> | MN818816 | Biebersteiniaceae |
| 10 | <i>Brassica nigra</i> | KT878383.1 | Brassicaceae |
| 11 | <i>Boswellia sacra</i> | KT934315.1 | Burseraceae |
| 12 | <i>Commiphora foliacea</i> | MH041484.1 | Burseraceae |
| 13 | <i>Commiphora gileadensis</i> | MH042752.1 | Burseraceae |
| 14 | <i>Commiphora wightii</i> | MF957201.1 | Burseraceae |
| 15 | <i>Azadirachta indica</i> | KF986530.1 | Meliaceae |
| 16 | <i>Carapa guianensis</i> | MF401522.1 | Meliaceae |
| 17 | <i>Cedrela odorata</i> | MG724915.1 | Meliaceae |
| 18 | <i>Entandrophragma caudatum</i> | MK058683.1 | Meliaceae |
| 19 | <i>Entandrophragma cylindricum</i> | KY923074.1 | Meliaceae |
| 20 | <i>Khaya madagascariensis</i> | MK058684.1 | Meliaceae |
| 21 | <i>Khaya senegalensis</i> | KX364458.1 | Meliaceae |
| 22 | <i>Swietenia macrophylla</i> | MH348156.1 | Meliaceae |
| 23 | <i>Swietenia mahagoni</i> | NC_040009.1 | Meliaceae |
| 24 | <i>Toona ciliata</i> | MG813875.1 | Meliaceae |
| 25 | <i>Xylocarpus granatum</i> | MH348155.1 | Meliaceae |
| 26 | <i>Xylocarpus moluccensis</i> | MH330688.1 | Meliaceae |
| 27 | <i>Xylocarpus rumphii</i> | MH330687.1 | Meliaceae |
| 28 | <i>Nitraria roborowskii</i> | MK347421 | Nitrariaceae |
| 29 | <i>Nitraria sibirica</i> | MK347422 | Nitrariaceae |
| 30 | <i>Nitraria tangutorum</i> | MK347423 | Nitrariaceae |
| 31 | <i>Peganum harmala</i> | MK347420 | Nitrariaceae |
| 32 | <i>Atalantia kwangtungensis</i> | MH329190.1 | Rutaceae |
| 33 | <i>Citrus aurantiifolia</i> | KJ865401.1 | Rutaceae |
| 34 | <i>Citrus depressa</i> | LC147381.1 | Rutaceae |
| 35 | <i>Citrus platymamma</i> | KR259987.1 | Rutaceae |
| 36 | <i>Citrus sinensis</i> | DQ864733.1 | Rutaceae |
| 37 | <i>Clausena excavata</i> | KU949003.1 | Rutaceae |
| 38 | <i>Glycosmis mauritiana</i> | KU949004.1 | Rutaceae |
| 39 | <i>Glycosmis pentaphylla</i> | KU949005.1 | Rutaceae |
| 40 | <i>Merrillia caloxylon</i> | KU949006.1 | Rutaceae |
| 41 | <i>Micromelum minutum</i> | KU949007.1 | Rutaceae |
| 42 | <i>Murraya koenigii</i> | KU949002.1 | Rutaceae |

| | | | |
|----|---------------------------------|------------|---------------|
| 43 | <i>Phellodendron amurense</i> | KY707335.1 | Rutaceae |
| 44 | <i>Zanthoxylum bungeanum</i> | KX497031.1 | Rutaceae |
| 45 | <i>Zanthoxylum piperitum</i> | KT153018.1 | Rutaceae |
| 46 | <i>Zanthoxylum schinifolium</i> | KT321318.1 | Rutaceae |
| 47 | <i>Zanthoxylum simulans</i> | MF716524.1 | Rutaceae |
| 48 | <i>Acer buergerianum</i> | KY419137.1 | Sapindaceae |
| 49 | <i>Acer catalpifolium</i> | MF179637.1 | Sapindaceae |
| 50 | <i>Acer davidii</i> | MK193786.1 | Sapindaceae |
| 51 | <i>Acer griseum</i> | KY511609.1 | Sapindaceae |
| 52 | <i>Acer laevigatum</i> | MF521832.1 | Sapindaceae |
| 53 | <i>Acer miaotaiense</i> | KX098452.1 | Sapindaceae |
| 54 | <i>Acer morrisonense</i> | KT970611.1 | Sapindaceae |
| 55 | <i>Acer sino-oblongum</i> | KY987160.1 | Sapindaceae |
| 56 | <i>Acer truncatum</i> | MF996341.1 | Sapindaceae |
| 57 | <i>Acer wilsonii</i> | MG012225.1 | Sapindaceae |
| 58 | <i>Aesculus wangii</i> | MF583747.1 | Sapindaceae |
| 59 | <i>Dimocarpus longan</i> | MG214255.1 | Sapindaceae |
| 60 | <i>Dipteronia dyeriana</i> | KT985457.1 | Sapindaceae |
| 61 | <i>Dipteronia sinensis</i> | KT878501.1 | Sapindaceae |
| 62 | <i>Dodonaea viscosa</i> | MF155892.1 | Sapindaceae |
| 63 | <i>Eurycorymbus cavaleriei</i> | MG813997.1 | Sapindaceae |
| 64 | <i>Koelreuteria paniculata</i> | KY859413.1 | Sapindaceae |
| 65 | <i>Litchi chinensis</i> | KY635881.1 | Sapindaceae |
| 66 | <i>Sapindus mukorossi</i> | KM454982.1 | Sapindaceae |
| 67 | <i>Xanthoceras sorbifolium</i> | KY779850.1 | Sapindaceae |
| 68 | <i>Ailanthus altissima</i> | MG799542.1 | Simaroubaceae |
| 69 | <i>Eurycoma longifolia</i> | MH751519.1 | Simaroubaceae |
| 70 | <i>Leitneria floridana</i> | KT692940.1 | Simaroubaceae |

Figure S1. Comparison of the borders of large single-copy (LSC), small single-copy (SSC), and inverted repeat (IR) regions among the chloroplast genomes of five species. JLB: junctions of LSC and IRb; JSB: junctions of SSC and IRb; JSA: junctions of SSC and Ira; JLA: junctions of LSC and Ira.

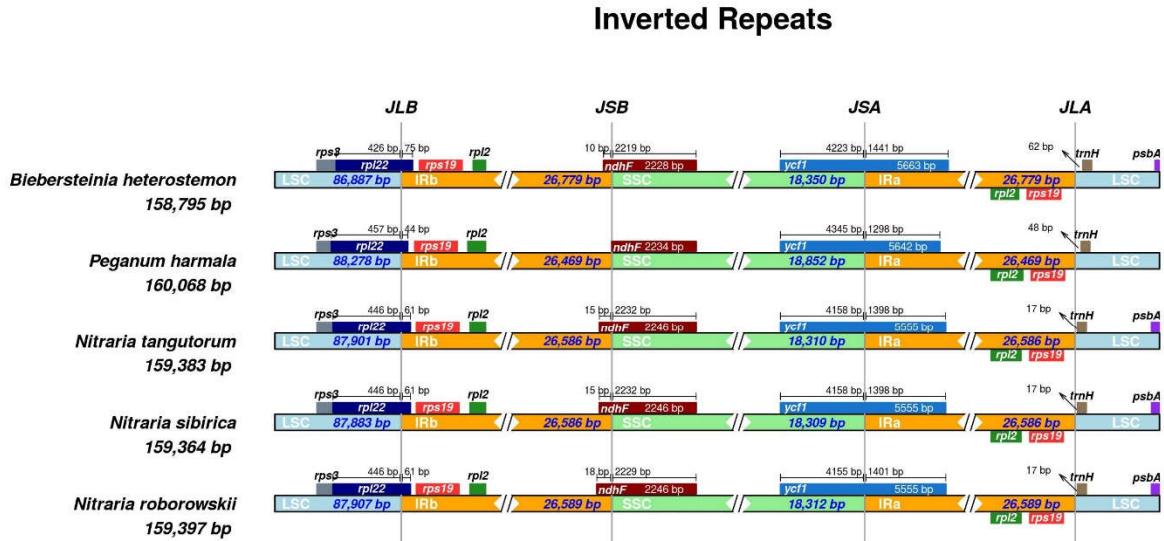


Figure S2. Sliding window analysis of nucleotide variability (pairwise divergence) among the five species. The number represented highly variable regions ($P_i > 0.10$). 1: *trnH-psbA*, 2: *matK-rps16*, 3: *psbK-psbI*, 4: *trnE-trnT*, 5: *trnF-ndhJ*, 6: *ndhD-ndhG*, and 7: *rrn23-trnA*.

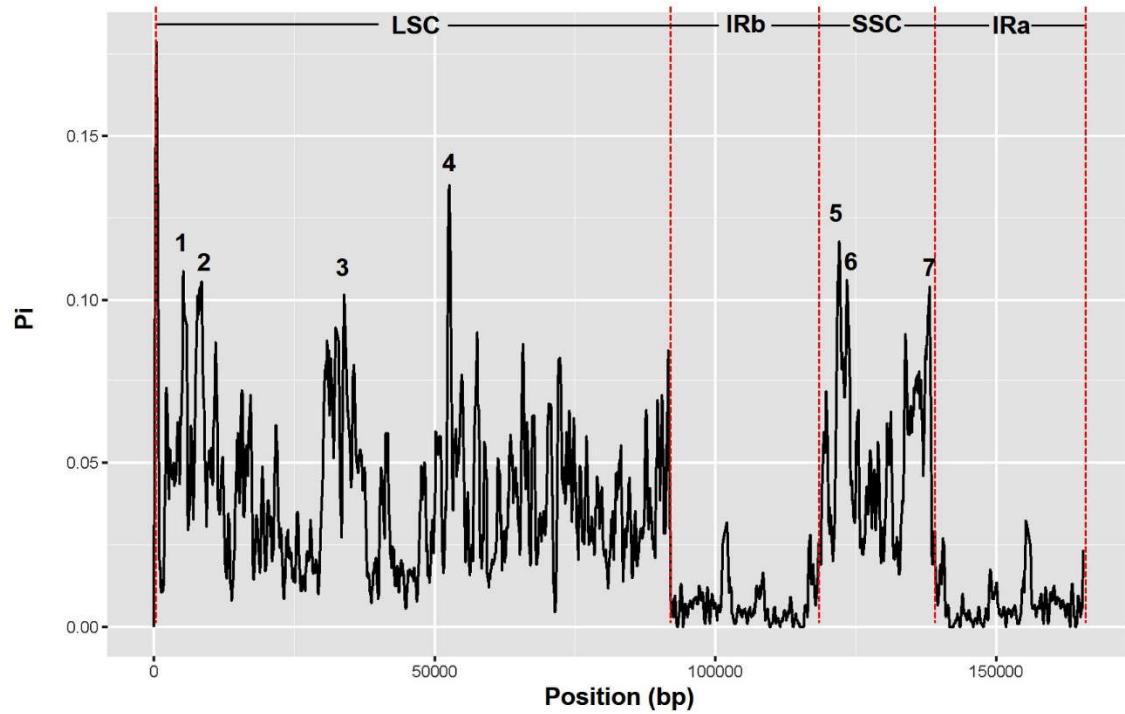


Figure S3. The phylogenetic relationships within Sapindales resolved by complete chloroplast genome. Numbers associated with the branches are ML bootstrap value (BS) and BI posterior probabilities (PP). Nodes without numbers are supported by 100/1.

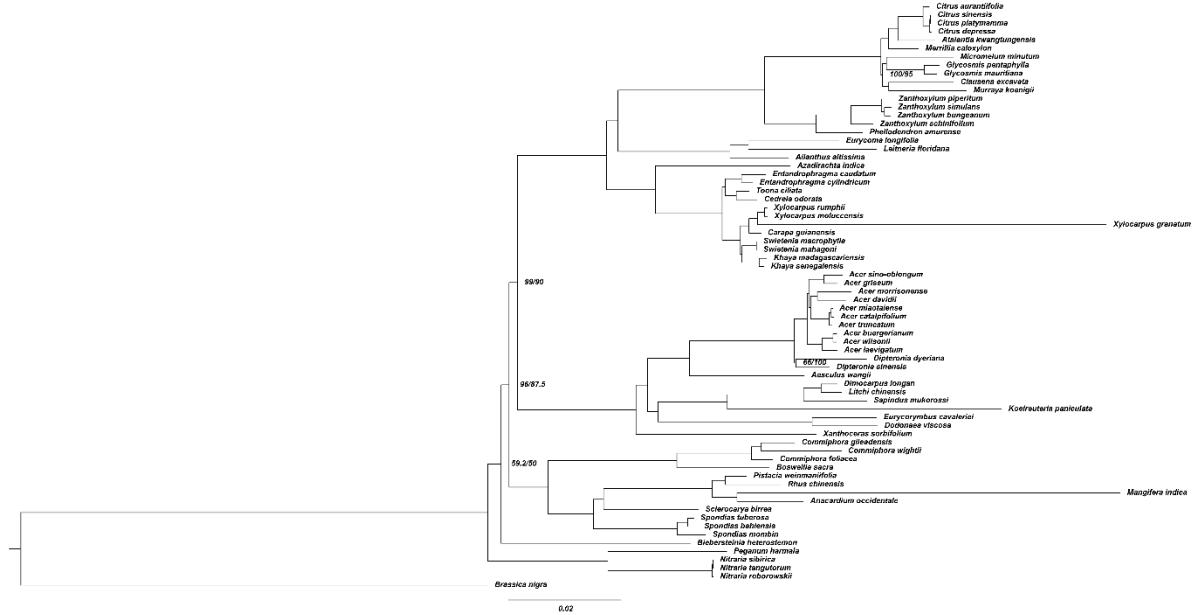


Figure S4. Amino acid sequence alignment of *clpP* gene for the five species. Red box indicates the gene structural changes domains.

| | | | | |
|--------------------------|---|--|---|-----|
| <i>B. heterostemon</i> : | MHLGLTYSATCQIYRICFVBPYASKGARTAPKGYSFILINRLYRERLLFLGCADVSEISNQLIGLMVYLSIENDTKDLY | : | 80 | |
| <i>P. harmala</i> : | MPIGVP-----KVPFRSGEEDA----- | SWDVVRLYRERLLFLGCADVSEISNQLIGLMVYLSIENDTKDLY | : | 63 |
| <i>N. tangutorum</i> : | MPIGVP-----KVPFRSGEEDA----- | SWDVVRLYRERLLFLGCADVSEISNQLIGLMVYLSIENDTKDLY | : | 63 |
| <i>N. roborowskii</i> : | MPIGVP-----KVPFRSGEEDA----- | SWDVVRLYRERLLFLGCADVSEISNQLIGLMVYLSIENDTKDLY | : | 63 |
| <i>N. sibirica</i> : | MPIGVP-----KVPFRSGEEDA----- | SWDVVRLYRERLLFLGCADVSEISNQLIGLMVYLSIENDTKDLY | : | 63 |
| | Mp6G6p | KVP5rSpGeedA | S5646yRLYRERLLFLGQe6DSEISNQLIGLMVYLSIENDTKDLY | |
| | | | | |
| <i>B. heterostemon</i> : | LFINSPGGWVTPGIAIYDTMQFVRPDVQTICMGLAASMGSFILVGGEITKRLAFTP H AVMIHQPIAF | FYEAQTGEFILEAB | : | 160 |
| <i>P. harmala</i> : | LFINSPGGWVTPGIAIYDTMQFVRPDVQTICMGLAASMGSFILVGGEITKRLAFTP H | FYEAQTGEFILEAB | : | 134 |
| <i>N. tangutorum</i> : | LFINSPGGWVTPGIAIYDTMQFVRPDVQTICMGLAASMGSFILVGGEITKRLAFTP H | FYEAQTGEFILEAB | : | 134 |
| <i>N. roborowskii</i> : | LFINSPGGWVTPGIAIYDTMQFVRPDVQTICMGLAASMGSFILVGGEITKRLAFTP H | FYEAQTGEFILEAB | : | 134 |
| <i>N. sibirica</i> : | LFINSPGGWVTPGIAIYDTMQFVRPDVQTICMGLAASMGSFILVGGEITKRLAFTP H | FYEAQTGEFILEAB | : | 134 |
| | LFINSPGGWVTPGIAIYDTMQFVRPDVQT6CMGLAASM S FILVGGEITKRLAFTP H | FYEAQTGEFILEAB | | |
| | | | | |
| <i>B. heterostemon</i> : | ELLKLRETLTRVYVQRTGKPLWVVSEDMERDVFMSATEAQAHGIVDLVAVG* | : | 211 | |
| <i>P. harmala</i> : | ELLKLRETLTRVYVQRTGKPLWVVSEDMERDVFMSATEAQAHGIVDLVAVG* | : | 185 | |
| <i>N. tangutorum</i> : | ELLKLRETLTRVYVQRTGKPLWVVSEDMERDVFMSATEAQAHGIVDLVAVG* | : | 185 | |
| <i>N. roborowskii</i> : | ELLKLRETLTRVYVQRTGKPLWVVSEDMERDVFMSATEAQAHGIVDLVAVG* | : | 185 | |
| <i>N. sibirica</i> : | ELLKLRETLTRVYVQRTGKPLWVVSEDMERDVFMSATEAQAHGIVDLVAVG* | : | 185 | |