Systematic study

Reasons for the study

• make a stable phylogeny for cycads
• evaluate utility of single-copy nuclear markers
• compare different gene tree-species tree reconciliation approaches and compare with concatenation
• estimate clade ages
• reconstruct ancestral areas
Study genus

- 10 genera, 331 species
- fossil record back to Lower Permian, peak abundance and diversity in Mesozoic
- ‘living fossils’ – many ancestral characters
- placement of *Bowenia, Dioon* and *Stangeria* not resolved
Methods

- 20 taxa (up to three species per genus)
- 5 single-copy markers (CyAG, COS26, GroES, GTP, HTS)
- PCR, sequencing, MAFFT alignment
- Evaluation of nucleotide substitution model (KAKUSAN)
- MP – concatenated dataset (jackknife + Bremer support)
- ML – non-partitioned analysis, 1 000 bootstrap rounds
- Species tree reconciliation
  - *BEAST
  - BEST
  - BUCKy – Bayesian concordance analysis
- Divergence age estimation – BEAST, calibrations (3 fossils)
- Biogeographic analyses – S-DIVA, DEC
Results

• MP (concatenated) – single fully resolved tree
• ML – same topology
• gene tree – species tree – also the same topology
• all analyses – high support except for nodes 15 and 16
• age estimation
  • crown node for Cycadales – 228 Mya
  • Zamiaceae crown node – 91 Mya
  • split between Old and New World (incl. Stangeria)– 71 Mya
  • crown nodes of all genera – late Miocene/Pliocene
Results – biogeographical analyses

• root note of the phylogeny
  • Australia – Mexico – Northern Central America (S-DIVA)
  • Australia – China – Northern Central America (DEC)
  • both – 2 dispersals, 1 extinction – BUT – low probabilities...

• crown node of *Cycas* (38)– Australia + China (vicariance)

• ...

• *Encephalartae* crown node (26)
  • dispersal to Africa from Australia
  • *Lepidozamia vs. Encephalartos* – vicariance Australia vs. Africa
Discussion

- complete congruence of 3 species tree estimation methods
- main phylogenetic outcomes – improved support for:
  - *Dioon* sister to all *Zamiineae*
  - *Bowenia* next branch in *Zamiineae*
  - *Stangeria* sister to *Microcycas/Zamia*
- most support came from CyAG, COS26 and GroES
- earliest known cycad fossils (300 Mya) congruent with estimation for crown age of modern Cycadales (230 Mya)
- Mesozoic – increased fossil diversity
- lack of fossils in S hemisphere – absent from Pangaea
- crown age for *Cycas* (12 Mya) – relatively recent dispersal to Africa, Australia and Pacific Islands
Discussion

• stem node of *Dioon* (Cretaceous) – Australia, Africa – broad distribution, today relict

• vicariance of *Encephalartos* (Africa) and *Lepidozamia* (Australia) in late Paleogene – unique pattern!

• Cretaceous/Tertiary boundary – major diversification time
  • branch lengths of nodes from ca. 75-60 Mya very short – rapid diversification – associated with drastic environmental changes

• late Eocene – climate cooling – elimination from higher latitudes of N hemisphere

• very recent species diversification (no generic crown node > 12 Mya)
Conclusions

• five nuclear genes – most congruent estimate of phylogeny
• evolutionary history and biogeography – supported by molecular and fossil data
• extant diversity is recent
• topology from concatenated dataset and gene tree – species tree reconciliations congruent