Systematic study

Salas-Leiva D.E.S. et al. (2013): Phylogeny of the cycads based on multiple single-copy nuclear genes: congruence of concatenated parsimony, likelihood and species tree inference methods. *Annals of Botany* 112(7): 1263–1278

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 Mesosoic
- ‘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