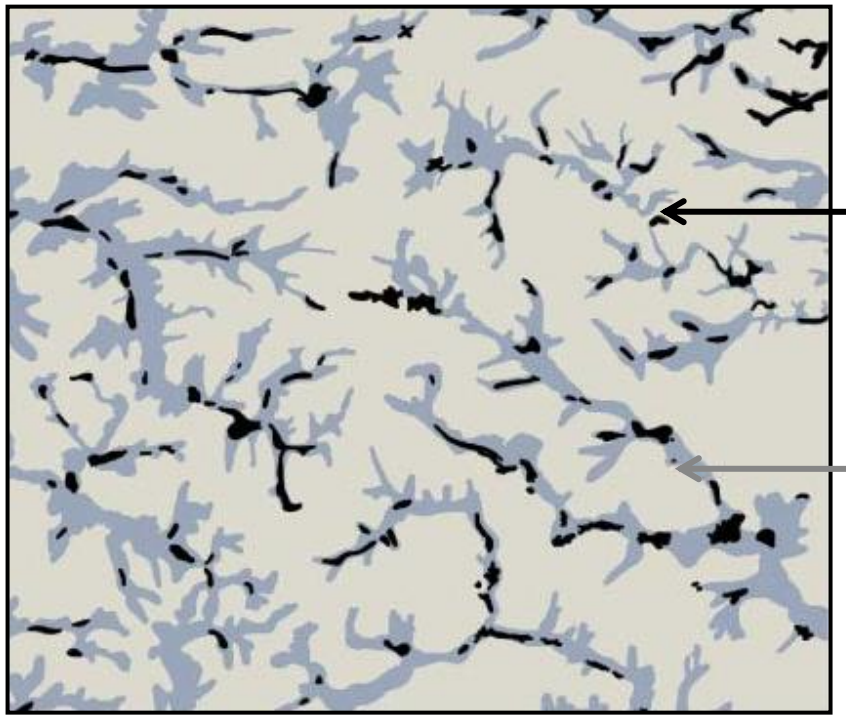
Genetic divergences and species boundaries in subterranean invertebrates 'Down Under'

Michelle T. Guzik

Cooper, S.J.B., Humphreys, W.F., Stevens, M.I., Javidkar, M., Harrison, S., King, R.A., Abrams, K., Harvey, M.S., Austin, A.D.

Calcrete aquifers of central Western Australia

- >200 calcium carbonate aquifers
- Enclosed micro caves that lie in ancient river valleys



Subterranean fauna





Stygofauna



Troglofauna

Images by C. Watts, K. Abrams, M. Harvey, T. Bradford

Significance of the region

- Evolutionarily valuable for understanding the history of Australia: living fossils
- Major conservation and management issues



Image by W. Humphreys

Exploring biodiversity

- Levels of diversity amongst fauna both between and within calcrete aquifers
- Examine species boundaries and ranges

Exploring biodiversity

- Species delineation is difficult in calcrete system
  - Difficulty sampling individuals (i.e. sample size low)
  - Large number of unidentified species
  - Highly derived morphological forms
  - Framework for taxonomy is poor (i.e. crustaceans)
  - Few experts available for morphological examination

Exploring biodiversity: methods

- Create a framework within which we can examine biodiversity
  - Robust criteria for delineating species

Images by K. Abrams and M. Harvey

Delineation of species

- Barcoding and DNA thresholds
  - Quick-sort specimens → genetically divergent lineages
  - Insects
    - Inter-specific divergences: > 4.4%
    - Intra-specific divergences: < 1%
  - Crustaceans
    - Inter-specific divergence: 16%
    - Intra-specific divergence: <14%


PNAS

DNA barcodes distinguish species of tropical Lepidoptera

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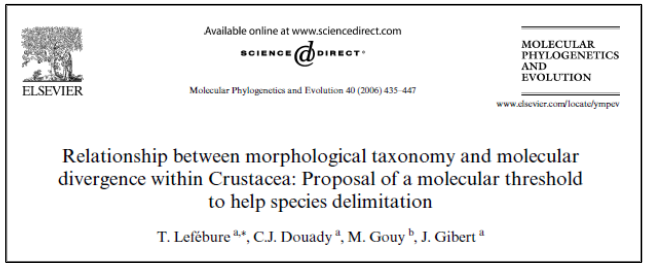
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Molecular Phylogenetics and Evolution 40 (2006) 435–447

MOLECULAR PHYLOGENETICS AND EVOLUTION

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Relationship between morphological taxonomy and molecular divergence within Crustacea: Proposal of a molecular threshold to help species delimitation

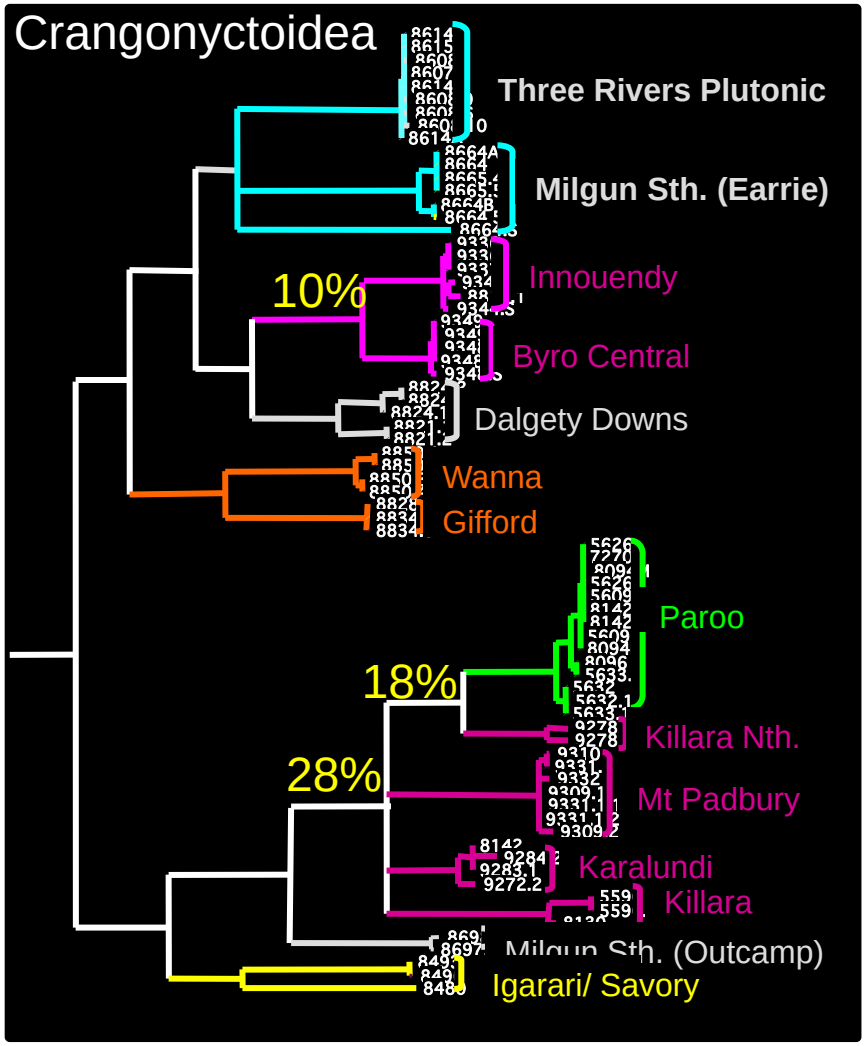
T. Lefébure [a,*], C.J. Douady [a], M. Gouy [b], J. Gibert [a]

Framework: *a priori* criteria

- COI sequencing to identify distinct lineages
  - Phylogenetic analyses
  - Molecular diversity
- Morphological comparisons
  - Taxonomy
- Geography
  - Species boundaries

Broad range: between aquifers

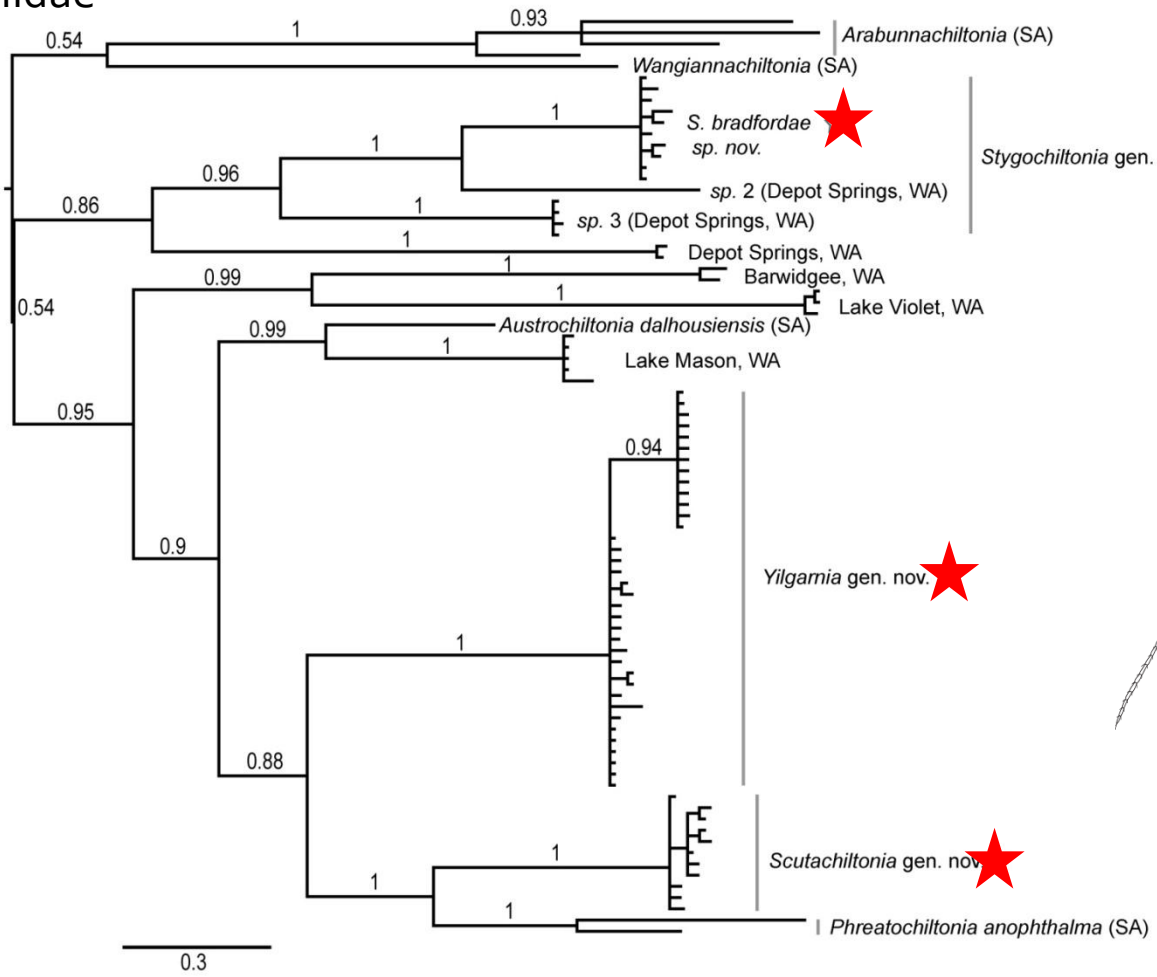
- Almost every CO1 mtDNA clade is restricted to a single and different calcrete aquifer
- Some sympatric lineages within aquifers
- Morphological data support separate species

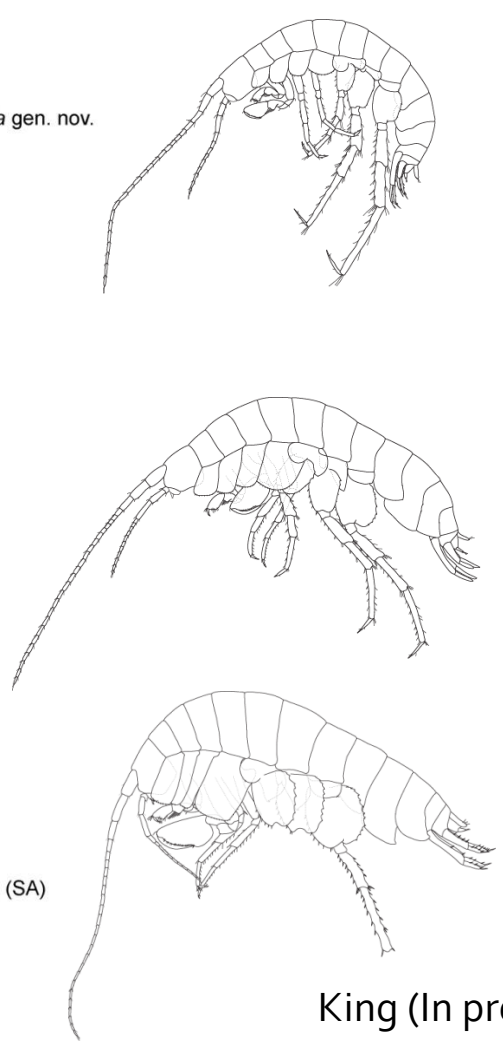


Cooper *et al.* (2007) *Mol. Ecol.*, (2008) *Invert. Syst.*; Guzik (2008) *Invert. Syst.*

Narrow range: within aquifers

Chiltoniidae





King (In press) *JCB*

New species from calcrete aquifers

Taxon group	DNA delineation (≥11%)	Published species	Geography (distinct aquifer)
Bathynellidae	18	9	8
Amphipoda	22	3	20
Aquatic Isopoda	24	2	15
Coleoptera	99	99	47
Copepoda	15	32	5
Terrestrial Isopoda	22	0	12
Collembola	30	0	14
Total	**230**	**145**	

Narrow range: within aquifers

- COI mtDNA sequencing
- Two calcrete aquifers
  - Aquifer 1: three beetle species (*n* >300)
  - Aquifer 2: three beetle species, one amphipod, one isopod (*n* <300)

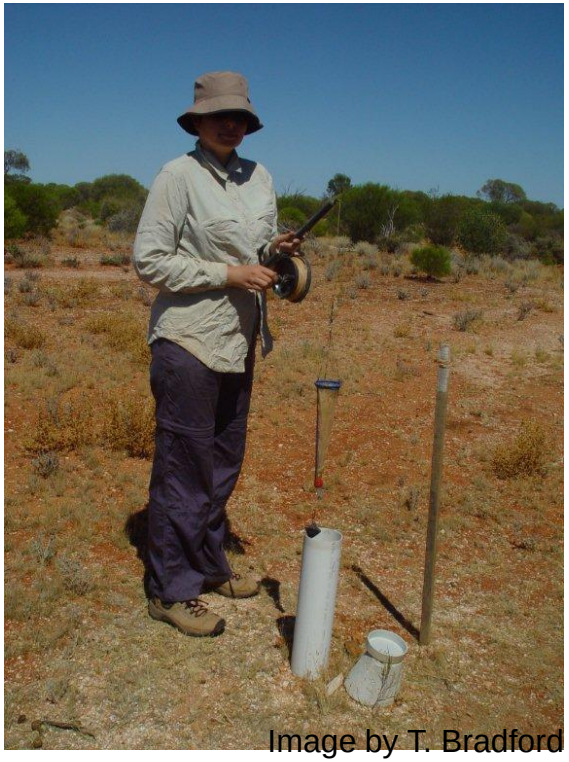
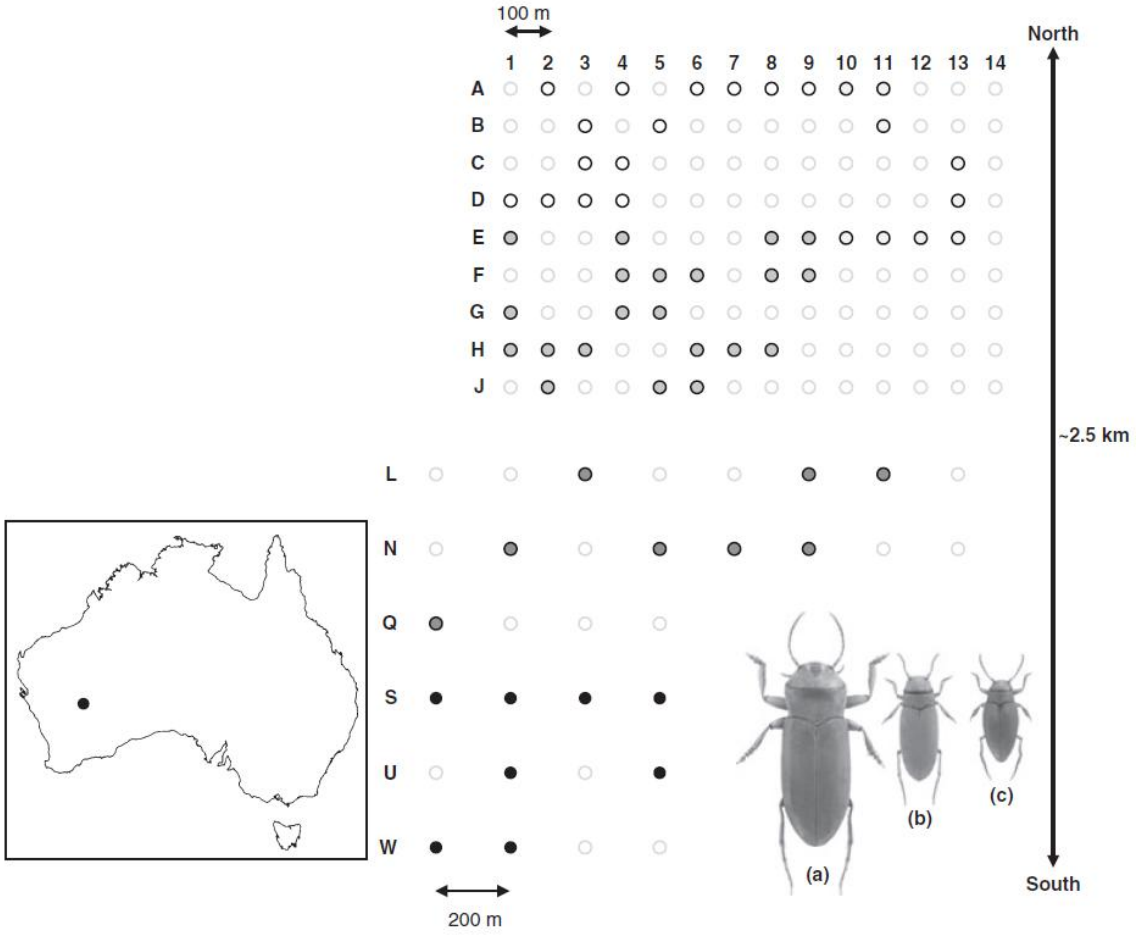
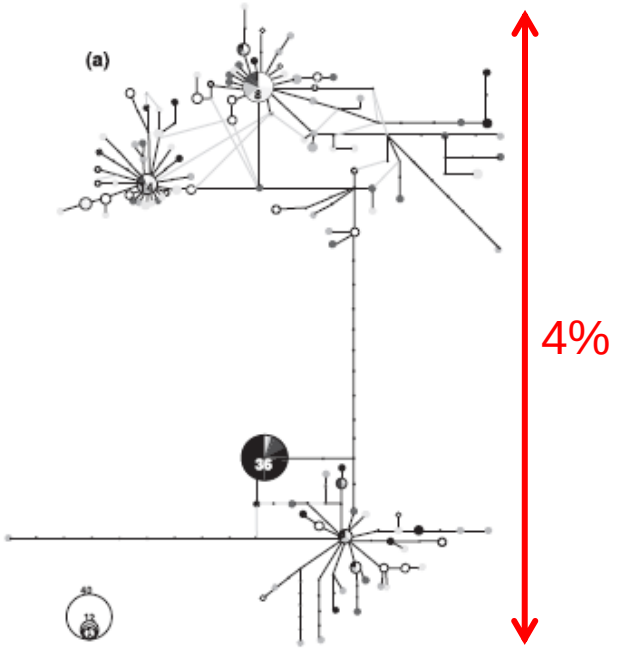


Image by T. Bradford

Aquifer 1

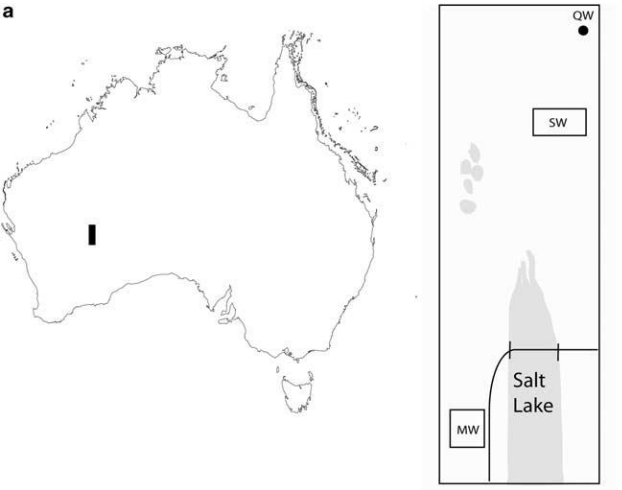


- Three known beetle species
- Very high genetic diversity
- Genetic structure and divergences

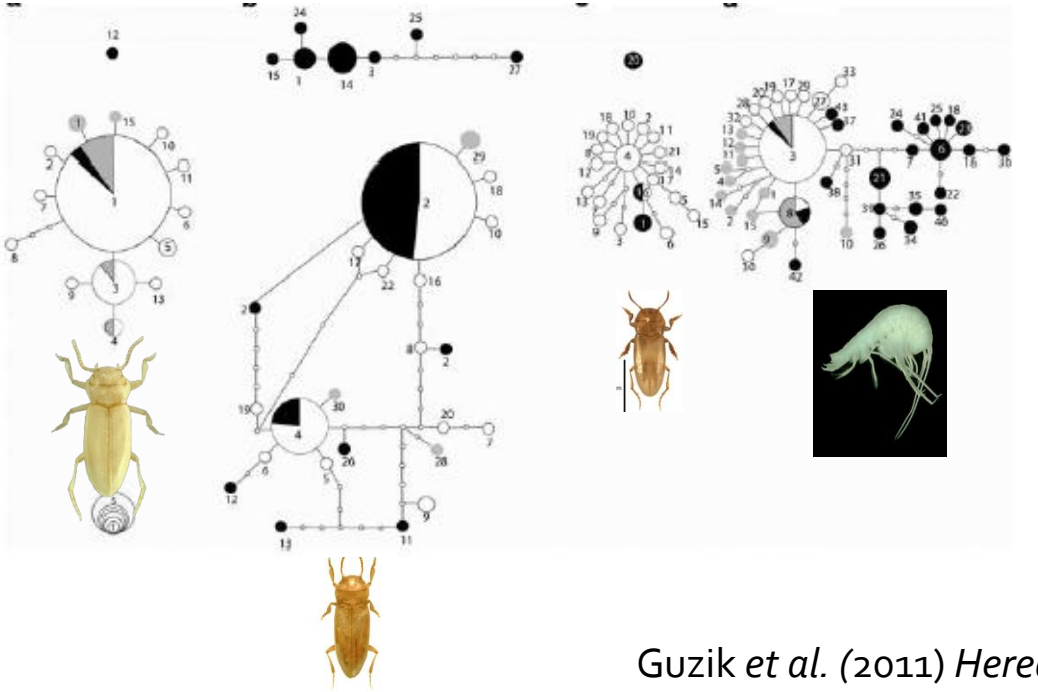


Guzik *et al.* (2009) *Mol. Ecol.*

Aquifer 2



- Three beetle species, one amphipod, one isopod
- High genetic diversity
- Strong population structure



Guzik *et al.* (2011) *Heredity*

Intra-calcrete DNA divergences

Aquifer	Taxon group	No. known species	Inter-specific %	Intra-specific %	Uncertain %
1	Coleoptera	3	9 - 16	0 - 4	
	Amphipoda	3	14 - 15	0 - 1	
2	Coleoptera	3	11 - 15	1 - 4	
	Amphipoda	1	-	0 - 2	
	Isopoda	?	-	-	19–21

Population level divergences

Genetic diversity

- Both within and between calcrete aquifers → high genetic divergences
  - Long term persistence of populations
    - Taxa isolated underground < 15 my
  - Large population sizes
  - Complex environment

Image by C. Watts

Exceptional biodiversity

- Prediction of ~1000 possible subterranean species in the Yilgarn alone
- DNA thresholds can be useful
  - But caution as intra-specific divergences can be high
- Combination of methods is useful especially in complex systems

Guzik *et al.* (2011) *Invert. Syst.*

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