More species than expected?

Ecological differentiation of cryptic species within an asexual protist morphospecies

Pavel Škaloud¹ & Fabio Rindi²

¹ Charles University in Prague, Department of Botany, Prague, Czech Republic
² Martin Ryan Institute, National University of Ireland, Galway, Ireland
Is everything everywhere?

Global Dispersal of Free-Living Microbial Eukaryote Species
Bland J. Finlay

Finlay & Clarke (1999); Finlay (2002)
- genetic variation in molecular markers reflects rather the accumulation of neutral mutations over historical time than the existence of morphologically indiscernible, cryptic species.
- the phenotype as the only proper feature to define real species of protists

Cyclidium glaucoma
Aims of the study

• Does the genetic diversity within protist morphospecies reflect an accumulation of neutral mutations?

*Klebsormidium flaccidum* – cosmopolitan, broadly distributed, asexual

• Mapping the morphological properties on the phylogeny of *K. flaccidum*

- 62 strains isolated from a variety of aero-terrestrial and aquatic habitats
- Genetic data: ITS rDNA & rbcL sequences
- Morphological data: width, growth habit, presence of a superficial layer of filaments, shape of release apertures in sporangia, zoospore germination, cell wall remnants
Bayesian phylogeny (ITS rDNA + rbcL)

- Two main clades resolved: A B
- 11 well-resolved lineages within *K. flaccidum* morphospecies
- Four morphologically different *Klebsormidium* species nested within *K. flaccidum*
Ancestral state reconstructions (MP)

**Average cell width**

- Partial usefulness of this character to characterize particular genetic lineages
- In some cases, genetically uniform strains considerably differ in their cell width
Ancestral state reconstructions (MP)

**Ability to produce a superficial layer of hydro-repellent filaments**

- Superficial layer completely absent in lineages A2, A9, and B4
Reproductive features (structure of release apertures and zoospore germination)

- distinct apertures
- indistinct apertures
- unipolar germination
- bipolar germination
Strong ecological preferences of the lineages to one of three habitat types:

- Natural substrates
- Artificial substrates
- Aquatic habitats
Ecological differentiation of cryptic species

- The genetic diversity within protist morphospecies really reflect the existence of cryptic species, which could be defined by their ecological preferences and slight morphological differences.
- The morphology alone is not sufficient to unambiguously discriminate among closely related protist species
- If the ecological differentiation of cryptic species is frequent in nature, the real species diversity of protists could be in fact much higher than estimated

*Orbulina universa*

Ecological significance of cryptic variation in Foraminifera: de Vargas et al. 1999, 2002
Speciation of asexual protists

• Diversification of asexual protists into the distinct, ecologically well defined cryptic species could be enabled by the process of ‘periodic selection’
Speciation of asexual protists – periodic selection

Single, genetically uniform species growing on natural substrates
Speciation of asexual protists – periodic selection

Mutations increase genetic diversity within the species
Speciation of asexual protists – periodic selection

Adaptive mutation could quickly spread and replace all organisms belonging to this species.
Speciation of asexual protists – periodic selection

The selection crashes the accumulated diversity back to near zero.
If a new mutant differs in its ecological niche, it could give rise to the new, ecologically defined species.
Speciation of asexual protists – periodic selection

Periodic selection will keep the species distinct, by purging the diversity only within and not between the ecologically differentiated species.
Conclusions

• Our findings clearly contradict the assumptions of Finlay (1999) and Fenchel and Finlay (2006) that the genetic variation in molecular markers only reflects the accumulation of neutral mutations.
• The phenotypic data should be combined with molecular background and ecological consequences.
• We consider that the permanent existence of genetically and ecologically well-defined cryptic species is enabled by the mechanism referred to as ‘periodic selection’
• To organize biological information in a meaningful fashion, any functional properties should be found to characterize the cryptic species.

Acknowledgements

• The study was supported by project No. 206/09/P291 of the Czech Science Foundation