Species delimitation in protists
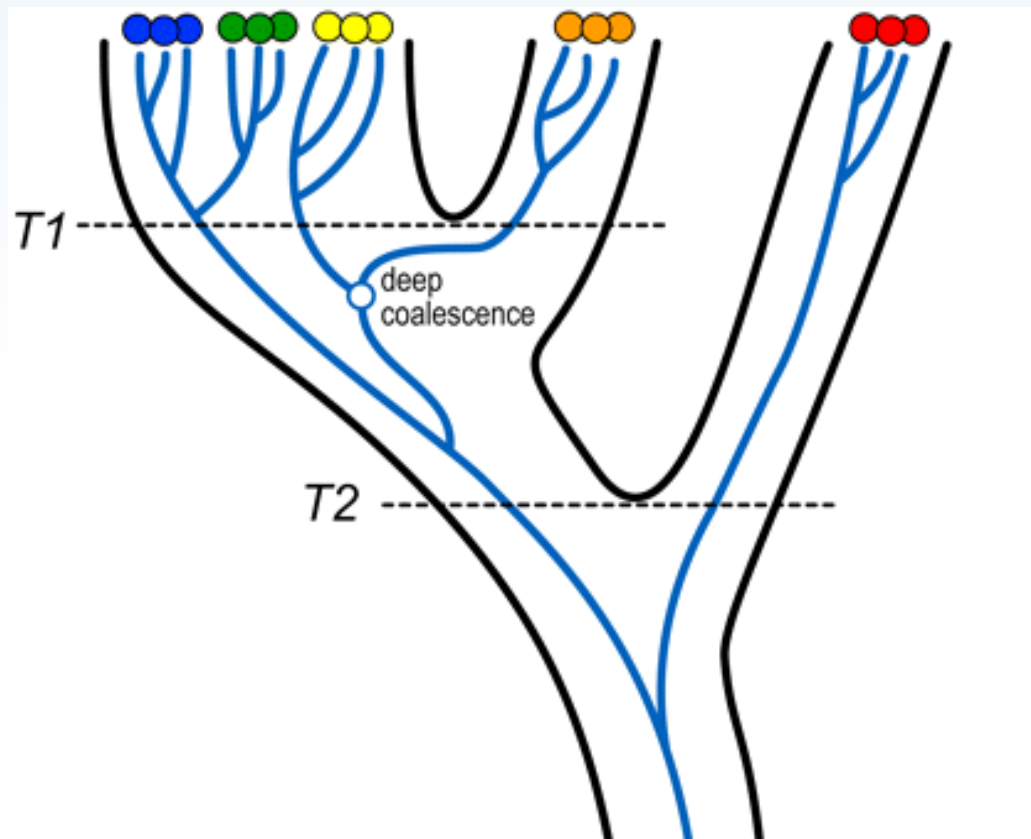

Pavel Škaloud,
Algal speciation & evolution lab
Charles University, Prague
Czech Republic

Species delimitation

- Importance of taxa delimitation

- Species concepts in protists
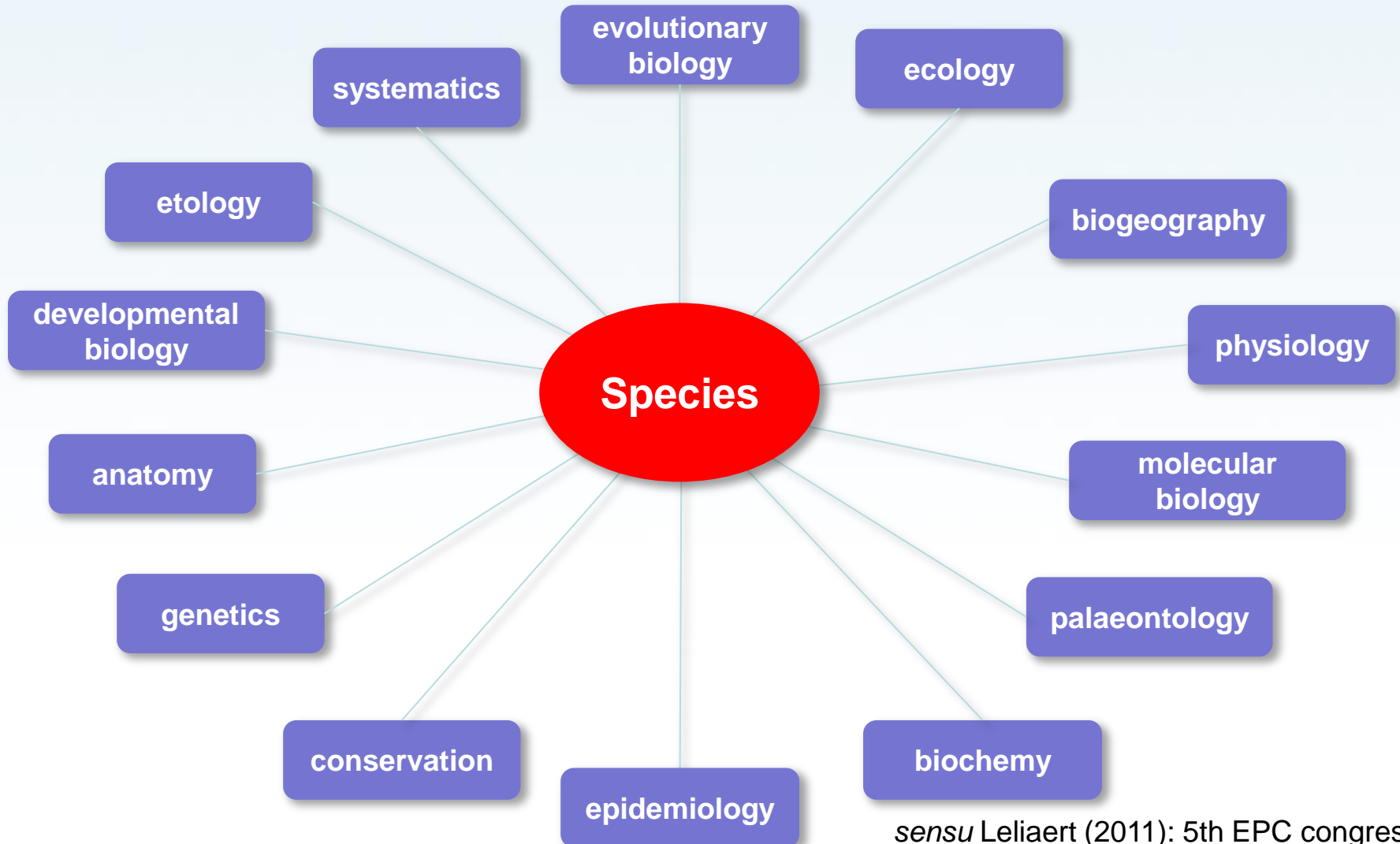
- Species delimitation in *Asterochloris*

Species

- Fundamental units of the systematics
 - Organisation of biodiversity to the well arranged system

biomodel.org.ua
Photos & layout by Grigoriy Kolomytsev

Species

- Fundamental units in all biological disciplines
- It is of a great importance to delimit the species correctly

sensu Leliaert (2011): 5th EPC congress

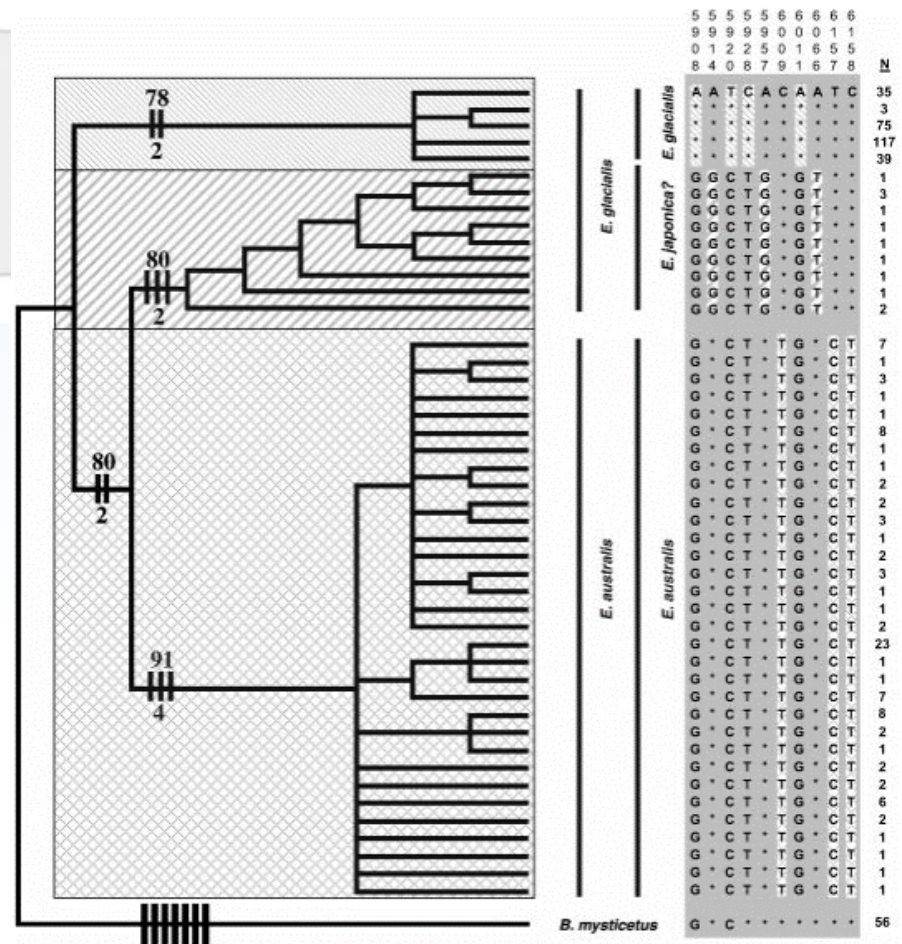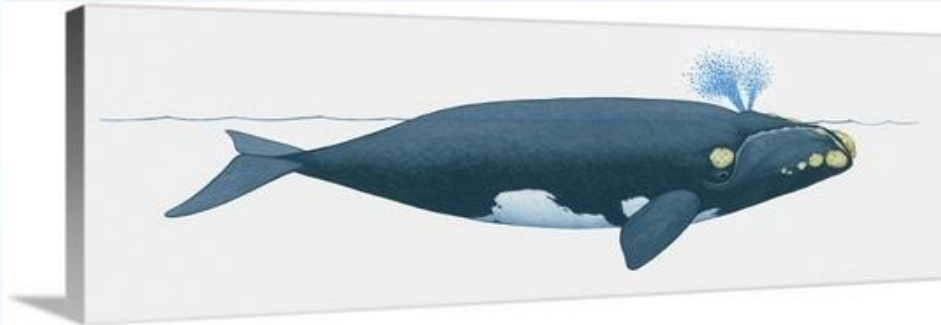# Species delimitation consequences

- Species as biodiversity indicators
  - Conservation management (biodiversity hotspots)

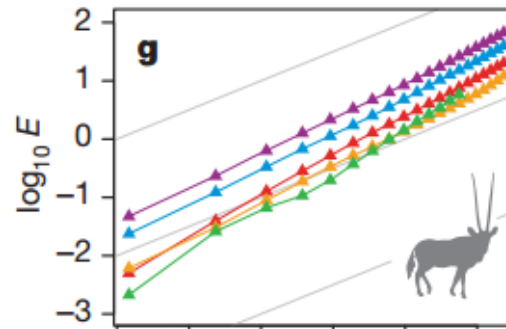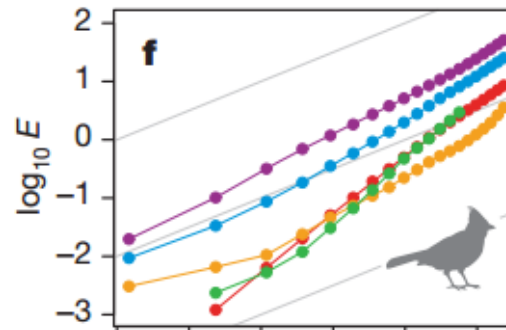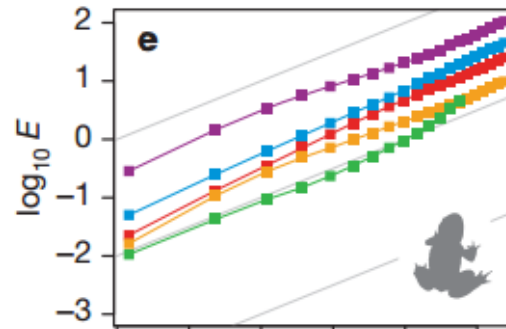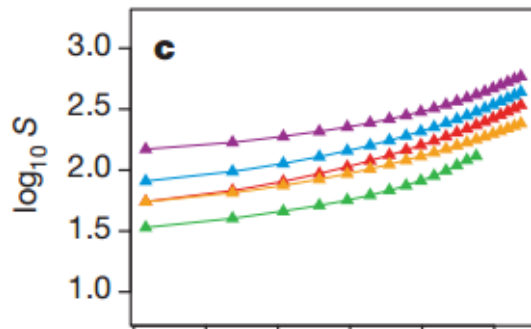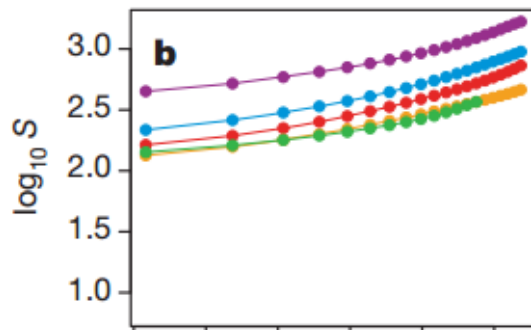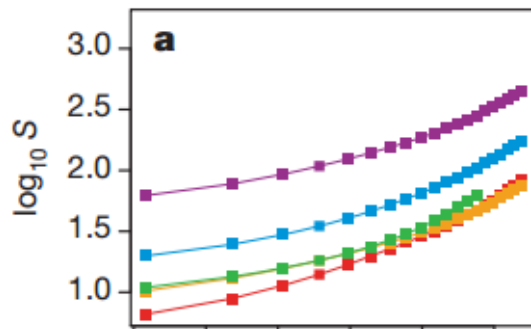HOTSPOTS MAP OF CONSERVATION INTERNATIONAL (NGO)
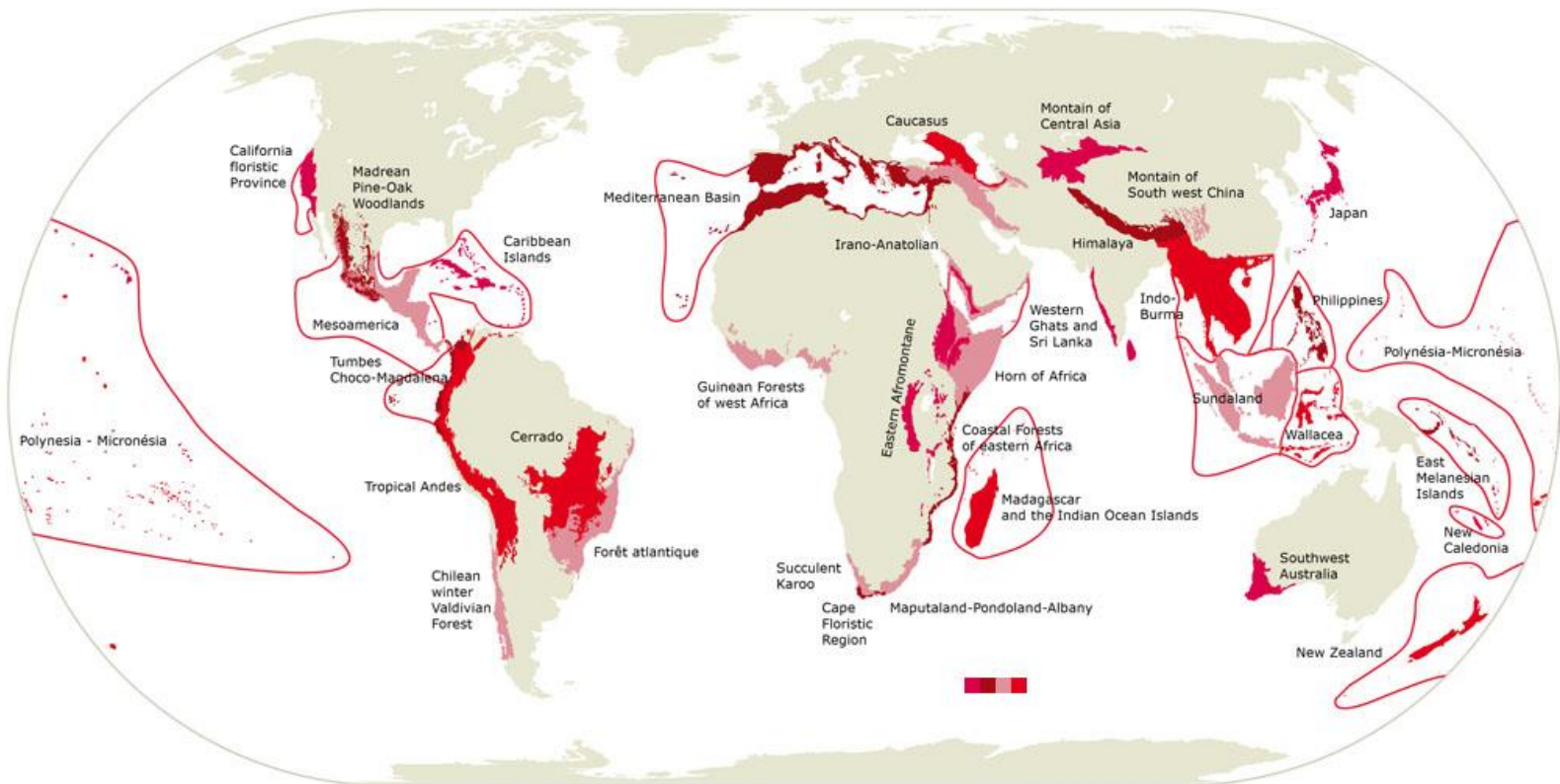
Species delimitation consequences

- Conservation
  - ➢ Japanese whale (*Eubalena japonica*): described in 2000, since 2008 registered as an endangered species

Rosenbaum et al. (2000): *Mol. Ecol.* 9: 1793-1802

Species delimitation consequences


Amphibians, Birds, Mammals
Africa, Eurasia, North America, South America, Australia

- Macroecology

  ➢ Species as fundamental units for testing general ecological hypotheses
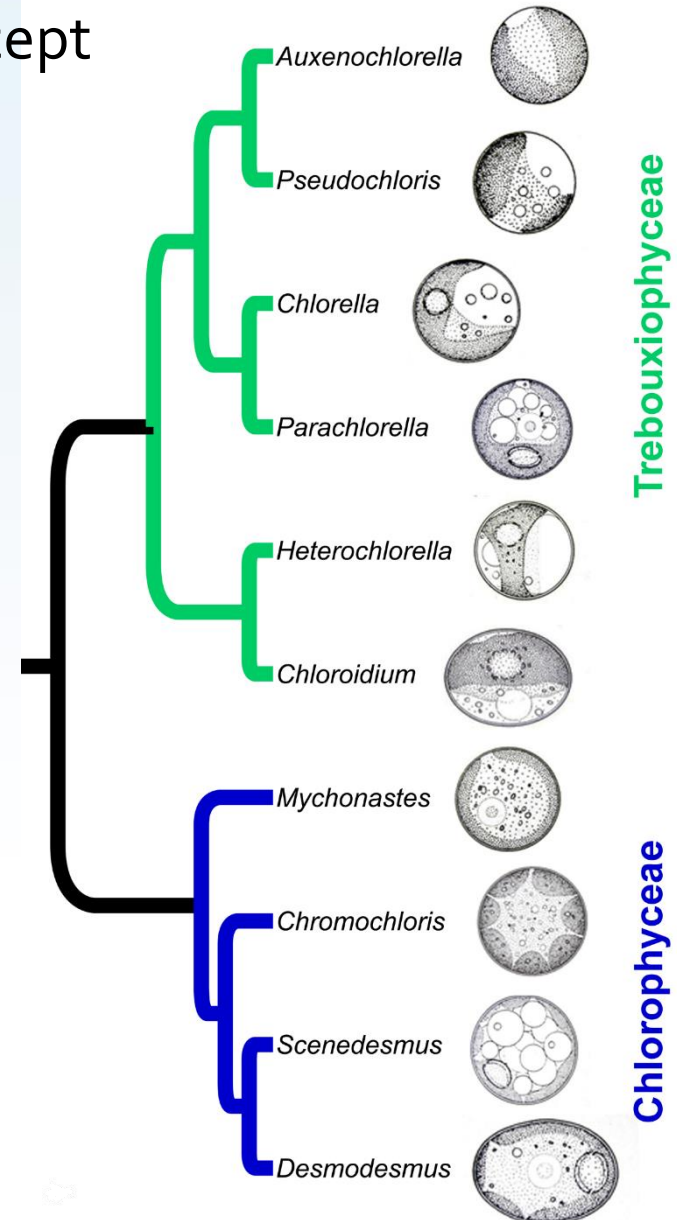
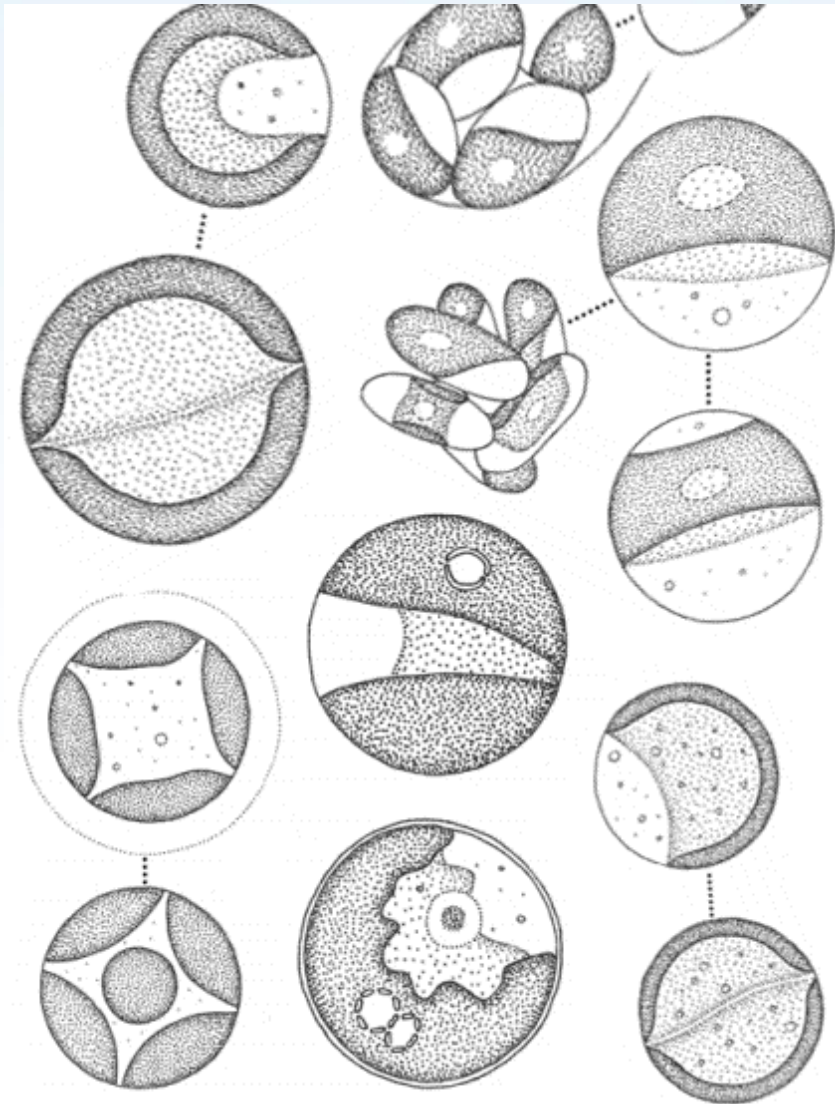Storch et al. (2012): *Nature* 488: 78-81

Species delimitation consequences

- Species numbers can be highly biased by species concepts employed

- Macroecology

  ➤ Species as fundamental units for testing general ecological hypotheses
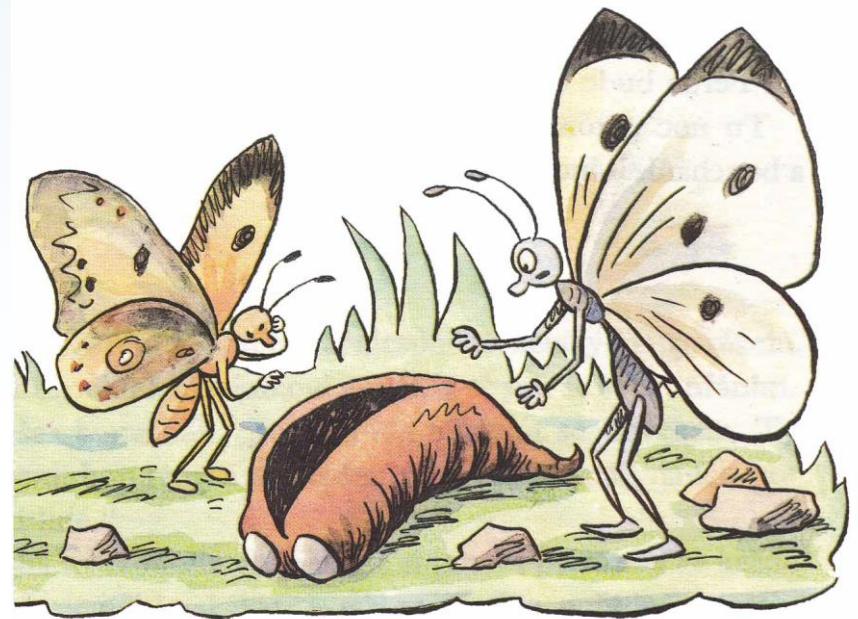
Species delimitation consequences

- *Chlorella* = morphological species concept

Species delimitation consequences

- *Chlorella* = broadly used in industry
  - sales of more than US$ 38 billion annually worldwide
  - strain selection based on lipid content and fatty acid profiles

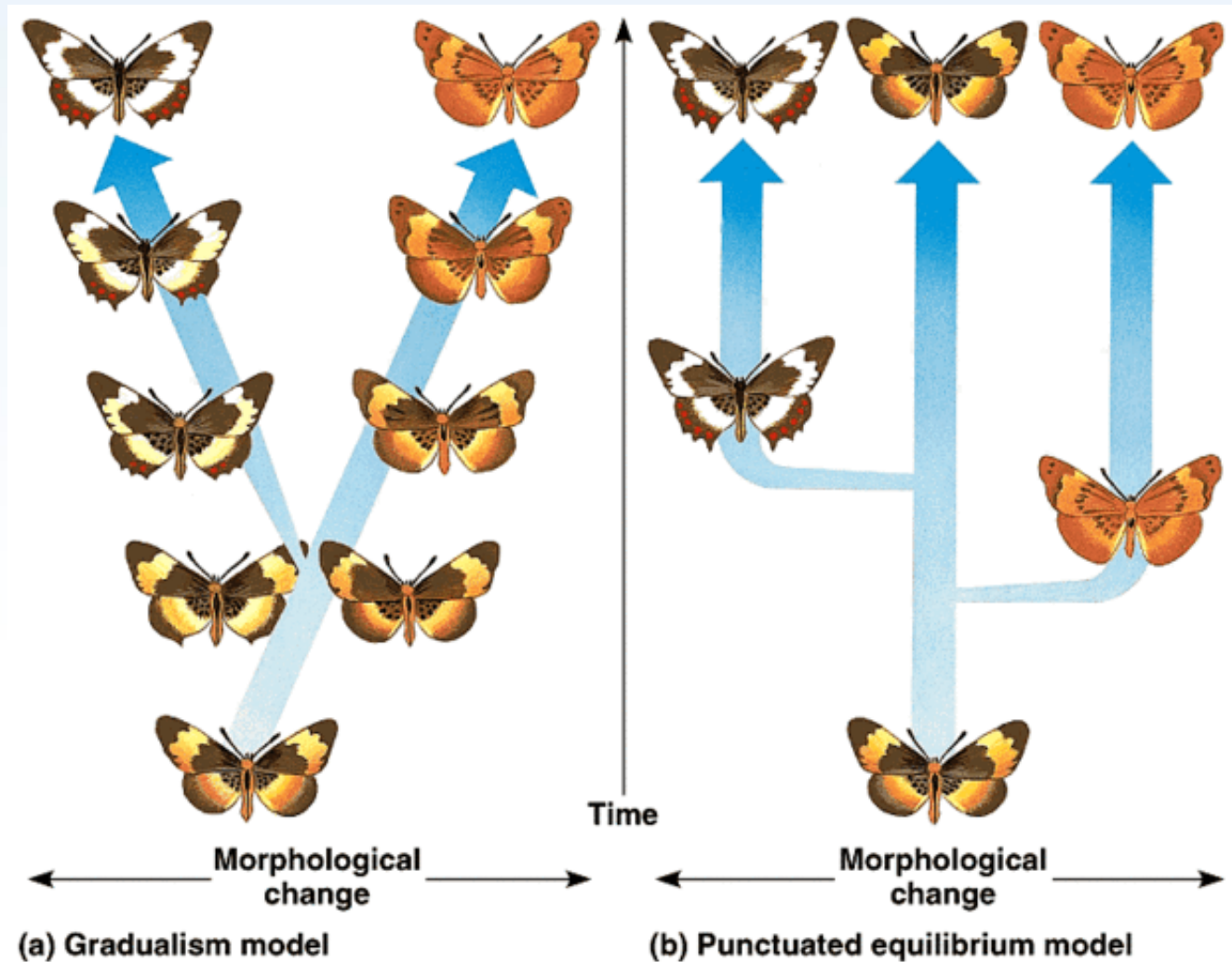|		EPA (omega-3)	18:1(9Z)	18:2(9Z,12Z)	18:3 (9Z,12Z,15Z)	9-Octadecanamid
Chlorella sp.	SAG 1.80	0	6	18	16	14
Chlorella sp.	SAG 3.83	0	10	14	15	14
Chlorella sp.	SAG 9.95	0	2	**20**	9	**19**
Chlorella sp.	SAG 15.93	0	2	**20**	17	**20**
Chlorella sp.	SAG 211-18	0	1	16	17	**21**
Chlorella sp.	SAG 211-6	0	1	11	**20**	**23**
Chlorella sp.	SAG 211-80	0	**19**	**41**	7	9
Chlorella sp.	SAG 241-80	0	1	11	**22**	17
Chlorella sp.	SAG 242.80	**24**	7	10	3	9
Chlorella sp.	SAG 69.94	9	**19**	12	**28**	0

Lang et al. (2011): *NBMC Plant Biol.* 11: 124

Species delimitation consequences

- *Chlorella* = registration as food products
  - ➤ list of permitted organisms (*Chlorella vulgaris, Ch. pyrenoidosa*)
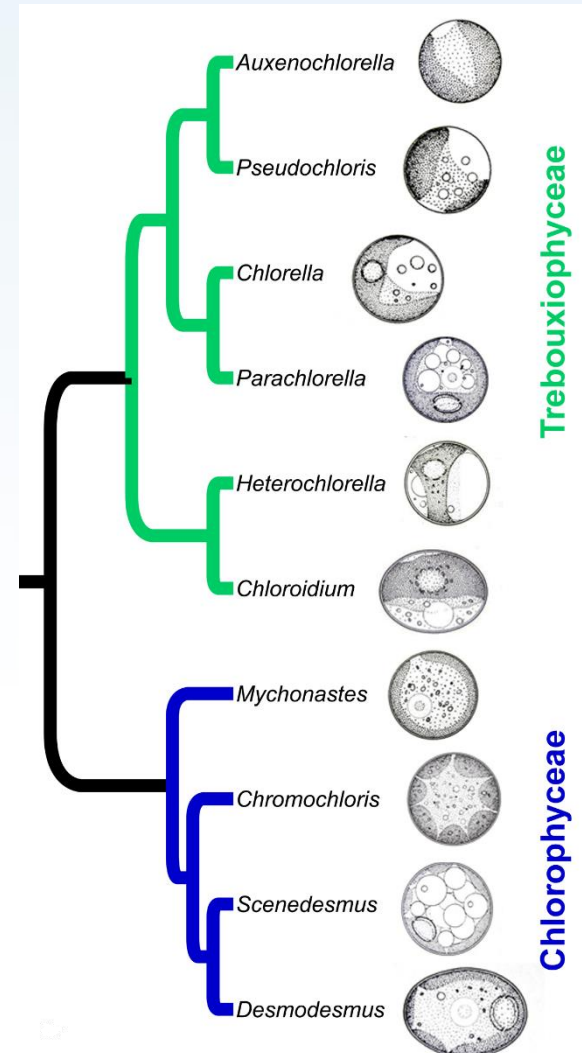
Species delimitation

- Difficulty of simple species definitions
 - Species are not rigid, but evolving entities

Time

Morphological change

(a) Gradualism model

Morphological change

(b) Punctuated equilibrium model

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Species concepts in protists

Biological species concept

Evolutionary significant unit

Cohesion species concept

Phylogenetic species concept

Polythetic species concept

Cladistic species concept

Recognition species concept

Genealogical concordance concept

Internodal species concept

Reproductive competition concept

Hennigian Species concept

Composite species concepts

Genetic species concept

Evolutionary species concept

Palaeospecies concept

Ecological species concept

Morphological species concept

Successional species concept

Non-dimensional species concept

Taxonomic species concept

Linnean species concept

Phenetic species concept

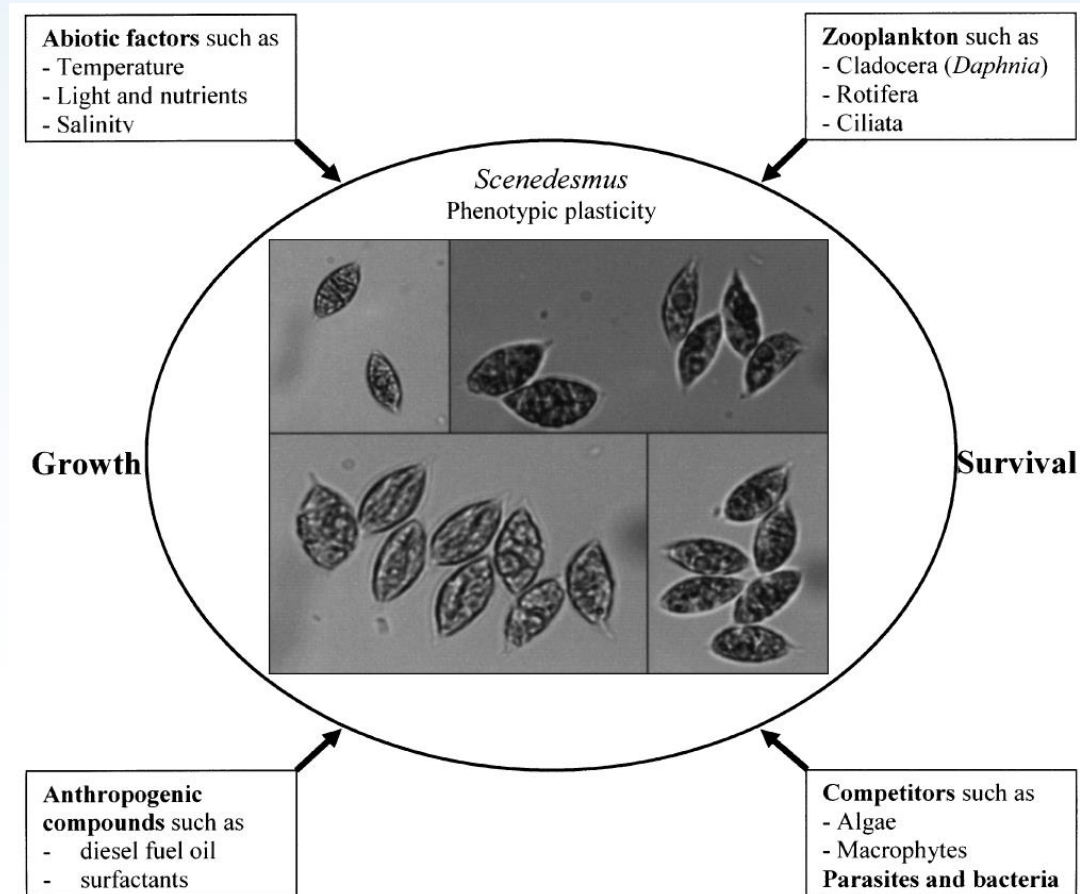Genotypic cluster definition

Agamospecies concept

Mayden (1997): In: *Species: the Units of Biodiversity*

Morphological species concept

- A tradition!
  - Still employed in some groups of algae
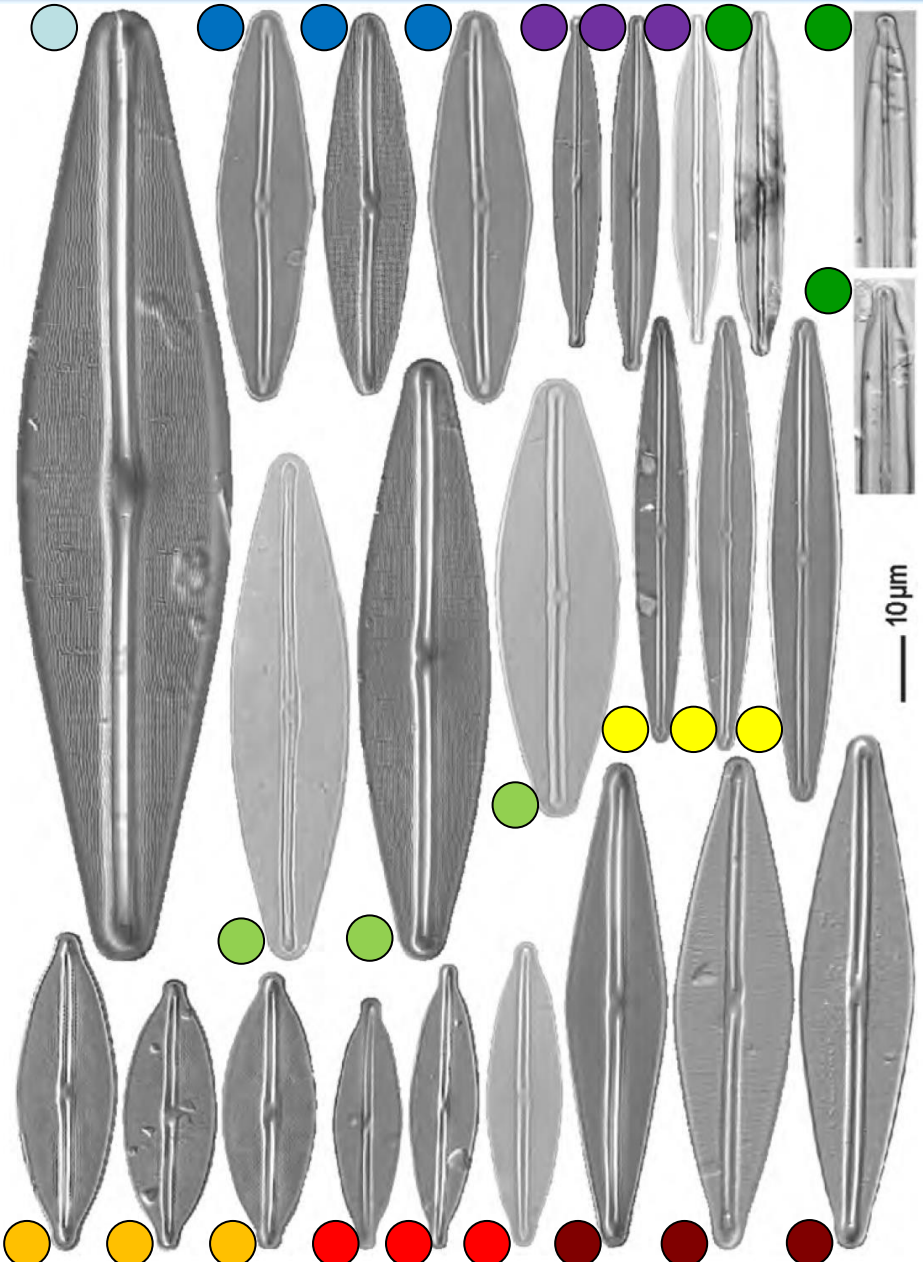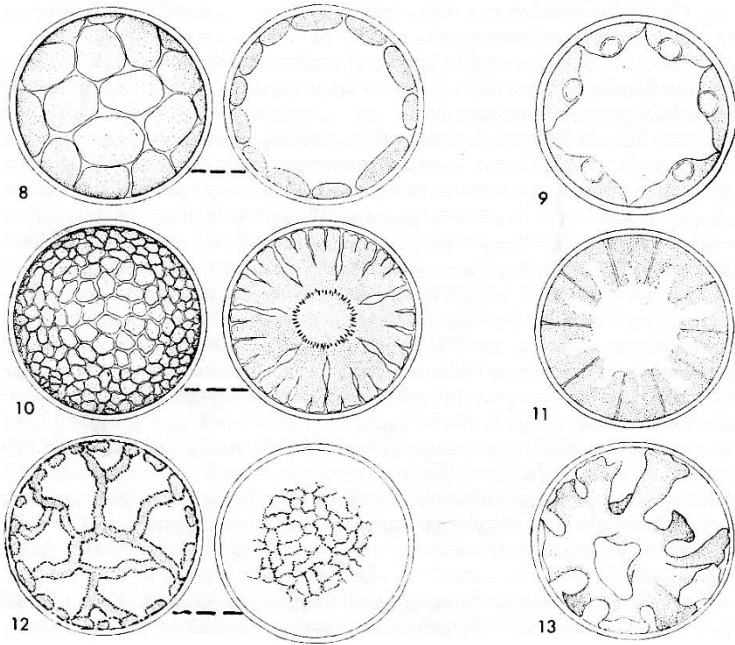
Morphological species concept

- Species delimited based on investigation of natural samples
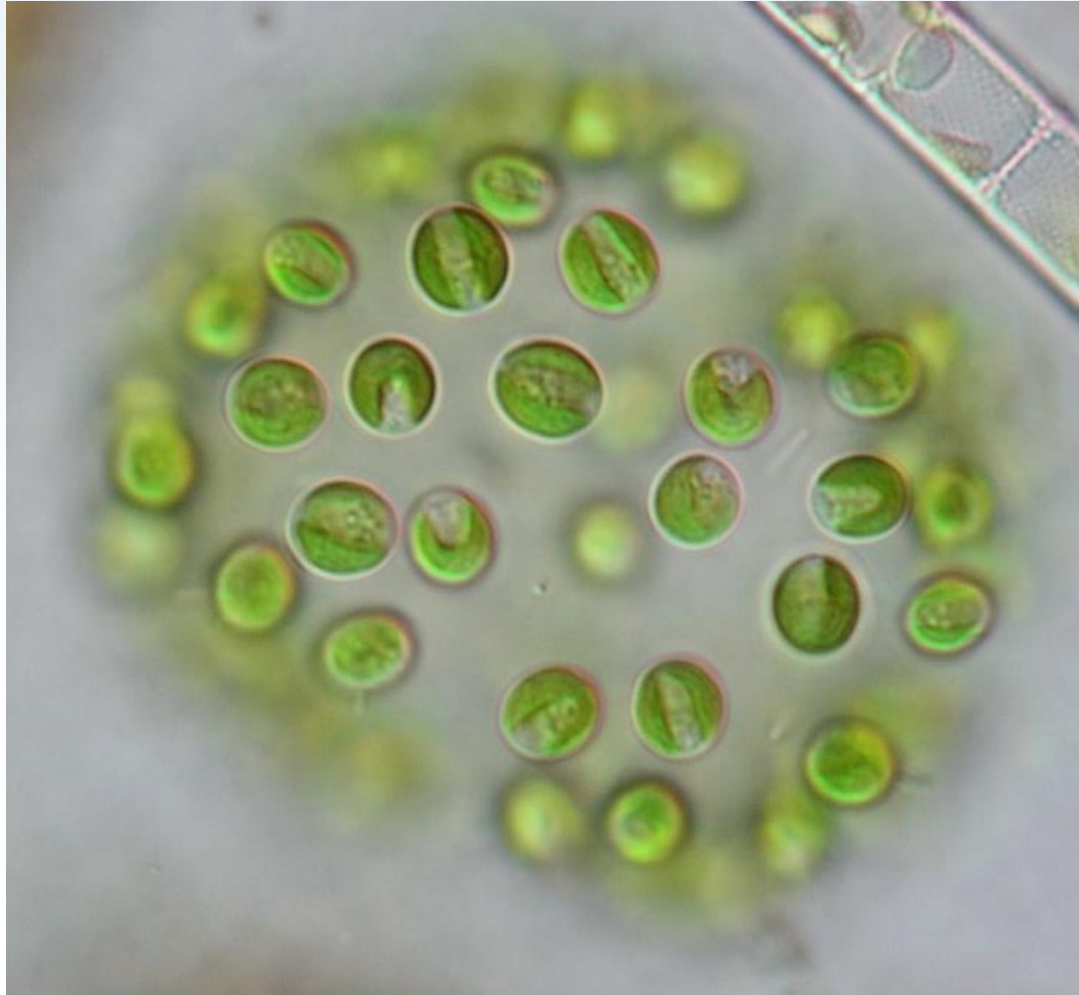 - No data on phenotypic plasticity (*Scenedesmus*)

Morphological species concept

- Species delimited based on investigation of cultured strains
  - Absence of traits manifested only in nature (*Microactinium*)
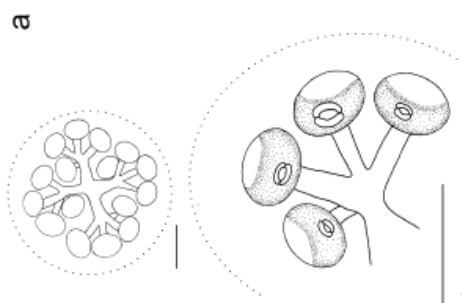
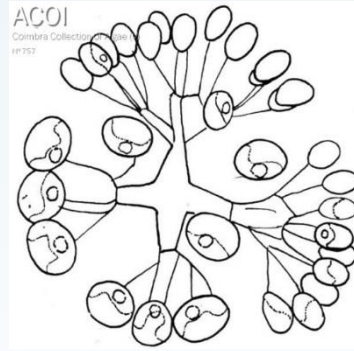# Morphological species concept

- Convergent morphological evolution
  - *Dictyosphaerium*

Morphological species concept

- Convergent morphological evolution
  - *Dictyosphaerium* (9 cryptic genera)
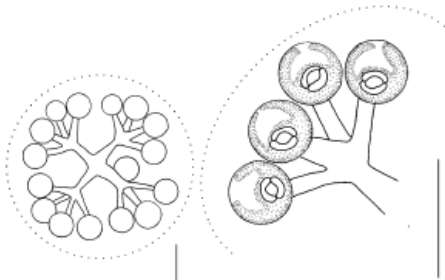

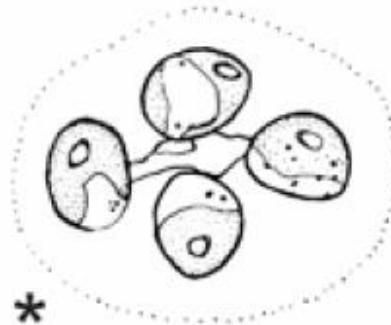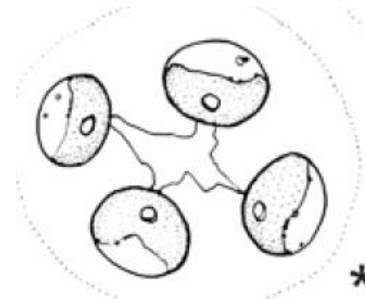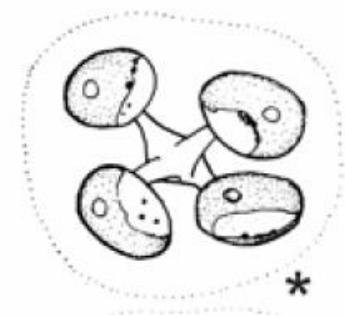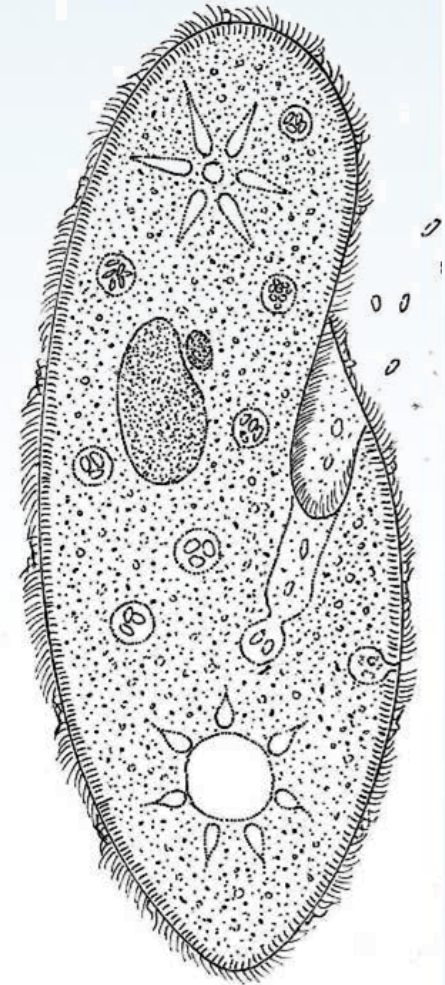*Dictyosphaerium* *Hindakia* *Mychonastes* *Chlorella* *Heynigia*
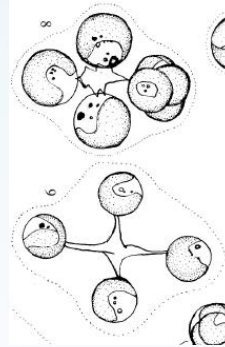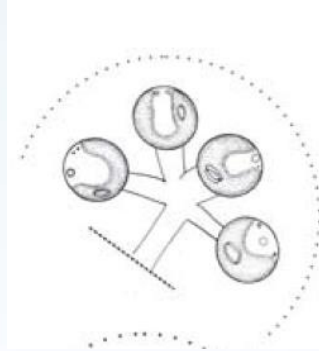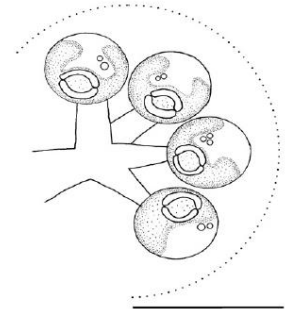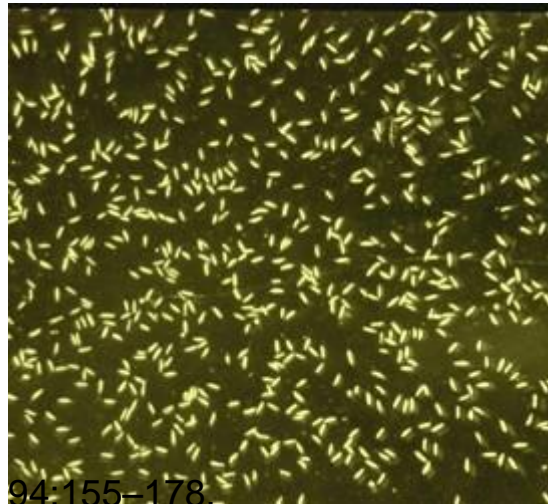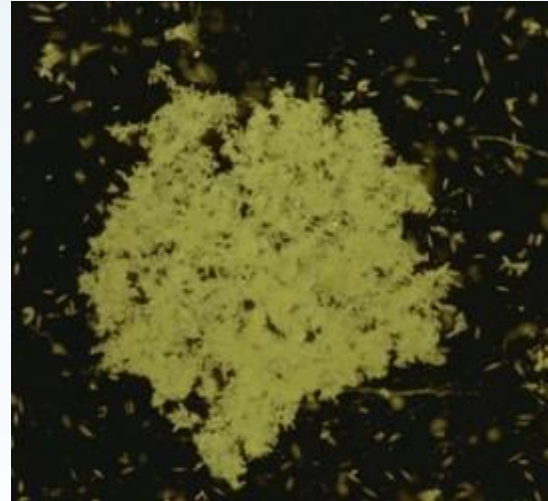
Mucidosphaerium *Compactochlorella* *Kalenjinia* *Masaia*
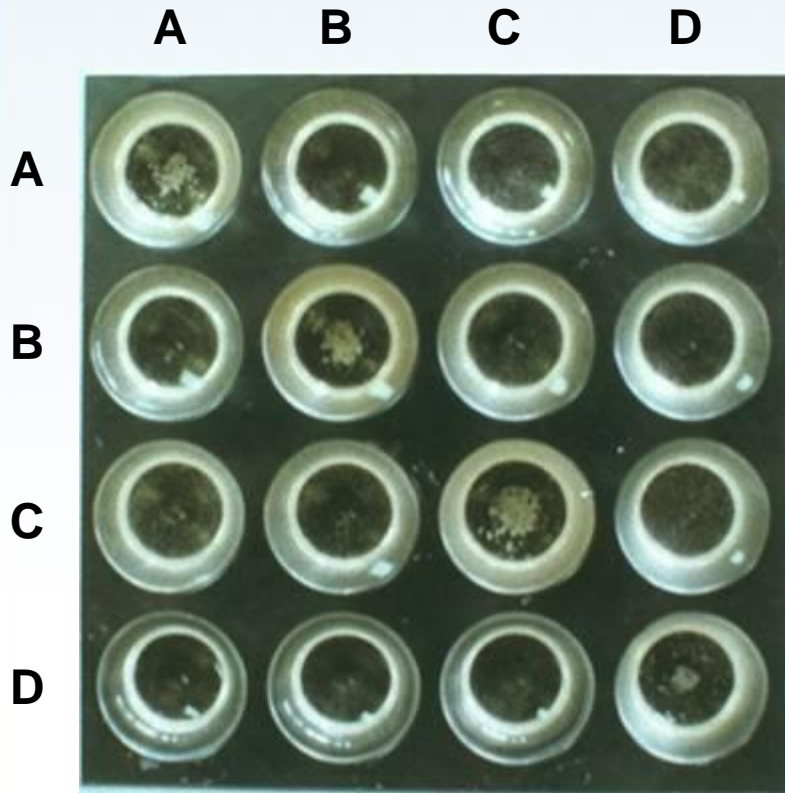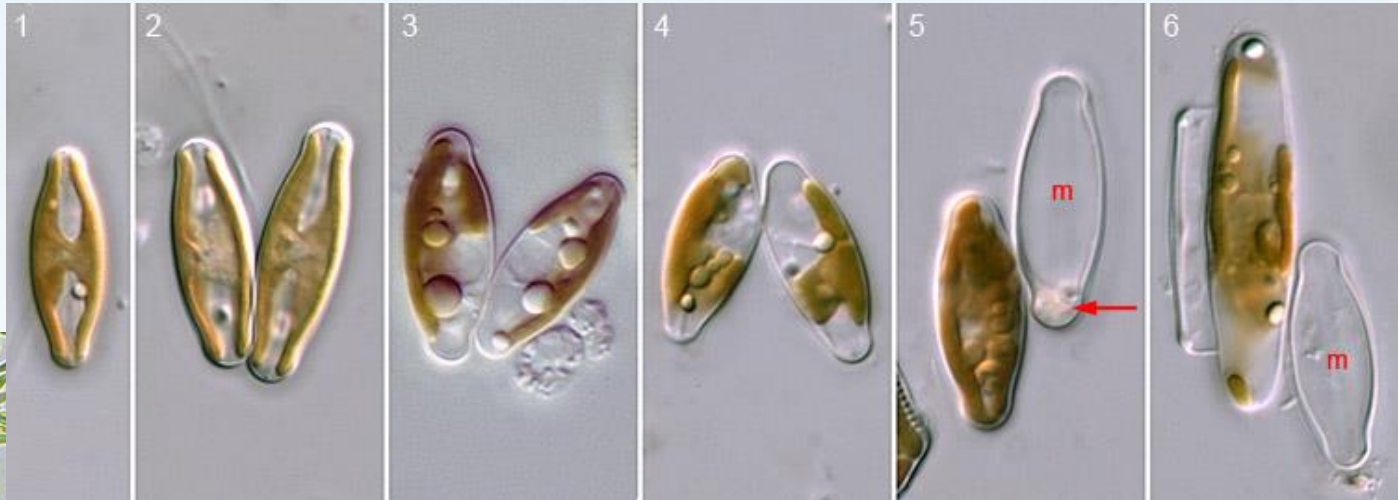
Biological species concept

- Applicable only on sexually reproducing organisms
  - ➢ cilliates (syngens in *Paramecium aurelia*)

Sonneborn (1975): *Trans. Amer. Microsc. Soc.*, 94:155–178.

Biological species concept
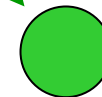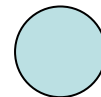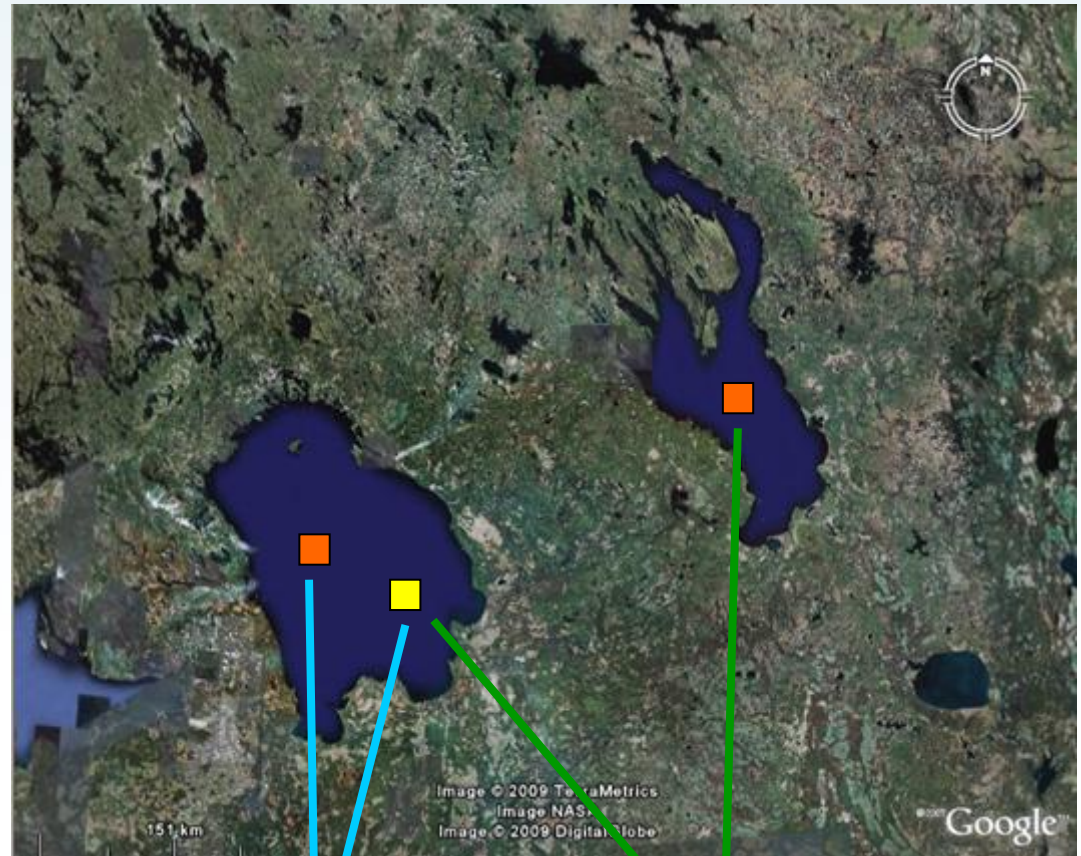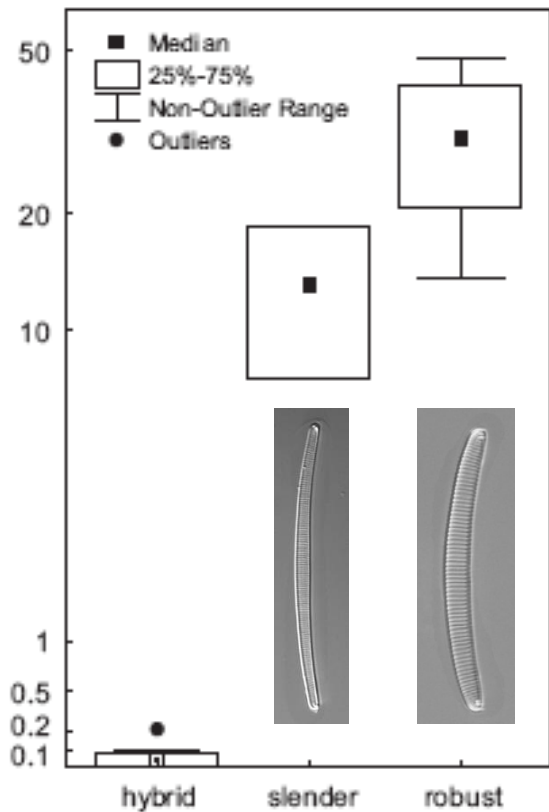
- Applicable only on sexually reproducing organisms
  - diatoms

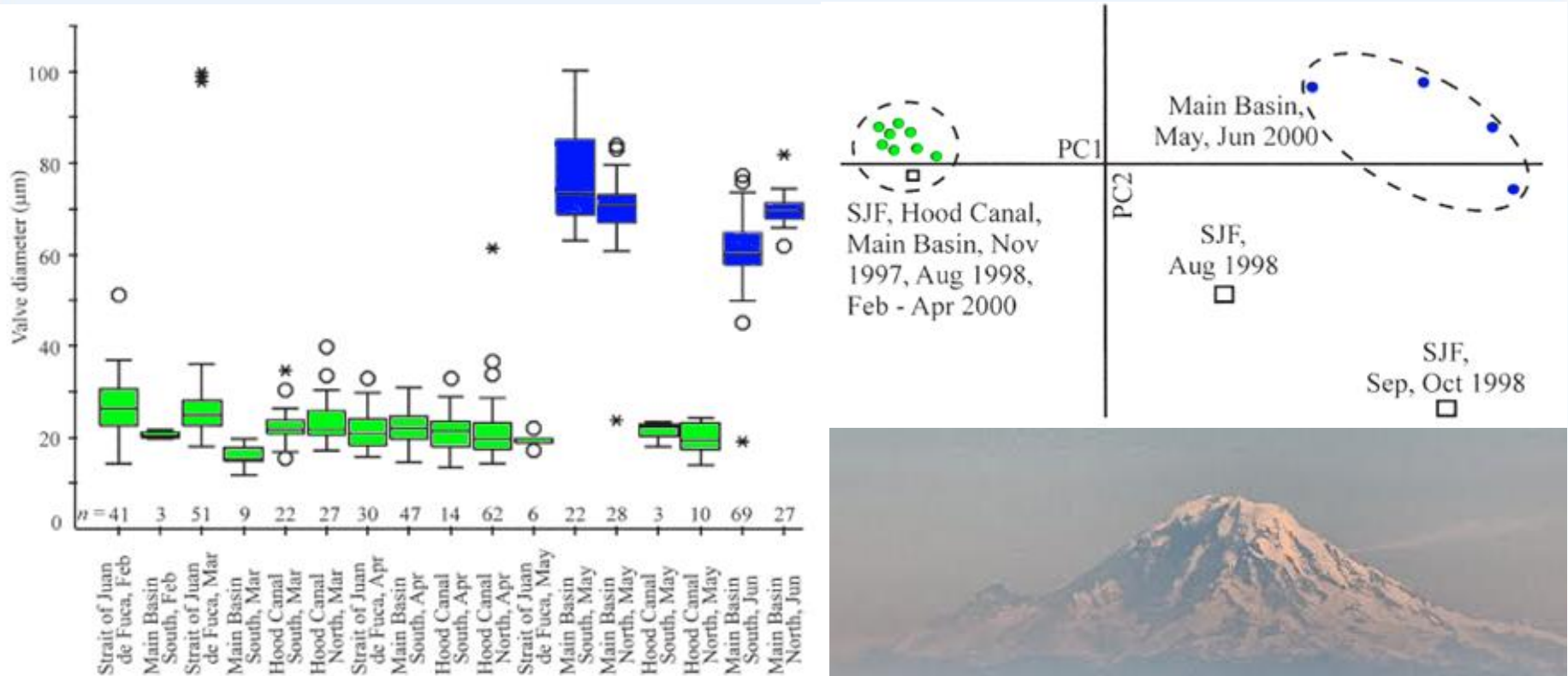# Biological species concept

- Often incomplete reproduction barriers
  - ➤ A problem of allopatric populations (*Eunotia*)
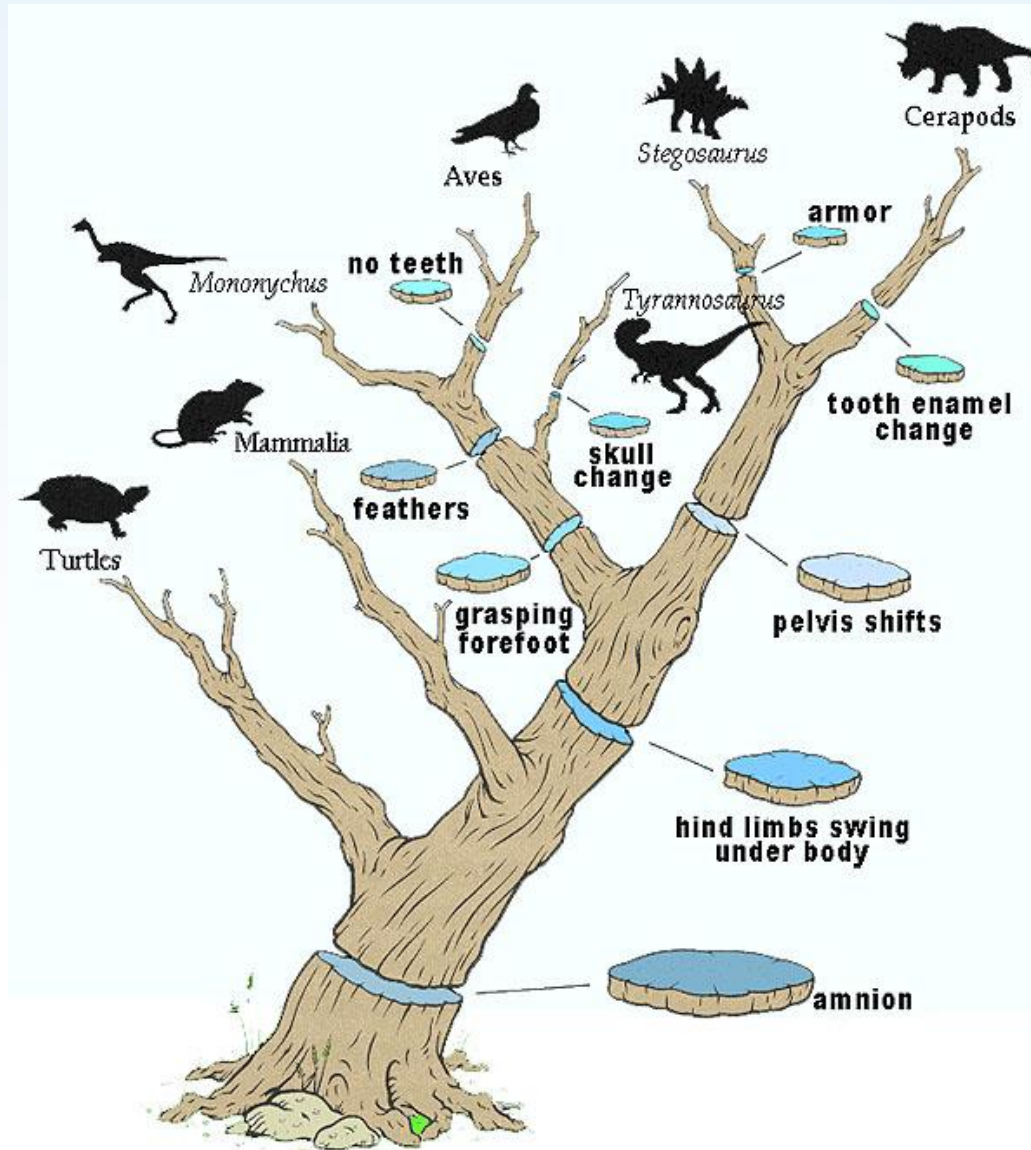

Vanormelingen et al. (2008): *Protist* 159: 73-90

Biological species concept

- Laboratory crossing experiments test the incompatibility
  - ➤ a problem of temporaral isolation (*Ditylum*)

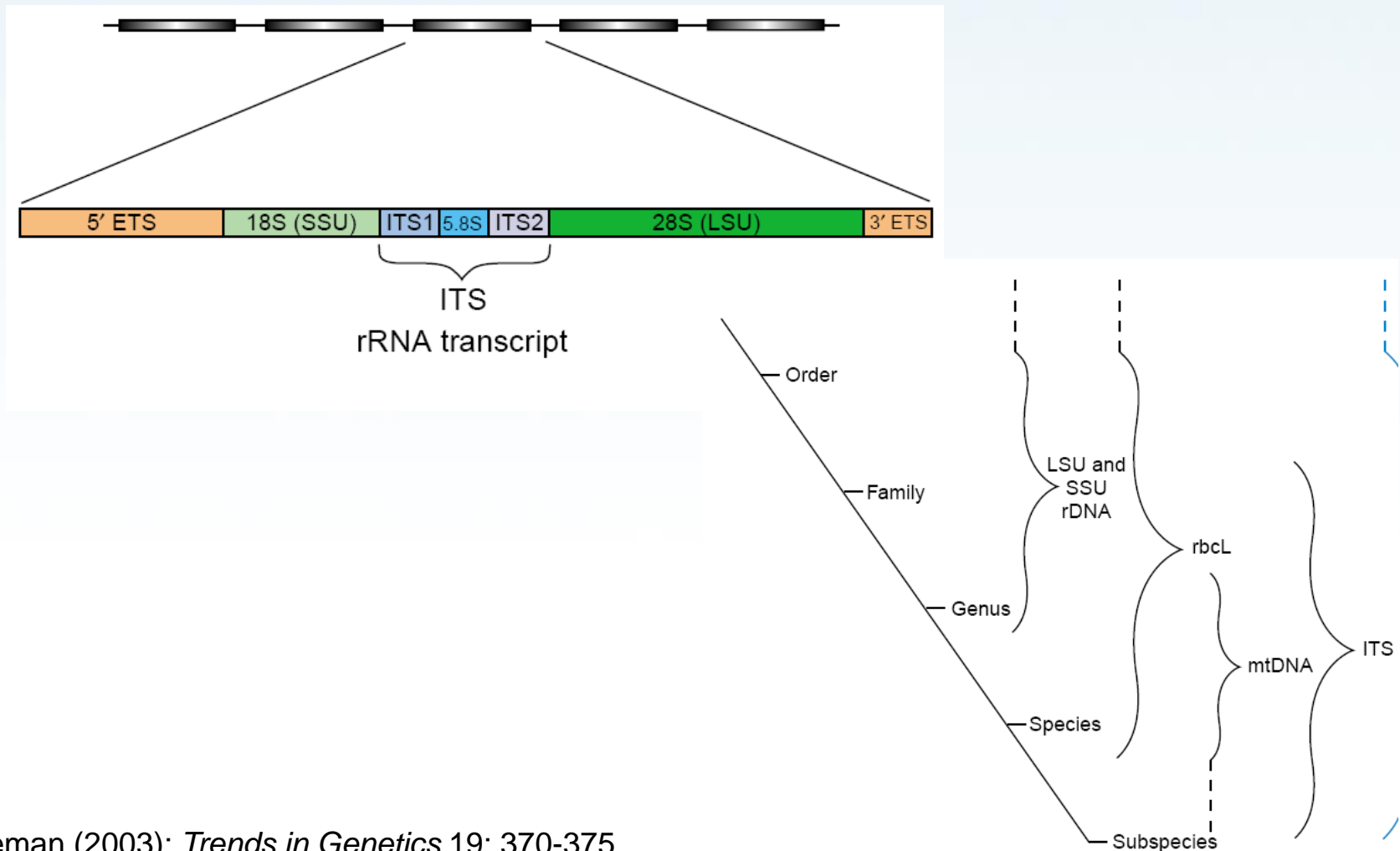Rynearson et al. (2006): *Limnol. Oceanogr.* 51: 1249-61

Phylogenetic species concept

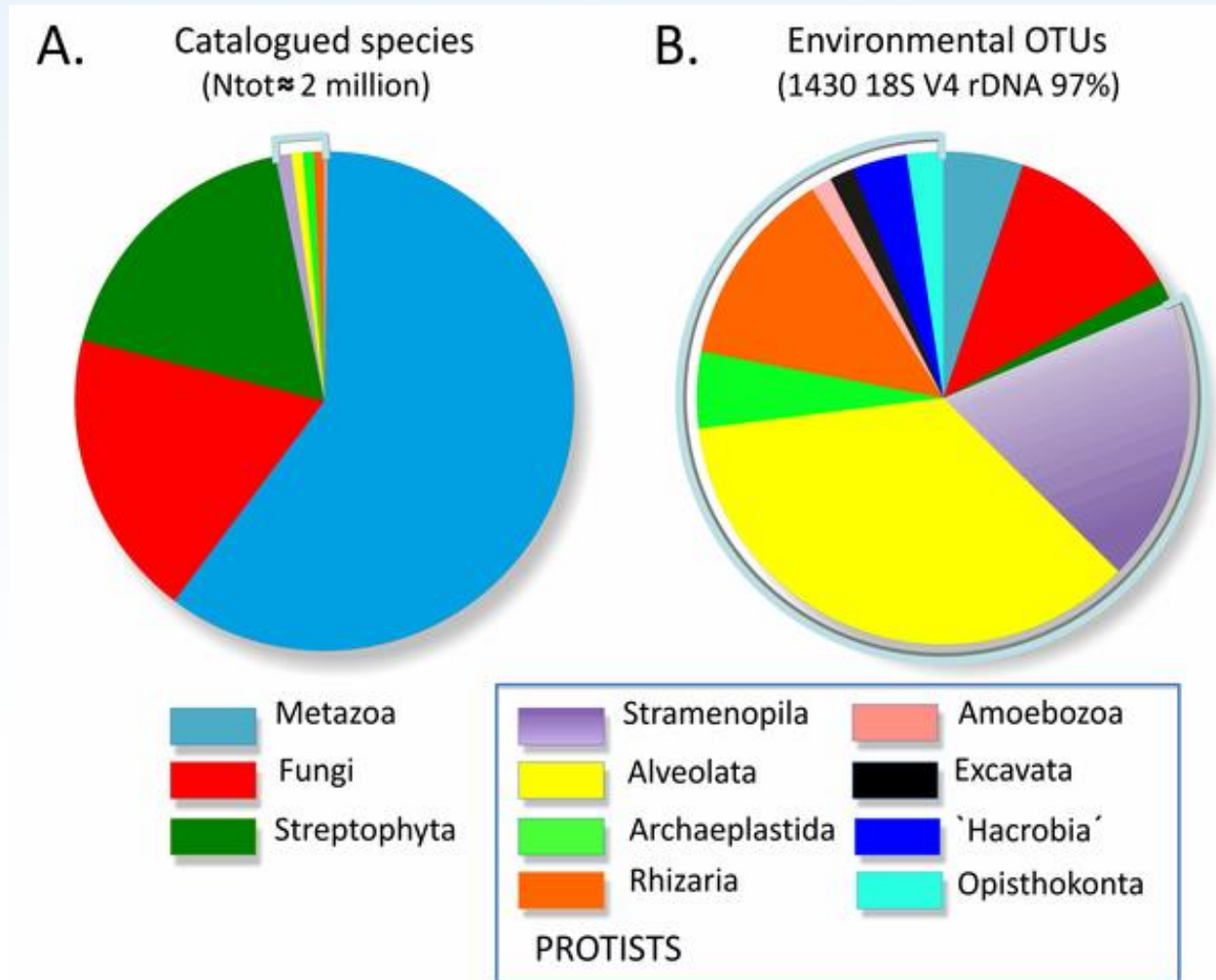- Based on tree topology (monophyly, branch lengths, supports)

Phylogenetic species concept

- Ribosomal operon as the frequently used marker

Coleman (2003): *Trends in Genetics* 19: 370-375

Phylogenetic species concept

- Cryptic diversity of protists

A. Catalogued species (Ntot ≈ 2 million)

B. Environmental OTUs (1430 18S V4 rDNA 97%)

Legend:
- Metazoa
- Fungi
- Streptophyta
- Stramenopila
- Alveolata
- Archaeplastida
- Rhizaria
- Amoebozoa
- Excavata
- `Hacrobia´
- Opisthokonta

PROTISTS

Phylogenetic species concept

- Cryptic diversity of protists
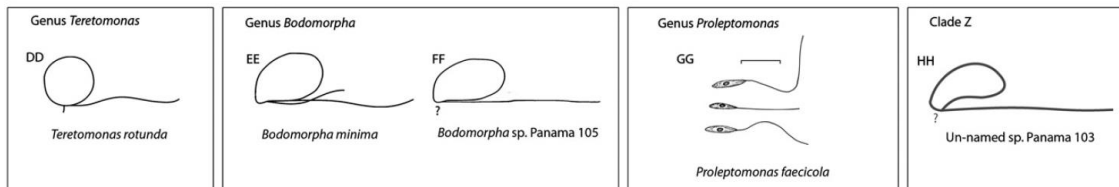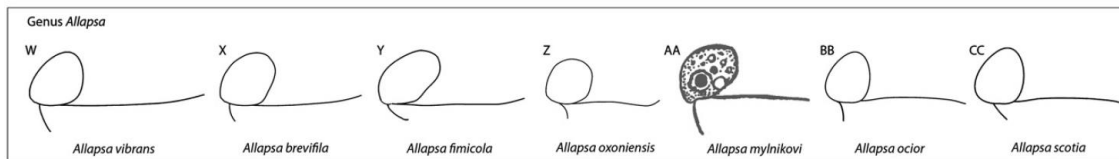 - glissomoads

Howe et al. (2009): *Protist* 160: 159-189
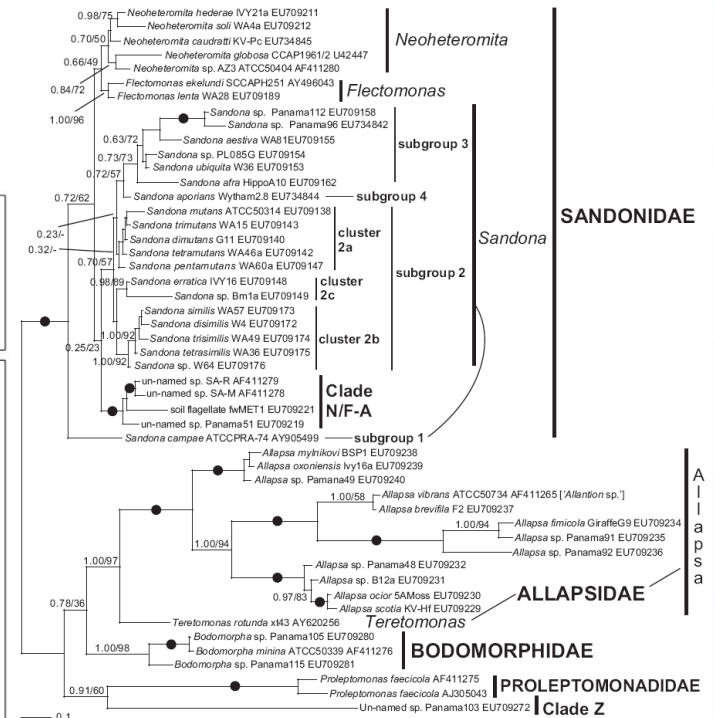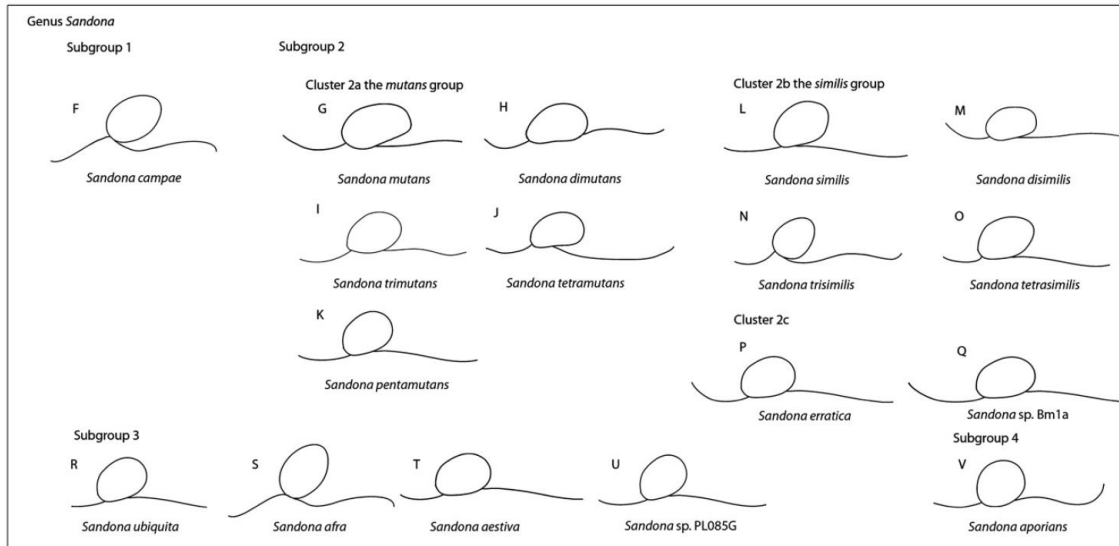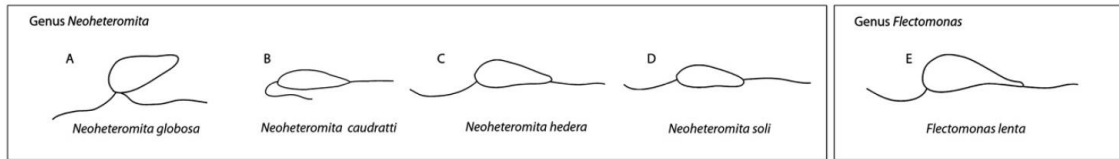
Phylogenetic species concept

- Cryptic diversity of protists
  - ➢ mere accumulation of neutral mutations?
  - ➢ the extent of genetic polymorphism is dependent on mutation rate (μ) and effective population size (N_e)

$$\Rightarrow \quad \theta = 4\,N_e\,\mu$$

Marine plankton – 10^{25} cells

Fenchel & Finlay (2006): *Phil. Trans. R. Soc. B* 361: 1965-1973

Phylogenetic species concept

- Cryptic diversity of protists (*Pentapharsodinium*)
  - ➢ population size: $\approx 2.9 \times 10^{14}$
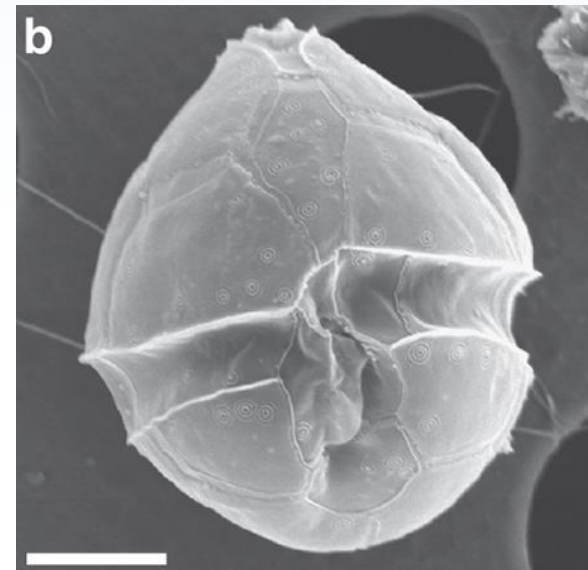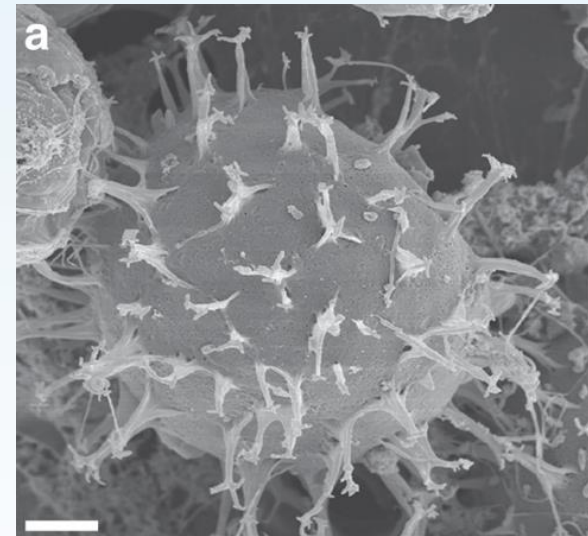 - ➢ $N_e \approx 178\text{-}1183$

Table 1. Estimates of contemporary effective population size (N_e) for samples of the marine dinoflagellate *P. dalei* revived from sediment cores (dated to 1922, 1960, 1985 and 2006) from Koljö Fjord, Sweden. Mean N_e ($\pm 95\%$ confidence intervals) was estimated for pairs of samples using ML [8] and moment estimators [6].

sample comparison	contemporary effective population size (N_e)	
	ML (95% CI)	moment (95% CI)
2006 – 1985	270 (161 – 544)	178 (98 – 343)
2006 – 1960	439 (252 – 924)	375 (195 – 793)
2006 – 1922	815 (401 – 2489)	1183 (476 – 5545)
1985 – 1960	264 (148 – 592)	342 (166 – 905)
1985 – 1922	305 (187 – 538)	652 (325 – 1528)
1960 – 1922	179 (109 – 320)	266 (134 – 570)

Watts et al. (2013): *Biol. Lett.* 9: 20130849

Phylogenetic species concept

- Based on tree topology (monophyly, branch lengths, supports)

  - ➢ Where to set species boundaries?
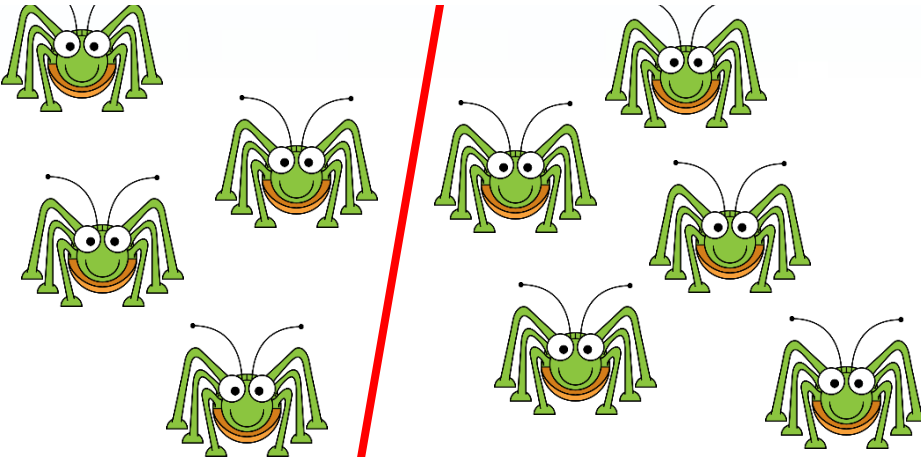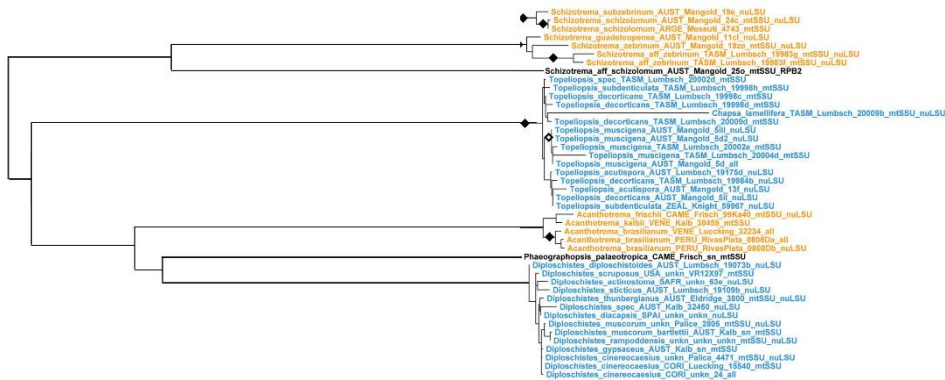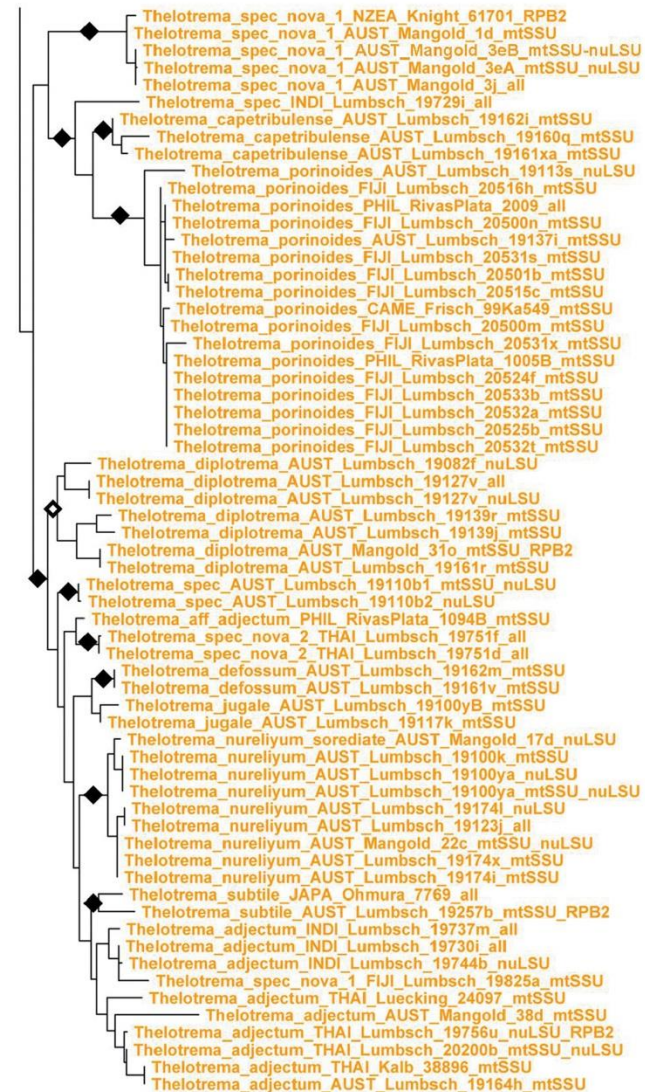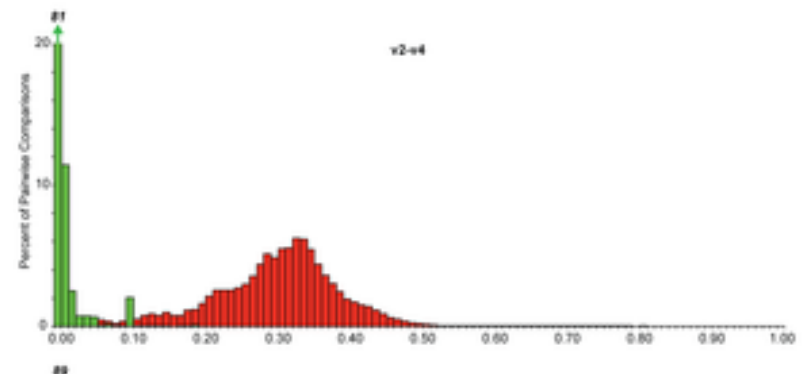
  - ➢ *Objective criteria?*

Fig. 8

DNA barcoding

- „barcoding gap"

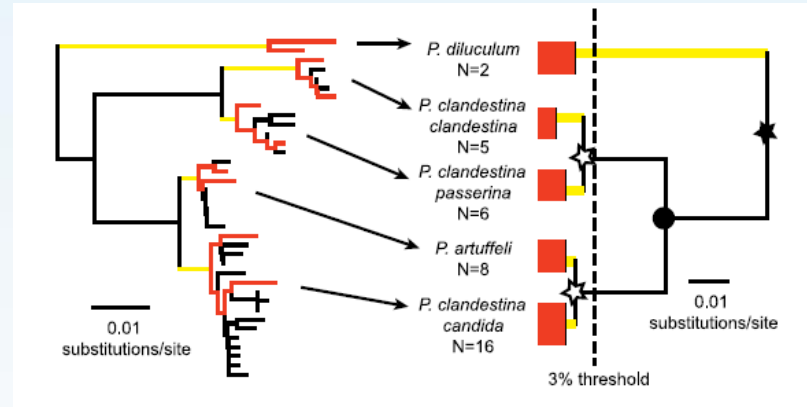Meyer & Paulay (2005): *PLoS Biol.* 3: e422
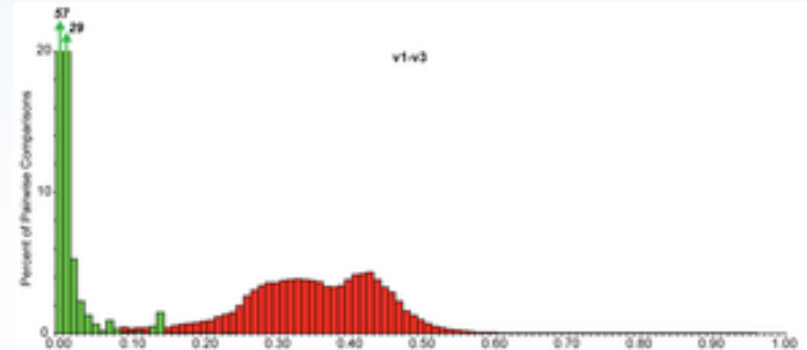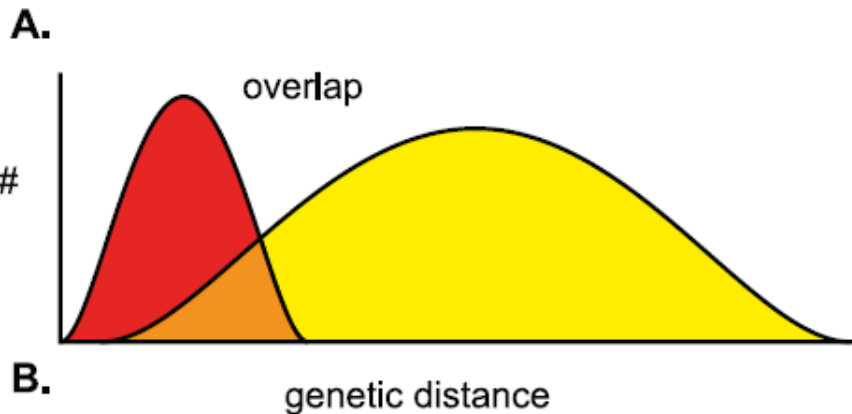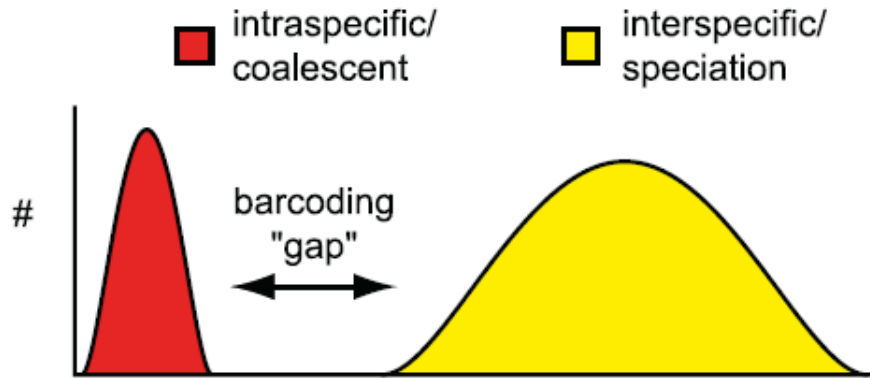
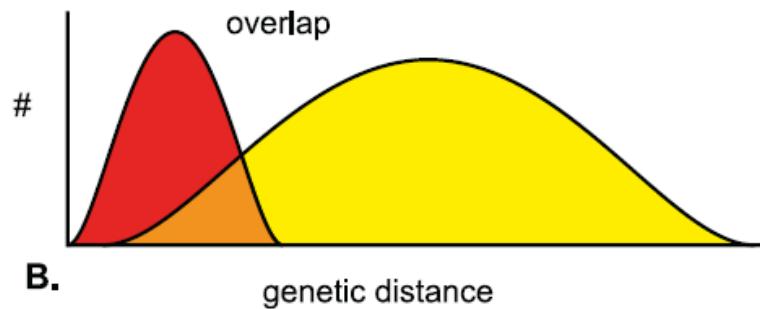# DNA barcoding

- More sampling – less obvious barcoding gap

Meyer & Paulay (2005): *PLoS Biol.* 3: e422
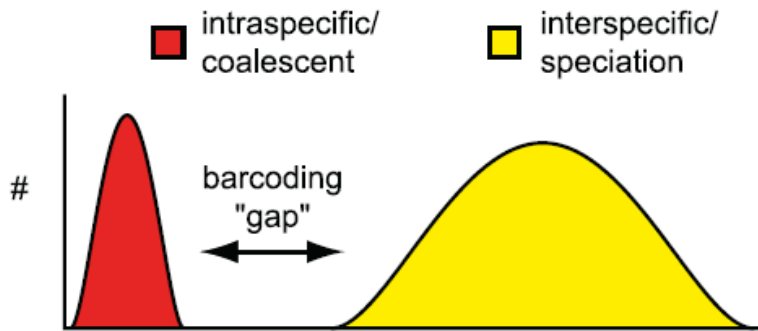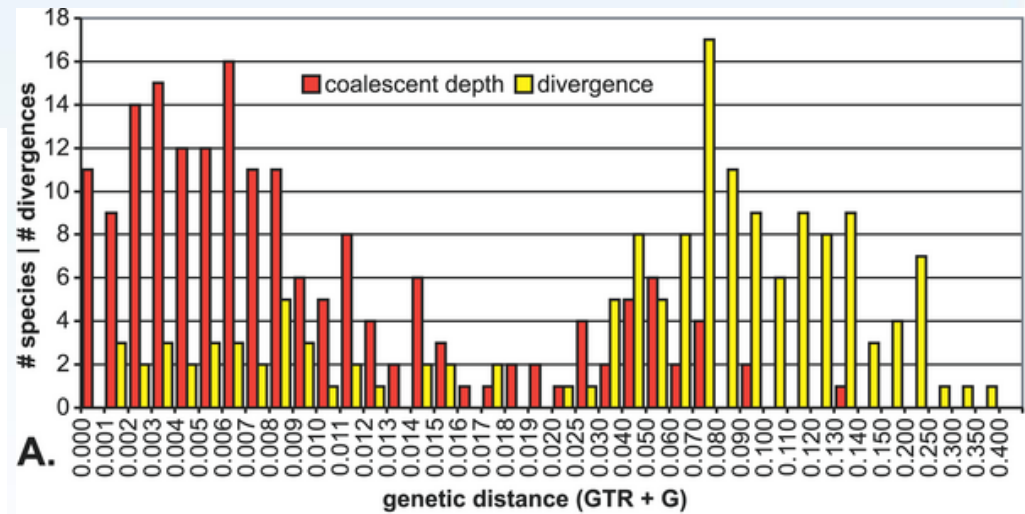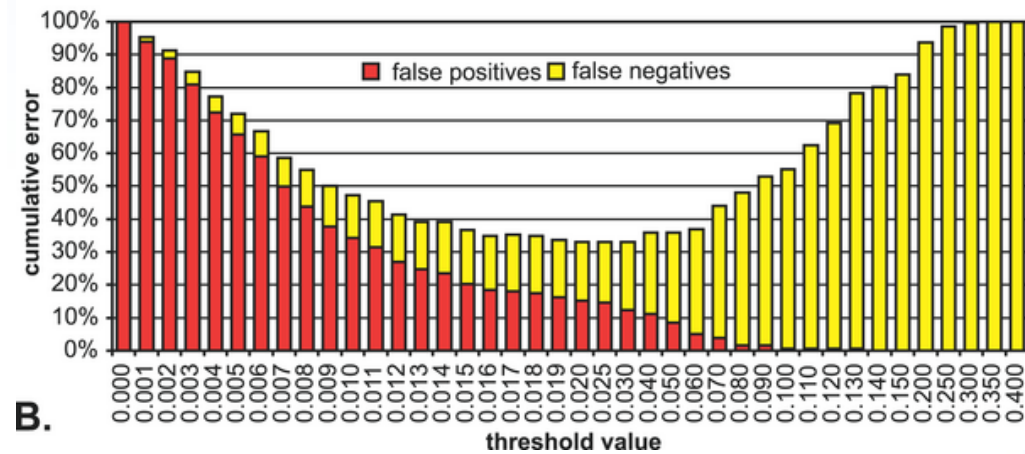
ABGD

- Automatic Barcode Gap Discovery
  - Alignment as an input file
  - Scanning a range of intraspecific divergence to find the barcode gap

Puillandre et al. (2011): *Mol Ecol* 21: 1864
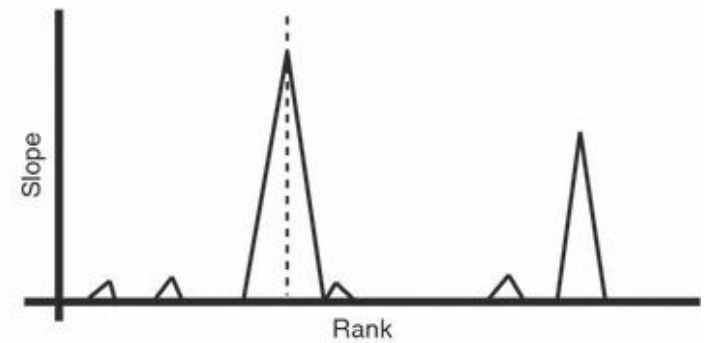
DNA barcoding

- Uneven mutation rates
  - ➤ tropics ~ temperate
  - ➤ islands ~ continents
  - ➤ free-living ~ symbionts

Wright et al. (2009): *Evolution* 63: 2275-2287

Zoller & Lutzoni (2003): *Mol. Phyl. Evol.* 29: 629-640
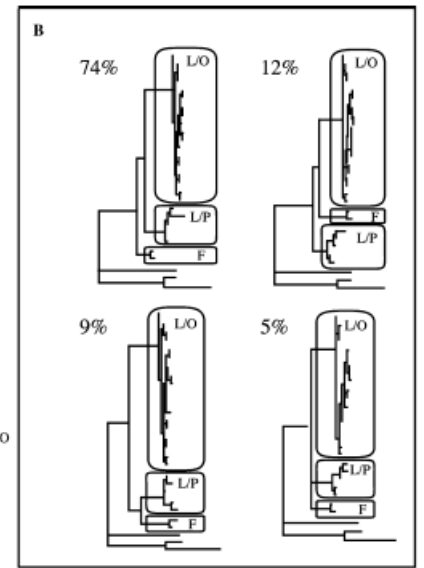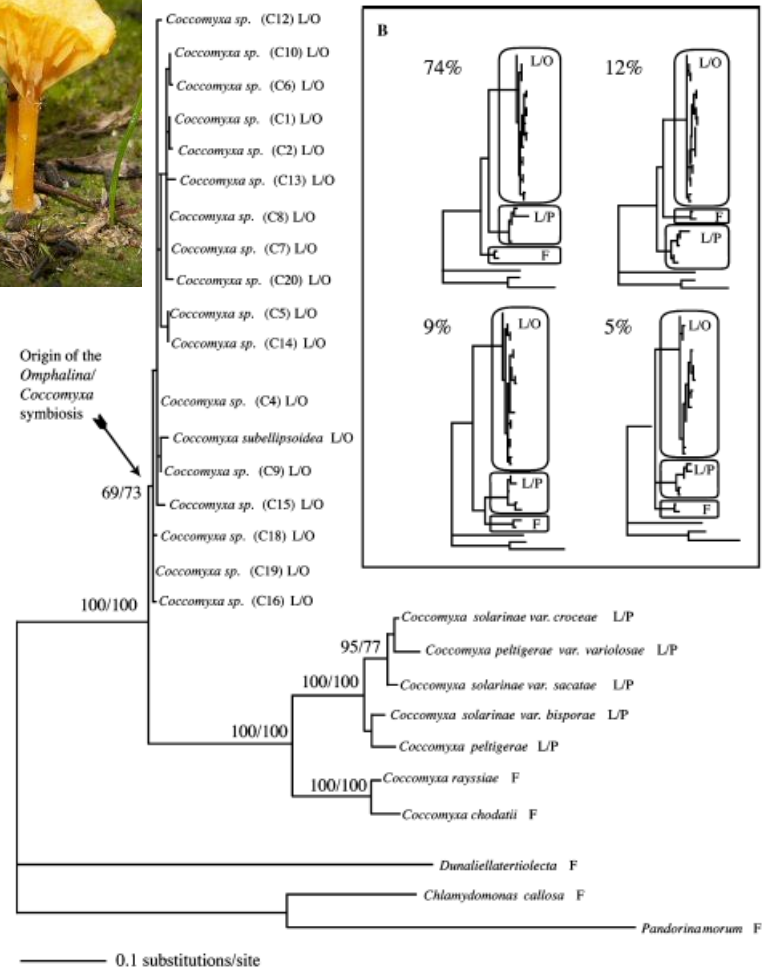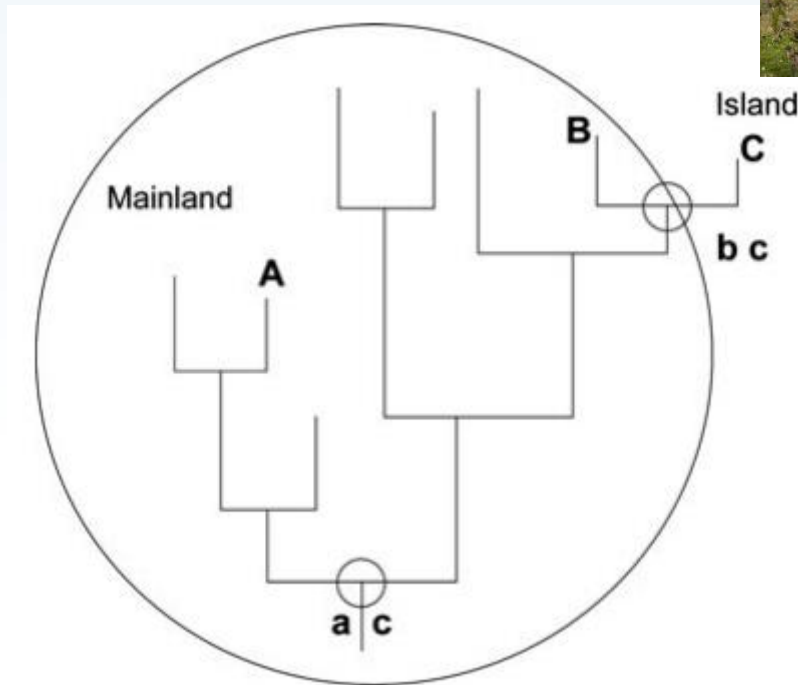
Good & Wake method

- test of *a priori* defined species

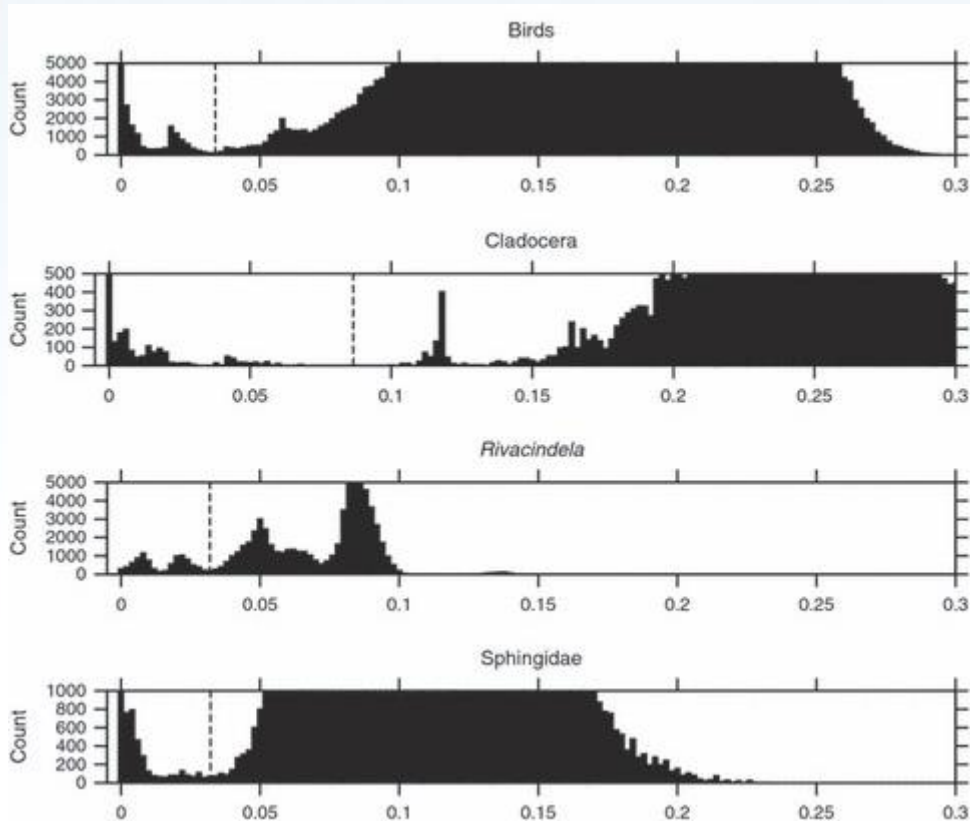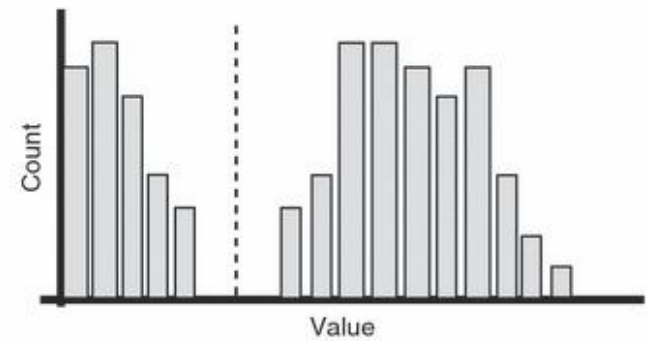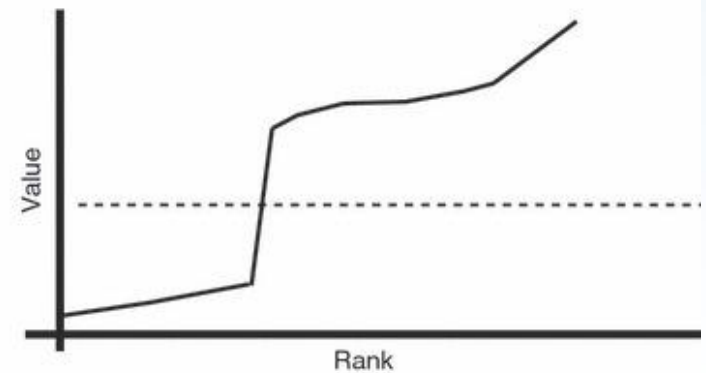- linear regression of genetic and geographic dostances should in single species go through the graph origin (gene flow with isolation-by-distance)

- Different regression indicates the presence of two distinct, genetically isolated species
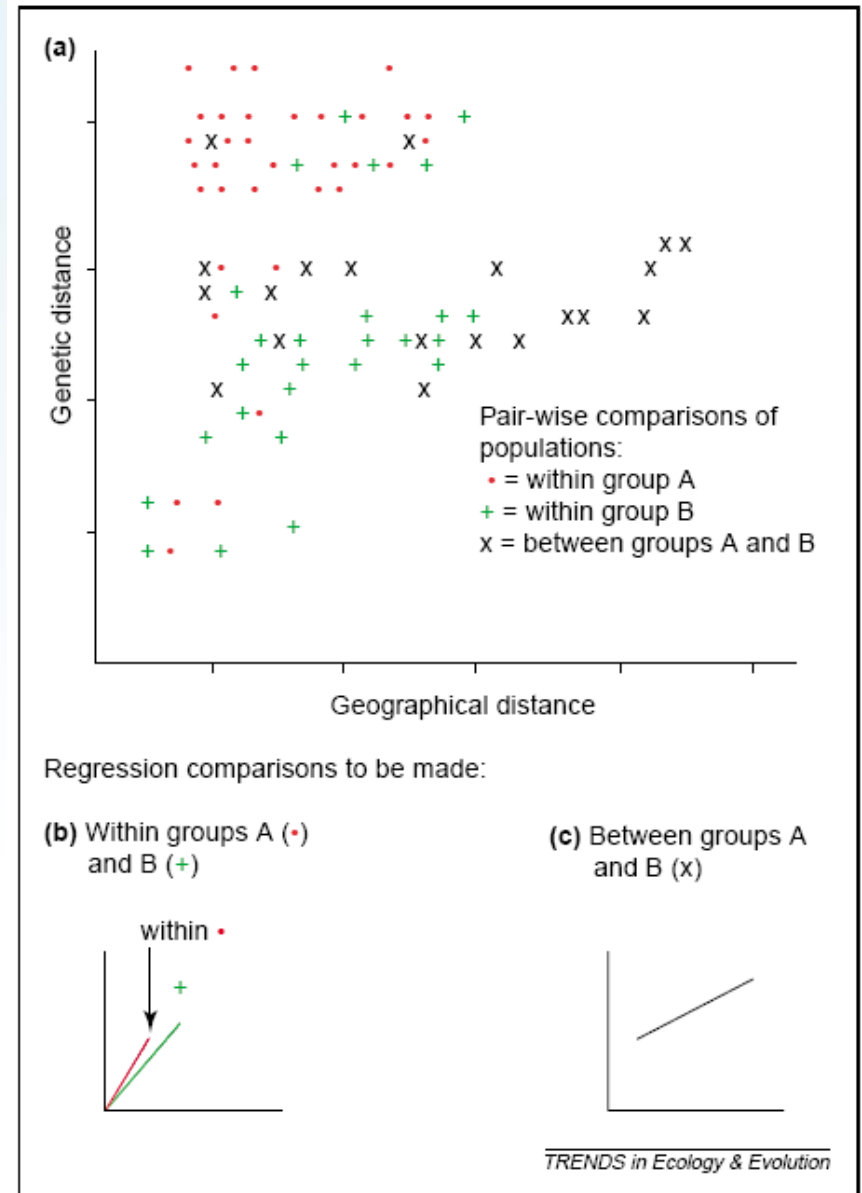
Sites & Marshall (2012): *Trends Ecol. Evol.* 18: 462-470

CBC concept

- Species delimited based on differences in conservative regions of the ITS2 molecule

Coleman (2007): *Nucleic Acids Res.* 2007: 1-8
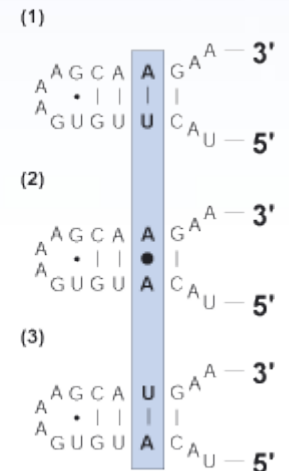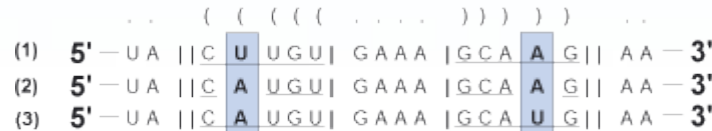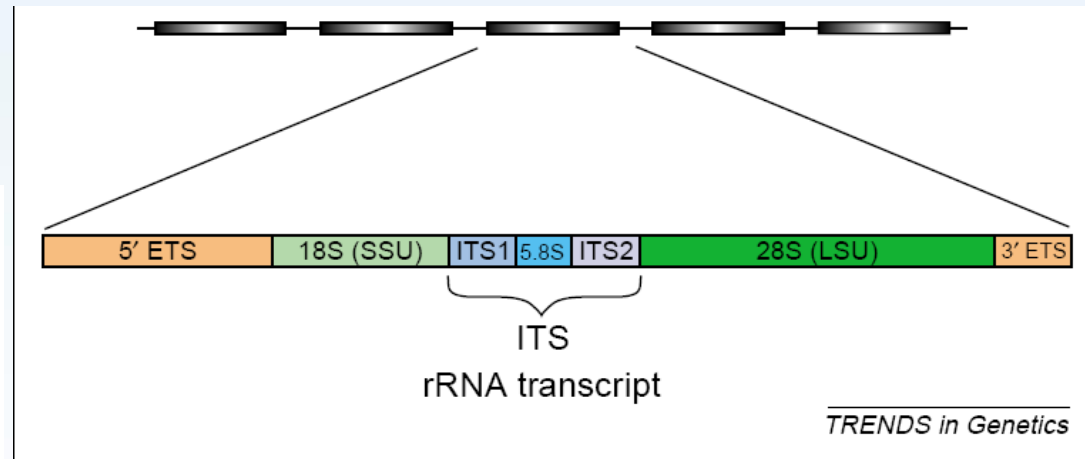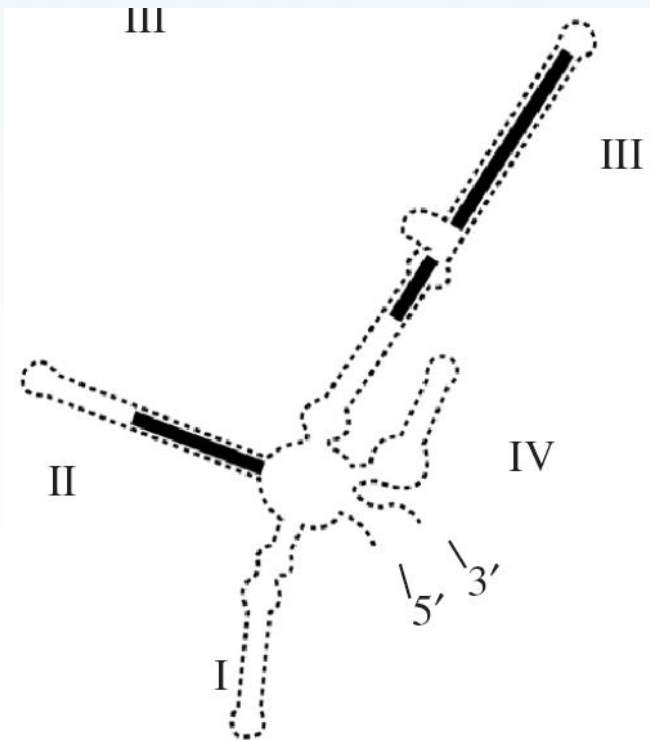
CBC concept

- Species delimited based on differences in conservative regions of the ITS2 molecule

  ➢ correlation between CBC and sexual compatibility (*Gonium*)
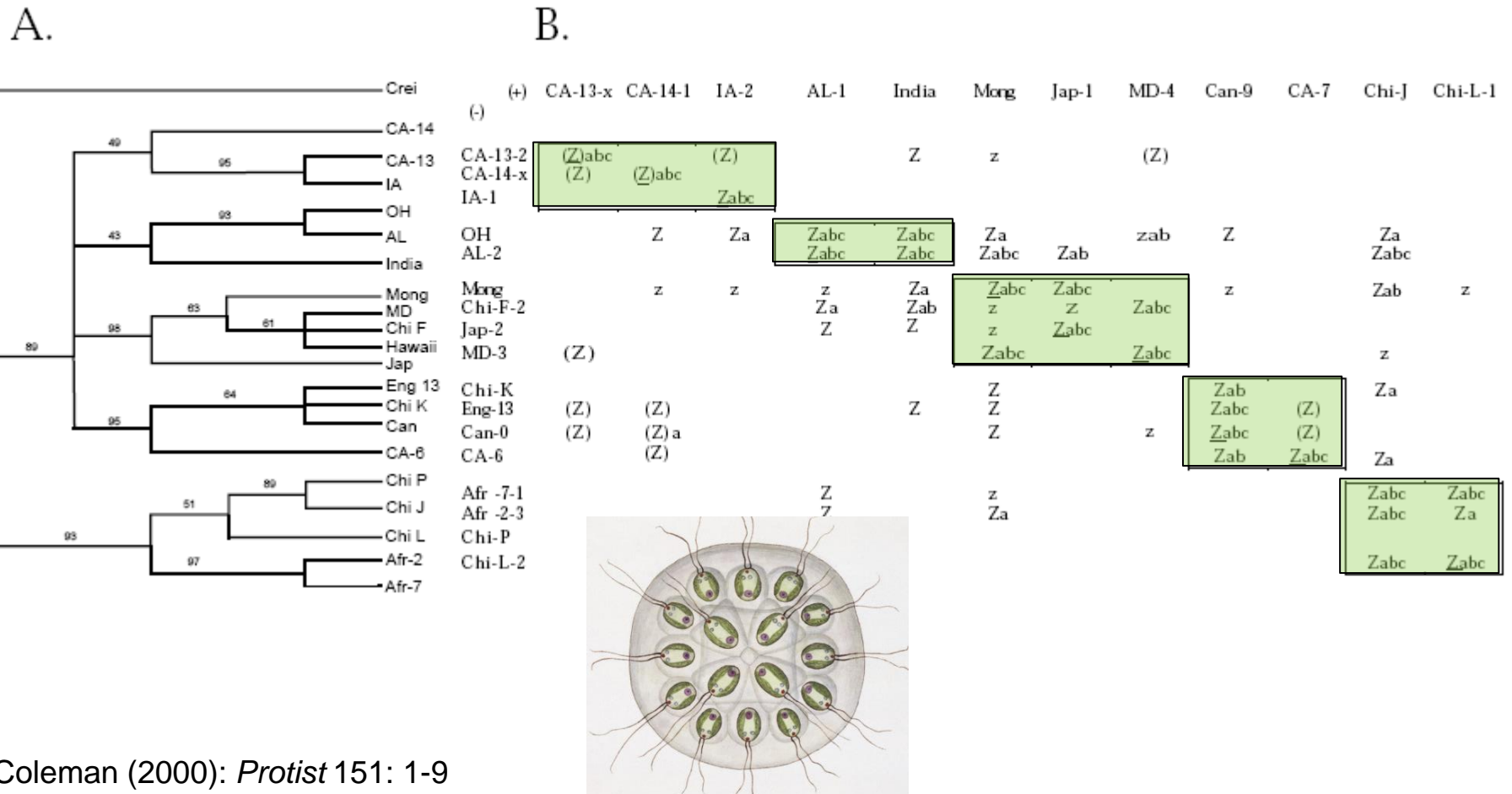
Coleman (2000): *Protist* 151: 1-9

CBC concept

- Species delimited based on differences in conservative regions of the ITS2 molecule

    ➤ not a universal concept

    ➤ CBCs as a measure of genetic relation, not species marker

Caisová et al. (2011): *BMC Evol. Biol.* 11: 262

ITS2-barcode concept

Demchenko et al. (2012):
Eur. J. Phycol. 47: 264
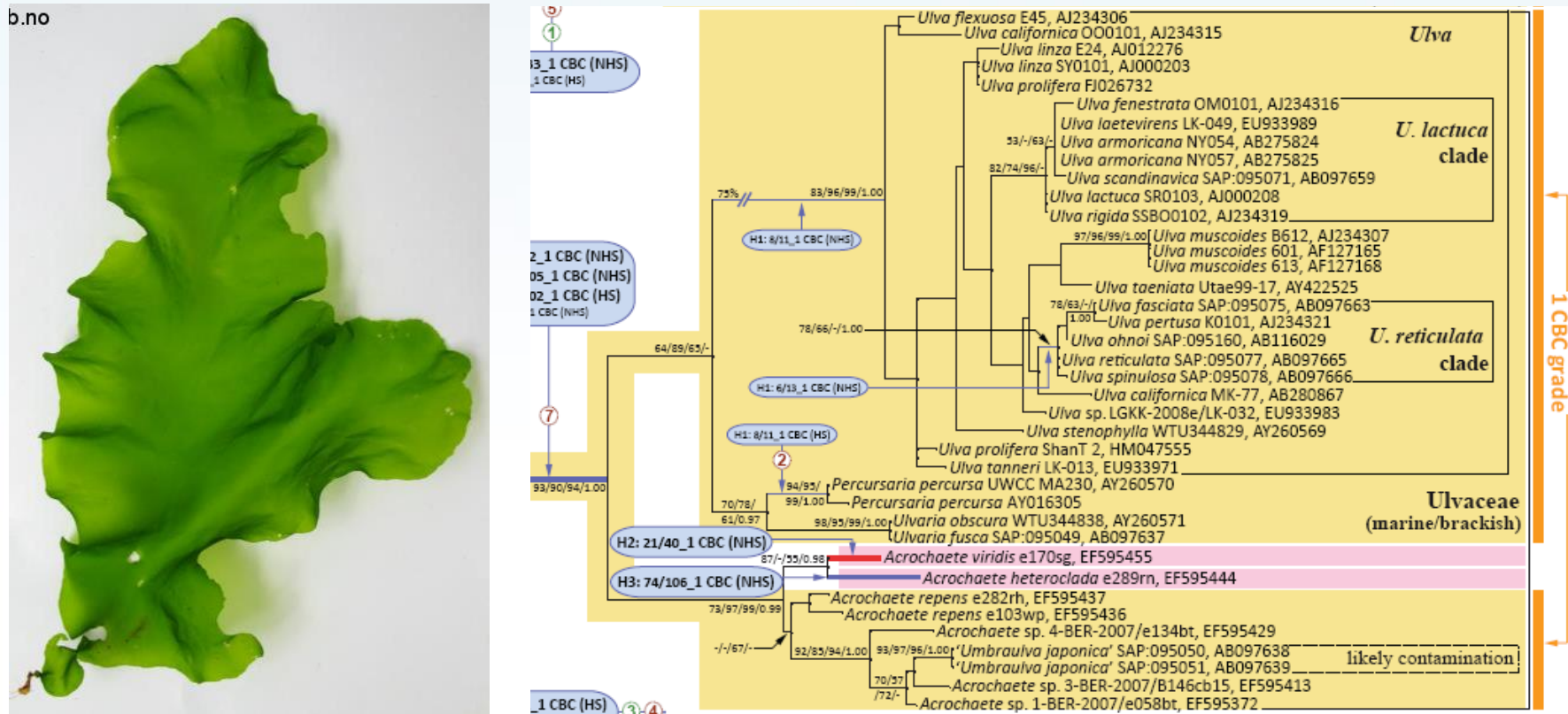
ITS2-barcode concept

- Again, CBCs is a measure of genetic relation, not species marker
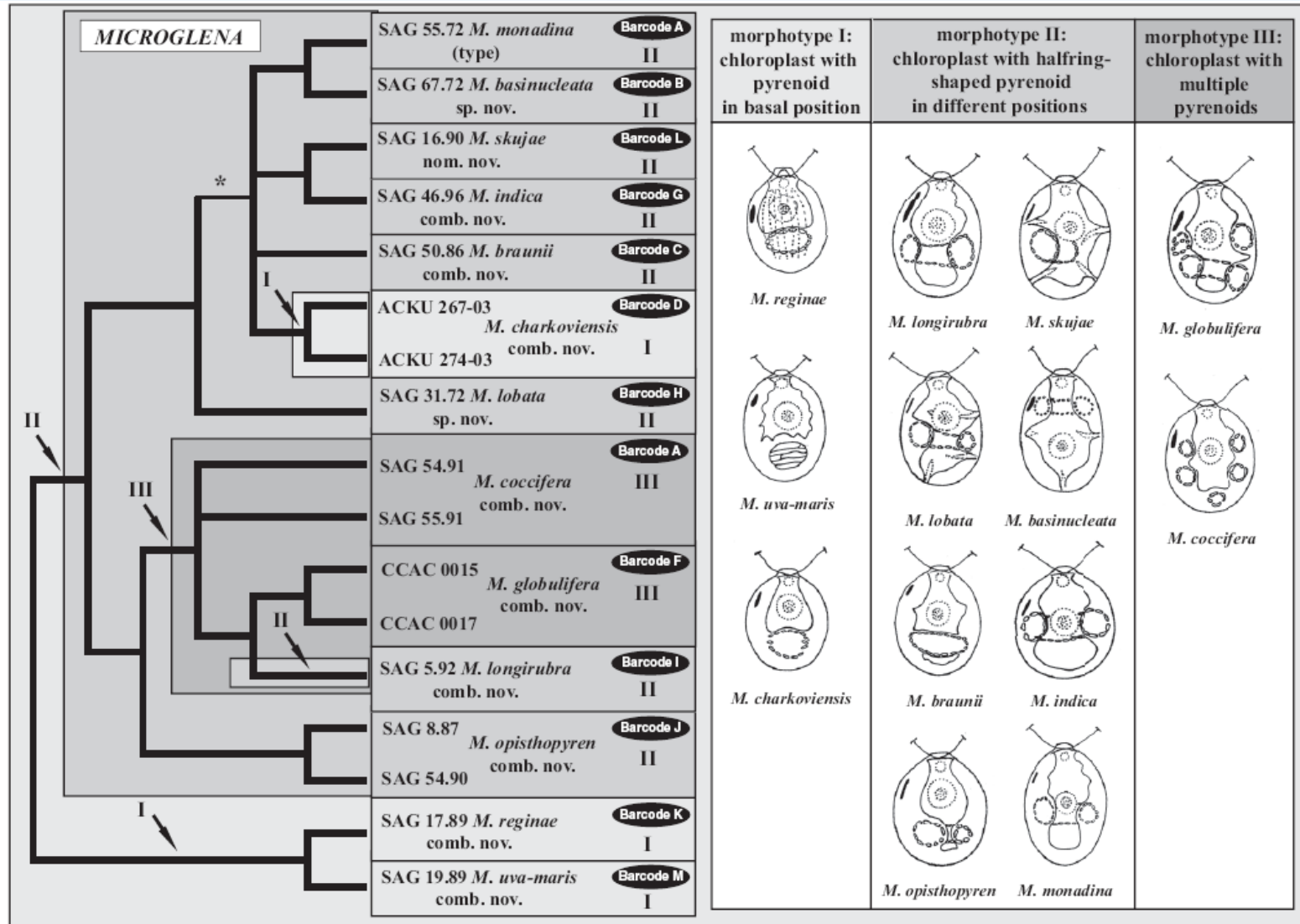
Coalescent theory

- Linking phylogenetics and population genetics
  - ➤ Identification of independently evolving lineages
  - ➤ GMYC, bPTP, ABGD, BP&P, ….

A   **B**   **C**   **A**   **B**   **C**
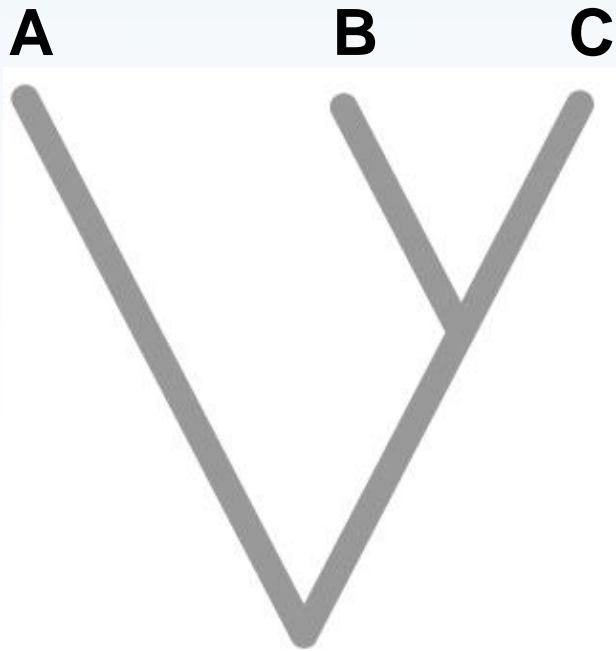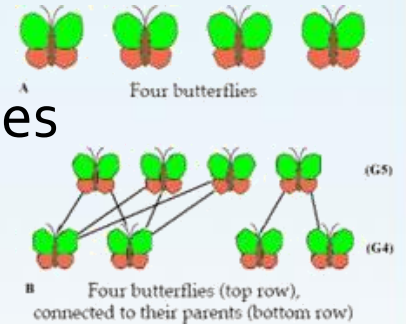
Phylogenetics
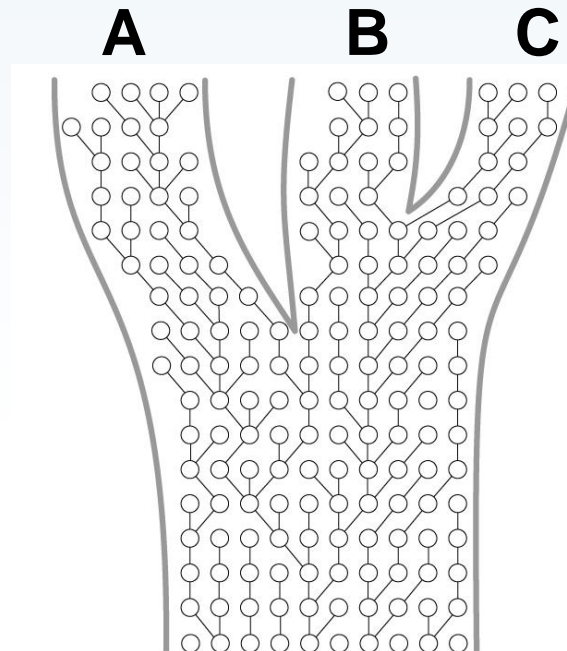
Population genetics
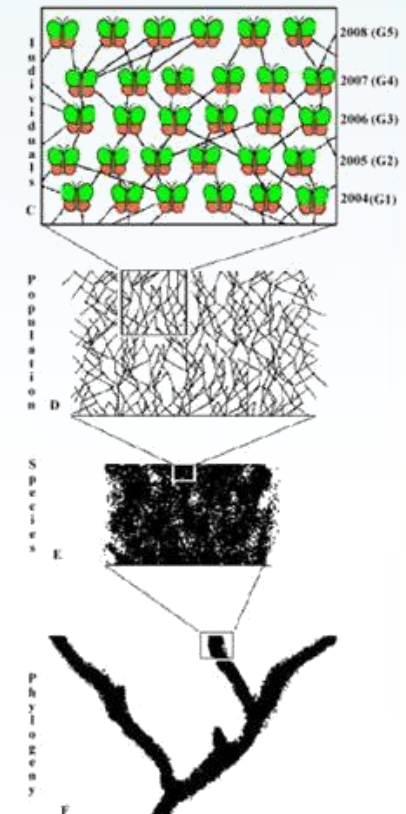
Coalescent theory

- Coalescence processes (Wright-Fisher)
  - ➤ allelic transfer to next generations
  - ➤ allelic frequences vary across generations
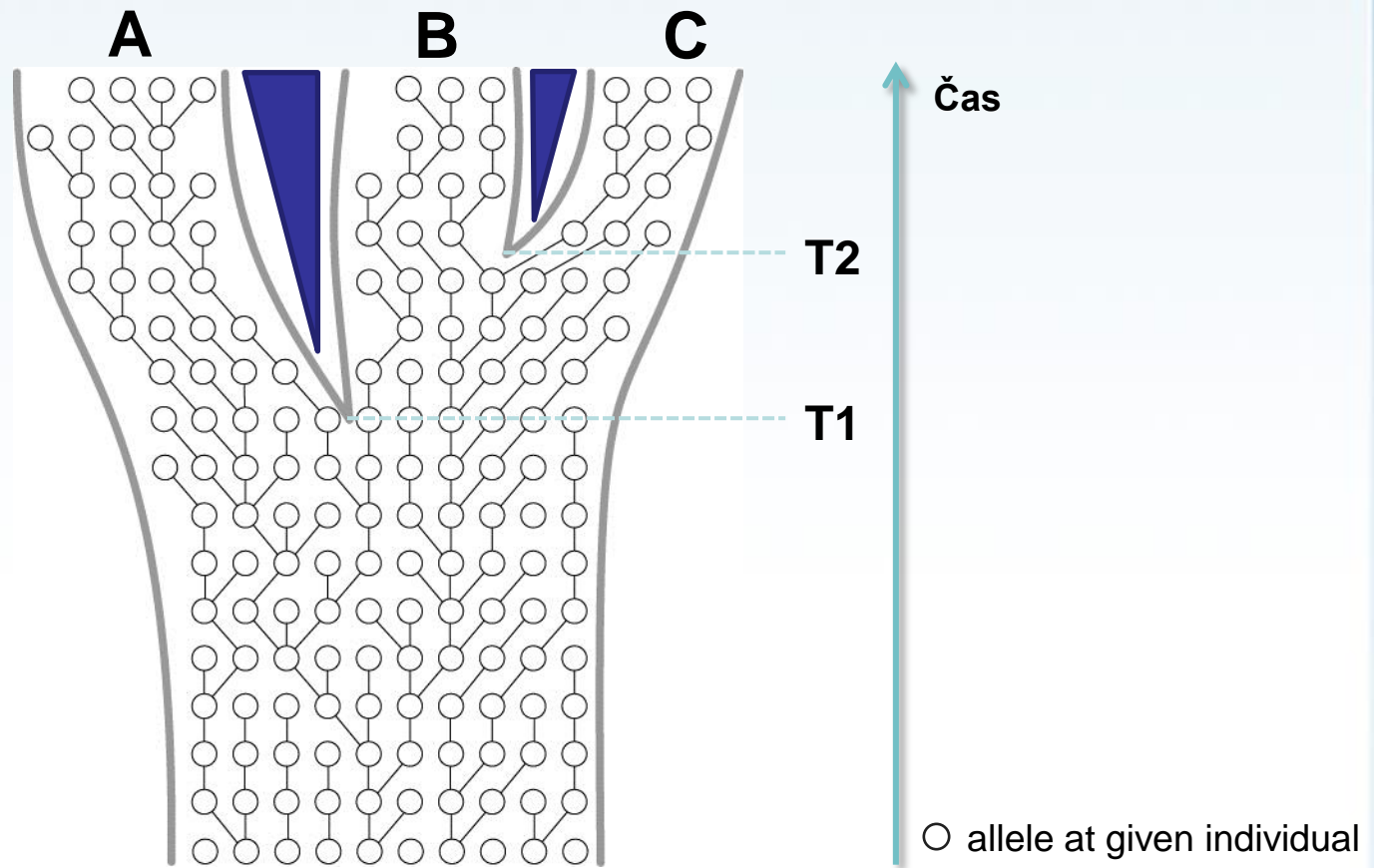
○ allele at given individual

Coalescent theory

- Coalescence processes (Wright-Fisher)
  - ➤ during the coalescence, gene tree topologies resolve the species as polyphyletic, paraphyletic, and monophyletic

paraphyletic species

Leliaert et al. (2014): *Eur. J. Phycol*

Coalescent theory

- Coalescence processes(Wright-Fisher)
  - during the coalescence, gene tree topologies resolve the species as polyphyletic, paraphyletic, and monophyletic

monophyletic species

Species delimited based on the difference between the inter- and intraspecific coalescence processes.

Leliaert et al. (2014): *Eur. J. Phycol*
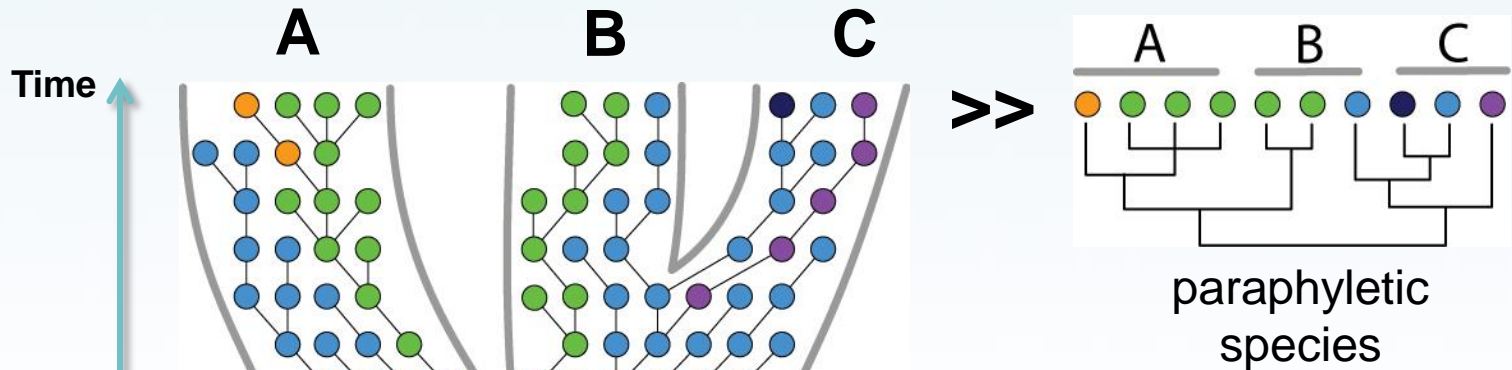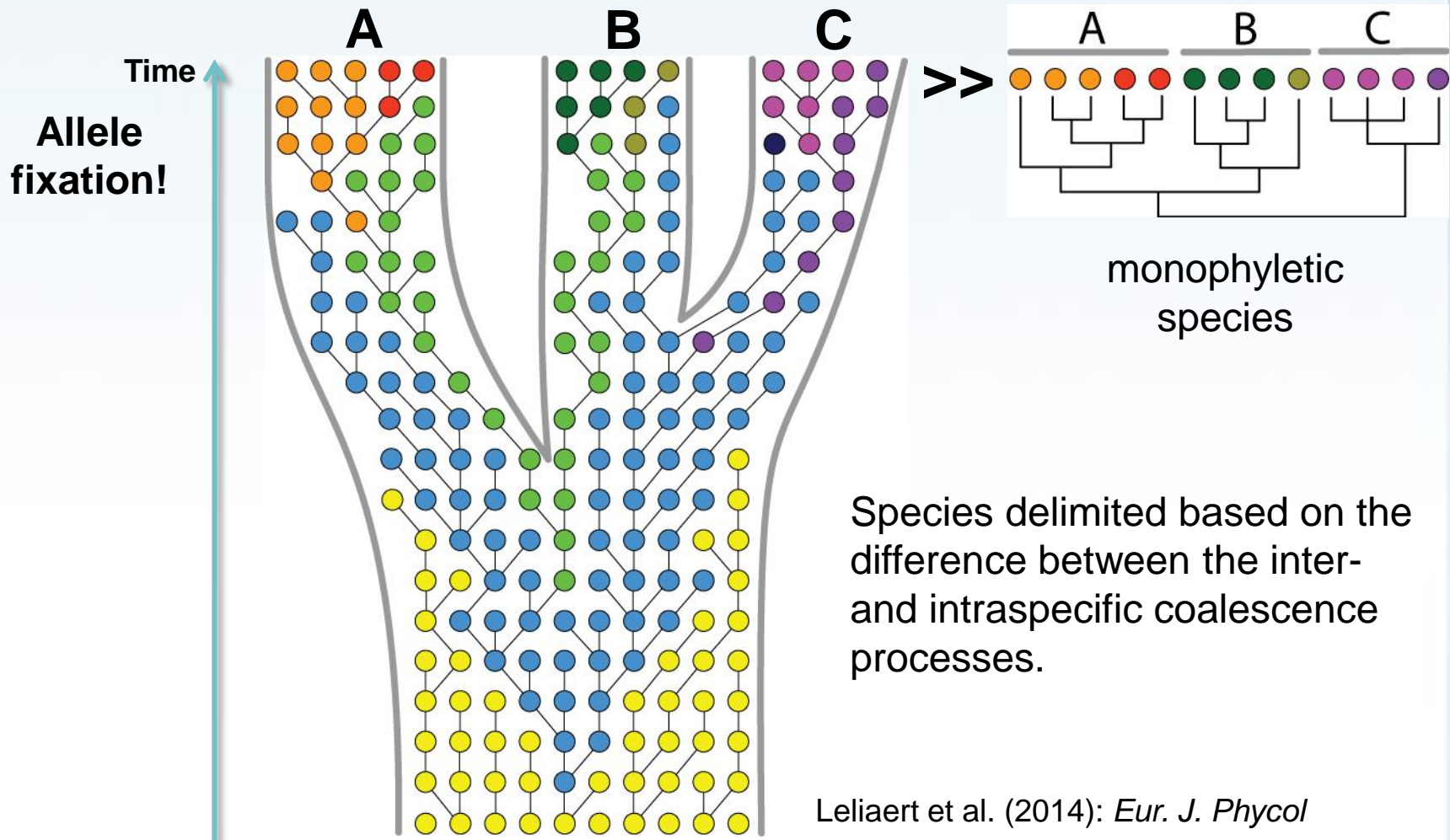
GMYC method

- Different branching patterns within and among species
  - A combination of species diversification (Yule model) and intraspecific coalescence models
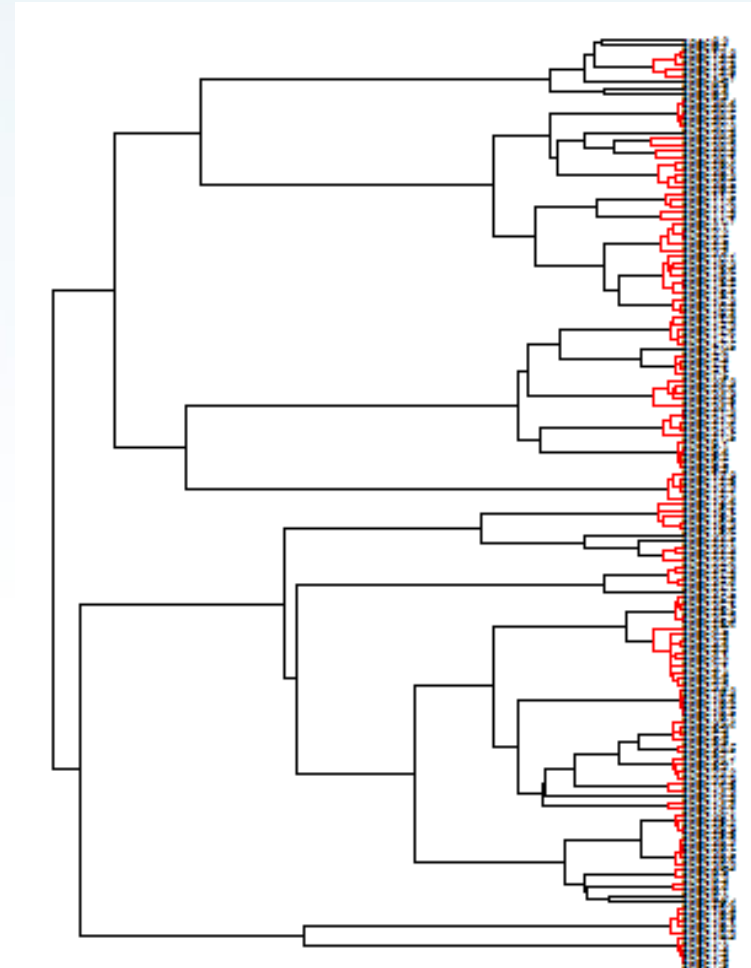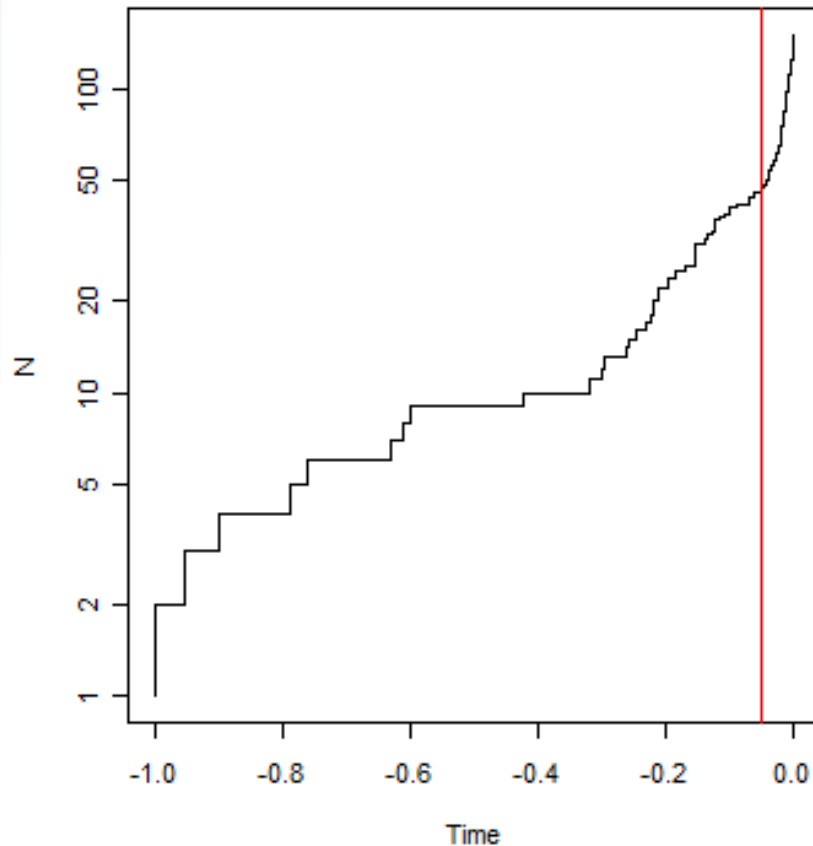
GMYC method

- Different branching patterns within and among species
  - ➤ statistical test, confidence interval

Interspecific branching   Intraspecific branching

```
>summary(result)


Result of GMYC species delimitation


method: single
likelihood of null model: 858.9326
maximum likelihood of GMYC model: 872.3755
likelihood ratio: 26.88581
result of LR test: 6.220952e-06***


number of ML clusters: 33
confidence interval: 31-34


number of ML entities: 46
confidence interval: 42-56


threshold time: -0.05174417
```

Confidence interval

bPTP

- Bayesian Poisson tree processes method
  - Similar to GMYC
  - No need of ultrametric tree
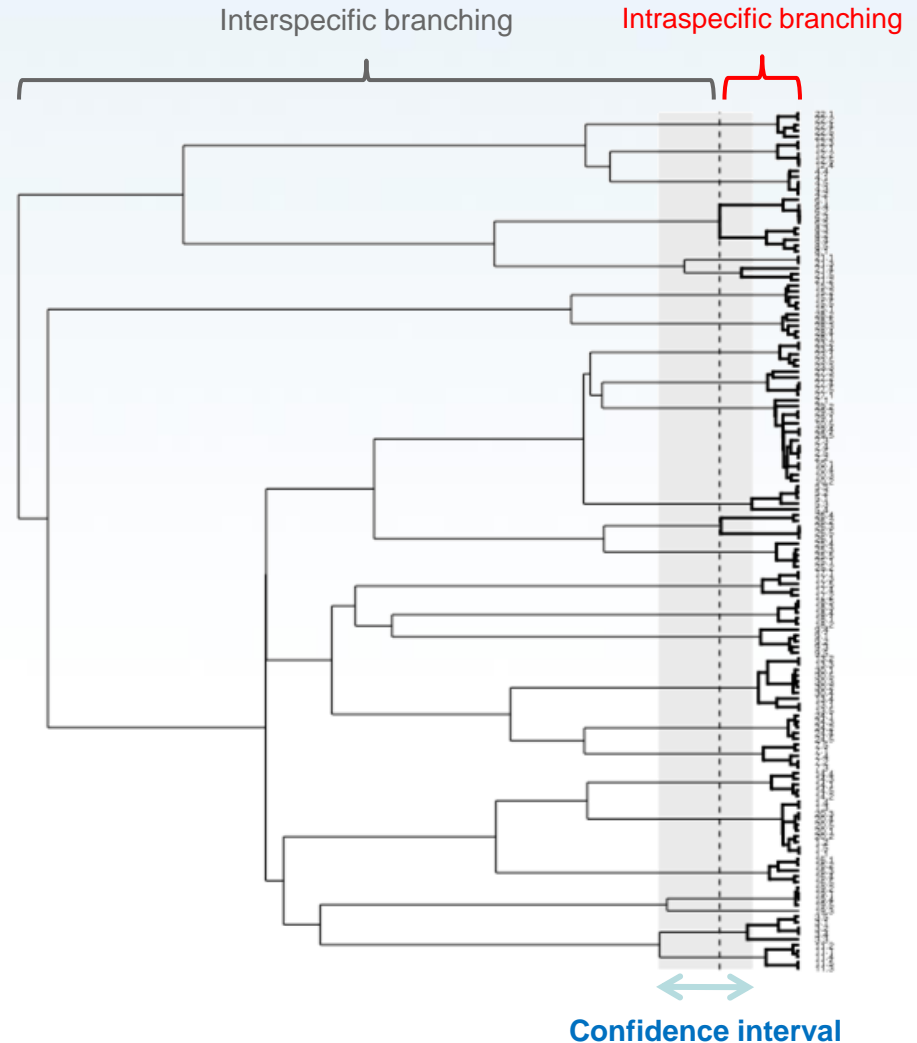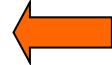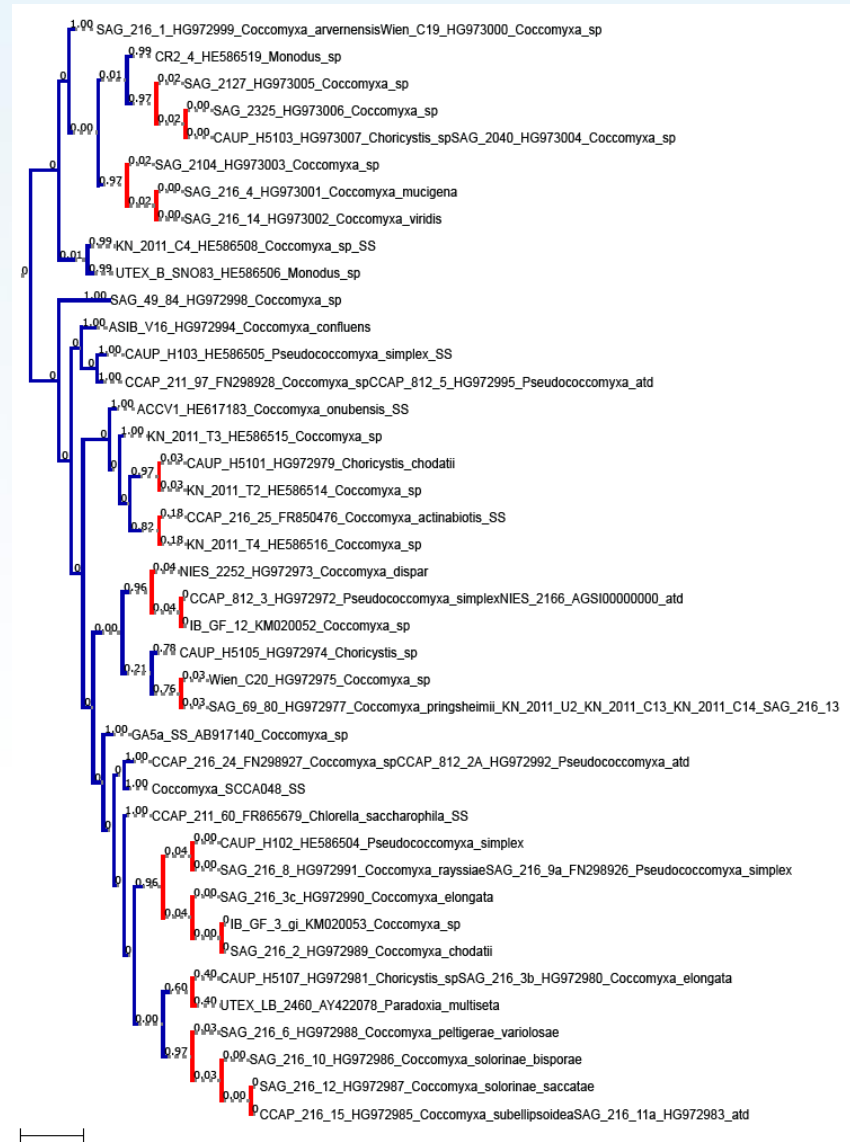 - Using directly the number of substitutions (instead of time) to simulate speciation and coalescent events
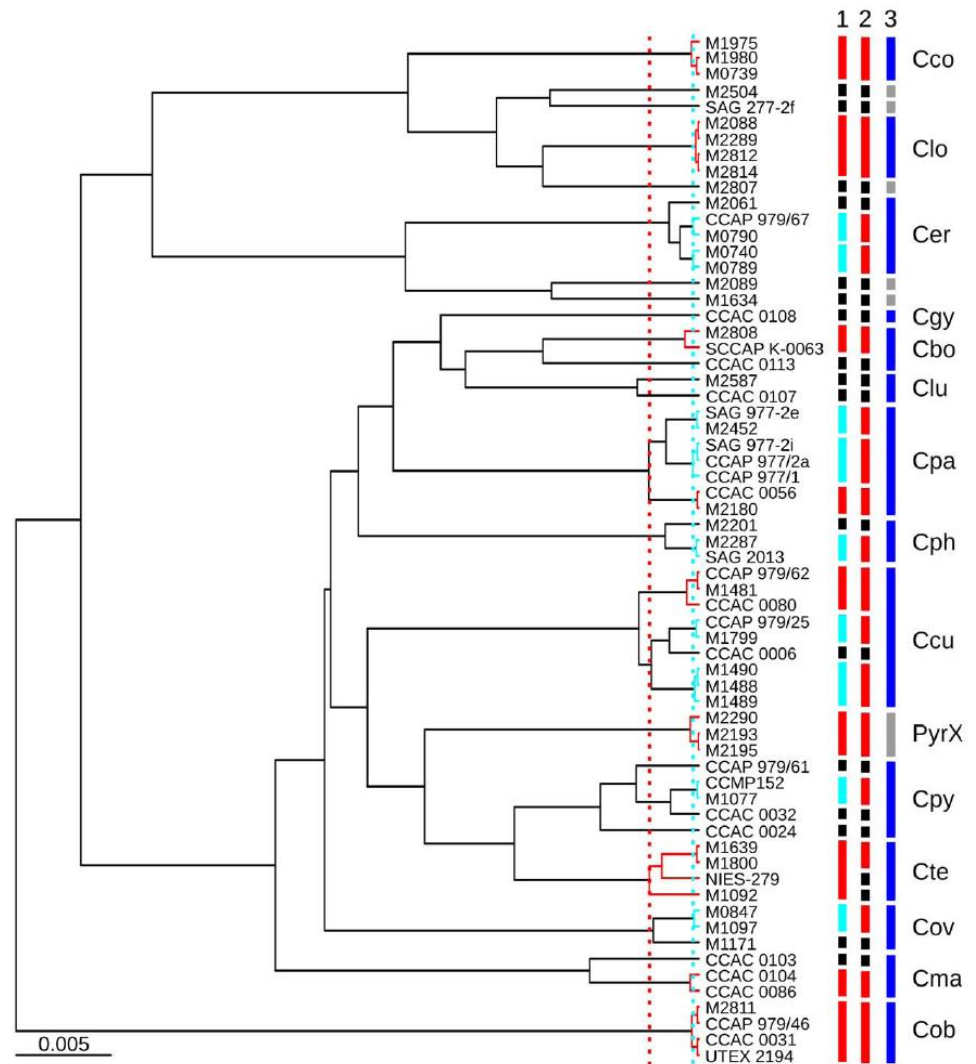
DNA-based species delimitation methods

| Reference	Existing species	Species after delimitation	No. of samples	No. of loci	Discovery approaches	Validation approaches
Avila et al. (2006)	6	>12	293	1	Statistical parsimony (NCA)	None
Barrett & Freudenstein (2011)	3	3	162	5	Morphological cluster analysis, PCA	BPP
Burbrink et al. (2011)	1	1	45	3	Structurama	BPP
Camargo et al. (2012)	3	1	505	4	None	spedeSTEM, BPP, ABC
Carstens & Dewey (2010)	3	7	42	6	None	spedeSTEM, Bayes Factors
Carstens & Satler (2013)	1	2	82	21	Structurama, Gaussian Clustering	spedeSTEM, BPP
Duminil et al. (2012)	Unknown	Unstated	103	7	Morphometric clustering; structure	None
Esselstyn et al. (2012)	13	18–19	413	1	GMYC	None
Florio et al. (2012);	1	2	111	1	Canonical variates analysis	None
Flot et al. (2010)	1		74	3	Haplowebs	None
Hamilton et al. (2011)	4	3	147	1	Combo WP and barcoding gap, monophyly, GMYC	
Kelly et al. (2008)	39	1	114	1	WP	None
Leaché & Fujita (2010)	1	3	51	6	Structure	BPP
Leavitt et al. (2012)	19	2	414	6	Structure	BPP, mean genetic distance
Leliart et al. (2009)	19	13	175	1	GMYC, statistical parsimony (NCA)—clades that exceed 95% cut off	None
Niemiller et al. (2012)	1	19	135	9	O'Meara clustering	BPP
Pons et al. (2006)	24	54	468	1	Parsimony network, PAA, CHA, WP, GMYC	None
Puillandre et al. (2009)	1	4	44	2	Elliptic Fourier analysis on shape to the mollusc shell; qualitative phylogenetic evidence	None
Puillandre et al. (2012)	43	27	1000	2	GMYC, ABGD	None

Carstens et al. (2013): *Mol. Ecol.* 22: 4369-83

Pitfalls of DNA-based species delimitation

- Incongruence between species delimited by GMYC and by those delimited by a combined molecular and morpho-logical aproach
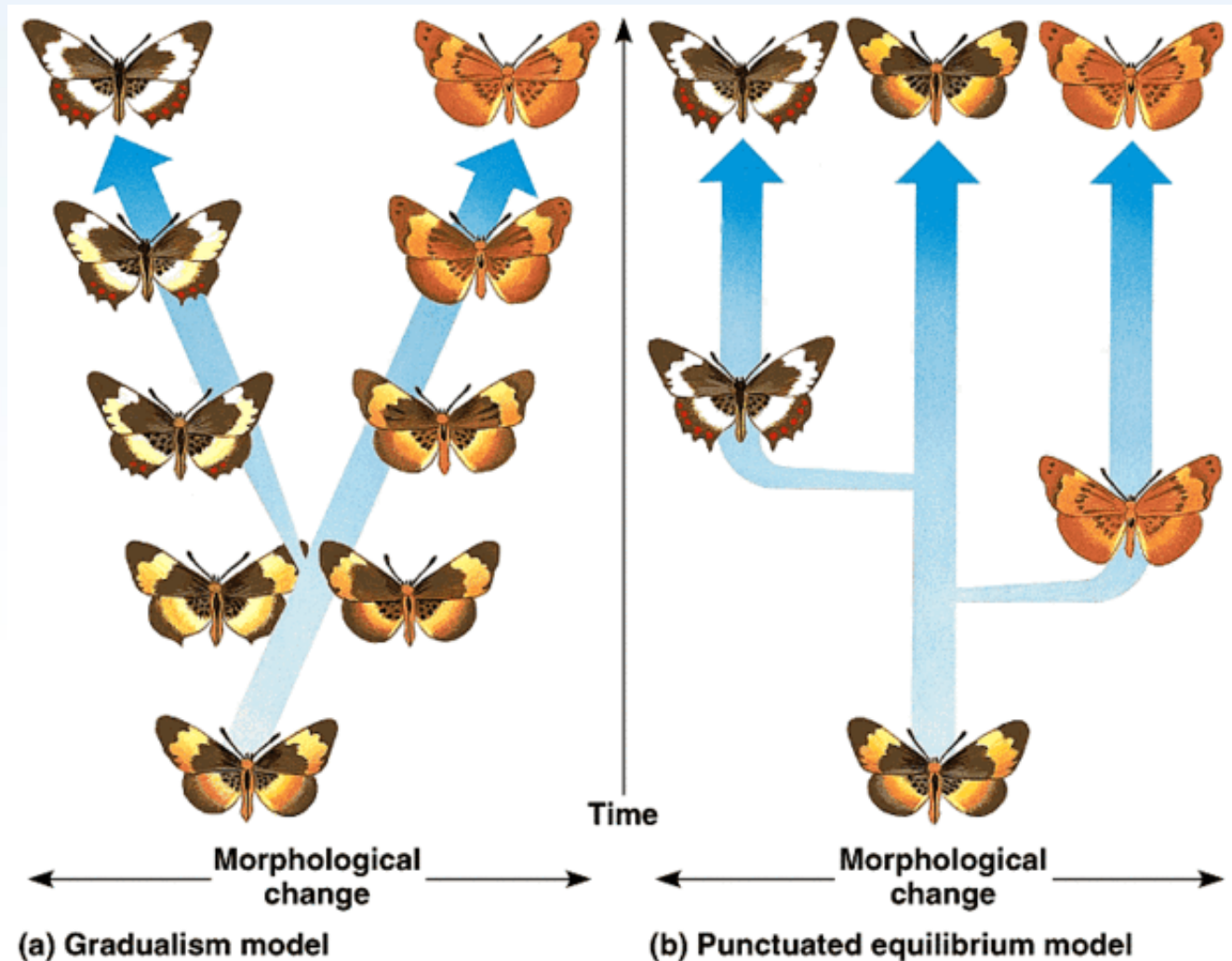
Pitfalls of DNA-based species delimitation

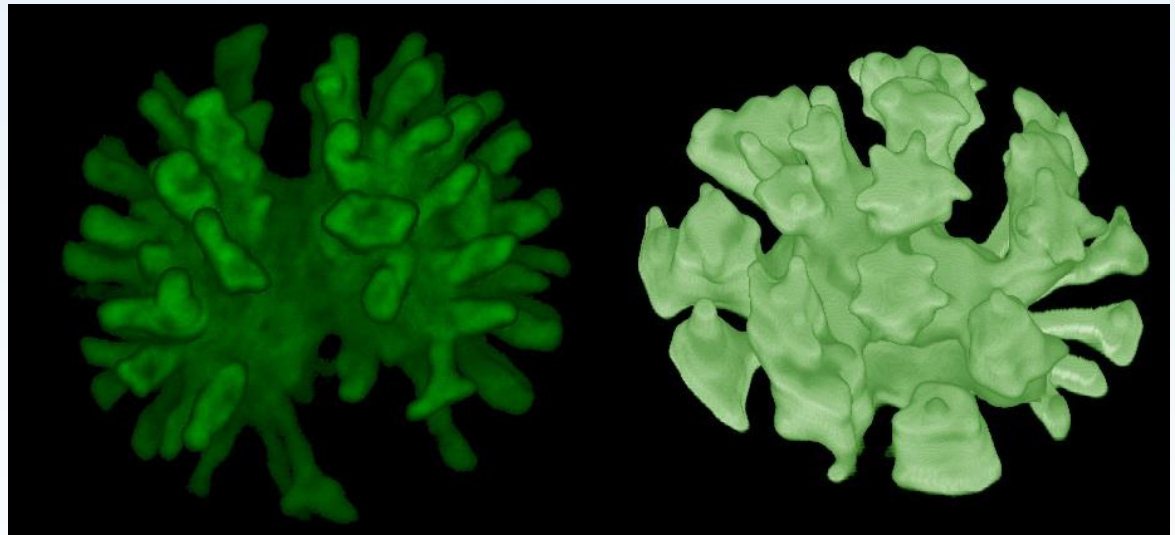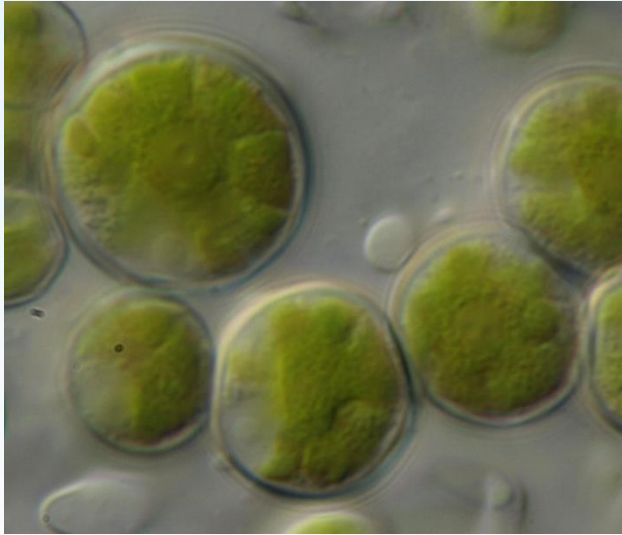- Problems with taxon sampling, using identical sequences

Malavasi et al. (2016): *Plos ONE* 11: e0151137.
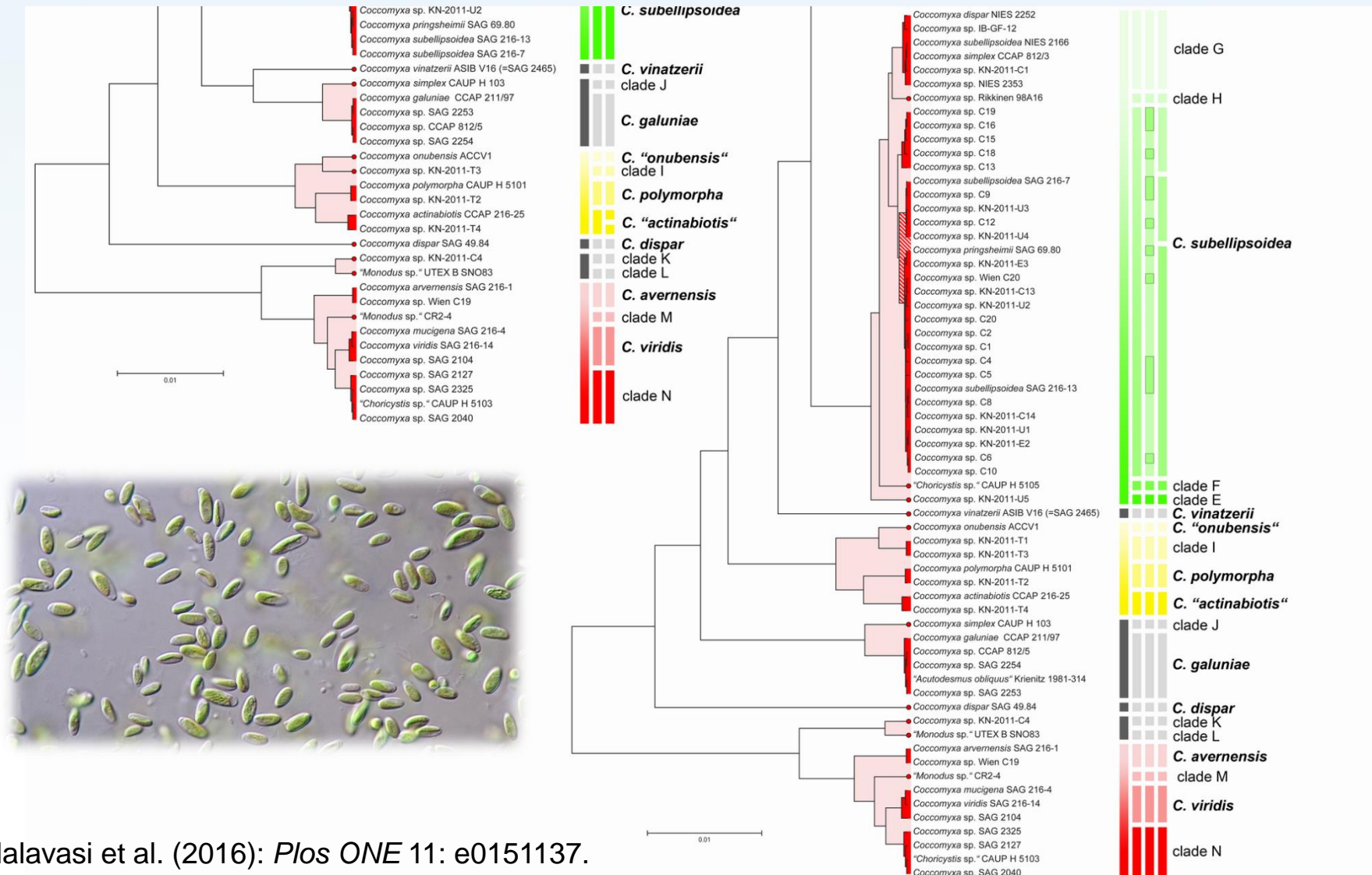
Universal species concept?

- Species are not rigid units, but evolving entities!
- A polyphasic, specific approach

Time

Morphological change

(a) Gradualism model

Morphological change

(b) Punctuated equilibrium model

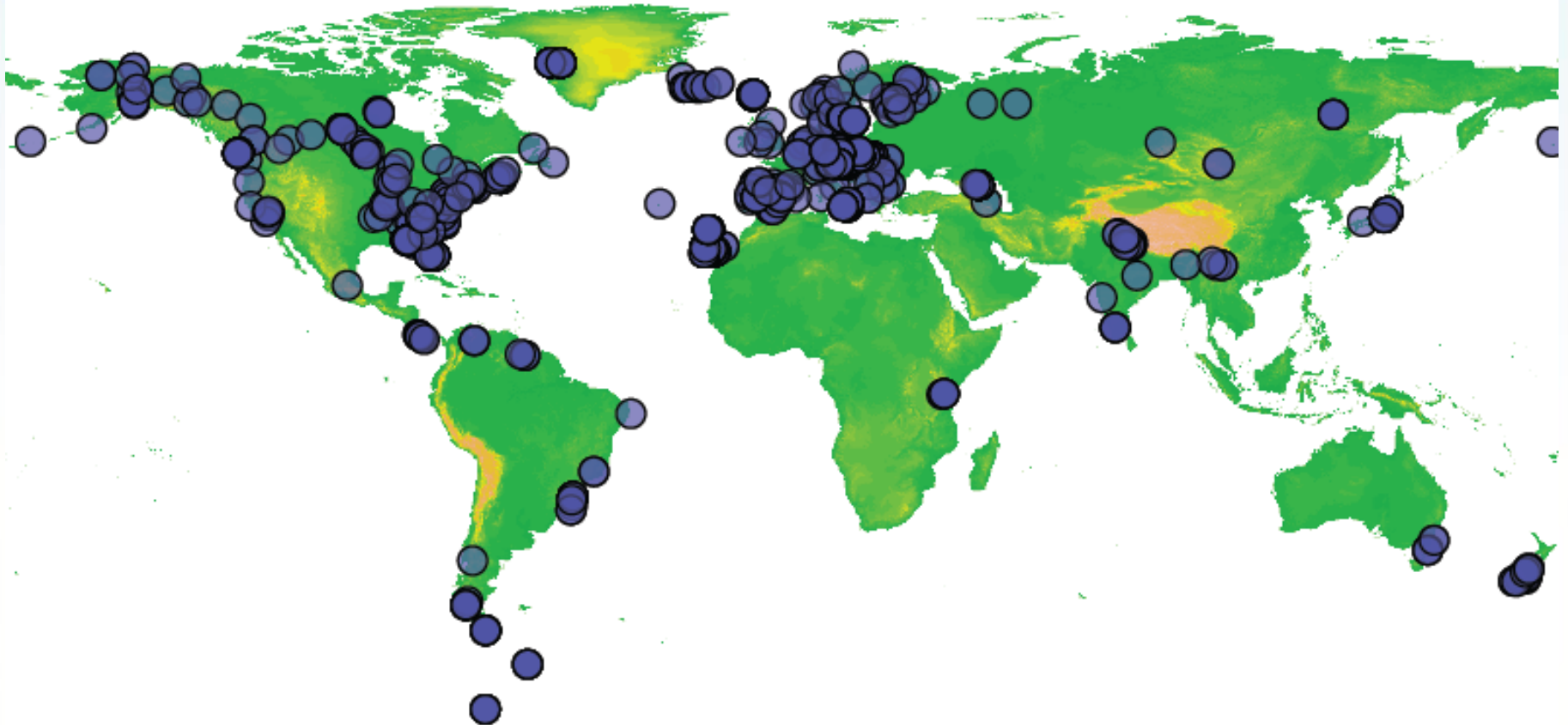Copyright © Pearson Education, Inc., publishing as Benjamin Cummings.

Species delimitation in *Asterochloris*

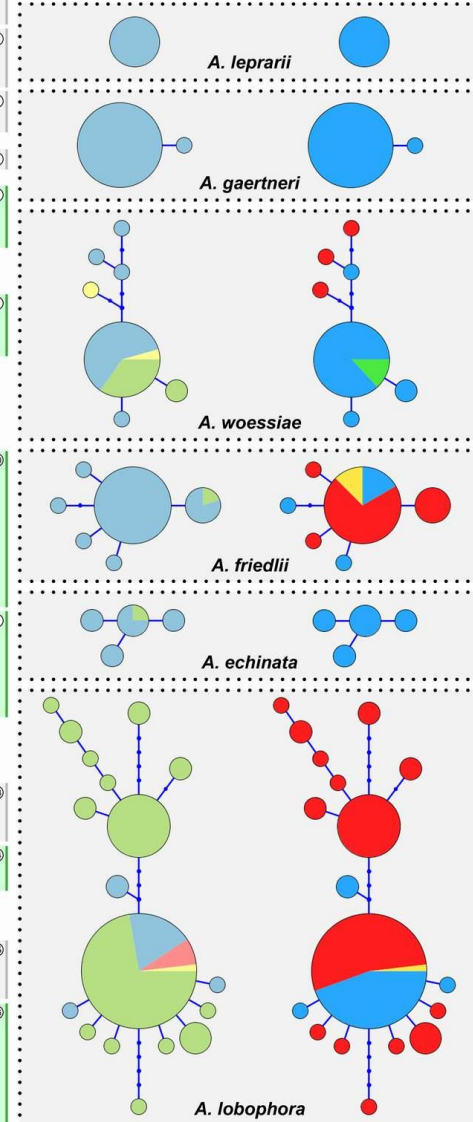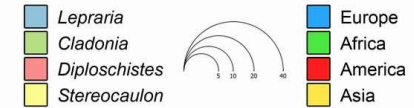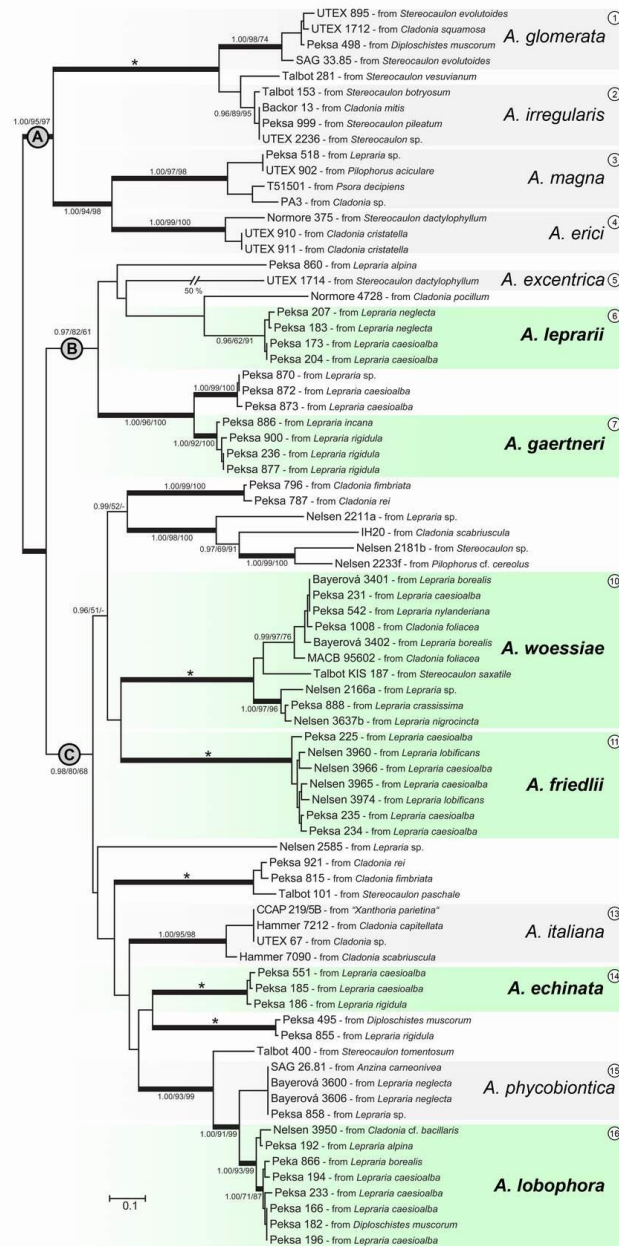- *Asterochloris – one of the most common lichen symbionts*

Species delimitation in *Asterochloris*

- A total of 1509 lichen samples
  - Algal ITS rDNA + actin sequences
  - Substrate data, mycobiont identity (ITS rDNA), climatic data
  - Culturing (morphological analyses)
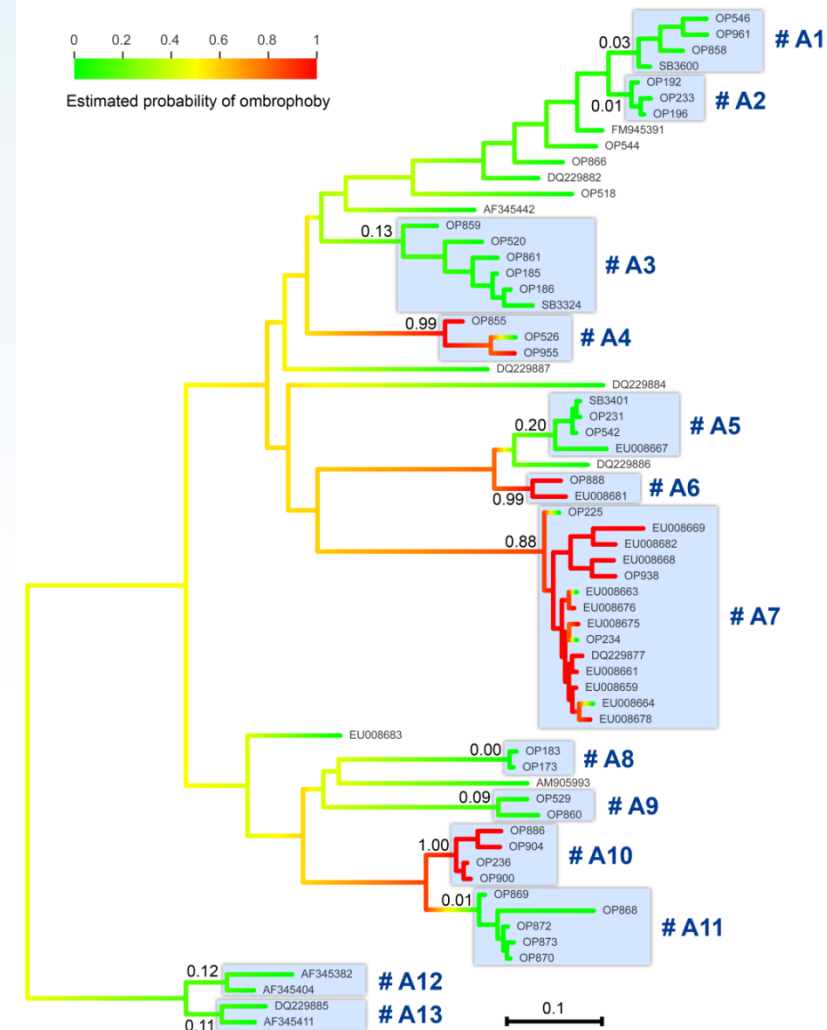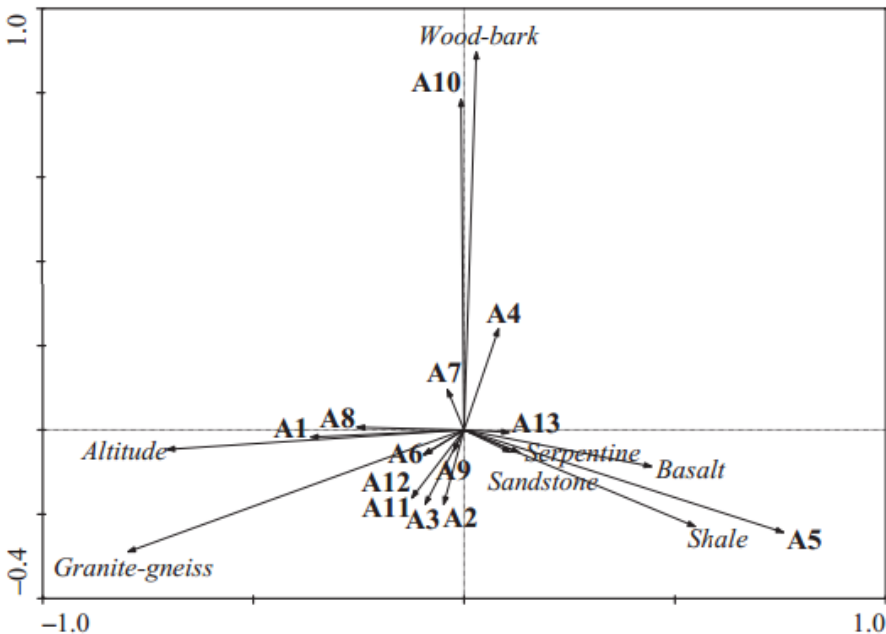
Species delimitation in *Asterochloris*

- molecular data
  (ITS rDNA + actin)

Škaloud et al. (2015): *J. Phycol.*
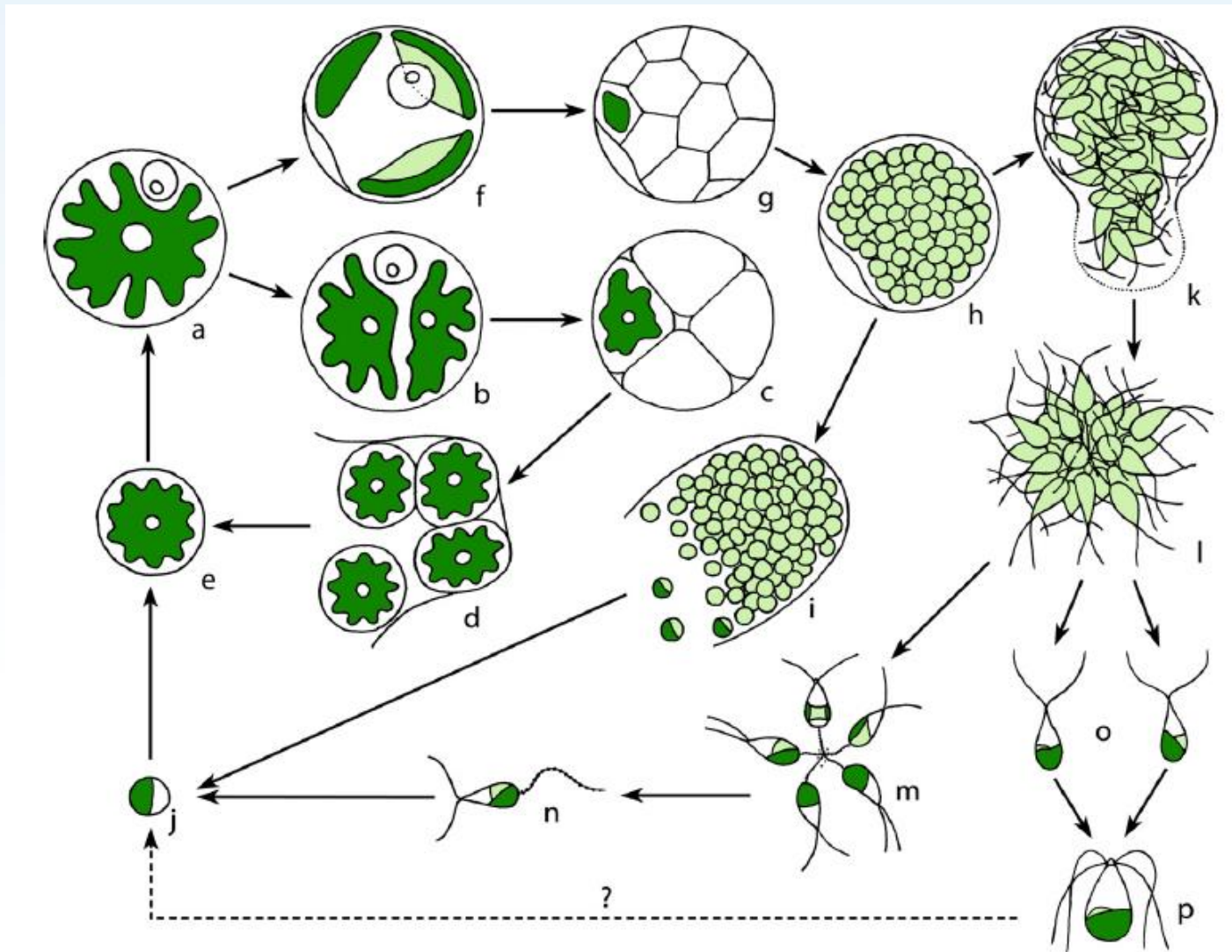51: 507-27

Species delimitation in *Asterochloris*

- ecology
  - substrate specificity, altitude, ombrotrophy

Trait	Pagel's λ		
	λ	Likelihood ratio	*P*-value
Exposure to rain	0.946	1.53	<0.0001
Altitude	0.045	1.01	<0.0001
Substrate type	0.652	1.05	0.0011

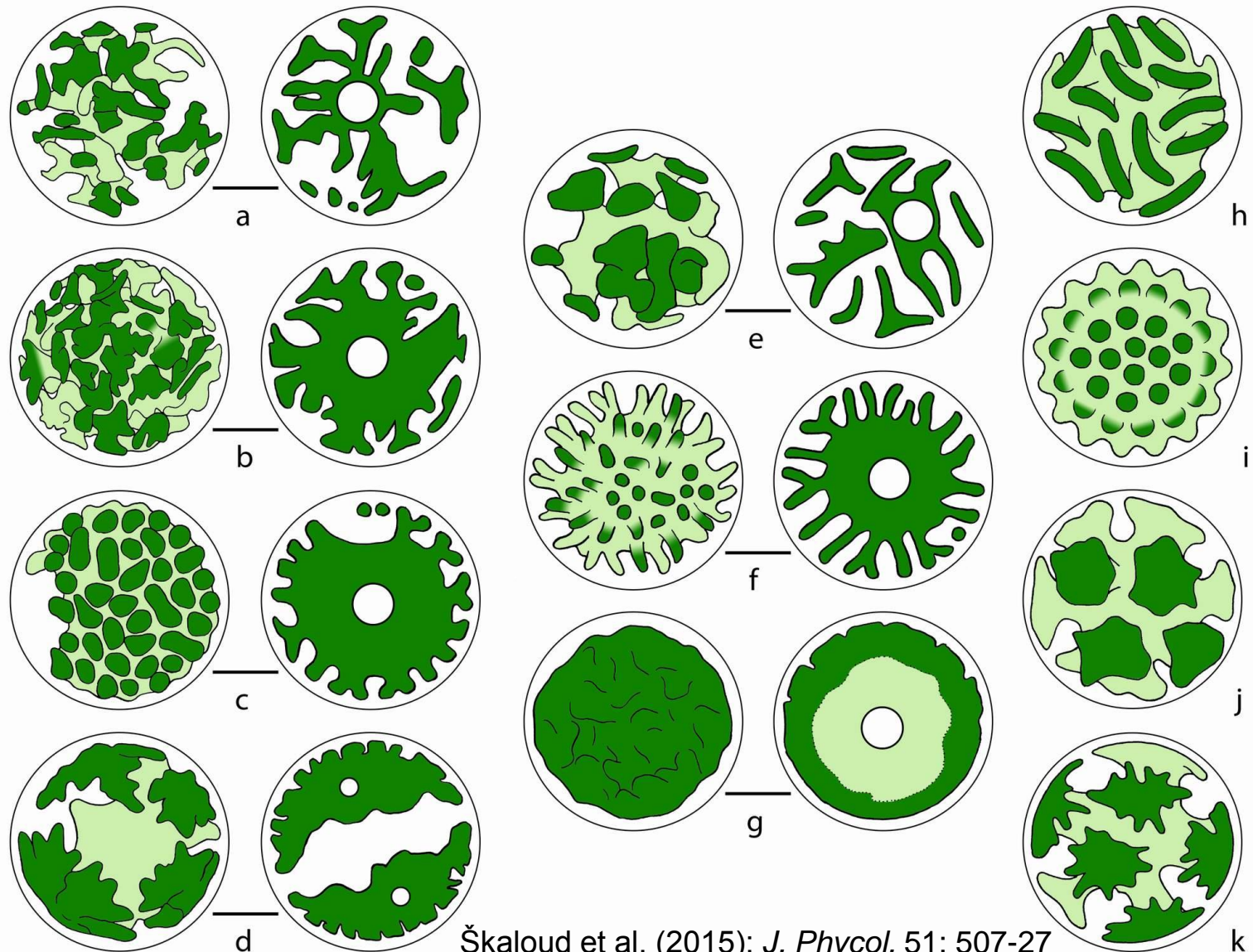Peksa & Škaloud (2011): *Mol. Ecol.* 20: 3936-48
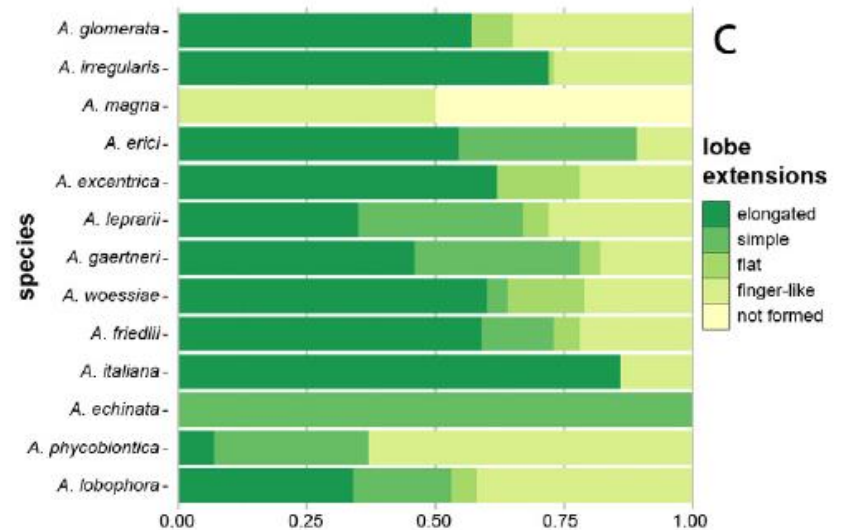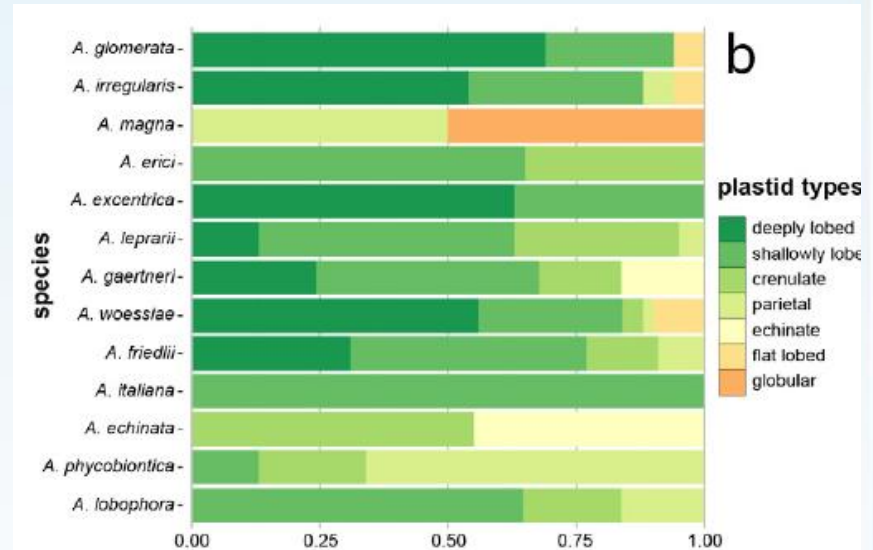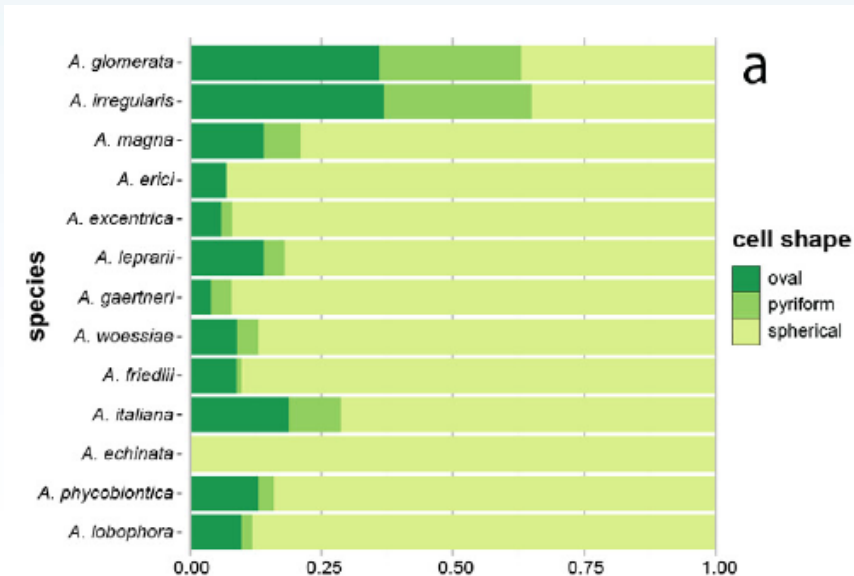
Species delimitation in *Asterochloris*

- Ontogeny
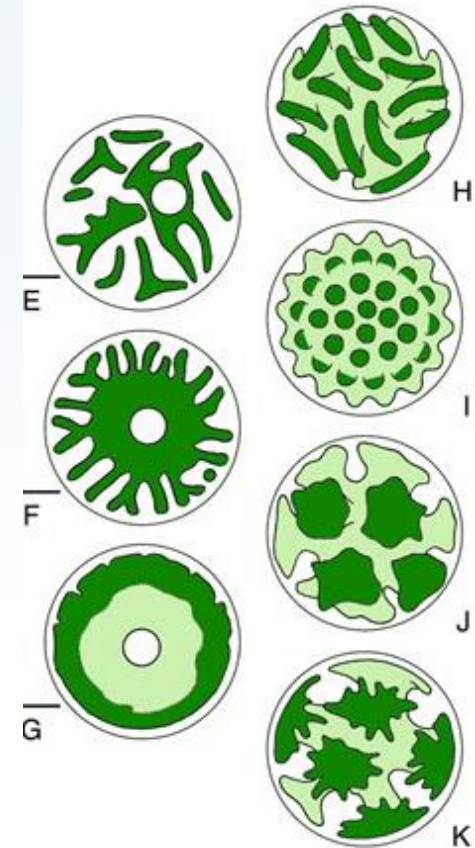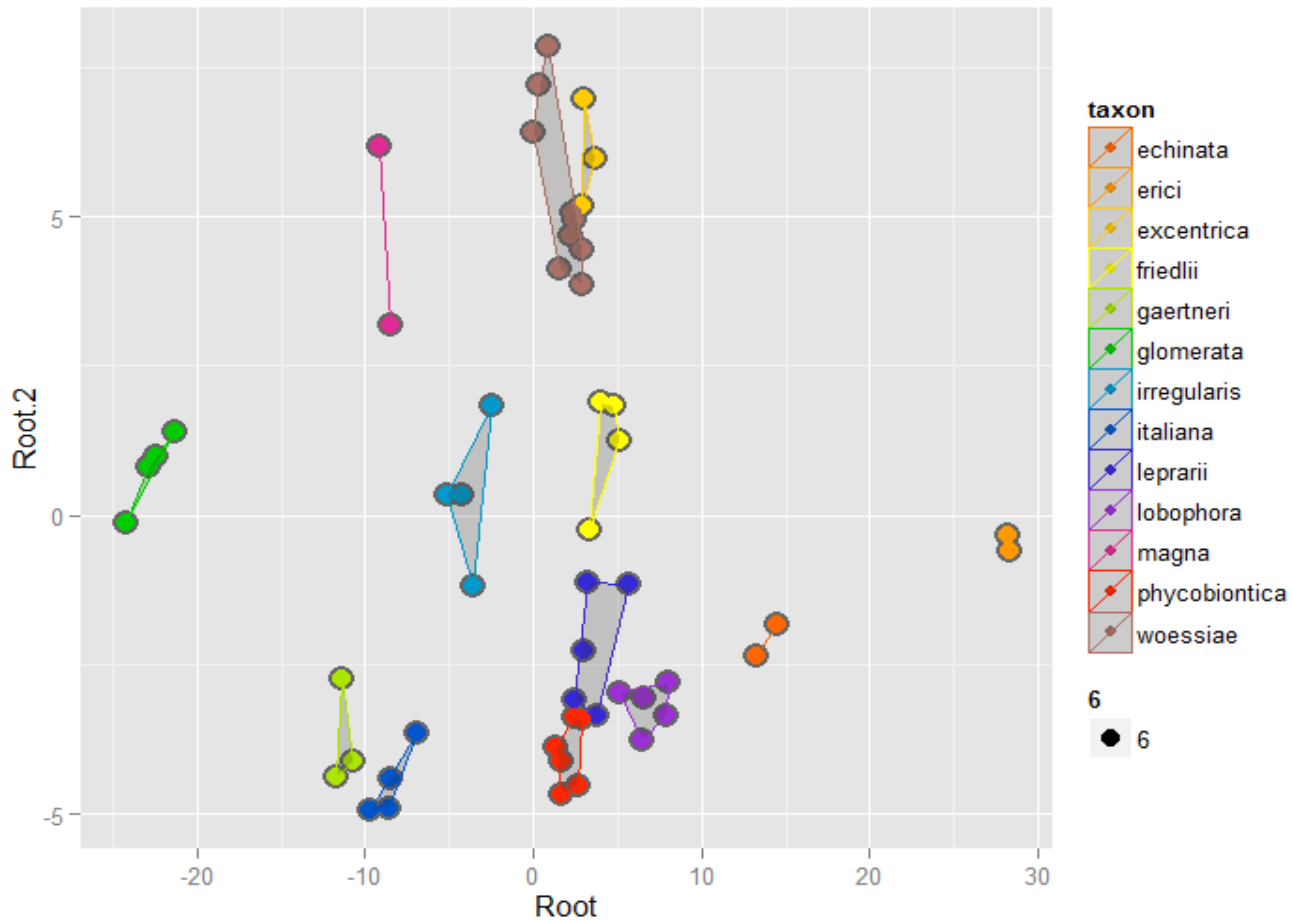
Species delimitation in *Asterochloris*

- Chloroplast morphology

Škaloud et al. (2015): *J. Phycol.* 51: 507-27

Species delimitation in *Asterochloris*

- Morphology
    - cell shape, cell dimensions, chloro-plast shape, number of zoo- and aplanospores

Škaloud et al. (2015): *J. Phycol.* 51: 507-27
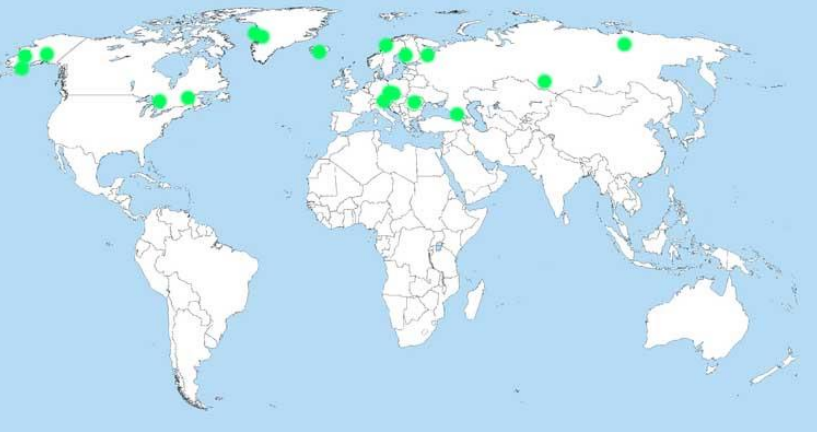
Species delimitation in *Asterochloris*

- Morphology – canonical discriminant analysis
  - cell shape, cell dimensions, chloroplast shape, number of zoo- and aplanospores
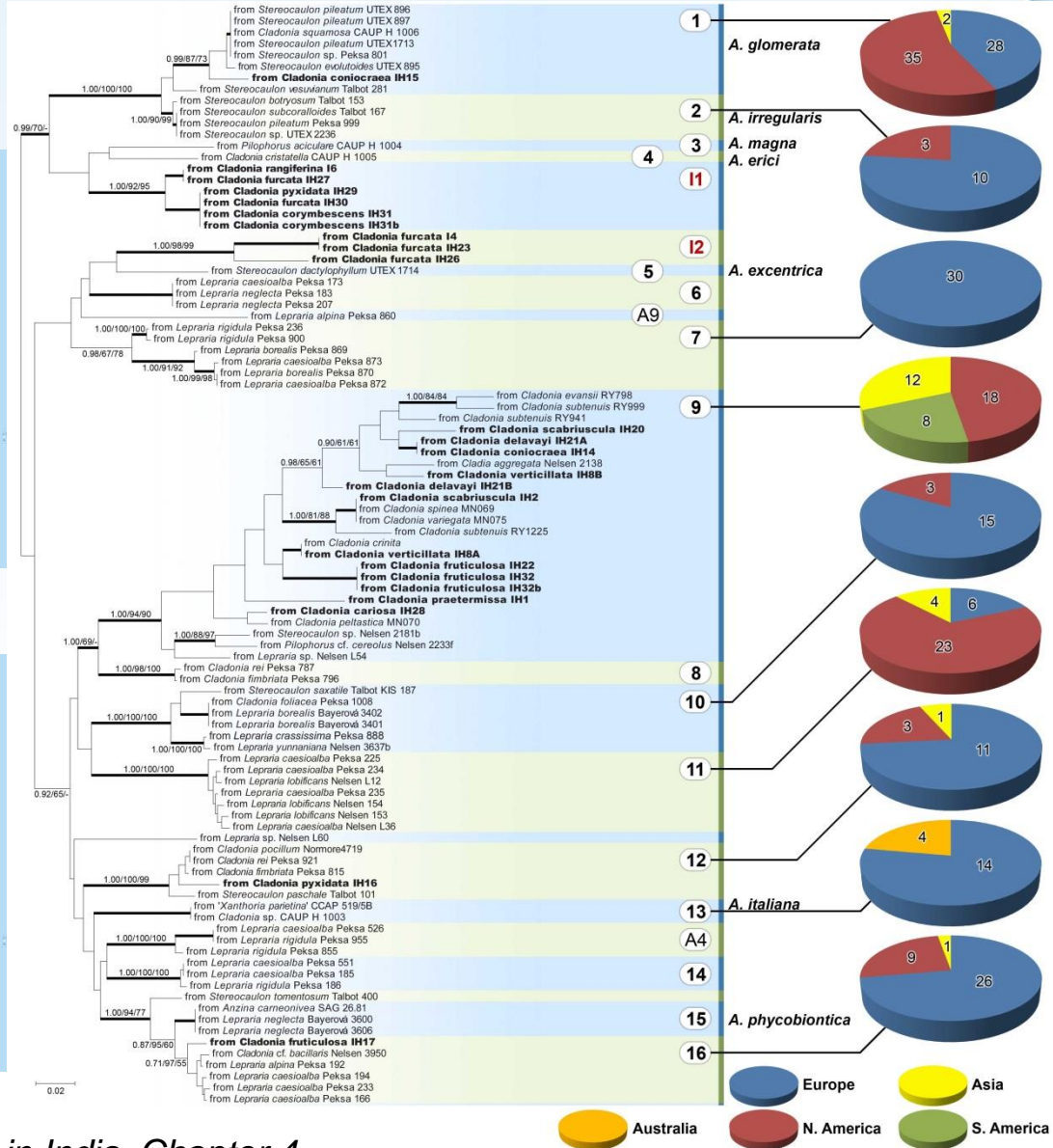
Species delimitation in *Asterochloris*
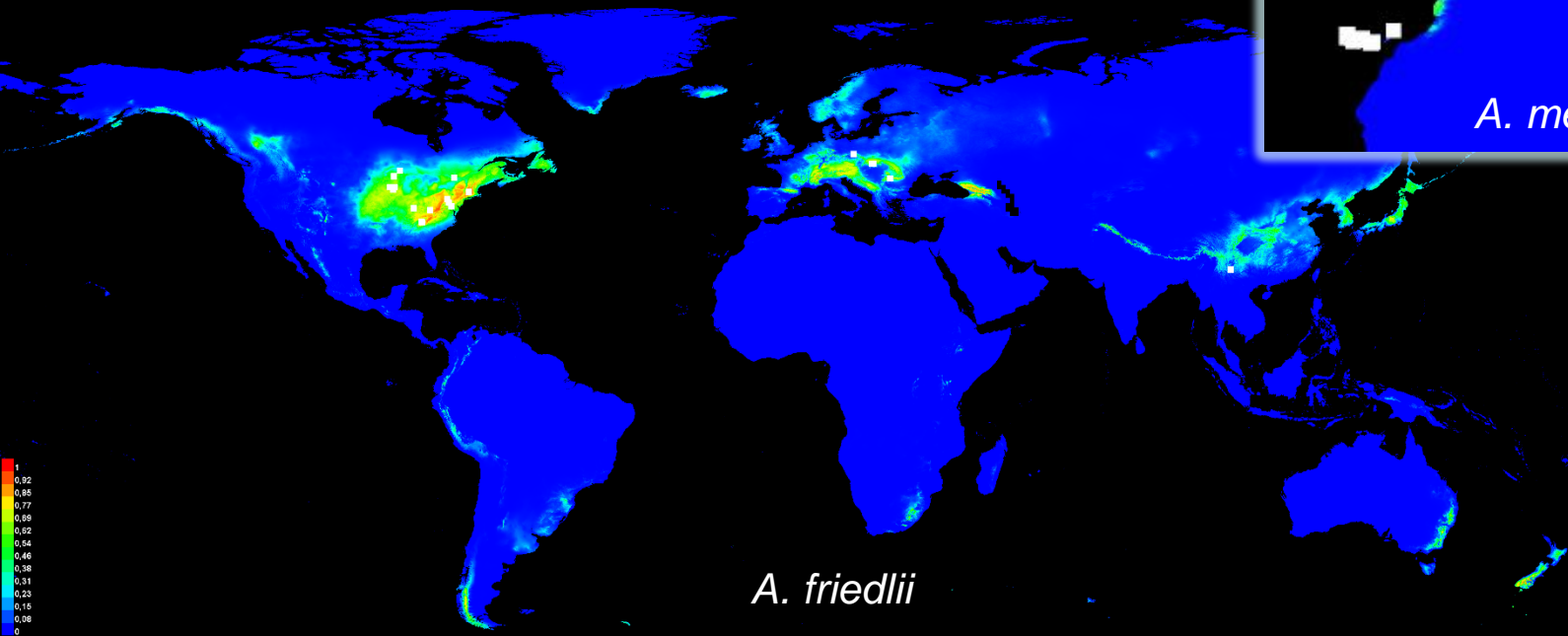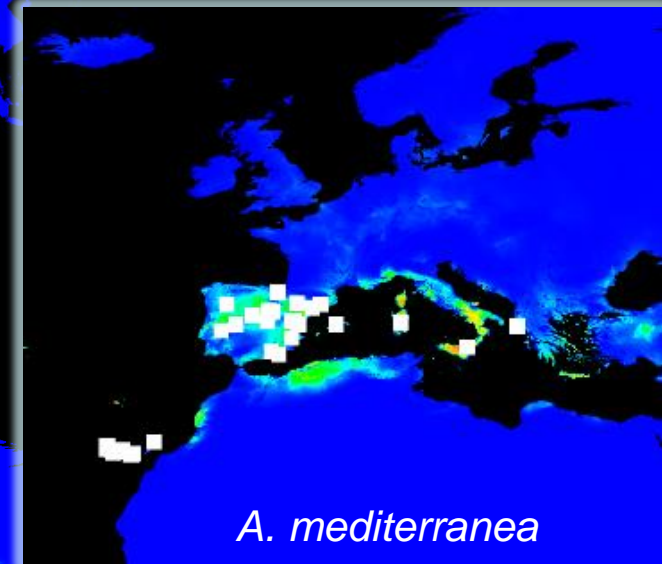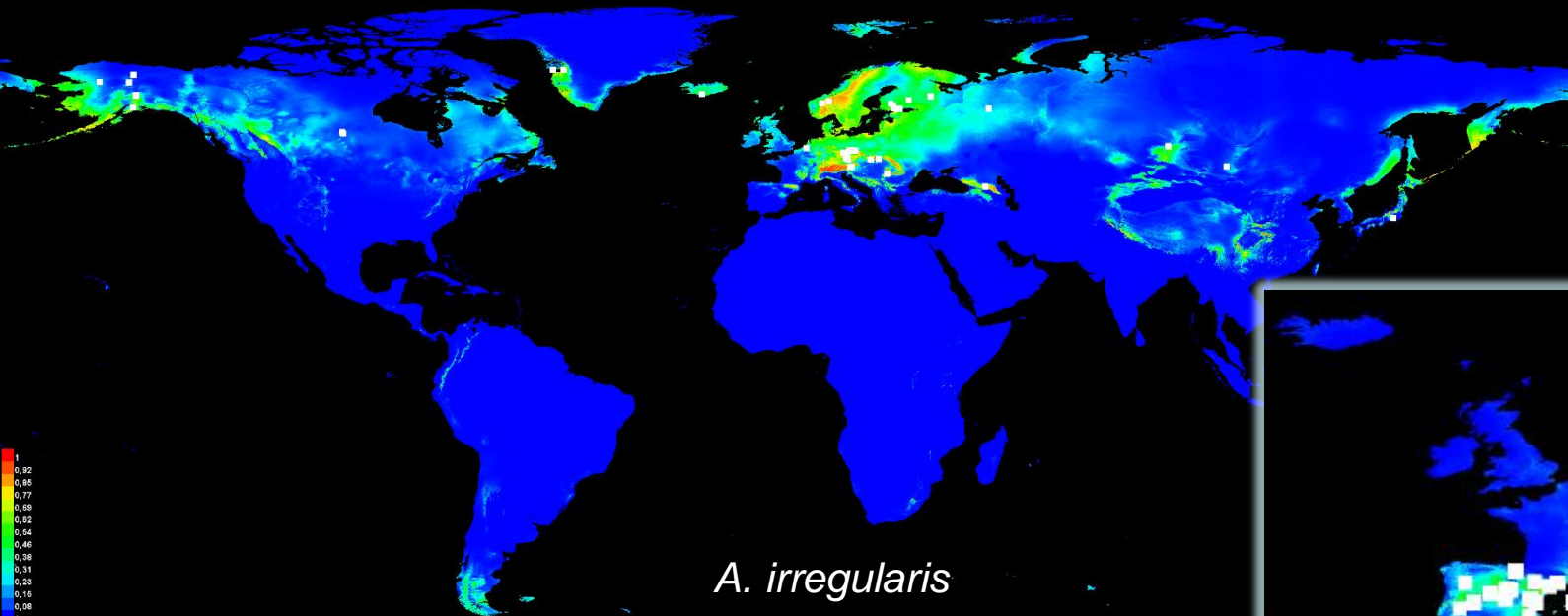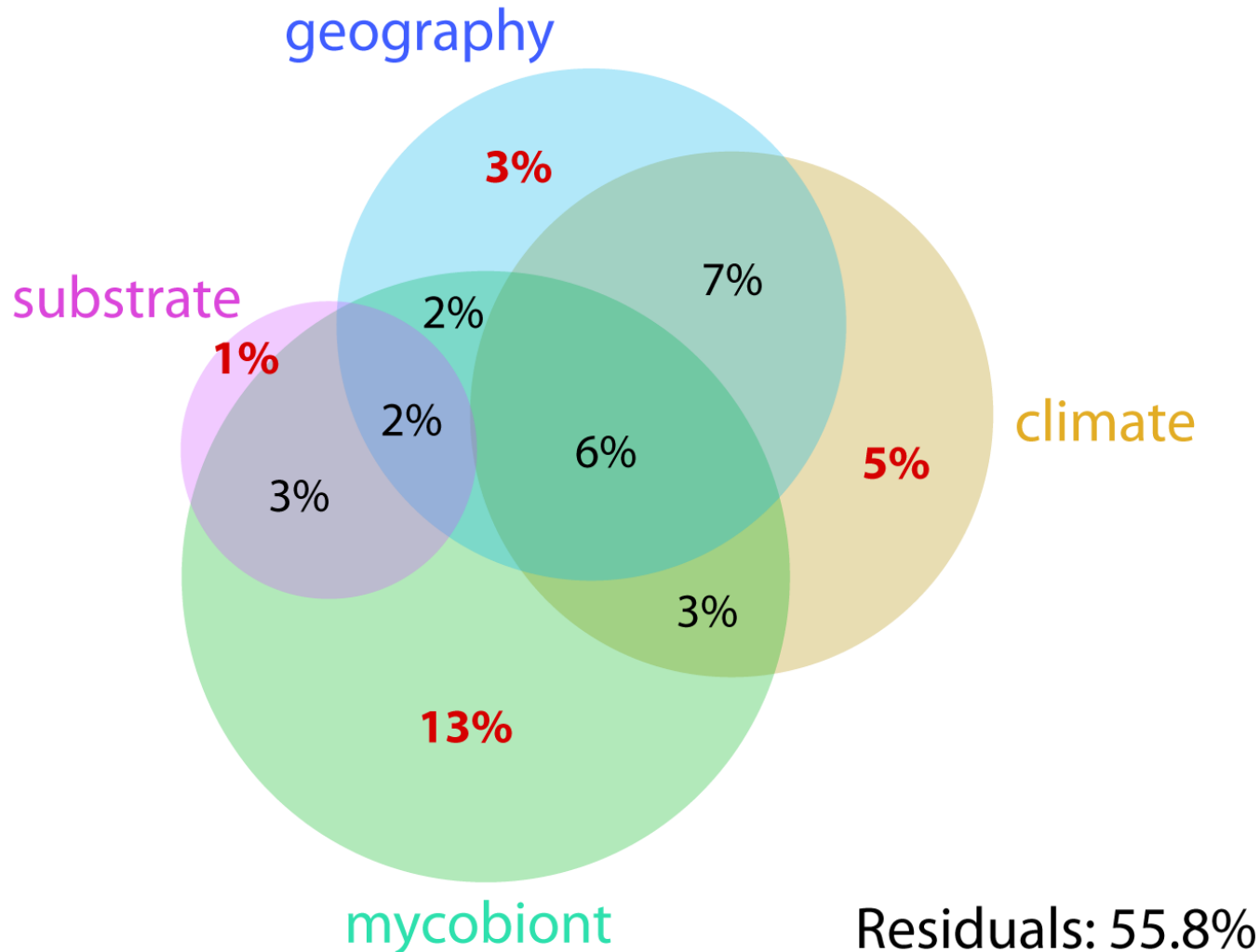
- Biogeography

A. irregularis

clade 9

Řídká et al. (2014): *Terricolous Lichens in India, Chapter 4*

- Biogeography: niche modelling

A. irregularis

A. mediterranea

A. friedlii

Species delimitation in *Asterochloris*

- Mycobiont selectivity

Venn diagram showing variance partitioning:
- geography: **3%**
- substrate: **1%**
- climate: **5%**
- mycobiont: **13%**
- geography ∩ climate: 7%
- geography ∩ substrate: 2%
- substrate ∩ geography (inner): 2%
- central overlap: 6%
- substrate ∩ mycobiont: 3%
- mycobiont ∩ climate: 3%
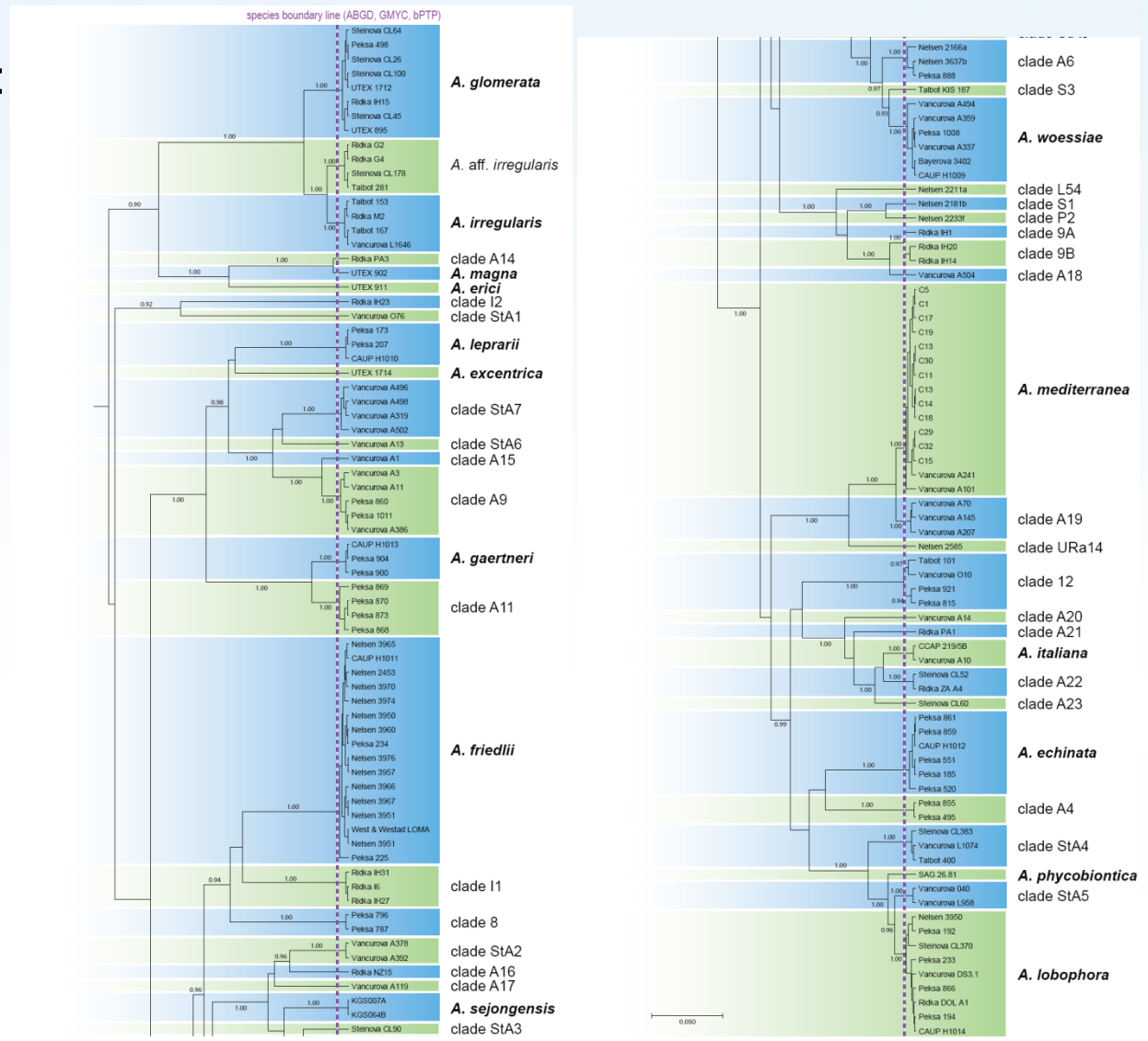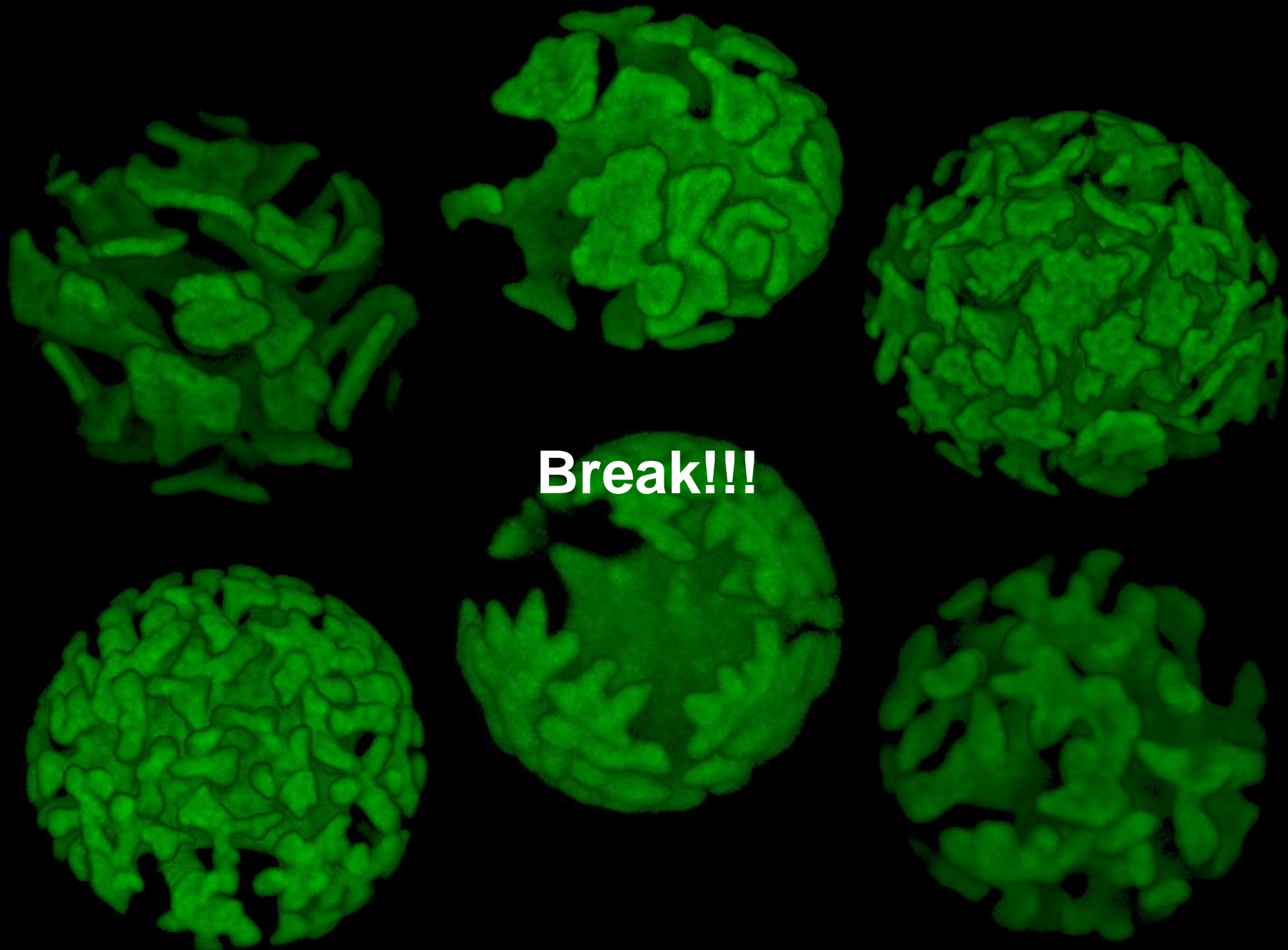
Residuals: 55.8%

Species delimitation in *Asterochloris*

- At the moment, 48 species lineages recognized

- Species delimitation:
  - Morphology
  - Ecology
  - Distribution
  - Mycobiont selectivity

Break!!!