Reconsidering the species problem in downy mildews – where are we now?

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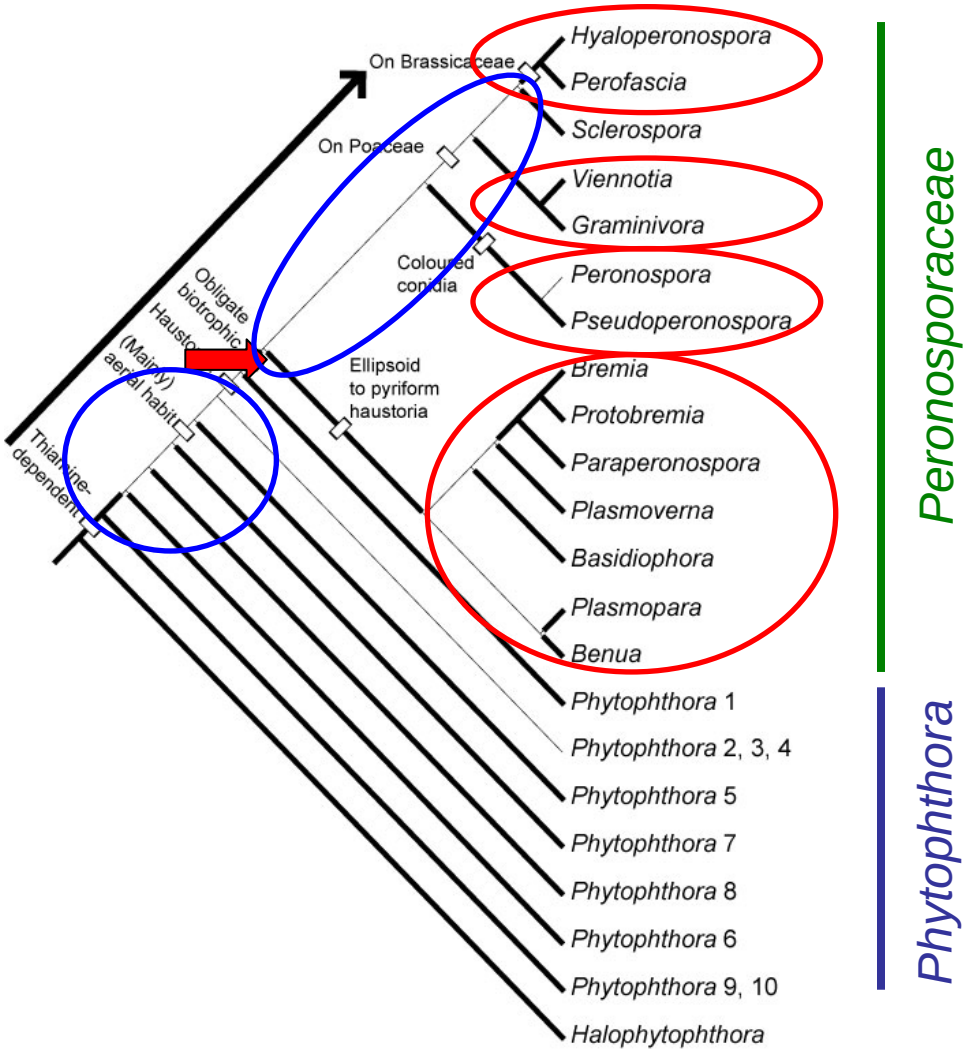
The current talk will focus on...

- historical competing species concepts of downy mildews, their implications and influence
- influence of recent (mainly molecular) evidence for the debate on a revised species concept in downy mildews
- the current status and shortcomings of knowledge on biodiversity of downy mildews
- the status of a molecular barcoding system

Multigene analysis of downy mildews

LSU (D1-D3, D7-D8), cox2, ß-tubulin, NADH (3921 bp)



- downy mildews (Peronosporaceae) are likely to be monophyletic
- downy mildews are rooted within a paraphyletic *Phytophthora*
- circumscription of important genera mostly resolved
- various subgroups of downy mildews highly supported
- relationships between these groups remains mostly unresolved

from Göker et al. (2007): How do obligate parasites evolve? A multi-gene phylogenetic analysis of downy mildews

What are important species problems in downy mildews?

- except for economically important species, most described species are little known and investigated
- lack of sound contemporary investigations on biodiversity
- lack of a sound reference for species identification
- uncritical use of species names
- species identification solely based on host association
- uncertainties about the host ranges of species
- how to delimit and define the species – is a narrow or wide species concept more appropriate (splitting vs. lumping)?
- is the popular and commonly applied “one host family – one parasite” concept appropriate?

What are the main reasons for the species problem?

- comparatively few morphological features available for species delimitation
- few morphological features are commonly variable and overlapping
- cryptic speciation appears to be common (genetically distinct entities lack morphological distinction) – shall they be formally classified?
- obligate parasites – cannot be cultured and investigated on artificial media
- experimentally difficult - many biological experiments which can be carried out in *Phytophthora* cannot practically be applied to downy mildews (crossing experiments, recognition reactions, nutrition requirements,...)
- host range can only be examined by time-consuming inoculation experiments

How many species do we have in downy mildews?

- species circumscription was in the past rather based on personal opinion than on facts
- highly deviating species estimates on downy mildews, depending on the species concept (narrow versus wide):
  - *Peronospora*: from c. 60 to more than 350
  - *Plasmopara*: from c. 80 to 120
  - *Bremia*: from 1 to c. 15

Brief history of species concepts in downy mildews

- **morphological (morphometric) species concept:**
- species delimitation based on morphological features/differences
- problem: few morphological features available; often no clear-cut morphological differences, but a morphological continuum/overlap
- morphological features often influenced by environment
- only few „species“ morphologically distinguishable
- each of these morphological „species“ would have a wide host range
- but: experimental data indicated narrow host range!
- due to these problems, practically, a purely morphological species concept was never applied in downy mildews

Brief history of species concepts in downy mildews

- **Gäumann‘s (1918, 1923) „biological“ species concept:**
- high host specialisation is considered the most important biological feature of species
- species delimitation primarily based on host species/genus, with a combination of morphological features/differences
- result: narrow “one host – one species“ concept - leads to a high number of accepted species (splitting approach)
- problems:
  - host specificity often not experimentally proven
  - morphometric differences between species given by Gäumann often very small, based on few (often single) specimens
  - species cannot be identified if host is unknown, new or unidentified
  - misleads to species identification only by host species
- not widely accepted by plant pathologists who preferred a wide species concept
- more widely applied by investigators of biodiversity

Brief history of species concepts in downy mildews

- **Yerkes & Shaw's (1959) "one host family-one species" concept:**
- accessions from the same host family are classified within a single species if not morphologically clearly distinct
- accessions from different host families are classified as distinct species, even if morphologically not clearly distinct
- result: wide "one host family – one species" concept – leads to few accepted species (lumping approach)
- convenient approach and therefore popular and widely accepted amongst plant pathologists and still commonly used
- problems:
  - misleads to species identification only by host family
  - untested assumption that downy mildews from the same host family are closely related – if not, non-related entities are classified under a single species
  - confusion about host ranges, inoculation sources, incomparable experiments etc.

Modern species concepts

- **Biological species concept (Ernst Mayr, 1982)**
- species are considered/defined as reproductive communities and separated by reproductive isolation
- practically not applicable in obligate parasitic downy mildews due to methodological difficulties (not culturable!)

- **Phylogenetic species concept**
- nowadays the dominant concept due to rapid progress in DNA sequencing techniques
- phylogenies (trees) are used for defining species
- species are defined as distinct, monophyletic entities
- reproductive isolation is mirrored by genetic distance

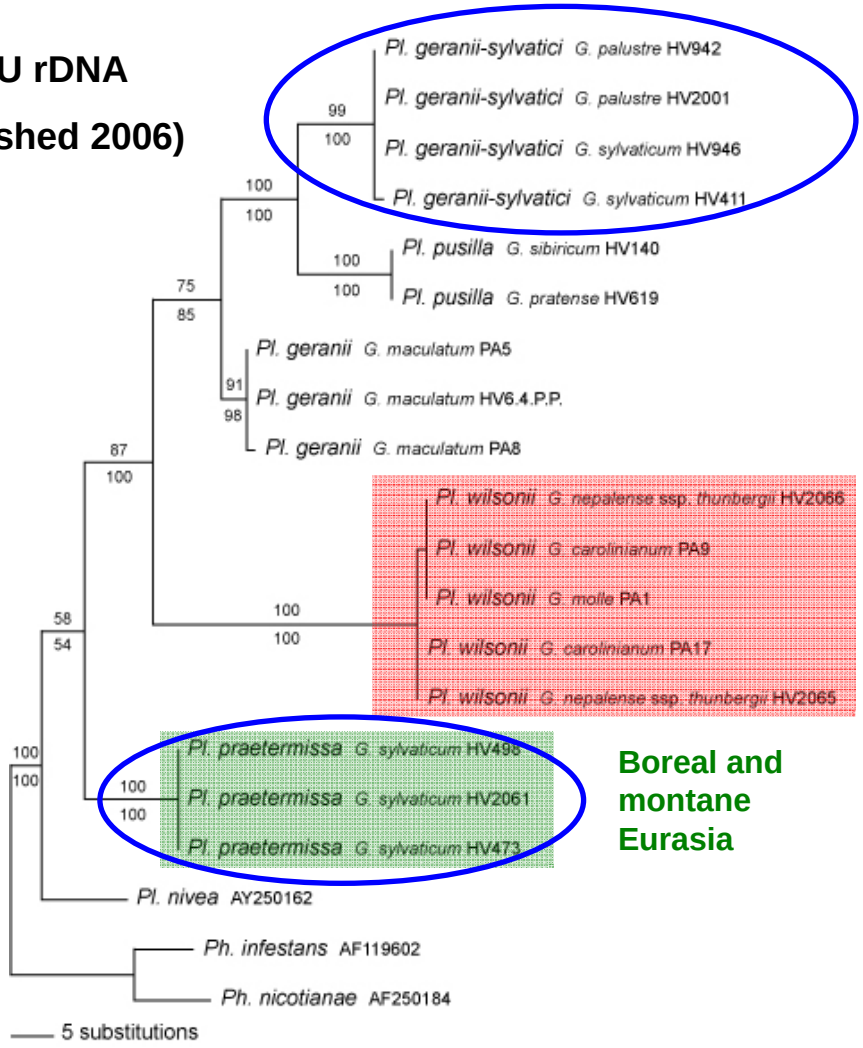
Evidence from recent investigations

- (1) detection of new, morphologically clearly distinct species by thorough re-investigations

Plasmopara on *Geranium*

nu LSU rDNA

(published 2006)



North America & Eastern Asia

Boreal and montane Eurasia

- 2 new species were revealed, which were clearly distinct
- new species are quite common, widespread and sympatric with already described species
- different species can infect the same host species even on the same host individual!
- remained undetected despite clear morphological differences - due to uncritical species determination based solely on host association!

MP tree from Voglmayr & al. (2006), *Mycological Research* 110: 633-645

Evidence from recent investigations

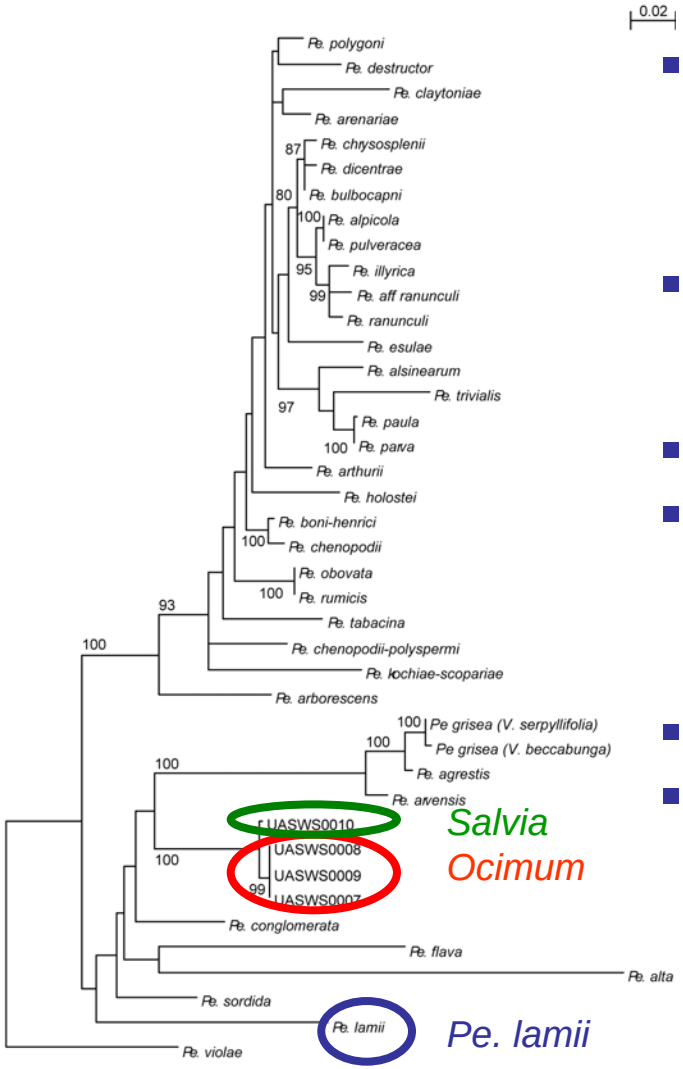
- (1) detection of new, clearly distinct species by thorough re-investigations
- (2) inappropriate species classification by uncritical adherence to the “one host family – one species” concept

The identity of the downy mildew of sweet basil (*Ocimum* spp.)

from Heller & Baroffio, http://www.db-acw.admin.ch/pubs/wa_cma_03_pub_492_d.pdf

- severe outbreaks of downy mildew of basil world-wide from about 2000 onwards.
- Identified as *Peronospora lamii* primarily on host family (Lamiaceae)
- *Peronospora lamii* supposed to be the sole species on Lamiaceae; type host: *Lamium*
- based on distribution records of *Peronospora lamii* on the various hosts, the pathogen was considered to be indigenous in most European, Asian and North American countries
- therefore, the sweet basil pathogen was not included in quarantine lists, promoting rapid spread via infected seed lots

The identity of the downy mildew of sweet basil (*Ocimum* spp.)



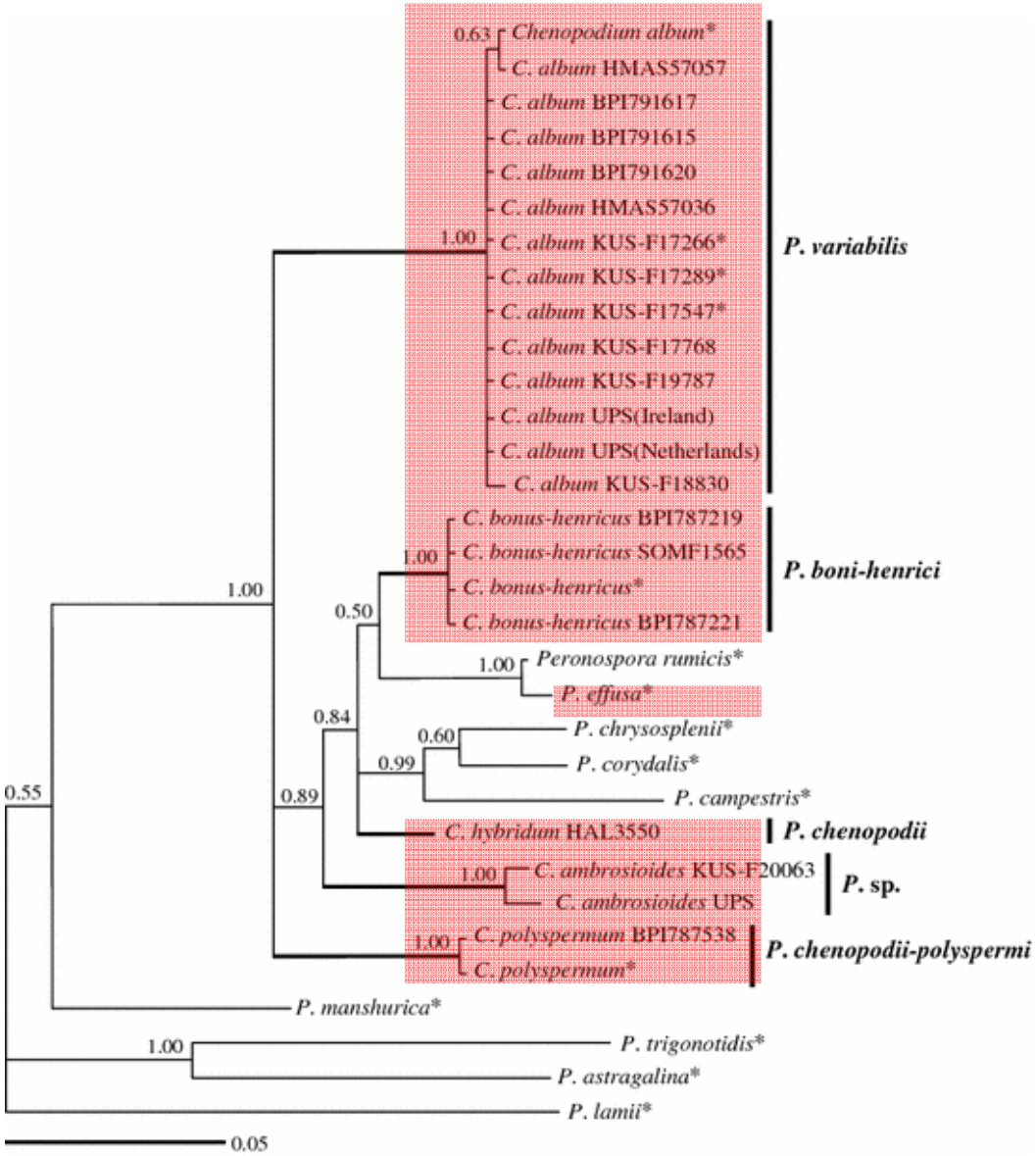
- molecular phylogenetic analyses (ITS rDNA) showed the *Ocimum-Peronospora* to be markedly distinct from *Peronospora lamii*! (Belbahri et al., 2005)
- close but probably not conspecific with the *Peronospora* from *Salvia* - should represent a distinct species
- the species could not be given a name
- problem: altogether, more than 30 *Peronospora* species were described from 23 genera of Lamiaceae, for which no molecular data are available!
- pathogen origin unclear (?Africa)
- recent outbreak on Painted Nettle (*Solenostemon scuttelarioides*), followed by rapid spread

tree from Belbahri et al. (2005), Mycological Research109: 841-848.

Evidence from recent investigations

- (1) detection of new, clearly distinct species by thorough re-investigations
- (2) inappropriate species classification by uncritical adherence to the “one host family – one species” concept
- (3) molecular evidence for a narrow species concept and the re-establishment of previously lumped species

Peronospora on Chenopodiaceae

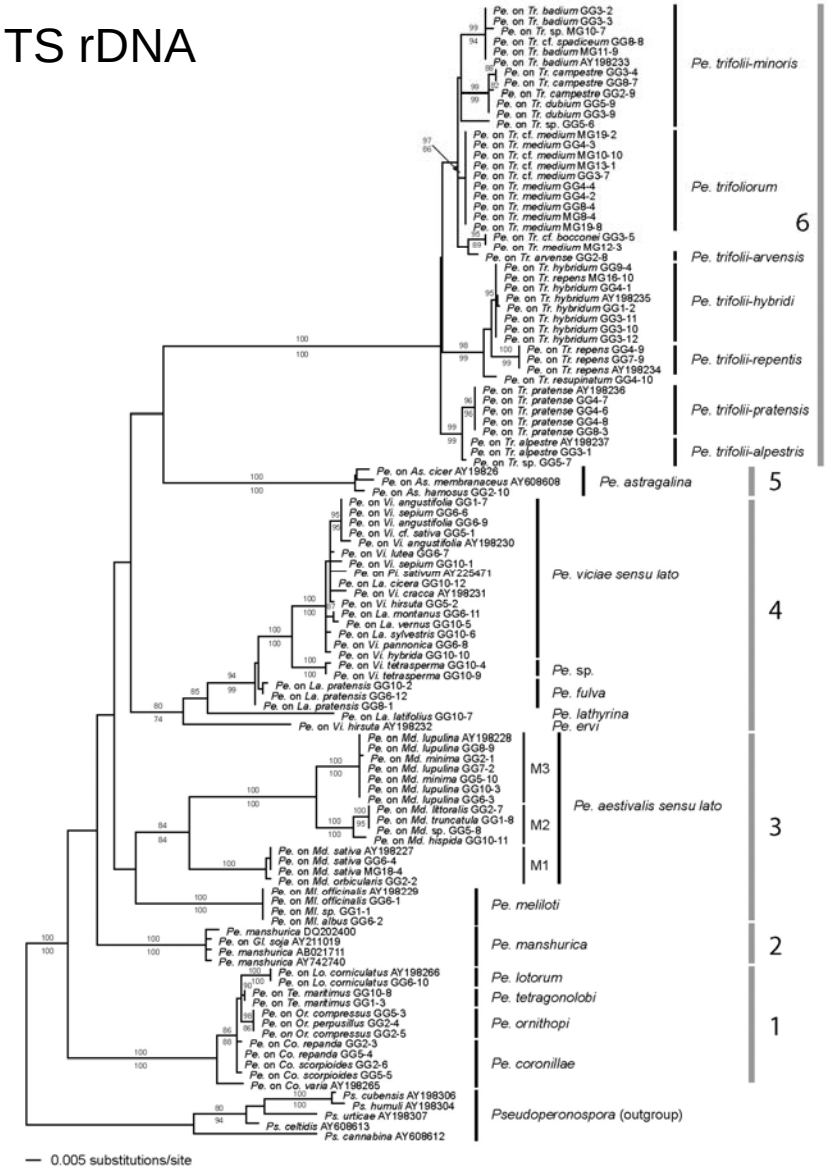


- *Peronospora* on Chenopodiaceae commonly treated as a single species (*Pe. farinosa*), following the concept of Yerkes & Shaw (1959)
- in phylogenetic analyses of DNA data, accessions from Chenopodiaceae are polyphyletic and not closely related
- high genetic distances between accessions from different hosts – evidence for high host specificity
- some subtle morphological differences present
- classification as a single species (*Pe. farinosa*) not tenable

tree from Choi et al. (2008), Mycopathologia 165: 155-164.

Peronospora on Fabaceae

ITS rDNA



- commonly two species accepted (*Pe. trifoliorum*, *Pe. viciae*) (de Bary 1864)
- accessions from different host genera/species are genetically distinct
- accessions from the same host are genetically homogeneous
- species on Fabaceae are not monophyletic
- high host specificity corroborated
- narrow species concept corroborated
- some nomenclatural problems require additional investigations

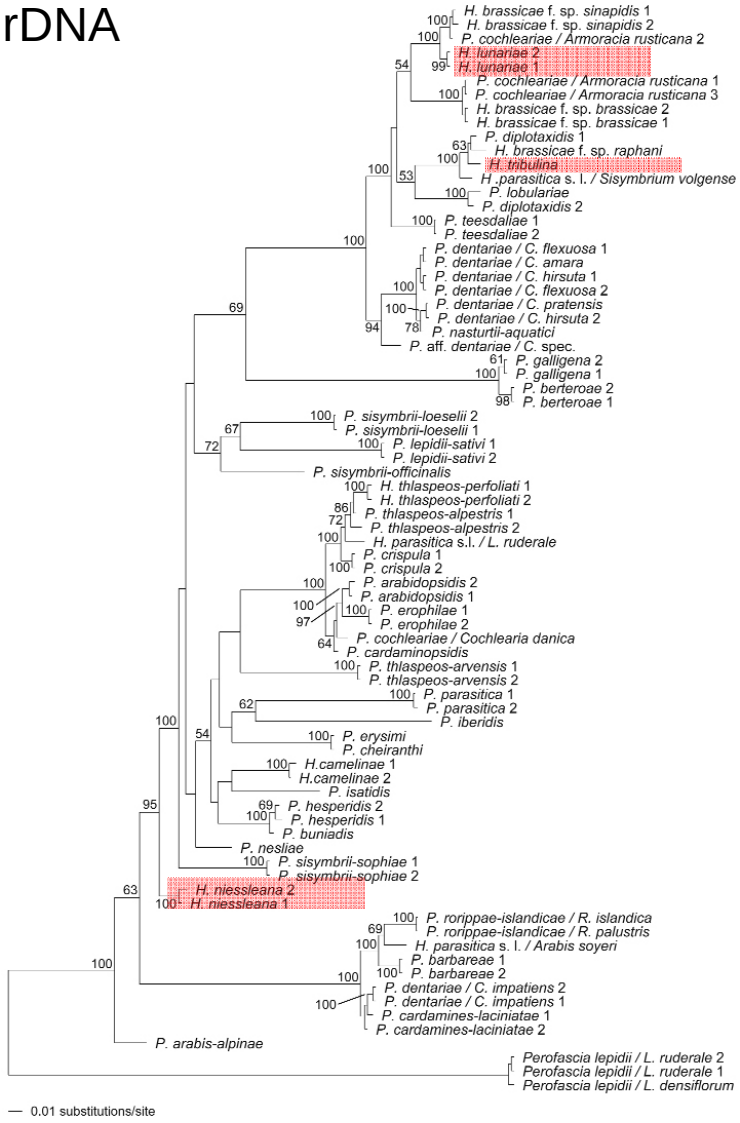
from García-Blázquez & al. (2008), Phylogeny of *Peronospora*, parasitic on Fabaceae, based on ITS sequences. Mycological Research, 112, 502-512

Hyaloperonospora – a case study for downy mildew speciation

- recently split from the genus *Peronospora* (Constantinescu & Fatehi 2002), recognising 6 morphologically distinct species
- numerous host species affected, mainly from *Brassicaceae*
- disagreement about the number of species (from 1 to more than 100!). Gäumann (1918, 1923) applied excessive splitting, whereas Yerkes & Shaw (1959) accepted only one species
- morphological delimitation often impossible
- often lumped into a single species (*H. parasitica*)
- species boundaries and host specificity often unclear
- ideal model group for investigating host-parasite cospeciation

Hyaloperonospora

ITS rDNA

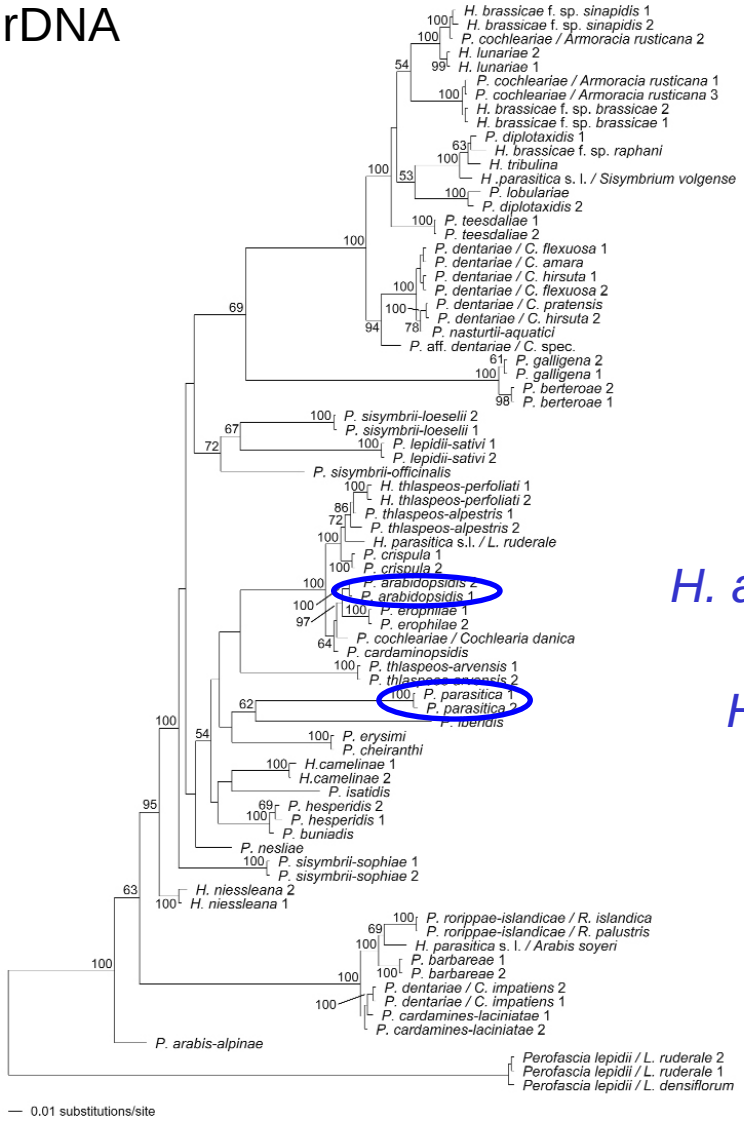


- Göker et al. (2004): morphologically clearly distinct taxa sensu Constantinescu & Fatehi (2002) are embedded within a paraphyletic "*H. parasitica*"
- accessions within a host species/genus genetically uniform
- genetic distances between host specific groups high and consistent
- evidence supports narrow species concept of Gäumann, but investigation included comparatively few accessions

from Göker & al. (2004), Phylogeny of Hyaloperonospora based on nuclear ribosomal internal transcribed spacer sequences

Hyaloperonospora

ITS rDNA



H. arabidopsidis

H. parasitica sensu stricto

Example for genetically distinct entities:

H. parasitica: on *Capsella bursa-pastoris* (type host)

H. arabidopsidis: on *Arabidopsis thaliana* (important species on a genetic model plant)

from Göker & al. (2004), *Mycological Progress* 3: 83-94.

Hyaloperonospora

ITS + nuLSU rDNA

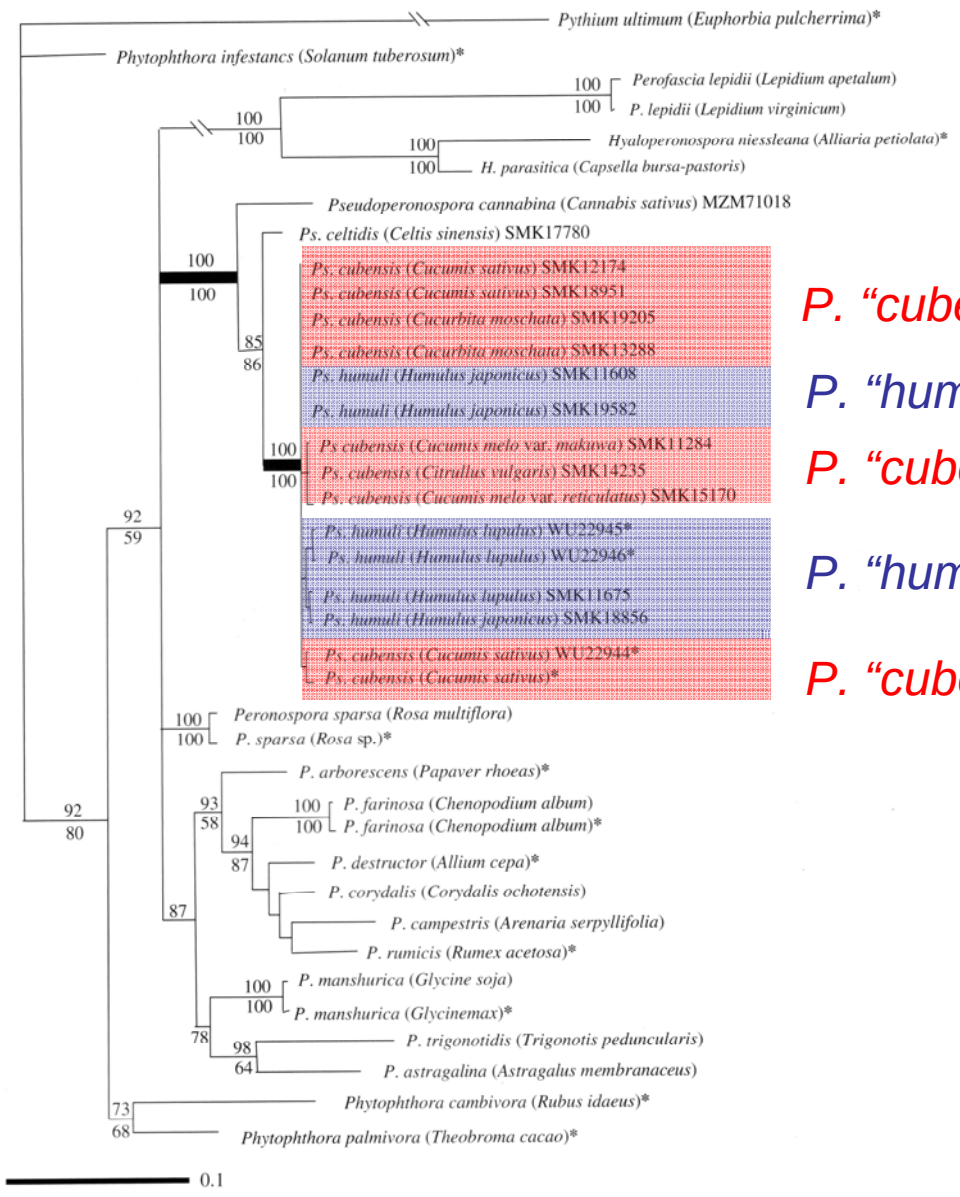
- extensive investigation using more accessions and sequence data (Göker et al., submitted) support previous results
- narrow species delimitation corroborated – high internal support
- the same host can be parasitised by more than one species (e.g. *Draba verna*)
- no evidence for hybridisation
- evidence for several undescribed species

from Göker & al. (submitted), Species delimitation in downy mildews: the case of Hyaloperonospora in the light of nuclear ribosomal internal transcribed spacer and large subunit sequences

Evidence from recent investigations

- (1) detection of new, clearly distinct species by thorough re-investigations
- (2) inappropriate species classification by uncritical adherence to the “one host family – one species” concept
- (3) molecular evidence for a narrow species concept and the re-establishment of previously lumped species
- (4) molecular evidence for a wide species concept and the lumping of species from different host families

Reevaluation of species: *Pseudoperonospora*



P. "cubensis"

P. "humuli"

P. "cubensis"

P. "humuli"

P. "cubensis"

- in *Pseudoperonospora* species were delimited based on host families
- *Pseudoperonospora humuli* on hop (Cannabaceae)
- *Pseudoperonospora cubensis* on cucumber/melon/pumpkin (Cucurbitaceae)
- little genetic and morphological differences between accessions from these 2 non-related families
- molecular evidence for conspecificity

ITS tree from Choi et al. (2005), *Mycological Research* 109: 841-848.

Species identification and molecular barcoding

- identification by molecular tools (sequences) highly reliable
- problem: there is still no consensus about the sequence region of choice
- the ITS rDNA region, a commonly used barcoding region for fungi and also *Phytophthora*, works well in *Peronospora* and *Hyaloperonospora* (especially ITS2)
- however, ITS cannot be used universally for downy mildews due to length polymorphism and presence of numerous repeats in some lineages, in combination with amplification and sequencing problems (e.g. in *Plasmopara*, *Bremia*).
- mitochondrial DNA has better candidates (e.g. cox): high resolution, high number of copies – can be amplified even in historic collections
- however, sequence data on mitochondrial DNA still highly fragmentary and not yet optimised. For downy mildews, specific well-working primers need to be developed for routine use
- species boundaries need to be clarified before a barcoding system can be implemented to avoid taxonomic confusion, which necessitates thorough taxonomic revisions

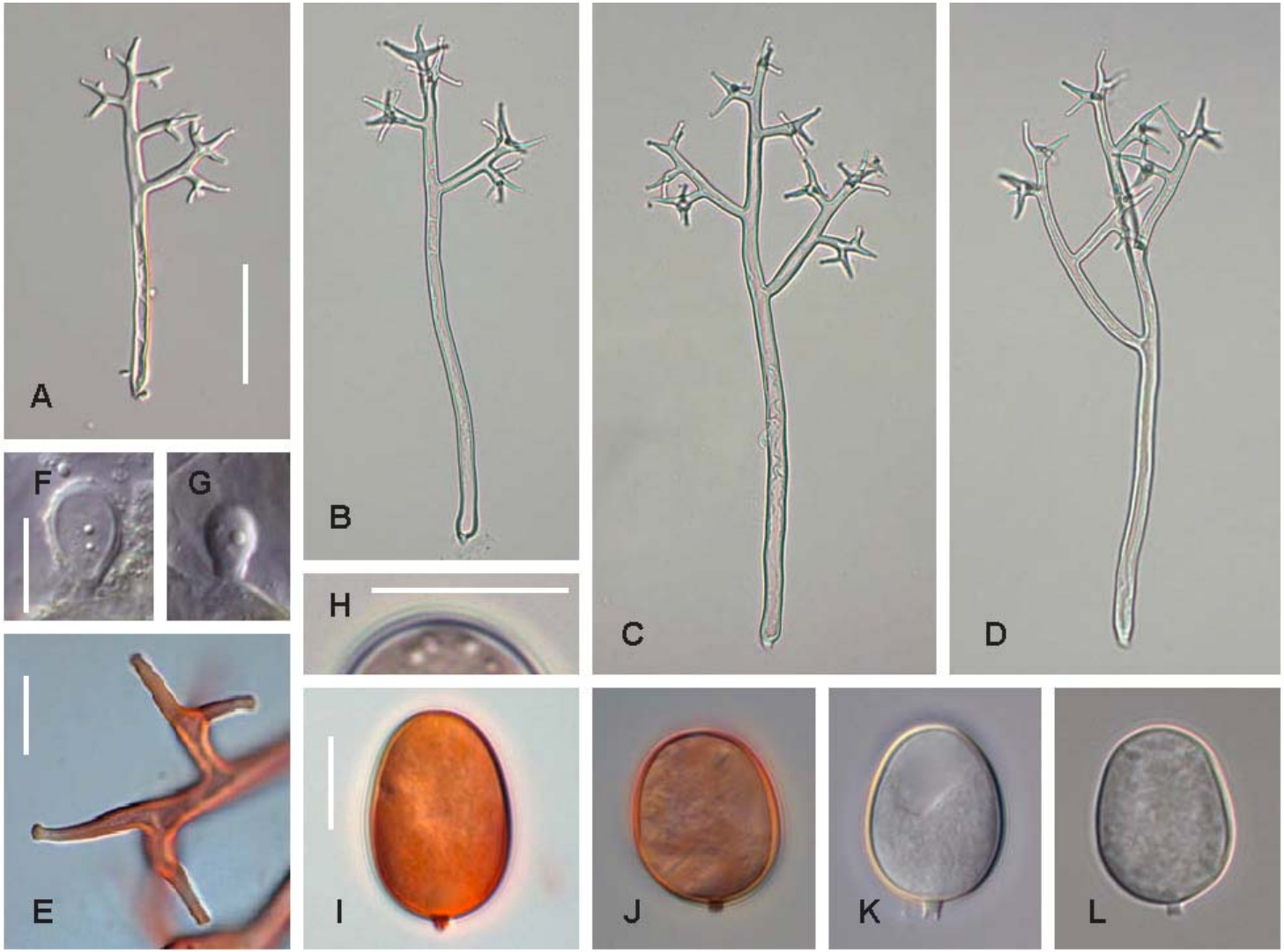
Conclusions

- applying a phylogenetic species concept, a narrow species circumscription seems to be more appropriate in most cases
- narrow host range should be a central factor for genetic isolation and speciation in downy mildews – strong genetic isolation barriers due to host specificity (no evidence for hybridisation, high genetic change)
- host jumps to unrelated hosts occurred frequently, followed by rapid genetic change
- the popular “one host family-one species” concept does not conform with a modern phylogenetic species concept. In addition, uncritical adherence to it can have severe practical consequences and problems (e.g. *Peronospora* on sweet basil)
- more appropriate to formally classify cryptic species
- methodologically, we currently rely on indirect evidence for genetic isolation by molecular data (mainly sequences). Most investigations are based on a single or few sequence regions

Conclusions

- for identification, molecular tools are most reliable and indispensable for downy mildews. For development of a reliable identification system (“barcoding”), additional investigations are needed
- for barcoding, the most important step is the choice of the region to be primarily used. As ITS is inappropriate for some important groups, mitochondrial DNA (cox?) may be a good candidate, which needs additional investigations
- a barcoding approach must be accompanied by thorough taxonomic revisions in order to clarify and stabilise species nomenclature
- additional investigations are also needed to appropriately document the biodiversity of downy mildews. Most detailed biodiversity investigations are more than 50 years old. Numerous distinct species still await description.

Thank you for your attention!



Plasmopara euphrasiae Voglmayr & Constantinescu (2008)

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