Thesis for degree of doctor of philosophy

Confusions in Fungal Systematics

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Abstract

The focus of this thesis has been on fungi with corticioid, polyporoid or stipitate stereoid sporocarps in the Agaricomycetes, but included are also two papers which are methodologically oriented. The family Podoscyphaceae, which is comprised of stipitate stereoid fungi, is studied with regard to representatives of five genera utilizing molecular marker LSU. Species in the family Podoscyphaceae are recovered in orders Agaricales, Hymenochaetales, Polyporales, Atheliales, and in one new order. The new order Stereopsidales, and the family Stereopsidaceae are described after molecular phylogenetic analyses of the nuclear genes *rpb2*, *tef1*, nLSU and nSSU, incorporating *Stereopsis radicans* and the new combination *Stereopsis globosum*, formerly *Clavulicium globosum*. *Clavulicium macounii*, which shares morphological traits with the Stereopsidales, is recovered as the sister lineage to the Stereopsidales or as sister to the Phallomycetidae, and is left *incertae sedis*. Polyporales is studied with genomic data and multi-locus phylogenies, but the species relationships are still difficult to resolve, especially with regard to corticioid and resupinate species. Steccherinaceae, which is comprised of resupinate polypores and resupinate hydnoid species, is studied in more detail. Genera *Antrodiella*, *Steccherinum* and *Junghunia*, are highly polyphyletic, showing once more that the morphological characters used to classify fungi have been misleading. Adding unidentified sequences to phylogenetic studies on any fungal group has an effect on the phylogenetic interpretation which can not be ignored, and it is recommended that significant BLAST hits are included in the phylogeny. Single copy genes inferred from proteomes of 50 Agaricomycotina species have slightly different evolutionary histories, and many splits can not be resolved.

Keywords: Agaricomycetes, order nova, family nova, comb. nova, orthology, homology, paralogy, gene tree incongruence, species tree, systematics, fungi, phylogeny.

Publications

This thesis is based on the work presented in the following papers and manuscripts. The papers are appended to the thesis, and are referred to by capital roman numbers in the text.

I E. Sjökvist, E. Larsson, U. Eberhardt, L. Ryvarden, and K.-H. Larsson. Stipitate stereoid basidiocarps have evolved multiple times. *Mycologia*, 104(5):1046–55, 2012. ISSN 0027-5514.

II R. H. Nilsson, M. Ryberg, K. Abarenkov, E. Sjökvist, and E. Kristiansson. The ITS region as a target for characterization of fungal communities using emerging sequencing technologies. *FEMS microbiology letters*, 296(1):97–101, July 2009. ISSN 1574-6968.

III O. Miettinen, E. Larsson, E. Sjökvist, and K.-H. Larsson. Comprehensive taxon sampling reveals unaccounted diversity and morphological plasticity in a group of dimitic polypores (Polyporales, Basidiomycota). *Cladistics*, 28(3):251–270, June 2012. ISSN 07483007.

IV M. Binder, A Justo, R. Riley, A. Salamov, F. Lopez-Giraldez, E. Sjökvist, A. Copeland, B. Foster, H. Sun, E. Larsson, K.-H. Larsson, J. Townsend, I. V. Grigoriev, D. S. Hibbett. Phylogenetic and Phylogenomic overview of the Polyporales. Accepted manuscript, Mycologia.

V E. Sjökvist, B. E Pfeil, E. Larsson, and K.-H. Larsson. Stereopsidales - a new order of mushroom forming fungi. Manuscript.

VI E. Sjökvist, Y. Bertrand, M. Töpel and B. E. Pfeil. Patterns of congruence in the gene trees of mushroom forming fungi. Manuscript.

ES was responsible for lab work in papers I, III, IV and V, together with EL. ES was responsible for writing papers I, V and VI, and contributed with comments on papers II, III and IV. The analytical work in paper II was shared equally between the authors. ES was main responsible for papers I, V and VI.

The contents of this thesis was produced under the guidance of Bernard E. Pfeil, Karl-Henrik Larsson and R. Henrik Nilsson

Contents

Svensk populärvetenskaplig sammanfattning

Alla kända namngivna arter klassificeras enligt ett hierarkiskt system, som sträcker sig från släkten, familjer, ordningar, klasser och fyla upp till domän. Klassificeringen bygger på släktskap och för svampar har indelningen länge varit baserat på morfologiska karaktärer så som hatt, fot, skivor, rör, och taggar. Indelningen av arter i ordningar och familjer baserat på morfologiska karaktärer stämmer dåligt överens med de släktträd som nyligen gjorts baserad på jämförelser av gener för tickor och skinnsvampar. Tickor karaktäriseras av att sporerna bildas i porer på undersidan av den sporbärande fruktkroppen. Gruppen skinnsvampar karaktäriseras av utbredda, skinnlika fruktkroppar med slät eller fintaggig yta. En nyare klassificering baserad på genetiska studier för alla grupper av svampar publicerades 2007 och sträcker sig från fyla till ordning, men omfattar inte familj- eller släktesnivå.

I den här avhandlingen har jag fokuserat på tickor, skinnsvampar och skinnsvampsliknande grupper med anknytning till ordningen Polyporales, en ordning som omfattar ca 2000 arter. Arbetet inehåller också artiklar om de problem som vi möter när vi gör släktskapsindelningar, både vad gäller metoder och tolkningen av data. Jag har tillsammans med andra kunnat visa att många arter placerade i släktena *Steccherinum*, *Antrodiella*, *Podoscypha*, *Cymatodema* och *Stereopsis*, inte alls är nära släkt, utan behöver föras in i andra släkten, ibland till och med i nya ordningar. En art av släktet *Stereopsis* bildar tilsammans med en tropisk art av släktet *Clavulicium* en ny ordning, som möjligen är syster till underklassen Phallomycetidae, vilken mest består av jordstjärnor, tryffelliknande arter och fingersvampar. En annan art av släktet *Clavulicium*, en nodramerikansk och nordeuropeisk art med utbredning i norra barrskogsbältet, har fortfarande ingen säker placering vad gäller släktskap, därför att våra analyser inte ger några statistiskt säkerställda re-

sultat. Jag presenterar tillsammans med andra ett släktträd för ordningen Polyporales där många släktskapsförhållanden fortfarande är oklara. I två av mina arbeten flyttas fokus från släktskapsförhållanden till själva metoderna för att bedöma släktskap. Anledningen till konflikter i resultaten och brist på signifikans diskuteras i den sista artikeln i avhandlingen.

Synopsis

This is a thesis on fungal systematics. Papers I, III, and V attempt to place taxa in an evolutionary framework based on molecular phylogenetics. The thesis also includes papers which are theoretically oriented, and where theories of phylogenetics and evolution are applied to empirical data sets. Paper II evaluates the effects on interpretation of molecular phylogenies when unidentified taxa in public databases are included in phylogenetic analyses of fungi. Paper IV evaluates molecular markers in Polyporales Gäum based on informativeness profiles, and ten Polyporales Gäum genomes, in addition to supermatrix analyses on a smaller number of markers and many taxa. Paper VI attempts to find the answerer to why the Agaricomycete tree is unresolved, and evaluates the alternative models of a tree versus the network representation of fungal evolution. The main focus of the thesis is on fungi with smooth or polyporoid hymenophore morphology in the Agaricomycetes Dowell.

Glossary

bracket fungi Fungi where the sporocarps forms shelves on trees or shrubs (fig. 1a paper III).

corticioid Fungi resembling those of genus *Corticium* Pers. go under the description corticioid. These have a sporocarp with a flat hymenium surface, and no stipe. They also go under the name crust fungi (fig. 1f paper III).

cystidia Sterile organ in the sporocarp, usually of a distinctive shape (fig 2a paper III).

gasteroid Enclosed sporocarp, such as for instance in puffballs and truffles.

gilled Lammellate.

hydnoid Resembling species of genus *Hydnum L.*, in the sense that the sporocarp bears spines or teeth where the spores are produced (fig. 1i paper III).

hymenophore Spore bearing structure of the sporocarp.

lamellate Having lamellae / gills where spores are formed.

polyporoid Species with sporocarps that have many pores.

poroid Having a surface with many pores.

sporocarp The structure of sexual reproduction, where the spores are formed, also known as fruiting-body and often equivalent to the mushroom of the fungus (fig. 1 paper III). Mushroom is the term for fleshy sporocarps.

1

A background to Agaricomycete systematics

The Agaricomycetes Dowell is perhaps the most conspicuous taxonomic group of fungi. It includes the edible *Agaricus bisporus* (J.E. Lange) Imbach (Button mushroom), the poisonous *Amanita virosa* (Fr.) Bertill. (Destroying angel), *Laetiporus sulphureus* (Bull.) Murrill (Sulfur shelf), and wood pathogens such as *Armillaria spp.*(Fr.) Staude and *Heterobasidion annosum* (Fr.) Bref. Many species have less showy sporocarps, and are by far more difficult to identify. Many more have only been recognised as Agaricomycetes Dowell from studies using molecular data.

In early classification of fungi, and up until recently, sporocarp morphology was central. The most recent major revision of fungal classification is that of Hibbett et al. [2007], which is based on many separate papers on fungal molecular phylogenies. A number of new classes and orders have been described from molecular studies since. Amongst these, two new orders were added to Agaricomycetidae Parmasto, bringing the number of orders in the Agaricomycetes Dowell up to 19.

The molecular classification by Hibbett et al. [2007] breaks completely with previous morphological classifications. In essence, it means that sporocarp morphology and hymenophore configuration no longer define any higher groups of fungi and that species with e.g., corticioid, polyporoid or gilled sporocarps, are now scattered among the orders of Agaricomycetes Dowell. Morphological characters are still given a key role in description or reclassification of fungal taxa, and the revision of Hibbett et al. [2007] included both morphological descriptions as well as cladistic delimitations. In this thesis I

will discuss Agaricomycetes Dowell in the sense of Hibbett et al. [2007].

1.1 Challenges in Agaricomycete classification - Morphology

Despite the struggles to describe higher taxa in Agaricomycetes Dowell using monophyly and morphology, none of the higher ranked taxa, i.e. subclasses and orders, have a single unambiguous morphological synapomorphy. Corticioid fungi are present in all orders of the Agaricomycetes Dowell [Binder et al., 2005, Hosaka et al., 2006, Larsson, 2007, Binder et al., 2010]. Fungi with a gilled, hydnoid or poroid hymenium are present in several orders [Larsson and Larsson, 2003, Matheny et al., 2007, Miettinen et al., 2012], and though the gasteroid fruiting-body forms, where the hymenium is enclosed, are mainly represented in the Phallomycetidae K. Hosaka, Castellano Spatafora, there are also examples of gasteroid forms in the Agaricales Underw., Boletales E.-J Gilbert, and Russulales Kreisel [Blackwell et al., 2007, Hosaka et al., 2006, Larsson and Jeppson, 2008, Wilson et al., 2011].

Micro-morphological characters are numerous among most taxa, but often so varying within even a genus that they become of little help in identification to order level. Nevertheless, some species have characters which are so distinct that the species can be placed directly in a genus whose order placement is known. One such example is the very special cystidia in some species in *Pluteus* Fr. Some characters, though not synapomorphies, are present exclusively in certain higher ranked taxa. For instance, setae (brown multi rooted cystidia) are only found in species in Hymenochaetales Oberw., and sulfocystidia are found in species in the Russulales Kreisel. Some generalizations can be made on fruiting body morphology still, and at a glance it is possible to make an educated guess as to where a species has its origin. Brown corticioid fungi for instance are most likely to belong in the Thelephorales Corner ex Oberw., at least if they have spiny spores. A lamellate fungus is probably member of Agaricales Underw. or Russulales Kreisel, bracket fungi are mostly occurring in the Polyporales Gäum or Hymenochaetales Oberw., and stipitate fungi with soft loosely attached poroid hymenia occur mainly in the Boletales E.-J Gilbert.

1.2 Challenges in Agaricomycete classification - Taxon sampling

Only a fraction of the described species have been sequenced for any molecular marker, and though there has been a rapid increase in sequences deposited in GenBank, there is an almost equal number of fungal sequences deposited where the species identity is unknown [Ryberg et al., 2009]. The setting for a molecular phylogeny is thus that we can never assume a complete taxon sample, even if we include all known extant species. Another complication when designing a phylogenetic study above genus level is the uncertainty of which species to include and where the sampling focus should be made. As is shown in studies I, III, IV, and V, the signal from molecular data shows that the genera previously included in the Aphyllophorales Rea, which are defined on morphological criteria, are very often poly- or paraphyletic, and even species which are quite similar may belong to different orders (I, III and V).

Complete taxon sampling of the ingroup increases the phylogenetic accuracy [Rannala et al., 1998], but even without complete taxon sampling an increase in taxon sampling has positive effects on the phylogenetic inference as shown from Angiosperm phylogenies and simulation studies [Zwickl and Hillis, 2002, Hillis, 1998, 1996, Pollock et al., 2010]. Townsend and Leuenberger [2011] offer an explanation for the positive effects of intensified taxon sampling from a mathematical perspective. When the taxon relationships are largely unknown increasing the taxon sampling may seem cumbersome, but a pragmatic approach of taxon sampling is offered in paper II. As a complement to including taxa with a high morphological resemblance, taxa with a high genetic resemblance can also be included regardless of the taxon identity. Paper II investigates the effect of including insufficiently identified sequences from public databases in molecular phylogenetic studies of fungi published during 2009.

1.3 Challenges in Agaricomycete classification - Choosing molecular markers

A small number of genes have been extensively used in fungal phylogenetic studies. The ribosomal genes, which are used in studies of all organism

groups, are also the most frequently sampled in fungi. The large and small sub units of the ribosomal genes (LSU and SSU) together with the protein coding genes *rpb1*, *rbp2* and *tef1*, were chosen by the fungal tree of life project (AFTOL1) as nuclear molecular markers [Spatafora, 2005]. Mitochondrial SSU and *atp6* were also chosen by AFTOL1, but are used to a lesser extent. Utilizing the same molecular markers over several groups is essential for a stable classification, as it is impossible to compare placement of taxa relative to one another otherwise. The classification of taxa within the Agaricomycetes Dowell are all based on molecular phylogenies of one or several of these molecular markers, where stated.

2

On the matter of Orthology

A goal in systematic studies is to describe evolutionary relationships between taxa. Therefore, when utilizing molecular sequence data for inferring species relationships, the aim is to use molecular markers that are orthologous (fig 2.1a) - genes descended through speciation. The concept of orthology springs from the concept of homology [Fitch, 2000]. Ribosomal genes are, due to concerted evolution, homogenized within the gene family, so that the genes are more similar within the gene family of a species than the repeats are to the sister taxon [Ganley and Kobayashi, 2007]. This makes the ribosomal genes well suited for phylogenetic studies, despite not being strictly orthologous. The coherence of the ribosomal genes has however been questioned [Rooney and Ward, 2005].

For any other marker the orthology assessment is even more dubious, unless the species tree is known. In fact, there is no guaranty of orthology even for single copy genes [Scannell et al., 2007]. Thus, despite some doubts as to the equivalence of orthology in the ribosomal genes, including at least one ribosomal gene in a study where orthology is not tested seems like a reasonable requirement. Using many markers and testing their topology against one another is the first step towards a stable species topology, but even markers that are orthologous can result in different trees. Table 2.1 summarizes potential origins of conflict between gene trees.

When comparing incongruent gene trees the challenge is to elucidate which of the above is the cause of conflict. When the origin of conflict is biological, the gene trees, even when correct, are expected to differ. When the origin of conflict is methodological, the inferred gene tree may be incorrect. Applying statistical supports such as bootstrap and posterior probabilities is

Table 2.1: A summary of biological processes and methodological errors that may lead to gene tree incongruence.

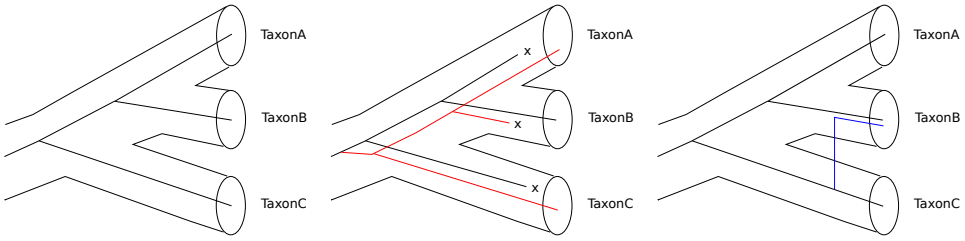
Biological origins of conflict	Methodological origins of conflict
Incomplete lineage sorting	Comparison between non homologous markers
Paralogy	Alignments include non homologous sites
Whole genome duplication	Undetected recombination
Hybridization events	Poor model choice
Rapid radiations	Poor taxon sampling
Introgression	
Lateral gene transfer	

one way of measuring the reliability of the gene tree. In a comparison between the phylogenetic signals of a few markers only, as in studies III and V, knowing which is the origin of conflict is impossible, as there are not enough markers to compare the signal between them.

2.1 Incomplete lineage sorting and paralogy

Paralogy and incomplete lineage sorting are similar in appearance. The interpreter may be decieved into thinking that the species tree split is as deep as the paralogous/deep coalescent split it corresponds to in the gene tree [Liu and Edwards, 2009, Degnan and Rosenberg, 2009]. Figure 2.1b illustrates gene duplication followed by random gene loss.

The events leading to these disparate signals are slightly different. Incomplete lineage sorting is due to the presence of multiple alleles at a locus at the time of speciation, and a loss of alleles after speciation [Kingman, 1982, Degnan and Rosenberg, 2009]. Paralogy, on the other hand, is due to a gene duplication, that is, a second locus coding for the same gene is created. At the time of speciation two or many gene copies are present, but all copies are not retained to present day [Fitch, 2000].



(a) (b) (c)

Figure 2.1: Species trees containing gene trees. a) An orthologous relationship between gene tree and species tree. b) A gene duplication in red, followed by gene loss represented by x, or incomplete lineage sorting, where a new allele is represented in red, followed by a loss of alleles, represented as x. c) Horizontal gene transfer (or introgression) represented as a blue line.

2.2 Whole genome duplication and rapid radiations

A special case of gene duplication is the whole genome duplication, which leads to multiple paralogous gene copies due to a period of gene loss following the duplication event [Scannell et al., 2007]. The duplicated genes will all trace back to the same event, but the loss of genes, though more frequent in some gene families, is random in time. Thus genome duplication will make species tree inference from gene trees much more difficult.

A rapid radiation is an event where many species/lineages are formed in such a short time interval that the appearance is that of a single speciation event leading to many species. Rapid radiations cause conflict among inferred gene trees for two reasons; the time for lineage sorting is decreased [Degnan and Rosenberg, 2006, Kubatko and Degnan, 2007], and the inference of the correct tree is more difficult, as there may be no time for informative mutations to occur between each speciation event [Whitfield and Lockhart, 2007].

2.3 Horizontal gene transfer and introgression

Horizontal gene transfers, are events where DNA is incorporated into the genome of a species from another species. This is in contrast to linear descent, where genes are inherited from the ancestor of a species. A special case of horizontal gene transfer is introgression where a species incorporates DNA from other species through hybridization and back crossing [Andersson, 1953, Rhymer and Simberloff, 1996, Soltis and Soltis, 2009]. The expected pattern from horizontal gene transfer is a gene tree relationship where the split between genes is more recent then the separation of the taxa (in a species tree) which are compared (fig 2.1c).

2.4 Inferring species trees from incongruent gene trees

There is at present no algorithm or program developed for telling incomplete lineage sorting and paralogy apart. From whole genome studies paralogy can be detected based on gene location [Scannell et al., 2007].

There are several programs that claim to make species trees out of gene trees, or out of genes, which deal with and compensate for incomplete lineage sorting [Kubatko et al., 2009, Liu et al., 2009]. Gene family evolution is usually explored by gene tree - species tree reconciliation methods, where prior knowledge of the species tree is assumed. These use either a parsimony approach to minimise gene duplications, or a birth-death process [Sennblad and Lagergren, 2009, Sung, 2011]. Introgression (HGT) is dealt with in a program by Linz et al. [2007], whereas hybridization is dealt with by programs STEM [Kubatko et al., 2009] and Padre [Lott et al., 2009]. However, no program can model all biological processes that lead to gene tree/species tree discordance.

2.5 Super-matrices, Super-trees, Super-networks, or The multispecies coalescent?

At present, several different approaches exist for the way in which species relationships are analysed and presented. There is however no way of analyzing genetic data that takes all evolutionary events causing conflict or incongruence between gene trees into account.

The argument for the super-matrix approach is based on the total evidence theory. What is often forgotten is the underlying assumption of total evidence [Queiroz et al., 1995]. Concatenating genes to make a species tree assumes that the genes share history and that the reason why many genes are needed is that they individually do not carry enough signal to resolve the species tree.

The super-tree approach follows the same principle as the super-matrix. Although the genes are allowed to evolve under separate tree models, the gene trees are later merged to form one species tree.

The super-network approach does not make any assumptions as to the cause of conflict between gene trees, but plots every split, so that reticulate events are mapped and a tree is not assumed.

The multispecies coalescent model, is based on the coalescent model and accounts for incomplete lineage sorting. However, this assumes that the only cause of conflict is incomplete lineage sorting.

2.6 Phylogenomic inference

With genome data increasing in availability [Grigoriev et al., 2012], it is becoming possible, at least for mycologists, to study evolutionary processes that cause major conflicts in the data for most groups. Yet, the phylogenomic studies published today are using concatenation to a large extent [Nery et al., 2012, Floudas et al., 2012, Morin et al., 2012], overshadowing any conflicts the data might hold, while still receiving high bootstrap values and resolved trees.

Why do concatenated datasets recieve higer bootstrap supports? Kumar et al. [2012] argues that with more data the accuracy does not increase but the variance decreases, leading to inflated p-values. A biological explanation might be that short branches inside the species tree give the lineage sorting process less time, increasing the risk of falsely supported trees, especially with

concatenation [Kubatko and Degnan, 2007]. For whatever reason, concatenation of genome data can lead to significant support of opposing theories in genome studies [Philippe et al., 2011]. Species tree reconstructions from genome data should preferably be estimated from gene trees in similar ways to those of the multi species coalescent model [Boussau et al., 2013], making use of the inparalogs. Investigating genetic congruence [Leigh et al., 2011b,a] is a step on the way towards a consistent classification, and a way to also infer biological processes that cause conflict.

3

Contributions to the field

As the classification of fungi is now being built on molecular phylogenetics, it is important to consider the value of phylogenies constructed from a few genes, where orthology has not been tested and gene tree incongruence not accounted for. The papers included in this thesis, which deal with molecular phylogenies, should be viewed as indicators of species relationships.

Papers I, III, IV, and V present species relationships of corticioid, polyporoid and stipitate stereoid species. The studies confirm that molecular data and morphological data correlate poorly, and that few if any recognizable morphological synapomorphies support the molecular species trees based on a few genes. With that in mind, the congruence of the molecular markers becomes even more important, because there is no morphological backbone to lean on. The congruence of molecular markers is explored in paper V, and explicitly studied in paper VI. Paper V also presents a new order, a new family and a new combination.

3.1 Paper summary

Paper I is a molecular phylogenetic study of stipitate stereoid taxa. Species of five genera in the Podoscyphaceae D.A. Reid are assigned to orders Agaricales Underw., Polyporales Gäum, Hymenochaetales Oberw. and Atheliales Jülich. One stipitate stereoid species, *Stereopsis radicans* (Berk.) D.A. Reid, cannot be assigned to an order, instead it forms a clade with the corticioid species *Clavulicium globosum*. This two taxon clade appears as sister to Phallomycetidae Hosaka, Castellano, Spatafora, Cantharellales Gäum and

the remaining Agaricomycetes Dowell.

Paper II deals with the impact of taxon sampling on gene tree inference. We re-analysed datasets with and the addition of incompletely identified sequences (IIS) from public databases. This study was done without regard to bootstrap support as the study was designed to copy and problematise phylogenetic studies as they are usually performed by molecular systematists. We show that including IIS has an effect on the tree inference, and we recommend the inclusion IIS in molecular phylogenetic studies of fungi.

Paper III is a study of resupinate polypores and hydnoids in the Polyporales. Molecular phylogenetic analyses of *Antrodiella* Ryvarden I. Johans., *Junghuhnia* Corda and *Steccherinum* Gray, from several markers, reveal a high morphological plasticity in the group.

Paper IV gives an overview of the Polyporales Gäum.

Paper V presents the order Stereopsidales Sjökvist, E. Larss., B.E. Pfeil K. H. Larss., the family Stereopsidace Sjökvist, E. Larss., B.E. Pfeil K. H. Larss., and the combination *Stereopsis globosum* Sjökvist. The two species from paper I which could not be assigned to order are studied in detail. Separate analyses of nuclear markers *rpb2*, *tef1*, LSU and SSU, each support a clade of *Stereopsis radicans*(Berk.) D.A. Reid and *Stereopsis globosum* Sjökvist (formerly *Clavulicium globosum* Hjortstam Ryvarden). A third species, *Clavulicium macounii* (Burt) Parmasto, which shares morphological characters with the *Stereopsis* clade, cannot assigned to an order, though in some analyses it appears as sister to the *Stereopsis* clade.

Paper VI explores the phylogenetic signals given by single copy genes, also normally treated as putative orthologs, and the reliability of these inferences. We are able to show that all single copy genes from 50 genomes have unique topologies, and that there is a lot of conflict among splits above order level. The correlation between bootstrap and genome support is low for the splits we investigated. Before any approach is taken to accommodate the gene trees in a species tree, processes that can lead to conflicts in the data should be explored. Paper VI presents a work-flow in which this can be surveyed.

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