Choosing one name for pleomorphic fungi: the example of Aspergillus v. Eurotium, Neosartorya and Emericella

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The latest International Code of Nomenclature of algae, fungi and plants (ICN, McNeill & al.*, 2012) requires that each fungal species have only one name. This change poses a dilemma for many fungal taxonomists, as many fungi are pleomorphic, having one or more generic names for sexual states associated with one or more asexual states. Here, to explore this general problem we focus on the asexual genus *Aspergillus.* This genus is characterised by a well defined asexual fruiting structure, but is very broad in concept, as it is associated with eleven sexual state genera (Geiser, in Med. Mycol. 47, Suppl. 1: S29. 2009; Houbraken & Samson, Stud. Mycol. 70: 1. 2011).

To address this taxonomic and nomenclatural problem for *Aspergillus*, two proposals have been published. One advocates applying the name *Aspergillus* to as many clades as possible that possess the asexual reproductive morphology characteristic of *Aspergillus* without having to include the asexual genus *Penicillium* Link (Houbraken & Samson, *ibid.* 2011). This we will refer to as "Wide Aspergillus". The second advocates recognizing the diversity in morphological and physiological phenotypes among these fungi, by maintaining the sexual names in some of these clades, restricting the name *Aspergillus* to one or more closely related clades, thereby preserving *Aspergillus* for many important species names (Pitt & Taylor, Mycologia 106: 1051. 2014). This approach we will refer to as "Narrow Aspergillus".

Although Wide Aspergillus has the natural appeal of preserving a large, important genus, phylogenetic problems exist and concern both exclusion and inclusion. In terms of exclusion, several taxa that possess *Aspergillus* anamorphs (e.g. the sexually defined genus *Sclerocleista* Subram. and the species, *A. clavatoflavus* Raper & Fennell*, A. zonatus* Kwon & Fennell*,* and *A. penicilliformis* Kamyschko) must be left outside Wide Aspergillus because including them would require also including the genus *Penicillium.* In terms of inclusion, some taxa that lack the Aspergillus anamorph (the genera *Polypaecilum* G. Sm. and *Phialosimplex* Sigler & al. and the species *Basipetospora halophila* (J.F.H. Beyma) Pitt & A.D. Hocking must be included in Wide Aspergillus to maintain monophyly. Also in terms of inclusion, some widely accepted, sexual genera, based on distinct sexual and physiological phenotypes associated with socially important attributes of spoilage, disease and research, i.e. *Eurotium* (F.H. Wigg.) Link*, Neosartorya* Malloch & Cain and *Emericella* Berk. respectively, lie inside Wide Aspergillus. In pursuit of a nomenclature that supports a large and diverse genus *Aspergillus*, a taxonomy has been proposed that ignores these biological and socially important differences (Samson & al., Stud. Mycol. 78: 141. 2014). As we show here, in

doing so, the normal process of using biological data to inform taxonomy, and then establishing a nomenclature to reflect taxonomy, has been reversed.

Alternatives to Wide *Aspergillus* are available, because valid names based on sexual morphology exist that recognize the phenotypic diversity of these fungi. Simply using this existing nomenclature would implement a taxonomy based on sound biological characters, but would result in the loss of the name *Aspergillus*. More particularly, because the type of *Aspergillus* resides in the *Eurotium* clade, both generic names cannot validly be maintained without a nomenclatural change. If the type of *Aspergillus* were moved from *Eurotium* to another biologically defined clade, it would be possible to achieve a nomenclature that reflects a sound biological taxonomy and also preserves *Aspergillus*. Such a change is readily accomplished by conservation. Based on phylogenetic analysis and social importance, *Aspergillus* subgenus *Circumdati* is the logical clade, and *A. niger* Teigh. the logical species (Pitt & Taylor, *ibid.* 2014). A proposal for a change in the type of *Aspergillus* is the subject of a separate submission (Pitt & Taylor, Taxon, in preparation or submitted.2015).

It is important to note that the proposed neotypification does not affect the debate between Wide and Narrow *Aspergillus*, because both proposals for redefining *Aspergillus* would be compatible with the change in type.

It is an axiom that taxonomy should be based on the best biology, both genetic and phenotypic, and that both should advise nomenclature. Recent phylogenetic analyses of fungi with *Aspergillus* anamorphs have resulted in two topologies, one developed by Houbraken & Samson (*ibid.* 2011) (Figure 1), and the other by Pitt & Taylor (*ibid.* 2014) (Figure 2). These topologies both indicate that support for the deep branches is weak, although support for the individual clades is often strong. The phylogenetic trees in Figures 1 and 2 were developed using the same data, originally obtained in a broad investigation of the *Aspergillaceae* (Houbraken & Samson (*ibid.* 2011). To focus on *Aspergillus*, Pitt and Taylor (*ibid.* 2014) reduced the taxon sampling to clades populated only by species with *Aspergillus* morphology plus the nearest outgroup, *Thermoascus* and allies.

Likelihood ratio testing using the Shimodairo Approximately Unbiased Test (Shimodaira, Syst. Biol. 51: 493. 2002) can be used with the sequence data studied in Houbraken and Samson (*ibid.* 2011) and Pitt and Taylor (*ibid.* 2014) to assess the size and inclusiveness of *Aspergillus* under the two proposals, and to compare the validity of the topologies in Figures 1 and 2. The first test involves the widest possible genus *Aspergillus*, which would include all species having an *Aspergillus* anamorph, i.e. of all white clades in Figures 1 and 2. Comparison of the most likely phylogenetic tree with no constraints with one where all *Aspergillus* species are constrained into one monophyletic branch results in rejection of the constrained, widest possible *Aspergillus* phylogeny (Table 1). To determine if the rejection was due to the inclusion of *Sclerocleista*, the most basal clade in Figure 1, S*clerocleista* was excluded from a second test. Again, the widest possible *Aspergillus* phylogeny was rejected (Table 1). Similarly, rejection resulted if *A. zonatus* or *A. penicilliformis* (but not *A. clavatoflavus*), was included in the widest possible *Aspergillus* (Table 1). Thus, it is not possible to make a monophyletic genus *Aspergillus* that includes all of the species, and only those species, possessing *Aspergillus* morphology.

The second test involves Wide *Aspergillus* and its inclusion of taxa lacking *Aspergillus* morphology (i.e. *Phialosimplex* and *Polypaecilum*). When Wide Aspergillus

was constrained to include only species with *Aspergillus* morphology by excluding *Phialosimplex* and *Polypaecilum*, the Shimadairo Approximately Unbiased test rejected the resulting phylogeny as significantly less likely than the unconstrained tree (Table 2), which also included *Penicillium* species.

The third test involves Wide Aspergillus and the inclusion of *Penicillium*, as shown in Figure 2. When Wide Aspergillus was constrained to exclude *Penicillium* species, although the resulting tree was slightly less likely than the most likely unconstrained tree, it was not significantly less likely.

. To summarize the results of likelihood ratio testing, attempts to bring together all of the species with *Aspergillus* morphology to make the widest possible, truly inclusive genus *Aspergillus* are rejected. Similarly, attempts to create a Wide Aspergillus that excludes taxa lacking the *Aspergillus* morphology, that is, *Phialosimplex* and *Polypaecilum*, are rejected. Finally a Wide Aspergillus that includes *Phialosimplex* and *Polypaecilum* but excludes *Penicillium* is not rejected. The most likely phylogeny is reflected in Figure 2, where Wide Aspergillus is rendered non-monophyletic by the inclusion of *Phialosimplex* and *Polypaecilum* as well as *Penicillium*.

Recently, Samson and colleagues (Samson & al. *ibid.* 2014) addressed these problems of non-monophyly of Wide Aspergillus. To solve the non-monophyly due to the inclusion of two genera lacking *Aspergillus* morphology, *Phialosimplex* and *Polypaecilum*, they simply applied the name *Aspergillus* to these fungi. Thus, Wide Aspergillus was enlarged to embrace fungi that lacked the defining morphology of *Aspergillus*. This approach could not be applied to the non-monophyly caused by *Penicillium*, because nothing would be gained by preserving the name *Aspergillus* at the cost of losing the name *Penicillium*. The phylogenies presented in support of Wide Aspergillus (Figure 1, Houbraken & Samson, *ibid.* 2011, Samson & al., *ibid.* 2014) show Wide Aspergillus and *Penicillium* to be reciprocally monophyletic, whereas the phylogeny presented here and in Pitt and Taylor (Figure 2, *ibid.* 2014) do not. We expected that differences in alignment are responsible, and there are two regions in Tsr1, where alignment is ambiguous. These comprise less than 2% of the alignment of all four gene regions, However, when we removed those regions, we still found Wide Aspergillus to be non-monophyletic due to the inclusion of *Penicillium*. We are at a loss to explain the differences in phylogenies and stand by ours (Figure 2) as the most likely topology. Albeit, as noted at the outset, branch support is weak for all of the basal branches (Figure 3).

Having presented the biological information, which supports Narrow Aspergillus over Wide Aspergillus, we now consider taxonomy and nomenclature. The monophyletic group comprising clades 2D, 2E and 2F (Figures 1 and 2), cannot be included in Wide Aspergillus due to the presence of species of *Phialosimplex* and *Polypaecilum* (Clade 2D in Figures 1 and 2), and those generic and species names should continue to be used. Excluding these genera also removes from Wide Aspergillus species in the teleomorph genera *Cristaspora* Fort & Guarro (2E) and *Eurotium* (2F). Species in *Eurotium* and the few other species in the current *Aspergillus* subgenus *Aspergilloides* (Gams et al., in Samson & Pitt, Adv. *Penicillium* Aspergillus System., Plenum Press, New York: 55. 1985; Houbraken & Samson, *ibid.* 2011) therefore should continue to be known by *Eurotium*, their well established, widely used, teleomorph name. The type species of *Cristaspora, C. arxii* Fort & Guarro, belongs in the same clade as *Aspergillus wentii* Wehmer (Houbraken & Samson, *ibid.* 2011), which belongs in the same clade as the

sexual genus *Chaetosartorya* (Peterson, in Samson & Pitt, Integration of Modern Taxonomic Methods for Classification of *Penicillium* and *Aspergillus*, Harwood, Amsterdam, 323. 2000). *Cristospora* is thus a synonym of *Chaetosartorya*: names in the genus *Chaetosartorya* should also continue to be used.   The remaining clades with *Aspergillus* anamorphs, 2A, 2B and 2C, form a monophyletic group in Figure 1 and might make a new "Intermediate Aspergillus". Thus constituted, the genus would embrace industrially important species [*A. niger* and *A. oryzae* (Ahlb.) Cohn, toxigenic species (*A. flavus* Link*, A. parasiticus* Speare*, A. ochraceus* K. Wilh.*, A. carbonarius* (Bainier) Thom], medically important species (*A. fumigatus* Fresen.), and scientific models [*A. nidulans* (Eidam) G. Winter, any of which could be neotypified as type of *Aspergillus*.   However, the new phylogenetic analyses presented here reveal that, in the most likely phylogeny, *Penicillium* prevents these clades from forming a monophyletic group (Table 2, Figures 2 and 3).   Therefore, only one of the clades 2A, 2B or 2C can be named *Aspergillus*. The justification for choosing *A. niger* as the neotype, in the clade representing *Aspergillus* subgenus *Circumdati,* has been discussed elsewhere (Pitt and Taylor 2014).

Narrow Aspergillus is consistent with both genotype, as measured by phylogenetic analysis, and phenotype, evidenced by the names based on distinct sexual morphologies that reflect equally distinct physiological attributes. However, the possibility exists that maintaining the genera *Eurotium, Chaetosartorya, Neosartorya* and *Emericella* would result in genera that embrace significantly less diversity than the neighboring genera. ****To test for this possibility, we will add results from Markus Goker here, which show that the genera *Eurotium, Chaetosartorya, Neosartorya* and *Emericella* are not too small, compared to the other closely related genera.

Table 1. Likelihood ratio tests with all taxa, clades 1-7

			Shimodairo-Hasagawa
Tree	-ln L	Diff -ln L	Approximately Unbiased
1	130838.63840	(best)	
2	131517.01639	678.37799	~0*
3	131489.20977	650.57137	~0*
4	131385.49278	546.85438	~0*
5	130936.80186	98.16346	0.0076*
6	130884.12206	45.48365	0.0691

* $P < 0.05$, indicating a significant difference in likelihoods between Tree 1 and another tree.

Tree 1 No constraints. This is the tree for which the data are most likely.

Tree 2 Constraint to make monophyletic all clades possessing species with Aspergillus anamorphs (2A, 2B, 2C, 2D, 2E, 2F, 3A, 4B, 4C, 7)

Tree 3 Constraint as in Tree 2 excluding Sclerocleista (7) from the monophyletic group (2A, 2B, 2C, 2D, 2E, 2F, 3A, 4B, 4C)

Tree 4 Constraint as in Tree 3 retaining only A. penicilliformis (3A) in addition to Broad Aspergillus (2A, 2B, 2C, 2D, 2E, 2F, 3A)

Tree 5 Constraint as in Tree 3 retaining only A. zonatus (4B) in addition to Broad Aspergillus (2A, 2B, 2C, 2D, 2E, 2F, 4B)

Tree 6 Constraint as in Tree 3 retaining only A. clavatoflavus (4C) in addition to Broad Aspergillus (2A, 2B, 2C, 2D, 2E, 2F, 4C)

Values for the Shimodaira Approximately Unbiased test are P values for null hypothesis of no difference between trees
Number of bootstrap replicates = 10000

Table 2. **Likelihood ratio tests with taxa in clades 1 and 2**

Tree	-ln L	Diff -ln L	Shimadairo-Hasagawa Approximately Unbiased
1	106903.77264	(best)	
2	106950.17747	46.40484	0.0475*
3	106903.85140	0.07876	0.5838

*$P < 0.05$, indicating a significant difference in likelihoods between Tree 1 and another tree.
Tree 1 No constraints. This is the tree for which the data are most likely.

   Tree 2 Constraint to make monophyletic all taxa in clade 2 possessing species with Aspergillus anamorphs (2A, 2B, 2C, 2E, 2F)
   Tree 3 Constraint to make monophyletic all taxa in clade 2, including species lacking Aspergillus anamorphs, i.e., Phialosimplex and Polypaecilum (2D) (2A, 2B, 2C, 2D, 2E, 2F
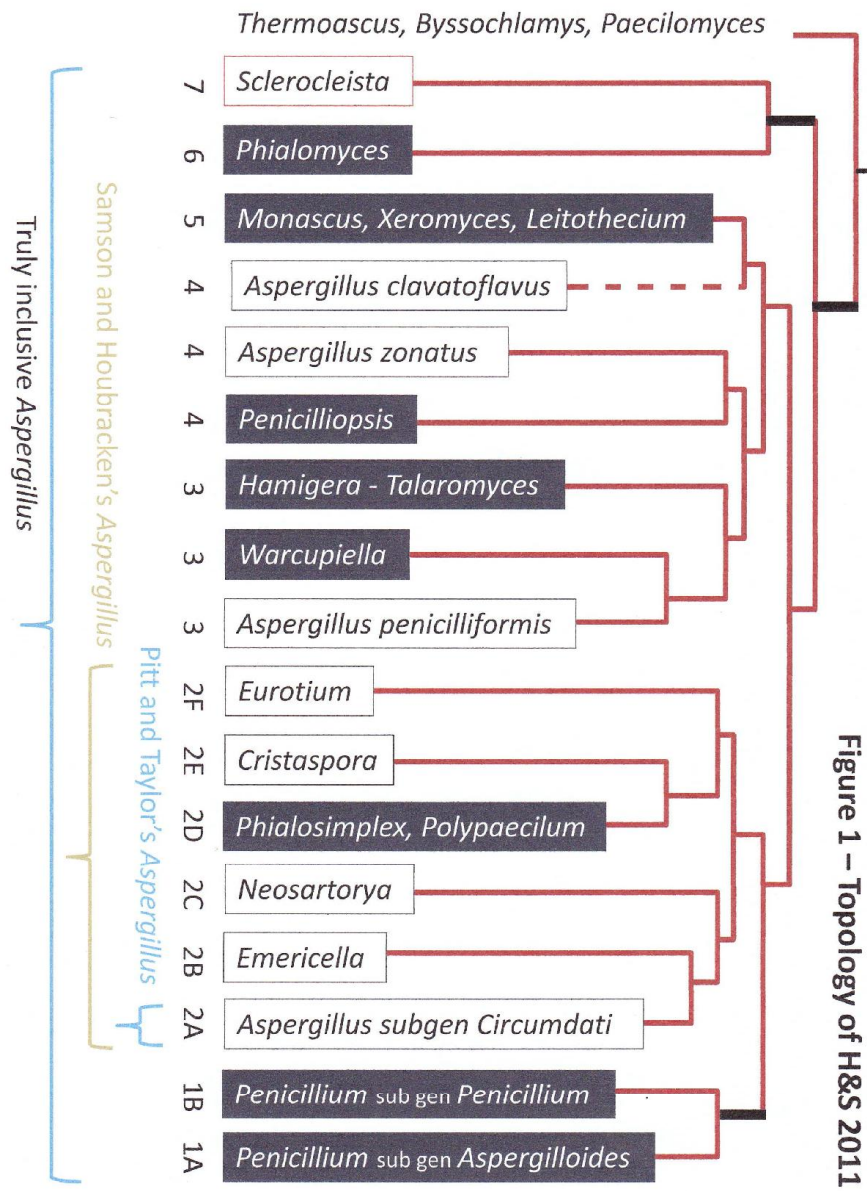
Figure 1. The *Aspergillus* problem in black and white. Tree topology based on that of Houbraken & Samson in Stud. Mycol. 78: 141. 2011. White clades have *Aspergillus* asexual morphology, black do not. The genus *Aspergillus* as "Narrow Aspergillus" encompassing just clade 2A, Aspergillus sub genus Circumdati (Pitt & Taylor, in Mycologia 106: 1051. 2014),as "Wide Aspergillus," clades 2A-2F, (Houbraken & Samson, *ibid.* 2011), or inclusive of all species with *Aspergillus* anamorphs, clades 1-7.
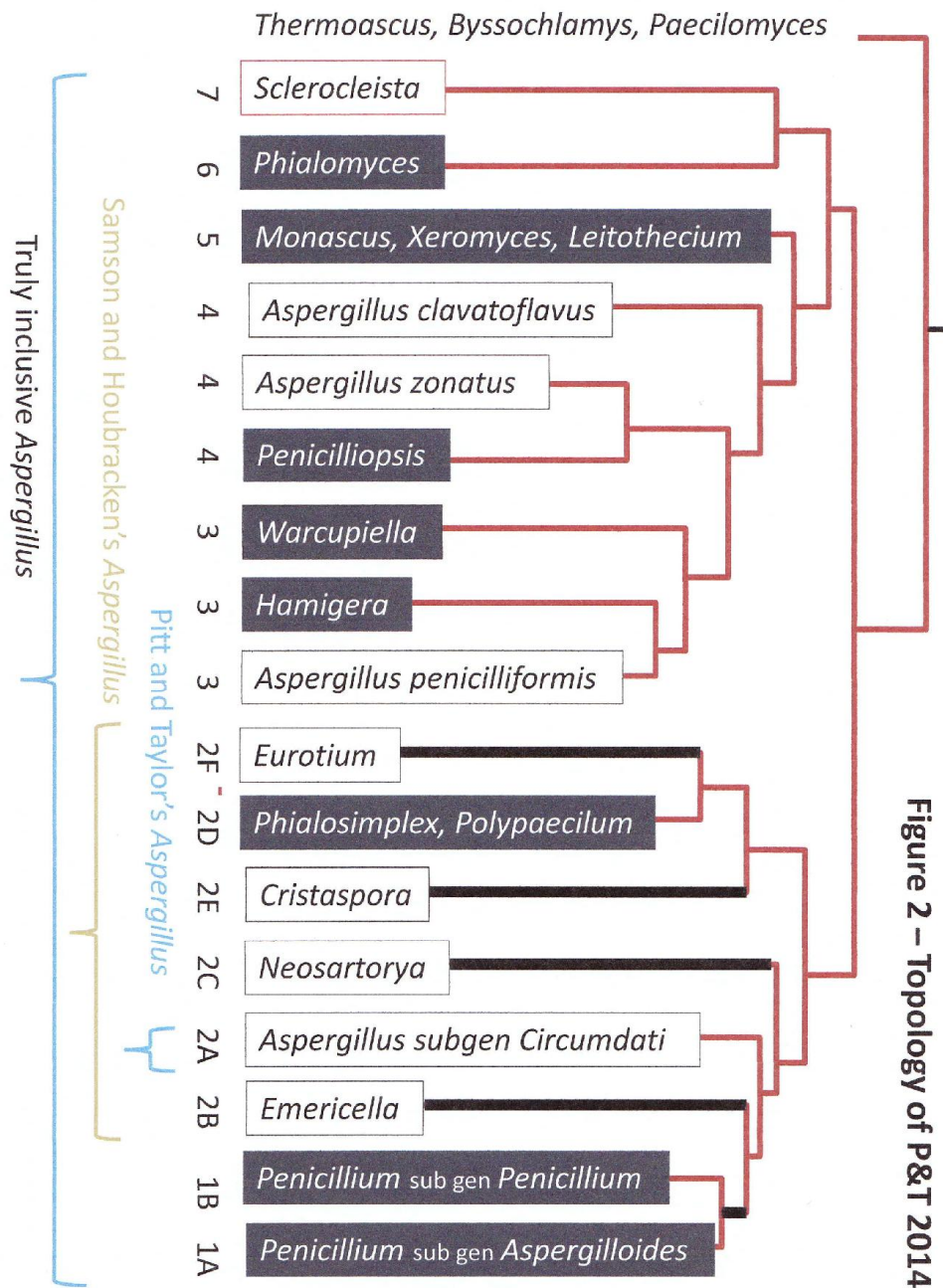
Figure 2. The *Aspergillus* problem in black and white. Tree topology based on that of Pitt & Taylor, in Mycologia 106: 1051. 2014. White clades have *Aspergillus* asexual morphology, black do not. The genus Aspergillus as "Narrow Aspergillus" encompassing just clade 2A, *Aspergillus* subgenus *Circumdati* (Pitt &

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Taylor, *ibid.* 2014),as "Wide Aspergillus," clades 2A-2F, (Houbraken & Samson, in Stud. Mycol. 78: 141. 2011), or inclusive of all species with *Aspergillus* anamorphs, clades 1-7.

Figure 3

Maximum likelihood analysis by RAxML including the most distant outgroup used by Houbraken and Samson 2011 and Samson et al 2014, *Talaromyces flavus.*

Most likely, unconstrained tree Note branches that make Broad Aspergillus non-monophyletic by uniting *Penicillium* with clades containing fungi with Aspergillus reproductive morphology (arrows).

Numbers are bootstrap support from maximum likelihood analysis for basal branches.
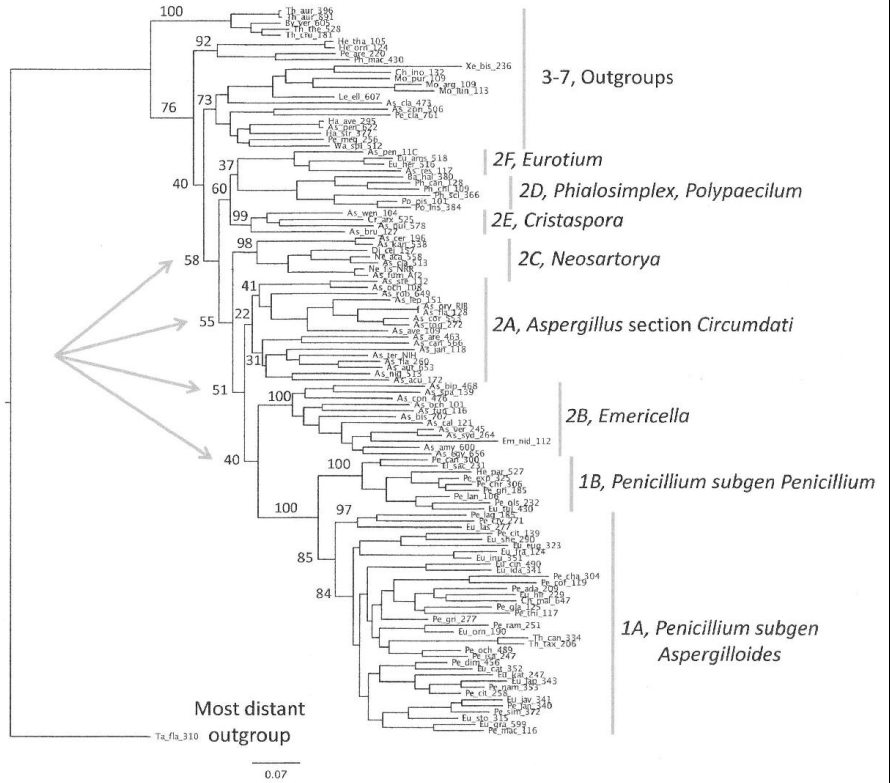
Figure 3. Phylogeny based on maximum likelihood analysis (RAxML) of four gene regions showing the most likely tree based on all taxa (Clades 1-7 and the outgroup). Outgroup selection from Houbraken & Samson, in Stud. Mycol. 78" 141. 2011. Note four, albeit weakly supported, branches (arrows) make Wide Aspergillus non-monophyletic by uniting clades with *Aspergillus* morphology with *Penicillium* (arrow). Bootstrap numbers are percentages of 1000 maximum likelihood RAxML phylogenies possessing that branch based on 1000 resampled datasets.

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