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ARTICLE

Considerations and consequences of allowing DNA sequence data as types of fungal taxa

Juan Carlos Zamora[1], Måns Svensson[1], Roland Kirschner[2], Ibai Olariaga[3], Svengunnar Ryman[1], Luis Alberto Parra[4], József Geml[5], Anna Rosling[6] , Slavomír Adamčík*, Teuvo Ahti, M. Catherine Aime, A. Martyn Ainsworth, László Albert, Edgardo Albertó, Alberto Altés García, Dmitry Ageev, Reinhard Agerer, Begoña Aguirre-Hudson, Joe Ammirati, Harry Andersson, Claudio Angelini, Vladimír Antonín, Takayuki Aoki, André Aptroot, Didier Argaud, Blanca Imelda Arguello Sosa, Arne Aronsen, Ulf Arup, Bita Asgari, Boris Assyov, Violeta Atienza, Ditte Bandini, João Luís Baptista-Ferreira, Hans-Otto Baral, Tim Baroni, Robert Weingart Barreto, Henry Beker, Ann Bell, Jean-Michel Bellanger, Francesco Bellù, Martin Bemmann, Mika Bendiksby, Egil Bendiksen, Katriina Bendiksen, Lajos Benedek, Anna Bérešová-Guttová, Franz Berger, Reinhard Berndt, Annarosa Bernicchia, Alona Yu. Biketova, Enrico Bizio, Curtis Bjork, Teun Boekhout, David Boertmann, Tanja Böhning, Florent Boittin, Carlos G. Boluda, Menno W. Boomsluiter, Jan Borovička, Tor Erik Brandrud, Uwe Braun, Irwin Brodo, Tatiana Bulyonkova, Harold H. Burdsall Jr., Bart Buyck, Ana Rosa Burgaz, Vicent Calatayud, Philippe Callac, Emanuele Campo, Massimo Candusso, Brigitte Capoen, Joaquim Carbó, Matteo Carbone, Rafael F. Castañeda-Ruiz, Michael A. Castellano, Jie Chen, Philippe Clerc, Giovanni Consiglio, Gilles Corriol, Régis Courtecuisse, Ana Crespo, Cathy Cripps, Pedro W. Crous, Gladstone Alves da Silva, Meiriele da Silva, Marjo Dam, Nico Dam, Frank Dämmrich, Kanad Das, Linda Davies, Eske De Crop, Andre De Kesel, Ruben De Lange, Bárbara De Madrignac Bonzi, Thomas Edison E. dela Cruz, Lynn Delgat, Vincent Demoulin, Dennis E. Desjardin, Paul Diederich, Bálint Dima, Maria Martha Dios, Pradeep Kumar Divakar, Clovis Douanla-Meli, Brian Douglas, Elisandro Ricardo Drechsler-Santos, Paul S. Dyer, Ursula Eberhardt, Damien Ertz, Fernando Esteve-Raventós, Javier Angel Etayo Salazar, Vera Evenson, Guillaume Eyssartier, Edit Farkas, Alain Favre, Anna G. Fedosova, Mario Filippa, Péter Finy, Adam Flakus, Simón Fos, Jacques Fournier, André Fraiture, Paolo Franchi, Ana Esperanza Franco Molano, Gernot Friebes, Andreas Frisch, Alan Fryday, Giuliana Furci, Ricardo Galán Márquez, Matteo Garbelotto, Joaquina María García-Martín, Mónica A. García Otálora, Dania García Sánchez, Alain Gardiennet, Sigisfredo Garnica, Isaac Garrido Benavent, Genevieve Gates, Alice da Cruz Lima Gerlach, Masoomeh Ghobad-Nejhad, Tatiana B. Gibertoni, Tine Grebenc, Irmgard Greilhuber, Bella Grishkan, Johannes Z. Groenewald, Martin Grube, Gérald Gruhn, Cécile Gueidan, Gro Gulden, Luis FP Gusmão, Josef Hafellner, Michel Hairaud, Marek Halama, Nils Hallenberg, Roy E. Halling, Karen Hansen, Christoffer Bugge Harder, Jacob Heilmann-Clausen, Stip Helleman, Alain Henriot, Margarita Hernandez-Restrepo, Raphaël Herve, Caroline Hobart, Mascha Hoffmeister, Klaus Høiland, Jan Holec, Håkon Holien, Karen Hughes, Vit Hubka, Seppo Huhtinen, Boris Ivančević, Marian Jagers, Walter Jaklitsch, AnnaElise Jansen, Ruvishika S. Jayawardena, Thomas Stjernegaard Jeppesen, Mikael Jeppson, Peter Johnston, Per Magnus Jørgensen, Ingvar Kärnefelt, Liudmila B. Kalinina, Gintaras Kantvilas, Mitko Karadelev, Taiga Kasuya, Ivona Kautmanová, Richard W. Kerrigan, Martin Kirchmair, Anna Kiyashko, Dániel G. Knapp, Henning Knudsen, Kerry Knudsen, Tommy Knutsson, Miroslav Kolařík, Urmas Kõljalg, Alica Košuthová, Attila Koszka, Heikki Kotiranta, Vera Kotkova, Ondřej Koukol, Jiří Kout, Gábor M. Kovács, Martin Kříž, Åsa Kruys, Viktor Kučera, Linas Kudzma, Francisco Kuhar, Martin Kukwa, T. K. Arun Kumar, Vladimír Kunca, Ivana Kušan, Thomas W. Kuyper, Carlos Lado, Thomas Læssøe, Patrice Lainé, Ewald Langer, Ellen Larsson, Karl-Henrik Larsson, Gary Laursen, Christian Lechat, Serena Lee, James C. Lendemer, Laura Levin, Uwe Lindemann, Håkan Lindström, Xingzhong Liu, Regulo Carlos Llarena Hernandez, Esteve Llop, Csaba Locsmándi, Deborah Jean Lodge, Michael Loizides, László Lőkös, Jennifer Luangsa-ard, Matthias Lüderitz, Thorsten Lumbsch, Matthias Lutz, Dan Mahoney, Ekaterina Malysheva, Vera Malysheva, Patinjareveettil Manimohan, Yasmina Marin-Felix, Guilhermina Marques, Rubén Martínez-Gil, Guy Marson, Gerardo Mata, P. Brandon Matheny, Geir Harald Mathiassen, Neven Matočec, Helmut Mayrhofer, Mehdi Mehrabi, Ireneia Melo, Armin Mešić, Andrew S. Methven, Otto Miettinen, Ana M. Millanes Romero, Andrew N. Miller, James K. Mitchell, Roland Moberg, Pierre-Arthur Moreau, Gabriel Moreno, Olga Morozova, Asunción Morte, Lucia Muggia, Guillermo Muñoz González, Leena Myllys, István Nagy, László G. Nagy, Maria Alice Neves, Tuomo Niemelä, Pier Luigi Nimis, Nicolas Niveiro, Machiel E. Noordeloos, Anders Nordin, Sara Raouia Noumeur, Yuri Novozhilov, Jorinde Nuytinck, Esteri Ohenoja, Patricia Oliveira Fiuza, Alan Orange, Alexander Ordynets, Beatriz Ortiz-Santana, Leticia Pacheco, Ferenc Pál-Fám, Melissa Palacio, Zdeněk Palice, Viktor Papp, Kadri Pärtel, Julia Pawlowska, Aurelia Paz, Ursula Peintner, Shaun Pennycook, Olinto Liparini Pereira, Pablo Pérez Daniëls, Miquel À. Pérez-De-Gregorio Capella, Carlos Manuel Pérez del Amo, Sergio Pérez Gorjón, Sergio Pérez-Ortega, Israel Pérez-Vargas, Brian A. Perry, Jens H. Petersen, Ronald H. Petersen, Donald H. Pfister, Chayanard Phukhamsakda, Marcin Piątek, Meike Piepenbring, Raquel Pino-Bodas, Juan Pablo Pinzón Esquivel, Paul Pirot, Eugene S. Popov, Orlando Popoff, María Prieto Álvaro, Christian Printzen, Nadezhda Psurtseva, Witoon Purahong, Luis Quijada, Gerhard Rambold, Natalia A. Ramírez, Huzefa Raja, Olivier Raspé, Tania Raymundo, Martina Réblová, Yury A. Rebriev, Juan de Dios Reyes García, Miguel Ángel Ribes Ripoll, Franck Richard, Mike J. Richardson, Víctor J. Rico, Gerardo Lucio Robledo, Flavia Rodrigues Barbosa, Cristina Rodriguez-Caycedo, Pamela Rodriguez-Flakus, Anna Ronikier, Luis Rubio Casas, Katerina Rusevska, Günter Saar, Irja Saar, Isabel Salcedo, Sergio M. Salcedo Martínez, Carlos A. Salvador Montoya, Santiago Sánchez-

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Ramírez, J. Vladimir Sandoval-Sierra, Sergi Santamaria, Josiane Santana Monteiro, Hans Josef Schroers, Barbara Schulz, Geert Schmidt-Stohn, Trond Schumacher, Beatrice Senn-Irlet, Hana Ševčíková, Oleg Shchepin, Takashi Shirouzu, Anton Shiryaev, Klaus Siepe, Esteban B. Sir, Mohammad Sohrabi, Karl Soop, Viacheslav Spirin, Toby Spribille, Marc Stadler, Joost Stalpers, Soili Stenroos, Ave Suija, Stellan Sunhede, Sten Svantesson, Sigvard Svensson, Tatyana Yu. Svetasheva, Krzysztof Świerkosz, Heidi Tamm, Hatira Taskin, Adrien Taudière, Jan-Olof Tedebrand, Raúl Tena Lahoz, Marina Temina, Arne Thell, Marco Thines, Göran Thor, Holger Thüs, Leif Tibell, Sanja Tibell, Einar Timdal, Zdenko Tkalčec, Tor Tønsberg, Gérard Trichies, Dagmar Triebel, Andrei Tsurykau, Rodham E. Tulloss, Veera Tuovinen, Miguel Ulloa Sosa, Carlos Urcelay, François Valade, Ricardo Valenzuela Garza, Pieter van den Boom, Nicolas Van Vooren, Aida M. Vasco-Palacios, Jukka Vauras, Juan Manuel Velasco Santos, Else Vellinga, Annemieke Verbeken, Per Vetlesen, Alfredo Vizzini, Hermann Voglmayr, Sergey Volobuev, Wolfgang von Brackel, Elena Voronina, Grit Walther, Roy Watling, Evi Weber, Mats Wedin, Øyvind Weholt, Martin Westberg, Eugene Yurchenko, Petr Zehnálek, Huang Zhang, Mikhail P. Zhurbenko, and Stefan Ekman[1]

[1]Museum of Evolution, Uppsala University, Norbyvägen 16, 75236 Uppsala, Sweden; corresponding author e-mail: jczamora@em.uu.se
[2]National Central University, Taoyuan City, Taiwan
[3]Universidad Rey Juan Carlos, 28933 Móstoles, Madrid, Spain
[4]Avenida Padre Claret 7, 5º G, 09400 Aranda de Duero, Burgos, Spain
[5]Naturalis Biodiversity Center, Vondellaan 55, 2332AA Leiden, The Netherlands
[6]Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, 75236 Uppsala, Sweden

*A first version of this text was prepared by the first eight authors and the last one. The other listed co-authors support the content, and their actual contributions varied from only support to additions that substantially improved the content. The full details of all co-authors, with their affiliations, are included in Supplementary Table 1 for reasons of clarity and space.

Abstract: Nomenclatural type definitions are one of the most important concepts in biological nomenclature. Being physical objects that can be re-studied by other researchers, types permanently link taxonomy (an artificial agreement to classify biological diversity) with nomenclature (an artificial agreement to name biological diversity). Two proposals to amend the International Code of Nomenclature for algae, fungi, and plants (ICN), allowing DNA sequences alone (of any region and extent) to serve as types of taxon names for voucherless fungi (mainly putative taxa from environmental DNA sequences), have been submitted to be voted on at the 11th International Mycological Congress (Puerto Rico, July 2018). We consider various genetic processes affecting the distribution of alleles among taxa and find that alleles may not consistently and uniquely represent the species within which they are contained. Should the proposals be accepted, the meaning of nomenclatural types would change in a fundamental way from physical objects as sources of data to the data themselves. Such changes are conducive to irreproducible science, the potential typification on artefactual data, and massive creation of names with low information content, ultimately causing nomenclatural instability and unnecessary work for future researchers that would stall future explorations of fungal diversity. We conclude that the acceptance of DNA sequences alone as types of names of taxa, under the terms used in the current proposals, is unnecessary and would not solve the problem of naming putative taxa known only from DNA sequences in a scientifically defensible way. As an alternative, we highlight the use of formulas for naming putative taxa (candidate taxa) that do not require any modification of the ICN.

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INTRODUCTION

Hawksworth *et al.* (2016) recently submitted a set of proposals to modify the *International Code of Nomenclature for algae, fungi, and plants* (ICN), aimed at allowing DNA sequences without vouchered specimens to serve as types for fungal taxon names. These proposals were first rejected by the Nomenclature Committee for Fungi (see Turland & Wiersema 2017) and subsequently by the XIX International Botanical Congress (IBC) in Shenzhen, China, in 2017. At the same time, a Special-purpose Committee on DNA sequences as types was proposed to explore and carefully discuss this issue, paving the way for further debate during the next IBC in Rio de Janeiro in 2023 (Turland *et al.* 2017).

However, apparently because of a perceived urgency in the establishment of a system for naming putative new taxa known only from DNA sequences, the same proposals were recently re-published (Hawksworth *et al.* 2018) with the intent that they be discussed and voted on at the forthcoming 11th International Mycological Congress (IMC11) in Puerto Rico in July 2018. The proposals aim at allowing the formal naming of fungal taxa only known by DNA sequences (the "dark matter fungi" of Grossart *et al.* 2016), by authorizing the DNA sequence itself to be the type of a taxon name in the absence of a specimen.

The ICN attempts to create "the provision of a stable method of naming taxonomic groups, avoiding and rejecting the use of names that may cause error and ambiguity or throw science into confusion" (Preamble 1). This provision relies on the use of the nomenclatural type, "the face — the desiccated, flattened face to be sure, but still the face — that is attached to the name of a species" (Daston 2004).

In our opinion, the fungal-specific amendments proposed to the ICN by Hawksworth *et al.* (2018) should be rejected on

the grounds that they would have major negative implications for fungal nomenclature and systematics, or more specifically, violate Preamble 1, promote irreproducible science, and fundamentally change the meaning of the type concept compared to how it has been applied during the last century. An informed debate is needed to avoid any unwanted effects of a rushed decision.

THE PROPOSALS

The proposals of Hawksworth *et al.* (2018) intend to insert a single article, Art. F.4.2, through proposal (F-005), followed by three recommendations, Rec. F.4A.1-3, through proposal (F-006). As only Art. F.4.2 would be mandatory, it is crucial to evaluate proposal (F-005) in particular detail: "(F-005) Insert a new paragraph after Art. F.4.1 as follows: F.4.2. In fungi, when DNA sequence data corresponding to a new taxon have been detected, but no physical specimen has been found to serve as the type of the name of the new taxon (Art. 8.1–8.4), the type may be composed of DNA sequence data deposited in a public repository."

The recommendations that follow suggest, in summary, that "the new taxon should be described with reference to a published phylogenetic analysis" (Rec. F.4A.1), that the new taxon "should be represented by multiple sequences obtained in independent studies" (Rec. F.4A.2), and that the sequence should derive from "the molecular regions that are appropriate for delimiting species" (Rec. F.4A.3). These are merely recommendations, however, and need not be followed (as emphasized by Turland & Wiersema 2017).

SPECIES *VERSUS* DNA SEQUENCES

It has been argued that "the *Code* serves only to regulate the valid publication of names, not to pass judgment on the scientific hypotheses embodied in names" (Herr *et al.* 2015). Although nomenclature can be seen as a "remarkable act of applied metaphysics" (Daston 2004), the circumscription of the taxa being named is a fundamentally scientific process. The proposal recommends that a new taxon "be described with reference to a published phylogenetic analysis" (Rec. F.4A.1 of proposal F-006). This wording implies that it is possible to first circumscribe a new taxon by phylogenetic analysis, then name the new taxon using a DNA sequence type that can be unequivocally associated with the new taxon. For the reasons outlined below, this may not readily be the case at the level of species in recombining organisms, which we suspect is where Art. F.4.2 is most frequently going to be applied.

Assuming that species are understood as somehow separately evolving units (e.g. de Queiroz 1998, 2005, 2007, Hey 2006), they can, sooner or later after formation, be detected using a variety of methods (often misleadingly termed 'species concepts'; Hey 2006), e.g. reproductive isolation (the 'biological species concept'), morphology, or genealogical monophyly with or without auxiliary criteria like concordance among genes (corresponding to the genetic versions of 'phylogenetic species concept'). During a simple divergence of one ancestral species into two daughter species, (nearly) neutral loci will inherit random samples of alleles from the ancestral species, some of which are likely to be shared across the daughter species (ancestral polymorphisms). Given time, ancestral alleles will go extinct randomly and new alleles will arise, in the most likely case causing species to appear non-monophyletic on the gene trees. Finally, species will achieve reciprocal monophyly on the gene trees. This process has been known and described in the literature for decades (e.g. Tajima 1983, Takahata & Nei 1985, Neigel & Avise 1986, Nei 1987, Pamilo & Nei 1988, Takahata 1989, Avise & Ball 1990, Hudson *et al.* 1992, Hey 1994, Harrison 1998, Avise 2000, Hudson & Coyne 2002, Rosenberg 2003, Coyne & Orr 2004, Naciri & Linder 2015) and has been elegantly explained and illustrated by, for example, Leliaert *et al.* (2014). The lag time from lineage divergence until reciprocal monophyly in neutral loci will depend on the effective population size, generation time, and population structure (Hudson 1990, Wakeley 2000) and its duration will vary stochastically between nuclear loci in recombining organisms (Hudson & Turelli 2003). Obviously, any species recognition protocol requiring reciprocal monophyly will only be able to detect the species long after they diverged (Hudson & Coyne 2002). Positive selection can substantially shorten the time it takes to remove ancestral polymorphisms and finally reach reciprocal monophyly. The proportion of the genome undergoing positive selection during and after speciation appears to be small, however, probably reaching at most a few per cent (e.g., 1.1 and 1.7 % of the genes in humans and chimpanzee, respectively; Bakewell *et al.* 2007). As an aside, the stochastic process finally leading to reciprocal monophyly in the individual genes also means that there cannot exist a universal divergence threshold for delimiting fungal (or other) species using DNA sequences, not for the very widely used internal transcribed spacer (ITS) region in fungi (e.g. Nilsson *et al.* 2008, Badotti *et al.* 2017), nor any other DNA region in any organism group (e.g. Meier *et al.* 2006 concerning metazoans).

Gene histories, a standard product in applied phylogenetics, cannot automatically be equated with the species history (e.g. Tajima 1983, Pamilo & Nei 1988, Maddison 1997, Knowles & Carstens 2007). There is no reason to think that any DNA region or any organism group is free of mechanisms that create a discordance between the gene and species histories. Such mechanisms have been found to be widespread across the tree of life (e.g. Sota & Vogler 2001, Rautenberg *et al.* 2008, Blanco-Pastor *et al.* 2012, Kutschera *et al.* 2014, Lamichhaney *et al.* 2015, Garrido *et al.* 2017, Kudryavtseva & Gladkikh 2017, Meyer *et al.* 2017, Parks *et al.* 2017, Peyrégne *et al.* 2017, Vd'ačný 2017). Incongruence between gene histories, demonstrating that at least some of them must be different from the history of the species, has indeed also been demonstrated to occur in the fungi (e.g. O'Donnell & Cigelnik 1997, Sung *et al.* 2007, Harder *et al.* 2013, Altermann *et al.* 2014, Saag *et al.* 2014, Stewart *et al.* 2014). A conflict between the gene histories and species history is not only caused by the randomness of genetic drift described above. Other mechanisms, all observed also in fungi, obscure relationships among taxa

and some (the first three) have the potential to cause non-identifiability of a single DNA sequence: the exchange of entire nuclei between heterospecific fungal syncytia, horizontal gene transfer, hybridization (sometimes followed by introgression or allopolyploidy), gene duplication (including also pseudogene and *numt* formation), and intra-individual variability in the ribosomal DNA repeat caused by limits to concerted evolution (Dean *et al.* 2005, Ruths & Nakhleh 2005, Jeffroy *et al.* 2006, Neafsey *et al.* 2010, Ellison *et al.* 2011, Lindner & Banik 2011, Roper *et al.* 2011, Hughes *et al.* 2013, Li *et al.* 2013, Lindner *et al.* 2013, Gladieux *et al.* 2014, Som 2014, Naciri & Linder 2015, Shapiro *et al.* 2016, Thiéry *et al.* 2016, Fourie *et al.* 2017, Li *et al.* 2017, Hughes *et al.* 2018, Steenkamp *et al.* 2018). Obviously, species delineations generated from a single marker cannot be evaluated using data from the same marker, because that would make the argument circular.

We conclude that a DNA sequence of an allele cannot be seen as "corresponding to" any taxon (the wording of the proposal), but represents the diversity of alleles of the gene from which it was derived. An allele cannot be expected to be unique to the species from which it was derived and we cannot know whether or not alleles are unique to a species when sequence data are only available from a single or a limited number of markers and individuals (e.g. the popular ITS barcode in fungi; Schoch *et al.* 2012, Badotti *et al.* 2017). "If species membership is contingent for organisms in general, it ought to be contingent for those chosen as the type specimens for their species" (Levine 2001). Having said that, some of these pitfalls are more easily detected and remedied when the number of markers is high and methods designed to handle them (including but not limited to versions of the 'phylogenetic analysis' prescribed by Rec. F.4A.1) are applied (Dupuis *et al.* 2012, Fujita *et al.* 2012).

IMPACT ON NOMENCLATURAL TYPES (SPECIMENS *VERSUS* DNA SEQUENCES)

An acceptance of the proposal would fundamentally alter the meaning of nomenclatural types. This is because instead of using a physical object as the type of a name, we would just use information from a character of the organism as the type. Indeed, the parallel to the designation of a DNA sequence as a type would be the designation of information extracted from organisms (specimens) as types, not with the designation of specimens as types. In other words, this would be akin to designating a sample of spore measurements as the type of an organism. It should be noted that the possibility to select a description as a type existed before the publication of the Berlin *Code* in 1988. However, this option was eventually rejected by the scientific community, and removed from the Berlin *Code* with this note in the Preface: "The provision that existed for a type to be a description under certain circumstances — something that many felt amounted to a repudiation of the type method — has been deleted from the *Code*" (Greuter *et al*. 1988: viii).

Names of taxa are applied to organisms, not to characters of those organisms. Therefore, a physical object should preferably serve as the type of a name, rather than the characteristics of that object. By allowing already extracted data, such as a DNA sequence, to serve as type instead of the source of the data, new information cannot be obtained when this is required (see below). In addition, we suspect that bypassing the current concept of a type is often unnecessary, because techniques exist to visualize fungal DNA with high specificity (Baschien *et al.* 2001, Behrens *et al.* 2003, Inácio *et al.* 2003, Baschien *et al.* 2008, Vági *et al.* 2014, Spribille *et al.* 2016). Although not yet standard parts of the mycological toolbox, such techniques can with relative ease be applied to locate physical specimens even for taxa that cannot currently be cultivated.

According to the ICN, a nomenclatural type is "that element to which the name of a taxon is permanently attached, whether as the correct name or as a synonym" (Art. 7.2). For species-level taxa and infraspecific taxa, which are the basic units in taxonomy, a type is "either a single specimen conserved in one herbarium or other collection or institution, or an illustration" (Art. 8.1). Why have researchers agreed to keep these definitions for such a long time? The answer is straightforward: because types are an almost never-ending source of information, as they can be analyzed by different people using different methods and thus provide new answers. Every time a type specimen is re-examined, there is an opportunity to extract new information, which may be useful for solving problems that are constantly arising as our knowledge increases. Most types are specimens (especially nowadays) because a specimen of any living organism is such a complex entity that it is hard to imagine us being able to extract all the possible information contained in it. These properties have already been considered in an editorial of *IMA Fungus* written by the President of the International Mycological Association (Seifert 2017). Therefore, even though the problem of non-unique characters used for diagnosis is not restricted to sequence data, the crucial distinction from morphological descriptions of biological type specimens is that having a DNA sequence as type virtually precludes the obtaining of any new information to resolve any taxonomic problems. In contrast, even illustrations, which are now accepted as types only in very specific situations (see Art. 40.5 for the current use of these) and increasingly falling into disuse, may be a source of overlooked information.

Epitype selection may be seen as a possible solution in the expected cases when the DNA sequence alone is insufficient for the precise application of the name of a taxon (Ryberg & Nilsson 2018). Epitypification was conceived as a practical solution in cases when the type of a name turns out to be ambiguous (ICN, Art. 9.8). Epitypes are frequently designated for old names, and they are not free of undesired problems affecting nomenclatural stability (Rindi *et al.* 2017). Epitypifications have to be based on an existing type, and are often being made because our knowledge or the present technology are the limits for extracting the needed information from the type that already exists. Those limitations may be overcome by other researchers or by new technologies in the future. For DNA sequence data, the type itself would always be the limiting bottleneck, regardless of the researcher's skills or the progress of science.

IMPACT ON NAMES OF TAXA AND FUTURE TAXONOMIC STUDIES

The main argument used by Hawksworth *et al.* (2016), to justify the urgency of allowing DNA sequences as types, is that taxa only known from DNA sequences "require scientific names in order to facilitate communication about them". While researchers indeed need names of taxa to communicate among colleagues and with the general public, those names are linked to information that makes them useful, like biology, distribution, ecology, morphology, physiology, pathology, etc. (Crous *et al.* 2015). In other words, we are using scientific names because they are meaningful to a wide range of people.

In addition, taxonomists are aware that an increased number of validly published names will not necessarily facilitate communication. On the contrary, in the not uncommon situation in which the same taxon has been named on several occasions, much confusion may arise until the identity of those names is finally settled. Indeed, taxa based solely on DNA sequences not precisely matching any of those present in public repositories have already been described and fallen into more or less immediate synonymy, because the necessary comparisons with previously described taxa were not undertaken (Gams 2016). The proposals would promote such bad practice.

An undesired side-effect that should also be considered is that, in practice, few researchers will be devoted to re-describing (or actually describing) species that have been previously named based on just a DNA sequence. This has several causes, but among them, there is an important bias in research journals disfavoring the publication of re-descriptions of already known taxa, *versus* the description of new taxa. Another reason is time constraints, since it is not uncommon that specialists do not have the time to properly describe all of the numerous undescribed species they are aware of. This makes them focus on those that are more likely to be published as new species and not on those that have been already described, even if previous descriptions are faulty or defective. Anyhow, having numerous names only based on DNA sequences and few descriptions of the actual organisms would create an enormous number of validly published names applied to taxa for which virtually no information exists.

RELIABILITY AND EXTENT OF DATA

The proposed Art. F.4.2. effectively means that any DNA sequence of any region and extent, generated by any procedure or taken from a public repository, could serve as the type of a name of a taxon somehow indicated to be new. In practice, the sequence selected as the type could range from an oligonucleotide to the entire genome. The proposal provides very little guidance, except for the recommendations that the type sequence should be represented by "multiple sequences" and that the selected marker should be "appropriate for delimiting species" (proposed Rec. F.4A.2, F.4A.3). It is not clear what 'multiple' means or how a marker is established as universally 'appropriate'. One can infer, however, that the 'appropriate' marker will, in most applications, be the ITS region, which has been dubbed as the primary barcode marker in fungi (Schoch *et al.* 2012).

A major concern is the reliability of the DNA sequence data (Bridge *et al.* 2003, Nilsson *et al.* 2006). PCR or cloning errors (including the introduction of chimeras), DNA degradation, and post-processing of chromatograms, have been shown to be a source of sequence variation in at least some groups (Haas *et al.* 2011, Sandoval-Sierra *et al.* 2014, Hughes *et al.* 2015, Strid *et al.* 2015, Aas *et al.* 2017, Nilsson *et al.* 2017, Thielecke *et al.* 2017, Bieker & Martin 2018). Such DNA sequences are not real and cannot be checked or corrected without access to a physical specimen or, as a minimum, access to the raw sequence reads (Tripp & Lendemer 2014). If accepted as types, this means mycology would embrace irreproducible science.

The concerns outlined here, in combination with the risk of comparing non-orthologous sequences or incompletely concerted copies of the ribosomal DNA, are really about scientific quality and not nomenclature *per se*. However, nomenclature assumes that taxa are first delineated, then named. The proposal, if implemented, would risk opening the floodgates to poor data and questionable scientific practice being translated into formally named taxa that will throw fungal taxonomy into paralysis and disrepute.

CANDIDATE NAMES

If we really want to strive for a comprehensive code of nomenclature able to cover all living organisms, it is necessary to consider the rules of the other existing codes of nomenclature. For our purposes, these are mainly the International Code of Zoological Nomenclature (ICZN; Ride *et al.* 1999) and the International Code of Nomenclature of Prokaryotes (ICNP; Parker *et al*. 2015). Also, it is important to consider the use of nomenclature by specialists in different taxonomic groups. In general, we think it is better to strive for standardization of rules instead of sharpening the differences between Codes. The goal should be to create a solid code of nomenclature that, some day, may perhaps cover all living organisms with all their peculiarities (e.g. the BioCode initiative; Greuter *et al.* 2011, http://www.bionomenclature.net/biocode2011.html).

An interesting formula concerning taxa that cannot be properly described under the rules of a code of nomenclature is the use of the term "*Candidatus*". Originally, this working term was proposed by Murray & Schleifer (1994), and soon after improved by Murray & Stackebrandt (1995) for "describing prokaryotic entities for which more than a mere sequence is available but for which characteristics required for description according to the Code are lacking". It was proposed because, under the rules of the ICNP, a prokaryotic organism can only be validly described if the type, which in this case is a living strain, can be conserved as an axenic culture. There are of course thousands of prokaryotic taxa that are not cultivable in such a way. Many of them can, however, be studied with regard to morphology, ecology, metabolism, DNA data, etc. For fungi, having such additional information for a particular cluster of DNA sequences (never a single one), or several

DNA regions from the same organism (ultimately and ideally, a complete genome), would be essential to ensure that a true taxon is being provisionally named, and to comply with basic scientific standards.

The *Candidatus* working term has proved to be a good solution for microbiologists who want to respect the rules of the ICNP as well as to apply useful names to certain taxa. Being aware that important information (e.g. a proper living strain as type) is lacking to allow a formal description, such taxa can be validated when the requirements of the ICNP are fulfilled. The best example of how well this alternative nomenclature works is the Candidate Phyla Radiation, a huge, well-known and well-communicated group of Bacteria that was proposed based on the combined information of hundreds of genomes, obtained from single cells as well as metagenomics (Hug *et al.* 2016, Danczak *et al*. 2017).

The alternative of using preliminary names for taxa only known from DNA data has already been proposed by Öpik *et al*. (2009) as "virtual taxa", by Taylor (2011) as "ENAS fungi", by Kõljalg *et al.* (2013) as "species hypothesis", and indeed also by Hibbett *et al.* (2011) as "candidate species". We think this is an interesting idea that should be further explored and discussed in the future. Such candidate names can be re-evaluated and possibly formally described in the future when enough information has become available to provide a good taxon description (see also Seifert 2017). Finally, they could be used with some freedom, since no specific rules within the codes of nomenclature apply for invalidly published names. If a major concern about fungi only known from DNA sequences is that "they do not enter names-based taxonomic databases" (see Herr *et al.* 2015), a reasonably easy solution would be to allow the registration of candidate or putative names in those databases, in the process making it clear that those names have not yet been validly published because one or more of the requirements for valid publication are lacking (e.g. http://www.bacterio.net/-candidatus.html for candidate names of prokaryotic taxa).

CONCLUSIONS

We consider the proposals by Hawksworth *et al.* (2018) highly problematic for the following reasons:

- DNA sequence types will have a very low information content; subsequent extraction of additional data or verification of the already extracted data will not be possible.
- Two different taxa may share identical DNA sequences at a given locus, even for already tested barcoding markers. Conversely, not all members of a species can be assumed to share the same DNA sequence at a specific locus.
- Intraspecific (or even intraindividual) differences in the DNA sequence of a marker may be comparable to or exceed interspecific differences.
- Some DNA sequences generated through different sequencing techniques may be artifacts and consequently not represent reality. The proposal does not say anything about data validation other than a recommendation that the DNA sequence should be represented by 'multiple sequences'.
- The proposal promotes the mechanical production of taxon names based on minor sequence divergence, without taking any other data (such as genetic variability or already described taxa) into account. Much downstream time will have to be spent by future mycologists gathering additional information.
- As taxa with DNA sequence types accumulate, the description of a new species will be increasingly difficult without DNA sequence data. Describing new species based on the morphology of unsequenced material will in practice not be feasible if the possibility exists that this species has been described based on a DNA sequence.
- Since the proposals allow any part of the genome to be used as a DNA type, situations in which different taxa may have been described using different parts of the genome will force researchers to sequence a variety of loci to establish whether an earlier name already exists. Likewise, a single taxon may be described as novel several times using different genomic regions as type. This will be impossible to detect without a specimen from which different genomic regions can be sequenced and may contribute to the description of unnecessary new names.

FINAL REMARKS

As discussed above, there are alternative ways of communicating the existence of taxa only known from DNA data, which do not require modifications to the ICN. Instead of allowing DNA data as types for taxon names, database registration of candidate names can be used for putative new taxa, when their existence has been made plausible based on various sources of information (including but not limited to DNA sequences). A functional system for environmental sequences under the *Candidatus* or species hypotheses approach could result from a carefully selected set of requirements to ensure high-quality data and reproducibility.

We submit that proposals F-005 and F-006, for the reasons outlined here, will not solve the problems they are intended to solve, disregard knowledge acquired through decades of research in the genetics of speciation, and will instead create confusion and substantial extra work for contemporary and future mycologists. We all have the responsibility to maintain the scientific standards of reproducibility as well as to provide well-considered rules for coming generations, so they can improve on our work and take appropriate, well-informed taxonomic decisions using all available information.

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REFERENCES

Aas AB, Davey ML, Kauserud H (2017) ITS all right mama: investigating the formation of chimeric sequences in the ITS2 region by DNA metabarcoding analyses of fungal mock communities of different complexities. *Molecular Ecology Resources* **17**: 730–741.

Altermann S, Leavitt SD, Goward T, Nelsen MP, Lumbsch HT (2014) How do you solve a problem like *Letharia*? A new look at cryptic species in lichen-forming fungi using Bayesian clustering and SNPs from multilocus sequence data. *PLoS ONE* **9**: e97556.

Avise JC (2000) *Phylogeography: the history and formation of species*. Cambridge, MA: Harvard University Press.

Avise JC, Ball RM (1990) Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Survey of Evolutionary Biology* **7**: 45–67.

Badotti K, Silva de Oliveira F, Garcia CF, Martins Vaz AB, Camargos Fonseca PL, *et al.* (2017) Effectiveness of ITS and sub-regions as DNA barcode markers for the identification of *Basidiomycota* (*Fungi*). *BMC Microbiology* **17**: 42.

Bakewell MA, Shi P, Zhang J (2007) More genes underwent positive selection in chimpanzee evolution than in human evolution. *Proceedings of the National Academy of Sciences of the USA* **104**: 7489–7494.

Baschien C, Manz W, Neu TR, Szewzyk U (2001) Fluorescence *in situ* hybridization of freshwater fungi. *International Review of Hydrobiology* **86**: 371–384.

Baschien C, Manz W, Neu TR, Marvanová L, Szewzyk U (2008) *In situ* detection of freshwater fungi in an alpine stream by new taxon-specific fluorescence *in situ* hybridization probes. *Applied and Environmental Microbiology* **74**: 6427–6436.

Behrens S, Rühland C, Inácio J, Huber H, Fonseca A, *et al.* (2003) *In situ* accessibility of small-subunit rRNA of members of the domains *Bacteria*, *Archaea*, and *Eucarya* to Cy3-labeled oligonucleotide probes. *Applied and Environmental Microbiology* **69**: 1748–1758.

Bieker VC, Martin MD (2018) Implications and future prospects for evolutionary analyses of DNA in historical herbarium collections. *Botany Letters*: DOI: 10.1080/23818107.2018.1458651.

Blanco-Pastor JL, Vargas P, Pfeil BE (2012) Coalescent simulations reveal hybridization and incomplete lineage sorting in Mediterranean *Linaria*. *PLoS ONE* **7**: e39089.

Bridge PD, Roberts PJ, Spooner BM, Panchal G (2003) On the unreliability of published DNA sequences. *New Phytologist* **160**: 43–48.

Coyne JA, Orr HA (2004) *Speciation*. Sunderland, MA: Sinauer Associates.

Crous PV, Hawksworth DL, Wingfield MJ (2015) Identifying and naming plant-pathogenic fungi: past, present and future. *Annual Review of Phytopathology* **53**: 247–267.

Danczak RE, Johnston MD, Kenah C, Slattery M, Wrighton KC, Wilkins MJ (2017) Members of the Candidate Phyla Radiation are functionally differentiated by carbon- and nitrogen-cycling capabilities. *Microbiome* **5**: 112.

Daston L (2004) Type specimens and scientific memory. *Critical Inquiry* **31**: 153–182.

Dean RA, Talbot NJ, Ebbole D, Farman ML, Mitchell TK, *et al.* (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* **434**: 980–986.

de Queiroz K (1998) The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations. In: *Endless forms: species and speciation* (Howard DJ, Berlocher SH, eds): 57–75. New York: Oxford University Press.

de Queiroz K (2005) Ernst Mayr and the modern concept of species. *Proceedings of the National Academy of Sciences, USA* **102**: 6600–6607.

de Queiroz K (2007) Species concepts and species delimitation. *Systematic Biology* **56**: 879–886.

Dupuis JR, Roe AD, Sperling FAH (2012) Multi-locus species delimitation in closely related animals and fungi: One marker is not enough. *Molecular Ecology* **21**: 4422–4436.

Ellison CE, Hall C, Kowbel D, Welch J, Brem RB, *et al.* (2011) Population genomics and local adaptation in wild isolates of a model microbial eukaryote. *Proceedings of the National Academy of Sciences, USA* **108**: 2831–2836.

Fourie G, Van der Merwe NA, Wingfield BD, Bogale M, Wingfield MJ, Steenkamp ET (2017) Mitochondrial introgression and interspecies recombination in the *Fusarium fujikuroi* species complex. *IMA Fungus* **9**: 37–48.

Fujita MK, Leaché AD, Burbrink FT, McGuire JA, Moritz C (2012) Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology and Evolution* **27**: 480–488.

Gams W (2016) Are old taxa without living authenticated cultures losing their status? *IMA Fungus* **7**: 72–73.

Garrido JL, Alcántara JM, Rey PJ, Medrano M, Guitián J, *et al.* (2017) Geographic genetic structure of Iberian columbines (gen. *Aquilegia*). *Plant Systematics and Evolution* **303**: 1145–1160.

Gladieux P, Ropars J, Badouin H, Branca A, Aguileta G, *et al.* (2014) Fungal evolutionary genomics provides insight into the mechanisms of adaptive divergence in eukaryotes. *Molecular Ecology* **23**: 753–773.

Greuter W, Burdet HM, Chaloner WG, Demoulin V,Grolle R, *et al.* (1988) *International Code of Botanical Nomenclature adopted by the Fourteenth International Botanical Congress, Berlin, July–August 1987*. [Regnum Vegetabile no. 118.] Königstein: Koeltz Scientific Books.

Greuter W, Garrity G, Hawksworth DL, Jahn R, Kirk PM, *et al.* (2011) Draft BioCode (2011): principles and rules regulating the naming of organisms. *Bionomina* **1**: 26–44; *Taxon* **60**: 201–212; *Bulletin of Zoological Nomenclature* **68**: 10–28.

Grossart HP, Wurzbacher C, James TY, Kagami M (2016) Discovery of dark matter fungi in aquatic ecosystems demands a reappraisal of the phylogeny and ecology of zoosporic fungi. *Fungal Ecology* **19**: 28–38.

Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, *et al.* (2011) Chimeric 16-rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Research* **21**: 494–504.

Harder CB, Læssøe T, Frøslev TG, Ekelund F, Rosendahl S, Kjøller R (2013) A three-gene phylogeny of the *Mycena pura* complex reveals 11 phylogenetic species and shows ITS to be unreliable for species identification. *Fungal Biology* **117**: 764–75.

Harrison RG (1998) Linking evolutionary pattern and process: the relevance of species concepts for the study of speciation. In: *Endless Forms: species and speciation* (Howard DJ, Berlocher SH, eds): 19–31. New York: Oxford University Press.

Hawksworth DL, Hibbett DS, Kirk PM, Lücking R (2016) (308–310) Proposals to permit DNA sequence data to serve as types of names of fungi. *Taxon* **65**: 899–900.

Hawksworth DL, Hibbett DS, Kirk PM, Lücking R (2018) (F-005-006) Proposals to permit DNA sequence data to be used as types of names of fungi. *IMA Fungus* **9**: v–vi.

Herr JR, Öpik M, Hibbett DS (2015) Towards the unification of sequence-based classification and sequence-based identification of host-associated microorganisms. *New Phytologist* **205**: 27–31.

Hey J (1994) Bridging phylogenetics and population genetics with gene tree models. In: *Molecular Ecology and Evolution: approaches and applications* (Schierwater B, Streit B, Wagner GP, DeSalle R, eds): 435–449. Basel: Birkhäuser.

Hey J (2006) On the failure of modern species concepts. *Trends in Ecology and Evolution* **21**: 447–450.

Hibbett DS, Ohman A, Glotzer D, Nuhn M, Kirk P, Nilsson RH (2011) Progress in molecular and morphological taxon discovery in *Fungi* and options for formal classification of environmental sequences. *Fungal Biology Reviews* **25**: 38–47.

Hudson RR (1990) Gene genealogies and the coalescent process. *Oxford Surveys in Evolutionary Biology* **7**: 1–43.

Hudson RR, Coyne JA (2002) Mathematical consequences of the genealogical species concept. *Evolution* **56**: 1557–1565.

Hudson RR, Slatkin M, Maddison WP (1992) Estimation of levels of gene flow from DNA sequence data. *Genetics* **132**: 583–589.

Hudson RR, Turelli M (2003) Stochasticity overrules the "three-times rule": genetic drift, genetic draft and coalescence times for nuclear loci versus mitochondrial DNA. *Evolution* **57**: 182–190.

Hug LA, Baker BJ, Anantharaman K, Brown CT, Probst AJ, *et al.* (2016) A new view of the tree of life. *Nature Microbiology* **1**: 16048.

Hughes KW, Petersen RH, Lodge DJ, Bergemann SE, Baumgartner K, *et al.* (2013) Evolutionary consequences of putative intra-and interspecific hybridization in agaric fungi. *Mycologia* **105**: 1577–1594.

Hughes KW, Morris SD, Reboredo Segovia A (2015) Cloning of ribosomal ITS PCR products creates frequent, non-random chimeric sequences – a test involving heterozygotes between *Gymnopus dichrous* taxa I and II. *MycoKeys* **10**: 45–56.

Hughes KW, Tulloss RE, Petersen RH (2018) A taxon cryptic with respect to *Amanita lavendula* is an apparent hybrid swarm that may have failed to undergo concerted evolution of the ribosomal repeat. *Mycologia* **110** (in press).

Inácio J, Behrens S, Fuchs BM, Fonseca A, Spencer-Martins I, Amann R (2003) *In situ* accessibility of *Saccharomyces cerevisiae* 26S rRNA to Cy3-labeled oligonucleotide probes comprising the D1 and D2 domains. *Applied and Environmental Microbiology* **69**: 2899–2905.

Jeffroy O, Brinkmann H, Delsuc, Philippe H (2006) Phylogenomics: the beginning of incongruence? *Trends in Genetics* **22**: 225–231.

Knowles LL, Carstens BC (2007) Delimiting species without monophyletic gene trees. *Systematic Biology* **56**: 887–895.

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, *et al.* (2013) Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* **22**: 5271–5277.

Kudryavtsev A, Gladkikh, A (2017) Two new species of *Ripella* (*Amoebozoa*, *Vannellida*) and unusual intragenomic variability in the SSU rRNA gene of this genus. *European Journal of Protistology* **61**: 92–106.

Kutschera VE, Bidon T, Hailer F, Rodi JL, Fain SR, Janke A (2014) Bears in a forest of gene trees: Phylogenetic inference is complicated by incomplete lineage sorting and gene flow. *Molecular Biology and Evolution* **31**: 2004–2017.

Lamichhaney S, Berglund J, Almen MS, Maqbool K, Grabherr M, *et al.* (2015) Evolution of Darwin's finches and their beaks revealed by genome sequencing. *Nature* **518**: 371–377.

Leliaert F, Verbruggen H, Vanormelingen P, Steen F, López-Bautista JM, *et al.* (2014) DNA-based species delimitation in algae. *European Journal of Phycology* **49**: 179–196.

Levine A (2001) Individualism, type specimens, and the scrutability of species membership. *Biology and Philosophy* **16**: 325–338.

Li Y, Jiao L, Yao YJ (2013) Non-concerted ITS evolution in fungi, as revealed from the important medicinal fungus *Ophiocordyceps sinensis*. *Molecular Phylogenetics and Evolution* **68**: 373–379.

Li Y, Yang R-H, Jiang L, Hu X-D, Wu Z-J, Yao Y-J (2017) rRNA pseudogenes in filamentous ascomycetes as revealed by genome data. *G3* **7**: 2695–2703.

Lindner DL, Banik MT (2011) Intragenomic variation in the ITS rDNA region obscures phylogenetic relationships and inflates estimates of operational taxonomic units in genus *Laetiporus*. *Mycologia* **103**: 731–740.

Lindner DL, Carlsen T, Nilsson RH, Davei M, Schumacher T, Kauserud H (2013) Employing 454 amplicon pyrosequencing to reveal intragenomic divergence in the internal transcribed spacer rDNA region in fungi. *Ecology and Evolution* **3**: 1751–1764.

Maddison WP (1997) Gene trees in species trees. *Systematic Biology* **46**: 523–536.

Meier R, Shiyang K, Vaidya G, Ng PKL (2006) DNA Barcoding and taxonomy in *Diptera*: A tale of high intraspecific variability and low identification success. *Systematic Biology* **55**: 715–728.

Meyer BS, Matschiner M, Salzburger W (2017) Disentangling incomplete lineage sorting and introgression to refine species-tree estimates for Lake Tanganyika cichlid fishes. *Systematic Biology* **66**: 531–550.

Murray RG, Schleifer KH (1994) Taxonomic notes: a proposal for recording the properties of putative taxa of prokaryotes. *International Journal of Systematic Bacteriology* **44**: 174–176.

Murray RG, Stackebrandt E (1995) Taxonomic note: implementation of the provisional status *Candidatus* for incompletely described procaryotes. *International Journal of Systematic Bacteriology* **45**: 186–187.

Naciri Y, Linder HP (2015) Species delimitation and relationships: the dance of the seven veils. *Taxon* **64**: 3–16.

Neafsey DE, Barker BM, Sharpton TJ, Stajich JE, Park DJ, *et al.* (2010) Population genomic sequencing of *Coccidioides* fungi reveals recent hybridization and transposon control. *Genome Research* **20**: 938–946.

Nei M (1987) *Molecular evolutionary genetics*. New York: Columbia University Press.

Neigel JE, Avise JC (1986) Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: *Evolutionary Processes and Theory* (Nevo E, Karlin S, eds): 515–534. New York: Academic Press.

Nilsson RH, Ryberg M, Kristiansoon E, Abarenkov K, Larsson KH, Urmas K (2006) Taxonomic realiability of DNA sequences in public sequence databases: a fungal perspective. *PLoS ONE* **1**: e59.

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson K-H (2008) Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics* **4**: 193–201.

Nilsson RH, Sánchez-García M, Ryberg M, Abarenkov K, Wurzbacher C, Kristiansson E (2017) Read quality-based trimming of the distal ends of public fungal DNA sequences is nowhere near satisfactory. *MycoKeys* **26**: 13–24.

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* **7**: 103–116.

Öpik M, Metsis M, Daniell TJ, Zobel M, Moora M (2009) Large-scale parallell 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytologist* **184**: 424–437.

Pamilo P, Nei M (1988) Relationships between gene trees and species trees. *Molecular Biology and Evolution* **5**: 568–583.

Parker CT, Tindall BJ, Garrity GM (2015) *International Code of Nomenclature of Prokaryotes*. *International Journal of Systematic and Evolutionary Microbiology*: DOI: 10.1099/ijsem.0.000778.

Parks MB, Wickett NJ, Alverson AJ (2017) Signal, uncertainty, and conflict in phylogenomic data for a diverse lineage of microbial eukaryotes (*Diatoms*, *Bacillariophyta*). *Molecular Biology and Evolution* **35**: 80–93.

Rautenberg A, Filatov D, Svennblad B, Heidari N, Oxelman B (2008) Conflicting phylogenetic signals in the SlX1/Y1 gene in *Silene*. *BMC Evolutionary Biology* **8**: 299.

Ride WDL, Cogger HG, Dupuis C, Kraus O, Minelli A, *et al.* (1999) *International Code of Zoological Nomenclature.* 4th edn. London: International Trust for Zoological Nomenclature.

Rindi F, Ryšánek D, Škaloud P (2017) Problems of epitypification in morphologically simple green microalgae: a case study of two widespread species of *Klebsormidium* (*Klebsormidiophyceae*, *Streptophyta*). *Fottea, Olomouc* **17**: 78–88.

Roper M, Ellison C, Taylor JW, Glass NL (2011) Nuclear and genome dynamics in multinucleate ascomycete fungi. *Current Biology* **21**: R786–R793.

Rosenberg NA (2003) The shapes of neutral gene genealogies in two species: probabilities of monophyly, paraphyly, and polyphyly in a coalescent model. *Evolution* **57**: 1465–1477.

Ruths D, Nakhleh L (2005) Recombination and phylogeny: effects and detection. *International Journal of Bioinformatics Research and Applications* **1**: 202–211.

Ryberg M, Nilsson RH (2018) New light on names and naming of dark taxa. *MycoKeys* **30**: 31–39.

Saag L, Mark K, Saag A, Randlane T (2014) Species delimitation in the lichenized fungal genus *Vulpicida* (*Parmeliaceae*, *Ascomycota*) using gene concatenation and coalescent-based species tree approaches. *American Journal of Botany* **101**: 169–2182.

Sandoval-Sierra JV, Martín MP, Diéguez-Uribeondo J (2014) Species identification in the genus Saprolegnia (Oomycetes): Defining DNA-based molecular operational units. *Fungal Biology* **118**: 559–578.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, *et al.* (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode for *Fungi*. *Proceedings of the National Academy of Sciences, USA* **109**: 6241–6246.

Seifert KA (2017) When should we describe species? *IMA Fungus* **8**: 37–39.

Shapiro BJ, Leducq J-B, Mallet J (2016) What is speciation? *PLoS Genetics* **12(3)**: e1005806.

Som A (2014) Causes, consequences and solution of phylogenetic incongruence. *Briefings in Bioinformatics* **16**: 536–548.

Sota T, Vogler AP (2001) Incongruence between mitochondrial and nuclear gene genealogy in the carabid beetles *Ohomopterus*. *Systematic Biology* **50**: 39–59.

Spribille T, Tuovinen V, Resl P, Vanderpool D, Wolinski H, *et al.* (2016) Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* **253**: 488–492.

Steenkamp ET, Wingfield MJ, McTaggart AR, Wingfield BD (2018) Fungal species and their boundaries matter – Definitions, mechanisms and practical implications. *Fungal Biology Reviews* **32**: 104–116.

Stewart JE, Timmer LW, Lawrence CB, Pryor BM, Peever TL (2014) Discord between morphological and phylogenetic species boundaries: incomplete lineage sorting and recombination results in fuzzy species boundaries in an asexual fungal pathogen. *BMC Evolutionary Biology* **14**: 38.

Strid Y, Ihrmark K, Stenlid J (2015) The primer fITS9 prevents chimera formation during fungal DNA amplification in a bark beetle DNA background. *Forest Pathology* **45**: 9–13.

Sung G-H, Sung J-M, Hywel-Jones NL, Spatafora JW (2007) A multi-gene phylogeny of *Clavicipitaceae* (*Ascomycota*, *Fungi*): Identification of localized incongruence using a combinational bootstrap approach. *Molecular Phylogenetics and Evolution* **44**: 1204–1223.

Tajima F (1983) Evolutionary relationships of DNA sequences in finite populations. *Genetics* **105**: 437–460.

Takahata N (1989) Gene genealogy in three related populations: consistency probability between gene and population trees. *Genetics* **122**: 957–966.

Takahata N, Nei M (1985) Gene genealogy and variance of interpopulational nucleotide differences. *Genetics* **110**: 325–344.

Taylor JW (2011) One fungus = one name: DNA and fungal nomenclature twenty years after PCR. *IMA Fungus* **2**: 113–120.

Thielecke L, Aranyossy T, Dahl A, Tiwari R, Roeder I, *et al.* (2017) Limitations and challenges of genetic barcode quantification. *Scientific Reports* **7**: 43249.

Thiéry O, Vasar M, Jairus T, Davison J, Roux C, *et al.* (2016) Sequence variation in nuclear ribosomal small subunit, internal transcribed spacer and large subunit regions of *Rhizophagus irregularis* and *Gigaspora margarita* is high and isolate-dependent. *Molecular Ecology* **25**: 2816–2832.

Tripp EA, Lendemer JC (2014) Sleepless nights: when you can't find anything to use but molecules to describe new taxa. *Taxon* **63**: 969–971.

Turland N, Wiersema J (2017) Synopsis of proposals on nomenclature – Shenzhen 2017: A review of the proposals concerning the *International Code of Nomenclature for algae, fungi, and plants* submitted to the XIX International Botanical Congress. *Taxon* **66**: 217–274.

Turland N, Wiersema J, Monro AM, Deng Y-F, Zhang L (2017) XIX International Botanical Congress: report of Congress action on nomenclature proposals. *Taxon* **66**: 1234–1245.

Vági P, Knapp DG, Kósa A, Seress D, Horváth ÁN, Kovács GM (2014) Simultaneous specific in planta visualization of root colonizing fungi using fluorescence *in situ* hybridization (FISH). *Mycorrhiza* **24**: 259–266.

Vd'ačný P (2017) Integrative taxonomy of ciliates: assessment of molecular phylogenetic content and morphological homology testing. *European Journal of Protistology* **61**: 388–398.

Wakeley J (2000) The effects of subdivision on the genetic divergence of populations and species. *Evolution* **54**: 1092–1101.

Peyrégne S, Boyle MJ, Dannemann M, Prüfer K (2017) Detecting ancient positive selection in humans using extended lineage sorting. *Genome Research* **27**: 1563–1572.