

A commonly quoted Chinese proverb states: ‘*The beginning of wisdom is to call things by their proper name.*’ This has been attributed to Confucius and, on more than one occasion, we have cited this aphorism to point out the importance of proper nomenclature in the scientific literature (Kroll et al., 2007; Raja et al., 2017a). In accordance with this notion, this journal has a policy that the full Latin binomial name (with authority) should be used for the organism studied in an article. However, in the Fungal Kingdom, there are noteworthy caveats. What if the current state of the science does not enable a full Latin binomial to be assigned? Would it not be better to describe the isolate only to the level in which there is a high degree of confidence? We believe that the answer is a resounding “yes” and that a conservative approach to assigning a Latin binomial of a fungus should be encouraged, especially in journals that focus on fungal metabolite chemistry.

Fungi are extremely diverse organisms in the tree of life. This diversity is seen in their ecology, evolution, morphology, physiology, and phylogeny. As a result of this variety in form and function, the number of species in the Fungal Kingdom has been estimated at well into the millions (Hawksworth and Lücking, 2017; Schmit and Mueller, 2007), possibly making them the second most diverse group of organisms on the planet (Purvis and Hector, 2000). However, taxonomic mycologists have only described approximately 135,000 to 150,000 species, likely to represent 10% at most of the total number of fungi in the world, even if only the most conservative estimates of their biological diversity are considered (Crous et al., 2021; Hibbett et al., 2016). This means that not only are the vast majority of fungi currently undescribed based on their morphology but also their DNA sequence data are not available. Furthermore, we do not have reliable molecular data for the type specimens of numerous described fungal names, while many type strains are neither obvious nor clearly indicated (Raja et al., 2017b).

In 2012, a group of mycologists proposed the ribosomal internal transcribed spacer (ITS) region as the official primary barcoding marker for the molecular identification of fungi (Schoch et al., 2012). Since then, there have been follow-up papers with suggestions about the precautions the scientific community needs to take when attempting to use the ITS region for species-level identification throughout the entire Fungal Kingdom (Hofstetter et al., 2019; Hongsanan et al., 2018; Inderbitzin et al., 2020; Lücking et al., 2020; Raja et al., 2017a, 2017b; Stadler et al., 2020). In brief, these studies report the following:

- a) The ITS region alone should not be used for a BLAST search;
- b) When carrying out a BLAST search, the use of the RefSeq database is recommended, which consists of sequence data from authentic fungal samples (holotype strains). Moreover, only sequences from published studies that are reliable and can be tracked to a journal should be used;
- c) Where possible, both morphological data and molecular data should always be used for species-level identification;
- d) That it is important to keep in mind that species of the top 20 fungal genera from which the highest number of natural products have historically been reported (including, for example, *Acremonium*, *Aspergillus*, *Alternaria*, *Fusarium*, *Ganoderma*, *Penicillium*, *Trichoderma*, and *Xylaria*) cannot be safely identified by ITS sequencing alone. Therefore, the use of “secondary barcodes”, i.e. single-copy protein coding regions, such as *RPB1*, *RPB2*, *TEF1-alpha*, *calmodulin*, *Beta tubulin*, among other regions, is advisable;
- e) That it is recommended to use Maximum Likelihood or other tree-based phylogenetic approaches, along with DNA barcoding, to place an unknown species into a phylogenetic clade, even without a species-level identification. For additional best practice advice on fungal identification, readers are encouraged to refer to the above-cited studies.

The ITS region basically has two different kinds of problems in some fungal groups. On the one hand, many species of a given genus often cannot be segregated using this non-coding part of the DNA (and, notably, it is not an essential gene that is undergoing evolutionary pressure). Examples are the *Daldinia concentrica* complex (Stadler et al., 2014), where many species belonging to the same group have almost identical ITS sequences, while in some important plant pathogenic genera, like *Ramularia*, there may be up to three dozen species that do not differ significantly in the ITS region but can easily be segregated based on secondary barcodes like *TEF1-alpha* or *Beta tubulin* (Videira et al., 2016). On the other hand, one of the first comprehensive studies using third generation sequencing techniques (Wibberg et al., 2020) recently demonstrated that the high quality genomes of some ecologically important Ascomycota, which are frequently isolated as endophytes and belong to some of the most prolific fungal metabolite producers, contain multiple copies of the ITS region. The ITS paralogues present in the same

genome may have a homology of less than 90%. Multiple copies of the ITS region are also seen in the mushroom genus *Laetiporus* (Lindner and Banik, 2011), but this phenomenon remains to be studied in other groups of fungi.

In many cases, identification of fungi to species-level may require a combination of morphology (phenotypic; including secondary metabolite profiles) and molecular sequence data from multiple genes (loci) to establish species boundaries (Lambert et al., 2019; Raja et al., 2017c). These so-called polythetic (comparative) taxonomic approaches are necessary to obtain an accurate species-level identification (Crous et al., 2021).

This recent paper also discusses a ten-step approach for identifying plant pathogenic fungi, which could be applied to the species-level identification of other Ascomycota and Basidiomycota in general. This level of taxonomic scrutiny is not always possible for articles submitted to *Phytochemistry*, as the fungal strain producing chemically-rich specialised metabolites could be new to science (Harms et al., 2021; Noumeur et al., 2020; Paguigan et al., 2016) or there may only be a few similar sequences available in the NCBI GenBank, making it difficult to determine the exact species identity of the specialised metabolite-producing strain (Chepkirui et al., 2019; Helaly et al., 2018; Paguigan et al., 2019). When new fungal species are encountered, the methods outlined by Aime et al. (2021) should be followed in order to publish these in mycology journals, allowing the taxonomic data to be both evaluated by and reported to experts in fungal taxonomy and systematics. Additional comments on fungal taxonomy and the sequence-based nomenclature of fungi are provided in a recent review (Lücking et al., 2021).

In conclusion, we advise being cautious with nomenclature and, where possible, we encourage greater collaborative efforts between mycologists and natural product chemists when determining the correct name for a fungal species (Raja et al., 2017b). We also suggest that it is not only permissible but also preferable to be conservative in providing a fungal identification, especially in the context of a journal that largely focuses on the chemistry of the organism. The most critical point can be summed up as: just because a BLAST search returns a hit, this does not mean it is the true identification of that specimen. An attempt to determine a fungal species by this method, particularly using only ITS sequences without referring to holotype sequences, is often about as accurate as if a natural product chemist tried to determine the absolute configuration of a new compound based solely on LC/MS data.

The difficulties outlined above of obtaining full taxonomic data for newly isolated and studied fungi should not, however, be used as an excuse for sloppy science. A reasonable effort is required to place isolates within the Fungal Kingdom, enabling comparisons with closely related organisms. The sequence data obtained from the fungi should be deposited in GenBank. When chemists encounter putative new species, they should work with mycologists to describe them as new, probably in a separate publication, following all the protocols and standards for new species descriptions (Aime et al., 2021) required by reputable mycology journals. Crucially, the isolate must be placed and conserved in a publicly-accessible mycology collection, allowing further study of its taxonomy and metabolism by other groups of researchers.

References

- Aime, C., Miller, A.N., Aoki, T., Cai, L., Crous, P.W., Hawksworth, D.L., Hyde, K.D., Kirk, P.M., Lücking, R., May, T.W., Malosso, E., Redhead, S.A., Rossman, A., Stadler, M., Thines, M., Yurkov, A., Schoch, C.L., 2021. How to publish a new fungal species, or name, version 3.0. *IMA Fungus* 12, 1–15. <https://doi.org/10.1186/s43008-021-00063-1>.
- Chepkirui, C., Cheng, T., Sum, W.C., Matasyoh, J.C., Decock, C., Praditya, D.F., Wittstein, K., Steinmann, E., Stadler, M., 2019. Skeletocutins AL: antibacterial agents from the Kenyan wood-inhabiting basidiomycete, *Skeletocutis* sp. *J. Agric. Food Chem.* 67, 8468–8475. <https://doi.org/10.1021/acs.jafc.9b02598>.
- Crous, P.W., Rossman, A.Y., Aime, C., Allen, C., Burgess, T., Groenewald, J.Z., Castlebury, L., 2021. Names of phytopathogenic fungi: a practical guide. *Phytopathology*. <https://doi.org/10.1094/PHYTO-11-20-0512-PER>.
- Harms, K., Milic, A., Stchigel, A.M., Stadler, M., Surup, F., Marin-Felix, Y., 2021. Three new derivatives of Zopfinol from *Pseudorhizophila mangenotii* gen. et comb. nov. *J. Fungi*. 7 (181) <https://doi.org/10.3390/jof7030181>.
- Hawksworth, D.L., Lücking, R., 2017. Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiol. Spectr.* 5, 79–95. <https://doi.org/10.1128/microbiolspec.FUNK-0052-2016>.
- Helaly, S.E., Ashrafi, S., Teponno, R.m.B., Bernecker, S., Dababat, A.A., Maier, W., Stadler, M., 2018. Nematicidal cyclic lipopeptide and a xanthocillin derivative from a phaeosporiaceae fungus parasitizing eggs of the plant parasitic nematode *Heterodera filipjevi*. *J. Nat. Prod.* 81, 2228–2234.

- <https://doi.org/10.1021/acs.jnatprod.8b00486>.
- Hibbett, D., Abarenkov, K., Koljalg, U., Opik, M., Chai, B., Cole, J.R., Wang, Q., Crous, P. W., Robert, V.A., Helgason, T., 2016. Sequence-based classification and identification of Fungi. *Mycologia* 108, 1049–1068. <https://doi.org/10.3852/16-130>.
- Hofstetter, V., Buyck, B., Eyssartier, G., Schnee, S., Gindro, K., 2019. The unbearable lightness of sequenced-based identification. *Fungal Divers.* 96, 243–284. <https://doi.org/10.1007/s13225-019-00428-3>.
- Hongsanan, S., Jeewon, R., Purahong, W., Xie, N., Liu, J.-K., Jayawardena, R.S., Ekanayaka, A.H., Dissanayake, A., Raspé, O., Hyde, K.D., 2018. Can we use environmental DNA as holotypes? *Fungal Divers.* 92, 1–30. <https://doi.org/10.1007/s13225-018-0404-x>.
- Inderbitzin, P., Robbertse, B., Schoch, C.L., 2020. Species identification in plant-associated Prokaryotes and Fungi using DNA. *Phytobiomes J.* <https://doi.org/10.1094/PBIOMES-12-19-0067-RVW>. PBIOMES-12-19-0067-RVW.
- Kroll, D.J., Shaw, H.S., Oberlies, N.H., 2007. Milk Thistle nomenclature: why it matters in cancer research and pharmacokinetic studies. *Integr. Canc. Ther.* 6, 110–119. <https://doi.org/10.1177/1534735407301825>.
- Lambert, C., Wendt, L., Hladki, A.I., Stadler, M., Sir, E.B., 2019. *Hypomontagnella* (*Hypoxylaceae*): a new genus segregated from *Hypoxylon* by a polyphasic taxonomic approach. *Mycol. Prog.* 18, 187–201. <https://doi.org/10.1007/s11557-018-1452-z>.
- Lindner, D.L., Banik, M.T., 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. <https://doi.org/10.3852/10-331>.
- Lücking, R., Aime, M.C., Robbertse, B., Miller, A.N., Aoki, T., Ariyawansa, H.A., Cardinali, G., Crous, P.W., Druzhinina, I.S., Geiser, D.M., Hawksworth, D.L., Hyde, K. D., Irinyi, L., Jeewon, R., Johnston, P.R., Kirk, P.M., Malosso, E., May, T.W., Meyer, W., Nilsson, H.R., Opik, M., Robert, V., Stadler, M., Thines, M., Vu, D., Yurkov, A.M., Zhang, N., Schoch, C.L., 2021. Fungal taxonomy and sequence-based nomenclature. *Nat. Microbiol.* 6, 540–548. <https://doi.org/10.1038/s41564-021-00888-x>.
- Lücking, R., Aime, M.C., Robbertse, B., Miller, A.N., Ariyawansa, H.A., Aoki, T., Cardinali, G., Crous, P.W., Druzhinina, I.S., Geiser, D.M., Hawksworth, D.L., Hyde, K. D., Irinyi, L., Jeewon, R., Johnston, P.R., Kirk, P.M., Malosso, E., May, T.W., Meyer, W., Opik, M., Robert, V., Stadler, M., Thines, M., Vu, D., Yurkov, A.M., Zhang, N., Schoch, C.L., 2020. Unambiguous identification of fungi: where do we stand and how accurate and precise is fungal DNA barcoding? *IMA Fungus* 1–32. <https://doi.org/10.1186/s43008-020-00033-z>.
- Noumeur, S.R., Teponno, R.B., Helaly, S.E., Wang, X.-W., Harzallah, D., Houbraken, J., Crous, P.W., Stadler, M., 2020. Diketopiperazines from *Batnamyces globulariicola*, gen. & sp. nov. (*Chaetomiaceae*), a fungus associated with roots of the medicinal plant *Globularia alypum* in Algeria. *Mycol. Prog.* 19, 589–603. <https://doi.org/10.1007/s11557-020-01581-9>.
- Paguigan, N.D., Raja, H.A., Day, C.S., Oberlies, N.H., 2016. Acetophenone derivatives from a freshwater fungal isolate of recently described *Lindgomyces madisonensis* (G416). *Phytochemistry (Oxf.)* 126, 59–65. <https://doi.org/10.1016/j.phytochem.2016.03.007>.
- Paguigan, N.D., Rivera-Chavez, J., Stempin, J.J., Augustinović, M., Noras, A.I., Raja, H. A., Todd, D.A., Triplett, K.D., Day, C., Figueroa, M., Oberlies, N.H., 2019. Prenylated Diresorcinols inhibit bacterial quorum sensing. *J. Nat. Prod.* 82, 550–558. <https://doi.org/10.1021/acs.jnatprod.8b00925>.
- Purvis, A., Hector, A., 2000. Getting the measure of biodiversity. *Nature* 405, 212–219. <https://doi.org/10.1038/35012221>.
- Raja, H.A., Baker, T.R., Little, J.G., Oberlies, N.H., 2017a. DNA barcoding for identification of consumer-relevant mushrooms: a partial solution for product certification? *Food Chem.* 214, 383–392. [10.1016/j.foodchem.2016.07.052](https://doi.org/10.1016/j.foodchem.2016.07.052).
- Raja, H.A., Miller, A.N., Pearce, C.J., Oberlies, N.H., 2017b. Fungal identification using molecular tools: a primer for the natural products research community. *J. Nat. Prod.* 80, 756–770. <https://doi.org/10.1021/acs.jnatprod.6b01085>.
- Raja, H.A., Paguigan, N.D., Fournier, J., Oberlies, N.H., 2017c. Additions to *Lindgomyces* (*Lindgomycetaceae*, *Pleosporales*, *Dothideomycetes*), including two new species occurring on submerged wood from North

- Carolina, USA, with notes on secondary metabolite profiles. *Mycol. Prog.* 16, 535–552. <https://doi.org/10.1007/s11557-017-1282-4>.
- Schmit, J.P., Mueller, G.M., 2007. An estimate of the lower limit of global fungal diversity. *Biodivers. Conserv.* 16, 99–111. <https://doi.org/10.1007/s10531-006-9129-3>.
- Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Fungal Barcoding, C., Fungal Barcoding Consortium Author, L., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci. U.S.A.* 109, 6241–6246. <https://doi.org/10.1073/pnas.1117018109>.
- Stadler, M., Læssøe, T., Fournier, J., Decock, C., Schmieschek, B., Tichy, H.-V., Peršoh, D., 2014. A polyphasic taxonomy of *Daldinia* (*Xylariaceae*). *Stud. Mycol.* 77, 1–143. <https://doi.org/10.3114/sim0016>.
- Stadler, M., Lambert, C., Wibberg, D., Kalinowski, J., Cox, R.J., Kolařík, M., Kuhnert, E., 2020. Intragenomic polymorphisms in the ITS region of high-quality genomes of the *Hypoxylaceae* (*Xylariales*, *Ascomycota*). *Mycol. Prog.* 19, 235–245. <https://doi.org/10.1007/s11557-019-01552-9>.
- Videira, S., Groenewald, J., Braun, U., Shin, H.-D., Crous, P.W., 2016. All that glitters is not *Ramularia*. *Stud. Mycol.* 83, 49–163. [10.1016/j.simyco.2016.06.001](https://doi.org/10.1016/j.simyco.2016.06.001).
- Wibberg, D., Stadler, M., Lambert, C., Bunk, B., Sproer, C., Rückert, C., Kalinowski, J., Cox, R.J., Kuhnert, E., 2020. High quality genome sequences of thirteen *Hypoxylaceae* (*Ascomycota*) strengthen the phylogenetic family backbone and enable the discovery of new taxa. *Fungal Divers.* 1–22. <https://doi.org/10.1007/s13225-020-00447-5>.

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