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Contributions to the taxonomy and distribution of *Tricholomella* (Lyophyllaceae) based on the basidiomata collected from Halkalı, İstanbul

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Abstract: Basidiomata of *Tricholomella constricta* (Fr.) Zerova ex Kalamees belonging to *Lyophyllaceae* are collected from Halkalı-İstanbul and studied using both morphologic and molecular methods. According to the classical systematic the genus *Tricholomella* Zerova ex Kalamees contains more than one species, such as *T. constricta* and *T. leucocephala*. Our studies found out that the two species are not genetically too different, but conspecific and a new description is needed including the members with- or without annulus. In this study, illustrations, a short discussion and a simple phylogenetic tree are provided.

Key words: Fungal taxonomy, ITS, Systematics, Turkey

İstanbul, Halkalı'dan toplanan bazidiyomalara göre *Tricholomella constricta* (Lyophyllaceae)'nın taksonomi ve yayılışına katkılar

Öz: *Lyophyllaceae* ailesine ait *Tricholomella constricta* (Fr.) Zerova ex Kalamees'in İstanbul-Halkalı'dan toplanan bazidiyomaları hem morfolojik ve hem de moleküler yöntemlerle çalışılmıştır. Klasik sistematiğe göre *Tricholomella* Zerova ex Kalamees genusu, *T. constricta* ve *T. leucocephala* gibi birden fazla tür içermektedir. Çalışmalarımız, bu iki türün genetik olarak birbirinden çok da farklı olmadığını, aynı tür içerisinde olduğunu ve annulus içeren ve de içermeyen türleri içerisine alan yeni bir deskripsiyon yapılması gerektiğini ortaya çıkarmıştır. Bu çalışmada arazi ve laboratuvar resimleri, kısa bir tartışma ve basit bir soyağacı verilmiştir.

Anahtar kelimeler: Fungal taksonomi, ITS, Sistematik, Türkiye

Introduction

Tricholomella Zerova ex Kalamees is monotypic and looks near to *Tricholoma*; saprotrophic on soil in forests and meadows in summer to autumn (Kalamees, 2004; Kirk et al., 2008). Basidiomata tricholomatoid; pileus convex to plane, smooth, cottony-tomentose, white to pale brownish, often olivaceous, yellowish to greyish. Lamellae emarginate to almost free, whitish. Stem whitish, smooth, tapered, fibrillose and dry. Veil whitish, membranous; smell and taste farinaceous. Basidiospores broadly ellipsoid to ovoid, typically echinulate; basidia siderophilous; cystidia absent; clamps present and pileipellis a cutis (Knudsen and Vesterholt, 2008).

The aim of this study is to contribute to the taxonomy and distribution of *Tricholomella* (Fr.) Zerova ex Kalamees. We aimed to found out the genetic similarity of *Tricholomella constricta* (Fr.) Zerova ex Kalamees and *T. leucocephala* (Bull.) Zerova ex Bon.

Materials and methods

Basidiomata were detected, photographed and collected from Halkalı-İstanbul on 23.12.2018 and 22.01.2019. Floristic elements, mycorrhizal relationships were noted in the field; sectioned from the pileus, lamellae and stipe; mounted in concentrated ammonia, subsequently stained with Congo red and later examined under Zeiss A2 Axio Imager trinocular research

microscope. Micro-slides of the pileipellis, basidia and the basidiospores were obtained and at least 25 measurements were made for each structures (Clémençon, 2009). Dried voucher specimens are kept at a personal fungarium of the Fatih Faculty of Education in the Trabzon University, Trabzon, Turkey. Morphological findings have been confirmed by the molecular analysis (ITS, GenBank code: MK957138) and a phylogenetic tree was produced. Total DNA was extracted from a dry specimen employing a modified protocol based on Murray and Thompson (1980). PCR reactions (Mullis and Faloona, 1987) included 35 cycles with an annealing temperature of 54 ºC, using primers ITS1F and ITS4 (White et al., 1990, Gardes and Bruns, 1993) to amplify the ITS rDNA region. PCR product was checked in a 1% agarose gel, and sequenced with primer ITS4. The chromatogram was checked searching for putative reading errors, and these were corrected. BLAST (Altschul et al., 1990) was used to select the most closely related sequences from the International Nucleotide Sequence Database Collaboration (INSDC) public databases. Sequences came mainly from Hofstetter et al. (2002), Consiglio et al. (2011), and Bellanger et al. (2015). Sequences first were aligned in MEGA 5.0 (Tamura et al., 2011) software with its Clustal W application and then corrected manually. The final alignment included 324/604 variable sites. The aligned dataset was loaded in MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003), where a Bayesian analysis was performed (model GTR+G, two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after 0.42 M generations, standard deviation having fell below 0.01. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML (Stamatakis, 2006) using the standard search algorithm (GTRMIX model, 2000 bootstrap replications). Significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Taxonomy

Lyophyllaceae

Tricholomella constricta (Fr.) Zerova ex Kalamees, Persoonia 14 (4): 446 (1992) [Syn. *Agaricus constrictus* Fr. = *Armillaria constricta* (Fr.) Gillet = *Calocybe constricta* (Fr.) Kühner ex Singer = *Echinosporella constricta* (Fr.) Contu = *Gyrophila constricta* (Fr.) Quél. = *Lepiota constricta* (Fr.) Quél. = *L. constricta* (Fr.) Rea = *Lyophyllum constrictum* (Fr.) Singer = *Melanoleuca constricta* (Fr.) Métrod= *Tricholoma constrictum* (Fr.) J.E.Lange = *T. constrictum* (Fr.) Ricken= *Tricholomella constricta* (Fr.) Zerova = *T. constrictum* (Fr.) Zerova ex Kalamees = *T. constrictum* (Fr.) Zerova]

Pileus 50-70 mm, convex to expanded or plane; sometimes slightly whitish, grey-yellowish on drying; umbo indistinct, sometimes umbilicate, slightly depressed when old, dirty, surface not very smooth, rough, sometimes partially eaten by insects. Lamellae crowded, white, broad. (L = 80-100, l = 2-5). Stipe 50-80 × 5-30 mm, cylindrical, generally curved; tapering towards the base, white, solid to stuffed, pruinose. Context white (Figure 1). Basidiospores strongly echinulate and typically elliptical, (8.1)8.5-10(10.5) × (5.3)6-6.7(7.3) µm, on average 9.3 × 6.3 µm (n = 50). Basidia clavate, 30-35 × 8-12 µm, generally 4- spored, rarely 2- spored. Cystidia absent. Pileipellis consists of an epicutis made up of 4.5-10.8 µm wide parallel hyphae with encrusting (Figure 2). Morphological findings are accordance with ITS sequence.

Specimens examined

Turkey, İstanbul, Halkalı, plantation, 23.12.2018, 41°02'45.44" N, 28°47'38.54" E, 100 m alt., Aytaç 026a; 22.01.2019, 41°02'45.57" N, 28°47' 39.02" E, Aytaç 026b, larch, spindles, needle tree, cherry laurel, bay tree.

Discussion

According to the traditional systematic based on the morphology, *Tricholomella* is not monotypic, but contains more than one species, such as *T. constricta* and *T. leucocephala* (Bon, 1999). *T. constricta* differs from *T. leucocephala* with the presence of a simple membranous annulus. Our studies found out that the two species are not genetically too different, but conspecific and a new description is needed including the members with- or without annulus. Before the present study *Tricholomella constricta* (Fr.) Zerova ex Kalamees was collected from Sarıkamış Allahukeber Mountains National Park (Kars) and studied according to morphological methods (Akçay, 2019). Our collection is from İstanbul-Halkalı; identified according to both molecular (Figure 3) and morphological methods and is the second record for the Turkish mycota (Keleş et al., 2014; Sesli and Denchev, 2014; Doğan and Kurt, 2016; Akata et al., 2018). The pileus of the collection from Istanbul is 50-70 mm, whitish, grey-yellowish, umbilicate, slightly depressed around the center when old. The pileus of the collection from Sarıkamış Allahuekber mountains is 20-60 mm, silky white, yellowish or greyish. The stipe of our collection is 50-80 × 5-30 mm, cylindrical, generally curved; tapering towards the base, white, solid to stuffed, pruinose. The Sarıkamış collection has 20-55 x 10-15 mm, white, slightly floccose-fibrillose, cylindrical or slightly tapered stipe.

Basidiospores of our collection are strongly echinulate and typically elliptical, 8.5-10 × 6-6.7 µm and the basidia clavate, 30-35 × 8-12 µm. The basidiospores of the other collection are hyaline, ellipsoid to oval and distinctly echinulate, 7-10 × 5-6 µm, while the basidia slenderly clavate and 25-35 x 6-8 µm (Akçay, 2019).

Figure 1. *Tricholomella constricta*: a, b and c. basidiomata (scale bars: 30 mm).

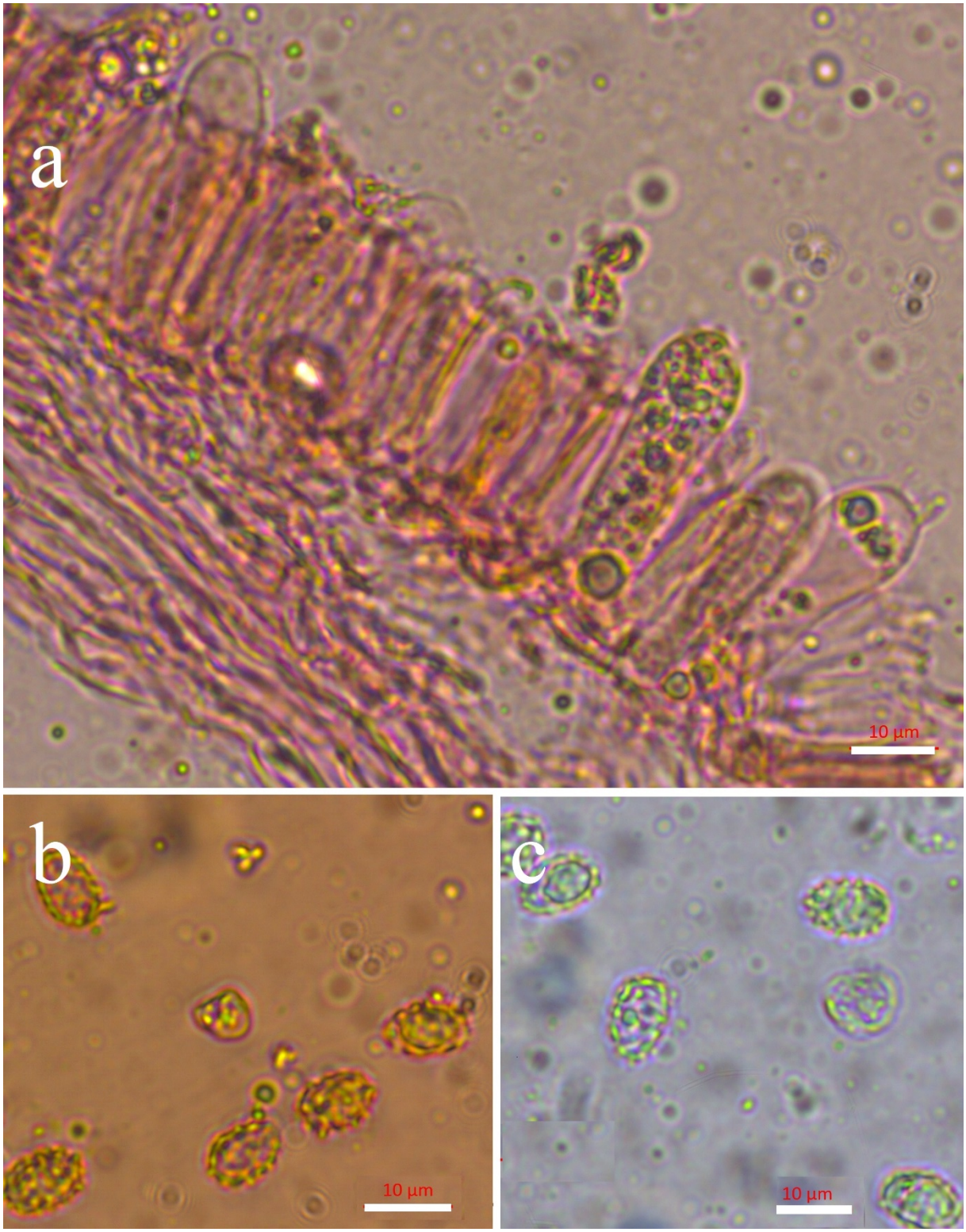

Figure 2. *Tricholomella constricta*: a. basidia, b and c. basidiospores (scale bars: 10 µm).

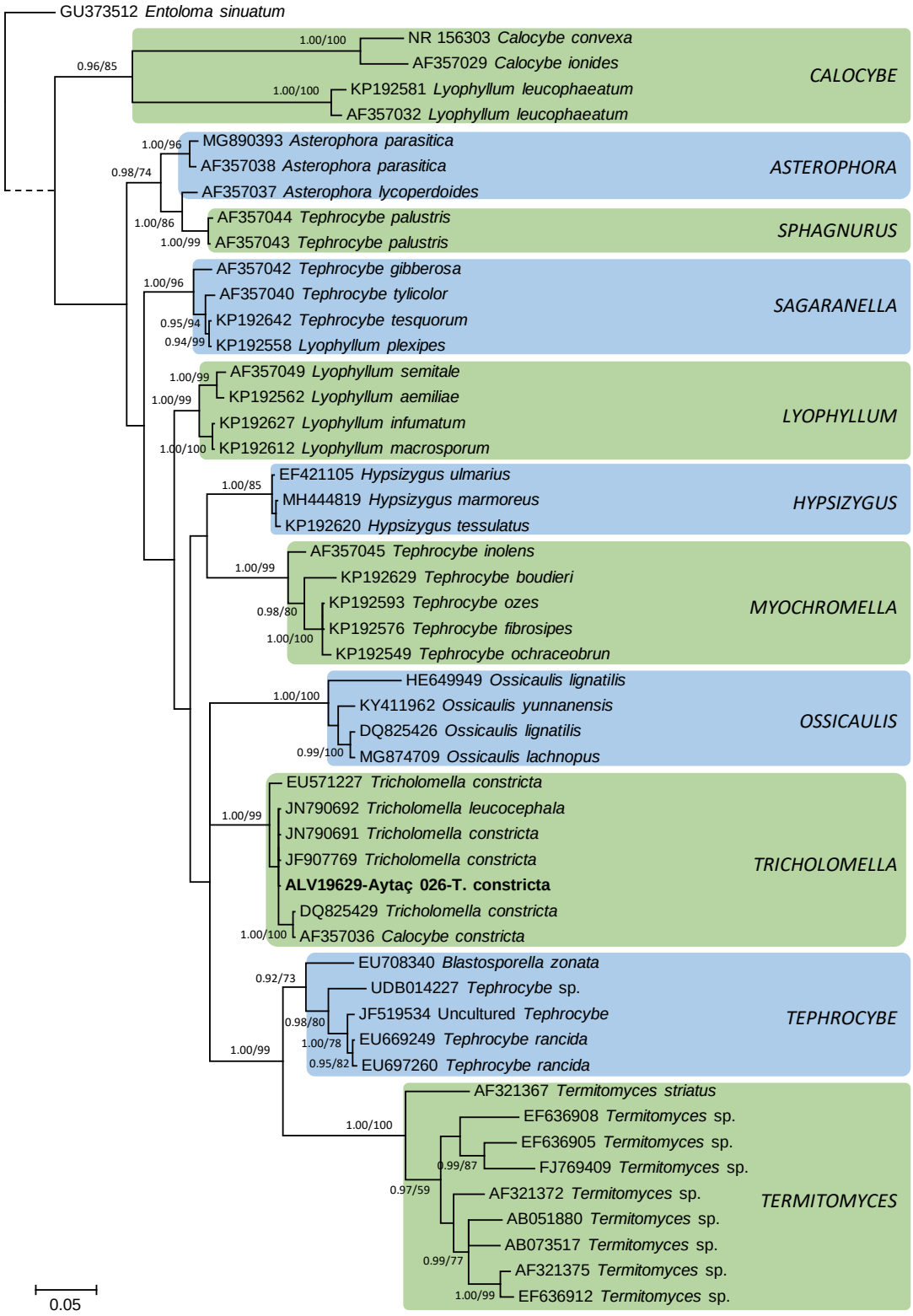

Figure 3. 50% Majority rule consensus ITS rDNA phylogram of selected genera of the family Lyophyllaceae obtained in MrBayes from 3150 sampled trees. Nodes were annotated if supported by >0.95 Bayesian PP (left) or >70% ML BP (right).

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References

Akata, I., Kabaktepe, Ş., Sevindik, M. and Akgül, H. (2018*).* Macrofungi determined in Yuvacık basin (Kocaeli) and its close environs. *Kastamonu University Journal of Forestry Faculty*, 18 (2) 152-163.

Akçay, M.E. (2019). A new edible macrofungus record for Turkey. *Journal of Natural & Applied Sciences of East*, 2 (1) 10-15.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, (215) 403-410.

Bellanger, J-M., Moreau, P-A., Corriol, G., Bidaud, A., Chalange, R., Dudova, Z. and Richard, F. (2015). Plunging hands into the mushroom jar: a phylogenetic framework for Lyophyllaceae (Agaricales, Basidiomycota). *Genetica*, 143 (2) 169-194.

Bon, M. (1999). Novitates - Tricholomatales (Marasmiaceae, Lyophyllaceae et Dermolomataceae). *Documents Mycologiques*, 29 (115) 33-34.

Clémençon, H. (2009). *Methods for Working with Macrofungi: Laboratory Cultivation and Preparation of Larger Fungi for Light Microscopy.* Germany: Erchtesgadener Anzeiger.

Consiglio, G., Orlandini, C., Setti, L., Moreno, G. and Alvarado, P. (2011). Il genere Tricholomella in Italia. *Rivista di Micologia*, (2) 135-155.

Doğan, H.H. and Kurt, F. (2016). New macrofungi records from Turkey and macrofungal diversity of Pozantı-Adana. *Turk J Bot*, (40) 209-217.

Gardes, M. and Bruns, T.D. (1993). ITS primers with enhanced specificity for Basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology*, (2) 113-118.

Hofstetter, V., Clémençon, H., Vilgalys, R. and Moncalvo, J-M. (2002). Phylogenetic analyses of the Lyophylleae (Agaricales, Basidiomycota) based on nuclear and mitochondrial rDNA sequences. *Mycological Research*, 106 (9) 1043-1059.

Kalamees, K. (2004). Palearctic Lyophyllaceae (Tricholomatales) in Northern and Eastern Europe and Asia. *Scripta Mycologica,* (18) 1-135.

Keleş, A., Demirel, K., Uzun, Y. and Kaya, A. (2014). Macrofungi of Ayder (Rize/Turkey) high plateau. *Biological Diversity and Conservation*, 7 (3) 177-183.

Kirk, P.M., Cannon, P.F., Minter, D.W. and Stalfers, J.A. (2008). *Authors of Fungal Names*. Wallingford, UK: CABI Bioscience.

Knudsen, H. and Vesterholt, J. (2008). *Funga Nordica. Agaricoid, Boletoid and Cyphelloid Genera.* Denmark: Nordsvamp.

Mullis, K. and Faloona, F.A. (1987). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymology*, (155) 335-350.

Murray, M.G. and Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, 8 (19) 4321-4325.

Ronquist, F. and Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, (19) 1572-1574.

Sesli, E. and Denchev, C.M. (2014). Onward (Continuously Updated). *Mycotaxon Webpage. Available online at http://www.mycotaxon.com/resources/weblists.html.*

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, (22) 2688-2690.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: Molecular volutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods*. Molecular Biology and Evolution*, 28 (10) 2731-2739.

White, T.J., Bruns, T.D., Lee, S. and Taylor, J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. M.A. Innis, D.H. Gelfand, J. Sninsky, T.J. White (Ed.) *PCR protocols: a guide to methods and applications. Academic*, (482 pp). San Diego.