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Protein coding gene, RPB2, better resolved the identity of *Trametes* species collected from Nigeria than ribosomal genes

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ABSTRACT

Trametes species is a cosmopolitan macrofungus and the identification of this particular genus has been chaotic over the years due to difficulty in separation of the species based on morphological characteristics. The present study reports the identification of twenty-five (25) specimens of *Trametes* spp collected from Akure, Nigeria using ribosomal marker and a protein-coding gene RPB2. The *Trametes* spp investigated were designated V01 to V026. Standard method employing Cetyltrimethyl ammonium (CTAB) was used to isolate the DNA of these macrofungi. PCR amplification of DNA and sequencing of the amplicons obtained from ribosomal gene (ITS) and protein coding gene (RPB2) was performed using standard methods. Phylogenetic analysis of the sequences obtained from ribosomal and the protein coding gene revealed that the 26 *Trametes* specimens belong to the following species: *Trametes elegans*, *T. polyzona*, *T. cubensis*, *T. lactinea,* and *T. cingulata*. The study revealed that the protein-coding gene, RPB2, resolved the identity of the *T. cingulata* collected from Nigeria better than ITS sequence data.

Keywords: *Trametes* species, Molecular Identification, Ribosomal gene, ITS, Protein coding gene, RPB2

Mushrooms could be edible, inedible and poisonous based on their composition. *Trametes* species is one of the groups of the inedible mushrooms. They are cosmopolitan polyporoid white rot fungi found on hard wood (Tomsosky *et al*., 2006). According to Gilbertson and Ryvarden (1987), *Trametes* spp can be found in almost all forest ecosystem throughout the world where they serve as decomposers. Moreover, they are economically important as bioremediator and biodegrader of cellulosic organic waste in the ecosystem (Wancura *et al*., 2024). Apart from their economic importance in the environment, some species of *Trametes* are important in medicine (Cui *et al*., 2011).

About fifty species of *Trametes* had been reported and are world wide in distribution (Kirk *et al*., 2008). Morphologically, *Trametes* spp is distinguished by a pileate basidiocarp, di- to trimitic hyphal systems and smooth non-dextroid spores (Ryvarden, 1991). One major challenge is to distinguish between members of this genus to species level. They are regarded as the most confused group of genera in Polyporaceae (Cui *et al*., 2011). According to Carlson *et al*. (2014), its species level taxonomy is still unsettled. Ryvarden (1991) had earlier reported that the genus represents a taxonomic chaos. Internal transcribed spacer (ITS) is commonly used in the molecular identification of fungi. Carlson *et al*. (2014) reported that ITS region

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for species delimitation resulted to poorly resolved phylogenies and unclear species boundaries especially in the *Trametes vesicolor* species complex.

The work of Ko and Jung (1999); Tomšovský *et al*. (2006); Justo and Hibbet (2011) and Oyetayo (2014) used ITS and nLSU sequences to study the phylogenetic relationship and placed *Trametes* spp in the core polyporoid clade. In a study by Carlson *et al*. (2014), it was observed that protein coding genes viz; RNA polymerase II subunit (RPB I and II) and Eukaryotic Translation Termination Factor (ETF1) better revealed the identity of *Trametes* species than ITS in separating the species in *T. versicolor* complex. The present study was aimed at distinguishing *Trametes* species collected from Nigeria using data gathered from ribosomal marker (ITS) and protein-coding gene (RPB2).

MATERIALS AND METHODS

Collection of *Trametes* specimens

Fruit bodies of *Trametes* species used for this study were collected from Oyo and Ondo States, Nigeria, between September 2012 and April 2015 and kept dry in well labeled, clean polythene paper. They were subsequently taken to the laboratory for further examination. Herbarium samples of *Trametes* species fruit bodies were kept at the herbarium of Institute of Microbiology, Chinese Academy of Sciences, Beijing.

DNA extraction from *Trametes* spp and sequencing

DNA was extracted from 26 *Trametes* specimens obtained from 19 fruit bodies from which ITS data had been earlier studied by Oyetayo (2014) and 7 new fruit bodies collected from September 2014 to April 2015. Standard DNA isolation protocol using Cetyltrimethyl ammonium (CTAB) lysis buffer (Zolan and Pukkila, 1986) was employed. PCR amplification and sequencing of the ITS region was performed with primers ITS4 and ITS5 (White *et al*., 1990, Gardes and Bruns 1993). The 6–7 region of RPB2, approximately 700–800 bp long, was amplified with primers RPB2-b6F and RPB2-b7.1R (Liu *et al*. 1999, Matheny 2005). DNA sequencing was performed using an ABI DNA sequencing machine (Applied Biosystems).

Sequence alignment and phylogenetic analyses

Alignments were performed using Clustal W package (Thompson *et al*., 1997). The aligned sequences were corrected manually and through focusing on gap positions. DNA sequence data were analyzed to provide pairwise percentage sequence divergence. The data obtained from the sequence alignment were used to draw tree diagrams using MEGA 4 Software. The two datasets were assembled to make an extended dataset that included all newly generated sequences plus publicly available sequences in GenBank and a core dataset included only the 26 isolates of *Trametes* species for the analysis. Analysis conducted with DAMBE (Data *Analysis* for Molecular Biology and Evolution) revealed that the 25 *Trametes* species collected from Nigeria belong to 5 different species. These were assembled individually for dataset from RPB2.

RESULTS AND DISCUSSION

Basic local alignment search tool (BLAST) was used to analyze the ITS sequences obtained. The analysis revealed the identity of 25 *Trametes* species as follows: *Trametes elegans* (10); *Trametes polyzona* (7) while the remaining 8 were not identified to the species level (Table 1). The use of DNA markers for direct identification of genomic sequence diversity to supplement genealogical information had been reported (Sheikh Kalukhi *et al*., 2023). Fig. 1

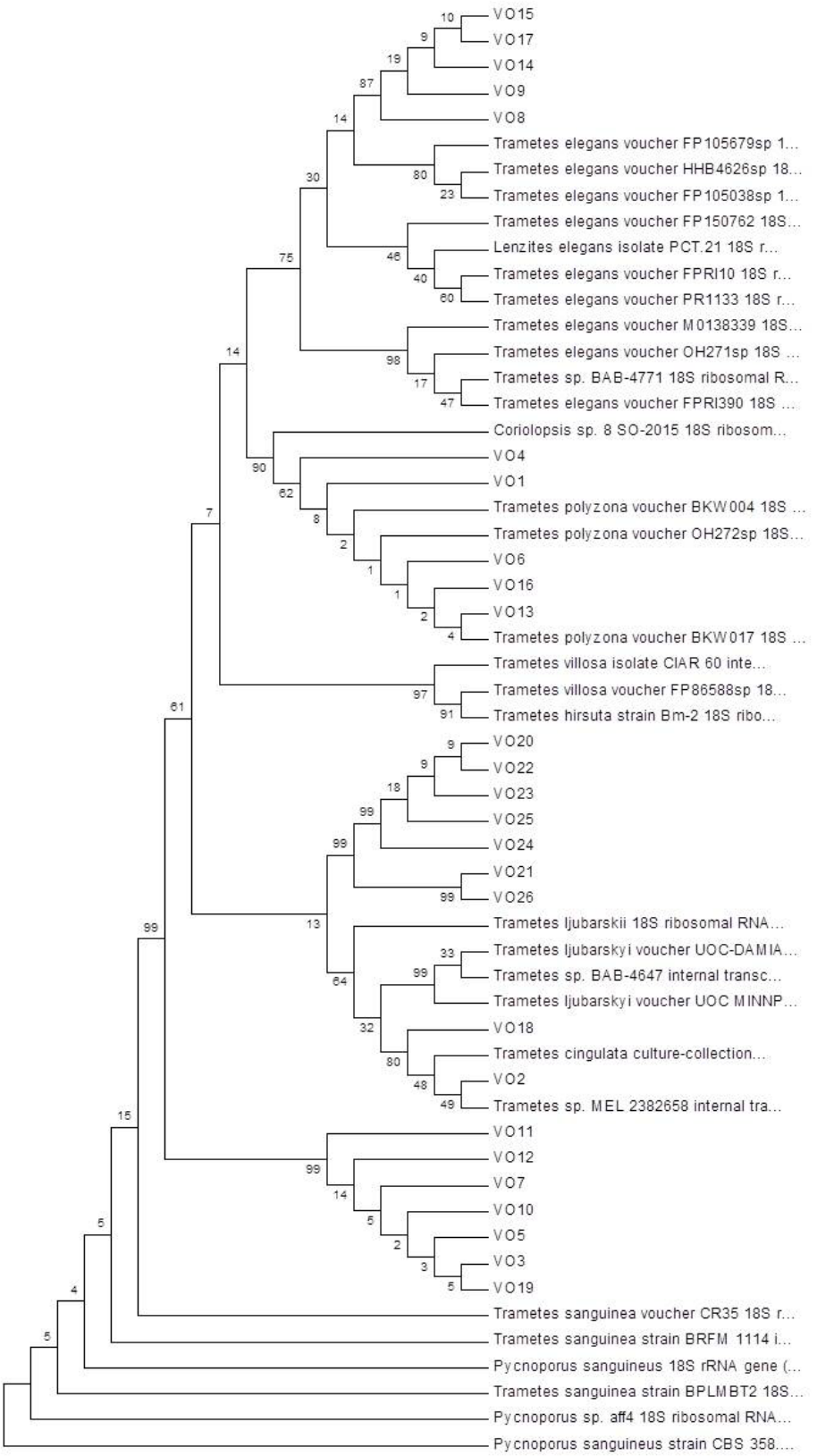

Fig. 1. Phylogenetic tree generated from ITS data set of *Trametes* species collected from Nigeria and sequences from GenBank

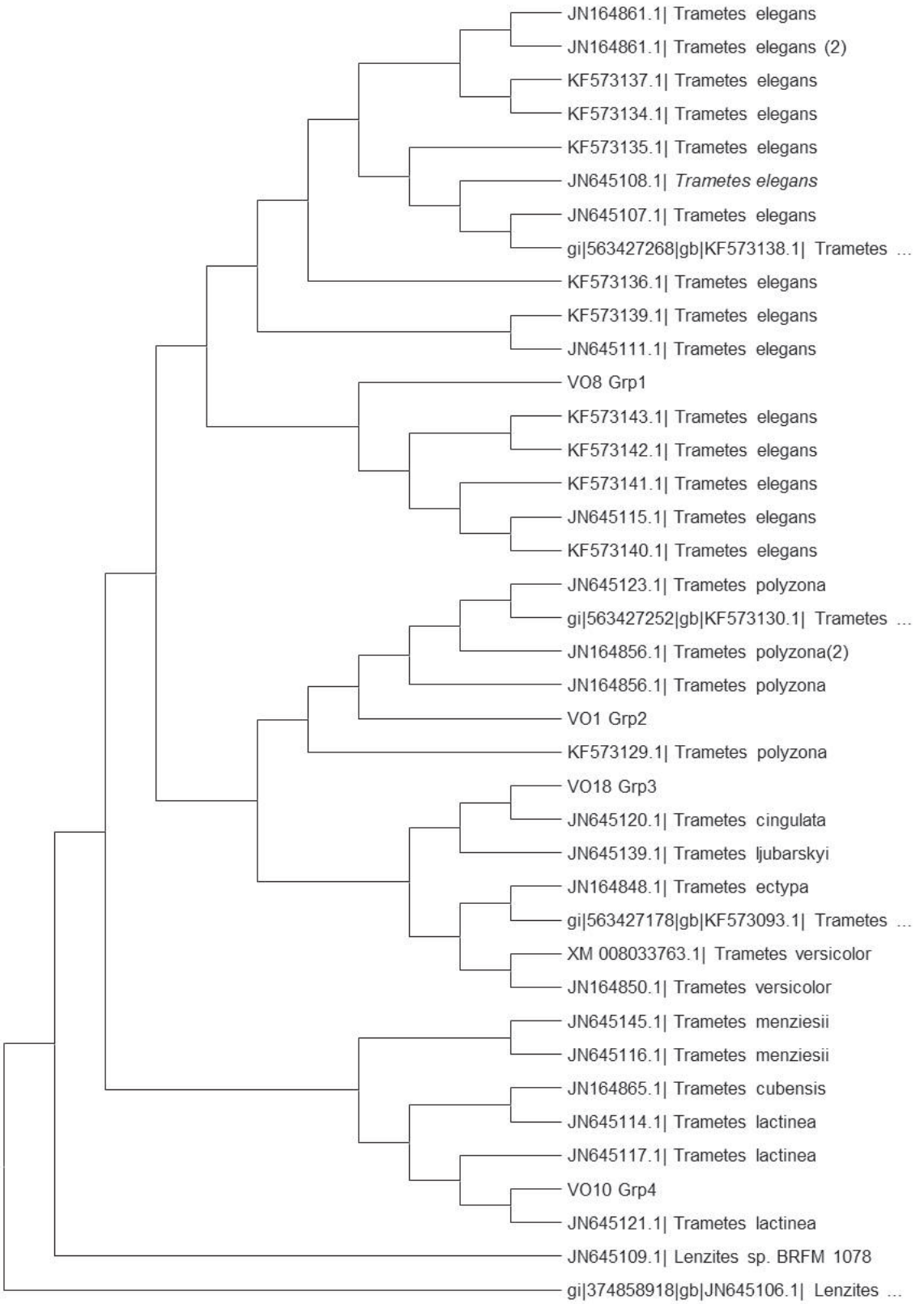

Fig 2. Phylogenetic tree of *Trametes* species collected from Nigeria as inferred by RPB2 sequences

Table 1. Resolution of the identity of *Trametes* species by Ribosomal marker (ITS) and Protein-Coding Gene (RPB2)

Group	Member	Identity	Resolution
1	VO8, 9, 14, 15, 17, 20, 22, 23, 24 and 25	*Trametes elegans*	ITS and RPB2
2	VO1, 4, 6, 13, 16, 21 and 26	*T. polyzona*	ITS and RPB2
3	VO2, 11, 12, and 18	*T. cingulata*	RPB2
4	VO3, 7 and 10	*T. lactinea*	RPB2
5	VO19	*Trametes* spp	ITS and RPB2

revealed that *Trametes* specimens VO-8, 9, 14, 15 and 17 were placed alongside *Trametes elegans* from GenBank. *Trametes* specimen VO-1, 4, 6, 13 and 16 were placed in the same clade as *Trametes polyzona*. However, the identity of the following *Trametes* specimens, VO-3, 5, 7, 10, 11, 12, 19, 20, 21, 22, 23, 24, 25 and 26 could not be resolved to species level (Fig. 1). Carlson *et al*. (2014) had earlier reported that commonly used molecular maker ITS used in molecular delimitation of fungi has low molecular variation in *Trametes* species.

Data analysis for molecular biology and evolution (DAMBE) software revealed that the 26 *Trametes* species belongs to 5 different species. These were grouped as follows: Group1 (VO-8, 9, 14, 15, 17, 20, 22, 23, 24, and 25); Group 2 (VO-1, 4, 6, 13, 16, 21 and 26); Group 3 (VO-2, 11, 12 and 18); Group4 (VO-3, 7 and 10) and Group5 (VO-19) (Table 1). Representative members of the above group sequenced using protein-coding gene (RPB2) gave better separation of the *Trametes* species from Nigeria into their respective species. RPB2 protein-coding gene respectively placed Group1 (VO8) and Group 2 (VO1) in the same clade with *Trametes elegans* and *Trametes polyzona* sequences obtained from NCBI GenBank. RPB2 further placed Group3 (VO18) in the same clade with *Trametes cingulata* (Fig. 2). In essence, the identity of *Trametes cingulata* and *Trametes lactinea* which were not revealed by ribosomal gene, ITS, was however well revealed by the protein coding gene, RPB2.

Studies by several Authors showed that molecular data inferred from nucleotide sequencing of different gene loci significantly changed the genus concept in Polyporaceae (Ko and Jung 1999; Tomšovský *et al*., 2006; Cui *et al*., 2011; Carlson *et al*., 2014; Ueitele *et al*., 2018; Olou *et al*., 2020). Moreover, the wide level of variation among members had been linked to the alleles that make them to survive in the environment (Yang *et al*., 2010). Burdon and Laine (2019) listed the following; mutation, sexual recombination, migration, gene flow, genetic drift, and selection as factors that promote genetic variation in fungi.

Conclusively, this study was the first research on comparison of ribosomal gene, ITS and protein coding gene, RPB2 in the identification of *Trametes* specimens collected in Ondo and Oyo States, Nigeria. Analysis with DAMBE (Data *Analysis* for Molecular Biology and Evolution) revealed that the 26 *Trametes* specimens collected from Nigeria belong to 5 different species. Protein coding gene, RPB2 identified the four (4) groups out of the five (5) groups of *Trametes* spp as follows: Group 1- *Trametes elegans*; Group 2- *Trametes polyzona*; Group 3- *Trametes cingulata*; Group 4- *Tametes lactinea*. However, the two genes (ITS and RPB2) could not identify group 5 to species level.

REFERENCES

1. Burdon, J.J. and A.L. Laine. 2019. *Evolutionary dynamics of plant-pathogen*

interactions. 384 p. Cambridge University Press. https://doi.org/10.1017/9781108625517.005.

2. Carlson, A.L., A. Justo and D.S. Hibbett. 2014. Species delimitation in *Trametes*: A comparison of ITS, RPB1, RPB2, TEF1 gene phylogenies. *Mycologia* **106:** 735-745.

3. Cui, D.Z., M. Zhao, H.Y. Yang, C.J. Wang, and H.B. Dai. 2011. Molecular phylogeny of *Trametes* and related genera based on internal transcribed spacer (ITS) and nearly complete mitochondrial small subunit ribosomal DNA (mt SSU rDNA) sequences. *African Journal of Biotechnology* **10(79):** 18111-18121.

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

5. Gilbertson, R.L. and L. Ryvarden. 1987. *North American polypores,* vol. 2. Synopsis Fungorum Special Volume. 885 p. Fungiflora, Oslo, Norway.

6. Justo, A. and D.S. Hibbett. 2011. Phylogenetic classification of *Trametes* (Basidiomycota, Polyporales) based on a five-marker dataset. *Taxon* **60(6):** 1567-1583.

7. Kirk, P. M., P.F. Cannon, D.W. Minter, and J.A. Stalpers. 2008. *Ainsworth and Bisby's Dictionary of the Fungi*. 10th Edition. 771 p. CABI Publishing.

8. Ko, K.S. and H.S. Jung. 1999. Molecular phylogeny of *Trametes* and related genera. *Anton Leeuw Int J G* **75:** 191-199.

9. Liu, Y.L., S. Whelen and B.D. Hall. 1999. Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerase II subunit. *Molec Biol Evol* **16:** 1799-1808.

10. Matheny, P.B. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). *Molec Phylogenet Evol* **35:** 1-20.

11. Olou, B.A., F.S. Krah, M. Piepenbring, N.S. Yorou, and E. Langer. 2020. Diversity of *Trametes* (Polyporales, Basidiomycota) in tropical Benin and description of new species *Trametes parvispora*. *MycoKeys* **65:** 25-47.

12. Oyetayo, V.O. 2014. Molecular Identification of *Trametes* species Collected from Ondo and Oyo States, Nigeria. *Jordan Journal of Biological Sciences* **7(3):** 165-169.

13. Ryvarden, L. 1991. *Genera of Polypores. Nomenclature and Taxonomy*. Synopsis fungorum 5. 363 p. Fungiflora, Oslo, Norway.

14. Sheikh Kalukhi, M., D. Ramezan, and A. Rahimian Boogar. .2023. Analysis of genetic diversity of *Trametes versicolor* isolates collected from Northern provinces of Iran using ISSR marker. *Mycologia Iranica* **10(2):** 45-52.

15. Thomson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The Clustal_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25:** 4876-4882.

16. Tomšovský, M., M. KolaĺÍk, S. Sylvie Pañoutová, and L. Homolka. 2006. Molecular phylogeny of European *Trametes* (Basidiomycetes, Polyporales) species based on LSU and ITS (nrDNA) sequences. *Verlagsbuchhandlung*, D-14129, Berlin. D-70176 Stuttgart.

17. Ueitele, I.S.E., P.M. Chimwamurombe, and N.P. Kadhila. 2018. Molecular Phylogeny of *Trametes* and Related Genera from Northern Namibia. *Jordan Journal of Biological Sciences* **11:** 99-105.

18. Wancura, G.C., M.T. Leite, L. Prestes, B. de P. Magalhaes, S. Kubeneck, and A.F.

Camargo. 2024. Application of mushrooms as a pollutant remediator. *Brazilian Applied Science Review Curitiba* **8(1):** 225-249.

19. Welti, S., P.A. Moreau, A. Favel, R. Courtecuisse, M. Haon, D. Navarro, S. Taussac, and L. Lesage-Meessen. 2012. Molecular phylogeny of *Trametes* and related genera, and description of a new genus *Leiotrametes*. *Fungal Diversity* **55(1):** 47-64.

20. White, T.J., T.D. Bruns, S.B. Lee, and J.W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR protocols: a guide to methods and applications, Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (eds). pp 315-322. Academic Press, New York.

21. Yang, X.L., G.J. Li, S.F. Li, and H.A. Wen. 2010. Genetic diversity of *Trametes versicolor* revealed by inter-simple sequence repeat markers. *Mycosystema* **29(6):** 886-892.

22. Zolan, M.E. and P.J. Pukkila. 1986. Inheritance of DNA methylation in *Coprinus cinereus*. *Mol Cell Biol* **6:** 195-200.