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# Molecular Validation of Two New Species of *Auricularia* from Maharashtra State of India

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***Abstract***

In the present study, two new records of non-gilled and jelly macrofungi *Auricularia novozealandica* and *A. srilankensis* have been described for the first time from the Maharashtra state of India. Genus of edible macrofungi *Auricularia*, which can be found as farmed or wild mushrooms. Certain species of *Auricularia* are used as nutrient-dense foods and medicines, with traditional Asian medicine giving them particular importance. Current study was conducted in 2022 and 2023 from the Madheghat and the adjoining area of Velha region of Maharashtra state. In this study, morphological analysis was carried out using visual observations and microscopic observations of the collected two specimens of the genus *Auricularia*. The specimens from macrofungal samples were identified based on molecular characters. The specimens were recognized based on morphological characters, the identification and validation were carried out through molecular testing. The DNA was extracted, where tissue was homogenized in liquid nitrogen for two minutes and then genomic DNA was recovered from lysis buffer. The nuclear ribosome's Internal Transcribed Spacer (ITS) region was amplified with reference to ITS1 and ITS4 primers using Polymerase Chain Reaction (PCR). Phylogenetics, have shown to be significant aids in accurately recognizing these fungi and have revolutionized fungal reclassification Molecular approaches, including nucleotides sequences of ITS region were characterized and compared with already existed sequences in National Center for Biotechnology Information (NCBI). GenBank and relevant literature were consulted to choose reference sequences and an outgroup. The ITS nucleotide sequencing data was used in a phylogenetic analysis to determine the taxonomic placement using BLASTn. After phylogenetic investigation, multiple fungal species were reclassified into other fungi. Accurate identification of the diverse species of fungi requires the use of molecular-based techniques. Thus, understanding macrofungi's

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|---|---|
| | biodiversity makes it possible to monitor changes caused by natural and human-caused processes and assess conservation. |
| **CC License**<br>CC-BY-NC-SA 4.0 | ***Keywords: Auricularia novozealandica: Auricularia srilankensis: Jelly fungi; Molecular validation; Madheghat; Maharashtra; India; Phylogeny*** |

## Introduction

*Auricularia* Bull. is a genus of jelly fungi belonging to the family Auriculariaceae (Order- Auriculariales) are distributed in tropical, subtropical and temperate regions. The genus is well recognized for their ecological, economic values and medicinal properties. Most of the species from genus plays an important role in degradation in forest ecosystems, usually inhabiting wood of angiospermic plants, such as dead trees, stumps, fallen trunks and branches, and rotten wood (Wu et al., 2021). The genus *Auricularia* Bull. ex Juss was described by Bulliard in 1787 (Khatua et al., 2022) and till ~ 15 species are known to exist globally (Looney et al., 2013). Globally, the jelly fungus *Auricularia* is a significant genus that grows on the woodlands of deciduous shrubs and trees. The fresh basidioma is gelatinous, auriform, lobbed frequently inverted, resupinate, and varies in colour from white to brownish. Morphologically, the genus is varied from other genera in the family. Macroscopically, it is identified by the presence of white to ochraceous basidiospores. This hymenial surface can be smooth, reticulate, or folded, and cylindrical to clavate basidia with three septations (phragmobasidia) (Alvarenga et al., 2015). One important genus of edible macrofungi is *Auricularia*, which can be found as farmed or wild mushrooms. Certain species of *Auricularia* are used as nutrient-dense foods and medicines, with traditional Asian medicine giving them particular importance (Wang et al., 2020). Worldwide production levels are possible because to the vast range of environments in which cultivated *Auricularia* species can be produced. Even though *Auricularia* species are primarily employed in the food sector, there is a good chance that they will be used to make therapeutic medications. Because the soluble sugar concentration of *Auricularia* species is relatively low, this is reflected in their flavor (Mau et al., 1998). Additionally, *Auricularia* has less fat than other edible mushrooms (Cheung, 2013). People with high blood cholesterol are advised to follow low-fat diets that are high in unsaturated fatty acids, which makes *Auricularia* an excellent dietary choice for them (Lichtenstein et al., 2006; Chen et al., 2011; Yu et al., 2023).

Mycological research, particularly study on fungal evolution and ecology, needs a solid and thorough fungal taxonomy and phylogeny to promote efficient and valuable communication among mycologists. The fungal systematics area has recently undergone multiple changes, ranging from early morphological classifications to an integrated taxonomy based on genetic phylogeny. These changes have occurred at various taxonomic levels, driven by breakthroughs in overcoming two fundamental challenges: appropriate and balanced sampling of genetic markers and taxa and reinterpreting the phylogenetic informativeness of multiple morphological and ecological features (Wang et al., 2016). DNA extraction and sequencing of specific genomic regions, such as the internal transcribed spacer (ITS) region, were performed to generate molecular fingerprints of the fungal specimens. DNA marker sequencing, often known as barcoding, has become a prominent method for various research, including species identification and molecular phylogenetic extrapolation (s et al., 2013; Savolainen et al., 2005). The mycological community has widely regarded the ITS region as the best identifier, with a high possibility of accurate identification for species in various fungal groups (Deng & Zhao, 2023). In this study, we employed advanced molecular techniques to identify and characterize wood-decaying fungi at the molecular level.

India has great biodiversity, among them Madheghat is in Western Ghats of Maharashtra state with its diverse sceneries and ecosystems has typical damp deciduous vegetation with a few patches of semi-evergreens (Champion & Seth, 1968). Few reports are offered on the diversity of Aphyllophorales in Maharashtra state of India; where, a total 256 Aphyllophoraceous fungal species are listed in the checklist of Maharashtra State, comprising 86 species from 20 non-poroid families and 170 species from 10 poroid families (Ranadive et al., 2011). A few notable species from the Pune District were reported. The dominant and frequent species including *Daedalea* sp. and *Ganoderma* sp. and *Phellinus* sp. etc. were reported (Ranadive et al., 2011). Genus *Auricularia* is reported in Pune, Aurangabad (Sambhajinagar), Ahmednagar, Gondia, and Raigad districts of Maharashtra state (Vaidya et al., 1991; Ranadive, 2013; Mendhe & Khobragade, 2017; Bhosale et al., 2019; Wakode & Ahuja, 2019; Ahmed et al., 2022). However, the detail information including molecular sequence data about *Auricularia* were lacking in the Maharashtra state. Earlier reports were based on morphological data instead of molecular sequence data of certain specimens. The fungal identification based on morphology might

occasionally produce inaccurate findings due to deceptive morphological traits (Olou et al., 2023). Therefore, in the present study molecular standpoints were consider and taken for the potential research to report a new record of Aphyllophorales from the India.

## Material And Methods

Study area. The Pune district falls between latitudes 17°54′ and 10°24′ North and longitudes 73°19′ and 75°10′ East. The district is 15.642 square kilometres in size. The districts of Ahmednagar in the northeast, Solapur in the southeast, Satara in the south, Raigad in the west, and Thane in the northwest encircle Pune district. With 5.10% of the state's total land area, it is the second biggest district in the state. The Pune district has considerable seasonal variations in temperature and rainfall conditions due to its location inside the tropical monsoon area. Pune's eastern section has hot, dry weather, whereas its western region enjoys a temperate climate. The current investigation is on the Velha region, part of the Pune District (18° 17' 46.824" N, 73° 38' 16.08" E.) in the Western Ghats of Maharashtra. A wide range of landscapes and ecosystems distinguishes the region. (Fig. 1). The vegetation in the Velha area is damp and deciduous, with a few semi-evergreen patches (Champion & Seth, 1968).

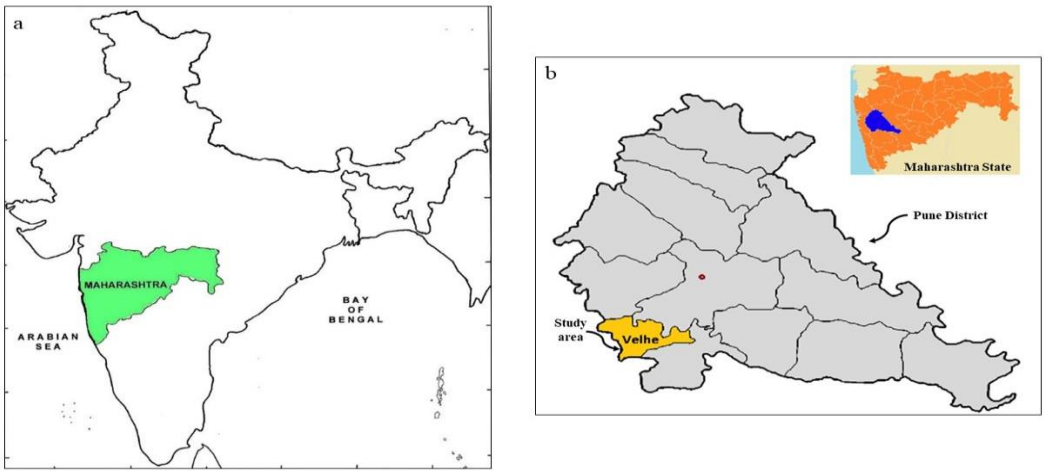


**Fig. 1.** Study area: a - Map of India showing inset Maharashtra, b - Map of Maharashtra State, showing the Pune District with highlighting study area Velha region (Source: https://images.app.goo.gl/ pgvvhzjb 4uukEzUR8).

*Collection, Examination, Isolation, and Preservation.* With the permission of the Maharashtra State Biodiversity Board, between 2022 and 2023, samples were collected every year from July to September at Madhe Ghat (Velha tehsil) in the Pune district of India (Table 1). These wood-rotting, basidiomycete-focused polypores are photographed in their natural environment. Small pieces of the fresh fruiting bodies are placed in paper bags for DNA extraction. Depending on the size of the fruiting bodies, they are air-dried for 1 to 2 days at room temperature. The dried fruiting bodies are used for morphological observations. The remaining fruiting bodies are stored in brown paper bags. The specimens are stored at Agharkar Research Institute's Ajrekar Mycological Herbarium, Pune, India.

**Table 1**List of collection localities with latitude and longitude

| Sr. No. | Collection localities | Latitude & Longitude |
|---|---|---|
| 1 | Madheghat Road | 18.2004209˚ 73.5775917˚ |
| 2 | Rajgad Fort Base | 18.6057611˚ 73.7880484˚ |
| 3 | Kelad | 18.2962682˚ 73.6374281˚ |
| 4 | Pasali | 18.2287414˚ 73.5904241˚ |

*Identification.* The microstructural examination was based on recorded morphological information such as size, shape, colour, surface texture, colour, attachment, and photographs of the specimens. For anatomical features, thin sections of basidiocarp were taken for comment under a compound light microscope using a sharp blade and mounted in 10% potassium hydroxide + 1% Phloxine aqueous solution in water (Olou et al., 2023) using Melzer's reagent + cotton blue. Microscopic examination was performed at 100× magnification under the Olympus compound microscope. Species were generally identified using identification keys developed in 1980 by Ryvarden & Johansen (1980), 1987 Gilbertson & Ryvarden (1990), and in 2010 Gorjón & Bericchia (2010). *DNA extraction.* Using the Buzina et al. (2001) method, tissue was homogenized in liquid nitrogen for two minutes and floated in a lysis buffer to recover genomic DNA from desiccated samples. The components of the lysis buffer were 3% Sodium Dodecyl Sulphate (SDS), 50 mM EDTA, and 100 mM Tris. The component tube was separated by centrifuging it for ten minutes at 10,000 g. Next, the DNA filtrate was cautiously shifted into a sterile 1.5 ml Eppendorf tube that was nuclease-free. The following step was adding phenol to the supernatant in an amount equal to that of chloroform and isoamyl alcohol (PCI). The Eppendorf tube is then entirely inverted to combine all of the ingredients. After that, the mixture was centrifuged (8000 g) for 12 minutes to extract the DNA-containing aqueous layer, which was then relocated into a fresh Eppendorf tube (1.5 ml). For the DNA precipitation, an equal volume of chilled isopropyl alcohol was added to the pooled aqueous layer. After being kept at -20 °C for 20 minutes, the tube was centrifuged (8000g) for 10 minutes to create a DNA pellet. The DNA pellet was again centrifuged at 8000g for five minutes after being cleaned with 70% cold ethanol (500µl). The remaining ethanol was evaporated, and the DNA pellet was dried at 55°C after removing the supernatant. Subsequently, it was again suspended in 40µl of 1x TE buffer.

*Polymerase Chain Reaction (PCR) amplification and purification.* The nuclear ribosome's ITS (Internal Transcribed Spacer) region was amplified using the ITS1 and ITS4 primers (White et al., 1990; Borde et al., 2021). One milliliter of DNA template (10 ng), 0.25 units of Taq DNA polymerase (Sigma-Aldrich, India), 2.5 milliliters of Taq DNA polymerase buffer (10X), one milliliter of deoxynucleotide triphosphate (dNTPs) at a concentration of 200 micrograms (Sigma-Aldrich, India), one milliliter of 10 pmol primer, and the remaining volume was made up with ultra-pure sterilized water. The extracted DNA of the specimens was amplified using an Eppendorf thermal cycler (Eppendorf, Hamburg, Germany). The parameters of the thermal cycler were set to: Initial denaturation occurred for 5 minutes at 95°C. There were 30 amplification cycles, with 1 minute of denaturation (95°C), 30 seconds of annealing (55°C), 1 minute of extension (72°C), and one final extension step lasting 7 minutes at 72°C. Next, the PCR products were purified using a PCR cleanup kit (Axygen Scientific Inc., CA, USA) to eliminate contaminants or unincorporated primers and nucleotides. An automated DNA sequencer, the ABI Avant 3100 (Applied Biosystems, USA), was used to sequence the purified PCR product from the cycle. The raw DNA sequencing data was modified and combined into a logical sequence using ChromasLite version 2.01. The resulting sequence data were deposited in the NCBI nucleotide sequence database.

*Phylogenetic Analysis.* Utilizing ITS sequencing data, we conducted a molecular phylogenetic analysis to ascertain the taxonomic and phylogenetic position of the collected specimen. Reference sequences and an out-group were selectively chosen from GenBank and pertinent literature (Chen et al., 2023). Subsequently, consensus sequences were generated using Chromas Pro software, and BLASTn examinations of the resulting ITS sequence were carried out to identify the fungal culture. To validate the identity further, we constructed a maximum likelihood (ML) phylogenetic tree with MEGA v.7.0, employing the Tamura-Nei parameter with a gamma distribution model for sequence evolution, as described by Saitou and Nei (1987) and Tamura et al. (2011). Nodal support was rigorously assessed through the analysis of 1000 bootstrap repetitions. The scale bar on the phylogenetic tree represents the number of anticipated substitutions per location. The phylogenetic tree was anchored with *Elmerina dimidiate* O18261 and *E. efibulata* Yuan 4525 for root determination. We confirmed the identification by situating *Auricularia srilanken*sis and *A. novozealandica* species within their respective clades using the ITS region-based ML phylogenetic tree.

**Result**

The samples were collected from the Madhe Ghat region of Velha tehsil of Pune district of Maharashtra State of India. *Terminalia chebula* Retz., *Memecylon umbellatum* Burm. f., *Artocarpus heterophyllus* Lam., *Mangifera indica* L., *Vitex negundo* L., *Ficus racemosa* L. and *Carrissa* carandas L. are chiefly found in the area along with small to medium-sized woody shrubs. The climatic conditions of the regions are quite favourable for the growth of fungi.

*Taxonomy*. *Auricularia novozealandica* (Fig. 2)
Occurrence: Decaying wood log of *Artocarpus heterophyllus* Lam.
Basidiomata: Jellylike when fresh, brown, solitary, substipitate; pileus cupulate, bulging ~14 cm, 0.6– 1.5 mm in thickness; upper side is covered with long soft hairs which turns brown after drying; hymenial side smooth, turns grey after dehydration.
Internal features: The section's medulla is prominently located in the center, and the abhymenial hairs are hyaline, thick-walled, and have a narrow lumen. The apical tips of the hairs have several septate lumens and are obtuse and tufted, measuring 100–320 × 7–9 µm. The hyphae have clamp connections and simple septa, measuring 0.5–3 µm in diameter in KOH; the basidia are clavate, transversely 3-septate, with oil guttules measuring 70–86 × 5–6 µm, and sterigmata are infrequently seen.
Spores: thin walled, smooth, hyaline usually with one or two large gutless (15–19) × (5–6.5) µm, L = 17.5µm, W= 5.6µm.
Distribution: New Zealand and Velha Tehsil, Maharashtra, India.

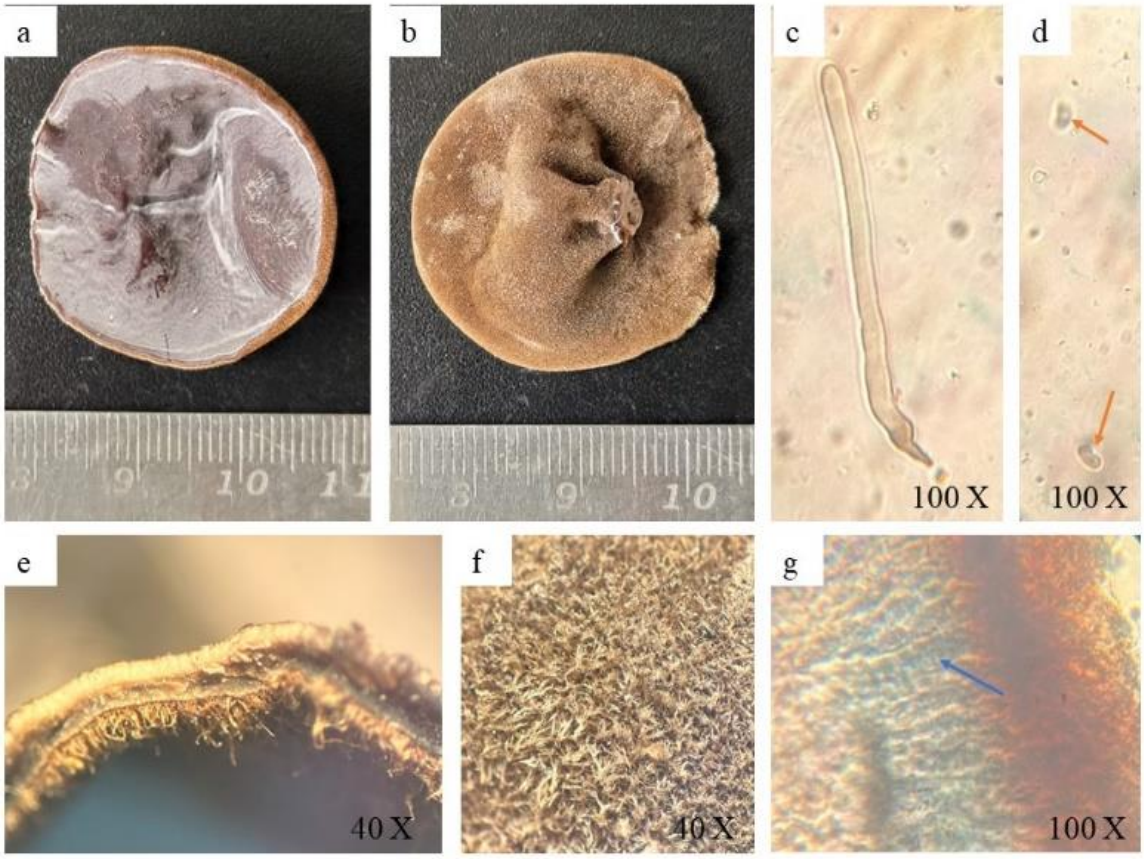


**Fig. 2.** *Auricularia novozealandica*: (a) Sporocarp- abhymenial surface (b) Sporocarp- hymenial surface, (c) Basidioles, (d) Spores, (e) V. S. of sporocarp (f) Abhymenial hairs (g) Basidia – blue arrow.

*Auricularia srilankensis* (Fig. 3)
Occurrence: Tree trunk of *Terminalia chebula* Retz.
Basidiomata: The fruiting body is gelatinous when fresh, colour is greyish blue to grey upon drying; free lobed pileus, with undulate margin; upper surface hairy, turning white to greyish violet upon drying; hymenophore surface with folds, turning dark greyish blue at dry state.
Internal features: The medulla is absent, the abhymenial hairs have a slightly swollen base, are hyaline, thick-walled, and have a narrow lumen. The hyphae have clamp connections and are clearly inflated, with a lumen in KOH that can reach 7 µm in diameter. The basidia are transversely 3-septate, clavate, and have some small oil guttules that measure 50–70 × 5–7 µm, usually with sterigmata observed. Cystoles are absent.

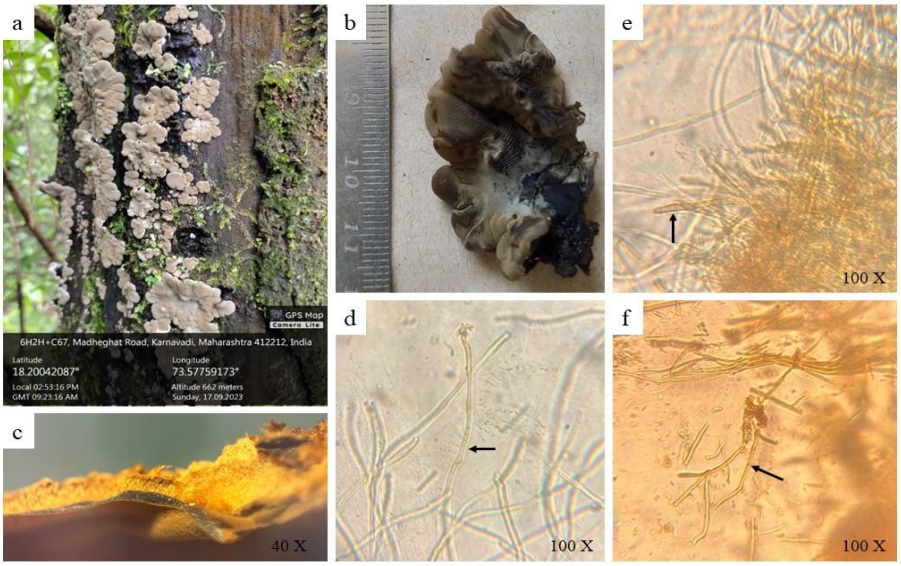


**Fig. 3.** *Auricularia srilankensis*: (a) Sporocarp- on field photo (b) Sporocarp- abhymenial surface, (c) V.S. of sporocarp with abhymenial hairs, (d) Generative hyphae– black arrow, (e) Basidia – black arrow (f) Skeletal hyphae– black arrow.

*Phylogenetic Analysis.* In the present investigation, identification of samples was done through both morphological and molecular characters. The morphological identification was complemented by a molecular identification on the basis of ITS sequences submitted in NCBI data basses, a total of 2 different *Auricularia* spp. was confirmed with molecular identification and used for the study (Fig. 4). The evolutionary Maximum likelihood tree was constructed using Mega 7 (Fig. 4). Results showed that the two non-gilled macrofungus *Auricularia* species have been first described from Maharashtra, India. The ITS sequences of the fungal specimens gathered (Table 2) were compared with those already in the GenBank database (using BLASTn) to identify them. Based on a mega blast search of the NCBI GenBank nucleotide database, the closest hits using the ITS sequence of *Auricularia srilankensis* had the highest similarity to *A. srilankensis* strains (CBS 145501, Dai 19520, Dai 19575, GenBank NR_171211; MZ647508.1, MZ647502.1; Identities= 449/450 (99.75%) respectively. Whereas, other species *A. novozealandica* had the highest similarity to *A. novozealandica* (strain PDD 83897, KX_022034.1; Identities= 472/481 (98%), one gap (0 %)). Phylogenetic analyses based on the entire ITS rDNA sequences supported the recognition of our isolates under the genus *Auricularia* (Fig. 4). Based on phylogenetic tree and DNA sequences, the collected fungal specimen was identified and confirmed to be member of *Auricularia novozealandica* (Fig. 4). The specimens have been submitted and deposited at Ajrekar Mycological Herbarium (AMH), ARI, Pune, India.

**Table 2** Nucleotide sequences and GenBank accession number of identified *Auricularia* species

| **Species Name** | **Code** | **ITS nucleotide sequence** | **GenBank accession number** |
|---|---|---|---|
| *Auricularia srilankensis* | FI1399 | 1 gaaacaacct cacacctgtg cacctttcg gttgtggcct cttgcggggc tgcttccgct<br>61 ttcacatgca actacttctg tcccgaatgt gctatactat ataaagtaac aactttcaac<br>121 aacggatctc ttggctctcg catcgatgaa gaacgcagcg aaatgcgata agtaatgtga<br>181 attgcagaat tcagtgaatc atcgaatctt tgaacgcatc ttgcgctcct tggtattcca<br>241 tggagcatgc ctgtttgagt gtcacgtaaa ccctcaccct tgcgatgtta cagtcgcccg<br>301 aggtggactt ggaccgtgcc gtagttggct cgtcctgaaa tgcattagct ggcgctttta<br>361 gagtgctggg cgacggtgtg ataattatct gcgccaatgc cctgggcctc ttcagctgtg<br>421 ccgcttacag tcgtccgcat ggacaactac | PP082777 |
| *Auricularia novozealandica* | FI1400 | 1 gctgtgcgcc ttttaccggg ctgcacgctg gagcaagacc ccacacctgt gcacctttc<br>61 ggttgcggct tcggtcgctg ccgcttttaa atgcaacaac tcagtctcga atgttaacaa | PP082779 |

| | | | |
|---|---|---|---|
| | | 121 aaccataaaa agtaacaact ttcaacaacg gatctcttgg ctctcgcatc gatgaagaac<br>181 gcagcgaaat gcgataagta atgtgaattg cagaattcag tgaatcatcg aatctttgaa<br>241 cgcatcttgc gctccttggt attccatgga gcatgcctgt ttgagtgtca cgtaaaccct<br>301 caccccctgcg atgtaacagt cgctcgcggt ggacttggac tgtgccgtaa ccggctcgtc<br>361 ttgaaatgca ttagctggcg cttttagagt gctgggcgac ggtgtgataa ttatctgcgc<br>421 caatgcctta ggcctcttca gcggtgctgc ttacagccgt ccctctgcgg acacattttt | |

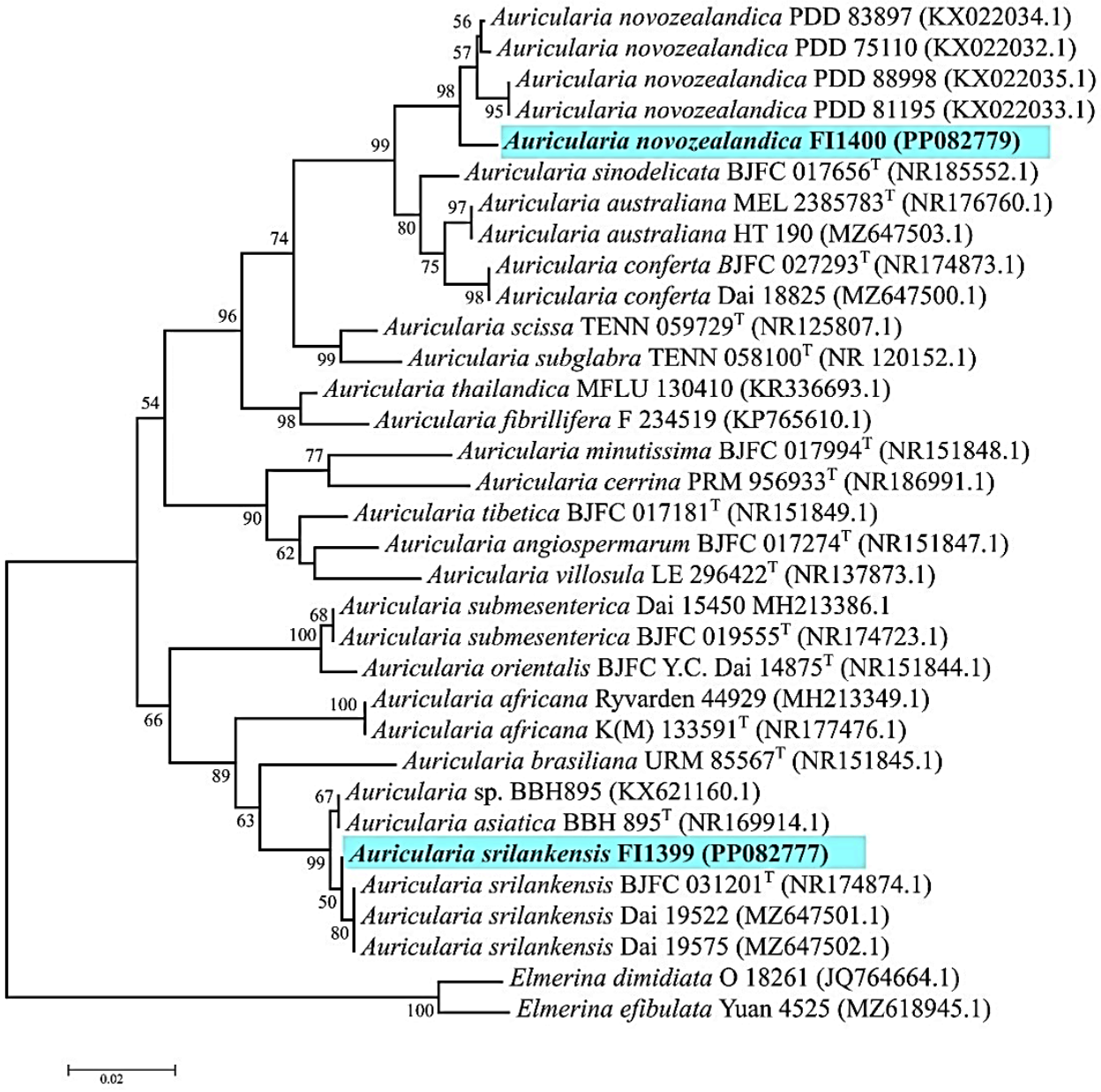


**Fig. 4. -** The placement of *Auricularia novozealandica* and *A. srilankensis* using a maximum-likelihood (ML) analysis of the combined ITS rDNA sequences employing the Tamura-Nei parameter with a gamma distribution model in MEGA 7 (Kumar S et al. 2016). The scale bar indicates the expected number of substitutions per site. The numbers provided on branches are frequencies with which a given branch appeared in 1000 bootstrap replications. The tree was rooted with *Elmerina dimidiate* O 18261 and *E. efibulata* Yuan 4525.

## Discussion

In the current investigation, two species of *Auricularia* were newly recorded from the Maharashtra state of India. Nucleotide ITS sequencing identified and confirmed two records, including *A. novozealandica* and *A. srilankensis*. Morphological characterization included fruiting body, hyphal nature, basidia, and spore characterization. The lack of trustworthy physical characteristics indicating a distinct genetic diversity among the species in the genus *Auricularia* has presented difficulties for taxonomic research in this area (Looney et al., 2013). The present study displays that even though the ITS region is recognized as widespread and apt for accurate identification (Schoch et al., 2012). The species were identified as *A. novozealandica* and *A. srilankensis* based on molecular validation. As per our acquaintance, this study has recorded the above species from Maharashtra, India, for the first time. Phylogeny and molecular taxonomy can potentially clarify the confusion caused by morphological plasticity. To resolve the two strains in this work, the phylogenetic tree's general topologies are inferred by the ITS utilizing the Neighbour-Joining Maximum Composite Likelihood method. A checklist published on order Aphyllophorales by Randive et al. (2011) recorded 256 species, including 170 from 10 poroid families and 86 from 20 non-poroid families. However, *A. novozealandica* and *A. srilankensis*. Previous reports on the order of Aphyllophorales recorded 20 species belonging to 8 families and 14 genera from 15 different localities in the Western Ghats of Pune district, Maharashtra. However, the present reported species were not listed in the earlier study, where specimens were identified based on morpho-taxonomical characteristics (Ranadive et al., 2013). In 2013, Randive overviewed an Aphyllophorales from India and A. *novozealandica* and *A. srilankensis* were not listed among 1175 species from 52 families and 190 genera of poroid and non-poroid Aphyllophorales. In recent studies, studied fungi have yet to be reported. (Ranadive, 2013; Hakimi et al., 2013; Yemul et al., 2019; Gore & Mali, 2023). In an earlier report, *Auricularia cornea* and *Auricularia nigricans* were reported from the Gujarat state of India; however, the currently described species were not reported in that checklist (Rajput et al., 2015). In another study, *Auricularia* auricula-judae, Auricularia delicata, Auricularia cornea, Auricularia nigricans and Auricularia mesenterica were reported; while, the currently studied species were missing in a checklist of the macrofungi of North East India (Roy et al., 2022). Table 3 shows, in recent studies, *Auricularia srilankensis* and *Auricularia novozealandica* were reported from Shrilanka and New Zealand, respectively (Wu et al., 2021). Nevertheless, no reports were designated for *A. novozealandica* and *A. srilankensis* from India. Therefore, it was observed that *A. novozealandica* and *A. srilankensis* were first time recorded in the Maharashtra state of India. After *Agaricus, Lentinula,* and *Pleurotus, Auricularia* is the fourth most widely grown genus of mushrooms (Chang, 1996). The relevance of this mushroom has grown quickly because of its nutritional benefits, therapeutic value, and widespread acceptance as a delicacy on the table. (Miles & Chang, 2004). Polysaccharides are abundant in the fruiting bodies of *Auricularia*. (De Silva et al., 2012a; Miles & Chang, 2004) and have anticoagulant, antioxidant, and blood sugar-lowering properties. (De Silva et al., 2012b; Mortimer et al., 2014). It may be possible to domesticate the new species, *A. novozealandica* and *A. srilankensis*, to create an edible fungus that thrives in tropical climes.

**Table 3** Earlier and current reports of *Auricularia srilankensis* and *Auricularia novozealandica*

| Species Name | Sample No. | Country | GenBank Accession Number | Reference |
|---|---|---|---|---|
| *Auricularia novozealandica* | PDD 81195 | New Zealand | KX022033 | Fang et al., 2021 |
| *Auricularia novozealandica* | PDD 88998 | New Zealand | KX022035 | Fang et al., 2021 |
| *Auricularia novozealandica* | PDD 75110 | New Zealand | KX022032 | Fang et al., 2021 |
| *Auricularia novozealandica* | PDD 83897, holotype | New Zealand | KX022034 | Fang et al., 2021 |
| ***Auricularia novozealandica*** | **FI1400** | **India** | **PP082779** | **Current Study** |
| *Auricularia srilankensis* | Dai 19522, holotype | Sri Lanka | MZ647501 | Fang et al., 2021 |
| *Auricularia srilankensis* | Dai 19575 | Sri Lanka | MZ647502 | Fang et al., 2021 |
| *Auricularia srilankensis* | Dai 19519 | Sri Lanka | MZ647507 | Fang et al., 2021 |
| *Auricularia srilankensis* | Dai 19520 | Sri Lanka | MZ647508 | Fang et al., 2021 |
| ***Auricularia srilankensis*** | **FI1399** | **India** | **PP082777** | **Current Study** |

## Conclusion

The Western Ghat region of India is a biodiversity hotspot. In the Pune District, the Velha region is a part of Maharashtra's Western Ghats. There needs to be more knowledge of fungi and molecular data. In the current study, macrofungi were assessed at the molecular level, and *Auricularia novozealandica* and *Auricularia srilankensis* (family Auriculariaceae) were discovered for the first time in Maharashtra state of India. Our

findings contribute to the growing knowledge of wood-rotting fungi, emphasizing the importance of molecular approaches in uncovering hidden diversity and functional attributes. The insights gained from this study have implications for both environmental conservation efforts and the development of innovative strategies for mitigating the impact of wood decay on structures of economic significance. Therefore, by understanding the biodiversity of macrofungi at the species and community levels, relevant authorities can assess the need for and effectiveness of conservation activities.

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