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Some New Records of *Trametes* (Polyporales, Basidiomycota); from Pakistan

Afshan Wahab^{1*}, Donald H. Pfister², Kathy LoBuglio², Siraj Ud Din¹, Abdul Nasir Khalid³

¹Department of Botany, University of Peshawar, KP, Pakistan

²Department of Organismic and Evolutionary Biology, Harvard University, 22 Divinity Avenue, Cambridge, MA 02138, USA

³Department of Botany, University of the Punjab, New Campus, 54590, Lahore, Pakistan

*Corresponding Author: Afshan Wahab, Department of Botany, University of Peshawar, KP, Pakistan,
Email: afshan.wahab2@gmail.com

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Abstract

Four different species of *Trametes* were collected during a field survey in Malakand district, Khyber Pakhtunkhwa, Pakistan. Based on morphological characteristics and the molecular data from internal transcribed spacer rDNA and large subunit nLSU, the species were identified as *T. elegans*, *T. gibbosa*, *T. hirsuta* and *T. trogii*. A literature review confirms *T. elegans*, *T. gibbosa* and *T. hirsuta* species to be the first records from Pakistan and comparison with herbarium specie confirms its identity.

Keywords

Basidiomycetes; Polypore; Taxonomy; Molecular Data

Introduction

Trametes Fr. (Fries, 1836) is genus in the family Polyporaceae with *Trametes suaveolens* (L.) Fr. as type species (Fries, 1838). It is characterized by the combination of pileate basidiocarps,

poroid hymenophore, trimitic hyphal system, thin-walled smooth basidiospores not reacting in Melzer's reagent and production of a white-rot to woods. It is a cosmopolitan genus present in almost all type of forest ecosystem in temperate, boreal, and tropical areas [1]. *Trametes versicolor* ("turkey tails") is among the most common and widespread species of mushroom-forming fungi. It has been recorded on, angiosperms (e.g., *Acer*, *Betula*, *Eucalyptus*, *Malus*, *Populus*, *Quercus*) and conifers (e.g., *Abies*, *Cupressus*, *Larix*, *Pinus*) [2,3]. Despite the high biodiversity of the Brazilian Amazonia, the knowledge about *Trametes* is still scarce, using hyphal types, Ryvarden classified 16 genera (*Cerrena*, *Corioloopsis*, *Cryptoporus*, *Daedaleopsis*, *Datronia*, *Earliella*, *Elmerina*, *Fomitella*, *Hexagonia*, *Lenzites*, *Megasporoporia*, *Microporus*, *Mollicarpus*, *Pycnoporus*, *Trametes* and *Trichaptum*) as belonging to the *Trametes* group and with only nine species reported: *T. cotonea*, *T. cubensis*, *T. lactinea*, *T. marianna* (Pers.) Ryvarden 1973, *T. maxima*, *T. membranacea*, *T. modesta*, *T. pubescens* (Schumach.) Pilát 1939, and *T. villosa* [4].

However, it is difficult to identify many species of *Trametes* and their related genera due to their highly similar morphological characteristics [1]. Thus, *Trametes* is regarded as one of the most confused group of genera in Polyporaceae. Actually, the current taxonomy of *Trametes* has been questioned by Zhang, et al., [5]. In recent years, mycologist have attempted to use sequence data to resolve the taxonomic problems in *Trametes* and in the related genera [6-9]. Zhang, et al., (2006) noted that the results based on molecular phylogenetic analysis are consistent with morphology in *Trametes*, and that ITS sequences are useful in distinguishing the genera with similar morphological characteristics [5].

In Pakistan, eleven *Trametes* species have been reported previously by Ahmad [10]. *Trametes cingulata* Berk, *T. corrugata* (Pers. Ex Fr.) Bres, *T. dickensii* Berk., *T. hispida* Bagl., *T. jubarksii* pilat., *T. incana* Berk., *T. lactinea* (Berk.) *T. roseola* Pat. and Har. *T. incerta* (Curr.) Cooke and *T. suaveolens* (L.) Fr. *T. Trogii* Berk., on logs and stumps of angiosperm and gymnosperm trees.

During our studies on the diversity of polyporoid fungi in Khyber Pakhtunkhwa Forests, an unreported *Tametes* species were found. The basidiomata were identified using morphological characters and sequence analyses of Internal Transcribed Spacer (ITS) rDNA region supported the identification. A detailed description of the species and a key to the Pakistani *Trametes* species are provided below.

Material and Methods

Macro-Microscopic observation

Collections of the basidiocarps are based on regular field visits to the forests of Malakand district, KP Pakistan during 2015-16. Important macroscopic features were noted from fresh

specimens using a field notebook. The specimens were dried using a fan heater with temperature adjusted at 40°C.

Macro and microscopic basidiocarp features were noted. Measurements and drawings were made from slide preparations mounted in a drop of lactic acid or Melzer's reagent or 5% KOH and Congo red on slides and were observed. Anatomical features were measured using calibrated Piximetre software connected to a B-350 Optika compound microscope through a Scope Image (H9D) microscopic camera and visualized on a computer screen.

Twenty basidiospores, basidia, and cystidia were measured and recorded as follows: Q = the length/width ratio of a single spore; Qe = average length/width ratio of all spores; Me = average $L \times W$ of all spores measured.

DNA extraction, amplification, sequencing and molecular phylogenetic analysis

About 50 mg of fungus were ground into fine powder using Qiagen plant mini-kit method. The universal primer pair ITS1F, ITS 5 and ITS 4 (Gardes, et al., 1993 and White, et al., 1990.) was used to amplify the rDNA 18s ITS (ITS1F+5.8s+ITS4) and LSU primer pair LROR, LR5 and LR7 (Vilgalys and Hester, 1990) was used to amplify the 28s larger sub-unit region in 25 µl reaction volumes following Gardes, et al., (1993) [11,12]. The PCR products were sequenced by Sanger Sequencing - Bauer Core Facility - Harvard University, USA. BLAST analysis was performed using the National Center for Biotechnology Information (USA) database, and closely matching sequences were downloaded for further phylogenetic analysis. Sequences were aligned using cypress science gateway (<https://www.phylo.org/>). Sequences are deposited in GenBank.

Results

Molecular phylogeny

Molecular Phylogenetic analysis

The ITSIF, ITS5 and ITS4 regions amplified were a 640bp and 1200bp LSU region were amplified by LROR, LR5 and LR7. The initial BLAST comparison of the Pakistani ITS+LSU sequences showed 100% identity and 99% query cover with *Trametes elegans* (JN164921.1 and JN164996.1), *T. gibbosa* (KC589144.1 and KX449481.1), *T. hirsuta* (JF439511.1 and FJ550367.1) and *T. trogii* (EU790491.1 and JN164936.1). From Genbank identical sequences were retrieved and were aligned through online muscle tool. The phylogenetic treatment clusters our Pakistan sequences with the *T. hirsuta*, *T. gibbosa*, *T. trogii* and *T. elegans* sequences into separate into their respective clades with a strong 99-100% of bootstrap value.

Using the online MUSCLE (version 3.8), these ITS+LSU sequences were aligned with the sequences generated in this study. Bootstrap analysis with maximum probability (ML) was performed in MEGA7 using Bio NJ and Neighbor-join. Estimates were made using the Maximum Composite Likelihood (MCL) criterion. Consequently, topology with the highest probability value for log was selected (Fig. 1).

For scaling the tree, number of replacements per site was used. It illustrates the genetic make-up of the taxa studied by including a consensus bootstrap tree constructed from 1000 replicates. The study was performed on 63 representative sequences. There were a total of 1056 positions in the final dataset after trimming both 5' and 3' ends of the aligned sequences. Of these, conserved were 530, variables were 263 and parsimonies informative were 204 and singletons were 59. The resulting tree have dataset of only 613 positions. The analysis included both the coding and non-coding positions. No account has been taken of those positions in which data was missing. In this phylogram *Daedalea dickinsii* was used as out group. Pakistani specimens are represented by ●.

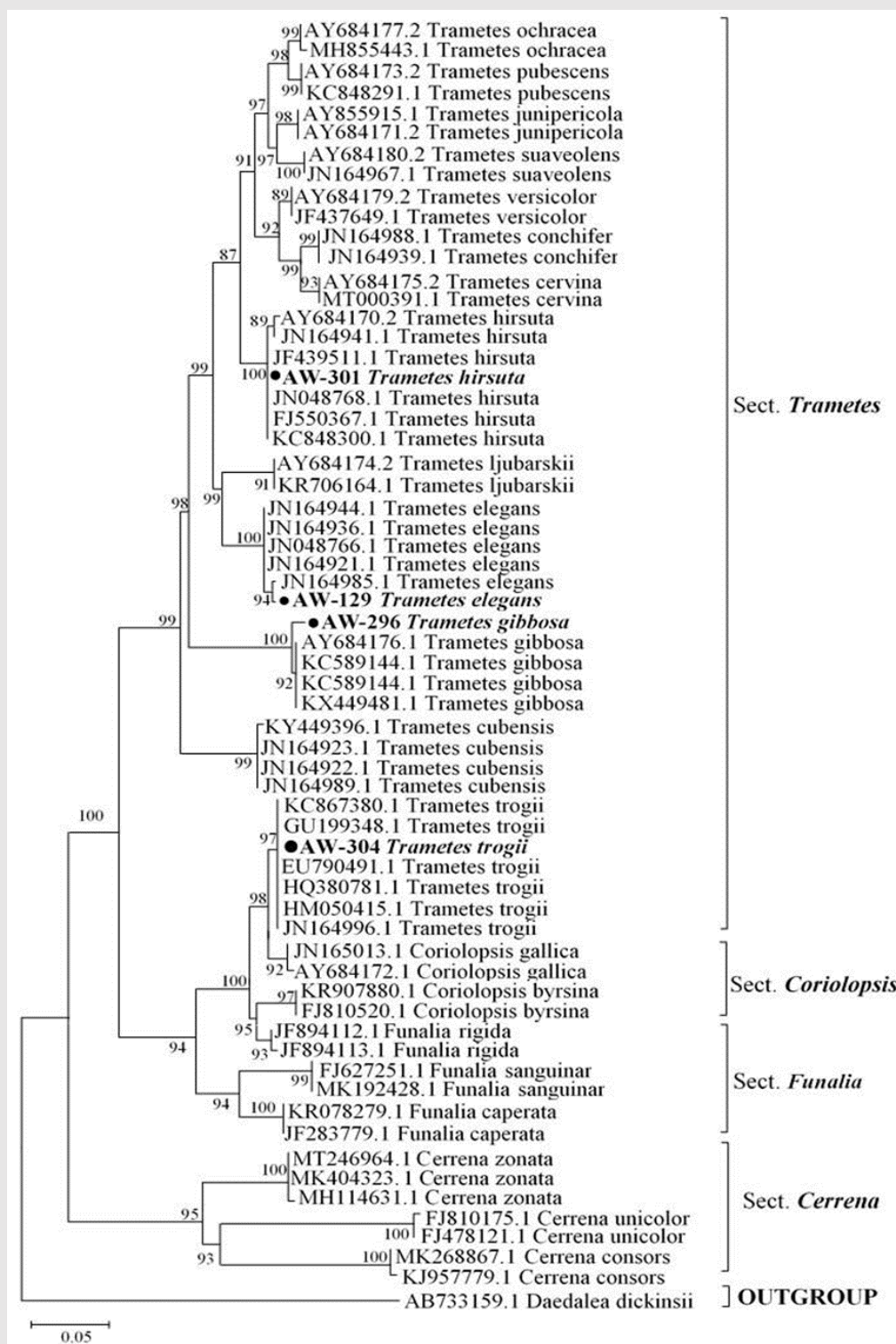


Figure 1: Phylogram obtained from ITS+LSU region of rDNA. The phylogenetic relationship of *T. hirsuta*, *T. gibbosa*, *T. trogii* and *T. elegans* was inferred from nrITS and rLSU sequences with the maximum log likelihood (-4593.92). The bootstrap values above branches are more than 50%. Pakistani collection is indicated by ●.

Taxonomy

Trametes elegans (Spreng.) Fr., Epicr. syst. mycol. (Upsaliae):492, (1838) Fig. 2.

Morpho-anatomical Description

Basidiocarp: pileus 7 cm long and 1-4 cm wide, annual to perennial, when fresh flexible and corky, more rigid when dry, sessile, attached laterally or centrally; Pileus very finely tomentose, circular to semi-circular, upper surface smooth, glabrous, concentrically zonate, white to grey. Stipe absent. Pore surface, white to pale cream in color; Pores up to 2 mm deep 1 - 3 per mm, up to 1 mm wide, semi-daedaloid, round to angular, very variable partly poroid. Context, up to 0.1 - 0.4 cm, thick near the base, white to pale cream, becomes woody hard when dried. Hyphal system trimitic; Generative hyphae, wide upto 2 - 4 μm , hyaline, thin walled with clamp; Binding hyphae, up to 5 μm wide, hyaline to pale yellow, sparsely branched, thick-walled; Skeletal hyphae, 3 - 7 μm in diameter, yellow to golden, thick-walled, unbranched, dominating. Cystidia as such not present but Binding hyphae project into the hymenium and may easily be interpreted as acute Cystidia until a section is squeezed and their true nature is revealed. Basidiospores, 5 - 7 \times 2 - 3 μm in diameter, cylindrical to oblong ellipsoid, thin-walled, smooth, hyaline.

Collection examined: Pakistan, Khyber Pakhtunkhwa, Malakand, Totakan, 626m asl, on dead wood of deciduous tree. September 8, 2015, A. Wahab (AW-129).

Substratum: On dead deciduous wood of all kinds.

Comments. The basidiocarp of this species is easy to document in the field because of narrow semi-dadeldoid pores and more yellowish at the pore surface and has a dark line between the lower context and the upper tomentum. However, the dark line may be weakly developed and it is necessary to take a section close to the base to verify its presence.

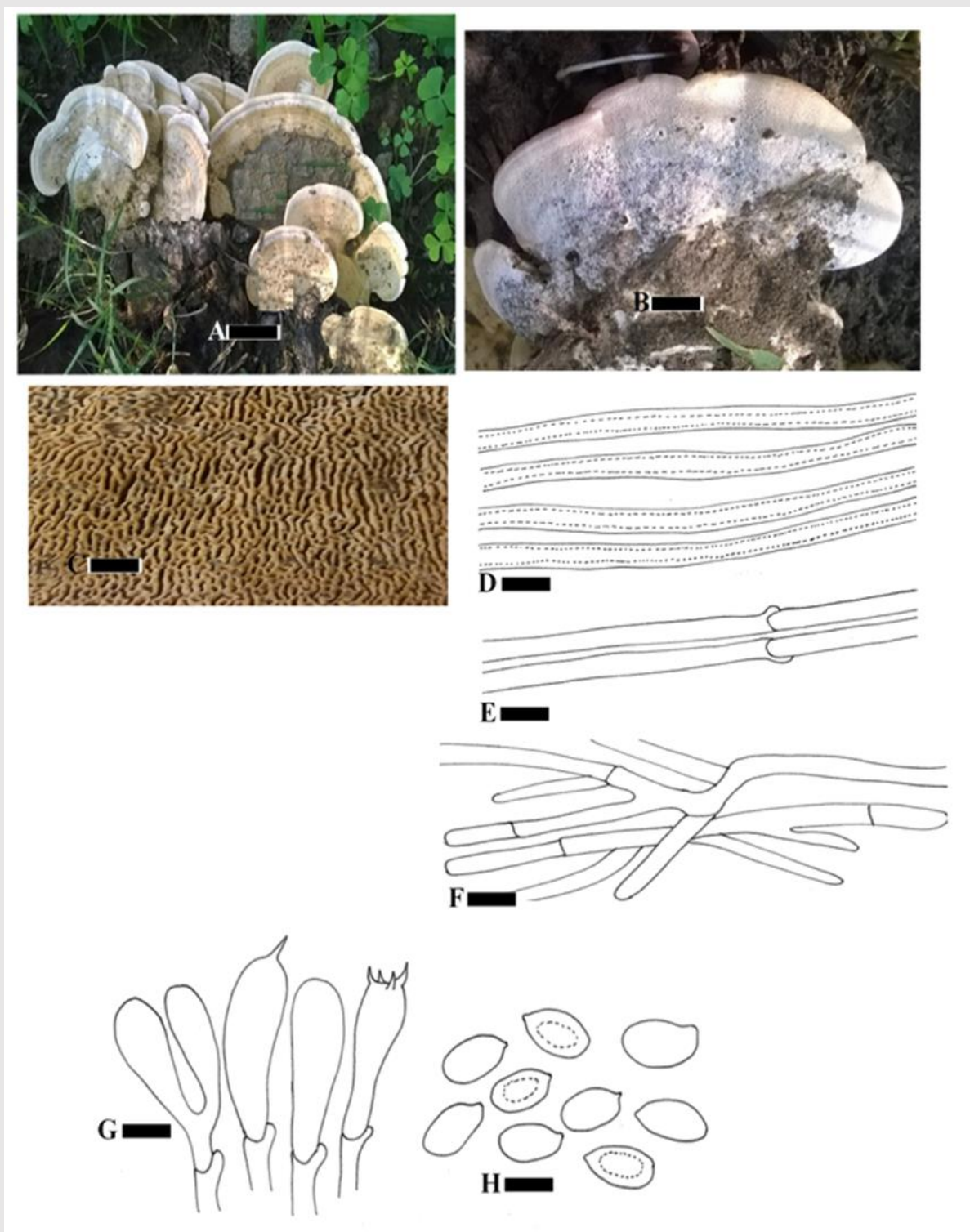


Figure 2: A-H. Morpho-anatomical features of *Trametes elegans* A-B. Basidiocarp; C. Pores; D. Skeletal hyphae; E. Generative hyphae; F. Binding hyphae; G. Basidia; H. Basidiospores; Scale bar: A-B=35mm, C=1-3/mm, D=2.5 μ m, E=2.5 μ m, F=2.1 μ m, G=3.5 μ m, H=1.8 μ m.

Trametes gibbosa (Pers.) Fr., *Epicrasis Systematis Mycologici*:492 (1838) Fig. 3.

Morpho-anatomical Description

Basidioscarps annual, usually semicircular in outline, tough and coriaceous, sessile to dimidiate, azonate; upper surface tomentose to glabrous and white at first, then cream to ochraceous or discolored pale brown, due to algae at the base often greenish, margin sharp, thick upto 1-4 cm at the base, wide up to 15 cm; pore surface, white to cream in colour when fresh, in old specimens becomes pale cream; pores, 1-5 mm long, 1-2 per mm, radially elongated, angular with entire dissepiments; Context, white in color, at the base thick up to 3 cm, tough-fibrous, dense, azonate; tube layer, thick up to 2 cm, with pore surface becomes concolorous. Hyphal system trimitic; Generative hyphae, 2-4 μ m in diameter, hyaline, branched, thin-walled, with clamps; Binding hyphae, 4-9 μ m in diameter, solid, thick-walled, hyaline, tortuous, 2-4 μ m in diameter; Skeletal hyphae, 13-19 \times 4-5 μ m in diameter, hyaline, nonseptate, thick-walled. Cystidia, lacking; fusoid cystidioles present. Basidia, 14-18 \times 3-5 μ m in diameter, clamp connection at base, clavate, having 4-sterigmata at apex. Basidiospores, 4-5 \times 2-2.5 μ m in diameter, hyaline, cylindrical to oblong ellipsoid, negative in Melzer's reagent, smooth.

Collection examined: Pakistan, Khyber Pakhtunkhwa, Malakand, Totakan, 626 m asl, on stem of *Olea ferruginea* Royle, August 8, 2016, A. Wahab (AW-296).

Substratum: On *Olea ferruginea* twig.

Comments: The Basidioscarp of this species is easy to distinguish by the flat white feature and having radially elongated pores.

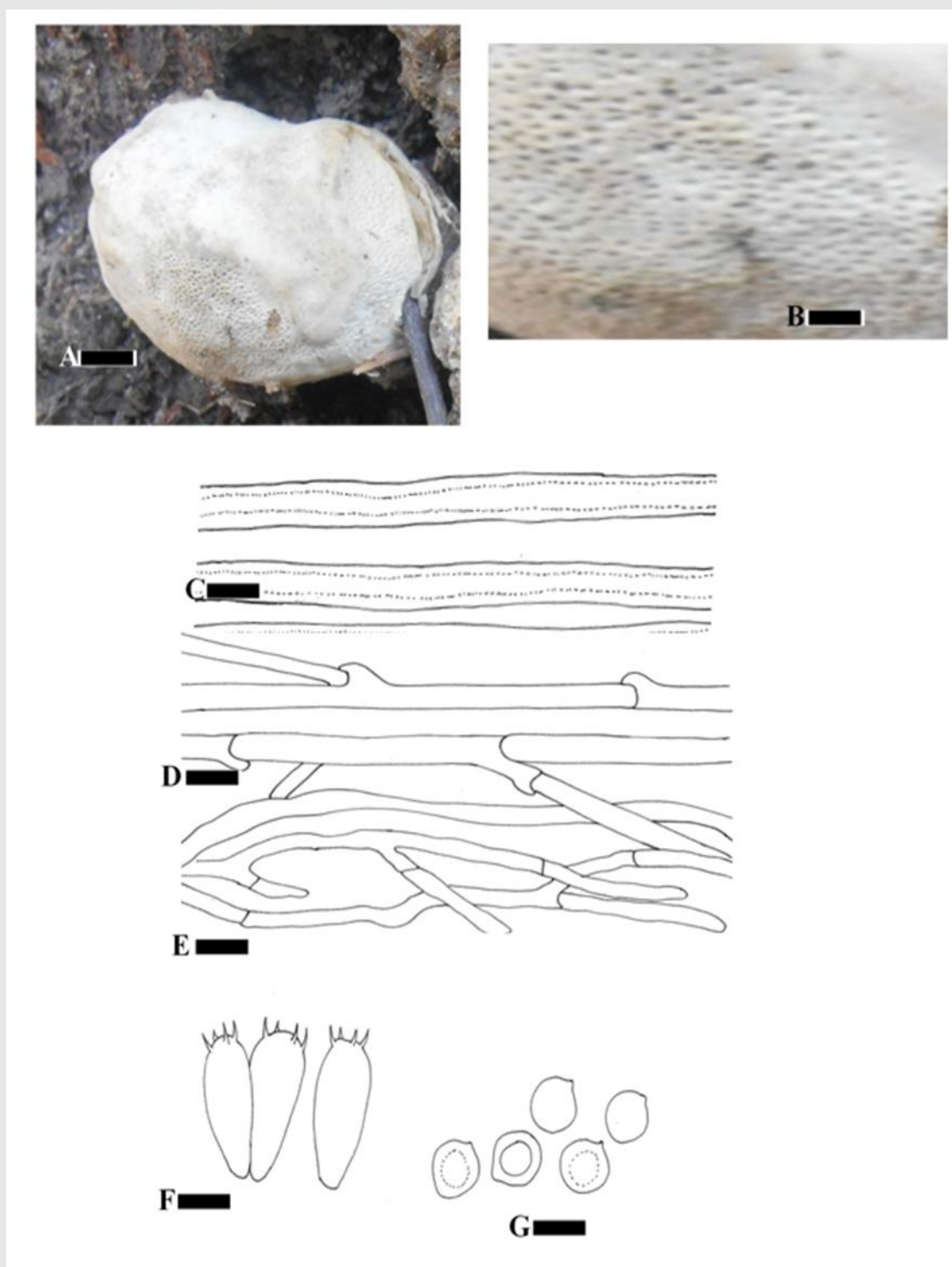


Figure 3: A-H Morpho-anatomical features of *Trametes gibbosa* A. Basidioscarp; B. Pores; C. Skeletal hyphae; D. Generative hyphae; E. Binding hyphae; F. Basidia; G. Basidiospores; Scale bar: A=23mm, B=1-2/mm, C=1.7 μ m, D=3.3 μ m, E=2.1 μ m, F=1.8 μ m, G=1.7 μ m.

Trametes hirsuta (Wulfen) Lloyd, Mycol. Writ. 7: 1319 (1924) Fig. 4.

Morpho-anatomical Description

Basidioscarps coriaceous when fresh, effused-reflexed, annual; pilei applanate to thick, dimidiate; upper surface, concentrically zonate hirsute, gray in color; margin tomentose, yellowish brown in color; Pore surface white-tan or cinereous in color; pores, 2 - 3 per mm, angular; Context, up to 1 mm thick, 6-7 cm long, thick up to 5 mm, 1-4 cm wide, duplex, soft-fibrous, the upper layer gray, from the lower part at the base parted by a thin black line; tube layer, thick up to 3 mm, becomes tortuous with lower context. Hyphal system trimitic; Contextual Generative hyphae, 2.5-2.9 μm thick, thin-walled, with clamp connection; Contextual Binding hyphae, 2-4 μm thick, thick-walled, much branched, nonseptate; Contextual Skeletal hyphae thick-walled, hyaline, often sinuous, with rare branching, nonseptate, 3-3.9 μm thick; tramal hyphae similar. Cystidia lacking; fusoid cystidioles present, 12-18 \times 3-5 μm . Basidia, 15-22 \times 5-7 μm in diameter, with a basal clamp, clavate, with 4 sterigmata. Basidiospores, up to 6-9 \times 2-2.5 μm in diameter, hyaline, smooth, cylindrical.

Type of Rot: Caused white rot to woods.

Collection examined: Pakistan, Khyber Pakhtunkhwa, Malakand, Totakan, 626m asl, on trunk of *Broussonetia papyrifera* (L.) Vent. August 8, 2016, A. Wahab (AW-301).

Substratum: On trunk of *Broussonetia papyrifera*.

Comments: The grey, hirsute upper surface and the greyish pore surface are characteristic of Basidioscarps of *T. hirsuta*.

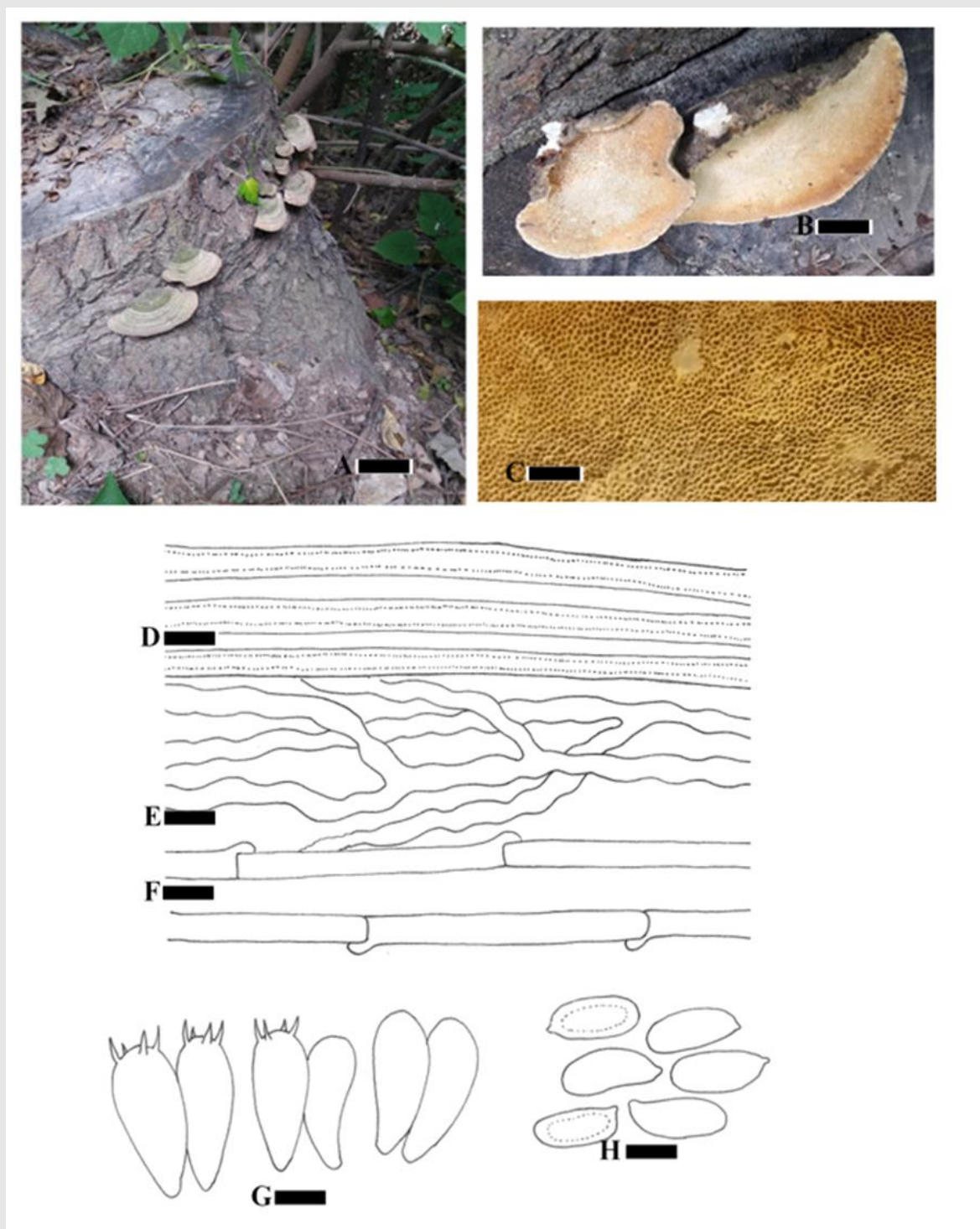


Figure 4: A-H Morpho-anatomical features of *Trametes hirsuta* A-B. Basidioscarp; C. Pores; D. Skeletal hyphae; E. Binding hyphae; F. Generative hyphae; G. Basidia; H. Basidiospores; Scale bar: A-B=41mm, C=2-3/mm, D=1.6 μ m, E=2 μ m, F=2 μ m, G=2 μ m, H=2 μ m.

Trametes trogii Berk., in Trog, Mittheil. d. schweiz. Naturf. Ges. in Bern 2: 52 (1850) Fig. 5.

Morpho-anatomical Description

Basidioscarps, 1.5-2 cm wide, up to 4-4.5 cm long, annual, sessile, tough-corky effused-reflexed or rarely resupinate; upper surface of pileus cream-buff to ochraceous-buff in color, azonate, coarsely hispid; margin sharp; pore surface, ochraceous buff; Pores, 1-2 per mm, angular, with thin dissepiments; tube layer, up to 9-10 mm deep, continuous with lower layer of Context and concolorous; Context, up to 2-4 mm thick, duplex, cream-buff, slightly dark in KOH. Hyphal system trimitic; Contextual Generative hyphae, 2-4 μ m in diameter, thin-walled with clamps, branched; Contextual Binding hyphae, 2-3.5 μ m in diameter, thick-walled, nonseptate, partly arboriform with an unbranched lower part and a branched upper part, partly tortuous with short side branches; Contextual Skeletal hyphae 4-6 μ m in diameter, thick-walled, infrequently branched, nonseptate, cyanophilous; hyphae trama, tramal Skeletal hyphae not common, thick-walled, cyanophilous and slightly dextrinoid; tramal Binding hyphae dominate, but are like those of the context. Cystidia or other sterile hymenial elements not seen. Basidia, 18-23 \times 6-7.5 μ m in diameter, clavate, basal clamp connection, with 4-sterigmata. Basidiospores Hyaline, smooth, cylindric, 10-12 \times 2-4 μ m in diameter.

Collection examined: Pakistan, Khyber Pakhtunkhwa, Malakand, Totakan, 626 m asl, on dead wood of deciduous tree. August 8, 2016, A. Wahab (AW-304) [13].

Substratum: On dead wood of deciduous tree.

Comments: This specimen is documented by tough-corky pileus having coarsely hispid upper surface.

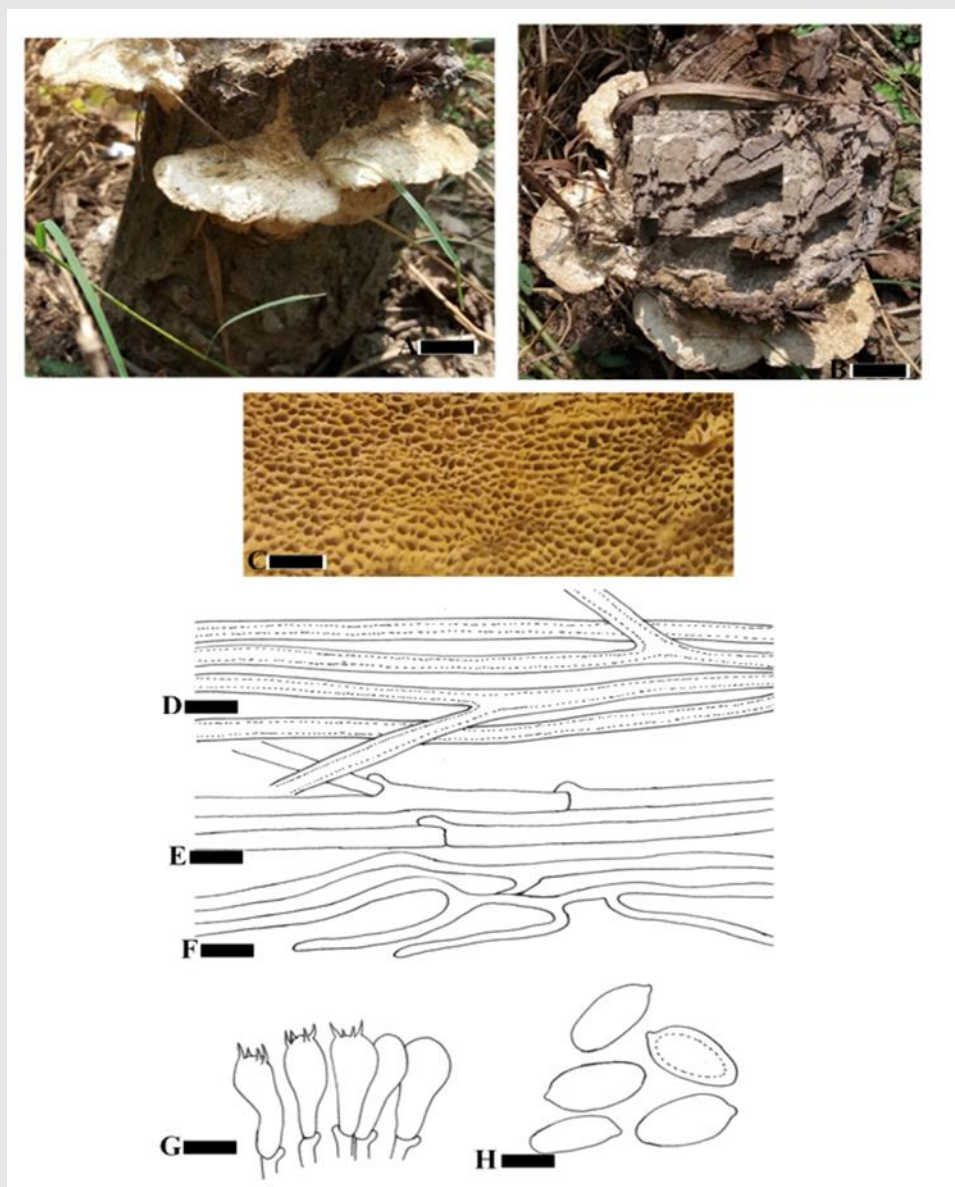


Figure 5: A-H Morpho-anatomical features of *Trametes trogii* A-B. Basidiocarp; C. Pores; D. Skeletal hyphae; E. Generative hyphae; F. Binding hyphae; G. Basidia; H. Basidiospores; Scale bar: A-B=15mm, C=1-2/mm, D=2 μ m, E=2 μ m, F=2 μ m, G=2.1 μ m, H=1.8 μ m.

Discussion

Previously ten *Trametes* species were reported from Pakistan that was found on logs and stumps of angiosperm and gymnosperm trees (Ahmad and Ahmad 1956, 1967, 1969 and 1972). But during our study on diversity of polyporoid fungi from KhyberPakhtunkhwa Forests Pakistan, an unreported four species of trametes were found i.e; *T. gibbosa onolea* twig, *T. hirsuta* on tree trunk of *Broussonetia papyrifera*, *Trametes elegans*, and *T. trogii* on dead deciduous wood.

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While the study confirms that species of trametes has been recorded on angiosperms (e.g., Acer, Betula, Eu calyptus, Malus, Populus, Quercus) and conifers (e.g., Abies, Cupressus, Larix, Pinus [2,14,15].

Trametes Fr. is genus in the family Polyporaceae with *Trametes suaveolens* (L.) Fr. as type species. The basidiocarp of this species is described by the pileate feature and with poroid hymenophore and trimitic hyphal system. These species caused white-rot to woods. Their basidiospores are smooth and thin-walled and shows negative result in Melzer's reagent. It is a diverse genus found in temperate, boreal, and tropical areas of almost all types of forest ecosystems. [15]. Previously from Pakistan Ten species of *Trametes* have been reported by Ahmad viz; *Trametes cingulata* Berk., *T. corrugata* (Pers. Ex Fr.) Bres., *T. dickensii* Berk., *T. hispida* Bagl., *T. incerta* (Curr.) Cooke., *T. ijubarksii* pilat., *T. incana* Berk., *T. lactinea* (Berk.) *T. roseola* Pat. & Har. and *T. suaveolens* (L.) Fr. on logs and stumps of angiosperm and gymnosperm trees.

Trametes elegans of genus *Trametes* in present study is illustrated as a new record from Pakistan. Its Basidiocarp is easily recognized in the field by short stipe-like base, corky and flexible when fresh, more rigid when dry. PILEUS semi-circular, upper surface white to grey and very finely tomentose, glabrous, smooth or concentrically zonate, margin thin, even or lobed. Basidiospores are cylindrical to oblong ellipsoid in shape, hyaline smooth and thin-walled, and $5-7 \times 2-3 \mu\text{m}$ in size [16]. The initial BLAST comparison of this collection shows 100% maximum identity with *Trametes elegans* (JN164921.1 and JN164996.1) from GENBANK. *Trametes elegans* belongs to section *trametes* in Trametoid clade [17]. The molecular phylogenetic tree reveals that *Trametes elegans* clusters with their respective clade with strong bootstrap value [1]. *T. elegans* showed resemblance to *Dadealea palisoti* Fr. from Guanggong China; voucher number HMAS 19763 (Teng 1953) in both specimens narrow semi-dadeloid pores were found and more yellowish on the pore surface and has a dark line between the upper tomentum and the lower context was also observed. *Trametes elegans* of genus *Trametes* in present study is defined and proved as a new record from Pakistan. Its Basidiocarp is easily recognized in the field by flexible and corky in texture when fresh, and when dried it becomes more rigid, with short stipe-like base. Its pileus is semi-circular, upper surface white to grey and glabrous, smooth or concentrically zonate and very finely tomentose. Its margin is thin, even or lobed. The basidiospores of this species are smooth, hyaline, cylindrical to oblong-ellipsoid, and thin-walled in structure and it is about $5-7 \times 2-3 \mu\text{m}$ in size [18]. The initial BLAST comparison of this collection shows 100% maximum identity with *Trametes elegans* (JN164921.1 and JN164996.1) from GENBANK. *Trametes elegans* belongs to section *Trametes* in Trametoid clade. The molecular phylogenetic tree reveals that *Trametes elegans* clusters with their respective clade with strong bootstrap value [1].

Trametes gibbosa (Pers.) Fr., is a member of *Trametes* and is illustrated as a new record for Pakistan. Basidiocarp is characterized by annual, sessile to dimidiate, usually semicircular, tough and coriaceous, upper surface at first tomentose to glabrous, azonate, at first white, often

greenish at the base due to algae; margin sharp. The Basidiospores is cylindrical to oblong ellipsoid, hyaline, smooth, and $4-5 \times 2-2.5\mu\text{m}$ in diameter. The ITS+LSU sequences of initial BLAST comparison of this collection shows 100% identity with *T. gibbosa* (KC589144.1 and KX449481.1). *T. gibbosa* belongs to section trametes clusters in Trametoid clade (Justo and Hibbett, 2011). The molecular phylogenetic tree reveals that *T. gibbosa* clusters with their respective clade with strong bootstrap value. Morphologically, *trametes speciesare* similar to Chinese collection of Harvard university herbaria. *Trametes gibbosa* shows morphological identification with *Daedalea gibbosa* Pers; locality; Heilongjiang, specimen number “HMAS 19761” deposited by (Teng 1956). Both having white to ochraceous, tough and coriaceous basidiocarp, pores distinctly radially elongated, angular with entire dissepiments; trimetric hyphal system; cystidia absent; basidiospores cylindrical to oblong ellipsoid, hyaline, smooth. *Trametes gibbosa* (Pers.) Fr., is also illustrated as a newly recorded species from Pakistan. The Basidiocarp is described by annual, sessile to dimidiate, tough to coriaceous, usually semicircular, at first the upper surface is glabrous and tomentose, azonate, at first white, often greenish at the base due to algae and its margin is sharp. The Basidiospores hyaline, smooth, and are cylindrical to oblong ellipsoid, and $4-5 \times 2-2.5\mu\text{m}$ in diameter. The ITS+LSU sequences of initial BLAST comparison of this collection show 100% identity with *T. gibbosa* (KC589144.1 and KX449481.1). *T. gibbosa* belongs to section trametes clusters in Trametoid clade (Justo and Hibbett, 2011). The molecular phylogenetic tree reveals that *T. gibbosa* clusters with their respective clade with strong bootstrap value [1].

Trametes hirsutais a member of *Trametes* and is illustrated as new record for Pakistan in this work. It is easily recognized in the field by, effused-reflexed, coriaceous when fresh Basidiocarps; pilei dimidiate, applanate to thick, upper surface hirsute, gray, concentrically zonate; margin yellowish-brown, tomentose, pore surface white to tan or cinereous, the pores are angular. The Basidiospores are cylindrical, hyaline, smooth, $6-9 \times 2-2.5 \mu\text{m}$ in diameter. The ITS+LSU sequences of initial BLAST comparison of this collection shows 100% identity with *T. hirsuta* (JF439511.1 and FJ550367.1). *T. gibbosa* belongs to section *Trametes* clusters in *Trametoid* clade (Justo and Hibbett, 2011). The molecular phylogenetic tree reveals that *Trametes hirsuta* clusters with their respective clade with strong bootstrap value [1]. While *T. hirsuta* has shown similarity to the herbarium specimen submitted by Yang 2009 under the voucher number 55017 from china. These specimens have grey, hirsute upper surface and the grayish pore surface were found in concentrically zonate basidiocarp. Pore surface white to tan; the pores are angular. *Trametes hirsuta* is another new record in *Trametes* section from Pakistan in this research. It is easily documented in the field by effused-reflexed, coriaceous when fresh Basidiocarps; pilei dimidiate, applanate to thick, concentrically zonate, upper surface hirsute, gray; margin yellowish-brown, tomentose, its pore surface is white to tan cinereous (ashy grey) in color, and the pores are angular. The Basidiospores are cylindrical, hyaline, smooth, and its size is about $6-9 \times 2-2.5\mu\text{m}$. The ITS+LSU sequences of initial BLAST comparison of this collection shows 100% identity with *T. hirsuta* (JF439511.1 and FJ550367.1). *T. gibbosa* belongs to section *Trametes*, clusters in *Trametoid clade* (Justo and

Hibbett, 2011). The molecular phylogenetic tree reveals that *Trametes hirsuta* clusters with their respective clade with strong bootstrap value (1).

Trametes trogii is a member of *Trametes* and is illustrated as a new record for Pakistan. This species is characterized by effused-reflexed or rarely resupinate and tough-corky Basidiocarps, the upper surface of pileus is coarsely hispid, cream-buff to ochraceous-buff. azonate; margin sharp, pore surface ochraceous buff. The Basidiospores are cylindrical, hyaline. Smooth, with 10-12 x 2.54 µm diameter in size [8]. The ITS+LSU sequences of initial BLAST comparison of this collection shows 100% identity with *T. trogii* (EU790491.1 and JN164936.1). *T. trogii* belongs to section *trametes* clusters in *Trametoid* clade. The molecular phylogenetic tree reveals that *T. trogii* clusters with their respective clade with strong bootstrap value [18]. While the *T. trogii* was similar in morphology to the white herb#3300 from Albany New York specimen (Anonymous 1910) in the herbarium. Both specimen have tough-corky pileus having coarsely hispid upper surface. *Trametes trogii* is also a new recorded species to Pakistan. The characteristic features of the basidiocarps of this species is effused-reflexed or rarely resupinate and tough-corky, the upper surface of pileus is azonate, cream-buff to ochraceous-buff in color and coarsely hispid in texture, with sharp margin, the pore surface is ochraceous buff. The Basidiospores are cylindrical, hyaline. Smooth, with 10-12 x 2.54 µm [8]. The ITS+LSU sequences of initial BLAST comparison of this collection shows 100% identity with *T. trogii* (EU790491.1 and JN164936.1). *T. trogii* belongs to section *Trametes* clusters in *Trametoid* clade. The molecular phylogenetic tree reveals that *T. trogii* clusters with their particular clade with a well-supported bootstrap value [1,17,18].

Conclusions

The ITS rDNA and 28S nLSU phylogenetic trees had similar topologies with *trametes* species forming different clades to *T. elegans*, *T. hirsute*, *T. gibbosa* and *T. trogii* with their respective *trametes* species (Fig. 1). On the other hand, all the analyzed *trametes* species were monophyletic in 18S rDNA and 28S nLSU tree, as previously suggested by Tomšovský et al. 2006 and Welti et al. 2012.

However, information regarding this species is still limited and the abilities of this species should be investigated further.

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