Short communication

Parvixerocomus matheranensis (Boletaceae), a new species from India

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ABSTRACT

A new species of *Parvixerocomus*, *P. matheranensis* belonging to *Boletioideae* of *Boletaceae* is described and illustrated from tropical region of Maharashtra, India. *P. matheranensis* is morphologically distinguished by small basidiomes having ruby red pileus with concolorous stipe, yellow hymenophore that stains blue to blackish blue on bruising, elongate ellipsoid to cylindrical basidiospores with inconspicuous suprahilar depression, ventricose to clavate cheilocystidia, ventricose to lageniform pleurocystidia. Further, extensive phylogenetic analyses based on five gene markers (nrITS, nrLSU, *rpb1*, *rpb2*, *tef1-α*) confirmed that *P. matheranensis* is distinct from its closest taxa *P. aokii* and *P. pseudoaokii* and also from other members of *Boletioideae*.

Keywords: *Boletioideae*, morphology, phylogeny, taxonomy

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The family *Boletaceae* Chevall. is a monophyletic collection of fleshy, sequestrate or pileate-stipitate mushrooms with lamellate or tubular hymenophore. In the last two decades, the morphotaxonomy of this family has been revised based on the multi-gene phylogenetic analyses that revealed several new genera and many new species (Binder & Bresinsky, 2002; Desjardin, Wilson, & Binder, 2008; Desjardin, Binder, Roekring, & Flegel, 2009; Li, Feng, & Yang, 2011; Zeng, Cai, & Yang, 2012; Zeng et al., 2013; Feng et al., 2012; Gelardi et al., 2013, 2019; Nuhn, Binder, Taylor, Halling, & Hibbett, 2013; Wu et al., 2014; Zhao, Wu, Feng, & Yang, 2014; Zhu et al., 2014). *Boletaceae* is well documented in temperate latitudes but less explored in tropics which have probably high number of taxa (Wu et al., 2016b). Although few remarkable boletes have been explored in India (Das, Chakraborty, Baghela, Singh, & Dentinger, 2015; Das, Chakraborty, Baghela, Singh, & Dentinger, 2016; Das, Chakraborty, & Vizzini, 2017; Das & Dentinger, 2015; Pradeep, Vrinda, Shibu, Varghese, & Kumar, 2015; Chakraborty, Parihar, Mehta, Baghela, & Das, 2017; Chakraborty, Vizzini, & Das, 2018; Parihar, Hembrom, Das, & Vizzini, 2018a; Parihar, Hembrom, Vizzini, & Das, 2018b), but the study on the family *Boletaceae* in India is deficient.

The genus *Parvixerocomus* G. Wu & Zhu L. Yang belonging to the subfamily *Boletioideae* is recently erected by Wu et al. (2016a) and it differs from other genera of *Boletioideae* by having smaller

basidiomes (20–40 mm), subdecurrent, yellow hymenophore without stuffed pores, context bluing on bruising and smooth, ovoid to ellipsoid basidiospores. *Parvixerocomus* is represented by only two species. *Parvixerocomus aokii* (Hongo) G. Wu, N.K. Zeng & Zhu L. Yang originally described as *Boletus aokii* was recorded from Japan and south China (Wu et al., 2016a). Another species *P. pseudoaokii* G. Wu, Kuan Zhao & Zhu L. Yang was reported from south western, south eastern and southern China (Wu et al., 2016a). In the present study, a new species of *Parvixerocomus*, *P. matheranensis* collected from Matheran Hills, Maharashtra, India is described based on morphological and molecular characteristics.

Matheran is a small hill station of the Western Ghats of Maharashtra, India situated at an elevation of 800 m asl and receives an annual rain fall of 2500–3000 mm. It is a mixed dry deciduous forest mainly dominated by *Memecylon umbellatum* Burm. f. belonging to the family *Melastomataceae*. Extensive explorations have been made to collect mushrooms samples from Matheran Hills during the period from 2015 to 2019. Several new taxa of mushrooms have been collected and *Parvixerocomus matheranensis* is one among them.

The morphological characters were studied from fresh specimens. A total of five collections were made and one specimen from each collection was studied. The colour codes and notations were referred from Methuen Handbook of Colour (Kornerup & Wanscher, 1978). All the micromorphological characters were observed with the help of a compound microscope MLX-B (Olympus, Tokyo) attached with a 5 MP Olympus camera. The thin hand-cut sections of dried samples were hydrated using 5% (w/v) KOH, stained with 1% (w/v) phloxine in distilled water, and Melzer's reagent. Total 30

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basidiospores from each specimen were measured and the range of measurements is followed by mean length and width ratio in parentheses. The ‘n’ represents the number of basidiospores measured and ‘s’ denotes number of specimens studied. The spore Quotient (Q) value was obtained by length divided by width of individual basidiospores and Qm was obtained by mean length divided by mean width of basidiospores. The holotype was deposited at Ajrekar Mycological Herbarium (AMH), Pune, India and the paratypes were maintained at ‘Matheran Mushroom Herbarium’ collection (MMH) of the department of Botany, Smt. Chandibai Himathmal Mansukhani College, Thane, India.

The fungal DNA was extracted by using CTAB method (Doyle & Doyle, 1987) and outsourced for PCR amplification and sequencing at Agile Life Science Technologies India, Pvt. Ltd. Mahape, Navi Mumbai. Total five DNA markers (nrITS, 28S rDNA, *rpb1*, *rpb2* and *tef1-α*) were amplified and sequenced using the primers: ITS1/ITS4 (White, Bruns, Lee, & Taylor, 1990), LROR/LR5 (Vilgalys & Hester, 1990; Moncalvo, Lutzoni, Rehner, Johnson, & Vilgalys, 2000), RPB1-B-F1/RPB1-B-R (Wu et al., 2014), RPB2-B-F1/ RPB2-B-R (Wu et al., 2014), EF1-B-F1/EF1-B-R (Wu et al., 2014) (Supplementary Table 1).

The chromatograms were curated and the contigs were prepared in Bioedit v 7.2.5 (Hall, 2011). All the sequences produced in this study were submitted to GenBank and the accession numbers were obtained (Supplementary Table 2).

The phylogenetic analysis includes total five DNA markers representing 279 sequences obtained from NCBI and five were generated during this study for new species (Supplementary Table 2). The NCBI deposited sequences used in combined dataset and for ITS phylogeny were selected on the basis of previous studies (Wu et al., 2014; Gelardi et al., 2015; Parihar et al., 2018b). For nrITS and combined dataset, *Austroboletus* species were taken as outgroup based on previous studies and is closely allied to *Parvixeroconus* (Wu et al., 2014; Gelardi et al., 2015). The sequences were analysed in two different datasets: 1) Combined dataset includes nrLSU, *rpb1*, *rpb2* and *tef1-α* with 73, 63, 48, and 65 sequences respectively (including four new sequences of current species) (Supplementary Table 2). 2) Second dataset includes 30 nrITS sequences (including one new sequence of current species). All the sequences were aligned using muscle programme in the MEGA v 7.0 software (Kumar, Stecher, & Tamura, 2016). The aligned sequences were further curated manually based on the ambiguous sites and ends were trimmed off to obtain the equal length and reduce the length biasness in matrix. Before combining the individual dataset, we performed the Partition Homogeneity test (Incongruence Length Difference- ILD) in PAUP 4.0b10 (Swofford, 2002). Based on our ILD test the obtained p value was 0.025 which is less than the significant value (0.05). So, the dataset can be combined. For combined tree the aligned sequences were concatenated by using software TaxonDNA (Sequence Matrix) v 1.7.8 (Vaidya, Lohman, & Meier, 2010). The phylogenetic analyses were performed with Maximum likelihood (ML) and Bayesian inference (BI) criteria. The ML phylograms were generated by using IQTree v 1.6.8 (Nguyen, Schmidt, Von Haeseler, & Minh, 2014). Best fit model of evolution was selected as TIM3e+R4 for combined dataset and TNe+G4 for nrITS dataset, according to Bayesian information criterion (BIC). Bayesian analysis was performed using Metropolis Coupled MCMC method in MrBayes v 3.2.6 (Ronquist et al., 2012). Two parallel chains were run for 2 million generations and standard deviation of split frequency was obtained less than 0.01 for both the datasets. The stationery and convergence were determined with Tracer v 1.5 (Rambaut, Suchard, Xie, & Drummond, 2014) in terms of log-likelihood of effective sample size. ML and Bayesian phylogenetic trees

were visualized in FigTree v 1.4.2 (<http://tree.bioed.ac.uk/software/figtree>). Statistical supports for the phylograms were determined in terms of bootstrap values (BS) and posterior probabilities (PP). The sequence alignments and phylogenetic trees were submitted to TreeBASE (accession URL: <http://purl.org/phylo/treebase/phylo/study/TB2:S27896>, <http://purl.org/phylo/treebase/phylo/study/TB2:S27897>).

Taxonomy

Parvixeroconus matheranensis P.B. Patil, Senthil., S.K. Singh & S.A. Vaidya, sp. nov. (Figs. 1–3)

MycoBank no.: MB 835719.

Diagnosis: Differs from *P. aokii* and *P. pseudoaokii* in dimensions of spores.

Type: INDIA, Maharashtra, Raigad District, Matheran Hills, 800 m asl, (18°58'48.00"N, 73°16'12.00"E), collected by P.B. Patil on 25 Jul 2017 (AMH 9976, Holotype).

Gene sequences ex-holotype: MH 521245 (ITS), MH 521253 (28S), MT140888 (*rpb1*), MT140889 (*rpb2*), MT140890 (*tef1-α*).

Etymology: The species epithet “*matheranensis*” refers to the place of collection.

Basidiomes small, boletoid, centrally stipitate. Pileus 8–40 mm diam, convex to applanate; surface uniformly ruby red (12C8, 12D8), subtomentose, dry, pruinose, glutinous when wet; margin incurved. Hymenophore subdecurrent often with a decurrent tooth running on the stipe apex; surface yellow (3A4, 3A5), bluing on bruising, pores more or less round to angular to irregular, 3–5 per mm, tubular. Stipe 22–35 × 1–3 mm, cylindrical, equal, slightly attenuated towards apex; surface concolorous with the pileus, staining blue on bruising, hollow, basal mycelium white. Pileal context thin, 2–4 mm thick, white, staining blue to blackish blue on bruising.

Basidiospores 6.5–10 × 3–4.5 μm, (Xm = 8.7±0.78 × 3.96±0.3 μm, Q = 1.8–2.7, Qm = 2.2±0.2, n = 30, s = 5), elongate ellipsoid to cylindrical, inequilateral, with inconspicuous suprahilar depression, brownish yellow, smooth, inamyloid. Basidia 25–38 × 5.5–9 μm, clavate, tetrasporic, bearing four short sterigmata, rarely bisporic. Cheilocystidia 21.5–53 × 7.5–12 μm, fusoid to clavate to ventricose, thin-walled. Pleurocystidia 22.5–72.5 × 8–20.5 μm, broadly subfusiform to ventricose to lageniform, with a long beak, thin walled, hyaline, often brownish to yellowish brown in KOH solution. Hymenophoral trama boletoid, lateral strata gelatinised, hyphae 6–7.5 μm diam, hyaline, thin-walled. Pileipellis an epithelium, consists of submoniliform to cylindrical cells, 18–48 × 4.5–14 μm, terminal cells narrow with acute apex. Pileal trama consists of intermingled hyphae 5–9 μm wide, thin-walled. Stipe trama consists of parallel hyphae 7.5–12 μm wide, thin-walled. Clamp connections absent.

Habitat and distribution: On soil, solitary to scattered, under the trees of *Memecylon umbellatum*. So far known only from Matheran Hills, Maharashtra, India.

Additional specimens examined: INDIA, Maharashtra, Raigad District, Matheran Hills (18°58'48.00"N, 73°16'12.00"E), 17 Jul 2015 (MMH 1011), 14 Aug 2016 (MMH 1012), 30 Jul 2017 (MMH 1013), 19 Aug 2018 (MMH 1014), 04 Aug 2019 (MMH 1015), Prashant B. Patil.

The closely related species *P. pseudoaokii* (Wu et al., 2016a) differs markedly from *P. matheranensis* by its yellowish red, greyish red to rose red pileus, distinctive ovoid basidiospores (Q = 1.4–1.89

Fig. 1. - *Parvixerocomus matheranensis* (AMH 9976, holotype). A, B: Basidiomata in its natural habitat. C: Basidiomata with scale bar. D: Pileal surface. E, F: Hymenophoral surface. G: Dissected basidiocarp showing bluish black colour (image taken immediately after sectioning). H: Pore surface staining bluish black on bruising.

vs 1.86–2.7) and slightly smaller pleurocystidia ($30\text{--}65 \times 8\text{--}15$ vs $22.5\text{--}72.5 \times 8\text{--}20.5 \mu\text{m}$). Another species *P. aokii* (Wu et al., 2016a) is differentiated from *P. matheranensis* in having orange red, vivid red to red pileus, larger, elongate ellipsoid basidiospores ($9\text{--}11$ vs $7\text{--}10 \mu\text{m}$ long), larger basidia ($32\text{--}42 \times 8\text{--}11$ vs $25\text{--}38 \times 5.5\text{--}8.5 \mu\text{m}$) and narrower pleurocystidia ($9\text{--}11$ vs $8\text{--}20.5 \mu\text{m}$ wide).

Parvixerocomus matheranensis morphologically resembles with *Boletus coccineinanus* Corner., *Boletus minimus* Zang & Huang., *Boletus patouillardii* Singer. and *Xerocomus tengii* Zang., Lin &

Huang. in having small basidiomes with red to dark red pileus. However, *B. minimus* is now synonymised with *B. patouillardii* (Zang & Huang, 2002; Zhou & Yang, 2008). *Boletus coccineinanus*, *B. patouillardii* and *Xerocomus tengii* distinctly differ morphologically from *P. matheranensis* in having basidiomes that are unchanging in colour on bruising (Zang, Lin, & Huang, 2002; Zhou & Yang, 2008). Further, *B. coccineinanus* (Zhou & Yang, 2008) has pinkish hymenophore and thick walled cells in the pileipellis. *Boletus patouillardii* differs from *P. matheranensis* in having shorter basidio-

Fig. 2. - *Parvixeroconus matheranensis* (AMH 9976, holotype). A: Basidiospores. B, C: Basidia. D–I: Pleurocystidia. J: Cheilocystidia. K: Pileipellis. L: Vertical section of stipitipellis. Bars: A, C, J, L 10 μ m; D, I 20 μ m.

spores ($7\text{--}8.5 \times 4\text{--}5 \mu\text{m}$; Zhou & Yang, 2008) and broadly ellipsoid to subfusiform to subcylindrical terminal cells of pileipellis with round to obtuse apices, while *X. tengii* has distinctly larger basidiospores ($15\text{--}16.5 \times 5\text{--}5.5 \mu\text{m}$; Zang, et al., 2002).

The combined data matrix alignment contained 2939 characters of which 1181 characters were parsimony informative sites. The number of characters and parsimony informative sites of individual gene markers in the combined data matrix alignment was provided (Supplementary Table 3). Based on combined analysis using ML and Bayesian methods we obtained similar tree topologies. The ML tree is supported with bootstrap and Bayesian tree with posterior probability values. In the combined dataset topology, *P. matheranensis* forms a sister clade to *P. aokii* and *P. pseudoaokii* with strong support (BS/PP-100/1) (Fig. 4).

The analyses using combined dataset revealed that the genus *Parvixeroconus* is sister to all the other genera of *Boletioideae* with strong support (99/1) (Fig. 4). Our study well supported the previous molecular as well as morphological findings, where the genus

Fig. 3. - *Parvixeroconus matheranensis* (AMH 9976, holotype). SEM micrograph of basidiospore.

Parvixeroconus was sister to rest of the genera of *Boletioideae* (Wu et al., 2014, 2016a). Further, in nrITS tree, *P. matheranensis* forms a sister clade to *P. pseudoaokii* clade with strong support (100/1) (Fig. 5). The sampling size for nrITS was restricted to closely allied genera of *Boletioideae*.

In our current study both morphological as well as molecular analyses revealed substantial variation and based on which, here we proposed *P. matheranensis* as a new species under the genus *Parvixeroconus*.

Disclosure

The authors declare no conflicts of interest.

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Fig. 4. - Phylogram of subfamily Boletoidae generated from the combined dataset of four nuclear genes (*nrLSU*, *rpb1*, *rpb2* and *tef1-α*) using Bayesian and Maximum likelihood analyses. Bootstrap values (BS) > 70 and Bayesian posterior probability (PP) values > 0.8 are given at the internodes.

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Fig. 5. - ITS Phylogram restricted to closely related genera of *Parvixeroconus* generated from Bayesian and Maximum likelihood analyses of ITS sequences. Bayesian posterior probability (PP) values > 0.7 and Bootstrap values (BS) > 80 are shown on the branches.

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