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Paleotropical distribution of the genus *Neotropicomus* A.C.Magnago, Alves-Silva & T.W.Henkel: a new species from India

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ABSTRACT
A novel species of *Neotropicomus* A.C.Magnago, Alves-Silva & T.W.Henkel is proposed from tropical India. *Neotropicomus indicus* sp. nov. is described as new, based on morphology and molecular phylogenetic studies. Detailed descriptions, colour photographs, and a phylogenetic tree based on ITS and 28S sequence analysis are presented. A taxonomic key to the described species of *Neotropicomus* is also provided. This forms the first report of the genus from the paleotropics.

KEY WORDS
India,
Boletaceae,
new species.

RÉSUMÉ
Répartition paléotropicale du genre Neotropicomus *A.C.Magnago, Alves-Silva & T.W.Henkel : une nouvelle espèce de l'Inde.*
Une espèce de *Neotropicomus* A.C.Magnago, Alves-Silva & T.W.Henkel provenant de l'Inde tropicale est décrite comme nouvelle pour la science sur la base de sa morphologie et d'études de phylogénies moléculaires. Une description morphologique détaillée et un arbre phylogénétique de l'ITS et de l'ARN 28S combinés sont présentés. Une clé taxonomique des espèces connues de *Neotropicomus* est aussi fournie. Cette étude donne un premier exemple du genre dans les paléotropiques.

MOTS CLÉS
Inde,
Boletaceae,
espèce nouvelle.

INTRODUCTION

Neotropicomus A.C.Magnago, Alves-Silva & T.W.Henkel is a recently described genus from Brazil, with *N. australis* A.C.Magnago as the type species. The name "Neotropicomus" indicates the occurrence of the genus in the tropics of the Western Hemisphere. The diagnostic features of the genus are epigeous basidiomata with smooth to rugulose pileus, tubulose-poroid pale olivaceous yellow hymenophore, smooth to minutely scabrous stipe surface, a pileus and a stipe context that does not change colour when bruised, phylloporoid type hymenophoral trama, and subfusoid to fusoid basidiospores with smooth walls (Magnago *et al*. 2022). The genus is so far represented by two species, *N. australis* and *N. parvogracilis* (T.W.Henkel & Husbands) A.C.Magnago & T.W.Henkel. *Neotropicomus australis* has been reported from Brazil's Atlantic Forest and was collected in the vicinity of *Guapira* Aubl. and *Pisonia* L. *Neotropicomus parvogracilis* was originally described as *Xerocomus parvogracilis* T.W.Henkel & Husbands from Guyana (Husbands *et al*. 2013; Magnago *et al*. 2022). *Neotropicomus* is placed in the subfamily Boletoideae Burnett closely related to *Tengioboletus* G.Wu & Zhu L. Yang (Magnago *et al*. 2022). Based on morphological studies and two-locus (ITS and 28S) phylogenetic analysis, we describe a unique species of *Neotropicomus* from India.

MATERIAL AND METHODS

Morphological studies

Basidiomata were collected from Palode, Thiruvananthapuram District, Kerala State. The collection locality was dominated by species of Dipterocarpaceae Blume. All basidiomata were photographed in the field, and macroscopic features were noted. The size, colour and colour change of the basidiomata after bruising were observed. Collected specimens were dried in a hot air oven (50-60°C) for 1-2 hours and used for microscopic studies. Microscopic examination of tissues taken from different parts of the fruit bodies were done with 1% Congo red and 1% phloxine. Excess stain was washed off with 5% KOH. Pigmentation of the different microscopic structures were noted by mounting in water. Length and breadth of basidiospores were noted. 'Q' value represents 'length/width ratio' of a basidiospore; 'Qm' is the average Q of all the basidiospores ± sample standard deviation. Basidiospores were also observed in Melzer's reagent and cotton blue for amyloidity, and cyanophiliy, respectively. Measurements of basidia, cystidia, and terminal cells of the pileipellis are presented as length × breadth. The specimens including the holotype are maintained at The Zamorin's Guruvayurappan College herbarium (ZGC), Kerala, India.

DNA extraction, PCR amplification and sequencing

REDExtract-N-Amp kit by Sigma-Aldrich Company was used for DNA extraction. ITS1F and ITS4R were the two primers used for the amplification of internal transcribed spacers (ITS) region, and LROR and LR7 were used for amplifying 28S of rDNA region (Vilgalys & Hester 1990; White *et al*. 1990; Magnago *et al*. 2022). Sequencing reaction was done by Barcode BioSciences Private Limited, Bangalore, India using ITS1F, ITS4R, LROR and LR7 sequencing primers. All the sequences generated during the study are deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank) with GenBank accession numbers: OQ633210 (ITS), OQ633194 (28S).

Phylogenetic analysis

Phylogenetic analyses were done using newly generated sequences (ITS nad28S) of *Neotropicomus*. The newly generated sequences were compared with the other available sequences in GenBank using BLASTN tool. The sequences that showed similarity in BLAST search and sequences from the study of Magnago *et al*. (2022) were retrieved from GenBank for constructing the data matrix. A concatenated dataset of ITS and 28S was prepared with the selected sequences, and aligned by using MAFFT online service (Katoh *et al*. 2019). Manual changes were made using MEGA X64 (Kumar *et al*. 2018). Details of the sequences used in this study are given in Table 1. The final dataset included 67 sequences from 49 taxa (25 ITS sequences and 42 LSU sequences). The outgroup selections were based on the previous study of Magnago *et al*. (2022). All the parameters were set to default in the software (Nguyen *et al*. 2015; Trifinopoulos *et al*. 2016; Kalyaanamoorthy *et al*. 2017; Hoang *et al*. 2018). Maximum Likelihood (ML) analysis was carried out using the IQTree program. IQTree program generated the phylogram by using the substitution model as auto with 1000 bootstrap in ultrafast approach (UFBoot) and SH-like approximate likelihood ratio test (SHaLRT). The obtained phylogenetic tree was viewed with FigTree v1.4.4 (Rambaut *et al*. 2018). Bootstrap values (BS) of ≥ 60% were displayed in the ML tree. Final aligned data matrix was deposited in TreeBase with accession number: 30382.

RESULTS

Family Boletaceae Chevall.
Genus *Neotropicomus*
A.C.Magnago, Alves-Silva & T.W.Henkel

Neotropicomus indicus sp. nov.
(Fig. 1)

Neotropicomus indicus sp. nov. is characterized by basidiomata with a smooth to minutely pruinose, reddish brown pileus, stipe surface finely pruinose at the apex, downward to the middle with small reddish brown squamules, subfusoid to fusoid basidiospores, and a cutis type of stipitipellis interrupted by caulocystidia clusters. The species differs from *N. australis* by its smaller basidiospores, larger cheilocystidia and stipitipellis structure.

Type material. — **India** • Kerala State, Thiruvananthapuram District, Palode; 6.VII.2022; holotype: ZGC[ZGCSN180]; GenBank: OQ633210 (ITS), OQ633194 (28S).

Etymology. — "*indicus*" indicates the distribution and collection locality of the species.

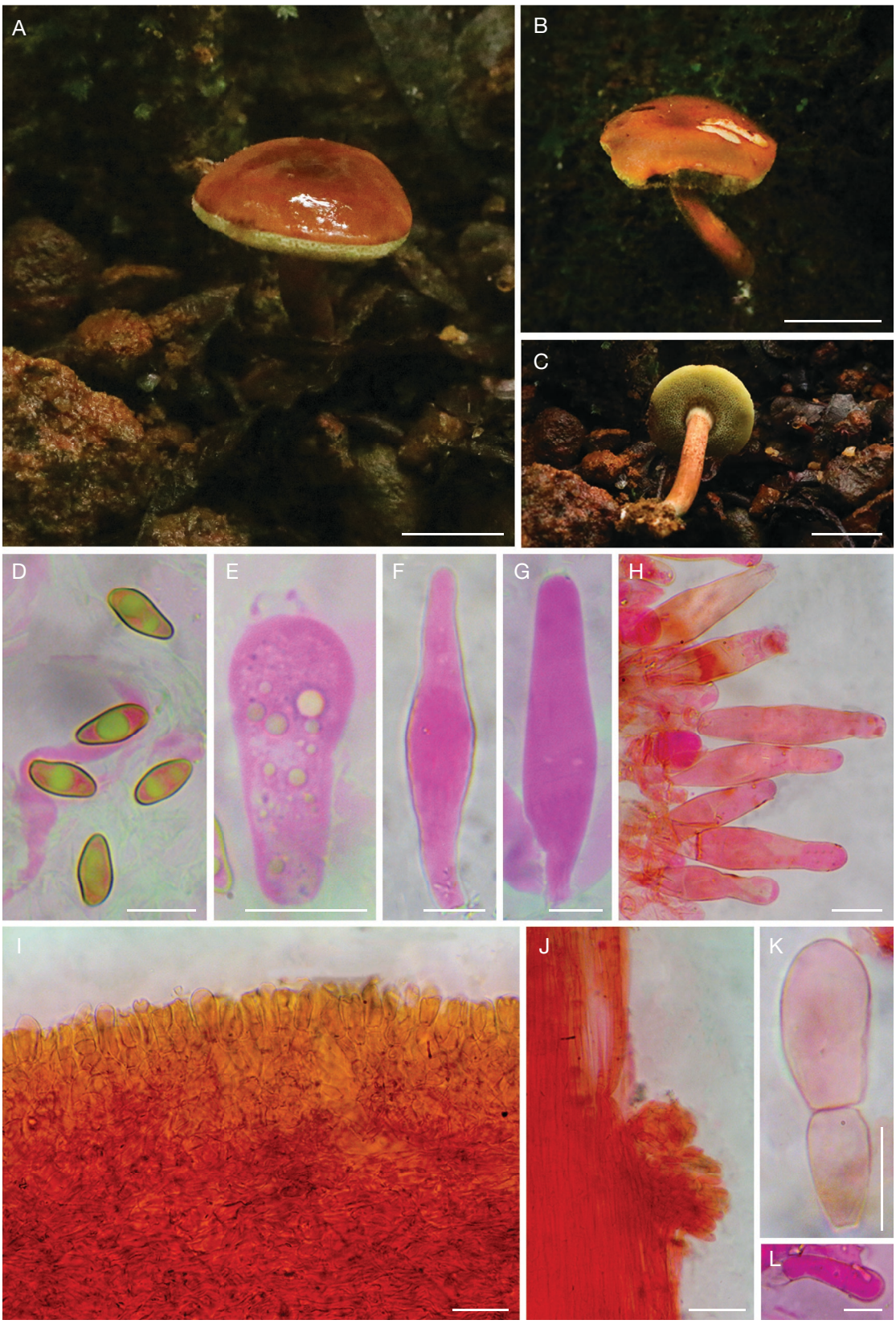

FIG. 1. — *Neotropicomus indicus* sp. nov. (from holotype): **A**-**C**, basidiomata; **D**, basidiospores; **E**, basidia; **F**, **G**, pleurocystidia; **H**, cheilocystidia; **I**, pileipellis; **J**, stipitipellis; **K**, terminal cells of pileipellis; **L**, caulocystidium. Scale bars: A-C, 20 mm; D-H, K, L, 10 µm; I, J, 20 µm.

Table 1. — Taxa, vouchers, localities, and GenBank accession numbers of the DNA sequences used in this study. New species shown in **bold**.

Taxon	Voucher	Locality	GenBank accession numbers	
			ITS	28S
Austroboletus albidus Yan C. Li & Zhu L. Yang	HKAS107148, holotype	China	–	NG088131
A. olivaceoglutinosus K.Das & Dentinger	HKAS57756	China	–	KF112383
Boletus fagacicola B.Feng, Y.Y.Cui, J.P.Xu & Zhu L.Yang	HKAS55975, holotype	China	JN563899	JN563853
B. reticuloceps (M.Zang, M.S.Yuan & M.Q.Gong) Q.B.Wang & Y.J.Yao	HKAS57671	China	–	KF112454
B. bainiugan Dentinger	HKAS52235	China	JN563905	KF112457
B. edulis Bull.	HMJAU4637	Russia	JN563894	KF112455
Brasilioporus olivaceoflavidus A.C.Magnago	FLOR 68467	Brazil	OM068901	–
B. olivaceoflavidus	FLOR 68468	Brazil	OM068902	–
B. olivaceoflavidus	VIES 9901322, holotype	Brazil	OM068903	–
B. rufonigricans (T.W. Henkel) A.C.Magnago & T.W.Henkel	G472	Guyana	KJ786658	–
B. simoniarum A.C.Magnago	VIES 9901327, holotype	Brazil	OM068905	OM068914
Hortiboletus amygdalinus Xue T. Zhu & Zhu L. Yang	HKAS54166, holotype	China	KT990581	KT990777
H. subpaludosus (W.F.Chiu) Xue T. Zhu & Zhu L. Yang	HKAS59608	China	KF112371	–
Imleria badia (Fr.) Vizzini	MB 03-098a	United States	–	KF030355
I. badia	Xb2	Germany	–	KF030357
I. obscurebrunnea (Hongo) Xue T. Zhu & Zhu L. Yang	HKAS52557	China	KC215207	KF112374
Indoporus shoreae A.Parihar, K.Das, Hembrom & Vizzini	AP 6693, holotype	India	MK123972	MK123973
I. shoreae	AP 6697	India	MK123977	MK123976
Neotropicomus australis A.C.Magnago	FLOR 68471	Brazil	OM068909	–
N. australis	ACM1138	Brazil	OM068907	OM068916
N. australis	VIES 9901328, holotype	Brazil	OM068906	–
N. australis	VIES 9901329	Brazil	OM068908	OM068917
N. indicus sp. nov.	ZGCSN180	India	**OQ633210**	**OQ633194**
N. parvogracilis (T.W.Henkel & Husbands) A.C.Magnago & T.W.Henkel	TH9209, holotype	Guyana	JQ751261	JQ751262
N. parvogracilis	TH8850, paratype	Guyana	JQ751263	HQ161865
Parvixerocomus aff. *aokii* (Hongo) G.Wu, N.K.Zeng & Zhu L. Yang	HKAS52633	China	–	KF112379
P. aokii	HKAS59812	China	–	KF112378
P. pseudoaokii G.Wu, K.Zhao & Zhu L. Yang	HKAS80480, holotype	China	–	KP658468
Porphyrellus castaneus Yan C. Li & Zhu L. Yang	HKAS52554, holotype	China	–	KT990697
P. castaneus	HKAS63076	China	–	KT990548
P. orientifumosipes Yan C. Li & Zhu L. Yang	HKAS53372, holotype	China	–	KT990629
Strobilomyces echinocephalus Gelardi & Vizzini	HKAS59420	China	–	KF112463
S. floccopus (Vahl) P. Karst.	AFTOL-716	United States	AY854068	AY684155
S. floccopus	AFTOL-716	United States	AY854068	AY684155
S. strobilaceus (Scop.) Berk.	WU0016537	Austria	–	KT990647
Tengioboletus glutinosus G.Wu & Zhu L. Yang	HKAS53425, holotype	China	–	KF112341
T. glutinosus	HKAS53452	China	–	KT990655
T. reticulatus G.Wu & Zhu L. Yang	HKAS52241	China	–	KT990657
T. reticulatus	HKAS53453, holotype	China	–	KT990656
Tylopilus dunensis A.C.Magnago & M.A.Neves	MAN216, paratype	Brazil	MF113418	OM068920
T. dunensis	MAN218, holotype	Brazil	MF113419	MF113428
T. felleus (Bull.) P. Karst.	HKAS54926	Germany	–	KF112411
T. felleus	HKAS90203	China	–	KT990545
T. violaceobrunneus Yan C. Li & Zhu L. Yang	HKAS89443, holotype	China	–	KT990702
Xanthoconium purpureum Snell & E.A.Dick	NY00720964	United States	–	KT990663
X. sinense G.Wu, Y.Y.Cui & Zhu L. Yang	HKAS77758, holotype	China	–	KT990665
Xerocomellus corneri Xue T. Zhu & Zhu L. Yang	HKAS52503	China	–	KT990668
X. communis Xue T. Zhu & Zhu L. Yang	HKAS50467	China	–	KT990670
Xerocomellus sp.	HKAS56311	China	KF112340	KF112170

Habitat. — Solitary, on ground in forest dominated by Dipterocarpaceae members.

MycoBank. — MB848296.

Description

Basidiomata small-sized. Pileus 20-30 mm broad, convex when young, becoming plano-convex on maturity; surface viscid when wet, smooth to minutely pruinose, reddish brown to grayish brown towards the centre; margin straight. Pileal context whitish, unchanging when cut. Hymenphore adnate, depressed around the apex of stipe; surface pale yellow to olive yellow; pores 1-2 per mm, angular, unchanging on bruising; tubes 2-4 mm long, concolourous to the hymenophore surface, unchanging when cut. Stipe 20-40 × 3-4 mm, central, almost equal; surface finely pruinose at the apex downward to the middle with small reddish brown squamules, whitish to pale yellowish in apex, reddish brown in middle to bottom in a cream background. Stipe context whitish, unchanging on bruising. Basal mycelium white.

Basidiospores 9-11 × 4-5 µm (Q = 1.8-2.75, Qm = 2.2), subfusoid to fusoid, thin- to slightly thick-walled (up to 0.5 µm), smooth, yellowish in water, inamyloid. Basidia 20-27 × 7-10 µm, clavate, thin-walled, 4-spored, hyaline with many oil droplets; sterigmata up to 3 µm long, hyaline with many oil droplets. Pleurocystidia 42-62 × 6-12 µm, narrowly fusiform to fusiform, thin-walled, hyaline, inamyloid. Cheilocystidia 28-50 × 7-10 µm,

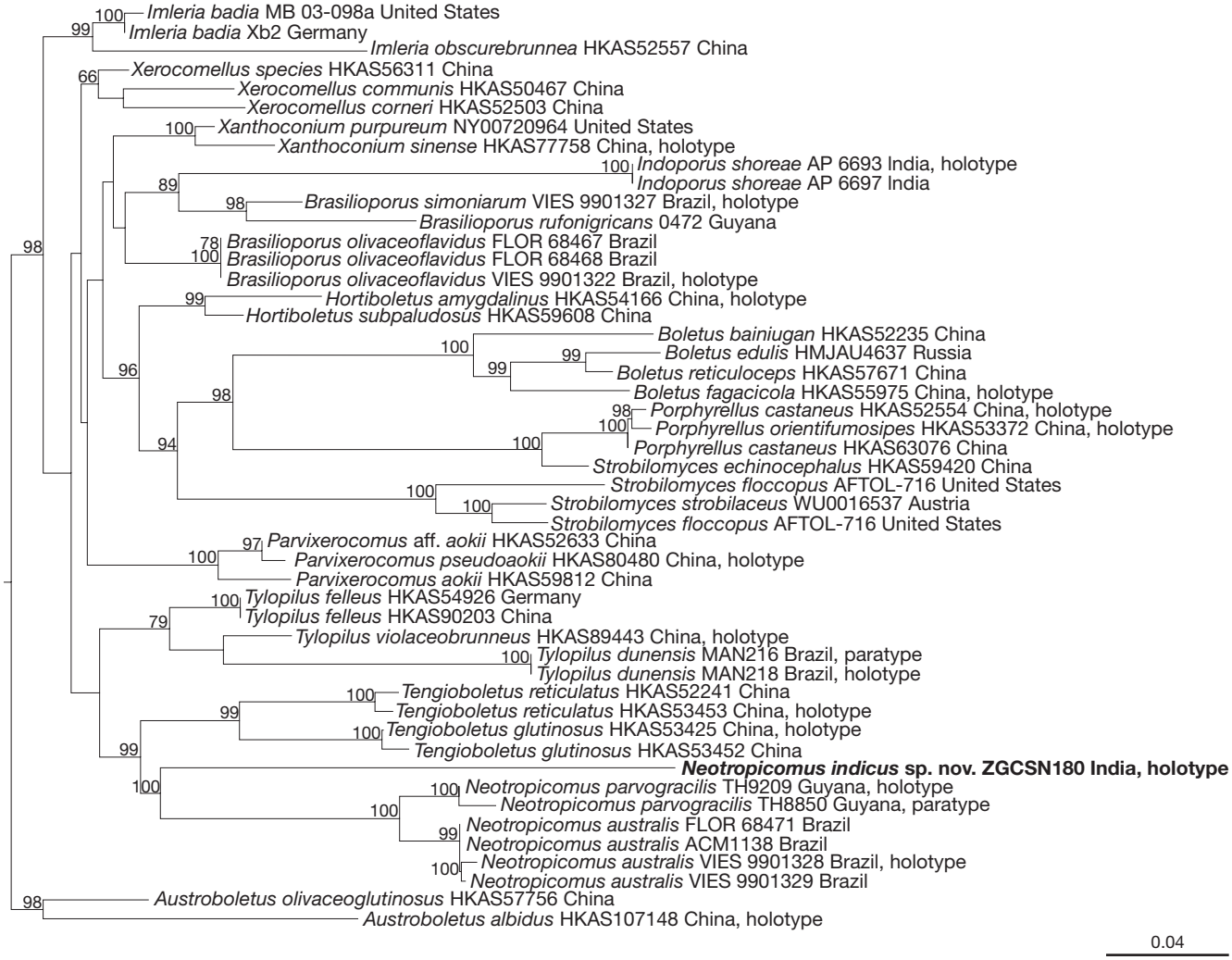

Fig. 2. — Phylogram inferred from combined ITS and 28S dataset using IQTree program. BS≥60% is indicated above or below the branches. Novel taxon is given in **bold**.

narrowly utriform, nearly fusiform, broadly clavate or nearly cylindrical, hyaline, inamyloid. Hymenophoral trama phylloporoid; hyphae 3-15 µm wide, thin-walled, hyaline. Pileipellis a trichodermium, made up of densely packed chains of terminal elements formed on subglobose cells; terminal elements 16-31 × 4-13 µm, nearly cylindrical or broadly clavate, some of them having subacute apex, thin-walled, inamyloid. Pileus trama tightly interwoven; hyphae 3-12 µm wide, thin-walled, hyaline, inamyloid. Stipitipellis a cutis interrupted by caulocystidia clusters; caulocystidia 13-28 × 4-7 narrowly clavate to clavate, rarely with subacute apex, thin-walled, inamyloid. Stipe trama parallel to interwoven; hyphae 3-12 µm wide, thin-walled. Clamp-connections absent.

Molecular analysis

The BLAST search of ITS and 28S sequences of *Neotropicomus indicus* sp. nov. showed maximum sequence similarity (95.73%) with an unidentified *Boletus* species (GenBank accession: MN580131). However, in the ML tree created by adding representatives of genera in the Boletoideae clade, *N. indicus* sp. nov. was found clustered with other *Neotropicomus* species with maximum bootstrap (BS) support (Fig. 2) with which the Indian collection formed a distinct lineage. *Tengioboletus* forms a sister clade to *Neotropicomus* with 99% bootstrap support.

Notes

Morphologically and phylogenetically, the new species can be comfortably placed in the recently described genus *Neotropicomus*. Morphologically *N. indicus* sp. nov. shows more resemblance with *N. australis* by having small basidiomata, reddish brown pileus which is viscid when wet, depressed hymenophore around the stipe apex, pruinose stipe surface, absence of colour change in pileal and stipe context, subfusoid to fusoid basidiospores and trichodermial type of pileipellis. However, *N. australis* differs from *N. indicus* sp. nov. in having larger basidiospores, ventricose pleurocystidia with long neck, smaller cheilocystidia, and a stipitipellis, which is a palisade of erect clusters of caulocystidia. *Neotropicomus indicus* sp. nov. also shows resemblance to *N. parvogracilis* by having basidiomata with a pileus which is viscid when wet, unchanging

Key to known *Neotropicomus* A.C.Magnago, Alves-Silva & T.W.Henkel species

1. Basidiospores smaller (9-11 × 4-5 µm); cheilocystidia larger (28-50 × 7-10 µm); stipitipellis a cutis interrupted by caulocystidia clusters; distributed in the tropical forest of eastern hemisphere ..... *N. indicus* sp. nov.
— Basidiospores larger; cheilocystidia smaller or absent; stipitipellis a trichodermial palisade; distributed in tropical forest regions of western hemisphere 2

2. Stipe surface finely pruinose to subscabrous on apex to middle; basidia 4-spored; cheilocystidia present (19-33 × 10-13 µm); basidiospores 12-14 × 4-5 µm ..... *N. australis* A.C.Magnago
— Stipe surface glabrous or scurfy-fibrillose and faintly longitudinally striate; basidia 2-spored; cheilocystidia absent; basidiospores 11-15 × 4-6 µm ..... *N. parvogracilis* (T.W.Henkel & Husbands) A.C.Magnago & T.W.Henkel

pileal and stipe context and a trichodermial type of pileipellis. However, *N. parvogracilis* differs from *N. indicus* sp. nov. by the presence of finely rugulose pileal surface, larger basidiospores, 2- to 4-spored basidia, absence of cheilocystidia and a stitpitipellis which is a trichodermial palisade.

Phylogenetic analysis based on ITS and 28S sequences placed the new species in *Neotropicomus* with maximum bootstrap support. However, *N. indicus* sp. nov. undoubtedly formed a separate lineage in the *Neotropicomus* clade.

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Declarations
The authors declare no competing interests.

REFERENCES

Hoang D. H., Chernomor O., Haeseler A. V., Minh B. Q. & Vinh L. S. 2018. — UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35 (2): 518-522. https://doi.org/10.1093/molbev/msx281

Husbands D. R., Henkel T. W., Bonito G. & Smith M. E. 2013. — New species of *Xerocomus* (Boletales) from the Guiana Shield, with notes on their ectomycorrhizal status and fruiting occurrence. *Mycologia* 105 (2): 422-435. https://doi.org/10.3852/12-146

Kalyaanamoorthy S., Minh B. Q., Wong W. T. F., Haeseler A. V. & Jermiin L. S. 2017. — Model Finder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587-589. https://doi.org/10.1038/nmeth.4285

Katoh K., Rozewicki J. & Yamada K. D. 2019. — MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20 (4): 1160-1166. https://doi.org/10.1093/bib/bbx108

Kumar S., Stecher G., Li M., Knyaz C. & Tamura K. 2018. — MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35 (6): 1547. https://doi.org/10.1093/molbev/msy096

Magnago A. C., Alves-Silva G., Henkel T. W. & Da Silveira R. M. 2022. — New genera, species, and combinations of Boletaceae from Brazil and Guyana. *Mycologia* 114 (3): 607-625. https://doi.org/10.1080/00275514.2022.2037307

Nguyen L. T., Schmidt H. A., Von Haeseler A. & Minh B. Q. 2015. — IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32 (1): 268-274. https://doi.org/10.1093/molbev/msu300

Rambaut A., Drummond A. J., Xie D., Baele G. & Suchard M. A. 2018. — Posterior summarization in Bayesian phylogenetics using tracer 1.7. *Systematic Biology* 67 (5): 901-904. https://doi.org/10.1093/sysbio/syy032

Trifinopoulos J., Nguyen L. T., Von Haeseler A. & Minh B. Q. 2016. — W-IQ-TREE: a fast-online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44 (W1): 232-235. https://doi.org/10.1093/nar/gkw256

Vilgalys R. & Hester M. 1990. — Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several species of *Cryptococcus*. *Journal of Bacteriology* 172 (8): 4238-4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

White T. J., Bruns T., Lee S. & Taylor J. 1990. — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, *in* Innis M. A., Gelfand D. H., Sninsky J. J. & White T. J. (eds), *PCR Protocols: A Guide to Methods and Applications*. Academic Press Inc., San Diego: 315-322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

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