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Research article

Recollection after 140 years and morpho-molecular re-characterization of Indian *Stromatoneurospora phoenix* (Kunze) S.C. Jong & E.E. Davis (Xylariaceae)

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Abstract: The present communication describes *Stromatoneurospora phoenix*, a stromatic macrofungi which was gathered from southern peninsula, India. We present here the detailed morphological description, illustration and nrITS-based phylogenetic estimations of this species. The species conspecificity with other counter species is also compared in details, recovering the species to Indian mycobiota after more than a century.
Keywords: Indian Botanic Garden - Macrofungi - Phylogeny - Recollection - Tamil Nadu - Taxonomy.

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INTRODUCTION

Mr. Sulpiz Kurz during his service as the Curator (Herbarium) of the Indian Botanic Garden, now renamed as Acharya Jagadish Chandra Bose Indian Botanic Garden (AJCBIBG), not only gathered floral collections but also made notable collections of Fungi from Burma (Myanmar) and various parts of India including the AJCBIBG, Howrah. Later his mycological specimens were identified by Frederick Currey (1874) and many collections of Kurz were established as the type specimens belonging to the AJCBIBG (Type locality) in the due course of time with the advancement of tools and technique. His most collections are preserved at Kew Fungurium (K) and among those collections 'Kurz 2650' (Holotype), which was identified as *Xylaria kurziana* Curr., is very significant and now it is regarded as *Stromatoneurospora phoenix* (Kunze) S.C. Jong & E.E. Davis, after re-examining with the help of SEM studies (Rogers & Ju 1992) and polyphasic taxonomic approach (Becker *et al*. 2020). This species was never recollected from India after the collection of Kurz, though collected from other parts of the globe (Dennis 1957, 1958, Jong & Davis 1973, Rogers *et al*. 1992, Martín *et al*. 1998, Becker *et al*. 2020).

During routine macrofungal surveys of the countryside in Tamil Nadu during rainy seasons (July & August) of 2013–2019, one of us (SP) collected interesting macrofungi belonging to *Xylariaceous* population, growing on the burnt bushes of *Saccharum* sp. in the cultivated fields. However, we failed to gather the specimens from Indian Botanic Garden, Calcutta/AJCBIBG, BSI, Howrah even after several attempts. After thorough macro- and micromorphological observations, ecological studies, consultation of relevant findings (Dennis 1957, 1958, Jong & Davis 1973, Rogers *et al*. 1992, Martín *et al*. 1998, Becker *et al*. 2020) and molecular phylogenetic estimation, some of the collected specimens were assigned as *Stromatoneurospora phoenix*. The reported species is not found in the records of Indian Xylariaceous mycobiota (Bilgrami *et al*. 1991, Sorbhoy *et al*. 1996, Jamaluddin *et al*. 2004, Pande 2008, http://www.cybertruffle.org.uk). A detailed macro- and micromorphology

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along with the nrITS-based phylogeny of this species are also being presented here for the first time from India.

MATERIAL AND METHODS

Morphology

Macromorphological characterizations were carried out in the field in broad daylight with the fresh fruiting bodies. Field photographs of fruiting bodies were taken with the help of Olympus C-5060 (wide zoom) camera. Fresh specimens were macromorphologically characterized in the field and their colour was noted using the Methuen Handbook of Colour (Kornerup & Wanscher 1978) as a guide. After recording the macromorphological characters a part of fruiting bodies were dried in sunlight as well as with a wooden drier while few were surface sterilized and kept separately for culture purpose at CAS Botany.

For micromorphological characterizations, 5% KOH solution was used for softening of tissues, and lactophenol, cotton blue, phloxine and 30% glycerol were used for staining. Melzer's reagent was chosen to observe the ascospores. Micro-morphological observations, measurements, drawings and photography were done under an Olympus CX41 light microscope equipped with a 100× objective (oil immersion), drawing tube, and photographic attachments. Spores were measured randomly from sixty ascospores in side view. Spore sizes are given herein as,

$$\text{Spore sizes} = SPa–(SPc)–SPb \times SPx–(SPz)–SPy$$

Where, SPa = minimum value for the length of the measured collections, SPb = maximum value for the length of measured collections, SPc = mean value for the length of measured collections and SPx = minimum value for the width of measured collections, SPy = maximum value for the width of measured collections, SPz = mean value for the width of the measured collections.

Quotient of spore indicates length/width ratio (Q = L/W) and is given as Q1–(Q3)–Q2 where Q1 = minimum quotient value amongst the measured collections, Q2 = maximum quotient value amongst the measured collections, Q3 = mean quotient value amongst the measured collections. All the specimens were deposited at Central National Herbarium (CAL). Herbarium codes follow Index Herbariorum (Thiers, continuously updated).

Scanning electron microscopy

The fungal sample for SEM analysis was prepared and imaged in their native form without any chemical treatment as described by Venkatesh Babu *et al*. (2018). The fungal spores were directly taken from the fruiting body and spread on to the sterile aluminum foil (4 × 4 mm; L × B) and subjected for simple air drying. The air-dried sample was sputter coated and imaged using Scanning Electron Microscope (Tescan Vega -3 w/EDX, Czech Republic).

Cultural studies

A part of stromata from the collection (SP-17-03) was utilized for culture purpose and after sterilization its internal tissues were transferred into Potato Dextrose Agar (PDA) medium in petri dish and incubated at 25°C. The mycelium growth appeared in and around of inoculated fresh tissue on the second day and petri dishes were found fully covered with mycelium in 9–11 days. The mycelium was white in colour with filamentous cottony consistency. The culture produced synnemeta in petri dish edges after 26 days. Young synnemeta in culture were found branched and light brown in colour.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from the 5 days old fungal culture following the method described by Möller *et al*. (1992). The nrITS gene region was amplified with primer pairs ITS-1F and ITS-4R (White *et al*. 1990). The PCR reaction was carried out in a final volume of 20 µl containing 10 µl 2x Emerald Amp GT PCR Master Mix (Takara, Japan), 10 p mol each of forward (ITS1) and reverse (ITS4) primers and, 80 ng of fungus genomic DNA. The PCR program includes 35 cycles with Initial denaturation at 95°C for 5 min followed by denaturation at 95°C for 1 min, primer annealing at 55°C for 1 min, primer extension at 72°C for 1 min and the final extension at 72°C for 10 min. The amplified product was analyzed on 1.2% agarose gel electrophoresis and stained with Ethidium Bromide. QIAquick Gel Extraction Kit (QIAGEN, Germany) was used to purify the PCR amplified products and then subjected to Sanger sequencing of both strands of PCR fragments in an automated DNA sequencer (ABI3730xl DNA Analyzer, Applied Biosystems, USA) using the same primers. Sequence Scanner Software v. 1 was used to check the sequence quality. Sequence alignment and required editing of the obtained sequences were carried out using Geneious v. 5.1 (Drummond *et al*. 2010). The final consensus sequences were deposited at GenBank to procure the accession numbers: MW227541 and MW227542 for the ITS sequence of *Stromatoneurospora phoenix*.

Sequence alignment and phylogenetic analysis

The nrITS sequences of *Stromatoneurospora phoenix* and outgroups were retrieved from the nBLAST search (Altschul *et al*. 1997), GenBank (Clark *et al*. 2016) and other relevant literature (Becker *et al*. 2020). The nrITS sequences were initially aligned using the online version (https://mafft.cbrc.jp/alignment/software/) of the multiple sequence alignment program MAFFT v. 7 (Katoh *et al*. 2019) using default settings and manually edited with MEGA v. 7 (Kumar *et al*. 2016). To eliminate ambiguously aligned positions in the alignment as objectively as possible, the on-line program Gblocks v. 0.91b (Talavera & Castresana 2007) was used. The program was run with settings allowing for smaller blocks, gaps within these blocks and less strict flacking positions. The single-locus dataset was phylogenetically analysed using the maximum likelihood (ML) method. A ML was carried out using in raxmlGUI v. 2.0 (Edler *et al*. 2021) with the GTRGAMMA substitution model. A ML analysis was executed applying the rapid bootstrap algorithm with 1000 replicates to obtain nodal support values. Branches with bootstrap values (BS) exceeding 50% were considered to be significantly supported.

RESULTS

Phylogeny

Our nrITS based phylogenetic analysis (Fig. 1) with 56 nrITS sequences (including the present species) resolved genus *Stromatoneurospora* with full support. Sequences (GenBank accession numbers: MW227541 and MW227542) derived from Indian collection are nested within the *S. phoenix* clade (indicated in blue arrow) consisting of Thailand (GenBank accession numbers: MT703666, MT703667, MT735133 and MT735134) and Mexican (GenBank accession number: AY909004) collections suggesting its strong similarity or conspecificity with the Asian and Mexican species: *Stromatoneurospora phoenix*.

Taxonomy

Stromatoneurospora phoenix (Kunze) S.C. Jong & E.E. Davis, *Mycologia* 65(2): 459 (1973) [Fig. 2 & 3]

Stromata annual, lignicolous gregarious, caespitose, broadly attached, weakly adnate, 2–8 × 4–13 mm, subglobose to bulbous ends with tapered stalk that deeply anchored on host, texture fleshy when fresh, hard and brittle on drying. Stromata exterior surface papillate due to small size black dotted ostioles, surface rough, pale orange (5A4) to light orange (5A5) when young then umber to apricot with maturity finally turning blackish brown to charcoal black on drying. Stromata interior homogenous, waxy in texture, chalky white (1A1). Perithecia minute, globose to subglobse to ellipsoid, embedded deep in stroma, opens as mamiform ostiole in ascomata surface, black brown.

Perithecia 295–600 × 245–500 µm, wall tissues narrow at base gradually widening towards ostiole; papillae 130–170 µm long, mostly embedded in white and waxy flesh, each perithecia are separated from the each other by up to 50 µm. Asci 8-spored, 118–176 × 9.5–13.0 µm, arranged in a uniseriate manner, cylindrical, clavate, septate at base, smooth, hyaline, spore bearing part 35–41 µm long, cube shaped; apical ring 3.5–4.5 × 1.5–2.0 µm, which turns blue in Melzer's reagent. Paraphyses up to 280 µm long and 6.5 µm wide, hyaline, septate, many, remain among the asci and visible well in young specimens. Ascospores 13–(15.8)–19 × 6–(7.8)–10 µm, Q = 1.49–(2.01)–2.37, fusiform to elliptic fusiform, (sometimes concave in one side), surface ornamented with longitudinal striation, hyaline when young gradually darkening to pale brown then converted into dark olive-brown, acyanophilic, inamyloid.

Specimens examined: India, Tamil Nadu, Vellore district, Nemeli Taluka, Mettu-Kunnathur village, 116 m, N 13º 2'13″ E 79° 31'11″, growing on half burnt *Saccharum officinarum* L. stalk, 15.12.2017, *S. Pichai*, SP-17-03 (CAL 1841); ibid., Nemeli Taluka, Mettur village, 118 m, N 13° 06'13″ E 79º 25'43″, growing on half burnt *Saccharum officinarum* L. stalk and half burnt *Phoenix sylvestris* (L.) Roxb., 04.11.2018, *S. Pichai*, SP-18-016 (CAL 1842).

Note: Morphological sketches like stromatic nature of fruiting bodies, brown-coloured longitudinally striated ascospores without any germ slits lying within stipitate cylindrical asci with amyloid apical apparatus (Jong & Davis 1973, Becker *et al*. 2020) make *Stromatoneurospora* unique within the family Xylariaceae. Micromorphological characters of Indian *Stromatoneurospora phoenix* such as: 3–5 septate tapered paraphysis (200–325 × 5.0–7.5 µm), unitunicate eight spored cylindrical asci (150–200 × 7.5–12.5 µm) and ellipsoid-fusiform shaped ascospores (13–19 × 6–10 µm) fully agrees its conformity with that of its selected localities like Surinam, Mexico, Thailand, Vietnam and Australian counterparts (Jong & Davis 1973, Martín *et al*. 1998, Becker *et al*. 2020) but few characters like absence of stalk or present as rudimentary form and host *Saccharum* sp. and *Phoenix sylvestris* (type burnt soil) are observed as acceptable variability in the present Indian collections. Moreover, our nrITS-based phylogeny strongly shows the conspecificity of our

Indian collections (represented by MW227541 and MW227542) to *Stromatoneurospora phoenix*, the Thailand and Mexican counterparts (Fig. 1).

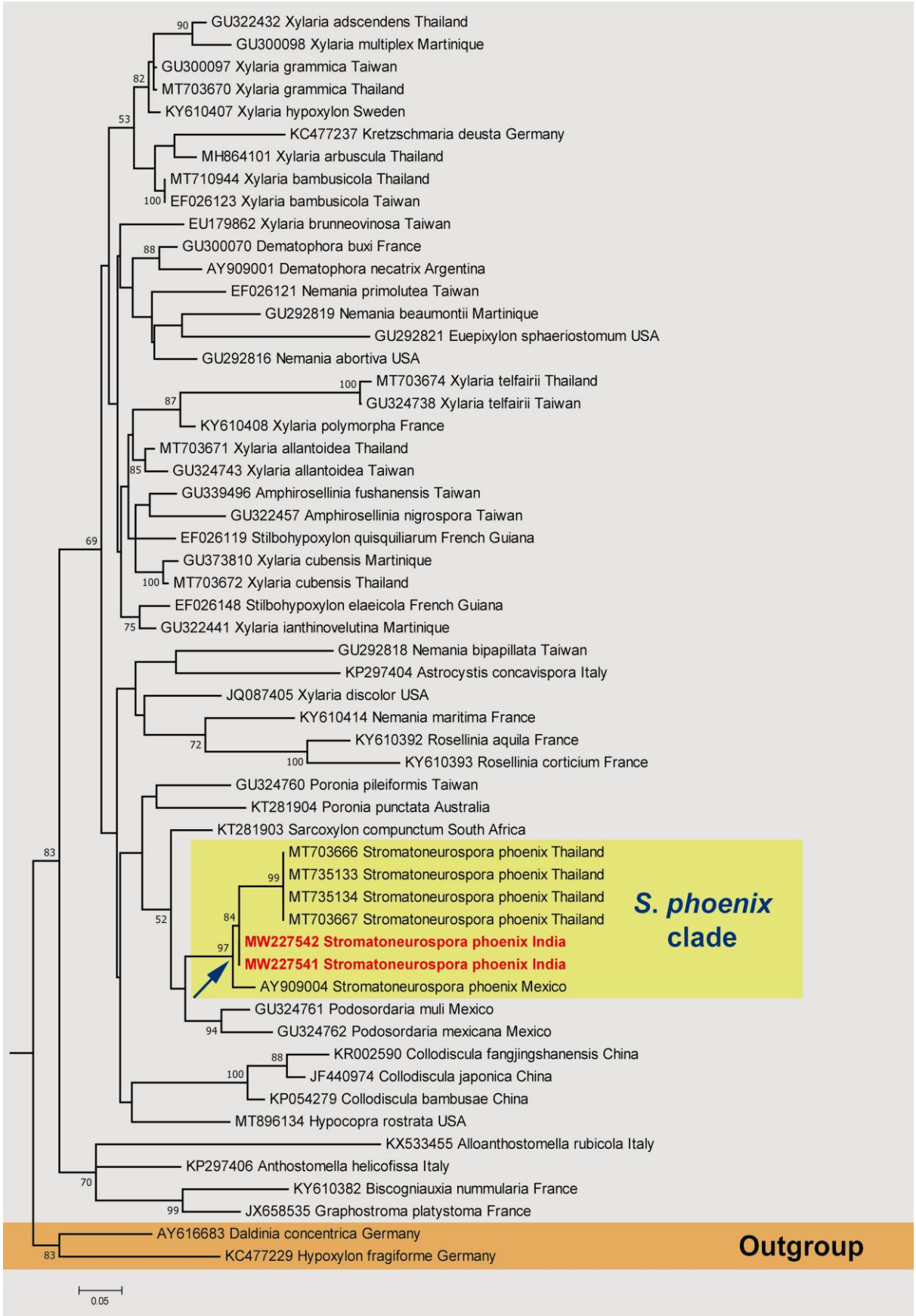

Figure 1. Phylogram generated from nrITS-DNA sequences: The evolutionary history was inferred by using the Maximum Likelihood (ML) method in raxmlGUI v. 2.0. Bootstrap support values (>50%) obtained from the ML analysis are shown above or below the branches at nodes.

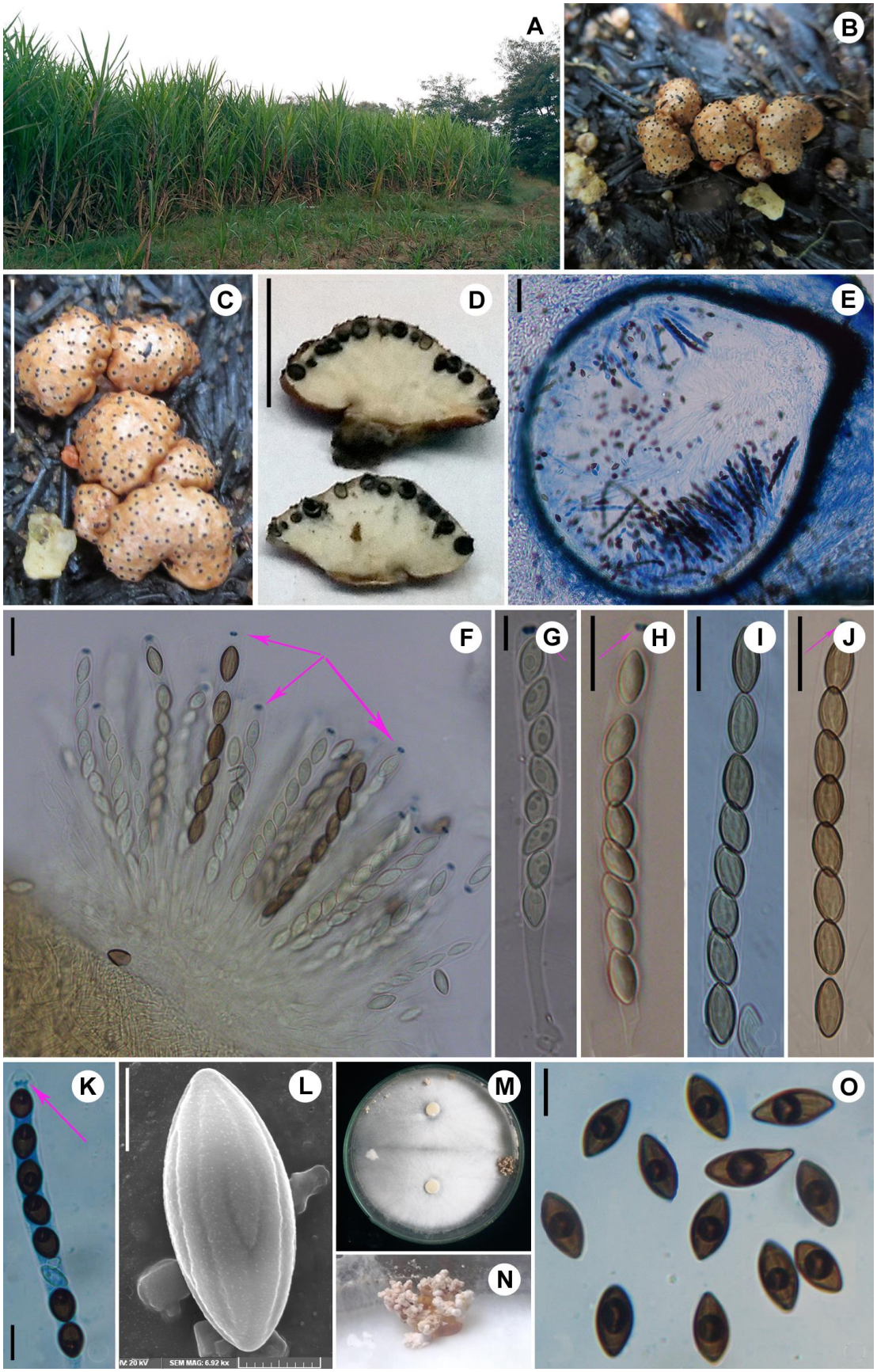

Figure 2. *Stromatoneurospora phoenix* (Kunze) S.C. Jong & E.E. Davis: **A**, Habitat; **B–C**, Habit showing stromata with details of surface and ostioles; **D**, Section through stromata showing arrangement of perithecia in stromatal tissue; **E**, Enlarged perithecia; **F**, Arrangement of Asci within perithecia; **G–J**, Developing Ascospore within Asci initially hyaline than yellowish brown (in Melzer's; Arrow showing apical apparatus changing into blue); **K**, Ascospores within Asci (in Cotton Blue); **L**, SEM image of Ascospore; **M**, 29 days old culture on PDA; **N**, Young synnema in PDA culture; **O**, Matured olive brown ascospores under light microscope. [Scale bars: C–D= 5 mm; E= 50 µm; F= 20 µm; G–K= 10 µm; O= 10 µm; L= 5 µm]

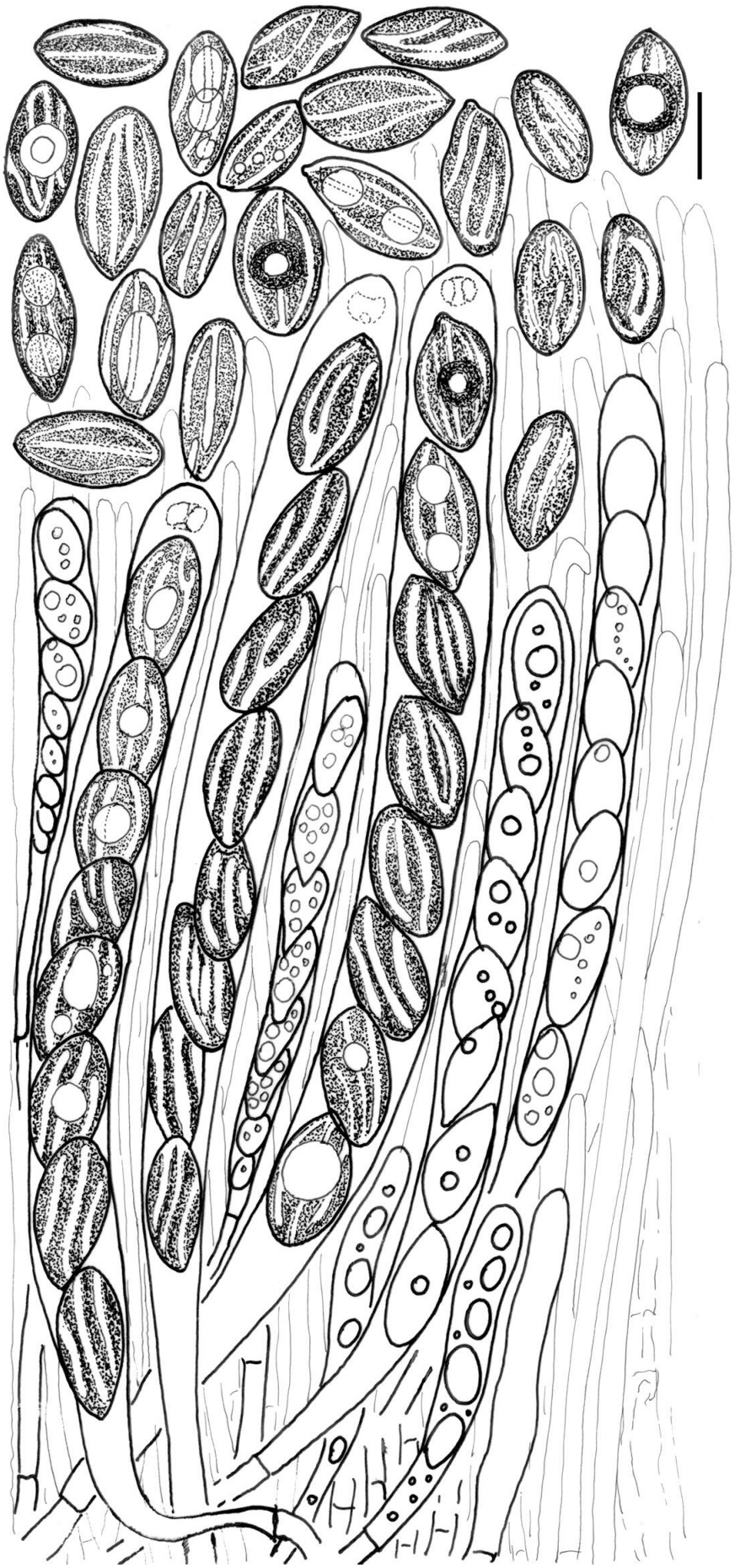

Figure 3. Arrangement of Ascospores within Asci and protective Paraphyses and details of Ascospores in *Stromatoneurospora phoenix* (Kunze) S.C. Jong & E.E. Davis. [Scale bar: 10 µm]

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REFERENCES

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W & Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389–3402.

Becker K, Wongkanoun S, Wessel A-C, Bills GF, Stadler M & Luangsa-ard JJ (2020) Phylogenetic and Chemotaxonomic Studies Confirm the Affinities of *Stromatoneurospora phoenix* to the Coprophilous Xylariaceae. *Journal of Fungi* 6(3): 144.

Bilgrami KS, Jamaluddin S & Rizwi AM (1991) *Fungi of India*. *Part III (List and References)*. Today & Tomorrow's Printers & Publishers, New Delhi, India, 798 p.

Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J & Sayers EW (2016) GenBank. *Nucleic Acids Research* 44: D67–D72.

Currey F (1874) On a Collection of Fungi made by Mr. Sulpiz Kurz, Curator of the Botanic Garden, Calcutta. *Transactions of the Linnean Society of London, 2nd Series, Botany* 1: 119–131.

Dennis RWG (1957) Further notes on tropical American Xylariaceae. *Kew Bulletin* 12: 297–332.

Dennis RWG (1958) Some Xylosphaeras of tropical Africa. *Revista de Biologia, Lisboa* 1: 175–208.

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Heled J, Kearse M, Moir R, Stones-Havas S, Sturrock S, Thierer T & Wilson A (2010) Geneious v. 5.1. Available from https://www.geneious.com

Edler D, Klein J, Antonelli A & Silvestro D (2021) Raxml GUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12: 373–377.

Jamaluddin S, Goswami MG & Ojha BM (2004) *Fungi of India 1989–2001*. Scientific Publishers, Jodhpur, India, 326 p.

Jong SC & Davis EE (1973) Stromatic neurosporas. *Mycologia* 65: 458–464.

Katoh K, Rozewicki R & Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20(4): 1160–1166.

Kornerup A & Wanscher JH (1978) *Methuen handbook of colour, 3rd Edition*. Methuen, London, 252 p.

Kumar S, Stecher G & Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33(7): 1870–1874.

Martín FS, Rogers JD & Ju Y-M (1998) Clave dicotómica para losgéneros de la familia Xylariaceae (Pyrenomycetes, Sphaeriales) de México. *Acta Botanica Mexicana* 42: 35–41.

Möller EM, Bahnweg G, Sandermann H & Geiger HH (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Research* 20(22): 6115–6116.

Pande A (2008) *Ascomycetes of Peninsular India*. Scientific Publishers, Jodhpur, India, 584 p.

Rogers JD & Ju Y-M (1992) *Hypoxylon rectangulosporum* sp. nov., *Xylaria psidii* sp. nov. and comments on taxa of *Podosordaria* and *Stromatoneurospora*. *Mycologia* 84(2): 166–172.

Rogers JD, Ju Y-M & Hemmes DE (1992) *Hypoxylon rectangulosporum* sp. nov., and comments on taxa of *Podosordaria* and *Stromatoneurospora*. *Mycologia* 84: 157–165.

Sorbhoy AK, Varshney JL & Agarwal DK (1996) *Fungi of India (1982–92)*. CBS Publishers & Distributors, New Delhi, India, 350 p.

Talavera G & Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* 56: 564–577.

Venkatesh Babu G, Perumal P, Muthu S, Pichai S, Narayan KS & Malairaj S (2018) Enhanced method for High Spatial Resolution surface imaging and analysis of fungal spores using Scanning Electron Microscopy. *Scientic Reports* 8: 16278.

White TJ, Bruns T, Lee SS & Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ & White TJ (eds.) PCR Protocols: a guide to method and applications. Academic Press, San Diego, pp. 315–322.