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Notes on Two New Records of Mycobiota in Japan, *Kostermansinda nana* and *Durella macrospora*

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Abstract Two new records of mycobiota in Japan were documented, i.e. *Kostermansinda nana* and *Durella macrospora.* While the latter clearly belongs to Helotiales, the phylogenetic position of the former was not clear based on BLAST search on ITS-5.8S, LSU, mtSSU, RPB1, RPB2, and EF1a.

Key words: barcode, *Durella macrospora*, Japan, *Kostermansinda nana*, mycobiota, taxonomy.

Introduction

In spite of continuous attempts in elucidation of mycobiota in Japan, a number of taxa have been remained unreported. In the present paper, we documented two new records of fungi scarcely known. To increase the value of their occurrence data, we also added genetic information for the future phylogenetic analysis.

Materials and Methods

For the isolation of *Kostermansinda nana*, single spore isolation method using Skerman's manipulator (Skerman, 1968) was used. Isolate of *Durella macrospora* was obtained from multispores discharged onto the agar as previously described (Itagaki *et al.*, 2019). Isolates were deposited in Biological Resource Center, National Institute of Technology and Evaluation (NITE-BRC, Tokyo, Japan, https://www.nite.go.jp/en/nbrc/cultures/index.html). Extracted DNA were deposited to the Center for Molecular Biodiversity Research, National Museum of Nature and Science. Coordinates followed WGS84 datum.

Extraction of DNA, PCR and sequencing followed Iatagaki *et al.* (2019). The following primer sets were used. For internal transcribed spacers (ITS1 and ITS2) and 5.8S ribosomal region (ITS-5.8S), ITS1 and ITS4 (for *Kostermansinda nana*) or ITS1Fand ITS4 (for *Durella macrospora*) (White *et al.*, 1990); for large subunit of ribosomal RNA, LR0R and LR5 (Vilgalys and Hester, 1990); for mitochondrial small subunit of ribosomal RNA, mrSSU1 and mrSSU3R (Zoller *et al.*, 1999); for DNA-directed RNA polymerase II subunit RPB1, RPB1-AF and RPB1-CR (Castlebury *et al.*, 2004); for partial sequence of the second largest DNA-directed RNA polymerase II subunit (RPB2), RPB2-P6F and RPB2-P7R (Liu *et al.*, 1999); for partial sequence of elongation factor 1 (EF1a), EF1-983F and EF2218R (Rehner and Buckley, 2005).

BLAST search was conducted based on the obtained sequences using the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PRO

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GRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome, visited on March 15, 2020). Only sequences matched with E = 0.0 and higher percent identity value were selected for discussion.

Results and Discussion

Descriptions

1. **Kostermansinda nana** (J.N.Kapoor & Munjal) G.Z.Zhao [as '*nanum*'], Journal of Shandong Agricultural University 34(3): 437. 2003. [Fig. 1]

≡ *Sclerographium nanum* J.N.Kapoor & Munjal, Indian Phytopath. 19(4): 352. 1967 [1966].

= *Kostermansinda minima* Cabello & Aramb., in Arambarri, Cabello & Mengascini, Mycotaxon 29: 32. 1987.

Mycelia immersed in the substrate, thick-walled, multi-septate, brown, forming synnemata. **Synnemata** upright, 200–300 × 20–40 µm, composed of dark-brown, multi-septate, smooth, unbranched hyphae of 2–3 µm wide. **Conidiophores** macronematous, thick-walled. **Conidiogenous cells** monoblastic, integrated, terminal, determinate. **Conidia** 20–30 × 12–15 µm, solitary, acrogenous, broadly ellipsoidal clavate, muriform, typically composed of three rows and 3–5 levels of cells with pale to dark-brown wall, arranged on one smaller hyaline basal cell. Germination occurs while the conidia are still on the conidiophores, and detached conidia germinate readily on artificial agar media.

Specimens examined. TNS-F-24817 (culture FC-1894 = NBRC 112551), 24818, and 24819 on bark of unidentified tree, Myoriki-ji, Iryuda, Odawara-shi, Kanagawa Pref. 2009-III-20. col. T. Hosoya; TNS-F-25226 and 25227 on unidentified log, Takizawa-en, Tanzawa, Kanagawa Pref.

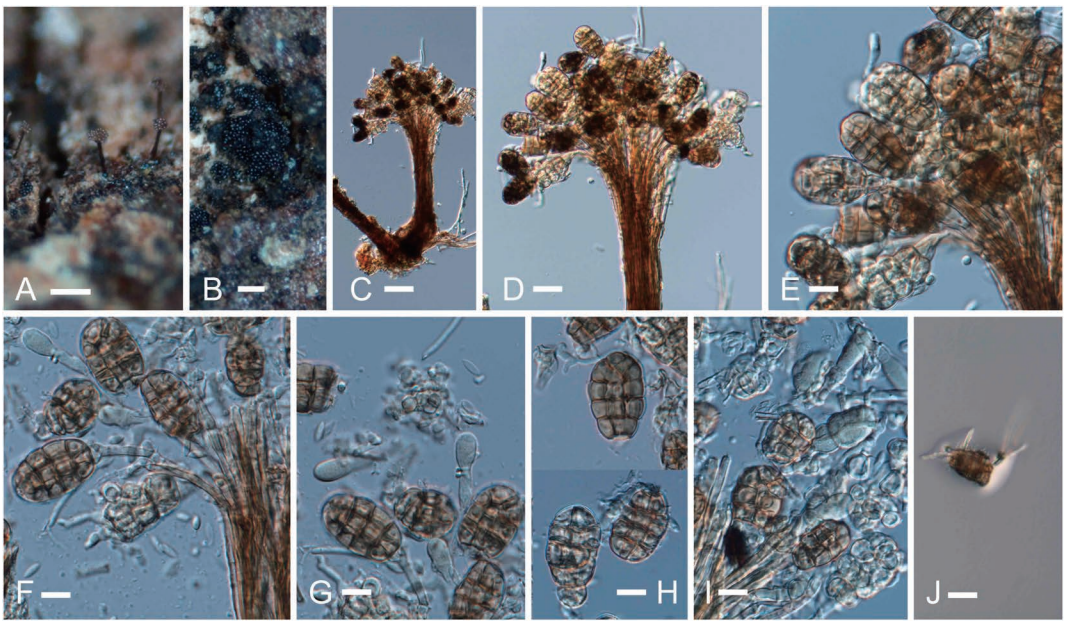

Fig. 1. *Kostermansinda nana* (TNS-F-24817). A. Synnemata. B. Spores detached and scattered on the substrate. C. Synnnemata connected with other synnema at the basal hyphae. D. Close up of the head apical region of the synnema. E. Close up of the head of synnema with a cluster of conidia. F. Crush mount of the conidia producing part. Note that the conidia are produced at the end of brown hyphae. G. Conidia and conidiogenous cells with terminal enlargement. H. Detached conidia, with globular, hyaline cell at the base. I. Juvenile conidia and end of conidiogenous cells. J. Germinating conidium on agar mediuma (potato dextrose agar). Scales: A–B = 100 µm, C–I = 10 µm, J = 20 µm

(35.398717, 139.183162), Oct., 1989. col. T. Hosoya; TNS-F-37038 (culture FC-2752 = NBRC 109679) on bark of living tree, Jinmuji, Zushi-shi, Kanagawa Pref., (33.43705, 132.637803), 2010–X–11. col. M. Yano.

Sequences registered. LC534830 (ITS-5.8S), LC534831 (LSU), LC535799 (mtSSU), LC535800 (RPB1), LC535801 (RPB2), and LC535802 (EF1α) obtained from NBRC 112551

Notes. Kostermansinda is a relatively newly described genus, containing small number of species. *Kostermansinda nana* has been collected several times in Japan, and previously known as *K. minima*, but no description was provided. The fungus has been known from Argentina (Arambarri *et al.*, 1987), India (Bhat, 2006), Taiwan (Chang, 1989) and Thailand (Kodsueb *et al.*, 2008), and this is the first occurrence report of *Kostermansinda* in Japan.

The phylogenetic position of *Kostermansinda* has remained unresolved, and it is disposed in "Ascomycota" in Index Fungroum (http://www.indexfungorum.org/names/Names.asp, visited on March 10, 2020), and inc. sedis in Wijayawardene *et al.* (2020). In the present study, BLAST search based on several genes are presented as follows (higher taxonomy followed Wijayawardene *et al.* 2020).

When ITS-5.8S was used for BLAST search, the only genus specified with E = 0.0 was *Entrophospora infrequnentan* (Glomeromycota) NY349 (U94714) with Max score 662, Query coverage 96%, and %Ident. 93.89% which is too low to evaluate taxonomic position based on the similarity.

LSU provided more genera as candidates in E = 0.0, but no genus was specified for %Ident ＞90%. Below %Ident ＜90%, *Funbolia* (Eriomycetaceae, order unknown), *Phellinocrescentia* (Eriomycetaceae, order unknown), *Tuckermanopsis* (listed as orthologous variant *Tuckermannopsis*; Parmeliaceae, Lecanorales), *Hypogymnia* (Parmeliaceae, Lecanorales), *Satchmopsis* Cochlearomycetaceae, Leotiales?), *Pleurosticta* (Parmeliaceae, Lecanorales), *Bryoria* (Parmeliaceae), *Bryocaulon* (Parmeliaceae), and *Heleiosa* (Eriomycetaceae, order unknown) were listed in this order.

For mtSSU, *Anisomeridium* sp. (Monoblastiaceae, Monoblastiales, Dothideomycetes) isolate e4012523987 (MH028639; Max score 710, Query coverage 97%, and %Ident. 83.78%), followed by *Megalotremis verrucosa* (Monoblastiaceae) (GU327694; 710, 86%, and 85.80%), *Trypetheliopsis kalbi* (Monoblastiaceae) (GU327703; 652, 86%, and 84.27%) were listed in the range of E = 0.0.

For RPB1, no matches were obtained with E = 0.0.

For RPB2, *Wiesneriomyces javanicus* (Wiesneriomycetaceae, Tubeufiales) isolate YMF 1.04036 (MK267285; 726, 94%, and 84.01%), *W. javanicus* isolate YMF 1.04044 (MK267286; 721, 94%, and 83.82%) were obtained.

For EF1α, many matches were obtained, but no match was presented for more than ＞90%Ident value. The highest match showed *Dictyocheirospora* (Dictyosporiaceae, Pleosporales).

The above results are a bit confusing, but allow preliminary consideration about the position of this mysterious fungus. Some of the BLAST search result suggest the phylogenetic relationship of *K. nana* with Dothideomycetes. This suggestion is also supported by brown to black, dictyosporous conidia often observed in this class.

Another possible interpretation brings up a doubt for the correct identification of the registered sequences. BLAST search listed some lichen members (members of Lecanorales). These sequences may have been originated from lichenicolous Dothideomycetes. The latter possibility can be eliminated by incorporating multiple sequences resulted from multiple specimens or examination of voucher specimen. However, due to the paucity of the genomic data for allied taxa, further analysis was not conducted.

2. **Durella macrospora** Fuckel, Jb. nassau. Ver. Naturk. 23–24: 281. 1870 [1869–70].

[Figs. 2, 3]

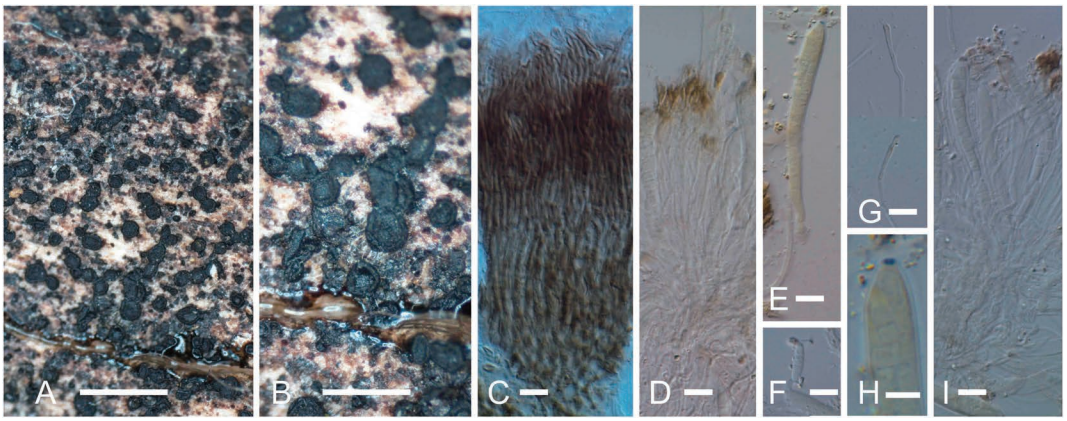

Fig. 2. *Durella macrospora* (TNS-F-61703). A–B. Dried specimens. C. Surface of ectal excipulum in crush mount. Note upper portion composed of undulating hyphae and lower portion composed of larger, thicker-walled cells. D. Brown epitheical structure. E. Ascus, F. Ascospore. G. Paraphyses apex, H. Ascal apex, I. Asci and undulating paraphyses in crush mount. Scales: A = 1 mm, B = 0.5 mm, C = 20 µm, D–I = 10 µm.

Apothecia superficial, gregarious, sessile, turbinate to shallowly concave, totally black when fresh, drying shallow to flat, attached to the neighboring apothecia, 120–250 µm in diameter. **Ectal excipulum** textura intricata, composed of undulating hyphae with elongate cells with brown-colored wall with thin, fewer septa 2–4 µm wide; wall becoming thicker toward the margin ending up in somewhat clavate terminal cell; cells shorter, thicker-walled and more prismatic (10–25 × 5–8 µm, with 1 µm thick wall) toward the base. **Asci** 60–88 × 7–9 µm, cylindrical clavate, arising from croziers; apex rounded; pore stained faintly in Melzer's reagent without 3% KOH pretreatment, greatly enhanced by 3% KOH pretreatment. **Ascospores** 10–19 × 3–4 µm (13.47 ± 2.24 × 3.37 ± 0.44 µm in average ± sd, n = 15), biseriate or biseriate only in upper region; clavate or ellipsoid, often narrower toward the pointed base, aseptate to three-septate. **Paraphyses** filiform, 1.5–2 µm wide at the base, undulate, simple or branched at the base or apical region, thicker-walled and enlarged toward the apex, up to 5 µm wide, brown-colored at the apex; almost the same length or slightly exceeding the asci, exudating brown, resinous matter forming epithecium.

Japanese name. Ko-kuro-sara-byotake

Specimen examined. TNS-F-61703 (culture FC-5679 = NBRC 114601), on unidentified wood, Renkoji, Tama-shi, Tokyo (35.636111, 139.4600), 2015-II-12. col. M. Nakajima.

Sequences registered. LC534832 (ITS-5.8S)

Notes. This is the first occurrence report of *Durella* in Japan. It is previously known mainly from Europe and America but so far no records from Asia (GBIF, https://www.gbif.org/, visited on April 25, 2020). Overall morphology agrees with *Durella connivens* (Fr.) Rehm (Hansen and Knudsen, 2000; Medardi, 2004; Luis *et al.*, 2017), but differs in the morphology of paraphyses and MLZ positive asci, and agrees with morphology of *D. macrospora* (Medardi, 2004). The BLAST search based on ITS-5.8S region shows almost perfect match with two sequences for *D. macrospora* [*D. macrospora* voucher G.M. 2015-04-05-4 (KY462813; Max score 1064, Query coverage 100%, and %Ident. 99.32%) and *D. macrospora* voucher H.B. 9974 (KY462812; 1064, 100%, 99.32%)]. When considering the 98.5% identify species limit (Nilsson *et al.*, 2012). The present specimen matches well with the previously reported sequences, and shows no doubt for species identity on molecular basis. However, some contradiction with the previous description given by Dennis (1956) are noted.

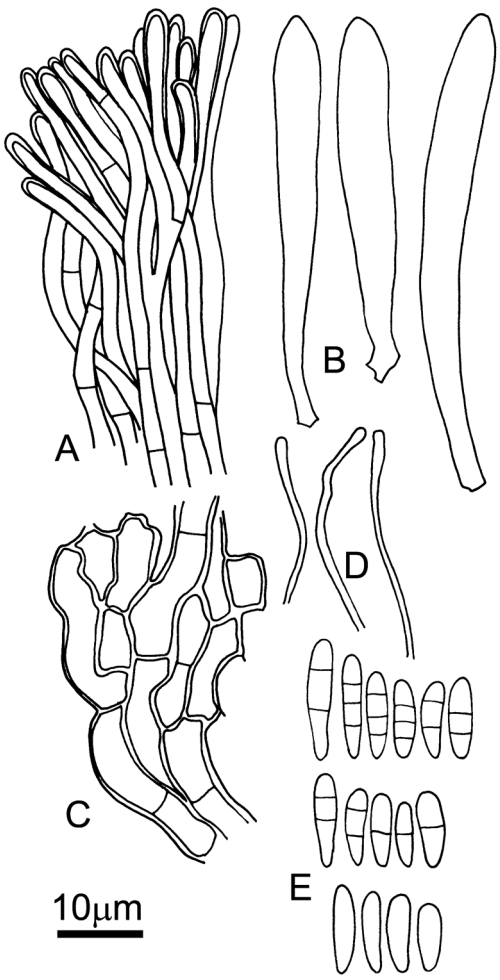

Fig. 3. *Durella macrospora* (TNS-F-61703). A. Marginal region of ectal excipulum in crush mount. B. Asci. C. Lower part of ectal excipulum in crush mount. D. Paraphyses. E. Ascospores.

Dennis (1956) described the asci as non-amyloid while our data and both data stored in NCBI shows amyloid reaction (https://www.ncbi.nlm.nih.gov/nucleotide/KY462813.1?report=genbank&log$=nuclalign&blast_rank=1&RID=6V48DVJS016; https://www.ncbi.nlm.nih.gov/nucleotide/KY462812.1?report=genbank&log$=nuclalign&blast_rank=2&RID=6V48DVJS016, accessed: 10 March 2020). Because the MLZ reaction is so faint, it is highly possible that Dennis (1956) overlooked the reaction. The illustrations and description of paraphyses is more branched, and more swollen in Dennis (1956) as compared with the present specimen (Fig. 3D). Nevertheless, we identified this species based on the high similarity of the barcoding region.

Johnston *et al.* (2019) included *D. macrospora* in their phylogenetic analysis of Leotiomycetes, and demonstated its close relationship with Arachnopezizaceae.

Baral (2016) placed the genus *Durella* in *Strossmayeria* lineage, next to Mollisiaceae, but Baral (2016) also pointed out the morphological differences between Mollisiaceae. We also agree with the morphological similarity of *Durella* in *Strossmayeria*, in particular ectal excipular structure, ascospores and paraphyses.

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