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Scleroderma capeverdeanum, a subhypogeous fungus new to Japan, collected from the *Eucalyptus* plantation in Ibaraki Prefecture

茨城県のユーカリ植栽地において採集された日本新産の半地下生菌，*Scleroderma capeverdeanum*

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Abstract

A subhypogeous sclerodermataceous fungus was collected in the *Eucalyptus* plantation of Ibaraki Prefecture, central Japan. Based on morphological observations and phylogenetic analyses using nuclear ribosomal DNA sequences, the present fungus was identified as *Scleroderma capeverdeanum*, belonging to Sclerodermataceae (Boletales). *Scleroderma capeverdeanum* is new to Japan and characterized by small-sized (4–10 mm in diam.), sessile basidiomata, yellowish to yellowish brown exoperidium with brownish, areolate to verrucose scales, and echinulate basidiospores. Japanese specimens of *S. capeverdearnum* are probably associated with introduced *E. globulus*, and therefore they are most likely exotic species introduced from Australia.

要旨

茨城県のユーカリ植栽地において、ニセショウロ型の半地下生菌の一種が採集された。本菌について、形態的特徴の観察および子実体より得られた核リボソームDNAの塩基配列を用いた系統解析を行った。その結果、本菌はイグチ目ニセショウロ科に属する *Scleroderma capeverdeanum* と同定された。本種は日本新産であり、子実体が小型（直径 4–10 mm）で無柄である点、外皮が黄色から黄褐色で、網目状からいぼ状にひび割れ、褐色を帯びた鱗片を有する点、そして担子胞子が針状突起に覆われる点により特徴づけられる。日本産標本は *Eucalyptus globulus* と共生し、オーストラリアからの外来種であると推察される。本菌の和名をユーカリキヒメニセショウロとする。

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Introduction

The genus *Scleroderma* Pers. is currently classified in the order Boletales based on molecular phylogenetic analyses (Binder & Bresinsky, 2002; Binder & Hibbett, 2006; Watling, 2006). Previously, ca. 40 species of the genus were recognized from tropical, subtropical to temperate regions of the world (Baseia et al., 2016; Guzmán, 1970; Guzmán et al., 2013; Nouhra et al., 2012; Ortiz-Rivero et al., 2021; Zhang et al., 2013, 2020). In Japan, fifteen species of *Scleroderma* were hitherto recorded (Kasuya et al., 2002; Kasuya & Guzmán, 2007; Yoshimi, 2002). The genus forms epigeous, subhypogeus or hypogeous basidiomata (Beaton & Weste, 1982; Giachini et al., 2000; Guzmán, 1970; Guzmán et al., 2013; Nouhra et al., 2012), and many species have been confirmed as ectomycorrhizal with a wide range of host plants such as Pinaceae, Fagaceae, Salicaceae and Myrtaceae (Mrak et al., 2017; Nouhra et al., 2012). Japanese species of *Scleroderma* were usually observed under betulaceous, fagaceous or pinaceous trees (Kasuya et al., 2002; Kasuya & Guzmán, 2007; Yoshimi, 2002).

Some species of *Eucalyptus* (Myrtaceae) were introduced from Australia to Japan early in the 20th century to meet the country's demand for pulpwood (Shibata & Yoshikawa, 1956). Recently, to supply the demand for gardening, greening, and feeds of Koala which are kept at zoos, several plantations of *Eucalyptus* spp. are maintained in Japan (Inuma et al., 2015; Komaki et al., 2000). *Eucalyptus* is an ectomycorrhizal tree genus and several species of *Scleroderma* have been known as its ectomycorrhizal symbionts (Bashir & Khalid, 2015; Beaton & Weste, 1982; Giachini et al., 2000; Rose et al., 1981). However, only two species, i.e., *S. cepa* Pers. and *S. flavidum* Ellis & Everh. have previously been recorded in exotic *Eucalyptus* plantations in Japan (Yoshimi, 2002). During the course of our recent studies of macrofungal diversity at the *Eucalyptus* plantation in Ishioka of Ibaraki Prefecture, central Honshu, Japan, three specimens of a subhypogeous sclerodermataceous fungus were collected. Based on morphological observations and phylogenetic analyses using nuclear ribosomal DNA sequences, the present fungus was identified as *Scleroderma capeverdeanum* M.P. Martín, M. Dueñas & Telleria. This species was originally described from Cape Verde, Macaronesia, off the coast of northwestern Africa (Crous et al., 2016) and it is known only from the type locality. Our morphological observations and phylogenetic analyses of the Japanese specimens revealed that it is the second distributional record of *S. capeverdeanum* in the world. Moreover, our study demonstrates that *S. capeverdeanum* is presumably associated with *Eucalyptus* spp. In this paper, morphological, phylogenetic and ecological characteristics of Japanese specimens of *S. capeverdeanum* are discussed.

Materials and methods

Sample collecting and morphological observations

Fresh subhypogeous basidiomata were collected among plant debris and rich soil in the exotic *Eucalyptus* plantation dominated by *E. globulus* Labill. at Otsuka, Ishioka, Ibaraki Prefecture (Fig. 1) during October to December, 2020. Specimens were photographed and observed macroscopically. Fresh basidiomata of specimens were dried using a food dehydrator (Snackmaster Express FD-60; Nesco/American Harvest, Milwaukee, WI, USA) under 46 °C for 46 hours. For light microscopy, hand-cut sections of both fresh and dried specimens were mounted in water, 3% KOH or 70% ethanol reagent. Dimensions of basidiospores were measured from water-mounted sections. More than 50 randomly selected basidiospores were measured under a light microscope at 1000× magnification. All

Fig. 1. Habitat in the locality of *Scleroderma capeverdeanum*. A: *Eucalyptus globulus* trees. B: Numerous fallen leaves and old bark of *E. globulus* are densely deposited on the ground.

measurements were performed with Photoruler 1.1.3 (http://inocybe.info/_userdata/ruler/PhotoRuler.html). In addition, the surface features of basidiospores were observed by scanning electron microscopy (SEM). For SEM, a small portion from glebal tissue was put onto double-sided adhesive tape on a specimen holder and coated with platinum-palladium using a JFC-1600 Ion Sputter Coater (JEOL, Tokyo, Japan). Specimen was examined with a JSM-6480LV SEM (JEOL, Tokyo, Japan) operating at 5 kV. Three specimens examined in this study were deposited at mycological herbaria of Ibaraki Nature Museum (INM) and National Museum of Nature and Science (TNS) in Japan.

DNA preparation, PCR and sequencing

DNA extraction, PCR and DNA sequencing were carried out according to the methods introduced by Kasuya et al. (2012). First, small fragments of glebal tissue from freshly collected samples were soaked in DMSO buffer (Seutin et al., 1991) with the addition of 100 mM Tris-HCl (pH 8.0) and 0.1 M sodium sulfite (Na_2SO_3) at 4 °C, following the procedures of Hosaka (2009), Hosaka & Castellano (2008), and Hosaka et al. (2010). DNA of the above specimens was extracted from the tissue fragments stored in DMSO buffer. DNA extractions used the modified CTAB extraction followed by glass milk purification methods as summarized by Hosaka (2009) and Hosaka & Castellano (2008). DNA sequence data were obtained from the nuclear ribosomal ITS region and a part of LSU. For amplifying the ITS region, the primer combination of ITS5 and ITS4 (White et al., 1990) was used. For amplifying the LSU, the combination of LR0R and LR5 (Vilgalys & Hester, 1990) was used. PCR were carried out using 20 µl reaction volume, each containing 1 µl genomic DNA, 1 µl dNTP (4 mM), 1 µl each primer (8 µM), 0.5 units Taq polymerase (Takara Bio Inc., Tokyo, Japan), 2 µl $MgCl_2$ (25 mM), and 2 µl bovine serum albumin (BSA). Cycling parameters for ITS region and LSU followed Kasuya et al. (2012). PCR products were electrophoresed in 1% agarose gels stained with ethidium bromide and visualized under UV light. When amplification bands were confirmed, PCR products were then purified using the ExoSap-IT (Millipore, Molsheim, France) and directly sequenced using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Norwalk, CT, USA), following the manufacturer's instructions. A total of two newly generated sequences from this study were deposited in GenBank (Table 1).

Phylogenetic analyses

Newly generated ITS and LSU sequences from the Japanese specimen were used for the phylogenetic analyses. Additionally, 42 ITS and 26 LSU sequences of sclerodermataceous fungi were retrieved from the NCBI GenBank databases (https://www.ncbi.nlm.nih.gov/) and included in the analyses (Table 1). DNA sequences were initially aligned using MUSCLE v.3.6 (Edgar, 2004a, b), followed by manual alignment in the data editor of BioEdit ver. 7.0.1 (Hall, 1999). A total of 36 ITS and 89 LSU nucleotide positions were respectively excluded from the analyses because of the presence of ambiguously aligned regions. Phylogenetic analyses were performed independently for ITS and LSU sequences under maximum parsimony (MP) and maximum likelihood (ML) criteria. MP analyses were conducted under the equally weighted parsimony criterion using PAUP version 4.0b10 (Swofford, 2002). The analyses were performed using the heuristic search option with tree-bisection-reconnection (TBR) and MULTREES option, and 1000 replicates of random addition sequence were conducted. Support for the individual nodes was tested by means of bootstrap analysis (Felsenstein, 1985) under the equally weighted parsimony criterion. A bootstrap analysis was based on 10,000 bootstrap replicates using the heuristic search option with TBR and MULTREES options with ten random additional sequences. ML analyses were performed using MEGA X (Kumar et al., 2018) after testing the best models. According to the lowest BIC (Bayesian Information Criterion) scores, Kimura 2-parameter (Kimura, 1980) with gamma distributed rate heterogenetic and a proportion of invariant sites (K2+G+I) and Tamura-Nei (Tamura & Nei, 1993) with gamma distributed rate heterogenetic and a proportion of invariant sites (TN93+G+I) were chosen as the optimal substitution models for the analyses of the ITS and LSU datasets, respectively. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. For the ML analyses, clade robustness was assessed using a bootstrap analysis with 1000 replicates. Sequences of *Pisolithus arhizus* (Scop.) Rauschert were selected for outgroups, which were strongly supported as the sister of the major clade containing the genus *Scleroderma* (Baseia et al., 2016; Binder & Hibbett, 2006; Hosaka, 2009; Phosri et al., 2009).

Results

Morphological observations

Japanese specimens were morphologically identical to *S. capeverdeanum* in their sessile, small-sized basidiomata (Fig. 2A–B), yellowish to yellowish brown exoperidium covered by brownish, areolate to verrucose scales (Fig. 2B–C), and globose, densely

Table 1. Specimen identification, voucher specimen and/or isolate numbers, origin and GenBank accession numbers for ITS and LSU sequences used for the present phylogenetic analyses

Species names	Herbarium voucher; isolate	Origin	GenBank accession no.		References
			ITS	LSU	
Pisolithus arhizus	Wat.139161; 92fbisPISOLI	Italy	FR748132	n/a	Phosri et al. (2012)
P. arhizus	588; n/a	n/a	n/a	AF336262	Binder & Bresinsky (2002)
Scleroderma areolatum	n/a; O3C_4	USA: New York	JX030282	n/a	n/a
S. areolatum	n/a; Scl_1-1-Qm1H	USA: New York	JX030288	n/a	n/a
S. areolatum	AWW211; n/a	USA	n/a	EU718149	Wilson et al. (2011)
S. aurantium	n/a; 8_5	China: Sichuan	HM237174	n/a	n/a
S. aurantium	HB#E02; HBS2	Pakistan	KF802172	n/a	Bashir & Khalid (2015)
S. bermudense	BZ3961; n/a	Belize	n/a	DQ644137	Louzan et al. (2007)
S. bermudense	n/a; BA 06.04.22	Guadeloupe	FR682092	n/a	Sene et al. (2015)
S. bovista	MG061001_01; n/a	Germany: Thuringia	JQ669943	n/a	Gube & Dörfelt (2012)
S. bovista	n/a	Japan	AB099901	n/a	Kanchanaprayudh et al. (2003)
S. bovista	Nara_ScB84e3; n/a	Japan: Shizuoka, Gotenba	AB211267	n/a	Nara (2006)
S. bovista	**TNS-F-82197 (= Kasuya B251); n/a**	**Japan: Yamanashi, Narusawa**	**OL764485**	**OL752409**	**Present study**
S. bovista	**TNS-F-82195 (= Kasuya B236); n/a**	**Japan: Nagano, Nagano**	**OL764484**	**OL752408**	**Present study**
S. bovista	**TNS-F-82200 (= Kasuya B804): n/a**	**Japan: Toyama, Toyama**	**OL764487**	**OL752412**	**Present study**
S. bovista	K80S09; n/a	New Zealand	GQ267487	n/a	Walbert et al. (2010)
S. bovista	Trappe26575; n/a	USA	EU819517	EU718178	Wilson et al. (2011)
S. bovista	n/a; O1Q_1	USA: New York	JX030277	n/a	n/a
S. bovista	W#1149; n/a	n/a	n/a	AF336264	Binder & Bresinsky (2002)
S. capeverdeanum	**TNS-F-82205 (= Kasuya B4288); n/a**	**Japan: Ibaraki, Ishioka**	**OL764491**	**OL752414**	**Present study**
S. capeverdeanum	MA-Fungi 87406*; n/a	Cape Verde: Santiago Island	KU747111	KU747110	Crous et al. (2016)
S. cepa	JMP0081; n/a	USA	EU819439	n/a	Hosaka (2009)
S. cepa	184; n/a	n/a	n/a	AF336265	Binder & Bresinsky (2002)
S. citrinum	n/a; ecmSC2	Czech	JX679368	n/a	n/a
S. citrinum	AWW212; n/a	USA	n/a	EU718151	Wilson et al. (2011)
S. columnare	CUB Microbiology KHS3; n/a	Thailand: Chachoengsao	AB459512	n/a	Disyatat et al. (2016)
S. columnare	MS43; n/a	n/a	n/a	AF336273	Binder & Bresinsky (2002)
S. dictyospora	MS55; n/a	n/a	n/a	AF336267	Binder & Bresinsky (2002)
S. dunensis	UFRN-Fungus 2551; n/a	Brazil	KU747116	n/a	Crous et al. (2016)
S. dunensis	UFRN-Fungus 2553; n/a	Brazil	KU747118	n/a	Crous et al. (2016)
S. dunensis	UFRN-Fungus 2206; n/a	Brazil	KU747121	n/a	Crous et al. (2016)
S. echinatum	MS34; n/a	n/a	n/a	AF336268	Binder & Bresinsky (2002)
S. laeve	ZLR46; n/a	China	MW553325	MW553729	Huang et al. (2021)
S. laeve	**TNS-F-82199 (= Kasuya B803); n/a**	**Japan: Ishikawa, Komatsu**	**n/a**	**OL752411**	**Present study**
S. laeve	MCA242; n/a	USA	EU718117	n/a	Wilson et al. (2011)
S. laeve	OSC27936; n/a	USA	EU718120	n/a	Wilson et al. (2011)
S. laeve	27936; n/a	n/a	n/a	DQ683003	Louzan et al. (2007)
S. meridionale	AWW218; n/a	USA	EU718121	EU718152	Wilson et al. (2011)
S. michiganense	E00278311; MICSCL2	USA:Wisconsin, Sheboygan	FM213347	n/a	Phosri et al. (2009)
S. michiganense	n/a; Scl_3-1-2L	USA: New York	JX030206	n/a	n/a
S. polyrhizum	n/a; 11_3	China: Sichuan	HM237173	n/a	n/a
S. polyrhizum	n/a; Scl_1-1-4Nc	USA: North Carolina	JX030195	n/a	n/a
S. polyrhizum	594; n/a	USA	n/a	DQ683000	Louzan et al. (2007)
S. radicans	PDD103563; n/a	New Zealand	n/a	KP191692	n/a
S. radicans	PDD103558; n/a	New Zealand	n/a	KP191693	n/a
S. septentrionale	J. Nitare (12.9.1986); SEPSCL2	Sweden: Norrbotten	FM213336	n/a	Phosri et al. (2009)
S. septentrionale	A.D. Parker (2.10.1997); SEPSCL3	USA: Wisconsin, Lone Rock	FM213338	n/a	Phosri et al. (2009)
S. sinnamariense	SINSCL_8; n/a	Thailand: Pranomprai, Roi Et	FM213363	n/a	Phosri et al. (2009)
S. sinnamariense	NAST-FB11; CMU53-210-2	Thailand	HQ687222	n/a	Siri-in et al. (2014)
S. sinnamariense	MS46; n/a	n/a	n/a	AF336270	Binder & Bresinsky (2002)
S. verrucosum	SV-5602; n/a	Burkina Faso	FJ840461	n/a	Sanon et al. (2009)
S. verrucosum	BCC-MPM2605; n/a	Spain: Catalonia	AJ629886	n/a	Phosri et al. (2007)
S. verrucosum	5; n/a	n/a	n/a	AF336271	Binder & Bresinsky (2002)
S. xanthochroum	AWW311; n/a	Malaysia	EU718126	EU718154	Wilson et al. (2011)
S. yunnanense	KUN-HKAS 79633A; Ji001A	China: Yunnan	JQ639040	n/a	Zhang et al. (2013)
S. yunnanense	KUN-HKAS 79633B; Ji001B	China: Yunnan	JQ639041	n/a	Zhang et al. (2013)
S. yunnanense	KUN-HKAS 79633C; Ji001C	China: Yunnan	JQ639042	n/a	Zhang et al. (2013)
***Scleroderma* sp.**	**TNS-F-82198 (= Kasuya B714); n/a**	**Japan: Ishikawa, Komatsu**	**OL764486**	**OL752410**	**Present study**
Scleroderma sp.	MEL2295738A; n/a	Australia	n/a	EU718181	Wilson et al. (2011)
Uncultured ectomycorrhizal fungus	n/a; L5001_Sclerod_Cam_Euc02	Cameroon	FR731681	n/a	Tedersoo et al. (2011)

*Holotype of *Scleroderma capeverdeanum.*

"n/a" means information not available. Sequences newly generated in the present study were shown in bold.

echinulate basidiospores (Fig. 2D–E). However, subhypogeous habit of Japanese specimens is different from the type specimen of *S. capeverdeanum*, which was described as epigeous (Crous et al., 2016). A detailed description and illustrations of the salient features of Japanese specimens are given below.

Phylogenetic analyses

The ITS dataset was consisted by 42 ingroup and 1 outgroup taxa. It had an aligned length of 865 characters including gaps, of which 36 characters were constant, 509 variable and phylogenetically uninformative, and 320 phylogenetically informative. The MP analysis of the ITS dataset yielded 10,000 most parsimonious trees, of which 149 trees were found in the first step of the heuristic search. Consistency index (CI), retention index (RI) and rescaled consistency index (RC) of the most parsimonious trees are 0.4487, 0.7205, 0.3233, respectively. The highest log likelihood of the

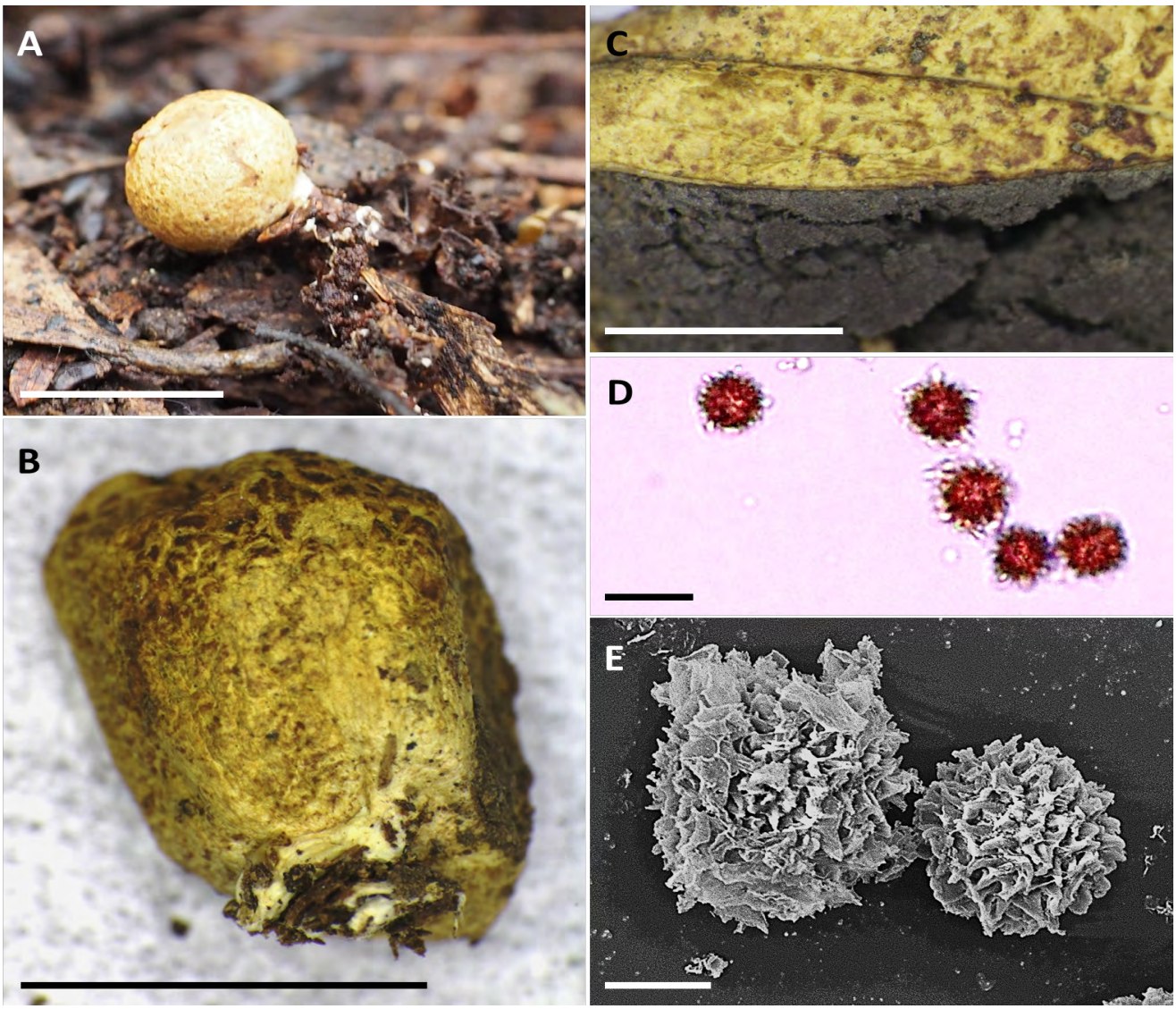

Fig. 2. Morphological features of *Scleroderma capeverdeanum*. A: Basidiomata in the natural habitat (INM-2-217835). B: Surface of mature basidioma (INM-2-217835). C: Vertical sections of mature basidiomata showing exoperidium and gleba (TNS-F-82205). D: Basidiospores (TNS-F-82205). E: Scanning electron microscopy image of basidiospores (TNS-F-82205). Bars: A–B = 1 cm; C = 5 mm; D = 10 µm; E = 5 µm.

resulting ML tree of the ITS dataset is -2446.64. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.5939)]. The rate variation model allowed for some sites to be evolutionarily invariable [(+I), 41.58% sites]. The MP and ML analyses resulted in trees that were identical in topology. Hence, only the MP tree of the ITS dataset is shown in Fig. 3. By MP and ML, ITS sequences generated from Japanese and Cape Verdean samples of *S. capeverdeanum* were placed within a strongly supported clade (MP BS = 100%, ML BS = 99%; Fig. 3) and were distinct from those of the other members of *Scleroderma*.

The LSU dataset was consisted by 26 ingroup and 1 outgroup taxa. It had an aligned length of 1006 characters including gaps, of which 89 characters were constant, 836 variable and phylogenetically uninformative, and 81 phylogenetically informative. The MP analysis of the LSU dataset yielded 10,000 most parsimonious trees, of which 36 trees were found in the first step of the heuristic search. CI, RI and RC of the most parsimonious trees are 0.3631, 0.5807, 0.2108, respectively. The highest log likelihood of the resulting ML tree of the LSU dataset is -2656.84. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.4036)]. The rate variation model allowed for some sites to be evolutionarily invariable [(+I), 63.46% sites]. The MP and ML analyses resulted in trees that were identical in topology. Hence, only the MP tree of the LSU dataset is shown in Fig. 4. By MP and ML, LSU sequences generated from Japanese and Cape Verdean samples of *S. capeverdeanum* were placed within a strongly supported clade (MP BS = 96%, ML BS = 100%; Fig. 4) and were distinct from those of the other members of *Scleroderma*.

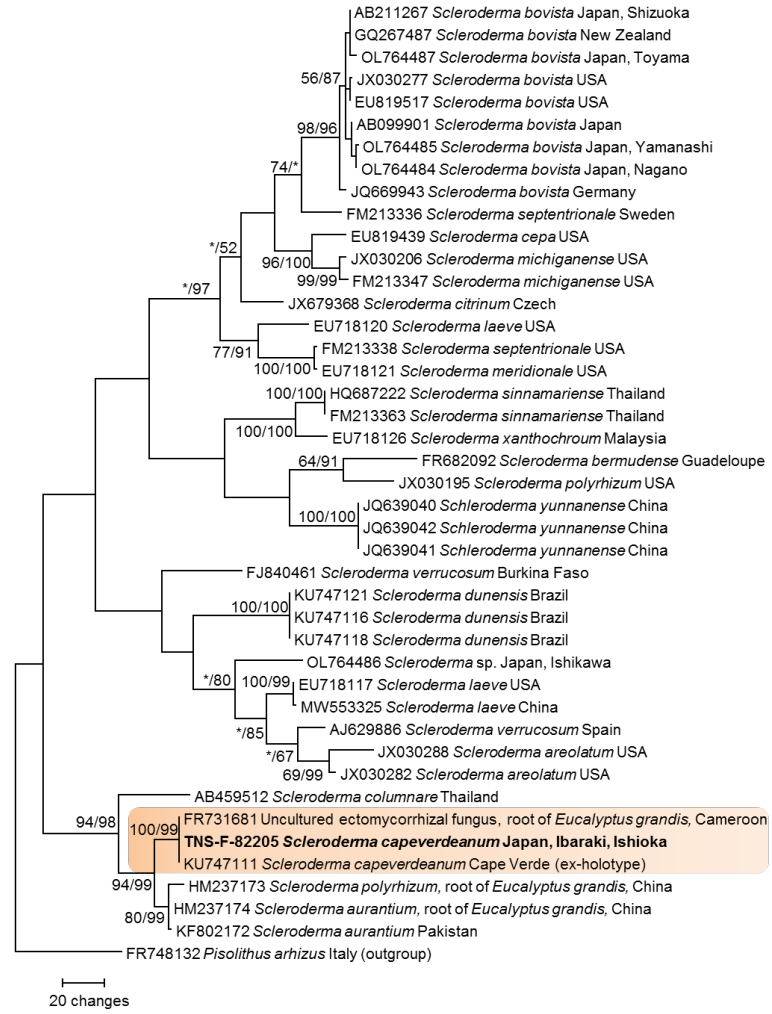

Fig. 3. Phylogenetic tree generated from maximum parsimony (MP) analysis based on the nuclear ribosomal ITS region of selected sclerodermataceous species. Bootstrap support values (BS) of MP and maximum likelihood (ML) greater than 50% are shown for each node (MP/ML), and BS less than 50% are indicated by an asterisk (*).

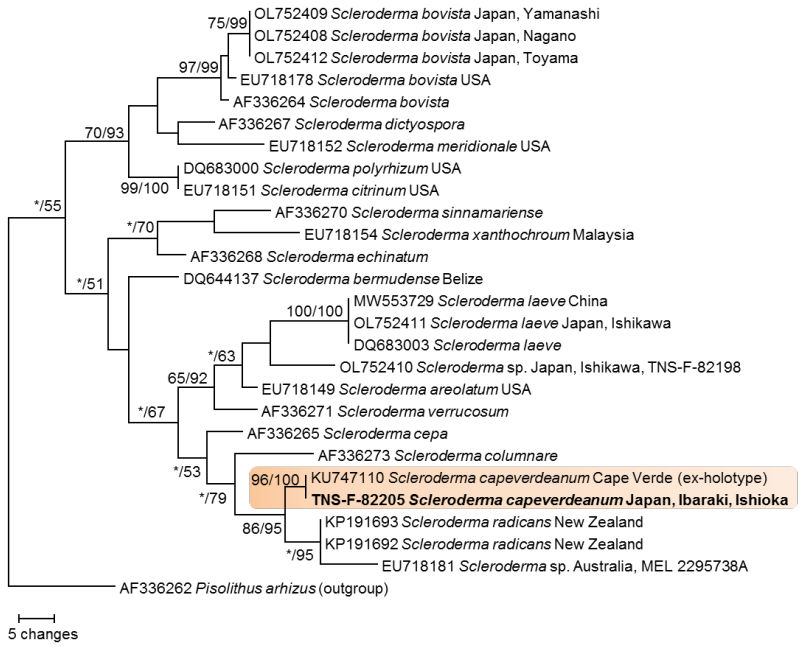

Fig. 4. Phylogenetic tree generated from maximum parsimony (MP) analysis based on the nuclear ribosomal LSU of selected sclerodermataceous species. Bootstrap support values (BS) of MP and maximum likelihood (ML) greater than 50% are shown for each node (MP/ML), and BS less than 50% are indicated by an asterisk (*).

Taxonomy

***Scleroderma capeverdeanum* M.P. Martín, M. Dueñas & Telleria**, in Crous et al., Persoonia 36: 413 (2016)

Fig. 2.

Description: Basidiomata (Fig. 2A–B) subhypogeous, globose, depressed globose to subglobose, small-sized, 4–10 mm broad, sessile, arising from whitish mycelial strands attached to plant debris and soil. Peridium ca. 0.5–1 mm thick, two-layered. Exoperidium (Fig. 2B–C) very thin, yellow to yellowish brown, covered by brown to dark brown, areolate to verrucose scales. Endoperidium papery, firm but brittle when dry, whitish. Apical part of peridium irregularly dehiscent and opening lacerate pore when mature. Gleba (Fig. 2C) compact when young, becoming powdery when mature, grayish. Basidiospores (Fig. 2D–E) globose, 8.5–11 µm in diam. including surface ornamentation, dark brown in 3% KOH, dark reddish brown in 70% ethanol reagent; surface densely echinulate composed of hyaline spines up to 1.5 µm long. Under SEM, spines of basidiospore surface irregularly covered with scaly to tabular materials (Fig. 2E). Basidia not observed. Rudimentary hyphae interwoven, septate, thin-walled, 4–8 µm broad, hyaline in 3% KOH and 70% ethanol reagent; surface sparsely covered with amorphous, hyaline gelatinous materials.

Habitat: Subhypogeous, usually solitary or small groups among plant debris and rich soil under *Eucalyptus globulus* trees (Fig. 1). Fruiting in the Japanese localities occurs in autumn to winter (October to December).

Specimens examined: JAPAN, Ibaraki Prefecture, Ishioka, Otsuka (alt. approx. 140 m asl.), October 12, 2020, coll. T. Kasuya, INM-2-217835; same place, October 12, 2020, coll. T. Kasuya, TNS-F-82205, GenBank accession no.: OL764491 (ITS), OL752414 (LSU); same place, December 21, 2020, coll. T. Kasuya, INM-2-217836.

Known distribution: Cape Verde (Crous et al., 2016) and Japan (new record).

Japanese name: *Yûkari-ki-hime-nise-shoro* ("yûkari" = *Eucalyptus*; "ki-hime" = yellowish, small-sized; "nise-shoro" = the Japanese name of *Scleroderma*).

Discussion

As outlined above, studied Japanese specimens were morphologically almost identical to the original description of *S. capeverdeanum* (Crous et al., 2016). However, basidiomata of Japanese materials were subhypogeous while the type specimen of the present species was described as epigeous (Crous et al., 2016). Numerous fallen leaves and old bark of *E. globulus* are densely deposited on the ground in the locality of Japanese specimens (Fig. 1B) and basidiomata of *S. capeverdeanum* were observed among these litter and rich soil, as subhypogeous nature. Although the detailed environmental information on the type locality of *S. capeverdeanum* was not shown by Crous et al. (2016), subhypogeous or epigeous nature of its basidiomata presumably depend on their habitat. The present species is morphologically similar to *S. flavidum* in its subhypogeous, sessile basidiomata with yellowish surface of peridium and echinulate basidiospores (Yoshimi, 2002). However, *S. flavidum* is clearly distinguishable from *S. capeverdeanum* by its larger basidiomata (3–5 cm in diam.; Yoshimi, 2002) and larger basidiospores (12–16 µm broad including surface ornamentation; Coccia et al., 1990). Another species having yellowish basidiomata, *S. sinnamariense* Mont. is also distinguished from *S. capeverdeanum* by its substipitate basidiomata and subreticulate basidiospores (Guzmán, 1970; Guzmán & Ovrebo, 2000). Although basidiospores of *S. cepa* are echinulate and it forms sometimes yellowish basidiomata, it is distinct from *S. capeverdeanum* because it has larger basidiomata (up to 4 cm broad; Guzmán, 1970), and thicker peridium (up to 3 mm; Coccia et al., 1990). Brownish scales on peridium appear in mature basidiomata of *S. capeverdeanum*. Although *S. verrucosum* (Bull.) Pers. also has similar verrucose scales on the peridium (Sims et al., 1995), it has larger basidiomata (up to 4 cm broad; Coccia et al., 1990; Guzmán, 1970) and larger basidiospores (11–15 µm in diam. including surface ornamentation; Yoshimi, 2002) than *S. capeverdeanum*.

Results of our phylogenetic analyses (Figs. 3, 4) show the monophyly of the present species, as indicated by Crous et al. (2016). Crous et al. (2016) reported that the holotype of *S. capeverdeanum* was collected under *Furcraea foetida* (L.) Haw. (Asparagaceae) and *Lantana camara* L. (Verbenaceae), and no relationships with *Eucalyptus* were shown in their original description. However, the ITS sequence of *S. capeverdeanum* from the root sample of *E. grandis* W. Hill in Cameroon (GenBank FR731681; Tedersoo et al., 2011) was identical with that of fruit body data (Fig. 3). In Cape Verde, there are exotic *Eucalyptus* plantations in wide range of islands in addition to introduced *F. foetida* and *L. camara* communities (Frahm et al., 1996). While it is unclear whether the mycorrhizal host of the present species is restricted to *Eucalyptus*, *S. capeverdeanum* presumably prefers the habitat dominated by eucalypt trees. Their potential association with *F. foetida* and *L. camara*, however, warrants further studies.

Based on the present ITS phylogeny (Fig. 3), *S. capeverdeanum* clusters with two sequences of root sample collected from *E. grandis* in China (GenBank HM237173 and HM237174), as *S. polyrhizum* (J.F. Gmel.) Pers. and *S. aurantium* (L.) Pers., and one sequence from Pakistan as *S. aurantium* (GenBank KF802172;

Bashir & Khalid, 2015), as a sister group. Moreover, phylogenetic tree of the LSU dataset (Fig. 4) shows that three sequences from Australia (GenBank EU718181; Wilson et al., 2011) as *Scleroderma* sp., and New Zealand (GenBank KP191692 and KP191693) as *S. radicans* Lloyd, are sister to *S. capeverdeanum*. Australian specimen housed at MEL (2295738A) is the voucher of EU718181, and it was collected on soil (grass nearby) under a rosaceous plant, *Prunus cerasifera* Ehrh. var. *atropurpurea* H. Jaeger. Although relationships between the above Australian specimen and any myrtaceous plants is not shown, KP191692 and KP191693 were obtained from two specimens (103558 and 103563 housed at PDD, respectively) identified as *S. radicans*, and both were collected from under *Leptospermum* (Myrtaceae) trees. These facts suggest that the *S. capeverdeanum* and its phylogenetically related taxa presumably have some type of preference to myrtaceous plants for their habitat. Although further studies on the ecological nature of *S. capeverdeanum* and its relatives are needed to clarify the relationship between them and their associated plants, we treat *S. capeverdeanum* as an introduced fungal species to Japan from outside because any native species of *Eucalyptus*, *Leptospermum*, *Furcraea* and *Lantana* are not known in the country. While it is unknown whether the origin of *S. capeverdeanum* is Cape Verde or not, this species was probably introduced to Japan from Australia with *E. globulus*. Further investigations of *Scleroderma* in worldwide eucalypt forests will demonstrate the origin and geographic distribution of *S. capeverdeanum*.

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