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## Short communication

# A new species of the genus Aleurodiscus sensu lato (Russulales, Basidiomycota) from Hachijo Island, Japan

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#### ABSTRACT

Aleurodiscus sagittisporus sp. nov. is described and illustrated. This species is characterized by producing basidiomata with a monomitic hyphal system, clampless-septate hyphae, arrowhead-shaped, amyloid, finely verrucose basidiospores, gloeocystidia, dendrohyphidium-like branched paraphysoid hyphae, and variously shaped swelling cells in the hymenium. Phylogenetic analyses based on nuclear rDNA LSU and ITS sequences revealed that the species is distinct from the lineage of Aleurodiscus s. str. and related genera in the Aleurodiscus s. lat. clade. Basidiomata of A. sagittisporus have been collected only from dead petioles attached to living trees of Livistona chinensis var. subglobosa on Hachijo Island, Japan.

Keywords: corticioid fungi, Livistona chinensis, molecular phylogeny, Stereaceae

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Hachijo Island (known locally as Hachijo-jima), located 287 km south of Tokyo, Japan (33°06' N, 139°47' E), is a small volcanic oceanic island and part of the Izu Islands. The climate is humid subtropical. In Aug 2010 and Sep 2011, the authors collected several specimens of an undescribed corticoid fungus on dead petioles of Livistona chinensis R. Br. ex Mart. var. subglobosa (Hassk.) Becc. (Arecaceae) planted in gardens and along roadsides at several sites on the island (Supplementary Fig. S1). This fungus is morphologically similar to taxa of Aleurodiscus Rabenh. ex J. Schröt. and related genera (Basidiomycota, Russulales, Stereaceae), except in its basidiospore morphology, which is clearly distinct. Here we describe the fungus as a new species of Aleurodiscus and discuss its phylogenetic position and ecological features.

The color and configuration of the hymenial surface and marginal zone were noted based on fresh and dried specimens. In the description, color names in quotation marks refer to Rayner (1970). For microscopic observations, a piece of a dried specimen was sectioned vertically using a razor blade. Sections were mounted in 3% (w/v) KOH, Melzer's reagent (Weresub, 1953), sulphobenzaldehyde reagent (SA) (Boidin, 1951), and distilled water. Microscopic elements of the basidiomata were drawn using a drawing tube (Y-IDT, Nikon Imaging, Tokyo, Japan) attached to the microscope (Eclipse Ni, Nikon Imaging). For each taxonomic element of each

specimen, 20 measurements were usually made in Melzer's reagent. Basidiospore surface structure was observed with a scanning electron microscope (SU1510, Hitachi, Tokyo, Japan) under 5 kV accelerating voltage, using dried specimens. Procedures for rehydrating, fixing, dehydrating, critical-point drying and sputter coating of the specimens followed Endo et al. (2019). The specimens and cultures examined in this study are deposited at the Tottori University Mycological Herbarium (TUMH) and the fungal culture collection (TUFC), respectively, in the Fungus/Mushroom Resource and Research Center (FMRC), Tottori University, Tottori, Japan.

All polyspore isolates examined in this study were obtained from voucher specimens. These isolates were grown on malt extract agar [MA, 1.5% (w/v) malt extract, Difco, Detroit, MI; 2% (w/v) Bacto agar, Difco] at 25 °C in the dark. To determine the optimum growth temperature, the isolates were grown on MA plates at eight different temperatures (5-40 °C).

The procedures for DNA extraction, PCR amplification, and sequencing analysis followed Maekawa et al. (2020). For PCR amplification and sequencing analysis, we used the primer pairs ITS5/ ITS4 (White et al., 1990) for the internal transcribed spacer (ITS) regions of nuclear rDNA and LR0R/LR5 (Hopple & Vilgalys, 1994) for the D1/D2 domain of the large subunit of the 28S nuclear rRNA (LSU). After assembling the bidirectional sequences, the ITS and LSU sequences of each of the 10 strains were deposited in the DNA Data Bank of Japan under the accession numbers LC754704-754713 and LC754714-754723, respectively.



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Phylogenetic analyses were performed using the combined LSU and ITS dataset. Taxon sampling of Aleurodiscus s. lat. and related taxa followed Wu et al. (2022) and included Stereaceae (Aleurodiscus s. lat.) and an outgroup (Table 1). We aligned sequences using MAFFT v. 7 (Katoh et al., 2019) under the "L-INS-i" algorithm. Because the resulting alignment included many ambiguous or gapped sites due to low homology among taxa, we trimmed the sequences. Manual trimming was mostly performed on the ITS2 region, where the newly described species showed a large amount of variation compared to related species. After manual trimming, the alignment was further trimmed using the software trimAl v. 1.2. (Capella-Gutiérrez et al., 2009) by using the "automated1" method. We included a total of 1212 sites of the alignment in our analyses, including 111 from ITS1, 55 from ITS2, 157 from 5.8S, and 885 from 28S. Each of ITS1, ITS2, 5.8S, and 28S was treated as a separate data block during model selection with ModelTest-NG v. 0.2.0 (Flouri et al., 2015; Darriba et al., 2020) and during phylogenetic analysis under the maximum likelihood (ML) and Bayesian inference (BI) methods. The best fitting substitution models were GTR+G+I for ITS1 and ITS2, HKY+G for 5.8S, and GTR+G+I for 28S. The ML phylogeny was inferred by using raxml-ng v. 1.1.0 (Kozlov et al., 2019) with 1,000 replicates for the bootstrap analysis for each branch. The BI analysis was run under the same partition schemes with MrBayes v. 3.2.7 (Ronquist et al., 2012). We ran two independents four-chain Markov chain Monte Carlo analysis for 3,000,000 generations. We checked for convergence by using Tracer v. 1.7.2 (Rambaut et al., 2018) and calculated the posterior probability for each branch under the 50% majority consensus tree after discarding the first 25% of trees as burn-in. The alignment and tree have been submitted to TreeBase (http://www.treebase.org; accession no. S30351).

Basidiomata of the present species are primarily characterized by having a monomitic hyphal system, clampless-septate hyphae, arrowhead-shaped, amyloid, finely verrucose basidiospores, gloeocystidia, dendrohyphidium-like branched paraphysoid hyphae, suburniform basidia, and variously shaped swelling cells in the hymenium. In addition, this species produces gloeoplerous hyphae with subhyaline oily contents, which have been observed in cultures of several taxa of *Aleurodiscus* and related genera. These morphological and cultural features indicate that the species belongs to *Aleurodiscus* s. lat.

Aleurodiscus s. lat. contains morphologically diverse species, and the following genera have been segregated based on morphological and/or phylogenetic analyses: Acanthobasidium Oberw. (Oberwinkler, 1966), Acanthophysellum Parmasto (Parmasto, 1967), Acanthophysium (Pilát) G. Cunn. (Cunningham, 1963), Aleurobotrys Boidin (Boidin et al., 1985), Aleurocystidiellum P.A. Lemke (Lemke, 1964a), Gloeosoma Bres. (Bresadola, 1920), and Stereodiscus Rajchenb. & Pildain (Rajchenberg et al., 2021). In addition, two allied genera, Acanthofungus Sheng H. Wu, Boidin & C.Y. Chien (Wu et al., 2000) and Neoaleurodiscus Sheng H. Wu (Wu et al., 2010), were established. However, recent phylogenetic analyses suggested that Aleurodiscus s. lat. is still polyphyletic (Wu et al., 2001; Dai & He, 2016; Wu et al., 2019; Rajchenberg et al., 2021; Wu et al., 2022) and that the clade of Aleurodiscus s. lat. included, in addition to the above genera, taxa of Aleurodiscus s. str., Conferticium Hallenb., Gloeocystidiellum Donk, Stereum Hill ex Pers., and Xylobolus P. Karst. (Wu et al., 2001; Dai & He, 2016; Rajchenberg et al., 2021; Wu et al., 2022). Our phylogenetic analyses based on nuclear rDNA LSU and ITS sequences also showed that Aleurodiscus s. lat. is polyphyletic and is intermixed with taxa of Acanthobasidium, Acanthofungus, Acanthophysellum, Acanthophysium, Boidinia Stalpers & Hjortstam, Conferticium, Gloeocystidiellum, Gloeocystidiopsis, Gloeosoma, Megalocystidium Jülich, Neoaleurodiscus, Stereum, and Xylobolus within the clade (Fig. 1). In these genera, no known species possess arrowhead-shaped (in frontal view) basidiospores like those produced by the present species (Figs. 2D, E, 3A). In our phylogenetic tree, the 10 accessions formed a strongly supported monophyletic clade (ML bootstrup/BI probability = 100/1) that is distinct from the lineage of Aleurodiscus s. str., to which the type species A. amorphus belongs. We could not identify any known genera suitable for this species within the Aleurodiscus s. lat. clade, although many subterminal nodes of the tree were not supported by high ML bootstrap values due to the high degree of divergence of rDNA LSU and ITS sequences between species (less than 90% sequence homology in most cases). Although this species can easily be delineated by ITS sequences, the interrelationships among the species within the clade remain unclear. Further phylogenetic studies are needed, including one to determine whether this species should be treated as an independent genus. Therefore, we describe the species as a new species of Aleurodiscus s. lat. as follows.

Aleurodiscus sagittisporus N. Maek., Y. Oba & R. Nakano, sp. nov. Figs. 2, 3.

MycoBank No.: MB 847629.

Diagnosis: This species is characterized by producing corticioid basidiomata, clampless-septate hyphae, numerous gloeocystidia, paraphysoid hyphae, usually urniform basidia and arrow-head-shaped, finely verrucose, amyloid basidiospores measuring  $14-17 \times 10-11.5 \ \mu$ m in frontal view, and by growing on dead petioles of *Livistona chinensis* var. *subglobosa*.

Holotype: JAPAN, Tokyo, Hachijo-machi, Sueyoshi, on dead petiole of *L. chinensis* var. *subglobosa*, 8 Sep 2011, collected by N. Maekawa and R. Nakano, TUMH 40363 (ex-holotype culture, TUFC 14455). Gene sequences ex-holotype: LC754708 (ITS), LC754718 (LSU).

Etymology: *"sagittisporus"* [*sagitti* (= sagittate) + *sporus* (= spore)] refers to having arrowhead-shaped basidiospores.

Description: Basidiomata annual, resupinate, adnate, occurring as small patches, then confluent; hymenial surface 'Rosy Buff', 'Rosy Vinaceous' to 'Pale Luteous', partly 'Orange' when fresh, 'Pale Luteous', 'Luteous' to 'Ochreous' when dry; margin 'Pale Luteous' to 'Luteous', thinning out, indeterminate; in vertical section 100–350 µm thick, subhyaline to pale yellow-brown, membranous, sometimes containing masses of crystals in the subicula. Hyphal system monomitic; hyphae 2–5 µm wide, smooth, thin- to slightly thick-walled (up to 0.5 µm), clampless septate, sometimes anastomosing. Paraphysoid hyphae 1.5-4 µm wide, sinuous, thin-walled, smooth, without a basal clamp, sometimes dendrohyphidium-like branched; branches sometimes anastomosing. Gloeocystidia 75- $213 \times 8.5$ – $12.5 \,\mu$ m, cylindrical, narrowly obclavate to tubular, occasionally branching at the apex, sometimes sinuous, smooth, thinwalled, without a basal clamp, numerous, mostly embedded but occasionally projecting up to 25 µm beyond the hymenial surface, positive to sulphobenzaldehyde (SA+). Swelling cells  $24-46 \times 9.5-$ 13 µm, various shaped, smooth, thin-walled, without a basal clamp, containing granular materials, present in the hymenium. Basidia 34.5–56 µm long, 7–9 µm wide at the upper part, 9.5–12.5 µm wide at the under part, suburniform to subclavate, occasionally with various shaped projections at under part, producing 4 sterigmata, without a basal clamp, containing granular materials. Basidiospores  $14-17 \times 10-11.5 \,\mu$ m, triangular to lanceolate in frontal view,  $14-17 \times 4.5-5.5 \,\mu$ m, banana-shaped to lunate in lateral view, finely verrucose, thin-walled, amyloid.

Table 1. Sequences in nrITS-LSU dataset

- · ·	··· 1 / . ·	Accession nos.	
Species	Voucher/strain nos.	ITS	nrLSU
Acanthobasidium bambusicola	He 2357	KU559343	KU574833
Acanthobasidium norvegicum	T623	-	AY039328
Acanthobasidium penicillatum	HHB13223	-	KU574816
Acanthobasidium phragmitis	CBS 233 86	_	AY039305
Acanthobasidium weirii	HHB12678	-	AY039322
Acanthofungus rimosus	Wu 9601-1	MF043521	AY039333
Acanthophysellum cerussatum	He 20120920-3	KU559339	KU574830
Acanthophysium bisporum	1614 T627	-	AY039327
Acanthophysium lividocaeruleum	FP-100292	_	AY039319
Aleurobotrys botryosus	He 2712	KX306877	KY450788
<i>.</i>	Wu 9302-61	-	AY039331
Aleurocystidiellum disciforme	He 3159	KU559340	KU574831
Aleurocystialelium subcruentatum	He 2886 T220	KU559341	KU5/484/
Aleurodiscus alpinus	Wu 1407-59	MF043522	MF043527
	Wu 1407–61	MF043523	MF043528
Aleurodiscus amorphus	Ghobad-Nejhad-2464	KU559342	KU574832
Aleurodiscus aurantius	T621	-	AY039317
Aleurodiscus bicornis	He 4201 Wu 1308_101	K 1 /06207 I C/33893	LC/33000
Aleurouiscus bicornis	Wu 1308-101 Wu 1308-125	LC433899	LC433906
Aleurodiscus canadensis	Wu 1207–90	KY706203	KY706225
Aleurodiscus cerussatus	He 2208	KX306874	KY450785
41	HHB11235	-	AY039321
Aleurodiscus dextrinoideophyses	EL25-97 He 4078	Ar506401	Ar506401 KY450792
mearouiscus uenrinoiueopriyses	He 4105	MH109050	KY450784
Aleurodiscus effusus	He 2261	KU559344	KU574834
Aleurodiscus fõrmosanus	Chen 2736	LC433894	LC433901
Alarma diama di	Chen 2748	LC433895	LC433902
Aleurodiscus gigasporus	WU 0108-15 He 2895	KY/06205	KY/06213 KU574927
Aueurouiscus grunnli	HHB14417	KU559363	KU574821
Aleurodiscus isabellinus	He 5283	MH109052	MH109046
Aleurodiscus mesaverdensis	FP-120155	KU559359	KU574817
Aleurodiscus oakesii	He 2243	KU559352	KU574840
Alguradisque parvisporus	HHB11890-A-sp	KU559365	KU574823
Aleuroaiscus parvisporus	Wu 1307-84 Wu 1307-88	LC433898	LC433904
Aleurodiscus pinicola	Wu 1106–16	MF043524	MF043529
L.	Wu 1308-54	MF043525	MF043530
Aleurodiscus sagittisporus	TUFC 13927	LC754704	LC754714
	TUFC 14450 TUFC 14454	LC754705	LC754715
	TUFC 14455	LC754707	LC754717
	TUFC 14456	LC754708	LC754718
	TUFC 14457	LC754709	LC754719
	TUFC 14458	LC754710	LC754720
	TUFC 14459 TUFC 14461	LC754711 LC754712	LC754721 LC754722
	TUFC 14462	LC754713	LC754723
Aleurodiscus senticosus	Wu 1209–7	MH596849	MF043531
	Wu 1209–9	MH596850	MF043533
Aleurodiscus sichuanensis	He 4935	LC430904	LC430907
Aleurodiscus subroseus	He 4807	MH396852 MH109054	MF043534 MH109048
Thear outseus subroseus	He 4895	LC430903	LC430910
Aleurodiscus tenuissimus	He 3575	KX306880	KX842529
Aleurodiscus thailandicus	He 4099	KY450781	KY450782
Aleurodiscus tropicus	He 3830	KX553875	KX578720
Aleurodiscus verrucosporus Aleurodiscus wakefieldiae	He 2580	KU559353	KU574841
1 Ilean outsetts Wattepterarae	FP-135654	KU559369	KU574829
Boidinia macrospora	Wu 9202-21	AF506377	AF506377
Bondarzewia mesenterica	DSM 108281	MK500942	MK500942
Conferticium rayum	CBS 321.00 NH13201	AF506381	AF506381
Gloeocystidiellum aspellum	LIN 625	AF506432	AF506432
Gloeocystidiellum compactum	Wu880615-21	AF506434	AF506434
Gloeocystidiellum formosanum	Wu9404-19	AF506439	AF506439
Gloeocystidiellum luridum	HK9808	AF506421	AF506421
Gioeocystiaiellum porosum Gloeocystiaiellum triste	WU 1608-176 KHI 10334	LC430905	LC430908
Gloeocystidiellum wakullum	Oslo-930107	AF506443	AF506443
Gloeocystidiopsis flammea	AH000219	AF506438	AF506438
	CBS 324.66	AF506437	AF506437
Gloeosoma mirabile	Dai 13281	KU559350	KU574839
Heterohasidion papuporum	ne 3733 91605	K 1450/87 K 1651502	K1450/91 K1651561
Megalocystidium chelidonium	LodgeSJ110.1	AF506441	AF506441
Megalocystidium diffissum	V.Spirin4244	MT477147	MT477147
Megalocystidium leucoxanthum	HK9808	AF506420	AF506420
Neoaleurodiscus fujii	He 2921	KU559357	KU574845
Staraodiscus limonisporus	WU 0807-41 CBS 125846	-	FJ799924 MH875266
Stereum complicatum	He 2234	KU559368	KU574828
Stereum hirsutum	JS18244	AF506479	AF506479
-	Wu 1109–127	LC430906	LC430909
Stereum ostrea	He 2067	KU559366	KU574826
Stereum reflexulum Stereum rugosum	EL48-97 NH11052	AF506480	AF506480
Stereum sanguinolentum	He 2111	KU559367	KU574827
Stereum subtomentosum	EL11-97	AF506482	AF506482
Xylobolus subpileatus	FP-106735	_	AY039309
Xylobolus frustulatus	He 2231	KU881905	KU574825

Bold shows newly obtained sequences. -: sequences not available.

## **Mycoscience**



**Fig. 1** – Maximum likelihood tree based on the LSU + ITS sequences of species of *Aleurodiscus* (s. lat.) and related genera. Values on branches show the maximum likelihood bootstrap value ( $\geq$ 50) and Bayesian inference posterior probability ( $\geq$ 0.90). Species names in bold indicate sequences of type specimens, and filled circles indicate sequences of type species in each genus.



**Fig. 2** – *Aleurodiscus sagittisporus* (TUMH 40363, holotype). A: *Livistona chinensis* tree with a dead petiole (arrowhead) hanging from the trunk, where basidioma was found. B: Basidioma. C: Hymenial surface, magnified. D: Frontal view of a basidiospore, focusing on the top surface (upper) and on the back surface showing an apiculus (arrowhead) in 3% KOH. E: SEM image of a basidiospore in frontal view showing the fine warts on the surface and a distinct apiculus (arrowhead). F: SEM image of a basidiospore in lateral view (arrowhead: apiculus). *Bars*: B 1 cm; C 1 mm; D 5  $\mu$ m; E, F 2  $\mu$ m.

Other specimens and cultures examined: JAPAN, Tokyo, Hachijo-machi (Hachijo Island), Nakanogo, on dead petiole of L. chinensis var. subglobosa, 19 Aug 2010, collected by Y. Oba [TUMH 40359 (TUFC 13927)]; on dead petiole of L. chinensis var. subglobosa, 7 Sep 2011, collected by N. Maekawa and R. Nakano [TUMH 40360, TUMH 40361, and TUMH 40362 (TUFC 14454)]; Hachijo-machi, Mitsune, on dead petiole of L. chinensis var. subglobosa, collected by N. Maekawa and R. Nakano [TUMH 40371 (TUFC 14462), TUMH 40372, and TUMH 40373]; Hachijo-machi, Okago, on dead petiole of L. chinensis, 8 Sep 2011, collected by N. Maekawa and R. Nakano [TUMH 40357 (TUFC 14450), TUMH 40358, TUMH 40368, and TUMH 40374]; Hachijo-machi, Sueyoshi, on dead petiole of L. chinensis var. subglobosa, 8 Sep 2011, collected by N. Maekawa and R. Nakano [TUMH 40364 (TUFC 14456), TUMH 40365 (TUFC 14457), TUMH 40366 (TUFC 14458), TUMH 40367 (TUFC 14459), TUMH 40369 (TUFC 14461), and TUMH 40370]. TUFC number in parentheses indicates isolate number.

Characteristics in culture: The optimum growth temperature for the polyspore isolates, TUFC 13927, TUFC 14456, and TUFC 14461, were 25–30 °C. These isolates could grow between 10 and 35 °C, but no visible growth was observed at 5 and 40 °C. Growth on MA was 3–7 mm at 25 °C for 24 h in the dark. Mycelial mats after 1 wk subhyaline to white; aerial mycelium cottony, partly woolly; margin distinct, raised, not even, usually with a fan-like extensions; odor not noticeable; reverse side of the mycelial mats white; agar not bleached; no fruiting after 6 wk. Marginal hyphae  $1-5 \mu m$  wide, thin-walled, clampless, sparsely branched. Aerial hyphae  $1-4 \mu m$ wide, thin-walled, clampless, sparsely branched, sometimes sparsely encrusted. Submerged hyphae  $1-8 \mu m$  wide, thin-walled, clampless, sometimes constricted at the septa of broader hyphae, partly encrusted, sometimes gloeoplerous with subhyaline oily contents.

Aleurodiscus sagittisporus is widely distributed on Hachijo Island (Supplementary Fig. S1). Basidiomata were collected only from dead petioles attached to living trees of *L. chinensis* var. subglobosa; they were not found on detached petioles. This species was not found on any other palm trees (*Arecaceae*), such as *Howea belmoreana* (C. Moore & F. Muell.) Becc., *Hyophorbe lagenicaulis* (L.H. Bailey) H.E. Moore, *Phoenix canariensis* Nabonnand, or *P. roebelenii* O'Brien. In addition, *A. sagittisporus* could not be observed on the fallen trunks or branches of any woody plants near individuals of *L. chinensis* on which its basidiomata occurred. These observations suggest that *L. chinensis* var. subglobosa is a specific host for *A. sagittisporus*. According to Index Fungorum (http://www.indexfungorum.org/names.asp, 6 Feb 2023), about



**Fig. 3** – Line-drawing of microscopic elements of basidioma of *Aleurodiscus sagittisporus* (TUMH 40357). A: Basidiospores in Melzer's reagent, the upper five in frontal view and the lower six in lateral view. B: Gloeocystidia. C: Basidia. D: Swelling cells produced in the hymenium. E: Paraphysoid hyphae. F: Hyphae. *Bars*: 10 μm.

200 species have been described as members of *Aleurodiscus* and related genera, but no species that occurs only on palm trees has been reported (Rogers & Jackson, 1943; Lemke, 1964a, 1964b; Ginns & Lefebvre, 1993; Núñez & Ryvarden, 1997; Gorjón et al., 2013; Dai & He, 2016; Dai, Zhao & He, 2017; Dai, Wu, et al., 2017; Tian et al., 2018; Wu et al., 2019; Rajchenberg et al., 2021; Wu et al., 2022). In Japan, *L. chinensis* var. *subglobosa* is distributed from the Nansei Islands to Kyushu (Yoshida et al., 2000) and is often planted

as a street tree in warm temperate to subtropical areas. We have looked for basidiomata of *A. sagittisporus* on natural and planted *L. chinensis* (var. *subglobosa* and var. *boninensis* Becc.) and other palm trees since 2011 in Kagoshima Prefecture (including Yakushima Island), Kochi Prefecture, Miyazaki Prefecture, Okinawa Prefecture (Okinawa, Ishigaki, and Iriomote Islands), and Tokyo (Hachijo Island and Ogasawara Islands), but so far this fungus has not been found outside Hachijo Island. To determine whether *A. sagittispo*- *rus* is endemic to Hachijo Island, further distribution surveys are required, including overseas.

### **Disclosures**

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of Japan.

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