Mycoscience xxx (2017) 1–7

Contents lists available at ScienceDirect

Mycoscience

journal homepage: www.elsevier.com/locate/myc

Short communication

Rediscovery of *Cyathus badius*, an 'extinct' species from the Bonin Islands, Japan

Rhudson Henrique Santos Ferreira da Cruz [a, b, *], Iuri Goulart Baseia [b], Kentaro Hosaka [c]

[a] Programa de Pós-Graduação em Sistemática e Evolução, Departamento de Botânica e Zoologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Lagoa Nova, Natal, 59078-970, Brazil
[b] Departamento de Botânica e Zoologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Lagoa Nova, Natal, 59078-970, Brazil
[c] Department of Botany, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba, Ibaraki, 305-0005, Japan

ARTICLE INFO

Article history:
Received 4 October 2016
Received in revised form
24 July 2017
Accepted 26 July 2017
Available online xxx

Keywords:
Bird's nest fungi
Endangered species
Ogasawara
Phylogeny
Taxonomy

ABSTRACT

The Bonin Islands, Japan, present a unique and endemic fauna and flora, however a large portion of these species, including fungi, are now considered endangered or extinct. During almost 80 y *Cyathus badius* was included in that statistics and no additional collections were recorded until a new expedition in 2015. Morphological comparisons with the holotype are consistent with the new specimen, and phylogenetic analyses based on ribosomal ITS, LSU and concatenated dataset placed *C. badius* in a highly supported clade with *C. parvocinereus*. Is proposed here to epitypify *C. badius* with illustrations, new morphological characters and DNA data.

The Bonin Islands (or Ogasawara Islands) are located ca. 1000 km south of Tokyo. Originated by volcanic activities on the Tertiary period, the Bonin Islands emerged from the Pacific Ocean ca. 1 million y ago (Kawakami, 2010).

The archipelago is divided into three groups: Mukojima, Chichijima and Hahajima Islands (from the north to south, respectively), and presents typical weather of monsoons, with long and dry summer, and moderate winter, with rain periods during May and Oct–Nov (Kato, Shibata, Yasui, & Nagamasu, 1999; Shimizu, 1995).

After the human colonization, with consequent environmental exploitation, the original vegetation has become disturbed, sparse and fragmented, with some humid forests in high altitudes. Subsequently alien species of woody plants, like the genus *Pinus*, covered part of the degraded area (Kato et al., 1999).

Shortly before the Second World War (1939–1945) two species of *Cyathus* Haller (Nidulariaceae, Agaricales, Basidiomycota) was collected and described from Chichijima Island, both in 1937: *Cyathus badius* Kobayasi (Kobayasi, 1937) and *C. boninensis* S. Ito & S. Imai (Ito & Imai, 1937). During the war the islands were evacuated and the residents have not returned to the islands (Chichijima and Hahajima) until 1968. With investment of Tokyo Metropolitan Government, some constructions were conducted and many cultivable fields were implanted in places around the island, assigning the other areas as a national park in 1972 (Kawakami, 2010; Shimizu, 1995).

As is often the case for the oceanic islands, fauna and flora of the Bonin Islands are unique and many species are considered endemic to the islands. However, a large proportion of flora and fauna in the islands are now considered endangered or in extreme case, extinct. According the Japanese Environmental Agency (Environmental Agency, 2016), both *Cyathus badius* and *C. boninensis* are included in the "extinct" category of the Red List of Threatened Fungi of Japan. The last and the only collections of both species were made in 1936, and no additional samples have been collected since then. In addition, the original collection area has now become a residential area.

1. Collection and sample preparation

During field expeditions to Chichijima and Hahajima Islands in

* Corresponding author. Programa de Pós-Graduação em Sistemática e Evolução, Departamento de Botânica e Zoologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Lagoa Nova, Natal, 59078-970, Brazil.
E-mail address: rhudsoncruz@yahoo.com.br (R.H.S.F. da Cruz).

https://doi.org/10.1016/j.myc.2017.07.004
1340-3540/

Nov 2015, some *Cyathus* species were collected in both islands. After careful examinations, one of the samples from Hahajima Island was identified as *C. badius*. This is the first record of this species after almost 80 y from the description of species (Kobayasi, 1937), changing the "extinct" status of this species from the Red List of Japan.

The collection methodology followed an adapted protocol from Lodge, Ammirati, O'Dell, and Mueller (2004). A total of six peridioles was stored in 2 mL Eppendorf® tubes filled with a modified DMSO buffer (Hosaka & Castellano, 2008) for DNA analyses, and the fruit bodies were dried at 45 °C for 24 h.

Morphological observations were made in the Mycology lab at the Department of Botany, National Museum of Nature and Science, Tsukuba, Japan. Macro-morphology was observed using a stereomicroscope Olympus SZX12 (Olympus, Tokyo, Japan) with capture camera. Micro-morphology was observed from longitudinal sections of the peridioles mounted in 5% (w/v) KOH and examined with the aid of a light microscope Olympus BX50, under 400 × magnification. Species identification followed Brodie (1984) and Kobayasi (1937). In the spore descriptions, "Qm" is the mean of the quotient of length ("L") and width ("W"), and "n" is the number of spores measured (Zhao, Desjardin, Soytong, & Hyde, 2008). The spore shape was in accordance with Bas (1969), and the color codes and names were defined using Kornerup and Wanscher (1978). The recent collection of *C. badius* was deposited in the fungal herbarium, Department of Botany, National Museum of Nature and Science, Tsukuba, Japan (TNS), the same herbarium where the holotype is deposited.

DNA from peridioles stored in DMSO buffer was extracted following the glass milk purification method from Hosaka and Castellano (2008) and Hosaka (2009). DNA sequences were obtained from the internal transcribed spacer (ITS) and large subunit (LSU) regions of the nuclear ribosomal DNA using the primer combinations ITS5–ITS4 (White, Bruns, Lee, & Taylor, 1990) and LR0R–LR5 (Vilgalys & Hester, 1990), for ITS and LSU, respectively.

PCR reactions were carried out using 10 μL reaction volumes following the EmeraldAmp® MAX PCR Master Mix manufacturer protocol (TaKaRa, Tokyo, Japan): 1 μL of genomic DNA from the original extracted concentration, 3.5 μL of water, 5 μL of EmeraldAmp® MAX PCR Master Mix and 0.25 μL of each primer. The thermal cycles were followed as previously described (Hosaka & Uno, 2013). PCR products were purified with illustra™ ExoProStar™ (GE Healthcare Life Sciences, Buckinghamshire, UK), and sequenced with Big Dye Terminator Cycle Sequencing Kit (Thermo Fisher Scientific Inc., Carlsbad, Canada) using the same primers described above. The obtained sequences were edited in the BioEdit ver. 7.2.5 (Ibis Biosciences, Carlsbad, Canada). Sequences of type and well identified species of *Cyathus* from Zhao et al. (2008), and those generated in this study (Table 1), were first aligned with Clustal algorithm and manually edited using MEGA – Molecular Evolutionary Genetics Analysis ver. 7.0.14 (Kumar, Stecher, & Tamura, 2016). Sequences from type material of *C. hortensis* R. Cruz & Baseia, *C. magnomuralis* R. Cruz & Baseia and *C. parvocinereus* R. Cruz & Baseia, only described with morphology by Cruz and Baseia (2014), and sequence of the LSU region of *C. lignilantanae* R. Cruz & M.P. Martín, described by Martín, Cruz, Dueñas, Baseia, and Telleria (2015), were included in the tree to update the phylogeny results with more sequences from types. *Crucibulum laeve* (Huds.) Kambly and *Nidula* sp., collected from Japan, was used together with sequences of *Cystoderma amianthinum* (Scop.) Fayod from GENBANK for rooting purposes.

Phylogenies were built using ITS and LSU datasets, and ITS-LSU combined data to evaluate the topology of all trees and understand the most accurate position of *Cyathus badius*.

Maximum Parsimony analysis (MP) was performed using PAUP* ver. 4.0a149 (Swofford, 2002), with trees calculated by Bootstrap method with heuristic search. Tree Bisection Reconnection (TBR) algorithm and *Multrees* option were in effect in PAUP*. Maxtrees was limited to 1000 trees and the trees were obtained via stepwise addition with 10,000 replications and random additional sequences repeated 10 times. The consensus tree included groups consistent with 50% majority-rule consensus, excluding groups with bootstrap proportions less than or equal to 5%. To assess homoplasy levels, the retention index, RI (Farris, 1989), consistency index, CI, and rescaled consistency index, RC (Kluge & Farris, 1969), were obtained.

Bayesian Analysis was performed using MrBayes ver. 3.1.2 (Ronquist & Huelsenbeck, 2003), with the substitution model T92 + G + I for ITS rDNA, and K2 + G for LSU rDNA region, chosen by MrModelTest ver. 2.2 (Nylander, 2004). The analysis used two different runs with four simultaneous MCMC simulations over 5 million generations. Trees were sampled at every 1000 generations discarding the first 25% first sampled trees as a burn-in stage to estimate posterior probabilities, observing the average standard deviation of split frequency values drops below 0.01. Obtained trees were viewed in FigTree ver. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree), exported as PDF and edited in INKSCAPE ver. 0.91 for Windows (https://inkscape.org). The sequence alignments are available at the TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S19947).

Table 1
– Taxon information and sequences accession numbers (GenBank).

Taxa	Origin	GenBank Accession numbers	
		ITS	LSU
Cystoderma amianthinum	Australia	KP311459	KP311339
Crucibulum laeve	Japan	KX906249	KX906256
Nidula sp.	Japan	KX906248	KX906255
Cyathus africanus[a]	Tanzania	DQ463347	DQ463330
C. annulatus[a]	Canada	DQ463351	DQ463332
C. badius[a]	Japan	KX906250	KX906257
C. berkeleyanus	China	DQ463355	–
C. colensoi[a]	India	DQ463344	–
C. crassimurus[a]	Hawaii	DQ463350	–
C. gansuensis	China	KC869661	KC691184
C. griseocarpus[a]	India	–	DQ463324
C. guandishanensis[a]	China	–	DQ463329
C. helenae[a]	Canada	–	DQ463334
C. hookeri	China	DQ463346	–
C. hortensis[a]	Brazil	KX906252	–
C. jiayuguanensis[a]	China	DQ463341	DQ463325
C. lanatus[a]	USA	–	DQ463337
C. lignilantanae[a]	Cape Verde	KX906254	KX906258
C. magnomuralis[a]	Brazil	KX906251	KX906259
C. olla f. *olla*[a]	Canada	DQ463345	DQ463327
C. olla f. *anglicus*[a]	USA	–	DQ463326
C. olla f. *brodiensis*	China	DQ463343	–
C. pallidus	China	DQ463356	DQ463336
C. parvocinereus[a]	Brazil	KX906253	KX906260
C. renweii[a]	China	DQ463352	DQ463333
C. setosus[a]	Jamaica	DQ463349	DQ463331
C. stercoreus	China	DQ463354	DQ463338
C. subglobisporus[a]	Thailand	EF613553	EF613554

[a] Refers to type specimen. New sequences obtained in this study are in bold.

2. Taxonomy

Cyathus badius Kobayasi, Botanical Magazine, Tokyo 51: 755 (1937). Figs. 1 and 2.

MycoBank no.: MB258093.

Peridium infundibuliform, (4.64–)6.72–9.28 mm in height, (3.84–)4.48–6.24 mm in width at the upper part, not expanded in the mouth or tapering abruptly at the base. Emplacement

Please cite this article in press as: da Cruz, R. H. S. F., et al., Rediscovery of *Cyathus badius*, an 'extinct' species from the Bonin Islands, Japan, Mycoscience (2017), https://doi.org/10.1016/j.myc.2017.07.004