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NOTE

Molecular characterization of stinkhorn fungus *Aseroë coccinea* Imazeki et Yoshimi ex Kasuya 2007 (Basidiomycota: Agaricomycetes: Phallales) from India

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The fungal family Phallaceae, commonly known as stinkhorn mushrooms, is a member of order Phallales in the division Basidiomycota. Although the *Dictionary of fungi* (Kirk et al. 2008) recognized 77 species under 21 genera, subsequent description of a new genus (Cabral et al. 2012), new species (Gogoi & Parkash 2015; Trierveiler-Pereira et al. 2017) and new distributional records (Gogoi & Parkash 2014; Kour et al. 2016) points to knowledge gap regarding the diversity and distribution of members within Phallaceae. One of the interesting genera of Phallaceae is the pantropical *Aseroë*, which is morphologically characterized as having a fruiting body consisting of pseudo-stipe that is partly covered at the top by a disc from the margin of which spring numerous, long, acute, fundamentally paired arms, which adopt a horizontal position at maturity; its gleba is located on the upper surface of the disc and adaxial faces of the arms (Dring 1980). While describing the species *A. coccinea*, Kasuya (2007) considered four valid species within the genus, namely, *A. arachnoidea*, *A. coccinea*, *A. floriformis* and *A. rubra*, however, recent phylogenetic studies (Cabral et al. 2012; Trierveiler-Pereira et al. 2014) suggested that *Aseroë* is not monophyletic and *A. arachnoidea* was transferred to *Lysurus* (Trierveiler-Pereira et al. 2014), while *A. floriformis* was transferred to *Abrachium* (Cabral et al. 2012). While there is no issue regarding the generic status of the name bearing type *Aseroë rubra*, generic status of *A. coccinea* has not been assessed using molecular methods.

In India, there are reports of *Lysurus arachnoideus* and *A. rubra* (Narasimhan 1932; Iyengar & Krishnamurthy 1954; Vasudeva 1962; Mohanan 2011a,b; Pradhan et al. 2012), however, there are no records of *A. coccinea*. In fact, to our knowledge, there are no reports of *A. coccinea* from anywhere outside its type locality in Japan. In the current communication, we provide the first report of *A. coccinea* from northern Western Ghats of India and provide its phylogenetic placement based on nuclear internal transcribed spacer region.

Six specimens of *A. coccinea* were observed at Khandobacha Mal (18.252ºN, 73.674ºE, 830m), at the base of Rajgad fort, Pune, India. Two specimens were collected in a clean bottle. A small piece of stipe from each specimen was preserved in absolute ethanol for

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Image 1. *Aseroë coccinea* from northern Western Ghats. (a–d) Basidiome of *Aseroë coccinea*: a & b—same individual from side and top view, respectively | e—cross section of arm showing a single chamber | f—basidiospores. © Vivek Bobade.

genetic study, while the specimens were dried for long term preservation. One of the collected specimens is deposited in the culture collection of Ajrekar Mycological Herbarium (AMH), National Fungal Culture Collection of India (NFCCI & FIS), Biodiversity and Palaeobiology Group, MACS-Agharkar Research Institute, Pune, India, under the accession number AMH 9967.

DNA was extracted from two specimens using QIAamp DNA Mini Kit following manufacturer's protocol. The nuclear gene encoding small-subunit ribosomal RNA (18S rRNA) was amplified using the primer pair A (5'-CCA ACC TGG TTG ATC CTG CCA GT-3') and B (5'-GAT CCT TCT GCA GGT TCA CCT AC-3') (Berger et al. 1998). The internal transcribed spacer (ITS) region in the nuclear ribosomal repeat unit was amplified using primer pair ITS1f (5'-CTT GGT CAT TTA GAG CGA AGT A-3') (Gardes & Bruns 1993) and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al. 1990), which included partial 18S, complete ITS1, complete 5.8S, complete ITS2 and partial 28S. Protocol for PCR amplification, PCR product purification and DNA sequencing follow Suranse et al. (2017) with the annealing temperature 55ºC for 18S and 50ºC for ITS. Sequences generated as a part of this study are deposited in GenBank under the accession numbers MK543504–MK543505 for 18S and MK541641–MK541642 for ITS.

Since limited genetic data were available for 18S gene, genetic analysis was performed only for ITS region.

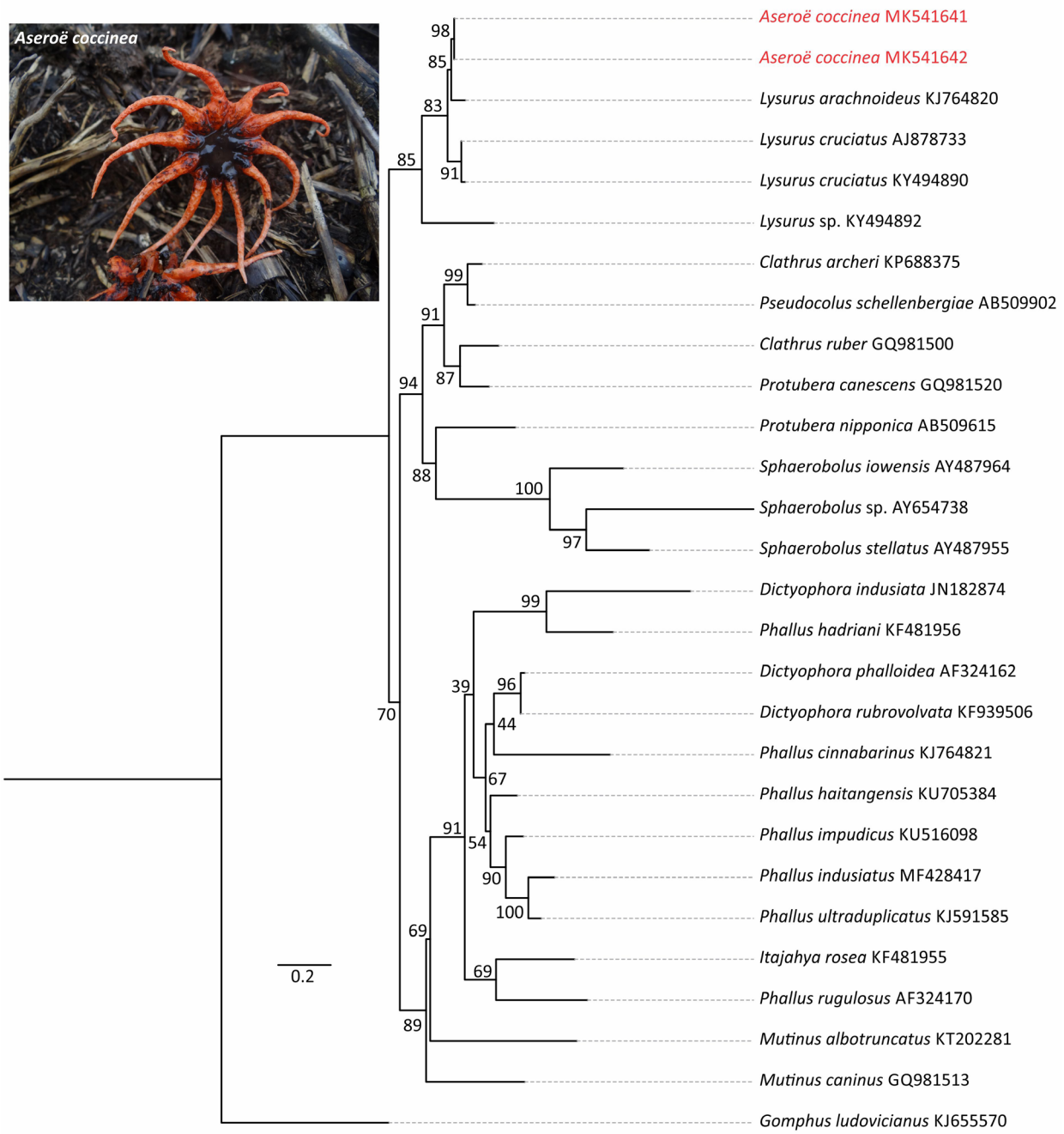

Figure 1. Maximum likelihood tree based on internal transcribed spacer region including 5.8S ribosomal RNA gene using the best nucleotide substitution model TNe+I+G4. *Gomphus ludovicianus* is used as an outgroup. Values along the nodes are percentage bootstrap based on 1000 replicates. GenBank accession numbers are provided after the species names.

Additional ITS sequences for Phallales were downloaded from NCBI database (https://www.ncbi.nlm.nih.gov/). *Gomphus ludovicianus* (Gomphales: Gomphaceae) was used as an outgroup following Trierveiler-Pereira et al. (2014). Sequences were aligned using MUSCLE (Edgar 2004) implemented in MEGA 7 (Kumar et al. 2016). Model for nucleotide substitution pattern was determined using ModelFinder (Kalyaanamoorthy et al. 2014) based on Bayesian Information Criterion (Schwarz 1978) and was used for constructing maximum likelihood tree in IQTree (Nguyen et al. 2015) with ultrafast bootstrap (Hoang et al. 2018) support for 1000 iterations. Phylogenetic tree was viewed and edited in FigTree (Rambaut 2009). Raw genetic p distance was calculated using MEGA 7 (Kumar et al. 2016).

Six fruiting bodies of *A. coccinea* were found

growing on donkey dung in dry deciduous forest floor at Khandobacha Mal. Basidiomes of four specimens are shown in Image 1a–d. Observed specimens showed following morphology. Basidiome terrestrial, gastrocarpic appearance, approximately 30–60 mm in height; gleba horizontally expanded, origin at the base of the arms, and at the top of the stipe covering the disc at the upper surface of receptacle, olivaceous green to dark green in color, mucoid, granular; receptacle white in color, cylindrical, spongy, hollow with single chamber, at the apex flattened to form a disc, about 10–12 mm in diameter; arms arise from the tip of receptacle, 25–50 mm in length, single arms arise from the margin of horizontal discoid portion, diameter at the base 5 mm in the middle 3mm and at the tip less than 1mm, single chambered (Image 1e), nonbifurcating, 9 (n = 1), 11 (n = 4) or 13 (n = 1) arms, it diameter at the base about 5mm, in the middle about 3mm, at the tip less than 1mm, red color on the dorsal surface, pale red to white ventrally, at maturity the arms are fully expanded; basidiospores hyaline (Image 1f), cylindrical or elongated in shape, mean spore dimensions were 4.4 (sd 0.3) × 1.9 (sd 0.2) µm , average spore quotient (length/width) of 2.4 (sd 0.2); spread gregarious as well as solitary; saprophytic, growing on donkey dung among the grasses close to the ground (epigeal).

Morphologically, the species closely resembles the original description of *A. coccinea* (see Kasuya 2007) except for the number of arms that varied from 9–13 in our specimens as appose to 7–9 arms reported in the original description. Further, the specimens we observed were slightly larger than the type of *A. coccinea*. The specimens in our collection differ from *A. rubra* in having nonbifurcating and single chambered arms versus bifurcating and several chambered arms in *A. rubra* (Kasuya 2007; Hemmes & Desjardin 2009). Further, the specimens in our collection differs from morphologically closely related species *Lysurus arachnoideus* in having dorsally reddish arms versus white arms in *L. arachnoideus* (Kasuya 2007; Hemmes & Desjardin 2009) and basidiospores larger and ellipsoid to cylindrical (Image 1f) 3.6–5.0 × 1.5–2.4 µm versus 2.5–3.5× 1.5 µm in *L. arachnoideus* (Kasuya 2007).

Our communication provides the first report of *A. coccinea* from northern Western Ghats of India and first report of this species under this nomen from outside its type locality in Japan. It is essential to note, however, that the earlier report of *L. arachnoideus* (as *Aseroë arachnoidea*) from Karnataka by Narasimhan (1932) needs a critical evaluation. Description of anomalous specimen of *L. arachnoideus* by Narasimhan (1932) with red colored arms has a close resemblance with the description of *A. coccinea* from our study. In fact, while describing the species *A. coccinea*, Kasuya (2007) pointed out that the description of Indian specimens provided by Narasimhan (1932) shares some characters with *A. coccinea*. We believe that the species collected by Narasimhan (1932) is same as the species in our collection, indicating that *A. coccinea* is distributed in both Maharashtra and Karnataka part of the Western Ghats. One notable difference among the specimens studied by Narasimhan (1932), type studied by Kasuya (2007) and our observation is the size of the fruiting bodies. Type studied by Kasuya (2007) is a small specimen as compared to the specimens we studied from northern Western Ghats, while the specimen dimensions provided by Narasimhan (1932) are very large. For instance, Kasuya (2007) reports the length of arm as 4 to 10 mm, we report it as 25–50 mm, while Narasimhan (1932) reports it as 38 to 40 mm. Because the number of specimens studied in all three studies are limited the variation in the size cannot be explained at the moment.

Genetically, *A. coccinea* from our study is sister taxon to *L. arachnoideus* (Figure 1). Nevertheless, the two species are separated by a raw genetic distance of 5.4%. This result is not very surprising, because even in the original description of *A. coccinea*, Kasuya (2007) pointed out close resemblance between the two species. The fact is, however, that *A. coccinea* is nested within the well supported *Lysurus* clade, suggests that the species needs to be transferred to the genus. In the current communication, we refrain from transferring *A. coccinea* to *Lysurus* because of two reasons, (1) there is need for detailed taxonomic study of the group, preferably including the material from the type locality, for delimiting the generic boundary for *Aseroë* by studying its type species *A. rubra* from throughout its distributional range and reassessment of *A. coccinea*, and (2) taxonomic sampling for the genetic analysis in our study is not adequate because of limited information available on 18S and ITS markers of family Phallaceae. Nevertheless, we make the data available for two genetic markers, 18S and ITS region, of specimens from our study, which can facilitate further comparative genetic studies on this group.

Our report of *A. coccinea*, along with its genetic information, suggests that there is a need for more exploratory surveys for understanding diversity, distribution and taxonomy of Phallales of India.

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