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Coltricia sharmae, a new species of Hymenochaetaceae from Indian Himalaya

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ABSTRACT: *Coltricia sharmae* is proposed here as a new species based on morphological characteristics and phylogenetic analyses of the internal transcribed spacer of the nuclear ribosomal DNA region. The combination of morphological and molecular data confirmed the novelty of the species and its infrageneric placement within genus *Coltricia*. This species was found in association with *Rhododendron arboreum* and *Quercus leucotrichophora* and mainly characterized by annual basidiome, regularly circular pileus, concentrically zoned with dark brown rings at the center and fade orange brown at the margins, irregularly angular pores and globose to sub-globose basidiospores.

KEY WORDS: *Coltricia abieticola*, nrITS, Phylogeny, Polypore, Taxonomy, Uttarakhand.

INTRODUCTION

Coltricia Gray is a genus within the order Hymenochaetales that has a global distribution (Larsson *et al*., 2006), with *C. perennis* (L.) Murrill serving as its type species. Most species in this genus are found on the ground and some have been linked to plant roots, suggesting a potential mycorrhizal relationship, while others have been found on wood (Tedersoo *et al*., 2007). The genus is primarily distinguished by its diverse basidiocarps, being resupinate, effuse-reflexed, pendent or stipitate and having a porioid or lamellate hymenial surface and monomitic hyphal system that lacks clamp connections. The basidiospores are generally pigmented and may be either smooth or ornamented (Corner, 1991; Dai, 2010; Ryvarden, 2004).

Coltricia has been relatively understudied in India, with only ten species of the genus reported in the country to date (Adarsh *et al*., 2018; Baltazar and Silveira, 2012; Kaur *et al*., 2016; Kour *et al*., 2015; Pongen *et al*., 2018; Patil *et al*., 2024). During macrofungal surveys in Ali-Bedni Bugyal trek, Uttarakhand, a unique species of *Coltricia* was collected from mixed broadleaf forests of the Didna region. Detailed macro- and micromorphological descriptions along with, nrITS-based phylogenetic analyses revealed this species to be distinct from other species in the genus *Coltricia*.

MATERIAL AND METHODS

Macro- and micromorphology

Fresh basidiomes were gathered and photographed in their natural setting using a Nikon D5300 camera. Macromorphological descriptions along with habitat and associated hosts were recorded from fresh specimens in the field, the freshly obtained specimens were thoroughly examined macromorphologically before being dried in a portable dyer so that micromorphological study could be conducted on them. The dryer was kept at a temperature between 45ºC to prevent the tissue from being burned. The terminology of macromorphology is in accordance with Vellinga (1988) and Heilmann-Clausen *et al*. (1998). Colour codes were designated according to Kornerup and Wanscher (1978). The various spot chemical tests (10% KOH, $FeSO_4$) for colour reaction on fresh specimens were performed on the pileus surface, stipe surface and context. Micromorphological characteristics were observed on sections mounted in 10% potassium hydroxide (KOH), 1% Congo red, and 1% phloxine, and observed under an Olympus BX43 microscope. Amyloidity was tested using Melzer's reagent (Largent *et al*., 1977). Microphotography was conducted with an Olympus CH33 microscope, and micromorphological elements were drawn using a camera lucida at 1000× magnification. For each specimen, 50 basidiospores were measured from each of the three specimens. Basidiospore dimensions were recorded as (min–) av.-min – av.-max (–max) length × (min–) av.-min – av.-max (–max) width, and Q = (min–) av. (–max) for the total basidiospores measured, with Q representing the ratio of length to width. At least 20 measurements were taken for basidia, excluding the length of sterigmata, and for the diameter of various hyphal arrangements. The holotype of the species has been deposited in Central National Herbarium (CNH), Howrah, West Bengal, (acronym CAL).

DNA Extraction, PCR amplification and sequencing

The fungal genomic DNA Mini Kit was utilized to extract nuclear genomic DNA from 100 mg of dried fruiting bodies. The ITS region of the nuclear ribosomal

DNA gene was amplified using the primer pairs ITS1 and ITS4 (White *et al*., 1990; Gardes and Bruns, 1993). PCR amplification was conducted in a 20 µl reaction volume, containing 1X Phire PCR buffer, 0.2 mM dNTPs, 1 µl DNA, 0.2 µl PhireHotstar II DNA polymerase enzyme, 0.1 mg/ml BSA, 3% DMSO, 0.5M betaine, and 5 pM of both forward and reverse primers. The PCR was performed in a thermal cycler (Applied Biosystems, Gene Amp PCR System 9700) with the following settings: 2 minutes at 96°C, followed by 30 cycles of 30 seconds at 96°C, 40 seconds at 50°C, and a final extension of 4 minutes at 60°C. PCR products were purified using the QIAquick Gel Extraction Kit (QIAGEN, Germany) and subsequently sequenced via Sanger sequencing using an automated DNA sequencer (AB13730xl DNA Analyzer, Applied Biosystems, USA) with the same primers used for amplification. All ITS sequences obtained were submitted to GenBank, and accession numbers were obtained.

Phylogenetic analysis

To ascertain the phylogenetic placement of the species, a phylogenetic analysis was conducted using nrITS sequence data. The dataset included 63 ITS sequences and reference sequences obtained from a BLAST search (Altschul *et al*., 1997) in GenBank (Clark *et al*., 2016) and relevant published phylogenies (Bian and Dai, 2017, 2020; Bian *et al*., 2022; Patil *et al*., 2024). *Fomitiporella chinensis* (Pilát) Y.C. Dai, X.H. Ji & Vlasák was selected as outgroup. The dataset was aligned using MAFFT v. 7.427 (Katoh and Standley, 2013) with default settings. The aligned dataset underwent Maximum Likelihood (ML) analysis in RAxML GUI 2.0 (Edler *et al*., 2021), with 1,000 bootstrap (BS) replicates performed to obtain nodal support values (Figure 1).

RESULTS

Phylogenetic inferences

Phylogenetic analysis reveals genetic closeness between *Coltricia sharmae* (represented by PQ050733 and PQ050734), *C. abieticola* Y.C. Dai (represented by KU360673, KU360674, KU364784 and KU364785), *C. weii* Y.C. Dai (represented by KX364796, KU360698 and KX364797) and C. *subperennis* (Z.S. Bi & G.Y. Zheng) G.Y. Zheng & Z.S. Bi (represented by KY693736 and OM959391) as they all nested within a same clade and *C. sharmae* well separated with a nodal support value of 100% (Figure 1).

TAXONOMIC TREATMENT

Coltricia sharmae Choudhary & Uniyal, ***sp. nov.***

Figs. 1–3

MycoBank no: MB855416

Diagnosis: Similar to *Coltricia abieticola* but differs by having smaller basidiome pileus (35–54 mm in diameter), regularly circular in shape with a less depressed concentrically zone with dark brown rings at centre and fade orange brown at the margins, irregularly angular pores and globose to sub-globose basidiospores.

Type: INDIA, Uttarakhand, Chamoli district, Lohajung, Kuling, Didna trek, 30°09'55.39"N & 79°38'00.03"E, elev. 2463 m, 06 August 2023, *Shikha Choudhary* and *Priyanka Uniyal* SC/PU/29 (CAL 2010, holotype).

GenBank Number: nrITS PQ050733

Etymology: In honor of Dr. Yash Pal Sharma for his invaluable contribution to the systematics of macrofungi in the Indian Himalayan region.

Description: Basidiomes annual, centrally stipitate, and can be solitary, leathery with fine hairs when fresh but becoming hard, corky, or brittle and lightweight when dried. Pileus generally circular, ranging from flat to depressed at centre, 35–54 mm in diameter. Upper surface finely velvety to tomentose, with dense hairs at the center, becoming smooth with age and varying in color, concentrically zoned from dark brown (7F7) to chestnut brown (7E5–7E8) at centre to light orange brown (5A5–5B7) at the margins when fresh, and turning brownish black (7F8) on drying. Margin incised, appressed when dry. Pore surface dark brown (7E7) at the centre to light orange brown at the margins (6C8). Pores irregularly angular, 3–6 per mm, dissepiments thin, entire. context dark brown (6D8) leathery, up to 3 mm thick; tubes 1–3 mm long, decurrent. Stipe concolorous to the pileus surface, cylindrical, velvety to tomentose, often branched near the apex, corky to leathery when dry, 30–52 × 5–8 mm, with a mostly up to 8 mm swollen tip.

Hyphal system monomitic, with generative hyphae having simple septa. Contextual hyphae golden brown, branched at wide angles, fairly thick-walled with a broad lumen, 4–5.6 µm wide. Stipe hyphae golden brown, thick-walled with a narrow lumen, notably narrower than the contextual hyphae, running parallel along the stipe, unbranched and 6.8–7.8 µm wide. Tramal hyphae pale yellow to buff yellow, slightly thick-walled, with wide lumen, moderately branched and loosely interwoven to subparallel, 7.7–10.6 µm wide. Cystidia and cystidioles absent. Basidia measuring 18.9–28.4 × 8.5–9.1 µm, broadly clavate, 4-spored. Basidioles slightly smaller, 15.3–22.5 × 5.6–7.8 µm, and similar in shape to basidia. Basidiospores (60/2/3) 6.7–**7.7**–9.6 × 4.9–**5.7**–7.7 µm (n=60, Q= 1.15–**1.35**–1.81) globose to subelliptical, golden brown, smooth, thick-walled, inamyloid.

Habit and Habitat: Terrestrial, solitary to gregarious under mixed forest of *Quercus leucotrichophora* A. Camus and *Rhododendron arboreum* Sm.

Distribution: Hitherto known only from India.

Additional specimen examined: India, Uttarakhand, Chamoli, Lohajung, Ajan top, 30˚07′28.63″N 79˚35′40.17″E, elev. 2384 m, 08 August 2023, *Shikha Choudhary* and *Priyanka Uniyal*, SC/PU/30. GenBank Number: nrITS PQ050734.

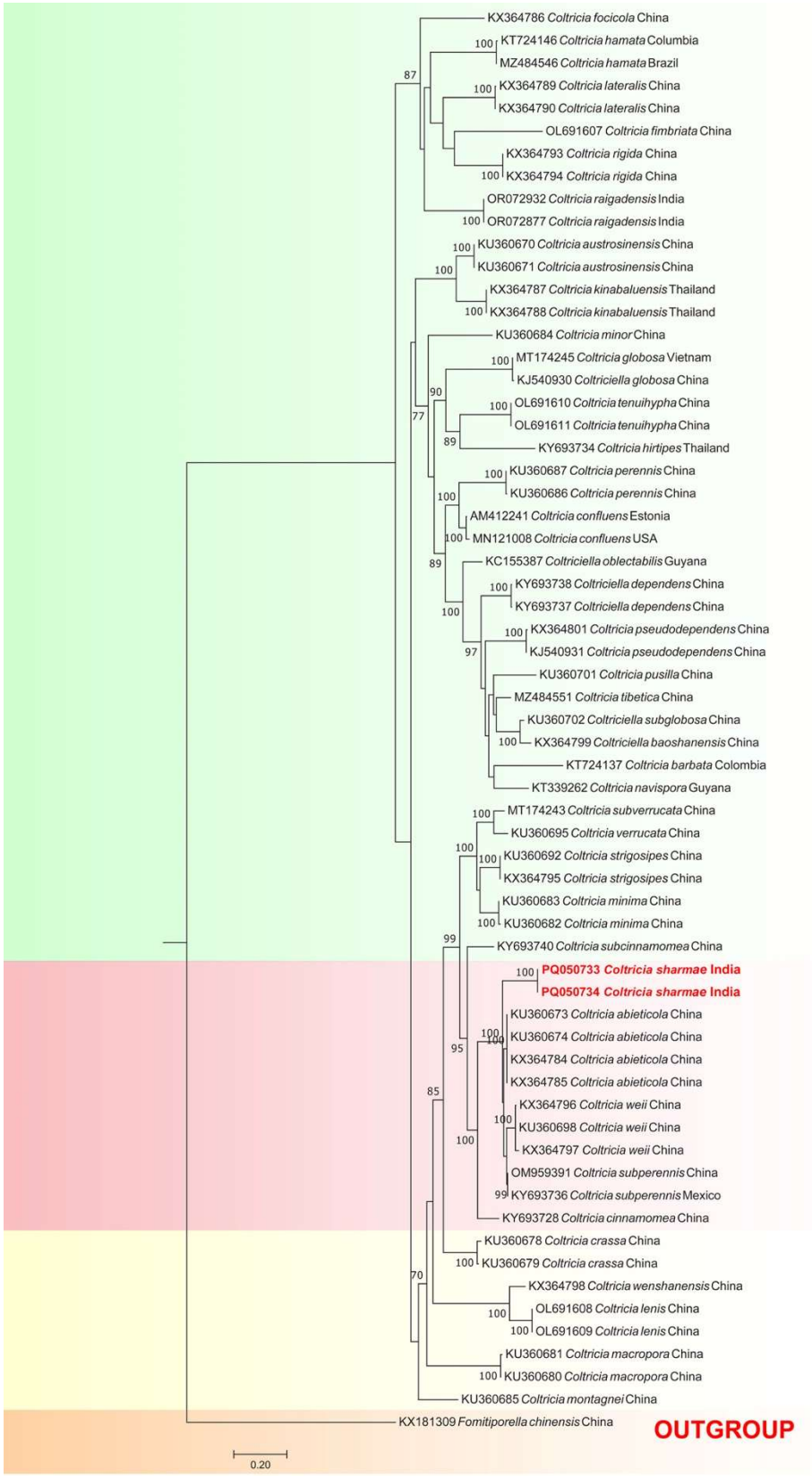

Fig. 1. Maximum Likelihood phylogenetic tree inferred from ITS-rDNA sequence data using GTR+GAMMA model of nucleotide evolution constructed in RAxML v.2.0.10. Branches are labelled with ML bootstrap support values (≥50 %). Sequence derived from ***Coltricia sharmae*** sp. nov. is shown as bold in the tree.

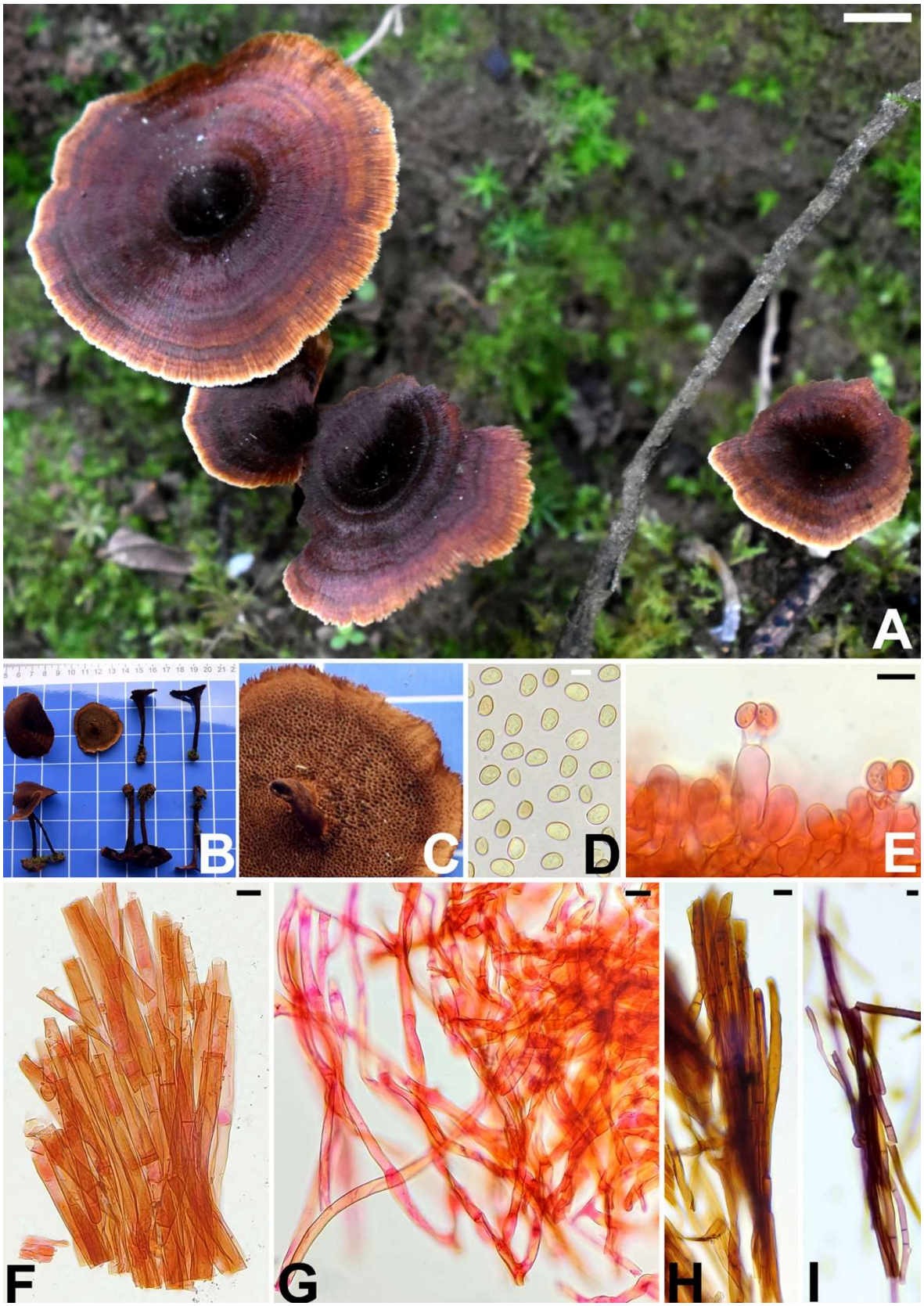

Fig. 2. ***Coltricia sharmae.:*** **A–C.** Fresh Basidiomes in the field and basecamp; **D.** Basidiospores; **E.** Hymenium showing basidia and basidioles; **F.** Tramal hyphae; **G&H.** Contextual hyphae; **I.** Stipe hyphae. Scale bars: A. = 10 mm, D–I. = 10 µm.

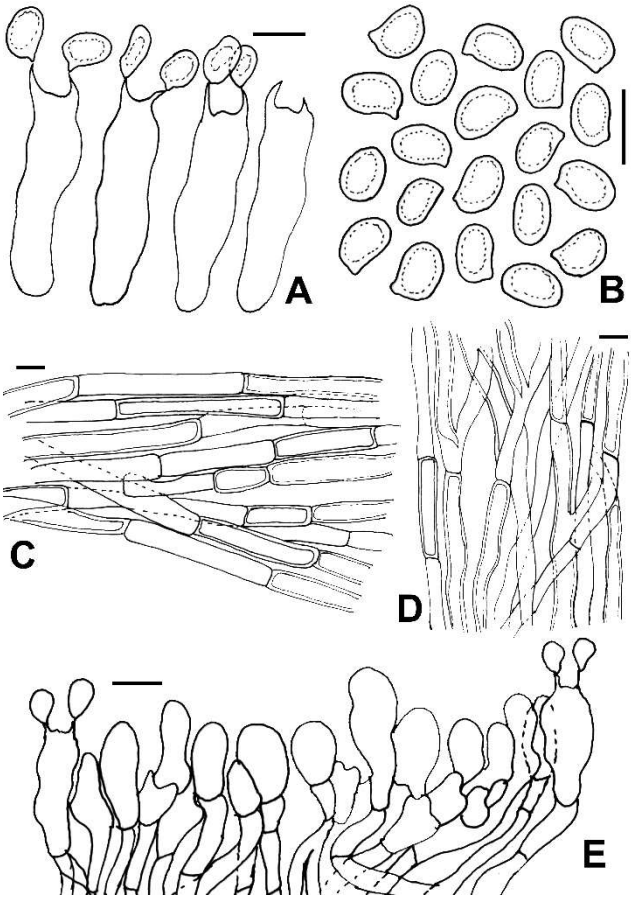

Fig. 3. ***Coltricia sharmae*** **A.** Basidia; **B.** Basidiospores; **C.** Contectual hyphae; **D.** Tramal hyphae; **E.** Hymenium showing basidia and basidioles. Scale bars: A–E. = 10µm.

DISCUSSION

Phylogenetically, the new taxon is close to *Coltricia abieticola*, *C. weii* and *C. subperennis*. However, *C. abieticola* can be differentiated from *C. sharmae* by its infundibuliform pileus (74 mm diam.), clay-buff when dry, margins curved down when dry; pore surface cinnamon to yellowish brown; angular pores; tube slightly paler and 0.8 mm long, solitary habitat and its association with *Abies* (Dai, 2010). *C. subperennis* can be segregated from the present species by its less circular to infundibuliform pileus (40 mm in diam.), snuff-brown to umber; angular, round, pores 3–5 per mm; *C. weii* can be differentiated from *C. sharmae* by its smaller pileus (30 mm diam.) rust-brown to dark reddish brown, smaller stipe (15 mm) (Dai, 2010). Our new species *C. sharmae* differs from *C. cinnamomea* by having smaller basidiome (40 mm diam.), flat to infuncibuliform, often confluent with a few adjacent ones, brown to deep reddish brown pileal surface with indistinct concentric zones, silky and curved down when dried, yellowish brown to rusty brown pore surface, with margins golden yellow and tubes reddish brown (Dai, 2010).

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