



Neofavolus subpurpurascens comb. nov., with new records from the Neotropics

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Abstract

Polyporus subpurpurascens is a rare Neotropical species characterized by deep purplish pilear surface and radially elongated pores. This study analyzed Neotropical specimens from Argentina, Bolivia and Brazil by applying detailed morphological examination and phylogenetic analyses. We conclude that *P. subpurpurascens* is a species of *Neofavolus*, and *N. subpurpurascens* is proposed as a new combination. New records from Argentina and Bolivia, a detailed description, and photographs of the species are included.

Introduction

Favolus E.M. Fries (1828: 44) and *Neofavolus* Sotome & T. Hatt. in Sotome *et al.* (2013: 249) are white rot polypore genera with similar annual flabelliform basidiomes that are laterally stipitate without black crust on the stipe surface. Microscopically, they are characterized by a dimitic hyphal system with skeletal-binding hyphae and hyaline thin-walled cylindrical basidiospores (Sotome *et al.* 2013). A glabrous pilear surface and a cutis pileipellis with agglutinated hyaline to brown generative hyphae differentiate *Neofavolus* from *Favolus*. In contrast, the pileipellis in *Favolus* is composed of non-agglutinated hyaline hyphae.

Originally, *Neofavolus* was exclusively described as having poroid basidiomes and being distributed in temperate areas. *Neofavolus mikawae* (Lloyd) Sotome & T. Hatt. in Sotome *et al.* (2013: 251) and *N. cremeoalbidus* Sotome & T. Hatt. in Sotome *et al.* (2013: 250) have angular pores and *N. alveolaris* (DC.) Sotome & T. Hatt. in Sotome *et al.* (2013: 250) have radially elongated pores. However, *N. suavissimus* (Fr.) J.S. Seelan, Justo & Hibbett in Seelan *et al.* (2015: 468), a subporoid lamellate species, was recently included in the genus *Neofavolus* (Seelan *et al.* 2015).

Polyporus subpurpurascens (Murrill) Ryvardeen (1985: 181) is a rare species described from Jamaica (Murrill 1907), characterized by a deep purple pilear surface. *Polyporus* is a polyphyletic genus (Sotome *et al.* 2008, Krüger *et al.* 2006, Sotome *et al.* 2011, Dai *et al.* 2014, Seelan *et al.* 2015) and the phylogenetic relationships of *P. subpurpurascens* remain unknown. We aim to perform accurate morphological and molecular analyses of specimens identified as *P. subpurpurascens* from Argentina, Bolivia and Brazil in order to access their phylogenetic position.

Material and Methods

Specimens

Specimens of several herbaria (ICN, LIL and CORD) collected from Northwest Argentina, Bolivia and South Brazil were included in this study. Freehand cross sections of dried materials mounted in Melzer's reagent, 5% KOH and/or 1% phloxine, lactophenol, cresyl blue and/or cotton blue –(CB) were observed under the microscope.

DNA extraction, PCR amplification and sequencing

DNA was extracted from the dried specimens using CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) and FH plant DNA kit II (Demeter Biotech Co., Ltd., Beijing, China),

following the manufacturers protocols. Primer pairs ITS4/ITS5 (White *et al.* 1990) and LR0R/LR7 (Vilgalys & Hester 1990) were used to amplify the nrITS and nrLSU regions, respectively, by a qualitative simplex polymerase chain reaction. The polymerase chain reaction (PCR) protocol for ITS was the following: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 94 °C for 40 s, 54 °C for 45 s, and 72 °C for 1 min, and final extension of 72 °C for 10 min. The PCR procedure for 28S was the following: initial denaturation at 94 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 1 min, and 72 °C for 1.5 min, and final extension of 72 °C for 10 min. The PCR products were purified and sequenced with the same primers at Beijing Genomics Institute in China.

Phylogenetic analyses

Sequences were manually edited using Geneious v. 11.1.4 (<http://www.geneious.com>, Kearse *et al.* 2012). The generated sequences, including related sequences downloaded from GenBank (Table 1), were aligned using Mafft v.7 (Katoh & Standley 2013). Besides, the Q-INS-I strategy was adopted for nrITS and G-INS-i, for nrLSU. The alignments were manually examined and adjusted with MEGA 6 (Tamura *et al.* 2013). We used a combined dataset with nrITS and nrLSU. The dataset was divided into four data partitions: ITS1, 5.8S, ITS2 and LSU. The best-fit model of nucleotide evolution for each partition was selected according to the Akaike Information Criterion and using jModelTest2 v.1.6 (Darriba *et al.* 2012; Guindon & Gascuel, 2003) as available in CIPRES Science Gateway 3.1 (Miller *et al.* 2010). The final alignments were submitted to TreeBASE (submission ID: 22972). Both Bayesian Inference (BI) and Maximum Likelihood (ML) phylogenetic analyses were carried out. BI was conducted using MrBayes 3.2.6 (as available in CIPRES Science Gateway 3.1) and implementing two independent runs, each with four chains and starting from random trees. The runs produced 20,000,000 generations and trees were sampled every 1000th generation. Twenty five percent of the sampled trees were discarded as burn-in, while the rest were used for calculating a 50% majority consensus tree and Bayesian Posterior Probabilities (BPP). ML trees were obtained using RAxML v.8.1.4 (Stamatakis 2014) from CIPRES Science Gateway. The analysis first involved 100 ML searches, each one starting from a randomized stepwise-addition parsimony tree in a GTRGAMMA model with no proportion of invariant sites and all the other parameters estimated by the software. We provided a partition file to force RAxML software to search for a separate evolution model in each dataset. Bootstrap support values (BS) were obtained with multi-parametric bootstrapping replicates under the same model, allowing the program halts bootstrapping automatically by the autoMRE option. A node was considered to be strongly supported if it showed a BPP 0.95 and/or BS 90%, while moderate support was considered when BPP < 0.95 and/or BS < 90%. Based on previous studies, *Trametes conchifer* (Schwein.) Pilát was used as outgroup (Zhou & Cui 2018).

TABLE 1. List of species, specimen-voucher information, geographic origin, and GenBank accession numbers of sequences used in the phylogenetic analyses in this study.

Species	Voucher	Origin	Genbank accession numbers		References
			nrITS	nrLSU	
<i>Datronia mollis</i>	RLG6304sp	USA	JN165002	JN164791	Justo & Hibbett (2011)
<i>Favolus acervatus</i>	Cui11053	China	KU189774	KU189805	Zhou <i>et al.</i> (2016)
<i>Favolus acervatus</i>	Dai10749b	China	KX548953	KX548979	Zhou & Cui (2018)
<i>Favolus brasiliensis</i>	TENN10242	Costa Rica	AB735976	AB368097	Sotome <i>et al.</i> (2013)
<i>Favolus brasiliensis</i>	INPA241452	Brazil	AB735977	AB735953	Sotome <i>et al.</i> (2013)
<i>Favolus emeric</i>	Cui10926	China	KU189776	KU189807	Zhou <i>et al.</i> (2016)
<i>Favolus emeric</i>	Yuan4410	China	KX548954	KX548980	Zhou & Cui (2018)
<i>Favolus niveus</i>	Cui11129	China	KX548955	KX548981	Zhou & Cui (2018)
<i>Favolus philippinensis</i>	Cui10941	China	KX548976	KX548998	Zhou & Cui (2018)
<i>Favolus philippinensis</i>	Dai7959	China	KX548977	KX548999	Zhou <i>et al.</i> (2016)
<i>Favolus niveus</i>	Dai13276	China	KX548956	KX548982	Zhou & Cui (2018)
<i>Favolus pseudobetulinus</i>	TRTC51022	Canada	AB587629	AB587620	Sotome <i>et al.</i> (2011)
<i>Favolus pseudobetulinus</i>	TFMF27567	Japan	AB587644	AB587639	Sotome <i>et al.</i> (2011)
<i>Favolus pseudoemeric</i>	Cui11079	China	KX548958	KX548984	Zhou & Cui (2018)
<i>Favolus pseudoemeric</i>	Cui13757T	China	KX548959	KX548985	Zhou & Cui (2018)
<i>Favolus roseus</i>	PEN33	Malaysia	AB735975	AB368099	Sotome <i>et al.</i> (2008)

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TABLE 1. (Continued)

Species	Voucher	Origin	Genbank accession numbers		References
			nrITS	nrLSU	
<i>Favolus spatulatus</i>	Dai13615	China	KU189775	KU189806	Zhou <i>et al.</i> (2016)
<i>Favolus spatulatus</i>	Cui8290	China	KX548969	KX548991	Zhou <i>et al.</i> (2016)
<i>Favolus subtropicus</i>	Cui4292	China	KX548970	KX548992	Zhou & Cui (2018)
<i>Favolus subtropicus</i>	Li1938	China	KX548971	KX548993	Zhou & Cui (2018)
<i>Favolus sp.</i>	MEL2382969	Australia	KP012829	KP012829	GenBank
<i>Lentinus tigrinus</i>	LE214778	Russia	KM411459	KM411475	Zmitrovich & Kovalenko (2016)
<i>Lentinus badius</i>	DED07668	Thailand	KP283480	KP283518	Seelan <i>et al.</i> (2015)
<i>Neofavolus alveolaris</i>	Dai11290	China	KU189768	KU189799	Zhou <i>et al.</i> (2016)
<i>Neofavolus alveolaris</i>	Cui9900	China	KX548974	KX548996	Zhou & Cui (2018)
<i>Neofavolus cremealbidus</i>	TUMH50006	Japan	AB735979	AB735956	Sotome <i>et al.</i> (2013)
<i>Neofavolus cremealbidus</i>	TUMH50008	Japan	AB735981	AB735958	Sotome <i>et al.</i> (2013)
<i>Neofavolus cremealbidus</i>	TUMH50009	Japan	AB735980	AB735957	Sotome <i>et al.</i> (2013)
<i>Neofavolus mikawae</i>	Cui11152	China	KU189773	KU189804	Zhou <i>et al.</i> (2016)
<i>Neofavolus mikawae</i>	Dai12361	China	KX548975	KX548997	Zhou & Cui (2018)
<i>Neofavolus suavissimus</i>	DSH2011	USA	KP283496	KP283525	Seelan <i>et al.</i> (2015)
<i>Neofavolus suavissimus</i>	LE202237	USA	KM411460	KM411476	Zmitrovich & Kovalenko (2016)
<i>Neofavolus subpurpurascens</i>	CG6241	Brazil	MH544274	MH544276	This study
<i>Neofavolus subpurpurascens</i>	CG6242	Brazil	MH544275	MH544277	This study
<i>Neofavolus subpurpurascens</i>	Robledo383	Argentina	—	MH544278	This study
<i>Neofavolus subpurpurascens</i>	Robledo390	Argentina	—	MH544280	This study
<i>Neofavolus sp.</i>	SV10	USA	KP283507	KP283526	Seelan <i>et al.</i> (2015)
<i>Neofavolus sp.</i>	M672	USA	KP283506	KP283524	Seelan <i>et al.</i> (2015)
<i>Polyporus arcularius</i>	Cui10998	China	KX548973	KX548995	Zhou <i>et al.</i> (2016)
<i>Polyporus brumalis</i>	Cui10750	China	KU189765	KU189796	Zhou <i>et al.</i> (2016)
<i>Polyporus tuberaster</i>	Dai12462	China	KU507580	KU507582	Zhou <i>et al.</i> (2016)
<i>Trametes conchifer</i>	FP106793sp	USA	JN164924	JN164797	Justo & Hibbett (2011)

Results

Phylogenetic analyses

The dataset contains 44 sequences and 2060 characters, including gaps. In total, 397 of them are parsimony informative; 1536, constant; and 474, variable. Additionally, TIM3+I+G, TPM2+I, HKY+G, and GTR+I+G were the evolutionary models selected for ITS1, 5.8S, ITS2 and LSU, respectively. The topology of the BI and ML analyses did not reveal any inconsistency in the supported clades, as is shown in the BI tree (Fig 1).

The phylogenetic analyses retrieved *Neofavolus* and *Favolus* as strongly-supported clades (BPP=1, BS =94, and BPP=0.99, BS=93, respectively). Furthermore, six lineages in the *Neofavolus* clade were also retrieved. Four of those lineages contained specimens of *N. alveolaris* (BPP=0.99, BS=98), *N. cremealbidus* (BPP=1, BS =100), *N. mikawae* (BPP=1, BS = 100), and *N. suavissimus* (BPP=1, BS =100). Besides, there was a lineage composed of American specimens of a not-yet-described species of *Neofavolus* (BPP=1, BS =96) that has already been detected (Seelan *et al.* 2015). Finally, a strongly-supported lineage (BPP=1, BS=100) composed of the six specimens collected in Argentina and Brazil was identified as *P. subpurpurascens*.

Taxonomy

Both phylogenetic and morphological analyses revealed that the specimens previously identified as *P. subpurpurascens* belong to the *Neofavolus* clade. As a result, we propose the following new combination:

Neofavolus subpurpurascens (Murril) Palacio & Robledo *comb. nov.*

Mycobank MB 826917

Basionym: *Hexagonia subpurpurascens* Murrill, North American Flora 9(1): 51 (1907).

Synonyms: *Polyporus subpurpurascens* (Murrill) Ryvardeen, Mycotaxon 23: 181 (1985); *Favolus subpurpurascens* (Murrill) Sacc. & Trotter, Syll. fung. (Abellini) 21: 357 (1912).

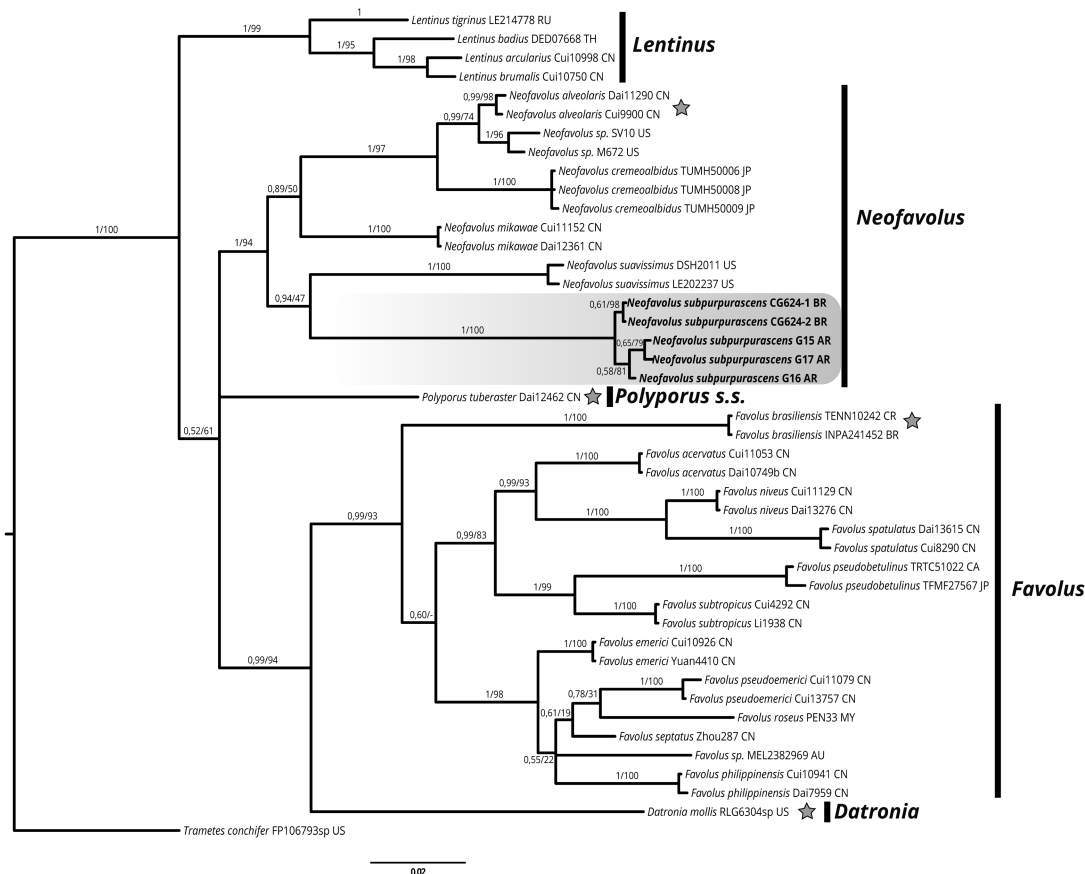


FIGURE 1. Phylogenetic relationships of *Neofavolus* and *Favolus* species inferred from nrITS and nrLSU sequences. Topology from Bayesian Inference analysis is presented. Bayesian posterior probabilities (before the slash markers) and Bootstrap support values (after the slash markers) are indicated. Red stars indicate type species of the genus.

Description:—Basidiome annual, fragile, centrally to laterally stipitate, solitary or clustered in small groups (2–3 basidiomes); pileus circular to reniform, centrally depressed, up to 2 cm diam., 0.1 cm thick, glabrous, azonate, light purplish, slightly tessellate when living, purplish ochraceous and tessellate when dry; stipe up to 2 cm long., 0.3 cm diam., light yellowish brown to purplish, with reddish dyes, more purplish and thicker towards the base, with a whitish attachment disc, smooth to longitudinally rugose when dry; margin acute, incurvated when dry; context thin (0.2 mm thick) to absent. Pore surface light brown yellowish, pores 1–2/mm, angular, hexagonal, radially elongated, decurrent, with hyphal pegs. Hyphal system dimitic: generative hyphae clamped, hyaline, thin walled, 3–6 µm diam., skeleto-binding hyphae arboriform, hyaline to yellowish, thick walled, with a wide lumen 3–6 µm diam. (in the principal stalk) or 2–3 µm diam. (in the branches), IKI–. Hyphal pegs 23–26 µm diam., composed of generative hyphae. Pileipellis present as a cutis composed of light brown, parallel, agglutinated, thick-walled generative hyphae, distinct from the contextual hyphae, which are hyaline, interwoven and non-agglutinated (Fig. 2). Basidia 18–22 × 6–8 µm, 4-sterigmate, clavate. Basidiospores 9–12 × 2.5–4 µm, Q= 3.2, n= 100/5, narrowly cylindrical, with a slightly supra-apical depression, smooth, thin walled, hyaline, guttulate, IKI–.

Geographical distribution:—*Neofavolus subpurpurascens* is a rare species, described from Jamaica and also recorded in Brazil (Coelho & Silveira 2014). This is the first record from Argentina and Bolivia.

Remarks:—*Neofavolus subpurpurascens* is a species recognized by its purplish basidiomes, compared to other *Neofavolus* taxa, which are white to cream or brownish (Sotome *et al.* 2013; Seelan *et al.* 2015). The specimens under study match the macro and microscopical descriptions based on the type collection provided by Ryvarden (1985) and (Coelho & Silveira 2014). Also we examined type material through NY virtual herbarium. Additionally, we found that the pilear surface is a cutis composed of light brown, parallel, agglutinated, thick-walled generative hyphae (Fig. 2c). Specimens:—ARGENTINA. Jujuy: Ledesma, Parque Nacional Calilegua, Mesada de las Colmenas, La Cascada trail, on dead twig, 1170 m, 23°42'1.5"S, 64°51'56.8"W, 6 March 2005, *Robledo 383* (CORD); *Robledo 385* (CORD); *Robledo 390* (CORD);—BOLIVIA. La Paz: Nor Yungas, Rio Yariza, 23 February 1956, *Singer B1346* (LIL);—BRAZIL. Rio Grande do Sul: Santa Maria, Seminários São José, 23 March 2007, *Coelho & Cortez 624–1* (ICN); *Coelho & Cortez 624–2* (ICN).

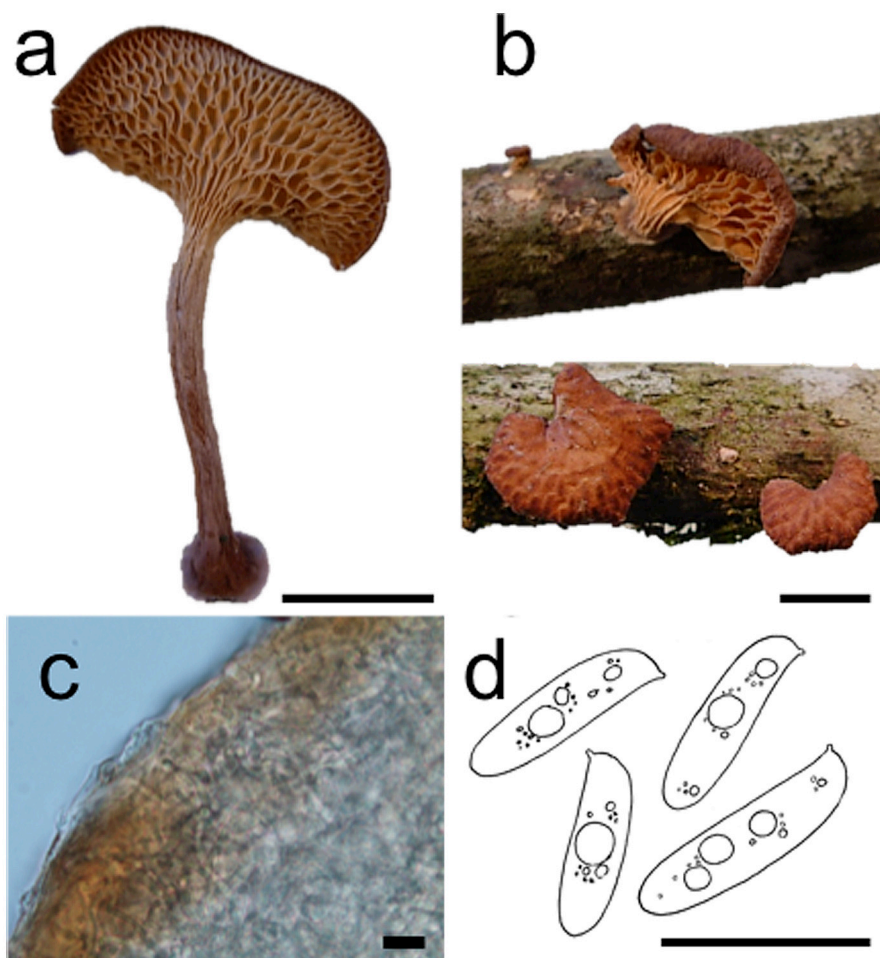


FIGURE 2. Morphological characteristics of *N. subpurpurascens*. **a.** pore surface detail. (Robledo383). **b.** pilear surface detail (Robledo390). **c.** pileipellis detail (CG624–1), cutis composed of light brown, parallel, agglutinated, thick-walled generative hyphae. **d.** basidiospores (CG624–1). Scale bar: **a,b**=1cm; **c,d**=10 μ m.

Discussion

Neofavolus subpurpurascens is the only neotropical species of *Neofavolus* and a rare species that has been collected few times in South America. Other *Neofavolus* species have been found in temperate regions (Sotome *et al.* 2013) but not in the tropics. Specifically, *Neofavolus alveolaris* and *N. suavissimus* are known in the Northern Hemisphere (North America, Europe and Asia), *N. mikawae* was recorded in China and Japan, and *N. cremeoalbidus* is restricted to temperate areas in Japan (Sotome *et al.* 2013).

Previously, *N. subpurpurascens* was mentioned as a member of the morphological group of *Favolus* (Ryvarden & Iturriaga 2003, Drechsler-Santos *et al.* 2008) and also suggested as a species of *Favolus* (Sotome *et al.* 2013, Coelho & Silveira 2014). However, none of those previous studies considered the pilear surface or the phylogenetic position of these species. In this study, we discovered that the pilear surface of *N. subpurpurascens* is a cutis composed of hyphae distinct from the contextual hyphae, as in other *Neofavolus spp.* (Sotome *et al.* 2013).

Therefore, the pilear surface is a useful and consistent feature to recognize *Neofavolus* species. Furthermore, the morphology of this surface has been highlighted as a strong feature in the classification of some polypore groups (Costa-Rezende 2016, Torres-Torres & Guzmán-Dávalos 2012). However, this surface in *Polyporus s.l.* is scarcely described. *Neofavolus* is the single genus in the *Polyporus s.l.* genera whose pilear surface has been accurately examined and compared. Considering the complexity of *Polyporus s.l.* species, pilear surface morphology is a recommended feature to validate and compare genera and species.

Since *Polyporus s.l.* is a polyphyletic and morphologically heterogeneous group, accurate morphological examination (e.g. Sotome *et al.* 2013, Palacio *et al.* 2017) may support the identification of morphological patterns.

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