Protistology 13 (3), 126–132 (2019)

Protistology

A new species of *Diderma* from Bidoup Nui Ba National Park (southern Vietnam)

Yuri K. Novozhilov, Ilya S. Prikhodko and Oleg N. Shchepin

Komarov Botanical Institute of the Russian Academy of Sciences, Laboratory of Systematics and Geography of Fungi, 197376 St. Petersburg, Russia

| Submitted June 6, 2019 | Accepted July 22, 2019 |

Summary

A new species of *Diderma*, described herein as *D. dalatense*, was found in ground leaf litter in mixed montane tropical forests (Bidoup Nui Ba National Park) of southern Vietnam. This species was discovered during intensive studies on the distribution and ecology of fungi and myxomycetes in tropical forests of Vietnam in the context of a long-term project Ecolan 1.2. The morphology of representative specimens was examined by light and scanning electron microscopy, and micrographs of relevant details are provided. *D. dalatense* has unique combination of morphological characters of the sporocarps among species of *Diderma* including very small (0.2–0.4 mm in diam.) dark-yellow, globose to subglobose sessile sporocarps, large conical or subglobose yellow columella, smooth peridium with three layers resembling that of *Leocarpus* and irregularly warted spores. In addition to the morphological description, partial sequences of three genetic markers of this new species (SSU, EF1A, COI) were obtained and submitted to GenBank. The stability of the taxonomic characters of the species was confirmed by several collections obtained in different localities of the National Park.

Key words: 18S rRNA, Amoebozoa, COI, EF1A, Myxogastria, myxomycetes, Paleotropics, slime molds

Introduction

Surveys of myxomycetes carried out in montane tropical forests of southern Vietnam in Bidoup Nui Ba National Park (BDB) by the first author in December 2014 in the context of a long-term project Ecolan 1.2 yielded a series of collections of the genus *Diderma* that could not be identified to a known species in the field. A colony of sporocarps with the same morphology was collected again in December 2017 in the same area. A thorough examination of these collections in the laboratory indicated that they did not fit any described species of *Diderma*. In this paper we describe a new species *Diderma* dalatense based on morphological traits of sporocarps and spores and provide partial sequences of 18S rRNA, COI and EF1A genes of this new species.

doi:10.21685/1680-0826-2019-13-3-2

Material and methods

Field sampling

This paper is based on field collections from BDB centered in the Bidoup Mountain massive (12°08′ N, 108°40′ E) in the southern Annamite Mountains, on the Dalat Plateau (Lam Dong Province). The topography of BDB is dominated by a range of high mountains, including Bidoup Mt. (2,287 m a.s.l.) and Gia Rich Mt. (1,922 m a.s.l.). The rainy season extends from May to October, and the dry season encompasses the period from December to April. The predominant vegetation of BDB is a montane evergreen forest, with small patches of coniferous forest and middle mountain mixed broadleaved deciduous polydominant forest including members of Fagaceae and Magnoliaceae along with *Pinus kesiya, P. dalatensis*, and *P. krempfii* (Kuznetsov et al., 2006; Tran, 2011). Voucher specimens are deposited in the myxomycete collection of the mycological herbarium in the Komarov Botanical Institute, Laboratory of Systematics and Geography of Fungi (LE).

Air-dried sporocarps were studied with a Zeiss motorized stereo microscope (DM) Discovery V20. All microscopic measurements and observations were made under a light microscope with differential interference contrast (LM) Zeiss Axio Imager A1 using the program Axio Vision 4.8.0.0 (Carl Zeiss Imaging Solutions). Size measurements for each specimen are given as the mean values of 25 spores (including spore ornamentation) with standard deviation (SD). Color notations in parentheses are from the ISCC-NBS color-name charts illustrated with centroid colors (Anonymous, 2012). Scanning electron micrographs were obtained with a JSM-6390 LA scanning electron microscope (SEM) at 10–15 kV using cryo-dried specimens mounted on copper stubs using double-sided sticky film and sputter-coated with gold.

DNA extraction and sequencing

Several dried sporocarps of a holotype of *Diderma dalatense* (LE 317550) were placed in a 2 ml safe-lock tube with a steel ball 3 mm diam. and frozen at –20 °C. After that the sample was crushed in TissueLyser LT homogenizer (QIAGEN). DNA was extracted with Proba-GS nucleic acid isolation kit (DNA-Technology, Russia) according to the manufacturer's protocol. Partial sequences of three genetic markers were obtained as described elsewhere (Shchepin et al., 2016): 18S rRNA gene (SSU, primers S2/SU19R), translation elongation factor 1-alpha gene (EF1A, primers PB1F/PB1R) and cytochrome c oxidase subunit 1 gene (COI, primers COMF/COMRs). Primer sequences and references are listed in Table 1.

Table 1. Primers used in this study.

Primer name	Sequence (5′–3′)	Authors
S2	TGGTTGATCCTGCCAGTAGTGT	Fiore-Donno et al., 2008
SU19R	TCGAGTAACAATTAGAGGACA	Fiore-Donno et al., 2012
PB1F	ACCCGTGAGCACGCTCTCCT	Novozhilov et al., 2014
PB1R	CGCACATGGGCTTGGAGGGG	Novozhilov et al., 2014
COMF	GCTCCTGATATGGCWTTTC	Liu et al., 2015
COMRs	CATGRAAWGCATATCWARACC	Modified from: Liu et al., 2015

Sequence chromatograms were examined in Unipro UGENE (Okonechnikov et al., 2012) and aligned to combine the forward and reverse reads. The resulting sequences were checked across GenBank Nucleotide collection using NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn) and deposited in GenBank. To demonstrate the level of genetic divergence of the new species, the obtained partial SSU and EF1a sequences were compared to the top of the BLAST output. Percentage of the genetic similarity of the sequences was calculated using usearch_global command in VSEARCH 2.13.4 (Rognes et al., 2016).

Results and Discussion

Diderma dalatense Novozh. Prikhodko et Shchepin, sp. nov. (Fig. 1)

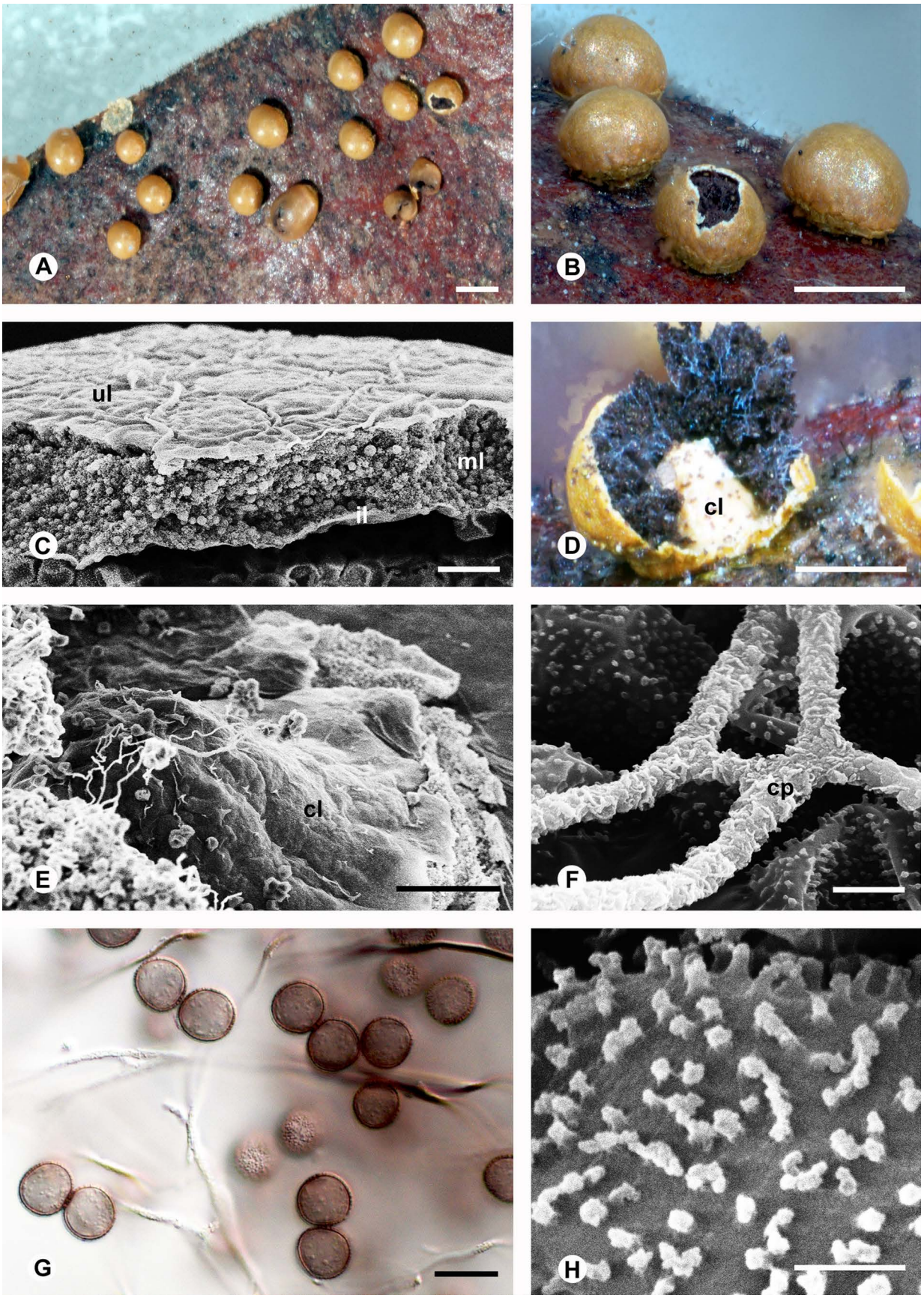
MycoBank **831244**

GenBank **MK968250 (SSU), MN052806 (COI), MN052807 (EF1A)**

Holotype LE 317550

Etymology. The specific epithet refers to the geographical region where all the collections of the new species were made and is derived from the name of the Dalat Plateau in Vietnam.

Macromorphology. Sporocarps grouped in small colonies (Fig. 1, A, B), sessile, about 0.2–0.4 mm diam. Sporotheca globose to subglobose (Fig. 1, B), brownish orange (54). Hypothallus membranous, inconspicuous, transparent. Peridium smooth, resembling that of *Leocarpus*, 20–30 μm in


A
B
ul
ml
il
C
cl
D
cl
E
cp
F
G
H

thickness, with three layers. The inner and the outer layers membranous, smooth and thin; the middle layer limy and fragile (Fig. 1, C, D). Dehiscence of the peridium irregular (Fig. 1, B).

Micromorphology. Columella present, large, conical, pale orange yellow (73) tapering at the center of the sporotheca (Fig. 1, D, E). Capillitium dense and reticulate, consisting of hyaline tubes (Fig. 1, F, G) covered by squamae seen under SEM (Fig. 1, F). Spores in mass brownish black (65), grayish reddish brown in LM (46), verruculose, irregularly warted (Fig. 1, G, H), (7.8–8.0)–(8.5–8.9) μm in diam. (mean: 8.37 μm, SD: 0.35, n = 30). Plasmodium unknown.

Habitat. Ground leaf litter of deciduous broad-leaved trees in a mixed broadleaved mountain tropical forest. The upper litter layer dries out fast, but the leathery leaves of trees of Fagaceae and Magnoliaceae decay slowly and may accumulate into thick layers, where a specific set of litter-inhabiting species was found including *D. dalatense*.

Known distribution. Known only from a few sites in BDB in Lam Dong Province of southern Vietnam.

Material examined. Vietnam, Lam Dong Province, BDB, Giang Ly Ranger Station, middle mountain broadleaved deciduous polydominant forest, in depression with numerous coarse wood debris, 12°11′12.0′′ N 108°40′32.0′′E, 1442 m. a.s.l., in upper layer of the ground leaf litter of *Quercus* sp. and *Litocarpus* sp., 12th Dec 2017, Yu. Novozhilov (LE 317550, Holotype). Middle mountain broad-leaved deciduous polydominant forest with Fagaceae, 12°11′07.8′′ N 108°40′32.2′′E, 1523 m. a.s.l., in upper layer of the ground leaf litter of *Quercus* sp., 13th Dec 2014, Yu. Novozhilov (LE 317449).

The features of the species, including the microscopic traits, were constant among the studied specimens.

Notes. *D. dalatense* belongs to the subgenus *Leangium* (Link) Rost., based on *Diderma floriforme* (Bull.) Pers. which includes species with cartilaginous, tough, shining peridium (Martin and Alexopoulos, 1969). The new species differs in a number of important characters from other species of *Diderma* that have sessile globose and subglobose sporangia with the yellowish, reddish brown or ochraceous three-layered (triple) peridium and developed columella: *Diderma velutinum* Bortnikov (Bortnikov et al., 2018) and *Diderma albocolumella* A.C.C. Bezerra et L.H. Cavalc. (Bezerra and Cavalcanti, 2010).

The former can be distinguished from *D. dalatense* for its larger sporocarps (0.5–0.9 mm diam. vs. 0.2–0.4 mm diam. in *D. dalatense*), pale yellow (89) or grayish greenish yellow (105) color of peridium, large, spherical or subspherical columella and large densely warted spores (mean: 12.15 μm, SD: 0.51, vs mean: 8.37 μm, SD: 0.35 in *D. dalatense*). In addition, partial SSU sequence of *D. dalatense* is only 79% similar to the published sequences of *D. velutinum*. *D. albocolumella* described from Brazil (Bezerra and Cavalcanti, 2010) is similar to the new species, because it has a triple peridium, a large spherical columella and warted spores. However, it has flattened hemispherical to discoid sporangia, white columella and larger spores (10.5–13.0).

Our results demonstrate the difficulties in reconciling molecules (DNA markers) with morphology (classical taxonomic descriptions) of this myxomycete morphospecies. *D. dalatense* has all morphological characters of *Diderma* including typical triple peridium with middle layer consisting of amorphous granular lime as well as limeless, filamentous branching and anastomosing capillitium. The color and texture of the outer layer of the peridium are similar to those of *Leocarpus fragilis* (Dicks.) Rostaf. However, the capillitium of *D. dalatense* consists only of hyaline tubules without calcareous nodes, which are characteristic of capillitium of *L. fragilis*. In our three-gene ML phylogeny (available at TreeBase with accession number S24769) the new species formed a long branch with an unresolved position within Physarales. Moreover, *D. dalatense* has a low level of genetic similarity to the other species of *Diderma* (Tables 2 and 3). This indicates a contradiction between the morphology of the new species and the molecular data. Further investigations are needed to verify whether the traditional separation of the genera within the order Physarales is justified well enough (Nandipati et al., 2012; Shchepin et al., 2016; Leontyev et al., 2019).

Fig. 1. *Diderma dalatense* holotype LE 317550. A – Scattered small sporocarps on leaf litter; B – sporocarps; C – triple peridium under SEM, the outer (ul), middle (ml), and inner (il) layers of the peridium; D – a sporocarp section with a large cone-shaped columella (cl); E – the columella under SEM; F – the surface of the capillitial threads (cp) under SEM; G – spores and threads of capillitium under LM; H – spore under SEM. Scale bars: A, B – 0.2 mm; C, G – 10 μm; D – 0.1 mm; E – 50 μm; F – 2 μm; H – 1 μm.

Table 2. Similarity of partial 18S rRNA gene sequences of *Diderma dalatense* to other Physarales calculated with VSEARCH.

Species	Similarity %	GenBank Accession #
Diderma fallax	82.9 – 79.7	JQ898089, KU198040, KR029660 and 10 more
Lepidoderma crustaceum	82.9	HE614619
Lepidoderma carestianum	82.5 – 80	HE614618, KY123438, JQ812618, AM231296
Lepidoderma peyerimhoffii	82.4 – 82.3	JQ812627, JQ898099
Diachea subsessilis	80.7 – 80.5	JQ031964, JQ900780
Didymium melanospermum	80.4 – 78.5	MG647913, KU577267
Diachea leucopodia	80.4 – 78.1	KP323370, KF743861, KM977849
Didymium dubium	80.4	KP323375
Diderma meyerae	80.3 – 79.5	KU198052, KR029671
Diderma niveum	80.2	KR029694
Lepidoderma alpestroides	80.2	JQ031998
Physaraceae sp.	80.1	MG429808
Mucilago crustacea	79.9 – 77.6	MH348905, HE614620, DQ903679
Physarum pusillum	79.8 – 79.4	MK336175, MK336174
Didymium sp.	79.8 – 76.4	MG647892, MG662518
Lepidoderma chailletii	79.8 – 76.4	JQ900774, KY123412, JQ898098 and 18 more
Didymium leptotrichum	79.5	MG662514
Didymium trachysporum	79.4 – 78.2	MG662513, MG647912
Leocarpus fragilis	79.1 – 77.9	MF352461, MG647916, MF352460
Didymium difforme	78.8	MG662515
Didymium flexuosum	78.8	MG429800
Didymium quitense	78.5	MG662516
Badhamia melanospora	78.2 – 76.3	MF352448, KC759039, KC759108 and 13 more
Didymium saturnus	77.9	MG677145
Didymium comatum	77.2	MG662512
Physarum cinereum	76.3	MF352471
Craterium minutum	76	MF352456, MF352455
Craterium leucocephalum	75	MF352454

Acknowledgments

The laboratory work was supported by grant from RFBR (Russian Foundation for Basic Research, 18–04–01232). Expeditions and field work of the first author were supported by the program Ecolan 1.2 of the Russian-Vietnamese Tropical Research and Technological Centre, the program "Biodiversity, ecology, structural and functional features of fungi and fungus-like protists" (AAAA-A19-119020890079-6) and the program AAAA-A19-119080990059-1 "Taxonomic diversity, ecology and physiological and biochemical features of fungi and fungus-like protists of Vietnam" of the Komarov Botanical Institute RAS. In addition, we acknowledge the use of equipment of the Core Facility Center "Cell and Molecular Technologies in Plant Science" at the Komarov Botanical Institute RAS (St. Petersburg).

References

Anonymous 2012. NBS/ISCC Colour System. Original and Improved 267 Colour Centroids. Washington, Inter-Society Colour Council. National Bureau of Standards. http://people.csail.mit.edu/jaffer/Color/Dictionaries (accessed May 2019).

Bezerra A.C.C. and Cavalcanti L.D.H. 2010. *Diderma albocolumella* (Myxomycetes), a new species in the Brazilian Atlantic Forest. Rodriguésia. 61, 1, 105–108.

Bortnikov F.M., Shchepin O.N., Gmoshinskiy V.I., Prikhodko I.S. and Novozhilov Y.K. 2018. *Diderma velutinum*, a new species of *Diderma* (Myxomycetes) with large columella and triple peridium from Russia. Botanica Pacifica. 7, 47–51.

Fiore-Donno A.M., Meyer M., Baldauf S.L. and Pawlowski J. 2008. Evolution of dark-spored Myxomycetes (slime molds): molecules versus

Table 3. Similarity of partial translation elongation factor 1-alpha (EF1A) gene sequences of *Diderma dalatense* to other Physarales calculated with VSEARCH.

Species	Similarity %	GenBank Accession #
Physarales sp.	91.2 – 90.6	MG430298, MG430297, MG430296, MG430295
Physarum album	90.9	EF513196
Physarum leucopus	90.7 – 90	MF352528, MF352529
Physarum melleum	90.7 – 90	MF352535, MF352534, MF352533
Craterium leucocephalum	90.7	MF352500
Physarum leucophaeum	90.7 – 89	KC473814, FJ546685
Diderma pseudotestaceum	90.2	KJ676602, KJ676604
Physarum contextum	90	MF352523
Physarum notabile	90	KC473820
Physarum polycephalum	90	AF016243
Physarum pusillum	90	KC473819
Badhamia nitens	89.8	MF352498
Badhamia panicea	89.7	FJ546661
Craterium minutum	89.5	MF352502
Physarum bivalve	89.4	MF352515
Lepidoderma carestianum	89.3 – 89	KY123391, KY123392, KY123393
Diderma velutinum	89.2	MH717085, MH717084
Diderma cattiense	89.1 – 88.9	KJ676603, KJ676601
Lepidoderma chailletii	88.9 – 88.6	KY123380, KY123382, KY123399 and 13 more
Lepidoderma tigrinum	88.7	EF513195
Fuligo septica	88.2	AY643817
Comatricha nigricapillitia	87.7	AY643818
Diderma europaeum	87.7	EF513191

morphology. Mol. Phylogenet. Evol. 46, 878–889.

Fiore-Donno A.M., Kamono A., Meyer M., Schnittler M., Fukui M., Cavalier-Smith T. 2012. 18S rDNA phylogeny of *Lamproderma* and allied genera (Stemonitales, Myxomycetes, Amoebozoa). PLoS One. 7, 4, e35359.

Kuznetsov A.N., Kuznetsova S.P. and Luong P. 2006. Vegetation of the Hon Ba Bidoup Mountain Range, Khanh Hoa Province, South Vietnam. In: Materials of Zoological and Botanical Studies in Bidoup and Hon Ba Mountains, Dalat Plateau, southern Vietnam. Ser. Bioraznoobrazie V'etnama (Eds: Korzun L.P., Rozhnov V.V. and Kalyakin M.V.). KMK Scientific Press, Moscow-Hanoi, pp. 9–115 (in Russian with English summary).

Leontyev D.V., Schnittler M., Stephenson S.L., Novozhilov Yu.K. and Shchepin O.N. 2019. Towards a phylogenetic classification of the Myxomycetes. Phytotaxa. 399, 209–238.

Liu Q.S., Yan S.Z. and Chen S.L. 2015. Further resolving the phylogeny of Myxogastria (slime molds) based on COI and SSU rRNA genes. Russ. J. Genet. 51, 46–53.

Martin G.W and Alexopoulos C.J. 1969. The Myxomycetes. Iowa City: Univ. of Iowa Press.

Nandipati S.C., Haugli K., Coucheron D.H., Haskins E.F. and Johansen S.D. 2012. Polyphyletic origin of the genus *Physarum* (Physarales, Myxomycetes) revealed by nuclear rDNA mini-chromosome analysis and group I intron synapomorphy. BMC Evol. Biol. 12, 166.

Novozhilov Y.K., Mitchell D.W., Okun M.V. and Shchepin O.N. 2014. New species of *Diderma* from Vietnam. Mycosphere. 5, 554–564.

Okonechnikov K., Golosova O. and Fursov M. 2012. Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics. 28, 1166–1167.

Rognes T., Flouri T., Nichols B., Quince C. and Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 4, e2584.

Shchepin O.N., Novozhilov Y.K. and Schnittler M. 2016. Disentangling the taxonomic structure of the *Lepidoderma chailletii-carestianum* species complex (Myxogastria, Amoebozoa): genetic and morphological aspects. Protistology. 10, 117–129.

Tran T.T.T. 2011. Spatial distribution and historical dynamics of threatened conifers of the Dalat Plateau, Vietnam. MSc thesis, University of Missouri.

Address for correspondence: Yuri K. Novozhilov. Komarov Botanical Institute of the Russian Academy of Sciences, Laboratory of Systematics and Geography of Fungi, Prof. Popov Street 2, 197376 St. Petersburg, Russia; e-mail: *YNovozhilov@binran.ru.*