Botanica Pacifica. A journal of plant science and conservation. 2018. 7(2): 47–51
DOI: 10.17581/bp.2018.07207

TAXONOMIC NOVELTIES

Diderma velutinum, a new species of *Diderma* (Myxomycetes) with large columella and triple peridium from Russia

Fedor M. Bortnikov[1]*, Oleg N. Shchepin[2], Vladimir I. Gmoshinskiy[1], Ilya S. Prikhodko[2] & Yuri K. Novozhilov[2]

Fedor M. Bortnikov[1]*
e-mail: f.m.bortnikov@gmail.com

Oleg N. Shchepin[2]
e-mail: ledum_laconicum@mail.ru

Vladimir I. Gmoshinskiy[1]
e-mail: rubisco@list.ru

Ilya S. Prikhodko[2]
e-mail: hypnothotep@gmail.com

Yuri K. Novozhilov[2]
e-mail: yurinovozhilov@gmail.com

[1] Lomonosov Moscow State University, Faculty of Biology, Mycology and Algology Dept., Moscow 119234 Russia

[2] V.L. Komarov Botanical Institute RAS, Saint-Petersburg, Russia

* corresponding author

Manuscript received: 07.09.2018
Review completed: 25.10.2018
Accepted for publication: 30.10.2018
Published online: 31.10.2018

ABSTRACT

A new species, *Diderma velutinum*, is described and illustrated by LM and SEM micrographs. Two specimens of this species were found in moist chamber cultures on the pieces of the bark of living tree of *Phellodendron amurense*, which was collected in July 2016 and July 2017 in the Kedrovaya Pad State Nature Biosphere Reserve (the Russian Far East). The main distinguishing features of this species are triple peridium, large spherical columella and ochraceous colour of sporangia clustered in dense groups. The partial 18S rRNA gene sequences of *D. velutinum* differ considerably from all sequences of *Diderma* species available to the moment.

Keywords: Amoebozoa, COI, DNA barcoding, EF1A, morphology, Physarales, SEM, taxonomy, 18S rRNA

РЕЗЮМЕ

Бортников Ф.М., Щепин О.Н., Гмошинский В.И., Приходько И.С., Новожилов Ю.К. *Diderma velutinum*, новый вид рода *Diderma* (Myxomycetes) с большой колонкой и трехслойным перидием из России. Новый вид *Diderma velutinum* описан и проиллюстрирован микрофотографиями, полученными с помощью светового и сканирующего электронного микроскопов. Два образца этого вида были получены методом влажных камер на фрагментах коры живого дерева бархата амурского (*Phellodendron amurense*), собранных в июле 2016 и июле 2017 года в государственном природном биосферном заповеднике «Кедровая Падь» (Приморский Край). Основными отличительными особенностями этого вида являются трехслойный перидий, большая сферическая колонка и охристая окраска спорангиев, собранных в плотные группы. Нуклеотидные последовательности фрагмента гена 18S рРНК нового вида показывают значительные отличия от имеющихся последовательностей других видов рода *Diderma*.

Ключевые слова: Amoebozoa, COI, EF1A, Physarales, ДНК-баркодинг, морфология, СЭМ, таксономия, 18S рРНК

Diderma is one of the three largest genera of myxomycetes and currently includes 82 species (Lado 2005–2018) of which 27 are recorded in Russia (unpublished data). During the study of biodiversity of myxomycetes in the Kedrovaya Pad State Nature Biosphere Reserve in 2016, in a moist chamber culture a collection of *Diderma* was found, which differed from all the described species of this genus. The second collection of this species was obtained in a moist chamber culture after additional sampling of substrates for moist chambers in July 2017 in the same locality.

In this paper we describe this new species of *Diderma*, including the morphological analysis of sporocarps and spores using the light (LM) and the scanning electron microscopy (SEM), and provide partial sequences of 18S rRNA, COI and EF1A genes.

MATERIAL AND METHODS

Isolates and morphology

The moist chamber cultures were used to obtain a new species. This new species was described and illustrated by the first author at the Department of Mycology and Algology of the Biological Faculty of the Lomonosov Moscow State University.

The sampling of the substrates for moist chamber cultures was carried out in July 2016 and 2017 in the same locality of the Kedrovaya Pad State Nature Biosphere Reserve. Small fragments of the bark of a living tree were cut out at about 1.5 m height, placed in paper bags and air-dried. In the laboratory, samples were evenly distributed over the bottom of Petri dishes above the filter paper and poured with distilled water. After a day, pH was measured with the pH-meter "Aquilon pH-420" and excess water was drained off. The cultures were incubated at room temperature (18–22°C) under diffused light for three months and checked for the presence of sporocarps of myxomycetes every 7–14 days, more often in the first 45 days. Sporocarps of the new species appeared within 50–70 days after the beginning of the moist chamber cultures. Plasmodium was seen only in the second setting of moist chamber cultures and after formation it quickly passed to sporangiogenesis. The macroscopic characteristics of air-dried sporocarps were studied with a Leica M80 stereomicroscope (with a

Leica IC 80 HD camera). Some photos were taken with a Nikon D5200 digital camera with a Nikkor 105 mm 1:2.8G ED lens. The microscopic features of peridium, capillitium and spores were studied with a Leica DM 2500 microscope. The microscopic structures were measured with the program LAS EZ v. 3.1.1. The scanning electron micrographs were obtained with a JEOL JSM-6380LA scanning electron microscope (SEM) after sputtering with gold-palladium. Colour notations in parentheses are from the ISCC-NBS colour-name charts illustrated with centroid colours (Anonymous 2012).

Analysis of partial 18S rRNA gene sequences

The first part of the 18S rRNA gene widely used as the most perspective DNA barcode for myxomycetes (Schnittler et al. 2017) was obtained for the both specimens of the new *Diderma* species using primers S1/SU19R as described elsewhere (Shchepin et al. 2016), as well as partial sequences of elongation factor 1 alpha gene (EF1A, primers PB1F/PB1R) and cytochrome c oxidase subunit I gene (COI, primers COMF/COMRs). All new sequences were deposited in GenBank. Additionally, to check the level of genetic divergence of the new species the same region of the 18S rRNA gene of five other *Diderma* species was sequenced and 136 sequences of the *Diderma* species were retrieved from the quality-checked sequence collection (Borg Dahl et al. 2017). Percentage of the genetic similarity of the sequences was calculated as 100 * (matching columns) / (alignment length – terminal gaps) using usearch_global command in VSEARCH 2.8.0 (Rognes et al. 2016).

TAXONOMY

***Diderma velutinum* Bortnikov, sp. nov.** Figs. 1–16
MycoBank: 827750
GenBank: MH714785, MH714786, MH717084–MH717087
Holotype: LE 318752

Description. Sporocarps grouped in small dense groups, globose or subglobose, often slightly angular from mutual compression, sessile, 0.5–0.9 mm in diameter, immature vivide orange (v.O 48), mature pale yellow (p.Y 89) to grayish greenish yellow (gy.gY 105). Hypothallus inconspicuous. Peridium three-layered, all layers closely appressed, break up together. Outer layer thin, membranous, translucent. Middle layer thick, fragile enough, yellowish-ochraceous, composed by closely adherent lime granules. Inner layer membranous, in upper part very thin and almost inconspicuous to absent, thicker and darker downwards, up to the dark hazel-brown, usually well distinguishable. Dehiscence not observed, but probably irregular. Columella concolorous with the peridium, well developed, large, spherical or subspherical, reaches about 1/2 (up to 2/3) height of sporangia, covered by thin membrane, filled with lime granules about 1.5 μm in diameter. Columella and peridium rarely with no more than 3–5 calcareous spike-like protuberances per sporangium that even more rarely can merge into column. Capillitium abundant, radiating from columella to inner peridium, but in most cases threads break at the ends and so spore mass is separated from peridium by free space. Capillitium threads hyaline, about 3–4.5 μm in diameter, wavy, branched and anastomosed, sometimes with membranous extensions at branching points, threads surface under SEM smooth or with small papulae. Spores in mass brownish black (brBlack 65), brown (d.Br 59) in transmitted light, sometimes slightly lighter on the one side, (10.6–) 12 (–13.2) μm in diameter (Mean: 12.15, SD: 0.51, n = 85), densely warted. Warts arranged irregularly, under SEM appear clavate. Plasmodium yellowish-ochraceous to deep yellow-orange.

Holotype: Russian Federation, Primorsky Territory, Kedrovaya Pad State Nature Biosphere Reserve, 43°06'47.3"N 131°30'57.5"E, 96 m a.s.l., mixed forest dominated by *Abies holophylla* Maxim., *Quercus mongolica* Fisch. ex Ledeb., *Phellodendron amurense* Rupr. and *Betula* sp., on bark of living *Phellodendron amurense* covered by mosses, in moist chamber culture (mcc), pH=5.83, bark sampling 19.07.2016, mcc starting 14.09.2016, sporangia sampling 2.11.2016 and 23.11.2016, leg. Bortnikov F.M., LE 318752. A fragment of the type material was deposited in the collection of Myxomycetes of the Department of Mycology and Algology of Biological Faculty of Lomonosov Moscow State University (No. 8240).

Paratype: the same location and substrate, in moist chamber culture, bark sampling 24.07.2017, mcc starting 9.02.2018, sporangia sampling 10.04.2018 and 30.04.2018, leg. Bortnikov F.M., LE 318753.

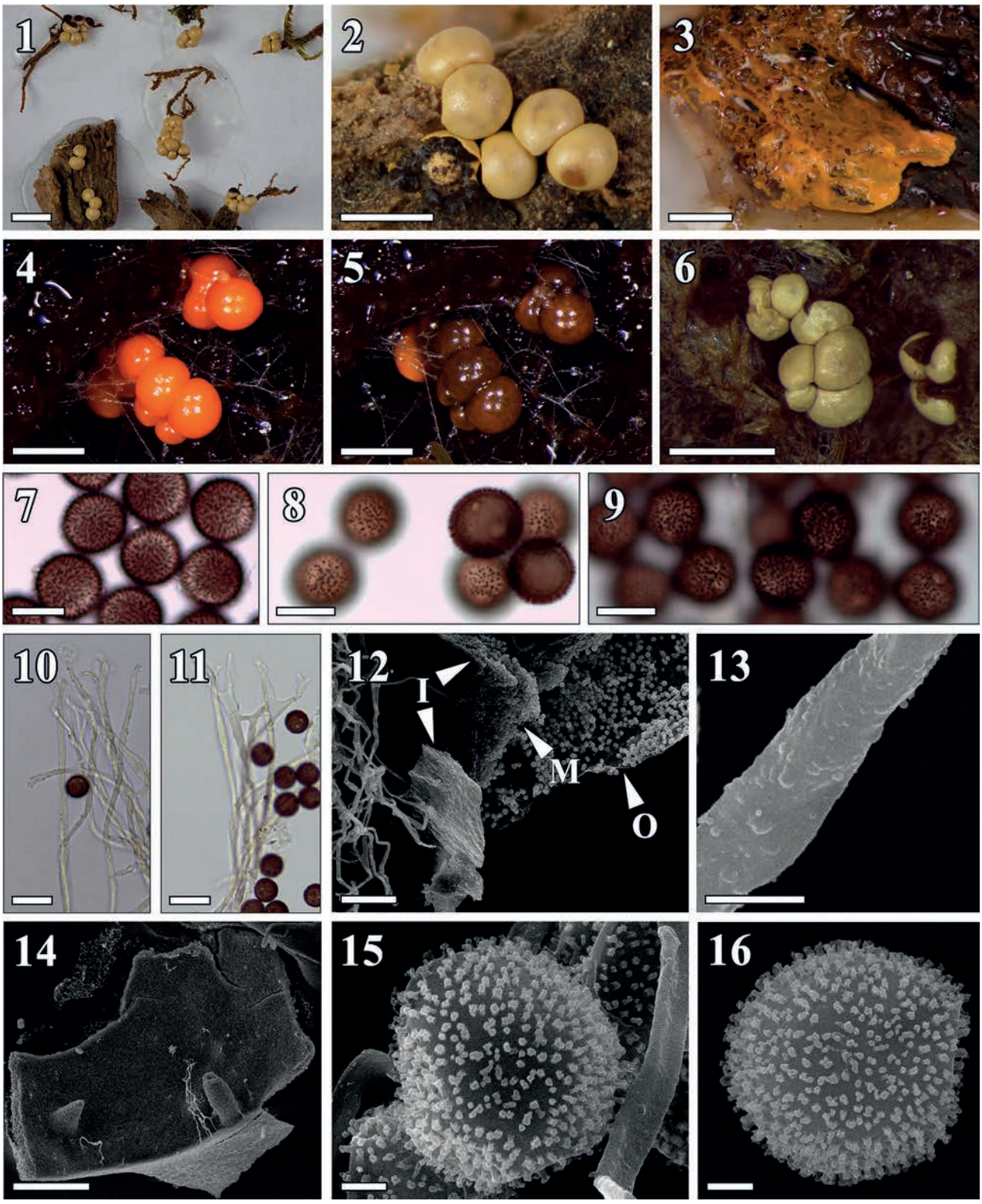
Etymology: from latin "velutinus" – velvety; reference to the substrate on which this species was found (*Phellodendron amurense* in Russian is also known as "Amur velvet" because of the soft surface of the cork bark).

DNA barcode. Comparison of the DNA barcode sequences of *Diderma velutinum* (first part of the 18S rRNA gene, GenBank accessions MH714785 and MH714786) to 143 sequences of 18 other species of *Diderma* showed that they are not more than 96 % similar to *Diderma* sequences available to the moment (Table 1), with *D. umbilicatum* being the closest match. Additionally, partial EF1A and COI gene sequences for holotype and paratype were located in the GenBank (MH717084–MH717087). Sequences of 18S rRNA gene and EF1A are identical in both specimens of *D. velutinum*, while COI have six mismatches.

DISCUSSION

The three-layered peridium with closely appressed layers, densely grouped subglobose to globose sporocarps which are bright orange when immature and yellowish-ochraceous when matured, the large spherical yellow columella, the hyaline colourless capillitium and warted spores about 12 μm in diameter are the diagnostic features of *Diderma velutinum*.

The new species differs in a number of important characters from other species that have sessile globose and subglobose sporangia with the yellowish, reddish brown or ochraceous peridium and more or less developed columella: *D. albocolumella* A.C.C. Bezerra & L.H. Cavalc., *D. ochraceum* Hoffm., *D. sauteri* (Rostaf.) E. Sheld., *D. simplex* (J. Schröt.) E. Sheld. and *D. testaceum* (Schrad.) Pers. (Bezerra & Cavalcanti 2010, Martin & Alexopoulos 1969) (Table 2). The former species described from Brazil (Bezerra & Cavalcanti 2010) is similar to the new species, because it has a triple peridium, a large spherical columella and warted spores 10.5–



Figures 1–16 *Diderma velutinum*: Holotype LE 318752 (Figs. 1, 2, 7, 8, 10, 12, 15) and paratype LE 318753 (Figs. 3–6, 9, 11, 13, 14, 16). 1. Dense small groups of sporocarp. 2. Sporocarps. Large spherical columella is seen in a sporocarp with broken peridium. 3. Plasmodium. 4–6. Different stages of maturation of sporocarps. 7–9. Spores under LM. 10, 11. Threads of capillitium under LM. 12. Triple peridium under SEM. The arrows indicate the inner (I), middle (M) and outer (O) layers of the peridium. 13. The surface of the capillitial thread under SEM. 14. The inner surface of the peridium under SEM. Outgoing threads of capillitium and calcareous protuberances are visible. 15. Spores and capillitial thread under SEM. 16. Spore under SEM. Scale bars: Figs. 1, 3. 2 mm; Fig. 2, 4–6. 1 mm, Figs. 7–9. 10 μm; Figs. 10–12. 20 μm; Figs. 13, 15, 16. 2 μm; Fig. 14. 100 μm.

Table 1. Similarity (%) of partial 18S rRNA gene sequences of *Diderma velutinum* to other *Diderma* species calculated with VSEARCH 2.8.0. Sequences obtained in present study are marked in bold.

Species	Similarity %	GenBank Accession
Diderma umbilicatum	96–95.9	**MH714787**, KP323371
D. meyerae	93.2–91.8	KR029670, KU198051, KU198050 and 15 more
D. microcarpum	93–92.2	KU198063, JQ898093, KU198060 and 12 more
D. niveum	92.4–90.5	KU198067, KU198068, KR029708 and 32 more
D. globosum var. *europaeum*	91–89.3	KU198043, KU198044, KU198047 and 16 more
D. montanum	89.1	KT358690
D. radiatum	86–85.8	KM977855, **MH714788**
D. hemisphaericum	85.3–84.1	**MH714789**, KM977853
D. deplanatum	85.3–76.4	KM977851, KF743863, KF743864
D. subochraceum	85.1	MG696637
D. fallax/*Lepidoderma peyerimhoffii*	85–84.7	KR029660, KU198040, JQ898089 and 14 more
D. testaceum	85	**MH714790**
D. alpinum	84.2–82.2	JQ898088, KR029646, KU198031 and 13 more
D. chondrioderma	84	KM977850
D. spumarioides	83.9–83.5	**MH714791**, **MH714792**, **MH714793**
D. pseudotestaceum	82.7	KJ659866, KJ659864, KJ659867
D. cor-rubrum	80.6	KT731233
D. cattiense	79.4	KJ659863, KJ659865
D. miniatum	73.8	KT731234

Table 2. Comparison of morphological characters of *Diderma velutinum* and five other similar species of *Diderma*

Characters	***Diderma velutinum***	***Diderma albocolumella***	***Diderma ochraceum***	***Diderma sauteri***	***Diderma simplex***	***Diderma testaceum***
Sporotheca diam. (mm)	0.5–0.9	0.3–0.8	0.4–1.0	0.6–1.0	0.2–0.8	0.7–1
Shape of sporotheca	globose to subglobose, often slightly angular	hemispheric-depressed to discoid, slightly umbilicated at the top	globose to subglobose, sometimes as short plasmodiocarps	globose to subglobose	subglobose, pulvinate or depressed	globose to subglobose, sometimes depressed to discoid
Colour of sporotheca	pale yellow to grayish and greenish yellow	bright yellowish-brown	deep ochraceous	pinkish-gray to light reddish-brown	brown or brick red to ochraceous	flesh-colored or light-pinkish-white
Peridium	three-layered	three-layered	double-layered	double-layered	single-layered	double-layered
Shape of columella	globose to subglobose, 1/2–2/3 of the sporotheca	subglobose, 1/3–1/4 of the sporotheca	poorly developed	small, often reduced	dome-shaped, often reduced	dome-shaped to hemispherical
Colour of columella	pale yellow	grayish white	NA	reddish-brown	brown or brick red to ochraceous	pinkish-brown or alutaceous
Colour of capillitium in transmitted light	hyaline	violet brown, with hyaline extremities	dark yellowish brown, with hyaline extremities	light violet or hyaline	hyaline to light-colored	hyaline to light-colored
Colour of spores in mass	brownish black	dark brown	black	black	dark brown	black
Colour of spores in transmitted light	brown	dark to yellowish brown	dark yellowish brown	dark brown	light violet brown	dark brown
Spore diam. (μm)	10.6–13.2	10.5–13.0	9.0–11.0	12–13	8–11	8–9
Spore ornamentation	irregularly covered with baculate warts	verrucose	densely and unevenly covered with large short warts	spinose	minutely warted or spinose, sometimes with groups of darker warts	almost smooth or minutely warted

13.0 μm in diameter. However, it has flattened sporangia grouped in more sparse groups. In addition, the peridium of *D. albocolumella* is bright yellowish-brown and capillitium violet-brown. *D. ochraceum* shares with *D. velutinum* the deep ochraceous colour of the peridium, but differs in its reduced columella, the double-layered peridium and smaller spores (9–11 μm *vs.* 11–13 in *D. velutinum*). In addition, the spore ornamentation with densely and unevenly distributed large short warts as seen with SEM (Schnittler et al. 2010, Moreno et al. 2018) differs from more evenly distributed baculate warts of *D. velutinum* (Fig. 15–16). *D. ochraceum* var. *izawae* Y. Yamam. & Nann.-Bremek. shares with *D. velutinum* spike-like outgrowths, which sometimes connect the columella and peridium but, as well as *D. ochraceum* Hoffm., it differs well by other attributes (Yamamoto & Nannenga-Bremekamp 1995).

The brown-reddish, yellowish and pale ochraceous spo-

rocarps of *D. sauteri*, *D. testaceum* and *D. simplex* are slightly similar to those of *D. velutinum*, but the former and the second species have the double-layered peridium and *D. simplex* has the single-layered peridium. In addition *D. testaceum* and *D. simplex* have smaller spores (8–9 and 8–11 µm respectively) (Martin & Alexopoulos 1969).

DNA barcode produced for *D. velutinum* in this study is at least 4 % different from all other *Diderma* sequences available to the moment, with an intraspecific sequence similarity threshold being estimated for dark-spored myxomycetes as 99.1 % similarity for the targeted 18S rDNA region (Borg Dahl et al. 2017).

Thus, we believe that the taxonomic status of our collections is undeniable, and they represent a new species for science.

ACKNOWLEDGEMENTS

The establishment of the collection of myxomycetes of the Department of Mycology and Algology of the Biological Faculty of the Lomonosov Moscow State University (MSU) was supported by a Russian Science Foundation project № 14-50-00029. Work on the collection and identification of the material was made in the framework of the State task of MSU, part 2 (topic number AAAA-A16-116021660). DNA sequencing was supported by Russian Foundation for Basic Research (project 18-04-01232 A) and state task of BIN RAS (AAAA-A18-118031290108-6). 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). The first author also thanks the staff of the Land of the Leopard National Park for the assistance in fieldwork organization.

LITERATURE CITED

Anonymous 2012. *NBS/ISCC Colour System. Original and Improved 267 Colour Centroids*. Washington, Inter-Society Colour Council. National Bureau of Standards. http://tx4.us/nbsiscc.htm (accessed July 2018).

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

Borg Dahl, M., A.D. Brejnrod, M. Unterseher, T. Hoppe, Y. Feng, Y.K. Novozhilov, S.J. Sørensen & M. Schnittler 2017. Genetic barcoding of dark-spored myxomycetes (Amoebozoa) – identification, evaluation and application of a sequence similarity threshold for species differentiation in NGS studies. *Molecular Ecology Resources* 18(2):306–318.

Lado, C. 2005–2018. *An on line nomenclatural information system of Eumycetozoa*. Real Jardin Botanico, CSIC. Madrid, Spain. http://www.nomen.eumycetozoa.com (accessed July 2018).

Martin, G.W. & C.J. Alexopoulos 1969. *The Myxomycetes*. University of Iowa Press, Iowa City, 561 pp.

Moreno, G., Á. López-Villalba, S.L. Stephenson & A. Castillo 2018. *Lepidoderma cristatosporum*, a new species of myxomycete from Australia. *Mycoscience* 59(5):386–391

Rognes, T., T. Flouri, B. Nichols, C. Quince & F. Mahé 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4:e2584. doi: 10.7717/peerj.2584

Schnittler, M., M. Unterseher, T. Pfeiffer, Yu. K. Novozhilov & A.M. Fiore-Donno 2010. Ecology of sandstone ravine myxomycetes from Saxonian Switzerland (Germany). *Nova Hedwigia* 90(3–4):277–302.

Schnittler, M., O.N. Shchepin, N.H.A. Dagamac, M. Borg Dahl & Y. K. Novozhilov 2017. Barcoding myxomycetes with molecular markers: challenges and opportunities. *Nova Hedwigia* 104(1–3):323–341.

Shchepin, O.N., Y.K. Novozhilov & M. Schnittler 2016. Disentangling the taxonomic structure of the *Lepidoderma chailletii-carestianum* species complex (Myxogastria, Amoebozoa): genetic and morphological aspects. *Protistology* 10(4):117–129.

Yamamoto, Y. & N.E. Nannenga-Bremekamp 1995. *Diderma ochraceum* var. *izawae*, a new Myxomycete from New Zealand. *Proceedings of the Royal Netherlands Academy of Arts and Sciences* 3(98):327–328.