Gromochytrium mamkaevae gen. & sp. nov. and two new orders: *Gromochytriales* and *Mesochytriales* (*Chytridiomycetes*)

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Key words

chytridiomycetes Gromochytriales Gromochytrium mamkaevae Mesochytriales Rhizophydium strain x-51 CALU **Abstract** During the last decade several new orders were established in the class *Chytridiomycetes* on the basis of zoospore ultrastructure and molecular phylogeny. Here we present the ultrastructure and molecular phylogeny of strain x-51 CALU – a parasite of the alga *Tribonema gayanum*, originally described as *Rhizophydium* sp. based on light microscopy. Detailed investigation revealed that the zoospore ultrastructure of this strain has unique characters not found in any order of *Chytridiomycetes*: posterior ribosomal core unbounded by the endoplasmic reticulum and detached from the nucleus or microbody-lipid complex, and kinetosome composed of microtubular doublets. An isolated phylogenetic position of x-51 is further confirmed by the analysis of 18S and 28S rRNA sequences, and motivates the description of a new genus and species *Gromochytrium mamkaevae*. The sister position of *G. mamkaevae* branch relative to *Mesochytrium* and a cluster of environmental sequences, as well as the ultrastructural differences between *Gromochytriales*.

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INTRODUCTION

Molecular phylogeny has dramatically changed chytrid taxonomy. Investigation of gene sequences of nearly any species or strain initiates a revision of neighbour taxa and often permits authors to establish new taxa of higher rank, e.g. family, order and class, divisions normally supported by zoospore ultrastructure. In the past few years we have seen several big changes in chytrid taxonomy: Letcher et al. (2006) described the Rhizophydium clade (James et al. 2000, 2006) as the order Rhizophydiales; Mozley-Standridge et al. (2009) established the order Cladochytriales from the Cladochytrium clade (James et al. 2006) and Simmons et al. (2009) described the clade formerly represented in phylogenetic trees (James et al. 2006) by Chytriomyces angularis as the order Lobulomycetales. "This removal of clades from the polyphyletic Chytridiales better reflected the diversity of the Chytridiomycota and began the corrective process of classifying the Chytridiomycetes (chytrids) into phylogenetic groups according to the best tools available." - wrote Longcore and Simmons in the introduction to the new order Polychytriales (Longcore & Simmons 2012: 276). This conclusion highlights the fact that we need molecular data for each traditionally described species of Chytridiomycetes to construct a meaningful and comprehensive classification of Chytridiomycetes.

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Rhizophydium is one of the largest genera of *Chytridiomycetes* known from the middle of the 19th century (Rabenhorst 1868). It accounts for more than 225 species, which were described from freshwater, primarily as parasites of algae, and from soil as saprotrophs (Longcore 1996, Letcher et al. 2004). The data on this genus were significantly expanded in recent investigations (Letcher et al. 2006, 2008) and reviewed in a comprehensive taxonomic summary and revision of the genus (Letcher & Powell 2012).

Nevertheless, the list of species investigated with modern methods is still far from being complete, and new data on the ultrastructure and molecular phylogeny of other strains are always important for understanding the huge morphological and genetic diversity of this genus. Moreover, the transmission electron microscopy (TEM) sometimes reveals peculiarities that can be used as new taxonomic characters, or may show the unimportance of some commonly accepted ultrastructural characters.

Here we present the ultrastructure and molecular phylogeny of an algal parasite, strain x-51 CALU, which was described in a preliminary study as '*Rhizophydium* sp.'. We show that zoospore ultrastructure of this strain differs from that of other described species, and includes characters not described in any orders of *Chytridiomycetes*. These morphological data confirm an isolated phylogenetic position of x-51 obtained from the analysis of 18S and 28S rRNA sequences, and serve as the basis for the description of a new species and genus. Sister position of the x-51 branch relative to a cluster of environmental sequences, which includes *Mesochytrium penetrans*, and the ultrastructural differences of x-51 and *Mesochytrium* zoospores prompt us to establish two new orders: *Gromochytriales* and *Mesochytriales*.

MATERIALS AND METHODS

Strain CALU x-51 was isolated from a water sample collected from a ditch by the highway near town Kirovsk, Leningrad

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Table 1 List of rRNA genes used in phylogenetic analysis.

Taxon	Isolate number	GenBank accession no.			Cumulative
		18S	5.8S	28S	length (%)
Outgroup: aphelids and rozellids					
Amoeboaphelidium protococcarum	CALU X-5	JX507298	JX507298	JX507298	99
Rozella allomycis Rozella sp	UCB 47-054 (AFTOL-ID 297) IEL 347 (AFTOL-ID 16)	AY 035838 AY 601707	AY997087 AY997086	DQ273803	99
Nozena sp.		AIGOIIGI	A1337000	DQ210100	30
Blastocladiomycota					
Blastocladiella emersonii		M54937	AY997032	X90411	98
Allomyces arbuscula	AFTOL-ID 300	AY552524	AY997028	AY552525	98
Physoderma maydis	AFTOL-ID 19	AY601708	AY997072	DQ273768	96
Neocallimastigomycota					
Neocallimastix sp.	GE13 (AFTOL-ID 638)	DQ322625	AY997064	DQ273822	97
Orpinomyces sp.	OUS1	AJ864616, AJ864475	AJ864475	AJ864475	98
Cyllamyces aberensis	EO14 (AFTOL-ID 846)	DQ536481 ELIQ10600	AY997042	DQ273829	100
20	uncultured	20010000			00
Monoblepharidomycetes					
Monoblepharella mexicana	BK 78-1 (AFTOL-ID 33)	AF164337	AY997061	DQ273777	98
Gonapodya prolifera		JGI v. 1.0	JGI v. 1.0	JGI v. 1.0	100
Hvaloraphidium curvatum	SAG 235-1 (AFTOL-ID 26)	Y17504	AY997055	DQ273771	91
PFE7AU2004	uncultured	DQ244008	1.1.001.000	242.0111	36
L73_ML_156	uncultured	FJ354068			22
Elev_18S_563	uncultured	EF024210			36
Gromochytrialos and nov					
Gromochytriaes, ord. nov.	CALU X-51	KF586842	KF586842	KF586842	99
kor_110904_17	uncultured	FJ157331			33
IIN1-34	uncultured		EU516964	EU516964	15
•• • · · · ·					
Mesochytriales, ord. nov.		E 1804140		E 1804153	37
WS 10-E02	uncultured	AJ867629		1 300 - 133	34
WS 10-E14	uncultured	AJ867630			36
WS 10-E15	uncultured	AJ867631			36
Spring_08	uncultured	JX069031			11
Spring_37 Spring_57	uncultured	JX069054			11
Spring_57 Spring_71	uncultured	JX069077			11
T2P1AeB05	uncultured	GQ995415			36
T2P1AeF04	uncultured	GQ995412			36
T3P1AeC03	uncultured	GQ995413			36
SAPA5 E7	uncultured	GQ995414 E M83310			36
P60E-9	uncultured	DQ104060			13
P60E-29	uncultured	DQ104068			14
Clones from a lake in China	uncultured	JX426910, JX426918,			7
		JX426923, JX426937,			
		JX426998, JX427002, IX427011			
Clones from Lake Bourget (BI74, B1,	uncultured	EF196711, EF196713,			20
B43, B44, B46-138, B49, B52, B56,		EF196728, EF196729,			
BI78, BI88, BI100, BI104, BI107,		EF196731, EF196734,			
BI121, BI123, BI15, BI72, BI76, B86 161, BI5)		EF196735, EF196738,			
Boo-101, BIS)		EF196751 EF196753			
		EF196755, EF196762,			
		EF196763, EF196765,			
		EF196775, EF196776,			
REESSR2005	upcultured	EF196786, EF196799			36
PFD6SP2005	uncultured	EU162637 3'-end			30
PFA12SP2005	uncultured	EU162643			36
Pa2007C10	uncultured	JQ689425			35
F08_SE1B	uncultured	FJ592495 3'-end			17
i ⊓JAR∠B-48 528-Ω25	uncultured	JF972676 FE586095			33
GA069	uncultured	HM486988			28
GF29312	uncultured	JX417945			16
PFG9SP2005	uncultured	EU162638			36
PA2009C3	uncultured	HQ191369	HQ191369		40
FA200900 PA2009D8	uncultured	HQ191400 HQ191406	HQ191400 HQ191406		40 40
PA2009E7	uncultured	HQ191286	HQ191286		40
Va2007BB6	uncultured	JQ689445			35
FV23_1H5	uncultured	DQ310332			29

Table 1 (cont.)

iss 5.8.5 2.8.6 Period Lobolenyes angularis LLLS (AFTCL-LD 500) AF164235 AF1877036 D2237815 Lobolenyes angularis PT0 FF432822 FF432822 FF432822 Lobolenyes angularis AF20 FF432822 FF432822 FF432822 CAUBIA AF20 FF432822 FF432822 FF432822 SSC-501/20 uncallured AF20000 FF432822 FF432822 SSC-501/20 uncallured AF20002 FF432822 FF432822 FF432823 SSC-501/20 uncallured H2010119 FF32781 AF387797 FF32882 D2237819 SSC-501/20 uncallured JJ311027 JJ311027 JJ311027 JJ311027 Synchybrin encobellown P-68 and Slukrov JJ74274, AF454282 JF79577 AF387867 JJ311027 Synchybrin encobellown JE130 (AFTCL-D 23) AF164278 AF3970737 AF46888 Colordorybrin encobellown JE130 (AFTCL-D 23) AF164278 AF3970737 AF46888 AF3970737 AF46888 <th>Taxon</th> <th>Isolate number</th> <th colspan="3">GenBank accession no</th> <th>Cumulative</th>	Taxon	Isolate number	GenBank accession no			Cumulative
Order Lockolmyce angularia LLE (A/TOL-ID DS) AF184253 AF184253 PL439319 EF439319			18S	5.8S	28S	length (%)
Labotomyce angulare JEL49 (H OLD D30) A+194.233 AV90703 JEL29 (H OLD D30) Labotomyce angulare AFD1 EF432819 EF432819 EF432819 Manacytykum kaenne AFD3 EF432819 EF432819 EF432819 Manacytykum kaenne AFD3 EF432819 EF432819 EF432819 DCV04 uncultured AF180202 EF432819 EU117013 EU117013 Strict AF18020 uncultured AF180202 EU117013 EU117013 EU117013 Strict AF18020 uncultured H2014919 EU117013 EU117013 EU117013 Strict AF18020 uncultured H2014919 EU117013 EU117013 EU117013 Strict AF18020 UF70570 UC214819 UC214819 UC214819 UC214819 Strict AF18020 UF70570 UC214819 UC214819 UC214819 UC214819 Strict AF18020 UF70570 UC214819 UC214819 UC214819 UC214819 Strict AF18020 UF70570 UC217819 UC214819 UC21481	Order Lobulomycetales					
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Other CladeChythiaes CladeActythiae AVS46683 AV997037 AVS46683 AV997037 AVS46683 CladeActythum sp. JEL326 AV340046 AV340041 D223316 Allochythum sp. JEL326 AV360040 AV997034 D2237316 Diplochytholim lagenatum JEL127 AH009044 AV49109 AV340081 Diplochytholim lagenatum JEL127 AH009045 D02373789 D2373789 Order Chytholides Pedcehythum dentatum JEL370 (AFTOL-ID 1539) AH009055 D0233843 D02373789 Chytromyces sp. JEL147 (AFTOL-ID 1539) AH009065 D02338483 D02373785 Chytromyces sp. JEL376 (AFTOL-ID 1530) D0538487 D0233848 D0237835 Chytromyces sp. JEL376 (AFTOL-ID 1537) D0538487 D0238498 D0233848 Chytromyces sp. JEL376 (AFTOL-ID 1537) D0538487 D0238498 D0233836 Chytromyces sp. JEL376 (AFTOL-ID 1537) D0538487 D0238498 D0233848 Chytromyces sp. JEL376 (AFTOL-ID 1231 D6538482 D0237378	Karlingiomyces asterocystis	JEL572	HQ901769		HQ901708	52
Order Classics AV546683 AV997037 AV546683 AV349081 Endochytrium sp. LEL325 AV540643 AV349081 AV349081 Molchytrium sp. LEL325 AV565844 AV997037 LE23511 Dipicityfitum isgenarium item LEL72 AH000049 EU2285111 Dipicityfitum isgenarium isgenar						
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neprodynamia, p. LE12 AH009044 AY340109 AY34003 Nowakowskiela sp. JEL127 (AFTOL-ID 146) AY655355 AY97065 D0227398 Order Chyridinales Podochyrhum dentatum JEL137 (AFTOL-ID 153) AH009055 D0236433 D02273838 Chyridinyces sp. JEL37 (AFTOL-ID 20) AY091709 AY997076 D02273838 Chyridinyces sp. JEL37 (AFTOL-ID 50) AY097076 D02273838 D02273838 Chyridinyces sp. JEL1367 (AFTOL-ID 540) AY0982825 AY097035 D02273838 Chyridinyces sp. JEL347 (AFTOL-ID 1537) DQ336487 D0336489 D02273836 Chyridinyces sp. JEL341 (AFTOL-ID 1537) DQ336487 D0336489 D02273836 Chyridinyces sp. JEL341 (AFTOL-ID 1531) DQ336482 D0336492 D02273854 Chyridinyces sp. JEL341 (AFTOL-ID 15431 DQ336447 AY1997083 D02273785 Phyrachyrithum planicom JEL342 (AFTOL-ID 1421 AY1997070 D02273776 D02273776 Spizelomyces sp. JEL350 (AFTOL-ID 152) AF164245 AY997075 </td <td>Nenbrochytrium sp</td> <td>JEL 324 (AT TOL-ID 031)</td> <td>AH009049</td> <td>A1337044</td> <td>EU828511</td> <td>97 /1</td>	Nenbrochytrium sp	JEL 324 (AT TOL-ID 031)	AH009049	A1337044	EU828511	97 /1
Display Display <t< td=""><td>Nephiochytridium lagenarium</td><td>JEL 125</td><td>AH009044</td><td>AY349109</td><td>AV349083</td><td>50</td></t<>	Nephiochytridium lagenarium	JEL 125	AH009044	AY349109	AV349083	50
Order Chytridiaes Podechythum dentatum JEL30 (AFTOL-ID 1539) AH009055 DG53650 DG273832 Chytriomyces sp. JEL37 (AFTOL-ID 1522) DG504633 DG273832 DG273832 Chytriomyces spinosus JEL97 (AFTOL-ID 1540) AY601709 AY997076 DG273839 Chytriomyces spinosus JEL97 (AFTOL-ID 1540) AY608708 DG236483 DG536487 DG238489 Chytriomyces spinosus JEL347.4 (AFTOL-ID 1531) DG536487 DG536489 DG238489 DG238489 DG238489 DG238489 DG238483 Chytriomyces spinosus JEL34 (AFTOL-ID 1531) DG358487 DG536498 DG237813 Rhizdium endosporangiatum JEL34 (AFTOL-ID 1531) DG35847 AY997083 DG273785 Spizeliomycetales JEL36 (AFTOL-ID 152) AY546884 AY997082 AY546892 Spizeliomycetales JEL35 (AFTOL-ID 182) AY546884 AY997070 DQ273776 DQ273776 Gaertneriomyces semiglobiferus BK1-10 AF164247 AY997075 DQ273776 DQ273776 Go247382 AY997078 DQ273783 DQ273783 </td <td>Nowakowskiella sp.</td> <td>JEL127 (AFTOL-ID 146)</td> <td>AY635835</td> <td>AY997065</td> <td>DQ273798</td> <td>98</td>	Nowakowskiella sp.	JEL127 (AFTOL-ID 146)	AY635835	AY997065	DQ273798	98
Order Chymoniaes Podechymin deniatum JEL30 (AFTOL-ID 1539) AH009055 DQ33650 DQ273838 Chytriomyces sp. JEL376 (AFTOL-ID 1532) DQ33483 DQ273832 Chytriomyces sp. JEL376 (AFTOL-ID 1532) DQ33483 DQ2737831 Chytriomyces sp. JEL187 (AFTOL-ID 139) AY697076 DQ273783 Chytriomyces sp. JEL187 (AFTOL-ID 139) DQ334487 DQ536498 DQ237831 Chytriomyces sp. WB235A (AFTOL-ID 1537) DQ5364481 DQ536499 DQ273831 Chytriomyces sp. JEL21 (AFTOL-ID 1531) DQ536484 DQ536499 DQ273813 Order Spiellomyces sp. JEL36 (AFTOL-ID 1531) DQ536473 AY997070 DQ273813 Order Spiellomyces sp. JEL36 (AFTOL-ID 152) AY46684 AY997075 DQ273775 Phitytochythum planicome JEL45 (AFTOL-ID 152) AY46684 AY997075 DQ273785 Powellomyces sp. JEL5 (AFTOL-ID 120 AY46684 AY997075 DQ273776 Tirpatricalcar arcicum AFTOL-ID 20 AF164247 AY997075 DQ273776 Geartneinomyce	Orden Obstrictions					
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Openal (a) Control (C) Control (C) CONTROL	Chytriomyces sp	IEL 378 (AFTOL-ID 1532)	DO536483	DQ33030	DQ273832	73
Chytriomyces spinosus JELB9 (AFTOL-ID 1540) AH009083 D0273833 Chytriomyces spinosus JELB9 (AFTOL-ID 1540) AY635825 AY997035 D0273833 Chytriomyces sp. WB236A (AFTOL-ID 1539) D0536486 D02536499 D0238483 Chytriomyces sp. JELB14 (AFTOL-ID 1531) D0536487 D02536499 D0273831 Rhizdidum endesporangiatum JEL221 (AFTOL-ID 1531) D0536482 D0273831 D0273831 Rhizdidum endesporangiatum JEL221 (AFTOL-ID 1531) D0536482 D0273831 D0273851 Rhizdidum endesporangiatum JEL27 (AFTOL-ID 1531) D02536473 AY997083 D0273785 Phytochytrium planicome JEL9 (AFTOL-ID 182) AY546684 AY997075 D0273776 Spizellomyces punctatus ATCC 48900 (AFTOL-ID 182) AF164247 AY997081 D0273787 Gaertneriomyces semiglobiferus BK91-10 AF164247 AY997081 D0273787. Gaertneriomyces semiglobiferus AFTOL-ID 043 AY635829 AY997073 D0273787. Grader Rhizophydiales JEL316 (AFTOL-ID 131) AF164272 AY997	Rhizoclosmatium sp	JEL 347-h (AFTOL -ID 20)	AY601709	AY997076	DQ273769	98
Orbitridiales sp. JEL 187 (AFTOL-ID 39) AY635825 AY997035 DQ273783 Chytriomyces sp. WB235A (AFTOL-ID 1536) DQ536448 DQ536498 DQ233836 Chytriomyces sp. JEL341 (AFTOL-ID 1537) DQ536487 DQ538498 DQ273831 Rhizoldum endosporangiatum JEL234 (AFTOL-ID 1531) DQ536482 DQ273831 Rhizoldum endosporangiatum JEL354 (AFTOL-ID 1531) DQ536482 DQ273785 Phytochytrium planicome JEL354 (AFTOL-ID 611) AY635827 AY997083 DQ273785 Order Spizellomyces punctatus ATCC 48900 (AFTOL-ID 182) AY546684 AY997092 AY546682 Powellomyces sp. JEL95 (AFTOL-ID 20) AF164245 AY997075 DQ273786 Gaetrneriomyces semiglobiferus AFTOL-ID 34 AF164247 AY997051 DQ273787. Grderer Rhizophyctials JEL 318 (AFTOL-ID 43) AY635829 AY997078 DQ273787. Cateromyces sp. JEL 318 (AFTOL-ID 43) AY635829 AY997078 DQ273778. Cateromyces sp. JEL 318 (AFTOL-ID 31) AF164247 AY997071 DQ273776. <td>Chvtriomvces spinosus</td> <td>JEL59 (AFTOL-ID 1540)</td> <td>AH009063</td> <td>11001010</td> <td>DQ273839</td> <td>86</td>	Chvtriomvces spinosus	JEL59 (AFTOL-ID 1540)	AH009063	11001010	DQ273839	86
Chytriomyces sp. WB236 (AFTOL-ID 1536) DG536486 DG536487 DG536487 DG536489 DG237836 Chytriomyces sp. JEL341 (AFTOL-ID 1531) DG336482 DG237836 DG237836 Rhizolidum endosporangiatum JEL21 (AFTOL-ID 1534) DG356484 DG356496 DG237835 Rhizolidum sp. JEL364 (AFTOL-ID 1534) DG356473 AY997083 DG237835 Phytochrytnium planicome JEL47 (AFTOL-ID 152) AY546684 AY997085 DG237875 Spizellomyces punctatus ATCC 48900 (AFTOL-ID 182) AY546684 AY997096 DG237876 Spizellomyces punctatus ATCC 48900 (AFTOL-ID 32) AF164245 AY997096 DQ273786 Triparticalear arcticum AFTOL-ID 696 DG356490 AY997086 DQ273787 Gaertneriomyces semiglobiferus AFTOL-ID 431 AY635820 AY997078 DQ273787 Catenomyces sp. JEL38 (AFTOL-ID 43) AY635820 AY997077 DQ273778 Catenomyces sp. JEL384 (AFTOL-ID 131) AF164272 AY997077 DQ273778 Catenomyces sp. JEL316 (AFTOL-ID 31)	Chvtridiales sp.	JEL187 (AFTOL-ID 39)	AY635825	AY997035	DQ273783	98
Chytriomyces hyalinus AFTOL-ID 1537 DG538487 DG538499 DQ273836 Chytriomyces sp. JEL341 (AFTOL-ID 1531) DG536482 DQ273831 DQ273834 Rhizidium endosporangiatum JEL221 (AFTOL-ID 1534) DQ536482 DQ273835 DQ273834 Rhizidium endosporangiatum JEL384 (AFTOL-ID 41) AY635827 AY997003 DQ273813 Order Spizellomyces punctatus ATCC 48900 (AFTOL-ID 182) AY546684 AY997075 DQ273765 Powellomyces sp. JEL95 (AFTOL-ID 182) AY546684 AY997092 AY546692 Powellomyces sp. JEL95 (AFTOL-ID 20) AFT64245 AY997096 DQ273776 Gaertneriomyces semiglobiferus AFT0L-ID 34 AY997091 DQ273776 Order Rhizophlyctidales AFTOL-ID 43) AY635829 AY997078 DQ273776 Cateomyces sp. JEL318 (AFTOL-ID 143) AY635830 AY997077 DQ273776 P04-43 uncultured AY642701 DC358485 DG536497 DQ273775 P04-78 JEL318 (AFTOL-ID 31) AF164272 Y997077 DQ273775 <	Chytriomyces sp.	WB235A (AFTOL-ID 1536)	DQ536486	DQ536498	DQ536493	98
Chytromyces sp. JEL341 (AFTOL-ID 1531) DGS36482 DG273831 Rhizidium endosporangiatum «Rhizophydiums sp. JEL324 (AFTOL-ID 1534) DGS36484 DG36496 DQ273834 wRhizophydiums sp. JEL34 (AFTOL-ID 628) DGS36473 AY997083 DQ273765 Phytotcohytrium planicome JEL47 (AFTOL-ID 628) DGS36473 AY997070 DQ273813 Order Spizellomyces punctatus ATCC 48900 (AFTOL-ID 182) AY546684 AY997092 AY546692 Powellomyces se JEL36 (AFTOL-ID 32) AF164245 AY997075 DQ273826 Gaertneriomyces semiglobiferus BK91-10 AF164247 AY997078 DQ273776 Grder Rhizophyctidales - Rhizophyctidales AFTOL-ID 34 AY635829 AY997078 DQ273787. Order Rhizophyctidales - JEL318 (AFTOL-ID 47) AY635820 AY997078 DQ273787. Order Rhizophyctidales - - AFTOL-ID 2006 DG536491 DQ273776 P34.43 uncultured AY642701 - - - 'Rhizophydium sp. JEL371 (AFTOL-ID 35) <td>Chytriomyces hyalinus</td> <td>AFTOL-ID 1537</td> <td>DQ536487</td> <td>DQ536499</td> <td>DQ273836</td> <td>98</td>	Chytriomyces hyalinus	AFTOL-ID 1537	DQ536487	DQ536499	DQ273836	98
Rhizophydium sp. JEL21 (AFTOL-ID 134) DGS36444 DQS36446 DQ23785 Phlytachythium planicome JEL354 (AFTOL-ID 41) AY635827 AY99703 DQ273813 Order Spizellomyces punctatus ATCC 48900 (AFTOL-ID 182) AY546684 AY997092 AY5466892 Powellomyces punctatus ATCC 48900 (AFTOL-ID 182) AY546684 AY997092 AY5466892 Powellomyces sp. JEL95 (AFTOL-ID 182) AY546684 AY997096 DQ273786 Gaetrneriomyces semiglobiferus BK91-10 AF164247 AY997051 DQ273776 Gaetrneriomyces semiglobiferus BK91-10 AF164247 AY997051 DQ273787 Order Rhizophlyctidacs JEL 318 (AFTOL-ID 43) AY635829 AY997078 DQ273787 Catenomyces sp. JEL318 (AFTOL-ID 43) AY635830 AY997033 DQ273776 Blyttiomyces helicus AFTOL-ID 2006 DQ536491 DQ273775 DQ273775 P34 4.3 uncultured AY642701 DQ273775 DQ273776 P34 4.3 uncultured AY642701 DQ273779 DQ273779	Chytriomyces sp.	JEL341 (AFTOL-ID 1531)	DQ536482		DQ273831	92
«Rhizophydium sp. JEL354 (AFTOL-ID 41) AY635827 AY997083 DQ27385 Phlycochytrium planicome JEL47 (AFTOL-ID 628) DQ536473 AY997070 DQ273813 Order Spizellomyces punctatus ATCC 48900 (AFTOL-ID 182) AY546684 AY997092 AY546692 Powellomyces sp. JEL95 (AFTOL-ID 32) AF164245 AY997096 DQ273786 Triparticaler arcticum AFTOL-ID 696 DC536480 AY997096 DQ273776 Gaertneriomyces semiglobiferus BK91-10 AF164247 AY997051 DQ273776 Gaertneriomyces semiglobiferus AFTOL-ID 34 Y997053 DQ273787 DQ273776 Order Rhizophydiales F HTOL-ID 47) AY635830 AY997033 DQ273776 Byltiomyces helicus AFTOL-ID 2006 DC536491 DQ273776 DQ273776 P34.43 uncultured AY642701 DQ273776 DQ273776 Pitzophydium sp. JEL371 (AFTOL-ID 131) AF164272 AY997071 DQ273776 Pdotiomyces narcesporum PLL38 (AFTOL-ID 22) AY6601710 AY997081 DQ273770	Rhizidium endosporangiatum	JEL221 (AFTOL-ID 1534)	DQ536484	DQ536496	DQ273834	100
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Spizellomyces punctatus ATCC 48900 (AFTOL-ID 182) AY546824 AY997092 AY546692 Powellomyces sp. JEL95 (AFTOL-ID 32) AF164245 AY997075 DQ273776 Triparticaler arcticum AFTOL-ID 966 DQ535480 AY997096 DQ273778 Gaertneriomyces semiglobiferus BK91-10 AF164247 AY997051 DQ273778 Order Rhizophlycitiales FTOL-ID 34 AY635829 AY997075 DQ273787. Catenomyces sp. JEL342 (AFTOL-ID 43) AY635830 AY997033 DQ273789 Blyttiomyces helicus AFTOL-ID 2006 DQ536481 AY997077 DQ273775 P34.43 uncultured AY642701 V V Order Rhizophydiales - - V V F/Rhizophlyditi's harderi JEL316 (AFTOL-ID 35) DQ536455 DQ536497 DQ273779 Rhizophydium sp. JEL316 (AFTOL-ID 22) AY601710 AY997081 DQ273770 Rhizophydium sp. JEL316 (AFTOL-ID 22) AY601710 AY997084 DQ273823 Kappamyces laurelensis AFTOL-ID 689	Order Spizellomycetales					
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Order Rhizophydiales	P34.43	uncultured	AY642701			36
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Fig. 1 Stages of the life cycle of *Gromochytrium mamkaevae* (x-51 CALU) on the host *Tribonema gayanum.* — a-c: LM images of living parasite on filament of host *Tribonema*, phase contrast. – a. Two cysts with a lipid globule; b. young sporangium with 3 lipid globules; c. mature sporangium contains zoospores. – d. Rhizoid in the host cell in TEM. – e. Drawing of the life cycle. — Abbreviations: cy = cyst; I = lipid globule; msp = mature sporangium; rh = rhizoid; sp = sporangium; spw = sporangium wall; ysp = young sporangium; zs = zoospores. — Scale bars: $a-c = 10 \mu m$; d = 2 μm .

Region (Russia) in the autumn of 1999 by B.V. Gromov, and maintained on the host culture of filamentous, freshwater yellow-green alga *Tribonema gayanum* Pascher CALU 20 cultivated on No. 1 liquid organic medium (Gromov & Titova 1991). A dual clonal culture was incubated at 25 °C under continuous illumination of 25 µmol photon m⁻²·s⁻¹ supplied by 40 W cool white fluorescent tubes.

For light microscopy, the parasite was examined with a Zeiss phase-contrast microscope.

For electron microscopy, the dual culture material was prefixed with 0.5 % OsO_4 for 10 min followed by 2.5 % glutaraldehyde in 0.05 M cacodylate buffer at 4 °C for 2 h. The samples were then incubated with buffered 1 % osmium tetroxide for 1 h at 4 °C. After centrifugation the pellet was dehydrated with a graded ethanol series, and embedded in Spurr's resin. Thin sections were stained with uranyl acetate and lead citrate, and examined with a Jeol 1011 electron microscope at 80 kV.

After inoculation of host strain with x-51, the cultures were incubated until the maximum infection of host cells was reached. Zoospores were then harvested by centrifugation and used directly for DNA extraction. The DNA was extracted with Diatom DNA Prep (IsoGen Lab, Moscow). The rRNA gene sequences were amplified using Encyclo PCR kit (Evrogen, Moscow) and a set of primers (Medlin et al. 1988, van der Auwera et al. 1994) and sequenced directly with Applied Biosystems 3730 DNA Analyzer. The assembled contig sequence was deposited in GenBank under accession number KF586842.

Molecular phylogenetic analysis

Ribosomal DNA sequences of x-51 were aligned with 113 OTUs from zoosporic fungi and closely related uncultured clones collected from the GenBank database. Sequences were selected based on the following scheme. First, all chytrid LSU genes that had sufficiently large length (> 2 000 bp) were added to the list of OTUs, and SSU genes were selected for all listed species. Second, all fragments of chytrid SSU and LSU rRNA genes were selected from cultured strains and environmental samples that occupied isolated positions on the distance tree. Third, all sequences of uncultured clones available in GenBank as of January 2013 were selected that grouped closely with x-51 CALU and *Mesochytrium penetrans* x-10 CALU. For environmental sample sequences that formed particularly long branches on the distance tree we performed an additional



Fig. 2 Bayesian phylogenetic tree based on concatenated rDNA sequences (18S, 5.8S, 28S). Node support values are given by Bayesian posterior probability (left of the vertical line) and Maximum Likelihood bootstrap support (right of the vertical line). Support values are omitted for nodes that score above 95 % in both analyses (edges drawn with thick lines) and nodes that score less than 50 % in both analyses (edges drawn with striated lines). The strain x-51 - *Gromochytrium mamkaevae* is highlighted with red. Two groups of nearly identical clones in the *Mesochytriales* clade are collapsed into single branches (represented by triangles).

verification step that involved breaking the sequence into two or more non-overlapping fragments that were then used as independent OTUs for preliminary phylogenetic analysis (data not shown). This method identified seven sequences (accession numbers: EU162637, EF196798, EF196785, EF196773, EF196750, FJ592495, HQ191339) from three independent environmental samples as potentially chimaeric. The parts of sequences EU162637 and FJ592495 that presumably have fungal source were retained; the remainder and the other four sequences were excluded from the phylogenetic analysis. To minimise missing data a small number of sequences was assembled by fusing or constructing a consensus of sequences from different isolates of the same species or by fusing partial sequences that have a 98–100 % overlap identity. The full list of consensus and chimaeric sequences constructed for the purpose of phylogenetic analysis is presented in Table 1 and Fig. 2. The sequences of early-branching fungal taxa – *Rozella allomycis* and *Amoeboaphelidium protococcarum* were chosen

 Table 2
 List of environmental clones of the Mesochytriales and Gromochytriales.

Name	GenBank accession no.	Habitat / Geographic location	Characterisation/Season	Reference
Gromochytrium mamkaevae CALU x-51	KF586842	Ditch near town Kirovsk, Leningrad Region	parasite of yellow-green alga <i>Tribonema gayanum</i>	This paper
Mesochytrium penetrans CALU x-10	FJ804149; FJ804153	Small lake in Karelia (Northern Europe)	parasite of green alga Chlorococcum minutum	Karpov et al. (2010)
528-025	EF586095	Opanuku Stream biofilm, Auckland, New Zealand		Dopheide et al. (2008)
PFD6SP2005, PFG9SP2005, PFF5SP2005, PFA12SP2005	EU162637, EU162638, EU162641, EU162643	Oligo-mesotrophic mountain Lake Pavin, France	May – June	Lefèvre et al. (2008)
BI74, B1, B43, B44, B46-138, B49, B52, B56, BI78, BI88, BI100, BI104, BI107, BI121, BI123, BI15, BI72, BI76, B86-161, BI5	EF196711, EF196713, EF196728, EF196729, EF196731, EF196734, EF196735, EF196738, EF196745, EF196749, EF196751, EF196753, EF196755, EF196762, EF196763, EF196765, EF196776, EF196776, EF196786, EF196799	Large mesotrophic alpine Lake Bourget, France	May – August	Lepère et al. (2008)
F08_SE1B	FJ592495	Cold-fumarole soil, Socompa Volcano, Andes (elev. 5824 m)	April	Costello et al. (2009)
P60E-9, P60E-29	DQ104060, DQ104068	Glacial ice from Tibetan plateau	150-yr-old ice	Zhang et al. (2009)
T2P1AeB05, T2P1AeF04, T3P1AeC03, T5P2AeC07	GQ995415, GQ995412, GQ995413, GQ995414	High-elevation soil not far from ice and snow	July – October	Freeman et al. (2009)
PA2009C3, PA2009B6, PA2009D8, PA2009E7	HQ191369, HQ191400, HQ191406, HQ191286	Oligo-mesotrophic mountain Lake Pavin, France	July	Monchy et al. (2011)
SAPA5_E7	FJ483310	Salt marsh, USA: RI	Summer	Mohamed & Martiny (2011)
ThJAR2B-48	JF972676	Air sample, Greece	October	Genitsaris (2011)
GA069	HM486988	Feces from a detritus-feeding crustacean <i>Gammarus tigrinus</i> ; Canada	September – October	Sridhar et al. (2011)
Spring_08, Spring_37, Spring_57, Spring_71	JX069031, JX069054, JX069067, JX069077	River site, Southern Alberta, Canada	Spring	Thomas et al. (2012)
Pa2007C10	JQ689425	Oligo-mesotrophic mountain Lake Pavin, France	April	Jobard et al. (2012)
Va2007BB6	JQ689445	Large brown-coloured humic and mesotrophic Lake Vassivière, France	May	Jobard et al. (2012)
WS 10-E02, WS 10-E14, WS 10-E15	AJ867629, AJ867630, AJ867631	Melted white snow water, alpine Lake Joeri XIII, Switzerland	-	Unpubl. data
GF29312	JX417945	Greenhouse soil, China	-	Unpubl. data
Seven clones from a freshwater lake in China	JX426910, JX426918, JX426923, JX426937, JX426998, JX427002, JX427011	Freshwater lake, China	-	Unpubl. data
kor_110904_17	FJ157331	Lake Koronia water column, Greece	Nov.	Genitsaris et al. (2009)
IIN1-34	EU516964	Alpine snow-covered soil, Alpes, Austria	-	Unpubl. data
Nineteen clones: E109_XXX, E107_XXX	KC561936-KC561954	High mountain soil Nepal	October	Naff et al. (2013)
Five clones: R11a_XX	KC561955-KC561959	Rocky Mountain talus snow, Colorado, USA	July – August	Naff et al. (2013)
Sixteen clones: ₫31a_XX, T31b_XX	KC561960-KC561975	Rocky Mountain talus snow, Colorado, USA	July – August	Naff et al. (2013)
NKS146	JX296576	Hyposaline soda lake Nakuru, Kenya, Fast Africa	November	Luo et al. (2013)

as outgroup (James et al. 2006, Karpov et al. 2013). Alignments were generated with MUSCLE (Edgar 2004) and refined manually using BioEdit (Hall 1999). After discarding ambiguously aligned nucleotide positions and concatenating the alignments of 18S, 5.8S and 28S rRNA genes, the alignment consisted of 4 850 positions. Tree search for the concatenated alignment was performed using the Bayesian method implemented by MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). The tree reconstruction used GTR+G12+I model and partition by genes (18S, 5.8S, and 28S) with all parameters unlinked, except the topology and branch lengths. Four independent runs of eight Markov Chain Monte Carlo (MCMC) were performed to evaluate the convergence. Chains were run for 10 million generations sampling trees every 1 000 generations after discarding the first 8 million as burn-in. Sampled trees were used to generate a majority rule consensus tree with Bayesian posterior probabilities. Bootstrap support values for the consensus tree reconstructed by MrBayes were generated using RAxML v. 7.2.6 (Stamatakis 2006) on the basis of 1 000 replicates under the GTR+G+I model.

RESULTS

Light microscopy

The parasite has a typical chytrid endogenous life cycle with tiny (~ 2 µm diam) zoospores that attach to the host cell surface, retract the flagellum and encyst. After the germ tube enters the host the zoospore cyst enlarges; a prominent lipid globule is clearly visible at this early stage (Fig. 1a). The young sporangium has homogenous contents with few lipid globules of different size (Fig. 1b), and the mature sporangium contains zoospores, which are released through an apical pore. The inoperculate sporangium is long ovoid (~ 18 × 10 µm diam) without a differentiated apical papilla (Fig. 1c). The apical pore varies in its dimensions: from narrow to as broad as the diameter of the sporangium or even broader (Fig. 1e). The delicate rhizoidal system is poorly visible, but can be estimated as weakly branched with short rhizoids emerging from a slender main axis (Fig. 1d, e). According to this description the fungus could be identified as Rhizophydium mammillatum (A. Braun) A. Fish. (1892) or, less likely, R. melosirae (1952) (Sparrow 1960, Letcher & Powell 2012), and therefore it was identified as R. mammillatum (Mamkaeva et al. 2006).

Molecular phylogeny

The rDNA sequences of strain x-51 occupy an isolated position in the tree (Fig. 2); its closest relatives are three uncultured clones: one from Lake Koronia in Greece (clone kor_ 110904 17), another from snow-covered soil in alpine Austria (clone IIN1-34), and one more from a hyposaline soda lake in Kenya, East Africa (Genitsaris et al. 2009, Kuhnert et al. 2012, Luo et al. 2013). Together these sequences form a new phylogenetic group. Among the described organisms, the closest relative of this group is Mesochytrium penetrans, which was classified in the Chytridiomycetes incertae sedis (Karpov et al. 2010). Mesochytrium penetrans is the only described species of a diverse group of uncultured fungi from soil, freshwater and hydrobiont gut samples collected from temperate zone of Eurasia and North America (Table 2). This group was recognised earlier as an order-level 'Novel clade I' within the Chytridiomycetes (Lefèvre et al. 2008, Jobard et al. 2012). Another name for 'clade I' is 'snow chytrids' ('Snow Clade 1' or SC1) according to Naff et al. (2013). The rDNA data places the clade uniting x-51 and the 'clade I' (Lefèvre et al. 2008) sister to Lobulomycetales (Simmons et al. 2009), albeit with relatively low support (Fig. 2). The distances inside the clusters of OTUs that contain x-51 and *M. penetrans* on the rDNA tree are comparable to the

distances inside the established orders of *Chytridiomycota*, and distances between the OTUs in these clusters and the members of *Lobulomycetales* are no less than the distances between different orders of *Chytridiomycota* (Fig. 2).

Zoospore ultrastructure

The spherical zoospore has a posterior flagellum and sometimes produces short anterior filopodia (Fig. 3c). A core of aggregated ribosomes is located in the posterior part of the cell. The ribosomal aggregation is relatively small and does not have surrounding endoplasmic reticulum (Fig. 3, 4). The ribosomes fill the space between the flagellar base and the nucleus and have no connection with nucleus, mitochondria or other membrane bounded organelles.

Several mitochondria with flat cristae reside at the cell periphery. A nearly central nucleus associates with anteriorly adpressed narrow microbody and a single large lipid globule anteriorly attached to the microbody (Fig. 3a). The anterior flat side of the lipid globule is bounded by a prominent fenestrated cisterna (rumposome) oriented to the cell exterior. Thus, the microbodylipid globule complex (MLC) contains a single microbody enveloping a large anterior lipid globule with fenestrated cisterna.

Endoplasmic reticulum cisternae are rare and are normally found at the cell periphery. A vesicle rich zone occupies an area from one side of the ribosomal core extending from the nucleus to the centriole (Fig. 3a, c). Several small vesicles with electron-opaque contents (dense bodies) are present in the cytoplasm of the anterior part of the cell.

Kinetid structure

The structure of the flagellar apparatus was investigated with serial sections of six released zoospores. The kinetosome and centriole are embedded in the ribosomal core (Fig. 3d, e, 4). The kinetosome is c. 400 nm long and composed of microtubular doublets (not triplets) with developed transitional fibers (props) (Fig. 4b-d). The flagellar transition zone is simple without transversal plate, but with a slightly inward curved diaphragm at the distal end of kinetosome (Fig. 4g). Two thin lines parallel to the peripheral microtubular doublets are present above the diaphragm, and seem to correspond to the spiral fiber, or cylinder (Fig. 4g). The centriole is about 100 nm long and lies at an angle of c. 30° to the kinetosome (Fig. 3e, 4b, c, e, f). The kinetosome is connected to the centriole by a broad fibrillar bridge composed of at least three thick connectors (Fig. 4d). The longest middle connector passes through the bottom of kinetosome to the side of centriole. The structure of interconnecting bridge seems to be an unstable character. The bridge looks rather broad and prominent, connecting the sides of kinetosome and centriole at the longitudinal sections (Fig 4e, f), but it is not visible at the corresponding transverse sections (Fig. 4b-d). Approximately 1/3 of all serial sections had the broad bridge connecting the sides of kinetosome and centriole and in 2/3 of the series the bridge connects the bottom of kinetosome to the side of centriole. The diagram (Fig. 5a) shows the more common state.

The kinetosome produces at least two microtubular roots. The anterior root consists of two microtubules and passes laterally in the direction of the lipid globule crossing the surface of fenestrated cisterna (Fig. 3a, 5). The posterior root is much shorter, composed of one or two microtubules and is directed right about the anterior root (Fig. 4a–d). Their origin is not clear: anterior root emerges in the vicinity of kinetosome, and posterior root appears somewhere in between the kinetosome and the centriole.

One more kinetosomal derivate, a spur, lies close to the outer surface of the kinetosome on the side opposite the centriole



Fig. 3 General ultrastructure of *Gromochytrium mamkaevae* (x-51 CALU) zoospore. — a. General disposition of nucleus and other organelles at LS; b. tangential section of fenestrated cisterna crossed by anterior microtubular root; c. pseudopodia at cell anterior; d, e. two consecutive sections of the kinetid. — Abbreviations: ar = anterior microtubular root; c = centriole; d = kinetosome diaphragm; db = dense bodies; fc = fenestrated cisterna; k = kinetosome; l = lipid globule; m = mitochondrion; mb = microbody; n = nucleus; ps = pseudopodia; rc = ribosomal core; tf = transitional fibers (props); vz = vesicular zone. — Scale bar on E: a = 300 nm; b, c = 400 nm; d, e = 200 nm.

(Fig. 4f, g). The spur is thin and short, projecting about 70–100 nm from the kinetosome into the ribosomal core (Fig. 4f).

A general scheme of zoospore ultrastructure is illustrated in Fig. 5a.

DISCUSSION

According to the morphology of strain x-51 at different life cycle stages it belongs to the genus Rhizophydium sensu Sparrow (1960). It has a simple thallus composed of inoperculate monocentric epibiotic elongated sporangium. It bears a single slightly branching rhizoidal axis. Judging by the shape of the sporangium and its dimensions this strain could be Rh. mammillatum, however, contrary to Rh. mammillatum, the sporangium of x-51 has no papilla. Our study has shown that zoospore ultrastructure of x-51 differs cardinally from that of Rhizophydium and other members of Rhizophydiales (Letcher et al. 2006, 2008). The order Rhizophydiales has 18 zoospore types that are rather different from each other, but none have a posterior ribosomal core without delimiting ER and mitochondria separated from MLC as in x-51. The MLC structure in the zoospore of x-51 has similarities with that of the recently established Gorgonomyces, which unlike other rhizophydiales has a close association of nucleus with microbody and lipid globule (Letcher et al. 2008), but in all other respects the zoospore of *Gorgonomyces* is different.

Molecular phylogeny places the strain x-51 far from *Rhizophydiales*, as a sister to 'clade l' – a cluster containing many environmental sequences of the *Chytridiomycetes* (Lefèvre et al. 2008, Jobard et al. 2012) besides a formally described species *Mesochytrium penetrans*, which was earlier shown to have a rather isolated position among the *Chytridiomycetes* (Karpov et al. 2010). The features that distinguish *Mesochytrium* are the partial penetration of the host cell by the sporangium and a zoospore with a unique ultrastructural organization.

Thus, we have to compare the zoospore structure of strain x-51 with that of *M. penetrans*. Two strains of *M. penetrans* (x-10 and x-46 CALU) were studied by electron microscopy, and 18S and 28S rRNA genes were sequenced for x-10 (Gromov et al. 2000, Karpov et al. 2010). Their general organization differs from that of x-51; unlike x-51 the *M. penetrans* has no ribosomal aggregation, its mitochondrion with MLC is enclosed by ER, a fenestrated cisterna faces the posterior of the cell, and a vacuole is present (Fig. 5b). At the same time, some morphological characters are similar in x-51 and x-10; both have small dense vesicles in the cytoplasm, which are common for the *Chytridiomycetes*; the kinetosomes lie at the same angle to each other and the flagellar transition zones contain a spiral



Fig. 4 Kinetid structure of *Gromochytrium mamkaevae* (x-51 CALU) zoospore. a-d. Selected serial TS of the kinetid from distal to proximal. View from flagellar base to top. Arrowhead on b shows a spiral fiber. Arrowheads on d mark the bridge between kinetosome and centriole; e-g. selected serial LS of the kinetid. Arrow on g shows a spiral fiber. — Abbreviations: c = centriole; d = kinetosome diaphragm; k = kinetosome; pr = posterior microtubular root; rc = ribosomal core; s = spur; tf = transitional fibers (props). — Scale bar on E: a-d = 300 nm, e-g = 200 nm.

element or a cylinder (Fig. 5). The kinetid structure also has some differences; x-51 has two microtubular roots which are absent in *M. penetrans*, a bridge in x-51 connects the bottom of kinetosome to the lateral surface of the centriole, not the lateral surfaces of kinetosome and centriole as in *M. penetrans* and the kinetosome of x-51 is composed of microtubular doublets. The spur structure and shape are also different; in x-51 the spur is inconspicuous and straight and in *M. penetrans* it is long and curved enclosing both the kinetosome and the centriole (Fig. 5).

We conclude, that the overall organization and kinetid structure of the zoospores of *M. penetrans* and x-51 differ considerably. According to the modern paradigm stemming from D. Barr's studies (e.g. Barr 1978, Barr & Hadland-Hartmann 1978, Powell 1978, Longcore 1995, 1996, Letcher et al. 2006, 2008, Simmons 2009), their zoospores certainly have enough peculiarities to separate them at the taxonomic level of order. Moreover, their zoospores can be regarded as having a unique organization among the chytridiomycetes. We have already shown this for *M. penetrans* (Karpov et al. 2010). For the strain x-51 the unique characters are: the posterior core of ribosomes is not bounded by ER membranes, mitochondria are not associated with MLC, and a bridge connects the bottom of kinetosome to centriole.

The nearest branch to the x-51/*Meshochytrium* cluster is the order *Lobulomycetales* (Fig. 2), a group that was recently established on the basis of SSU and partial LSU gene phylogeny and ultrastructural analysis of zoospores (Simmons et al. 2009). In the previous study, the 18S and 28S sequences of *M. penetrans* (strain x-10 CALU) also placed this strain as a sister lineage to *Lobulomycetales* but with a rather low support (Karpov et al. 2010). 'Snow chytrids' were also suggested as a deep divergent branch sister to *Lobulomycetales* (Naff et al. 2013). In the present study the increased taxon sampling through the addition of environmental sequences results in better support for the sister group position of the x-51/*Meshochytrium* cluster relative to *Lobulomycetales* (Fig. 2).

Zoospores of Lobulomycetales (Lobulomyces angularis, Clydaea vesicula and Maunachytrium keaense) differ from those of x-51 and Meshochytrium in a number of ways: kinetids of lobulomycetes have parallel centrioles, an electron-opaque plug is present in the flagellar transition zone, and no spur or flagellar roots are found; the ribosomal core in Lobulomycetes is bounded by the ER, and the vacuole and 1–2 lipid globules lie posteriorly (Simmons et al. 2009). The presence of a rumposome (fenestrated cisterna) was noted in the text, but not shown in the pictures of the above cited article, therefore its precise position is unknown for *Lobulomycetales*.

Thus, our morphological data strongly support an isolated position of x-51/*Meshochytrium* cluster on the phylogenetic tree.

Taxonomy

An isolated position of Mesochytrium was shown by 18S+28S rRNA gene phylogeny and zoospore morphology of two strains: x-46 CALU (Gromov et al. 2000) and x-10 (Karpov et al. 2010), and recapitulated by molecular phylogenetic analysis in the present paper. The sequence of *M. penetrans* clusters with a large number of environmental sequences forming a clear monophyletic branch with good statistical support (Fig. 2). Molecular phylogenetic analysis of this genus does not reveal family or ordinal level affinity of *M. penetrans*, consequently in the previous paper we referred to it as incertae sedis (Karpov et al. 2010). Here we have a better resolved tree with a number of environmental sequences and a new neighbour of this branch that includes isolate x-51. Because of the molecular phylogeny of M. penetrans and CALU x-51, together with each having a unique organisation of zoospores, we establish new orders and families for both, plus a new genus and species for CALU x-51.

Gromochytriales Karpov & Aleoshin, ord. nov. — MycoBank MB805305

Zoospore with posterior ribosomal aggregation not bounded by endoplasmic reticulum. Microbody-lipid complex adpressed to the nucleus and containing a single microbody enveloping a large anterior lipid globule with anteriorly oriented fenestrated cisterna. Several mitochondria are separated from MLC. Small dense bodies present in peripheral cytoplasm. Kinetosome and centriole embedded in posterior side of the ribosomal core. *Flagellar transition zone* contains a spiral fiber, or a cylinder. Centriole at an angle of c. 30° to kinetosome; bottom of kinetosome connected by a broad fibrillar bridge to centriole. Anterior and posterior microtubular roots and a short straight spur associated with kinetosome.



Fig. 5 General scheme of zoospore structure. — a. *Gromochytrium mamkaevae* (x-51 CALU); b. *Mesochytrium penetrans* (x-10 CALU). Arrows show the spiral fiber in flagellar transition zone (b: after Karpov et al. (2010) with modified abbreviations).— Abbreviations: ar = anterior microtubular root; br = bridge between kinetosome and centriole; c = centriole; d = kinetosome diaphragm; db = dense bodies; er = endoplasmic reticulum; fc = fenestrated cisterna; gf = girdle fiber; k = kinetosome; I = lipid globule; m = mitochondrion; mb = microbody; n = nucleus; pr = posterior microtubular root; ps = pseudopodia; rc = ribosomal core; s = spur; tf = transitional fibers (props); v = vacuole; ve = veil; vz = vesicular zone.

Gromochytriaceae Karpov & Aleoshin, fam. nov. — MycoBank MB805306

Type genus. Gromochytrium Karpov & Aleoshin.

Description as for *Gromochytriales*: simple thallus with inoperculate, monocentric, epibiotic sporangium having endogenous development and single slightly branching rhizoidal axis.

Gromochytrium Karpov & Aleoshin, gen. nov. — MycoBank MB805307

Type species. Gromochytrium mamkaevae Karpov & Aleoshin.

Simple thallus with inoperculate, monocentric, epibiotic sporangium having endogenous development and single slightly branching rhizoidal axis. *Zoospore* with posterior ribosomal aggregation unbounded by endoplasmic reticulum. Microbodylipid-complex adpressed to the nucleus and contains a single microbody enveloping a large anterior lipid globule with anteriorly oriented fenestrated cisterna. Several mitochondria are separated from MLC. Small dense bodies present in peripheral cytoplasm. Kinetosome and centriole embedded in posterior side of the ribosomal core. *Flagellar transition zone* contains a spiral fiber, or a cylinder. Centriole at an angle of c. 30° to kinetosome; bottom of kinetosome connected by a broad fibrillar bridge to centriole. Anterior and posterior microtubular roots and a short straight spur associated with kinetosome composed of microtubular doublets.

Gromochytrium mamkaevae Karpov & Aleoshin, sp. nov. — MycoBank MB805308, GenBank KF586842; Fig. 1–5a

Etymology. Genus named in honour of Boris V. Gromov, a prominent Russian microbiologist, and species named in honour of his spouse, colleague and co-author, Kira A. Mamkaeva.

Mature inoperculate epibiotic sporangium long ovoid (18 × 10 μ m) without papillae. *Zoospores* released through apical pore. Delicate, weakly branched rhizoidal system with short rhizoids emerging from a slender main axis. *Zoospores* 2 μ m diam with single lipid globule.

Specimen examined. Russia, Leningrad Region, ditch near town Kirovsk, parasite of *Tribonema gayanum*. Holotype x-51 presented by fixed specimen embedded in resin block for electron microscopy. Deposited in CALU (Biological Faculty of St. Petersburg State University, St. Petersburg 199034, Russia).

Mesochytriales Karpov & Aleoshin, *ord. nov.* — MycoBank MB805303

Zoospores with unique ultrastructural organisation; centriole at an angle of c. 30° to kinetosome; ribosomes dispersed through the cytoplasm; mitochondrion and MLC surrounded by rough endoplasmic reticulum.

Mesochytriaceae Karpov & Aleoshin, fam. nov. — MycoBank MB805304

Description as for *Mesochytriales*. Sporangium inoperculate, monocentric, epibiotic, endogenous, semi absorbed by host cell.

Mesochytrium B.V. Gromov, Mamkaeva & Pljusch. Nova Hedwigia 71: 159. 2000, emend. Karpov

Type species. Mesochytrium penetrans B.V. Gromov, Mamkaeva & Pljusch.

Zoosporangium sessile, partially penetrating host cell. Delicate branched rhizoids emerge near the sporangial base. Zoospores spherical to oval with single lipid globule and dispersed ribosomes. Microbody-lipid-complex composed of a single mitochondrion and a single lipid globule partially covered with microbody and posterior fenestrated cisterna; centriole with veil at an angle of c. 30° to kinetosome, the two being connected by a broad, dense fibrillar bridge. *Flagellar transition zone* contains a spiral fiber. *Resting spore* endobiotic, spherical with smooth thick wall.

Mesochytrium penetrans B.V. Gromov, Mamkaeva & Pljusch. Nova Hedwigia 71: 159. 2000, emend. Karpov

Sporangium pyriform $10-14 \times 6-7.5 \mu m$ with thin smooth wall and apical papilla. *Zoospores* spherical 2–2.5 μm diam with

a 5–14 µm long flagellum. Parasite of green alga *Chlorococ-cum minutum*.

Specimen examined. Small lake Pryazha in Karelia, parasite of *Chloro-coccum minutum.* Holotype CALU x-46.

Diversity and abundance of Mesochytriales and Gromochytriales in nature

The fact that Mesochytrium penetrans and Gromochytrium mamkaevae have thus far not been found during environmental DNA studies indicates that these species are not prevalent in the sampled ecosystems, at least not during the time of sampling. This fact emphasizes the incompleteness of our current knowledge of chytrid diversity and the importance of collecting new samples for exhaustive description of fungal diversity. At the same time, some of the undescribed species from the Mesochytriales clade that are represented by almost identical clones were repeatedly recovered in several environmental samples. Such clusters are formed by clones shown on Fig. 2 as small black triangles: one is presented by PFG9SP2005, PA2009C3, PA2009B6, PA2009D8 (Lefèvre et al. 2008, Monchy et al. 2011), another by PFF5SP2005, PFD6SP2005 (3'-end), Pa2007C10 and 20 clones are from Lake Bourget (Lefèvre et al. 2008, Lepère et al. 2008, Jobard et al. 2012), collected during the course of several years from lakes in France. Moreover, the clones of Mesochytriales from Lake Bourget form a substantial fraction of all fungal clones in the sample, which implies that their zoospores were ubiquitous during the time of sampling. It is likely that the abundance of Mesochytriales may vary by season. Ribosomal DNA clones of Mesochytriales accounted for about 50 % of the number of fungal rDNA clones from Lake Pavin (France) in spring and summer seasons (Lefèvre et al. 2008, Jobard et al. 2012), but they were not detected there in autumn (Lefèvre et al. 2007). Similarly to M. penetrans and G. mamkaevae, these clones probably can be attributed to parasites of algae. The diversity and abundance of rDNA clones from undescribed members in these environmental samples suggest that members of the Mesochytriales may play an important role as regulators of phytoplankton populations (Lefèvre et al. 2008, Lepère et al. 2008, Genitsaris et al. 2009, Monchy et al. 2011).

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