

SUPPLEMENTARY MATERIAL

Rediscovery and morpho-molecular characterization of three astome ciliates, with new insights into eco-evolutionary associations of astomes with their annelid hosts

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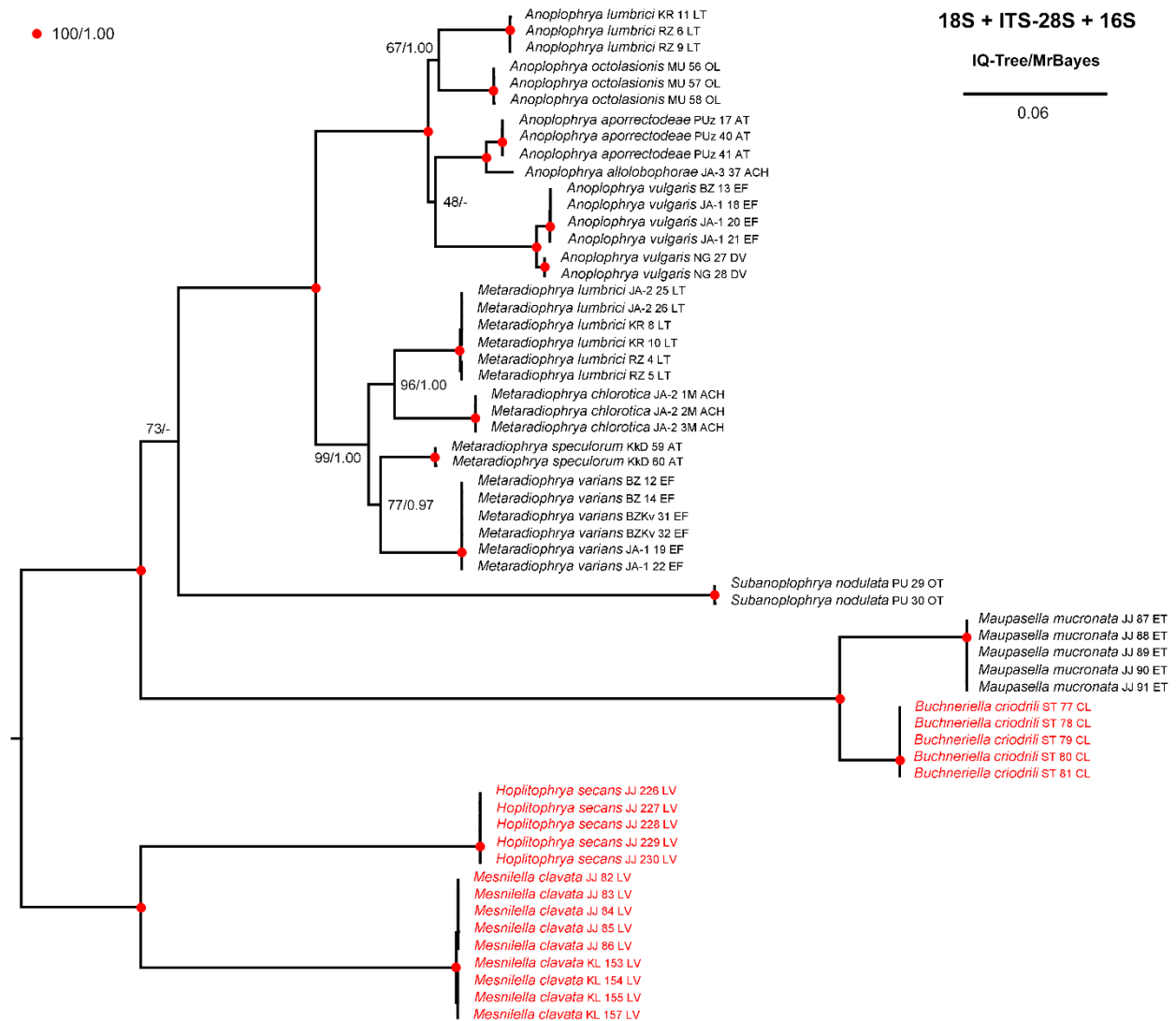


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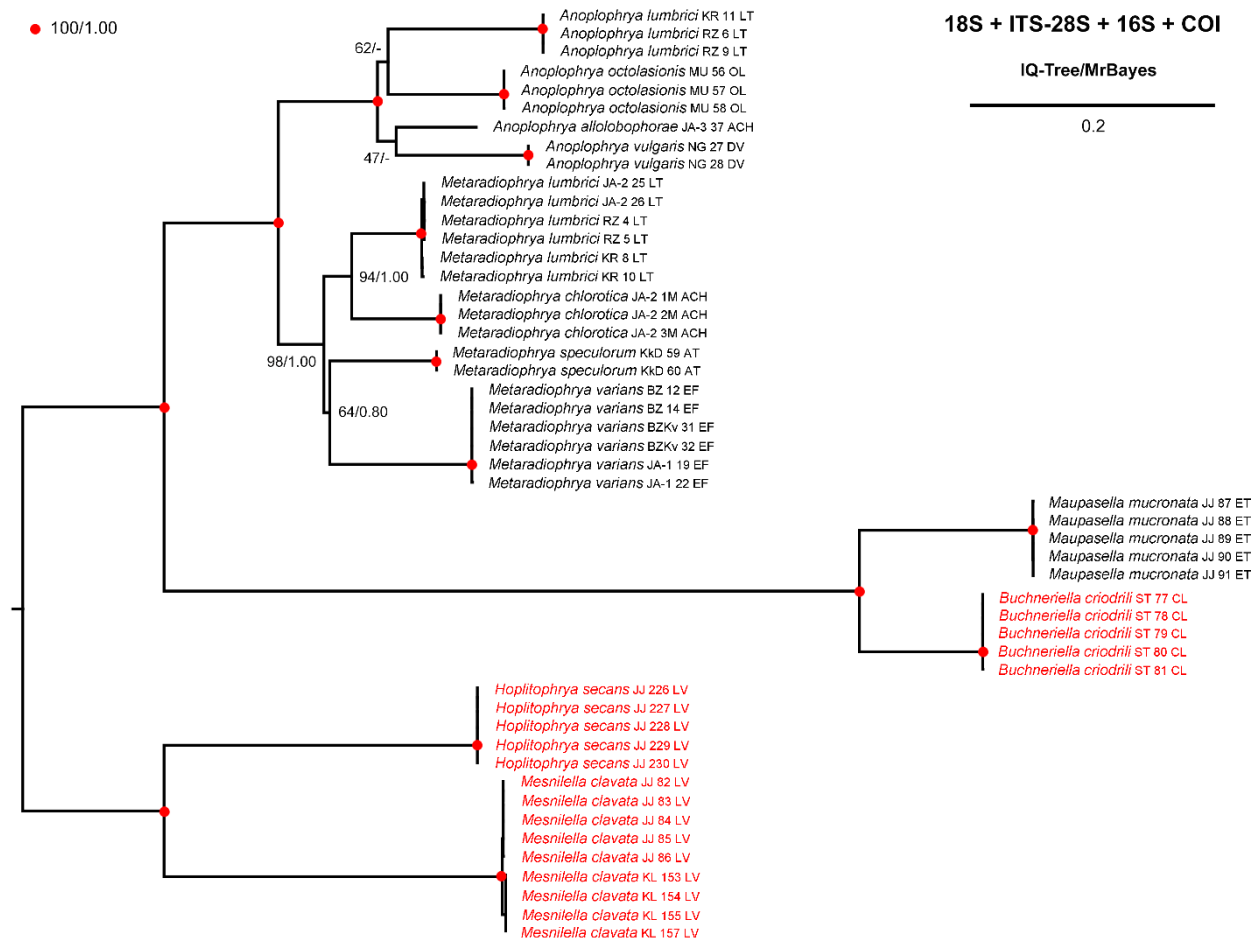
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Supplementary Fig. S1. Phylogenetic tree of the subclass Astomatia based on the nuclear 18S rRNA and ITS-28S rRNA gene sequences as well as the mitochondrial 16S rRNA gene sequences, showing the systematic positions of astome ciliates isolated from *Lumbriculus variegatus* and *Criodrilus lacuum*. Bootstrap values for maximum likelihood conducted in IQ-TREE and posterior probabilities for Bayesian inferences conducted in MrBayes were mapped onto the best scoring IQ-Tree. Sequences marked in red were obtained during this study. Fully statistically supported nodes are marked with red solid circles. Dash (–) indicates a mismatch in tree topologies. The scale bar denotes six substitutions per one hundred nucleotide positions



Supplementary Fig. S2. Phylogenetic tree of the subclass Astomatia based on the nuclear 18S rRNA and ITS-28S rRNA gene sequences as well as the mitochondrial 16S rRNA gene and COI sequences, showing the systematic positions of astome ciliates isolated from *Lumbriculus variegatus* and *Criodrilus lacuum*. Bootstrap values for maximum likelihood conducted in IQ-TREE and posterior probabilities for Bayesian inferences conducted in MrBayes were mapped onto the best scoring IQ-Tree. Sequences marked in red were obtained during this study. Fully statistically supported nodes are marked with red solid circles. Dash (–) indicates a mismatch in tree topologies. The scale bar denotes two substitutions per ten nucleotide positions

Supplementary Table S1. List of taxa with GenBank accession numbers of corresponding mitochondrial 18S rRNA gene sequences included in dataset 1.

Taxon	GeBank	Taxon	GeBank	Taxon	GeBank
Astomatia		Astomatia		Hymenostomatia	
<i>Almophrya bivacuolata</i>	HQ446281	<i>Mesnilella clavata</i> KL 154 LV	PQ238893	<i>Tetrahymena australis</i>	X56167
<i>Anoplophrya allolobophorae</i> JA-3 37 ACH	MZ048824	<i>Mesnilella clavata</i> KL 155 LV	PQ238894	<i>Tetrahymena borealis</i>	M98020
<i>Anoplophrya aporrectodeae</i> PUz 17 AT	MZ048825	<i>Mesnilella clavata</i> KL 157 LV	PQ238895	<i>Tetrahymena hegewischi</i>	X56166
<i>Anoplophrya aporrectodeae</i> PUz 40 AT	MZ048826	<i>Metaracoelophrya intermedia</i>	HQ446278	<i>Tetrahymena rostrata</i>	AF364042
<i>Anoplophrya aporrectodeae</i> PUz 41 AT	MZ048827	<i>Metaracoelophrya</i> sp. 1	HQ446277	Peniculia	
<i>Anoplophrya lumbrici</i> KR 11 LT	MN121061	<i>Metaracoelophrya</i> sp. 2	HQ446279	<i>Paramecium tetraurelia</i>	X03772
<i>Anoplophrya lumbrici</i> KR 9 LT	MZ048831	<i>Metaradiophrya chlorotica</i> JA-2 1M ACH	MZ048835	<i>Frontonia vernalis</i>	U97110
<i>Anoplophrya lumbrici</i> RZ 6 LT	MN121062	<i>Metaradiophrya chlorotica</i> JA-2 2M ACH	MZ048836	<i>Lembadion bullinum</i>	AF255358
<i>Anoplophrya octolasionis</i> MU 56 OL	MZ048828	<i>Metaradiophrya chlorotica</i> JA-2 3M ACH	MZ048837	Peritrichia	
<i>Anoplophrya octolasionis</i> MU 57 OL	MZ048829	<i>Metaradiophrya lumbrici</i> JA-2 25 LT	MN121068	<i>Campanella umbellaria</i>	AF401524
<i>Anoplophrya octolasionis</i> MU 58 OL	MZ048830	<i>Metaradiophrya lumbrici</i> JA-2 26 LT	MN121069	<i>Carchesium polypinum</i>	AF401522
<i>Anoplophrya vulgaris</i> BZ 13 EF	MZ048834	<i>Metaradiophrya lumbrici</i> KR 8 LT	MN121070	<i>Epicarchesium abrae</i>	DQ190462
<i>Anoplophrya vulgaris</i> JA-1 18 EF	MZ048832	<i>Metaradiophrya lumbrici</i> KR 10/1 LT	MN121071	<i>Epistylis galea</i>	AF401527
<i>Anoplophrya vulgaris</i> JA-1 20 EF	MN121065	<i>Metaradiophrya lumbrici</i> RZ 4 LT	MN121074	<i>Epistylis plicatilis</i>	AF335517
<i>Anoplophrya vulgaris</i> JA-1 21 EF	MN121066	<i>Metaradiophrya lumbrici</i> RZ 5 LT	MN121075	<i>Pseudovorticella punctata</i>	DQ190466
<i>Anoplophrya vulgaris</i> NG 27 DV	MZ048833	<i>Metaradiophrya</i> sp.	HQ446279	<i>Vorticella campanula</i>	AF335518
<i>Anoplophrya vulgaris</i> NG 28 DV	MN121067	<i>Metaradiophrya speculorum</i> HkD 59 AT	MW182012	<i>Vorticella microstoma</i>	DQ868347
<i>Buchneriella criodrilii</i> ST 77 CL	PQ238896	<i>Metaradiophrya speculorum</i> HkD 60 AT	MW182013	<i>Zoothamnium arbuscula</i>	AF401523
<i>Buchneriella criodrilii</i> ST 78 CL	PQ238897	<i>Metaradiophrya varians</i> BZ 12 EF	MN121076	<i>Zoothamnium pluma</i>	DQ662854
<i>Buchneriella criodrilii</i> ST 79 CL	PQ238898	<i>Metaradiophrya varians</i> BZ 14 EF	MN121077	Scuticociliatia	
<i>Buchneriella criodrilii</i> ST 80 CL	PQ238899	<i>Metaradiophrya varians</i> BZkv 31 EF	MZ048838	<i>Ancistrum crassum</i>	HM236340
<i>Buchneriella criodrilii</i> ST 81 CL	PQ238900	<i>Metaradiophrya varians</i> BZkv 32 EF	MZ048839	<i>Boveria subcylindrica</i>	FJ848878
<i>Durchoniella brasili</i>	FN998990	<i>Metaradiophrya varians</i> JA-1 19 EF	MN121078	<i>Cinetochilum ovale</i>	FJ870103
<i>Durchoniella legeridubosqui</i>	FN998995	<i>Metaradiophrya varians</i> JA-1 22 EF	MN121079	<i>Conchophthirus acuminatus</i>	OR127221
<i>Durchoniella</i> sp.	FN998993	<i>Njinella prolifera</i>	HQ446276	<i>Conchophthirus curtus</i>	OR127229
<i>Eudrilophrya complanata</i>	HQ446280	<i>Paraclautilocola constricta</i>	HQ446275	<i>Conchophthirus unionis</i>	OR127243

Taxon	GeBank	Taxon	GeBank	Taxon	GeBank
<i>Hoplitophrya secans</i> JJ 226 LV	PQ238882	<i>Paraclausilocola elongata</i>	HQ446274	<i>Cyclidium glaucoma</i>	Z22879
<i>Hoplitophrya secans</i> JJ 227 LV	PQ238883	<i>Pennarella elegantia</i>	PP109353	<i>Dexiotricha granulosa</i>	KF878931
<i>Hoplitophrya secans</i> JJ 228 LV	PQ238884	<i>Subanoplophrya nodulata</i> PU 29 OT	MN121063	<i>Dexiotricha</i> sp. 1	KF878932
<i>Hoplitophrya secans</i> JJ 229 LV	PQ238885	<i>Subanoplophrya nodulata</i> PU 30 OT	MN121064	<i>Haptophrya dugesiarum</i>	OL752521
<i>Hoplitophrya secans</i> JJ 230 LV	PQ238886	Apostomatia		<i>Haptophrya planariarum</i>	OL752480
<i>Maupasella mucronata</i> KDo 33 ET	MW182008	<i>Gymnodinioides pitelkae</i>	EU503534	<i>Haptophrya schmidtearum</i>	OL752526
<i>Maupasella mucronata</i> KDo 34 ET	MW182009	<i>Hyalophysa lwoffii</i>	EU503538	<i>Philaster apodigitiformis</i>	FJ648350
<i>Maupasella mucronata</i> KDo 35 ET	MW182010	<i>Vampyrophrya pelagica</i>	EU503539	<i>Philaster sinensis</i>	KJ815049
<i>Maupasella mucronata</i> KDo 36 ET	MW182011	Hymenostomatia		<i>Philasterides armatalis</i>	FJ848877
<i>Mesnilella clavata</i> JJ 82 LV	PQ238887	<i>Bromeliophrya brasiliensis</i>	AJ810075	<i>Pleuronema coronatum</i>	AY103188
<i>Mesnilella clavata</i> JJ 83 LV	PQ238888	<i>Colpidium campylum</i>	X56532	<i>Pleuronema setigerum</i>	FJ848874
<i>Mesnilella clavata</i> JJ 84 LV	PQ238889	<i>Glaucoma chattoni</i>	X56533	<i>Uronema marinum</i>	GQ465466
<i>Mesnilella clavata</i> JJ 85 LV	PQ238890	<i>Glaucomides bromelicola</i>	AJ810077	<i>Uronemella filificum</i>	EF486866
<i>Mesnilella clavata</i> JJ 86 LV	PQ238891	<i>Ichthyophthirius multifiliis</i>	U17354		
<i>Mesnilella clavata</i> KL 153 LV	PQ238892	<i>Ophryoglena catenula</i>	U17355		

Supplementary Table S2. Characterization and origin of nuclear and mitochondrial sequences of astome ciliates included in datasets 2 and 3.

Species	Specimen	Host species	Locality ^a	18S rRNA gene	ITS region and 28S rRNA gene	16S rRNA gene	COI gene
<i>Anoplophrya allolobophorae</i>	JA-3 37 ACH	<i>Allolobophora chlorotica</i>	JA-3	MZ048824	MZ048775	MZ048789	MZ044303
<i>Anoplophrya aporrectodeae</i>	PUz 17 AT	<i>Aporrectodea tuberculata</i>	PUz	MZ048825	MZ048776	MZ048790	–
	PUz 40 AT	<i>Aporrectodea tuberculata</i>	PUz	MZ048826	MZ048777	MZ048791	–
	PUz 41 AT	<i>Aporrectodea tuberculata</i>	PUz	MZ048827	MZ048778	MZ048792	–
<i>Anoplophrya octolasionis</i>	MU 56 OL	<i>Octolasion lacteovicinum</i>	MU	MZ048828	MZ048779	MZ048793	MZ044304
	MU 57 OL	<i>Octolasion lacteovicinum</i>	MU	MZ048829	MZ048780	MZ048794	MZ044305
	MU 58 OL	<i>Octolasion lacteovicinum</i>	MU	MZ048830	MZ048781	MZ048795	MZ044306
<i>Anoplophrya lumbrici</i>	KR 9 LT	<i>Lumricus terrestris</i>	KR	MZ048831	MZ048782	MZ048796	MZ044307
	KR 11 LT	<i>Lumricus terrestris</i>	KR	MN121061	MN897871	MZ048797	MZ044308
	RZ 6 LT	<i>Lumricus terrestris</i>	RZ	MN121062	MN897872	MZ048798	MZ044309
<i>Anoplophrya vulgaris</i>	JA-1 18 EF	<i>Eisenia andrei</i>	JA-1	MZ048832	MZ048783	MZ048799	–
	JA-1 20 EF	<i>Eisenia andrei</i>	JA-1	MN121065	MN897875	MZ048800	–
	JA-1 21 EF	<i>Eisenia andrei</i>	JA-1	MN121066	MN897876	MZ048801	–
	NG 27 DV	<i>Dendrobaena veneta</i>	NG	MZ048833	MN897877	MZ048802	MZ044310
	NG 28 DV	<i>Dendrobaena veneta</i>	NG	MN121067	MN897878	MZ048803	MZ044311
	BZ 13 EF	<i>Eisenia andrei</i>	BZ	MZ048834	MN897879	MZ048804	–
<i>Buchneriella criodrili</i>	ST 77 CL	<i>Criodrilus lacuum</i>	ST	PQ238896	PQ240662	PQ249023	PQ247159
<i>Buchneriella criodrili</i>	ST 78 CL	<i>Criodrilus lacuum</i>	ST	PQ238897	PQ240663	PQ249024	PQ247160
<i>Buchneriella criodrili</i>	ST 79 CL	<i>Criodrilus lacuum</i>	ST	PQ238898	PQ240664	PQ249025	PQ247161
<i>Buchneriella criodrili</i>	ST 80 CL	<i>Criodrilus lacuum</i>	ST	PQ238899	PQ240665	PQ249026	PQ247162
<i>Buchneriella criodrili</i>	ST 81 CL	<i>Criodrilus lacuum</i>	ST	PQ238900	PQ240666	PQ249027	PQ247163
<i>Hoplitophrya secans</i>	JJ 226 LV	<i>Lumbriculus variegatus</i>	JJ	PQ238882	PQ240648	PQ249009	PQ247145
<i>Hoplitophrya secans</i>	JJ 227 LV	<i>Lumbriculus variegatus</i>	JJ	PQ238883	PQ240649	PQ249010	PQ247146

Species	Specimen	Host species	Locality ^a	18S rRNA gene	ITS region and 28S rRNA gene	16S rRNA gene	COI gene
<i>Hoplitophrya secans</i>	JJ 228 LV	<i>Lumbriculus variegatus</i>	JJ	PQ238884	PQ240650	PQ249011	PQ247147
<i>Hoplitophrya secans</i>	JJ 229 LV	<i>Lumbriculus variegatus</i>	JJ	PQ238885	PQ240651	PQ249012	PQ247148
<i>Hoplitophrya secans</i>	JJ 230 LV	<i>Lumbriculus variegatus</i>	JJ	PQ238886	PQ240652	PQ249013	PQ247149
<i>Mesnilella clavata</i>	JJ 82 LV	<i>Lumbriculus variegatus</i>	JJ	PQ238887	PQ240653	PQ249014	PQ247150
<i>Mesnilella clavata</i>	JJ 83 LV	<i>Lumbriculus variegatus</i>	JJ	PQ238888	PQ240654	PQ249015	PQ247151
<i>Mesnilella clavata</i>	JJ 84 LV	<i>Lumbriculus variegatus</i>	JJ	PQ238889	PQ240655	PQ249016	PQ247152
<i>Mesnilella clavata</i>	JJ 85 LV	<i>Lumbriculus variegatus</i>	JJ	PQ238890	PQ240656	PQ249017	PQ247153
<i>Mesnilella clavata</i>	JJ 86 LV	<i>Lumbriculus variegatus</i>	JJ	PQ238891	PQ240657	PQ249018	PQ247154
<i>Mesnilella clavata</i>	KL 153 LV	<i>Lumbriculus variegatus</i>	KL	PQ238892	PQ240658	PQ249019	PQ247155
<i>Mesnilella clavata</i>	KL 154 LV	<i>Lumbriculus variegatus</i>	KL	PQ238893	PQ240659	PQ249020	PQ247156
<i>Mesnilella clavata</i>	KL 155 LV	<i>Lumbriculus variegatus</i>	KL	PQ238894	PQ240660	PQ249021	PQ247157
<i>Mesnilella clavata</i>	KL 157 LV	<i>Lumbriculus variegatus</i>	KL	PQ238895	PQ240661	PQ249022	PQ247158
<i>Maupasella mucronata</i>	KDo 33 ET	<i>Eiseniella tetraedra</i>	KDo	MW182008	MW181992	–	MZ044312
	KDo 34 ET	<i>Eiseniella tetraedra</i>	KDo	MW182009	MW181993	–	MZ044313
	KDo 35 ET	<i>Eiseniella tetraedra</i>	KDo	MW182010	MW181994	–	MZ044314
	KDo 36 ET	<i>Eiseniella tetraedra</i>	KDo	MW182011	MW181995	–	MZ044315
<i>Meteradiophrya chlorotica</i>	JA-2 1M ACH	<i>Allolobophora chlorotica</i>	JA-2	MZ048835	MZ048784	MZ048805	MZ044316
	JA-2 2M ACH	<i>Allolobophora chlorotica</i>	JA-2	MZ048836	MZ048785	MZ048806	MZ044317
	JA-2 3M ACH	<i>Allolobophora chlorotica</i>	JA-2	MZ048837	MZ048786	MZ048807	MZ044318
<i>Metaradiophrya lumbrici</i>	JA-2 25 LT	<i>Lumricus terrestris</i>	JA-2	MN121068	MN897880	MZ048808	MZ044319
	JA-2 26 LT	<i>Lumricus terrestris</i>	JA-2	MN121069	MN897881	MZ048809	MZ044320
	KR 8 LT	<i>Lumricus terrestris</i>	KR	MN121070	MN897882	MZ048810	MZ044321
	KR 10/1 LT	<i>Lumricus terrestris</i>	KR	MN121071	MN897883	MZ048811	MZ044322
	RZ 4 LT	<i>Lumricus terrestris</i>	RZ	MN121074	MN897884	MZ048812	MZ044323

Species	Specimen	Host species	Locality ^a	18S rRNA gene	ITS region and 28S rRNA gene	16S rRNA gene	COI gene
<i>Metaradiophrya varians</i>	RZ 5 LT	<i>Lumricus terrestris</i>	RZ	MN121075	MN897885	MZ048813	MZ044324
	BZ 12 EF	<i>Eisenia andrei</i>	BZ	MN121076	MN897886	MZ048814	MZ044325
	BZ 14 EF	<i>Eisenia andrei</i>	BZ	MN121077	MN897887	MZ048815	MZ044326
	JA-1 19 EF	<i>Eisenia andrei</i>	JA-1	MN121078	MN897888	MZ048816	MZ044327
	JA-1 22 EF	<i>Eisenia andrei</i>	JA-1	MN121079	MN897889	MZ048817	MZ044328
	BZkv 31 EF	<i>Eisenia andrei</i>	BZkv	MZ048838	MZ048787	MZ048818	MZ044329
	BZkv 32 EF	<i>Eisenia andrei</i>	BZkv	MZ048839	MZ048788	MZ048819	MZ044330
<i>Metaradiophrya speculorum</i>	HkD 59 AT	<i>Aporrectodea tuberculata</i>	HkD	MW182012	MW181996	MZ048820	MZ044331
	HkD 60 AT	<i>Aporrectodea tuberculata</i>	HkD	MW182013	MW181997	MZ048821	MZ044332
<i>Subanoplophrya nodulata</i>	PU 29 OT	<i>Octolasion tyrtaeum</i>	PU	MN121063	MN897873	MZ048822	–
	PU 30 OT	<i>Octolasion tyrtaeum</i>	PU	MN121064	MN897874	MZ048823	–

^a For locality codes and further details, see Obert et al. (2021, 2022).

Supplementary Table S3. Characterization of newly obtained nuclear and mitochondrial sequences of astome ciliates.

Species	Specimen	Host species	Locality	18S rRNA gene	ITS region and 28S rRNA gene	16S rRNA gene	COI gene
<i>Hoplitophrya secans</i>	JJ 226 LV	<i>Lumbriculus variegatus</i>	Jurské jazierko	PQ238882	PQ240648	PQ249009	PQ247145
<i>Hoplitophrya secans</i>	JJ 227 LV	<i>Lumbriculus variegatus</i>	Jurské jazierko	PQ238883	PQ240649	PQ249010	PQ247146
<i>Hoplitophrya secans</i>	JJ 228 LV	<i>Lumbriculus variegatus</i>	Jurské jazierko	PQ238884	PQ240650	PQ249011	PQ247147
<i>Hoplitophrya secans</i>	JJ 229 LV	<i>Lumbriculus variegatus</i>	Jurské jazierko	PQ238885	PQ240651	PQ249012	PQ247148
<i>Hoplitophrya secans</i>	JJ 230 LV	<i>Lumbriculus variegatus</i>	Jurské jazierko	PQ238886	PQ240652	PQ249013	PQ247149
<i>Mesnilella clavata</i>	JJ 82 LV	<i>Lumbriculus variegatus</i>	Jurské jazierko	PQ238887	PQ240653	PQ249014	PQ247150
<i>Mesnilella clavata</i>	JJ 83 LV	<i>Lumbriculus variegatus</i>	Jurské jazierko	PQ238888	PQ240654	PQ249015	PQ247151
<i>Mesnilella clavata</i>	JJ 84 LV	<i>Lumbriculus variegatus</i>	Jurské jazierko	PQ238889	PQ240655	PQ249016	PQ247152
<i>Mesnilella clavata</i>	JJ 85 LV	<i>Lumbriculus variegatus</i>	Jurské jazierko	PQ238890	PQ240656	PQ249017	PQ247153
<i>Mesnilella clavata</i>	JJ 86 LV	<i>Lumbriculus variegatus</i>	Jurské jazierko	PQ238891	PQ240657	PQ249018	PQ247154
<i>Mesnilella clavata</i>	KL 153 LV	<i>Lumbriculus variegatus</i>	Kráľovská lúka	PQ238892	PQ240658	PQ249019	PQ247155
<i>Mesnilella clavata</i>	KL 154 LV	<i>Lumbriculus variegatus</i>	Kráľovská lúka	PQ238893	PQ240659	PQ249020	PQ247156
<i>Mesnilella clavata</i>	KL 155 LV	<i>Lumbriculus variegatus</i>	Kráľovská lúka	PQ238894	PQ240660	PQ249021	PQ247157
<i>Mesnilella clavata</i>	KL 157 LV	<i>Lumbriculus variegatus</i>	Kráľovská lúka	PQ238895	PQ240661	PQ249022	PQ247158
<i>Buchneriella criodrili</i>	ST 77 CL	<i>Criodrilus lacuum</i>	Stupavský potok	PQ238896	PQ240662	PQ249023	PQ247159
<i>Buchneriella criodrili</i>	ST 78 CL	<i>Criodrilus lacuum</i>	Stupavský potok	PQ238897	PQ240663	PQ249024	PQ247160
<i>Buchneriella criodrili</i>	ST 79 CL	<i>Criodrilus lacuum</i>	Stupavský potok	PQ238898	PQ240664	PQ249025	PQ247161
<i>Buchneriella criodrili</i>	ST 80 CL	<i>Criodrilus lacuum</i>	Stupavský potok	PQ238899	PQ240665	PQ249026	PQ247162
<i>Buchneriella criodrili</i>	ST 81 CL	<i>Criodrilus lacuum</i>	Stupavský potok	PQ238900	PQ240666	PQ249027	PQ247163