Diversity and eco-evolutionary associations of endosymbiotic astome ciliates with their lumbricid earthworm hosts

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**SUPPLEMENTARY TABLE 1** | Characterization of collection sites of earthworm species examined for the presence of astome ciliates.

| Locality no. | Locality code | Collection date | Collection site | GPS coordinates | Host species                  | No. of specimens |
|--------------|---------------|-----------------|-----------------|-----------------|-------------------------------|-----------------|
| Decomposing plant material | | | | | | |
| 1 | BZ | 05/19/2018 | Decomposing plant material from a compost heap, south part of the Botanical Garden, Karlova Ves, Bratislava | 48°08'43.5"N 17°04'21.1"E | *Eisenia andrei* | 60 |
| 2 | BZkv | 09/12/2019 | Decomposing plant material from a compost heap, west part of the Botanical Garden, Karlova Ves, Bratislava | 48°08'51.0"N 17°04'26.5"E | *Eisenia andrei* | 50 |
| 3 | JA-1 | 06/28/2018 | Decomposing plant material and humous soil from a garden compost heap, Jakubská ulica street, Rača, Bratislava | 48°12'10.9"N 17°09'05.7"E | *Eisenia andrei* | 60 |
| 4 | NG | 06/30/2018 | Humous soil with high content of decomposing plant material from a garden at the foothill of the Malé Karpaty Mts., Na Grunte street, Nové mesto, Bratislava | 48°11'44.4"N 17°07'47.5"E | *Dendrobaena veneta* | 25 |
| 5 | PUh | 12/04/2019 | Decomposing material under fallen trees, poplar forest in the vicinity of the Pusté Úľany village, Galanta district | 48°13'08.2"N 17°34'43.0"E | *Lumbricus rubellus* | 20 |
| Floodplain and waterlogged soils | | | | | | |
| 6 | KR | 10/03/2017 | Floodplain soil from a riparian, willow-poplar forest near the Karlova Ves branch of the Danube river, Bratislava | 48°08'47.5"N 17°04'08.0"E | *Lumbricus terrestris* | 50 |
| 7 | AMc | 10/14/2019 | Floodplain soil from a riparian, willow-poplar forest near the Danube river, Devín, Bratislava | 48°10'46.0"N 16°58'39.6"E | *Aporrectodea tuberculata* | 25 |
| 8 | HkD | 10/14/2019 | Moist soil near the confluence of the Danube and Morava rivers, foot of the cliff of the Devín Castle, Bratislava | 48°10'28.0"N 16°58'37.0"E | *Aporrectodea rosea* | 5 |
| 9 | LS | 10/14/2019 | Floodplain soil from a meadow near the Danube river, Devín, Bratislava | 48°10'15.3"N 16°59'04.3"E | *Aporrectodea tuberculata* | 15 |
| 10 | KDo | 09/15/2019 | Waterlogged soil around the Banský potok stream in an urban oak-hornbeam forest, Knižková dolina valley, Bratislava, Malé Karpaty Mts. | 48°13'37.0"N 17°07'13.1"E | *Oulactosion lacteum* | 10 |
| 11 | KDo | 09/29/2019 | Moist soil in the vicinity of the Banský potok stream in an urban oak-hornbeam forest, Knižková dolina valley, Bratislava, Malé Karpaty Mts. | 48°12'60.0"N 17°08'21.7"E | *Dendrobaena octaedra* | 15 |
| 12 | CBk | 10/06/2019 | Waterlogged soil around the Šenkársky potok stream in an urban oak-hornbeam forest, Bratislava, Malé Karpaty Mts. | 48°13'50.2"N 17°08'21.9"E | *Lumbricus terrestris* | 20 |
| 13 | MB | 10/27/2019 | Moist soil from an urban oak-hornbeam forest in the vicinity of Malá Baňa, Bratislava, Malé Karpaty Mts. | 48°13'14.7"N 17°07'45.6"E | *Fitzingeria platyura* | 1 |
| Locality no. | Locality code | Collection date | Collection site                                                                 | GPS coordinates                  | Host species                                      | No. of specimens |
|-------------|---------------|-----------------|---------------------------------------------------------------------------------|----------------------------------|---------------------------------------------------|-----------------|
| 14          | PU            | 07/02/2018      | Upper 50 cm turf layer in the riparian zone of the Rašelinisko pond in the vicinity of the Pusté Úľany village, Galanta district | 48°13'21.9"N 17°34'49.9"E       | Octolasion tyrtaeum                               | 5               |
| 15          | RZ            | 06/06/2017      | Agricultural soil from a garden, Šúrska ulica street, Rendez, Bratislava         | 48°11'57.6"N 17°10'25.0"E        | Lumbricus terrestris                              | 50              |
| 16          | JA-2          | 11/28/2018      | Agricultural soil from a garden, Jakubská ulica street, Rača, Bratislava        | 48°12'12.2"N 17°09'03.1"E        | Allolobophora chlorotica                         | 10              |
| 17          | FNS           | 12/09/2019      | Soil from a grassland in the vicinity of the Faculty of Natural Sciences, Comenius University, Karlova Ves, Bratislava | 48°08'56.7"N 17°04'21.2"E        | Lumbricus terrestris                              | 25              |
| 18          | PUz           | 06/20/2018      | Agricultural soil from a garden, Spodná ulica street, Pusté Úľany village, Galanta district | 48°13'41.0"N 17°34'48.6"E        | Aporrectodea tuberculata                         | 20              |
| 19          | PUz           | 09/14/2019      | Agricultural soil from a garden, Spodná ulica street, Pusté Úľany village, Galanta district | 48°13'41.0"N 17°34'48.6"E        | Allolobophora chlorotica                         | 10              |
| 20          | PUz           | 11/28/2019      | Agricultural soil from a field in the vicinity of the Pusté Úľany village, Galanta district | 48°13'19.2"N 17°34'57.1"E       | Aporrectodea tuberculata                         | 20              |
| 21          | JA-2          | 06/28/2018      | Loamy soil with fallen needles in the surroundings of a garden wall, Jakubská ulica street, Rača, Bratislava | 48°12'12.2"N 17°09'03.1"E        | Lumbricus terrestris                              | 50              |
| 22          | JA-3          | 09/18/2019      | Soil from a garden, Jakubská ulica street, Rača, Bratislava                     | 48°12'11.4"N 17°09'05.3"E        | Allolobophora chlorotica                         | 10              |
| 23          | HO            | 11/08/2019      | Soil from a garden, Horská ulica street, Nové mesto, Bratislava                | 48°11'52.1"N 17°08'03.3"E        | Aporrectodea trapezoides                         | 10              |
| 24          | MU            | 10/13/2019      | Soil from a garden, Moskovská ulica street, Staré mesto, Bratislava             | 48°09'05.0"N 17°07'18.2"E        | Octolasion lacteovicinum                         | 20              |
| 25          | BZ            | 05/19/2018      | Soil from the east part of the Botanical Garden, Karlova Ves, Bratislava         | 48°08'41.9"N 17°04'24.6"E        | Lumbricus terrestris                              | 10              |

* Dates are given as mo/d/yr.

b Identified as Eisenia fetida by Obert and Vďačný (2019) on the basis of morphological data. However, the identification within the E. fetida complex is specified to E. andrei given the mitochondrial COI and ND1 sequences.
**SUPPLEMENTARY TABLE 2** | Primers used for amplification of five molecular markers analyzed in astome ciliates and their earthworm hosts.

| Molecular marker                     | Organism group | Primer name | Primer sequence (in 5' to 3' direction) | Reference                  |
|--------------------------------------|----------------|-------------|-----------------------------------------|----------------------------|
| 18S rRNA gene                        | Astome ciliates| Euk A       | AAC CTG GTT GAT CCT GCC AGT             | Medlin et al. (1988)       |
|                                      |                | Euk B       | TGA TCC TTC TGC AGG TTC AC             | Medlin et al. (1988)       |
| 16S rRNA gene                        | Astome ciliates| 16S-mtSSU-F | TGT GCC AGC AGC CGC GG T AA           | van Hoek et al. (2000)    |
|                                      |                | 16S-mtSSU-R | CCC MTA CCR GTA CCT TGT GT             | van Hoek et al. (2000)    |
| ITS region and 28S rRNA gene         | Astome ciliates| ITS-F       | GTA GGT GAA CCT GCG GAA GGA TCA TTA   | Miao et al. (2008)         |
|                                      |                | LO-R        | GCT ATC CTG AGR GAA ACT TCG            | Pawlowski (2000)           |
| Cytochrome c oxidase subunit I       | Astome ciliates| F388dT      | TGT AAA ACG ACG GCC AGT GGW KCB AAA GAT GTW GC | Strüder-Kypke and Lynn (2010) |
|                                      |                | R1184dT     | CAG GAA ACA GCT ATG ACT ADA CYT C      | Strüder-Kypke and Lynn (2010) |
|                                      |                | F388dT-mod20| GGT TCC AAA GAT GTT GCD TA             | Present study\(^b\)       |
|                                      |                | R1184dT-mod21| AGG GTG ACC GAA AAA TCA RAA           | Present study\(^b\)       |
| Earthworms                           |                | LCO 1490    | GGT CAA CAA ATC ATA AAG ATG TTG G      | Foermer et al. (1994)     |
|                                      |                | HCO 2198    | TAA ACT TCA GGG TGA CCA AAA AAT CA    | Foermer et al. (1994)     |
| NADH-ubiquinone oxidoreductase chain 1| Earthworms    | tRNA-Leu-ND1-LumbF2 | GAA TAG TGC CAC AGG TTT AAA C | Pérez-Losada et al. (2009) |
|                                      |                | tRNA-Leu-ND1-LumbR1b | TTA ACG TCA TCA GAG TTA TC | Pérez-Losada et al. (2009) |

\(^a\) The barcoding D1/D2 domains of the 28S rRNA gene were amplified.

\(^b\) Based on conserved regions of astome COI sequences amplified with the F388dT and R1184dT primers.
SUPPLEMENTARY TABLE 3 | Conditions of PCR reactions used for amplification of five molecular markers analyzed in astome ciliates and their earthworm hosts.

| Molecular marker | Organism group | PCR program | Cycling (denaturation, annealing, extension) | Final extension | Reference |
|------------------|----------------|-------------|---------------------------------------------|-----------------|-----------|
| 18S rRNA gene    | Astome ciliates| 95 °C/15 min| 30 cycles: 95 °C/45 s, 55 °C/60 s, 72 °C/150 s | 72 °C/10 min    | Vďačný et al. (2011) |
| 16S rRNA gene    | Astome ciliates| 94 °C/3 min | 5 cycles: 94 °C/30 s, 50 °C/60 s, 68 °C/75 s | 68 °C/10 min    | Rataj and Vďačný (2020) |
|                  |                |            | 35 cycles: 94 °C/30 s, 60 °C/60 s, 68 °C/75 s |                 |           |
| ITS region and 28S rRNA gene<sup>a</sup> | Astome ciliates| 95 °C/15 min| 35 cycles: 95 °C/45 s, 55 °C/60 s, 72 °C/150 s | 72 °C/10 min    | Vďačný et al. (2011)<sup>b</sup> |
| Cytochrome c oxidase subunit I (COI) | Astome ciliates| 94 °C/4 min | 5 cycles: 94 °C/45 s, 45 °C/75 s, 72 °C/90 s | 72 °C/8 min     | Rataj and Vďačný (2020) |
|                  |                |            | 35 cycles: 94 °C/45 s, 55 °C/75 s, 72 °C/90 s |                 |           |
| NADH-ubiquinone oxidoreductase chain 1 (ND1) | Earthworms  | 95 °C/5 min | 40 cycles: 95 °C/30 s, 50 °C/90s, 72 °C/180 s | 72 °C/10 min    | Kolicka (2019) |
|                  | Earthworms     | 94 °C/4 min | 5 cycles: 94 °C/45 s, 45 °C/75 s, 72 °C/90 s | 72 °C/8 min     | Rataj and Vďačný (2020) |
|                  |                |            | 35 cycles: 94 °C/45 s, 55 °C/75 s, 72 °C/90 s |                 |           |

<sup>a</sup> The barcoding D1/D2 domains of the 28S rRNA gene were amplified.

<sup>b</sup> Modified from Vďačný et al. (2011) by adding five cycles.
**SUPPLEMENTARY TABLE 4** | List of astome ciliates with GenBank accession numbers of corresponding 18S rRNA gene sequences included in Phylogenetic Interaction-adjusted Similarity Analyses.

| No. | Taxon                             | GenBank entry |
|-----|-----------------------------------|---------------|
| 1   | Almophrya bivacuolata             | HQ446281      |
| 2   | Anoplophrya allolobophorae        | MZ048824      |
| 3   | Anoplophrya aporrectodeae         | MZ048825      |
| 4   | Anoplophrya lumbrici              | MN121062      |
| 5   | Anoplophrya marylandensis         | AY547546      |
| 6   | Anoplophrya octolasionis          | MZ048828      |
| 7   | Anoplophrya vulgaris              | MN121065      |
| 8   | Eudrilophrya complanata           | HQ446280      |
| 9   | Maupasella mucronata              | MW182008      |
| 10  | Metaracoelophys intermedia        | HQ446278      |
| 11  | Metaracoelophys sp.               | HQ446282      |
| 12  | Metaracoelophys sp.               | HQ446277      |
| 13  | Metaradiophys sp.                 | HQ446279      |
| 14  | Metaradiophys chlorotica          | MZ048835      |
| 15  | Metaradiophys lumbrici            | MN121068      |
| 16  | Metaradiophys speculorum          | MW182012      |
| 17  | Metaradiophys varians             | MN121076      |
| 18  | Njinella prolifera                | HQ446276      |
| 19  | Paraclausilocola constricta       | HQ446275      |
| 20  | Paraclausilocola elongata         | HQ446274      |
| 21  | Subanoplophrya nodulata           | MN121063      |
| No. | Species            | Mucronata | Vulgaris | Octolasioni | Lumbrici | Allolobophorae | KDo | JA | LT | ET | OL |
|-----|--------------------|-----------|----------|-------------|----------|----------------|-----|----|----|----|----|
| 1   | Anoplophrya spp.    | 0.03171   | 0.03222  | 0.03222     | 0.03222  | 0.03222        | 0.03222 | 0.03222 | 0.03222 | 0.03222 | 0.03222 |
| 2   | Metaradiophrya      | 0.03171   | 0.03222  | 0.03222     | 0.03222  | 0.03222        | 0.03222 | 0.03222 | 0.03222 | 0.03222 | 0.03222 |
| 3   | Anoplophrya spp.    | 0.03171   | 0.03222  | 0.03222     | 0.03222  | 0.03222        | 0.03222 | 0.03222 | 0.03222 | 0.03222 | 0.03222 |
| 4   | Metaradiophrya      | 0.03171   | 0.03222  | 0.03222     | 0.03222  | 0.03222        | 0.03222 | 0.03222 | 0.03222 | 0.03222 | 0.03222 |
| 5   | Anoplophrya spp.    | 0.03171   | 0.03222  | 0.03222     | 0.03222  | 0.03222        | 0.03222 | 0.03222 | 0.03222 | 0.03222 | 0.03222 |
| 6   | Metaradiophrya      | 0.03171   | 0.03222  | 0.03222     | 0.03222  | 0.03222        | 0.03222 | 0.03222 | 0.03222 | 0.03222 | 0.03222 |
| 7   | Anoplophrya spp.    | 0.03171   | 0.03222  | 0.03222     | 0.03222  | 0.03222        | 0.03222 | 0.03222 | 0.03222 | 0.03222 | 0.03222 |
| 8   | Metaradiophrya      | 0.03171   | 0.03222  | 0.03222     | 0.03222  | 0.03222        | 0.03222 | 0.03222 | 0.03222 | 0.03222 | 0.03222 |
| 9   | Anoplophrya spp.    | 0.03171   | 0.03222  | 0.03222     | 0.03222  | 0.03222        | 0.03222 | 0.03222 | 0.03222 | 0.03222 | 0.03222 |
| 10  | Metaradiophrya      | 0.03171   | 0.03222  | 0.03222     | 0.03222  | 0.03222        | 0.03222 | 0.03222 | 0.03222 | 0.03222 | 0.03222 |

Note: Specimen code consists of a locality code: an inside code, and an abbreviation of host name. Locality codes are summarized in Supplementary Table 1.
| No. | Specimen | Pairwise distances of ACH |
|-----|----------|--------------------------|
| 1   |          |                          |
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| 35  |          |                          |
| 36  |          |                          |
| 37  |          |                          |
| 38  |          |                          |

Note: Specimen code consists of a locality code, an inside code, and an abbreviation of host name. Locality codes are summarized in Supplementary Table 1.
| No. | Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 |
|-----|---------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1   | Anoplophora belgicae JG 27 BOJ   | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 2   | Anoplophora belgicae JG 30 BOJ   | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 3   | Anoplophora aequatorialis PL 36 BT | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 4   | Anoplophora aequatorialis PL 38 BT | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 5   | Anoplophora belgicae HK 28 LT    | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 6   | Anoplophora belgicae HK 20 LT    | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 7   | Anoplophora belgicae HK 18 LT    | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 8   | Anoplophora belgicae HK 17 LT    | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 9   | Anoplophora belgicae HK 16 LT    | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 10  | Anoplophora belgicae HK 15 LT    | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 11  | Anoplophora belgicae HK 14 LT    | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 12  | Anoplophora belgicae HK 13 LT    | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 13  | Anoplophora belgicae HK 12 LT    | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 14  | Anoplophora belgicae HK 11 LT    | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 15  | Anoplophora belgicae HK 10 LT    | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 16  | Anoplophora belgicae HK 9 LT     | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 17  | Anoplophora belgicae HK 8 LT     | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 18  | Anoplophora belgicae HK 7 LT     | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 19  | Anoplophora belgicae HK 6 LT     | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 20  | Anoplophora belgicae HK 5 LT     | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 21  | Anoplophora belgicae HK 4 LT     | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 22  | Anoplophora belgicae HK 3 LT     | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 23  | Anoplophora belgicae HK 2 LT     | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 24  | Anoplophora belgicae HK 1 LT     | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 25  | Anoplophora belgicae HK 0.5 LT   | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 26  | Anoplophora belgicae HK 0.1 LT   | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 27  | Anoplophora belgicae HK 0.01 LT  | 0.00000 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

Note: Species code consists of a locality code: an isolate code, and an abbreviation of host name. Locality codes are summarized in Supplementary Table 1.

SUPPLEMENTARY TABLE 1 (Supplementary Table 1)
| No. | Species | Pairwise 1 | Pairwise 2 | Pairwise 3 | Pairwise 4 | Pairwise 5 | Pairwise 6 | Pairwise 7 | Pairwise 8 | Pairwise 9 | Pairwise 10 | Pairwise 11 | Pairwise 12 | Pairwise 13 | Pairwise 14 | Pairwise 15 | Pairwise 16 | Pairwise 17 | Pairwise 18 |
|-----|---------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 1   | Anoplophrya heterospila 17' 20' ADH | 1.01836 | 0.34559 | 0.25584 | 0.34839 | 0.24186 | 0.25030 | 0.20157 | 0.27600 | 0.28023 | 0.26091 | 0.26776 | 0.24394 | 0.25007 | 0.25235 | 0.20285 | 0.20285 | 0.16975 |
| 2   | Anoplophrya heterospila 17' 20' ADH | 0.34559 | 0.33784 | 0.24590 | 0.24590 | 0.24592 | 0.24592 | 0.23910 | 0.20329 | 0.20447 | 0.19601 | 0.19396 | 0.19026 | 0.19416 | 0.17803 | 0.17803 | 0.16416 | 0.16416 |
| 3   | Anoplophrya heterospila 17' 20' ADH | 0.25584 | 0.24590 | 0.24592 | 0.24592 | 0.23910 | 0.20329 | 0.20447 | 0.19601 | 0.19396 | 0.19026 | 0.19416 | 0.17803 | 0.17803 | 0.16416 | 0.16416 |
| 4   | Anoplophrya heterospila 17' 20' ADH | 0.34839 | 0.24592 | 0.24592 | 0.23910 | 0.20329 | 0.20447 | 0.19601 | 0.19396 | 0.19026 | 0.19416 | 0.17803 | 0.17803 | 0.16416 | 0.16416 |
| 5   | Anoplophrya heterospila 17' 20' ADH | 0.24186 | 0.24592 | 0.23910 | 0.20329 | 0.20447 | 0.19601 | 0.19396 | 0.19026 | 0.19416 | 0.17803 | 0.17803 | 0.16416 | 0.16416 |
| 6   | Anoplophrya heterospila 17' 20' ADH | 0.25030 | 0.23910 | 0.20329 | 0.20447 | 0.19601 | 0.19396 | 0.19026 | 0.19416 | 0.17803 | 0.17803 | 0.16416 | 0.16416 |
| 7   | Anoplophrya heterospila 17' 20' ADH | 0.20157 | 0.20329 | 0.20447 | 0.19601 | 0.19396 | 0.19026 | 0.19416 | 0.17803 | 0.17803 | 0.16416 | 0.16416 |
| 8   | Anoplophrya heterospila 17' 20' ADH | 0.27600 | 0.20157 | 0.20447 | 0.19601 | 0.19396 | 0.19026 | 0.19416 | 0.17803 | 0.17803 | 0.16416 | 0.16416 |
| 9   | Anoplophrya heterospila 17' 20' ADH | 0.28023 | 0.20157 | 0.20447 | 0.19601 | 0.19396 | 0.19026 | 0.19416 | 0.17803 | 0.17803 | 0.16416 | 0.16416 |
| 10  | Anoplophrya heterospila 17' 20' ADH | 0.26091 | 0.20157 | 0.20447 | 0.19601 | 0.19396 | 0.19026 | 0.19416 | 0.17803 | 0.17803 | 0.16416 | 0.16416 |

Note: Pairwise code numbers are of a locality code, at site code, and an abbreviation of host name. Locality codes are summarized in Supplementary Table 1.
| No. | Specimen | Name                      | locality codes | Isolate codes | Abbreviation of host name |
|-----|----------|---------------------------|----------------|---------------|--------------------------|
| 1   |          | Anoplophrya albidolophorae | NA             | NA            |                          |
| 2   |          | Anoplophrya vulgans        | NA             | NA            |                          |
| 3   |          | Anoplophrya vulgaris       | NA             | NA            |                          |
| 4   |          | Anoplophrya lumbrici       | NA             | NA            |                          |
| 5   |          | Anoplophrya lumbrici       | NA             | NA            |                          |
| 6   |          | Metaradiophrya varians     | NA             | NA            |                          |
| 7   |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 8   |          | Metaradiophrya chlorotica  | NA             | NA            |                          |
| 9   |          | Anoplophrya octosax at MU | NA             | NA            |                          |
| 10  |          | Anoplophrya octosax at MU | NA             | NA            |                          |
| 11  |          | Maupasella murocana        | NA             | NA            |                          |
| 12  |          | Maupasella murocana        | NA             | NA            |                          |
| 13  |          | Maupasella murocana        | NA             | NA            |                          |
| 14  |          | Metaradiophrya chlorotica  | NA             | NA            |                          |
| 15  |          | Metaradiophrya chlorotica  | NA             | NA            |                          |
| 16  |          | Metaradiophrya chlorotica  | NA             | NA            |                          |
| 17  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 18  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 19  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 20  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 21  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 22  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 23  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 24  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 25  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 26  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 27  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 28  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
| 29  |          | Metaradiophrya lumbrici    | NA             | NA            |                          |
## Occurrence of eleven Astome ciliates in sixteen earthworm species.

### Localities

**Locality no.** | **Locality code** | **Host species** | **Endosymbiont species** | **Supplementary Table 1**
--- | --- | --- | --- | ---
1 | BZ | Eisenia andrei | Subanoplophrya nodulata, Maupasella mucronata | 10
2 | BZ | Eisenia andrei | Metaradiophrya chlorotica, Lumbrici | 10
3 | JA-1 | Eisenia andrei | Metaradiophrya varians, Anglophrya allolobophorae, Aporrectodea rosea | 10
4 | NG | Dendrobaena veneta | Metaradiophrya lumbrici, Metaradiophrya speculorum, Octolasion lacteum | 10
5 | PUh | Lumbricus rubellus | | 10
6 | KR | Lumbricus terrestris | | 10
7 | AMc | Aporrectodea tuberculata | | 10
8 | HKD | Aporrectodea rosea | | 10
9 | LS | Aporrectodea tuberculata | | 10
10 | KDo | Dendrobaena octaedra, Eiseniella tetraedra, Octolasion sp. | | 10
11 | KDo | Lumbricus terrestris | | 10
12 | CBk | Bimastos rubidus, Eiseniella tetraedra, Octolasion sp. | | 10
13 | MB | Fitzingeria platyura | | 10
14 | PU | Octolasion tytaeum | | 10
15 | RZ | Lumbricus terrestris | | 10
16 | JA-2 | Allolobophora chlorotica | | 10
17 | FNS | Lumbricus terrestris | | 10
18 | PUz | Aporrectodea tuberculata | | 10
19 | PUz | Aporrectodea trapezoides, Aporrectodea tuberculata, Lumbricus terrestris | | 10
20 | PUz | Aporrectodea tuberculata | | 10
21 | JA-2 | Lumbricus terrestris | | 10
22 | JA-3 | Allolobophora chlorotica, Aporrectodea trapezoides | | 10
23 | HO | Lumbricus terrestris | | 10
24 | MU | Octolasion lacteovicinum | | 10
25 | BZ | Lumbricus terrestris | | 10

Presence is designated by plus (+). Colors denote the individual ecological group of earthworms (yellow = epigeic, green = anecic, and blue = endogeic).

For locality codes, see Supplementary Table 1.
**SUPPLEMENTARY TABLE 1** | Characterization and origin of the mitochondrial ND1 gene sequences of earthworms analyzed in the present study.

| Species                          | Specimen no. | Locality no. | Locality                                      | GenBank no.       |
|----------------------------------|--------------|--------------|-----------------------------------------------|-------------------|
| Allolobophora chlorotica         | JA-2 6       | 16           | Garden, Jakubská ulica, Rača, Bratislava      | MZ056758          |
| Allolobophora chlorotica         | JA-3 8       | 22           | Garden, Jakubská ulica, Rača, Bratislava      | MZ056759          |
| Allolobophora chlorotica         | PUz 16       | 19           | Garden, Spodná ulica, Pusté Úľany village    | MZ056760          |
| Aporrectodea rosea               | Hkd 22       | 8            | Foot of the cliff of the Devín Castle, Bratislava | MZ056761          |
| Aporrectodea trapezoides         | JA-3 7       | 22           | Garden, Jakubská ulica, Rača, Bratislava      | MZ056762          |
| Aporrectodea trapezoides         | PUz 9        | 19           | Garden, Spodná ulica, Pusté Úľany village    | MZ056763          |
| Aporrectodea tuberculata         | Amc 19       | 7            | Willow-poplar forest near the Danube river, Devín, Bratislava | MZ056764          |
| Aporrectodea tuberculata         | Amc 20       |              |                                               | MZ056765          |
| Aporrectodea tuberculata         | Hkd 21       | 8            | Foot of the cliff of the Devín Castle, Bratislava | MZ056766          |
| Aporrectodea tuberculata         | Pu 29        | 20           | Field in the vicinity of the Pusté Úľany village | MZ056771          |
| Dendrobaena octaedra             | Kdo 5        | 10           | Oak-hornbeam forest, Knížková dolina valley, Bratislava, Malé Karpaty Mts. | MZ056773          |
| Eisenia andrei                   | Bz 1         | 1            | Botanical Garden, Karlova Ves, Bratislava      | MZ056774          |
| Eisenia andrei                   | Ja-1         | 3            | Jakubská ulica, Rača, Bratislava              | MZ056775          |
| Eiseniella tetraedra             | CBk 14       | 12           | Oak-hornbeam forest, Bratislava, Malé Karpaty Mts. | MZ056776          |
| Eiseniella tetraedra             | Kdo 3        | 10           | Oak-hornbeam forest, Knížková dolina valley, Bratislava | MZ056777          |
| Eiseniella tetraedra             | Kdo 4        |              | Bratislava, Malé Karpaty Mts.                 | MZ056778          |
| Fitzingeria platyura             | MB 27        | 13           | Oak-hornbeam forest, Malá Baňa, Bratislava, Malé Karpaty Mts. | MZ056779          |
| Lumbricus rubellus               | Puh 30       | 5            | Poplar forest in the vicinity of the Pusté Úľany village | MZ056780          |
| Lumbricus rubellus               | Puh 31       |              |                                               | MZ056781          |
| Lumbricus terrestris             | Bz           | 25           | Botanical Garden, Karlova Ves, Bratislava      | MZ056782          |
| Lumbricus terrestris             | Fns 32       | 17           | Grassland, Comenius University, Bratislava     | MZ056783          |
| Lumbricus terrestris             | Ho 28        | 23           | Garden, Horská ulica, Nové mesto, Bratislava   | MZ056784          |
| Lumbricus terrestris             | Ja-2         | 21           | Garden wall, Jakubská ulica, Rača, Bratislava  | MZ056785          |
| Lumbricus terrestris             | Kdo 15       |              | Oak-hornbeam forest, Knížková dolina valley, Bratislava, Malé Karpaty Mts. | MZ056786          |
| Lumbricus terrestris             | Kr           | 6            | Willow-poplar forest, Karlova Ves, Bratislava  | MZ056787          |
| Lumbricus terrestris             | Rz           | 15           | Garden, Šúrska ulica, Rendez, Bratislava      | MZ056788          |
| Lumbricus terrestris             | Pu 10        | 19           | Garden, Spodná ulica, Pusté Úľany village    | MZ056789          |
| Octolasion lacteovicinum         | Mu 23        | 24           | Garden, Moskovská ulica, Staré mesto, Bratislava | MZ056790          |
| Octolasion lacteovicinum         | Mu 24        |              |                                               | MZ056791          |
| Octolasion lacteovicinum         | Mu 25        |              |                                               | MZ056792          |
| Octolasion lacteum               | Ls 18        | 9            | Meadow near the Danube river, Devín, Bratislava | MZ056793          |
| Octolasion sp.                   | CBk 12       | 12           | Oak-hornbeam forest, Bratislava, Malé Karpaty Mts. | MZ056794          |

For locality codes and further details, see Supplementary Table 1.
**SUPPLEMENTARY TABLE 1** | Characterization and origin of the mitochondrial COI gene sequences of earthworms analyzed in the present study.

| Species                        | Specimen no. | Locality no. | Locality                                      | GenBank no.   |
|--------------------------------|--------------|--------------|-----------------------------------------------|---------------|
| *Allolobophora chlorotica*     | JA-2 6       | 16           | Garden, Jakubská ulica, Rača, Bratislava      | MZ044869      |
| *Allolobophora chlorotica*     | JA-3 8       | 22           | Garden, Jakubská ulica, Rača, Bratislava      | MZ044870      |
| *Allolobophora chlorotica*     | PUz 16       | 19           | Garden, Spodná ulica, Pusté Úľany village     | MZ044871      |
| *Aporrectodea rosea*           | HkD 22       | 8            | Foot of the cliff of the Devin Castle, Bratislava | MZ044872      |
| *Aporrectodea trapezoides*     | JA-3 7       | 22           | Garden, Jakubská ulica, Rača, Bratislava      | MZ044873      |
| *Aporrectodea trapezoides*     | PUz 9        | 19           | Garden, Spodná ulica, Pusté Úľany village     | MZ044874      |
| *Aporrectodea tuberculata*     | LS 17        | 9            | Meadow near the Danube river, Devin, Bratislava | MZ044875      |
| *Dendrodrilus rubidus*         | CBk 13       | 12           | Oak-hornbeam forest, Bratislava, Malé Karpaty Mts. | MZ044876      |
| *Eisenia andrei*               | BZ 1         | 1            | Botanical Garden, Karlova Ves, Bratislava      | MZ044877      |
| *Eisenia andrei*               | JA-1         | 3            | Jakubská ulica, Rača, Bratislava              | MZ044878      |
| *Eiseniella tetraedra*         | CBk 14       | 12           | Oak-hornbeam forest, Bratislava, Malé Karpaty Mts. | MZ044879      |
| *Eiseniella tetraedra*         | KDo 3        | 10           | Oak-hornbeam forest, Knižková dolina valley,   | MZ044880      |
| *Eiseniella tetraedra*         | KDo 4        |              | Bratislava, Malé Karpaty Mts.                 | MZ044881      |
| *Fitzingeria platyura*         | MB 27        | 13           | Oak-hornbeam forest, Malá Baňa, Bratislava, Malé Karpaty Mts. | MZ044882      |
| *Lumbricus rubellus*           | PUh 30       | 5            | Poplar forest in the vicinity of the Pusté Úľany village | MZ044883      |
| *Lumbricus rubellus*           | PU 31        |              |                                               | MZ044884      |
| *Lumbricus terrestris*         | BZ           | 25           | Botanical Garden, Karlova Ves, Bratislava      | MZ044885      |
| *Lumbricus terrestris*         | FNS 32       | 17           | Grassland, Comenius University, Bratislava     | MZ044886      |
| *Lumbricus terrestris*         | HO 28        | 23           | Garden, Horská ulica, Nové mesto, Bratislava   | MZ044887      |
| *Lumbricus terrestris*         | JA-2         | 21           | Garden wall, Jakubská ulica, Rača, Bratislava  | MZ044888      |
| *Lumbricus terrestris*         | KDo 15       | 10           | Oak-hornbeam forest, Knižková dolina valley,   | MZ044889      |
| *Lumbricus terrestris*         | KR           | 6            | Willow-poplar forest, Karlova Ves, Bratislava  | MZ044890      |
| *Lumbricus terrestris*         | RZ           | 15           | Garden, Šúrska ulica, Rendez, Bratislava       | MZ044891      |
| *Lumbricus terrestris*         | PUz 10       | 19           | Garden, Spodná ulica, Pusté Úľany village     | MZ044892      |
| *Octolasion lacteovicinum*     | MU 23        | 24           | Garden, Moskovská ulica, Staré mesto, Bratislava | MZ044893      |
| *Octolasion lacteovicinum*     | MU 24        |              |                                               | MZ044894      |
| *Octolasion lacteovicinum*     | MU 25        |              |                                               | MZ044895      |

For locality codes and further details, see Supplementary Table 1.
SUPPLEMENTARY FIGURE 1 | Phylogenetic network computed from NADH-ubiquinone oxidoreductase chain 1 (ND1) sequences of all studied lumbricid earthworms, using the neighbornet algorithm and the uncorrected distances in SplitsTree ver. 4. Sequences in bold face were downloaded from GenBank and served for the classification of the earthworm species studied. For GenBank accession numbers of newly obtained sequences, see Supplementary Table 11. The scale bar indicates three substitutions per one hundred nucleotide positions.
SUPPLEMENTARY FIGURE 2 | Phylogenetic network computed from cytochrome oxidase c subunit I (COI) sequences of lumbricid earthworms carrying astome ciliates with the neighborNet algorithm and the uncorrected distances in SplitsTree ver. 4. Sequences in bold face were downloaded from GenBank and served for the classification of the earthworm species carrying astome ciliates. For GenBank accession numbers of newly obtained sequences, see Supplementary Table 12. The scale bar indicates three substitutions per one hundred nucleotide positions.
SUPPLEMENTARY FIGURE 3 | Putative secondary structure of the ITS2 molecule of *Almophrya bivacuolata* (HQ446281, host earthworms: *Alma emini / Alma nilotica*).
Putative secondary structure of the ITS2 molecule of *Anoplophrya aporrectodeae* (samples PUz 17 AT, PUz 40 AT, and PUz 41 AT, host earthworm: *Aporrectodea tuberculata*).
SUPPLEMENTARY FIGURE 5 | Putative secondary structure of the ITS2 molecule of *Anoplophrya allolobophorae* (sample JA-3 37 ACH, host earthworm: *Allolobophora chlorotica*).
SUPPLEMENTARY FIGURE 6 | Putative secondary structure of the ITS2 molecule of *Anoplophrya lumbrici* (samples RZ 6 LT, KR 9 LT, and KR 11 LT, host earthworm: *Lumbricus terrestris*).
SUPPLEMENTARY FIGURE 7 | Putative secondary structure of the ITS2 molecule of *Anoplophrya octolasionis* (samples MU 56 OL, MU 57 OL, and MU 58 OL, host earthworm: *Octolasion lacteovicinum*).
SUPPLEMENTARY FIGURE 8 | Putative secondary structure of the ITS2 molecule of *Anoplophrya vulgaris* (samples BZ 13 EF, JA-1 18 EF, JA-1 20 EF, JA-1 21 EF, NG 27 DV, and NG 28 DV, host earthworms: *Eisenia fetida* complex and *Dendrobaena veneta*).
SUPPLEMENTARY FIGURE 9 | Putative secondary structure of the ITS2 molecule of Eudrilophrya complanata (HQ446280, host earthworm: Eupolytoreutus sp.).
SUPPLEMENTARY FIGURE 10 | Putative secondary structure of the ITS2 molecule of *Maupasella mucronata* (samples KDo 33 ET, KDo 34 ET, KDo 35 ET, and KDo 36 ET, host earthworm: *Eiseniella tetraedra*).
SUPPLEMENTARY FIGURE 11 | Putative secondary structure of the ITS2 molecule of *Metaracoeolophrya* sp. 1 (HQ446277, host earthworms: *Alma ermini* / *Alma nilotica*).
SUPPLEMENTARY FIGURE 12 | Putative secondary structure of the ITS2 molecule of *Njinella prolifera* (HQ446276, host earthworm: *Eupolystoreutes* sp.).
SUPPLEMENTARY FIGURE 13 | Putative secondary structure of the ITS2 molecule of *Paraclausilocola constricta* (HQ446275, host earthworm: *Eupolytoreutus* sp.).
SUPPLEMENTARY FIGURE 14 | Putative secondary structure of the ITS2 molecule of *Subanoplophrya nodulata* (samples PU 29 OT and PU 30 OT, host earthworm: *Octolasion tyrtaeum*).
SUPPLEMENTARY FIGURE 15 | Putative secondary structure of the ITS2 molecule of *Metaradiophrya chlorotica* (samples JA-2 1M ACH, JA-2 2M ACH, and JA-2 3M ACH, host earthworm: *Allolobophora chlorotica*).
SUPPLEMENTARY FIGURE 16 | Putative secondary structure of the ITS2 molecule of *Metaradiophrya lumbrici* (samples RZ 4 LT, RZ 5 LT, KR 8 LT, KR 10 LT, JA-2 25 LT, and JA-2 26 LT, host earthworm: *Lumbricus terrestris*).
SUPPLEMENTARY FIGURE 17 | Putative secondary structure of the ITS2 molecule of *Metaradiophrya speculorum* (samples HkD 59 AT and HkD 60 AT, host earthworm: *Aporrectodea tuberculata*).
SUPPLEMENTARY FIGURE 18 | Putative secondary structure of the ITS2 molecule of *Metaradiophrya varians* (samples BZ 12 EF, BZ 14 EF, JA-1 19 EF, JA-1 22 ED, BZkv 31 EA, and BZkv 32 EA, host earthworms: *Eisenia fetida* complex).
SUPPLEMENTARY FIGURE 19 | Phylogenetic trees based on 18S rRNA, ITS region, and D1/D2-28S rRNA gene sequences of astome ciliates isolated from lumbricid earthworms. The positions of *Metaradiophrya* species conflict between the Phycas/neighbor-joining trees (A) and MrBayes/IQTree (B). The discrepancy is very likely caused by the plesiomorphic trap, as thoroughly discussed by Obert and Vďačný (2019, 2020). Bootstrap values for neighbor-joining and maximum likelihood IQTree analyses as well as posterior probabilities for Bayesian interference performed in MrBayes and Phyicas were mapped onto the respective trees. Fully statistically supported nodes are marked with red solid circles. Dashes indicate statistical support below 50%. For specimen codes and further details, see Supplementary Table 1. Scale bars denote the fraction of substitutions.
SUPPLEMENTARY FIGURE 20 | Phylogenetic trees based on 18S rRNA, ITS region, D1/D2-28S rRNA, and COI gene sequences of astome ciliates isolated from lumbricid earthworms. Note that there is a conflict between the Phycas tree (A) and MrBayes/IQTree (B) in the position of some Anoplophrya and Metaradiophrya species. Bootstrap values for maximum likelihood IQTree analyses as well as posterior probabilities for Bayesian interference performed in MrBayes and Phycas were mapped onto the respective trees. Fully statistically supported nodes are marked with red solid circles. Dashes indicate statistical support below 50%. For specimen codes and further details, see Supplementary Table 1. Scale bars denote the fraction of substitutions.
SUPPLEMENTARY FIGURE 21 | Phylogenetic trees based on 16S, 18S, 28S rRNA, ITS region sequences (A) and on 16S, 18S, 28S rRNA, ITS region, and COI sequences (B) of astome ciliates isolated from lumbricid earthworms. Posterior probabilities for Bayesian inference performed in Phycas and MrBayes as well as bootstrap values for maximum likelihood analyses conducted in IQTrees were mapped onto the 50%-majority rule consensus Phycas trees. Fully statistically supported nodes are marked with red solid circles. Dashes indicate statistical support below 0.50 or 50%. For specimen codes and further details, see Supplementary Table 1. Scale bars denote the fraction of substitutions.
>Anoplophrya_lumbriciu_KR_11_LT

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>Anoplophrya_vulgaris_BZ_13_EF

---

>Anoplophrya_rubella_BZ_13_EF

---

>Anoplophrya_octolasioni

---

>Anoplophrya_vulgaris_BZ_13_EF

---

>Anoplophrya_vulgaris_MU_56_OG

---

>Anoplophrya_vulgaris_MU_56_OG

---

>Anoplophrya_vulgaris_BE_13_EF

---

>Anoplophrya_vulgaris_JA_16_EF

---
Metaradiophrya_lumbrici_JA2_26_LT
Metaradiophrya_lumbrici_JA2_26_LT
Metaradiophrya_speculorum_BZ_14_EF
Metaradiophrya_speculorum_BZ_14_EF
Metaradiophrya_speculorum_HkD_59_AT
Metaradiophrya_speculorum_HkD_60_AT
Metaradiophrya_lumbrici_JA2_26_LT
Metaradiophrya_lumbrici_JA1_22_EF
Metaradiophrya_lumbrici_JA1_19_EF
Metaradiophrya_lumbrici_JA1_22_EF

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