**Supplementary material**

**Table S1**. Sequences of Monogenoidea species used in the phylogenetic analyses, their specific host (and acronym), voucher numbers and collection locality data. CHIOC= Coleção Helmintológica do Instituto Oswaldo Cruz.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parasite species** | **Host species (or taxonomic group) and acronym** | **Host voucher number** | **Locality with**  **country, state, river (and freshwater ecorregion)** | **CHIOC number –voucher of parasite** | **28S**  **Accession**  **number** | **18S**  **Accession**  **number** |
| ***Characithecium*** |  |  |  |  |  |  |
| *C. triprolatum* | *O. perdido* ***OPE*** | ZUFMS5461 | Brazil, Mato Grasso do Sul, Perdido River (Paraguay) | 39528A | OM397873 | --- |
| *C. triprolatum* | *O. perdido* ***OPE*** | ZUFMS5461 | Brazil, Mato Grasso do Sul, Perdido River (Paraguay) | 39528B | OM397874 | --- |
| *C. triprolatum* | *O. jacuiensis* ***OJA*** | Not cataloged | Brazil, Rio Grande do Sul, Taquari River, (Laguna dos Patos) | 39527 | OM397875 | OM397866 |
| *C. triprolatum* | *O. hepsetus* ***OH*** | UFRGS18571 | Brazil, São Paulo, Ribeira de Iguape River (Ribeira de Iguape) | 39526 | OM397876 | --- |
| *C. triprolatum* | *O. brevioris* ***OBR*** | UFRGS24283 | Brazil, Rio Grande do Sul, Taquari River (Laguna dos Patos) | 39525 | OM397877 | --- |
| *C. triprolatum* | *O. argenteus* ***OAR*** | UFRGS19745 | Brazil, Minas Gerais, Doce River, (Northeastern Mata Atlantica) | 39524 | OM397878 | --- |
| *C. triprolatum* | *O. paranensis* ***OPA*** | UFRGS24341 | Brazil, Paraná, Ivaí River, (Upper Paraná) | 39523 | OM397879 | --- |
| *C. triprolatum* | *O. oligolepis* ***OO*** | UFRGS27218 | Brazil, Rio Grande do Sul, Ibicuí River (Lower Uruguay) | 39522 | OM397880 | --- |
| *C. quadratum* | *O. jenynsii* ***OJE*** | UFRGS22006 | Brazil, Rio Grande do Sul, Lagoa Corvina (Tramandaí-Mampituba) | 39521 | OM397881 | --- |
| *C. quadratum* | *O. robustus* ***OR*** | UFRGS17248 | Brazil, Rio Grande do Sul, Fortaleza Lagoon (Tramandaí-Mampituba) | 39520 | OM397882 | --- |
| *C. quadratum* | *O. oligolepis* ***OO*** | UFRGS23402 | Brazil, Rio Grande do Sul, Ibicuí River (Lower Uruguay) | 39519 | OM397883 | --- |
| *C. quadratum* | *O. oligolepis* ***OO*** | UFRGS24012 | Brazil, Rio Grande do Sul, Uruguai River (Lower Uruguay) | 39518 | OM397884 | --- |
| *C. quadratum* | *O. robustus* ***OR*** | UFRGS17248 | Brazil, Rio Grande do Sul, Fortaleza Lagoon (Tramandaí-Mampituba) | 39520 | OM397885 | --- |
| *C. robustum* | *O. longirostris* ***OL*** | UFRGS25342 | Brazil, Paraná, Iguaçu River (Iguaçu) | 39517 | OM397886 | --- |
| *C. robustum* | *O. jenynsii* ***OJE*** | UFRGS17472 | Brazil, Rio Grande do Sul, Tramandaí River (Tramandaí-Mampituba) | 39516 | OM397887 | --- |
| *C. chascomusensis* | *O. hepsetus* ***OH*** | UFRGS18700 | Brazil, São Paulo, Ribeira de Iguape river (Ribeira de Iguape) | 39505 | OM397888 | --- |
| *C. chascomusensis* | *O. hepsetus* ***OH*** | UFRGS18757 | Brazil, Rio de Janeiro, Paraibuna river (Paraíba do Sul) | 39504 | OM397889 | --- |
| *C. chascomusensis* | *O. hepsetus* ***OH*** | UFRGS18901 | Brazil, Rio de Janeiro, Silva Jardim (Fluminense) | 39503 | OM397890 | OM397867 |
| *C. chascomusensis* | *O. acutirostris* ***OAC*** | LBP10185 | Brazil, Minas Gerais, Mucuri River (Northeastern Mata Atlantica) | 39502 | OM397891 | --- |
| *C. chascomusensis* | *O. argenteus* ***OAR*** | LBP17391 | Brazil, Minas Gerais, São Francisco River (São Francisco) | 39501 | OM397892 | --- |
| *C. chascomusensis* | *O. jenynsii* ***OJE*** | Not cataloged | Brazil, Rio Grande do Sul, Jacuí River (Laguna dos Patos) | 39500 | OM397893 | --- |
| *C. costaricensis* | *Astyanax mexicanus* ***AM*** | UFRGS23111 | Mexico | 39512A | OM397894 | --- |
| *C. costaricensis* | *Astyanax mexicanus* ***AM*** | UFRGS23111 | Mexico | 39512B | OM397895 | --- |
| *C. costaricensis* | *Astyanax mexicanus* ***AM*** | UFRGS23111 | Mexico | 39512C | OM397896 | --- |
| *C. longianchoratum* | *O. jacuiensis* ***OJA*** | Not cataloged | Brazil, Rio Grande do Sul, Jacuí River (Laguna dos Patos) | 39515 | OM397897 | OM397868 |
| *C. longianchoratum* | *O. jacuiensis* ***OJA*** | Not cataloged | Brazil, Rio Grande do Sul, Jacuí River (Laguna dos Patos) | 39515 | OM397898 | --- |
| *C. longianchoratum* | *O. jacuiensis* ***OJA*** | Not cataloged | Brazil, Rio Grande do Sul, Jacuí River (Laguna dos Patos) | 39515 | OM397899 | --- |
| *C. longianchoratum* | *O. robustus* ***OR*** | Not cataloged | Brazil, Rio Grande do Sul, Guaiba Lake (Laguna dos Patos) | 39513 | OM397900 | --- |
| *C. longianchoratum* | *O. hepsetus* ***OH*** | UFRGS18522 | Brazil, São Paulo, Eldorado River (Ribeira de Iguape) | 39514 | OM397901 | --- |
| *C. chelatum* | *O. varii* ***OV*** | UFRGS22700 | Brazil, Rio Grande do Sul, São Marcos River (Laguna dos Patos) | 39511 | OM397902 | --- |
| *C. chelatum* | *O. argenteus* ***OAR*** | UFRGS19745 | Brazil, Minas Gerais, Doce River (Northeastern Mata Atlantica) | 39510 | OM397903 | --- |
| *C. chelatum* | *O. oligolepis* ***OO*** | UFRGS27218 | Brazil, Rio Grande do Sul, Ibicuí River (Lower Uruguay) | 39509 | OM397904 | OM397869 |
| *C. chelatum* | *O. planaltinae* ***OPL*** | LBP17054 | Brazil, Distrito Federal, Paranaíba River (Upper Paraná) | 39508 | OM397905 | OM397870 |
| *C. chelatum* | *O. paranensis* ***OPA*** | UFRGS24341 | Brazil, Paraná, Ivaí River, (Upper Paraná) | 39507 | OM397906 | --- |
| *C. chelatum* | *O. longirostris* ***OL*** | UFRGS25342B | Brazil, Paraná, Iguaçu River (Iguaçu) | 39506 | OM397907 | OM397871 |
| *C. paranapanemense* | *Psalidodon paranae* | - | Brazil, São Paulo, Middle Paranapanema River (Upper Paraná) |  | MZ408907 | --- |
| *C. paranapanemense* | *P. paranae* | - | Brazil, São Paulo, Middle Paranapanema River (Upper Paraná) |  | MZ408902 | --- |
| *C. paranapanemense* | *P. paranae* | - | Brazil, São Paulo, Middle Paranapanema River (Upper Paraná) |  | MZ408908 | --- |
| *Jainus hexops* | *Astyanax dissensus* ***AD*** | UFRGS17473 | Brazil, Rio Grande do Sul, Tramandaí River (Tramandaí-Mampituba) | 39499 | OM397908 | OM397872 |
|  | *Astyanax dissensus* ***AD*** | UFRGS17473 | Brazil, Rio Grande do Sul, Tramandaí River (Tramandaí-Mampituba) | 39499 | *---* | OM397872 |
| *Vancleaveus janauacaensis* | Siluriformes | - | Peru | - | KP056247 | --- |
| *Demidospermus prolixus* | Siluriformes | - | Brazil | - | KY766955 | --- |
| *Demidospermus anus* | Siluriformes | - | Brazil | - | KY766957 | --- |
| *Demidospermus rhinelepisi* | Siluriformes | - | Brazil | - | MG001324 | --- |
| *Ancyrocephalinae* gen. sp*.* | Siluriformes | - | Peru | - | KP056224 | --- |
| *Ancyrocephalus paradoxus* | Cyprinidae | - | Slovakia | - | AJ969952 | --- |
| *Actinocleidus recurvatus* | Cyprinidae | - | Slovakia | - | AJ969951 | --- |
| *Cleidodiscus pricei* | Cyprinidae | - | Czech Republic | - | AJ969939 | --- |
| *Urocleidus similis* | Cyprinidae | - | Czech Republic | - | AJ969938 | --- |
| *Cacatuocotyle papilionis* | *Astyanax lacustris* | - | Brazil | - | MG832889 | --- |
| *Unilatus unilatus* | Siluriformes | - | Brazil | - | MF102106 | --- |
| *Unilatus unilatus* | Siluriformes | - | Japan | - | LC104307.1 | --- |
| *Trinigyrus peregrinus* | Siluriformes | - | Japan | - | LC104308.1 | --- |
| *Heteropriapulus heterotylus* | *Pterygoplichthys ambrosettii* | - | Brazil | - | MF116370.1 | --- |
| *Heteropriapulus anchoradiatus* | *P. ambrosettii* | - | Brazil | - | MF116371.1 | --- |
| *Heteropriapulus simplex* | *P. ambrosettii* | - | Brazil | - | MF116372.1 | --- |
| *Ameloblastella chavarriai* | Siluriformes | - | Mexico | - | KP056251.1 | --- |
| *Unibarra paranoplatensis* | Siluriformes | - | Peru | - | KP056219.1 | --- |
| *Quadriacanthus clariadis* | Siluriformes | - | Sudan | - | KX685952.1 | --- |
| *Thaparocleidus vistulensis* | Cyprinidae | - | United Kingdom | - | AJ969941.1 | --- |

**Table S2.** Pairwise distance matrix (uncorrected p-distances, %) for *Characithecium* species based on 28S rDNA.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **p-distance based on 28S rDNA sequences** | | | | | | | | | | | | | | | | | | | | | |
|  | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** |
| **1** | *C. triprolatum* OPL |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **2** | *C. triprolatum* OPE | 0.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **3** | *C. triprolatum* OJA | 0.00 | 0.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **4** | *C. triprolatum* OH | 0.00 | 0.00 | 0.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **5** | *C. triprolatum* OBR | 0.00 | 0.00 | 0.00 | 0.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **6** | *C. triprolatum* OAR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **7** | *C. triprolatum* OPA | 0.37 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **8** | *C. triprolatum* OO | 1.15 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 1.06 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **9** | *C. quadratum* OJE | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.31 | 0.96 |  |  |  |  |  |  |  |  |  |  |  |  |
| **10** | *C. quadratum* OR1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.26 | 0.80 | 0.00 |  |  |  |  |  |  |  |  |  |  |  |
| **11** | *C. quadratum* OO1 | 0.74 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.79 | 1.33 | 0.63 | 0.52 |  |  |  |  |  |  |  |  |  |  |
| **12** | *C. quadratum* OO2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.26 | 0.80 | 0.00 | 0.00 | 0.52 |  |  |  |  |  |  |  |  |  |
| **13** | *C. quadratum* OR2 | 0.37 | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 0.63 | 1.27 | 0.00 | 0.31 | 0.95 | 0.31 |  |  |  |  |  |  |  |  |
| **14** | *C. robustum* OL | 10.49 | 9.67 | 9.67 | 9.73 | 9.73 | 9.70 | 10.04 | 10.52 | 12.08 | 9.73 | 10.42 | 9.73 | 12.52 |  |  |  |  |  |  |  |
| **15** | *C. robustum* OJE | 10.49 | 13.04 | 13.04 | 13.04 | 13.04 | 13.09 | 13.59 | 14.32 | 13.09 | 13.04 | 14.05 | 13.04 | 13.54 | 0.00 |  |  |  |  |  |  |
| **16** | *C. chascomusensis* OH1 | 10.98 | 10.01 | 10.01 | 10.07 | 10.07 | 10.04 | 10.39 | 10.86 | 12.52 | 10.07 | 10.78 | 10.07 | 12.97 | 0.26 | 0.37 |  |  |  |  |  |
| **17** | *C. chascomusensis* OH2 | 10.98 | 10.01 | 10.01 | 10.07 | 10.07 | 10.04 | 10.39 | 10.86 | 12.52 | 10.07 | 10.78 | 10.07 | 12.97 | 0.26 | 0.37 | 0.00 |  |  |  |  |
| **18** | *C. chascomusensis* OH3 | 10.98 | 10.01 | 10.01 | 10.07 | 10.07 | 10.04 | 10.39 | 10.86 | 12.52 | 10.07 | 10.78 | 10.07 | 12.97 | 0.26 | 0.37 | 0.00 | 0.00 |  |  |  |
| **19** | *C. chascomusensis* OAC | 10.98 | 10.01 | 10.01 | 10.07 | 10.07 | 10.04 | 10.39 | 10.86 | 12.52 | 10.07 | 10.78 | 10.07 | 12.97 | 0.26 | 0.37 | 0.00 | 0.00 | 0.00 |  |  |
| **20** | *C. chascomusensis* OAR | 10.98 | 10.01 | 10.01 | 10.07 | 10.07 | 10.04 | 10.39 | 10.86 | 12.52 | 10.07 | 10.78 | 10.07 | 12.97 | 0.26 | 0.37 | 0.00 | 0.00 | 0.00 | 0.00 |  |
| **21** | *C. chascomusensis* OJE | 11.02 | 11.02 | 11.02 | 11.02 | 11.02 | 11.02 | 11.54 | 12.25 | 11.07 | 11.02 | 12.08 | 11.02 | 11.54 | 0.40 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **22** | *C. costaricensis* AM1 | 5.08 | 5.08 | 5.08 | 5.08 | 5.08 | 5.08 | 5.74 | 6.58 | 5.08 | 5.08 | 6.45 | 5.08 | 5.08 | 3.15 | 3.15 | 3.86 | 3.86 | 3.86 | 3.86 | 3.86 |
| **23** | *C. costaricensis* AM2 | 9.65 | 10.32 | 10.32 | 10.32 | 10.32 | 10.35 | 10.75 | 11.35 | 10.35 | 10.32 | 11.13 | 10.32 | 10.76 | 3.34 | 3.45 | 3.71 | 3.71 | 3.71 | 3.71 | 3.71 |
| **24** | *C. costaricensis* AM3 | 10.42 | 10.75 | 10.75 | 10.75 | 10.75 | 10.75 | 11.17 | 11.79 | 10.79 | 10.75 | 11.60 | 10.75 | 11.21 | 3.54 | 3.26 | 3.94 | 3.94 | 3.94 | 3.94 | 3.94 |
| **25** | *C. longianchoratum* OJA1 | 12.76 | 11.23 | 11.23 | 11.27 | 11.27 | 11.27 | 11.61 | 12.18 | 14.09 | 11.27 | 11.96 | 11.27 | 14.54 | 3.06 | 4.29 | 3.35 | 3.35 | 3.35 | 3.35 | 3.35 |
| **26** | *C. longianchoratum* OJA2 | 11.81 | 10.54 | 10.54 | 10.54 | 10.54 | 10.57 | 10.90 | 11.46 | 13.14 | 10.54 | 11.20 | 10.54 | 13.57 | 4.26 | 5.56 | 4.57 | 4.57 | 4.57 | 4.57 | 4.57 |
| **27** | *C. longianchoratum* OJA3 | 11.81 | 10.47 | 10.47 | 10.54 | 10.54 | 10.49 | 10.82 | 11.38 | 13.14 | 10.54 | 11.20 | 10.54 | 13.57 | 4.23 | 5.56 | 4.54 | 4.54 | 4.54 | 4.54 | 4.54 |
| **28** | *C. longianchoratum* OR | 8.03 | 8.03 | 8.03 | 8.03 | 8.03 | 8.03 | 8.79 | 8.03 | 8.03 | 8.03 | 9.59 | 8.03 | 8.03 | 6.45 | 6.45 | 7.28 | 7.28 | 7.28 | 7.28 | 7.28 |
| **29** | *C. longianchoratum* OH | 12.45 | 10.95 | 10.95 | 10.95 | 10.95 | 10.99 | 11.32 | 11.03 | 13.69 | 10.95 | 11.62 | 10.95 | 14.12 | 4.57 | 6.00 | 4.88 | 4.88 | 4.88 | 4.88 | 4.88 |
| **30** | *C. chelatum* OV | 9.24 | 9.15 | 9.15 | 9.15 | 9.15 | 9.19 | 9.72 | 10.45 | 9.19 | 9.15 | 10.22 | 9.15 | 9.68 | 1.76 | 1.76 | 1.31 | 1.31 | 1.31 | 1.31 | 1.31 |
| **31** | *C. chelatum* OAR | 11.64 | 10.52 | 10.52 | 10.52 | 10.52 | 10.55 | 10.89 | 11.43 | 13.12 | 10.52 | 11.21 | 10.52 | 13.56 | 1.08 | 1.49 | 0.81 | 0.81 | 0.81 | 0.81 | 0.81 |
| **32** | *C. chelatum* OO | 11.64 | 10.42 | 10.79 | 10.52 | 10.52 | 10.48 | 10.82 | 11.31 | 13.12 | 10.52 | 11.21 | 10.52 | 13.56 | 1.07 | 1.49 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 |
| **33** | *C. chelatum* OPL | 11.92 | 12.15 | 12.15 | 12.15 | 12.15 | 12.15 | 13.17 | 12.15 | 12.15 | 12.15 | 12.15 | 12.15 | 12.38 | 0.64 | 0.85 | 0.64 | 0.64 | 0.64 | 0.64 | 0.64 |
| **34** | *C. chelatum* OPA | 11.05 | 10.12 | 10.12 | 10.12 | 10.12 | 10.15 | 10.49 | 11.00 | 12.59 | 10.12 | 10.81 | 10.12 | 13.03 | 1.64 | 2.27 | 1.36 | 1.36 | 1.36 | 1.36 | 1.36 |
| **35** | *C. chelatum* OL | 11.64 | 10.02 | 10.38 | 10.12 | 10.12 | 10.09 | 10.43 | 10.89 | 12.59 | 10.12 | 10.81 | 10.12 | 13.03 | 1.35 | 1.49 | 1.08 | 1.08 | 1.08 | 1.08 | 1.08 |
| **36** | *J. hexops* | 17.83 | 17.94 | 17.94 | 17.94 | 17.94 | 17.94 | 18.42 | 19.26 | 18.01 | 17.94 | 18.90 | 17.94 | 18.50 | 13.66 | 15.02 | 13.22 | 13.22 | 13.22 | 13.22 | 13.22 |
| **37** | *C. paranapanemense* MZ408907 | 0.00 | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 0.62 | 1.28 | 0.31 | 0.31 | 0.94 | 0.31 | 0.62 | 12.57 | 13.04 | 13.00 | 13.00 | 13.00 | 13.00 | 13.00 |
| **38** | *C. paranapanemense* MZ408908 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.46 | 1.45 | 0.00 | 0.00 | 0.93 | 0.00 | 0.46 | 5.66 | 5.66 | 6.24 | 6.24 | 6.24 | 6.24 | 6.24 |
| **39** | *C. paranapanemense* MZ408902 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.35 | 1.10 | 0.00 | 0.00 | 0.71 | 0.00 | 0.35 | 13.09 | 13.09 | 13.59 | 13.59 | 13.59 | 13.59 | 13.59 |
| **40** | *Cacatuocotyle papilionis* MG832889 | 18.54 | 19.04 | 19.04 | 19.04 | 19.04 | 19.11 | 19.57 | 19.11 | 19.91 | 19.04 | 19.96 | 19.04 | 19.90 | 12.08 | 13.11 | 12.50 | 12.50 | 12.50 | 12.50 | 12.50 |

*(Continued)*

**Table S2.** *(Continued.)*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** | **29** | **30** | **31** | **32** | **33** | **34** | **35** | **36** | **37** | **38** | **39** | **40** |
| **21** | *C. chascomusensis* OJE |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **22** | *C. costaricensis* AM1 | 3.86 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **23** | *C. costaricensis* AM2 | 2.95 | 0.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **24** | *C. costaricensis* AM3 | 2.95 | 0.00 | 2.05 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **25** | *C. longianchoratum* OJA1 | 5.19 | 6.52 | 5.96 | 6.16 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **26** | *C. longianchoratum* OJA2 | 5.67 | 6.52 | 5.92 | 6.99 | 1.07 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **27** | *C. longianchoratum* OJA3 | 5.67 | 6.52 | 5.92 | 6.99 | 1.07 | 0.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **28** | *C. longianchoratum* OR | 7.28 | 7.73 | 7.12 | 7.12 | 0.66 | 0.66 | 0.66 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **29** | *C. longianchoratum* OH | 6.16 | 7.27 | 6.32 | 7.39 | 1.35 | 0.27 | 0.27 | 0.00 |  |  |  |  |  |  |  |  |  |  |  |  |
| **30** | *C. chelatum* OV | 1.38 | 5.88 | 4.09 | 4.31 | 5.11 | 5.11 | 5.11 | 6.44 | 5.64 |  |  |  |  |  |  |  |  |  |  |  |
| **31** | *C. chelatum* OAR | 1.22 | 5.88 | 4.81 | 5.06 | 3.07 | 4.26 | 4.26 | 6.44 | 4.57 | 0.00 |  |  |  |  |  |  |  |  |  |  |
| **32** | *C. chelatum* OO | 1.22 | 5.88 | 4.81 | 5.06 | 3.06 | 4.26 | 4.23 | 6.44 | 4.57 | 0.00 | 0.00 |  |  |  |  |  |  |  |  |  |
| **33** | *C. chelatum* OPL | 0.94 | 5.61 | 5.46 | 5.56 | 2.66 | 5.44 | 5.44 | 3.09 | 5.44 | 0.00 | 0.00 | 0.00 |  |  |  |  |  |  |  |  |
| **34** | *C. chelatum* OPA | 2.06 | 5.88 | 5.19 | 5.43 | 3.67 | 4.88 | 4.88 | 7.28 | 5.20 | 0.86 | 0.53 | 0.53 | 0.64 |  |  |  |  |  |  |  |
| **35** | *C. chelatum* OL | 1.22 | 5.88 | 4.44 | 4.70 | 3.36 | 3.95 | 3.93 | 6.44 | 4.26 | 0.00 | 0.27 | 0.26 | 0.64 | 0.81 |  |  |  |  |  |  |
| **36** | *J. hexops* | 12.98 | 12.11 | 14.23 | 13.61 | 14.47 | 13.90 | 13.90 | 19.18 | 14.45 | 11.50 | 13.66 | 13.66 | 18.68 | 13.79 | 13.10 |  |  |  |  |  |
| **37** | *C. paranapanemense* MZ408907 | 11.02 | 5.08 | 10.82 | 11.27 | 14.62 | 13.66 | 13.66 | 8.03 | 14.22 | 9.15 | 13.62 | 13.62 | 10.68 | 13.08 | 13.09 | 17.65 |  |  |  |  |
| **38** | *C. paranapanemense* MZ408908 | 6.62 | 5.17 | 4.56 | 4.83 | 6.78 | 6.78 | 6.78 | 8.03 | 7.39 | 6.83 | 6.83 | 6.83 | 10.60 | 6.83 | 6.83 | 14.40 | 0.00 |  |  |  |
| **39** | *C. paranapanemense* MZ408902 | 11.07 | 5.08 | 11.65 | 12.71 | 15.48 | 15.03 | 15.03 | 8.03 | 15.73 | 9.15 | 14.32 | 14.32 | 10.58 | 13.69 | 14.32 | 20.34 | 0.00 | 0.00 |  |  |
| **40** | *Cacatuocotyle papilionis* MG832889 | 14.05 | 14.72 | 13.58 | 14.94 | 9.50 | 7.94 | 7.94 | 7.86 | 7.52 | 12.86 | 12.08 | 12.08 | 14.25 | 12.99 | 11.60 | 16.93 | 19.06 | 12.00 | 22.16 |  |

**Table S3.** Principal diagnostic morphological and morphometric characteristics of *Characithecium* species based on literature (Mendoza-Franco *et al.*, 2009; Rossin and Timi, 2015; Gallas *et al.*, 2016) and examined material. Average size followed by minimum and maximum size in parentheses. μm = micrometers; n= number of specimens.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Character/species | *C. costaricensis* | *C. triprolatum* | *C. chascomusensis* | *C. robustum* | *C. quadratum* | *C. chelatum* | *C. longianchoratum* |  |
| Body length (μm) | 280  (215–370; n= 11) **a** | 426 (322–555; n= 11) **c** | 611 (480–754; n= 20) **b** | 842 (606–1000; n= 11) **b** | 631 (498–752; n= 10) **b** | 337 (270–426; n= 10) **b** | 450 (351–540; n= 20) **b** | Literature information (**a, b, c**) |
| **Haptor structures** | | | | | | | |
| Ventral anchor length (μm) | 34 (33–35; n= 19) **a** | 37 (32–40; n= 12) **c** | 40 (31–44; n= 20) **b** | 43 (39–48; n= 11) **b** | 43 (40–46; n= 10) **b** | 44 (41–46; n= 10) **b** | 56 (51–61; n= 20) **b** |
| Dorsal anchor length (μm) | 28 (28–29; n= 10) **a** | 28 (22–35; n= 12) **c** | 36 (27–42; n= 20) **b** | 35 (32–37; n= 11) **b** | 34 (32–36; n = 10) **b** | 29 (26–31; n= 10) **b** | 34 (30–38; n= 20) **b** |
| Ventral bar shape | Straight (n= 10) **d** | Straight (n= 25) **d** | U-shaped (n= 60) **d** | V-shaped (n= 15) **d** | U-shaped (n= 15) **d** | V-shaped (n= 57) **d** | U-shaped (n= 23) **d** | Material examined in present study (**d**) and checked in the Literature (**a, b, c**) |
| Medial suture in ventral bar | Absent (n= 10) **d** | Absent (n= 25) **d** | Present (n= 60) **d** | Present (n= 15) **d** | Present (n= 15) **d** | Present (n= 57) **d** | Present (n= 23) **d** |
| Postero-medial  projection in ventral bar | Present (n= 10) **d** | Present (n= 25) **d** | Absent (n= 60) **d** | Absent (n= 15) **d** | Absent (n= 15) **d** | Absent (n= 57) **d** | Absent (n= 23) **d** |
| **Reproductive structures** | | | | | | | |
| Rings in the MCO | ½ – 1 (n= 10) **d** | 1 (n= 25) **d** | 3 – 4 (n= 60) **d** | 2 ½ (n= 15) **d** | 2 (n= 15) **d** | 1 ½ (n= 57) **d** | 2 (n= 23) **d** |
| Accessory piece shape | Rod-shaped (n= 10) **d** | Pincer-shaped (n= 25) **d** | Clamp-shaped (n= 60) **d** | Pincer-shaped (n= 15) **d** | Clamp-shaped (n= 15) **d** | Pincer-shaped (n= 57) **d** | Clamp-shaped (n= 23) **d** |
| Vaginal opening | Ventral - middle of the body (n= 10) **d** | Ventral - middle of the body (n= 25) **d** | Marginal and sinistral (n= 60) **d** | Ventral - middle of the body (n= 15) **d** | Marginal and sinistral (n= 15) **d** | Marginal and sinistral (n= 57) **d** | Ventral - middle of the body (n= 23) **d** |

**a** Mendoza-Franco *et al.* (2009)

**b** Rossin and Timi (2015)

**c** Gallas *et al.* (2016)

**d** present study