Research article

Title: Phylogenetic placement and contamination screening of Amoebozoa genomic data from the Protist 10,000 Genomes (P10K) Database

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Abstract

Background: Genomic data are essential for uncovering the evolutionary history, ecological roles, and diversity of life. Yet, microbial eukaryotes like Amoebozoa, an ancient and morphologically diverse lineage, remain critically underrepresented in genomic repositories. This has limited our ability to address fundamental questions in eukaryotic evolution. The Protist 10,000 Genomes (P10K) initiative seeks to fill this gap by generating and compiling genome- and transcriptome-level data for a wide range of microbial eukaryotes. To ensure the reliability of these resources, accurate taxonomic identification and contamination screening are vital. In this study, we aimed to assess the taxonomic consistency and integrity of the P10K database with a phylogenetic-based approach using Amoebozoa as a case study.

Results: Through SSU rDNA/rRNA and COI phylogenetic reconstructions this study confirmed several initial taxonomic identifications provided in the P10K database, resolved ambiguities at higher taxonomic levels, and corrected misassignments among morphologically similar but phylogenetically distant taxa. Moreover, the contamination screening using SSU rDNA/rRNA revealed several amoebozoan data that are contaminated by sequence from other eukaryotic taxa, representing contaminated genomic assemblies.

Conclusion: Phylogenetic placement coupled with contamination screening enabled us to distinguish the higher-quality Amoebozoa datasets currently available in the P10K database from those requiring decontamination or additional sequencing before downstream use. These findings serve as a reference for the future use of these data and as a guide for further sequencing efforts aimed at expanding the taxonomic diversity of Amoebozoa represented at the genomic level. By applying a phylogenetic survey to the Amoebozoa data, we present a framework that can be extended to other microbial eukaryote lineages. Addressing imprecise taxonomic identifications and contamination in certain P10K datasets, as well as data reproducibility, will further enhance the value of this unprecedented genomic resource for protists, with significant potential to illuminate the evolution and diversification of eukaryotic life.

Keywords: Amoebozoa, Arcellinida, Eukaryotic diversity, Comparative genomics, Genomic database curation

Introduction

Genomic-level data are essential for advancing our understanding of the evolution of life on Earth [1, 2]. High-quality genome and transcriptome sequences enable comparative analyses that reveal patterns of genomic evolution, including gene family expansions, horizontal gene transfers, and changes in genome organization and regulatory systems [3, 4]. Such data also clarify phylogenetic relationships through multi-gene and genome-scale reconstructions [5, 6]. Moreover, genomic analyses uncover ecological interactions by identifying metabolic pathways, symbiotic associations, and genetic adaptations to specific environmental conditions [7]. Consequently, genomics has become central to studying life's diversity and complexity.

While genomics has progressed rapidly for major eukaryotic groups such as plants, animals, fungi, and other traditional model systems, most microbial eukaryotes (commonly referred to as protists) remain vastly underrepresented [1, 2]. Although they comprise most of eukaryotic diversity, the majority of lineages within this highly diverse paraphyletic assemblage still lack genomic data [2, 5]. Over the past decade, several initiatives have contributed to fill this gap, such as the Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP) for marine microbial genomics [8], the Tree of Life Programme for eukaryotic genome sequencing [9], and the One Thousand Plant Transcriptomes initiative (1KP) for plants, including single-celled algae [10]. Similarly, the Protist 10,000 Genomes (P10K) initiative aims to address the underrepresentation of microbial eukaryotes in genomic databases by generating new genomic data (i.e., genomes and transcriptomes) and compiling previously available data from a wide array of lineages, coupled with taxonomic identification and decontamination procedures [2]. This large-scale effort, consolidated in the P10K database, represents an unprecedented genomic resource for protists and potentially provides a valuable foundation for achieving a comprehensive and integrative view of eukaryotic evolution.

However, to fully exploit the potential of this newly available genomic resource for protists, three key challenges must be considered: (1) accurate taxonomic identification, (2) the presence of contaminated genomic data resulting from non-target eukaryotic contamination, and (3) data interpretation and reproducibility [11, 12]. Specifically, the taxonomic identification of the P10K database relies on small subunit ribosomal DNA (SSU rDNA) retrieval and BLAST similarity searches against curated SSU databases, including SILVA, PR^2, and NCBI's NT (nucleotide sequence) database

91 [2]. Although informative, a BLAST-based approach is less accurate than phylogeny-based methods
92 for taxonomic identification and may lead to a less precise classification of the target groups. Regarding
93 contamination, protists typically inhabit environments with high eukaryotic microbial diversity, several
94 prey on other organisms, many host endosymbionts, and most are often difficult to isolate as
95 monoeukaryotic cultures or single cells [13–15]. As a result, genomic-level sequencing efforts might
96 produce contaminated assemblies that contain not only the genome of the target organism but also
97 sequences from other eukaryotic organisms [16, 17]. While P10K performs a decontamination strategy
98 to remove bacterial, archaeal, viral, fungal, and other eukaryotic contaminants from ciliate data; for
99 other protist groups, only bacterial, archaeal, and viral contaminants are filtered out [2]. Finally, the
100 P10K database currently lacks photo-documentation and detailed information, for instance, about the
101 sequencing platforms used for each sample (i.e., Illumina, Oxford Nanopore, or PacBio Sequel II),
102 which are essential for deeper interpretation and reproducibility of the data. In this context, accurate
103 taxonomic identification, assessment of eukaryotic contamination, and the availability of more detailed
104 sample information are most essential for the effective downstream use of data in the P10K database.
105 Here, we present a survey of the taxonomic identification and contamination screening of the P10K
106 database using Amoebozoa as a case study. Amoebozoa is an ancient clade of heterotrophic
107 organisms, estimated to have originated around 1.5 billion years ago, and it exhibits remarkable
108 morphological and ecological diversity [13, 15, 18]. Because Amoebozoa are heterotrophic and often
109 share habitats with a wide range of eukaryotic organisms, making them particularly prone to sequence
110 contamination, they serve as an ideal model group for evaluating the accuracy of taxonomic
111 identification and contamination assessment in the P10K database. In short, we retrieved all publicly
112 available Amoebozoa genomes from the P10K database, representing the three major amoebozoan
113 clades; Tubulinea, Evosea, and Discosea [13]. From these data, we conducted phylogenetic analyses
114 using two commonly employed molecular markers: small subunit ribosomal DNA/RNA (SSU
115 rDNA/rRNA or 18S) and cytochrome c oxidase subunit I (COI, also known as CO1 or cox1). This
116 phylogenetic investigation enables the phylogenetic placement and contamination screening of
117 Amoebozoa genomic data present in the P10K database, leading to the identification of higher-quality
118 data, while also highlighting those that require decontamination or additional sequencing prior to
119 downstream analyses. Additionally, we present some strategies that can mitigate contamination during
120 sample preparation and discuss how the availability of more detailed metadata directly associated to

121 each sample can improve the interpretation, usability, and reproducibility of P10K data. Ultimately, this
122 study serves as a proof of concept for using a phylogenetic framework to improve taxonomic
123 identification and contamination assessment within the P10K dataset, as an approach that can be
124 extended to other taxa represented in the database.
125
126 **Methods**
127
128 *Datasets construction*
129
130 We retrieved genomic-level assemblies for the 201 amoebozoans from the P10K database based
131 on the taxonomic annotations provided on the platform (**Supplementary Information - Table S1**). We
132 constructed a small subunit ribosomal DNA/RNA (SSU rDNA/rRNA) dataset considering all 201 of these
133 assemblies. Further, we constructed a cytochrome c oxidase subunit I (COI) dataset focusing only on
134 the testate amoebae order Arcellinida (Tubulinea), since this marker is well sampled for this lineage as
135 it has been traditionally used for phylogenetics in arcellinids. To construct datasets for the small subunit
136 ribosomal DNA/RNA (SSU rDNA/rRNA) and cytochrome c oxidase subunit I (COI) markers, we
137 extracted sequences from these assemblies using similarity searches implemented in BLAST+
138 v2.16.0+ and a custom Python script (**Supplementary Information - File S1**). For each marker, the
139 script automated the creation of BLAST databases using makeblastdb and performed local blastn
140 searches for each query sequence in a multi-FASTA file against each Amoebozoa genomic data.
141 Specifically, the script executed the commands *makeblastdb -in P10KID.fasta -dbtype nucl* and *blastn*
142 *-query marker_query -db P10K.fasta -outfmt [script_default_choice]* (**Supplementary Information -**
143 **File S1**). The script retrieved the top five hits per genomic data file, extracting each aligned region along
144 with 1000 bp of upstream and downstream flanking sequence. This approach enabled recovery of
145 extended SSU and COI regions suitable for downstream phylogenetic analyses and compatible with
146 Amoebozoa SSU and COI data available in the PR^2 database [19] and NCBI. The orientation of each
147 retrieved sequence was assessed, and sequences were reverse complemented when necessary to
148 match the strand of the original query. For the query sequences, we used SSU rDNA/rRNA data from
149 the PR^2 database for three representative species of the major amoebozoan clades: *Arcella vulgaris*
150 WP (Tubulinea; GenBank: HM853762.1), *Dictyostelium discoideum* (Evosea; GenBank: AM168040.1),

151 and *Acanthamoeba castellanii* Neff (Discosea; GenBank: U07416.1). As the COI query sequence, we
152 considered the sequence of *Arcella uspiensis* (SRR5396453).
153 To build a phylogenetically informative datasets, we combined the retrieved sequences with
154 previously published SSU rDNA/rRNA and COI datasets for Amoebozoa, as well as sequences from
155 the PR^2 database for SSU [13, 15, 19, 20]. For SSU, we curated a non-redundant dataset broadly
156 representative of the major lineages in Amoebozoa (Tubulinea, Evosea, and Discosea) and for COI a
157 dataset broadly representative of the major lineages in Arcellinida, for both markers excluding
158 environmental sequences. This strategy ensured a robust and interpretable dataset for our phylogenetic
159 framework. From a preliminary phylogenetic reconstruction, we curated the SSU and COI datasets
160 used to generate the main trees in this study. This initial analysis allowed us to identify and remove
161 identical or highly similar sequences that resulted from retrieving the top five BLAST+ hits per genome.
162 It also enabled visual inspection of the tree to detect SSU sequences that either failed to cluster within
163 Amoebozoa or formed unusually long branches. Many of these were short sequences (<200 bp) and
164 were excluded from downstream analyses, while some long branches corresponded to full-length
165 sequences that likely represented contaminants. These were retained for further contamination
166 screening.
167 For contamination screening, we focused on the SSU rDNA/rRNA dataset. After the initial
168 phylogenetic reconstruction (see *Phylogenetic reconstructions* section), any SSU sequences from
169 P10K genome assemblies that represented long branches or did not branch within the Amoebozoa
170 clade were selected for further analysis. These sequences were subjected to additional BLAST+
171 searches against the PR^2 database, a curated SSU resource representing eukaryotic diversity, using
172 our custom script and the same parameters described above (**Supplementary Information - File S1**).
173 To perform this search locally, we downloaded the complete PR^2 database and used it as the reference
174 for the BLAST+ similarity search. This approach allowed us to retrieve SSU sequences from the PR^2
175 database that were similar to those of the putative contaminant eukaryotes and to assign their
176 taxonomic affiliations through subsequent phylogenetic analyses.
177
178
179 *Phylogenetic reconstructions*
180

181 All phylogenetic reconstructions in this study were based on multiple sequence alignments (MSAs)
182 generated using MAFFT v7.490 with the E-INS-I algorithm and 1000 refinement iterations. Alignments
183 were produced with the following command: *mafft --genadpair --maxiterate 1000 input.fasta >*
184 *output_aligned.fasta*. Automated alignment trimming was performed with trimAl v1.2, using the
185 command *trimal -in input_aligned.fasta -out output_aligned_trimmed.fasta -keepheader -gt [threshold]*,
186 where the gap threshold was set to 0.3 for SSU rDNA/rRNA and 0.5 for COI. Phylogenetic trees were
187 inferred from the trimmed alignments using the maximum likelihood method implemented in IQ-TREE
188 v2.3.6, with ModelFinder for model selection and node support assessed via 1,000 ultrafast bootstrap
189 replicates and 1,000 SH-aLRT tests. The analysis was executed with the command *iqtree2 -s*
190 *aligned_trimmed.fasta -alrt 1000 -bb 1000 -m TEST*.
191
192 **Results and discussion**
193
194 *Phylogeny-based taxonomic identification of P10K amoebozoan data*
195
196 Amoebozoan small subunit ribosomal DNA/RNA (SSU rDNA/rRNA) sequences were successfully
197 retrieved from 151 of the 201 genomic datasets available for Amoebozoa in the P10K database
198 (**Supplementary Information - Table S1**). Given the established use of the Cytochrome c oxidase
199 subunit I (COI) marker in Arcellinida phylogenetics, we also specifically targeted this marker for
200 arcellinid taxa. Arcellinid COI sequences were successfully recovered from 40 of the 59 genomic data
201 available for Arcellinida (**Supplementary Information - Table S1**). Phylogenetic analyses based on
202 SSU rDNA/rRNA and COI were largely congruent, supporting most of the original taxonomic
203 assignments in the P10K database (**Figs. 1 and 2**). Moreover, they enabled a more precise
204 identification for 43 taxa, including a refined classification at the genus and family levels for taxa initially
205 classified only at the family or higher levels (**Figs. 1 and 2**; **Supplementary Information - Table S1**).
206 As species-level identification typically requires extensive morphological and morphometric data in
207 addition to molecular evidence, we adopted a conservative approach and assigned identifications at
208 the genus level based on our phylogenetic results. However, it is worth noting that several genomic
209 data from the P10K database originate from well-established cultures of widely used and shared strains,
210 some originally available in the NCBI database, for which detailed morphological data are available in

211 the literature, allowing confident species-level identifications (**Figs. 1 and 2**; **Supplementary**
212 **Information - Table S1**).
213 Notably, seven original taxonomic assignments from the P10K database were not corroborated
214 by our phylogenetic reconstructions (**Figures 1 and 2**; **Supplementary Information - Table S1**). This
215 was most apparent within Arcellinida, where misidentifications primarily involved closely related or
216 morphologically similar genera and families. Although Arcellinida are well known for their test (shell),
217 which provides informative taxonomic characters, convergent evolution has led to similar shell
218 morphologies across distantly related lineages. For example, species of *Difflugia* (Difflugiidae),
219 *Hyalosphenia* (Hyalospheniidae), and *Netzelia* (Netzelliidae) often possess rounded, ovoid, or
220 elongated shells, which can lead to misidentification if other shell features (e.g., aperture shape,
221 composition) or cellular characteristics are not considered [21, 22]. Consistent with this, our analyses
222 revealed that several taxa initially assigned to Difflugiidae (infraorder Longithecina) belong to
223 Hyalospheniidae (Hyalospheniformes) or Netzelliidae (Sphaerothecina) (**Supplementary Information**
224 **- Table S1**). These infraorders are distantly related, with their last common ancestor estimated to have
225 lived over 500 million years ago [15, 23], making such misassignments evolutionarily significant.
226 Outside Arcellinida, only one notable case of misidentification was observed: *Pessonella* sp. PRA-29,
227 a culture originally submitted to the American Type Culture Collection (ATCC) under the genus
228 *Pessonella*, was later described as a new genus and species, *Armaparvus languidus*, representing the
229 correct taxonomic identification for this organism [24].
230
231 *Contamination screening of the P10K amoebozoan data*
232
233
234 Non-amoebozoan SSU sequences were retrieved from 58 of the 201 genomic datasets available
235 for Amoebozoa in the P10K database (**Supplementary Information - Table S1**). The phylogenetic
236 analysis of these non-amoebozoan SSU and SSU sequences from the PR^2 database reveals a
237 widespread contamination of the P10K amoebozoan dataset by a taxonomically diverse set of
238 eukaryotic lineages, mirroring the ecological complexity of the environments these protists inhabit
239 (**Figure 3 and Supplementary Information - Table S1**). Among the contaminant groups were fungi
240 and metazoans, including sequences from arthropods and nematodes, likely introduced via soil

241 particles, organic debris, or sample handling procedures (**Figure 3**). SSU sequences affiliated with
242 ciliates were particularly abundant, with representatives spanning multiple clades such as Vorticellidae,
243 *Coleps*, and *Pseudomicrothorax* (**Figure 3**). Additional contaminants included lineages within the
244 Stramenopiles such as *Paraphysomonas* and *Poterioochromonas*, as well as centrohelid heliozoans
245 (Haptista) and cercozoans (Rhizaria) (**Figure 3**). Several photosynthetic eukaryotes represented
246 another major group of contaminants, including land plants, especially angiosperms of the Fabaceae
247 family, likely introduced through pollen or plant debris, as well as green algae from the Chlorophyceae
248 (e.g., *Chlamydomonas*, *Hyalomonas*) and Zygnematophyceae, the closest relatives of land plants
249 (**Figure 3**). Collectively, these findings demonstrate that contamination in amoebozoan assemblies is
250 both frequent and taxonomically widespread, encompassing multiple branches of the eukaryotic tree.
251
252
253 *Quality assessment of the P10K amoebozoan data*
254
255 Based on the results from single-marker retrieval, phylogenetic inference, and quality assessment,
256 we were able to evaluate the current state of Amoebozoa genomic data in the P10K database. As a
257 relative measure, we can consider samples to be of relatively higher quality if they contain the SSU
258 marker (and COI for arcellinids), show no signs of contamination based on SSU phylogenetic
259 reconstruction, and have a BUSCO completeness score of at least 50% (**Figure 4; Supplementary**
260 **Information - Table S1**). These samples are more likely to yield meaningful results in downstream
261 analyses, including phylogenomics and comparative genomics (**Figure 4; Supplementary**
262 **Information - Table S1).** It is important to note, however, that the SSU-based contamination screening
263 we used as an exploratory assessment of the amoebozoan P10K data has limitations and cannot, on
264 its own, detect all sources of potential contamination. Therefore, incorporating additional markers for
265 further screening is advisable before using the data in downstream analyses. Similarly, while we chose
266 a relatively low BUSCO threshold (≥50%) to include a broader range of potentially useful genomic data.
267 More stringent completeness cutoffs, as well as additional quality metrics such as genome contiguity,
268 N50 values, and assembly size, are required depending on the downstream applications of the data
269 and should be considered accordingly. On the other hand, the P10K samples lacking key markers,
270 flagged as contaminated based on SSU phylogenetic inference, or with BUSCO scores below 50%

271 represent more incomplete and lower-quality genomic data (**Figure 4; Supplementary Information -**
272 **Table S1**). In this context, several of the amoebozoan genomic data available in the P10K database
273 require decontamination or further sequencing prior to downstream analysis (**Figure 4D;**
274 **Supplementary Information - Table S1**). Finally, this quality assessment of the P10K genomic
275 resource highlights that several major Amoebozoa lineages remain unsampled at the genomic level
276 and serves as a useful guide for targeted sampling efforts aimed at expanding the taxonomic diversity
277 sampled for genomic data.
278
279
280 *Widespread Contamination in Protist Genomes: Sources, Impacts, and Mitigation Strategies*
281
282 While the widespread contamination identified in amoebozoan genome assemblies is certainly
283 undesirable, it is consistent with the natural ecological context not only of Amoebozoa but of microbial
284 eukaryotes in general. Protists typically inhabit complex microbial communities, including soil, biofilms,
285 freshwater and marine sediments, mosses, and decaying organic matter, where they coexist and
286 interact with a wide diversity of organisms [13, 21, 25]. Like many free-living protists, amoebozoans are
287 predatory and feed on bacteria, algae, fungi, and other eukaryotic cells, or form close physical
288 associations with them, such as transient or stable endosymbiotic relationships [13, 21]. These
289 ecological interactions, combined with the technical difficulty of isolating single amoebozoan cells free
290 from other microbial associates, make the presence of contaminant sequences in genome assemblies
291 not only possible but likely [16]. Many species cannot be maintained in long-term axenic or
292 monoeukaryotic cultures, and even those that can often require extensive purification efforts to
293 eliminate co-cultured organisms [26].
294 Contaminant sequences can significantly impact genomic-level downstream analyses. They may
295 compromise gene prediction, reveal artifactual patterns of gene family evolution, mislead functional
296 annotations, and introduce biases in comparative genomic studies [4, 27, 28]. More specifically,
297 contamination can lead to overestimation of genomic complexity and distorts analyses of gene family
298 evolution by introducing homologs or paralogs from unrelated lineages, which can result in artificial
299 expansions or contractions of gene families and misrepresentation of evolutionary trajectory studies [4,
300 27, 28]. Functional annotation is similarly affected, as contaminant sequences may be erroneously

301 assigned to the target genome, leading to inaccurate inferences about metabolic capabilities, signaling
302 pathways, or ecological roles [4, 27, 28]. Similarly, assemblies that include sequences from diverse
303 eukaryotic contaminants may cluster incorrectly in phylogenomic datasets, leading to artifactual
304 phylogenetic trees [29]. Technically, contamination can also affect genome completeness metrics by
305 artificially increasing the number of expected genes detected. This may create the false impression of
306 high assembly quality and completeness, even when substantial portions of the assembly are derived
307 from non-target organisms [16]. Ultimately, unaddressed contamination undermines efforts to draw
308 biologically meaningful conclusions about evolution, diversity, and functional biology from genomic
309 data.

310 Given these challenges, several strategies that are not reported to be used by the P10K initiative
311 have been successfully used to reduce contamination in protist genomic-level data generation efforts,
312 including for Amoebozoa [13, 15, 23]. For taxa that can be cultivated, growing cultures through multiple
313 generations can help eliminate contaminant organisms that were initially co-isolated with the target
314 taxon [13, 15, 23]. Other effective practices include visual inspection of cultures to detect fungi or small
315 eukaryotes, filtration of culture media, and rigorous sterile handling during DNA and RNA extraction.
316 For species that must be isolated as single cells from environmental samples, useful techniques include
317 repeated transfers of the cell through filtered sterile water followed by overnight starvation in sterile
318 medium [13, 15, 23]. These procedures allow cleaning of the cells and digestion of prey items, reducing
319 the risk of capturing genetic material from non-target organisms derived from their environment or food
320 source.

321 Even when applying these methods, it is not possible to guarantee that genomic data will be
322 completely free from contamination, particularly when working with environmentally derived specimens.
323 Therefore, comprehensive screening of genome assemblies remains essential. When contamination is
324 detected, identifying the phylogenetic affinities of non-target sequences can guide decisions on data
325 curation and inform subsequent analyses [29]. As corroborated in this study, combining single marker-
326 based phylogenetic screening with genome-level examination provides a powerful and generalizable
327 strategy for distinguishing genuine genomic content from artifactual sequences, especially SSU that
328 have been traditionally sequenced from diverse eukaryotes and for which comprehensive curated
329 databases like PR^2 are available. Importantly, this strategy couples the strengths of likelihood-based
330 phylogenetics, which outperform similarity-based approaches such as BLAST by incorporating explicit

331 models of molecular evolution and statistically grounded inference [30, 31]. These features allow for
332 more accurate reconstruction of evolutionary relationships, particularly among divergent or closely
333 related taxa, where mere sequence similarity may be misleading [31, 32]. This approach serves as a
334 guide for data curation and ensures that genomic data accurately represent the biology and evolutionary
335 histories of the target protist lineages.
336
337 *Reproducibility of the P10K data*
338
339 Currently, the P10K database lacks photo-documentation and detailed metadata directly
340 associated with each sample, which impairs both the reproducibility and deeper interpretation of the
341 data. In particular, the absence of voucher images of specimens or cultures from which the data were
342 derived prevents taxonomic confirmation and revision. Without such reference material, especially for
343 uncultivable organisms, it becomes impossible to attempt re-isolation of the target taxa for additional
344 sequencing efforts aimed at generating more complete genomic data. Moreover, the integration of
345 morphological documentation with phylogenetic analyses would not only improve taxonomic accuracy
346 and robustness, but also enable more comprehensive, integrative discoveries that combine molecular
347 and morphological information. Ideally, the database could include voucher photographs and, when
348 possible, images of multiple specimens of the same taxon from environmental samples or cultures. This
349 would facilitate further morphometric analyses and potentially contribute to the utility of the P10K
350 dataset for researchers who study protists using both molecular and morphological approaches.
351 Another limitation affecting data accessibility and reproducibility is the lack of clear information on the
352 specific sequencing platforms used for each sample (e.g., Illumina with short or long-insert libraries,
353 Oxford Nanopore, or PacBio Sequel II). Since each platform has characteristic error profiles and biases,
354 this metadata is critical for downstream analyses and informed interpretation of the genomic data. Thus,
355 the incorporation of photo-documentation and detailed metadata for each sample could substantially
356 contribute to the scientific value, reproducibility, and long-term impact of the P10K database.

357 **Conclusions**

358 This study presents a comprehensive phylogenetic assessment of taxonomic assignments and
359 contamination across Amoebozoa genomic datasets in the P10K database. By using SSU rDNA/rRNA

360 and COI markers, we confirmed many of the original classifications, refined others, and identified
361 multiple cases of misidentification within morphologically similar lineages. Additionally, we uncovered
362 widespread contamination by diverse eukaryotic lineages, including fungi, metazoans, green algae, and
363 other protists. These findings highlight the ecological complexity of protist-associated environments and
364 the inherent challenges of obtaining contamination-free genomic data for target lineages. Despite these
365 challenges, our results demonstrate that single-marker phylogenetic screening, particularly using SSU,
366 provides a reliable and scalable strategy for verifying taxonomic identity and for an exploratory detection
367 of contamination. By improving the taxonomic resolution and reliability of available genome assemblies,
368 this work contributes to downstream evolutionary, ecological, and functional genomic studies of
369 Amoebozoa enabled by the genomic resources available in the P10K database. More broadly, our
370 framework offers a practical and generalizable approach for curating the growing volume of genomic
371 data from protists. As genomic resources for microbial eukaryotes continue to expand, phylogenetically
372 aware data curation efforts, alongside the strategies to minimize contamination and improve data
373 reproducibility discussed here, will be critical to ensuring data accuracy. Accordingly, the P10K project
374 already envisions improving the reliability of its genomic data in future developments by providing, for
375 instance, bioinformatic tools for multiple sequence alignment and phylogenetic analysis, made available
376 through the P10K database. Ultimately, this will maximize the impact of the genomic data available
377 through the P10K initiative in addressing major biological questions, including the origins of complex
378 traits, symbioses, multicellularity, and the diversification of eukaryotic life on Earth.

379 **Availability of data and materials**

380 All compiled and curated SSU and COI datasets associated with this manuscript are publicly available
381 on FigShare at https://doi.org/10.6084/m9.figshare.29814947

382 **Competing interests**

383 The authors declare that they have no competing interests

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Authors' contributions

Alfredo L. Porfirio-Sousa: Conceptualization, Data curation, Visualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Robert E. Jones**: Investigation, Data curation, Writing – review & editing. **Matthew W. Brown:** Investigation, Writing – review & editing. **Daniel Lahr:** Investigation, Writing – review & editing. **Alexander K. Tice:** Conceptualization, Data curation, Visualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Resources, Supervision, Funding acquisition.

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Declaration of generative AI in scientific writing

During the preparation of this work the authors used ChatGPT GPT-4o to improve the readability and language of the first draft of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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References

1. Lewin HA, Robinson GE, Kress WJ, Baker WJ, Coddington J, Crandall KA, et al. Earth BioGenome Project: Sequencing life for the future of life. Proceedings of the National Academy of Sciences. 2018;115:4325–33. https://doi.org/10.1073/pnas.1720115115.

2. Gao X, Chen K, Xiong J, Zou D, Yang F, Ma Y, et al. The P10K database: a data portal for the protist 10 000 genomes project. Nucleic Acids Research. 2024;52:D747–55. https://doi.org/10.1093/nar/gkad992.

3. Brown MW, Tice AK. A genetic toolbox for marine protists. Nat Methods. 2020;17:469–70. https://doi.org/10.1038/s41592-020-0794-z.

4. Schoenle A, Francis O, Archibald JM, Burki F, Vries J de, Dumack K, et al. Protist genomics: key to understanding eukaryotic evolution. Trends in Genetics. 2025;0. https://doi.org/10.1016/j.tig.2025.05.004.

5. Burki F, Roger AJ, Brown MW, Simpson AGB. The New Tree of Eukaryotes. Trends in Ecology & Evolution. 2020;35:43–55. https://doi.org/10.1016/j.tree.2019.08.008.

6. Williamson K, Eme L, Baños H, McCarthy CGP, Susko E, Kamikawa R, et al. A robustly rooted tree of eukaryotes reveals their excavate ancestry. Nature. 2025;640:974–81. https://doi.org/10.1038/s41586-025-08709-5.

7. López-García P, Moreira D. The Syntrophy hypothesis for the origin of eukaryotes revisited. Nat Microbiol. 2020;5:655–67. https://doi.org/10.1038/s41564-020-0710-4.

8. Keeling PJ, Burki F, Wilcox HM, Allam B, Allen EE, Amaral-Zettler LA, et al. The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP): Illuminating the Functional Diversity of Eukaryotic Life in the Oceans through Transcriptome Sequencing. PLOS Biology. 2014;12:e1001889. https://doi.org/10.1371/journal.pbio.1001889.

9. The Darwin Tree of Life Project Consortium. Sequence locally, think globally: The Darwin Tree of Life Project. Proceedings of the National Academy of Sciences. 2022;119:e2115642118. https://doi.org/10.1073/pnas.2115642118.

10. Leebens-Mack JH, Barker MS, Carpenter EJ, Deyholos MK, Gitzendanner MA, Graham SW, et al. One thousand plant transcriptomes and the phylogenomics of green plants. Nature. 2019;574:679–85. https://doi.org/10.1038/s41586-019-1693-2.

11. Lahr DJ. An emerging paradigm for the origin and evolution of shelled amoebae, integrating advances from molecular phylogenetics, morphology and paleontology. Mem Inst Oswaldo Cruz. 2021;116:e200620. https://doi.org/10.1590/0074-02760200620.

12. Ribeiro GM, Lahr DJG. Survival in a Changing World: The role of transcriptomics and the urgent need for genomes to understand Arcellinida's adaptive capabilities. Acta Protozoologica. 2025;2024 Volume 63, Special Issue / Early View.

13. Kang S, Tice AK, Spiegel FW, Silberman JD, Pánek T, Čepička I, et al. Between a Pod and a Hard Test: The Deep Evolution of Amoebae. Molecular Biology and Evolution. 2017;34:2258–70. https://doi.org/10.1093/molbev/msx162.

14. Onsbring H, Tice AK, Barton BT, Brown MW, Ettema TJG. An efficient single-cell transcriptomics workflow for microbial eukaryotes benchmarked on Giardia intestinalis cells. BMC Genomics. 2020;21:448. https://doi.org/10.1186/s12864-020-06858-7.

15. Porfirio-Sousa AL, Tice AK, Morais L, Ribeiro GM, Blandenier Q, Dumack K, et al. Amoebozoan testate amoebae illuminate the diversity of heterotrophs and the complexity of ecosystems throughout geological time. Proceedings of the National Academy of Sciences. 2024;121:e2319628121. https://doi.org/10.1073/pnas.2319628121.

16. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 2015;25:1043–55. https://doi.org/10.1101/gr.186072.114.

17. Astashyn A, Tvedte ES, Sweeney D, Sapojnikov V, Bouk N, Joukov V, et al. Rapid and sensitive detection of genome contamination at scale with FCS-GX. Genome Biol. 2024;25:60. https://doi.org/10.1186/s13059-024-03198-7.

18. Eme L, Sharpe SC, Brown MW, Roger AJ. On the Age of Eukaryotes: Evaluating Evidence from Fossils and Molecular Clocks. Cold Spring Harb Perspect Biol. 2014;6:a016139. https://doi.org/10.1101/cshperspect.a016139.

19. Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, et al. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. Nucleic Acids Research. 2013;41:D597–604. https://doi.org/10.1093/nar/gks1160.

20. Ribeiro GM, Useros F, Dumack K, González-Miguéns R, Siemensma F, Porfírio-Sousa AL, et al. Expansion of the cytochrome C oxidase subunit I database and description of four new lobose testate amoebae species (Amoebozoa; Arcellinida). European Journal of Protistology. 2023;91:126013. https://doi.org/10.1016/j.ejop.2023.126013.

21. Kosakyan A, Gomaa F, Lara E, Lahr DJG. Current and future perspectives on the systematics, taxonomy and nomenclature of testate amoebae. European Journal of Protistology. 2016;55:105–17. https://doi.org/10.1016/j.ejop.2016.02.001.

22. González-Miguéns R, Todorov M, Blandenier Q, Duckert C, Porfirio-Sousa AL, Ribeiro GM, et al. Deconstructing *Difflugia*: The tangled evolution of lobose testate amoebae shells (Amoebozoa: Arcellinida) illustrates the importance of convergent evolution in protist phylogeny. Molecular Phylogenetics and Evolution. 2022;175:107557. https://doi.org/10.1016/j.ympev.2022.107557.

23. Lahr DJG, Kosakyan A, Lara E, Mitchell EAD, Morais L, Porfirio-Sousa AL, et al. Phylogenomics and Morphological Reconstruction of Arcellinida Testate Amoebae Highlight Diversity of Microbial Eukaryotes in the Neoproterozoic. Current Biology. 2019;29:991-1001.e3. https://doi.org/10.1016/j.cub.2019.01.078.

24. Schuler GA, Brown MW. Description of Armaparvus languidus n. gen. n. sp. Confirms Ultrastructural Unity of Cutosea (Amoebozoa, Evosea). Journal of Eukaryotic Microbiology. 2019;66:158–66. https://doi.org/10.1111/jeu.12640.

25. Burki F, Sandin MM, Jamy M. Diversity and ecology of protists revealed by metabarcoding. Current Biology. 2021;31:R1267–80. https://doi.org/10.1016/j.cub.2021.07.066.

26. Kosakyan A. Towards testate amoebae genomics and beyond, a wish list…. Acta Protozoologica. 2025;2024 Volume 63, Special Issue:41–7.

27. Francois CM, Durand F, Figuet E, Galtier N. Prevalence and Implications of Contamination in Public Genomic Resources: A Case Study of 43 Reference Arthropod Assemblies. G3 (Bethesda). 2020;10:721–30. https://doi.org/10.1534/g3.119.400758.

28. Cornet L, Baurain D. Contamination detection in genomic data: more is not enough. Genome Biol. 2022;23:60. https://doi.org/10.1186/s13059-022-02619-9.

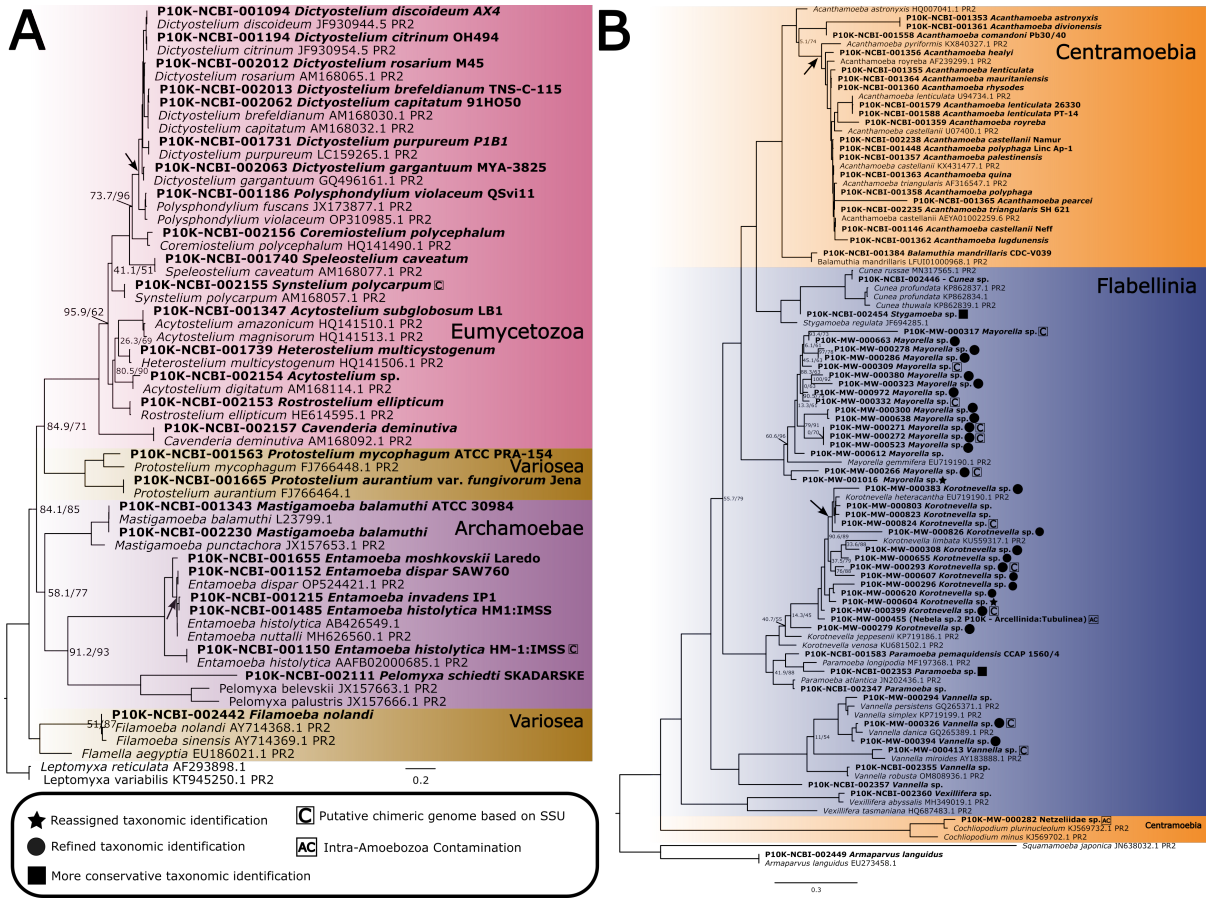
29. Tice AK, Žihala D, Pánek T, Jones RE, Salomaki ED, Nenarokov S, et al. PhyloFisher: A phylogenomic package for resolving eukaryotic relationships. PLOS Biology. 2021;19:e3001365. https://doi.org/10.1371/journal.pbio.3001365.

30. Yang Z, Rannala B. Molecular phylogenetics: principles and practice. Nat Rev Genet. 2012;13:303–14. https://doi.org/10.1038/nrg3186.

31. Emms DM, Kelly S. SHOOT: phylogenetic gene search and ortholog inference. Genome Biology. 2022;23:85. https://doi.org/10.1186/s13059-022-02652-8.

32. Smith SA, Pease JB. Heterogeneous molecular processes among the causes of how sequence similarity scores can fail to recapitulate phylogeny. Brief Bioinform. 2017;18:451–7. https://doi.org/10.1093/bib/bbw034.

504 **Figures**



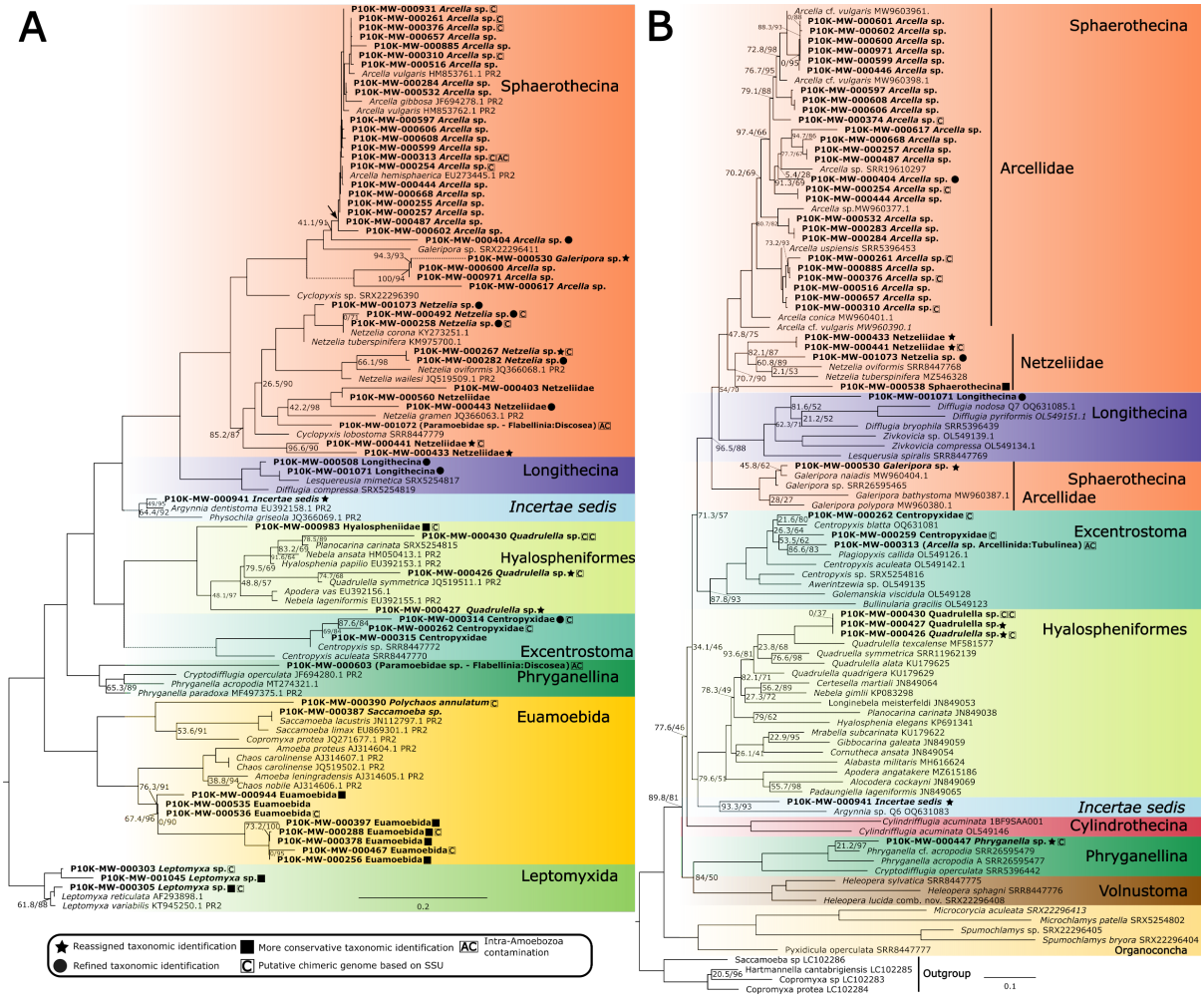
505

506 **Figure 1. The phylogenetic placement of Amoebozoa genomic data from the P10K focused on**
507 **the major groups Evosea and Discosea.** Maximum-likelihood phylogenetic trees constructed from
508 the Small Subunit ribosomal RNA (SSU) inferred from a subset of the curated dataset presented in
509 Figure S1. **A.** Focuses on the major Amoebozoa group Evosea. Phylogenetic reconstruction was
510 conducted using IQ-TREE v2.3.6, with ModelFinder identifying the best-fit substitution model
511 (TIM2+F+R4). **B.** Focuses on the major Amoebozoa group Discosea. Phylogenetic reconstruction was
512 conducted using IQ-TREE v2.3.6, with ModelFinder identifying the best-fit substitution model
513 (GTR+F+G4). Node support was assessed using both ultrafast bootstrap (UFBoot) and the
514 Shimodaira–Hasegawa approximate likelihood ratio test (SH-aLRT). Support values are reported as
515 SH-aLRT / UFBoot, with values ≥80/95 considered indicative of strong support. For clarity, high-support
516 values are omitted in this figure, as well as support values for nodes above the nodes indicated by the
517 arrows, which are represented mostly by flat branches. The complete tree, including all support values,
518 is shown in Figures S2 and S3. Stars indicate genomic data reassigned to a different taxonomic identity
519 than reported in the P10K database. Filled circles mark indicate cases with refined taxonomic
520 resolution. Filled squares denote more conservative identifications (e.g., genus or family level) rather
521 than the more specific genus or species-level identification originally provided in the P10K database.
522 'C' indicates putative chimeric genomes containing sequences from multiple eukaryotes and 'AC'
523 denotes intra-Amoebozoa contamination.

524

525

526



527

528 **Figure 2. The phylogenetic placement of Amoebozoa genomic data from the P10K focused on**
529 **the major group Tubulinea. A.** The maximum-likelihood phylogenetic tree constructed from the Small
530 Subunit ribosomal RNA (SSU) inferred from a subset of the curated dataset presented in Figure S1,
531 focuses on the major Amoebozoa group Tubulinea. Phylogenetic reconstruction was conducted using
532 IQ-TREE v2.3.6, with ModelFinder identifying the best-fit substitution model (TIM3e+G4). The length of
533 branches depicted as dashed lines have been reduced by 50% for presentation purposes. **B.** The
534 maximum-likelihood phylogenetic tree of cytochrome c oxidase subunit I (COI) inferred from a curated
535 dataset generated in the present study, focusing on Arcellinida order (Tubulinea:Amoebozoa)
536 comprising COI sequences retrieved from genomes and transcriptomes available in the P10K
537 database, along with reference sequences made available by previous. Phylogenetic reconstruction
538 was conducted using IQ-TREE v2.3.6, with ModelFinder identifying the best-fit substitution model
539 (GTR+F+I+G4). Node support was assessed using both ultrafast bootstrap (UFBoot) and the
540 Shimodaira–Hasegawa approximate likelihood ratio test (SH-aLRT). Support values are reported as
541 SH-aLRT / UFBoot, with values ≥80/95 considered indicative of strong support. For clarity, high-support
542 values are omitted in this figure, as well as support values for nodes above the one indicated by the
543 arrow, which are represented mostly by flat branches. The complete tree, including all support values,
544 is shown in Figures S4 and S5. Stars indicate genomic data reassigned to a different taxonomic identity
545 than reported in the P10K database. Filled circles mark indicate cases with refined taxonomic
546 resolution. Filled squares denote more conservative identifications (e.g., genus or family level) rather
547 than the more specific genus or species-level identification originally provided in the P10K database.
548 'C' indicates putative chimeric genomes containing sequences from multiple eukaryotes and 'AC'
549 denotes intra-Amoebozoa contamination.

550

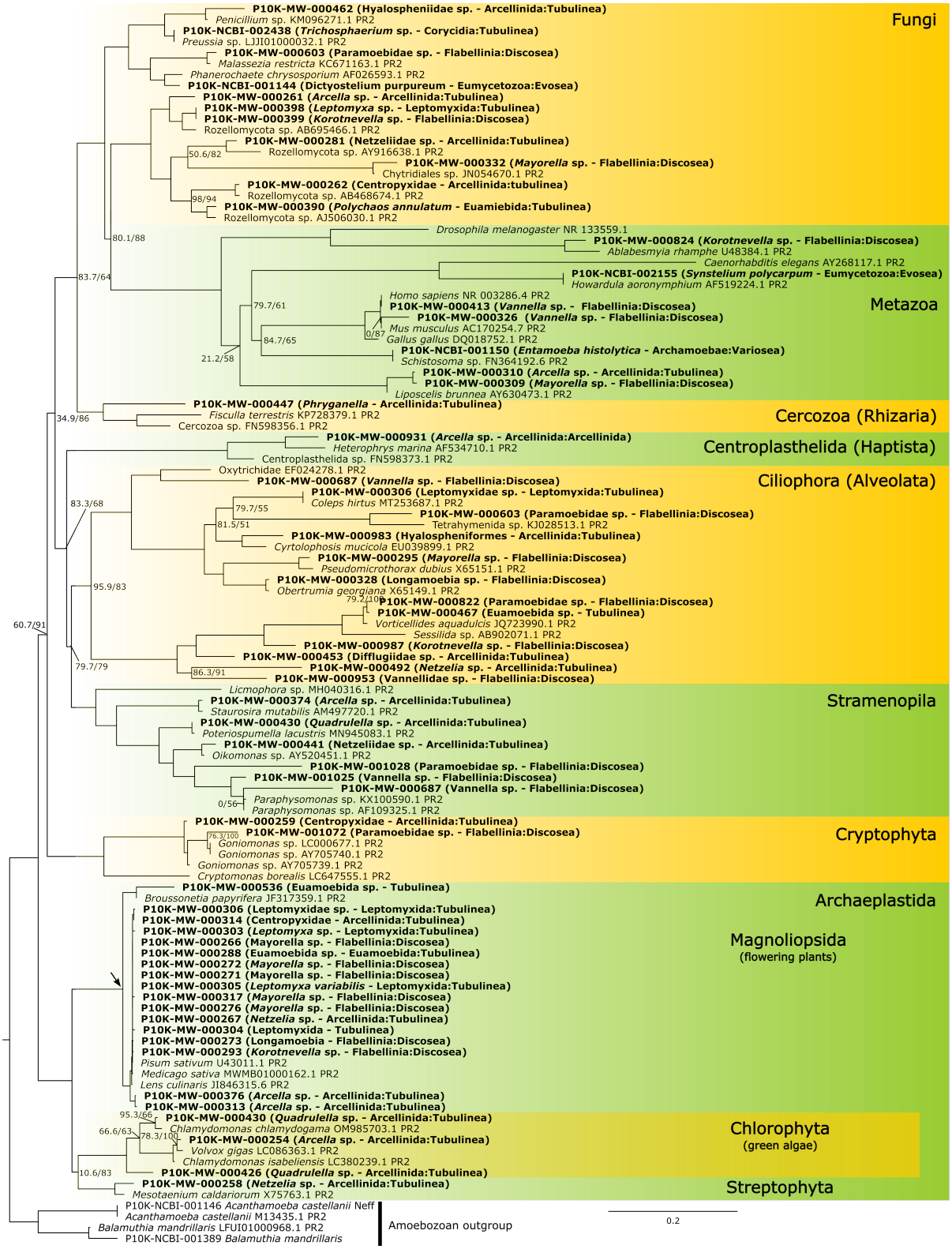


Figure 3. Contamination-screening phylogenetic tree. The maximum-likelihood phylogenetic tree constructed from the Small Subunit ribosomal RNA (SSU), inferred from a curated dataset generated in the present study, comprises SSU sequences of putative non-Amoebozoan contaminants retrieved from genomes and transcriptomes available in the P10K database, along with reference sequences from the PR^2 database (indicated by PR^2 IDs), identified through BLAST+ similarity searches using the putative contaminant SSU sequences as queries. Phylogenetic reconstruction was conducted using IQ-TREE v2.3.6, with ModelFinder identifying the best-fit substitution model (TN+F+I+G4). Node

support was assessed using both ultrafast bootstrap (UFBoot) and the Shimodaira–Hasegawa approximate likelihood ratio test (SH-aLRT). Support values are reported as SH-aLRT / UFBoot, with values ≥80/95 considered indicative of strong support. For clarity, high-support values are omitted in this figure, as well as support values for nodes above the one indicated by the arrow, which are represented mostly by flat branches. The complete tree, including all support values, is shown in Figure S6. The taxonomic identification of Amoebozoa taxa corresponding to the displayed P10K ID codes is shown in parentheses.

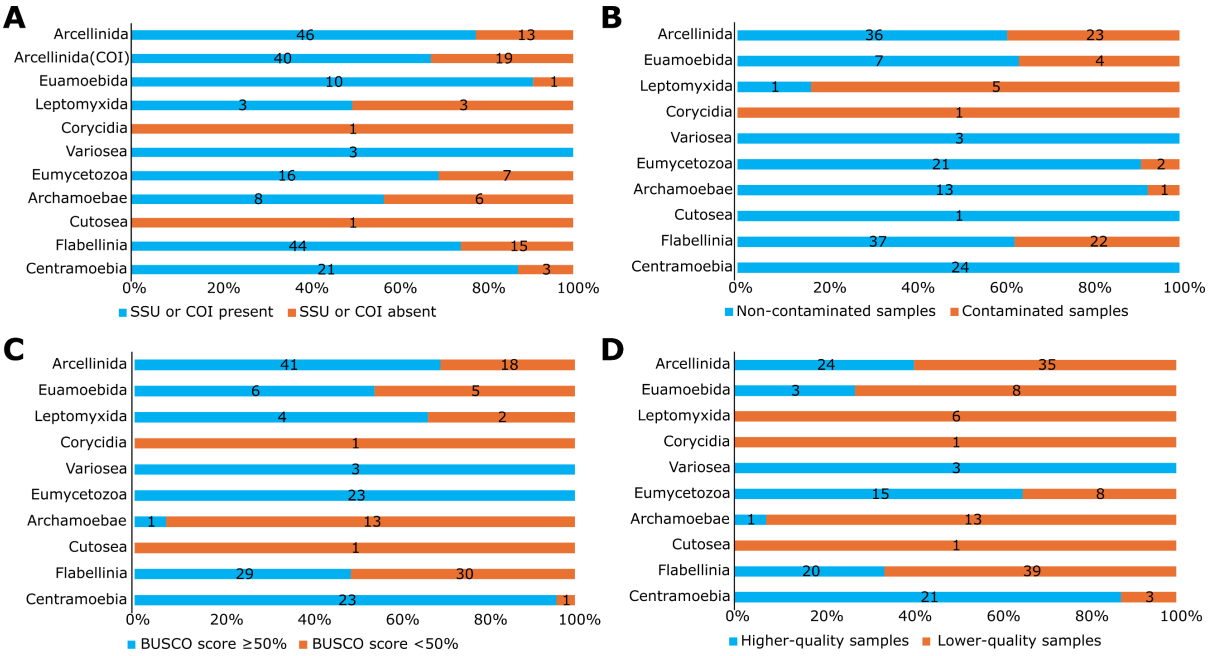
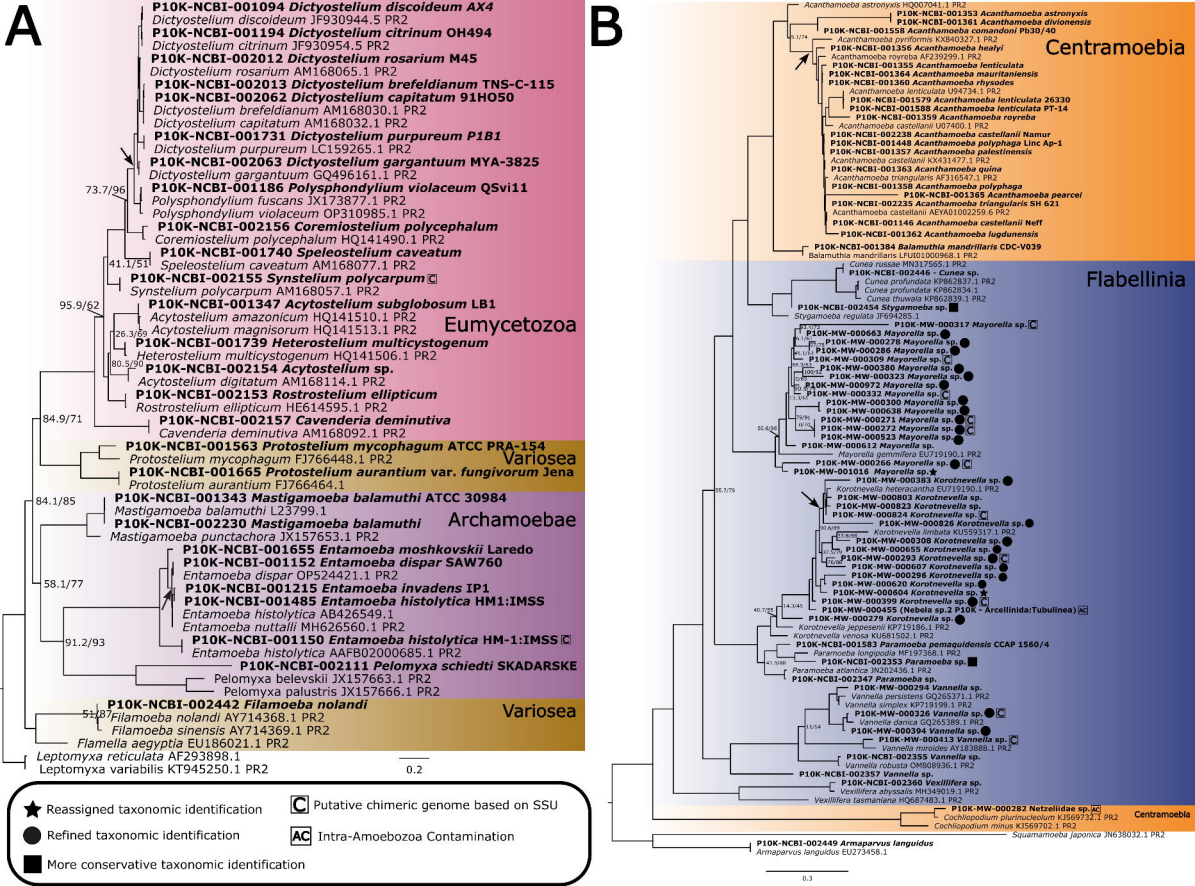


Figure 4. Summary of Amoebozoa P10K database sample counts by taxonomic group, focusing on the key parameters used to evaluate the data. A. presence of SSU or COI. **B.** BUSCO completeness score. We considered the BUSCO score of each sample as originally provided in the P10K database and reported in Gao et al. (2024). **C.** contamination by another eukaryotic lineage. **D.** sample quality assessment. Higher-quality samples are defined as those with SSU (and COI in the case of Arcellinida), a BUSCO score ≥50%, and no contamination identified based on the SSU. Lower-quality samples are those that require decontamination or further sequencing prior to reliable downstream analysis. The bars represent the total number of Amoebozoa samples in the P10K database available for each taxonomic group. The samples considered as higher quality are highlighted in the Supplementary Information – Table S1.


A
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Dictyostelium discoideum JF930944.5 PR2
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Dictyostelium citrinum JF930954.5 PR2
P10K-NCBI-002012 Dictyostelium rosarium M45
Dictyostelium rosarium AM168065.1 PR2
P10K-NCBI-002013 Dictyostelium brefeldianum TNS-C-115
P10K-NCBI-002062 Dictyostelium capitatum 91HO50
Dictyostelium brefeldianum AM168030.1 PR2
Dictyostelium capitatum AM168032.1 PR2
P10K-NCBI-001731 Dictyostelium purpureum P1B1
Dictyostelium purpureum LC159265.1 PR2
P10K-NCBI-002063 Dictyostelium gargantuum MYA-3825
Dictyostelium gargantuum GQ496161.1 PR2
73.7/96
P10K-NCBI-001186 Polysphondylium violaceum QSvi11
Polysphondylium fuscans JX173877.1 PR2
Polysphondylium violaceum OP310985.1 PR2
P10K-NCBI-002156 Coremiostelium polycephalum
Coremiostelium polycephalum HQ141490.1 PR2
41.1/51
P10K-NCBI-001740 Speleostelium caveatum
Speleostelium caveatum AM168077.1 PR2
P10K-NCBI-002155 Synstelium polycarpum
Synstelium polycarpum AM168057.1 PR2
95.9/62
P10K-NCBI-001347 Acytostelium subglobosum LB1
Acytostelium amazonicum HQ141510.1 PR2
Acytostelium magnisorum HQ141513.1 PR2
Eumycetozoa
26.3/69
P10K-NCBI-001739 Heterostelium multicystogenum
Heterostelium multicystogenum HQ141506.1 PR2
80.5/99
P10K-NCBI-002154 Acytostelium sp.
Acytostelium digitatum AM168114.1 PR2
P10K-NCBI-002153 Rostrostelium ellipticum
Rostrostelium ellipticum HE614595.1 PR2
84.9/71
P10K-NCBI-002157 Cavenderia deminutiva
Cavenderia deminutiva AM168092.1 PR2
P10K-NCBI-001563 Protostelium mycophagum ATCC PRA-154
Protostelium mycophagum FJ766448.1 PR2
Variosea
P10K-NCBI-001665 Protostelium aurantium var. fungivorum Jena
Protostelium aurantium FJ766464.1
84.1/85
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Mastigamoeba balamuthi L23799.1
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Mastigamoeba punctachora JX157653.1 PR2
Archamoebae
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Entamoeba dispar OP524421.1 PR2
58.1/77
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Entamoeba histolytica AB426549.1
Entamoeba nuttalli MH626560.1 PR2
91.2/93
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Entamoeba histolytica AAFB02000685.1 PR2
P10K-NCBI-002111 Pelomyxa schiedti SKADARSKE
Pelomyxa belevskii JX157663.1 PR2
Pelomyxa palustris JX157666.1 PR2
P10K-NCBI-002442 Filamoeba nolandi
51/87
Filamoeba nolandi AY714368.1 PR2
Filamoeba sinensis AY714369.1 PR2
Variosea
Flamella aegyptia EU186021.1 PR2
Leptomyxa reticulata AF293898.1
Leptomyxa variabilis KT945250.1 PR2
0.2
B
Acanthamoeba astronyxis HQ007041.1 PR2
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P10K-NCBI-001361 Acanthamoeba divionensis
P10K-NCBI-001558 Acanthamoeba comandoni Pb30/40
Acanthamoeba pyriformis KX603271.1 PR2
Centramoebia
P10K-NCBI-001356 Acanthamoeba healyi
Acanthamoeba royreba AF239299.1 PR2
P10K-NCBI-001355 Acanthamoeba lenticulata
P10K-NCBI-001364 Acanthamoeba mauritaniensis
P10K-NCBI-001360 Acanthamoeba rhysodes
Acanthamoeba lenticulata U94734.1 PR2
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P10K-NCBI-001588 Acanthamoeba lenticulata PT-14
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Acanthamoeba castellanii U07400.1 PR2
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P10K-NCBI-001448 Acanthamoeba polyphaga Linc Ap-1
P10K-NCBI-001357 Acanthamoeba palestinensis
Acanthamoeba castellanii KX431477.1 PR2
P10K-NCBI-001363 Acanthamoeba quina
Acanthamoeba triangularis AF316547.1 PR2
P10K-NCBI-001358 Acanthamoeba polyphaga
P10K-NCBI-001365 Acanthamoeba pearcei
P10K-NCBI-002235 Acanthamoeba triangularis SH 621
Acanthamoeba castellanii AEYA01002259.6 PR2
P10K-NCBI-001146 Acanthamoeba castellanii Neff
P10K-NCBI-001362 Acanthamoeba lugdunensis
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Balamuthia mandrillaris LFU01000968.1 PR2
Cunea russae MN317565.1 PR2
P10K-NCBI-002446 - Cunea sp.
Cunea profundata KP862837.1 PR2
Cunea profundata KP862834.1
Cunea thuwala KP862839.1 PR2
P10K-NCBI-002454 Stygamoeba sp.
Stygamoeba regulata JF694285.1
Flabellinia
P10K-MW-000317 Mayorella sp.
P10K-MW-000663 Mayorella sp.
P10K-MW-000278 Mayorella sp.
P10K-MW-000286 Mayorella sp.
P10K-MW-000309 Mayorella sp.
P10K-MW-000380 Mayorella sp.
P10K-MW-000323 Mayorella sp.
P10K-MW-000972 Mayorella sp.
P10K-MW-000332 Mayorella sp.
P10K-MW-000300 Mayorella sp.
P10K-MW-000638 Mayorella sp.
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Mayorella gemmifera EU719190.1 PR2
P10K-MW-000266 Mayorella sp.
P10K-MW-001016 Mayorella sp.
P10K-MW-000383 Korotnevella sp.
Korotnevella heteracantha EU719190.1 PR2
P10K-MW-000803 Korotnevella sp.
P10K-MW-000823 Korotnevella sp.
P10K-MW-000824 Korotnevella sp.
P10K-MW-000826 Korotnevella sp.
Korotnevella limbata KU559317.1 PR2
P10K-MW-000308 Korotnevella sp.
P10K-MW-000655 Korotnevella sp.
P10K-MW-000293 Korotnevella sp.
P10K-MW-000607 Korotnevella sp.
P10K-MW-000298 Korotnevella sp.
P10K-MW-000620 Korotnevella sp.
P10K-MW-000604 Korotnevella sp.
P10K-MW-000399 Korotnevella sp.
P10K-MW-000455 (Nebela sp.2 P10K - Arcellinida:Tubulinea)
P10K-MW-000279 Korotnevella sp.
Protostelia jeppesenii KP719186.1 PR2
Korotnevella venosa KU681502.1 PR2
P10K-NCBI-001583 Paramoeba pemaquidensis CCAP 1560/4
Paramoeba longipodia MF197368.1 PR2
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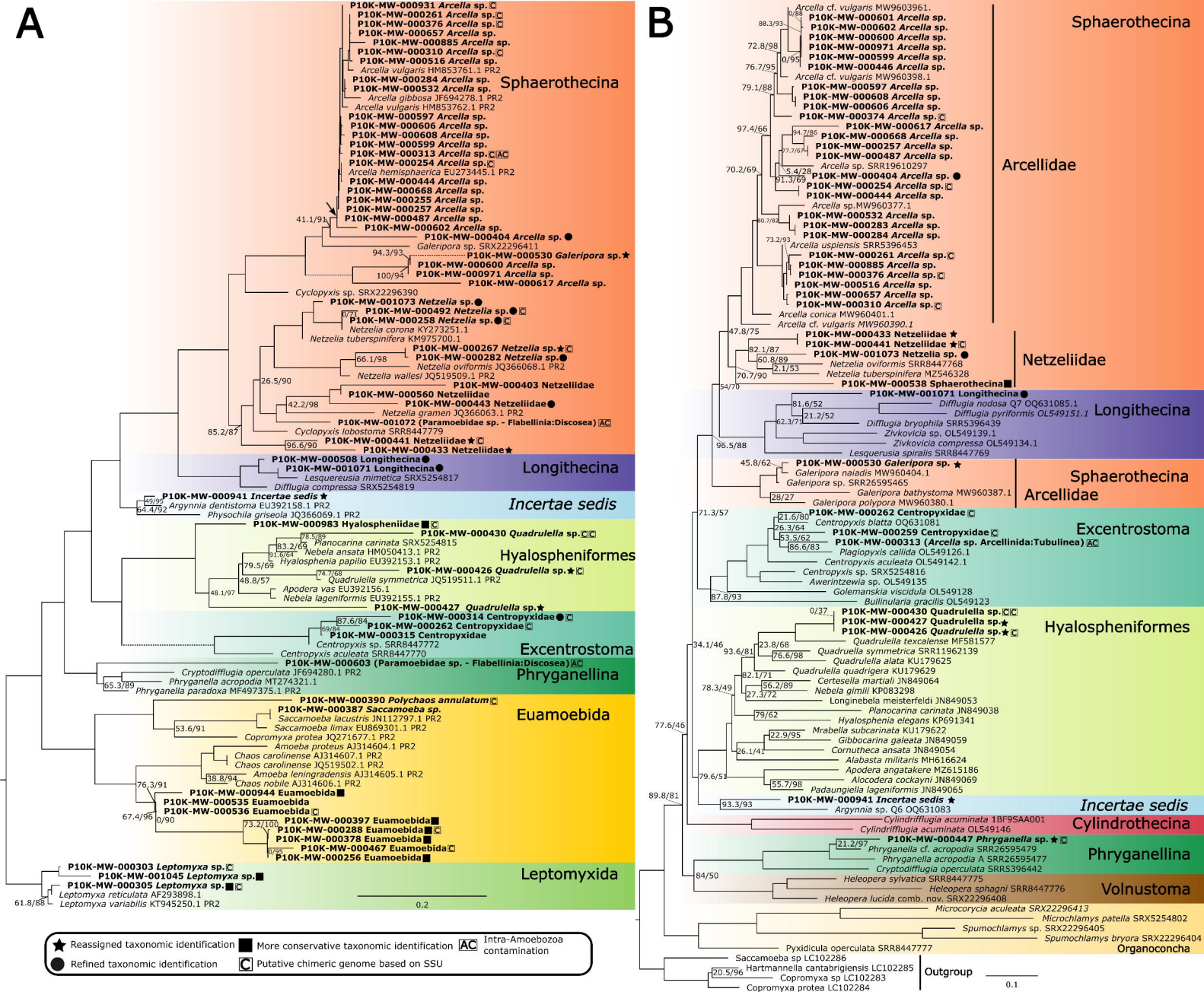
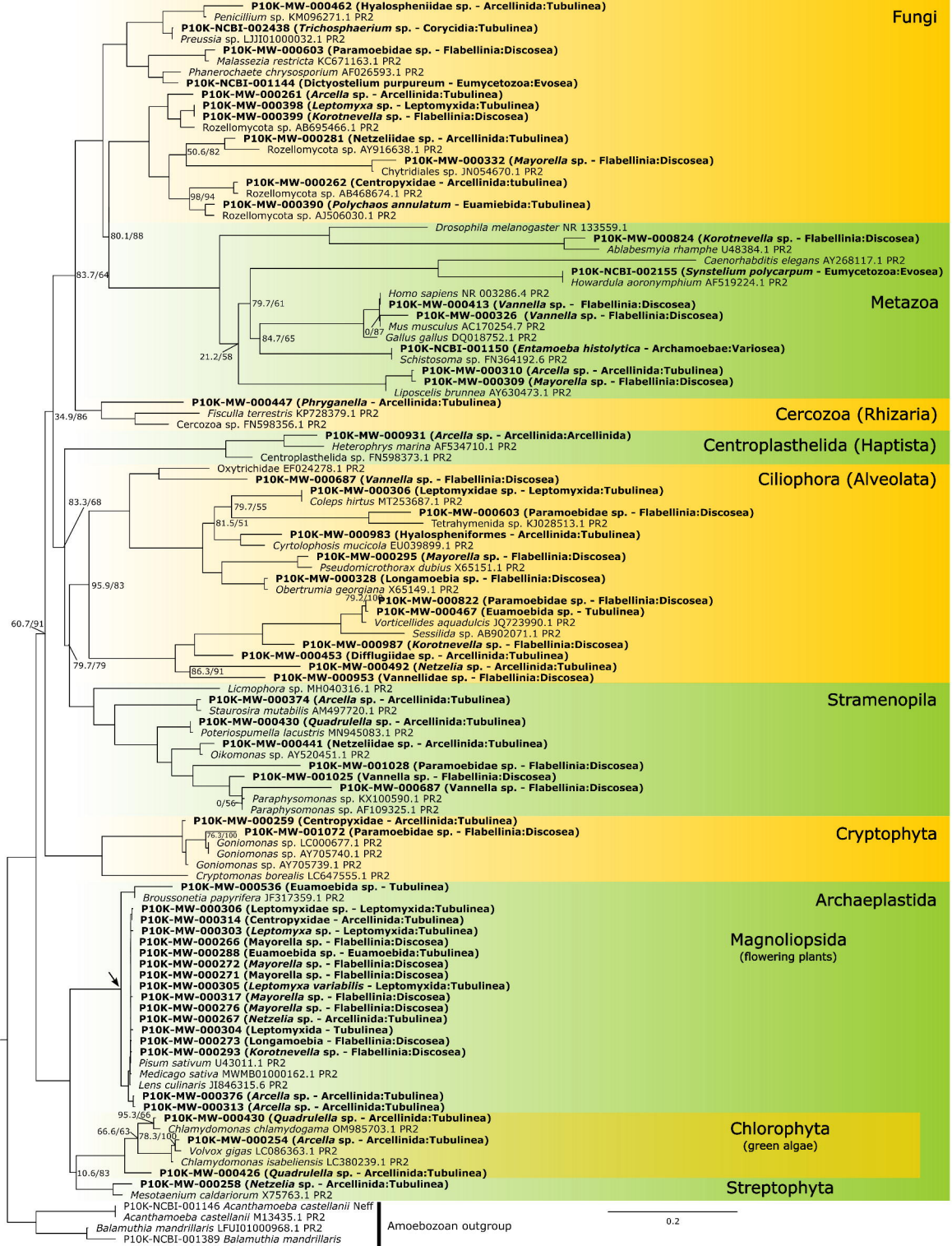
Vannella robusta OM608936.1 PR2
P10K-NCBI-002357 Vannella sp.
P10K-NCBI-002360 Vexillifera sp.
Vexillifera abyssalis MH349019.1 PR2
Vexillifera tasmaniana HQ687483.1 PR2
P10K-MW-000282 Netzeliidae sp.
Centramoebia
Cochliopodium plurinucleolum KJ569732.1 PR2
Cochliopodium minus KJ569702.1 PR2
Squamamoeba japonica JN638032.1 PR2
P10K-NCBI-002449 Armaparvus languidus
Armaparvus languidus EU273458.1
0.3
Reassigned taxonomic identification
Refined taxonomic identification
More conservative taxonomic identification
C Putative chimeric genome based on SSU
AC Intra-Amoebozoa Contamination


A
B
Sphaerothecina
Arcellidae
Netzeliidae
Longithecina
Incertae sedis
Hyalospheniformes
Excentrostoma
Phryganellina
Euamoebida
Leptomyxida
Cylindrothecina
Volnustoma
Organoconcha
Outgroup
Reassigned taxonomic identification
Refined taxonomic identification
More conservative taxonomic identification
Putative chimeric genome based on SSU
Intra-Amoebozoa contamination


Fungi
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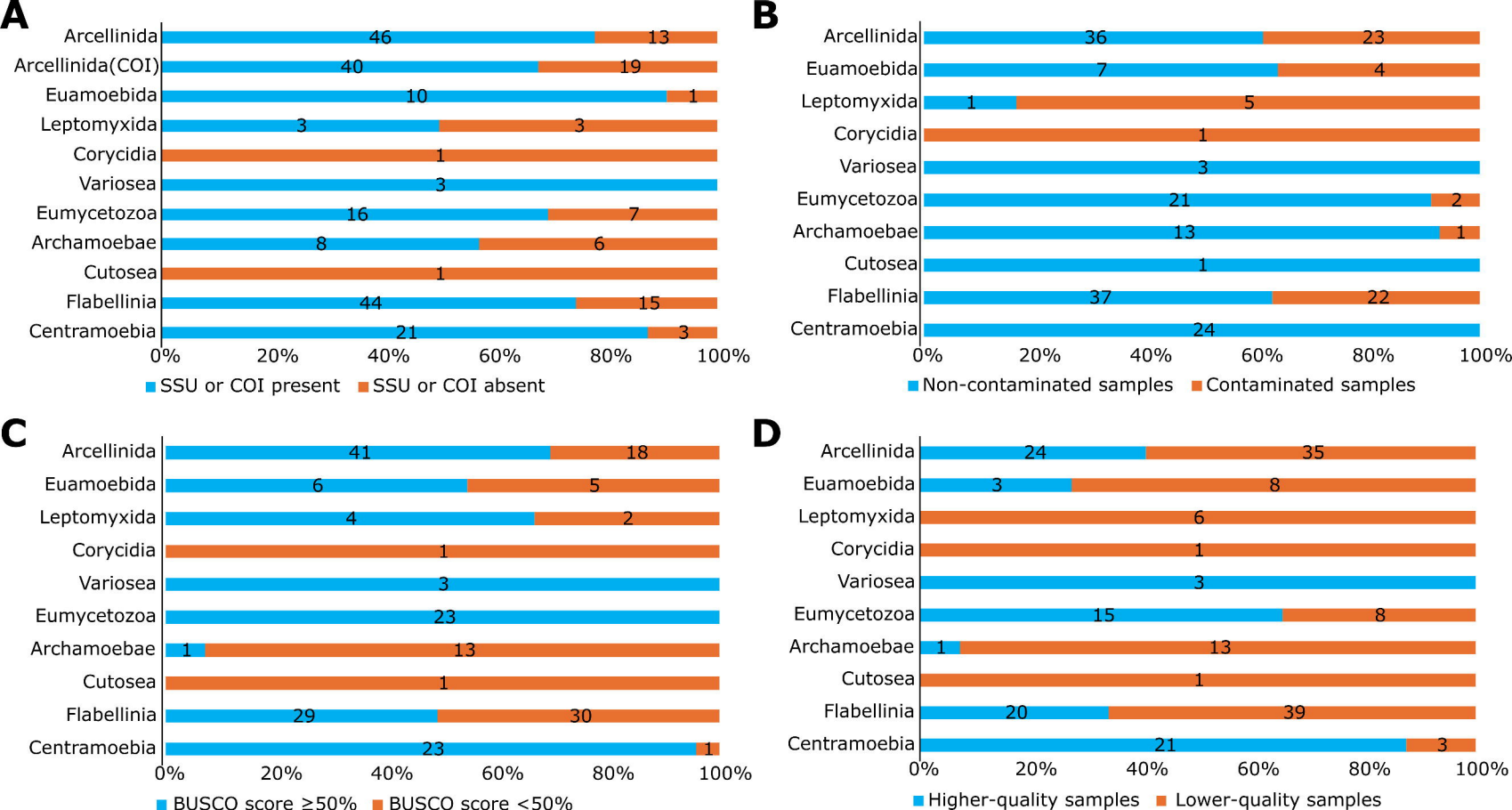
Penicillium sp. KM096271.1 PR2
P10K-NCBI-002438 (Trichosphaerium sp. - Corycidia:Tubulinea)
Preussia sp. LJJI01000032.1 PR2
P10K-MW-000603 (Paramoebidae sp. - Flabellinia:Discosea)
Malassezia restricta KC671163.1 PR2
Phanerochaete chrysosporium AF026593.1 PR2
P10K-NCBI-001144 (Dictyostelium purpureum - Eumycetozoa:Evosea)
P10K-MW-000261 (Arcella sp. - Arcellinida:Tubulinea)
P10K-MW-000398 (Leptomyxa sp. - Leptomyxida:Tubulinea)
P10K-MW-000399 (Korotnevella sp. - Flabellinia:Discosea)
Rozellomycota sp. AB695466.1 PR2
P10K-MW-000281 (Netzeliidae sp. - Arcellinida:Tubulinea)
50.6/82
Rozellomycota sp. AY916638.1 PR2
P10K-MW-000332 (Mayorella sp. - Flabellinia:Discosea)
Chytridiales sp. JN054670.1 PR2
P10K-MW-000262 (Centropyxidae - Arcellinida:tubulinea)
98/94
Rozellomycota sp. AB468674.1 PR2
P10K-MW-000390 (Polychaos annulatum - Euamiebida:Tubulinea)
Rozellomycota sp. AJ506030.1 PR2
80.1/88
Metazoa
Drosophila melanogaster NR 133559.1
P10K-MW-000824 (Korotnevella sp. - Flabellinia:Discosea)
Ablabesmyia rhamphe U48384.1 PR2
Caenorhabditis elegans AY268117.1 PR2
P10K-NCBI-002155 (Synstelium polycarpum - Eumycetozoa:Evosea)
Howardula aoronymphium AF519224.1 PR2
83.7/64
79.7/61
Homo sapiens NR 003286.4 PR2
P10K-MW-000413 (Vannella sp. - Flabellinia:Discosea)
P10K-MW-000326 (Vannella sp. - Flabellinia:Discosea)
0/87
Mus musculus AC170254.7 PR2
Gallus gallus DQ018752.1 PR2
84.7/65
P10K-NCBI-001150 (Entamoeba histolytica - Archamoebae:Variosea)
21.2/58
Schistosoma sp. FN364192.6 PR2
P10K-MW-000310 (Arcella sp. - Arcellinida:Tubulinea)
P10K-MW-000309 (Mayorella sp. - Flabellinia:Discosea)
Liposcelis brunnea AY630473.1 PR2
P10K-MW-000447 (Phryganella - Arcellinida:Tubulinea)
Cercozoa (Rhizaria)
34.9/86
Fisculla terrestris KP728379.1 PR2
Cercozoa sp. FN598356.1 PR2
P10K-MW-000931 (Arcella sp. - Arcellinida:Arcellinida)
Centroplasthelida (Haptista)
Heterophrys marina AF534710.1 PR2
Centroplasthelida sp. FN598373.1 PR2
Ciliophora (Alveolata)
Oxytrichidae EF024278.1 PR2
P10K-MW-000687 (Vannella sp. - Flabellinia:Discosea)
P10K-MW-000306 (Leptomyxidae sp. - Leptomyxida:Tubulinea)
83.3/68
79.7/55
Coleps hirtus MT253687.1 PR2
P10K-MW-000603 (Paramoebidae sp. - Flabellinia:Discosea)
81.5/51
Tetrahymenida sp. KJ028513.1 PR2
P10K-MW-000983 (Hyalospheniformes - Arcellinida:Tubulinea)
Cyrtolophosis mucicola EU039899.1 PR2
P10K-MW-000295 (Mayorella sp. - Flabellinia:Discosea)
Pseudomicrothorax dubius X65151.1 PR2
95.9/83
P10K-MW-000328 (Longamoebia sp. - Flabellinia:Discosea)
Obertrumia georgiana X65149.1 PR2
79.2/100
P10K-MW-000822 (Paramoebidae sp. - Flabellinia:Discosea)
P10K-MW-000467 (Euamoebida sp. - Tubulinea)
60.7/91
Vorticellides aquadulcis JQ723990.1 PR2
Sessilida sp. AB902071.1 PR2
P10K-MW-000987 (Korotnevella sp. - Flabellinia:Discosea)
P10K-MW-000453 (Difflugiidae sp. - Arcellinida:Tubulinea)
79.7/79
P10K-MW-000492 (Netzelia sp. - Arcellinida:Tubulinea)
86.3/91
P10K-MW-000953 (Vannellidae sp. - Flabellinia:Discosea)
Stramenopila
Licmophora sp. MH040316.1 PR2
P10K-MW-000374 (Arcella sp. - Arcellinida:Tubulinea)
Staurosira mutabilis AM497720.1 PR2
P10K-MW-000430 (Quadrulella sp. - Arcellinida:Tubulinea)
Poteriospumella lacustris MN945083.1 PR2
P10K-MW-000441 (Netzeliidae sp. - Arcellinida:Tubulinea)
Oikomonas sp. AY520451.1 PR2
P10K-MW-001028 (Paramoebidae sp. - Flabellinia:Discosea)
P10K-MW-001025 (Vannella sp. - Flabellinia:Discosea)
P10K-MW-000687 (Vannella sp. - Flabellinia:Discosea)
0/56
Paraphysomonas sp. KX100590.1 PR2
Paraphysomonas sp. AF109325.1 PR2
P10K-MW-000259 (Centropyxidae - Arcellinida:Tubulinea)
Cryptophyta
76.3/100
P10K-MW-001072 (Paramoebidae sp. - Flabellinia:Discosea)
Goniomonas sp. LC000677.1 PR2
Goniomonas sp. AY705740.1 PR2
Goniomonas sp. AY705739.1 PR2
Cryptomonas borealis LC647555.1 PR2
Archaeplastida
P10K-MW-000536 (Euamoebida sp. - Tubulinea)
Broussonetia papyrifera JF317359.1 PR2
Magnoliopsida
(flowering plants)
P10K-MW-000306 (Leptomyxidae sp. - Leptomyxida:Tubulinea)
P10K-MW-000314 (Centropyxidae - Arcellinida:Tubulinea)
P10K-MW-000303 (Leptomyxa sp. - Leptomyxida:Tubulinea)
P10K-MW-000266 (Mayorella sp. - Flabellinia:Discosea)
P10K-MW-000288 (Euamoebida sp. - Euamoebida:Tubulinea)
P10K-MW-000272 (Mayorella sp. - Flabellinia:Discosea)
P10K-MW-000271 (Mayorella sp. - Flabellinia:Discosea)
P10K-MW-000305 (Leptomyxa variabilis - Leptomyxida:Tubulinea)
P10K-MW-000317 (Mayorella sp. - Flabellinia:Discosea)
P10K-MW-000276 (Mayorella sp. - Flabellinia:Discosea)
P10K-MW-000267 (Netzelia sp. - Arcellinida:Tubulinea)
P10K-MW-000304 (Leptomyxida - Tubulinea)
P10K-MW-000273 (Longamoebia - Flabellinia:Discosea)
P10K-MW-000293 (Korotnevella sp. - Flabellinia:Discosea)
Pisum sativum U43011.1 PR2
Medicago sativa MWMB01000162.1 PR2
Lens culinaris JI846315.6 PR2
P10K-MW-000376 (Arcella sp. - Arcellinida:Tubulinea)
P10K-MW-000313 (Arcella sp. - Arcellinida:Tubulinea)
95.3/66
P10K-MW-000430 (Quadrulella sp. - Arcellinida:Tubulinea)
Chlorophyta
(green algae)
66.6/63
78.3/100
Chlamydomonas chlamydogama OM985703.1 PR2
P10K-MW-000254 (Arcella sp. - Arcellinida:Tubulinea)
Volvox gigas LC086363.1 PR2
Chlamydomonas isabeliensis LC380239.1 PR2
10.6/83
P10K-MW-000426 (Quadrulella sp. - Arcellinida:Tubulinea)
P10K-MW-000258 (Netzelia sp. - Arcellinida:Tubulinea)
Streptophyta
Mesotaenium caldariorum X75763.1 PR2
P10K-NCBI-001146 Acanthamoeba castellanii Neff
Acanthamoeba castellanii M13435.1 PR2
Balamuthia mandrillaris LFUI01000968.1 PR2
P10K-NCBI-001389 Balamuthia mandrillaris
Amoebozoan outgroup
0.2


A
Arcellinida 46 13
Arcellinida(COI) 40 19
Euamoebida 10 1
Leptomyxida 3 3
Corycidia 1
Variosea 3
Eumycetozoa 16 7
Archamoebae 8 6
Cutosea 1
Flabellinia 44 15
Centramoebia 21 3
0% 20% 40% 60% 80% 100%
SSU or COI present
SSU or COI absent
B
Arcellinida 36 23
Euamoebida 7 4
Leptomyxida 1 5
Corycidia 1
Variosea 3
Eumycetozoa 21 2
Archamoebae 13 1
Cutosea 1
Flabellinia 37 22
Centramoebia 24
0% 20% 40% 60% 80% 100%
Non-contaminated samples
Contaminated samples
C
Arcellinida 41 18
Euamoebida 6 5
Leptomyxida 4 2
Corycidia 1
Variosea 3
Eumycetozoa 23
Archamoebae 1 13
Cutosea 1
Flabellinia 29 30
Centramoebia 23 1
0% 20% 40% 60% 80% 100%
BUSCO score ≥50%
BUSCO score <50%
D
Arcellinida 24 35
Euamoebida 3 8
Leptomyxida 6
Corycidia 1
Variosea 3
Eumycetozoa 15 8
Archamoebae 1 13
Cutosea 1
Flabellinia 20 39
Centramoebia 21 3
0% 20% 40% 60% 80% 100%
Higher-quality samples
Lower-quality samples