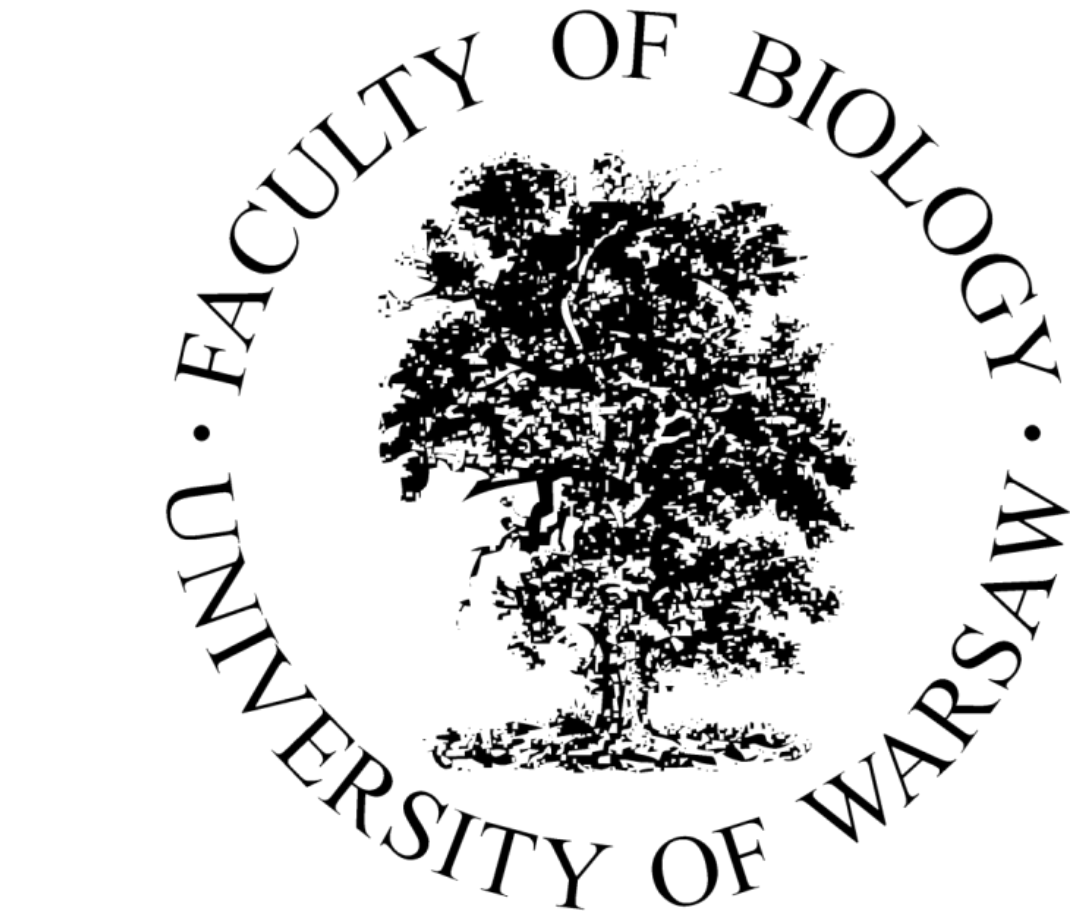


Occurrence of *Prototheca* spp. in aquatic environments in Poland

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INTRODUCTION

Prototheca species are saprophytic, and occasionally pathogenic, unicellular, achlorophyllous algae ubiquitously distributed in nature.

Aquatic niches contaminated with the *Prototheca* algae may provide a source of infection for vertebrates either through direct contact with or traumatic inoculation. The aim of this study was to cross-sectionally examine the occurrence of the *Prototheca* microalgae in water environments in Poland.

METHOD

A total of 362 samples were collected from freshwater and artificial water reservoirs across Poland between October 2018 and March 2020. Liquid and semisolid samples were spread on the *Prototheca* Isolation Medium (PIM) plates, after either concentration through centrifugation and resuspension in sterile water or pre-incubation in liquid PIM. Plates were incubated at 30°C for 2-5 days. Colonies suspected of being *Prototheca* spp., upon macro- and micromorphology observations, were subjected to species-level identification, by using PCR-RFLP and/or sequence analysis of the *cytb* gene. For selected *Prototheca* spp. strains, three rDNA loci (LSU, SSU, and ITS) were PCR-amplified and sequenced. The assimilation profiles of novel species were examined using API®20C AUX system (bioMérieux, France).

RESULTS

Of the samples collected, 51 (14%) yielded *Prototheca* growth with species isolation rates of 33.3%, 23.5%, 13.7%, 9.8%, 5.8%, 3.9%, and 1.9% for *P. wickerhamii*, *P. pringsheimii*, *P. cerasi*, *P. bovis*, *P. ciferrii*, *P. cookei*, and *P. zopfii*, respectively. Upon *cytb*- and rDNA-derived phylogenies, four strains named PK1, PK2, PK6, and W3 were clearly distant from all *Prototheca* species described so far. Their *cytb* gene sequences of those strains showed less than 90.7% similarity to each other and less than 95.4% to all other *Prototheca* species. Morphologically, the four strains were similar to *P. moriformis*, yet capsule was observed only in one strain (W3).

Prevalence of *Prototheca* algae in water samples:

Of 362 samples collected, 299 (82.6%) were liquid and 63 (17.4%) were solid samples. Almost half of the samples originated from 163 natural stagnant water environments (17 lakes, 145 ponds, 1 wetland; 163/362; 45%). The remaining samples were collected either from 14 flowing water bodies (114/362; 31.5%) or from 22 artificial reservoirs (85/362; 23.5%).

Fifty one (14.1%) samples collected from 10 environmental sites in all provinces except Warmia-Masuria yielded growth of *Prototheca* spp. The strains cultured were of both aqueous and solid origin (37 vs. 14 strains). They were recovered most frequently from rivers and streams (40/114 samples or 35.1%; 30 aqueous and 10 solid samples), with only the Vistula River accounting for 23 strains (23/51 or 45.1%; 17; 6). The most *Prototheca*-abundant was the urban Vistula River corridor in Warsaw, from where 19 (37.3%; 19/51) strains were retrieved. Seventeen (33.3%) *Prototheca* strains were isolated from minor rivers in Subcarpathia (Wielopolka, 7), Łódź (Strawa, 3), Masovia (Służewiecki Stream, 2; Narew, 2; Omulew, 1), and Lesser Poland (Foluszowy Stream, 1; Bystra, 1). The prevalence of the algae in stagnant waters of lakes and ponds was 9-fold lower than in flowing waters (8/164 or 4.9%; 4 water and 4 solid samples). Even less frequent was the *Prototheca* isolation from artificial reservoirs (3/84 or 3.6%). The only three strains were isolated from the Żerański canal (2 strains) and a municipal fountain in Warsaw (one strain), both located in central Poland (Masovia).

The overall isolation rate of *Prototheca* spp. from water environments was 14.1% (51/362), with the within-site prevalence ranging from 0% (16 sites) to 100% (2 sites). The highest proportion of *Prototheca*-positive samples was observed in Łódź (3/8 or 37.5%), followed by Lesser Poland (5/23 or 21.7%), Subcarpathia (7/35 or 20%), Warmia-Masuria (2/16 or 12.5%), Masovia (29/253 or 11.5%), and Kuyavia-Pomerania voivodeships (1/10 or 10%). No *Prototheca* spp. were detected only in the utmost south east of Poland (Podlasie).

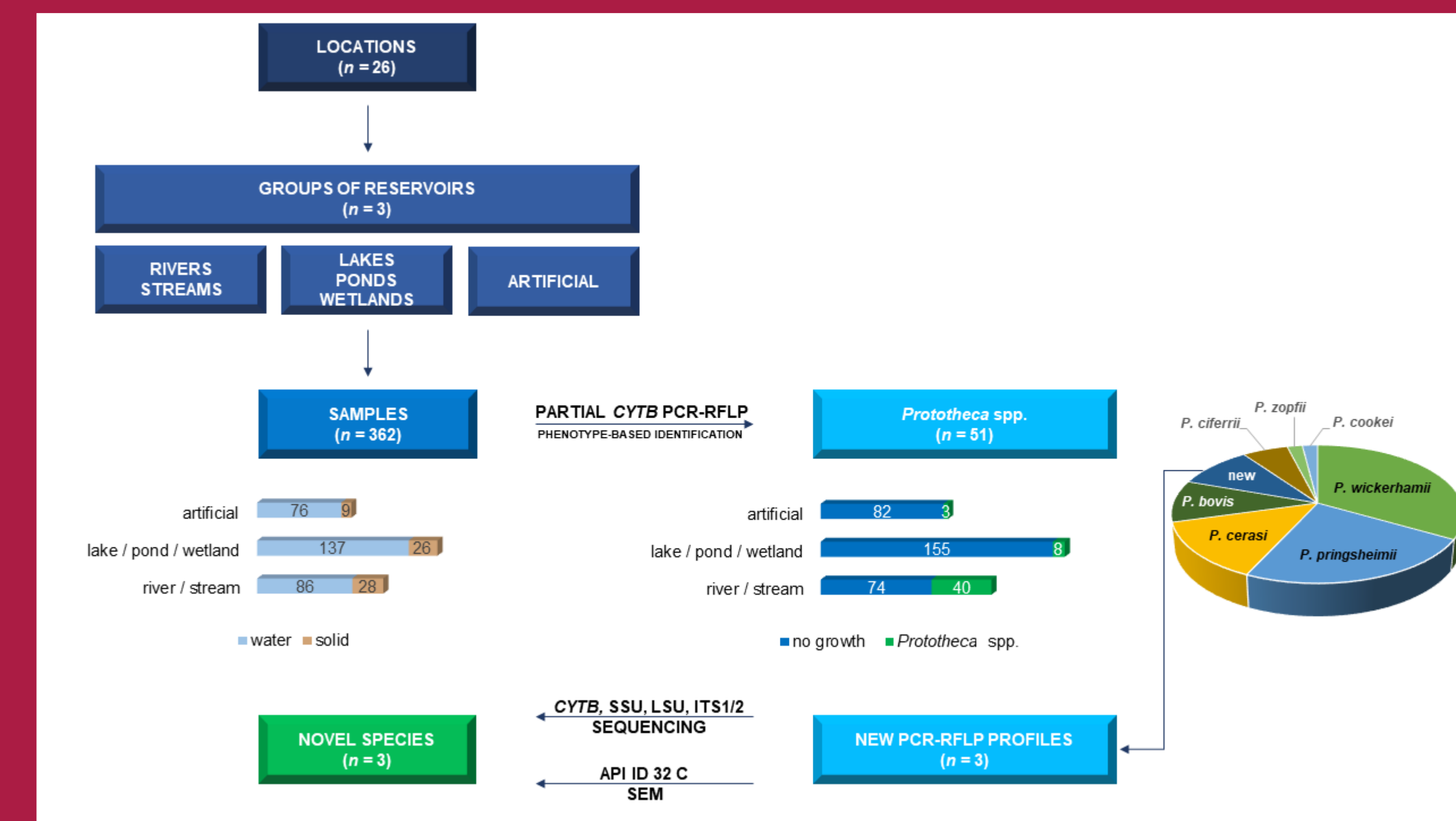


Fig. 1. A flowchart depicting the sampling strategy and identification procedure of cultured *Prototheca* isolates. API ID 32 C – API identification strips for yeasts, Biomerieux; SEM – scanning electron microscopy.

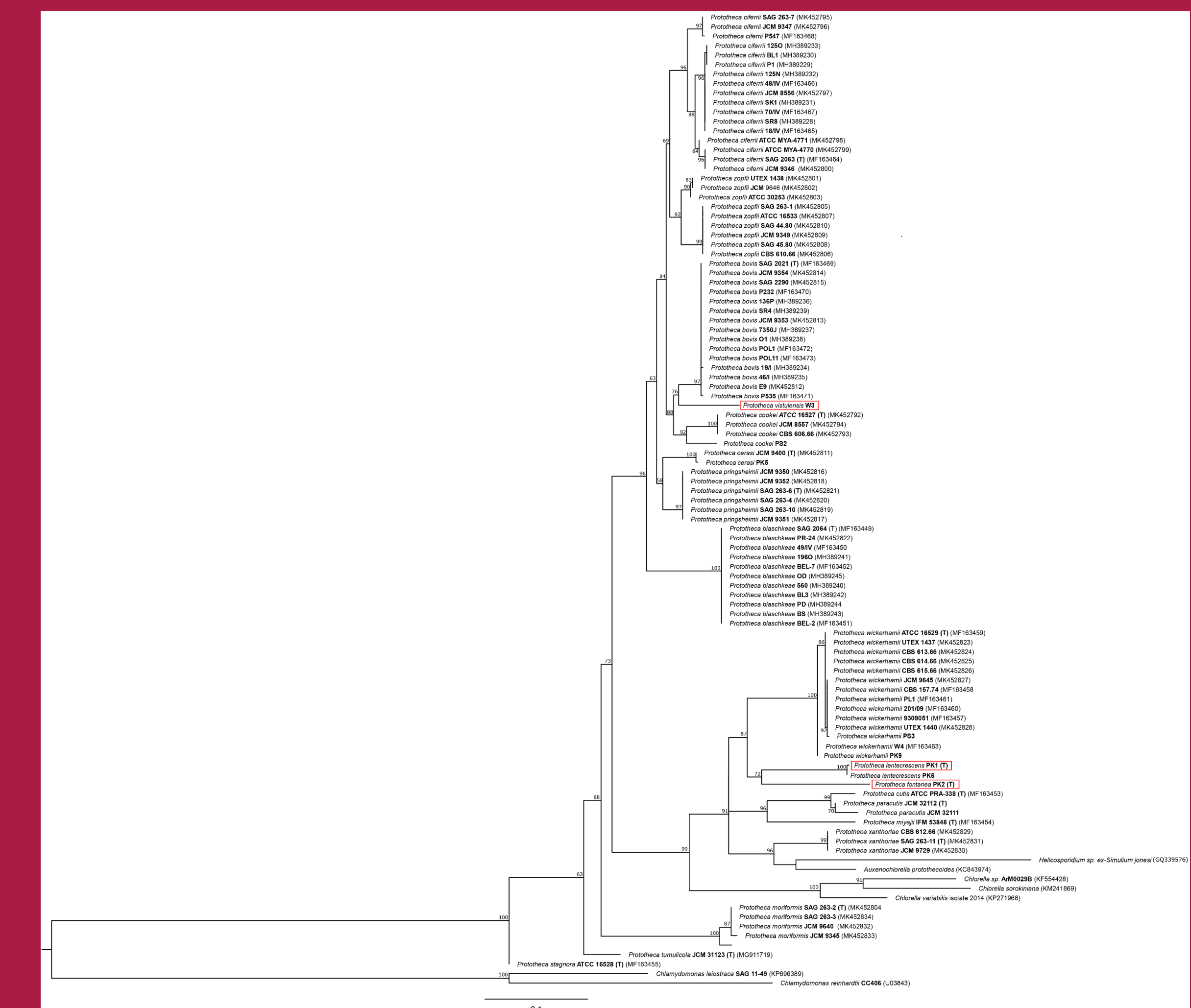


Fig. 5. Phylogenetic tree constructed by maximum likelihood analysis based on CYTB sequences. Numbers at the nodes are ML bootstrap values (bs) above 50%. The phylogram was rooted to *Chlamydomonas leiostraca* (SAG 11-49) and *Chlamydomonas reinhardtii* (CC 406). Scale bar indicates one substitution per 10 nucleotide positions.

CONCLUSIONS

Since the mid-1980s, this study provides the first extensive investigation into the occurrence of *Prototheca* spp. in water sources. A relatively low isolation rate of *Prototheca* algae may be somewhat surprising. It seems inconsistent with a belief of the environmental ubiquity of the algae, that has long lingered in the literature. Still, given a low recovery rate, the species diversity of the *Protothecae* can be considered high.