ARPHA Conference Abstracts 4: e64932
doi: 10.3897/aca.4.e64932

Conference Abstract

Development of specific markers for the detection of *Tolypella canadensis* eDNA in water samples

Christina Wiebe‡, Petra Nowak‡, Hendrik Schubert‡

‡ Rostock University, Rostock, Germany

Corresponding author: Christina Wiebe (christina.wiebe@uni-rostock.de)

Received: 24 Feb 2021 | Published: 04 Mar 2021

Citation: Wiebe C, Nowak P, Schubert H (2021) Development of specific markers for the detection of *Tolypella canadensis* eDNA in water samples. ARPHA Conference Abstracts 4: e64932. https://doi.org/10.3897/aca.4.e64932

Abstract

Assessing the biodiversity of an ecosystem plays a major role in ecosystem management. However, proper determination on species-level is often tricky when morphological features are scarce and especially rare species require huge sampling efforts to be detected in the aquatic realm. As an alternative to conventional methods, environmental samples can be examined via the eDNA method, allowing for large-scale integration as well as taxa resolution independent from expression of morphological characters. However, to apply this technique genetic markers that are specific to a species or at least a genus are required. Such markers until now have been successfully developed only for a few well studied taxonomic groups like, e.g., fishes and amphibians, but are still missing for others, especially plants and algae (e.g. Bista et al. 2017).

This project focusses on the development of species-specific markers for the macrophytic green algae *Tolypella canadensis* (Characeae, Charophyta), a rare alga preferring deep water and known so far mainly from remote places. *Tolypella canadensis* is a circumpolar species and prefers oligotrophic lakes, where it grows in depths up to 13 m (Langangen 2002; Romanov and Kopyrina 2016). In addition, proper determination of Tolypella-species is a field of a few specialists, further complicating monitoring or even detection of this rare species.

The design of the species-specific primers was based on reference nucleotide sequences of the chloroplast genes *rbc*L, *psb*C and *atp*B and of the ribosomal internal transcribed spacer regions ITS1 and ITS2, obtained from GenBank (Perez et al. 2017). To determine the specificity of the newly designed primers, DNA isolates obtained from *T. canadensis* specimens collected from the Torneträsk (Sweden, 2018) and other charophyte species were prepared in different proportions. The sensitivity of the primers was experimentally assayed by using serial dilutions of *T. canadensis* DNA. Additionally, a mock test comprised of a sample with the DNA of several charophyte species was conducted and finally, the markers were tested on environmental samples from the Torneträsk.

Tolypella canadensis-specific primers of the ITS2 region yielded positive PCR amplifications of one single band when *T. canadensis* was present in a sample. Cross-amplification was not found during the mock test; other charophyte species did not yield positive amplification. The eDNA samples from the Torneträsk validated the performance of the ITS2 marker.

The *T. canadensis*-specific marker designed in this project was proven to be sensitive and accurate. It could be recommended as a useful tool to detect the presence of *T. canadensis* DNA, even at low concentration and in complex samples containing other charophyte species.

Keywords

Tolypella canadensis, Characeae, eDNA, genetic marker, ITS2

Presenting author

Christina Wiebe

Presented at

1st DNAQUA International Conference (March 9-11, 2021)

References

- Bista I, Carvalho GR, Walsh K, Seymour M, Hajibabaei M, Lallias D, Christmas M, Creer S (2017) Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. Nature communications 8: 14087. https://doi.org/10.1038/ncomms14087
- Langangen A (2002) *Tolypella canadensis* Sawa (Charales), a charophyte new to the flora of Russia, with remarks on its ecology and distribution. J. Nat. Mus., Nat. Hist. Ser. 171: 132-175.

- Perez W, Casanova M, Hall J, McCourt R, et al. (2017) Phylogenetic congruence of ribosomal operon and plastid gene sequences for the Characeae with an emphasis on *Tolypella* (Characeae, Charophyceae). Phycologia 2: 230-237.
- Romanov R, Kopyrina L (2016) *Tolypella canadensis* (Charales, Charophyceae) in Asia: final evidence of its circumpolar distribution. Nova Hedw. (3)423-427.