

SPECIES ANALYSIS OF ULVA SP. BASED ON CLASSICAL AND MOLECULAR TAXONOMY

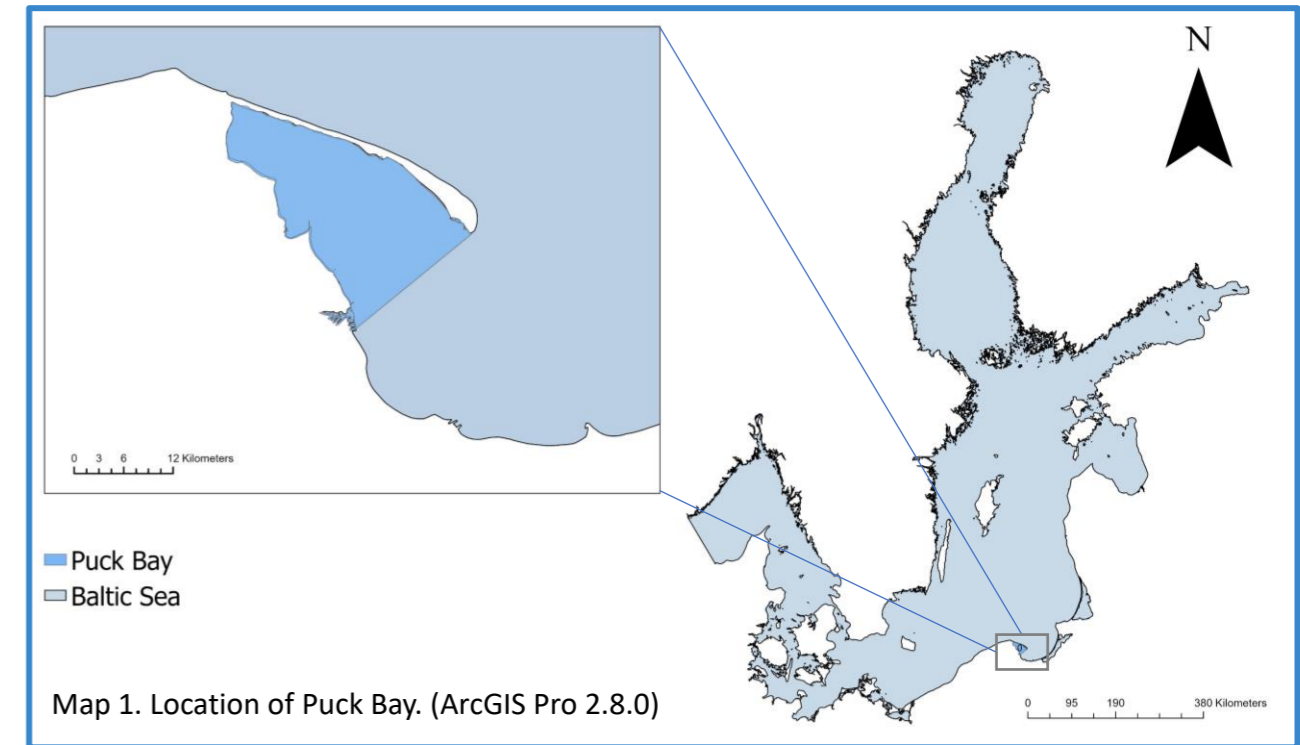
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INTRODUCTION

Puck Bay is characterized by a large diversity of environmental conditions. This is a region potentially rich in representatives of the genus *Ulva*. However, identification at the species level is usually difficult due to the multitude of observed morphological forms. Therefore, as part of the work, selected representatives of *Ulva intestinalis* Linnaeus and *Ulva prolifera* O.F.Müller were characterized. The methods of classical taxonomy were based on the analysis of morphological features, while molecular taxonomy on the identification of appropriate barcodes. A key element of the work was testing effectiveness of universal primers applied for particular loci of the genus *Ulva* from Puck Bay.



METHODOLOGY

STEP 1. COLLECTION OF TAXONS

Research material in the form of thallus *Ulva* spp. collected in August-September 2021 in the coastal zone of Puck Bay.

STEP 2. MORPHOLOGICAL IDENTIFICATION

Cleaned thalli were identified using a Nikon light microscope Eclipse E400 under 40-100x magnification. Photographic documentation was made using Delta Optical DLT-Cam PRO 20 MP USB cameras 3.0. Selected photos are shown in Fig. 1-6.

STEP 3. MOLECULAR IDENTIFICATION

- Genetic material was isolated from the frozen material by precipitation. Based on literature data, loci (*tufA*, *rbcL*, *28S*, *5S*, *rps2*) with appropriate discriminatory power were selected.
- The primers used for the study were selected based on the literature, i.e.: *tufGF4* x *tufAR* (Saunders and Kucera, 2010; Fama et al., 2002), *GrbcIFi* x *1385R/RBCL2* (Saunders and Kucera, 2010; Manhart, 1994), *28s_F(LSU-D2/D3)/T16N* x *28s_R(LSU-D2/D3)/T24U* (Saunders and Kucera, 2010; Harper and Saunders, 2001), *5S-F* x *5S-R* (Shimada et al., 2008), *rps2-trnL-F1* x *rps2-trnL-R1* (Liu et al., 2020)
- The isolated DNA was sent to Genomed's external laboratory to perform direct sequencing of selected DNA fragments.
- The sequences were compared with the NCBI database. Phylogenetic trees were made and clades were determined for the selected loci. Selected trees are shown in Figure 7-8.

RESULTS

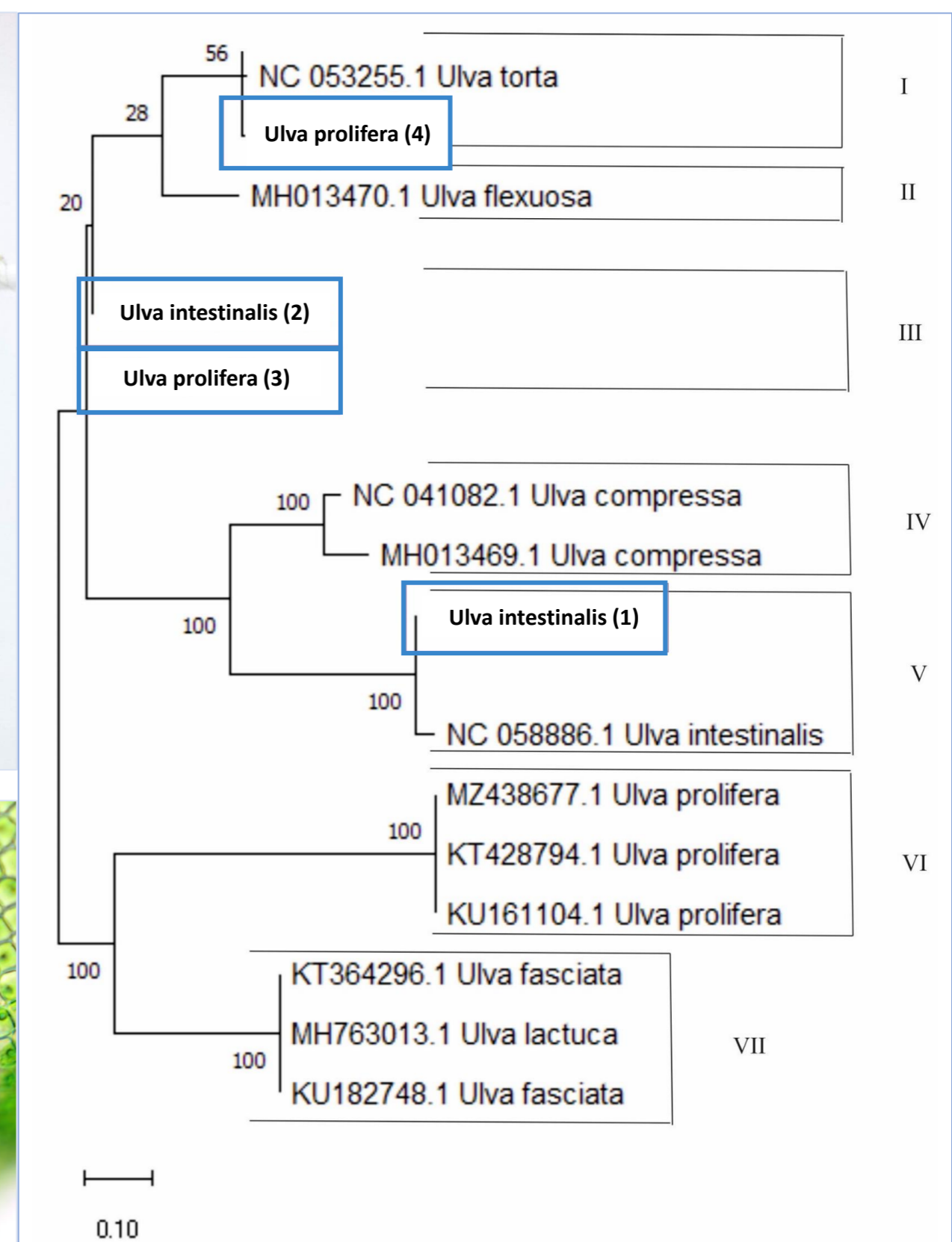


Fig. 7. Phylogenetic tree based on model Tamura-Nei for locus *rps2* for genus *Ulva*; Clades: I - *c. torta*, II - *c. flexuosa*, III - *c. intestinalis/prolifera*, IV - *c. compressa*, V - *intestinalis*, VI - *prolifera*, VII - *fasciata/lactuca* (Mega 11).

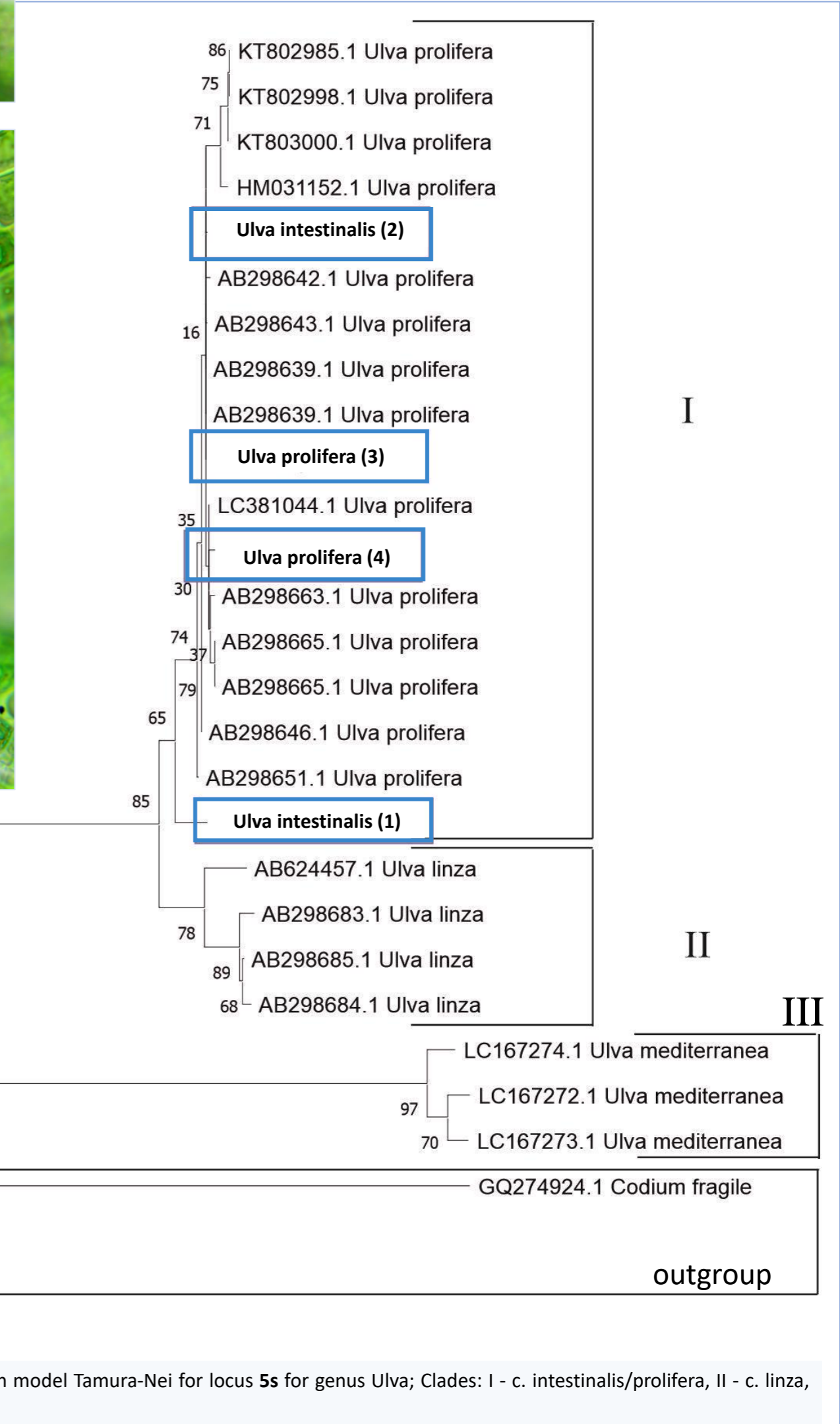
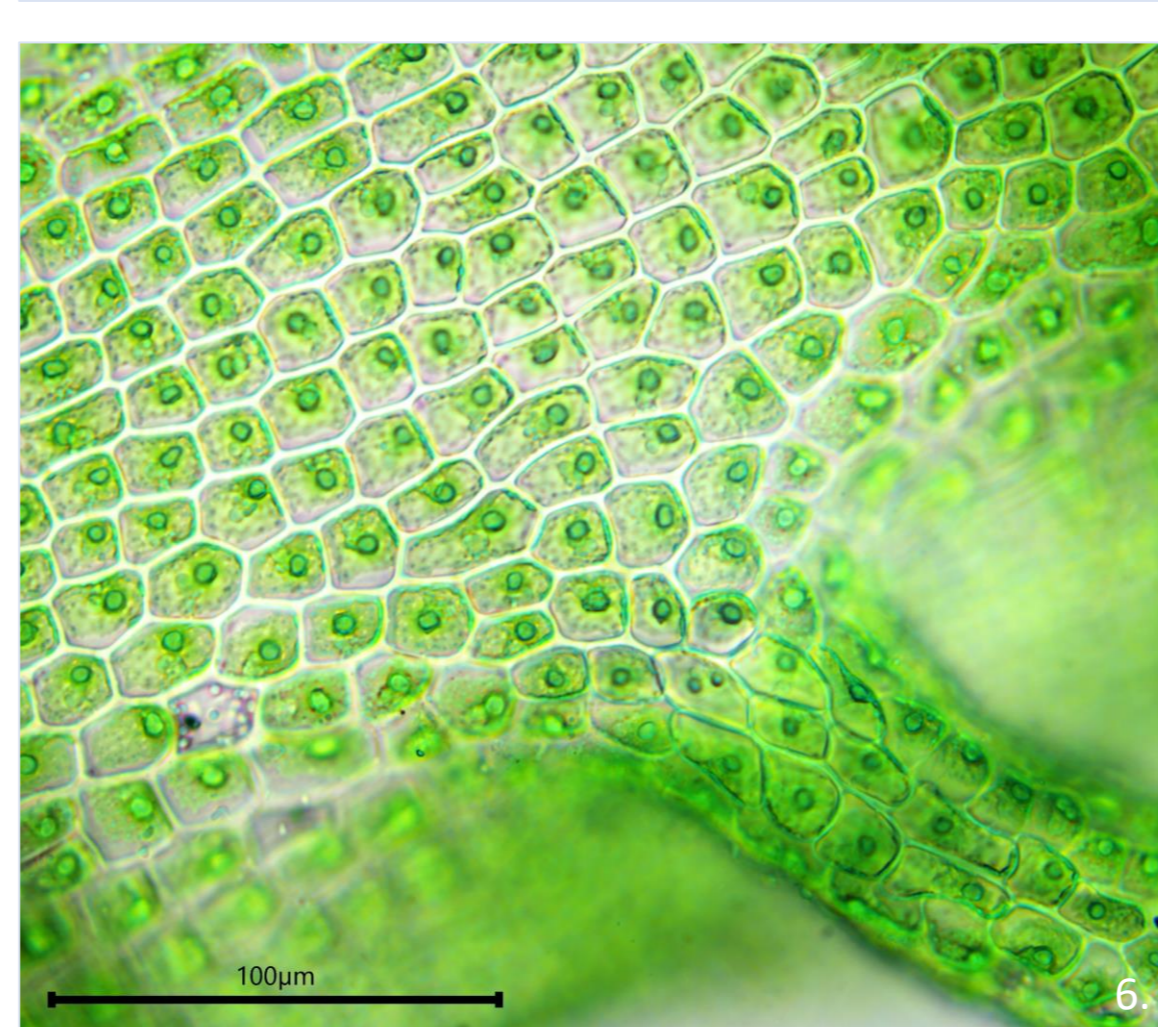
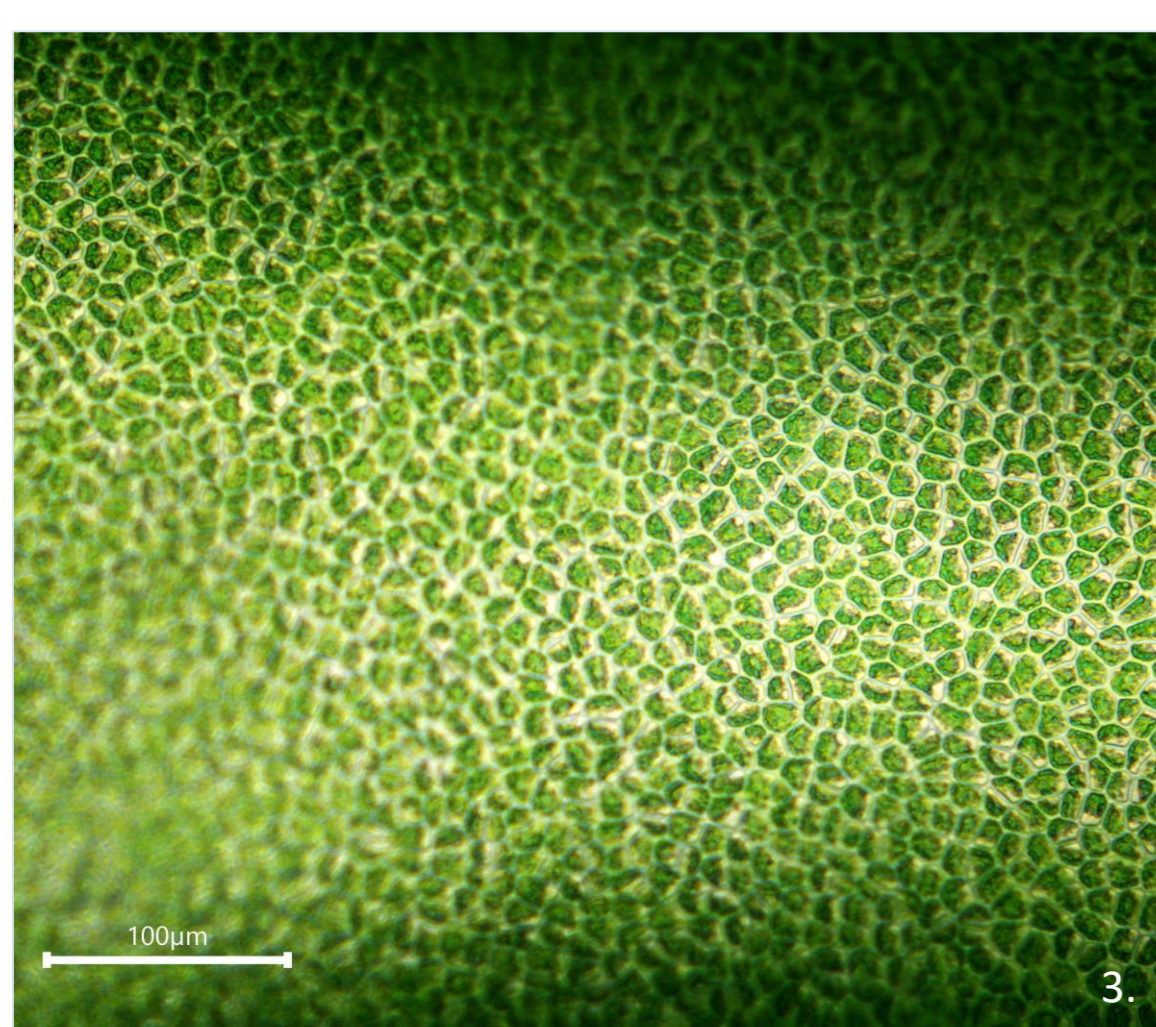
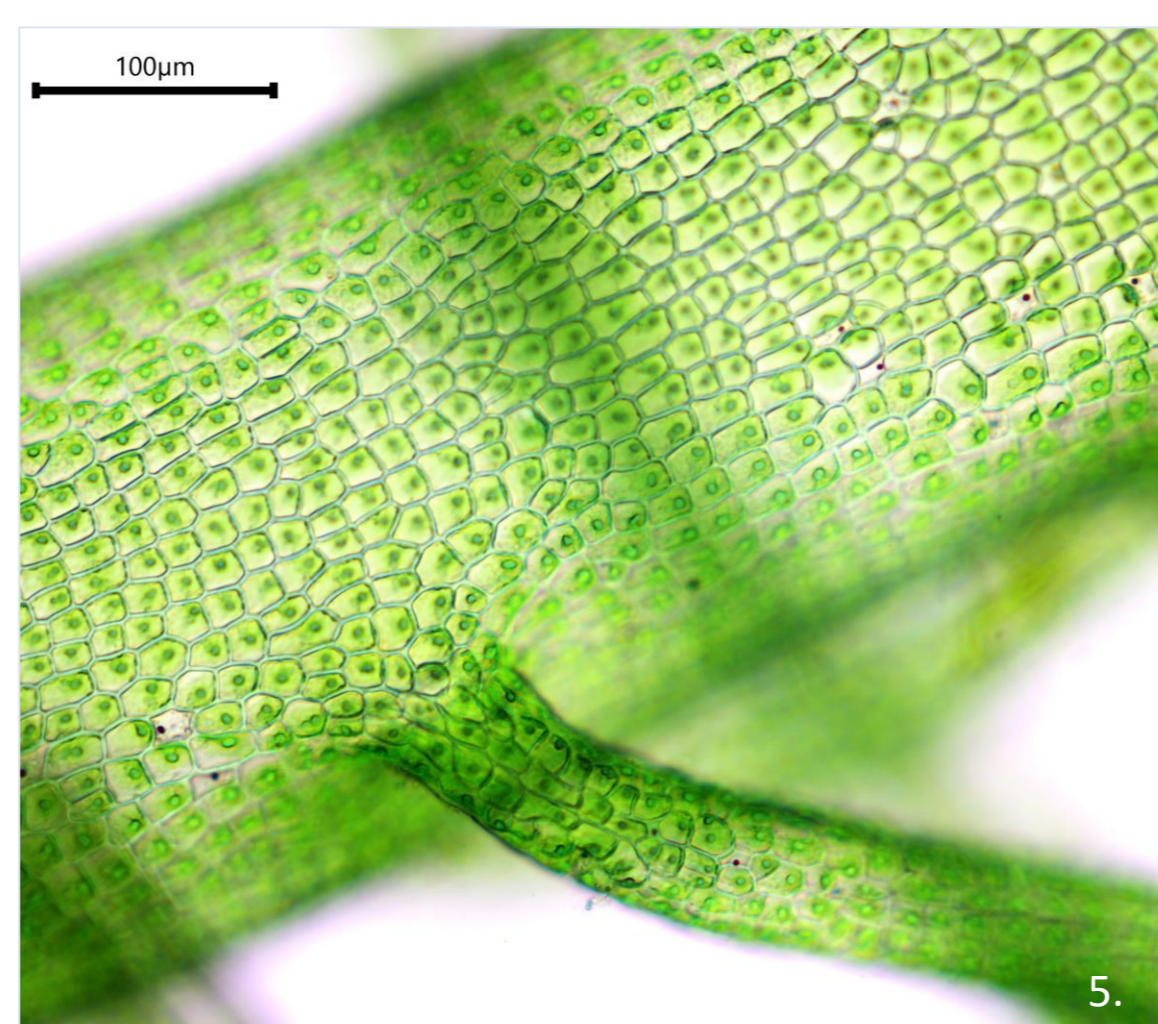


Fig. 8. Phylogenetic tree based on model Tamura-Nei for locus *5s* for genus *Ulva*; Clades: I - *c. intestinalis/prolifera*, II - *c. linza*, III - *c. mediterranea* (Mega 11).

Figure 1-3. *Ulva intestinalis* Linnaeus: 1 - typical tubular, strongly wrinkled thalli of various widths, characterized by the lack of branches; 2 - thallus enlarged x 40 with characteristic "folds"; 3 - irregularly arranged polymorphic cells with one pyrenoid at x 400 magnification; (Photos: W.Chudzik)

Figure 4-6. *Ulva prolifera* O.F.Müller: 4 - typical tubular narrow thallus with numerous proliferations; 5 - thallus enlarged x 40 with characteristic proliferations; 6 - usually regular rectangular cells with one pyrenoid arranged in rows at x 400 magnification; (Photos: W.Chudzik)

CONCLUSIONS

- Based on the analyzes carried out, it was found that for the Baltic *Ulva* spp. the best barcodes for direct taxonomic identification, ranked according to the discriminant power, are **5s>rps2>tufA>28s>rbcL**.
- The limiting factor in the case of molecular taxonomy may be the lack of a sufficient number of deposited sequences in the database.
- Recently introduced barcoding sites, such as *rps2* (Liu et al., 2020) strengthened the discriminatory power.
- Both methods of identification - morphological and genetic - complement each other, usage of both methods increased credibility

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