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# Revision of herbarium specimens of freshwater *Enteromorpha*-like *Ulva* (Ulvaceae, Chlorophyta) collected from Central Europe during the years 1849–1959

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## Abstract

This paper presents results concerning the taxonomic revision of *Ulva* taxa originating from herbarium specimens dating back to 1849–1959. A staining and softening mixture was applied to allow a detailed morphometric analysis of thalli and cells and to detect many morphological details. The study focused on individuals collected exclusively from inland water ecosystems (having no contact with sea water). Detailed analysis concerned the following items: structures of thalli and cells, number of pyrenoids per cell, configuration of cells inside the thallus, occurrence or not of branching thallus, shapes of apical cells, and shape of chromatophores. The objective of the study was to confirm the initial identifications of specimens of *Ulva* held in herbaria. The study sought to determine whether saltwater species of the genus *Ulva*, e.g., *Ulva compressa* and *U. intestinalis*, could have been found in European freshwater ecosystems in 19th and 20th centuries. Moreover, the paper presents a method for the initial treatment of voucher specimens for viewing stained cellular structures that are extremely vulnerable to damage (the oldest specimens were more than 160 years old). The use of a glycerine-based softening mixture allowed the detailed analysis of the anatomic structure of cells as well as the determination of the number and size of pyrenoids, i.e., some of the key taxonomic features of *Ulva* taxa. Most of specimens were identified as belonging to the species *U. flexuosa*. Marine species such as *Ulva intestinalis* or *U. compressa* were not confirmed to occur in the inland water ecosystems of Central Europe in the 19th and 20th centuries.

**Key words:** green algae; macroalgae; softening solution; Ulvales

## Introduction

*Ulva* Linnaeus (1753: 1163) (syn. *Enteromorpha* Link, 1820: 5) (Chlorophyta, Ulvophyceae, Ulvales) is a genus of green macroalgae widespread in multiple aquatic environments (Wolf *et al*. 2012). Thalli are very different in shape and structure. The genus *Ulva* comprehends species with blade-like thalli, generally flat, corrugated at the edges and composed of two layers of cells (*Ulva* morphology, e.g., *U. lactuca* Linnaeus (1753: 1163) and species with tubular thallus, intestine-like, composed of one layer of cells, showing a hollow centre, simple or branched (*Enteromorpha* morphology, e.g., *U. prolifera* O.F. Müller (1778: 7) (Starmach 1972). Today, the list of accepted taxa of the genus *Ulva* contains 132 items with other 455 taxa awaiting taxonomic validation (Guiry & Guiry 2015).

Several *Ulva* species are cosmopolitan, living in the littoral zone of most marine ecosystems, including estuaries (Fletcher 1996, Bäck *et al*. 2000). Species of *Ulva* exhibit broad tolerances to a range of abiotic factors including water salinity, nutrients concentration, and temperature (Poole & Raven 1997). The ability of *Ulva* species to live in extreme environmental conditions results in the presence of this genus of macroalgae as the most frequent group of plants on rocks and other substrates in most coastal areas (Bäck *et al*. 2000). *Ulva* taxa are epilithic on natural (e.g., rocks, shells and wood) to anthropogenic (e.g., breakwaters and ship hulls) substrates (Chapman 1956). These macroalgae can form underwater meadows that are diverse in structure and species composition and usually occurring at a depth of more than 2 m (Adams 1994, McAvoy & Klug 2005). Many *Ulva* species thrive in high nutrient conditions, and their abundance in an area may indicate high concentrations of nitrate, ammonium, and phosphate in water. Some species can also be used as bioindicators for heavy metal contamination (Rybak *et al*. 2012a, 2012b, 2013). *Ulva* species are often involved in macroalgae blooms ("green tide") in coastal areas. Eutrophication of water has been identified as the main factor controlling *Ulva* 'green tide' events (Perrot *et al*. 2014). This opportunistic macroalgal genus is therefore

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