DNA SEQUENCING OF LINNAEUS'S *ULVA COMPRESSA*, *U. INTESTINALIS*, AND *U. LINZA* (ULVACEAE, CHLOROPHYTA) AND OTHER *ULVA* TYPE SPECIMENS[1]

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Abstract

The names published by Linnaeus in *Species Plantarum* represent the foundation of modern plant taxonomy. Despite their systematic value however, very few of Linnaeus's original type specimens have been analyzed using modern DNA sequencing technologies. Here we performed high-throughput sequencing on Linnean and other type specimens of the sea lettuce genus *Ulva*, a group of ecological and commercial importance. Chloroplast and mitochondrial genomes were assembled for Linnaeus's *U. compressa*, *U. intestinalis*, *U. lanceolata* and *U. linza* as well as Kützing's *Phycoseris smaragdina* type specimens. Phylogenetic analyses of these data showed that the names *U. compressa* and *U. intestinalis* have been correctly applied, but *U. linza* and *U. lanceolata* were misapplied. *Ulva linza* is the earliest available name for the European species currently called *U. pseudocurvata*. The correct name for the globally distributed species previously known as *U. 'linza'* is *Ulva smaragdina* (Kützing) comb. nov. The names *Ulva lanceolata, U. crispata* and *Phycoseris olivacea* do not represent distinct species, instead being heterotypic synonyms of *U. compressa*, and *P. planifolia* is a heterotypic synonym of *U. intestinalis*. These results demonstrate that genetic characterization of type material can unequivocally resolve longstanding taxonomic debates over scientific names.

KEYWORDS: ITS, mitochondrial genome, plastid genome, *rbc*L, *tuf*A, *Ulva crispata, Ulva lanceolata, Ulva smaragdina* comb. nov.

Abbreviations: HTS, high-throughput sequencing; ITS, internal transcribed spacer; PCR, polymerase chain reaction; tufA, translation elongation factor EF-Tu

INTRODUCTION

The most valuable specimens in herbaria include type specimens, the original specimens on which species names are based (Turland et al., 2025). The current specimen-based voucher system was codified in the Cambridge rules (Merrill, 1930) to start with the publication of Linnaeus's *Species Plantarum* (Linnaeus, 1753), the foundation of modern botanical nomenclature. Linnaeus provided 5,940 binomials of plants, fungi and algae from around the world. Since then, hundreds of thousands of new species have been proposed, all of them scaffolded upon Linnaeus's original work. Despite their importance, very few of Linnaeus's specimens have been sequenced (Andreasen et al., 2014; Chomicki & Renner, 2015; Hughey et al., 2019; Salojärvi et al., 2024). The challenge faced by the many taxonomists who followed Linnaeus has been to determine accurately his intent based on very brief descriptions, often only two or three words, and very little accurate habitat or geographical information. The description for the green seaweed species *Ulva intestinalis* (Linnaeus, 1753: 1163), for example, is simply ~~stated~~ "*tubulosa simplex*" or simple tube, and the habitat was "*mari omni*", every sea. This created and continues to create taxonomic uncertainty, leading to the misapplication of species names. For example, a recent genetic analysis of the Linnean type specimen of *Ulva lactuca* (Linnaeus, 1753: 1163), the generitype, showed that it was a warm water species not found in northern European waters, where it has been incorrectly reported for more than 250 years (Hughey et al., 2019). *Ulva* specimens previously assigned to *U. lactuca* were actually *U. fenestrata* (Postels & Ruprecht, 1840: 21, Pl. XXXVII), based on material from the North Pacific Ocean. Hughey et al. (2019) were able to correct the misapplication of the name because the type specimens had been carefully maintained in herbaria for hundreds of years.

Ulva species, previously split into two genera, *Ulva* and *Enteromorpha* (Hayden et al., 2003), are blades that are two cells thick or tubes one cell thick. Both show a high level of

phenotypic plasticity, compounding the difficulty of identifying species based on morphology. Beyond being able to identify species in the field or lab, the misapplication of names is a serious issue since *Ulva* is commercially important as well as playing significant ecological and environmental roles (Bruhn et al., 2011; Peter et al., 2024). Species of *Ulva* are globally important in marine ecosystems and food webs, and are increasingly used as bioindicators, animal feed, and human food (Hofmann et al., 2024). Maintaining accurate taxonomy, grounded in type specimens, is therefore potentially critical to science, conservation, and industrial use.

Of the nine species of *Ulva* named by Linnaeus in *Species Plantarum*, four are currently classified in the genus: *U. compressa* (Linnaeus, 1753: 1163), *U. intestinalis*, *U. lactuca*, and *U. linza* (Linnaeus, 1753: 1163). Despite detailed morpho-anatomical studies and the DNA sequencing of thousands of contemporary specimens worldwide, only *U. lactuca* has been sequenced, whilst the molecular identities of the type specimens of *U. compressa*, *U. intestinalis*, and *U. linza* are unknown. The only objective method to determine the correct application of these *Ulva* names is to sequence their type specimens as has been demonstrated repeatedly in the past eight years (Hanyuda and Kawai, 2018, Hughey et al. (2018, 2019, 2021a, b, 2024), Hughey and Gabrielson, 2022; Maggs et al., 2024). We, therefore, performed high-throughput sequencing (HTS) on the epitype specimens of *U. compressa*, *U. intestinalis* and *U. linza* (Figure 1) from Oxford University Herbaria (OXF; herbarium acronyms follow Index Herbariorum online [Thiers, 2025]). In addition, to test the taxonomic conclusions of previous workers, we sequenced type specimens of heterotypic synonyms of these species from OXF and Leiden (L): the epitype specimen (Figure 1c) of *U. lanceolata* (Linnaeus, 1767: 719), the lectotype specimen (Figure 2c) of *Phycoseris smaragdina* (Kützing, 1843: 297), and the holotype specimens (Figure

2) of *Phycoseris olivacea* (Kützing, 1843: 297), *Phycoseris planifolia* (Kützing, 1843: 297) and *Ulva crispata* (Bertoloni, 1810: 63).

MATERIALS AND METHODS

DNA Extraction, Genome Sequencing and Assemblies

DNA from the *Ulva* and *Phycoseris* type specimens was extracted using approximately 5 x 5 mm samples of algal thalli following established protocols (Hughey et al., 2021; Lindstrom et al., 2011) and strict precautionary guidelines for working with herbarium specimens (Hughey et al., 2012). The 150 bp paired-end libraries of the type specimens of *Ulva compressa, U. intestinalis, U. lanceolata, U. linza,* and *Phycoseris smaragdina* were constructed with the KAPA HyperPrep Kit (Roche, Basel, Switzerland) according to the manufacturer's instructions and sequenced on an Illumina NovaSeq X Plus (San Diego, California, USA). The resulting reads were filtered using the default BBDuk settings, trimmed on the right and left ends, then saved with a minimum length of 36 bp in Geneious Prime 2019.1.3 (Biomatters Limited). The organellar genomes from each type specimen were assembled with the map to reference function in Geneious Prime using the Low Sensitivity / Fastest setting with the filtered reads and a contigs file generated from a *de novo* assembly using MEGAHIT 1.2.9 with kmers 39–141 bp (Li et al., 2015). The references were as follows: *U. compressa* (GenBank accession numbers MK069586 and MT916929), *U. intestinalis* (GenBank accession numbers MZ571476 and PQ777150), *U. 'gigantea'* (GenBank accession numbers KU179356 and MT179350), and *U. 'linza'* (GenBank accession numbers KU189740 and KX058323). '' represents species that are incorrectly identified. The gaps in the

organellar genomes were closed by iteration using the Medium – Low Sensitivity / Fast or Low Sensitivity / Fastest settings with the map to reference function in Geneious Prime. The accuracy of the final complete or partial organellar genome assemblies were confirmed using the map to reference function in Geneious Prime. Draft genome annotations were performed using the default settings in GeSeq 2.03 designating the GenBank accession numbers above as reference genomes (Tillich et al., 2017). Organellar genome annotations were finalized with manual adjustments to start and stop gene positions according to NCBI ORFfinder and Sequin 15.5 (Benson et al., 2018).

PCR of *tuf*A, *rbc*L, and ITS 1 markers

PCR amplifications of DNA from the type specimens of *Phycoseris olivacea*, *P. planifolia*, *P. smaragdina,* and *Ulva crispata* were performed on a MJ Research Minicycler PTC-150 with a heated lid using the parameters: 94ºC for 3 min, followed by 40 cycles of 94ºC for 30 s, 50ºC for 45 s, and 72ºC for 60 s, and a final extension of 72ºC for 7 min. The reactions were carried out in 25 µl volumes containing 12.5 µl Amplitaq Gold 360 Master Mix (ThermoFisher Scientific), 5 µl Q-solution (Qiagen), 1 µl each of forward and reverse primer solutions in 33 µM concentrations (Integrated DNA Technologies), 0.5 µl of TopTaq DNA Polymerase (Qiagen), and 5 µl of undiluted DNA from the above DNA extractions. Four primer pairs were used, two *rbc*L primer pairs UlvaInnerrbcLFor with UlvaOuterrbcL-Rev primers (Hughey et al., 2021) and UlvaMiddle with UlvaMiddle-Rev, one *tuf*A primer pair 704tufA-For with 822tufA-Rev, and one ITS1 primer pair UlvaITS1-For with UlvaITS1-Rev (Maggs et al., 2024). Amplified

products were Sanger sequenced by Functional Biosciences, Inc. Forward and reverse sequencing reads were manually assembled and the primers removed manually.

Phylogenetic and phylogenomic analyses

Representative *Ulva tuf*A, *rbc*L, ITS and 5S marker sequences were downloaded from GenBank (Table S1) and aligned with the type specimen sequences using MAFFT v7.450 (Katoh et al., 2002; 2013) with the E-INS-i algorithm,1PAM/k=2 scoring matrix, and a 2 gap open penalty. The chloroplast and mitochondrial genome sequences were downloaded from GenBank (Table S2) and aligned with type sequences using the auto algorithm setting with the same options above in MAFFT. Phylogenetic analyses of the marker sequences were performed online (http://iqtree.cibiv.univie.ac.at/) with the auto substitution model using ModelFinder (Kalyaanamoorthy et al., 2017), 1000 ultrafast bootstrap replicates (Hoang et al., 2018), and the approximate bayes test (abayes) in W-IQ-TREE 3.0.1 (Minh et al., 2020; Nguyen et al., 2015; Trifinopoulos et al., 2016; Wong et al., 2025). Model selections were as follows: *tuf*A and *rbc*L (TIM3+F+I+G4), ITS (TIM+F+I+G4), and 5S (GTR+F+I+G4). Phylogenomic analyses were executed by cmd using IQ-TREE with the settings cited above. Model selections were as follows: chloroplast (GTR+F+I+G4) and mitochondrial (TIM3+F+G4). The trees were visualized with TreeDyn 198.3 at Phylogeny.fr (Dereeper et al., 2008) using outgroups from the Ulvaceae: *Percursaria 'percursa'*, *Ulvaria 'obscura var. blyttii'* and *Umbraulva 'japonica'* for the marker phylogenies and midpoint rooted for the phylogenomic inferences.

Microscopy

The *Ulva compressa* and *U. 'prolifera'* specimens were viewed on Wild dissection and Leitz compound microscopes and photographed through the oculars with an Olympus Tough TG7. The *'U. prolifera'* specimen was removed with tweezers and heated to 70ºC in a domestic detergent for 1 minute and then mounted in 50% Karo solution, infilled with 100% Karo.

RESULTS

Botanists misunderstood Linnaeus's *Ulva linza* for centuries

Organellar genome assemblies obtained from the epitype of *U. linza* (Table S3) did not match sequences identified as *U. linza* in any of the public nucleotide sequence databases. *Ulva 'linza'* was a well-known seaweed easily recognized by its elongated frilly ribbons which are bilayered with hollow margins (Brodie et al., 2007). Phylogenomic and phylogenetic marker analyses of the *U. linza* epitype sequences resolved it in a fully or strongly supported clade containing the holotype of *U. pseudocurvata* (Koeman & van den Hoek, 1981) and sequences in GenBank incorrectly called *U. 'gigantea'* (Figures 3, 4, and S1–S3). Since *U. linza* has priority of publication (ICN Principle III [Turland et al., 2025]), the name *U. pseudocurvata* is here placed into synonymy under *U. linza*. The distribution of authentic *U. linza* as confirmed by DNA sequences is Belgium, England, France, Germany, Ireland, Netherlands, Northern Ireland, Sweden, and New Brunswick, Canada.

Ulva linza Linnaeus 1753: 1163

Epitype (herein designated): OXF: *Tremella marina fasciata*, Sheerness, Kent, England, 1721–1741, *leg.* Johann Jakob Dillenius. Originally designated as 'typotype' by L. M. Irvine on note attached to sheet dated xii.1966; epitype supports the lectotype: Dillenius, 1742: 46, Plate 9, Figure 6 (designated by J. Brodie and L. M. Irvine in Spencer et al., 2009).

Comment: Jarvis (2007: 907) noted that the lectotypification and epitypification of *Ulva linza* by Hayden et al. (2003) was not effective as the phrase "designated here" or an equivalent was not stated as required by Article 7.11 after 1 January 2001 (Turland et al., 2025). That was remedied by J. Brodie and L. M. Irvine in Spencer et al. (2009) for the lectotypification of *Ulva linza*, but not the epitypification, which is remedied here.

Heterotypic synonym: Note that listed here are only the heterotypic synonyms of *U. linza* reported in AlgaeBase (Guiry & Guiry, 2025) whose type specimens have been sequenced.

Ulva pseudocurvata Koeman & C.Hoek 1981: 19, Figures 2–31.

The species previously, but incorrectly, assigned the epithet '*linza*', therefore needs a validly published name. We began our search with the heterotypic synonyms of *U. 'linza'*. The earliest available name is *U. lanceolata*, but assembly of the partial chloroplast and mitochondrial genomes, and ribosomal cistron, showed that *U. lanceolata* is identical or nearly identical to sequences from the epitype of *U. compressa* (Figures 3, 4, S1–S3, and Table S3). The next available name by priority is *U. crispata* (Figure 2d). We surmised correctly that this type specimen might be in L in Kützing's herbarium, as Kützing (1843) reported seeing it. To test its synonymy we sequenced regions of the *rbc*L and *tuf*A genes, and the nuclear internal transcribed spacer 1 (ITS1) region from the holotype of *U. crispata*. The *rbc*L and *tuf*A markers

were identical to those of the epitype of *U. compressa* and the ITS1 sequence differed by one SNP. Phylogenetic analyses of the three markers confirmed the placement of *U. crispata*, with *U. lanceolata*, as a synonym of *U. compressa* (Figures 3, S1 and S2).

Phycoseris smaragdina* Kützing is the oldest available name for *U. 'linza'

The next earliest name treated as conspecific with *U.* '*linza*' by various workers was *Phycoseris smaragdina* described from Venice, Italy. We performed the same targeted PCR sequencing and phylogenetic analyses as above on two *P. smaragdina* individuals from an envelope in L containing a gathering of ten individuals (Figure 2c). In all analyses both *P. smaragdina* individuals were positioned in a clade with sequences deposited in GenBank under the name *Ulva 'linza'* (Figure 3, S1 and S2). Because targeted sequencing of the two individuals of *P. smaragdina* indicated that this species was the likely name for specimens identified as *U.* '*linza*', we analyzed one of the individuals of *P. smaragdina* that was Sanger sequenced using HTS, herein designated as the lectotype (see below). The sequencing generated partial chloroplast and mitochondrial genomes (Table S3), ITS region, and the 5S gene sequences. Phylogenomic and phylogenetic analysis of the *U. smaragdina* sequences supported the PCR results (Figures 3, 4, and S1–S3). The *P. smaragdina* marker sequences were identical or highly similar to 144 *tuf*A, 22 *rbc*L, and 62 ITS sequence accessions in GenBank deposited under the name *U.* '*linza*' (Figures S5-S7 and Tables S4–S6). To further confirm these results, we performed an analysis of the 5S sequence of *P. smaragdina* and found it to be resolved in the '*linza*' branch of the *Ulva linza-procera-prolifera* (LPP) complex (Figure S8 and Table S7). These sequencing data confirm *P. smaragdina* is the correct name to apply to the globally distributed species incorrectly called *U.* '*linza*'. We therefore propose the following new combination for the more than 200

sequenced specimens cited in GenBank, as well as specimens identified as *U.* '*linza*' in earlier literature.

Ulva smaragdina (Kützing) Hughey, Maggs, van der Loos, S.A.Harris, & P.W.Gabrielson *comb. nov.* (Figure 2c)

Basionym: *Phycoseris smaragdina* Kützing 1843: 297.

Lectotype (herein designated): L: L.4144346, the individual in Fig. 2C indicated by an asterisk (*), 1835, Venice, Italy, collector unknown.

Comment: There are two sheets, L.4144342 and L.4144346 each labeled *Phycoseris smaragdina* and with the same locality, Venedig = Venice (Italy) given in the original protologue (Kützing, 1843: 297) along with the year of collection, 1835. The former has a packet with three specimens; the latter a packet with ten specimens, two of which were sequenced. We are designating the HTS sequenced specimen as the lectotype.

Linnaeus's *Ulva compressa* and *U. intestinalis* were correctly applied

Assembly of the Linnaean *U. compressa* epitype data yielded the complete chloroplast and partial mitochondrial genomes as well as the ribosomal cistron (Table S3). Phylogenomic analysis of the chloroplast and mitochondrial genomes, as well as phylogenetic analyses of *tuf*A, *rbc*L and ITS *U. compressa* epitype sequences and representative *Ulva* sequences downloaded from GenBank (Tables S1 and S2), resolved *U. compressa* in a strongly supported clade. Also in this clade was the epitype of *U. lanceolata*, and the holotypes of *Phycoseris curvata* (Kützing, 1845: 245), *Phycoseris gigantea* (Kützing, 1843: 298), *P. olivacea*, *U. crispata*, and DNA

sequences deposited in GenBank as *U. compressa* (Figures 3, 4, and S1–S3). The findings that *Ulva lanceolata*, *U. crispata* and *P. olivacea* were conspecific with *U. compressa* disagrees with previous taxonomic conclusions based on morpho-anatomy (Bliding, 1963; Silva et al., 1996). *Ulva lanceolata* and *U. crispata* were both incorrectly considered heterotypic synonyms of *U. linza*, and the status of *P. olivacea* was unknown (Guiry & Guiry, 2025). The type specimens of *P. gigantea* and *P. curvata* had been correctly assigned to *U. compressa* (Maggs et al., 2024).

The epitype of *U. compressa* was mounted onto a herbarium sheet along with seven other specimens (Figure 1a). Gross observation of the epitype indicated the presence of numerous light rust-coloured and branched strands pressed underneath and intermixed with the larger, tubular thalli of *U. compressa* (Figures S4a–S4c). Examination of the rust-coloured thallus using light microscopy indicated it possessed longitudinal and transversely oriented cells with single pyrenoids (Figures S4d and S4e), and was thus tentatively identified as *U. prolifera* (Müller, 1778). Upon further analysis of the HTS *U. compressa* reads, we confirmed the presence of this second species of *Ulva* in the sample. Phylogenetic analysis of *tuf*A, *rbc*L and ITS marker sequences of this *Ulva* resolved it in a fully supported clade with sequences deposited in GenBank as *U. 'prolifera'* (Figures 3, S1 and S2). Sequences of *U. 'prolifera'* specimens in this clade however do not correspond to those of the *U. prolifera* epitype and therefore require further investigation to determine the correct name to apply to this light rust-coloured entity. Note that the single quotes around a specific epithet above indicate a misapplied name.

Ulva compressa Linnaeus 1753: 1163

Epitype (herein designated): OXF: *Tremella marina tenuissima* & *compressa*, Bognor, Sussex, England?, 1721–1741, *leg.* Johann Jakob Dillenius; epitype supports the lectotype: Dillenius, 1742: 48, Plate 9, Figure 8 (designated in Blomster et al., 1998).

Comment: The Hayden et al. (2003) epitypification of *Ulva compressa* was not effective, lacking the required "designated herein" or equivalent as required after 1 January 2001 by Article 7.11 of the ICN (Turland et al., 2025), which is remedied here.

Heterotypic synonyms: Note that listed here are only the heterotypic synonyms of *U. compressa* reported in AlgaeBase (Guiry & Guiry, 2025) whose type specimens have been sequenced.

Ulva lanceolata Linnaeus 1767: 719

Epitype: (herein designated) OXF: *Tremella marina*, *porri folio*, in Oceano, 1721–1741, no collector. Supports lectotype: Dillenius, 1742: 46, Plate 9, Figure 5 (designated by J. Brodie and L. M. Irvine in Spencer et al., 2009).

Ulva crispata Bertoloni 1810: 63

Holotype: L: L.4144461, Lunae Portu (Golfo della Spezia, Italy), no date, on rocks or Fuci, *leg*. Antonio Bertoloni.

Phycoseris curvata Kützing 1845: 245

Holotype: L: L.0054993, Ostsee (Rügen, Germany), no date, collector unknown.

Note: The above specimen was called the lectotype, but there is only one sheet of three specimens in L labeled *Phycoseris curvata* (Maggs et al., 2024, figure 2b), and the left and center specimens are mirror images of those illustrated by Kützing (1856, Pl. 20).

Phycoseris gigantea Kützing 1843: 298

Holotype: L: L.0795872, Rügen, Germany, no date, collector unknown.

Phycoseris olivacea Kützing 1843: 297

Holotype: L: L.414340, Venedig (Venice, Italy), no date, collector unknown.

Ulva intestinalis ....

Assembly of the Linnaean *U. intestinalis* epitype data resulted in complete chloroplast and partial mitochondrial genomes as well as the ribosomal cistron (Table S3). Phylogenomic, *tuf*A, *rbc*L and ITS marker phylogenetic analyses fully resolved the *U. intestinalis* epitype in a clade with sequences deposited in GenBank as *U. intestinalis* and with the holotype of *Phycoseris planifolia* (Figures 3, 4 and S1–S3). The taxonomic status of *P. planifolia* was previously unknown (Silva et al., 1996).

Ulva intestinalis Linnaeus 1753: 1163

Epitype (herein designated): OXF, *Tremella marina tubulosa*, Woolwich, London, England?, 1721–1741, *leg.* Johann Jakob Dillenius. Supports the lectotype: Dillenius, 1742: 47, Plate 9, Figure 7, designated in Blomster et al. (1998).

Comment: The Hayden et al. (2003) epitypification of *Ulva intestinalis* was not effective, lacking the required "designated herein" or equivalent as required after 1 January 2001 by Article 7.11 of the ICN (Turland et al., 2025), which is remedied here.

Heterotypic synonym: Note that listed here are only the heterotypic synonyms of *U. intestinalis* reported in AlgaeBase (Guiry & Guiry, 2025) whose type specimens have been sequenced.

Phycoseris planifolia Kützing 1843: 297

Holotype: L: L.4144341, Timavo River, Malfacone, Italy, 1835, collector unknown. See Figure 2b for a photograph of the holotype.

DISCUSSION

Our results demonstrate the value of preserving and curating herbarium specimens. Without the specimens in OXF and L, botanists would only have educated guesses about the identity and original intent of Linnaeus and Kützing regarding the species discussed here. This study demonstrates that detailed genetic analyses of type specimens are very valuable in determining the correct application of algal names. Of the five *Ulva* species described by Linnaeus (1753, 1767) that are still classified in the genus, only two, *U. compressa* and *U. intestinalis*, have generally been correctly applied historically to specimens that had been examined by morphology and anatomy, as well as to recently collected and DNA sequenced *Ulva* specimens. The remaining names were misapplied, including the generitype, *U. lactuca* (Hughey et al., 2019). *Ulva lanceolata* was incorrectly considered a heterotypic synonym of *U. linza*, itself a

misapplied name. It is a later heterotypic synonym of *U. compressa*. *Ulva linza* appears to have been consistently misapplied after its original description. It is the earliest available name for the species currently called *U. pseudocurvata.* The earliest available name for specimens currently, but incorrectly, called *U. 'linza'* is *Ulva smaragdina*. Thus, three of the five earliest names of *Ulva* species have been consistently misapplied for over 250 years, with consequences for the many commercial uses of *Ulva*, which are increasing globally.

The first DNA sequences published from historical *Ulva* type specimens were by Hanyuda and Kawai (2018) and Hughey et al. (2018). Hanyuda and Kawai analyzed the lectotype of *U. australis* (Areschoug, 1854: 370) using Sanger sequencing of the plastid-encoded *rbc*L gene and the nuclear encoded ITS2. Hughey et al. (2018) analysed the holotype of *U. expansa* (Setchell & Gardner, 1920: 285) using HTS that yielded an incomplete mitogenome, but also the plastid encoded genes *rbc*L and *tuf*A and the ribosomal cistron, all markers used for phylogenetic analyses of *Ulva* species. Subsequent papers sequencing DNA from historical *Ulva* type specimens have followed this pattern of using a combination of Sanger sequencing and HTS (Hughey et al., 2019, 2021,a, b, 2024) or sometimes only Sanger sequencing (Hughey & Gabrielson, 2022; Maggs et al., 2024) to correctly apply names. To date 42 historical *Ulva* type specimens have yielded DNA sequences (Table S8), as well as one species of *Monostroma* (Ulvales [Maggs et al., 2024]). This paper contributes another eight *Ulva* type specimens to the growing list that have been analyzed and includes the oldest algal specimens to have been genome sequenced, four of them dated from 1721–1741. Based on the findings from this investigation and the previous analyses highlighted above, we strongly advocate for the continued DNA sequencing of type specimens of all validly published *Ulva* from the 19th and early 20th century to be pursued.

AlgaeBase (Guiry & Guiry, 2026) is the best source for the current application of *Ulva* names, as well as *Phycoseris* and *Enteromorpha* names that have not been transferred to *Ulva*. This includes validly published names whose status is listed as unknown on AlgaeBase. However, sequence databases, e.g., GenBank, frequently do not update the names used by the original depositors of sequences. Those using these sequences in phylogenetic, or other analyses, need to compare their sequences to the type specimens sequences deposited in GenBank to ensure the correct application of names.

Despite DNA sequencing of type specimens being in its infancy, we have the methodology and technologies to widely apply this to algal, fungal and plant herbarium specimens, especially name-bearing type specimens. The amount of material required for both Sanger sequencing and low-input HTS is similar to or less than that used for classical microscopic anatomical studies. For most *Ulva* species such morpho-anatomical investigations have not resulted in the correct application of historical names (Chávez-Sánchez et al., 2019; Kang et al., 2019). What is needed is a collaborative effort between herbarium curators and plant molecular systematists to sequence historical type specimens. This would enhance the value of herbaria as repositories of plant, algal and fungal biodiversity and provide those studying the ecology, evolution, biology, and filing for patents of these organisms the fundamental knowledge of the correct, consistent application of names.

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Figure 1. Type specimens of the Linnaean names *Ulva compressa*, *U. intestinalis*, *U. lanceolata and U. linza*. Images showing habits of four Linnaean types in Oxford University Herbaria (OXF). (a) Historia Muscorum-XXXIII (OXF), IX.8. *Tremella marina tenuissima* & *compressa*, epitype of *Ulva compressa* Linnaeus. (b), Historia Muscorum-XXXII (OXF), IX.7. *Tremella marina tubulosa*, lectotype of *Ulva intestinalis* Linnaeus. (c) Historia Muscorum-XXXI (OXF),

IX.5 and IX.6. The printed labels of 6. *Tremella marina fasciata* and 5. *Tremella marina, Porri folio* were interchanged. The above specimen labeled *Tremella marina fasciata* is the epitype of *Ulva lanceolata* Linnaeus and the lower specimen on the sheet labeled *Tremella marina, Porri folio* is the epitype of *Ulva linza* Linnaeus. (d) Descriptions of the interchanged labels by Linda Irvine.

Figure 2. Type specimens of *Phycoseris olivacea*, *P. planifolia*, *P. smaragdina* and *Ulva crispata.* Images showing habits of four type specimens in L (Leiden Herbarium). (a) L.4144340, *P. olivacea* holotype. (b) L.4144341, *P. planifolia* holotype. (c) L.4144346, *P. smaragdina* lectotype, * indicates specimen used for HTS. (d) L.4144461, *Ulva crispata* holotype with label.

Figure 3. Phylogram of *Ulva* species and type specimens inferred from IQ-TREE maximum likelihood analysis of *tuf*A gene sequences. Phylogram based on *tuf*A gene sequences showing that the names *U. compressa* and *U. intestinalis*, were correctly applied, however *U. crispata*, *U. lanceolata*, and *U. linza* were misapplied. It also shows that *U. smaragdina* is the correct binomial for the misapplied name *U. 'linza'* and that *Phycoseris olivacea* and *P. planifolia* are not distinct species, rather synonyms of *U. compressa* and *U. intestinalis*. Outgroup *Ulvaria obscura* var. *blytii* (family Ulvaceae). Species names in bold indicate type specimens analyzed in this study; * taxon whose type specimen has not been sequenced; ' ' specific epithet incorrectly applied to the GenBank sequence; taxa with neither an * or ' ' corresponds to a sequenced type specimen or the sequenced type specimen is indicated. Abayes (IQTREE) branch support values

shown at nodes when ≥0.75. Bootstrap percentages (ultrafast IQTREE nreps=1,000) shown at nodes when ≥75%; -- indicates less than ≥0.75 abayes and/or bootstrap support.

Figure 4. Midpoint rooted phylogram of *Ulva* species and type specimens inferred from IQ-TREE maximum likelihood analysis of complete chloroplast genome sequences. Phylogram based on chloroplast genome analysis shows that the names *U. compressa* and *U. intestinalis*, were correctly applied, however *U. lanceolata* and *U. linza* were misapplied. Species names in bold indicate type specimens analyzed in this study; * taxon whose type specimen has not been sequenced; ' ' specific epithet incorrectly applied to the GenBank sequence; taxa with neither an * or ' ' corresponds to a sequenced type specimen or the sequenced type specimen is indicated. Abayes (IQTREE) branch support values shown at nodes when ≥0.75. Bootstrap percentages (ultrafast IQTREE nreps=1,000) shown at nodes when ≥75%; -- indicates less than ≥0.75 abayes and/or bootstrap support.