Lab Activity: Tools and Concepts for Exploring Algal Biodiversity: Using Dichotomous Keys in Reverse to understand the Morphological Species Concept

Developed by: Dr. Brian Wysor, Roger Williams University, Department of Biology, Marine Biology & Environmental Science

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Learning Objectives

By the end of this laboratory activity, students should be able to:

1. Describe 3 criteria by which species can be differentiated from one another.
2. Define the biological and morphological species concepts and highlight the limitations of their application to different organismal groups.
3. Systematically compare the morphology of algae specimens to decide whether they constitute one or more than one species.
4. Use dichotomous keys to identify diagnostic morphological, cytological, anatomical, and/or reproductive characters useful for distinguishing species of algae.

Assessment Method

Students will produce 3 illustration sets that highlight diagnostic morphological features to distinguish one or more species.

Instructor Notes

Materials or supplies required: Macroalgae samples from one to two sites (can be collected by students if time permits, or as homework for students to bring to lab); plastic baggies for sample collection; waders and snorkeling gear, as appropriate; razor blades for preparing hand sections; microscope slides, coverslips and cytological stains, if used.

Equipment required: Compound light microscopes (with camera, if available); seawater, sorting trays, thumb pipettes and watch bowls.

Techniques required (those which are not taught during the activity but students must already have a working knowledge): Microscopy skills, hand sectioning, use of dichotomous keys.

Time required: The basic laboratory activity can be completed in a single 2-3 hours laboratory period, but the development of the biodiversity portfolio project in which intra- and/or interspecies morphological variation is documented will be developed over the course of a 3-4 week laboratory sequence.

Anticipated audience: 1) intro majors course 2) upper level majors course 3) non-majors course 4) graduate course 5) outreach

Notes to supplement background information.

***Limitations to applying the biological species concept*:**

1. You collect 2 fresh, living specimens that appear similar and they are thought to be one and the same species. Unbeknownst to you they are both male/female/vegetative.
   a. **In this case, it is impossible to apply the biological species concept because there is no potential for successful crossing if only males, only females are non-reproductive specimens are available for study.**
2. You uncover specimens from a private herbarium, long forgotten but well preserved in a dry storage cabinet. Specimens date to the late 1800s, before the industrial revolution. No living representatives of these species are available – in fact, the original collection site has been filled in, and is now a parking lot for a marina. The original site is destroyed and nearby sites are so highly disturbed that finding contemporary specimens seems unlikely.
   a. **In this case, it is impossible to apply the biological species concept because there appear to be no living specimens, and the possibility of evaluating reproductive compatibility is impossible with dead specimens.**

Post Lab Activities *ANSWERS*

1. **Describe the challenges to the identification of marine macroalgae using morphology.**
   a. Complex and highly specific vocabulary can be daunting for students new to phycological exploration. The use of imprecise vocabulary, while more recognizable (e.g., worm-like), can make interpretations challenging.
   b. Convergence in morphology.
      i. two species appear identical, even though they are genetically distinct. Absent genetic, behavioral, environmental or other sources of data it may be impossible to know that multiple species are present. This can result in an underestimate of species richness.
   c. Phenotypic plasticity.
      i. a single species has broad morphological variation such that different individuals might be confused as two or more species. This may be the consequence of different environmental pressures (e.g., light, water velocity, nutrient availability, grazing pressure), heteromorphic life histories phases or hybridization. This can result in an overestimate of species richness.

2. **Describe the consequence of species mis-identification in terms of the interpretations of studies reliant on accurate species identifications.**
   a. Collecting a species known to have a compound with important medical, industrial or cosmetic properties may be problematic if morphological convergence clouds species identification. In other words, time and energy may be wasted, and the compound never encountered, if a look-alike species is collected.
   b. Understanding evolutionary patterns becomes problematic when a common morphology is resolved on two different branches of a phylogenetic tree.
   c. Under- or overestimating species richness may have consequences for setting conservation priorities.

d. The inability to detect a species invasion could prove devastating if a non-native species is not held in check by local competitors and predators.

3. Describe what you need to do to be successful in this course.

a. Commit to vocabulary by using context in dichotomous keys and by referencing available phycological glossaries.
b. Consult dichotomous keys, reference books and primary literature to understand morphological and reproductive variation within and between species.
c. Consult multiple references (not just a single source) to enhance understanding.
d. Make illustrations to re-inforce diagnostic features of local species.
e. Appreciate that species concepts are hypotheses, and that few specimens will represent all aspects of a species description.
f. Approach species determinations cautiously, understanding that specimen morphology will deviate from species descriptions in minor to substantial ways and that additional data drawn from other sources (literature, DNA, crossing studies) may be necessary to confirm species identity.
g. Curate specimens responsibly so that you can begin to understand whether environmental stimuli might explain morphological variation (i.e., do all of the specimens from protected habitats have thinner cell walls than the same species collected from higher energy habitats).

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Introduction. Biodiversity matters. The ability to distinguish among different, discrete biological entities is important for numerous reasons including:

- the recognition of indicator species, which use the presence of one species to infer something about an environmental condition or community composition (Anderson 1992),
- monitoring the impacts of environmental disturbance (e.g., as a consequence of global climate change, coastal development, the introduction of non-native species),
- the use of biotechnological and natural products that might require patents or at least an easy way to find the source of a compound or product after initial characterization, and/or,
- making predictions about organismal biology and ecology using species phylogenies.

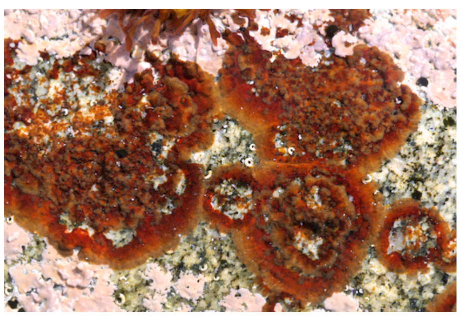
Numerous criteria have been elaborated to define species as discrete, recognizable biological entities. A species operates to preserve the genotype of an organism or organismal lineage in the midst of different evolutionary mechanisms (i.e., gene flow, genetic drift, mutation and natural selection), which can both reinforce or diversify organismal genotypes. In studies of algal diversity, several species concepts are used and the application of a particular concept is largely based on the type of research question that is being addressed.

Biological species concept. Long heralded as the holy grail of species concepts, the biological species concept is predicated on the establishment of reproductive isolation and the cessation of gene flow among populations. While this is fairly easy to apply in concept, it is an impractical gauge for the determination of species when the goal is to characterize a community for its constituent species. Consider the following scenarios:

1. You collect 2 fresh, living specimens that appear similar and they are thought to be one and the same species. Unbeknownst to you they are both male/female/vegetative.
2. You uncover specimens from a private herbarium, long forgotten but well preserved in a dry storage cabinet. Specimens date to the late 1800s, before the industrial revolution. No living representatives of these species are available – in fact, the original collection site has been filled in, and is now a parking lot for a marina. The original site is destroyed and nearby sites are so highly disturbed that finding contemporary specimens seems unlikely.

Morphological species concept. The morphological species concept defines species on the basis of shared likenesses in phenotype. The study of morphology is central to the interaction of natural historians and field biologists with the specimens they study. Indeed, noticing, handling and collecting specimens involves morphological study whether conscious or not. But, like the biological species concept, problems can and do emerge and these are often due to a poor understanding of the range of morphological variation within a species. Consider the following scenarios.

1. The tropical brown alga, *Padina jamaicensis,* is a morphologically variable species that occurs as fan-shaped expanded blades on coral reefs. In areas in which grazing pressure from herbivorous fish is high, however, *P. jamaicensis* occurs as small prostrate blades that develop as part of a turf community (Lewis et al. 1987).
2. Species of the tropical green algal genus *Caulerpa* are distinguished on the basis of the morphology of assimilator branchlets. In a single specimen (which also happens to be a single, giant, multi-nucleate cell) grown over a depth gradient, branchlets at the surface are spherical, while those grown at depth are flattened and saucer-shaped (Ohba et al. 1992).
3. As part of an effort to document the species richness of a temperate cold water marine flora from the western North Atlantic, you scrape a small brown algal crust from the surface of an intertidal rock. When you return to the same site 6 months later to ascertain seasonal variation, you fail to observe the crusts because they are covered by meadows of the sausage weed, *Scytosiphon lomentaria* (Littler & Littler 1983).



Ralfisoid crust
© seaweed.ie

Scytosiphon lomentaria
© Francisco Rodriguez (Faluke)

4. A problematic bloom of a monostromatic, blade-forming sea lettuce is discovered to be the same species as a monostromatic, tubular gut weed through comparative DNA sequencing (Blomster et al. 2002). Contrarily, molecular investigations reveal that sea lettuce biodiversity is under estimated because numerous species are morphologically indistinguishable (e.g., O'Kelly et al. 2010).

As the final scenario above suggests, the limitations of both the biological and morphological species concept are commonly resolved with reference to additional sources of data. Increasingly systematists, ecologists and natural historians rely on molecular data derived from DNA barcoding, molecular phylogenetic and genomic studies to sort out problems. Be that as it may, the morphological concept is still heavily relied upon because it is the way in which organisms are detected in the field in the first place, or in the laboratory when cultures are seeded from environmental sources. Thus, despite the limitations to morphological interpretations, it is essential for the student of phycology to be able to decipher morphological traits as a means to a preliminary species identification.

Learning Objectives

By the end of this laboratory activity, students should be able to:

5. Describe 3 criteria by which species can be differentiated from one another.
6. Define the biological and morphological species concepts and highlight the limitations of their application to different organismal groups.
7. Systematically compare the morphology of algae specimens to decide whether they constitute one or more than one species.
8. Use dichotomous keys to identify diagnostic morphological, cytological, anatomical, and/or reproductive characters useful for distinguishing species of algae.

Essential terminology

Biological species	Diagnostic character	Morphological plasticity
Character	Dichotomous	Morphological species
Character state	Morphological convergence	Species concept

Pre-lab Assignments

1. Rate each concept using the following key:

1 = I have never heard of it.
2 = I have heard of it but do not understand it.
3 = I think I understand it partially.
4 = I know and understand it.
5 = I can explain it to a friend.

a. Species concept ______

b. Biological species concept ______

c. Morphological species concept ______

d. Character ______

e. Character state _______

Laboratory Activity

Scientists who specialize in the study of biodiversity and classification of that diversity are known as systematists. Systematists, evaluate organismal diversity using a variety of methods to try to make organismal characterization as objective as possible. You can use some of the standard techniques of the systematist in order to understand the diversity of organisms in your local flora, and organisms that might initially appear very similar to one another may soon be easily distinguished when you know how and what to compare. When systematists compare species to one another, they need to compare "apples to apples," and "oranges to oranges." In other words, systematists first identify a common trait for comparison (known as a character), and then define the variants of that trait (known as the character states), for a set of organisms.

Students of phycology can identify characters and their corresponding character states using dichotomous keys. Dichotomous means forking or splitting into two. Thus, in a dichotomous key, the user is basically asked a series of yes/no questions (i.e., a question with 2 possible answers), which corresponds to a list of characters or character states that can be followed until an endpoint is reached. The endpoint is a possible species identification. Here is an example from Villalard-Bohnsack (2003).

1. Thallus calcareous (encrusted with lime, including calcium and magnesium carbonates), very hard; usually pink, but ranging from purplish-red to chalky white……………………………2
1. Thallus not calcareous……………………………………………………………………..14

In this case, the character is "calcareous," and the possible character states are either, "yes" (calcified) or "no" (not calcified). It would also be possible to define "calcareous" by the amount of calcium carbonate present, such that character states for "calcareous" could include, "heavily calcified," "moderately calcified," "lightly calcified," and "not calcified." For any given flora, the more closely related the species are under consideration, the more likely it is that a given character will have more than two character states.

Continuing with the example, if you decide that your specimen is calcareous (i.e., you can answer "yes" to the first statement and "no" to the second statement), then you advance to step 2, and if you decide your specimen is not calcareous (i.e., you can answer "no" to the first statement, and "yes" to the second statement), then you advance to step 14.

2. (Calcareous Thallus) Stiff upright tufts; with beaded axes and branches formed by chains of calcified articulated segments………………………………………………*Corallina officinalis*
2. (Calcareous Thallus) Calcareous crust or disc…………………………………………….3

14. (Non-calcareous) Non-calcareous red crust or film attached to rocks, shells or other algae, or eelgrass……………………………………………………………………………………15
14. Thallus upright or small and cushion-like, but neither crustose nor calcareous……………...21

You can see from this very simple example, that with only two steps through the key, a species determination is possible. Of course, this happens here, because the marine flora of RI (the focus of Villalard-Bohnsack's key), only has one articulated coralline red alga in the flora, and this morphology is very different from all other species; in other words, there are no other articulated coralline red algae known in the flora with which to confuse *Corallina officinalis*. As you advance through a dichotomous key it will likely get harder and harder to answer the dichotomies because, the more traits that are shared between organisms, the more similar they appear. When keying out algae, you will commonly need to examine microscopic details to confirm species determinations. Using dichotomous keys takes practice because specimens are rarely perfect representatives of their written descriptions and interpreting the nuanced meaning of technical vocabulary common in dichotomous keys is challenging. Phycological glossaries should be within easy reach whenever using a dichotomous key (Table 1). Through repetition you will come to understand what the author of a given key means when she uses imprecise terms (e.g., wiry, feathery, worm-like, etc.) to describe the morphology of species you might encounter in your flora. However, such imprecision will be unique to each key, such that you may need to reset expectations when using dichotomous keys prepared by different authors.

Table 1. Phycological Glossaries. Precision in morphological descriptions is essential to accurate species determination, and has resulted in the elaboration of a vocabulary that may be daunting for students new to the discipline. Fortunately, numerous on-line and print resources are available to navigate the nuanced vocabulary of phycology. In addition to glossaries that are often found at the end of books on phycology, these print and on-line resources are excellent sources to clarify unfamiliar terminology.

1. Guiry, M.D. & Guiry, G.M. 2017. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://algaebase.org/search/glossary/; searched on 12 May 2017.
2. Hine, A. E. 1977. *A Glossary of Phycological Terms for Students of Marine Macroalgae*. St. Alden's in the Weeds, Miami, 91.
3. Mathieson, A. C. & Dawes, C. J. 2017. *Seaweeds of the Northwest Atlantic*. University of Massachusetts Press, Amherst, pp. 669-685.
4. Smithsonian Tropical Research Institute. 2017. *Caribbean Phycology Resources*: *Bilingual glossary of phycological terms*. https://www.stri.si.edu/sites/taxonomy_training/future_courses/2009_Biological_glossary_Phycology.html; searched on 12 May 2017.

To get some practice working with dichotomous keys and applying your nuanced understanding of systematic comparisons of morphology, examine pairs of the following specimens. For each individual species, find the name at the end point of the dichotomous key. Then, work backwards through the key, and record the morphological characteristics of each species. Sketch each species under consideration, being sure to highlight every trait that you extract from the dichotomous key. An example is shown for western north Atlantic rockweeds, *Fucus vesiculosus* and *Ascophyllum nodosum*, with reference to Villalard-Bohnsack (2003).

Fucus vesiculosus

- Golden to dark brown in color
- Large, up to 70 cm long and 1.5 cm wide
- Attached to rocks, commonly in the mid- to low-intertidal
- Paired vesicles present
- Dicthotomous branching
- Tips of branches forming receptacles (inflated vesicles)
- Mid-rib present

Ascophyllum nodosum

- Branching irregular
- Axes with short side branchlets
- No mid-rib
- Single vesicles present on main axis
- Receptacles on side branches
- Dark brown, yellow or greenish in color
- Large, up to 100cm long or longer
- Attached to rocks, commonly in the mid- to low-intertidal

Next, use the two sets of characteristics for a given species pair to identify the common features for comparison, noting the utility of each character to differentiate among the two species. These features are the **characters**, while the variation present between species are the **character states**.

Table 2. Common basis for comparison deduced from morphological descriptions highlighted in a dichotomous key. Note, not all features identified in the key are useful to distinguish among species, regardless of their utility in describing the nature of an individual species.

Basis for comparison (character)	*Fucus vesiculosus* traits (character state)	*Ascophyllum nodosum* traits (character state)	Comments on Utility
1. Color	Golden to dark brown in color	Dark brown, yellow or greenish in color	Feature overlaps; may not be useful to distinguish these species on the basis of color alone
2. Size	Up to 70 cm	Up to 100 cm or more	Feature overlaps; may not be useful to distinguish these species on the basis of size alone
3. Substratum	Attached to rocks	Attached to rocks	Identical trait; cannot distinguish on the basis of substratum
4. Ecological distribution	Mid- to low intertidal	Mid- to low intertidal	Identical trait; cannot distinguish on the basis of ecological distribution
5. Presence of inflated vesicles	Present, paired (usually)	Present, single	Feature is distinctive, and therefore could be used to differentiate between species based on the nature of inflated vesicles.
6. Mid-rib	Present	Absent	Feature is distinctive, and therefore could be used to differentiate between species based on the presence/absence of mid-rib.

Now that you have elaborated the basis for comparison, illustrate both species in a side-by-side comparison and highlight only those features that distinguish one species from the other. You should make reference to live or preserved specimens before finalizing your illustrations, both to enhance your drawing by observing natural variation and to identify deviations from the written description.

As you can tell from the example above, identifying a meaningful set of character states to distinguish among species is challenging because species can vary extensively in the phenotypic expression of its genotype. While humans are very good at recognizing subtle variation in some populations (i.e., it is often the basis of discrimination in human societies), the range of intra- and inter-specific morphological variation is less well understood in algae. As you characterize the differences between species pairs below using the dichotomous key, you will also find it useful to consult other references for genus and species descriptions that reveal other elements of organismal biology or natural history that wouldn't necessarily be detected as part of morphological examination. Such traits might include the nature of internal anatomy, life history patterns, pigment composition, the nature of dispersal stages, or reproductive and vegetative developmental biology, among others. The key to understanding species richness in marine algae is to consider all of these traits, collectively, in the characterization and recognition of a species.

Commonly confused, but easy to distinguish in external morphology

Ulva vs. *Ulvaria*
Ulva vs. tubular *Ulva* (i.e, "*Enteromorpha"*)
Phyllophora vs. *Coccotylus*

Commonly confused, but easy to distinguish in internal anatomy

Gracilaria sp. vs. *Aghardiella subulata*
Grateloupia turuturu vs. *Palmaria palmata*
Punctaria vs. *Petalonia*

Commonly confused

Chondrus vs. *Gymnogrongrus*
Protomonostroma vs. *Monostroma* vs. *Ulvaria* vs. *Kornmannia*

References:

Blomster, J., Back, S., Fewer, D. P., Kiirikki, M., Lehvo, A., Maggs, C. A. & Stanhope, M. J. 2002. Novel morphology in *Enteromorpha* (Ulvophyceae) forming green tides. *Am. J. Bot.* **89**:1756-63.

Cianciola, E. N., Popolizio, T. R., Schneider, C. W. & Lane, C. E. 2010. Using Molecular-Assisted Alpha Taxonomy to Better Understand Red Algal Biodiversity in Bermuda. *Diversity* **2**:946-58.

De Clerck, O., Guiry, M. D., Leliaert, F., Samyn, Y. & Verbruggen, H. 2013. Algal taxonomy: a road to nowhere? *J. Phycol.* **49**:215-25.

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Huisman, J. 2016. The Age of Discovery is still with us. *J Phycol* **52**:37-9. **[to be read prior to the start of this or other lessons on the biodiversity/discovery of algae]**

Littler, M. M. & Littler, D. S. 1983. Heteromorphic life-history strategies in the brown alga *Scytosiphon lomentaria* (Lyngb.) Link. . *J. Phycol.* **19**:425-31.

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Villalard-Bohnsack, M. 2003. *Illustrated Key to Seaweeds of New England, 2nd Edition.* Rhode Island Natural History Survey, 149p.

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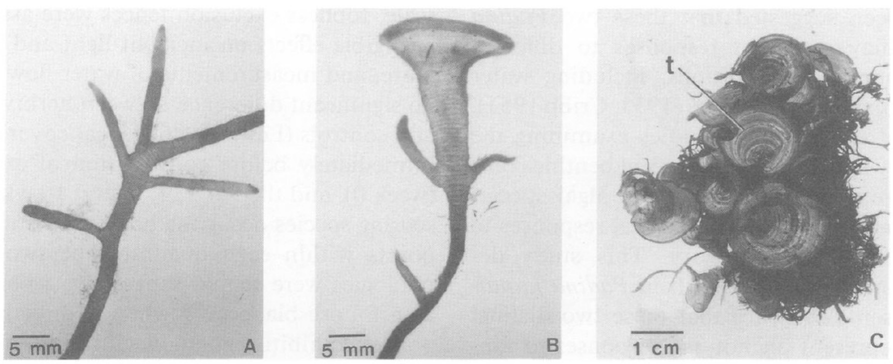

FIG. 1. Morphological plasticity in *Padina jamaicensis*. (A) Prostrate turf growth form found in reef areas heavily grazed by herbivorous fishes. These flattened, uncalcified plants exhibit adventitious branching, and plants are tightly attached to the substratum by uniseriate rhizoids produced from the ventral portion of the thallus. (B) Early development of foliose blades under experimentally reduced grazing intensity. Transformation from the turf form into the foliose form is accompanied by upward growth and deposition of calcium carbonate along the upper surfaces of the developing fan-shaped blades. (C) Foliose form of *P. jamaicensis* after 8 wk of reduced herbivory. Erect blades grow from a semicircular, inrolled margin of meristematic cells. Concentric bands of tetrasporangia (t) are visible as dark lines on upper blade surfaces.

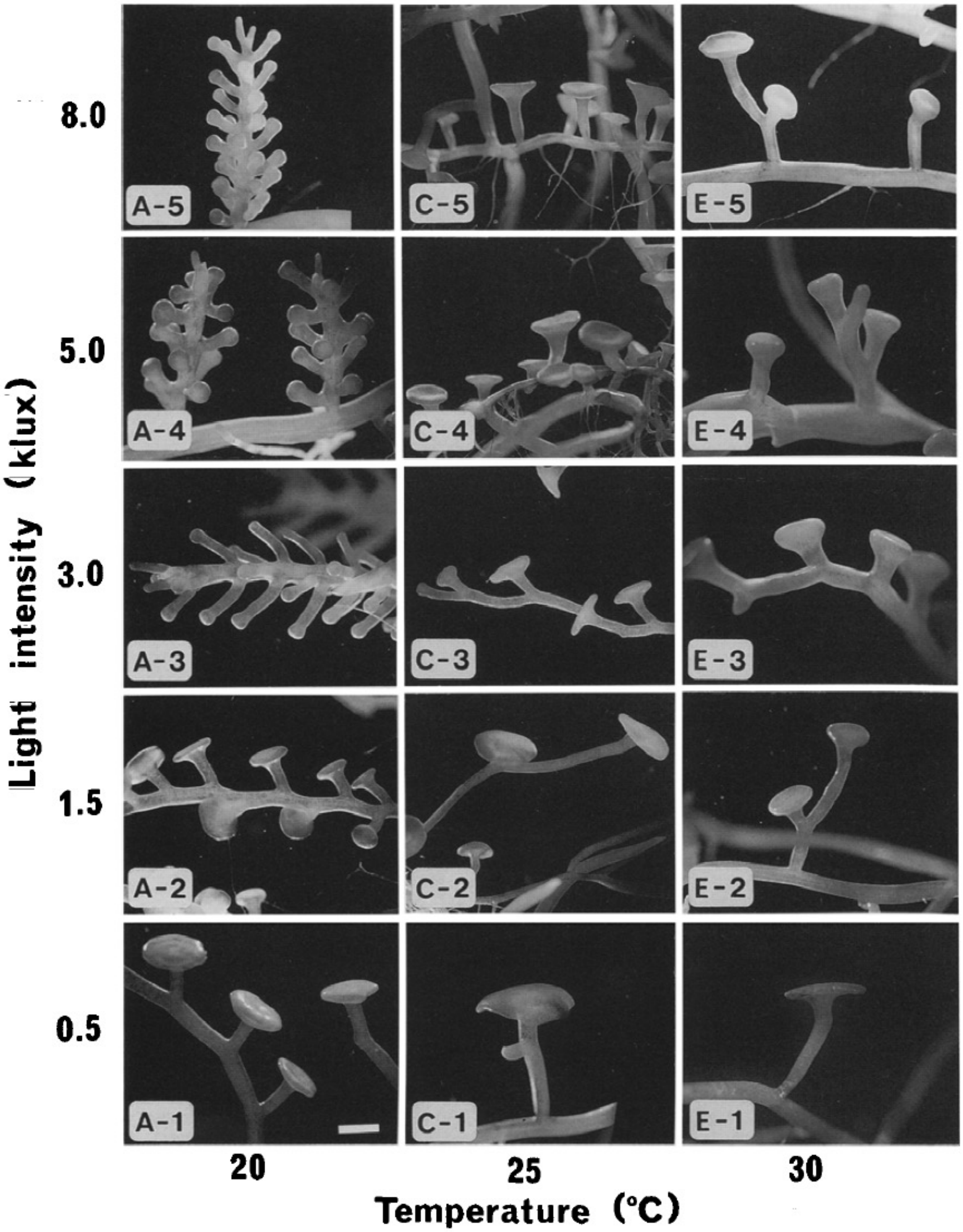

Fig. 26 Morphogenetic responses of *C. racemosa* var. *peltata* to various temperatures and light intensities. Scale bar: 2 mm. Refer to Fig. 1 for explanation of symbols.

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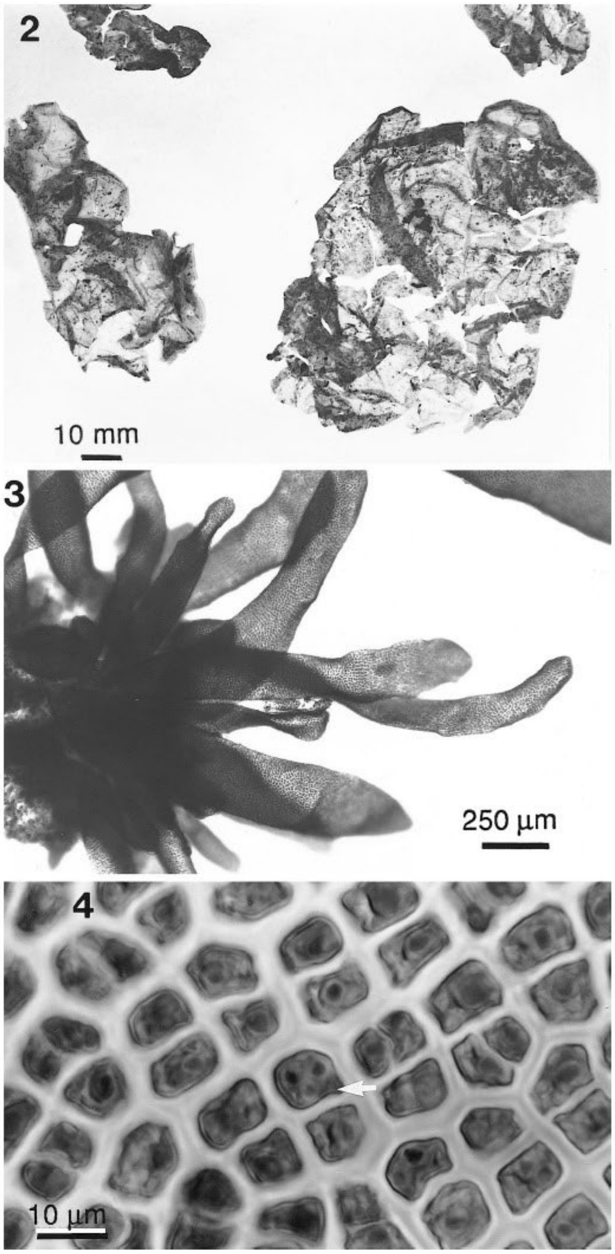

Figs. 2–4. Field-collected and cultured material from green tides in Finland. **2.** Herbarium specimens of monostromatic sheet-like green seaweed from green tide at Olkiluodonvesi, Finland, on 5 June 1996. **3.** Bloom-forming sheet-like green alga grown in culture has given rise from callus-like tissue to unbranched tubular outgrowths indistinguishable from *Enteromorpha intestinalis*. **4.** Tubular green alga in culture, showing small cells with 1–3 pyrenoids (arrow).

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<u>Post-lab Assignments</u> **Name: ________________________**

In addition to the submission of 3 illustration sets that highlight diagnostic morphological features to distinguish one or more species, address the following questions, given your experience in lab today:

1. Describe the challenges to the identification of marine macroalgae using morphology.

2. Describe the consequence of species mis-identification in terms of the interpretations of studies reliant on accurate species identifications (hint, consider the scenarios presented on p. 1 and 2, above). In support of your response, find an article from the primary literature that:
    a. documents the presence of morphological convergence or phenotypic plasticity. Briefly, summarize the nature of the problem (including reference to characters and character states) and then describe how the investigator(s) resolves the problem.

b. features a species of algae or group of algae for which understanding species-level identification is essential for achieving some other (biological, ecological, environmental, economic or public health) goal.

3. Describe what you need to do to be successful in this course as it relates to species identification.

4. Concept Check! Re-Rate each concept using the following key:

1 = I have never heard of it.
2 = I have heard of it but do not understand it.
3 = I think I understand it partially.
4 = I know and understand it.
5 = I can explain it to a friend.

a. Species concept _____

b. Biological species concept _____

c. Morphological species concept _____

d. Character _____

e. Character state ______