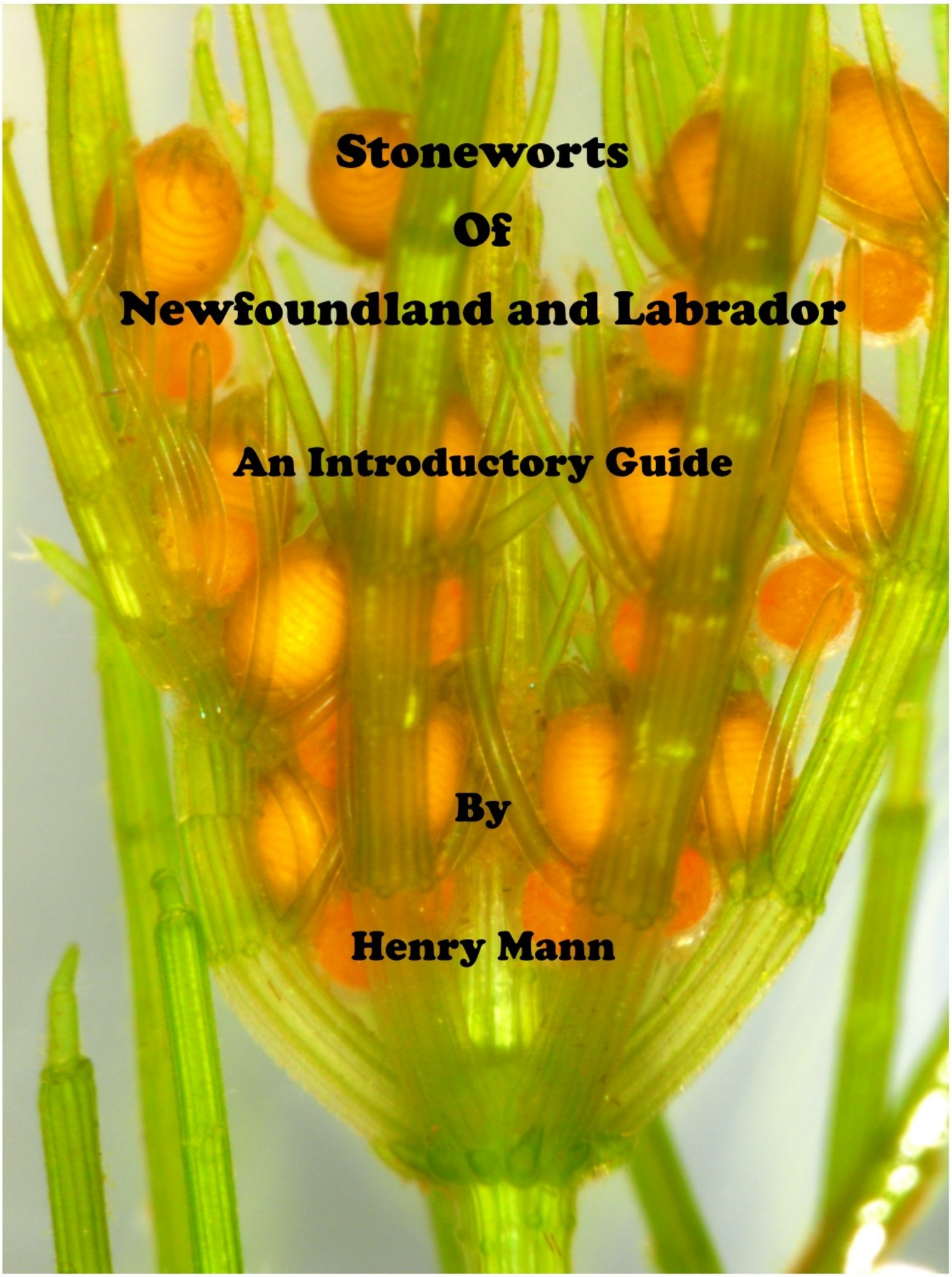
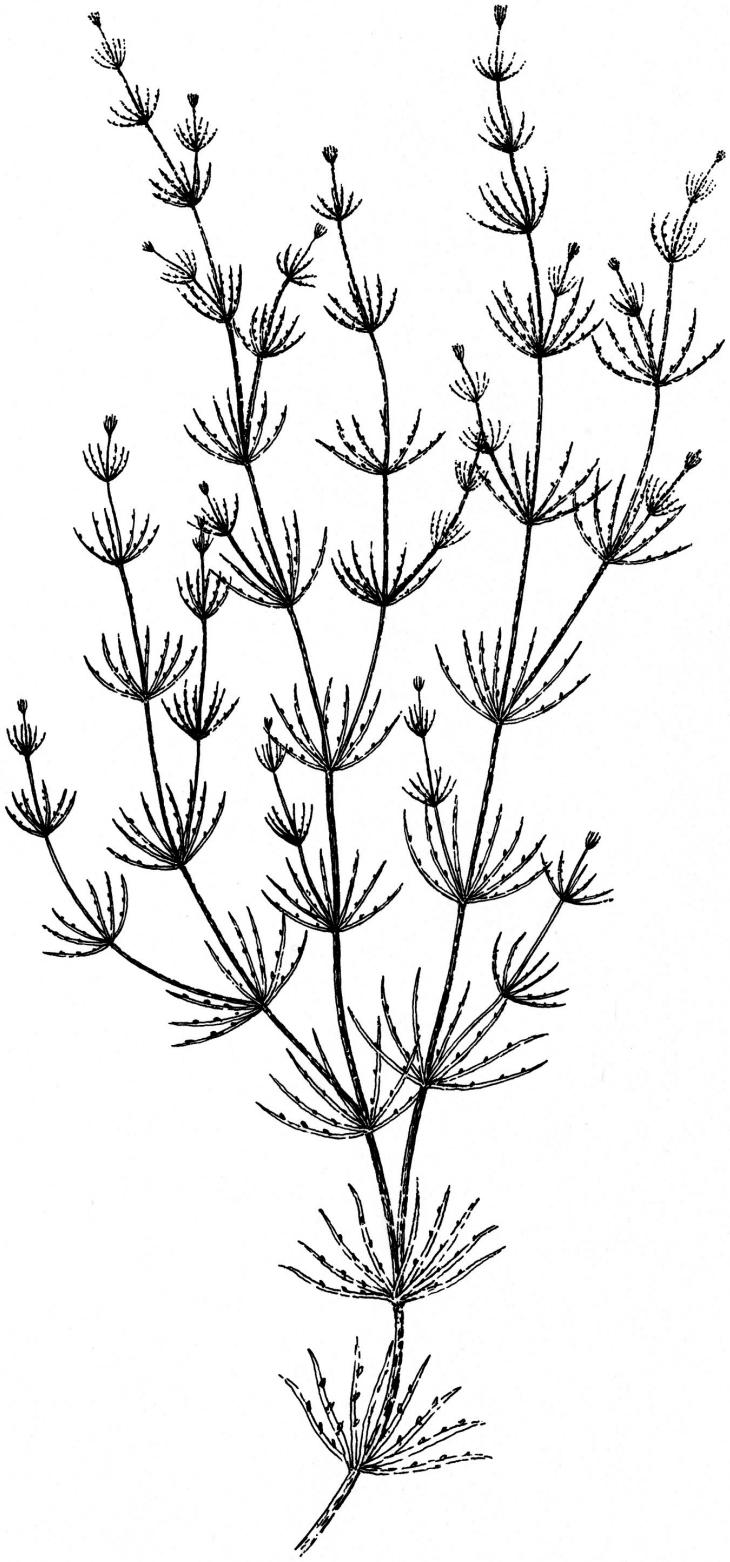


Stoneworts
Of
Newfoundland and Labrador

An Introductory Guide

By

Henry Mann



Chara WH

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In cooperation with The Herbarium (SWGC)
Grenfell Campus
Memorial University of Newfoundland and Labrador
Cornerbrook, Newfoundland, Canada, A2H 6P9

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2022

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Front Cover: A branchlet whorl of *Chara virgata* with mature antheridia and oogonia. HEM

Back Cover:: *Chara virgata* fertile branchlets. HEM

This work is dedicated to the late
Dr. M. V. S. Raju (Sethu), a kind
and generous Botany Professor who
introduced me to the fascinating
world of plants and mentored
me through my graduate years.

And to Phyllis,
who kept house and home together
during my many hours, days and years in the field,
and before a microscope and computer.

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(Ancillary resources are available as a separate file to supplement this guide for individuals who may become actively interested in the study of charophytes.)

Much fresh and brackish water harbouring charophytes occurs in rivers, streams, lakes, ponds, backwaters and lagoons throughout Newfoundland and Labrador. Humber River above Deer Lake.

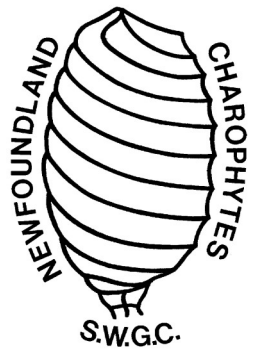
Preface

Aquatic plants are less studied and less well known than the surrounding terrestrial floras. This is partially due to being less accessible and often viewed only from a distance. To some they inhabit uncomfortable wet muddy places hosting hordes of blackflies, deerflies and mosquitoes. Some aquatic plants have pretty showy flowers held above the water surface which may be sought by wildflower enthusiasts and nature photographers. However, a great many aquatics do not produce eye-catching blossoms or other features inviting investigation from a distance. A considerable number are totally submerged, therefore out of sight and out of mind. Stoneworts are among these. Many aquatics have no obvious value to the human economy and therefore there has been less incentive to study their structure, properties, or ecology. Yet we now know that the stoneworts play a tremendously important ecological role in our aquatic habitats and they do directly translate into significant and pleasurable benefits to many Newfoundlanders. Hunters, fishermen, trappers, anglers, wildlife managers, ecologists and naturalists all have a reason for knowing about and recognizing our apparently lowly charophytes.

This work is intended as a "How To" guide for the beginner, allowing the novice to become acquainted with the charophytes and our local species before delving into the more technical literature. It is not intended for the practicing charophytologist or already knowledgeable specialist although there may also be small bits of information contained herein for such individuals especially relating to the Newfoundland condition. Many topics and details that are not immediately relevant for an initial introduction are omitted or only briefly mentioned.

In my early years teaching botany courses at the Sir Wilfred Grenfell Campus of Memorial University, I searched for locally available living material to use in student laboratories and discovered two species of stoneworts in a fen pond adjacent to the campus buildings. It soon became apparent that little was known about this group in Insular Newfoundland, almost nothing in Labrador, and my interest took off from there. No good North American source for introducing the novice to these unique "plants" was available although considerable scientific literature existed, some older and difficult to access, some very technical with much specialized jargon, and some even contradictory and confusing. Fortunately some of the earlier European literature was a great help in bridging the gap and providing the incentive to delve further into the structure and biology of this strange and ancient group of primary producers. It is my hope that this modest guide will encourage and assist future naturalists to become familiar with our stoneworts and help to flesh out the paucity of information in this province and across Canada. There is a considerable storehouse of botanical treasures and pleasures in our ponds, lakes, streams and rivers, and the oft neglected and unknown stoneworts are prominent among these.

Henry Mann
Pasadena, NL
January 2022



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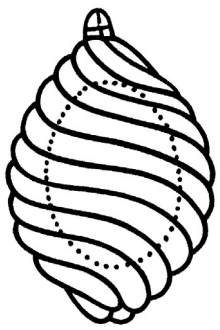
I: Introduction

Stoneworts, also known as charophytes, are submerged aquatics which superficially appear to be small versions of some vascular plants such as the horsetails, but which are really unique members of the green algae (Chlorophyta) in the Family Characeae. Their apparent "stems", "roots", and "leaves" are not equivalent to these structures in the mosses or in the vascular plants. They are an ancient group with a fossil record going back in excess of 400 million years before the first land plants appeared. Long before the dinosaurs, they flourished in shallow fresh and brackish waters. No modern forms are truly marine.

Worldwide there are six groups of living charophytes, the genera *Chara*, *Nitella*, *Tolypella*, *Nitellopsis, Lamprothamnium*, and *Lychnothamnus*. Lamprothamnium is not known from North America. The most recent North American list (Scribailo and Alix 2010) reports 84 species. Whether these will all prove to be good species will require considerable future investigation. Currently 11 of the 84 species are known from the Province of Newfoundland and Labrador, 7 in the genus *Chara*, 3 in the genus *Nitella* and 1 in the genus *Tolypella*. The variation within the Newfoundland species has not yet been adequately assessed. As the flora of our provincial waters becomes better known, we may expect the discovery of more species and be able to describe more sub-species/varieties.

Stoneworts are small slender "plants" rooted in the aquatic substrate by fine thread-like rhizoids. Branching stems extend upward into the waters. They normally range in length from a few centimeters to 30 cm or more depending on the species and the growth conditions. All genera are based on a similar body plan, a main axis (stem) which is composed of nodes (joints) and internodes (the stem sections between joints). At the nodes two features occur, branches and branchlets. Branches are divisions of the main stem to produce a more bushy plant (Figure I:1). Stems of some species branch more than others. In all charophytes whorls of branchlets occur at the stem nodes. These are the structures on which the sex organs (gametangia) are produced (Figure I:2). Branchlets vary structurally in the three genera *Chara*, *Nitella* and *Tolypella* and are among the chief features distinguishing the three genera from one another. The unique sex organs (gametangia) when present are major distinguishing features of the stonewort group since nothing else with similar structures can be found amongst green plants or algae. These and other features used to identify genera and species will be discussed and described under each of our three NL genera. Potential new discoveries in the province are also suggested. Although technically the term "plant" is only used for mosses and vascular plants, not algae, we still sometimes refer to them as "plants" because of the charophytes' large size and appearance unlike that of the thalli of most green algae, but similar to the higher plants.

Since there is no other green plant-like group that is structured like the charophytes, a unique terminology has developed to describe their features. It is necessary for beginners to become familiar with these terms since they are commonly used, often without explanation in the literature. Such terminology is kept to a minimum in this introductory work and is explained when used in context and as diagrams, illustrations and photos.



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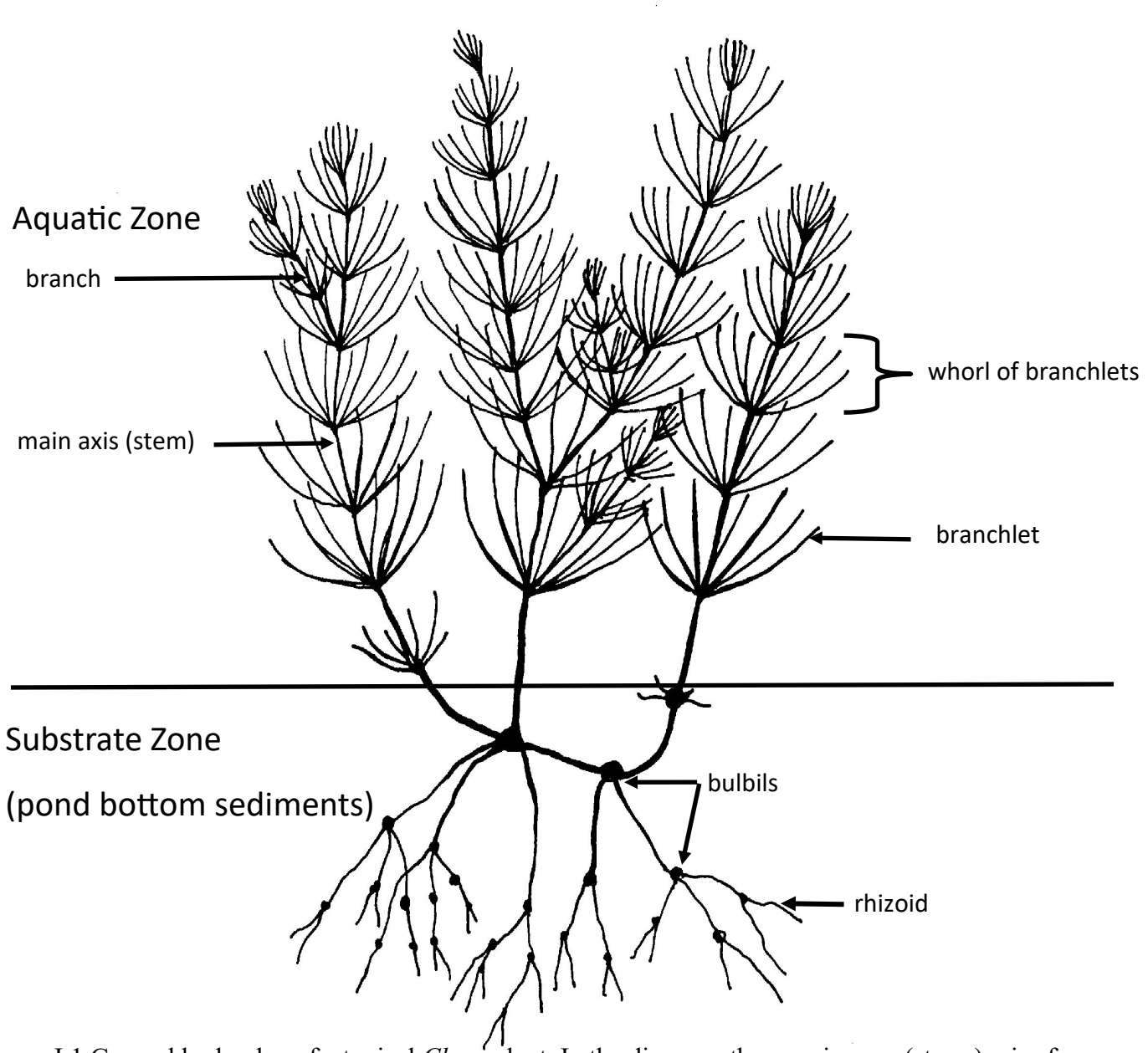


Figure I:1 General body plan of a typical *Chara* plant. In the diagram, three main axes (stems) arise from rhizoids in the substrate. The two main axes on the left exhibit branching. At each of the stem nodes a whorl of branchlets occurs. Enlarged nodes in the substrate (bulbils) contain stored starches and are branching points giving rise to a number of green axes (stems) above the substrate. WH

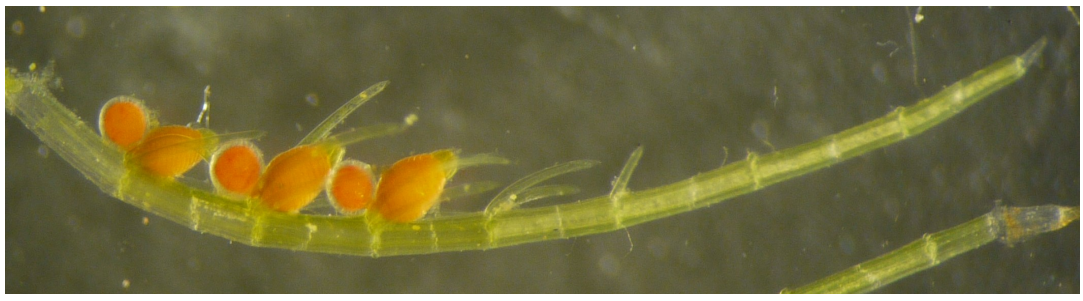


Figure I:2 An individual Chara branchlet with male and female reproductive structures (gametangia). HEM

Typical surface view of a serene Newfoundland pond with little indication of what lies below in its depths. HEM

To investigate stoneworts one must search the bottom of water bodies by whatever means are appropriate in the situation.

PM

II. The Genus Chara

Of our three Newfoundland genera, *Chara* is the most common and also has the most complex structure. Once familiar with the genus *Chara*, it can easily be distinguished from our other two genera *Nitella* and *Tolypella*. If the axis (stem) surface is covered with elongate cells (a cortex) then the specimen is a *Chara*. However, a few Chara species which have no cortication occur worldwide, but which still have all the other diagnostic features of the genus Chara. None of these have yet been reported for NL.

General Appearance

Like all charophyte shoots, *Chara* has a main axis ("stem") which is divided into nodes (joints) and internodes (the stem portions between nodes). At each node a whorl of branchlets is produced (Figure II:1). The branchlets themselves are also organized into nodes and internodes. At the branchlet nodes smaller bract cells are usually produced. Also at these nodes the male and female reproductive structures (gametangia) are located when present (Figure II:2). In *Chara* the male gametangia (antheridia) occur below the female gametangia (oogonia) in species that have both male (♂) and female (♀) gametangia on the same plants (monoecious species). Some *Chara* species have antheridia and oogonia on separate plants (dioecious species). At the main axis nodes below the branchlets are also produced one or two rows of cells known as stipulodes (Figures II:3 and II:6). Their arrangement and shape are useful identification features. The main axes often may produce side branches at the nodes which have the same structure as described above. Branching produces a more divided "bushy" type of *Chara* structure. In the pond substrate (mud, sand, etc.) the axes produce a series of fine treads (rhizoids) which anchor the plant. At nodes of substrate axes and/or on rhizoids, enlarged starch-filled structures known as bulbils may be produced (Figure 1:1). In some species bulbils are characteristic (e.g. *C. aspera*) and diagnostic for identification.

Axis Cortication

In the genus *Chara* the axis internodes are each composed of one long large central cell sheathed around on its outside by smaller cells known as cortical cells. The arrangement of the cells in this surface cortex is an important identification feature. It is important for the novice to become acquainted with the common patterns and their terminology (Figures II:4 and II:5). Elongated cortical cells occur in rows running between two axis nodes. Two types of rows may be distinguished, rows in which the elongated cells have smaller cells (spine cells) where their ends meet (primary cortical rows), and rows where there are no spine cells where their ends meet (secondary cortical rows). Normally there is one primary row for each branchlet. The haplostichous condition occurs when there are only primary rows with spine cells and no secondary rows. The axis is diplostichous when the primary rows are separated by one secondary row, and triplostichous when the primary rows are separated by two secondary rows. One way of avoiding this technical terminology is to refer to the stem as having a single cortex, double cortex or triple cortex, however, the haplo-, diplo-, triplo- terminology is most often used in most publications. In addition, sometimes the primary cortical rows are more prominent (thicker) than the secondary rows (tylacanthous) and sometimes less prominent (aulacanthous). If primary and secondary rows are more or less equally prominent, the pattern is termed isostichous (Figure II 5). These three features are often also useful for species identification.

The smaller cortical cells in the primary rows are called spine cells (Figure II:6). Often there is only one spine cell where two elongated primary cells abut, but in some species a cluster of two or more may occur. The shape, size and number of spine cells are characteristic of the species and are useful for identification. Some terms used to describe spine cells include rudimentary (tiny, insignificant, difficult to see), globular (tiny, rounded), papillate/papillous (little short nipples), elongate (long), spinulose (long, thin, spiny), etc. Often spine cell length is compared to the width of the main axis (stem) on which they are located (e.g. shorter, as long as, up to twice the length, etc.). It is best to examine the spine cells in the younger mature internodes

behind the growing tip of the stem. In the older stems they are often lost or obscured.

It seems like it should be easy with a stereoscope to determine whether a Chara axis has only primary rows of cells with no secondary rows (haplostichous), each primary row alternating with one secondary row (diplostichous) or one primary row alternating with two secondary rows (triplostichous). However, often as growth occurs there is some cortex cellular rearrangement of secondary row cells, especially where their ends overlap, to obscure whether there are two secondary rows or really only one. It is often best to examine younger internodes behind the growing tip if uncertainty exists. Also young actively growing specimens are easier to determine than older more senescent material. Two of our Newfoundland Chara species are haplostichous (*C. canescens* and *C evoluta*). Two are dipolstichous (*C. contraria* and *C. vulgaris*). Three are triplostichous (*C. aspera*, *C. globularis*, and *C. virgata*).

There is a Chara species not yet reported for Newfoundland/Labrador, but likely to be found here in the future, which has no cortex over its large stem internodal cells or on branchlets, so might be superficially confused as a *Nitella*, but it has all of the other important taxonomic features of a Chara (*Chara braunii*). It will be described at the end of Section III (III:8). Occasionally Chara material with partially absent or abnormally appearing main axis cortication can be found. When growing such in culture it will often revert to typical cortication of the species indicating that the abnormality was caused by environmental factors during growth.

Branchlets

Branchlets arise in whorls at axes nodes. In *Chara* the branchlets, like the main axes, are also corticated, covered with elongated cells into nodes and internodes (Figure II:7). Nodes are sites where gametangia are located along with associated bract-like cells. Each branchlet ends in one or more naked cells which have no cortication. These naked end cells are often short, but sometimes in some varieties may become quite elongate. Also in some varieties the cortication may become disorganized or entirely absent producing naked branchlets. It is often not clear whether these unusual features are genetic or due to environmental growth factors. Sampling in a number of different locations in the same water body (deeper, shallower, still water, wave-washed conditions, shaded, exposed, solitary, mixed with other vegetation, etc.) may provide some clues as to whether unusual variations may be ecologically induced or perhaps genetic. Also water quality parameters such as nutrient richness, salinity, pollution, as well as the population density of herbivore invertebrates that feed on charophytes (e.g. scuds, beetles, crayfish, etc.) may induce some structural (morphological) variation from one water body to another within the same species. Considerable morphological variation is common within the charophytes, especially in the cortication features of *Chara*, and it is often difficult to determine the roles played by the environment and that of genetics. Growing unusual material in culture is often helpful in this regard.

At the branchlet nodes, smaller cells known as bract cells (bracts), and bracteoles are produced Figure II:7). Bract cells may be tiny or elongate depending on the species and position. A bract-like pair which arise on either side at the base of an oogonium are termed bracteoles. They are often longer than other bracts at the node. In dioecious species (e.g. Chara aspera) an additional cell occurs where the antheridium is normally located in monoecious species at the base of the oogonium, and is termed a bractlet (Figure II:8).

Gametangia

Gametangia are produced at the branchlet nodes as are small cells (bracts, bracteoles and bractlets) associated with the gametangia.

Male gametangia (antheridia) are globular structures normally enclosed by eight triangular shield cells (scutes) arranged edge to edge to form the outside wall of the globe (Figure II:9). On the inside of the shield cells are filaments which produce the motile sperm. At maturity the antheridia burst and the sperm swim to fertilize the eggs inside the oogonia. In *Chara*, antheridia tend to be yellow-green to bright orange at maturity.

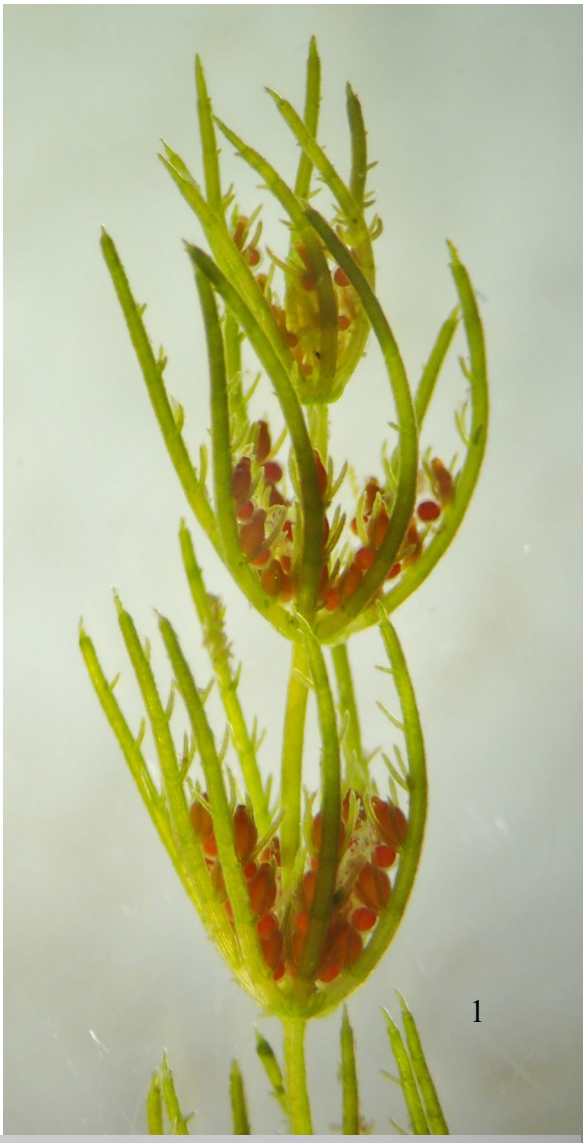
In monoecious *Chara* species antheridia are located directly below the oogonia, usually singly, or sometimes in pairs. In dioecious *Chara*s they occur at branchlet nodes only on male plants. Only two of our known Newfoundland *Chara* species are dioecious, *C. aspera* and *C. canescens*. For beginning studies antheridial structure is not very important for identification purposes, except perhaps size, but in advanced studies their microscopic features can be significant.

The female gametangium is termed an oogonium. In our monoecious *Chara* species it is situated above the antheridium at branchlet nodes (Figure II:10). A central large egg cell is surrounded by five cells which spiral clockwise upward from the base to enclose the egg. At the upper tip of each of these spiral cells, a smaller cell is produced so the oogonium is capped by five smaller cells forming a "crown" termed a coronula. Oogonia may vary in colour from yellow-green to bright orange in our species of *Chara*. In Chara, oogonia are terete, that is, they are round in cross section and when viewed directly from the tip or directly from the base. Oogonia size and shape and the size and shape of the coronula are often characteristic of the species. After fertilization the inner egg develops into a spore (oospore) forming a thick tough outer wall and turning dark, usually brown to black. Eventually the outer enveloping spiral cells disintegrate and the spores accumulate in the pond substrate to germinate into new plants when conditions are favorable, some remaining dormant for years. Spores exhibit the grooves and ridges left by the five spiral cells that once surrounded the oogonium. In advanced studies microscopic features of the oospore wall are sometimes utilized including the number of ridges from the base to the tip and markings on the groove surfaces (membranes). Determining membrane ornamentation requires high power compound microscope examination. SEM examination provides finer, more detailed structure, but is beyond the scope of introductory charophytology. Oospore membrane ornamentation is especially significant in taxonomic studies of the genus *Nitella* , less so in the genus *Chara*.

Rhizoids and Bulbils

The bases of the upright *Chara* axes penetrate the muddy substrate, some growing horizontal and spreading. At the nodes of these buried "stems" prominent enlargements known as bulbils may be present in some species (Figure II: 11). These are storage sites of nutrients and starches and can produce new shoots in favorable conditions. They are often sought after by ducks and geese for their starch content. From these subterranean stem nodes a fine meshwork of cellular threads (rhizoids) penetrate the substrate. In some species these rhizoids produce white spherical bulbils, a distinguishing feature of our *Chara aspera*. Bulbils may also be found in other charophyte genera.

Figure II:1 General features of a Chara "plant". 1. A branching plant with whorls of branchlets at the axis (stem) nodes. 2. Enlarged details of a main axis (stem). 3. Cross section of a main axis (stem). 4. One branchlet enlarged with attached sex organs (gametangia) at the branchlet nodes. MIG



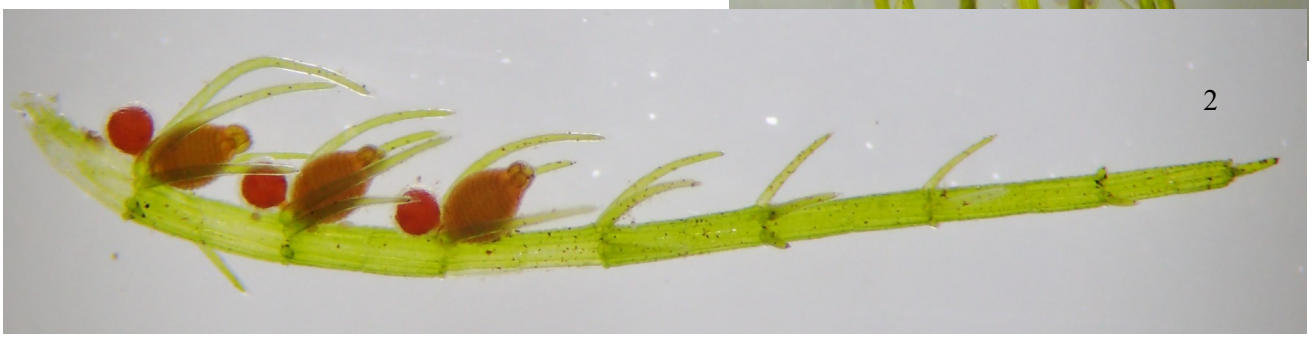


Figure II:2 Photos of a live Chara stem tip. 1. Stem (axis) tip with three whorls of branchlets. 2. Enlarged single branchlet with mature male and female sex organs (gametangia). HEM

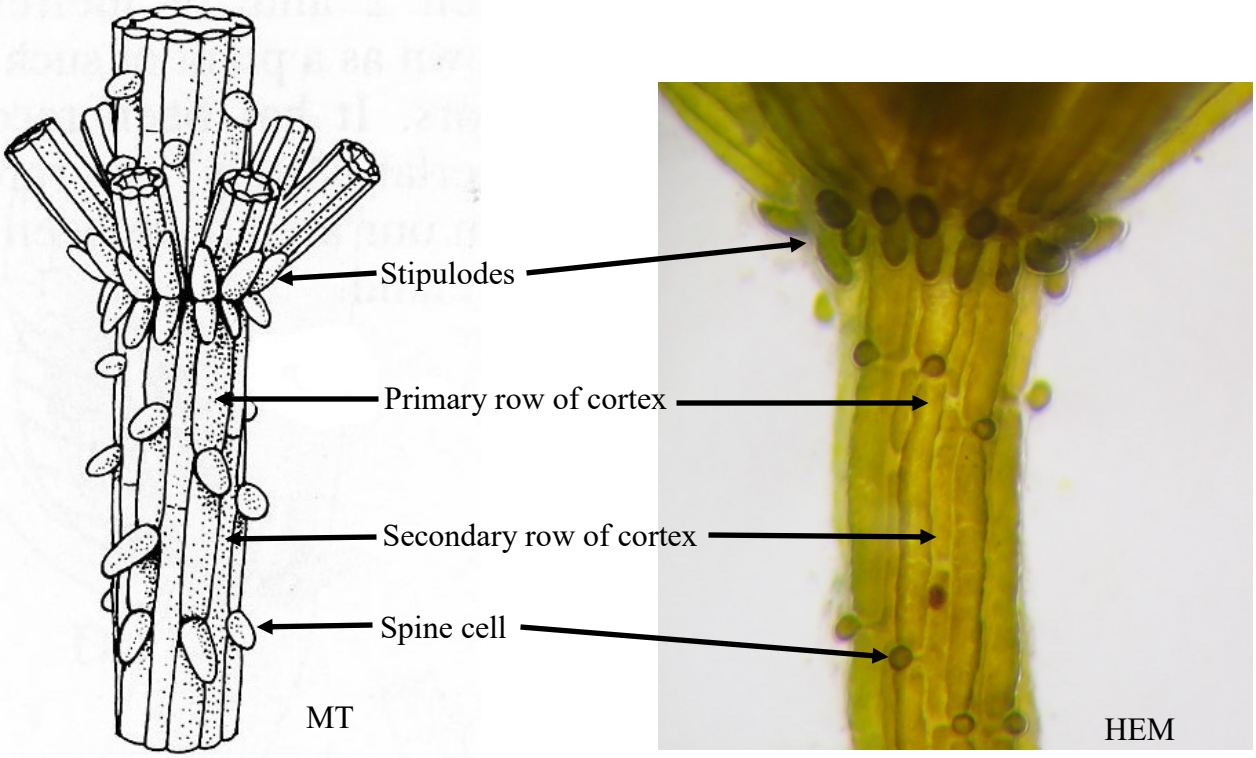


Figure II:3 Above: Labelled diagram and photo of a portion of the main axis (stem) of a Chara featuring stipulodes, spine cells and rows of cortex cells of the axis. Below: An unlabelled photo showing similar features.

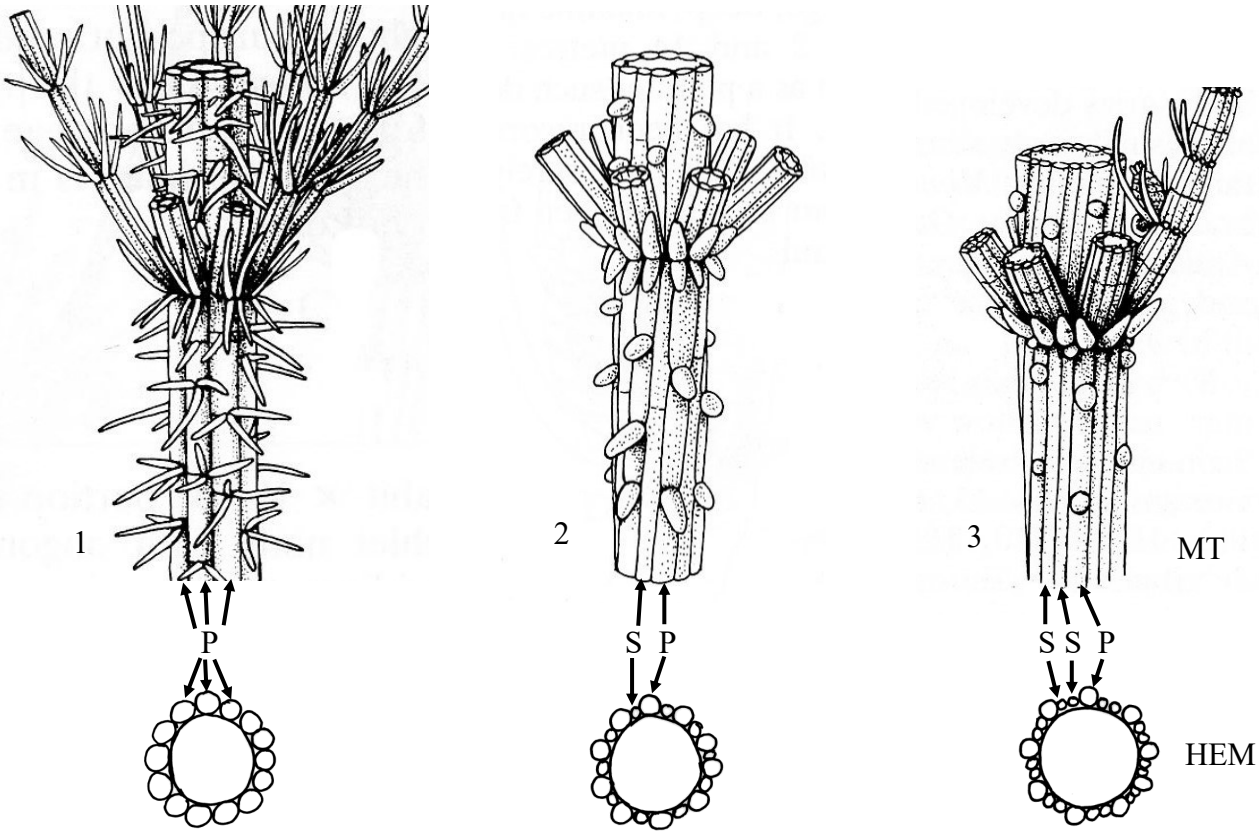
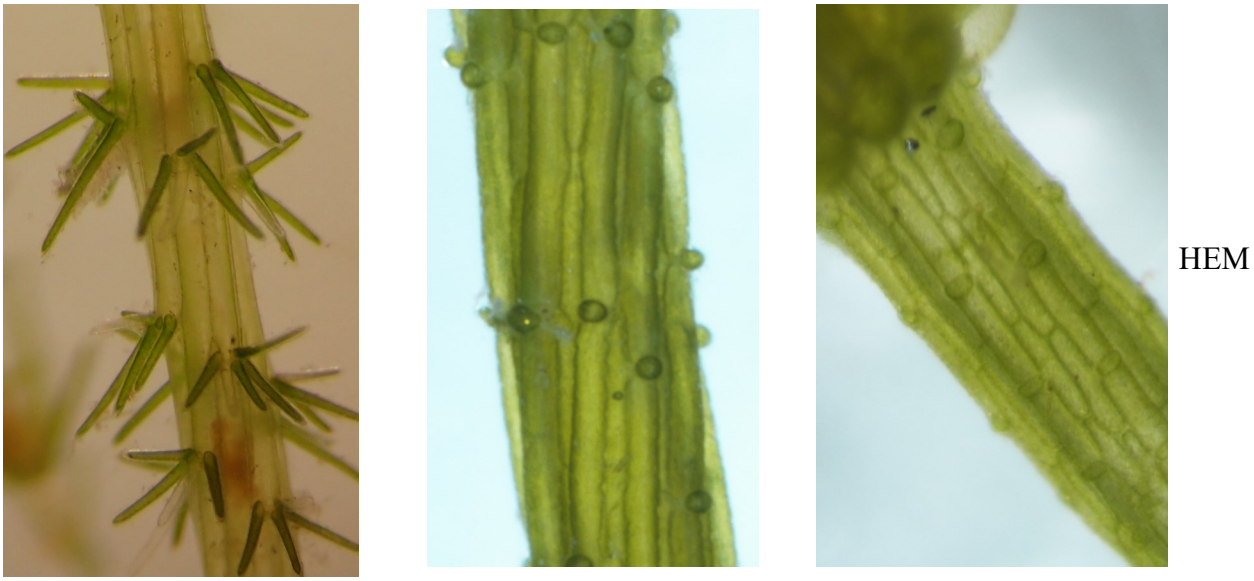


Figure II:4 Portions of three Chara axes in side view showing different cortical cell arrangement patterns. Beneath each is a cross section view of the main axis. For clarity, spine cells have been omitted on the cross sections. 1. All cortex rows in this species have spine cells, all are primary (P) rows. This condition is known as “haplostichous”. 2. Each primary row (P) is separated by one secondary row (S). This condition is known as “diplostichous”. 3. In this species, each primary row (P) is separated by two secondary rows (S). This condition is known as “triplostichous”. Below are photographic examples of the three cortex patterns illustrated above. Note that in example 1 spine cells are in clusters in the primary rows; in 2 and 3 they are singly produced in the primary rows.



Tylacanthous Aulacanthous Isostichous

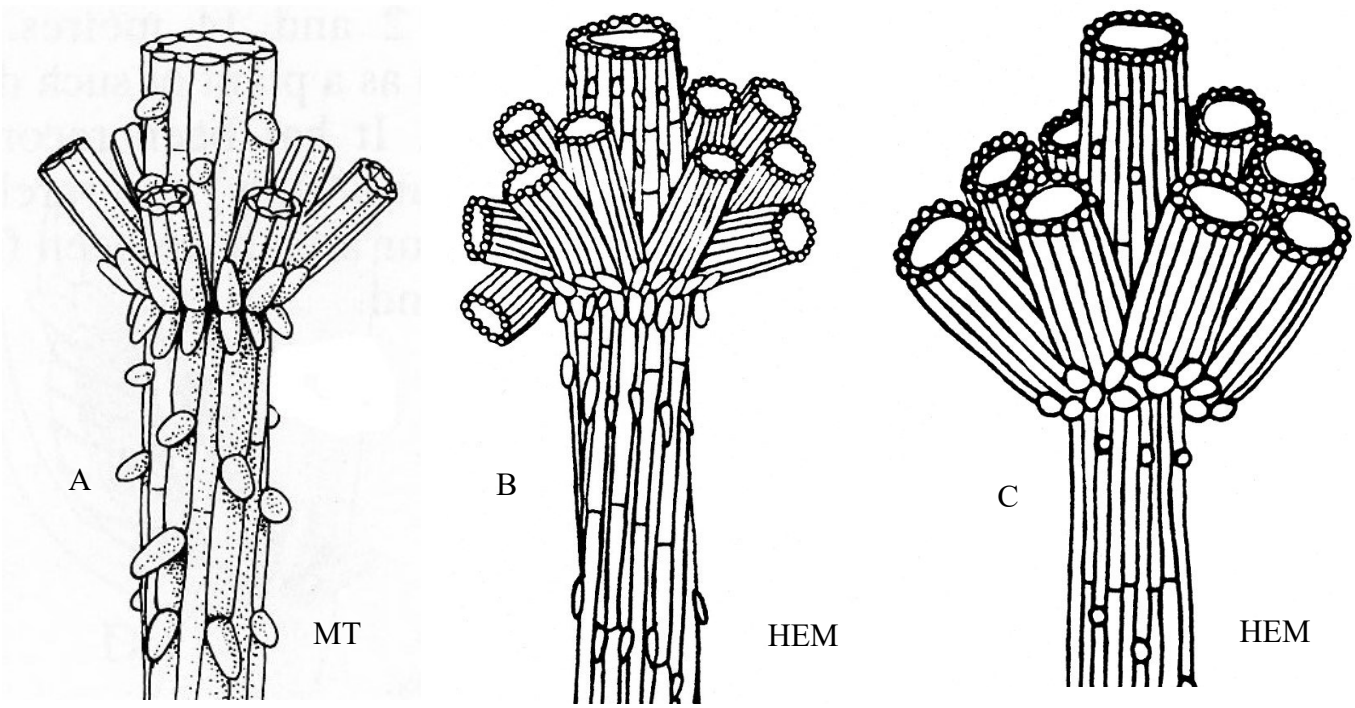
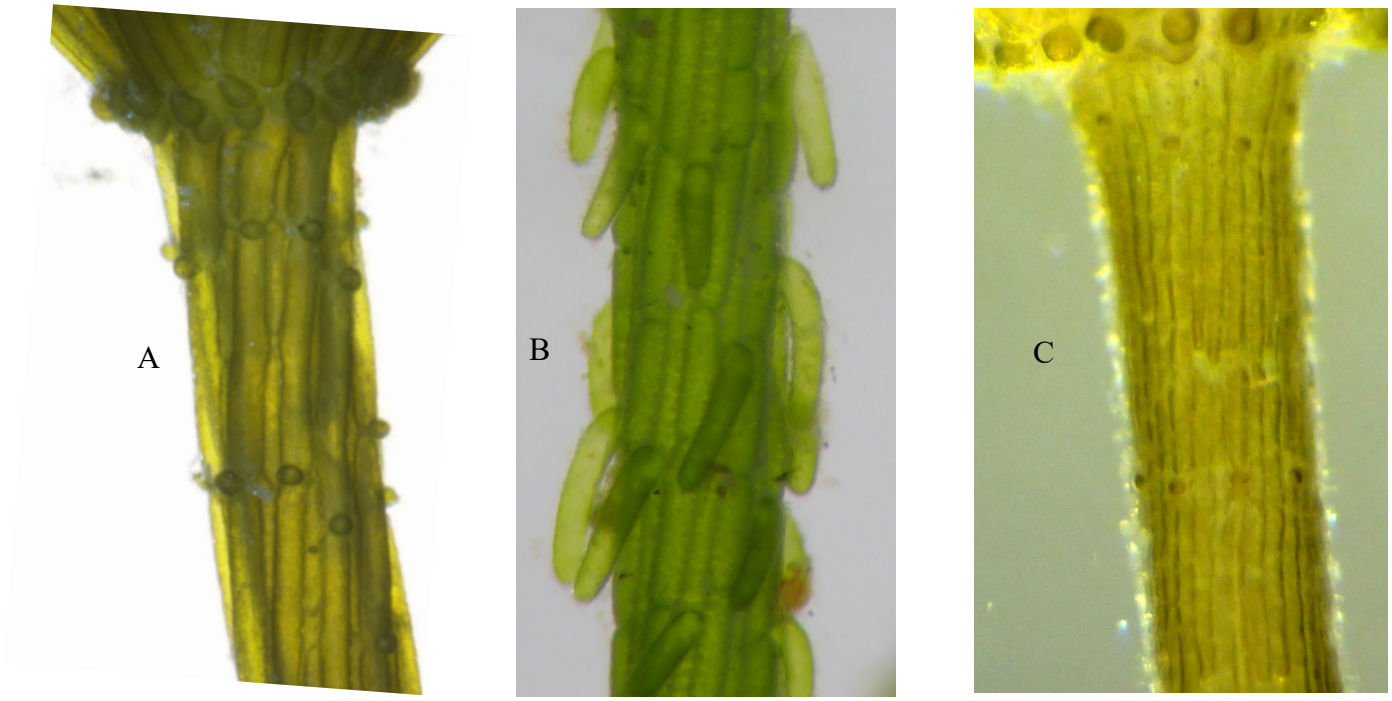


Figure II:5 Comparative size of primary and secondary cortex rows on the main axis (stem) of Chara. A. The "tylacanthous" condition when primary rows are more prominent (larger) than secondary rows. This example is also "diplostichous". B. The "aulocanthous" condition when secondary rows are more prominent than primary rows. This example is also "diplostichous". C. The "isostichous" condition when primary and secondary rows are the same size. This example is "triplostichous". Below is a photographic example of each term. HEM



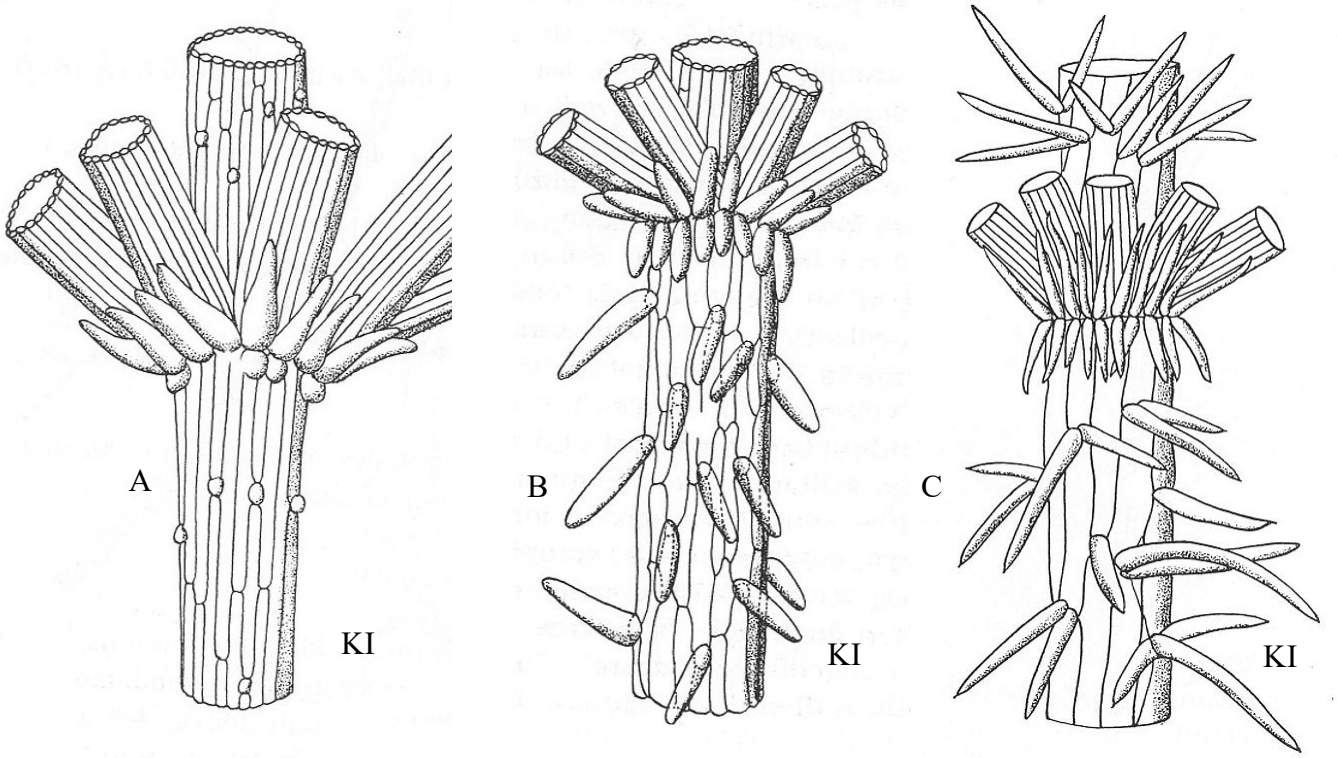
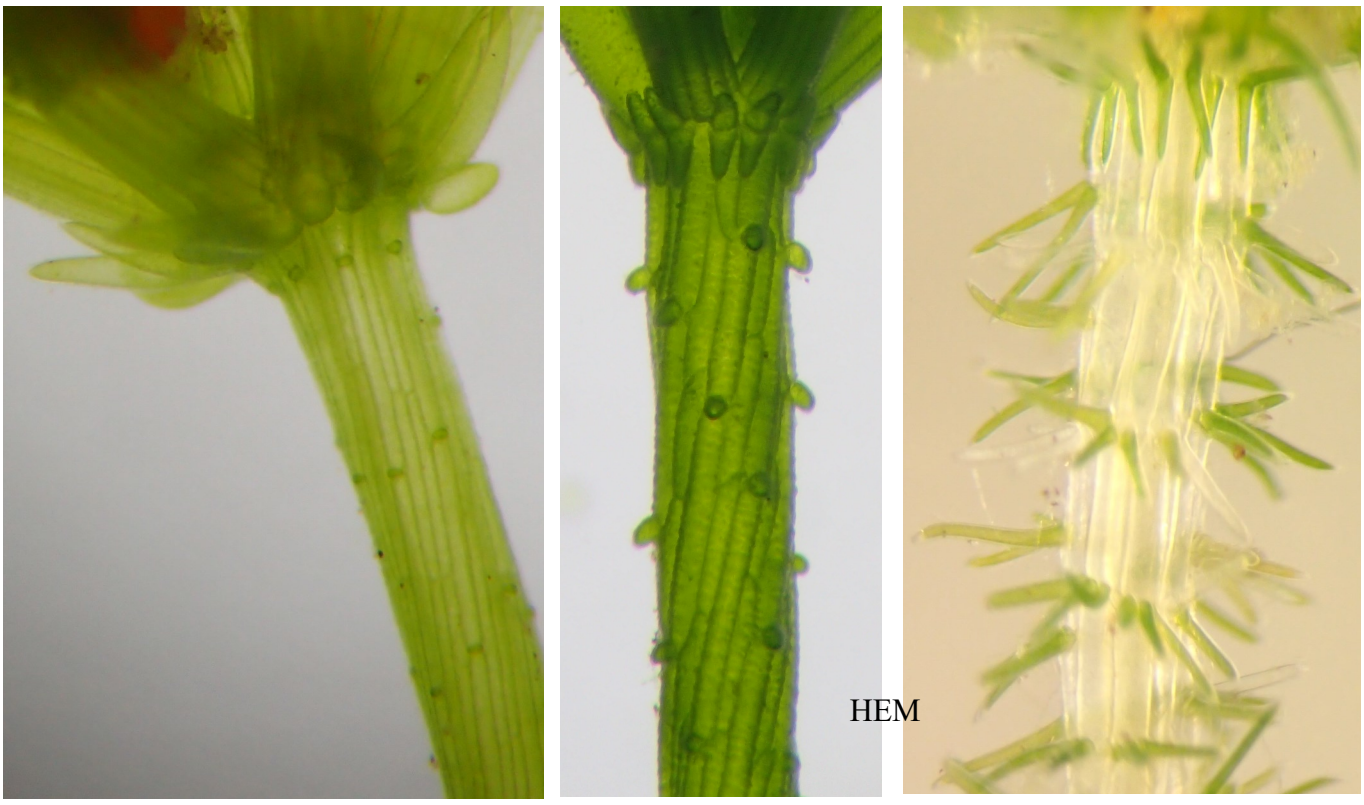


Figure II:6. Above: Spine cell and stipulode examples. A. Spine cells produced singly, rudimentary/ globular. Stipulodes, two rows, upper elongate conical, lower row, globular/rudimentary. B. Spine cells produced singly, elongate, up to width of stem, tips rounded. Stipulodes in two rows, upper slightly longer than lower, round tipped. C. Spine cells single and in clusters of two or three, elongate to slightly longer than stem width, sharply pointed. Stipulodes narrow spiny, sharp tipped, upper row longer than lower. Below: Photo examples of above features.



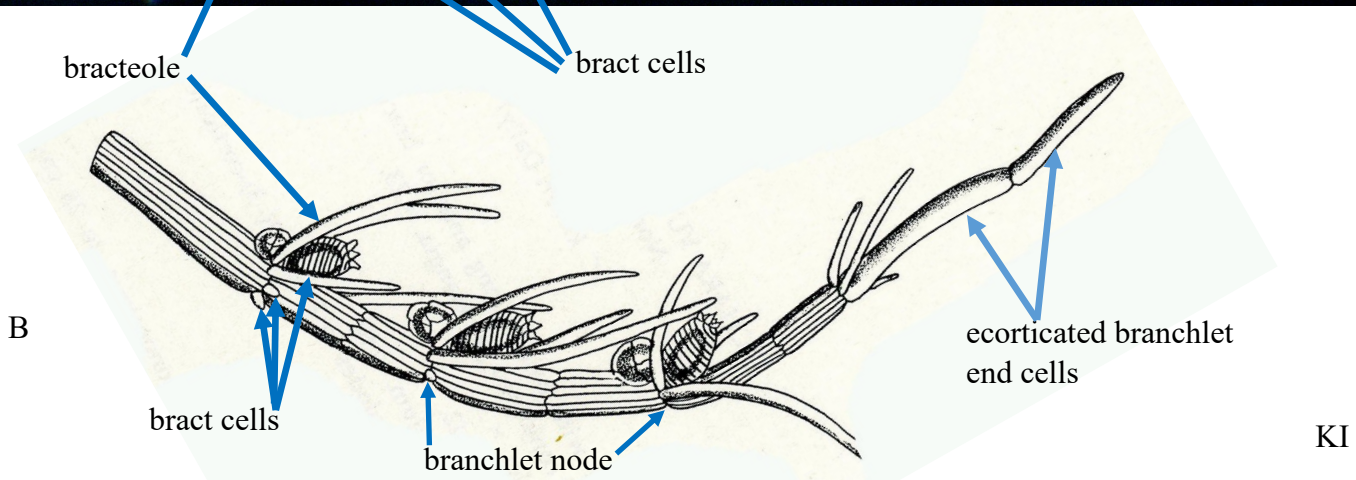


Figure II:7 Branchlet features. A. Enlargement of two branchlet nodes with antheridia located beneath oogonia. Bracteoles are two cells on either side at the base of the oogonia. The other cells around the branchlet are termed bracts. The length of bracteoles and bracts vary with the different species and are often characteristic. B. Illustration of an entire typical branchlet showing three fertile nodes and two non-corticated (naked/ecorticate) end cells.

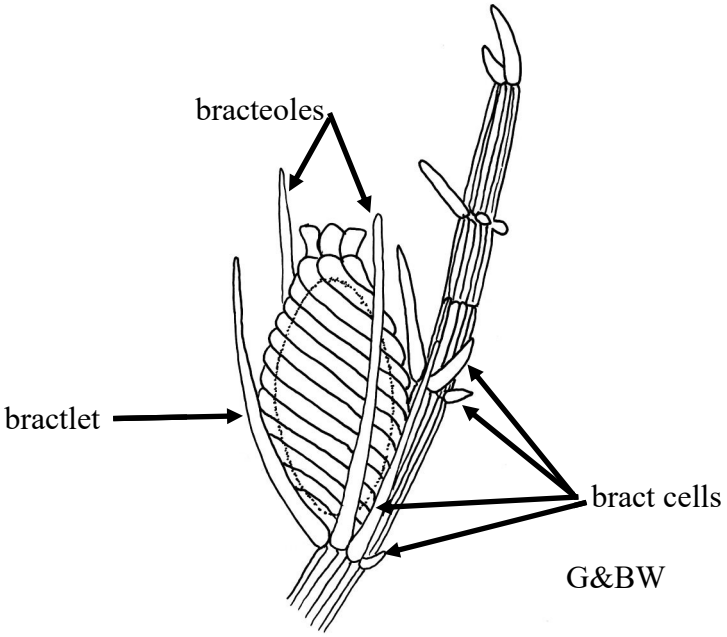
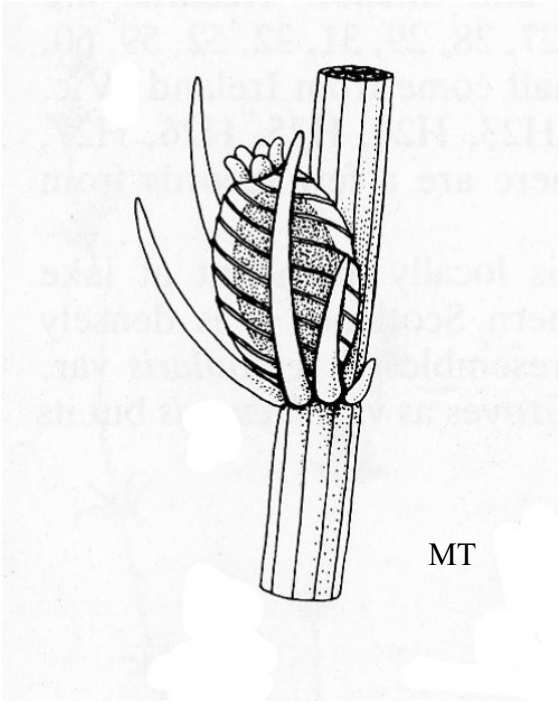


Figure II:8 Above: In dioecious species (e.g. *Chara aspera*) female plants produce only oogonia. In the place where an antheridium would occur in monoecious species, a bract-like cell is produced, termed a bractlet. Below: An unlabelled illustration showing bracteoles, bract cells (bracts), and a bractlet.



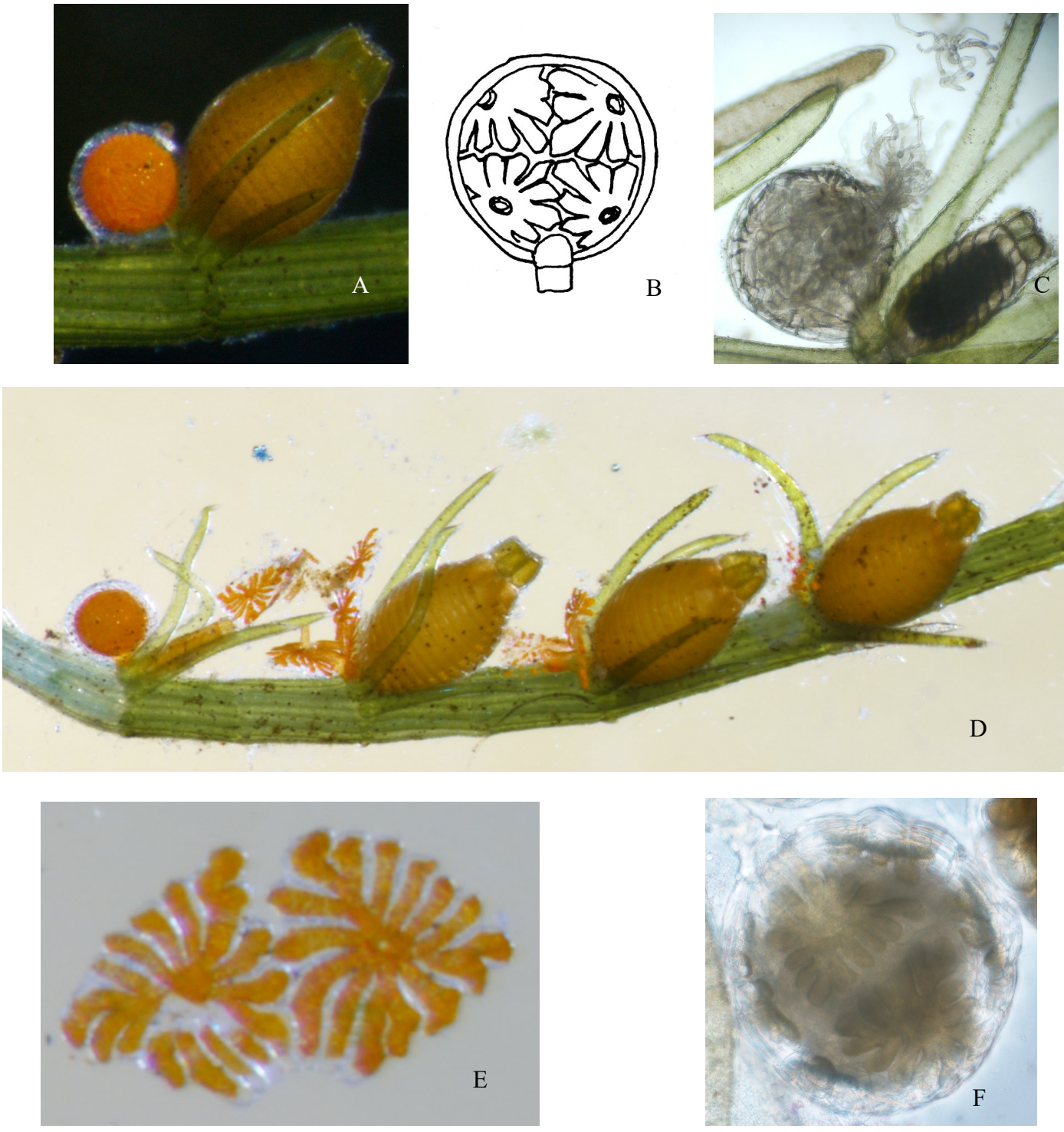


Figure II:9 Antheridium features: A. Mature antheridium located at base of mature oogonium. B. Illustration showing four of the eight triangular shield cells (plates/scutes) forming the wall of the globular structure. C. An antheridium beginning to burst releasing filaments with sperm cells. D. Shield cell remnants at three branchlet nodes, the fourth antheridium still intact. E. Enlarged photo of two shield cells. F. Photo of a liquid preserved antheridium with two shield cells fully visible. HEM

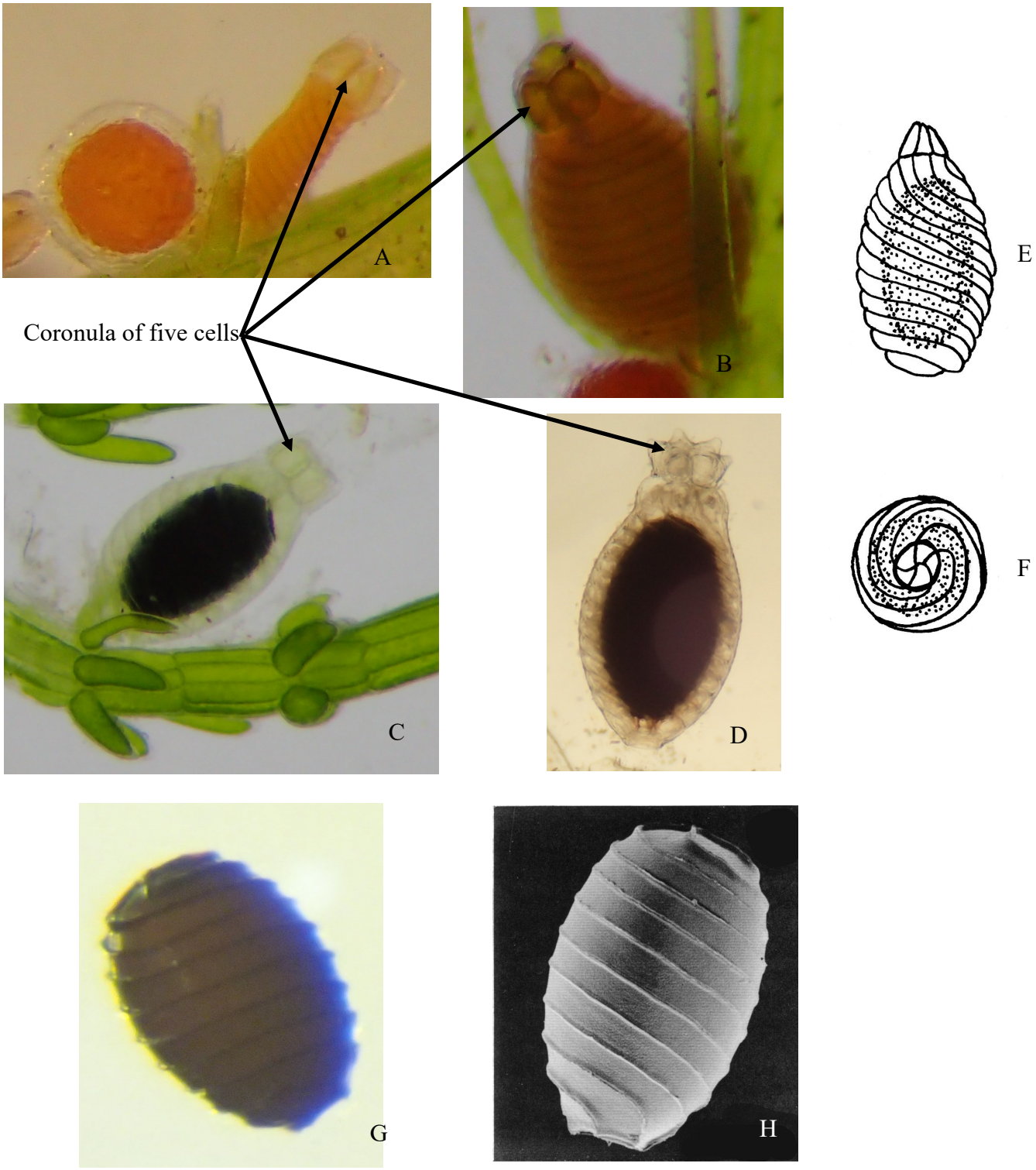
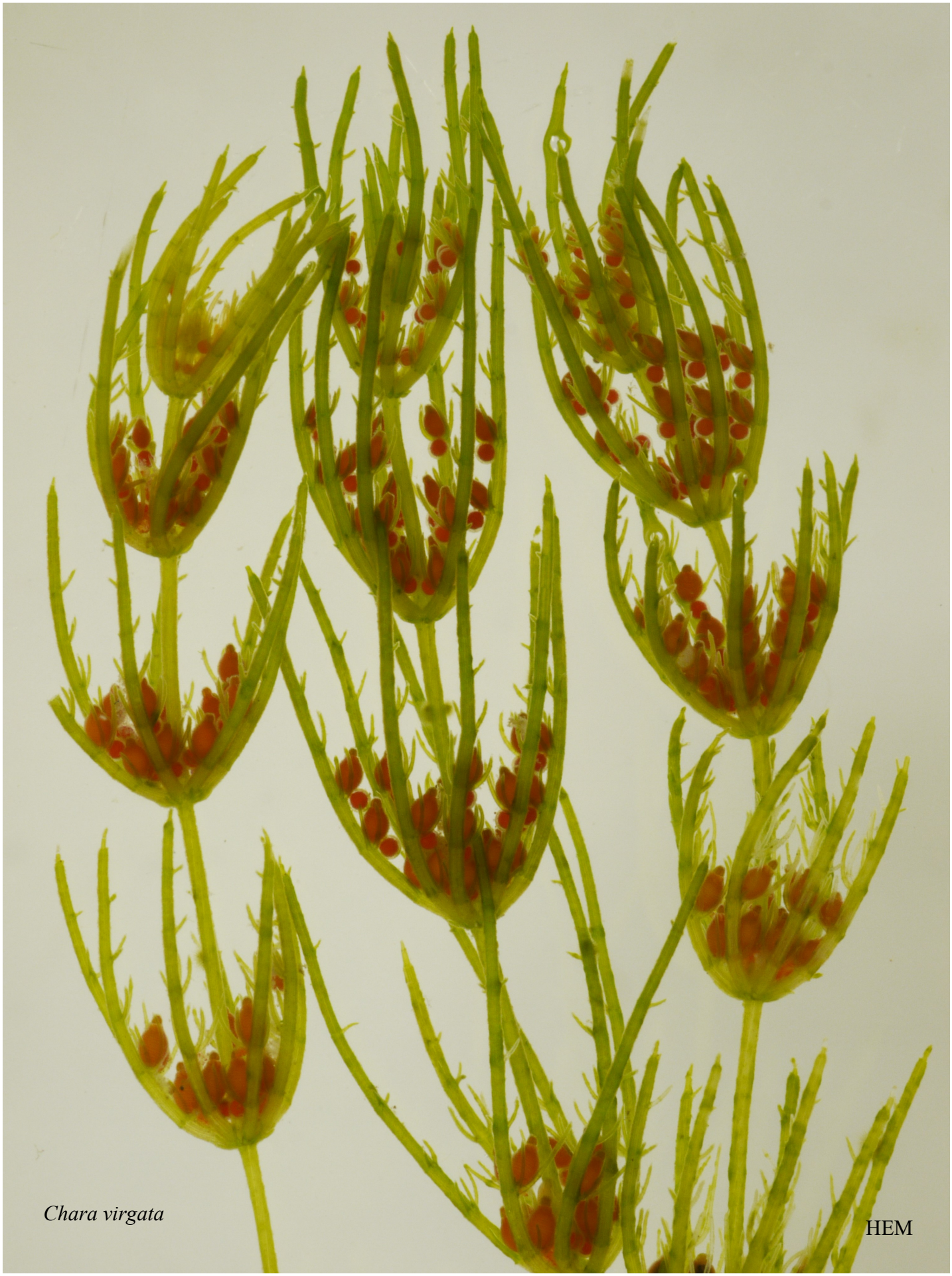


Figure II:10 Features of oogonia and oospores: A. Immature oogonium with mature antheridium. B. Mature oogonium. C. and D. Oogonia with internal mature oospores. E. Illustration of an oogonium showing prominent spiral cells surrounding the interior egg cell (stippled). F. Top (apical) view showing that the Chara oogonium is round (terete) in cross section, and featuring the characteristic five coronula cells at the apex. G. A light microscope photo of an oospore free of the spiral oogonial cell envelope. Note the grooves between the spiral ridges where the spiral oogonial cells were located. H. An SEM photo of a Chara oospore. HEM

Figure II: 11 Common bulbil types: A. Chara shoot with bulbils on the lower stems in the substrate. B. Photo enlargement of bulbils in A. C. Lower portion of a Chara with bulbils and rhizoids in a tight cluster. D. The most common type of bulbil is composed of a cluster of tiny cells at a subterranean stem node, also as in A, B, and C. E. Another type of bulbil is composed of a large single spherical cell (often clustered) attached to the thin transparent rhizoids in the substrate. This type is characteristic of *Chara aspera*. F. Enlargement photo of two *Chara aspera* bulbils. HEM



Chara virgata

HEM

III: Description of Newfoundland Chara Species

The following Chara species are currently known from the province Newfoundland and Labrador and are described in this section:

Chara aspera

Chara canescens

Chara contraria

Chara evoluta

Chara globularis

Chara virgata

Chara vulgaris

In addition, one Chara species (***Chara braunii***) without axis or branchlet cortication, which is not yet known from the province, will also be described. There is a high probability that this cosmopolitan species may be discovered here in the future. It has all the attributes of Chara except a total lack of cortication so it may initially confuse the novice.

III:1 *Chara aspera* Willd.

Dioecious. Triplostichous (axes cortication with primary rows separated by two secondary rows). However, cortication often tends to become irregular especially in older internodes sometimes appearing diplostichous. Tylacanthous (primary cortical rows more prominent than the secondary rows), although sometimes on older axes becoming isostichous. Spine cells are single, prominent from quite short to as long or longer than the axis diameter. Stipulodes are elongate, the upper row longer than the lower. Both spine cells and stipulode cells tend to have slightly expanded (bulbous) bases. Bract cells and bracteoles are shorter or only slightly longer than the oogonia. Bracts tend to be shorter than antherida diameter in male plants. Considerable variation in bract and bracteole lengths occurs in different collections. Oogonia have sharply pointed diverging coronula tips. Spherical whitish "chalky" bulbils up to 1 mm in diameter on the rhizoids are characteristic of the species.

In Newfoundland it is only known from 6 sites along the southwest coast of the Island in brackish coastal waters (Mann and Nambudiri 2005), and in one coastal freshwater pond (Cape Anguille Lighthouse Pond). Currently considered rare in NL (S Rank 2 = may be at risk). Only known from the Northern Hemisphere, North America, Europe, Asia and North Africa.

Figure III:1A. *Chara aspera* ♀ stem cluster with some mature orange oogonia. HEM

Figure III:1B. 1. Axis tip with very immature oogonia at branchlet nodes.
2. Axis tip with mature oogonia.
3. Magnified very young internode with prominent stipulodes and spine cells. HEM

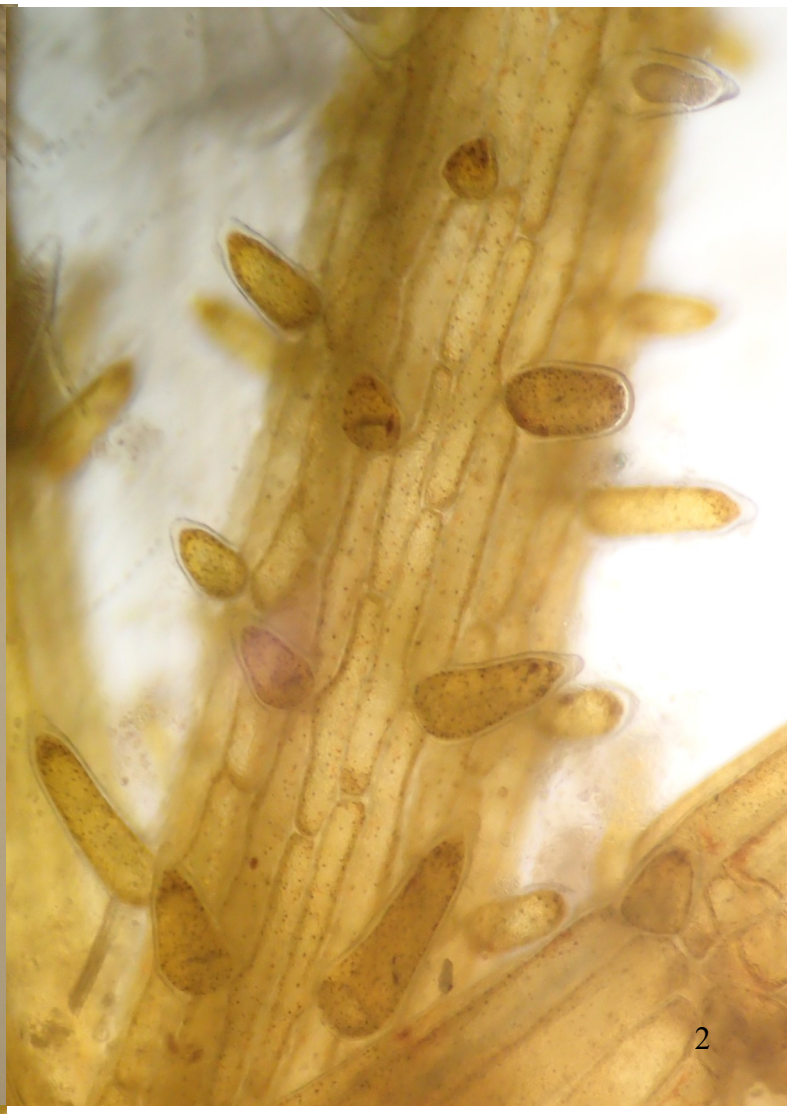
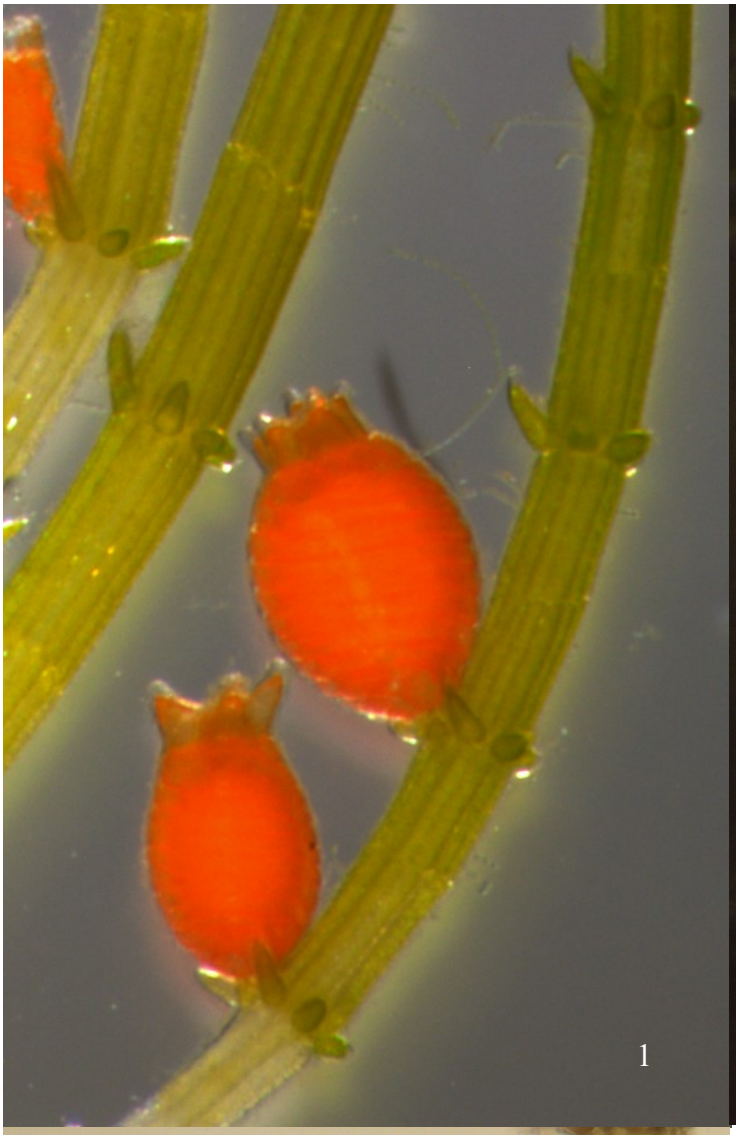


Figure III:1C. 1. Axis with irregular cortication not clearly exhibiting the triplostichous condition. 2. Axis clearly exhibiting the triplostichous and tylacanthous conditions. 3. Portion of axis below a node showing the enlarged bulbous nature of spine cell and stipulode cell bases. Cells of the upper stipulode row are somewhat longer than the lower row cells.

These three photos are from liquid preserved material which removes the green chlorophyll color of living material. HEM



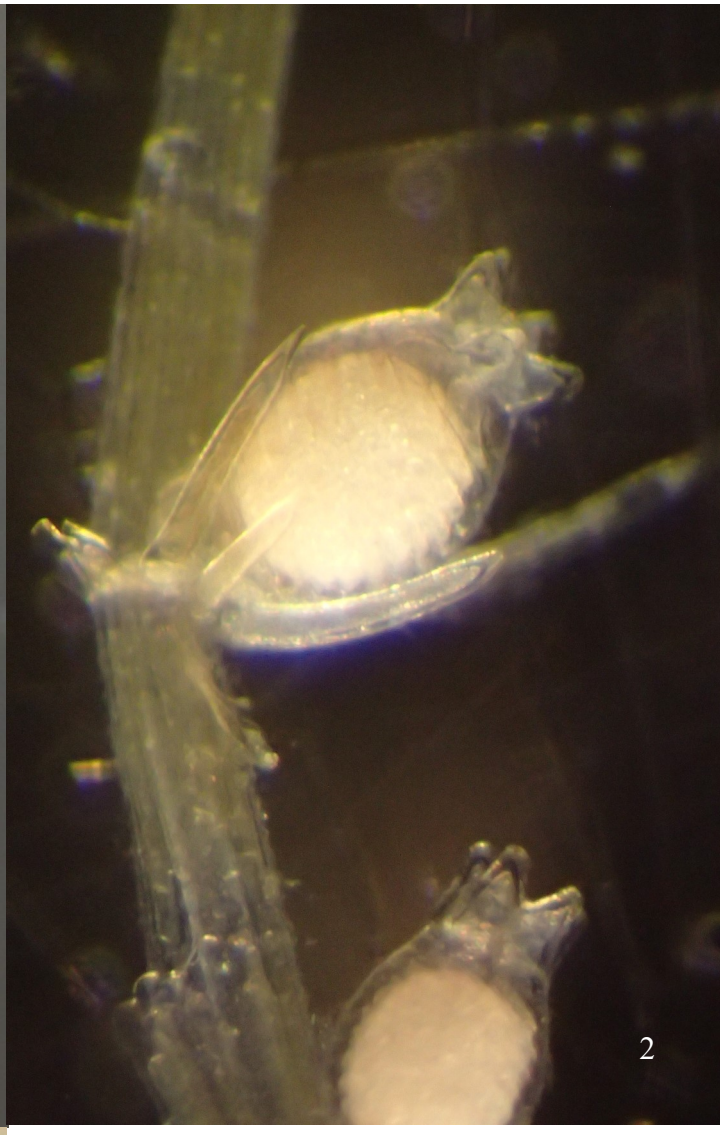


Figure III:1D 1. Mature oogonia in live material. 2. Mature oogonia in liquid preserved material. 3. Developing oospore in oogonium in liquid preserved material. Note the sharply pointed and diverging coronula tips in all three photos. Also note that the bracteoles in photo 1 are very reduced, but in 2 and 3 they are better developed and longer. This feature seems to be quite variable in different collections. HEM

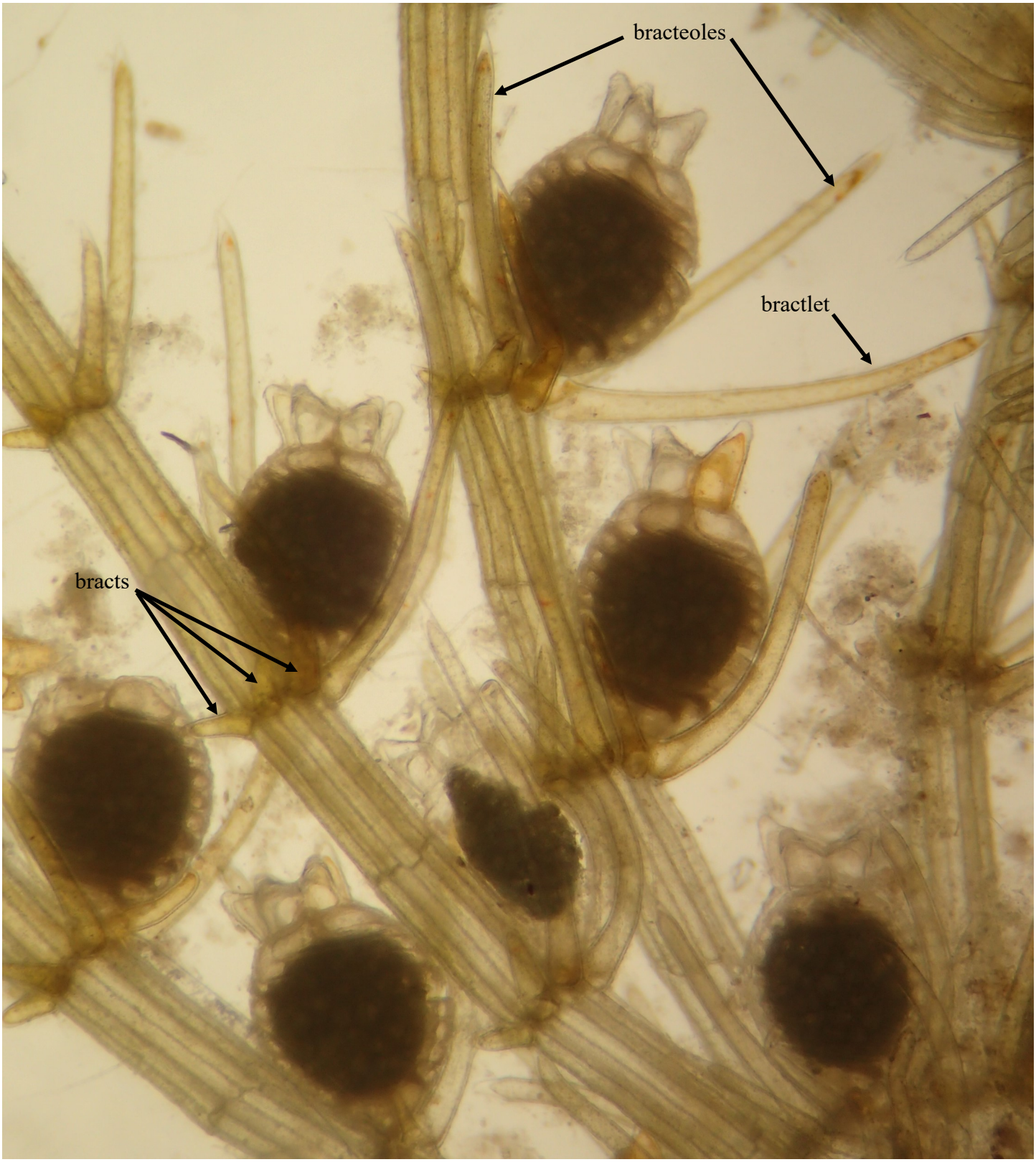


Figure III:1E. Several branchlets with oogonia containing developing oospores. Bracteoles and bractlets are as long or longer than the oogonium while posterior bract cells (bracts) are quite short. A "bractlet" is a cell in dioecious species which is located where the antheridium would occur in monoecious species (i.e. it is absent in monoecious species). Liquid preserved material. HEM

Figure III:1F. Highly magnified antheridium from liquid preserved material. Bracts are much reduced in this specimen, but may be as long as the antheridium diameter in some. Living colour of the antheridium would be bright orange. HEM

Figure III:1G. Upper photo illustrates spherical white bulbils up to one mm in diameter characteristic of *Chara aspera*. Currently, no other species is known to occur in NL with this type of bulbil. Lower photo is a highly magnified cluster of bulbils and transparent rhizoids on which they are produced. HEM

Figure III:1H. Coastal “barachois” ponds in the Grand Bay West area, Port aux Basque. These are also known as “salt ponds”. “Barachois” is a local term of the Canadian Atlantic region referring to saline types of coastal ponds/lagoons separated from the ocean by a sand bar but receiving occasional influx of marine water and spray thus producing fluctuating levels of dissolved salts in these habitats. *Chara aspera* as well as *C. evoluta* and *C. canescens* occur in these waters.

Upper photo is Rocky Barachois Pond. Lower photo is Saltwater Pond. HEM

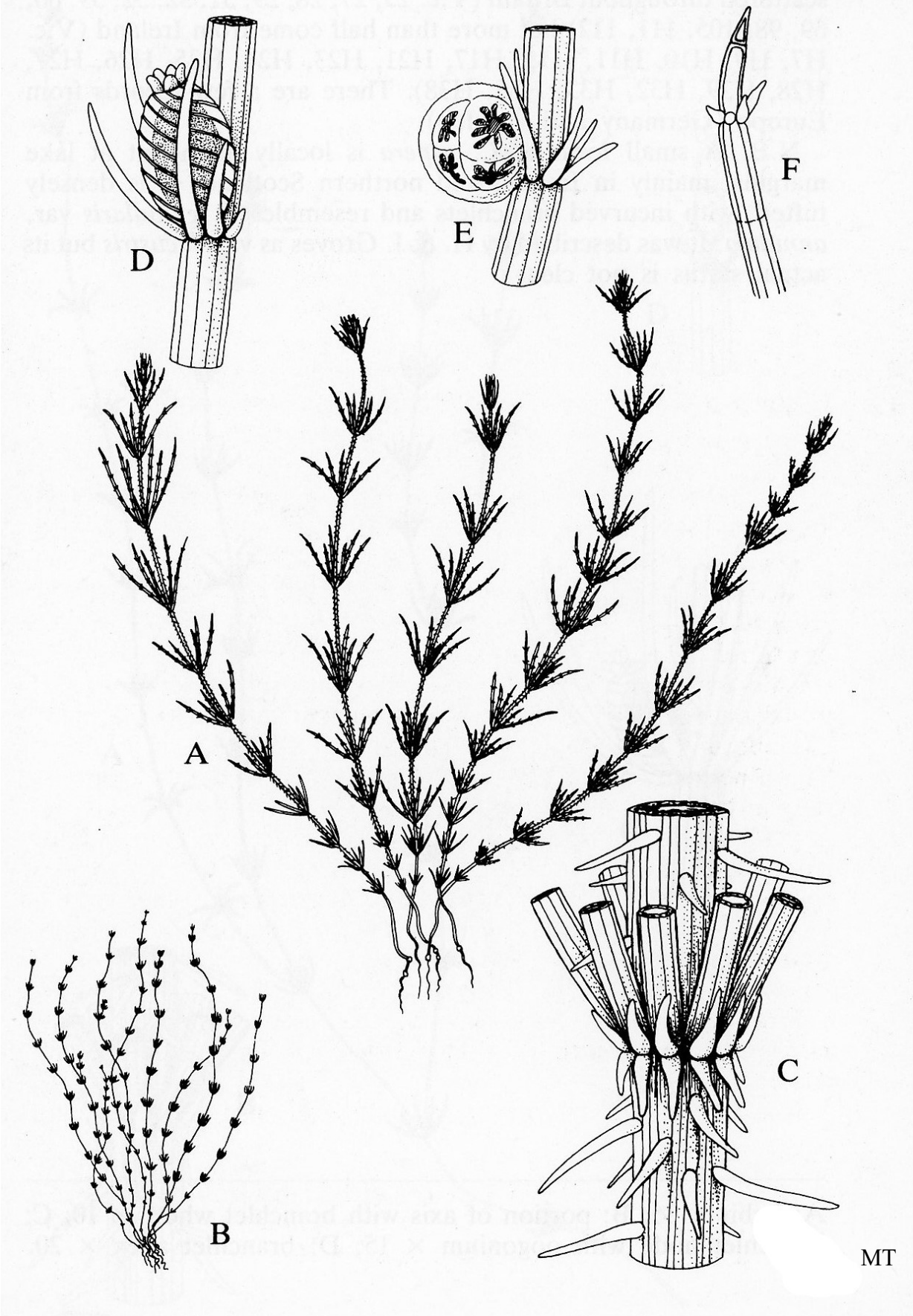


Figure III:1I. *Chara aspera* illustrations from J.A. Moore. 1986. Charophytes of Great Britain and Ireland. BSBI Handbook No. 5, Botanical Society of the British Isles. London, UK.

III:2 *Chara canescens* Desv. & Lois.

Dioecious. Parthenogenetic. Only female plants known from North America. Male plants with antheridia known from parts of Europe and Asia.

Cortication haplostichous, more neatly so and regular compared to *C. evoluta*.. Spine cells are elongate and acute tipped, occurring in clusters of 4 to 6 giving the axes a bristly appearance. All or most spine cells of a cluster tend to be equally long compared to one long and one or two shorter as in *C. evoluta*. Stipulodes are well developed and elongate in both rows, the upper slightly longer than the lower. Coronulas are smaller than those of *C. evoluta* (less than 180 micrometers wide). In deeper lagoons the axes may be 30 cm or more in length whereas in shallow ephemeral coastal pools plants may only be a few centimeters.

C. canescens is a brackish water species, to date only collected in coastal southwest Newfoundland at four sites, tthree coastal saline lagoons/barachois, (Grand Bay West Barachois, St. George's Pond and Gravels Pond) and one in shallow ephemeral pools in the Aguathuna Limestone Quarry on Port au Port, subject to sea spray. Globally it is known only from the northern hemisphere in North America, Europe, Asia and Africa. Considered Rare in Newfoundland (S Rank 1 – at risk).

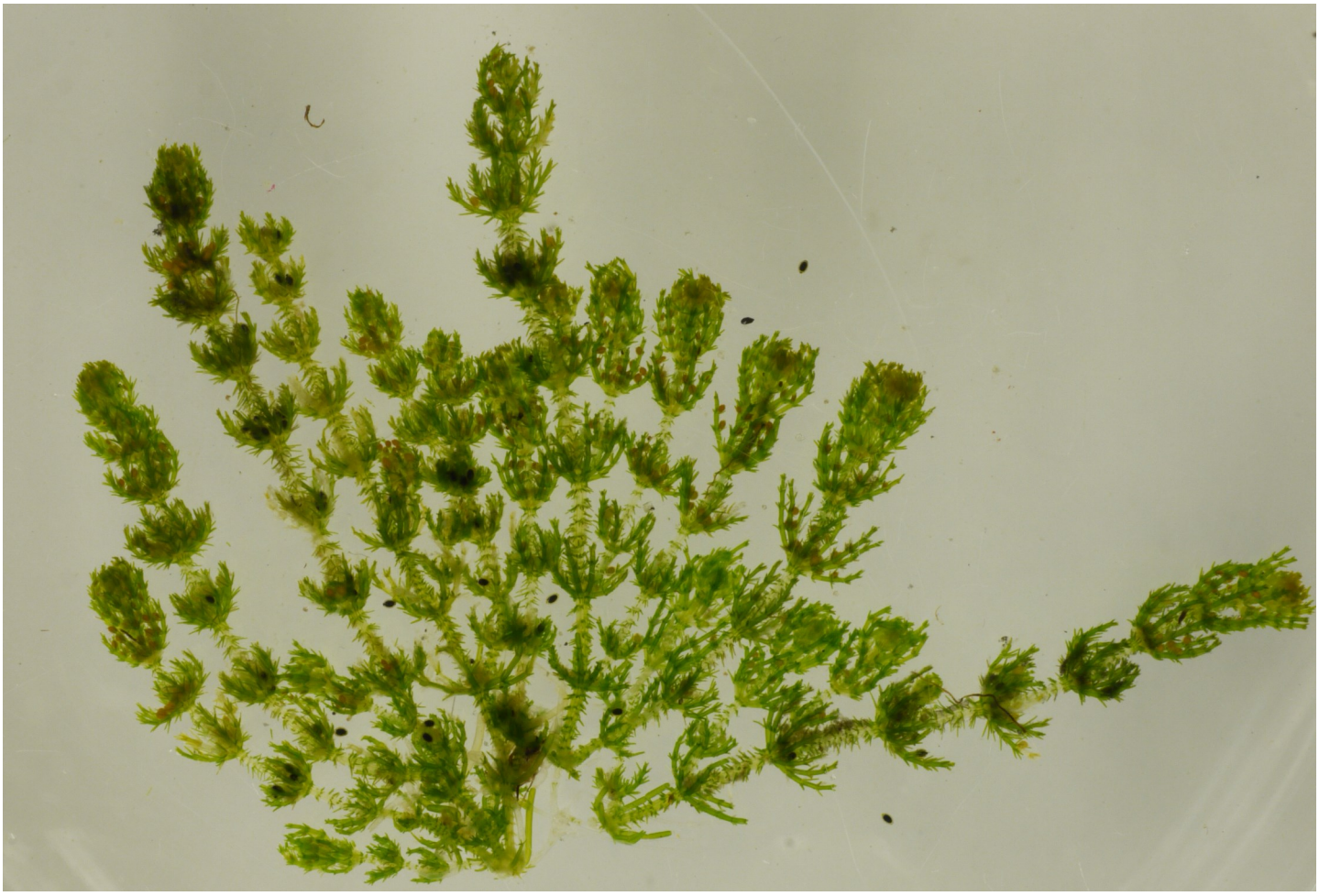


Figure III:2A *Chara canescens* stem cluster. A compact plant with axes up to 10 cm long growing in shallow waters of about 30 cm near shore in The Gravels Lagoon, Port au Port Peninsula. Internodes short and axes very bristly appearing. HEM

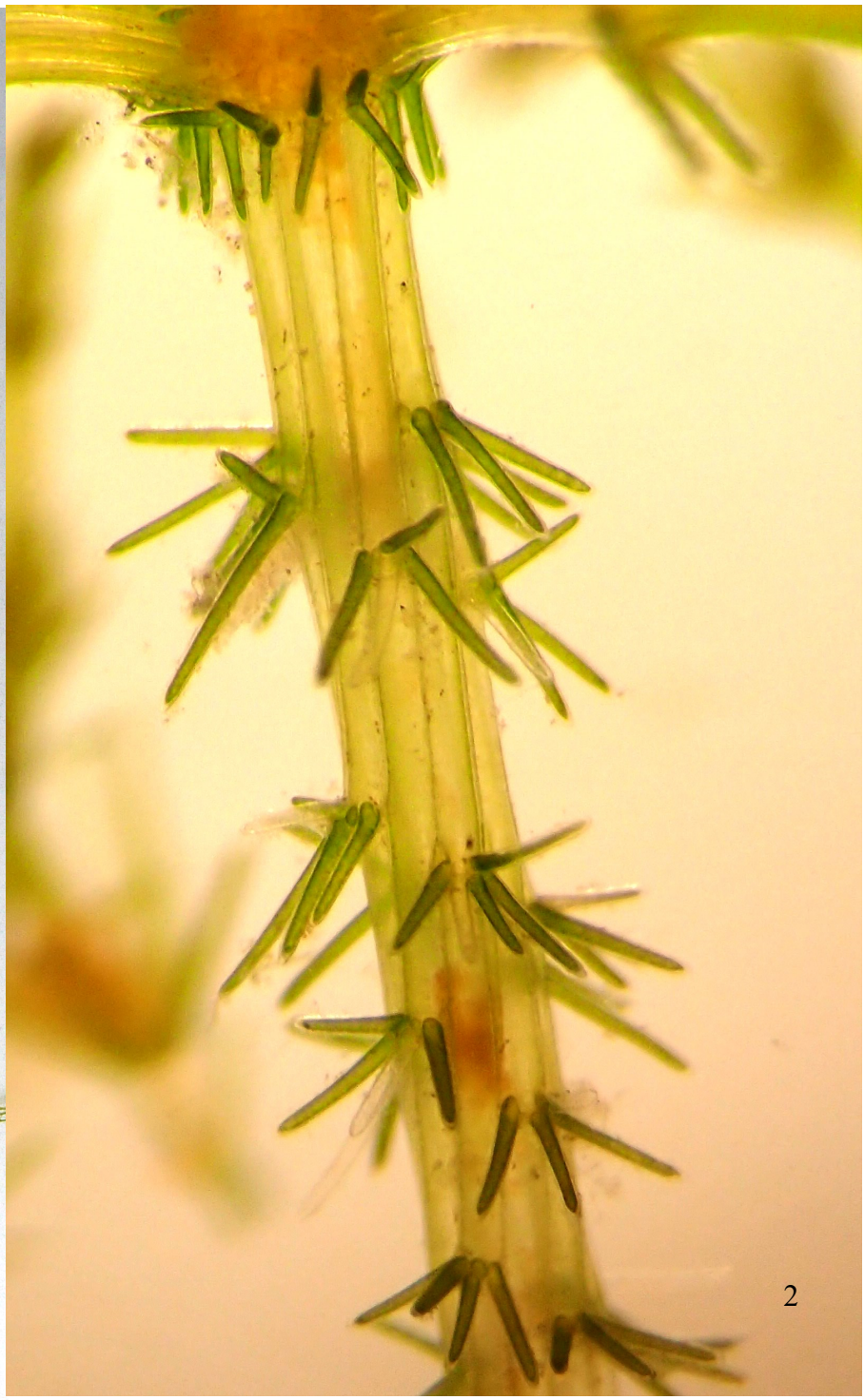
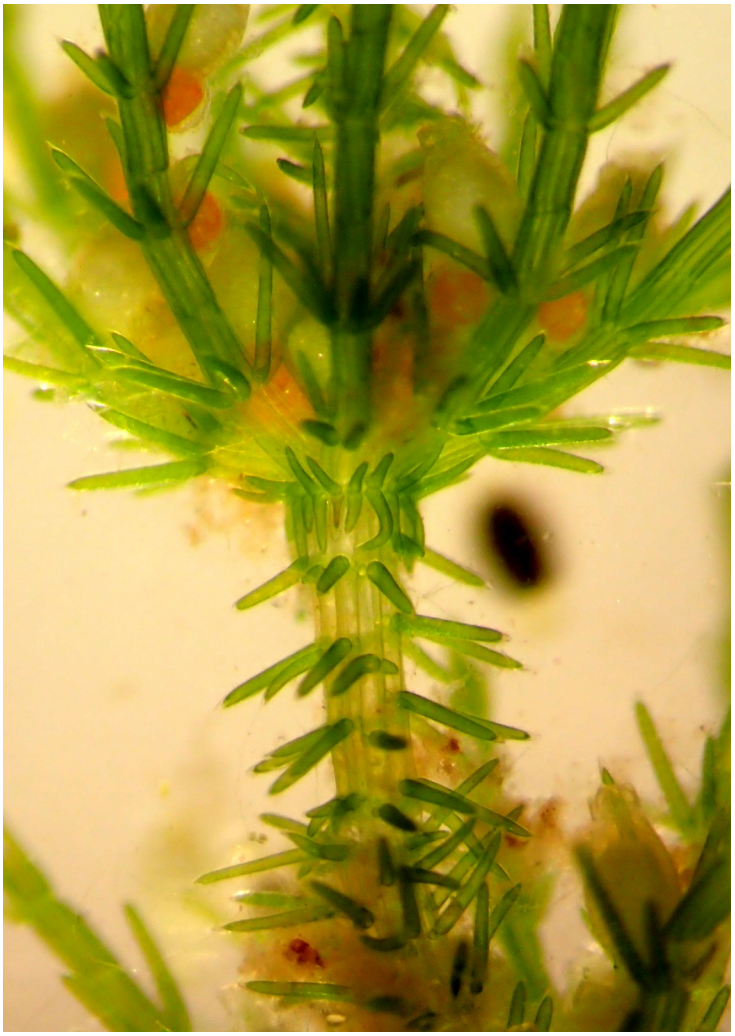


Figure III:2B 1. Elongated stems of about 30 cm from plants growing in deeper 2 meter waters of The Gravels Lagoon.
2. Haplostichous cortex. Stem cortical cells are often pale green to almost colourless with bright green spine cells and stipulodes. Spine cells in clusters of 2 to 4, all usually elongate. HEM



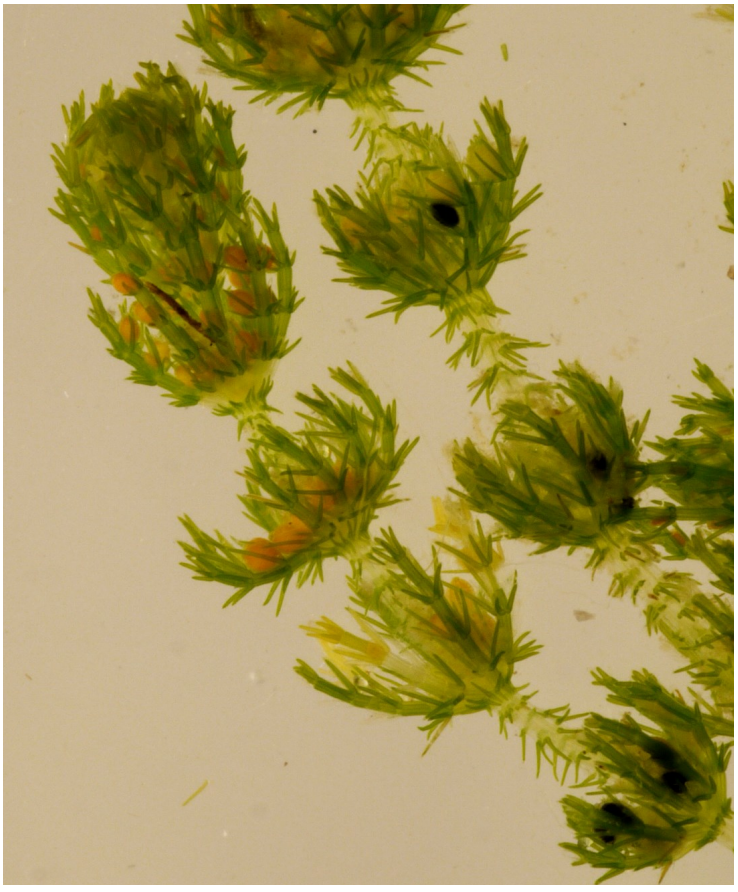
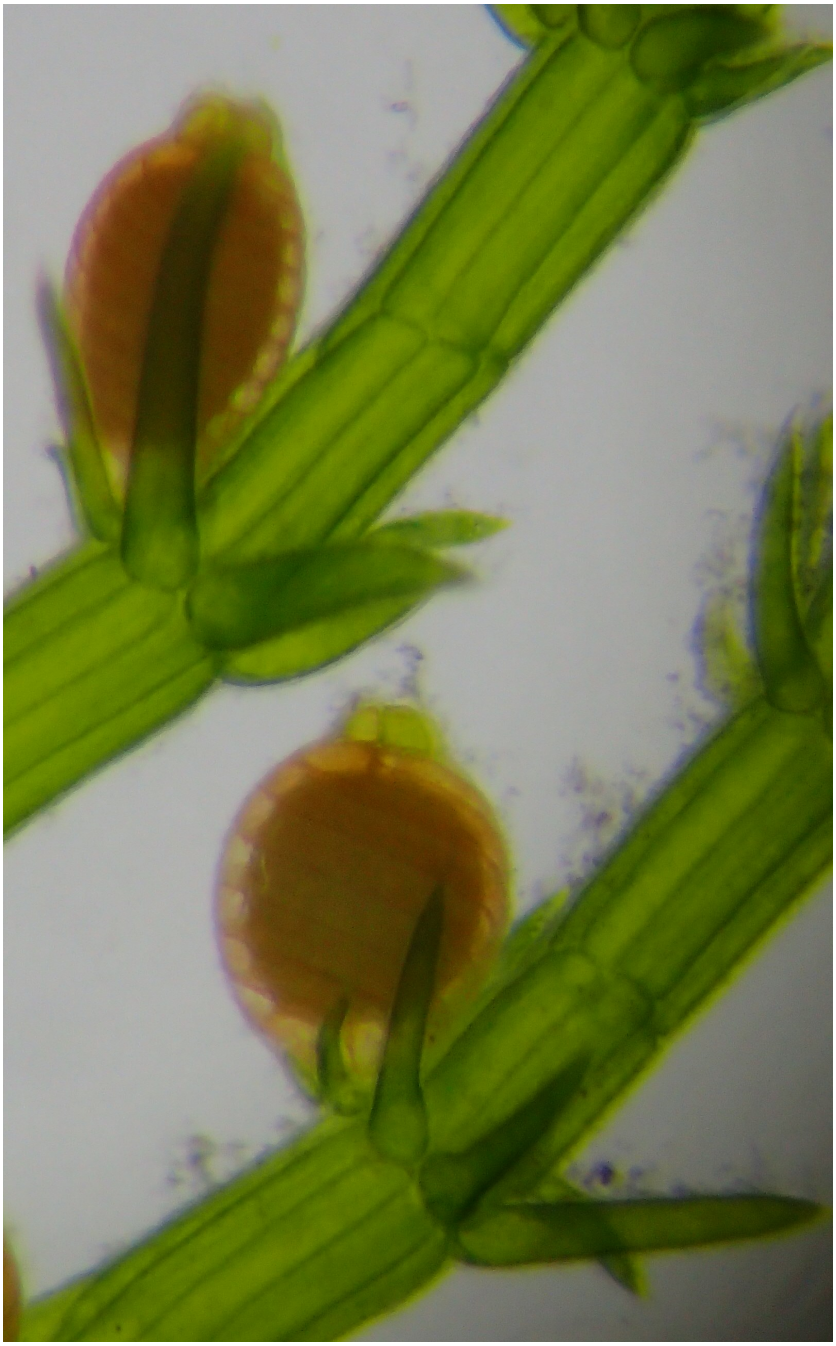


Figure III:2C Three views of *Chara canescens* axes. HEM



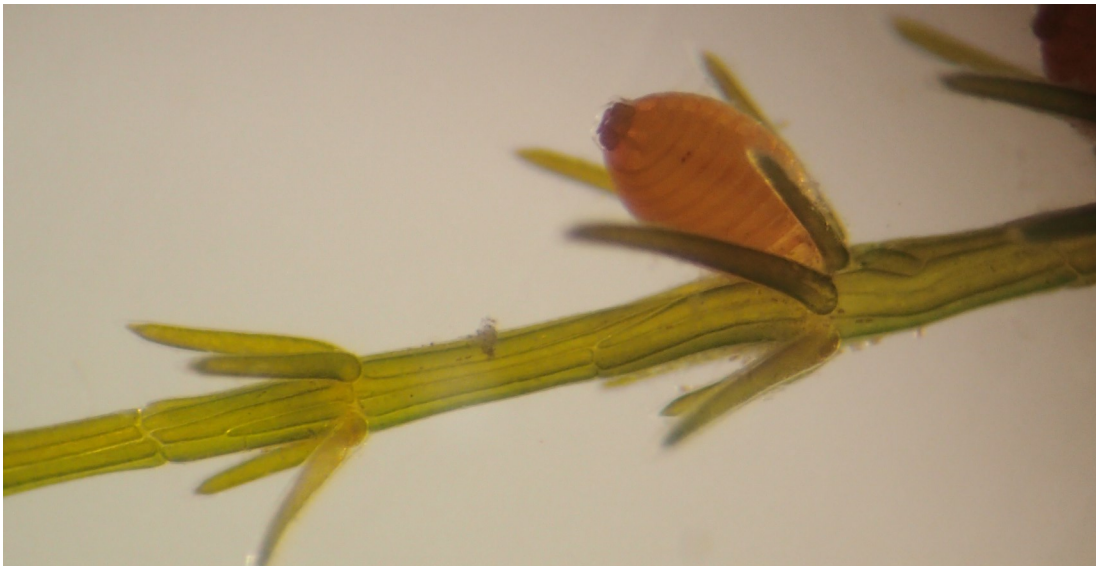


Figure III:2D

Three views of *C. canescens* branchlets with mature oogonia.

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Chara evoluta

Chara canescens

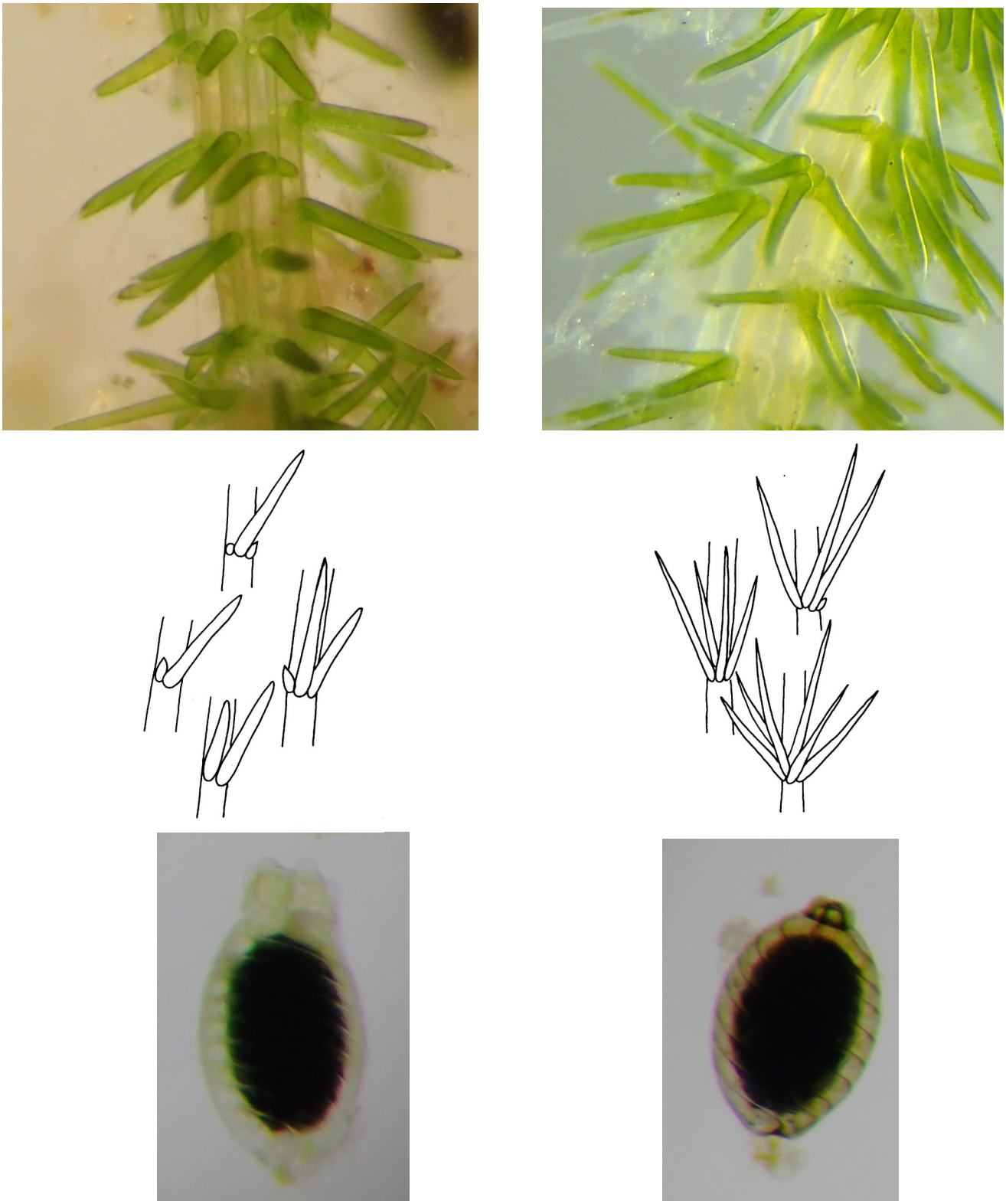


Figure III:2E Some differences between *C. evoluta* (left column) and *C. canescens* (right column) in addition to the monoecious/dioecious condition. Top and center: *C. evoluta* has spine cells singly or in clusters of two or three. One of a cluster is long and the other two often shorter, sometimes tiny. *C. canescens* has clusters of spine cells often from two to five with all usually long. Lower: *C. evoluta* has a large coronula compared to *C. canescens*. HEM

Figure III:2F Upper photo: The coastal Aguathuna Limestone Quarry, Port au Port Peninsula is an unusual habitat for C. canescens and C. contraria. Lower photo: Shallow ephemeral pools dot the abandoned quarry floor often less than 10 cm deep harbouring both species in warm basic fresh waters. HEM

Figure III:2G Miniature *C. canescens* plants with 3 to 4 cm long stems seem to flourish in the warm shallow pools of the Aguathuna Limestone Quarry. They are partially submersed in the flocculent organic ooze of microscopic flora and fauna. Mature oogonia and ripe oospores can be readily observed in the field with a hand lens.

HEM

Figure III:2H Upper photo: St. Georges Pond, a shallow lagoon pond receiving some salinity from the estuary and harbouring *C. canescens* as well as *C. evoluta*. Lower photo: A coastal saltwater pond (barachois) at Grand Bay West, Port aux Basques. HEM

III:3 *Chara contraria* A. Braun ex Kutz.

Monoecious. Axial cortex diplostichous (each primary row alternating with one secondary row). Tylacanthous (primary rows more prominent than secondary rows). Axes and branchlets commonly encrusted. Spine cells are solitary and usually short, papillate or rounded, although in some more uncommon varieties/forms may be longer than stem diameter. Stipulodes are well developed, but short, round tipped or slightly conical pointed, in two rows, often slightly unequal, the upper row sometimes slightly more developed than the lower row. Ripe oospores black. Usually distinguished from *C. vulgaris* by the tylacanthous condition, stipulode differences and black ripe oospores.

C. contraria is a common widespread diplostichous species occurring in a wide range of freshwater habitats, generally in more nutrient rich waters, calcareous ponds and in slightly saline conditions. Globally it is a widespread cosmopolitan species.

Figure III:3A HEM

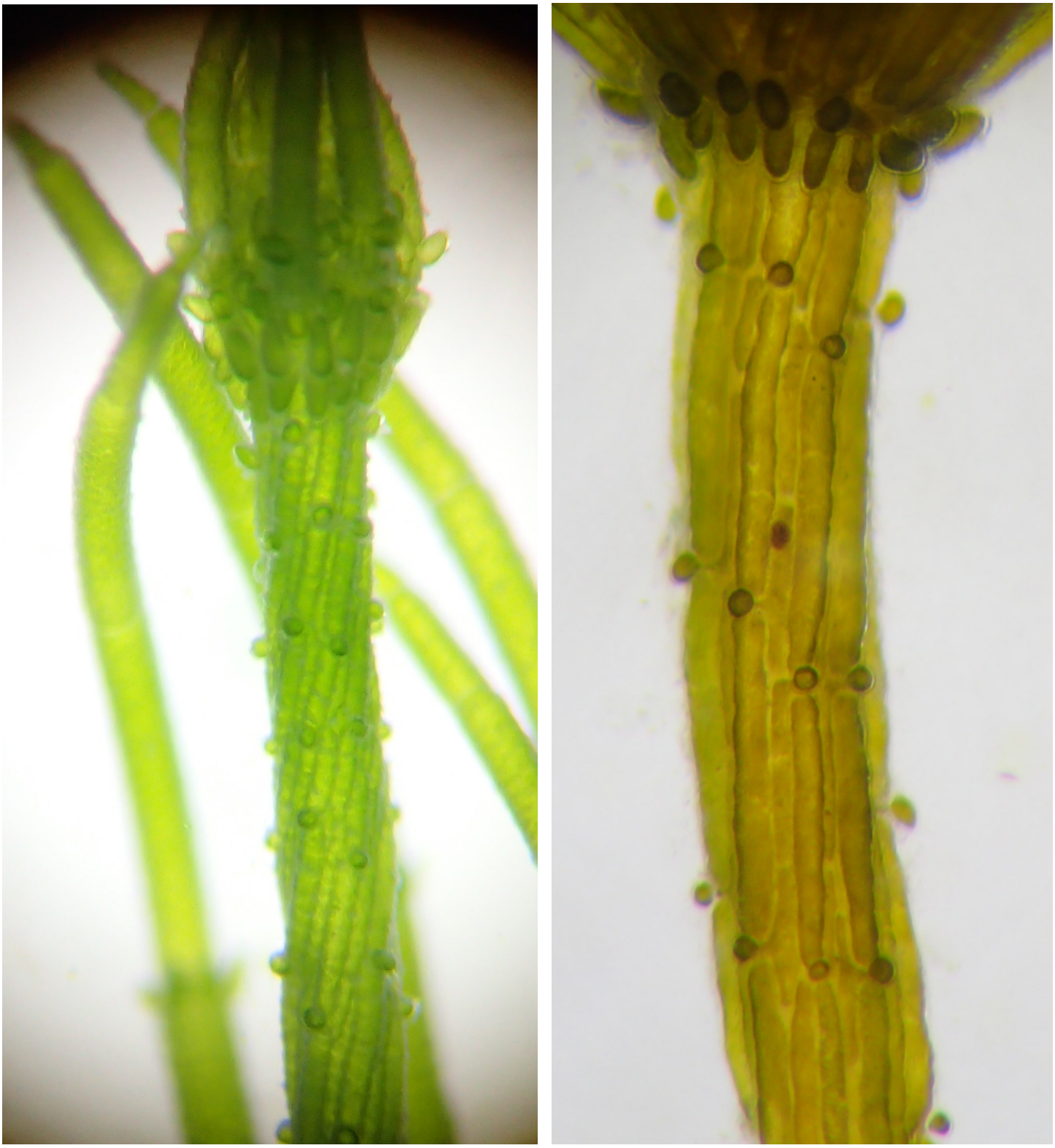


Figure III:3B Left: Young shoot tip showing diplostichous tylacanthous condition. Right: Older tylacanthous internode . Spine cells are papillate in these specimens. HEM

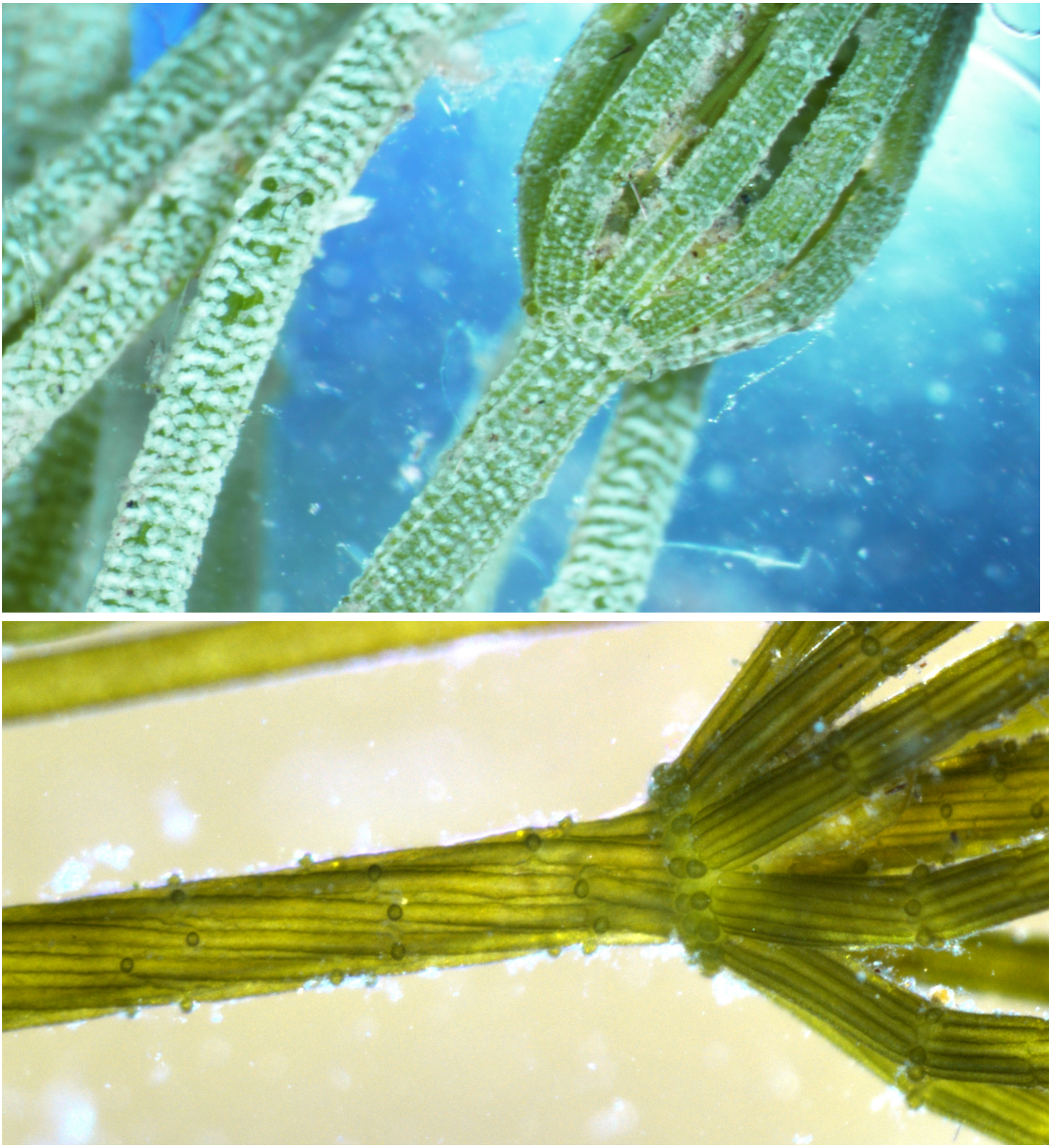


Figure III:3C Upper photo: *C. contraria* is often encrusted with a calcium carbonate surface obscuring cellular detail required for identification purposes. Lower photo: This is a specimen from the same collection as above which has been soaked in a weak acid (vinegar) to remove the encrustation. The diplostichous tylacanthous condition can now be clearly determined. HEM

Figure II:3D Two photos of part of an internode and a node. Spine cells are short, but more elongate than the pappillate cells in previous photos. Stipulodes are more conical pointed than those of *C. vulgaris* and often upper and lower cells are unequal. HEM

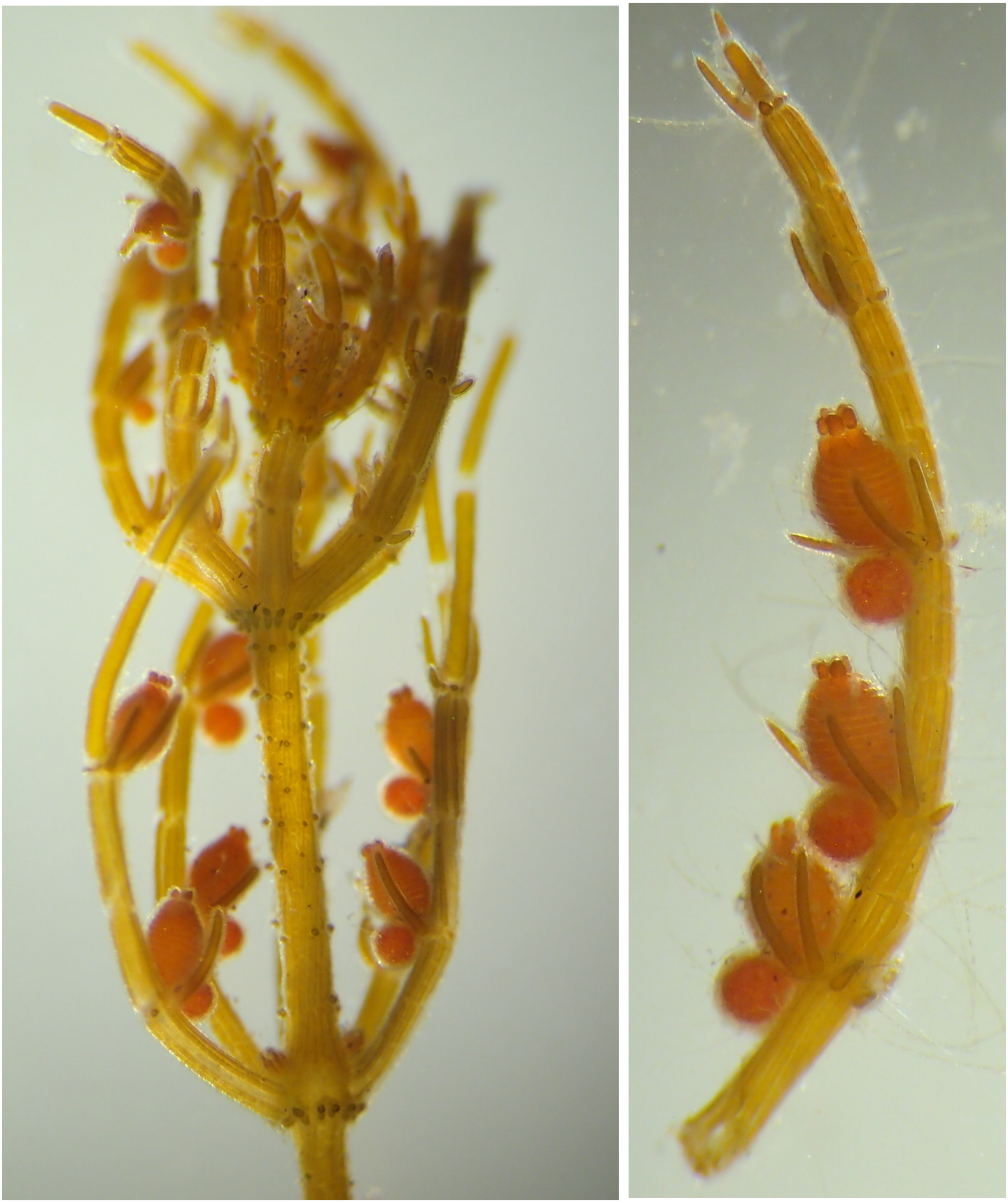


Figure III:3E Some live material of this species often has a yellow-green colour. Actively growing young material will be typically bright green. Left photo: Several branchlet whorls and internodes at a stem tip. Spine cells are tiny, papillate. Right photo: Single branchlet enlarged with mature gametangia. HEM

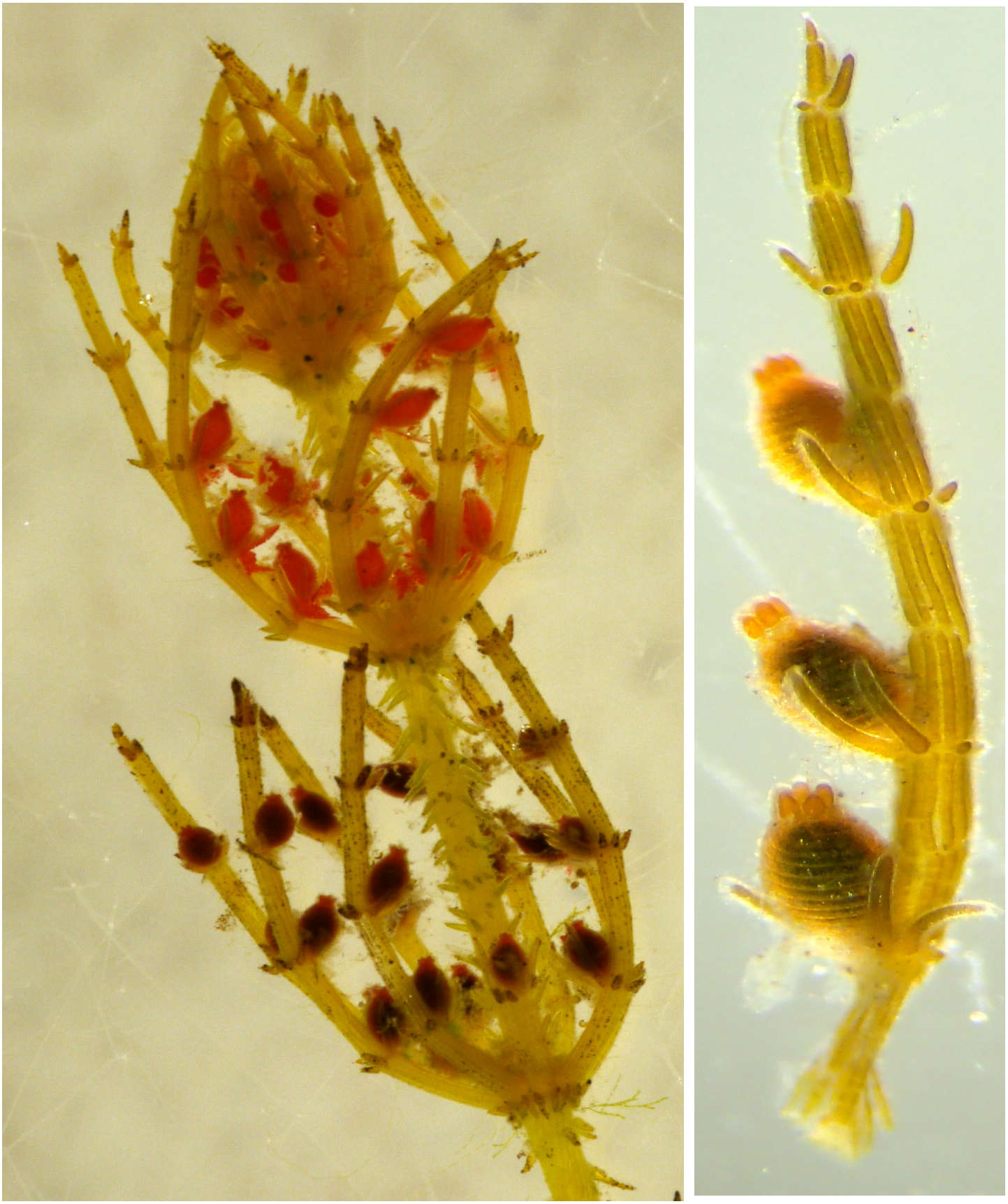


Figure III:3F Left photo. Three branchlet whorls at stem tip. Spine cells somewhat elongated up to 3/4 stem diameter. Right photo: Enlarged single branchlet. Bracteoles and anterior bract cells are shorter than the mature oogonia, posterior bract cells are short or papillate. In some collections bracteoles and anterior bract cells may exceed oogonia. HEM

Figure III:3G 1. Oogonium with mature black oospore from liquid preserved material. 2. Oogonium with mature oospore from living material. 3. Oospore which has shed its outer oogonium, from live material. HEM

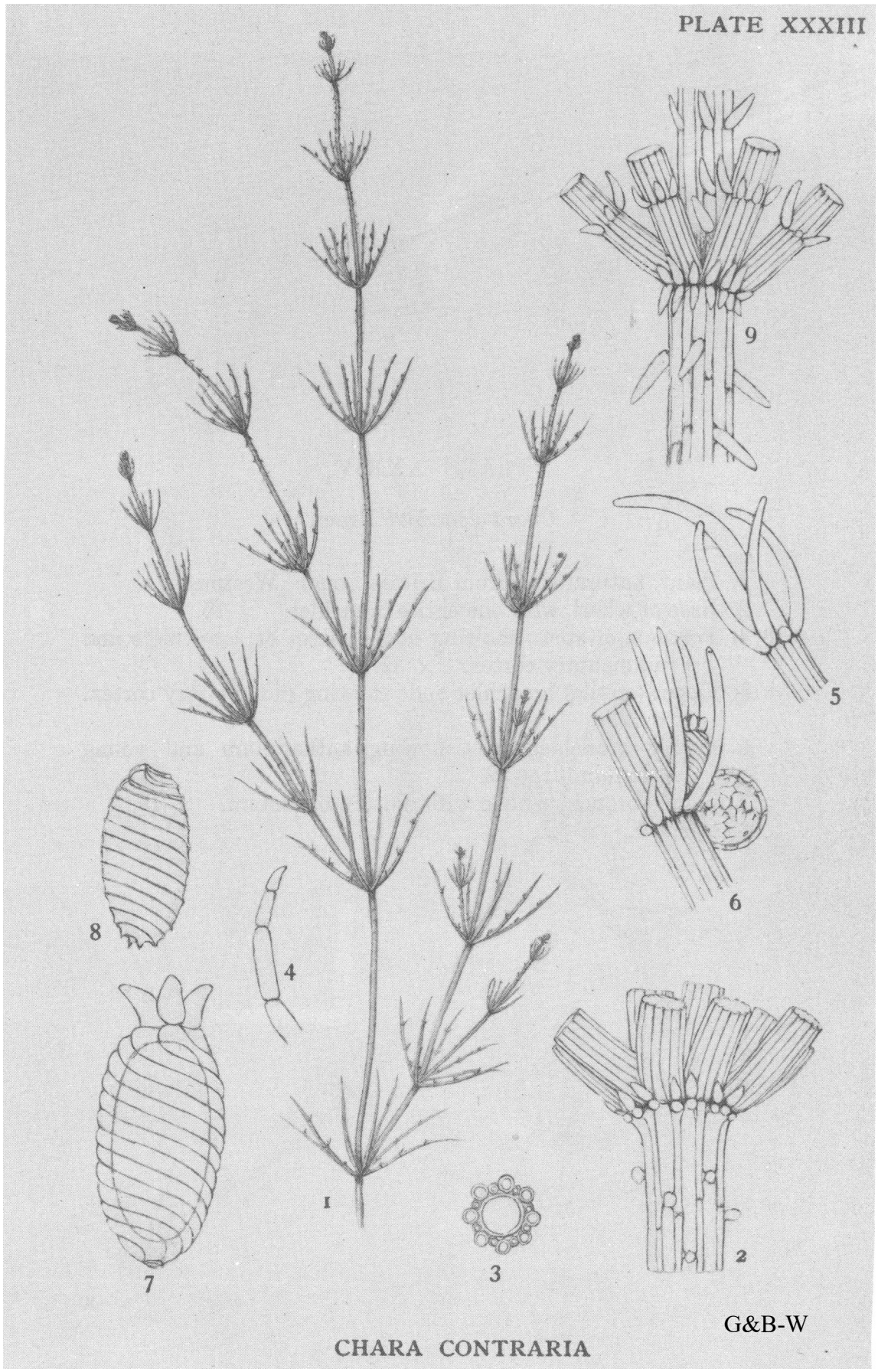


Figure III:3H *Chara contraria* illustrations from J. Groves and G.R. Bullock-Webster. 1924. The British Charophyta, Volume II, Chareae. The Ray Society, London.

Figure III:3I Upper photo: A fen stream near Corner Brook, NL depositing calcium carbonate marl and hosting mats of highly encrusted *Chara contraria* in its shallow water. Lower photo: A calcium rich pond with *C. contraria* in the Corner Brook region. HEM

Chara contraria var. *hispidula* Braun

This variety keys to *C. contraria* via being monoecious, tylacanthous and with similar gametangial measurements. Morphologically it chiefly differs in extremely long stipulodes, spine cells and bracteoles. Spine cells are often 2 to 3 times the diameter of the main axis especially in actively growing terminal whorls with young and maturing antheridia and oogonia. Stipulodes are much longer than those of typical *C. contraria* (e.g. var. *contraria*), the lower row often longer than the uppers. Bracteoles may be 2 to 3 times longer than mature oogonia. Only one location is known from NL, a stagnant shallow backwater channel of the Middle Barachois River near the village of Cartyville on the southwest coast of the Island. The water is slightly brackish.

This variety has not been described for Canada and apparently not described in detail for North America although several collections are reported from the USA and Mexico. A complete description with location and habitat is provided in the publication by Mann et al. 202x.

Figure III:3J Collection site of var. *hispidula* in a shallow backwater channel of the Middle Barachois River , southwest Insular Newfoundland. CH

Figure III:3K Actively growing tip of var. *hispidula* with mature antheridia and oogonia. Branchlets tend to spiral clockwise (upward from right to left). HEM

Figure III:3L Young vegetatively growing axis tip in culture showing features of typical *C. contraria* with tylacanthous diplostichous cortication and papillate spine cells. HEM

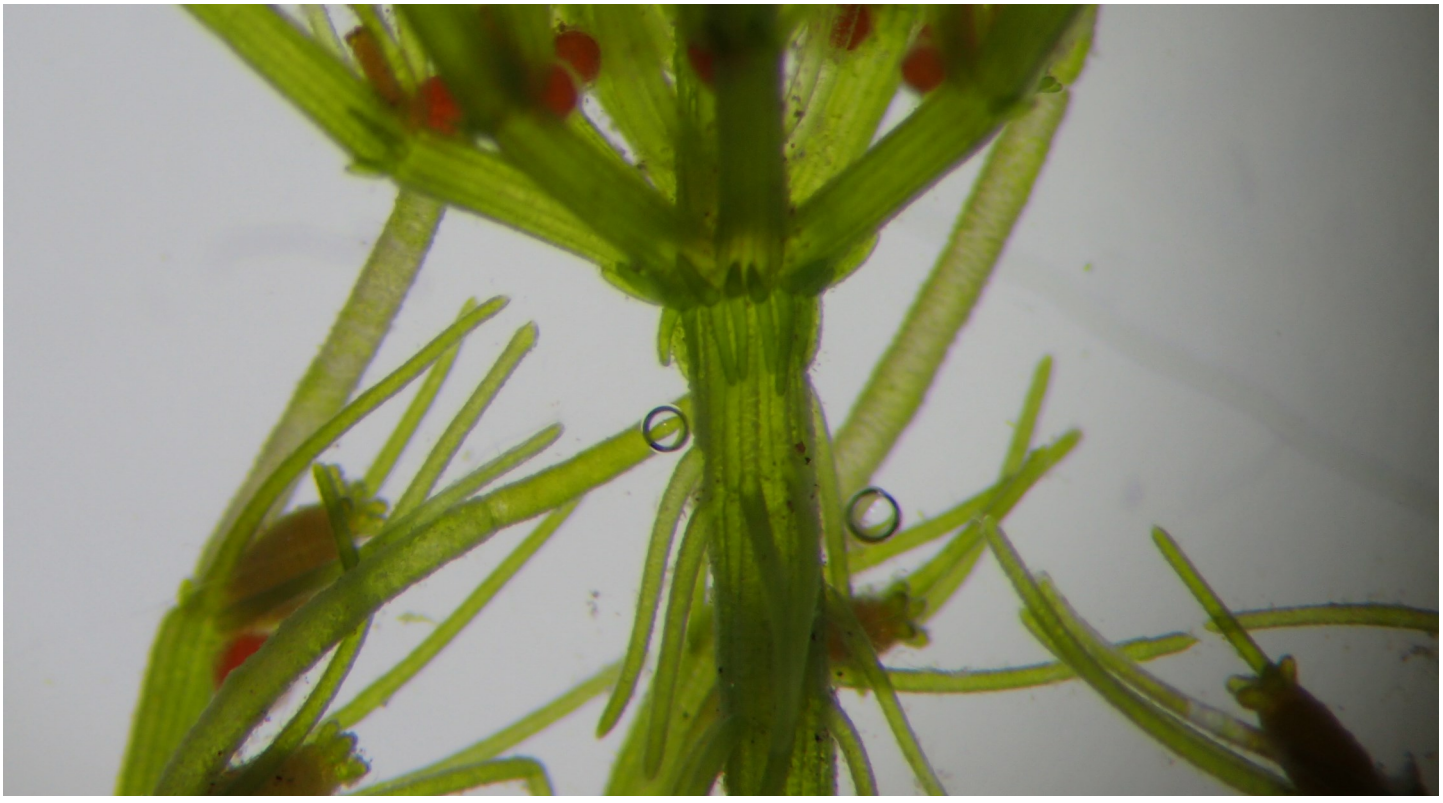


Figure III:3M Axis showing elongate spine cells and elongate stipulodes, the lower stipulodes sometime exceeding the upper in length. HEM

Figure III:3N Branchlets of var. *hispidula* with elongate bracteoles and anterior bract cells. Upper photo with mature oogonia, lower photo with immature oogonia. HEM

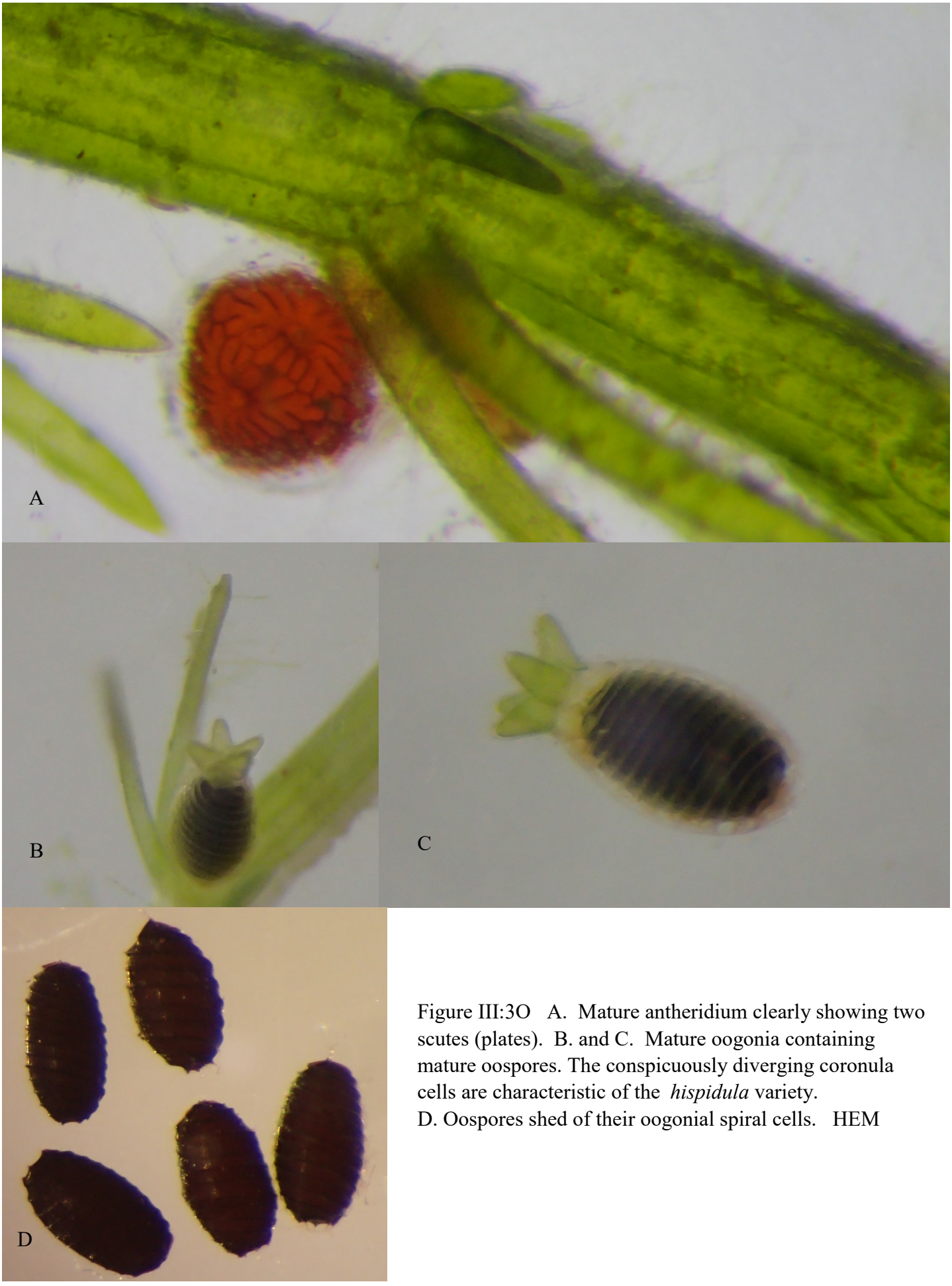


Figure III:3O A. Mature antheridium clearly showing two scutes (plates). B. and C. Mature oogonia containing mature oospores. The conspicuously diverging coronula cells are characteristic of the *hispidula* variety.
D. Oospores shed of their oogonial spiral cells. HEM

III:4 *Chara evoluta* T.F. Allen

Monoecious. Cortication on main axes is haplostichous (all rows of cells are primary rows). Sometimes on older axes occasional secondary cells may occur providing a false impression of being diplostichous. Spine cells are elongate, sometimes singular, but more often in clusters of two or three. Characteristically at least one of the spine cells of a cluster will be long, the other one or two being shorter, sometimes tiny and rudimentary. Two rows of elongate acute stipulodes are produced, the upper row slightly longer than the lower.

C. evoluta can readily be distinguished from *C. canescens* which is dioecious, but no male plants with antheridia are known from North America. Oospores are produced by parthenogenesis in *C. canescens*. Also size of coronulas differ as well as spine cell clusters.

C. evoluta is currently considered a North American endemic species. Its distribution is mostly limited to the western half of North America and uncommon in the eastern part of the continent (Mann and Nambudiri 2005). It is a species of brackish saline waters only known in NL from eight coastal lagoons and salt ponds (barachois) on the southwest coast of the Island from Grand Bay West to the Port au Port Peninsula. Considered rare in NL (S Rank 2 – may be at risk).

Figure III:4A Branching *Chara evoluta* stems with a bristly appearance, especially in the younger internodes. Orange antheridia and oogonia and those with internal black oospores prominently visible. HEM

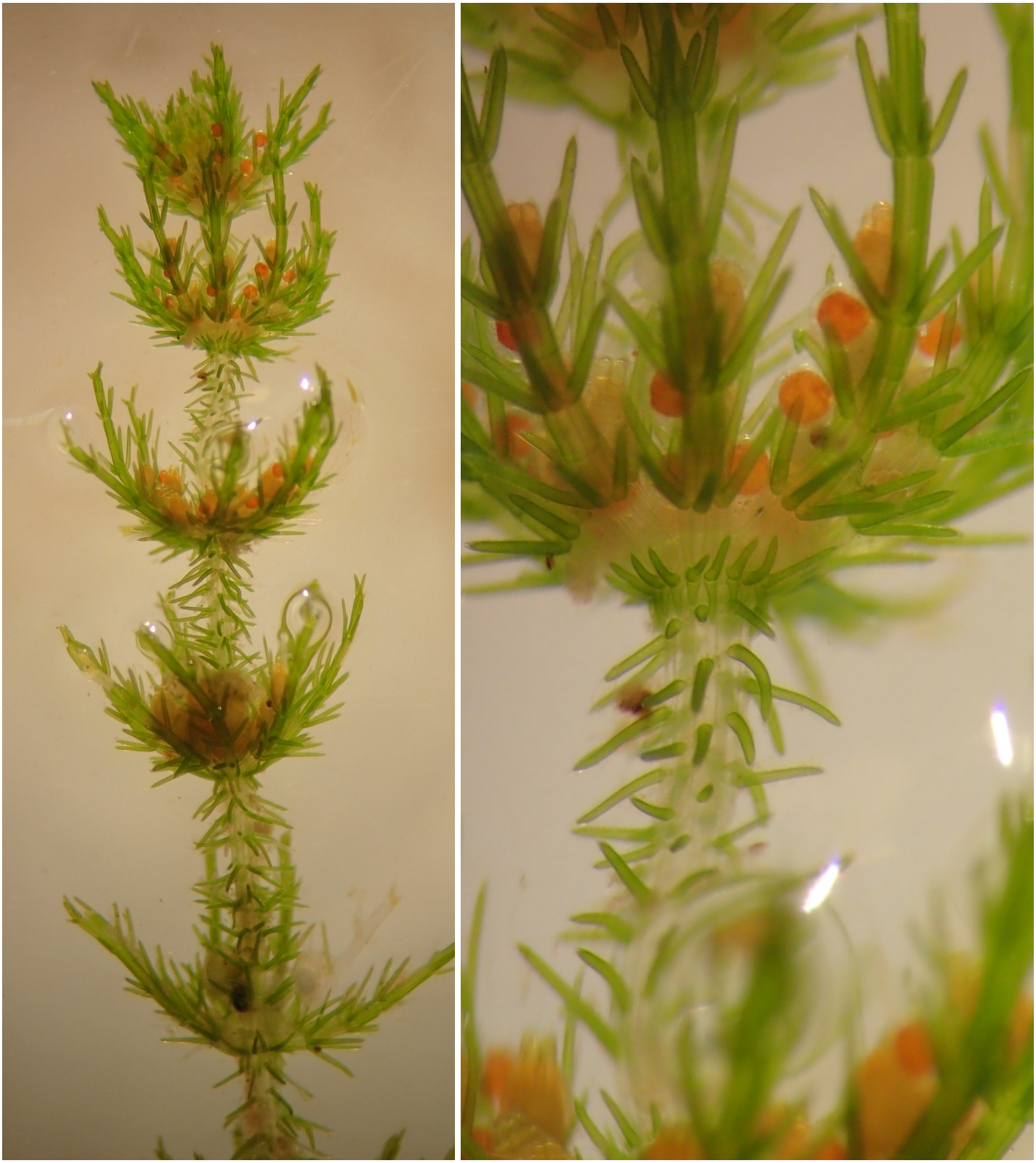


Figure III:4B Two views of *C. evoluta* axes with mature antheridia and oogonia. HEM

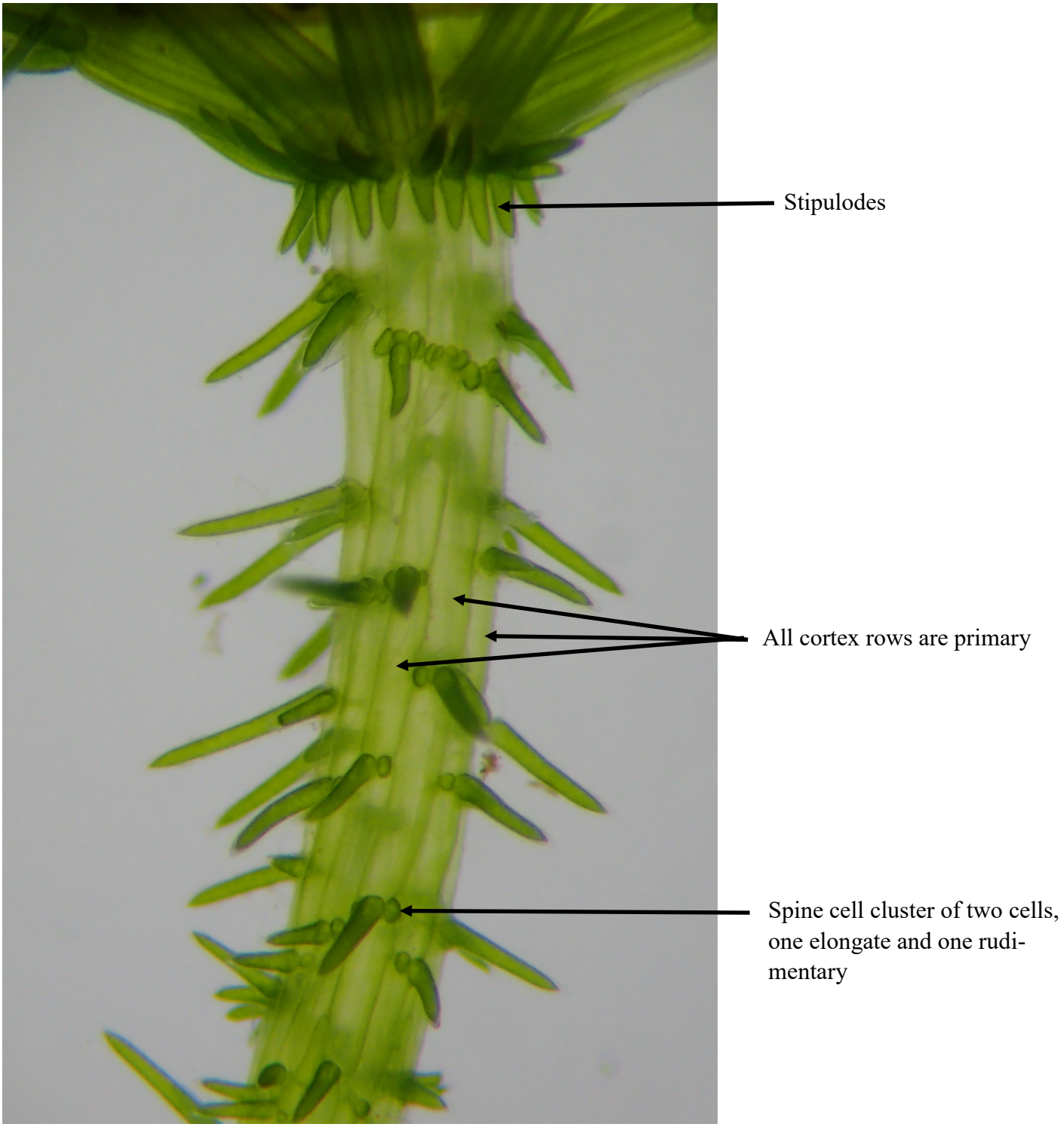
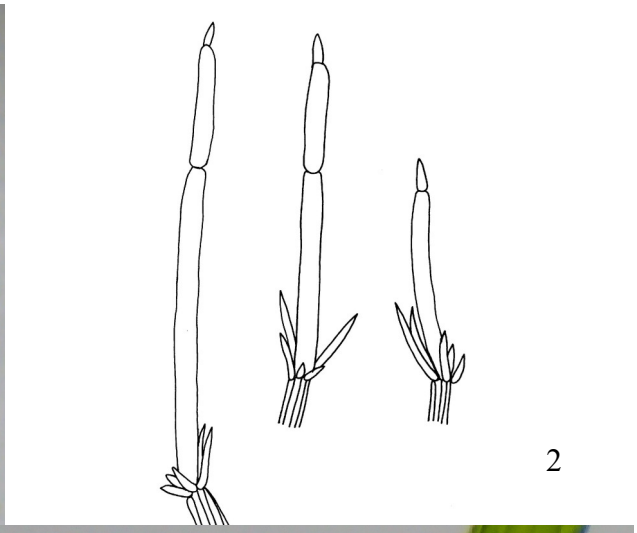


Figure III:4C Typical enlarged axis (stem). All stem cortex rows are primary, producing spine cells (haplostichous). Spine cells are acute tipped, up to as long as stem width, and produced singly, but mostly in clusters of two or three. Characteristically one spine cell of a cluster is longer and often at least one of the shorter cells are small and rudimentary. Stipulodes are well developed in two rows and often longer than those shown in this photo. HEM

Figure III:4D Two branchlets with mature gametangia. Remnants of stipulodes present. HEM



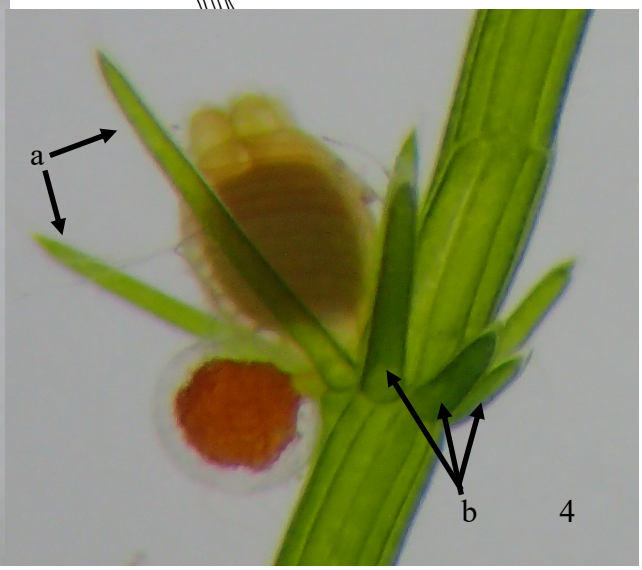
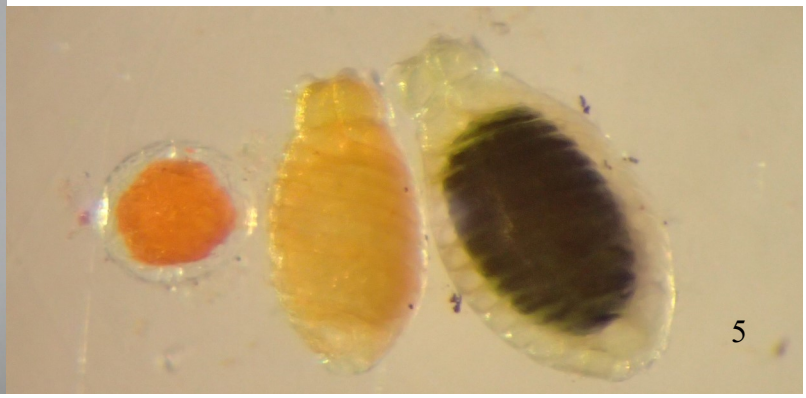
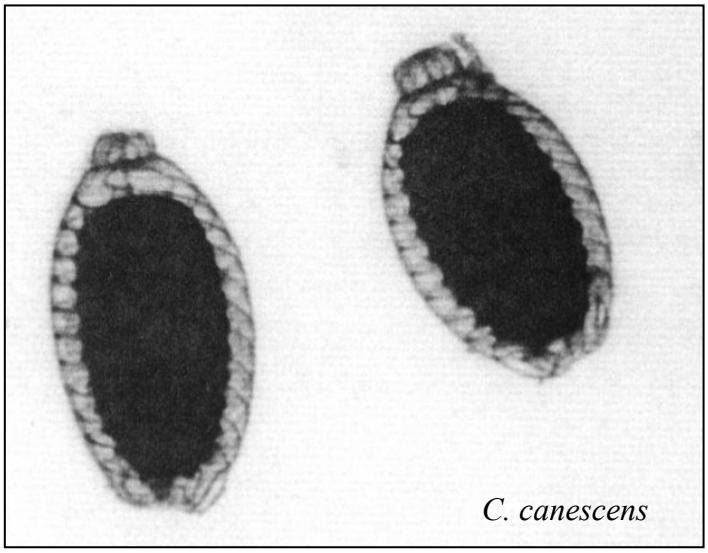


Figure III:4E 1. Typical branchlets with mature antheridia and oogonia. 2. Occasionally the ecorticated branchlet end cells may be elongated. 3. Sometimes branchlets may be quite short with only one or two fertile nodes. These oogonia contain mature black oospores. 4. Mature antheridium and oogonium with bracteoles (a) and bracts (b). 5. Comparative sizes of an antheridium, a mature oogonium and an oogonium with a mature black oospore inside. Note the comparatively large prominent coronula of this species. HEM





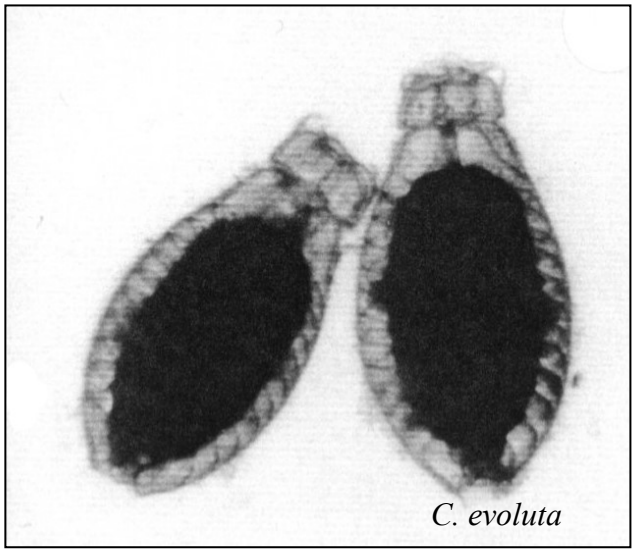


Figure III:4F Upper photos: Comparison of *C. canescens* and *C. evoluta oogonia and* coronulas. *C. evoluta* has a distinctly larger coronula.

Lower photo: *Chara evoluta* is a species inhabiting brackish coastal waters in Newfoundland. This is a shallow coastal brackish lagoon at the neck of the Port au Port Peninsula (The Gravels) containing both *Chara evoluta* and *Chara canescens.* HEM

III:5 *Chara globularis* Thuill. (= *Chara fragilis* Desv.)

Monoecious. Triplostichous. Isostichous (primary and secondary cortex rows the same size). Spine cells are solitary and rudimentary, difficult to see, best seen in very young axes, usually absent or not visible on older axes. Stipulodes in two rows, but very rudimentary and globular. Bract cells and bracteoles shorter than the oogonium.

Less common than *C. virgata* in NL. Found in a broad range of freshwaters, often more nutrient rich or mildly brackish. Often encrusted and a more yellow-green colour.

C. globularis is very similar to *C. virgata* and sometimes difficult to distinguish. Under certain ecological conditions (e.g. brackish waters and/or heavy grazing by invertebrates, etc.) *C. virgata* may appear morphologically like *C. globularis*. However, under cultural conditions it will return to its tylacanthous appearance. *C. globularis* can usually be distinguished by its very rudimentary upper stipulodes, its isostichous cortex and bracteoles shorter than the oogonia. If there appears to be at least some elongation of the upper stipulodes one should suspect it may be ecologically modified *C. virgata* especially if axis cortication tends to be tylacanthous in very young internodes.

Figure III:5A *Chara globularis*. A single axis showing typical growth form. HEM

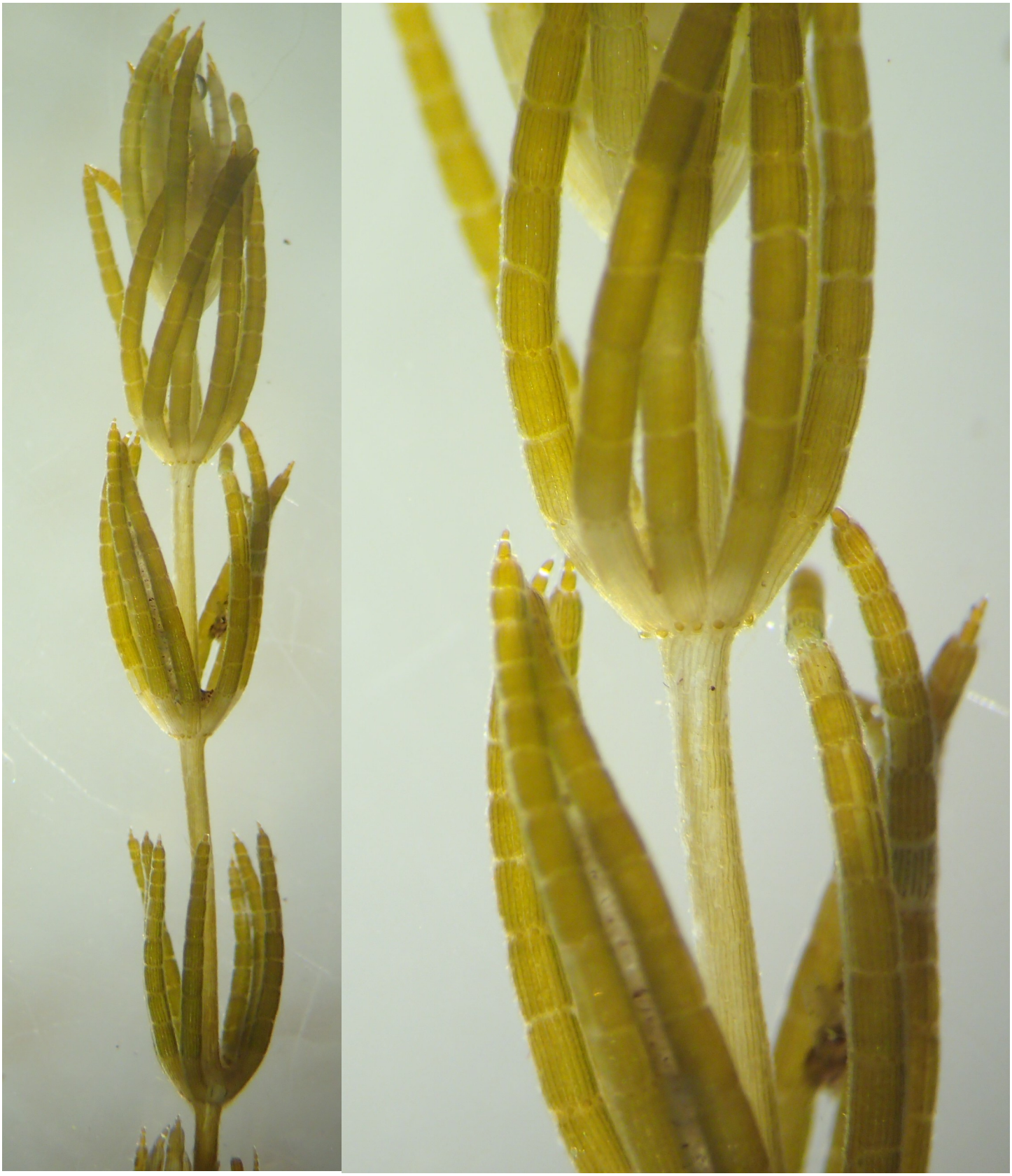


Figure III:5B *Chara globularis* has a smooth appearance without prominent spine cells, stipulodes or bracts, these being small or rudimentary. HEM

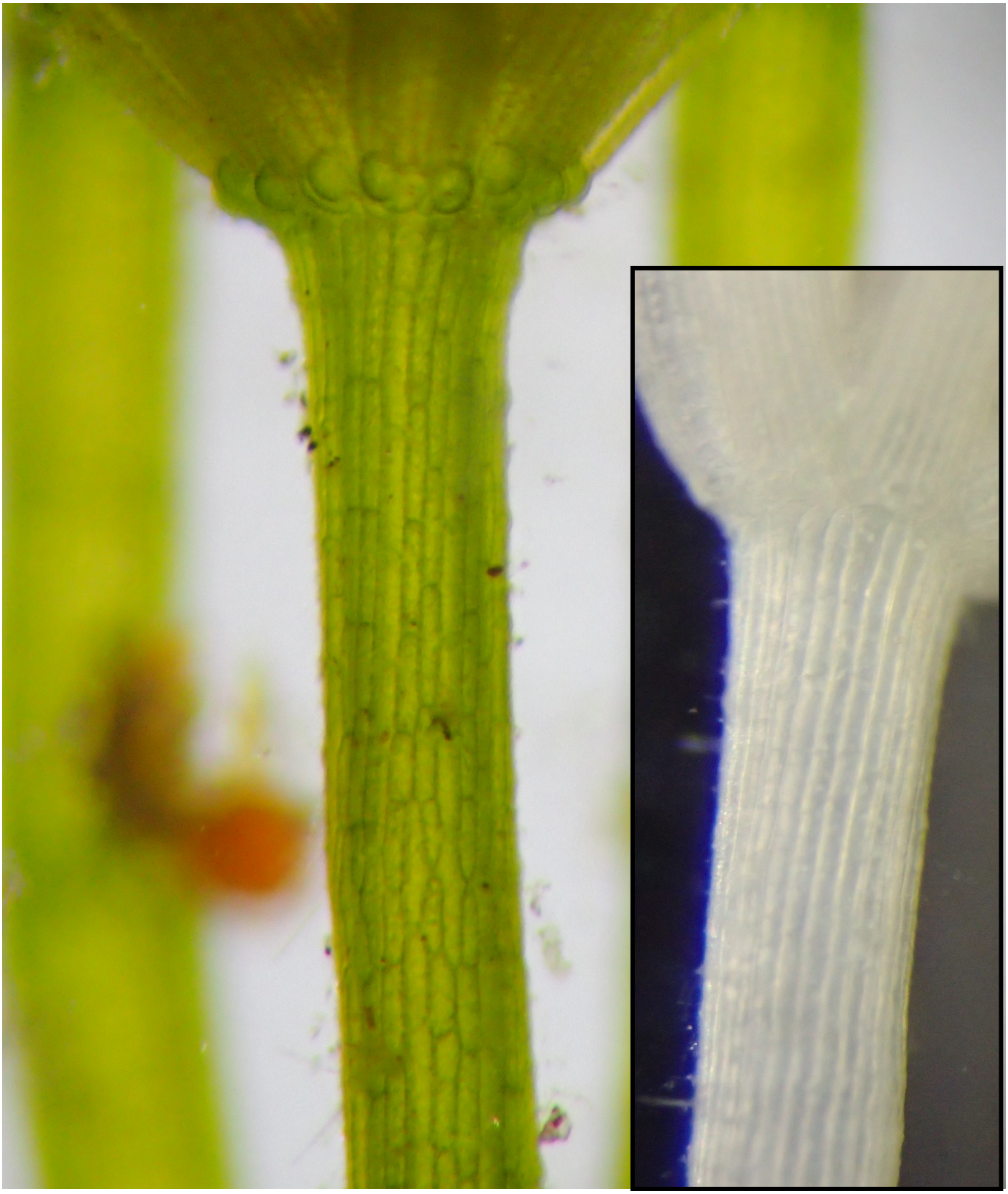


Figure III:5C Stem cortex is triplostichous, but primary and secondary cortex cells are about the same size and therefore difficult to distinguish. Spine cells are tiny and difficult to see except in very young internodes. Inset photo is of liquid preserved material clearly shows all cortical rows are of equal size (isostichous). Stipulodes of this species are globular to rudimentary. HEM

Figure III:5D 1. Young actively growing stem tip with immature gametangia. 2. Two branchlets with immature gametangia, bracteoles short, and most bracts rudimentary. HEM

Figure III:5E Branchlets with immature oogonia. Compare relative coronula size to that of a fully mature oogonium in the following Figure F. HEM

Figure III:5F 1. A mature oogonium. 2. A short branchlet with four mature oogonia and four mature antheridia. The branchlet and oogonia have some surface calcareous encrustation as well as epiphytic filamentous blue-green algal growth. Axes and branchlets in this species often have a surface encrustation when growing in calcareous waters. 3. Mature oogonium with fertilized internal egg darkening into the black oospore at maturity.

Photos 1 and 2 are of liquid preserved material therefore normal living colours are absent. Photo 3 is of live material. HEM

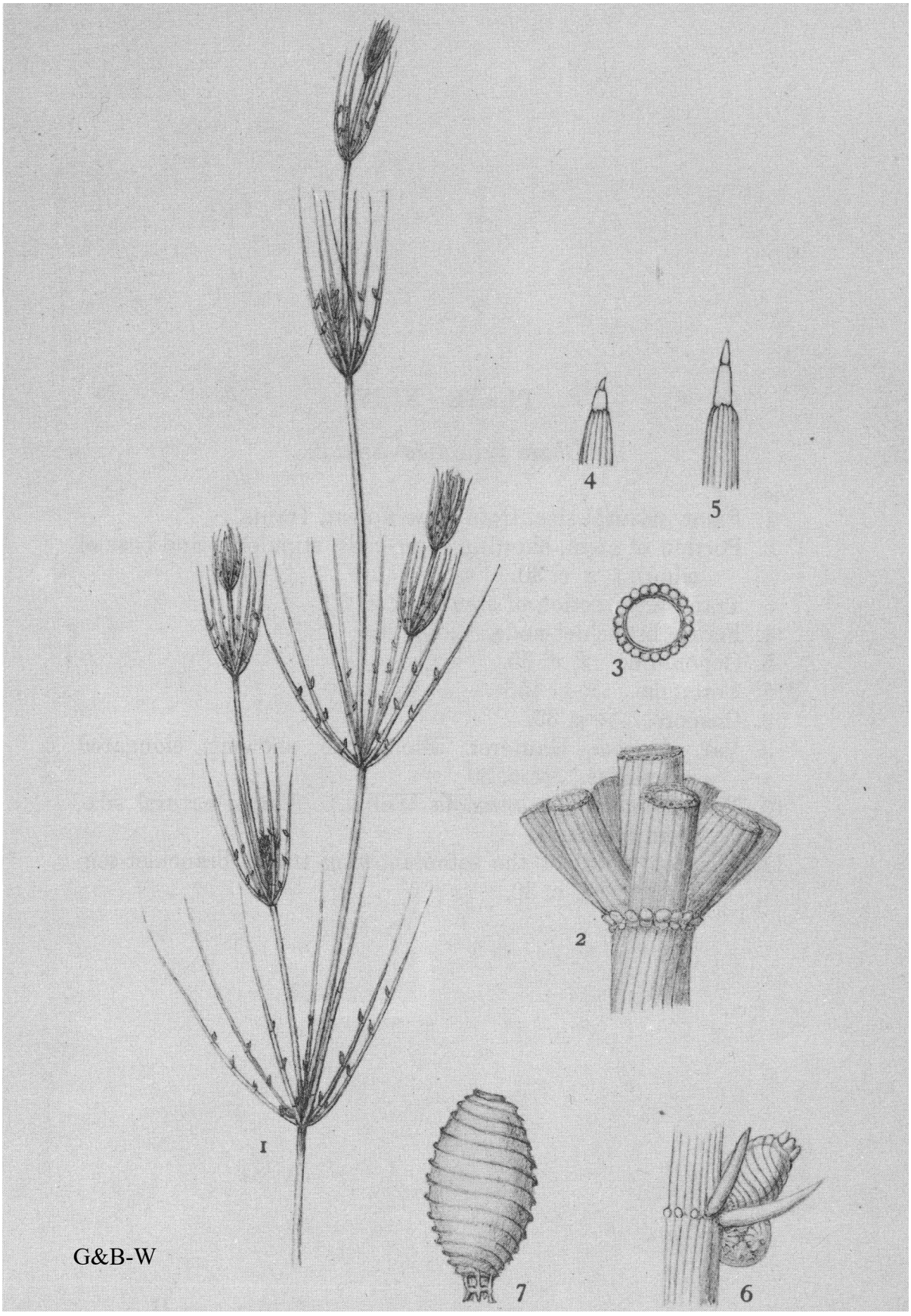


Figure III:5G *C. globularis* illustrations from J. Groves and G.R. Bullock-Webster. 1924. "The British Charophyta", Vol. II, Chareae. The Ray Society, London.

Figure III:5H A boreal pond in a limestone rich area near Corner Brook harbouring *Chara globularis*, *Chara virgata, Chara vulgaris* and *Chara contraria*. HEM

III:6 *Chara virgata* Kutz. (= *Chara delicatula* Agardh., = *Chara globularis* var. *virgata* f. *virgata* (Kutz.) R.D. Wood)

Monoecious. Triplostichous. Tylacanthous. Spine cells are tiny and rudimentary. Upper row of stipulodes are slightly elongate, or sometimes up to diameter of axes, occasionally more. Lower row of stipulodes obscure to globular. Plants are often bright green and not encrusted especially in oligotrophic acidic waters. Bracteoles are usually longer than the oogonia.

This is our most common Chara found throughout Newfoundland and Labrador. It has a broad ecological tolerance and can be found in low nutrient acid waters to more nutrient rich waters of a basic pH, and even in slightly brackish conditions. It is globally cosmopolitan.

C. virgata can sometimes be confused with *C. globularis*. See comments under that species.

Figure III:6A *Chara virgata* growth habit. Several bulbils at base of stems. HEM

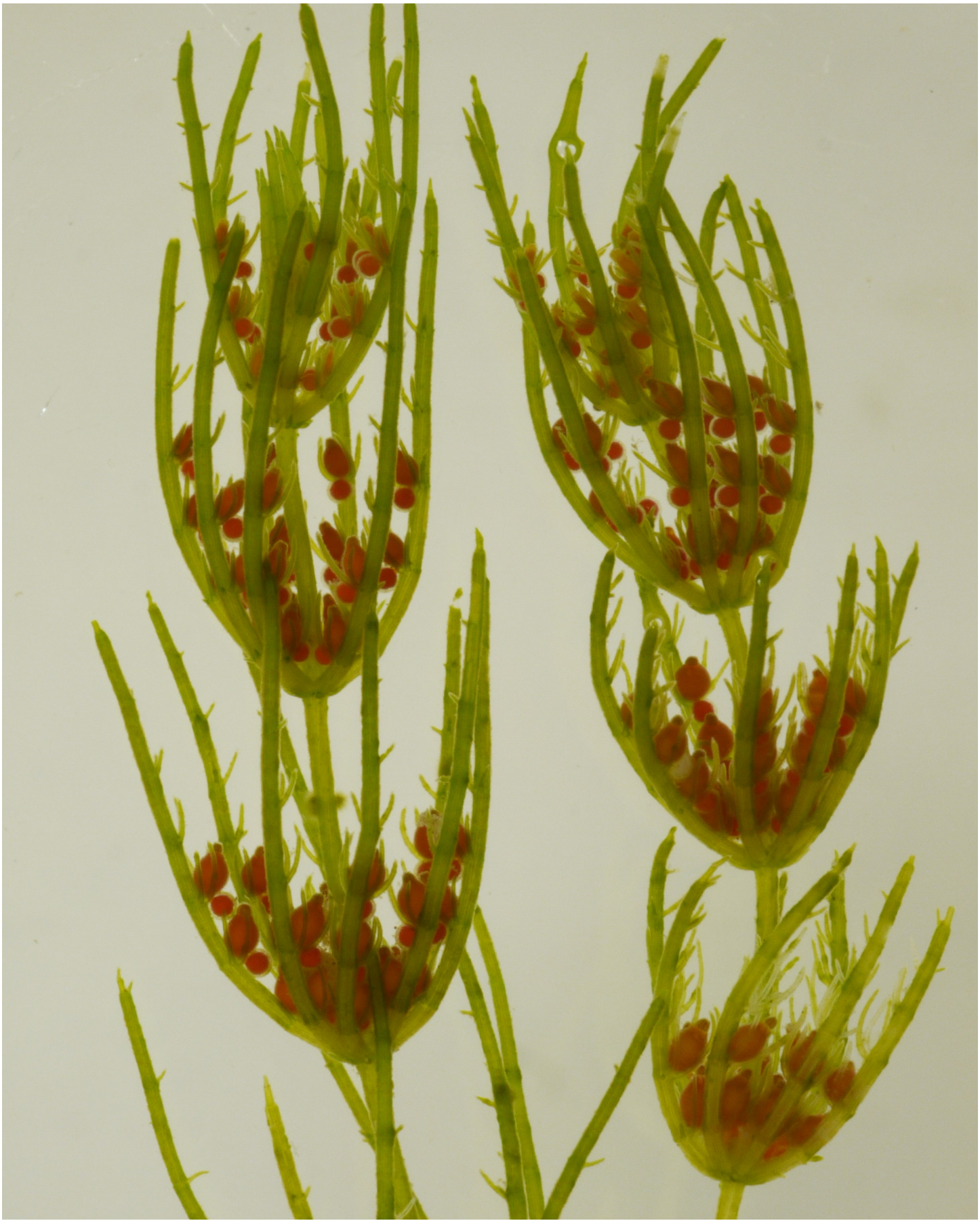


Figure III:6B Upper terminal portion of two stems. Branchlets with mature antheridia and oogonia. HEM

Figure III:6C Four views of axis cortication and stipulodes. Cortication is triplostichous and tylacanthous, usually distinctly so. Spine cells are tiny, papillate to rudimentary, but usually readily visible especially in younger internodes. Upper stipulode cells are elongate, sometimes only slightly so (upper left photo) to distinctly so as in other photos, occasionally quite long and exceeding axis diameter. Lower stipulode cells remain tiny, globular to obscure. HEM

Figure III:6D Upper stipulodes quite long in this specimen, exceeding stem diameter. Bracteoles and anterior bract cells longer than oogonia, bracteoles sometimes twice the length. Posterior bract cells short to rudimentary. HEM

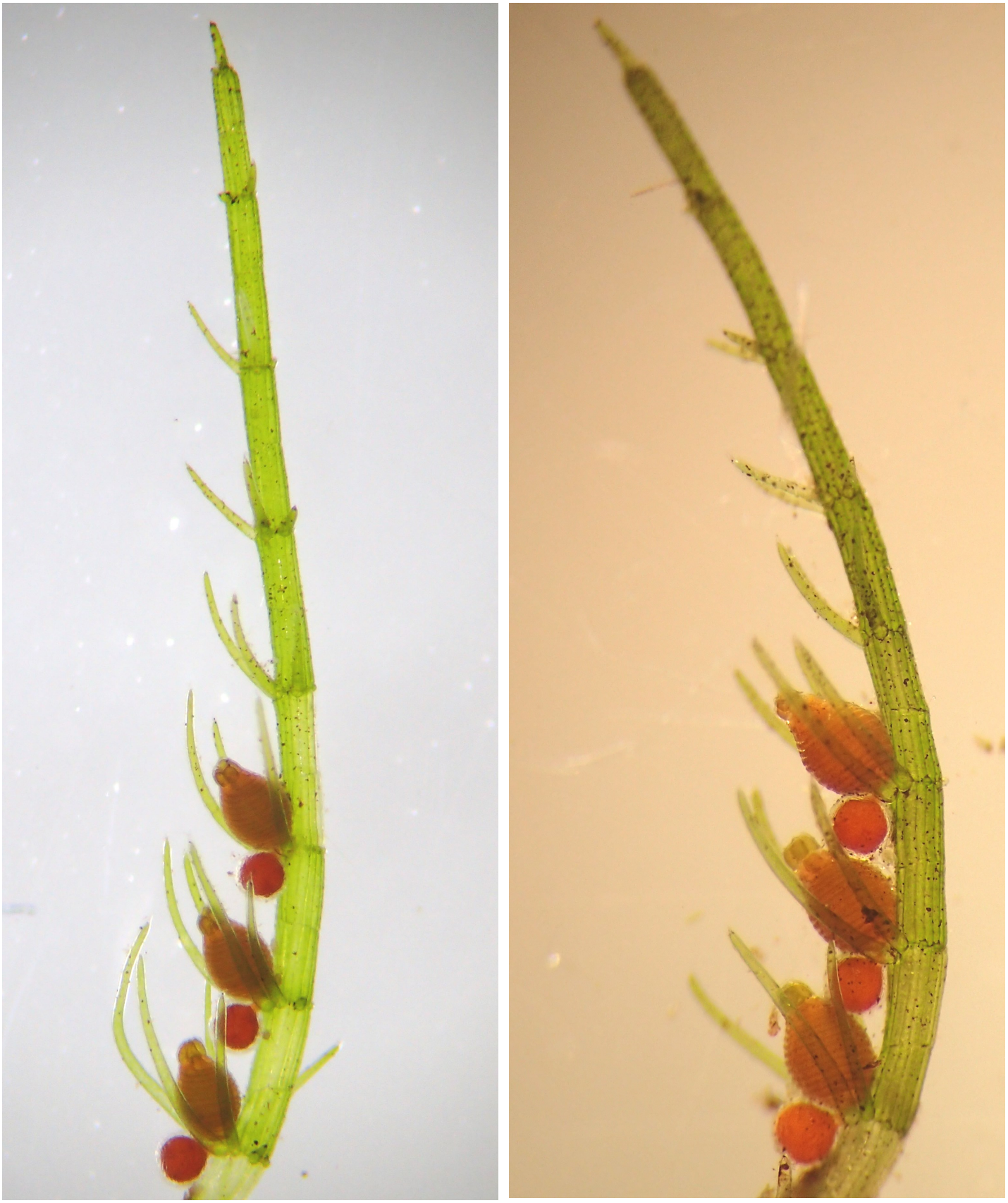


Figure III:6E Two branchlets with mature gametangia. HEM

Figure III:6F A portion of two branchlets with typical mature gametangia and typical bracteoles and bract cells. HEM

Figure III:6G Upper photo: Two branchlet nodes with mature gametangia. Bracteoles and anterior bract cells are relatively short in this specimen. Lower photo: Close-up of an oogonium with mature black oospore. Coronula cells of this species are large and prominent, often with tips converging/ coming close together (connivent). Some blue-green algal filaments are epiphytic on the branchlet and oogonial base. HEM

Figure III:6H *C. virgata* is our most common Chara with the broadest ecological tolerance range. It occurs in acid low nutrient peatland pools (upper photo) to more nutrient rich waters of relatively neutral pH and low calcium content (lower photo), to calcareous waters, and even slightly brachish waters. This "Lobelia/Potamogeton lake" (Rapid Pond, Humber Valley) hosts *C. virgata* as well as *Nitella flexilis*. HEM

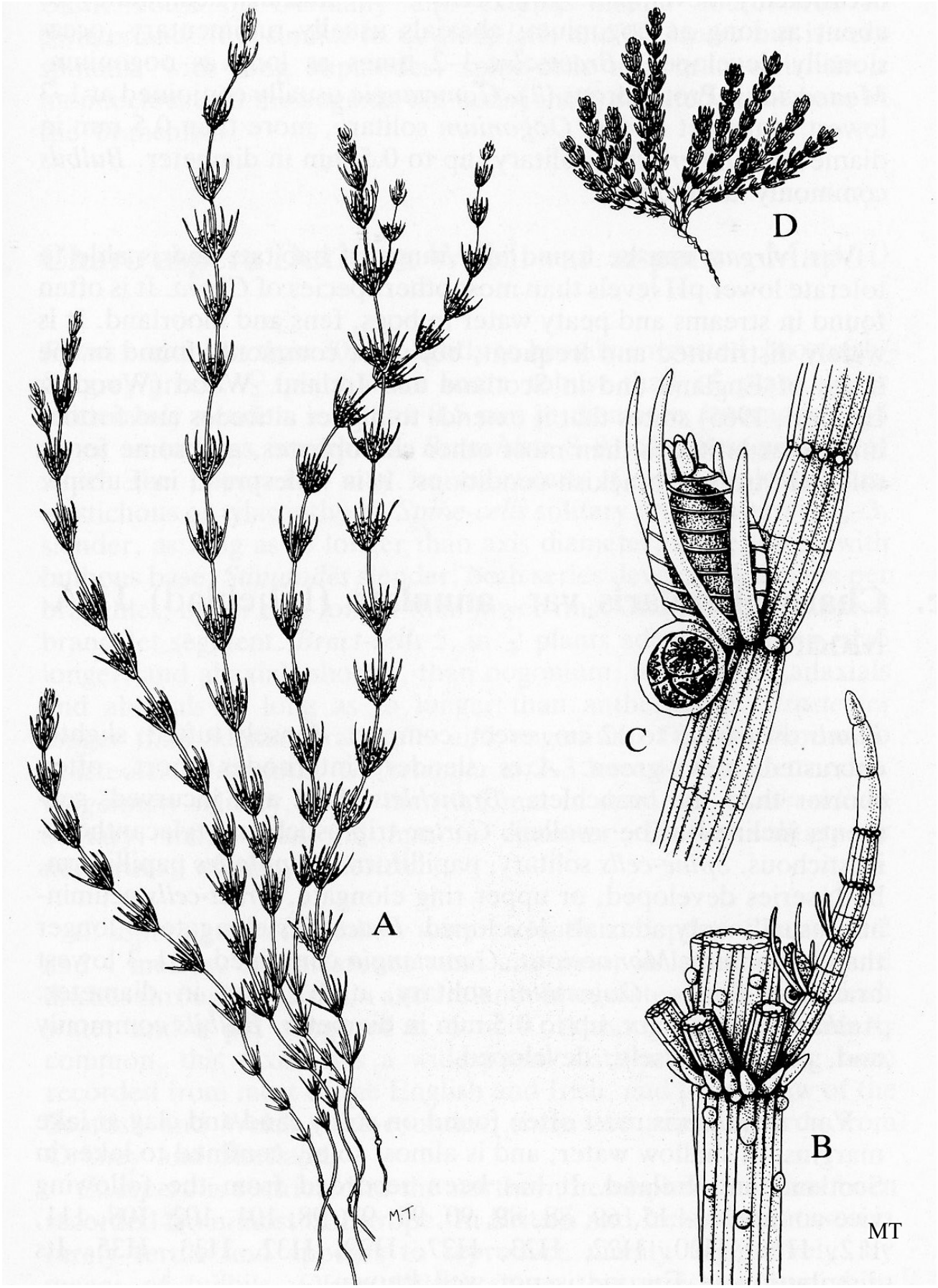


Figure III:6I *Chara virgata*. Illustrations from J.A. Moore. 1986. "Charophytes of Great Britain and Ireland". BSBI Handbook No. 5, Botanical Society of the British Isles, London.

Figure III:6J *Chara virgata* occurs in most of our oligotrophic to mesotrophic ponds.

III:7 *Chara vulgaris* L.

Monoecious. Axial cortex diplostichous. Aulacanthous (secondary rows of cells more prominent than the primary rows) therefore the spine cells appear to occur in grooves or furrows. Axes often encrusted. Spine cells solitary, small and rounded, or sometimes short and well developed or longer and slightly bent. Stipulodes well developed in two equal rows, tips blunt to rounded. Mature oospores are brown. Considerable variation within this species. Branchlets sometimes with elongated uncorticated end cells and /or elongated bract cells. A common diplostichous Chara in nutrient rich fresh waters, sometimes slightly brackish. Often found in shallow ephemeral waters, pools, ditches, puddles, ponds. Globally widely distributed and common (cosmopolitan).

Usually readily distinguishable from C. contraria by the aulacanthous condition, stipulode shape and brown ripe oospores.

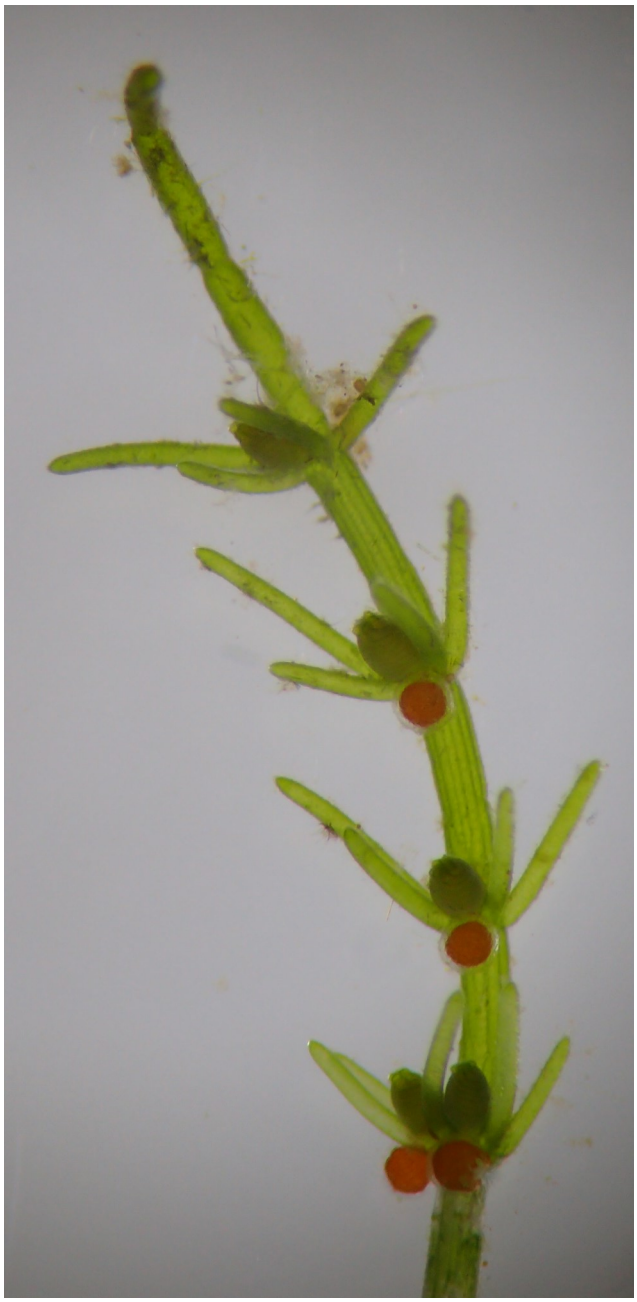


Figure III:7A A typical *C. vulgaris* axis and one branchlet enlarged. Note the bracts and bracteoles exceeding the oogonia and the occasional two antheridia and oogonia at a node. HEM

Figure III:7B *C. vulgaris* variety *longibracteata* has the ecorticated end cells of the branchlets elongated into whip-like projections. Bractioles and bracts are also very elongate. There is considerable structural variation in *C. vulgaris* and it is yet to be determined which variants are good genetic varieties and which are due mainly to environmental factors. HEM

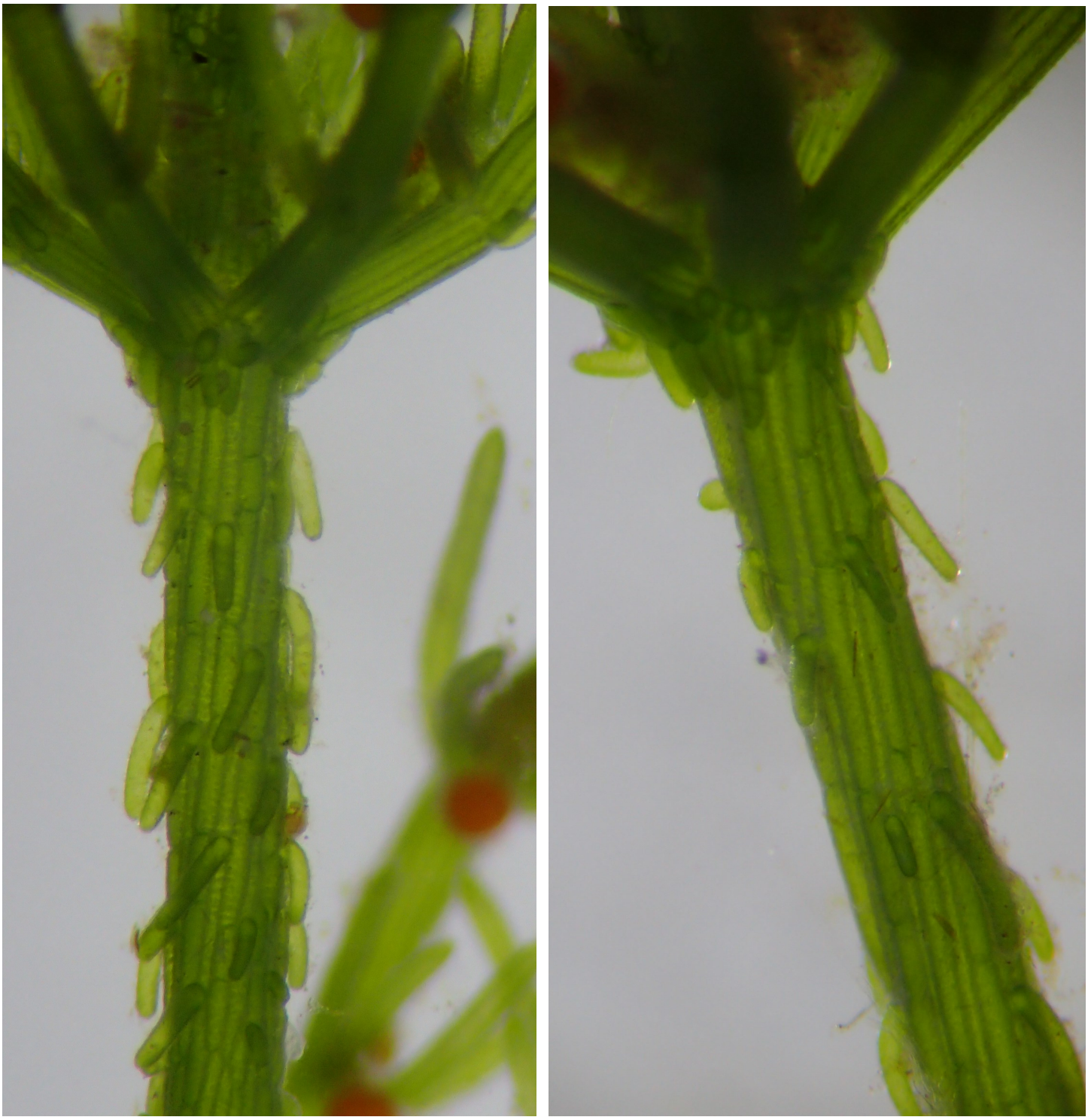


Figure III:7C One of the chief features distinguishing *C. vulgaris* from *C. contraria* is the aulacanthous cortication on the axes. *C. contraria* is tylacanthous. These photos show the primary cortex cells with spine cells are smaller/narrower than the secondary cells. The bent nature of the spine cells is often also characteristic. The length of spine cells is quite variable in this species, occasionally longer, but most often shorter than in these photos. HEM

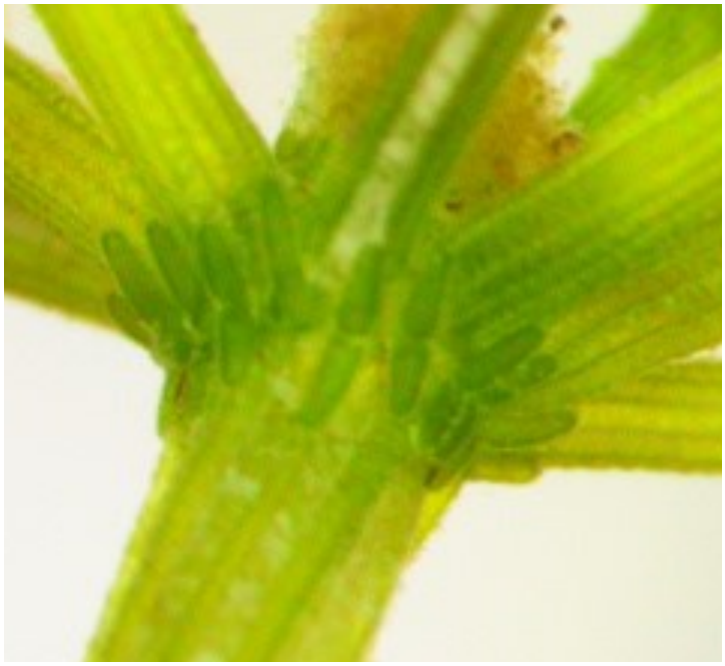


Figure III:7D 1. Actively growing stem tip of several internodes with maturing antheridia, but very immature oogonia. 2. and 3. Stipulodes are fairly regularly produced, upper and lower approximately similar in shape and size, and stipulode tips are blunt and rounded. HEM

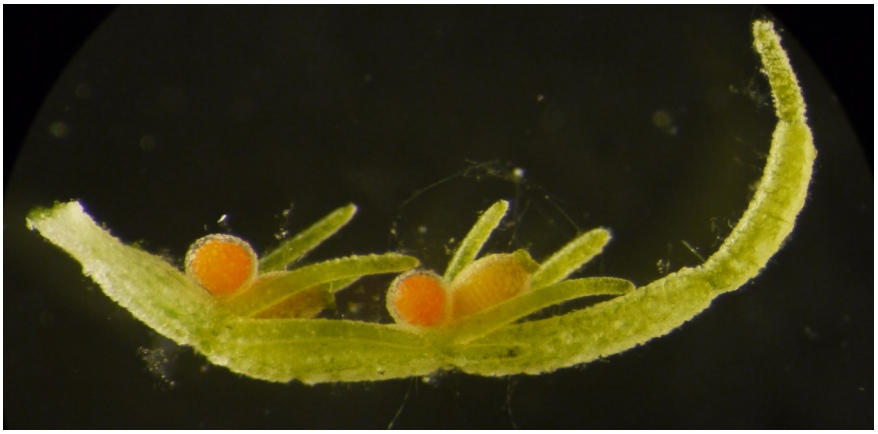


Figure III:7E Upper two photos: branchlets with mature antheridia and oogonia. Bracteoles and bracts exceed the gametangia, but are relatively short in these specimens when compared to some other collections. Lower photo: A branchlet showing some encrustation, especially on the uncorticated end segments. Plants growing in calcium rich water can often become highly encrusted and require acid removal of the lime layer in order to see cellular structures clearly. HEM

Figure III:7F Upper photo: Highly encrusted branchlet tips from plants growing in calcareous waters. Lower photo: Young not encrusted growth in non-calcareous waters. HEM

Figure III:7G Upper photo: A shallow pool recently formed in a bulldozed industrial site in Corner Brook. Water depth is only about 15 cm, but the bottom is almost entirely covered with *C. vulgaris*. This species is commonly found in shallow puddles, pools and roadside ponds of disturbed areas and attests to its rapid dispersal potential and its ability to quickly colonize disturbed sediments. Bottom photo: Close-up of *C. vulgaris* dense growth on pool bottom. HEM

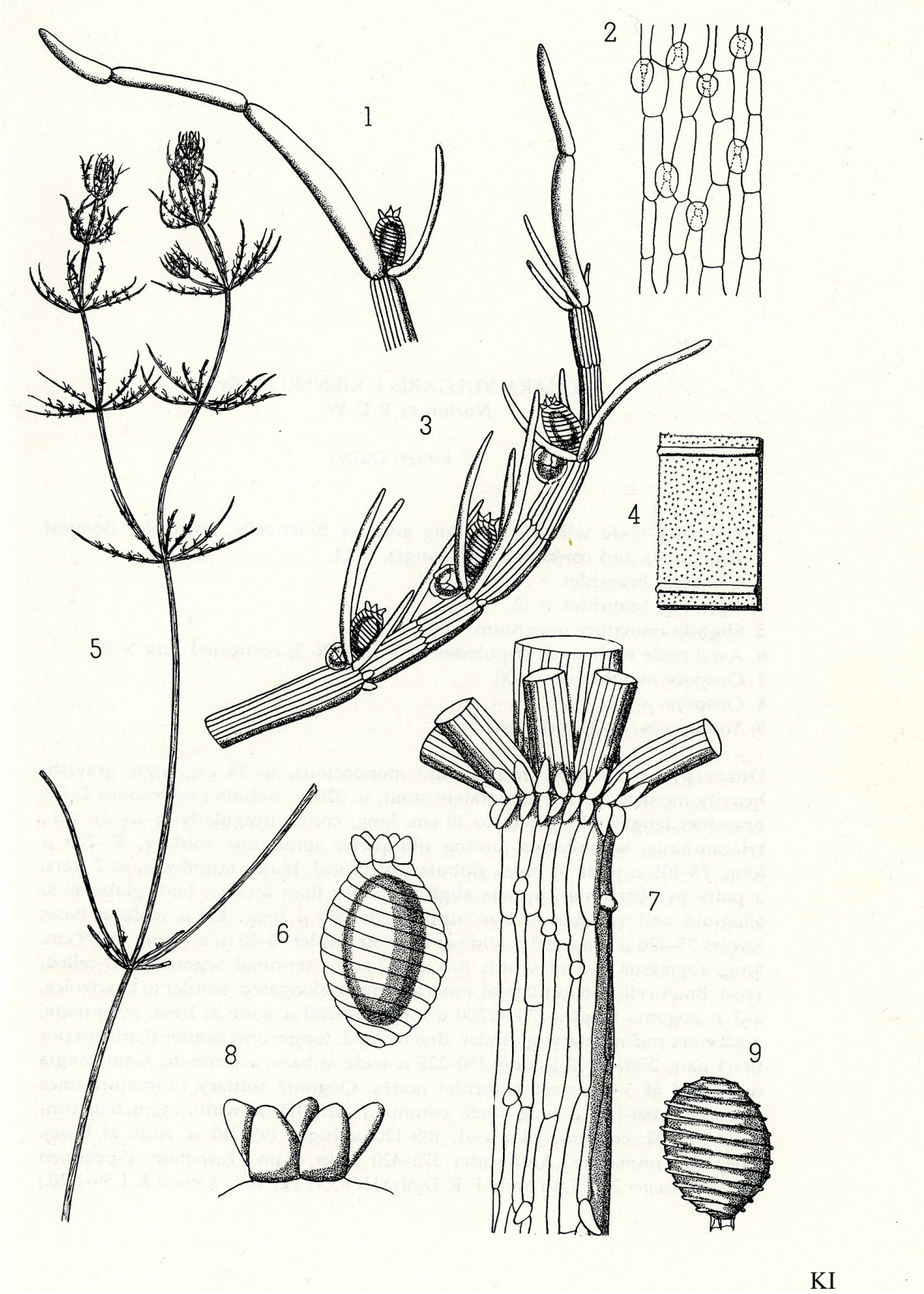


Figure III:7H Illustrations of *Chara vulgaris* from R.D. Wood and K. Imahori. 1964. "Iconograph of the Characeae", J. Cramer, Weinheim. Stipulodes in our material are often somewhat longer than illustrated in figure 7 above (see Figure III:7C).

III:8 *Chara braunii* Gmelin

C. braunii is not yet known from NL, but has been reported from Maine and Quebec and has a good chance of also being located in NL. This distinctive monoecious Chara is a bright green tufted plant without a cortex on axes or branchlets, so superficially might be mistaken for a Nitella. However, when examined it has all the basic features of a Chara, stipulodes, undivided branchlets with bract cells, and five coronula cells. It has only one row of pointed, somewhat inflated stipulodes. It is reported to grow in fresh nutrient rich waters and slightly brackish waters. Often found growing as small clustered plants in shallow pools and pond margins. It is a globally cosmopolitan species.

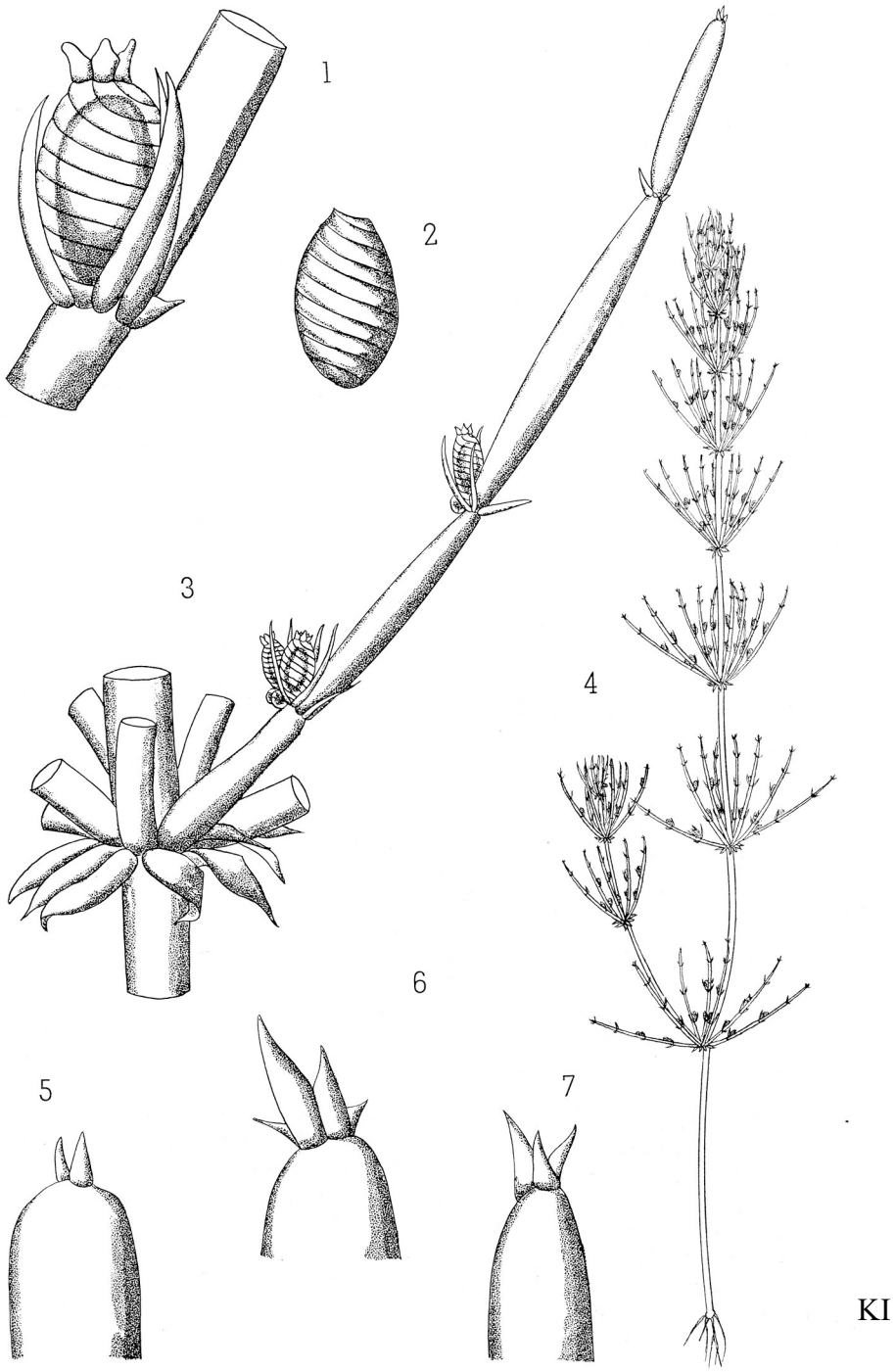


Figure III:8A *Chara braunii* illustrations from: R.D. Wood and K. Imahori. 1964. Iconograph of the Characeae, J. Cramer, Weinheim.

Figure III:8B Photo is from the shallow sandy shores and bays of eastern Grand Lake near the community of Howley, NF. Along with the connecting shallows of Sandy Lake, the lakes have not been studied for their stonewort floras and may yield interesting finds in the future. The charophyte floras of all Newfoundland's and Labrador's larger freshwater bodies are currently unknown. Additionally, coastal ponds, barachois, and brackish lagoons may also still yield some unreported species and/or varieties. HEM

IV: The Genus *Nitella*

General Appearance

Like *Chara*, the genus *Nitella* has jointed axes of nodes and internodes (Figure IV:1). Unlike *Chara*, *Nitella* axes are "naked", having no cortication on either axes or branchlets. No stipulodes occur. The stem and branchlets have a green translucent colour when not encrusted. Encrustation, when it occurs is usually banded with encrustation bands alternating with non-encrusted bands along the stem (Figures IV: 2 and 3). Branchlets arise in a whorl at the axes nodes as in *Chara*. However, unlike Chara, in *Nitella* the branchlets divide by forking into two approximately equal segments one or more times, depending on the species. The term furcate (= forked) is often used. The ultimate end segments of the furcating branchlets are called dactyls (= fingers). The dactyls may be composed of one or more cells depending on the species. The number of times the branchlets divide (fork) and the number of cells in the dactyls (fingers) are important for identification (Figures IV: 4, 5, and 6). The internodes of *Nitella* branchlets are termed "rays", the dactyls being the ultimate terminal rays. Gametangia (antheridia and oogonia) are located at the joints (nodes) where forking occurs, usually a single antheridium above and one, two, or three oogonia beside or below in monoecious species. Sometimes forking of branchlets without much elongation growth produces dense compact clusters (heads) with or without some sticky mucus-like substance (Figure IV:7). In *Nitella*, branching of the main axes is quite common to produce "bushy" plants. Branches arise at the nodes as do the branchlets. Rhizoids are produced in the substrate.

Figure IV: 1 A branching cluster of *Nitella opaca* axes with mature antheridia. HEM

Figure IV:2 Several *Nitella flexilis* axes with whorls of once furcating branchlets. No gametangia are present .

HEM

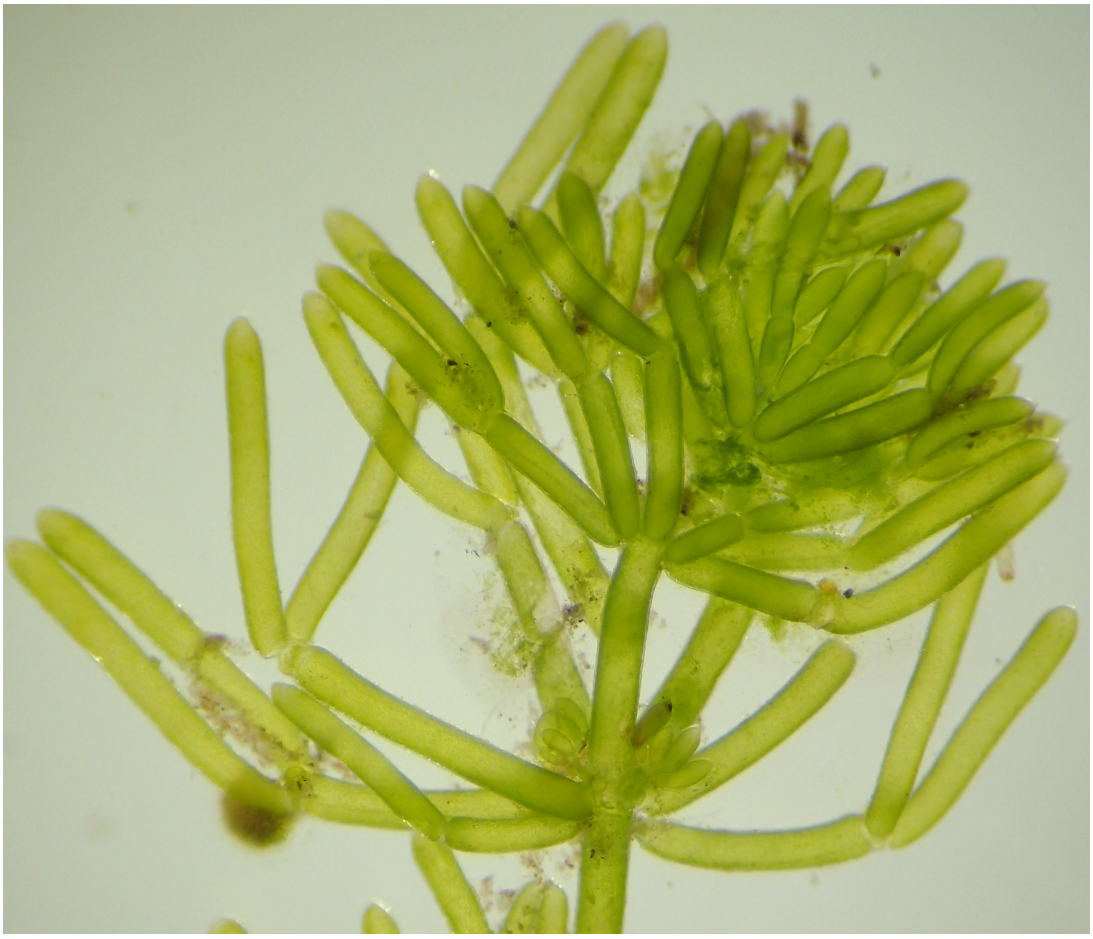


Figure IV:3A

Young axis tip showing absence of cortication, absence of stipulodes and typical bright green translucent colour. Branchlets are once divided (once furcate) into two dactyls each. No evidence of encrustation.

HEM

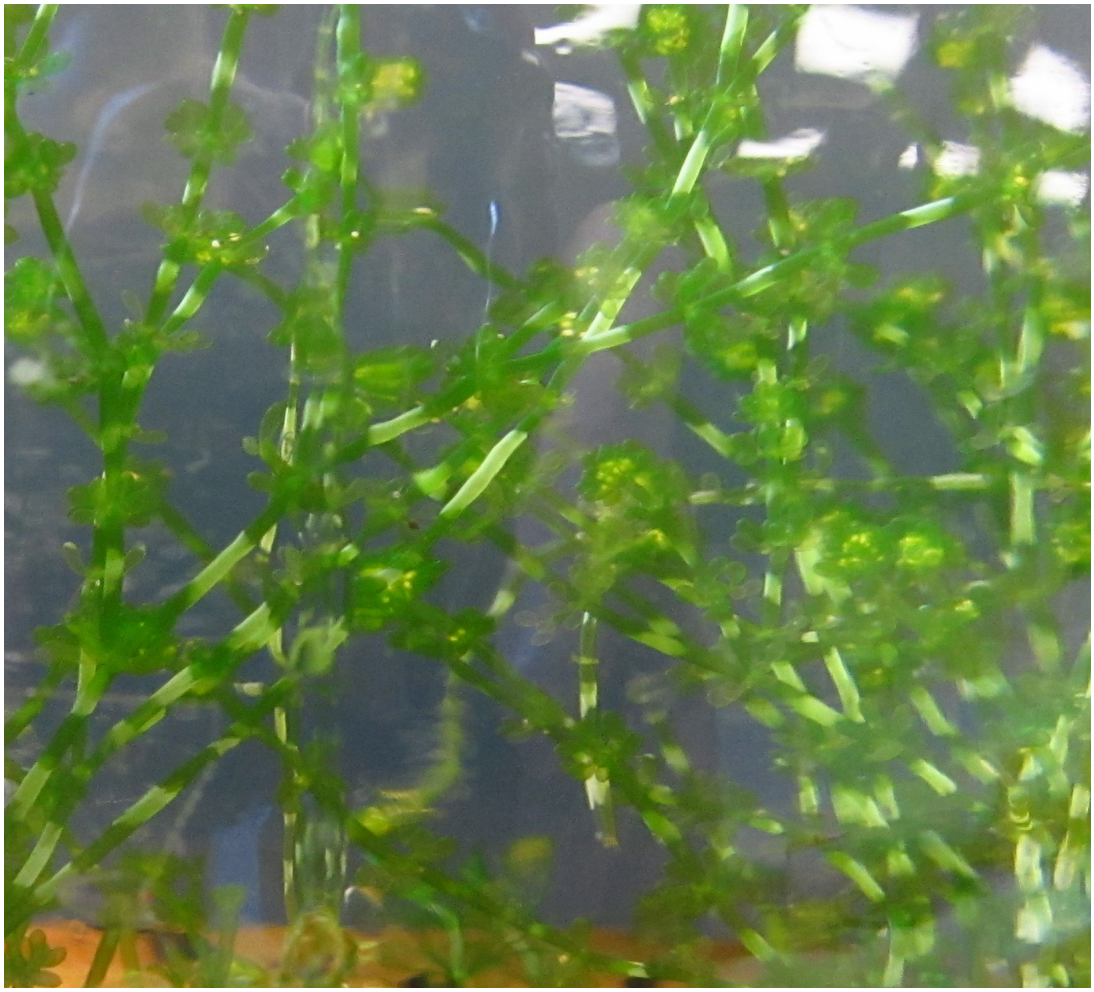


Figure IV:3B

Nitella axes in culture exhibiting banded incrustation. Often axes show little or no encrustation in our common species growing in acidic and nutrient poor waters.
HEM

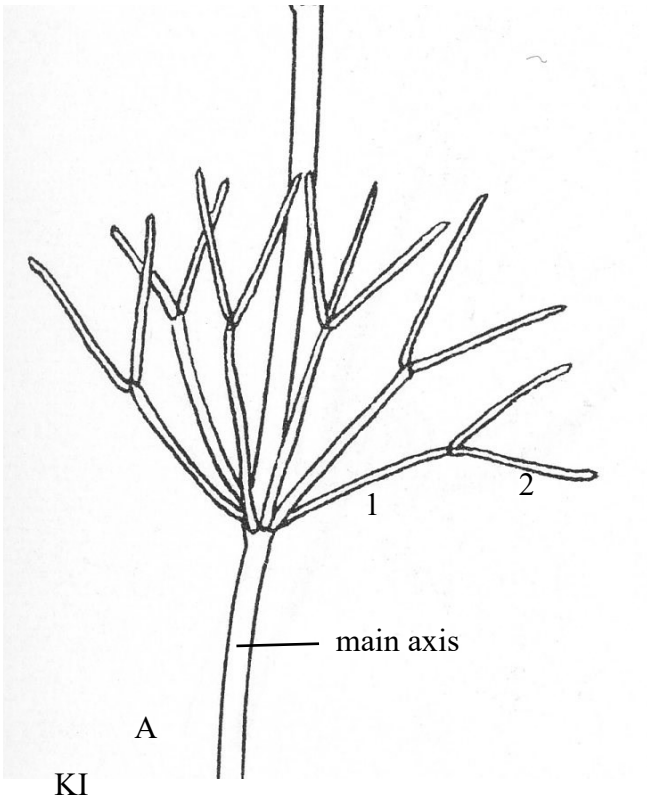
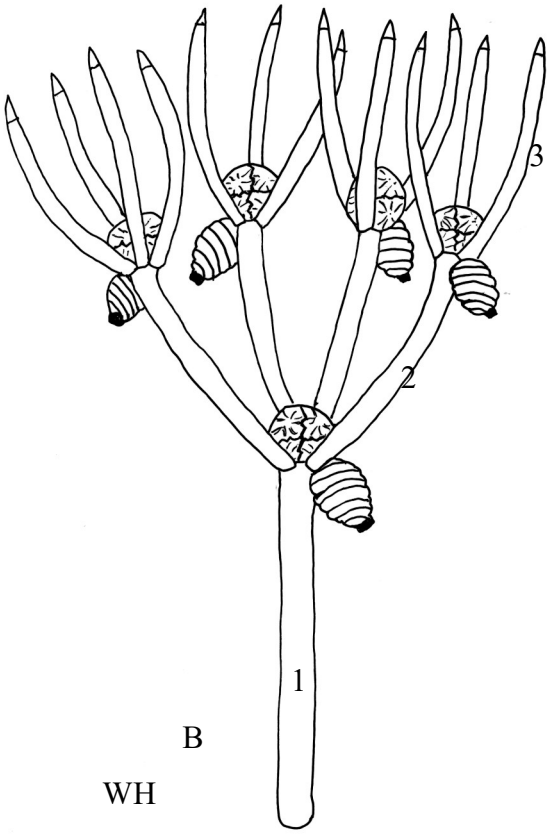


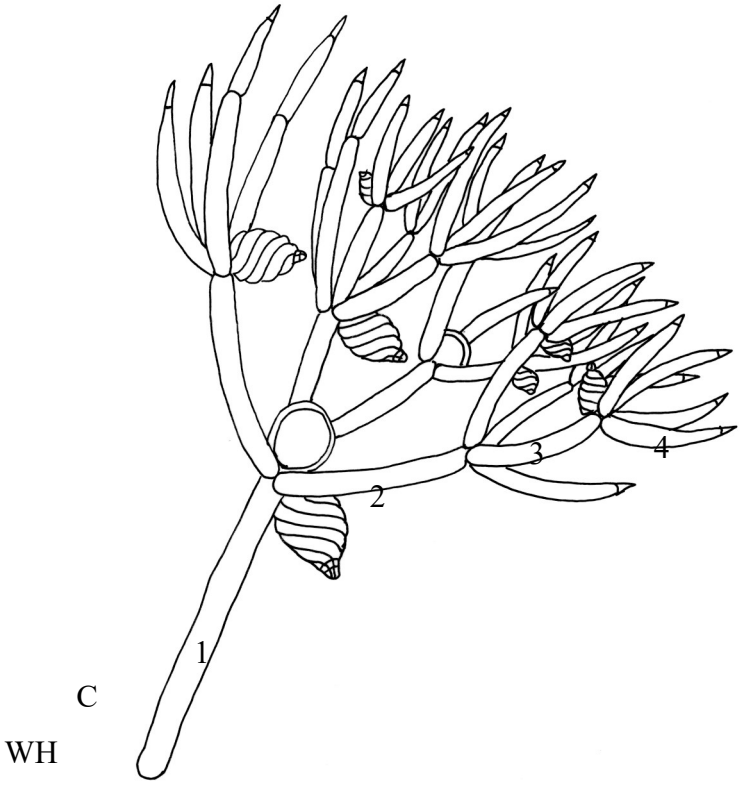
Figure IV:4 A. One node of a Nitella axis with six branchlets. Each branchlet is once divided into two or three dactyls. Each dactyl is one-celled.

B. One entire branchlet of a Nitella species which divides (furcates) twice, the second furcation producing three or four two-celled dactyls.

C. One entire branchlet of a Nitella which furcates two to three times. Dactyls are two, occasionally three-celled.

Each segment of a branchlet is termed a ray. The basal ray attached to the main axis is termed the primary ray, the next ray is secondary, then tertiary and then quaternary if there are three furcations. The quaternary rays are the dactyls. (terminal rays are termed dactyls). On the diagrams the branchlet rays are numbered as follows: primary-1, secondary-2, tertiary-3, quaternary-4. Rarely a few species may divide 4 times producing quinary rays (dactyls).





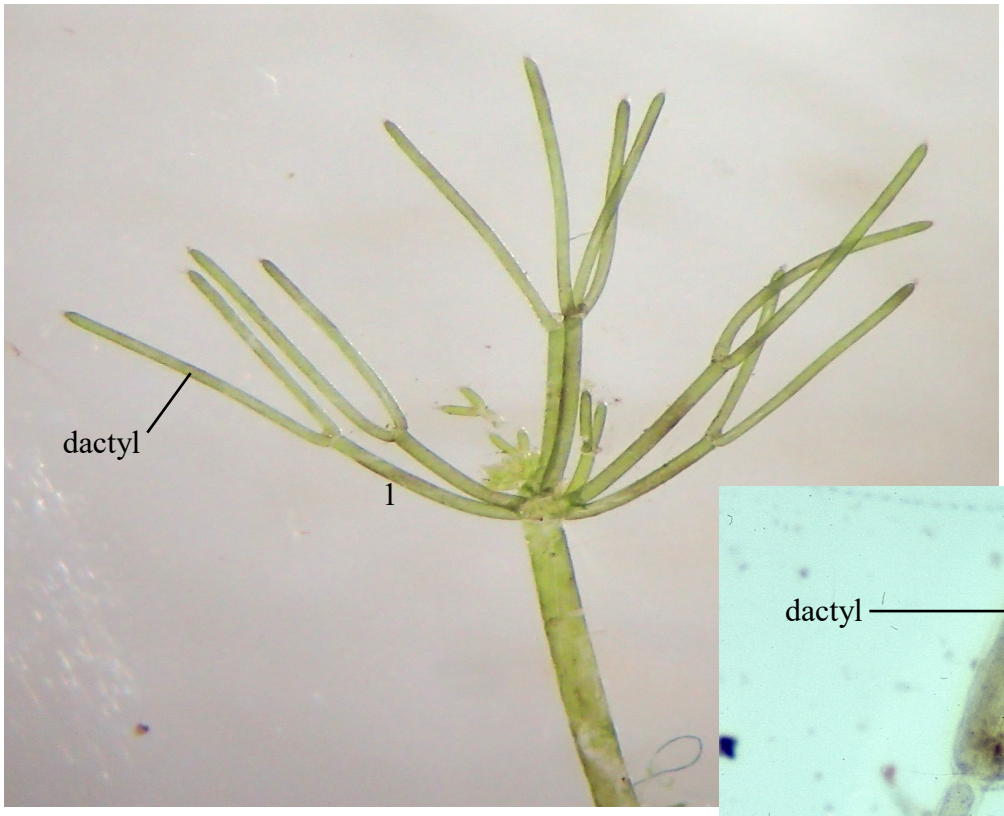


Figure IV:5A

Once furcate branchlets as in *N. flexilis* and *N. opaca*.

Primary ray labelled 1.

HEM

Figure IV:5B

Twice furcate branchlet as in *N. confervacea* . Photo of liquid preserved material. Primary ray labelled 1.

HEM

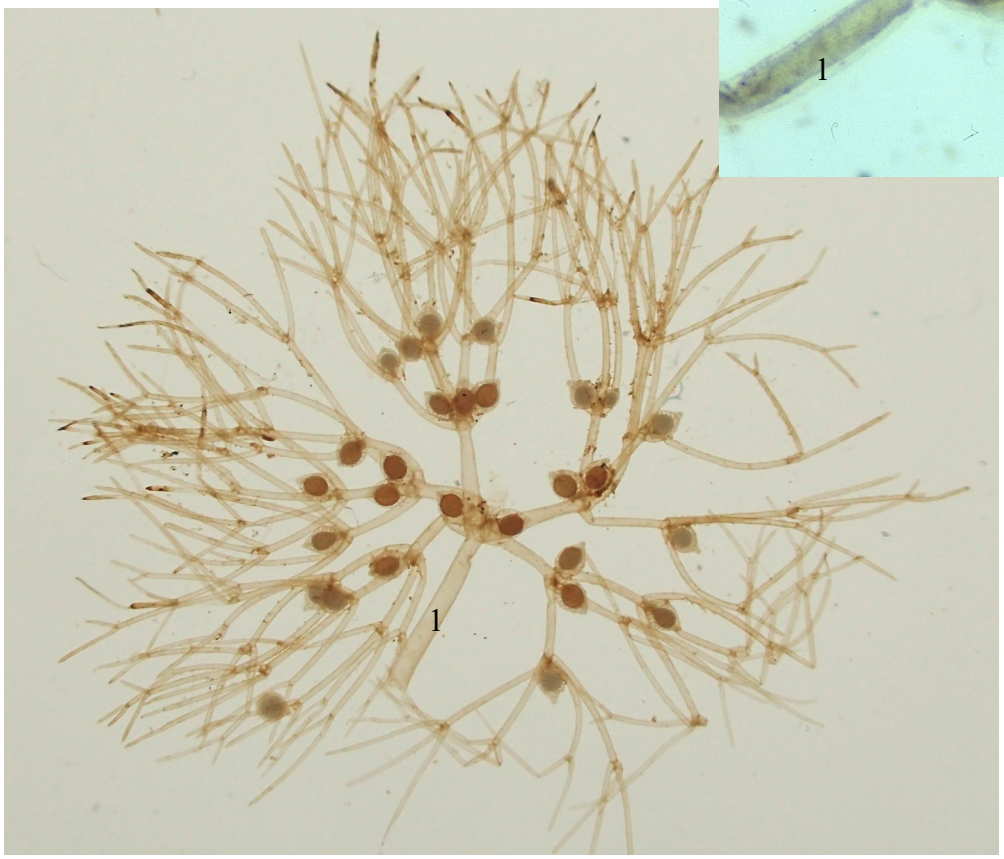


Figure IV:5C

A branchlet of a Nitella exhibiting three to four times division. Primary ray is labelled 1. Liquid preserved material.

HEM

Figure IV:6 Illustration of a Nitella in which branchlets are twice furcate. A plate from Groves and Bullock-Webster. 1920. British Charophyta, Volume I, Nitelleae. The Ray Society, London.

Figure IV:7 *Nitella opaca* ♂

A. A branching axis with terminal short branchlet whorls compacted into “heads”.

B. An enlarged “head” of compacted short branchlets with mature antheridia.

C. Compared to A and B, a more lax arrangement of elongated terminal branchlet whorls in some collections of this species.

HEM

Gametangia and Spores

Nitella species may be either monoecious or dioecious, a major feature distinguishing similar species.

Antheridia in *Nitella* are similar in structure to those in *Chara*. Their colour varies at maturity from greenish-yellow to bright orange to reddish-orange depending on the species. Their size at maturity (diameter) is sometimes a useful taxonomic (identification) feature (Figure IV:8).

Oogonia differ from those of *Chara* in several ways. Like *Chara*, the oogonium in *Nitella* is structured with a large central egg cell surrounded by five elongated narrow cells spiralling upward from right to left (clockwise). However, at its tip each spiralling cell produces two tiny cells so that the resulting coronula is composed of ten cells in two tiers rather than the five of *Chara* (Figure IV: 9). The ten coronula cells are very small and difficult to see without proper magnification and lighting. In some *Nitella* species the coronula is deciduous (may fall off at maturity) so younger oogonia should always be examined (Figures IV:10-11). A second major difference is that in *Chara* the oogonium is round (terete) in cross section, and when viewed from the coronula end. In *Nitella* when viewed from the coronula end, the oogonium is laterally compressed (somewhat flattened). Oogonia may be yellow-green to orange or reddish when mature. Their mature size may be a useful identification feature.

Mature oospores shed of their enveloping cells vary in colour from golden to brown, reddish-brown or black depending on degree of maturity and species. Like oogonia they are flattened when viewed terminally (in cross section). Their size and number of ridges from base to tip may be useful in identification.

In Nitella, oospore surface appearance (membrane decoration) is often important for identification, and many keys involve determining membrane features of mature oospores. This requires a compound microscope with magnifications of 100x to 400x. (Very advanced studies use an SEM, but that is beyond the scope of introductory charophytology as discussed in this guide, Figure IV:13). However, our common *Nitella* species can often be identified without the need of determining membrane decoration.

For anyone reasonably familiar with the use of a compound microscope, the technique of examining oospore membrane decoration is fairly simple. Several fully ripe oospores should be chosen and removed from their enveloping spiral cells. These are placed in a drop of water on a microscope slide, a cover slip is added and with gentle pressure and tapping with the eraser end of a pencil the oospores are crushed to split open. Often a bit of sideways sliding of the cover slip will spread and help flatten the membranes. Flattened sections are examined at 400x by up and down focussing to determine the pattern present.

Numerous types of membrane patterns have been described in the literature at the light microscope level. For practical purposes they fall into four main categories; (a) smooth, unornamented with no distinctive features visible, (b) granulate, a rough dotted appearance of fine to coarse granules arranged in various ways, (c) tuberculate, large, coarse protuberances of various shapes, and (d) reticulate, ridges or tuberculate features forming a mesh network (Figure IV:12). Sometimes a judgement must be made when these types appear to overlap (e.g. when does a coarse granulate surface become tuberculate? or when does a granular or tuberculate pattern become sufficiently organized to be considered reticulate?). Also immature oospores sometimes do not clearly show the mature pattern. Despite the sometimes arbitrary judgemental nature required, usually the features visible at 400x with the compound light microscope are sufficient for identification.

Figure IV:8 A. Tip of *Nitella opaca* ♂ axis with mature antheridia terminal in branchlet furcation. HEM B. Illustration of an antheridium showing four antheridial plates (scutes). WH C. Two antheridia, the larger exhibiting four scutes. HEM

Figure IV: 9 A. Lateral view of a mature Nitella ogonium. HEM B. Top view of a mature Nitella oogonium showing flattened structure and ten coronula cells. HEM C. A once furcate branchlet with a terminally situated antheridium and two laterally situated oogonia. WH. D. Oogonia situated laterally at branchlet furications. Note enlarged spiral cell tips and the absence of coronulas which have already been shed at this stage. HEM

Figure IV:10 A. Compound microscope photo of a liquid preserved mature antheridium and two immature oogonia (one out of focus in background). B. Greatly magnified same oogonium. Two spiral cells are each terminated by two coronula cells, one above the other. HEM

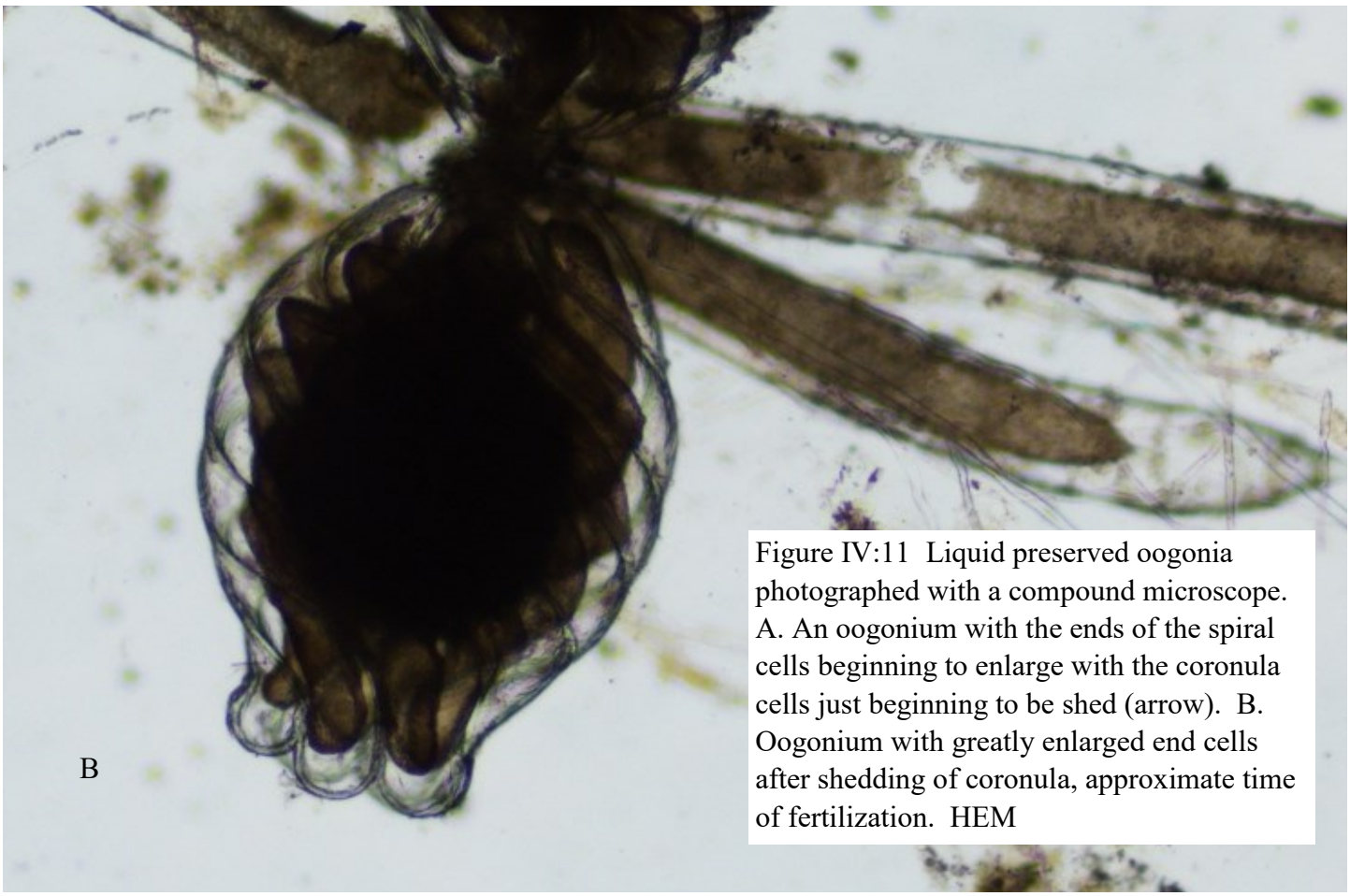
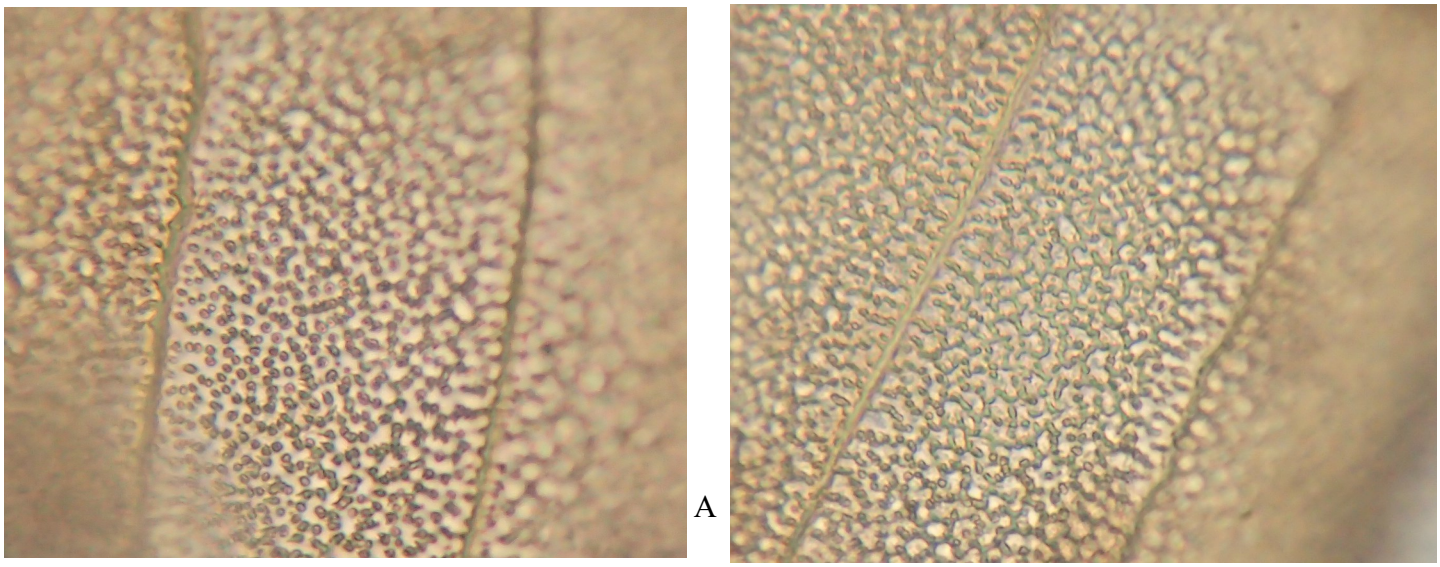


Figure IV:11 Liquid preserved oogonia photographed with a compound microscope. A. An oogonium with the ends of the spiral cells beginning to enlarge with the coronula cells just beginning to be shed (arrow). B. Oogonium with greatly enlarged end cells after shedding of coronula, approximate time of fertilization. HEM



A

B

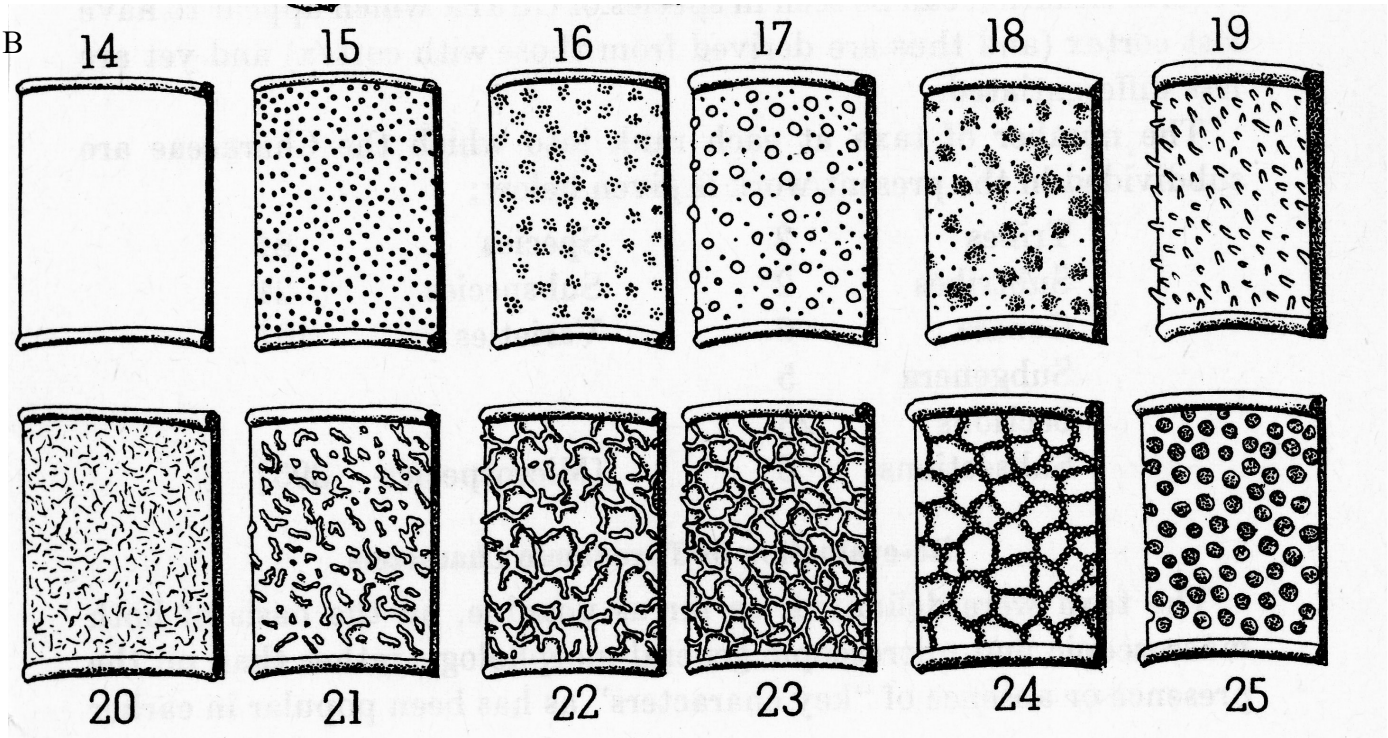


Figure IV:12 A. Two membrane photos at 400x of the same Nitella oospore. The left photo exhibits a coarse granular pattern, while the right photo shows an imperfect reticular pattern. HEM

B. Some basic membrane decoration patterns as illustrated in Wood (1965). 14. smooth, not ornamented, 15-19 various granular patterns, 20 felty, fibrous, 21 tuberculate, 22 imperfectly reticulate, 23-24 reticulate, 25 pitted. KI

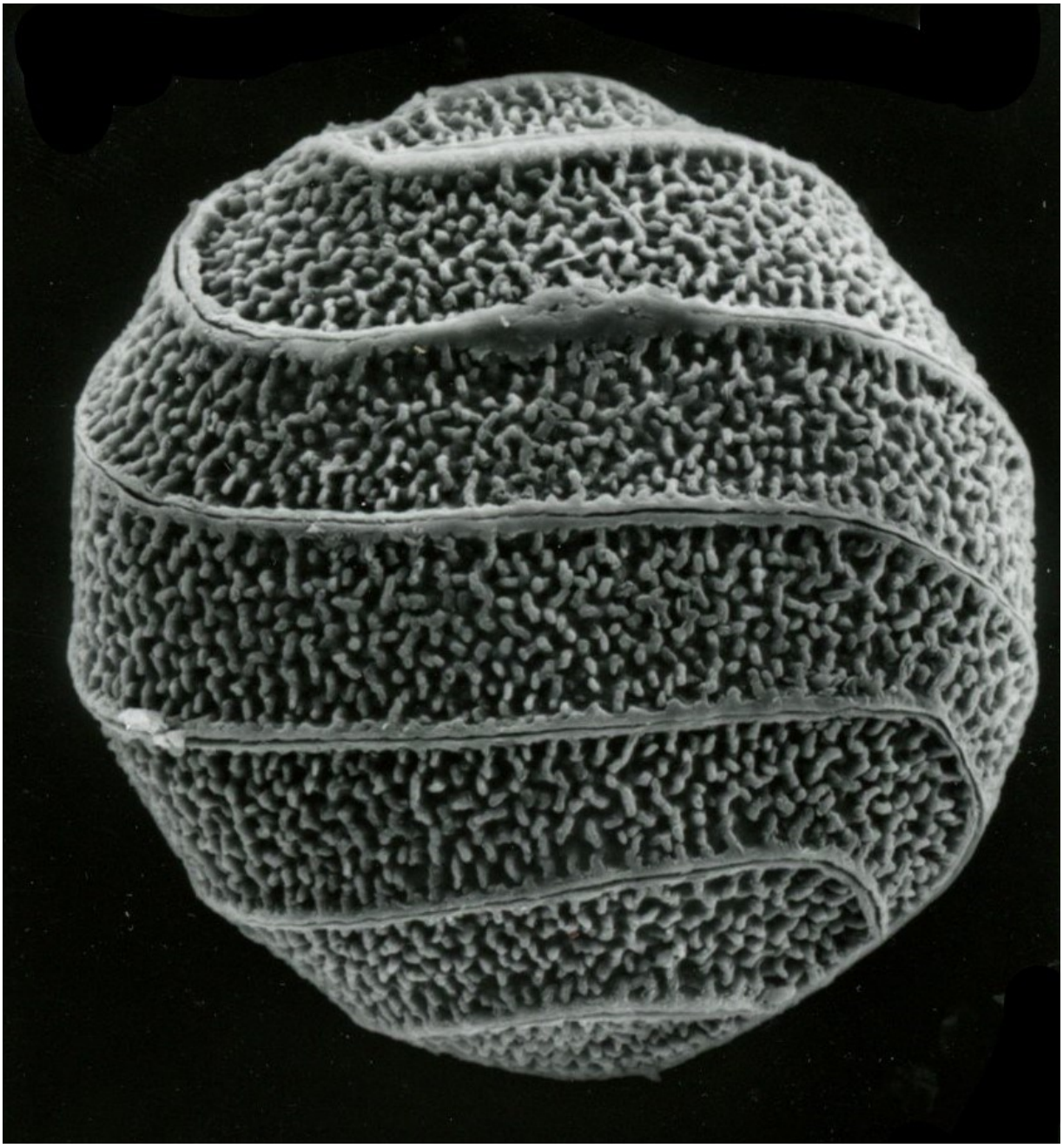


Figure IV: 13 An SEM photograph of a *Nitella macounii* oospore exhibiting a tuberculate membrane decoration. EMVN

Nitella Species Descriptions

Nitella flexilis (L.) Agardh

Monoecious*. Axes normally from 10 cm to an excess of 30 cm with a fresh green appearance. Usually not encrusted, or only slightly so. Branchlets fork (furcate) once into 2 – 3 one-celled dactyls, dactyls sometimes of unequal size. Variable appearance, from lax slender plants (Figure IV:14) to more stout robust plants, to plants with branchlets clustered into heads; probably related, at least in part, to environmental conditions such as depth, flowing water, etc. (Figure IV:15-16).

Common throughout Newfoundland and Labrador mainly in nutrient poor oligotrophic waters, often in deep ponds and lakes, although reports also occur from slightly brackish waters. Globally cosmopolitan.

* Overwintering or very early season plants may have only immature antheridia before oogonia develop and so may be mistaken for dioecious *N. opaca* (Figure IV:17).Also antheridia are shed (disintegrate) early so plants later in the season may only have oogonia or be with developing oospores, therefore appearing to be female plants of *N. opaca*. Sterile plants cannot be distinguished from *N. opaca* and should be treated as *N. flexilis* (s.l.), or N. flexilis (Agg.) (Agg.=aggregate).

Figure IV:14 Several sterile axes of *Nitella flexilis*, relatively slender lax features. HEM

Figure IV:15 A branching axis of Nitella flexilis with terminal branchlet whorls compacted into fertile heads. HEM

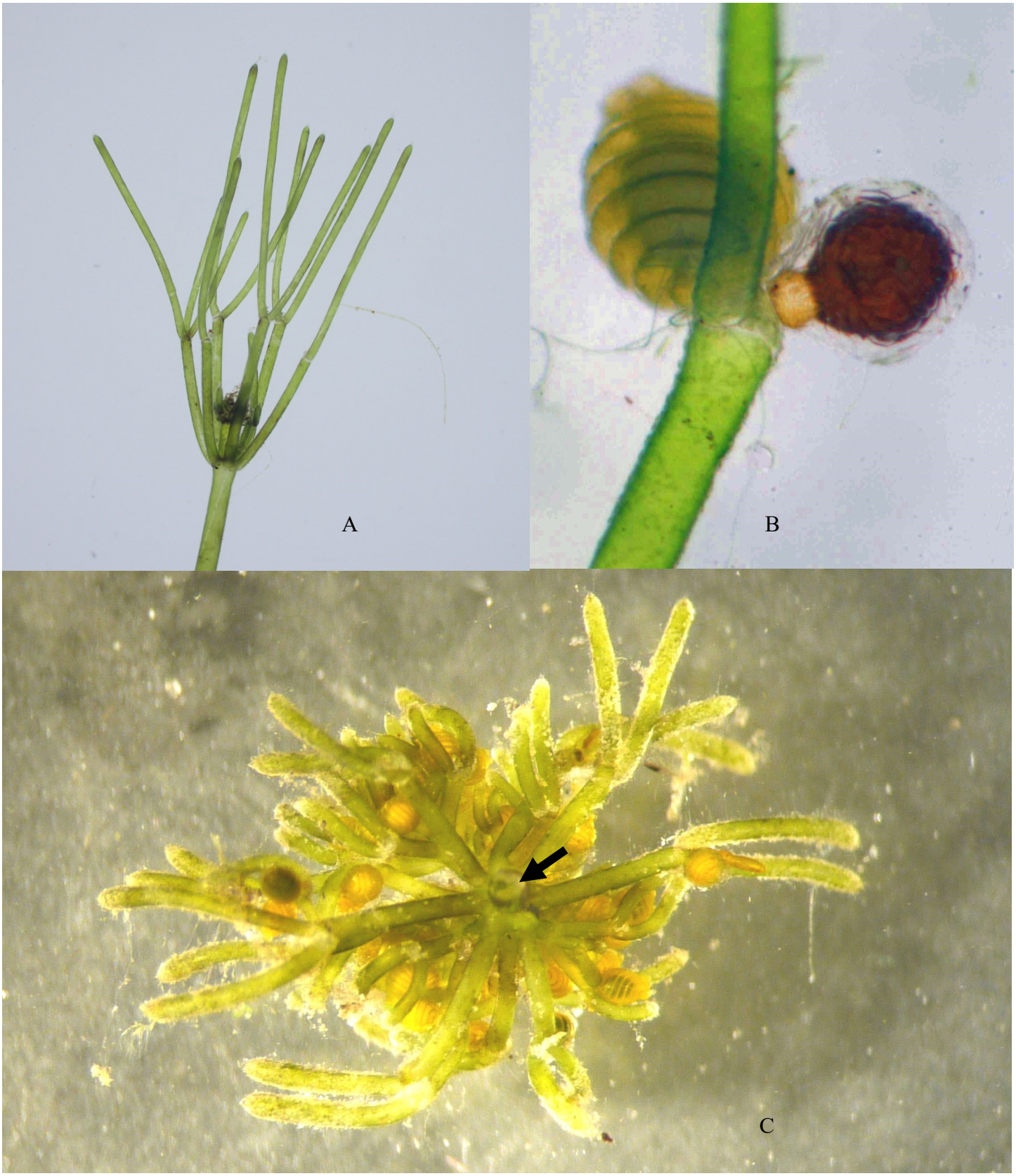


Figure IV:16. A. Once furcate sterile branchlets. HEM. B. Enlarged oogonium and antheridium at branchlet furcation, only one dactyl visible. AL. C. Branchlet whorls radiating outward from a cross section of the main axis (arrow). Oogonia with maturing oospores developing, antheridia no longer present. HEM

Figure IV:17 Young branchlet whorl with maturing antheridia easily mistaken for dioecious N. opaca because very immature oogonia (arrow) are sometimes infrequent and often overlooked.

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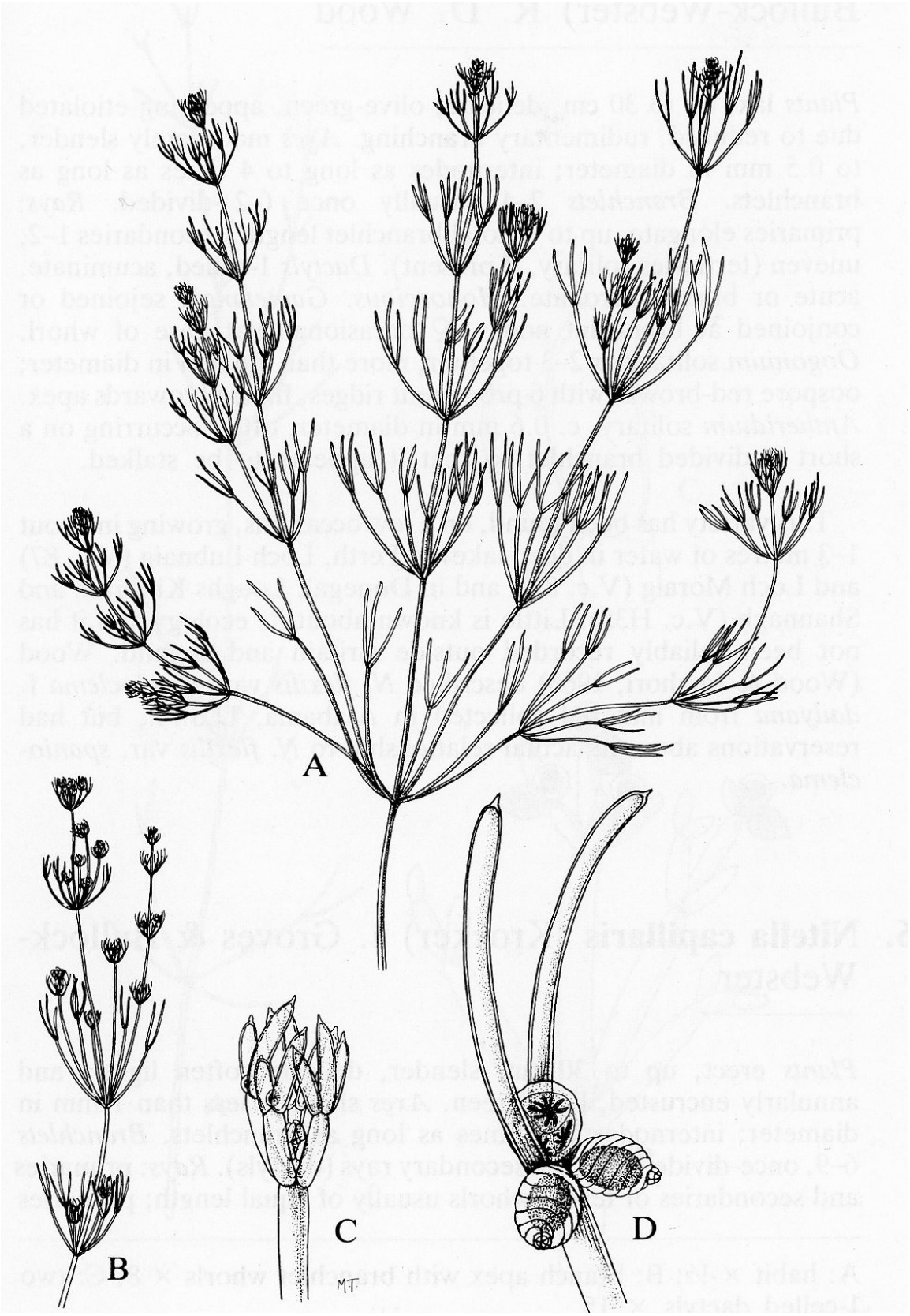

Figure IV:18 Illustrations of *Nitella flexilis* from J.A Moore, 1986. Charophytes of Great Britain and Ireland. BSBI Handbook No. 5. Botanical Society of the British Isles, London, UK. MT

Nitella opaca* (Bruzelius) Agardh

Dioecious. Fresh green plants normally without encrustation. Branchlets once furcate into 2 or 3 one-celled dactyls. Sterile plants without gametangia are not distinguishable from monoecious *N. flexilis*. Plants without gametangia are referred to as *Nitella flexilis* (s.l.) or "*Nitella flexilis* aggregate". (s.l. = sensu lato, meaning "in the broad sense"). Originally this species was considered a variation of *N. flexilis* before current acceptance that monoecious and dioecious taxa constitute separate species. When gametangia are present, size differences may help to distinguish the two species (G.O. Allen 1950).

	N. flexilis	*N. opaca*
Antheridium diameter	500-625 µm	650-775 µm
Oospore length	500-600, av. 550 µm	350-450, av. 400 µm
Oospore width	av. 450 µm	av. 375 µm

Common in NL, especially in oligotrophic nutrient poor waters. Globally cosmopolitan.

* Every effort should be made to collect both male and female plants at the same time and location, especially at the time when antheridia are mature prior to their disintegration.

Figure IV:19 A liquid preserved cluster of *N. opaca* ♂ axes arising from a common source in the substrate. Liquid preservation maintains morphological structure well, but the bright green color becomes washed out. Compare to living specimen Figure IV:1. HEM

Figure IV:20 Nitella opaca ♂ with mature antheridia situated terminally in the branchlet furcations. Enlarged insert photo exhibits two antheridial plates (scutes). HEM

Figure IV:21 Nitella opaca ♀. Oogonia situated laterally at branchlet furcations. Tips of spiral cells have enlarged and coronulas are no longer present.

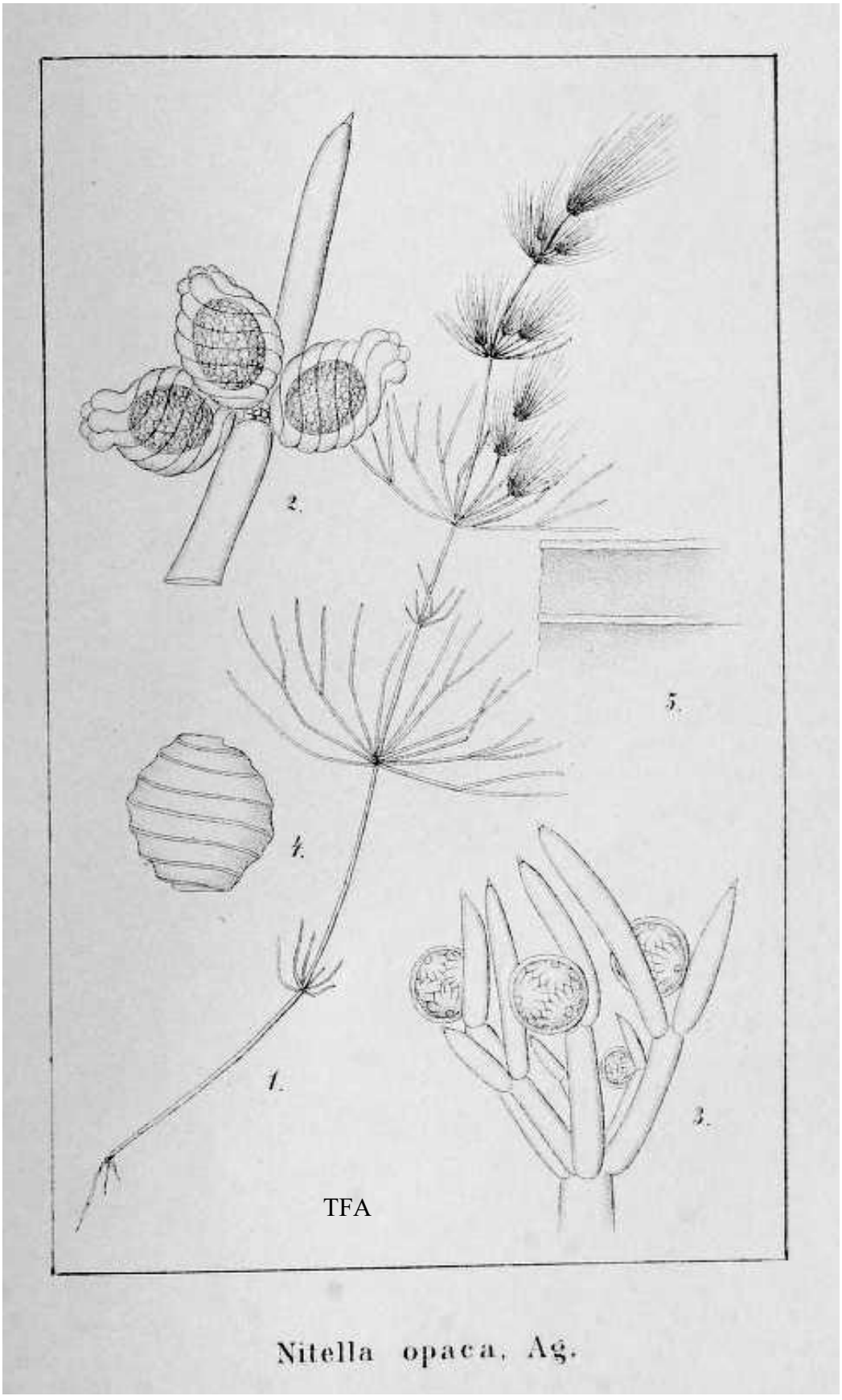

Figure IV:22 Illustrations of Timothy Field Allen (T.F. Allen. 1888. The Characeae of North America. Part II. New York Academy of Sciences, NY.)

***Nitella confervacea* (Breb.) A. Braun** (= *N. batrachosperma* Braun)

Monoecious. One of the smallest Nitellas; a tiny delicate species only about 3-5 cm in length, much branched, tight clusters of branchlets often forming "heads" (Figure IV:23). Heads sometimes with a hint of mucus. Branchlets dividing (furcating) twice, occasionally three times. Each dactyl of two cells, the small end cell sharply pointed. Gametangia common at lowest branchlet node, less common at second branchlet node. Could be easily mistaken for a similar, but somewhat larger species *Nitella gracilis* which has not yet been reported for NL (*N. gracilis* is a slightly larger plant to 10 cm or more with some dactyls 3-celled and gametangia common at all branchlet furcations).

Currently known in NL only from three locations; Upper Codroy Estuary (slightly brackish) on muddy sediment in 1-2 meters of water, on sandy shallow shores of Deer Lake (partly sand covered), and in Labrador in sandy/muddy shallows of Gosling Lake. Considered rare in NL (S Rank 1 – at risk), but because of its tiny size it may be more common than is now known. Globally a cosmopolitan species.

Figure IV:23 Branching liquid preserved axes of *Nitella confervacea*. Distinct heads formed with some mucus. Scale bar in mm. HEM

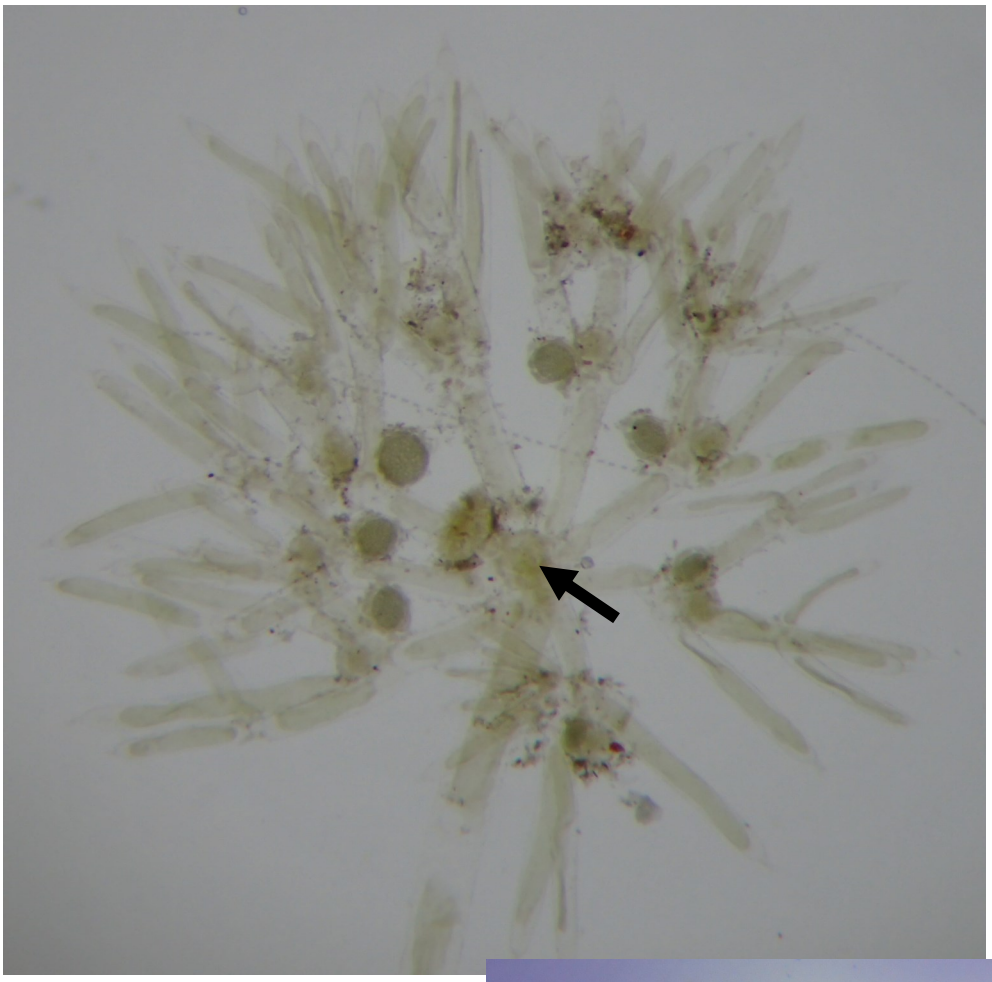

Figure IV:24

Nitella confervacea. Liquid preserved unstained whorl of branchlets radiating outward from a cross section of a main axis (arrow) as seen with a stereomicroscope. Structures indistinct. HEM

Similar whorl of branchlets like upper photo, but stained with toluidine blue. Branchlets and gametangia much more distinct. HEM

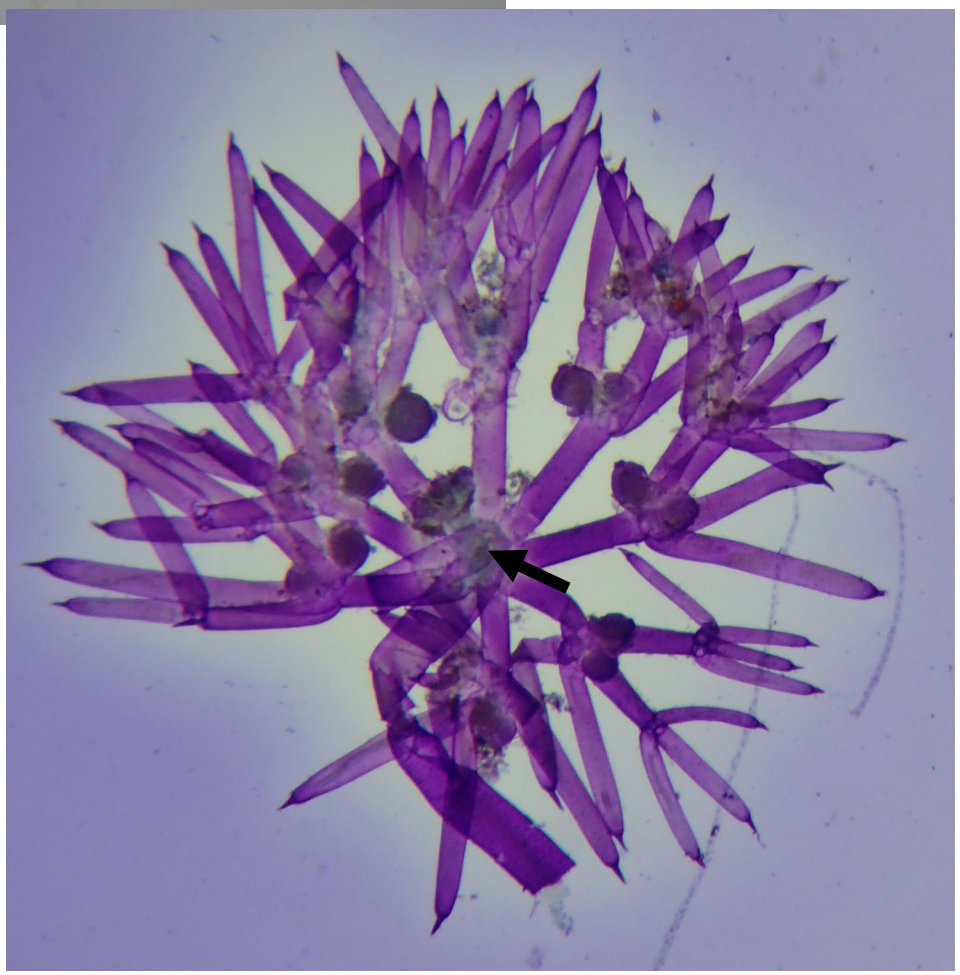

Figure IV:25

Upper photo: two branchlets stained with toluidine blue and viewed with stereo microscope. Rays labelled: primary ray-1, secondary ray-2, tertiary ray-3 (dactyl).

Lower photo a single branchlet also stained, and as viewed with a compound microscope (transmitted light). Rays labelled as above.

HEM

Figure IV:26 Upper photo: two-celled dactyls with tiny conical end cells as seen with a stereomicroscope. Lower photo: greatly enlarged tips of three dactyls with tiny conical end cells as seen with a compound microscope. Both photos of liquid preserved material stained with toluidine blue. HEM

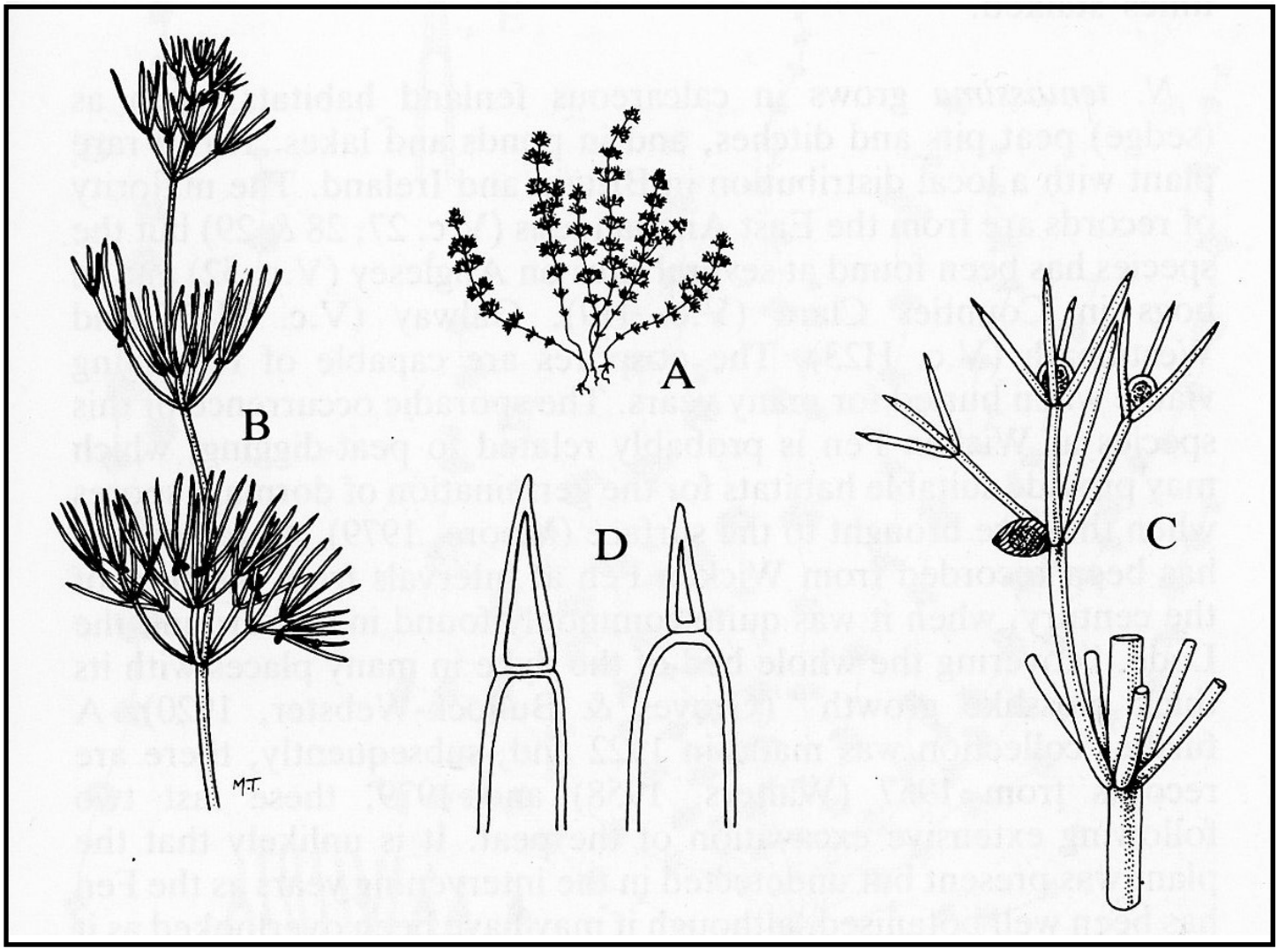

Figure IV:27 *Nitella confervacea.* Illustrations from: J.A. Moore. 1986. Charophytes of Great Britain and Ireland. B.S.B.I. Handbook No. 5., Botanical Society of the British Isles, London, UK. MT

V: The Genus Tolypella

General Appearance

Like all charophytes, the basic structure consists of stem nodes and internodes with whorls of branchlets arising at the nodes. Branchlet whorls are often compacted into tight clusters (heads) sometimes referred to as "bird's nests". Division (branching) of the main axes (stems) is common in Tolypella to produce bushy plants. Like *Nitella*, *Tolypella* has naked axes and branchlets without any cortication (Figure V:1).

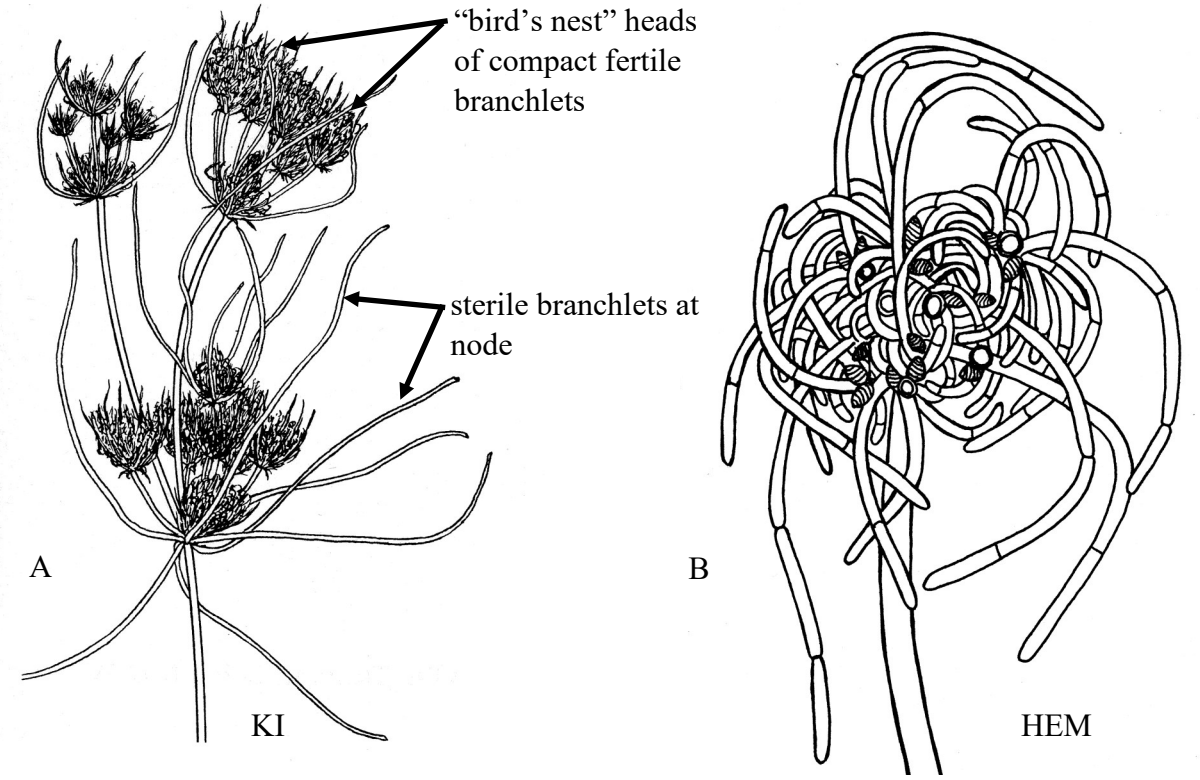

Figure V:1 A. Upper portion of a branching axis illustrating sterile branchlets at nodes and tight fertile clusters of branchlets organized into "bird's nests". B. An enlarged "bird's nest" of fertile branchlets.

Tolypella produces branchlets of two types at the main axis nodes, sterile ones that do not exhibit gametangia and smaller fertile ones compacted into "bird's nests" with antheridia and oogonia. The sterile ones arising at the nodes are often elongate and undivided. The fertile branchlets in compact heads are small, often bent or curled and with a larger, more prominent central main axis (ray) and smaller side divisions (lateral rays) (Figure V:2). They do not obviously fork (furcate) into two roughly equal parts as in *Nitella.* The end cells of the branchlet rays are important for identification, whether rounded at the tip (obtuse) or sharply pointed (acute), (Figure V:11).

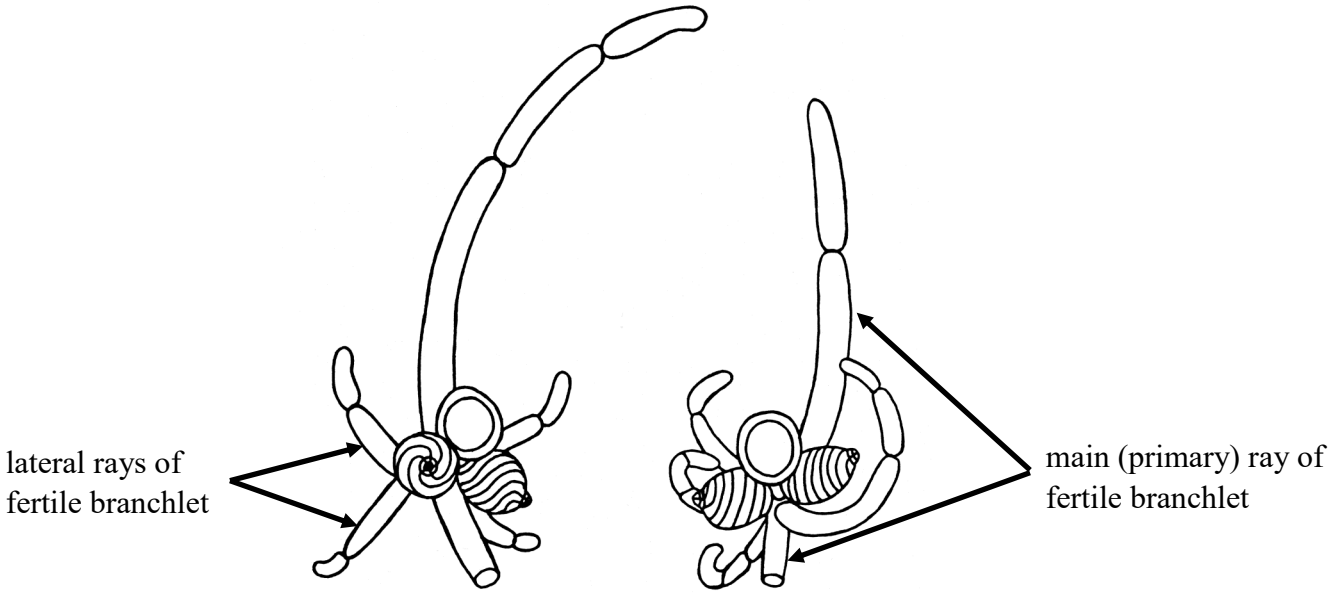

Figure V:2 Two fertile branchlets, each with four lateral rays produced at the first node of the primary ray. HEM

Gametangia

A single antheridium flanked by several oogonia occurs at the base of the lateral rays and at the base of the branchlets where they attach to the main stem (axis). Antheridia in *Tolypella* are globular and similar to those of *Nitella* except that they are often each attached by a visible short stalk. Oogonia are also attached on short stalks, careful observation required to distinguish them. Like *Nitella* there are ten coronula cells, but unlike *Nitella*, oogonia are terete (round) in cross section rather than flattened (Figure V:3). Oospores are also terete and may vary with the species from golden-yellow, to brown, to reddish. Oospore size may be useful for identification. The known species of Newfoundland Tolypella and others likely to be found in Newfoundland are all monoecious.

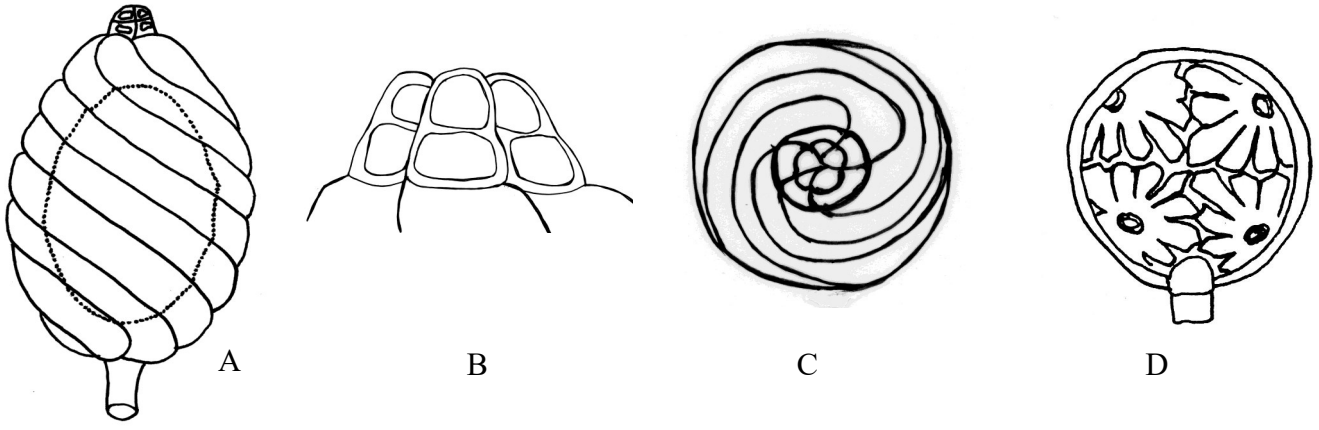

Figure V:3 A. Side (lateral) view of an oogonium showing stalk at base. B. Enlarged side view of coronula showing two tiers of cells (ten cells in total). C. Apical view of oogonium showing ten coronula cells and the terete (round) shape of the oogonium. D. An antheridium showing four triangular shields (plates or scutes/scuta) and a stalk which is often longer than illustrated. HEM

Description of Tolypella Species

Genus Tolypella: Branching naked axes (stems) without cortication; no stipulodes. "Bird's nest" clusters (heads) of fertile branchlets; branchlets composed of main (primary) rays and smaller lateral rays. Coronula of two tiers of five cells (total 10). Oogonia and oospores terete (round) in cross section.

Only one species of Tolypella has been located in Newfoundland and Labrador to date, *Tolypella glomerata*. Other species may be discovered provincially in the future and those most likely are discussed after the following descriptions.

***Tolypella glomerata* (Desv.) Leonh.**

Monoecious. Plants 5-20 cm long, usually grayish encrusted. Longer sterile undivided branchlets of 3-5 cells occur at the nodes. The fertile branchlets at the nodes are short, incurved and clustered into compact heads ("bird's nests"). Fertile branchlets have a main central end ray of 3-4 cells, the end cell has a blunt rounded tip. From the main central ray, smaller lateral rays of 3-4 cells are produced at a node whose end cell tips are also rounded. Cells of the rays are somewhat "sausage" shaped. Antheridia and oogonia are clustered at the branchlet nodes, sometimes also with oogonia at the branchlet base. Oospores are brown with a granular membrane. (For details of this species refer to the Mann 1994 paper)

All three NL collections are coastal, two in shallow ephemeral pools and one in a small pond, all subject to some salt spray from the ocean. All are in calcareous areas with a high water pH and relatively high conductivity, but still in the freshwater range. To date collections are all from the general Port au Choix to Eddie's Cove East area. Listed as Rare in NL (S Rank 1 = at risk). Globally cosmopolitan.

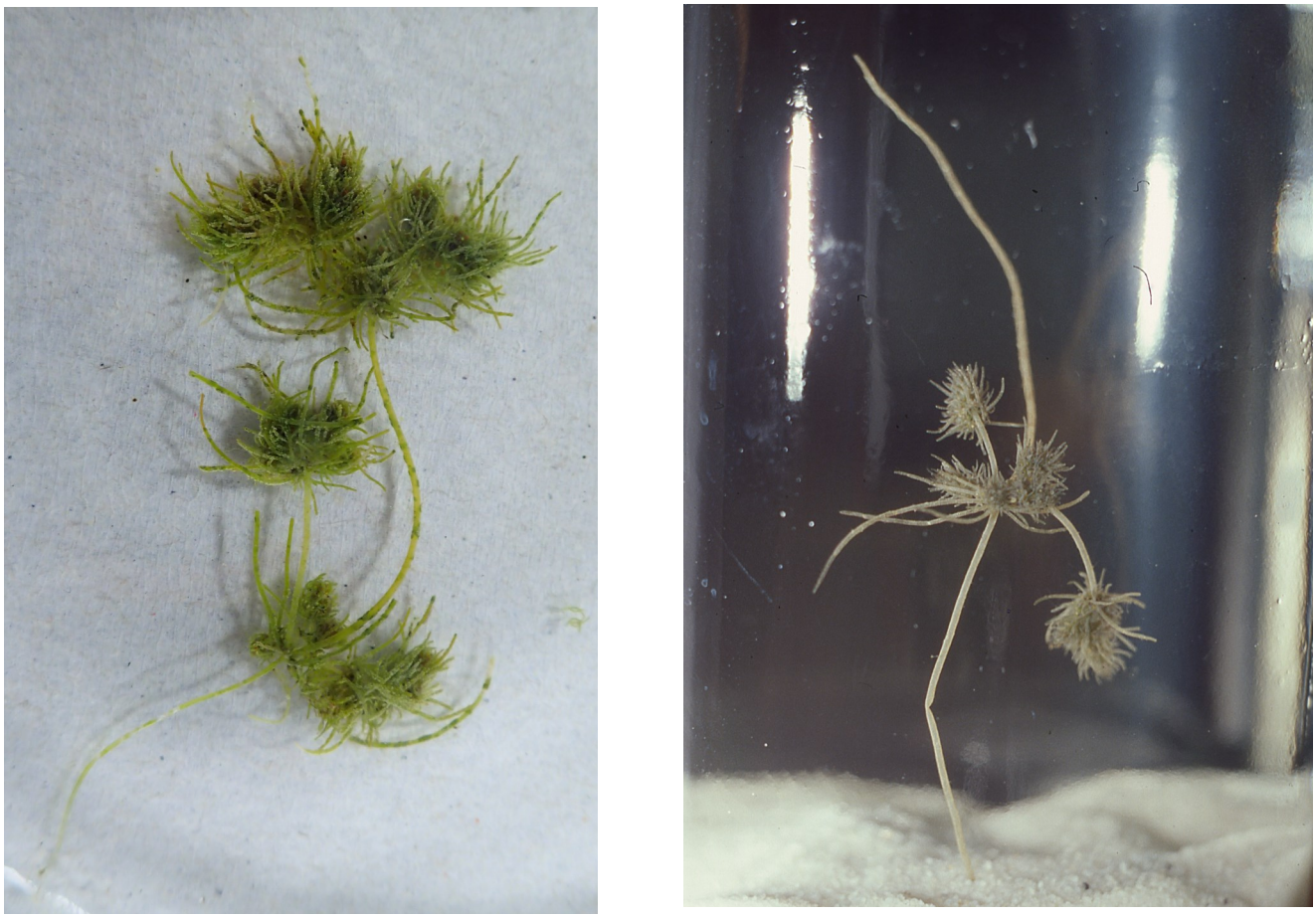

Figure V:4 Left: A live *T. glomerata* plant with several "bird's nest" heads (also see Figure V:15). Right: A plant growing in a culture jar, somewhat greyish and encrusted. HEM

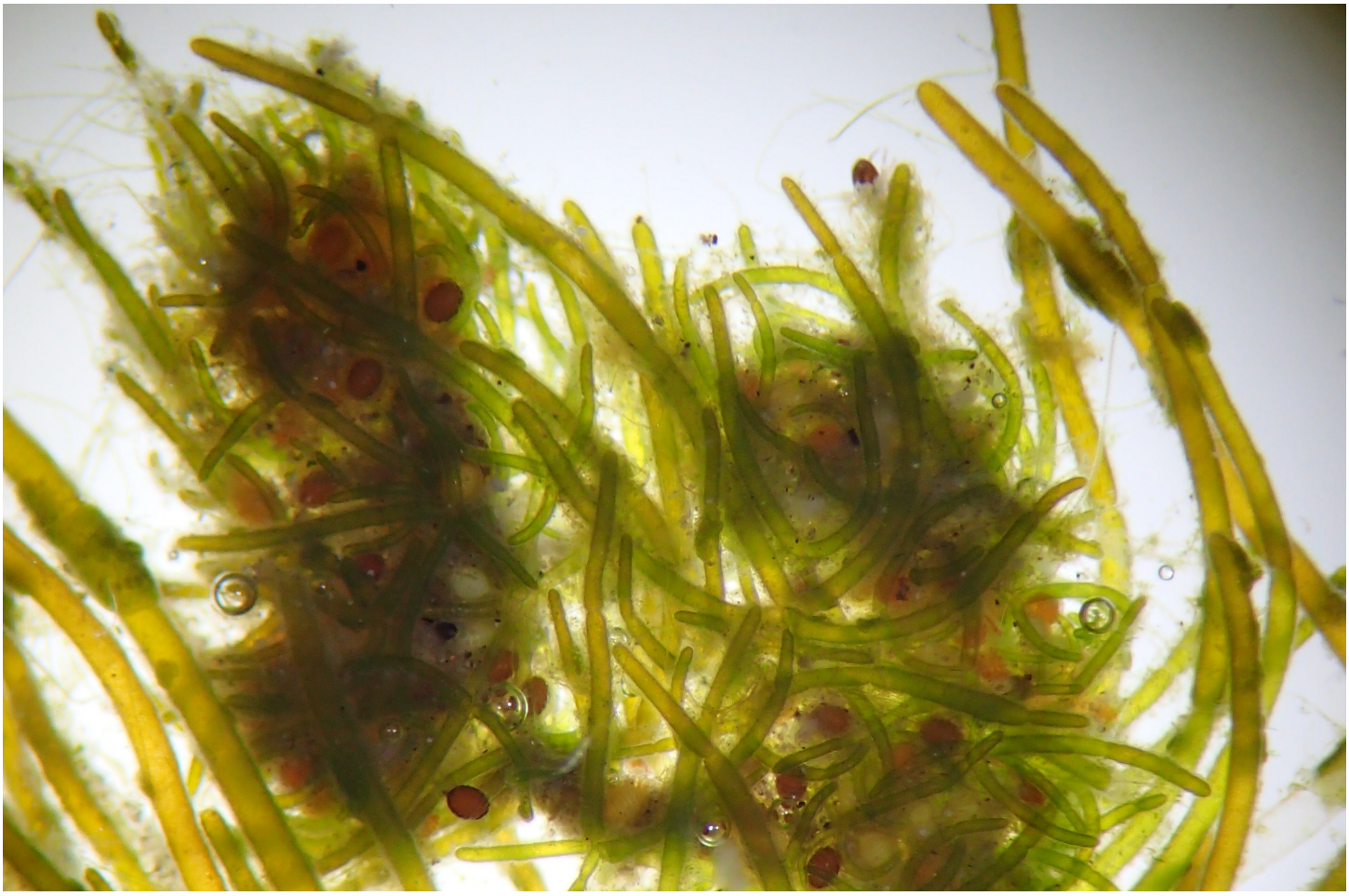

Figure V:5 Close-up view of several smaller clusters of fertile branchlets comprising the larger 'bird's nest" heads. HEM

Figure V:6 Left: A portion of a smaller fertile branchlet cluster. Right: Three of the tiniest fertile branchlets from the center of a cluster. A mature separate oospore also present in this photo. HEM

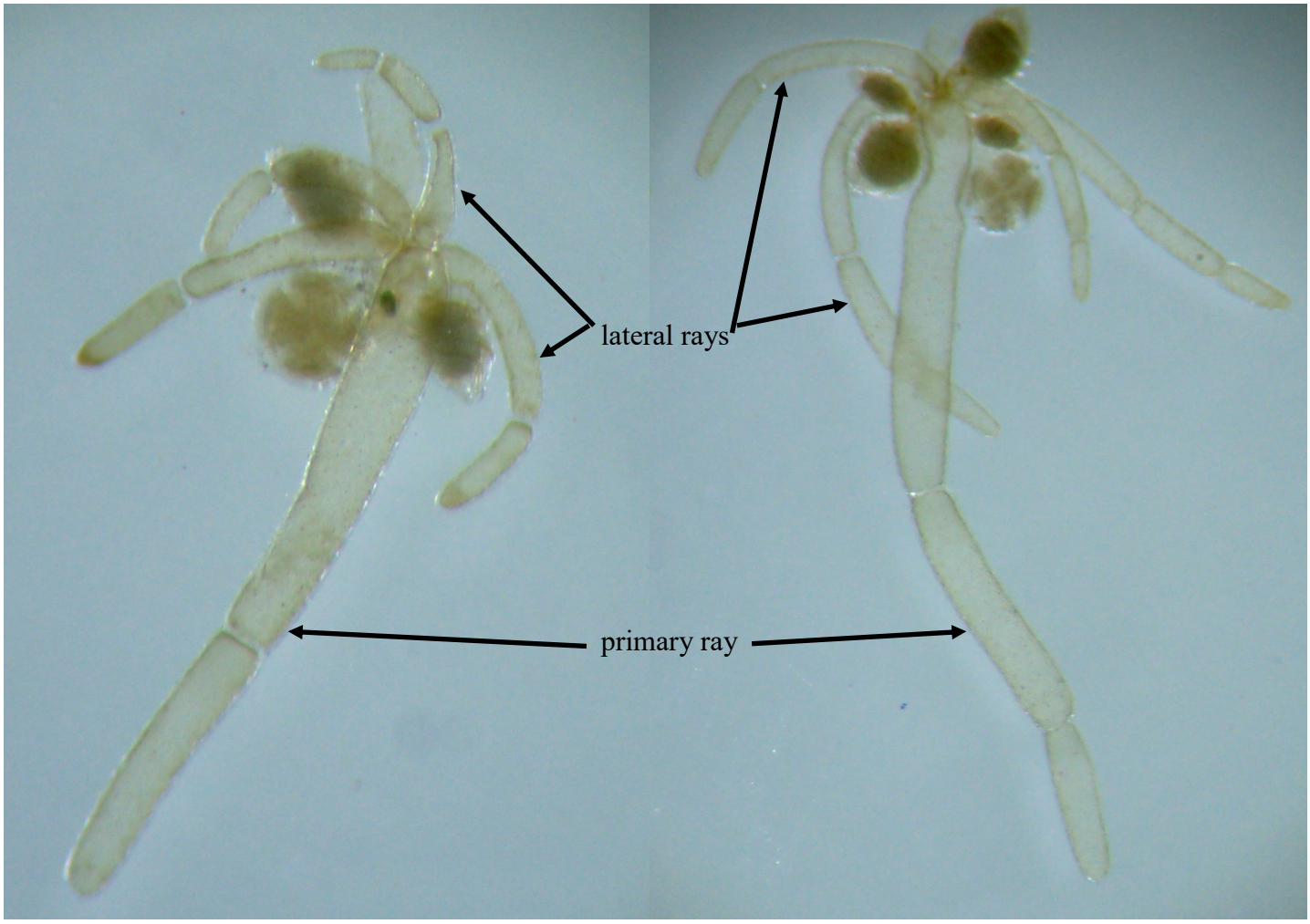

Figure V:7 Three examples of typical fertile branchlets of *T. glomerata*. Lateral rays are often only produced at the first node of the primary ray . Clusters of gametangia occur at the node with lateral rays and at the base of the primary ray where it joins the stem.

Insert photo is the tip of a ray that has not had the calcareous encrustation removed.

All three branchlets are from liquid preserved material.

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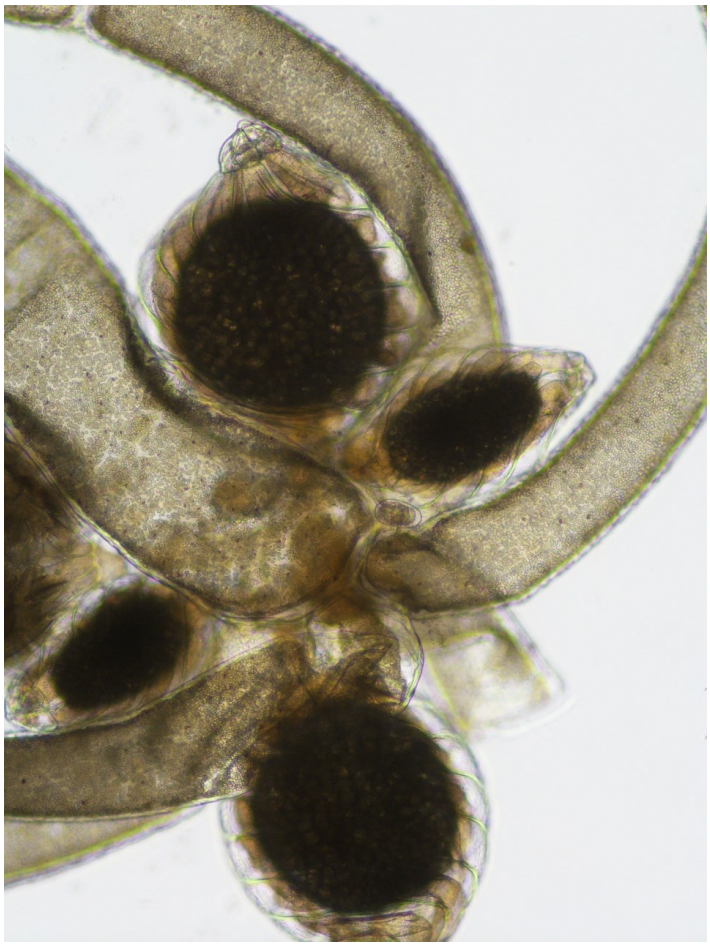

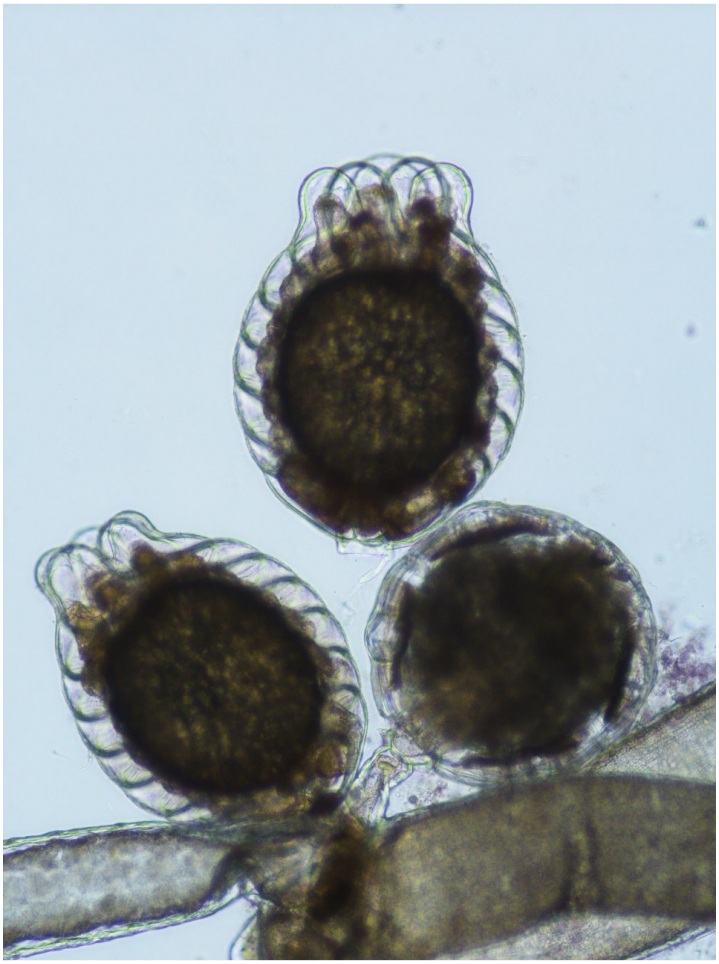

Figure V:8 Photos taken from liquid preserved material with a compound microscope.

Upper left: Two mature and two immature oogonia. Upper oogonium showing two-tiered coronula.

Upper right: An immature oogonium beside an antheridium. Four of the eight antheridial plates can be distinguished.

Lower left: Two mature oogonia and an antheridium. Coronulas have been shed and the tips of the spiral cells that were beneath the coronula have swollen indicating fertilization is occuring, or has occurred. The stalk of the antheridium is visible.

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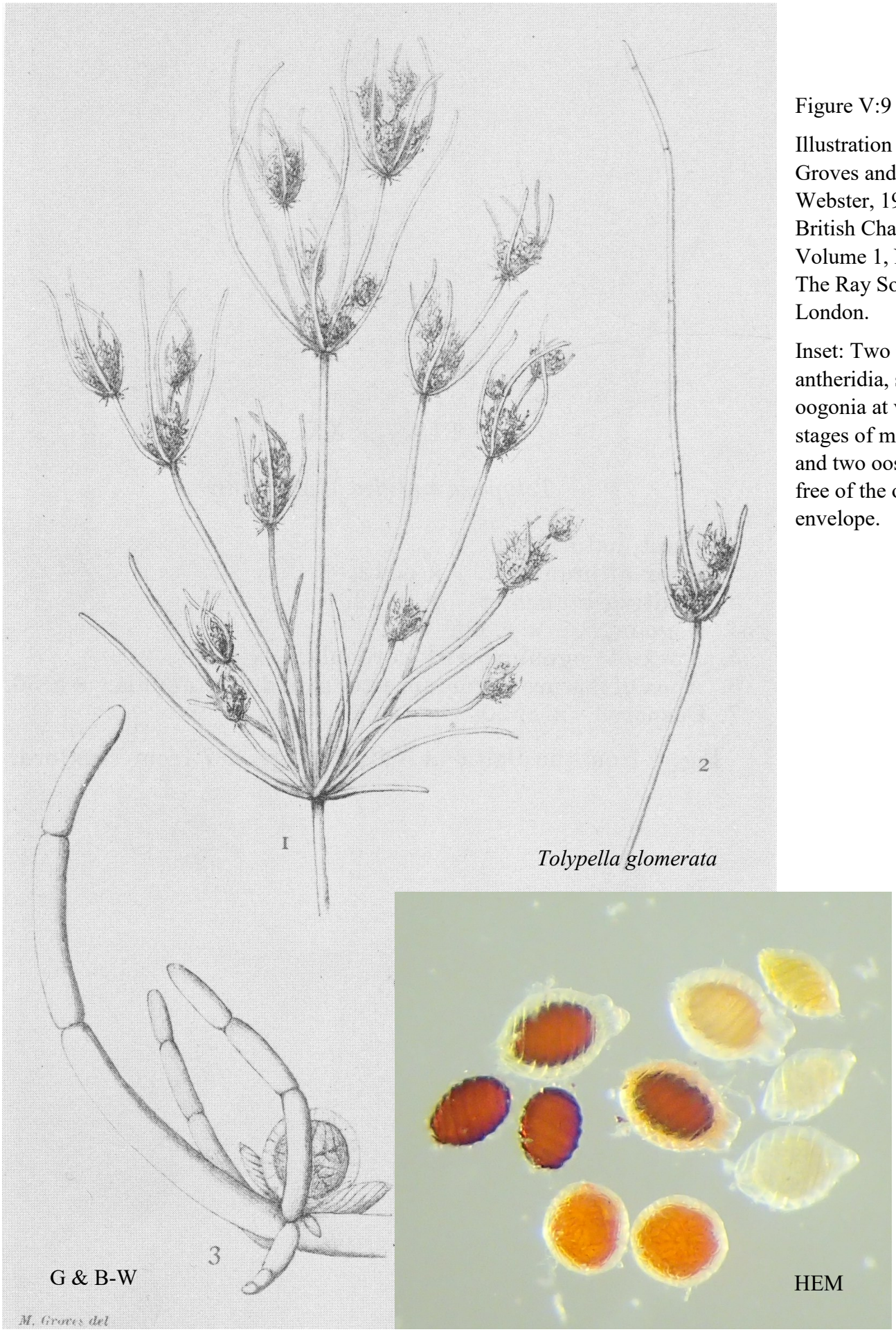


Tolypella glomerata

Figure V:9

Illustration from Groves and Bullock-Webster, 1924. The British Charophyta, Volume 1, Nitelleae. The Ray Society, London.

Inset: Two mature antheridia, several oogonia at various stages of maturity, and two oospores-free of the oogonial envelope.

Figure V:10 Upper photo: coastal pond at Sam's Cove on road to Spirity Cove, Great Northern Peninsula containing *Tolypella glomerata*, *Chara contraria* and *Chara globularis*. Pond is in limestone area about 50 meters from seashore. Lower photo: A shallow ephemeral pool on the coastal limestone barrens at Port au Choix, Great Northern Peninsula containing *T. glomerata*. HEM

Figure V:12 Upper photo: Shallow coastal roadside pool in the limestone barrens at the tip of the Great Northern Peninsula containing *Tolypella glomerata.* Lower photo: Flocculent organic substrate of the above pool with several Tolypella plants emerging above the organic ooze. HEM

Tolypella Species With The Potential To Occur In NL.

Tolypella species can be divided into two groups (sections) based on the shape of the end cells of the branchlet rays. Our known NL species *T. glomerata* is in Section Tolypella.

Section Tolypella (also known as **Section Obtusifolia** in the older literature) - species having end cells of branchlets with rounded (obtuse) tips (Figure V:11A).

Tolypella nidifica is very similar to *T. glomerata*, but tends to be a brackish water species, usually not encrusted and with larger antheridia and oogonia than *T. glomerata*. Ripe oospores have a deep wine-red colour.

Tolypella normaniana is a small Tolypella of brackish waters without sterile branchlets and brown to black oospores, antheridia and oogonia smaller than *T. nidifica*.

Both these species occur in Northern Europe, and *T. nidifica* in Greenland, in similar ecological conditions to ours, so amphi-atlantic occurrence appears potentially possible. (See Langangen 2007)

Section Rothia (also known as **Section Acutifolia** in the older literature) - species in which branchlet end cells are tiny and sharply pointed/acute (Figure V:11B).

T. intricata, *T. prolifera* and *T. canadensis* occur both on the adjacent North American mainland and in Northern Europe so occurrence in NL might be expected.

Two recent works on North American Tolypella recognize nine species and provide keys:

Perez, William. 2014. A systematic Revision of North American Tolypella A. Braun (Charophyceae, Charophyta). PhD Thesis, City University of New York. (Key on page 150*)

Perez, W., Hall, J.D., McCourt, R.M., and Karol, K.C. 2014. Phylogeny of North American Tolypella (Charophyceae, Charophyta) based on Plastid Sequences With a Description of Tolypella Ramosissima sp. Nov. Journal of Phycology 50: 776-789. (Key on page 787*)

* These keys are difficult to interpret without a thorough understanding of the details in their associated references. Initially for new finds from our area it is advisable to use the Canadian Tolypella Key as provided in Section IX of this guide.

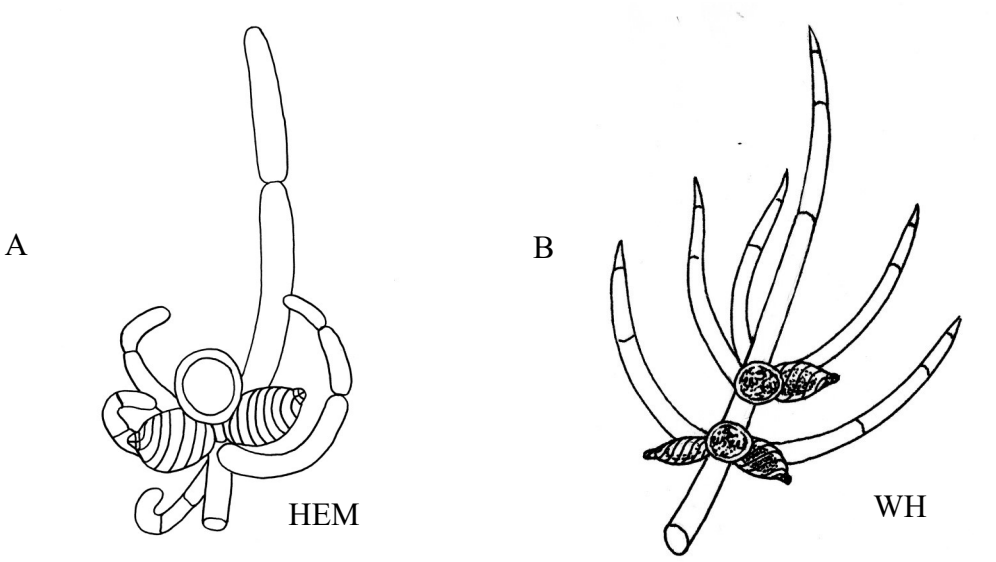


Figure V:11 A. **Section Tolypella** having rounded tips to the end cells of the branchlet rays. B. **Section Rothia** having sharply pointed (acute) end cell tips.

***Tolypella intricata* Leonhardi** and ***Tolypella prolifera* Leonhardi**

Both species occur in the adjacent North American mainland and so may potentially also be present in Newfoundland and Labrador.

T. prolifera is normally distinguishable from *T. intricata* by being a more robust plant with an axis diameter up to 2 mm, and sterile branchlets are undivided. Antheridia diameter is about 300 µm. *T. intricata* has an axis diameter of up to 1 mm and at least some sterile branchlets are divided. Antheridia diameter is about 400 µm.

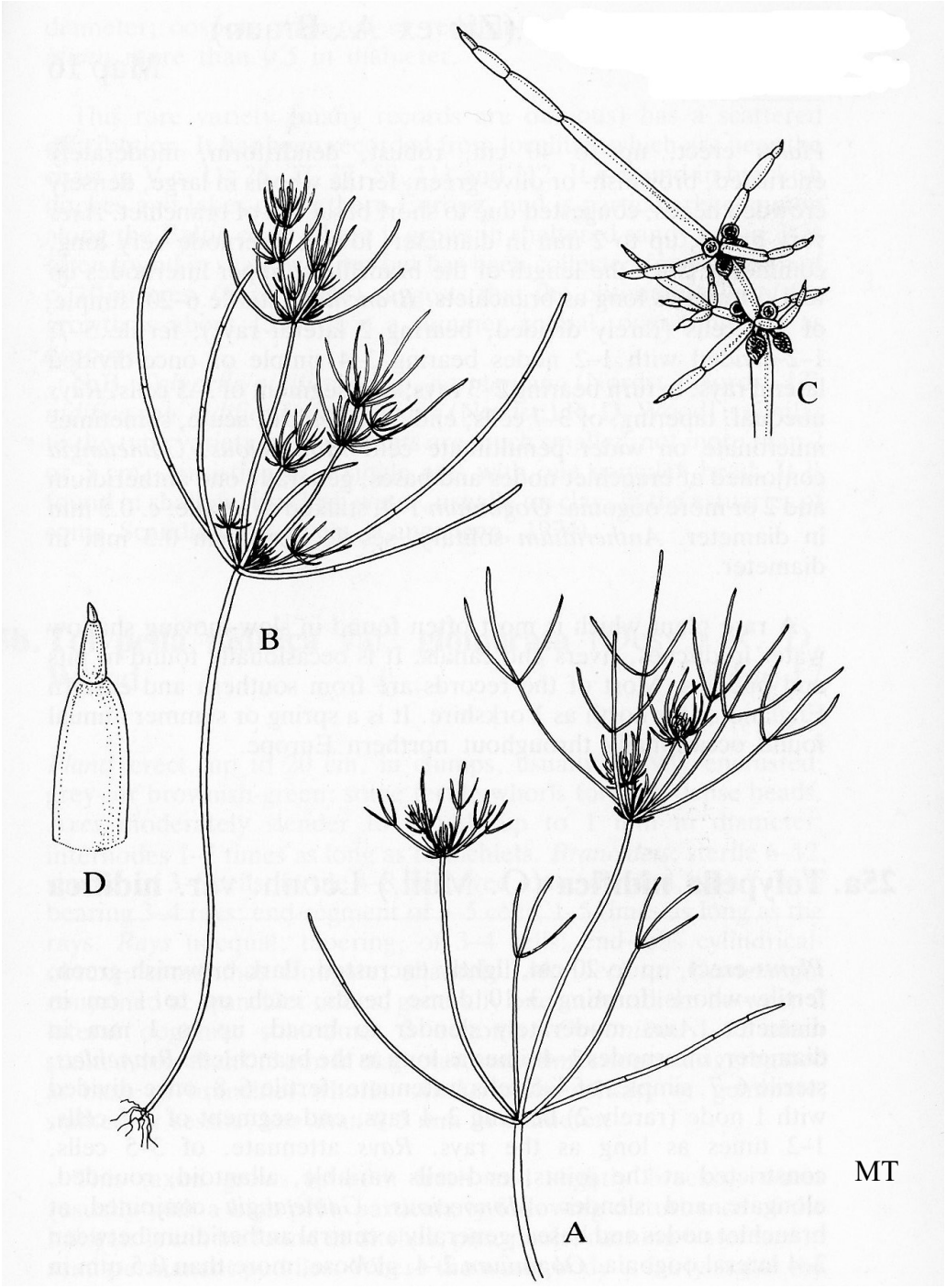


Figure V:12 A. *T. intricata* with divided sterile branchlets. B, C and D. *T. prolifera*.

Illustrations from : J.A. Moore. 1986.Charophytes of Great Britain and Ireland. BSBI Handbook No. 5, Botanical Society of The British Isles, London, UK.

Tolypella canadensis Sawa

T. canadensis was first described by T. Sawa in 1973* from Ontario. Subsequent investigations by Anders Langangen (2007**) have determined it to be a circumpolar species of oligotrophic cold waters across NA, Europe and Asia. With future field work it can also be expected to be found in Newfoundland and Labrador.

T. canadensis are small plants to 8 cm, sometimes more, Axes are up to 400 µm in diameter. Plants are dark grass-green, not encrusted. Sterile branchlets are undivided; branchlets of sterile whorls bend upward somewhat stiffly towards the stem. Branchlet end cells are distinctly mucronate. Antheridia are borne terminally; antheridial diameters 385-495 µm (Sawa 1973), or up to 400 µm (Langangen 2007).

* Sawa, T. 1973. Two new species of Tolypella (Characeae) from North America. Journal of Phycology 9: 472-482.

** Langangen, A. 2007. Charophytes of the Nordic Countries, Saeculum ANS, Oslo, Norway.

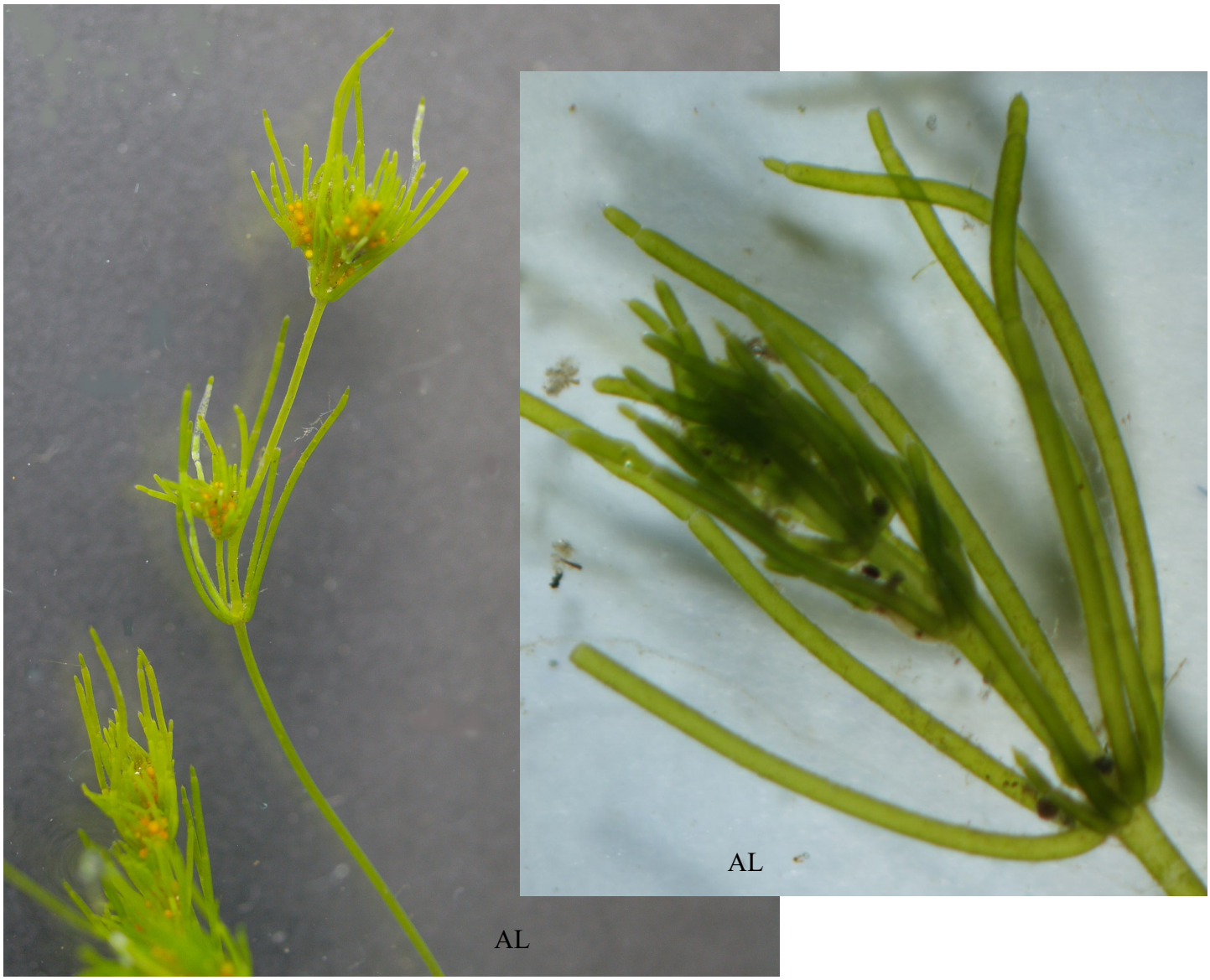


Figure V:13 Live *Tolypella canadensis*. Photos courtesy of Anders Langangen, Oslo, Norway.

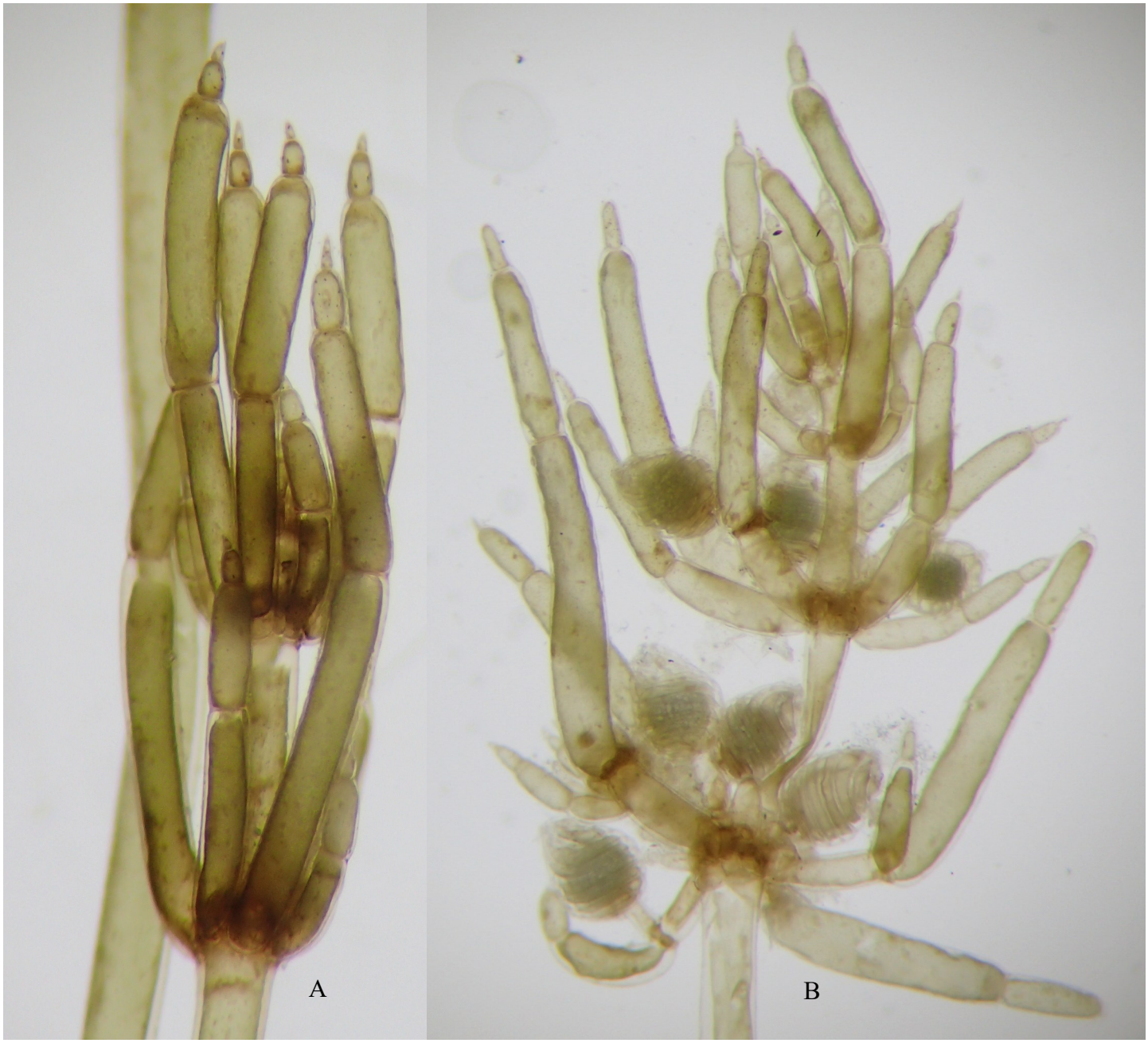


Figure V:14 *Tolypella canadensis*. A. Two sterile branchlet whorls with characteristic upward pointing undivided branchlets. Branchlets tipped by a tiny mucro. B. Terminal portion of an axis with fertile whorls of branchlets. Liquid preserved material supplied by Anders Langangen, Oslo Norway. HEM

Figure V:15 *Tolypella glomerata*. A branching axis with “bird nests”. Plant is lightly encrusted. HEM

VI: The Genus *Nitellopsis*

Nitellopsis obtusa (Desv.) J. Groves (Starry Stonewort)

Nitellopsis obtusa is a deep water species native to Europe and Asia. It is only known in North America from the St. Lawrence River, the Great Lakes Region rivers, and more recently spreading into inland lakes of states bordering the Great Lakes and in Ontario. It has been suggested that it was originally introduced to North America via commercial international shipping. It is known to inhabit fresh and brackish waters. *N. obtusa* is not likely to be found in Newfoundland or Labrador, but the possibility cannot be ruled out entirely. Most of our deep waters especially the fiords have not been sampled for charophytes. It is a dioecious species, entirely naked/ecorticate (without cortication) and without stipulodes, but like *Chara* has five coronula cells. Antheridia are very large, about a millimeter in diameter. The presence of characteristic star-shaped bulbils in the substrate is diagnostic. Apparently only male plants of this dioecious species are known from North America.

In North America there is much recent interest in Starry Stonewort including published literature and internet videos. Its vigorous growth and spread has categorized it as an invasive species in inland lakes and rivers where it clogs swimming areas and interferes with boating and fishing.

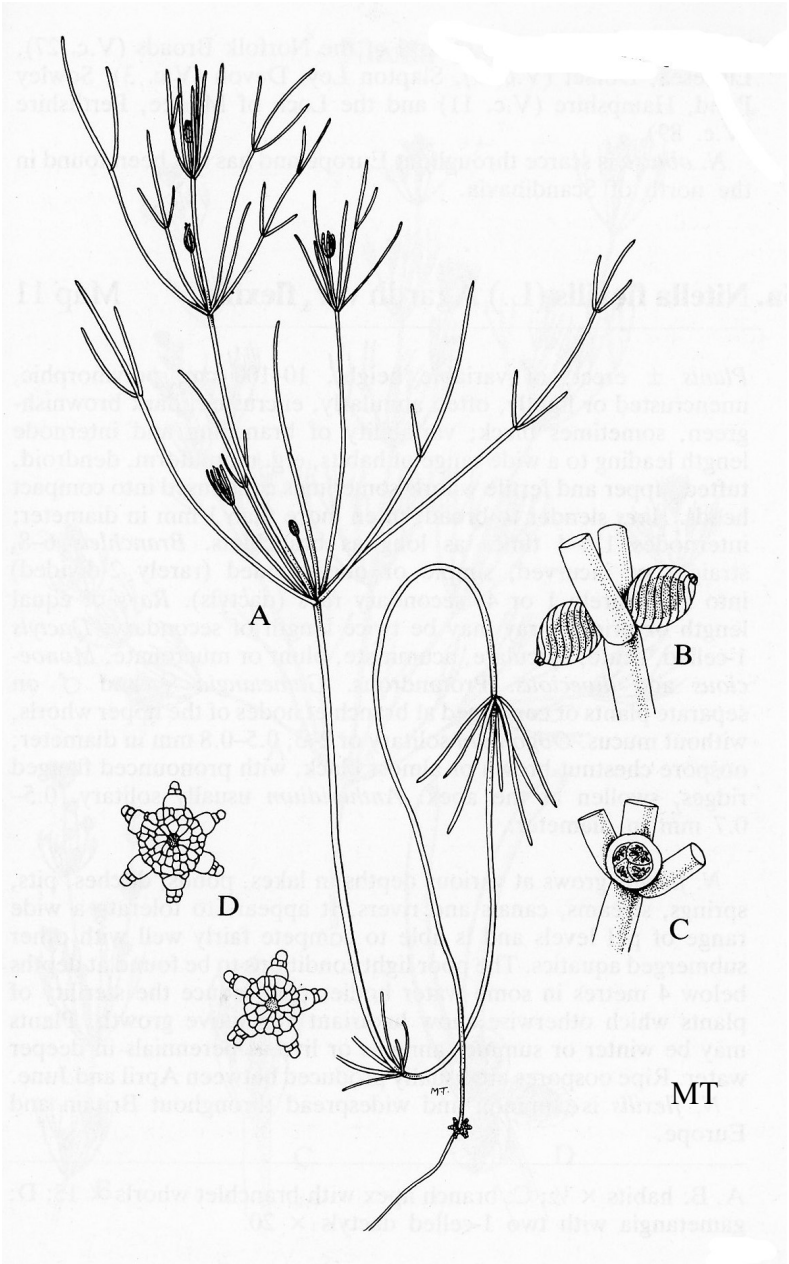
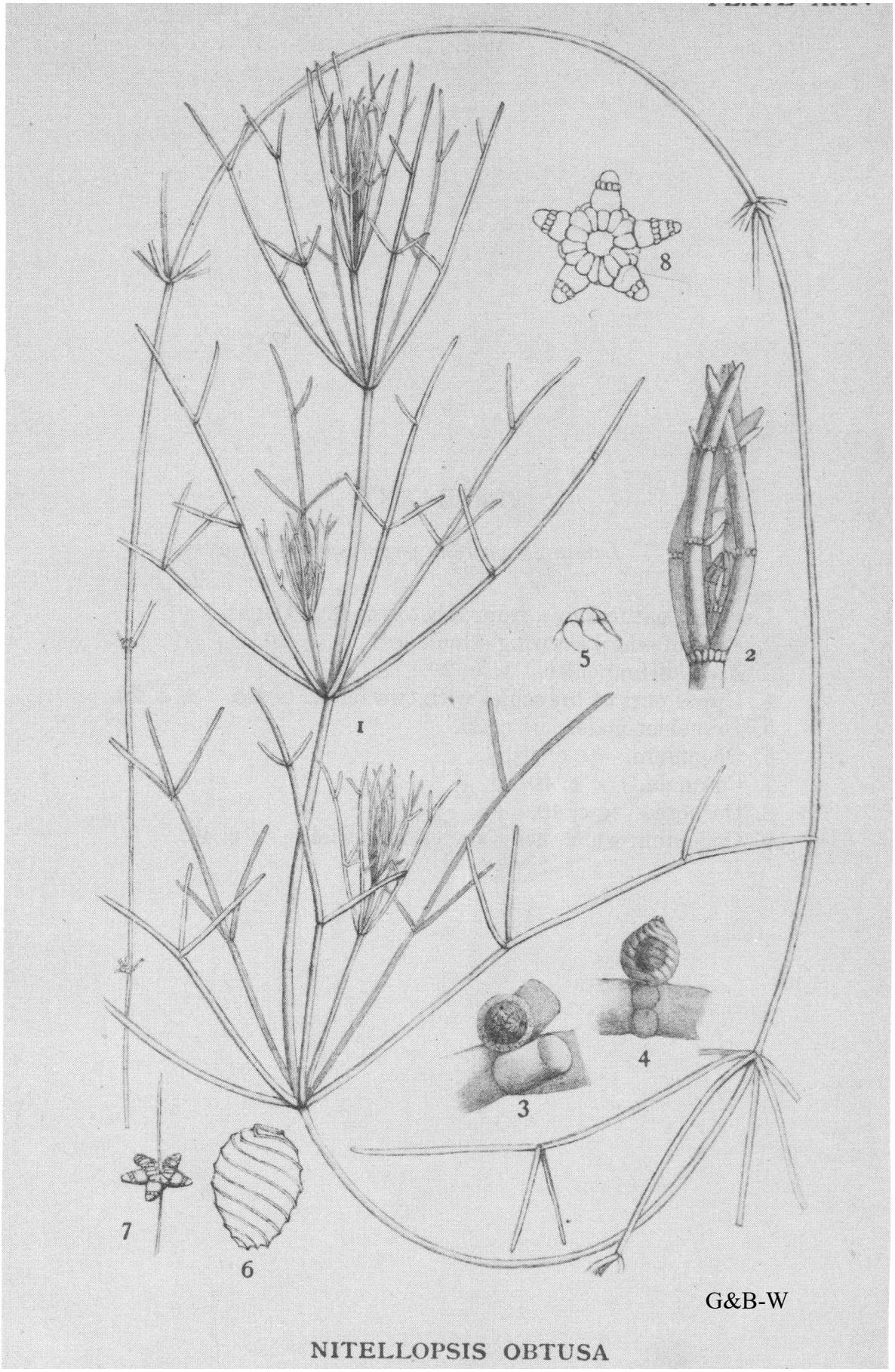


Figure VI: 1 *Nitellopsis obtusa.* Illustrations from J. A. Moore. 1986. "Charophytes of Great Britain and Ireland". BSBI Handbook No. 5, Botanical Society of the British Isles, London.



VI:2 Nitellopsis obtusa illustration from Groves and Bullock-Webster. 1924. The British Charophyta. Volume II, Chareae. The Ray Society, London, UK.

VII: The Genus *Lamprothamnium*

Lamprothamnium papulosum (Wallr.) J. Groves

Lamprothamnium papulosum is a European and North African species not reported for North America. It is a brackish water species occurring in Scandinavian countries in waters and climates similar to our own in Newfoundland so presence here in brackish coastal lagoons is potentially possible. It has the appearance of a naked, ecorticated *Chara* with compacted whorls of branchlets at the tips forming elongate "foxtails". It has one row of elongate acute stipulodes pointing downward at the branchlet nodes. It mainly differs from Chara by having the antheridium attached above the oogonium, not beneath as in *Chara.*

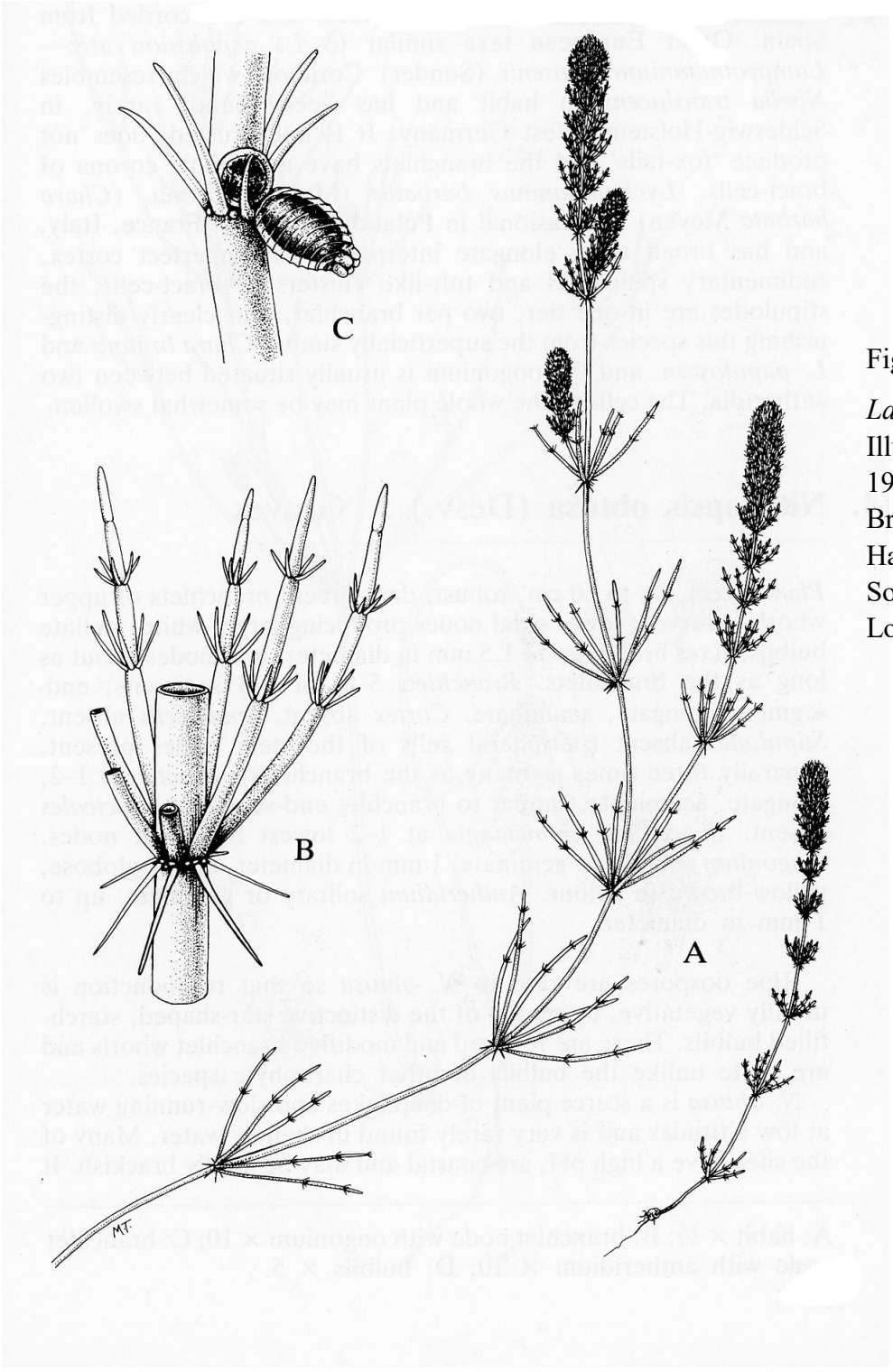


Figure VII:1

Lamprothamnium papulosum. Illustrations from J.A. Moore. 1986. "Charophytes of Great Britain and Ireland". BSBI Handbook, No. 5, Botanical Society of the British Isles, London.

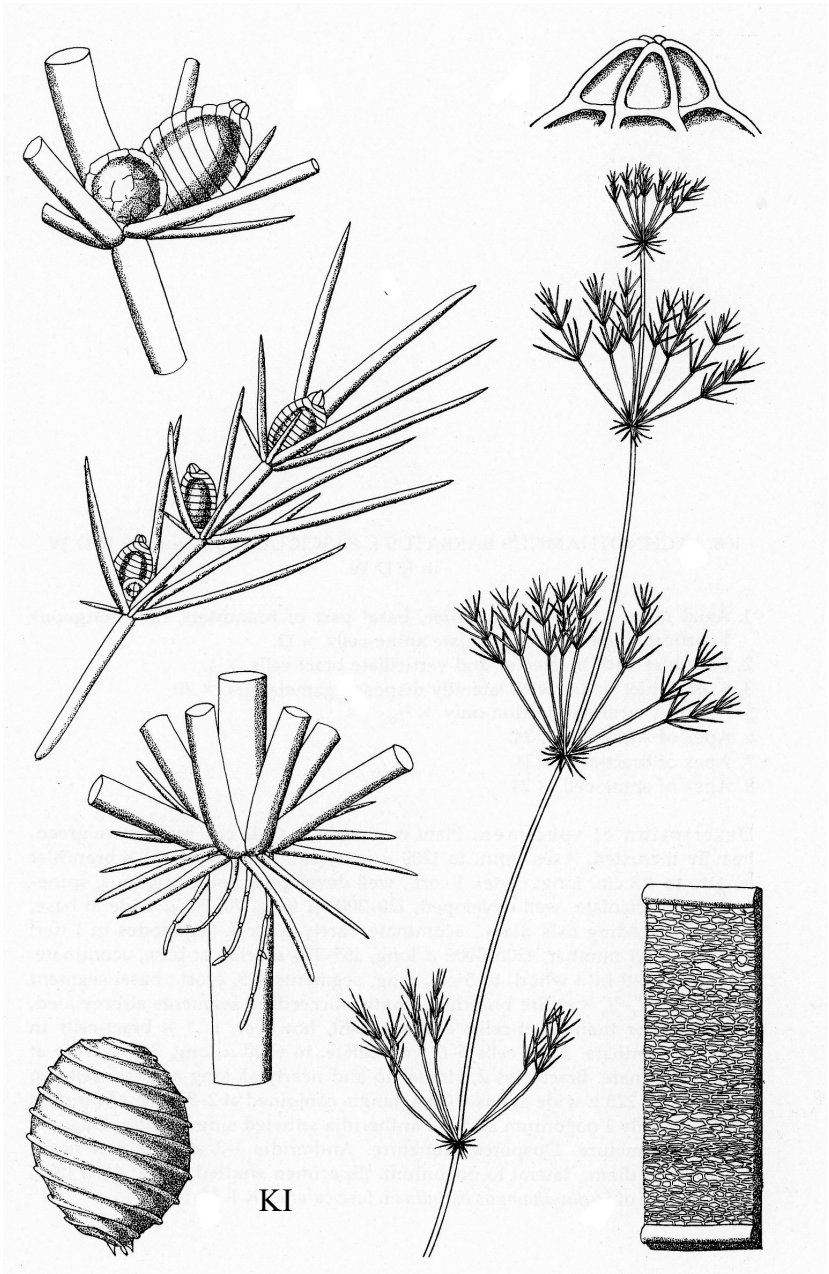
VIII: The Genus Lychnothamnus

***Lychnothamnus barbatus* (Meyen) Leonh.**

Lychnothamnus barbatus is a species of Europe, Asia, and Australia only recently reported from North America in freshwater lakes of Wisconsin and Minnesota*. It generally inhabits mesotrophic waters, but also occasionally oligotrophic and eutrophic conditions. The species has a distinctive morphology of ecorticated branchlets with elongated basal segments and shorter distal segments with whorls of bracts and bracteoles at the nodes. Stipulodes are in a single row and prominently elongate. Axis cortication is often present, but usually incomplete or rudimentary. Spine cells are single or in clusters of 2-3 when present. Characteristic is the arrangement of gametangia at branchlet nodes, typically an oogonium flanked laterally by two antheridia. Oospore membranes are finely and elongate reticular.

Potential habitat for this species occurs in NL so it is included in this guide as a future possibility.

* Karol, K.G. et al. 2017. First discovery of the charophycean green alga *Lychnothamnus barbatus* (Charophyceae) extant in the New World. American Journal of Botany 104(7): 1108-1116.



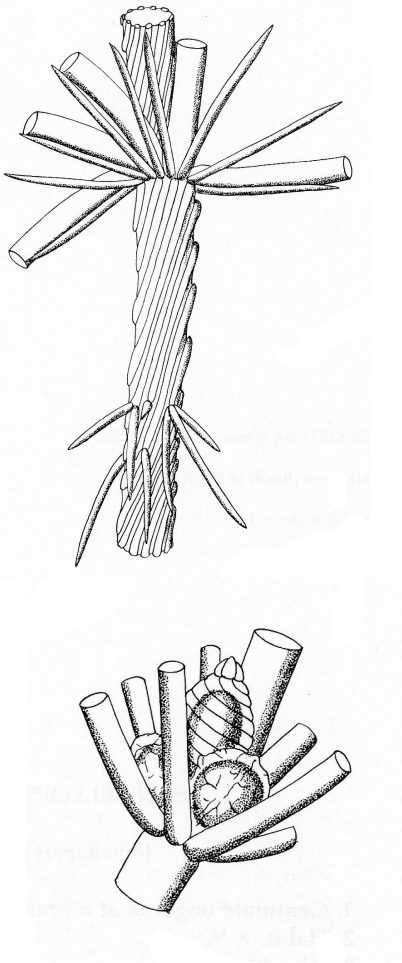


Figure VIII:1 Illustrations from R.D. Wood and K. Imahori, 1964. Iconograph of the Characeae. J. Cramer, Weinheim.

IX: Charophyte Keys

Biological keys are literary tools to facilitate the identification of species. None are perfect or without problems. Their usefulness is entirely dependent upon three factors, the skill of the key creator, the knowledge of the user, and the quality of the material available. Often keys are created by specialists for other specialists and are not very useable by beginners, students and naturalists. It is therefore incumbent upon neophytes to thoroughly become knowledgeable of the group's structure, ecology, taxonomy and terminology to successfully utilize many of the keys in the published literature. Thirdly, availability of good specimen material exhibiting all necessary identification features is a major asset in keying out specimens. For charophytes this requires careful and diligent collecting of material, and sometimes re-collecting at a later date if necessary gametangial features are not present (e.g. mature antheridia, mature oospores, etc.).

Normally keys are structured dichotomously; the user is provided two choices (a dichotomy) at each step. Which of the choices made will then lead to the next step (next dichotomy/couplet), and so on. If the specimen being keyed does not clearly exhibit the features specified in a couplet, then a difficulty arises. It may then be necessary to follow both choices to see which best leads to an identity that represents the material in hand. Finally, upon arriving at a tentative identification with the key, good detailed literature descriptions prepared by authorities should be consulted as a check to determine whether the specimen actually fits within the range of that species for all of its features. It is often easy to make incorrect identifications if this confirmation step is not carried out thoroughly.

Biological keys can be formatted in several ways. Those prepared for this guide have numbered choices within each couplet (1a, 1b; 2a, 2b; etc.), the two choices are always together, and couplets are indented alternately to facilitate the ease of use. Some keys in other publications use fully unnumbered couplets, with successive indentation for following couplets, and the two couplet choices may be far apart with other couplets between. This can work well if the key is rather short and can fit on a single page. However, many-paged keys in this format can be inconvenient to use. Figure IX:1 provides an example of an unnumbered indented key.

The keys in this guide are regional keys. They include all species known from the region for which they were created (e.g. the NL key), or species known for the region plus those that are most likely to be encountered in the future (e.g. the Canadian Chara key). Species that are considered "southern" species and are less likely to extend their range into Canada are not included.

Currently there is no good, up-to-date "easy to use" key to the entire North American charophyte flora. Although not including a few more recently described species, G. O. Allen (1954) and R. D. Wood (1948) are still essential references for the genus Nitella. C. B. Robinson's 1906 monograph of the genus Chara summarizes the early charophyte work of T. F. Allen. The keys in the two volume work by R. D. Wood and K. Imahori (A Revision of the Characeae, 1964, 1965) are useful , but only for the seasoned charophytologist with a sound grasp of charophyte history, taxonomy and nomenclature. Wood's 1967 "Charophytes of North America" key has some use initially if one recognizes that often it only leads to a group of species many of which are now recognized as distinct species sometimes under different synonyms. Nevertheless, the above works will remain foundational references to the North American charophyte flora even after a new revision with updated keys is produced at some future date. In the meantime it is hoped that this introductory guide will provide a reasonably accurate tool for the identification of charophytes in the Atlantic Canadian region.

Stem-cortex two-ranked (diplostichous)
  Secondary cortical-cells the more prominent (aulacanthous)
    Spine-cells solitary ..... *C. vulgaris*
    Spine-cells two or three together
      Spine-cells mostly geminate, one above the other ... *C. rudis*
      Spine-cells two or three: when geminate, side by side ..... *C. hispida*
  Primary cortical-cells the more prominent (tylacanthous)
    Dioecious ..... *C. tomentosa*
    Monoecious
      Spine-cells and stipulodes short, rather blunt: posterior bract-cells rudimentary ..... *C. contraria*
      Spine-cells and stipulodes long and acute: posterior bract-cells developed.
        Spine-cells solitary ..... *C. baltica*
        Spine-cells in fascicles ..... *C. aculeolata*
Stem-cortex three-ranked (triplostichous)
  Dioecious
    Spine-cells conspicuous
      Solitary ..... *C. aspera*
      Fasciculate (usually three or four) ..... *C. desmacantha*
    Spine-cells rudimentary
      Branchlets of male plants strongly connivent: branchlets of 8 or 9 segments: no bulbils ..... *C. connivens*

Figure IX:1 (above) A portion of an unnumbered indented key. The two alternative choices (couplets) are those with the same indentation, e.g. Stem-cortex two-ranked (diplostichous) and Stem-cortex three-ranked (triplostichous). They may be far apart in the key or close together, depending on how the key is structured. More difficult to use than numbered keys especially in lengthy keys of many pages.

Key to the Worldwide Genera of Extant Charophytes

(Indented Key Adapted from Groves and Bullock-Webster, The British Charophyta)

Coronula of oogonium composed of 10 cells in two tiers.

Antheridia apical in the furcations of the branchlets above the oogonia. Branchlets furcating into roughly equal rays. Ripe oospore laterally compressed ……..**Nitella**

Antheridia lateral at the furcations of the branchlets. Branchlets with main central ray and smaller lateral rays. Ripe oospore terete in cross section……..**Tolypella**

Coronula of oogonium composed of 5 cells in one tier.

Stipulodes absent. Branchlets of 2-3 very long segments. Bract cells 1-2, very long …

……...**Nitellopsis**

Stipulodes always present, sometimes rudimentary. Branchlets of 4 or more segments. Bract cells normally 4 or more.

Oogonium normally situated below the antheridium. Stem ecortcate…… .**Lamprothamnium**

Oogonium and antheridium situated side by side. Stem cortcate…….…….**Lychnothamnus**

Oogonium situated above the antheridium. Stem corticate or ecorticate* ……...**Chara**

**Chara braunii* is the only fully ecorticate NA Chara

Key to the Genera of Charophytes of North America

Charophytes are represented by six extant genera worldwide. Only five of these are known from North America; Chara, Nitella,, Tolypella, Nitellopsis, and Lychnothamnus*. The genus Lamprothamnium has not been validly reported for this continent. Only the first three are currently known from Newfoundland and Labrador. Following is a simple key to the five NA genera.

1a Coronula of 10 cells in two tiers, branchlets divided …….2

1b Coronula of 5 cells in one tier, branchlets not divided, but having bract cells at the node….3

2a Branchlets forking (furcating) into more-or-less equal rays, one or more times, ripe oospores laterally compressed (slightly flattened) ……..…….**Nitella**

2b Branchlets with a larger main central ray and smaller lateral rays, ripe oospores round in cross section (terete) ……**Tolypella**

3a Plants without cortex……...4

3b Plants with cortex (sometimes imperfect) …….....5

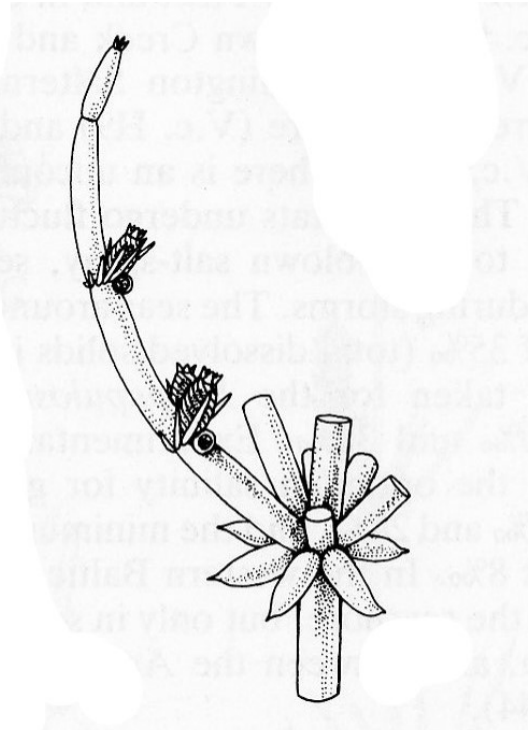
4a Plants without cortex, lacking stipulodes, producing large white star-shaped bulbils at lower stem nodes …….....**Nitellopsis**

4b Plants without cortex, with one tier of stipulodes, not producing large white star-shaped bulbils ……..***Chara braunii*****

5a Stipulodes in a single tier, oogonia flanked by two lateral antheridia …...**Lychnothamnus***

5b Stipulodes produced in two tiers, antheridia single, oogonia above antheridia .....**Chara****

* Lychnothamnus has only recently been reported for North America in Minnesota and Wisconsin lakes (Karol et al. 2017. First discovery of the charophycean green alga *Lychnothamnus barbatus* (charophyceae) extant in the New World. American Journal of Botany 104(7): 1108-1116.)



** Chara braunii is our only entirely ecorticate Chara, it has a single row of stipulodes, but all other features of the genus Chara (Figure IX:2).

Figure IX: 2 Node of *Chara braunii* with one branchlet and one row of stipulodes. Both the stems and branchlets are ecorticate. MT

Key to Currently Known NL Charophytes

1a Coronula of 5 cells in one tier, cortical cells present on the main axis, stipulodes present, (*Chara*)……5
1b Coronula of 10 cells in two tiers of 5 each, stipulodes absent ……..2

2a Oogonia and oospores round in cross-section, branchlets consisting of a main axis with smaller laterals (*Tolypella*) ……*Tolypella glomerata* (Desv.) Leonh.
2b Oogonia and oospores somewhat flattened in cross-section, branchlets forking' more or less equally, one or more times (*Nitella*)……..3

3a Unbranched end segments of branchlets (dactyls) of more than one cell, end cell tiny and pointed ……..*Nitella confervacea* (Breb.) A. Braun
3b Unbranched end segments of branchlets (dactyls) of one cell ……4

4a Plants monoecious ……*Nitella flexilis* (L.) Agardh
4b Plants dioecious ……*Nitella opaca* Agardh

5a Axial cortex composed only of primary rows of cells (all contain spine cells = haplostichous), axes bristly with elongate spine cells ……..6
5b Axial cortex of primary rows alternating with one or two secondary rows without spine cells, (diplo- or triplostichous), axes not greatly bristly …….....7

6a Plants monoecious, coronula width over 180 µm ……..*Chara evoluta* T.F. Allen
6b Plants dioecious with only oogonia, no male plants with antheridia known for North America ……*Chara canescens* Desv. & Lois. In Lois.

7a Axial cortex with each primary row alternating with one secondary row (diplostichous) ……..8
7b Axial cortex with each primary row alternating with two secondary rows (tripostichous)……. 10

8a Secondary cortex rows of axis more prominent than primary rows …*Chara vulgaris* L.
8b Primary cortex rows of axis more prominent than secondary rows ……9

9a Spine cells shorter than axis diameter, often papillate, individual stipulode cells much shorter than axis diameter ……*Chara contraria* A. Braun ex Kütz.
9b Spine cells longer than axis diameter, up to thrice the length, individual stipulode cells up to axis diameter or more in length ……*Chara contraria* var. *hispidula* Braun

10a Plants dioecious, upper and lower stipulodes well developed, rhizoids with tiny white spherical bulbils……*Chara aspera* Deth. ex Willd.
10b Plants monoecious, stipulodes, especially lower ones, rudimentary or globular, rhizoids without tiny white spherical bulbils ……..11

11a Primary cortical rows more prominent than secondary rows (tylacanthous), upper row of stipulodes more elongate than lower row……*Chara virgata* Kütz.
11b Primary and secondary cortical rows equal in size (isostichous), both upper and lower stipulodes rudimentary or globular……*Chara globularis* Thuill.

Nitella Key

This key includes the 15 species known to occur on the adjacent NA mainland and which are the most likely to be reported for NL in the future. Currently only *N. confervacea*, *N. flexilis*, and N. *opaca* are known for NL.

1a Branchlet dactyls of one cell……2

1b Branchlet dactyls of more than one cell……9

2a Branchlets 2 – 3 furcate, oospore membrane strongly tuberculate to incompletely reticulate …… *N. macounii*

2b Branchlets once furcate, oospore membrane smooth, granular, or weakly tuberculate…3

3a Plants dioecious ……4

3b Plants monoecious ……5

4a Oospore membrane smooth ……. *N. opaca*

4b Oospore membrane granular ……*N. obtusa*

5a Branchlets of fertile whorls of two different types, some divided and others not divided, dactyls short, inflated, acute, tapering to a sharp point, (appearing similar to those of *N. dilatata*), membrane granular ……..*N. clavata*

5b Branchlets of fertile whorls all similar, membranes smooth, granular, or tuberculate …. .6

6a Oospore membranes smooth …….. 7

6b Oospore membranes granular to tuberculate …….. 8

7a Dactyls short, inflated, acuminate, tips tapering to a sharp point, whorls of branchlets forming dense heads around stem, upper coronula cells conspicuously elongate ….*N. dilatata*

7b Datyls elongate, not inflated, tips blunt to acute, plant more loosely spreading, upper row of coronula cells not conspicuously elongate ……*N. flexilis*

8a Oospore membrane finely granular, branchlets 6 – 8 at a node, branchlet apices acuminate, oospore length 240 – 300 µm …….*N. accuminata*

8b Oospore membrane granular and tuberculate, branchlets 2 – 4 at a node, branchlet apices acute, oospore length 475 – 500 µm …….*N. spanioclema*

9a Two types of branchlets at stem nodes, smaller accessory branchlets in two whorls above and below the primary longer branchlet whorl ……*N. hyalina*

9b All branchlets similar and in a single whorl…….. 10

10a Small plants, seldom over 15 cm high (usually less than 10 cm), stems 200 – 450 µm in diameter, dactyls of normal length ……11

10b Larger, robust plants, 15 cm or more in height, stems 600 µm or more in diameter, with at least some very short (small) dactyls ……...13

11a Oospore membrane reticulate, gametangia not present at first branchlet furcation ……*N. tenuissima*

11b Oospore membrane granular, gametangia produced at first branchlet furcation…….12

12a Plants miniature, rarely over 5 cm high, dactyls 2 celled, gametangia produced mainly at first branchlet furcation, rarely at second furcation……*N. confervacea*

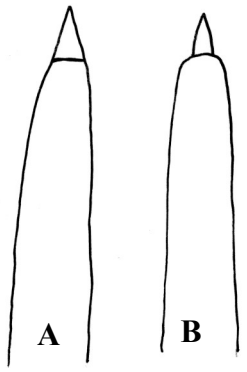
12b Plants usually 10 cm or more high, dactyls 2 – 3 celled, gametangia produced at all branchlet furcations…… *N. gracilis*

13a Dactyls of normal size (long, large), not greatly shortened, end cell a distinct mucro*, oospore membrane reticulate, oospores 300 – 350 µm long……...*N. mucronata* (here includes *N. furcata* of Wood, 1965)

13b At least some dactyls very short (abbreviated), tiny end cell of dactyl conical, but not a distinct mucro* ……. 14

14a Oospore membrane reticulate, oospore about 200 µm long ……...*N. microcarpa*

14b Oospore membrane granular to incompletely reticular, oospore about 400 µm long ……*N. megacarpa*



* Figure IX:3 Distinction between a mucro and conical dactyl end cell.

A. When the base of the tiny pointed end cell is about as broad as the end of the cell below it (the penultimate cell), it is considered a conical dactyl end cell. (the penultimate cell tapers at its tip to the width of the base of the conical end cell).

B. When the penultimate cell is somewhat rounded at its end and the tiny pointed end cell's base is much narrower, it is considered a mucro (i.e. the dactyl is mucronate).

Canadian Chara Key

This key includes the following Chara species which are known from Canada or which have the potential to occur in this region: *C. aspera, C. braunii, C. canescens, C. contraria, C. evoluta, C. foliolosa, C. globularis, C. haitensis, C. hornmanii, C. hydropitys, C. keukensis, C. longifolia, C. virgata, C. vulgaris,* and *C. zeylanica*.

1a With some cortication on stems or branchlets ……2
1b Without cortication on stems and branchlets ……...*C. braunii*

2a Branchlets corticated, at least in part ……...3
2b Branchlets naked, without cortication ……7

3a Basal branchlet segment naked, without cortication ……...4
3b Basal branchlet segment corticated ……9

4a Stipulodes in one tier ……*C. hydropitys*
4b Stipulodes in two tiers ……5

5a Gametangia sejoined (antheridia and oogonia alternating at branchlet nodes, antheridia with 8 plates/scutes ……..*C. foliolosa*
5b Gametangia conjoined (antheridia and oogonia together at branchlet nodes ……..6

6a Antheridia with 4 plates/scutes (tetrascutate)……*C. zeylanica*
6b Antheridia with 8 plates/scutes (octoscutate) ……...…*C. haitensis*

7a Small plants to 15 cm high, stem 400 µm in diameter, monoecious ……*C. keukensis*
7b Large plants to 30 cm high, stems 600 – 1100 µm in diameter, dioecious ……8

8a Stipulodes 2 or more per branchlet, spine cells long and abundant ….*C. hornmanii*
8b Stipulodes one per branchlet, spine cells obscure, rudimentary or absent …….. *C. longifolia*

9a All stem cortical cell rows primary (haplostichous) ……...10
9b Stem cortex with one or two secondary rows of cells between primary rows (diplo- or triplostichous) ……11

10a Plants monoecious ……..*C. evoluta*
10b Plants dioecious, (plants with antheridia are not known from NA) …...*C. canescens*

11a Stem cortex with one secondary row alternating with each primary row (dilostichous) ……...12
11b Stem cortex with two secondary rows alternating with each primary row (triplostichous) ……13

12a Primary cortical rows more prominent than secondary rows ……*C. contraria*
12b Secondary cortical rows more prominent than primary rows ……*C. vulgaris*

13a Plants dioecious, spherical white bulbils produced on fine transparent rhizoids ……...*C. aspera*
13b Plants monoecious, without spherical white bulbils on fine transparent rhizoids ……14

14a Upper row of stipulodes elongate, sometimes only slightly, lower stipulode row globular-obscure, cortex tylacanthous, bracteoles longer than oogonia ……*C. virgata*

14b Both rows of stipulodes globular-obscure, cortex isostichous, bracteoles shorter than oogonia ……..*C. globularis*

Key to Known Canadian Tolypella Species

1a Branchlet end cells obtuse, rounded ……...*T. glomerata* (including *T. longicoma* and *T. comosa*)

1b Branchlet end cells sharply pointed, acute and/or mucronate ……..…2

2a Sterile branchlets usually divided ……..*T. intricata* (including *T. fimbriata* and *T. intertexta*)

2b Sterile branchlets simple, undivided……3

3a Robust plants to 35 cm high, axes stout, 1000 – 2500 µm in diameter, usually greyish lime encrusted, branchlet end cells conical/acute, but not distinctly mucronate, fertile branchlets curving inward to produce a tangled "bird's nest", antheridia produced laterally, antheridia about 300 µm in diameter, ranging to 375 µm……. *T. prolifera*

3b Small plants to 8 cm high, sometimes more, axes to 400 µm in diameter, dark grass green, not encrusted, branchlet end cells distinctly mucronate, branchlets of fertile whorls bending upward toward stem, antheridia borne terminally, antheridia 385 – 495 µm in diameter ……..*T. canadensis*

Reformatted *Nitella* Key of G.O. Allen, 1954

1a All branchlets at a node of the same kind ……2

1b Branchlets at a node of more than one kind ……...33

2a Dactyls of one cell…….3

2b Dactyls of more than one cell……..17

3a Heads without mucus……4

3b Heads with mucus……...14

4a Dactyls obtuse (or abruptly pointed) ……5

4b Dactyls acute ……..10.

5a Dioecious ……..6

5b Monoecious……....7

6a Membrane smooth …… N. opaca

6b Membrane granulate …… N.obtusa

7a Oospore about 400 µm long …….8

7b Oospore small, about 275 µm …….. N. mexicana

8a Scars left by fallen gamatangia, membrane boldly ornamented, branchlets 2 – 4 at a node ……. ……N. spanioclema

8b No such scars left by fallen gametangia, membranes smooth or granular, branchlets 6 – 8 at a node ……9

9a Membrane smooth …… N. flexilis

9b Membrane coarsely granular …… N. californica

10a Dioecious ……..... N. missouriensis

10b Monoecious ……..11

11a Branchlets usually once furcate……12

11b Branchlets once to three times furcate……. ……N. macounii

12a Upper tier of coronula cells elongate ……...N. dilatata

12b Upper tier of coronula cells not elongate ……..13

13a Oospore 240 – 300 µm long, membrane finely granulate …….. N. accuminata

13b Oospore about 400 µm long, membrane boldly granulate ……. N. allenii (N. greenii)

14a Stems very stout (about 2 mm diameter) ……..15

14b Stems medium or slender ……..16

15a Dioecious……...N. cernua

15b Monoecious …….N. praelonga

16a Oospore membrane tuberculate ……...N. capillaris

16b Oospore membrane reticulate ……..N. bastinii

17a Most dactyls of two cells……...18

17b Most dactyls of more than two cells ……N. capillata

18a Dactyls of normal size (large) ……...19

18b Some or all dactyls short (small) ……..31

19a Branchlets twice or more divided……..20

19b Branchlets once divided (occasionally twice)……...29

20a Plants without mucus ……....21

20b Plants with mucus……..27

21a Medium size plants, up to 20 cm high……...22

21b Very small plants, up to about 10 cm high……24

22a Sterile branchlets more than once divided ……23

22b Sterile branchlets once divided……N. capitellata

23a Oospore membrane reticulate ……..N. mucronata

23b Oospore membrane granulate……...N. gracilis

24a Oospore membrane reticulate……25

24b Oospore membrane granulate……....26

25a First fork sterile, oogonia solitary…….N. tenuissima

25b First fork only fertile, oogonia aggregate…….N. pygmaea

26a Branchlets usually 8 …….N. confervacea

26b Branchlets usually 6 …….N. leibergii

27a Plants very small, up to about 6 cm high…….N. minuta

27b Plants medium size, up to about 15 cm high…….28

28a Fertile branchlets not in small heads……... N. intermedia

28b Fertile branchlets in small heads……..N. asagrayana

29a sterile dactyls not minute, oospore membrane granulate……30

29b Sterile dactyls minute, oospore membrane reticulate ……..N. axillaris

30a Oogonia aggregate……N. annularis

30b Oogonia solitary……...N. morongii

31a Ogonia solitary…….N. oligospira

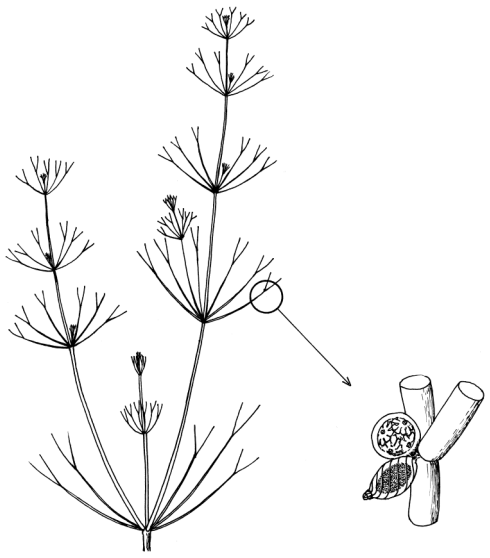
31b Oogonia aggregate…….32

32a Oospore small, about 200 µm long……..N. microcarpa

32b Oospore large, about 400 µm long ……..N. megacarpa

33a Dactyls of one cell……N. clavata

33b Dactyls of more than one cell……...N. hyalina



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On the Long Range Mountains of Gros Morne National Park, Western Newfoundland, June 4, 2015

X: Ecology of Newfoundland Charophytes

Significance

Apparently insignificant and therefore poorly known, stoneworts have a surprising impact in our waters which directly affect several activities cherished by many Newfoundlanders. Most of our many lakes, ponds and streams are nutrient poor and therefore not very productive in supporting a great diversity or numbers of plants, animals and fishes. Our common stoneworts, on the other hand, prefer these clean, unpolluted waters of low nutrient content, often carpeting the bottoms of such waters, producing miniature "forests" that harbour a great variety and numbers of aquatic insects and invertebrates which constitute a major food source for ducks, geese, shorebirds and fish. Although common in shallower waters, some can grow to greater depths than normal rooted aquatic plants and therefor increase the productive zones in large, deeper water bodies. Some remain active in winter under the ice producing food and oxygen for aquatic animals when other greenery is absent. In addition to providing habitat for invertebrates, they are also eagerly sought after and eaten by ducks and geese for their nourishing starch rich reproductive structures and bulbils. Undigested spores are one of the means of dispersal by birds to new water bodies. Spores and bulbils are also spread to other water bodies by adhering to feathers and muddy feet of both waterfowl and shore birds (Figure X:1).. Some species produce a brittle limestone coating on their stems which along with hard tough spores are a source of roughage aiding food processing in the crops of waterfowl.

From stoneworts to aquatic invertebrates, to fish, birds, mammals, and humans, this food web encompasses all life in the natural world. All organisms are interconnected with their environment and with each other. So the productivity of mink, otter, muskrat, beaver, even foxes, birds and many other species is indirectly enhanced by stoneworts for the benefits and pleasures of hunters, trappers and nature lovers. Hunters and fishermen owe much to stoneworts as the basis for their enjoyable sports and pastimes. Our ponds would be much poorer places without these little green wonders.

Besides productivity, stoneworts also have other important ecological attributes. They are known as aquatic pioneer species, often among the first vegetation to colonize newly formed ponds, whether naturally produced or man-made. Their tough spores can remain buried and dormant for years, even decades, ready to germinate when favorable conditions occur. Forming dense stands on pond and steam bottoms, they restrict the movement of sediments and considerably aid in the clearing of turbid waters, benefitting the growth and survival of other plants, fish and invertebrates. In addition there is evidence that some species may release chemical compounds which inhibit the growth of phytoplankton thereby reducing or moderating algal blooms, especially blue-green algae. Some species have a distinct strong skunky odour giving rise to the common names, "muskgrass" and "skunkweed".

The group has only been utilized directly by humans in a minor way as farm fertilizer, cattle feed, filtering agents, and other incidental historical uses. Recent evidence indicates that they may be useful in phytoremediation efforts to accumulate and remove heavy metals such as zinc, cadmium and lead from water-bodies.

Habitats and Distribution.

In many ways our climate and aquatic conditions are similar to the northern Europe Scandinavian countries and we can group our charophyte habitats in somewhat similar ways (Langangen 2007). All but one of our currently known species also occur in northern Europe.

"Chara Lakes" are found in calcareous areas of limestone rock and occur mostly on the west coast of the Island but also in other scattered areas on the Island and in Labrador (Figure X:2). The waters are usually low in nutrients, but have high concentrations of dissolved calcium and a neutral to high pH. The common stoneworts of these waters are usually *Chara contraria* and *Chara vulgaris*, and often *Chara virgata* and *Chara globularis*

Figure X:1 Ducks and Geese, along with shorebirds (sandpipers, plovers, yellow legs, etc.) are the chief dispersal agents for stoneworts, as they visit surrounding bodies of water. HEM

Figure X: 2 Upper photo: Outflow stream from Watson's Pond, one of the calcareous "blue ponds" near Corner Brook. The slow stream has deposited pale whitish calcium carbonate forming a marl fen rich in calcium loving vascular plants. The stream contains highly encrusted *Chara contraria.* Lower Photo: Blue Pond south of Corner Brook situated in a limestone area. Contains *Chara contraria.* Marl deposits create varying shades of blue in the clear waters. HEM

which have broad mineral and pH tolerances. *Chara* species in these waters are usually highly encrusted with a surface layer of calcium carbonate. Calcium rich waters of streams and pools in fen peatlands also commonly support one or more of the above species.

"Lobelia Lakes" are waters with low mineral content (oligotrophic lakes), low in nutrients, and acidic with a pH below 7 (figure X:3A).. They do not support many vascular plants, but often support extensive stands of charophytes of *Nitella flexilis*, *Nitella opaca* and/or *Chara virgata*. Very acid bog pools and alpine barrens ponds with coloured (humic) waters are often devoid of charophytes, but if not too acid may harbour *Chara virgata* and/or *Nitella opaca (Figure X:4)*. On the other hand, peatland fen pools in limestone rich areas though low in nutrients have a more neutral pH, lusher growth of vascular plant species and tend to harbour Charas including *C. virgata* and *C. contraria* (Figure X:3B).

Lobelia Lakes intergrade with more nutrient rich (mesotrophic) lakes known as "Potamogeton Lakes" supporting a more lush growth and variety of vascular plant species such as yellow water lily, pondweeds, and others (Figure X:5). Our "mesotrophic" waters are still much more nutrient poor and harbour fewer higher plant species than those of adjacent Maritime Provinces in areas with richer better developed soils and in comparison may still be considered in the upper oligotrophic range. In the more mesotrophic Newfoundland lakes (Potamogeton Lakes) charophytes may be more scattered where they must compete with vascular species in shallower areas. In deeper waters beyond the normal depth of the vasculars, extensive meadows of *Nitella* may occur. *Chara virgata* and *Chara globularis* have a broad nutrient and pH tolerance and are often common in both Lobelia and Potamogeton Lakes.

Brackish Coastal Waters form another major aquatic habitat for Newfoundland charophytes. Coastal lagoons and ponds with some periodic sea water influx contain fluctuating saline levels somewhere between fresh and fully marine (Figure X:6). No charophytes are known to be truly marine, but some like our *Chara evoluta*, *Chara canescens* and *Chara aspera* flourish in these brackish waters, the first two can only be found there. *Chara aspera* has a reported broader tolerance and can inhabit fresh waters, and to date has only been found once in non-brackish waters in Newfoundland. *Chara contraria* and *Chara vulgaris* occur in mineral rich fresh waters, but not in the very saline brackish waters that the three above prefer. *Chara globularis* and *Chara virgata* sometimes occur in slightly brackish conditions. Brackish coastal waters may prove to be a significant source of new species discoveries for this province.

Coastal freshwater ponds usually harbour some of our most common charophytes (Figure X:7). In addition, small shallow or ephemeral coastal pools occur near the coast with basically fresh water, but with slightly more mineral nutrients due to minor aerial influx of marine spray and fog. Some of these pools may dry or partially dry in July-August. *Tolypella glomerata* has only been found in such habitats and in some small costal ponds. *Chara globularis* and *Chara contraria* sometimes also occur.

Charophytes may be found in slow moving rivers and streams, but are usually absent from fast-flowing waters (Figure X:8). However, even in fast flowing channels there are often calmer side pools away from the currents which often harbour species such as *C. virgata*, *C.vulgaris*, *C. contraria*, *Nitella flexilis* and *N. opaca*. The Nitellas are often found in such habitats in waters that are very low in nutrients and dissolved materials and often with a pH less than 7.

Considerable waters of disturbed sites around and in human population centers and along roadways exist harbouring stoneworts (Figure X:9). Charophytes are easily spread and are early colonizers of disturbed waters and if conditions are favorable they almost invariable appear. Some species can tolerate high mineral content of waters, but tend not to favour polluted waters, especially high levels of phosphorous.

Since only a small portion of our Newfoundland freshwaters have been sampled for charophytes, the probability of extending distributions is certain and discovery of unreported species is very likely. Our larger

Figure X:3 Upper photo: First Pond on Bottom Brook, a low nutrient "Lobelia Lake"

Lower photo: Fen pools with calcareous influence creating a more diverse vascular flora and harbouring *Chara virgata* and *C. contraria*. HEM

Figure X:4 Upper photo: Shallow pond in a bog peatland. Waters are acid, low in nutrients, and the substrate is a mucky organic peat often devoid of vasculars and charophytes.

Lower photo: Ponds on alpine barrens and plateaus are often nutrient poor and low in both vasculars and charophytes especially in areas of acidic bedrocks. Pools in basic bedrock areas (e.g. peridotite) often harbour some charophytes. HEM

Figure X:5 Upper photo: Rapid Pond, Humber Valley is a transition pond from "Lobelia" to "Potamogeton", more rich in vasculars and harbouring *Chara virgata* and *Nitella opaca.* Lower Photo: A pond rich in vascular species, Ramea Island, south coast of Insular Newfoundland. HEM

Figure X:6 Upper photo: A shallow brackish coastal lagoon (St. Georges Pond) with some influx of sea water from the St. Georges Estuary. This lagoon harbours *C. evoluta*, *C. canescens* and *C. virgata.* Lower photo: Cape Anguille Lighthouse Pond, a coastal pond in a calcareous area, freshwater, but with some elevated conductivity due to salt spray, a high pH, and harbouring *Chara aspera* and *C. contraria*. HEM

Figure X:7 Upper photo: A coastal freshwater pond at New Ferolle, high pH and elevated conductivity due to seawater spray, harbouring *Chara virgata* and containing high concentrations of invertebrate herbivores. When collected *C. virgata* appearing as *C. globularis*, but upon culturing returned to typical *C. virgata* morphology. A potential study site.

Lower photo: Shallow ephemeral coastal pools on the limestone barrens at Cape Riche harbouring *Tolypella glomerata*. HEM

Figure X:8 Upper photo: Hughes Brook, Humber Arm, a fast flowing stream with calmer side pools harbouring *C. virgata*, *C. contraria*, *C. vulgaris*, *C. globularis* and *Nitella flexilis.*

Lower photo: One of the several branches and side channels of the Grand Codroy River where it enters the Estuary. Some small amount of seawater influence here. A potentially rich area for further charophyte finds. *Chara aspera*, *Nitella flexilis*, as well as the rarely encountered *Nitella confervacea* are known from this area. HEM

Figure IX:9 Upper photo: A pool in an abandoned roadside quarry near McIvers, south shore, Humber Arm, harbouring *Chara contraria.*

Lower photo: A shallow ephemeral roadside ditch pool containing *C. contraria* and tadpoles. Part of this pool has dried and the white dry calcareous encrustation of Chara is apparent. HEM

lakes and deeper waters have not been investigated and much coastal fresh and brackish water needs to be surveyed. Only the western half of the Island has seen much collecting. Five of our currently known species are only known from a few sites and are considered provincially rare. (*C. aspera, C. canescens, C. evoluta, N. confervacea, T. glomerata*). Finding other sites of their preferred habitats may be profitable. We can expect to discover in our flora species of *Chara*, *Nitella* and *Tolypella* that occur on the mainland in neighbouring regions of the U.S.A., the Canadian Maritimes and Quebec which have not yet been reported for our province. Upon discovery of material that does not fit into our current known species concepts, consultation of the more detailed literature would be necessary and/or consulting a charophyte specialist for assistance.

Charophytes are no respecters of political boundaries or sometimes even continents so the whole of North America as defined in the Scribailo and Alix (2010) list should be considered when determining unknown and unfamiliar species. Charophyte spores are tiny, microscopic, extremely resistant to damage or desiccation, and can remain viable for many years. They are easily distributed even great distances by waterfowl, shore birds, animals that frequent aquatic and wetland habitats, and various human conveyances or activities. Almost invariably if suitable aquatic habitat exists, it will eventually be colonized by one or more species. Even in recent history conveyance from Europe of *Nitellopsis obtusa* into the St. Lawrence Seaway by commercial shipping has been proposed and documented. A number of our common charophytes are cosmopolitan, also occurring in Europe and elsewhere. A northern relationship between North America and Europe has been demonstrated for both vascular plants and charophytes. The European literature is important and useful in advanced studies of the charophyte flora, especially for Newfoundland and Labrador whose historical, geographical and biological connections with Europe are well known.

Chara contraria growing in a shallow pool in a disturbed roadside area. The stonewort is highly encrusted. The pool is also home to numerous black tadpoles. HEM

XI: Collecting, Examining, Recording and Preserving Charophytes

Charophytes can be found in most of our fresh waters and some species in brackish waters. They occur in shallow pools, ditches, streams, rivers, shorelines, backwaters, lagoons, and in deeper ponds and lakes. Initially the most useful collecting equipment for shallow waters consists of boots or waders, and a common long-handled garden rake. To extend distance, a throw-in drag (grapnel) on a rope can be employed (Figure XI:1 and XI:9). Various designs can easily be homemade with whatever materials are available. For deeper waters a weighted drag on a rope can be slowly pulled along the bottom from a canoe or boat. Commercial bottom samplers for very deep waters can sometimes be loaned from colleges, universities or government departments. For those skilled in SCUBA diving, charophytes in deeper waters can be hand collected, photographed and studied under normal growing and habitat conditions.

Whenever possible, material collected should include reproductive structures (gametangia) usually visible with the naked eye or a hand lens as tiny greenish, orange or black globular or egg-shaped structures. Sometimes male and female gametangia are located on the same plant (monoecious species), and sometimes male and female gametangia are on separate plants (dioecious species). With dioecious species an effort should be made to locate both male and female plants. Rhizoidal material growing in the substrate should be collected to determine if characteristic bulbils are present (e.g. *Chara aspera*, etc.).

Most features useful for the identification of stoneworts require magnification. Specimens can be examined fresh in the field with a 10x and 20x hand lens, but for critical study fresh material is best placed in plastic bags or containers with collected water and transported to a home base to be carefully examined and compared with the literature for identification. If available, a 10x to 40x zoom stereomicroscope will greatly assist in examination, however, any good stereoscope will do (Figure XI:2). Containers for transport should be kept cool and out of direct sunlight in the shade to prevent excessive temperature rise resulting in “cooked” material.

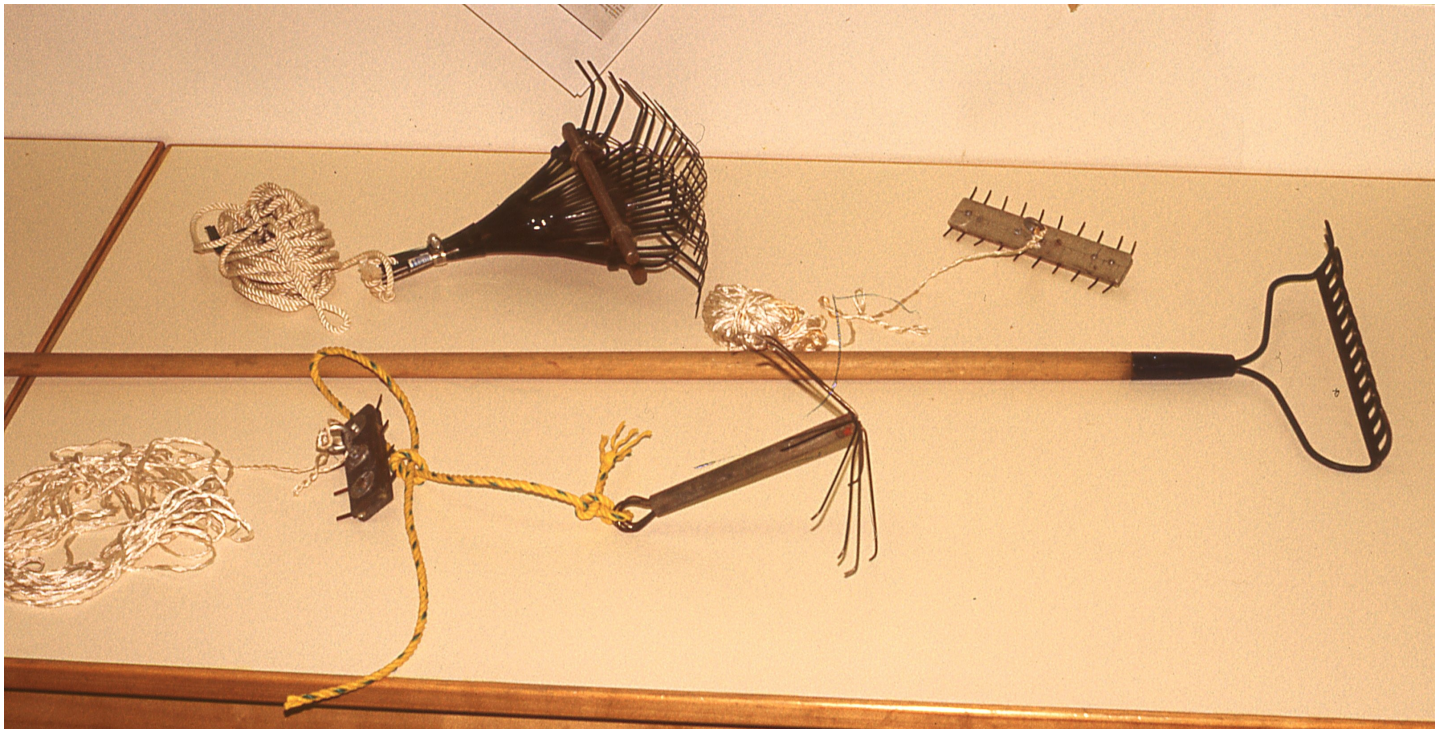


Figure XI:1 Some examples of simple collecting tools from shore or a boat, a common garden rake and four types of home-made grapnels. HEM

Figure XI:2 A basic stereomicroscope. Inset: A standard 10x and a 20x hand lens (loupe), essential for field examination of charophyte material. HEM

Some charophytes, especially those growing in calcium rich waters, produce a hard lime coating (calcium carbonate) on their axes and branchlets. This is the source of two of their common names, “brittleworts” and “stoneworts”. This coating when quite thick can make it difficult to determine cellular structure and arrangement, such as the axial cortex pattern in the genus Chara. In such cases it is preferable to dissolve away this limestone by placing the material to be examined in an acid such as vinegar or one of the other commonly available relatively weak acids (Figure XI:3).

It is always advisable that good written field records be kept of collections and collection sites. As much detail as possible should be recorded, but especially date, collection number, location (map or GPS if possible), type of water body, depth of collection, associated plants, and so on. A pencil written note on good quality paper with the same collection number as recorded in the field notebook should be placed in the container with the collected material and should always be retained with the material. Preferably water quality parameters should also be recorded, but this will depend on the purpose of the collection and the equipment and materials available (e.g. water pH, salinity/conductivity, colour, temperature, transparency, etc.). With modern digital camera technology it is easy to photographically record collection site features as well as actual collected material in the field. Even if interest in this group is only at the hobby or naturalist level, it is advisable to keep a preserved specimen collection in addition to written records. Often as interest increases and more species or varieties are collected, correspondence and specimen exchange with other enthusiasts will ensue. As well, difficult or new finds may be sent to specialists for confirmation or identification.

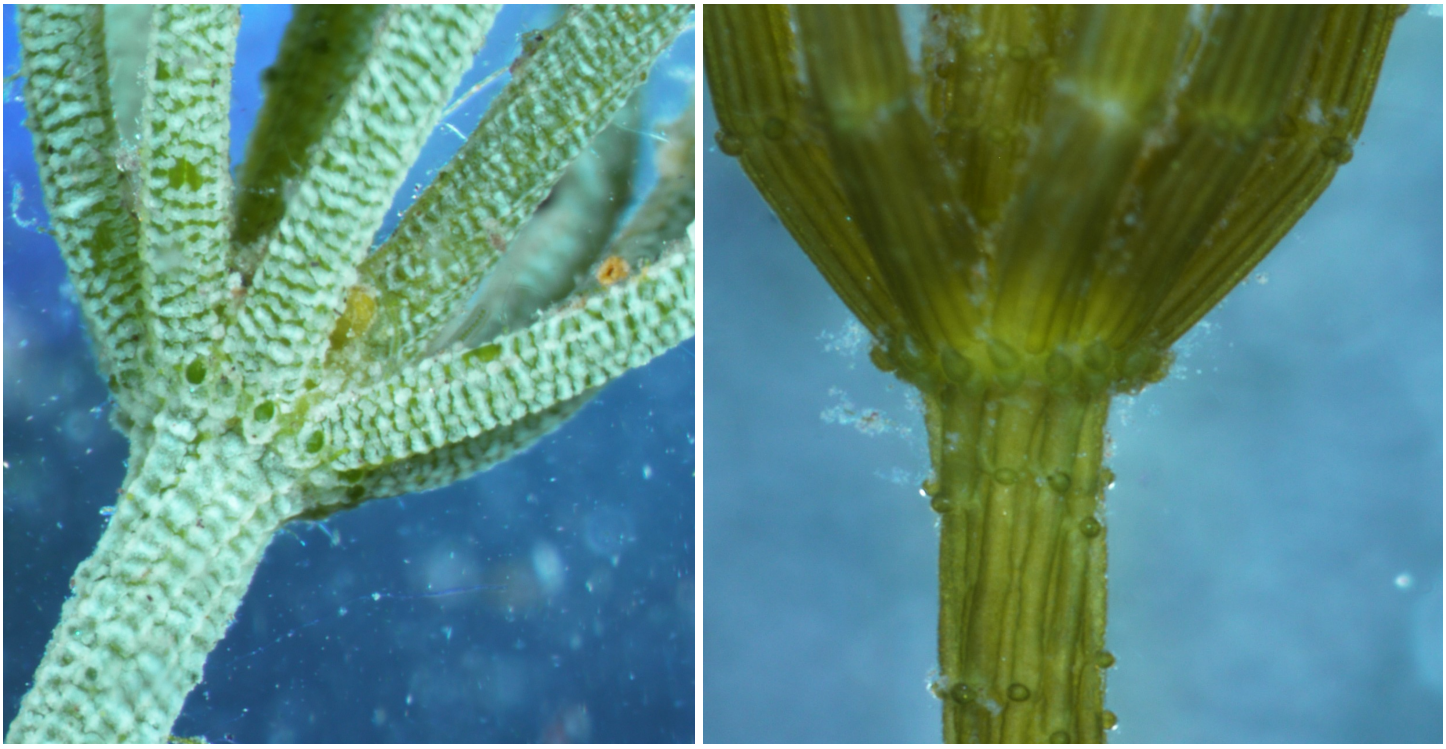


Figure XI:3 Left: *Chara contraria* highly encrusted when collected and difficult to determine cortication pattern. Right: Same collection after removing encrustation by immersing material in common household vinegar. HEM

Charophytes are most easily identified with live fresh material, secondly as liquid preserved material, and with most difficulty as dried material. It is always preferable to make identifications from live material. If material is to be kept for future reference or study, it may be preserved in several ways. Liquid preservation in 4 to 5 % formalin has been traditionally used, but skin contact and inhalation of fumes should be avoided. Alcohol (60 – 70 %) will retain structure well, although live colour will be washed out. Ethyl alcohol (ethanol) is commonly used as "methylated spirits" or denatured ethyl alcohol, however, common rubbing alcohol (isopropyl alcohol) is also quite satisfactory and readily available in pharmacies. Liquid preserving containers are bulky, heavy and may need to be topped up with the preservative fluid periodically because of evaporation around the lids. Alternately, pressed and dried herbarium sheets of stoneworts may be prepared much in the same manner as is done in the higher plants (but only gently flattened!). Such a collection is lighter, less bulky, requires no preservative, is easily stored and will last indefinitely if kept dry (Figure XI:4). Dry material, however, is brittle, easily damaged and difficult to examine critically for cellular structure until one becomes experienced with such flattened dried material. Rehydration of dried material can often be adequately accomplished by soaking in a weak acid or heated in water with a drop or two of liquid dish detergent. However, it is not as easy to interpret as liquid preserved material.

Standard herbarium sheets as utilized by colleges, universities, museums and other scientific institutions are of heavy special paper sized 290 mm by 415 mm (11.5 by 16.5 inches). However, for a naturalist's home collection, the common page size 216 by 279 mm (8.5 by 11 inches) of good quality white heavy paper (or card stock) is quite adequate, inexpensive and available everywhere. Also standard page-sized folders, boxes and binders are readily available. Because delicate and finely divided aquatic charophyte material has little rigid support, it is difficult to arrange on a paper sheet out of water. The paper sheet is submerged in a flat shallow pan of water, then the specimen is floated above the sheet, spread out to show its branching form, and the paper is very slowly raised and pulled out of the water and allowed to drain (Figure XI:5). It can then be

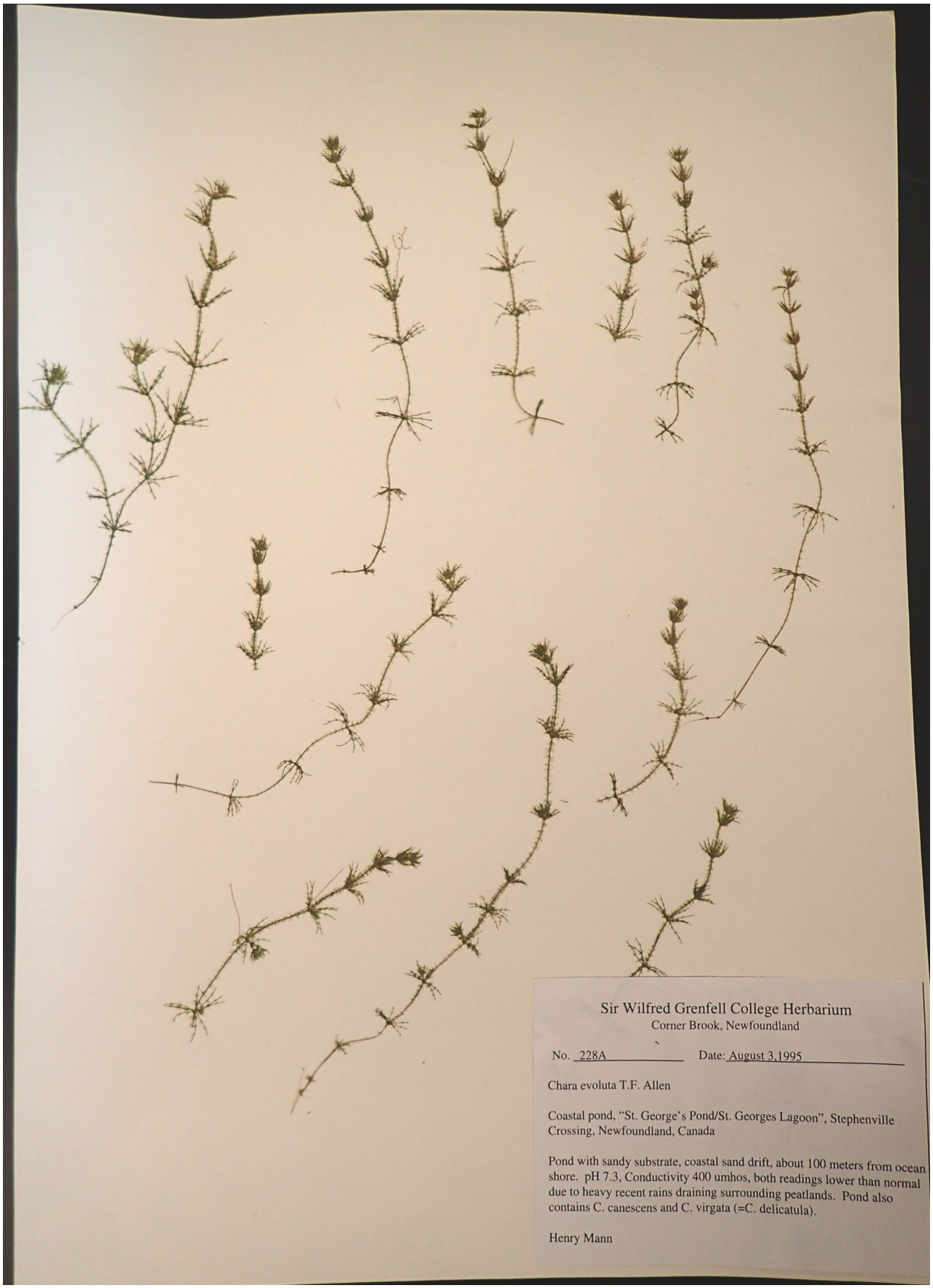


Figure XI:4 Pressed specimens of *Chara evoluta* on a standard herbarium sheet. HEM

stacked on a flat surface between layers of newspaper or other absorbent paper and allowed to air dry. Before placing a specimen sheet between newspaper layers, a sheet of household wax paper should be placed over the wet specimen to prevent it sticking to the newspaper above it. A gentle fan and/or gentle heat can speed up the drying process. Normal plant presses and driers can be used if available, but press charophytes gently. There are numerous variations of these procedures which can easily be adapted to suit an individual's needs and situation. Once dried, the sheets should be labelled with their collection data and stored in a dry location. The field book when full should be stored with the specimen collection.

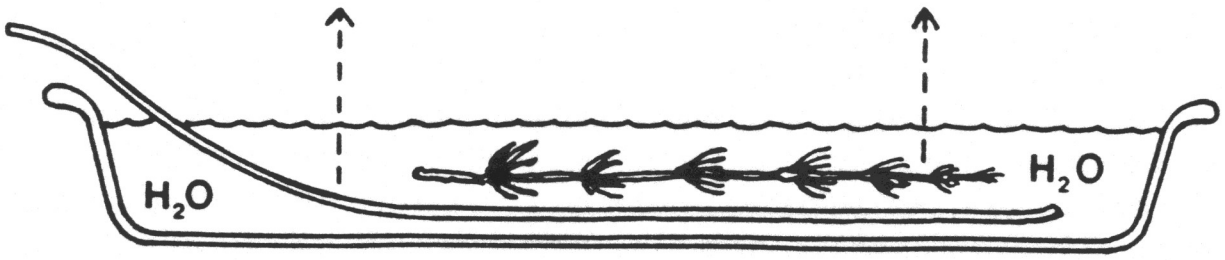


Figure XI:5 Floating charophyte material above a paper mounting sheet in a shallow pan of water. After spreading the material over the paper, the sheet is slowly pulled and lifted out of the water and allowed to drain excessive water before pressing and drying. WH

Anyone considering study of this group will also require a compound microscope in addition to a stereoscope to examine cellular features such as oospore wall ornamentation (Figure XI:6). A calibrated eyepiece is required to make microscopic measurements necessary for charophyte description and identification. Even a SEM is utilized by advanced researchers, but for purposes of this introductory work, 10x and 20x hand lenses supplemented by a stereoscope and a compound microscope are quite adequate. Often most features necessary for basic identification can be determined with a stereoscope in the range of 10x to 40x.

One feature of charophytes which may initially cause some difficulty for the novice is the considerable structural variation which may occur within species. Some of this variation is environmentally induced while some may be genetic. It is generally recognized that in flowing water and in deeper water with low light conditions, charophytes tend to be longer, more slender, more lax, and have fewer reproductive structures, sometimes none. While in shallow brighter waters with wave action they tend to be more compact, shorter, stouter and with more gametangia. Other environmental factors that may affect stonewort morphology include presence of aquatic herbivore invertebrates, salinity, water temperature, nutrient concentration, and others. Sometimes quite a range of variation can be found within the same water body or between adjacent water bodies. As one becomes more familiar with the charophyte flora and the range of variation, questions will arise whether observed variations are sufficient to warrant new variety name designations. Growing charophytes indoors under controlled conditions is sometimes helpful in distinguishing between environmental and genetically caused variation.

It is still not clear in several cases whether some closely related and named morphological species are in fact separate species or variants (varieties) within a larger complex. Future genetic analysis and interbreeding studies may shed some light on these matters.

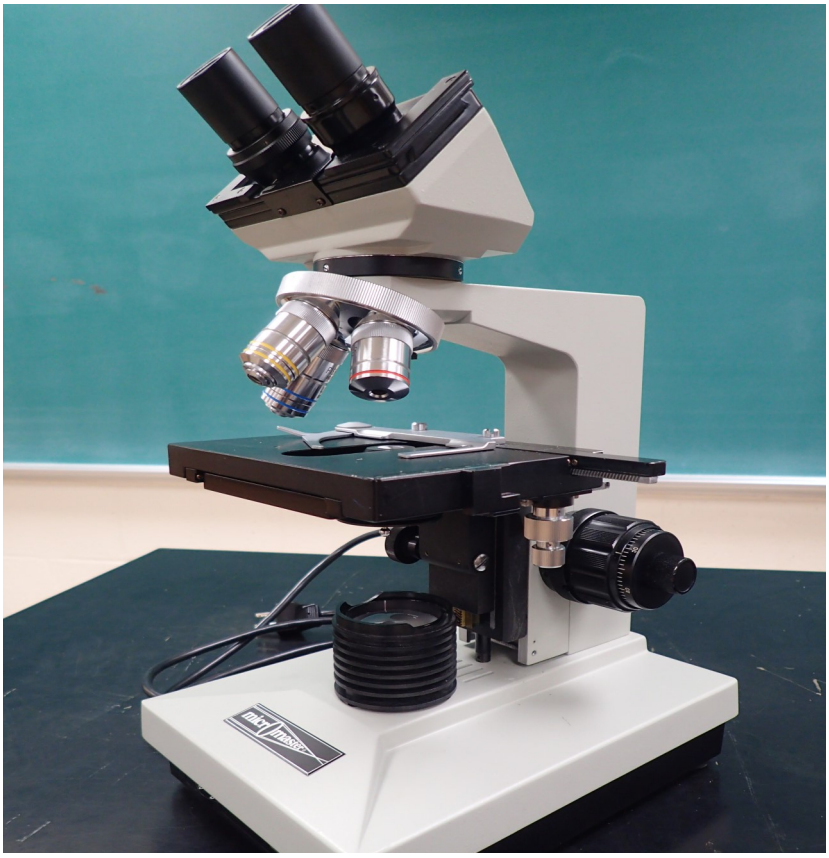


Figure XI:6 A basic compound microscope with 40x, 100x and 400x magnifications. HEM

Photographing Charophytes

Currently digital photography is replacing many line drawings formerly used extensively to illustrate biological materials. However, there will always be a place for good drawings because of their clarity, simplicity, and the ability to combine features into a single illustration with optimal orientation, something often difficult to achieve with photographs, especially at the microscopic level.

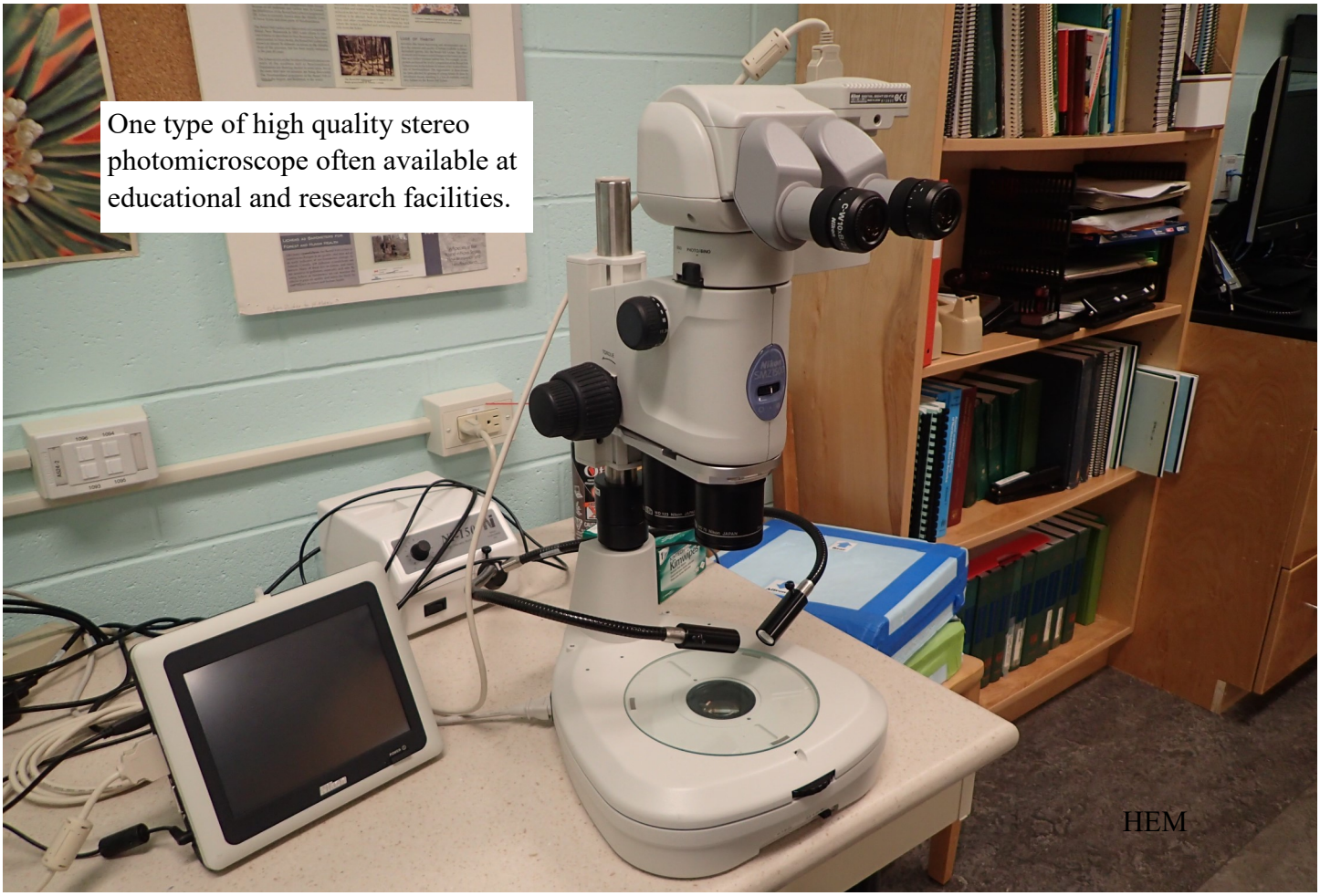
Photographing charophytes requires some magnification beyond that of the normal eye. Most important features such as cortication, stipulodes, spine cells, and coronulas require a 10x to 40x magnification to be observed clearly. Macrophotography can be used to provide general overviews of charophyte morphology using inexpensive "point and shoot" cameras. However, to determine cellular organization and structure, photographing with a stereoscope or compound microscope is necessary. Dedicated high-performance photomicroscopes are often available at educational and research institutions, however, normal digital cameras and basic microscopes can be utilized to provide reasonably adequate photos for charophyte recording and identification at the introductory level. With some trial and error lighting and focus manipulations, digital photos can even be obtained in the 10x to 400x range by hand-holding a camera on the eyepiece of a microscope (Figure XI:7). Some of the photos in this guide have been obtained in this manner, others with more dedicated equipment. Although many photos used in this guide are not of the highest quality, they are intended to convey basic information about the major features of charophytes, not necessarily to promote photographic skills. Hopefully they are found suitable for their primary focus.

Photographing charophyte details can be a time consuming process, microscopically selecting good representative material, cleaning it and the water in which it is immersed, orienting it in a dish or on a slide to best observe the intended structures, and adjusting the lighting and background. Material immersed in water will move with the slightest vibration of the microscope and depth of field is very shallow at higher

magnifications. Sometimes it is advantageous to slightly flatten material in a film of water between two slides or between inner and outer petri dish halves. Live material photos are always preferable, but sometimes liquid preserved material which has its normal colours bleached out needs to be used. Such material often does not provide sufficient cell contrast for adequate photos. A stain such as Toluidine Blue can help to clearly show cell structure in such bleached material (Figure XI:8). No doubt other water based dyes will also work. The concentration of stain to use and the length of time that the material should remain in the stain solution needs to be determined by trial and error to prevent over staining. Some of the photos in this guide use such a technique. The source of photographs and illustrations used in this guide are indicated by abbreviated initials which are fully acknowledged and explained in the section " Photograph and Illustration Sources".

Figure XI:7 Top photo: A "point and shoot" camera used for some charophyte photos in this guide. Bottom photo: After adjusting lighting, background, focus and magnification, adequate photos in the 10x to 40x range can often be achieved by simply holding the camera on the eyepiece of the microscope. A thorough understanding of the camera's capability is a distinct asset. HEM

Figure XI:8 Left photo: Liquid preserved material of Nitella without much colouration. Right photo: Same material stained with Toluidine Blue dye. After some trial and error manipulation it can readily be determined how long the material needs to be immersed in the stain to prevent under or over staining. HEM



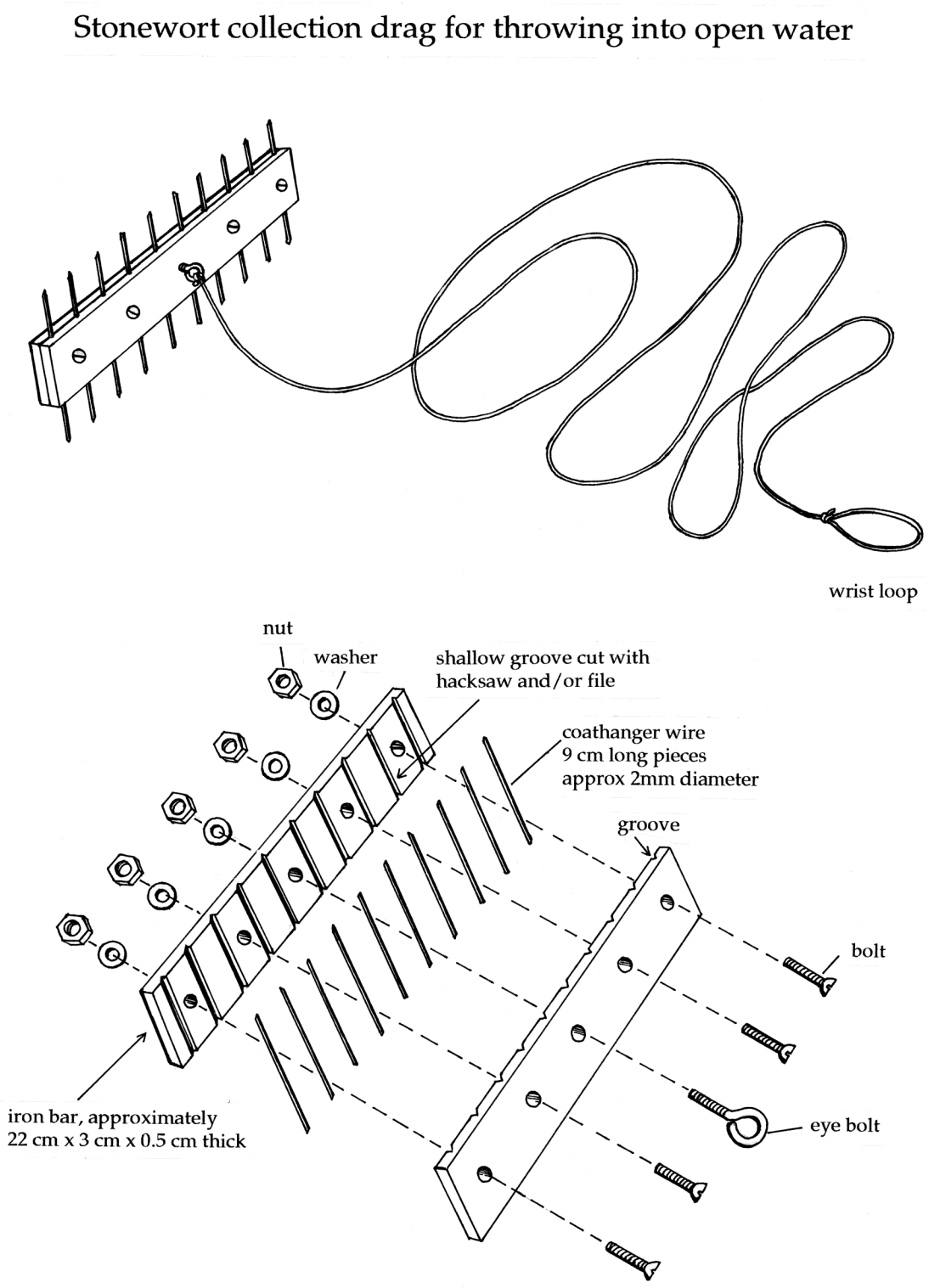


Figure XI:9 Example of the construction of a homemade grapnel. Many other designs are limited only by one's imagination. WH

Several salt ponds (barachois) on the west coast at Point au Mal have not been investigated for charophytes and are potential sites for new finds. HEM

XII: Growing Charophytes

Some charophyte species can readily be grown indoors in containers on windowsills, others with less success. Sometimes it is just as simple as collecting material with some of its natural substrate and water, and placing it in a glass container (Figure XII:1).. A north or east facing window is preferable because direct south or west sunlight during the afternoon may cause small glass containers to overheat. This could be overcome by some partial shading during the heat of the day. If window space is not available, daylight grow-lights will suffice, but a light period of twelve hours or more should be employed to encourage the production of gametangia.

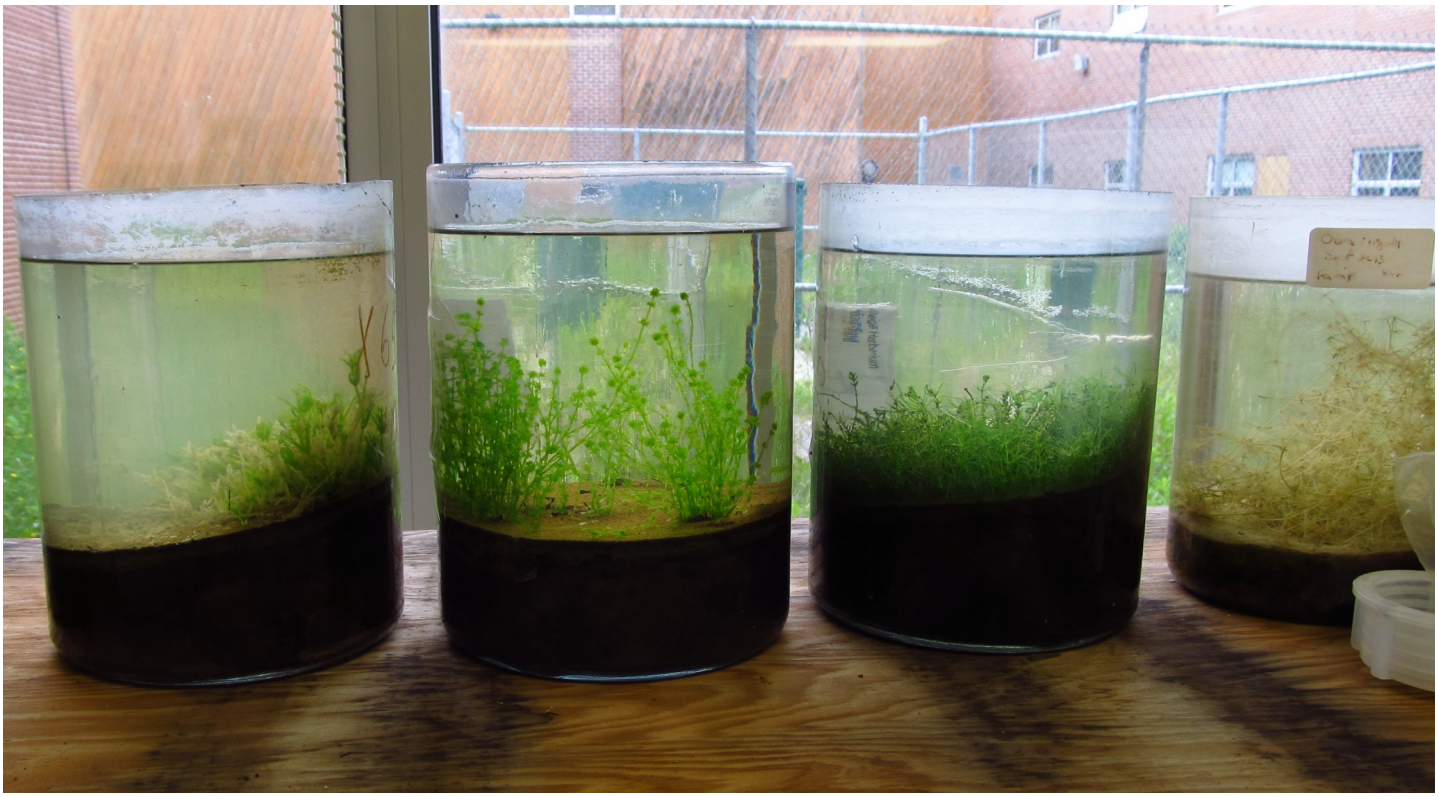


Figure XII: 1 Jars of charophytes on a window ledge. HEM

If repeated culturing is anticipated it is preferable to locate some fine sandy-loam soil free of rocks, possibly along stream banks or in alluvial flats. The wet soil may be steamed or cooked to sterilize it. Institutions often have equipment known as autoclaves for sterilization purposes, but this can readily be done at home (outdoors!) on a camp stove or barbecue in a covered pot. Avoid containers or utensils made of copper as some charophytes do not tolerate well even tiny amounts of copper in soil or water. Good clean pure water should be used. If tap water is used, let the tap run for a few minutes to reduce any copper or lead from the pipes. If water is chlorinated, allow it to stand in an open container at room temperature for a few days to allow gassing off of most of the chlorine before use.

When collecting charophytes for container transplanting, always obtain the lower portions of the plants with a mass of rhizoids located within the substrate. If there is much branch growth, some can be trimmed back to encourage new growth from the base (Figure XII:2).

Clear transparent glass containers with wide mouths or small glass aquaria are preferable, but food-grade transparent plastic containers have also been successfully utilized. A 3 to 5 cm layer of sandy loam soil on the bottom is adequate substrate. The muddy water created by the planting process will gradually sediment out and clear. When topping up containers with water, slowly pour water onto a shallowly submerged container lid or other flat object (e.g. a glass/plastic petri dish, etc.) to prevent excessive stirring up of bottom sediments.

Figure XII:2 A stonewort plant with some subterranean stems with bulbils and/or rhizoids can often easily be transplanted into substrate in a jar or aquarium. Several stems may be pruned off near the base in a very bushy plant. HEM

Knowledge of the conditions where a species normally flourishes will help to successfully culture that species. For example, if the species normally grows in brackish waters, it may be preferable to adjust the culture water to that approximate salinity by adding a few pinches of sea salt normally used in the kitchen. If the species grows in calcareous marl ponds, it may be advantageous to add a bit of common agricultural limestone to the soil. Chemical soil and water analysis can help to determine natural conditions if equipment and time are available, however, common-sense approximation is usually quite adequate, especially for casual growers and beginners. Many of our common stoneworts have quite a tolerance range for soil and water conditions and if toxic substances are avoided will often flourish with little maintenance, happily adjusting to their environment.

Using some of these simple procedures, cultures have been maintained in excess of thirty years, often with considerable neglect other than topping up with water as needed (Figure XII:3). If a culture appears to be declining, re-culturing into fresh substrate and water will often rejuvenate it. Sometimes growth will be slow so patience is required.

Another method consists of collecting bottom substrate from water bodies and keeping these in small jars on a windowsill (Figure XII:4). Charophyte shoots may emerge from germinating oospores in the sediment sometimes very quickly and sometimes after prolonged periods. At one time a pan of wet substrate was left outdoors to freeze solid over winter. In spring two species of charophyte sporelings emerged in the wet neglected pan confirming the tenacity and survivorship of this ancient group of aquatics. Much enjoyment and knowledge can be obtained by individuals with the interest and patience to grow these marvelous denizens of the shallows and the deep.

Figure XII:3 Chara growing in an a normal fish tank aquarium with aquarium lighting. This culture was maintained with minimum maintenance for many years. HEM

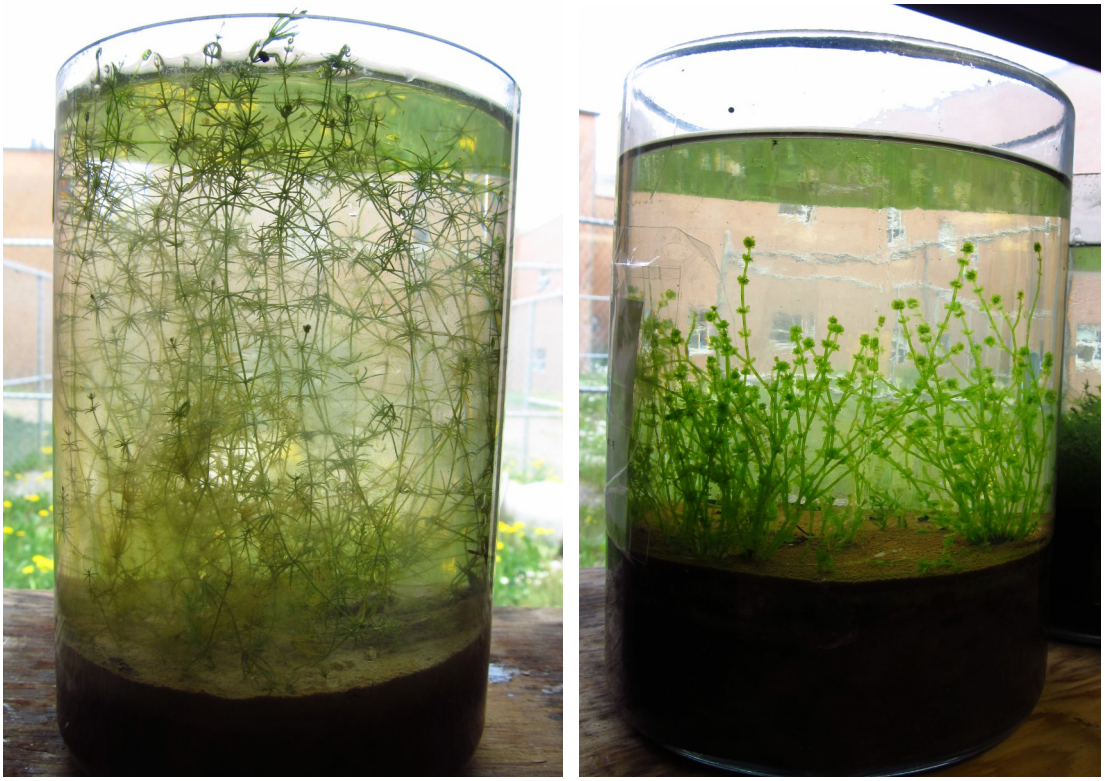


Figure XII:4

Two charophyte cultures in jars on window ledges. HEM

Windowsill culture of
Nitella clavata. HEM

XIII: Selected Charophyte Literature For Beginners

Because this work is intended for beginners in charophytology, only a very few sources of immediate relevance are provided. The English European works listed below will provide good additional general information to supplement this guide. Unfortunately there are no such comparable recent works for North America. Other more detailed European works also exist in English, German, Dutch, French and Russian.

A. European Sources in the English Language:

Allen, G.O. 1950. British Stoneworts. The Haslemere Natural History Society, Surrey, U.K.
(Highly recommended for the novice. Nothing comparable exists in North America. It is highly readable and explanatory. Although out of print, it can be obtained as a loan through libraries or second hand copies purchased on the internet.)

Groves, J. and G.R. Bullock-Webster. Volume I Nitelleae: 1920, Volume II Chareae: 1924. The Ray Society, London, U.K. A 1971 edition by the Johnson Reprint Corporation, New York has bound the two into a single volume.
(A more detailed work on charophyte structure, development and identification of British charophytes, but worth studying as an overview of the group. Although almost a century old it is still a good survey of the basics and history of the stoneworts in the English language. May be obtained as a loan through a local library.)

Langangen, A. 2007. Charophyes of the Nordic Countries. Saeculum ANS, Oslo, Norway.
(A good recent overview of northern European charophytes. All of our species except *Chara evoluta* are included.)

Moore, J.A. 1986. Charophyes of Great Britain and Ireland. BSBI Handbook No. 5. Botanical Society of the British Isles (BSBI), London, U.K.
(A well illustrated introduction to charophytes for naturalists. However scientific names used should be checked against the more current NA list by Scribailo and Alix, 2010.
This is a small inexpensive book, highly recommended, which includes most of our species.

Schubert, H. and I. Blindow (Editors). 2003. Charophytes of the Baltic Sea. Koeltz Scientific Books, Konigstein, Germany. (A good modern overview of most of our species.)

Urbaniak, J. and M. Gabka. 2004. Polish Charophytes: An Illustrated Guide to Identification. Uniwesytet Przyrodniczy we Wrodawiu, Wroclaw, Poland. (Available as a downloadable PDF file. Highly photographically illustrated.)

B. The Author's Charophyte Publications:

Mann, H. 1982. Ancient Pond Worts. The Osprey 13(1): 1-5.

Mann, H. 1989. Charophytes of Insular Newfoundland. Canadian Field-Naturalist 103(1): 34-42.

Mann, H. 1990. The Stoneworts of Newfoundland I: Introduction. The Osprey 21(2): 57-61. (Intended as the lead article in a charophyte series for naturalists and students, but the planned subsequent articles were never produced. This guide will hopefully fill that gap.)

Mann, H. 1994a. The Genus Tolypella (Characeae) in Insular Newfoundland. Canadian Field-Naturalist 108 (3): 297-304.

Mann, H. 1994b. Charophytes of Crooked Lake, Saskatchewan. Canadian Field-Naturalist 108(4): 413-427.

Langangen, A., J.B. Hansen and H. Mann. 1996. The Charophytes of Greenland. Cryptogamie Algologie 17 (4): 239-257.

Mann, H. 1998. Newfoundland Charophytes. In: Assessing the state of the environment in Gros Morne National Park. Edited by D.W. Anions and A.R. Berger, Gros Morne National Park, Rocky Harbour, NL. Report 011, pp. 74-77.

Mann, H., V.W. Proctor and A.S. Taylor. 1999. Towards a Biogeography of North American Charophytes. Australian Journal of Botany 47: 445-458.

Mann, H. 2000. General Status of Charophytes on the Island of Newfoundland. Report for the Newfoundland Rare Plant Project, Endangered Species and Biodiversity Section, Inland Fish and Wildlife Division, Government of Newfoundland and Labrador, St. John's. 11 pp.

Mann, H. and M.V.S. Raju. 2002. First Report of the Rare Charophyte Nitella macounii (T.F. Allen) T.F. Allen in Saskatchewan and Western Canada. Canadian Field-Naturalist 116(4): 559-570.

Mann, H. 2004. Charophytology in Newfoundland, Canada. Nieuwsbrief Kranswieren 8(13): 9-13. (A general overview of Newfoundland and Canadian charophytology as invited by the editor of this Dutch publication.)

Mann, H. 2004. Of Rocks, Ponds, Ducks and Stoneworts. Sarracenia 12(2): 20-25.

Mann, H. and M.V.S. Raju. 2005. A New Tadpole Shrimp, Triops longicaudatus in Saskatchewan. Blue Jay 63(2): 94-97.
(Includes some ecological observations on charophytes.)

Mann, H. and E.M.V. Nambudiri. 2005. Charophytes of Insular Newfoundland II: Chara evoluta and Chara canescens. Canadian Field-Naturalist 119(1): 26-37.

Mann, H., C. Hanel, A. Langangen, and P. Nowak. 202x. *Chara contraria* var. *hispidula* Braun (Charales) in Newfoundland, Canada a new variety described from North America. Botany Letters (submitted for publication)

C. North American Sources:

Scribailo, R.W. and M.S. Alix. 2010. A Checklist of North American Characeae. Charophytes 2(1): 38-52.
(The most comprehensive and recent list of NA charophytes. Initially just useful for the novice as an overview of the species that inhabit NA and their older synonyms. The details of taxonomy and biogeography will only become important once the novice gains a good understanding of the group. Much future research will still be required to validate some of the listed species, however, it is an important milestone reference for this continent. Also in the current guide the author will indicate those species that exist in adjacent regions and which could potentially be discovered in the future in Newfoundland and Labrador.)

Proctor, Vernon W. (Dr. V. W. Proctor is the acknowledged authority on North America charophytes and well known internationally. His many publications are perhaps a bit technical for novice charophytologists, but as one advances in the discipline, they become essential references for an in-depth understanding of the group.)

Wood, R.D. 1967. Charophytes of North America. University of Rhode Island, Kingston, R.I.
(This is a brief summary for North America from the Wood and Imahori large two volume work, "A Revision of the Characeae"(1964, 1965).This brief work is useful if one understands that the taxonomy utilized by Wood has since advanced as represented by the most recent checklist by Scribailo and Alix (2010). (For example Wood considered that similar monoecious and dioecious forms were the same species, now

considered to be separate species by most charophytologists e.g. *C. globularis/virgata* and *N. flexilis/opaca* are now considered separate species, etc.). Wood's key could be useful in tracking down new Newfoundland discoveries if used in conjunction with the section pp. 60 – 62 , (Other Names Associated With Each Species In American Literature), and the 2010 checklist. Wood's works are tremendously important resources, however, an understanding of their true significance is only possible once a thorough grounding in charophyte structure and taxonomy has been achieved.

van Raam, J. 2008. Bibliography of Extant and Fossil charophytes (Charales) up to year 2008. 287 page pdf document. International Research Group on Charophytes (IRGC). www.sea.ee/irgcharophytes/
(This massive 287 page document lists all worldwide charophyte references up to the year 2008, including all of the North American scientific and technical literature. Although not initially relevant to novices for whom this guide is intended it is only mentioned as general information. North American publications since 2008 can be searched for and/or accessed via the internet. Among the North American authors, the works of T.F.Allen, R.D. Wood, and V.W. Proctor stand out as milestones. ***Some North American papers directly relevant to Newfoundland and Labrador charophytes can be found in the references of the author's papers.)***

D. International Research Group on Charophytes (I.R.G.C.)
This international group publishes a newsletter and maintains a website www.sea.ee/irgcharophytes/ providing recent information on research, publications, regional groups, newsletter archives and members lists. Also meetings and field trips are organized in various countries and continents. Anyone interested in charophytes might consider an affiliation.

Plate IV.

NITELLA FLEXILIS. Ag.

XIV: Acknowledgements

My fascination with stoneworts has become more of a hobby than an academic pursuit, a reason to get out into nature to explore places and habitats that otherwise may have gone unnoticed and unappreciated. As such there are literally hundreds of individuals who have been part of the process and to whom I am grateful, too many to name individually, friends, relatives, acquaintances, naturalists, colleagues, correspondents and students. You know who you are and I have been privileged to travel this pathway with your interest and fellowship.

I thank Vernon W. Proctor of Lubbock, Texas who provided much information and insight into the study of charophytes and also provided necessary encouragement in the early years.

Anders Langangen of Oslo, Norway provided considerable information, specimens and fruitful correspondence over the years. His studies were inspiring.

I am grateful to Paul Hamilton and Michel Poulin for accommodations and access to the national charophyte collection at the Canadian Museum of Nature in Ottawa.

The Grenfell Campus of Memorial University of Newfoundland and Labrador provided the infrastructure, time and incidental funding that allowed this ongoing inquiry to flourish.

Thanks to Dmitry Sveshnikov, Grenfell Herbarium Curator (SWGC) for access to the herbarium and for technical manuscript assistance.

A number of individuals supplied me with collected Newfoundland and Labrador charophyte specimens. These include John Maunder, Claudia Hanel, Nathalie Djan-Chékar, Wally Skinner, Monique Vassallo, Wolfgang Maas, Stan Pieda Jr., Carol Gilbert, Maria Howell, Todd Howell.

I am grateful to the following publishers who permitted the use of illustrations: The Botanical Society of the British Isles (J.A. Moore. 1986. Charophytes of Great Britain and Ireland); The Ray Society. (1920, 1924. The British Charophyta) ; Schweizerbart and Borntraeger Science Publications (www.borntraeger-cramer.de) (R.D. Wood and K. Imahori. 1964, 1965. A Revision of the Characeae).

The following individuals permitted the use of their photos, Anders Langangen, John Maunder, Claudia Hanel, Nathalie Djan-Chékar, Phyllis Mann.

Patricia Baines provided access to the CFS Atlantic Forestry Centre Herbarium (CDFN), Corner Brook, NL.

Julissa Roncal provided access to the Memorial University of Newfoundland Ayre Herbarium (NFLD), St. John's, NL.

WH

Upper photo: Shallow richly vegetated wetlands frequently visited by waterfowl and shorebirds usually contain pools with charophytes. Lower photo: Charophytes are often among the first colonizers of human -made and/or beaver-made ponds.

XV: Photograph and Illustration Sources

The following abbreviations were used to identify the sources of photos and illustrations.

AL - Anders Langangen, Oslo, Norway

CH - Claudia Hanel, Ecosystem Management Ecologist (Botany), Fisheries, Forestry, and Agriculture, Government of Newfoundland and Labrador, Corner Brook, NL.

EMVN - E.M. (Vasu) Nambudiri, from publication by Mann and Nambudiri 2005.

G&B-W - Groves, J. and G.R. Bullock-Webster, 1920, 1924, Volumes I and II, The British Charophyta, The Ray Society, London. Used with the permission of The Ray Society, London.

HEM - photos and illustrations of the author.

JM - John Maunder, Curator Emeritus, The Rooms Provincial Museum (NFM), St. John's, NL.

KI - Illustrations by Kozo Imahori from R.D. Wood and K. Imahori, A Revision of the Characeae, J. Cramer, Weinheim. Volume 1: 1965, Monograph of the Characeae; Volume 2: 1964, Iconograph of the Characeae. Permision to use by Schweizerbart and Borntraeger Science Publications, Stuttgart, Germany (www.borntraeger-cramer.de).

MIG - Migula, W. 1907 (1909?). Kryptogamen-flora von Deutschland, Deutsch-Osterreich und der Schweiz. Band II. Algen. 2. Teil. Rhodophyceae, Phaeophyceae, Characeae. Hugo Bermuhler Verlag, Berlin-Lichterfelde. In the public domain.

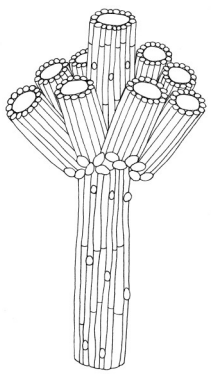
MT - Margaret Tebbs from J.A. Moore. 1986. Charophytes of Great Britain and Ireland. B.S.B.I. Handbook No. 5, Botanical Society of the British Isles, London, UK. Permission granted by the Botanical Society of the British Isles, London, UK.

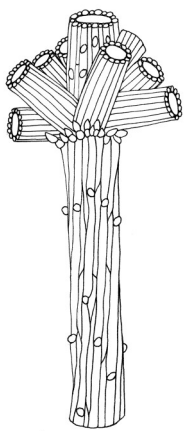
NDC - Nathalie Djan-Chékar, Collections Manager (Natural History), The Rooms Provincial Museum (NFM), St. John's, NL.

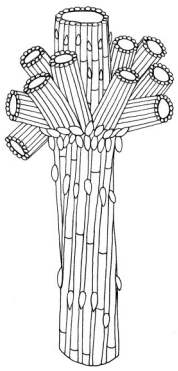
PM - Phyllis Mann, Pasadena, NL.

TFA - Timothy Field Allen. 1888. The Characeae of America. Part II. New York Academy of Sciences, NY. In the public domain.

WH - Warwick Hewitt, Graphic Artist, Sir Wilfred Grenfell College, Memorial University of Newfoundland, Corner Brook, Newfoundland, Canada. From the Grenfell Campus MUN Herbarium Archives. Utilized with permission.







WH

Water Lobelia and Yellow Pond Lily are common vasculars in the shallows of many oligotrophic to mesotrophic ponds in NL.

HEM

XVI: Glossary

Plural forms of selected terms are in brackets.

acuminate – gradually tapering to a sharp point.

aggregate – a combination of several taxa.

antheridium (antheridia) – The male reproductive organ producing the male gamete (sperm).

Atlantic Canada – the four provinces of Nova Scotia, New Brunswick, Prince Edward Island, and Newfoundland and Labrador.

aulacanthous – having a stem cortex where the secondary cortical cells are more prominent than the primary cells.

axial cortex – the cortex on the main axes (stems) as opposed to the cortex on the branchlets.

axis (axes) – the main stem on which whorls of branchlets are borne.

bird's nest – a compact cluster of fertile branchlets as in the genus Tolypella.

bog – an acidic peatland characterized by the presence of sphagnum mosses.

brackish – waters more saline than freshwaters, but less saline than marine waters.

bract/bract cell – a small cell growing outward at the branchlet nodes.

bracteole – one of a pair of bract-like cells arising on either side at the base of the oogonium.

bractlet – a single bract-like cell at the base of the oogonium in dioecious species occupying the location where the antheridium would be located in a monoecious species.

branch – a division of the main axis (stem).

branchlet – a structure produced in whorls at the stem nodes on which gamatangia are produced.

bulbil – an enlarged structure of starch-rich cells, single-celled or in clusters, at stem or rhizoid nodes.

bulbous – rounded or enlarged at one end.

calcium carbonate – the main mineral in limestone rock formed from calcium combining with carbon dioxide ($CaC0_3$).

central ray – the larger main portion of a dividing branchlet on which smaller lateral rays are produced at the nodes, as in Tolypella.

charophytology – the discipline of charophyte study.

coronula – a cluster of small cells at the tip of the oogonium produced by divisions at the terminal ends of the spiral cells.

cortex – a sheath of smaller cells covering the large stem internodal cells as in the genus Chara and in the genus Lychnothamnus.

cortical cell – an elongate or a spine cell of the cortex.

corticate – having a cortex.

couplet – a pair of contrasting leads in a dichotomous key.

dactyl – the terminal ray in a branchlet of Nitella composed of one or more cells.

dichotomous key – a key which always presents two contrasting choices (leads) in couplets.

dichotomy – two contrasting choices.

dioecious – male and female gametangia produced on separate male and female plants of the same species.

diplostichous – having a cortex where primary rows are separated by one secondary row.

distal – toward the tip.

ecorticate – lacking a cortex.

eutrophic – waters rich in plant nutrients.

extant – currently existing; currently living.

fen – a wet peatland less acidic and more nutrient rich than a bog and characterized by mosses, sedges and grasses.

fertile – having gametangia (antheridia and/or oogonia) present.

forking – dividing into two, more or less, equal parts.

fresh water – water very low in dissolved salts.

furcate – divided (forked) into two, more or less, equal segments.

furcation – equal division or forking.

gametangium (gametangia) – a reproduction (sex) organ; an antheridium or oogonium.

globular – a spherical appearance.

granular – having a rough surface of separated dots (grains) of various sizes and/or various spacings.

haplostichous – having a cortex of only primary rows.

head – a compact cluster of fertile branchlets as sometimes produced in the genus Nitella.

humic waters – waters high in dissolved organic compounds (humic acids) which often stain the waters yellowish or brown.

imperfect cortication – cortication only partially present on a stem (axis) or branchlet.

indented key – a key which distinguishes two leads of a couplet by equal indentation rather than by number.

Insular NL – the island portion of the province of Newfoundland and Labrador.

internode – the portion of a stem or branchlet between two nodes.

isostichous – having primary and secondary cortical rows of equal prominence/size.

key – a literary biological device helpful in identification, printed or digital.

lateral – arising from the side.

lateral ray – a smaller ray arising at a node of the primary ray, as in the genus Tolypella.

lead – one of the two contrasting statements of a key couplet.

lime – a white or pale-grey calcium compound derived from limestone.

limestone – sedimentary rock with a high calcium content.

marine water – waters high in dissolved salts; ocean water.

Maritime Provinces – the provinces of Nova Scotia, New Brunswick, and Prince Edward Island, not including Newfoundland and Labrador.

membrane – the outer wall of an oospore which may be variously ornamented.

mesotrophic – waters only moderately rich in plant nutrients.

monoecious – having male and female gametangia produced on the same plant.

mucro – a tiny pointed end cell.

mucronate – having a mucro at the tip of a branchlet.

mucus – gelatinous material sometimes enclosing gametangia in the genus Nitella.

NA – North America.

naked cell – a cell without covering cortication.

NL – the province of Newfoundland and Labrador (i.e. Insular Newfoundland and Labrador).

node – a joint in a stem (axis) or branchlet.

obtuse – blunt or rounded at the tip

octoscutate – having an antheridium composed of eight scutes or shields.

oligotrophic – waters very low in dissolved salts and in plant nutrients.

oogonium (oogonia) – the female gametangium

oospore – the fertilized egg cell (zygote).

oospore membrane – the outer wall of the oospore which may be variously ornamented.

papillate/papillous/papillose – having short rounded nipple-like projecting cells.

peatlands – general term for wetlands with an accumulation of peat, i.e. bogs and/or fens.

penultimate cell – the cell below the one in question, i.e. the cell to which the terminal cell is attached.

pH – the acidity or alkalinity of a solution.

pioneer species – species that are early colonizers of disturbed habitats.

primary cortex – cortex rows that include spine cells.

primary ray – the basal segment of a branchlet in Nitella or Tolypella.

ray – a segment of a branchlet in Nitella and Tolypella.

reticulate – having a mesh-like appearance.

rhizoid – fine transparent hair-like filaments from nodes or bulbils of stems in the substrate.

rudimentary – small and incompletely or only partially developed.

saline – waters having considerable dissolved salts.

secondary ray – the ray attached to the primary ray in Nitella.

scute – an often triangular shield cell forming part of the wall of an antheridium.

secondary cortical cell – a cortical cell located between the primary rows.

SEM – Scanning Electron Microscope

senescent – aged; material no longer in prime condition, no longer clearly exhibiting features important for identification.

shield cell – often triangular cell forming part of the wall of an antheridium, i.e. a scute.

s.l. (sensu lato) – broadly speaking/in the broad sense; combined species under one name which some authorities consider to be separate.

spine cell – outward projecting cell in the primary cortex rows.

spinulose – like a thin sharply pointed spine.

stem – used as a synonym for a main axis.

sterile – a plant without gametangia.

stipulode – a cell from the one or two whorls of cells produced at the axis nodes of Chara, Lamprothamnium. and Lychnothamnus.

substrate – the sediments on the bottom of a water body in which charophytes are "rooted".

taxon (taxa) – a named group in a classification system.

taxonomy – the science of classifying and naming living organisms.

terete – rounded in cross section

tetrascutate – having an antheridium composed of four shield cells (scutes).

thallus (thalli) – the body of "plants" that do not have true stems, roots or leaves, e.g. algae.

tier – layer or row placed one above the other

triplostichous – cortex with two secondary rows of cells alternating with the primary rows.

tuberculate membrane – oospore membrane with large irregularly shaped raised protuberances.

turbid – murky or muddy

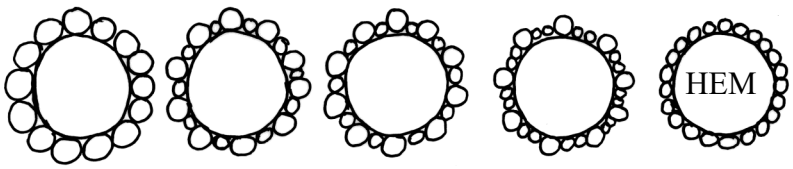
tylacanthous – cortex with primary cell rows more prominent (larger) than secondary rows.

unnumbered key – indented key with unnumbered leads.

whorl – a ring of similar structures around the stem (axis), e.g. branchlets or stipulodes.

XVII: About the Author

Henry Mann is a naturalist, teacher, and retired biology professor. He taught in the Saskatchewan public schools, at Memorial University of Newfoundland St. John's Campus, and at the Sir Wilfred Grenfell Campus in Corner Brook. He lives in Pasadena, Newfoundland with his wife Phyllis. His interests include gardening, hiking, snowshoeing, nature photography, and other nature and plant-related activities. He enjoys studying the distribution, taxonomy and ecology of algae called stoneworts, primitive freshwater "plants" also known as Charophytes.



Somewhere in the wilds of Newfoundland

XVIII: Indices

A. Index to Species Featured in This Guide

Chara aspera *27*
Chara braunii *91*
Chara canescens *37*
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WH

Pools in a sub-alpine plateau peatland in the vicinity of Nain, Labrador.

XIX: APPENDICES

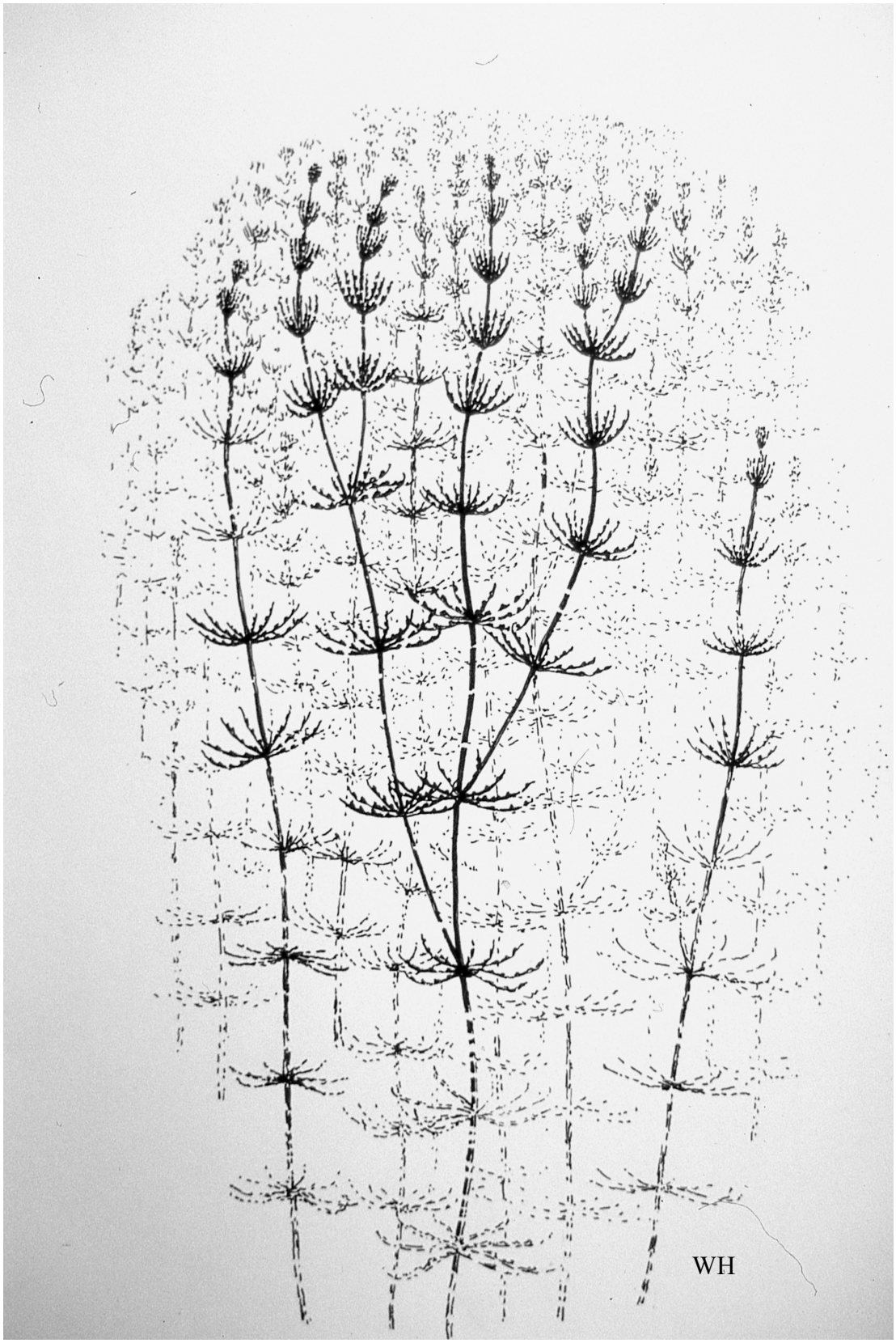

Illustration of Underwater Chara “forest”.

Appendix Contents

A Listing of Canadian Charophytes by Province and Territory

B List of Labrador Charophytes

A: Listing of Canadian Charophytes by Province and Territory

These lists should not be considered definitive or exhaustive. They only include specimens the author has seen or reports in the published literature. No doubt there are many regional or local herbaria, as well as personal collections, which contain unrecorded charophytes. In general, the Canadian charophyte flora is poorly investigated and documented. Much work needs to be done on occurrence, distribution, taxonomy and ecology of the group. The paucity of information in Atlantic Canada suggests that many more species and varieties will be reported should detailed collection and field studies take place. The greatest number of species are reported for Ontario and Quebec, provinces with the largest population, the most education and academic institutions and the most aquatic studies. But even here, knowledge of the charophyte flora is sketchy and few actual voucher specimens are available in herbaria for detailed study. Much opportunity exists for students and naturalists across the country to contribute to our knowledge of these significant aquatic macrophytes.

The species names used correspond to the most recent North American list of Scibailo and Alix, 2010. Because older collections often used now outdated synonyms on their labels, these are included in parentheses following the currently accepted name, e.g. *Chara globularis* is the current binomial for the former *C. fragilis*, *C. virgata* for former C. delicatula, *C. vulgaris* for former C. foetida, etc. For *Nitella flexilis* (monoecious) and *Nitella opaca* (dioecious), when gametangia were not present, "s.l. (sensu lato)- sterile" is indicated because the two cannot be distinguished reliably without reproductive structures. Scientific nomenclature is continually in a state of flux, especially so since the advent of DNA analysis. It is suspected that most of the species listed will remain as "good" species, while a few may become variants (varieties) within a species, and perhaps others will be split into separate species as our understanding increases.

If voucher specimens are known to exist in herbaria, the herbarium code is provided after the listed charophyte. Following are the codes used and the names of the institutions:

CANA – Canadian Museum of Nature, Ottawa

SWGC – Grenfell Herbarium, Grenfell Campus, M.U.N., Corner Brook

NY – New York Botanical Garden, New York

NBM – New Brunswick Museum, Saint John

ALA – University of Alaska, Fairbanks

NBV – Rijksherbarium, Leiden, Netherlands

NFLD – Phycological Herbarium, Memorial University, St. John's

NFM – Newfoundland Provincial Museum, The Rooms, St. John's

DAO – Agriculture and Agrifoods Canada, Ottawa

If the species is known from literature reports, the citations are also provided. A cited reference list is provided at the end of this Appendix A.

Because it is geographically intertwined with British Columbia and the Yukon, Alaska is also included.

Future stonewort studies in the Maritimes and Newfoundland/Labrador must also consider the charophyte floras of the eastern USA in addition to the large neighbouring provinces of Quebec and Ontario. Waterfowl and shorebird north-south migrations have undoubtedly dispersed and introduced more southerly or westerly reported species into our region wherever suitable aquatic habitat exists.

Charophytes of Newfoundland and Labrador *a,b

Chara aspera	SWGC, CANA
Chara canescens	SWGC, CANA, NFM, NFLD
Chara contraria	SWGC, CANA
Chara contraria var. hispidula	SWGC, NFM, NFLD
Chara contraria var. inconnexa	SWGC, CANA
Chara evoluta	SWGC, CANA, NFM, NFLD
Chara globularis (fragilis)	SWGC, CANA
Chara virgata (delicatula)	SWGC, CANA, NFLD
Chara vulgaris (foetida)	SWGC, CANA, NFLD
Nitella confervacea	SWGC, CANA
Nitella flexilis (s.l.-sterile)	SWGC
Nitella flexilis (monoecious)	SWGC, CANA
Nitella opaca (dioecious)	SWGC, CANA
Tolypella glomerata	SWGC, CANA

*a See publications of H. Mann in "XIII: Selected Literature" section of this guide.
*b Refer to Appendix B for a separate listing of Labrador charophytes.

Charophytes of Nova Scotia

Chara contraria	(Cape Breton)	SWGC
Chara globularis (fragilis)	(Cape Breton)	CANA
Chara globularis (fragilis)	(Sable Island)	CANA
Chara virgata (delicatula)	(Sable Island)	CANA
Nitella flexilis (s.l.-sterile)	(Sable Island)	CANA
Nitella flexilis (s.l.-sterile)	(West Bridgewater)	CANA
Nitella opaca	(Sable Island)	CANA

Charophytes of Prince Edward Island

No Records

Charophytes of New Brunswick*

Chara aspera	NBM
Chara braunii	NBM
Chara globularis (fragilis)	CANA, NBV
Chara virgata (delicatula)	CANA, NBM
Nitella dilatata	Allen 1954, Wood 1948
Nitella flexilis	NBV
Nitella flexilis (s.l.-sterile)	CANA, NBM
Nitella flexilis/acuminate/dilatata group (monoecious)	NBM

* Refer to "Ancillary Charophyte Files" for details of NBM and MBV charophytes.

Charophytes of Quebec*

Chara braunii	CANA, NBV, Corillion 1995
Chara contraria	CANA
Chara fragifera (? needs verification)	Corillion 1995
Chara globularis (fragilis)	CANA, Corillion 1995
Chara virgata (delicatula)	CANA
Chara vulgaris (foetida)	CANA, Corillion 1995
Chara zeylanica	NBV, Corillion 1995
Nitella clavata	Allen 1954
Nitella confervacea (brachyosperma)	CANA
Nitella dilatata	Corillion 1995
Nitella flexilis (monoecious)	CANA, Corillion 1995
Nitella flexilis (s.l.-sterile)	CANA
Nitella gracillis	Wood 1948
Nitella macounii	NY, Frame 1977
Nitella megacarpa	Corillion 1995
Nitella microcarpa	Wood 1948, Wood 1965
Nitella mucronata	Corillion 1995
Nitella obtusa	CANA, NBV, NY, Frame 1977, Wood 1948
Nitella opaca	CANA
Nitella tenuissima (transilis)	Corillion 1995
Tolypella prolifera	NY, Frame 1977

*Refer to "Ancillary Charophyte Files" for the Corillion (Lachavanne) 1995 communication.

Charophytes of Ontario

Chara aspera	CANA
Chara braunii	CANA
Chara contraria	CANA
Chara foliolosa (zeylanica)	CANA 030312
Chara globularis (fragilis)	CANA
Chara haitensis (zeylanica)	CANA 15941
Chara keukensis	Robinson 1906
Chara virgata (delicatula)	CANA
Chara vulgaris (foetida)	CANA
Nitella acuminata	CANA, Allen 1954
Nitella dilatata	Allen 1954
Nitella flexilis (monoecious)	CANA
Nitella flexilis (s.l.-sterile)	CANA
Nitella furcata (mucronata)	CANA, Frame and Sawa 1975
Nitella gracillis	CANA, NBV
Nitella hyalina	Nichols et al. 1988, 1989
Nitella intermedia	Frame and Sawa 1975
Nitella macounii	CANA, Allen 1954, Wood 1948
Nitella obtusa	Sawa and Frame 1974

Nitella opaca (dioecious)	CANA
Nitella spanioclema	Sawa 1973, Sawa and Frame 1974
Nitella tenuissima (transilis)	Frame 1977, Frame and Sawa 1975, Sawa and Frame 1974
Nitellopsis obtusa	Geis et al. 1981, Nichols et al. 1989
Tolypella prolifera	CANA, SWGC
Tolypella canadensis	Sawa 1973, Sawa and Frame 1974, Frame and Sawa 1975
Tolypella glomerata (comosa)	Sawa 1974, Sawa and Frame 1974, Frame and Sawa 1975
Tolypella intricata (intertexta)	NY, Frame 1977

Charophytes of Manitoba

Chara braunii	NBV
Chara contraria	NBV
Chara globularis (fragilis)	CANA
Chara vulgaris (foetida)	NBV
Nitella flexilis (s.l.-sterile)	CANA
Tolypella intricata (intertexta)	NBV

Charophytes of Saskatchewan

Chara aspera	CANA, Mann 1994
Chara aspera var. macounii	Robinson 1906, Wood 1965, Mann 1994
Chara braunii (coronata)	SWGC, CANA, NFM
Chara canescens	CANA, Mann 1994
Chara contraria	SWGC, CANA, Mann 1994
Chara evoluta	CANA, NBV
Chara globularis (fragilis)	SWGC, CANA, Mann 1994
Chara longifolia (buckellii)	Hammer and Heseltine 1988*
Chara virgata (delicatula)	CANA, Mann 1994
Chara vulgaris (foetida)	SWGC, CANA
Chara vulgaris var. longibracteata	SWGC, CANA, Mann 1994
Nitella dilatata	SWGC
Nitella macounii	SWGC, CANA, NFM, Mann 2002
Nitella sp. (flexilis/opaca ?)	Mann 1994
Tolypella glomerata	SWGC, CANA, Mann 1994
Tolypella prolifera	SWGC, CANA, Mann 1994

* Also see V.T. Hammer communication in “Ancillary Charophyte Files”

Charophytes of Alberta

Chara aspera	CANA
Chara contraria	SWGC, CANA, NBV
Chara evoluta	Robinson 1906
Chara globularis (fragilis)	SWGC, CANA, NBV
Chara virgata (delicatula)	CANA
Chara vulgaris var. longibracteata	Allen 1951

Tolypella glomerata	CANA
Tolypella prolifera	SWGC, CANA

Charophytes of British Columbia*

Chara aculeolata (? possibly C. contraria var. hispidula)	CANA
Chara aspera	CANA
Chara braunii	SWGC, CANA, Allen 1951
Chara canescens	SWGC, CANA, Allen 1951
Chara contraria	NBV, Allen 1951
Chara evoluta	CANA, Allen 1951, NBV
Chara globularis (fragilis)	CANA, Allen 1951, NBV
Chara longifolia (buckellii)	SWGC, NBV, Allen 1951, Frame and Sawa 1975
Chara virgata (delicatula)	CANA, Allen 1951, NBV
Chara vulgaris (foetida)	NBV
Nitella clavata	CANA, Allen 1951
Nitella flexilis (monoecious)	SWGC, CANA, NBV, Allen 1951
Nitella flexilis (s.l.-sterile)	CANA
Nitella opaca (dioecious)	CANA, NBV
Tolypella prolifera	Allen 1951

*Refer to "Ancillary Charophyte Files" for Malcolm Martin correspondence regarding B.C. collections.

Charophytes of the Yukon

Chara aspera	CANA
Chara contraria	CANA, NBV
Chara globularis (fragilis)	CANA
Chara virgata (delicatula)	CANA, NBV
Nitella flexilis	DAO, Cody 1998
Nitella gracillis	NBV

Charophytes of the North West Territories (pre-Nunavut, e.g. before 1999)

Tolypella canadensis	SWGC, CANA, Langangen 1999
Tolypella prolifera	Sawa 1974
Chara globularis (fragilis)	CANA
Chara aspera var. macounii	CANA
Chara contraria	CANA

Chara virgata (delicatula)	CANA
Nitella flexilis (s.l.-sterile)	CANA

Charophytes of Alaska, U.S.A.

Nitella flexilis	NY
Nitella flexilis (s.l.-sterile)	CANA
Nitella opaca	CANA
Tolypella canadensis	CANA(6625), ALA, Langangen 1999

Summary List of Canadian Charophyte Species*

Chara aspera
Chara braunii
Chara canescens
Chara contraria
Chara evoluta
Chara foliolosa (zeylanica)
Chara globularis (fragilis)
Chara haitensis (zeylanica)
Chara keukensis
Chara longifolia (buckellii)
Chara virgata (delicatula)
Chara vulgaris (foetida)
Chara zeylanica
Nitella acuminata
Nitella clavata
Nitella confervacea
Nitella dilatata
Nitella flexilis (monoecious)
Nitella gracilis
Nitella hyalina
Nitella macounii
Nitella megacarpa
Nitella microcarpa
Nitella mucronata (furcata)
Nitella obtusa
Nitella opaca
Nitella spanioclema
Nitella tenuissima (transilis)
Nitellopsis obtusa
Tolypella canadensis
Tolypella glomerata (comosa/longicoma)
Tolypella intricata (intertexta/fimbriata)
Tolypella prolifera

*Some species are only known from a very few locations with few voucher specimens available.

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Appendix B: List of Labrador Charophytes

Following is a listing of Labrador charophyte collections compiled up to and including the year 2019. Very little is known about Labrador charophytes and no thorough surveys have been made. Only six species are reported to date and it is suspected that lakes, ponds, rivers and streams as well as limestone waters and brackish lagoons will contain a number of other species/varieties.

The following herbaria have been surveyed for Labrador specimens: **SWGC** = Grenfell Campus Herbarium, M.U.N., Corner Brook; **NFLD** = M.U.N. Herbarium, St. John's; **NFM** = Provincial Museum Division, The Rooms, St. John's**; CDFN** = Canadian Forest Service Atlantic, Forestry Center Botanical Hebarium, Corner Brook; **CANA** = Canadian Museum of Nature, Ottawa. No collections from Labrador are currently known to exist in **NFLD** or **CANA**.

The abbreviation s.l. (sensu lato = "in the broad sense") has been utilized in *Nitella flexilis* specimens when no gametangia are present. It is not possible to distinguish monoecious *N*. *flexilis* from dioecious *N. opaca* in specimens without gametangia. In earlier works these two species were often considered as variations of *N. flexilis*.

These six species have been reported as per the following collections:

Chara contraria A. Br. Ex Kutz.
Chara globularis Thuill. (= C. fragilis Desv.)
Chara virgata Kutz. (= C. delicatula Agardh)
Nitella confervacea (Breb.) A. Braun
Nitella flexilis (L.) Agardh
(*Nitella flexilis* (L.) Agardh (s.l.) – 8 collections without gametangia)
Nitella opaca Agardh

One of these species*, Nitella confervacea*, is only known from three sites in NL, two on the Island and one in Labrador which would position it in the rarity S Rank – 1. However, this is a miniature species, one of the smallest of the Nitellas and could easily be under collected. Globally it is a widespread cosmopolitan species. The other charophytes currently known from Labrador are all common cosmopolitan species.

The following individuals provided information used in the creation of this compilation:

Patricia Baines (CDFN)
Nathalie Djan-Chekar (NFM)
Claudia Hanel (NFM)
John Maunder (NFM)
Julissa Roncal (NFLD)

Compilation by Henry Mann (SWGC)

Labrador Charophytes

Collection Number: 238 Herbarium SWGC

Date: August 14, 1996

Species: *Nitella flexilis* (s.l.), lax, thin, elongate Nitella, once furcate, often unequally, apices acute, no gametangia present

Location: Pinware River, Labrador, Topo map 12P/10, Edition 2, WH 277323, river broad and shallow at this point

Details: fine brown sand substrate, material collected in about 1.0 meter of water. Dissolved solids = 16 umhos/cm (very dilute), pH ~ 7.0

Collectors: Henry Mann

Labrador Charophytes

Collection Number: 257 a Herbarium SWGC

Date: August 2, 1998

Species: *Nitella flexilis*, monoecious, once furcate, heavily covered with epiphytes

Location: Gosling Lake, Labrador, Topo Map 13F, Edition 2, PQ7324

Details: Sandy mud substrate, windswept shallow lake margins easily stirred by wave action. Collected in 0.75 meters of water.

Collectors: Henry Mann

Labrador Charophytes

Collection Number: 258 Herbarium SWGC

Date: August 2, 1998

Species: *Nitella flexilis* s.l., no gametangia, once furcate

Location: Topo Map Winokapau Lake 13E, Edition 3, NQ1104

Details: Pond south of TLH 202 Km west of Happy Valley, rocky/mud bottom, yellow water lilies main emergent. pH = 7.2, Conductivity = 10 umhos/cm (very low!)

Collectors: Henry Mann

Labrador Charophytes

Collection Number: 259a Herbarium SWGC

Date: August 4, 1998

Species: *Nitella flexilis* (s.l.), no gametangia

Location: Jean Lake, Wabush, Labrador, north end of lake, Topo Map Lac Opocopa 23B, Edition 3, FU4465

Details: collection depth 1 – 2 meters, sandy/mud substrate, emergents *Equisetum fluviatile*, *Potamogeton* sp. water pH = 7.5 - 8.0, conductivity 725 umhos/cm

Collectors: Henry Mann

Labrador Charophytes

Collection Number: 259b no material collected, but recorded in field.

Date: August 4, 1998

Species: *Chara virgata,* (=*C. delicatula*)

Location: Jean Lake, north end, Topo Map Lac Opocopa 23B, Edition 3, FU4465

Details: sandy /mud bottom, 1 – 2 meters of water, emergent vegetation *Equisetum fluviatile, Potamogeton* sp., pH = 7.5 – 8.0, conductivity = 725 umhos/cm

Collectors: Henry Mann

Labrador Charophytes

Collection Number: 260 Herbarium SWGC

Date: August 4, 1998

Species: *Nitella flexilis* (s.l.), with mature oospores

Location: 17 Km west of Lab City on TLH, = 0.5 Km east of Quebec/Labrador border, Topo Map Lac Opocopa 23B, Edition 3, FU2759

Details: pond with *Nitella* and *Isoetes*, yellow water lily emergents

Collectors: Henry Mann

Labrador Charophytes

Collection Number: 261 Herbarium SWGC

Date: August 5, 1998

Species: Nitella opaca, dioecious

Location: Esker Road, about 3 Km in on Esker Road from TLH junction.

Details: Gravel pit pond, shallow pond margins near gravel embankments, mature antheridia and oogonia present, no mature oospores seen

Collectors: Henry Mann

Labrador Charophytes

Collection Number: 262 Herbarium SWGC

Date: August 7, 1998

Species: *Nitella flexilis*, monoecious

Location: Churchill River Lagoon directly south of Happy Valley/Goose Bay, Topo Map Goose Bay 13F Edition 2, PQ7807

Details: specimens growing in tufts of *Vaucheria* sp. pH = 7.0, conductivity 20.0 umhos/cm

Collectors: Henry Mann

Labrador Charophytes

Collection Number: 263 Herbarium SWGC

Date: August 8, 1998

Species: Nitella flexilis (s.l.) non-fruiting material, once furcate

Location: Goose River backwater by bridge on road to Northwest River. Topo Map Goose Bay 13F, Edition 2, PQ7319

Details:

Collectors: Henry Mann

Labrador Charophytes

Collection Number: TH040805-21a (Labrador Straits Botanical Initiative) Herbarium NFM

Date: 5/8/2004 (D/M/Y)

Species: Chara globularis (=C. fragilis), non-fruiting material

Location: Labrador Straits, Forteau, ~1.5 km directly W of S end of community, on top of Forteau Hill, shore at east end of pond, ~ 350 m S of highway, UTM NAD 27, 21 501766E 5700839N

Details: wet open pond shore in limestone area, substrate 4 cm moss litter over well divided organic soil, over rocks

Collectors: T. Hart, T. Keats, B. Barney

Labrador Charophytes

Collection Number: TH040805-21b Herbarium NFM

Date: 5/8/2004 (D/M/Y)

Species: *Chara virgata* (= *C. delicatula*)

Location: Labrador Straits, Forteau, 1.5 km directly W of S end of community, on top of Forteau Hill, Shore at east end of pond, ~350m S of highway, UTM: NAD27 21 501766E 5700839N

Details: wet, open pond shore in limestone area; substrate 4 cm moss litter over well divided organic soil over rocks.

Collectors: T. Hart, T. Keats, B. Barney

Labrador Charophytes

Collection Number: TH040805-21c Herbarium NFM

Date: 5/8/2004 (D/M/Y)

Species: *Chara contraria* (fruiting, monoecious)

Location: Labrador Straits, Forteau, 1.5 km directly W of S end of community, on top of Forteau Hill, shore at east end of pond, ~350m S of highway, UTM: NAD 27 501766E 5700839N

Details: wet open pond shore in limestone area; substrate 4cm moss litter over well-divided organic soil, over rocks.

Collectors: T. Hart, T. Keats, B. Barney

Labrador Charophytes

Collection Number: CH 040801-1 Herbarium NFM

Date: 1/8/2004 (D/M/Y)

Species: *Nitella opaca* (fruiting, dioecious, only a few antheridia)

Location: Labrador Straits, Forteau Brook Valley, First Pond, beach on W shore. UTM: NAD 27 21 502200E 5705700N

Details: Washed upon beach, original rooting spot unknown

Collectors: C. Hanel

Labrador Charophytes

Collection Number: CH 040803-22 Herbarium NFM

Date: 3/8/2004 (D/M/Y)

Species: *Chara globularis* (= *C. fragilis*) non-fruiting material

Location: Labrador Straits, L'Anse au Loup, ~4km NW of S of highway, along Battery Trail, ~500m from trailhead UTM: NAD 27 21 514976E 5711036N

Details: shallow water near shore of pond, with very soft mud bottom and limestone slabs higher on shore; water level very low

Collectors: C. Hanel, T. Keats, R. Belbin

Labrador Charophytes

Collection Number: CH 040826-2 Herbarium NFM

Date: 26/8/2004 (D/M/Y)

Species: *Nitella flexilis* (s.l.) (non-fruiting material)

Location: Labrador Straits, Pinware Pond on headwaters of Pinware River, along inflowing brook, ~400m from the mouth of brook at NE corner of the pond, UTM: NAD: 27 21 532097E 5778482N

Details: In shallow water of brook where it is wide and slow; substrate sandy to muddy

Collectors: C. Hanel, T. Keats, R. Belbin, T. Hart

Labrador Charophytes

Collection Number: TH 040802-14 Herbarium NFM

Date: 2/8/2004 (D/M/Y)

Species: *Chara contraria* (fruiting, monoecious)

Location: Labrador Straits, L'Anse au Clair, ~1 km W of community on top of plateau, E shore of pond which is the water supply for L'Anse au Clair, ATV trail 2m above water level, UTM: NAD 27 21 494151E 5698025N

Details: disturbed ATV trail in tall meadow/tuckamore dominated by small shrubs, along shore of pond, substrate a moist 3-4 cm thick dark organic horizon over 3-4 cm light brown sandy clay

Collectors: T. Hart, T. Keats, M. Flynn

Labrador Charophytes

Collection Number: 38 Herbarium SWGC

Date: 13/07/2001

Species: Fragments of encrusted dead material, *Chara virgata* and *Chara contraria*

Location: Seaplane Lake, East of L'Anse au Clair, NAD 83 500201 5700321

Details: muddy limestone/sandstone shores

Collectors: John Maunder

Labrador Charophytes

Collection Number: 128 Herbarium NFM

Date: 09/08/97 (D/M/Y)

Species: *Chara globularis* (= *C. fragilis*)

Location: Beverly Lake, Labrador City, Labrador, just NW of main stop lights

Details: sandy bottom

Collectors: John Maunder

Labrador Charophytes

Collection Number: 2681 and 2686 Herbarium CDFN

Date: September 5, 2000

Species: Nitella flexilis (s.l.)

Location: Southwest Brook, Labrador, Route 516, at TLH bridge crossing, shallow shore pool, 25 meters downstream from bridge

Details: Lax sterile plants in muddy substrate

Collectors: Patricia Baines

Labrador Charophytes

Collection Number: 2687 Herbarium CDFN

Date: September 5, 2000

Species: Nitella flexilis (s.l.)

Location: Southwest Brook, Labrador, Route 516, at TLH bridge, upstream on the southwestern side of brook, slow moving water 1 meter from shore

Details: Lax sterile plants, no gametangia

Collectors: Patricia Baines

Labrador Charophytes

Collection Number: 257 b Herbarium SWGC

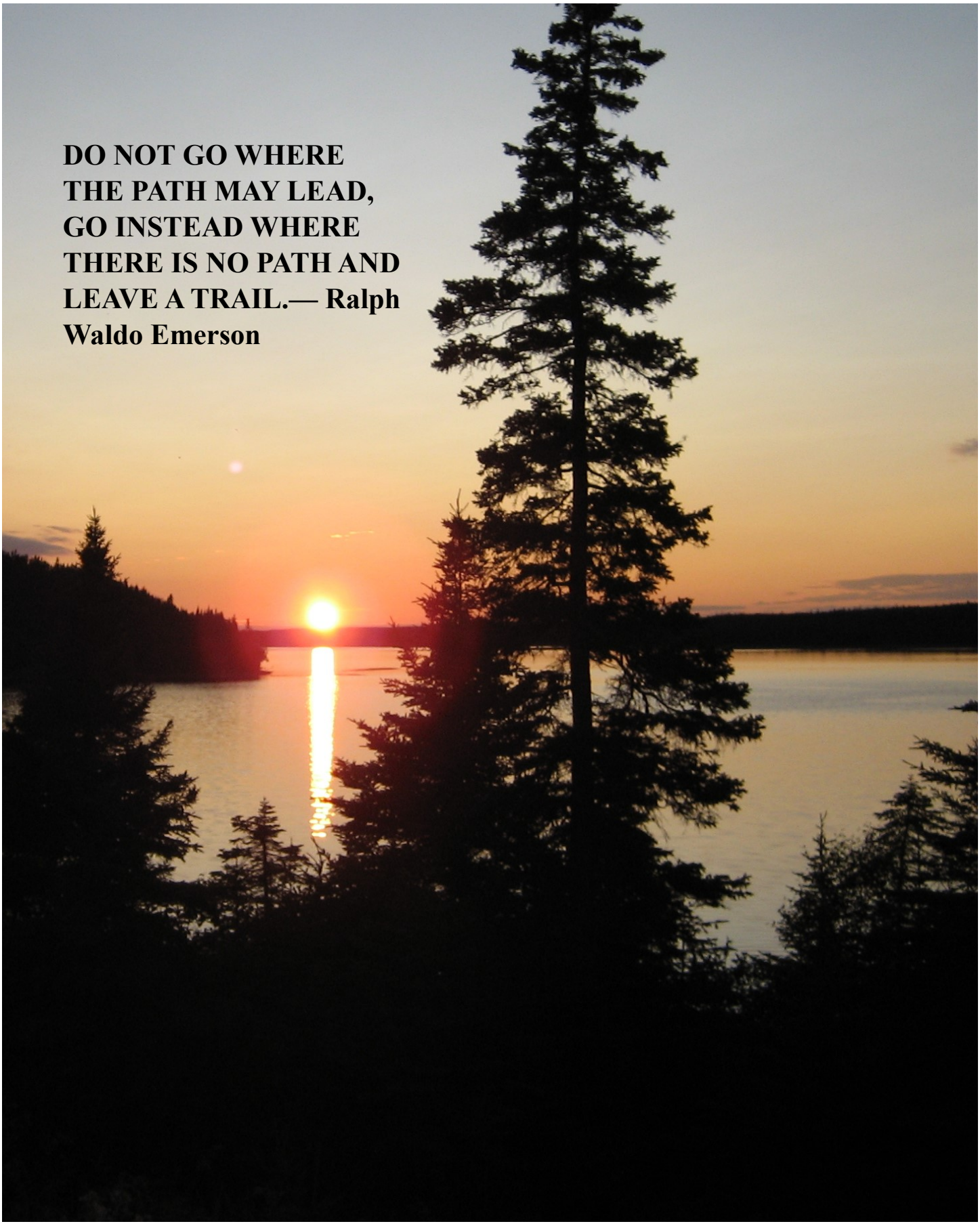
Date: August 2, 1998

Species: Nitella confervacea, monoecious

Location: Gosling Lake, Labrador, Topo Map Goose Bay 13F, Edition 2, PQ7324

Details: Shallow, less than 1 meter depth, sandy, muddy shoreline, various aquatic vascular plants present. Plants with mature antheridia and oogonia, mature oospores not seen, dactyls two–celled, tiny acute end cell, branchlets twice furcate, gametangia mainly at lowest branchlet furcation, plants miniature/tiny, only 1-2 cm.

Collectors: Henry Mann


DO NOT GO WHERE
THE PATH MAY LEAD,
GO INSTEAD WHERE
THERE IS NO PATH AND
LEAVE A TRAIL.— Ralph
Waldo Emerson

Through photographs, illustrations, and descriptions, students and nature lovers can enter the hidden world of Charophytes, aquatic "plants" which inhabit the fresh and brackish waters across Newfoundland and Labrador, as well as around the globe.

NEWFOUNDLAND CHAROPHYTES
S.W.G.C.