Mary J. Beilby · Michelle T. Casanova

The Physiology of Characean Cells

Springer

The Physiology of Characean Cells

Mary J. Beilby • Michelle T. Casanova

The Physiology of Characean Cells

Springer

Mary J. Beilby
School of Physics
The University of New South Wales
Sydney
New South Wales
Australia

Michelle T. Casanova
Centre for Environmental Management
University of Ballarat
Mt Helen
Victoria
Australia

Royal Botanic Gardens
Melbourne
Australia

ISBN 978-3-642-40287-6 ISBN 978-3-642-40288-3 (eBook)
DOI 10.1007/978-3-642-40288-3
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2013951109

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Dedicated to Alan Walker (1929 – 2013),
who inspired and helped to shape this book

Preface

When I was selecting my Honours project in 1970, Biophysics was my second choice after Solid State Physics. Looking back, this was a key decision in my life, which would have turned out very differently, if I got my first choice.

My Honours project was not particularly successful. I was trying to measure *Chara* membrane impedance with a bridge, which was far to slow to get meaningful results. John Smith took over the project and computerised the measurements and data collection (one of the first such experiments in Australia). Impedance measurements later became the main theme of Hans Coster's group (School of Physics, University of NSW). However, I did enjoy my Honours work and decided to do my Ph.D. on *Chara* action potential (AP). I applied the Nobel-Prize winning Hodgkin–Huxley equations that describe the squid axon AP to *Chara* excitation. My 6 years with Hans and other members of the group, John Smith, Terry Chillcott, David Bell, Bob Aschcroft and others, taught me practical basis of electrophysiology. I could make up circuits for voltage clamp and current sources, eliminate earth loops and electrical noise, match impedance of circuits and manufacture microelectrodes. The *Chara* membrane itself was seen as a region of fixed charges to be modelled by a circuit. The *Chara* AP was an interesting phenomenon to be explained, but we did not ponder what use it might be to the plant.

During my Ph. D. work, I did a lot of modelling of the experimental data. I experienced great satisfaction, when the models "fitted" and gave predictive insights into the process. On the other hand, I have also become aware of limitations of models and experimental and theoretical artefacts.

I got my first postdoctoral job by word of mouth with Alan Walker, School of Biological Sciences, Sydney University. I started getting more education in biology. We worked on amine transport and chloride/proton symporter, the former important in plant nutrition and the latter in turgor maintenance and salt tolerance. This was a great time to meet many important workers in electrophysiology and plant physiology (and future close associates): AB Hope, Geoff Findlay, FA Smith, Rob Reid, Mary Bisson, Steve Tyerman, Gunter Kirst, John Cram, and Tony Larkum.

My next postdoctoral job was with Enid MacRobbie at School of Botany, University of Cambridge, UK. I have learned in my Honours year that computer-controlled data logging can be a very powerful technique. I enlisted my husband Bruce's help and we built our first computer-controlled electrophysiology set-up.

The high speed (for plants) of current–voltage (*I*/*V*) scans allowed us to record the sigmoidal *Chara* proton pump *I*/*V* characteristics, as well as the large conductance K^+ channel *I*/*V* profile with typical negative conductance regions. We also tackled the tonoplast electrical characteristics, using the permeabilisation technique. These were really interesting years, meeting with new colleagues John Cork, Teruo Shimmen, Mike Blatt and Mark Tester. Our daughter Kiri was born in 1984.

After 6 years in Cambridge I have returned to Alan Walker's group at Sydney University. Bruce and I have built a second version of our computer-controlled experimental set-up. I have worked on Na^+/K^+ transport with Alan Walker and Stephen McCulloch and mastered cell compartment manipulation with Virginia Shepherd and perfusion (especially after my trip to Japan visiting Teruo Shimmen and Tetsuro Mimura). Together with Alan Walker we further developed modelling of the *I*/*V* characteristics of both intact and modified cells. I joined Mary Bisson to investigate the high pH state, involving H^+/OH^- channels.

In 1992 I became a lecturer at School of Physics, University of NSW, Sydney. I have become interested in salt tolerance and sensitivity and compared the response to increased osmolarity and salinity of salt-sensitive *Chara australis* and very salt-tolerant *Lamprothamnium* sp. My group (Virginia Shepherd, later Alan Walker and Sabah Al Khazaaly) uses computer-controlled electrophysiological set-up, cell compartment modification and rigorous modelling of the data. Working with *Lamprothamnium*, we have characterised Cl^- and K^+ channels in the hypotonic turgor regulation. We were the first to document the involvement of extracellular sulphated polysaccharide mucilage in ion transport and salt tolerance. In the hypertonic turgor adjustment the proton pump is activated by both decrease in turgor and increase in Na^+ concentration. In contrast, the proton pump in *Chara* is inactivated by increase in Na^+ concentration, cell undergoes spontaneous APs and putative activation of H^+/OH^- channels erodes the proton electromotive force needed to expel sodium from the cytoplasm.

With Mary Bisson and Virginia Shepherd I also started to work on sea algae *Ventricaria* (*Valonia*) *ventricosa* from the Chlorophyta branch of the phylogenetic tree. The enormous contrast in the Characeae and Valoniceae electrophysiology underlines the similarity of Characeae and higher plants and their value as simplified, easy-to-manipulate model.

In the last decade I joined the International Research Group on Charophytes and Plant Signaling and Behaviour society, completing my evolution from seeing the cell membrane just as a circuit, to appreciating the beauty and complexity of the whole Characeae cells and plants and their survival strategies. The first chapter of the book by Michelle Casanova introduces the morphology, systematics and ecology of the Characeae. If I only had this chapter when I started my work on Characeae! The Chaps. 2 and 3 summarise electrophysiology and transport in single cells in steady state and under stress. Chapter 4 shows that specialised cells, joined cells and whole Characeae plants also provide excellent model systems. The book is aimed at research students and researchers who want to use the Characeae system. It will also be useful for electrophysiologists working on higher plants.

I want to thank all my colleagues for teaching me so much and for enthusiastic collaborations, my husband Bruce for providing such great computer set-up, and my daughter for putting up with me, while preoccupied with research. I would like to dedicate this book to my three mentors: Hans Coster, Alan Walker and Enid MacRobbie.

Sydney, Australia Mary J. Beilby

Foreword

Early in life I decided I wanted to be a biologist, but I was a little fearful that all the questions would be answered, and problems solved, before I became capable of working in science myself. Now, of course, I realise that the more you find out, the more questions you have. Humanity would have to change before there was an end to enquiry.

I remember the first charophyte I found, in Llangothlin Lagoon, *Chara australis*. Finding it made me ask questions about what charophytes were doing in Australian systems and how their ecology might differ from the ecological dogma generated in Northern Hemisphere studies.

I surveyed the Northern Tablelands of New South Wales for charophytes, worked on their taxonomy (simply to be able to determine how many species we had), and undertook experimental ecology to write my Ph.D. thesis under Margaret Brock. During that time I was able to visit Vernon Proctor in Texas and started to understand some of the basic problems and potentials of research into family Characeae. On that visit to USA, I met European charophyte palaeontologists Monique Feist and Carles Martin-Closas, taxonomist Henry Mann and geneticists Ken Karol and Rick McCourt, people who were to have a role in my continued work on Characeae. Attendance at the International Phycological Congress in Melbourne introduced me to David John and his work on charophyte oospores, a strand of research that I continue to this day.

During my Ph.D. studies I worked as a tutor in Botany, learning more about plant identification, morphology, botanical history and communication by teaching first and second year botany students and trying to engender enthusiasm and passion for plant diversity and morphology. I honed my drawing and communication skills by teaching and communicating to all levels of society, within and outside of the university, from landcare groups to specialised conference audiences. Ian Parbery (fungal taxonomy) and Wal Whalley (grass taxonomy) were my supervisors while Margaret was on study leave, so little surprise that taxonomic questions became part of my vocabulary.

Postdoctoral employment on ecological projects on biomanipulation (the relationship between water-plant and algal abundance in shallow lakes), farm dams and the process of plant establishment in wetlands led to a series of publications on water plant ecology, but I managed to retain some study of charophytes in all these projects.

Taxonomic projects continue to the present day, since the need for a good taxonomy underpins all other research on charophytes. Grants from the Australian Biological Resources Study and the "Bush Blitz" program have funded the work. Visits to herbaria in Sweden, Germany, the Netherlands, Austria, Hungary, France and the UK in 2012 allowed me to solidify species concepts and typification in the Characeae. Sampling trips all over Australia (avoiding crocodiles in the Northern Territory, following wombat trails in the Coorong, and dodging snakes and kangaroos from Western Australia to coastal New South Wales) have allowed my treatment on the Australian species to be somewhat comprehensive. Who said taxonomy was boring?

My current lifestyle revolves around farming, writing about charophyte taxonomy and ecological research on wetlands. I also find time to contribute to the management and conservation of charophytes and their habitats by participating in local community, government and non-government management groups. Volunteer work to nominate charophytes and their habitats as "endangered" under the Ecological Protection and Biodiversity Conservation Act has resulted in conservation listing that should protect charophyte diversity in the future, even in a changing climate. Living on the farm and in a farming community engenders a practical perspective and gives visiting charophyte experts a different experience of Australian charophyte research.

I am currently employed by the Royal Botanic Gardens Melbourne as a botanist and by Ballarat University as a wetland hydro-ecologist. I hope this book will enthuse more people and give them the basic knowledge to answer their own questions about charophytes.

My efforts here are dedicated to my husband Anthony Casanova and son Robert, who know more about charophytes than they ever really wanted to, and in memory of my grandmothers Sylvester Mary Davis and Helen Katherine Bryant Atkinson.

Mt Helen, Australia Michelle T. Casanova

List of Symbols and Acronyms

dV/dt	Initial volume flow in transcellular osmosis experiment (Eq. 2.11)
$[X]_o$, $[X]_i$	Concentrations of transported ion X outside and inside (e.g. $[K^+]_o$)
6CF	6-Carboxyfluorescein
A	Area exposed to each medium in a symmetrical arrangement
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
Amt/Mep/Rh	Proteins constituting the ammonium transporter family
AP	Action potential
APW	Artificial pond water
ATP	Adenosine triphosphate
ATPase	Pump powered by ATP
AZ	Acetazolamide
BX	Bromoxynil
C_9	Nonyltriethylammonium
CCCP	Carbonyl cyanide m-chlorophenylhydrazone
CHL1	First NRT1 gene identified
DAG	Diacylglycerol
DCCD	Dicyclohexylcarbodiimide
DCMU	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
DCPIP	2,6-Dichlorophenolindophenol
DES	Diethyl stilbestrol
DFCM	Double fixed charge membrane model
DIC	Dissolved inorganic carbon
DMO	5,5-Dimethyloxazolidine-2,4-dione
DNA	Deoxyribonucleic acid
DNP	2,4-Dinitrophenol
EDAC	1–Ethyl -3-(3-dimethylamino-propyl) carbodiimide
EGTA	Ethylene glycol tetraacetic acid
E_j	Nernst potential of ion j
ER	Endoplasmic reticulum
EZA	Ethoxyzolamide

F	Faraday constant: 96,485.33 C/mol
F	Fluorescence yield
F'_m	Maximum fluorescence yield
FITC	Fluorescein isothiocyanate
G_{bkg}	Background conductance
GFP	Green fluorescent protein
GHK	Goldman, Hodgkin, Katz model
H^+/OH^- state	H^+or OH^- channels are the dominant transporter
HGSS	Hansen, Gradmann, Sanders, Slayman model for the proton pump
HMM	Heavy mero-myosin
HK	Hexokinase
I	Current density
IAA	Indole-3-acetic acid
IAc	Iodoacetamide
IP_3	Inositol-1,4,5,-triphosphate
IP_6	Inositol-1,2,3,4,5,6-hexakisphosphate
$I_{symport}$	Symport current (eqn. 2.10)
I_X	Current due to ion X
J_i	Flux of ion i
K^+ state	K^+ channels are the dominant transporter
k_1–k_4	Rate parameters in the symport model
k_{io} and k_{oi}	Voltage dependent rate constants in HGSS model
K_m	Michaelis–Menten parameter
Lp	Hydraulic conductivity
ME	2-Mercaptoethanol
MIFE	Microelectrode ion flux estimation
MIP	Major intrinsic protein
mRNA	Messenger RNA
MSX	L-methionine-D, L-sulphoximine
N	Scaling factor in HGSS model: 2×10^{-8}
NAXT	Nitrate exporter
NEM	*N*-ethyl maleimide
NMR	Nuclear magnetic resonance
NPA	1-*N*-naphthylphthalamic acid
NPQ	Non-photochemical quenching
NRT1, NRT2	Subgroups of plant nitrate transporter
N_xP_x	Number of X ion channels times their permeability
PAT	Polar auxin transport
pCMBS	p-chloromercuribenzenesulfonate
pCMPS	p-chloromercuriphenysulfonate
PD	Potential difference
PEG	Polyethylene glycol
pH_c	Cytoplasmic pH
pH_v	Vacuolar pH

Pi	Inorganic phosphate
PIP	Plasma membrane intrinsic protein
PIP_2	Phosphatidylinositol 4,5-biphosphate
pK_a	Negative base-10 logarithm of the acid dissociation constant of a solution
PLC	Phospholipase C
P_{o+}, P_{o-}	Boltzmann distributions of open probabilities
PPase	Pump powered by pyrophosphate
PPi	Pyrophosphate
R	Universal gas constant: 8.314 JK^{-1} mol
R_a, R_b, R_n	Resistances of cell a, cell b and the node between them
RGDS	Arg-Gly-Asp-Ser peptide
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPD	Receptor potential
SH	Sulfhydryl group
SHAM	Salicylhydroxamic acid
SW	Seawater
T	Temperature in kelvin
TEA	Tetraethylammonium
TIP	Tonoplast intrinsic protein
UV	Ultraviolet
V	Membrane PD
V_{50+}, V_{50-}	Half activation potentials
V_m	Michaelis–Menten parameter
VP	Variation potential
Y'	Quantum yield
YIGSR	Tyr-Ile-Gly-Ser-Arg pentapeptide
z_g	Number of gating charges
z_j	Valency of ion j
γ_l, γ_p	Fractional areas of lipid and protein pathway (Eq. 2.12)
$\Delta\mu_{Cl}$	Electrochemical chloride ion gradient
$\Delta\mu_H$	Electrochemical proton gradient
$\Delta\pi_0$	Difference in osmotic potential between the two chambers (Eq. 2.11)
κ_{io} and κ_{oi}	Voltage independent rate constants in HGSS model
σ_l, σ_p	Reflection coefficients in water transport in lipid and protein pathway (Eq. 2.12)
ϵ	The elastic modulus of the cell wall

Contents

1 The Characean Plant 1
1.1 General Morphology 1
1.2 Morphology of Individual Species 7
1.2.1 The *Chara corallina/australis* Group of Species 7
1.2.2 The *Lamprothamnium papulosum/macropogon* Group of Species 9
1.2.3 The *Lamprothamnium succinctum* Group of Species 11
1.2.4 *Lamprothamnium inflatum* 11
1.2.5 Species of *Tolypella* 14
1.2.6 Species of *Nitellopsis* 14
1.2.7 Species of *Nitella* Suitable for Physiological Studies 16
1.3 Cellular Structures and Processes 18
1.3.1 Cells 18
1.3.2 Gametangia 19
1.3.3 The Oospore 20
1.3.4 The Germinating Oospore 22
1.4 Characean Names 22
1.4.1 Systematics and the Concept of Species 23
1.4.2 Individual Species' Stories 25
1.4.3 Lessons to be Learned 28
1.5 Ecology 28
1.5.1 Distribution and Abundance 28
1.5.2 Processes Controlling Characean Distribution 29
1.5.3 Ecological Role of Characean Plants 30
1.5.4 Abiotic Environmental Factors 31
1.5.5 Culturing Characean Plants 34
1.5.6 Life History 35
1.5.7 Population Ecology 35
1.5.8 Application of Ecological Knowledge 37
References 37

2 Detached Cells in Steady State: Electrophysiology and Transport . . . 43
2.1 Introduction 44
2.2 Cell Compartments and Their Modification 50

2.2.1 Cell Wall and Extracellular Mucilage 50
2.2.2 Cytoplasm 52
2.2.3 Vacuole 57
2.3 Plasma Membrane PD 58
2.3.1 Pump State 58
2.3.2 Background State 60
2.3.3 K^+ State 66
2.3.4 H^+/OH^- State 68
2.3.5 Temporal Interaction Between the States 71
2.4 Spatial Interaction Between States: "pH Banding" 72
2.4.1 Carbon Supply for Photosynthesis 74
2.4.2 Charasomes 75
2.4.3 Circulating Currents 77
2.4.4 Establishment and Collapse of Banding 77
2.4.5 Interaction with Cytoplasmic Streaming and AP 80
2.5 Other Plasma Membrane Transporters 82
2.5.1 Cl^- Transport 82
2.5.2 Nitrogen Transport 83
2.5.3 Phosphate Transport 89
2.5.4 Na^+/K^+ Transport 92
2.5.5 Water Transport 94
2.6 Tonoplast PD and Transporters 97
2.6.1 Introduction 97
2.6.2 Permeabilised and Perfused Cells 98
2.6.3 Cytoplasmic Droplets 100
References 105

3 Electrophysiology of the Detached Cell Under Stress 115
3.1 Introduction 116
3.2 Mechanical Stress or Electrical Stress 118
3.2.1 Receptor Potential Differences (RPDs) 118
3.2.2 Action Potentials 124
3.2.3 Voltage Clamp to Negative Levels 130
3.2.4 Wound (Death) Response 132
3.2.5 Testing of Herbicides 137
3.3 Osmotic and/or Saline Stress 137
3.3.1 Introduction 137
3.3.2 Electrophysiology of Hypertosmotic Regulation in Salt-Tolerant Characean Cells 140
3.3.3 Hypotonic Regulation in Salt-Tolerant Characean Cells 145
3.3.4 Osmotic and Salt Stress in Salt-Sensitive Characean Cells 148
3.3.5 Na^+ Fluxes at the Time of Salt Stress 151
3.3.6 Control of Water Transport by Osmolarity 152
References 156

4 The Whole Plant and Cell-to-Cell Transport 165
4.1 Introduction 166
4.2 Cytoplasmic Streaming 167
4.2.1 Characean Cell Started the Research into Streaming Mechanism 167
4.2.2 Actin and Myosin in Characeae 168
4.2.3 Some Factors Affecting Streaming 171
4.2.4 Calcium Concentration and Action Potential 173
4.3 Cell-to-Cell Transport 175
4.3.1 Structure of the Nodal Complex and Plasmodesmata 175
4.3.2 Transport and Its Controlling Factors 178
4.3.3 IAA Transport 186
4.4 Gravitropism 186
4.4.1 Cytoplasmic Streaming 186
4.4.2 Rhizoids and Protonemata 187
4.5 Cell Walls 189
4.6 Characeae in Phytoremediation 190
References 191

Appendix 195

Index 197

The Characean Plant

1

Abstract

The aim of this chapter is to give physiologists a thorough grounding in the morphology, taxonomy and ecology of the characean plant. The morphology of characean plants is depicted and explained, with specific examples of the morphological characteristics of different species or species groups that are used in physiological studies. The details of characean cellular structure in growing plants and in the reproductive organs are reviewed. The history of taxonomy and nomenclature is outlined, along with the most recent approaches to systematics (and what name to use for characean plants in physiological studies), and finally the patterns of characean plant distribution and requirements for growth in natural situations are explained and related to the culture and growth of characean plants for physiological studies.

1.1 General Morphology

The characean thallus (or plant body) is similar in appearance and size to the plant body of other submerged plants such as *Ceratophyllum* or *Myriophyllum*. Characean plants consist of long photosynthetic stem-like structures (axes) anchored in the soil, with whorls of leaf-like organs (branchlets) along the stem (Fig. 1.1a). Close examination reveals that the structure of characean plants is very different to that of flowering plants. Instead of roots they have colourless rhizoids, instead of leaves they have whorls of branchlets of limited growth, instead of stems they have an axis of giant cells joined end on end and instead of flowers and fruit they have relatively simple reproductive structures (the oogonium and antheridium, Fig. 1.1b) that produce gametes. The product of fertilisation of the gametes is an oospore (Fig. 1.1c) rather than a seed.

The thallus of characean plants is essentially filamentous. The axes (stems) are made up of long, multinucleate, single cells interrupted by multicellular nodes (Fig. 1.2). There is no development of tissues such as parenchyma, although the axial nodes approach such an arrangement (Sect. 4.3). Several organs of limited

M.J. Beilby and M.T. Casanova, *The Physiology of Characean Cells*,
DOI 10.1007/978-3-642-40288-3_1,

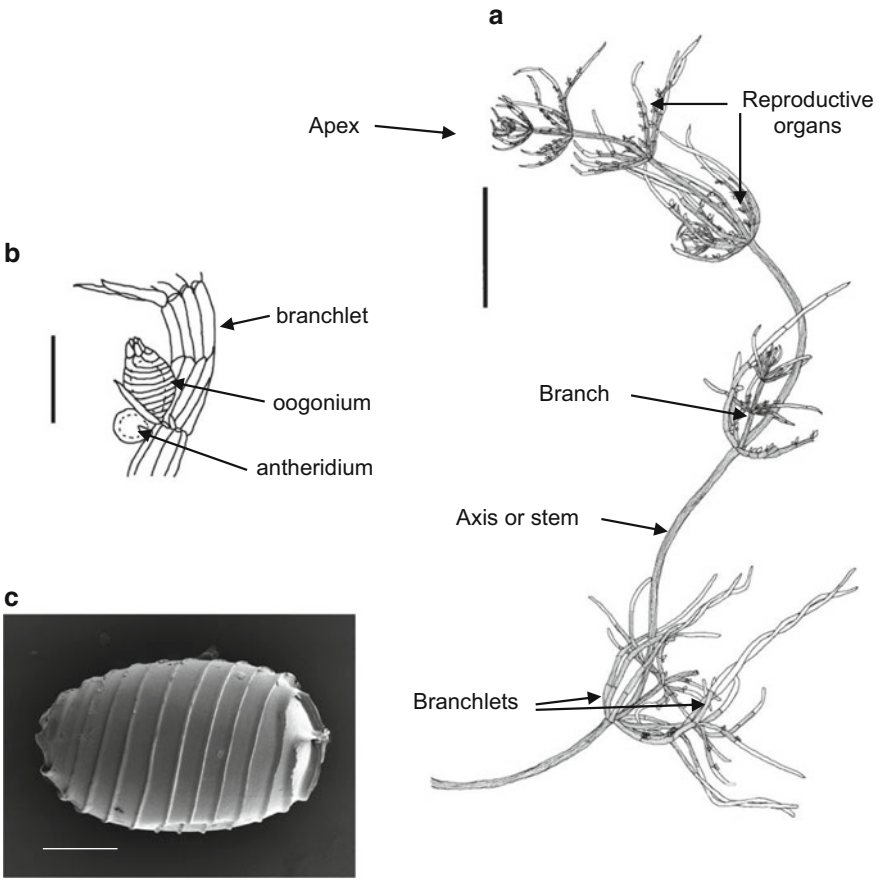

Fig. 1.1 Thallus of a characean plant (*Chara* sp. r862). (**a**) Stem showing whorls of branchlets, scale bar = 10 mm. (**b**) Reproductive organs, scale bar = 1 mm. (**c**) Scanning electron micrograph of an oospore of *Chara* sp. (r862), scale bar = 200 μm

growth (branchlets, stipulodes and cortical filaments) arise in whorls at the nodes (Fig. 1.2). Branchlets are the "leaf-like" organs that occur in spreading whorls, and below these there are often whorls of smaller cells called stipulodes. In many species of *Chara*, the stipulodes occur in two whorls, the upper whorl pointing upwards and the lower whorl pointing downwards (Fig. 1.2a, b). In the genera *Lamprothamnium* and *Tolypella*, and some species of *Chara*, the axial node can also be the site of gametangial development (Fig. 1.2c).

Branchlet arrangement (Fig. 1.3) and morphology (Fig. 1.4) varies among the genera but is characterised by elongate multinucleate cells interrupted by multicellular branchlet nodes. Other cellular structures can be produced at the branchlet nodes, namely bract cells (Figs. 1.3a, b, and 1.4a, b), secondary and tertiary (*et seq.*) branchlet segments or rays (Figs. 1.3c, d, and 1.4c, d), cortical filaments (Fig. 1.4a) and gametangial initials (Fig. 1.4). Some species of *Chara* have elongate bract cells

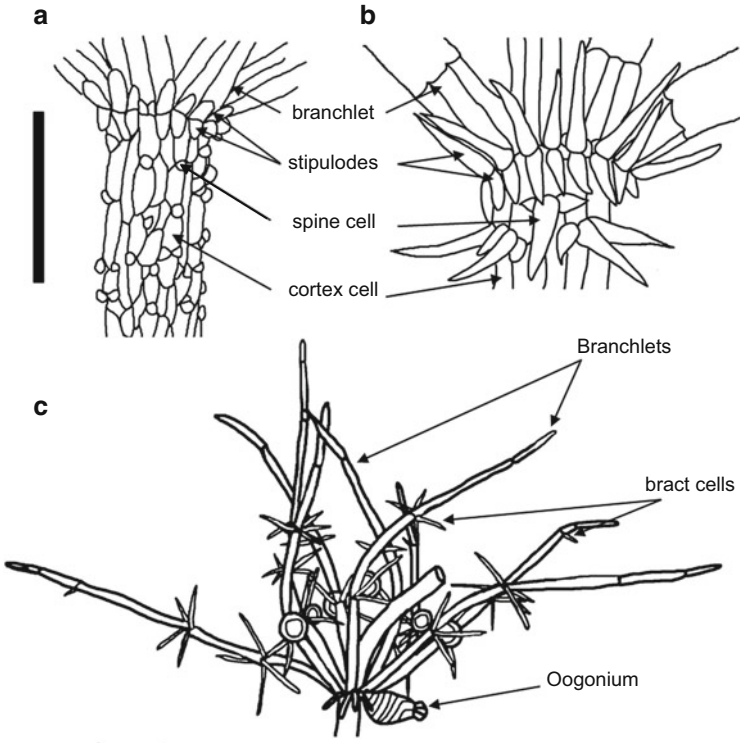

Fig. 1.2 Characean axial nodes. Scale bar = 1 mm. (**a**) *Chara* sp. (r822) node with two rows of stipulodes, short cortical cells and small spine cells. (**b**) *Chara canescens* (r020) node with longer stipulodes and spines cells. (**c**) *Lamprothamnium* sp. (r870) with no cortication, oogonium and stipulodes below the base of the whorl, and spreading bract cells at the branchlet nodes

in whorls (verticillate) at the branchlet nodes, as well as at the apices of the branchlets (Fig. 1.3a). Other species produce unilateral bract cells (Fig. 1.4a). *Lamprothamnium* exhibits the same overall branchlet morphology as *Chara*, but the bract cells are generally inserted at angles of 45–90° to the branchlet, forming a "cage-like" structure around the nodal complexes (Figs. 1.3b and 1.4b). *Nitella* species have branchlets that are divided or forked (furcate) into separate rays or segments (Fig. 1.4c), which can be very evenly arranged (Fig. 1.3d) or irregular (Fig. 1.3c). Some species of *Nitella* can produce more than one whorl of branchlets at the nodal complexes, a condition referred to as "heteroclemous" (Fig. 1.3d). *Tolypella* branchlets are different from those in other genera, usually with a central pluricellulate ray, secondary rays and clusters of gametangia (Fig. 1.4d).

The gametangial initial cell produces the gametangia (oogonia and/or antheridia), bracteoles and sometimes a bractlet (Fig. 1.5). Oogonia have a striped appearance with a small but distinctive crown of cells (coronula) at their apex. Oogonia vary in colour from green to bright orange, and in older parts of the thallus the fertilised oospore within the oogonium becomes darker as it matures. The antheridia can also be green, but are often orange to red in colour, and in dioecious