The Choanoflagellates

Evolution, Biology and Ecology

Choanoflagellates have three distinctive claims to fame: they are the closest, living, unicellular relatives of animals; they are a major component of aquatic microbial foodwebs; and one group is remarkable for its siliceous basket-like coverings.

This landmark book offers a unique synthesis of over 40 years of choanoflagellate research. Key areas are covered, from the phylogenetic evidence supporting the sister-group relationship between choanoflagellates and Metazoa, to choanoflagellate distribution and diversity in marine and freshwater environments. The structure and assembly of choanoflagellate loricae is also presented together with a full discussion of a novel example of 'regulatory evolution', suggesting that the switch from nudiform to tectiform cell division and lorica production was achieved by a sudden reorganisation of existing structures and mechanisms.

Providing an authoritative summary of what is currently known about choanoflagellates, this title will serve as a foundation upon which future research and discussion can take place.

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CAMBRIDGE
UNIVERSITY PRESS

Shaftesbury Road, Cambridge CB2 8EA, United Kingdom

One Liberty Plaza, 20th Floor, New York, NY 10006, USA

477 Williamstown Road, Port Melbourne, VIC 3207, Australia

314–321, 3rd Floor, Plot 3, Splendor Forum, Jasola District Centre, New Delhi – 110025, India

103 Penang Road, #05–06/07, Visioncrest Commercial, Singapore 238467

Cambridge University Press is part of Cambridge University Press & Assessment, a department of the University of Cambridge.

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www.cambridge.org
Information on this title: www.cambridge.org/9780521884440

First published 2015

A catalogue record for this publication is available from the British Library

Library of Congress Cataloging-in-Publication data
Leadbeater, Barry S. C.
The choanoflagellates : evolution, biology, and ecology / Barry S. C. Leadbeater, University of Birmingham, UK.
pages cm.
Includes bibliographical references and index.
1. Choanoflagellates. 2. Protozoa. I. Title.
QL368.C5L43 2014
579.4–dc23 2014022201

ISBN 978-0-521-88444-0 Hardback

Contents

Preface

Choanoflagellates have enjoyed an unusual degree of celebrity since Henry James-Clark (in 1867) first showed that they bore a remarkable morphological resemblance to the choanocytes of a sponge. This similarity gave rise to the hypothesis that sponges, and, by implication, other animals, evolved from a choanoflagellate-like ancestor. For a while it appeared that colonial choanoflagellates might be a missing link between unicellular protozoa and animals. However, it is only in the last 20 years that molecular phylogenetic studies have provided substantive support for choanoflagellates being the sister group to all Metazoa, which makes them the closest, known, living, unicellular relatives of animals.

Ecologically, choanoflagellates have also enjoyed an important, if less high-profile, reputation. They are universally distributed in aquatic habitats and are a major contributor to aquatic microbial foodwebs (the microbial loop). What appears at first sight to be a rather uniform and unadventurous protistan group turns out to be a diverse and fascinating collection of organisms. In particular, variations in lorica morphology have led to the extensive distribution and diversification of loricate choanoflagellates in the oceans of the world. Detailed ultrastructural studies, particularly on lorica construction and assembly, have also revealed many novel features of cell biological interest.

It can now be said with confidence that choanoflagellates have come of age. Recent molecular studies have rekindled their fashionable status and the time has come to consolidate an extensive body of literature into book format. This is a particularly good time at which to undertake the task since, for the moment at least, the various disciplines can still be treated as a coherent whole. In the future it is likely that the subject will fragment as individual disciplines become ever more detailed and specialised. A book on choanoflagellates at this juncture will not only provide a summary of knowledge to date, but will also serve as a foundation upon which future research and discussion can take place.

The three disciplines on which this book is based have different historical backgrounds. Cell biology has grown out of traditional light microscopy dating back to the nineteenth century. The advent of electron microscopy permitted more detailed investigations of lorica morphology and the intricacies of lorica production and assembly. Ecological studies are also rooted in the descriptive work of the nineteenth and early twentieth centuries, but have been greatly augmented by quantitative and, more recently, molecular investigations. Molecular and evolutionary studies, although having their roots in the late twentieth century, are essentially a feature of the twenty-first century. Choanoflagellates, as a member of the supergroup Opisthokonta, have fared particularly well in phylogenetic analyses, being regularly recovered as the monophyletic sister group to Metazoa. Purely molecular studies, particularly those based on entire genomes, are still in their infancy.

This book has two basic aims. The first is to provide an authoritative summary of what is currently known about choanoflagellates (Chapters 1, 2, 3, 9 and 10). The second is to provide a detailed account of loricate choanoflagellates with particular reference to their requirement for silicon and the production and assembly of the basket-like lorica (Chapters 4–8). This includes a full discussion for the first time of a novel example of 'regulatory evolution', whereby it is suggested that the switch from nudiform to tectiform cell division and lorica production was achieved by a sudden reorganisation of existing structures and mechanisms rather than the emergence of new structures and mechanisms.

Serendipity has played a large part both in my research of choanoflagellates and in writing this book. My first encounter with choanoflagellates was accidental. I was a phycologist working on calcified nanoplankton when, by fortune, on a field excursion to Norway in 1970, I netted loricate choanoflagellates instead of coccolithophorids. In spite of treating choanoflagellates as a hobby I subsequently managed to isolate several species in culture and from that time on I was hooked. The writing of this book has also been dependent on good fortune. First, in receiving financial assistance from the Leverhulme Trust for an Emeritus Fellowship, and second for the generosity of the School of Biosciences, University of Birmingham in providing office and laboratory facilities long after I had retired from active teaching.

Since my interest in choanoflagellates began over 40 years ago, there are many colleagues and past students worldwide whom I wish to thank for assistance, advice, collaboration and hospitality. I thank John Dodge for having first introduced me to flagellates during my postgraduate research. The opportunity to carry out postdoctoral work at Leeds University gave me the opportunity to work with Irene Manton FRS, who had a profound influence on the course of my research career. I am grateful to Helge Thomsen, a fellow traveller who has remained a colleague and friend since we first met in Denmark in 1971. I thank Professor Sergey Karpov for research collaboration and generous hospitality on numerous exchange visits between Birmingham and St Petersburg. His excellent work in Birmingham led to three joint publications and some of his TEM images are used in this book (see Figure and Table Credits).

I am sincerely grateful to many scientific colleagues who contributed expertise in disciplines outside my own area of competence. In this category come Malcolm Davies, who collaborated with work on silicon metabolism and whose untimely death came before completion of the work and publication of the results. Jackie Parry provided me with generous assistance on choanoflagellate ecophysiology and the kinetics of continuous culture. John Blake brought a mathematical mind to bear on the hydrodynamics of filter feeding in choanoflagellates. Sandra Baldauf and Martin Carr introduced me to the world of molecular phylogeny and biosystematics. Oliver Smart, Dov Stekel and QiBin Yu kindly developed the computer graphic model used for constructing costal patterns in choanoflagellate loricae.

Choanoflagellate research has taken me to many parts of the world. I would particularly like to thank Yves LeGal and the staff at the Concarneau Marine Biology Station for so generously making available their facilities for countless field excursions to the Brittany coast over 30 years. I am grateful to Rick Wetherbee and Jeremy Pickett-Heaps FRS for hosting my 1996 sabbatical stay in the Department of Botany, University of Melbourne which proved to be one of the most productive and enjoyable periods of research during my working life. I took part on two sea-going collections in Icelandic waters arranged by the late Thorunn Thordardottir of the Marine Research Institute, Reykjavik. Ray Leakey, while working at the British Antarctic Survey, kindly arranged for me to partake in a sea-going cruise to the Southern Ocean and South Georgia.

I am thankful to Peter Holland FRS for convening the first choanoflagellate workshop in Reading in 2002; Nicole King and colleagues for hosting two subsequent workshops (2009 and 2011) at UC Berkeley; and Hartmut Arndt, Frank Nitsche and colleagues for hosting the fourth workshop in Cologne (2013). These meetings have provided a convivial forum for the exchange of results, discussion and techniques and have served to nucleate and develop a global 'choanoflagellate community'.

I gratefully acknowledge fellow scientists, doctoral students and generations of undergraduates who have contributed to my work on flagellates in general and choanoflagellates in particular. They include (in alphabetical order): Christi Adams, Lisa Armstrong, Jenny Bailey, Nik Berovic, Kurt Buck, Rong Cheng, Stephen Coupe, Sheila Crosbie, Stephen Day, Angela de Rosa, Ilana Fleetwood, Jan Frösler, Richard Geider, Anne Hartley, Ruhana Hassan, Magali Henouil, Sarah Innes, Seamus Jackson, Harriet Jones, Michelle Kelly, Christopher Kent, Vicki Lawson, Sally Leys, Malwin Malinowski, Sharon McCready, Linda Medlin, Pamela Merrick, Elaine Mitchell, Carol Morton, Rich Norris, Ken Oates, Helen Peterkin, Michala Pettitt, Julie Pickett-Heaps, Steve Price, Daniel Richter, David Roberts, Roy Sladden, Nancy Slater, David Small, Jahn Throndsen, Lesley Tomkins, Phillipa Towlson, Peter Whittle and Mehreen Yousuf.

I am indebted to countless members of the British Society for Protist Biology, the International Society of Protistologists, the British Phycological Society and the Systematics Association for a working lifetime of meetings, discussion, collaborations and hospitality. In particular I would like to thank: Keith Vickerman FRS, who on many occasions supported applications to the Royal Society and elsewhere for assistance with funding; John Green and the late Mary Parke FRS of the Marine Biological Association, Plymouth for their expertise and collaboration on flagellates in general; Bob Andersen, Hartmut Arndt, Tom Cavalier-Smith FRS, Richard Crawford, David Hibberd, Harvey Marchant, Michael Melkonian, Øjvind Moestrup, Jackie Parry, David (Paddy) Patterson, Tony Walsby FRS and the late Tyge Christensen, Dennis Greenwood, Michael Peel and Frank Round for so much help over many years as colleagues and friends.

I gratefully acknowledge the generosity of the following colleagues for providing me with illustrations or unpublished information and for giving me permission to include them in this book: Eve Boucaud, Thomas Bürglin, Martin Carr, William Gurske, David Hibberd, John van den Hoff, Sergey Karpov, Áron Kiss, David Lazarus, Ray Leakey, Sally Leys, Sunshine Menezes, Leonel Mendoza, Cynthia McKenzie, Frank Nitsche, N. V. Nemtseva, Franco Novarino, Marcus Paul,

Michala Pettitt, A. O. Plotnikov, E. A. Selivanova, Emma Steenkamp, Daniel Stoupin and Helge Thomsen. I am grateful to Chris Sheppard, Head of Special Collections at the University of Leeds, for permitting me to refer to the Irene Manton archive located in the Brotherton Library.

I owe an enormous debt of gratitude to Mrs Joyce Kent for the hours of work she devoted to organising the illustrative work for this book. Jennifer Neumann kindly assisted with the translation of German texts. I am thankful to Martin Carr, Nicole King, Nicholas Leadbeater, Frank Nitsche and Jackie Parry for reading and commenting on individual chapters. I am especially grateful to Emeritus Professor Michael Sleigh for scrupulously reading a complete draft of the script and for making many helpful comments and suggestions with respect to its scientific and editorial content. The text has been greatly improved by his wise counsel. I am thankful to colleagues at Cambridge University Press, particularly Dominic Lewis, Jessica Murphy and Ilaria Tassistro, for their patience in waiting so long for the final text.

Finally, I am deeply indebted to my wife, family, colleagues and friends for their patience and support during my research career and while writing this book. Deadlines have come and gone so often that, short of showing them a copy of the book itself, they will think this project was a figment of my imagination.

Note on Terminology, Taxonomy and Nomenclature

TERMINOLOGY

Choanoflagellate studies, starting with James-Clark's publication in 1866, are about to celebrate their sesquicentenary. In the intervening period the subject has acquired a substantial vocabulary of terminology. Since the relevant terminology has accumulated in a piecemeal fashion there are many examples of different terms with the same meaning and of general terms being used in specific contexts.

The policy that has been adopted in this book is to retain the use of general terms, such as theca (restrictive organic covering) and lorica (siliceous basket-like covering), that have been traditionally used in a 'choanoflagellate' context. Definitions of these and many other terms can be found in the appropriate chapters and/or in the glossary towards the end of this book. Creating new names or adopting infrequently used terms has been resisted on the grounds that they could confuse the reader and might distract from understanding the more important aspects of the text.

TAXONOMY AND NOMENCLATURE

The author is acutely aware that, at the time of writing, protist taxonomy and nomenclature are undergoing a major upheaval for a variety of reasons. The situation has been compounded by the phylogenetic revolution in taxonomy. Traditional taxonomy based on morphology has been the norm for choanoflagellates until recently. For craspedid choanoflagellates this has generally been unsatisfactory because many morphological features are ill-defined or variable at the light microscopical level and most species have polymorphic life cycles that are still unknown. For acanthoecids (loricate taxa) the situation is better because the pattern of costae comprising the lorica provides a set of well-defined morphological characters.

The introduction of molecular phylogeny to taxonomy will, no doubt, be of the greatest benefit in the long term. However, combining morphological and molecular phylogenies is proving much more difficult than was at first appreciated. For a start, it is most desirable that comprehensive data sets from both morphological and molecular sources should be available which, unfortunately, is often not the case. Incongruencies between morphological and molecular data are particularly problematical. If species can only be identified by molecular sequence data this not only presents problems for specialists without molecular facilities but may also invalidate the taxonomy of closely related species. As discussed in Chapter 10, phylogenetic systematics also challenges the Linnaean rank-based system of nomenclature that has underpinned much protistan taxonomy and nomenclature to date. The result is a confusion of hierarchical ranks and names. These are uncertain times and it is not obvious when or if stability will return.

Like it or not taxonomy and nomenclature underpin much biological research. Species names recorded in this text have been obtained from publications. Only occasionally have original names been changed in this text, for instance species of *Diplotheca* have been altered to *Didymoeca* for reasons explained in Chapter 7 and two species of *Savillea* have been combined because they co-occur within a single clonal culture (see Chapter 6).

The loricate choanoflagellate *Stephanoeca cupula* (Leadbeater 1972) Thomsen 1988 presents an anomalous situation that requires special mention. The original name given to this species was *Pleurasiga cupula* (Leadbeater, 1972c). The type illustrations (Figs 16 and 17 in Leadbeater, 1972c) show a cup-shaped lorica with anterior chamber comprising 8-10 longitudinal and two transverse costae, one forming the anterior ring and the second in an intermediate position. Subsequently, Thomsen (1988) transferred this species to the genus *Stephanoeca* and expanded the description by including

more specimens. However, some of these specimens (Figs 16–19 in Thomsen, 1988) differed from the original material in that they possessed an additional transverse costa in the anterior chamber and the anterior intermediate transverse costa is located outside the longitudinal costae, a feature not displayed by the original specimens. It is now judged that the two forms of *S. cupula* are probably not the same species. However, until the second form can be re-named the two forms are designated in this text as *Stephanoeca cupula sensu* Leadbeater (1972c) for the original form and *Stephanoeca cupula sensu* Thomsen (1988) for the second form.

Current authorities are appended to lists of choanoflagellates and other species recorded in their respective indexes. For most names applied to higher ranks the publication by Adl *et al*. (2012) has been used. Their classification, proposed on behalf of the International Society of Protistologists, makes use of the oldest valid name that describes each group irrespective of its status. Thus the scheme incorporates an eclectic assortment of taxonomic nomenclature and spellings.

This book is about choanoflagellate evolution, cell biology and ecology and therefore taxonomy and nomenclature are not of primary importance. However, as it now seems likely that there will be major changes in nomenclature and phylogeny within the coming years, it is hoped that the underlying themes of this book will outlive whatever taxonomic and systematic changes might occur in the future.