SYSTEMATICS AND BIOLOGY OF GRANIA (ANNELIDA: CLITELLATA: ENCHYTRAEOIDEA) OF THE BERMUDA ISLANDS

by

Jan Maureen Locke

A thesis submitted in conformity with the requirements for the degree of Master of Science
Graduate Department of Zoology
University of Toronto

© Copyright by Jan Maureen Locke 1998
The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L’auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L’auteur conserve la propriété du droit d’auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-45419-3
ABSTRACT

Systematics and Biology of Grania (Annelida, Clitellata, Enchytraeidae)
of the Bermuda Islands

MSc 1998
Jan M. Locke
Department of Zoology, University of Toronto

The diversity and distribution of the marine enchytraeid genus Grania present
within intertidal and subtidal coastal habitats of the Atlantic island of Bermuda are
investigated. Two new species, Grania laxarta and Grania hylae, are recorded and
Grania bermudensis and Grania americana are redescribed from Bermuda, Florida
and the Caribbean. These recent descriptions incorporate the new taxonomic
character information of bipartite sac male apparatus and the anterior cerebral
statocyst. This statocyst is the first description within clitellate annelids. These new
informative characteristics were combined with a compilation of familiar characters
for the genus to produce a dichotomous key for eight species of Grania and two
species of the genus Randidrilus from the western Atlantic and Caribbean. Grania
species from these areas were also used in a phylogenetic study to determine
possible evolutionary relationships among the Bermudian species. An evolutionary
hypothesis of dispersal by currents, the Gulf Stream in particular, was suggested for
the species of Grania that occupy Bermuda, due to its volcanic, oceanic island
history. The combination of good taxonomic information with characteristically small
ranged species enable Grania to be a model taxon for zoogeographic studies of
benthic marine invertebrates.
ACKNOWLEDGMENTS

The encouragement and patience given to me by my advisor and friend Kathryn Coates, is deeply appreciated. I was extremely fortunate to be surrounded by her wealth of expert knowledge. I do hope some of it rubbed off. Dr. Doug Currie, my adjunct supervisor provided valuable assistance and was a great contact in Toronto during my period of research abroad. I am also grateful to Mr. Don Stacey of the Royal Ontario Museum, for his patience and pointing me in the right direction when it came to "worm work". My research was conducted at the Bermuda Biological Station for Research, Inc. as part of the BBSR graduate intern program. Thank you to the faculty and staff of BBSR for their kindness during my time there.

Several individuals contributed continued support during this work: my parents Donna and Wallace Locke, my friends Michael Stevens, Jason Ritter, Jamey Penney-Ritter, Ruth Kelty and Astrid Schnetzer for her German translations and assistance in the final preparations. I am also grateful to Mr. Rudy Villadiego for electron microscopy technical assistance and Hyla Tibbitts and Linda Nelson who provided detailed illustrations of Grania that I truly appreciated.

I gratefully acknowledge the financial support provided by, University of Toronto Open Masters Fellowship 1996-1998; The Canadian Associates of the Bermuda Biological Station; The Roger Thayer Stone Fellowship, The Munson Foundation and Hank Trapido-Rosenthal through the Bermuda Biological Station for Research. Support and funding for research facilities were also provided by National Science Foundation grants # DEB-9615211 and # DBI 9724335.
DEDICATION

For Donna and Wallace
# TABLE OF CONTENTS

| TITLE PAGE | .......................................................... | i |
| ABSTRACT   | ......................................................................................... | ii |
| ACKNOWLEDGEMENTS | .................................................................................. | iii |
| DEDICATION | ........................................................................................ | iv |
| TABLE OF CONTENTS | ................................................................................... | v |
| LIST OF TABLES | .......................................................................................... | viii |
| LIST OF FIGURES | ................................................................................... | ix |

1. GENERAL INTRODUCTION .......................................................... 1

   The Genus ............................................................................. 5

   History .................................................................................. 6

   Morphology ............................................................................. 13

   Habitat and Distribution ...................................................... 17

   Ecology .................................................................................. 18

   Reproductive Biology ............................................................. 18

   Copulation ............................................................................. 20

   Taxonomic Difficulties ............................................................ 21

   Objectives ............................................................................. 22

   References ............................................................................. 24

2. REDESCRIPTIONS OF GRANIA AMERICANA, G. BERMUDENSIS AND DEScriptions OF TWO NEW SPECIES OF GRANIA (ANNELIDA: CLITELLATA: ENCHYTRAЕIDAE) FROM BERMUDA ................................................. 31

   Abstract ................................................................................ 31

   Introduction ........................................................................... 33
Key for Grania ......................................................................................................................... 140
Annotated Systematic List ...................................................................................................... 142
References .............................................................................................................................. 146

5. RELATIONSHIPS AND ORIGINS OF GRANIA SPECIES IN BERMUDA: 
PHYLOGENETIC ANALYSIS OF GRANIA (ANNELIDA: CLITELLATA: 
ENCHYTRAЕIDAE) FROM EASTERN NORTH AMERICA THE CARIBBEAN 
AND ANTARCTICA .................................................................................................................. 149

Abstract ................................................................................................................................. 149
Introduction ............................................................................................................................. 150
Material and Methods ........................................................................................................... 152
  Taxa ...................................................................................................................................... 152
  Characters .......................................................................................................................... 154
  Analysis .............................................................................................................................. 154
Results ........................................................................................................................................ 157
Discussion ............................................................................................................................... 157
  Characters .......................................................................................................................... 159
  Zoogeography .................................................................................................................... 159
  Vicariance .......................................................................................................................... 160
  Dispersal to Bermuda .......................................................................................................... 162
  Endemic Species ................................................................................................................ 164
  Evolution of Characters ...................................................................................................... 164
References .............................................................................................................................. 167

6. GENERAL DISCUSSION .................................................................................................... 171

Future Work with the Genus ................................................................................................. 173
References .............................................................................................................................. 175

SUMMARY ............................................................................................................................. 176
LIST OF TABLES

CHAPTER 2

Table 1. Site location and substrate description for *Grania laxartia*, *Grania americana*, *Grania hylae* and *Grania bermudensis*. For more complete Carrie Bow Cay data, refer to Erséus, 1990b. 37

CHAPTER 5

Table 1. List of taxa analyzed, species distribution, and source of character data (CE, Christer Erséus; KAC, Kathryn Coates). 153

Table 2. Character list and states. 155

Table 3. Character and state matrix used in phylogenetic analysis of *Grania*. 156
LIST OF FIGURES

CHAPTER 1

Figure 1. The North Atlantic islands of Bermuda. 2

Figure 2. Historical time line for species of the genus *Grania*. Grey areas emphasize taxonomic uncertainties. 7

Figure 3. Representative of the genus *Grania*. Scale equals 250 µm. 14

Figure 4. General morphological illustrations showing main taxonomic characters for recognizing and identifying species of *Grania*. Scale equals 100 µm. 16

CHAPTER 2

Figure 1. Location of four *Grania* species within coastal North American, Bermudian and Caribbean waters. ■ G. *americana*, ▲ G. *laxarta*, ● G. *hylae*, ◆ G. *bermudensis*. 35
Figure 2. *Grania laxarta*, new species from Bermuda. Ventrolateral view of whole mounted specimen; prostomium to segment XIV, illustrating position and relative sizes of diagnostic structures. Scale equals 100 μm.

Figure 3. *Grania laxarta*: setae from various segments, showing broad base and slight toe. Scale equals 20 μm.

Figure 4. *Grania laxarta*: dorsal view of spermatheca in V. Scale equals 20 μm.

Figure 5A. *Grania laxarta*: ventral view of male duct system showing sperm funnel, vas deferens and penial apparatus. Scale equals 20 μm.

Figure 5B. *Grania laxarta*: lateral view of penial apparatus. Scale equals 20 μm.

Figure 6. Comparison of *Grania* species. Measurements and counts are shown as averages. *Grania bermudensis* and *G. americana* spermathecae are shown here at one half the magnification used for *G. hylae* and *G. laxarta*. 
Figure 7. *Grania hylae*, new species from Bermuda. Lateral view of whole mounted specimen; prostomium to segment XIV. Scale equals 100 μm.

Figure 8. *Grania hylae*, setae from different segments. Scale equals 20 μm.

Figure 9. *Grania hylae*, lateral view of anterior brain vesicle with one inclusion. Scale equals 20 μm.

Figure 10. *Grania hylae*, lateral view of spermatheca in V, with dorsal pore. Scale equals 20 μm.

Figure 11A. *Grania hylae*, lateral view of male duct system showing sperm funnel, vas deferens and penial apparatus. Scale equals 20 μm.

Figure 11B. *Grania hylae*, photo of live specimen showing stylet orientation within vasa deferentia and bipartite penial apparatus. Scale equals 20 μm.
Figure 12. *Grania bermudensis*, specimen from Bermuda. Dorsolateral view of whole mounted specimen, from prostomium to segment XIV. Scale equals 100 μm.

Figure 13. *Grania bermudensis*, setae from various segments. Scale equals 20 μm.

Figure 14. *Grania bermudensis*, lateral view of spermatheca with epidermal papilla. Scale equals 20 μm.

Figure 15A. *Grania bermudensis*, male duct system and penial apparatus. Scale equals 20 μm.

Figure 15B. *Grania bermudensis*, live specimen with thick muscular area of vas deferens. Scale equals 20 μm.

Figure 15C. *Grania bermudensis*, dorsolateral view of bipartite penial apparatus. Scale equals 20 μm.

Figure 16. *Grania americana*, specimen from Bermuda. Ventrolateral view of whole mounted specimen from prostomium to segment XIV. Scale equals 100 μm.
Figure 17. *Grania americana*, setae from various segments. Scale equals 20 μm.

Figure 18. *Grania americana*, dorsal view of anterior brain vesicle showing inclusions. Scale equals 20 μm.

Figure 19. *Grania americana*, dorsolateral view of spermatheca with glands at ectal pore. Scale equals 20 μm.

Figure 20A. *Grania americana*, dorsolateral view of male duct system and penial apparatus. Scale equals 20 μm.

Figure 20B. *Grania americana*, lateral view of penial apparatus. Scale equals 20 μm.

CHAPTER 3

Figure 1. *Grania americana* whole mount illustration, emphasizing anterior segments and the prostomial (p) region that is occupied by the statocyst (so) anterior to the blood vessel (bv) bifurcation (bb). Scale equals 100 μm.
Figure 2. Light microscopy of chambered statocyst (so) anterior to blood vessel (bv) bifurcation, prostomial connectives(c) are visible and three of four statoliths are indicated by arrows. Scale equals 20 μm.

Figure 3. Longitudinal section through prostomial region. Narrow, spiraled extension (e) and anterior attachment to body wall by membrane (mb) are visible. Scale equals 20 μm.

Figure 4. Lateral cells (lp) of statocyst vesicle. Two regions of cilia (c), lateral sensory and supporting cells (lp), membrane attachment (a) to body wall and dorsal blood vessel (bv) are visible. Magnification 3750X.

Figure 5. Ciliated sensory cell with four basal bodies indicated by arrows. Microvilli are shown to the left of asterisks and a statolith(s) is visible. Transverse cilia (c) within chambers are also shown. Magnification 14000X.

Figure 6. Sensory cell with cilia originating from apex indicated by arrow. Magnification 5900X.
Figure 7. Cellular secretions indicated by arrowheads. Magnification 7500X.

Figure 8. Anterior glial cell (gc) and cilia (c) within individual statolith (s) chambers which are separated by a double membrane (indicated by arrow). Magnification 9333X.

Figure 9. Spiraled medial extension of bilobed brain showing dorsal muscle (dm) location of cilia (c) and blood vessel (bv). Magnification 2400X.

Figure 10. Cilia (c) running transversely within the medial spiral extension. Magnification 15469X.

Figure 11. Posterior region of brain showing muscle (m), membrane junctions (indicated by arrows) and cilia (c). Magnification 8455X.

Figure 12. Longitudinal section through prostomial region of *Grania americana*. Arrowheads indicate epidermal ciliated papilla. Scale equals 20 μm.
CHAPTER 4

Figure 1. General morphology of Grania, based on Grania longiducta, illustrating main diagnostic characters. Scale equals 100 µm. 128

Figure 2. Distribution of Grania species within the Western Atlantic and Caribbean. 130

Figure 3. Spermathecae, morphological illustrations. A. Grania america, with glands (g) at ectal pore, B. Grania hylae with narrow ectal duct (e), and C. Grania laxarta with distinct dilation (d) of ectal duct. Also illustrated are ampulla (a) and ental duct (en). Scale equals 20 µm. 133

Figure 4. Male apparatuses A. Bulbous gland of Grania laxarta and B. Randirillus codensis, and C. Bipartite sac of Grania bermudensis. Note anterior gland (ag), stylet (st) and vasa deferentia (v). Scale equals 20 µm. 134

Figure 5. A. Position of ventral setae (s) in worm body. B. Setae illustrating heel (h) and toe (t) characteristic of some species. Scale equals 20 µm for B only. 136
Figure 6. Chambered statocyst (cv) anterior to blood vessel (bv) bifurcation. Three of four statoliths (s) are clearly shown. Scale equals 20 μm.

Figure 7. Male duct system of A. Grania americana and B. Grania bermudensis, illustrating vasa deferentia (v), muscular modifications (mm), sperm funnel (sf), bulbous gland (bg) and bipartite sac (bp). Scale equals 20 μm.

CHAPTER 5

Figure 1. Phylogenetic tree depicting relationships among species of the genus Grania. Character changes are mapped on the tree (CI 0.75; RI 0.84; RC 0.63).

Figure 2. The main surface currents of the Atlantic and Caribbean Oceans.
The Atlantic, subtropical islands of Bermuda are located one thousand kilometers off the coast of North Carolina, USA. The Bermuda pedestal rises from the floor of the North American basin and for the purposes of this study will be considered in close association zoogeographically with North America (Fig. 1). Running between these two land masses is the Gulf Stream. This oceanic river is a combination of the Equatorial Current from Africa, which runs through the Caribbean and circles the Gulf of Mexico, exiting through the Straits of Florida, and the Antilles Current which flows by the northern coasts of Puerto Rico and Cuba to Florida. These two currents join to form the Gulf Stream as they flow northward along Florida. The Gulf Stream continues to meander northeasterly before turning eastward off the coast at Cape Hatteras, to move across the Atlantic. The Gulf Stream aids in dispersal of the larvae of Caribbean species within its warmer water as it travels northward between Bermuda and the eastern coastal United States. The cold eddies produced by the eastern meanders of the Gulf Stream rotate counterclockwise toward the shores of Bermuda, and over the years a Caribbean fauna has developed. For many Caribbean species, Bermuda is the northernmost limit (Cavaliere et al., 1992).

Many species of enchytraeids (Annelida: Clitellata) are marine (Healy and Coates, in press) but the majority of genera inhabit terrestrial environments. Marine species found within the family Enchytraeidae are in the genera Achaeta
Figure. 1 The North Atlantic islands of Bermuda.
Vejdovský, 1878; *Enchytraeus* Henle, 1837; *Lumbricillus* Ørsted, 1844;
*Stephensoniella* Cernosvitov, 1934 (amended, Coates, 1983); *Marionina*
Michaelson, 1889, *Grania* Southern, 1913; and *Randidrilus* Coates & Erséus,
1985. Only *Stephensoniella*, *Grania* and *Randidrilus* are strictly marine. Of the
marine genera, five are reported from Bermuda, *Achaeta*, *Enchytraeus*,
*Stephensoniella*, *Marionina* and *Grania* (Erséus and Lasserre, 1976; Healy and
Coates, in press).

The Enchytraeidae of Bermuda has been understudied, with only four
publications referring to the islands fauna. Initially, Moore (1902) described
*Enchytraeus marina* from the shores of Bermuda, this species has since been
assigned to *Stephensoniella* (Coates, 1983). Moore’s work was followed several
years later by Lasserre & Erséus (1976) on the habitat and distribution of marine
Enchytraeidae and Tubificidae in Bermuda, and notes of the families, Naididae
and Megascolecidae (= Acanthodrilidae). *Grania macrochaeta bermudensis* was
also described from the island in that study. Giere (1979) further investigated the
oligochaete fauna of Bermuda, and reported *G. m. bermudensis* and five new
tubificids. More recently Healy and Coates (in press) show that the species
diversity of the Enchytraeidae in Bermuda is much higher than previous studies
indicated. This is not very surprising considering the documented work
conducted on enchytraeids in Bermuda.

The genus *Grania* is an ubiquitous, wholly marine, genus of clitelates
belonging to the family Enchytraeidae. Within marine habitats *Grania* is
frequently the dominating enchytraeid (Brinkhurst, 1982; Erséus, 1990).
However, its diversity in all marine habitats, from the abyssal to intertidal, is in the early stages of investigation. The genus has been virtually neglected within the southwestern Atlantic region of North America. The literature documents only six species of *Grania* from this extensive area, *Grania americana* Kennedy, 1966 from Florida, *Grania longiducta* (Erséus & Lasserre, 1976) from Massachusetts, *Grania monospermatheca* Erséus & Lasserre, 1976 from Massachusetts and Florida (Coates & Erséus, 1985) and Coates & Erséus’ (1985) more recent accounts of *Grania atlantica*, *Grania levis*, and *Grania reducta* from the northeast US continental shelf.

A single endemic taxon of *Grania* has been reported from Bermuda. *Grania macrochaeta bermudensis* was originally recorded as a subspecies of *G. macrochaeta* (Pierantoni, 1901) (Erséus & Lasserre, 1976). Studies of North American and European coastal enchytraeids of the Atlantic have led to descriptions of other subspecies of *Grania macrochaeta* designated by Erséus and Lasserre (1976) as separate species (Coates & Erséus, 1985; Locke & Coates, 1998). Considering the instability of Erséus and Lasserre’s (1976) polytypic species groupings and the changes in the taxonomy of *G. macrochaeta* (Locke & Coates, 1998) the status of *G. m. bermudensis* is in need of reevaluation. Other studies (Coates, 1984) have indicated that *G. m. bermudensis* has a unique and complex genital apparatus which was not noted in the original description. Observations of the type specimens (Coates, pers. comm.) also revealed undescribed, unique characteristics of the spermathecae of this species. Thus the morphology of *G. m. bermudensis* also requires
detailed reconsideration. New material of *G. m. bermudensis* was sought in order to redescribe the species and better determine its distribution in Bermuda.

The diversity of worms present on such an isolated and geologically young island poses some interesting questions about their dispersal patterns and more generally about colonization of Bermuda. Considering Bermuda’s young geological age, the most recent emergence was 52-34 MYA (Morris *et al.*, 1977), how did the genus colonize this isolated island and what sort of divergence patterns does it exhibit?

THE GENUS

*Grania* Southern, 1913


The genus *Grania* was originally described from Clare Island, northwestern Ireland, with the discovery of *Grania maricola* Southern 1913, in Clew Bay. Years before the establishment of the genus, however, species of this lineage were described within existing taxa (Fig. 2). This set the trend for the early taxonomic history of the genus, several synonymies and misidentifications later, the concept of *Grania* would emerge and grow to hold well over forty species (Fig. 2).

**HISTORY**

Twelve years before Southern's (1913) designation of *Grania*, an Italian taxonomist, Pierantoni (1901) described the enchytraeid, *Enchytraeus macrochaetus* from the Gulf of Naples, Italy. In 1903, Pierantoni reassigned this species to the genus *Michaelsena* Ude, 1896, hence, *Michaelsena macrochaeta*. Subsequently, Southern (1913) established the genus *Grania* following the discovery of *Grania maricola* in Ireland. Pierantoni (1915) believed *Grania maricola* was synonymous with *Michaelsena macrochaeta*. Stephenson (1930) disagreed with Pierantoni and pronounced them separate species, but belonging in the genus *Michaelsena*. However, Nielsen and Christensen (1959) declared *Michaelsena* an artificial genus and both *Michaelsena maricola* and *Michaelsena macrochaeta* were subsumed in the genus *Enchytraeus*. Nielsen and Christensen (1959, p. 91) noted that both *E. maricola* and *E. macrochaeta* should be placed in a separate genus, *Grania* being the most reasonable, but failed to do this.
<table>
<thead>
<tr>
<th>YEAR</th>
<th>SPECIES PRESENT</th>
<th>TAXONOMIC EVENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1966</td>
<td>G. mira</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. pusilla</td>
<td>reinstated as species</td>
</tr>
<tr>
<td>1997</td>
<td>G. integra</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. lassemeri</td>
<td>new species</td>
</tr>
<tr>
<td>1998</td>
<td>G. hirsuticauda</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. carchinii</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. angustinasus</td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td>G. longistyla</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. allata</td>
<td>new species</td>
</tr>
<tr>
<td>1990</td>
<td>G. erasii</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. crassiducta</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. bykane</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. ascophora</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G. hyperoadenia</td>
<td></td>
</tr>
<tr>
<td>1985</td>
<td>G. reducata</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. atlantica</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. longiducta</td>
<td>reinstated</td>
</tr>
<tr>
<td>1981</td>
<td>G. incerta</td>
<td>new species</td>
</tr>
<tr>
<td>1980</td>
<td>G. paucipina</td>
<td>redescribed</td>
</tr>
<tr>
<td>1977</td>
<td>G. ovitheca</td>
<td>new species/subspecies</td>
</tr>
<tr>
<td></td>
<td>G. m. trichaeta</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>YEAR</th>
<th>SPECIES PRESENT</th>
<th>TAXONOMIC EVENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1976</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G. monospermatheca</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. variochaeta</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G. postcrilochea</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. monochaeta</td>
<td></td>
</tr>
<tr>
<td>1974</td>
<td>G. pusilla</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. macrochaeta</td>
<td>new species</td>
</tr>
<tr>
<td></td>
<td>G. maricola</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G. americana</td>
<td></td>
</tr>
<tr>
<td>1971</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1967</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1966</td>
<td>G. maricola</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G. americana</td>
<td></td>
</tr>
<tr>
<td>1959</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1930</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1915</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1913</td>
<td>Grania maricola</td>
<td></td>
</tr>
<tr>
<td>1904</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1903</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1901</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Historical time line for species of the genus *Grania*. Grey areas emphasize taxonomic uncertainties.
In 1966, Kennedy redescribed the genus *Grania*, placing both *G. maricola* and *G. macrochaeta* within it, along with a new species *G. americana* from Florida, USA. Lasserre (1966) also rehabilitated *Grania* with *G. maricola* and *G. macrochaeta* as two separate species and transferred *Michaelsena postclitellochaeta* Knöllner, 1935 to the genus.

Before its reinstatement as a genus (Kennedy, 1966; Lasserre, 1966), *Grania*, had been in synonymy for over half a century. The genus now had four included species, *G. maricola*, *G. macrochaeta*, *G. americana* and *G. postclitellochaeta*. *Grania* was primarily defined by setal characteristics and species were separated by their setal distributions, a character that did not significantly distinguish the first three of the four described species. These had ventral setae occurring in segments VI, V and IV, respectively. Very few species specific characters were recognized for earlier descriptions of the genus and this would prove to be a problem with the addition of more species. This confusion is evidenced by the subspecies Lasserre designated.

In 1967, Lasserre proposed that due to the similarities among a number of species, *G. macrochaeta* should have four subspecies: *G. m. macrochaeta*, *G. m. maricola*, *G. m. americana* and *G. m. roscoffsris* Lasserre, 1967. He maintained that with the inclusion of *Grania postclitellochaeta* (Knöllner, 1935) and *Grania monochaeta* (Michaelsen, 1888), transferred (Lasserre, 1967) from the genus *Enchytraeus*, within the same genus it would be inconsistent to regard the various subspecies of *G. macrochaeta* as equally different. In 1974, Erséus reinstated *G. macrochaeta*, *G. maricola*, *G. americana* and *G. roscoffsris* as
distinct species after studying their type material. The character basis for this change is not discussed in detail, perhaps distinctions were quite obvious from the material. In addition to this Erséus also described G. *pusilla* Erséus, 1974, from Sweden.

A new genus, *Hemigrania*, was erected by Lasserre (1971) based on the absence of peptonephridia in some *Grania*-like species. Peptonephridia are usually hollow, postpharyngeal extensions of the gut wall, which probably have a digestive function. Lasserre (1971) transferred *Grania postclitellochaeta*, *Grania monochaeta* and *Marionina principissae* (Michaelsen, 1907), previously transferred from *Michaelsenia* (Nielsen & Christensen, 1959, p.109) to *Hemigrania*. However, Erséus and Lasserre (1976) synonymized *Hemigrania* with *Grania* because they found that no species of *Grania* have peptonephridia.


Erséus and Lasserre proposed a tentative classification of the genus based on two characters, distribution of setae and spermathecal morphology. This resulted in the recognition of two polytypic species groupings within *Grania macrochaeta* (Pierantoni, 1901) and *G. postclitellochaeta*. These were *Grania*
 macrosoma-bermudensis-pusilla and G. postclitellochaet-a-longiducta. Erséus and Lasserre (1976) also considered G. americana a nomen dubium, due to poor quality of the type material and discussed including it in the G. macrosoma polytypic grouping (p.123). Jamieson (1977) has since added G. m. trichaeta to the polytypic G. macrosoma grouping. By 1977, ten species and four subspecies were included in the genus. Considering the low species richness, restricted distributional information and lack of species specific characters used in descriptions, the logic to Lasserre's subspecies categories can be recognized.

Generic stability after 1976, is indicated by several species descriptions and redescriptions (Fig. 2). Coincidentally there was also general recognition that marine oligochaetes were more diverse and numerous than previously supposed, based largely on renewed interest in the group and increased sampling. Erséus (1977) described Grania ovitheca from the west coast of Sweden and noted the taxonomic importance of the penial bulb for recognizing species of Grania; Erséus and Lasserre (1977) redescribed Grania monochaeta (Michaelsen, 1888); and Erséus (1980) described G. parvitheca from Ascension Island, South Atlantic. Coates and Erséus (1980) reported the first Grania species from Pacific North America with the redescription of G. paucispina and description of Grania incerta. Up to this point in the taxonomic history of Grania, published accounts related solely to species identifications and distribution. Coates (1984) expanded our knowledge of structural diversity within the reproductive system of Grania. Attention to and emphasis on the morphology of male reproductive structure and species specific penial bulb types provided
valuable taxonomic information. Species distinctions were more easily made following this additional morphological information. Descriptions increased dramatically and redescriptions helped to reduce taxonomic confusion within the genus.

Scientists began to expand their collecting efforts, broadening the known geographical distribution and species diversity of *Grania*. Coates and Erséus (1985) described three new species of *Grania* from the coastal northwest Atlantic, *Grania reducta*, *Grania levis* and *Grania atlantica*. They redescribed *G. monospermatheca* and recalled *G. longiducta* from synonymy. Coates and Erséus (1985) provided the only study from the Atlantic coast of North America since Erséus and Lasserre’s (1976) account from Massachusetts, and Lasserre’s (1971) work at Woods Hole, Beaufort, USA. In Australia, the first records of *Grania* emerged with the discovery of *Grania macrochaeta tricheta* Jamieson, 1977 from Wistari and Heron Reefs. Coates (1990) described six new species from Albany, Western Australia, *G. ersei*, *G. crassiducta*, *G. bykane*, *G. ascophora*, *G. hyperoadenia* and *G. hastula*, and redescribed *G. principissae* (Michaelsen, 1907). Erséus (1990) recorded three new species from Hong Kong, *G. hongkongensis*, *G. inermis*, and *G. stilifera*. Coates and Stacey (1993) described four new species from Rottnest Island, Western Australia, *G. longistyla*, *G. alliata*, *G. vacivasa* and *G. conjuncta*. Rota and Erséus (1996) described six new species from McMurdo Sound and Terra Nova Bay, Antarctica, *G. hirsuticauda*, *G. acanthochaeta*, *G. carchinii*, *G. angustinasus*, *G. antarctica* and *G. algida*. The authors also recorded an interesting morphological
character they referred to as the “head organ” in some of the newly described species. A re-examination of *G. monochaeta* was undertaken by Rota and Erséus (1997) and another two new species, *G. lasserrei* and *G. stephensoniana*, were found in material from South Georgia, Antarctica. In 1997, Coates and Stacey described two new species of *Grania* from Darwin Harbour, Northern Australia, *G. integra* and *G. eurystila*. The most recent descriptions (Locke & Coates, 1998) are from the rocky intertidal of Ireland, these include a new species, *Grania mira* and a redescription of *G. pusilla* as a distinct species. The redescription restricted the concept of *G. macrochaeta sensu lato* as discussed by Locke and Coates (1998).

The rate of description of new species has elevated in recent years. In 1967, only six species of *Grania* were known, whereas there are now over 40 described species (Fig. 2). The localities of these records cover all seven continents, Antarctica, North America, South America, Europe, Australia, Asia and Africa. Species recorded from more than one locality do seem somewhat restricted by continent, although the deep sea *G. atlantica* is an exception having been recorded from four continents (North America, Europe, South America, Africa). The distribution patterns of the genus most likely reflect the areas that have been sampled rather than the actual species distribution (Rota & Erséus, 1996). It seems only a matter of looking for these enchrytraeids to increase their known distribution and diversity.

Species descriptions dominate the taxonomic history of the genus *Grania*. Although species richness within the genus has accelerated in recent years,
knowledge of basic biology and ecology still await adequate investigation. Hypotheses of evolutionary relationships within *Grania* species have never been proposed. Phylogenetic studies are entirely lacking for the genus and this is more than likely due to the lack of abundant characters for distinguishing species. The determination of new taxonomic characters would benefit our understanding of ancestral relationships within the genus.

**MORPHOLOGY**

*Grania* are infaunal segmented worms, characterized by a long slender body-form and movements that are very nematode-like due to their thick cuticle (Fig. 3). On average, sexually mature specimens of *Grania* are 7 mm in length and 0.12 mm in width. They are easily recognized by their straight, pointed setae that occur singly, the ectal tip protruding beyond the epidermis. Generally, these setal “bundles” are located both ventrally and laterally, although lateral setae are often reduced or absent anterior to the clitellum. There are also records of species totally lacking setae (Coates & Erseus, 1985). Probably because the species live in an environment with limited light, body wall pigmentation is not found in *Grania* so that they are somewhat translucent. This, combined with their small size, enables the microscopical observation of internal structures in live specimens.
Figure 3. Representative of the genus *Grania*. Scale equals 250 μm.
The anterior-most part of the worm body is the prostomium which may have a sensory function. A septum or body division is visible between this area and the peristomium, called segment 1, noted as I, in oligochaete annelids. The mouth, brain and dorsal blood vessel bifurcation are located in the peristomium (Fig. 4). An opening to the exterior of the worm, the head pore, is also present dorsally on the peristomium. Progressing posteriorly the worm anatomy is as follows: pharynx in II-III; paired reproductive structures, spermathecae, in V; paired pharyngeal glands, in IV-VII. A ventral nerve cord, dorsal and ventral blood vessels and a ciliated intestine travel the length of the worm. There is a transition of the dorsal blood vessel to an intestinal sinus in posterior segments. Nephridia are present in most species, usually not occurring before VIII. The clitellum occupies XII; this glandular region surrounds the genital pores and gonadal region. Free floating paired sperm funnels in XI are connected via vasa deferentia to the male penial apparatuses in XII (Fig. 4). Testes and ovaries are found in XI and XII, respectively. The posterior end of the worm consists of a post-segmental pygidium, at the anterior boundary of this is the growth region of the worm where new mesoderm and segments are produced.

Species identification is based mainly on morphology of the reproductive organs. A general practice in clitellate morphological description is to denote the segment numbers by Roman numerals (e.g. IX, for segment nine) and septa by Arabic numerals (e.g. 10/11, for septum between X and XI).
Figure 4. General morphological illustration showing main taxonomic characters and relative sizes. Scale equals 100 μm.
HABITAT AND DISTRIBUTION

*Grania* species are known from both lower intertidal and nearshore subtidal sediments, less than 100 m in depth (Rota & Erséus, 1996). An exception is *Grania atlantica*, a deep sea species recorded at a range of 744-1796 m from the Atlantic Ocean (Coates & Erséus, 1985). The genus is especially abundant where organic matter accumulates among permeable, well-oxygenated, coarse, biogenic sediments. *Grania* are usually found in microhabitats that are protected from extreme wave action. Healy (1996) has recorded them from a wave exposed, rocky shoreline where they were protected within crevices and algal material. Although species of *Grania* are characteristic of coarse, calcareous substrates, some species are also abundant in fine, silty, seagrass sediments and sediments of algal turfs (Healy, 1996; Locke & Coates, 1998; Locke & Coates, in Review). Organic matter within the sediments consists of diatoms, meiofaunal invertebrates and decomposing plant material.

A larval stage is not known to occur in *Grania*, and according to accepted generalizations about clitellates, *Grania* undergoes direct development. The potential for long distance dispersal via larval forms is therefore very limited. However, morphologically similar populations in continental and remote island habitats have been observed (Locke & Coates, in Review) suggesting that there is either regular genetic exchange among these or very slow divergence rates. Another possibility is decoupling of genetic and morphological divergence between the isolated populations. If the last is the case, this would have
significant implications for taxonomic and systematic procedures appropriate to these marine organisms as morphology would not reflect species boundaries.

ECOLOGY

Similar to other clitellate worms, *Grania* feed on microorganisms such as bacteria, fungi, cyanobacteria and diatoms (pers. obs.) present within the detrital sands they inhabit. They act as important nutrient recyclers, degrading organic debris that is washed ashore (Giere, 1975). Increased organic content usually leads to the explosion of aerobic bacteria resulting in oxygen depletion. In this respect, *Grania* are probably undervalued bioturbators as their burrowing brings oxygen into the sediment. Other burrowing invertebrates that share the same habitat are Nematoda, Sipunculida, other meiofaunal invertebrates, Tubificidae and the marine enchytraeid genus *Marionina*.

REPRODUCTIVE BIOLOGY

Information regarding the basic biology and ecology of marine enchytraeids, *Grania* in particular, is minimal (Giere & Pfannkuche, 1982). This may be due to their relatively small size. However, some conclusions can be drawn from their morphology. *Grania* are hermaphroditic, with male and female reproductive organs occurring simultaneously in the same individual. In *Grania*, the glandular clitellum occurs in XII and the male and female reproductive organs are paired, in most species. *Grania monospermatheca* Erséus and Lasserre, 1976 and an undescribed species from Mexico (Coates, pers. com.) are the only
known exceptions, each individual having only one dorsally opening spermatheca.

The male reproductive system consists of paired testes present in XI, the segment immediately preceding the segment possessing the male pores. Sperm produced from these are enclosed and stored within a sperm sac derived from septum 11/12, which extends posteriorly as far as XX, sperm migrate from this sac through the coelom to the sperm funnels in XI. Each sperm funnel is connected to a male penial apparatus in segment XII via a vas deferens. The vas deferens may have muscular modifications. In addition, the penial apparatus of some species may contain a thin, possibly cuticular tube, referred to as a stylet, which originates within the ental end of the vas deferens and may extend through the penial apparatus, tapering toward the male pore. In maturing worms, those in which the setae are missing from XII, an external male pore is seen with the beginning of an invagination, however, no vasa deferentia or other penial apparatuses are visible (pers. obs.). The male system usually forms prior to the female system (pers. obs.).

The female reproductive system in *Grania* consists of paired structures called spermathecae, which are located in segment V of the worm. These are of variable shapes among species. Each spermatheca consists of an ectal duct which connects the spermathecal ectal pore to a sac-like ampulla. The spermathecal ectal pore can be located dorsally or laterally depending on the species. In maturing worms, the invagination of the spermathecal ectal duct originates from the body wall (pers. obs.). Sperm are transferred from a
partnered worm through the ectal duct where they are stored in the walls of the ampulla as sperm rings until needed for fertilization of the worm's eggs. The spermathecae are connected to the gut via an ental duct. The function of such a connection is unknown, although Westheide (in press) speculates unused sperm are transferred to the gut via the ental duct, where they are resorbed. The spermathecal ampulla arises as a bump from the endoderm of the intestine in immature individuals (pers. obs.). Therefore, a connection to the gut may be a vestige in the developmental stages of the spermathecae and may not have a function in mature individuals. Paired ovaries are present within XII and an egg sac, derived from septum 12/13, often extends posteriorly as far as XXII, more mature eggs are visible at the posterior end of the sac. Eggs exit the body of the worm through a ventrolateral female pore, located immediately posterior to 12/13.

COPULATION

During copulation, each of the partnered worms presumably extends an everted portion of the male apparatus from the male pore in XII; in some species the stylet may act as a supportive structure for the transfer of sperm from each of the male apparatuses to the paired female spermathecae. Sperm are stored within the spermathecae until fertilization. Prior to fertilization, _Grania_ are generally thought to secrete mucous from the glandular tissue of their clitellum, this mucous forms a ring around the body wall. Eggs produced from paired ovaries in XII and stored in the posterior egg sac, are deposited within the
mucous ring when they are extruded from the female pore at 12/13. The worm backs its way out of the mucous surrounding its body, until this reaches the fifth segment. Here, sperm are released from the spermathecal ectal pore into the mucous to fertilize the eggs. The mucous is then shed anteriorly from the parent in the form of a cocoon where the eggs are left to develop into juveniles.

**TAXONOMIC DIFFICULTIES**

The family Enchytraeidae is distinguished from other families mainly by genital segment position combined with setal features. There is no generally accepted subfamilial classification of the genera (Coates, 1989). Classification above the family level will not be used herein, due to the lack of substantiated phylogenetic support for such classifications (Stephenson, 1930; Jamieson, 1981, 1988; Brinkhurst, 1994).

Taxonomic difficulties abound within the family. Published species descriptions are often too short and based on too few characters. Earlier descriptions were also published in a variety of languages, Italian, French and German, thus poor or incomplete translations may account for some of the confusion. The lack of distinct descriptive characteristics for species during the pioneering efforts of earlier *Grania* taxonomists may provide an explanation for the taxonomic exigency within this genus.

Initially, *Grania* species were distinguished from one another based only on setal distribution, thus a single taxonomic character was relied upon. However, the importance of internal structures such as reproductive organs has
become recognized within the last 25 years (Erséus, 1974; Coates, 1984). Species with very different reproductive structures often exhibit similar setal patterns, therefore setal distribution is not an adequate and reliable specific taxonomic character. Unfortunately, clitellates are externally simple and as organisms of such small size only have so many anatomical characters that can be used to separate them from one another. Recent taxonomic character advances and increased species knowledge (Coates, 1984; Rota and Erséus, 1996, 1997) are serving to promote better species descriptions, and recognition of varieties as valid species. The head organ recently observed by Rota and Erséus (1996, 1997) in certain deep sea species from the Antarctic and in *G. atlantica*, may prove both interesting and informative as not only a descriptive character but also an important phylogenetic character.

**OBJECTIVES**

**The objectives of this study are:**

To determine the status of the enchytraeid genus *Grania* in Bermuda, redescribing and determining a rank for the taxon, *G. m. bermudensis*, reported from the islands and describing any new species found. Exploring certain aspects of distribution, biology and evolutionary history within the genus.

To further investigate the "head organ" reported in Antarctic *Grania* species by Rota and Erséus (1996, 1997); first determining its presence in any readily available species of the genus; and then investigating the "head organ" as a possible taxonomic character by revealing its structural make-up and
relationship to the brain. To determine its function in *Grania*, and elucidate its evolutionarily significant within the Annelida.

To construct a dichotomous key for *Grania* species of the Western Atlantic. The key may increase awareness of marine microdrile clitellates, *Grania* species in particular, and their distribution along the coast. To make this information widely available to both enchytraeid specialists and microfaunal biologists.

To discover relationships among the species of *Grania* by constructing a phylogeny of the species that inhabit the Northwest Atlantic coastline. A phylogeny of *Grania* species will provide a basis for answering questions relating to evolution and dispersal. A search for new taxonomically useful characters for descriptive and phylogenetic purposes will be conducted, and existing characters will be reassessed.

The thesis has an expanded introduction and discussion. Each of the four intermediate chapters deal with the objectives outlined above and are formatted as manuscripts for submission to scientific journals.
REFERENCES


REDESCRIPTIONS OF GRANIA AMERICANA, G. BERMUDENSIS AND
DESCRIPTIONS OF TWO NEW SPECIES OF GRANIA (ANNELIDA:
CLITELLATA: ENCHYTRAEOIDEA) FROM BERMUDA

Citation and authority for new species:

Contribution of JML: Described Grania laxarta and Grania hylae; contributed to descriptions of Grania bermudensis and Grania americana; developed ideas for introduction, taxonomic remarks, arguments and discussion.

ABSTRACT
The diversity of the genus Grania (Clitellata: Enchytraeidae) in Bermuda has not been fully investigated over the range of ecologically available habitats. Previously, Grania macrochaeta bermudensis Erséus and Lasserre, 1976 was the only recorded species from subtidal coarse sands of the Bermuda islands, but anecdotal information suggested there were additional, abundant species living in Bermuda. Herein, four species, G. bermudensis, G. americana Kennedy, 1966, G. laxarta, new species and G.
*hylae*, new species, are described from intertidal and subtidal biogenic calcium carbonate sands of Bermuda. *Grania bermudensis* is designated as a separate species rather than as a subspecies of *G. macrochaeta* (Pierantoni, 1901). *Grania americana* and *G. laxarta* are also reported from the Caribbean. *Grania americana* and *G. laxarta* are distinguished by their distinctive spermathecal structures and by the presence of a penial stylet in the latter. *Grania bermudensis* and *G. hylae* can be easily separated from their Bermudian congeners by the dorsal position of spermathecal pores. *Grania bermudensis* has a much larger spermathecal ampulla than *G. hylae* and an external epidermal bump at the spermathecal pore. Their male duct systems have similar bipartite penial apparatuses but differ in development of the wall of the vasa deferentia. *Grania bermudensis* has heavily muscled vasa deferentia but in *G. hylae* these are only slightly muscled and nucleate. Limited muscularization of the vasa deferentia is also seen in *G. laxarta*. Both *G. americana* and *G. hylae* have anterior vesicles associated with their brain. These descriptions extend basic knowledge of diversity, distribution and taxonomy of *Grania* species of the North Atlantic. They also provide new information on the structural diversity of male duct systems with elongate sac-like invaginations, stylets, and highly muscled vasa deferentia, and on the structure and taxonomic distribution of statocyst-like sensory structures.
INTRODUCTION

The taxonomic diversity in Bermuda, of the abundant and widespread marine oligochaete genus *Grania* Southern, 1913 (Annelida: Clitellata: Enchytraeidae) has been poorly investigated, as are most of the marine Enchytraeidae (Healy & Coates, in press). Only one species, *Grania bermudensis* Erséus and Lasserre, 1976, has been described from Bermuda. Originally it was described as a subspecies of *G. macrochaeta* (Pierantoni, 1901) (Erséus & Lasserre, 1976; Locke & Coates, 1998). However, a great degree of anatomical variability in *Grania* collected from Bermuda has been recognized for some time (Erséus, pers. comm.). Furthermore, there are only four publications referring primarily to the Enchytraeidae of Bermuda (Moore, 1902; Lasserre & Erséus, 1976; Giere, 1979; Healy & Coates, in press) and the most recent of these shows the species diversity is much higher than previous studies indicated.

The single species described from Bermuda was reported prior to the recognition of a variety of important taxonomic characters for *Grania* species. In a detailed examination, Coates (1984) provided new criteria for categorizing forms of the male duct system found in the genus. Such characteristics have allowed the distinction of several new species (e.g., Coates & Erséus, 1985; Erséus, 1990a; Coates & Stacey, 1993). Recently, another interesting structural character has been revealed by Rota and Erséus (1996). In a few new species from the Antarctic and subantarctic, a structure was observed that was termed the head organ (Rota & Erséus, 1996, 1997). This head organ was described as
a vesicular body which contained refractile inclusions and was located within the peristomium just anterior to the brain. Such advances in recognition of morphological characters have resulted in increased knowledge of specific diversity and provide additional clues that can be applied to uncovering evolutionary relationships among species of _Grania._

Observations of type specimens of _G. bermudensis_ revealed hitherto undescribed, diagnostic characteristics of the spermathecae of this species. This combined with additional information about the male ducts of _G. bermudensis_ (Coates, 1984) suggested that, indeed, this species was in need of redescription.

A major zoogeographic element in the Bermuda fauna is species with Caribbean and coastal southern U.S. distributions (Fig. 1) (Sterrer, 1986). _Grania americana_ is the only described species of _Grania_ with such a distribution. It was initially reported from the shores of Florida and Bimini (Kennedy, 1966) but, there have been no subsequent records. Erséus and Lasserre (1976) stated _G. americana_ must be considered a nomen dubium due to the poor condition of the type material and the lack of detail in the original description, but also suggested it was very similar to _G. bermudensis_, implying that _G. americana_ might also be a subspecies of _G. macrochaeta_. Lasserre (1967) had designated _G. americana_ as a subspecies of _G. macrochaeta_ along with many other species of _Grania_. The basis for this designation as discussed by Locke and Coates (1998) is probably largely historical and possibly eurocentric. Oligochaetes have long been considered a lineage that originated
Figure 1. Location of four *Grania* species within coastal North American, Bermudian and Caribbean waters. ■ *G. americana*, ▲ *G. laxarta*, ○ *G. hylae*, ● *G. bermudensis*. 
and diversified in non-marine habitats, and thus a low species diversity in marine systems was expected. Erseus (1974) did indicate G. americana and G. macrochaeta were probably separate species based on definite morphological differences, but that further information about intraspecific variation of each taxon was needed. New taxonomic characteristics and complete redescriptions are presented to substantiate species differences among G. americana, G. bermudensis and G. macrochaeta sensu stricto. Redescriptions of the first two taxa and a survey of Grania species in Bermuda were undertaken in order to advance understanding of the diversity, zoogeography and evolution of marine oligochaetes.

MATERIALS AND METHODS

Sediments were collected from eight subtidal and three intertidal sites within Bermuda. Intertidal sites were Ferry Point Bridge, Whalebone Bay and Rocky Hill Park, where samples were taken from an accumulation of sand inside dissolutions of limestone rock and rocky limestone beach areas, respectively. Subtidal collections were from Castle Harbour: Eastern Castle Roads, Rushy Island, and a patch reef 100 m from Rushy Island; ENE and SE Paget Island, St. George’s Harbour; Ferry Reach; NW Pearl Island, Great Sound; NE entrance, Smith’s Sound; and Bailey’s Bay. Other specimens were collected by C. Erseus, from Hutchinson Island, Florida; Fowey Rocks, Miami, Florida; North of Angelfish Key, Florida Keys; and Carrie Bow Cay, Belize (Table 1).
Table 1. Site location and substrate description for *Grania laxarta*, *Grania americana*, *Grania hylae*, and *Grania bermudensis*. For more complete Carrie Bow Cay data, refer to Erseus, 1990b.

<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>Position</th>
<th>Description</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bermuda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whalebone Bay</td>
<td>Dec. '77</td>
<td>32°21'90&quot;N 64°42'75&quot;W</td>
<td>intertidal fine to coarse</td>
<td><em>G. laxarta, G. americana</em></td>
</tr>
<tr>
<td>Ferry Point Bridge</td>
<td>96-'98</td>
<td>32°21'70&quot;N 64°42'80&quot;W</td>
<td>intertidal dissolution tubes</td>
<td><em>G. laxarta, G. americana</em></td>
</tr>
<tr>
<td>Rocky Hill Park</td>
<td>Oct. '98</td>
<td>32°22'60&quot;N 64°41'40&quot;W</td>
<td>intertidal coarse with rocks</td>
<td><em>G. hylae, G. americana</em></td>
</tr>
<tr>
<td>Pearl Island</td>
<td>Aug. '77</td>
<td>32°17'50&quot;N 64°50'19&quot;W</td>
<td>2.5 m fine</td>
<td><em>G. laxarta, G. americana</em></td>
</tr>
<tr>
<td>Ferry Reach 1</td>
<td>Nov. '77</td>
<td>32°22'05&quot;N 64°41'44&quot;W</td>
<td>0.2 m medium to coarse</td>
<td><em>G. laxarta, G. americana</em></td>
</tr>
<tr>
<td>Ferry Reach 2</td>
<td>Dec. '77</td>
<td>32°22'05&quot;N 64°41'44&quot;W</td>
<td>0.5 m medium to coarse</td>
<td><em>G. laxarta</em></td>
</tr>
<tr>
<td>NW Smith's Sound</td>
<td>Dec. '77</td>
<td>32°22'14&quot;N 64°39'21&quot;W</td>
<td>4 m fine with seagrass</td>
<td><em>G. laxarta</em></td>
</tr>
<tr>
<td>Castle Island</td>
<td>Aug. '77</td>
<td>32°20'40&quot;N 64°40'25&quot;W</td>
<td>15 m medium to coarse</td>
<td><em>G. hylae, G. bermudensis</em></td>
</tr>
<tr>
<td>Bailey's Bay</td>
<td>Aug. '77</td>
<td>32°20'94&quot;N 64°43'30&quot;W</td>
<td>6.5 m fine to medium</td>
<td><em>G. hylae, G. americana</em></td>
</tr>
<tr>
<td>ENE Paget Island</td>
<td>Dec. '77</td>
<td>32°22'38&quot;N 64°38'57&quot;W</td>
<td>17 m medium to coarse</td>
<td><em>G. hylae</em></td>
</tr>
<tr>
<td>SE Paget Island</td>
<td>Dec. '77 / Mar. '98</td>
<td>32°22'35&quot;N 64°39'30&quot;W</td>
<td>8 m, 15 m medium to coarse</td>
<td><em>G. hylae, G. bermudensis</em></td>
</tr>
<tr>
<td>E Castle Roads</td>
<td>Aug. '77</td>
<td>32°20'30&quot;N 64°40'20&quot;W</td>
<td>8 m coarse</td>
<td><em>G. bermudensis</em></td>
</tr>
<tr>
<td>Rushy Island</td>
<td>Aug. '77</td>
<td>32°20'18&quot;N 64°40'45&quot;W</td>
<td>8-9 m coarse</td>
<td><em>G. bermudensis</em></td>
</tr>
<tr>
<td>Patch Reef</td>
<td>Aug. '77</td>
<td>32°20'19&quot;N 64°40'26&quot;W</td>
<td>8-9 m coarse</td>
<td><em>G. bermudensis</em></td>
</tr>
</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>Longitude</th>
<th>Position</th>
<th>Description</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fowey Rocks</td>
<td>Nov. '77</td>
<td>25°35.5'N</td>
<td>80°05.8'W</td>
<td>3 m coarse</td>
<td>G. hylaee</td>
</tr>
<tr>
<td>N. Angelfish Key</td>
<td>Nov. '77</td>
<td>25°10'N</td>
<td>80°13'02&quot;W</td>
<td>6 m Thalassia, fine</td>
<td>G. americana</td>
</tr>
<tr>
<td>Hutchinson Island</td>
<td>Nov. '72 / May '73</td>
<td>27°20'24&quot;N</td>
<td>80°13'04&quot;W</td>
<td>10 m shell rubble</td>
<td>G. americana</td>
</tr>
<tr>
<td>Carrie Bow Cay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cbc-85-2</td>
<td>Nov. '85</td>
<td>all stations</td>
<td>all stations</td>
<td>1.5 m medium to coarse</td>
<td>G. laxarta, G. americana</td>
</tr>
<tr>
<td>cbc-85-2-14</td>
<td>Nov. '85</td>
<td></td>
<td></td>
<td>0.5 m medium to coarse</td>
<td>G. americana</td>
</tr>
<tr>
<td>cbc-85-15</td>
<td>Nov. '85</td>
<td></td>
<td></td>
<td>0.2 m Thalassia, rubble</td>
<td>G. americana</td>
</tr>
<tr>
<td>cbc-85-26</td>
<td>Nov. '85</td>
<td></td>
<td></td>
<td>1 m muddy</td>
<td>G. laxarta</td>
</tr>
<tr>
<td>cbc-85-36</td>
<td>Nov. '85</td>
<td></td>
<td></td>
<td>0.2 m grey, coarse</td>
<td>G. americana</td>
</tr>
<tr>
<td>cbc-85-41</td>
<td>Nov. '85</td>
<td></td>
<td>1 m</td>
<td>medium to coarse</td>
<td>G. laxarta</td>
</tr>
<tr>
<td>cbc-85-57</td>
<td>Nov. '85</td>
<td></td>
<td>1 m</td>
<td>medium to coarse</td>
<td>G. americana</td>
</tr>
<tr>
<td>cbc-85-65</td>
<td>Nov. '85</td>
<td></td>
<td>0.1 m</td>
<td>Thalassia, coarse</td>
<td>G. laxarta</td>
</tr>
<tr>
<td>cbc-85-68</td>
<td>Nov. '85</td>
<td></td>
<td>0.3 m</td>
<td>coarse, rubble</td>
<td>G. americana</td>
</tr>
</tbody>
</table>
Specimens collected by the authors in Bermuda were obtained by washing samples on a 300 \( \mu m \) sieve and then sorting the residue in seawater using a dissecting microscope. Specimens were fixed in Kahle's fluid, stained lightly with borax carmine, dehydrated and cleared, and mounted in Canada balsam for morphological observations. Only mature specimens were identified. Specimens of all \textit{Grania} species were also observed while alive.

Type specimens of \textit{G. americana} (USNM 33005) and \textit{G. bermudensis} (USNM 53202), Castle Island, were borrowed from the United States National Museum of Natural History (USNM) (Smithsonian Institution), Washington, D. C., and examined for identification of and comparison to the new specimens. Whole mounted specimens of \textit{G. americana}, \textit{G. bermudensis}, \textit{Grania monospermatheca}, \textit{Erseus} and \textit{Lasserre}, 1976, and \textit{Grania atlantica}, \textit{Coates} & \textit{Erseus}, 1985 and new species were also examined from the collections of K.A. Coates and C. Erseus.

Observations of specimens were made using differential interference contrast light microscopy and videomicroscopy. Thirty-five mm photography was done with a Wild MPS46 photoautomat on Kodak Technical Pan film ASA 100. Drawings were made with the aid of a drawing tube. An Olympus AX70 Provis microscope and Toshiba 3CCD camera with Image-Pro Plus\textsuperscript{TM} software were utilized for video microscope image capture analysis and morphological measurements. Measurements were also taken with the use of Jandel Video Analysis Software (JAVA) on live images and with the aid of an ocular micrometer.
Measurements for *G. americana* and *Grania laxart*a, new species, were made from Bermuda and Carrie Bow Cay whole mounts; for *Grania hylae*, new species, and *G. bermudensis* measurements were taken from Bermuda whole mounts. Terminology for the head organ was adapted from Rota and Erseús (1996, 1997) and for penial bulb types from Coates (1984). Observations of the "head organ", indicate that a more appropriate name for this structure would be the anterior brain vesicle.

**Abbreviations used in the figures**

- a, ampulla; ab, anterior brain vesicle; b, brain; bu, bipartite union of sac-like penial apparatus; bv, blood vessel; bw, body wall; c, clitellar gland cells; cc, ciliated canal; co, sperm funnel collar; cp, conical protrusion of spermathecal duct; cv, cellular vas wall; e, spermathecal ectal duct; ed, ectal dilation; eg, ectal gland of spermatheca; en, spermathecal ental duct; ep, epidermal papillae; g, gut; h, setal heel; hp, head pore; i, invagination at male pore; lp, lateral posterior seta; lpc, lateral postclitellar seta; m, muscle; mk, muscle knot on vas deferens; p, male pore; pa, penial apparatus; pg, penial gland; ph, pharyngeal glands; pj, pad-like glandular junction; pr, prostomium; px, pharynx; ri, refractile inclusion; rs, rigid contracted sac; sb, subesophageal ganglion; sf, sperm funnel; sm, spiral of muscle; sp, spermatheca; spp, spermathecal pore; sr, sperm ring; st, stylet; t, setal toe; v, vas deferens; vc, vas deferens constrictions; vp, ventral posterior seta; vpc, ventral postclitellar seta; vs, ventral seta.
RESULTS

*Grania* Southern, 1913

For a recent synonymy of the genus refer to Locke and Coates, 1998.

**New Species Descriptions**

*Grania laxarta*, new species

Figs. 2-6; Table 1

**Holotype:** USNM ****, stained, whole mounted specimen, collected from the type locality, Feb. 1996 by KAC.

**Type Locality:** Ferry Point Bridge, Bermuda. 32°21'70"N, 64°42'80"W. Intertidal course biogenic sands within dissolution tubes.

**Paratypes:** three whole mounted specimens, USNM **** Ferry Reach, Bermuda, BBS-77-38.6, C. Erséus, 1977; USNM **** Carrie Bow Cay, Belize, CBC 85-41, C. Erséus, 1985; and Bermuda Aquarium, Museum and Zoo, BAMZ **** Ferry Reach, Bermuda, BBS-77-41.4, C. Erséus, 1977.

**Other Material examined:** Approximately 45 live specimens from the type locality collected by the authors, over the period of 1996-1998. Twenty-six whole mounts from type locality, collected (KAC) February and March, 1996. Fourteen whole mounts collected by C. Erséus from Bermuda. Five whole mounts collected from Carrie Bow Cay, Belize collected by C. Erséus (Table 1).
Description: **External characteristics:** Live specimens are translucent white with a pink tinge due to colored blood within their vessels. Preserved length 4.7-8.3 mm (n=18, $\bar{x}$=6.7, s=1.1). Number of segments 33-64 (n=20, $\bar{x}$=50.0, s=9.3) (Fig. 2). Diameter at 4/5 90.0-160.0 μm (n=29, $\bar{x}$=129.4, s=15.9), diameter at clitellum 100.0-198.2 μm (n=26, $\bar{x}$=138.0, s=18.3). Dorsal head pore located in anterior region of peristomium. Setae present from IV ventrally and XVI-XVIII laterally (Fig. 3); setae absent in XII of mature worms. Ventral setae anterior to the clitellum appear more robust and thicker than postclitellar setae. Setae are pointed distally and somewhat broad at base and with a curve and slight toe present at the proximal end, no distinct heel (Fig. 3). Length of anterior ventral setae 38.0-56.0 μm (n=16, $\bar{x}$=48.4, s=5.1); midventral setae 34.0-50.0 μm long (n=28, $\bar{x}$=42.0, s=4.35); postclitellar lateral setae 38.0-48.0 μm long (n=18, $\bar{x}$=42.4, s=2.8). In ten posterior-most segments ventral setae ranged from 37.3-75.5 μm (n=9, $\bar{x}$ = 45.7, s=11.7) and lateral setae were 36.0-45.2 μm in length (n=11, $\bar{x}$=42.5, s=3.2). Spermathecal pores paired, lateral, immediately posterior to 4/5. Clitellum extends from mid XI to mid XIII. Male pores ventral, in line of setae, located at about midpoint of XII. Female pores more lateral than male pores just posterior to 12/13.

**Internal somatic characteristics:** Brain narrow anteriorly and indented posteriorly. No anterior brain vesicle present. Pharyngeal glands very small: small dorsolateral pair at 4/5; two ventral paired lobules in V and one dorso-lateral pair in 5/6; in VI, two or three paired lobules ventro-lateral and one pair
Figure 2. *Grania laxarta* new species from Bermuda. Ventrolateral view of whole mounted specimen; prostomium to segment XIV, illustrating position and relative sizes of diagnostic structures. Scale equals 100 μm.
Figure 3. *Grania laxarta*, setae from various segments, showing broad base and slight toe. Scale equals 20 μm.
lateral at 6/7 (Fig. 2). Coelomocytes numerous anteriorly, not found posterior to clitellum. Patterned chloragogen cells covering posterior regions of the intestine, which often contains pinnate diatoms. Nephridia not seen. Dorsal blood vessel transition to blood sinus in XXVI. No glands on ventral nerve cord.

**Internal reproductive characteristics:** Spermathecae (Fig. 4) paired in anterior of V, ampulla with narrow, ventrolateral, ental connection to esophagus; connection more ventral than line of ectal spermathecal pores. Spermathecal ectal duct has large spherical dilation midway between pore and ampulla (Fig. 4). Ectal duct runs at about 90° from body wall to dilation, where it turns 90° to run parallel to body wall. The ectal duct then penetrates the ampulla as a cone-like protrusion (Fig. 4). The diameter of the ectal duct dilation is 18.0-32.6 μm (n=54, \( \bar{x} = 25.0, s=3.0 \)) (Fig. 4). Width of spermathecal ectal duct 10.0-21.9 μm (n=46, \( \bar{x} = 15.5, s=2.7 \)) between dilation and ampulla and 10.0-14.0 μm (n=31, \( \bar{x} = 11.2, s=1.5 \)) between dilation and ectal pore. Ampulla roughly spherical, greatest diameter 26.0-50.0 μm (n=54, \( \bar{x} = 42.5, s=4.7 \)). Rings of sperm in wall of ampulla of reproductive specimens. No glands at ectal pore (Fig. 4). Sperm funnels 80.0-160.0 μm long (n=25, \( \bar{x} = 112, s=23.5 \)), 25-55 μm maximum width (n=25, \( \bar{x} = 41.4, s=9.2 \)) or length about three times width; attached sperm with straight head (acrosome & nucleus) (Fig. 5A). Laterally paired vasa deferentia possess a muscular “knot” at one quarter their total length, near sperm funnel (Fig. 5A); muscle bands run around vas and constrict it, but there are no obvious elongate muscle bands. Vas confined to XI-XIII. Width of vas 6.3-8.8 μm (n=26, \( \bar{x} = 7.6, s=0.8 \)) both before and after “knot”. Penial apparatus with small gland and
Figure 4. *Grenia laxarta*, dorsal view of spermatheca in V. Scale equals 20 µm.
Figure 5A. *Grania laxarta*, ventral view of male duct system showing sperm funnel, vas deferens and penial apparatus. Scale equals 20 μm.
lateral aglandular sac formed by extended invagination of male pore, muscles extend from body wall to outer wall of sac (Fig. 5B). Vas enters gland from dorsal, lateral side, extends through glandular material to medial side. Thin walls of vas deferens have pronounced nuclei where stylet begins. Stylet extends from ectal end of vas deferens, tapering toward blind end of sac-like extended invagination of male pore. Length of stylet 30.0-47.7 μm (n=12, x=33.9, s=6.5); maximum width of stylet 1.7 μm (measured for only two specimens) (Fig. 5B). Sperm sac extends from XVIII to XXI. Egg sac extends to XXIII, with primary oocytes located at posterior end of sac; these can be recognized by the absence of a nuclear envelope and presence of aligned, condensed chromosomes.

Etymology: The species is named for the unique shape of the spermathecal ectal duct. From the Latin, laxare, meaning to widen, combined with artus, meaning joints.

Taxonomic remarks: The distribution of the lateral setae is reported as XVI-XVIII. One specimen was an exception with lateral setae beginning in XXV; it was also lacking ventral setae from XVIII-XXIII. The ventral setal distribution for G. laxarta is similar to G. bermudensis, G. americana and G. hylae, new species (Fig. 6) from the Northwest Atlantic, all of which have ventral setae beginning in segment IV. The last three species are easily distinguished from G. laxarta by dorsal spermathecal pores and a muscular sac-like penial apparatus in both G. bermudensis and G. hylae and by the absence of both a penial stylet and muscular vas deferens (Coates, 1984) and presence of glands at the spermathecal ectal pore in G. americana. Furthermore, G. bermudensis, G.
Figure 5B. *Gria laxarta*, lateral view of penial apparatus. Scale equals 20 μm.
<table>
<thead>
<tr>
<th></th>
<th>Grania laxarta</th>
<th>Grania hylae</th>
<th>Grania bermudensis</th>
<th>Grania americana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (mm)</td>
<td>6.7</td>
<td>8</td>
<td>7.8</td>
<td>10.8</td>
</tr>
<tr>
<td>Number of segments</td>
<td>50</td>
<td>52</td>
<td>51</td>
<td>64</td>
</tr>
<tr>
<td>Diameter IV/V (μm)</td>
<td>129.4</td>
<td>155.9</td>
<td>180.7</td>
<td>168.7</td>
</tr>
<tr>
<td>Setal distribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ventral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lateral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spermathecal pores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dorsal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lateral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spermathecal shape</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glands at spermaticheal pore</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Glands on nerve cord</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Head organ</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Sperm funnel</td>
<td>L3XW</td>
<td>L2.5XW</td>
<td>L4XW</td>
<td>L8XW</td>
</tr>
<tr>
<td>Vas deferens muscular</td>
<td>yes knot</td>
<td>thin with constrictions</td>
<td>yes spiral and thick</td>
<td>no</td>
</tr>
<tr>
<td>Penial apparatus type</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 6. Comparison of Grania species. Measurements are shown as averages. Grania bermudensis and G. americana spermathecae are shown here at one half the magnification used for G. hylae and G. laxarta.
*hylae* and *G. americana* each have very different spermathecal morphologies, they lack the distinct dilation of the spermathecal ectal duct that is present in *G. laxarta*. Such a dilation of the spermathecal ectal duct is only reported in one other species of *Grania* from the Northwest Atlantic, *G. monospermatheca*. The ectal duct dilation of *G. monospermatheca* occurs immediately after the ampulla and is not as distinctly bulb-like as the medial dilation of the spermathecal ectal duct in *G. laxarta*. *Grania monospermatheca* can also readily be distinguished from *G. laxarta* by the lack of lateral setae and the presence of a single spermatheca with a dorsal pore instead of a pair of spermathecae that open laterally.

**Habitat and distribution:** Intertidal pools with accumulations of sand, in eroded limestone rock, dissolution tubes. Also occurs subtidally. Found in sand samples with debris along with *G. americana*, various Tubificidae, Nematoda and Sipunculida. Sand size for samples ranged from fine (occurring with *Thalassia testudinum* Koenig) to medium, coarse calcareous coral sand. Known from Ferry Point Bridge, Whalebone Bay, Pearl Island, Ferry Reach and Smith’s Sound, Bermuda and Carrie Bow Cay, Belize.

*Grania hylae*, new species

Figs. 6, 7-11; Table 1

**Holotype:** USNM ****, stained, whole mounted specimen, collected from the type locality, BBS-77-42 by C. Erséus, 1977.
Type Locality: ENE of Paget Island, Bermuda, 32°22’38”N, 64°38’57”W. Subtidal in 17 m, medium to coarse coral sand.


Other Material examined: Live material from Rocky Hill Park, Bermuda. Fourteen whole mounted specimens from the collection of C. Erséus, from three different localities in Bermuda, and Fowey Rocks, Florida, USA (Table 1).

Description: External characteristics: fixed specimen length 5.3-11.5 mm (n=7, \( \bar{x} = 8.0, s = 2.1 \)). Number of segments 41-61 (n=6, \( \bar{x} = 52, s = 8.1 \)) (Fig. 7). Diameter at 4/5 139.5-192.0 \( \mu \text{m} \) (n=7, \( \bar{x} = 155.9, s = 17.3 \)), diameter at clitellum 154.0-196.0 \( \mu \text{m} \) (n=7, \( \bar{x} = 171.4, s = 17.3 \)). Head pore in anterior of peristomium. Ventral setae from IV and laterally from XV-XVII (Figs 7 & 8). No setae in XII of mature specimens. Length of anterior ventral setae 37.0-66.4 \( \mu \text{m} \) (n=10, \( \bar{x} = 51.3, s = 8.9 \)); midventral setae 49.8-91.0 \( \mu \text{m} \) (n=35, \( \bar{x} = 71.8, s = 8.5 \)); postclitellar lateral setae 59.8-80.0 \( \mu \text{m} \) long (n=31, \( \bar{x} = 69.6, s = 5.8 \)). In ten posterior-most segments ventral setae 53.7-71.0 \( \mu \text{m} \) long (n=17, \( \bar{x} = 62.8, s = 5.2 \)) and lateral setae 49.0-74.0 \( \mu \text{m} \) (n=14, \( \bar{x} = 62.2, s = 8.4 \)). Setal shaft widening proximally, with sloping toe, distinct heel absent (Fig. 8). Spermathecal pores paired, dorsal, immediately posterior to 4/5, distance between pores 46.0-37.5 \( \mu \text{m} \) (n=2, \( \bar{x} = 41.8, s = 6.0 \)), no epidermal papilla at pore. Clitellum extending from
Figure 7. *Grania hylae*, new species from Bermuda. Lateral view of whole mounted specimen; prostomium to segment XIV. Scale equals 100 μm.
Figure 8. *Grania hylae*, setae from different segments. Scale equals 20 μm.
post XI to mid XIII. Male pores ventro-lateral at midpoint of XII. Female pores posterior to 12/13, opening just lateral to line of male pores.

**Internal somatic characteristics:** Brain indented posteriorly with narrow transition to small anterior brain vesicle. Membrane bound vesicle lies below head pore, with one refractile inclusion visible (Fig. 9). Pharyngeal glands present in segments IV through VI; two pairs at 4/5, one lateral and one ventral, three paired lobules in V, two pairs ventral and one pair dorsal at 5/6, three pairs lying laterally in VI and one pair at 6/7. No nephridia observed. Dorsal blood vessel transition to blood sinus at XX or XXI. Gland on ventral nerve cord in XIV.

**Internal reproductive characteristics:** Spermathecae lie dorsal to pharyngeal glands in segment V (Fig. 10). Spermathecal ectal duct of uniform, narrow width with slight curvature toward pore, length of duct about 1.2X maximum diameter of ampulla. Glands lacking at ectal pore. No protrusion of duct tissue into ampulla. Spermathecal ampulla small and round to ovoid, maximum diameter 32.0-52.0 μm (n=11, \( \bar{x} = 43.8, s = 6.3 \)); ampullae lie close together, dorsal to gut, sometimes even touching; sperm rings often present (Fig. 10). Narrow ental connection of spermathecal ampulla to esophagus. Sperm funnel length two and a half times the width, with non-granular section posterior to collar. Walls of vasa deferentia constricted at three almost equally spaced points immediately posterior to junction with sperm funnel; with slight muscularization ectal to constrictions; vas possess large, obvious nuclei along length, diameter 6.7-10.0 μm (n=13, \( \bar{x} = 8.4, s = 0.9 \)). Stylet originating within the vas deferens, extending to and into the penial apparatus (Figs 11A & B). Stylet
Figure 9. *Grania hylae*, lateral view of anterior brain vesicle with one inclusion. Scale equals 20 μm.
Figure 10. *Grania hylae*, lateral view of spermatheca in V, with dorsal pore. Scale equals 20 μm.
long, 55.4-70.9 μm (n=6, \( \bar{x} = 61.8.1, s=7.1 \)). Vas terminates at ental end of the ental saccate part of the bipartite penial apparatus, which lies parallel to body wall and gut. This sac may appear longitudinally ridged or smooth, depending on whether it is contracted or relaxed. At anterior end of sac, there is an acute 90° bend that unites the ridged, contracted sac to an erect extension of the male pore (standing more or less perpendicular to the body wall). Muscles run from the body wall to insert on both the longitudinal sac and onto the lateral wall of the perpendicular extension (Figs 11A & B). Sperm sac extends to XVIII. Egg sac extends to XXII.

**Etymology:** Named hylae in appreciation of Hyla Tibbitts’ dedication and keen interest in the subject matter of her illustrations.

**Taxonomic remarks:** *Grania hylae* is similar to *G. bermudensis* in subtidal habitat and spermathecal characteristics. Both have dorsal spermathecal pores and round to ovoid ampullae, however the average maximum ampulla diameter for *G. hylae* is only half that for *G. bermudensis*. An epidermal bump at the ectal pore is also seen only in *G. bermudensis* as well as a widening of the ectal duct near the ampulla. *Grania hylae* possesses an anterior brain vesicle which is lacking in *G. bermudensis*. Equally great differences between the two species are seen in the male duct systems. The walls of the vas deferens in *G. bermudensis* are highly muscularized whereas those of *G. hylae* have only a thin muscle layer. The maximum width of muscular vas deferens in *G. bermudensis* averages 22.9 μm and the non-muscular areas 12.3 μm; *G. hylae* has an
Figure 11A. *Grania hylae*, lateral view of male duct system showing sperm funnel, vas deferens and penial apparatus. Scale equals 20 μm.
Figure 11B. *Grania hylae*, photo of live specimen showing stylet orientation within vasa deferentia and bipartite penial apparatus. Scale equals 20 μm.
average vas width of 8.4 μm. Both species do possess somewhat similar bipartite sac-like penial apparatuses.

Habitat and distribution: Subtidal, found in fine, medium and coarse coral sand, many Tubificidae also present. Intertidal, rocky limestone beach area, medium to course sand, with *G. americana*, Tubificidae and Nematoda. Known from Rocky Hill Park, Castle Island, Bailey’s Bay, and ENE and SE Paget Island, Bermuda and Fowey Rocks, Miami, Florida, USA (Table 1).

Species Redescriptions

*Grania bermudensis* Erséus and Lasserre, 1976

Figs. 6, 12-15; Table 1

*Grania macrochaeta bermudensis* Erséus and Lasserre, 1976: 122-124, fig. 3.


Type Material: Holotype USNM 53202; paratypes USNM 53203; whole mounts. Collected by P. Lasserre, November 12, 1973.

Type Locality: Castle Island, Bermuda 32°20’40”N, 64°40’25”W. Coarse coral sand with ripple marks, 8 m depth.

Other material examined: Live specimens from the Southeastern tip of Paget Island, Bermuda, collected March, 1998 by JML. Eleven whole mounted specimens from the collections of C. Erséus collected from two sites in Bermuda, including the type locality (Table 1).
Description: **External characteristics:** Length of preserved specimens 5.8-10.1 mm (n=7, \( \bar{x} = 7.8, \ s = 1.4 \)). Number of segments 31-59 (n=7, \( \bar{x} = 51.4, \ s = 9.4 \)). Diameter at 4/5 138.0-216.0 µm (n=6, \( \bar{x} = 180.7, \ s = 27.5 \)), diameter at clitellum 159.0-258.9 µm (n=7, \( \bar{x} = 217.9, \ s = 36.4 \)) (Fig. 12). Ventral setae from IV, lateral setae from XVI-XX (n=7). No setae in XII of mature specimens.

Ventral setae at IV-V 48.0-72.0 µm (n=14, \( \bar{x} = 59.2, \ s = 7.2 \)); length of all other ventral setae 69.0-92.2 µm (n=32, \( \bar{x} = 80.6, \ s = 5.9 \)); postclitellar lateral setae 75.0-96.0 µm (n=19, \( \bar{x} = 83.4, \ s = 6.3 \)) (Fig. 13). In ten posterior most segments ventral setae 68.9-74.4 µm (n=6, \( \bar{x} = 71.5, \ s = 1.8 \)) and lateral setae 64.7-75.6 µm (n=6, \( \bar{x} = 70.3, \ s = 3.5 \)). Setae thin with blunt proximal part possessing a slight downturned toe, no distinct heel present (Fig. 13). Head pore mid-dorsal, in anterior portion of peristomium. Spermathecal pores paired, dorsal just posterior to 4/5, with thickened, glandular, epidermal papilla posterior to each spermathecal pore. Dorsal spermathecal pores approximately 60.0-64.4 µm apart (n=2, \( \bar{x} = 62.1, \ s = 3.1 \)). Clitellum extends from posterior XI to mid XIII. Male pores ventro-lateral at mid XII. Female pores located more lateral than male pores, just posterior to 12/13.

**Internal somatic characteristics:** Brain indented posteriorly. Anterior brain vesicle lacking. Pharyngeal glands present as free dorso-lateral lobes on anterior faces of 4/5 through 6/7; ventral lobes, one pair in IV, two paired lobules in V and three paired lobules in VI (Fig. 12). Nephridia not observed in live specimens, however fixed material showed five to seven pairs of complete
Figure 12. *Grania bermudensis*, specimen from Bermuda. Dorsolateral view of whole mounted specimen, from prostomium to segment XIV. Scale equals 100 μm.
Figure 13. *Grania bermudensis*, various setae. Scale equals 20 μm.
nephridia in segments posterior to egg sac; small overall, with small funnels, about four pairs in posterior most segments and about two to three pairs near egg sac. Dorsal blood vessel transition to blood sinus as far back as XX. One specimen with small gland on ventral nerve cord in XIV.

**Internal reproductive characteristics:** Spermathecae paired in V with narrow ental connection of ampulla to esophagus (Fig. 12). Ampulla large and round, walls very grainy, maximum diameter 42.9-106.5 μm (n=13, \( \bar{x}=80.7 \), s=23.5); sperm rings often present (Fig. 14). Glands lacking at spermathecal ectal pore. Spermathecal ectal duct diameter wider closest to ampulla, diameter ranges from 10.5-19.5 μm (n=12, \( \bar{x}=14.9 \), s=3.0). Ental duct runs ventro-lateral to reach wall of gut, width about one half ampullar width. Sperm funnel length about four times width, with non-staining, aglandular region posterior to collar (Fig. 15A). Vas deferens thick and muscular along about one-third of length. Vas deferens relatively wide at sperm funnel, but lacking muscle on walls; vas walls then progress to a spiral of muscle bands first wound tightly around circumference of vas; spiral then unwinds so that the thick muscles run almost along length of the duct; transition to thin-walled and narrow duct lacking muscle before pad-like glandular junction with the penial apparatus (Fig. 15A).

Maximum diameter of longitudinally muscled part is 18.0-25.5 μm (n=8, \( \bar{x}=22.9 \), s=2.6) (Fig. 15B), non-muscular ectal and ental vas deferens diameter 7.5-18.0 μm (n=9, \( \bar{x}=12.3 \), s=3.2). Stylet extends within ectal end of vas deferens causing that portion to be curved (Figs 15A & C). Stylet length 91.8-130.1 μm (n=6, \( \bar{x}=107.9 \), s=16.8); maximum width at origin within vas deferens 7.3 μm
Figure 14. *Grania bermudensis*, lateral view of spermatheca with epidermal papilla. Scale equals 20 μm.
Figure 15A. *Grenia bermudensis*, male duct system and penial apparatus. Scale equals 20 μm.
Figure 15B. *Grania bermudensis*, live specimen with thick muscular area of vas deferens. Scale equals 20 μm.
Figure 15C. *Grania bermudensis*, dorsolateral view of bipartite penial apparatus. Scale equals 20 μm.
(one specimen); stylet width along length 3.0-4.2 μm (n=3, \( \bar{x} = 3.4 \), s=0.7).

Bipartite penial apparatus consists of an erect sac-like extension from the male pore running to a lateral, anterior-posterior oriented, longitudinally ridged, sac lying parallel to the body wall; stylet terminates at pore on pad in posterior, ental end of ridged sac, and can be protruded through pore and into penial apparatus (Fig. 15C). Extensions of muscles from the body wall run to the erect extension and the lateral sac, and the whole of the penial apparatus can be everted through the male pore at the body wall. Sperm sac extends to XIX. Egg sac extends to XXI.

Taxonomic remarks: The only recorded species of *Grania* in Bermuda prior to this study, *G. bermudensis*, was originally described as a subspecies of *G. macrochaeta* by Er séus and Lasserre (1976). The original description of the new subspecies was brief and indefinite about some characteristics, which in part explains why *G. bermudensis* was considered part of *G. macrochaeta* sensu lato. The grouping of four subspecies, *G. m. macrochaeta*, *G. m. bermudensis*, *G. m. pusilla* and *G. m. trichaeta* was recently reevaluated as taxonomically invalid (Locke and Coates, 1998) and it is probably also polyphyletic. *Grania pusilla* has been fully redescribed. This redescription of *G. bermudensis* provides details that were omitted from the original description, about dorsal spermathecal pore position and male duct structures. The distribution of lateral setae is amended from previous records of XVIII-XIX to XVI-XX.

The dorsal spermathecal pore position, bipartite penial apparatus and highly muscularized vas deferens separate this species from *G. macrochaeta*
sensu stricto (Locke & Coates, 1998) and other Bermudian congeners (Fig. 6). *Grania hylae* does share comparable modifications of the first two character states with *G. bermudensis*, however *G. hylae* lacks the extreme muscular modifications of the vas deferens. *Grania bermudensis* is named here as a distinct species.

**Habitat and distribution:** Subtidal, medium to coarse coral sand and gravel. Not found intertidally. Found in samples with Tubificidae. Known only from East Castle Roads, Rushy Island, a patch reef 100 m south of Rushy Island and Paget Island, Bermuda.

*Grania americana* Kennedy, 1966

Figs. 6, 16-20; Table 1

*Grania americana* Kennedy, 1966: 404-405, fig 3.


*Grania americana:* Erséus, 1974: 90-93, table 1.

*Grania americana* nomen dubium: Erséus and Lasserre, 1976: 123

**Type Material:** Holotype, USNM 33005, paratype USNM 33039. Whole mounts. Collected by M.L. Jones, August 27, 1962.

**Type Locality:** 0.5 km from North Entrance point, West side of North Bimini, 25° 43.2'N; 79° 19.0'W.
Other material examined: Five stained, whole mounted specimens, USNM ****, Pearl Island, Bermuda; USNM ****, Ferry Reach, Bermuda; USNM **** Hutchinson Island, USA; and USNM **** and ***** Carrie Bow Cay, Belize. Approximately 55 live specimens from Ferry Point Bridge, Bermuda, collected by the authors over the period of 1996-1998. Twenty-four whole mounted specimens from the collections of C. Erséus, collected from Bermuda, Carrie Bow Cay, Belize, Angelfish Key, Florida Keys, and Hutchinson Island, Florida (Table 1).

Description: External characteristics: Live specimens long bodied with pink blood in vessels, posterior segments yellowish to light brown due to colored granules within chloragogen cells. Prostomium pointed with anterior thin spot; with many multi-ciliate epidermal papillae. Fixed length 8.7-12.6 mm (n=10, \( \bar{x} = 10.8, s = 1.4 \)). Number of segments 60-74 (n=9, \( \bar{x} = 64, s = 4.8 \)). Diameter at 4/5 140.0-196.5 \( \mu \text{m} \) (n=15, \( \bar{x} = 168.7, s = 18.7 \)). Clitellum diameter 150.0-208.9 \( \mu \text{m} \) (n=15, \( \bar{x} = 188.1, s = 19.1 \)) (Fig. 16). Setae from IV ventrally and XVI-XX laterally (n=13); no setae in XII of mature specimens. Setae broad at base with sloping toe, slight heel present in posterior setae (Fig. 17). Anterior ventral setae 32.6-53.3 \( \mu \text{m} \) long (n=10, \( \bar{x} = 44.2, s = 8.2 \)); midventrals from 53.2-60.8 \( \mu \text{m} \) in length (n=8, \( \bar{x} = 56.3, s = 2.4 \)). In ten posterior most segments ventral setae 56.6-71.3 \( \mu \text{m} \) long (n=21, \( \bar{x} = 63.4, s = 3.7 \)) and lateral setae 49.2-64.1 \( \mu \text{m} \) long (n=16, \( \bar{x} = 55.6, s = 4.2 \)). Head pore at anterior of peristomium. Spermathecal pores
Figure 16. *Grania americana*, specimen from Bermuda. Ventrolateral view of whole mounted specimen from prostomium to segment XIV. Scale equals 100 µm.
Figure 17. *Grania americana*, setae from various segments showing heel (h) and toe (t). Scale equals 20 μm.
paired, lateral, posterior to 4/5. Clitellum extension XII-XIII. Male pores, in line with ventral setae at mid XII. Female pores at 12/13.

**Internal somatic characteristics:** Brain indented posteriorly. Antero-medial brain vesicle present (Fig. 18) and divided into two distinct compartments, each containing two inclusions for a total of four. Inclusions in each compartment located at different levels, maximum diameter of inclusions 2.5-2.8 μm (n=6, $\bar{x}=2.7$, s=0.1) (Fig. 18). Total length across both compartments of head organ 20.5 μm (one specimen). Coelomocytes flattened and ovoid. Chloragogen cells, especially in posterior segments, very distinctive with peripherally clumped granules giving the cells the doughnut appearance of red blood cells, granules reddish-brown by transmitted light in both live and fixed specimens; outer surface of intestine, as a result, with leopard-spot pattern. Pharyngeal glands present as two paired lobules at 4/5; within V, two lobules ventral to spermathecae and one pair dorso-lateral in posterior of V; three laterally paired lobules in VI and one pair at 6/7. Nephridia observed in posterior segments, often unpaired, with small elongate funnel. Solitary internalized setae present in very few segments. Dorsal blood vessel transition around XXVI-XXVIII. Glands on nerve cord at XIV and/or XV.

**Internal reproductive characteristics:** Spermathecae (Fig. 16) paired in V with ental duct connecting ampulla to esophagus. Ampulla oviform with a maximum diameter of 42.0-90.0 μm (n=51, $\bar{x}=68.5$, s=11.8). Spermathecal ectal duct with two glands at pore, one small and one large (Fig. 19). Ectal duct of spermatheca consists of two distinct parts: shorter part immediately adjacent to
Figure 18. *Grania americana*, dorsal view of anterior brain vesicle showing inclusions. Scale equals 20 μm.
Figure 19. *Gmania americana*, dorsolateral view of spermatheca with glands at pore. Scale equals 20 μm.
body wall with external glands and with wide canal; ental or proximal part with canal occluded by glandular cells that run as longitudinal segments around a narrow canal (Fig. 19). Spermathecal ectal duct distal width 8.7-14.1 μm (n=6, \( \bar{x} = 10.8, s = 2.1 \)), ectal duct medial (max) 28.5-83.7 μm (n=8, \( \bar{x} = 42.7, s = 17.4 \)), proximal portion of ectal duct 21.2-46.0 μm in width (n=21, \( \bar{x} = 34.0, s = 6.7 \)).

Spermathecal ectal duct length 54.0-94.7 μm (n=16, \( \bar{x} = 74.3, s = 15.7 \)). Sperm rings present in walls of ampulla (Fig. 19). Sperm funnel length about eight times width, often displaced from XI to XIII (Figs 16 & 20A). Sperm funnel with a non-staining aglandular region immediately posterior to the collar; more posteriorly glandular tissue loose, giving funnel a very irregularly lobed surface (Fig. 20A). Sperm heads straight. Vas deferens non-muscular, 6.8-11.4 μm wide (n=8, \( \bar{x} = 9.6, s = 1.7 \)). Penial apparatus relatively uncomplicated with a distinct bulbous gland and an elongate, saccate dorsal extension from the male pore which extends along the lateral side of the gland (Fig. 20B); vas enters gland from dorso-medial side and runs to the lateral side, extending to the elongate extension. Ventrally, near epidermal pore, penial apparatus is narrower with very little glandular material and surrounded by very strong elaboration of the muscle bands of the body wall (Fig. 20B). Stylet absent. Sperm sac extends to XXI. Egg sac extends to XXVII.

Taxonomic remarks: Kennedy's (1966) original description of G. *americana* differs in a number of respects from the above description. This is probably due to Kennedy's shortage of good specimens, which limited collection of both morphometric and structural data. Kennedy did provide approximate
Figure 20A. *Grania americana*, dorsolateral view of male duct system and penial apparatus. Scale equals 20 μm.
Figure 20B. *Grania americana*, lateral view of penial apparatus. Scale equals 20 μm.
measurements for the overall length of the species (10 mm), the number of segments (50), and the ratio of length to width of the sperm funnels (several times longer than wide). However, the description was based on two specimens only. These were missing a few segments from their posterior ends and were eventually mounted in a macerating medium (pers. obs.).

The original description of *G. americana* states that the dorsal setae begin at XVI or XVIII (Kennedy, 1966). In our redescription, the “dorsal” setae are described as lateral and the range of segments at which they begin is extended to XVI - XX. The presence of pharyngeal glands in VII (Kennedy, 1966; Erséus, 1974) was not seen in the type material when it was re-examined by us. The peptonephridia originally noted for *G. americana* have since been determined to be absent from the genus *Grania* (Erséus & Lasserre, 1976) and what Kennedy observed, most likely, were the thick bundles of ducts running from the pharyngeal glands to the dorsal pharyngeal pad.

In more recent literature the taxonomic importance of penial apparatus structure (Coates, 1984) has become apparent, and therefore, *G. americana* which had a brief subspecies status, is not a subspecies of *G. macrochaeta* (sensu Lasserre, 1967). The penial apparatus of *G. americana* has a distinct bulbous gland, an elongate, saccate, epidermal invagination and is lacking a penial stylet. *Grania americana* also has ectal glands on the spermathecal ectal duct at the pore, contrary to Erséus and Lasserre’s (1976) statement that these were absent in *Grania*. Similar glands have been noted in other recently described species (Coates and Erséus, 1980; Erséus, 1980; Rota and Erséus,
1997) and the generic description amended accordingly. These glands are still visible in the type material of *G. americana*.

*Grania americana* differs from other Bermudian congeners by the possession of glands at the spermathecal ectal pore, lack of penial stylet and muscular vas deferens (Fig. 6), and the overall length of *G. americana* is much greater than that of the other species described from Bermuda. Also, *G. americana* possesses an anterior brain vesicle, as discovered and described as the “head organ” in other species of *Grania* by Rota and Erséus (1996, 1997). This organ is located in the anterior of the peristomium, just below the boundary with the prostomium, as an anterior, medial bulbous extension of the brain. Four refractile bodies are visible within the organ. Previous accounts of this structure in recent species descriptions (Rota and Erséus, 1996, 1997) have also noted the presence of four refractile bodies. However, Rota and Erséus (1996) also noted that some damaged specimens showed fewer inclusions or none. Possibly this organ has a chemosensory or geotactic function (Locke, In review, Chapter 3). This structure has only been reported for eight subtidal species of *Grania*, five from the Ross Sea, Antarctica, two from South Georgia in the subantarctic, and one from the western Atlantic. It is possible that *G. americana* shares a unique apomorphy with this subgroup of *Grania*, but is an intertidal representative of this lineage.

**Habitat and distribution:** Coarse intertidal sand within dissolutions at Ferry Point Bridge, Bermuda and rocky limestone beach area at Whalebone Bay and Rocky Hill Park, Bermuda. Habitat at type location subtidal, Kennedy (1966)
records that the original specimens were collected “off the coast”. Found in
samples with *G. laxarta*, various Tubificidae and Nematoda. Sampled from fine
to coarse sand, *Thalassia* beds, and shell rubble. Known from off the coast of
Bimini, Bahamas; Panama City, Miami and Hutchinson Island, Florida, USA;
Whalebone Bay and Rocky Hill Park, Bermuda; and Carrie Bow Cay, Belize.

**DISCUSSION**

This is the first addition to our knowledge of the species diversity within
*Grania* for the Northwestern Atlantic since Coates and Erséus (1985). Many
other species of the North Atlantic remain to be described (pers. obs.). Further
studies will assist in our understanding of the distribution, speciation patterns,
and evolution of this genus.

The distributions of *Grania* species found in Bermuda pose interesting
questions about speciation and dispersal patterns, especially due to the
relatively young geological age of Bermuda. *Grania americana*, *G. laxarta* and
*G. hylae* are all known from the Caribbean and Bermuda. This distribution
pattern is considered typical for Bermudian fauna (Sterrer, 1986). Absence of a
free swimming larval stage in the development of these worms makes it difficult
to explain how regular genetic exchange between Bermudian and coastal
populations is maintained. Genetic studies of Bermudian and Caribbean coastal
populations could reveal information about effective population sizes, frequency
of colonization events and whether the interchange is unidirectional, as would
seem likely due to prevailing currents. Morphological measurements for different
localities of the same species did not demonstrate notable differences.

The intertidal pools formed within dissolution tubes at Ferry Point Bridge
created an intertidal refuge for *G. laxarta* and *G. americana* rich in detrital matter. Healy (1996) noted the importance of crevices within the rocky intertidal as moist
micro-climates during low tide. The dissolutions serve the same purpose as
Healy's rocky crevices in that they protect from wave disturbance, displacement
by water currents and retain organic matter, but have high oxygen levels (Healy,
1996), conditions in which enchytraeids thrive (Healy & Coates, in press). These
small pots or tubes may also effectively isolate adjacent populations as the rock
surface between is very exposed and barren. This is in contrast to the subtidal
habitat occupied by *G. bermudensis* and *G. hylae*. These species are protected
from wave action by the greater depth of the water in which they are found and
the habitat is relatively continuous. The subtidal sites in Bermuda were located
to the side of major channels into bays and harbours. Tidal currents through
these openings bring nutrients and detrital matter to the areas, and these get
deposited in the slower water and back eddies that border the main channel.

The presence of *G. laxarta* and *G. americana* within the same samples
throughout the year conflicts with existing ideas about spatial separation and
ecological divergence between congeneric species. There is some evidence,
other than morphological discontinuities, to suggest that co-occurring populations
of the two species are biologically isolated by temporal separation of
reproductive periods. High numbers of mature specimens of each species were found at different times of the year.

The muscular, bipartite penial apparatuses found in *G. bermudensis* and *G. hylae* are different from the many types of glandular, bulb-like apparatuses previously described for *Grania* species (Coates, 1984). This form of penial apparatus also appears to be associated with species which possess dorsal spermathecal pores. It also is found in *G. monospermatheca*, which possesses a single dorsally displaced spermathecal pore. Coates (1984) had previously designated a penial bulb “Type 6” as glandular penial bulbs reduced or absent, aglandular sac often absent; with a long penial stylet plus considerable modifications to other parts of the vasa deferentia; representative taxa were, *G. bermudensis* and *G. monospermatheca*. This grouping included more structural diversity then the other “types” recognized. Additional information from the species described herein is considered and Coates (1984) “Type 6” is revised. Type 6 is described as: Bipartite, saccate penial apparatus lacking a distinct glandular bulb; saccate part with horizontal and erect parts. Stylet long and contained within vasa deferentia. Muscular modifications of the vasa deferentia. Spermathecal pores dorsal. Representative taxa: *G. bermudensis*, *G. monospermatheca*, *G. hylae*, and two undescribed specimens from Australia (Coates, 1984). Documentation of dorsal spermathecal pores and complex penial apparatuses in *G. stillifera* Erseus, 1990 and *G. inermis* Erseus, 1990 present the possibility of their being included within the Type 6 grouping also.
Other forms originally “lumped” in Type 6 await further clarification and typification.

The presence of an anterior brain vesicle in *G. americana* and *G. hylae* are the first records in readily collected species. This structure, first reported as the “head organ” by Rota and Erséus (1996), has only been found in one other species from the North Atlantic, *G. atlantica* (pers. obs. and Rota and Erséus, 1996). The function of the anterior brain vesicle remains unknown, but it appears to be statocyst-like in structure (pers. obs.). Phylogenetic studies of *Grania* species which possess this structure may provide ecological and functional hypotheses for the origin of such a structure in clitellates.

Although Erséus and Lasserre (1976) stated their understanding of the morphology of *Grania* had improved, they still believed that differences between distinct populations were small, sufficient only to justify their recognition as subspecies. However, Erséus and Lasserre (1976, p.131) were not fully confident about their species groupings as they stated that with further studies the reinstatement of their subspecies to species could easily be made. Erséus and Lasserre, 1976 also noted the importance of spermathecae in the taxonomy of enchytraeids. Locke and Coates (1998) resurrected *G. pusilla* from subspecies status; herein, *G. bermudensis* is recognized as even more distinctive and *G. americana* is resurrected from nomen dubium (Lasserre & Erséus, 1976) to full species status. The grouping *G. macrochaeta* sensu lato is too broad (Erséus, 1977). The variability of the details of spermathecal structures among these species plus the additional information relating the male
duct systems of the *Grania* species (Coates, 1984) provides sufficient information for the redesignation of all these *G. macrochaeta* subspecies as separate species. Only *Grania macrochaeta trichaeta* Jamieson, 1977 awaits a full redesription.
REFERENCES


Locke, J.M. In review. Ultrastructural studies of the statocyst of the marine enchytraeid Grania americana (Annelida, Clitellata).


Chapter 3

ULTRASTRUCTURAL STUDIES OF THE STATOCYST OF THE MARINE ENCHYTRAeid GRANIA AMERICANA (ANNELIDA: CLITELLATA)

ABSTRACT

Within the peristomium of the enchytraeid Grania americana Kennedy, 1966, lies a dorsal vesicular extension of the cerebral ganglion. Transmission electron and light microscopy were used to conduct a study of this region of the cerebral ganglion. The vesicular extension contains up to four electron dense granules, each in a separate, membrane bound chamber, surrounded by numerous sensory cilia. Cilia within the vesicle and the cerebral ganglion possess an 8+2 microtubule arrangement. Gravity receptor systems in some molluscs and polychaetes have vesicular statocysts containing statoliths surrounded by cilia. The ultrastructure of this element of the central nervous system in G. americana is consistent with statocyst structure of these other invertebrates, although it is a more complex, multi-chambered system. This is the first record of a statocyst in the Clitellata.

INTRODUCTION

In the continuing search for more characteristics to use in microdrile clitellate taxonomy and phylogenetic analysis, ultrastructural investigations can be very helpful. One region that may yield valuable information is the central
nervous system, in particular the supraoesophageal ganglion and its neural connectives. An example of this are the mushroom bodies found in polychaetes and arthropods (Strausfeld et al., 1996) which are used to support deep phylogenetic relationships of these taxa ( Strausfeld et al., 1995 ). Although recent reviews (Cook, 1971; Golding, 1974; Golding and Whittle, 1977; Mill, 1978; Jamieson, 1981; Jamieson, 1988, 1992) have provided information regarding general aspects of the clitellate nervous system, details are insubstantial. Accounts of the enchytraeid nervous system have focused on neurosecretion within the genus Enchytraeus Henle, 1937, (Deuse-Zimmerman, 1960; Gersch & Ude, 1967; Bianchi, 1974; Golding, 1974; Golding and Whittle, 1975, 1977) or have been derived from general clitellate morphology.

The brain of the interstitial, marine enchytraeid, Grania Southern, 1913, is large relative to the small size of the animal. It is a posteriorly bilobed structure which, as in other clitellates, connects to the subesophageal ganglion via circumpharyngeal connectives (pers. obs.; Mill, 1978). Recently, attention was drawn to the enchytraeid cerebral ganglion by Rota and Erséus (1996, 1997) who observed a new structure in the peristomium of certain Grania species. The structure, which Rota and Erséus (1996, 1997) termed the "head organ", is located just anterior to the brain in seven of nine subtidal Grania species reported from the Antarctic and subantarctic seas and in one widespread deep sea species, Grania atlantica Coates & Erséus, 1985. Only field preserved organisms were available for their studies. The head organ was described by Rota and Erséus (1996) as a middorsal vesicular body located within the
peristomium, containing a few refractile inclusions and it was supposed that it had a sensory function. Since the initial reports, it has been found in *Grania americana* Kennedy, 1966 and possibly also in *Grania hylae* (Locke & Coates, in press). Both *G. americana* and *Grania hylae* are readily available along the shores of Bermuda.

An investigation was undertaken to explore the structure and possible function of the "head organ" (c.f. Rota and Erséus, 1996,1997) in *G. americana*. This should prove valuable in the search for more characters to test evolutionary hypotheses of the Enchytraeidae and Clitellata and to determine their position among the Annelida (Rouse & Fauchald, 1995, 1997; Purschke, 1997; Westheide, 1997). This will also, undoubtedly, assist evolutionary and phylogenetic studies of *Grania*.

**MATERIALS AND METHODS**

Specimens of *G. americana* were collected intertidally from Ferry Point Bridge, Bermuda (32°21'90"N 64°24'70"W) for electron and light microscopy. Whole mounted material stained with borax carmine and mounted in Canada balsam from the collections of C. Erséus also was observed. For transmission electron microscopy (TEM) preservation, the anterior six segments were trimmed and fixed in either a) 2.5% glutaraldehyde in 0.2M phosphate buffer (7.0 pH); or b) 2.5% glutaraldehyde in seawater, followed by postfixation in 1% osmium tetroxide. Fixed specimens were then dehydrated in alcohol and embedded in Spurr's resin. For light microscopy, whole mounts were observed and sections
approximately 1μm were cut on a Reichert-Jung 'Ultracut E' microtome using glass knives, and stained with 1% toluidine blue in 1% sodium borate. An Olympus AX70 Provis microscope and a Toshiba 3CCD camera with Image-Pro Plus™ software were used for video microscope image capture analysis and morphological measurements. For TEM, ultra-thin sections approximately 50-80 nm thick, cut with diamond knives were collected on 300-mesh copper grids. Grids were stained for 30 min with 2% uranyl acetate in methanol, rinsed in distilled water and placed for 5 min in Reynolds' lead citrate, before rinsing again in distilled water. Sections were examined using a Hitachi H-7000 electron microscope, operated at 75 KV.

RESULTS

LIGHT MICROSCOPY

Light microscopy of whole mounts and thick sections revealed the presence of a membrane bound vesicle anterior to the brain, situated between the laterally paired, anterior, prostomial connectives (Fig. 1). This structure was present in all mature whole mounted and live specimens that were examined (over 40 specimens). The chestnut-shaped, chambered vesicle lies within the peristomium beneath a large epidermal coelomic pore (called the head pore in enchytraeids), dorsal and anterior to the anterior blood vessel bifurcation (Fig. 2). Longitudinal sections show the vesicle to be elliptical in shape from anterior to posterior. It is attached to the posterior bilobed brain by a narrow, spiraled
Figure 1. *Grania americana* whole mount illustration, emphasizing anterior segments, the prostomial region (p) and the chambered statocyst (so) anterior to the dorsal blood vessel (bv) bifurcation (bb). Scale equals 100 μm.
Figure 2. Light microscopy of chambered statocyst (so) anterior to blood vessel (bv) bifurcation, prostomial connectives (c) are visible and three of four statoliths are indicated by arrowheads. Scale equals 20 μm.
extension and anteriorly is secured to the dorsal body wall in the peristomium by a membrane or septum lying at the posterior of the prostomium (Fig. 3; see also Rota & Erséus, 1997, fig. 4). The distance across the vesicle is 20.5-22.7 μm (n=4, \( \bar{x} = 21.8 \), s=1.0) and dorsal to ventral depth is 10.7-12.5 μm (n=4, \( \bar{x} = 11.7 \), s=0.8). The anterior to posterior dimension of the elliptical vesicle is 28.7-32.1 μm (n=4, \( \bar{x} = 30.9 \), s=1.5). The posterior region of the vesicle is composed of a number of cells. Cells are absent from the anterior region of the vesicle. The vesicle consists of four compartments, each of which houses a single globular granule, which is refractile in whole mounts (Fig. 2) and densely stained by toluidine blue in sodium borate in sections (Fig. 3). Two granules lie in the left half of the vesicle and two in the right, on each side the granules are located on different levels. The maximum diameter of the granules is 2.5-5.5 μm (n=12, \( \bar{x} = 3.1 \), s=1.07). Four refractile granules were consistently observed in all the material examined by light microscopy.

**TRANSMISSION ELECTRON MICROSCOPY**

Anteriorly the vesicle is divided into four double membrane-bound chambers. The electron dense granule in each chamber is surrounded by numerous cilia. Four cells are present on each side of the vesicle, two of which are possibly supporting cells and the other two ciliated sensory cells (Fig. 4). Cilia extend from the lateral sensory cells toward the granules. Two multi-ciliated sensory cells containing numerous mitochondria were seen in the posterior region of the vesicle in a lateral section. Of the posterior ciliated sensory cells,
Figure 3. Longitudinal section through prostomial region. Narrow, spiraled extension (e) to bilobed brain (b) above blood vessel (bv), anterior attachment of statocyst to body wall by membrane (mb) and statoliths (s) are visible. Scale equals 20 μm.
Figure 4. Lateral cells (lp) of statocyst vesicle. Two regions of cilia (c), lateral sensory and supporting cells (lp), membrane attachment (a) to body wall and dorsal blood vessel (bv) are visible. Magnification 3750X.
one had four ciliary basal bodies, and the other only one basal body (Fig. 5). No ciliary rootlets were seen. Microvilli surround the base of each cilium (Fig. 5). Approximately 15 cilia seem to originate from a depression in the cell apex (Fig. 6). Cilia from the posterior sensory cells project forward toward the individually chambered granules, apparently with one posterior cell serving each granule. The longest length of cilium observed is 3.0 μm. The cilia possess an 8 peripheral and 1 central doublet arrangement with no singlets visible. Diameter of cilia cross sections is 0.2-0.3 μm (n=18, $\bar{x}$=0.3, s=0.03). Globular secretions were visible near the cell membrane of the posterior ciliated sensory cell containing four basal bodies (Fig. 7). The anterior lateral portion of the vesicle possesses a glial cell associated with the vesicle's anterior attachment (Fig. 8).

The attachment of the vesicle with the brain is narrower than the vesicle. It appears as a spiraling cellular bundle with muscle present dorsally and beneath which lie several cells with numerous mitochondria (Fig. 9). In this region cilia of 8+2 doublet arrangement are present as bundles that run transverse within two membrane bound chambers (Fig. 10).

Compact bundles of cilia with the same 8+2 microtubule arrangement are also present where the posterior bilobes of the brain secure onto a muscular attachment. Membrane junctions are visible between the posterior bundle of cilia and the muscular attachment (Fig. 11).
Figure 5. Ciliated sensory cell with four basal bodies indicated by arrows. Microvilli are shown to the left of asterisks and a statolith (s) is visible. Transverse cilia (c) within chambers are also shown. Magnification 14000X.
Figure 6. Sensory cell with cilia originating from apex indicated by arrow. Magnification 5900X.
Figure 7. Cellular secretions indicated by arrowheads. Magnification 7500X.
Figure 8. Anterior glial cell (gc) and cilia (c) within individual statolith (s) chambers which are separated by a double membrane (indicated by arrow). Magnification 9333X.
Figure 9. Spiraled medial extension of bilobed brain showing dorsal muscle (dm), location of cilia (c) and dorsal blood vessel (bv). Magnification 2400X.
Figure 10. Cilia (c) running transversely through the medial spiral extension. Magnification 15469X.
Figure 11. Posterior region of brain showing muscle (m), membrane junctions (indicated by arrows) and cilia (c). Magnification 8455X.
Three tufts of cilia on the anterior ventral peripheral region of the prostomium are visible in light sections. These equally spaced groups of multiple cilia project from cells of the epidermis through the cuticle (Fig. 12).

**DISCUSSION**

A statocyst is any sense organ that aids orientation with respect to gravity. Typically statocysts are composed of a chamber containing granules of higher density than the fluid within the chamber. The granules, or statoliths, are in contact with receptor cells of the chamber walls. Within invertebrates, the most common statocyst receptor cells are ciliated sensory cells (Bundelmann, 1988) but in some nemertines and turbellarians the receptor systems are double membrane chambers composed of specialized receptor cells which are not ciliated (Reiger *et al.*, 1991; Turbeville, 1991).

Gravity receptor systems with ciliated sensory cells are reported specifically from bivalve and nudibranch molluscs, where they are located in the muscular foot, associated with the pedal ganglion (Barber & Dilly, 1969; Chia *et al.*, 1981; Morton, 1985), in hydromedusae, associated with the tentacular bases (Singla, 1975), and in Ctenophora in the apical organ (Aronova, 1974; Bundelmann, 1988).

Certain tubicolous and burrowing polychaetes also are frequently mentioned in general references as having statocysts; these include species of families Arenicolidae, Terebellidae, Paraonidae (genus *Aricia*), Orbiniidae, and Sabellidae (Bullock, 1965; Dales, 1967; Mill, 1978; Storch & Schlötzer-Schrehardt, 1988). However, primary
Figure 12. Longitudinal section through prostomial region of *Grania americana*. Arrowheads indicate epidermal ciliated papilla. Scale equals 20 μm.
literature references were only found for light microscope observations of such structures in Sabellidae (Buddenbrock, 1913), and ultrastructural studies for Terebellidae (Heimler, 1983) and Arenicolidae (Titova & Kharkeevitch, 1973; Kharkeevitch & Titova, 1974; Nowak, 1978). Paired subepidermal, ciliated statocysts in the polychaete Arenicola marina (Linnaeus, 1758) are reported as occurring dorsally in the buccal segment (Titova & Kharkeevich, 1973; Kharkeevich & Titova, 1974; Nowak, 1978). These statocysts are open to the environment by narrow canals, formed from epidermal cells, but may be closed in older individuals. The statoliths of A. marina consist of sand grains presumably brought into the statocyst chamber through the epidermal opening. Receptor cells lining the statocyst chamber or vesicle bear up to six cilia, and are interspersed with supporting cells (Titova & Kharkeevich, 1973). Paired, open, ciliated statocysts have also been found on the first peristomial segment in the terebellid, Lanice conchilega (Pallas, 1766). Sensory cells bear 5 or 6 cilia which lack rootlets (Heimler, 1983; Storch & Schlötzer-Schrehardt, 1988). The epidermal origins of these sensory structures are clear due to their continuity with this layer. The only known taxon of interstitial polychaetes reported as possessing statocysts is Protodrilus (Polychaeta: Protodrilidae) (Salensky, 1907; Purschke, 1990a). However, these "so called statocysts" have since been determined not to function as statocysts (Purschke, 1990b).

Prior to the discovery of the “head organ”, typical statocysts with consolidated statoliths were not reported within the Clitellata (Jamieson, 1992). Rota and Erséus (1996, p. 181) state: “In this phylum [Annelida], no such structure has ever been observed as deep inside the body and closely adjacent
to the cerebral ganglion as the *Grania* head organ." They proposed that the head organ "may indeed appear like a complex statocyst" (p.181). The "head organ" of *G. americana* is a complex multicellular, ciliated statocyst composed of sensory and supportive cells. The refractile inclusions observed by Rota and Erséus (1996) are electron dense granules or statoliths surrounded by numerous sensory cilia. The statolith composition is still unknown, but they are possibly secreted by the associated sensory cells (Fig. 8). The electron density of the granules suggest that they are unlikely to be composed of lipid.

The statocyst of *G. americana* is multi-chambered, each statolith is housed within a separate chamber. If the vesicle is envisioned with right and left lateral divisions, each of the two statoliths in each lateral division are located at different levels; the two statoliths of the right side are located posteriordorsal and anterio-ventral, respectively, and the two statoliths on the left side are posterioventral and anteriodorsal (Fig. 2). Sections into one lateral half showed at least two posterior sensory cells and two lateral sensory cells, with lateral supporting cells. Assuming the vesicle is symmetrical, these cells would be present on both the right and left sides. Thus, it is proposed that each chambered statolith is surrounded by cilia from at least one posterior sensory cell and one lateral sensory cell.

One possibility for how the structure functions is that the four statoliths work in combination to signal different orientations of the worms head. A glial cell is present anteriorly within each lateral division and possibly aids in
transmission of information from the statocyst structure to muscles which effect changes in orientation.

The multiciliate sensory cells are typical epithelial cells with microvilli surrounding the cilia. The term “stereocilia” is deeply embedded in the literature for referring to longer filamentous microvilli that surround the base of cilia. This term is confusing. These are not cilia: they lack a basal body, are incapable of movements and do not have a doublet microtubule arrangement, all of which are characteristic of cilia. This misleading term arose before the advent of electron microscopy, which has since proven that “stereocilia” are microvilli, membrane bound cytoplasmic extensions.

At least four cilia originate from one cell, as is evidenced by the number of ciliary basal bodies. However, due to the number of cilia (15) seen originating from the cell apex (Fig. 6) and surrounding each granule, it is likely that each cell has more than four cilia. It is possible that more ciliated sensory cells may be involved, however my observations do not support this.

In a few invertebrate statocysts, presence of one or more statoliths within one chamber has been observed (Bundelmann, 1988). However, the statocyst of G. americana is multi-chambered, with each chamber holding it's own statolith. Nemertine worms also are known to have mulitple (2-6) statoliths, each enclosed in a separate cell cavity, however these are not ciliated organs (Brüggemann & Ehlers, 1981). Four statoliths housed in separate ciliate chambers may represent a highly evolved equilibrium receptor system and indicate sophisticated burrowing behaviours.
Cilia within the supraesophageal ganglion have been reported previously for the oligochaete clitellates (Zimmerman, 1971; Golding and Whittle, 1975). In four aquatic oligochaetes, including an unidentified enchytraeid referred to as *Enchytraeus* “alba,” for which no micrographs were shown, cilia associated with extracerebral and sensory cells in the anterior and posterior brain regions have been noted (Golding & Whittle, 1975). These cilia had a 9+2 arrangement and no pigment cells or granules were associated with the ciliary bundles. Due to the compact bundled appearance of these cilia, they seem similar to those found in the posterior region of the brain in *G. americana*, however the microtubule arrangement differs (Fig. 10). The proposed photoreceptor function for these cerebral cilia (Golding & Whittle, 1975) remains uncertain (Jamieson, 1981). Jamieson (1992) suggested instead a statocyst function based on the presence of fine particles within the space separating the cilia. However, in the figures given in Golding and Whittle (1975), I do not observe the “fine particles” noted by Jamieson, and therefore disagree with his suggestion that these cells form a statocyst. The sensory function of these bundles of cilia is still unknown.

The irregular microtubule arrangement of the sensory cerebral cilia of *G. americana* is found in other clitellates. Storch (1972) described cilia of sensory cells with an 8+2 microtubule arrangement on the prostomium of the lumbriculids, *Lumbriculus variegatus* and *Rynchelmis limosella* [Note in Mill, 1978, p.72 this species is reported as a priapulid, with its name misspelled as “Rychelmis”]. In *L. variegatus*, cilia running parallel to the body wall commonly are reported with microtubule arrangements of 9+0 and 8+2 (Knapp & Mill, 1971;
Mill, 1978; Jamieson, 1988). In *R. limosella* cilia were also observed to have an 8+2 arrangement of microtubules, the central 2 often form a doublet which also is sometimes the case in *Lumbriculus* (Jamieson, 1981; Storch, 1972). Cilia on cerebral cells in *L. variegatus* arise in a deep depression of the cell apex and the apical part of the cell is filled with mitochondria with glycogen rosettes (Storch, 1972). This corresponds with ciliary basal body positions and organelles observed in the ciliated sensory cells associated with the statocyst of *G. americana* (Figs 5&6).

The multi-ciliated papillae projecting from the epicuticle of the prostomium of *G. americana* can also be seen on whole-mounted material and are more numerous than the three observed in sections. These papillae may have a chemosensory or thigmosensory function and are like those found in polychaetes and other oligochaetes (Jamieson, 1981).

Other sensory structures known on the prostomial region of annelids are photoreceptors and the chemosensory nuchal organs of polychaetes. Both ciliary and rhabdomeric photoreceptors (Zahid & Golding, 1974; Eakin & Hermans, 1988; Wilmer, 1990) occurring epidermally and cerebrally are reported in annelids. There is less diversity reported for photoreceptors of clitellates (Jamieson, 1988). Within the Enchytraeidae there are only records of epidermal photoreceptors in *Enchytraeus fragmentosus* Bell, 1958 (Bradke, 1962). Photoreceptors are also known to occur in the epidermis of the lumbricid *Eisenia fetida* (Savigny, 1826) and in ganglionic swellings and in the posterior cerebral ganglion of the lumbricid *Lumbricus terrestris* Linneaus, 1758 (Jamieson, 1981).
The photoreceptors described for enchytraeids and lumbricids do not possess granules or a closed vesicular structure like the statocyst of G. americana. Also the Grania species possessing statocysts are unpigmented, subtidal and/or infaunal inhabitants. Light is probably not a significant cue for orientation within the sediments, although it may be an important cue to remaining below the sediment surface in intertidal or shallow water habitats.

**PHYLOGENETIC SIGNIFICANCE**

The nuchal organ has been proposed as the single anatomical synapomorphy for the Class Polychaeta of the annelids (Rouse & Fauchald, 1995; Rouse & Fauchald, 1997; Purschke, 1997); therefore, it is important to evolutionary hypotheses of annelids to know whether the statocyst of G. americana has a similar origin to nuchal organs. It should be noted that molecular data do not support recognition of either Polychaeta or Annelida as monophyletic (Trapido-Rosenthal et al., 1997; McHugh, 1997; Giribet & Ribera, 1998) and structural homologues of chemosensory nuchal organs have not been found in the clitellates (Purschke, 1997).

Nuchal organs are epidermal structures and are usually paired dorsally on the prostomium or peristomium. They may be permanent invaginations or form eversible pockets. They are formed by supporting and ciliated sensory cells of the epidermis (Storch & Schlötzer-Schrehardt, 1988). They differ from the statocyst of G. americana, in that they do not possess granules, and they are ciliated pits, bands or folded lobes on or connected to the exterior surface of the
animal. Their diversity and rather general characterization even suggests they might not be well defined homologues for the polychaetes.

The position of the head pore in relation to the statocyst in *G. americana*, suggests the possibility of origination of the statocyst of *Grania* from an epidermal invagination, as in the polychaete *A. marina*, which in fully developed specimens is evidenced only by the surface opening and a submerged membrane-bound vesicle, the connection having disappeared. Developmental studies of the head pore and vesicle could prove interesting.

*Grania americana* is the only reported intertidal annelid species with an anterior cerebral statocyst. The statocyst of the various *Grania* species may well be a synapomorphy for the genus, and provide clues to invasions of shallow water or deep water habitats by *Grania* species. *Grania hylae*, a shallow subtidal species from Bermuda also has a “head organ” but with only one granule. Phylogenetic analyses may show the origin of this structure to be distinct from that in *G. americana*. This region of the central nervous system of *Grania* species remains a potential source of more taxonomic and phylogenetic characters and has a previously unrecognized complexity.

It is likely that some known species other than those already reported possess statocysts. However, since seeing these structures is dependent upon preservation and orientation of the organism, as well as the intention of looking for them, their observation in other species of *Grania* awaits specific reexamination and collection of additional specimens.
REFERENCES


Microfauna Marina 4:121-133.

body: its functional roles, evolutionary enigmas and mistaken identities. In:

Strausfeld, N.J., R.S. Gomez and L.C. Hansen. 1996. Neural arrangements are
evolutionarily conserved in olfactory and sensory association centers of

Paraphyly of the annelids: all annelids are not segmented worms. Bull.
Mar. Sci. 60:624.

Microscopic anatomy of invertebrates. 3:285-328.

organization of the statocyst of the lugworm Arenicola marina. J. Evol.
Biochem. Physiol. 9:445-452.


A KEY TO THE SPECIES OF GRANIA (ENCHYTRAЕIDAE: CLITELLATA) OF EASTERN NORTH AMERICA AND NEARBY COASTAL AND CARIBBEAN POPULATIONS

ABSTRACT

The marine, clitellate genus *Grania* (Southern, 1913) is a globally prevalent group of enchytraeids within intertidal and shallow subtidal habitats, but seems more diverse in tropical and subtropical locations. Eight species have been described from eastern, coastal North America and the nearby Atlantic island of Bermuda. Due to the rising numbers of species within the genus over the last twenty years and an increase in known distributions, a basic, artificial, dichotomous key using anatomical characters of *Grania* is needed for species identification. The first key of this sort is presented here for species of the genus *Grania* with known distributions within Eastern North America and the neighboring island of Bermuda.

INTRODUCTION

Enchytraeids are characterized morphologically by their setal shapes and reproductive structures. When present, setae are straight, sigmoid or bent, rarely possessing a nodulus and rarely forked. Spermathecae have an anterior location (segment V) and male pores are on segment XII, glandular sperm
funnels and a male penial apparatus are also present. Within marine habitats there are only seven known genera (Healy & Coates, in press), as opposed to about 22 terrestrial and freshwater genera. The genera with marine species are, *Achaeta* Vejdovský, 1878, *Enchytraeus* Henle, 1837; *Lumbricillus* Örsted, 1844, *Stephensoniella* Cernosvitov, 1934 (amended, Coates, 1983); *Marionina* Michaelson, 1889, *Grania* Southern, 1913; and *Randidrilus* Coates & Erséus, 1985; all of which have been recorded from the Western Atlantic.

**GRANIA OF THE NORTHWEST ATLANTIC**

The coastal habitats of the Western Atlantic have escaped major exploration of the marine enchytraeid Clitellata. Within the cursory taxonomic surveys of marine enchytraeids, the genus *Grania* has been diverse and relatively abundant (Kennedy, 1966; Lasserre, 1971; Erséus and Lasserre, 1976; Lasserre & Erséus, 1976; Coates and Erséus, 1985). These reports include species found along eastern North American coastlines, on the nearby Atlantic island of Bermuda, and in the Caribbean. It is expected that many new species remain to be discovered in this region as new habitats are explored and taxonomic character information improves.

*Grania* are infaunal, (sometimes interstitial) worms. Generally they are found in coarse biogenic sediments both intertidally and subtidally. The worms are microscopic, with an average length of approximately 7 mm and diameter of 0.12 mm. *Grania* are distinguished from other marine enchytraeids by their robust, straight, pointed setae which occur singly both ventrally and laterally.
The setae may be lacking completely in some species. The unpigmented body wall of *Grania* is rigid, due to the thick cuticle they possess causing their movements to be stiff and inflexible, like a nematode. The diagnostic characteristics for species are the shape and distribution of setae, the spermathecal shape and structure of the male duct system: the sperm funnel, vasa deferentia and penial apparatus (Fig. 1). Specimens must be mature to be identified to species.

*Grania americana* Kennedy, 1966, was the first record of an enchytraeid in a continental shelf habitat of Atlantic North America (Coates & Erséus, 1985). A few years later, an extensive report by Lasserre (1971) working at the Marine Biological Laboratories of Woods Hole, Massachusetts, recorded three subtidal enchytraeid species, one of which was *Hemigrania postclitellochaeta*. The genus *Hemigrania* has since been disbanded (Erséus and Lasserre, 1976) and *Grania postclitellochaeta* does not occur in North American waters (Coates & Erséus, 1985). However, the area sampled by Lasserre, remains the most northern known limit for *Grania* in eastern North America. In 1976, Erséus & Lasserre made additions to the North Atlantic fauna with records of *Grania monospermatheca* Erséus & Lasserre, 1976, from Cape Cod, Massachusetts, and *Grania bermudensis* Erséus & Lasserre, 1976 (Locke & Coates, in press) from the Atlantic sub-tropical island of Bermuda. Coates and Erséus (1985) described three new species, *G. reducta*, *G. atlantica* and *G. levis*, from off the northeast USA. The enchytraeid genus, *Randidrilus*, also described from the North American coast by Coates and Erséus (1985) is quite similar to *Grania* in
Figure 1. General *Grania* morphology, based on *Grania longiducta*, illustrating main diagnostic characters. Scale equals 100 μm.
morphology and distribution. Due to its common occurrence in samples with Eastern North American *Grania*, the two *Randidrilus* species of the region are also included in the key presented here. The most recently reported species from the Western Atlantic region are *G. laxarta* and *G. hylae* from Bermuda (Locke & Coates, in press) (Fig. 2).

Cook and Brinkhurst (1973) published a report on the marine oligochaetes of the Northeastern United States in which they presented information in standard couplet key format for four families, Enchytraeidae, Naididae, Tubificidae and Megascolecidae (= Acanthodrilidae). *Grania* was represented within the key by a single species, *Hemigrania postclitellochaeta* (Knöllner, 1935). As noted above, the taxonomic status of this species has since been amended (Coates & Erséus, 1985). A modern, comprehensive key does not exist for *Grania* of the northwestern Atlantic or any other enchytraeids for that matter, as recent key-makers (Brinkhurst, 1986; Milligan, 1996; Brinkhurst & Kathman, pers. com.) have avoided this task. Many *Grania* species are now described from this extensive region (Coates & Erséus, 1985; Locke & Coates, in press) and, most definitely, there are several more species awaiting discovery beyond the current northern limit for *Grania* in Eastern North America.

The following key has been designed to assist in the identification of the 11 species of *Grania* and *Randidrilus* recorded from the eastern coast of North America, Bermuda and the Caribbean. Information in the key should prove beneficial for distinguishing species already present and enable the recognition of species never before described from the region.
Figure 2. Distribution of *Grania* species within the Western Atlantic and Caribbean.
RECOGNITION OF GRANIA

Combinations of characters can be used to distinguish the marine enchytraeid genera. Where I have included them, unique characteristics are indicated in bold in the following descriptions of genera. These characteristics may not occur in each species now placed in one of these genera.

_Achaeta_ species totally lack setae and many have _setal glands_; spermathecae are in V or extend posteriorly to X and are without an ental connection to the esophagus; the brain is convex posteriorly; sperm sac is small and compact and penial apparatus is formed by several small, gland like structures.

_Enchytraeus_ species possess paired, unbranched peptonephridia; setae are straight with 2 or more in a bundle; glands are present at the spermathecal ectal pore; brain is rounded posteriorly and the penial apparatus may be either a single compact gland or a row of glands along the ventral body wall.

_Lumbricillus_ species have _lobed seminal vesicles_; body sometimes with colored pigment; usually sigmoid setae, more than two per bundle; glands are present at spermathecal ectal pore; and the brain is incised posteriorly.

_Marionina_ species have straight or sigmoid setae, and sometimes no setae, single, paired or multiple per segment; spermathecae are small, with glands along spermathecal ectal duct and at pore; brain incised or rounded posteriorly; nephridial canal extending anterior to coelomic septum; and anterior _dorsal blood vessel bifurcation posterior to peristomium with lateral loops_.

_Stephensoniella_ species have bundles of only two or three slightly sigmoid
setae; single compact penial glands; large compact pharyngeal glands and thickened septa; and spermatheca usually with globose or saccate diverticulum.

Randidrilus species have single, ventrolateral setae present as far forward as II; one or two pairs of spermatheca; and penial apparatuses with two separate, compact glandular masses.

Grania species have stout, single setae, sperm rings; elongate posteriorly extending sperm sacs & egg sacs; and complex penial apparatuses, sometimes with stylets.

DEFINITIONS AND DIAGNOSTIC CHARACTERS

ampulla: the bulbous portion of the spermatheca located in V. Sperm donated by another worm is stored here until needed for fertilization. It is attached to the gut by an ental duct and opens to the exterior of the worm by way of the ectal duct. Sperm rings are often visible within the ampulla (Fig. 3).

bi-partite sac: type of penial apparatus, present in XII. Consists of two parts joined at an angular bipartite union. One part is an erect invagination of the male pore, it unites with a ridged contracted sac which can expand. Stylet penetrates the ridged sac (Fig. 4).
Figure 3. Spermathecae, morphological illustrations. A. *Grania americana* with glands (g) at ectal pore, B. *Grania hylae* with narrow ectal duct (e), and C. *Grania laxarta* with distinct dilation (d) of ectal duct. Also illustrated are the ampulla (a) and ental duct (en). Scale equals 20 µm.
Figure 4. Male apparatuses A. Bulbous gland of *Grania laxarta* and B. *Randidrilus codensis*, and C. Bipartite sac of *Grania bermudensis*. Note anterior gland (ag), stylet (st) and vasa deferentia (v). Scale equals 20 μm.
bulbous gland: type of penial apparatus, present in XII. Consists of a glandular portion and an extended invagination of the male pore. Stylet may be present (Fig. 4).

clitellum: in mature specimens extends from XI-XIII as glandular epidermal cells of the body wall. Male reproductive organs are present within the area. No setae are present on segment XII (Fig. 1).

ectal duct: part of the spermathecae in V. Connects the external spermathecal pore to the ampulla. Sperm from a donating worm is transported through the ectal duct to the ampulla (Fig. 3).

ental duct: portion of spermatheca in V. Attaches the ampulla to the gut, the function for such a connection, if any, is unknown (Fig. 3).

ectal glands: present in some species at the spermathecal ectal duct pore in V (Fig. 3).

head pore: unpaired opening to the exterior, occurs dorsally within the anterior region of the peristomium.

penial apparatus: male reproductive system located from XI-XII. It is a paired system of free floating sperm funnels, extending from 11/12, each attached to a bulbous gland or bipartite sac by way of a vas deferens. A stylet may be present (Fig. 4).

setae: straight, pointed, rod-like projections, tips of which protrude from the body wall. Epidermal in origin. Occur singly, generally four “bundles” occur per segment, two ventral and two dorsal. (Fig. 5).
Figure 5. A. Position of ventral setae (s) in worm body. B. Setae, illustrating heel (h) and toe (t) characteristic of some species. Scale equals 20 μm for B only.
spermathecae: present in V. Female reproductive structures, consist of three main parts: ampulla, ectal duct and ental duct (Fig. 3).

statocyst: anterior vesicular portion of bi-lobed brain. Oriented under head pore and in front of anterior blood vessel bifurcation. Statoliths should be visible (Fig. 6).

stylet: tapering tube-like structure associated with the male penial apparatus of some species. Present in the bulbous gland or within the vas deferens of a bipartite sac apparatus (Fig. 4).

vasa deferentia: paired tubular extensions that join sperm funnels to penial apparatus. Sperm travel through the ciliated canal. Muscular modifications are often present as well as heavy nucleation. Present in XI-XIII. Muscular modifications may be present as, constrictions, knot, spiral, and/or longitudinal bands (Fig. 7).

MATERIALS AND METHOD

Recognition of the characters in the key requires a compound microscope. Either live specimens or dehydrated, cleared, stained and mounted specimens can be used. The following dichotomous key should be used in conjunction with the definitions and diagnostics section. The numbers in parentheses after the main couplet indicate the couplet from which that particular point in the key was reached.

The construction of the key was based on examination of whole-mounted material of Grania and Randirilus from the United States National Museum of
Figure 6. Chambered statocyst (cv) anterior to blood vessel (bv) bifurcation. Three of four statoliths (s) are clearly shown. Scale equals 20 µm.
Figure 7. Male duct system of A. *Grania bermudensis* and B. *Grania americana*, illustrating vasa deferentia (v), muscular modifications (mm), sperm funnel (sf), bulbous gland (bg), bipartite sac (bp) and stylet (st). Scale equals 20 µm.
Natural History (USNM), Washington, D. C. and the Royal Ontario Museum (ROM), Toronto, Ontario. These included *Grania levis* USNM 96509, *Randirilus quadririthecatus* USNM 96498 and *R. codensis* ROMIZ 12177 (5 specimens).

The remaining specimens of North American *Grania* observed were from the collections of C. Erséus, K. A. Coates and personal collections.

**KEY FOR GRANIA**

The hierarchy of the following key is wholly artificial.

1. A. Ventral setae occurring before clitellum............................................2
   B. Ventral setae absent or only post-clitellar........................................7

2. A. No glands present at spermathecal ectal pore.................................5
   (1) B. Glands present at spermathecal ectal pore......................................3

3. A. Statocyst present, anterior to brain,
   Penial apparatus with single bulbous gland
   Stylet absent.................................................................*Grania americana*
   (2) B. Statocyst not present,
   Bulbous gland divided into anterior and posterior glands
   Stylet absent............................................................................4

4. A. Two pair of spermathecae present in V and VI,
   Ventral seta in II, absent in III to XXI-XXVII,
   Lateral setae absent..............................................................*Randirilus quadririthecatus*
   (3) B. One pair of spermathecae in V,
   Ventral setae from II,
   Lateral setae absent..............................................................*Randirilus codensis*
5. A. Spermathecal ectal pores dorsal,
   Penial apparatus a bi-partite sac, stylet present......................6
   B. Spermathecal ectal pores lateral,
   Penial apparatus bulbous, stylet absent.............................10

6. A. Extreme muscular modifications of vasa deferentia,
    No statocyst anterior to the brain...................................Grania bermudensis
   B. Muscular constrictions of vasa deferentia,
    Statocyst anterior to brain........................................Grania hylae

7. A. Lateral, paired spermathecal pores,
    Penial apparatus bulbous...............................................8
    B. Dorsal, single spermathecal pore,
    Penial apparatus a bi-partite sac.................................Grania monospermatheca

8. A. Penial stylet absent................................................Grania reducta
    B. Penial stylet present................................................9

9. A. Setae lacking entirely.................................................Grania levis
    B. Setae only post-clitellar........................................Grania longiducta

10. A. Bipartite spermathecal ectal duct
    Statocyst present..................................................Grania atlantica
    B. Distinct dilation midway along spermathecal ectal duct,
    Statocyst absent ..................................................Grania laxarta
**ANNOTATED SYSTEMATIC LIST**

*Grania americana* Kennedy, 1966. Type locality: 0.5 km from North Entrance point West side of North Bimini. Intertidal. Known from Florida, USA, Ferry Point Bridge and Whalebone Bay, Bermuda and Carrie Bow Cay, Belize. Refer to Locke and Coates (in press).

*Grania atlantica* Coates & Erséus, 1985. Type locality: Continental slope off Massachusetts, 1102 m. Subtidal. Known from off New Jersey, SW of Ireland (NE Atlantic), N of Surinam, off Guinea (W Africa) and Luanda, Anglola. Refer to Coates and Erséus (1985). Additional comments on the species: The anterior bilobed brain region consists of a thin extension which connects to an anterior statocyst, previously referred to as the “head organ” (Rota & Erséus, 1996). The statocyst differs from that of *G. americana* in that the statocyst vesicle appears more elongate in shape than bulbous and the medial extension is much longer (pers. obs.). Three to four statoliths are apparent, although their position seems to differ from *G. americana*. The statocyst was not visible in the majority of material. It was observed in only 3 of 21 specimens.
Grania bermudensis Lasserre, & Erséus, 1976. Type locality; Castle Island, Bermuda 32°20'40"N, 64°40'25"W, coarse coral sand with ripple marks, 8 m. Subtidal. Known only from East Castle Roads, Rushy Island, a patch reef 100 m south of Rushy Island and Paget Island, Bermuda. Refer to Locke and Coates (in press).

Grania hylae Locke and Coates, in press. Type locality: ENE of Paget Island, Bermuda, 32°22'38"N. 64°38'57"W, medium to coarse coral sand, 17 m. Intertidal and Subtidal. Known from Rocky Hill Park, Castle Island, Bailey’s Bay, and SE Paget Island, Bermuda and Fowey Rocks, Miami, Florida, USA. Refer to Locke and Coates (in press).

Grania laxarta Locke and Coates, in press. Type locality: Ferry Point Bridge, Bermuda. 32°21'70"N, 64°42'80"W, coarse biogenic sands within dissolution tubes. Intertidal. Known from Whalebone Bay, Pearl Island, Ferry Reach and Smith’s Sound, Bermuda and Carrie Bow Cay, Belize. Refer to Locke and Coates (in press).

Grania longiducta Erséus & Lasserre, 1976. Type locality: Cape Cod Bay, Massachusetts, 17.1 m. Subtidal. Known from George’s Bank (SE of Massachusetts), off New Jersey, off Delaware, Maryland. Refer to Coates and Erséus (1985). Additional comments on the species: Within the anterior region of the bilobed brain a narrow, possibly spiraled extension was visible, however a statocyst was apparently lacking anterior to this in 13 specimens examined.

Grania monospermatheca Erséus & Lasserre, 1976. Type locality: Cape Cod Bay, Massachusetts, sand with some gravel, 3.4 m. Subtidal. Known from off New Jersey, Maryland, North Carolina, NE of Oregon Inlet, Beaufort, North Carolina and Biscayne Bay, Florida. Refer to Coates and Erséus (1985). Additional comments on the species: The penial apparatus is classified here as a bipartite sac which lacks a bulbous gland and contains a long stylet within a modified vas deferens, also known as Type 6 (Coates, 1984; Locke & Coates, in press). A statocyst was apparently lacking in the 40 specimens observed.

Grania reducta Coates & Erséus, 1985. Type locality: Off Maryland, medium to coarse sand, 29 m. Subtidal. Known from Delaware/Maryland, and off New Jersey. Refer to Coates and Erséus (1985). Additional comments on the species: Statocyst apparently lacking (2 specimens).
Randidrilus codensis Coates and Erséus, 1985. Type locality: Cape Cod Bay, Massachusetts, USA; 6.7 m. Subtidal. Known from off of New Jersey and Maryland, USA. Refer to Coates and Erséus (1985). Additional comments on the species: Statocyst not observed (6 specimens).

Randidrilus quadrithecatus Coates and Erséus, 1985. Type locality: off New Jersey, medium to course sand, 58 m. Subtidal. Known from Maryland. Refer to Coates and Erséus (1985). Additional comments on the species: Statocyst apparently lacking from Holotype.
REFERENCES


Chapter 5

RELATIONSHIPS AND ORIGINS OF GRANIA SPECIES IN BERMUDA:
PHYLOGENETIC ANALYSIS OF GRANIA (ANNElDA: CLITELLATA:
ENCHYTRAeIDAE) FROM EASTERN NORTH AMERICA, THE CARIBBEAN
AND ANTARCTICA

ABSTRACT

Four species of the genus Grania are found in Bermuda; one is endemic and the others are also known from the Caribbean region. There have been no investigations of evolutionary relationships among the species of Grania from the Caribbean and adjacent North Western Atlantic. Characteristics and distribution of the Bermuda species suggest a number of routes to development of the extant fauna. Phylogenetic analyses of seventeen Grania species from eastern North America, nearby Atlantic and Caribbean islands, Antarctica and Ireland are used to develop hypotheses about the origins and evolution of the Bermudian fauna. The recent and relatively complete descriptions of these species allow their inclusion in a phylogenetic study. Based on one most parsimonious tree three Bermuda/Caribbean species form a clade with the North American species Grania monospermatheca and the Irish species Grania mira. A clade of South Atlantic, Bermuda/Caribbean and Antarctic species is also represented in Bermuda by Grania americana. Species relationships and distributions also suggest speciation has occurred in Bermuda. The phylogeny for Grania in the
Atlantic, suggests dispersal via oceanic currents to Bermuda, the Gulf Stream in particular, vicariance corresponding to opening of the North Atlantic and South Atlantic Oceans, and speciation of isolated populations consistent with founder events on remote islands.

**INTRODUCTION**

The strictly marine clitellate genus *Grania* is cosmopolitan. *Grania* species have been described from seven continents, primarily from coastal habitats. The genus is also known from the Atlantic oceanic island of Bermuda and from a number of islands in the Caribbean. These enchytraeids are commonly found in shallow water, inhabiting coarse, oxygenated substrates, but are also known from muddy, shallow or intertidal, substrates and from very fine substrates of the deep ocean.

The absence of a larval stage in *Grania* limits genetic exchange between widely separated populations, such as those of oceanic islands and the continents. This developmental pattern also suggests, more generally, poor dispersal capabilities overall and corresponding to this many of the known species of *Grania* have very limited geographic ranges. Isolated areas may harbor a number of endemic species (Coates, 1990; Coates & Stacey, 1993, 1997; Rota & Erseus, 1996). Species of *Grania* inhabiting Bermuda are also found within the Caribbean and Florida in contrast to the distribution patterns suggested above. However, one species *Grania bermudensis* is known only
from Bermuda, suggesting recent, local speciation and loss of genetic continuity with the ancestor.

There have been various systematic problems with the genus on a morphological level since its establishment by Southern in 1913. These problems have originated mainly from the lack of distinctive characters for species or species groups. This has also posed a significant limitation to phylogenetic analyses. However, improved observation and reporting of the details of internal structures is allowing reliable distinctions among species. Reproductive structures, the spermathecae and the male apparatus (Coates, 1984), have proven valuable in more recent studies, allowing redescriptions and reinstatements of earlier described species (Coates & Erseus, 1985; Locke & Coates, 1998a; Locke & Coates, in press). Other new species identifications have revealed new possibilities for taxonomic characters, in particular the statocyst (c.f. head organ, Rota & Erseus, 1996, 1997) and the bipartite penial apparatus (Locke & Coates, in press). Both of these morphological characters were initially described for new species but later found to occur within established species. Better knowledge of species diversity and distribution as well as of characteristics allows these first phylogenetic analyses, and as a result, a basis for developing evolutionary hypotheses.

Until 1986, phylogenetic methods had rarely been employed on aquatic oligochaetes (Coates, 1989). Coates (1986, 1987 a, b, 1989) has carried out phylogenetic studies of enchytraeids, but *Grania* was not specifically included in these analyses. Locke and Coates (1998b) used phylogenetic analyses of
Grania species of Australia to determine speciation patterns. However, no strong hypotheses about species zoogeographic patterns for this globally distributed genus have been published.

Bermuda is an oceanic island surrounded by the Sargasso Sea, located in the Northwestern Atlantic. The Bermuda pedestal is approximately 110 million years old, although the most recent emergence occurred 52-34 MYA (Morris, et al., 1977). The marine invertebrate fauna of Bermuda includes few species not found in the West Indies or other Caribbean regions (Ekman, 1953). Briggs (1974) claims there has not been sufficient time since the low sea levels during the Pleistocene glaciation (2-2.5 MYA) for evolutionary change in Bermuda and that despite its isolated position it will exhibit a low level of endemism. However, it is possible that motile species in shallow water might survive these sea level changes and would have, potentially a much longer period of inhabitation of Bermuda.

MATERIALS AND METHODS

Taxa

Taxa included in the analysis were: 17 Grania species from the Western Atlantic [North America (NA), Bermuda (BDA), and the Caribbean (CAR)], Ireland (IRE) and Western Antarctica (ANT) (Table 1). Two Randidrilus species (Enchytraeidae) from North America and Lumbricillus lineatus (Müller, 1774), a cosmopolitan enchytraeid species, were also included in the analysis as outgroup taxa.
Table 1. List of taxa analyzed, species distribution, and source of character data (CR, Christer Erséus; KAC, Kathryn Coates).

<table>
<thead>
<tr>
<th>TAXA</th>
<th>DISTRIBUTION</th>
<th>SOURCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grania atlantica</td>
<td>NA</td>
<td>CE &amp; KAC collections</td>
</tr>
<tr>
<td>Grania reducta</td>
<td>NA</td>
<td>CE &amp; KAC collections</td>
</tr>
<tr>
<td>Grania levis</td>
<td>NA</td>
<td>USNM 96509</td>
</tr>
<tr>
<td>Grania longiducta</td>
<td>NA</td>
<td>CE &amp; KAC collections</td>
</tr>
<tr>
<td>Grania monospermatheca</td>
<td>NA</td>
<td>CE &amp; KAC collections</td>
</tr>
<tr>
<td>Grania americana</td>
<td>NA, BDA</td>
<td>CE &amp; KAC collections</td>
</tr>
<tr>
<td>Grania laxarta</td>
<td>BDA, CAR</td>
<td>CE &amp; KAC collections</td>
</tr>
<tr>
<td>Grania bermudensis</td>
<td>BDA</td>
<td>CE &amp; KAC collections</td>
</tr>
<tr>
<td>Grania hylae</td>
<td>NA, BDA</td>
<td>CE &amp; KAC collections</td>
</tr>
<tr>
<td>Grania mira</td>
<td>IRE</td>
<td>KAC collections</td>
</tr>
<tr>
<td>Grania hirsuticauda</td>
<td>ANT</td>
<td>Rota &amp; Erséus, 1996</td>
</tr>
<tr>
<td>Grania angustinasus</td>
<td>ANT</td>
<td>Rota &amp; Erséus, 1996</td>
</tr>
<tr>
<td>Grania acanthochaeta</td>
<td>ANT</td>
<td>Rota &amp; Erséus, 1996</td>
</tr>
<tr>
<td>Grania antarctica</td>
<td>ANT</td>
<td>Rota &amp; Erséus, 1996</td>
</tr>
<tr>
<td>Grania algida</td>
<td>ANT</td>
<td>Rota &amp; Erséus, 1996</td>
</tr>
<tr>
<td>Grania lasserrei</td>
<td>ANT</td>
<td>Rota &amp; Erséus, 1997</td>
</tr>
<tr>
<td>Grania stephensoniana</td>
<td>ANT</td>
<td>Rota &amp; Erséus, 1997</td>
</tr>
<tr>
<td>Randidrilus codensis</td>
<td>NA</td>
<td>ROMIZ 12177</td>
</tr>
<tr>
<td>Randidrilus quadrirhecatus</td>
<td>NA</td>
<td>USNM 96498</td>
</tr>
<tr>
<td>Lumbricillus lineatus</td>
<td>NA</td>
<td>KAC collections</td>
</tr>
</tbody>
</table>
**Characters**

Morphological characters and states were compiled from specimens and literature (Coates & Erséus, 1985; Rota & Erséus, 1996, 1997; Locke & Coates, 1998a) (Table 1). Characters (Table 2) were derived from setal form and distribution, female reproductive organs (spermathecae), the male duct system (vasa deferentia and male apparatuses) and the statocyst associated with the anterior brain region. Six binary and six multi-state characters were coded (Table 2). Type material from the United States National Museum of Natural History (USNM), Washington, D. C. (USNM) and the Royal Ontario Museum (ROMIZ) (Table 1) as well as new material was examined.

**Analysis**

The phylogenetic analysis of a 20 taxa X 12 character data matrix with a minimum of 18 and a maximum of 55 possible transitions, was performed using PAUP 3.1.1 (Swofford, 1993) (Table 3). The data matrix was prepared in MacClade 3.02 (Maddison and Maddison, 1992). Characters were treated as unordered with equal weights. Tree searches were performed using heuristic and branch and bound methods. Zero-length trees were collapsed and MULPARS was activated. *Lumbricillus lineatus* was defined as the outgroup for rooting the tree.
Table 2. Character list and states.

**Setae**
1. Ventral setae: 0. before clitellum, 1. postclitellar, 2. absent
2. Lateral setae: 0. absent, 1. begin same as ventrals, 2. begin more posterior than ventrals
3. Ventrals in II: 0. absent, 1. present

**Spermathecae**
4. Number of spermathecae: 0. one pair, 1. two pairs, 2. single
5. Ectal pore: 0. dorsal, 1. lateral
6. Bipartite ectal duct: 0. absent, 1. present
7. Glands at ectal pore: 0. absent, 1. present

**Vasa Deferentia**
8. Muscular modifications of vas deferens: 0. absent, 1. slight constriction or knot, 2. thick extensions

**Penial Apparatus**
9. Penial apparatus: 0. bulbous, 1. bipartite
10. Bulbous gland: 0. absent, 1. single, 2. double
11. Stylet: 0. absent, 1. present

**Statocyst**
12. Number of statoliths: 0. absent, 1. one statolith, 2. 2-4 statoliths
Table 3. Character and state matrix used in *Grania* phylogenetic analysis.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Characters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>123456789012</td>
</tr>
<tr>
<td><em>Grania atlantica</em></td>
<td>02001100 0002</td>
</tr>
<tr>
<td><em>Grania reducta</em></td>
<td>10001000 0000</td>
</tr>
<tr>
<td><em>Grania levis</em></td>
<td>20001000 0110</td>
</tr>
<tr>
<td><em>Grania longiducta</em></td>
<td>12001000 0110</td>
</tr>
<tr>
<td><em>Grania monospermatica</em></td>
<td>10020102 1210</td>
</tr>
<tr>
<td><em>Grania americana</em></td>
<td>02001110 0102</td>
</tr>
<tr>
<td><em>Grania laxarta</em></td>
<td>02001001 0110</td>
</tr>
<tr>
<td><em>Grania bermudensis</em></td>
<td>02000002 1210</td>
</tr>
<tr>
<td><em>Grania hylae</em></td>
<td>02000001 1211</td>
</tr>
<tr>
<td><em>Grania mira</em></td>
<td>02001001/20100</td>
</tr>
<tr>
<td><em>Grania hirsuticauda</em></td>
<td>02001000 0002</td>
</tr>
<tr>
<td><em>Grania angustinasus</em></td>
<td>02001000 0102</td>
</tr>
<tr>
<td><em>Grania acanthochaeta</em></td>
<td>02001000 0102</td>
</tr>
<tr>
<td><em>Grania antarctica</em></td>
<td>02001000 0002</td>
</tr>
<tr>
<td><em>Grania algida</em></td>
<td>02001000 0102</td>
</tr>
<tr>
<td><em>Grania lasserrei</em></td>
<td>02001100 0102</td>
</tr>
<tr>
<td><em>Grania stephensoniana</em></td>
<td>02001000 0102</td>
</tr>
<tr>
<td><em>Randidrilus codensis</em></td>
<td>00101010 0000</td>
</tr>
<tr>
<td><em>Randidrilus quadrithecatus</em></td>
<td>00111010 0000</td>
</tr>
<tr>
<td><em>Lumbricillus lineatus</em></td>
<td>01101010 0000</td>
</tr>
</tbody>
</table>
RESULTS

Analyses

Both the heuristic and branch and bound methods yielded one most parsimonious tree with a length of 24 steps (Fig. 1). The tree had a CI of 0.75, an RI of 0.84 and a RC of 0.63 (Maddison and Maddison, 1992). The monophyly of Grania is well supported by the absence of ventral setae in segment II (2), and the absence of glands at the spermathecal ectal pore (7). This latter character, however, is reversed in G. americana. The relationships among the Antarctic species, G. hirsuticauda, G. angustinasus, G. acanthochaeta, G. antarctica, G. algida and G. stephensoniana and to the clade of G. americana, G. atlantica and G. lasserrei were unresolved.

DISCUSSION

Four distinct clades are recognizable among the Grania taxa included in these analyses. The grouping of North American G. reducta, G. longiducta and G. levis is supported by a pattern of having only postclitellar ventral setae (1), although these are lost in G. levis. The Bermuda/Caribbean species form a clade with G. mira and G. monospermatheca which is supported by muscular modifications of the vasa deferentia (8). Support for the clade of Antarctic species including G. americana and G. atlantica is the presence of a statocyst with two or more statoliths (12); the G. americana, G. atlantica and G. lasserrei clade is supported by the presence of a bipartite spermathecal ectal duct (6), but originates from a polytomy of all other Antarctic species that were included.
Figure 1. Phylogenetic tree depicting relationships among species of the genus *Grania*. Character changes are mapped on the tree (CI 0.75; RI 0.84; RC 0.63).
Characters

The basis for the recognition/designation of character states for the Antarctic species were the recent descriptions by Rota and Erseus (1996, 1997). These relatively short descriptions proved confusing for the vas deferens musculature (8). Rota and Erseus (1996) described certain Antarctic specimens as having muscular vas deferens 7-9 μm in width, but no illustrations of these ducts were included for these species. In the better studied species used in the phylogenetic analyses muscular vasa deferentia were only noted when the general width of the vas deferens was much greater than 10 μm, for example 18-25 μm is the range seen in G. bermudensis. Frequently the textual information on the Antarctic species was not fully corroborated by the illustrations provided and the structural interpretations of Rota and Erseus (1996, 1997) are questioned. The relatively short descriptions given for the Antarctic species are fine for separating species, although the new species from the Antarctic are not distinguished by unique characteristics, but they are not adequate for determining evolutionary relationships among taxa.

Two other characters, form of the male pore invagination and spermathecal ampullar form, require further analysis of specimens to ensure consistent records and recognition of states. The states described in the literature are not presented within the data set.

Zoogeography

Due to the relatively young age of Bermuda and to its volcanic, mid-ocean origins, the presence here of species, confined to shallow water, would seem to
be attributable to dispersal rather than vicariant speciation. However, relationships within and among the lineages represented in Bermuda fit the patterns of origin of the North and South Atlantic Ocean basins, and the separation of Ireland and the rest of Europe from the North American plate (Hallam, 1981).

This phylogeny provides evidence for all of vicariant (plate tectonics), dispersal, and local/island (dispersal based) speciation hypotheses.

Vicariance

There appears to have been a widespread ancestral taxon in the Caribbean and North Atlantic. From this, three lineages have diverged, one which currently is represented in the northwest Atlantic by *G. levis*, *G. longiducta* and *G. reducta*, the “cold water” species. A second which includes a northwest Atlantic species, *G. monospermatheca* which ranges from Florida to the Carolinas, the Caribbean-Bermuda species, *G. laxarta, G. hylae, G. bermudensis* and an Irish species, *G. mira*, the “warmer water” species. The third lineage is the South Atlantic-subtidal and Caribbean lineage, including the Antarctic species, cosmopolitan *G. atlantica* and *G. americana* also from Bermuda.

The first mentioned lineage, now found in colder waters, seems to have diverged first. What geographical barrier or event separated the widespread species into northwestern and southern components is unknown. However, the patterns of cold currents running south along the coast and the warm Gulf
Stream running north, further offshore, are effective physical and ecological barriers to genetic continuity between the northern and southern faunas.

The warm water and southern lineages, subsequently diverged into Caribbean and South Atlantic lineages. Again the effective barriers are not immediately obvious and present day distributions of these taxa do overlap in the Caribbean due to the presence there and in Bermuda of *G. americana*.

The inclusion in the “warmer water” lineage of *G. mira* in Ireland may seem ecologically anomalous. Geographically the vicariant event leading to its origin is recognizable as the separation of Ireland and Europe from North America as the Atlantic Ocean basin spread. Geological evidence fits this scenario very well. The splitting of Pangea into Gondwanaland and Laurasia caused what is now Ireland to move from an area closely associated with what is now the Caribbean about 160 MYA (Hallam, 1981). It is very likely that the influence of the Gulf Stream on Ireland has provided the warm conditions which have allowed the persistence of this lineage in Ireland.

The relationships among the taxa of the South Atlantic-subtidal and Caribbean lineage are not well resolved, so that it is difficult to suggest evolutionary scenarios. However, if divergence of lineages (speciation) is assumed to occur most frequently when there are geographic barriers or separation, then the presence of *G. americana* in the Caribbean may be secondary in that it succeeded diversification of this lineage. Supporting this hypothesis is the sister-group relationship between *G. americana* and *G. atlantica* (and possibly other taxa) which is a very widespread species in the
deeper Atlantic. The distribution of *G. atlantica* is very different from what is known for other species of *Grania* and it may represent the widespread ancestral species from which the several South Atlantic taxa diverged.

**Dispersal to Bermuda**

The only obvious explanation for the presence of the species now occurring in Bermuda is long distance dispersal of some life stage. Since its origin, Bermuda has always been surrounded by deep water and it has always been distant from the continental shallow water habitats. The Gulf Stream which originates in the Caribbean is an obvious dispersal agent for the *Grania* species found in the Caribbean and Bermuda (Fig. 2). As noted above, this current also extends near Ireland, as the North Atlantic Drift, where the sister of the Bermuda-Caribbean clade, *G. mira* is found. There is no evidence for a widespread, deep and shallow water taxon as the ancestor to the species found in Bermuda nor does it seem likely that the Caribbean species crawled into the depths and then resurfaced in Bermuda.

At this time, there is little evidence to substantiate hypotheses of long distance transport of oligochaetes as adults, juveniles, or cocoons, but that is what the morpho-species with populations in Bermuda and along the coasts of the Caribbean and mainland south eastern North America suggest (Locke & Coates, in press). Also, the indistinguishable morphologies of *G. americana*, *G. laxarta* and *G. hylae* between the Caribbean and Bermuda populations suggests genetic continuity—regular introductions from the Caribbean into the Bermudian gene pool. A mechanism for a similar flow from Bermuda to the Caribbean is
Figure 2. The main surface currents of the Atlantic and Caribbean Oceans.
difficult to imagine as there are no ocean surface currents running in that direction. The employment of molecular methodology would best determine if these morpho-species actually include cryptic species.

**Endemic Species**

Dispersal must also be responsible for introduction of *G. bermudensis* or its ancestor into Bermuda but it has diverged, to a point where conspecificity with a Caribbean/North American taxon is not recognized. It is interesting that the sister species of *G. bermudensis* is *G. monospermatheca* which has a more northern distribution rather than a Caribbean pattern. This distribution may limit transport or dispersal to Bermuda to vanishingly rare events. Thus, for this taxon, *G. monospermatheca*—*G. bermudensis* (and possibly other taxa), dispersal to Bermuda acted as the vicariant event preceding speciation (see Nelson & Platnick, 1981). As the most recent emergence of Bermuda as an island is thought to be only about 52-34 MYA. Divergence of *G. bermudensis* must have happened between this time period and the present. If rates of genetic exchange are high enough and dispersal is an important factor to distributions of marine oligochaetes, significant divergence of the Bermuda populations of other species may never occur.

**Evolution of Characters**

*Grania bermudensis, G. monospermatheca, and G. hylae* all share dorsal spermathecal pores and modified male duct systems. These seem to have evolved from an ancestor that had lateral spermathecal pores and a modified vasa deferentia. This ancestor is shared with *G. laxarta* and *G. mira*, both of
which also have distinct spermathecal ectal ducts and modified, slightly muscular male duct systems. Other species, not mentioned in this study, that share dorsal spermathecal pores and possibly a bipartite, sac-like, penial apparatus are recorded from Hong Kong (Erséus, 1990). As the Pacific is much older than the Atlantic and is the obvious source for widespread taxa that moved into the Atlantic at its origin, we might well expect species with muscular ducts in that ocean as well.

The independent evolution of a statocyst (12) within G. hylae and the lineage occupied by G. americana, suggests that this structure may not be homologous in these two species. Personal observations of other Grania species have indicated that this anterior brain region is more complex than previously anticipated. More studies of these structures will no doubt provide interesting taxonomic and evolutionary results.

**Evolutionary Ecology**

*Grania americana* belongs to a separate lineage from the other Bermudian species, which is particularly interesting because it is often found in the same samples with G. laxarta (Locke and Coates, in press). These are the only species of *Grania* commonly found together in the same microhabitats, intertidal dissolution tubes. Thus, phylogenetic divergence here is probably correlated with ecological divergence. This is an issue that is relatively underexplored because of the lack of robust phylogenies for most invertebrates but which is central to establishing strategies for conserving biological diversity. This is in contrast to hypotheses of sympatric speciation with little ecological
divergence as suggested by Locke and Coates (1998b) for some Australian species of *Grania*.
REFERENCES


Hydrobiol. 115:45-50.

Coates, K.A. 1986. Redescription of the oligochaete genus *Propapus*, and


180:17-33.


Müller, O.F. 1774, *Vermium terrestrium et fluviatilum*. Heineck and Faber, Havniae and Lipsiae.


Chapter 6

GENERAL DISCUSSION

General knowledge of diversity within the enchytraeid genus *Grania* has been furthered with the descriptions of two new species *Grania laxa* and *Grania hylae* and two complete species redescriptions for *Grania bermudensis* and *Grania americana* from Bermuda, North America and the Caribbean. The taxonomy of the polytypic species is one step nearer to being resolved.

New character information for these species benefit studies of the taxonomy and systematics of the genus. In particular these studies of *G. americana* allowed the first description of a statocyst in a clitellate annelid. The first key to *Grania* species of Eastern North America will contribute to the development of taxonomic and distributional information for the genus, essential elements of faunistic and environmental impact studies. This is the first key for *Grania* species based on reliable taxonomy. The first successful phylogenetic study of species of the genus *Grania* utilized the new character information and revealed the necessity of accurate and complete taxonomic descriptions. The first hypotheses of relationships, dispersal and diversification of *Grania* species were developed from the phylogeny.

The new species records from the intertidal and shallow subtidal habitats of Bermuda, Belize, and southern Florida is an important contribution to the known diversity and distribution of *Grania*. These descriptions also supply baseline data for ongoing studies of biodiversity. With increased species
descriptions, character information used to distinguish between species must also broaden. This means more detailed accounts of species rather than shorter descriptions, as well as greater attention to diagnostic or unique characteristics, are needed.

Improved taxonomic information will provide a better means of identifying collections and determining the status of recognized species as evolutionary entities. Careful studies also ensure the historical taxonomic difficulties of past *Grania* studies will not be revisited. The statocyst in *G. americana* and *G. hylae* and the male apparatus, including the bipartite sac as seen in *G. bermudensis* and *G. hylae*, will provide a valuable suite of characters to ongoing taxonomic and evolutionary studies of the genus. The presence of the bipartite sac in the Bermuda species has enabled the male apparatus “type 6” described by Coates (1984) for this penial apparatus structure to be refined and redescribed (Locke & Coates, in press). The statocyst of *G. americana*, revealed by ultrastructural investigations, is the first confirmed record of such a sensory structure within the clitellate annelids and may have significant implications for the ongoing and controversial phylogenetic studies concerning the monophyly of the Annelida (Jamieson, 1992; Rouse & Fauchald, 1995, 1997; Purschke, 1997; Westheide, 1997). Determination of these characters in other species of this widely occurring genus will better distinguish existing species and help in the resolution of evolutionary relationships within *Grania* and zoogeographic patterns of marine invertebrates.
The recent emergence of Bermuda from the Atlantic Ocean 52-34 MYA (Morris et al., 1977) and the near shore inhabitant *Grania* species present on such an isolated island provide a model for studying long distance dispersal. Without a doubt the Gulf Stream has been and is a major contributing force behind dispersal to Bermuda. Species present in Bermuda, excluding the endemic *Grania bermudensis*, are also found within the Western Caribbean and Florida.

**Future work with the genus**

Additional taxonomic studies in the Caribbean and along the North Atlantic Coast, particularly from George's Bank and north of Cape Cod would provide missing species information, filling in the evolutionary and distributional gaps for the genus *Grania*. Incorporation of *Grania* species in environmental and ecological studies based on use of the new key may also contribute more information regarding distribution and diversity of known species.

An examination of the genetic relationship between populations of *Grania* from Bermuda and conspecifics from Florida, the Caribbean and along the Atlantic Coast would enhance information regarding morphological relationships of species known from both island and continental populations.

The composition of the statoliths within the anterior cerebral statocyst of *G. americana*, is unknown. Studies of these structures with elemental x-ray EM may reveal more about their composition. The continued search for new taxonomic characters will certainly involve further investigation of the brain
region within *G. americana* and other species of *Grania*. Developmental studies of the statocyst and its possible association with the head pore would determine the origin of the statocyst.

Phylogenetic studies that also incorporate Pacific species and additional species as above would broaden our understanding of the origins of *Grania*. A combination of morphological, molecular and phylogenetic data would provide a more substantial basis for looking at speciation patterns and mechanisms.
REFERENCES


SUMMARY

- Two new species, *Grania laxarta* and *Grania hylae* were described from Bermuda, Belize and Florida.
- *Grania macrochaeta bermudensis* was redescribed as a distinct species, *Grania bermudensis*. The taxonomy of the polytypic grouping of *Grania macrochaeta* was therefore reinforced as invalid.
- *Grania americana* was completely redescribed and new taxonomic characteristics for the genus were recorded in this species.
- The known distribution of *Grania americana* increased with records from Bermuda and Belize.
- A new morphological characteristic of the male duct system in *Grania* was described as a bisaccate penial apparatus. Resulting also in the redes cription of Type 6 penial apparatus (Coates, 1984). This type of penial apparatus is recognized as being associated with dorsal, spermathecal pores.
- A statocyst was described for *Grania americana*. This is the first discription of a statocyst within the Clitellata and is among one of the most complex statocyst structures known for invertebrates. This is also a new characteristic for distinguishing species of *Grania*.
- A basic dichotomous key to *Grania* species of North America, Bermuda and Caribbean distribution was compiled. This is the first for anywhere that has been based on reliable taxonomic descriptions.
A phylogeny for species of *Grania* of the Western Atlantic and Caribbean was proposed. An hypothesis of dispersal from the Pacific Ocean into the Caribbean and North America is presented.

Dispersal and vicariant speciation are hypothesized as possible distributional forces accounting for the presence of the *Grania* species in Bermuda, North America and Ireland.

The phylogeny clearly indicates that a polytypic species *G. macrochaeta* including *G. americana* and *G. bermudensis* is untenable evolutionarily.