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DOCTORAL DISSERTATION

A Molecular Approach to Questions in the Phylogeny of Planktonic Sarcodines

by

Linda Angela Amaral Zettler

September 1996

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A Molecular Approach to Questions in the Phylogeny of Planktonic Sarcodines

by

Linda Angela Amaral Zettler

Sc.B. Aquatic Biology
Brown University, 1990

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
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A Molecular Approach to Questions in the Phylogeny of Planktonic Sarcodines

by

Linda Angela Amaral Zettler

Submitted to the Department of Biology in August, 1996 in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

Abstract

The Acantharea and the Polycystinea are two classes of sarcodines (Sarcodina) which are exclusively planktonic and occur strictly in oligotrophic marine environments. Although these protists have been the topic of research since Ernst Haeckel's systematic investigations of samples from the H. M. S. Challenger Expedition, many aspects of their phylogeny and systematics remain poorly resolved. Part of the problem is that the criteria used in systematics of these groups until now has emphasized morphological elements which may be similar due to convergence rather than common ancestry. The application of molecular biology to the field of biological oceanography offers alternative approaches to reexamining sarcodine phylogeny with the goal of producing classifications which reflect evolutionary history.

The relationships of the Acantharea and the Polycystinea (order Spumellarida) to other protists were investigated using phylogenetic analyses of small-subunit ribosomal RNA (SSU rRNA) genes. Members of these two classes have been traditionally grouped into the common superclass Actinopoda based on their specialized pseudopodia called axopodia. Sequences from two orders of Acantharea (Symphyacanthida and Chaunacanthida) and four representatives from the order Spumellarida and the class Polycystinea (one solitary and three colonial spumellaria) were aligned against 25 other eukaryotic SSU rRNA sequences extracted from a data base of more than 800 eukaryotic sequences and subjected to distance, maximum parsimony and maximum likelihood analyses. SSU rRNA-based phylogenies do not support the common ancestry of the Acantharea and the Polycystinea, implying that the superclass Actinopoda is artificial and should be discarded. The respective monophyly of the Acantharea and the Polycystinea were supported in all analyses accomplished. The origin of the sequences was confirmed by in situ hybridization experiments.

SSU rRNA gene sequences for the solitary spumellarian Thalassicolla nucleata were compared from individuals collected from the Sargasso Sea and the Pacific Ocean. Sequences from pooled individuals showed primary structure differences which were consistent with genus-level variation reported in the literature for unrelated taxa. These results indicate that there may be different strains of this genus which are morphologically identical or that perhaps there may be allelic variation within a given individual.

The evolutionary relationships between the solitary T. nucleata and seven colonial spumellaria were analyzed to determine whether the two families of colonial spumellaria (Collosphaeridae and Sphaerozoidae) form a monophyletic evolutionary assemblage. Phylogenies inferred from distance and maximum likelihood methods did not support the monophyly of the colony-forming spumellaria. Parsimony methods did support the monophyly of the colonial spumellaria but with very low bootstrap support. The monophyly of members from the Collosphaeridae family was supported in all analyses with 100% bootstrap support while only distance analyses supported the monophyly of the Sphaerozoidae. The possibility that coloniality has evolved more than once in the Spumellarida has been suggested from observations of the fossil record. However, contrary conclusions have been reached from studies based on skeletal morphogenesis. The results obtained from molecular analyses question the utility of coloniality as a reliable phylogenetic marker. Sequence variation within the SSU rRNA genes of the Spumellarida appears to be sufficient enough for continued fine-scaled comparisons between existing morphospecies.

The branching patterns within three of the four orders of the Acantharea were examined using additional SSU rRNA gene sequence data from representatives of the Symphyacanthida, Chaunacanthida and Arthracanthida. The results from this analysis revealed a phylogeny which placed one representative of the Symphyacanthida (Haliommatidium sp.) branching among the Arthracanthida. An examination of the cytological features of Haliommatidium sp. in the literature revealed morphological similarities it shares with the Arthracanthida that could corroborate this result. The variability within acantharian SSU rDNA was significantly less than that observed in spumellaria, and may prove less useful in establishing relationships at taxonomic categories below the order level.

Name and Title of Thesis Advisor: David A. Caron, Associate Scientist with tenure

This dissertation is dedicated to my grandmother,

Guiseppina Quattrocchi Cavallaro

1902-1987

whose

formal education ended after

first grade, when an earthquake destroyed her school.

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I am sincerely indebted to the many people who made the work accomplished during this dissertation not only possible but also very enjoyable over the past five years. Perhaps the person who has had the most influence on my academic career has been my advisor David Caron. My first introduction to Woods Hole and WHOI were largely the result of a desire to work with Dave, a world expert on the biology of planktonic sarcodines, and learn more about a group of protists for which I had, until that point, studied only the fossil remains. I am grateful for his guidance and enthusiasm to allow me to pursue a research topic peripheral to other projects in his lab.

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Anderson also assisted in radiolarian identifications and took part in numerous e-mail and telephone conversations regarding aspects of radiolarian biology.

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I especially want to acknowledge my family. My parents have always made education a very high priority in their lives and I am grateful for the sacrifices they have endured on my behalf. Finally, my husband and best friend Erik Zettler has been a continuous source of encouragement and support for the duration of my dissertation and it is to him that I owe my deepest thanks.

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Introduction

Planktonic sarcodines are a heterogeneous group of single-celled aquatic eukaryotes which include amoebae, foraminifera, and actinopods. While many planktonic sarcodines share similar ecological niches in marine and freshwater environments, it is unclear to what extent they share a common evolutionary history. Taxonomic frameworks that encompass members of the planktonic sarcodines are equivocal. This is largely because many of the morphological characters upon which these classification schemes were built are probably not phylogenetically meaningful. Some taxonomic schemes which were created at the turn of the century have seen little revision since their creation and are still in use.

Planktonic sarcodines are grouped within the subphylum Sarcodina based on the possession of pseudopodia during some part of their life cycle. The validity of this grouping has been questioned and current opinion largely regards it as an artificial taxon (Corliss 1984; Page 1987; Cavalier-Smith 1993). Further taxonomic divisions of the Sarcodina based on specialized pseudopodial structures such as axopodia, possessed by members of the superclass Actinopoda, have also come under scrutiny (Cavalier-Smith 1993). The question of the monophyly of the Actinopoda remains largely unresolved.

The application of molecular biological techniques to protistan systematics provides an independent means of examining existing systematic frameworks based on classical approaches. This thesis considers the evolutionary relationships between and among two currently-recognized actinopod classes, the Acantharea and the Polycystinea, based on sequence analysis of small-subunit ribosomal RNA genes. The reconstruction of phylogenies based on SSU rRNA genes aims to establish the relatedness of the Acantharea and Polycystinea to other eukaryotes and ultimately to provide information for further development of "natural" classification schemes within these classes.

General Background on Acantharia and Polycystine Radiolaria

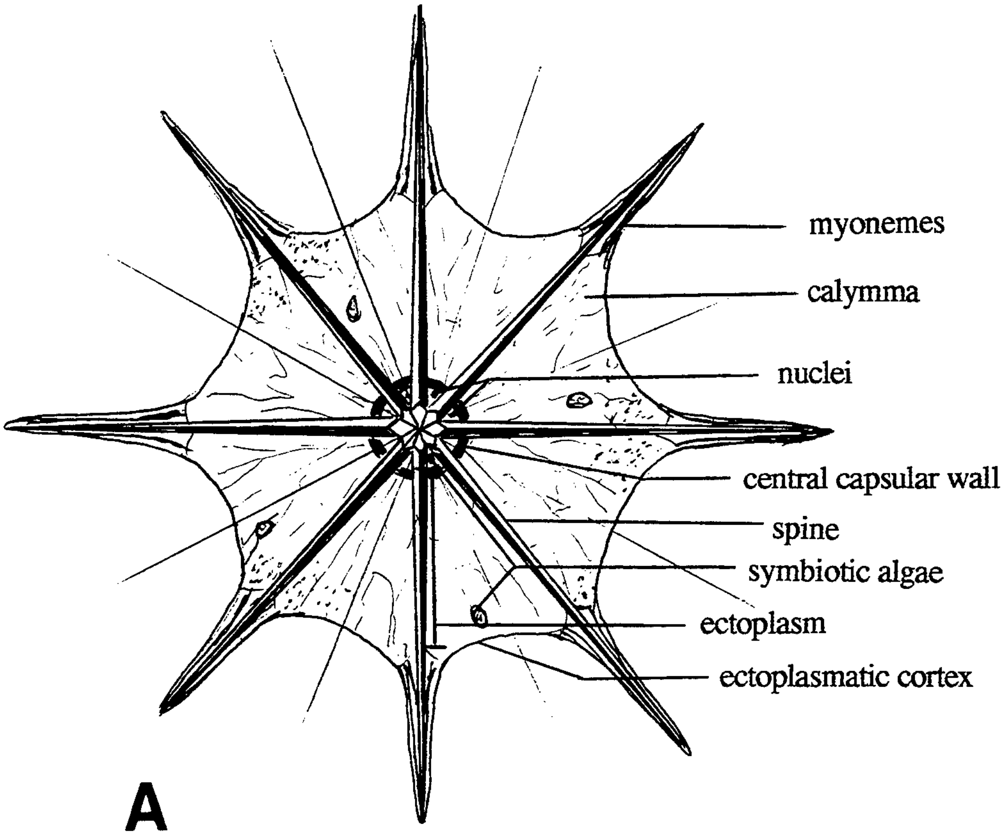
Acantharea and Polycystinea are two classes of axopod-bearing protists which are strictly planktonic and found exclusively in open-ocean oligotrophic environments (see Fig.

1). Among the larger planktonic sarcodines, the acantharia and particularly the colony-forming polycystines are perhaps the most conspicuous of the zooplankton found in the open ocean owing to the bloom-like conditions often created by the Acantharea and due to the conspicuous visibility of large macroscopic colonies of spumellarian members of the Polycystinea. Acantharia range in size from 50-800 µm in diameter, solitary spumellarians range from 10 µm to several centimeters in diameter, and colonial spumellaria have been reported up to three meters in length. Despite an often visible presence in the plankton, sampling methods and preservation techniques have led to underestimates of living acantharian and spumellarian abundances and ultimately underestimation of their importance in marine environments (Swanberg 1979; Michaels 1988).

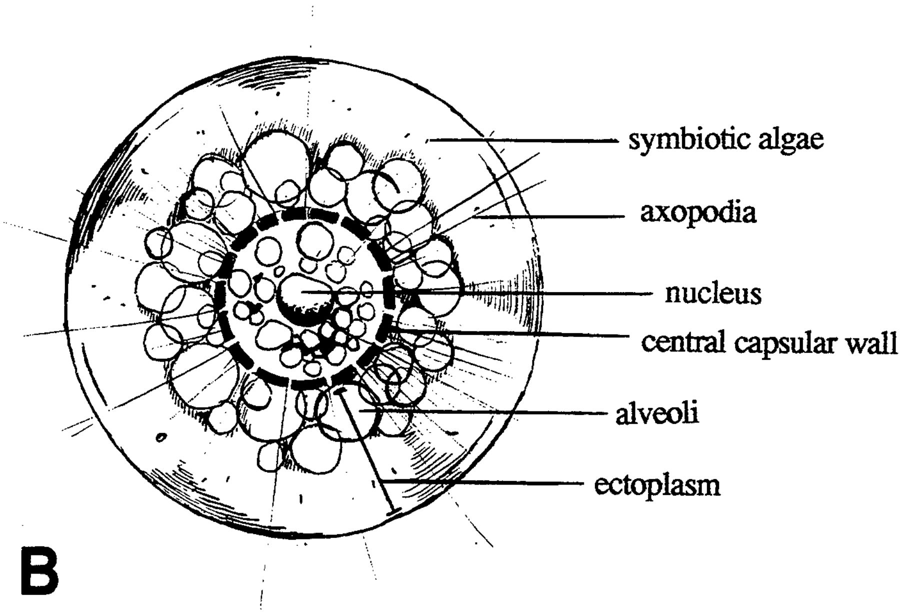
Although living polycystines have received limited study, their fossilized skeletons have not. Many polycystines including the colonial spumellaria considered in this thesis possess siliceous skeletons which are preserved in marine sediments. The polycystines offer the longest geological and widest biogeographical ranging microfossils available for study by micropaleontologists (Casey et al. 1983). The long fossil record left behind by shell-bearing Polycystinea has precipitated a suite of research spanning paleoclimatological, paleoecological, and evolutionary studies (e. g. Riedel and Sanfilippo 1971; Kellogg and Hays 1975; Lazarus 1983).

Unlike the Polycystinea, members of the Acantharea, which possess shells of strontium sulfate, are absent from the fossil record and have thus received far less attention than the Polycystinea. Despite a lack of geological utility, the acantharia have been the topic of various kinds of biological research. Since they possess unique non-actin myonemes, they have been intensively studied by cell biologists (Febvre 1990 and the references therein). As the sole protistan utilizers of strontium sulfate as the major structural component of their skeletons, acantharia serve an important function in the strontium cycle of the world oceans (Bernstein et al. 1987). Since members of the

Fig. 1. A schematic of hypothetical **A.** acantharian and **B.** spumellarian cells indicating cell structural features of the two different sarcodines.


myonemes
calymma
nuclei
central capsular wall
spine
symbiotic algae
ectoplasm
ectoplasmatic cortex

A


symbiotic algae
axopodia
nucleus
central capsular wall
alveoli
ectoplasm

B

Acantharea and Polycystinea often live in association with symbiotic algae, they also play a role in both primary productivity and microbial food-web dynamics (Michaels 1988; Caron and Swanberg 1990; Caron et al. 1995).

The inability to culture acantharia and polycystines through successive generations in the laboratory has been an important impediment to their study. Our inability to maintain sarcodines in laboratory culture and the care which must be taken in collecting these fragile organisms has resulted in a fragmentary understanding of their biology and more fundamentally, their relationships to other organisms. However, despite recalcitrance to laboratory culturing, acantharia and polycystines have been the objects of study for over a century and a half.

Historical Perspective: Haeckel's Radiolaria

One of the first described radiolaria (Meyen 1834) was a colonial polycystine spumellarian, belonging to the spicule-bearing genus Sphaerozoum. Ehrenberg (1838) erected the first classification for the radiolaria and is credited as giving the Polycystinea their name, which was derived from descriptions of spherical latticed-shells found of fossil polycystines. Earliest reports of acantharia were made by Müller (1858) who grouped the acantharia and the radiolaria together based on a shared radial disposition of the pseudopodia possessed by both groups. The highly-ordered geometrical pattern of spine orientation formed by acantharian cells has been called Müller's Law in recognition of his early observations of acantharian skeletal architecture (see Chapter 4, Fig. 1C). Thus, from the very early stages of their study, shared morphological similarities of the Acantharea and the Polycystinea united these two sarcodine groups into a common taxonomic category.

The first exhaustive accounts of both acantharian and polycystine systematics were those of Ernst Haeckel after the H. M. S. Challenger Expedition. Haeckel (1883, 1887) combined the Acantharia (modern-day Acantharea) and the Spumellaria and the Nassellaria (now classified collectively in the class Polycystinea) along with the fourth "legion", the Phaeodaria (Phaeodarea), into the "class" Radiolaria. He further united the Acantharia and the Spumellaria (Spumellarida) into the now defunct "subclass" Porulosida based on the shared characteristics of the distribution and size of the pores in the central capsule wall. In 1909, the Acantharea and Polycystinea were grouped along with other sarcodines based on the structure of their pseudopodia into what was first created as a "class" by Calkins called the Actinopoda (Calkins 1909). The Actinopoda, still persists in modern classifications but has been elevated to a superclass (Fig. 2). The superclass Actinopoda was originally created to encompass all sarcodines which possessed microtubule-supported pseudopodia termed axopodia.

Historical Perspective: Acantharea

Following the establishment of the Actinopoda in 1909, Schewiakoff (1926) promoted the Acantharia to the level of subclass based on the differences he noted in the capsule membrane of acantharia from the radiolaria and the absence of central capsules in certain acantharia. He also revised lower level acantharian systematics to take into account cytological features. The "subclass" Acantharia was elevated to the level of superorder by Enriques (1931). He called the new superorder "Birefrangentia" based on the birefringant properties of the strontium sulfate-containing acantharian skeleton. The current status of the "class" level of organization currently given to the Acantharea was first proposed by Tregouboff (1953) and has been accepted by other specialists since that time.

Historical Perspective: Polycystinea

The remainder of the historical review will be restricted to polycystine systematics because only a limited group of spumellarian representatives from the class Polycystinea were addressed in this thesis.

Huxley (1851) further elaborated on the work of Meyen and assigned all colonial spumellaria to the species Thalassicolla punctata. However, Müller disagreed with the

Fig. 2. Taxonomic position of the planktonic sarcodines examined in this thesis.

- Kingdom: Protista
  - Phylum: Sarcomastigophora
    - Subphylum: Sarcodina
      - Superclass: Actinopoda
        - Class: **Acantharea (acantharia)**
          - Order: **Symphyacanthida**
            - Family: **Pseudolithidae**
              - Genus: **Haliommatidium**
                - ***Haliommatidium*** **sp.**
          - Order: **Chaunacanthida**
          - Order: **Arthracanthida**
            - Suborder: **Sphaenacanthina**
              - Family: **Acanthometridae**
                - Genus: **Acanthometra**
                  - ***Acanthometra*** **sp.**
        - Class: **Polycystinea (radiolaria)**
          - Order: **Spumellarida**
            - Suborder: **Sphaerocollina**
              - Family: **Thalassicollidae**
                - Genus: **Thalassicolla**
                  - ***Thalassicolla nucleata***
              - Family: **Sphaerozoidae**
                - Genus: **Collozoum**
                  - ***Collozoum pelagicum***
                  - ***Collozoum serpentinum***
                - Genus: **Sphaerozoum**
                  - ***Sphaerozoum punctatum***
                - Genus: **Rhaphidozoum**
                  - ***Rhaphidozoum acuferum***
              - Family: **Collosphaeridae**
                - Genus: **Collosphaera**
                  - ***Collosphaera globularis-huxleyi***
                - Genus: **Acrosphaera**
                  - ***Acrosphaera (circumtexta?)***
                - Genus: **Siphonosphaera**
                  - ***Siphonosphaera cyathina***

Based on Levine et al. (1980) and Lee et al. (1985).

inclusion of the colonial radiolaria within the single genus Thalassicolla which was already known to contain the solitary spumellarian T. nucleata. Müller was the first to differentiate between the solitary and colonial spumellaria which he called the Solitaria and the Polyzoa respectively (Müller 1858; Strelkov and Reshetnyak 1971).

Haeckel was the next taxonomist to substantially revise colonial spumellarian systematics based on the presence or absence of skeletal features. He described 84 species, 17 genera and 3 families. After Haeckel's 1862-1887 systematic revisions of the spumellarian polycystines, further taxonomic revisions were largely the efforts of Brandt (1885, 1905) and his students. According to Strelkov and Reshetnyak (1971), Brandt disagreed with many aspects of Haeckel's classification, most importantly of which was his separation of the colonial spumellarian radiolaria into different orders. Brandt created a separate taxon which included all colonial radiolaria into the one group called the Sphaerozoa and reduced the number of species, genera and families proposed by Haeckel. Brandt kept the two families of colonial spumellarians recognized in modern classifications, the Collosphaeridae and the Sphaerozoidae. These two families were grouped into the suborder Polycyttaria by Haecker in 1908. Haecker also divided the Spumellaria into two additional suborders, the Sphaerellaria, which contained solitary shell-bearing forms and the Collodaria which contained either skeletonless or spicule- bearing solitary forms such as Thalassicolla. Further systematic revisions were carried out by Hilmers (1906), Breckner (1906), and Popofsky (1908) later by Tregouboff (1953). The latest revision of the colonial spumellaria has been carried out by Strelkov and Reshetnyak (1971).

Modern Day Classifications and Taxonomic Perspectives

Although not formally recognized, some revisions have been suggested in the recent literature to reflect a more "natural" classification scheme for the Actinopoda. Under current classification schemes recognized by the Committee in Systematics and Evolution of the Society of Protozoologists (Levine et al., 1980), the Actinopoda is a superclass which includes four classes: the Acantharea, Polycystinea, Phaeodarea and Heliozoea. However, recognizing the diversity within the Actinopoda, Cavalier-Smith (1987) elevated the taxon Actinopoda to a "parvkingdom". He further recommended a division of the Actinopoda into the phyla "Radiozoa" and "Heliozoa" in order to account for recognized differences between the Heliozoea (Heliozoa) on the one hand and the Acantharea, Polycystinea and Phaeodarea (Radiozoa) on the other. While recognizing its diversity, Cavalier-Smith maintained the taxon Actinopoda suggesting that it might be monophyletic. More recently, Corliss (1994) adopted Cavalier-Smith's "Radiozoa" and the further divisions of the subphylum Acantharia with the class Acantharea and the subphylum Radiolaria with the classes Polycystinea and Phaeodarea.

The classification scheme used throughout this thesis does not incorporate the most recent suggestions as indicated above since no real consensus has been reached on the appropriate revisions, but instead adopts the last formally revised classification of the Protozoa made by the Committee on Systematics and Evolution of the Society of Protozoologists (Levine et al., 1980) (see Fig. 2). The Levine et al.(1980) classification scheme is used for higher level classifications wherein phyla, subphyla and superclasses end in "a"; classes end in "ea"; subclasses in "ia"; orders in "ida"; and suborders in "ina". The classification scheme of Strelkov and Reshetnyak (1971) has been used for the colonial spumellaria and that found in Lee et al. (1985) for the solitary spumellaria and the acantharia since these schemes address systematic groupings below the suborder level whereas Levine et al. (1980) stops at suborder-level classification.

As mentioned earlier, the work described herein attempts to use newly-developed molecular methods as independent tools for examining sarcodine phylogenetic relationships and producing classifications which reflect these phylogenetic relationships. There are many reasons for choosing ribosomal RNA molecules to address the phylogenetic and systematic questions posed above. These include their ubiquitous occurrence among all living organisms, their functional uniformity, and absence of lateral gene transfer (Olsen et al. 1986; Sogin et al. 1986; Field et al. 1988). Ribosomal RNA molecules possess both very conserved and very variable regions which allow for nucleotide base pair alignments between both closely and distantly-related organisms (Gobel et al. 1987; Sogin and Gunderson 1987). In addition to these features, the current data base for rRNA gene sequences is one of the largest of its kind, and so allows for comparisons between many different organisms (Neefs et al. 1991; De Rijk et al. 1992).

Yet another advantage of rRNA-based analysis is the potential for constructing phylogenetic oligonucleotide probes based on the gene sequences of the organism of interest. Such oligonucleotide probes have been conjugated to reporter molecules and used as molecular probes in conjuction with fluorescence and transmitted light microscopy to distinguish between different kingdoms and even different species (DeLong et al. 1989; Amann et al. 1990). Ribosomal RNA probes can also provide a means of verifying sequences obtained from organisms collected from the environment (as opposed to laboratory grown cultures). Planktonic sarcodines have resisted laboratory culture through successive generations and have consequently been difficult to study. The fact that planktonic sarcodines must be collected each time more samples were needed was one of the most challenging aspects of this thesis. Since rRNA is a very abundant in the cytoplasm of cells it provides many targets for in situ hybridizations using rRNA probes. These probes can be a valuable tool for verifying gene sequences obtained from organisms from the environment.

This thesis is organized into four chapters which are written in manuscript form. In Chapter 1, I first present the overall phylogenetic placement of the Acantharea and Polycystinea among other eukaryotes in a broad-based SSU rRNA phylogenetic analysis. In this analysis I include two representatives from the Acantharea (one Symphyacanthid representative and one Chaunacanthid representative) and four representatives of the

Spumellarida [one solitary (*Thalassicolla nucleata*) and three colonial spumellaria (*Collosphaera globularis-huxleyi*, *Sphaerozoum punctatum* and *Collozoum serpentinum*]. In addition to phylogenetic reconstructions, data on *in situ* verification of the acantharian and spumellarian sequences is presented using acantharian and colonial spumellarian-specific oligonucleotide probes. This chapter also addresses the issue of the monophyly of the Actinopoda.

In Chapter 2, I present a comparison between the *Thalassicolla nucleata* sequence from Chapter 1 which was obtained from the Sargasso Sea with 4 additional *T. nucleata* sequences derived from Pacific samples. This chapter addresses variability within a single species and reexamines the known species in the genus *Thalassicolla* in view of the SSU rRNA sequence data obtained from specimens collected from geographically different locations.

In Chapter 3, I focus more closely on the relationships among the Spumellarida (Sphaerocollina) and more specifically, address the validity of coloniality as a legitimate phylogenetic character. In addition, to the spumellarian taxa used in Chapter 1, data from *Collozoum serpentinum*, *Rhaphidozoum acuferum*, *Acrosphaera* (*circumtexta?*) and *Siphonosphaera cyathina* are also utilized. Phylogenies are inferred using the same homologous positions as in the sequence alignment of Chapter 1 and using additional sites by restricting the analysis to just the colonial spumellaria.

In Chapter 4, I consider the branching patterns within the Acantharea using SSU rRNA gene sequence data from two representatives each of three orders of Acantharea, the the Symphyacanthida, the Chaunacanthida and the Arthracanthida.

In Chapter 5, I summarize the conclusions of the thesis. Appendix A includes the alignment and sequence positions used in phylogenetic analyses presented in Chapters 1, 3 and 4 along with proposed locations of the secondary structure helices.

REFERENCES

Amann, R. I., L. Krumholz and D. A. Stahl. 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. Journal of Bacteriology **172:** 762-770.

Bernstein, R. E., P. R. Betzer, R. A. Feely, R. H. Byrne, M. F. Lamb and A. F. Michaels. 1987. Acantharian fluxes and strontium to chlorinity ratios in the north Pacific Ocean. Science **237:** 1490-1494.

Brandt, K. 1885. Die koloniebildenden Radiolarien (Sphaerozoëen) des Golfes von Neapel und der angrenzenden Meeresabschnitte. Fauna und Flora Golfes Neapel **13:** 1-276.

Brandt, K. 1905. Zur systemik der koloniebildenden Radiolarien. Zool. Jahrb. Suppl.: 311-352.

Breckner, A. 1906. Beiträge zur Kenntnis der koloniebildenden Radiolarien mit Nadeln (Sphaerozoida). Ph.D. thesis, Christian-Albrechts Univ.

Calkins, G. N. 1909. Protozoölogy. Lea & Febiger.

Caron, D. A., A. F. Michaels, N. R. Swanberg and F. Howes. 1995. Primary productivity by symbiont-bearing planktonic sarcodines (Acantharia, Radiolaria, Foraminifera) in surface waters near Bermuda. Journal of Plankton Research **17:** 103-129.

Caron, D. A. and N. R. Swanberg. 1990. The ecology of planktonic sarcodines. Reviews in Aquatic Sciences **3:** 147-180.

Casey, R. E., R. C. Wigley and A. M. Perez-Guzman. 1983. Biogeographic and ecologic perspective on polycystine radiolarian evolution. Paleobiology **9:** 363-376.

Cavalier-Smith, T. 1987. The origin of eukaryote and archaebacterial cells. Ann. N.Y. Acad. Sci. **503:** 17-54.

Cavalier-Smith, T. 1993. Kingdom Protozoa and its 18 phyla. Microbiological Reviews **57:** 953-994.

Corliss, J. O. 1984. The kingdom Protista and its 45 phyla. BioSystems **17:** 87-126.

Corliss, J. O. 1994. An interim utilitarian ("user-friendly") hierarchical classification and characterization of the protists. Acta Protozoologica **33:** 1-51.

De Rijk, P., J.-M. Neefs, Y. Van de Peer and R. De Wachter. 1992. Compilation of small ribosomal subunit RNA sequences. Nucleic Acids Research **20:** 2075-2089.

DeLong, E. F., F. S. Wickham and N. R. Pace. 1989. Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. Science **243:** 1360-1363.

Ehrenberg, C. 1838. Über die bildung der kreidfelsen und kreidemergels durch unsichtbare organismen. Abh. Kgl. Akad. Wiss. Jahrb. Berlin 59-147.

Enriques, P. 1931. Saggio du una classificazione dei Radiolari. Arch. Zool. It. **16:** 978-994.

Febvre, J. 1990. Phylum Actinopoda Class Acantharia, p. 363-379. *In* Lynn Margulis, John Corliss, Michael Melkonian and David J. Chapman [ed.], Handbook of Protoctista. Jones and Bartlett Publishers.

Field, K. G., G. J. Olsen, D. J. Lane, S. J. Giovannoni, M. T. Ghiselin, E. C. Raff, N. R. Pace and R. A. Raff. 1988. Molecular phylogeny of the animal kingdom. Science **239:** 748-753.

Gobel, U. B., A. Geiser and E. J. Stanbridge. 1987. Oligonucleotide probes complementary to variable regions of ribosomal RNA discriminate between Mycoplasma species. Journal Gen. Microbiol. **133:** 1969-1974.

Haeckel, E. 1883. Über die Ordnugen der Radiolarien, Sitzungsber. Jenaischen Gesellsch. Med. Naturwiss., Jahrg 1883, 1-19.

Haeckel, E. 1887. Report on Radiolaria collected by H. M. S. Challenger during the years1873-1876, p. 1-1760. *In* C. W. T. a. J. Murray [ed.], The Voyage of the H. M. S. Challenger. vol. 18. Her Majesty's Stationary Office.

Haecker, V. 1908. Tiefsee-Radiolarien. Allgemeiner Teil. Form und Formbildung bei den Radiolarien. Wiss. Ergeb. Deutsch. Tiefsee-Exp. Dampfer "Valdivia" 1898-1899 **14:** 477-706.

Hilmers, C. 1906. Zur Kenntnis der Collosphaeriden. Ph.D. thesis, Christian-Albrechts Univ.

Huxley, T. H. 1851. Zoological notes and observations made on board the H. M. S. Rattlesnake. The Annals and Magazine of Natural History **viii:** 433-442.

Kellogg, D. E. and J. D. Hays. 1975. Microevolutionary patterns in late Cenozoic radiolaria. Paleobiology **1:** 150.

Lazarus, D. 1983. Speciation in pelagic Protista and its study in the planktonic microfossil record: a review. Paleobiology **9:** 327-340.

Lee, J. J., S. H. Hutner and E. C. Bovee. 1985. An illustrated guide to the protozoa. Society of Protozoologists.

Levine, N. D., J. O. Corliss, F. E. G. Cox, G. Deroux, J. Grain, B. M. Honigberg, G. F. Leedale, A. R. I. Loeblich, J. Lom, D. Lynn, E. G. Merinfeld, F. C. Page, G. Poljansky, V. Sprague, J. Vavra and F. G. Wallace. 1980. A newly revised classification of the protozoa. Journal of Protozoology **27:** 37-58.

Meyen, F. 1834. Beiträge zur Zoologie, gesammelt auf einer Reise um die Erde. Nova Act. Acad. Leop. Carol. **5:** 125-218.

Michaels, A. F. 1988. Vertical distribution and abundance of Acantharia and their symbionts. Marine Biology **97:** 559-569.

Müller, J. 1858. Über die Thalassicollen, Polycystinen und Acanthometren des Mittelmeeres, p. 1-62. *In* F. Dummler [ed.], Abhandlungen der Königlische Akademie der Wissenschaften zu Berlin.

Neefs, J.-M., Y. Van de Peer, P. De Rijk, A. Goris and R. De Wachter. 1991. Compilation of small ribosomal subunit RNA sequences. Nucleic Acids Research **19 (Supplement):** 1987-2015.

Olsen, G. J., D. J. Lane, J. Giovannoni and N. R. Pace. 1986. Microbial ecology and evolution: a ribosomal RNA approach. Annual Review of Microbiology **40:** 337-365.

Page, C. F. 1987. The classification of 'naked' amoebae (Phylum Rhizopoda). Arch. Protistenkd. **133:** 199-217.

Popofsky, A. 1908. Die radiolarien der Antarktis (mit ausnahme der tripyleen), p. 237-261. Deutsche Südpolar-Expedition 1901-1903.

Riedel, W. and A. Sanfilippo. 1971. Cenozoic Radiolaria from the western tropical Pacific, Leg 7, p. 1529-1672. *In* E. L. W. e. al. [ed.], Initial Reports of the D.S.D.P. vol. 7. U. S. Gov. Print. Off.

Schewiakoff, W. 1926. Die Acantharia des Golfes von Neapel. Fauna und Flora, p. 1-755. *In* G. Bardi [ed.], Fauna und Flora des Golfes von Neapel. R. Friedlander und Sohn.

Sogin, M. L., H. J. Elwood and J. H. Gunderson. 1986. Evolutionary diversity of eukaryotic small-subunit rRNA genes. Proceedings of the National Academy of Science **83:** 1383-1387.

Sogin, M. L. and J. H. Gunderson. 1987. Structural diversity of eukaryotic small subunit ribosomal RNAs. Annals of the New York Academy of Sciences **503:** 125-139.

Strelkov, A. A. and V. V. Reshetnyak. 1971. Colonial Spumellarian Radiolarians of the World Oceans, p. 295-418. Radiolarians of the Ocean, Reports of the Soviet Expeditions. vol. IX. Nauka Leningrad Department.

Swanberg, N. R. 1979. The ecology of colonial radiolarians: their colony morphology, trophic interactions and associations, behavior, distribution and the photosynthesis of their symbionts. Ph.D. thesis, Woods Hole Oceanographic Institution and Massachusetts Institute of Technology,

Tregouboff, G. 1953. Classe des Acanthaires, p. 271-320. *In* P. P. Grassé [ed.], Traité de Zoologie. Masson et Cie.

Chapter 1

Phylogenetic Relationships between the Acantharea and the Polycystinea (Spumellarida) Inferred from 16S-like Ribosomal RNA Gene Analyses: A Molecular Perspective on Haeckel's Radiolaria

ABSTRACT The evolutionary relationship of the Acantharea and the Polycystinea (Sarcodina) to other protists was investigated using comparative small-subunit ribosomal RNA (SSU rRNA) gene analyses. While current opinion regards the Acantharea as a separate class distinct from its original Haeckelian inclusion among the "Radiolaria" ("Radiolaria" sensu lato: Polcystinea, Phaeodarea and Acantharea), most investigators continue to support the hypothesis that the Acantharea and the Polycystinea share common ancestry, as revealed by their inclusion among the superclass Actinopoda (Calkins 1909). A major impediment to using a molecular approach to ascertain whether the Actinopoda represents a formal evolutionary assemblage has been an inability to culture many of these protists in the laboratory. We collected and maintained actinopods of the classes Acantharea and Polycystinea to obtain reproductive specimens highly enriched with DNA in order to facilitate DNA extraction and sequencing. The origin of the sequences described herein were confirmed by in situ hybridization experiments. The results from molecular phylogenetic analyses inferred from SSU rRNA gene sequences do not support a shared history between the Acantharea and the Polycystinea. However, the monophyly of the Acantharea and the separate monophyly of the Polycystinea (Spumellarida) are well supported by our molecular phylogenetic analyses. The acantharian lineage branches among crown organisms while the polycystine lineage diverges prior to the radiation of the crown groups. In view of our findings, we conclude that the Actinopoda does not represent a monophyletic evolutionary assemblage and recommend that this taxonomic designation be discarded.

One morphological feature which members of the Sarcodina share is the presence of a pseudopod during some part of their life cycle. Further taxonomic division is based on the structure of these pseudopodia. Even though pseudopod-bearing protists are grouped together, there are morphological and molecular data indicating that they are polyphyletic. At the morphological level, authors have argued that a lack of morphological characters (amoeboid-form) fails to provide firm support that a group of organisms shares common ancestry (Bovee and Jahn 1973; Lee et al. 1985). At the molecular level, the polyphyly of the sarcodines has been revealed by small-subunit ribosomal DNA (SSU rDNA) analyses which place various groups of the sarcodines branching at different parts of the evolutionary tree of life (Clark and Cross 1988; Hinkle and Sogin 1993).

In the superclass Actinopoda (Calkins 1909), all members possess specialized microtubule-stiffened pseudopodia called axopodia. The taxon Actinopoda has been maintained as a phylogenetic assemblage in the most recent considerations of protistan systematics with agreement that certain heliozoa should be removed (e.g. pedinellids and heliomonads) (Cavalier-Smith 1993; Corliss 1994). The classes currently represented in this superclass include the Acantharea, Polycystinea, Phaeodarea, and Heliozoea.

One of the major distinctions between the Acantharea and the Polycystinea is the composition, architecture, and symmetry of the skeleton, when present. All acantharia form skeletons composed of monocrystals of strontium sulfate which come together at the center of the cell in a symmetrical fashion known as Müller's Law (Müller 1858). Polycystine skeletons, when present, are typically siliceous and exhibit a range of morphologies from simple spicules to more elaborate latticed shells possessing radial spines. Despite these differences, the common use of radial symmetry in cell-body plan and shell architecture often gives members of the Acantharea and the polycystine order Spumellarida a superficially similar appearance.

Although the term "radiolaria" is now often reserved as an informal taxonomic descriptor for members of the Polycystinea (Spumellarida and Nassellarida) and the Phaeodarea only, the term was originally used by biologists to include members of the class Acantharea, as well. While the term "Radiolaria" was actually coined by Johannes Müller (1858), Ernst Haeckel is credited as being the first of the early taxonomists to do an extensive description of acantharian and radiolarian systematics.

Of the 4,417 species of organisms described from collections of the Challenger Expedition, 3,508 of them were new species of Radiolaria identified by Ernst Haeckel (Haeckel 1887; Anderson 1983). In his classification scheme of the class "Radiolaria", Haeckel included four legions: the Acantharia, the Spumellaria, the Nassellaria and the Phaeodaria. This classification was later modified (Deflandre 1952; Deflandre 1953; Tregouboff 1953; Goll and Merinfeld 1979) to exclude the Acantharia (Acantharea) from the Radiolaria (the Polycystinea which included Spumellarida and Nassellarida, and the Phaeodarea). Despite some taxonomic revision, many of Haeckel's original descriptions of the Challenger Radiolaria persist today. Modern systematists, while placing acantharia in a class distinct from polycystines and phaeodaria generally agree that these classes share common ancestry and should be united within the Actinopoda (Levine et al. 1980; Lee et al. 1985; Febvre 1990; Cavalier-Smith 1993; Corliss 1994).

In all phaeodaria, polycystines and members of the acantharian order Arthracanthida, the central capsule or capsular wall divides the cell into an intracapsular region and an extracapsular region. The intracapsular region includes the nucleus, mitochondria, golgi and other major cellular machinery while the extracapsular region contains the axopodial network of the cell. Phaeodarian and polycystine radiolaria possess pores in their central capsules whereas acantharia do not.

The main features which have lead taxonomists to infer the relatedness of the Acantharea and Polycystinea are the presence of axopodia and occurrence of a central capsule, although additional cytological similarities have been noted. Most of these similarities occur between members of the Acantharea and the Polycystinea belonging to the order Spumellarida. Most acantharia are polynucleated, but the occurrence of a single nucleus in the acantharian genus Haliommatidium has been argued as a feature it shares with the polycystines, most of which have only one nucleus. Furthermore, some authors have observed an apparent similarity between the "gelatinous pellicle" of some Sphaerellarina, a suborder in the Spumellarida, and that of some acantharia (Hollande and Enjumet 1960; Massera Bottazzi 1978). Finally, the existence of strontium sulfate crystals (a skeleton-building material thought to be used only by members of the Acantharia) in some adult vegetative colonial spumellarian radiolaria and in apparently all swarmer cells of spumellaria has been suggested as potential evidence of their common ancestry (Dogel 1950; Hollande and Martoja 1974; Anderson 1981; Cavalier-Smith 1993).

Members of the Actinopoda are among the remaining protistan groups which lack any DNA sequence information with which to support or challenge the above views. We sequenced the small-subunit ribosomal RNA genes of representatives of the Acantharea and Polycystinea to determine if their assumed shared ancestry based on morphological features is supported at a molecular level. Since the taxa chosen for this study are thought to be among the most closely related of the four major classes included in the Actinopoda, this study further addresses the suitability of higher taxon-designations as Actinopoda and rekindles the debate over the best definition for "radiolaria".

MATERIALS AND METHODS

Sample Collection. All specimens were collected by divers by hand using glass or polycarbonate jars. Specimens were maintained in 0.22 µm Millipore-filtered Sargasso Sea water in glass culture tubes with brine shrimp (*Artemia salina*) as food until sacrificed for molecular analysis. All individuals were given sample designations prior to identification, and then order or genus-level classifications were made. All acantharia were collected off the southwestern coast of Bermuda in September 1994. Acantharian samples used in this paper were *Haliommatidium* sp. (BBSR 235: Order: Symphyacanthida, Family: Pseudolithidae) and Chaunacanthid 218 (BBSR 218: Order: Chaunacanthida). Polycystine radiolarian specimens, all from the order Spumellarida, were collected in a similar fashion off the southwestern coast of Bermuda on multiple dates. One solitary and three colonial spumellaria were used in this study. Solitary spumellarian *Thalassicolla nucleata* (BBS 3: Family: Thalassicollidae) was collected in May 1992, colonial spumellarian *Collosphaera globularis-huxleyi* (BBSR 173: Family: Collosphaeridae) was collected in May 1994, and colonial spumellarians *Sphaerozoum punctatum* (CR4: Family: Sphaerozoidae) and *Collozoum serpentinum* (CR16: Family: Sphaerozoidae) were collected in May 1995. Specimens used for *in situ* hybridizations were collected in September and October of 1995 in the same location.

DNA Extraction, Amplification, Cloning and Sequencing. In order to enrich for sarcodine DNA, whenever possible, reproductive acantharian and spumellarian radiolarian specimens were sacrificed at a point in their life cycle just prior to swarmer cell release from the central capsule. In the case of the acantharia, single individuals were collected upon formation of cysts that were generated prior to swarmer cell release. The rationale behind collecting the specimens at this point in their life cycle was twofold: first, there is a natural amplification of DNA which occurs within the organism at this time and second, many species of spumellarian radiolaria and acantharia either consume or expel endocytoplasmic symbiotic algae before swarmer formation, thereby reducing the potential of amplifying non-target DNA.

Individual central capsules or cysts were rinsed several times in 0.22 µm-Millipore filtered seawater followed by a final MilliQ-water rinse prior to placement in buffer solution. T. nucleata specimens were processed by pooling 2 central capsules, placing them in lysis buffer (40 mM EDTA, 50 mM Tris pH 8.3, 0.75 M Sucrose) and freezing at -20°C until further processed. Cells were lysed with proteinase K (10 mg/ml) and 20% SDS and then incubated at 55°C until lysis was complete. Genomic DNA was extracted with phenol, phenol/chloroform/isoamyl alcohol and chloroform/isoamyl alcohol, precipitated with ethanol and resuspended in TE buffer according to standard protocols.

The remaining samples were subjected to the following more streamlined protocols designed to minimize loss of DNA by minimizing the number of transfer steps (e.g. organic extractions and ethanol precipitation). Central capsules or individual cysts were rinsed as above, placed in modified 1X PCR Buffer (50 mM KCl, 10 mM Tris, pH 8.3, 2 mM $MgCl_2$, 0.001% Gelatin, 1.0% NP40 (Sigma)), stored frozen at -70°C and then heated at 95°C for 10 minutes to lyse cells and liberate DNA. Between 1 µl and 5 µl of a 20µl sample lysed in this manner was then used directly in PCR amplifications and typically yielded strong products.

T. nucleata, C. globularis-huxleyi and Haliommatidium sp. 16S-like rDNAs were amplified using PCR and eukaryotic primers specific to the ends of the molecule (Medlin et al. 1988). T. nucleata rDNA was cloned into M13 single strand phage, several clones were pooled and the resulting template was sequenced using Sequenase 2.0 (US Biochemical; Cleveland, OH) enzyme and existing rDNA primers (Elwood et al. 1985; Medlin et al. 1988). Two additional primers were designed and synthesized (Indiana University; Bloomington, IN) to obtain a full length sequence of T. nucleata (690FTnucl, 5'-AGAGGTGAAATTCAAG-3'; 690RTnucl, 5'- CTTGAATTTCACCTCT-3').

Collosphaera globularis-huxleyi and Haliommatidium sp. rDNA PCR products were cloned into a double-stranded TA plasmid vector pCRII (Invitrogen; San Diego, CA) and plasmid DNA for sequencing was obtained using the Magic MiniPrep system (Promega; Madison, WI). Double-stranded sequencing of both the entire forward and reverse strands of the rDNA coding regions was accomplished using the Sequenase version 2.0 kit and methods.

Oligonucleotide Probe Design. In situ whole-cell hybridizations using oligonucleotide probes complementary to the sarcodine SSU rRNA were carried out in order to verify that the sequence data was derived from the sarcodine DNA and not from a contaminating source such as algal symbionts or prey material. Oligonucleotide probes were designed which were unique to the acantharian sequence data and separate probes were designed which were unique to the colonial spumellarian sequence data. At the time of design of these probes, at least single-stranded sequence data was available representing three orders of Acantharea; Arthracanthida, Chaunacanthida and Symphyacanthida (Haliommatidium sp.), with which to search for signature sequences for designing probes (Chapter 4). For the colonial spumellarian radiolaria, at least single-stranded sequence information from C. globularis-huxleyi and Rhaphidozoum acuferum (see Chapter 3) was available.

Probes were designed which would target either the Acantharea or the colonial spumellaria (Fig. 1). Only colonial spumellarian probes were designed because it was not possible to find signature sequences which were sufficiently unique (having at least three base pair mismatches against any other SSU sequences in the RDP database) to design probes which would identify both the solitary radiolarian T. nucleata and the two colonials. The biotin-labeled probes designed for acantharian samples were as follows: A497bio, 5'-TCATTCCAATCAACTCAC-3'; A899bio, 5'-TCGTCATACAAAGGTCCA-3'. The probes designed for colonial spumellarian samples were as follows: R906bio, 5'-AAC-

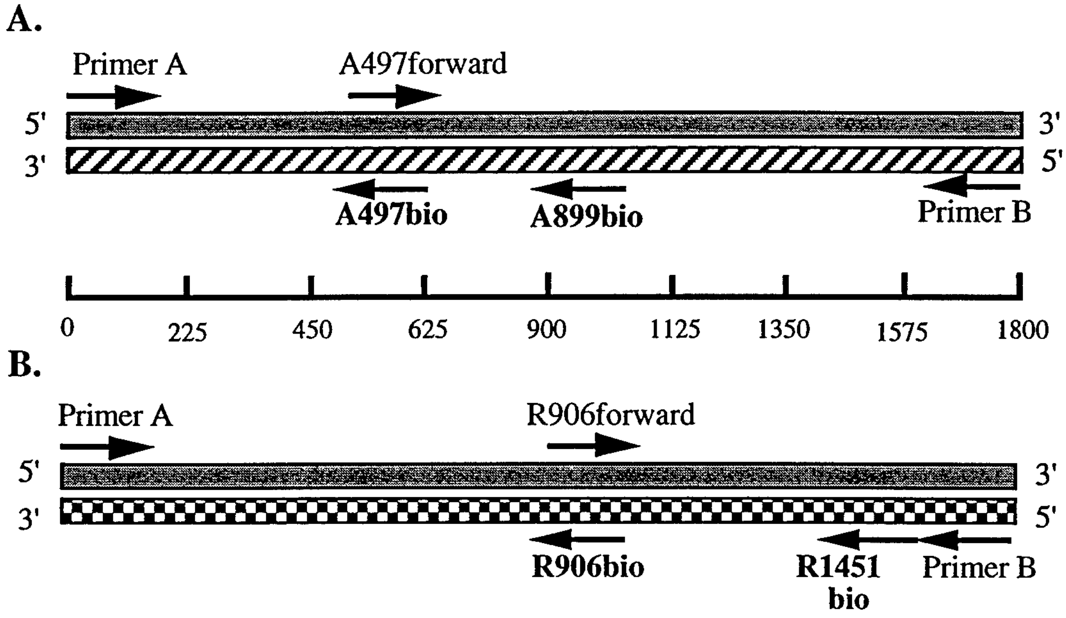


FIG. 1. A. The locations of acantharian-specific oligonucleotide probes (in bold) A497bio and A899bio used in in situ hybridization experiments of acantharia. The complement of A497bio was synthesized (denoted as A497forward in the schematic) and used in combination with Primer B in PCR reactions to later specifically amplify the acantharian gene fragment approximately 1,300 bp in length. Primer A was then used in combination with A899bio to obtain an overlapping fragment to the previous one approximately 900 bp in length to obtain the rest of the gene. B. The locations of colonial spumellarian-specific oligonucleotide probes (in bold) R906bio and R1451bio used in in situ hybridization experiments of colonial spumellaria. The complement of R906bio was synthesized (denoted as R906forward in the schematic) and used in combination with Primer B in PCR reactions to specifically amplify the gene fragment approximately 900 bp in length. Primer A was used in combination with R1451bio to obtain an overlapping fragment to the previous one approximately 1,450 bp in length to obtain the rest of the gene.

GATAAAATACTAATA-3'; R1451bio, 5'-TATTGTAGCCCGTGCGCT-3'. All probes were analyzed by Check Probe (RDP; University of Illinois, Urbana) for potential homology to other SSU rRNA sequences in the database before syntheses were carried out. The oligonucleotide probes A899bio and R1451bio were synthesized by Eppendorf (Madison, WI) and oligonucleotide probes A497bio and R906bio were synthesized by Cruachem (Foster City, CA). The following eukaryote-specific, 5'-biotinylated probes were used as positive controls: EUK502Rbio; 5'ACCAGACTTGCCCTCC-3' (Amann et al. 1990) and EUK1209Rbio; 5'-GGGCATCACAGACCTG-3' (Giovannoni et al. 1988). These probes will hybridize with all known eukaryotic SSU rRNA.

In situ Hybridizations. In situ hybridizations were carried out using both fluorescence and colorimetric detection methods. The latter technique was necessary for the colonial spumellarian samples due to severe autofluorescence occurring in these organisms. Fluorescence in situ hybridizations on acantharia were carried out as described in Lim et al. (1993) using biotinylated probes and detection with FITC-avidin solution (20 µg/ml in 100 mM $NaHCO_3$-buffered saline, pH 8.2; (Vector Laboratories, Inc.; Burlingame, CA)). Acantharia were fixed for 1 hour at 4°C in 1X Histochoice (Amresco; Solon, OH) fixative diluted in 0.22 µm-filtered Sargasso seawater. Individuals were then transferred to gel-subbed-slides, overlaid with 0.05% agarose and allowed to dry overnight. Probe was added to a final concentration of 5 ng/µl. Probe treatments consisted of a negative control (incubation in fluorescein-labeled avidin with no probe added), a positive control (biotinylated EUK 1209Rbio added), and two separate acantharian-specific probe treaments using A497bio and A899bio oligonucleotides respectively.

Hybridizations were carried out at 42°C for 6-8 hours and subsequent washes were done at 45°C. Cells were mounted in Citifluor immersion oil (Citifluor, Ltd.; London, England) and viewed on a Zeiss Axiophot equipped for epifluorescence microscopy. Epifluorescence photomicrographs were taken with an integral camera system using a fluorescein isothiocyanate (FITC) filter set combination consisting of a 450-490 nm band-pass excitation filter; a 510 nm long-pass dichroic mirror; and a 515-565 nm band-pass emission filter. Fuji 100 ASA Provia color slide film was used for fluorescence pictures. All exposure times for a set of samples (i. e. negative control, positive control, taxon-specific probes) were kept constant so that the relative intensity was indicative of probe binding. Transmitted light photomicrographs were also taken of the same specimens using Kodak ASA 160 Tungsten film.

Colorimetric-based in situ hybridizations were carried out on colonial spumellarian samples using the Gibco BRL In Situ Hybridization and Detection System (Life Technologies; Frederick, MD) with the following modifications for use with rRNA and larger sarcodines. Colonies were preserved in 1X Histochoice with 95% ethanol added in a ratio of 4:1. Colonies were preserved for 1 hour at 4°C, transferred to 70% ethanol and held overnight at 4°C. Aliquots of preserved central capsules from a single colony were placed on silanated glass slides (Midwest Scientific; St. Louis, MO) and allowed to air dry. Slides were then baked at 65°C for 1 hour to remove endogenous alkaline phosphatase activity. Hybridizations were carried out in 50 µl-capacity Probe-Clip "Press-to-Seal" incubation chambers and holders (Midwest Scientific; St. Louis, MO). Four probe treatments were carried out using central capsules from the same colony: a negative control incubation (streptavidin-alkaline phosphatase conjugate with no probe added), a negative probe control (A899bio acantharian probe added), a positive probe control (EUK 502bio and EUK 1209bio added), and a colonial spumellarian probe treatment (R906bio and R1451bio added). All probe treatments contained final total probe concentrations of 1 ng/µl.

Hybridizations were conducted according to the manufacturer's instructions for "DNA Detection" with the above modifications and the omission of any steps specifically required for DNA targets. Slides were hybridized for 8 hours and probe detection was carried out according to manufacturer's protocol with levamisole (Sigma; St. Louis, MO) added at 200 µg/ml upon addition of alkaline phosphatase conjugate to further eliminate any potential endogenous alkaline phosphatase activity. Developed slides were permanently mounted in Crystal/Mount (Biomeda; Foster City, CA) and observed on a Zeiss standard microscope equipped with phase microscopy. Transmitted light photomicrographs of samples were taken with an Olympus OM4-T camera using Kodak 160 speed Tungsten film.

Direct Sequencing of PCR Products. Upon achieving successful in situ hybridizations, further amplifications were accomplished using group-specific probes as primers in PCR reactions to specifically amplify and sequence sarcodine rDNA. The acantharian probe A899bio was used as a reverse primer in combination with Medlin amplification-primer A (Medlin et al. 1988) to specifically amplify the first 900 base pairs of acantharian SSU rRNA genes from the chaunacanthid sample BBSR 218 (See Fig. 1). The complement of probe A497 (non-biotinylated) was synthesized (Cruachem) and used in combination with Medlin amplification primer B (Medlin et al. 1988) to amplify a gene fragment approximately 1,300 base pairs in length which overlapped the primer A/A899bio amplification fragment.

Likewise for the colonial spumellaria, the complement of probe R906bio was synthesized (Cruachem) and the primer A/R1451bio and R906/primer B primer-pair amplifications were carried out on colonial spumellarian samples CR4 and CR16 (See Fig. 1). All PCR fragments were purified using the Wizard PCR Prep system (Promega; Madison, WI). Direct sequencing of PCR products was accomplished using reagents from the Sequitherm Long Read Sequencing Kit (Epicentre Technologies; Madison, WI) along with the Sequitherm Cycle sequencing protocol developed by Li-Cor which consisted of 5 minutes of denaturation at 95°C prior to 30 cycles of 20 sec at 95°C, 30 sec at 60°C, and 1 minute at 70°C using a Perkin Elmer 2400 Thermo Cycler. Sequenced templates were run out on a Licor model 4000L sequencer. Gel images were transferred from Licor to BioImage

(Millipore Corp.; Ann Arbor, MI) and sequences were analyzed using the Millipore BioImage DNA Sequence Film Reader software.

Phylogenetic Analysis. The 16S-like rRNA sequences of acantharian and radiolarian samples were aligned against a subset of the total eukaryotic alignment data base (Olsen et al. 1992). The 31 taxa included in this study are listed in Table 1. Sequences were aligned by eye using the Olsen Multiple Sequence Alignment Editing program with regard to primary and secondary structural conservation. 1,369 positions were used in the phylogenetic analyses. A distance matrix based on pairwise distances was created for the data set and a phylogenetic tree was inferred from these data by the method of Olsen (Olsen 1988). One hundred bootstrap replicates were conducted and a consensus tree was obtained using PHYLIP 3.5 (Felsenstein 1985). Phylogenetic trees were also inferred by the maximum likelihood method in conjuction with the fastDNAml program (Olsen et al. 1994) using a generalized two parameter model of evolution (Kishino and Hasegawa 1989) and maximum parsimony method using PAUP, version 3.1.1 (Swofford 1991). The maximum parsimony tree was obtained from a consensus of 100 bootstrap replications which were conducted using a heuristic search option with random addition sequence, 10 replicates and the tree bisection-reconnection algorithm. Identical phylogenetic analyses as those described above were also performed with Phreatamoeba balamuthi removed from the data set, in order to determine stability of the relative branching of the acantharia and the polycystine radiolaria. In these analyses, the same alignment and sequence positions were used as in those analyses including Phreatamoeba balamuthi in the data set.

RESULTS

In situ hybridization experiments confirmed the origin of the acantharian and spumellarian sequences (Fig. 2). Acantharian specific probes were found to specifically hybridize to the acantharia (Fig. 2, panels F and H) and not to colonial spumellaria (Fig. 2, panel J).

Table 1. Percent G + C content and taxonomic affinities of the taxa used in this study.

Species	SSU rDNA G+C (%)	Taxonomic affinity
Theileria annulata	45	Apicomplexa
Symbiodinium pilosum	45	Dinoflagellida
Oxytricha granulifera	46	Ciliophora
Blepharisma americanum	47	Ciliophora
Porphyridium aerugineum	48	Rhodophyta
Stylonema alsidii	46	Rhodophyta
Emiliana huxleyi	50	Haptophyta
Labyrinthuloides minuta	44	Labyrinthulid
Ochromonas danica	45	Chrysophyceae
Cafeteria roenbergensis	47	Bicosoecids
Chlamydomonas reinhardtii	50	Chlorophyte
Oryza sativa	51	Plantae
Acanthamoeba castellanii	52	Amoebida
Hartmanella vermiformis	49	Amoebida
Athelia bombacina	47	Fungi (Eumycota)
Blastocladiella emersonii	46	Fungi (Eumycota)
Mnemiopsis leidyi	47	Animalia
Diaphanoeca grandis	44	Choanoflagellate
Phreatamoeba balamuthi	47	Amoeba

Table 1. (cont.)

Species	SSU rDNA G+C (%)	Taxonomic affinity
Paulinella chromatophora	48	Filosea
Euglypha rotunda	45	Filosea
Haliommatidium sp.	44	Symphyacanthida
Chaunacanthid 218	45	Chaunacanthida
Dictyostelium discoideum	42	Dictyostelida
Physarum polycephalum	52	Plasmodial Slime Molds
Thalassicolla nucleata	36	Spumellarida
Collosphaera globularis-huxleyi	35	Spumellarida
Sphaerozoum punctatum	37	Spumellarida
Collozoum serpentinum	38	Spumellarida
Entamoeba gingivalis	34	Amoebida
Naegleria gruberi	48	Schizopyrenida

Likewise, colonial spumellarian probes specifically hybridized with colonial spumellaria (Fig. 2, panel L).

The % G + C content of the SSU rRNA gene for Haliommatidum sp. and Chaunacanthid 218 were 44% and 45% respectively, which was similar to many of the other taxa used in the analyses (Table 1). However, spumellarian % G+ C content values (35% - 38%) were similar to that of Entamoeba gingivalis (34%) and were low relative to typical eukaryotic values which are usually around 50%. Gene lengths in base pairs (bp) for acantharian and spumellarian samples were typical for eukaryotic SSU rRNA genes. Haliommatidum sp. and Chaunacanthid 218 were 1788 bp and 1778 bp. Lengths of genes for spumellaria were as follows: T. nucleata, 1770 bp; C. globularis-huxleyi, 1797 bp ; S. punctatum, 1788 bp; C. serpentinum, 1798 bp.

The phylogenetic trees inferred by the distance-matrix, maximum parsimony (Fig. 3) and maximum likelihood (data not shown) methods clearly rejected a common ancestry between these two groups of actinopods. Numbers at nodes represent bootstrap values as a percentage of 100 resamplings of the data set. Only bootstrap values greater than 50% are shown and represent relative measures of confidence. Both the distance and parsimony trees placed the spumellarian radiolaria branching as a diverging lineage below the "crown" groups (Knoll 1992), those taxa representing major eukaryotic assemblages simultaneously radiating from the node labeled with a bootstrap value of 85/65. Both methods revealed a poorly resolved branching point for the acantharia (Haliommatidum sp. and Chaunacanthid 218) among the crown radiation. The relative positions of the acantharia and the polycystine radiolaria were not affected by removal of Phreatamoeba balamuthi (Fig. 4). Removal of Phreatamoeba balamuthi from the data set resulted in higher bootstrap support values for the node leading to the crown (89/98).

A low bootstrap support value of 67% was obtained for the branching of the spumellaria with Entameoba gingivalis in the parsimony analysis, but this support was not observed in

FIG. 2. In situ hybridization of Histochoice-preserved specimens using oligodeoxynucleotide probes complementary to the 16S-like (small-subunit) ribosomal RNA sequences of acantharia (A-H) and colonial spumellaria (I-L). For both acantharian and colonial spumellarian cells, probes conjugated to biotin were detected by either fluoroisothiocyanate (FITC)-avidin or streptavidin-alkaline phosphatase-conjugated secondary labels. For the acantharian cells, hybridization detection was carried out using epifluorescence microscopy with settings specific for FITC excitation (panels B, D, F, H). Colonial spumellarian cells were viewed using phase contrast microscopy and hybridizations were detected colorimetrically using the localized, purple precipitate of the enzymatic reaction of alkaline phosphatase on nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolylphosphate (BCIP) substrates. Panels A-H depict four different acantharian cells of the same species with corresponding phase and epifluorescence photomicrographs of the same cell. Scale bars represent 75 µm in panels A-H. Panels A and B show the negative control to which only FITC-avidin was added. Note the minimal background fluorescence of the cell under epifluorescence (B). Panels C and D show the positive control treatment to which a eukaryotic-specific probe designed to target all eukaryotes (EUK 1209R) was added. Panels E and F and G and H show the probing of cells with two different acantharian probes (F, A497; H, A899), both designed against members of three different orders of acantharia (i.e. these probes should hybridize with all species within these orders of acantharia). Panels I through L show hybridization results for single individuals within the same colony. Panel I shows the negative control to which only the streptavidin-alkaline phosphatase-conjugated secondary label was added. Panel J shows a negative probe control treatment to which an acantharian probe was added. Panel K shows the results of the positive control hybridization with eukaryote probes (EUK502R and EUK1209R) and panel L shows hybridization with colonial spumellarian probes (R906 and R1451). Scale bars represent 35 µm in panels I through L.

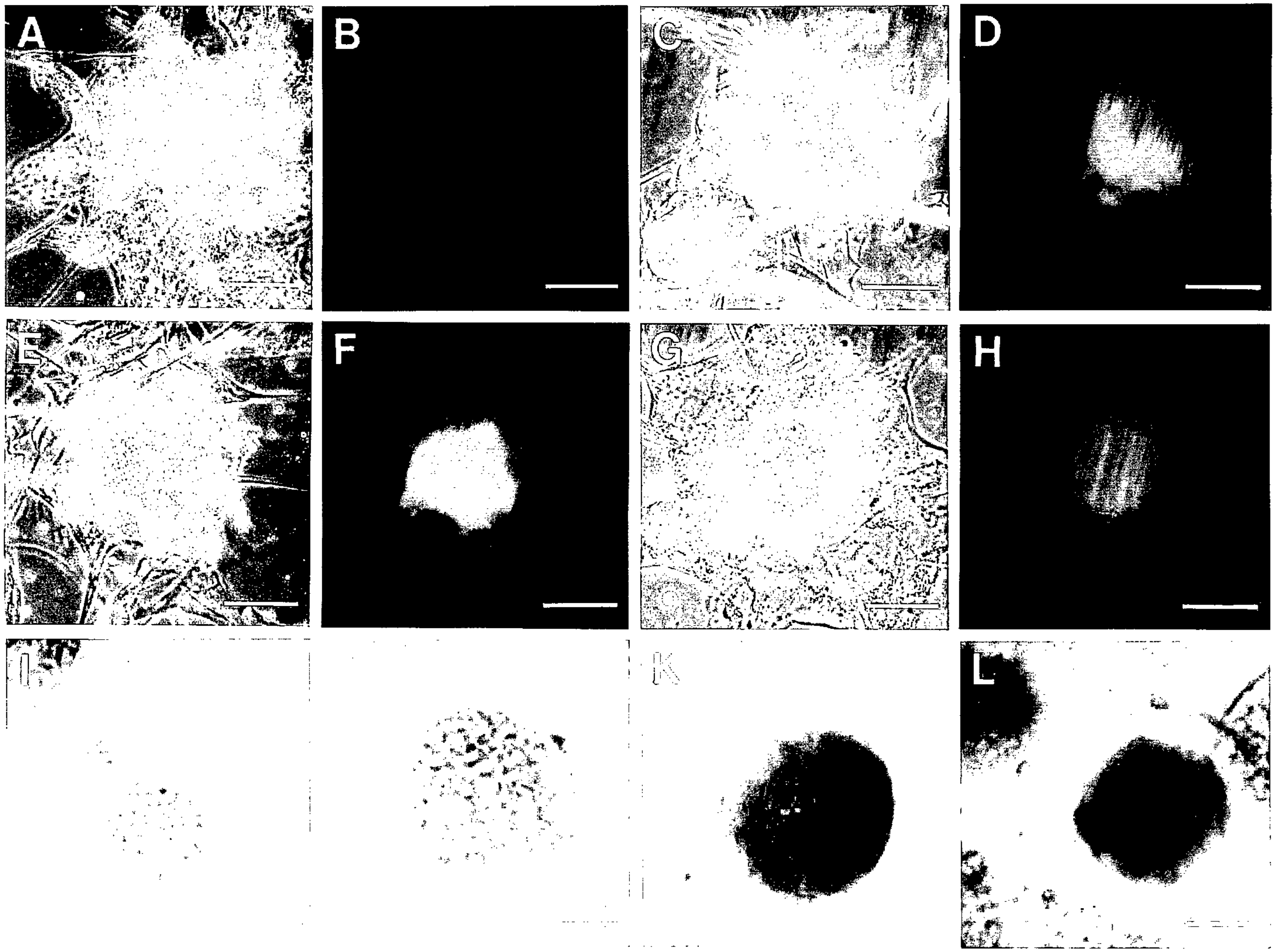

A
B
C
D
E
F
G
H
I
K
L

FIG. 3. The inferred phylogeny for the acantharia and the spumellarian radiolaria. A distance tree is shown. Numbers at the nodes represent bootstrap values, given as a percentage of 100 resamplings of the data. Bootstrap values for distance analyses are given above the line, whereas maximum parsimony values are below. A dash indicates that the bootstrap value for that node was below 50% in the method used for phylogeny reconstruction. The bar insert corresponds to 10 changes per 100 nucleotide positions. Only horizontal components of the tree are measures of evolutionary distance.

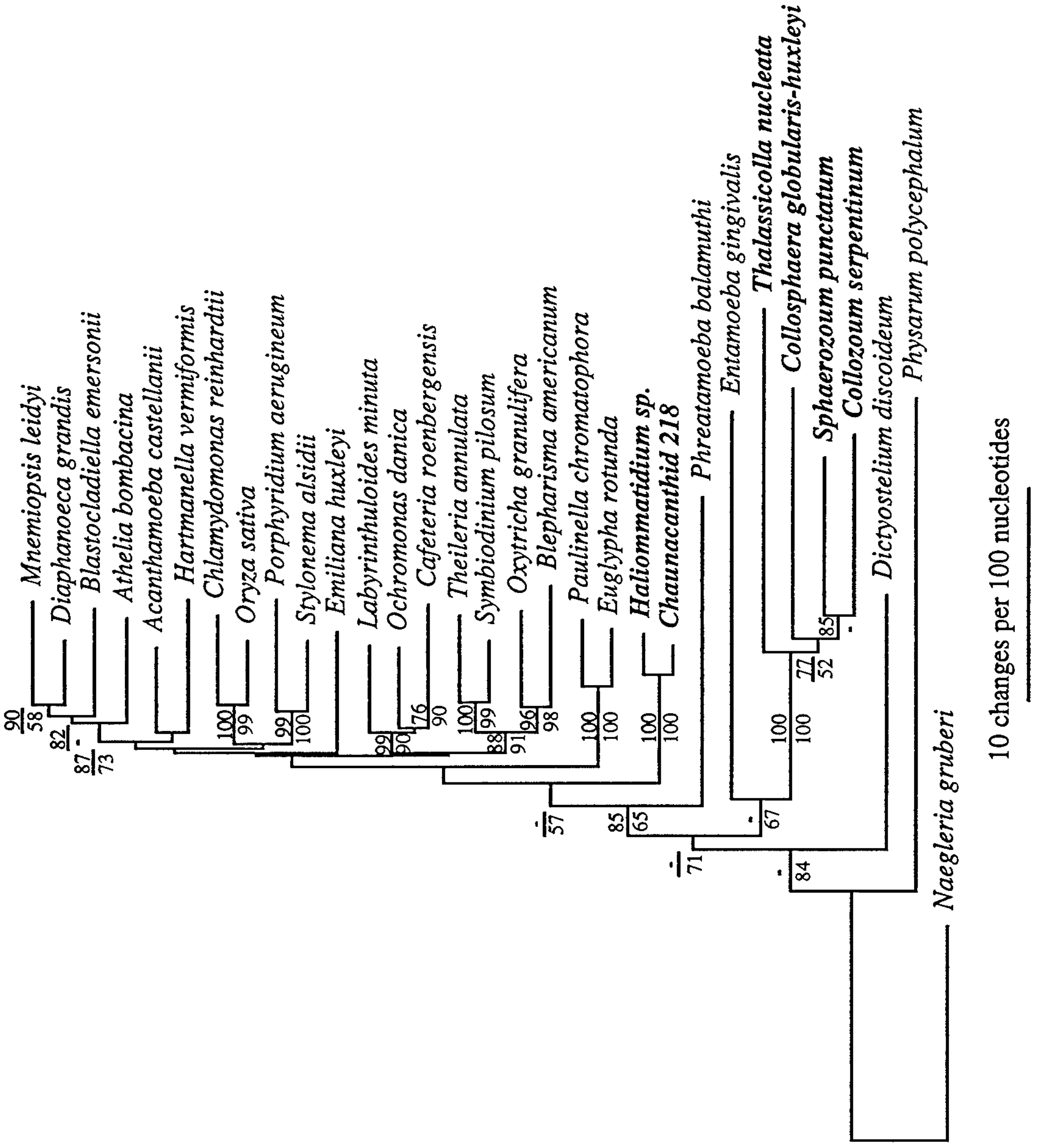
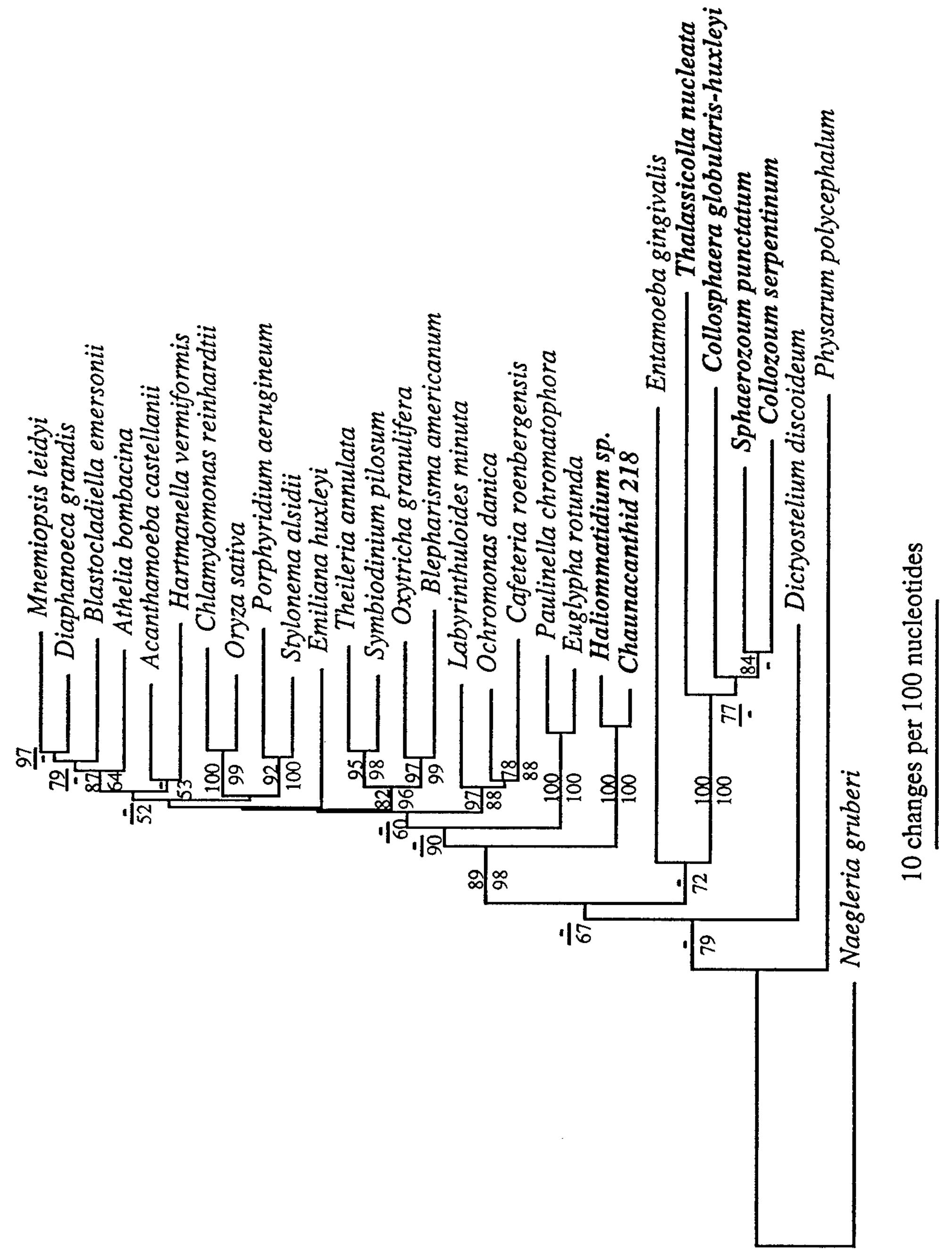

Mnemiopsis leidyi
Diaphanoeca grandis
Blastocladiella emersonii
Athelia bombacina
Acanthamoeba castellanii
Hartmanella vermiformis
Chlamydomonas reinhardtii
Oryza sativa
Porphyridium aerugineum
Stylonema alsidii
Emiliana huxleyi
Labyrinthuloides minuta
Ochromonas danica
Cafeteria roenbergensis
Theileria annulata
Symbiodinium pilosum
Oxytricha granulifera
Blepharisma americanum
Paulinella chromatophora
Euglypha rotunda
Haliommatidium sp.
Chaunacanthid 218
Phreatamoeba balamuthi
Entamoeba gingivalis
Thalassicolla nucleata
Collosphaera globularis-huxleyi
Sphaerozoum punctatum
Collozoum serpentinum
Dictyostelium discoideum
Physarum polycephalum
Naegleria gruberi
90
58
82
-
87
73
100
99
99
100
99
90
76
90
100
99
88
91
96
98
100
100
100
100
-
57
85
65
-
71
100
100
-
67
77
52
85
-
-
84
10 changes per 100 nucleotides

FIG. 4. The inferred phylogeny for the acantharia and the spumellarian radiolaria after the removal of Phreatamoeba balamuthi from the data set. A distance tree is shown. Numbers at the nodes represent bootstrap values, given as a percentage of 100 resamplings of the data. Bootstrap values for distance analyses are given above the line, whereas maximum parsimony values are below. A dash indicates that the bootstrap value for that node was below 50% in the method used for phylogeny reconstruction. The bar insert corresponds to 10 changes per 100 nucleotide positions. Only horizontal components of the tree are measures of evolutionary distance.


Mnemiopsis leidyi
Diaphanoeca grandis
Blastocladiella emersonii
Athelia bombacina
Acanthamoeba castellanii
Hartmanella vermiformis
Chlamydomonas reinhardtii
Oryza sativa
Porphyridium aerugineum
Stylonema alsidii
Emiliana huxleyi
Theileria annulata
Symbiodinium pilosum
Oxytricha granulifera
Blepharisma americanum
Labyrinthuloides minuta
Ochromonas danica
Cafeteria roenbergensis
Paulinella chromatophora
Euglypha rotunda
Haliommatidium sp.
Chaunacanthid 218
Entamoeba gingivalis
Thalassicolla nucleata
Collosphaera globularis-huxleyi
Sphaerozoum punctatum
Collozoum serpentinum
Dictyostelium discoideum
Physarum polycephalum
Naegleria gruberi
10 changes per 100 nucleotides

the distance analysis nor the topology of the maximum likelihood analysis (data not shown). No other potential immediate common ancestors were indicated by these data. The position of the spumellaria relative to other groups branching below the crown varied between the distance and parsimony analyses. Therefore, the exact branching order of the spumellarian radiolaria also remains unresolved at this time. The monophyly of the acantharia and the monophyly of the spumellaria, however, were well supported (100% in all cases).

The branching patterns within the spumellaria in the distance and the parsimony analyses both showed the solitary spumellarian T. nucleata branching prior to the colonial spumellaria. Although the bootstrap support for this node was barely above 50% in the parsimony analysis, a higher bootstrap value was obtained (77%) with distance methods. The relationship between the solitary and colonial spumellaria has been examined using a larger suite of spumellarian taxa and additional nucleotide sites in Chapter 3.

DISCUSSION

The relative positions of the acantharia and the spumellaria in molecular phylogenetic trees indicate that the presence of axopodia, a capsule membrane and the ability to metabolize strontium sulfate should be reconsidered as reliable phylogenetic markers. Our molecular study of acantharian and spumellarian phylogeny strongly agrees with what has been speculated in the literature over the past several years: axopodia have evolved more than once and most likely represent convergent structures created in response to similar ecological constraints through evolutionary time (Merinfeld 1978; Shulman and Reshetnyak 1980; Merinfeld 1981; Reshetnyak 1981a). An independent evolution of axopodia within the chromistan Pedinellea of the Heliozoea already has been suggested on morphological grounds (Cavalier-Smith 1993). Given the results of this study, retention of the superclass Actinopoda seems inappropriate, as does the adoption of the new phylum Radiozoa, which has been described as a modern-day Radiolaria sensu lato (Cavalier-Smith 1993; Corliss 1994).

The presence of a central capsule and the ability to secrete strontium sulfate have been described as two synapomorphies defining the Radiozoa (Cavalier-Smith 1993). However, the central capsule found in spumellaria and that which exists in one order of Acantharea (the Arthracanthida) have been shown to differ (Massera Bottazzi 1978; Reshetnyak 1981a). The acantharian central capsule in this order is non-perforated and of ectoplasmic origin while that of the polycystines and the phaeodaria is perforated and located between the ectoplasm and the endoplasm. Furthermore, the presence of central capsules in the Arthracanthida, which are considered to be more derived than other orders of acantharia which lack central capsules (Hollande et al. 1965; Strelkov and Reshetnyak 1974; Reshetnyak 1981a), suggests that "central capsules" may have evolved more than once.

The occurrence of strontium sulfate in both acantharia and spumellaria is another feature often cited as evidence of their common ancestry. Vegetative adults of colonial spumellaria are known to house crystals of strontium sulfate in their central capsules and the biflagellated swarmers of all spumellarian radiolaria examined thus far contain crystals of strontium sulfate in membrane bound vesicles. However, metabolism of strontium sulfate is not unique to the acantharia and spumellarian radiolaria. Crystals of strontium sulfate have been observed in the desmid alga Closterium littorale (Raven et al. 1986), in Chara, the "stonewort" freshwater plant, and in loxodid ciliates (Fenchel and Finlay 1986). The role of strontium sulfate in Chara and the loxodid ciliates is apparently graviperception (Fenchel and Finlay 1986; Raven et al. 1986). A similar function has been proposed in the desmid algae (Raven et al. 1986). This function apparently has never been proposed for the membrane-bound crystals found in spumellarian swarmers. Instead, Anderson (1981) has suggested that strontium sulfate crystals may serve a function in buoyancy control but admits that silica or calcium compounds, which occur at higher concentrations in sea water, would be better candidates for this purpose. Anderson also suggested that strontium may be of some physiological importance to the spumellaria but does not elaborate on what this requirement might be. One possibility is that strontium serves a similar function in spumellaria as in some gastropods where it is required for proper shell development (Bidwell et al. 1986). A caveat to the potential importance of strontium in spumellarian skeletal development, however, is that even spumellarian species which lack skeletal material, like T. nucleata and Collozoum spp., have swarmers with crystalline strontium sulfate inclusions. Furthermore, the lack of strontium sulfate crystals in acantharian swarmer cells, seems inconsistent with the idea that strontium sulfate serves a similar function in both the Acantharea and the spumellarian polycystines.

While most of the literature has favored a common ancestry of the acantharia and the spumellaria, a series of papers published in Russian during the early 1980's argued against this idea (Shulman and Reshetnyak 1980; Reshetnyak 1981a; Reshetnyak 1981b). These papers describe several morphological features as unique to the Acantharea. These major features include the existence of a skeleton of strontium sulfate, not merely crystals of the compound as are found in some spumellaria, organized in a highly geometrical fashion according to Müller's Law. Also thought to be unique to Acantharea is the cytoplasmic feature called the calymma which, along with the ectoplasmic cortex and the non-actin containing myonemes, forms a "hydrostatic apparatus" thought to render acantharia capable of movement in the vertical direction. These authors concluded that the axopodial system was not a reliable phylogenetic marker, and defended their argument by comparison of ultrastructural studies of the axopodial systems in different groups of Actinopoda (Hollande 1953; Cachon and Cachon 1964; Febvre 1971; Cachon and Cachon 1972; Febvre 1972; Cachon et al. 1973; Febvre 1973).

In brief, the Russian authors proposed that axopodial systems evolved independently several times in evolution as amoeboid-like protists were going from benthic to pelagic modes of existence. The authors pointed out differences in the axopodial systems of various classes within the Actinopoda in support of their interpretation of the ultrastructural data provided by the French investigators cited above. They pointed out structural differences in the axoneme (the microtubular shaft which stiffens axopodia) and differences in the size and location of the axoplast (the microtubule-organizing center of the axoneme) between taxa which they say is suggestive of convergence not homology. Finally, they mention the presumed artificial grouping of actinophrid and centrohelid heliozoa (which posses very different axoneme structures) in support for their argument. For more details and diagrams comparing actinopod axopodial systems the reader is referred to the Russian literature cited above for which fairly complete translations are available from LAZ. The results from the molecular work described in this thesis support the major claims made by these Russian authors.

The absence of strontium sulfate in swarmer cells of Acantharea, as mentioned above, is noteworthy in this discussion because it suggests yet another difference between the respective requirements of acantharian and spumellarian swarmers. Given the fact that strontium sulfate crystals are thought to be involved in buoyancy control, their absence in acantharian swarmers, and the fact the acantharia are understood to reproduce at depth (Reshetnyak 1981a), the following scenario is consistent with what is currently understood about acantharian biology: Perhaps the need for strontium sulfate crystals for buoyancy control in acantharian swarmers is overcome by the ability of acantharia to regulate their depth in the water column via their "hydrostatic apparatus", allowing the acantharian to sink to the desired depth for release of its swarmer cells. In addition, many species of acantharia form cysts also composed of strontium sulfate which aid in the sinking of swarmer cells to depth. The greater density of strontium sulfate relative to silicon dioxide may also explain why polycystine radiolaria, which can possess siliceous skeletons, utilize the heavier strontium sulfate in their swarmers. Interestingly, phaeodarian radiolaria which live deeper in the water column do not have the capacity to metabolize strontium sulfate and, like acantharia, lack strontium sulfate-containing swarmer cells. Whatever function served, the presence of strontium sulfate in these marine protists and its singular utilization as the structural compound in the skeletons of acantharia deserves further scrutiny in the evolution of this group as do their unique non-actin-containing myonemes.

Which protists, then, share most recent ancestry with the Acantharea? The branching pattern of the acantharia was strikingly shallow relative to that of the spumellarian radiolaria, possibly suggesting that the acantharia diversified more recently than the spumellaria. The most recent common ancestor of the Acantharea could possibly be found among actinopods which have been placed among the incertae sedis. Among them we find such specimens as Podactinelius sessilis (Schröder 1907), possibly the only living benthic acantharian, which was described aboard the Deutschen Südpolar-Expedition of 1901-1903. This genus was once included as a separate order Actineliida in the class Acantharea. However, since the last publication of the Committee on Systematics and Evolution of the Society of Protozoologists, it been relegated to an uncertain taxonomic affinity. This genus possesses spines of strontium sulfate (400-500) which are not arranged in the characteristic geometric pattern observed in all Acantharea.

The determination of the nearest relative of the Spumellarida remains equally challenging. If the long branches occurring in the spumellarian lineage may be interpreted as evidence of their ancient origins it may be difficult to determine the phenotype of the most recent common ancestor of the Spumellarida. Although the fossil record of spicule-bearing forms (Sphaerozoidae) extends to the Lower Oligocene (Bjørklund and Goll 1979) and that of the Collosphaeridae to the base of the Miocene (Riedel 1967), even more ancient origins are possible in view of the existence of extant skeletonless forms which would not be preserved in the sediments. As an alternative hypotheses, the long branch lengths of the spumellaria may be explained as the result of a rapidly evolving lineage.

It is assumed that the Nassellarida which represent the second order included in the Polycystinea are closely related to the Spumellarida (Cachon et al. 1990). The molecular phylogenetic position of the Phaeodarea is also unknown and deserves consideration. As for Haeckel's Radiolaria and the definition of the Radiolaria sensu lato (Polcystinea, Phaeodarea and Acantharea), continued use of this definition in anything but a historical perspective, and the biological implications behind it appear unjustified in view of the results described herein.

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REFERENCES

Amann, R. I., B. J. Binder, S. W. Olson, R. Devereux and D. A. Stahl. 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Applied and Environmental Microbiology **56:** 1919-1925.

Anderson, O. R. 1981. Radiolarian fine structure and silica deposition, p. 347-379. *In* T. L. Simpson and B. E. Volcani [ed.], Silicon and Siliceous Structures in Biological Systems. Springer-Verlag.

Anderson, O. R. 1983. Radiolaria. Springer-Verlag.

Bidwell, J. P., J. A. Paige and A. M. Kuzirian. 1986. Effects of Strontium on the embryonic development of Aplysia californica. Biol. Bull. **170:** 75-90.

Bjørklund, K. R. and R. M. Goll. 1979. Internal skeletal structures of Collosphaera and Trisolenia: A case of repetitive evolution in the Collosphaeridae (Radiolaria). Journal of Paleontology **53:** 1293-1326.

Bovee, E. C. and T. L. Jahn. 1973. Taxonomy and phylogeny, p. 38-52. *In* K. W. Joen [ed.], The Biology of Amoeba. Academic Press.

Cachon, J. and M. Cachon. 1964. Les mouvements de ceclose dans les axopodes d'Acanthaires. Leur role lors la nutrition et de la locomotion. Bull. Inst. oceanogr. **61:** 3-8.

Cachon, J. and M. Cachon. 1972. Le système axopodial des Radiolaires Sphaeroides. I. Centroaxoplastidae. Arch. Protistenk. **114:** S. 51-64, 291-307.

Cachon, J., M. Cachon and K. W. Estep. 1990. Phylum Actinopoda Classes Polycystina (= Radiolaria) and Phaeodaria, p. 334-346. *In* Lynn Margulis, John Corliss, Michael Melkonian and David J. Chapman [ed.], Handbook of Protoctista. Jones and Bartlett Publishers.

Cachon, J., M. Cachon, C. Febvre-Chevalier and J. Febvre. 1973. Déterminisme de l'edification des systèmes microtubulaires stéréoplasmiques d'Actinopods. Arch. Protistenk. **115:** S. 137-153.

Calkins, G. N. 1909. Protozoölogy. Lea & Febiger.

Cavalier-Smith, T. 1993. Kingdom Protozoa and its 18 phyla. Microbiological Reviews **57:** 953-994.

Clark, C. G. and G. A. M. Cross. 1988. Small-subunit ribosomal RNA sequence from Naegleria gruberi supports the polyphyletic origin of the amoebas. Molecular Biology and Evolution **5:** 512-518

Corliss, J. O. 1994. An interim utilitarian ("user-friendly") hierarchical classification and characterization of the protists. Acta Protozoologica **33:** 1-51.

Deflandre, G. 1952. Classe des Radiolaires, p. 303-313. *In* J. Piveteau [ed.], Traité de Paléontologie. vol. 1. Masson.

Deflandre, G. 1953. Radiolaires fossiles, p. 389-436. *In* P. P. Grassé [ed.], Traité de Zoologie. vol. 1. Masson.

Dogel, V. A. 1950. Novye dannye po filogenii radiolyarii (New data on the phylogeny of Radiolaria). Zoologicheski Zhurnal (Zoological Journal) **29:** 562-565.

Elwood, H. J., G. J. Olsen and M. L. Sogin. 1985. The small-subunit ribosomal RNA gene sequences from the hypotrichous ciliates Oxytricha nova and Stylonychia pustulata. Mol. Biol. Evol. **2:** 399-410.

Febvre, J. 1971. Le myonème d'Acanthaire: Essai d'interprétation ultrastructurale et cinétique. Protistologica **7:** 379-391.

Febvre, J. 1972. Le cortex ectoplasmique des Acanthaires I. - Les systèmes maillés. Protistologica **8:** 169-178.

Febvre, J. 1973. Le cortex des Acanthaires II. Ultrastructure des zones de jonction entre les pièces corticales. Protistologica **9:** 87-94.

Febvre, J. 1990. Phylum Actinopoda Class Acantharia, p. 363-379. *In* Lynn Margulis, John Corliss, Michael Melkonian and David J. Chapman [ed.], Handbook of Protoctista. Jones and Bartlett Publishers.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolùtion **39:** 783-791.

Fenchel, T. and B. J. Finlay. 1986. The structure and function of Müller Vesicles in the loxodid cilates. Journal of Protozoology **33:** 69-79.

Giovannoni, S. J., E. F. DeLong, G. J. Olsen and N. R. Pace. 1988. Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial cells. Journal of Bacteriology **170:** 720-726.

Goll, R. M. and E. G. Merinfeld. 1979. Radiolaria, p. 673-684. *In* R. W. Fairbridge and Jablonski, D. [ed.], The Encyclopedia of Paleontology. Dowden, Hutchinson & Ross.

Haeckel, E. 1887. Report on Radiolaria collected by H. M. S. Challenger during the years1873-1876, p. 1-1760. *In* C. W. T. a. J. Murray [ed.], The Voyage of the H. M. S. Challenger. vol. 18. Her Majesty's Stationary Office.

Hinkle, G. and M. L. Sogin. 1993. The evolution of Vahlkampfiidae as deduced from 16S-like ribosomal RNA analysis. Journal of Eukaryotic Microbiology **40:** 599-603.

Hollande, A. 1953. Complement sur la cytologie des Acanthaires et des Radiolaires, p. 1089-1100. *In* [ed.], Traité de Zoologie, Anatomie, Systématique, Biologie. vol. 1.

Hollande, A., J. Cachon and M. Cachon-Enjumet. 1965. Les modalités de l'enkystement présporogénétique chez les Acanthaires. Protistologica **1:** 91-112.

Hollande, A. and M. Enjumet. 1960. Cytologie, Évolution et Systématique des Sphaeroïdés (Radiolaires). Arch. Museum Paris **VII:** 1-134.

Hollande, A. and R. Martoja. 1974. Identification du cristalloide des isospores de radiolaires a un cristal de célestite ($SrSO_4$) détermination de la constitution du cristalloide par voie cytochimique et a l'aide de la microsonde électronique et du microanalyseur par émission ionique secondaire. Protistologica **10:** 603-609.

Kishino, H. and M. Hasegawa. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and branching order in Hominoidea. Journal of Molecular Evolution. **29:** 170-179.

Knoll, A. H. 1992. The early evolution of eukaryotes: A geological perspective. Science **256:** 622-627.

Lee, J. J., S. H. Hutner and E. C. Bovee. 1985. An Illustrated Guide to the Protozoa. Society of Protozoologists.

Levine, N. D., J. O. Corliss, F. E. G. Cox, G. Deroux, J. Grain, B. M. Honigberg, G. F. Leedale, A. R. I. Loeblich, J. Lom, D. Lynn, E. G. Merinfeld, F. C. Page, G. Poljansky, V. Sprague, J. Vavra and F. G. Wallace. 1980. A newly revised classification of the protozoa. Journal of Protozoology **27:** 37-58.

Lim, E. L., L. A. Amaral, D. C. Caron and E. F. DeLong. 1993. Application of rRNA-based probes for observing marine nanoplanktonic protists. Applied and Environmental Microbiology **59:** 1647-1655.

Massera Bottazzi, E. 1978. Systematic-ecological aspects of Radiolaria with special reference to Acantharia. Boll. Zool. **45:** 133-144.

Medlin, L., H. J. Elwood, S. Stickel and M. L. Sogin. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene **71:** 491-499.

Merinfeld, E. G. 1978. Ecological considerations in the interpretations of morphological convergences among the oceanic planktonic sarcodines. Biosystems **10:** 29-30.

Merinfeld, E. G. 1981. Phylogenetic relationships among the Actinopoda. VI International Congress of Protozoology 244.

Müller, J. 1858. Über die Thalassicollen, Polycystinen und Acanthometren des Mittelmeeres, p. 1-62. *In* F. Dummler [ed.], Abhandlungen der Königlische Akademie der Wissenschaften zu Berlin.

Olsen, G. J. 1988. Phylogenetic analysis using ribosomal RNA, p. 793-812. *In* H. F. Noller Jr. and K. Moldave [ed.], Methods in Enzymology. Ribosomes. vol. 164. Academic Press.

Olsen, G. J., H. Matsuda, R. Hagstrom and R. Overbeek. 1994. fastDNAml: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. Computational Applied Biosciences **10:** 41.

Olsen, G. J., R. Overbeek, N. Larsen, T. L. Marsh, M. J. McCaughey, M. A. Maciukenas, W.-M. Kuan, T. J. Macke, Y. Xing and C. R. Woese. 1992. The ribosomal database project. Nucleic Acids Research **20 (Supplement):** 2199-2200.

Raven, J. A., F. A. Smith and N. A. Walker. 1986. Biomineralization in the Charophyceae sensu lato, p. 125-139. *In* B. S. C. Leadbeater and R. Riding [ed.], Biomineralization in Lower Plants and Animals. vol. 30. Clarendon Press.

Reshetnyak, V. V. 1981a. Evolution and phylogeny, p. 224. *In* [ed.], Acantharia (Acanthariea, Protozoa) from the World Ocean. vol. 123. Nauka, SSSR.

Reshetnyak, V. V. 1981b. Sovreminnoe predstavlenie ob akantariyakh (Akantaria) i ixk polozhenie v sisteme prosteishik (Living Acantharia and their position in the Protozoans), p. 17-21. *In* V. A. Krasheninnikov [ed.], Sistematika evolutisiya i stratigraficheskoe znachenie radiolyarii (Systematics, evolution and stratigraphic significance of Radiolaria). Nauka.

Riedel, W. R. 1967. Subclass Radiolaria, p. 291-298. *In* W. B. Harland [ed.], The Fossil Record. Geol. Soc. Lond.

Schröder, O. 1907. Eine gestielte Acanthometride Podactinelius sessilis nov. gen. sp. Deutsche Südpolar-Expedition 1901-1903 **9:** 227-236.

Shulman, S. S. and V. V. Reshetnyak. 1980. Realno li cushchestvovanie v cisteme prosteishikh nadklassa Actinopoda? (About the possibility of the superclass Actinopoda existence in the system of Protozoa?). Printsipy postroeniya makrosistemy odnokletochnykh zhivotnykh (Principles of the Construction of the Macrosystem of the Unicellular Animals) 94: 23-41.

Strelkov, A. A. and V. V. Reshetnyak. 1974. General Characteristics and Radioecology of the Strontium Organisms, Acantharian Radiolarians, p. 95-191. *In* G. G. Polikarpov [ed.], Chemoradiology of the Pelagical and Benthal (metals and their radionuclei in hydrobionts and in their environment medium). Naukova Dumka.

Swofford, D. L. 1991. PAUP: Phylogenetic Analysis Using Parsimony.

Tregouboff, G. 1953. Classe des Acanthaires, p. 271-320. *In* P. P. Grassé [ed.], Traité de Zoologie. Masson et Cie.

Chapter 2

Insights on the Diversity within a "species" of Thalassicolla (Spumellarida) Based on Small-Subunit Ribosomal RNA Gene Sequencing

ABSTRACT. We compared small-subunit ribosomal RNA gene sequences of samples from solitary spumellarian radiolarian Thalassicolla nucleata collected from the Sargasso Sea and the Pacific Ocean. Sequences derived from these separate locations showed variability in both length and base-pair composition which is consistent with genus-level variation reported in the literature for other taxa. The seven existing descriptions of Thalassicolla species, including T. nucleata, are discussed in view of these molecular findings and with reference to our current understanding of the physiology and life cycle of the spumellarian radiolaria.

Supplementary key words. Actinopoda, Polycystinea, radiolarian, sarcodine

Little systematic revision has occurred in the genus Thalassicolla since its first representative, Thalassicolla nucleata was described by Thomas Huxley in 1851. The solitary spumellarian T. nucleata along with many colonial spumellaria, all to which Huxley assigned the name Thalassicolla punctata, were among the first described living polycystine radiolaria. Thalassicolla punctata was later dissolved by Johannes Müller, but T. nucleata was retained and is still recognized as a valid species today.

The six other species of the genus Thalassicolla were all proposed by Ernst Haeckel primarily from specimens collected aboard the H. M. S. Challenger (Haeckel 1887). These species included the following: T. pellucida, T. spumida, T. zanclea, T. australis, T. maculata and T. melacapsa. Haeckel used qualities of the central capsule such as wall texture, color and size as the major distinguishing features upon which to separate species of Thalassicolla. Curiously, of the seven known species of Thalassicolla, only four, T. pellucida, T. spumida, T. melacapsa and T. nucleata appear to be mentioned in the literature since Haeckel's first reports, and no systematic revisions of the species of Thalassicolla have occurred since Haeckel's time.

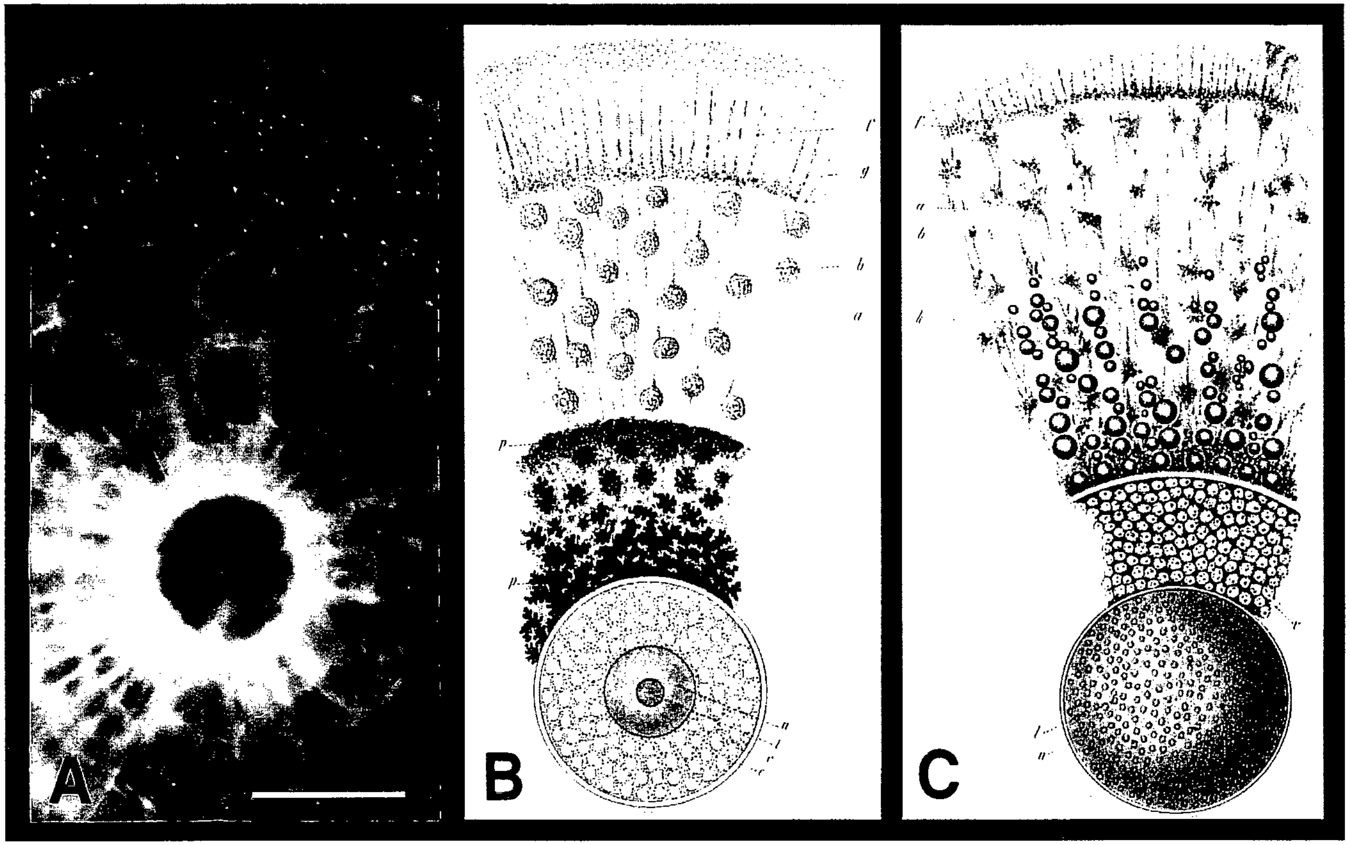
As more information about the physiology and life cycle of this genus has been obtained, the validity of some of Haeckel's species descriptions have been questioned. Most of these studies have been carried out on the single species T. nucleata.

Since its original description, T. nucleata has become a model organism for research into the biology of the spumellarian radiolaria (Anderson 1978; Herring 1979; Anderson 1983). Thalassicolla nucleata is a large (3 - 5 mm) solitary, spumellarian polycystine found ubiquitously in tropical and subtropical surface waters of open-ocean communities including the Sargasso Sea and the Pacific Ocean, as well as other locations. Thalassicolla nucleata lacks a skeleton and represents one of the simplest examples of polycystine cell architecture: a single central capsule, usually enveloped by a dark opaque layer, which is in turn surrounded by a matrix of highly-alveolated, symbiont filled extracapsular material (see Fig. 1A).

Given the rather simple cell-architecture of T. nucleata, it seems possible that the criteria used by Haeckel to distinguish between different species of Thalassicolla may not have been reflected on the genetic level. The morphological differences noted by Haeckel may have been the result of the physiological state of the cell, life cycle effects, or environmental influences. Ultimately it may not be possible to distinguish between species of Thalassicolla based on morphological criteria alone.

We approached the question of species diversity in the genus Thalassicolla by comparing gene sequences of the small subunit ribosomal RNA (SSU rRNA) in T. nucleata collected from different locations to determine if there are genetic differences which are not associated with morphological details at the species level. Two features of the life history make T. nucleata well-suited for molecular phylogenetic study. First, although it cannot be reared through successive generations, T. nucleata readily undergoes swarmer formation in the laboratory during which time the dark opaque layer surrounding the central capsular region of the cell is shed revealing a milky-white capsule beneath it (Anderson 1978). Swarmer formation marks the onset of reproduction in the cell, at which time the intracapsular DNA concentration is significantly increased and divided among swarmer cells. This "natural" amplification of DNA within the cell greatly facilitates retrieval of DNA for molecular analysis. Second, like many spumellarian radiolaria, T. nucleata lives in association with symbiotic algae which are believed to enhance survival of species in oligotrophic environments (Anderson 1978; Anderson and Botfield 1983). These algae might normally complicate separation of host DNA from symbiont DNA, but the symbionts in polycystines are physically excluded from the central capsular region by the capsular membrane. Dissection of the central capsule away from the rest of the extracapsular material which houses symbionts, along with sacrificing the cell immediately prior to

Fig. 1. **A.** A transmitted-light photomicrograph of a typical specimen of Thalassicolla nucleata showing the spherical central capsule covered by a dark opaque layer and surrounded by an alveolated extracapsular material. Bar = 0.4 mm. **B.** A drawing of T. maculata by Ernst Haeckel (from Haeckel 1887, Pl. 1 Fig.4). After Haeckel: c, The central capsule; v, vacuoles filling this capsule; n, the central nucleus; l, the concentric nucleolus; g, the voluminous calymma, a small radial piece of which is only presented; a, the large alveoles; b, peculiar exoplasmatic bodies; p, black pigment in the inner zone; f, the retracted pseudopodia in the outer zone of the calymma. **C.** A drawing of T. melacapsa also by Ernst Haeckel (from Haeckel 1887, Pl. 1 Fig. 5). After Haeckel: n, The large nucleus; l, numerous small nucleoli inside the nucleus; v, the vacuoles filling up the central capsule and separated by black pigment; a, large alveoles of the calymma; k, oil globules; b, exoplasmatic bodies; f, the retracted pseudopodia in the outer zone of the calymma.


A
B
f
g
h
a
p
p
C
f
a
b
k

swarmer release, therefore greatly enhances amplification of host DNA for further molecular analyses.

MATERIAL AND METHODS

Thalassicolla nucleata cells were collected in glass jars by divers. Cells were maintained in 0.22 µm Millipore-filtered sea water in glass culture tubes and fed brine shrimp (Artemia salina) as food. T. nucleata samples were collected in the Sargasso Sea approximately 4 miles off the southeast coast of Bermuda and in the North Pacific Central Gyre along a transect from Portsmouth, Oregon to Honolulu, Hawaii.

Central capsules of the polycystine radiolarian cells which contain the nucleus, as well as other cellular machinery, were physically separated from extracapsular material which contained endosymbiotic algae at a time in their life cycle immediately before swarmer release. The T. nucleata sequence derived from the Sargasso Sea sample designated TnucBBS 3 was obtained from central capsules of two individuals. The Thalassicolla sequences obtained from the Pacific, designated TnW10.79, TnW10.74 , TnW10.72, and TnW10.10, were four different clones derived from a single sample which contained 17 pooled central capsules. Total DNA from Sargasso Sea-collected specimens was extracted, rDNA was amplified, cloned and sequenced as described in Chapter 1. Pacific collected T. nucleata were extracted using the same protocols as the Sargasso Sea-collected specimens. However, amplified rDNA (after Saiki et al. 1988) from Pacific samples was cloned into a double-stranded TA plasmid vector pCRII (Invitrogen) and purified plasmid template DNA for sequencing was obtained using the Magic MiniPrep system (Promega). To minimize sequencing error, double stranded sequence of both the entire forward and reverse strands of the rDNA coding regions was obtained using the Sequenase version 2.0 kit and methods or Sequitherm (Epicentre) kit and Li-Cor automated sequencing methods (Li-Cor).

The 16S-like rRNA sequences of Thalassicolla were aligned against a larger eukaryotic data set by eye with regard to primary and secondary structural conservation using the

Olsen Multiple Sequence Alignment Editing program (Olsen et al. 1992). Absolute percent differences were calculated as a percentage of dissimilarity between pairs of the five T. nucleata sequences. Percent dissimilarity values were obtained by dividing the absolute number of base pair differences between pairs of taxa by the length of the longer sequence of the pair, counting gaps and ambiguities as a single difference, and representing the resulting value as a percentage of 100.

RESULTS

The SSU rRNA sequences for five representatives of T. nucleata are listed in Fig. 2. Based on this alignment, T. nucleata sequences showed variability at 66 positions scattered over the entire length of the gene. The percent dissimilarity values of these sequences are listed in Table 1. The amount of genetic variation found among samples of T. nucleata small-subunit rRNA gene ranged from 0.45% to 2.54%. The largest dissimilarity values of 2.54% were seen between the sequence from the Sargasso and two sequences from the Pacific sample. The sequences derived from the Pacific sample were more similar to each other than any of the four were to the sequence derived from Sargasso. The gene lengths in base pairs (bp) for the sequences presented in this paper are as follows: TnucBBS3, 1770 bp; TnW10.79, 1771 bp; TnW10.74 , 1765 bp; TnW10.72, 1771 bp.; TnW10.10, 1771 bp.

DISCUSSION

The degree of variability seen in the T. nucleata sequence data exceeds that expected within a given species and is comparable to that seen between different genera or within genera of other protistan taxa in the literature (Sogin et al. 1986; Manhart et al. 1995). While all the specimens used in this study fit the morphological description of T. nucleata, it is possible that different strains of T. nucleata exist which cannot be distinguished based on morphological criteria. Alternatively, the individuals collected as T. nucleata may have included other species indistinguishable from T. nucleata at the light microscope level. Yet

Table 1. Percent dissimilarity for rDNA sequences derived from different samples of Thalassicolla nucleata.

Sample #'s	BBS3	W10.79	W10.74	W10.72	W10.10
BBS3	0	2.37	2.32	2.54	2.54
W10.79		0	1.98	0.45	0.62
W10.74			0	1.92	2.03
W10.72				0	0.62
W10.10					0

Fig. 2. The alignment of 16S-like rRNA sequences of Sargasso Sea-collected T. nucleata (TnucBBS3) and four sequences derived from a pooled sample of T. nucleata from the Pacific (TnW10.79, 74, 72 and 10). Sequence identity is represented by dots and nucleotide abbreviations follow the IUB code.

```
TnucBBS3     1 AACCUGGUUGAUCCUGCCAGUAGUCAUACGCUAACAUUAAAGAUUAAGC
TnW10.79     1 .................................................
TnW10.74     1 .................................................
TnW10.72     1 .................................................
TnW10.10     1 .................................................

TnucBBS3    50 CAUGCAUGUACGAGUAUACAAUUACCAUUUUAAACUGCGUAAAGCUCAU
TnW10.79    50 ................A.A....U.........................
TnW10.74    50 ................A.--.............................
TnW10.72    50 ................A.A....U.........................
TnW10.10    50 ................A.A....U.........................

TnucBBS3    99 UAUAUCAGUUCUAACAUCUUAGGAAUACAAAAAAGAUGGAUAUUAGUGC
TnW10.79    99 ............................G-....A.C............
TnW10.74    97 ............................U-....A.C............
TnW10.72    99 ............................G-....A.C............
TnW10.10    99 ............................G-....A.C............

TnucBBS3   148 UAAUUCUACAAUCAAUACAUUUAUAACGUCUAAUUUU-UUAGACUAAAU
TnW10.79   147 ................................U....AA....U.....
TnW10.74   145 ....................................A.-....U.....
TnW10.72   147 ................................U....AA....U.....
TnW10.10   147 ................................U....AA....U.....

TnucBBS3   196 UACUGAGUAUCAAAAGUACGACUAUCUGAAUUCUUAAUAUUUACUGGUU
TnW10.79   196 .................G...............................
TnW10.74   193 .................................................
TnW10.72   196 .................G...............................
TnW10.10   196 .................G...............................

TnucBBS3   245 ACACUACAGAGUGAUAGUUCUAUUUAGUGACUGACCCAUCAGUUGUUCU
TnW10.79   245 .................................................
TnW10.74   242 .................................................
TnW10.72   245 .................................................
TnW10.10   245 .................................................

TnucBBS3   294 AUUAUGUAGUGAAUUAUUGAGGCUGAAACGGGUAGCGGAGAAUUAGGGU
TnW10.79   294 .................................................
TnW10.74   291 .................................................
TnW10.72   294 .................................................
TnW10.10   294 .................................................

TnucBBS3   343 UCCGUUCCGGAGAAAGAGCCUGCGAAACGGCUACUACAUCUAAGGAAGG
TnW10.79   343 .................................................
TnW10.74   340 .................................................
TnW10.72   343 .................................................
TnW10.10   343 .................................................

TnucBBS3   392 CAGCAGGCGNGUAAAUUAUUCAAUUCUAAAUCAGAGAGUUAGUAACAAU
TnW10.79   392 .........C.......................................
TnW10.74   389 .........C.......................................
TnW10.72   392 .........C........C..............................
TnW10.10   392 .........C.......................................
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```
TnucBBS3    441 AUAUUACGAUGUUAAACCUUAGGGUUAAAUUAUUAUAUUGAGGAUAGAU
TnW10.79    441 .................................................
TnW10.74    438 .................................................
TnW10.72    441 .................................................
TnW10.10    441 .................................................

TnucBBS3    490 UAUUUAUUUAUACGAUUGACUAUAGGAGGGCAAGUCUGGUGCCAGCAGC
TnW10.79    490 .................................................
TnW10.74    487 .......A.........................................
TnW10.72    490 .................................................
TnW10.10    490 .................................................

TnucBBS3    539 CGCGGUAAUACCAGCUCCAAUAGUGUAUGCUAACGUUGUUGCAGUUACA
TnW10.79    539 .................................................
TnW10.74    536 .................................................
TnW10.72    539 .................................................
TnW10.10    539 .................................................

TnucBBS3    588 AAGCUCGUAGUUGGUCUAUUAUGARUUUUAUUUAUAUAUAGUAUAAUUG
TnW10.79    588 ........................A........................
TnW10.74    585 ........................A..........G.............
TnW10.72    588 ........................A........................
TnW10.10    588 ........................A........................

TnucBBS3    637 UACUAUUAUUGACAAUGCCUAAAUCUUACUUAGAACAUGUACUAUGUUG
TnW10.79    637 ........................................CU...A...
TnW10.74    634 C.......C................................U...A...
TnW10.72    637 ........................................CU...A...
TnW10.10    637 ........................................CU...A...

TnucBBS3    686 AAAYUUACUUUUCGACAUUUCCUCAUGUUUGUUAUUACUUUGAAAAAAU
TnW10.79    686 ...U...........U.......U.........................
TnW10.74    683 ...U...U.......U.......U.........................
TnW10.72    686 ...U...........U.......U.........................
TnW10.10    686 ...U...........U.......U.........................

TnucBBS3    735 UAUGUUGAUUUAAGAGAAGAAAUGAUAUUGUACUAUAGUACAGAAUAAU
TnW10.79    735 .................................................
TnW10.74    732 .............................................C...
TnW10.72    735 .................................................
TnW10.10    735 ......................C..........................

TnucBBS3    784 ACUUGAAGAUCUCAGUAAAACUUAAACUUUUGGAUACUGGUGUARUGCU
TnW10.79    784 ..................G.........................A....
TnW10.74    781 ............................................A....
TnW10.72    784 ..................G.........................A....
TnW10.10    784 ..................G.........................A....

TnucBBS3    833 CUUUAGAGUUAGCUGAAGAUAUUAAUAUUUUAGCGWUAGAGGUGAAAUU
TnW10.79    833 ...................................U.............
TnW10.74    830 ...................................U.............
TnW10.72    833 ...................................U.............
TnW10.10    833 ...................................U.............
```

```
TnucBBS3    882  CAAGAAUCGUUAUAAGAUUAACAAGUGCCAAAGCAAUUAUCUAAGAUUA
TnW10.79    882  U................................................
TnW10.74    879  ......................U..........................
TnW10.72    882  .................................................
TnW10.10    882  ........A........................................

TnucBBS3    931  AUUCAUUGAUCAAGAACGUAAGUUGAAGGAUUGAAGACGAUCAGAUACC
TnW10.79    931  .................................................
TnW10.74    928  .................................................
TnW10.72    931  .................................................
TnW10.10    931  .................................................

TnucBBS3    980  GUCGUAAUCUCAAUUGUAAACUAUAUCAACUAGGGAUUAACAACUGUUU
TnW10.79    980  .................................................
TnW10.74    977  .................................................
TnW10.72    980  .................................................
TnW10.10    980  .................................................

TnucBBS3   1029  UUUAUGACAUUGUUGGCACCUUGUGAGAAAUUAGAGUUCUCAGAUUCCG
TnW10.79   1029  .................................................
TnW10.74   1026  C................................................
TnW10.72   1029  .................................................
TnW10.10   1029  .................................................

TnucBBS3   1078  GGGGGAGUAUGGUUGCAAGUCUGAAACUUAAAGGAAUUGACGGAAGGGC
TnW10.79   1078  .................................................
TnW10.74   1075  .................................................
TnW10.72   1078  .................................................
TnW10.10   1078  .................................................

TnucBBS3   1127  ACCACAAGUUGUGGAUACUGUGGCUUAAUUUGACUCAACACUGGAAAAC
TnW10.79   1127  .................................................
TnW10.74   1124  .................................................
TnW10.72   1127  .................................................
TnW10.10   1127  .................................................

TnucBBS3   1176  UUACCAGGUCCAGACAUAUUUAGGAUUGACAGAUUAAUAGCCCUGUCCU
TnW10.79   1176  ..................GC.............................
TnW10.74   1173  .................................................
TnW10.72   1176  ..................GC.............................
TnW10.10   1176  ..................GC.............................

TnucBBS3   1225  GAUUUUGUGGCUGGUGGUGCAUGGCCGUUCUUAGUUGGUGAAGUGAUUU
TnW10.79   1225  .................................................
TnW10.74   1222  .................................................
TnW10.72   1225  .........................................G.......
TnW10.10   1225  .................................................

TnucBBS3   1274  GUCUGGUUUAUUCCGUUAACGAACGAGACUAUUACCAAUAAAUAGUAAG
TnW10.79   1274  ..........................U......................
TnW10.74   1271  .................................................
TnW10.72   1274  .................................................
TnW10.10   1274  .................................................
```

```
TnucBBS3   1323 YACUGCA--UUAGCAGUGUGAUUACUUCUUAGAGGGACUGGUGAUACAU
TnW10.79   1323 CG...U.UU..C.....................................
TnW10.74   1320 U....U-.A...A....................................
TnW10.72   1323 CG..AUGUU..C.....................................
TnW10.10   1323 CG...UGUU..C...C.................................

TnucBBS3   1370 AAGUUACUGGAGGCAAGUUGCAAUGACAGGUCUGUGAUGCCCUUAGAUG
TnW10.79   1372 .................................................
TnW10.74   1368 .................................................
TnW10.72   1372 .................................................
TnW10.10   1372 .................................................

TnucBBS3   1419 UACUGGGCCGCGCACGGGAUACAACAGGGGAGAUAAUAUGUACAUUUAA
TnW10.79   1421 ........U...........................A.........-..
TnW10.74   1417 ........U...........................A.........-..
TnW10.72   1421 ........U...........................A.........-..
TnW10.10   1421 ........U...........................A.........-..

TnucBBS3   1468 ACAUAAUUUGACAAUAAUAUUGUAACCGYGAAUCUGUCUUUAAUAUGGA
TnW10.79   1469 ............................U.......C............
TnW10.74   1465 ............................U.......C............
TnW10.72   1469 ............................U.......C............
TnW10.10   1469 .U..........................U.......C............

TnucBBS3   1517 AUUGCACUAUGCAAUUUUACACAUAAACUAGGAAUAUCUUGUAAGUACA
TnW10.79   1518 .....G...........................................
TnW10.74   1514 .................................................
TnW10.72   1518 .....G...........................................
TnW10.10   1518 .....G...........................................

TnucBBS3   1566 UGUCAUAAUCGUGUUCUGAAUGCGUCCCUGUCCUUUGUACACACCGCCC
TnW10.79   1567 .................................................
TnW10.74   1563 .....C...........................................
TnW10.72   1567 .................................................
TnW10.10   1567 .................................................

TnucBBS3   1615 GUCGCUCCUACCGAUUGGAUGAGAUGGUGAGUAAAUCUUAAUGAUUGAA
TnW10.79   1616 .................................................
TnW10.74   1612 .................................................
TnW10.72   1616 .................................................
TnW10.10   1616 ...............C.................................

TnucBBS3   1664 GUUAUACUGUAAAGUUGAAKGUCAGUUAUAUAUAUUUGCAAACUAAACU
TnW10.79   1665 ....A......U....A..G.............................
TnW10.74   1661 ...........U.......G.............................
TnW10.72   1665 ....A......U.......G.............................
TnW10.10   1665 ...........U...A...G.............................


TnucBBS3   1713 AUUUAGAGGAAGGAGAAGUCGUAACAAGGUUUCCGUAGGUGAACCUGCA
TnW10.79   1714 .................................................
TnW10.74   1710 .................................U...............
TnW10.72   1714 .................................U...............
TnW10.10   1714 .................................................
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TnucBBS3   1762 GAAGGAUCA
TnW10.79   1763 .........
TnW10.74   1759 .........
TnW10.72   1763 .........
TnW10.10   1763 .........
```

a third possibility is that T. nucleata possesses multiple copies of its SSU rRNA genes which differ in both length and base pair composition. The last of these three possibilities is difficult to address because individuals were pooled when samples were collected. This would make it impossible to determine the source of heterogeneity (e.g. interspecific vs. intraspecific variability). The first two possibilities require a better understanding of the criteria used in defining species of Thalassicolla and are addressed below.

In reviewing the original species descriptions made by Haeckel, it seems likely that at least some of Haeckel's species were probably descriptions of different physiological states of a given species or descriptions of individuals infected by parasitic dinoflagellates. For example, dinoflagellate infections are known to occur in T. nucleata (Chatton 1920; Hollande 1974) and were observed during this study in a number of T. nucleata specimens that were not observed to undergo swarmer formation but instead erupted with dinoflagellate parasites. In all cases, such individuals of T. nucleata lacked symbionts and possessed a yellowish-orange color to the central capsule which was visible beneath the dark covering of the central capsule. While all of these infected T. nucleata specimens (possessing yellowish-orange central capsules) were observed in the Sargasso, Haeckel makes similar references to cell-types with such yellowish-colored central capsules in T. nucleata (which is a cosmopolitan species) and also in another species in the Pacific (namely, T. maculata Fig. 1B). These details are noteworthy because of the prevalence with which we encountered specimens of this description during various trips to the Sargasso Sea. In addition, Haeckel described T. maculata as possessing no zooxanthellae. It seems possible that parasitism may also occur in the Pacific and that T. maculata is just a description of a stage in the parasitism of T. nucleata. Although parasitism appears to occur in Thalassicolla collected from other geographic locations, information for its frequency in the Pacific is poorly documented.

The presence or absence of an opaque layer surrounding the central capsule rendering it colorless was another criterion Haeckel used to define species of Thalassicolla. It is noteworthy that in two of the species descriptions of Thalassicolla in which Haeckel described members with colorless central capsules (T. pellucida and T. australis), he also reported an absence of zooxanthellae. This is interesting because complete loss of the extracapsular material can happen when an individual is sufficiently agitated, as might occur during ingestion, excessive wave action or excessive agitation in net tows (Verworn 1891; Gamble 1909; O. R. Anderson, personal communication). When individuals shed their dark extracapsular material, they also shed their symbionts and may require some time before regenerating the opaque layer and acquiring a new population of symbionts.

The size of the central capsule also has been used by Haeckel to delineate species of Thalassicolla, as in his description of T. melacapsa (Fig. 1C). Likewise, this feature is a questionable taxonomic criterion because of possible variability originating from non-genetic origins. For example, the diameter of the central capsule in Thalassicolla has been observed to change within an individual, possibly in response to physiological condition (O. R. Anderson, Amaral Zettler, personal observation). Furthermore, many of Haeckel's descriptions make reference to the "patchy" appearance of the opaque-layer surrounding the central capsule, however, this characteristic may also be attributed to nutritional status and variation in light intensity (O. R. Anderson, personal observation).

Since we lack type specimens and even drawings of all of the original species of Thalassicolla described by Haeckel, it is impossible to determine whether or not the above observations are important in determining the actual number of species for the genus. We do not consider this to be an exhaustive study of the species diversity of the genus Thalassicolla. However, we obtained notable differences at the level of the SSU rRNA gene which raises the question of what defines the species T. nucleata. Due to the manner in which these samples were collected it is impossible to know if these differences represent intraspecific (multiple alleles of the rDNA gene within one species) or interspecific variability (different genes of different species). This question could be addressed by examining a single individual or preferably several individuals separately. With the current sequence information in hand, genus specific-primers could be designed to further explore the extent to which the morphological criteria used in Haeckel's species designations reflect reliable phenotypic markers for distinguishing between different species of Thalassicolla.

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REFERENCES

Anderson, O. R. 1978. Light and electron microscopic observations of feeding behavior, nutrition, and reproduction in laboratory cultures of Thalassicolla nucleata. Tissue and Cell **10:** 401-412.

Anderson, O. R. 1983. Radiolaria. Springer-Verlag.

Anderson, O. R. and M. Botfield. 1983. Biochemical and fine structure evidence for cellular specialization in a large spumellarian radiolarian Thalassicolla nucleata. Marine Biology **72:** 235-241.

Chatton, E. 1920. Les Peridiniens parasites. Morphologie, reproduction, éthologie. Arch. Zool. Exp. Géné. **59:** 1-475.

Gamble, F. W. 1909. The Radiolaria, p. 94-153. *In* E. R. Lankester [ed.], A Treatise on Zoology. Adam and Charles Black.

Haeckel, E. 1887. Report on Radiolaria collected by H. M. S. Challenger during the years 1873-1876, p. 1-1760. *In* C. W. T. a. J. Murray [ed.], The Voyage of the H. M. S. Challenger. vol. 18. Her Majesty's Stationary Office.

Herring, P. J. 1979. Some features of the bioluminescence of the radiolarian Thalassicolla sp. Marine Biology **53:** 213-216.

Hollande, A. 1974. Données ultrastructurales sur les isospores de radiolaires. Protistologica **10:** 567-572.

Huxley, T. H. 1851. Zoological notes and observations made on board the H. M. S. Rattlesnake. The Annals and Magazine of Natural History **viii:** 433-442.

Manhart, J. R., G. A. Fryxell, M. C. Villac and L. Y. Segura. 1995. Pseudo-nitzschia pungens and P. multiseries (Bacillariophyceae): Nuclear ribosomal DNAs and species differences. Journal of Phycology **31:** 421-427.

Olsen, G. J., R. Overbeek, N. Larsen, T. L. Marsh, M. J. McCaughey, M. A. Maciukenas, W.-M. Kuan, T. J. Macke, Y. Xing and C. R. Woese. 1992. The ribosomal database project. Nucleic Acids Research **20 (Supplement):** 2199-2200.

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science **239:** 487-491.

Sogin, M. L., A. Ingold, M. Karlok, H. Nielsen and J. Engberg. 1986. Phylogenetic evidence for the acquisition of ribosomal RNA introns subsequent to the divergence of some of the major Tetrahymena groups. The EMBO Journal **5:** 3625-3630.

Verworn. 1891. Thalassicolla. Pflüger's Archiv f. Physiologie **li:** 118.

Chapter 3

Towards a Molecular Phylogeny of Colonial Spumellarian Radiolaria[1]

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[1] The classification scheme of Strelkov and Reshetnyak (1971) was used for classifications at the family-level and below and that of Levine et al. (1980) for higher-level classifications.

ABSTRACT. Throughout their history of classification, the colonial spumellarian radiolaria have been grouped together taxonomically on the basis of their ability to form colonies. A molecular phylogenetic basis for this grouping, however, has never been explored. We used small-subunit ribosomal RNA gene sequence data to examine whether the colonial spumellarian radiolaria (Polycystinea) constitute a monophyletic evolutionary assemblage. Representatives from two spumellarian families known to form colonies, the Sphaerozoidae and the Collosphaeridae were considered in this study and included the following taxa: Sphaerozoidae: Collozoum pelagicum; Collozoum serpentinum; Rhaphidozoum acuferum; Sphaerozoum punctatum; and Collosphaeridae: Collosphaera globularis-huxleyi; Acrosphaera (circumtexta?); and Siphonosphaera cyathina. The results from our molecular phylogenetic analyses do not strongly support the monophyly of the colonial spumellarian radiolaria yet do not completely eliminate this possibility either. Coloniality may have arisen more than once among the Spumellarida or existing solitary Spumellarida may have once possessed colonial forms. All molecular analyses supported the monophyly of the Collosphaeridae but only distance analyses supported the monophyly of the Sphaerozoidae. The idea that coloniality appeared more than once in spumellarian evolution is contrary to current opinion based on skeletal morphogenesis studies but has been suggested from studies of the fossil record.

Supplementary key words. Acrosphaera, Collosphaeridae, Collozoum, colonial radiolaria, planktonic sarcodine, Siphonosphaera, Sphaerozoidae

Colonial spumellarian radiolaria are holoplanktonic sarcodines (Subphylum Sarcodina, Class Polycystinea) which occur exclusively in open ocean oligotrophic environments. As in all polycystines, each cell is physically separated into the endocytoplasm and the ectocytoplasm by a porous proteinaceous capsular wall. The capsular wall, together with the major cellular machinery it encloses, (the nucleus, mitochondria, golgi, endoplasmic reticulum, vacuoles, and oil droplets), is referred to as the central capsule. In colonial spumellarian radiolaria, thousands of individual central capsules extend their pseudopodia into a shared gelatinous extracapsular matrix which connects the cells and also typically houses numerous symbiotic algae.

As "multicellular" entities, the colonial spumellaria are macroscopic and have been reported to reach lengths of up to three meters, making them very conspicuous components of tropical and subtropical pelagic marine environments (Anderson and Swanberg 1981). Despite a visible presence in the plankton, their fragile nature and resistance to laboratory culture has left many unanswered questions regarding colonial spumellarian biology, including the reasons for colony formation. Apart from isolated reports of colony formation by phaeodaria of the family Tuscaroridae (Haecker 1908; Swanberg 1979), the spumellaria are the only other "radiolaria" sensu stricto (Polycystinea and Phaeodarea) which form colonies.

The "colonial radiolaria" are restricted to two families within the order Spumellarida; the Sphaerozoidae and the Collosphaeridae. In the Sphaerozoidae, skeletal material is either lacking or else composed of several silicate spicules of varying degrees of complexity. The most recent systematic treatment of the colonial spumellaria (Strelkov and Reshetnyak 1971) divides the Sphaerozoidae into three genera, Collozoum, Sphaerozoum, and Rhaphidozoum. The genus Collozoum possesses either simple spines (Strelkov and Reshetnyak 1971) or no skeleton. Members of the genus Sphaerozoum contain characteristic paired-triradiate spines, while Rhaphidozoum representatives have both simple and radiate spines. Species designations are typically based on the structure of these spines, when present, or the morphology of the central capsular wall, as in the case of species within the genus Collozoum.

All members of the family Collosphaeridae are characterized by siliceous, spherical latticed shells having varying degrees of ornamentation. Strelkov and Reshetnyak (1971) divided the Collosphaeridae into three tribes, the Collosphaerini, the Acrosphaerini and the Siphonosphaerini, in order to maintain a more "natural" system of classification. In brief, Collosphaerini possess smooth surfaces on both the inner and outer portions of the shell, Acrosphaerini have a spine-covered outer surface of the latticed shell, and the Siphonosphaerini have latticed shells whose pores are either partially or completely elongated into tube-like projections.

It is generally assumed that the members of the colonial spumellaria were derived from a single common ancestor and that the ability to form colonies has arisen only once in their evolution (Strelkov and Reshetnyak 1971; Anderson and Swanberg 1981). Some authors have suggested that colonial spumellaria are part of a life cycle stage of solitary forms which undergo multiple binary fission of their central capsule to form colonies or perhaps that they are different stages of the same species (Brandt 1902; Hollande and Enjumet 1953; Swanberg 1979). Solitary forms are, in fact, known for some members of the Sphaerozoidae. The genus name Thalassophysa, for example, is used when referring to the solitary stage of the various members of Collozoum. In the taxa examined in this study, Thalassophysa sanguinolenta is the name given to the solitary stage of the colonial Collozoum pelagicum (Brandt, 1902). C. serpentinum is also recognized as having a solitary stage (Swanberg, 1979). Solitary forms have, however, never been observed for members of the Collosphaeridae. While members of the Collosphaeridae have left behind a fossil record, only individual shells are found in the marine sediments. Therefore, it is unknown whether or not fossil collosphaerids also produced colonies but it assumed that they did. Likewise, we are working under the assumption that Thalassicolla has evolved from a solitary ancestor and that the genus is not capable of forming colonies. All available information in the literature and personal observation indicates that the genus is strictly solitary, however, the possibility that Thalassicolla evolved from a colonial ancestor and has now lost the character of coloniality, cannot be excluded.

Due to the application of molecular biological techniques, scientists now have a novel means of exploring the question of coloniality in spumellarian evolution. We sequenced the small-subunit ribosomal RNA (SSU rRNA) genes of representatives from both families of Spumellarida known to form colonies in order to examine the origins of coloniality and investigate the evolutionary relationships among the colonial spumellaria.

MATERIAL AND METHODS

Colonial spumellarians were collected in glass jars by divers. Colonies were maintained in 0.22 µm Millipore-filtered seawater in glass culture tubes with brine shrimp (Artemia salina) as food. All samples were collected approximately 4 miles off the southeast coast of Bermuda on the dates listed below. Samples were typically given individual sample designations prior to identification. The following samples were included in this study, with sample designation and collection date following the species identification: Collozoum pelagicum (BBSR 2, November, 1993); Rhaphidozoum acuferum (BBSR 7, November, 1993); Collosphaera globularis-huxleyi (BBSR 173, May, 1994); Sphaerozoum punctatum (CR 4 , May, 1995); Acrosphaera (circumtexta?) (CR 6, May, 1995); Collozoum serpentinum (CR 16, May, 1995); Siphonosphaera cyathina (October, 1995). C. pelagicum consisted of a section of a vegetative (non-reproductive) colony. All other samples consisted of pooled or single central capsules from a single reproductive colony.

In all but the C. pelagicum sample, colonies were held until the early stages of onset of swarmer production. At that time, central capsules were physically separated from extracapsular material which contained endosymbiotic algae by repeated micropipeting.

The rationale behind sacrificing individuals at that point in their life cycle was twofold: first, a natural amplification of DNA occurs within the organism at that time as multiple copies of the genome are made in preparation for swarmer formation. Second, many species either consume or expel endocytoplasmic symbiotic algae immediately prior to swarmer formation thereby reducing the potential of amplifying non-target DNA. Individual central capsules were pipetted through several 0.22 µm-Millipore filtered seawater rinses followed by a final MilliQ-water rinse prior to placement in a modified 1X PCR buffer solution which consisted of 50 mM KCl, 10 mM Tris, pH 8.3, 2 mM $MgCl_2$, 0.001% Gelatin, and 1.0% NP40 (Sigma; St. Louis, MO). Samples were then stored frozen at -70°C. Samples used for molecular analyses were heated at 95°C for 10 minutes to lyse cells and liberate DNA. An aliquot of the lysed sample was used directly in PCR amplification reactions (Saiki et al. 1988). Sequences from R. acuferum and C. globularis-huxleyi samples were obtained from cloned products (Chapter 1). Sequence information obtained from these two samples was then used to design "colonial spumellarian"-specific primers which were effective in amplifying SSU rRNA genes of different genera.

Sequence data from the remaining samples were obtained from directly sequencing PCR products amplified using a combination of colonial spumellarian specific primers and Medlin primers (Medlin et al. 1988). These colonial spumellarian-specific primers were synthesized as described in Chapter 1. The nucleotide sequences are: forward primer R906, 5'-TATTAGTATTTRTCGTT-3'; reverse primer R1451bio, 5'-TATTGTAG-CCCGTGCGCT-3' (previously used as a probe in in situ verification experiments in Chapter 1). PCR reactions consisted of 3 minutes of denaturation at 95°C followed by 30 amplification cycles each consisting of 94°C for 1 minute, 42°C for 1 minute and 72°C for 2 minutes. Two separate 100 µl PCR reactions typically provided enough template for sequencing reactions. PCR reactions were then pooled prior to purification using the Wizard PCR Kit (Promega; Madison, WI) to obtain purified DNA for direct sequencing.

Direct sequencing of PCR products was accomplished using IR-labeled primers and reagents from the Sequitherm Long-Read Sequencing Kit (Sequitherm; Madison, WI), along with the Sequitherm Cycle sequencing protocol developed by Li-Cor (Lincoln, NE) which consisted of 5 minutes of denaturation at 95°C prior to 30 cycles of 20 sec at 95°C (30 sec for plasmid DNA), 30 sec at 60°C, and 1 minute at 70°C using a Perkin Elmer 2400 thermo-cycler. Double stranded sequencing of the entire forward and reverse strands of the rDNA coding regions was conducted for cloned products. For directly-sequenced PCR products, double-stranded read for the all but the primer-specified ends were obtained.

The 16S-like rRNA sequences of colonial spumellaria were aligned against a subset of the total eukaryotic alignment data base (Olsen et al. 1992). Sequences were aligned by eye using the Olsen Multiple Sequence Alignment Editing program with regard to primary and secondary structural conservation. The same positions were used in this analysis as were used in the data set analyzed in Chapter 1 (1,368 total sites minus one site which became a gap when certain taxa were removed). In addition to colonial spumellaria, the alignment also included the solitary spumellarian Thalassicolla nucleata (Chapter 1) and acantharian outgroups Haliommatidium sp. and Chaunacanthid 218 (Chapter 1). In reality, however, no clearly appropriate outgroups exist for the spumellaria at the time of the writing of this manuscript since the spumellarian sequences are extremely divergent and are unrelated to any other taxa for which SSU rRNA sequence data is available.

The colonial spumellarian sequences were also analyzed independently of an outgroup (in "unrooted" networks) in order to include more sites in the analysis (an expanded number of homologous sites which included 1,635 positions). Molecular phylogenetic relationships were inferred for both data sets using distance (Olsen 1988), maximum parsimony (Swofford 1991) and maximum likelihood (Olsen et al. 1994) methods. The robustness of the tree topologies obtained were examined using 100 bootstrapping resamplings for all three methods and additionally for the maximum parsimony method using decay analyses (Bremer 1988).

The decay analyses were accomplished by first doing an exhaustive search using PAUP 3.1.1 (Swofford, 1991) to obtain the length of the most parsimonious tree, and then sequentially adding steps to the value of the shortest tree found using the initial upper bound setting of the branch and bound search option. Resulting trees constructed at each additional step-allowance were then consensed in a strict consensus tree, and the order in which various clades "decayed" was compared.

RESULTS

Photomicrographs of the skeletal structures of spicule-bearing and skeleton-bearing colonial spumellaria used in this study are shown in Fig. 1. Species identifications were straightforward with the following two exceptions. Sample number BBSR 173 was best described as Collosphaera globularis-huxleyi, owing to features of the latticed shell possessed by this specimen (see Fig. 1), which appeared to exhibit qualities shared by both C. globularis and C. huxleyi. Haeckel (1887) asserted that these two species of Collosphaera formed intergrades. Therefore a combined species (globularis-huxleyi) description for this sample seemed most appropriate given the qualities of the shell morphology. Sample number CR 6 is Acrosphaera. The species designation was difficult to ascertain but is probably A. circumtexta. The length in base pairs and % G + C content of the SSU rRNA genes of spumellaria used in this study are listed in Table 1.

The results obtained from the three different phylogenetic methods used in this study did not identify a single common tree (Fig. 2). Distance methods failed to clearly segregate the solitary spumellarian T. nucleata from the colonial spumellaria. Maximum parsimony was the only method which segregated the colonial spumellaria from the solitary spumellarian T. nucleata, with low (61%) but significant bootstrap support. Weak support for the node uniting all the colonial spumellaria was also identified in the parsimony tree by the decay

Table 1. The gene lengths in base pairs (bp) and % G + C content of spumellaria used in this study.

		SSU rDNA
Species	Length (bp)	% G + C
Thalassicolla nucleata	1770	36
Rhaphidozoum acuferum	1813	39
Sphaerozoum punctatum	1788	37
Collozoum pelagicum	1792	38
Collosphaera globularis-huxleyi	1797	35
Acrosphaera (circumtexta?)	1803	35
Siphonosphaera cyathina	1791	36
Collozoum serpentinum	1798	38

Fig. **1**. Photomicrographs of voucher sections of shell-bearing and spicule bearing colonies taken of samples used in this study. **A.** Acrosphaera (circumtexta?). Note the ridge-like structures often connected with thin bars. The spines, which characterize members of this genus, did not photograph well in this specimen . **B**. Siphonosphaera cyathina. Note the cylindrical, short tube-like projections which characterize the genus. In S. cyathina the tube-like projections are irregularly dispersed and sometimes terminate with a folded-back distal edge. **C**. Rhaphidozoum acuferum. This species is characterized by having both simple and radiate spines as the ones shown in this panel. **D**. Collosphaera globularis-huxleyi. A portion of the latticed-shell of this specimen reveals smooth inner and outer surfaces which characterize members of this genus. This specimen was given a species designation of C. globularis-huxleyi because while most of the pore and bar dimensions matched those reported for C. globularis a small number of specimens possessed shapes more similar to C. huxleyi. E. Sphaerozoum punctatum. This specimen shows the paired triradiate spicules possessed by this genus. The spines of S. punctatum are often barbed as seen in this photograph. Note the numerous crystal inclusions of the swarmers within the central capsule of this reproductive individual. Scale bar = 48 μm for all panels.

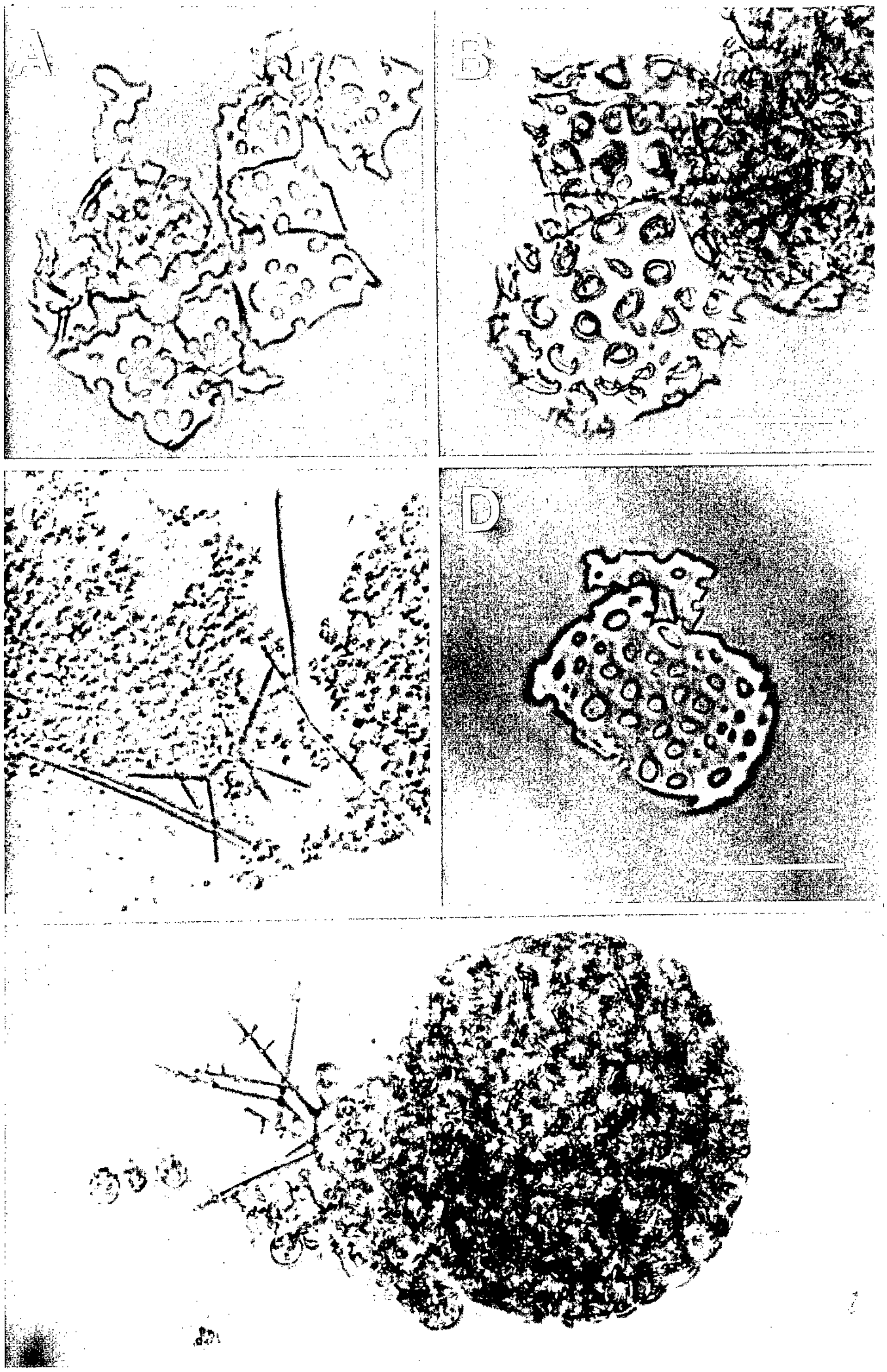
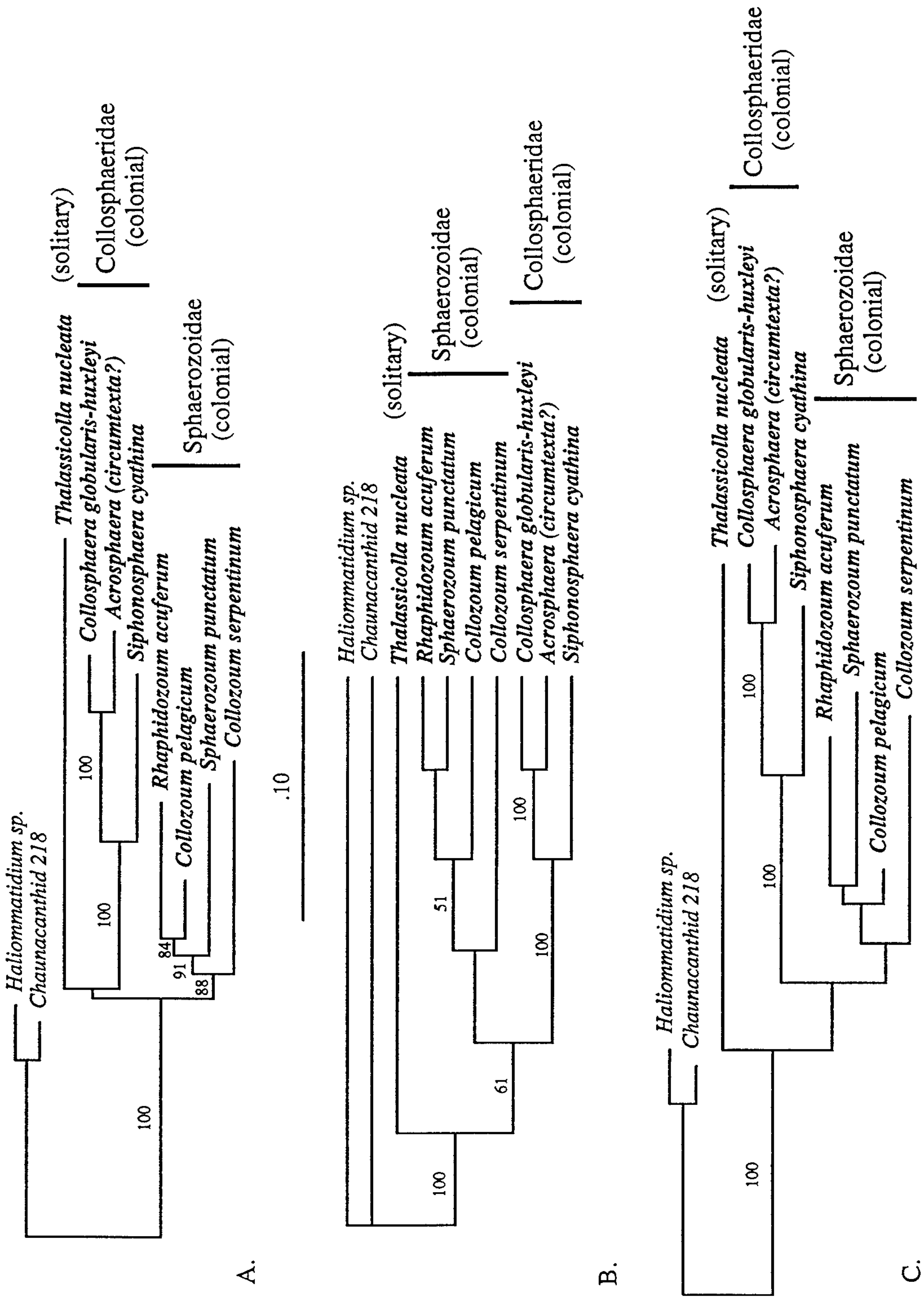

A
B
D

Fig. **2**. Phylogenetic reconstructions for solitary and colonial spumellarians using acantharian outgroups Haliommatidium sp. and Chaunacanthid 218 inferred from: **A.** distance, **B**. maximum parsimony and **C**. maximum likelihood methods. There were 1,368 positions used in the phylogenetic analyses. All bootstrap values were computed separately for 100 resamplings of the three respective data sets. Only bootstrap values greater than 50 % are shown. The evolutionary distances are indicated by the bar insert (distance and maximum likelihood) which represents 10 changes per 100 nucleotides.

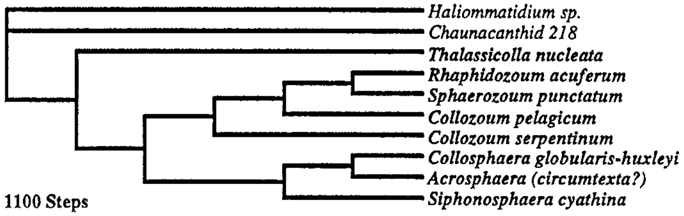

A.
Haliommatidium sp.
Chaunacanthid 218
Thalassicolla nucleata
(solitary)
Collosphaera globularis-huxleyi
Acrosphaera (circumtexta?)
Siphonosphaera cyathina
Collosphaeridae
(colonial)
Rhaphidozoum acuferum
Collozoum pelagicum
Sphaerozoum punctatum
Collozoum serpentinum
Sphaerozoidae
(colonial)
100
100
100
84
91
88
.10
B.
Haliommatidium sp.
Chaunacanthid 218
Thalassicolla nucleata
(solitary)
Rhaphidozoum acuferum
Sphaerozoum punctatum
Collozoum pelagicum
Collozoum serpentinum
Sphaerozoidae
(colonial)
Collosphaera globularis-huxleyi
Acrosphaera (circumtexta?)
Siphonosphaera cyathina
Collosphaeridae
(colonial)
100
61
51
100
100
C.
Haliommatidium sp.
Chaunacanthid 218
Thalassicolla nucleata
(solitary)
Collosphaera globularis-huxleyi
Acrosphaera (circumtexta?)
Siphonosphaera cyathina
Collosphaeridae
(colonial)
Rhaphidozoum acuferum
Sphaerozoum punctatum
Collozoum pelagicum
Collozoum serpentinum
Sphaerozoidae
(colonial)
100
100
100
.10

analysis in which collapse of this node occurred after only 3 steps. Maximum likelihood methods yielded the same topology as maximum parsimony but the branching of T. nucleata separate from the colonial spumellaria was not well-supported by bootstrapping analysis. A likelihood ratio test was conducted (data not shown) but failed to find a significant difference between the distance, maximum parsimony and maximum likelihood tree topologies.

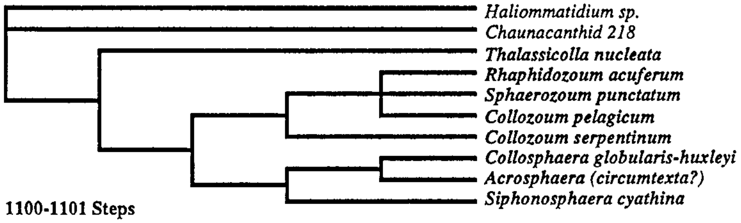
The monophyly of the Collosphaeridae was well supported in all methods for both the analyses with acantharian outgroups (Fig. 2 A - C) and the "unrooted" networks shown in Fig. 4 (A - C) (based on bootstrap values of 100% in all cases). Likewise, in both decay analyses (Figs. 3 & 5), the node leading to the Collosphaeridae was the last to collapse, implying robust support for this clade. The branching pattern within the Collosphaeridae consistently placed S. cyathina branching prior to the divergence of C. globularis-huxleyi and A. (circumtexta?) in all methods used. The strong support for the grouping of C. globularis-huxleyi and A. (circumtexta?) was revealed in the decay analysis of a consensus tree (Fig. 3), in which it required an additional 45 steps before the Collosphaeridae clade completely collapsed.

The separation of the remaining two families (the Sphaerozoidae and the Thalassicollidae) belonging to the suborder Sphaerocollina was not clearly supported in all cases. The bootstrap support values for these latter two families varied dramatically in the distance analysis relative to the maximum parsimony and maximum likelihood analyses (Fig. 2, A, C). The distance analysis clearly isolated the Sphaerozoidae from the Collosphaeridae and T. nucleata (bootstrap value of 88 on the branch leading to the Sphaerozoidae). Although the maximum parsimony and maximum likelihood methods supported a separate ancestry for the Sphaerozoidae distinct from the Collosphaeridae, the low bootstrap support for the parsimony and maximum likelihood tree topologies indicate poor support for the Sphaerozoidae as a distinct clade.

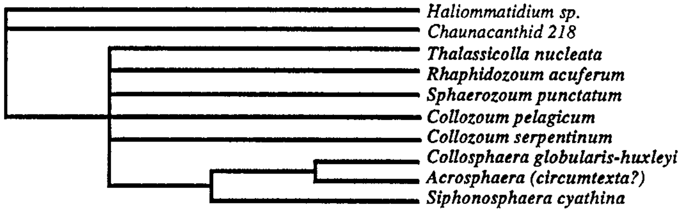
Fig. 3. Results from a decay analysis of the most parsimonious tree obtained from an exhaustive search. The number of additional steps required to produce the consensus trees with progressive degrees of collapse of major nodes is shown to the bottom left of each corresponding tree.



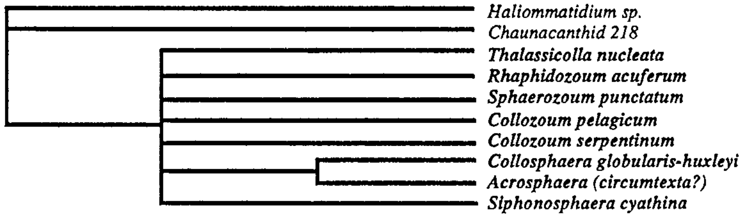
1100 Steps



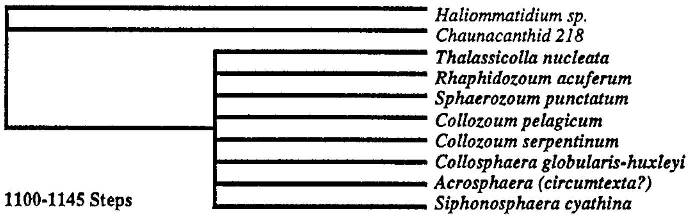
1100-1101 Steps



1100-1103 Steps



1100-1144 Steps



1100-1145 Steps

Fig. **4.** Three "unrooted" trees obtained from **A.** distance, **B.** maximum parsimony and **C.** maximum likelihood methods using additional (1,635) positions in analyses. Only bootstrap values greater than 50 % are shown. The evolutionary distances are indicated by the bar insert (distance and maximum likelihood) which represents 10 changes per 100 nucleotides.

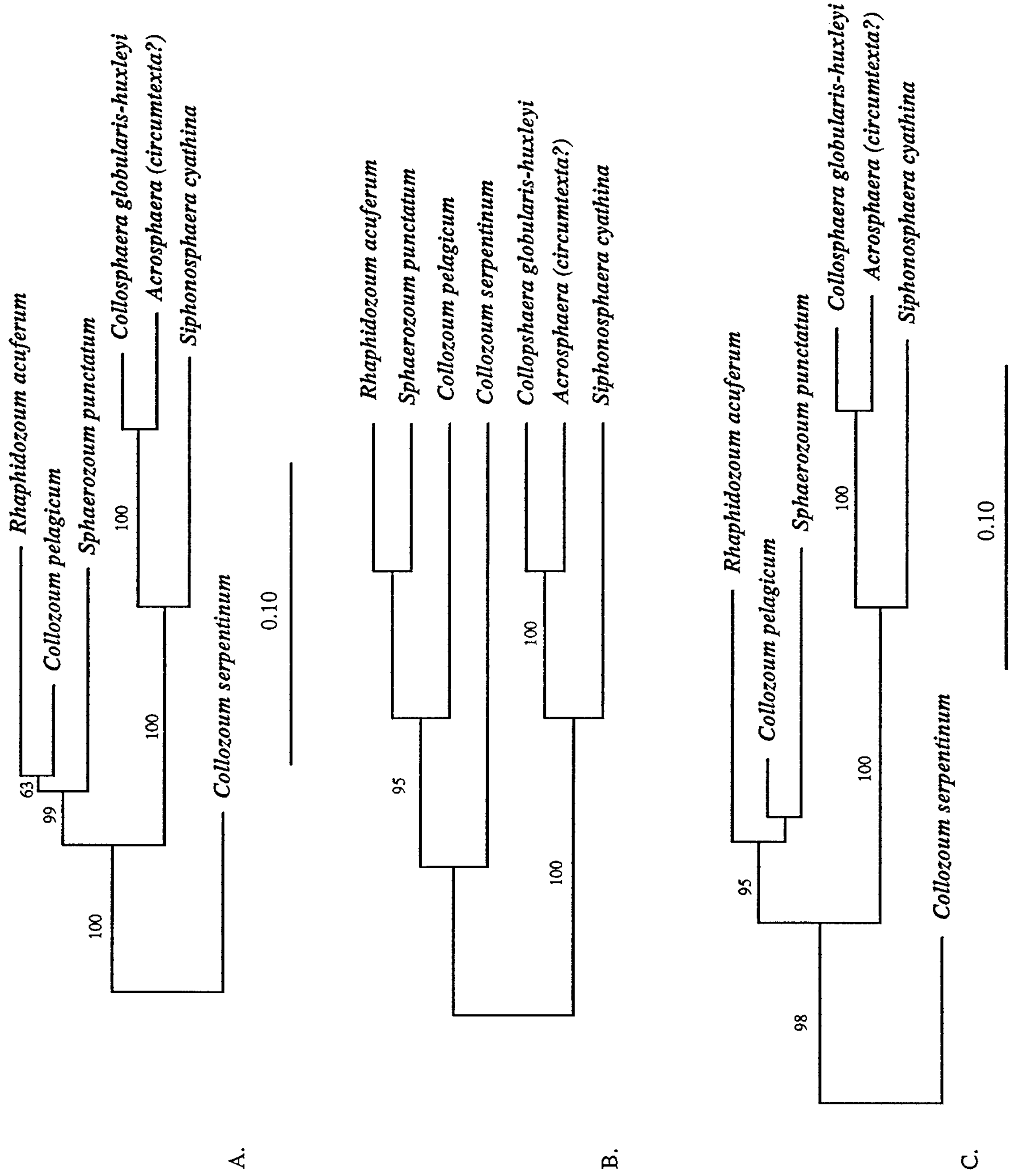
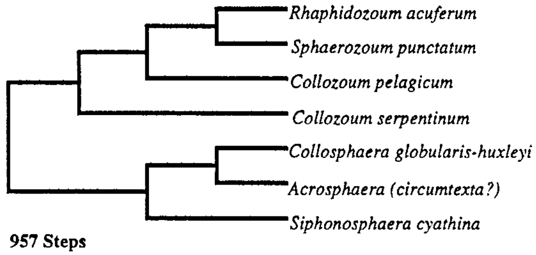
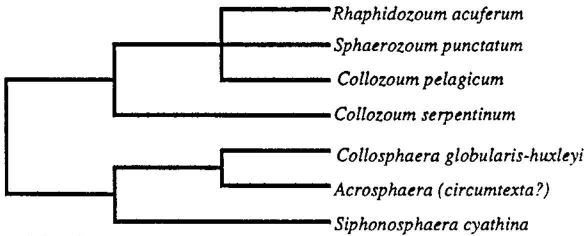

A.
Rhaphidozoum acuferum
Collozoum pelagicum
Sphaerozoum punctatum
Collosphaera globularis-huxleyi
Acrosphaera (circumtexta?)
Siphonosphaera cyathina
Collozoum serpentinum
63
99
100
100
100
0.10
B.
Rhaphidozoum acuferum
Sphaerozoum punctatum
Collozoum pelagicum
Collozoum serpentinum
Collopshaera globularis-huxleyi
Acrosphaera (circumtexta?)
Siphonosphaera cyathina
95
100
100
C.
Rhaphidozoum acuferum
Collozoum pelagicum
Sphaerozoum punctatum
Collosphaera globularis-huxleyi
Acrosphaera (circumtexta?)
Siphonosphaera cyathina
Collozoum serpentinum
95
100
100
98
0.10

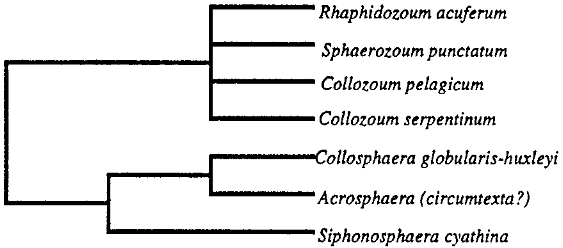
Fig. **5**. Results from a decay analysis of the most parsimonious tree obtained from an exhaustive search restricting the taxa to just the colonial spumellaria. The number of additional steps required to produce the consensus trees with progressive degrees of collapse of major nodes is shown to the bottom left of each corresponding tree.



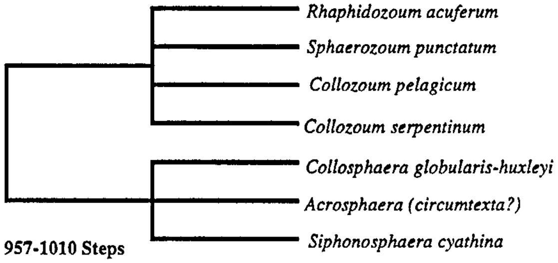
957 Steps



957-958 Steps



957-969 Steps



957-1010 Steps

The support for branching order within the Sphaerozoidae also varied depending on the method of phylogenetic inference. In general, the branching patterns within the Sphaerozoidae were poorly resolved in the parsimony and maximum likelihood consensus trees (not shown). However, there was weak support for the grouping of R. acuferum, C. pelagicum and S. punctatum in the parsimony consensus tree (bootstrap value of 51%). The highest bootstrap support values were obtained from distance analyses and supported a branching order which separated the two species of Collozoum, placing one species branching early in the Sphaerozoidae and another sharing common ancestry with spicule-bearing genus R. acuferum. This tree also separated the two more commonly occurring, spicule-bearing species S. punctatum and R. acuferum.

The results from "unrooted networks" (Fig. 4, 5) provided limited additional information on the branching patterns within the Sphaerozoidae. Although the branching order was not better resolved by restricting the analysis to just the colonial spumellaria, better branching support emerged from the maximum likelihood analysis, which agreed with parsimony analysis, and placed the R. acuferum together with S. punctatum and C. pelagicum (bootstrap value of 95%).

DISCUSSION

Phylogenetic reconstructions based on SSU rRNA coding regions challenge existing theories regarding the evolutionary history of the colonial spumellarian radiolaria. The data from molecular phylogenetic analyses indicate that the ability to form colonies may have evolved more than once in the evolution of the spumellarian radiolaria. The distance matrix method produced a tree topology which could not resolve the branching order of T. nucleata, a solitary spumellarian, relative to the two families of colonial spumellaria examined. The maximum likelihood tree did not show strong support for the branching order of T. nucleata relative to the two colonial families. Only a bootstrap value of 61% obtained in the maximum parsimony analysis alone separated the colonial spumellaria from

T. nucleata. Furthermore, the weak support for the monophyly of the colonial spumellaria was identified in a decay analysis in which the most parsimonious tree collapsed the node separating T. nucleata from the representatives of the Sphaerozoidae after only 3 steps. All of the above indicate that the node separating the colonial spumellaria from the solitary T. nucleata is not very robust.

The geological records of the colonial spumellaria have been used to yield information on their evolution. Based on observations from the fossil record, Bjørklund and Goll (1979) have suggested that coloniality may have evolved independently in the Collosphaeridae and the Sphaeorozoidae. These authors argued that there is no evidence for the common ancestry of the Collosphaeridae and Sphaerozoidae in the fossil record. They state that the first occurrence of Sphaerozoidae in the fossil record is much earlier (Lower Oligocene) than the Collosphaeridae (basal Miocene) and that the distributions of the Sphaerozoidae are typically high-latitude whereas the Collosphaeridae originated and diversified from equatorial regions. More importantly, these authors assert that because the first occurrences of the Collosphaeridae in the fossil record are abrupt and characterized by fully formed lattice shells, it is probable that latticed shells were not the result of fusion of the spicules.

The conclusions made by the above authors based on the fossil record, however, appear to be difficult to test rigorously. Since skeleton-forming colonial spumellaria are not preserved in their colonial form in the fossil record, it is impossible to know which fossil forms actually produced colonies. In fact, this very problem lead Haeckel to give different species names to some shell-bearing fossil forms which were later found to be synonyms of colony-forming spumellaria. Furthermore, the existence of solitary-stages of Sphaerozoidae and the occurrence of spicule-bearing spumellaria which have never been observed to form colonies brings into question the phylogenetic importance of colony formation. For example it has been suggested that the genus Collozoum may have members which all have solitary stages (Swanberg, 1979). Given the relative phylogenetic positions obtained in the distance analysis for the two Collozoum species, both of which have been cited as having solitary stages, we might conclude that coloniality may not be a definitive phylogenetic character. Likewise, while T. nucleata has never been observed to form colonies, we have to consider the possibility that its exclusively solitary habit may be a secondarily derived characteristic. If this is the case, similar arguments could be used for the existence of other exclusively solitary spumellaria so it is unclear that this question can be easily resolved even with additional sequence data from solitary forms.

The absence of solitary forms in the Collosphaeridae, however, is noteworthy. Strelkov and Reshetnyak (1971) hypothesized that the skeleton of the Collosphaeridae is derived from an ancestor with spines which merged to form a skeletal structure. A similar perspective on the possible phylogenetic relationships of the colonial spumellaria was reached by Anderson and Swanberg (1981) in their analysis of skeletal morphogenesis in representatives from the Collosphaeridae. These authors proposed a mechanism for shell deposition in colonial spumellaria which involved the precursory production of "cytokalymma" (differentiated extracapsular cytoplasm), followed by deposition of "organic nucleation centers" which serve as the matrix for the developing silicate shell. The authors described two methods of shell morphogenesis (bridge-growth and rim-growth) which they submitted could account for the variations in pore characteristics and shell ornamentation such as spines and tubules. Like Strelkov and Reshetnyak, these authors suggested that shell-bearing forms evolved from a spicule-bearing ancestor and that lattice shells are the result of the fusion of bar-like elements.

The phylogenetic reconstructions carried out in this study unanimously supported the monophyly of shell-bearing colonial spumellaria belonging to the family Collosphaeridae. Strong support was identified by both high bootstrap values (100% in all cases) and robust Bremer (decay analysis) support. Branching patterns within the Collosphaeridae indicate that Siphonosphaera diverged prior to the split of Collosphaera and Acrosphaera. Evidence from physiological and electron microscopy studies indicates that the tubelike-projections seen in members of the genus Siphonosphaera may be the result of silicification after cytoplasmic streaming which is exhibited by all members of the spumellaria, as well as many other protista (Cachon and Cachon 1972; Anderson and Swanberg 1981; Anderson 1981). While the tube-like projections displayed by S. cyathina are very symmetrical, other species of the genus possess tubular ornamentation which is irregular and bears a striking resemblance to cytoplasmic shapes created during cytoplasmic streaming (Anderson and Swanberg 1981).

The observed divergence of Siphonosphaera prior to Collosphaera and Acrosphaera is contrary to an hypothesis presented by Strelkov and Reshetnyak (1971). These authors speculated that members of the genus Collosphaera represent a more primitive line of decent and that Acrosphaera and Siphonosphaera represent more derived forms. They argued that the smooth latticed skeletons possessed by the members of the genus Collosphaera represent more primitive features than the more elaborate skeletons of the genus Acrosphaera, which have a spiny appearance or those of Siphonosphaera which possess tube-like projections. Anderson and Swanberg (1981) also stated that spines and tubule ornamentation are most likely more derived features. However, if cytoplasmic streaming is fundamental in the formation of the tube-like projections possessed by Siphonosphaera, one can imagine that these structures may have arisen any time in evolution and possibly even more than once.

The monophyly of the Sphaerozoidae was well-supported in the distance analysis (bootstrap value of 88% leading to this family) however parsimony and maximum likelihood methods generated tree topologies which were in general poorly supported by the bootstrapping method.. A well-supported branching pattern was also identified within the Sphaerozoidae using distance methods. The branching pattern for the distance analysis separated the two *Collozoum* species indicating a separate ancestry for the two *Collozoum* taxa. This pattern indicates that a secondary loss of skeletal material (i.e. spicules) occurred within *C. pelagicum*.

A similar conclusion about secondary skeletal loss was reached by Strelkov and Reshetnyak (1971). These authors proposed that the absence of skeletal elements is a secondary phenomenon and that the common ancestor of the Sphaerozoidae was spicule-bearing. They apparently attributed the secondary loss of skeletal elements to the fact that members of genus *Collozoum*, which are typically free of any skeletal material, are very infrequently found to possess simple spicules in their cytoplasm. These authors fail to consider that the occurrence of these spicules may be due to ingestion of other spumellaria or other spicule-bearing protists (Anderson, personal communication). Therefore, absence of skeletal features (which largely defines the genus *Collozoum*) may not be a reliable phylogenetic marker.

Collozoum serpentinum differs most noticeably from *C. pelagicum* by the characteristics of its central capsule. The central capsule in *C. serpentinum* is elongated and often forms twisted loops whereas in *C. pelagicum* it is characterized by digitform apophyses which are often branching at the ends. Interestingly, in maximum parsimony and maximum likelihood analyses of the "unrooted" network phylogenies, *C. pelagicum* was observed to branch with *R. acuferum* and *S. punctatum*, both spicule-bearing colonials. Although perhaps only coincidental, the shape of the apophyses on the central capsule of *C. pelagicum* bears a crude resemblance to the spicules of *R. acuferum* and *S. punctatum* suggesting a possible evolutionary connection between these apophyses and the radiate spicules possessed by *Rhaphidozoum* and *Sphaerozoum*.

Based on our molecular results, the diversity within the Spumellarida, both solitary and colonial forms, should not be understated. Molecular phylogenetic analyses of the SSU rRNA genes in this study revealed diversity within the colonial and solitary spumellaria that rivals that observed in many other protist groups analyzed to date. An explanation for such divergence within this order is wanting given our incomplete understanding of generation times and other factors which would affect the rate at which these protists evolved.

Other questions remain concerning the degree of variability within an individual colony. In a recent study, Petrushevskaya and Swanberg (1990) examined the morphological variability in the Collosphaeridae. These authors concluded that much of the variability seen within a colony is due to environmental differences and that sexual reproduction is probably absent in colonial radiolaria. However, geologists have reported "hybridizations" in Collosphaeridae which they believe are attributable to sexual reproduction (Bjørklund and Goll 1979). The question of whether or not colonial spumellarians are indeed entirely clonal can now be examined by comparing the SSU rRNA genes from several individuals from the same colony.

While this molecular study has not resolved of the issue of the evolution of coloniality among the spumellaria, it has raised the question of the importance of this character in determining relationships among the Spumellarida. This analysis, while far from a complete molecular diagnosis, has revealed potential avenues for further exploration into colonial spumellarian evolution. We believe that the molecular tools designed during this work will be helpful in determining the extent to which morphological variability seen in colonial spumellarians is reflected at the genetic level. Such studies as this will hopefully provide much-needed insights into the life history of these morphologically and genetically diverse protists.

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REFERENCES

Anderson, O. R. 1981. Radiolarian fine structure and silica deposition, p. 347-379. *In* T. L. S. a. B. E. Volcani [ed.], Silicon and Siliceous Structures in Biological Systems. Springer-Verlag.

Anderson, O. R. and N. R. Swanberg. 1981. Skeletal morphogenesis in some living collosphaerid radiolaria. Marine Micropaleontology **6:** 385-396.

Bjørklund, K. R. and R. M. Goll. 1979. Internal skeletal structures of Collosphaera and Trisolenia: A case of repetitve evolution in the Collosphaeridae (Radiolaria). Journal of Paleontology **53:** 1293-1326.

Brandt, K. 1902. Beiträge zur Kenntnis der Colliden. Arch. Protistenkd. **1:** 59-88.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution **42:** 795-803.

Cachon, J. and M. Cachon. 1972. Les modalités du dépot de la Silice chez les Radiolaires. Arch. Protistenk. **114:** 1-13.

Haeckel, E. 1887. Report on Radiolaria collected by H. M. S. Challenger during the years1873-1876, p. 1-1760. *In* C. W. T. a. J. Murray [ed.], The Voyage of the H. M. S. Challenger. vol. 18. Her Majesty's Stationary Office.

Haecker, V. 1908. Tiefsee-Radiolarien. Allgemeiner Teil. Form und Formbildung bei den Radiolarien. Wiss. Ergeb. Deutsch. Tiefsee-Exp. Dampfer "Valdivia" 1898-1899 **14:** 477-706.

Hollande, A. and M. Enjumet. 1953. Contribution à l'étude biologique des Sphaerocollides (Radiolaires Collodaires et Radiolaires Polycyttaires) et de leurs parasites. Ann. des. sci. Nat. Zool. **15:** 99-183.

Levine, N. D., J. O. Corliss, F. E. G. Cox, G. Deroux, J. Grain, B. M. Honigberg, G. F. Leedale, A. R. I. Loeblich, J. Lom, D. Lynn, E. G. Merinfeld, F. C. Page, G. Poljansky, V. Sprague, J. Vavra and F. G. Wallace. 1980. A newly revised classification of the protozoa. Journal of Protozoology **27:** 37-58.

Medlin, L., H. J. Elwood, S. Stickel and M. L. Sogin. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene **71:** 491-499.

Olsen, G. J. 1988. Phylogenetic analysis using ribosomal RNA, p. 793-812. *In* H. F. Noller Jr. and K. Moldave [ed.], Methods in Enzymology. Ribosomes. vol. 164. Academic Press.

Olsen, G. J., H. Matsuda, R. Hagstrom and R. Overbeek. 1994. fastDNAml: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. Computational Applied Biosciences **10:** 41.

Olsen, G. J., R. Overbeek, N. Larsen, T. L. Marsh, M. J. McCaughey, M. A. Maciukenas, W.-M. Kuan, T. J. Macke, Y. Xing and C. R. Woese. 1992. The ribosomal database project. Nucleic Acids Research **20 (Supplement):** 2199-2200.

Petrushevskaya, M. G. and N. R. Swanberg. 1990. Variability in skeletal morphology of colonial Radiolaria (Actinopoda: Polycystinea: Collosphaeridae). Micropaleontology **36:** 65-85.

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science **239:** 487-491.

Strelkov, A. A. and V. V. Reshetnyak. 1971. Colonial Spumellarian Radiolarians of the World Oceans, p. 295-418. *In* [ed.], Radiolarians of the Ocean, Reports of the Soviet Expeditions. vol. IX. Nauka Leningrad Department.

Swanberg, N. R. 1979. The ecology of colonial radiolarians: their colony morphology, trophic interactions and associations, behavior, distribution and the photosynthesis of their symbionts. Ph.D. thesis, Woods Hole Oceanographic Institution and Massachusetts Institute of Technology.

Swofford, D. L. 1991. PAUP: Phylogenetic Analysis Using Parsimony.

Chapter 4

Phylogenetic Relationships among Three Orders of Acantharea Based on SSU rRNA Gene Sequencing

SUMMARY

The phylogeny of the Acantharea was examined using small-subunit ribosomal RNA (SSU rRNA) gene sequence analysis of two previously sequenced (Chapter 1) acantharia along with additional representatives from the Symphyacanthida, Chaunacanthida and the Arthracanthida. Our previous studies showed that Acantharea form a monophyletic group branching as an independent protist lineage among crown groups but not directly related to any of them. The results from this more in-depth molecular analysis of the branching patterns within the Acantharea revealed a phylogeny which is not entirely consistent with morphology-based phylogenies. In particular, the phylogenetic placement of Haliommatidium sp. was in disagreement with its current taxonomic placement among the Symphyacanthida. In molecular analyses described herein, Haliommatidium clustered with members of the order Arthracanthida. Apart from cyst formation and number of axopodial exit pores, Haliommatidium sp. shares several morphological features with the Arthracanthida which support these molecular results.

Key words: Acantharea, evolution, molecular phylogeny, small-subunit ribosomal RNA

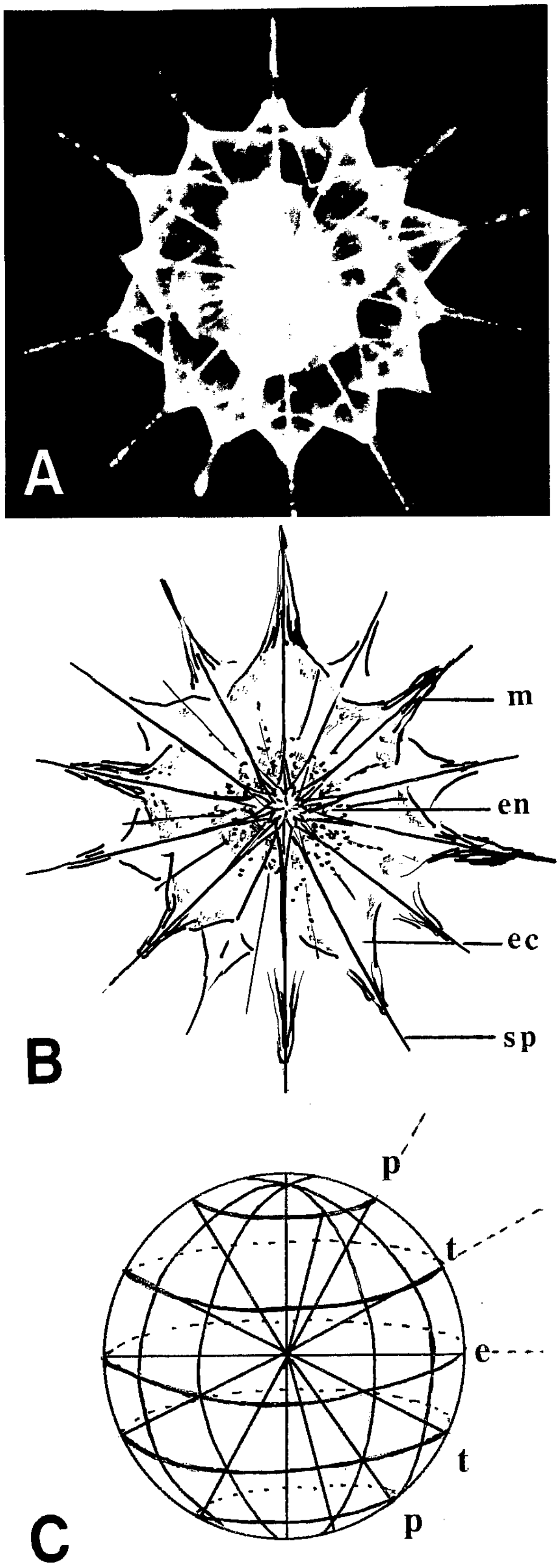
INTRODUCTION

Members of the class Acantharea are heterotrophic planktonic sarcodines which are common components of open ocean environments. In addition to their role as consumers, acantharia also contribute to primary productivity in the ocean via their symbioses with eukaryotic algae. Acantharia typically occur at densities of 10 cells l^{-1} (Caron and Swanberg 1990), however, they have occasionally been found to dominate the biomass of microzooplankton during "bloom-like" conditions (Merinfeld 1969; Massera Bottazzi and Andreoli 1981; Febvre 1990) where densities from 30-35 cells l^{-1} have been reported (Michaels 1988). While acantharian abundances in the world oceans have been underestimated in the past improved methods of sampling and preservation (Michaels 1988) are revealing their abundances in the plankton.

Acantharian cells are divided into the endoplasm and the ectoplasm (see Fig. 1 A, B), which are separated by a capsular wall in one order of acantharia, the Arthracanthida. The ectoplasm is encompassed by the periplasmic cortex or outer pellicle, and also the outermost layer, the calymma. The calymma houses the characteristic acantharian non-actin-filaments called myonemes. The myonemes are contractile bundles located around the tips of the skeletal spines and are postulated to contribute to active vertical motion in the acantharia (Febvre 1981; Reshetnyak 1981; Febvre and Febvre-Chevalier 1982).

Acantharia are further distinguished from other protists on the basis of the Müllerian-arrangement of spines and their skeletal composition. The acantharian skeleton is organized in a highly symmetrical fashion known as Müller's law, in which 10 diametric or 20 radial spines come together at the center of the cell to form a characteristic geometric pattern (see Fig. 1C). The acantharia are the only protists known to construct skeletons of monocrystals of strontium sulfate (Schröder 1907; Hollande and Cachon-Enjumet 1963; Massera-Bottazzi and Vinci 1965), although other protistan groups are known to metabolize strontium sulfate or similar alkaline earth compounds (Fenchel and Finlay 1986;

Fig. 1. **A.** An unidentified acantharian specimen. **B.** Schematic diagram showing the location of the myonemes (m), endoplasm (en), ectoplasm (ec) and spines (sp). **C.** Müllerian arrangement of spines (after Febvre, 1990). (p) polar spine; (t) tropical spine; (e) equatorial spine.


A
m
en
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t
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C

Raven et al. 1986). As such, acantharia play a role in the cycling of strontium in the ocean (Bernstein et al. 1987) and some attempts have been made to use the levels of Sr ^{90} incorporated into acantharian skeletons as a means of measuring radioactivity in the oceans (Schreiber and Ortalli 1964; Strelkov and Reshetnyak 1974).

Like other skeleton-bearing sarcodines, the acantharia were first classified on the basis of their skeletal morphology (Müller 1858; Hertwig 1879; Haeckel 1888). These authors all considered Acantharia as members of the "Radiolaria", a now defunct formal taxonomic term whose original definition encompassed them (see Chapter 1 for a more in-depth discussion of the differences between Acantharea and Polycystinea). Schewiakoff (1926) is credited with establishing a classification scheme which incorporated aspects of acantharian cytology and skeletal morphology. His 1926 monograph first recognized the Acantharia as distinct from the "radiolaria" (Polycystinea and Phaeodarea) and still serves as the foundation of modern-day classifications. Despite the need for systematic revision noted in the latest protistology reviews of the acantharia (Cachon and Cachon 1985; Febvre 1990) the past decade has seen very little systematic revision within the Acantharea. The latest treatments of the group include Trebougoff (1953) and Reshetnyak (1981) (in Russian).

There are 150 species, 50 genera, 20 families and 4 orders of acantharia reported in the most recent literature (Febvre 1990). Morphology-based systematic work requires the labor-intensive and time-consuming techniques of treatment of specimens with sulfuric acid prior to observation under the light microscope or use of electron-microscopy to determine species-level identifications. Furthermore, the phylogenetic significance of the some of the criteria used in distinguishing between different taxa (such as nature of the central juncture of the spines (after treatment with sulfuric acid)) have not been challenged. Since the acantharia lack a fossil record, there are few alternative methods available for comparing how well existing systematic schemes reflect phylogenetic relationships.

Ribosomal RNA-based phylogenetic approaches offer an alternative means of inferring relationships within the Acantharea. Recent cloning and sequencing efforts of small-subunit ribosomal RNA genes (Chapter 1) show a branching of Acantharea among crown groups. In this paper, we examine more closely the branching pattern of three orders of Acantharia in an effort to compare existing taxonomic frameworks with the results from this study.

MATERIALS AND METHODS

One very practical problem with the methods used in making accurate identifications of acantharia is that they typically destroy cytoplasmic material in the process. This makes microscopic identification at the light-microscope level difficult and in some cases only allows for order or family-level identifications with confidence. However, in certain groups, especially within the Arthracanthida, as well as, the Symphyacanthida (such as Haliommatidium), there are representatives which can be identified live to genus-level due to very distinctive features.

Individuals were given sample numbers prior to identification. In this study, all identifications were made by Dr. A. F. Michaels (Bermuda Biological Station for Research, Inc., Bermuda) who is a specialist in acantharian biology. Acantharian samples used in this paper were: Arthracanthid 205 (Order: Arthracanthida, Suborder: Sphaenacanthina, Family: Acanthometridae, Acanthometra sp.), Arthracanthid 206, (Order: Arthracanthida), Symphyacanthid 211 (Order: Symphyacanthida), Chaunacanthid 217 (Order: Chaunacanthida), and Chaunacanthid 218 (Order: Chaunacanthida), and Symphyacanthid 235 (Order: Symphyacanthida, Family: Pseudolithidae, Haliommatidium sp.).

All specimens were collected in glass or polycarbonate jars by divers off the southwestern coast of Bermuda in September 1994. Specimens were maintained in 0.22 µm Millipore-filtered Sargasso Sea water in glass culture tubes with brine shrimp (Artemia salina) as food until sacrificed for molecular analysis. Whenever possible, reproductive acantharia, which are often characterized by cyst-formation, were sacrificed for molecular analyses. The rationale for using reproductive individuals was to obtain samples that were highly enriched with sarcodine DNA over non-target DNA's such as prey or symbiotic algal DNA which may be present in the sample.

Individual central capsules or cysts were passed through several 0.22 μm-Millipore filtered seawater rinses followed by a final MilliQ (distilled, deionized)-water rinse. Specimens were then placed in a modified 1X PCR buffer solution which consisted of 50 mM KCl, 10 mM Tris, pH 8.3, 2 mM $MgCl_2$, 0.001% Gelatin, and 1.0% NP40 (Sigma; St. Louis, MO). Cells were then stored frozen at either -20°C or -70°C. Samples for molecular analyses were heated at 95°C for 10 minutes to lyse cells and liberate DNA. An aliquot of the lysed sample was used directly in PCR amplification reactions (Saiki et al. 1988). Typically anywhere between 1 and 5 μl of a 20μl sample lysed in this manner yielded strong PCR amplifications.

Arthracanthid 206, Chaunacanthid 217 and Symphyacanthid 235 (Haliommatidium sp.) 16S-like rDNAs were amplified using PCR and eukaryotic primers specific to the ends of the molecule (Medlin, 1988) and subsequently cloned into a double-stranded TA plasmid vector pCRII (Invitrogen; San Diego, CA). Plasmid DNA was purified using Promega Wizard Midiprep (Promega; Madison. WI) kit and methods. Remaining samples, Arthracanthid 205 (Acanthometra sp.), Symphyacanthid 211, and Chaunacanthid 218 SSU rRNA genes were PCR-amplified in two overlapping fragments using one acantharian-specified primer in combination with either the forward or reverse Medlin primer to yield a final full length product. These acantharian-specific primers were synthesized as described in Chapter 1 and consisted of the forward primer A497, 5'GTGAGTTGATTGGAATGA-3' and the reverse primer A899, 5'-TCGTCATACAAAGGTCCA-3'.

All PCR fragments were purified using the Wizard PCR Prep system (Promega; Madison, WI). Direct sequencing of PCR products as well as cloned plasmid DNA was accomplished using reagents from the Sequitherm Long Read Sequencing Kit (Epicentre Technologies; Madison, WI) along with the Sequitherm Cycle sequencing protocol developed by Li-Cor which consisted of 5 minutes of denaturation at 95°C prior to 30 cycles of 20 sec at 95°C (30 sec for plasmid DNA), 30 sec at 60°C, and 1 minute at 70°C using a Perkin Elmer 2400 Thermo Cycler. Sequenced templates were run out on a Licor model 4000L sequencing machine.

Gel images were transferred from Licor to BioImage (Millipore Corp; Ann Arbor, MI) and sequences were analyzed using the BioImage DNA Sequence Film Reader software. The 16S-like rRNA sequences of acantharian samples were aligned against a subset of the total eukaryotic alignment data base (Olsen et al. 1992). Sequences were aligned by eye using the Olsen Multiple Sequence Alignment Editing program with regard to primary and secondary structural conservation. Phylogenetic analyses employed distance (Olsen 1988), maximum parsimony (Swofford 1991) and maximum likelihood (Olsen et al. 1994) methods. The sites used in this analysis included 1,368 positions and was identical to the one used in the analyses to infer the phylogenetic placement of acantharia relative to the polycystine radiolaria (Chapter 1). The 1 base pair difference (e.g. 1,369 positions used in Chapter 1 compared with 1,368 positions used in this study) is due to one site becoming a gap when the data set was restricted to the acantharian and two polycystine spumellarian sequences. Thalassicolla nucleata and Collosphaera globularis-huxleyi were used as outgroups in the analyses. Bootstrap (Felsenstein 1985) and decay (Bremer 1988) analyses were conducted to provide a means of relative branch support.

RESULTS

All phylogenetic reconstructions accomplished yielded identical tree topologies. However, bootstrap values obtained for the three methods differed and are indicated on the nodes of the consensus parsimony tree shown in Fig. 2. Distance bootstrap values are listed on top, parsimony in the middle, and maximum likelihood on the bottom. In general, all analyses favored the branching of the Symphyacanthida with the Chaunacanthida and segregated these two orders from the Arthracanthida. Haliommatidium sp., currently classified as a symphyacanthid, was observed to branch with the Arthracanthida in all analyses. Haliommatidium sp. branched with Acanthometra sp. with moderate support in all analyses but the distance analysis, wherein the branch order between Acanthometra sp., Haliommatidium sp. and Arthracanthid 206 was poorly resolved.

In addition to bootstrapping, the stability of branching was tested further in a decay analysis depicted in Fig. 3. The decay analysis was accomplished by first performing an exhaustive search using PAUP 3.1.1 to obtain the length of the most parsimonious tree, and then sequentially adding steps to the value of the shortest tree found using the initial upper bound setting of the branch and bound search option. Resulting trees constructed at each additional step-allowance were then consensed in a strict consensus tree, and the order in which various clades "decayed" was compared.

The trees depicted in Fig. 3 show the single most parsimonious tree obtained which was 608 steps long, followed by the strict-consensus trees from 609, 614, 615 and 621 steps respectively. After 1 additional step (608-609), the node joining Haliommatidium sp. and Acanthometra sp. collapsed. Six steps (608-614) were required for the collapse of the chaunacanthid clade. After 7 steps (608-615) the connection between the chaunacanthid clade and Symphyacanthid 211 was lost. Complete loss of structure in the acantharian lineage resulted after 13 steps (608-621) with the collapse of the "arthracanthid" clade (including Haliommatidium sp.).

Fig. 2. The most parsimonious tree inferred from of an exhaustive search using maximum parsimony. Distance and maximum likelihood analyses yielded the same tree topology. Three sets of bootstrap values are given for each method as follows: distance (top value), maximum parsimony (middle value), maximum likelihood (bottom value). Only values greater than 50% are shown, the dash for the node leading to Arthracanthid 205 and Symphyacanthid 235 indicates a distance bootstrap value which was less than 50%. Acantharian sequences are indicated in bold.

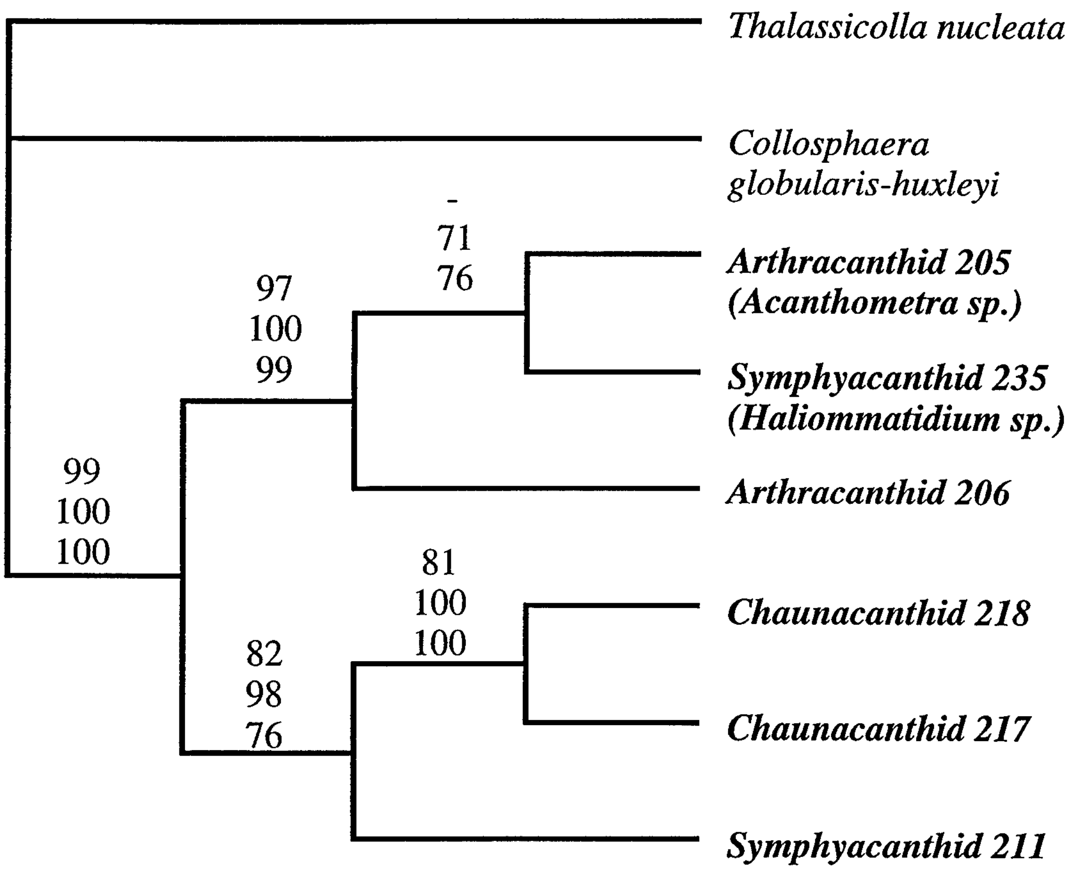
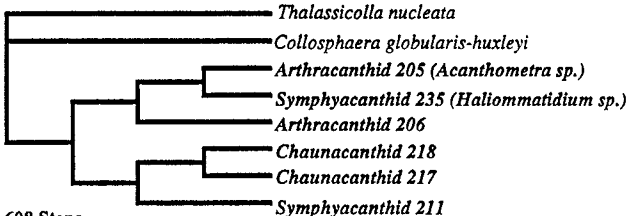
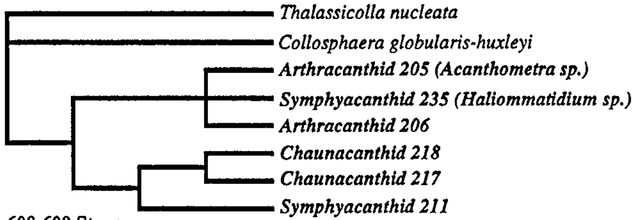

Thalassicolla nucleata
Collosphaera globularis-huxleyi
-
71
76
Arthracanthid 205 (Acanthometra sp.)
97
100
99
Symphyacanthid 235 (Haliommatidium sp.)
Arthracanthid 206
99
100
100
81
100
100
Chaunacanthid 218
82
98
76
Chaunacanthid 217
Symphyacanthid 211

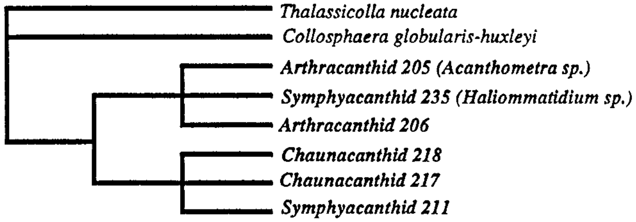
Fig. 3. The results of a decay analysis conducted using the most parsimonious tree from an exhaustive PAUP search. Strict consensus trees obtained after 1 step (608-609), 6 steps (608-614), 7 steps (608-615), and 13 steps (608-621) indicate the order of decay of the major clades in the acantharian lineage. Acantharian sequences are represented in bold.



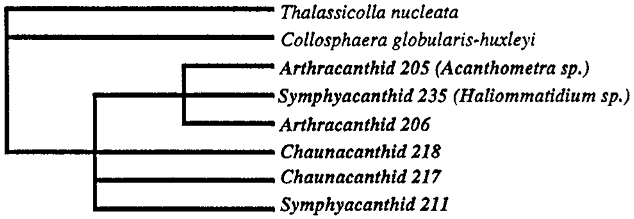
608 Steps



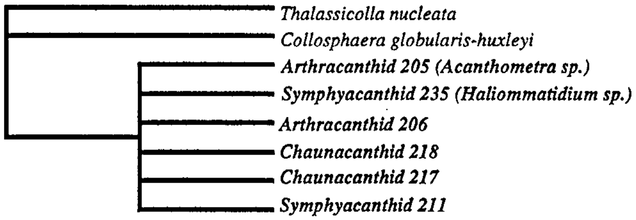
608-609 Steps



608-614 Steps



608-615 Steps



608-621 Steps

DISCUSSION

The current classification scheme for the Acantharea, based on morphological criteria established by Schewiakoff in his 1926 monograph, has been accepted with little formal systematic revision since that time (Reshetnyak 1981; Febvre 1990). Some specialists acknowledge that members of the symphyacanthid family Pseudolithidae, of which Haliommatidium is a member, require taxonomic reevaluation (Cachon and Cachon 1982; Cachon and Cachon 1985) but no formal revisions have been suggested to date. The results from this study found the placement of members of the Arthracanthida and the Chaunacanthida to be consistent with the systematic scheme proposed by the above authors. However, the results for the Symphyacanthida indicate that some of the morphological criteria used in defining the symphyacanthid clade are not reliable phylogenetic markers.

One of the major results of this study was the branching of Symphyacanthid 235 (Haliommatidium sp.) with Arthracanthid 205 (Acanthometra sp.) and an unidentified arthracanthid, Arthracanthid 206 within the arthracanthid clade. This result was well-supported by both the bootstrapping and decay analysis results. Reexamination of the literature available on the morphology and cytology of Haliommatidium, however, reveals some salient features shared by Haliommatidium and members of the Arthracanthida which substantiate this result.

The features which distinguish members of the Arthracanthida from other orders of acantharia include the following: the existence of a central capsule; a well-defined body plan possessing latticed or armored shells; the presence of a small number of apertures in the calymma for the axopodia to exit, and an increase in the number of myonemes compared to other orders (from 24-40). In considering these criteria, there are several morphological features of Haliommatidum which might place it among the Arthracanthida.

When we compare the above features to those found in Haliommatidium we see that

Febvre (1990) makes note of a very conspicuous central capsular wall in Haliommatidium as is seen in most Arthracanthida. In addition, Haliommatidium forms a latticed shell through the fusion of the apophyses on its spines, similar to those that can be seen in members of the Arthracanthida. Furthermore, Haliommatidium is known to possess 23-34 myonemes as compared with the 8-12 myonemes possessed by other members of the order Symphyacanthida (Strelkov and Reshetnyak 1974).

One difference between Haliommatidium and members of the Arthracanthida lies in the number of apertures for the exit of axonemes which number between 30-40 in the family Pseudolithidae whereas there are many fewer in the Arthracanthida. Another difference between Haliommatidium and members of the Arthracanthida is that Haliommatidium forms a cyst prior to swarmer formation, whereas no members of the Arthracanthida form cysts. The cysts formed by Haliommatidium, however, develop differently than those of other cyst-forming Symphyacanthida such as members of the Astrolithiidae.

Other differences exist between Haliommatidium and other Symphyacanthida members. For example, one of the distinguishing features of the Symphyacanthida is the inability of the central skeletal mass to be dissociated with sulfuric acid treatment. The central body of members of the genus Haliommatidium can be dissociated by sulfuric acid treatment whereas dissociation does not occur in most other Symphyacanthida. Finally, another striking difference found in Haliommatidium that is not seen in any other acantharian let alone symphyacanthid, is a single large nucleus during the trophic stage of the organism instead of the many nuclei observed in all other types of vegetative acantharia. The evolutionary significance of this mononuclear condition seen in Haliommatidium remains enigmatic. In any event, many morphological features possessed by Haliommatidium set it apart from other symphyacanthids.

The branching of Symphyacanthid 211 relative to the chaunacanthid clade is also noteworthy. The data indicate that the Symphyacanthida diverged prior to the

Chaunacanthida. This hypothesis is contrary to what has been suggested based on the morphological data alone (Schewiakoff 1926; Strelkov and Reshetnyak 1974; Reshetnyak 1981). These authors suggest that based on myoneme number and skeletal complexity, the Symphyacanthida are probably more derived than the Chaunacanthida. However, given that the Symphyacanthida (as it is currently defined) was shown to be polyphyletic in this analysis, the branching order of the Symphyacanthida relative to the Chaunacanthida is best determined only after the analysis of additional symphyacanthid sequence data.

The results from this study revealed that analysis of SSU rRNA genes may prove useful in future taxonomic revision within the Acantharea, at least at the order level of taxonomic organization. The additional acantharian order the Plegmacanthida (Reshetnyak 1981) (not yet formally recognized) along with representatives from the Holacanthida and representatives of a once proposed fifth order, the Actineliida (Levine et al. 1980) await molecular investigation.

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REFERENCES

Bernstein, R. E., P. R. Betzer, R. A. Feely, R. H. Byrne, M. F. Lamb and A. F. Michaels. 1987. Acantharian fluxes and strontium to chlorinity ratios in the north Pacific Ocean. Science **237:** 1490-1494.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution **42:** 795-803.

Cachon, J. and M. Cachon. 1982. Actinopoda, p. 553-568. *In* S. P. Parker [ed.], Synopsis and Classification of Living Organisms. McGraw Hill Book Company.

Cachon, J. and M. Cachon. 1985. Class Acantharea, p. *In* J.J. Lee, S. H. Hutner and E. C. Bovee [ed.], An Illustrated Guide to the Protozoa. Society of Protozoologists.

Caron, D. A. and N. R. Swanberg. 1990. The ecology of planktonic sarcodines. Reviews in Aquatic Sciences **3:** 147-180.

Febvre, J. 1981. The myoneme of the Acantharia (Protozoa): A new model of cellular motility. Biosystems **14:** 327-336.

Febvre, J. 1990. Phylum Actinopoda Class Acantharia, p. 363-379. *In* Lynn Margulis, John Corliss, Michael Melkonian and David J. Chapman [ed.], Handbook of Protoctista. Jones and Bartlett Publishers.

Febvre, J. and C. Febvre-Chevalier. 1982. Motility processes in Acantharia (Protozoa). I.- Cinematographic and cytological study of the myonemes. Evidence for a helix-coil mechanism of the constituent filaments. Biol. Cell **44:** 283-304.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution **39:** 783-791.

Fenchel, T. and B. J. Finlay. 1986. The structure and function of Müller Vesicles in the loxodid cilates. Journal of Protozoology **33:** 69-79.

Haeckel, E. 1888. Die Radiolarien (Rhizopoda, Radiolaria). III. Die Acantharien oder Actipyleen Radiolarien, p. 1-31. *In* G. Reimer [ed.], Monographie des Radiolarien.

Hertwig, R. 1879. Der organismus der Radiolarien. Jenaische Denkschriften **2:** 129-277.

Hollande, A. and M. Cachon-Enjumet. 1963. Sur la constitution chimique des spicules d'Acanthaires. Bull. Inst. océanogr. Monaco **60:** 1-4.

Levine, N. D., J. O. Corliss, F. E. G. Cox, G. Deroux, J. Grain, B. M. Honigberg, G. F. Leedale, A. R. I. Loeblich, J. Lom, D. Lynn, E. G. Merinfeld, F. C. Page, G. Poljansky, V. Sprague, J. Vavra and F. G. Wallace. 1980. A newly revised classification of the protozoa. Journal of Protozoology **27:** 37-58.

Massera Bottazzi, E. and M. G. Andreoli. 1981. "Blooming" of Acantharia (Protozoa-Sarcodina) in the southern Atlantic Ocean. Quad. Lab. Tecnol. **3:** 637.

Massera-Bottazzi, E. and A. Vinci. 1965. Verifica della legge di Müller e osservazioni sulla struttura mineralogica delle spicole degli Acantari (Protozoa). L'Ateneo Parmense, Acta Naturalia **1:** 1-12.

Merinfeld, E. G. 1969. Enxames de Acantários em águas neríticas. 21st Annual Meeting of the Brazilian Society for the Progress of Science 21: 455-456.

Michaels, A. F. 1988. Vertical distribution and abundance of Acantharia and their symbionts. Marine Biology **97:** 559-569.

Müller, J. 1858. Über die Thalassicollen, Polycystinen und Acanthometren des Mittelmeeres, p. 1-62. *In* F. Dummler [ed.], Abhandlungen der Königlische Akademie der Wissenschaften zu Berlin.

Olsen, G. J. 1988. Phylogenetic analysis using ribosomal RNA, p. 793-812. *In* H. F. Noller Jr. and K. Moldave [ed.], Methods in Enzymology. Ribosomes. vol. 164. Academic Press.

Olsen, G. J., H. Matsuda, R. Hagstrom and R. Overbeek. 1994. fastDNAml: a tool for constructing of phylogenetic trees of DNA sequences using maximum likelihood. Computational Applied Biosciences **10:** 41.

Olsen, G. J., R. Overbeek, N. Larsen, T. L. Marsh, M. J. McCaughey, M. A. Maciukenas, W.-M. Kuan, T. J. Macke, Y. Xing and C. R. Woese. 1992. The ribosomal database project. Nucleic Acids Research **20 (Supplement):** 2199-2200.

Raven, J. A., F. A. Smith and N. A. Walker. 1986. Biomineralization in the Charophyceae sensu lato, p. 125-139. *In* B. S. C. Leadbeater and R. Riding [ed.], Biomineralization in Lower Plants and Animals. vol. 30. Clarendon Press.

Reshetnyak, V. V. 1981. Evolution and phylogeny, p. 224. *In* [ed.], Acantharia (Acanthariea, Protozoa) from the World Ocean. vol. 123. Nauka, SSSR.

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science **239:** 487-491.

Schewiakoff, W. 1926. Die Acantharia des Golfes von Neapel. Fauna und Flora, p. 1-755. *In* G. Bardi [ed.], Fauna und Flora des Golfes von Neapel. R. Friedlander und Sohn.

Schreiber, B. and I. Ortalli. 1964. Animali <<estrattori>> di Sr nel plancton marino: gli acantari. Nuovo Cimento no. **1:** 79-85.

Schröder, O. 1907. Eine gestielte Acanthometride Podactinelius sessilis nov. gen. sp. Deutsche Südpolar-Expedition 1901-1903 **9:** 227-236.

Strelkov, A. A. and V. V. Reshetnyak. 1974. General Characteristics and Radioecology of the Strontium Organisms, Acantharian Radiolarians, p. 95-191. *In* G. G. Polikarpov [ed.], Chemoradiology of the Pelagical and Benthal (metals and their radionuclei in hydrobionts and in their environment medium). Naukova Dumka.

Swofford, D. L. 1991. PAUP: Phylogenetic Analysis Using Parsimony.

Tregouboff, G. 1953. Classe des Acanthaires, p. 271-320. *In* P. P. Grassé [ed.], Traité de Zoologie. Masson et Cie.

Chapter 5

Conclusions

The work described in this thesis is significant for its contribution to eukaryotic molecular phylogeny and to the field of acantharian and polycystine biology. The sequence data obtained are the first SSU rRNA genes to be obtained from the Acantharea and Polycystinea respectively. They represent the first molecular genetic work to be accomplished on representatives of either of these two classes. The writing of this thesis and subsequent publication of the contents of its chapters as manuscripts in scientific journals will hopefully instigate further applications of molecular approaches to answering questions in the biology of these understudied protists.

The most significant result from this thesis comes with the finding that based on SSU rRNA gene-based phylogenies, the Acantharea and the Polycystinea do not share common ancestry (Chapter 1). These results imply that the taxon Actinopoda (as well as any other taxon uniting Acantharea and Polycystinea such as Cavalier-Smith's newly proposed "Radiozoa") is not monophyletic and should be discarded in future systematic revisions of the Sarcodina. These results are actually in agreement with speculations made by a variety of authors who have submitted that axopodia are convergent structures which are probably the result of ecological constraints placed on organisms possessing a common planktonic mode of existence (Cachon and Balamuth 1977; Merinfeld 1978; Shulman and Reshetnyak 1980; Merinfeld 1981; Reshetnyak 1981). The results from Chapter 1 also support the monophyly of the Acantharea and at least the separate monophyly of the order Spumellarida of the Polycystinea.

The extent to which SSU rRNA genes differ within a given species of Thalassicolla nucleata was the focus of Chapter 2. The amount of variation observed within a species of this genus collected from the Sargasso Sea and the Pacific Ocean was higher than one might expect for a single species, with the highest values falling at levels observed at the genus level in other taxa. Perhaps given the amount of divergence displayed within the spumellarian SSU rRNA genes sequenced during this thesis, these values should not be surprising.

Whether this degree of genetic variation warrants new species or strain designations in the Thalassicolla genus is unclear but raises the important question of what defines a species in Thalassicolla. The existing species designations made by Haeckel for Thalassicolla are suspect because they occur so infrequently in the literature following their initial descriptions, and also because they include a total of seven species, four of which are lacking in symbionts. Given our present understanding of the feeding behavior within Thalassicolla and the importance of symbiont-derived nutrition, it seems that some of these species may not be valid. These facts in combination with morphological changes associated with parasitism, differences in physiological condition, and external factors such as excessive wave agitation, may have contributed to the morphological features used by Haeckel to describe different species of this genus.

The third chapter examined the phylogeny of the colonial spumellaria and attempted to determine whether or not the colonial radiolaria represent a monophyletic evolutionary assemblage. One robust conclusion drawn from this chapter was the monophyly of the Collosphaeridae, which is comprised of shell-bearing colonial forms. Representatives of three genera from this family grouped together with bootstrap values of 100% in all analyses accomplished. These results are exciting because they suggest a potential for determining further relationships between the Collosphaeridae and comparing them to phylogenies derived from the polycystine fossil record. These results also suggest a potential for establishing a phylogeny based classification for the Collosphaeridae.

Representatives of the two families of Spumellarida known to form colonies used in these analyses indicated that the colonial spumellaria may not be monophyletic. Because the different methods employed in reconstructing phylogenies did not yield the same answer, I cannot be fully confident of this result. The monophyly of the colonial spumellaria was supported in only one of the methods (maximum parsimony) and with low bootstrap support (61%). Given these results, it appears that coloniality may not serve as a reliable phylogenetic marker.

All of these results come with the overwhelming revelation of the high sequence divergence exhibited by the Spumellarida. However, Hillis et al. (1996) discuss several possibilities that might account for the observed differences in heterogeneity rates seen within a given gene. Among them are differences in DNA repair efficiency and differences in exposure to mutagens, both of which may explain some of the source of this variability. It may be that the spumellaria, as planktonic organisms, are subjected to high levels of UV damage since they typically occur in the surface portions of water column. If spumellaria lack a means of protecting themselves from UV or else do not possess adequate DNA repair mechanisms to efficiently repair damaged DNA, this might explain some of the observed sequence divergence.

Furthermore, the low % G + C content found in the spumellarian sequences may make them more susceptible to thymine-dimer formations created during exposure to UV which may be difficult to repair with existing DNA Repair mechanisms. However, if UV radiation is acting as a selective force in the % G + C content of these organisms, we would expect to see high % G + C content not the low values observed thus far in the Spumellarida. This scenario has been proposed by Singer and Ames (1970) to account for the high % G + C content in bacteria inhabiting high UV-exposed environments. It seems equally likely that members of the Spumellarida may have evolved mechanisms to deal with UV and that the long branch lengths observed in the spumellarian phylogenies are attributable to other reasons such as long divergence times or fast organismal generation times.

The final chapter of this thesis examined the evolutionary relationships between three orders of Acantharea. The results from this work were consistent with the morphology-based systematics in that they supported the monophyly of the Chaunacanthida and the Arthracanthida. The exception was in the phylogenetic placement of Haliommatidium sp. with the Arthracanthida. While this result is contrary to its current taxonomic position among the Symphyacanthida, it is less surprising when one reexamines the morphological features that Haliommatidium sp. shares with the Arthracanthida. Given this result, the formation of cysts (an ability possessed by Haliommatidium sp. but not members of the Arthracanthida) may not be a reliable phylogenetic marker whereas myoneme number and presence of a central capsule wall may be. In any event, it appears that the Acantharia are a more recently divergent lineage that are not closely related to any known protistan group for which there is currently SSU rRNA sequence information.

The molecular approach using an SSU rRNA-based method for reconstructing phylogenies of the Acantharea and the Polycystinea has proven to be a fruitful one. Within the spumellaria, especially, there appears to be sufficient sequence variation to make fine-scaled comparisons between existing morphospecies. The variability within the acantharian SSU rDNA was significantly less that of the spumellaria, and may prove less useful in establishing differences at the species level. The design of acantharian and colonial spumellarian oligonucleotide probes and primers accomplished during this thesis, will assist in further efforts to establish a phylogeny-based systematic framework for both of these protistan groups. The application of the oligonucleotide probes also holds potential for addressing ecological questions surrounding the life cycle and distributions of these elusive protists.

REFERENCES

Cachon, M. and W. Balamuth. 1977. The Sarcodina, p. 190-196. *In* S. H. Hutner [ed.], Protozoological Actualities. 5th International Congress of Protozoology.

Hillis, D. M., B. K. Mable and C. Moritz. 1996. Applications of molecular systematics: The state of the field and a look to the future., p. 515-543. *In* D. M. Hillis, C. Moritz and B. K. Mable [ed.], Molecular Systematics. Sinauer Associates, Inc.

Merinfeld, E. G. 1978. Ecological considerations in the interpretations of morphological convergences among the oceanic planktonic sarcodines. Biosystems **10:** 29-30.

Merinfeld, E. G. 1981. Phylogenetic relationships among the Actinopoda. VI International Congress of Protozoology 244.

Reshetnyak, V. V. 1981. Evolution and phylogeny, p. 224. *In* [ed.], Acantharia (Acanthariea, Protozoa) from the World Ocean. vol. 123. Nauka, SSSR.

Shulman, S. S. and V. V. Reshetnyak. 1980. Realno li cushchestvovanie v cisteme prosteishikh nadklassa Actinopoda? (About the possibility of the superclass Actinopoda existence in the system of Protozoa?). Printsipy postroeniya makrosistemy odnokletochnykh zhivotnykh (Principles of the Construction of the Macrosystem of the Unicellular Animals) 94: 23-41.

Singer, C. E. and B. N. Ames. 1970. Sunlight ultraviolet and bacterial DNA base ratios. Science **170:** 822-826.

APPENDIX A: SEQUENCE ALIGNMENT

```
"Sept4aln" on  5-SEP-96 15:33:41

  1 (1369mask):  1,369 homologous sites included in phylogenetic analyses
  2 (MNELEIDY):  L10826:Mnemiopsis leidyi:Ctenophore
  3 (DIAGRAND):  L10824:Diaphanoeca grandis:Choanoflagellate
  4 (ATHBOMBA):  M55638:Athelia bombacina:fungus:basidiomycete
  5 (BLAEMERS):  M54937:Blastocladiella emersonii
  6 (CHLREINH):  M32703:Chlamydomonas reinhardtii:Chlorophyte:Volvocales
  7 (ORYSATIV):  X00755:Oryza sativa (rice):chlorophyte
  8 (PORAERUG):  L27635:Porphyridium aerugineum:rhodophyte:Bangiophycideae
  9 (ACACAST1):  M13435:Acanthamoeba castellanii:"Amoebida"
 10 (PHRBALAM):  L23799:Phreatamoeba balamuthi
 11 (STYALSID):  L26204:Stylonema alsidii:Rhodophyte:Porphyridiaceae
 12 (EMIHUXLE):  L04957:Emiliana huxleyi:Haptophyte
 13 (OCHDANIC):  M32704:Ochromonas danica:stramenopile:chrysophyte
 14 (CAFROENB):  L27633:Cafeteria roenbergensis:stramenopile:bicosoecid
 15 (LABMINUT):  L27634:Labyrinthuloides minuta:stramenopile:labyrinthulid
 16 (OXYGRANU):  X53486:Oxytricha granulifera:ciliate:hypotrich
 17 (BLEAMERI):  M97909:Blepharisma americanum:ciliate:heterotrich
 18 (HARVERMI):  M95168:Hartmanella vermiformis:"Lobosa"
 19 (THEANNUL):  M64243:Theileria annulata:apicomplexa:Coccidia piroplasm
 20 (SYMPILO1):  M88518:Symbiodinium pilosum:dinoflagellate:Zooxanthellales
 21 (ZBBSR205):  Arthracanthid 205 (Acanthometra sp.)
 22 (ZBBSR206):  Arthracanthid 206
 23 (ZBBSR235):  Symphyacanthid 235 (Haliommatidium sp.)
 24 (ZBBSR218):  Chaunacanthid 218
 25 (ZBBSR217):  Chaunacanthid 217
 26 (ZBBSR211):  Symphyacanthid 211
 27 (Pchroma ):  Paulinella chromatophora SSU rRNA, X81811
 28 (EUGROTUN):  X77692:Euglypha rotunda CCAP 1520/1:Sarcodina
 29 (ENTOGING):  Entamoeba gingivalis (st.ATCC30927)
 30 (DICDISCO):  K02641:Dictyostelium discoideum:dictyostelids
 31 (ZTnucl   ):  Thalassicolla nucleata (BBS3) from the Sargasso Sea
 32 (W10Z79   ):  Thalassicolla "nucleata" clone number 79 collected from the Pacific sample W10
 33 (W10Z74   ):  Thalassicolla "nucleata" clone number 74 collected from the Pacific sample W10
 34 (W10Z72   ):  Thalassicolla "nucleata" clone number 72 collected from the Pacific sample W10
 35 (W10Z10   ):  Thalassicolla "nucleata" clone number 10 collected from the Pacific sample W10
 36 (ZBBSR7   ):  Rhaphidozoum acuferum
 37 (ZBBSR173):  Collosphaera globularis-huxleyi
 38 (Siphcyan):  Siphonophaera cyathina
 39 (ZBBSR2   ):  Collozoum pelagicum
 40 (ZCR4     ):  Sphaerozoum punctatum
 41 (ZCR16    ):  Collozoum vermiformi
 42 (ZCR6A    ):  Acrosphaera (circumtexta?)
 43 (PHYPOLYC):  X13160:Physarum polycephalum:physarids
 44 (NAEGRUBE):  M18732:Naegleria gruberi:schizopyrenids:vahlkampfiids
 45 (euk heli):  possible secondary structure helices
 46 (rad     ):
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"Sept4aln" on  5-SEP-96 15:33:41

 Posi-   Sequence
 tion:   identity:   Data:

    1   1 1369mask  --------------------------1111111111111111111111111111111111111------------------------------------1  1369mask
    1   2 MNELEIDY  CAACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGAUUAAgccAugcAUGUCUAAguauaaaCU----------------uuuauacU-GUG  MNELEIDY
    1   3 DIAGRAND   AACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGAUUAAgccAugcAUGUCUAAguauaaaUGAC--------------uuuauacA-GUG  DIAGRAND
    1   4 ATHBOMBA   AACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGAUUAAgccAugcAUGUCUAAguauaaaCAAA--------------uuuguacU-GUG  ATHBOMBA
    1   5 BLAEMERS   UACcugguUGAuccugccagUaguCauaugcuUGUcuAaAAGAUUAAgccAugcAUGUCUAAguauaaaCUGC--------------uuuauacAAGUG  BLAEMERS
    1   6 CHLREINH   UACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGAUUAAgccAugcAUGUCUAAguauaaaCUGC---------------uuauacU-GUG  CHLREINH
    1   7 ORYSATIV   UACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGAUUAAgccAugcAUGUGCAAguAugaaCUAA--------------uucgAacU-GUG  ORYSATIV
    1   8 PORAERUG   AACcugguUGAuccugccagUuauGauaugcuUGUcucaAAGAUUAAgccAugcAUGUGUAAguauaaaUC----------------uuuauacG-GUG  PORAERUG
    1   9 ACACAST1   UACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGAUUAAgccAugcAUGUCUAAguauaagCUUG--------------uuuauacG-GCG  ACACAST1
    1  10 PHRBALAM   AACcCgguCGAuccugccAgUAGUCAUAUGCUUGUguuaAAGAUUAAgccAugcAUGCCUAAguaCAAaCUAU---------------uCUuauG-GUA  PHRBALAM
    1  11 STYALSID  CAACcugguUGAuccugccagUggu-auagucuUGUcuCaAAGAUUAAgccAugcAUGUCUAAguauaaaCAAA--------------ucuauauU-GUG  STYALSID
    1  12 EMIHUXLE   AACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGAUUAAgccAugcAUGUCUAAguauaAgCGA----------------cuauacU-GUG  EMIHUXLE
    1  13 OCHDANIC   AACcugguUGAuucugccagUag-CauacgcuUGUcucaAAGAUUAAgccAugcAUGUCUAAguauaagCAAU-------------cuuuauac--GUG  OCHDANIC
    1  14 CAFROENB   AACcugguUGAuccugccagUaguCauacgcuUGUcucaAAGAUUAAgccAugcAUGUCUAAGUAUAAGCGA------------------A-A---GCG  CAFROENB
    1  15 LABMINUT   AACcugguUGAuccugccagUaguCauacgcuCGUcucaAAGAUUAAgccAugcAUGUCUAAgugcaagCA----------------cuuguacU-GUG  LABMINUT
    1  16 OXYGRANU   AACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGACUAAgccAugcAUGUCUAAguauaaAU-----------------GuuauacA-GUG  OXYGRANU
    1  17 BLEAMERI   AACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGAUUAAgccAugcAUGUCUAAguauaaGCA----------------AuuauacG-GCG  BLEAMERI
    1  18 HARVERMI   AACcugguUGAuccugccagUagUCauaugcuUGYcucaGAGAUUAAgccAugcAUGUCUAAguauaagCUAU--------------cuuauacA-GCG  HARVERMI
    1  19 THEANNUL   AACcugguUGAuccugccagUaguCauaugcuUGUcuuaAAGAUUAAgccAugcAUGUCUAAguauaagCU----------------uuuauauG-GUG  THEANNUL
    1  20 SYMPILO1    ACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGAUUAAgccAugcAUGUCUCAguAuaAgCU----------------uCuaCacG-GCG  SYMPILO1
    1  21 ZBBSR205        gguUGAuccugccagUaguCauaugcuUGUcucaAAGACUAAgccAugcAUGUCUAAguuuaagCGA---------------uuuaaacG-GUG  ZBBSR205
    1  22 ZBBSR206   AACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGACUAAgccAugcAUGUCUAAguuuaagCGA---------------uuuaaacG-GUG  ZBBSR206
    1  23 ZBBSR235   AACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGACUAAgccAugcAUGUCUAAguuuaagCGA---------------uuuaaacG-GUG  ZBBSR235
    1  24 ZBBSR218   AACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGAUUAAgccAugcAUGUCUAAguuuaagCGA---------------uuuaaacA-GCG  ZBBSR218
    1  25 ZBBSR217   AACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGAUUAAgccAugcAUGUCUAAguuuaagCGA---------------uuuaaacA-GCG  ZBBSR217
    1  26 ZBBSR211   AACcugguUGAuccugccagUaguCauaugcuUGUcucaAAGACUAAgccAugcAUGUCUAAguuuaagCGA---------------uuuaaacA-GCG  ZBBSR211
    1  27 Pchroma    AACcugguUGAuccggccagUaguCauaugcuUGUcucaAAGAUUAAgccAugcAUGUCUAAguauaagCAAC--------------uuuauacU-GCG  Pchroma
    1  28 EUGROTUN   AACcugguUGAuccugccagUa-aCauaugcuUGUcucaAAGACUAAgccAugcAUGUCUAAguauaaaCAAC--------------uuuauacU-GUG  EUGROTUN
    1  29 ENTOGING                            auaugcuGAUGuuaAAGAUUAAgccAugcAUGUGUAAGuAuAAAGACC---------------AAGaaGG-AUG  ENTOGING
    1  30 DICDISCO   UAAcugguUGAuccugccagUaguCauaugcuUGUCucaAAGAUUAAgccAugcAUGUCUAAguauaaAUUC----------------uuguacG-AUG  DICDISCO
    1  31 ZTnucl    CAACcugguUGAuccugccagUaguCauacgcUAACAuuaAAGAUUAAgccAugcAUGUACGAguaUACA-------------------AUuaccAUUUU  ZTnucl
    1  32 W10Z79     AACcugguUGAuccugccagUaguCauacgcUAACAuuaAAGAUUAAgccAugcAUGUACGAgUaaAAA-------------------AuuUccAUUUU  W10Z79
    1  33 W10Z74     AACcugguUGAuccugccagUaguCauacgcUAACAuuaAAGAUUAAgccAugcAUGUACGAguaaA---------------------AuuaccAUUUU  W10Z74
    1  34 W10Z72     AACcugguUGAuccugccagUaguCauacgcUAACAuuaAAGAUUAAgccAugcAUGUACGAgUaaAAA-------------------AuuUccAUUUU  W10Z72
    1  35 W10Z10     AACcugguUGAuccugccagUaguCauacgcUAACAuuaAAGAUUAAgccAugcAUGUACGAgUaaAAA-------------------AuuUccAUUUU  W10Z10
    1  36 ZBBSR7     AACcugguUGAuccugccagUaguCauacacUUACUuuaAAGAUUAAgccAugcAUGUCUUAaugCuuugUU------------UUugUaacau----U  ZBBSR7
    1  37 ZBBSR173   AACcugguUGAuccugccagUaguCauauacUUGUCuuUAAGAUUAAgccAugcAUGUCUCAguuCuUuaAUU-------------UugUaaac--AAU  ZBBSR173
    1  38 Siphcyan     CcugguUGAgccugccagUaguCauaUUcUUGUCuuaAAGAUUAAgccAugcAUGUCUCAgUuCuucGAUU-------------ggUaCaCc--A-U  Siphcyan
    1  39 ZBBSR2     AACcugguUGAgccugccagUaguCauacacUUGUCuuaAAGAUUAAgccAugcAUGUCUAAaugCuUuGUU--------------UaUaUaau--AUU  ZBBSR2
    1  40 ZCR4       AACcugguUGACccugccagUaguCauacacUUGUCuuaAAGAUUAAgccAugcAUGUCUCAaugCuuUgUUUA-------------UUuUaac--AUU  ZCR4
    1  41 ZCR16      AACcugguUGAgccugccagUaguCauacacUUGUCuaaAAGAUUAAgccAuguAUGUCUAUgUaCUUcGAUA--------------gUCCuUc--AUU  ZCR16
    1  42 ZCR6A      aaCcugguUGAgccugccagUaguCauauacUUGUCucaAAGAUUAAgccAugcAUGUCUCAguuCuUACacU-------------UUguAUAa--AAU  ZCR6A
    1  43 PHYPOLYC   UACcugguUGAuccugccagUagu-guaugcuUCUccuaAAGACUAAgccAugcAUGUCUCCGAAUAGAAGAG------------CAAGU-CUCU-CUG  PHYPOLYC
    1  44 NAEGRUBE   UACcugguUGAuccugccagUaCu-auaugcuUGUCucaAAGCCUAAgccAugcAAAUGUAAGauCAAUCauaugcgguuUCggccguguauAau-GAU  NAEGRUBE
    1  45 euk heli       1      2    1        3           4            5            6                            6        euk heli
    1  46 rad           ---->   ---><---- --- ------>    --->       --- --->      ----->                    <-----        rad
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"Sept4aln" on 5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

104   1 1369mask  11111111111111111111111111111111111111111111---------------------------------111111111-1111111111111  1369mask
104   2 MNELEIDY  AAACUGCGAAUGGcucAUUARAUcagUUAUCguCuAUUugaUUGUGCCC--------------------------UUACUAcaugG-AuaAcCGuagUAA  MNELEIDY
104   3 DIAGRAND  AAACUGCGAAUGGcucAUUAAAUcagUUAUAguuuAUUugaUAGUAC----------------------------UUACUAcUugG-AuaAcCGuggUAA  DIAGRAND
104   4 ATHBOMBA  AAACUGCGAAUGGcucAUUAAAUcagUUAUAguuuAUUugaUgAUACC---------------------------UUACUAcaugG-AuaAcuGuggUAA  ATHBOMBA
104   5 BLAEMERS  AAACUGCGAAUGGcucAUUAAAUcagUUAUAauuuAUUugauAGUACCCC------------------------AUUACUAcUugG-AuaAcCGuggUAA  BLAEMERS
104   6 CHLREINH  AAACUGCGAAUGGcucAUUAAAUcagUUAUAguuuAUUugaUgGUACC----------------------------UACUAcUcgG-AuaAcCGuagUAA  CHLREINH
104   7 ORYSATIV  AAACUGCGAAUGGcucAUUAAAUcagUUAUAguuugUUgaugGUACG-----------------------------UGCUAcUcgG-AuaAcCGuagUAA  ORYSATIV
104   8 PORAERUG  AAACUGCGRAUGGcucAUUAAAAcagUCAUAauuuACUygaUaGUACC----------------------------UUUUAcUugG-AuaCcCcuagUAA  PORAERUG
104   9 ACACAST1  AGACUGCGGAUGGcucAUUAAAUcagUUAUAguuuAUUugauGGUCUCUUUUGUCUU--------------UUUUUACCUAcUugG-AuaAcCGuggUAA  ACACAST1
104  10 PHRBALAM  AAACUGCGGACGGcucCAUAGAUcagUAAUAguucgUUcagugAUUUGAA-------------------------AAAGUAcUugG-AuaAcCcuguUAA  PHRBALAM
104  11 STYALSID  AAACUGCGAAUGGcucAUUAAAAcagUUAUAauuuAUUugauAGUACC----------------------------UACUAcUugG-AuaAcCGuagUAA  STYALSID
104  12 EMIHUXLE  AAACUGCGAAUGGcucAUUAAAUcagUUAUGguuuAUUugaUGGUACCU---------------------------UGCUAcUugG-AuaAcCGuagUAA  EMIHUXLE
104  13 OCHDANIC  AAACUGCGAAUGGcucAUUAUAUcagUUAUAguuuCUUugaugGUCCU----------------------------UGCUAcUugG-AuaAcCGuagUAA  OCHDANIC
104  14 CAFROENB  AAACUGCGAAUGGcucAUUAUAUcagUUAUAguuuAUUugaUAGUUU-----------------------------UCCUAuAugG-AuaAcCGurgUAA  CAFROENB
104  15 LABMINUT  AAACUGCGAAUGGcucAUUAUAUcagUUAUAguuuAUUugauAGAUUUC---------------------------UACUAcUugG-AuaAcCGuagUAA  LABMINUT
104  16 OXYGRANU  AAACUGCGAAUGGcucAUUAAAAcagUUAUAguuuAUUugauAAUCGA----------------------------AUUUAcaugG-AuaAcCGuggUAA  OXYGRANU
104  17 BLEAMERI  AGACUGCGAAUGGcucAUUAAAAcagUUAUAguuuAUUugcuAGACG------------------------------UUUAuaugG-AuaAcCGuagUAA  BLEAMERI
104  18 HARVERMI  AAACUGCGAAUGGcucAUUAAAUcagUUAUAguCuACUugaucGUAUC---------------------------UUCUUAcAugG-AuaAcCGuagUAA  HARVERMI
104  19 THEANNUL  AAACUGCGAAUGGcucAUUACAAcagUUAUAguuuAUUugauGUUCG----------------------------UUUCUAcaugG-AuaAccGugcUAA  THEANNUL
104  20 SYMPILO1  AAACUGCGAAUGGcucAUUAAAGcagUUAUAauuuAUUUgaugGUCA----------------------------CUGCUAcAugG-AuaAcuGuggUAA  SYMPILO1
104  21 ZBBSR205  AGACUGCGGACGGcucAUUAUAUcagUUAUAgUuuAUUugaUGGUCGA---------------------------UUACUAcaagG-AuaACCGuagGAA  ZBBSR205
104  22 ZBBSR206  AGACUGCGGACGGcucAUUAUAUcagUUAUAgUuuAUUugaUGGUCGA---------------------------UUACUAcaagG-AuaACCGuagGAA  ZBBSR206
104  23 ZBBSR235  AGACUGCGGACGGcucAUUAUAUcagUUAUAgUuuAUUugaUGGUCGA---------------------------UUACUAcaagG-AuaACCGuagGAA  ZBBSR235
104  24 ZBBSR218  AGACUGCGGACGGcucAUUAUAUcagUUAUAgUuuAUUugaUGGUCGA---------------------------UUACUAcaagG-AuaACCGuagUAA  ZBBSR218
104  25 ZBBSR217  AGACUGCGGACGGcucAUUAUAUcagUUAUAgUuuAUUugaUGGUCGA---------------------------UUACUAcaagG-AuaACCGuagUAA  ZBBSR217
104  26 ZBBSR211  AGACUGCGGACGGcucAUUAUAUcagUUAUAgUuuAUUugaUGGUCGA---------------------------UCACUAcaagG-AuaACCGuagUAA  ZBBSR211
104  27 Pchroma   AAACUGCGUACAGcucAUUAUAUcagCAAUAauuuAUUugaUGGUUUC---------------------------UUACUAcUugG-AuaAcCGuagUAA  Pchroma
104  28 EUGROTUN  AAACUGCGUACAGcucAUUAUAUcagCAAUUauuuAUUugaUGGUACC---------------------------UUACUAcaugG-AuaAcCGuggUAA  EUGROTUN
104  29 ENTOGING  AAACUGCGGACGGcucAUUAGAAcagUUAUaAuuucUuugauuAGUAC-----------------------------CAUAcaagGAAuaGcuuuguGAA  ENTOGING
104  30 DICDISCO  AAACUGCAGACGGcucAUUACAAcagUGAUaAacUAAuagacuuUCGGGUU-----------------------UUACCU-uUugG-AuaAccGcAgUAA  DICDISCO
104  31 ZTnucl    AAACUGCGUAAAGcucAUUAUAUcagUUCUAacA-UCUuaggaauaCA----------------------------AAAAagaugG-AuAUuAgugCUAA  ZTnucl
104  32 W10Z79    AAACUGCGUAAAGcucAUUAUAUcagUUCUAacA-UCUuaggaauaCG-----------------------------AAAaaaCgG-AuAUuAgugCUAA  W10Z79
104  33 W10Z74    AAACUGCGUAAAGcucAUUAUAUcagUUCUAacA-UCUuaggaauaCU-----------------------------AAAaaaCgG-AuAUuAgugCUAA  W10Z74
104  34 W10Z72    AAACUGCGUAAAGcucAUUAUAUcagUUCUAacA-UCUuaggaauaCG-----------------------------AAAaaaCgG-AuAUuAgugCUAA  W10Z72
104  35 W10Z10    AAACUGCGUAAAGcucAUUAUAUcagUUCUAacA-UCUuaggaauaCG-----------------------------AAAaaaCgG-AuAUuAgugCUAA  W10Z10
104  36 ZBBSR7    AGAUUGCGGACGGcucAUUAUAAcagUUUUUauuuaaUUgauauuaU----------------------------UUUUUAGgugG-AuaCGCGuggUAA  ZBBSR7
104  37 ZBBSR173  AGAUUGCGAAUGGcucAGUAUAAcagUUACCauuuGUUugaauGACU-----------------------------AUUUAugugG-AuaAcuUUggAAU  ZBBSR173
104  38 Siphcyan  AGAUUGCGAAUGGuuuAGUAUAAcagBUUCUauAuUCUugaauAANU-----------------------------UUCUUugugG-AuaAcugCggGAA  Siphcyan
104  39 ZBBSR2    AGAUUGCGGACGGcucAUUAUAAcagUUAUUguAuUaUugaGAuUAC----------------------------UUUGUAGgugG-AuaCcuGuggUAA  ZBBSR2
104  40 ZCR4      AGAUUGCAAAUGGcucAUUAUAAcagUUAUUauAuUAUugagauuAU----------------------------UUUAUAGgugG-AuACuUaugaGAA  ZCR4
104  41 ZCR16     AGACUGCAGAUGGcucAUUAUAUcagUUUUUguAugCUugacAUUAU----------------------------CUUAUAugugG-AuaCcuGuggAAA  ZCR16
104  42 ZCR6A     AGAUUGCAAAUUGcucAGUAUAAcagUUACCauuGGUUugaauAAUA-----------------------------GUUUAugugG-AuaAcuUUggAAU  ZCR6A
104  43 PHYPOLYC  AAUCUGCGAACGGcucCGCAUACcagUUGUAAACCAUAGCAAGCAAGCCGCGUUGUUGCCGCAAGGCGACGGCGCGGUUCAcAGgG-AuaAccCuggUAA  PHYPOLYC
104  44 NAEGRUBE  AGUCUGUGGAAGGcucAUUAUAACagUUAUacucc-uAgcCAcuggAAAG---------------------------UUUAcaagG-AuaCcAcCguUAA  NAEGRUBE
104  45 euk heli                7         8              9                                                10            euk heli
104  46 rad                    -->       --->    --------------->                                  ----   -------->     rad
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"Sept4aln" on  5-SEP-96 15:33:41

 Posi-   Sequence
 tion:   identity:   Data:

 204   1 1369mask  11111111111111111111-------------------------------------------------------------------------------  1369mask
 204   2 MNELEIDY  UUcuaGAgCuaAUAcaugCGAAAAGuccc-GACuucU-------------------------------------ggaA-gggaUGUAU-uuauuagaUU  MNELEIDY
 204   3 DIAGRAND  UUcuaGAgCuaAUAcaUgCGAAAAGgccc-GACuGkUU------------------------------------gGgA-ggguUGUAU-uuauuagaUA  DIAGRAND
 204   4 ATHBOMBA  UUcuaGagCuaAUAcaugCAUUAAAgccccGACuucU-------------------------------------ggga-gggguGUAU-uuauuagaUA  ATHBOMBA
 204   5 BLAEMERS  UUcuaGAgCuaAUAcaUgCACUCAAguccuGACcgCUUGU---------------------------------cgGAA-gggauGCAG-uuaUuagaUA  BLAEMERS
 204   6 CHLREINH  UUcuaGAgCuaAUAcgUgCGCACA-accc-GACuucU-------------------------------------Ggaa-ggguCGUAU-uuauuagauA  CHLREINH
 204   7 ORYSATIV  UUcuaGAgCuaAUAcgUgCAACAAAcccc-GACuucC-------------------------------------Ggga-ggggCGCAU-uuauuagaUA  ORYSATIV
 204   8 PORAERUG  UUcuagAgCuaAUAcaUgCGCCAAcaucc-GACucRC--------------------------------------grA-ggaugGUAU-uuauuAg--C  PORAERUG
 204   9 ACACAST1  UUcuaGAgCuaAUAcaUgCGCAAGGuccc-GAgcgcgggggcagggcuUCAC---ggcccuguccucGCAugcgcAGA-gggaUGUAU-uuauuAgguu  ACACAST1
 204  10 PHRBALAM  UUguagAgCuaAUAcaUgCACCGACggccuGAucggGU--------------------------------GAccgaGa-gggucGCAC-UUGUCUUAAU  PHRBALAM
 204  11 STYALSID  -CcuaGAgCuaAUAcrUgCCUAAAcgccc-GACUCAC--------------------------------------GAA-gggugGUAU-uuauuAGAUA  STYALSID
 204  12 EMIHUXLE  UUcuaGAgCuaAUAcaUgCAGGAGUuccc-GAcuCAC-------------------------------------ggA--gggaUGUAU-uuauuagauA  EMIHUXLE
 204  13 OCHDANIC  UUcuaGAgCuaAUAcaUgCAGCAAUcccu-GAcuUA--------------------------------------ggAA-agggUGUAC-uuauuagaUA  OCHDANIC
 204  14 CAFROENB  UUcuaGAgCuaAUAcaUgCACGAAGuccG-GAucccUC----------------------------------gggaRA-AggaUGCGU-uuauuagauA  CAFROENB
 204  15 LABMINUT  UUcuaGAgCuaAUAcaUgCAACAAAgccc-aUU--------------------------------------------u-gggcUGUAU-uuauuagauA  LABMINUT
 204  16 OXYGRANU  UUcuaGAgCuaAUAcaugCUGGUUagccu-GACuuuU-----------------------------------GUggaA-gggcuGUAU-uuauuAgaUA  OXYGRANU
 204  17 BLEAMERI  UUcuaGAgCuaAUAcaugCUGGUua-cgc-U---------------------------------------------GU-gcguaGUAU-uuauuAgauA  BLEAMERI
 204  18 HARVERMI  AUcuaGAgCuaAUAcaUgCUAAAAAuccuGGAcgcgGC-------------------------------AAcgcgGAA-gggaCGAGU-uuauuAGAU-  HARVERMI
 204  19 THEANNUL  UUguaGggCuaAUAcaugUUCGAG-gcca-UU-----------------------------------------------uggcGGCGu-uuauuagaCC  THEANNUL
 204  20 SYMPILO1  UUcuaGagCuaAUAcaUgCACCAAaaccc-AACuucG------------------------------------CAgaa-ggguuGUAU-uuauuAgaUA  SYMPILO1
 204  21 ZBBSR205  UUcuaGAGCuaAUAcuugCACUUcaAggu-gacUU-------------------------------------AAAUgu-uaccugCAC-uuauuagaUU  ZBBSR205
 204  22 ZBBSR206  UUcuaGAGCuaAUAcuugCACUUcaAAcu-gaUUU-----------------------------------------Uu-cagAugCAC-uuauuagaUU  ZBBSR206
 204  23 ZBBSR235  UUcuaGAGCuaAUAcuugCACUUcaAggu-gauUU-------------------------------------AAACau-caucugCAC-uuauuagaUU  ZBBSR235
 204  24 ZBBSR218  UUcuaGAGCuaAUAcuugCACUUcaAaac-acUUU----------------------------------------UUg-uguuugCAC-uuauuagaUU  ZBBSR218
 204  25 ZBBSR217  UUcuaGAGCuaAUAcuugCACUUcaAaac-acUUU----------------------------------------UUg-uguuugCAC-uuauuagaUU  ZBBSR217
 204  26 ZBBSR211  UUcuaGAGCuaAUAcuugCACUCcaAaaa-caUU-------------------------------------------u-guuuugCAC-uuauuagaUU  ZBBSR211
 204  27 Pchroma   UUcuaGAgCuaAUAcaUgCGCAAA-AccugGACucuug-----------------------------------cgggaAcgggUGUAU-uuauuagaUA  Pchroma
 204  28 EUGROTUN  UUcuaGAgCuaAUAcaugCACAAAguccc-AAcucuUgc----------------------------------ggggA-gggauGUAU-uuauuagaUA  EUGROTUN
 204  29 ENTOGING  UAauaaagauaAUAcuugAG--ACGAuccuguucUAUUACUAG--------------------------------AAUAG--GCGCAU-UUCgaacagg  ENTOGING
 204  30 DICDISCO  AUc-gGggCuaAUAcaUaCAAGCGAUggguGAcugGC----------------------------------AAcggAA-gcucAGCGA-uuauuagCau  DICDISCO
 204  31 ZTnucl    UUCuacAaUCaAUAcauuuAUAACgucuaaUUUU----------------------------------------------uuagac-U-aaauUAC--u  ZTnucl
 204  32 W10Z79    UUCuacAaUCaAUAcAuuuAUAACgucuauuUUU---------------------------------------------aauagau-U-aaauUAC--u  W10Z79
 204  33 W10Z74    UUCuacAaUCaAUAcAuuuAUAACgucuaAUUU-----------------------------------------------Auagau-U-aaauUAC--u  W10Z74
 204  34 W10Z72    UUCuacAaUCaAUAcAuuuAUAACgucuauuUUU---------------------------------------------aauagau-U-aaauUAC--u  W10Z72
 204  35 W10Z10    UUCuacAaUCaAUAcAuuuAUAACgucuauuUUU---------------------------------------------aauagau-U-aaauUAC--u  W10Z10
 204  36 ZBBSR7    UUcuaGAGCuaAUAcauGUCAUAAAaauUguauaCUUUUUUAUAUGAU----------------------------UuauauUguu-U-AugUuUu--u  ZBBSR7
 204  37 ZBBSR173  UGcuUCagCuaAUAcaugGU-ACUuAuauuuaaAUCUAUUAUUUUGAU--------------------------uugUaUauaGaU-UUGauUUu---u  ZBBSR173
 204  38 Siphcyan  UUuuUcagCuaAUAcaugGU-A--cuau-guauaAAUAUAUAUUUGAU--------------------------Auguauau-ggCUGUuaUuUUu--u  Siphcyan
 204  39 ZBBSR2    UUcuaGagCuaAUAcauGUC-GCUAaug-GUauaUUUUUUAUU----------------------------------uauU-GAGcauUUauuugu--u  ZBBSR2
 204  40 ZCR4      AAuuauUaAAaAUAuauGAUUUUAaaauAuuUaUU------------------------------------------UAUu-UggCauuuauuugc--u  ZCR4
 204  41 ZCR16     UCcuaGagCuaAUAcaugCC-AG-aaauAAuauUUCUAUUUUGUA--------------------------------UaUu-gGGCauuuAUuu-C--u  ZCR16
 204  42 ZCR6A     UUcuUUagCuaAUAcaugGU-ACAauauauaua-uaCCU-AUAAAUGUAUUAUAUU-------------------GAuauguauauAG-CAUUUUu--u  ZCR6A
 204  43 PHYPOLYC  UUcugAggCuaAUAcAAgAACGUACCAcc-cgcuuCGACCCGUAA---------------------------GGGGAgggcgggg-gu-ugu-gugACC  PHYPOLYC
 204  44 NAEGRUBE  CUgcAgCgAua-UAcuugUUCCCUUCGgg-guGG---------------------------------------------uaauaguAu-uugUGCugaa  NAEGRUBE
 204  45 euk heli          10                 11                                                  1-1                1  euk heli
 204  46 rad         <--------   ----      ---------->                                         <---------   ----------  rad
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"Sept4aln" on  5-SEP-96 15:33:41

Posi-  Sequence
tion:  identity:   Data:

 304    1 1369mask  ----------------------------------------------------------------------------------------------------  1369mask
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 304    3 DIAGRAND  AAAA-accaaC----AUU----------------------------------------------------------------------------------  DIAGRAND
 304    4 ATHBOMBA  AAAA-accaaC---gcggcUC-------------------------------------------------------------------------------  ATHBOMBA
 304    5 BLAEMERS  CAAA-accagC---cccggGCA------------------------------------------------------------------------------  BLAEMERS
 304    6 CHLREINH  AAAG-gccAGC---cgggcUCU------------------------------------------------------------------------------  CHLREINH
 304    7 ORYSATIV  AAAG-gCugACg--cgggcUCC------------------------------------------------------------------------------  ORYSATIV
 304    8 PORAERUG  CGCA-accaGC---cgggcUU---g---------------------------------------------------------------------------  PORAERUG
 304    9 ACACAST1  AAAA-accagCg--gcaggggucaGC--------------------------------------------------------------------------  ACACAST1
 304   10 PHRBALAM  UCAC-AgugccccGgAACUGAGGCUGUUCGACGUGGUAGGGGAGGACGCUGAAUGGGGCUGGUAGAAACAACuGgggguauAAAACCAAGGAGGAAGCAA  PHRBALAM
 304   11 STYALSID  CAAA-accaaCc--ggcUU---------------------------------------------------------------------------------  STYALSID
 304   12 EMIHUXLE  AGAA-ACcaa-A--CCGGU---------------------------------------------------------------------------------  EMIHUXLE
 304   13 OCHDANIC  -GAA-ACCaaug--gggG----------------------------------------------------------------------------------  OCHDANIC
 304   14 CAFROENB  -CAA--CCUUC-----------------------------------------------------------------------------------------  CAFROENB
 304   15 LABMINUT  -GAA-ACcaaug--cagggUUU------------------------------------------------------------------------------  LABMINUT
 304   16 OXYGRANU  ACAA-ACcaaua--UUCCCCG-------------------------------------------------------------------------------  OXYGRANU
 304   17 BLEAMERI  AAA--CCCAacg--gggGCGA-------------------------------------------------------------------------------  BLEAMERI
 304   18 HARVERMI  UAAAAaccGAC----accucuccGC---------------------------------------------------------------------------  HARVERMI
 304   19 THEANNUL  UAAA-accAA-a--ccgcUU--------------------------------------------------------------------------------  THEANNUL
 304   20 SYMPILO1  CAGA-accaUCG--caggCUC-------------------------------------------------------------------------------  SYMPILO1
 304   21 ZBBSR205  UACC-aucaAug--CU-CUUC-------------------------------------------------------------------------------  ZBBSR205
 304   22 ZBBSR206  UACC-aCcaAug--CU-CUUC-------------------------------------------------------------------------------  ZBBSR206
 304   23 ZBBSR235  UACC-aCcaAug--CU-CUUC-------------------------------------------------------------------------------  ZBBSR235
 304   24 ZBBSR218  CCCC-aCcaAug--CC-UUU--------------------------------------------------------------------------------  ZBBSR218
 304   25 ZBBSR217  CCCC-aCcaAug--CC-UUU--------------------------------------------------------------------------------  ZBBSR217
 304   26 ZBBSR211  CCCC-aCcaAug--CC-CAU--------------------------------------------------------------------------------  ZBBSR211
 304   27 Pchroma   AAAA-accaaCG--cguccuC-------------------------------------------------------------------------------  Pchroma
 304   28 EUGROTUN  CAAA-accaaua--ccaccUC-------------------------------------------------------------------------------  EUGROTUN
 304   29 ENTOGING  aAUGUAGAAAAG----------------------------------------------------------------------------------------  ENTOGING
 304   30 DICDISCO  uCU--AccaaUg--ccUU----------------------------------------------------------------------------------  DICDISCO
 304   31 ZTnucl    gagU-aucaAAA--GUA-----------------------------------------------------------------------------------  ZTnucl
 304   32 W10Z79    gagU-aucaAAA--GUG-----------------------------------------------------------------------------------  W10Z79
 304   33 W10Z74    gagU-aucaAAA--GUA-----------------------------------------------------------------------------------  W10Z74
 304   34 W10Z72    gagU-aucaAAA--GUG-----------------------------------------------------------------------------------  W10Z72
 304   35 W10Z10    gagU-aucaAAA--GUG-----------------------------------------------------------------------------------  W10Z10
 304   36 ZBBSR7    gAAa-aucaACA-UGUUACG--------------------------------------------------------------------------------  ZBBSR7
 304   37 ZBBSR173  auCaaacUAAAA-UGUG-----------------------------------------------------------------------------------  ZBBSR173
 304   38 Siphcyan  aUUaaauUAAAA--UUUA----------------------------------------------------------------------------------  Siphcyan
 304   39 ZBBSR2    auCaaAcCAAAA-UGUU-----------------------------------------------------------------------------------  ZBBSR2
 304   40 ZCR4      UuCaaAcCAAAA-UGUU-----------------------------------------------------------------------------------  ZCR4
 304   41 ZCR16     auuAaGcCAGAG-UGAG-----------------------------------------------------------------------------------  ZCR16
 304   42 ZCR6A     auCaaacCAAAA-UGUG-----------------------------------------------------------------------------------  ZCR6A
 304   43 PHYPOLYC  CAGG-UCGCAAA-UAUUAACUG------------------------------------------------------------------------------  PHYPOLYC
 304   44 NAEGRUBE  GCCUAgcuauug--UAaccuAGUUUUc-------------------------------------------------------------------------  NAEGRUBE
 304   45 euk heli  2                                                                                                     euk heli
 304   46 rad       -------->                                                                                             rad
```

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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

 404    1 1369mask  ----------------------------------------------------------------------------------------------------  1369mask
 404    2 MNELEIDY  ----------------------------------------------------------------------------------------------------  MNELEIDY
 404    3 DIAGRAND  ----------------------------------------------------------------------------------------------------  DIAGRAND
 404    4 ATHBOMBA  ----------------------------------------------------------------------------------------------------  ATHBOMBA
 404    5 BLAEMERS  ----------------------------------------------------------------------------------------------------  BLAEMERS
 404    6 CHLREINH  ----------------------------------------------------------------------------------------------------  CHLREINH
 404    7 ORYSATIV  ----------------------------------------------------------------------------------------------------  ORYSATIV
 404    8 PORAERUG  ----------------------------------------------------------------------------------------------------  PORAERUG
 404    9 ACACAST1  ----------------------------------------------------------------------------------------------------  ACACAST1
 404   10 PHRBALAM  AAAAGCCAUAACCCGGCGAUGGCcuugguGgAAACCUCUGGGCUCAAgguuguuAuUAuGuUcAuuguGGccuCUCGGGGUUAUUUUGAAUGUAGUAAUA  PHRBALAM
 404   11 STYALSID  ----------------------------------------------------------------------------------------------------  STYALSID
 404   12 EMIHUXLE  ----------------------------------------------------------------------------------------------------  EMIHUXLE
 404   13 OCHDANIC  ----------------------------------------------------------------------------------------------------  OCHDANIC
 404   14 CAFROENB  ----------------------------------------------------------------------------------------------------  CAFROENB
 404   15 LABMINUT  ----------------------------------------------------------------------------------------------------  LABMINUT
 404   16 OXYGRANU  ----------------------------------------------------------------------------------------------------  OXYGRANU
 404   17 BLEAMERI  ----------------------------------------------------------------------------------------------------  BLEAMERI
 404   18 HARVERMI  ----------------------------------------------------------------------------------------------------  HARVERMI
 404   19 THEANNUL  ----------------------------------------------------------------------------------------------------  THEANNUL
 404   20 SYMPILO1  ----------------------------------------------------------------------------------------------------  SYMPILO1
 404   21 ZBBSR205  ----------------------------------------------------------------------------------------------------  ZBBSR205
 404   22 ZBBSR206  ----------------------------------------------------------------------------------------------------  ZBBSR206
 404   23 ZBBSR235  ----------------------------------------------------------------------------------------------------  ZBBSR235
 404   24 ZBBSR218  ----------------------------------------------------------------------------------------------------  ZBBSR218
 404   25 ZBBSR217  ----------------------------------------------------------------------------------------------------  ZBBSR217
 404   26 ZBBSR211  ----------------------------------------------------------------------------------------------------  ZBBSR211
 404   27 Pchroma   ----------------------------------------------------------------------------------------------------  Pchroma
 404   28 EUGROTUN  ----------------------------------------------------------------------------------------------------  EUGROTUN
 404   29 ENTOGING  ----------------------------------------------------------------------------------------------------  ENTOGING
 404   30 DICDISCO  ----------------------------------------------------------------------------------------------------  DICDISCO
 404   31 ZTnucl    ----------------------------------------------------------------------------------------------------  ZTnucl
 404   32 W10Z79    ----------------------------------------------------------------------------------------------------  W10Z79
 404   33 W10Z74    ----------------------------------------------------------------------------------------------------  W10Z74
 404   34 W10Z72    ----------------------------------------------------------------------------------------------------  W10Z72
 404   35 W10Z10    ----------------------------------------------------------------------------------------------------  W10Z10
 404   36 ZBBSR7    ----------------------------------------------------------------------------------------------------  ZBBSR7
 404   37 ZBBSR173  ----------------------------------------------------------------------------------------------------  ZBBSR173
 404   38 Siphcyan  ----------------------------------------------------------------------------------------------------  Siphcyan
 404   39 ZBBSR2    ----------------------------------------------------------------------------------------------------  ZBBSR2
 404   40 ZCR4      ----------------------------------------------------------------------------------------------------  ZCR4
 404   41 ZCR16     ----------------------------------------------------------------------------------------------------  ZCR16
 404   42 ZCR6A     ----------------------------------------------------------------------------------------------------  ZCR6A
 404   43 PHYPOLYC  ----------------------------------------------------------------------------------------------------  PHYPOLYC
 404   44 NAEGRUBE  ----------------------------------------------------------------------------------------------------  NAEGRUBE
 404   45 euk heli                                                                                                        euk heli
 404   46 rad                                                                                                             rad
```

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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:  Data:

504   1 1369mask  ----------------------------------------------------------------------------------------------------  1369mask
504   2 MNELEIDY  ------------------------------------------------------------------gacgc------UUUuCgguGAUucAua-auaaCU  MNELEIDY
504   3 DIAGRAND  ----------------------------------------------------------------------------UUUCuugguGAAucAua-auaaCU  DIAGRAND
504   4 ATHBOMBA  ------------------------------------------------------------------gccgc-----UCCYuugguGAUucAua-auaaCU  ATHBOMBA
504   5 BLAEMERS  ------------------------------------------------------------------Accggg-UUUUCUguugguGAUucAug-CuaaCU  BLAEMERS
504   6 CHLREINH  ------------------------------------------------------------------gcccg-----ACCUGCgguGAaucAug-auaaCU  CHLREINH
504   7 ORYSATIV  ------------------------------------------------------------------gcccg----cUGAUCcgAuGAUucAug-auaaCU  ORYSATIV
504   8 PORAERUG  ------------------------------------------------------------------ccc--g-----UUUu-gguGAGUcAUa-auaaCU  PORAERUG
504   9 ACACAST1  ------------------------------------------------------AA-uggccccu-gccAA---ACACUCcuggugaUucAUa-guaaCU  ACACAST1
504  10 PHRBALAM  AACCGAAAGCAACUCUAUCAGUUUGGUUCGGAUGUCCGUUAAUCCUGCGUGGCCAGCGCGUUUGGGACUCCa-ggGGacagGgCgAaACgAggcaauuCA  PHRBALAM
504  11 STYALSID  ------------------------------------------------------------------gccgU------UUUuugguGAAUCAUg-guaaCU  STYALSID
504  12 EMIHUXLE  -----------------------------------------------------------------cUCC--GG---uugCGUGCUGAgucAua-auaaCU  EMIHUXLE
504  13 OCHDANIC  ----------------------------------------------------------------CAAcccu-----UUguuUGGUGAUucAua-guaaUU  OCHDANIC
504  14 CAFROENB  ----------------------------------------------------------------------------AACACAGGUGAaucAua-auaaCC  CAFROENB
504  15 LABMINUT  ------------------------------------------------------------------UcccuGg---uguugUGGUGAgucAua-auaaCU  LABMINUT
504  16 OXYGRANU  --------------------------------------------------------------------UGUC----uauugUGACGACucAUa-auaaCU  OXYGRANU
504  17 BLEAMERI  -----------------------------------------------------------------------c----ccuguGGAGGAaucAUa-auaaCU  BLEAMERI
504  18 HARVERMI  ---------------------------------------------------------------GAggggaggu---AGCACAgguGAUucACa-guaaCY  HARVERMI
504  19 THEANNUL  ------------------------------------------------------------------gcgguGU-------CCgguGAUucAua-auaaaU  THEANNUL
504  20 SYMPILO1  ----------------------------------------------------------------UGccu-------gGUUGugguGAUucAUg-auaaCU  SYMPILO1
504  21 ZBBSR205  --------------------------------------------------------------------------GGAGCUUcgAugauucAua-guaaUU  ZBBSR205
504  22 ZBBSR206  --------------------------------------------------------------------------GGAGCUUcgGugAuucAua-guaaUU  ZBBSR206
504  23 ZBBSR235  --------------------------------------------------------------------------GGAGCUUcgGugAuucAua-guaaUU  ZBBSR235
504  24 ZBBSR218  --------------------------------------------------------------------------AUGGCUUcgGugAaucAua-guaaUU  ZBBSR218
504  25 ZBBSR217  --------------------------------------------------------------------------AUGGCUUcgGugAaucAua-guaaUU  ZBBSR217
504  26 ZBBSR211  --------------------------------------------------------------------------UGGGCUUcgGugAaucAua-guaaUU  ZBBSR211
504  27 Pchroma   --------------------------------------------------------------------UggggcgGUUGUuugguGAUucAua-auaaCU  Pchroma
504  28 EUGROTUN  ------------------------------------------------------------------ggugg----CuauuCUgguGACucAua-auaaCU  EUGROTUN
504  29 ENTOGING  ---------------------------------------------------------------------------AAGUUUAUUAAGAAAAAG-AACAAA  ENTOGING
504  30 DICDISCO  ----------------------------------------------------------------------CG---gguUuuggGUgauACcGa-auaaUA  DICDISCO
504  31 ZTnucl    -----------------------------------------------------------------------CGACUAUC-ugaAuUcuuaAUa-uuuAC-  ZTnucl
504  32 W10Z79    -----------------------------------------------------------------------CGACUAUC-ugaAuUcuuaAUa-uuuAC-  W10Z79
504  33 W10Z74    -----------------------------------------------------------------------CGACUAUC-ugaAuUcuuaAUa-uuuAC-  W10Z74
504  34 W10Z72    -----------------------------------------------------------------------CGACUAUC-ugaAuUcuuaAUa-uuuAC-  W10Z72
504  35 W10Z10    -----------------------------------------------------------------------CGACUAUC-ugaAuUcuuaAUa-uuuAC-  W10Z10
504  36 ZBBSR7    ------------------------------------------------------------------GCAUAAUGGUGGA-ugauuCCuaaUUa-UcaAUU  ZBBSR7
504  37 ZBBSR173  ------------------------------------------------------------------CGUACAUUAUGGA-AGguuuauaaUUa-uUACUA  ZBBSR173
504  38 Siphcyan  ------------------------------------------------------------------UAUAUUKUUUUGA-GGauuuUuaaUUa-UuaCCU  Siphcyan
504  39 ZBBSR2    ------------------------------------------------------------------UCAGCAUUAUGGA-UgAuuCauaguaa-UguUU-  ZBBSR2
504  40 ZCR4      ------------------------------------------------------------------UUAUCAUUAUGGA-UgAuuCaUaguaa-UguUU-  ZCR4
504  41 ZCR16     ------------------------------------------------------------------UUAAUACUUUGgA-uGaauCaUaaUAA-UU-CU-  ZCR16
504  42 ZCR6A     -----------------------------------------------------------------UGUACAUCA-UG----GAAGguuuauaa-UUGUU-  ZCR6A
504  43 PHYPOLYC  -------------------------------------------------------------GGAGUGGCc----acacgaucUGACCACCAUA-CCAAAC  PHYPOLYC
504  44 NAEGRUBE  ------------------------------------------------ggGUGUGGCAACAUAUUCGGGG----GAUUAGGAAUCGACCGCUAGCAGGUG  NAEGRUBE
504  45 euk heli                                                                                             12         euk heli
504  46 rad                                                                               <----------------     rad
```

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"Sept4aln" on  5-SEP-96 15:33:41

Posi-  Sequence
tion:  identity: Data:

604  1 1369mask  ------------------------------------------------------------------------11111111-1111111111111111111  1369mask
604  2 MNELEIDY  GU--UYGAAucgcA--uggcCCU-----------------------------------CGUgccggcga-UGUUucaUUC-gAguUUcugCccuAUCAac  MNELEIDY
604  3 DIAGRAND  UG--GCGAAucgcA--ugguCUU------------------------------------GUgccggcga-UGAAucaUUC-aaauUUcugCccuAUCAac  DIAGRAND
604  4 ATHBOMBA  UC--UCGAAucgcA--uggcCUU------------------------------------GCgccggcga-UGCUucaUUC-aaauAUcugCccuAUCAac  ATHBOMBA
604  5 BLAEMERS  UU--GCGGAucgcA--uagcCUUU-----------------------------------GUgcuggcga-CGGgucaUUC-aaauUUcugCccuAUCAac  BLAEMERS
604  6 CHLREINH  UC--ACGAAucguA--ugggCUC------------------------------------GUcccgacga-UGuUucaUUC-aaauUUcugCccuAUCAac  CHLREINH
604  7 ORYSATIV  CG--ACGGAucgcA--cggcCCU-----------------------------------CGUgccggcga-CGcaucaUUc-aaauUUcugCccuAUCAac  ORYSATIV
604  8 PORAERUG  UU--UCGGAucgca----GC------------------------------------------AAugcga-CGuUucaUUC-aaauUUcugCccuAUCAac  PORAERUG
604  9 ACACAST1  CUU-UCGGAucgcA--UUCAuguccucCUU-GU-------------------------ggggacggcga-CGauUcaUUC-aaauUUcugCccuAUCAac  ACACAST1
604 10 PHRBALAM  AA--GCUGAucgcu--uucUA--------------------------------------ACgagggcga-CAcacugUUc-gaauUCcugAccuAUCAac  PHRBALAM
604 11 STYALSID  UU--UCGGAucgcA--uggc------------------------------------UUUUUgucggcga-CGAaucaUUC-aaauUUcugCccuAUCAac  STYALSID
604 12 EMIHUXLE  GC--UCGAAucgcA--cggc------------------------------------UCUACgccggcga-UGGUucaUUC-aaauUUcugCccuAUCAgc  EMIHUXLE
604 13 OCHDANIC  UU---CGGAucgau-----cuU--------------------------------------Cgg-aucga-UGcaucaUUC-aaguUUcugCccuAUCAgc  OCHDANIC
604 14 CAFROENB  GA--GCGAAccucG---------------------------------------------AU---Agagg-UGGUucaUUC-aaguUUcugCccuAUCAgc  CAFROENB
604 15 LABMINUT  AA--GCGAAucgcAG-uggcUUC-------------------------------------Ggccggcga-UGAaucaUUC-aaguUUcugCccuAUCAgc  LABMINUT
604 16 OXYGRANU  GA--UCGAAucgcAu-ggacUUU--------------------------------------guccgcga-UACaucaUUC-aaguUUcugCcccAUCAgc  OXYGRANU
604 17 BLEAMERI  UA--GCGAAcucgA--------------------------------------------------cgUag-UGAagcaUUC-aaguUUcugCccuAUCAgc  BLEAMERI
604 18 HARVERMI  GA--UCGGAucgu---cggcCCC--------------------------------------gccgacga-CgauGcaUAC-gAguUUcugCccuAUCAac  HARVERMI
604 19 THEANNUL  AU--GCGAaucgu-----acU----------------------------------------CUguacga-uGUaucaUUC-aaguUUcugAccuAUCAgc  THEANNUL
604 20 SYMPILO1  CG--AUGAAucguGu--ggcUU--------------------------------------Ggccgacga-UGcaucUUUC-aaguUUcugAccuAUCAgc  SYMPILO1
604 21 ZBBSR205  UA--GCGGAucgcUa-cGACUUC--------------------------------------GguCgcga-CGCUucaUUC-AaauUUcugCccuAUCAgc  ZBBSR205
604 22 ZBBSR206  UA--GCGGAucgcUA-cGGCUUC--------------------------------------GgCCgcga-CGCUucaUUC-AaauUUcugCccuAUCAgc  ZBBSR206
604 23 ZBBSR235  UA--GCGGAucgcUa-cGACUUC--------------------------------------GguCgcga-CGCUucaUUC-AaauUUcugCccuAUCAgc  ZBBSR235
604 24 ZBBSR218  UA--GCGGAucCACA--gccUUC--------------------------------------GggcGCga-CGCUucaUUC-AaauUUcugCccuAUCAgc  ZBBSR218
604 25 ZBBSR217  UA--GCGGAucCACA--gccUUC--------------------------------------GggcGCga-CGCUucaUUC-GaauUUcugCccuAUCAgc  ZBBSR217
604 26 ZBBSR211  UA--GCGGAucCAua--gccUUC--------------------------------------Gggcgaga-CGCUucaUUC-AaauUUcugCccuAUCAgc  ZBBSR211
604 27 Pchroma   UU--UCGGAucgcA--cggcCUCGC------------------------------------gcuggcga-CAGUucaUUC-aaauUUcugCccuAUCAgc  Pchroma
604 28 EUGROTUN  GA--UCGAAucgcA--uggcCUC------------------------------------GUgcuggcga-UAGUucaUUC-aaauUUcugCccuAUCAgc  EUGROTUN
604 29 ENTOGING  UUU-ACA-Auugua--GaaaugAAAUA--------------------------------cauuuGacaa-GGaaucaaUgaaaAuAUcugAucuAUCAac  ENTOGING
604 30 DICDISCO  UU--GCAGAucgag--gauUU-------------------------------------aucuucgaCAa--gucu-a-CU-GUguCAcugCccuAUCAac  DICDISCO
604 31 ZTnucl    ------UGGuuacA----CUAC----------------------------------------AGAguga-uaguucuaUU-UAguGAcugAcccAUCAgu  ZTnucl
604 32 W10Z79    ------UGGuuacA----CUAC----------------------------------------AGAguga-uaguucuaUU-UAguGAcugAcccAUCAgu  W10Z79
604 33 W10Z74    ------UGGuuacA----CUAC----------------------------------------AGAguga-uaguucuaUU-UAguGAcugAcccAUCAgu  W10Z74
604 34 W10Z72    ------UGGuuacA----CUAC----------------------------------------AGAguga-uaguucuaUU-UAguGAcugAcccAUCAgu  W10Z72
604 35 W10Z10    ------UGGuuacA----CUAC----------------------------------------AGAguga-uaguucuaUU-UAguGAcugAcccAUCAgu  W10Z10
604 36 ZBBSR7    UU----GUAucgcAU-UUUAAC----------------------------------------AUUgcga-CuaauCauuU-uugaaucugAccuAUCAgu  ZBBSR7
604 37 ZBBSR173  ----ACCGAuugcU----UUAC-----------------------------------------AAgcgg-CAauucaUUA-aaauUUcugAccuAUCAgu  ZBBSR173
604 38 Siphcyan  ----ACAGAuugcU----GUAC----------------------------------------UA-gugg-CAauucaUUU-aAguUUcugAccuAUCAgu  Siphcyan
604 39 ZBBSR2    ----GGAGAAcgcU----UUAA----------------------------------------UACgugA-CAaaucaUuC-aAguUUcugAccuAUCAgu  ZBBSR2
604 40 ZCR4      ----GGAGAAcgcUU---CGAU-----------------------------------------AUgcgG-CAgauC-uucaCgguUUcugAccuAUCAgu  ZCR4
604 41 ZCR16     ------UUGucacaU---CACGGUAU-------------------------------------ACguggUUAg-ucaUUc-gAauUUcugAccuAUCAgu  ZCR16
604 42 ZCR6A     ACUAACAGAuugcU----UUAC-----------------------------------------AAgugg-CAauucaUUA-AaauUUcugAccuAUCAgu  ZCR6A
604 43 PHYPOLYC  GG--UUA-Uccgcuu-CgaaaGCuucgGUGAGUA-----------------ACGGcggauuucUgggugg-CUCUCGCugu-GugcUUcugAccuAUCAac  PHYPOLYC
604 44 NAEGRUBE  CCU-UCGGGCGCGGGAAAGUGaauUaaCAAGGUUUUCaUaaggcCUUUCAGGUUUgcuuuUuCUAGUGG-ccaggcAGag-gaguUUcuUAccuAUCAgc  NAEGRUBE
604 45 euk heli              1--3                                                  13            9       8  14     15  euk heli
604 46 rad               --------->                                            <------ <-------------- <--- --->   --  rad
```

```
"Sept4aln" on  5-SEP-96 15:33:41

Posi-  Sequence
tion:  identity:  Data:

705  1 1369mask  1111111111111111111111-111111111111111111-1111111111111111111111-1111111111111111111111111111111111  1369mask
705  2 MNELEIDY  uUUCGaugguaagGUAUUGGcuu-accaugguGACAACgggU-AAcggagaauuAGgguucGAUu-ccggagAggGagccCGAGAAACggcuAccACAuc  MNELEIDY
705  3 DIAGRAND  uUUCGaugguaagGUAGUGGcuu-accauggUUGCAACgggU-AAcggagaauuAGgguucGAUu-ccggagAggGagccUGAGAAACggcuAccACAuc  DIAGRAND
705  4 ATHBOMBA  uUUCGaugguaggAUAGAGGccu-accaugguUUCAACgggU-AAcggggaauAAGGguucGAUu-ccggagAggGagccUGAGAAACggcuAccACAuc  ATHBOMBA
705  5 BLAEMERS  uUUCGacgguaggAUAGAGGccu-accgugguGAUAACgggU-AAcggggaauaAGgguuuGAUu-ccggagAggGagccUGAGAAACggcuAccACAuc  BLAEMERS
705  6 CHLREINH  uUUCGaugguaggAUAGAGGccu-accaugguGGUAACgggU-GAcggaggauuAGgguucGAUu-ccggagAggGagccUGAGAGAUggcuAccACAuc  CHLREINH
705  7 ORYSATIV  uUUCGaugguaggAUAGGGGccu-accaugguGGUGACgggU-GAcggagaauuAGgguucGAUu-ccggagAggGagccUGAGAAACggcuAccACAuc  ORYSATIV
705  8 PORAERUG  uUUCGacgguaggGUAGUGGccu-accgugguGUUUACgggU-AAcggagaauuAGgguucGAUu-ccggagAggGagccUGAGAAAUggcuAccACAuc  PORAERUG
705  9 ACACAST1  uUUCGaugguaggAUAGAGGccu-accaugguCGUAACgggU-AAcggagaauuAGgguucGAUu-ccggagAggGagccUGAGAAAUggcuAccACUuc  ACACAST1
705 10 PHRBALAM  u--CGaugguaggAUAGUGGccu-accaugguUAUaACgggU-AAcggggaAucAGggCucGAUu-ccggagAggGagccUGAGAAACggcuAccACUuc  PHRBALAM
705 11 STYALSID  uUUCGaugguaggGUAGUGGccu-accaugguGUUCACgggU-GAcggggaauuAGgguucGAUu-ccggagAggGagccUGAGAAACggcuAccACAuc  STYALSID
705 12 EMIHUXLE  uUUCGaugguaggAUAGAGGccu-accauggcGUUAACgggU-AAcggagaauuAGgguucGAUu-ccggagAggGagccUGAGAAAUggcuAccACAuc  EMIHUXLE
705 13 OCHDANIC  uUUGGaugguaggGUAUUGGccu-accauggcAUUAACgggU-AAcggagaauuAGgguucGAUu-ccggagAggGagccUGAGAAAUggcuAccACAuc  OCHDANIC
705 14 CAFROENB  uUUCGaugguaggGUAUUGGccu-accauggcGUUAACgggU-AAcggagaauuAGgguucGAUu-ccggagAggGagccUGAGAAACggcuAccACAuc  CAFROENB
705 15 LABMINUT  uGUCGaugguaguGUAUUGGacu-accauggcGUUAACgggU-GAcggagaauuAGgguucGAUu-ccggagAggGagccUGAGAGACggcuAccACAuc  LABMINUT
705 16 OXYGRANU  uUUCGaugguaguGUAUUGGacu-accauggcUUUAACgggU-AAcggaggauuAGgguucGAUu-ccggagAggGagccUGAGAAACggcuAccACAuc  OXYGRANU
705 17 BLEAMERI  uUUCGaugguaguGUAUUGGacu-accauggcGAUGACgggU-GAcggagaauuAGgguucGAUu-ccggagAggGagccUGAGAAAUggcuAccACAuc  BLEAMERI
705 18 HARVERMI  uUUAGaugguagaGUAUCGGucu-accaugguCGUAACgggU-GAcggggrauuAGgguucGAUu-ccggagAggGagccUGAGAAACggcuAccACUuc  HARVERMI
705 19 THEANNUL  uUUGGAcgguaggGUAUUGGccu-accgGggcAACGACgggU-AAcggggaauuAGgguucGAUu-ccggagAggGagccUGAGAAACggcuAccACAuc  THEANNUL
705 20 SYMPILO1  uUCCGacgguagggUAUGGGccu-accguggcAAUGACgggU-AAcggagaauuAGgguuuGAUu-ccggagAggGagccUGAGAAACggcuAccACAuc  SYMPILO1
705 21 ZBBSR205  uUUCGaugguaguGUAUUGGacu-accauggcUUUGACgggU-AAcggagaauuAGgguucGAUu-ccggagAggGagccUGAGAAACggcuAccACAuc  ZBBSR205
705 22 ZBBSR206  uUUCGaugguaguGUAUUGGacu-accauggcUUGGACgggU-AAcggagaauuAGGGuucGAUU-ccggagAggGagccUGAGAAACggcuAccACAuc  ZBBSR206
705 23 ZBBSR235  uUUCGaugguRguGUAUUGGacu-accauggcUUUGACgggU-AAcggagaauuAGGGuucGAUU-ccggagAggGagccUGAGAAACggcuAccACAuc  ZBBSR235
705 24 ZBBSR218  uUUCGaagguaguGUAUUGGacu-accuuggcUUUGACgggU-AAcggagaauuAGGGuucGAUU-ccggagAggGagccUGAGAAACggcuAccACAuc  ZBBSR218
705 25 ZBBSR217  uUUCGaagguaguGUAUUGGacu-accuuggcUUUGACgggU-AAcggagaauuAGGGuucGAUU-ccggagAggGagccCGAGAAACggcuAccACAuc  ZBBSR217
705 26 ZBBSR211  uUUCGaagguaguGUAUUGGacu-accuuggcAUUGACgggU-AAcggagaauuAGGGuucGAUU-ccggagAggGagccUGAGAAACggcuAccACAuc  ZBBSR211
705 27 Pchroma   uUUCGacgguaguGUAGAGGacu-accguggcGUUAACgggUGCAcggagaauuAGgguucGAUu-ccggagAggGagccUGAGAAACggcuAccACAuc  Pchroma
705 28 EUGROTUN  uUUCGaugguaguGUAAAGGacu-accauggcUAUAACgggU-AAcggggaauuAGgguucGAUu-ccggagAggGagccUGAGAAAUggcuAccACAuc  EUGROTUN
705 29 ENTOGING  uA--GuugguaguAUAGAGGacu-accaagguUAUAACggaU-AAcgaGaaauuAGgguuuGACA-ucggagAagGagcuUUCAAAAUggcuAcuACUuc  ENTOGING
705 30 DICDISCO  uUUCGaugguaCgGUAUUGGcCu-accaugguUGUAACgggU-AAcggggaauuAGgguucGAUu-ccggagAggGagccUGAGAAAUggcuAccACUuc  DICDISCO
705 31 ZTnucl    uG--UucUauUauGUAGUGAauU-auUgaggcUGAAACgggU-AGcggagaauuAGgguucCGUu-ccggagAaAGagccUGCGAAACggcuACuACAuc  ZTnucl
705 32 W10Z79    uG--UucUauUauGUAGUGAauU-auUgaggcUGAAACgggU-AGcggagaauuAGgguucCGUu-ccggagAaAGagccUGCGAAACggcuACuACAuc  W10Z79
705 33 W10Z74    uG--UucUauUauGUAGUGAauU-auUgaggcUGAAACgggU-AGcggagaauuAGgguucCGUu-ccggagAaAGagccUGCGAAACggcuACuACAuc  W10Z74
705 34 W10Z72    uG--UucUauUauGUAGUGAauU-auUgaggcUGAAACgggU-AGcggagaauuAGgguucCGUu-ccggagAaAGagccUGCGAAACggcuACuACAuc  W10Z72
705 35 W10Z10    uG--UucUauUauGUAGUGAauU-auUgaggcUGAAACgggU-AGcggagaauuAGgguucCGUu-ccggagAaAGagccUGCGAAACggcuACuACAuc  W10Z10
705 36 ZBBSR7    uA--GuACguaCuGUAUUGGaCu-acUGaggcGUUGACgggU-AAcAgagaauuAGgguucUGUu-cCggagAggGagcCUGAGAAAUAgcuAcuACAuc  ZBBSR7
705 37 ZBBSR173  uA--AuuagUagCGUCUUUAUcu-uuugaggcAUUUACgggU-AAcAgagaauuAGgguCcUAUu-cCggagAggGaguCUGCGAAAUAgcuAccACAuc  ZBBSR173
705 38 Siphcyan  uA--AuuaguugCGUCUUGUAca-auugaggcAUUAACgggU-AAcAgagaauuAGgguucUGUu-cCggagAggGaguCUGAGAAAUAgcuGccACAuc  Siphcyan
705 39 ZBBSR2    uA--GuAaguaguGUAUUGGacu-acuGaggcAUUGACgggU-AAcAgagaauuAGgguucUGUu-cCggagAggGagcCUGAGGAACAgcuAcuACAuc  ZBBSR2
705 40 ZCR4      uA--GucaguaguGUACUGGacu-acugaagcGUUGACgggU-AAcAgagaauuAGgguucUGUu-cCggagAagGagcCUGAGAAACAgcuAcuAUAuc  ZCR4
705 41 ZCR16     uA--GuGagUaguAUAUAGGacu-UuuGaggcAUAUACaggU-AAcggagaauuAGgguucCAAu-ccggagAagGagcCUGCGAGACAgcuAcuACAuc  ZCR16
705 42 ZCR6A     uA--AuuagUUgCGUCUUGAUcU-UuugaggcAUUCACgggU-AAcAgagaauuAGgguCcUGUu-cCggagAggGaguCUGCGAAAUAgcuAccACAuc  ZCR6A
705 43 PHYPOLYC  uA--GauggcagCGUAACGGAcAugccaugguAACAACgggU--AcagaggauAAGGguucGAUc-cuggagAgugggcCUGAGAGAUUgcucacACUuc  PHYPOLYC
705 44 NAEGRUBE  uC--GuUguuuguUUAAAGGaca-aacCAggcUUUGACgggU--AcggggaauCAGUguucGAUu-ccggagAggGagcCUGAGAAAUCgcuAccACAuc  NAEGRUBE
705 45 euk heli          16               16   15       14      17                16    7   18            18       19  euk heli
705 46 rad       ->   ------->       <--------<--      <--    -------->   <---   -----<-- ------>        <------   --  rad
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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

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806    2 MNELEIDY   cAAggaAGGCAgcaggcGCGCAAauuaccC-AAucccGAUUC---ggggA-gguaguGACAAUAAAUAAcguugcag-gCgcc---------------AA  MNELEIDY
806    3 DIAGRAND   cAAggaAGGCAgcaggcGCGCAAauuaccC-AAuccuGACAC---agggA-gguaguGACAAUAAAUAAcaauacag-ggccu---------------UU  DIAGRAND
806    4 ATHBOMBA   cAAggaAGGCAgcaggcGCGCAAauuaccC-AAucccGACAC---ggggA-gguaguGACAAUAAAUAAcaauauag-ggcucUU--------------U  ATHBOMBA
806    5 BLAEMERS   cAAggaAGGCAgcaggcGCGCAAauuaccC-AAuccuGAUAC---agggA-gguaguGACAAUAAAUAAcgaugcgg-ggccUU---------------U  BLAEMERS
806    6 CHLREINH   cAAggaAGGCAgcaggcGCGCAAauuaccC-AAucccGACAC---ggggA-gguaguGACAAUAAAUAAcaauaccg-ggcgcU---------------U  CHLREINH
806    7 ORYSATIV   cAAggaAGGCAgcaggcGCGCAAauuaccC-AAuccuGACAC---ggggA-gguaguGACAAUAAAUAAcaauaccg-ggcgcUU--------------U  ORYSATIV
806    8 PORAERUG   cAAggaAGGYAgcaggcGCGCAAauuaccC-AAuccuGACGC---agggA-gguaguGACAAGAAAUAAcaauggGA-gaccUU---------------U  PORAERUG
806    9 ACACAST1   uAAggaAGGCAgcaggcGCGCAAauuaccC-AAucccGACAC---ggggA-gguaguGACAAUAAAUAAcaauacaggCgcucGAU-------------A  ACACAST1
806   10 PHRBALAM   cAAggaAGGCAgcaggcGCGUAAauuacuc-cCucgcGACAG---cggAgggguaguCACGACAAAUACcaaGgaa--aaccgccUUU-----------g  PHRBALAM
806   11 STYALSID   cAAggaAGGCAgcaggcGCGCAAauuaccC-AAuccuGACUC---agggA-gguaguGACAAAAAAUAAcgaacgga-GgcuuU---------------U  STYALSID
806   12 EMIHUXLE   cAAggaAGGCAgcaggcGCGUAAauugccCGAAuccuGACAC---agggA-gguaguGACAAGAAAUAAcaauacag-ggcuaU---------------U  EMIHUXLE
806   13 OCHDANIC   cAAggaAGGCAgcaggcGCGUAAauuaccC-AAuccuGACAC---agggA-gguaguGUCAAUAAAUAAcaauguug-ggccuUC---------------  OCHDANIC
806   14 CAFROENB   cAAggaAGGCAgcaggcGCGUAAauuaccC-AAuccuAAAGC---agggA-gguaguGACAAGAAAUACcgaCgggu-gacUU-----------------  CAFROENB
806   15 LABMINUT   cAAggaAGGCAgcaggcGCGUAAauuaccC-AAuccuGAUAC---ggggA-gguaguGACAAUAAAUAAcaauacgg-ggcccA---------------U  LABMINUT
806   16 OXYGRANU   uAAggaAGGCAgcaggcGCGUAAauuaccC-AAuccuGACUC---agggA-gguaguGACAAGAAAUAAcggAccga-agcC------------------  OXYGRANU
806   17 BLEAMERI   uAAggaAGGCAgcaggcGCGCAAauuaccC-AAuccuAACUC---agggA-gguaguGACAAGAAAUAAcaacgcgg-ggc-------------------  BLEAMERI
806   18 HARVERMI   cAAggaAGGCAgcaggcGCGCAAauuaccC-AAucccGACAC---ggggA-gguaguGACAAGAAAUACcauuguaaUgucuccu-------------cG  HARVERMI
806   19 THEANNUL   uAAggaAGGCAgcaggcGCGCAAauuaccC-AAuccuGACAC---agggA-gguaguGACAAGAAAUAAcaauacgg-ggcuU-----------------  THEANNUL
806   20 SYMPILO1   uAAggaAGGCAgcaggcGCGCAAauuaccC-AAuccuGACAC---agggA-gguaguGACAAGAAAUAAcaauacag-ggcau----------------C  SYMPILO1
806   21 ZBBSR205   uAAggaAGGCAgcaggcGCGCAAauuaccC-AAucccAACGC---ggggA-gguaguGACAAGAAAUAAcaacaacu-aaccu-------------AUUU  ZBBSR205
806   22 ZBBSR206   uAAggaAGGCAgcaggcGCGCAAauuaccC-AAucccAACGC---ggggA-gguaguGACAAGAAAUAAcaacaacu-aaccu-------------AUUU  ZBBSR206
806   23 ZBBSR235   uAAggaAGGCAgcaggcGCGCAAauuaccC-AAucccAACGC---ggggA-gguaguGACAAGAAAUAAcaacaacu-aaccu-------------AUUU  ZBBSR235
806   24 ZBBSR218   uAAggaAGGCAgcaggcGCGCAAauuaccC-AAuccuAACACU--agggA-gguaguGACAAGAAAUAAcaacagcu-aacuu-------------AAUA  ZBBSR218
806   25 ZBBSR217   uAAggaAGGCAgcaggcGCGCAAauuaccC-AAuccuAACACU--agggA-gguaguGACAAGAAAUAAcaacagcu-aacuu-------------AAUA  ZBBSR217
806   26 ZBBSR211   uAAggaAGGCAgcaggcGCGCAAauuaccC-AAuccuAACACAUAagggA-gguaguGACAAGAAAUAAcaacagcu-aacuu-------------AGUU  ZBBSR211
806   27 Pchroma    cAAggaAGGCAgcaggcGCGCAAauuaccC-AAuCcuGACAC---agUgA-gguaguGACUAGAAAUAAccauaccg-ggcuUUUA--------------  Pchroma
806   28 EUGROTUN   cAAggaAGGCAgcaggcGCGCAAauuaccC-AAuccuGACAC---agggA-gguaguGACAAGAAAUAAcgauaccg-ggccUUA--------------A  EUGROTUN
806   29 ENTOGING   uAAggaAGGCAgcaggcGCGUAAauuaccC-ACuuuuAACAG---aaagA-gguaguGACGACAAAUAAcucUauuc-uuuAAC----------------  ENTOGING
806   30 DICDISCO   uACggaAGGCAgcaggcGCGCAAauuacuC-AAucccAAUAC---ggggA-aguaguGACAAUAAAUAUcaauAccu-AuccUU---------------U  DICDISCO
806   31 ZTnucl     uAAggaAGGCAgcaggcGNGUAAauuaUUC-AAuucuAAAUC---agagA-GUuaguAACAAUAUAUUACgauguuA-aaccU----------------U  ZTnucl
806   32 W10Z79     uAAggaAGGCAgcaggcGCGUAAauuaUUC-AAuucuAAAUC---agagA-GUuaguAACAAUAUAUUACgauguuA-aaccU----------------U  W10Z79
806   33 W10Z74     uAAggaAGGCAgcaggcGCGUAAauuaUUC-AAuucuAAAUC---agagA-GUuaguAACAAUAUAUUACgauguuA-aaccU----------------U  W10Z74
806   34 W10Z72     uAAggaAGGCAgcaggcGCGUAAauuaCUC-AAuucuAAAUC---agagA-GUuaguAACAAUAUAUUACgauguuA-aaccU----------------U  W10Z72
806   35 W10Z10     uAAggaAGGCAgcaggcGCGUAAauuaUUC-AAuucuAAAUC---agagA-GUuaguAACAAUAUAUUACgauguuA-aaccU----------------U  W10Z10
806   36 ZBBSR7     uAAggaAGGCAgcaggcGCGUAAauuaccC-AAuuuuGAUAA---agagA-gguaguGACAAGAAAUCAcaauauuu-UUccU----------------U  ZBBSR7
806   37 ZBBSR173   uAAggaAGGCAgcaggcACGUAAauuaccC-AAuuUuGAUUA---gUagA-gguaguGACAAUAUAUCAuaauAUuu-ugccU----------------A  ZBBSR173
806   38 Siphcyan   uAAggaAGGCAgcaggcGCGAAAauuaccC-AAuuUuGAUGA---aUagA-gguaguGACAAGAAAUCAcaauauuA-uggcA----------------U  Siphcyan
806   39 ZBBSR2     uAAggaAGGCAgcaggcGCGUAAauuaccC-AAuucuGAUAC---agagA-gguaguGACAAGAAAUCAcaauAUuu-ugCcu----------------U  ZBBSR2
806   40 ZCR4       uAAggaACGCAgcaggcGCGUAAauuaccC-AAuucuGAUAA---agagA-gguaguGAUAAGAAAUCAcaauauuu-UaccU----------------U  ZCR4
806   41 ZCR16      uAAggaAGGCAgcaggcGCGUAAauuaccC-AUuucgGAUCA---cgagA-gguaguGACAAGAAAUCAcaaCAUuu-ugccU----------------U  ZCR16
806   42 ZCR6A      uAAggaAGGCAgcaggcACGUAAauuaccC-AAuuUuGAUUA---gUagA-gguaguGACAAUAUAUCAuaauauau-UgccG----------------A  ZCR6A
806   43 PHYPOLYC   uAAggaAGGCAgcaggcGCGCAAcguucCC-AUugggCAAAG---cucgA-gggcgUUAGGGGACAUAUgaaugccu-gccUU-----------------  PHYPOLYC
806   44 NAEGRUBE   uAAggaCGGCAgcaggcGCGCAAauuaccC-AAucucAAUAC---gaggA-gguaguGACAAGCUAUAGUgaCucCAcaCCAUUCGGUGGGGAGGUAUUG  NAEGRUBE
806   45 euk heli      19        5              20                   2-0                        2-1                       euk heli
806   46 rad        -><---     <-----      ------    --->        <---  ------            --------------->                  rad
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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:  Data:

 906   1 1369mask  -----11111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111  1369mask
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 906   3 DIAGRAND  UagguuuuguaAuugGAAugagUACAAUCUAAAUCUCUUAACGAGUAACAauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  DIAGRAND
 906   4 ATHBOMBA  CgggucuuauaAuugGAAugagUACAAUUUAAAUCCCUUAACGAGGAACAauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  ATHBOMBA
 906   5 BLAEMERS  UggccAucgcaAuugGAAuGagUACAAUUUAAAUCCCUUAACGAGGAACAauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  BLAEMERS
 906   6 CHLREINH  CgcgucugguaAuugGAAugagUACAAUCUAAAUCCCUUAACGAGGAUCCauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  CHLREINH
 906   7 ORYSATIV  AgugucugguaAuugGAAugagUACAAUCUAAAUCCCUUAACGAGGAUCCauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  ORYSATIV
 906   8 PORAERUG  UgguuG-AccaCuugGAAygagUACAUUUUAAAUCACUUAACGAGUAUCCAuuggagGGCAAGWcuggUgCCAGCAGCCGCGGUAAuUccagcuccaauA  PORAERUG
 906   9 ACACAST1  AgagucuuguaAuugGAAugagUACAAUUUAAACCCCUUAACGAGUAACAauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  ACACAST1
 906  10 PHRBALAM  gugguuu-ucCAuugGAAu-agcAGAAUUCAAACCCCUCUGCAAGUAACAAuuggagGGCAAGUcuggUgCCAGCaGCCGCGGUAAuUccagcuccaaGA  PHRBALAM
 906  11 STYALSID  CaaguGuccguCuugGAAuGagAACAAUUUAAAUCCCUUAUCGAGUAACCAcuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccagUa  STYALSID
 906  12 EMIHUXLE  UuagucuuguaAuugGAAugagUACAAUUUACAUCUCUUCACGAGGAUCAauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  EMIHUXLE
 906  13 OCHDANIC  -gggucugacaAuugGAAugagAACAAUUUAAAUCCCUUAUCGAGGAUCAauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  OCHDANIC
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 906  15 LABMINUT  CgggucuuguaAuugGAAugagUACAAUCUAAAUCCCUUAACGAGGAUCAauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  LABMINUT
 906  16 OXYGRANU  UAUguuucggGAuugCAAugagUAGAAUUUAAACCCCUUUACGAGGAUCAauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  OXYGRANU
 906  17 BLEAMERI  UUUgucuugcgAuugGAAugagUUAAGUGUAAAAGCCUUU-CGAGGACCCacuggagGGCAAGUcu-gUgCCAGCAGCCGCGGUAAuUcCagcuccagua  BLEAMERI
 906  18 HARVERMI  AgaggcuuacgaUugGAAugagUACAAUUUAAAUCCCUUAACGAGUAACGauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  HARVERMI
 906  19 THEANNUL  AAagucuuguaAuugGAAugaUGGGAAUUUAAACCUCUUCCAGAGUAUCAauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  THEANNUL
 906  20 SYMPILO1  CaugucuuguaAuugGAAugagUAGAAUUUAAACCCCUUUAUGAGUAUCAauugUagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuCcaaua  SYMPILO1
 906  21 ZBBSR205  CaggugaguugAuugGAAugagUACAAUCUAAACACCUUAACGACUAUCCauuggagGGCAAGCcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  ZBBSR205
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 906  23 ZBBSR235  CaggugaguugAuugGAAugagUACAAUCUAAACACCUUAACGACUAUCCauuggagGGCAAGCcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  ZBBSR235
 906  24 ZBBSR218  CaagugaguugAuugGAAugagUACAAUCUAAACACCUUAACGA-ACUCCauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  ZBBSR218
 906  25 ZBBSR217  CaagugaguugAuugGAAugagUACAAUCUAAACACCUUAACGA-ACUCCauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  ZBBSR217
 906  26 ZBBSR211  UaagugaguugAuugGAAugagUACAAUCUAAACACCUUAACGA-AUUCCauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaua  ZBBSR211
 906  27 Pchroma   -UggucugguAauggGAAugagUGUAAUUUAAAUCCCUAUGCGAGGAUCCacuggagGGCAAGUcuggUgCCAGCAGCCGCG-UAAuUccagcuccagua  Pchroma
 906  28 EUGROTUN  UCggucugguaAucgGAAugagUACAAUUUAAAUCCCUUAACGAGGAUCCacuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccagua  EUGROTUN
 906  29 ENTOGING  AAaaagaauUgA-agGAAugaACGGAACGUACAUAGUUUUGUGA-AAGCAAuuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaaUa  ENTOGING
 906  30 DICDISCO  UUgg-agggCaAuugAAAugaACACAAAUUAAAACUCUUAAUUA-ACACAauugragGGCAAGUcuggUgCCAGCRGCCGCGGUAAuUccagcuccaaua  DICDISCO
 906  31 ZTnucl    AGgguuAaauUauuAUAUugaGGAUAGAUUAUUUAUUUAUACGAUUGACUauAggagGGCAAGUcuggugCCAGCAGCCGCGGUAAuaccagcuccAauA  ZTnucl
 906  32 W10Z79    AGgguuAaauUauuAUAUugaGGAUAGAUUAUUUAUUUAUACGAUUGACUauAggagGGCAAGUcuggugCCAGCAGCCGCGGUAAuaccagcuccAauA  W10Z79
 906  33 W10Z74    AGgguuAaauUauuAUAUugaGGAUAGAUUAUUUAUAUAUACGAUUGACUauAggagGGCAAGUcuggugCCAGCAGCCGCGGUAAuaccagcuccAauA  W10Z74
 906  34 W10Z72    AGgguuAaauUauuAUAUugaGGAUAGAUUAUUUAUUUAUACGAUUGACUauAggagGGCAAGUcuggugCCAGCAGCCGCGGUAAuaccagcuccAauA  W10Z72
 906  35 W10Z10    AGgguuAaauUauuAUAUugaGGAUAGAUUAUUUAUUUAUACGAUUGACUauAggagGGCAAGUcuggugCCAGCAGCCGCGGUAAuaccagcuccAauA  W10Z10
 906  36 ZBBSR7    AGggUUaaauAauugUAUugaGAACAGUGUACCAAUUUUACCGAUAAACUauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuccaauA  ZBBSR7
 906  37 ZBBSR173  UUgguUaUgUAauugUAUUgaGAACAAUAUAAAUAUAUUAUCUAUAAGUUAGuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuucaAAA  ZBBSR173
 906  38 Siphcyan  AUGgcuaaauAauugUAUugaAAAAMAUGUAGAAAUUUUAUCUAUAAGCUaGuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcucuaAuA  Siphcyan
 906  39 ZBBSR2    AGggUuaagUAauugUAUugaGAACAGAGUAGCCAUUUUAUCGAUAAACUauuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcucuaauG  ZBBSR2
 906  40 ZCR4      AGgguUaaguAauugAAUugaGAACACAGUAGCCAUAUUACCAAUAAACUauuggagGGCAAGUcuggUgCCAGCAGCUGCGGUAAuUccagcuccaauG  ZCR4
 906  41 ZCR16     AAAgguaaaUAUuugUACugaGAACAAAUUAGAUAUUUUAUCGAUAAACAauuagagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcucuaauG  ZCR16
 906  42 ZCR6A     UUgguUauguAauugUAUugaGAACAAUGUAAAUAUCUUAUCUAUAAGUUAGuggagGGCAAGUcuggUgCCAGCAGCCGCGGUAAuUccagcuucaAAA  ZCR6A
 906  43 PHYPOLYC  AU-ggugggcaAuucAAAugggACUGUUUUAAACAUCCUAUCGAGUAACAauuagagGACAAGUcuggUgCCAGCACCCGCGGUAAuUccagcucuaaua  PHYPOLYC
 906  44 NAEGRUBE  UCUUCugACgaUuuUCCAugaUUUGGGUGUAGAUAACCCUUAGAGUAGCCAuuggagGAAAAGUcuggUgCCAGCACCCGCGGUAAuUccagcuccaaGa  NAEGRUBE
 906  45 euk heli         21          4                                22           23                    23    22       euk heli
 906  46 rad        <------------   <---                             ------>       ---- ->               <----<------ <  rad
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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

1007   1 1369mask   1111111111111111111111111111111111111111111--------------------------------------------------------- 1369mask
1007   2 MNELEIDY   gcguauauuAAAGUUGuuGcAgUUAAAAAgCucGUAGUUGGAUUUCGGAA-------------------------------------------------- MNELEIDY
1007   3 DIAGRAND   gcguauauuAAAGUUGuuGcagUUAAAAAgCucGUAGUUGGAUUUCUGUU-------------------------------------------------- DIAGRAND
1007   4 ATHBOMBA   gcguauauuAAAGUUGuuGcagUUAAAAAgCucGUAGUUGAACUUCAGGC-------------------------------------------------- ATHBOMBA
1007   5 BLAEMERS   gcguauauuAAAGUUGuuGcagUUAAAAAgCucGUAGUUGAAUUUCAGGG-------------------------------------------------- BLAEMERS
1007   6 CHLREINH   gcguauauuUAAGUUGuuGcAgUUAAAAAgCucGUAGUUGGAUUUCGGGU-------------------------------------------------- CHLREINH
1007   7 ORYSATIV   gcguauauuUAAGUUGuuGcAgUUAAAAAgCucGUAGUUGGACCUUGGGC-------------------------------------------------- ORYSATIV
1007   8 PORAERUG   gcguauauaAAAGUUGuuGcagUUAAAAAgCucGUAGUCGGAGAUGGGGC-------------------------------------------------- PORAERUG
1007   9 ACACAST1   gcguauauuAAAGUUGuuGcAgUUAAAAAgCucGUAGUUGGAUCUAGGGA-------------------------------------------------- ACACAST1
1007  10 PHRBALAM   guguAuacuaAAGCUGuuGcg-UUAAAACgCucGUAGUUGAAUUCCAACACCCGGUUUCAAGUUGAAUGGAAAGAAGGGUAUAUUAGUAUUUAGAGGGAG PHRBALAM
1007  11 STYALSID   gcguauauuAAAGUUGuuGcagUUAAAAAgCucGUAGUCGAACCUCGGGC-------------------------------------------------- STYALSID
1007  12 EMIHUXLE   gcguauauuAAAGUUGuuGcAgUUAAAACgCucGUAGUCGGAUUUCGGGG-------------------------------------------------- EMIHUXLE
1007  13 OCHDANIC   gcguauAcuAAAGUUGuuGcAgUUAAAAAgCucGUAGUUGAAAUUCUGAU-------------------------------------------------- OCHDANIC
1007  14 CAFROENB   gcguauauuAAAGUUGuuGcAgUUAAAAAgCucGUAGUUGAAUUUCUGRR-------------------------------------------------- CAFROENB
1007  15 LABMINUT   gcguauauuAAAGUUGuuGcAgUUAAAAAgCucGUAGUUGGAUUUCUGGU-------------------------------------------------- LABMINUT
1007  16 OXYGRANU   gcguauauuAAAGUUGuuGcagUUAAAAAgCucGUAGUUGGAUUUCUGGG-------------------------------------------------- OXYGRANU
1007  17 BLEAMERI   gcguauauuAAAGUUGuuGcagUUAAAAAgCucGUAGUUGAAGCUCUGCG-------------------------------------------------- BLEAMERI
1007  18 HARVERMI   gcguauAuuAAAGUUGuuGcagUUAAAAAgCucGUAGUUGGAUUUCG-GA-------------------------------------------------- HARVERMI
1007  19 THEANNUL   gcguauauuAAAAUUGuuGcagUUAAAAAgCucGUAGUUGAAUUUCUGC--------------------------------------------------- THEANNUL
1007  20 SYMPILO1   gcguauauuAAAGUUGuuGcggUUAAAAAgCucGUAGUUGGAUUUCUGUUG------------------------------------------------- SYMPILO1
1007  21 ZBBSR205   gcguauacuAAUGUUGuuGcAgUUAAAAAgCucGUAGUCGGAUUUCAGU--------------------------------------------------- ZBBSR205
1007  22 ZBBSR206   gcguauacuAAUGUUGuuGcAgUUAAAAAgCucGUAGUCGGAUUUCAGU--------------------------------------------------- ZBBSR206
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1007  24 ZBBSR218   gcguauacuAAUGUUGuuGcAgUUAAAAAgCucGUAGUCGGAUUUCAGU--------------------------------------------------- ZBBSR218
1007  25 ZBBSR217   gcguauacuAAUGUUGuuGcAgUUAAAAAgCucGUAGUCGGAUUUCAGU--------------------------------------------------- ZBBSR217
1007  26 ZBBSR211   gcguauacuAAUGUUGuuGcAgUUAAAAAgCucGUAGUCGGAUUUCAGU--------------------------------------------------- ZBBSR211
1007  27 Pchroma    gcguauauuAAAAUUGuuGcAgUUAAAANgCucGUAGUUGGAUUUCUGG--------------------------------------------------- Pchroma
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1007  29 ENTOGING   guauauAUUAAAGUUGuuGugaUUAAAAGgCucGUAGUUGAAUGAAGAUACUA----------------------------------------------- ENTOGING
1007  30 DICDISCO   gcauauacuAAAGUUGuuGcAgUUAAAAAgCucGUAGUUGAAGUUUAAGG-------------------------------------------------- DICDISCO
1007  31 ZTnucl     guguaugcuAACGUUGuuGcAgUUACAAAgCucGUAGUUGGUCUAUUAUGA------------------------------------------------- ZTnucl
1007  32 W10Z79     guguaugcuAACGUUGuuGcAgUUACAAAgCucGUAGUUGGUCUAUUAUGA------------------------------------------------- W10Z79
1007  33 W10Z74     guguaugcuAACGUUGuuGcAgUUACAAAgCucGUAGUUGGUCUAUUAUGA------------------------------------------------- W10Z74
1007  34 W10Z72     guguaugcuAACGUUGuuGcAgUUACAAAgCucGUAGUUGGUCUAUUAUGA------------------------------------------------- W10Z72
1007  35 W10Z10     guguaugcuAACGUUGuuGcAgUUACAAAgCucGUAGUUGGUCUAUUAUGA------------------------------------------------- W10Z10
1007  36 ZBBSR7     guguaugcuAACAUUGuuGcAgUUAAAAAgCucGUAGUCGAAUUUUUAAGG------------------------------------------------- ZBBSR7
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1007  38 Siphcyan   gUUuaugcuAACAAUGuuGcAAUUAAAAAgCucAUAGUCGAAUAUUUCAGA------------------------------------------------- Siphcyan
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1007  41 ZCR16      guguaugcuAACAUUGuuGcAgUUAAAAAgCucCUAGUCGAAUAUGUUAGG------------------------------------------------- ZCR16
1007  42 ZCR6A      guauauguuAACAMUGcuGcAAUUAAAAAgUucAUAGUCGAACUUUUCAGA------------------------------------------------- ZCR6A
1007  43 PHYPOLYC   gcauacguuAAAGUUGuuGcggUUAAAACgcucGUAGUCGGCUCCAGACCUUCAGAGCCUUGAU------------------------------------ PHYPOLYC
1007  44 NAEGRUBE   gcguauauuAAUACUGcuGuagUUAAAACgCccGUAGUAUACCUAAGAGUGGGUGUGUAGUAA------------------------------------- NAEGRUBE
1007  45 euk heli     3              24            25                                                                      euk heli
1007  46 rad        ---------       -- -->       ---->                                                                     rad
```

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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

1107   1 1369mask  ----------------------------------------------------------------------------------------------------  1369mask
1107   2 MNELEIDY  ---------------------------------------------------------------CCGGCC-G-A-UUGGUCCG------------------  MNELEIDY
1107   3 DIAGRAND  ---------------------------------------------------------------ACUGGAGG-G-GUGGUCCGCC----------------  DIAGRAND
1107   4 ATHBOMBA  ---------------------------------------------------------------CUGGUU-G-G-GCGGUCCGCU----------------  ATHBOMBA
1107   5 BLAEMERS  -----------------------------------------------------------CUGUCUUUGU-C-A-CCUGGCCACU----------------  BLAEMERS
1107   6 CHLREINH  ---------------------------------------------------------------GGGGUG-G-U-GCGGUCCGCC----------------  CHLREINH
1107   7 ORYSATIV  ---------------------------------------------------------------GCGGCCGG-G-CCGGUCCGCC----------------  ORYSATIV
1107   8 PORAERUG  ---------------------------------------------------------------GGUG-GAS-A-UYGUGCU-----------------GG  PORAERUG
1107   9 ACACAST1  ---------------------------------------------------------------CGCGCAUU-U--CAAGCGCCC------GUGUCGUCGG  ACACAST1
1107  10 PHRBALAM  CAAUCCUUUUAAGUAUUGAUGUAUCAGUUCACCGUUUAACUCCGGGGAAGGCUAUCCACU-----CUAAUGCCUUAUUCCCGCCA---------------  PHRBALAM
1107  11 STYALSID  ---------------------------------------------------------------CCGG-GAC-A--CGGUCC------------------U  STYALSID
1107  12 EMIHUXLE  ---------------------------------------------------------------CGGG-CCG-A-CCGGUCUGC-----------------  EMIHUXLE
1107  13 OCHDANIC  ---------------------------------------------------------------UUCG-GAU-G-UCGGUCGGCCC---------------  OCHDANIC
1107  14 CAFROENB  ---------------------------------------------------------------CUcG-CCG-AGNC-GGCGGNCUU--------------  CAFROENB
1107  15 LABMINUT  ---------------------------------------------------------------AGGA-GUG-A-CCGUUCCGAACUU-------------  LABMINUT
1107  16 OXYGRANU  ---------------------------------------------------------------AGGGUGCC-A--AUGUCCGCUCGUUUG----------  OXYGRANU
1107  17 BLEAMERI  --------------------------------------------------------------GGGCGGGUC-G-CUGGGCCUCGGUCU------------  BLEAMERI
1107  18 HARVERMI  --------------------------------------------------------------AGGUCUUUA-GU--GGUCCGC-----------------  HARVERMI
1107  19 THEANNUL  ----------------------------------------------------------------------------------------------------  THEANNUL
1107  20 SYMPILO1  ---------------------------------------------------------------AGGAUGA----CCGGUCCGCCUUCU----------GG  SYMPILO1
1107  21 ZBBSR205  ---------------------------------------------------------------AGGCUCAG---UU-UAGUCUUCAU-------------  ZBBSR205
1107  22 ZBBSR206  ---------------------------------------------------------------AGGCUCAG---UU-AAGUCU-CUUCU-----------  ZBBSR206
1107  23 ZBBSR235  ---------------------------------------------------------------AGGCUCAG---UU-UAGUCUUCAU-------------  ZBBSR235
1107  24 ZBBSR218  ------------------------------------------------------------AUUAGG--------U----CCACCUACUC-----------  ZBBSR218
1107  25 ZBBSR217  ------------------------------------------------------------AUUAGG--------U----CCACCUACUC-----------  ZBBSR217
1107  26 ZBBSR211  ------------------------------------------------------------AUUG----CAGA--U--GCACACCUCUU------------  ZBBSR211
1107  27 Pchroma   --------------------------------------------------------------------UUGA--CCGGG-CAC-----------------  Pchroma
1107  28 EUGROTUN  ------------------------------------------------------------GAACCAAAUUGG-CCCGUUCC-C-U-UU------------  EUGROTUN
1107  29 ENTOGING  ----------------------------------------------------------------------------------------------------  ENTOGING
1107  30 DICDISCO  ----------------------------------------------------------------UUUA------CCGGG-----UUUAUGU---CAUU--  DICDISCO
1107  31 ZTnucl    ----------------------------------------------------------------------------------------------------  ZTnucl
1107  32 W10Z79    ----------------------------------------------------------------------------------------------------  W10Z79
1107  33 W10Z74    ----------------------------------------------------------------------------------------------------  W10Z74
1107  34 W10Z72    ----------------------------------------------------------------------------------------------------  W10Z72
1107  35 W10Z10    ----------------------------------------------------------------------------------------------------  W10Z10
1107  36 ZBBSR7    ----------------------------------------------------------------------------------------------------  ZBBSR7
1107  37 ZBBSR173  ----------------------------------------------------------------------------------------------------  ZBBSR173
1107  38 Siphcyan  ----------------------------------------------------------------------------------------------------  Siphcyan
1107  39 ZBBSR2    ----------------------------------------------------------------------------------------------------  ZBBSR2
1107  40 ZCR4      ----------------------------------------------------------------------------------------------------  ZCR4
1107  41 ZCR16     ----------------------------------------------------------------------------------------------------  ZCR16
1107  42 ZCR6A     ----------------------------------------------------------------------------------------------------  ZCR6A
1107  43 PHYPOLYC  ----------------------------------------------------------------------------------------------------  PHYPOLYC
1107  44 NAEGRUBE  ----------------------------------------------------------------------------------------------------  NAEGRUBE
1107  45 euk heli                                                                                                          euk heli
1107  46 rad                                                                                                               rad
```

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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

1208   1 1369mask  ----------------------------------------------------------------------------------------------------  1369mask
1208   2 MNELEIDY  ---------UCC--UCUG-------------------------------------------------------GAUCGUG-UACUGAUC-----------  MNELEIDY
1208   3 DIAGRAND  --------------UCA--------------------------------------------------------CGGCGAG-CACUGCUA-----------  DIAGRAND
1208   4 ATHBOMBA  ------------U--AA--------------------------------------------------------CGGCGUG-UA-CUGUC-----------  ATHBOMBA
1208   5 BLAEMERS  ------------GAGCCUUURCGGCCAGUG-------------------------------------------UUGCUUU-CG-GGUGC-----------  BLAEMERS
1208   6 CHLREINH  --------------UC---------------------------------------------------------UGGUGUG-CACUGCUC-----------  CHLREINH
1208   7 ORYSATIV  --------------UCA--------------------------------------------------------CGGCAGG-CA-CCGAC-----------  ORYSATIV
1208   8 PORAERUG  GGCAACU------------------------------------------------------------------CGG--UG-YG-GUGUU-----------  PORAERUG
1208   9 ACACAST1  GUCAAACCGGCGACUG---CG-UUG--GCGUUGCGGGC--UCGGUCCGUCGGUGGACCCUCGUGGUCUUAAU-CGGCGUGUCAACCGGC-CCGCCCGUCC  ACACAST1
1208  10 PHRBALAM  ----------------------GCUCUUAAUUGAGUCUUGGAUAUAC---------------------------------------CACCCGGACCGAUA  PHRBALAM
1208  11 STYALSID  GGCAAAGU-----------------------------------------------------------------CAGCGUC-UGUGUCUU-----------  STYALSID
1208  12 EMIHUXLE  ----CGAU-----------------------------------------------------------------GGGUAUG-CACUGGCC-----------  EMIHUXLE
1208  13 OCHDANIC  --CAAG-------------------------------------------------------------------GGGUUAG-UACCGUCU-----------  OCHDANIC
1208  14 CAFROENB  -------------------------------------------------------------------------UGGNNSACCACUCCG------------  CAFROENB
1208  15 LABMINUT  ------------------------GAUUGUUCGUGUAUUGUGUUUU------------------------------------------------------  LABMINUT
1208  16 OXYGRANU  ------------------------------------CGUGUGCAGC------------------------------------------------------  OXYGRANU
1208  17 BLEAMERI  -----------GGU--------------------------------------------------------------------------------------  BLEAMERI
1208  18 HARVERMI  ----------------------------------------------------------------------------------------------------  HARVERMI
1208  19 THEANNUL  ----------------------------------------------------------------------------------------------------  THEANNUL
1208  20 SYMPILO1  GUGUGUAUCUGGCU--------------------------------------------------------------------------------------  SYMPILO1
1208  21 ZBBSR205  UGUAAGAUCUA-----------------------------------------------------------------------------------------  ZBBSR205
1208  22 ZBBSR206  AUUGAGUUCUU-----------------------------------------------------------------------------------------  ZBBSR206
1208  23 ZBBSR235  UGUAAGAUCUA-----------------------------------------------------------------------------------------  ZBBSR235
1208  24 ZBBSR218  UGUUGUAGCGU-----------------------------------------------------------------------------------------  ZBBSR218
1208  25 ZBBSR217  UGUUGUAGCGU-----------------------------------------------------------------------------------------  ZBBSR217
1208  26 ZBBSR211  CGAGGCAGC-------------------------------------------------------------------------------------------  ZBBSR211
1208  27 Pchroma   GUCGGCGUCCU-------------------------------------------------------------UCGGGCCGUGUGUGCG------------  Pchroma
1208  28 EUGROTUN  -------------------------------------------------------------------------CGGGUAUGA------------------  EUGROTUN
1208  29 ENTOGING  ----------------------------------------------------------------------------------------------------  ENTOGING
1208  30 DICDISCO  -U--ACCACUUC---------------GU-GGUUAA----------------------------------------------------------------  DICDISCO
1208  31 ZTnucl    ------------------------RUUUUAUUUAUAUAUAG-UAUA------------------------------------------------------  ZTnucl
1208  32 W10Z79    ------------------------AUUUUAUUUAUAUAUAG-UAUA------------------------------------------------------  W10Z79
1208  33 W10Z74    ------------------------AUUUUAUUUAUGUAUAG-UAUA------------------------------------------------------  W10Z74
1208  34 W10Z72    ------------------------AUUUUAUUUAUAUAUAG-UAUA------------------------------------------------------  W10Z72
1208  35 W10Z10    ------------------------AUUUUAUUUAUAUAUAG-UAUA------------------------------------------------------  W10Z10
1208  36 ZBBSR7    ------------------------GGAUCAAUUUUGUGUGG-UCUC------------------------------------------------------  ZBBSR7
1208  37 ZBBSR173  ------------------------UUAUAAUUUUUAUGUGA-UCAU------------------------------------------------------  ZBBSR173
1208  38 Siphcyan  ------------------------UAUUCAAUUUUGUAUGG-UCCU------------------------------------------------------  Siphcyan
1208  39 ZBBSR2    ------------------------GAAUCAAUUUUAUGUGG-UAUU------------------------------------------------------  ZBBSR2
1208  40 ZCR4      ------------------------AAAUCAAUUUUAUGUGA-UCCU------------------------------------------------------  ZCR4
1208  41 ZCR16     ------------------------GAAUCAUUUUUAUGUGA-UCUU------------------------------------------------------  ZCR16
1208  42 ZCR6A     ------------------------UUUUUAUUUUUAUGAGU-UCAU------------------------------------------------------  ZCR6A
1208  43 PHYPOLYC  ------------------------UCGGGACACUGGGUCAG-CUGC------------------------------------------------------  PHYPOLYC
1208  44 NAEGRUBE  ------------------------UUAGUUUUACCAGAGGACGGUUGGCGAGAGUUUAUC--------------------------------ACUCUUG-  NAEGRUBE
1208  45 euk heli                                                                                                        euk heli
1208  46 rad                                                                                                             rad
```

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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

1311   1 1369mask  ----------------------------------------------------------------------------------------------------  1369mask
1311   2 MNELEIDY  ------------------------------------------------------------------------------GGUCUGUU-CUU------CUUC  MNELEIDY
1311   3 DIAGRAND  -----------------------------------------------------------------------------UCCUCUGUGACAU------CCUC  DIAGRAND
1311   4 ATHBOMBA  -----------------------------------------------------------------------------UGACUGG-GUCUU------ACCU  ATHBOMBA
1311   5 BLAEMERS  -----------------------------------------------------------------------------GGGCCC--UUUAUUU----CUGC  BLAEMERS
1311   6 CHLREINH  -----------------------------------------------------------------------------UGCUCC--ACCUUC-----CUGC  CHLREINH
1311   7 ORYSATIV  -----------------------------------------------------------------------------CUGCUCG-ACCCUU-----CUGC  ORYSATIV
1311   8 PORAERUG  -------------------------------------------------------------------------------CCGCC--GCCUU-----CCUC  PORAERUG
1311   9 ACACAST1  C----------------------------------------CU--CCUUCUG-GAUUCCCGUUCCU-GCUAUUGAGUUAGUGGGGACGUCA-----CAGG  ACACAST1
1311  10 PHRBALAM  AGGAAUGGGGGGAAUAAGGACUAAAGGUUUACCUUGAACAAAUUAGAGUGAUUCAGGCAGGCUUUUUUAAGCCCUUGAAUACAUUAGCAUGGGACAAACA  PHRBALAM
1311  11 STYALSID  -------------------------------------------------------------------------------GGG----CCUUU-----UUCC  STYALSID
1311  12 EMIHUXLE  --------------------------------------------------------------------------------GGCGCGUCCUU----CCACC  EMIHUXLE
1311  13 OCHDANIC  -----------------------------------------------------------------------------GUUC--GGAAUCAU----CCUCG  OCHDANIC
1311  14 CAFROENB  -----------------------------------------------------------------------------CAA-----GUUCAU----C----  CAFROENB
1311  15 LABMINUT  -----------------------------------------------------------------------------CUCC----AGCCAU----CCUU-  LABMINUT
1311  16 OXYGRANU  -----------------------------------------------------------------------------GGCGCCCUU-CCAU----CCUUC  OXYGRANU
1311  17 BLEAMERI  -----------------------------------------------------------------------------GACCUUCCUCGCAU----CCAUC  BLEAMERI
1311  18 HARVERMI  -----------------------------------------------------------CCCUUCGGGGAGCGGGUCACUGGCC--UCCUA----UGUUC  HARVERMI
1311  19 THEANNUL  ------------------------------------------------------------------------UGCAUUGCUUGUGUCCCU-----CUGGG  THEANNUL
1311  20 SYMPILO1  ------------------------------------------------------------------------------------CAGCCUUGA--CAUCU  SYMPILO1
1311  21 ZBBSR205  ---------------------------------------------------------------------------------UAUGGGCCUG----CUUCU  ZBBSR205
1311  22 ZBBSR206  ---------------------------------------------------------------------------------UAUGGGCCUG----CUUCU  ZBBSR206
1311  23 ZBBSR235  ---------------------------------------------------------------------------------UAUGGGCCUG----CUUCU  ZBBSR235
1311  24 ZBBSR218  ------------------------------------------------------------------------------------GGCUCUUUA--CUUCU  ZBBSR218
1311  25 ZBBSR217  ------------------------------------------------------------------------------------GGCUCUUUA--CUUCU  ZBBSR217
1311  26 ZBBSR211  ------------------------------------------------------------------------------------ACCUCGAUA--CUUCU  ZBBSR211
1311  27 Pchroma   -----------------------------------------------------------------------------------UCCUGCGUUUGCCAUCC  Pchroma
1311  28 EUGROTUN  ----------------------------------------------------------------------GUGCCGCUUUGAGUUUGUCAU----CCUUC  EUGROTUN
1311  29 ENTOGING  ------------------------------------------------------------------------------UUGAAAAGG--------CUUUU  ENTOGING
1311  30 DICDISCO  ----------------------------------------------------------------------AUCGACACCGGUAUCUCUUU-----CUUAA  DICDISCO
1311  31 ZTnucl    --------------------------------------------------AUUGUA-CUAUUAUUGACAAUGCCU--------------------AAAUC  ZTnucl
1311  32 W10Z79    --------------------------------------------------AUUGUA-CUAUUAUUGACAAUGCCU--------------------AAAUC  W10Z79
1311  33 W10Z74    --------------------------------------------------AUUGCA-CUAUUACUGACAAUGCCU--------------------AAAUC  W10Z74
1311  34 W10Z72    --------------------------------------------------AUUGUA-CUAUUAUUGACAAUGCCU--------------------AAAUC  W10Z72
1311  35 W10Z10    --------------------------------------------------AUUGUA-CUAUUAUUGACAAUGCCU--------------------AAAUC  W10Z10
1311  36 ZBBSR7    --------------------------------------------------AAAUGA-CC-GCAAGAAAUAU-----GAU----------------UUUUC  ZBBSR7
1311  37 ZBBSR173  --------------------------------------------------AAUGGA-UU-AUAAAGAAGUUG------AUUU-------------AUCUU  ZBBSR173
1311  38 Siphcyan  --------------------------------------------------AUUGGA----UUAAAAAAAUC-----GAAUU--------------AUUUU  Siphcyan
1311  39 ZBBSR2    --------------------------------------------------AAUGAA-CUGCAGAGGAAUAUG-----AU----------------UUAUC  ZBBSR2
1311  40 ZCR4      --------------------------------------------------AAUGAA-CA-ACAAAAAAAUA----UGAU----------------UUAUC  ZCR4
1311  41 ZCR16     --------------------------------------------------AUUCAA-CU-ACCAAGAAAUUC----GAU----------------UAUUC  ZCR16
1311  42 ZCR6A     --------------------------------------------------AAUGAA-UU-GUAAAGAAGUUG-----AUUU--------------AUCUU  ZCR6A
1311  43 PHYPOLYC  -----------------------------------UCUUCUCCAAGAGCGCGCCAACUACUGAUGAUAGGGGACGUCGGUCAAUCGUGCCCUGGGCUGUC  PHYPOLYC
1311  44 NAEGRUBE  ----------------------------------------------------UUUGCCU-ACUUUUGGUAGACUUUAGUCGGGCUGGAUCUUUGGUCCUC  NAEGRUBE
1311  45 euk heli                                                                                                        euk heli
1311  46 rad                                                                                                             rad
```

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"Sept4aln" on  5-SEP-96 15:33:41

Posi-    Sequence
tion:    identity:   Data:

1411   1 1369mask  -----------------------------------------------------------------111111111111111111111111111111111--  1369mask
1411   2 MNELEIDY  GCGAAG-ACC--GCGU--GUGCC-CUUAACU-GGG-U-GG-GC------GYGGGAUUC--GC--GACGUUUACUUURAAAAAAUUAGAGUGUUCAAAGC-  MNELEIDY
1411   3 DIAGRAND  UCAAAG-UUUUAGCGG--GUGCA-CUUAGUU-GUG-U-GG-CU------GKUGAAUUU--GG--GAUSUUUACUUUGAAAAAAUUAGAGUGUUCAAAGC-  DIAGRAND
1411   4 ATHBOMBA  CUUGGU-GAGCCG-GC--GUGCC-AUUUAUU-UGG-U-GC-GU------CGGGGAACC--AG--GACUUUUACCUUGAGAAAAUUAGAGUGUUCAAAGC-  ATHBOMBA
1411   5 BLAEMERS  UGGGUG-CU-CC-UUUGGGUUGG-CUUUUG--CUAG--CUCGGGGG----AUGUCUGCCCAG--CACGUUUACUUUGAAAAAAUUAGAGUGUUCAAAGC-  BLAEMERS
1411   6 CHLREINH  CGGGGA-CGGGC--UC--CUGGG-CUUCACU-GUC-U-GG-GA------CUCGGAGUC--GG--CGAGGUUACUUUGAGUAAAUUAGAGUGUUCAAAGC-  CHLREINH
1411   7 ORYSATIV  CGGCGA-UGCGC--UC--CUGGC-CUUAACU-GGC-C-GG-GUU-----CGUGCCUCC--GG--CGCCGUUACUUUGAAGAAAUUAGAGUGCUCAAAGC-  ORYSATIV
1411   8 PORAERUG  GGUGUGA---GCGGUG--C-UGG-CAUUAAG-UUG-U-UG-G-UA---GCGUGAGCGCC--G---ACUUUUACUGUGAAAAAAUUAGAGUGUUCAAAGC-  PORAERUG
1411   9 ACACAST1  GGGCUCA---UCG-UCGUC-AUG-CA-AAU--GGCGGCGGUGGGUC--CCUGGGGCCC--AG--AUCGUUUACCGUGAAAAAAUUAGAGUGUUCAAAGC-  ACACAST1
1411  10 PHRBALAM  CAUCCCAAAAACAACCUGGGUCGGGUGGCACCGGGGUUGGAGGUUUGAUAUGAGAGGAGAUUAUGAGUAGGUGGUUAUGAUCAUUUGUGCAAUCAAAAGU  PHRBALAM
1411  11 STYALSID  UGGCGAA----CUUGU--C-CGCUCUUAACU-GAG-U-GG-GC-----UUGGUAGUC---AG--GACGUUUACUGUGAAAAAAUUAGAGUGUUCAAAGC-  STYALSID
1411  12 EMIHUXLE  CGGAGA----CCGCGC--CUACU-CUUAACU-GAG-C-GG-GC-----GCGGGAGAC---GG--GUCUUUUACUUUGAAAAAAUCAGAGUGUUUCAAGC-  EMIHUXLE
1411  13 OCHDANIC  AGAGGAA----CACGU--CUGUC-AUUCAGUUGA--U-GG-GC-----GUGGGAUUC---UC--GUCUUUUACUGUGAGUAAACUAGAGUGUUCAAAGC-  OCHDANIC
1411  14 CAFROENB  ----------CYcgNC----------------------------------CUUCGGG---GC--GUCAUUUACUGUGAAAAAAUUAGAGUGUUUAAAGC-  CAFROENB
1411  15 LABMINUT  GUGGAGA---ACUUUU--CUUGC-AUUAAUUUGU--A-GG-GAU-----UGGGA-CCC--GC--AUCGUUUACUGUGAAAAAAUUAGAGUGUUUAAAGC-  LABMINUT
1411  16 OXYGRANU  UGUUAAC----GUUUC--UUGGU-AUUCAUUUAC--U-GG-UUU--------CGGGCUCAG---AUACUUUACCUUGAGAAAAUUAGAGUGUUCCAGGC-  OXYGRANU
1411  17 BLEAMERI  UGAGCAA----CGGCU--CCGGC-AUUAACUUGU--C-GC-UGU--------CGUGAUCAG---GUACUUUACCUUGAGCAAAUGAGAGUGUUCCAGGC-  BLEAMERI
1411  18 HARVERMI  CU--AAC---GGUCCU--CAUCC-GC-GAGG-GUG-G-GG-AAU-----CAACCGCUA--GG--AUCGUUUACUUUGAGGAAAUUAGAGUGUUCAAAGC-  HARVERMI
1411  19 THEANNUL  GUCUGUG--CAUGUGG--CUUUU-UUCGGACGGA---------------GUUUCUUUGU-CUG--AAUGUUUACUUUGAGAAAAUUAGAGUGCUCAAAGC-  THEANNUL
1411  20 SYMPILO1  UCCUAAAGAACGUAU---CU-GC-ACUUCAUUGU--------------GUGGUGCGGUAUUUAGGACAUUUACCUUGAGGAAAUUAGAGUGUUUCAAGC-  SYMPILO1
1411  21 ZBBSR205  UUGAC-AGAAACUUC---UAUGU-UAUUCAUUUA-------GC-----GUGGGUAGCGACUGU-CUCUUUUACUUUGAGAAAAUUAGAGUGUUCAAAGC-  ZBBSR205
1411  22 ZBBSR206  UUGAC-AGAAACUUC---UAUGU-UAUUCAUUUA-------GC-----GUGGGUAGCGACUGU-CUCUUUUACUUUGAGAAAAUUAGAGUGUUCAAAGC-  ZBBSR206
1411  23 ZBBSR235  UUGAC-AGAAACUUC---UAUGU-UAUUCAUUUA-------GC-----GUGGGCAGCGACUGU-CUCUUUUACUUUGAGAAAAUUAGAGUGUUCAAAGC-  ZBBSR235
1411  24 ZBBSR218  UUAAC-AGAAUCUUU---CAUCG-CAUUAAUUUA-------CG-----GUGUUUGGGGCCUGU-UUCUUUUACUUUGAGAAAAUUAGAGUGUUCAAAGC-  ZBBSR218
1411  25 ZBBSR217  UUAAC-AGAAUCUUU---CAUCG-CAUUAAUUUA-------CG-----GUGUUUGGGGCCUGU-UUCUUUUACUUUGAGAAAAUUAGAGUGUUCAAAGC-  ZBBSR217
1411  26 ZBBSR211  UUGAC-AGAAACAUU---CAUCG-CCUUCAUUGG-------UG-----GUGUUUUGGGACUGU-CUCUUUUACUUUGAGAAAAUUAGAGUGUUCAAAGC-  ZBBSR211
1411  27 Pchroma   UUCCA-GAGAGUUGU---GCCUA-CCGGCUCGUU--C-GG-CAG----GGCGCUGCGAUCUGG-AUCUUUUACUUUGAAAAAAUUAGAGUGUUCCAAGC-  Pchroma
1411  28 EUGROTUN  CAGAG-AGCUGCGUC---UACCG-UUUACUCGGC--C-GG--------ACGUAUGCGAUCUGG-AUCUAUUACUUUGAAAAAAUUAGAGUGUUUAAAGC-  EUGROTUN
1411  29 ENTOGING  CUUUUUAU-A-----------CAAAAAGAGAAGUU---UAGUGAAACAAU---AGAAGAAGGAAAUGGAUUACUUUGAAUAAAAUAGAGUGUUUAAAGC-  ENTOGING
1411  30 DICDISCO  UAG-UU------------CAGCUUGUAUUAUCUUU-------------GAUAGUGCUUGUUUGGACAUUUCACUGUGAGAAAAUUGUGGUGUUUAAAGC-  DICDISCO
1411  31 ZTnucl    UUACUUAGAACAUGUACUAUGUUGAAAYUUACUUUUC-----------GACAUUUCCUCAUGUUUGUUAUUACUUUGAAAAAAUUAUGUUGAUUUAAG--  ZTnucl
1411  32 W10Z79    UUACUUAGAACAUGUCUUAUAUUGAAAUUUACUUUUC-----------GAUAUUUCCUUAUGUUUGUUAUUACUUUGAAAAAAUUAUGUUGAUUUAAG--  W10Z79
1411  33 W10Z74    UUACUUAGAACAUGUAUUAUAUUGAAAUUUAUUUUUC-----------GAUAUUUCCUUAUGUUUGUUAUUACUUUGAAAAAAUUAUGUUGAUUUAAG--  W10Z74
1411  34 W10Z72    UUACUUAGAACAUGUCUUAUAUUGAAAUUUACUUUUC-----------GAUAUUUCCUUAUGUUUGUUAUUACUUUGAAAAAAUUAUGUUGAUUUAAG--  W10Z72
1411  35 W10Z10    UUACUUAGAACAUGUCUUAUAUUGAAAUUUACUUUUC-----------GAUAUUUCCUUAUGUUUGUUAUUACUUUGAAAAAAUUAUGUUGAUUUAAG--  W10Z10
1411  36 ZBBSR7    UUACUCCAUUUUUUUUCACCAAUGGAAUUCAUUUUCU-----------GAUAGUGAAGUAAGUUUGAAUUUACUUUGAGAAAAUUAGAGUGUUCAAAAC-  ZBBSR7
1411  37 ZBBSR173  AAUAUAAAUAGUAUUACUCUGUUAGCAUUAAUUUUCU-----------GACAGUAA-UUAAAGUAAUUUUUACUUUGAAAAAAUUAGAGUGUUCAAAGC-  ZBBSR173
1411  38 Siphcyan  AGUUUAUAUUUAUUUAUACUGUUGGCAUUAAUUUUUU-----------GAUAGUGAUUUAAA-UUUGUUUCACUUUGGAAAAAUGAGAGUGUUUUAAGC-  Siphcyan
1411  39 ZBBSR2    UUAUUUUAUAUUUUUGCACCUUUGGAAUUCGUUUUCU-----------GAUAGUGGAAUAAGUUUGUGUUUACUUUGAGAAAAUUAGAGUGUUCAAAGC-  ZBBSR2
1411  40 ZCR4      UUAUUUUAUGCUUAUACACCUUUGAAAUUCAUUUUUU-----------GAUGGUGGACUGAUUUUGUGUUUACUUUGAGAAAAUUAGAGUGUUCAAAAC-  ZCR4
1411  41 ZCR16     CUAUUAUUCGAAGAUGCAAGGUUGGAAUUUAUUUUCC-----------AAUCAUUGAAAUAAUGUAAUUUUACUUUGAAAAAAAUAGAGUGUUUUAGAC-  ZCR16
1411  42 ZCR6A     AAUUUAUAUAGUCUUACUCUGUUAGCAUUAAUUUUCU-----------GACAGUAA-UUAAAGUUAUUUUUACUUUGAAAAAAUUAGGGUGUUCUAAGC-  ZCR6A
1411  43 PHYPOLYC  UCCCCCCCGGGGGAGUCACAGGGUCGUUCAGCGGUCU-----------UCGCGAUCGCGGUUCGGCUCGGCUCGGGGUACCAAUCACCAUGAUUAAACC-  PHYPOLYC
1411  44 NAEGRUBE  GUCUGACAGUUGCUAC--GUACUUACUUA-CCACGGUUCAUCCGU---GAGGCCC-UU----GG-CUUGCAACUGUAAAUAAAUCGUUGUGCUUAAAGC-  NAEGRUBE
1411  45 euk heli                                                                                                             euk heli
1411  46 rad                                                                                                                  rad
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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

1511   1 1369mask   -----------------------------------------------------------------11111111111111111111111111---------  1369mask
1511   2 MNELEIDY   AGGC---AGU--------------------------------------CGCUY----GAAUAU--CUCAGCAUGGAAUAAUAGAAUAGGAC--UUUGG-U  MNELEIDY
1511   3 DIAGRAND   AGGC---UAUU-------------------------------------UGCUU----GAAUAU--AUUAGCAUGGAAUAAUGGAAUAGGAC--UUUGG-U  DIAGRAND
1511   4 ATHBOMBA   AGGC---UUA--------------------------------------CGCCU----GAAUAC--AUUAGCAUGGAAUAAUAAAAUAGGACG-UGCGG-U  ATHBOMBA
1511   5 BLAEMERS   AGGC---UUUUUUUA---------------------------------AGCUU----GAAUAU--CUUAGCAUGGAAUAAUAGAAUAGGAC--UCUGG-U  BLAEMERS
1511   6 CHLREINH   AGGC---CUA--------------------------------------CGCUC---UGAAUAC--AUUAGCAUGGAAUAACACGAUAGGAC--UCUGG--  CHLREINH
1511   7 ORYSATIV   AAGC---CAU--------------------------------------CgcuC---UGGAUAC--AUUAGCAUGGGAUAACAUCAUAGGAU--UCCGG-U  ORYSATIV
1511   8 PORAERUG   AGGC---GUU--------------------------------------UGCUG---UGAAUAC--AUUAGCAUGGGAUAUUGGAA-GAAGC--UUUUG--  PORAERUG
1511   9 ACACAST1   AGGC---AGAUCCAAUUUUC----------------------------UGCCA--CCGAAUAC--AUUAGCAUGGGAUAAUGGAAUAGGAC--CCUGUCC  ACACAST1
1511  10 PHRBALAM   UAUAGUAGCCUCAAUGGUUCCGUUUGGUUUUCCUGAGGGGGCUCGGACUUGGUCA-UCUGUAG-GUAUUUCA-GGGGUGAUGGGCUCCGGUCUGUUAU-U  PHRBALAM
1511  11 STYALSID   AGGC---CUUC-------------------------------------AGCUA---UGAAUAC--ACUAGCAUGGAAUAAUAGAAUAGGAC--UUGGG-U  STYALSID
1511  12 EMIHUXLE   AGGC---AGU--------------------------------------CGCUC--UUGCAUGG--AUUAGCAUGGGAUAAUGAAAUAGGAC--UCUGG-U  EMIHUXLE
1511  13 OCHDANIC   AGAC---AUCA-------------------------------------UGUCA--UUGAAUAC--GUUAGCAUGGAAUAAUAAGAUAGGAC--CUUGG-U  OCHDANIC
1511  14 CAFROENB   AGGC---UUUA-------------------------------------GGCU----UGAAUAC--AUUAGCAUGGAAUAAUGAGAUAGGAC--CA-RG-U  CAFROENB
1511  15 LABMINUT   AGGC---AAU--------------------------------------CGCU----UGAAUAC--AUUAGCAUGGAAUAAUAAGAUAGGAC--UUUGG-U  LABMINUT
1511  16 OXYGRANU   AGGC---UUG--------------------------------------CGC----CGGAAUAC--AUUAGCAUGGAAUAAUAGAAUAGGAC--UUUAG-U  OXYGRANU
1511  17 BLEAMERI   AGGC---UUG--------------------------------------GGC----CUGAAUCG--UCCAGCAUGGAAUAAUAGAAGAGGAC--UGGGC-U  BLEAMERI
1511  18 HARVERMI   AGGC---GUAA-----------------------------------CUCGCCU--CCGAAUAC--GUUAGCAUGGGAUAAUGGAAUACGAC--UUCGG-U  HARVERMI
1511  19 THEANNUL   AGGC---UUU--------------------------------------CGCCU---UGAAUAG--UUUAGCAUGGAAUAAUAAAGUAGGAC--UUUGG-U  THEANNUL
1511  20 SYMPILO1   AAGC---GAU--------------------------------------UGCC---UUGAAUAC--AUUAGCAUGGAAUAAUAAGAUAGGAC--CUCAG-U  SYMPILO1
1511  21 ZBBSR205   AGGU---AGA--------------------------------------UGCC----UGAAUA---UUACUCUUGGAAUAAUGCUAUAAGAC--UUUGG-U  ZBBSR205
1511  22 ZBBSR206   AGGU---AUU--------------------------------------CGCC----UGAAUA---UUACUCUUGGAAUAAUGCUAUAAGAC--UUUGG-U  ZBBSR206
1511  23 ZBBSR235   AGGU---AGA--------------------------------------UGCC----UGAAUA---UUACUCUUGGAAUAAUGCUAUAAGAC--UUUGG-U  ZBBSR235
1511  24 ZBBSR218   GGGU---UUU--------------------------------------CGCC----UGGAUA---UUACUCUUGGAAUAAUAAUAUAGGAC--UUUGG-U  ZBBSR218
1511  25 ZBBSR217   GGGU---UUU--------------------------------------CGCC----UGGAUA---UUACUCUUGGAAUAAUAAUAUAGGAC--UUUGG-U  ZBBSR217
1511  26 ZBBSR211   GGGU---UUU--------------------------------------CGCC----AGAAUA---UUACUCUUGGAAUAAUACUAUAGGAC--UCCGG-U  ZBBSR211
1511  27 Pchroma    AAGC---UUA--------------------------------------CGCUU---UGAAUAC--AUUAGCAUGGAAUAAUAACAUAGGAC--UUCUG-U  Pchroma
1511  28 EUGROTUN   AGGC---UUA--------------------------------------UGCUG---UGAAUAC--AUUAGCAUGGAAUAAUAAUUUAGGAC--UUUGG-U  EUGROTUN
1511  29 ENTOGING   AAAAC--A-A--------------------------------------UGUUAA--UGAAUAA--UGAAGCAUGGGACAAUAAGAAGGAGA--UUUGA--  ENTOGING
1511  30 DICDISCO   AGGC---GUC-------------------------------------UCGCC----UGAUCUUU-UGCAGCAUGGUAUGAUGAAACAUGAC--AU-----  DICDISCO
1511  31 ZTnucl     AGAAG--AAA--------------------------------------UGAU----AUUGUAC--UAUAGUACAGAAUAAUACUUGAAGAU--CUCAG-U  ZTnucl
1511  32 W10Z79     AGAAG--AAA--------------------------------------UGAU----AUUGUAC--UAUAGUACAGAAUAAUACUUGAAGAU--CUCAG-U  W10Z79
1511  33 W10Z74     AGAAG--AAA--------------------------------------UGAU----AUUGUAC--UAUAGUACAGAACAAUACUUGAAGAU--CUCAG-U  W10Z74
1511  34 W10Z72     AGAAG--AAA--------------------------------------UGAU----AUUGUAC--UAUAGUACAGAAUAAUACUUGAAGAU--CUCAG-U  W10Z72
1511  35 W10Z10     AGAAG--AAA--------------------------------------CGAU----AUUGUAC--UAUAGUACAGAAUAAUACUUGAAGAU--CUCAG-U  W10Z10
1511  36 ZBBSR7     AGUC---CGUCU----------------------------------CACGGC----UGAUUAG--GAAAGCAUGGAAUGAUGAGUAACGAC--AGUGGUU  ZBBSR7
1511  37 ZBBSR173   AGUC---GUU--------------------------------------CGAA----AUUAUUC--UAAAGCACGGAAUGAUAAGUAAUGAU--GCUAG-U  ZBBSR173
1511  38 Siphcyan   AGUC---GUU--------------------------------------CGUU----AUUAUUC--UAAAGUAUGGAAUGAUAAGUAAUGAU--GUUGG-C  Siphcyan
1511  39 ZBBSR2     AGUC---UUA--------------------------------------CGGC----UUAAUAU--UAAAGCAUGGAAUGAUAAAUAAUGAC--AUCGG-U  ZBBSR2
1511  40 ZCR4       AGCC---UUUG-------------------------------------CGGA----UUUAUAU--UAAAGCAUGGAAUGAUAAAUAAUGAC--AUCGG-C  ZCR4
1511  41 ZCR16      AAUC---UUA--------------------------------------UGGA----UGUAUAU--UAAAGCAUAGAAUGGUAGAUAAUGAC--AUCAG-U  ZCR16
1511  42 ZCR6A      GGUC---GUA--------------------------------------CGAU----AUUGUUC--CAAAGCAAGGAAUAAUAAGUAAUGAU--GUUAG-U  ZCR6A
1511  43 PHYPOLYC   -GUAGU-GACCAAAGC----------------------------ACGUCUUUAGA-CGGGCACGGCACAGCAUGGGACGAAACGCACCGGG--CUCGC-C  PHYPOLYC
1511  44 NAEGRUBE   GGGC---UAUGA-------------------------------UACUCUGCC----AGAGCGA--UUUAGCAUGGGACUGCAGAGUAGCUG-UAUUUG-A  NAEGRUBE
1511  45 euk heli                                                                                                           euk heli
1511  46 rad                                                                                                                rad
```

```
"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:  Data:

1611    1 1369mask  ----------------------------------------------111111-111111111111111111-----------------------------  1369mask
1611    2 MNELEIDY  -----CUUA--UUUUGUUGGUUUC-------------CGAGACC-GAAGUAA-ugauuaaUAgggacaGuu-----------------------------  MNELEIDY
1611    3 DIAGRAND  -----UCUA--UUUUGUUGGUUUC-------------UAGGACU-GAAGUAA-ugauuaauagggacaGuu-----------------------------  DIAGRAND
1611    4 ATHBOMBA  -----UCUA--UUUUGUUGGUUUC-------------UAGAGUC-GCCGUAA-ugauuaauAgggauaGuu-----------------------------  ATHBOMBA
1611    5 BLAEMERS  -----UCUA--UUUUGUUGGUUUC-------------UGGGACU-GGAGUAAAugauuaauAgggauaGuu-----------------------------  BLAEMERS
1611    6 CHLREINH  -----CCUA--UC-UGUUGGUCUG-------------UGGGACC-GGAGUAA-ugauuaaGAggguaGuc-----------------------------  CHLREINH
1611    7 ORYSATIV  -----CCUA--UUGUGUUGGCCUU-------------CGGGAUC-GGAGUAA-ugauuaauagggacaGuc-----------------------------  ORYSATIV
1611    8 PORAERUG  -----CUUA---UUCGUUGGUU-G-------------AUAGGCY-GGAAGCA-uggucaaGAggaacgGuc-----------------------------  PORAERUG
1611    9 ACACAST1  U----CCUAUUUUCAGUUGGUUUUGGCA------GCGCGAGGACUAGGGUAA-ugauuaauagggauaGuu-----------------------------  ACACAST1
1611   10 PHRBALAM  CC----------------------------------UGAGG---UAUUCGGA-ugauugaggggu CuAAccAGCAAUGGUUAUAUUAUGAGUUUGUAUGA  PHRBALAM
1611   11 STYALSID  -----UCUA--UUUUGUUGGUUUC-------------UAGGACC-GAAGUAA-ugauuaaUAgggauaGuu-----------------------------  STYALSID
1611   12 EMIHUXLE  G-----CUA--UUUUGUUGGUUU-------------CGAACACC-GGAGUAA-ugauuaACAgggacaGuc-----------------------------  EMIHUXLE
1611   13 OCHDANIC  ------CUA--UUUUGUUGGUU---------------UGACUCC-AAGGUAA-ugauuaauAgggacaGuu-----------------------------  OCHDANIC
1611   14 CAFROENB  -----CGUA--UUUUGUUGGUU---------------GCGCGAU-UCGGUAA-ugauuaauAgggacaGuu-----------------------------  CAFROENB
1611   15 LABMINUT  A-----CUA--UUUUGUUGGUUU--------------GCAUACC-AAAUUAA-ugauCaaCAggaacaGuuU----------------------------  LABMINUT
1611   16 OXYGRANU  ------CUC--CUUUGUUGGUUC-------------GAGGGACU-GAAGUAA-ugauuaauAgggauaGuc-----------------------------  OXYGRANU
1611   17 BLEAMERI  ------C-C--AUUUAUUGGUGU-------------UAUGGCGC-UUAGUAA-ugauuaauagggauaGuu-----------------------------  BLEAMERI
1611   18 HARVERMI  -----CUUG--UUUCGUUGGUUUCG------------CUUGGCU-GAAGUAA-ugauugaUAgggacaGuu-----------------------------  HARVERMI
1611   19 THEANNUL  -----UCUA--UUUUGUUGGUUU-------------UAGGUACC-AAAGUAA-ugguuaauAggaacaGuu-----------------------------  THEANNUL
1611   20 SYMPILO1  -----UCUA--UUUUGUUGGUUU-------------CUAGAGCU-GAGGUAA-uggucgauAgggauaGuu-----------------------------  SYMPILO1
1611   21 ZBBSR205  -----UCUA--AUGUAUUGGUGAU-------------UGGGACC-AGAGUAA-ugauugauAgggacgGuu-----------------------------  ZBBSR205
1611   22 ZBBSR206  -----UCUA--AUGUAUUGGUGAU-------------UGGGACC-AGAGUAA-ugauugauAgggacgGuu-----------------------------  ZBBSR206
1611   23 ZBBSR235  -----UCUA--AUGUAUUGGUGAU-------------UGGGACC-AGAGUAA-ugauugauAgggacgGuu-----------------------------  ZBBSR235
1611   24 ZBBSR218  -----UCUU--UUUUGUUGGUGACU------------UAGAACC-GAAGUAA-ugauugauAgggacaGuu-----------------------------  ZBBSR218
1611   25 ZBBSR217  -----UCUU--UUUUGUUGGUGACU------------UAGAACC-GAAGUAA-ugauugauAgggacaGuu-----------------------------  ZBBSR217
1611   26 ZBBSR211  -----UCUU--UUUUGUUGGUGACU------------UAGGACC-GGGGUAA-ugauugauAgggacaGuu-----------------------------  ZBBSR211
1611   27 Pchroma   -----UCUA--UUUUGUUGGUUUCU-------------AGAGCC-GAAGUAA-ugauugaUAgggauaGuu-----------------------------  Pchroma
1611   28 EUGROTUN  -----UCUA--UUUUGUUGGUUUC-------------UAGGACU-GAAGUAA-ugauugaUAgggauaGuu-----------------------------  EUGROTUN
1611   29 ENTOGING  AAG-----------------------------------GAUUU-CGAGAAGA-AgauuaaAAggaauaaau-----------------------------  ENTOGING
1611   30 DICDISCO  -----UUUA--CGCUAUUGGUUU-------------GCGUUUA-AAGUGUAA-ugauuaauAgggaUgGau-----------------------------  DICDISCO
1611   31 ZTnucl    AAAA--CUU-AAACUUUU-------------------GGAUAC-UGGUGUAR-ugCucUUUAgaguuagcu-----------------------------  ZTnucl
1611   32 W10Z79    AAGA--CUU-AAACUUUU-------------------GGAUAC-UGGUGUAA-ugCucUUUAgaguuagcu-----------------------------  W10Z79
1611   33 W10Z74    AAAA--CUU-AAACUUUU-------------------GGAUAC-UGGUGUAA-ugCucUUUAgaguuagcu-----------------------------  W10Z74
1611   34 W10Z72    AAGA--CUU-AAACUUUU-------------------GGAUAC-UGGUGUAA-ugCucUUUAgaguuagcu-----------------------------  W10Z72
1611   35 W10Z10    AAGA--CUU-AAACUUUU-------------------GGAUAC-UGGUGUAA-ugCucUUUAgaguuagcu-----------------------------  W10Z10
1611   36 ZBBSR7    AAAA-UUUU-----UUUUGGUUUU------------CAGUGGC-CAGUGUAA-ugauGaCUaaaguUaguc-----------------------------  ZBBSR7
1611   37 ZBBSR173  UAA--UAUA-----UAAUGGUUU-------------GAGAGGU-UGAUGUAA-uUauUUCUagaGUuUUuc-----------------------------  ZBBSR173
1611   38 Siphcyan  UUA--AUUU-----GAUUGGUUU-------------GAGAGGU-UGAUGUAA-ugauuUaUACgGUgauuc-----------------------------  Siphcyan
1611   39 ZBBSR2    UAA--UUUU-----UGUUGGUUU-------------GAGAGGC-UGAUGUAA-ugauuUaUAgaguUAguc-----------------------------  ZBBSR2
1611   40 ZCR4      UAA--UUUU-----UGUUGGGAU-------------GAGGAGC-UGAGGUAA-ugauuUaUagaguCaGuc-----------------------------  ZCR4
1611   41 ZCR16     UAAA-UGUC-----UGAUGGUUU-------------GAGAGGU-UGAUGUAA-ugauuUaUAgAgUUUGuc-----------------------------  ZCR16
1611   42 ZCR6A     UAA--UUUU-----UAAUGGUUA-------------GAGAGGU-UGAUGUAA-uUaCUUaUaaaGUuUuuc-----------------------------  ZCR6A
1611   43 PHYPOLYC  U-----------UUUUUUUGCGGGGG------------CGUGA-CUCGGUAA--AAGCGAAAgggau-guuc----------------------------  PHYPOLYC
1611   44 NAEGRUBE  GCGAAGGUU-GCACCUCGGUGUGGCUGGAGCUUG--GUACAGC-GCUUGUAA-uggAgcUCAggG-----------------------------------  NAEGRUBE
1611   45 euk heli                                                                   26                                  euk heli
1611   46 rad                                                            ----------------->                              rad
```

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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

1712    1 1369mask  ---------------------------------------------------------------------------------------------------- 1369mask
1712    2 MNELEIDY  ---------------------------------------------------------------------------------------------------- MNELEIDY
1712    3 DIAGRAND  ---------------------------------------------------------------------------------------------------- DIAGRAND
1712    4 ATHBOMBA  ---------------------------------------------------------------------------------------------------- ATHBOMBA
1712    5 BLAEMERS  ---------------------------------------------------------------------------------------------------- BLAEMERS
1712    6 CHLREINH  ---------------------------------------------------------------------------------------------------- CHLREINH
1712    7 ORYSATIV  ---------------------------------------------------------------------------------------------------- ORYSATIV
1712    8 PORAERUG  ---------------------------------------------------------------------------------------------------- PORAERUG
1712    9 ACACAST1  ---------------------------------------------------------------------------------------------------- ACACAST1
1712   10 PHRBALAM  AUGAUUAUGAUUGCUCUUAUAGUCGACAAAGACUUCACGUGUCGGGCUUUCAGUCUUAGGACCAUCCAUUAAGACUCAGUCGUUUUGGUUGUAGAAAUGC PHRBALAM
1712   11 STYALSID  ---------------------------------------------------------------------------------------------------- STYALSID
1712   12 EMIHUXLE  ---------------------------------------------------------------------------------------------------- EMIHUXLE
1712   13 OCHDANIC  ---------------------------------------------------------------------------------------------------- OCHDANIC
1712   14 CAFROENB  ---------------------------------------------------------------------------------------------------- CAFROENB
1712   15 LABMINUT  ---------------------------------------------------------------------------------------------------- LABMINUT
1712   16 OXYGRANU  ---------------------------------------------------------------------------------------------------- OXYGRANU
1712   17 BLEAMERI  ---------------------------------------------------------------------------------------------------- BLEAMERI
1712   18 HARVERMI  ---------------------------------------------------------------------------------------------------- HARVERMI
1712   19 THEANNUL  ---------------------------------------------------------------------------------------------------- THEANNUL
1712   20 SYMPILO1  ---------------------------------------------------------------------------------------------------- SYMPILO1
1712   21 ZBBSR205  ---------------------------------------------------------------------------------------------------- ZBBSR205
1712   22 ZBBSR206  ---------------------------------------------------------------------------------------------------- ZBBSR206
1712   23 ZBBSR235  ---------------------------------------------------------------------------------------------------- ZBBSR235
1712   24 ZBBSR218  ---------------------------------------------------------------------------------------------------- ZBBSR218
1712   25 ZBBSR217  ---------------------------------------------------------------------------------------------------- ZBBSR217
1712   26 ZBBSR211  ---------------------------------------------------------------------------------------------------- ZBBSR211
1712   27 Pchroma   ---------------------------------------------------------------------------------------------------- Pchroma
1712   28 EUGROTUN  ---------------------------------------------------------------------------------------------------- EUGROTUN
1712   29 ENTOGING  ---------------------------------------------------------------------------------------------------- ENTOGING
1712   30 DICDISCO  ---------------------------------------------------------------------------------------------------- DICDISCO
1712   31 ZTnucl    ---------------------------------------------------------------------------------------------------- ZTnucl
1712   32 W10Z79    ---------------------------------------------------------------------------------------------------- W10Z79
1712   33 W10Z74    ---------------------------------------------------------------------------------------------------- W10Z74
1712   34 W10Z72    ---------------------------------------------------------------------------------------------------- W10Z72
1712   35 W10Z10    ---------------------------------------------------------------------------------------------------- W10Z10
1712   36 ZBBSR7    ---------------------------------------------------------------------------------------------------- ZBBSR7
1712   37 ZBBSR173  ---------------------------------------------------------------------------------------------------- ZBBSR173
1712   38 Siphcyan  ---------------------------------------------------------------------------------------------------- Siphcyan
1712   39 ZBBSR2    ---------------------------------------------------------------------------------------------------- ZBBSR2
1712   40 ZCR4      ---------------------------------------------------------------------------------------------------- ZCR4
1712   41 ZCR16     ---------------------------------------------------------------------------------------------------- ZCR16
1712   42 ZCR6A     ---------------------------------------------------------------------------------------------------- ZCR6A
1712   43 PHYPOLYC  ---------------------------------------------------------------------------------------------------- PHYPOLYC
1712   44 NAEGRUBE  ---------------------------------------------------------------------------------------------------- NAEGRUBE
1712   45 euk heli                                                                                                       euk heli
1712   46 rad                                                                                                            rad
```

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"Sept4aln" on  5-SEP-96 15:33:41

Posi-    Sequence
tion:    identity:   Data:

1812   1 1369mask   --------------------------------11111111111111111111-1111111111111111111111111111111111111111111111  1369mask
1812   2 MNELEIDY   --------------------------------GGGggcaUUCGUAuuucauUg-UCAgagGUGAAAuucUUGGAUuUaugaaaGACGAACUUCUgcGAAA  MNELEIDY
1812   3 DIAGRAND   --------------------------------GGGggcaUUAGUAuuuAauUg-UCAgagGUGAAAuucUUGGAUuUauGaaaGACUAACUACUgcGAAA  DIAGRAND
1812   4 ATHBOMBA   --------------------------------GGGggcaUUGGUAuugagucg-CUAgagGUGAAAuucUUGGAUugacucaaGACCAACUAUUgcGAAA  ATHBOMBA
1812   5 BLAEMERS   --------------------------------GGGggcaUUAGUAuuuAAuUg-UCAgagGUGAAAuucUUGGAUuUaGGaaaGACUAACUUCUgcGAAA  BLAEMERS
1812   6 CHLREINH   --------------------------------GGGggcaUUCGUAuuccguUg-UCAgagGUGAAAuucUUGGAUuUacggaaGACGAACAUCUgcGAAA  CHLREINH
1812   7 ORYSATIV   --------------------------------GGGggcaUUCGUAuuucauag-UCAgagGUGAAAuucUUGGAUuuaugaaaGACGAACAACUgcGAAA  ORYSATIV
1812   8 PORAERUG   --------------------------------GGGggcaCUCGUAuuucaUUg-UCAgagGUGAAAuucUUGGAUuUUgaaaGACGAACCGCUgcGAAA  PORAERUG
1812   9 ACACAST1   --------------------------------GGGggcaUUAAUAuuuAauUg-UCAgagGUGAAAuucUUGGAUuUauGaaaGAUUAACUUCUgcGAAA  ACACAST1
1812  10 PHRBALAM   AAUCGAAACGUUAACCAAAUAAUAGGACAGCCGGGguggUUAAUACuuccgug-CUAgggGUGAAAuccUAUGAUuacggaaAGAUUAACUAGUgcGAAA  PHRBALAM
1812  11 STYALSID   --------------------------------GGGggcaUUCGUAuuucauUg-UCAgagGUGAAAuucUUGGAUuUaugaaaGACGAACAACUgcGAAA  STYALSID
1812  12 EMIHUXLE   --------------------------------AGGggcaCUCGUAuuccgcCg-AGAgagGUGAAAuucUCAGACcAgcggaaGACGAACCACUgcGAAA  EMIHUXLE
1812  13 OCHDANIC   --------------------------------GGGgguaUUCGUAuucAauUg-UCAgagGUGAAAuucUUGGAUuUauGgaaGACGAACUACUgcGAAA  OCHDANIC
1812  14 CAFROENB   --------------------------------GGGgguaUUCAUAuuuaauUg-UCAgagGUGAAAuucUUAGAUuUauuaaaGAUGAACUACUgcGAAA  CAFROENB
1812  15 LABMINUT   --------------------------------GAGgauaUUCGUAugaACAUg-UCAgagGUGAAAuucUUGGAUuUUGAucaGACGAACUACUgcGAAA  LABMINUT
1812  16 OXYGRANU   --------------------------------GGGggcaUUAGUAuuuaauUg-UCAgagGUGAAAuucUCGGAUuUguuaaaGACUAACUUAUgcGAAA  OXYGRANU
1812  17 BLEAMERI   --------------------------------GGGggcaUUUGUAuuuaauUg-UCAgagGUGAAAuucUAUGAUuUauuaaaGACAAACUUAUgcGAAA  BLEAMERI
1812  18 HARVERMI   --------------------------------GGGggcaUUAGUAuuuaguUg-UCAgagGUGAAAuucUAGGAUuUacuaaaGACUGACCAAUgcGAAA  HARVERMI
1812  19 THEANNUL   --------------------------------GGGggcaUUCGUAuuuaacUg-UCAgagGUGARAuucUUAGAUuUguuaaaGACGAACUACUgcGAAA  THEANNUL
1812  20 SYMPILO1   --------------------------------GGGggcaUUCGUAuuuaacUg-UCAgagGUGAAAuucUUGGAUuUguuaaaGACGGACUACUGcGAAA  SYMPILO1
1812  21 ZBBSR205   --------------------------------GGGgucuUACGUACugcaaag-CGAgagGUGAAAuucUUGGACcuuuguaUGACGAACAACUgcGAAA  ZBBSR205
1812  22 ZBBSR206   --------------------------------GGGgucuUACGUACugcaaag-CGAgagGUGAAAuucUUGGACcuuuguaUGACGAACAACUgcGAAA  ZBBSR206
1812  23 ZBBSR235   --------------------------------GGGgucuUACGUACugcaaag-CGAgagGUGAAAuucUUGGACcuuuguaUGACGAACAACUgcGAAA  ZBBSR235
1812  24 ZBBSR218   --------------------------------GGGgucaUUCGUACugcaaag-CGAgagGUGAAAuucAUGGACcuuuguaUGACGAACUACUgcGAAA  ZBBSR218
1812  25 ZBBSR217   --------------------------------GGGgucaUUCGUACugcaaag-CGAgagGUGAAAuucAUGGACcuuuguaUGACGAACUACUgcGAAA  ZBBSR217
1812  26 ZBBSR211   --------------------------------GGGgucaUUCGUACugcaaag-CGAgugGUGAAAuacAUGGACcuuuguaUGACGAACUACUgcGAAA  ZBBSR211
1812  27 Pchroma    --------------------------------GGGggugCUAGUAuu-cagcggCCAgagGUGAAAuucUUGGAUucgcugaaGACUAACUACUgcGAAA  Pchroma
1812  28 EUGROTUN   --------------------------------GGGggugCUAGUAuuccacgg-CCAgagGUGAAAuucUUGGAUucguggaaGACUAACUACUgcGAAA  EUGROTUN
1812  29 ENTOGING   --------------------------------GGGguaaUUUAGAAaaagaug-GGAgaGGUGAAAAucCAUGAUcaucuuuAGAUAAACGAGAgcGAAA  ENTOGING
1812  30 DICDISCO   --------------------------------GGGggugUUCAUAuugguggg-CGAgagGUGAAAuucGUUGACccuaucaaGAUGAACUUCUgcGAAA  DICDISCO
1812  31 ZTnucl     --------------------------------GAAgauaUUAAUAuuUuagcg-WUAgagGUGAAAuucAAGAAUcguuaUaaGAUUAACAAGUgcCAAA  ZTnucl
1812  32 W10Z79     --------------------------------GAAgauaUUAAUAuuUuagcg-UUAgagGUGAAAuuuAAGAAUcguuaUaaGAUUAACAAGUgcCAAA  W10Z79
1812  33 W10Z74     --------------------------------GAAgauaUUAAUAuuUuagcg-UUAgagGUGAAAuucAAGAAUcguuaUaaGAUUAACUAGUgcCAAA  W10Z74
1812  34 W10Z72     --------------------------------GAAgauaUUAAUAuuUuagcg-UUAgagGUGAAAuucAAGAAUcguuaUaaGAUUAACAAGUgcCAAA  W10Z72
1812  35 W10Z10     --------------------------------GAAgauaUUAAUAuuUuagCg-UUAgagGUGAAAuucAAGAAUcAuuaUaaGAUUAACAAGUgcCAAA  W10Z10
1812  36 ZBBSR7     --------------------------------GGAgguAUUAGUAuuuugucg-UUAgaaGUGAAAuucUGGGAUcgauaaaaGACGAACAACUgcGAAG  ZBBSR7
1812  37 ZBBSR173   --------------------------------GGAggGAUUAGUAuuuuAUcg-UUAgaaGUGAAAuucUUAGAUugUGaaaaGAUUAACUACAgcAAAU  ZBBSR173
1812  38 Siphcyan   --------------------------------GGAgguAUUCGUAuuuuGucg-UUAgaaGUGAAAuucUUGGAUugaGagaaGGCAAACAAUAgcAAAA  Siphcyan
1812  39 ZBBSR2     --------------------------------GGAgguaUUAGUAuuuugucg-UUAgaaGUGAAAuucCGGGAUcgacaaaaGACUAACAACUgcGAAA  ZBBSR2
1812  40 ZCR4       --------------------------------GGAgguaUUAGUAuuuUaUcg-UUAgaAGUGAAACucUCGGAUcgUuUaaaGACUAACAACUgcAAAA  ZCR4
1812  41 ZCR16      --------------------------------GAAgAuaUUAGUAuuuugucg-UUAgagGUGAAAuucUGGGAUcgacaaaaGAUUACCAAGAgcGGAA  ZCR16
1812  42 ZCR6A      --------------------------------GGAggGAUUAGUAuuuuGUcg-UUAgaaGUGAAAuucUUGGAUugUGaaaaGAUUAACUACAgcAAAU  ZCR6A
1812  43 PHYPOLYC   --------------------------------GAGggugACCGAAuugcuggg-CGAgugGUGAAAuacGUUGACccuagcaaGUCGACCAAAGgcGUAA  PHYPOLYC
1812  44 NAEGRUBE   -------------------------------uGAggcccCGGGUACcaugagg-CUAgagGUGAAAuucUGAGACccucaugUGACCAACUAAGgcGAAA  NAEGRUBE
1812  45 euk heli                                      27          28      29       29         28              30       euk heli
1812  46 rad                                           --->      ------->    -->     <--      <-------           -->      rad
```

```
"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

1912   1 1369mask   1111111---111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111  1369mask
1912   2 MNELEIDY   gcAUUug---ccAAggAuguuuuCAuuaaucaAgaAcGAAAguuggagGCUCGAAgacgAUCAGAUACcgucCUAGuuccaacCAUAAACGAUgccgucU  MNELEIDY
1912   3 DIAGRAND   gcAUUug---ccAAggAuguuuuCRuuaaucaAgaAcGAAAguuagggGAUCGAAgacgAUCAGAUACcgucGUAGucuuaacCAUAAACUAUgccgacU  DIAGRAND
1912   4 ATHBOMBA   gcAUUug---ccAAggAuguuuuCauuaaucaAgaAcGAAGguuagggGAUCGAAaacgAUCAGAUACcguuGUAGucuuaacAGUAAACUAUgccgacU  ATHBOMBA
1912   5 BLAEMERS   gcAUUug---ccAAggAuguuuuCauuaaucaAgaAcGAAAguuaaggGAUCGAAgaugAUUAGAUACcaucGUAGucuuaacCAUAAACGAUgccgacC  BLAEMERS
1912   6 CHLREINH   gcAUUug---ccAAggAuacuuuCAuugaucaAgaAcGAAAguuggggGCUCGAAgacgAUUAGAUACcgucGUAGucucaacCAUAAACGAUgccgacU  CHLREINH
1912   7 ORYSATIV   gcAUUug---ccAAggAuguuuuCauuaaucaAgaAcGAAAguuggggGCUCGAAgacgAUCAGAUACcgucCUAGucucaacCAUAAACGAUgccgacC  ORYSATIV
1912   8 PORAERUG   gcGUUug---ccAAggAcguuuuCAuugaucaAgaAcGAAAguuagggGAUCGAAgacgAUCAGAUACcgucGUAGucuuaacCAUAAACUAUgccGacU  PORAERUG
1912   9 ACACAST1   gcAUCug---ccAAggAuguuuucauuaaucaAgaAcGAAAguuagggGAUCGAAgacgAUCAGAUACcgucGUAGucuuaacCAUAAACGAUgccgacC  ACACAST1
1912  10 PHRBALAM   gcAUUcc---acAAggaUguuuuCAuuaaucaAgaAcGAAAguuggggGAUCGAAgacgAUCAGAUACcgucGUAGucucaacCAUAAACUAUgccGaCC  PHRBALAM
1912  11 STYALSID   gcAUCug---ccAAggAugucuuCAuugaucaAgaAcGAAAguuagggGAUCGAAgacgAUCAGAUACcgucGUAGucuuaacCAUAAACUAUgccGacU  STYALSID
1912  12 EMIHUXLE   gcAUUug---ccAGggAuguuuuCACugaucaAgaAcGAAAguuagggGAUCGAAgacgAUCAGAUACcgucGUAGucuuaacCAUAAACCAUgccgacU  EMIHUXLE
1912  13 OCHDANIC   gcAUUua---ccAAggAuguuuuCauuaaucaAgaAcGAAAguuagggGAUCGAAgaugAUUAGAUACcaucGUAGucuuaacCAUAAACUAUgccgacU  OCHDANIC
1912  14 CAFROENB   gcAUUua---ccAAggAuguuuUCAuuaaucaAgaAcGAAAgucagggGAUCGAAgaggAUUAGAUACccucGUAGucuugacCAUAAACUAUgccgacU  CAFROENB
1912  15 LABMINUT   gcAUUua---ucAAggAuguuuuCAuuAaucaAgaAcGAAAguuagggGAUCGAAgaugAUUAGAUACcaucGUAGucuuaacCAUAAACUAUgccgacU  LABMINUT
1912  16 OXYGRANU   gcAUUug---ccAAggAuguuuuCauuaaucaAgaAcGAAAguuagggGAUCAAAgacgAUCAGAUACcgucCUAGucuuaacCAUAAACUAUgccgacU  OXYGRANU
1912  17 BLEAMERI   gcAUUug---ccAAggAuguuuuCauuaaucaAgaAcGAAAguuaaggGAUCAAAgacgAUCAGAUACcgucGUAGucuuaacCAUAAACUAUgccgacU  BLEAMERI
1912  18 HARVERMI   gcAUUug---ccAAggAuguuuuCAuuaaucaAgaAcGAAAguaggggGAUCGAAgacgAUCAGAUACcgucGUAGucucuacCGUAAACUAUgccGacC  HARVERMI
1912  19 THEANNUL   gcAUUug---ccAAggAuguuuuCauuaaucAAgaAcGAAAguuagggGAUCGAAgacgAUCAGAUACcgucGUAGuccuaacCAUAAACUAUgccgacU  THEANNUL
1912  20 SYMPILO1   g-AUUug---ccAAggAuguuuuCauugaucaAgaAcGAAAguuagggGAUCGAAgacgAUCAGAUACcgucCUAGucuuaacCAUAAACUAUgccAacU  SYMPILO1
1912  21 ZBBSR205   gcAUUgg---acUAggAcguuccCguugaucaAgaAcGAAAguuggggGAUCGAAgacgAUCAGAUACcgucGUAGucucaacCAUAAACUAUgccAgcU  ZBBSR205
1912  22 ZBBSR206   gcAUUgg---acUAggAcguuccCguugaucaAgaAcGAAAguuggggGAUCGAAgacgAUCAGAUACcgucGUAGucucaacCAUAAACUAUgccAgcC  ZBBSR206
1912  23 ZBBSR235   gcAUUgg---acUAggAcguuccCguugaucaAgaAcGAAAguuggggGAUCGAAgacgAUCAGAUACcgucGUAGucucaacCAUAAACUAUgccAgcU  ZBBSR235
1912  24 ZBBSR218   gcAUUug---acUAggAuguuccCguugaucaAgaAcGAAAguuggggGAUCGAAgacgAUCAGAUACcgucGUAGucucaacCAUAAACUAUgccAgcU  ZBBSR218
1912  25 ZBBSR217   gcAUUug---acUAggAuguuccCguugaucaAgaAcGAAAguuggggGAUCGAAgacgAUCAGAUACcgucGUAGucucaacCAUAAACUAUgccAgcU  ZBBSR217
1912  26 ZBBSR211   gcAUUug---acUAggAuguuccCguugaucaAgaAcGAAAguuggggGAUCGAAgacgAUCAGAUACcgucGUAGucucaacCAUAAACUAUgccAgcU  ZBBSR211
1912  27 Pchroma    gcAUUca---ccAAggAugucuuCUuuaaucaAgaAcGAAAguuggggGAUCGAAgacgAUCAGAUACcgucGUAGucucaacCAUAAACUAUaccgacU  Pchroma
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1912  29 ENTOGING   gcAUUuu---acUCaauuauAuucAuuaaucAAgaAcGAAAguuagggGAACAAAgacgAUCAGAUACcgucGUAGuccuaacUAUAAACGAUguuAacC  ENTOGING
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1912  31 ZTnucl     gcAAUua---ucUAagAuuaauucAUUgaUcaAgaAcGUAAguugaAgGAUUGAAgacgAUCAGAUACcgucGUAAuCucaauUGUAAACUAUaucaacU  ZTnucl
1912  32 W10Z79     gcAAUua---ucUAagAuuaauucAUUgaUcaAgaAcGUAAguugaAgGAUUGAAgacgAUCAGAUACcgucGUAAuCucaauUGUAAACUAUaucaacU  W10Z79
1912  33 W10Z74     gcAAUua---ucUAagAuuaauucAUUgaUcaAgaAcGUAAguugaAgGAUUGAAgacgAUCAGAUACcgucGUAAuCucaauUGUAAACUAUaucaacU  W10Z74
1912  34 W10Z72     gcAAUua---ucUAagAuuaauucAUUgaUcaAgaAcGUAAguugaAgGAUUGAAgacgAUCAGAUACcgucGUAAuCucaauUGUAAACUAUaucaacU  W10Z72
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1912  36 ZBBSR7     gcAGAGg---ccUAgaAuuUacuuuUuGaucaAgaAcGUAAguuggggGAUCGAAgacgAUCAGAUACcgucGUAGucucaacUUUAAACUAUaccGUcU  ZBBSR7
1912  37 ZBBSR173   gcAUUCA---ucUAggCUaUAuuuUUUUauCaAgaAcGUAAguuggggGAUCGAAgacgAUCAGAUACcgucGUAGucucaacUAUAAACUAUaucAacU  ZBBSR173
1912  38 Siphcyan   gcAUUCa---ucUAgaaucUAcUCCuUggucaAgaAcGUAAguugUggGAUCGAAgacgAUCAGAUACcgucGUAGucUcaacUAUAAACAAUauuAauU  Siphcyan
1912  39 ZBBSR2     guAUUug---ccUAgaAuCUgcucCUugaucaAgaAcGUAAguuggggGAUCGAAgacgAUCAGAUACcgucGUAGucucaacUUUAAACUAUaucAgcU  ZBBSR2
1912  40 ZCR4       gcAUUua---ccUAgaAuauucuCCuUgaucaAgaAcGUAAguugaggGAUCGAAgacgAUCAGAUACcgucGUAGucucaacUGUAAACUAUaccGgcU  ZCR4
1912  41 ZCR16      gcAUUug---CcUAgaAUUUcCuCUuUgaucaAgaAcGUAAguugaGgGAUCGAAgacgAUCAGAUACcgucGUAGuGucaacUGUAAACUAUauuAgcU  ZCR16
1912  42 ZCR6A      gcAUUCA---ucUAggaUaUAuuuCuUUAuCaAggAcGUAAguugggUGAUCGAAgacgAUCAGAUACcgucGUAGUcucaacUAUAAACAAUAUUAACU  ZCR6A
1912  43 PHYPOLYC   gcAGUca---ucAAgggcauuccCGuuGAUCAAgagcGAAAguuaaggGUUCGAAgacgAUCAGAUACcgucGUAGucuuaacUAUAAAUGAUgcAgacC  PHYPOLYC
1912  44 NAEGRUBE   gcUGUCGUgggccACCACAaGcuCguCUAucaGggAcAAAAguuggggGAUCGAAgacgAUUAGAUACcgucGUAGucccaacUAUAAACGAUaccAacC  NAEGRUBE
1912  45 euk heli   30      27        26           25           31          32           32       31               33     euk heli
1912  46 rad        <-   <------  <-- -- ---   <---   ---    ------>       --->         <---    <------          ----->    rad
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"Sept4aln" on  5-SEP-96 15:33:41

Posi-    Sequence
tion:    identity:   Data:

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2012   3 DIAGRAND   cggGAucggugug-GUGAAA-------------------------------------------------------------------------------  DIAGRAND
2012   4 ATHBOMBA   aggGAucggacaacCUCAAUUUU----------------------------------------------------------------------------  ATHBOMBA
2012   5 BLAEMERS   aggGAucgggcguCAUGUAGUUAUUCACAUA--------------------------------------------------------------------  BLAEMERS
2012   6 CHLREINH   aggGAuuggcagaUgUUCUUUU-----------------------------------------------------------------------------  CHLREINH
2012   7 ORYSATIV   aggGAucggcggaUgUUGCUUA-----------------------------------------------------------------------------  ORYSATIV
2012   8 PORAERUG   gggGAuugguggaCguuCCAA-U----------------------------------------------------------------------------  PORAERUG
2012   9 ACACAST1   agcGAuuaggAgaCGUUGAAUACAAAACAccacAUCggcGCggucguccuuGgcgucugucccuUUCAACgggggcaggcgcgagggcgguuUAgccCg  ACACAST1
2012  10 PHRBALAM   agAGAucggaAgacGCUGUCCUAGAAGGAC---------------------------------------------------------------------  PHRBALAM
2012  11 STYALSID   gggGAucgguagcuUCUUUUCA-----------------------------------------------------------------------------  STYALSID
2012  12 EMIHUXLE   aggGAuuggaggaUGUUCCAUU-----------------------------------------------------------------------------  EMIHUXLE
2012  13 OCHDANIC   aggGAuugguggAcGUU--GUA-----------------------------------------------------------------------------  OCHDANIC
2012  14 CAFROENB   cagGAuuggcaccc-UU-UUUU-----------------------------------------------------------------------------  CAFROENB
2012  15 LABMINUT   aggGAuuggcggAcGUUGUCUA-----------------------------------------------------------------------------  LABMINUT
2012  16 OXYGRANU   aggGAucggagg-cgCGCUUU------------------------------------------------------------------------------  OXYGRANU
2012  17 BLEAMERI   agaGAuuggagg-UgCCAUUAA-----------------------------------------------------------------------------  BLEAMERI
2012  18 HARVERMI   aggGAuccggAga-GUUUGCAUGGA--------------------------------------------------------------------------  HARVERMI
2012  19 THEANNUL   agAGAuuggaggucguCagU-------------------------------------------------------------------------------  THEANNUL
2012  20 SYMPILO1   agAGAuuggaggucguUAC--------------------------------------------------------------------------------  SYMPILO1
2012  21 ZBBSR205   aggGAucggaggaAguuAUU-------------------------------------------------------------------------------  ZBBSR205
2012  22 ZBBSR206   aggGAucggaggaCguuACA-------------------------------------------------------------------------------  ZBBSR206
2012  23 ZBBSR235   aggGAucggaggaAguuAUU-------------------------------------------------------------------------------  ZBBSR235
2012  24 ZBBSR218   aggGAuuggaagaAgau-CA-------------------------------------------------------------------------------  ZBBSR218
2012  25 ZBBSR217   aggGAuuggaagaAgau-CA-------------------------------------------------------------------------------  ZBBSR217
2012  26 ZBBSR211   aggGAuuggugggUguuACU-------------------------------------------------------------------------------  ZBBSR211
2012  27 Pchroma    gagGAuccguggaCGUCUAAU------------------------------------------------------------------------------  Pchroma
2012  28 EUGROTUN   cagGAucagugaaCGUUGCAUU-----------------------------------------------------------------------------  EUGROTUN
2012  29 ENTOGING   aagGAuuggauaaaUACAUUAAUACAUUUUGUAUUG-----------------------------------UCAUUGUAACUUGUUACAAGAGAAUAGA  ENTOGING
2012  30 DICDISCO   aggGAucgguuaaaaUUUUUU------------------------------------------------------------------------------  DICDISCO
2012  31 ZTnucl     aggGAuuaacaaCUGUUUUUU------------------------------------------------------------------------------  ZTnucl
2012  32 W10Z79     aggGAuuaacaaCUGUUUUUU------------------------------------------------------------------------------  W10Z79
2012  33 W10Z74     aggGAuuaacaaCUGUUUCUU------------------------------------------------------------------------------  W10Z74
2012  34 W10Z72     aggGAuuaacaaCUGUUUUUU------------------------------------------------------------------------------  W10Z72
2012  35 W10Z10     aggGAuuaacaaCUGUUUUUU------------------------------------------------------------------------------  W10Z10
2012  36 ZBBSR7     aggGGuuggcaCACGUUUUU-------------------------------------------------------------------------------  ZBBSR7
2012  37 ZBBSR173   aggGAuuagcgAUUAUUUUU-------------------------------------------------------------------------------  ZBBSR173
2012  38 Siphcyan   aggGAuuagcgAAUGUUUAU-------------------------------------------------------------------------------  Siphcyan
2012  39 ZBBSR2     aggGGuuggcaAAUGUUUAU-------------------------------------------------------------------------------  ZBBSR2
2012  40 ZCR4       aggGGuuagcaAAUGUUUAU-------------------------------------------------------------------------------  ZCR4
2012  41 ZCR16      aGguGuuggcaAAUGUUUAA-------------------------------------------------------------------------------  ZCR16
2012  42 ZCR6A      aggGAuuagcgAAUAUGUUUA------------------------------------------------------------------------------  ZCR6A
2012  43 PHYPOLYC   aggGAuAggaCAGUGUCCA--------------------------------------------------------------------------------  PHYPOLYC
2012  44 NAEGRUBE   gagUAuuugggaagaCACUAUC-----------------------------------------------------------------------------  NAEGRUBE
2012  45 euk heli         34                                                                                              euk heli
2012  46 rad        ------------->                                                                                         rad
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"Sept4aln" on  5-SEP-96 15:33:41

Posi-    Sequence
tion:    identity:    Data:

2112   1 1369mask  -------------1111111111111111-1111111111111-1111111111111111111111111111111111111111111111111111111  1369mask
2112   2 MNELEIDY  ------------AAGGCuccuucggCAcg-cUAUgaGAAAucA--AAgacUUCGgguUcCgGggGgaguAuguucGCAAgaaugAAacuuAAaggaAUU  MNELEIDY
2112   3 DIAGRAND  ------------UUAGCcgcaucggCAcc-gUAUgaGAAAucA--AAguuUUUGgguUcugGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  DIAGRAND
2112   4 ATHBOMBA  -----------GAUGUguuguucggCAcc-uUACgaGAAAucA--AAgucUUUGgguUcugGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  ATHBOMBA
2112   5 BLAEMERS  -----------AAAGACgcgcccggCAcc-uUUUgaGAAAucA--AAguuUUUGgguUcugGggGgaguAuggucGCAAggcugAAacuuAAaggaAuU  BLAEMERS
2112   6 CHLREINH  -----------GAUGAcucugccagCAcc-uUAUgaGAAAucA--AAguuUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  CHLREINH
2112   7 ORYSATIV  -----------UAGGAcuccgccggCAcc-uUAUgaGAAAucA--AAgucUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  ORYSATIV
2112   8 PORAERUG  ------------CUgacuccaucagCAcc-cUAUgaGAAAucA--AaguGUUUGgguUcugGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  PORAERUG
2112   9 ACACAST1  guggCACCGGUGAAUGACucCcuagCAgc-uUGUgaGAAAucA-UAAgucUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  ACACAST1
2112  10 PHRBALAM  ----------GCGUGGcucCuccggCACcuUUGAgaGAAAucAUUGAGUGUCuGggcUUcgGggGgaguAuggucGCAAggcugAaacuuAAaggaAUU  PHRBALAM
2112  11 STYALSID  -----------AAU-AagcuaucggCAcc-cUAAgaGAAAucA--AAguCUUUGgguUcugGggGgagCAuggucGCAAggcugAAACuuAAaggaAUU  STYALSID
2112  12 EMIHUXLE  -----------UGUGACuccuucagCAcc-uUUCggGAAAcuA--AAgucUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  EMIHUXLE
2112  13 OCHDANIC  -----------AUUgACuccaucagCAcc-uUAUgaGAAAucA--AAgucUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAagaaAUU  OCHDANIC
2112  14 CAFROENB  ------------AACAgggugucagCAcu-gUAUgaAAAAucA--AAgucUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  CAFROENB
2112  15 LABMINUT  -----------UAUgACUccgccagCAcc-uCAUgaGAAAucA--AAgucUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  LABMINUT
2112  16 OXYGRANU  -------------AUCcgccuucggCAcc-uUAUgaGAAAucA--AAgucUUUGgguUcugGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  OXYGRANU
2112  17 BLEAMERI  -----------AAGUAcUccuucagCAuc-uUCCgaGAAAucA--AAgucUUUGgguUcugGggGgaguAuggucGCAAgacugAAacuuAAaggaAuU  BLEAMERI
2112  18 HARVERMI  -------------UGACucCccgggCAcc-uUGUgaGAAAucA-UAAguGUUUGgguUuugGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  HARVERMI
2112  19 THEANNUL  --------UuuUacgacuCCuucagCACc-uUGAgaGAAAucA--AAgucUUUGgguUcugGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  THEANNUL
2112  20 SYMPILO1  --------UCAUacgaCUccuucagCACc-uUAUgaGAAAucA--AAguCUUUGgguUccgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  SYMPILO1
2112  21 ZBBSR205  ---------GUUUCgacuccuucggCAcc-uUAUgaGAAAucA--AAgcAUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  ZBBSR205
2112  22 ZBBSR206  ---------UGUUCgacuccuucggCAcc-uUAUgaGAAAucA--AAgcAUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  ZBBSR206
2112  23 ZBBSR235  ---------GUUUCgacuccuucggCAcc-uUAUgaGAAAucA--AAgcAUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  ZBBSR235
2112  24 ZBBSR218  ---------CAUACgucucuuucagCAcc-uUAUgaGAAAucA--AAgcAUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  ZBBSR218
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2112  27 Pchroma   ----------CUUCGACuccaugggCAcu-cUAUgaGAAAucA--AAgucUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  Pchroma
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2112  31 ZTnucl    ------------AUGACAuuguuggCAcc-uUGUgaGAAAuuA--GAguuCUCAgauUcCgGggGgaguAuggUuGCAAgUcugAAacuuAAaggaAUU  ZTnucl
2112  32 W10Z79    ------------AUGACAuuguuggCAcc-uUGUgaGAAAuuA--GAguuCUCAgauUcCgGggGgaguAuggUuGCAAgUcugAAacuuAAaggaAUU  W10Z79
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2112  34 W10Z72    ------------AUGACAuuguuggCAcc-uUGUgaGAAAuuA--GAguuCUCAgauUcCgGggGgaguAuggUuGCAAgUcugAAacuuAAaggaAUU  W10Z72
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2112  36 ZBBSR7    ------------UUGACUUugucagAAcc-uUAUgaGAAAucA--AAgCGUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  ZBBSR7
2112  37 ZBBSR173  ------------UAAACU-uguuagAAcc-uCAAgaGAAAucA-AGAguGUCUGgauUcCGGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  ZBBSR173
2112  38 Siphcyan  ------------AAAACAUuguuagAAcc-uAAAgaGAAAucA--AAguAUUUGgauUcCGGggGgaguAugguuGCAAggcugAAacuuAAaggaAUU  Siphcyan
2112  39 ZBBSR2    ------------AUGACUUuguuagAAcc-uUAUgaGAAAucA--AAgcGUUUGgguUcCgGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  ZBBSR2
2112  40 ZCR4      ------------AUGCCAUuguuagAAcc-uUUGgaGAAAucA--CAgcGUUUGgguUcCgGggGaaguAugguuGCAAggcuaAAacuuAAAggaAUU  ZCR4
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2112  42 ZCR6A     ------------UAUACU-uguuagAAcc-uCAAgaGAAAucA-AGAguAUCUGgauUcCGGggGgaguAuUgucGCAAggcUgAAacuuAAaggaAUU  ZCR6A
2112  43 PHYPOLYC  ----------UCUCGACUCUuccgG-Acc-uU-GgaGAAAucA-CGAgucUAUGGguUcugGggGgaguAuggucGCAAggcugAAacuuAAaggaAUU  PHYPOLYC
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2112  45 euk heli                           34        35    35         3-3       24      36             36        2     euk heli
2112  46 rad                        <--------  ----   ->    <-     <----   -- <----- ---------->   <----  ----  <---     rad
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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

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2213    3 DIAGRAND  GacGgaagggcaCCACCa-ggaguGGagcCugcGgcUUAAuuugACUCAACAcgggGAAACUCAccaggUCCAGAcauagUAAGGAUUgaCagauuGAGa  DIAGRAND
2213    4 ATHBOMBA  GacGgaagggcaCCACCa-gguguGGagcCugcggcUUAAuuugACUCAACAcggggAAACUCAccaggUCCAGAcauaaCUAGGAUUgaCagauuGAUa  ATHBOMBA
2213    5 BLAEMERS  GacGgaagggcaCCACCa-gguguGGagcCugcggcUUAAuuugACUCAACAcgggGAAACUCAccaggUCCAGAcauggUAAGGAUUgaCagauuGAAa  BLAEMERS
2213    6 CHLREINH  GacGgaagggcaCCACCa-ggcguGGagcCugcggcUUAAuuugACUCAACAcgggGAAACUUAccaggUCCAGAcacgggaAGGAUUgaCagauuGAGa  CHLREINH
2213    7 ORYSATIV  GacGgaagggcaCCACCa-ggcguGGgcCugcggcUUAAuuugACUCAACAcgggGAAACUUAccaggUCCAGAcauagCAAGGAUUgaCagacuGAGa  ORYSATIV
2213    8 PORAERUG  GacGgaagggcaUCACCg-ggcguGGagcCugcggcUUAAuuugACUCAACAcgggGAAACUUAccaggUCCAGAcgaagUUAGGAUUgaCagauuGAGa  PORAERUG
2213    9 ACACAST1  GacGgaagggcaCCACCa-ggaguGGagcCugcggcUUAAuuugACUCAACAcgggGAAACUUAccaggUCCGGAcauagUAAGGAUUgaCagauuGAUa  ACACAST1
2213   10 PHRBALAM  GACGgaagggcaCCACCa-ggaguGGagcCugcg-CUuAAuuugACUCAACAcgggGAACCUUAccaggUCCAGAcaguaGAAGGAUUgaCagaUuGAGA  PHRBALAM
2213   11 STYALSID  GacGgaagggcaCCACCa-ggaguGGagcCugcggcUUAAuuugACUCAACAcgggGAAACUUAccaggUCCAGAcauagUAAGGAUUgaCagauUGAGa  STYALSID
2213   12 EMIHUXLE  GacGgaagggcaCCACCA-ggaguGGagcCugcggcUUAAuuugACUCAACAcgggGAAACUUAccaggUCCAGAcauugUGAGGAUUgaCagauuGAGa  EMIHUXLE
2213   13 OCHDANIC  GacGgaagggcaCCACCA-ggaguGGagcCugcggcUUAAuuugACUCAACAcgggGAAACUUAccaggUCCAGAcauagUGAGGAUUgaCagauuGAGa  OCHDANIC
2213   14 CAFROENB  GacGgaagggcaCCACCA-ggaguGGagccugcGgCUUAAuuugACUCAACAcgggGAAACUUAccaggUCCAGAcauggUGAGGAUUgaCagauuGAAa  CAFROENB
2213   15 LABMINUT  GacGgaagggcaCCACCA-ggaguGGagcCugcggcUUAAuuugACUCAACAcgggAAAACUUAccaggUCCAGAcauagUAAGGAUUgaCagauuGAGa  LABMINUT
2213   16 OXYGRANU  GacGgaagggcaCCACCa-ggaguGGagcUugcggcUCAAuuugACUCAACAcgggAAAACUUAccaggUCCAGAcauagUGAGGAUUgaCagauuGAUa  OXYGRANU
2213   17 BLEAMERI  GacGgaagggcaCCACCa-ggaguGGcA-UGgcggcUUAAuuugACUCAACAcgggGAAACUUAccaggUCCAGAcauggGAAGGAUUgaCagauuGAUa  BLEAMERI
2213   18 HARVERMI  GacGgaagggcaCCACCa-ggaguGGaacCugcGgcUUAAuuugACUCAACAcgggGAAACUUAcgaggUCAGGAcacugUGAGGAUUgaCagauuGAAa  HARVERMI
2213   19 THEANNUL  GacGgaagggcaCCACCA-ggcguGGagc-ugcggcUUAAuuugACUCAACAcgggGAAACUCAccaggUCCAGAcaaagGAAGGAUUgaCagauuGAUa  THEANNUL
2213   20 SYMPILO1  GacGgaagggcaCCACCA-ggaguGGagcCugcggcUUAAuuugACUCAACAcgggGAAACUUAccaggUCCAGAcauagUAAGGAUUgaCagauuGAUa  SYMPILO1
2213   21 ZBBSR205  GacGggagggcaCCACCa-ggaguGGagccuguggcUUAAuuugACUCAACAcgggAAAACUUAccaggUCCGGAcauagUAAGGAUUgaCagauuGAAa  ZBBSR205
2213   22 ZBBSR206  GacGggagggcaCCACCa-ggaguGGagccuguggcUUAAuuugACUCAACAcgggAAAACUUAccaggUCCGGAcauagUAAGGAUUgaCagauuGAAa  ZBBSR206
2213   23 ZBBSR235  GacGggagggcaCCACCa-ggaguGGagccuguggcUUAAuuugACUCAACAcggrAAAACUUAccaggUCCGGAcauagUAAGGAUUgaCagauuGAAa  ZBBSR235
2213   24 ZBBSR218  GacGggagggcaCCACCa-ggaguGGagccugcggcUUAAuuugAUUCAACAcgggAAAACUUAccaggUCCGGAcauggGAAGGAUUgaCagauuGAAa  ZBBSR218
2213   25 ZBBSR217  GacGggagggcaCCACCa-ggaguGGagccugcggcUUAAuuugAUUCAACAcgggAAAACUUAccaggUCCGGAcauggGAAGGAUUgaCagauuGAAa  ZBBSR217
2213   26 ZBBSR211  GacGggagggcaCCACCa-ggaguGGagccugcggcUUAAuuugAUUCAACAcgggAAAACUUAccaggUCCGGAcauggGAAGGAUUgaCagauuGAAa  ZBBSR211
2213   27 Pchroma   GacGgaagggcaCCACCA-ggaguGGagcCugcggcUUAAuuugACUCAACAcgggAAAACUUAccaggUCCAGAcauagUAAGGAUUgaCagauuGAag  Pchroma
2213   28 EUGROTUN  GacGgaagggcaCCACCa-ggaguGGagcCugcGgcUUAAuuugACUCAACAcgggAAAACUUAccaggUCCAGAcauagUGAGGAUUgaCagauuGAAg  EUGROTUN
2213   29 ENTOGING  GacGgaagggca-CACCa-ggaguGGagcCugcggcUUAAuuugACUCAACAcgggAAAACUUAccaagACCGAAcauaAgaAGGAAUgaCagauuAAAg  ENTOGING
2213   30 DICDISCO  GacGgaagggca-CACAAUgaaguGGagcCugcggcUUAAuuugACUCAACUcgggAAAACUUAccaagCUAAGAuauagUAAGGAUUgaCagacuAAAa  DICDISCO
2213   31 ZTnucl    GacGgaagggcaCCACAa-gUuguGGauAcuguggcUUAAuuUgACUCAACAcUggAAAACUUAccagGUCCAGAcauaUUUAGGAUUgacagAUuAAUA  ZTnucl
2213   32 W10Z79    GacGgaagggcaCCACAa-gUuguGGauAcuguggcUUAAuuUgACUCAACAcUggAAAACUUAccagGUCCAGAcauagcUAGGAUUgacagAUuAAUA  W10Z79
2213   33 W10Z74    GacGgaagggcaCCACAa-gUuguGGauAcuguggcUUAAuuUgACUCAACAcUggAAAACUUAccagGUCCAGAcauaUUUAGGAUUgacagAUuAAUA  W10Z74
2213   34 W10Z72    GacGgaagggcaCCACAa-gUuguGGauAcuguggcUUAAuuUgACUCAACAcUggAAAACUUAccagGUCCAGAcauagcUAGGAUUgacagAUuAAUA  W10Z72
2213   35 W10Z10    GacGgaagggcaCCACAa-gUuguGGauAcuguggcUUAAuuUgACUCAACAcUggAAAACUUAccagGUCCAGAcauagcUAGGAUUgacagAUuAAUA  W10Z10
2213   36 ZBBSR7    GacGgaagggcaCCACAa-gguguGGaUccuguggcUUAAuuUgACUCAACAcUggGAAACUCAccagGUCCGGAcauaggUAGGACUgaCagauuGAUA  ZBBSR7
2213   37 ZBBSR173  GacGgaagggcaCCACAa-gauguGGaUucuguggcUCAAuuUgAUUCAACAcUggAAAGCUUAucagGUCUAGAcauuggaaCUAUAAaCagACuGAUA  ZBBSR173
2213   38 Siphcyan  GacGgaagggcaCCACAa-gUuguGUaUucuguggcUUAAuuugACUCAACAcaggAAAACUUAccagGUCUAGAcauaggaAUUAUUgaCggaUuUAUA  Siphcyan
2213   39 ZBBSR2    GacGgaagggcaCCACAa-gAuguGGaUccuguggcUUAAuuUgACUCAACAcUggAAAACUCAccagGUCCAGAcauaggUAGGAUUgaCagauuGAUA  ZBBSR2
2213   40 ZCR4      GacGgaagggcaCCACAa-gAuguGGaUucuguggcUUAAuuUgACUCAACAcUggAAAACUUAucagGUCCAGAcauaggUAGGAUUgaCagaUuGAUA  ZCR4
2213   41 ZCR16     GacGgaagggcaCCACAA-gauguGGaUccuguggcUUAAuuUgACUCAACAcUgaAAAAUUUAccUgGUCCGGAcauagCUUAGAUUgAUagauuGGUA  ZCR16
2213   42 ZCR6A     GacGgaagggcaCCACAa-gauguGGaUucuguggcUCAAuuugAUUCAACAcaggAAAGCUUAuuagGUCCAGAcauaGUAACUAUAgaCagaCuGAUA  ZCR6A
2213   43 PHYPOLYC  GacGgaagggca-CACAa-agaguGGAACCugcggcUUAAuuugACUCAACAcgggAAAACUCAccaggUCCGGAuacaCGUAUGAAAguCaagcuGAAa  PHYPOLYC
2213   44 NAEGRUBE  GacGgaaaggcaCCACCA-ggaguGGaguCugcggcUUAAuucgACUCAACAcgggGAAACUCAccaggUCAGGAcacaAGUUUGAUUgaCagguuAAUa  NAEGRUBE
2213   45 euk heli        37            38        39         40          40         41          42            43          euk heli
2213   46 rad        ---------->     ------>  --------->   --->        <---        ---->      ---->        -- --->        rad
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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

2313   1 1369mask   1111111111111111111111111111111111111111111111111111111111111111111-11111111111111111111111111111-11  1369mask
2313   2 MNELEIDY   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccuuaaccu-Gc  MNELEIDY
2313   3 DIAGRAND   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccWuaaccu-Gc  DIAGRAND
2313   4 ATHBOMBA   gCucuUucAUGAUUuuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgAUAACGaacGAGAccuuaaccu-Gc  ATHBOMBA
2313   5 BLAEMERS   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguuCguggauUGAuuuguCuggUU-AAUUccgAUAACGaacGAGAccuugaucu-Gc  BLAEMERS
2313   6 CHLREINH   gCucuUucUUGAuucugugGGuggUggugcauggccguuCuuAguuggugggUUGCcuugucAggUU-GAUUccGGUAACGaacGAGAccucagccu-Gc  CHLREINH
2313   7 ORYSATIV   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGCGAuuugucuggUU-AAUUccgUUAACGaacGAGAccucagccu-Gc  ORYSATIV
2313   8 PORAERUG   gCucuUucUUGAUUcuuugGGuggUggugcauggccguuCuuAguuGguggaGUGAuuuguCuggUU-GAUUccgUUAACGaacGAGAccuuaaccu-Gc  PORAERUG
2313   9 ACACAST1   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccuuaaccu-Gc  ACACAST1
2313  10 PHRBALAM   gCucuUucUAGAUUugcugGGuggUggugcauggccguuCuuAguuCgugguUUGAauuguCugUGUCAAUUccgCUAACGaacGAGAccuuaaccu-Gc  PHRBALAM
2313  11 STYALSID   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccuuaaccu-Gc  STYALSID
2313  12 EMIHUXLE   gCucuUucUUGAUUcgaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccgcagccu-Gc  EMIHUXLE
2313  13 OCHDANIC   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccccugccu-Gc  OCHDANIC
2313  14 CAFROENB   gGucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgAUAACGaacGAGAccuccgccu-Ac  CAFROENB
2313  15 LABMINUT   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccucagccu-Gc  LABMINUT
2313  16 OXYGRANU   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccuuagccu-Ac  OXYGRANU
2313  17 BLEAMERI   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgAUAACGaacGAGAccuuaaccu-Gc  BLEAMERI
2313  18 HARVERMI   gCucuUucUUGAUUcagugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccuuaaccu-Gc  HARVERMI
2313  19 THEANNUL   gCucuUucUUGAUUcuuugGGuggUggugcauggccguuCuuAguuGguggaGUGAuuuguCuggUU-AAUUccgUUAACGaacGAGAccuuaaccu-Gc  THEANNUL
2313  20 SYMPILO1   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccuuaaccu-Gc  SYMPILO1
2313  21 ZBBSR205   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccucagccu-Gc  ZBBSR205
2313  22 ZBBSR206   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccucagccu-Gc  ZBBSR206
2313  23 ZBBSR235   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccucagccu-Gc  ZBBSR235
2313  24 ZBBSR218   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccucagccu-Gc  ZBBSR218
2313  25 ZBBSR217   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccucagccu-Gc  ZBBSR217
2313  26 ZBBSR211   gCucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccucagccu-Gc  ZBBSR211
2313  27 Pchroma    A-ucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccucgAccu-Gc  Pchroma
2313  28 EUGROTUN   a-ucuUucUUGAUUcuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAccucgaccu-Gc  EUGROTUN
2313  29 ENTOGING   gAucuUucAUGAuuGuaugGGGagUggugcaUGgccguuCuuAguuGguggaAUGAuuuguCAggUU-AAUUccGGUAACGaacGAGAcuuaaAccu-Au  ENTOGING
2313  30 DICDISCO   gAucuUucAUGAUUcuaugAGuggUggugcauggucguuCuuAguugguggaGCGAuuugucuggUC-AAUUccgAUAACGgacGAGAccucgaccu-Gc  DICDISCO
2313  31 ZTnucl     gCCcugucCUGAUUUugugGCuggUggugcauggccguuCuuAguuggugaaGUGAuuugucuggUU-UAUUccgUUAACGaacGAGAcUauuAccA-A-  ZTnucl
2313  32 W10Z79     gCCcugucCUGAUuuugugGCuggUggugcauggccguuCuuAguuggugaaGUGAuuugucuggUU-UAUUccgUUAACGaacGAUAcUauuAccA-A-  W10Z79
2313  33 W10Z74     gCCcugucCUGAUUUugugGCuggUggugcauggccguuCuuAguuggugaaGUGAuuugucuggUU-UAUUccgUUAACGaacGAGAcUauuAccA-A-  W10Z74
2313  34 W10Z72     gCCcugucCUGAUuuugugGCuggUggugcauggccguuCuuAguuggugagGUGAuuugucuggUU-UAUUccgUUAACGaacGAGAcUauuAccA-A-  W10Z72
2313  35 W10Z10     gCCcugucCUGAUuuugugGCuggUggugcauggccguuCuuAguuggugaaGUGAuuugucuggUU-UAUUccgUUAACGaacGAGAcUauuAccA-A-  W10Z10
2313  36 ZBBSR7     guucuUucUUGAUucuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAcuaucaUuu-Gc  ZBBSR7
2313  37 ZBBSR173   gCGcuCuCUUGAuuuugugGGuUgUggugcauggccguuCuuAguugguggUGUGAUuugucuggUU-AAUUccgUUAACGaacGAGAcuauCuAuu-Gu  ZBBSR173
2313  38 Siphcyan   gCucuUucUUAAuuuugugGGUUguggugcauggccguuCCuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaauGAGGcuauuaUCu-Gu  Siphcyan
2313  39 ZBBSR2     gCuuuUucUUGAUucuaugGGuggUggugcauggucguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAcuaucaCuu-Ac  ZBBSR2
2313  40 ZCR4       gUuuuUucUUGAUucuaugGGuggUggugcauggccguuCuuAguuggugaaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAcuUccaCAu-Ga  ZCR4
2313  41 ZCR16      ggucuUCcUUGAUUuuaugGGuggUggugcauggccguuCuuAguugguggaGUGAuuugucuggUU-AAUUccgUUAACGaacGAGAcuauuaCuu-Gc  ZCR16
2313  42 ZCR6A      gCucuCucUUGAUUGugugGGuggUggugcauggccguuGuuAguugguggUGUGAUuugucuggUU-AAUUccgUUAACGaacGAGAcuauCUAuu-Au  ZCR6A
2313  43 PHYPOLYC   gAcuuUacUCAAUGAuguaAGuggUggUgcAUggucguuCUUAguuCguggaUUGAuuuguCuggUU-UAUUccgAUAACGagcGAGACcccgGCGUUCc  PHYPOLYC
2313  44 NAEGRUBE   gCccuUucUUGAUUGugugGUGggUagugcaUggccguuUCCAguuCguggaGUGAucuguCuugUU-AAUUcagAUAACGaacGAGAccuaagccu-u-  NAEGRUBE
2313  45 euk heli        43         42             44          45   46       46   47         47       45     48     4-9   euk heli
2313  46 rad         <--- --      <----  --------------------> -->----->    <------->       <--      <--    ----->------  rad
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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

2413   1 1369mask   111-1111--------------------------------------------------------------------------------------------  1369mask
2413   2 MNELEIDY   uaa-AUagugA--cacgguuc-------------------------------------------------------------------------------  MNELEIDY
2413   3 DIAGRAND   uaa-AUaguuA--cacGauau-------------------------------------------------------------------------------  DIAGRAND
2413   4 ATHBOMBA   uaa-AUagacA--ggccggcU-------------------------------------------------------------------------------  ATHBOMBA
2413   5 BLAEMERS   uaa-UUaguug--ggcuugcauUU----------------------------------------------------------------------------  BLAEMERS
2413   6 CHLREINH   uaa-AUagucA--gcaucgc--------------------------------------------------------------------------------  CHLREINH
2413   7 ORYSATIV   uaa-CUagcua--ugcggagC-------------------------------------------------------------------------------  ORYSATIV
2413   8 PORAERUG   uaa-CUaguGG--auuGaa----ucu--------------------------------------------------------------------------  PORAERUG
2413   9 ACACAST1   uaa-AUAugcC-gcgcuaacccGUCCAUCAAAACCCAUGCGUGgcucacgcgguccgCUgcgggguggugucgcu----UCGC-----------------  ACACAST1
2413  10 PHRBALAM   uaa-AUaAuGUUGGGC--AGAGGCCUUUGGUUUgauuAAguuucuuuuaUCGGGGCAACUCGGUuagaggggauGGagucUUAUCAAACUCUAGUucaaa  PHRBALAM
2413  11 STYALSID   uaa-AUagcgA--cgcgaa---------------------------------------------------------------------------------  STYALSID
2413  12 EMIHUXLE   uaa-AUagcGA--Cgcga----------------------------------------------------------------------------------  EMIHUXLE
2413  13 OCHDANIC   uaa-CUagucg--UcuGaaugc------------------------------------------------------------------------------  OCHDANIC
2413  14 CAFROENB   uagUCUagaug-uuucgucu--------------------------------------------------------------------------------  CAFROENB
2413  15 LABMINUT   uaa-AUagu-G-ugcauauc--------------------------------------------------------------------------------  LABMINUT
2413  16 OXYGRANU   uaa-CUaguCGAAc-c-aaUCUC-----------------------------------------------------------------------------  OXYGRANU
2413  17 BLEAMERI   uaa-CUagucG-UcucuuGCC-------------------------------------------------------------------------------  BLEAMERI
2413  18 HARVERMI   uaa-AUaguc-A-cgcgaacCgguccGCAA----------------------------------------------------------------------  HARVERMI
2413  19 THEANNUL   uaa-AUag-gguacgggAauaAGUUU--------------------------------------------------------------------------  THEANNUL
2413  20 SYMPILO1   uaa-AUag-uuAcauguaaccUC-----------------------------------------------------------------------------  SYMPILO1
2413  21 ZBBSR205   uaa-AUagu-gucauuuucuuc------------------------------------------------------------------------------  ZBBSR205
2413  22 ZBBSR206   uaa-AUagu-gucauuuucuuc------------------------------------------------------------------------------  ZBBSR206
2413  23 ZBBSR235   uaa-AUagu-gucauuuucuuc------------------------------------------------------------------------------  ZBBSR235
2413  24 ZBBSR218   uaa-AUag-uuagauu--cug-------------------------------------------------------------------------------  ZBBSR218
2413  25 ZBBSR217   uaa-AUag-uuagauu--cug-------------------------------------------------------------------------------  ZBBSR217
2413  26 ZBBSR211   uaa-AUaguc-acaUcu-cu--------------------------------------------------------------------------------  ZBBSR211
2413  27 Pchroma    uaa-AUaggggccGaauccU--------------------------------------------------------------------------------  Pchroma
2413  28 EUGROTUN   uaa-AUaguu--cuUgucauc-------------------------------------------------------------------------------  EUGROTUN
2413  29 ENTOGING   uaa-UUaguUGCauuuGAaauggAAAUGCA----------------------------------------------------------------------  ENTOGING
2413  30 DICDISCO   uaa-CUaguaG-UAUU-UAUUAGUCGAUAUAGACGAUAGCUUUUCUGGGGUUUGGAAUGAUUUCGGUCAUCUCCU-------------------------  DICDISCO
2413  31 ZTnucl     uAa-aUaguaa--gyacugcA-------------------------------------------------------------------------------  ZTnucl
2413  32 W10Z79     uAa-aUaguaa--gcgcuguAU------------------------------------------------------------------------------  W10Z79
2413  33 W10Z74     uAa-aUaguaa--guacugu--------------------------------------------------------------------------------  W10Z74
2413  34 W10Z72     uAa-aUaguaa--gcgcuAuGU------------------------------------------------------------------------------  W10Z72
2413  35 W10Z10     uAa-aUaguaa--gcgCuguGU------------------------------------------------------------------------------  W10Z10
2413  36 ZBBSR7     uaa-AUagcaG---Uaauuuuu------------------------------------------------------------------------------  ZBBSR7
2413  37 ZBBSR173   UUa-UUCgcaU---C-auaaU-------------------------------------------------------------------------------  ZBBSR173
2413  38 Siphcyan   Uaa-UUCgcgU----aauaaUU------------------------------------------------------------------------------  Siphcyan
2413  39 ZBBSR2     uaa-AUaguaC----auuuuuU------------------------------------------------------------------------------  ZBBSR2
2413  40 ZCR4       uaa-AUggcaC----aAuuu--------------------------------------------------------------------------------  ZCR4
2413  41 ZCR16      uaa-AUaguau----aaucAC-------------------------------------------------------------------------------  ZCR16
2413  42 ZCR6A      Uaa-UUCguaU----uauaaU-------------------------------------------------------------------------------  ZCR6A
2413  43 PHYPOLYC   uaa-UAGGGGU--GGC-AGCCAGACCGGUCGCAAGACAGGUUAGCUC-----------------------------------------------------  PHYPOLYC
2413  44 NAEGRUBE   uaa-CUAGCCGUAGGC---CUUUUCCUUCGGGGAAGGGUUAGUUUGUCGGAACAGGUUUC-----------------------------------------  NAEGRUBE
2413  45 euk heli             5-0                                                                                         euk heli
2413  46 rad        --->  -------------->                                                                                rad
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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:  Data:

2513  1 1369mask  --------------------------------------------------------------------------------------11111111111111  1369mask
2513  2 MNELEIDY  ----------------------------------------------------------------UUUgaaccgug--GCUc----acuUCuuagaggGAc  MNELEIDY
2513  3 DIAGRAND  -----------------------------------------------------------------UCguauGgug--GACa----acuUCuuagaggGAc  DIAGRAND
2513  4 ATHBOMBA  -----------------------------------------------------------------UUUgcugguc-GCGgu----AcuUCuuagaggGAc  ATHBOMBA
2513  5 BLAEMERS  ------------------------------------------------------------------augcaagu-AAUcca----acuUCuuagagaGAc  BLAEMERS
2513  6 CHLREINH  ----------------------------------------------------------------ACCUgcggu--gcGCCg----acuUCuuagaggGAc  CHLREINH
2513  7 ORYSATIV  ----------------------------------------------------------------CAUCCcuccgc-aGCUa----gcuUCuuagaggGAc  ORYSATIV
2513  8 PORAERUG  -----------------------------------------------------------------UCggauuGaauGGA------acuUCuuagaggGAc  PORAERUG
2513  9 ACACAST1  -------------------------ggcgacgucaucccgcCGGCAGGGCCcgggUccgugugggcGGUAggguuCggcguCCgugCUUCuuagaggGAc  ACACAST1
2513 10 PHRBALAM  cCAUUCUCCUCGACguuugaUUGggguauuuguUaucgggGCAAcucgguUauaaguauuuCAGUCUUAUGCAAAGAAUGGACCAaCuUCuuagagg-Ac  PHRBALAM
2513 11 STYALSID  ------------------------------------------------------------uCUUCGUauucgc-g-GAC--A-c-gcuUCuuagaggGAc  STYALSID
2513 12 EMIHUXLE  -----------------------------------------------------------------acCCUCCguucg--cUGGA-gcuUCuuagaggGAc  EMIHUXLE
2513 13 OCHDANIC  ---------------------------------------------------------------CUAGgcauug-aGAUCcg----acuUCuuagaggGAc  OCHDANIC
2513 14 CAFROENB  ------------------------------------------------------------------GCgga----cggUa-ryg-ucuUCcuagaggGAc  CAFROENB
2513 15 LABMINUT  ---------------------------------------------------------------------GAAAgaaugugUUcg-acuUCuuagaggGAc  LABMINUT
2513 16 OXYGRANU  --------------------------------------------------------------------CGA---uuggCUG-CG-acuUCuuagaggGAc  OXYGRANU
2513 17 BLEAMERI  ----------------------------------------------------------------------Aaaga---gUC-Gg-acuUCuuagaggGAc  BLEAMERI
2513 18 HARVERMI  ----------------------------------------------------------------gggcuCAAAguucgcgGCUCgacuUCuuagaggGAc  HARVERMI
2513 19 THEANNUL  ------------------------------------------------------------------CUACuguccc-guUa-uc-GcuUCuuagaggGAc  THEANNUL
2513 20 SYMPILO1  -------------------------------------------------------------------gguuacau---gGG-Ca-acuUCuuagaggGAc  SYMPILO1
2513 21 ZBBSR205  --------------------------------------------------------------------UGAagau---gUg-c--acuUCuuagaggGAc  ZBBSR205
2513 22 ZBBSR206  --------------------------------------------------------------------GGAagau---gUg-c--acuUCuuagaggGAc  ZBBSR206
2513 23 ZBBSR235  --------------------------------------------------------------------UGAagau---gUg-c--acuUCuuagaggGAc  ZBBSR235
2513 24 ZBBSR218  -----------------------------------------------------------------CACGUuagga--uuUu-a--acuUCuuagaggGAc  ZBBSR218
2513 25 ZBBSR217  -----------------------------------------------------------------CACGUuagga--uuUu-a--acuUCuuagaggGAc  ZBBSR217
2513 26 ZBBSR211  ------------------------------------------------------------------UUUUagagU--ugug-C--acuUCuuagaggGAc  ZBBSR211
2513 27 Pchroma   -----------------------------------------------------------------CU----ggau-uGgguccG-ccuUCuuagaggGAc  Pchroma
2513 28 EUGROTUN  ----------------------------------------------------------------GUGAgauggcU-ggaU-----acuUCuuagaggGAc  EUGROTUN
2513 29 ENTOGING  ------------------------------------------------------GAUAAUCCUAucauuAUUAGa-aauAAUAAUacuACuuaaaggGAC  ENTOGING
2513 30 DICDISCO  ----------------------------------------GCUUCAAGGAGUGUGUAGUCUGACUCGAUAGGUACGAAUU-AAA-acuUCuuagaggGAc  DICDISCO
2513 31 ZTnucl    ------------------------------------------------------------------UUAgcagug-uGAuu----acuUCuuAgAggGAc  ZTnucl
2513 32 W10Z79    -----------------------------------------------------------------UUUCgcagug-uGAuu----acuUCuuAgAggGAc  W10Z79
2513 33 W10Z74    -----------------------------------------------------------------AUUAacagug-uGAuu----acuUCuuAgAggGAc  W10Z74
2513 34 W10Z72    -----------------------------------------------------------------UUUCgCagug-uGAuu----acuUCuuAgAggGAc  W10Z72
2513 35 W10Z10    -----------------------------------------------------------------UUUCgcaCug-uGAuu----acuUCuuAgAggGAc  W10Z10
2513 36 ZBBSR7    ------------------------------------------------------------------UAAUaaaaa-uuUGu----gcuUCuuagAagGAc  ZBBSR7
2513 37 ZBBSR173  -----------------------------------------------------------------UUAUuuAGAa--uuuu----gcUUCuUUgAgaAAc  ZBBSR173
2513 38 Siphcyan  ------------------------------------------------------------------AGAUuAGug-uuUUu----gcUUCuuUaAgAAAc  Siphcyan
2513 39 ZBBSR2    ------------------------------------------------------------------UAAAUagaa-UauUu----gcuUCuuagAggGAc  ZBBSR2
2513 40 ZCR4      -----------------------------------------------------------------UUACAUagaA-uCAUu----gcuUCuuauAgGGAc  ZCR4
2513 41 ZCR16     -----------------------------------------------------------------UCCUGUAGga-uuguA----gcuUCuuagAggGAc  ZCR16
2513 42 ZCR6A     -----------------------------------------------------------------UUAUuuAGAa--uaUu----gcUUCuuUgAgaAAc  ZCR6A
2513 43 PHYPOLYC  -----------------------------------------------------------------GCCACCUGAA-GUUAU----GCUUCuuagACGUAU  PHYPOLYC
2513 44 NAEGRUBE  ----------------------------------------------------GGCCUGUUCCAAAACCUACGAGACUUUUGUCA-GCUUCuuaaaggGAc  NAEGRUBE
2513 45 euk heli                                                                                   50         49        euk heli
2513 46 rad                                                                              <----------------  <------  -  rad
```

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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

2613   1 1369mask  111----------------------------------------------------------------------------------------111111111  1369mask
2613   2 MNELEIDY  uau-cg-gu-------------------------------------------------------------------------uUCAaaccgauggAAGUu  MNELEIDY
2613   3 DIAGRAND  uau-cg-gu-------------------------------------------------------------------------GUUUAaccgauggAAGUu  DIAGRAND
2613   4 ATHBOMBA  ugu-ca-gc-------------------------------------------------------------------------GUCUAgcugacggAAGUu  ATHBOMBA
2613   5 BLAEMERS  uGc-ug-gc-------------------------------------------------------------------------GUUUAgccagAggAAGUu  BLAEMERS
2613   6 CHLREINH  uau-ug-gc-------------------------------------------------------------------------GUUUAgccaauggAAGUa  CHLREINH
2613   7 ORYSATIV  uAu-gg-cc-------------------------------------------------------------------------GUUUAggccaCggAAGUu  ORYSATIV
2613   8 PORAERUG  ACc-cu-gg-------------------------------------------------------------------------G-CAAccaggAGgAGGUu  PORAERUG
2613   9 ACACAST1  ugc-ugCgc-------------------------------------------------------------------------GCCUAgccagcggAAGUu  ACACAST1
2613  10 PHRBALAM  uau-uu-gcUC-------------------------------------------------------------------------CCAgcaaauggAAGUu  PHRBALAM
2613  11 STYALSID  uau-ccguGAU-----------------------------------------------------------------------UACCAacggauggAAGUu  STYALSID
2613  12 EMIHUXLE  Aac-uu-gu-------------------------------------------------------------------------CUUCAacaaguGgAAGUu  EMIHUXLE
2613  13 OCHDANIC  uuu-ug-gu-------------------------------------------------------------------------GACUAaccaaaggAAGUu  OCHDANIC
2613  14 CAFROENB  uau-gg-uc-------------------------------------------------------------------------GUUUAggccauggAAGUu  CAFROENB
2613  15 LABMINUT  Auu-uc-gg-------------------------------------------------------------------------UUUUAccggaaGgAAGUu  LABMINUT
2613  16 OXYGRANU  uuu-gu-gG-------------------------------------------------------------------------GCCAAAcacaaggAAGUu  OXYGRANU
2613  17 BLEAMERI  uuu-g--gg-------------------------------------------------------------------------gGCUAcuccaaggAAGUu  BLEAMERI
2613  18 HARVERMI  uau-ucgcACCCG-----------------------------------------------------------------------CCAgcgaauggAAGUu  HARVERMI
2613  19 THEANNUL  uuu-gc-gguU-----------------------------------------------------------------------AUAAaucgcaaggAAGUu  THEANNUL
2613  20 SYMPILO1  uuu-gu-gu-------------------------------------------------------------------------GUCUAacgcaaggAAGUu  SYMPILO1
2613  21 ZBBSR205  uAc-uu-gUGA-----------------------------------------------------------------------AACAACcaagCggAAGCu  ZBBSR205
2613  22 ZBBSR206  uAc-uu-gUGA-----------------------------------------------------------------------AACAACcaagCggAAGCu  ZBBSR206
2613  23 ZBBSR235  uAc-uu-gUGA-----------------------------------------------------------------------AACAACcaagCggAAGCu  ZBBSR235
2613  24 ZBBSR218  uAc-gc-guC------------------------------------------------------------------------UACAAacgcgAggAAGCu  ZBBSR218
2613  25 ZBBSR217  uAc-gc-guC------------------------------------------------------------------------UACAAacgcgAggAAGCu  ZBBSR217
2613  26 ZBBSR211  uAc-gu-gACC-----------------------------------------------------------------------UACAAGcgcgAggAAGCu  ZBBSR211
2613  27 Pchroma   uau-cggAUGG-------------------------------------------------------------------------UGUAccgguggAAGUC  Pchroma
2613  28 EUGROTUN  uau-cg-gu-------------------------------------------------------------------------GGAUAgccgauggAAGUu  EUGROTUN
2613  29 ENTOGING  ACG-ua-ucAAUCucuuuuuUAuacaGuaGauaauAAUUUGUGUUAUUUUUGAAUAauaaaAauuauAuaGuguaUCaaaaagaAAAgauaAAGGAAGCG  ENTOGING
2613  30 DICDISCO  uAccugcCU---------------------------------------------------------------------------CAAgcaggCggAAGUc  DICDISCO
2613  31 ZTnucl    ugg-ug-auA------------------------------------------------------------------------CAUAAguuacuggAGGCA  ZTnucl
2613  32 W10Z79    ugg-ug-auA------------------------------------------------------------------------CAUAAguuacuggAGGCA  W10Z79
2613  33 W10Z74    ugg-ug-auA------------------------------------------------------------------------CAUAAguuacuggAGGCA  W10Z74
2613  34 W10Z72    ugg-ug-auA------------------------------------------------------------------------CAUAAguuacuggAGGCA  W10Z72
2613  35 W10Z10    ugg-ug-auA------------------------------------------------------------------------CAUAAguuacuggAGGCA  W10Z10
2613  36 ZBBSR7    ugg-cu-uug------------------------------------------------------------------------CCuaaaUUgcuggAAGUu  ZBBSR7
2613  37 ZBBSR173  uag-Au-uug------------------------------------------------------------------------CUuaaaAAGcuggAAGUg  ZBBSR173
2613  38 Siphcyan  uag-uu-uug------------------------------------------------------------------------CCuaaaAAgcuggAAGUu  Siphcyan
2613  39 ZBBSR2    ugg-uu-uug------------------------------------------------------------------------UAuaaaUUgcuggAAGUu  ZBBSR2
2613  40 ZCR4      ugg-cu-uug------------------------------------------------------------------------CAuaaaUUgcuggACGAu  ZCR4
2613  41 ZCR16     Ugg-cU-uuu------------------------------------------------------------------------CAUgaaUCgcuUgAAGUu  ZCR16
2613  42 ZCR6A     uag-Au-uuG------------------------------------------------------------------------CUUAAaaaGcuggAAGUU  ZCR6A
2613  43 PHYPOLYC  Cag-ag-ccG-------------------------------------------------------------------------AUAAgguucuUGAAAu-  PHYPOLYC
2613  44 NAEGRUBE  uuc-auucGUAA-----------------------------------------------------------------------ACUAggaugaggAAGAu  NAEGRUBE
2613  45 euk heli      51                                                                                     51         euk heli
2613  46 rad       --------->                                                                              <-------   <  rad
```

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"Sept4aln" on 5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

2713    1 1369mask   111111111111111111111111111111111111111111111111111111111111111111111111111-------------------------  1369mask
2713    2 MNELEIDY   ugaggCaaUaacAggucUgugAugccCuuaGAUGUucuggGCCacAcgcgCguuACACugaugaagCCAGCGAGUAUAUCG-------------------  MNELEIDY
2713    3 DIAGRAND   ugaggCaaUaacAggucUgugAugccCuuaGAUGUucuggGCCgcACgcgCgcuACACugacaaggACAACGAGUUUAUAA-------------------  DIAGRAND
2713    4 ATHBOMBA   ugaggCaaUaacAggucUgugAugccCuuaGAUGUucuggGCCgcAcgcgCgcuACACugacagagCCAGCGAGUAUUUUU-------------------  ATHBOMBA
2713    5 BLAEMERS   ugaggCaaUaacAggucUgugAugccCuuaGAUGUucuggGCCgcAcgcgCgcuACACugauaaggUCAGCGAGUUUCAACUUGCUGGCUUAAGUUGAGU  BLAEMERS
2713    6 CHLREINH   ugaggCgaUaacAggUcugugAugccCuuaGAUGUucuggGCCgcAcgcgCgcuACACugacgcgACCAACGAGCCUAU---------------------  CHLREINH
2713    7 ORYSATIV   ugaggCaaUaacAggUcugugAugccCuuaGAUGUucuggGC-gcAcgcgCgcuACACugaugUaUCCAACGAGUAUAUAG-------------------  ORYSATIV
2713    8 PORAERUG   ugagGCaaUaacAggUcugugAugccCuuaGAUGUucuggGCCgcAcgcgCgcuACACugaugcauGCAGCGAGUGUA----------------------  PORAERUG
2713    9 ACACAST1   ugaggCaaUaacAggUcugugAugccCuuaGAUGUucuggGCCgcAcgcgCgcuACACugauuaaUCCAACGAGUCCGCUUCAAUCGAGGCGCGAUG-CC  ACACAST1
2713   10 PHRBALAM   ugaggCaaUaacAggucUgugaugcccuucgAUGCccuggGCCgCAcgcgCgcuACACugauuggguGAAUUAGCAuguguACAccccAcuuuuuccuug  PHRBALAM
2713   11 STYALSID   ugaggCaaUaacAggUcugugAugccCuuaGAUGUucuggGCCgcAcgcgCgcuACACugaugcaUUCAACGAGUA------------------------  STYALSID
2713   12 EMIHUXLE   cgcggCaaUaacAggUcugugAugccCuuaGAUGUucuggGCCgcAcgcgCgcuACACugAugcaCUCAACGAGUCUAUCA-------------------  EMIHUXLE
2713   13 OCHDANIC   aggggCaaUaacAggUcugugAugccCuuaGAUGUccuggGCCgcAcgcgCgcuACACugAcacaUGCAGCGAGUUCUU---------------------  OCHDANIC
2713   14 CAFROENB   ggaggCaaUaacAggUcugugAugccCuuaGAUGUccuggGCCgcAcgcgCgcuACACugGcuggUGCAACAAGUGUCUACA------------------  CAFROENB
2713   15 LABMINUT   ugaggCaaUaacAggUcugugAugccCuuaGAUGUucuggGCCgcAcgcgCgcuACAAugAugaaUUCAACGAGUUUAUAA-------------------  LABMINUT
2713   16 OXYGRANU   ugaggCaaUaacAggUcugugAugccCuuaGAUGUccuggGCCgcAcgcgUgcuACACugacgcauACAGCGAGUACGUC--------------------  OXYGRANU
2713   17 BLEAMERI   uaaggCaaUaacAggUcugugAngccCuuaGANG-ccuggGCCgcAcgcgCgcuACACugaugcagGCAGUAAGCCCU----------------------  BLEAMERI
2713   18 HARVERMI   ugaggCaaUaacAggUcugugAugccCuuaGAUGUucucgGCCgcACgcgCguuACACugacggagUCAACGAGCGUUUUU-------------------  HARVERMI
2713   19 THEANNUL   uaaggCaaUaacAggUcugugAugccCuuaGAUGUccuggGCUgcAcgcgCgcuACACugaugcguUCAUCGAGUUUA----------------------  THEANNUL
2713   20 SYMPILO1   ugaggCaaUaacAggucUgugAugccCuuaGAUGUccuggGCUgCAcgcgCgcuACACugaugcgCUCAACGAGUUUGCAA-------------------  SYMPILO1
2713   21 ZBBSR205   ugaggCaaUaacAggUcugugAugccCuuaGAUGUucuggGCCgcAcaugggcuACACuggugcaUUCAACAAGCAUUAUAA------------------  ZBBSR205
2713   22 ZBBSR206   ugaggCaaUaacAggUcugugAugccCuuaGAUGCucuggGCCgcAcaugggcuACACuggugcaUUCAACAAGCAUAAUCA------------------  ZBBSR206
2713   23 ZBBSR235   ugaggCaaUaacAggUcugwgAugccCuuaGAUGUucuggGCCgcAcaugggcuACACuggugcaUUCAACAAGCAUUAUAA------------------  ZBBSR235
2713   24 ZBBSR218   ugaggCaaUaacAggUcugugAugccCuuaGAUGUucuggGCCgcAcgcgggcuACACugguguuUUCAACAAGCCUUAUUU------------------  ZBBSR218
2713   25 ZBBSR217   ugaggCaaUaacAggUcugugAugccCuuaGAUGUucuggGCCgcAcgcgggcuACACugguguuUUCAACAAGCCUUAUUU------------------  ZBBSR217
2713   26 ZBBSR211   ugaggCaaUaacAggUcugugAugccCuuaGAUGUucuggGCCgcAcgcgggcuACACugguguuUUCAACAAGCCUAGUAC------------------  ZBBSR211
2713   27 Pchroma    cgaggCaaUaacAggucUgugAugccCuuaGAUGUucuggGCCgcAcgcgCgcuACACugacacguUCAUCAAGUUUACAAC------------------  Pchroma
2713   28 EUGROTUN   cgaggCaaUaacAggUcugugAugccCuuaGAUGUucuggGCUgcACgcgCgcuACACugauacguUCAUCGAGCUCUA---------------------  EUGROTUN
2713   29 ENTOGING   uuaagCaaUaacAgguCugugAUGCCCUUAGACAUcuuggGCUgcAcgcgCgcuACAAugAaaauACUAAAGAGUAGUACCUUACUAGUGGAUUUUUAU  ENTOGING
2713   30 DICDISCO   cgaggCaaUaacAggUcugugAugccCuuaGAUACcuuggGCCgcAcgcgCgcuACAAuguaggaaACAAAAAGGCU-----------------------  DICDISCO
2713   31 ZTnucl     aguUgCaaUgacAggUcugugAugccCuuaGAUGUAcuggGCCgcGcacggGauACAACaggggAgAUAAUAUGUACAUUUAAA----------------  ZTnucl
2713   32 W10Z79     aguUgCaaUgacAggUcugugAugccCuuaGAUGUAcuggGCUgcGcacggGauACAACaggggAgAUAAAAUGUACAUU-AAA----------------  W10Z79
2713   33 W10Z74     aguUgCaaUgacAggUcugugAugccCuuaGAUGUAcuggGCUgcGcacggGauACAACaggggAgAUAAAAUGUACAUU-AAA----------------  W10Z74
2713   34 W10Z72     aguUgCaaUgacAggUcugugAugccCuuaGAUGUAcuggGCUgcGcacggGauACAACaggggAgAUAAAAUGUACAUU-AAA----------------  W10Z72
2713   35 W10Z10     aguUgCaaUgacAggUcugugAugccCuuaGAUGUAcuggGCUgcGcacggGauACAACaggggAgAUAAAAUGUACAUU-AAA----------------  W10Z10
2713   36 ZBBSR7     gguagCaaUaacAggUcugugAugccCuuaGAUGUUcuggGCAgcGcacgggCuACAAUaggagAgUAACUGAGUUUGUU-CAA----------------  ZBBSR7
2713   37 ZBBSR173   UguagCaaUaacAggUcugugAugccCuUaGAUGUUcuggGCAgcGcacgggCuACAAUAggaGaaUUAGUACGCUUAC--CAU----------------  ZBBSR173
2713   38 Siphcyan   gguagCaAUaacAggUcugugAugccCauCGACGUUuuggGCAgcGcacgggCuACAAUAGgagAaUUACUAUGUUUAC--UAU----------------  Siphcyan
2713   39 ZBBSR2     gguagCaaUaacAggUcugugAugccCuuaGAUGUUcuggGCAgcGcacgggCuACAAUagaagagUAAGUGAGUUUGUU-CGA----------------  ZBBSR2
2713   40 ZCR4       ggUagCaaUaacAggUcugugAugccCuuaGAUGUUcuggGUAgcGcacgggCuACAAUagaaGAgUAAAGAAGUUUGGG-UAA----------------  ZCR4
2713   41 ZCR16      aguagCaaUaacAggUcugugAugccCuuaGAUGUCcUggGCRgcGcacgggCuACAAUaggaGAGUAAUUUAGCUUACU-CUA----------------  ZCR16
2713   42 ZCR6A      UguagCaaUaacAggUcugugAugccCuuaGAUGUUcuggGCAgcGcacgggCuACAAUaagaGAAUCAGUACGCUUAC--UAU----------------  ZCR6A
2713   43 PHYPOLYC   -gggUUaaUaacAggucAguCAUGccCuuaGAUGUucuggGCCgcAcgcgCGUUACAAUggcaUgUAAAACGAGUGCUUGAACAAGGCGUC---------  PHYPOLYC
2713   44 NAEGRUBE   uuaggCCAUaacAggucugugAUGcuCuuAGAUGUccuggGCUgcAcgcgUacuACAAuaacgguACCAGCGAGCGCUAugguuuuauAACC--------  NAEGRUBE
2713   45 euk heli    48            44                    41          39          52                                        euk heli
2713   46 rad        ----- <------------------- --      <----   <- ---- ---     ------->                                   rad
```

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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

2813   1 1369mask   ---------------------------------------------------------11111111--1111--11111--1111-1111111111111  1369mask
2813   2 MNELEIDY   ---------------------------------------------------------ccuUcacc--GGAA--ggug-C-gggU-AAUCUUGUGAAAc  MNELEIDY
2813   3 DIAGRAND   ---------------------------------------------------------ccuUcacc--GGUA--ggugCCUgggU-AAUCUUUUGAGCc  DIAGRAND
2813   4 ATHBOMBA   ---------------------------------------------------------ccuUggcc--GGAA--ggucU--gggU-AAUCUUGUGAAAc  ATHBOMBA
2813   5 BLAEMERS   UUCGGCUCGGCUUAAGCUGGGAUU---------------------------------ccuUggcc--GUGA--ggucU--gggU-AAUCUUUUGAAAc  BLAEMERS
2813   6 CHLREINH   ---------------------------------------------------------ccuUggcc--GAGA--ggccC--gggU-AAUCU-UGUAAAC  CHLREINH
2813   7 ORYSATIV   ---------------------------------------------------------ccuggUcc--GACA--ggccC--gggU-AAUCUUGGGAAAU  ORYSATIV
2813   8 PORAERUG   ---------------------------------------------------------ccuUgguc--GGAA--ggccU--gggG-AAUCUUGUGAAAa  PORAERUG
2813   9 ACACAST1   -GU-UGGGGUCAAACCCAACU--------------GUGUC-GCUGUCCUCGAUCGCGccugggcc--GAUA--ggucc--gggU-AAUCUUUGCAAAU  ACACAST1
2813  10 PHRBALAM   uucuucucGAAA-------gggaggggcagggaggaggCggggAUAAauguaCCUACccuGaguc--GAAA--gacuU-CaggU-AACCUGCUGAAac  PHRBALAM
2813  11 STYALSID   ----------------------------------------------------UUCAUccuUgguc--GGAA--ggccU--gggU-AAUCUUUUGAAAU  STYALSID
2813  12 EMIHUXLE   ---------------------------------------------------------ccuUgacc--GAGA--ggucC--gggU-AAUCUUUUGAAAU  EMIHUXLE
2813  13 OCHDANIC   ---------------------------------------------------------ccuUgUcc--GAAA--ggUcU--gggU-AAUCUUGUCAAUG  OCHDANIC
2813  14 CAFROENB   ---------------------------------------------------------cguUggccUCGAGA--ggccU--gcgC-AAUCUUG-GAACG  CAFROENB
2813  15 LABMINUT   ---------------------------------------------------------ccuUgguu--GAAA--agccU--gggU-AAUCUUUUGAACU  LABMINUT
2813  16 OXYGRANU   ---------------------------------------------------------ccagcucc--GCGA-ggCagc--uggU-AAUC--AGCAAUa  OXYGRANU
2813  17 BLEAMERI   ---------------------------------------------------------ccgCggca--GAAA-AugccC--cggU-AACCU--UCAAAC  BLEAMERI
2813  18 HARVERMI   ---------------------------------------------------------ccuUgccc--GACA--gggcU--gggU-AACCUCCUGAAAc  HARVERMI
2813  19 THEANNUL   --------------------------------------------------------UccuUggcc--GAGA--ggccU--gggU-AAUCU--UUAGUa  THEANNUL
2813  20 SYMPILO1   --------------------------------------------------------AucuUgccU--GAAA--UggcC--gggU-AAUCUUUUUAAAA  SYMPILO1
2813  21 ZBBSR205   ---------------------------------------------------------ccuUagcc--GAAG--gguuU--gggU-AAUCU-UUGANAU  ZBBSR205
2813  22 ZBBSR206   ---------------------------------------------------------ccuUaacc--GAAG--gguuU--gggU-AAUCU-UUGAAAU  ZBBSR206
2813  23 ZBBSR235   ---------------------------------------------------------ccuUagcc--GAAG--gguuU--gggU-AAUCU-UUGAAAU  ZBBSR235
2813  24 ZBBSR218   ---------------------------------------------------------ccuUgacc--GAAA--ggucU--gggU-AAUCU-UUGAAAU  ZBBSR218
2813  25 ZBBSR217   ---------------------------------------------------------ccuUgacc--GAAA--ggucU--gggU-AAUCU-UUGAAAU  ZBBSR217
2813  26 ZBBSR211   --------------------------------------------------------UccuGgacc--GAAA--gguuU--gggU-AAUCU-UUGAAAU  ZBBSR211
2813  27 Pchroma    ---------------------------------------------------------cuuGgCuc--GAAA--ggCcU--gggU-AAUCUUUUAAAAa  Pchroma
2813  28 EUGROTUN   ---------------------------------------------------------ccuUgacu--GAAA--agucU--gggU-AAUCUUCUCAACg  EUGROTUN
2813  29 ENTOGING   UCCAUUA--------------UAUUGUAUAAUGGAGUAAAAAGAAACAGUAGUAGUac-AAAuuu--GAAA--aaaG---GAguAAAACU-CAAAAAa  ENTOGING
2813  30 DICDISCO   ---------------------------------------------------------ccuggucc--GGAA--ggauu--gggU-AAUCAUUUGAAUu  DICDISCO
2813  31 ZTnucl     ---------------------------------------------------------caUAauuU--GACAA-UaauA--UugU-AACCG--YGAAUC  ZTnucl
2813  32 W10Z79     ---------------------------------------------------------caUAauuU--GACAA-UaauA--UugU-AACCG--UGAAUC  W10Z79
2813  33 W10Z74     ---------------------------------------------------------caUAauuU--GACAA-UaauA--UugU-AACCG--UGAAUC  W10Z74
2813  34 W10Z72     ---------------------------------------------------------caUAauuU--GACAA-UaauA--UugU-AACCG--UGAAUC  W10Z72
2813  35 W10Z10     ---------------------------------------------------------uaUAauuU--GACAA-UaauA--UugU-AACCG--UGAAUC  W10Z10
2813  36 ZBBSR7     ---------------------------------------------------------cauUguuu--GAGAA-gaacU--gugG-AAUCU--UAAAAC  ZBBSR7
2813  37 ZBBSR173   ---------------------------------------------------------cuuaAUuc--GAAAA-gaCCu--gagA-AAUCA--UGAAAC  ZBBSR173
2813  38 Siphcyan   ---------------------------------------------------------cuuaauuc--GAAAA-gaguu--gagU-AAUCA--UGAAGG  Siphcyan
2813  39 ZBBSR2     ---------------------------------------------------------cUuagcuc--GAAAA-gggcu--gUgG-AAUCU--UAAAAU  ZBBSR2
2813  40 ZCR4       ---------------------------------------------------------cauAgcuc--AAAAA-gggcC--gugA-AAUCU--UAAAAC  ZCR4
2813  41 ZCR16      ---------------------------------------------------------cUuagcUC--GAAAA-AUgcu--aUgA-AAUCU--UUAAAC  ZCR16
2813  42 ZCR6A      ---------------------------------------------------------uUGAauuc--gAAAc-gaau---GUgA-AAUCA--UGAAAC  ZCR6A
2813  43 PHYPOLYC   ---------------------------------------------------------ccaCggcc--GAAA--ggucG--uggU-AACC--CUUAGUC  PHYPOLYC
2813  44 NAEGRUBE   ---------------------------------------------------------ccuUauccu-AaUa--ggauu--gggA-AAACUUUUCAAAC  NAEGRUBE
2813  45 euk heli                                                                 53               53                    euk heli
2813  46 rad                                                               -------->       <---------                  rad
```

```
"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:   Data:

2913    1 1369mask  11111111111111111111111111111111111111111111111111111111111-11111-111111111111111111111111111111111  1369mask
2913    2 MNELEIDY  uucaucgUGCUGGGGAUAgaccaUUGCAAUUAUuggucUUGAACGAGGAauuccUAGUAa-gcacg-agUCAUCAAcucgugcuGAUUACGUCCCugccc  MNELEIDY
2913    3 DIAGRAND  cuugycgUGCUGGGGCUAgaucUUUGUAAUUUUCgaucUUCAACGAGGAauuccuAGUAa-gcgug-auUCAUCAGaucgcguuGAUUACGUCCCugccc  DIAGRAND
2913    4 ATHBOMBA  ucugucgUGCUGGGGAUAgagcaUUGCAAUUAUugcucUUCAACGAGGAauaccuAGUAa-gcgca-agUCAUCAGcuugcguuGAUUACGUCCCugccc  ATHBOMBA
2913    5 BLAEMERS  cuuaucgUGAUGGGGAUUgaccUUUGUAAUUAUCggucAUGAACGAGGAauaccuAGUAa-gugug-ggUCAUCAAcccacgcuGAUUACGUCCCugccc  BLAEMERS
2913    6 CHLREINH  cgcgucgUGAUGGGGAUagauuaUUGCAAUUAUuagucuUCAACGAGGAaugccuAGUAa-gcgcg-agUCAUCAGcucgcguuGAUUACGUCCCugccc  CHLREINH
2913    7 ORYSATIV  uUcaucgUGAUGGGGAUagaucaUUGCAAUUGUuggucuUCAACGAGGAaugccuAGUAa-gcgcg-agUCAUCAGcucgcguuGACUACGUCCCugccc  ORYSATIV
2913    8 PORAERUG  ugcaucgUGCUGGGGAUagauuaUUGUAAUUAUuaaucuUCAACGAGGAaugccuUGUAa-gcgca-agUCAUCAGcuugcguuGAAUACGUCCCugccc  PORAERUG
2913    9 ACACAST1  uuaaucgUGCUGGGGAUagaucaUUGUAAUUAUugaucuUCAACGAGGAauuccuAGUAa-gcgcg-agUCAUCAGcucgcguuGAUUACGUCCCugccc  ACACAST1
2913   10 PHRBALAM  cuaaucgUGAUGGGGAUaggccUUUGCAAUUAUUggccuUAAACGAGGAauuccuAGUAggcCGCCUAGUCAUC--MKUGCgccGAUUACGUCCCugccc  PHRBALAM
2913   11 STYALSID  ugcaucgUGCUGGGGAUagauuaUUGGAAUUAUuaaucuUGAACGAGGAauuccuUGUAa-gcgug-agUCAUCAGcucgcguuGAAUACGUCCCugccc  STYALSID
2913   12 EMIHUXLE  ugcaUcgUGAUGGGGAUAgauuaUUGCAACUAUuaaucUUCAACGAGGAauuccUAGUAa-gcgug-ugUCAUCAGcgcacguuGAUUACGUCCCugccc  EMIHUXLE
2913   13 OCHDANIC  ugugUcgUGAUAGGGAUAgauuaUUGCAAUUAUuaaucUUGAACGAGGAauuccUAGUAa-augcg-ggUCAUCAGcucgcguuGAUUACGUCCCugccc  OCHDANIC
2913   14 CAFROENB  ccagCcgUGAUGGGGAUAgguuCUUGCAAUUUUUaauCUUCAACGAGGAauuccUAGUAa-aCgca-arUCAUCAGcuugcAuuGAUUACGUCCCugccc  CAFROENB
2913   15 LABMINUT  uucgUcgUGAUGGGGCUAgaccCUUGCAAUUAUUggucUCCAACGAGGAauuccUAGUAa-aCgca-agUCAUCAGcuugcAuuGAUUACGUCCCugccc  LABMINUT
2913   16 OXYGRANU  ugcgucgUGAUGGGGAUAgaucuUUGGAAUUAUagaucUUGAACGAGGAauuccuAGUAa-gcgca-agUCAUUACcuugcgcuGAUUAAGUCCCugccc  OXYGRANU
2913   17 BLEAMERI  ugcaucgUGAUGGGGAUUgacuCUUGGAAUUAUUggucAUCAACGAGGAauuccuAGUAa-aCgca-agUCAUCAAcuugcAuuGACUACGUCCCugccc  BLEAMERI
2913   18 HARVERMI  uccgucgUGAUGGGGAUagaucaUUGCAAUUAUugaucuUGAACGAGGAauuccuAGUAa-gcgcg-agUCAUCAAcucgcgcuGAUUACGUCCCugccc  HARVERMI
2913   19 THEANNUL  cgcaucgUGAUGGGGAUCgauuaUUGCAAUUGUuaaucGUGAACGAGGAaugccUAGUAu-gcgca-agUCAUCAGcuugugcaGAUUACGUCCCugccc  THEANNUL
2913   20 SYMPILO1  UgcaucgUGAUGGGGAUAgaucaUUGCAAUUAUugaucUUCAACGAGGAauuccUAGUAa-gcgcg-aguCAUCAgcucgugcuGAUUACGUCCCugccc  SYMPILO1
2913   21 ZBBSR205  ugcaccgUGCUUGGGAUAgacuaUUGCAAUUUUuagucUUCAACGAGGAauuccuAGUAa-aCgca-agUNAUCAGcuugcAuuGAUUACGUCCCugccc  ZBBSR205
2913   22 ZBBSR206  ugcaccgUGCUUGGGAUAgacuaUUGCAAUUUUuagucUUCAACGAGGAauuccuAGUAa-aCgca-agUCAUCAGcuugcAuuGAUUACGUCCCugccc  ZBBSR206
2913   23 ZBBSR235  ugcaccgUGCUUGGGAUAgacuaUUGCAAUUUUuagucUUCAACGAGGAauuccuAGUAa-aCgca-agUCAUCAGcuugcAuuGAUUACGUCCCugccc  ZBBSR235
2913   24 ZBBSR218  aacaccgUGCUUGGGAUAgacuaUUGCAAUUUUuagucUUCAACGAGGAauuccuAGUAa-aCgca-agUCAUUAGcuugcAuuGAUUACGUCCCugccc  ZBBSR218
2913   25 ZBBSR217  aacaccgUGCUUGGGAUAgacuaUUGCAAUUUUuagucUUCAACGAGGAauuccuAGUAa-aCgca-agUCAUUAGcuugcAuuGAUUACGUCCCugccc  ZBBSR217
2913   26 ZBBSR211  aacaccgUGCUUGGGAUAgacuaUUGCAAUUUUuagucUUCAACGAGGAauuccuAGUAa-aCgca-agUCAUUAGcuugcAuuGAUUACGUCCCugccc  ZBBSR211
2913   27 Pchroma   cgugucgUGAUGGGGAUAgauuaUUGUAAUUAUugaucUUCAACGAGGAauuccUAGUAa-aCgcg-ggUCAUCAAcccgnAuuGAUUACGUCCCugccc  Pchroma
2913   28 EUGROTUN  cguaucgUGAUGGGGAUagauuaUUGUAAUUUUugaucuUCAACGAGGAauuccuAGUAa-aCgcg-cguCAUUAacgcgcAuuGAUUACGUCCCugccc  EUGROTUN
2913   29 ENTOGING  uuAuAcaUGACAGGGAUuaaugaUUGAAAUUAUuuguuaUGAACGAGGAauuccuUGUAa-aAuug-ugUCAUUAAcauaaGuuGAAUACGUCCCugccc  ENTOGING
2913   30 DICDISCO  uccuacgUAACUGGGCUugaucUUUGUAAUUAUUgaucaUAAACGAGGAauuccUUGUAa-gcgua-agUCAUUACcuuaugcuGAAUAUGUCCCugccc  DICDISCO
2913   31 ZTnucl    uGucuuuAAUAUGGAAUugCACuaUGCAAUUUu-aCAcaUAAACUAGGAauaUcuUGUAa-gUaca-ugUCAUAAUcguguUcuGAAUGCGUCCCugucc  ZTnucl
2913   32 W10Z79    uGccuuuAAUAUGGAAUugCgCuaUGCAAUUUu-acAcaUAAACUAGGAauaUcuUGUAa-gUaca-ugUCAUAAUcguguUcuGAAUGCGUCCCugucc  W10Z79
2913   33 W10Z74    uGccuuuAAUAUGGAAUugCACuaUGCAAUUUu-aCAcaUAAACUAGGAauaUcuUGUAa-gUaca-ugUCACAAUcguguUcuGAAUGCGUCCCugucc  W10Z74
2913   34 W10Z72    uGccuuuAAUAUGGAAUugCgCuaUGCAAUUUu-acAcaUAAACUAGGAauaUcuUGUAa-gUaca-ugUCAUAAUcguguUcuGAAUGCGUCCCugucc  W10Z72
2913   35 W10Z10    uGccuuuAAUAUGGAAUugCgCuaUGCAAUUUu-acAcaUAAACUAGGAauaUcuUGUAa-gUaca-ugUCAUAAUcguguUcuGAAUGCGUCCCugucc  W10Z10
2913   36 ZBBSR7    uCuucuuUAUAUGGAAUcacgCuUUGCAACUUUaCcgugUAAACUAGGAauaccuAGUAa-guguU-uguCAUCAacgUacgcuGAAUACGUCCCugccc  ZBBSR7
2913   37 ZBBSR173  uuAuuuAUAAUUGGGCUuacgCaUuGAAGUUgUuAuguaCAAACCAGGAauaucuAGUAa-auAga-uguCAUUAacauuGauuGAAUAUGUCCCugccu  ZBBSR173
2913   38 Siphcyan  uGAcuuAUAAUUGGGCUuacaCaUUGAAAUUUUuUuguaUAAACCAGGAauaUcuAGUAa-gUcaa-GguCAAUAacAuugUuuGAAUGCGUCCCugccu  Siphcyan
2913   39 ZBBSR2    uuuuyuuUAUAUGGAAUcasgCUUUGAAAUUUUAAcgugUAAACUAGGAauaCcuAGUAa-gugua-uguCAUCAacgugcauuGAAUAUGUCCCugccc  ZBBSR2
2913   40 ZCR4      uGuucuAUAUAUGGAAUuacGCuUuGAAAUUaUaCGguaUAAACCAGGAauaCcuAGUAa-guguA-uguCACUAacgCguacuGAAUACGUCCCugccc  ZCR4
2913   41 ZCR16     GAuucuUUAUUUGUAAUcaugCuUUGAAAUUUUaAcaugUAAACUAGGAauaucCAGUAa-guguU-UguCAUCAacUUgcacuGAAUACGUCCCugccc  ZCR16
2913   42 ZCR6A     uuACuuACAAUUGGGCUuacgCaUuGAAAUUaUuAuguaCAAACCAGGAauaucuAGUAa-aUaga-uguCAUUAacauugCuuGGAUAUGUCCCugccu  ZCR6A
2913   43 PHYPOLYC  cCugcuUUGACUGGGACAgaucUUUGCAAUUAUUggucUCAAACGAGGAauuuuuAGUAa-Ucgca-ggUCAUUAAccugcgUuGAAUGCGUCCCugccc  PHYPOLYC
2913   44 NAEGRUBE  accguuAUGACAGGGAUcgaggaUUGGAAC-Au-ccucgUGAACGAGGAauuccUAGUAa-gCgug-guUCAUCAUaccacAuuGAUUACGUCCCUgccu  NAEGRUBE
2913   45 euk heli     52              54             54              38          55              55                     euk heli
2913   46 rad       <------          ----->        <------          <-----    ---------->     <--------           <----  rad
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"Sept4aln" on  5-SEP-96 15:33:41

 Posi-    Sequence
 tion:    identity:   Data:

3013    1 1369mask   1111111111111111111111111111111---111111111111111111111111------------------------------------------  1369mask
3013    2 MNELEIDY   uuuguACACACCGCCCGUCGCuacuAccGAUU--gaaugguUUaguGAgaUcUCGGGAuuggcGAcgc--cAugcy-----GCG-----A-gg------c  MNELEIDY
3013    3 DIAGRAND   uuuguACACACCGCCCGUCGCuacuAccGAUU--gaaugguUUaguGAgaUuUUGGGAcugacAcggg--uAgguuG----GCA-----ACaac-----c  DIAGRAND
3013    4 ATHBOMBA   uuuguACACACCGCCCGUCGCuacuAccGAUU--gaauggcUUaguGAggucUCCGGAuuggcUUugu---ggAgccg---GAA-----Acggc-----A  ATHBOMBA
3013    5 BLAEMERS   uuuguACACAcCGcCCGUCGCuacuAccGAUU--ggauggcUUaguGAgaUuUUGGAuugccgggug---Auugca----GCA-----Augcg-----a  BLAEMERS
3013    6 CHLREINH   uuuguACACACCGCCCGUCGCuccuAccGAUU--gggugUgCUgguGAagugUUCGGAuugagcUUggc----ug----ggGCA-----Accug-----g  CHLREINH
3013    7 ORYSATIV   uuuguACACACCGCCCGUCGCuccuAccGAUU--gaaugguCCgguGAagugUUCGGAucgcggcgAcg--gggg---cgg-UUC----Gcc-------g  ORYSATIV
3013    8 PORAERUG   uuuguACACACCGCCCGUCGCuccuAccGAUU--ggaugguCCgguGAaaugUUCGGCcugugcuug-cuUUg------gggCA-----A--cucu--gg  PORAERUG
3013    9 ACACAST1   uuuguACACACCGCCCGUCGCuccuAccGAUU--gaaugguCCgguGAaaucCUCGGAgccguggCCucuAcgcaauccggGCAAccgggCuugugaggU  ACACAST1
3013   10 PHRBALAM   uuuGUACACACCGCCCGUCGCuccuAccGAUU--ggaugauCCgguGAagauAACGGAugUGUaaaguauaggacccUUggg---ggcUU-gcucucUUg  PHRBALAM
3013   11 STYALSID   uuuguACACACCGCCCGUCGCuccuAccGAUU--gaaugguCCgguGAaaugUUCGGAuugggacgU----auACUgccgguUUU-----acu------g  STYALSID
3013   12 EMIHUXLE   uuuguACACACCGCCCGUCGCuccuAccGAUU--gaaugauCCgguGAggCcCCCGGAcugcggcgCcg--cAgcugg----UUC----Uccag-----c  EMIHUXLE
3013   13 OCHDANIC   uuuguACACACCGCCCGUCGCaccuAccGAUU--gaaugauUCgguGAaaCuUUCGGAccgugguUcuu--gccac------UUC-----Ggu------g  OCHDANIC
3013   14 CAFROENB   uuuguACACACCGCCCGUCGCaccuAccGAUU--ggaugguCCgguGAagccCUCGGAucg-Gccu-U-------------GA-C-------cuc----c  CAFROENB
3013   15 LABMINUT   uuuguACACACCGCCCGUCGCaccuAccGAUU--gaaugguCCgguGAaaucUUCGGAuugagacUUg---AuuuC-----UUC---ACGGA------a  LABMINUT
3013   16 OXYGRANU   uuuguACACACCGCCCGUCGCuccuAccGAUUUCgagugguCCgguGAacCuUUUGGAcugcgcgaggc-------------CCC---G----------A  OXYGRANU
3013   17 BLEAMERI   uuuguACACACCGCCCGUCGCuccuAccGAUUUCgagugauGAgguGAauCcUCCGGAcugcacac----------------CUC---------------  BLEAMERI
3013   18 HARVERMI   uuuguACACACCGCCCSUCGCuccuAccGAUU--gaacgguCCggCGAgaacUCCGGAuggucggCacg-caggg------GUCA---A---A------c  HARVERMI
3013   19 THEANNUL   uuuguACACACCGCCCGUCGCuccuAccGAUC--gagugauCCgguGAauUaUUCGGAccGUgaugu-----uccc-----GUC---------------A  THEANNUL
3013   20 SYMPILO1   uuuguACACACCGCCCGUCGCuccuAccGAUU--gagugauCCgguGAauUaUUCGGAcugacgcagu----gCucag------CUU----cu------g  SYMPILO1
3013   21 ZBBSR205   uuuguACACACCGCCCGUCGCuccuAccGAUU--ggaugauuCgguAAgCuuUUgggAuugaUUaug-----uauuUg----------ucaUu------g  ZBBSR205
3013   22 ZBBSR206   uuuguACACACCGCCCGUCGCuccuAccGAUU--ggaugauuCgguAAgCuuUUgggAuugaUUacg-----uaUUg-----------ucaUu------g  ZBBSR206
3013   23 ZBBSR235   uuuguACACACCGCCCGUCGCuccuAccGAUU--ggaugauuCgguAAgYuuUUgggAuugaUUaug-----uaucUg----------ucaUu------g  ZBBSR235
3013   24 ZBBSR218   uuuguACACACCGCCCGUCGCuccuAccGAUU--ggaugauuCgguAAgCucUUgggAuugaUugAC-----gacCUG-----CAUg-ucA--------G  ZBBSR218
3013   25 ZBBSR217   uuuguACACACCGCCCGUCGCuccuAccGAUU--ggaugauuCgguAAgCucUUgggAuugaUugAC-----gacCUG-----CAUg-ucA--------G  ZBBSR217
3013   26 ZBBSR211   uuuguACACACCGCCCGUCGCuccuAccGAUU--ggaugauuCgguAAgCucUCgggAuugaUgg-Cc----agcugc-----CACg-uguag------c  ZBBSR211
3013   27 Pchroma    uuuguACACACCGCCCGUCGCuacuAccGAUU--ggacguuUUgguGAgGCucAGGGAauguag--cc---ugcggug---guUC-------g------c  Pchroma
3013   28 EUGROTUN   uuuguACACACCGCCCGUCGCuacuAccGAUU--ggaugguUUaguGAgguuCACGGAcUguug--ccUCUGGUUGGU----UUC--CUUC--------C  EUGROTUN
3013   29 ENTOGING   uuuguACACACCGCCCGUCGCuccuAccGAUU--gaauaaAGAgguGAaAUucuaGGAuuguuuu----------------UGAG---------------  ENTOGING
3013   30 DICDISCO   uuugUACACACCGCCCGUCGCuccuAccGAUC--gaaugauACgguAAagUuaacGGAuCguuuUauCU----GUGGC---AACACU-------------  DICDISCO
3013   31 ZTnucl     uuuguACACACCGCCCGUCGCuccuAccGAUU--ggauGagAUgguGAguaaauCuUAaugauugaA------guuAu----acU---guaAagu-----  ZTnucl
3013   32 W10Z79     uuuguACACACCGCCCGUCGCuccuAccGAUU--ggauGagAUgguGAguaaauCuUAaugauugaA------guuAa----acU---guAuagu-----  W10Z79
3013   33 W10Z74     uuuguACACACCGCCCGUCGCuccuAccGAUU--ggauGagAUgguGAguaaauCuUAaugauugaA------guuau----acU---guauagu-----  W10Z74
3013   34 W10Z72     uuuguACACACCGCCCGUCGCuccuAccGAUU--ggauGagAUgguGAguaaauCuUAaugauugaA------guuaA----acU---guAuagu-----  W10Z72
3013   35 W10Z10     uuuguACACACCGCCCGUCGCuccuAccGAUC--ggauGagAUgguGAguaaauCuUAaugauugaA------guuau----acU---guauagu-----  W10Z10
3013   36 ZBBSR7     uuuguACACACCGCCCGUCGCuccuAccGAUU--ggaUgagUUgguGAguggauuGGagaaaCugcU------gucuu----gcU---gcaagau-----  ZBBSR7
3013   37 ZBBSR173   uuuguACACACCGCCCGUCGCuccuAccGAUU--gaauaCgUUgguGAuuggauUgGAuaAAgAGAU------Aucuu-----CUA--AAaagaC-----  ZBBSR173
3013   38 Siphcyan   uuuguACACACCGCCCGUCGCuccuAccGAUC--ggauAagCUaguGAUugaauuGGaugAggAguu------aGGuu----AcU---gAaaGAu-----  Siphcyan
3013   39 ZBBSR2     uuuguACACACCGCCCGUCGCuccuAccGAUU--ggaugAgUUgguGAgugaauuGGAgUaaCAgcU------aucuu----ucU---gaaagau-----  ZBBSR2
3013   40 ZCR4       uuuguACACACCGCCCGUCGCuccuAccGAUU--gaauGagUUgguGAguUgauuGGagCgaCGgcU------aucuu----GCA---AAaagau-----  ZCR4
3013   41 ZCR16      uuuguACACACCGCCCGUCGCuccuAccGAUU--ggaugUgUUgguGAgugaUuuGGagaaauGgcu------auuuU-----CUU-GAUUagau----g  ZCR16
3013   42 ZCR6A      uuuguACACACCGCCCGUCGCuccuAccGAUU--gaauaCaUUgguGAUugaauUGGAuaAAgAGAu------aucCU-----CUU--AAAUg------a  ZCR6A
3013   43 PHYPOLYC   uuuguACACACCGCCCGUCGCugcuAccGAUU--ggguuUuACaguUACGCGUUCGGAggAcgauguuuuCgggucu---UAAC---gggcuc------C  PHYPOLYC
3013   44 NAEGRUBE   uuuguACACACCGCCCGUCGCuccuAccGAUG--ggacgaaGAgauGaaCcuggCGGAccGAaccG----------------------------------  NAEGRUBE
3013   45 euk heli   37                     5-6          57     58     59       60                                         euk heli
3013   46 rad        -----                ---- -->       ------> ---> ------>   ------->                                   rad
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"Sept4aln" on  5-SEP-96 15:33:41

Posi-   Sequence
tion:   identity:  Data:

3114   1 1369mask  ------------------111-1111-11111-11111111111111111111111111111111111111111111111111111111111--------  1369mask
3114   2 MNELEIDY  a---CggcgCCgcc-gaGAAC-UUgC-ucAAa-cuUGaucauuuAGAggAaguaAAAGUCGUAACAAGGuuucCguagguGAaccugcAgaagGAUCAAG  MNELEIDY
3114   3 DIAGRAND  C----gccugAguu-ggGAAC-UUgC-ucAAa-cuUGaucauuuAGAggAaguaAAAGUCGUAACAAGGuuucCguagguGAaccugcAgaagGAUCAAG  DIAGRAND
3114   4 ATHBOMBA  A-Cccgc-gUUgcu-gaGAAG-CUga-ucAAa-cuUGgucauuuAGAggAaguaAAAGUCGUAACAAGGuuucCguaggUGAAccugcAgaagGAUCAAG  ATHBOMBA
3114   5 BLAEMERS  A---cac-uuggc--gaGAAA-UUgC-ucAAa-cuUGgucaucuAGAggAaguaAAAGUCGUAACAAGGuuuccguaggUGAAccugcggaagGAUCAUU  BLAEMERS
3114   6 CHLREINH  c---cUU---gcuu-gaGAAG-UUca-uuAAa-ccCUcCcaccuAGAggAaggaGAAGUCGUAACAAGGuuuccguaggUGAAccugcggaagGAUCAUU  CHLREINH
3114   7 ORYSATIV  c-ccccgAcgucgc-gaGAAG-UCca-uuGAa-ccUUaucauuuAGAggAaggaGAAGUCGUAACAAGGuuuccguaggUGAAccugcggaagGAUCAUU  ORYSATIV
3114   8 PORAERUG  c------gagugc--ggAAAG-UUca-uuAAa-ccUUaucaucuAGAggAaggaGAAGUCGUAACAAGGuCucCguagguGAaccugcAgaAgGAUCAAG  PORAERUG
3114   9 ACACAST1  CUCCCCUUuu-GgcggcGAAG-UCga-uuGAa-ccUUaccauuuAGAggAaggaGAAGUCGUAACAAGGucuccguaggUGAAccugcggaggGAUCAUU  ACACAST1
3114  10 PHRBALAM  gguuuuauauuuuC-caGAAG-UUau-cuAAa-ccUUauuaucuAGAggAaggaGAAGUCGUAACAAGGuuucuguagguGAaccugcagaagGAUCAAG  PHRBALAM
3114  11 STYALSID  g---cGauUcgucccgaGAAG-UUca-uuAAa-ccUUaucauuuAGAggAaggaGAAGUCGUAACAAGGuuucCguagguGAaccugcAgaagGAUAGA   STYALSID
3114  12 EMIHUXLE  C---gcgAcgccgc-rgGAAG-CUgU-ccGAa-ccUUaucauuuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggUGAAccugcAgaagGAUCAAG  EMIHUXLE
3114  13 OCHDANIC  g---ugagAaucgu-ggAAAG-UUaU-uuAAa-ccUCaucauuuAGAggAagguGAAGUCGUAACAAGGuuuccguaggUGAAccugcggaagGAUCAUU  OCHDANIC
3114  14 CAFROENB  g---gUgAUaggAc-gaGAAG-UAgg-cuAAa-ccUUaccaucuAGAggAagguGAAGUCGUAACAAGGuuucCguaggUGAAccugcAgaagGAUCAA   CAFROENB
3114  15 LABMINUT  a---acgCUguuuu-aaAAAG-UUga-uuAAa-ccUUaccauuuAGAggAagguGAAGUCGUAACAAGGuuucCguaggUGAAccugcAgaagGAUCAAG  LABMINUT
3114  16 OXYGRANU  -----gccuugugc-ggAAAA-UCAA-guAAa-ccAuaucacuuAGAggAaggaGAAGUCGUAACAAGGuuuccguaggUGAAccugcAgaagGAUCAUU  OXYGRANU
3114  17 BLEAMERI  --------gugugu-ggGAAG-UUgA-guAAa-ccUUaucacuuAGAggAaggaGAAGUCGUAACAAGGuAucuguaggUGAAccugcagaAgGAUCAAG  BLEAMERI
3114  18 HARVERMI  ccuguguccgUgc--caGAAG-CUgu-ucGAA-ccUUaccguuuAGAggAaggaGAAGUCGUAACAAGGUauccGua-gUGAaCcugCAgaAgGAUCAAG  HARVERMI
3114  19 THEANNUL  g---ggaacgucUA-ggGAAG-UUuU-guGAa-ccUUaucacuuAAAggAaggaGAAGUCGUAACAAGGuuucCguaggUGAAccugcAgaagGAUCAAG  THEANNUL
3114  20 SYMPILO1  g---AcguugcguU-ggAAAG-UUuC-auGAa-ccUUaucacuuAGAggAaggaGAAAUCGUAACAAGGuuucCguaggUGAAccugcAgaagGAUCAAG  SYMPILO1
3114  21 ZBBSR205  a---cgguacauUuuaaCAAc-uuaa-UcAAa-ccUaaucaucuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggUGAAccugcAgaagGNUCAAG  ZBBSR205
3114  22 ZBBSR206  a---caUugcguUuuaaCAAc-uuaa-UcAAa-ccUaaucaucuAGAggAaggaGAAGUCGUAACAAGGuuucUguaggUGAAccugcAgaagGAUCAAG  ZBBSR206
3114  23 ZBBSR235  a---cgguacauUuuaaCAAc-uuaa-UcAAa-ccUaaucaucuAGAggAaggaGAAGUCGUAACAAGGuuucYguaggUGAAccugcAgaagGAUCAAG  ZBBSR235
3114  24 ZBBSR218  A---cgG-A-UUuc-gaCAAc-uuga-UcAAa-ccUaaucaucuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggUGAAccngcAgaagGAUCAAG  ZBBSR218
3114  25 ZBBSR217  A---cgG-A-UUuc-gaCAAc-uuga-UcAAa-ccUaaucaucuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggUGAAccugcAgaagGAUCAAG  ZBBSR217
3114  26 ZBBSR211  u----gAAUuuUuc-gaAAAc-uuga-UcAAa-ccUaaucaucuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggUGAAccugcAgaagGAUCAAG  ZBBSR211
3114  27 Pchroma   cacugcgggcAugc-auGAAC-UgaA-AcAAa-ccUGagcgucuAGAggAaguaAAAGUCGUAACAAGGuuucCgua-gUGAACcugcAgaagGAUCA    Pchroma
3114  28 EUGROTUN  U---UUggcaUacU-g-GAAG-Ugac-CcAAa-cuUGaucaucuAGAggAaguaAAAGUCGUAACAAGGuuucCgua-gUGAACcugcAgaagGAUCA    EUGROTUN
3114  29 ENTOGING  -----caauugaaaaUgaAAA-uagaUGuGAa-ccUCCuuauuuAGAggAaggaGAAGUCGUAACAAGGuuuccguaggUGAAccugcggaagGAUC     ENTOGING
3114  30 DICDISCO  -----GAUauaaau-UaAAAg-uuaU-uuAAa-ucUCauuguuuAGAggAaggaGAAGUCGUAACAAGGuauccguaggUGAAccugcggaugGAUCAUU  DICDISCO
3114  31 ZTnucl    --UGAAKGucaguu-auAUaU-auuu-gcAAa-cuAAAcuauuuAGAggAaggaGAAGUCGUAACAAGGuuuccguaggUGAAccugcAgaa          ZTnucl
3114  32 W10Z79    --UAAAGGucaguu-auAUaU-auuu-gcAAa-cuAAAcuauuuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggUGAAccugcAgaagGAUCAAG  W10Z79
3114  33 W10Z74    --UGAAGGucaguu-auAUaU-auuu-gcAAa-cuAAAcuauuuAGAggAaggaGAAGUCGUAACAAGGuuucuguaggUGAAccugcagaagGAUCAAG  W10Z74
3114  34 W10Z72    --UGAAGGucaguu-auAUaU-auuu-gcAAa-cuAAAcuauuuAGAggAaggaGAAGUCGUAACAAGGuuucuguaggUGAAccugcagaagGAUCAAG  W10Z72
3114  35 W10Z10    --AGAAGGucaguu-auAUaU-auuu-gcAAa-cuAAAcuauuuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggUGAAccugcAgaagGAUCAAG  W10Z10
3114  36 ZBBSR7    ----UGGUguaCUuuuuuAAa-auuu-gcAAa-uuAAAuuuucuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggUGAAccugcAgaagGAUCAAG  ZBBSR7
3114  37 ZBBSR173  ----AUCAAAAcG--GuaAAc-auuu-gaAAa-cuAGAuuauuuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggUGAAccugcAgaagGAUCAAG  ZBBSR173
3114  38 Siphcyan  ----aacAuuAug--GuUAAg-auuu-gCUAa-cuAGAuuauuuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggNGAAccugcAgaagGAUCAAG  Siphcyan
3114  39 ZBBSR2    ----UGUUguAUuu-UuAAAa-auuu-gcAAa-cuAGAuuaucuAGAggAaggaGAAGUCGUAACAAGGuuucCguagguGAAccugcAgaagGAUCAAG  ZBBSR2
3114  40 ZCR4      ---UAUUguGAUuu-UuuAAa-auuU-acAAa-cuAGAuuauuuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggUGAAccuNcAgaagGNUCAaN  ZCR4
3114  41 ZCR16     g--uAGUACAauuu--uuAAa-aUuu-gcAAg-cuAGAuuaucuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggNGAAccugcAgaagGAUCAAG  ZCR16
3114  42 ZCR6A     u---aGCAAAGcG--GuaAAC-auuu-gUAAa-cuAGAuuauuuAGAggAaggaGAAGUCGUAACAAGGuuucCguaggUGAAccngcAgaagGAUCAAG  ZCR6A
3114  43 PHYPOLYC  ----ggggcgucgGccAAAGU-UCUA-ACUa--cuGUaCggccuAGAggAagcaGAAGUCGUAACAAGGuaaUcguaggUGAAccugcgUuugGAUCAUU  PHYPOLYC
3114  44 NAEGRUBE  ------CAAgguAA-ggGAAA-ccag-uuAAa-ucUCuucgucuGUAggAaggaAAAGUCGUAACAAGGucuUcguaggUGAAccugcgUaggGAUCAUU  NAEGRUBE
3114  45 euk heli              60          59      58     57        5-6                        60          60            euk heli
3114  46 rad              <--------    <-------  <---  <------  <--  ----                 ---------->  <----------          rad
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"Sept4aln" on  5-SEP-96 15:33:41

 Posi-   Sequence
 tion:   identity:   Data:

 3214    1 1369mask  ---------  1369mask
 3214    2 MNELEIDY  CUUGGAUCC  MNELEIDY
 3214    3 DIAGRAND  C          DIAGRAND
 3214    4 ATHBOMBA  C          ATHBOMBA
 3214    5 BLAEMERS  A          BLAEMERS
 3214    6 CHLREINH  G          CHLREINH
 3214    7 ORYSATIV  G          ORYSATIV
 3214    8 PORAERUG  C          PORAERUG
 3214    9 ACACAST1  A          ACACAST1
 3214   10 PHRBALAM  C          PHRBALAM
 3214   11 STYALSID             STYALSID
 3214   12 EMIHUXLE  CUUGGAUCC  EMIHUXLE
 3214   13 OCHDANIC  -          OCHDANIC
 3214   14 CAFROENB             CAFROENB
 3214   15 LABMINUT  C          LABMINUT
 3214   16 OXYGRANU  A-         OXYGRANU
 3214   17 BLEAMERI  C-         BLEAMERI
 3214   18 HARVERMI  C          HARVERMI
 3214   19 THEANNUL  C          THEANNUL
 3214   20 SYMPILO1  C          SYMPILO1
 3214   21 ZBBSR205  C          ZBBSR205
 3214   22 ZBBSR206  C          ZBBSR206
 3214   23 ZBBSR235  C          ZBBSR235
 3214   24 ZBBSR218  C          ZBBSR218
 3214   25 ZBBSR217  C          ZBBSR217
 3214   26 ZBBSR211  C          ZBBSR211
 3214   27 Pchroma              Pchroma
 3214   28 EUGROTUN             EUGROTUN
 3214   29 ENTOGING             ENTOGING
 3214   30 DICDISCO  U          DICDISCO
 3214   31 ZTnucl               ZTnucl
 3214   32 W10Z79    C          W10Z79
 3214   33 W10Z74    C          W10Z74
 3214   34 W10Z72    C          W10Z72
 3214   35 W10Z10    C          W10Z10
 3214   36 ZBBSR7    C          ZBBSR7
 3214   37 ZBBSR173  C          ZBBSR173
 3214   38 Siphcyan  C          Siphcyan
 3214   39 ZBBSR2    C          ZBBSR2
 3214   40 ZCR4      c          ZCR4
 3214   41 ZCR16     C          ZCR16
 3214   42 ZCR6A     C          ZCR6A
 3214   43 PHYPOLYC  A          PHYPOLYC
 3214   44 NAEGRUBE  U          NAEGRUBE
 3214   45 euk heli             euk heli
 3214   46 rad                  rad
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16. Abstract (Limit: 200 words)

The relationships of the Acantharea and the Polycystinea (order Spumellarida) to other protists were investigated using phylogenetic analyses of small-subunit ribosomal RNA (SSU rRNA) genes. These two classes are included in the superclass Actinopoda based on specialized pseudopodia called axopodia. SSU rRNA-based phylogenies do not support the common ancestry of the Acantharea and the Polycystinea, implying that the superclass Actinopoda is artificial and should be discarded. All analyses supported the monophyly of the Acantharea and the separate monophyly of the Polycystinea. The origin of the sequences was confirmed by *in situ* hybridizations.

The SSU rRNA-based method was also useful in fine-scaled comparisons. Sequence comparisons for the solitary spumellarian *Thalassicolla nucleata* from the Sargasso Sea and the Pacific Ocean indicate there may be different species which are morphologically identical or that there may be allelic variation in a given individual. Analyses between the solitary *T. nucleata* and seven colonial spumellaria indicate that the colonial spumellaria may not be monophyletic. The results from examination of branching patterns within three of the four orders of the Acantharea placed the symphyacanthid *Haliommatidium* sp. branching among the Arthracanthida, indicating that the Symphyacanthida are not monophyletic as defined by morphology-based systematics.

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