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MOLECULAR PHYLOGENY OF CALCAREOUS SPONGES USING 18S RRNA AND 28S RRNA SEQUENCES

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ABSTRACT

In a recently published work, the phylogeny of Calcispongia was investigated using 18S rRNA data and morphological characters, supporting the monophyly of Calcispongia and their subdivision into Calcaronea and Calcinea, and proposing parsimony-based evolutionary scenarios for a few morphological characters. In a search for a better understanding of body plan evolution in Calcispongia, especially regarding symmetries and the organization of the aquiferous system, the present study analyzes a new dataset of 28S rRNA sequences, alone and in combination with the 18S rRNA data. Independent analyses of the two datasets provide corroboration of the monophyly of Calcispongia, Calcinea and Calcaronea, and of several clades within Calcaronea. The combined analyses of 18S and 28S rRNA provide a better resolution of the phylogeny which leads us to refine hypotheses of morphological character evolution. The asconoid aquiferous system would be primitive for the Calcispongia, with independent acquisitions of the syconoid and leuconoid types within Calcinea and Calcaronea. There is confirmation of the primitive nature of axial symmetry in Calcispongia, with two independent inferred losses, within Calcinea and Calcaronea.

KEY WORDS

Calcarea, Calcispongia, evolution, Metazoa, phylogeny, Porifera, axial symmetry, 18S rRNA, 28S rRNA.

INTRODUCTION

The study of calcareous sponges is particularly interesting with respect to body plan evolution, due to the high level of variability of their levels of organization. Indeed, Calcispongia is the only sponge taxon where all four known different types of poriferan aquiferous systems are present, namely asconoid, syconoid, sylleibid and leuconoid aquiferous systems. These four states constitute a clear morphocline, with complexity of the aquiferous system increasing from asconoid to leuconoid. The traditional evolutionary interpretation considered the asconoid and simplest type of organization as the primitive state, from which the remaining ones would be derived (*e.g.* HAECKEL, 1872; DENDY & ROW, 1913; BOROJEVIC, 1979). Rejection of this "gradist" view has been the exception among spongologists (but see BURTON, 1963).

A high taxonomic value was once attributed to this character; for example HAECKEL (1872), divided the Calcispongia into Ascones, Leucones, and Sycones. POLÉJAEFF (1883) split the calcareous sponge between Homocoela (all internal cavities lined by choanocytes: basically the asconoid species) and Heterocoela (some internal cavities lined by pinacocytes: basically the syconoid sylleibid and leuconoid species). Alternatively, the classification scheme used in more recent works, where the Calcispongia are subdivided into the two subclasses Calcinea and Calcaronea, attributes a higher taxonomic value to cytological and embryological characters and a lower taxonomic value to the aquiferous system (MANUEL *et al.,* 2002).

The calcareous sponges also display a great variability in the architecture of their mineral skeleton (while the spicules themselves are relatively homogenous). For example the sponge body may or not be covered by a protective layer of spicules disposed in a tangential orientation, and the presence of such a cortex has been classically considered as a derived character (see BOROJEVIC, 1979; BOROJEVIC *et al.,* 1990, 2000). The whole skeleton architecture may or not display radial symmetry, a character especially important for the comprehension of diversity and evolution of body symmetries among Metazoa.

The modern tools of phylogenetic reconstruction offer the opportunity to formulate explicit hypotheses of character evolution, based on the principle of parsimony. The first molecular phylogeny of the Calcispongia was recently published by MANUEL *et al.* (2003). In this work, the morphology-based classification adopted in Systema Porifera (HOOPER & VAN SOEST, 2002) was evaluated in comparison with the phylogenetic trees, and evolutionary scenarios were proposed for a few key morphological characters. Among the results, a great amount of homoplastic change was evidenced for the evolution of the aquiferous system, as for the evolution of the skeletal architecture (with an instance of hypothesized loss of the cortex).

Most strikingly, the occurrence of axial symmetry was shown to be probably primitive for the Calcispongia, suggesting a reconsideration of the widely admitted view of axial symmetry, and polarity, as a synapomorphy of Eumetazoa (= non sponge metazoans).

However this pioneer work was based on the analysis of only one molecular (18S rRNA) dataset. Other data sources are thus warranted in order to corroborate the 18S rRNA results, as well as to help increasing the resolution of the trees.

Here we reanalyze the 18S rRNA data, together with a new dataset of 28S rRNA sequences for the same taxonomic sampling. The two datasets are first analyzed separately in order to evaluate tree reliability from taxonomic congruence, and then in combination in a total evidence approach. The result of the combined analysis is used for reconstruction of character evolution.

Tab. 1. Classification of the genera of Calcispongia included in the molecular dataset, according to BOROJEVIC *et al.* (1990, 2000, 2002a,b,c) and VACELET *et al.* (2002a,b). Correspondence with the alternative classification scheme (POLÉJAEFF, 1883): (1) Homocoela (2) Heterocoela.

subclass Calcinea Bidder, 1898

- Order Clathrinida Hartman, 1958
  - Family Clathrinidae Minchin, 1900
    - ***Clathrina*** Gray, 1867 (1)
  - Family Soleneiscidae Borojevic *et al.,* 2002a
    - ***Soleneiscus*** Borojevic *et al.* (2002a) (1)
  - Family Leucaltidae Dendy and Row, 1913
    - ***Leucaltis*** Haeckel, 1872 (2)
  - Family Leucettidae Borojevic, 1968
    - ***Leucetta*** Haeckel, 1872 (2)

subclass Calcaronea Bidder, 1898

- Order Leucosoleniida Hartman, 1958
  - Family Leucosoleniidae Minchin, 1900
    - ***Leucosolenia*** Bowerbank, 1864 (1)
  - Family Sycettidae Dendy, 1892
    - ***Sycon*** Risso, 1826 (2)
  - Family Grantiidae Dendy, 1892
    - ***Grantia*** Fleming, 1828 (2)
    - ***Leucandra*** Haeckel, 1872 (2)
  - Family Jenkinidae Borojevic *et al.,* 2000
    - ***Anamyxilla*** Poléjaeff, 1883 (2)
  - Family Heteropiidae Dendy, 1892
    - ***Sycettusa*** Haeckel, 1872 (2)
    - ***Vosmaeropsis*** Dendy, 1892 (2)
  - Family Amphoriscidae Dendy, 1892
    - ***Paraleucilla*** Dendy, 1892 (2)
  - Family Lelapiidae Dendy and Row, 1913
    - ***Grantiopsis*** Dendy, 1892 (2)
- Order Baeriida Borojevic *et al.,* 2000
  - Family Baeriidae Borojevic *et al.,* 2000
    - ***Leuconia*** Grant, 1841 (2)
- Order Lithonida Vacelet, 1981
  - Family Petrobionidae Borojevic, 1979
    - ***Petrobiona*** Vacelet and Lévi, 1958 (2)

MATERIALS AND METHODS

Procedures used for genomic DNA extraction, PCR amplification and DNA sequencing are described in Manuel *et al.* (2003). For partial 28S rRNA amplification, a pair of primers was designed to permit amplification of approximately 950 bp: primer 28SC (5'-CGTGAGGGAAAGATGAAAAGCACT-3') and primer 28S31 (5'-CTCCTTAGCGGATT CCGACTTC-3').

A classification of the sampled genera of Calcispongia, based on Systema Porifera (Hooper & Van Soest, 2002) is given in Tab. I. All species sampled, including outgroup taxa, are listed in Tab. II, with respective accession numbers of 18S and 28S rRNA sequences in GenBank. Fifteen 28S rRNA sequences from calcareous sponges are new from this work.

Tab. II. Sequence sources and GenBank accession numbers (in bold for new sequences from this work; voucher specimens are the same as in Manuel *et al.,* 2003). *Tripedalia* and *Atolla* have been used as scyphozoan representatives respectively in the 18S and 28S rRNA datasets, and the two sequences have concatenated to generate a "Scyphozoa" terminal taxon in the combined dataset. The same operation has been done for the two gastropods *Nerita* and *Aplysia.*

Species name	Taxonomic group	GenBank 18S	GenBank 28S
Mycale fibrexilis	Demospongiae	AF100946	AY026376
Suberites ficus	Demospongiae	AF100947	AY026381
Anamyxilla torresi	Calcispongia	AF452020	**AY563536**
Leuconia nivea	Calcispongia	AF182191	**AY563534**
Clathrina cerebrum	Calcispongia	U42452	**AY563541**
Grantia compressa	Calcispongia	AF452021	**AY563538**
Grantiopsis heroni	Calcispongia	AF452019	**AY563539**
Leucaltis clathria	Calcispongia	AF452016	**AY563542**
Leucandra aspera	Calcispongia	AF452022	**AY563535**
Leucetta chagosensis	Calcispongia	AF182190	**AY56354**3
Leucosolenia sp.	Calcispongia	AF100945	AY026372
Paraleucilla sp.	Calcispongia	AF452023	**AY563540**
Petrobiona massiliana	Calcispongia	AF452026	**AY563533**
Soleneiscus radovani	Calcispongia	AF452017	**AY563544**
Sycettusa sp.	Calcispongia	AF452025	**AY563530**
Sycon ciliatum	Calcispongia	L10827	**AY563532**
Sycon raphanus	Calcispongia	AF452024	**AY563537**
Vosmaeropsis sp.	Calcispongia	AF452018	**AY563531**
Mnemiopsis leidyi	Ctenophora	AF293700	AY026373
Beroe ovata	Ctenophora	AF293694	AY026369
Antipathes galapagensis	Cnidaria	AF100943	AY026365
Atolla vanhoeffeni	Cnidaria		AY026368
Tripedalia cystophora	Cnidaria	L10829	
Nerita albicilla	Bilateria	X91971	
Aplysia californica	Bilateria		AY026366
Mus musculus	Bilateria	X00686	X00525

Sequence Alignment and Taxonomic Sampling

Sequences from Calcispongia were aligned together with a number of outgroup taxa sequences available in GenBank for both 18S and 28S rRNA sequences, from Demospongiae, Cnidaria, Ctenophora, and Bilateria (see Tab. II).

Alignments were first done automatically by using the GeneWork program (Intelligenetics), and then manually for optimization of the alignment, using the SeaView program package (GALTIER *et al.,* 1996). Ambiguously aligned regions were treated as described in MANUEL *et al.* (2003).

The final 18S rRNA alignment contains 1885 positions, of which 1237 are constant, and 407 are parsimony informative. The 28S rRNA alignment contains 962 characters, of which 502 are constant, and 335 are parsimony informative. The alignments are accessible from the corresponding author upon request.

Phylogenetic Analyses

All phylogenetic analyses were performed using Paup 4.0b6 (SWOFFORD, 1998). For Maximum Parsimony (MP) analyses, characters were treated as unordered and equally weighted.

MP trees were computed using heuristic searches with 20 replicates of random taxon addition sequence and TBR branch swapping. For the Maximum Likelihood (ML) analyses of nucleotide data, we used a GTR (General Time Reversible) + G + I model. This model was chosen because it is one of the most general models available for phylogenetic reconstruction from nucleotide data using ML. Among site variation was estimated using a discrete approximation to the gamma distribution with 8 rate categories. Parameters were estimated with Paup 4.0b6 from the result of a MP heuristic search, using the command lscores. The estimated parameters were: for the 18S rRNA data set: shape = 0.412, pinvar = 0.161, rmatrix = (1.54 2.52 0.99 1.34 6.05); for the 28S rRNA data set: shape = 0.278, pinvar = 0, rmatrix = (0.7 2.73 0.87 0.94 7.70); for the combined data set: shape = 0.31, pinvar = 0.106, rmatrix = (1.13 2.56 0.89 1.2 6.74). The heuristic search was done with 10 replicates of taxon random addition sequence. Gaps in molecular sequences were treated as missing data. Branch support was tested with bootstrapping (FELSENSTEIN, 1985) (500 replicates for NJ and MP, 100 replicates for ML). Reconstitutions of character evolution were done using Mac Clade 3.0 (MADDISON & MADDISON, 1992), with the option "soft polytomies".

RESULTS

A 28S rRNA dataset was generated with the same taxonomic sampling of calcareous sponge species than in the 18S rRNA dataset previously analyzed by MANUEL *et al.* (2003). The two datasets were first analyzed independently, with the same sampling of outgroup taxa. The results (ML tree and NJ, MP and ML bootstrap values) are shown in Fig. 1A (18S dataset) and 1B (28S dataset). A strict consensus of 18S and 28S ML trees is shown in Fig. 1C.

Both ribosomal RNA datasets converge on the monophyly of Calcispongia (highly supported by bootstrap values in all analyses, excepted in the NJ analysis of the 28S rRNA dataset, in which Calcispongia is non monophyletic), Calcaronea, and Calcinea. There is conflict between the two ML trees about relationships among the Calcinea, but the strict consensus of MP minimal trees from both 18S and 28S rRNA agree in branching *Soleneiscus* at the base of Calcinea. Among Calcaronea, four clades are shared between 18S and 28S ML trees: (*Sycon ciliatum, Vosmaeropsis, Sycettusa*), (*Leuconia, Petrobiona*), (*Anamyxilla, Leucandra, Sycon raphanus*), (*Grantiopsis, Paraleucilla*). All of these clades are well supported by bootstrap values for both datasets (with the following criterion: bootstrap values > 80 % in both MP and ML analyses), excepted for (*Grantiopsis, Paraleucilla*) which is not significantly supported by the analyses of 28S rRNA data. There is conflict between the two datasets about the relationships between these four clades and the two remaining calcaronean terminal taxa, *Grantia* and *Leucosolenia*. The genus *Sycon* (with only two species sampled, in the present study) is polyphyletic in all analyses.

The results of the combined analyses of 18S and 28S rRNA data are shown in Fig. 1D. Five clades are well supported in the combined analyses and were absent from the strict consensus of separated analyses (Fig. 1C): (*Leucandra, Sycon raphanus*), (*Grantia, Anamyxilla, Leucandra, Sycon raphanus*), (*Grantia, Anamyxilla, Leucandra, Sycon raphanus, Grantiopsis, Paraleucilla*), (*Leucaltis, Leucetta*), and (*Clathrina, Leucaltis, Leucetta*). All of these clades are actually found in the ML analysis of the 28S rRNA dataset (Fig. 1B), and not the 18S rRNA dataset (Fig. 1A). There is a strong increase in bootstrap values between the 28S rRNA analyses and the combined analyses for three of these clades: (*Grantia, Anamyxilla, Leucandra, Sycon raphanus*), (*Grantia, Anamyxilla, Leucandra, Sycon raphanus, Grantiopsis, Paraleucilla*), and (*Clathrina, Leucaltis, Leucetta*).

It is remarkable that asconoid species (*Leucosolenia, Soleneiscus, Clathrina*) are basal within both Calcinea and Calcaronea, even if the basal position of *Leucosolenia* in Calcaronea is not supported by bootstrap values.

The ML tree resulting from the analysis of the combined dataset was used for the optimization of two body plan characters deserving particular attention, namely the types of organization of the aquiferous system (Fig. 2A) and the absence or presence of axial symmetry (Fig. 2B). According to the reconstruction, the asconoid aquiferous system would be primitive for the Calcispongia, with the two main remaining states syconoid and leuconoid appearing convergently within Calcaronea and Calcinea. There would be a unique acquisition of a syconoid aquiferous system in Calcaronea, followed by four independent acquisitions of the leuconoid type within this clade. Axial symmetry would be present in the last common ancestor of Calcispongia, with two subsequent losses, one among Calcaronea in the common branch of (*Leuconia* + *Petrobiona*), and one among Calcinea in the branch of *Leucetta*.

DISCUSSION AND CONCLUSIONS

Whenever several datasets are available for the same taxonomic sampling, they may either be analysed independently, and the resulting topologies compared to check for taxonomic congruence, or alternatively the datasets may be combined in a total evidence approach. The respective advantages of both approaches have been extensively discussed elsewhere (*e.g.* LEVASSEUR & LAPOINTE, 2001; CHEN *et al.,* 2003). Independent analyses offer the opportunity of evaluating the reliability of phylogenetic hypotheses, because the recurrent occurrence of a given monophyletic grouping in trees reconstructed from several independent datasets is a strong indication that it is due to a true historical signal (and not just the effect of particular bias in a given dataset).

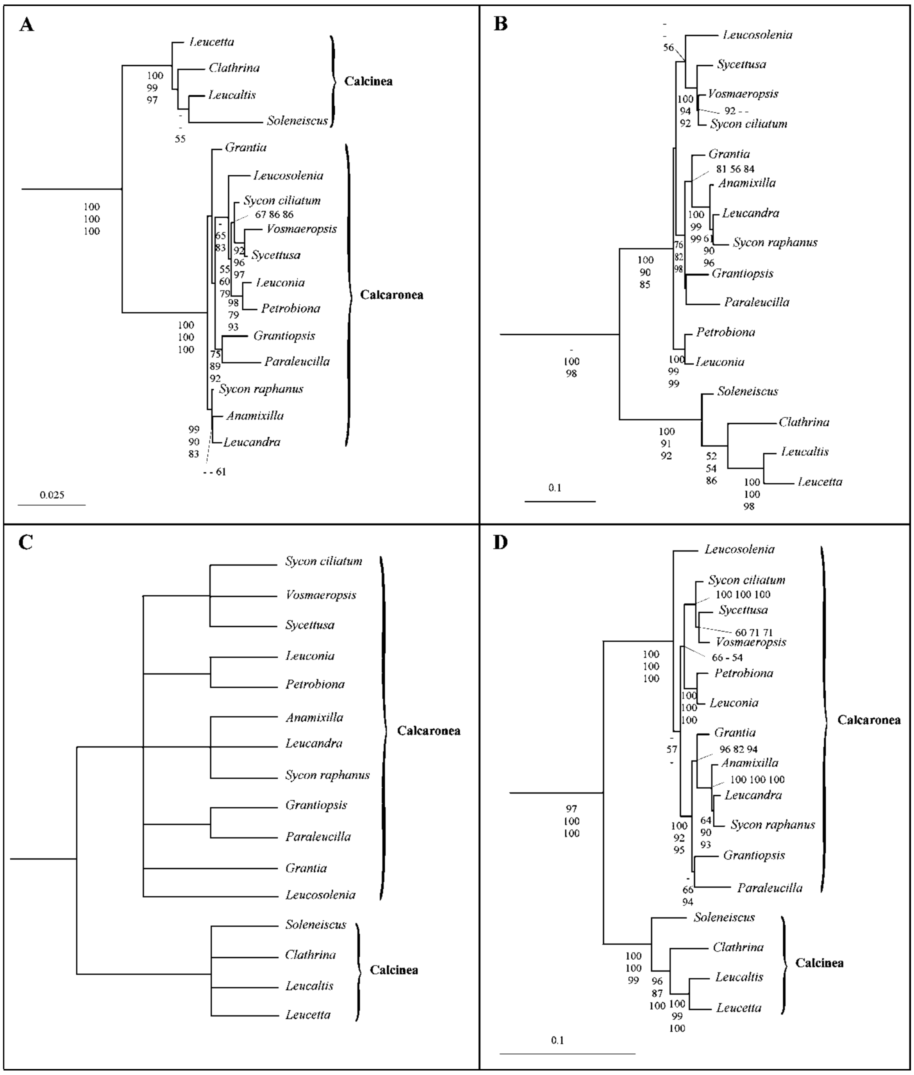

Fig. 1. Relationships within Calcispongia as inferred from 18S and 28S rRNA. **1A,** ML optimal tree from the 18S rRNA dataset (one tree, - lnL = 8168.05895). Bootstrap values, when > 50 %, indicated below the branches, in the following order: NJ, MP, ML. **1B,** ML optimal tree from the 28S rRNA dataset (one tree, - lnL = 5805.39056). Bootstrap values, when > 50 %, indicated below the branches, in the following order: NJ, MP, ML. **1C,** strict consensus of the two preceding trees. **1D,** ML optimal tree from the combined 18S and 28S rRNA dataset (two trees but with no difference within Calcispongia, - lnL = 14268.06544). Bootstrap values, when > 50 %, indicated below the branches, in the following order: NJ, MP, ML. The scale bar in Fig. 1A, 1B and 1D indicates the number of nucleotide substitutions per position in the sequence.

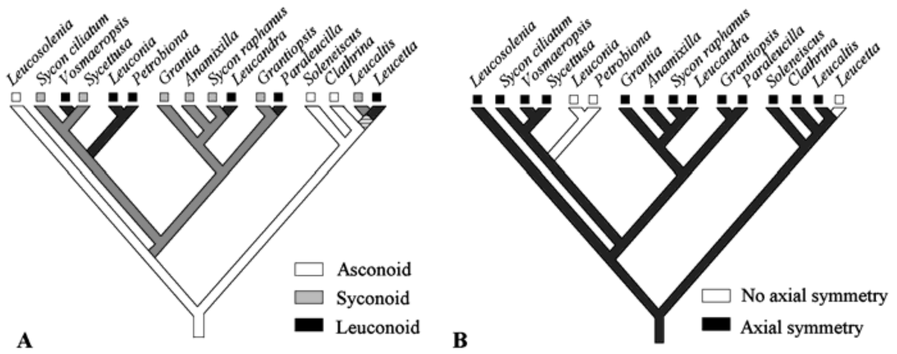

Fig. 2. Evolution of two morphological characters as reconstructed on the topology resulting from ML analysis of combined 18S and 28S rRNA data (tree in Fig. 1D). **2A,** organization of the aquiferous system. **2B,** axial symmetry of the skeleton architecture along the body axis. Note that even taxa showing no axial symmetry along the axis ("no axial symmetry" in the figure) display in fact radial symmetry of the skeleton around the oscula. Horizontal lines indicate indetermination of character state in the branch. The squares below taxon names give character state in the considered taxon. Character transformations have been reconstructed by MacClade (option "soft polytomies").

In the present study, the two main topological results emerging from the analyses of 18S rRNA data in MANUEL *et al.* (2003) are independently confirmed by the 28S rRNA dataset: (i) the calcareous sponges (Calcispongia) form a highly supported monophyletic group; and (ii) there is high support for their subdivision into two monophyletic subgroups, Calcinea and Calcaronea (in consistence with the classification scheme adopted in Systema Porifera, see MANUEL *et al.,* 2002). As discussed in details in MANUEL *et al.* (2003), this result implies rejection of an alternative classification scheme that was first proposed by POLÉJAEFF (1883), and followed by several authors, even until the second half of the 20th century: the subdivision of Calcispongia between Homocoela and Heterocoela (based upon the organisation of the aquiferous system). The monophyly of Calcinea and Calcaronea is consistent with the taxonomic distribution of several cytological and embryological characters, *e.g.* nucleus basal in choanocytes in Calcinea vs. apical in Calcaronea, and coeloblastula larva in Calcinea vs. amphiblastula larva in Calcaronea. However the polarisation of such characters is not possible in the current state of knowledge so that there is no unambiguous synapomorphy of Calcinea or Calcaronea.

Within Calcaronea, the 28S rRNA dataset provides independent confirmation of the major lines of phylogenetic structure, as reconstructed by the analyses of the 18S rRNA dataset, especially when the 28S ML tree (this study, fig. 1B) is compared to the 18S (MP and ML) trees in MANUEL *et al.* (2003, Fig. 6): the former differs from the latter only with respect to the position of *Leucosolenia* and the clade (*Leuconia* + *Petrobiona*). Differences in 18S rRNA tree topology between MANUEL *et al.* (2003) and the present study are due exclusively to a different sampling of outgroup taxa (while the alignment is exactly the same). Such topological instability of 18S rRNA trees at this level is probably due to the low number of informative characters in the 18S dataset. Noteworthy, the polyphyly of *Sycon* is confirmed by the 28S rRNA data, with *Sycon ciliatum* falling in a clade with the Heteropiidae (*Vosmaeropsis* and *Sycettusa*) and *Sycon raphanus* falling in a clade with *Leucandra aspera* and *Anamyxilla torresi*, exactly as suggested also by the 18S rRNA data.

Within Calcinea there appears to be a conflict between the two datasets, at least when the results of ML analyses are compared (MP analyses of the two molecular datasets give congruent results with *Soleneiscus* branching at the base of Calcinea, result not shown). However it must be noticed that in the 18S rRNA dataset there are only 8 informative characters at the Calcinea level, while there are 35 in the 28S rRNA dataset.

The total evidence approach is complementary to the independent analyses, with the underlying assumption that combining several dataset allows reinforcement of the phylogenetic signals and neutralisation of differently oriented homoplasy. Such an effect is exemplified in the present work, where three clades that are found in the 28S rRNA ML tree, and not in the 18S rRNA ML tree, are distinctly better supported in the combined ML analysis than they are in the 28S rRNA ML analysis. Overall, the combined dataset provides a better supported topology than either the 18S or the 28S data alone.

For this reason the tree resulting from the ML analysis of combined small and large rRNA subunits has been used for reconstructing character evolution; but it must be noticed that a few nodes are not supported by bootstrap indices in this tree. This is the case for the basal position of *Leucosolenia* within Calcaronea, a result with strong influence on the reconstitution of the evolution of the aquiferous system. The basal position of *Leucosolenia* is also found in the strict consensus of MP minimal trees, from the combined dataset and from the 28S rRNA data alone. In the latter case, a clade grouping all Calcaronea excepted *Leucosolenia* is supported by 81 % of MP and 56 % of ML bootstraps (results not shown because *Leucosolenia* is not basal in the 28S rRNA optimal ML tree, fig. 1B).

As a consequence of the basal position of *Leucosolenia* in Calcaronea and *Soleneiscus* and *Clathrina* in Calcinea, the asconoid type of aquiferous system is reconstructed as primitive for Calcispongia (Fig. 2A). In MANUEL *et al.* (2003), the primitive state for this character could not be determined because the topology derived from 18S rRNA and morphology was less resolved. While keeping in mind the poor statistical support for the position of *Leucosolenia*, this result is consistent with the traditional assumption that the asconoid (and homocoel) aquiferous system is the most primitive (HAECKEL, 1872; DENDY & ROW, 1913; TUZET, 1973; BOROJEVIC, 1979; BOROJEVIC *et al.,* 1990, 2000). This idea classically relied on the simplicity of the asconoid organization, and also on morphogenetic data in *Sycon*, where an asconoid olynthus stage precedes the acquisition of the syconoid adult morphology, both after larval metamorphosis and in the process of sponge reconstitution after cell dissociation.

Interestingly, the new topology also suggests a unique acquisition of the syconoid aquiferous system in Calcaronea (in addition to a similar event in Calcinea). The leuconoid aquiferous system would have been acquired repeatedly from the syconoid one among Calcaronea. This pattern can appropriately be called parallelism, and possibly reflects the effect of selective pressure acting in syconoid sponges to increase the ratio (surface of choanoderm / sponge volume). Another implication of this hypothesis is that the leuconoid organization of demosponge and hexactinellid aquiferous systems is not homologous with the leuconoid aquiferous system in any calcareous sponge.

With respect to other poriferan lineages, the evolutionary plasticity of the aquiferous system in calcareous sponges, as revealed by the high number of homoplastic changes for this character, is remarkable. Identification of molecular mechanisms controlling the organization of the aquiferous system in asconoid, syconoid and leuconoid representatives of the Calcispongia would undoubtedly provide valuable insights on this interesting aspect of sponge body plan evolution.

The evolution of another important character, absence or presence of axial symmetry, may have a more general implication for the understanding of body plan evolution at the base of metazoan tree. When this character is mapped on the Calcispongia tree (Fig. 2B), there is confirmation of the main result from MANUEL *et al.* (2003) that the axial symmetry is primitive in Calcispongia, with its absence being derived at least two times independently.

Axial symmetry and body polarity have been widely considered to be fundamental evolutionary acquisitions of Eumetazoa (non-sponge metazoans), together with «true tissues», muscle and nervous system (see for example FINNERTY, 1998; KNOLL & CARROLL, 1999), and instances of axial symmetry in sponges (such as *Sycon*) used to be presented as exceptions. This position cannot be hold anymore and the evolutionary status of axial symmetry and polarity in sponges should be seriously reconsidered in research programs dealing with the early evolution of body plans, and especially symmetries and polarities, in Metazoa. Not only the Calcispongia, but also hexactinellids and many demosponges, are axially symmetrical. Currently it is not possible to assess if axial symmetry and body polarity is homologous within sponges, and between sponges and Eumetazoa, but this possibility should not be excluded *a priori*. Again, the investigation of genetic mechanisms underlying morphogenesis may provide precious insights on this question. Several homeobox genes have been already characterized in one species of Calcispongia (MANUEL & LE PARCO, 2000), and we believe that calcareous sponges deserve particular attention in the field of comparative developmental genetics.

We explored elsewhere (MANUEL *et al.,* 2003) the phylogenetic potential of morphological characters for the resolution of internal relationships within Calcispongia. It appeared that very little resolution could be obtained from the analysis of available morphological data alone, so Calcispongia is typically the kind of taxon for which molecular phylogeny is the most powerful tool, both for the elaboration of a classification reflecting the phylogenetic history, and as support for the formulation of evolutionary scenarios of character transformations. Future works should primarily aim at a more complete sampling of calcareous sponge diversity, especially among the Calcinea. Currently our attention is also focalized on the *Sycon* problem. A taxonomic revision of this "genus" (starting with European species) is in preparation, together with the phylogenetic positioning of the commonest European species based on molecular data.

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