CHAPTER ONE

Deep Phylogeny and Evolution of Sponges (Phylum Porifera)

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Contents

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Abstract

Sponges (phylum Porifera) are a diverse taxon of benthic aquatic animals of great ecological, commercial, and biopharmaceutical importance. They are arguably the earliest-branching metazoan taxon, and therefore, they have great significance in the reconstruction of early metazoan evolution. Yet, the phylogeny and systematics of sponges are to some extent still unresolved, and there is an on-going debate about the exact branching pattern of their main clades and their relationships to the other non-bilaterian animals. Here, we review the current state of the deep phylogeny of sponges. Several studies have suggested that sponges are paraphyletic. However, based on recent phylogenomic analyses, we suggest that the phylum Porifera could well be monophyletic, in accordance with cladistic analyses based on morphology. This finding has many implications for the evolutionary interpretation of early animal traits and sponge development. We further review the contribution that mitochondrial genes and genomes have made to sponge phylogenetics and explore the current state of the molecular phylogenies of the four main sponge lineages (Classes), that is, Demospongiae, Hexactinellida, Calcarea, and Homoscleromorpha, in detail. While classical systematic systems are largely congruent with molecular phylogenies in the class Hexactinellida and in certain parts of Demospongiae and Homoscleromorpha, the high degree of incongruence in the class Calcarea still represents a challenge. We highlight future areas of research to fill existing gaps in our knowledge. By reviewing

sponge development in an evolutionary and phylogenetic context, we support previous suggestions that sponge larvae share traits and complexity with eumetazoans and that the simple sedentary adult lifestyle of sponges probably reflects some degree of secondary simplification. In summary, while deep sponge phylogenetics has made many advances in the past years, considerable efforts are still required to achieve a comprehensive understanding of the relationships among and within the main sponge lineages to fully appreciate the evolution of this extraordinary metazoan phylum.

Key Words: sponges; Porifera; non-Bilateria; phylogeny; evolution; evo-devo; Demospongiae; Hexactinellida; Homoscleromorpha; Calcarea

1. Introduction

Sponges are sessile aquatic organisms that inhabit most marine and many freshwater habitats. Adult sponges are of large ecological importance as, for example, filter-feeders and bioeroders (Bell, 2008) and have considerable commercial/biopharmaceutical value (Faulkner, 2002). Their systematics, phylogeny, evolution, and taxonomy have often been proven difficult to reconstruct because many sponges possess only a few systematically/phylogenetically informative morphological characters, and some skeletal traits, which for a long time served as the sole basis for sponge systematics, are prone to homoplasies (reviewed in Erpenbeck and Wörheide, 2007) and relatively variable as a function of local environmental conditions (Maldonado *et al.*, 1999). Nevertheless, significant progress has been achieved in recent years (e.g. Cárdenas *et al.*, 2009, 2011; Dohrmann *et al.*, 2011, 2012; Morrow *et al.*, 2012; Voigt *et al.*, 2012b).

Because of their early-branching position in the animal tree of life (Philippe *et al.*, 2009; Pick *et al.*, 2010), sponges are instrumental in the on-going efforts to better understand the main trajectories of early animal evolution and to decipher the paleogenomics of the last common ancestor of animals (Taylor *et al.*, 2007). Additionally, other non-bilaterian taxa (i.e. Placozoa, Cnidaria, and Ctenophora) and their relationships to each other and to the Bilateria have gained substantial interest as they are of great importance for understanding the evolution of key metazoan traits (Miller, 2009). The statement "*Nothing in biology makes sense except in the light of (a) phylogeny*" (modified after Dobzhansky, 1973) is especially true for the non-bilaterian part of the animal tree of life.

This review is intended to summarize the current state of the debate on the phylogenetic relationships within and among the main sponge lineages and their relationships to other non-bilaterian animals. Erpenbeck and Wörheide (2007) reviewed the then current status of the molecular

phylogeny of sponges. They concluded with the statement that "*Coming years will bring the science of sponge systematics closer to its long-awaited goal of a fully consistent phylogeny*". Since then, numerous phylogenies have been published, and the reconstruction of deep-level animal relationships has shifted from the analyses of single or a small number of genes to phylogenomic approaches analyzing dozens to hundreds of genes (e.g. Hejnol *et al.*, 2009; Philippe *et al.*, 2009) and complete mitochondrial genomes (e.g. Lavrov *et al.*, 2008)—we might now ask the question: are we there yet?

2. Higher-Level Non-bilaterian Relationships

In recent years, several contradicting hypotheses about higher-level non-bilaterian relationships have been published (reviewed by Edgecombe *et al.*, 2011; Philippe *et al.*, 2011). Conflicting results among studies addressing non-bilaterian relationships are not completely unexpected because such studies attempt to reconstruct cladogenetic events that occurred hundreds of millions of years ago (Ma), possibly as early as the Cryogenian (~650 Ma, Peterson *et al.*, 2008; Erwin *et al.*, 2011). Resolving such ancient splits with molecular sequence data is always difficult because of phylogenetic signal erosion along long terminal branches caused by multiple substitutions (saturation) ("non-phylogenetic signal", see Philippe *et al.*, 2011), which is often combined with short internal branches along which little phylogenetic signal has accumulated (see Rokas and Carroll, 2006). In such cases, the phylogenetic signal along those short internal branches is too low to achieve high statistical support (see Felsenstein, 1985). As a consequence of these difficulties, the relationships of the non-bilaterian taxa, including the origin of Porifera, remain among the most important open questions concerning the higher-level relationships of the Metazoa (Edgecombe *et al.*, 2011; Telford and Copley, 2011).

Due to reductions in sequencing costs, increasing amounts of DNA sequence data have been generated in genome and transcriptome sequencing projects in recent years, and then included in "phylogenomic" analyses. Phylogenomics, described by Eisen and Fraser (2003) as the "intersection of evolution and genomics", currently uses either data from fully sequenced genomes or more commonly, due to the lower resource demands, from expressed sequence tag (EST)/transcriptome sequencing projects to build large alignments (supermatrices) (Philippe and Telford, 2006). Phylogenomics should be distinguished from multi-gene analyses (e.g. Sperling *et al.*, 2009), which typically include fewer than 30 genes that are selected before rather than after sequencing.

In an early phylogenomic study, Rokas *et al.* (2005) used 50 protein-coding genes to reconstruct animal evolution and found that non-bilaterian

relationships were unresolved. The authors concluded that these cladogeneses, which most likely occurred several million years before the Bilateria diversified during the "Cambrian Explosion" (Peterson *et al.*, 2008), happened so fast (possibly within about 20 million years) that it is very difficult, if not impossible, to resolve these relationships with sequence data from extant organisms (Rokas and Carroll, 2006).

Dunn *et al.* (2008) applied a much broader phylogenomic approach by analyzing 150 genes, focusing on the relationships within Bilateria. Here, they added a large amount of new EST data for many taxa, which led to increased resolution in this part of the tree. However, non-bilaterian taxa were not extensively sampled, and only a few representatives of Porifera, Cnidaria, and Ctenophora were included. The most publicized result of this study was the position of the Ctenophora as a sister group to the remaining Metazoa, including sponges. A follow-up study from the same group added some additional non-bilaterian taxa (including Placozoa) and reconstructed very similar relationships (Hejnol *et al.*, 2009). Pick *et al.* (2010) significantly improved the taxon sampling of the Dunn *et al.* (2008) dataset. They added EST data from 18 additional non-bilaterian species, including previously unsampled placozoans and sponges but used the same genes and phylogenetic reconstruction methods. In contrast to the findings of Dunn *et al.* (2008), they found that monophyletic sponges branched off first, followed by Ctenophora as the sister group to the remaining metazoans (Cnidaria, Placozoa, Bilateria). The non-bilaterian relationships, although not highly supported by posterior probabilities, were stable regardless of whether only choanoflagellates (the closest living relatives of Metazoa, see e.g. Carr *et al.*, 2008), or the full set of outgroups used by Dunn *et al.* (2008) that included the more distantly related Fungi, were included in the analyses. Pick *et al.* (2010) thus concluded that the early-branching position of Ctenophora found by Dunn *et al.* (2008) was an artefact of insufficient taxon sampling leading to long branch attraction (LBA, *sensu* Felsenstein, 1978, a phenomenon where taxa with long branches are attracted to each other in phylogenetic analyses without being truly related). This conclusion was further corroborated by Philippe *et al.* (2011).

Schierwater *et al.* (2009) published a combined analysis of nuclear protein-coding and mitochondrial genes with morphological characters. They recovered a clade of diploblastic (i.e. non-bilaterian) animals (within which Placozoa branched off first) as the sister group to the triploblastic Bilateria, which led the authors to derive far-reaching conclusions about the evolution of characters such as the nervous system and to propose a "modernized Urmetazoa hypothesis". However, according to topology tests (Shimodaira and Hasegawa, 1999), their preferred tree was not significantly better than competing hypotheses. Furthermore, it was recently shown that their supermatrix contained genes with questionable orthology, frameshift errors, point mutations, as well as biological and *in silico* contaminations (Philippe *et al.*, 2011). An analysis of the same dataset after correction of

errors resulted in a different tree topology in which the diploblastic clade was no longer supported (Philippe *et al.*, 2011).

Finally, Philippe *et al.* (2009) published a study based on 128 genes and the most comprehensive sampling of non-bilaterian taxa at that date. Their results revived traditional views on deep animal relationships in that they recovered a highly supported monophyletic Porifera (discussed in more detail below) as sister to the remaining Metazoa, as well as a clade uniting Ctenophora and Cnidaria (=Coelenterata) as sister to the Bilateria, with Placozoa the sister to the Eumetazoa (Coelenterata+Bilateria) clade (see Fig. 1.1). From the morphological perspective, this tree is plausible because if ctenophores or placozoans would have branched off prior to sponges (i.e. form the sister group to the remaining metazoans including sponges),

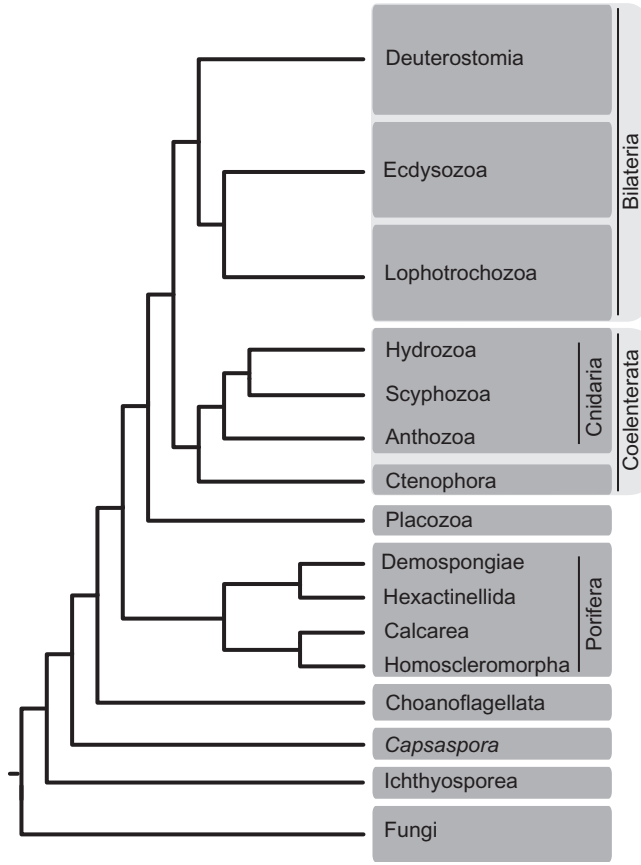

Figure 1.1 Consensus of higher-level phylogenetic relationships of Metazoa, including relationships to non-metazoan relatives (redrawn from Philippe *et al.*, 2009).

cytological features of, for example, sponges—such as choanocyte-like cells, presumably shared by choanoflagellate protists and the hypothetic animal ancestor—would have been lost in Ctenophora or Placozoa and independently in the ancestor of Cnidaria and Bilateria, being only retained by the common ancestor of sponges.

2.1. The status of phylum Porifera: Monophyletic or paraphyletic?

Porifera is morphologically well supported as a monophylum within the Metazoa as judged by the main biphasic life cycle, filter-feeding habits in combination with a sessile adult form, pinacocytes, choanocytes, and aquiferous system (e.g. Böger, 1983; Ax, 1996; Reitner and Mehl, 1996), although exceptions to the classical sponge bauplan exist (e.g. some sponges lack a larval stage and/or mineral skeleton, carnivorous sponges lack choanocyte chambers and an aquiferous system, see Erpenbeck and Wörheide, 2007). The extant classes within the phylum Porifera are also morphologically well defined—Calcarea (calcareous sponges or calcisponges) produce extracellular calcite spicules, Hexactinellida (glass sponges) are characterized by triaxonic silica spicules and adult tissues largely formed by syncytia, and Demospongiae possess monaxonic, tetraxonic, and/or polyaxonic silica spicules, and/or collagen-derived skeletal structures (e.g. spongin fibres and filaments, masses of collagen fibrils) (Hooper and Van Soest, 2002d). Recently, the Homoscleromorpha, which were considered as a subgroup of demosponges at different taxonomic levels (Hooper and Van Soest, 2002d), have received special attention (see below) and are now regarded as a separate class (e.g. Gazave *et al.*, 2010b, 2012).

The monophyly of the extant sponge classes is generally supported by molecular data (Erpenbeck and Wörheide, 2007; Gazave *et al.*, 2012), but the phylogenetic relationships among them and to other metazoan taxa are still regarded as contentious. Morphological analyses have supported different scenarios of relationships within a monophyletic Porifera (see Erpenbeck and Wörheide, 2007 for a summary), while molecular studies, beginning in the early 1990s, predominantly suggested that sponges might be a paraphyletic assemblage sharing a grade of construction rather than common ancestry (see Table 1.1).

Lafay *et al.* (1992) were among the first to investigate higher-level sponge phylogeny with molecular sequence data, using about 400 bp of 28S rDNA. Their analyses suggested that sponges are paraphyletic (with high support) and they also found paraphyletic demosponges. Calcarea was reconstructed as the sister group to Ctenophora, but this inference was not robustly supported. Furthermore, Hexactinellida and Bilateria were not included. Cavalier-Smith *et al.* (1996) analyzed 450 near-complete eukaryotic 18S rDNA sequences, including about 100 animal species, and they also found

Table 1.1 Non-exhaustive summary of molecular phylogenetic studies that include statements about sponge mono- versus paraphyly

Author	Molecular marker	Inference method (Model)	Sponge lineages included (number of taxa)				Bilateria included	Monophyly, support	Paraphyly, support
			Demospongiae	Homoscleromorpha	Hexactinellida	Calcarea			
Lafay *et al.* (1992)	Partial 28S rDNA	NJ, MP, ML	9	–	–	2	No		Yes, high
Cavalier-Smith *et al.* (1996)	18S rDNA	NJ, MP, ML	4	–	1	3	Yes		Yes, low
Van de Peer and de Wachter (1997)	18S rDNA (secondary structure)	Distance	2	–	–	2	Yes		Yes, low (BS 67)
Zrzavy *et al.* (1998)	18S rDNA	MP	3	–	–	3	Yes		Yes, low
Collins (1998)	18S rDNA	MP, ME, ML	5	1	1	3	Yes		Yes, low
Kruse *et al.* (1998)	cPKC	NJ	2	–	1	1	Yes		Yes, low
Schütze *et al.* (1999)	*Hsp*70, cPKC, calmodulin, tubulin	NJ	2	–	1	1	Yes		Only with *Hsp*70 and cPKC, low
Kim *et al.* (1999)	18S rDNA		3	–	–	3	Yes	Unresolved	Unresolved
Adams *et al.* (1999)	18S rDNA (secondary structure)	MP, ME, ML	7	–	2	4	No		Yes, low
Medina *et al.* (2001)	18S rDNA, 28S rDNA	ML with KH tests	2	–	1	1	Yes	Equivocal	Equivocal
Peterson and Eernisse (2001)	18S rDNA	MP	8	–	2	4	Yes		Yes, low
Rokas *et al.* (2003)	a-tubulin, b-tubulin, EF-2, *HSP90*, *HSP70*	ML	4	–	1	2	Yes	Unresolved	Unresolved
Manuel *et al.* (2003)	18S rDNA	MP, ML	9	–	2	17	Yes	Equivocal	Equivocal
Rokas *et al.* (2005)	50 genes	ML, MP, BI	1	–	1	1	Yes	Unresolved	Unresolved
Borchiellini *et al.* (2001)	18S rDNA	NJ, MP	12	–	5	2	No		Yes, high (BS: MP 83/Nj 85)
Peterson and Butterfield (2005)	Seven nuclear housekeeping genes	MP, ML, distance	3	–	–	2	Yes		Yes, medium (BS 76)
Peterson *et al.* (2005)	Seven nuclear housekeeping genes, mtDNA COI, 18S rDNA	MP	3	–	–	2	yes		Yes, low (BS 62)

Sperling *et al.* (2007)	Seven nuclear housekeeping genes	Partitioned BI	9	1	–	2	Yes		Yes, high
Dohrmann *et al.* (2008)	18S rDNA, 28S rDNA, 16S rDNA (mt)	Partitioned BI, secondary structure	6	2	32	4	No	Yes, low (PP 0.6/0.7, BS 74)	
Dohrmann *et al.* (2009)	18S rDNA, 28S rDNA, 16S rDNA (mt)	Partitioned BI, secondary structure	6	2	43	4	No	Yes, low-moderate (PP 0.59/0.84)	
Sperling *et al.* (2009)	Seven nuclear housekeeping genes	BI (CAT-GTR)	20	2	3	4	Yes		Yes, low (PP 0.65/0.71)
Sperling *et al.* (2010)	Seven nuclear housekeeping genes	BI (CAT-GTR)	20	2	3	5	Yes		Yes, moderate to low (PP 0.92/0.75)
Philippe *et al.* (2009)	128 genes	BI (CAT)	4	1	2	2	Yes	Yes, high (Bayesian BS 96)	
Pick *et al.* (2010)	150 genes	BI (CAT)	6	2	3	2	Yes	Yes, moderate (PP 0.91)	
Erwin *et al.* (2011)	Seven nuclear housekeeping genes, 18S rDNA, 28S rDNA	Partitioned BI (GTR)	14	2	–	5	Yes		Yes, high

Abbreviations: MP, maximum parsimony; ML, maximum likelihood; BI, Bayesian inference; NJ, neighbour-joining; KH, Kishino-Hasegawa test; PP, posterior probability; BS, bootstrap support; mt, mitochondrial; CAT, CAT model; GTR, general time-reversible model; rDNA, ribosomal DNA; KH, Kishino-Hasegawa.

sponge paraphyly with Calcarea identified as a sister to Ctenophora; however, this was again poorly supported. Van de Peer and de Wachter (1997) were the first to use RNA secondary structure to guide alignment of 18S rDNA sequences, and they investigated about 500 eukaryote species. The authors did not focus on animal phylogeny, although they also found sponges to be paraphyletic with Calcarea as a sister group to Ctenophora, but with low support; no hexactinellids were included. Koziol *et al.* (1997) analyzed a protein-coding gene, *Hsp*70, from three sponges and one bacterium and recovered a completely unresolved tree. Similarly, using the same gene, Borchiellini *et al.* (1998) were unable to resolve the branching order between Cnidaria, Ctenophora, and the three classes of Porifera with convincing support, as their results were highly dependent on the tree reconstruction method. Subsequent studies using protein-coding genes (Kruse *et al.*, 1998; Schütze *et al.*, 1999) have generally provided low support for paraphyletic sponges, but these studies suffered from poor taxon sampling, and they only used simple distance methods.

Zrzavy *et al.* (1998) recovered sponge paraphyly from a combined analysis of 18S rDNA and morphology. The authors reported that siliceous sponges diverged early (although hexactinellids were not included). They used maximum parsimony for tree reconstruction and provided no statistical support measures; sponges were recovered as monophyletic when the morphological data were analyzed alone. Adams *et al.* (1999) also analyzed 18S rDNA and again recovered a weakly supported sister group relationship between Calcarea and Ctenophora, while Cnidaria was identified as sister to a siliceous sponge clade (Demospongiae + Hexactinellida). Collins (1998), also using 18S rDNA, found Demospongiae + Hexactinellida as the sister group to the remaining Metazoa but could not resolve the position of Calcarea and Ctenophora with convincing support. Further rDNA analyses by Kim *et al.* (1999) and Medina *et al.* (2001), the latter including 28S sequences in addition to 18S sequences, likewise found no unambiguous support for either sponge mono- or paraphyly. Another 18S rDNA analysis (Borchiellini *et al.*, 2001) supported paraphyletic sponges with Calcarea as the sister group of eumetazoans, followed by Demospongiae and then Hexactinellida. Their preferred topology received relatively high bootstrap support, but the authors only used simple distance and parsimony algorithms, and Bilateria were not included. Their proposition to elevate Calcarea to the phylum level did not find wide acceptance.

Peterson and Eernisse (2001) conducted a similar study to that of Zrzavy *et al.* (1998) using maximum parsimony to analyze 18S rDNA and morphology, this time including hexactinellid sequences. Their results were similar to those of Zrzavy *et al.* (1998), but again, statistical support for the paraphyly hypothesis was not assessed. In another 18S rDNA study, which focused on the phylogeny of Calcarea, Manuel *et al.* (2003) found no convincing support for either sponge mono- or paraphyly and concluded

that "18S rRNA alone is inefficient for resolving sponge [. . .] monophyly". In summary, early molecular studies produced many conflicting hypotheses regarding sponge interrelationships (summarized in Table 1.1) while analyses based on morphology consistently support sponge monophyly (see above). However, those molecular studies were based on only one or a few genes (often partial) with little phylogenetic signal, often missed some important in-group taxa, and frequently suffered from systematic biases.

Rokas *et al.* (2003) were among the first to analyze multiple protein-coding genes from non-bilaterian animals but failed to resolve their relationships, including sponge mono- or paraphyly. They concluded that none of these genes contains sufficient phylogenetic signal to resolve deep metazoan phylogeny.

Peterson *et al.* (2004) used seven nuclear housekeeping genes to investigate metazoan evolution, and this was followed by a series of studies that steadily increased taxon sampling of the same set of genes and always found sponges to be paraphyletic (Peterson and Butterfield, 2005; Peterson *et al.*, 2005; Sperling *et al.*, 2007, 2009, 2010). These authors are among the strongest proponents of the sponge paraphyly hypothesis, and they derived far-reaching conclusions about early animal evolution from it. Peterson *et al.* (2005) and Peterson and Butterfield (2005) only included Calcarea and Demospongiae, and paraphyly (with Calcarea closer to Eumetazoa) was not strongly supported (Table 1.1). Sperling *et al.* (2007) added more demosponges and a homoscleromorph and found the latter as sister to Eumetazoa with good support (see also below for further discussion of this grouping). However, hexactinellids were still missing from their data set, preventing a relevant test of sponge monophyly. Also, very distantly related outgroups (one plant, one fungus) were used, which might have introduced a bias (see Philippe *et al.*, 2011).

Dohrmann *et al.* (2008, 2009) investigated the phylogeny of Hexactinellida using 18S and 28S rDNA (also 16S rDNA, but no outgroups were included for this partition) and found monophyletic sponges (albeit with low support) and a highly supported sister group relationship between Calcarea and Homoscleromorpha (see below for further discussion of this grouping). Hexactinellida were shown to be closely related to demosponges; although the latter were paraphyletic with respect to the former, this was attributed to insufficient taxon sampling of Demospongiae.

Sperling *et al.* (2009) again increased the taxon sampling of their housekeeping gene dataset by including among others another homoscleromorph and three hexactinellids. Although their topology was similar to that of their previous study (Sperling *et al.*, 2007), with Hexactinellida recovered as sister to Demospongiae, critical nodes were not well supported under their best-fitting substitution model. In particular, the nodes responsible for sponge paraphyly, that is, the positions of Calcarea and Homoscleromorpha as successive sister groups to Eumetazoa, only had 0.65 and 0.71 Bayesian

posterior probability. Sperling *et al.* (2010) added another calcareous sponge, which resulted in increased support for sponge paraphyly (0.92 for the position of Calcarea). The same gene-sampling was used by Erwin *et al.* (2011) in combination with rDNA sequences, and their analysis recovered a Calcarea + Homoscleromorpha clade (consistent with Dohrmann *et al.*, 2008; Philippe *et al.*, 2009) as sister to Eumetazoa. However, Erwin *et al.* (2011) did not use the substitution model identified by Sperling *et al.* (2009) as best-fitting for the nuclear housekeeping genes and, even more surprisingly, included less demosponges than in Sperling *et al.* (2010) and removed the Hexactinellida altogether.

Sperling *et al.* (2010) also analyzed sponge micro RNAs (miRNAs), a set of novel molecular markers that have been proven valuable in studies of bilaterian relationships (Sperling and Peterson, 2009). None of seven (out of eight) demosponge-specific miRNAs were found in any of the hexactinellid, calcarean, or homoscleromorph small RNA libraries, so miRNAs could not contribute to resolving the mono- versus paraphyly issue. However, the presence of miR-2019 only in the Hexactinellida and Demospongiae supports their sister group relationship (Sperling *et al.*, 2010).

Philippe *et al.* (2009) were the first to apply a phylogenomic approach to the problem of sponge paraphyly. They included the most comprehensive sampling of non-bilaterian taxa to date, including all four extant sponge classes, for a set of 128 genes and recovered sponge monophyly with high support. Within Porifera, they found a sister group relationship of Hexactinellida and Demospongiae, and this "Silicea *sensu stricto*" clade was sister to a Homoscleromorpha + Calcarea clade (see Fig. 1.1), although the latter was less well supported than in Dohrmann *et al.* (2008). Pick *et al.* (2010) recovered a similar topology from their extended dataset from the Dunn *et al.* (2008) study (see above), although sponge monophyly was not as highly supported.

Due to the present lack of complete mitochondrial genome data from Calcarea and unique modes of mtDNA evolution in Calcarea and Hexactinellida (see below), mitogenomics could not yet contribute significantly to evaluations of the interrelationships of the four sponge classes. Studies based on mitochondrial genomes consistently find a sister group relationship between Homoscleromorpha and Demospongiae (Wang and Lavrov, 2007; Lavrov *et al.*, 2008), but resolving higher-level relationships of non-bilaterians using mitochondrial (genome) data has proven to be generally difficult (Lavrov, 2007).

In summary, the majority of studies that have suggested sponge paraphyly provide non-significant support for this hypothesis and/or are hampered by insufficient data (particularly taxon sampling) and/or methodological shortcomings (e.g. simple distance methods for phylogeny reconstruction). Although the final verdict is still open, the congruence of phylogenetic hypotheses derived from independent data types represents the strongest evidence to support one of these alternatives (Pisani *et al.*,

2007). Consequently, the reconstruction of poriferan relationships provided by Philippe *et al.* (2009) and corroborated by Pick *et al.* (2010) and Philippe *et al.* (2011) represents—with respect to the monophyly of sponges—the working hypothesis that is at present preferred by us (Fig. 1.1). Sponge monophyly is (a) supported by currently the largest amount of phylogenomic data (in terms of amino acid positions and in-group taxon sampling) (Philippe *et al.*, 2009; Pick *et al.*, 2010) and (b) is congruent with cladistic analyses of morphological characters (e.g. Böger, 1983; Ax, 1996; Reitner and Mehl, 1996). It should be noted, however, that the alleged sister group relationship of Calcarea and Homoscleromorpha, as reported from recent molecular studies (Dohrmann *et al.*, 2008; Philippe *et al.*, 2009; Pick *et al.*, 2010; Erwin *et al.*, 2011) is presently difficult to support by morphological synapomorphies (see discussion below).

2.2. Why is the phylogenetic status of sponges important for understanding early animal evolution?

In the sponge paraphyly scenario (Fig. 1.2), either Calcarea or Homoscleromorpha or both are more closely related to the rest of the metazoans than to Demospongiae and Hexactinellida. The possible position of Homoscleromorpha as a sister to Eumetazoa has received special attention because these are the only sponges which possess a basement membrane (Fig. 1.3) with evidence of the presence of type-IV collagen in this layer (Boute *et al.*, 1996), which is traditionally considered to define "true" epithelia and might then be interpreted as a synapomorphy of Homoscleromorpha and Eumetazoa (Sperling *et al.*, 2007). Consequently, Homoscleromorpha were included in the Epitheliozoa (a clade combining Eumetazoa and Placozoa, Ax, 1996) by Sperling *et al.* (2009). This scenario opens the possibility that "true" epithelia and developmental mechanisms involved in epithelial patterning and morphogenesis would have appeared before the emergence of Eumetazoa, which is consistent with a conserved function of *Wnt* signalling in epithelial morphogenesis in Homoscleromorpha and Eumetazoa (Ereskovsky *et al.*, 2009; Windsor and Leys, 2010).

The most remarkable feature of the sponge body plan is the aquiferous system, a system of internal canals in which water is pumped from the external medium to chambers lined by choanocytes (flagellate filtering cells). In the paraphyly scenario, the aquiferous system and the choanocytes would have to be interpreted most parsimoniously as ancestral features of Metazoa, implying that the most recent common ancestor of all extant animals was a sponge-like organism (Fig. 1.2). In this scenario, non-sponge metazoans are derived from a sponge-like ancestor through loss of poriferan attributes (Fig. 1.2). Maldonado (2004) proposed that such a step could have involved a neotenic evolution from a poriferan-like larval stage and Nielsen

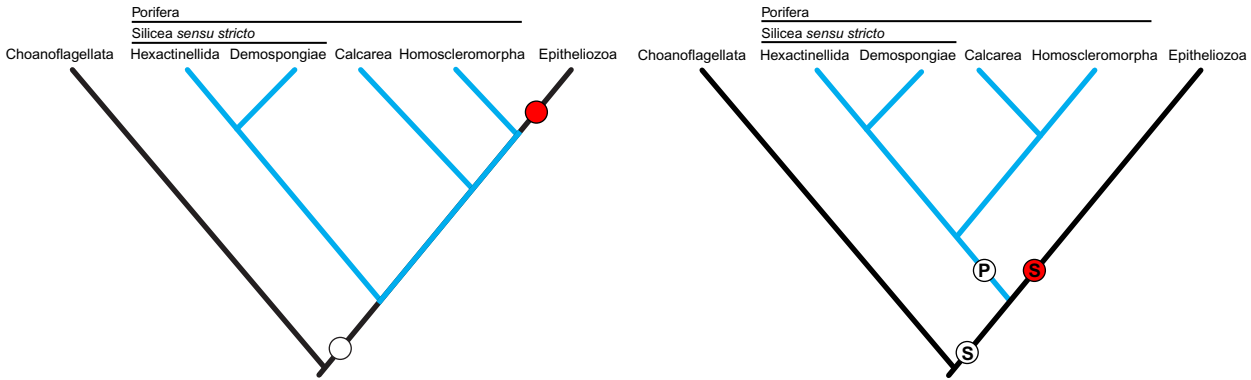

Figure 1.2 Alternative scenarios for the higher-level relationships of extant sponges. *Left*: sponge paraphyly (e.g. Sperling *et al.*, 2009). According to this scenario, a sponge-like body plan (white circle) was acquired in the last common ancestor of Porifera and Epitheliozoa (*sensu* Ax, 1996; = Cnidaria, Ctenophora, Placozoa, Bilateria) and subsequently lost (red circle) from the last common ancestor of Epitheliozoa. Alternative paraphyly scenarios exist mainly in earlier studies, where homoscleromorphs were often not included (see text for details). *Right*: sponge monophyly (e.g. Philippe *et al.*, 2009). According to this scenario, the sponge-like body plan (white circle) was acquired either in the stem lineage of Porifera (P) or, if choanocytes are considered homologous to choanoflagellate cells as judged by outgroup comparison to the well-established sister group of the Metazoa, the Choanoflagellata (see text for details), in stem-group metazoans (S). The latter scenario would require one gain and one loss (indicated by white/red dots marked with S), as in the paraphyly hypothesis.

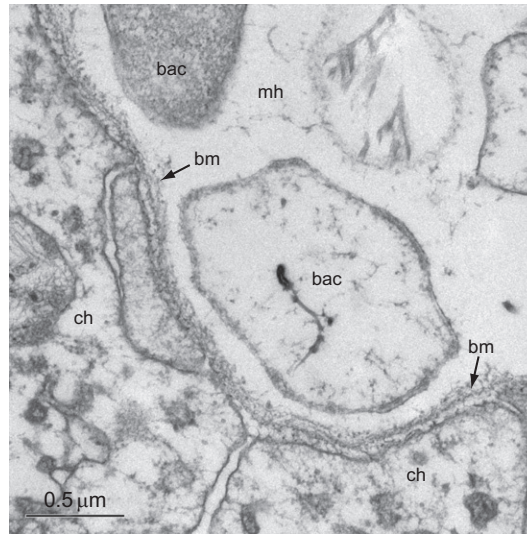

Figure 1.3 Details of the basement membrane (bm) reinforcing the proximal side of the choanocyte layer (ch) in the homoscleromorph *Corticium candelabrum*. Note the abundant intercellular bacteria (bac) and collagen fibrils in the sponge mesohyl (mh) adjacent to the choanocytes (ch).

(2008) suggested it to be a homoscleromorph-like larva that became sexually mature.

In contrast, in the monophyly hypothesis, with sponges as the sister group of all other metazoans, there are two options to make inferences about the metazoan ancestor. These depend on whether sponge choanocytes are considered as homologous to choanoflagellate cells or not. Based on the well-established sister group relationship of Choanoflagellata and Metazoa (King *et al.*, 2008) homology appears most likely, but seemingly different functional properties and ultrastructural differences led some authors to consider that gross morphological similarities could be rather convergences (see discussion in Woollacott and Pinto, 1995; Karpov and Leadbeater, 1998; Philippe *et al.*, 2009). If the latter is true, then the poriferan body plan with, for example, its aquiferous system was at a minimum present only in the stem group of extant sponges (Fig. 1.2) and no immediate inferences can be made about the metazoan ancestor. If sponge choanocytes are indeed homologous to choanoflagellate cells, then the stem group of extant metazoans could well have been a filter-feeding sponge-like organism.

Furthermore, the sponge monophyly scenario either implies that a basement membrane and, consequently, "true" epithelia as classically recognized were present in the last common ancestor of the Metazoa and were subsequently lost in sponge lineages other than Homoscleromorpha

(Fig. 1.2; see also discussion in Lavrov, 2007) or evolved convergently in Homoscleromorpha and Eumetazoa (see Fig. 3 in Philippe *et al.*, 2009). Loss of a basement membrane has been described in some Turbellaria (Plathyhelminthes, Brusca and Brusca, 2003), indicating that such a loss is indeed possible. Recent findings by Leys and Riesgo (2012) indicated that type-IV collagen, a major constituent of a basement membrane, is more ubiquitously distributed in different sponge lineages (found also in demosponges and calcareans) than previously appreciated. Type-IV collagen thus has likely been acquired in stem-group metazoans (as suggested by Aouacheria *et al.*, 2006). Whether the basement membrane then is a more recent independent innovation of homoscleromorphs and eumetazoans, in both cases involving the co-option of type-IV collagen, or symplesiomorphic for the Metazoa is unsolved at present and more research is needed to address these issues.

3. Mitochondrial DNA in Sponge Phylogenetics

Mitochondria—the energy-producing organelles present in most eukaryotic cells—contain their own genome (mt-genome or mtDNA), which is separate from that of the nucleus. For technical and historical reasons, mtDNA has been one of the favourite molecular markers in animal phylogenetic, population genetic, and biogeographic studies as it provides convenient access to a set of orthologous genes with few or no introns, little or no recombination, usually uniparental inheritance, and high evolutionary rates (for a review see Moritz *et al.*, 1987). Although complete sequences of animal mtDNA have been determined since the early 1980s (e.g. Anderson *et al.*, 1981), the first complete mitochondrial genomes of sponges were only published in 2005 (Lavrov *et al.*, 2005). Since then, complete mitochondrial genome sequences have been determined for ~30 sponges, and current projects aim to bring this number into the 100s. Here, we describe the general organization of mtDNA in sponges and we review a few studies that inferred phylogenies based on mitochondrial sequences. Our focused attention on mtDNA is due to its unique role in animal phylogenetics and our advanced knowledge of its genomic organization in sponges.

3.1. The mitochondrial genomes of sponges

Studies of the mitochondrial genomes of sponges have produced two main unexpected outcomes. First, the study of mtDNA from a few species of demosponges revealed its unique organization, which is different from that in bilaterian animals (Lavrov *et al.*, 2005). Second, a sampling of additional mtDNA from Demospongiae as well as from Hexactinellida, Calcarea, and

Homoscleromorpha showed distinct modes and rates of mitochondrial genome evolution in each of these groups.

So far, most mitochondrial genomes have been determined for the class Demospongiae, including at least one genome for each traditionally recognized order in this group (Lavrov *et al.*, 2005; Erpenbeck *et al.*, 2007d, 2009; Belinky *et al.*, 2008; Lukic-Bilela *et al.*, 2008; Wang and Lavrov, 2008; Ereskovsky *et al.*, 2011). Mitochondrial genomes in Demospongiae are characterized by the retention of several ancestral features (e.g. shared with non-metazoan eukaryotes), including a minimally modified genetic code, the presence of extra genes, conserved structures of tRNA genes, and the existence of multiple non-coding regions (Lavrov *et al.*, 2005). At the same time, some variation has been found in their size, gene content, and gene order (Wang and Lavrov, 2008). The rate of nucleotide substitutions in demosponges is low, although a significant acceleration in evolutionary rates occurred in the Keratosa (G1) lineage (Wang and Lavrov, 2008). However, it is unclear whether this acceleration was restricted to a certain period in the history of the Keratosa or represents an on-going process, as several species with very different morphologies are separated by small mitochondrial genetic distances (Erpenbeck *et al.*, 2009).

Although most of the determined mitochondrial genomes come from the class Demospongiae, the best sampled group is the Homoscleromorpha, considering the ratio of published complete mitochondrial genomes (14) to the number of described species (<100) (Wang and Lavrov, 2007, 2008; Gazave *et al.*, 2010b). The mitochondrial genomes of homoscleromorphs are similar overall to those of demosponges and retain the same ancestral genomic features. However, two different mitochondrial organizations have been found within this group (Gazave *et al.*, 2010b) corresponding to the families of spiculate and aspiculate homoscleromorphs (Plakinidae and Oscarellidae, respectively; see below). Interestingly, one or two introns are present in the gene for cytochrome oxidase subunit 1 (*cox1* or COI) of several species of Plakinidae (Gazave *et al.*, 2010b), in the same positions where introns have also been found in the demosponge family Tetillidae (Szitenberg *et al.*, 2010). Their location in the standard DNA bar coding primer sites in *cox1* greatly complicates the amplification of this gene as a marker for sponge species identification.

Hexactinellida is currently represented by mitochondrial genomes of three species: *Iphiteon panicea*, *Sympagella nux* (Haen *et al.*, 2007), and *Aphrocallistes vastus* (Rosengarten *et al.*, 2008), although several additional genomes are forthcoming. Mitochondrial genomes in this group show a distinctly different organization that is superficially similar to that of bilaterian animals (Haen *et al.*, 2007). In particular, Bilateria and Hexactinellida share a change in the mitochondrial genetic code and unusual tRNA structures that are unknown outside these groups. Additionally, glass sponges are characterized by phylogenetically diverse and extensive usage of translational frameshifting in mitochondrial translation (Haen *et al.*, unpublished data).

The mitochondrial genome of calcareous sponges remains poorly characterized. However, a partial mitochondrial genome of the calcinean sponge *Clathrina clathrus* has been reported but has not yet been published (Lavrov *et al.*, 2006; Kayal *et al.*, 2010). Preliminary data indicate that this genome is highly unusual and exhibits a very high rate of sequence evolution. In addition to this genomic sequence, several mitochondrial genes of the calcinean *Leucetta chagosensis* were obtained from its cDNA library. The rate of mitochondrial sequence evolution in this group appears to be much higher than in other sponges (Voigt *et al.*, 2012a). Consequently, a first study on the intraspecific variation of the *cox3* gene in *Leucetta chagosensis* suggests that mitochondrial genes are very useful for phylogeographic studies of Calcarea (Voigt *et al.*, 2012a).

3.2. Inferring sponge phylogeny from mtDNA

Although poriferan mtDNA likely evolves as a single locus, its individual genes display different rates of sequence evolution (Wang and Lavrov, 2008) and so may be more or less appropriate for a specific phylogenetic inference. However, many studies utilized only *cox1* in sponge phylogenetics (e.g. Erpenbeck *et al.*, 2002, 2007a; Nichols, 2005; Cárdenas *et al.*, 2011). We note, however, that other genes, in particular *cob*, have been shown to be more phylogenetically informative (Farias *et al.*, 2001; Lavrov *et al.*, 2008) and some regions in the mtDNA genome may appear to be more informative than others for a particular group (e.g. Rua *et al.*, 2011).

To date, several studies have used complete mtDNA sequences to study the phylogenetic relationships of sponges. In particular, Lavrov *et al.* (2008) investigated demosponge relationships using 21 complete mt genomes representing all recognized orders in the group and Gazave *et al.* (2010b) used 14 complete and 2 partial mitochondrial genomes to study the relationships within Homoscleromorpha. In addition, individual mitochondrial genomes have been used in several other studies (Haen *et al.*, 2007; Belinky *et al.*, 2008; Lavrov *et al.*, 2008; Lukic-Bilela *et al.*, 2008; Erpenbeck *et al.*, 2009; Ereskovsky *et al.*, 2011). Phylogenetic results from these studies are described in the following sections of this review.

4. The Current Status of the Molecular Phylogeny of Demospongiae

4.1. Introduction to Demospongiae

Demosponges inhabit most aquatic habitats, including all oceans from the intertidal to the abyss, from the tropics to the polar seas, and (almost) all types of freshwater habitats. This diversity in habitats is reflected in their

taxonomic diversity. Demosponges are by far the most diverse group of Porifera, comprising about 85% of all extant sponge species.

Demosponges comprise cellular (i.e. not syncytial) Porifera possessing spongin (sometimes greatly reduced) whose mineral skeleton (if present) consists of either monaxonic, tetraxonic, or polyaxonic, -but never triaxonic-, siliceous spicules, and/or occasionally a calcareous basal skeleton. The mineral skeleton can be partially or entirely replaced by an organic skeleton consisting of spongin; alternatively, the skeleton may be reduced to its minimal expression in some demosponges, which only contain abundant collagen fibrils in their mesohyl. So far, there has been no evidence of the presence of a basal lamina as reported for Homoscleromorpha. Molecular data indicate that the definition of Demospongiae in the Systema Porifera (Hooper and Van Soest, 2002d) is now outdated because the inclusion of homoscleromorph sponges within Demospongiae has been rejected based on molecular and cytological data (see other parts of this article).

4.2. Taxonomic overview

In Boury-Esnault's (2006) review of the literature on the evolution of demosponges, the transition from an emphasis on morphology to an emphasis on genetics is described and how different data sets, analytical methods, and interpretations over the decades have resulted in many different classifications is discussed. With the advent of molecular techniques in sponge systematics (Kelly-Borges *et al.*, 1991), one of the first molecular phylogenies (e.g. Lafay *et al.*, 1992) indicated that the then-accepted classifications, which were based on morphology (e.g. Halichondrida, Van Soest *et al.*, 1990), lacked significant support from the molecular data.

Among the most important recent contributions to our understanding of the relationships between the demosponge taxa is the congruence between nuclear and mitochondrial gene trees. As both are reconstructed from independent loci, which have potentially different evolutionary histories and substitution patterns (see, e.g. Moore, 1995), congruent topologies provide strong evidence for accuracy. This congruence should be regarded as more important in phylogenies than high bootstrap support values, which indicate only the support from the underlying data of a single dataset (e.g. Felsenstein, 1985).

One of the first phylogenies directly targeting the deeper demosponge relationships was the work of Borchiellini *et al.* (2004), who expanded the 18S and 28S rDNA data set of Manuel *et al.* (2003) to include representatives of almost all accepted demosponge orders (*sensu*, Hooper and Van Soest, 2002d). Subsequent analyses with complete mitochondrial genomes of selected demosponge taxa (Lavrov *et al.*, 2008; Wang and Lavrov, 2008) provided important support for the new understanding of the deeper phylogenetic splits in demosponges as it revealed a high level of congruence

with the nuclear gene trees. However, the number of taxa analyzed for these studies was relatively low (1–2 species per order) compared to the diversity of demosponges and the uncertain monophyly of many orders. In addition, nuclear housekeeping gene data contradict some aspects of these new nuclear ribosomal and mitochondrial phylogenies (Sperling *et al.*, 2009) (see below).

Despite this finding, surprisingly little new insight into the deeper splits among demosponge lineages has been published since the last review (Erpenbeck and Wörheide, 2007) until the work of Morrow *et al.* (2012), who unravelled phylogenetic relationships of the "G4" clade (see below; see also Fig. 1.4, and for a new definition of the higher demosponge clades

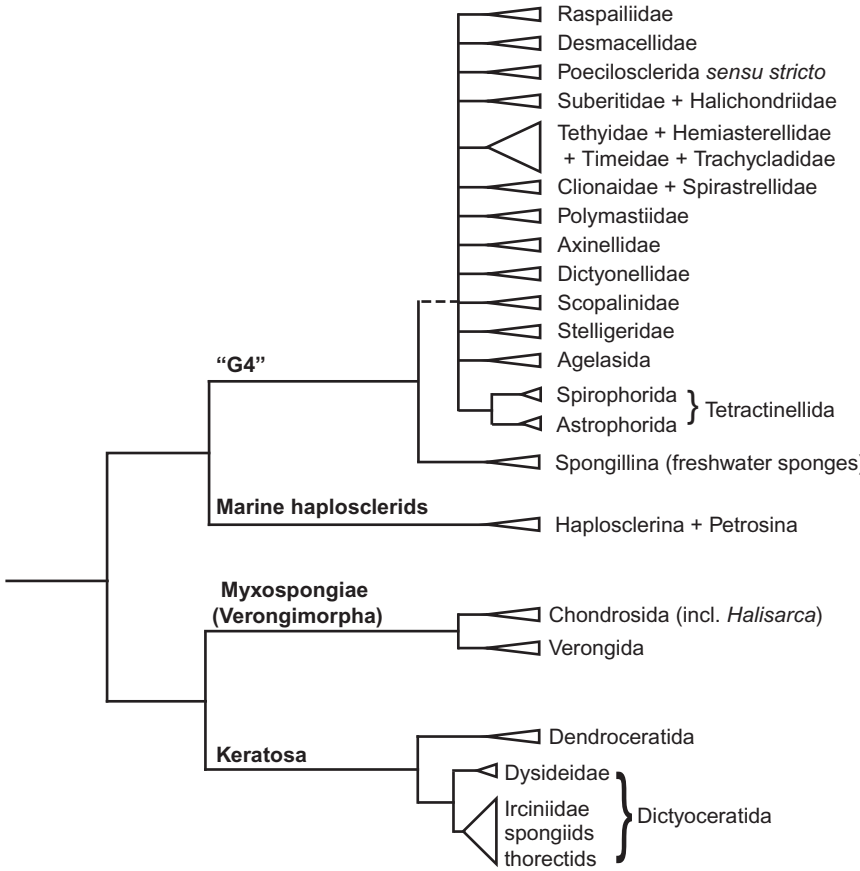

Figure 1.4 Overview of the current phylogenetic relationships of Demospongiae as evident from nuclear ribosomal and mitochondrial gene trees (see the text for details). The names of the deeper clades are adopted from Borchiellini *et al.* (2004), Boury-Esnault (2006) and Erpenbeck *et al.*, (2012b).

see the contribution of Cárdenas *et al.,* Chapter 2, this volume). Most new publications focused particularly on the phylogenetic relationships on shallower levels including species (e.g. López-Legentil and Pawlik, 2008), genera (e.g. Pöppe *et al.*, 2010), families (e.g. Gazave *et al.*, 2010a), and orders (e.g. Cárdenas *et al.*, 2011), leaving many questions about deep demosponge phylogeny unaddressed.

Among the (mostly) aspiculate demosponges, the orders Verongida, Halisarcida, and Chondrosida (with a single genus containing siliceous spicule elements) form a clade termed Myxospongiae ("G2", Borchiellini *et al.*, 2004 also termed "Verongimorpha", (Erpenbeck *et al.*, 2012b)), as revealed by ribosomal (Addis and Peterson, 2005; Nichols, 2005; Schmitt *et al.*, 2005; Holmes and Blanch, 2007; Redmond *et al.*, 2007), mitochondrial (Nichols, 2005; Rot *et al.*, 2006; Lavrov *et al.*, 2008; Wang and Lavrov, 2008), and nuclear housekeeping gene data (Sperling *et al.*, 2009; Fig. 1.4). The monogeneric Halisarcida do not possess any skeletal elements besides collagenous fibrils. Such askeletal taxa are likewise found in Verongida (*Hexadella*), which includes sponges possessing spongin skeletons, and in Chondrosida, which include askeletal (*Chondrosia*), spiculose (*Chondrilla*), and spongin skeleton-possessing (e.g. *Thymosia*) taxa.

The orders Dictyoceratida and Dendroceratida form a clade termed Keratosa (clade "G1", Borchiellini *et al.*, 2004; Addis and Peterson, 2005; Schmitt *et al.*, 2005; Lavrov *et al.*, 2008; Wang and Lavrov, 2008; Fig. 1.4). Both orders include sponges with a spongin skeleton lacking authigenic (produced by the organism itself) mineral elements (with the exception of the coralline sphinctozoan *Vaceletia*; see below).

Molecular phylogenies, based on both nuclear ribosomal and mitochondrial data, suggest a deep split between (mostly) spiculose and (mostly) aspiculose demosponges (Borchiellini *et al.*, 2004; Addis and Peterson, 2005; Nichols, 2005; Schmitt *et al.*, 2005; Holmes and Blanch, 2007; Redmond *et al.*, 2007; Lavrov *et al.*, 2008; Wang and Lavrov, 2008). While ribosomal RNA data initially could not unambiguously resolve whether Keratosa and Myxospongiae are sister taxa (as suggested by 18S phylogenies) or Keratosa are sister group to all other demosponges (as suggested by 28S phylogenies) (Borchiellini *et al.*, 2004), subsequent trees reconstructed from mitochondrial genomes suggested the former, although support values were low (Lavrov, 2007; Lavrov *et al.*, 2008). Consequently, sponges (mostly) without a mineral skeleton might form a sister group to the (mostly) spiculose sponges (Fig. 1.4). However, analyses of nuclear housekeeping genes suggested that Myxospongiae are the sister group to all other Demospongiae (Sperling *et al.*, 2009), albeit with low support. Therefore, the deepest split among the demosponge lineages currently remains unresolved.

The clade of (mostly) spiculose sponges contains the majority of the demosponge taxa. Nuclear ribosomal and mitochondrial data univocally suggest that marine species of the order Haplosclerida (i.e. two of its suborders, Petrosina

and Haplosclerina) form a sister group to all other spiculose sponges (Fig. 1.4). This marine Haplosclerida clade corresponds to a clade termed "G3" in the nuclear ribosomal analyses of Borchiellini *et al.* (2004) and is congruent with several other nuclear and mitochondrial gene trees (Schmitt *et al.*, 2005; Holmes and Blanch, 2007; Lavrov *et al.*, 2008; Wang and Lavrov, 2008). DNA data repeatedly demonstrated that marine Haplosclerida do not form a clade with their freshwater counterparts (suborder Spongillina), which form a clade with all remaining demosponge taxa subsequently termed "G4" that is the sister group to all other (mostly spiculose) demosponges (see Borchiellini *et al.*, 2004; Addis and Peterson, 2005; Nichols, 2005; Itskovich *et al.*, 2007; Redmond *et al.*, 2007; Lavrov *et al.*, 2008; Wang and Lavrov, 2008). In contrast, marine and freshwater Haplosclerida form a well-supported clade in phylogenetic reconstructions based on nuclear housekeeping genes (Sperling *et al.*, 2009).

The "G4" clade is the by far most diverse among the higher taxa of demosponges and comprises besides the freshwater sponges a wide range of taxa that are morphologically classified into the orders Astrophorida, Spirophorida, Poecilosclerida, Hadromerida, Halichondrida, and Agelasida (Fig. 1.4). A plethora of molecular gene trees (discussed below) indicates the non-monophyly of several of these orders, and the resolution of the "G4" clade into a new classification and subsequent re-interpretation of morphological characters is the focus of several recent studies (e.g. Erpenbeck *et al.*, 2012a; Morrow *et al.*, 2012).

Molecular data corroborated the earlier views, particularly after introducing cladistic character analyses in sponge systematics (Van Soest, 1990), that a division of demosponges into the subclasses "Ceractinomorpha" and "Tetractinomorpha" is invalid (Hooper, 1984; Van Soest, 1984). Those two subclasses were based primarily on reproductive features (see, e.g. Van Soest, 1991) and were consequently disregarded in the last major classification of sponge genera (Systema Porifera, Hooper and Van Soest, 2002d). They were subsequently abandoned because of a lack of molecular support (Boury-Esnault, 2006).

4.3. Molecular phylogenetics

4.3.1. Keratosa

Dictyoceratida and Dendroceratida are sister groups that form the Keratosa. In dendroceratids, the fibre skeleton is frequently (but not always) dendritic and arises from a basal plate, and it is generally less dense than in dictyoceratids. Dictyoceratids possess anastomosing and, in comparison to dentroceratids, mostly denser fibre networks.

4.3.1.1. Dendroceratida

All dendroceratid sponges possess eurypylous choanocyte chambers. Eurypylous choanocyte chambers connect directly with inhalant and exhalant canals, whereas diplodal choanocyte chambers connect only via a so-called

prosodus or aphodus. Morphologically, Dendrocertida are divided into two families, Darwinellidae, which possess a mostly dendritic skeleton (i.e. the skeletal elements branch, but do not rejoin) and Dictyodendrillidae, which possess a mostly anastomose skeleton (i.e. the skeletal elements branch and rejoin and might form a network [reticulum]) (Bergquist and Cook, 2002b). Published molecular data on dendroceratids are scarce (see, e.g. Borchiellini *et al.*, 2004), but recent results based on nuclear (28S rDNA) and mitochondrial (*cox1*) sequences support the monophyly of Dendroceratida while rejecting the monophyly of the two traditionally recognized families (Erpenbeck *et al.*, unpublished data).

4.3.1.2. Dictyoceratida

Dictyoceratida is composed of the families Dysideidae, Irciniidae, Thorectidae, and Spongiidae. Thorectidae and Spongiidae are mostly distinguishable by the presence of laminated (Thorectidae) or homogeneous (Spongiidae) spongin fibre bark, while Irciniidae have characteristic collagenous filaments, which impart a rubber-like consistency (Cook and Bergquist, 2002). All of these three families have diplodal choanocyte chambers, while members of the fourth family, the Dysideidae, have eurypylous choanocyte chambers, which has led to speculations about a dendroceratid origin of the Dysideidae (see, e.g. Bergquist, 1980). However, recent research based on 28S rDNA and *cox1* sequences supports the dictyoceratid origin of dysideid sponges (Erpenbeck *et al.*, 2012b). Most molecular phylogenies published so far are consistent with monophyly of Dictyoceratida (e.g. Borchiellini *et al.*, 2004; Nichols, 2005; Holmes and Blanch, 2007; Redmond *et al.*, 2007; Lavrov *et al.*, 2008; Wang and Lavrov, 2008; Wörheide, 2008), although taxon sampling is still somewhat limited (but see also Erpenbeck *et al.*, 2012b). Dysideidae are probably a sister group to the remaining dictyoceratids (congruent with Borchiellini *et al.*, 2004; but see also Sperling *et al.*, 2009), indicating monophyly of dictyoceratids with diplodal choanocyte chambers. Within this clade, Irciniidae form a monophyletic group, while the validity of Spongiidae and Thorectidae is still to be resolved (Erpenbeck *et al.*, 2012b). A striking discovery was that *Vaceletia*, a coralline sponge with sphinctozoan bauplan that had until then been placed in its own order Verticillitida, also falls within the Dictyoceratida based on 18S and 28S rDNA data (Wörheide, 2008). This finding was subsequently corroborated by complete mtDNA data (Wang and Lavrov, 2008). Thus, *Vaceletia* appears to be the only recent keratose sponge with an authigenic, although secondary, mineral skeleton.

4.3.2. Myxospongiae (Verongimorpha)

Myxospongiae comprise taxa of the orders Chondrosida, Halisarcida, and Verongida. Myxospongid synapomorphies are mainly cytological, for example, the orientation and position of the accessory centriole, the nuclear

apex, and the Golgi apparatus relative to each other, as observed in the ultrastructure of epithelial and larval cells (Maldonado, 2009). Molecular data indicate a sister group relationship between Chondrosida and Halisarcida (Boury-Esnault, 2006), with the Verongida more distantly related. However, the exact branching pattern is dependent on the uncertain taxonomic status of the Chondrosida (see below). On grounds of the cytological and molecular congruencies, Maldonado (2009) elevated the clade formed by these three orders to the subclass level (Myxospongia).

4.3.2.1. Chondrosida

Among the myxospongid taxa, chondrosids possess a marked cortex with fibrillar collagen (Boury-Esnault, 2002). Its four genera display a wide range of skeletal features, such as the possession of siliceous spicules (*Chondrilla*), an irregular, sparse network of small nodal fibres (e.g. *Thymosia*), or only collagen fibrils in the mesohyl (*Thymosiopsis*). Nevertheless, Chondrosida do not appear to be monophyletic in molecular trees. While the molecular data support a close relationship between *Chondrilla*, *Thymosia*, and *Thymosiopsis* (Vacelet *et al.*, 2000), neither nuclear ribosomal (Borchiellini *et al.*, 2004) nor mitochondrial data (Erpenbeck *et al.*, 2007a) group these three genera with *Chondrosia* (see also Nichols, 2005).

4.3.2.2. Halisarcida

The monogeneric Halisarcida (*Halisarca*) include sponges without a skeleton but with a highly organized ectosomal and subectosomal collagen as well as tubular and branched choanocyte chambers (Bergquist and Cook, 2002c). According to recent molecular data, Halisarcida form a clade with *Chondrilla*, *Thymosia*, and *Thymosiopsis*, indicating the non-monophyly of chondrosids (Erpenbeck *et al.*, unpublished data). Recently the Halisarcida have been merged with the Chondrosida (Ereskovsky *et al.*, 2011).

4.3.2.3. Verongida

Verongida is the largest order within the Myxospongiae. Verongid sponges are characterized by the presence of spongin fibres in all but one genus, with a generally well-laminated bark, a dark cellular pith (=fine inclusions) (Bergquist and Cook, 2002d), and the production of bromotyrosines (see Erpenbeck and Van Soest, 2007 for a discussion). Verongida are classified into four families, of which the Ianthellidae possess eurypylous choanocyte chambers while the other three families Aplysinidae, Aplysinellidae, and Pseudoceratinidae (with diplodal choanocyte chambers) differ based on their skeletal characteristics (Bergquist and Cook, 2002d).

Monophyly of Verongida has been demonstrated in a series of analyses (Borchiellini *et al.*, 2004; Nichols, 2005; Holmes and Blanch, 2007; Kober and Nichols, 2007; Redmond *et al.*, 2007). Current rDNA internal transcribed spacer (ITS) data indicate the non-monophyly of several verongid families as the monogeneric Pseudoceratinidae (*Pseudoceratina*) form the

sister group to *Verongula* (Aplysinidae), while the other Aplysinidae branch earlier (*Aplysina*) (Erwin and Thacker, 2007). Other Aplysinidae (Aiolochroia, although Aplysinidae insertae sedis, Bergquist and Cook, 2002a) form a sister group to Ianthellidae (Erwin and Thacker, 2007). Mitochondrial (*cox1*) and 28S rDNA data support the findings of Erwin and Thacker (2007), indicating Ianthellidae monophyly and the non-monophyly of aplysinid and aplysinellid sponges (Erpenbeck *et al.*, 2012b).

4.3.3. Marine Haplosclerida

Haplosclerida is regarded as an evolutionarily successful taxon with respect to diversity and habitat (e.g. Van Soest and Hooper, 2002b). The skeleton of marine haplosclerids displays a (partial) isodictyal reticulation (i.e. triangular meshes with sides of one spicule length) of diactinal (two rayed) spicules. Marine Haplosclerida are currently classified into the suborders Haplosclerina and Petrosina with three families each. Their molecular phylogeny is still one of the largest mysteries in demosponge systematics. Molecular data have so far been unable to confirm the morphological classification, including the monophyly of the marine haplosclerid suborders, families, and even genera (particularly the species-rich genera *Haliclona* and *Callyspongia*, see, e.g. McCormack *et al.*, 2002; Erpenbeck *et al.*, 2004; Itskovich *et al.*, 2007; Raleigh *et al.*, 2007; Redmond *et al.*, 2007; Redmond and McCormack, 2008; Voigt *et al.*, 2008). Reasons for these discrepancies are still unknown, although an elevated substitution rate in comparison to the other demosponge orders has been detected for the nuclear ribosomal genes (Erpenbeck *et al.*, 2004) and occasionally in mitochondrial genes (Erpenbeck *et al.*, 2007d), which can cause tree reconstruction artefacts but may not entirely explain the incongruent branching patterns. In recent years, attempts to resolve marine haplosclerid phylogeny found congruency of topologies reconstructed from ribosomal RNA (including a study on the suitability of ITS, Redmond and McCormack, 2009) and mitochondrial markers. These congruencies diminish the possibility of reconstruction artefacts as a source of the contradictions to morphology and strengthen the need for a revised marine haplosclerid classification. An analysis of 28S rDNA (McCormack *et al.*, 2002), 18S rDNA (Redmond *et al.*, 2007), including secondary structure analyses (Redmond and McCormack, 2008; Voigt *et al.*, 2008), and two different fragments of *cox1* (Itskovich *et al.*, 2007; Raleigh *et al.*, 2007) suggest the presence of a large clade including several intermixed *Callyspongia* (Callyspongiidae) and *Haliclona* (Chalinidae) species, while most petrosids, niphatids, and phloeodictyids branch earlier.

4.3.4. The "G4" clade

The remaining demosponge taxa form a clade designated as "G4" by Borchiellini *et al.* (2004) (also termed "Democlavia" by Sperling *et al.*, 2009). It comprises by far the largest taxonomic diversity of demosponges. Molecular data suggest that most of the morphologically defined orders are

not monophyletic and a recent study of Morrow *et al.* (2012) led to a new classification of the "G4" clade based on analyses of mitochondrial and nuclear DNA sequences.

The order Halichondrida occupies a pivotal position in the history of demosponge phylogeny (for an overview see Erpenbeck *et al.*, 2012a). After Van Soest and Hooper reported inconsistencies in the current classifications of Poecilosclerida and Axinellida, respectively (Hooper, 1984; Van Soest, 1984), and cladistic character analyses were introduced in sponge systematics (Van Soest, 1990), both authors independently concluded that the distinction between Ceractinomorpha and Tetractinomorpha is unparsimonious and suggested the re-merging of the order "Axinellida" with Halichondrida. Monophyly of the re-defined order Halichondrida and its five families (Van Soest and Hooper, 2002a) could not be demonstrated in morphological (see Erpenbeck, 2004), biochemical (Erpenbeck and Van Soest, 2005), or molecular data sets (see, e.g. Morrow *et al.*, 2012). In fact, halichondrid polyphyly has been repeatedly demonstrated, since both ribosomal RNA (Lafay *et al.*, 1992) and biochemical data (Van Soest and Braekman, 1999) suggested a close relationship between Agelasida and axinellids (later corroborated with several independent molecular data sets, see Erpenbeck *et al.*, 2006). Molecular data also demonstrated that the family Dictyonellidae (Van Soest *et al.*, 1990), which was mostly defined based on the absence of specific characters, consisted of unrelated taxa (Nichols, 2005), and its nominal genus *Dictyonella* did not form a clade with Halichondriidae. Axinellidae has been reported as polyphyletic in molecular phylogenies and this is also the case of its nominal genus *Axinella* (Alvarez *et al.*, 2000; see Gazave *et al.*, 2010a for a recent review). Similar, other taxa included in Axinellidae, such as *Reniochalina*, *Ptilocaulis*, and *Phakellia*, do not form a monophyletic group with *Axinella* in all molecular phylogenies (Erpenbeck *et al.*, 2007b,c, 2012; Holmes and Blanch, 2007; Morrow *et al.*, 2012). Halichondrida are also polyphyletic due to a close relationship between Halichondriidae and the hadromerid Suberitidae repeatedly that emerged from molecular analyses (e.g. Chombard and Boury-Esnault, 1999; McCormack and Kelly, 2002; Erpenbeck *et al.*, 2004, 2005b, 2012; Morrow *et al.*, 2012).

The order Hadromerida, which has been frequently targeted for molecular analyses but is often too weakly represented with respect to the diverse spicule and skeletal shape (Kelly-Borges *et al.*, 1991; Chombard and Boury-Esnault, 1999; Borchiellini *et al.*, 2004), was eventually shown to be paraphyletic with respect to Poecilosclerida (Nichols and Barnes, 2005; Kober and Nichols, 2007; Morrow *et al.*, 2012). Likewise, Poecilosclerida (Hooper and Van Soest, 2002c) itself has been found to be polyphyletic based on molecular markers. This taxon was established on the basis of chelae microscleres, which are present in most of the Poecilosclerida genera. Other non-chelae bearing taxa, such as Raspailiidae or Desmacellidae, are

assigned to Poecilosclerida due to skeletal similarities other than chelae (see Hooper and Van Soest, 2002c for details). However, mitochondrial data revealed that chelae-bearing Poecilosclerida are unrelated to chelae-lacking Raspailiidae, some Desmacellidae and several microcionid taxa (Erpenbeck *et al.*, 2007a), which was later supported by ribosomal RNA analyses (Erpenbeck *et al.*, 2007b,c; Morrow *et al.*, 2012).

The polyphyly of lithistid demosponges has been accepted for a longer time. Lithistid sponges are characterized by the presence of irregular articulated choanosomal siliceous spicules called desmas that interlock and form a rigid skeleton in most fossil and recent genera. Based on this feature, they were grouped together as Order Lithistida Schmidt, 1870. However, polyphyly of this order had been suspected for about a century (see, e.g. Pisera and Lévi, 2002), which has been supported by molecular data (e.g. Kelly-Borges and Pomponi, 1994) and is accepted in the most recent morphological classification (Hooper and Van Soest, 2002d). Lithistid sponges are currently divided into 13 extant families (Pisera and Lévi, 2002). While the phylogenetic relationships of all extant lithistid taxa have yet to be fully resolved, molecular data demonstrated that several triaene-bearing lithistid sponges fall into the Tetractinellida (see below) (Cárdenas *et al.*, 2011).

4.3.4.1. Spongillina (freshwater sponges)

The monophyly of freshwater sponges has been supported by molecular data in several analyses (e.g. Addis and Peterson, 2005; Itskovich *et al.*, 2007; Redmond *et al.*, 2007; Voigt *et al.*, 2008). However, its nominal family Spongillidae was found to be paraphyletic (see below), particularly with respect to Lubomirskiidae, the Lake-Baikal endemic family (Itskovich *et al.*, 1999, 2008; Addis and Peterson, 2005; Meixner *et al.*, 2007; Redmond *et al.*, 2007). Monophyly of Lubomirskiidae has been suggested based on *cox1* and tubulin intron analyses (but see also Schröder *et al.*, 2003; Itskovich *et al.*, 2006), but more recent *cox1* and 18S rDNA data contradict this hypothesis (e.g. Itskovich *et al.*, 2007; Meixner *et al.*, 2007). Nevertheless, support in the latter analysis is rather low and newer ITS2 data again strongly support Lubomirskiidae monophyly (Itskovich *et al.*, 2008).

Many members of the family Spongillidae (e.g. Itskovich *et al.*, 2008) and several of its genera, such as *Ephydatia*, have been found to be paraphyletic (e.g. Addis and Peterson, 2005; Meixner *et al.*, 2007). This indicates the need for a revised taxonomy of freshwater sponges (Addis and Peterson, 2005; Harcet *et al.*, 2010a). Several endemic taxa are thought to have been derived from widespread Spongillidae, such as *Spongilla* or *Ephydatia* (or Erpenbeck *et al.*, 2011 for Lake Tanganyika sponges; see, e.g. Meixner *et al.*, 2007 for Lake Baikal).

The phylogenetic position of the remaining freshwater sponge families is unresolved as they are clearly underrepresented in current gene trees. In most analyses, the Metaniidae *Corvomeyenia* splits first from all Spongillina

(e.g. Addis and Peterson, 2005; Itskovich *et al.*, 2007; Redmond *et al.*, 2007; Voigt *et al.*, 2008), and in several analyses, the lithistid *Vetulina* (Vetulinidae) is a sister group to freshwater sponges (see, e.g. Addis and Peterson, 2005; Itskovich *et al.*, 2007).

4.3.4.2. Tetractinellida

Molecular data support the monophyly of Tetractinellida, which include the orders Astrophorida and Spirophorida (Vacelet *et al.*, 2000; Borchiellini *et al.*, 2004; Addis and Peterson, 2005; Nichols, 2005; Erpenbeck *et al.*, 2007a; Holmes and Blanch, 2007; Itskovich *et al.*, 2007; Redmond *et al.*, 2007; Lavrov *et al.*, 2008; Wang and Lavrov, 2008; Morrow *et al.*, 2012). Morphologically, the Tetractinellida are distinguished by the possession of tetractine (four rayed) megascleres, which have one ray clearly prolonged and the remaining three approximately evenly short (triaenes). Several lithistid sponges have been found to fall into the Tetractinellida, among them *Aciulites* sp. (Scleritodermidae), *Theonella* sp. and *Discodermia dissoluta* (Theonellidae), and *Corallistes* sp. (Corallistidae) (e.g. Addis and Peterson, 2005; Nichols, 2005; Itskovich *et al.*, 2007).

4.3.4.2.1. Astrophorida

Astrophorida are conventionally (leaving apart the lithistids) divided into five families (Hooper and Van Soest, 2002b). The most comprehensive molecular phylogeny of Astrophorida was recently published by Cárdenas *et al.* (2011), who extended an earlier study on the taxonomic status of the family Geodiidae (Cárdenas *et al.*, 2009) by additional astrophorid representatives. They found that the astrophorid suborders Euastrophorida and Streptosclerophorida are polyphyletic (as indicated earlier by Chombard *et al.*, 1998); likewise, the families Geodiidae, Ancorinidae, and Pachastrellidae as well as many genera are polyphyletic (Cárdenas *et al.*, 2011). The combined analysis of 28S and *cox1* results in the following phylogenetic hypothesis, which is significantly congruent with earlier analyses based on a much lower taxon sampling (Chombard *et al.*, 1998; Nichols, 2005):

A well-supported geodinid clade has been recovered, including three *Geodia* subclades termed "*Geodia*", "*Depressogeodia*", and "*Cydonium*" (following the PhyloCode) as well as *Ecionemia*, *Rhabdastrella*, and *Stelletta* species, which are currently classified as Ancorinidae. This geodinid clade is a sister group to a calthropellid clade (*Calthropella*) and an erylinid clade (including Erylus, Penares, and Pachymatisma, see also Cárdenas *et al.*, 2007). Together, the geodinid and erylinid + calthropellid clade form a (poorly supported) geodiid clade, which is a sister to Pachastrellidae (*Poecillastra* + *Pachastrella* + *Triptolemma*).

Sister to this geodiid + pachastrellid clade are Ancorinidae and several lithistid families, such as Corallistidae, Phymaraphiniidae, and Theonellidae, as well as the pachastrellid genera, *Characella*, and *Dercitus*. The remainder of

pachastrellids branch earlier in the Astrophorida phylogeny. For these, Cárdenas *et al.* (2011) suggest a new family, designated as Vulcanellidae (for *Vulcanella* and *Poecillastra*), and the resurrection of the families Theneidae (for *Thenea* spp.) and Thoosidae for *Alectona millari*, which is currently classified in Alectonidae (Hadromerida). Maldonado (2004) suggested that the occurrence of discotriaenes (triaenes in which the short rays form a single disc) in the larva of *Thoosa* and *Alectona* indicate that those genera did not belong to the family Clionaidae (Order Hadromerida) and suggested transferring them to the order Astrophorida. A subsequent molecular study (Borchiellini *et al.*, 2004) corroborated the suggestion that *Alectona millari* was more closely related to members of the order Astrophorida than to representatives of Hadromerida. Another alectonid, *Neamphius*, also falls into Astrophorida (Cárdenas *et al.*, 2011).

Although the deeper splits of this phylogeny are weakly supported or unsupported, it provides important clues about demosponge character evolution. It also reminds us that even taxa that are relatively rich in complex characters compared to other demosponges are prone to character misinterpretations resulting in unrecognized homoplasies.

4.3.4.2.2. Spirophorida

Even 5 years after the last review of the field (Erpenbeck and Wörheide, 2007), Tetillidae is still the only Spirophorida family with published data for molecular phylogenetics. This is probably due to the encrusting or excavating habit of Samidae and Spirasigmidae, which are more prone to DNA contamination than the more massive tetillids. Therefore, the monophyly of Spirophorida lacks confirmation from molecular data, but as their sigmaspire microscleres are unique among Demospongiae, this hypothesis might remain unchallenged (see Hooper and Van Soest, 2002a). Tetillidae so far appear to be monophyletic, as *Tetilla* and *Cinachyrella* form a clade in several larger phylogenies (e.g. Nichols, 2005; Redmond *et al.*, 2007). The largest phylogenetic contribution to Tetillidae is based on an analysis of a mitochondrial intron in the Tetillidae (Szitenberg *et al.*, 2010). The tree derived from the corresponding *cox1* fragment displays the genera *Cinachyrella*, *Tetilla*, and *Craniella* as non-monophyletic. However, additional data are necessary to verify and explain these outcomes.

4.3.4.3. Agelasids + axinellids + raspailids + dictyonellids + heteroxyids

Agelasida possess spicules with spines arranged into verticills. They contain the Astroscleridae, which have a calcareous basal skeleton (Wörheide, 1998), and the soft-bodied monogeneric (*Agelas*) Agelasidae (see also Parra-Velandia, 2011 for internal relationships of Caribbean species of this family). The close relationship of the families Astroscleridae *s.s.* (*Astrosclera*) and Agelasidae was repeatedly demonstrated with molecular (Chombard *et al.*, 1997; Alvarez *et al.*, 2000; Nichols, 2005) and biochemical data (see

review in Wörheide, 1998). Nevertheless, recent molecular data indicate paraphyly of the Astroscleridae, suggesting an *Agelas* + *Astrosclera* clade to which other Astroscleridae (*Stromatospongia vermicola* + *Ceratoporella nicholsoni*) form a sister group (Parra-Velandia, 2011).

Molecular data have repeatedly indicated a close relationship between Agelasida and axinellid taxa, especially several *Axinella* species and *Stylissa* (see Erpenbeck *et al.*, 2006 for details). Polyphyly of *Axinella* was first demonstrated with 28S rDNA (Alvarez *et al.*, 1998), and additional 18S data indicate at least three separate clades of *Axinella* with *A. damicornis*, *A. verrucosa*, and *A. corrugata* in a sister group to Agelasida together with *Cymbastela cantharella* (Gazave *et al.*, 2010a, who subsequently termed this clade "Cymbaxinella"). However, *Cymbastela* has been demonstrated to be polyphyletic (Alvarez *et al.*, 2000; Alvarez and Hooper, 2010; Erpenbeck *et al.*, 2012a), and *C. cantharella* might be unrelated to the type species *C. stipitata* for which a close relationship to Agelasida has never been shown.

In addition, molecular analyses also group some raspailid taxa (Poecilosclerida) with this Agelasida/axinellid assemblage. The 28S rDNA sequences of *Amphinomia* are almost identical with their agelasid sequences of the clade (Erpenbeck *et al.*, 2007c); furthermore, molecular data found "*Eurypon* cf. *clavatum*" closely related to Agelasida/Axinellidae (besides *Prosuberites laughlini*; Hadromerida: Suberitidae and *Hymerhabdia typica*; Halichondrida: Bubaridae) (Nichols, 2005; Itskovich *et al.*, 2007; Morrow *et al.*, 2012). Agelasida were re-defined based on the new taxon composition (Morrow *et al.*, 2012).

All raspailiid taxa so far investigated with molecular markers, including its nominal genus *Raspailia*, are unrelated to Poecilosclerida *s.s.* and form a clade with the axinellids *Ptilocaulis* and *Reniochalina* (Erpenbeck *et al.*, 2007a; Holmes and Blanch, 2007), with the heteroxyid halichondrid *Didiscus* (Erpenbeck *et al.*, 2007b), and with the former hadromerid (*incertae sedis*) family Sollasellidae (Van Soest *et al.*, 2006; Erpenbeck *et al.*, 2007b). Molecular analyses show that *Raspailia* (*s.s.*), *Eurypon*, *Sollasella*, *Aulospongus*, and *Ectyoplasia* form a Raspailiinae clade, while several other *Raspailia* subgenera, for example, *Parasyringella*, do not appear to be monophyletic.

Gazave *et al.* (2010a) recovered two additional *Axinella* spp. clades: one clade, subsequently termed "Axinellidae", including the type species *Axinella polypoides*, *Dragmacidon*, and other *Axinella* (including *Axinella dissimilis* and *Axinella aruensis*); and the other, subsequently termed "*Acanthella*", including *Axinella cannabia* and the dictyonellids *Acanthella acuta* and *Dictyonella*. In previous molecular analyses, *Acanthella* was the only dictyonellid with close relationships to the nominal genus *Dictyonella*, and it formed a clade with the axinellid *Cymbastela* (including the type species *C. stipitata*) and the halichondriid *Axinyssa* (Alvarez *et al.*, 2000; Erpenbeck *et al.*, 2005b). This clade now forms with *Phakellia* and the lithistid *Desmanthus* a re-defined family Dictyonellidae (Morrow *et al.*, 2012).

4.3.4.4. Halichondridae + Suberitidae

Ribosomal and mitochondrial genes indicate a close relationship between Halichondriidae (order Halichondrida) and Suberitidae (order Hadromerida) (Chombard and Boury-Esnault, 1999; McCormack and Kelly, 2002; Erpenbeck *et al.*, 2004, 2005b, 2012), although this lacks support from evaluation of Elongation Factor 1 alpha and biochemical analyses (Erpenbeck and Van Soest, 2005; Erpenbeck *et al.*, 2005a). *Axinyssa* is the only halichondrid without molecular phylogenetic affinities with this Halichondriidae + Suberitidae clade. *Axinyssa* is also the only halichondriid without an ectosomal skeleton—a feature shared by *Acanthella* and *Dictyonella*, which are close relatives based on molecular phylogenies (see above). Molecular analyses also support the morphological distinction of the genus *Johannesia* from *Vosmaeria* (Gerasimova *et al.*, 2008).

4.3.4.5. Polymastiidae

Polymastiidae, albeit so far only represented by *Polymastia* spp., form a monophyletic group in several 28S phylogenies (Nichols, 2005), sometimes in the form of a sister group to the Halichondridae + Suberitidae clade (Kober and Nichols, 2007; Morrow *et al.*, 2012).

4.3.4.6. Clionaidae + Spirastrellidae

Nichols' (2005) 28S analysis resulted in a spiraster-bearing Clionaidae + Spirastrellidae clade including *Spirastrella*, *Diplastrella* (both Spirastrellidae), *Cliona*, *Pione*, and *Cervicornia* (all Clionaidae). Neither of these two families was found to be monophyletic. Another 28S analysis based on the D2 fragment corroborated these results, finding the genera *Cliona* and *Spheciospongia* to be non-monophyletic and *Cliothosa* nested within *Cliona* (Barucca *et al.*, 2007). Nevertheless, this fragment could not support Clionaidae as monophyletic because *Diplastrella* falls in this clade (see also Kober and Nichols, 2007; Morrow *et al.*, 2012). Finally, 28S analyses placed *Placospongia* (Placospongidae) in an unsupported sister group relationship to Clionaidae (Nichols, 2005; Kober and Nichols, 2007).

4.3.4.7. Tethyidae + hemiasterellids

Analyses of 28S support a clade combining Tethyidae with several hemiasterellid species (*Axos cliftoni*, *Adreus* spp.), although neither family has been found to be monophyletic (Nichols, 2005; Kober and Nichols, 2007; see Heim *et al.*, 2007a,b,c also for other tethyid species phylogenies). Other hemiasterellid taxa, such as *Stelligera* and *Paratimea*, fall outside this clade (Nichols, 2005; Morrow *et al.*, 2012) and form with the heteroxyid *Halicnemia* a re-erected Family Stelligeridae (Morrow *et al.*, 2012). As hemiasterellid taxa show similarities to several other demosponge families (Hooper, 2002), the polyphyletic status of this group is not surprising. Nichols (2005), Kober and Nichols (2007), and Morrow *et al.* (2012)

recovered a close relationship of Timeidae to Tethyidae + hemiasterellids (which is morphologically supported by the presence of asterose microscleres) to which *Trachycladus* (Trachycladidae) is the sister group.

4.3.4.8. Poecilosclerida *sensu stricto* (primary chelae-bearing poecilosclerids)

Poecilosclerida is the largest order of sponges with respect to the numbers of families and genera (Hooper and Van Soest, 2002d), but it is the least studied by means of molecular systematics. Nuclear ribosomal and mitochondrial analyses recently revealed that several taxa classified as poecilosclerids, all of them lacking the Poecilosclerida-characteristic chelae microscleres, do not form a clade with most non-chelae-bearing poecilosclerids (Erpenbeck *et al.*, 2007a,b,c). However, these Poecilosclerida *sensu stricto* may contain taxa with an assumed secondary loss of chelae, such as *Tedania* (Tedaniidae), which groups within chelate poecilosclerids (Erpenbeck *et al.*, 2007a).

Nevertheless, the suborders of the chelae-bearing Poecilosclerida, Mycalina, Microcionina, and Myxillina (see Hooper and Van Soest, 2002c) could not be supported by molecular data (e.g. Nichols, 2005). Podospongiidae are Mycalina *incertae sedis* based on an interpretation that the protorhabd of spinorhabds is potentially a sigmancistra derivative (Kelly and Samaai, 2002), and the sequences of the podospongiids *Negombata* and *Diacarnus* form a monophyletic group in *cox1* analyses. However, molecular data so far do not support a clade combining Podospongiidae with other Mycalidae, but rather with myxillids (Nichols, 2005; Schmitt *et al.*, 2005; Rot *et al.*, 2006; Itskovich *et al.*, 2007; Morrow *et al.*, 2012).

4.4. Future work

It is evident that in recent years, our understanding of the phylogenetic relationships of demosponges has greatly improved, particularly due to congruence between mitochondrial and nuclear data. However, recruitment of additional, independent markers is clearly needed, especially to contribute to the resolution of the deeper splits. Nevertheless, many details of morphological character evolution remain unclear. The widespread inconsistency between gene trees and morphology-based taxonomy in marine Haplosclerida is probably among the most difficult issues to solve in demosponge phylogeny.

Additionally, there are many taxa for which the traditional placement has been rejected based on molecular data, and most of them currently await a new assignment, including dictyonellids, such as the relationships of *Svenzea* and *Scopalina* to other "G4" sponges. These taxa were studied intensively on the species level (e.g. Blanquer *et al.*, 2005; Blanquer and Uriz, 2008, 2010) and are currently placed in a newly erected Family Scopalinidae (Morrow *et al.*, 2012). Likewise, the phylogenetic position of *Biemna* and other

desmacellids is unresolved (see also Mitchell *et al.*, 2011; Morrow *et al.*, 2012), as well as most taxa of Poecilosclerida *sensu stricto*.

Increased taxon sampling of Axinellidae, Raspailiidae, Agelasida, Dictyonellidae, and (former) Heteroxyidae are needed to fully appreciate the emerging classification schemes from molecular data. Distinguishing between raspailids and axinellid species is difficult and subjective (Alvarez and Hooper, 2010) and might be complicated by hybridization (Alvarez *et al.*, 2007). Furthermore, the analysis of Morrow *et al.* (2012) provides a new view on the classification, but mostly fails to provide a robust resolution of the phylogenetic relationships of the major clades; additional analyses using slow-evolving molecular markers are desirable.

5. The Current Status of the Molecular Phylogeny of Hexactinellida

5.1. Introduction to Hexactinellida

Hexactinellida (glass sponges) are exclusively marine and siliceous sponges largely restricted to the deep sea, with a few notable exceptions, such as massive glass sponge reefs found in SCUBA-accessible depths off the Canadian west coast (e.g. Conway *et al.*, 2001; Krautter *et al.*, 2001; Cook *et al.*, 2008) and population of sublittoral caves in the Mediterranean by one species (*Oopsacas minuta*: Vacelet *et al.*, 1994; Bakran-Petricioli *et al.*, 2007). Currently, 623 extant species are considered valid according to the World Porifera Database (Van Soest *et al.*, 2011), but because the deep sea is still to a large extent unexplored and vast museum collections await revision by a limited number of experts, this is probably a gross underestimate of the actual diversity of this group (Reiswig, 2002). Glass sponges are remarkably distinct from other sponges in many aspects of their biology (reviewed in Leys *et al.*, 2007). In particular, their syncytial tissue organization and triaxonic spicule symmetry clearly distinguish them from the other three major sponge groups and make them one of the best-supported higher-level metazoan monophyla (Mehl, 1992). They also differ from other sponges because they generally have a richer set of morphological characters, displaying a complex skeletal anatomy and a vast array of different spicule types that provide a wealth of information for the taxonomy of the group.

5.2. Taxonomic overview

The current classification of extant Hexactinellida (Dohrmann *et al.*, 2011; Hooper *et al.*, 2011) recognizes two subclasses, characterized by distinct types of microscleres: Amphidiscophora (with amphidiscs) and Hexasterophora

(with hexasters). Amphidiscophora contains a single extant order, Amphidiscosida, with three families: Hyalonematidae, Pheronematidae, and the monogeneric Monorhaphididae. With 16 families in four orders, Hexasterophora is much more diverse. Within this subclass, two main types of skeletal organization are distinguished: lyssacine, that is, mainly composed of unfused spicules (which is also the sole type of skeletal organization found in Amphidiscophora), and dictyonine, that is, with rigid skeletons (dictyonal frameworks) composed of fused six-rayed (hexactine) megascleres in addition to loose spiculation. The lyssacine hexasterophorans (Rossellidae, Euplectellidae, and Leucopsacidae) are placed in a single order Lyssacinosida. The dictyonine taxa are divided into three orders: Aulocalycoida (Aulocalycidae and Uncinateridae) and Lychniscosida (Aulocystidae and the monogeneric Diapleuridae) are rare, species-poor groups; the majority of dictyonine genera are placed in the Hexactinosida, including Euretidae, Tretodictyidae, Farreidae, Dactylocalycidae, Aphrocallistidae, and the monogeneric Auloplacidae, Fieldingiidae, Craticulariidae, and Cribrospongiidae. While most genera and families of Hexactinellida are morphologically well-defined taxa, order-level relationships, relationships between the families and intrafamilial relationships (e.g. division of larger families into subfamilies) are difficult to resolve with morphological data (Dohrmann *et al.*, 2008).

5.3. Molecular phylogenetics

5.3.1. Current status

Although nuclear and mitochondrial sequences of a few glass sponge species have become available since the early 1990s (see Table 1.1), the internal phylogenetic relationships of this group were only recently investigated with molecular data. The first molecular phylogenetic study of Hexactinellida (Dohrmann *et al.*, 2008) included 34 species from 27 genera, 9 families, and 3 orders (Amphidiscosida, Hexactinosida, and Lyssacinosida) and was based on nuclear 18S, partial nuclear 28S, and partial mitochondrial 16S rDNA sequences. As expected from morphological predictions, monophyly of Hexactinellida and of its two subclasses was highly supported. Furthermore, and in contrast to the molecular phylogenies of Demospongiae and Calcarea (see the respective sections above and below), the reconstructed relationships within these groups are also remarkably congruent with the taxonomic classification—all but one genera and all families with more than one species included were found to be monophyletic. Also, almost all of the included species of Hexactinosida formed a highly supported clade corresponding to the Sceptrulophora (Mehl, 1992), a taxon that was only recently formally introduced (Dohrmann *et al.*, 2011). As the name suggests, its members are characterized by the possession of sceptrules, a distinct class of spicules that is

regarded as synapomorphic and that occurs in different variations, most commonly scopules or clavules (see Dohrmann *et al.*, 2011). In contrast, the Hexactinosida as a whole were found to be non-monophyletic because the only included sceptrule-lacking species (*Iphiteon panicea*; Dactylocalycidae) either formed the sister group to the Lyssacinosida or was nested within that group, depending on the substitution models employed. However, these results were not totally unexpected given that monophyly of Hexactinosida and Lyssacinosida had been called into question before on purely morphological grounds (Mehl, 1992).

In a follow-up study based on increased taxon sampling of the same markers, Dohrmann *et al.* (2009) resolved the position of *Iphiteon* as sister to Lyssacinosida, supporting monophyly of the latter (it should be noted, however, that additional, so far unsampled, dictyonal taxa might be nested within Lyssacinosida [see below]). Since then, the taxonomic sampling of Hexactinellida has been increased to 50 species (38 genera, 10 families, 3 orders), and the rDNA dataset was supplemented with an additional marker, *cox1* (Dohrmann *et al.*, 2011, 2012). Below we discuss the relationships within Sceptrulophora and Lyssacinosida based on the combined analysis of rDNA and *cox1* sequences from these two studies (Fig. 1.5).

The subdivision of Sceptrulophora into Scopularia and Clavularia (Mehl, 1992), based on the presence of scopules (most taxa) or clavules

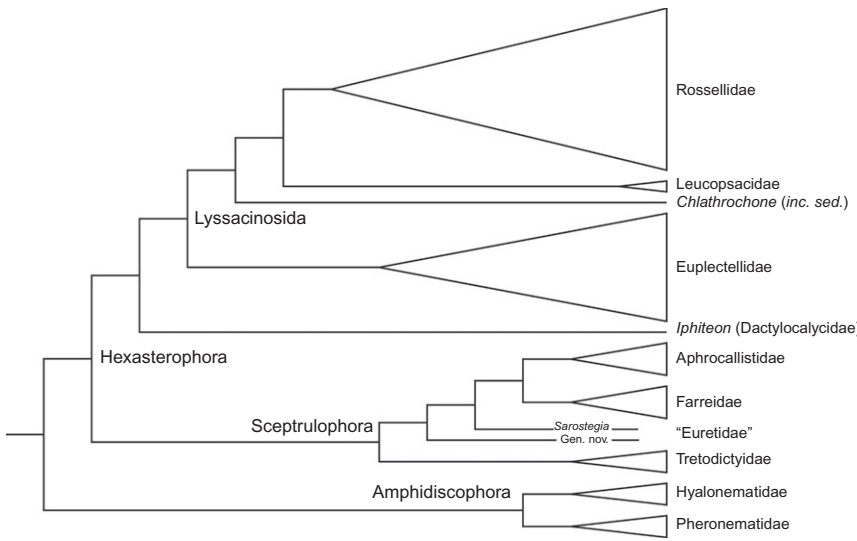

Figure 1.5 Overview of current knowledge about higher-level phylogeny of Hexactinellida. Based on maximum likelihood analysis of combined 18S, 28S, 16S rDNA, and *cox1* sequences (~4600 bp). See Dohrmann *et al.* (2011, 2012) for details. Gen. nov., yet-to-be described new genus of "Euretidae"; *inc. sed.*, Lyssacinosida *incertae sedis*.

(restricted to Farreidae) is strongly rejected by the molecular data. Instead, Farreidae (=Clavularia) is nested within a paraphyletic "Scopularia" as sister group to the Aphrocallistidae (see Dohrmann *et al.*, 2011). Monophyly of "typical" Farreidae is highly supported, but the monospecific genus *Sarostegia*, which appeared somewhat misplaced in this family, clearly groups outside this clade and was consequently moved to Euretidae, consistent with earlier classifications (Dohrmann *et al.*, 2011). Although the exact position of *Sarostegia* remains unclear due to low bootstrap support, a grouping with the other included euretid is consistent with the molecular data (Dohrmann *et al.*, 2011). However, because Euretidae is particularly speciose and morphologically diverse and because there seem to be no potentially apomorphic characters uniting its genera, more taxa need to be sampled to test the monophyly of this family. Monophyly of the similarly species-rich Tretodictyidae, which is so far resolved as the sister group to all remaining sceptrulophorans, is currently only moderately supported by molecular data. However, this family is morphologically well characterized, so it can be expected that molecular support will solidify with inclusion of additional genera. Finally, monophyly of Aphrocallistidae, a species-poor but highly abundant family that includes the only extant examples of reef-building sponge species (see above), is highly supported by both morphological and molecular evidence. However, reciprocal monophyly of its two constituent genera could not be demonstrated by the combined molecular data, a result that is somewhat puzzling and might be related to gene-tree—species-tree conflict (Dohrmann *et al.*, 2011).

Among the Lyssacinosida, monophyly of all three families is highly supported by the combined DNA sequence data. While for the Euplectellidae (the "venus-flower basket" family) this result was expected from morphology, in case of the Rossellidae (the most speciose family of Hexactinellida) and the small family Leucopsacidae (three genera) this can be viewed as a positive surprise because morphological autapomorphies of these taxa are hard to pin down. In contrast, at the intrafamilial level, the situation is more "typical" for sponges: molecular data do not support any of the currently recognized subfamilies of Rossellidae (Rossellinae, Lanuginellinae) or Euplectellidae (Euplectellinae, Corbitellinae, Bolosominae). However, with the exception of Lanuginellinae (see Dohrmann *et al.*, 2012), these taxa are either negatively defined (Rossellinae=non-Lanuginellinae) or defined based on homoplasy-prone characters (Dohrmann *et al.*, 2009, 2012). On the interfamilial level, Euplectellidae has been identified as the sister group of the remaining lyssacinosidans, among which the unplaced monospecific *Clathrochone* is sister to a Rossellidae + Leucopsacidae clade. Although Tabachnick (2002) apparently favours a closer relationship of Leucopsacidae to Euplectellidae, the reasons for this proposal are unclear; it remains to be shown what (if any) morphological characters would support or contradict the higher-level molecular phylogeny of Lyssacinosida.

5.4. Future work

Although the taxon sampling achieved so far is already fairly comprehensive, it is heavily skewed towards Hexasterophora. Relationships within Hyalonematidae and Pheronematidae (Amphidiscophora) should be further investigated by incorporating additional taxa, and the phylogenetic position of *Monorhaphis* (Monorhaphididae), which is famous for its up to 3 m long giant anchor spicule, remains to be determined.

Within Hexasterophora, taxon sampling of the dictyonal groups still needs improvement. Of special importance are the sceptrule-lacking Lychniscosida, Aulocalycoida, and Dactylocalycidae. These taxa are crucial for understanding skeletal evolution because their dictyonal frameworks differ considerably from those found in Sceptrulophora. Mehl (1992) rejected a closer relationship of Lychniscosida—a relict group that was highly diverse and reef-building in the Mesozoic—to other dictyonal sponges, instead proposing a position within Lyssacinosida, which remains to be tested with molecular data. While Lychniscosida is morphologically well supported, this is not the case for Aulocalycoida—although members of this group display a similar type of framework, constructional differences between the families (Hooper and Van Soest, 2002d; Janussen and Reiswig, 2003; Leys *et al.*, 2007; Reiswig and Kelly, 2011) raise doubts about their homology, and even monophyly of the families is not well established. In Uncinateridae, the presence of scopules in *Tretopleura* has been confirmed (Dohrmann, personal observation), and molecular data indicate a nested position of this genus within Sceptrulophora (Dohrmann, unpublished data). Therefore, at least the Uncinateridae, or parts thereof, belong in Sceptrulophora; the position and status of Aulocalycidae still remain elusive. Dactylocalycidae only consists of the already sampled *Iphiteon* (see above) and the type genus *Dactylocalyx*; if molecular data can confirm monophyly of this family and its position as sister to Lyssacinosida remains stable, Dactylocalycidae should best be classified in a separate order. Finally, within Sceptrulophora, monophyly and intergeneric relationships of Euretidae and Tretodictyidae need to be further investigated (see above), and the positions of the four monogeneric families remain to be resolved.

Within Lyssacinosida, intrafamilial relationships of Rossellidae and Euplectellidae are in need of further clarification. A dense taxon sampling comprising the majority of genera will be required to determine if the molecular phylogeny supports morphologically diagnosable clades that could be classified as subfamilies; if this is not the case, subfamilies should be abandoned among Lyssacinosida. Finally, inclusion of *Hyaloplacoida* (*incertae sedis*) might support the designation of a fourth family, if this taxon groups with *Clathrochone* (see above), which can be predicted from their similar spiculation (see Hooper and Van Soest, 2002d).

6. The Current Status of the Molecular Phylogeny of Homoscleromorpha

6.1. Introduction to Homoscleromorpha

Homoscleromorpha is a small group of marine sponges (< 100 described species), the monophyly of which is well accepted on the basis of their general organization and the shared features of their cytology and embryology. Their affinities to other sponges, however, are less clear and have recently been questioned. Traditionally, homoscleromorph sponges were considered as a family or a suborder of the subclass Tetractinellida of the class Demospongiae mainly due to the shared presence of siliceous tetractinal-like calthrops (Lévi, 1956). This small group, however, progressively appeared to be problematic. In recent molecular phylogenetic studies that recovered sponges as monophyletic, Homoscleromorpha appears to be most closely related to Calcarea (Dohrmann *et al.*, 2008; Philippe *et al.*, 2009; Pick *et al.*, 2010), although support for this is only moderate to low in the latter two studies (see Part 2 of this chapter and Table 1.1 for alternative relationships that have been proposed). This grouping has been claimed earlier to be consistent with similarities of spicule shape and gross larval morphology in these two groups (Grothe, 1989; Van Soest, 1990), but these morphological similarities are rather superficial (and therefore of limited phylogenetic value); so far, no clear-cut morpho-anatomical characters appear to support this clade (Gazave *et al.*, 2010b). Recently, Gazave *et al.* (2012) considered sponges to be monophyletic, formally raised the Homoscleromorpha to class-level and proposed the presence of cross-striated rootlets in larval ciliated cells of both cinctoblastula (Homoscleromorpha) (Boury-Esnault *et al.*, 2003), amphiblastula (Calcaronea), and calciblastula (Calcinea) (Gallissian and Vacelet, 1992; Ereskovsky and Willenz, 2008) as a possible synapomorphy of Homoscleromorpha and Calcarea.

Homoscleromorpha are often encrusting or lobate with a smooth surface, and they usually occur at shallow depths, but a few have been recovered from abyssal depths. Homoscleromorph sponges display a large number of characters that distinguish them from Demospongiae (Muricy and Diaz, 2002; Uriz *et al.*, 2003; Uriz, 2006; Maldonado and Riesgo, 2007; 2008b; Ereskovsky *et al.*, 2009; Ereskovsky, 2010; Gazave *et al.*, 2010b). They are characterized by an aquiferous system with sylleibid-like or leuconoid organization with eurypylous, diplodal, or aphodal choanocyte chambers. These sponges possess a unique type of tetractine spicules (calthrops), distinguishable from calthrops of the Demospongiae and their derivatives by their small size, ramification (lophose calthrops), and/or reduction (diods and triods) of one to all four actines. These spicules are secreted not only within sclerocytes (as in the demosponges) but also within epithelial cells, showing a unique

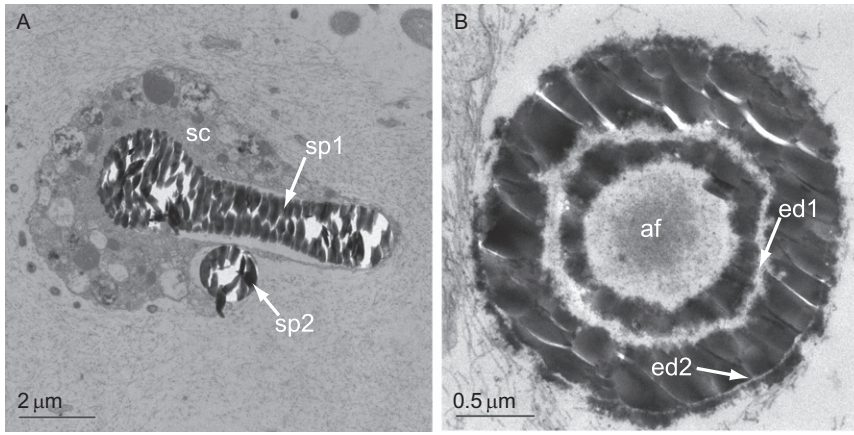

Figure 1.6 (A) A sclerocyte (sc) of the homosclerophorid *Corticium candelabrum*, showing an intracellular spicule (sp1) and another (sp2) that appears to be in the process of extrusion to the surrounding mesohyl. (B) Cross-section of a spicule belonging to *C. candelabrum* in an early stage of silicification. This still growing intercellular spicule has an axial filament (af) and two concentric extra-axial organic deposits (ed1, ed2) between the silica layers. Modified from Maldonado and Riesgo (2007).

silicification process characterized by amorphous axial filaments and two concentric extra-axial organic layers (Maldonado and Riesgo, 2007; Fig. 1.6). These spicules do not form a well-organized skeleton. Homoscleromorpha possess flagellated exopinacocytes and endopinacocytes, unique flagellated apopylar cells, an incubated cinctoblastula larva with cross-striated ciliary rootlets that are surprisingly derived from the accessory centriole (a unique feature in Porifera), and asynchronous spermatogenesis that occurs inside of spermatic cysts. Another feature of the Homoscleromorpha is that they are the only sponge lineage in which adult cell layers are underlain by a basement membrane containing type-IV collagen and *zonula adherens* cell junctions. However, whether the epithelium of the larval stage, although reported (Boury-Esnault *et al.*, 2003), always has a basement membrane remains discussed (see discussion in Maldonado and Riesgo, 2008b).

6.2. Taxonomic overview

Since 1995, Homoscleromorpha has been composed of a single order (Homosclerophorida) with a single family (Plakinidae) and seven genera (*Oscarella*, *Plakina*, *Plakortis*, *Plakinastrella*, *Corticium*, *Pseudocorticium*, and *Placinolopha*) (Boury-Esnault *et al.*, 1995; Hooper *et al.*, 2002; Van Soest *et al.*, 2011). The genera have been distinguished based on four morphological characters (Diaz and Soest, 1994; Muricy and Diaz, 2002): the

presence or absence of a siliceous skeleton; the presence or absence of a cortex associated with the architecture of the aquiferous system and the type of choanocyte chambers; if spicules are present, they are characterized based on the number of spicule size classes; and the presence and type of ramification in the actins of the calthrops. Molecular phylogenetics have recently changed the taxonomic system (see below), now two families (Plakinidae, Oscarellidae) are accepted, with five genera (68 species) and two genera (17 species), respectively (Hooper *et al.*, 2011).

6.3. Molecular phylogenetics

The internal relationships within this group have recently been investigated using molecular data for six of the seven valid genera (Gazave *et al.*, 2010b), resulting in a revision of the suprageneric classification in the World Porifera Database (Van Soest *et al.*, 2011). Based on the congruence of the results from mitochondrial, nuclear, and chemical markers (Gazave *et al.*, 2010b; Ivaniševic *et al.*, 2010), it has been proposed that the subdivision of Homoscleromorpha, which was abandoned in 1995 (Boury-Esnault *et al.*, 1995), into Oscarellidae (aspiculate genera, including the genus *Oscarella*) and Plakinidae (spiculate genera) should be restored (see Fig. 1.7). It was only after the designation of a new genus, *Pseudocorticium*, which is similar in histological traits to the spiculate genus *Corticium* but devoid of a mineral skeleton like *Oscarella* (Solé-Cava *et al.*, 1992), that it was proposed to merge

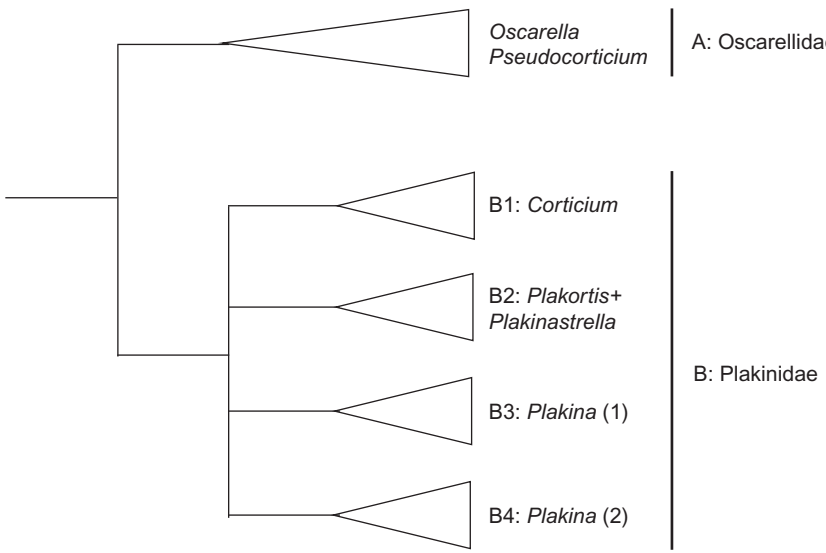

Figure 1.7 Internal relationships of Homoscleromorpha.

all the homoscleromorphs into a single family, the Plakinidae, with *Pseudocorticium* as an aspiculate morph of *Corticium*. Thus, the absence of a skeleton in *Oscarella* and *Pseudocorticium* was phylogenetically non-informative (Boury-Esnault *et al.*, 1995). Recent molecular phylogenetic results (Gazave *et al.*, 2010b) have challenged this view, as they supported a sister group relationship of *Pseudocorticium* and *Oscarella*. This hypothesis implies that the cortex, aquiferous system organization, and external morphological similarities of *Corticium* and *Pseudocorticium*, previously interpreted as synapomorphies, represent convergent characters. In contrast, the presence or absence of spicules in these two genera can be considered as diagnostic. In addition, mitochondrial gene arrangement consistently gives strong support for this scenario (Wang and Lavrov, 2007, 2008; Gazave *et al.*, 2010b). Indeed, the mitochondrial genomes of the Oscarellidae species share a specific gene order, the presence of *tatC*, as well as genes for 27 tRNAs (Wang and Lavrov, 2007; Gazave *et al.*, 2010b), whereas the species included in the Plakinidae clade share the lack of *tatC* as well as the lack of 20 of the 25 tRNA genes typically found in demosponges (Wang and Lavrov, 2008; Gazave *et al.*, 2010b). In Oscarellidae, the monophyly of the genus *Oscarella* has not been confirmed by all molecular analyses. However, the hypothesis of a paraphyletic *Oscarella* as suggested by 18S and mitochondrial data sets (Gazave *et al.*, 2010b) needs further testing with the inclusion of more *Oscarella* species. Within the family Plakinidae, a more robust hypothesis is obtained based on 28S data, congruent with the morphologically well-defined genera *Plakortis*, *Corticium*, and *Plakinastrella* and validating morphological characters as diagnostic for these clades (Muricy and Diaz, 2002). In contrast, regardless of the genetic marker and analytical method used, the genus *Plakina* appears paraphyletic with two of the four *Plakina* species being more closely related to *Corticium*. This scenario is not surprising based on the lack of clear apomorphic characters, which has already led several authors to question the monophyly of this genus (Muricy *et al.*, 1996, 1998; Muricy and Diaz, 2002; Gazave *et al.*, 2010b). Other molecular and morphological analyses of extant species are needed to resolve this issue and propose a subdivision into several genera. Yet, the presence of several characters (i.e. well-developed mesohyl, well-differentiated ectosome, large subectosomal cavities, and tetralophose calthrops) has been proposed to support a clade uniting *Plakina jani* and *Plakina trilopha* (Gazave *et al.*, 2010b). At a higher taxonomic level, molecular analyses support the grouping of *Plakortis* and *Plakinastrella*. A synapomorphy of this clade could be the absence of lophose spicules, which are present in all the other spiculate genera.

6.4. Future work

Molecular analyses reject the monophyly of *Plakina*, which should be tested using a larger taxon sampling. Additional data are also needed to resolve the question of the phylogenetic status of the genus *Oscarella*. Also, more

detailed studies of *Pseudocorticium* and *Oscarella* species are needed, and the phylogenetic position of *Placinolopha*, the only genus not yet included in any dataset, should be determined.

7. The Current Status of the Molecular Phylogeny of Calcarea

7.1. Introduction to Calcarea

Calcareous sponges (Class Calcarea) include about 675 accepted extant species (Van Soest *et al.*, 2011), which are exclusively marine. They occur mostly in shallow waters; only a few species are known from the deep sea (for an overview see, e.g. Rapp *et al.*, 2011). In contrast to the intracellularly formed siliceous spicules found in the other sponge classes, Calcarea are characterized by calcium carbonate spicules that are excreted to the extracellular space (Manuel *et al.*, 2002; Sethmann and Wörheide, 2008). In most Calcarea, the skeleton is exclusively composed of free spicules, but some species additionally possess a rigid basal skeleton of fused or cemented spicules (Manuel *et al.*, 2002). Three basic spicule types can be distinguished depending on the numbers of actines: diactines, triactines, and tetractines. Variation in spicule morphology is limited compared to other sponges (Manuel, 2006). Four different types of aquiferous systems occur in Calcarea. In asconoid Calcarea, all internal cavities are lined with choanocytes (this organization is referred to as homocoel). In syconoid, sylleibid and leuconoid Calcarea, choanocytes occur in choanocyte chambers, and parts of the internal cavities (inhalant and exhalant canals or the atrium) are lined with pinacocytes (heterocoel organization). In the traditional taxonomy, the arrangement of the spicules and the organization of the aquiferous system are important characters (Manuel, 2006). All species of Calcarea are viviparous (Manuel *et al.*, 2002).

7.2. Taxonomic overview

Calcarea is divided into two subclasses: Calcinea and Calcaronea. This subdivision is supported by several characters: the position of the nucleus in the choanocytes (basal in Calcinea, apical in Calcaronea), development (eversion of stomoblastula in Calcaronea), larval types (coeloblastula in Calcinea, amphiblastula in Calcaronea), the spicule type that is built first during ontogenesis (Calcinea: triactines; Calcaronea: diactines) (Bidder, 1898; Hartman, 1958; Manuel *et al.*, 2002; Manuel, 2006), and different values of $\delta^{13}C$ isotopes in the spicules (Reitner, 1992; Wörheide and Hooper, 1999). Several autapomorphies for each subclass can also be found in the secondary structure of the 18S rRNA (Voigt *et al.*, 2008).

The definition of orders, families, and genera is based on characters of skeletal architecture and the aquiferous system (Manuel, 2006). The classification of Calcarea is mainly typological and not based on phylogenetic analyses (Erpenbeck and Wörheide, 2007). Unsurprisingly then, the first phylogenetic analysis of morphological characters showed only little resolution below the subclass level, suggesting a high level of homoplasy (Manuel *et al.*, 2003).

In the following, we refer to the latest taxonomic revisions at the subclass level for Calcinea (Borojevic *et al.*, 1990) and Calcaronea (Borojevic *et al.*, 2000). Importantly, the classification is based on the idea of gradual evolution and that extant Calcarea represent different evolutionary "steps", from sponges with a simple, asconoid, and olynthus-like organization to more complex forms through several intermediate stages on different evolutionary paths (reviewed and illustrated by Manuel, 2006).

Calcinea contains two orders, Murrayonida and Clathrinida. The order Murrayonida comprises Calcinea with a reinforced calcite skeleton, calcareous plates, or spicule tracts. Only a few species belong to this order (three families, three genera, four species, Van Soest *et al.*, 2011). Order Clathrinida includes the majority of calcinean species (6 families, 16 genera, 160 species, Van Soest *et al.*, 2011), with skeletons that are only composed of free spicules.

In Calcaronea, three orders are recognized: Leucosolenida, Lithonida, and Baerida. Leucosolenida contains the majority of calcaronean species (9 families, 43 genera, 467 species, Van Soest *et al.*, 2011). Their skeleton is composed of free spicules without calcified non-spicular reinforcements (Borojevic *et al.*, 2000). Lithonida comprises a small number of calcaronean species with reinforced skeletons (2 families, 6 genera, 20 species, Van Soest *et al.*, 2011). Baerida is a similarly small group (3 families, 8 genera, 17 species, Van Soest *et al.*, 2011). Sponges of this order have skeletons formed exclusively or in substantial parts by microdiactines (Borojevic *et al.*, 2000).

7.3. Molecular phylogenetics

7.3.1. Current status

Only a few molecular studies have aimed at resolving relationships of the entire class by analysis of small and large subunit ribosomal RNA genes (Manuel *et al.*, 2003, 2004; Dohrmann *et al.*, 2006; Voigt *et al.*, 2012b). An overview of the relationships according to Voigt *et al.* (2012b) is shown in Fig. 1.8. A common outcome of these studies is the monophyly of Calcarea and its subclasses Calcinea and Calcaronea, while relationships below the subclass level strongly conflict with the classification system described above. Many of the supraspecific taxa cannot be recovered as monophyletic (e.g. Dohrmann *et al.*, 2006; Voigt *et al.*, 2012b), and the phylogenetic

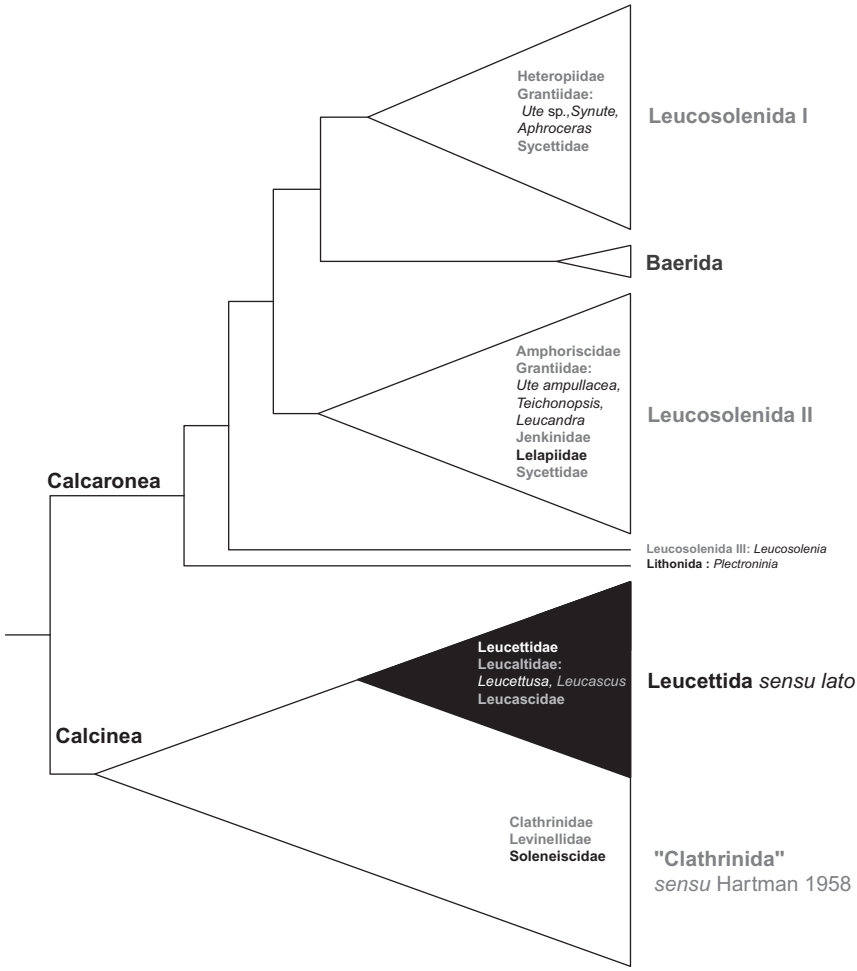

Figure 1.8 Relationships of Calcarea inferred from ribosomal RNA-gene sequences (see Voigt *et al.*, 2012b for details). Taxa that are not monophyletic are shown in grey. When other members of a family also occur in a separate clade, the genus or species names are given.

hypotheses that were brought forward contradict the scenarios of morphological evolution that are the foundations of the current taxonomic system (see above).

7.3.1.1. Calcinea

In Calcinea, the orders Clathrinida and Murrayonida are not monophyletic. Instead, homocoel (asconoid) genera without a cortex form a paraphyletic grade leading to a clade containing all included Calcinea with a cortex

(Voigt *et al.*, 2012b). The included species of Murrayonida do not group together and are nested within the clade of cortex-bearing Clathrinida (Dohrmann *et al.*, 2006; Voigt *et al.*, 2012b), which includes all sampled species from families Leucettidae and Leucascidae, as well as the sampled heterocoel members of Leucaltidae (*Leucaltis* and *Leucettusa*). The genus *Leucetta* (Leucettidae) is not monophyletic, and *Ascandra*, a homocoel member of Leucaltidae, is more closely related to other homocoel Calcinea than to *Leucaltis* or *Leucettusa* (Voigt *et al.*, 2012b). Relationships among homocoel Calcinea are not resolved, as many nodes are poorly supported (Dohrmann *et al.*, 2006; Voigt *et al.*, 2012b). Within this paraphyletic group, the family Clathrinidae and the genus *Clathrina* are not monophyletic (Dohrmann *et al.*, 2006; Voigt *et al.*, 2012b).

The clade of cortex-bearing Calcinea can be classified by broadening the definition of the order Leucettida (Hartman, 1958) to include Calcinea with a cortex and heterocoel organization. This order was rejected by Borojevic *et al.* (1990) and merged with Clathrinida. These authors instead suggested independent gains of a cortex in Leucaltidae, Leucettidae + Leucascidae, and Murrayonida. However, molecular phylogenies reject the monophyly of Leucaltidae, Leucascidae, and Murrayonida (Dohrmann *et al.*, 2006; Voigt *et al.*, 2012b), thereby contradicting this evolutionary scenario. Instead, Leucettida *sensu lato* can be defined as Calcinea with a cortex, which would also include Murrayonida. The asconoid aquiferous system of *Ascaltis* may be interpreted as a secondary simplification within this clade, a hypothesis that needs to be tested further (Voigt *et al.*, 2012b).

In summary, despite discrepancies with the classification of Borojevic *et al.*, an evolution from simple to more complex forms in Calcinea is supported by molecular phylogenies (Manuel *et al.*, 2003; Dohrmann *et al.*, 2006). However, the suggested independent evolutionary paths in Leucaltidae and Murrayonida are rejected (Voigt *et al.*, 2012b).

7.3.1.2. Calcaronea

In Calcaronea, the order Leucosolenida is paraphyletic because it includes species of the order Baerida (Dohrmann *et al.*, 2006; Voigt *et al.*, 2012b). Baerida is also paraphyletic as far as the classical taxonomy is concerned, as it includes the hyper-calcified sponge *Petrobiona massiliana*, which is currently classified in the order Lithonida (Manuel *et al.*, 2003; Dohrmann *et al.*, 2006; Voigt *et al.*, 2012b). However, the grouping in Baerida is also supported by morphological characters, indicating misclassification of this genus (Manuel *et al.*, 2003). The only other included lithonid (*Plectroninia neocaledoniense*) is the sister taxon to all other Calcaronea (Dohrmann *et al.*, 2006), which has led to the speculation that the rigid basal skeleton of fused spicules in this species might be an ancestral character of Calcaronea (Dohrmann *et al.*, 2006). However, this hypothesis needs further testing by inclusion of more species of Lithonida (Dohrmann *et al.*, 2006). The

asconoid species *Leucosolenia* sp. branches off after *Plectroninia* (Dohrmann *et al.*, 2006), which calls into question the primitive state of the asconoid aquiferous system in this subclass because *Plectroninia* has a leuconoid aquiferous system.

The remaining Calcaronea form the sister clades (Leucosolenida I + Baerida) and Leucosolenida II (Voigt *et al.*, 2012b). Leucosolenida I includes all sampled Heteropiidae (*Sycettusa*, *Syconessa*, *Grantessa*), two *Sycon* species (*S. capricorn* and *S. ciliatum*), and some species from Grantiidae with giant cortical diactines (*Ute* sp., *Synute* and *Aphroceras*, Voigt *et al.*, 2012b). Within this clade, Heteropiidae and *Sycettusa* are not monophyletic (Dohrmann *et al.*, 2006; Voigt *et al.*, 2012b). Giant cortical diactines also occur in the heteropiid genera *Heteropia* and *Paraheteropia* (Borojevic *et al.*, 2000), which were not included in molecular analyses. A closer relationship between such Grantiidae and Heteropiidae and between *Sycon* and Grantiidae has been suggested before (e.g. Borojevic, 1965; Borojevic *et al.*, 2000). However, both *Sycon* and Grantiidae are polyphyletic according to molecular data (Manuel *et al.*, 2003; Dohrmann *et al.*, 2006; Voigt *et al.*, 2012b). Other *Sycon* species and *Ute ampullacea* are included in Leucosolenida II (Voigt *et al.*, 2012b). Leucosolenida II also includes species from the families Amphoriscidae, Jenkinidae, and Lelapiidae and from some additional genera of Grantiidae (*Grantia*, *Teichonopsis,* and *Leucandra*). Besides Lelapiidae, which is only represented by the genus *Grantiopsis*, these families are not monophyletic (Manuel *et al.*, 2003, 2004; Dohrmann *et al.*, 2006; Voigt *et al.*, 2012b).

The morphological evolution in Calcaronea is poorly understood. As mentioned above, the early-branching position of *Plectroninia* might imply that the common ancestor of the subclass was not asconoid as suggested before (e.g. Borojevic *et al.*, 2000; Manuel, 2006), but was leuconoid with a rigid skeleton of fused spicules (Dohrmann *et al.*, 2006). The syconoid aquiferous system is the most frequent in the included taxa (see, e.g. Voigt *et al.*, 2012b). Ancestral character state reconstruction suggests that leuconoid aquiferous systems evolved several times independently (Manuel *et al.*, 2003, 2004; Voigt *et al.*, 2012b). A cortex might have evolved early in Calcaronea, possibly before or after the split of *Leucosolenia*, and several syconoid taxa lacking a cortex (e.g. *Sycon, Syconessa*) might have lost it secondarily (e.g. Voigt *et al.*, 2012b). However, these inferences have to be treated with caution, as inclusion of additional taxa might result in a different conclusion.

7.4. Future work

In summary, molecular data suggest that morphological evolution in this taxonomically difficult class of sponges is even more complex than anticipated based on previous studies. Approaches to resolve the phylogeny of Calcarea will be more problematic than in other sponges because the

classical taxonomy is of limited value as a framework to guide taxon sampling. Additionally, taxon-specific revisions (e.g. Klautau and Valentine, 2003) need to be treated with caution because they possibly do not consider monophyletic groups, which in turn can hamper the recognition of potential morphological synapomorphies.

An alternative phylogenetic classification of Calcarea cannot yet be established from molecular phylogenies, although the recognition of monophyletic Leucettida *sensu lato* in Calcinea may provide a starting point. Until a classification based on a better understanding of morphological character evolution is available, it appears crucial to include DNA data in any taxonomic study and to include all available taxa of the subclass of the target species. Future molecular phylogenetic studies should include many more species, but not only from the still unsampled families and genera. It would also be desirable to extend and test the results obtained from ribosomal RNA data with independent phylogenetic markers, such as mitochondrial genes.

With such additional data at hand, remaining questions will have to be addressed: In Calcinea, the validity of Leucettida *sensu lato* must be tested, and the relationships of the asconoid Clathrinida remain to be resolved. The positions of *Burtonulla*, a heterocoel genus of Levinellidae, and *Paramurrayona* (Murrayonida) with respect to Leucettida *sensu lato* should be determined. In Calcaronea, inclusion of members of the families Lepidoleuconidae and Trichogypsiidae is needed to further test the monophyly of Baerida (*sensu* Manuel *et al.*, 2003), and among Leucosolenida, the position and monophyly of the still unsampled Achramorphidae and Sycanthidae needs to be tested. Additional taxa are also required to shed light on the phylogenetic affinities of Heteropiidae, certain *Sycon* species, and Grantiidae of the genera *Ute*, *Synute*, and *Aphroceras*. In this context, the inclusion of Heteropiidae with giant cortical diactines would be especially interesting, as the resemblance in skeletal architecture between certain Heteropiidae and Grantiidae has been recognized before (Borojevic, 1965; Borojevic *et al.*, 2000, 2002). In Leucosolenida II (Voigt *et al.*, 2012b), the connections between species of Jenkinidae, Amphoriscidae, Grantiidae, Sycettidae, and Lelapiidae need to be clarified. Finally, the monophyly of Minchinellidae (Lithonida) needs to be tested.

8. The Evolution of Sponge Development

With a phylogeny mostly based on molecular markers that are independent of morphological characters, it is now possible to map traits, trace their origin, and define shared ancestral features of Porifera and, more generally, Metazoa. In particular, the analysis of development in a phylogenetic framework may identify some of the key innovations that

accompanied the origin of the Metazoa. The use of embryonic development to reconstruct early animal evolution dates back to Haeckel's Gastraea theory (Haeckel, 1874), which was largely inspired by embryonic and adult sponges. As multicellular animals evolved from a protist ancestor, cells had to acquire different identities, specialize in particular functions and become organized into tissues and organs to form a macroscopic, coordinated organism. Such crucial attributes of multicellularity evolved through the assembly of a primordial metazoan developmental program, which was then modified to produce the large diversity of body plans found across the Metazoa. By comparing embryonic development in the different branches of the metazoan tree, we can attempt to reconstruct the first animal developmental program and understand the core traits that underpin multicellularity in animals. In this endeavour, it is crucial to examine the arguably earliest-branching extant metazoan taxon—Porifera.

Animal embryonic development progresses through three major steps: (1) blastulation or cleavage—from the zygote, cell divisions produce a multicellular embryo of generally undifferentiated cells called blastomeres; (2) gastrulation—spatial redistribution and initial differentiation of the blastomeres delineate embryonic germ layers and symmetry; and (3) organogenesis—differentiation and patterning of the germ layers into organs and along one or two axes of symmetry. The reproductive process in the phylum Porifera shows astonishing complexity and diversity. Development in sponges seems to occur similarly to other metazoans, which can be illustrated by examining the model demosponge *Amphimedon queenslandica* (Haplosclerida) (Degnan *et al.*, 2009). After a period of cleavage, segregation of the primary cell layers (termed gastrulation), patterning along an anterior–posterior (AP) axis, and cell differentiation give rise to a typical parenchymella larva with an obvious axis of symmetry and at least eleven differentiated cell types, apparently organized into three concentric layers in *A. queenslandica* (Leys and Degnan, 2001; 2002). As an example of embryonic patterning, pigment cells scattered throughout the outer layer migrate to the posterior pole and are organized into a photosensory ring. The competent *A. queenslandica* larva responds to light and biochemical settlement cues, settles on its anterior end and, during metamorphosis, the aquiferous system of the juvenile sponge is formed. Despite the similarities between sponge and eumetazoan development, the extent to which the processes are homologous has been long debated—in particular, regarding gastrulation, germ layers, and symmetry. Sponges have long been interpreted as having no true tissues or organs and hence representing a primitive animal body plan.

While there are excellent recent reviews analyzing the large diversity of embryogenesis in sponges (Leys, 2004; Maldonado, 2004; Leys and Ereskovsky, 2006; Ereskovsky, 2010), our purpose here will be to focus on developmental traits that are informative in a phylogenetic framework in order to gain insight into the ancestral sponge developmental program. We will point out certain reproductive traits whose phylogenetic value has been

revised, including the mode of reproduction and spermatozoon ultrastructure. Additionally, we will discuss other traits that are comparable to other animals and phylogenetically informative, including larval form and gastrulation, we will briefly review relevant molecular analyses of sponge embryogenesis, and we will discuss what these features can tell us about ancestral sponge development.

8.1. Differences in the mode of reproduction and spermatozoon ultrastructure are not synapomorphies of higher-level sponge clades

For many years, the externally developing oviparous condition versus the brooding viviparous condition was assumed to represent a strong phylogenetic signal. Therefore, by finding relative congruence between these reproductive features and some skeletal features, Lévi (1957, 1973) established the first modern taxonomic classification of Demospongiae, discriminating three large lineages: Homosclerophorida (or Homoscleromorpha, brooding sponges with minute tetractinal to diactinal spicules), Tetractinomorpha (with tetraxonic spicules and derived forms, without spongin, typically oviparous), and Ceractinomorpha (without tetraxonic spicules, with variable levels of spongin, typically viviparous). As previously discussed in this chapter, the advent of molecular methods has revealed that Tetractinomorpha and Ceractinomorpha are not monophyletic, suggesting that oviparity evolved independently multiple times from viviparous ancestors (Borchiellini *et al.*, 2004).

It was thought until recently that the absence of a "true acrosome" was the rule in sponge spermatozoa with the notable exception of the homoscleromorph sponges, which have rounded or C-shaped simple acrosomes (reviewed in Reiswig, 1983; Boury-Esnault and Jamieson, 1999; Riesgo and Maldonado, 2009). This feature has often been proposed to support a closer relationship between eumetazoans and homoscleromorph sponges relative to that of the other three major sponge lineages. However, it is not as phylogenetically informative as once thought. Indeed, a large conical acrosome has also been documented in the calcaronean *Sycon calcaravis* (Nakamura and Okada, 1998), and the most atypical and complex spermatozoon known in demosponges so far belongs to the poecilosclerid *Crambe crambe* (Riesgo and Maldonado, 2009). The elongated V-shaped spermatozoon has a sophisticated acrosomal complex with an associated organelle called a perforatorium, which is far more complex than homoscleromorph acrosomes. The prevailing idea that the organization of the spermatozoon would have increased in complexity in the animal lineage (e.g. Franzén, 1987; Reunov, 2001) has hence been disproved by the discovery of both "simple" and "complex" spermatozoa in Porifera. The absence of an acrosome in most sponges might be a derived condition related to particular mechanisms mediating the process of oocyte fertilization.

8.2. Diversity in sponge larval types: The parenchymella larva may be ancestral to Demospongiae

Sponge embryonic development typically gives rise to a larval stage, with up to eight major larval types clearly identified to date (see Fig. 1.9), in addition to three other described larvae that are difficult to categorize (e.g. Maldonado and Riesgo, 2009). These major larval types are defined according to not only differences in their final morphology and cytology but also a distinctive embryogenesis (reviewed in Maldonado and Bergquist, 2002;

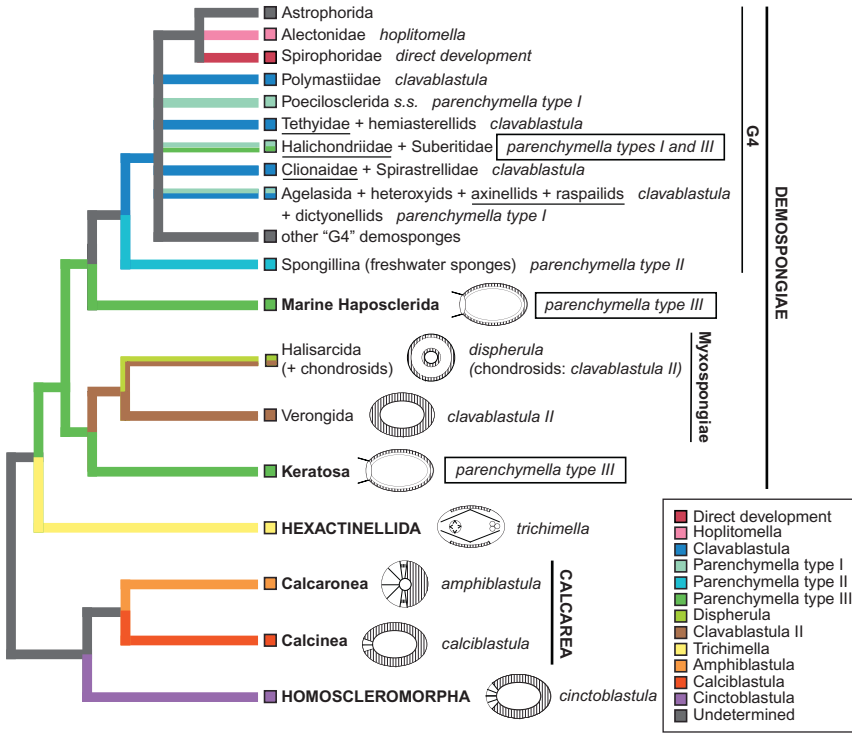

Figure 1.9 Larval types traced onto the consensus sponge phylogeny. Striped regions indicate ciliated cells in the larval schematics. Character states at ancestral nodes were reconstructed using Mesquite (Maddison and Maddison, 2011), and a parsimony criterion of optimality with multistate coding of all the larval types in a single character. Although they have the same name, clavablastulae from the G4 clade and those from the Myxospongiae clade were treated as separate characters (Myxospongiae: clavablastula II) as these hollow entirely ciliated larvae are most likely not homologous (Maldonado and Bergquist, 2002). The resulting tree features type III parenchymella as the ancestral demosponge larval type. Where more than one taxon is included at the tips of the branches, the group where the indicated larval type is found is underlined.

Maldonado, 2004; Ereskovsky, 2010). The larva of certain invertebrates with divergent adult body plans, such as echinoderms or ascidians, display a core set of fundamental animal synapomorphic traits and gene expression patterns that are lacking in the adult form. Similarly, although sponges have little in common with other animals as adults, their larvae are more readily comparable with eumetazoans. Hence, sponge larval development may be the only stage that is evolutionarily conserved with other animals, illustrating the importance of analyzing larval evolution in this group.

In hexactinellid sponges, an elongated and ciliated trichimella larva has been described that is highly differentiated along an AP axis including in its ciliation (Fig. 1.9). It contains a large syncytium formed by cell fusion (Okada, 1928; Boury-Esnault *et al.*, 1999; Leys *et al.*, 2006). The ovate and ciliated cinctoblastula larva of homoscleromorphs is in essence a monolayered epithelium differentiated into three distinct regions along an AP axis with at least five cell types (Boury-Esnault *et al.*, 2003; Ereskovsky, 2010). In the subclass Calcinea of calcareous sponges, an ovate and ciliated coeloblastula (i.e. hollow) called a calciblastula consisting of one cell layer with one or two cell types is released into the water column (Minchin, 1900; Johnson, 1979; Amano and Hori, 2001; Maldonado and Bergquist, 2002). Calcaronean sponges (the other subclass of Calcarea) have an amphiblastula larva with anterior ciliated micromeres, posterior macromeres, and four "cellules en croix" (cross cells) that might be phototactic (Tuzet and Grassé, 1973; Franzen, 1988; Amano and Hori, 1992; Leys and Eerkes-Medrano, 2005).

In contrast to the other three major sponge clades, there is great larval diversity among demosponges, but most members of this group release a highly differentiated parenchymella larva (described above for *A. queenslandica*) (Harrison and De Vos, 1991; Maldonado and Bergquist, 2002; Maldonado and Riesgo, 2008a). The parenchymella type shows some morphological variability regarding ciliation and cytology, the phylogenetic significance of which remains unexplored. Some parenchymellae are entirely and homogeneously covered by equally long cilia or have a small region at the posterior pole devoid of cilia (herein considered to be type I). In freshwater sponges, the parenchymella contains a large cavity probably involved in osmoregulation (type II). Other parenchymellae have a bare posterior pole surrounded by a ring of pigmented cells with long cilia, which functions as an organ-like photoreceptory structure (as in *A. queenslandica*, type III).

Demosponge subclasses, as well as some orders and genera, appear to be paraphyletic, but four major clades have been detected: Keratosa (G1), Myxospongiae (G2), marine Haposclerida (G3), and a large unnamed clade termed G4, with G1 + G2 and G3 + G4 being relatively well supported (Borchiellini *et al.*, 2004 and discussed earlier in this chapter). Type III parenchymella larvae are well documented in the Keratosa clade both among dictyoceratids and dendroceratids (Woollacott and Hadfield, 1989; Maldonado *et al.*, 2003; Ereskovsky and Tokina, 2004; Mariani *et al.*, 2005)

and in the marine haplosclerid clade (including *A. queenslandica*) (Woollacott, 1993; Fromont, 1994; Maldonado and Young, 1999; Leys and Degnan, 2001; Mariani *et al.*, 2005; Fig. 1.9). In the Myxospongiae clade, both verongids and chondrosids have ciliated coeloblastula (hollow) larvae (termed clavablastulae) that develop externally (Usher and Ereskovsky, 2005; Maldonado, 2009; Fig. 1.9), while halisarcids release a ciliated dispherula larva (Lévi, 1956). Depending on the level of cell ingression into the blastocoel, the dispherula larva may be coeloblastula-like or parenchymella-like (Gonobobleva and Ereskovsky, 2004; Ereskovsky, 2010). In the large G4 clade, which includes many paraphyletic orders, poecilosclerids mainly have type I parenchymella (Bergquist *et al.*, 1970, 1977; Wapstra and van Soest, 1987; Mariani *et al.*, 2005), freshwater sponges have type II (Brien, 1973; Saller, 1988), and halichondrids have types I and III (Woollacott, 1990; Maldonado and Young, 1996; Fig. 1.9). Clavablastula and hoplitomella larvae and direct development are also found in this clade; these clavablastulae are unlikely to be homologous to those found in the Myxospongiae clade (Maldonado and Bergquist, 2002; Maldonado and Riesgo, 2008a). Thus, as the very distinctive type III parenchymella is definitely present in three of the four demosponge clades (and the Myxospongiae are sister to the Keratosa; Fig. 1.9), it is most parsimonious to propose that it is the ancestral form for Demospongiae, with other larval types derived from it (e.g. types I and II parenchymella, dispherula, clavablastula). The analysis shown in Fig. 1.9 suggests a tentative pattern of phylogenetic relationships for these larval forms and supports type III parenchymella as the ancestral demosponge larval type.

Parenchymella sub-epithelial layers have been described in dictyoceratids (Keratosa; e.g. Ereskovsky and Tokina, 2004), halichondrids (G4; Brien, 1973), freshwater sponges (G4; Brien, 1973), and marine haplosclerids (e.g. Woollacott, 1993; Leys and Degnan, 2001). In poecilosclerids (G4), three layers are described, but the intermediate layer is particularly wide (Boury-Esnault, 1976; Bergquist and Green, 1977). Thus, as it is present in the three demosponge clades with parenchymella larvae, it is likely that the intermediate layer was present in the ancestral parenchymella. As it arises long after gastrulation and a third cell layer is absent from other sponge classes, it is unlikely that the third layer is related to the mesoderm germ layer of bilaterians. It probably represents a patterning event that arose in this lineage.

8.3. Sponge gastrulation as the morphogenetic movements during embryogenesis

Gastrulation can be defined as the movement of cells in the embryo to form the primary germ layers (Brusca *et al.*, 1997). It occurs after cleavage and is a key step in development because the multicellular animal becomes

organized into two or three cell layers and along one or two axes of symmetry. Eumetazoans become either "diploblastic", with two germ layers (ectoderm and endoderm), or "triploblastic", with three germ layers (ectoderm, mesoderm, endoderm). It is increasingly evident from molecular data that gastrulation, germ layer formation, and axial patterning were associated in the cnidarian–bilaterian ancestor (Lee *et al.*, 2006). As endoderm gives rise to the gut, gastrulation is also associated with gut formation. This part of the definition has made it problematic to define gastrulation in sponges or to agree on whether they undergo gastrulation at all (Rasmont, 1979; Ereskovsky, 2010) as sponges feed using a specialized aquiferous system with no known homology to the eumetazoan gut. Furthermore, there are two phases of reorganization of cell layers that have been documented in all sponge lineages except Hexactinellida: during embryogenesis and during metamorphosis. In the latter phase, an "inversion of germ layers" results in the formation of the aquiferous system, which is the analogue of a sponge "gut" (Amano and Hori, 1996; Leys and Degnan, 2002), and some authors argue that this is gastrulation (Brien, 1973; Simpson, 1984). Other authors, however, have described gastrulation as the earlier cellular movements that follow cleavage and result in the embryonic "germ" layers in certain sponges (Lévi, 1956; Efremova, 1997; Boury-Esnault *et al.*, 1999; Leys and Degnan, 2002; Maldonado and Bergquist, 2002; Leys, 2004; Maldonado, 2004). We favour the latter interpretation, based on the association of cell movements during embryogenesis with the formation of primary cell layers and axial patterning as well as developmental timing (Leys and Degnan, 2002; Maldonado, 2004). We do not, however, argue that gastrulation occurs during embryogenesis in every sponge lineage but rather that this was the case in the ancestral sponge, with some lineages possibly conserving this trait and modifications in other lineages.

In the context of the demosponge common ancestor having a type III parenchymella larva (a plausible possibility discussed above), formation of the primary cell layers would have likely occurred through the migration of cells resulting in an outer layer of micromeres and a central core of macromeres (Borojevic and Lévi, 1965; Leys and Degnan, 2002). It is unclear whether micromeres migrate outwards, macromeres migrate inwards, or both. In the hexactinellid sponge *Oopsacas minuta*, cellular reorganization occurs by cellular delamination—oriented unequal cleavage resulting in micromeres outside and macromeres inside—a gastrulation mode described in hydrozoans (Cnidaria) (Okada, 1928; Boury-Esnault *et al.*, 1999; Leys *et al.*, 2006). These are the strongest cases for gastrulation in sponges, as these processes occur at the end of cleavage and coincide with the formation of two cell layers and the appearance of polarity in the embryo. In the case of demosponges, molecular expression data from *A. queenslandica* provide additional evidence that this is true gastrulation (discussed below).

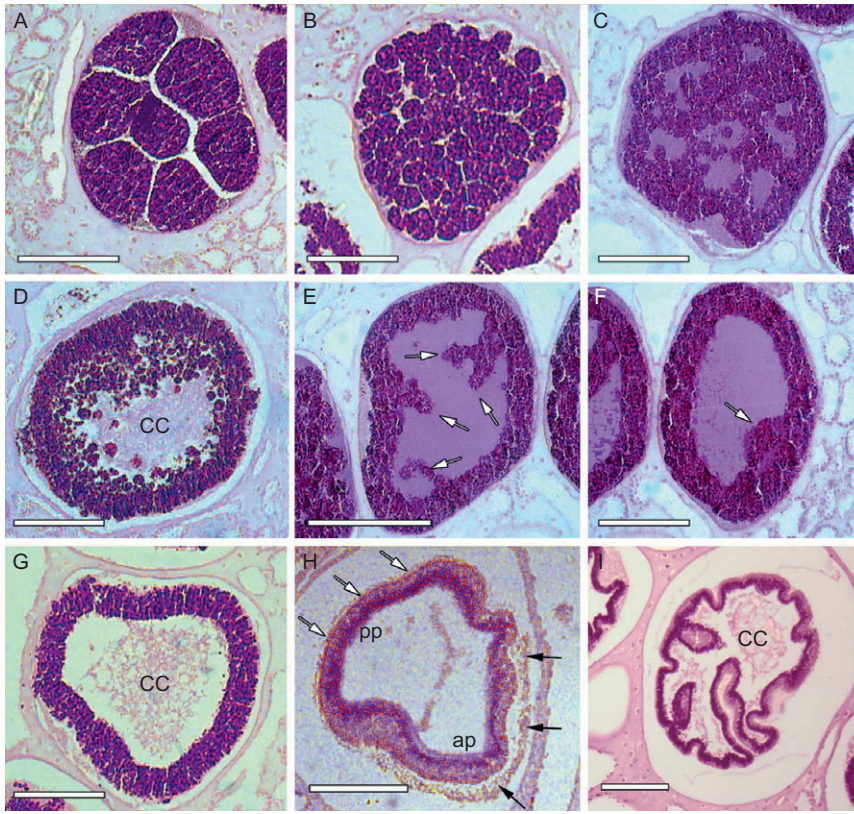

Figure 1.10 Blastomere reorganization during the gastrulation process of the homosclerophorid *Corticium candelabrum*, through which the solid blastula becomes a hollow embryo by multipolar outward cell migration (i.e. multipolar egression). (cc) larval cavity, filled with symbiotic bacteria and collagen fibrils. (pp) posterior and (ap) anterior embryo pole, which will become the posterior and anterior larval pole, respectively, relative to the direction of swimming. Scale bars: 100 μm. Modified from Maldonado and Riesgo (2008b).

In homoscleromorphs, cells of the solid blastula migrate to the outer region of the embryo to form one cell layer during a unique process called multipolar egression (Boury-Esnault *et al.*, 2003; Maldonado and Riesgo, 2007; Fig. 1.10). These cell movements follow cleavage. For some authors, this process differs from gastrulation in that the resulting embryo apparently consists of one uniform cell layer and lacks polarity (Ereskovsky, 2010). However, this remarkable reorganization of the embryo marks the onset of polarization and regionalization processes in the embryo, suggesting that it is akin to gastrulation (Maldonado and Riesgo, 2007).

A highly unusual morphogenetic phenomenon occurs during the embryogenesis of calcaronean sponges when the coeloblastula with cilia facing inwards everts. This process maintains one cell layer and occurs at the end of cleavage when the embryo is already polarized (Franzen, 1988; Leys and Eerkes-Medrano, 2005). As neither of these types of morphogenetic movements have a parallel elsewhere in the Metazoa and/or result in two cell layers, it is difficult to equate them with gastrulation at this point in time.

The calcinean calciblastula larvae released from the adult sponge can be interpreted as the blastula stage with gastrulation occurring later because these larvae appear to be less differentiated than in other lineages (Maldonado, 2004). They are primarily composed of a uniform layer of ciliated cells. No morphogenetic movement occurs during embryogenesis, but cells ingress into the hollow larva while it is free-swimming before metamorphosis begins (Borojevic, 1969). This process is akin to eumetazoan gastrulation by multipolar ingression. Such putative gastrulation after larval release is reminiscent of the continuing "gastrulation" of the swimming planula larva of the cnidarian *Nematostella vectensis* (Magie *et al.*, 2007).

8.4. Molecular evidence for homology between sponge and eumetazoan development

Although sponge embryology has been studied since the nineteenth century, a concerted effort to understand the genetic mechanisms underlying sponge development, and hence to determine the homology or lack thereof of sponge and eumetazoan developmental mechanisms, has begun in detail only in the last decade. In particular, sequencing of the genome of the haplosclerid demosponge *A. queenslandica* and comparison with data from other sponges and early-branching phyla have enabled a large leap in our understanding of the nature of the ancestral metazoan genome (Srivastava *et al.*, 2010b).

Embryogenesis in well-studied bilaterian model organisms, such as vertebrates or *Drosophila*, is governed by a common set of genetic tools, primarily transcription factors and signalling pathways, which are found at all levels of the developmental program. Transcription factors directly switch genes on or off in a specific manner while signalling pathways transmit signals between cells. Comparative genomic analyses have shown that the large majority of gene classes encoding developmental proteins arose with animals (Larroux *et al.*, 2007, 2008; Simionato *et al.*, 2007; Gazave *et al.*, 2009; Richards and Degnan, 2009; Adamska *et al.*, 2010; Bridgham *et al.*, 2010; Srivastava *et al.*, 2010a,b). However, the *A. queenslandica* genome only has a fraction of the genes that are shared by most eumetazoans. This simpler genetic toolkit may represent secondary loss in this lineage or it may reflect a simpler developmental program in the animal ancestor. The presence of these animal developmental genes in sponges strongly supports homology between the embryogeneses of all metazoans.

Regardless, in order to determine the ancestral sponge developmental gene content, more data from all four classes are needed. Fortunately, ESTs from other sponges have already been sequenced (Nichols *et al.*, 2006; Gazave *et al.*, 2009; Labepie *et al.*, 2009; Philippe *et al.*, 2009; Harcet *et al.*, 2010b; Pick *et al.*, 2010).

The expression of transcription factors and signalling pathway components in *A. queenslandica* embryogenesis suggests that sponge and eumetazoan development are homologous. These genetic tools appear to be used in a similar manner in this sponge as they are in other animals. In some cases, conservation of gene function between sponges and bilaterians (*Drosophila* or vertebrate) has been shown (Coutinho *et al.*, 2003; Richards *et al.*, 2008; Bridgham *et al.*, 2010; Hill *et al.*, 2010). The localized expression of the signalling molecule *WntA* just prior to the segregation of cell layers in *A. queenslandica* suggests that early morphogenetic movements in demosponges may be homologous to eumetazoan gastrulation (Adamska *et al.*, 2007a). Expression patterns of *WntA* and *TGF-beta* suggest a role for these signalling ligands in axial patterning during gastrulation (Adamska *et al.*, 2007a, 2011). This proposed role, shared with cnidarians and bilaterians, awaits confirmation by functional gene studies but does suggest that the primary body axes of sponge and eumetazoan larvae are homologous (Adamska *et al.*, 2007a, 2011). Similarly, the canonical Wnt signalling pathway as well as the TGF-beta and Hedgehog-like pathways appear to pattern the photosensory ring, the only organ-like structure in the larva (Adamska *et al.*, 2007a,b, 2010, 2011). Expression analyses in the homoscleromorph sponge *Oscarella lobularis* suggest that the Wnt signalling pathway also has a conserved function in metazoan epithelial patterning and morphogenesis (Labepie *et al.*, 2009; see also Windsor and Leys, 2010). The Notch pathway also seems to fulfil a similar role in *A. queenslandica* as it does in eumetazoans, determining different cell fates of daughter cells during cell division (Richards *et al.*, 2008). The expression of transcription factors in certain cell lineages suggests that they contribute to the gene regulatory networks that govern cell fate determination and differentiation, as they do in eumetazoans (e.g. Larroux *et al.*, 2006; Fahey *et al.*, 2008; Gauthier and Degnan, 2008; Richards *et al.*, 2008; Bridgham *et al.*, 2010; Holstien *et al.*, 2010; Srivastava *et al.*, 2010a; Larroux, unpublished data).

These data come with certain caveats. It is often difficult to make sense of expression data because we know little about the functions of different larval cells and have no embryonic cell lineage data. Additionally, there have been no studies demonstrating the function of genes in sponge embryogenesis. However, advances with pharmacological disruption of signalling pathways and RNA inhibition in sponge adults and juveniles (Lapébie *et al.*, 2009; Windsor and Leys, 2010; Rivera *et al.*, 2011) are promising and suggest we may have success in applying these tools to the study of sponge embryogenesis in the near future.

8.5. Ancestral sponge development

Despite the important differences between the embryogeneses of different sponge groups, development in the Porifera essentially follows similar steps to those in eumetazoans and can thus point us towards a reconstruction of ancestral animal development. Larval types and "gastrulation" modes vary greatly between Hexactinellida, the two subclasses of Calcarea, Homoscleromorpha, and Demospongiae. Within the first four clades, however, different species seem to develop largely in the same manner (although data are limited to one of the five orders for Hexactinellida). In contrast, there is a great deal of diversity within the Demospongiae, but we proposed in the second section that parenchymella-type development could be ancestral. Hence, by comparing hexactinellids, calcaroneans, calcineans, homoscleromorphs, and demosponges with parenchymellae, we can propose some hypotheses regarding development in the poriferan common ancestor.

Cell movement in development is by no means exclusive to Metazoa and does not entail homology of animal developmental traits. For example, *Volvox* spp., multicellular algae, "gastrulate" by inverting their cell layer using cytoplasmic bridges (Viamontes and Kirk, 1977), a process resembling the inversion of calcaronean sponges. Nonetheless, gastrulation is a central step in eumetazoan embryogenesis. The debate regarding gastrulation in sponges must be considered within the context of a sponge developmental program that incorporates a number of eumetazoan attributes, and it thus seems most parsimonious to infer homology of gastrulation across Metazoa. While there is some evidence for gastrulation in demosponges and hexactinellids, the homology of morphogenetic movements in homoscleromorph and calcareous sponges with gastrulation remains a matter of discussion. Determining whether sponges truly gastrulate awaits further testing and molecular data. It is worth noting, however, that in contrast to the low diversity in the modes of gastrulation in bilaterians, which mainly gastrulate by invagination, cnidarians display a large variety of gastrulation modes, some of which are unique to the phylum (Byrum and Martindale, 2004). This could also be the case in sponges, which have had a longer time to evolve than cnidarians, and inversion and multipolar egression may one day be accepted in textbooks as modes of gastrulation. It could also be that more plastic embryogenesis in sponges (with less developmental constraints than other animals) enabled certain lineages to lose the gastrulation step or shift its timing. If sponge embryonic cell movements are revealed to be homologous to eumetazoan gastrulation, the ancestral poriferan and metazoan mode of gastrulation would have probably been through cell migration rather than invagination, based on sponge and cnidarian gastrulation (Price and Patel, 2004). Furthermore, as the process of gastrulation is intimately linked to the formation of germ layers in eumetazoans, demosponge, and hexactinellid germ layers would likely correspond to endoderm and ectoderm.

If homoscleromorph and calcareous sponges are sister groups, as suggested by the molecular phylogenies discussed above, it is interesting to note that the larvae from both of these groups are hollow and single layered, although their development and state of differentiation are quite different. Likewise, in Silicea *sensu stricto* (the sister group of the Calcarea + Homoscleromorpha clade, see above), the hexactinellid- and parenchymella-type demosponge larvae are similar because they are solid and highly differentiated. The trichimella of hexactinellids, the parenchymella proposed to be ancestral to Demospongiae, the cinctoblastula of homoscleromorphs, and the calciblastula or amphiblastula of calcareous sponges are all non-feeding (i.e. lecithotrophic) and ciliated larvae with a clear AP axis. Along with the similar nature of the planula larva of cnidarians (although the planulae of some anthozoans are planktotrophic), it is most parsimonious to postulate that both the poriferan and metazoan common ancestors had such a larva in their life history.

The multiplication of sponge developmental models, with for example *Oscarella* (Homoscleromorpha) (Nichols *et al.*, 2006; Ereskovsky *et al.*, 2009), *Sycon* (Calcarea) (Manuel and Le Parco, 2000; Adamska *et al.*, 2011), and *Ephydatia* (Demospongiae) (Elliott and Leys, 2003; Funayama *et al.*, 2005) species, promises to advance our understanding of ancestral sponge embryogenesis. Although we have not discussed it in this review, most of the molecular research on sponge developmental mechanisms nowadays is actually undertaken on sponge adults, juveniles, or cell culture (e.g. Adell *et al.*, 2003; Perovic *et al.*, 2003; Funayama *et al.*, 2005, 2010; Gazave *et al.*, 2008; Wiens *et al.*, 2008; Labepie *et al.*, 2009; Windsor and Leys, 2010). This body of research has considerably advanced our understanding of sponge and ancestral metazoan development. In conjunction, we propose that efforts to study the molecular basis of sponge embryogenesis should be renewed in order to make significant progress towards understanding the fundamental characters of sponge and animal development.

9. Conclusions and Outlook

Based on the discussion in this chapter, it is clear that deep sponge phylogenetics has come a long way in recent years. Large-scale phylogenomic analyses have so far rejected the hypothesis that sponges are paraphyletic; instead, several studies are consistent with the notion of monophyletic Porifera. It has also become clear from evolutionary developmental studies of sponges that sponge larvae share traits and complexity with eumetazoans and that the simple sedentary adult lifestyle of sponges probably reflects some degree of secondary simplification.

An unexpected sister-group relationship between the former demosponge group Homoscleromorpha—now considered the fourth extant sponge class—and the Calcarea within monophyletic Porifera has been suggested in a few studies. Although this relationship has not yet received unequivocal support, and clear morphological synapomorphies remain to be identified, this would shed some new light on the evolution of some of the key traits of sponges as well as on the early evolution of the Metazoa. Type-IV collagen, previously only thought to occur in Homoscleromorpha and Eumetazoa, now appears to be plesiomorphic for the Metazoa because it has recently been found in Calcarea and Demospongiae too (its presence in the Hexactinellida remains to be detected). Monophyletic sponges with a Calcarea + Homoscleromorpha clade would either suggest that the basement membrane is also plesiomorphic for the Metazoa and is now found in the Homoscleromorpha and Eumetazoa but lost from the other sponge lineages or that it convergently evolved in Homoscleromorpha and Eumetazoa. In either case, a basement membrane would not be synapomorphic for an "Epitheliozoa" clade (Homoscleromorpha + Placozoa + Eumetazoa). A Calcarea + Homoscleromorpha clade also has important implications for the evolution of spiculogenesis in sponges. It would either imply that silica spiculogenesis is plesiomorphic for Porifera and was lost in Calcarea or that it evolved several times independently in sponges (see also Maldonado and Riesgo, 2007). Both Demospongiae and Hexactinellida produce their spicules around an axial filament, which in demosponges contains silicatein. However, while silicatein was apparently characterized in a single hexactinellid species, *Crateromorpha meyeri* (Müller *et al.*, 2008), other studies have failed to demonstrate that classical silicateins are ubiquitously involved in spiculogenesis in other hexactinellids (Ehrlich *et al.*, 2010; Veremeichik *et al.*, 2011). Clearly, more work is needed, but the results have so far called into question the homology of spiculogenesis in Silicea *sensu stricto*. Additionally, the Homoscleromorpha appear to secrete their silica spicules differently than Demospongiae, but their spiculogenesis awaits more detailed study.

While most of the higher-level relationships in Demospongiae appear resolved and corroborated by independent molecular markers, the "mixed-bag" "G4" clade still represents a serious challenge, as many relationships within this clade await robust resolution (but see Morrow *et al.*, 2012). Higher-level relationships in Hexactinellida appear largely congruent with previous morphological systematics, but some critical taxa (such as Lychniscosida and Aulocalycidae) await to be included in molecular studies. The Homoscleromorpha are clearly distinct from the demosponges, and their internal phylogeny is largely resolved, although taxon sampling could be improved. The phylogeny of Calcarea remains largely unresolved because molecular phylogenies are highly incongruent with the taxonomic system based on morphological characters. Here, probably the most work is needed to fully understand the basis for this incongruence. Calcarea are also among

the few non-bilaterian taxa where no complete mitochondrial genome has yet been sequenced.

As discussed above, we have made great progress in deep sponge phylogenetics, but we still have a long way to go to achieve a comprehensive understanding of the relationships among and within the main sponge lineages, which will be crucial to fully appreciate the evolution of this extraordinary metazoan phylum.

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