

Analysis of evolutionary, biogeographical and taxonomic patterns of nucleotide composition in demosponge rRNA

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The ribosome is the location of protein translation and therefore a pivotal macromolecular complex for all organisms. The RNA molecules involved in the formation and functioning of the ribosome (rRNA) are partially single-stranded (loops) and partially double-stranded (helices or stems) as a result of pairing of complementary regions in either their own or other rRNA subunits. This pattern provides the rRNA with a secondary structure crucial for its functionality. The stability of these secondary structures is mediated by their base compositions: a helix rich in G-C pairs possesses a higher thermodynamic stability than an A-T rich counterpart. However, the base composition of these structures is neither homogeneous throughout the molecule nor throughout the demosponge taxa. Here, we present patterns of biased nucleotide composition in demosponge 28S rDNA. We analyse their correlation in respect to environment and taxonomy. We find significantly higher G+C contents in haplosclerid demosponges compared to other orders and investigate evidence for an association between water temperature and rRNA base composition in demosponges.

INTRODUCTION

The frequency of the four nucleotide types in organismal DNA has been shown to be variable among lineages. In particular, the different ratios of the G-C as opposed to the A-T Watson-Crick nucleotide pairs have attracted the interest of evolutionary biologists (see Mooers & Holmes (2000) for a detailed review on G+C composition studies). The G+C content of genomes is, for example, relatively low in invertebrates but highly variable for protists and algae. Bacterial genomes, which are among the best studied for G+C content (see Galtier et al. (1999) for a systematic context) display the highest variation in G+C content with ranges between 25 and 75% (see Mooers & Holmes, 2000). One reason for those differences might be the general mutational bias in DNA, which results from the preference of one nucleotide pair above the other (Eyre-Walker, 1993). Another reason for the pronounced variance in nucleotide frequency is natural selection (Smit et al., 2006). Natural selection might favour a higher percentage of G-C bonds in a DNA strand because this nucleotide pair forms more energetically stable bonds compared to A-T bonds. Although this bias appears plausible for organisms that live in extreme environmental conditions, a direct correlation between genomic G+C content and environment is not always evident. Selection for higher G+C contents has been shown for UV exposure of bacteria (Singer & Ames, 1970), but thermophilic bacteria do not have a significantly higher

G+C content than mesophilic bacteria (Galtier & Lobry, 1997; see also Nakashima et al., 2003), although G+C rich DNA (and RNA) chains would be more heat-stable than their G+C poorer counterparts. In addition, *Drosophila* species of colder climates can have a higher G+C content than their warmer climate congeners (Rodriguez-Trelles et al., 1999; Mooers & Holmes, 2000).

Apparently, selection towards higher G+C is more evident in single genes and gene-fragments than in entire genomes. Such bias can be seen in rRNA genes (rDNA), which code for rRNA molecules that undergo a complex folding into a secondary structure of single-strands ('loops') and double-strands ('stems' or 'helices', see Figure 1); these form tertiary (and higher) structures in the final ribosome. The ribosome's shape, which is important for its functionality, is mediated in particular (but not exclusively) by rRNA helices. Therefore, there is considerable evolutionary pressure on maintaining a functional shape of secondary structure maintained by helices within the rRNA molecule. In thermophilic bacteria, helix regions possess a higher G+C rate than mesophilic bacteria, presumably in order to maintain rRNA function in extreme environments (Galtier & Lobry, 1997). For vertebrates it has been shown that warm-blooded species have a higher overall G-C content in rRNA than their cold-blooded counterparts. However, this difference is not concentrated in the paired regions of the molecule, suggesting that thermal adaptation is not the cause of the nucleotide differences among vertebrate lineages (Wang et al., 2006). To our knowledge,

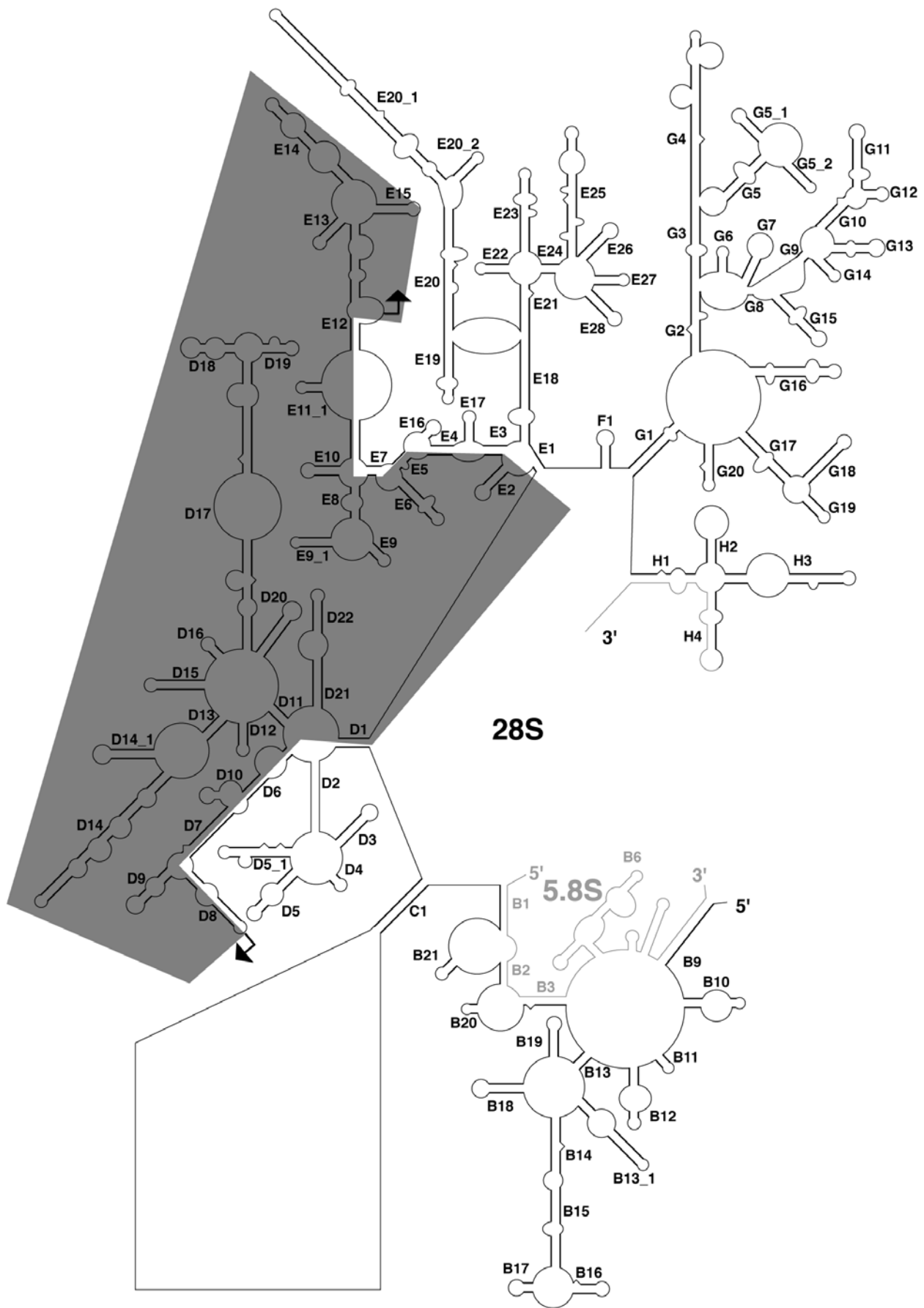


Figure 1. Schematic view of LSU. The pairing nucleotide sites (helices) are given in thicker lines, single stranded regions with thinner lines. The section included in our alignments is shaded in grey and flanked by arrows. Hypervariable sites of other sections are marked with dashed lines.

the correlation between environmental temperature and stem-rDNA G+C content has rarely been analysed among invertebrates. In this study we aim to seek evidence for such a correlation in early-branching Metazoa.

RESULTS AND DISCUSSION

Sponges are particularly suitable for this type of analysis. As sessile marine invertebrates they are poikilothermic and surrounded by a medium of easily measurable temperature without sudden changes. Demosponges are also cosmopolitan and present in all marine habitats. Furthermore, a considerable number of demosponge large sub-unit 28S (LSU) rDNA sequences are published with their secondary structure readily assessed (Erpenbeck et al., 2007a). The latter were used to align the LSU sequences and to identify the position of stems in the transcribed and assembled rRNA molecule (see Figure 1). We analysed the data of Nichols (2005), which comprises a relatively long LSU fragment (>1400 characters) and a suitable representation of demosponge orders (11 out of 15) (Figure 2). G+C contents of stems and loops were calculated with PAUP 4.0 (Swofford, 2002) and statistically analysed with ANOVA using Statistica for Windows 6.1 (Statsoft, Tulsa, OK, USA).

First, we tested for taxonomical bias in the rRNA helix and loop nucleotide compositions throughout the 106 demosponge sequences. We observed a strongly increased helix G+C content in the taxa of the order Haplosclerida (67.8%; SD=1.4; N=13) compared to all other demosponges pooled (62.6%; SD=2.1; N=83). Haplosclerid sponges contained a significantly higher G+C content in stem regions ($F_{5,71}=45.53$, $P<0.001$) compared to all other orders tested (Bonferroni test: $P<0.001$). Astrophorids also contained a significantly higher G+C content than dictyoceratids, poecilosclerids and hadromerids but not halichondrids. There was no significant difference in G+C content among dictyoceratids, poecilosclerids, hadromerids or halichondrids (Bonferroni test: $P>0.05$). These findings are in congruence with earlier observations, in which Haplosclerida displayed higher substitution rates and different secondary structure features compared to all other demosponge rRNA, in addition to increased substitution rates in mitochondrial DNA (Erpenbeck et al., 2004, 2007b). The order Haplosclerida comprises the highest biodiversity of all sponges in terms of species and habitats (van Soest & Hooper, 2002), perhaps caused by higher evolutionary rates that allowed members of this taxon to open up new ecological niches. The resulting structural diversity of the ribosomal rRNA is stabilized by increased G+C contents in the helices.

There were also significant differences in the nucleotide composition of the loops among orders ($F_{5,71}=45.00$, $P<0.001$). G+C content was highest in the astrophorids, haplosclerid and halichondrids and did not differ significantly among these orders (Bonferroni test: $P>0.05$). G+C content was significantly lower in the poecilosclerid than astrophorids (Bonferroni test: $P=0.006$). The dictyoceratids had a significantly lower G+C content than all other orders (Bonferroni test: $P<0.001$). The increased amount of loop adenosine in taxa of all orders (33.7–40.4%) is usual for unpaired sites in ribosomal loop partitions (Telford et al., 2005).

In addition to assessing variation in G+C content among orders we also tested for the effect of the environment on G+C substitutions in demosponge LSU rDNA loops and stems. For this approach, species were divided into two simplified groups of 'cold' (approximately temperate/mediterranean and polar) and warm (tropical) locations, in which minimum and maximum temperatures were least overlapping (see Appendix 1). Temperature charts for the species locations have been taken from the NOAA Satellite and Information Service (<http://nodc.noaa.gov/OC5/WOA01F/tsearch.html>). This simplification certainly bears the risk of experimental error, which, however, cannot entirely be excluded due to the lack of knowledge on optimal growth temperatures for the individual sponge species. Collection depth of 30 m (opposed to surface, 10 m, 50 m, or deeper as alternative options) has been assumed to approximate the temperature conditions of the taxon set best. For this depth we chose the annual mean temperature (as opposed to maximum and minimum temperature as their influence has not been estimated yet).

For the analysis we followed the method of Charette et al. (2006) and compared our pool of 'cold water' species with their closest known warm-water relative. For our data set this resulted in the formation of a maximum of 15 species pairs, which included seven congeneric pairs (pairs A–G, see Appendix 1), five confamilial pairs (pairs H–L) and three pairs of species within the same order (pairs M–O). When there were multiple candidate species for a matched pair, species with name lowest in alphabetical order were chosen (considered as a random choice). Differences in G+C content between 'cold water' and 'warm water' species were tested for significance with a paired *t*-test (aka *t*-test for dependent samples) using Statistica for Windows v. 6.1. The loop G+C content of 'cold' and the 'warm' species did not differ significantly ($t=1.427$, $P=0.175$, $N=15$), but the helix G+C content was significantly higher among the species in the 'warm' group compared to the 'cold' group ($t=2.303$, $P=0.037$, $N=15$). This result would support the hypothesis of evolutionary pressure on more stable RNA helices by increasing the ratio of G+C pairs.

In any event, the G+C content does not necessarily reflect the number of triple bonds in a given stretch of RNA. G can form the 'wobble'-bonds with uracil (U), which are double bonds and considerably weaker than the G-C triple bonds. As C only binds with G, the frequency of C in paired sites is a direct indicator for the number of triple hydrogen-bonds in RNA secondary structure. In fact the samples originating from warmer waters exhibit a higher C content (28.4%; SD=0.03, N=76) than their 'cold'-group counterparts (27.3%; SD=0.01; N=13) (*t*-test, $P<0.05$).

Nevertheless, there are clear pitfalls in our analysis, which are frequently neglected by other analyses: sponge systematics frequently suffers from ambiguous classification paired with incongruent (and inconsistent) molecular phylogenies. This phenomenon affects our present study as well.

First, freshwater sponges are morphologically classified with the marine Haplosclerida (van Soest & Hooper, 2002), but molecular (predominantly rDNA) analyses provide evidence against the monophyletic relationship of marine and freshwater sponges (e.g. Borchiellini, 2004; Addis &

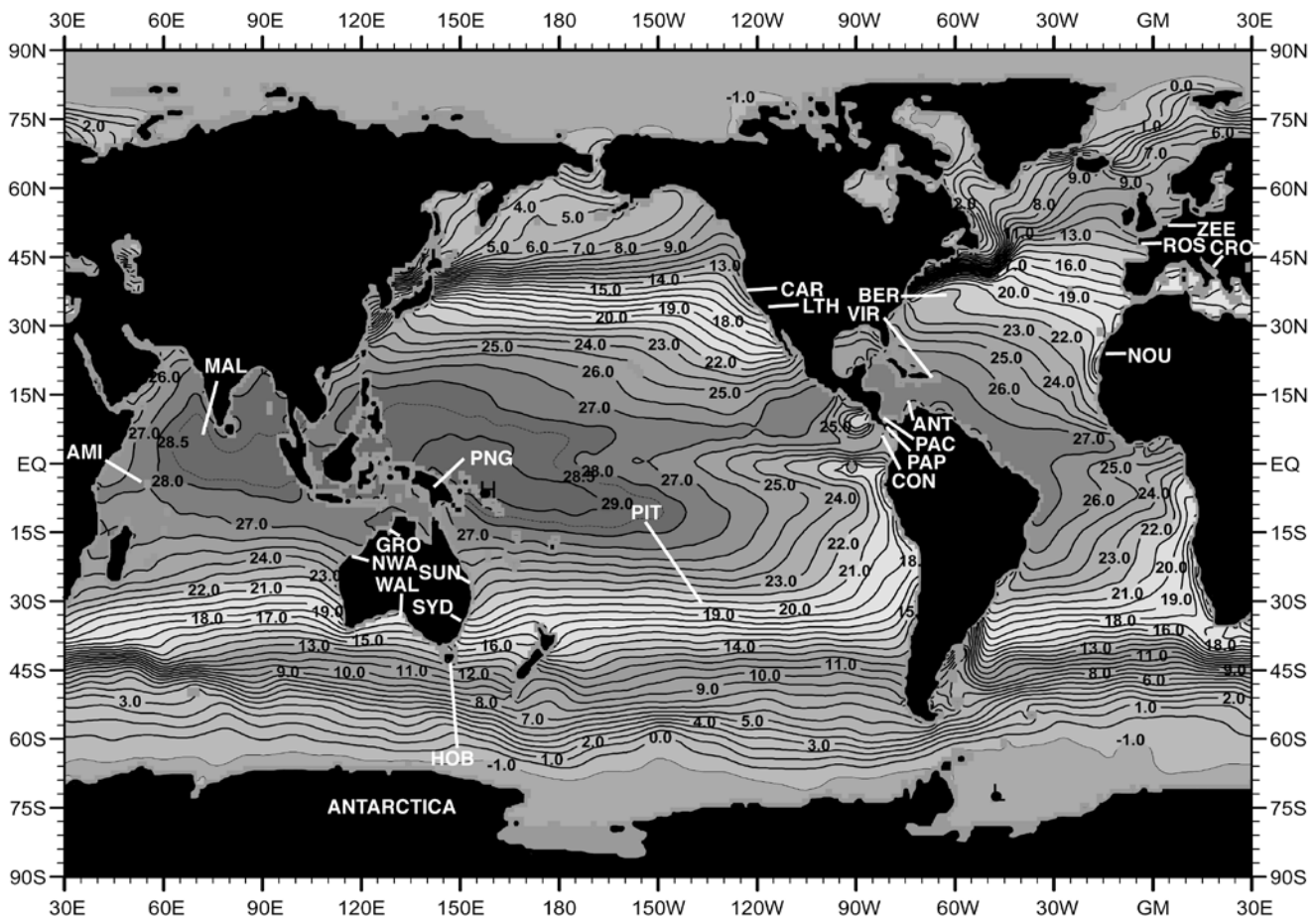


Figure 2. Plot of the sampling locations (three letter codes) of the 95 non-haplosclerid demosponges against the water temperatures used as basis for raw division into 'cold' and 'warm' water regions (annual mean, 30 m, source: NOAA Satellite and Information Service). Locality codes: AMI, Seychelles, Amirantes; ANT, Curacao, Netherlands Antilles; BER, Bermuda; CAR, Carmel, California; CON, Contreras Island, Panama; CRO, Croatia; GRO, Dampier and Onslow WA, Australia; HOB, Hobart, Australia; LTH, Lake Tahoe, California, USA; MAL, Maldives; NOU, Nouadhibou, Mauritania; NWA, North West Cape, WA, Australia; PAC, Panama (Caribbean); PAP, Panama (Pacific); PIT, Pitcairn Islands; PNG, Papua New-Guinea; ROS, Roscoff; SUN, Sunshine Coast, QLD, Australia; SYD, Sydney Heads, Australia; VIR, Virgin Islands; WAL, Waldergrove Islands, Australia; ZEE, Zeeland, Netherlands.

Peterson, 2005; Nichols, 2005; Redmond et al., 2007). Inclusion of the Haplosclerida pair into our statistics might infer a conceptual error even if the classification of this group still awaits final acceptance.

Secondly, several other taxon pairs are not recovered at their expected positions: Both *Polymastia* sp. 1 and *Geodia barretti*, cluster distantly from their congeners in the original phylogenetic tree (Nichols, 2005), which may raise some doubt on their suitability for this analysis. While *G. barretti* can be exchanged against *G. papyracea* from our taxon set, there is no sequence to replace *Polymastia* sp. 1.

When the haplosclerid sponge pair is excluded from the analysis the result is marginally non-significant using an α of 0.05 ($t=2.011$, $P=0.066$, $df=13$). Likewise if the taxon-pair including *Polymastia* sp.1 is disregarded the result is marginally non-significant ($t=-1.788$, $P=0.099$, $df=12$). It must be noted, however, that the exclusion of data points decreases the power of the sample set, which effects small data sets in particular. All tests, however, revealed a probability below 0.1, which due to the small sample size suggests an association between G+C content and environment although this warrants further research.

We therefore find some evidence of a trend towards increased evolutionary pressure on stem triple hydrogen bonds, but more exhaustive research is needed, in particular of sequences of verified entity with unambiguous phylogenetic origin. Answering molecular evolutionary questions using published sequences generated for a different purpose, however, remains problematic, especially in sponges, as long as contamination and misidentification problems are not resolved. Nevertheless, we can deduce from our analysis on evolutionary nucleotide composition in demosponge rRNA that taxonomic patterns are clearly present and there is evidence that environmental patterns might exist.

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Appendix 1. Taxa, locations, GenBank accession numbers and nucleotide frequencies of *Nichols* (2005) data set. Nucleotide frequencies are split up in helix and loop partitions.

Order	Family	Species	Accession number	Location	Temp	Pair	Helix A	Helix C	Helix G	Helix T	Helix C+G	Loop A	Loop C	Loop G	Loop T	Loop A+T
Agelastida	Agelastidae	<i>Agelas</i> sp.	AY561929	Papua New Guinea	warm		0.155	0.282	0.344	0.219	0.626	0.368	0.147	0.288	0.196	0.565
Agelastida	Astroscleridae	<i>Astrosclera volleyana</i>	AY561928	Papua New Guinea	warm		0.153	0.284	0.346	0.217	0.630	0.371	0.147	0.287	0.196	0.566
Astrophorida	Ancorinidae	<i>Ecionemia</i> sp.	AY561938	Panama Caribbean	warm		0.145	0.296	0.351	0.208	0.647	0.349	0.171	0.297	0.182	0.531
Astrophorida	Ancorinidae	<i>Holoxea</i> sp.	AY561942	Papua New Guinea	warm		0.144	0.302	0.351	0.203	0.653	0.351	0.175	0.291	0.182	0.533
Astrophorida	Ancorinidae	<i>Metaphlus</i> sp.	AY561940	Papua New Guinea	warm		0.142	0.301	0.357	0.200	0.657	0.346	0.171	0.301	0.182	0.528
Astrophorida	Ancorinidae	<i>Rhabdastrella</i> sp.	AY561939	Papua New Guinea	warm		0.144	0.301	0.355	0.200	0.655	0.346	0.175	0.297	0.182	0.528
Astrophorida	Geodidae	<i>Geodia barretti</i>	AY561877	Sweden	cold	A	0.152	0.279	0.364	0.205	0.643	0.369	0.159	0.277	0.196	0.565
Astrophorida	Geodidae	<i>Geodia media</i>	AY561937	Panama Pacific	warm	A	0.138	0.297	0.363	0.202	0.659	0.353	0.171	0.294	0.182	0.535
Astrophorida	Geodidae	<i>Geodia papiraca</i>	AY561879	Panama Caribbean	warm		0.163	0.281	0.348	0.209	0.628	0.365	0.156	0.280	0.199	0.564
Astrophorida	Incertae Sedis	<i>Lamellonoplia</i> sp.	AY561941	Papua New Guinea	warm		0.141	0.299	0.356	0.204	0.656	0.349	0.171	0.294	0.185	0.535
Chondrosida	Chondrillidae	<i>Chondrilla australiensis</i>	AY561867	Henderson Island/Pitcairn Island	warm	B	0.122	0.309	0.373	0.196	0.681	0.361	0.165	0.295	0.179	0.540
Chondrosida	Chondrillidae	<i>Chondrilla</i> sp.	AY561868	Australia, Sydney Heads	warm	B	0.153	0.297	0.347	0.203	0.645	0.372	0.175	0.291	0.161	0.533
Chondrosida	Chondrillidae	<i>Chondrosia</i> sp.	AY561866	Papua New Guinea	warm		0.158	0.298	0.340	0.204	0.638	0.404	0.156	0.266	0.174	0.578
Dicyoceratida	Dysideidae	<i>Dysidea alberta</i>	AY561954	Bermuda	warm		0.166	0.285	0.334	0.215	0.619	0.400	0.140	0.253	0.207	0.607
Dicyoceratida	Dysideidae	<i>Lamellodysidea chlorea</i>	AY561957	Papua New Guinea	warm		0.163	0.291	0.334	0.212	0.625	0.398	0.141	0.254	0.208	0.606
Dicyoceratida	Spongiidae	<i>Spongia</i> sp. 4	AY561951	Papua New Guinea	warm		0.157	0.294	0.337	0.212	0.631	0.400	0.140	0.253	0.207	0.607
Dicyoceratida	Spongiidae	<i>Spongia</i> sp. 1	AY561953	Papua New Guinea	warm		0.155	0.296	0.339	0.210	0.635	0.401	0.141	0.254	0.204	0.606
Dicyoceratida	Thorectidae	<i>Luffianella</i> sp.	AY561952	Papua New Guinea	warm		0.166	0.285	0.337	0.213	0.621	0.404	0.126	0.249	0.221	0.625
Dicyoceratida	Thorectidae	<i>Thorectidae</i> 1	AY561955	Papua New Guinea	warm		0.161	0.295	0.335	0.209	0.631	0.400	0.133	0.253	0.214	0.614
Dicyoceratida	Undetermined	<i>Dicyoceratida</i> 1	AY561958	Panama Pacific	warm		0.157	0.293	0.339	0.211	0.633	0.404	0.126	0.249	0.221	0.625
Hadromerida	Clonaidae	<i>Cliona celata</i>	AY561891	France, Bretagne, Roscoff/Le Paradis	cold	C	0.173	0.260	0.338	0.229	0.598	0.370	0.157	0.278	0.196	0.566
Hadromerida	Clonaidae	<i>Cliona delirix</i>	AY561889	Panama Caribbean	warm	C	0.171	0.271	0.339	0.218	0.611	0.365	0.159	0.284	0.191	0.557
Hadromerida	Clonaidae	<i>Cliona orientalis</i>	AY561887	Australia, WA, Dampier	warm		0.169	0.269	0.341	0.220	0.610	0.362	0.159	0.287	0.191	0.553
Hadromerida	Clonaidae	<i>Cliona</i> sp.	AY561886	Papua New Guinea	warm		0.158	0.279	0.339	0.224	0.617	0.367	0.157	0.287	0.189	0.556
Hadromerida	Clonaidae	<i>Pione velans</i>	AY561890	Australia, WA, Dampier	warm		0.163	0.273	0.357	0.207	0.630	0.365	0.162	0.278	0.195	0.559
Hadromerida	Hemiasterellidae	<i>Axos</i> sp.	AY561902	Australia, WA, Onslow	warm		0.174	0.263	0.347	0.216	0.611	0.362	0.147	0.294	0.197	0.559
Hadromerida	Hemiasterellidae	<i>Hemiasterella</i> sp. 1	AY561901	Australia, Sunshine Coast	warm		0.159	0.277	0.338	0.226	0.615	0.371	0.147	0.287	0.196	0.566
Hadromerida	Hemiasterellidae	<i>Hemiasterella</i> sp. 2	AY561946	Papua New Guinea	warm		0.144	0.301	0.354	0.201	0.654	0.367	0.168	0.294	0.171	0.538
Hadromerida	Hemiasterellidae	<i>Hemiasterella</i> sp. 3	AY561947	Australia, WA, NW-Cape	warm		0.139	0.295	0.363	0.203	0.658	0.367	0.157	0.290	0.185	0.552
Hadromerida	Hemiasterellidae	<i>Stelligera</i> sp.	AY561949	Papua New Guinea	warm	D	0.160	0.288	0.340	0.212	0.628	0.349	0.143	0.304	0.203	0.552
Hadromerida	Hemiasterellidae	<i>Stelligera stuposa</i>	AY561950	France, Bretagne, Roscoff/Ar Tourin	cold	D	0.167	0.280	0.347	0.206	0.627	0.341	0.163	0.308	0.188	0.529
Hadromerida	Placospongiidae	<i>Placospongia</i> sp.	AY561896	Papua New Guinea	warm		0.160	0.278	0.335	0.227	0.613	0.367	0.150	0.287	0.196	0.563
Hadromerida	Polymastidae	<i>Polymastia imagnata</i>	AY561922	Antarctica	cold	E	0.159	0.281	0.340	0.220	0.621	0.374	0.147	0.287	0.192	0.566
Hadromerida	Polymastidae	<i>Polymastia paclynastia</i>	AY561924	USA, California, Carmel	cold	F	0.167	0.275	0.339	0.219	0.614	0.374	0.139	0.287	0.199	0.573
Hadromerida	Polymastidae	<i>Polymastia</i> sp. 1	AY561897	Australia, WA, Dampier	warm	E	0.168	0.263	0.342	0.227	0.605	0.389	0.142	0.288	0.179	0.569
Hadromerida	Polymastidae	<i>Polymastia</i> sp. 2	AY561923	Australia, WA, NW-Cape	warm	F	0.164	0.272	0.342	0.222	0.614	0.378	0.154	0.292	0.176	0.554
Hadromerida	Polymastidae	<i>Pseudonachya</i> sp.	AY561965	Panama Caribbean	warm		0.162	0.283	0.335	0.220	0.617	0.372	0.140	0.284	0.204	0.575
Hadromerida	Polymastidae	<i>Spinularia spinularia</i>	AY561921	Mauritania, Nouadhibou, off Banc d'Arguin	warm		0.170	0.268	0.345	0.218	0.612	0.369	0.149	0.287	0.195	0.564
Hadromerida	Spirastrellidae	<i>Diphastrella megastellata</i>	AY561893	Panama Caribbean	warm		0.158	0.279	0.339	0.224	0.617	0.367	0.154	0.287	0.192	0.559

Hadromerida	Spirastrellidae	<i>Diplastrella spinglobata</i>	AY561894	Seychelles, Amirantes	warm	0.169	0.273	0.338	0.221	0.610	0.362	0.159	0.287	0.191	0.553
Hadromerida	Spirastrellidae	<i>Spirastrella hartmani</i>	AY561895	Panama Caribbean	warm	0.170	0.270	0.338	0.223	0.607	0.363	0.155	0.289	0.194	0.556
Hadromerida	Spirastrellidae	<i>Spirastrella</i> sp.	AY561892	Australia, WA, Dampier	warm	0.168	0.275	0.343	0.214	0.618	0.363	0.157	0.285	0.196	0.559
Hadromerida	Subertiidae	<i>Aaptos</i> cf. <i>lithoplaga</i>	AY561909	USA, Virgin Islands, St Croix	warm	0.176	0.253	0.336	0.235	0.589	0.374	0.139	0.283	0.203	0.577
Hadromerida	Subertiidae	<i>Aaptos</i> sp.	AY561908	Australia, WA, Dampier	warm	0.177	0.248	0.338	0.237	0.586	0.369	0.142	0.284	0.206	0.574
Hadromerida	Subertiidae	<i>Carlospongia reticulata</i>	AY561914	Australia, SA, Waldegrave Isl.	cold	0.177	0.260	0.334	0.229	0.594	0.374	0.139	0.283	0.203	0.577
Hadromerida	Subertiidae	<i>Prosuberites laugliini</i>	AY561927	Panama Caribbean	warm	0.166	0.276	0.344	0.214	0.620	0.381	0.139	0.283	0.196	0.577
Hadromerida	Subertiidae	<i>Protosuberites denhartogi</i>	AY561916	Netherlands, Zeeland, Neeltje Jans	cold	0.178	0.261	0.342	0.219	0.603	0.366	0.143	0.283	0.208	0.573
Hadromerida	Subertiidae	<i>Protosuberites</i> sp. nov.	AY561915	Panama, Contreras Island,	warm	0.169	0.275	0.354	0.202	0.629	0.363	0.158	0.277	0.201	0.565
Hadromerida	Subertiidae	<i>Pseudosuberites</i> sp. 2	AY561913	Panama Caribbean	warm	0.176	0.264	0.336	0.224	0.600	0.371	0.139	0.287	0.203	0.573
Hadromerida	Subertiidae	<i>Pseudosuberites</i> sp. 3	AY561917	Papua New Guinea	warm	0.163	0.271	0.335	0.231	0.606	0.371	0.139	0.287	0.203	0.573
Hadromerida	Subertiidae	<i>Pseudosuberites</i> sp. 1	AY561919	Mauritania, Nouadhibou, off Banc d'Arguin,	warm	0.182	0.260	0.341	0.218	0.601	0.363	0.147	0.284	0.205	0.568
Hadromerida	Subertiidae	<i>Rhizaxinella</i> sp.	AY561910	Australia, Hobart	cold	0.166	0.263	0.328	0.243	0.590	0.377	0.137	0.278	0.208	0.585
Hadromerida	Subertiidae	<i>Suberites ficus</i>	AY026381	taken from GenBank	n/a	0.168	0.273	0.329	0.230	0.601	0.374	0.133	0.287	0.206	0.580
Hadromerida	Subertiidae	<i>Suberites</i> sp.	AY561912	USA, California, Carmel	cold	0.184	0.253	0.327	0.235	0.581	0.374	0.136	0.283	0.206	0.580
Hadromerida	Subertiidae	<i>Teopios aurantiaca</i>	AY561911	Netherlands Antilles, Curacao, Schottegat	warm	0.184	0.255	0.331	0.231	0.585	0.377	0.141	0.278	0.204	0.581
Hadromerida	Subertiidae	<i>Teopios</i> cf. <i>austaliensis</i>	AY561918	Australia, WA, Dampier	warm	0.174	0.258	0.348	0.219	0.607	0.363	0.140	0.284	0.212	0.576
Hadromerida	Tethyidae	<i>Laxothelpha dampierensis</i>	AY561905	Australia, WA, Dampier	warm	0.171	0.275	0.339	0.216	0.613	0.365	0.149	0.287	0.199	0.564
Hadromerida	Tethyidae	<i>Stallithelpha ingens</i>	AY561899	Australia, WA, NW-Cape	warm	0.163	0.278	0.333	0.227	0.610	0.371	0.150	0.287	0.192	0.563
Hadromerida	Tethyidae	<i>Tethya californiana</i>	AY561900	USA, California, Carmel	cold	0.166	0.284	0.340	0.210	0.624	0.364	0.152	0.288	0.197	0.561
Hadromerida	Tethyidae	Tethyidae 1	AY561904	Papua New Guinea	warm	0.165	0.275	0.333	0.227	0.608	0.371	0.147	0.287	0.196	0.566
Hadromerida	Tethyidae	<i>Telhytinea stellagrandis</i>	AY561920	Maldives, Rashdoe Atoll, Kuramathi	warm	0.169	0.275	0.325	0.231	0.600	0.371	0.143	0.287	0.199	0.569
Hadromerida	Timeidae	<i>Timea</i> cf. <i>centrifera</i>	AY561906	Australia, WA, Dampier	warm	0.170	0.263	0.345	0.222	0.608	0.369	0.145	0.284	0.202	0.571
Hadromerida	Timeidae	<i>Timea lowchayi</i>	AY561871	Australia, Bass Reef	warm	0.154	0.295	0.342	0.209	0.637	0.374	0.147	0.287	0.192	0.566
Hadromerida	Timeidae	<i>Timea</i> sp.	AY561907	Australia, Sunshine Coast	warm	0.162	0.279	0.337	0.222	0.615	0.371	0.139	0.287	0.203	0.573
Halichondrida	Axinellidae	<i>Axinella</i> sp. 2	AY561931	Panama Caribbean	warm	0.146	0.299	0.357	0.198	0.655	0.353	0.154	0.304	0.189	0.542
Halichondrida	Axinellidae	<i>Axinellidae</i> 1	AY561930	Papua New Guinea	warm	0.148	0.299	0.355	0.198	0.653	0.357	0.154	0.301	0.189	0.545
Halichondrida	cf. Axinellidae	<i>Axinellidae</i> 2	AY561932	Papua New Guinea	warm	0.145	0.298	0.357	0.200	0.655	0.349	0.154	0.308	0.189	0.538
Halichondrida	Desmoxiidae	<i>Dubiscus</i> sp.	AY561948	Papua New Guinea	warm	0.152	0.292	0.347	0.209	0.640	0.346	0.143	0.301	0.209	0.556
Halichondrida	Dictyonellidae	<i>Acanthella</i> sp.	AY561936	Panama Pacific	warm	0.145	0.296	0.358	0.201	0.654	0.353	0.161	0.301	0.185	0.538
Halichondrida	Dictyonellidae	<i>Scopalinia nuelzleri</i>	AY561872	Panama Caribbean	warm	0.153	0.297	0.344	0.206	0.642	0.371	0.154	0.287	0.189	0.559
Halichondrida	Halichondriidae	Halichondriidae sp. 1	AY561926	Papua New Guinea	warm	0.151	0.285	0.347	0.217	0.632	0.360	0.143	0.304	0.192	0.552
Halichondrida	Halichondriidae	Halichondriidae sp. 2	AY561933	Papua New Guinea	warm	0.147	0.296	0.354	0.203	0.650	0.352	0.144	0.306	0.197	0.549
Halichondrida	Halichondriidae	Halichondriidae sp. 3	AY561935	Papua New Guinea	warm	0.149	0.294	0.348	0.209	0.642	0.378	0.139	0.287	0.196	0.573
Halichondrida	Unidentified	Halichondrida 1	AY561934	Papua New Guinea	warm	0.145	0.299	0.349	0.206	0.648	0.351	0.144	0.309	0.196	0.547
Haplosclerida	Callyspongiidae	<i>Callyspongia</i> sp.	AY561863	Papua New Guinea	warm	0.127	0.299	0.366	0.208	0.665	0.376	0.121	0.316	0.188	0.564
Haplosclerida	Chalinidae	<i>Haliclona</i> sp.	AY561862	Papua New Guinea	warm	0.127	0.302	0.363	0.208	0.665	0.379	0.119	0.316	0.186	0.565
Haplosclerida	Haplosclerina	Haplosclerida 2	AY561856	Papua New Guinea	warm	0.132	0.316	0.367	0.185	0.684	0.354	0.137	0.326	0.182	0.537
Haplosclerida	Haplosclerina	Haplosclerina 1	AY561855	Papua New Guinea	warm	0.135	0.313	0.365	0.187	0.679	0.372	0.123	0.323	0.182	0.554
Haplosclerida	Haplosclerina	Haplosclerina 2	AY561854	Papua New Guinea	warm	0.129	0.319	0.365	0.187	0.684	0.382	0.123	0.312	0.182	0.565
Haplosclerida	Haplosclerina	Haplosclerina 3	AY561858	Papua New Guinea	warm	0.129	0.300	0.366	0.205	0.666	0.337	0.154	0.329	0.179	0.516
Haplosclerida	Haplosclerina	Haplosclerina 4	AY561860	Papua New Guinea	warm	0.127	0.302	0.368	0.203	0.670	0.354	0.137	0.326	0.182	0.537
Haplosclerida	Haplosclerina	Haplosclerina 5	AY561861	Papua New Guinea	warm	0.123	0.325	0.374	0.178	0.699	0.358	0.140	0.319	0.182	0.540
Haplosclerida	Petrosiidae	<i>Petrosia</i> sp.	AY561859	Panama Caribbean	warm	0.137	0.303	0.357	0.203	0.660	0.372	0.126	0.312	0.189	0.561

Appendix 1. (Continued).

Haplosclerida	Petrosiidae	<i>Xestospongia</i> sp.	AY561853	Papua New Guinea	warm	0.129	0.321	0.375	0.175	0.696	0.351	0.144	0.326	0.179	0.529
Haplosclerida	Phlocodictyidae	<i>Oreamapta</i> sp.	AY561857	Papua New Guinea	warm	0.127	0.313	0.374	0.186	0.687	0.369	0.137	0.313	0.179	0.549
Haplosclerida	Spongillidae	<i>Spongilla lacustris</i>	AY561873	USA, California, Lake Tahoe	cold	0.153	0.292	0.353	0.202	0.645	0.364	0.161	0.287	0.189	0.552
Haplosclerida	Undetermined	Haplosclerida 1	AY561852	Papua New Guinea	warm	0.125	0.319	0.375	0.181	0.694	0.347	0.144	0.333	0.175	0.523
Homosclerophorida	Plakinidae	<i>Plakinastrella</i> sp. 1	AY561869	Panama Caribbean	warm	0.155	0.302	0.339	0.204	0.640	0.374	0.150	0.294	0.182	0.556
Homosclerophorida	Plakinidae	<i>Plakinastrella</i> sp. 2	AY561870	Panama Caribbean	warm	0.154	0.302	0.342	0.202	0.643	0.374	0.150	0.294	0.182	0.556
Lithistids	Scleritodermidae	<i>Aciculites</i> sp.	AY561945	Panama Caribbean	warm	0.138	0.299	0.361	0.202	0.659	0.353	0.175	0.290	0.182	0.535
Poecilosclerida	cf. Raspaillidae	Raspaillidae 1	AY561885	Papua New Guinea	warm	0.164	0.274	0.330	0.232	0.604	0.371	0.157	0.279	0.192	0.563
Poecilosclerida	Coelosphaeridae	<i>Lissodendoryx topsentii</i>	AY561876	USA, California, Carmel	cold	0.145	0.287	0.352	0.216	0.640	0.374	0.150	0.290	0.185	0.559
Poecilosclerida	Crambeidae	<i>Crambe crambe</i>	AY561883	Croatia	cold	0.172	0.269	0.343	0.216	0.612	0.369	0.152	0.280	0.199	0.567
Poecilosclerida	Crambeidae	<i>Monanchora arbuscula</i>	AY561882	Panama Caribbean	warm	0.167	0.277	0.347	0.210	0.624	0.369	0.163	0.280	0.188	0.557
Poecilosclerida	Ietrochoitidae	<i>Ietrochota hirsutiflata</i>	AY561884	Panama Caribbean	warm	0.168	0.281	0.340	0.211	0.621	0.365	0.156	0.280	0.199	0.564
Poecilosclerida	Isodictyidae	<i>Coelocarteria singaporensis</i>	AY561874	Papua New Guinea	warm	0.145	0.300	0.349	0.206	0.649	0.372	0.161	0.284	0.182	0.554
Poecilosclerida	Isodictyidae	<i>Coelocarteria</i> sp.	AY561875	Papua New Guinea	warm	0.143	0.297	0.354	0.206	0.652	0.371	0.161	0.287	0.182	0.552
Poecilosclerida	Mycalidae	<i>Mycala fibroxilis</i>	AY026376	USA, Woods Hole	cold	0.157	0.279	0.341	0.223	0.620	0.371	0.157	0.279	0.192	0.563
Poecilosclerida	Podospongiidae	<i>Diacarus spinipoculum</i>	AY561881	Papua New Guinea	warm	0.155	0.279	0.342	0.224	0.621	0.371	0.154	0.287	0.189	0.559
Poecilosclerida	Tectaniidae	<i>Tectania ignis</i>	AY561878	Panama Caribbean	warm	0.163	0.269	0.352	0.216	0.621	0.365	0.159	0.280	0.195	0.560
Spirophorida	Tetillidae	<i>Cinaclayrella</i> sp.	AY561943	Papua New Guinea	warm	0.139	0.303	0.359	0.199	0.663	0.357	0.168	0.297	0.178	0.535
Spirophorida	Tetillidae	<i>Tetilla arb</i>	AY561944	USA, California, Carmel	cold	0.146	0.295	0.353	0.206	0.647	0.360	0.168	0.279	0.192	0.552
Verongida	Aplysiniellidae	<i>Aplysinella</i> sp.	AY561865	Papua New Guinea	warm	0.157	0.297	0.343	0.203	0.641	0.394	0.144	0.257	0.204	0.599
Verongida	Aplysinidae	<i>Aplysina fistularis</i>	AY561864	Panama Caribbean	warm	0.157	0.299	0.342	0.202	0.641	0.391	0.151	0.257	0.201	0.592
Verongida	Pseudoceratimidae	<i>Pseudoceratina</i> sp.	AY561956	Papua New Guinea	warm	0.157	0.294	0.334	0.215	0.628	0.404	0.126	0.249	0.221	0.625