

## Supplementary Tables and Figures

**Supplementary Table S1.** Detailed annotation information of *Agelas nakamura* mitochondrial genome.

Feature	Nucleotide size (bp)	Position	Product	Start codon	Stop codon
cox1	1569	1-1569	cytochrome c oxidase subunit 1	ATG	TAA
tRNA-Ser (TGA)	80	1647-1726	tRNA-Ser		
tRNA-Ala (TGC)	72	1740-1811	tRNA-Ala		
tRNA-Cys (GCA)	72	1850-1921	tRNA-Cys		
nad1	975	2022-2996	NADH dehydrogenase subunit 1	ATG	TAG
tRNA-Leu (TAA)	71	3083-3153	tRNA-Leu		
tRNA-Leu (TAG)	72	3265-3336	tRNA-Leu		
tRNA-Tyr (GTA)	72	3359-3430	tRNA-Tyr		
tRNA-Met (CAT)	72	3439-3510	tRNA-Met		
tRNA-Met (CAT)	71	3537-3607	tRNA-Met		
nad2	1461	3669-5129	NADH dehydrogenase subunit 2	ATG	TAA
nad5	1866	5170-7035	NADH dehydrogenase subunit 5	ATG	TAA
tRNA-Ile (GAT)	71	7077-7147	tRNA-Ile		
tRNA-Phe (GAA)	71	7186-7256	tRNA-Phe		
rns	1275	7257-8531	s-rRNA		
tRNA-Gly (TCC)	72	8532-8603	tRNA-Gly		
tRNA-Val (TAC)	73	8605-8677	tRNA-Val		
rnl	2587	8784-11370	l-rRNA		
cox2	744	11782-12525	cytochrome c oxidase subunit 2	ATG	TAA
atp8	285	12575-12859	ATP synthase F0 subunit 8	ATG	TAA
atp6	735	12987-13721	ATP synthase F0 subunit 6	GTG	TAA
tRNA-Arg (TCT)	73	13845-13917	tRNA-Arg		
cox3	786	13994-14779	cytochrome c oxidase subunit 3	ATG	TAA
tRNA-Gln (TTG)	73	14838-14910	tRNA-Gln		
tRNA-Trp (TCA)	71	14950-15020	tRNA-Trp		
cob	1146	15092-16237	cytochrome b	ATG	TAG
tRNA-Thr (TGT)	72	16330-16401	tRNA-Thr		
atp9	237	16549-16785	ATP synthase F0 subunit 9	ATG	TAA
tRNA-Ser (GCT)	84	16990-17073	tRNA-Ser		
tRNA-Lys (TTT)	73	17101-17173	tRNA-Lys		
tRNA-Asn (GTT)	71	17239-17309	tRNA-Asn		
tRNA-Met (CAT)	71	17377-17447	tRNA-Met		
tRNA-His (GTG)	73	17456-17528	tRNA-His		
tRNA-Pro (TGG)	72	17569-17640	tRNA-Pro		
nad4	1470	17661-19130	NADH dehydrogenase subunit 4	ATG	TAA
tRNA-Glu (TTC)	73	19184-19256	tRNA-Glu		

nad6	573	19254-19826	NADH dehydrogenase subunit 6	GTG	TAA
nad3	357	19919-20275	NADH dehydrogenase subunit 3	ATG	TAG
tRNA-Arg (TCG)	71	20474-20544	tRNA-Arg		
nad4L	300	20545-20844	NADH dehydrogenase subunit 4L	ATG	TAA

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**Supplementary Table S2.** Detailed information of 38 Demospongiae sponge mitochondrial genomes for phylogenetic analysis.

Accession	Organism	Order	Superfamily	Family	Genus	Length	AT%
OQ363829	<i>Agelas nakamurai</i>	Agelasida	Agelasida	Agelasidae	Agelas	20885	62.4
NC_010213.1	<i>Agelas schmidtii</i>	Agelasida	Agelasida	Agelasidae	Agelas	20360	61.7
NC_008944.1	<i>Amphimedon queenslandica</i>	Haplosclerida	Haplosclerida	Niphatidae	Amphimedon	19960	56.1
NC_010203.1	<i>Aplysina fulva</i>	Verongiida	Verongiida	Aplysinidae	Aplysina	19620	67.1
NC_053937.1	<i>Aplysina gerardogreeni</i>	Verongiida	Verongiida	Aplysinidae	Aplysina	19620	67.2
NC_006894.1	<i>Axinella corrugata</i>	Axinellida	Axinellida	Axinellidae	Axinella	25610	57.1
JQ302310.1	<i>Baikalospongia intermedia</i>	Spongillida	Spongillida	Lubomirskiidae	Baikalospongia	28227	58.8
NC_031192.1	<i>Cacospongia mycofijiensis</i>	Dictyoceratida	Dictyoceratida	Thorectidae	Cacospongia	16227	62.9
NC_010206.1	<i>Callyspongia plicifera</i>	Haplosclerida	Haplosclerida	Callyspongiidae	Callyspongia	18846	64.3
NC_010208.1	<i>Chondrilla aff. nucula CHOND</i>	Chondrillida	Chondrillida	Chondrillidae	Chondrilla	19282	66.4
NC_010198.1	<i>Cinachyrella kuekenthali</i>	Tetractinellida	Tetractinellida	Tetillidae	Cinachyrella	18089	64
NC_069646.1	<i>Cliona patera</i>	Clionaida	Clionaida	Clionaidae	Cliona	19133	67
NC_027520.1	<i>Crella elegans</i>	Poecilosclerida	Poecilosclerida	Crellidae	Crella	18543	63.9
NC_010210.1	<i>Ectyoplasia ferox</i>	Axinellida	Axinellida	Raspailiidae	Ectyoplasia	18312	69.5
NC_010202.1	<i>Ephydatia muelleri</i>	Spongillida	Spongillida	Spongillidae	Ephydatia	23929	59.6
NC_016431.1	<i>Eunapius subterraneus</i>	Spongillida	Spongillida	Spongillidae	Eunapius	24850	59.2
NC_006990.1	<i>Geodia neptuni</i>	Tetractinellida	Tetractinellida	Geodiidae	Geodia	18020	66.5
NC_037391.1	<i>Halichondria okadai</i>	Suberitida	Suberitida	Halichondriidae	Halichondria	20722	64.2
NC_014876.1	<i>Halisarca harmelini</i>	Dendroceratida	Dendroceratida	Halisarcidae	Halisarca	20591	65.4
EU237484.1	<i>Hippospongia lachne</i>	Dictyoceratida	Dictyoceratida	Spongiidae	Hippospongia	16755	63
NC_021422.1	<i>Hyattella sinuosa</i>	Dictyoceratida	Dictyoceratida	Spongiidae	Hyattella	16422	62.7
NC_022450.1	<i>Hymeniacidon sinapium</i>	Suberitida	Suberitida	Halichondriidae	Hymeniacidon	23435	65.5
NC_010216.1	<i>Igernella notabilis</i>	Dendroceratida	Dendroceratida	Dictyodendrillidae	Igernella	20310	72
NC_010207.1	<i>Iotrochota birotulata</i>	Poecilosclerida	Poecilosclerida	Iotrochotidae	Iotrochota	19112	64.4
NC_013662.1	<i>Ircinia strobilina</i>	Dictyoceratida	Dictyoceratida	Irciniidae	Ircinia	16414	63
NC_013760.1	<i>Lubomirskia baikalensis</i>	Spongillida	Spongillida	Lubomirskiidae	Lubomirskia	28958	58.5
KR911863.1	<i>Petrosia ficiformis</i>	Haplosclerida	Haplosclerida	Petrosiidae	Petrosia	18887	63.7

NC_025335.1	<i>Poecillastra laminaris</i>	Tetractinellida	Tetractinellida	Vulcanellidae	Poecillastra	18413	64.3
NC_023834.1	<i>Polymastia littoralis</i>	Polymastiida	Polymastiida	Polymastiidae	Polymastia	21719	69
NC_010209.1	<i>Ptilocaulis walpersi</i>	Axinellida	Axinellida	Axinellidae	Ptilocaulis	18865	71.4
NC_018360.1	<i>Rezinkovia echinata</i>	Spongillida	Spongillida	Lubomirskiidae	Rezinkovia	28614	58.7
NC_010496.1	<i>Suberites domuncula</i>	Suberitida	Suberitida	Suberitidae	Suberites	26300	64.5
NC_065020.1	<i>Terpios hoshinota</i>	Suberitida	Suberitida	NA	Terpios	20504	63.5
NC_006991.1	<i>Tethya actinia</i>	Tethyida	Tethyida	Tethyidae	Tethya	19565	70.6
MN587873.1	<i>Thoosa mismalolli</i>	Tetractinellida	Tetractinellida	Thoosidae	Thoosa	19019	66.7
NC_010204.1	<i>Topsentia ophiraphidites</i>	Suberitida	Suberitida	NA	Topsentia	19763	66.3
NC_010218.1	<i>Vaceletia</i> sp.	Dictyoceratida	Dictyoceratida	Verticillitidae	Vaceletia	20658	70.6
NC_010211.1	<i>Xestospongia muta</i>	Haplosclerida	Haplosclerida	Petrosiidae	Xestospongia	18990	66.1

**Supplementary Table S3.** Information on 17 mitochondrial genomes of Demospongiae sponges for evolutionary analysis.

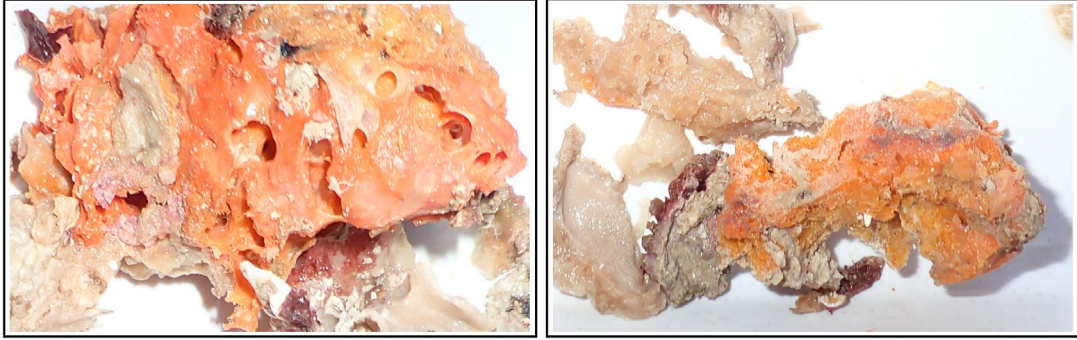
Accession	Organism	Order	Superfamily	Family	Genus	Length	AT%
OQ363829	<i>Agelas nakamurai</i>	Agelasida	Agelasida	Agelasidae	Agelas	20885	62.4
EU237475.1	<i>Agelas schmidtii</i>	Agelasida	Agelasida	Agelasidae	Agelas	20360	61.7
EU237474.1	<i>Amphimedon compressa</i>	Haplosclerida	Haplosclerida	Niphatidae	Amphimedon	18564	59.2
EU237477.1	<i>Callyspongia plicifera</i>	Haplosclerida	Haplosclerida	Callyspongiidae	Callyspongia	18846	64.3
EU237479.1	<i>Cinachyrella kuekenthali</i>	Tetractinellida	Tetractinellida	Tetillidae	Cinachyrella	18089	64
KR911862.1	<i>Crella elegans</i>	Poecilosclerida	Poecilosclerida	Crellidae	Crella	18543	63.9
EU237480.1	<i>Ectyoplasia ferox</i>	Axinellida	Axinellida	Raspailiidae	Ectyoplasia	18312	69.5
EU237481.1	<i>Ephydatia muelleri</i>	Spongillida	Spongillida	Spongillidae	Ephydatia	23929	59.6
AY320032.1	<i>Geodia neptuni</i>	Tetractinellida	Tetractinellida	Geodiidae	Geodia	18020	66.5
MH756603.1	<i>Halichondria panicea</i>	Suberitida	Suberitida	Halichondriidae	Halichondria	19571	63.2
EU237486.1	<i>Iotrochota birotulata</i>	Poecilosclerida	Poecilosclerida	Iotrochotidae	Iotrochota	19112	64.4
KR911863.1	<i>Petrosia ficiformis</i>	Haplosclerida	Haplosclerida	Petrosiidae	Petrosia	18887	63.7
EU237488.1	<i>Ptilocaulis walpersi</i>	Axinellida	Axinellida	Axinellidae	Ptilocaulis	18865	71.4
AY320033.1	<i>Tethya actinia</i>	Tethyida	Tethyida	Tethyidae	Tethya	19565	70.6
MN587873.1	<i>Thoosa mismalolli</i>	Tetractinellida	Tetractinellida	Thoosidae	Thoosa	19019	66.7
EU237482.1	<i>Topsentia ophiraphidites</i>	Suberitida	Suberitida	NA	Topsentia	19763	66.3
EU237490.1	<i>Xestospongia muta</i>	Haplosclerida	Haplosclerida	Petrosiidae	Xestospongia	18990	66.1

**Supplementary Table S4.** Best partition scheme and model for BI phylogenetic analysis.

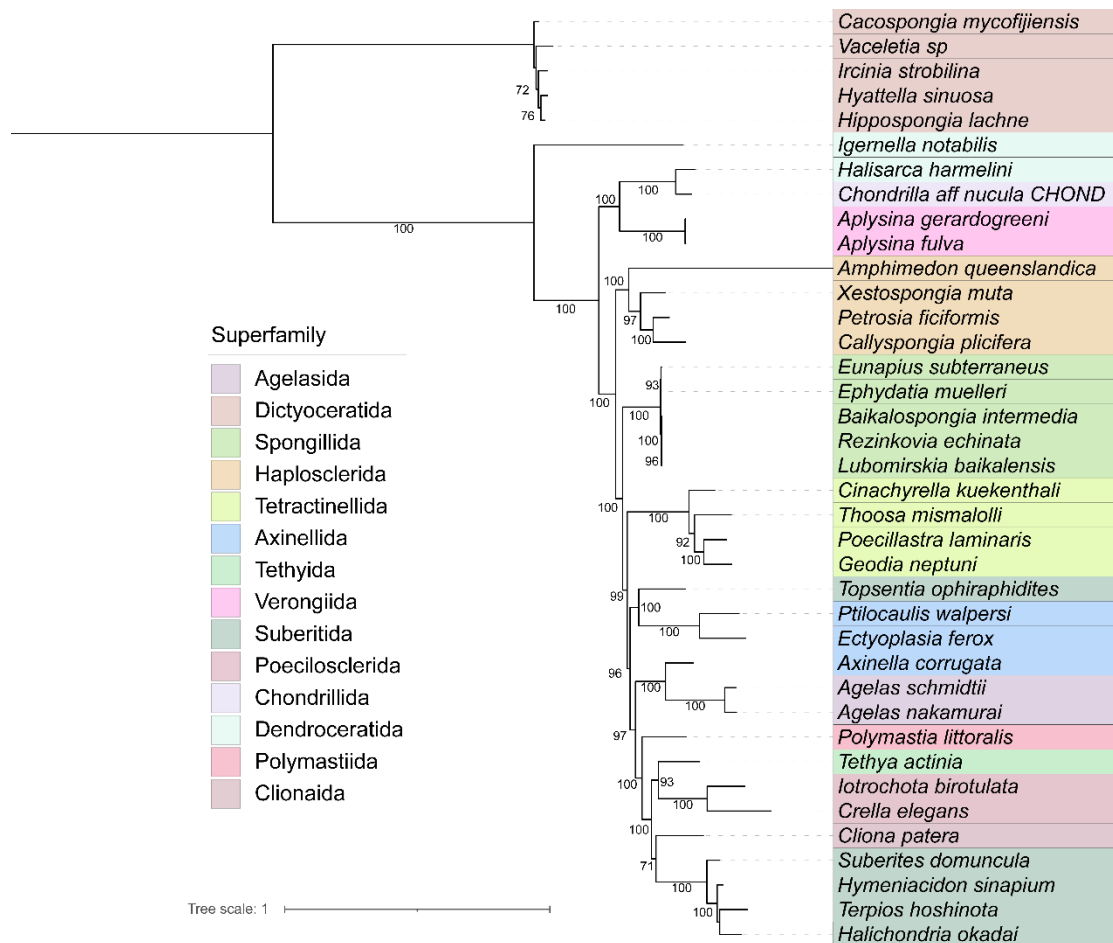
Subset partitions	Best model
P1: (atp6, cox2, cox3, nad1)	GTR+F+I+G4
P2: (atp8)	HKY+F+I+G4
P3: (atp9)	GTR+F+I+G4
P4: (cox1)	GTR+F+I+G4
P5: (cytb, nad2)	GTR+F+I+G4
P6: (nad3, nad4L, nad4, nad5, nad6)	GTR+F+I+G4

**Supplementary Table S5.** Best partition scheme and model for ML phylogenetic analysis.

Subset partitions	Best model
P1: (atp6, cox3)	GTR+F+R4
P2: (atp8)	TPM3u+F+I+I+R3
P3: (atp9)	TIM+F+I+G4
P4: (cox1)	TIM2+F+I+G4
P5: (cox2, nad1, nad3, nad4, nad5, nad6)	GTR+F+I+I+R4
P6: (cytb, nad2, nad4L)	GTR+F+I+I+R4

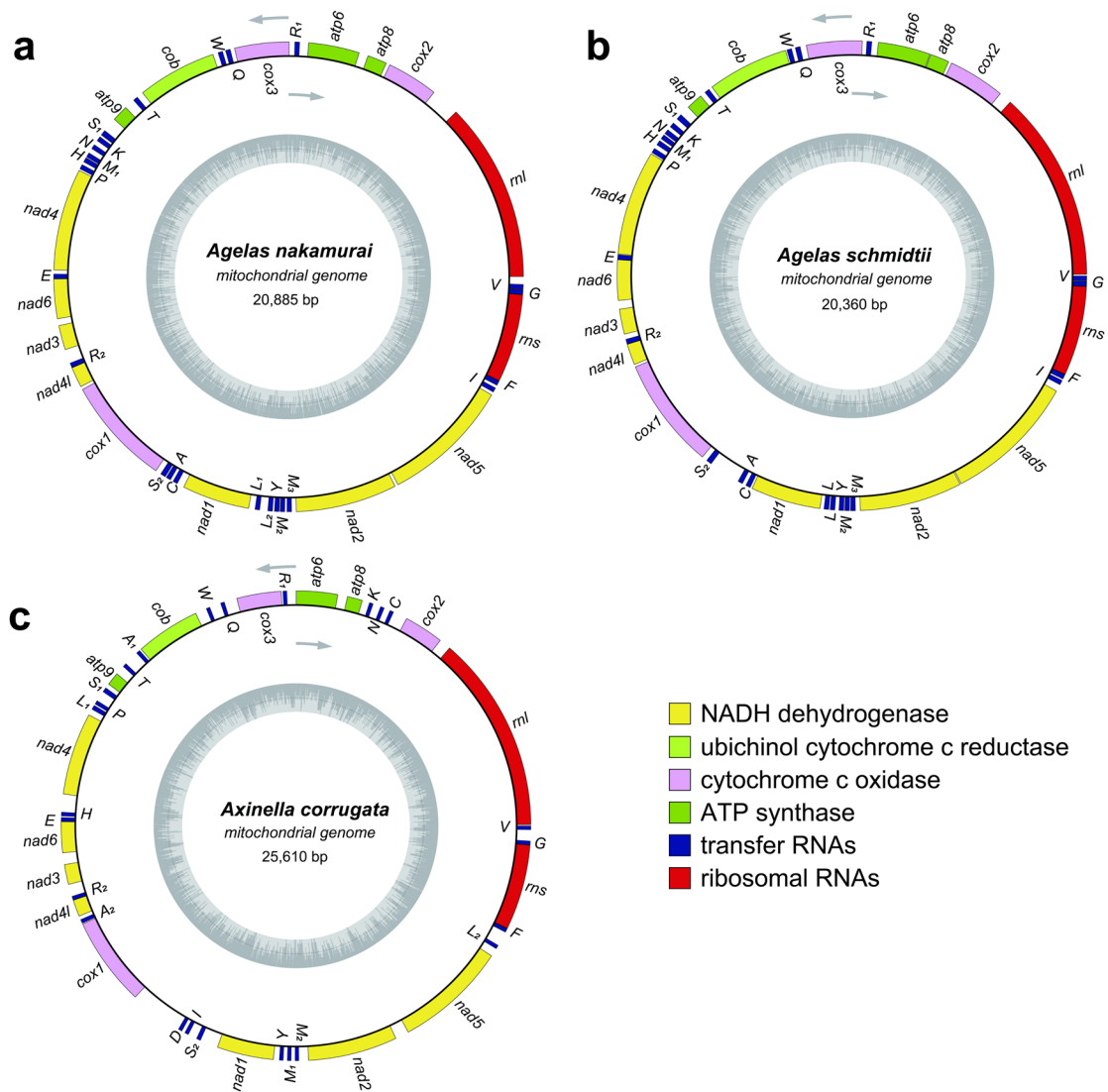


**Supplementary Figure S1.** Sponge tissue sample placed on the dissection tray.

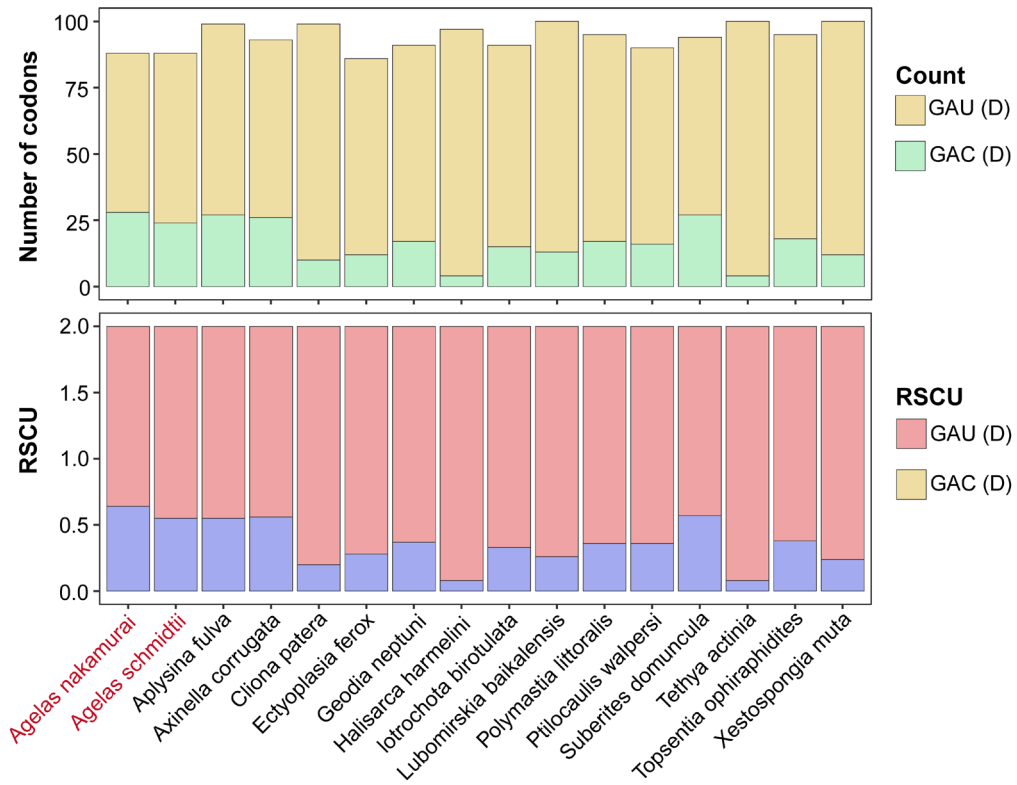


**Supplementary Figure S2.** ML phylogenetic tree of 38 Demospongiae sponges constructed using mitochondrial genome PCGs. *Hippospongia lachne*, *Hyattella sinuosa*, *Ircinia strobilina*, *Vaceletia* sp. and *Cacospongia mycofijiensis* were used as outgroups for tree construction. The numbers adjacent to the nodes indicate the percent posterior probability values, with only values exceeding 70 being shown. Leaf labels are color-coded to represent different superfamilies.





**Supplementary Figure S3.** Mitogenome maps of *Agelas nakamurai*, *Agelas schmidtii*, and *Axinella corrugata*. (a), (b) and (c) represent the complete mitogenome maps of *Agelas nakamurai*, *Agelas schmidtii*, and *Axinella corrugata*, respectively. Distinct colors correspond to various genes. The tRNA genes are denoted by single-letter abbreviations representing the accepted amino acid. The gray inner ring represents the GC content of the sequence.



**Supplementary Figure S4.** Number of aspartic acid codons and RSCU in 16 Demospongiae mitochondrial PCGs.