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First Complete Mitochondrial Genome Sequence from a Box Jellyfish Reveals a Highly Fragmented Linear Architecture and Insights into Telomere Evolution

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Abstract

Animal mitochondrial DNAs (mtDNAs) are typically single circular chromosomes, with the exception of those from medusozoan cnidarians (jellyfish and hydroids), which are linear and sometimes fragmented. Most medusozoans have linear monomeric or linear bipartite mitochondrial genomes, but preliminary data have suggested that box jellyfish (cubozoans) have mtDNAs that consist of many linear chromosomes. Here, we present the complete mtDNA sequence from the winged box jellyfish *Alatina moseri* (the first from a cubozoan). This genome contains unprecedented levels of fragmentation: 18 unique genes distributed over eight 2.9- to 4.6-kb linear chromosomes. The telomeres are identical within and between chromosomes, and recombination between subtelomeric sequences has led to many genes initiating or terminating with sequences from other genes (the most extreme case being 150 nt of a ribosomal RNA containing the 5′ end of *nad2*), providing evidence for a gene conversion–based model of telomere evolution. The silent-site nucleotide variation within the *A. moseri* mtDNA is among the highest observed from a eukaryotic genome and may be associated with elevated rates of recombination.

Key words: *Alatina moseri*, Cnidaria, Cubozoa, gene conversion, inverted repeat, nucleotide diversity.

Introduction

The archetypal animal mitochondrial DNA (mtDNA) is a 15- to 20-kb circular molecule (Boore 1999). Medusozoan cnidarians (jellyfish and hydroids) are among the few metazoans whose mtDNAs depart significantly from this "traditional" architecture. Staurozoans, scyphozoans, and most hydrozoans have linear monomeric mitochondrial genomes with lengths of ~16 kb; certain *Hydra* species have mtDNAs that are segmented into two 8-kb linear chromosomes; and all investigated members of the Cubozoa (box jellyfish) have mitochondrial genomes that are segmented into several linear molecules of ≤4 kb (Warrior and Gall 1985; Bridge et al. 1992; Ender and Schierwater 2003; Kayal and Lavrov 2008; Voigt et al. 2008; Kayal et al. 2011).

Although nearly complete mtDNA data are available from a diversity of medusozoans (Kayal et al. 2011), whole mitochondrial genome sequences, including telomeres, exist for just three: the scyphozoan *Aurelia aurita* and the hydrozoans *Hydra oligactis* and *H. magnipapillata* (Shao et al. 2006; Kayal and Lavrov 2008; Voigt et al. 2008). The telomeres from each of these mitochondrial genomes form an inverted repeat (IR), and in *H. oligactis* and *H. magnipapillata*, duplicate genes and pseudogenes are found in the subtelomeric regions. Medusozoans, therefore, provide a unique opportunity for understanding the evolution of linear chromosomes, telomeres, and mtDNA structural stability. To address all three of these topics, we present the complete mitochondrial genome sequence from

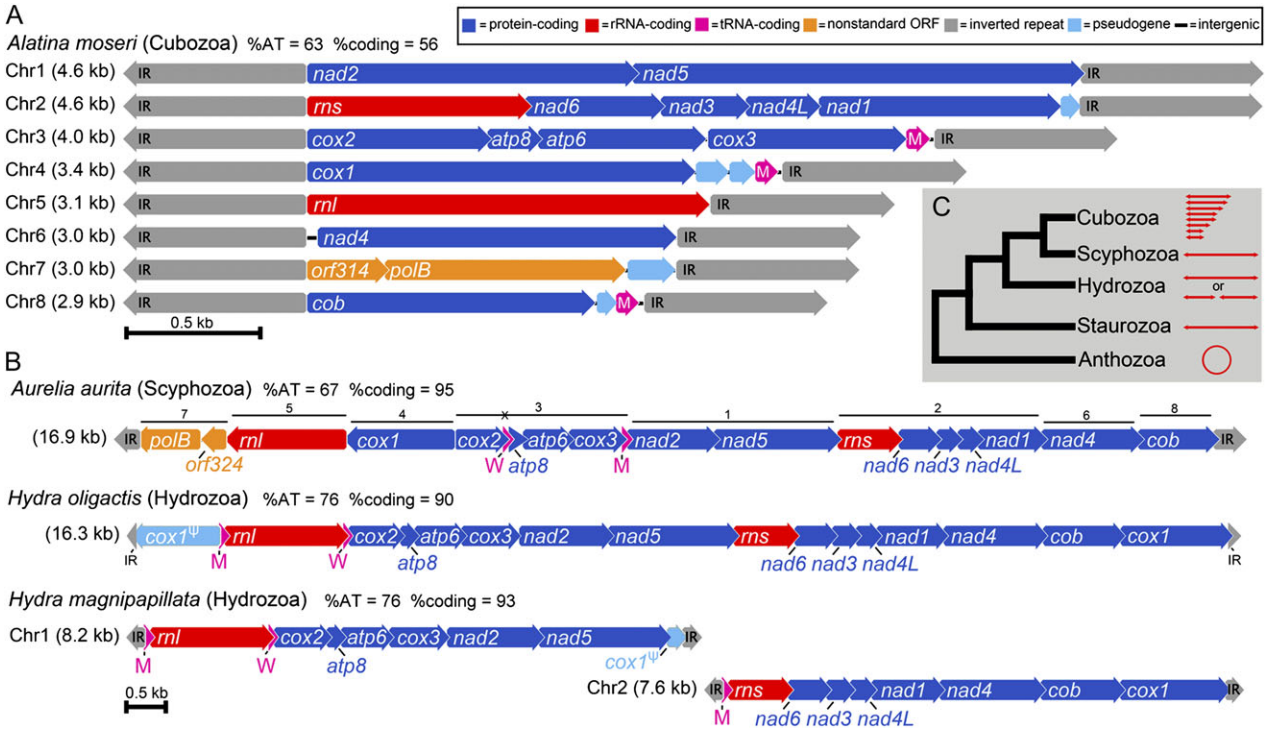

Fig. 1.—Mitochondrial genome architectural diversity within Medusozoa. (*A* and *B*) Genetic maps of completely sequenced medusozoan mtDNAs. The genomic AT content and coding composition are indicated next to the taxon name. mtDNA lengths are shown adjacent to the mitochondrial chromosomes (Chr). Synteny between the mtDNA of *Alatina moseri* and that of *Aurelia aurita* is marked with horizontal black lines, which have labels corresponding to *A. moseri* chromosome numbers. See figure 2 for details on the *A. moseri* mtDNA pseudogenes. (*C*) Hypotheses on the evolution of medusozoan mtDNA architecture. Phylogenetic relationships based on Collins et al. (2006, 2008) and Cartwright et al. (2008). Note: cnidarian mtDNA protein-coding genes are translated using the minimally derived genetic code (UAG = Tryptophan).

the winged box jellyfish *Alatina moseri* (the first from a cubozoan). This genome has a highly fragmented linear structure and inflated levels of silent-site genetic diversity; moreover, subtelomeric recombination has changed the actual proteins encoded in the genome, causing genes to initiate or terminate with short regions of subtelomeric genes from other chromosomes.

Eighteen Genes: Eight Chromosomes

The *A. moseri* mitochondrial genome contains 18 unique genes distributed over eight linear-mapping chromosomes, which range from 2.9–4.6 kb. The architectural and compositional features of this genome and how they compare with the mtDNAs from other medusozoans are shown in figure 1*A* and *B*, respectively. *Alatina moseri* has among the most fragmented mitochondrial genomes observed from animals (for other examples, see Suga et al. 2008; Shao et al. 2009; Cameron et al. 2011). The complete mitochondrial genome assembly for *A. moseri* supports earlier analyses of this taxon and six other cubozoan species (*Carukia barnesi*, *Carybdea marsupialis*, *Carybdea xaymacana*, *Chironex fleckeri*, *Chiropsalmus quadrumanus*, and *Tripedalia cystophora*), which were indicative of highly subdivided linear mtDNA architectures (Ender and Schierwater 2003; Kayal et al. 2011). It also reinforces the hypothesis that within Cnidaria, the shift from a single linear mtDNA to a fragmented one occurred at least twice: in the Cubozoa and within the Hydrozoa (fig. 1*C*; Voigt et al. 2008; Kayal et al. 2011). Outside of medusozoans, linear fragmented mtDNAs have been observed in various protists, including green algae (Borza et al. 2009; Smith et al. 2010), fungi (Valach et al. 2011), and the ichthyosporean *Amoebidium parasiticum* (Burger et al. 2003)—one of the closest unicellular relatives of animals (Lang et al. 2002).

The mtDNA gene complement of *A. moseri* closely parallels those from other medusozoans (fig. 1*B*). It includes 16 standard mitochondrial genes, representing 13 proteins, 2 ribosomal RNAs (rRNAs), and 1 transfer RNA (tRNA) (for secondary structure diagrams of the functional RNAs, see supplementary fig. S1, Supplementary Material online), and two nonstandard ones: *polB*, which codes for a putative beta DNA polymerase, and *orf314*, which may have DNA-binding properties (Kayal et al. 2011). *polB* and *orf314* are found in other medusozoan mtDNAs, except those of hydroidolinan hydrozoans, and are believed to have originated from a mitochondrial plasmid (Shao et al. 2006). Both of these genes are located on chromosome 7, where no other

full-length gene is encoded, which is interesting when considering that they are thought to have arisen from a selfish element. Only one type of tRNA is encoded in the *A. moseri* mitochondrial genome: $trnM_{CAU}$. It is present in three identical copies, each located on a different chromosome, and has characteristics that suggest a role in initiation rather than elongation. Although all cnidarians have reduced mitochondrial tRNA-coding suites, *A. moseri* is the first medusozoan known to lack an mtDNA-encoded $trnW_{UCA}$. In the mtDNAs of octocorals, the absence of a mitochondrial $trnW_{UCA}$ is associated with a complete lack of "UGA" codons. In *A. moseri*, however, half of the tryptophan residues within protein-coding mtDNA are represented by UGA codons, implying that either a nucleus-encoded mitochondrial-targeted $trnW_{UCA}$ or nonstandard wobble properties of an imported $trnW_{UGG}$ is compensating for the lost mitochondrial version of the gene. A 20-nt sequence that can be folded into a stem-loop structure was found in the telomeric regions immediately adjacent to the coding DNA (supplementary fig. S2, Supplementary Material online); putative stem-loop structures have been identified in the noncoding regions of other medusozoan mtDNAs and were proposed to be potential control regions (Kayal et al. 2011).

A Gene Conversion–Based Model for Mitochondrial Telomere Evolution

The 16 telomeres of the eight *A. moseri* mitochondrial chromosomes are identical in sequence and found in opposing orientations so they make up an IR (fig. 1*A*). Telomeres arranged as IRs are a hallmark of linear organelle genomes (Nosek et al. 2004) and are found in the three other medusozoan mtDNAs for which telomeric data are available (fig. 1*B*). However, the subtelomeric regions of the *A. moseri* mtDNA also contain substantial amounts of other repeated sequence; in this case, fragments of genes found on other mitochondrial chromosomes (fig. 2). For example, based on our annotations, the beginning of the *rnl* gene is found within the 5′ ends and adjoining intergenic regions of six different protein-coding genes, meaning that the first 15–150 nt of these loci are identical and the amino termini of these proteins are presumably translated from a rRNA (for *rnl* secondary structure diagram, see supplementary fig. S1, Supplementary Material online), a statement supported by both the conserved amino acid sequence of these genes and the *rnl* secondary structure model (supplementary fig. S1, Supplementary Material online), but protein sequencing data will be needed to confirm this. Similarly, the 3′ end of *cox3* is identical in sequence, but in a different reading frame, to the 3′ end of *cob*, and this same *cox3*/*cob* segment and an additional portion of *cob* are found in the intergenic DNA beside *cox1* (fig. 2). Even more extreme, the 3′ end of *nad4* contains tracts that show sequence identity to *nad1*, *trnM*, and *rnl*. Fragmented and sometimes duplicate genes are present in the subtelomeric mtDNA of *H. oligactis* and *H. magnipapillata* (fig. 1*B*) but to a much lesser extent than in *A. moseri*; and a cursory scan of other linear DNAs reveals that this is a reoccurring, but poorly understood, feature of organelle telomeres, including those of nucleomorph DNAs and mitochondrial plasmids (table 1) (Burger et al. 2000; Handa 2008; Moore and Archibald 2009; Hikosaka et al. 2010; Pérez-Brocal et al. 2010).

The inverted architecture of the *A. moseri* mitochondrial telomeres and partial duplication of adjacent coding and noncoding regions (fig. 2) resemble the expansion of the large IR region of plastid genomes (Plunkett and Downie 2000). One proposed mechanism for plastid genome IR expansion is based on gene conversion (Goulding et al. 1996). In this model, recombination between two IRs allows for the branch migration of a Holliday junction across the IR boarder into nonidentical sequence; the branch migration stalls but not before a short stretch of heteroduplex DNA is formed. Resolution of the heteroduplex occurs by sequence correction against either of the two strands and can lead to an expansion (or reduction) of the IR (Goulding et al. 1996). Similarly, in telomerase-lacking cells of the yeast *Kluyveromyces lactis*, it was shown that a nuclear DNA marker present at only one telomere can spread to all other nuclear telomeres in the cell via subtelomeric gene conversion (McEachern and Iyer 2001); it was also shown that these same cells can maintain the lengths of their telomeres through recombination (McEachern and Blackburn 1996), which is one of the primary strategies for the lengthening of telomeres in telomerase-independent systems, including mitochondria (Nosek et al. 2004; Tomaska and Nosek 2009). When the *A. moseri* telomeres and their abutting loci are aligned (fig. 2), it is clear how gene conversion could have generated the subtelomeric regions. Subtelomeric gene conversion can also explain why, as a whole, linear DNAs with terminal IRs tend to have duplicate genes or gene fragments at their ends (table 1). Moreover, in most systems, gene conversion is biased toward GC (Marais 2003), which may help explain why in *A. moseri* the sequence that appears to be most frequently involved in conversion events (*rnl*) is also among the more GC-rich genes in the mitochondrial genome. Overall, in the subtelomeric mtDNA of *A. moseri*, a recombination-based mechanism of telomere maintenance appears to have been taken to an extreme so that fragments of genes introduced to a chromosome end by recombination have actually become embedded in the coding regions of preexisting genes.

Unprecedented Levels of mtDNA Diversity

We measured the mitochondrial genetic diversity between three different isolates of *A. moseri*: two from Hawaii

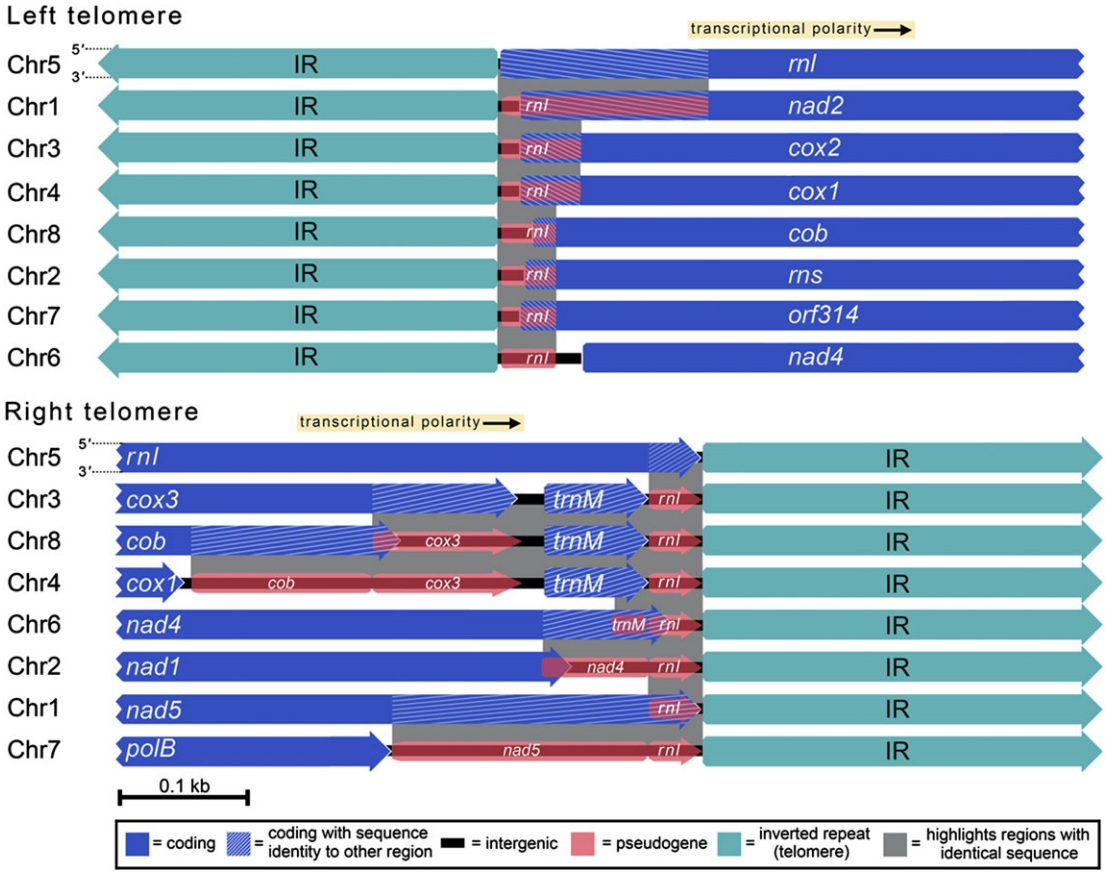

Fig. 2.—Alignment and architecture of the *Alatina moseri* mitochondrial telomeric and subtelomeric regions. The *Alatina moseri* mitochondrial genome is divided into eight chromosomes (Chr), each of which has two telomeres: left and right (based on genetic map in fig. 1*A*). The telomeres (turquoise; length = 750 nt; not to scale) are identical in sequence and found in opposing orientations so that they make up an IR. The subtelomeric regions are composed of coding (dark blue) and noncoding (black) regions and contain fragments of genes (red) from neighboring chromosomes. Gray highlighting denotes sequence identity between different subtelomeric regions; when the sequence identity includes coding DNA, these regions are striped.

(collected in the same location and on the same evening) and one from Australia. All three isolates had identical mitochondrial genome architectures, including gene and telomere organizations. Their mtDNA sequences, however, differed substantially, especially at silent sites (table 2). The average number of nucleotide differences per site (π) between the mtDNAs from the two Hawaiian isolates was 2.5%, rising to 5% at telomeric positions and 10% at synonymous sites ($\pi_{synonymous}$ varied from 6.5% (*atp8*) to 15.5% (*nad6*) among protein-coding loci). Similar amounts of silent-site variation were observed when the almost complete mtDNA sequence of the Australian specimen was added to the analysis ($\pi_{synonymous}$ = 7.9%; $\pi_{telomere}$ = 4.4%), meaning that on average the two Hawaiian mtDNAs were more different from each other than they were from that of the Australian isolate. Mean diversity at the more functionally constrained sites (i.e., amino acid replacement and rRNA-coding positions) was between 0.3% and 1.8% for both the Hawaiian and combined data sets (table 2).

Animal mitochondrial genomes are renowned for having high levels of within-species silent-site diversity (largely due to high substitution rates) (Bazin 2006; Lynch et al. 2006; Piganeau and Eyre-Walker 2009), but the *A. moseri* mtDNA is in the upper extreme of what has been documented, making it among the most genetically diverse eukaryotic genomes observed to date—see Lynch and Conery (2003) for a compilation. Recombination rates often scale positively with genetic diversity and mutation rates (Eyre-Walker 1993; Nachman 2001; Hellmann et al. 2003), which is significant because sequence data from the *A. moseri* mitochondrial telomeres and subtelomeres suggest that these regions are recombinogenic, although the high levels of variation found in other parts of the genome are probably associated with a high mutation rate. An inflated π_{silent} can also result from a large effective genetic population size. In this context, it is noteworthy that *Alatina*, unlike most box jellyfish, can be found at considerable ocean depths (e.g., Morandini 2003) and is thought to only come to shallow water when breeding (e.g., Arneson and Cutress 1976;

Table 1
Examples of Linear Mitochondrial Genomes with Fragmented or Duplicate Genes Are Their Termini

Taxon	Lineage	Genome Size (kb)	Number of Chromosomes	Telomere Architecture	Genes or Pseudogenes Located at Both Ends	Reference
Alatina moseri	Medusozoa	29	8	IR	*cob*, *cox3*, *nad4*, *nad5*, *rnl*, *trnM*	Present study
Candida viswanathii	Fungi	39	1	IR	*atp6*, *trnM*	Valach et al. (2011)
Hydra magnipapillata	Medusozoa	16	2	IR	*cox1*, *trnM*	Voigt et al. (2008)
Hydra oligactis	Medusozoa	16	1	IR	*cox1*	Kayal and Lavrov (2008)
Ochromonas danica	Stramenopiles	41	1	IR	*nad11*, *trnC*, *trnI*, *trnM*, *trnQ*	GenBank accession NC_002571
Proteromonas lacertae	Stramenopiles	49	1	IR	36 genes (12 proteins, 22 tRNAs, and 2 rRNAs)	Pérez-Brocal et al. (2010)
Tetrahymena pyriformis	Ciliophora	47	1	IR	*rnl*-a, *rnl*-b, *trnL*, *trnY*	Burger et al. (2000)
Theileria equi	Alveolata	8	1	IR	*cox3*, *rnl*-d, *rnl*-e	Hikosaka et al. (2010)

Yanagihara et al. 2002). Moreover, previous genetic diversity analyses of *rnl* revealed no geographic structure between Hawaiian and Australian specimens, suggesting that they constitute a single large population (Bentlage et al. 2010).

Other medusozoans harbor moderate amounts of intra- and interspecific mtDNA variation (Cunningham and Buss 1993; Dawson and Jacobs 2001; Schroth et al. 2002). Anthozoan cnidarians, however, are one of the few animal lineages that contain very little intraspecific mtDNA diversity (Shearer et al. 2002; Hellberg 2006). Unlike medusozoans and most other animals, octocorallian anthozoans have a mitochondrial-encoded *msh1* gene (a homologue of the bacterial DNA repair gene *mutS*), which may be linked to their low mtDNA substitution rates (van Oppen et al. 1999; Abdelnoor et al. 2006). Similarly, in land plants, *msh1* is allied with low rates of mtDNA evolution (Davila et al. 2011). Moreover, *msh1* mutants of *Arabidopsis thaliana* have inflated mitochondrial recombination rates as compared with the wild type, and they also show high levels of asymmetrical genetic exchange, resulting from gene conversion (Davila et al. 2011). It has been hypothesized that within the phylum Cnidaria, there was an abrupt shift from slow to fast rates of mtDNA evolution in the stem of Medusozoa (Hellberg 2006), which may be connected to the loss of an *msh1* gene in medusozoans: This gene is absent from all available medusozoan mtDNAs and a blast scan of 6 Giga bases of *A. moseri* nuclear DNA data failed to uncover a nuclear copy of *msh1*. The potential loss of *msh1* in *A. moseri*, and medusozoans as a whole, could explain both the high levels of genetic diversity and subtelomeric gene conversion in this group.

Materials and Methods

Specimens of *A. moseri* (sometimes called *Carybdea alata*) were collected from Waikiki (O'ahu, HI) as previously described (Bentlage et al. 2010). Complete mtDNA sequences were generated for two different Hawaiian specimens: one using Roche 454 (GS FLX Titanium) sequencing (GenBank project SRX017292) and the other with ABI SOLiD

Table 2
mtDNA Diversity between Three Isolates of *Alatina moseri*

	Hawaiian Isolates[a]				Hawaiian and Australian Isolates[a]			
Loci	***N***	***S***	**Indels**	**π (%)**	***N***	***S***	**Indels**	**π (%)**
rRNA-coding genes	2,573	25	6	0.97	1,676	23	5	0.74
Synonymous sites "standard genes"[b]	2,918	298	—	10.20	2,536	300	—	7.90
Synonymous sites (*orf314*, *polB*)	303	25	—	8.25	287	30	—	6.96
Nonsynonymous sites "standard genes"[b]	8,941	30	1	0.34	7,766	43	1	0.40
Nonsynonymous sites (*orf314*, *polB*)	969	17	2	1.75	912	15	0	1.10
Telomeres[c]	742	36	2	4.89	637	41	2	4.36

NOTE.— *N*, number of nucleotide sites (comprises all sites in the nucleotide alignment, including those with indels); *S*, number of polymorphic (i.e., segregating) sites; π, average number of pairwise nucleotide differences per site (calculated with DnaSP v5.10.01; Librado and Rozas 2009); and indels: insertion–deletion events (consecutive indels were counted as a single event).

[a] Two specimens of *A. moseri* were collected from Waikiki, O'ahu, HI (i.e., Hawaiian isolates) and one from Osprey Reef in the Coral Sea, Queensland, Australia. See Bentlage et al. (2010) for details.

[b] Includes all protein-coding genes except *orf314* and *polB*.

[c] Includes only 1 of the 16 telomeres.

sequencing (GenBank project SRX063443). Reads corresponding to mtDNA were mined from the 454 and SOLiD data sets using BlastN (v2.2.25+) (Altschul et al. 1990) with the *A. aurita* mitochondrial genome as a query. Mined reads were assembled with Geneious Pro v5.4.6 (Biomatters Ltd, Auckland, NZ), and then extended using the raw 454 or SOLiD data with CodonCode Aligner v3.7.1.1 (CodonCode Corporation, Dedham, MA). The data from both sequencing platforms independently gave the same assembly results: eight linear-mapping mtDNA chromosomes with 25- to 250-fold coverage. For both the 454 and SOLiD assemblies, there was an abrupt decrease in sequence coverage at the extreme ends of the contigs, which is consistent with these regions representing the ends of the mitochondrial chromosomes—a similar conclusion was made with the assembly of the *H. magnipapillata* mtDNA (Voigt et al. 2008). Standard polymerase chain reaction and Sanger sequencing of DNA isolated from a third specimen of *A. moseri* (from Osprey Reef in the Coral Sea, Queensland, Australia) confirmed the sequence and arrangement of the 454 and SOLiD assemblies. The mtDNA diversity between the different specimens of *A. moseri* was calculated with DnaSP v5.10.01 (Librado and Rozas 2009). The *A. moseri* mitochondrial genome is deposited in GenBank under accession numbers JN642329–JN642344.

Supplementary Material

Supplementary figures S1 and S2 are available at *Genome Biology and Evolution* online (http://www.gbe.oxfordjournals.org/).

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