The complete mitochondrial genome of the intertidal spider (*Desis jiaxiangi*) provides novel insights into the adaptive evolution of the mitogenome and the evolution of spiders

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**Abstract**

**Background:** Although almost all extant spider species live in terrestrial environments, a few species live fully submerged in freshwater or seawater. The intertidal spiders (genus *Desis*) built silk nests within coral crevices can survive submerged in high tides. The diving bell spider, *Argyroneta aquatica*, resides in a similar dynamic environment but exclusively in freshwater. Given the pivotal role played by mitochondria in supplying most energy for physiological activity via oxidative phosphorylation and the environment, herein we sequenced the complete mitogenome of *Desis jiaxiangi* to investigate the adaptive evolution of the aquatic spider mitogenomes and the evolution of spiders.

**Results:** We assembled a complete mitogenome of the intertidal spider *Desis jiaxiangi* and performed comparative mitochondrial analyses of data set comprising of *Desis jiaxiangi* and other 45 previously published spider mitogenome sequences, including that of *Argyroneta aquatica*. We found a unique transposition of *trnL2* and *trnN* genes in *Desis jiaxiangi*. Our robust phylogenetic topology clearly deciphered the evolutionary relationships between *Desis jiaxiangi* and *Argyroneta aquatica* as well as other spiders. We dated the divergence of *Desis jiaxiangi* and *Argyroneta aquatica* to the late Cretaceous at ~ 98 Ma. Our selection analyses detected a positive selection signal in the *nd4* gene of the aquatic branch comprising both *Desis jiaxiangi* and *Argyroneta aquatica*. Surprisingly, *Pirata subpiraticus*, *Hypochilus thorelli*, and *Argyroneta aquatica* each had a higher Ka/Ks value in the 13 PCGs dataset among 46 taxa with complete mitogenomes, and these three species also showed positive selection signal in the *nd6* gene.

**Conclusions:** Our finding of the unique transposition of *trnL2* and *trnN* genes indicates that these genes may have experienced rearrangements in the history of intertidal spider evolution. The positive selection signals in the *nd4* and *nd6* genes might enable a better understanding of the spider metabolic adaptations in relation to different environments. Our construction of a novel mitogenome for the intertidal spider thus sheds light on the evolutionary history of spiders and their mitogenomes.

**Keywords:** Mitogenome, Phylogeny, Evolution, Positive selection, *Desis jiaxiangi*, *Argyroneta aquatica*

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the forces that drive the evolution of the mitochondrial genome (mitogenome) provides crucial information, as this evolution is affected by a range of factors that in turn influence the information content of the genome. The mitogenome of metazoans is a circular double-stranded DNA comprising 37 generally conserved genes, including 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), and 2 ribosomal RNAs (rRNAs; 16S and 12S), which are essential for various mitochondrial functions [9]. Each mitochondrion has its own systems for replication, transcription, and translation. Proteins encoded by the 13 PCGs are related to oxidative phosphorylation, a critical process in producing ATP (adenosine triphosphate) to maintain the energy supply of cells using oxygen and simple sugars [10].

As of April 2020, there were records for 88,254 complete mitogenomes for animals in the NCBI database (https://www.ncbi.nlm.nih.gov/nuclecore/?term=mitochondrion+complete+genome). Of them, 72,151 records were for 6129 vertebrate species and 10,559 records were for 5428 arthropod (invertebrate) species. Compared to vertebrates, however, studies on invertebrate mitogenomes are relatively limited [6].

Spiders are highly diverse and play an important role in various ecosystems. With about 50,000 known species [11], spiders form a distinctive, megadiverse, and ancient clade. Our study provides new mitogenomic resources for up to 19 days [16, 18] or even up to 23 days (unpublished data). This unusual biological characteristic makes Desis spiders amazing. Their ecological habitat, intertidal zones, is one of the most stressful environments on earth, with dynamic changes in salinity, pH, temperature, and oxygen concentrations [21]. Mitochondria play an important role in aerobic respiration through oxidative phosphorylation [22]. However, no mitogenome assembly has been reported for the family Desidae in general or for the genus Desis specifically (as of December 2020). Such studies have the potential to shed light on the molecular mechanisms behind spiders’ adaptation to harsh aquatic environments and their evolution.

Given the critical role played by mitochondria in aerobic respiration and the environment of aquatic spiders, here we explore the adaptive evolution of spider mitogenomes and phylogenetic relationships in spiders. Taking advantage of new sequencing technologies and rapid development in bioinformatics, we sequenced and assembled the complete mitogenome of Desis jiaxiangi Lin, Li & Chen, 2020 from Hainan Island, China [23, 24]. To explore the evolution of aquatic spiders, we combined data from all 45 publicly available spider mitogenomes, including that of the fully freshwater Argyroneta aquatica. We analyzed this large data set to infer the evolutionary relationships and divergence times of spiders. Using the dated phylogenetic tree, we performed selection analyses on each PCG across the 46 spider species, with a focus on the clade containing terrestrial and aquatic (Desis jiaxiangi and Argyroneta aquatica) lineages, to detect potential positive selection acting on this aquatic clade. Our study provides new mitogenomic resources and enhances understanding of the molecular mechanisms behind adaptation to the aquatic environment among intertidal spiders and the evolution of spiders.

Results

Summary of the mitogenome assembly and annotation

The primary mitogenome of Desis jiaxiangi (see Additional file 2: Fig. S1) assembled by MitoZ was about 14,600 bp (Fig. 1). To validate the assembled sequence, we manually revised the sequence with the mitogenomes of four close species (Agelenia silvatica Olinger, 1983, Argyroneta aquatica, Telamonia vlijmi Prószyński, 1984, and Dolomedes angustivirgatus Kishida, 1936) using BLAST. After detailed comparisons, we successfully amplified uncertain regions by PCR with the two pairs of designed primers. We then successfully assembled and annotated the mitogenome of Desis jiaxiangi.

The final complete mitogenome of Desis jiaxiangi (GenBank accession no. MW178198) was 14,610 bp, including 13 PCGs, 22 tRNA genes, 2 rRNA genes, and a noncoding region (D-loop). The majority of...
genes were encoded on the heavy strand; few genes were encoded on the light strand (for more details, see Table 1 and Fig. 1). The GC content of the entire mitogenome was 23%, and the length of the tRNAs ranged from 49 to 99 bp (Table 1).

In the mitogenome of *Desis jiaxiangi*, the initiation codon of most PCGs was ATT (5), ATA (3), TTG (3) or ATG (1); the *cox1* was initiated with non-canonical codon (see Table 1). Conversely, the termination codons were TAA (6), TAG (5), T (2).

**Mitochondrial gene rearrangement**

We revealed gene rearrangements from Mandibulata ancestor to Chelicerata ancestor, Mesothelae, RTA (retrolateral tibial apophysis) clade, and other major clades (Fig. 2). Compared to the gene order of Mandibulata
ancestor, the trnL2 of Chelicerata ancestor and Mesothe-
lae changed its position to a new location between nd1 and trnL1, and also moved from heavy to light strand. More inversion and transpositions were observed in Mygalomorphae (Fig. 2). The lineages in Entelegynae have a major gene order (Fig. 2). Within the RTA clade, Agelena silvatica, Argyroneta aquatica, and Desis jiaxiangi are classified as marronoids (Fernandez et al. [25]; Wheeler et al. [12]). The gene orders found in these three marronoid spider mitogenomes were clearly different from one another. The mitogenome structures of the two aquatic spiders, Argyroneta aquatica and Desis jiaxiangi, were very similar, except for the unique transposition of trnL2 and trnN. In addition, trnN was encoded from the light strand instead of the heavy strand in these two aquatic species. However, compared to their terrestrial

| Gene     | From | To   | Spacer(+)/Overlap(−) | Length(bp) | Start | Stop  | Strand |
|----------|------|------|----------------------|------------|-------|-------|--------|
| tRNA^Met | 1    | 67   | 0                    | 67         |       |       | H      |
| nd2      | 55   | 1014 | −13                  | 960        | ATA   | TAG   | H      |
| tRNA^tp  | 1048 | 1111 | 33                   | 64         |       |       | H      |
| tRNA^sr  | 1078 | 1146 | −34                  | 69         |       |       | L      |
| tRNA^ys  | 1132 | 1230 | −15                  | 99         |       |       | L      |
| cox1     | 1198 | 2739 | −33                  | 1542       | GTA   | TAA   | H      |
| cox2     | 2744 | 3406 | 4                    | 663        | TTG   | TAA   | H      |
| tRNA^ys  | 3403 | 3469 | −4                   | 67         |       |       | H      |
| tRNA^ap  | 3452 | 3516 | −18                  | 65         |       |       | H      |
| atp8     | 3514 | 3657 | −3                   | 144        | ATA   | TAG   | H      |
| atp6     | 3651 | 4319 | −7                   | 669        | ATG   | TAA   | H      |
| cox3     | 4326 | 5111 | 6                    | 786        | TTG   | TAG   | H      |
| trnL2     | 5111 | 5170 | −1                   | 60         |       |       | H      |
| nd3      | 5171 | 5518 | 0                    | 348        | ATT   | TAA   | H      |
| tRNA^ln  | 5519 | 5568 | 0                    | 50         |       |       | H      |
| tRNA^uc(LUR) | 5719 | 5781 | 150                  | 63         |       |       | L      |
| tRNA^NA  | 5787 | 5847 | 5                    | 61         |       |       | H      |
| trnL2     | 5844 | 5892 | −4                   | 49         |       |       | H      |
| trnL2     | 5896 | 5969 | 3                    | 74         |       |       | H      |
| trnL2     | 5939 | 5998 | −31                  | 60         |       |       | H      |
| trnL2     | 5978 | 6031 | −21                  | 54         |       |       | L      |
| nd5      | 6030 | 7670 | −2                   | 1641       | ATT   | TAA   | L      |
| trnL2     | 7658 | 7719 | −13                  | 62         |       |       | L      |
| nd4      | 7720 | 8998 | 0                    | 1,279      | TTG   | T     | L      |
| nd4      | 8952 | 9269 | −47                  | 318        | ATT   | TAA   | L      |
| tRNA^ho  | 9265 | 9319 | −5                   | 55         |       |       | L      |
| nd6      | 9329 | 9754 | 9                    | 426        | ATA   | TAG   | H      |
| tRNA^ae  | 9753 | 9814 | −2                   | 62         |       |       | H      |
| cyt     | 9804 | 10,911 | −11                  | 1,108      | ATT   | T     | H      |
| tRNA^uc(LUR) | 10,935 | 10,993 | 23                  | 59         |       |       | H      |
| tRNA^hu  | 10,993 | 11,060 | −1                  | 68         |       |       | H      |
| nd1      | 11,038 | 11,946 | −23                  | 909        | ATT   | TAG   | L      |
| trnL2     | 11,951 | 12,003 | 4                   | 53         |       |       | L      |
| 16S rRNA   | 12,005 | 13,022 | 1                   | 1,018      |       |       | L      |
| trnL2     | 13,023 | 13,077 | 0                   | 55         |       |       | L      |
| 12S rRNA   | 13,078 | 13,765 | 0                   | 688        |       |       | L      |
| trnL2     | 13,767 | 13,828 | 1                   | 62         |       |       | L      |
| D-loop   | 13,829 | 14,610 | 0                   | 782        |       |       | -      |

1 H and L refer to the heavy (majority) and light (minority) strand, respectively
counterpart *Agelena silvatica*, more significant changes were visible. For example, in the freshwater and intertidal spiders, *trnL2* was inserted after *nd3*; *trnA* and *trnN* were interchanged; *trnS1*, *trnR*, and *trnE* were accordingly located after *trnA*. Unlike the major lineages of Enteleagnae that had *trnN* in the light strand, the freshwater spider *Argyroneta aquatica* had *trnN* in the heavy strand. However, the gene orders of all PCGs were conserved within Araneae, given that gene order changes occurred only in tRNA genes (for more details, see Fig. 2).

**Phylogenetic and divergence time analyses**

The BI and ML phylogenetic trees, based on amino acid sequence data from 13 PCGs derived from the 46 spider mitogenomes (see Additional file 1: Table S1), were identical (see Fig. 3). Both the BI and ML trees strongly supported the monophyly of the suborder Mesothelae, the infraorders Mygalomorphae and Araneomorphae. Both the BI and ML trees also strongly supported the lineage containing Agelenidae, Desidae, and Dictynidae, which belonged to the marronoid clade within Araneae. Our findings support all of the major backbone lineages within Araneae, recovering a deep split between the two suborders, Mesothelae and Opisthothelae (Mygalomorphae and Araneomorphae). The most diverse Araneomorphae lineage encompasses Hypochilidae, Synspermiata, Araneoidea and RTA clade. These evolutionary relationships are consistent with a previous study based on transcriptome data [25]. Because of the lack of public mitogenome data for the family Filistatidae, the Hypochilidae clade is closed to the clade of Synspermiata. According to previous studies [12, 25], within Araneomorphae, the Hypochilidae + Filistatidae cluster is sister to Synspermiata and constitutes the sister group of all other clades in Araneomorphae. Our findings also support this view (for more details, see Fig. 3).

Based on the three-fossil-calibrated phylogeny (Fig. 4), we dated the most recent common ancestor (MRCA) of *Desis jiaxiangi* and *Argyroneta aquatica* to the late Cretaceous at ~ 98 Ma (95% confidence interval: 73–122 Ma).

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**Fig. 2** Gene arrangements in spider mitogenomes. The last three species belong to the marronoid clade within the RTA group. The rearranged genes are color-coded, and one color indicates one rearranged gene. **“•”** refers to genes on the light (minority) strand.
Selection analyses

Of all values of Ka and Ka/Ks calculated from the 13 PCGs of the 46 spider mitogenomes examined, atp8 had the highest averages of Ka and Ka/Ks. This implies that atp8 might have evolved more quickly than the other PCGs in the spider mitogenomes (see Fig. 5). Conversely, cox1 had the lowest averages of Ka and Ka/Ks. When Ka/Ks was compared among the different lineages for all 13 PCGs dataset (free-ratios model), all the Ka/Ks values are lower than 1. Interestingly, Pirata subpiraticus, Hypochilus thorelli, and Argyroneta aquatica (the freshwater spider) had higher Ka/Ks values than other species, whereas the intertidal spider (Desis jiaxiangi) had a relatively lower Ka/Ks value (Fig. 6).

Furthermore, the results from both the branch and branch-site models showed significant positive selection on the ancestral branch of Desis jiaxiangi and Argyroneta aquatica with nd4 (p < 0.05; Table 2). There was no significant positive selection signal from both the branch and branch-site models when testing Pirata subpiraticus, Hypochilus thorelli, Argyroneta aquatica, Desis jiaxiangi and Agelena silvatica as the foreground independently (Tables 3, 4, 5, 6, 7). Nevertheless, the nd6 showed significant positive selection in the branch-site model in the three species with the highest Ka/Ks value (i.e., Pirata subpiraticus, Hypochilus thorelli, Argyroneta aquatica) when tested independently (p < 0.05; Tables 3, 4, 5).

Discussion

The complete mitogenome of Desis jiaxiangi is comparable in size to those of other spiders. There is no obvious mitogenome expansion or contraction within available spider mitogenomes during the diversification process. Although mitogenome rearrangements are very common in spider lineages [26] and also in other arthropods, such as crabs [27] and beetles [28], the unique tRNA gene rearrangements were detected in the mitogenomes of the two aquatic spider species, Argyroneta aquatica (family: Dictynidae) and Desis jiaxiangi (family: Desidae). Compared to spider lineages in the clade Entelegynae, the translocation of trnL2 and trnN was identified in Desis jiaxiangi, and trnN changed the linked strand...
in *Argyrnota aquatica*. More novel mitogenomes from these two families (Desidae and Dictynidae), to which *Argyrnota aquatica* and *Desis jiaxiangi* belong, respectively, are thus needed to determine whether these gene arrangements are the shared gene order pattern within each of these two spider families.

These two species within the superfamily Dictynoidea can be used as good models for comparative genome studies, because they occupy completely different ecological environments. *Argyrnota aquatica* lives almost entirely in freshwater, whereas *Desis jiaxiangi* resides in intertidal zones and can survive long periods submerged.
in seawater during high tides. Desis spiders are so-called semiaquatic spiders, as they hunt in intertidal zones during low tides like terrestrial spiders [29]. Therefore, Desis spiders must adapt to both aquatic and terrestrial conditions. Desis formidabilis was confirmed to have high hemolymph, the spider’s blood, in concentrations similar to those of marine crustaceans [30]. This feature is very well adapted to intertidal zones. The hydrofuge hairs on the bodies of aquatic spiders can form a thin air film, which enables them to use a physical gill or plastron respiration to exchange oxygen and carbohydrates [29, 31]. Meanwhile, Desis marina has lower respiration rates than other spider species [17]. In general, many organisms highly diverged in the opposite osmotic pressures...
induced by freshwater or seawater. While these two aquatic genera (*Argyroneta* and *Desis*) occupy different habitats, they have similar strategies and morphological features to prevent water from entering the book lungs and tracheae [32, 33].

It is noted that the terrestrial counterpart, *Agelena silvatica*, has many more gene rearrangements than the aquatic spiders. As the majority of terrestrial spiders in Entelegynae share the similar gene order (see Fig. 2), the significant gene transpositions in *Agelena silvatica* are uncertain. Further mitogenome sequencing of spiders in the marronoid clade is thus required.

The molecular dating analysis estimated that the MRCA of *Desis* and *Argyroneta* spiders diverged around 98 Ma, which is consistent with the extreme age (> 90 Myr) of major adaptations of spiders with aquatic lifestyles (superfamily Dictynoidea) [32]. This result

### Table 2 Chi-square tests based on the branch model and branch-site model analyses when testing the clade comprising both *Desis jiaxiangi* and *Argyroneta aquatica* as the foreground

| Gene  | p-value in branch model | p-value in branch-site model |
|-------|-------------------------|-----------------------------|
| atp6  | 0.239                   | 0.072                       |
| atp8  | NA                      | 1.000                       |
| cox1  | 0.006                   | 0.061                       |
| cox2  | 0.386                   | 0.991                       |
| cox3  | 0.028                   | 0.365                       |
| cyt6  | 0.034                   | 0.739                       |
| nd1   | 0.260                   | 0.277                       |
| nd2   | 0.538                   | 0.587                       |
| nd3   | 0.960                   | 1.000                       |
| nd4   | 0.020                   | 0.017                       |
| nd4l  | 0.745                   | 0.598                       |
| nd5   | 0.269                   | 0.415                       |
| nd6   | 0.618                   | 0.243                       |

NA: not applicable. A significant positive selection is in bold

### Table 3 Chi-square tests based on the branch (*Pirata subpiraticus*) model and branch-site model analyses

| Gene  | p-value in branch model | p-value in branch-site model |
|-------|-------------------------|-----------------------------|
| atp6  | 0.589                   | 1.000                       |
| atp8  | NA                      | 1.000                       |
| cox1  | 0.551                   | < 0.0001                    |
| cox2  | 0.205                   | 1.000                       |
| cox3  | 0.971                   | 0.212                       |
| cyt6  | 0.288                   | 0.047                       |
| nd1   | 0.933                   | 0.991                       |
| nd2   | 0.128                   | 0.811                       |
| nd3   | 0.019                   | 0.411                       |
| nd4   | 0.005                   | 0.193                       |
| nd4l  | 0.791                   | 1.000                       |
| nd5   | 0.285                   | 0.615                       |
| nd6   | 0.543                   | 0.009                       |

NA, not applicable. A significant positive selection is in bold

### Table 4 Chi-square tests based on the branch (*Hypochilus thorelli*) model and branch-site model analyses

| Gene  | p-value in branch model | p-value in branch-site model |
|-------|-------------------------|-----------------------------|
| atp6  | 0.055                   | 1.000                       |
| atp8  | NA                      | 0.419                       |
| cox1  | 0.089                   | 1.000                       |
| cox2  | 0.704                   | 0.999                       |
| cox3  | 0.766                   | 0.150                       |
| cyt6  | 0.651                   | 1.000                       |
| nd1   | 0.658                   | 0.500                       |
| nd2   | 0.801                   | 1.000                       |
| nd3   | 0.640                   | 0.298                       |
| nd4   | 0.741                   | 1.000                       |
| nd4l  | 0.161                   | 0.640                       |
| nd5   | 0.151                   | 1.000                       |
| nd6   | 0.708                   | 0.009                       |

NA, not applicable. A significant positive selection is in bold

### Table 5 Chi-square tests based on the branch (*Argyroneta aquatica*) model and branch-site model analyses

| Gene  | p-value in branch model | p-value in branch-site model |
|-------|-------------------------|-----------------------------|
| atp6  | 0.778                   | 0.124                       |
| atp8  | NA                      | 1.000                       |
| cox1  | 0.990                   | 0.941                       |
| cox2  | 0.948                   | 0.933                       |
| cox3  | 0.018                   | 0.696                       |
| cyt6  | 0.008                   | 0.078                       |
| nd1   | 0.097                   | 0.420                       |
| nd2   | 0.399                   | 1.000                       |
| nd3   | 0.551                   | 1.000                       |
| nd4   | 0.553                   | 0.066                       |
| nd4l  | 0.804                   | 0.351                       |
| nd5   | 0.716                   | 1.000                       |
| nd6   | 0.252                   | 0.004                       |

NA, not applicable. A significant positive selection is in bold
presents a helpful timeline for clarifying the evolution of aquatic spiders.

Our phylogenetic analyses provided a robust phylogeny for spiders. Most tree nodes with high support values (bootstrap = 100, posterior probability = 1) reconstructed the family relationships, such as Liphistiidae, Tetragnathidae, and Araneidae. The sister relationships are also revealed by high support values, for example, between Desidae and Dictynidae (bootstrap = 80, posterior probability = 0.99). Therefore, PCGs (amino acid sequences) in mitochondria can be considered reliable molecular markers for phylogenetic analyses of various spiders.

Without a doubt, all 13 PCGs in the mitochondria of living creatures are important to their aerobic metabolism. Positive selection may provide important functional details associated with adaptation to new environments [5]. For example, positive selection in nd4, cytb, and atp8 is assumed to have played a critical role in the origin of flight in bats to meet the huge change in energy demand [34]. Positive selection in atp6, nd2, and nd4 has been found for galliforms’ adaptation to high altitudes [22]. Our selective analyses revealed significant positive selection signals in nd4 on the branch of two aquatic spider lineages, Argyroneta aquatica and Desis jiaxiangi. In the spider mites (Tetranychus truncates and Tetranychus pueraricola), a positive selection on nd4 and atp6 was also detected and assumed to be associated with the different climate adaptations [35]. The peptides encoded by nd4 constitute mitochondrial complexes I, which participate in oxidative phosphorylation (OXPHOS); the amino acid changes within nd4 also affect the efficiency of ATP synthesis [35]. Like the high-altitude environments of galliforms, aquatic environments are short of oxygen, which implies association between gene positive selection and adaptation to energy metabolism in aquatic environments [22, 36]. While our study did not directly test the adaptation to aquatic environment, our results can inspire further investigation of aquatic adaptations of spider evolution.

The atp8 gene with the highest averages of both Ka and Ka/Ks suggests more changes in amino acids. This is also consistent with the previously identified Ka/Ks ratios in other spiders [37], insects [36] and fishes [38]. The higher Ka/Ks values indicate that the atp8 gene might have experienced more relaxed selective constraints and accumulated more mutations, thus it would be more likely to lose its function [36]. The cox1 gene with lowest Ka and Ka/Ks value suggests that it might have experienced more strong evolutionary pressures [36]. Therefore, cox1 has been widely used as barcoding marker for reconstructing phylogenies of spiders and other taxa. In the free-ratios model, Ka/Ks values for all 13 PCGs are less than 1 (Fig. 6), suggesting that purifying selection may have predominated the evolution of mitogenomes, as shown in spider mites [35] and insects [36].

Surprisingly, the much higher Ka/Ks values were detected in Pirata subpiraticus, Hypochilus thorelli and Argyroneta aquatica. This finding indicates that these three species may have higher nonsynonymous substitution rates than other examined species in this study. The higher nonsynonymous mutations suggest that these

### Table 6 Chi-square tests based on the branch (Desis jiaxiangi) model and branch-site model analyses

| Gene  | p-value in branch model | p-value in branch-site model |
|-------|-------------------------|-----------------------------|
| atp6  | 0.081                   | 0.331                       |
| atp8  | NA                      | 0.009                       |
| cox1  | 0.335                   | 0.206                       |
| cox2  | 0.001                   | 0.541                       |
| cox3  | 0.676                   | 0.038                       |
| cyt b | 0.032                   | 0.518                       |
| nd1   | 0.278                   | 0.786                       |
| nd2   | 0.609                   | 0.824                       |
| nd3   | 0.022                   | 0.999                       |
| nd4   | 0.941                   | 1.000                       |
| nd4l  | 0.968                   | 0.993                       |
| nd5   | 0.230                   | 1.000                       |
| nd6   | 0.057                   | 0.702                       |

NA, not applicable. A significant positive selection is in bold

### Table 7 Chi-square tests based on the branch (Agelela silvatica) model and branch-site model analyses

| Gene  | p-value in branch model | p-value in branch-site model |
|-------|-------------------------|-----------------------------|
| atp6  | 0.299                   | 0.480                       |
| atp8  | NA                      | 0.034                       |
| cox1  | 0.457                   | 1.000                       |
| cox2  | 0.056                   | 0.171                       |
| cox3  | 0.079                   | 0.631                       |
| cyt b | 0.489                   | 0.129                       |
| nd1   | 0.445                   | 1.000                       |
| nd2   | 0.979                   | 0.865                       |
| nd3   | 0.909                   | 1.000                       |
| nd4   | 0.041                   | 1.000                       |
| nd4l  | 0.557                   | 1.000                       |
| nd5   | 0.084                   | 1.000                       |
| nd6   | 0.359                   | 0.983                       |

NA, not applicable. A significant positive selection is in bold
species may have experienced more relaxed evolutionary constraints [36] and might have fixed more slightly beneficial amino acid changes [22]. When testing these three species and the intertidal species as the foreground independently, the nd4 gene had no significant signal from both the branch and branch-site models. This may be because the aquatic spider species (Argyroneta aquatica or Desis jiaxiangi) existed in the background branches when analysed. In other words, a significant positive selection signal in nd4 can be revealed only when the foreground branch contained both Desis jiaxiangi and Argyroneta aquatica. However, the nd6 gene were detected to have significant signal in the branch-site models, but not from both the branch and branch site models which demonstrate a strong positive selection, in the three species, Pirata subpiraticus, Hypochilus thorelli and Argyroneta aquatica (p < 0.05; Tables 3, 4, 5). The nd6 gene may play an essential role in each evolutionary process of these three species with the highest Ka/Ks values. The peptides encoded by nd6 involve in catalytic synthesis of ATP, and changes within atp6 have been shown to link to the differences in metabolism [35]. Among these three spiders, Pirata subpiraticus and Argyroneta aquatica are in the RTA clade, while Hypochilus thorelli is in the Hypochilidae clade. Pirata subpiraticus is a pond wolf spider with a relatively large body shape and a quick predation ability in paddy field [39]. Hypochilus thorelli is considered to be the most primitive araneomorph spider species that builds the lampshade-shaped web. Its preferred habitat is close to a stream and well-shaded ledges [40]. The positive selection signals on nd6 found in these three species may be relevant to their adaptations on energetic requirements. However, further studies are needed to test this hypothesis.

Mitochondria, as the powerhouses of the cell may also be related to the foraging strategies of spiders. Web-building spiders are ambushers that use their webs as traps to catch prey, whereas hunting spiders have to actively search for prey [41]. Hunting spiders may thus require more energy to find prey than ambushing spiders. Neither Argyroneta aquatica nor Desis jiaxiangi use webs for foraging; instead, both have to run or swim quickly in search of prey. These spiders also stay under water for long periods in oxygen-limited environments [16, 32]. Thus, these aquatic spiders should be very efficient at metabolizing energy to meet the demands of energy consumption. Positive selection on spider mitochondrial PCGs may have shaped the evolution of aquatic spider lineages.

Similarly, recent research on intertidal chiton has also detected positive selection on their mitochondrial PCGs, which may help the chiton adapt to contrasting environments [21]. Although our analyses revealed significant positive selection signals in nd4 on the branch of two spider species, Argyroneta aquatica and Desis jiaxiangi, our data set did not cover all spider lineages; hence, more studies of the mitogenomes of Desidae, Dictynidae, and other families are required. Furthermore, the higher Ka/Ks values and the positive selection on the nd6 gene in Pirata subpiraticus, Hypochilus thorelli and Argyroneta aquatica warrant for more studies. Because of limited data, comparative genome studies on spider lineages remain largely underexploited. It is hoped that our study stimulates more genome studies on spiders, especially aquatic spiders, with the expectation of revealing more details on the molecular mechanisms behind the adaptation of intertidal spiders to marine environments.

**Conclusions**

The fact that Desis spiders can live under seawater at high tides shows how well they have adapted to extremely harsh conditions and how they are able to tolerate limited oxygen and seawater salinity. Here we provide a complete mitogenome sequence of spiders from the family Desidae. The different mitogenome orders of Argyroneta aquatica and Desis jiaxiangi imply they have undergone a different divergent evolution in the gene order of their mitogenome. Both BI and ML phylogenetic analyses supported the close relationship between Argyroneta aquatica and Desis jiaxiangi, and our dating analyses revealed that they diverged in the late Cretaceous. The positive selection acting on nd4 on the branch consisting of both Argyroneta aquatica and Desis jiaxiangi may affect the efficiency of their ATP synthesis. The three species, Pirata subpiraticus, Hypochilus thorelli and Argyroneta aquatica, have higher Ka/Ks values than other species in the 13 PCGs dataset and have been detected positive selection signals on nd6. This interesting finding may be relevant to their adaptations on energetic requirements. More in-depth analysis on these clades could build up our knowledge towards their metabolic adaptations. In summary, our study probably provides molecular evidence of the evolution of an aquatic lifestyle and presents a new genetic resource for phylogenetic studies in spiders.

**Methods**

**Sample collection**

In December 2018, we visited intertidal zones in Sanya City, Hainan Province, China. During low tides, we carefully searched coral crevices in the intertidal zone and collected 13 specimens of Desis jiaxiangi (see Additional file 2: Fig. S1) in the location (N18.21999°, E109.51128°, 3 m a.s.l.). Voucher specimens were deposited in the Marine Bank of the China National GeneBank (CNGB voucher nos. Des_001 to Des_013), Shenzhen, China.
Assembly and annotation of the complete mitogenome of *Desis jiaxiangi*

We extracted total DNA from the whole body of one specimen (Des_002) for traditional whole-genome shotgun sequencing using a Puregene Tissue Core Kit A (Qiagen, Germantown, MD, USA). We constructed the library with a short-insert size of 500 bp using standard protocols (Illumina, San Diego, CA, USA). We sequenced with paired-end reads (150 bp in length) on an Illumina Hiseq X Ten platform at BGI-Wuhan, China. The raw sequencing data was filtered by SOAPnuke [42] to trim adapter, low quality, high N base ratio and etc. Finally, we acquired about 100 Gb of clean reads after the sequencing and data filtering. We used all of these clean data as an input file to the Python3-based mitogenome assembly toolkit MitoZ [43] for a primary mitogenome assembly of *Desis jiaxiangi*.

Subsequently, we performed a BLAST search to compare this mitogenome assembly with corresponding mitogenome data of four relatively close spider species from RTA clade: *Agelena silvatica* (GenBank Accession no. KX290739.1), *Argyroneta aquatica* (No. NC_026863.1), *Dolomedes angustivirgatus* (No. NC_031355.1), and *Telamonia viljini* (No. KJ598073.1). We manually annotated the conserved regions of our assembled *Desis* mitogenome based on the other four mitogenomes. For the uncertain regions of the assembled *Desis* mitogenome, we designed two pairs of primers (see Additional file 1: Table S2) using Primer Premier 5 (Premier Biosoft, Palo Alto, CA, USA) to obtain the missing sequences using PCR. To validate the sequences of the uncertain regions, we extracted genomic DNA from the leg tissue of the specimens using a Puregene Tissue Core Kit A (Qiagen). We performed PCR in 50 µL volume tubes, each containing 25 µL 2× Taq PCR MasterMix (Tiangen Biotech, Beijing, China), 2 µL of each primer (10 µM), and 2 µL genomic DNA (100 ng/µL), with 19 µL double-distilled water. The PCR protocol was as follows: pre-denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, elongation at 72 °C for 1 min 40 s, and a final elongation at 72 °C for 10 min. A Veriti Thermal Cycler (Applied Biosystems, Carlsbad, CA, USA) was used for the PCR. We checked the PCR products by electrophoresis using 1% agarose gels.

After successfully amplifying the uncertain regions, we obtained the final assembly of the complete mitogenome of *Desis jiaxiangi*, which was annotated with MITOS2 [44] on MITOS WebServer (http://mitos2.bioinf.uni-leipzig.de/index.py). The final mitogenome sequence was visualized with MitoZ in the visualize module [43].

Comparative analysis of mitochondrial gene orders is a powerful method of revealing ancient events in the process of species evolution [45]. After completely annotating the mitogenome of *Desis jiaxiangi*, we compared its gene order with the available 45 complete spider mitogenomes (see Additional file 1: Table S1) from the NCBI GenBank.

Phylogenetic analyses and estimation of divergence time

We prepared a data set comprised the novel mitogenome of *Desis jiaxiangi* and the complete mitogenomes of 45 other spider species (see detailed species names in Additional file 1: Table S1) downloaded from the NCBI GenBank. We performed phylogenetic analyses of this data set using Bayesian inference (BI) and maximum likelihood (ML) methods. We aligned the nucleotide and amino acid sequences of the 13 PCGs from each mitogenome using the multiple sequence alignment program Clustal Omega in EMBL-EBI [46]. We performed a best-fit model selection of amino acid replacement using ProtTest-3.4.2 [47]. Based on Akaike’s information criterion, MtREV +1 +G was chosen as the best model for both inference methods. We reconstructed the phylogenetic tree with the ML method using PhyML 3.0 [48]. The node support values were estimated with 100 replicates and other parameters as the default. We also performed BI using MrBayes v3.2.6 [49] to compare the topologies of the ML phylogenetic trees. MCMC algorithm parameters were set for two independent runs with four chains (one cold chain and three heated chains) for 10,000,000 cycles. The sample frequency parameter was set at 1000 for sampling each chain every 1000 cycles. The first 25% of the runs were discarded as burn-in. We used FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) to visualize the derived BI and ML trees.

We used the Bayesian MCMCTree program in PAML package v4.9j [50] to estimate divergence times. Optimized parameters were as follows: clock = independent rates, model = HKY85, nsample = 20,000, burn-in = 2,000. We used calibration points from recommended fossils and a related time in a recent spider fossil review [51]. Given the limited spider species with available mitogenomes, we used three fossils to calibrate the phylogenetic tree: *Palaeothele montceauensis* (299–304 Ma) for the Mesothelae stem, *Eoplectreurys gertschi* (164–175.1 Ma) for the Synspermiata stem, and *Montsecarachne amicorum* (125–129.4 Ma) for the Synspermiata crown. As the fossil of *Almolinus ligula* (43–47.8 Ma) in the Salticidae crown can be placed in the superfamily Hisponinae [51], and no sample mitogenome is available for this superfamily, this fossil was not used for calibration in the present work.
Selection analyses
To evaluate potential adaptive evolution in the mitochondrial genes of intertidal Desis spiders, we performed positive selection analyses using PAML4.9j [50]. Synonymous substitutions in protein-coding sequences cannot cause changes in amino acids, which are typically found in the third, or sometimes first, position of a codon [52]. We thus used a gene-level approach based on the ratio of nonsynonymous (Ka) to synonymous (Ks) substitution rates to detect potential positive selection signal on PCGs across closely related or divergent species [52, 53]. A Ka/Ks ratio of 1, <1, or >1 in protein-coding sequences may be interpreted as a neutral mutation, a negative (purifying) selection, or a positive (diversifying) selection, respectively [53]. To investigate the variation in nucleotide substitution rates in spider mitogenomes, we retrieved all 13 mitochondrial PCGs (nd2, cox1, cox2, atp8, atp6, cox3, nd3, nd5, nd4, nd4l, nd6, cyt b, and nd1) from each annotated mitogenome of the 46 spider species examined. We aligned each gene separately with the codon-based model in the Muscle module of MEGA7 [54]. Ambiguous regions in each alignment were removed with Gblocks v0.91b [55]. Ka, Ks, and the Ka/Ks ratio across all 13 PCGs were calculated with KaKs_Calculator v2.0 [53].

To examine positive selection pressure on individual PCGs of the 46 spider species, we generated conserved blocks from codon alignments of each PCG using Gblocks v0.91b. We ran both the branch and branch-site models in the CodeML program of PAML on those codons from the conserved blocks. We used the phylogenetic topology inferred from PhyML as the guide tree for PAML analyses. In branch models, the free Ka/Ks ratio model is allowed to vary among branches to detect positive selection on the foreground branch. In branch-site models, the one-ratio model assumes that all branches have an identical Ka/Ks ratio, whereas the two-ratio model assumes that the foreground branch has a different Ka/Ks ratio from the background branches. The Ka/Ks ratios in branch-site models are thus allowed to vary both among sites and across branches to detect positive selection on a few sites along the foreground branch. The branch-site model A null fixed all Ka/Ks ratios to 1, whereas the branch-site model A (positive selection model) did not fix the Ka/Ks ratio. The branch-site model A was used to detect positive selection sites along the lineages of aquatic spiders (i.e., the foreground branch). The presence of a site with Ka/Ks ratio >1 is suggested when the positive selection model A fits the data significantly better than the corresponding model A null.

We first used the free-ratios model (branch model) to calculate the average Ka/Ks ratio for all 13 PCGs in each lineage to represent their evolutionary rate of mitogenomes. We paid particular attention to two aquatic species, the intertidal spider (Desis jiaxiangi) and the freshwater spider (Argyroneta aquatica), which have aquatic habitats, whereas the other species are almost all limited to land. For this purpose, we marked the ancestral branch containing both Desis jiaxiangi and Argyroneta aquatica as the foreground branch and the rest of the species as the background branches. To compare the results, we marked the branches of Pirata subpiraticus, Hypochilus thorelli, Argyroneta aquatica, Desis jiaxiangi and Agelen a silvatica separately to test these five species independently. We ran the branch models (one-ratio model vs. two-ratio model) and branch-site models (null model A vs. model A) as two pair models using likelihood ratio tests and chi-square tests, respectively, to detect whether the positive selection signals were significant. The null hypothesis assumed that all branches had a common Ka/Ks ratio. An alternative hypothesis assumed that the ancestral branch had an independent Ka/Ks ratio that differed significantly from the background branches with a common Ka/Ks ratio. If this hypothesis has a better fit than the null hypothesis, we considered the occurrence of positive selection in the ancestor of aquatic spiders in certain gene(s) when p < 0.05. Positive selection does not usually generate the function on the whole length of the target genes, and only a few sites can reflect the positive selection signal. The branch-site models further identified the positive site(s), and the Bayes empirical Bayes analysis was used to calculate posterior probabilities to detect those sites under positive selection.

Abbreviations
ATP: Adenosine triphosphate; BI: Bayesian inference; CNCB: China National GeneBank; ML: Maximum likelihood; MRCA: Most recent common ancestor; PCG: Protein-coding gene; rRNA: Ribosomal RNA; RTA: Retrolateral tibial apophysis; tRNA: Transfer RNA.

Supplementary Information
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Additional file 1: Table S1. Reported complete mitogenomes from the NCBI database. Table S2. Primers used to amplify uncertain fragments.

Additional file 2: Fig. S1. Morphology and habitat of the intertidal spider, Desis jiaxiangi.

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Authors' contributions

DL and QS conceived this project. FL, YL, ZW, CB, XZ and SG performed data analysis. FL wrote the manuscript. DL, QS and FL revised the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

The final complete mitogenome sequences of Desis jiaxiangi in this manuscript are deposited in NCBI with Accession Number MW178198.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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