Next generation sequencing yields complete mitogenomes of Leopard whipray (Himantura leoparda) and Blue-spotted stingray (Neotrygon kuhlii) (Chondrichthyes: Dasyatidae)

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Kang-Ning Shen[1], Chih-Wei Chang[2,3], Shiou-Yi Tsai[4], Shan-Chun Wu[4], Zi-Han Lin[4], Yen-Fan Chan[4], Ching-Hung Chen[5], Chung-Der Hsiao[4], and Philippe Borsa[6]

[1] Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan
[2] Department of Biology, National Museum of Marine Biology and Aquarium, Checheng, Pingtung, Taiwan
[3]Graduate Institute of Marine Biodiversity & Evolutionary Biology, National Dong Hwa University, Checheng, Pingtung, Taiwan
[4] Department of Bioscience Technology, Chung Yuan Christian University, Chung-Li, Taiwan
[5] WeThink Biotech Inc., Taoyuan, Taiwan
[6] IRD-UMR 250, eMtroPI c/o Universitas Udayana, Denpasar, Indonesia

Abstract
The Leopard whipray (*Himantura leoparda*) and Blue-spotted stingray (*Neotrygon kuhlii*) are distributed in the Indian and West Pacific Ocean and considered as species complexes based on morphological and molecular evidence. In this study, we used the next-generation sequencing method to decipher the two complete mitogenomes of *H. leoparda* and *N. kuhlii*. The assembled mitogenomes had a length of 17,690 bp in *H. leoparda* and 17,974 bp in *N. kuhlii*, and showed 78% identity to each other. Both mitogenomes had the typical vertebrate arrangement, including 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNAs genes and the non-coding control region. The control region, which was 1931-bp long in *H. leoparda* and 2243-bp long in *N. kuhlii* was located between the *tRNA-Pro* and *tRNA-Phe* loci. The overall GC content was 40.3% in *H. leoparda* and 39.8% in *N. kuhlii*. The complete mitogenome sequences of *H. leoparda* and *N. kuhlii* provide important molecular data for further phylogenetic and evolutionary analyses of these two stingray species complexes.

Keywords
Blue-spotted stingray; leopard whipray; mitogenome; next generation sequencing

The population genetics of stingrays are among the most poorly understood among vertebrates because of the limited geographic sampling and the occurrence of cryptic species (Beheregaray, 2008). The two stingray species targeted by this study belong to the family Dasyatidae, which comprises about 70 species in 9 genera A recent taxonomic revision of ocellated whipray species complex revealed at least four biological species (Arlyza et al., 2013), including three known species *Himantura leoparda*, *H. uarnak*, *H. undulata*, and a newly described cryptic species *H. tutul* (Borsa et al., 2013). Sequencing the mitogenome of stingrays is important to understand their molecular phylogenetics, phylogeography, population genetics, and evolution, especially when addressing complexes of cryptic species such as the *H. uarnak* species complex.

Samples of *H. leoparda* (a voucher specimen here numbered 329) and *N. kuhlii* (voucher no. 330) were collected from, respectively Taiwan and Cebu, Philippines. The methods for genomic DNA extraction, library construction and next generation sequencing followed Shen et al. (2014). Using the Geneious v. 8 (Auckland, New Zealand) commercial software, about 0.16% (10,058 out of 6,280,529) and 0.02% raw reads (1082 out of 5,005,674) were isolated from total genomic reads and de novo assembled to produce a single, circular form of complete mitogenome with about an average of 185_ and 22_ coverage for *H. leoparda* and *N. kuhlii*, respectively.

The assembled mitogenomes of *H. leoparda* (GenBank accession no. KR019776) and *N. kuhlii* (GenBank KR019777) had a total length of, respectively, 17,690 base pairs (bp) and 17,974 bp and showed 78% identity to each other. In addition, the mitogenome sequence of the *N. kuhlii* specimen from this study showed 97.6% identity to a previously sequenced specimen of the *N. kuhlii* species complex (Chen et al., 2014). The control region, with a length of 1931 bp in *H. leoparda* and 2243 bp in *N. kuhlii*, is located between tRNA-Pro and tRNA-Phe.

The protein-coding, rRNA and tRNA genes of the mitogenome were predicted using DOGMA (Wyman et al., 2004), ARWEN (Laslett & Canback, 2008), MITOS (Bernt et al., 2013) and MitoAnnotator (Iwasaki et al., 2013) tools. For *H. leoparda*, 6 of 13 protein-coding genes were inferred to terminate with an incomplete stop codon of either T– – (COX2, ND3, ND4 and CYTB) or TA– (ATP6 and COX3). For *N. kuhlii*, 4 of 13 protein-coding genes were inferred to terminate with an incomplete stop codon of either T– – (COX2, ND3 and ND4) or TA– (ND2). The longest protein-coding gene was the gene encoding ND5 (1848 bp in *H. leoparda* and 1842 bp in *N. kuhlii*), whereas the shortest was the gene encoding ATP8 (168 bp in both species). The two ribosomal RNA genes, *12S rRNA* (968 bp for *H. leoparda* and 963 bp for *N. kuhlii*) and *16S rRNA* (1687 bp for *H. leoparda* and 1701 bp for *N. kuhlii*), were located between tRNA-Phe and tRNALeu (UAA) and separated by tRNA-Val.

To examine the phylogenetic positions of *H. leoparda* and *N. kuhlii*, we used MEGA6 (Tamura et al., 2013) to construct a maximum-likelihood tree (with 500 bootstrap replicates) of the complete mitogenomes of 16 species from 11 different genera in the Chondrichthyes. *Chimaera monstrosa* (Chimaeridae) was used as outgroup for tree rooting. Result showed that *H. leoparda* was closely related to *H. granulata* and *N. kuhlii* with high bootstrap support (Figure 1).

In summary, the complete mitogenomes of *H. leoparda* and of the *N. kuhlii* clade that occurs in the Philippines were decoded for the first time; they provided important information for further phylogenetic and evolutionary analysis in stingrays.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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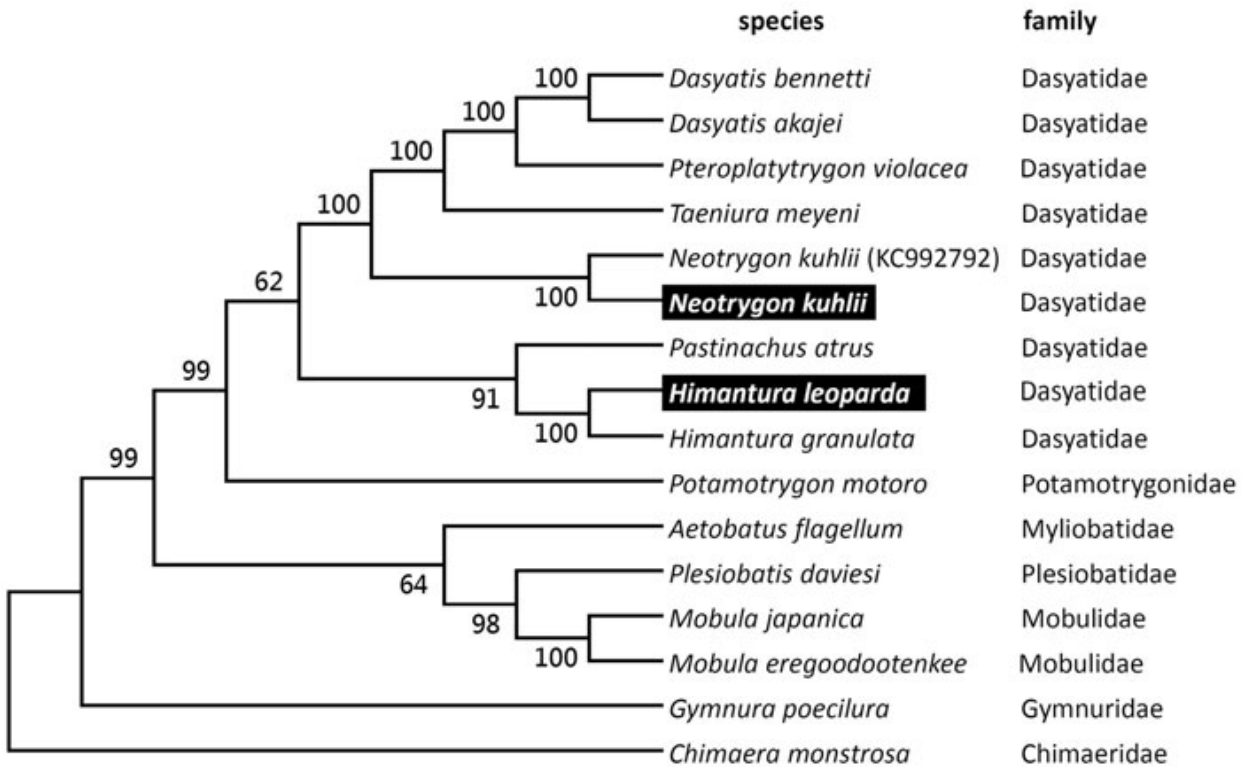

Figure 1. Phylogenetic placement of *Himantura leoparda* and *Neotrygon kuhlii* based on the complete mitogenome. The phylogenetic tree was constructed using the maximum-likelihood algorithm, with 500 bootstrap replicates. Complete mitogenome sequences were downloaded from GenBank; the GenBank accession numbers for the sequences were: KC196067 (*Dasyatis bennetti*), KC526959 (*Dasyatis akajei*), KJ641617 (*Pteroplatytrygon violacea*), JX827260 (*Taeniura meyeni*), KC992792 (*Neotrygon kuhlii*), KR019777 (*Neotrygon kuhlii*), HG942172 (*Pastinachus atrus*), KR019776 (*Himantura leoparda*), KF751650 (*Himantura granulata*), KF709642 (*Potamotrygon motoro*), KF482070 (*Aetobatus flagellum*), AY597334 (*Plesiobatis daviesi*), JX392983 (*Mobula japanica*), KM361353 (*Mobula eregoodootenkee*), KJ617038 (*Gymnura poecilura*) and AJ310140 (*Chimaera monstrosa*). Highlighted black: two new mitogenomes announced in present note.