



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

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► **To cite this version:**

Kang-Ning Shen, Chih-Wei Chang, Shou-Yi Tsai, Shan-Chun Wu, Zi-Han Lin, et al.. Next generation sequencing yields complete mitogenomes of Leopard whiplay (*Himantura leoparda*) and Blue-spotted stingray (*Neotrygon kuhlii*) (Chondrichthyes: Dasyatidae) . Mitochondrial DNA Part A, 2016, 27, pp.2613-2614. 10.3109/19401736.2015.1041119 . ird-01312741v1

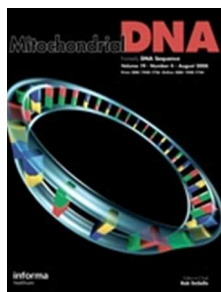
HAL Id: ird-01312741

<https://ird.hal.science/ird-01312741v1>

Submitted on 9 May 2016 (v1), last revised 9 May 2016 (v2)

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Journal:	<i>Mitochondrial DNA</i>
Manuscript ID:	Draft
Manuscript Type:	Mitogenome Announcements
Date Submitted by the Author:	n/a
Complete List of Authors:	Shen, Kang-Ning; National Taiwan Ocean University, Center of Excellence for the Oceans Chang, Chih-Wei; National Museum of Marine Biology and Aquarium, Department of Biology Tsai, Shiou-Yi; Chung Yuan Christian University, Department of Bioscience Technology Wu, Shan-Chun; Chung Yuan Christian University, Department of Bioscience Technology Lin, Zi-Han; Chung Yuan Christian University, Department of Bioscience Technology Chan, Yen-Fan; Chung Yuan Christian University, Department of Bioscience Technology Chen, Ching-Hung; WeThink Biotech INC, Hsiao, Chung-Der; Chung Yuan Christian University, Department of Bioscience Technology Borsa, Philippe; Universitas Udayana,
Keywords:	Leopard whiplay, Blue-spotted stingray, next generation sequencing, mitogenome

MITOGENOME ANNOUNCEMENTS

~~Next generation sequencing yields the complete mitogenome of Leopard whiplay~~
(*Himantura leoparda*) and Blue-spotted ~~stingray~~ ^{maskray} (*Neotrygon kuhlii*) (Chondrichthyes:
Dasyatidae)

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,*}, 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 9220 eMtropi c/o Universitas Udayana, Denpasar, Indonesie
Institut de recherche pour le développement,

* corresponding to : Chung-Der Hsiao, Department of Bioscience Technology, Chung
Yuan Christian University, 200 Chung-Pei Rd., Chung-Li, Taiwan 32023
e-mail address: cdhsiao@cycu.edu.tw

Abstract

The Leopard whiplay (*Himantura leoparda*) and Blue-spotted stingray (*Neotrygon kuhlii*) are stingray distributed in the Indian and West Pacific Ocean and considered as species complex by morphological and molecular evidences. In this study, we used the next-generation sequencing method to decode the complete mitogenome of *H. leoparda* and *N. kuhlii*. The assembled mitogenome, consisting of 17,690 bp for *H. leoparda* and 17,974 bp for *N. kuhlii*, shows 78% identities each other. Both mitogenomes follow the typical vertebrate arrangement, including 13 protein coding genes, 22 transfer RNAs, 2 ribosomal RNAs genes and a non-coding control region of D-loop. D-loop contains 1,931 bp (*H. leoparda*) and 2,243 bp (*N. kuhlii*) length is located between tRNA-Pro and tRNA-Phe. The overall GC content is 40.3% for *H. leoparda* and 39.8% for *N. kuhlii*. The complete mitogenome of *H. leoparda* and *N. kuhlii* provides essential and important DNA molecular data for further phylogenetic and evolutionary analysis for stingray species complex.

Keywords: Leopard whiplay, Blue-spotted stingray, mitogenome, next generation sequencing

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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 targeted stingray species in this study ^{belong to} are classified ^{encompasses} in the family Dasyatidae which ^{encompassing} encompasses nine genera and about 70 species. A recent taxonomic revision of ocellated whipray species complex comprised at least four biological species mostly from the Coral Triangle (Arlyza et al., 2013), including three known species *Himantura leoparda*, *H. uarnak*, *H. undulata* and another newly described cryptic species *H. tutul* sp. nov. (Borsa et al., 2013). The ^{development} establishment of stingray mitogenome ^S is important to understand their population genetics, molecular phylogenetic, phylogeography and evolution ~~for stingray species complex.~~

Samples *H. leoparda* (voucher no. 329) and *N. kuhlii* (voucher no. 330) were collected from Taiwan and Cebu, the Philippines, respectively. The methods for genomic DNA extraction, library construction and next generation sequencing were followed by our previous publication (Shen et al., 2014). By using commercial software (Geneious V8, Auckland, New Zealand), about 0.16% (10,058 out of 6,280,529) and 0.02% raw reads (1,082 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 185 X and 22 X coverage for *H. leoparda* and *N. kuhlii*, respectively. The assembled mitogenome of *H. leoparda* and *N. kuhlii*, consisting of 17,690 bp (GenBank: KR019776) and 17,974 bp (GenBank: KR019777), both showing 78% identities each other. In addition, the mitogenome of *N. kuhlii* decoded in this study show 97.6% identity to previously reported *N. kuhlii* species complex (Chen et al., 2014). D-loop contains 1,931 bp in *H. leoparda* and 2,243 bp in *N. kuhlii* and locates between tRNA-Pro and tRNA-Phe.

The protein coding, rRNA and tRNA genes of mitogenome were predicted by using DOGMA (Wyman et al., 2004), ARWEN (Laslett and Canback, 2008), MITOS (Bernt et al., 2013) and MitoAnnotator (Iwasaki et al., 2013) tools. For *H. leoparda*, six of 13

protein-coding genes is inferred to termination with an incomplete stop codon of either T- - (COX2, ND3, ND4 and CYTB) or TA- (ATP6 and COX3). For *N. kuhlii*, four of 13 protein-coding genes is inferred to termination with an incomplete stop codon of either T- - (COX2, ND3 and ND4) or TA- (ND2). The longest one is ND5 gene (1,848 bp for *H. leoparda* and 1,842 bp for *N. kuhlii*) in all protein coding genes, whereas the shortest is ATP8 gene (168 bp for both species). The two ribosomal RNA genes, 12S rRNA gene (968 bp for *H. leoparda* and 963 bp for *N. kuhlii*) and 16S rRNA gene (1,687 bp for *H. leoparda* and 1,701 bp for *N. kuhlii*), are located between tRNA-Phe and tRNA-Leu (UAA) and separated by tRNA-Val.

To validate the phylogenetic position of *H. leoparda* and *N. kuhlii*, we used MEGA6 (Tamura et al., 2013) software to construct a Maximum likelihood tree (with 500 bootstrap replicates) containing complete mitogenomes of 16 species derived from 11 different genus in Dasyatidae. *Chimaera monstrosa* derived from Chimaeridae was used as outgroup for tree rooting. Result shows *H. leoparda* is closely related to *H. granulata* and *N. kuhlii* is closely related to its species complex (Chen et al., 2014) with high bootstrap value supported (Figure 1). In conclusion, the complete mitogenome of *H. leoparda* and *N. kuhlii* are decoded for the first time in this study and provides essential and important DNA molecular data for further phylogenetic and evolutionary analysis for stingray species complex.

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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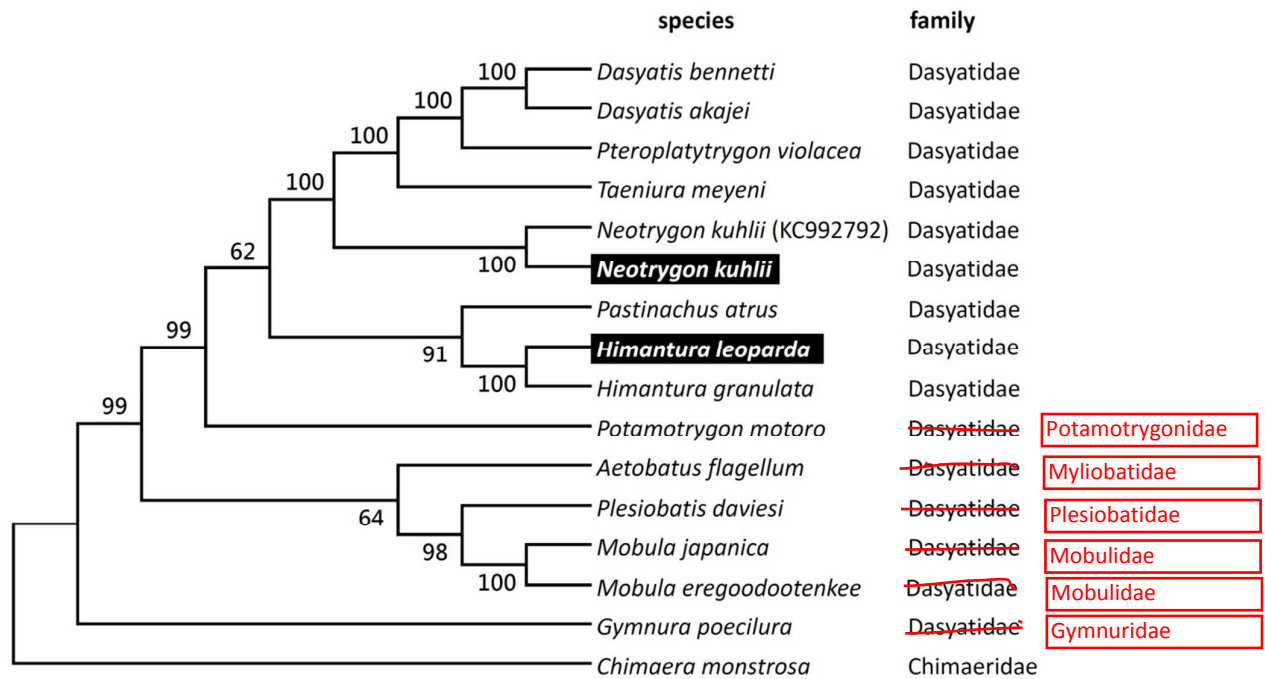


Figure 1. Molecular phylogeny of *Himantura leoparda* and *Neotrygon kuhlii* in ~~Dasyatidae~~ based on complete mitogenome. The complete mitogenome ~~is~~ downloaded from GenBank and the phylogenetic tree is constructed by Maximum likelihood method with 500 bootstrap replicates. The gene's accession number for tree construction is listed as follows: *Dasyatis bennetti* (KC992792), *Neotrygon kuhlii* (NC_021767; present study), *Pteroplatytrygon violacea* (KJ641617), *Taeniura meyeri* (JX827260), *Neotrygon kuhlii* (KC992792), *Neotrygon kuhlii* (KR019777), *Pastinachus atrus* (HG942172), *Himantura leoparda* (KR019776), *Himantura granulata* (KF751650), *Potamotrygon motoro* (KF709642), *Aetobatus flagellum* (KF482070), *Plesiobatis daviesi* (AY597334), *Mobula japanica* (JX392983), *Mobula eregoodootenkee* (KM361353), *Gymnura poecilura* (KJ617038) and *Chimaera monstrosa* (AJ310140).

Highlighted black: two new mitogenomes announced in present note.