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DNA sequences and morphological variation in *Lophiodes iwamotoi* Ho, Serét & Shao, 2011 based on new material from New Caledonia

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Abstract

Iwamoto's anglerfish *Lophiodes iwamotoi* is recorded from New Caledonia for the first time. Study of molecular features further support the validity of the species. Moloecular sequence data from the cytochrome c oxidase subunit-I and Rhodopsin loci, along with morphological variation are provided, as well as information on its fresh coloration.

Key words: Pieces, Teleotsei, Lophiiformes, Lophiidae, New Caledonia

Introduction

Ho *et al*. (2011) documented the species of the family Lophiidae from the South Pacific Ocean, recognizing seven valid species and describing two new species from Polynesia. One of these, *Lophiodes iwamotoi* Ho, Serét & Shao, 2011 was described on the basis of five specimens collected from the Savannah Seamount of Polynesia at depths of 706-710 m in 1977. These specimens, despite being in good condition, were strongly faded due to long-term preservation.

Recently, the authors examined fish specimens collected during the 39-day exploratory cruise (campaign: EXBODI) by the R/V *Alis* deployed by the French Oceanographic Fleet on seamounts off New Caledonia, in which nine lophiid specimens were recognized. Among them, four are identified as *L. iwamotoi*. Of the remaining five, three were identified as *L. mutilus* (Alcock 1894) and two as *Lophiomus setigerus* (Vahl 1797).

The purpose of the present work is to record this recently described species and its congeners represented in the New Caledonian ichthyofauna. A molecular comparison was undertaken to obtain further data regarding the validity of the species. We discuss this DNA sequence data as well as morphological variation in *L. iwamotoi*, and provide fresh color information on this species for the first time (Fig. 1).

Material and methods

Nine lophiid specimens were included in the morphological and molecular examination. Whole genomic DNA was extracted from fin clips or muscle tissue of specimens using the Qiagen DNeasy extraction kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Fragments of two protein-coding genes (cytochrome c oxidase subunit-I [COI] and rhodopsin [Rhod]) located in mitochondrial and nuclear genomes, respectively, were amplified and sequenced for this study. Protocols for collecting molecular data follow those outlined in Ward *et al.* (2005) for COI and Chen *et al.* (2003) for Rhod. Collected DNA sequences were deposited in NCBI GenBank (see Fig. 2 for the accession numbers of the corresponding gene sequences). Compiled sequences were manually aligned based on the inferred amino acid translation using Se-Al v2.0a11 (available at http://tree.bio.ed.ac.uk/software/seal/).

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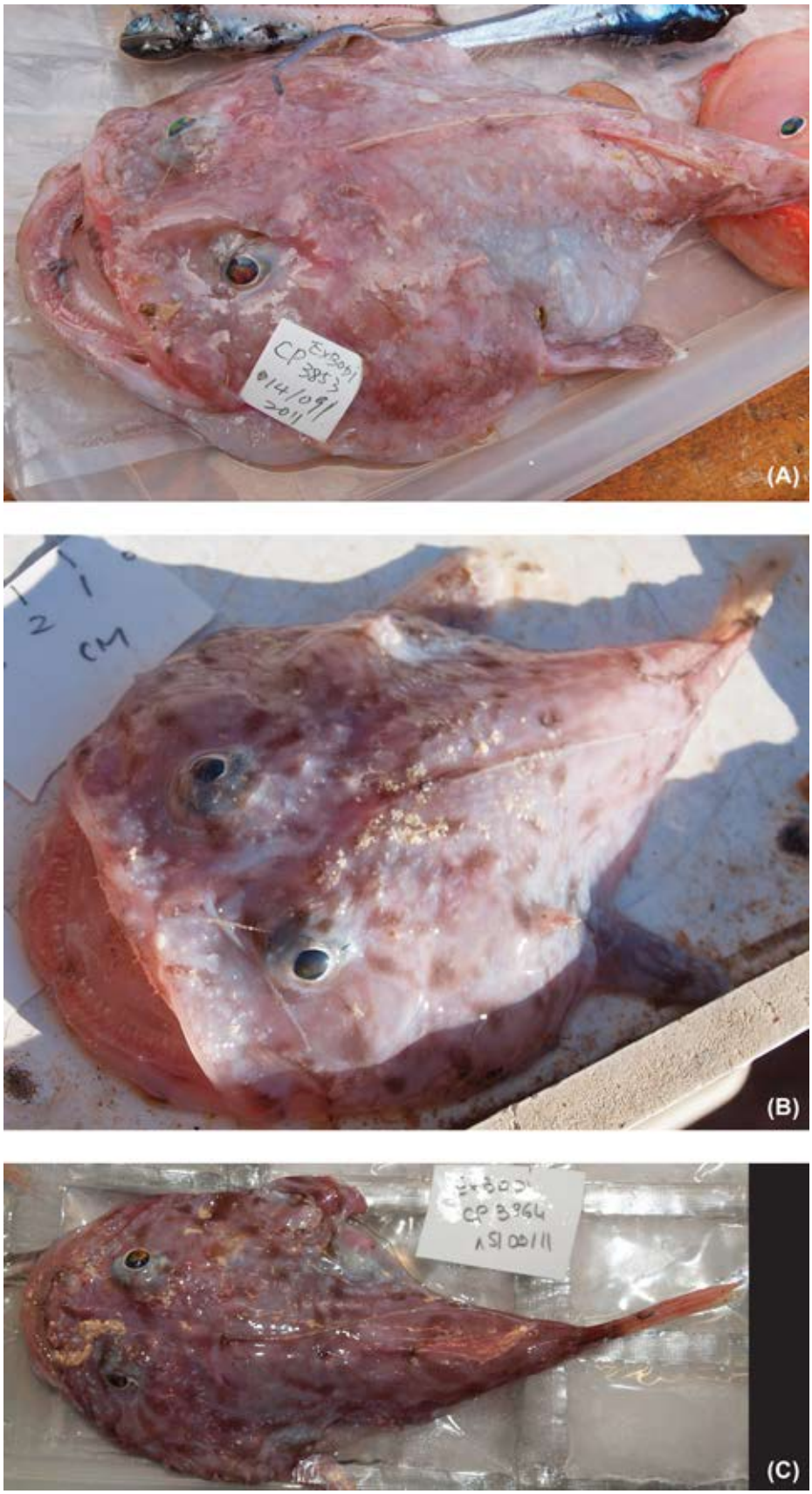

FIGURE 1. Freshly caught specimens of *Lophiodes iwamotoi*. A. ASIZP 73486, 248 mm SL. B. ASIZP 73489, 247 mm SL. C. ASIZP 73488, 154 mm SL. Not to scale.

Methods for taking counts and proportional measurements generally followed Caruso (1981, 1983). Specimens examined are deposited in the fish collection of the Academia Sinica (ASIZP). Meristic and morphometric values of the four newly collected specimens compared to those of the type-series are provided in Table 1.

TABLE 1. Morphomeric data of the type-series and four additional specimens of *Lophiodes iwamotoi*.

	Types	New Caledonian specimens	
Standard length (mm SL)	169–226 (n=4)	145–248 (n=3)	76.7 (n=1)
Proportion as %SL			
Head length (HL)	36.2–37.2	36.6–38.3	43.5
Illicial length	23.0–26.7	19.8–23.5	25.4
2nd dorsal-spine length	27.2–31.7	29.1–30.5	27.4
3rd dorsal-spine length	56.9–70.8	66.5–68.8	44.3
5th dorsal-spine length	10.5–13.1	13.0–17.5	10.7
Tail length	26.3–29.0	25.3–29.8	32.7
Proportion as % HL			
Head width	52.1–53.8	51.8–53.0	56.3
Head depth	65.8–67.9	62.4–66.7	65.0
Snout length	55.4–57.5	57.6–59.6	58.1
Snout width	19.2–22.5	19.3–23.3	19.5
Distance between inner sphenotic spines	41.1–42.5	40.4–41.7	45.2
Distance between posterior frontal spines	41.0–43.2	43.2–44.6	45.2
Distance between pterotic and sphenotic spines	19.2–20.5	18.4–20.3	19.5
Distance between quadrate and anterior palatine spines	67.1–72.6	64.5–68.8	50.9
Distance between opercular and subopercular spines	41.6–47.4	41.5–43.9	46.4

Results and discussion

Among the species in the *Lophiodes mutilus*-species group (*sensu* Caruso, 1981), *L. iwamotoi* is closest to *L. mutilus* in having a long third dorsal-fin spine, a simple small escal bulb, and many tendrils on the third dorsal-fin spine. Several characters have been used to readily separate it from *L. mutilus*: 19–20 pectoral-fin rays (vs. 15–18; mostly 16), a much longer third dorsal-fin spine (56.9–70.8% SL vs. 36.6–63.9% SL) that reaches the caudal peduncle, but a relatively short fifth dorsal-fin spine (10.5–13.1% SL vs. 16.9–29.3% SL) that reaches the origin of the soft dorsal fin.

The New Caledonian specimens agree well with the original description, except for the following variations. The inner frontal spine is present in all type-specimens and in the two smaller New Caledonian specimens, but is strongly reduced in two larger specimens. The same change of the inner frontal spine morphology was also observed in other members of the *L. mutilus* species group (Ho, pers. obs.), implying that an ontogenic change of this particular character might be common in *Lophiodes* species. The inner sphenotic spines are strongly reduced in the 248 mm specimen.

The illicium is much shorter in the 248 mm specimen, only 19.8% SL (vs. 23.0–26.7% SL in type series), whereas that of the 226 mm holotype is 26.7% SL. The length of the illicium in the 154 mm specimen is also below the average value for the type-series. No trend in growth in the spine is observed. The 248 mm specimen has a relatively long fifth dorsal-fin spine at 17.5% SL (vs. 10.5–13.1% SL in the type-series). However, the spine reaches the origin of the dorsal fin when fully laid back, identically to the type-series.

Several proportional values are different in the 76.7 mm specimen: it has a longer head (43.5% SL), a wider head (56.5% HL), a shorter 3rd dorsal-fin spine (44.3% SL) that reaches only the middle portion of dorsal-fin base, a longer tail (32.7% SL), a greater distance between inner sphenotic spines (45.2% HL), a greater distance between

frontal spine (45.2% HL), and a smaller distance between quadrate and anterior palatine spines (50.9% HL). However, it has 19 pectoral-fin rays and similar coloration with the other three specimens; its molecular features also support our identification. All these differences may be attributed to changes in growth.

(A) Cytochrome c oxidase subunit - I

Species	Nucleotide position
specimen (Genbank no.)	1111112222222223333333333444444444444445555555666667 2347780122688990233667899023345556678890456899024 60 3874797658158140139098734805844692510351684847018 60
L. mutilus	
EXB088 (KF060329)	TAGTTTACCGCCGGCGCGCACATTCTCACCTGCGCGATTCTATCTGAGCGT
EXB089 (KF060330)	.....TA.....A.
EXB110 (KF060331)	.....G.....
L. iwamotoi	
EXB097 (KF060332)	ATACCCGTTCTTAATATATGTGCCTCAGTTCCGATAGCCTCGGACCCATCC
EXB111 (KF060333)	ATACCCGTTCTTAATATATGTGC.TCAGTTCCGATAGCCTC.GACCCATCC
EXB158 (KF060334)	ATACCCGTTCTTAATATATGTGCCTCAGTTCCGATAGCCTC.GACCCATCC
EXB182 (KF060335)	ATACCCGTTCTTAATATATGTGC.TCAGTTCCGATAGCCTC.GACCCATCC

(B) Rhodopsin

Species	Nucleotide position
specimen (Genbank no.)	13344555688 581528167834 125464619716
L. mutilus	
EXB088 (KF060336)	GCGCCACTCAGT
EXB089 (KF060337)	....Y.....A.
EXB110 (KF060338)	..R...G..C..
L. iwamotoi	
EXB097 (KF060339)	C.AATT...C.G
EXB111 (KF060340)	C.AATT...C.G
EXB158 (KF060341)	C.AATT...C.G
EXB182 (KF060342)	CTAATT.CTC.G

FIGURE 2. Polymorphic nucleotide sites at the cytochrome c oxidase subunit-I locus (A) and Rhodopsin locus (B) in two *Lophiodes* species collected from seamounts off New Caledonia. Diagnostic nucleotides of *L. iwamotoi* to *L. mutilus* are highlighted. Numeration of nucleotide sites starts from first nucleotide of the corresponding genes. The sequences used for the examination were deposited in NCBI Genbank. Their accession numbers are given.

Few knobs are found on the dorsal surface of the humeral spine in the type-series, but three blunt spines are present on the two smaller specimens and absent in two larger specimens. Ho *et al.* (2011) mentioned that "although the humeral spines are quite variable in most species of *Lophiodes*, ranging from simple to multifid, all specimens in the type series of *L. iwamotoi* have a simple humeral spine with few low knobs on it." We confirm that *L. iwamotoi* has a simple humeral spine, especially in larger specimens. Arrangement and form of other spines on the head bones in the New Caledonian material are as recorded in the originally described specimens of the species.

Figure 1 shows the coloration of the freshly caught New Caledonian specimens. The 248 mm specimen (Figs. 1A–B) is uniformly light brown dorsally and pale gray ventrally, with a pale posterior pectoral-fin margin. The

remaining specimens have many small irregular black patches on a light brown background; these patches faded after preservation.

Distribution of *L. iwamotoi* is now extended westward from the Society Islands to southeastern New Caledonia, and the depth range is extended to 460–780 m.

From the sample collection data and molecular evidence from both mitochondrial and nuclear gene markers (see below) (Fig. 2), the two *Lophiodes* species are suggested to be genetically and reproductively isolated, living sympatrically at least on seamounts of the South Durand Bank. It is notably that one of our *Lophiomus setigerus* specimens was collected at a depth of 815–970 m, which represents the deepest record for the species.

No nucleotide insertions or deletions were found in our aligned sequences for the samples of the two *Lophiodes* species. Fifty-one and 12 variable sites were observed along the 630 bp and 828 bp sequenced fragments for the COI and Rhod loci, respectively. This represents 7.82% and 0.84 % of interspecific nucleotide divergence (evaluated by uncorrected pairwise *p*-distance) from COI and Rhodopsin genes. A few single nucleotide polymorphism sites (SNPs) were detected among the samples of the same species (Fig. 2). In addition to morphological characters, the two *Lophiodes* species co-occuring in New Caledonian waters can be unambiguously distinguished from each other by four and more diagnostic nucleotides along the fragment of the two genes sequenced (Fig. 2).

Specimens examined. *Lophiodes iwamotoi*: ASIZP 73486 (248 mm), stn. EXB 097, CP3853, Banc Sud Durand, 22°18'S, 168°46'E, 692 m, 14 Sep. 2011. ASIZP 73487 (76.7 mm), stn. EXB 111, CP3854, Banc Sud Durand, 22°18'S, 168°45'E, 570 m, 14 Sep. 2011. ASIZP 73488 (154 mm), EXB 158, stn. CP3864, Banc de L'Orne/Walpole, 22°22'S, 168°57'E, 460–708 m, 15 Sep. 2011. ASIZP 73489 (247 mm), stn. EXB 182, CP3871, Banc Ellet, 22°53'S, 169°25'E, 580–780 m, 16 Sep. 2011. *Lophiodes mutilus*: ASIZP 73483 (107 mm SL) and ASIZP 73484 (56.3 mm), stn. EXB 088, CP3852, Banc Sud Durand, 22°17'S, 168°43'E, 582 m, 14 Sep. 2011. ASIZP 73485 (177 mm), stn. EXB 110, CP3854, Banc Sud Durand, 22°18'S, 168°45'E, 570 m, 14 Sep. 2011. *Lophiomus setigerus*: ASIZP 73490 (121 mm), stn. EXB532, CP3834, Au large passe Yaté, 22°06'S, 167°04'E, 257–258 m, 9 Sep. 2011. ASIZP 73491 (235 mm), stn. EXB538, CP3844, Au large passe de la sarcelle, 22°20'S, 167°22'E, 815–970 m, 10 Sep. 2011.

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