間隙性針紐虫類ノーレンバーグサザレヒモ (新称) (紐形動物・単針類) の沖縄からの報告

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Interstitial hoplonemertean *Ototyphlonemertes norenburgi* (Nemertea: Monostilifera) from Okinawa, Japan

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Abstract. The interstitial monostiliferous hoplonemertean *Ototyphlonemertes norenburgi* Kajihara, Tamura & Tomioka, 2018 has been known only by the original description from Vietnam. We confirmed the species' distribution ranging to Japanese waters by comparison of cytochrome *c* oxidase subunit I (COI) gene sequences from specimens collected in Onna, Okinawa. The morphology of the Okinawan specimens is briefly described with photomicrographic images. This is the first record of *O. norenburgi* from Japan, representing a second report of the species in the world, as well as the fifth congener known from the country.

Introduction

The genus *Ototyphlonemertes* Diesing, 1863 consists of 33 species of mesopsammic monostiliferous hoplonemerteans, dwelling exclusively in interstices of coarse sand grains in intertidal and shallow subtidal zones (Kirsteuer 1977; Norenburg 1988a, b; Leasi et al. 2016). From Japan, four congeners have been reported so far: *O. martynovi* Chernyshev, 1993, *O. nikolaii* Chernyshev, 1998, and *O. dolichobasis* Kajihara, 2007 from Otsuchi Bay (Shimomura et al. 2001; Kajihara 2007) and *O. ani* Chernyshev, 2007 from Shirahama and Gesashi (Leasi et al. 2016; Kajihara et al. 2018). In this paper, we report *O. norenburgi* Kajihara, Tamura & Tomioka, 2018 from Okinawa as the fifth member of the genus in Japanese waters.

Material and Methods

Fourteen specimens were collected at a beach (26°28′13.92″N, 127°49′47.13″E) in Onna, Okinawa-jima, Okinawa, Japan by H. Yamasaki on 12 October 2015 following the method of Corrêa (1953). Morphological observation, DNA extraction, PCR amplification, and sequencing were performed following those of Kajihara et al. (2018). Cytochrome oxidase subunit I (COI) sequences (588 bp) were determined from 12 of the 14 specimens and deposited in DDBJ with accession numbers LC333563–LC333574. Haplotype network was constructed by TCS ver. 1.2.1 (Clement et al. 2000) based on statistical parsimony (Templeton et al. 1992), using the 12 sequences from Okinawa along with two sequences of *O. norenburgi* from Vietnam, LC310997 (from the holotype) and LC310998 (from paratype).

Results

Ototyphlonemertes norenburgi Kajihara, Tamura & Tomioka, 2018

[New Japanese name: Nōrenbāgu-sazarehimo]
(Fig. 1)

Material examined. Fourteen specimens, all destroyed during DNA extraction.

Description. Body length 2.7–4.2 mm (mean 3.5 mm, n = 14), width 0.11–0.17 mm (mean 0.13 mm, n = 14). Epidermis whitish (Fig. 1A); cephalic furrow post-cerebrally; tissues around brain reddish; cirri present in both anterior and posterior ends of body. Statoliths bipartite (Fig. 1B), each granule 5–8 µm in diameter (mean 7 µm, n = 14); statocyst 20–28 µm in short-axis diameter (n = 9). Proboscis anterior chamber with wart-like papillae (Fig. 1C); diaphragm short (Fig. 1C); two accessory stylet pouches, lateral to central-stylet basis, each containing mostly two stylets (n = 8), but three (n = 2) and four stylets (n = 1) were also found; in one specimen, two and three accessory stylets were found in each pouch; accessory stylets mostly directing forward, but occasionally backward; middle chamber bulbous; stylet smooth (Fig. 1C), 21–34 µm long; basis 24–29 µm long, 6–11 µm wide (n = 13); basis length/width

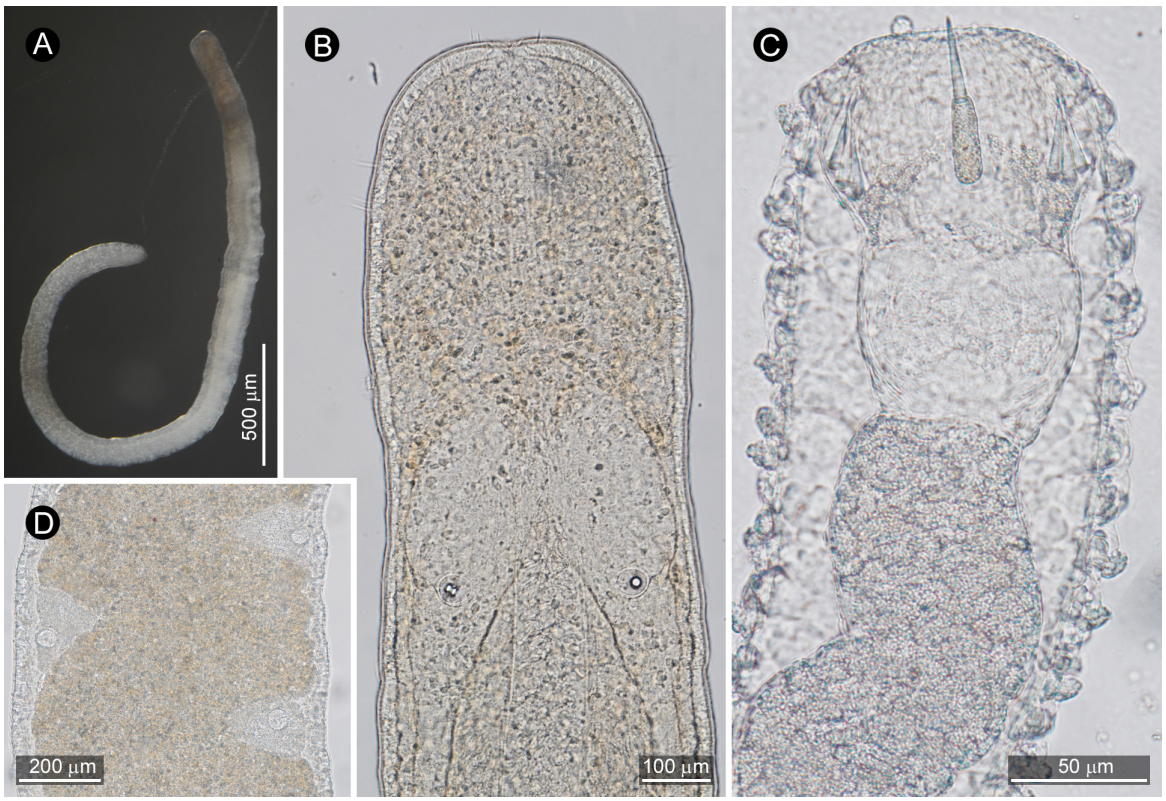

Fig. 1. *Ototyphlonemertes norenburgi* Kajihara, Tamura & Tomioka, 2018. A, Entire animal, anesthetized in $MgCl_2$ solution; B, photomicrograph of head; C, photomicrograph of stylet apparatus; D, photomicrograph of intestinal region showing oocytes.
図 1. ノーレンバーグサザレヒモ (新称). A, 麻酔した状態での全体図 ; B, 頭部顕微鏡写真 ; C, 針装置の顕微鏡写真 ; D, 腸部顕微鏡写真 , 卵母細胞を示す .

ratio 2.6–4.1 (n = 13); posterior chamber opaque, without specialized anterior portion. Intestine variously yellowish, whitish, olive, or colorless; intestinal diverticula shallow, occasionally alternating with oocytes (Fig. 1D). One of 14 individuals observed possessed mature oocytes, up to 100 µm in diameter in squeezed state, accounting for 18–27% of body width (Fig. 1D).

Genetics. Five haplotypes were detected from 14 sequences included in the analysis (12 from Okinawa, 2 from Vietnam). They differed up to 0.0136 (*p*-distance) and 0.0139 (K2P). A major haplotype was shared by seven individuals from Okinawa and one paratype specimen from Vietnam (Fig. 2). Six steps different from this major haplotype was the holotype sequence of *O. norenburgi* from Vietnam, with which one Okinawan individual shared the haplotype.

Distribution. So far known from Dam Ngoai, Vietnam (Kajihara et al. 2018), and Onna, Okinawa-jima Island (present study).

Remarks. *Ototyphlonemertes norenburgi* belongs to the *O. duplex* species group, which is characterized by having two-granular statoliths, smooth central stylet, thick central-stylet basis, short

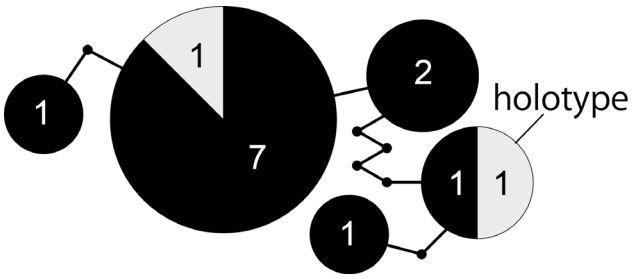

Fig. 2. Statistical parsimony network for five haplotypes detected among 14 *Ototyphlonemertes norenburgi* specimens (12 from Okinawa, 2 from Vietnam). Numbers in each circle (pie chart) indicate sample size, which is proportional to the size of each pie diagram. Okinawan and Vietnamese haplotypes are indicated by black and light gray, respectively.
図 2. ノーレンバーグサザレヒモ 14 個体 (沖縄産 12 個体 , ベトナム産 2 個体) に見出された 5 つのハプロタイプの統計的節約ネットワーク . 円の中の数字はサンプル数を表し , 個々の円はサンプルサイズに比例した円グラフになっている . 沖縄とベトナムのハプロタイプはそれぞれ黒色と明灰色で示す .

proboscis diaphragm, and bulbous proboscis middle chamber (Envall & Norenburg 2001; Kajihara et al. 2018). The Okinawan specimens, up to 4.2 mm in length, were smaller than the Vietnamese type specimens, which were 7.8–9.0 mm long.

Measurements of statocysts, statoliths, stylet and basis length and width mostly overlap with those in other members of the *O. duplex* species group. Without barcoding sequence data, the present specimens could not have been positively identified to the species level.

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間隙性針紐虫類ノーレンバーグサザレヒモ（新称）（紐形動物・単針類）の沖縄からの報告

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要旨. 間隙性単針類のノーレンバーグサザレヒモ（新称）はこれまでベトナムからの原記載で知られるのみであった．我々は沖縄県恩納村から得られた標本の COI 配列の比較から，本種が日本にも分布していることを確認した．沖縄産個体の形態を顕微鏡写真画像と共に簡潔に記載した．本研究はノーレンバーグサザレヒモの日本からの初記録であると同時に，本種の世界から 2 番目の記録，かつ本邦から 5 番目の本属種の報告となる．

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