Short Notes

The Southernmost Record of *Nipponacmea fuscoviridis* (Patellogastropoda: Lottiidae) from Iriomote Island, Okinawa

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Nipponacmea fuscoviridis (Teramachi, 1949) is enormously abundant in the intertidal zone throughout the temperate area of the Japanese islands (southern Hokkaido and southward: Sasaki & Okutani, 1993). It also occurs in part of the Ryukyu Islands, but known subtropical localities are extremely few in number. In the literature, reliable records exist only from four of the Nansei Islands, viz. Amami-Oshima Island, Kakeroma Island, Tokashiki Island and Kunigami, Okinawa Island (Sasaki & Okutani, 1993; Kubo & Kurozumi, 1995).

In 2004 one of us (T. S.) surveyed the molluscan fauna of Iriomote Island, Okinawa (Sasaki *et al*., 2006) and unexpectedly collected specimens of the genus *Nipponacmea,* which is mainly distributed in temperate areas. As a result of morphological examinations they were identified as *N. fuscoviridis* based on shell characters, animal pigmentation and radular sac configuration. In addition, molecular phylogenetic analysis confirmed that the material is genetically conspecific with temperate population of *N. fuscoviridis*. We report this interesting range extension in this paper.

Material and Methods

Ten specimens of *Nipponacmea fuscoviridis* were collected alive from steep sides of huge smooth stones standing on a sandy beach at Haemida, Iriomote Island, Okinawa (locality F of Sasaki *et al*., 2006: fig. 1). A single specimen was fixed in 99.5% ethanol and used for molecular phylogenetic analysis, and the rest of the samples were fixed in 10% formalin and preserved in 70% ethanol. The animal was dissected under a binocular microscope and photographed. The methods of DNA extraction, PCR amplification, sequencing, alignment and phylogeny reconstruction were the same as those already described by Nakano and Ozawa (2004) and Nakano (2006). All specimens were deposited in the University Museum, The University of Tokyo (registration number: UMUT RM29321-29330) and the sequences of 12S rRNA, 16S rRNA and COI genes from the specimen UMUT RM29321 (Fig. 1A–B) were registered in Gen Bank (accession numbers AB263732-263734).

Results

Morphology: The shell is flattened with a low anteriorly directed apex (Fig. 1A). The surface is marked with a radial pattern over a dark green ground colour and ornamented with radial rows of fine elongate granules with no radial riblets. The inside is glossy and dark greenish in the center (the exposed surface of the M-1 shell layer: see Fuchigami & Sasaki, 2005 for definition), dull greenish white in the muscle scar and surrounding area (M+1 layer), and fringed by a dark striped shell margin (M+2 and M+3 layers) (Fig. 1B).

The epithelia of the cephalic tentacles are dark-coloured, but the remaining areas of the animal, including the head and the lateral wall of the foot, lack black pigmentation completely (Fig. 1C). The radular sac forms loops on the right anterior and posterior sides of the visceral mass (r1, r2: Fig. 1D). The radular teeth have the formula 0-3-0-3-0; the cusps of all teeth are sharply pointed. The colour of the gonad was not observed.

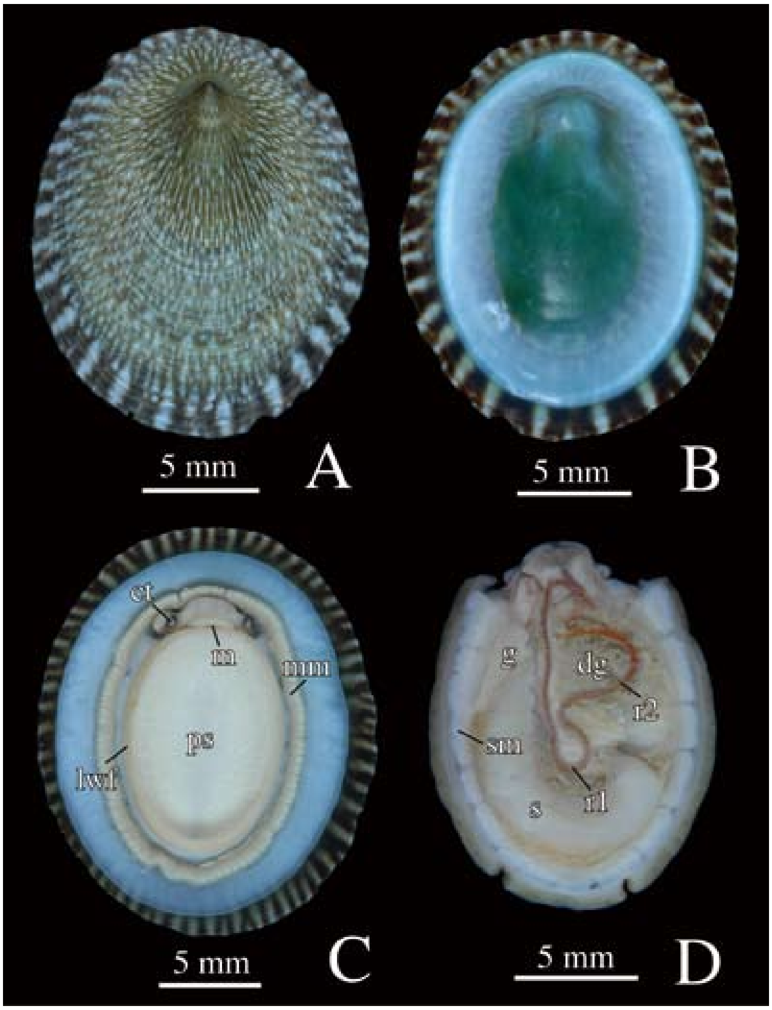

Fig. 1. *Nipponacmea fuscoviridis* from Haemida, Iriomote Island, Okinawa. **A.** Dorsal view of the shell. **B.** Ventral view of the shell. **C.** Ventral view with the animal. **D.** The dorsal view of the animal with part of the visceral mass, ctenidium and anterior mantle removed. Abbreviations: ct – cephalic tentacle; dg – digestive gland; g – gonad; lwf – lateral wall of foot; m – mouth; mm – mantle margin; ps – pedal sole; r1 – posterior loop of radular sac; r2 – right anterior loop of radular sac; s – stomach; sm – shell muscle. A-B: UMUT RM29321, C: UMUT RM29322, D: UMUT RM29323.

Molecular phylogenetic analyses: Neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses were performed on the data set comprising 1248 base pairs of 12S rRNA, 16S rRNA and COI genes of 12 lottiid samples, including three *Lottia* species as outgroups. The resulting tree revealed the following relationships with high statistical support: (1) species of *Nipponacmea* form a robust monophyletic group distinct from *Lottia*; (2) within *Nipponacmea*, *N. gloriosa* branches off first; (3) the remaining groups are split into two clades, *habei-teramachii-nigrans* and *schrenckii-concinna-fuscoviridis-radula*; (4) the specimen from Iriomote Island forms a clade with *N. fuscoviridis* from Fukui Prefecture with 100% posterior probabilities and bootstrap values. These results suggest the specimen from Iriomote Island is correctly identified as *N. fuscoviridis*, at least at the mitochondrial gene level.

Discussion

The identification of *Nipponacmea fuscoviridis* from Iriomote Island, Okinawa was verified by both anatomical and molecular evidence. In shell characters, the sculpture of radially arranged fine granules without prominent riblets is diagnostic of *N. fuscoviridis*. In addition, the finely radiating colour pattern is also consistent with typical features of the species. In anatomical characters, the absence of black pigmentation except on the cephalic tentacles and the radular sac forming double loops are also clear-cut diagnostic characters of the species (see Sasaki & Okutani, 1993 for comparison among *Nipponacmea* species). Furthermore, the molecular phylogenetic analyses using 1248 base pairs of 12S rRNA, 16S rRNA and COI genes (Fig. 2) revealed robust support for its identification as *N. fuscoviridis* with high posterior probabilities and bootstrap values. Pairwise molecular distances among nine *Nipponacmea* specimens show that intraspecific distance was low (1.7%) while the interspecific distance of two specimens of *N. fuscoviridis* (model-based distance using Kimura's 2 parameter method) was greater (> 5%).

The occurrence of *N. fuscoviridis* on Iriomote Island represents a remarkable southward range extension for the species. The previously known distribution ranges from southern Hokkaido to the Kerama Islands west off Okinawa, which are located approximately 370 km north of Iriomote Island. In the literature, there are only four recorded localities in the subtropical Ryukyu Islands, *viz*. Amami-Oshima Island, Kakeroma Island, Tokashiki Island (Sasaki & Okutani, 1993: table 1) and Kunigami, Okinawa Island (Kubo & Kurozumi, 1995: 19). Thus, the southernmost limit of the species is now updated by the new record from Iriomote Island.

The dispersal route of *N. fuscoviridis* into Iriomote Island remains uncertain, although the origin of *Nipponacmea* is inferred to be the temperate area of the Japanese Islands (Nakano & Ozawa, 2004, 2007). Southward migration within the Ryukyu Islands seems likely based on the closeness to other existing localities. Another hypothetical possibility is long-distance migration by way of a continental route along the coasts of Korea and China. However, there is no actual support for this scenario, because *N. fuscoviridis* has never been reliably reported from Korea (the specimen illustrated in Min *et al.*, 2003 as *N. fuscoviridis* is actually a variant of *N. radula*) or China (e.g. Lü, 2004). The phylogeographic relationships of Asian *Nipponacmea* will need to be tested by more extensive sampling and molecular work in the future.

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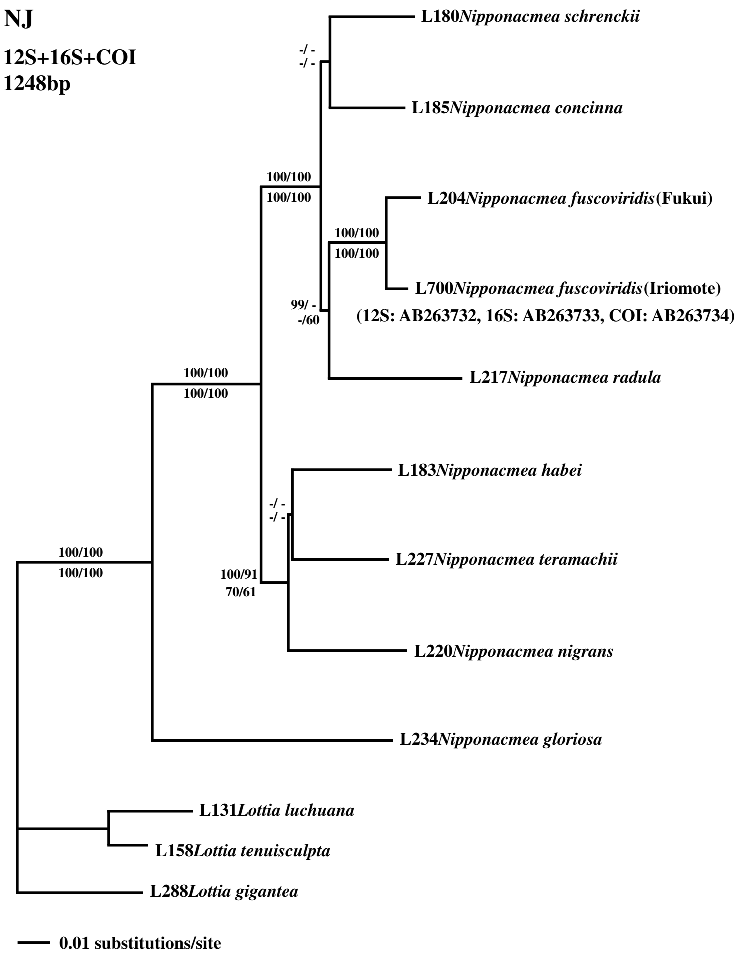

Fig. 2. NJ tree of *Nipponacmea* and *Lottia* including *N. fuscoviridis* from Fukui Prefecture and Iriomote Island (specimen UMUT RM29321; accession numbers of Gen Bank = AB263732 for 12S rRNA, AB263733 for 16S rRNA and AB263734 for COI). Values on each branch represent posterior probabilities/bootstrap (NJ) (above) and bootstrap (MP)/(ML) (below).

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沖縄県西表島から採集されたクサイロアオガイの南限新記録

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著者の一人佐々木が2004年に西表島の貝類を調査した際に，クサイロアオガイと思われるアオガイ属の１種を採集した。アオガイ類は日本列島の温帯域に多産する分類群であるが，琉球列島では稀である。この興味深い標本を形態およびミドコンドリアDNA（12S, 16S, COI）の両者から検討した結果，西表産の標本はクサイロアオガイに確実に同定されることが確認された。この産地は従来知られていたクサイロアオガイの南限（慶良間列島渡嘉敷島）から約370 kmも離れて孤立しており，分布の最南端記録である。本種がどのような経路を経て西表島に到達したかという点は大変興味深い問題であるが，今後の検討課題である。