Note

Molecular-based method to distinguish sympatric deep-sea whelks, *Buccinum striatissimum* and *Buccinum tenuissimum* in the Sea of Japan

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Abstract: *Buccinum striatissimum* and *Buccinum tenuissimum* are commercially important whelks in the Sea of Japan. *B. striatissimum* is very similar to *B. tenuissimum* and their morphological variations make the identification of these species difficult, especially in case that these species are caught together because they are subject to the same environmental factors. In order to develop an easy and objective method to delineate these species, we utilized the difference of mitochondrial 16SrDNA of these two species. As a result, we could easily distinguish these two species by PCR-RFLP method using the difference of this region and *Asel* restriction endonuclease. In addition, we succeeded in distinguishing sympatric samples of these species, which suggests that these two species are likely to be reproductively isolated although the possibility of hybridization can not be completely ruled out.

Key words: *Buccinum*, sympatry, 16SrDNA, PCR-RFLP, the Sea of Japan

In the Sea of Japan, four *Buccinum* species (*Buccinum bayani* Jousseaume, 1883; *Buccinum striatissimum* Sowerby, 1899; *Buccinum tenuissimum* Kuroda in Teramachi, 1933; *Buccinum tsubai* Kuroda in Teramachi, 1933) are commercially important for the deep bottom fisheries (Kato 1979). In particular, *B. tenuissimum* is the most valuable among them (Kato 1981, Maeda & Doi 2006). However, the wide range of morphological variations often makes the identification of these species difficult (Amano & Watanabe 2001, Kato 1979), especially in cases where these species are caught together because they are subject to the same environmental factors. *B. striatissimum* usually inhabits shallower regions (200–590 m) than *B. tenuissimum* (350–1550 m), and they also show sympatry around 400–500 m (Iguchi et al. unpublished data, Kato et al. 1979). It is usually easy to distinguish these two species based on shell thickness and depth of suture (Iguchi et al.

2007), but *B. striatissimum* shows very thin shell thickness and is very similar to *B. tenuissimum* in places where they are caught together (Fig. 1). In such a case, it is very difficult to distinguish these species without enough taxonomical experiences. Even if they could be distinguished by morphological differences, it would be unclear whether they are genetically different from each other considering their ambiguous mor-

Fig. 1. Shells of sympatric *Buccinum tenuissimum* (left) and *Buccinum striatissimum* (right).

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B_tenuissimum1	121	GACCTATCGAGCTTGAAAAAAACTAGTAGAGTAA	GTA	TTAATGATATCA	AAAAATTA
B_tenuissimum2	121	GACCTATCGAGCTTGAAAAAAACTAGTAGAGTAA	GTA	TTAATGATATCA	AAAAATTA
B_striatissimum1	121	GACCTATCGAGCTTGAAAGAAACTAGTAGAGTAA	-	TAATTAATGATATCA	AAAAATTA
B_striatissimum2	121	GACCTATCGAGCTTGAAAGAAACTAGTAGAGTAA	-	TAATTAATGATATCA	AAAAATTA
B_striatissimum3	121	GACCTATCGAGCTTGAAAGAAACTAGTAGAGTAA	-	TAATTAATGATATCA	AAAAATTA

B_tenuissimum1	181	CTATTAGAAATTTTAGTTGGGGCGACTAAGGAACAAAAAAGCTTCCTTTAAAAATTA	A		
B_tenuissimum2	181	CTATTAGAAATTTTAGTTGGGGCGACTAAGGAACAAAAAAGCTTCCTTTAA	G	AATTA	
B_striatissimum1	180	CTATTAGAAATTTTAGTTGGGGCGACTAAGGAACAAAAAAGCTTCCTTTAAAAATTA	A		
B_striatissimum2	180	CTATTAGAAATTTTAGTTGGGGCGACTAAGGAACAAAAAAGCTTCCTTTAAAA	C	TAA	
B_striatissimum3	180	CTATTAGAAATTTTAGTTGGGGCGACTAAGGAACAAAAAAGCTTCCTTTAAAAATTA	A		
B_tenuissimum1	241	AACTAAACAAGTATTGATCCAAAACATTT	-	GATTAAAGGAATTAGTTACCGTAGGGATA	A
B_tenuissimum2	241	AACTAAACAAGTATTGATCCAAAACATTT	-	GATTAAAGGAATTAGTTACCGTAGGGATA	A
B_striatissimum1	240	AACTAAACAAGTATTGATCCAAAACATTTT	G	ATTAAAGGAATTAGTTACCGTAGGGATA	A
B_striatissimum2	240	AACTAAACAAGTATTGATCCAAAACATTTT	G	ATTAAAGGAATTAGTTACCGTAGGGATA	A
B_striatissimum3	240	AACTAAACAAGTATTGATCCAAAACG	T	TTTGATTAAAGGAATTAGTTACCGTAGGGATA	A

Fig. 2. Alignment of 5 sequences of the 16SrDNA region of *Buccinum striatissimum* and *Buccinum tenuissimum*. Numbers to the left of each block denote the position in the matrix. Each name corresponds to the sequence of the accession number available from the DNA Data Bank of Japan below; B_tenuissimum1: AB183330; B_tenuissimum2: AB183331; B_striatissimum1-3: AB183334–AB183336. Predicted restriction site of *AseI* is shown by asterisks.

phological variations. Thus, an easy and objective method to delineate these species should be developed. In a previous study, we found that *B. striatissimum* is clearly different from *B. tenuissimum* in mitochondrial 16SrDNA sequence analysis (Iguchi et al. 2007). By utilizing this difference, we tried to develop a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method to distinguish sympatric *B. striatissimum* and *B. tenuissimum*.

In order to search for the sequence to distinguish *B. striatissimum* and *B. tenuissimum*, we aligned five 16SrDNA sequences of these two species reported in our previous study (Iguchi et al. 2007) using the computer software CLUSTAL X (Thompson et al. 1997). The alignment is shown in Fig. 2, which demonstrates that *B. striatissimum* is different from *B. tenuissimum* in the sequence of ATTAAT (Fig. 2). Accordingly, we selected *AseI* as a candidate restriction endonuclease for our PCR-RFLP analysis. Three individuals of each typical *B. striatissimum* and *B. tenuissimum* samples (see Iguchi et al. 2007) were collected from the Sea of Japan (*B. striatissimum*, 36°30.92'N, 136°01.04'E, depth 300 m; *B. tenuissimum*, Toyama Bay). All specimens were kept in a freezer (−20°C) prior to analysis. DNA extraction, PCR procedures followed our previous methods (Iguchi et al. 2004). A total of 15 µL of each PCR product was digested with 6-bp-recognizing restriction endonuclease, *AseI* (recognition sequence: AT/TAAT) at 50°C over night. Treated PCR products were used for 2% agarose gel electrophoresis, and visualized with ethidium bromide under ultraviolet light. In addition, we collected sympatric samples of one individual of *B. striatissimum* and three individuals of *B. tenuissimum* from the Sea of Japan (36°17.78'N, 135°30.89'E, depth 520 m). Species identification was mainly based on the depth of suture (Iguchi et al. 2007). These samples were also treated as described above to perform PCR-RFLP analysis.

As expected from the result of alignment, three individuals of *B. striatissimum* showed two bands (200-bp, 261-bp) and

Fig. 3. PCR-RFLP analysis of the 16SrDNA region of typical *Buccinum striatissimum* and *Buccinum tenuissimum*. Lane designations are as follows: M, 100-bp DNA ladder (BioLabs); 1–3, *B. striatissimum*; 4–6, *B. tenuissimum*.

Fig. 4. PCR-RFLP analysis of the 16SrDNA region of sympatric *Buccinum striatissimum* and *Buccinum tenuissimum*. Lane designations are as follows: 1–3, *B. tenuissimum*; 4, *B. striatissimum*.

three ones of *B. tenuissimum* a single band (462-bp, Fig. 3). Thus, we can distinguish these two species by this PCR-RFLP method using mitochondrial 16SrDNA region and *AseI* restriction endonuclease. In addition to these six samples, six individuals of *B. striatissimum* and 11 ones of *B. tenuissimum* collected from the Sea of Japan had the same types of sequences characterized by “ATTAAT” in *B. striatissimum* and “GTTAAT” in *B. tenuissimum* (Iguchi et al. 2007, unpublished data). Thus, this PCR-RFLP method is reliable approach to

distinguish these two species. The result of PCR-RFLP analysis of sympatric samples of *B. striatissimum* and *B. tenuissimum* is shown in Fig. 4. In the lane of *B. tenuissimum*, single band was detected, and in the lane of *B. striatissimum*, two bands were found. The result strongly supports that *B. striatissimum* and *B. tenuissimum* clearly show sympatry, which suggests that these two species are likely to be reproductively isolated. However, considering the low number of specimens used in this study, more samples should be analyzed by using the method developed here and direct-sequencing because introgressive hybridization might be occurring between these two species in sympatric area. PCR-RFLP method is rapid and cost-effective method (Borgo et al. 1996, Hosoi et al. 2004). The method developed here would enable us not only to distinguish these commercially important deep-sea whelks, but also to examine the large number of samples for analysis of genetic structure of *Buccinum* species easily.

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