

**Shell and appendages variability in two allopatric ostracod species  
seen through the light of molecular data**

*Ivana Karanovic*

Department of Life Science, College of Natural Sciences, Hanyang University, Seoul  
04763, Republic of Korea

Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 49, 7001,  
Hobart, Tasmania, Australia

*ivana@hanyang.ac.kr*

*Pham Thi Minh Huyen*

Department of Life Science, College of Natural Sciences, Hanyang University, Seoul  
04763, Republic of Korea

*Hyunsu Yoo*

Marine Environmental Research and Information Laboratory (MERIL), 17, Gosan-ro,  
148 beon-gil, Gunpo-si, Gyeonggi-do, 15180, Republic of Korea

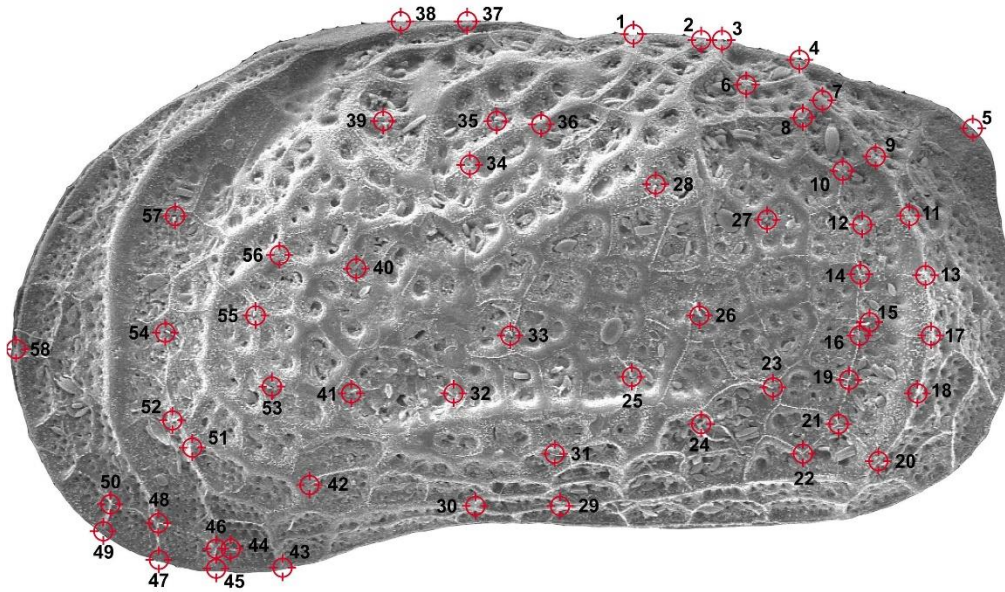
*Yuriko Nakao*

Department of Earth & Environmental Sciences, College of Humanities and Sciences,  
Nihon University, Sakurajousui 3-25-40, Setagayaku, Tokyo, 156-8550, Japan

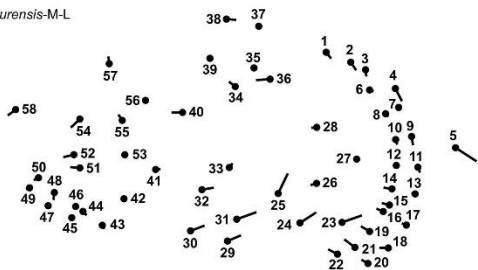
*Akira Tsukagoshi*

Department of Geoscience, Faculty of Science, Shizuoka University, Ohya 836, Suruga-  
ku, Shizuoka City, 422-8529 Japan

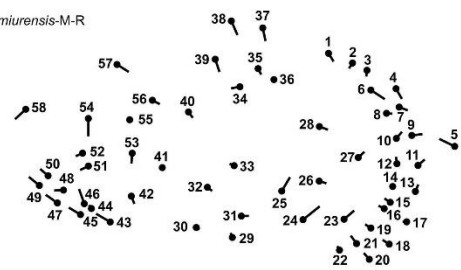
**Supplementary material**



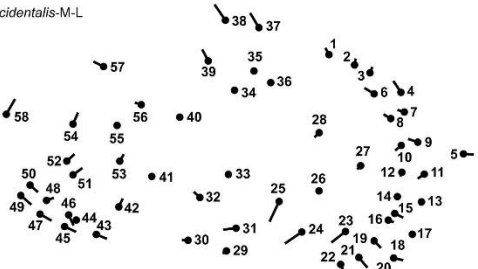
*miurensis*-M-L



*miurensis*-M-R



*occidentalis*-M-L



*occidentalis*-M-R

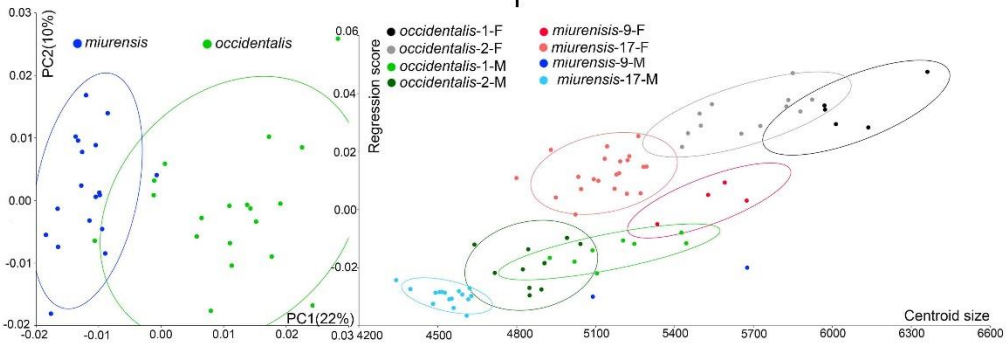
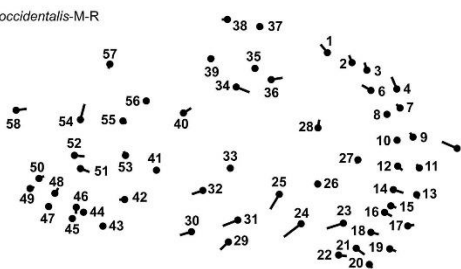


FIGURE S1 Landmark-based geometric morphometric shape analyses of the male valves. Target symbols with numbers on the left valve scanning electron microscope image of *Ishizakiella occidentalis* sp. nov. (top) represent landmarks. Middle graph: shape changes (scaled 5×) associated with species and valves relative to the mean shape of the sample consisting of males only. The bottom left scatter plot shows delimitation of species (male dataset) in the principal component analysis (based on the landmarks for specimens collected from localities 1, 2, 9, and 17). The bottom right scatter plot is a visualization of allometry analysis of the shell dataset for both sexes. Ellipses represent 95% confidence intervals, and numbers between brackets represent percentages of total variance for the first two eigenvectors.

Revised diagnosis of *Ishizakiella miurensis*: Valves are asymmetrical with most prominent shape changes in the posterior (LM 5 displaced outwardly on the LV and inwardly on the RV) and dorsal regions (LMs 37 and 38 displaced antero-dorsally on the LV and antero-ventrally on the RV). The postero-dorsal region, defined as shape changes of LM group 1–8, is shifted in the postero-dorsal direction; postero-ventral region (LMs 11–22) is more inwardly displaced, and LMs in the antero-ventral region (LMs 43–50) are shifted either ventrally on the LV or anteriorly on the RV. LMs 23–25 and 31 are all displaced posteriorly. LM 28 is shifted ventrally, and LM 27 dorsally.

The size of the shape changes in females of LM are almost the same between the valves, in males RV has more prominent shifts than the LV, especially in the antero-ventral and postero-ventral region.

TABLE S1 PCR protocols and primer pairs used for the amplification of studied DNA markers

Markers	Name of primer	Primer sequences (5'-3')	PCR settings	Reference		
Mitochondrial marker	COI	F1	TTTGGGAAGCTCTCTYAGYGCC	95°C-3 min; [ 95°C-45s; 45°C-1s; 72°C-1.5 min] 40 cycles; 72°C- 3 min; 4°C- ∞	This study	
		R1	TAYACTGTYCAACCRGTRCC			
	ITS	ITS1	TCCGTAGGTAGGTGAACCTGCGG	95°C-2 min; [94°C -30s; 52°C-40s; 72°C-1.5 min] 40 cycles; 72°C- 10 min; 4°C- ∞		White et al., 1990
		ITS3	GCATCGATGAAGAACGCAGC			
		ITS4	TCCTCCGCTTATTGATATGC			
	ITS5	GGAAGTAAAAGTCGTAACAAGG				
Nuclear markers	28S rDNA	vv	AAGGTAGCCAAATGCCTCGTCATC	95°C-5 min; [94°C -30s; 50°C-1 min; 72°C-1 min] 35 cycles; 72°C- 5 min; 4°C- ∞	Hillis & Dixon, 1991	
		xx	GTGAATTCTGCTTCACAATGATAG GAAGAGCC			
		ee	ATCCGACTAAGGAGTGTGTAACAA CTCACC			
		mm	GAGCCAATCCTTATCCCGAAGTTA CGGATC			
	18S rDNA	F1	TACCTGGTTGATCCTGCCAG	94°C-3 min; [ 94°C-30s; 48°C-30s; 72°C- 1 min] 40 cycles; 72°C- 5 min; 4°C- ∞	Yamaguchi & Endo, 2003	
		R9	GATCCTTCCGCAGGTTACCTAC			
		F2	CCTGAGAAACGGCTRCCACAT			
		R8	ACATCTRAGGGCATCACAGACC			

TABLE S2 Within and between species K2P distances for each studied marker

	<i>miurensis/occidentalis</i>	<i>miurensis</i>	<i>occidentalis</i>
COI	0.169	0.022	0.042
ITS	0.006	0	0.003
28S/em	0.003	0.001	0
28S/vx	0.001	0.001	0.001
18S	0.001	0.001	0.001

TABLE S3 *COI* K2P distances within (diagonal) and between populations (below diagonal)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1	0.00																				
2	0.07	0.01																			
3	0.17	0.16	/																		
4	0.06	0.07	0.15	/																	
5	0.05	0.06	0.13	0.01	/																
6	0.07	0.04	0.15	0.06	0.05	/															
7	0.06	0.04	0.17	0.07	0.06	0.02	/														
8	0.14	0.17	0.14	0.14	0.13	0.16	0.16	/													
9	0.17	0.17	0.09	0.14	0.13	0.14	0.15	0.10	0.02												
10	0.17	0.17	0.08	0.13	0.12	0.14	0.14	0.11	0.03	/											
11	0.17	0.16	0.07	0.13	0.12	0.14	0.14	0.10	0.02	0.00	/										
12	0.17	0.17	0.08	0.13	0.12	0.14	0.14	0.11	0.03	0.01	0.04	/									
13	0.19	0.18	0.10	0.15	0.14	0.15	0.15	0.11	0.02	0.02	0.02	0.02	/								
14	0.18	0.17	0.06	0.14	0.13	0.15	0.15	0.11	0.03	0.02	0.01	0.01	0.03	/							
15	0.19	0.18	0.10	0.16	0.15	0.16	0.16	0.12	0.03	0.03	0.02	0.03	0.00	0.03	/						
16	0.19	0.18	0.10	0.16	0.15	0.16	0.16	0.12	0.03	0.03	0.02	0.03	0.00	0.03	0.00	/					
17	0.17	0.17	0.10	0.15	0.14	0.15	0.15	0.10	0.02	0.03	0.03	0.03	0.01	0.04	0.01	0.01	0.01				
18	0.19	0.18	0.10	0.15	0.14	0.15	0.15	0.11	0.02	0.02	0.02	0.02	0.00	0.03	0.00	0.00	0.00	0.01	/		
19	0.18	0.16	0.09	0.16	0.15	0.17	0.17	0.12	0.06	0.06	0.06	0.06	0.04	0.06	0.05	0.05	0.05	0.05	0.04	/	
20	0.18	0.16	0.10	0.15	0.14	0.16	0.17	0.12	0.07	0.07	0.07	0.07	0.05	0.07	0.06	0.06	0.06	0.06	0.06	0.01	/

TABLE S4 Linear measurement (in  $\mu\text{m}$ ) of valve lengths (number of valves between brackets). LV, left valve;  
RV, right valve

Locality	Females		Males	
	LV	RV	LV	RV
1. Samcheok	113.27 (5)	114.62 (4)	105.2 (6)	102.46 (8)
2. Tanegashima	108.2 (12)	105.71 (5)	98.59 (9)	96.69 (10)
9. Aomori	107.75 (2)	106.63 (3)	105.3 (1)	109.15 (2)
17. Tokyo Bay	99.32 (19)	98.48 (17)	91.84 (19)	91.75 (15)