Pore distributional patterns of *Loxoconcha ikeyai* (Crustacea: Ostracoda: Loxoconchidae) from the Lower Pleistocene Kakio Formation in Kanagawa Prefecture, central Japan

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Abstract The ostracod genus *Loxoconcha* is widely known from modern shallow-marine and brackish water environments and also as fossils in Upper Cenozoic strata. This study examined pore distributional patterns of *Loxoconcha ikeyai* from the Lower Pleistocene Kakio Formation in Kanagawa Prefecture, central Japan, and classified 18 *Loxoconcha* species and one species in the related-genus *Loxocorniculum* in Japan and its adjacent areas. We identified two groups (Group A and B), analyzing the distribution of pores over the entire valve and below the eye tubercle. In the adult stage, the members of Group A have a total of 83–88 pores per valve, with an L-shaped pore pattern below the eye tubercle. Group B has 89–112 pores per valve, and an upside-down L-shaped pore pattern below the eye tubercle. With 83 pores per valve and an L-shaped pore pattern below the eye tubercle in the adult stage, *L. ikeyai* is included in Group A. Biogeographical data and fossil records indicate that *L. ikeyai* and the other species of Group A appeared in the Northwest Pacific around 3 Ma. We presented additional evidence for the successful diversification of the genus *Loxoconcha* in the Northwest Pacific since the Pliocene.

Key words: Ostracoda, *Loxoconcha ikeyai*, Pleistocene, pore, Northwest Pacific

Introduction

The ostracod genus *Loxoconcha* (Podocopida, Loxoconchidae) is widely distributed in shallow-marine and brackish water environments from tropical to subarctic regions (e.g., Athersuch and Horne, 1984; Athersuch et al., 1989; Horne, 2003). *Loxoconcha* is one of the most diverse ostracod genera mainly in shallow-marine areas and is common along the coast of Japan (e.g., Ishizaki, 1968; 1971; Okubo, 1980; Yajima, 1982; Ikeya and Suzuki, 1992; Zhou, 1995; Tanaka and Ikeya, 2002; Ozawa, 2010; Le and Tsukagoshi, 2014). Ishii et al. (2005) and Ozawa and Ishii (2008) investigated the pore and phylogeny of the 17 species in this genus and one species in its related-genus *Loxocorniculum* in the same family, primarily from Japan. They examined the number, distribution, and differentiation of pores on the carapace throughout ontogeny to determine the phylogenetic relationships among *Loxoconcha* and *Loxocorniculum* species.

However, the phylogenetic relationships of several *Loxoconcha* species in Japan and its adjacent areas have not yet been determined by pore analyses. The ostracod fauna from the Pacific coast of Japan has diversified since the Pliocene, including many *Loxoconcha* species (e.g., Ishizaki, 1983; Zhou, 1995; Nakao et al., 2001; Yasuhara and Irizuki, 2001; Iwata and Irizuki, 2008; Tanaka et al., 2012; Yamaguchi et al., 2012). Therefore, the detailed phylogenetic examination of *Loxoconcha* species in this area is important for elucidating the global evolutionary history of podocopids during the Cenozoic.
This study examined the pore distributional patterns of *Loxoconcha ikeyai* using fossil specimens from the Lower Pleistocene Kakio Formation in Kanagawa Prefecture, central Japan. We used the differentiation of distributional pattern of pores (DDP analysis of Kamiya, 1997) and pore distributional pattern below the eye tubercle analyses (PBE analysis of Ishii et al., 2005) to re-assign the classification of the genera *Loxoconcha* and *Loxocorniculum*. We focused on *L. ikeyai* found primarily in the Pleistocene strata on the Pacific coast of Kanagawa Prefecture in central Japan, and briefly discuss its biogeography and ecology.

**Materials and Methods**

Fossil specimens of *Loxoconcha ikeyai* (Fig. 1) were extracted from a siltstone sample RM-016729 taken from an exposure in the Tama Hills (37°35′42″N, 136°30′19″E; leg. the late Y. Kuwano, Japan) of the Lower Pleistocene (ca. 1.5–1.4 Ma; Takano, 1994; Suzuki and Murata, 2011) Kakio Formation of the Kazusa Group in Kawasaki City, Kanagawa Prefecture, central Japan (Figs. 2 and 3). This locality is the same as Loc. “Yamaguchi” (Otuka, 1932) in the bed of a small river in Yamaguchi, Asao-ku, Kawasaki City, Kanagawa Prefecture, central Japan. This sample was collected by Dr. Y. Kuwano in the 1940s. This outcrop is no longer visible on the river bed because of residential development in the last half century. This sample is repositioned in the sample collections of the National Museum of Nature and Science, Tsukuba, Japan, and is identified by numbers with the prefix RM (i.e., raw material collection for microfossil analysis).

We examined the number, morphology, and distributional pattern of pores (Fig. 4) as well as the carapace ornamentation morphology of the adult and A-1 juvenile stages of *L. ikeyai* using a scanning electron microscope (SEM), JEOL-JSM-5310 at the National Museum of Nature and Science, Tsukuba, Japan. Ostracods grow by molting (ecdysis) like other crustaceans such as shrimps, and podocopid ostracods usually undergo eight molt (instar) stages between egg and adult (e.g., Athersuch et al., 1989). The juvenile stage just prior to the adult stage is called ‘A-1’ (Fig. 5C caption, and Fig. 6), because herein ‘A’ refers to the last adult stage (i.e., the first sexually mature stage; e.g., Athersuch et al., 1989).

**Outline of the pore analyses**

Ostracod pores on the carapace are a type of sensory receptor, and based on histological studies of living species (e.g., Okada, 1981, 1982) the pore distributional pattern on the carapace surface indicates the distributional pattern of the nerve cells below the carapace. Beginning at the embryo stage in arthropods, the relationship

![Fig. 1. SEM images of *Loxoconcha ikeyai*. A, female right valve, external lateral view. B, female left valve, external lateral view. These specimens were obtained from the sample RM-016729 of the Lower Pleistocene Kakio Formation in Kanagawa Prefecture, central Japan (Fig. 2).](image-url)
Pore distributional patterns of *Loxoconcha ikeyai* (Crustacea: Ostracoda: Loxoconchidae) between the distribution of both the sensory receptors and the *Distal-less* homeobox gene (Mittmann and Scholtz, 2001) suggests that the distributional pattern of ostracod pores is conservatively controlled by genes (Tsukagoshi, 2007).

Tsukagoshi (1990) first proposed the reconstruction of ostracod phylogeny using the interspecific differentiation of pore pattern distribution for species in the podocopid genus *Cythere*. He demonstrated ontogenetic differences in the pore distributional pattern among 14 species, obtaining greater differences in later molting stages (the A-2 and A-1 juveniles and the adult). He concluded that the interspecific differentiation in the pore distributional patterns during ontogeny reflected the phylogeny of this genus. He estimated the phylogenetic relationship among these species based on the molting stage, first differentiating the pore distributional patterns in the A-2 juvenile to adult stages from those of other species.

Tsukagoshi (2001) pointed out that the pore-analysis based on phylogeny of Tsukagoshi (1990) was supported by a molecular phylogenetic analysis for approximately 600 base pairs of the mitochondrial gene cytochrome oxidase I. The results demonstrated that interspecific similarities or differences in the pore distributional patterns can be used to examine the phylogenetic relationships among ostracod species (e.g., Tsukagoshi, 2001). Tsukagoshi’s (1990) research on *Cythere* was followed by several studies of the phylogenetic relationships of species within other ostracod genera (e.g., Irizuki, 1993; Kamiya, 1997; Ishii *et al.*, 2005; Smith and Kamiya, 2005; Sato and Kamiya, 2007). Kamiya (1997) named the phylogenetic reconstruction method proposed by Tsukagoshi (1990) the “dif-
Differentiation of distributional pattern of pores analysis” (DDP analysis).

Furthermore, Ishii et al. (2005) and Ozawa and Ishii (2008) divided the 17 species in the genus *Loxoconcha* and one species in the related-genus *Loxoconiculum* in the same family into Groups A and B, based on analysis of the pore distributional pattern below the eye tubercle in later molting stages (A-3 juvenile to adult). Ishii et al. (2005) first proposed the PBE analysis for *Loxoconcha* species, and concluded that this division (i.e., Groups A and B) reflected phylogenetically related groups. Using only the PBE of the adult stage (i.e., the pattern of common pores named \(v, w, x, y\), and \(z\) in and around the common pattern of polygonal reticulations named \(A\) to \(J\) in this genus), we can immediately determine if a species belongs to Group A or B. At the adult stage, Group A species (a total of 83–88 pores on a valve; 13 species) have an “L-shaped” PBE pattern, and Group B species (89–112 pores on a valve; 5 species) have an “upside-down L-shaped” PBE pattern.

Previously these two pore-based methods (the DDP and PBE analyses) were used for phylogenetic assessments of *Loxoconcha* species. Pores

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**Fig. 4.** Close-up view of SEM images of pores of two *Loxoconcha* species. A, B, normal pores in central area (A) and mid-dorsal marginal area (B) on left valve of *Loxoconcha ikeyai*, obtained from the sample RM-016729 of the Lower Pleistocene Kakio Formation in Kanagawa Prefecture, central Japan. C, normal pores in antero-dorsal area around eye tubercle on left valve of *Loxoconcha kamiyai* from the Lower Pleistocene Omma Formation in Toyama Prefecture, central Japan (MPC-03676 in the National Science Museum, Tsukuba, Japan), modified from Ozawa and Ishii (2008).

**Fig. 5.** A, Distributional pattern of pores in adult left valve of *Loxoconcha ikeyai*. B, C, distributional patterns of pores in left valve below the eye tubercle (PBE) at the adult (B) and A-1 juvenile (C) stages. The codes for pores (\(v\)–\(z\)) and for reticulations (\(A\)–\(L\)) were designated by Ishii et al. (2005) in the PBE analysis. A black circle and triangle indicate a normal pore and a marginal pore, respectively. A shaded oval represents the eye tubercle. A dotted area oval labelled ‘ms’ represents the muscle scar area.
Pore distributional patterns of *Loxoconcha ikeyai* (Crustacea: Ostracoda: Loxoconchidae) are relatively well-preserved on the carapaces of not only living ostracods but also of modern dead or fossil ostracods without soft parts (e.g., appendages and copulatory organs), enzymes and nucleic acids. However, future determination of phylogenetic relationships would ideally be obtained by analyses of both carapace pore distribution and DNA sequence (e.g., Kamiya, 1997; Tsukagoshi, 2007). Detailed principles, procedures, strengths, and problems with determining phylogeny based on ostracod pore distributions are outlined in the following studies: Tsukagoshi (1988, 1989, 1990, 1996, 2001, 2007), Kamiya and Hazel (1989), Irizuki (1993), Ikeya and Yamaguchi (1993), Kamiya (1997), Ishii *et al.* (2005), Smith and Kamiya (2005), Tsurumi and Kamiya (2007), and Le and Tsukagoshi (2014).

**Results**

We examined the pore distributional patterns on the valves of *Loxoconcha ikeyai* (Fig. 5). The DDP analysis showed that this species has a total of 83 pores (normal and marginal pores) per valve in the adult stage (Fig. 5A). Marginal pores (5 µm in diameter) are situated on the marginal infold. Normal pores are larger (with sieve plates 10–20 µm in diameter) than marginal pores, and are found on all parts of the carapace except the marginal area. The PBE analysis for the adult and A-1 juvenile stages of *L. ikeyai* revealed the “L-shaped” pore pattern noted by Ishii *et al.* (2005) (pores v–z in Fig. 5B). This differed from the “upside down L-shaped” pattern also noted in their study. The PBE analysis for the A-1 juvenile stage revealed an “upside down L-shaped” pattern (v–z in Fig. 5C), which differs from another “L-shaped” pattern in their study. Based on the results of the PBE analysis by Ishii *et al.* (2005) and Ozawa and Ishii (2008), the 17 *Loxoconcha* species and one *Loxocorniculum* species were divided into two groups, A and B. Based on the PBE result and the total number of pores on the adult, *L. ikeyai* belongs to Group A, which includes seven species with 83 pores (Fig. 6).

Within Group A, *L. japonica* and *L. lillijeborgii* have the same total number of pores in the adult stage as *L. ikeyai* (Fig. 6), but the three species differ in pore distribution; *L. ikeyai* has two missing pores and two additional pores as compared to *L. japonica* (Fig. 7A) and *L. lillijeborgii* (Fig. 7B), respectively. A difference of a single pore in the total number of pores in the adult stage between *L. ikeyai* and *L. harimensis* was observed, with two missing pores in the dorsal and posterior areas and three additional pores in the posterior area of *L. ikeyai* (Fig. 7C).

**Discussion**

Based on the results of the DDP and PBE analyses for the adult phase, *L. ikeyai* is included in
one subgroup of Group A (Loxoconcha hattorii, L. kattoi, L. tosaensis, L. epeterseni, L. kitanipponica, L. zamia and L. prolaeta; Fig. 6). These eight species have the same total number of normal and radial pores in the adult and A-1 juvenile stages (83 and 72 pores, respectively; Fig. 6) and the same distributional pattern. The similarity of the surface ornamentation of L. ikeyai and the other seven species in the group supports this assessment. These eight species in this subgroup show (1) the existence of concentric rows of clear reticulations parallel to the anterior, ventral, and posterior marginal areas, (2) relatively distinct eye tubercles, and (3) a slightly swollen ala in the postero-ventral area.

Our compiled data indicate L. ikeyai appeared on the Pacific coast of southwestern Japan in the Late Pliocene (ca. 2.7 Ma; age data from Iwatani et al., 2011; Fig. 3). Today this species is mainly distributed in upper shelf areas in the Pacific off southwestern Japan (Zhou, 1995, unpublished data). Ishii et al. (2005) showed that the present diversification of Japanese Loxoconcha depended on the success of Group A. Most species in Group A appeared around Japan during or following the Late Pliocene, and diversified within a relatively short geological period. They developed various modes of life, with particular adaptations to phytal (seagrasses and marine algae) habitats. Among the species shown in Fig. 6, L. japonica and two other species in Group A are phytal dwellers (e.g., Ozawa and Ishii, 2008). The successful diversification of Group A was related to the prevalence of marine flowering plants, e.g., Zostera, during the Late Cenozoic (Ishii et al., 2005). The remainder of the taxa in both groups are bottom dwellers, including L. ikeyai (Ishii et al., 2005). The seagrass beds must have provided new and stable habitats not only for phytal-dwelling but also for bottom-dwelling species (Kamiya, 1988; Ishii et al., 2005).

In contrast, Group B contains several species that have lived in the area around Japan since the early Miocene (Irizuki et al., 2004; Ishii et al., 2005). The main habitats of these species are currently brackish-water areas, where the number of ostracod species is more limited than in marine environments because of unstable and variable conditions such as salinity (e.g., Ikeya and Shiozaki, 1993; Ikeya et al., 1995; Nakao and Tsukagoshi, 2002, 2008; Izuzuki et al., 2008). Group B is not as diverse as Group A despite its long his-
Pore distributional patterns of *Loxoconcha ikeyai* (Crustacea: Ostracoda: Loxoconchidae) which may be attributable to habitat conditions (Ishii et al., 2005). Our examination of the pore distributional patterns on *L. ikeyai* provided additional evidence for the successful diversification of the genus *Loxoconcha* in the Northwest Pacific since the Pliocene.

Based on the carapace shape and water-depth of the habitat, *Loxoconcha ikeyai* is considered to be a bottom-dwelling species. According to Kamiya (1988), who identified morphological difference between bottom-dwelling and phytal-dwelling taxa, the bottom-dwelling ostracods have carapaces with the rectangle lateral view and the triangle posterior view, while the phytal-dwelling taxa possess carapaces with the circle lateral view and the rugby-ball shaped posterior view. The triangle posterior view of *L. ikeyai* indicates that the species is a bottom-dwelling species. Our inference is supported by the water depth where *L. ikeyai* is found. This species was reported mainly from seafloor sediments at or deeper than ca. 100 m water depth deeper than the photic zone (Zhou, 1995, unpublished data). On the seafloors deeper than the photic zone, marine algae and plants, and phytal-dwelling ostracods would be difficult to live.

**Taxonomic Note**

Family Loxoconchidae Sars, 1926
Genus *Loxoconcha* Sars, 1926
*Loxoconcha ikeyai* Zhou, 1995
(Figs. 1A, B, 4A, B)

*Loxoconcha ikeyai*: Zhou, 1995, pl. 5, figs. 14-a, b, 15; Nakao et al., 2001, p. 141, fig. 11-3; Iwatani and Irizuki, 2008, p. 68, fig. 6-18; Ozawa, 2009, p. 234, table 1; Iwatani et al., 2011, p. 280, fig. 5-3.

*Occurrence*: Fossil, Pliocene and Pleistocene, Japan; Upper Pliocene Takanabe Formation (Iwatani and Irizuki, 2008; Iwatani et al., 2011) of Northwest Pacific coast; Lower Pleistocene Takanabe Formation (Iwatani et al., 2011), Ukari and Hijikata Formations (Nakao et al., 2001), Kakio Formation of Northwest Pacific coast (this study; sample no. RM-016729), Lower Pleistocene stratum on the Cheju Island of East China Sea (Zhou, 1995), Middle Pleistocene Naganuma Formation of Northwest Pacific coast (Ozawa, 2009). Recent, off southwestern Japan; Northwest Pacific east of Tanegashima Island off Kyushu from surface sediment obtained at 254 m water depth, Hyuga-nada region southeast of Kyushu from surface sediment obtained at 36–140 m depths, Bungo-suido region east of Kyushu from surface sediment obtained at 102–189 m depths, in Tosa Bay south of Shikoku from surface sediment obtained at 106–162 m depths, southwest of Kii Peninsula (Tanabe City) of Honshu from surface sediment obtained at 102–309 m depths, and Kumano-nada region southeast of Kii Peninsula of Honshu from surface sediment obtained at 100 m depth (Zhou, 1995). For detailed data of Recent sample localities, see Zhou (1995).

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