Variation in genetics, morphology, and recruitment in the invasive barnacle *Amphibalanus eburneus* (Gould, 1841) in the southern Korean peninsula

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Abstract

The ivory barnacle *Amphibalanus eburneus* is a marine crustacean, which presents near-cosmopolitan distribution due to extensive introduction and exhibits a wide spectrum of phenotypic variation. To elucidate geographical differentiation among populations through invasion, we investigated variation in genetic structure, shell morphology, and recruitment pattern for *A. eburneus*, from the southern Korean Peninsula where it has been established since the late 1980s. We selected samples from four populations in corresponding ecologically relevant regions representing all surrounding South Korean waters. From these we amplified the mitochondrial genetic marker cytochrome oxidase subunit I (COI) from 57 individuals and performed a populational genetic analyses with 11 additional GenBank sequences to evaluate population structure. To examine morphological variation, we applied two-dimensional landmark-based geometric morphometrics to the scutum and tergum for 148 and 151 individuals, respectively. Furthermore, we estimated the density and plate occupancy of year-old individuals in the field to compare recruitment responses among localities. We detected 33 haplotypes among the four locations belonging to three distinct clades based on moderate intraspecific pairwise genetic distance (≥3.5%). The haplotypes in these clades were not locality-specific in their distribution. In contrast, we did detect interpopulation variation in opercular shape and morphospace structure, and one population could be separated from the rest based on its distinct tergum morphotype alone. This morphologically distinct population was also differentiated by displaying the lowest mean recruitment density and level of plate occupancy. Our results indicate that although there is no relationship between molecular variation in the COI gene and geographic regions in South Korea, association with locality for operculum morphology, and recruitment response suggest ecological adaptation by this barnacle in a new habitat.

Materials & Methods
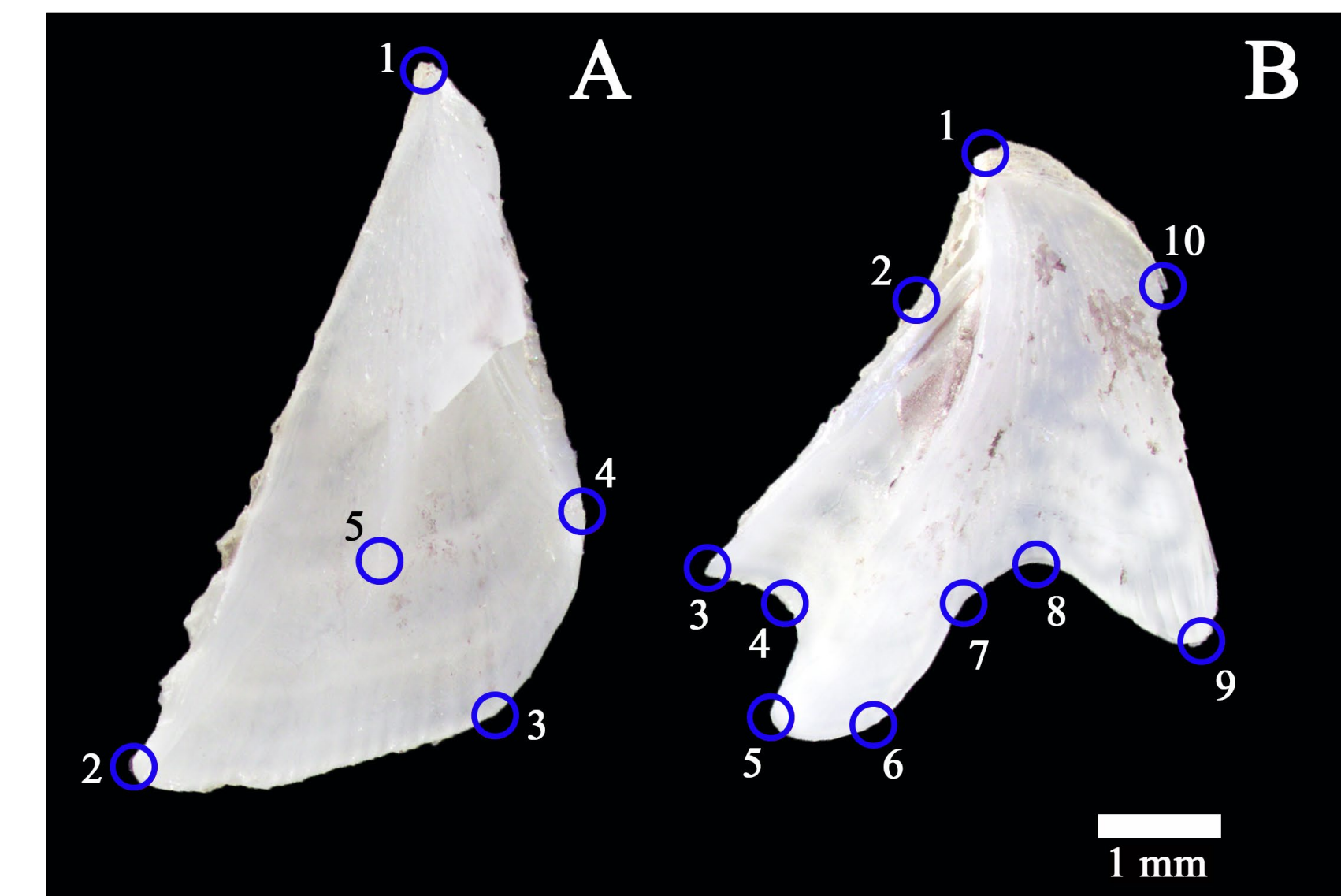
(1) Monitoring survey and sample collection
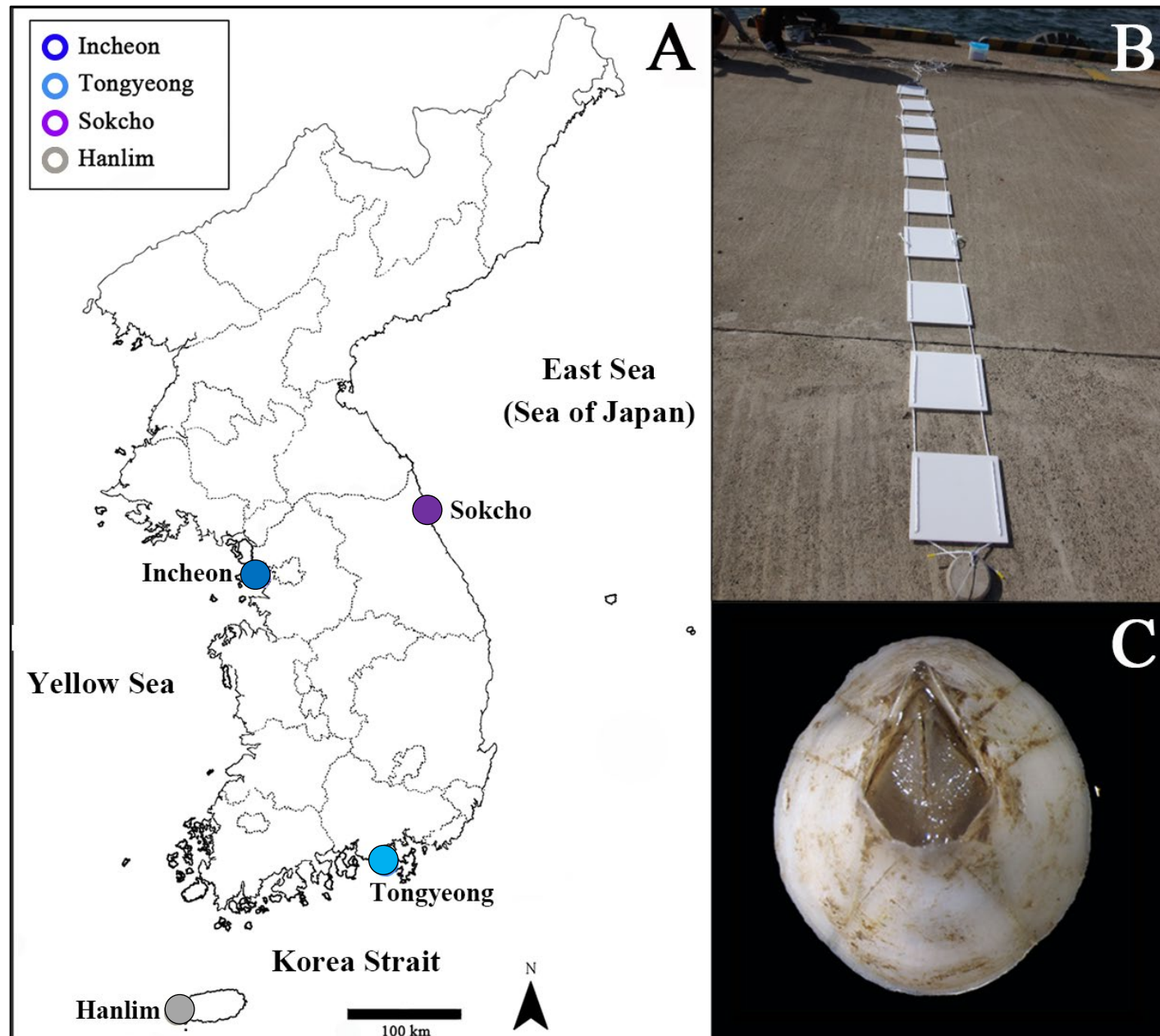
Fig. 1. (A) Map of the Korean Peninsula displaying collection sites of *A. eburneus* for this study. Blue circle: Incheon; sky blue circle: Tongyeong; Violet circle: Sokcho; grey circle: Hanlim (B) Photographs of 10 acrylic attachment plates for monitoring (C) Photograph of *A. eburneus* in dorsal view.

Fig. 2. *Amphibalanus eburneus* colony formed on the attachment plate of Hallim Port.

- Monitoring survey and sample collection were performed in four localities of the southern Korean Peninsula, Incheon (37°27′41.4″N, 126°36′49.8″E), Tongyeong (34°49′38.1″N, 128°26′03.5″E), Sokcho (38°13′36.7″N, 128°35′19.6″E), and Hanlim (33°25′11.2″N, 126°15′40.1″E) harbors (Fig. 1A).
- Attachment plate for monitoring is composed of 10 acrylic plates of 30×30cm size (Fig. 1B), and installed in April 2020 each locality and exposed to water depth of more than 3m for a year.
- In April 2021, 60, 55, 61, and 82 adult individuals were collected from the plates, respectively (Fig. 2).

(2) Genetic and geometric morphometric analyses

Fig. 3. (A) Ventral view of the left scutum with anatomical references for landmark digitization (marked by blue circles). (B) Ventral view of the left tergum with anatomical points for landmark digitization.

- Universal primer (Geller et al., 2013) were used to amplify mitochondrial cytochrome c oxidase partial (mtCOI) gene.
- Sixty eight sequences including NCBI ones were employed for the genetic analysis.
- TCS algorithm-based PopART (Clement et al., 2000) was used to generate haplotype diversity (Hd) and haplotype network.
- Photographs of five and ten anatomical references of scutum and tergum were taken under dissecting microscope.
- Two TPS files for the scutum (148 individuals) and the tergum (151 individuals) were separately generated to evaluate the geometric variation in size and shape among populations, including the asymmetry in side-by-side pairs (matching symmetry between left and right parts).
- All geometric morphometric (GM) analyses (Procrustes one-way ANOVA, PCA) were performed using Morpho J software (Klingenberg, 2011).
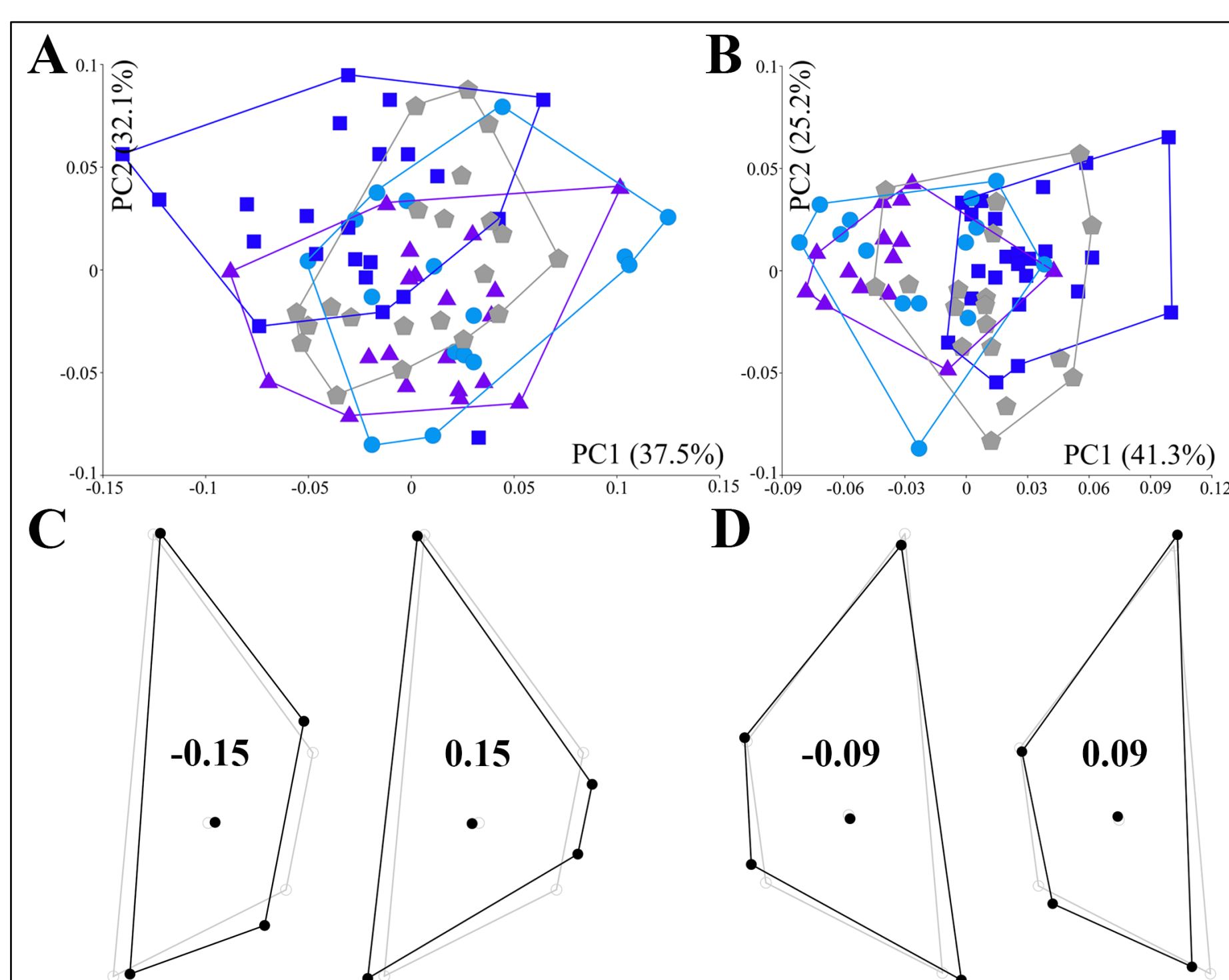
Results & Discussion
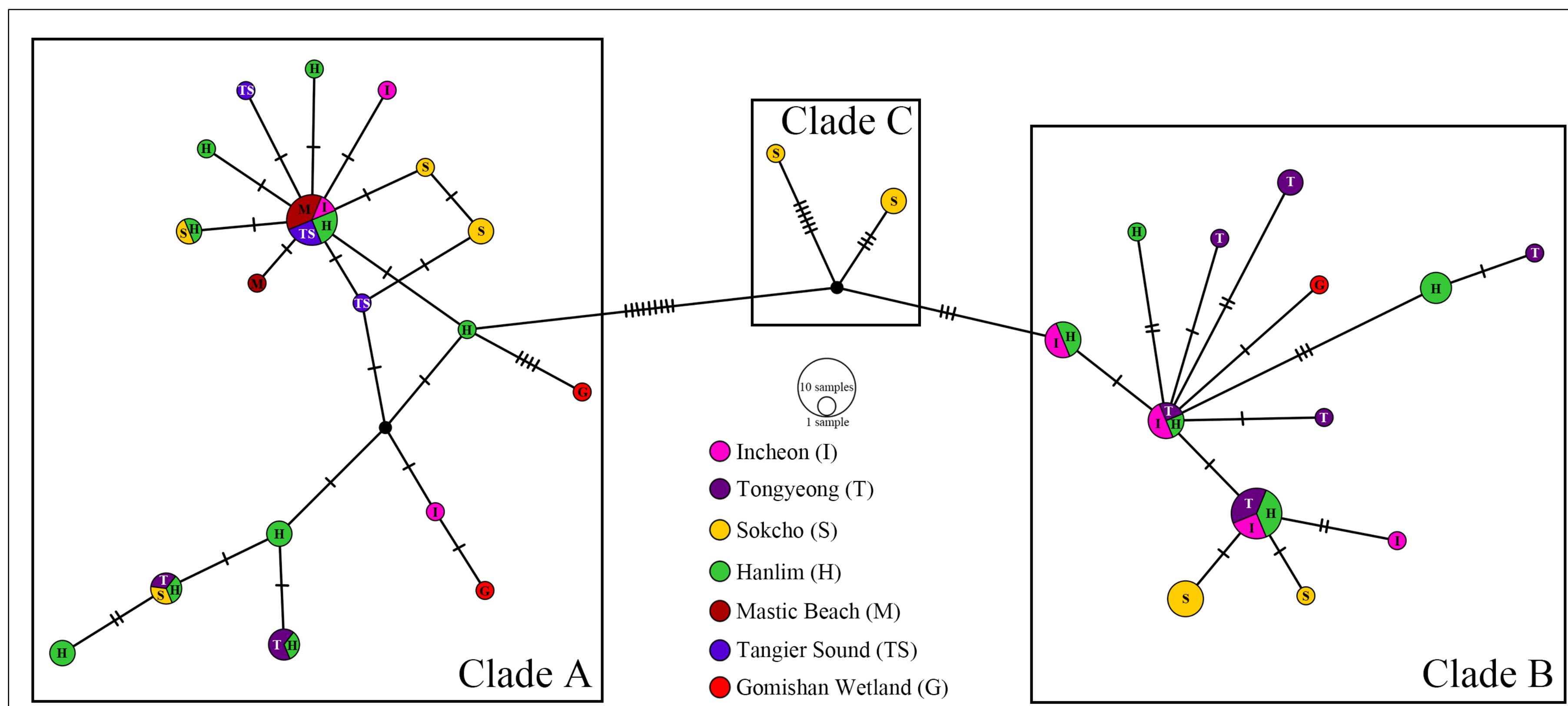
Fig. 4. TCS haplotype network generated using 68 mtCOI sequences of *A. eburneus*. Different colors and alphabets in each circle indicate different collecting sites. Sizes of nodes and pie segments are proportional to haplotype frequency. Vertical parallel lines of the network represent the number of substitutions.

- The TCS network (Fig. 4) detected 33 haplotypes (Hd = 0.961, π = 0.016) from all obtained sequences, forming three distinct clades (A, B, and C) based on moderate intraspecific pairwise genetic distance (≥3.5%).
- The haplotypes in these clades were not locality-specific in their distribution (Fig. 4).
- The ANOVA results showed that the individuals significantly varied in terms of size of the scutum (F = 7.99, p < 0.0001) and tergum (F = 3.58, p < 0.0001).
- The effect of asymmetry side-by-side was significant, contributing to the variations in the shape of the scutum (F = 5.49, p < 0.0001) and tergum (F = 6.33, p < 0.0001).
- The population most significantly contributed to the variations in the shape of the scutum (F = 7.39, p < 0.0001) and tergum (F = 8.23, p < 0.0001).
- The PCA on the left and right scutum datasets did not reveal apparently distinguishable clustering among the populations (Fig. 5).
- The PCA on the right tergum dataset revealed apparently distinguishable clustering in the Tongyeong populations (Fig. 6B, sky blue circles) showing relatively narrower tergum than mean shape (Fig. 6D).

★ **Brief discussion**
- The clade separation in the haplotype network was not locality-specific, and nearly all haplotypes of the populations were randomly placed in the three clades, indicating *A. eburneus* has been introduced in all directions of the Korean Peninsula without significant genetic differentiation suggesting that dispersal and/or delivery are intermixed. As such *A. eburneus*'s invasion success could be primarily attributed to its wide range of adaptability in various salinity and water temperatures, comparable to other introduced balanomorph species.
- Our results might indicate a possible genetic relationship between the Korean and Caspian populations, although the latter region is geographically isolated at a large distance from the Far East.
- Our LBGM analysis revealed substantial geometric variations in the opercular plates of *A. eburneus*, which are reported for the first time in the study of barnacles.
- In our dataset, individuals from the Sokcho population (Fig. 6A and B) showed obvious asymmetry in the tergum, forming a distinct cluster in the left and right morphospace of the PCA biplot. The two sides of the opercular plate have likely traced independent evolutionary paths in terms of shape, which may lead to incorrect population or species identification depending on which of the two sides is selected for taxonomic examination.
- Regarding population differentiation, the Tongyeong population could be clearly differentiated from the rest based on variations in the shape of the tergum on both sides of the opercular plate.
- Despite the inability to predict major factors driving the morphological variation in *A. eburneus* in Tongyeong population, we demonstrated an unique phenotypic form of *A. eburneus* among diverse genotypes at a single location.

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Kim et al. (2022): http://dx.doi.org/10.7717/peerj.14002

Fig. 5. Principal component analysis of residuals of the scutum shape coordinate. (A) Scatter plot of the left scutum. Principal components 1 and 2 are indicated on x-axis and y-axis, respectively. (B) Scatter plot of the right scutum. Blue squares: Incheon individuals; sky blue circles: Tongyeong individuals; violet triangles: Sokcho individuals; grey pentagons: Hanlim individuals. (C) Wireframes of shape change in the left scutum (black line) corresponding to the PC score against the mean shape (grey line). (D) Wireframes of shape change in the right scutum.
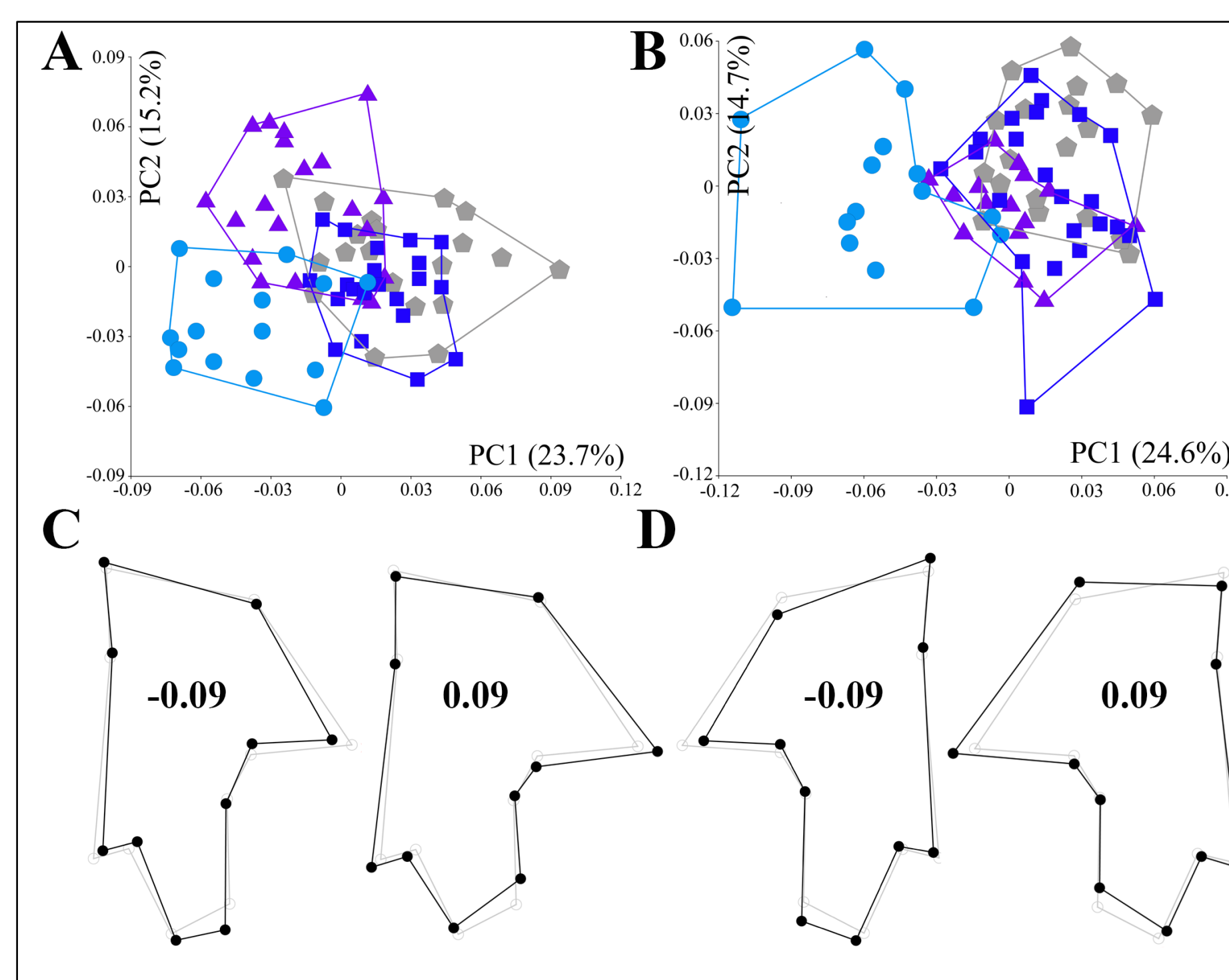
Fig. 6. Principal component analysis of residuals of the tergum shape coordinate. (A) Scatter plot of the left tergum. Principal components 1 and 2 are indicated on x-axis and y-axis, respectively. (B) Scatter plot of the right tergum. Blue squares: Incheon individuals; sky blue circles: Tongyeong individuals; violet triangles: Sokcho individuals; grey pentagons: Hanlim individuals. (C) Wireframes of shape change in the left tergum (black line) corresponding to the PC score against the mean shape (grey line). (D) Wireframes of the shape change in the right tergum.

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