Population connectivity of *Dascyllus trimaculatus* among the different MPAs in Zamboanga del Norte using geometric morphometric analysis

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Abstract. The study of connectivity between populations is an important element for the design of ecologically coherent networks of marine protected areas (MPAs). Geometric morphometrics, a tool widely used in studying shape variation in fish populations, was utilized to analyze the three-spot damsel (*Dascyllus trimaculatus*) in different MPAs in the Northern Zamboanga Peninsula. A total of eleven (11) sets of landmarks were measured using Tps Dig v.2 to determine the patterns of connectivity in the different National Integrated Protected Areas System (NIPAS) sites. Applying R software with the Geomorph package, Generalized Procrustes Analysis (GPA) was used. Generated relative warps were used to determine the different body shape variations between fish samples. Relative warp scores were subjected to Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA). The variability of about 47% was explained by two principal components. It was observed that clusters from the scatterplot were overlapping which suggests that populations in terms of morphology were connected. These overlapping clusters are more likely to share and exchange populations with each other. Local management for these sites within the Zamboanga del Norte MPAs must be integrated. A more holistic approach must be done to fully comprehend the connectivity pattern among the studied population. Molecular approaches like microsatellites coupled with morphometric analysis may provide accurate results.
Key Words: *Dascyllus trimaculatus*, fish morphology, landmark-based, marine protected area.

Introduction. Tropical countries like the Philippines are home to many reef fishes including three-spot damsel *Dascyllus trimaculatus*. Most coral reef fishes exhibit a bipartite life with a pelagic larval phase and a benthic adult phase. In case of *D. trimaculatus*, it has relatively short pelagic larval stages for about 22-26 days (Wellington & Victor 1989; Bernardi et al 2003; Leray et al 2010). Its recruits settle into anemones and spend a few months as juveniles before migrating to the reef. Juveniles that survive in the anemones have greater survivorship in the transition from the anemone to coral reefs (Salas et al 2019). Through its larval stage, fishes mostly travel away compared to its adult phase. Ocean currents carry the larvae, while the adults actively swim from one location to another (Holbrook & Schmitt 1997).

Ecological spatial connectivity is the movement and dispersal of eggs, spores, larvae, and adult individuals between different entities (Carr et al 2017). Determining this dispersal ability serves as an important task for understanding the ecology of populations and the predictions relating to the population distribution and dynamics (Botsford et al 2009; Weersing & Toonen 2009). This will serve as a tool for identifying the source and sink populations to properly manage them, especially in an identified marine protected area (MPA). The role that marine protected areas (MPAs) can play in promoting the health of our oceans and seas has been recognized at the highest levels (WCPA/IUCN 2007). The study of connectivity between populations is an important element for the design of ecologically coherent networks of MPAs (Balbar & Metaxas 2019; Salas et al 2020; Podda & Porporato 2023).

Zamboanga del Norte is a province in the Philippines located in Northern Mindanao. It is bordered to the north and west by the Sulu Sea, to the east by Misamis Occidental, and to the south by Zamboanga del Sur. It has a total of three registered islands under the National Integrated Protected Areas System (NIPAS) Act of the Philippines namely, Aliguay Island Protected Landscape & Seascape (AIPLS), Murcielagos Island Protected Landscape and Seascape (MIPLS), and Selinog Island Protected Landscape and Seascape (SIPLS).

A common way of studying marine connectivity can be done by utilizing genetic markers by directly studying the population structure, but this comprises delicate and complex reactions and is usually financially expensive (Ilustre et al 2014; Gagnaire et al 2015). An alternative way of inferring connectivity is by the use of morphometric data analysis. Geometric morphometric (GM) can either be a landmark-based analysis (using a set of landmarks to describe the object or specimen) or an outline-based analysis (using the margin of the specimen) (Richtsmeier et al 2002). This powerful technique can capture differences in structures that are not easily observed through traditional types of measurements or by the naked eye. It has greatly improved stock identification and discrimination in fish (Cadrin & Friedland 1999; Dierickx et al 2023). Thus, GM has been used in various studies on fish population biology (Santos & Quilang 2012; Luo 2024).

In this study, landmark-based geometric morphometrics was utilized to investigate population differences in body shapes of *D. trimaculatus* from the MPAs in Zamboanga del Norte. It also aims to identify whether these morphometric differences can produce distinct population signatures in the studied *D. trimaculatus* from the different MPAs. This will help the local authorities of Zamboanga del Norte specifically the respective protected area management office, to enhance their existing management and conservation measures.

Material and Method

Description of the study sites. There is a total of three (3) established NIPAS sites (represented by blue circles) in the Zamboanga del Norte, namely: SIPLS, AIPLS, and MIPLS (Figure 1). An adjacent site was also added for the sample collection, specifically the Dapitan mainland.

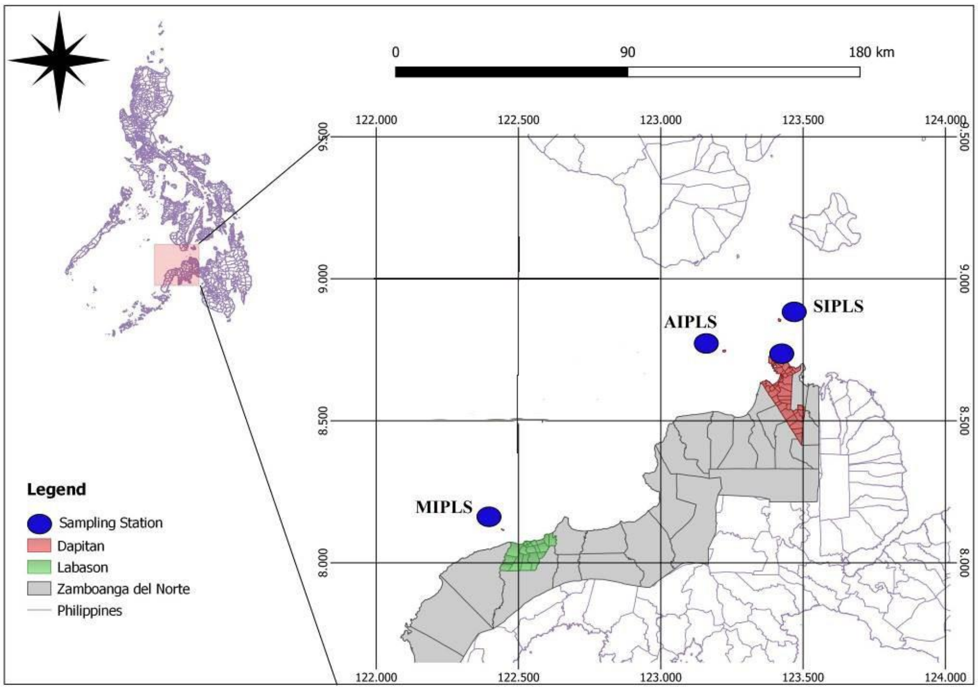

Figure 1. Map of Northern Zamboanga Peninsula showing locations of the sampling sites from Selinog Island.

Sample collection and digitization. Samples of *D. trimaculatus* were collected from the different MPAs and sampling locations from the months of April to September 2022.

Prior to the collection of samples, the study was presented during the Protected Area Management Bureau (PAMB) meeting for each protected area site. Permits were also gathered from the local government unit.

Fish samples were subjected to morphological examination. A photograph was taken prior to the fin and tissue sample extraction. Geometric morphometrics were performed on the digital pictures of the collected samples. Image acquisition of fishes were done using a DSLR (Fujifilm X-A7) camera mounted on a tripod for stability and uniform focus. Standard positioning was followed so as to show their natural position when in water.

Sample digitization. Fish samples were subjected to morphological examination. The landmark-based geometric morphometric analysis involves analyzing the pictures taken using the software Tps Dig Version 2.12. The software allowed the processing of the landmark data statistically. The figure below shows the homologous plotted anatomical landmarks utilized in the analysis of the variations in *D. trimaculatus* (Figure 2).

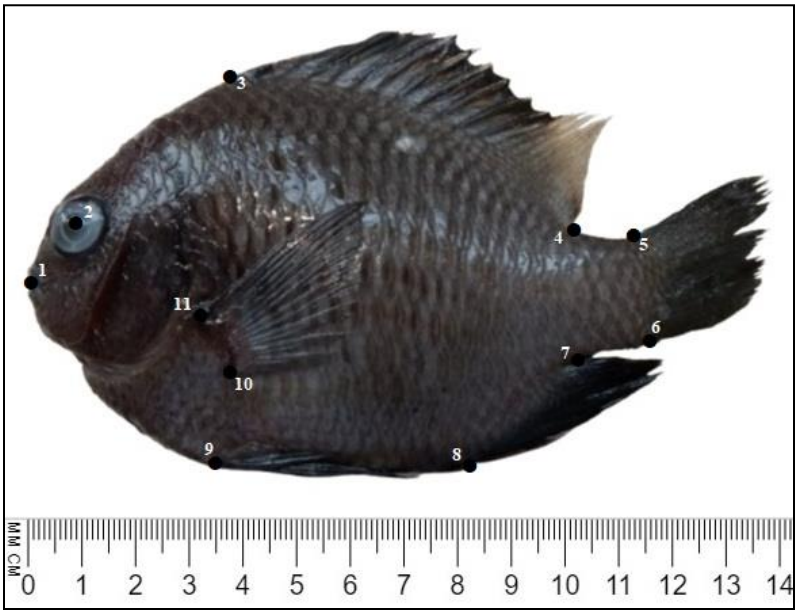

Figure 2. Set of 11 landmarks to be used: (1) anterior tip of the snout, (2) center of orbit, (3) and (4) anterior and posterior insertion of the dorsal fin, (5) and (6) upper and lower insertion of the caudal fin, (7) and (8) posterior and anterior insertion of anal fin, (9) insertion of the ventral fin, (10) and (11) lower and upper insertion of pelvic fin.

Statistical analysis. From their digitized landmarks, the geometric conformations composed of x and y coordinates were converted first into shape variables before subjecting them to statistical analyses of shape variation. Applying R software with the Geomorph package, generalized Procrustes analysis (GPA) was utilized. Generated relative warps were used to determine the different body shape variations between fish samples. Relative warp scores were subjected to principal component analysis (PCA) and discriminant function analysis (DFA) to further analyze the differences between fish samples collected from the different MPAs in Zamboanga del Norte.

Results. A total of seventy (70) *D. trimaculatus* samples were collected from the different MPAs in Zamboanga del Norte, including the adjacent Dapitan mainland. Some areas provided fewer fish samples due to the minimal availability of the studied species. Figure 3 shows the linked landmarks measured and analyzed for Procrustes analysis.

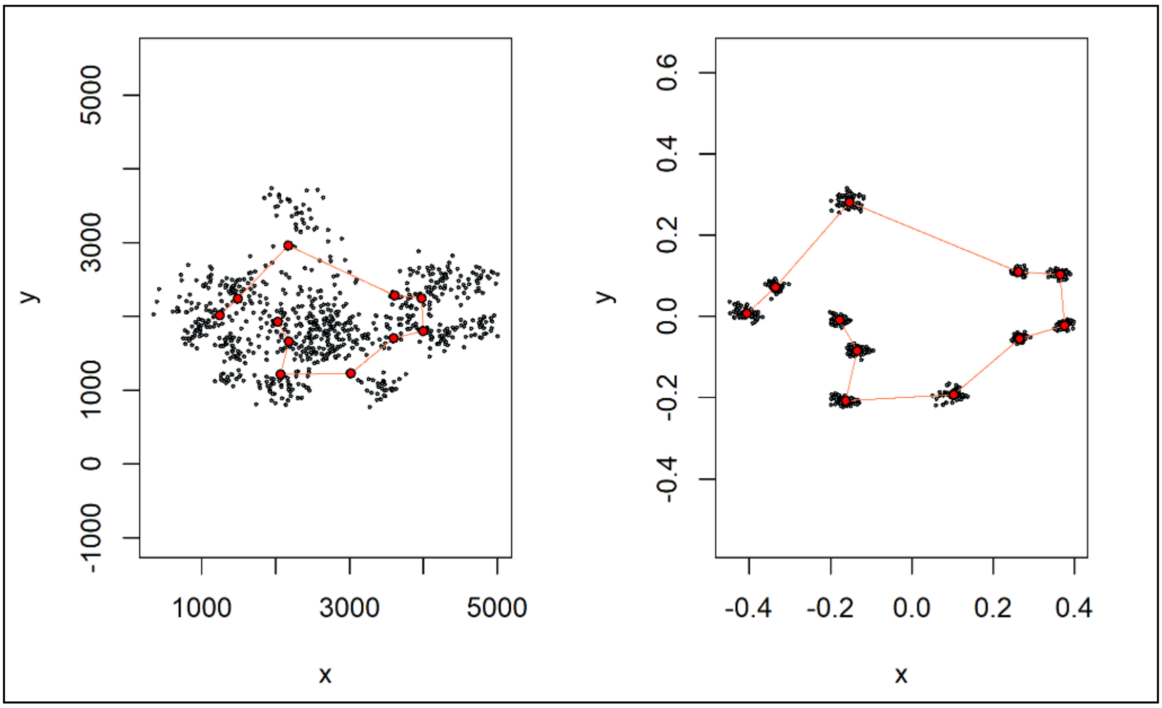

Figure 3. Landmarks for Procrustes analysis of the collected samples.

Principal component and discriminant function analyses. The results revealed eighteen (18) components, which were utilized to determine the differences in fish morphology (Figure 4). Two characters (PC1 and PC2) constituted 47% of the total variation across sites. Principal component 1 (PC1) explained 30.17 % of the total variance while principal component 2 (PC2) explained 17.41 % of the total variance. Scree plots of the eigenvalues of the principal components were also shown (Figures 5 and 6).

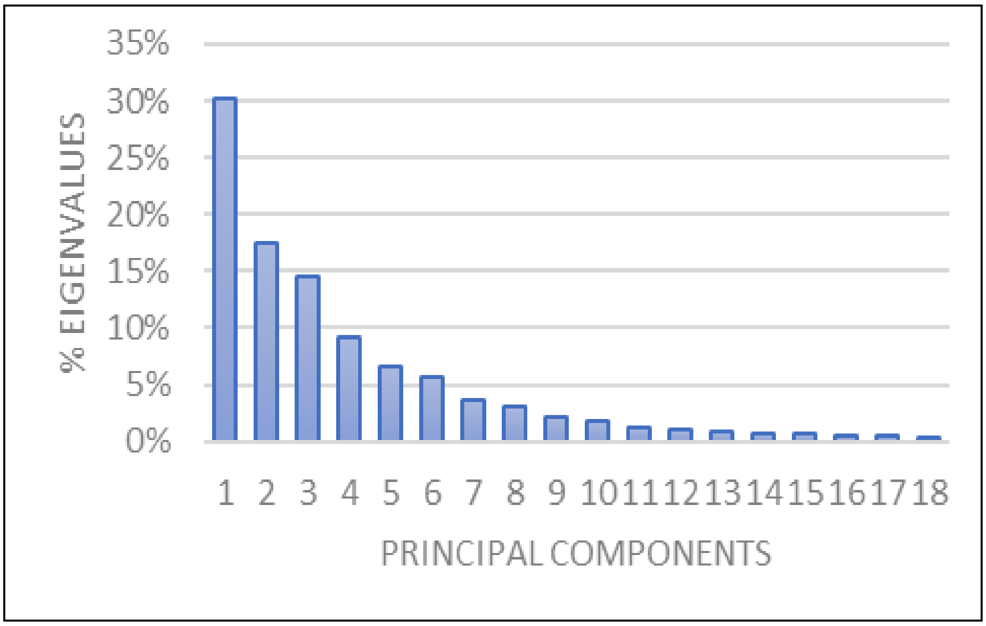

Figure 4. Principal component values against percent eigenvalues of *D. trimacullatus*.

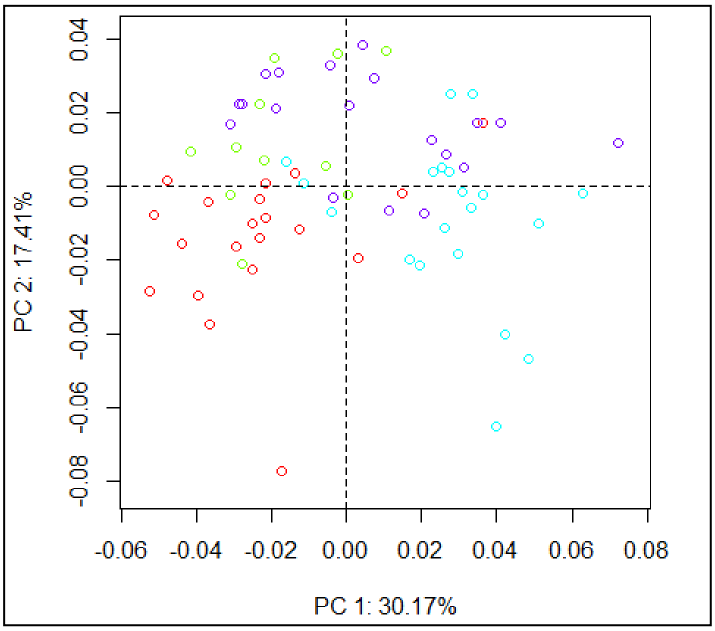

Figure 5. Scatter-plot from principal components analysis (PCA) of *D. trimacullatus*. Principal component 1 (PC1) explained 30.17 % of total variance while principal component 2 (PC2) explained 17.41 % of the total variance.

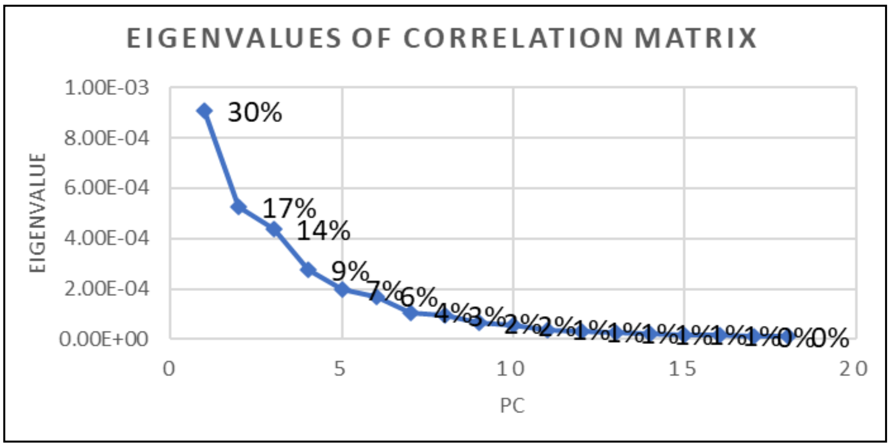

Figure 6. Scree plot of the eigenvalues of the principal components.

Figure 7 provides us with an idea of how the differences in fish morphology are identified and differentiated from each other. The figure visually presented us the differences between the four study areas in terms of body shape. The four character traits were also presented in the vector deformity at both PC1 and PC2 minimum and maximum values explaining shape variations. The same set of characters was utilized for the DFA. Similar to the results from PCA, no clear groupings were formed (Figure 8). However, they formed a homogenous big cluster. Some samples were observed to be separated from the cluster, but are still not distinctly detached.

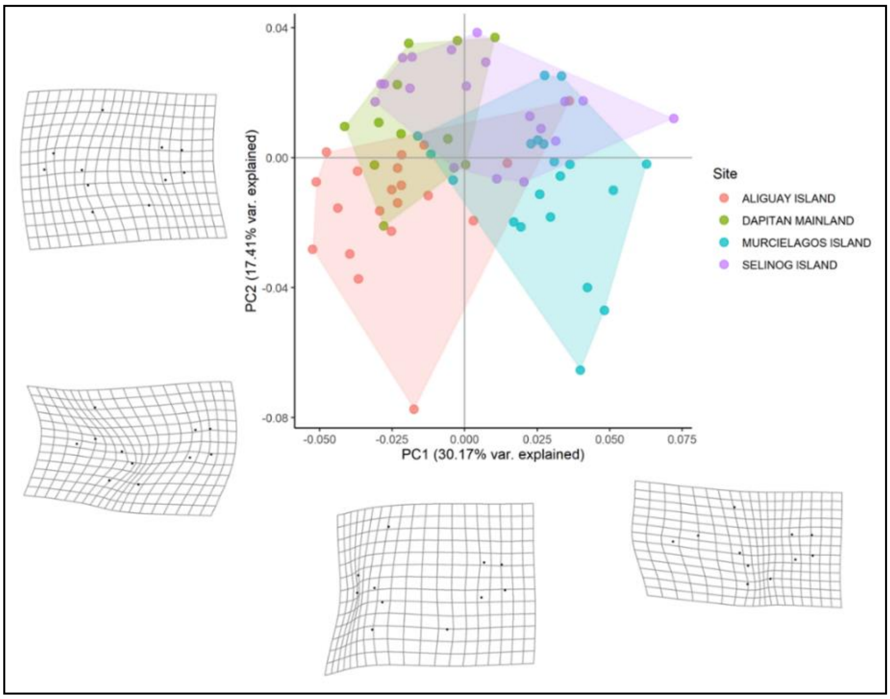

Figure 7. Principal components analysis of four population; (pink) AIPLS, (green) Dapitan mainland, (aqua) MIPLS, and (purple) SIPLS of *D. trimacullatus* scatterplot with vector deformity at PC 1 and PC 2 minimum and maximum value.

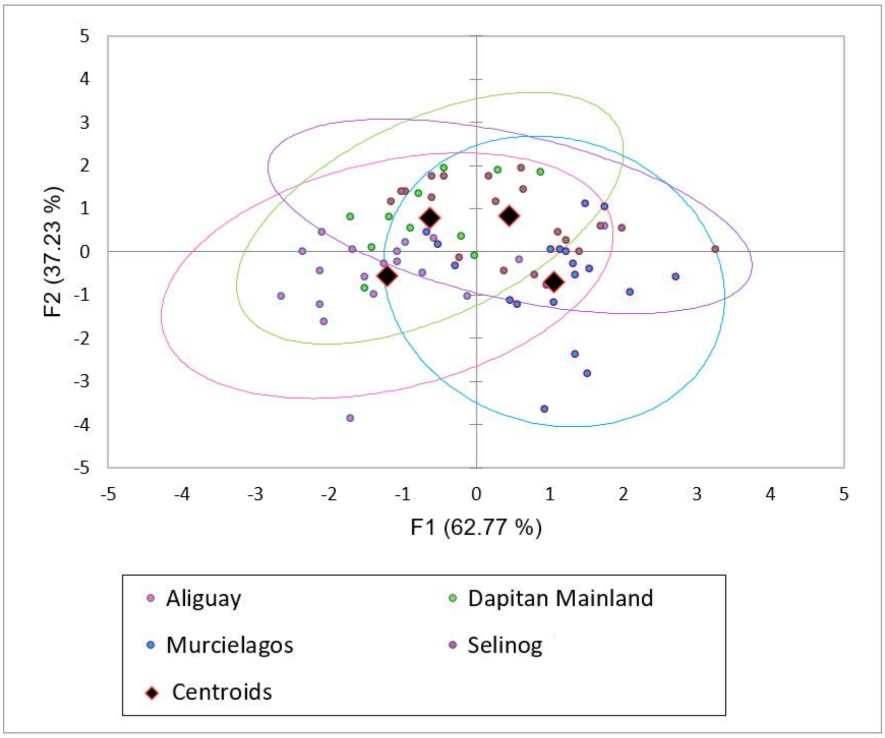

Figure 8. Discriminant function analysis of the *D. trimacullatus* from different sampling population; (pink) AIPLS, (green) Dapitan mainland, (aqua) MIPLS, and (purple) SIPLS.

Discussion. The analyses indicate a general morphological homogeneity among *D. trimaculatus* populations across the different study sites in Zamboanga del Norte. The PCA results showed that the variation in fish morphology could largely be explained by just two principal components, PC1 and PC2, which together accounted for nearly half of the total variance. The low eigenvalue further supports the notion of minimal morphological differences among the populations.

The absence of distinct morphological differences might be due to the oceanographic conditions affecting the region. Specifically, the currents moving southwest from the Bohol Sea during the Northeast monsoon are affected by rising water levels in the Pacific side and the influence of the Sulu Sea currents during the reversal from the Southwest monsoon likely facilitate gene flow and exchange between populations. South China Sea current influenced Sulu Sea waters as it passed through the Northern Zamboanga Peninsula area through Dipolog Strait and eventually to the Bohol Sea in the Southwest monsoon. This constant movement and mixing could contribute to the observed morphological homogeneity (Wyrtki 1961; Meñez et al 2006; U.S. Navy 2007; Han et al 2009; Cabrera et al 2011; Nakajima et al 2023; Naguit 2015).

Similar findings have been reported in other regions. For example, Binashikhbubkr et al (2022) observed morphological homogeneity among populations on the east coast of Peninsular Malaysia. Similarly, Ilustre et al (2014) found no significant morphological distinctions among populations in the Bohol Sea, suggesting that population exchanges likely occur in these areas.

These observations highlight the role of ocean currents in shaping the population structure and morphology of marine species. The exchange facilitated by these currents may prevent significant morphological divergence among populations, leading to the homogeneity observed in *D. trimaculatus*.

Conclusions. The study attempts to utilize a geometric morphometric approach on *Dascyllus trimaculatus* to imply population connectivity among marine protected areas in Zamboanga del Norte, as a basis for management. Data from principal component analysis and discriminant function analysis revealed that clusters were overlapping, which suggests that populations in terms of morphology were connected. These overlapping clusters are more likely to share and exchange genetic materials with each other. Local management for these sites within the Zamboanga del Norte marine protected areas must be integrated with each other. A more holistic approach must be done to fully comprehend the connectivity pattern among the studied population. Molecular approaches like microsatellites coupled with morphometric analysis may provide accurate results.

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Conflict of interest. The authors declare that there is no conflict of interest.

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