The relationship between weight and morphometric size of genus *Margarites* (Mollusca) in Manado coast, North Sulawesi

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Abstract. This study aims to analyze the relationship between the weight and morphometric parameters of the genus *Margarites*. Sampling used quadrate line transect, and the morphometric data were determined using ImageJ software, while data analysis used principle component analysis (PCA) to reduce the data, then applied a multiple regression. Results showed that FERET ANGLE variables influenced the weight of the genus *Margarites* so these variables have a very important role in the growth of the genus. All morphometric variables could be included in the model ($p < 0.05$) to explain the weight variable.
Key Words: ferret angle, imageJ, multiple regression, PCA.

Introduction. Gastropods (Mollusca) have an important role in the coastal ecosystems and are one of the non-fish living resources with high diversity. They are widely distributed in various marine habitats spreading in almost all continents (Ruppert et al 2004; Strong et al 2008). Mollusca comprises of about 120,000 species (80% Gastropod, 15% Bivalvia) and thus is the second most diverse animal phylum occurring in all major environments except aerospace. It is also the leading phylum concerning endangered and vulnerable species. Mollusca are bilateral (or secondarily asymmetrical) Protostomia (Lophotrochozoa) (Haszprunar 2001). Also, their diversity in all aspects of morphology, physiology, behavior, genetics, and ecology is remarkable. Most species are equipped with external spicules or shell (plates) and a foot, a mantle cavity with gills, a ciliary or muscular gliding sole (foot), a visceral portion with heart and excretory organs, and an alimentary tract with rasping tongue (radula). The body cavity is mesenchymal with a gonopericaridal coelomic system. Development starts with a spiral cleavage, mostly followed by (often modified) a trochophore-like larva (Byrne 2011). The shell is made of dissolved carbon. Gastropods move using their belly muscle (Hidayat et al 2004). They are distributed from the coastline to the deep sea, but mostly inhabit shallow waters. In the littoral zone, gastropods are influenced by tides (Suwignyo et al 2005; Bulahari et al 2019). In areas with strong waves and currents, they generally live in dense populations (Sotto & von Cosel 1982).

According to Nurracmi & Marwan (2012), gastropods are closely related to the availability of organic matter in the bottom substrate. Estimates of the about 85,000 (minimum 50,000, maximum 120,000) described species of Mollusca with approved names can be used to determine the number of gastropod species (Chapman 2009).

According to Georgiev et al (2009), the abundance of gastropods is subject to the availability of food such as detritus and macroalgae, and they prefer habitats that are protected from currents, waves, and direct sunlight. The occurrence of molluscs is dependent upon the availability of nutrients as important materials in a biological function that can limit the productivity in an ecosystem, such as growth (Santoso 2017).

This research activity has the following objectives: (1) to know and estimate the weight and morphometry of the genus *Margarites*, (2) to analyze the morphometric relationships in Manado Beach.

Material and Method. The study was carried out from July to September 2022 in Groin of Malalayang Beach, Malalayang district, Manado, North Sulawesi (Figure 1). Sampling site selection used a purposive random sampling based on the topographic condition with the focus on the beach ridge in the littoral area of Manado beach by placing 2 stations, the left part of Groin and the right part of Groin (Figure 1).

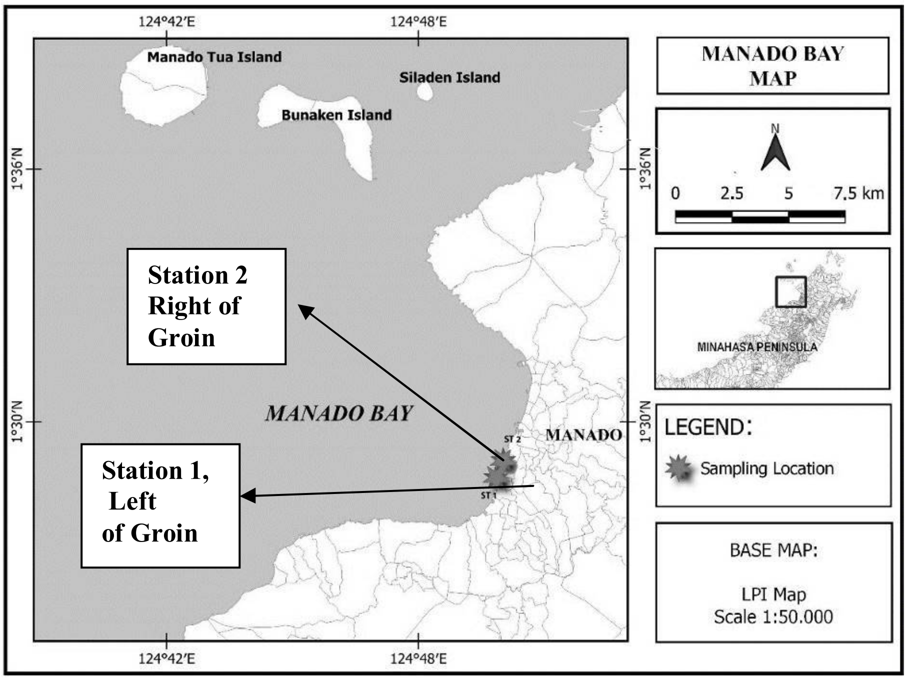

Figure 1. Study site.

As many as 108 individuals of genus *Margarites* (Mollusca) were randomly collected using a small scoop at the lowest tide. All samples were preserved in alcohol 70% and brought to the Laboratory of the Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Manado. The samples were then decantated and put into one liter of 4% formaldehyde added with one gram of rose Bengal for staining, then filtered using a set of sieves to obtain the desired mesh size. The individual weight of the sample was obtained using an MH-Series pocket scale-typed multifunction electronic scale, while the morphometric measurements used image-J software.

Data analysis. As mentioned above, morphometric data were analyzed using ImageJ software. The parameters measured are described as follows:

AA = the selected value is in square pixels. An area is a unit that is calibrated, such as square millimeters, square centimeters, and others;

PE = perimeter is the length of the selection's outer boundary;

CI = circ. (circle): 4п * area/perimeter ^ 2. A value of 1.0 indicates a perfect circle. Getting closer to 0.0 indicates an elongated shape. The value may not be valid for very small particles;

F = feret size is based on mean statistics after rotating the object through all possible different angles. Feret diameter is the longest distance between two points along the selected area, also known as the maximum caliper; Feret X and feret Y are the coordinates of the initial feret diameter (on X and Y axes):

FX = feret X is the coordinate of the initial feret diameter (on X axis);

FY = feret Y is the coordinate of the initial feret diameter (on Y axis);

FA	=	feret angle is the feret value (0-180 degrees), the angle between the feret diameter and the line parallel to the X axis of the image;
MF	=	MinFeret is the minimum caliper diameter;
AR	=	AR (aspect ratio): major_axis / minor_axes;
RO	=	rotation (roundness): 4 * area / (π * major_ axis ^ 2), or the reciprocal of the aspect ratio;
SO	=	solidity: area / convex area.

The morphometric data were reduced using principle component analysis (PCA) to reduce the data that possibly yield collinearity. Euclidean distance is the metric mostly used to estimate the similarity of two factors. The formula of the Euclidean distance is the squared root o difference between 2 vectors (Wurdianarto et al 2014) as follows:

$$d(i,j)=\sqrt{\sum_{k=1}^{n}(x_{ik}-x_{jk})^2}$$

where: d(i,j) = dissimilarity degree); n = number of vectors, x_{ik} = input vector; x_{jk} = output vector. Data analysis used Excel 2007 and version 14-add-in XLSTAT 2014.5.03 Final Incl. Patch & Serial-MPT [ATOM].

Multiple regression analysis. The reduced measurement data of genus Margarites were analyzed using a multiple regression approach (Sokal & Rohlf 1981) to measure the relationship between variables as follows:

$$Y_i = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_4X_4+\beta_5X_5+\varepsilon$$

$$i = 1, 2, ..., n$$

where: Y_i (weight) is a dependent variable and X_i (morphometric variable) is an independent variable, with the hypothesis (H_0): $\beta_1 = \beta_2 = \beta_3 = ... = \beta_i = 0$ that any morphometric variable does not influence the weight, or the hypothesis (H_1) that there is at least one morphometric variable (X) influencing the weight.

Results. The correlation coefficient estimation in PCA used the Pearson method. Tables 1 and 2 demonstrate the observation data before and after reduction.

Table 1

Observation data before reduced

Variable	*Observations*	*Obs. with missing data*	*Obs. without missing data*	*Minimum*	*Maximum*	*Mean*	*Std. deviation*
AREA	108	0	108	0	1.61	0.69	0.39
PERIM.	108	0	108	0.03	6.48	3.89	1.48
CIRC.	108	0	108	0.18	1.00	0.57	0.19
FERET	108	0	108	0.01	1.99	1.15	0.46
FERET_X	108	0	108	3.86	18.90	11.52	4.43
FERET_Y	108	0	108	2.02	13.38	7.56	3.31
FERET_ANGLE	108	0	108	67.92	153.44	96.06	14.77
MIN_FERET	108	0	108	0.01	1.24	0.78	0.27
AR	108	0	108	1.00	4.73	1.43	0.36
ROUND	108	0	108	0.21	1.00	0.72	0.09
SOLIDITY	108	0	108	0.73	1.00	0.94	0.04

Table 2

Observation data after reduced

Variable	*Observations*	*Obs. with missing data*	*Obs. without missing data*	*Minimum*	*Maximum*	*Mean*	*Std. deviation*
AREA	108	0	108	0.0001	1.6130	0.6934	0.3881
PERIM.	108	0	108	0.0250	6.4760	3.8922	1.4849
FERET	108	0	108	0.0120	1.9910	1.1520	0.4595
FERET_ANGLE	108	0	108	67.9200	153.4350	96.0630	14.7681
MIN_FERET	108	0	108	0.0090	1.2350	0.7844	0.2730

Table 1 and Table 2 demonstrate that the mean value of FERET ANGLE is higher than that of other variables indicating that the FERET ANGLE variable influences the weight of the genus *Margarites* and thus it has a very important role in the growth of this genus.

Based on the correlation matrix (Tables 3 and 4), it is apparent that several variables have relationships with each other. Low correlation is shown in Tables 3 and 4, with black color and a correlation coefficient below 0.70, namely CIRC, FERET X, FERET Y, FERET ANGLE, AR, ROUND, and SOLIDITY. It means that the 7 variables could be reduced. However, in the PCA context, these 7 variables could be explained through their positions on axis 1. The position approaching axis 1 indicates the strength of the effect. The closer to the correlation circle is, the higher the effect on the model so these variables are the potential to be included in the model, while the position approaching axis 2 and far from the correlation circle indicates a weak effect and thus, the variables are potential to be removed from the model. Figure 2 shows the AR, SOLIDITY, and ROUND approach to axis 2 (F2) so that these variables could be reduced.

The sufficiently close relationship (Tables 3 and 4, blue color) is indicated by a correlation coefficient above 0.70, namely PERIM, FERET, and MIN FERET, so these variables were included in the model. The correlation between variables could be divided into positive correlation and negative correlation. The former indicates that the change in a variable has resulted in the other shift in the same direction. In other words, an increase in the morphometric variable will result in a weight increment. The latter indicates that an increase in one variable will make the other decrease. According to Sugiyono (2012), the correlation coefficient ranges from 0.00 to 1.000, the higher the coefficient is, the stronger the ability of x can explain the y variable.

Figures 2 and 4 indicate that the variability on axis 1 to axis 2 reaches 0.6098, meaning that the data variance could be explained up to axis 2 as much as 60.98%, while the rest, 39.02% could be explained by axis 3 to 8. If the axis 3 and 4 are included to describe the variance, it will reach 84.53%. It means that PCA could be relied upon to analyze the relationship between weight and the morphometric variables.

Table 5 shows the cumulative eigenvalue obtained from axes F1 and F2, 60.98%, reaches 84.53% in F4; after the data have been reduced (Table 6), the cumulative eigenvalue rises to 95.05 from axis F1 to F2 indicating that 95% of the data variability could be explained up to axis 2 (F2) and rests 5% by axis 3 to F5, so that the PCA could be relied on to analyze the relationship between weight and the morphometric variables. According to UCLA (2022), the eigenvalue represents the total number of variances that can be explained by the major component used. It could be positive or negative in theory, but it explains positive variances in practice. If the eigenvalue is bigger than 0, it is a good indicator. Since the variance cannot be negative, the negative eigenvalue indicates that the model cannot be conditioned. The eigenvalue approaching 0 indicates multicollinearity because all variances can be taken by major components. Duriš et al (2021) stated that the determination of the number of main components is sufficient to represent the original variables based on variability and the transfer of the original data to a new base. The number of major components (MC) is determined to maintain information (eigenvalues, which explain e.g., 90% of variability). Based on Kaiser's rule, the major components used have to have bigger values than mean eigenvalue with data standard, the mean equals to 1.

Figures 2 and 3 clearly show that the FERET ANGLE position is closer to the correlation circle indicating that the variable could potentially be included in the model, while Figures 4 and 5 demonstrate that the 19th observation resides at different positions in the quadrat. It means that the projection of the initial variable in the correlation circle before reduction (Figure 4) is different from that after reduction (Figure 5). This difference could result from variable removals, such as Round, Solidity, AR, Feret Y, Circ, Feret X, and AR. The Perim, AREA, Feret, and Min FERET variables close to the correlation circle (Figures 2 and 3) indicate their significantly positive correlation (r approaches 1), while the Solidity and Round variables show a significantly negative correlation (r approaches -1). Moreover, the FERET_ANGLE, FERET-X, CIRC., and FERET-Y variables that are far from the correlation circle indicate a weak correlation.

Multiple regression analysis indicates that the morphometric variables, area (X1) and perimeter (X2), give a positive effect on the weight increment, while other variables, FERET, FERET ANGLE, and MIN FERET give a negative effect on the weight growth. The squared R of 0.876706 indicates that X1, X2, X3, X4, and X5 could explain the Y variable as much as 88%, and the rest 12% by other factors.

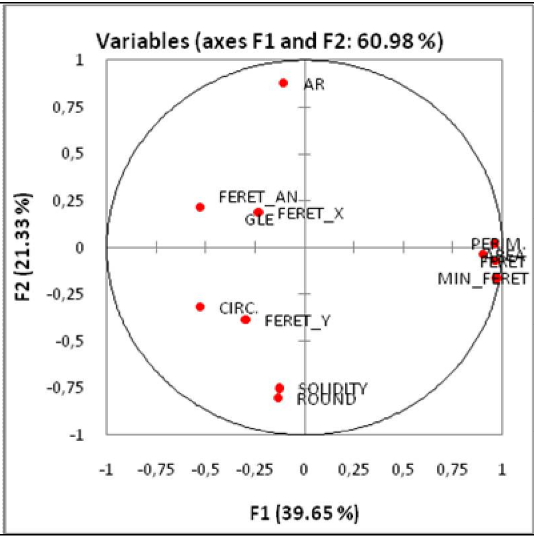

Figure 2. Correlation cycle before reduction.

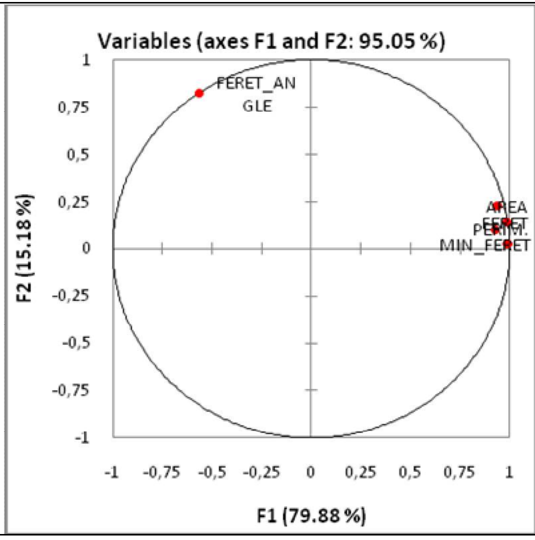

Figure 3. Correlation cycle after reduction.

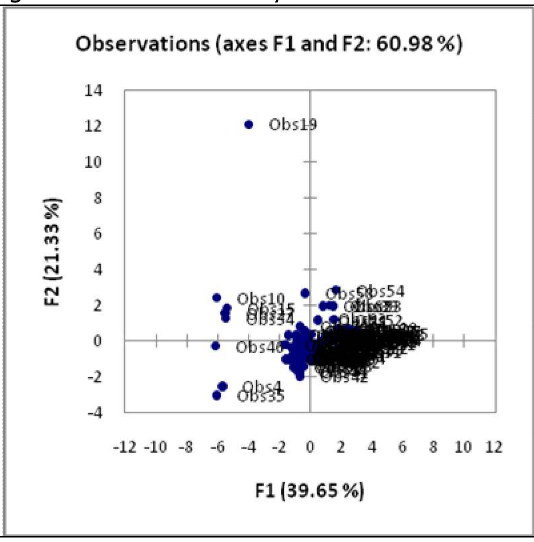

Figure 4. Distribution of genus *Margarites* on the biplot of the main axis (F1 and F2) before reduction

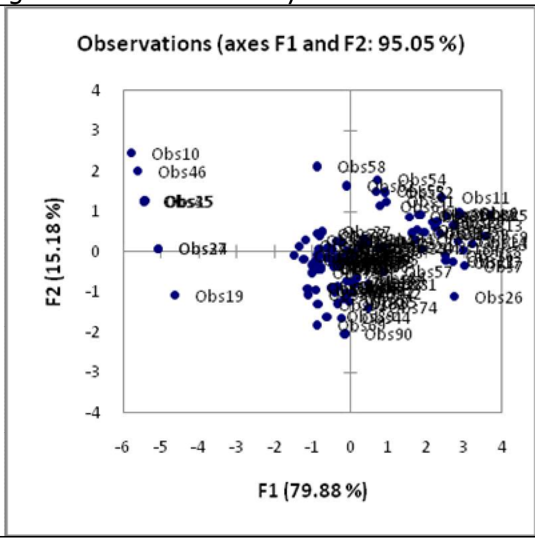

Figure 5. Distribution of genus *Margarites* on the biplot of the main axis (F1 and F2) after reduction.

If 1) the squared R is large (approaches to 1), the standard error is usually small, and if it is 100% or 1, the epsilon value equals to 0; 2) the squared R value is a measure of the linear correlation level between the dependent variable and the independent variable. The R value could be positive or negative (between -1 and +1), but for more than 2 variables, R value is always positive (between 0 and 1). Larger R value (negative or positive) indicates a stronger correlation. The present study found the R value of 0.936326 indicating very strong correlation; 3) the significance value of F 1.02E-44 (Table 7) indicates that the estimated model rejects the hypothesis (H0). It means that there is at least one morphometric parameter (X) influencing the body mass (Y).

Table 3

Correlation matrix (Pearson (n)) before reduced

Variables	*AREA*	*PERIM.*	*CIRC.*	*FERET*	*FERET_X*	*FERET_Y*	*FERET_ANGLE*	*MIN_FERET*	*AR*	*ROUND*	*SOLIDITY*
AREA	**1.00**	0.81	-0.17	0.97	-0.26	-0.28	-0.35	0.91	-0.02	-0.25	0.10
PERIM.	0.81	**1.00**	-0.67	0.89	-0.17	-0.27	-0.43	0.91	-0.17	-0.08	-0.21
CIRC.	-0.17	-0.67	**1.00**	-0.32	-0.04	0.22	0.31	-0.39	0.00	0.00	0.63
FERET	0.97	0.89	-0.32	**1.00**	-0.23	-0.25	-0.45	0.98	-0.11	-0.17	0.03
FERET_X	-0.26	-0.17	-0.04	-0.23	**1.00**	0.00	-0.04	-0.22	0.10	-0.04	-0.09
FERET_Y	-0.28	-0.27	0.22	-0.25	0.00	**1.00**	-0.18	-0.19	-0.14	0.21	0.23
FERET_ANGLE	-0.35	-0.43	0.31	-0.45	-0.04	-0.18	**1.00**	-0.53	0.12	-0.12	-0.01
MIN_FERET	0.91	0.91	-0.39	0.98	-0.22	-0.19	-0.53	**1.00**	-0.21	-0.03	0.02
AR	-0.02	-0.17	0.00	-0.11	0.10	-0.14	0.12	-0.21	**1.00**	-0.85	-0.49
ROUND	-0.25	-0.08	0.00	-0.17	-0.04	0.21	-0.12	-0.03	-0.85	**1.00**	0.32
SOLIDITY	0.10	-0.21	0.63	0.03	-0.09	0.23	-0.01	0.02	-0.49	0.32	**1.00**

Table 4

Correlation matrix (Pearson (n)) after reduced

Variables	*AREA*	*PERIM.*	*FERET*	*FERET_ANGLE*	*MIN_FERET*
AREA	**1**	0.8087	0.9651	-0.3548	0.9142
PERIM.	0.8087	**1**	0.8911	-0.4294	0.9063
FERET	0.9651	0.8911	**1**	-0.4452	0.9759
FERET_ANGLE	-0.3548	-0.4294	-0.4452	**1**	-0.5348
MIN_FERET	0.9142	0.9063	0.9759	-0.5348	**1**

Table 5

The eigenvalue before reduced

	F1	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*	*F9*	*F10*	*F11*
Eigenvalue	4.36	2.35	1.50	1.09	0.89	0.46	0.22	0.08	0.03	0.01	0.01
Variability (%)	39.65	21.33	13.60	9.95	8.13	4.18	1.96	0.74	0.28	0.11	0.06
Cumulative %	39.65	60.98	74.58	84.53	92.66	96.85	98.81	99.55	99.83	99.94	100.00

Table 6

The eigenvalue after reduced

	F1	*F2*	*F3*	*F4*	*F5*
Eigenvalue	3.99	0.76	0.20	0.04	0.01
Variability (%)	79.88	15.18	3.94	0.83	0.18
Cumulative (%)	79.88	95.05	98.99	99.82	100.00

Table 7

ANOVA of the morphometric parameters and the weight of genus *Margarites*

	df	*SS*	*MS*	$F_{calc.}$	*Significance* $F_{tab.}$
Regression	5	20.48833	4.097665	145.0587	1.02E-44
Residual	102	2.881329	0.028248		
Total	107	23.36965			

Table 8 demonstrates that p values in all morphometric parameters are < 0.05, meaning that all of the morphometric variables could remain in the model and be relied upon to explain the body mass. Sarkar & Krupanidhi (2020) observed the weight of the aquatic and terrestrial gastropods with 10 individuals of each family using the regression analysis, the determination coefficient (r^2), and standard errors found a high F value. Roth & Mercer (2000) stated that the morphometric approach facilitates the quantitative variability analysis in the form of dimension to explain the relationship between the morphometric variables and the body mass.

Table 8

Multiple regression analysis of morphometric variables

	Coefficients	*Standard error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	1.1760	0.1973	5.9613	0.0000	0.7847	1.5674
AREA	3.1122	0.1943	16.0197	0.0000	2.7268	3.4975
PERIM.	0.0666	0.0277	2.4017	0.0181	0.0116	0.1217
FERET	-0.9651	0.3025	-3.1906	0.0019	-1.5650	-0.3651
FERET_ANGLE	-0.0003	0.0015	-0.1913	0.8487	-0.0032	0.0026
MIN_FERET	-2.2125	0.3579	-6.1824	0.0000	-2.9224	-1.5027

The equation is:

$$Y = 1.176049 + 3.112157X1+0.066641X2-0.96507X3-0.00028X4-2.21255X5$$

where: Y = weight; X1 = area; X2 = perimeter; X3 = FERET; X4 = FERET ANGLE; X5 = MIN FERET.

Urra et al (2007) found that the morphometry of Caenogastropod species cannot be differentiated based on the meristic characteristics (number of circles and columella folds), shell thickness, or shell weight, but they have a significant difference in shell form and body weight. *Adelomelon ancilla* has a fusiform shell (small opening and high-peaked shell) to hold a smaller body mass than *Odontocymbiola magellanica* having a round shape (bigger opening and low-edged shell).

Conclusions. The PCA analysis has reduced the Circ, Feret X, Feret Y, Ar, Round, and Solidity variables from the model, while the Perim, Area, Feret, and Min Feret were strong variables to determine the relationship between weight and the morphometric size. Based on the multiple regression analysis the variables AREA (X1) and PERIMETER (X2) give a positive effect on the weight, while the variables Feret (X3), Feret Angle (X4), and Min Feret (X5) give a negative effect on the weight.

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

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