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Research article

Otolith morphometry and scanning electron microscopy analysis of three fish species from the Black Sea

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Abstract: In this study, it was aimed to determine the otolith morphology and morphometry of *Merlangius merlangus*, *Trachurus mediterraneus* and *Mullus barbatus* inhabiting at different depths. The fish samples were obtained from the Ordu coast of the Black Sea. Sagittal otoliths of the individuals were carefully removed. Otolith length, width, perimeter, and area were measured, and values of form factor, roundness, aspect ratio, circularity, rectangularity, and elipticity of sagittal otoliths were evaluated for each species. According to the results, there was a difference between the right and left sagittal otoliths in specimens of each population inhabiting different depths. The left sagittal otoliths were preferred for analysis. Moreover, there was a statistical difference in shape indices values among fish species. On the contrary, there was no statistical difference between the rectangularity values of *T. mediterraneus* and *M. barbatus*. The otolith morphology of fish species were analysed by scanning electron microscopy. The sagittal otolith was elliptic to lanceolated for *T. mediterraneus*, elliptic to oval for *M. barbatus* and lanceolated for *M. merlangus*. According to the results, separating stocks using otolith morphometry and shape indices in fish populations was a possible and feasible method. Therefore, otolith morphometry research should also be planned for other species inhabiting at different depths and ecological conditions.

Keywords: Otolith morphometry, Sagittal otolith morphology, Scanning electron microscopy, Shape indices.

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Introduction

The inner ear of teleosts is complicated, and this structure consists of canals, sacs and ducts filled with endolymph. Each otic sac contains three pairs of otoliths of calcium carbonate structure named sagitta, lapillus and asteriscus. Generally, in most species, sagitta is the largest otolith and this structure is largely used in age estimation. Although the otoliths have similar functions, they have different form, size, growth, weight, and chemical composition for each species (Karlou-Riga, 2000). Nevertheless, otoliths are generally considered as taxonomical and biological archives as they reflect growth and development of species (Zorica et al., 2010). Therefore, the otoliths are used in various studies such as age and growth, examination of stomach contents, identification of predator-prey relationships with removed otoliths from the stomach, and stock discrimination. In recent years, the digital technology was rapidly developed, so fishery biologists started using shape analysis to identify stocks by means of morphometric characters of fish or otoliths and to study geographical variations in fish populations (Tuset et al., 2008; Zorica et al., 2010; Yoraz, 2015; Zengin et al., 2015; Çiçek et al., 2021; Milošević et al., 2021; Mohamed et al., 2023).

Several studies emphasized the analysis of otoliths of marine and freshwater fishes in a detailed way and introduction of otolith morphology in terms of preparation of otolith atlas. Otolith morphology is used the different field such as fish biology, fish anatomy, description of new fish species, the taxonomic revision of fish taxon, determination of phylogenetic relationship, ecomorphology studies, determination of relationship between fish growth, otolith growth, especially on identification of correlation between the growth of alive or fossil species (Tuset et al., 2008).

Trachurus mediterraneus, *Merlangius merlangus* and *Mullus barbatus* are the most important economic fish species consumed gladly by consumers in Türkiye. There are many studies on Mediterranean horse mackerel (Bostancı, 2009; Viva et al., 2015; Yedier et al., 2018; Yedier and Bostancı, 2020), on whiting (Tayhan, 2014; Yoraz, 2015; Süer, 2016) and on red mullet (Aguirre and Lombarte, 1999; Süer, 2008; Morat et al., 2012; Yoraz, 2015; Viva et al., 2015; Süer, 2016; Mohamed et al., 2019; Milošević et al., 2021; Mohamed et al., 2023). There are very few studies on determination otolith morphology using scanning electron microscopy and shape indices on these species, especially *T. mediterraneus* and *M. merlangus*. For this purpose, the otolith morphology of three important species inhabiting different depths was presented with scanning electron microscopy and examined by their shape indices. This study was realized to determine otolith shape indices and otolith morphometry of fish species inhabiting at different depths. In addition, the variations in otolith patterns were determined and it also analysed otolith biometry of three fish species that are pelagic, benthopelagic and demersal in this study.

Materials and Methods

Trachurus mediterraneus (Mediterranean horse mackerel), *Merlangius merlangus* (Whiting) and *Mullus barbatus* (Red mullet) individuals were obtained from the fishermen from Ordu coast in the Black Sea. These species inhabit at different depths and *T. mediterraneus* is pelagic, *M. merlangus* is benthopelagic, *M. barbatus* is demersal fish species. Total length (TL) of all specimens were measured to the nearest 0.1 cm. Sagittal otolith pairs were removed, cleaned, and stored dry before examination. The clean and undamaged otolith pairs were photographed and analysed. The otolith length (OL, mm), width (OW, mm), perimeter (P, mm) and area (A, mm^2) were measured using Leica S8APO brand microscope and computer-connected camera system. 'Leica Application Suit' software. Otolith length was defined as the longest axis between anterior and posterior otolith edge, and width as the distance from dorsal to ventral edge taken perpendicular to the length through the otolith focus.

The shape indice values such as form factor (FF), roundness (RD), aspect ratio (AR), circularity (C), rectangularity (R) and ellipticity (E) were calculated with the formulas according to Tuset et al. (2003), Ponton (2006), Zorica et al. (2010), Skeljo and Ferri (2012). The formulas were given in Table 1.

Table 1. The formulas of shape indices.

$F_F = (4 \,.\, \pi.\, A) / P^2$	$C = P^2 / A$
$R_D = (4 \,.A) / (\pi \,.\, OL^2)$	$R = A / (OL.OW)$
$A_R = OL / OW$	$E = (OL – OW) / (OL+OW)$

Form factor (FF) gives information about the similarity of various features of a perfect circle. Roundness (RD) is the ratio of the actual area to the area of a circle of the same length. As this factor grows, the shape of the otolith becomes more circular. The aspect ratio (AR) is the ratio between the otolith length and otolith width. This indice expresses the shape tendency of the otolith. Rectangularity refers to changes in the length and width of the otolith relative to its area. Ellipticity indicates whether the changes in the otolith axes are proportional (Tuset et al., 2003; Zorica et al., 2010; Skeljo and Ferri, 2012).

The descriptive statistics were determined for sagittal otolith pairs of fish species. Whether there was a difference between right and left sagittal otoliths was tested by Paired t-test. Kolmogorov-Smirnov test was used to determine whether all variables were normally distributed. Six shape indice values were compared among fish species inhabiting different depths by ANOVA. The statistical analyses were performed by Minitab 16.0 and the Excel software.

The otolith morphology of the left sagittal otoliths was viewed using Scanning Electron Microscope (SEM). The left sagittal otoliths were photographed on the distal and proximal side.

Results

A total of 220 individuals (*T. mediterraneus*, n=105, *M. merlangus*, n=100 and *M. barbatus*, n=105) were examined in this study. The total length ranged between 11.9-16.4 cm for *T. mediterraneus*, 10.8-18.7 cm for *M. merlangus* and 11.0-17.7 cm for *M. barbatus*, respectively. The general measurements of sagittal otolith pairs were shown in Figures 1-3.

The descriptive statistics of otolith width (OW), length (OL), perimeter (P) and area (A) were shown in Table 2. Generally, the left otolith measurements were higher than the right ones. According to the Paired t-test results, there was a statistical difference between left and right sagittal otoliths for all fish species ($P < 0.05$). For this reason, the left otolith was preferred for further analysis.

Table 2. The descriptive statistics of otolith measurements (R: Right otolith, L: Left otolith, WO: Otolith width, OL: Otolith length, P: Otolith perimeter, A: Otolith area).

		T. mediterraneus **Mean±S.E.** **Min.-Max.**	***M. merlangus*** **Mean±S.E.** **Min.-Max.**	***M. barbatus*** **Mean±S.E.** **Min.-Max.**	
OW	**R**	2.708 ± 0.0192	2.452 ± 0.0344	2.041 ± 0.0148	**P < 0.05**
		2.310-3.110	1.869-3.110	1.712-2.440	
	L	**2.773 ± 0.0187**	**2.512 ± 0.0367**	**2.117 ± 0.0173**	
		2.354-3.239	1.883-3.296	1.812-2.611	
OL	**R**	4.814 ± 0.0382	7.393 ± 0.125	2.874 ± 0.0206	**P < 0.05**
		3.952-5.450	5.336-9.731	2.311-3.410	
	L	**4.903 ± 0.0391**	**7.525 ± 0.132**	**2.935 ± 0.0237**	
		4.009-5.679	5.379-9.973	2.383-3.481	
P	**R**	12.370 ± 0.0931	17.499 ± 0.295	8.585 ± 0.0673	**P < 0.05**
		10.115-13.852	12.612-23.732	6.726-10.388	
	L	**12.866 ± 0.0959**	**18.100 ± 0.317**	**8.995 ± 0.0796**	
		10.698-15.055	13.051-24.651	7.235-11.595	
A	**R**	8.932 ± 0.118	13.276 ± 0.394	4.153 ± 0.0549	
		6.459-11.055	5.379-9.973	3.014-5.775	
	L	**9.300 ± 0.123**	**13.893 ± 0.427**	**4.319 ± 0.0656**	**P < 0.05**
		6.673-12.204	7.735-22.865	2.706-5.967	

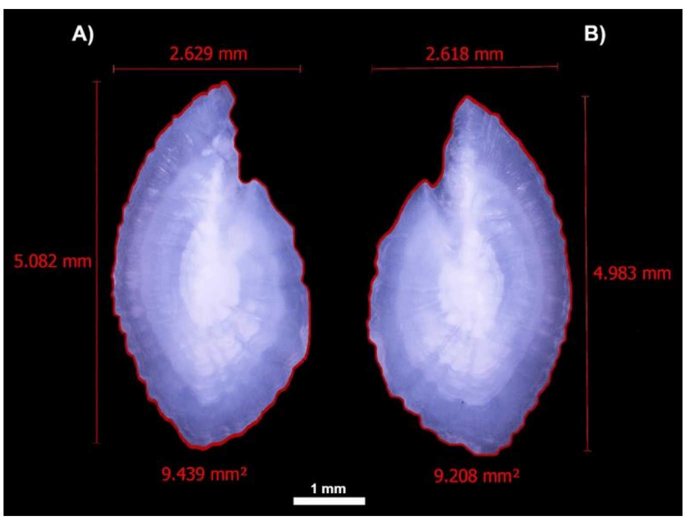

Figure 1. The otolith measurements of sagittal otolith pairs (A: Left otolith, B: Right otolith) for *Trachurus mediterraneus*.

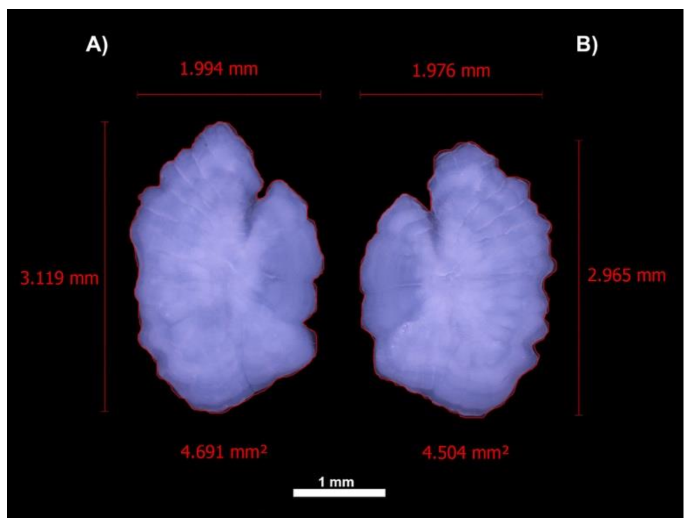

Figure 2. The otolith measurements of sagittal otolith pairs (A: Left otolith, B: Right otolith) for *Mullus barbatus*.

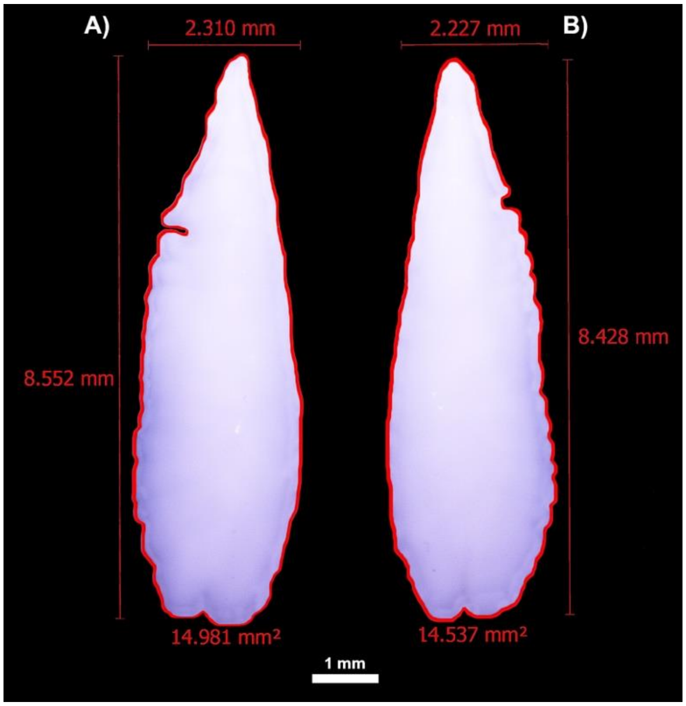

Figure 3. The otolith measurements of sagittal otolith pairs (A: Left otolith, B: Right otolith) for *Merlangius merlangus*.

The shape indice values of left sagittal otoliths were given in Table 3. The form factor, roundness, aspect ratio, circularity, and elipticity showed significant differences among three species (ANOVA, P<0.001). There was no statistical difference between rectangularity values of *T. mediterraneus* and *M. barbatus* (ANOVA, P>0.05) but the difference was statistically important between *M. merlangus* and the other fish species (ANOVA, P<0.001).

Table 3. The descriptive statistic values of left sagittal otolith shape indices of three fish species

	T. mediterraneus **Mean±S.E. Min.-Max.**	***M. merlangus*** **Mean±S.E. Min.-Max.**
Form factor	$0.672^{a} \pm 0.0025$ 0.576 - 0.724	$0.502^{c} \pm 0.0044$ 0.427 - 0.766
Roundness	$0.074^{b} \pm 0.0002$ 0.064 - 0.080	$0.055^{c} \pm 0.0004$ 0.047 - 0.085
Aspect ratio	$1.767^{b} \pm 0.0078$ 1.621 - 1.989	$2.987^{a} \pm 0.0195$ 2.532 - 3.524
Circularity	$17.869^{c} \pm 0.0681$ 16.554 - 20.832	$24.040^{a} \pm 0.182$ 15.659 - 28.056
Rectangularity	$0.681^{b} \pm 0.0018$ 0.644-0.729	$0.720^{a} \pm 0.0045$ 0.655-1.011
Elipticity	$0.276^{b} \pm 0.0020$ 0.237-0.330	$0.497^{a} \pm 0.0024$ 0.433-0.557

The Scanning Electron Microscope (SEM) images of left sagittal otoliths of *T. mediterraneus*, *M. merlangus* and *M. barbatus* were presented in Figures 4-6. Tuset et al. (2008) was used in describing the morphology of left sagittal otoliths.

Sagittal otolith of *T. mediterraneus* is elliptic to lanceolated. The dorsal margin of otolith is sinuate to entire-smooth. The cauda is straight, tubular, and ending far from the posterior margin in the smallest otoliths. It is ending close to the posterior-ventral margin in the largest otoliths. The anterior region is broad and pointed and the rostrum is short to long in this region. The antirostrum is broad, short, and round to blunt or poorly defined. The posterior region is round to oblique. The position of sulcus acusticus is median and heterosulcoid and ostial (Tuset et al., 2008) (Figure 4).

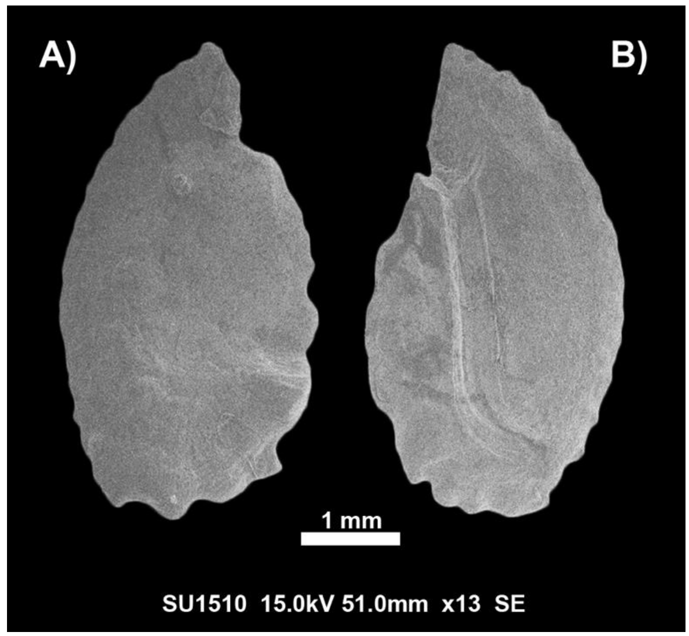

Figure 4. The SEM images of left sagittal otoliths on distal side (A) and proximal side (B) for *Trachurus mediterraneus*.

The sagittal otolith of *M. barbatus* has elliptic to oval and its margins are crenate to irregular. The cauda of the otolith is curved, tubular, significantly flexed from the middle region. It is ending close to the posterior margin. The rostrum is irregular or broad, short, blunt, and pointed. The antirostrum of otolith is broad, pointed, and short. The posterior region of sagittal otolith is round to angled-irregular. The sulcus acusticus is heterosulcoid and ostial. Its position is median (Tuset et al., 2008) (Figure 5).

The sagittal otolith of *M. merlangus* is lanceolated and the anterior region of otolith is more globose than the posterior region. The cauda of the otolith is tubular and straight. The anterior region of the otolith is round to irregular, and the posterior region is sharply lanceolated. The sulcus acusticus position is median and it is pseudoostiocaudal and heterosulcoid (Tuset et al., 2008) (Figure 6).

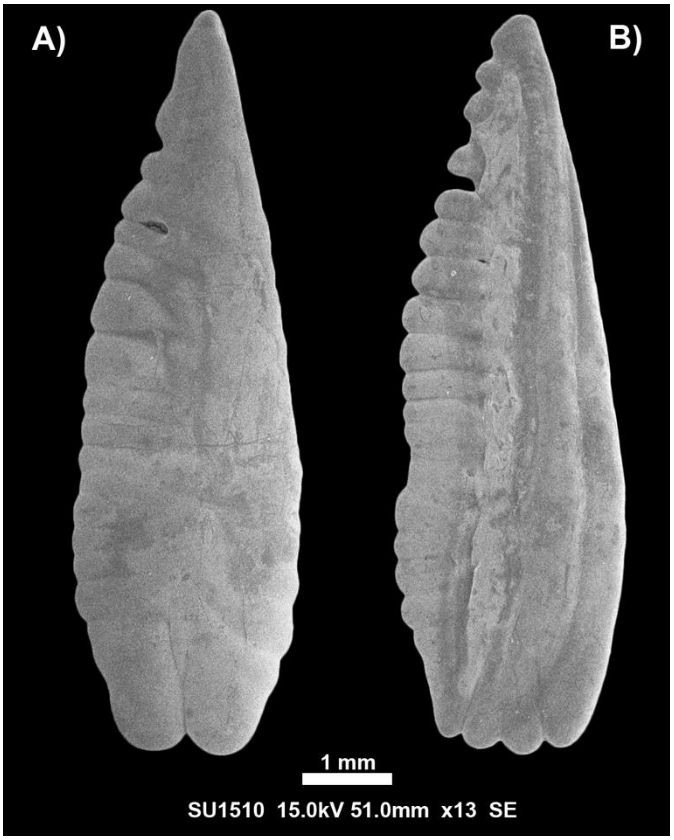

Figure 5. The SEM images of left sagittal otoliths on distal side (A) and proximal side (B) for *Merlangius merlangus*.

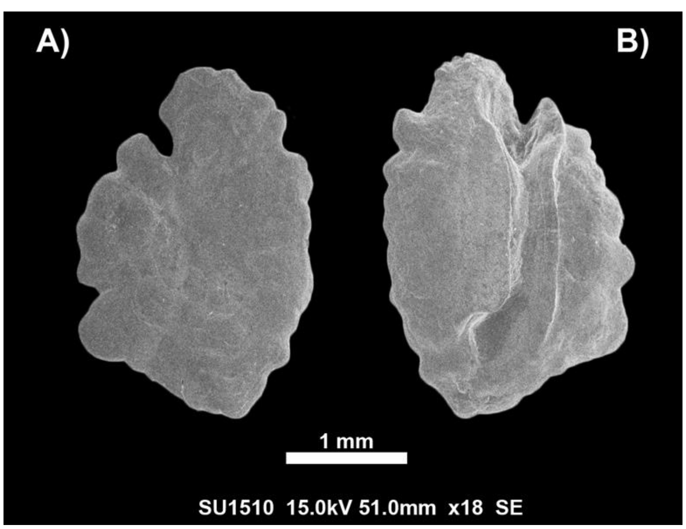

Figure 6. The SEM images of left sagittal otoliths on distal side (A) and proximal side (B) for *Mullus barbatus*.

Discussion

The morphology of fish otoliths is highly variable from species to species. These structures are ranging from the simple disc to the irregular shape. Zorica et al. (2010) informed that the sagittal otoliths were the most used in comparative taxonomy studies because of their form, weight, growth, consistency, and chemical composition have a distinctive degree of interspecific variation. Therefore, their structure, composition and form is evaluated together for morphology of otoliths.

The six shape indices values calculated using sagittal otolith variables differed among three species (P<0.001). While otolith length was increasing, the values of form factors and roundness were generally decreasing for *M. merlangus*. The form factor and roundness values were the highest for *M. barbatus*. The value of aspect ratio was proportionally increasing with otolith length. The aspect ratio was ranged from the highest to lowest value for *M. merlangus*, *T. mediterraneus* and *M. barbatus* (Table 3). The elipticity was the highest for *M. Merlangus.* The rectangularity value was the lowest for *M. barbatus* (P<0.001). On the other hand, circularity and form factor indices provided the highest values for *T. mediterraneus* while roundness value was the lowest for *M. merlangus*. Tuset et al. (2008) reported that circularity values were ranged in 19.4-24.0 for *M. merlangus*, 15.4-18.6 for *T. mediterraneus* and 16.9-17.3 for *M. barbatus* for Mediterranean Sea population, respectively. The rectangulary values were 0.5 for *M. merlangus* and ranged from 0.3-0.4 for *T. mediterraneus* and 0.1-0.2 for *M. barbatus*, respectively. In the present study, there were statistically differences among otolith shape indices of three species (P<0.001) (Table 3). In addition, the circularity and the rectangularity values were higher than the previous results of Tuset et al. (2008). This result may be due to the different sizes of the fish individuals. The shape of otoliths is related to the biological and ecological behaviour of the species (Tuset et al., 2003), therefore results are different for two studies.

The potential for using otolith morphometry as a tool for stock identification or species classification increased with the development of image analysis systems (Tuset et al., 2003). The power of otolith shape analysis to distinguish the specified groups is very important. Tayhan (2014) reported that there are three different groups of otoliths in whiting in his study and that the shape of the otoliths is affected by both genetic and environmental changes. Çiçek et al. (2020) stated that although some differences were detected between species belonging to the Mugilidae family in terms of morphological and morphometric features, it was not possible to determine the species based on these features. Campana and Casselman (1993) explained that otolith shape may change depending on growth rate. In this case, it was evaluated that the difference in otolith morphology detected between fish individuals from two different regions occurred due to the change in growth rate (Süer, 2016). Başçınar and Atılgan (2016) revealed that the otoliths of anchovy in Rize and Samsun stocks were similar, but the otoliths of the Ukrainian stock showed significant differences. This study, conducted using otolith shape analysis, played an important role in revealing the regional differences of anchovies living in Ukraine.

This study on otolith shape analysis constitutes an example of morphological research in other fish species. This study not only develops new strategies for fisheries management and conservation of fish species, but also contributes to fish ecology. The results of this study make otolith shape analysis a valuable tool in the field of fisheries science and population studies (Özpiçak et al., 2019). In this study, otolith morphology and otolith shape indices were analysed for bilateral symmetrical three fish species inhabiting the different depths in Black Sea. We think that morphological characteristics should determine for different ecological conditions, depth, niche, and the other species.

Ethical Approval
All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflicts of Interest
The authors declare that they have no conflict of interest.

Funding Statement
The author does not declare any fund.

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