MEIO EXTREME

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# A new species of *Ligiarctus* (Tardigrada, Arthrotardigrada) from the Brazilian continental shelf, Southwestern Atlantic Ocean

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Abstract A new marine arthrotardigrade, *Ligiarctus alatus* sp. nov. found in sediments of the Brazilian continental shelf (100-150 m depth) in the Southwestern Atlantic Ocean, is described in this study. This new species was recorded from sites located in the major oil extraction basins in Brazil (Campos and Potiguar basins). Within the subfamily Floractinae, the genus Ligiarctus characteristically presents primary clavae bent backwards, occupying the reduced lateral edges of the head. The new species is clearly distinguishable from L. eastwardi, the only known species of the genus, by the presence of internal distal notches on all claws, and six aliform cuticular expansions (frontal ala, two anterolateral alae, two posterolateral alae and caudal ala) with continuous digitiform procuticular supports (caesti). Ligiarctus eastwardi has only a caudal ala without caesti and internal notches are present on external claws only. The same pattern of cuticular expansions and caesti exhibited by the new species also occurs in the

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genus *Florarctus*, increasing the difficulty of defining the taxonomy of the subfamily Florarctinae, and forcing the emendment of the generic diagnosis of *Ligiarctus*.

**Keywords** Arthrotardigrada · Florarctinae · Ligiarctus alatus sp. nov. · Meiobenthos · Sublittoral

### Introduction

Tardigrades (phylum Tardigrada) are micrometazoans that occur worldwide in terrestrial, freshwater and marine environments. Marine tardigrades, with a body length of 80 to 800 µm, constitute a much neglected group of meiobenthic organisms, with only approximately 200 known species (mostly intertidal) representing approximately 16% of all tardigrade species (Kaczmarek et al. 2015). According to Kaczmarek et al. (2015), the lack of knowledge available regarding marine tardigrades is particularly severe in the Southern hemisphere, namely in poorly surveyed, deep sublittoral and abyssal zones. So far, studies of Tardigrada in deep sea were not performed for the Southwestern Atlantic Ocean where tardigrades are only known from shallow intertidal and sublittoral zones (da Rocha et al. 2013). For nature management purposes, the need for species inventories is especially justified in changing marine environments such as the Brazilian coast, which is a region strongly subjected to oil extraction. Thus, in the frame of a project aiming to assess the impact of this activity on natural resources and marine communities, a new and interesting arthrotardigrade was recorded from two sites located in the major oil extraction basins in Brazil (Campos and Potiguar basins, Southwestern Atlantic Ocean).

The new species reported in this paper, *Ligiarctus alatus* sp. nov., was collected from sediment samples at 100–150 m

depth, and belongs to the rare and monotypic bathyal genus Ligiarctus. The genus Ligiarctus was erected by Renaud-Mornant (1982) through the description of L. eastwardi from the Northwestern Atlantic Ocean (North Carolina coast, U.S.A.) collected at bathyal depth (400 m b.s.l.) in fine sand and constitutes the only record of the genus until now. The presence of massive primary clavae in males and a discshaped caudal cuticular expansion (ala) were the main attributes considered by Renaud-Mornant (1982) when describing the new genus. Additionally, the presence of an internal distal notch on external claws has also been described as a peculiar characteristic of the genus Ligiarctus. With the description of the genus Ligiarctus, Renaud-Mornant (1982) established a new subfamily, Florarctinae, within the much-diversified family Halechiniscidae, which included the genus Florarctus (Delamare-Deboutteville & Renaud-Mornant 1965). Later, a third genus, Wingstrandarctus, was assigned to the subfamily and an emended diagnosis of the family, Halechiniscidae, was proposed (Kristensen 1984). According to Kristensen (1984), genera of the subfamily Florarctinae share the presence of cuticular aliform expansions (alae); a complete set of cephalic sense organs; the presence of four digits with external calcar on claws; external digits with peduncles, and two spheroid seminal receptacles in females, each one with an S-shaped genital duct. The description of L. alatus sp. nov. provides new insights into the phylogeny of Florarctinae. Furthermore, the present number of marine tardigrade species recorded in Brazil has now increased to 30 (da Rocha et al. 2013: Santos et al. 2017).

# Material and methods

Sublittoral sediment samples were collected by box corer in two localities of the Brazilian continental shelf (Southwestern Atlantic Ocean), in the major oil extraction basins of the country: Campos Basin (22°10'S, 40°20'W), Rio de Janeiro State, in 2009, at 100 m depth, and Potiguar Basin (4°37'S, 36°45' W), Rio Grande do Norte State, in 2009, at 150 m depth in the transition zone between the continental shelf, characterized to be very narrow in the Potiguar Basin (Vital et al. 2010), and the continental slope. In both localities the environment is very heterogeneous with the presence of canyons and rhodolith beds in the vicinity, the sediment type is represented by bioclastic and litoclastic sand. Samples were preserved in 4% buffered formaldehyde for later study. At the laboratory, sediments were washed and sieved through a 40 µm mesh sieve. Tardigrades were sorted under a dissecting microscope and transferred to microslides with coverslips. Specimens were permanently mounted in pure glycerine with formalin (ca. 1%) or in glycerol that, after a period of several days to evaporate to glycerin, were sealed with nail varnish. Measurements, given in micrometers (µm) were made under  $100 \times$  oil immersion, using a Zeiss Axioscope 40 Phase Contrast Microscope (PHC) and a Zeiss Axioimager 2 Differential Interference Contrast Microscope (DIC), both equipped with digital cameras and using Zen Imaging Software (from Zeiss). All photomicrographs were made in DIC. The new species was compared with the original description of *Ligiarctus eastwardi* Renaud-Mornant, 1982.

# Results

#### Systematics

Phylum: Tardigrada Doyère, 1840.

Class: Heterotardigrada Marcus, 1927.

Order: Arthrotardigrada Marcus, 1927.

Family: Halechiniscidae Thulin, 1928 (emended by Grimaldi de Zio et al. 1990).

Subfamily: Florarctinae Renaud-Mornant, 1982 (emended by Kristensen 1984).

Genus: Ligiarctus Renaud-Mornant, 1982.

**Diagnosis (emended)** Florarctinae with narrow head. Wide implantation of the primary clavae that are bent backwards, occupying the reduced lateral edges of the head. With lateral and caudal aliform lobate expansions, sometimes restricted to the caudal aliform expansion. A short frontal ala is frequently present. Procuticular processes (caesti) either absent or present inside the alae. An internal distal notch is present on claws of all four digits or on claws of external digits only.

*Type species: Ligiarctus eastwardi* Renaud-Mornant, 1982. *Ligiarctus alatus* sp. nov. (Figs. 1-5; Table 1).

**Type locality** Potiguar Basin (4°37'S, 36°45'W), Rio Grande do Norte State, Brazil, in the Brazilian continental shelf at 150 m below sea level. The sediment type is bioclastic and litoclastic sand with medium to fine grain size.

**Material examined and type repository** Holotype: adult female (slide C.VII-84) collected from the Potiguar Basin, mounted in glycerine and deposited in the collection of P. Fontoura (Department of Biology, Faculty of Sciences, University of Porto, Portugal). Allotype: adult, male (slide TARD-UFRPE-02-27) collected from the Potiguar Basin, mounted in glycerine deposited in the collection of Clélia Rocha (Laboratório de Meiofauna, Departamento de Biologia, Universidade Federal Rural de Pernambuco, Brazil). Paratypes mounted in glycerine: eight specimens, four females and four juveniles, collected from the Potiguar Basin (slides TARD-UFRPE-01-42, TARD-UFRPE-01-43 and TARD-UFRPE-02-27), deposited in the collection of Clélia Rocha (Laboratório de Meiofauna, Departamento de Fig. 1 Drawing of *Ligiarctus alatus* sp. nov. (ventral view): ala - antero-lateral ala; bv – bacterial vesicle; ca - caudal ala; cae caestus; cE - cirrus *E*; ec – external cirrus; fa – frontal ala; go- gonopore; ic – internal cirrus; lc – lateral cirrus *A*; pb – pharyngeal bulb; pc – primary clava; pIV – sense organ IV; pla – postero-lateral ala; sc – secondary clava; sv – seminal vesicle; th – cuticular thickening; scale bar = 50  $\mu$ m



Biologia, Universidade Federal Rural de Pernambuco, Brazil) and one female (slide C.VII-85) deposited in the collection of P. Fontoura (Department of Biology, Faculty of Sciences, University of Porto, Portugal); and two juveniles collected from Campos Basin (slides C.VII-86 and C.VII-87), mounted in glycerol and deposited in the collection of P. Fontoura (Department of Biology, Faculty of Sciences, University of Porto, Portugal).

**Diagnosis** *Ligiarctus* with ovoid body, finely punctated cuticle with marked dorsal folds. Body surrounded by six alae with fine punctation and digit-shaped procuticular processes (caesti). Alae consist of frontal ala; pair of antero-lateral alae, pair of postero-lateral alae and caudal ala. Continuous caesti, absent in the frontal ala and less evident in the anterior portion of antero-lateral ala, have several elongated processes with single or bifid tips reaching the external edge of alae. Tubular primary clavae bent backwards as typical of the genus and secondary clavae present. Four digits with claws present in adults. External digits with hook-shaped peduncles. All claws with external calcars and internal distal notches. Females with two seminal receptacles, each consisting of spheroid vesicles and S-shaped genital ducts.

**Etymology** The specific name alludes to the presence of welldeveloped cuticular aliform expansions surrounding the lateral margin of the body, alatus = having wings, from the Latin word ala = wing.

**Description of the holotype** Female with mature ovary, with ovoid body 163.5  $\mu$ m long (195.7  $\mu$ m including alae) and 68.3  $\mu$ m wide between the second and third pair of legs (Figs. 1 and 2a). Head (48.1  $\mu$ m wide between primary clavae) clearly distinct from the rest of the body. Eye spots not observed. Head with complete set of eleven cephalic appendages. Unpaired median cirrus, 14.8  $\mu$ m long, with cirrophore (about 2.4  $\mu$ m long), scapus (5.7  $\mu$ m long) and flagellum (6.7  $\mu$ m long). Paired internal cephalic cirri inserted dorsally on the frontal edge of the head are 23.8  $\mu$ m long, each one bearing cirrophore (about 4.3  $\mu$ m long), scapus (8.0  $\mu$ m

Table 1 Measurements (in µm) of selected morphological structures for specimens of Ligiarctus alatus sp. nov. (Holo -Holotype; Allo - Allotype; SD -Standard deviation: Range refers to the smallest and largest measured specimen/structure; N number of specimens/structures measured)

	FEMALES adults		MALE adult	JUVENILES 4-toed
STRUCTURES	Holo	Mean ± SD (Range); N	Allo	Mean ± SD (Range); N
Body length	195.7	179.8 ± 13.9 (162.6–195.7); 5	145.8	136.0 ± 23.6 (105.2–158); 6
Body without caudal ala	163.5	151.1 ± 10.5 (136.5–163.5); 5	124.7	122.0 ± 31.6 (85.8–173.5); 6
Body width	68.3	$79.6 \pm 9.9 \ (68.3 - 89.9); 4$	67.7	68.0 ± 18.0 (45.0–86.9); 6
Median cirrus	14.8	$13.3 \pm 2.4$ (11–15.9); 4	12.1	12.9 ± 3.7 (10.3–15.6); 2
Internal cirri	23.8	23.5 ± 2.4 (20–26.2); 5	13.2	17.0 ± 4.1 (11.0–22.5); 6
External cirri	25.4	22.5 ± 2.89 (18.3–25.4); 5	16.7	16.7 ± 5.5 (10.2–25.1); 6
Lateral cirri A	26.1	20.7 ± 5.8 (13.1–26.1); 4	7.5	22.8 ± 6.5 (13.9–28.8); 4
Primary clavae	44.0	$40.3 \pm 4.8$ (35.4–45); 4	49.1	38.7 ± 8.8 (27.0–48.4); 5
Primary clavae width	3.9	$3.8 \pm 0.72$ (2.6–4.4); 5	4.1	3.2 ± 0.5 (2.4–3.7); 5
Primary clavae / cirri A	1.7	2.1 ± 0.8 (1.5–3.4); 4	6.5	1.8 ± 0.3 (1.6–2.4); 4
Cirri E	32.5	33.7 ± 2.5 (31.4–37.6); 5	27.3	25.8 ± 8.6 (18.2–39.3); 5
Leg I spine	10.7	10.6 ± 0.82 (10.0–12); 5	?	10.3 ± 2.4 (8.1–12.9); 3
Leg II spine	7.4	$7.4 \pm 0.07$ (7.4–7.5); 2	?	7.7 ± 5.0 (4.1–11.3); 2
Leg III spine	2.5	5.8 ± 2.9 (2.5–7.5); 3	?	?
Leg IV sense organ	12.6	12.5 ± 1.2 (11.2–13.7); 3	9.4	10.5 ± 1.9 (9.1–12.0); 2
Frontal ala	4.5	4.8 ± 0.26 (4.5–5.0); 3	?	$3.4 \pm 0.94$ (2.5–4.7); 5
Caudal ala	30.9	27.2 ± 8.3 (13.5–35); 5	21.7	22.3 ± 5.4 (15.4–29.5); 6
Antero-lateral ala	22.9	21.4 ± 3.7 (17–25); 5	?	27.3 ± 8.7 (19.6–36.8); 3
Postero-lateral ala	28.5	25.7 ± 5.1 (18.1–29.4); 4	16.8	20.7 ± 9.4 (11.0–37.8); 6
Legs I: Digit 1	11.4	10.7 ± 0.6 (10.1–11.4); 4	8.0	9.3 ± 1.1 (8.6–10.5); 3
Digit 2	13.9	13.9 ± 1.4 (12.3–15.7); 5	10.0	12.9 ± 0.9 (11.9–13.6); 3
Digit 3	14.6	13.3 ± 1.4 (11.3–14.6); 5	10.5	13.4 ± 1.2 (12.6–14.8); 3
Digit 4	12.5	10.2 ± 1.9 (7.7–12.5); 5	7.5	10.1 ± 1.1 (9.0–11.3); 3
Leg IV: Digit 1	11.9	10.2 ± 1.0 (9.6–11.9); 5	11.0	$7.3\pm3.9\;(3.710.9)\;4$
Digit 2	17.4	14.7 ± 1.5 (13.5–17.4); 5	15.2	8.3 ± 3.5 (4.9–12.7); 4
Digit 3	17.3	14.7 ± 1.7 (12.8–17.3); 5	?	8.8 ± 4.1 (4.9–13.6); 4
Digit 4	12.3	10.3 ± 1.4 (9.2–12.3); 4	11.5	7.3 ± 4.0 (3.7–11.6); 4
Gonopore - Anus	17.8	15.6 ± 2.0 (13.0–17.8); 5	6.5	-

long) and flagellum (11.5 µm long). Paired external cephalic cirri 25.4 µm long, each with cirrophore (about 3.9 µm long), scapus (6.0 µm long) and flagellum (15.5 µm long), are inserted in a ventro-lateral head lobe, under the lateral cirri and primary clavae. Lateral cirrus A and primary clava share a common short pedestal (about 4.2 µm long). The lateral cirrus A, inserted dorsally in relation to the primary clava, is 21.9 µm long (scapus and flagellum are 8.6 and 13.3 µm long respectively). The tubular primary clava, 44.0 µm long with a diameter of 3.9 µm (in the medial section), has a van der Land body at its base, and is bent backwards as typical of the genus (Figs. 1, 2a and 3a, black asterisk, 3d, white arrowhead). Hardly visible, elongate secondary-clavae are present at each side of the mouth cone (Fig. 3b, white arrowhead). Subterminal mouth located in a protruded mouth cone which is subdivided by cuticular folds into several annular portions (Fig. 3c). The mouth is followed by a long buccal tube (52.8  $\mu$ m long) connected with the pharyngeal bulb by a thickening. Small ovoid pharyngeal bulb (14.5  $\times$  12.2  $\mu$ m) with three thin placoid bars. Very long thin stylets (about 77.1 µm long) with very small furca and indistinct stylet supports (Fig. 3d). Laterally to the posterior region of the buccal cone there are two cuticular thickenings (Fig. 3b, black arrowhead). Bacterial vesicles are present in the margin of the ventro lateral head lobes (Fig. 4a, arrowheads).

Cuticle finely punctated (about 16 pillars/10 µm) with marked dorsal folds (Fig. 2b). Lateral margin of the body surrounded by well-developed cuticular aliform expansions (alae) with fine punctation and digit-shaped procuticular processes (caesti). Alae consist of frontal ala; pair of antero-lateral alae, pair of postero-lateral alae and caudal ala (Figs. 1, 2a and 4b, c). Continuous caesti have several elongated processes with single or bifid tips reaching the external edge of alae (Figs. 1 and, 4b, c). These elongated caesti processes are less evident in the anterior portion of antero-lateral alae. Frontal ala weakly developed (4.5 µm high), without caesti, spreading Fig. 2 Ligiarctus alatus sp. nov. a. Habitus, ventral view (scale bar = 50  $\mu$ m); b. Dorsal view showing cuticular folds, median cirrus (black arrowhead) and cirrus E (white arrowhead) (scale bar = 10  $\mu$ m)



between the internal cephalic cirri and having the frontal edge slightly convex (Fig. 3d). Cirrophores and proximal portion of the scapus of internal cephalic cirri fused with frontal ala. Antero-lateral alae (about 23  $\mu$ m high) with a median deep indentation, spreading from the base of pedestals bearing primary clava and lateral cirrus *A* to the third pair of legs (Fig. 2a). Postero-lateral alae (about 28  $\mu$ m high) with a median slight indentation, spreading between the third and fourth leg pairs and slightly overlapping with antero-lateral alae (Figs. 2a and 4b). Bilobed caudal ala (about 40  $\mu$ m wide and 31  $\mu$ m high) with a median indentation about 10  $\mu$ m deep inserted between the hind legs and well separated from postero-lateral alae (Fig. 4c).

Paired cirri E (Fig. 2b, white arrowhead), inserted dorsally over legs IV, each one bearing cirrophore, slightly corrugated scapus and flagellum (respectively 2.5, 6.6 and 23.4  $\mu$ m long).

Telescopic legs consisting of coxa, femur, tibia and tarsus ending in four digits with proximal wrinkles and distal claws. Internal digits longer than external ones; this character is particularly evident on the fourth pair of legs (in leg IV digit 1, the most anterior digit, is 11.9  $\mu$ m long, digit 2 is 17.4  $\mu$ m, digit 3 is 17.3  $\mu$ m, and digit 4 is 12.3  $\mu$ m). Strong hook-shaped peduncles with developed proximal pads are present at the base of external digits (Fig. 4d arrowheads). Sensorial spines present on all legs (10.7; 7.4; 2.5, and 12.6  $\mu$ m long on leg I, II, III and IV respectively). Sense organs in leg I, II, III undivided with blunt tips (Fig. 5a white arrowhead). Leg IV sense organ consists of a tubular spine with a van der Land body at the base, separated by a constriction with a light refracting unit from a very small distal enlarged portion (Fig. 5b arrowhead). Claws with external calcars. An internal distal notch, as characteristic of the genus, is present on all claws (Fig. 5a black lines). Internal claws with short and difficult to see accessory points are slightly smaller than simple external claws.

Rosette-shaped gonopore opens 17.8  $\mu$ m from the anus. The gonopore is surrounded by a ring of cuticular folds (Fig. 5c). Two vesicular seminal receptacles with S-shaped ducts opening postero-laterally the gonopore are present.

**Remarks** Measurements of specimens of the new species are given in Table 1. The allotypic male, the only male found, has

Fig. 3 Ligiarctus alatus sp. nov. a. Detail of the head showing cephalic appendages: internal cirrus (black arrowhead), external cirrus (white arrowhead), primary clava (black asterisk), lateral cirrus (white asterisk); b. Ventral view of the head showing the secondary clava (white arrowhead), cuticular thickenings (black arrowhead) and external cirrus (black asterisk); c. Detail of the mouth cone showing cuticular folds; d. Anterior portion of the body showing the frontal ala, buccal apparatus, internal cirrus (black arrowhead) and primary clava (white arrowhead). Scale bars =  $10 \ \mu m$ 



a crescent shaped gonopore 6.5  $\mu$ m distant for the anus (Fig. 5d). The male although smaller than females has primary clavae relatively more developed (39.4% of body length in the

allotypic male vs 26.9% in the holotypic female). However, the caudal ala of the female is broader (22.1  $\mu$ m wide, about 18% of body length in the male vs 25% in the holotypic

**Fig. 4** *Ligiarctus alatus* sp. nov. **a**. *anterior* portion of the body showing bacterial vesicles (*black arrowheads*); **b**. Detail of the postero-lateral ala with caesti; **c**. Detail of the caudal ala with caesti, a seminal receptacle (*black arrowhead*) and part of a seminal duct (*white arrowhead*) are visible. **d**. Detail of the fourth pair of legs showing the peduncles on external digits (*black arrowheads*). Scale bars = 10 μm



Fig. 5 *Ligiarctus alatus* sp. nov. a. Detail of the legs showing legs I, II and III sense organs (*white arrowheads* numbered 1, 2 and 3, respectively) and indentations on claws (*black lines*); b. Detail of the sense organ on leg IV (*black arrowhead*); c. Female gonopore (go) and anus (an); d. Male gonopore (go) and anus (an). Scale bars = 10  $\mu$ m



female). Juveniles, with four digits on each leg but without a visible gonopore, are similar to adults.

## Discussion

The new species, Ligiarctus alatus sp. nov., shares the presence of the same type of primary clavae, aliform expansions and internal distal notches on claws with L. eastwardi. However, contrary to L. eastwardi, which only has a caudal ala, the new species exhibits a complete set of alae (6): frontal ala, paired antero-lateral alae, paired postero-lateral alae and caudal ala. Moreover, with exception of the frontal ala, in the new species, alae have procuticular processes (caesti) that are absent in the type species of the genus. The caudal ala in L. eastwardi is disc-shaped while in the new species it is bilobed. On the other hand, in L. alatus sp. nov., all the claws have internal distal notches while in L. eastwardi they are only present on external claws. The two species also differ in some quantitative characters. Primary clavae are more developed (52  $\mu$ m long and 6  $\mu$ m wide) and cirri *E* is longer (35  $\mu$ m long) in the holotypic male of L. eastwardi (body length: 90 µm long) compared to the allotypic male of the new species (which has a primary clava 49.1 µm long and 4.1 µm wide, and a cirrus E 27.3 µm long; body length 124.7 µm long without caudal ala). In Florarctinae smaller primary clavae and a broader caudal ala are characteristic of females (see Renaud-Mornant 1976; de Zio Grimaldi et al. 1999; Jørgensen et al. 2014). As the description of L. eastwardi was based on the examination of only five specimens, four males and one adult of unknown sex, knowledge of female *Ligiarctus* was not available until now. Nonetheless, the above-mentioned differences do in fact characterize different species and cannot be attributed to sexual dimorphism.

Until now the genus Ligiarctus, exhibiting simplified aliform expansions and claws, and missing H-shaped secondary clavae, was considered more plesiomorphic than the other Florarctinae genera Florarctus and Wingstrandarctus (Renaud-Mornant 1982, 1989, Kristensen 1984). The shape of secondary clavae was barely visible in L. alatus sp. nov., described here, therefore it is necessary to confirm the presence and/or form of this character in future studies. Nevertheless, the complete set of cuticular expansions present in the new species and the presence of well-developed caesti, as in the genus Florarctus, bring new insights into the phylogeny of Florarctinae. On the other hand, these characters increase the difficulty of defining the taxonomy of this subfamily and ultimately forced a revision of the generic diagnosis of Ligiarctus. Nonetheless, the validity of the genus Ligiarctus is still supported by the important apomorphies previously highlighted by Renaud-Mornant (1987), such as the strong development and peculiar implantation of primary clavae, bent backwards, occupying the lateral edges of the head and also the presence of internal distal notches on claws.

Contrary to the other Florarctinae genera, *Florarctus* and *Wingstrandarctus*, that were recorded worldwide, mainly from shallow subtidal zones (see Kaczmarek et al. 2015), sampling sites of the new species are consistent with the

former record of the genus *Ligiarctus*, which shows a tendency towards a bathyal distribution restricted, until now, to the Atlantic Ocean. It is clear that the finding of *L. alatus* sp. nov. alone does not allow evaluating the impact of oil extraction on marine life and, in particular, on tardigrade fauna; however, it adds one more piece to understand this complex puzzle.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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