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MRI Investigations on Venomous Glands of Brown Bullhead, *Ameiurus nebulosus* (Lesueur, 1819) (Actinopterygii: *Ictaluridae*)

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Authors' Contribution
CD and CM supervised the research and designed the experiments. CD, RC, CM CL, AC and FP drafted the manuscript. VM, CM and RC did analysis and anatomical description. FP asessed biological material. CL performed image processing. RVFT did MRI translation. AŞF analysed MRI.

Key words
Fin spine, Anatomical topography, High resolutions, Defence mechanism, Venomous gland.

ABSTRACT

In this study we analysed the shape and topography of venomous glands in Brown bullhead (*Ameiurus nebulosus*) through MRI investigations. The main reason of this investigation was to provide a better understanding of the defence mechanism of this species. The presence, topography and structure of venomous glands made brown bullhead one of the top invasive species spreading its habitat worldwide. The specimens were collected from Stejeriş Lake, Cluj County. MRI investigations were conducted within the National Center for Magnetic Resonance (NCMR), Babeş-Bolyai University, Faculty of Physics. For anatomical investigations, RARE (Rapid Acquisition with Refocused Echoes) and Turbo RARE High Resolution protocols were used, based on the echo-type RF pulse sequence. In transversal sections, organs, blood vessels, and bone formations appear unchanged in shape, size, appearance and location. For the studied species, the venomous glands, together with the first radius of the pectoral and dorsal fins, as well as the muscular fascicles attached to the radius, are forming the venomous apparatus. Triangular positioning of pectoral and dorsal spines and also of their afferent venomous glands represents an efficient defensive mechanism against aquatic, terrestrial and aerial predators. Using MRI techniques for anatomical investigations of venomous glands in Brown bullhead proved to be very precise both in identifying the shape and the topography of the venomous glands. Also by using MRI techniques we highlighted the complex defence mechanism that Brown bullhead has developed against its natural predators.

INTRODUCTION

Venomous organisms have been extensively studied since ancient times. Initially, methods in which humans could defend themselves from these animals were investigated and recent studies have focused on how the venom of these species can be used in the pharmaceutical industry. It is well known that venom can be the precursor of many synthetic drugs used to treat various conditions. Bee venom (apitoxin) can be successfully used as an adjuvant to Parkinson's disease therapies (Cho *et al*., 2012; Alvarez-Fischer *et al*., 2013) and viper venom in combination with ACE inhibitors, ARBs and some calcium channel blockers contribute to lowering high blood pressure and reducing the risk of stroke (Patlak, 2004). Some peptides derived from arachnid venom have an analgesic effect, selectively inhibiting Ca^{2+} and Na^{+} ions or glutamate-ionotropic receptors (De Lima *et al*., 2010). In some cases, venom involves neural substrates and has been used in tests to treat epilepsy (Nelson, 2004; Baby and Jency, 2012). Practically, all of these studies follow the use of seemingly noxious substances for purposes favorable to mankind.

From a toxicity point of view, one of the least studied categories is venomous fish. Specialty literature mentions over 2500 species of venomous fish (Wright, 2012a), each of them having specific anatomical formations of venom production, storage and elimination. Some species have venal glands associated with specialized

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dentition (*Meiacanthus* spp.) (Smith-Vaniz and Allen, 2011). Other fish species use the bones of the fins for the expulsion of venom (*Acanthuridae* spp., *Apistidae* spp.; *Aploactinidae* spp.; *Batrachoididae* spp.; *Caracanthidae* spp.; *Gnathanacanthidae* spp.; *Neosebastidae* spp.; *Scathophagidae spp*.; *Scorpaenidae* spp.; *Sebastidae* spp.; *Setarchidae* spp.; *Siganidae* spp.; *Synanceiidae* spp.; *Tetrarogidae* spp.) (Smith and Wheeler, 2006; Wright, 2012a). Siluriformes (*Ictaluridae*) are also included in this category (Wright, 2012b). Last, but not least, some species use bones of the opercular apparatus or flat bones, such as cleitrum, as a venomous spine (*Trachinidae* spp.) (Dehaan *et al*., 1991; Strempel and Ceballos, 2012), *Uranoscopidae spp*. (Huet *et al*., 1999). Regardless of anatomical structure and positioning of the venomous glands of fish, they are heavily studied for the use of venom in the treatment of various diseases.

Sometimes, common fish species are venomous and dangerous. The same situation is also found in the case of Brown bullhead, *Ameiurus nebulosus*. The species is originary from North America and it is found today in most of Romania's inland waters. Brown bullhead is part of the invasive species and its presence in natural waters and also in systematic fisheries is undesired. This is the reason why the legislation of most European countries prohibits the release of this species in natural habitats (Nowak *et al*., 2010). Its predatory and destructive character leads to the numerical reduction of other endemic species due to adaptability to environmental conditions, resistance to various stressors and morphophysiological features (Rojo, 2013).

The factors that contribute to the area extension of this species are both physiological (good adaptability to poor medial conditions, such as low level of dissolved oxygen and high temperature variations) and ethological (brown bullhead has a gregarious behavior). When these factors are added to the anatomical peculiarities, especially the presence of venomous glands at fin level of the first hard ray, it provides safety and protection in the natural environment, where brown bullhead has few predators.

The purpose of this study was to investigate topographically the disposition of venomous glands at brown bullhead by using MRI techniques.

MATERIALS AND METHODS

The brown bullhead specimens were captured from Stejeriş Lake, Cluj County. Five specimens were used for MRI investigations. MRI investigations were conducted at the National Magnetic Resonance Center (NRMC) at Babes-Bolyai University from Cluj-Napoca, Faculty of Physics. NRMC is organized as a Multi-User Research Base. The Bruker Biospec 7.0 Tesla scanner used in our determinations, based on non-invasive investigations, has the ability to provide a combination of functional and anatomical *in vivo* type data. During the anatomical and functional investigations of brown bullhead, the specimens were anesthetized with clove oil and placed inside the superconducting magnet.

The scanner has a magnetic field of 7.04 Tesla generated by a superconducting magnet operating at 4.2 Kelvin degrees. During the investigations, the brown bullhead specimens were maintained at 300°Kelvin, vital functions being monitored (ECG, temperature, respiration). A 40 mm diameter radio frequency coil has been used. It provides the best ratio between active volume and RF performance that can produce images of the highest resolution. For the general anatomical investigations and for the area investigation, the RARE protocols (Rapid Acquisition with Refocused Echoes) and TurboRARE High Resolution based on the RF pulse sequence of the echo type were used (Tables I, II). These protocols allow the acquisition of a high number of sections of images in a relatively short time, thus avoiding artefacts caused by animal movement. Using a homogenization and gradient unit (HGU) that generates a magnetic field gradient of approximately 1 Tesla / m, it was possible to obtain images with a section thickness of 500 microns and a resolution of up to 76 micrometres.

Table I.- Protocol (I) for MRI investigations in brown bullhead (*Ameiurus nebulosus*).

NR exp. (scan)	Protocol type	TR (ms)	TE (ms)	Flip angle (ϑ°)-	FOV (cm)	Slice thickness (mm)
3	RARE-T1	1300	9	180	4	1
4	TurboRARE-high-res	2500	36	180	4	1
5	TurboRARE-high-res	2500	36	180	2.5	0.5
6	TurboRARE-high-res	2500	36	180	3.4	0.8
7	TurboRARE-high-res	3916.5	36	180	3.3	0.8
8	TurboRARE-high-res	3916.5	36	180	2.9	0.8
9	TurboRARE-high-res	3916.5	36	180	2.9	0.5

Table II.- Protocol (II) for MRI investigations in Brown bullhead (*Ameiurus nebulosus*).

Interslice distance (mm)	Matrix	Resol. read (cm/pixel)	Resol. P1 (cm/pixel)	Averages	Scan time
1.5	256x256	0.0156	0.0156	1	1m 2s 400ms
1.5	384x384	0.0104	0.0104	5	10 m
0.75	384x384	0.0065	0.0065	5	10 m
1.3	384x384	0.0089	0.0078	5	10 m
1.2	384x384	0.0086	0.0076	7	21m 55s 957ms
1.2	384x384	0.0076	0.0083	7	21m 55s 957ms
0.8	384x384	0.0076	0.0083	7	21m 55s 957ms

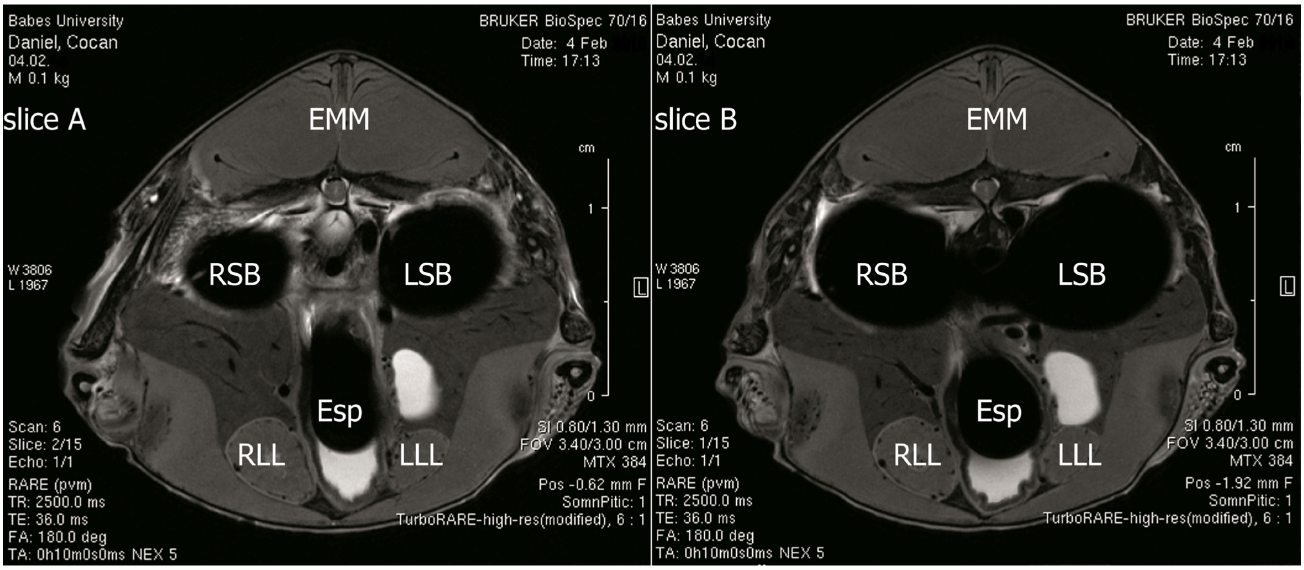

Fig. 1. Axial section of brown bullhead (*Ameiurus nebulosus*). Slice scanned cranial to the first dorsal fin. A, slice 2/15; B, slice 1/15.

RESULTS

In cross sections (Fig. 1) or axial exposure, organs, blood vessels and bone formations appear unchanged in shape, size, aspect and location. The epaxial median muscle (EMM), found on both sides of the median plane, specific for the species with two dorsal fins, (Richter-Moritz, 2017) is highlighted in the dorsal part.

The myomeres are well defined and separated by concentric myosepta (connective tissue). The two groups of epaxial muscles are separated on the medial plane by a longitudinal mioseptum in which the dorsal apophyses of the vertebrae are also found. On the top of the coelomic cavity, ventral to the vertebral column, the obtained MRI reveals the presence of two swim bladders [RSB (right swim bladder) - LSB (left swim bladder)] and laterally, on both sides (subtegumentary), a thin layer of connective tissue (CT), as well as reserves of adipose tissues. The same MRI images allow identification of the esophagus (Esp) along with the two lobes of the liver [RLL (right liver lobe) – LLL (left liver lobe)] ventrally, in the median plane. At the same time, the image obtained also allows the identification of two adipose deposits. One is situated laterally, left to the esophagus (Esp) and dorsal to the left lobe of the liver (LLL). The second is disposed in the medial plane, ventral to the esophagus (Esp), between the two lobes of the liver (Fig. 1). Similar to the situation described above, MRI proved to be an effective method in the visualization of adipose tissue for *Ctenopharyngodon idella*, *Scophthalmus maximus*, *Pseudosciaena crocea*, *Trachinotus ovatus* and *Oreochromis niloticus* (Wu *et al*., 2015).

Also in axial section, on the ventrolateral sides of the trunk, the MRI image highlights the presence of an ovoid, well-defined, piriform and low density formation. It is well delimited and it exhibits a progressive increase in diameter on successive sections. This ovoid formation represents the venomous pectoral gland, disposed at the base of the first hard ray of the pectoral fins. Similar aspects could also be identified in the case of images obtained in the coronal plane (Fig. 2).

The MRI examination on sagittal section allows both the identification of an oval shape, a low density formation (venomous gland), as well as the identification of a hard formation which represents the first hard ray of the pectoral fins (Fig. 3). This study reveals similar anatomical aspects as in the case reported for Siluriformes by Wright (2012a).

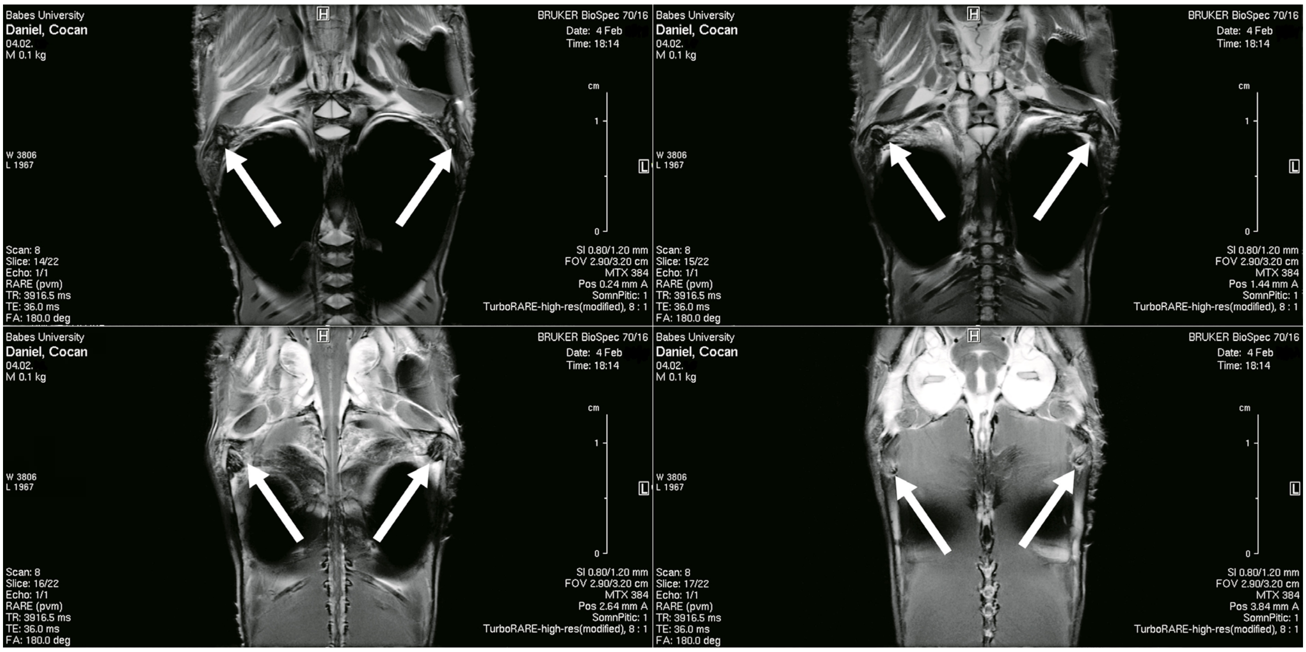

Fig. 2. Venomous pectoral glands of Brown bullhead (*Ameiurus nebulosus*). Different aspects in successive slices from ventral to dorsal part (section in coronal plane).

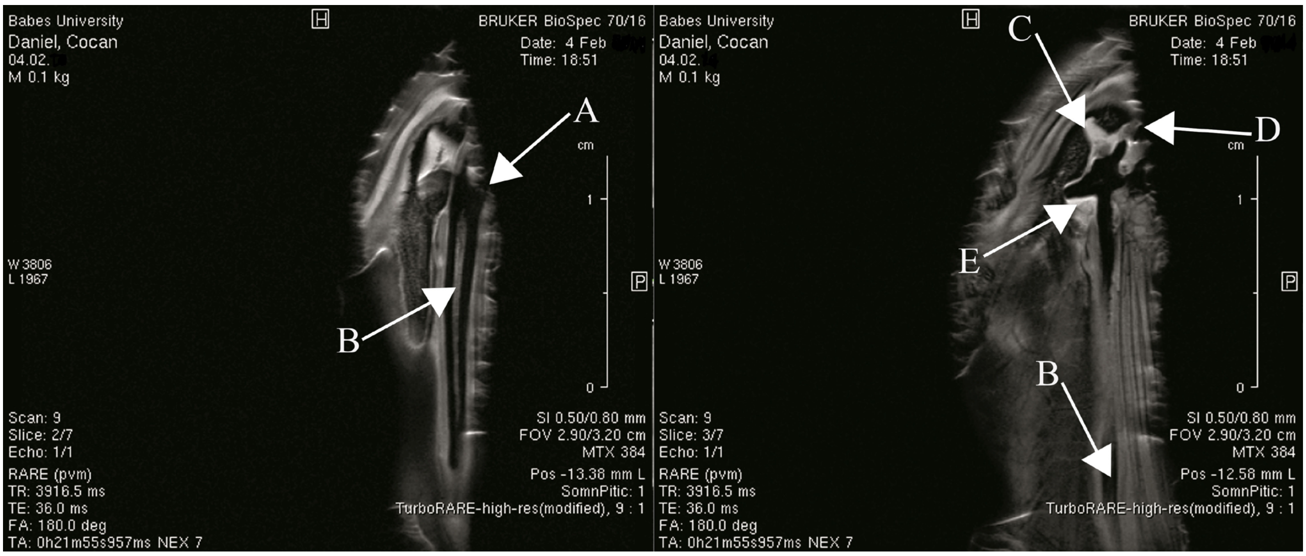

Fig. 3. Morphology of venomous pectoral glands of Brown bullhead (*Ameiurus nebulosus*) in sagittal section. A, venomous pectoral gland; B, pectoral spine; C, anterior process; D, dorsal process; E, ventral process.

The presence of pectoral spines in the *Ameiurus nebulosus* is reported by Rojo (2013), which describes these formations as the result of the calcification of the first pectoral radius. The MRI image obtained on sagittal section allows the identification of three processes oriented in three different directions (dorsal, ventral and anterior) at the base of the pectoral spine. The presence of these processes has also been reported in the literature for the species *Ictalurus lambda* (Hubbs and Hibbard, 1951), *Clarias gariepinus* and *Heteropneustes fossilis* (Satora *et al*., 2008). Muscle bundles are inserted at the level of the three bone processes mentioned above. Their contraction has the role of positioning the pectoral spines in a perpendicular angle on the body (Miano *et al*., 2013; Moldowan *et al*., 2015). In addition to the important defensive role it presents, it seems that pectoral spines in many species also play a role in interspecific communication by issuing high frequency sounds (Fine *et al*., 1997; Fine and Ladich 2003; Kaatz *et al*., 2010).

The MRI image, in the cranial-caudal sense, allows the identification of a well-defined globular formation, dorsally disposed to the vertebral column (Fig. 4), in the medial-sagittal plane.

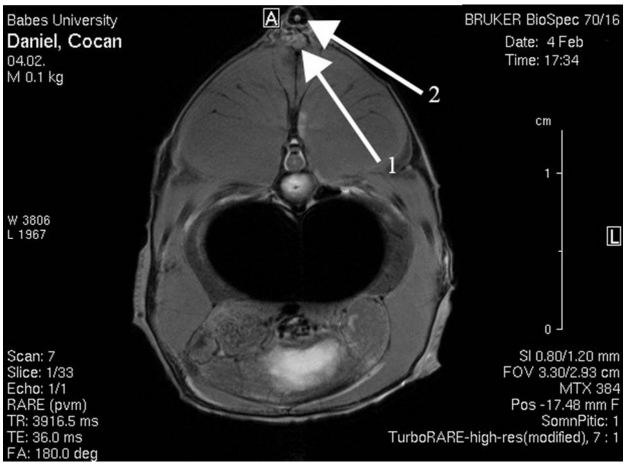

Fig. 4. Anatomical topography of venomous dorsal gland in axial section. Arrow 1, venomous dorsal gland; Arrow 2, dorsal spine.

DISCUSSION

Successive axial exposures allow this formation to be identified as the dorsal venomous gland, which in the *Ameiurus nebulosus* is located at the base of the first hard ray of the dorsal fin. As in the case of pectoral fins, in the dorsal fin, the first ray suffers intense calcification processes. In the case of the studied species, the venomous glands, together with the first hard ray of the pectoral and dorsal fins and the muscular fascicles attached to the radius form the venomous apparatus. Similar issues have been reported in the literature for *Noturus gladiator* (Egge and Simons, 2011) and *Noturus stigmosus* (Wright, 2012a). Unlike the studied species, for the *Scatophagus argus*, the venomous apparatus is more complex, consisting of 11 dorsal spines, two ventral spines and four anal spines, which are associated with the same amount of venomous glands (Cameron and Endean, 1970).

When in an erect position, in brown bullhead, as in other catfish species, the dorsal spine together with the two pectoral spines become a mechanism of protection, increasing practically the dimensions of the body over the ingestion capability of predators (Halstead *et al*., 1953; Endler, 1986; Haddad, 2000; Fine and Ladich, 2003; Bosher *et al.,* 2006; Rojo, 2013). Furthermore the same defense mechanism is described in the literature in the case of individuals of the species *Diodontidae* (Leis, 2006; Byeon *et al.*, 2011).

If the predator performs the attack, due to the pressure and rupture of the spines that have penetrated the tissues of the predator, the venomous apparatus discharges a certain quantity of venom (Blomkalns and Otten, 1999; Satora *et al*., 2008).

Unlike the brown bullhead (*Ameiurus nebulosus*), which presents a dorsal spine and two pectoral spines, the literature only describes the presence of the pectoral spines in African catfish (*Clarias gariepinus*) (Satora *et al*., 2008). Haddad and Gadig (2005) reported only the presence of the dorsal spine in case of sharks from the *Heterodontidae, Squalidae* and *Dalatiidae* families. From these three shark families listed, the presence of a venomous gland at the base of the dorsal spine (Halstead, 1988) is reported only for individuals belonging to *Heterodontidae* and *Squalidae*.

Three spines (2 pectorals and 1 dorsal spine) were highlighted in the studied species, whereas for *Synanceja horrida* and *Synanceja verrucosa*, the number of spines on the body surface is higher (17-19 spines). They are distributed in 12-14 dorsal spines, 3 anal spines and 2 pelvic spines. A venomous gland (Phoon and Alfred, 1965) is placed at the base of each spine. A very large number of spines on the body surface is also reported in the case of *D. eydouxii, D. hystrix, D. holocanthus, D. liturosus* and *D. nicthemerus* (Leis, 2006).

The common length of brown bullhead is usually between 20 to 25 centimeters (Muus and Dahlström, 1968), having almost the same size as other prey fish (rudd, bream, prussian carp). Even though brown bullhead and the species mentioned above share the same ecological niche and are gregarious, they have different defence mechanisms. The presence of scales on rudd, bream and prussian carp is in fact a simple defence mechanism, meanwhile the pectoral and dorsal spines and venomous apparatus of brown bullhead is a complex defence mechanism. Scales are a passive defence mechanism while spines and venomous glands are an active defence mechanism because of muscular contraction when the species is in danger. In this case the dorsal and pectoral spines are positioned in a triangular formation (Rojo, 2013) making it very hard to be caught by predators. The efficiency of this triangular formation and venomous apparatus is increased when brown bullhead is attacked by predators who used their mouths to catch prey (cormorant, pelican, pike, zander, turtle, otter). In case of predators who use their claws to catch the prey (Canadian bald eagle, white-tailed eagle), the defence mechanism is less effective (Jackman *et al.*, 1999).

CONCLUSIONS

The use of MRI technique in the anatomical investigation of venomous glands in brown bullhead (*Ameiurus nebulosus*) proved to be of real use, providing precise information on the shape, size and topography of these glands.

In the studied species, the presence of these three spines, 1 dorsal and 2 pectoral, is an anatomical defensive adaptation against predators. *Ameiurus nebulosus* has a very strong and efficient defensive mechanism regardless of predator. Unlike other species, in brown bullhead, each described spine (first hard ray) has a venomous gland at its base. All the three spines develop the venomous apparatus.

Statement of conflict of interest

The authors declare no conflict of interest.

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