

## SHORT COMMUNICATION

# Anatomical and histochemical features of the vegetative organs of *Dioscorea polystachya* (Dioscoreaceae)

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## ABSTRACT

We investigated the anatomical and histochemical features of *Dioscorea polystachya* (Dioscoreaceae), an important food crop in China. The primary structures of *D. polystachya* include vertical tubers, adventitious roots, twining aerial stems, and leaves with bulblets. The vertical tubers have storage parenchyma with scattered vascular bundles, a cortical meristem zone, and phellem. The cortical meristem zone acts as a cambium to thicken the tubers. The twining stems and petioles both have a sclerenchyma layer with vascular bundles, while the blades have palisade tissues and spongy tissues covered by a cuticle. The roots have stele, an endodermis, and an exodermis. The scattered vascular bundles and phellem in the vertical tubers, the sclerenchyma layers and cuticles in the stems and leaves, and the endodermis and exodermis in the roots facilitated the adaptation of *D. polystachya* to terrestrial environments. The cortical meristem zone thickens the tubers, which improves cultivation techniques. These structural features help to clarify the complex taxonomy, evolutionary history, and phylogenetic relationships of *D. polystachya*.

**Keywords:** anatomy; *Dioscorea polystachya*; histochemistry; morphology; primary structure

## INTRODUCTION

The Chinese yam (*Dioscorea polystachya*, Dioscoreaceae) is an important food crop and ingredient in Chinese traditional medicine (Ding and Gilbert 2000; Babil et al. 2013; He et al. 2014; Chen et al. 2016). This species has a long history of use in East Asia and is extensively cultivated across the region (Ding and Gilbert 2000; Kawasaki et al. 2008; Zhou et al. 2008; Babil et al. 2013; Chen et al. 2016). Cultivars of the *D. polystachya* are highly genetically diverse, with large variations in ploidy among groups; these differences across cultivars facilitate *D. polystachya* cultivation and breeding (Zhou et al. 2008; Babil et al. 2013; Chen et al. 2016). The vegetative organs of *D. polystachya* include adventitious roots and cylindric vertical tubers or vertical rhizomes, which may reach depths of 1–2 m underground (Moseley et al. 1993; Ding and Gilbert 2000; Raman et al. 2014; Yang et al. 2020). *Dioscorea polystachya* have right-twining aerial stems or vines with bulblets, aerial tubers, or seed tubers in the leaf axils (Ding and Gilbert 2000; Walck et al. 2010; Matsumoto et al.

2015). The vertical tubers, or tuber segments and bulblets, are used for vegetative propagation in agricultural settings (Xie et al. 1993; He et al. 2014; Walck, Cofer and Hidayati 2010; Liu 2014; Matsumoto et al. 2015).

Several studies have explored the morphology, anatomy, and histochemistry of various *Dioscorea* species, including *D. polystachya*. The structures investigated have included seedlings, vertical tubers, bulblets, adventitious roots, aerial stems, and leaves (Martin and Ortiz 1963; Degras and Mathurin 1978; Cao and Hu 2003; Zhu et al. 2010b; Tajuddin et al. 2013; Degbeu et al. 2019). In *D. polystachya*, as well as *D. rotundata*, *D. sansibarensis*, *D. alata*, *D. trifida*, *D. nipponnica*, and *D. bulbifera*, vertical tubers and bulblets have tuber-heads with accessory buds that produce new individuals (Rao and Tan 1976; Wickham et al. 1981, 1982; Hamadina 2012). In these species, tuber heads have a suberized periderm, a cortex, and scattered vascular bundles throughout the amyloiferous parenchyma (Martin and Ortiz 1963; Xie et al. 1993; Cao and Hu 2003; Zhu et al. 2010b;

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Hong 2012; Liu 2014). In addition, sclerenchyma layers are found under the periderm in *D. polystachya*, *D. balcanica*, *D. bulbifera* (Raman et al. 2014), *D. alata* (Degras and Mathurin 1978), and *D. esculenta* (Wickham et al. 1981). Amyloplast sedimentation was observed in the growing vertical tubers of *D. polystachya*, suggesting that this species has graviperception abilities (Kawasaki et al. 2008). Seedlings of *D. florilunda* and *D. zingiberensis* consist of a radicle, an epicotyl, adventitious roots, aerial shoots with bulblets, and vertical tubers (Martin and Ortiz 1963; Cao and Hu 2003; Zhu et al. 2010b). A cortical meristem is present in the tuber head and distal vertical tubers of *D. zingiberensis*, *D. rotundata* (Cao et al. 2003a,b; Ile et al. 2006; Li et al. 2010), and *D. cayenensis-rotundata* (Degbeu et al. 2019). The aerial stems of *D. subhastata*, *D. florilunda*, *D. hispida*, *D. alata* and *D. sansibarensis* have peripheral collenchyma, cortex, vascular bundles, and pith (Martin and Ortiz 1963; Tan and Rao 1974; Tajuddin et al. 2014; Sheikh and Kumar 2017; Tenorio et al. 2017); shoot tips have three cellular areas in *D. alata* (Barrao et al. 2014). Roots have an endodermis in *D. florilunda* (Martin and Ortiz 1963). The leaves of *D. alata*, *D. zingiberensis*, and *D. hispida* possess palisade tissue and stomata (Zhu et al. 2010a,b; Xiao et al. 2013; Tajuddin et al. 2014; Sheikh and Kumar 2017).

Although the anatomical characters of the vertical tubers and bulblets of the *D. polystachya* have been studied (Xie et al. 1993; Liu 2014; Raman et al. 2014), aerial stems, aerial leaves, and adventitious roots have been little investigated, and the few available studies did not use modern histochemical staining techniques (Brundrett, et al. 1988, Brundrett et al. 1991; Xiang et al. 2019; Yang et al. 2011, 2019a,b, 2020; Zhang et al. 2017; Zhang et al. 2018). To address this knowledge gap, we aimed to analyze the structural and histochemical features of the *D. polystachya* during development. We aimed to clarify the growth habits of this species in order to improve cultivation techniques. Therefore, in this study, we characterized *D. polystachya* anatomy, including the vertical tubers, bulblets, aerial stems and leaves, and adventitious roots. Our results may also inform future studies of this complex taxon, particularly with respect to its evolutionary history and phylogenetic relationships with congeners (Ding and Gilbert 2000; Zhou et al. 2008; Babil et al. 2013; Chen et al. 2016).

## MATERIALS AND METHODS

### Plant cultivation and sampling

*Dioscorea polystachya* samples were collected in Shennongjia, Hubei Province, China on 25 October 2019. Fifty bulblets, 50 vertical tubers, and 50 vertical-tuber segments from these plants were cultivated in sand-filled pots beginning on 1 March 2020; plants were watered with 1/4 Hoagland

nutrient solution to keep the sand moist. Plants were grown in ambient conditions with an artificial light intensity of approximately 3500 lx and a 14:10 h (light: dark) photoperiod; artificial light was provided by LED devices. Ambient temperature ranged from 18–32°C, and relative humidity was 55–85% (Duan et al. 2020). On 20 March 2020, we collected 5 samples of buds, aerial stems, aerial leaves, and adventitious roots from each of the sprouting bulblets, vertical tubers, and vertical-tuber segments. On 6 June 2020, we repeated the sampling process, collecting five samples of each of the vertical tubers, aerial stems, aerial leaves, and adventitious roots growing on each of the sprouting bulblets, vertical tubers, and vertical-tuber segments. On 6 July 2020, the sampling process was repeated, collecting five samples of each of the vertical tubers produced by each of the sprouting bulblets, vertical tubers, and vertical-tuber segments. These samples represented the different developmental phases of the *D. polystachya* throughout the growing season. Finally, on 6 October 2020, we collected five samples of vertical tubers, aerial stems, aerial bulblets, aerial leaves, and adventitious roots from each of the sprouting bulblets, vertical tubers, and vertical-tuber segments. This sample reflected the state of the *D. polystachya* in the harvest season. Samples were fixed in formaldehyde-alcohol-acetic acid (FAA) immediately after collection (Ruzin 1999).

### Microscopy and histochemistry

The bulblets produced adventitious roots at about 0.7–1.8 cm long, while the bud bases and vertical tubers produced adventitious roots at about 2.5–21.2 cm long. Once roots were produced, root tissues were then sectioned freehand under a stereoscope (JSZ6, JNOEC, China), using a two-sided razor blade. Root sections were cut at 10 mm, 20 mm, and 30 mm from the apex. Cross-sections were taken of the buds produced by the sprouting bulblets, vertical tubers, and vertical-tuber segments. Cross-sections or longitudinal sections were also taken of the proximal, medial, and distal ends of the vertical tubers produced by the sprouting bulblets, vertical tubers, and vertical-tuber segments throughout the growth season. Cross-sections were also taken of mature aerial stems, bulblets, petioles, and blades. Sections were divided into three identical groups, each containing 3–6 identical sections. Each group of sections was stained using a different dye: 0.1% (w/v) Sudan red 7B, to test for suberin in the cell walls (Brundrett et al. 1991); 0.1% (w/v) berberine hemisulfate-aniline blue to test for Casparian bands and lignin in the cell walls (Brundrett et al. 1988; Seago et al. 1999); and 0.05% (w/v) toluidine blue O to visualize tissue structures (Ruzin 1999; Peterson, Peterson, and Meville 2008). All specimens were washed with sterile water 2–3 times, mounted with sterile water, and then examined using bright-field microscopy under a Leica DME microscope (Germany). Specimens

were photographed with a digital camera and a micrometer (Nikon E5400, Japan). Specimens stained with BAB were viewed under ultraviolet light on an Olympus IX71 epifluorescence microscope (Japan) with excitation filter G 365 nm, absorption filter barrier U-WB (blue light), dichromatic mirror DM 500, compensation excitation filter BP 450–480, and compensation absorption filter BA 515. BAB-stained specimens were photographed with a digital camera and a micrometer (RZ200C-21, Ruizhi Corp., China) (Yang et al. 2011).

## RESULTS

### Vertical tubers and bulblets

The vertical tubers and bulblets of *D. polystachya* had tuber heads with accessory buds covered by scale leaves (Fig. 1A). Vertical tubers, vertical-tuber segments, and bulblets sprouted twining stems, vertical tubers, and adventitious roots (Fig. 1B,C); vertical tubers and bulblets died after sprouting, which have mature vegetative organs. Vertical tubers had pith with scattered vascular bundles, a parenchymal cortex, and phellem; secretory cavities were scattered throughout the parenchymal tissues (Fig. 1D–H). At a very young stage, the cortex of the vertical tuber was composed of parenchymal tissues. During the vigorous growth stage in the summer, a meristem zone formed

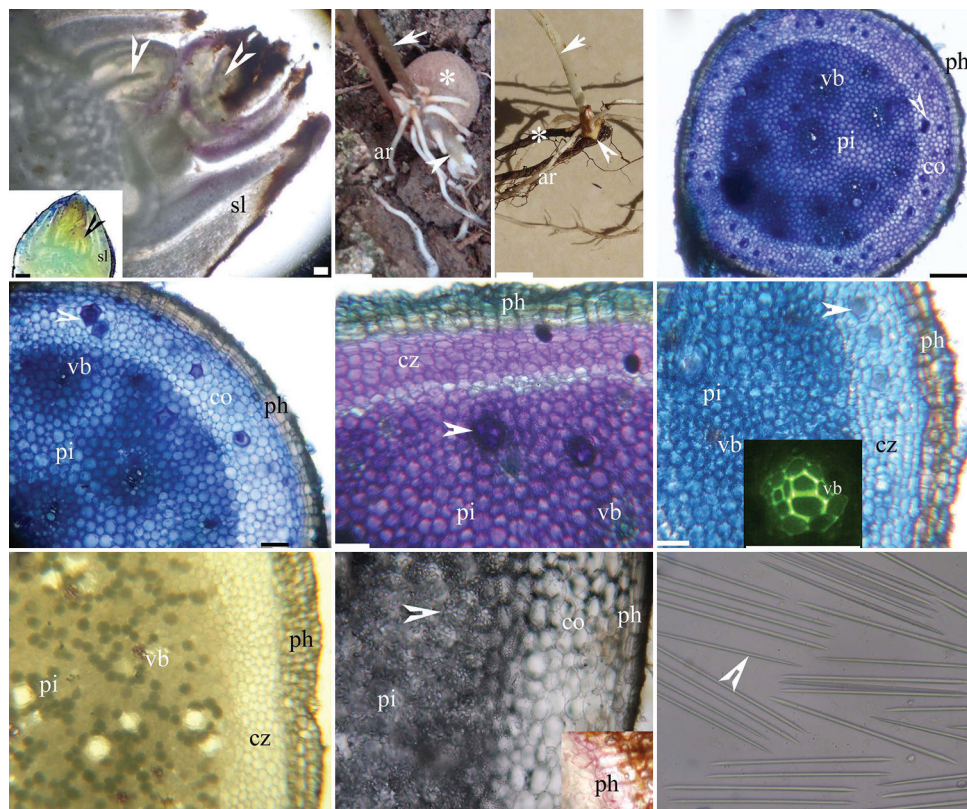
along the periphery of the cortex, and the vertical tubers thickened (Fig. 1F–H). The storage parenchymal cells in the vertical tubers contained amyloplast and raphides; the cortex cells stopped dividing in autumn (Fig. 1I, J). and the phellem became suberized (Fig. 1I, inset).

### Twining stems and leaves

Twining stems had a sclerenchyma ring internal to the cortex, with scattered vascular bundles delimiting the central pith (Fig. 2A,B); the twining-stem epidermis had a cuticle. The petioles had a sclerenchyma ring internal to a chloroplast-containing cortex; the sclerenchyma ring in the petioles contained scattered vascular bundles delimiting a central pith (Fig. 2C,D). The petiole epidermis had a cuticle. The blades had a vein with vascular bundles. The adaxial side of each blade had palisade tissue, while the abaxial side of each blade had spongy tissue; both sides of the blade were covered with by a cuticle (Fig. 2E,F).

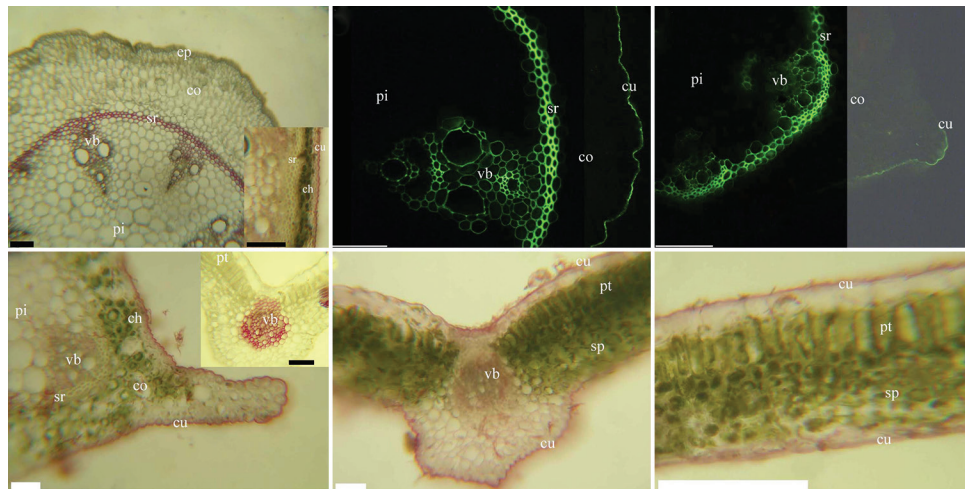
### Adventitious roots

At about 10 mm from the apex, the adventitious roots had a protoxylem with a few central protoxylem and metaxylem vessels, a suberized and lignified endodermis and exodermis with Casparian bands, a cortex, and a rhizodermis (Fig. 3A–C). At about 20 mm from the apex, the endodermis contained some suberin and lignin as well as passage cells; the endodermis had obvious Casparian

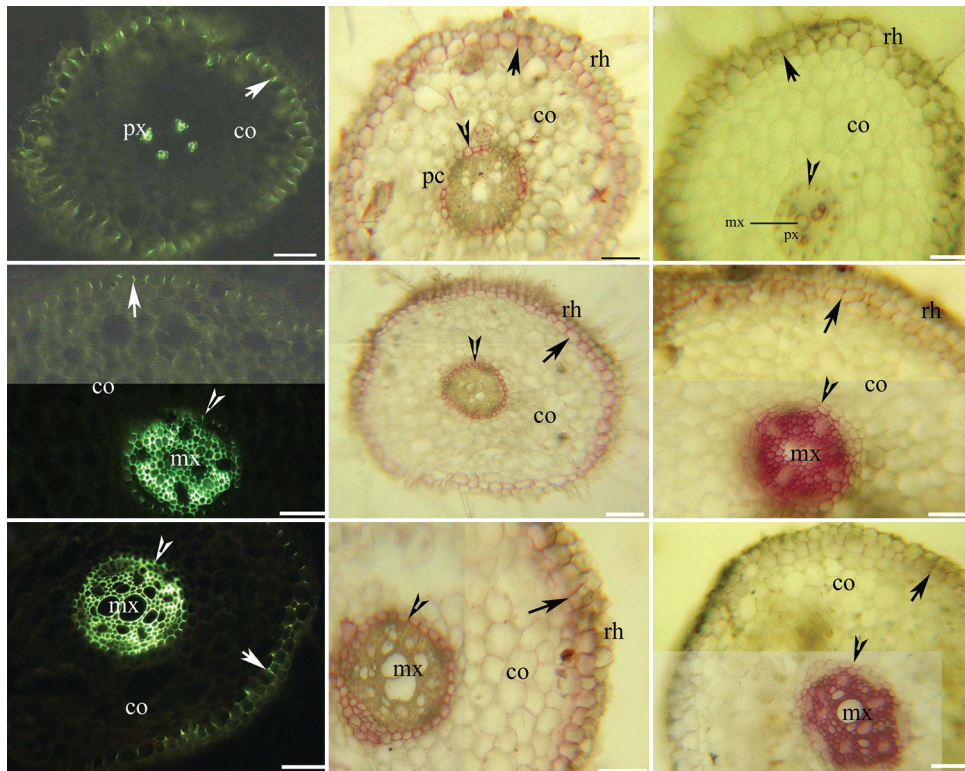


**Fig 1.** Anatomy of vertical tubers and bulblets of *D. polystachya*





**Fig 2.** Anatomy of twining stems and leaves



**Fig 3.** Anatomy of adventitious roots

bands, and the pith had obvious metaxylem (Fig. 2B, 3D–F). At about 30 mm from the apex, the pith had a fully developed metaxylem, while the endodermis and exodermis had suberin and less lignin (Fig. 3G–I).

## DISCUSSION

The buds that formed on the tuber heads of *D. polystachya* vertical tubers and bulblets produced twining aerial stems, vertical tubers, and adventitious roots, consistent with observations in *D. alata*, *D. bulbifera*, *D. esculenta*, *D. nipponica*,

*D. rotundata*, *D. sansibarensis*, *D. trifida*, and *D. zingiberensis* (Rao and Tan, 1976; Wickham et al. 1981, 1982; Xie et al., 1993; Ile et al., 2006; Hamadina, 2012; Hong 2012; Liu, 2014). Vertical tubers of *D. polystachya* had a central storage parenchyma with scattered vascular bundles, a cortical meristem zone, and phellem; the cortical meristem zone ceased dilation and appeared as cortex tissue in the autumn. Similar vertical tuber structures were reported in *D. alata*, *D. balcanica*, *D. cayenensis*, *D. esculenta*, *D. floribunda*, *D. nipponica*, *D. rotundata*, *D. spiculiflora*, *D. transversa*, *D. trifida*, and *D. zingiberensis* (Martin and Ortiz, 1963; Ayensu, 1972;

Degras and Mathurin, 1978; Wickham et al. 1981, 1982; Cao and Hu, 2003; Cao, Lin, and Hu 2003a,b; Ile et al., 2006; Li et al., 2010; Raman et al., 2014). As is common in most plants, the phellem of *D. polystachya* included suberin, but not lignin (Fahn, 1990; Evert, 2006; Crang et al. 2019). In contrast, *D. alata*, *D. balcanica*, *D. esculenta* and *D. polystachya* have sclerenchyma layers (Ayensu, 1972; Degras and Mathurin, 1978; Wickham et al. 1981; Raman et al., 2014); however, this was not observed in our samples.

Similar to *D. alata*, *D. belophylla*, *D. cinnamomifolia*, *D. hispida*, *D. sansibarensis*, and *D. zingiberensis*, the twining stems and petioles of *D. polystachya* had a sclerenchyma layer with vascular bundles, and the blades had both palisade tissue and spongy tissue (Tan and Rao, 1974; Tajuddin et al., 2013; Xiao et al., 2013; Sheikh and Kumar, 2017; Tenorio et al., 2017). However, the twining stems of *D. stegelmanniana*, *D. ovata*, and *D. campestris* had two layers of vascular bundles, with the outer layer containing sclerenchyma, while the pith of *D. ovata* and *D. campestris* stems had sclereids, in contrast to parenchyma of *D. polystachya* (Tenorio et al., 2017). The sclerenchyma layer in the stem, which is also found in *Cardamine hupingshanensis*, *Cynodon dactylon*, *Paspalum distichum*, *Phalaris arundinacea*, and *Zizania latifolia*, may increase mechanical strength (Yang et al. 2011, 2014; Zhang et al., 2017; Xiang et al., 2019). The sclerenchymatous tissue in the tuber phellem of *D. alata*, *D. balcanica*, and *D. esculenta*, and in the stem pith of *D. ovata* and *D. campestris*, may help the plant tissues to adapt to arid environments (Ayensu, 1972; Degras and Mathurin, 1978; Wickham et al. 1981; Raman et al., 2014; Tenorio et al., 2017). However, *D. polystachya* lacks the sclerenchyma layers under the phellem in the tubers and the peripheral collenchyma under the epidermis of the aerial stems (Martin and Ortiz 1963; Tan and Rao 1974; Tajuddin et al. 2014; Sheikh and Kumar 2017; Tenorio et al. 2017).

The stele in the *D. polystachya* roots have protoxylem and metaxylem, and the endodermis and exodermis are both one layer, each containing Casparian bands, suberin lamellae, and small amounts of lignin. This root structure is common in other perennial plants, including *Camellia sinensis*, *C. dactylon*, *Hydrocotyle sibthorpioides*, *P. arundinacea*, *Z. latifolia*, and *Alternanthera philoxeroides* (Evert, 2006; Yang et al., 2011, 2014, 2015; Hu et al., 2016; Zhang et al., 2017; Crang et al. 2019). In contrast, the exodermises of *C. dactylon*, *P. arundinacea*, and *Z. latifolia* have two or more layers. The endodermis, exodermis, cuticle, and phellem act as barriers, blocking water and ions from penetrating the tissue, but allowing passage to individual cells (Enstone et al. 2003; Yang et al., 2011, 2014, 2015; Zhang et al., 2017; Zhang et al. 2018), the barriers in the *D. polystachya* consist of cuticle, phellem, endodermis and exodermis.

## CONCLUSIONS

*Dioscorea polystachya* plants consist of vertical tubers, adventitious roots, twining aerial stems, and leaves with bulblets. The vegetative propagules of the vertical tubers and bulblets have tuber heads with buds. Anatomically, the vertical tubers contain storage parenchyma with scattered vascular bundles, a cortical meristem zone, and phellem. The cortical meristem zone acts as a cambium in the dicotyledon to thicken the tubers. Twining stems and petioles have a sclerenchyma layer with vascular bundles, and blades have differentiated tissues. The roots have primary xylem, as well as an endodermis and exodermis with Casparian bands, suberin lamellae, and lignin. These structural features help to clarify the complex taxonomy, evolutionary history, and phylogenetic relationships of *D. polystachya*. The structural features such as scattered vascular bundles and phellem in the vertical tubers, the sclerenchyma layers and cuticles in the twining stems and leaves, and the endodermis and exodermis in the roots facilitated the adaptation of *D. polystachya* to terrestrial environments. The cortical meristem zone thickens the tubers and may provide a basis for improving agricultural use.

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## Author contributions

All authors contributed to this study design, conception and data collection. Cunyu Zhou and Hanyu Gong characterized the morphological, anatomical and histochemical alternations and wrote the manuscript. Xia Zhang, Youzhi Li, Chongnan Zhao, Huanhuan Xiong, Teng Li and Mengdi Zhang assisted stain solution preparation. Zhiguo Jiang and Chaodong Yang prepared cross sections and analyzed the root anatomical structures and histochemical components. Cunyu Zhou supervised the experiments and revised the manuscript. All authors read and approved the final manuscript.

## Conflict of interests

The authors declare there are no conflicts of interest related to this article.

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