

Emendation of the genus *Bactrodesmiastrum* (Sordariomycetes) and description of *Bactrodesmiastrum monilioides* sp. nov. from plant debris in Spain

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Abstract *Bactrodesmiastrum monilioides* sp. nov. is described and illustrated from the twig of an unidentified plant collected in Spain. This fungus is characterized by sporodochial conidiomata with moniliform conidiophores, its conidia being solitary, broadly navicular to obovoid, mostly 2-septate, dark brown, with a black band at the septa and basal cell pale brown to brown. Analysis of the ITS region and D1/D2 domains of the LSU of rDNA sequences confirms the placement of this fungus in *Bactrodesmiastrum* (Sordariomycetes). Because the genus *Bactrodesmiastrum* was originally characterized by solitary or aggregated conidiophores reduced to a single brown monoblastic conidiogenous cell, an emended description of the genus is provided to accommodate the novel species. A key to species of *Bactrodesmiastrum* is provided.

Taxonomical novelties *Bactrodesmiastrum monilioides* Hern.-Rest., Gené & R.F. Castañeda sp. nov.

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Introduction

During a survey of anamorphic fungi from plant debris collected in two natural areas in the Eurosiberian region in the north of Spain, two strains of an interesting fungus were collected and isolated in pure culture. The fungus showed some similarity with members of the genus *Bactrodesmiastrum* Hol.-Jech., but differed mainly by the production of rather long moniliform conidiophores in sporodochial conidiomata. *Bactrodesmiastrum* was described on the basis of *B. obscurum* Hol.-Jech., the type species, and characterized by solitary or aggregate conidiophores reduced to dark brown conidiogenous cells, which could be conical-cylindrical, lageniform or subulate, and by the production of dark brown, smooth-walled phragmoconidia (Holubová-Jechová 1984). Currently, the genus includes two other saprophytic species, viz. *B. obovatum* (M. Caldusch et al.) J. Mena et al. and *B. pyriforme* Hern.-Rest. et al. (Hernández-Restrepo et al. 2013). However, none of these fungi showed the type of conidiogenous apparatus observed in the Spanish specimens.

Sequence analyses of the internal transcribed spacers (ITS) and the D1/D2 region of the large subunit (LSU) of the rDNA of the two strains confirmed a close phylogenetic relationship with *Bactrodesmiastrum* species. Therefore, the combination of morphological features supported by molecular evidences allows us to describe a new species of *Bactrodesmiastrum*. An emended description of the genus is provided to accommodate the novel fungus, and a key to the species concerned is also provided.

Materials and methods

Isolation and identification of fungi

Samples of decomposing wood, leaves and bark were collected from the forest floor at the Ordesa y Monte Perdido National Park and at the Picos de Europa National Park, two locations in northern Spain. Samples were treated according to Castañeda-Ruiz (2005) and Hernández-Restrepo et al.(2013). To achieve pure cultures, conidia from the specimens growing on the natural substratum were transferred to potato carrot agar (PCA; 20 g potatoes, 20 g carrots, 20 g agar, 1 L distilled water) and oatmeal agar

(OA; 30 g oat flakes, 20 g agar, 1 L distilled water), and incubated at 25 °C in the dark. In the description, colour notations in parentheses are based on Kornerup and Wanscher (1984). Microscopic features were examined in lactic acid wet mounts using an Olympus CH2 light microscope (Olympus Corporation, Tokyo, Japan). Photomicrographs were obtained with a Zeiss Axio Imager M1 light microscope (Carl Zeiss Meditec, Göttingen, Germany) and a Jeol JSM-6400 scanning electron microscope (JEOL, Ltd., Tokyo, Japan).

Representative specimens of the fungi examined were deposited in the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands (CBS), in the Faculty of

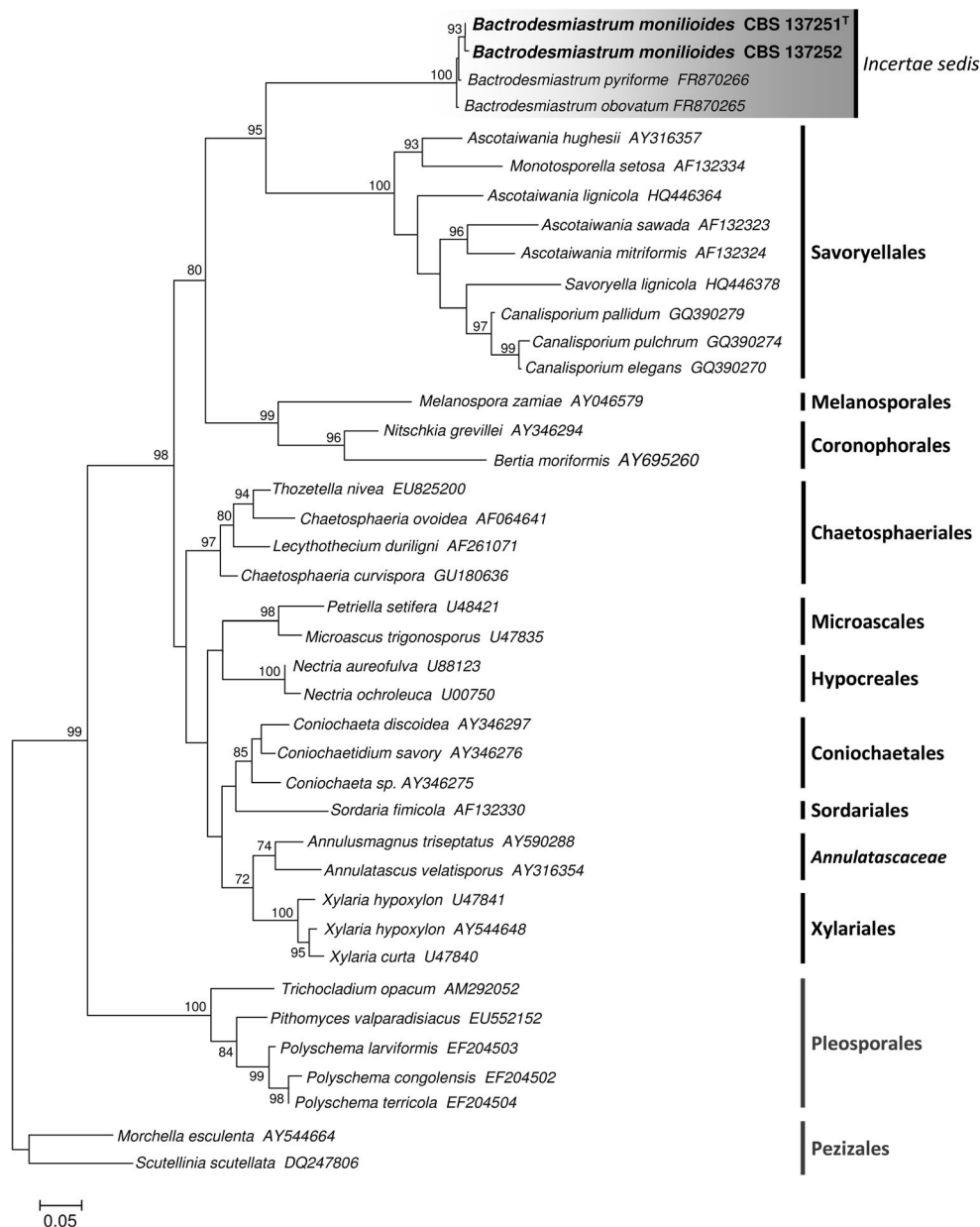


Fig. 1 Maximum likelihood tree constructed with the D1/D2 sequences of the LSU rDNA. Branch length is proportional to distance. Bootstrap values >70 % are indicated near the internodes. Orders or families of the Sordariomycetes are in bold. [†]type strain

Medicine, Reus, Spain (FMR), and in the Martin-Luther-University, Halle, Germany (HAL).

DNA extraction, sequencing and phylogenetic analysis

DNA was extracted from pure cultures using the PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol. The ITS region and D1/D2 domain of the LSU of the rDNA were amplified with the primer pairs ITS5/ITS4 and NL1/NL4, respectively, following the protocols described by Cano et al. (2004) and Gilgado et al. (2005). PCR products were purified and sequenced at Macrogen Corp. Europe (Amsterdam, the Netherlands). The program SeqMan 7.0 was used to obtain consensus sequences. BLAST sequence identity searches (Altschul et al. 1990) were carried out to compare the investigated strains (CBS 137251 and CBS 137252) with other fungi deposited in GenBank.

A phylogenetic analysis using the D1/D2 sequences was performed to assess the relationships of our strains with *Bactrodesmiastrum* species and other members of the Sordariomycetes, and species with some morphological similarity such as those of the genus *Polyschema* Upadhyay in the Dothideomycetes. Sequences used in the analysis were retrieved from GenBank from previous studies (Shenoy et al. 2010; Boonyuen et al. 2011; Réblová and Seifert 2011; Hernández-Restrepo et al. 2013). Sequences of *Morchella esculenta* and *Scutellinia scutellata* (Pezizomycetes) were used as outgroups.

Alignments of the sequences were performed first in MEGA5 (Tamura et al. 2011) using its Clustal W application, followed by manual adjustments with a text editor. The phylogenetic study was carried out with maximum likelihood analysis (ML) using MEGA5 software. Tamura-Nei with gamma distribution was the best-fit nucleotide substitution model, and we used nearest-neighbor interchange (NNI) as heuristic model. Confident branch support was defined as bootstrap values (bs) ≥ 70 % from a maximum likelihood search with 1000 replicates.

Sequences were deposited in GenBank with the following accession numbers: KF771878 (ITS) and KF771879 (D1/D2) for CBS 137251, and KF771876 (ITS) and KF771877 (D1/D2) for CBS 137252. The alignment was deposited in TreeBASE (submission number S16337).

Results

The BLASTN search using the D1/D2 sequences of the strains CBS 137251 and CBS 137252 showed a

similarity of 98–99 % with *B. pyriforme* (FR870265 and HE646637) and *B. obovatum* (FR870266). The ITS sequences of the strains CBS 137251 and CBS 137252 revealed that the closest hits were FR870263 and HE646636 corresponding to *B. pyriforme* with a similarity between 86 and 87 %, and FR870264 corresponding to *B. obovatum* with a similarity of 87 %. While the D1/D2 sequences were not useful for separating the investigated strains from the other two *Bactrodesmiastrum* species, the ITS sequences revealed that they could be considered to represent a different species.

The D1/D2 domain alignment contained 40 sequences including the two outgroups, and 597 characters were used in the phylogenetic analysis. The groups obtained in the ML tree agreed with several lineages revealed in previous phylogenetic assessments, including ten orders (i.e., Chaetosphaeriales, Coniochaetales, Coronophorales, Hypocreales, Melanosporales, Microascales, Savoryellales, Sordariales, and Xylariales in Sordariomycetes and Pleosporales) and a family (Annulatasaceae). The resulting phylogenetic tree (Fig. 1) placed the two investigated isolates in an *incertae sedis* clade (100 % bs) of Sordariomycetes, exclusively formed by *Bactrodesmiastrum* species. This appeared as the sister clade of Savoryellales, but both clades appeared separated by considerable branch lengths.

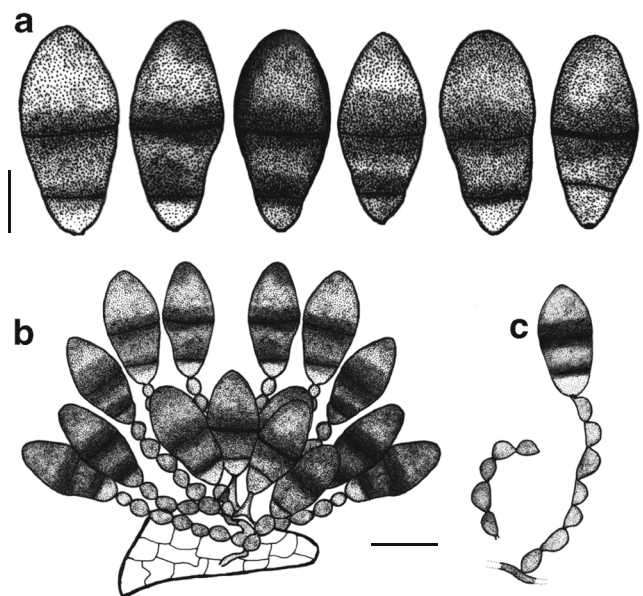


Fig. 2 *Bactrodesmiastrum monilioides* (CBS 137251). **a–c** Conidia, sporodochium and moniliform conidiophores from the natural substratum. Bars **a**=10 μ m; **b**, **c**=20 μ m

Taxonomy

Bactrodesmiastrum Hol.-Jech., Folia Geobot. Phytotax. 19: 103, 1984.

Type species: *Bactrodesmiastrum obscurum* Hol.-Jech., Folia Geobot. Phytotax. 19: 105, 1984.

Emended description: *Colonies* effuse, blackish brown to black. *Mycelium* partly immersed in the substratum, composed of branched, septate, pale brown, smooth hyphae. *Conidiophores* semi-macronematous or macronematous, mononematous, simple, usually reduced to conidiogenous cells arranged singly or aggregated in small groups, or conidiophores arising from

pulvinate to subpustulate, scattered and black sporodochial conidiomata; conidiophores from sporodochia simple or branched, mostly moniliform, composed of globose, subglobose or clavate cells centrally or eccentrically connected, pale brown to brown, smooth-walled. *Conidiogenous cells* monoblastic, determinate, integrated, terminal, usually cylindrical to conical with truncate apex and swollen base, mid-brown to dark brown, smooth-walled; conidiogenous cells from sporodochial conidiophores globose, subglobose or clavate, pale brown to brown. *Conidial secession* schizolytic. *Conidia* solitary, dry, acrogenous, clavate, obovoid, pyriform or navicular,

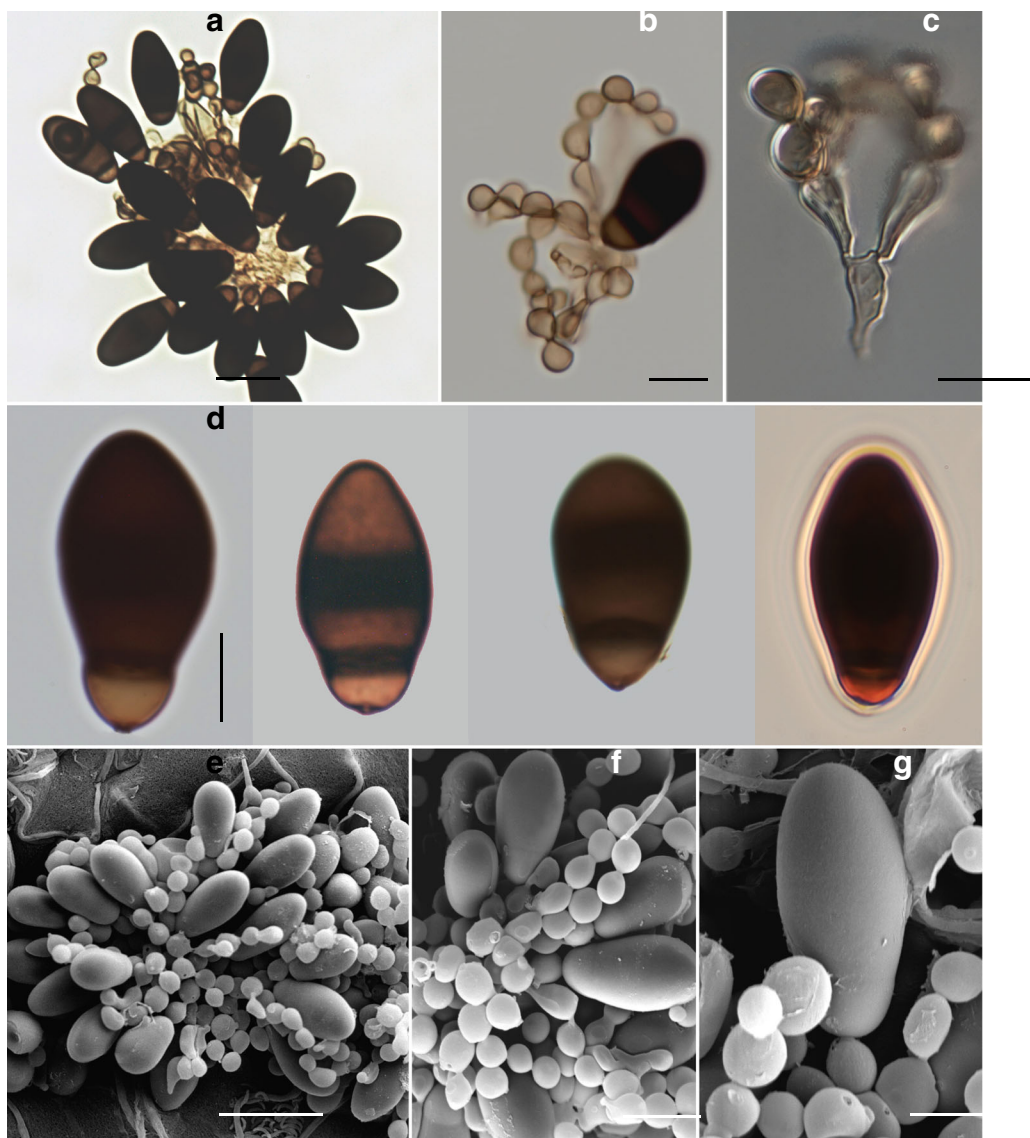


Fig. 3 *Bactrodesmiastrum monilioides* (CBS 137251). **a–d** Conidiogenous apparatus from the natural substratum. **e–g** Conidiogenous apparatus from culture. **a, e** Sporodochia. **b, f, g** Moniliform conidiophores with conidia. **c** Detail of a conidiophore showing basal racket cells. **d** Conidia. Bars **a**= 20 μ m; **b–d, f, g**= 10 μ m; **e**= 25 μ m

2–5-septate, obconico-truncate at the base, brown, with paler proximal cells and darker distal cells, often with black bands around the septa, smooth-walled. *Sexual morph* unknown.

Bactrodesmiastrum monilioides Hern.-Rest., Gené & R.

F. Castañeda, sp. nov. (Figs. 2–3)

MycoBank: MB 805963

Etymology: Latin, *monilioides*- referring to the catenulate swollen cells, constricted at the septa, forming the conidiophores.

Colonies on the natural substratum effuse, dark brown or black. *Mycelium* superficial and immersed, composed of septate, cylindrical to torulose, branched, pale brown, smooth-walled, 3–5 µm wide hyphae. *Conidiomata* sporodochial, pulvinate to subpustulate, scattered, superficial, black, 85–190 µm diam. *Conidiophores* macronematous, mononematous, compact or loosely fasciculate, erect or prostrate, simple or branched, mostly moniliform, with globose, subglobose, ellipsoidal or clavate cells, 4–6 µm wide, centrally or eccentrically connected, branches sometimes arise from basal racket cells, pale brown to brown, smooth-walled, 15–75×3–6 µm; conidiophores reduced to a single conidiogenous cell occasionally present. *Conidiogenous cells* monoblastic, integrated, mostly terminal, globose, subglobose, ellipsoidal or clavate, occasionally subulate or lageniform, pale brown to brown, smooth-walled, 5–8×4–6 µm. *Conidia* broadly navicular to obovoid, mostly 2-septate, apical cell bigger than the others, basal cell broadly obconico-subtruncate and pale brown, distal cells dark brown, with a black band around the septa, smooth-walled, 24–35×15–18 µm, basal scar 1–1.5 µm wide.

Culture: Colonies on OA and PCA at 25 °C attaining 24–25 mm after 14 days, granulose with black spots at the centre, glabrous or slightly cottony and white towards the periphery; reverse dark blond (5E4). Sporulation after 7 days. Sporodochia similar to those observed on the natural substratum, but producing smaller conidia, 25–26 × 10–12.5 µm.

Holotype: **SPAIN. Aragón**, Ordesa y Monte Perdido National Park, near Añisclo Canyon, 42°35'17,57"N; 0°01'37,39"O, 1316 masl, on a decaying twig of an unidentified plant, 19 June 2009, *M. Hernández-Restrepo, Julio Mena-Portales* and *Josep Cano*. HAL 2613 F (Holotype), FMR H-10756 (Isotype). Cultures ex-type: CBS 137251, FMR 10756.

Additional specimen examined: **SPAIN. Asturias**, Picos de Europa National Park, Poncebos Municipality, Cares river, AS-264 Road, 43°16'34,10"N; 4°50'2,56"

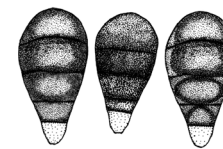
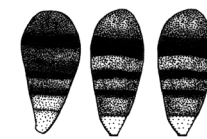
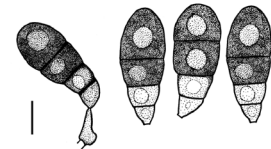
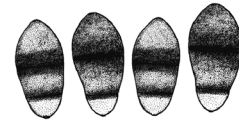
O; 199 masl, on a decaying twig of an unidentified plant, 14 July 2010, *M. Hernández-Restrepo, Julio Mena-Portales* and *Josep Guarro* (CBS 137252, FMR 11337).

Discussion

The novel species *B. monilioides* is easily distinguishable from all other *Bactrodesmiastrum* species by its moniliform conidiophores arranged in sporodochia, and by its broadly navicular, 2-septate conidia. Diagnostic features to distinguish the currently accepted species of *Bactrodesmiastrum* are provided in the key below. The morphology observed in *B. monilioides* can cause some confusion with the identification of this fungus, since other genera such as *Polyschema* or *Bactrodesmium* Cooke show similar conidiogenous structures. However, the members of *Polyschema* can be differentiated by their monotretic or polytretic conidiogenous cells (Ellis 1976), and the type species of the genus, *P. terricola* Upadhyay, as well as two other species of *Polyschema*, *P. congolensis* Reising & Kiffer and *P. larviformis* (Fairman) M.B. Ellis, having been proved to belong to the order Pleosporales (Dothideomycetes) (Shenoy et al. 2010). By contrast, *Bactrodesmiastrum* has monoblastic conidiogenous cells, and phylogenetically its species show an unresolved position within the subclass Hypocreomycetidae (Sordariomycetes), appearing as a sister clade of the Savoryellales (Fig. 1) as previously published (Hernández-Restrepo et al. 2013). Most species of *Bactrodesmium* also show monoblastic conidiogenous cells, although none of them have the type of conidiogenous apparatus observed in the novel species, and the conidia of *Bactrodesmium* have rhexolytic secession and usually a wider basal scar (3–5 µm wide versus 1–1.5 µm wide in *B. monilioides*). It is noteworthy that the phylogeny of *Bactrodesmium* remains uncertain; the type species, *B. abruptum* (Berk. & Broome) E.W. Mason & S. Hughes, has never been sequenced, and the few molecular studies on some *Bactrodesmium* species have suggested that it is a poly-phyletic genus (Hernández-Restrepo et al. 2013). Based on our data, the D1/D2 sequence of a Spanish specimen morphologically identified as *Bactrodesmium pallidum* M.B. Ellis fits into the Savoryellales (Hernández-Restrepo 2013), while *B. abruptum*, according to MycoBank (www.mycobank.org), is a dothideomycetous fungus.

Key to the species of *Bactrodesmiastrum*

1. Sporodochial conidiomata present; conidiophores mostly moniliform; conidia mostly 2-septate, broadly navicular to obovoid, 24–35 × 15–18 μm, dark brown, with a black band around the septa and a pale brown basal cell.....***B. monilioides***
- 1'. Sporodochial conidiomata absent; conidiophores otherwise, usually reduced to a single conidiogenous cell.....2
2. Conidia mostly 3-septate, clavate, obovoid to pyriform, 29–35 × 11–14 μm, brown, with two darker distal cells and two paler proximal cells***B. obscurum***
- 2'. Conidia mostly 4-septate3
3. Conidia broadly clavate or obovate, 22.5–33.5 × 12–15 μm, blackish brown at the apex and progressively paler towards the base***B. obovatum***
- 3'. Conidia pyriform or obovoid, 20–50 × 14–28 μm, dark brown to black, with 1–2 paler proximal cells.....***B. pyriforme***



(Bars = 10 μm)

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

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