The Phylogenetic Relationship Revealed Three New Wood-Inhabiting Fungal Species From Genus *Trechispora*

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Wood-inhabiting fungi play a significant role in wood degradation and the cycle of matter in the ecological system. In the present study, three new wood-inhabiting fungal species, *Trechispora bambusicola*, *Trechispora fimbriata*, and *Trechispora fissurata* sp. nov., are nested in *Trechispora*, which are proposed based on a combination of morphological features and molecular evidence. Sequences of internal transcribed spacer (ITS) and large subunit (nLSU) regions of the studied samples were generated, and the phylogenetic analyses were performed with maximum likelihood, maximum parsimony, and Bayesian inference methods. The phylogenetic analyses inferred from ITS showed that *T. bambusicola* was sister to *Trechispora stevensonii*, *T. fimbriata* grouped with *Trechispora nivea*, and *T. fissurata* grouped with *Trechispora echinospora*. The phylogenetic tree based on ITS + nLSU sequences demonstrated that *T. bambusicola* formed a single lineage and then grouped with *Trechispora rigida* and *T. stevensonii*. *T. fimbriata* was sister to *T. nivea*. *T. fissurata* grouped with *Trechispora thelephora*.

Keywords: Hydnodontaceae, phylogeny, taxonomy, wood-inhabiting fungi, Yunnan Province

INTRODUCTION

*Trechispora* P. Karst. (Hydnodontaceae, Trechisporales) was typified with *Trechispora onusta* P. Karst. (Karsten, 1890). It is characterized by the resupinate to effused basidiomata with smooth to hydnoid to poroid hymenophore, a monomitic or dimitic hyphal structure with clamped generative hyphae having typical ampullaceous septa, and short cylindric

Abbreviations: ITS, internal transcribed spacer; nLSU, large subunit; SWFC, herbarium of Southwest Forestry University, Kunming, China; KOH, 5% potassium hydroxide; CB, Cotton Blue; CB$^{-}$, acyanophilous; IKI, Melzer’s reagent; IKI$^{-}$, both inamyloid and indextrinoid; L, mean spore length (arithmetic average for all spores); W, mean spore width (arithmetic average for all spores); Q, variation in the L/W ratios between The studied specimens, n (a/b), number of spores (a) measured from given number (b) of specimens, spore measurements do not include ornamentation; CTAB, cetyltrimethylammonium bromide; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; MP, maximum parsimony; ML, maximum likelihood; BI, Bayesian inference; TBR, tree-bisection reconnection.
basidia and smooth to verrucose or aculeate basidiospores (Karsten, 1890; Bernicchia and Gorjón, 2010). About 49 species are currently known in Trechispora worldwide (Liberta, 1966, 1973; Larsson, 1994, 1995, 1996; Ryvarden, 2002; Trichiès and Schultheis, 2002; Miettinen and Larsson, 2006; Ordynets et al., 2015; Xu et al., 2019) and Index Fungorum\(^1\) and MycoBank\(^2\).

Larsson (2007) addressed the classification of corticioid fungi and revealed that Trechispora farinacea (Pers.) Liberta grouped with Trechispora hymenocystis (Berk. and Broome) K.H. Larss., in which both species nested within the family Hydnodontaceae Jülich. Based on the large subunit nuclear ribosomal RNA gene (nLSU) datasets, Albee-Scott and Kropp (2010) supported to transfer Hydnodon thelephorus (Lév.) Banker to Trechispora as Trechispora thelephora (Lév.) Ryvarden. The order Trechisporales was studied employing the internal transcribed spacer (ITS) and nLSU regions, in which it suggested that Pseudomyces Jülich, Sistotremastrum J. Erikss., Subulicystidium Parmasto, and Trechispora belonged to a highly supported clade and Trechispora belongs to Hydnodontaceae and was closely related to Brevicillicium K.H. Larss. and Hjortstam (Tellería et al., 2013). A phylogenetic study of Trechispora was addressed and demonstrated that Trechispora cyatheae Ordynets, Langer and K.H. Larss. and Trechispora echinocristallina Ordynets, Langer and K.H. Larss. clustered into Trechispora as new members, inferred from the combined data of the ITS and LSU datasets (Ordynets et al., 2015). The phylogeny of Trechisporales was inferred from a combined dataset of ITS-nLSU sequences and showed that Pseudomyces, Scytinopogon Singer, and Trechispora grouped together and nested within family Hydnodontaceae (Liu et al., 2019). Phylogram generated from analysis of ITS sequence dataset of Trechispora showed that Trechispora echinospora Tellería was sister to the clade formed by Trechispora araneosa (Hohn. and Litsch.) K.H. Larss., T. farinacea, T. hymenocystis, and Trechispora mollusca (Pers.) Liberta with a low support (Phookamsak et al., 2019). The ITS + nLSU dataset comprised 22 species and revealed that Trechispora yunnanensis C.L. Zhao formed a monophyletic lineage within Trechispora and was closely related to Trechispora byssinella (Bourdot) Liberta and Trechispora laevis K.H. Larss. (Xu et al., 2019).

During the studies on wood-inhabiting fungi in southern China, three species of Trechispora could not be assigned to any described species. Obtaining sequences from the new taxa, the authors examine taxonomy and phylogeny of three new species within the genus Trechispora, based on the ITS and nLSU sequences.

### MATERIALS AND METHODS

#### Morphology

The studied specimens are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, China. Macro-morphological descriptions were based on field notes. Color terms follow Petersen (1996). Micromorphological data were obtained from the dried specimens and observed under a light microscope following Dai (2012). The following abbreviations were used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB− = acyanophilous, IKI = Melzer’s reagent, IKI− = both inamyloid and indextrinoid, L = mean spore length (arithmetic average for all spores), W = mean spore width (arithmetic average for all spores), Q = variation in the L/W ratios between the studied specimens, n(a/b) = number of spores (a) measured from given number (b) of specimens, spore measurements do not include ornamentation.

#### Molecular Phylogeny

Cetyltrimethylammonium bromide (CTAB) rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to obtain genomic deoxyribonucleic acid (DNA) from dried specimens, according to the manufacturer’s instructions following Zhao and Wu (2017). ITS region was amplified with primer pair ITS5 and ITS4 (White et al., 1990). Nuclear LSU region was amplified with primer pair LR0R and LR7\(^3\). The polymerase chain reaction (PCR) procedures for ITS and nLSU following Zhao and Wu (2017). The PCR products were purified and directly sequenced at Kunming Tisingke Biological Technology Limited Company, Kunming, Yunnan Province, China. All newly generated sequences were deposited at GenBank (Table 1).

Sequencer 4.6 (GeneCodes, Ann Arbor, United States) was used to edit the DNA sequence. Sequences were aligned in MAFFT 7\(^4\) using the “G-INS-I” strategy and manually adjusted in BioEdit (Hall, 1999). The sequence alignment was deposited in TreeBase (submission ID 25879). Sequences of Fibrodontia alba Yurchenko and Sheng H. Wu and Fibrodontia gossypina Parmasto retrieved from GenBank were used as an outgroup in the ITS + nLSU analyses by following Ordynets et al. (2015).

Maximum parsimony (MP) analyses were applied to the ITS + nLSU dataset sequences. Approaches to phylogenetic analysis followed Zhao and Wu (2017), and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford, 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with tree-bisection reconnection (TBR) branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein, 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree generated. Datamatrix was also analyzed using maximum likelihood (ML) approach with RAxML-HPC2 through the Cipres Science Gateway with GTR + I + G molecular evolution model\(^5\) (Miller et al., 2009). Branch support (BS) for ML analysis was determined by 1000 BT replicates.

\(^1\)http://www.indexfungorum.org/Names/Names.asp
\(^2\)https://www.mycobank.org/Biolomics.aspx?Table=Mycobank&Page=200&ViewMode=Basic
\(^3\)http://lutzonilab.org/nuclear-ribosomal-dna/
\(^4\)http://mafft.cbrc.jp/alignment/server/
\(^5\)www.phylo.org
### TABLE 1 | List of species, specimens, and GenBank accession numbers of sequences used in this study.

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References:
- Yurchenko and Wu (2014)
- Unpublished
- Larsson et al. (2004)
- This study
- Ordynets et al. (2015)
- Telleria et al. (2013)
- Ordynets et al. (2015)
- Telleria et al. (2011)
- Larsson et al. (2004)
- Telleria et al. (2015)
MrModeltest 2.3 (Nylander, 2004) was used to determine the best-fit evolution model (GTR + I + G) for each data set for Bayesian inference (BI) of the phylogeny. BI was calculated with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Four Markov chains were run for two runs from random starting trees for 1 million generations and trees were sampled every 100 generations; the first one-fourth of generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received ML BT values > 75%, MP BT values > 75%, or Bayesian posterior probabilities (PP) > 0.95.

RESULTS

Molecular Phylogeny
In the ITS dataset, the sequences from 43 fungal specimens representing 25 species were included. The dataset had an aligned length of 1034 characters, of which 521 characters
FIGURE 2 | Maximum Parsimony strict consensus tree illustrating the phylogeny of three new species and related species in *Trechispora* based on ITS + nLSU sequences. Branches are labeled with maximum likelihood bootstrap values > 70%, parsimony bootstrap proportion values > 50%, and Bayesian posterior probabilities > 0.95, respectively.

are constant, 86 are variable and parsimony-uninformative, and 427 are parsimony-informative. MP analysis yielded 26 equally parsimonious trees (TL = 2048, CI = 0.4561, HI = 0.5439, RI = 0.6174, RC = 0.2816). Best model for the ITS dataset estimated and applied in the Bayesian analysis: GTR + I + G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in a similar topology to MP analysis, with an average standard deviation of split frequencies = 0.009985 (BI).

The phylogeny (Figure 1) inferred from ITS sequences showed that *Trechispora bambusicola* was sister to *Trechispora stevensonii* (Berk. and Broome) K.H. Larss, and *Trechispora fimbriata* grouped with *Trechispora nivea*. *T. fissurata* grouped with *T. echinospora* Telleria, M. Dueñas, I. Melo, and M.P. Martín.

In the ITS + nLSU dataset, it included sequences from 49 fungal specimens representing 27 species. The dataset had an aligned length of 2256 characters, of which 1387 characters are constant, 188 are variable and parsimony-uninformative, and 681 are parsimony-informative. MP analysis yielded 100 equally parsimonious trees (TL = 2811, CI = 0.4963, HI = 0.5037, RI = 0.6409, RC = 0.3180). Best model for the ITS dataset estimated and applied in the Bayesian analysis: GTR + I + G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in a similar topology to MP analysis, with an average standard deviation of split frequencies = 0.009991 (BI).

The phylogenetic tree (Figure 2) inferred from ITS + nLSU sequences demonstrated 27 species of *Trechispora* and revealed that *T. bambusicola* formed a single lineage and then grouped with *Trechispora rigida* (Berk.) K.H. Larss. and *T. stevensonii*. 

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Zhao and Zhao Three Potential New Ectomycorrhizal Species

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**Taxonomy**

*Trechispora bambusicola* C.L. Zhao, sp. nov.

*MycoBank no.:* MB 838612 (Figures 3, 4).

Holotype—China, Yunnan Province, Pu’er, Laiyanghe National Forest Park, on dead bamboo, 30 September 2017, CLZhao 3305 (SWFC).

Etymology—*Bambusicola* (Lat.): referring to occurrence on bamboo stump.

**Basidiomata**

Annual, adnate, soft, and fragile, without odor or taste when fresh, becoming granulose upon drying, up to 15 cm long and 5 cm wide, 50–300 µm thick. Hymenial surface odontoid, aculei cylindrical to conical, blunt, 0.3–0.5 mm long, white to cream when fresh, turn to cream to buff upon drying. Margin white to cream.

**Hyphal structure**

Monomitic, generative hyphae with clamp connections, hyaline, thick-walled, up to 0.7 µm, richly branched, 2–3 µm in diameter, IKI−, CB−; hyphae unchanged in KOH.

**Hymenium**

Cystidia and cystidioles absent; basidia shortly cylindrical to subclavate with median constriction, with 4–sterigmata and a basal clamp connection, 9–13 × 2.5–5 µm, basidioles dominant, in shape similar to basidia, but slightly smaller.

**Basidiospores**

Ellipsoid, hyaline, thick-walled, ornamented, sparse aculei, sharp, IKI−, CB−, (2.6−)2.9–3.5(−3.9) × 2−2.7 µm, L = 3.18 µm, W = 2.41 µm, Q = 1.26–1.38 (n = 60/2).

**Type of rot**

White rot.

**Additional specimen examined**

China, Yunnan Province, Pu’er, Laiyanghe National Forestry Park, on dead bamboo, 30 September 2017, CLZhao 3302 (SWFC).

*Trechispora fimbriata* C.L. Zhao, sp. nov.

*MycoBank no.:* MB 838613 (Figures 5, 6).

Holotype—China, Yunnan Province, Pu’er, Jingdong County, Wuliangshan National Nature Reserve, on the angiosperm trunk, October 5, 2017, CLZhao 4154 (SWFC).

Etymology—*Fimbriata* (Lat.): refers to the fimbriate margin of the basidiomata.

**Basidiomata**

Annual, adnate, without odor or taste when fresh, becoming fragile upon drying, up to 10 cm long and 3 cm wide, 100–200 µm thick. Hymenial surface hydnoid, with aculei, cylindrical, blunt, 0.4–0.7 mm long, white to pink when fresh, turn to pink to buff upon drying. Margin white to cream, thinning out, fimbriate.

**Hyphal system**

Monomitic, generative hyphae with clamp connections, hyaline, thick-walled, up to 0.6 µm, branched, 2–4 µm in diameter, IKI−, CB−; hyphae unchanged in KOH.

**Hymenium**

Cystidia and cystidioles absent; basidia shortly cylindrical with median constriction, with 4–6 sterigmata and a basal clamp connection, 7–11.5 × 3.5–5 µm, basidioles dominant, in shape similar to basidia, but slightly smaller.

**Basidiospores**

Ellipsoid, hyaline, thick-walled, ornamented, sparse aculei, sharp, IKI−, CB−, (2.5−)3–3.6(−3.8) × 2.4–3.2 µm, L = 3.25 µm, W = 2.63 µm, Q = 1.17–1.38 (n = 90/3).

**Type of rot**

White rot.
Additional specimens examined
China, Yunnan Province, Yuxi, Xinping County, Mopanshan National Forestry Park, on living tree of angiosperm, August 9, 2018, CLZhao 7969 (SWFC); on angiosperm trunk, October 15, 2018, CLZhao 9006 (SWFC).

*Trechispora fissurata* C.L. Zhao, sp. nov.
*MycoBank no.:* MB 838614 ([Figures 7, 8](#)).

Holotype—China, Yunnan Province, Puer, Jingdong County, Wuliangshan National Nature Reserve, on angiosperm trunk, October 6, 2017, CLZhao 4571 (SWFC).

Etymology—*Fissurata* (Lat.): refers to the cracking fissures on hymenial surface.

**Basidiomata**
Annual, adnate, without odor or taste when fresh, becoming cracking upon drying, up to 8 cm long and 4.5 cm wide, 400–800 μm thick. Hymenial surface hydnoid, with aculei, cylindrical to conical, sharp, 0.5–0.9 mm long, cream to straw yellow when fresh, turn to cream to yellow upon drying. Margin cream to yellow.

**Hyphal system**
Monomitic, generative hyphae with clamp connections, hyaline, thick-walled, up to 0.8 μm, branched, 2.5–5 μm in diameter, IKI−, CB−; hyphae unchanged in KOH.

**Hymenium**
Cystidia and cystidioloes absent; basidia shortly clavate to tubular, with 4-sterigmata and a basal clamp connection, 8–10.5 × 2.5–4.5 μm, basidioles dominant, in shape similar to basidia, but slightly smaller.
**Basidiospores**
Ellipsoid, hyaline, thick-walled, ornamented, dense aculei, sharp, IKI−, CB−, (3−)3.3−4(−4.3) × (2.5−)2.8−3.5(−3.9) μm, $L = 3.67 \ μm$, $W = 3.19 \ μm$, $Q = 1.13−1.17$ ($n = 60/2$).

**Type of rot**
White rot.

**Additional specimen examined**
CHINA, Yunnan Province, Yuxi, Xiping County, Mopanshan Forestry Park, on the fallen angiosperm branch, January 17, 2017, CLZhao 995 (SWFC).

**DISCUSSION**
Phylogenetically, Phookamsak et al. (2019) introduced the phylogram generated from BI analysis of ITS sequence dataset of *Trechispora* sequences and included most taxa in this genus, in which it implied the phylogenetic relationship among species of *Trechispora*. In the present study, based on the ITS sequences (Figure 1), *T. bambusicola* was sister to *T. stevensonii* (Berk. and Broome) K.H. Larss; *T. fimbriata* grouped with *T. nivea*; *T. fissurata* grouped with *T. echinospora* Telleria, M. Dueñas, I. Melo, and M.P. Martín. Further ITS + nLSU dataset (Figure 2) revealed that *T. bambusicola* formed a single lineage and then grouped with *T. rigida* and *T. stevensonii*; *T. fimbriata* was sister to *T. nivea*; *T. fissurata* grouped with *T. thelephora*. However, *T. rigida* differs in its dirty white to buff hymenophore (Larsson, 1996). *T. stevensonii* is separated from *T. bambusicola* by the smooth to hydnoid hymenophore and larger basidiospores (4−4.5 × 3−3.5 μm; Larsson, 1995). *T. nivea* differs from *T. fimbriata* by the white to light ochraceous hymenial surface (Persoon, 1794). *T. echinospora* differs from *T. fissurata* by the farinaceous to grandinioid hymenophore and larger, globose basidiospores (3.3−4 × 2.8−3.5 μm; Phookamsak et al., 2019) and *T. thelephora* differs in its pileate to stipitate
with light yellow brown surface and larger (4–5 × 3.4–4.5 µm; Albee-Scott and Kropp, 2010).

In the present study, three new species, *T. bambusicola*, *T. fimбриата*, and *T. fissurata* spp. nov. are found from rotten wood. Morphologically, *T. bambusicola* is similar to *T. cyatheae* Ordynets, Langer and K.H. Larss. by sharing the characteristics of soft and fragile basidiomata. However, *T. cyatheae* differs from *T. bambusicola* by having farinaceous to grandinoid hymenophore and thin-walled generative hyphae (Ordynets et al., 2015).

*Trechispora fimбриата* has similar characteristics of having the fimbriate margin with *Trechispora canariensis* Ryvarden and Liberta, *Trechispora clancularis* (Park.-Rhodes) K.H. Larss., *Trechispora microspora* (P. Karst.) Liberta, *Trechispora stellulata* (Bourdot and Galzin) Liberta, and *Trechispora subhelvetica* (Parmasto) Liberta. However, *T. canariensis* differs in its arachnoid to pelliculose hymenophore and larger basidiospores (5–7 × 3–3.5 µm; Ryvarden and Liberta, 1978); *T. clancularis* differs in the poroid to irtipoid hymenophore and slightly cyanophilous basidiospores (Larsson, 1994); *T. stellulata* differs in the arachnoid to byssoid hymenophore with whitish hymenial surface (Liberta, 1966); and *T. subhelvetica* differs in the narrower basidiospores (3–4 × 2–2.5 µm; Parmasto, 1965).

*Trechispora fissurata* resembles several species with similar features of having the hydnyd hymenophore and a monomitic hyphal system: *T. nivea* (Pers.) K.H. Larss., *T. stevensonii* (Berk. and Broome) K.H. Larss., and *Trechispora verruculosa* (G. Cunn.) K.H. Larss., but *T. nivea* by the white to pale ochraceous hymenial surface and thin-walled generative hyphae encrusted with granular crystals (Bernicchia and Gorjón, 2010); *T. stevensonii* by the white to ochraceous hymenial surface and larger basidiospores (4–4.5 × 3–3.5 µm; Larsson, 1995); *T. verruculosa* by the slightly cyanophilous and larger basidiospores (4.5–5.5 × 3.5–4.5 µm; Larsson, 1996).

Currently, eight species of *Trechispora* have been reported from China (Dai, 2011; Xu et al., 2019), *Trechispora alnicola*, *Trechispora cloxaeraens*, *T. farinacea*, *T. microspora*, *T. nivea*, *Trechispora polygonospora* Ryvarden, *Trechispora subphaesporpha* (Litsch.) Liberta, and *T. yunnanensis*, and one species of *T. yunnanensis* was found in Yunnan Province of China and it differs from three new species by having a smooth to farinaceous hymenial surface and larger basidiospores (7–8.5 × 5–5.5 µm; Xu et al., 2019). Three new taxa do not closely group together in phylogenetic trees, and morphologically, *T. bambusicola* differs from *T. fimбриата* and *T. fissurata* by having granulose bisadiomata with cream to buff hymenial surface and growth on dead bamboo. *T. fimбриата* differs in its fimbriate margin of the basidiomata with pink to buff hymenial surface.

In addition, the ectomycorrhizal fungi (EcM) play an important role in ecosystems based on their mutualistic association with many groups of plants (Heijden et al., 2015). Vanegas-León et al. (2019) discovered the Trechisporales basidiomes and root colonization from *T. thelephora* based on ITS + nLSU phylogenetic analysis (Figure 2), which implied that both species have close evolutionary relationship. However, *T. fissurata* grows on deeply decayed wood, and *T. thelephora* is a soil-inhabiting fungus. Therefore, future investigations in both inhabiting types are needed to determine whether the natural selection or other factors pushes the different direction on inhabiting soil/wood among *Trechispora*.

In the habitat and distribution, Hibbett et al. (2014) revealed that most species of *Trechispora* is considered as soil-inhabiting. Later, some species were found on deeply decayed wood fungi (Bernicchia and Gorjón, 2010; Dai, 2011). However, some species in *Trechispora* are a typical feature of ectomycorrhizal fungi as frequently forming basidiomes on soil (Dunham et al., 2007; Vanegas-León et al., 2019). In the neotropical and subtropical region, the ectomycorrhizal basidiomes are found; however, the researches on the new taxa related to wood-decaying fungi of *Trechispora* from China are poorly reported. Further studies may focus on the relationships between...
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Three Potential New Ectomycorrhizal Species

FIGURE 8 | Microscopic structures of Trechispora fissurata (drawn from the holotype). (A) Basidiospores. (B) Basidia and basidioles. (C) Section of hymenium. Bars: (A) 5 µm; (B,C) 10 µm.

the plants and species from Trechispora and try to better understand the evolutionary directions between soil-inhabiting and decayed wood fungi of Trechispora; many fungal studies on phylogeny and application were from these areas, which will be useful to push future researches for the genus Trechispora (Dai, 2011; Cui et al., 2019; Shen et al., 2019; Zhu et al., 2019; Richter et al., 2019; Angelini et al., 2020; Bao et al., 2020).

DISCLOSURE
All the experiments undertaken in this study comply with the current laws of the People's Republic of China.

DATA AVAILABILITY STATEMENT

AUTHOR CONTRIBUTIONS
C-LZ collected the species. WZ performed the molecular phylogenetic analyses. Both authors were responsible for the morphological analysis and description of the collections, planned, organized, and evaluated critically the experimental parts, wrote the manuscript, contributed to the article, and approved the submitted version.

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REFERENCES


Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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