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**Opinion**

# Do you believe in "new" fungal species?

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**Abstract**
Fungal taxonomy has undergone a transformation in recent decades, propelled by the advent of molecular methodologies that have fundamentally reshaped traditional morphology-based species delineation. This paper examines the progression of fungal classification systems, with a focused discussion on integrative taxonomic frameworks, including Genealogical Concordance Phylogenetic Species Recognition (GCPSR) and coalescent-based methods. These molecular advancements have revealed extensive cryptic diversity, as exemplified by revisions within the *Colletotrichum gloeosporioides* species complex. While DNA barcoding—particularly through the Internal Transcribed Spacer (ITS) region—has accelerated species discovery, the potential for taxonomic inflation highlights the need for robust multi-locus and population-genetic analyses. The conceptualization of "new" fungal species is critically evaluated through an eco-evolutionary lens, emphasizing the imperative for a multidisciplinary synthesis of molecular, morphological, and ecological data. Furthermore, this paper emphasizes the importance of equitable global access to molecular technologies and infrastructural resources, particularly in biodiverse yet under-resourced regions such as India. Such inclusivity is vital to fostering broader engagement in fungal systematics and ensuring more comprehensive biodiversity assessment and conservation efforts.

**Keywords**: Biodiversity, Fungal Taxonomy, Molecular Identification, Mycology, Species Delimitation

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## 1. Introduction
Fungi occupy a pivotal role in ecosystems, agriculture, medicine, and industry (Manoharachary et al., 2005; Solomon, Tomii, & Dick, 2019). Despite their ecological and economic significance, accurate fungal identification remains a persistent challenge, historically reliant on the expertise of trained mycologists (Hibbett et al., 2016; Lücking et al., 2020). In recent years, public interest in fungi has surged (Hyde et al., 2018), driven by factors such as the

popularization of foraging, heightened awareness of mould-related health concerns, and growing engagement with biodiversity conservation. This trend is reflected in digital search behaviour, where individuals increasingly seek guidance on fungal identification through diverse methodologies.

**Taxonomy**, the science of classifying organisms based on evolutionary relationships, serves as a cornerstone of biological research. It provides a systematic framework for understanding biodiversity, tracing evolutionary lineages, and standardizing scientific communication (Godfray & Knapp, 2004). The discipline is structured around three fundamental components: identification, nomenclature, and classification, each contributing to the organization and interpretation of biological diversity (Fig. 1).

**Identification** involves the empirical process of assigning organisms to established taxonomic categories using morphological, molecular, or integrative approaches. Traditional tools such as dichotomous keys and microscopic analysis are now complemented by DNA-based techniques (Hyde, Abd-Elsalam, & Cai, 2011), enhancing accuracy and efficiency.

**Nomenclature** refers to the standardized naming of organisms, governed by international codes (Winston, 2018) such as the *International Code of Nomenclature for algae, fungi, and plants* (ICN; Turland et al., 2018). Fungal species, for instance, are designated using binomial nomenclature (e.g., *Aspergillus niger*), ensuring consistency across scientific literature.

**Classification** involves the hierarchical arrangement of organisms into taxonomic ranks, from broad domains (e.g., Eukarya) to specific species, based on shared morphological or genetic traits (Ruggiero et al., 2015). This system reflects evolutionary relatedness and facilitates comparative biological studies.

Together, these components support biological taxonomy, enabling the systematic documentation and conservation of biodiversity. Precise taxonomic identification is indispensable across applied disciplines—including ecology, agriculture, medicine, and conservation biology—where it informs evidence-based decision-making and policy formulation (Lyal et al., 2008).

## 2. History of Biological Classification

The systematic classification of living organisms based on shared characteristics has ancient origins, yet it underwent a profound transformation in the 18$^{th}$ century through the work of Carl Linnaeus (1707–1778), widely regarded as the father of modern taxonomy (Reid, 2009). Prior to Linnaeus, classification systems were largely inconsistent and lacked standardized principles. Early attempts by Greek scholars, such as Aristotle's division of animals into categories based on the presence of blood (ἔναιμα/enaima) and bloodlessness (ἄναιμα/anaima) or habitat (Tasić, 2017), demonstrated rudimentary efforts but remained devoid of a unifying framework.

Linnaeus revolutionized biological classification by introducing binomial nomenclature, a system in which each species is assigned a two-part Latinized name (genus + species epithet), ensuring global consistency in scientific communication (Schuh, 2003). His foundational work, Systema Naturae (1735), underwent successive expansions and established a hierarchical classification system that organized life into nested ranks—Kingdom, Class, Order, Genus, and Species. Although later revisions introduced additional ranks (e.g., Phylum, Family),

Linnaeus's structure remains the backbone of modern taxonomy (Reid, 2009). His contributions not only standardized nomenclature but also provided a systematic approach that facilitated comparative biology and evolutionary studies.

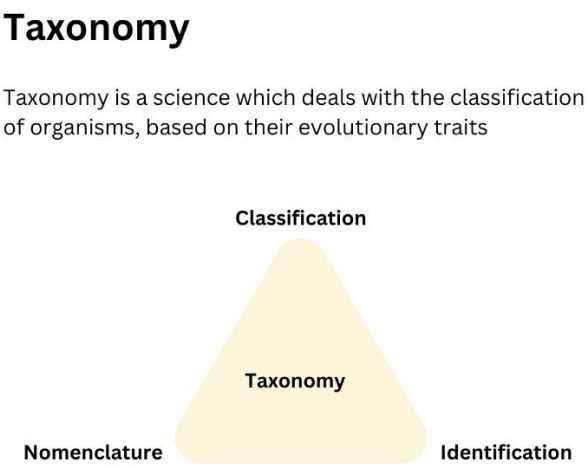


**Fig. 1** - The three foundational components of taxonomy—identification, nomenclature, and classification—are illustrated as integral elements forming the basis of taxonomic practice. Together, they facilitate the scientific understanding and organisation of biological diversity based on evolutionary relationships.

**3. Traditional classification of fungi and fungi-like organisms**

Early taxonomic systems classified fungi and fungi-like organisms within the Kingdom Plantae, primarily due to superficial morphological similarities, including sessile growth patterns, cell wall composition, and absorptive mode of nutrition. The influential classification scheme proposed by Ainsworth et al. (1973) formalized this arrangement by establishing a fungal subkingdom under Plantae (Fig. 2), representing the dominant phylogenetic paradigm of the pre-molecular era. This conventional taxonomy persisted as the principal organizational framework until molecular phylogenetic analyses fundamentally reconceptualized fungal evolutionary relationships (Hibbett et al., 2007; Shenoy et al., 2007), demonstrating the polyphyletic nature of these historically grouped organisms.

**4. Molecular taxonomy**

The advent of molecular phylogenetics revolutionized microbial taxonomy when Woese & Fox (1977) pioneered the use of 16S ribosomal RNA (rRNA) sequences to reconstruct prokaryotic evolutionary relationships. Their work delineated three primary lineages—Eubacteria, Archaebacteria (now known as Archaea), and Eukaryotes—establishing a molecular framework that superseded traditional morphology-based classifications. This paradigm shift culminated in the formal proposal of the three-domain system (Bacteria, Archaea, and Eukarya) by Woese et al. (1990), displacing the classical five-kingdom model. Subsequent analyses by Wainright et al. (1993) further transformed fungal systematics by demonstrating, through small-subunit rRNA phylogenies, that fungi share a closer evolutionary affinity with animals than with plants. These findings necessitated a fundamental revision of fungal classification to align with phylogenetic evidence.

The standardization of fungal molecular systematics was significantly advanced by White et al. (1990), who developed universal primers for amplifying and sequencing ribosomal RNA genes (18S, 5.8S, 28S, and the ITS region). These protocols became foundational for fungal phylogenetics, enabling robust species identification and taxonomic delineation across diverse lineages. A major reclassification of fungi was undertaken by Hibbett et al. (2007), who constructed a comprehensive phylogeny of 195 taxa, leading to the recognition of novel phyla (e.g., Blastocladiomycota, Glomeromycota, and Neocallimastigomycota). This framework emphasized clade-based classification over morphological traits, reflecting the increasing influence of genomic data in reshaping taxonomic boundaries.

At the species level, DNA barcoding has emerged as a critical tool, with the ITS region established as the universal fungal barcode (Schoch et al., 2012). Its interspecific variability permits rapid and precise identification, even for environmental DNA or non-culturable specimens, facilitating the discovery of cryptic diversity.

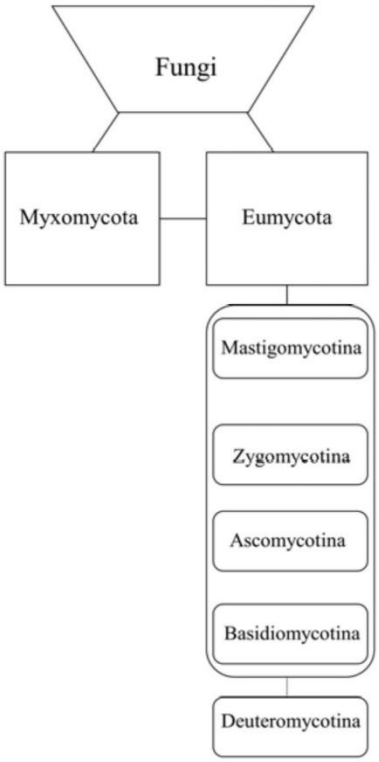


**Fig. 2** - Traditional fungal classification, as outlined by Ainsworth et al. (1973), grouped fungi and fungi-like organisms under the Kingdom Plantae, dividing them into Myxomycota (slime molds) and Eumycota (true fungi). The Eumycota included the following groups: Mastigomycotina, Zygomycotina, Ascomycotina, Basidiomycotina, and Deuteromycotina, based on morphological and reproductive characteristics (Source: Shenoy et al. (2007)/Fungal Diversity/CC BY)

**5. What is a species?**

The delimitation of fungal species remains a subject of ongoing debate, often characterized by the adage: "*A species is what a fungal taxonomist says it is*"! This statement emphasizes the inherently expert-driven nature of fungal taxonomy, where species boundaries are frequently obscured by morphological plasticity and inconsistent molecular thresholds. Consequently,

taxonomic decisions often rely on the integrative assessment of polyphasic data by specialized researchers.

From an evolutionary standpoint, species are conceptualized as independently evolving metapopulation lineages occupying distinct branches on the phylogenetic tree (Cai et al., 2011). This framework prioritizes genetic divergence and reproductive isolation over phenotypic similarity—a critical distinction in fungal systematics, where cryptic speciation is pervasive and traditional morphological traits frequently fail to delineate evolutionarily significant units (Chethana et al., 2021; Ekanayaka et al. 2025; Jayawardena et al., 2021). The prevalence of morphologically indistinguishable yet genetically divergent lineages has necessitated a shift in paradigm toward molecular, ecological, and phylogenetic criteria for recognizing fungal species.

**6. Species recognition in fungi: Concept vs. Recognition**

Species recognition in fungal systematics involves the operational application of diagnostic criteria to delineate discrete evolutionary lineages (Cai et al., 2011). While theoretical species concepts (evolutionary, biological, and phylogenetic) establish the conceptual framework for species boundaries, recognition methods provide the practical methodologies for empirical species delimitation. Contemporary fungal taxonomy employs three principal approaches (Fig. 3).

**Morphological Species Recognition (MSR)**: This conventional approach relies on phenotypic characteristics, including spore morphology, colony pigmentation, and reproductive structures. While historically fundamental in mycological taxonomy, MSR demonstrates significant limitations due to phenotypic plasticity and the prevalence of morphologically cryptic but genetically divergent species complexes.

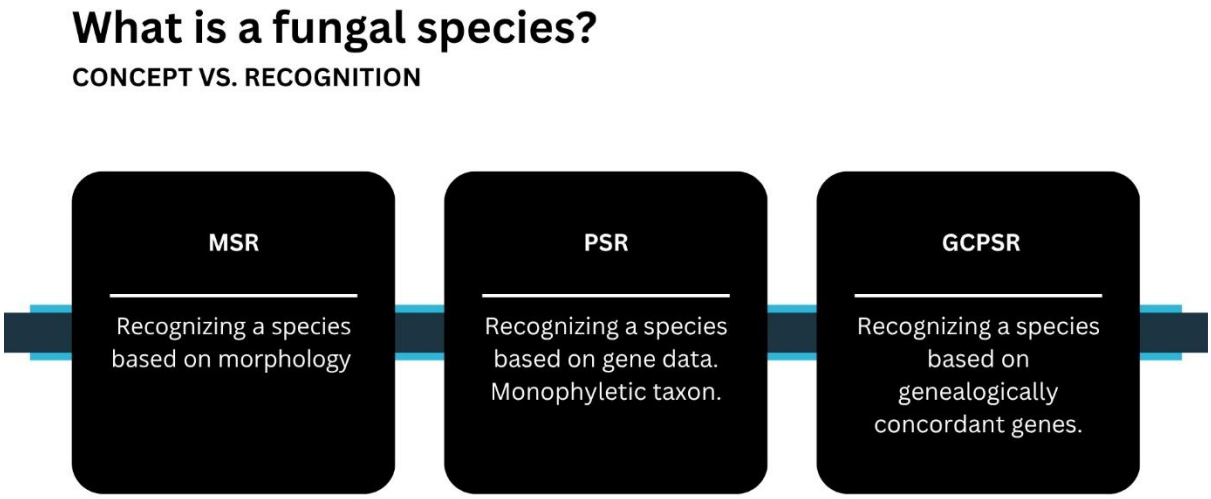


**Fig. 3** - Fungal species recognition approaches: Morphological Species Recognition (MSR) uses phenotypic traits; Phylogenetic Species Recognition (PSR) employs monophyletic gene trees; and Genealogical Concordance Phylogenetic Species Recognition (GCPSR) requires concordance across multiple gene genealogies. These complementary approaches provide operational criteria for species delimitation in fungi.

**Phylogenetic Species Recognition (PSR)**: This molecular approach defines species as monophyletic clades supported by nucleotide sequence data from one or more genetic loci. PSR offers superior resolution compared to morphological methods, particularly in taxonomically challenging groups where phenotypic characters show inadequate diagnostic variation.

**Genealogical Concordance Phylogenetic Species Recognition (GCPSR)**: This rigorous multilocus method requires a concordant phylogenetic signal across multiple unlinked gene genealogies to validate species boundaries (Taylor et al., 2000). By incorporating independent genetic evidence, GCPSR minimizes arbitrary taxonomic splitting while providing robust support for species-level distinctions.

## 7. A case study in *Colletotrichum gloeosporioides* species complex

*Colletotrichum gloeosporioides* species complex presents a paradigmatic case study in fungal taxonomy, demonstrating the critical limitations of morphological species recognition (MSR) and the transformative impact of molecular phylogenetic approaches. Historically, this phytopathogenic genus was delineated through MSR based on conidial morphology, appressorial characteristics, and colony phenotypes (Cannon et al., 2008). Typification efforts by Cannon et al. (2008) employed rigorous morphotaxonomic analyses of lectotype and epitype specimens, evaluating conidial dimensions, cultural growth patterns on standardized media (PDA, PCA), and conidiogenesis. While ribosomal rRNA gene (ITS) phylogenies initially supported the placement of the epitype within a monophyletic *C. gloeosporioides* clade, subsequent multilocus analyses revealed significant taxonomic oversimplification.

Phoulivong et al. (2010) conducted a reassessment of this species complex through GCPSR. Their analysis of five unlinked loci from 25 tropical fruit isolates demonstrated that none clustered with the *C. gloeosporioides* epitype. Instead, these are segregated into distinct, well-supported clades corresponding to *C. asianum*, *C. fructicola*, *C. siamense*, and *C. kahawae* - each meeting GCPSR criteria through consistent genealogical exclusivity across loci. This work fundamentally challenged the presumed ubiquity of *C. gloeosporioides* in tropical pathosystems, revealing widespread misidentification stemming from morphological convergence.

The taxonomic resolution advanced substantially through a comprehensive GCPSR analysis of >150 isolates using concatenated sequences from ACT, CAL, CHS-1, GAPDH, and ITS loci by Weir et al. (2012). Bayesian phylogenies separated the morphospecies into 22 phylogenetically distinct lineages, each demonstrating strong nodal support and multilocus concordance. This study established that traditional MSR dramatically underestimated species diversity in the complex; host associations and geographic distributions required complete reappraisal; and GCPSR provided the necessary analytical framework for robust species delimitation in morphologically cryptic taxa.

## 8. Author's experience with *Colletotrichum siamense* "species complex"

The taxonomic resolution of *Colletotrichum siamense* "species complex" was significantly advanced through the work of Sharma et al. (2015), who applied GCPSR to address long-standing ambiguities. By employing the phylogenetically informative *ApMat* marker, the authors uncovered substantial cryptic diversity within the complex. Their multilocus phylogenetic analyses revealed distinct, well-supported clades, including the novel taxon *C.*

*communis*, demonstrating the power of GCPSR to delineate species boundaries in morphologically challenging groups.

Liu et al. (2016) expanded upon this foundation by integrating GCPSR with coalescent-based species delimitation methods, offering a more nuanced perspective on evolutionary relationships within the complex. While GCPSR evaluates lineage independence through concordance across unlinked gene genealogies, coalescent approaches such as the Generalized Mixed Yule Coalescent (GMYC) and Poisson Tree Processes (PTP) incorporate population genetic processes—including incomplete lineage sorting and gene tree discordance—into probabilistic models of species divergence. Notably, their analyses did not support the splitting of *C. siamense sensu lato* into multiple species, challenging earlier assumptions and highlighting the risks of taxonomic over-splitting based solely on phylogenetic topology.

**9. Coalescent-based methods in defining fungal species boundaries**

Coalescent-based methods have become essential tools in fungal taxonomy, providing statistically robust approaches to species delimitation by explicitly modeling the evolutionary processes underlying gene tree variation (Carbone & Kohn, 2004; Carbone et al., 2007; Dissanayake et al., 2024; Maharachchikumbura et al., 2021; Mead et al., 2021; Parnmen et al., 2012; Pereira & Phillips, 2024; Pereira et al., 2023; Singh et al., 2015; Steenwyk et al., 2024; Stewart et al., 2014). Recent applications of techniques such as GMYC (Generalized Mixed Yule Coalescent), PTP (Poisson Tree Processes), and STACEY have successfully delineated species boundaries in taxonomically complex fungal groups, including *Diaporthe* (Dissanayake et al., 2024; Pereira & Phillips, 2024; Pereira et al., 2023), *Aspergillus* (Bian et al., 2022; Carbone et al., 2007; Steenwyk et al., 2024), and *Colletotrichum* (Liu et al., 2016). These methods have proven particularly effective at identifying cryptic species that cannot be distinguished through traditional morphological examination or standard multilocus phylogenetic analyses.

However, some of these studies have also revealed important limitations in GCPSR, notably the potential for over-splitting when minor genetic variations are erroneously interpreted as evidence of speciation (e.g., Liu et al. 2016). The strength of coalescent-based methods lies in their ability to account for biological complexities such as incomplete lineage sorting and gene tree discordance, making them especially valuable for resolving species boundaries in recently diverged lineages with high genetic variability.

**10. Rethinking the “New” in New fungal species**

The accelerated pace of fungal species discovery, facilitated by DNA-based technologies, has substantially expanded our knowledge of fungal biodiversity. However, this rapid proliferation has raised legitimate concerns regarding taxonomic validity, as many newly described taxa rely on limited molecular evidence without sufficient phylogenetic support, population-level analyses, or corroborating ecological and morphological data (Stengel et al., 2022; Fig. 4). While molecular methods like GCPSR and coalescent-based approaches have revolutionized species delimitation, their effectiveness depends on rigorous, multidimensional application. When implemented in isolation or with incomplete datasets, these techniques risk taxonomic inflation and potentially compromise the integrity of species recognition.

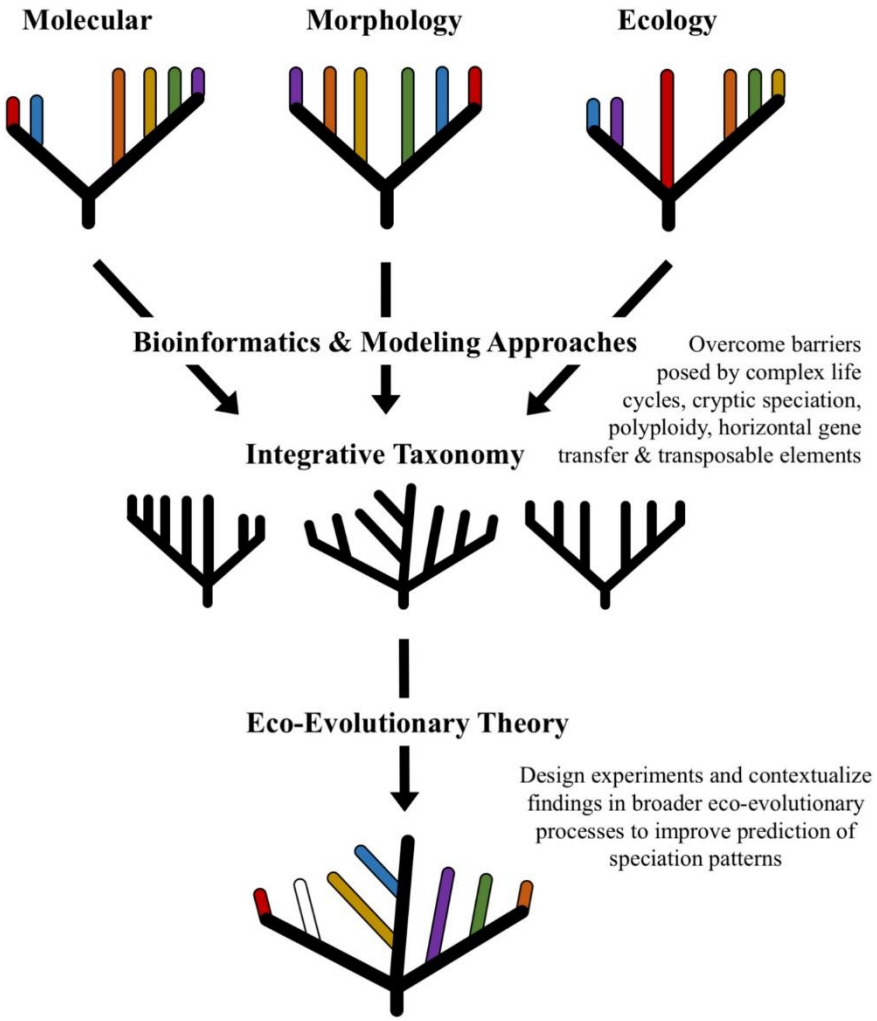


**Fig. 4** - Integrative taxonomic framework combining molecular, morphological, and ecological data through bioinformatic and modeling approaches to address challenges including cryptic speciation, polyploidy, and horizontal gene transfer. This eco-evolutionary approach enhances species boundary resolution by incorporating evolutionary context (Source: Stengel et al. (2022)/Frontiers in Microbiology/CC BY)

Contemporary fungal taxonomy increasingly requires integrative, eco-evolutionary frameworks that combine molecular systematics with morphological characterization, ecological data, and advanced bioinformatic analyses (Fig. 4). Such comprehensive approaches are particularly crucial in the genomic era, where biological complexities - including cryptic speciation, horizontal gene transfer, and ecological specialization - challenge conventional taxonomic paradigms. Multidisciplinary species concepts that incorporate population genomics, phenotypic plasticity, and niche differentiation can establish evolutionarily significant and biologically meaningful species boundaries (Stengel et al., 2022) with practical applications across agriculture, biotechnology, and conservation biology.

However, significant disparities persist in global research capacity, particularly affecting mycologists in developing nations who face constraints in sequencing technologies, computational resources, technical training, and sustained funding. The prohibitive costs of molecular reagents, limited high-throughput infrastructure, and restricted access to genomic databases create barriers to equitable participation in taxonomic research. Yet, the potential of

strategic initiatives, including international partnerships, regional sequencing centers, open-access bioinformatics platforms, and mentorship programs, to bridge these gaps and foster inclusive participation in fungal systematics is promising and should instill hope and optimism.

The future of fungal taxonomy is promising, with a growing recognition of the ecological and economic importance of fungi. This understanding is fostering more collaborative, interdisciplinary approaches. As the field advances, success will hinge not only on technological innovation but also on cultivating diverse, globally connected research communities. These communities, with their shared knowledge and collaborative spirit, will be instrumental in reconstructing the fungal tree of life with unprecedented accuracy and biological relevance.

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**Declaration**
There is no conflict of interest.

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