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刘俊丽

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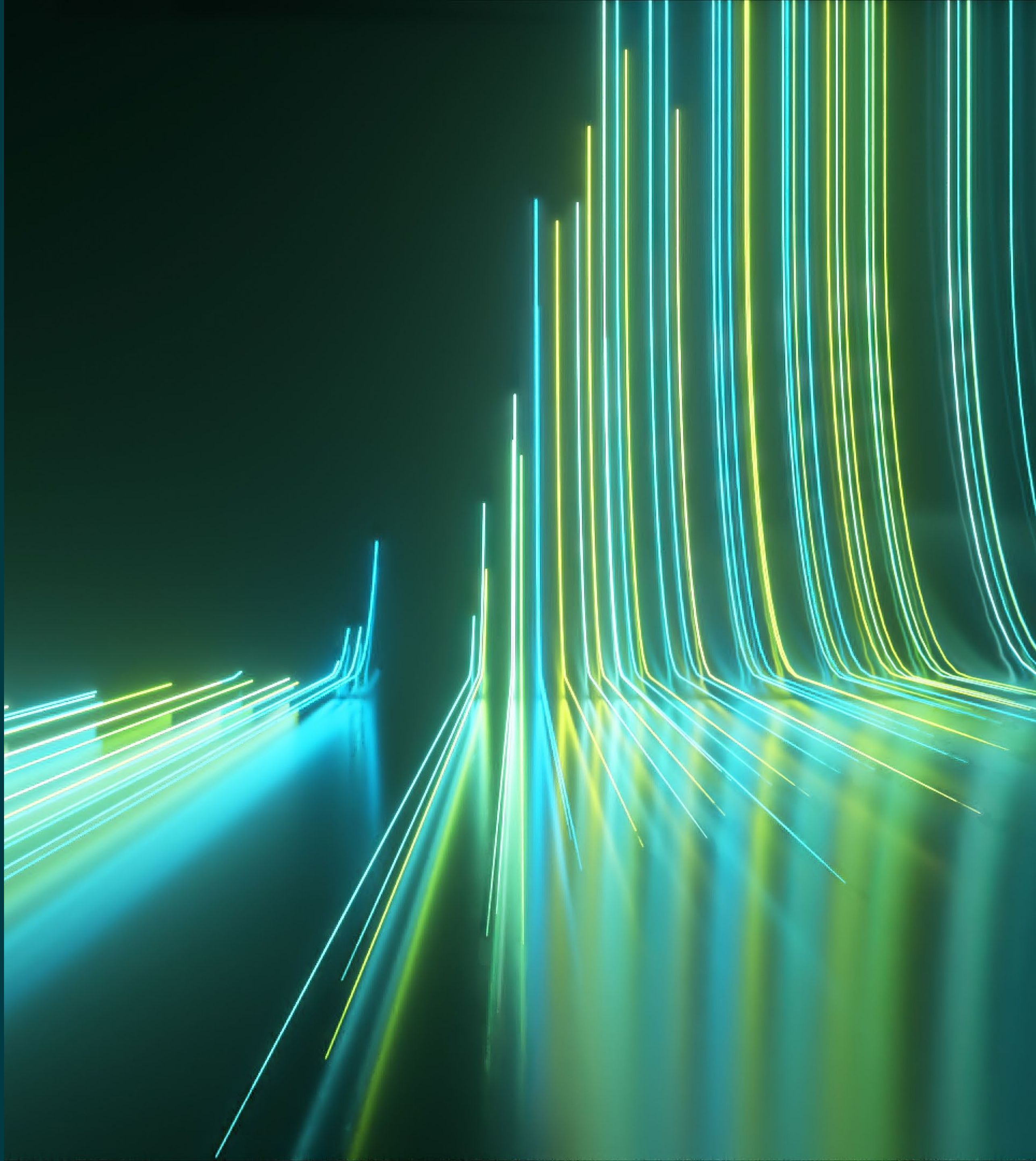

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RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

The 2019-new coronavirus epidemic: Evidence for virus evolution

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Abstract

There is a worldwide concern about the new coronavirus 2019-nCoV as a global public health threat. In this article, we provide a preliminary evolutionary and molecular epidemiological analysis of this new virus. A phylogenetic tree has been built using the 15 available whole genome sequences of 2019-nCoV, 12 whole genome sequences of 2019-nCoV, and 12 highly similar whole genome sequences available in gene bank (five from the severe acute respiratory syndrome, two from Middle East respiratory syndrome, and five from bat SARS-like coronavirus). Fast unconstrained Bayesian approximation analysis shows that the nucleocapsid and the spike glycoprotein have some sites under positive pressure, whereas homology modeling revealed some molecular and structural differences between the viruses. The phylogenetic tree showed that 2019-nCoV significantly clustered with bat SARS-like coronavirus sequence isolated in 2015, whereas structural analysis revealed mutation in Spike Glycoprotein and nucleocapsid protein. From these results, the new 2019-nCoV is distinct from SARS virus, probably trasmitted from bats after mutation conferring ability to infect humans.

KEYWORDS
coronavirus, epidemiology, macromolecular design, SARS coronavirus

1 | INTRODUCTION

The family *Coronaviridae* comprises a group of large, single, plus-stranded RNA viruses isolated from several species, and it is previously known to cause the common cold and diarrheal illnesses in humans.[1,2] In 2003, a new coronavirus (severe acute respiratory syndrome coronavirus [SARS-CoV]) was associated with the SARS outbreak.[1,2] Recently, a new coronavirus (2019-nCoV) has emerged in the region of Wuhan (China) as a cause of severe respiratory infection in humans. Since December 2019, different cases of pneumonia of unknown origin associated with permanence at the Wuhan market in China have been reported.[3,4] A new coronavirus, named 2019-nCoV, belonging to the *Orthocoronavirinae* subfamily, distinct from MERS-CoV and SARS-CoV, was described.[5] To date, a total of 1975 pneumonia cases have been confirmed in China (the State Council Information Office in Beijing, capital of China, 26 January 2020).[6,7] Animal to human transmission is considered the origin of epidemics, as many patients declared to have visited a local fish and wild animal market in Wuhan in November. Quite recently, evidence has been gathered for the animal to the human and interhuman transmission of the virus.[7,8]

Although prompt diagnosis and patient isolation are the hallmarks for initial control of this new epidemic, molecular epidemiology, evolutionary models, and phylogenetic analysis can help estimate genetic variability and the evolutionary rate, which in turn have important implications for disease progression as

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Silvia Angeletti and Massimo Ciccozzi contributed equally to this study.

well as for drug and vaccine development. In this short report, we provide a phylogenetic tree of the 2019-nCoV and identify sites of positive or negative selection pressure in distinct regions of the virus.

2 | MATERIAL AND METHODS

The complete genomes of 15 2019-nCoV sequences have been downloaded from GISAID (https://www.gisaid.org/) and GenBank (http://www.ncbi.nlm.nih.gov/genbank/). A dataset has been built using five highly similar sequences for SARS, two sequences for the Middle East respiratory syndrome (MERS), and five highly similar sequences for bat SARS-like coronavirus. The percentage of similarity has been identified using a basic local alignment search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi); eventually duplicated sequences have been excluded from the datasets. The dataset including 27 sequences has been aligned using multiple sequence alignment online tool[9] and manually edited using BioEdit program v7.0.5.[10]

Maximum likelihood (ML) methods were employed for the analyses because they allow for testing different phylogenetic hypotheses by calculating the probability of a given model of evolution generating the observed data and by comparing the probabilities of nested models by the likelihood ratio test. The best-fitting nucleotide substitution model was chosen by jModeltest software.[11] ML tree was reconstructed using generalized time-reversible plus gamma distribution and invariant sites (+G+I) as an evolutionary model using MEGA-X.[12]

The adaptive evolution server (http://www.datamonkey.org/) was used to find eventual sites of positive or negative selection. For this purpose, the following test has been used: fast unconstrained Bayesian approximation (FUBAR).[13] This test allowed us to infer the site-specific pervasive selection, the episodic diversifying selection across the region of interest, and to identify episodic selection at individual sites.[14] The statistically significant positive or negative selection was based on *P* value less than .05.[14]

Homology models have been built relying on the website SwissModel.[15] Structural templates have been searched and validated using the software available within the SwissModel environment and HHPred.[16] Homology models have been validated using the QMEAN tool.[17] Three-dimensional structures have been analyzed and displayed using PyMOL.[18] To map the structural variability of the N, E, S, and M regions of the virus and their sites under selection pressure, homology modeling has been applied to the sequence of 2019-nCoV.

3 | RESULTS

The ML phylogenetic tree, performed on whole genome sequences, is represented in Figure 1. In the tree, MERS virus sequences formed a distinct clade (clade I) from Bat SARS-like coronavirus, SARS virus, and the 2019-nCoV clustering together in clade II. This clade includes

4 | DISCUSSION

The data reported above show that the new 2019-nCoV significantly clustered with a sequence from the bat SARS-like coronavirus isolated in 2015. Moreover, in the phylogenetic tree, these two sequences are separated from the other bat SARS-like coronavirus sequences, suggesting that this bat SARS-like coronavirus is homologous and genetically more similar to the 2019-nCoV than to the other sequences of Bat SARS-like coronavirus. This supports the hypothesis that the transmission chain began from the bat and reached the human. All other genomic sequences represented in the phylogenetic tree, also including SARS and MERS coronavirus, clustered separately, thus excluding the fact that the virus involved in the actual epidemic could belong to these subgenuses. The structural analysis of two important viral proteins, the nucleocapsid and the spike-like nucleoprotein (protein S), confirmed the significant similarity of the new coronavirus with the bat-like SARS coronavirus and its difference from SARS coronavirus.

From the selective pressure and structural analysis, mutations of surface proteins, as the spike protein S, and of nucleocapsid N protein conferring stability to the viral particle have been shown. The viral spike protein is responsible for virus entry into the cell after by binding to a cell receptor and membrane fusion, two key steps in viral infection and pathogenesis. The N protein is a structural protein involved in virion assembly, playing a pivotal role in virus transcription and assembly efficiency. Mutation of these proteins could determine two important characteristics of the coronavirus isolated during the 2019-nCoV epidemic: a higher ability to infect and enhanced pathogenicity than the bat-like SARS coronavirus but lower pathogenicity than SARS coronavirus. These features can explain the 2019-nCoV zoonotic transmission and its initial lower severity than SARS epidemic. These results do not exclude the fact that further mutation due to positive selective

confirmed by FUBAR analysis, suggesting that the S region could be highly conserved.

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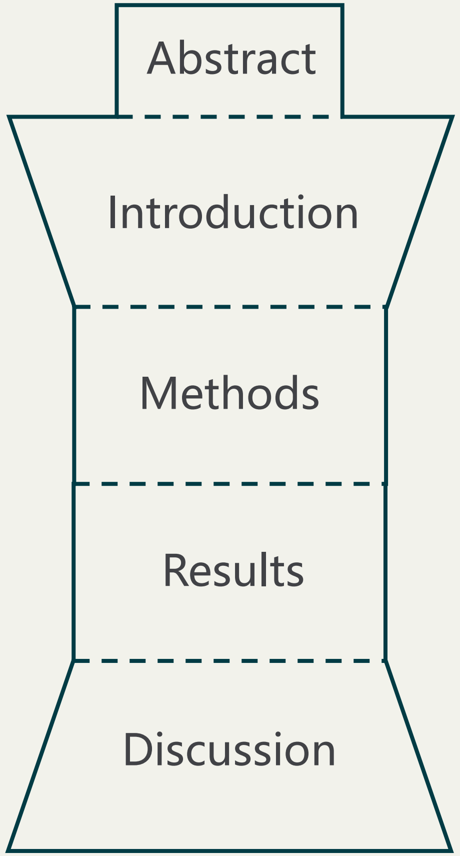


Abstract

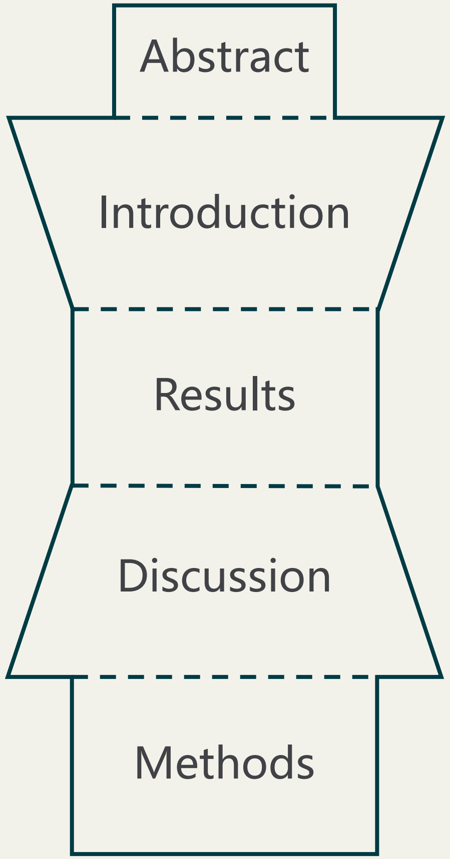
Introduction

Methods

Results

Discussion

原创论文的其他结构



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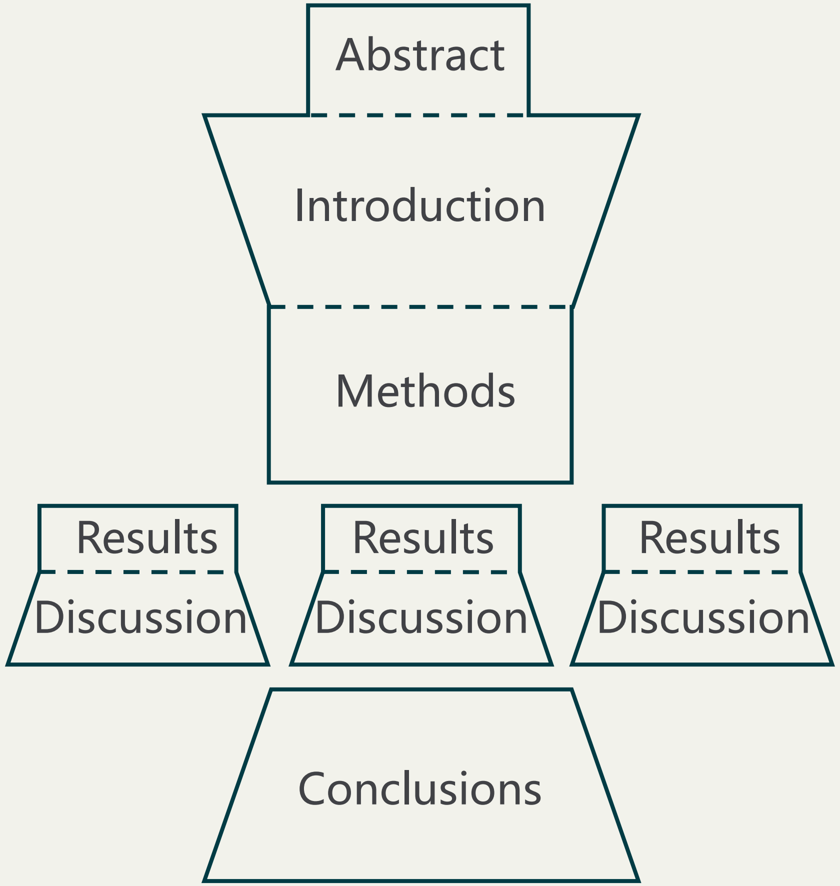


AIM(RaD)C

标题

A	**Accuracy** **准确**	本篇文章要传达的最主要的信息
B	**Brevity** **概括**	避免过多细节&无用的字眼
C	**Clarity** **清晰**	关键词！关键词！关键词！

标题的句式

名词短语式

由名词性短语构成，有效强调关键词

e.g., *Ecological responses to recent climate change*

强调研究主题和研究对象

主副式

将标题分割为两个或多个名词短语，用于希望表达的内容层次较多时

e.g., *One-dimensional nanostructures: synthesis, characterization, and application*

主标题强调研究对象，副标题强调研究目的

陈述句式

直接在题目中给出完整的研究结论

e.g., *Acrylamide is formed in the Maillard reaction*

提出结论，在文章中加以证明

疑问句式

使用探讨性的疑问句来引发读者兴趣

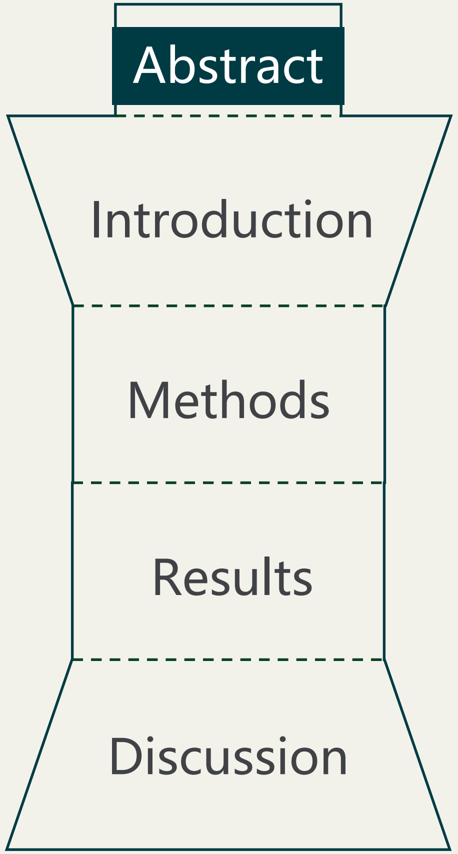
e.g., *Which way for genetic-test regulation?*

提出问题，在文章中回答

摘要——关键词！关键词！关键词！

摘要是整篇文章的高度浓缩 （150-300字）：

- 背景
- 目标/假设
- 简述方法
- 主要结果
- 结论及意义



撰写摘要的原则

简短

注意摘要的字数限制

完整

摘要需涵盖文章的全部部分（IMRaD）

满足读者需求

撰写摘要是需考虑读者希望从摘要中获得什么信息

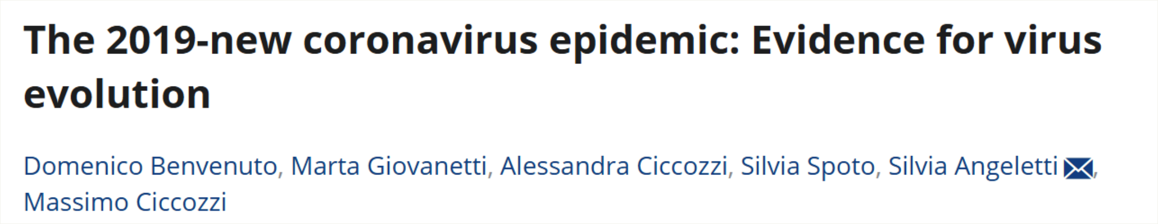
一致性

与正文内容保持一致——不提及正文中没有提及的信息

概括

从目标导向——摘要是“摘取要点”

摘要：优秀范例



The 2019-new coronavirus epidemic: Evidence for virus evolution

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There is a worldwide concern about the new coronavirus 2019-nCoV as a global public health threat. In this article, we provide a preliminary evolutionary and molecular epidemiological analysis of this new virus. A phylogenetic tree has been built using the 15 available whole genome sequences of 2019-nCoV, 12 whole genome sequences of 2019-nCoV, and 12 highly similar whole genome sequences available in gene bank (five from the severe acute respiratory syndrome, two from Middle East respiratory syndrome, and five from bat SARS-like coronavirus). Fast unconstrained Bayesian approximation analysis shows that ……differences between the viruses. The phylogenetic tree showed that 2019-nCoV significantly clustered with bat SARS-like coronavirus sequence isolated in 2015, whereas structural analysis revealed mutation in Spike Glycoprotein and nucleocapsid protein. From these results, the new 2019-nCoV is distinct from SARS virus, probably trasmitted from bats after mutation conferring ability to infect humans.

背景

目标

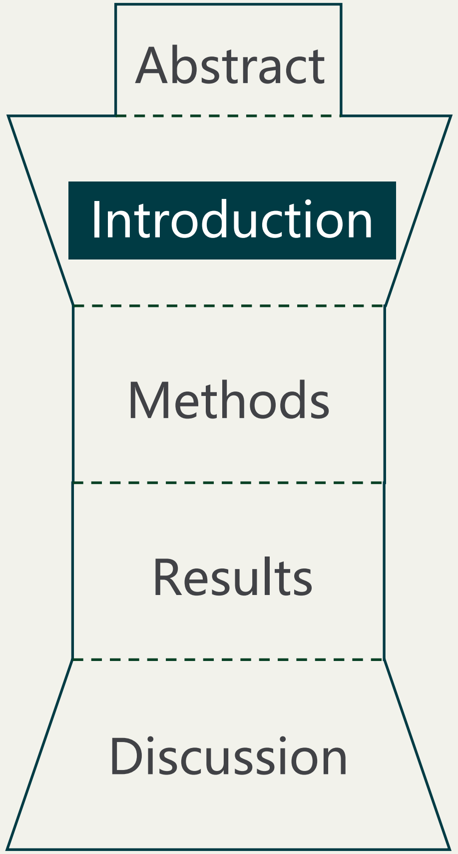
方法

结果

结论

引言

- 整体研究的背景
- 详细描述研究的结果与其他相关的研究
- 阐述还未解决的问题
- 描述开展此研究的原因以及目的
- 让非此领域的专家也可以理解研究的基本原理



引言——阐述研究目的

研究目的是将研究任务具现化

Aims

研究中希望实现的宏观目的

Objectives

研究中将达成的数条具体任务

重要性　创新性

材料与方法

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- 提供统计学方法

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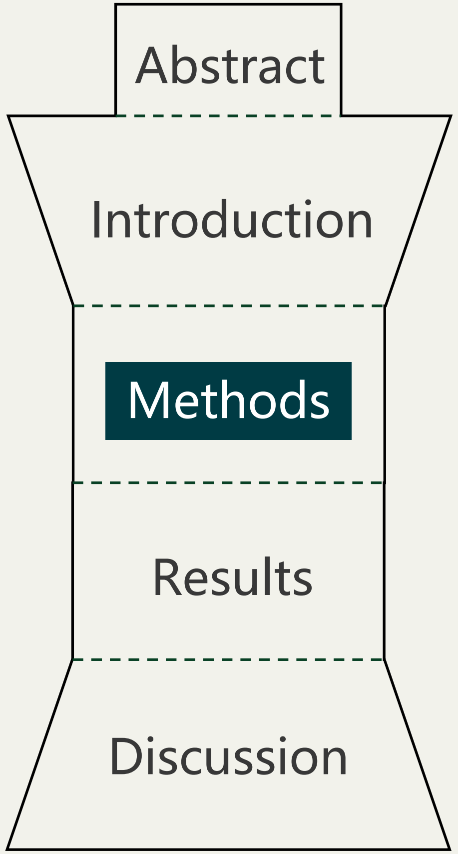

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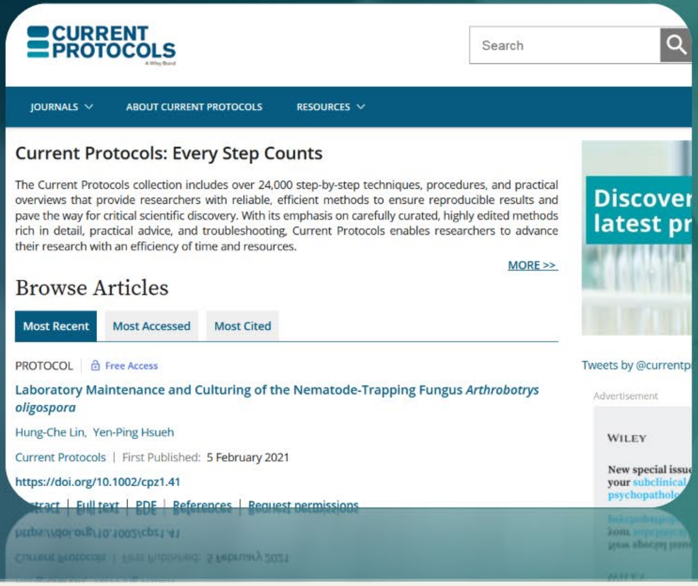

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结果

逻辑

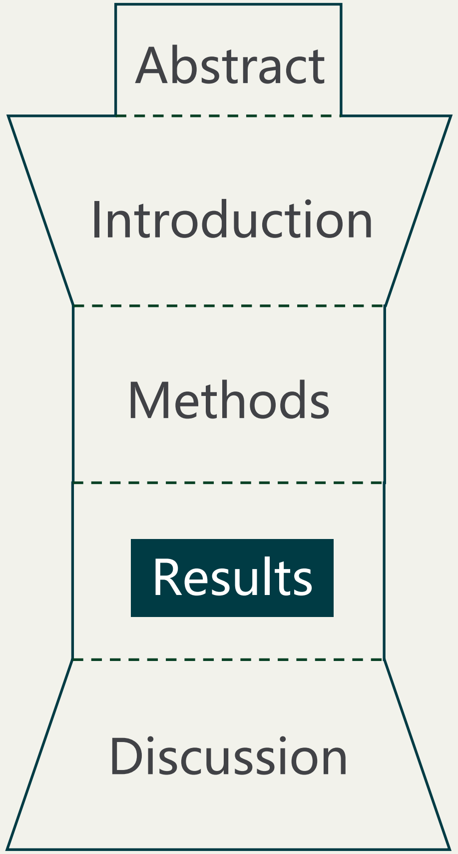
以逻辑和清晰的方式，展示和解释实验

关注细节

务必要引用每个/所有图表并描述实验结果

可信/可重复

提供统计分析以及清晰地阐述重要的数据

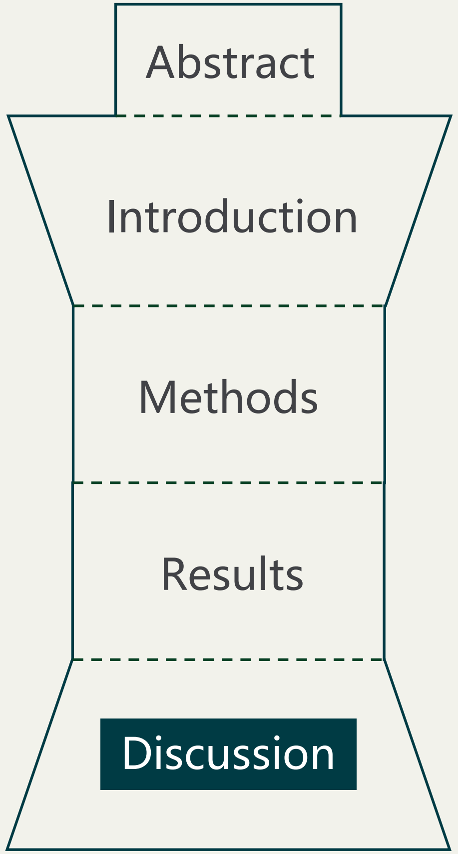


讨论——写作要点

- 快速总结研究发现
  - **不要**重复“结果”
- 结果需要结合更广的背景、范围展开讨论
  - 结果是否否定了前人的研究？为什么？
- 结论是如何促进这一领域进展的？
- 研究的局限性，对未来研究的影响？

实事求是，不要过度解读，也不要妄自菲薄。

撰写“讨论”是一种艺术，不要吝于请教他人寻求帮助！



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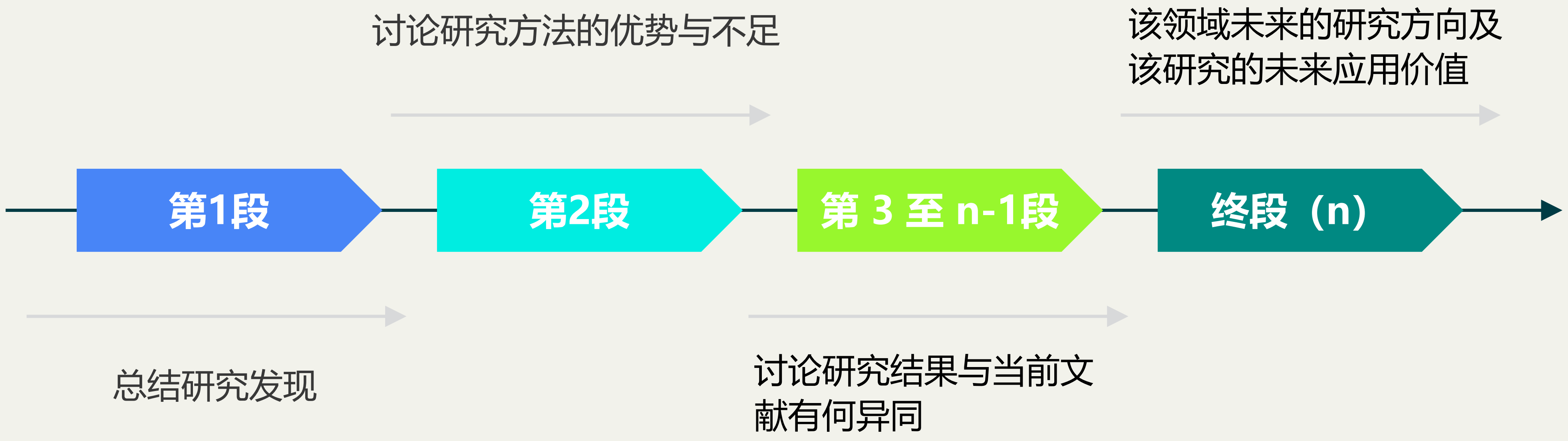

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该领域未来的研究方向及
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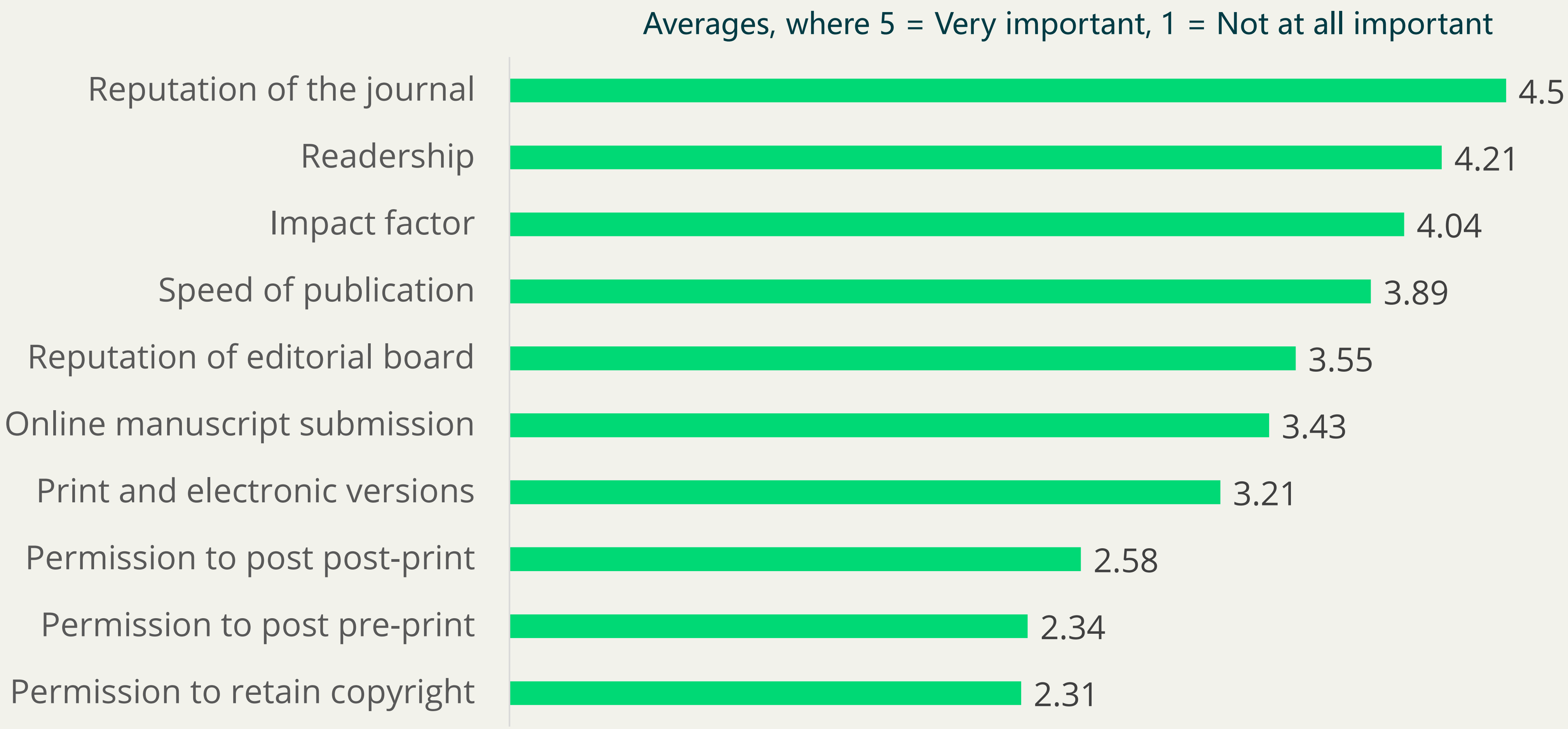

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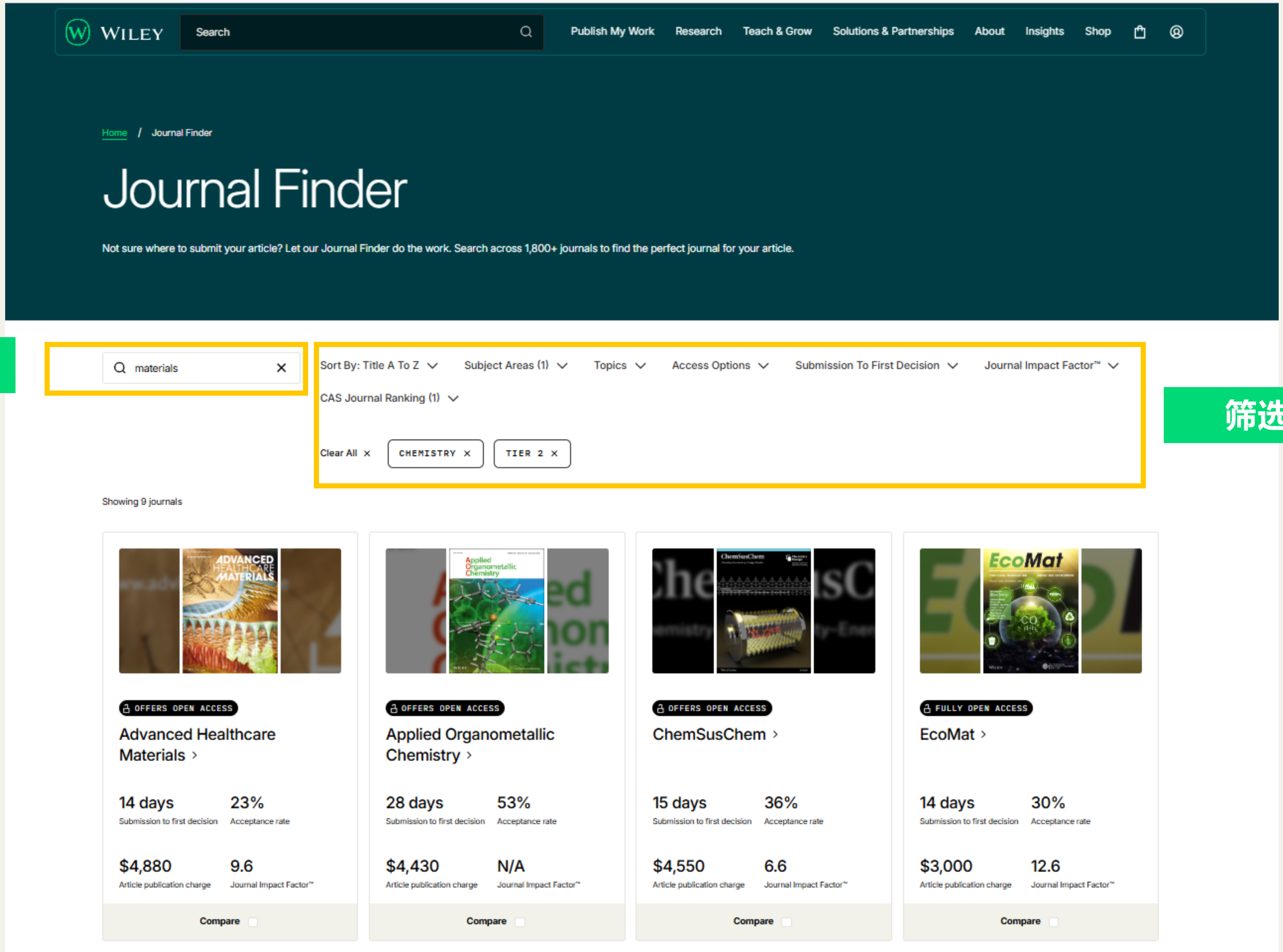

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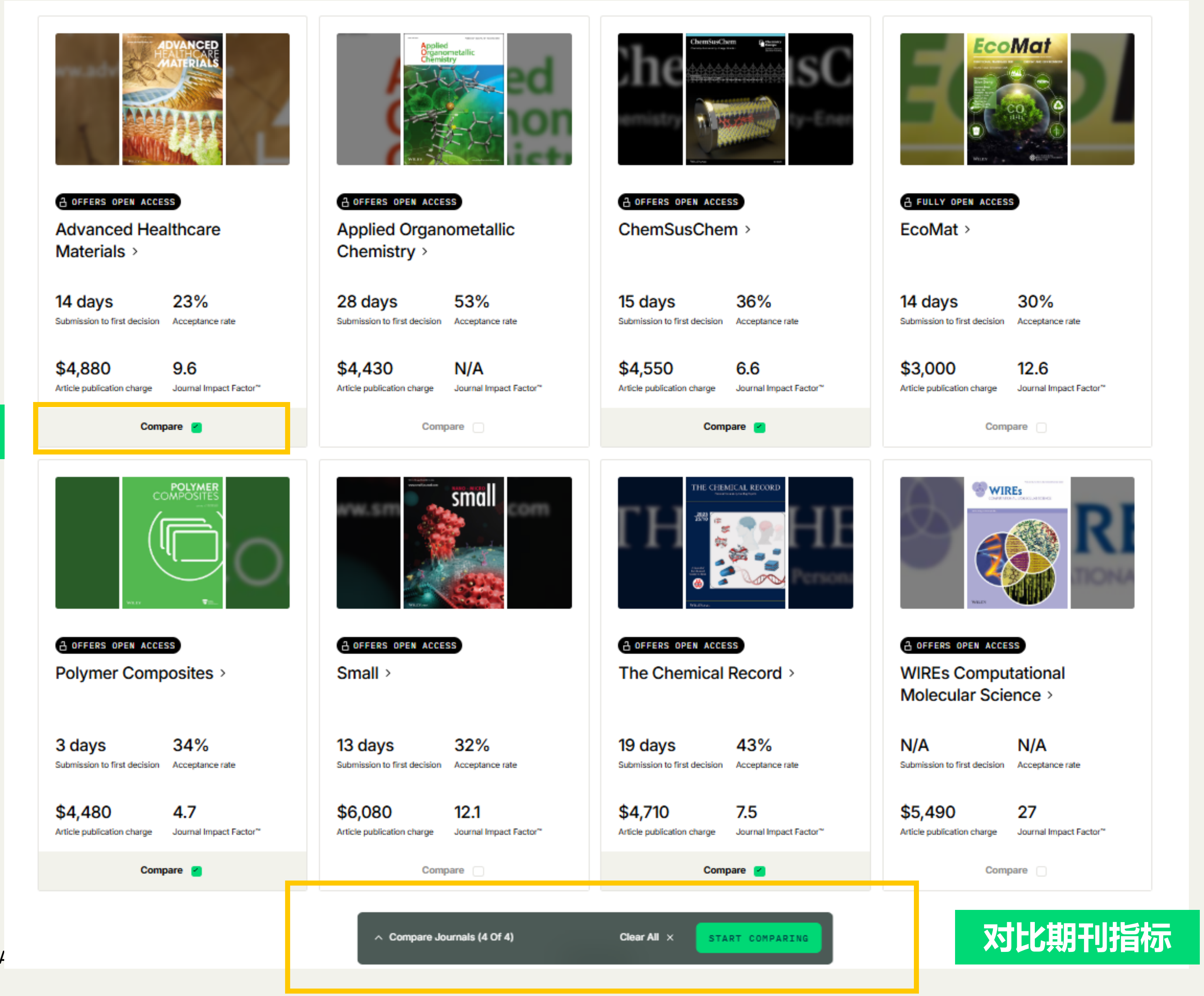

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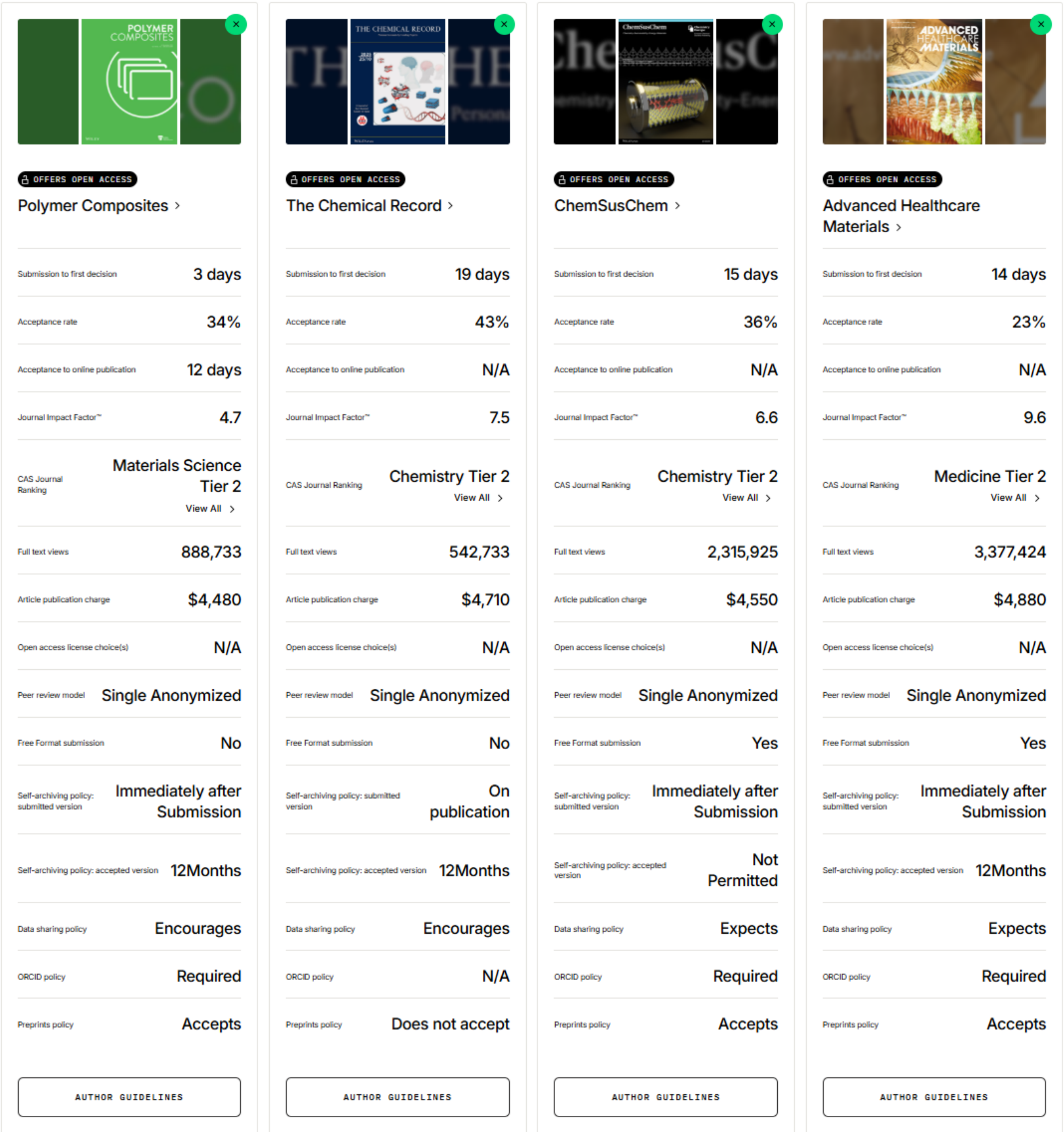

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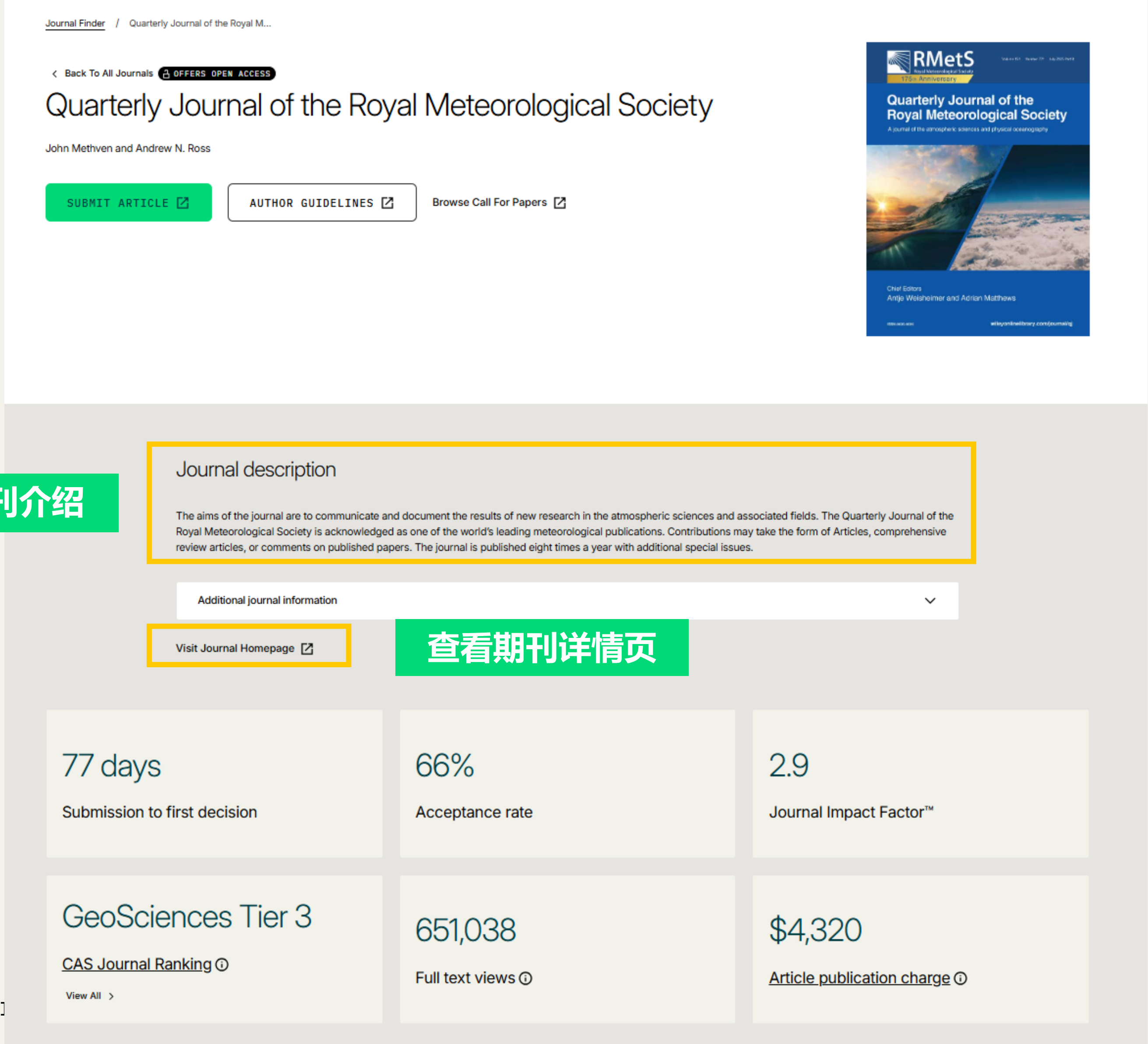

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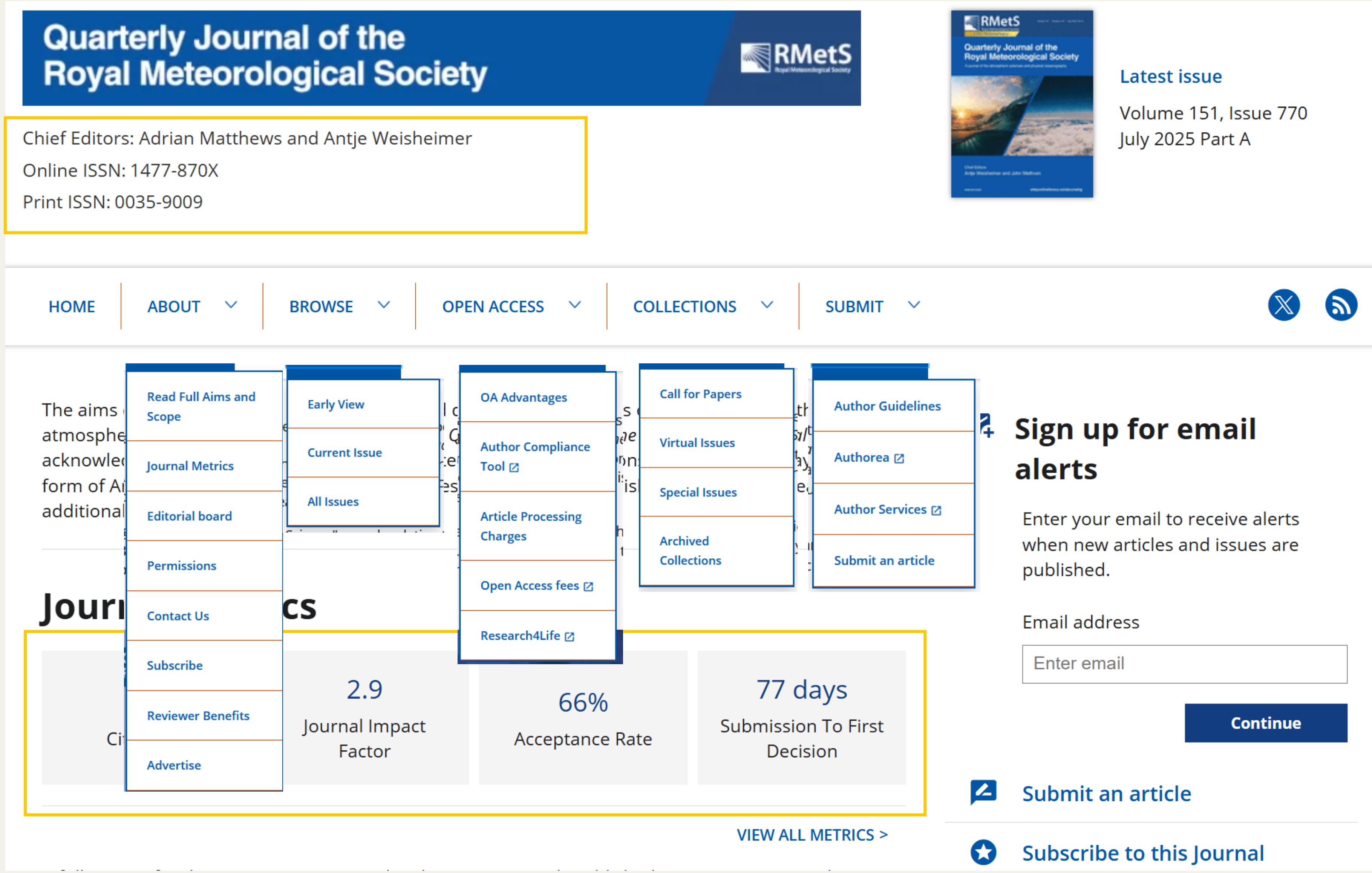

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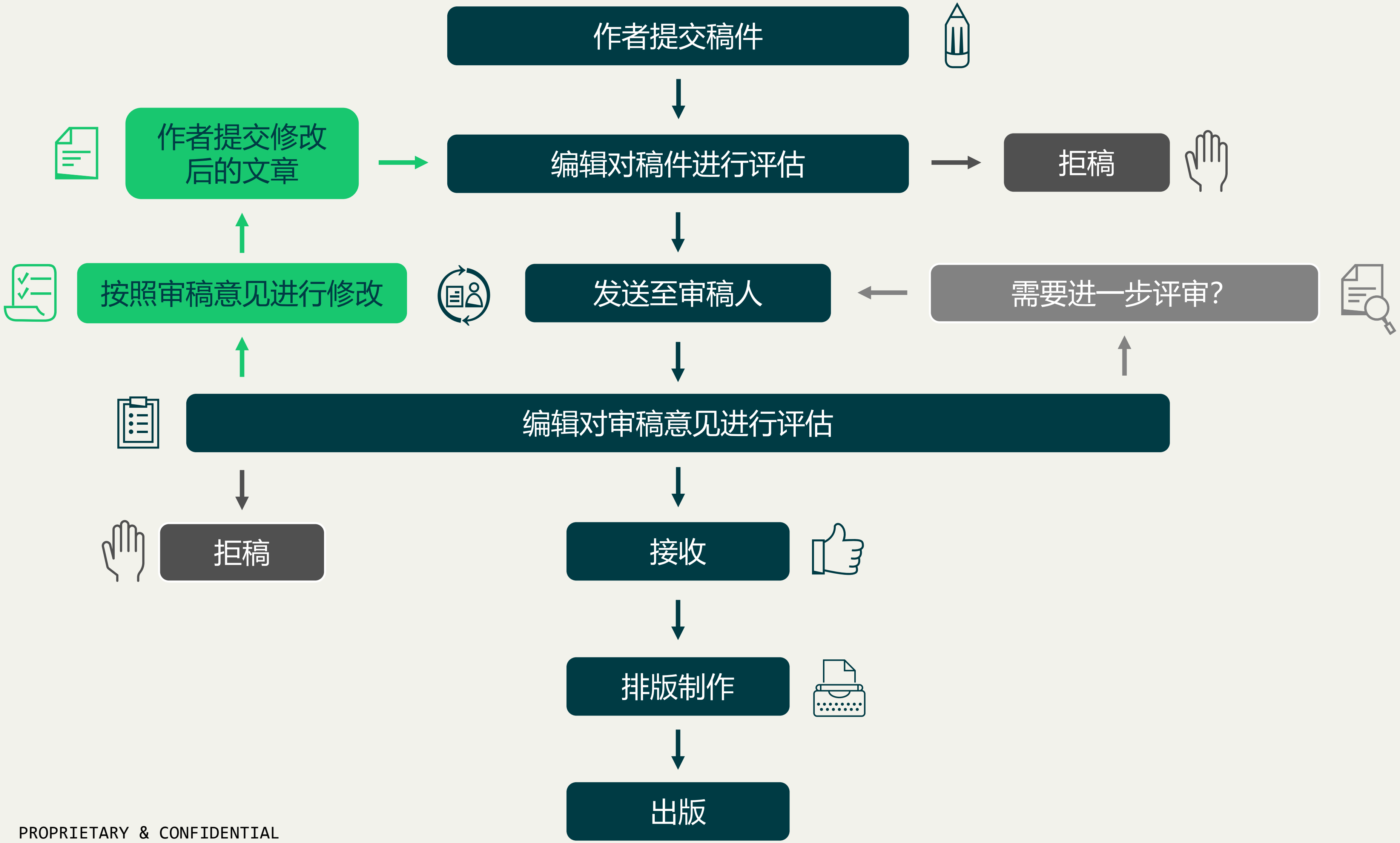

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Cover Letter

Dear xxxx editors,

I hope that xxxx will consider the above paper. This manuscript researches on the sensory properties of fragrant and non-fragrant rice and their flavour contributors. This manuscript shows that the demarcation between fragrant and non-fragrant rice, based on their 2-acetyl-1-pyrroline contents, is not as clear-cut as suggested in the literature. 2-Acetyl-1-pyrroline can contribute to the aroma of non-fragrant rice at concentrations below its limit of detection. However, much higher concentrations exert relatively small effects because the dose-response response is logarithmic, with a log-log plot showing a gradient of only 0.37. This results in difficulty for assessors trying to distinguish the two types of rice by sensory analysis.

This paper has not been submitted to any other journal and all the authors listed above are aware that this paper is to be submitted to xxxx.

Yours sincerely,

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Dear Editor,

We have modified our manuscript and added data as requested by the reviewers and addressed their comments point by point below. We hope that this will make the manuscript suitable for publication in Biotechnology Journal.
Best wishes,
XXX

Replies to the referee's comments:

Reviewer 1 Comments:

1) The authors describe that ladA is disrupted by a homolog of A. niger pepD. It would be interesting to determine the fate of the original locus of that gene. Was pepD duplicated or translocated? Is it expressed?
Response: We agreed with the referee's comment and performed an additional experiment to find out, if the ladA gene rearrangement has results from the pepD gene duplication and insertion or chromosomal translocation. Based on PCR data, it seems that a chromosomal translocation has occurred between the pepD and ladA loci resulting in disruption of both genes. We did not check for pepD transcription, as the translocation is mapped in the middle of the pepD coding region and therefore the correct transcript very likely cannot be formed.

2) Additionally, the authors fail to construct a clean ladA deletion strain, which is necessary in order to show, that mere the lack of L-Arabitol-Dehydrogenase is responsible for the observed phenotype.
Response: We did not aim to knock out LadA in 1M parent strain and compare it with the 3M-43 mutant. The approach was rather the opposite. We complemented the mutant with the parent ladA gene, confirmed restored LadA enzymatic levels and checked the complemented strain for extracellular production. We showed that L-arabitol dehydrogenease activity, once reverted back, resulted in extracellular hemicellulases expression similar to that observed in the parent strain. Therefore, these data indicate on direct link between the intrecellular levels of L-arabitol dehydrogenase which regulates the L-arabitol flux in the fungal cell and transcription/secretion of a number of hemicellulases. We don't exclude that other mutations accumulated in the strain during mutagenesis may contribute to its improved productivity but the major effect observed resulted from the ladA locus rearrangement. And the ladA gene, homologous or heterologous, alone is able to revert the phenotype back to the parent train.

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- 将这些实验作为今后研究方向并在结论中陈述

当不同意审稿人意见时

In particular, the authors should be considered that from previous literature (see the list below) seems that the 2-AP is an odour active compounds peculiar of the fragrant rice due to the genetic pattern. With this consideration, my doubt is: can the formation of the 2-AP in non-fragrant rice is due to the drying process or cooking process?

A discussion about this will improve the results of the manuscript.

There are studies that report 2-AP only in the aromatic rice because of their genetic characteristics. See the works reported below

Louis M. T. Bradbury et al. A perfect marker for fragrance genotyping in rice. Molecular Breeding 2005 16: 279-283

Melissa A. Fitzgerald et al. Is there a second fragrance gene in rice? Plant Biotechnology Journal 2008 6, pp. 416-423

Michael J. Kovach et al. The origin and evolution of fragrance in rice (Oryza sativa L.). PNAS 2009 106, pp. 14444-14449

Melissa A. Fitzgerald et al. Not just a grain of rice: the quest for Quality Trends in Plant Science 2009 14 pp.134-139

Response: Thanks for your comment, and valuable literatures. Maillard reaction could be an explanation of 2-AP generation in non-fragrant rice during drying and cooking but the reaction yield should be very low. Moreover, 2-AP can also be accumulated in non-fragrant rice through a BADH2 independent pathway during planting. Please see the discussion below, and this is also added into the discussion of manuscript for clarification (from Line 486-539).

In current study, popcorn attributes were noticed in boiled non-fragrant rice and 2-AP was detected in raw non-fragrant rice, which indicated that 2-AP present in not only boiled non-fragrant rice, but also uncooked non-fragrant rice. Moreover, 2-AP was reported in raw and boiled non-fragrant rice by some literature research which supported the observation in current study. Several researches determined 2-AP in non-fragrant rice with simultaneous distillation extraction (SDE; rice was boiled during SDE) (Buttery et al., 1983; Buttery et al., 1986; Widjaja et al., 1996a); Buttery et al. (1988) used Dynamic headspace extraction (Tenax) extracted 2-AP in boiled non-fragrant rice; Maraval et al. (2010) and Mathure et al. (2014) detected 2-AP in raw non-fragrant rice with SPME. Therefore, investigation of 2-AP origin in non-fragrant rice is important to understand 2-AP generation mechanism and improve non-fragrant fla-
vour. Since it was reported that 2-AP could be generated
biosynthesis and Maillard reaction, there are three potential
can lead to 2-AP generation in non-fragrant rice: from biosy
rice planting; from Milliard reaction during post-harvest p
ing drying, dehusking, polishing and storage; and from Ma
during boiling.

Synthesis of 2-AP during fragrant rice planting has been stu
decades. Currently, two 2-AP generation pathways were pr
grant rice: the BADH2 dependent pathway, and the BADH
pathway. It is reported that 2-AP generation can be explain
function of enzyme BADH2, which is due to the gene *ba*
through in fragrant rice through a deletion of eight base pai
seven base pairs in exon 2 (Fitzgerald, McCouch & Hal
Handoko, Pather, Lisa Methven & Elmore, 2017). Hence
amino acids metabolic compound, is dehydrated to 1-pyrrol
rice rather than GABA through BADH2 catalysation, an
roline is acetylated to 2-AP (Bradbury, Gillies, Brushe
Henry, 2008). Whereas, a BADH2 independent 2-AP bios
way in fragrant rice was proposed according to a positive c
found between 2-AP and an amino acids metabolic produ
5-carboxylate (P5C) in fragrant rice (Huang et al. 2008
BADH2 independent pathway was proposed as ornithine,
and proline can metabolite to generate P5C, then P5C was
pyrroline, finally, 2-AP is generated through acetylation of
However, although there is no research that provides an evi
the correlation between P5C and 2-AP in non-fragrant rice
is rarely detected in non-fragrant rice in many researches, t
P5C and methylglyoxal, the key intermediates of 2-AP sy
ported by Huang et al.(2008). Therefore, generation of sm
2-AP from P5C during planting can be a potential explan
fragrant rice.

Several studies reported 2-AP change in fragrant rice durin

process. Wongpornchai et al. (2004) compared 2-AP content in fragrant rice after drying with several different drying conditions and the effect of drying methods on 2-AP content during storage. It was reported that 2-AP was lost during drying and storage, and high drying temperature and

phosphate buffer. Hence, boiling may provide a condition for 2-AP generation through Maillard reaction. However, Yoshihashi (2002) measured 2-AP content in fragrant rice after heating at 90 °C without water and boiling with water for 8, 10, 12, 14 min and conclude that 2-AP cannot generated during cooking or postharvest process in fragrant rice. It was reported that boiling can produce least Maillard reaction products than deep frying and baking (Semedo Tavares, et al., 2018). And because of the instability of 2-AP, generated 2-AP during boiling may not great for detectable.

Therefore, biosynthesis of 2-AP followed by BADH2 independent pathway can be a potential explanation of small amount of 2-AP presence in non-fragrant rice; on the other hand, generation through Maillard reaction during postharvest process and cooking is theoretically possible, however, several researches reported a decrease of 2-AP during drying, storage and cooking in fragrant rice, therefore, the generation of 2-AP in non-fragrant rice during drying and cooking may difficult be observed because of the instability of 2-AP.

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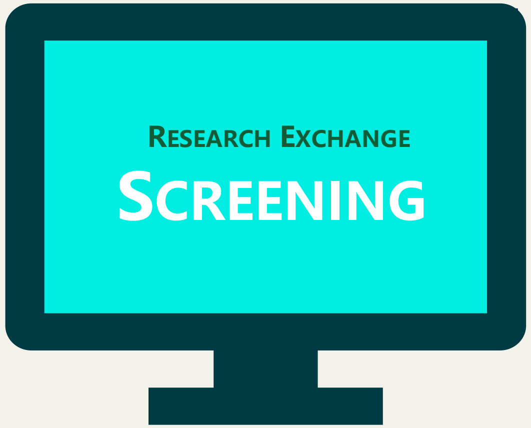

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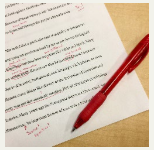

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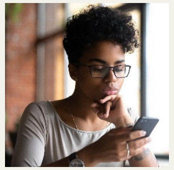

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