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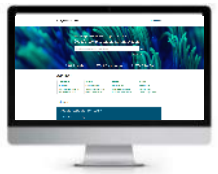

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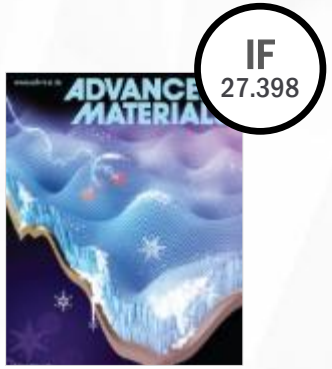

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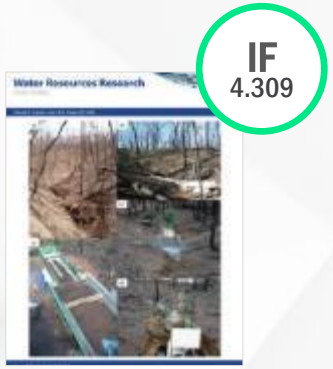

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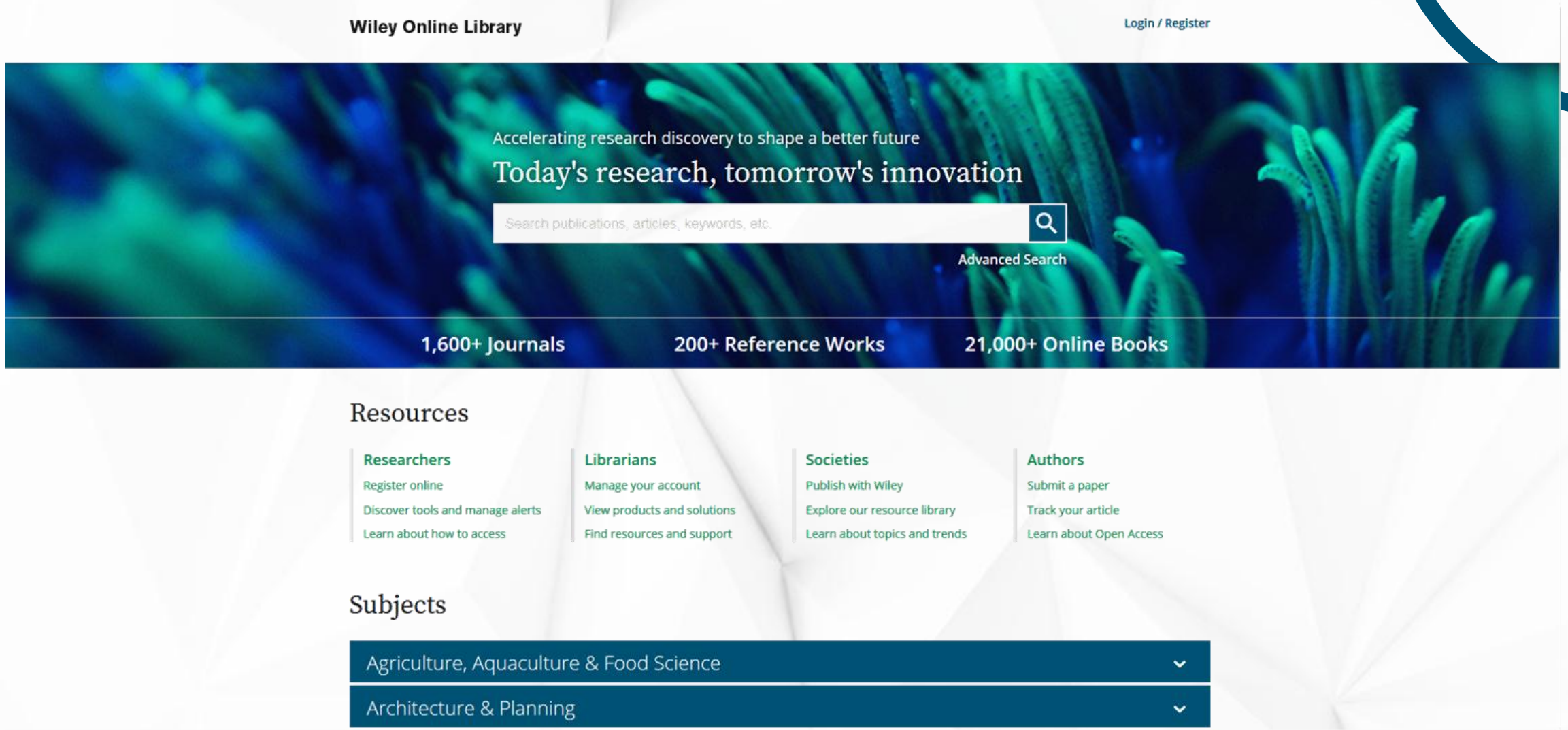

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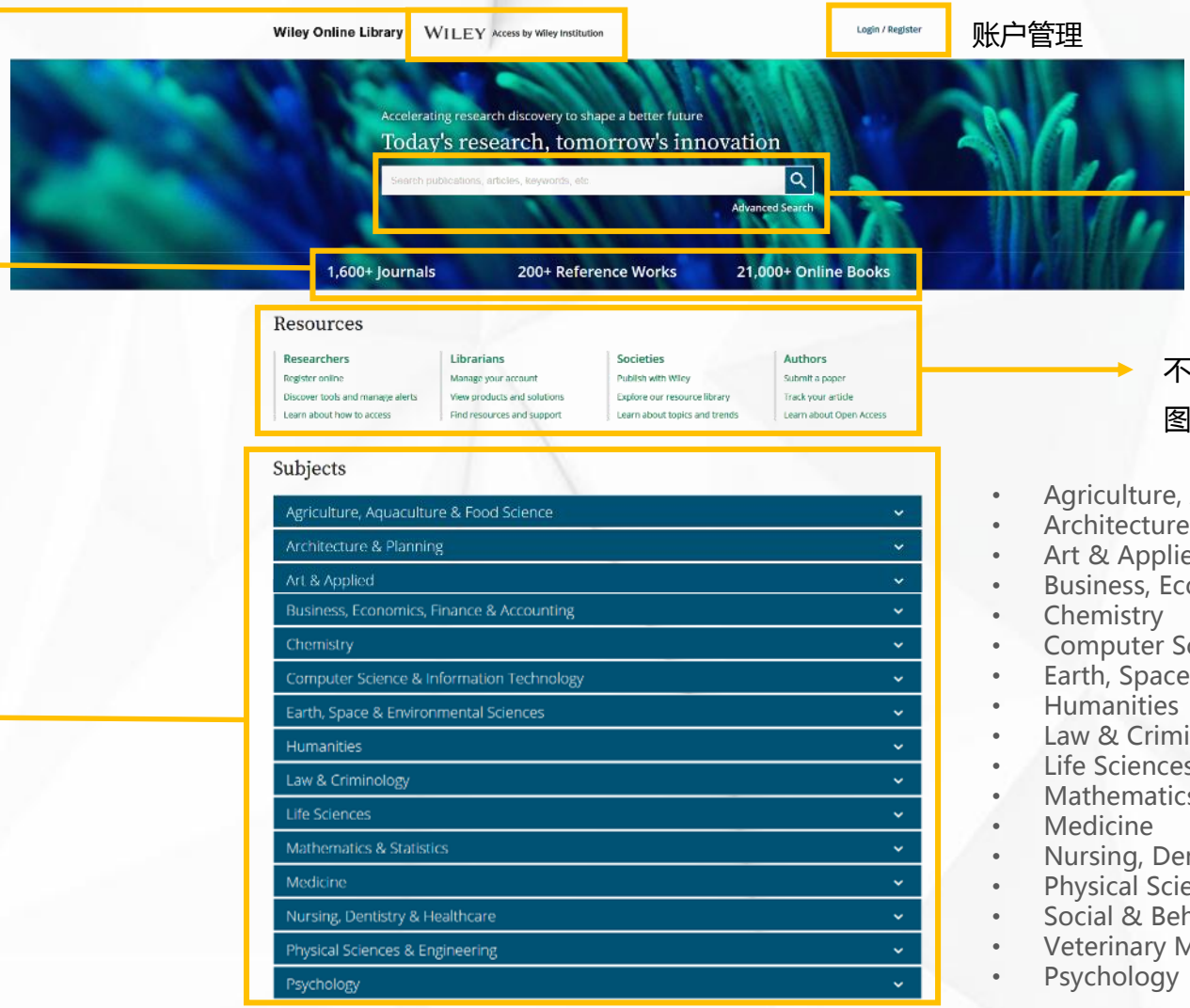

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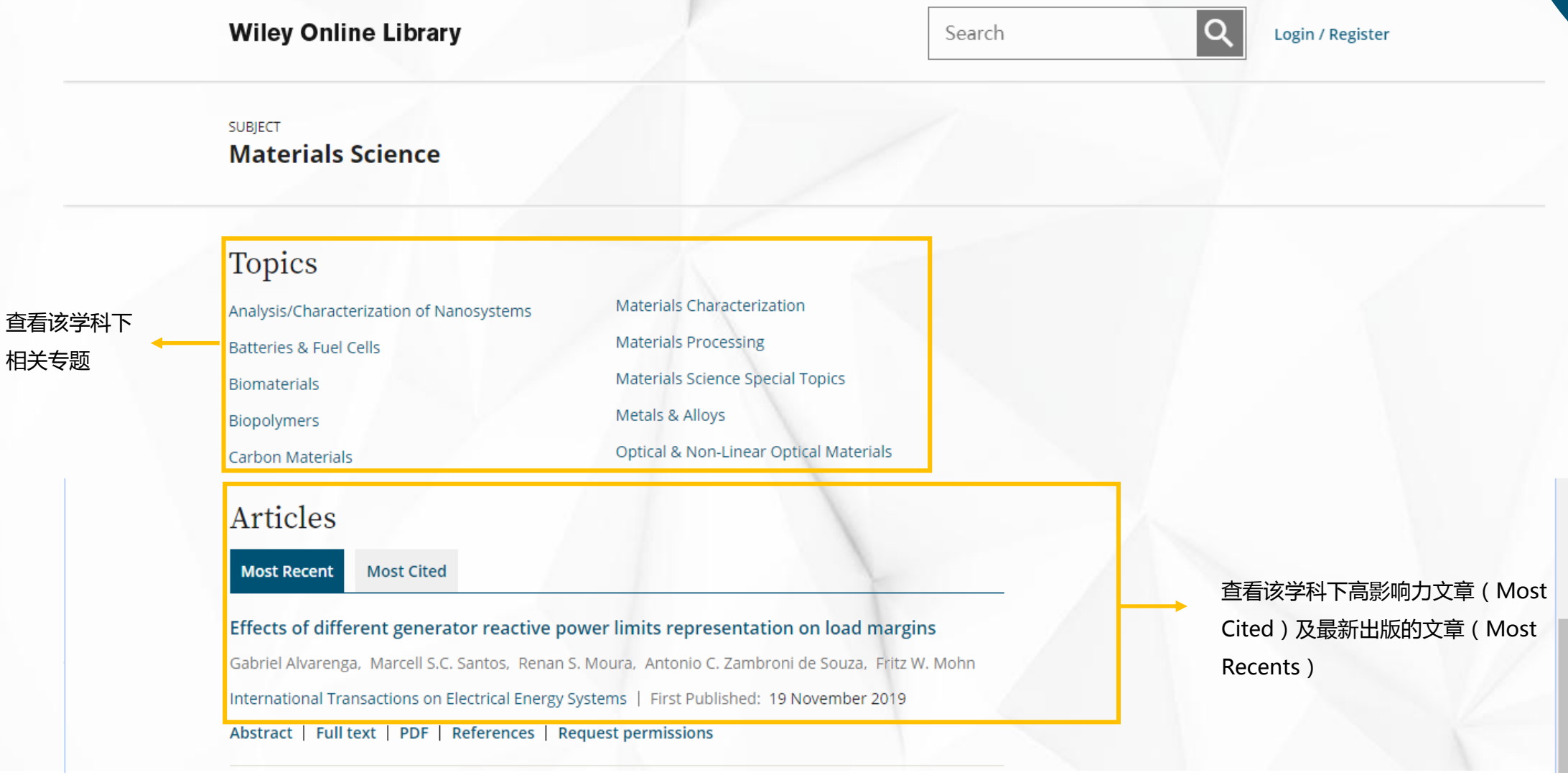

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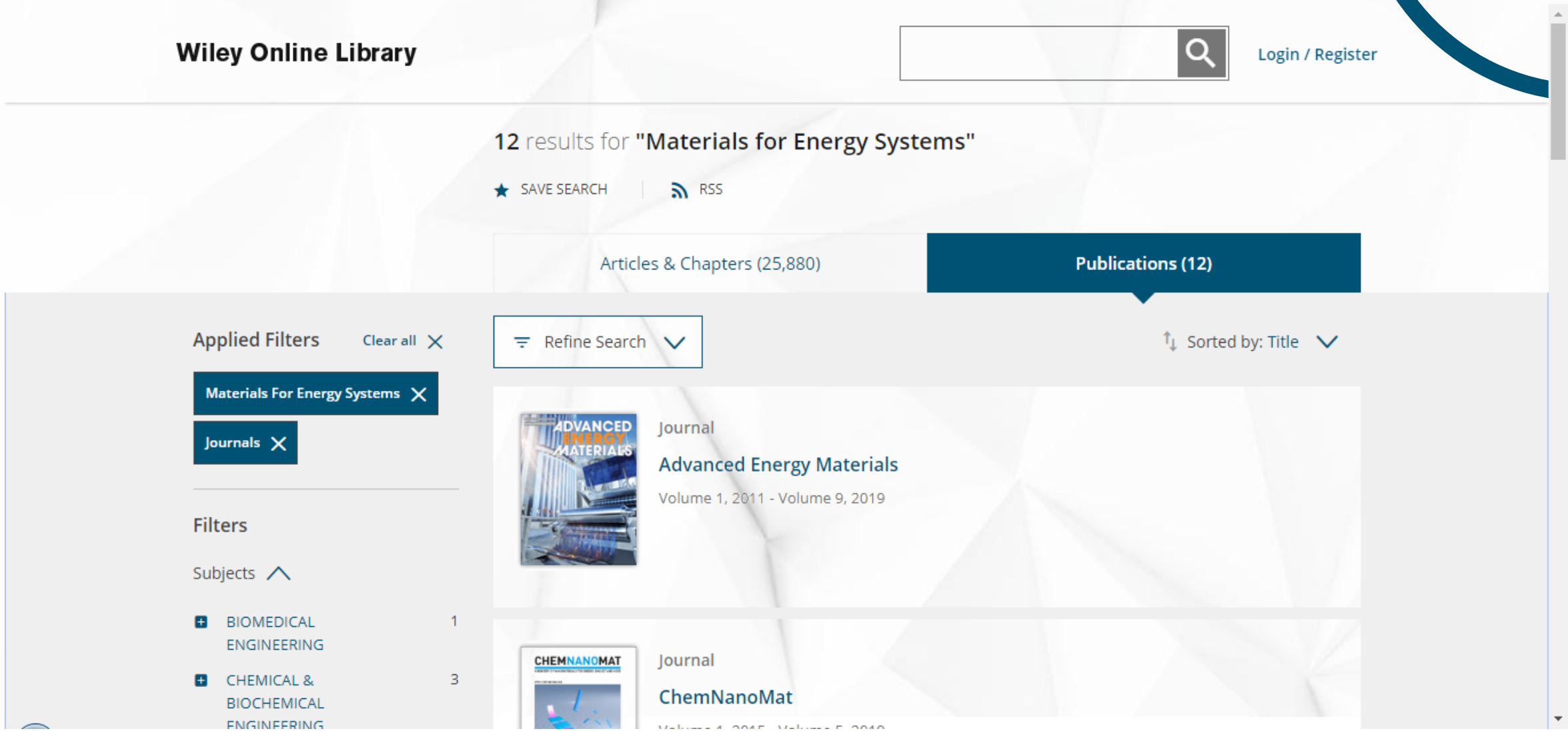

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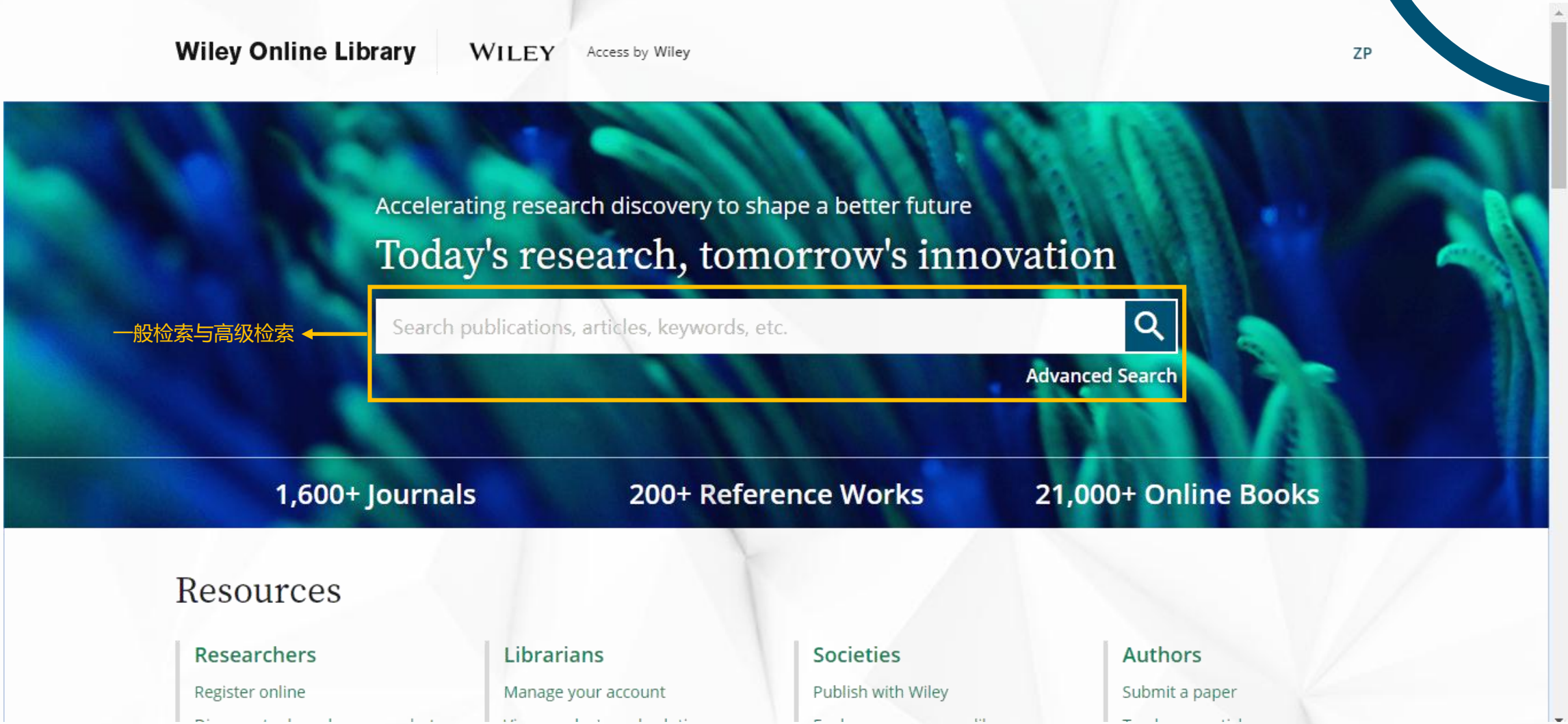

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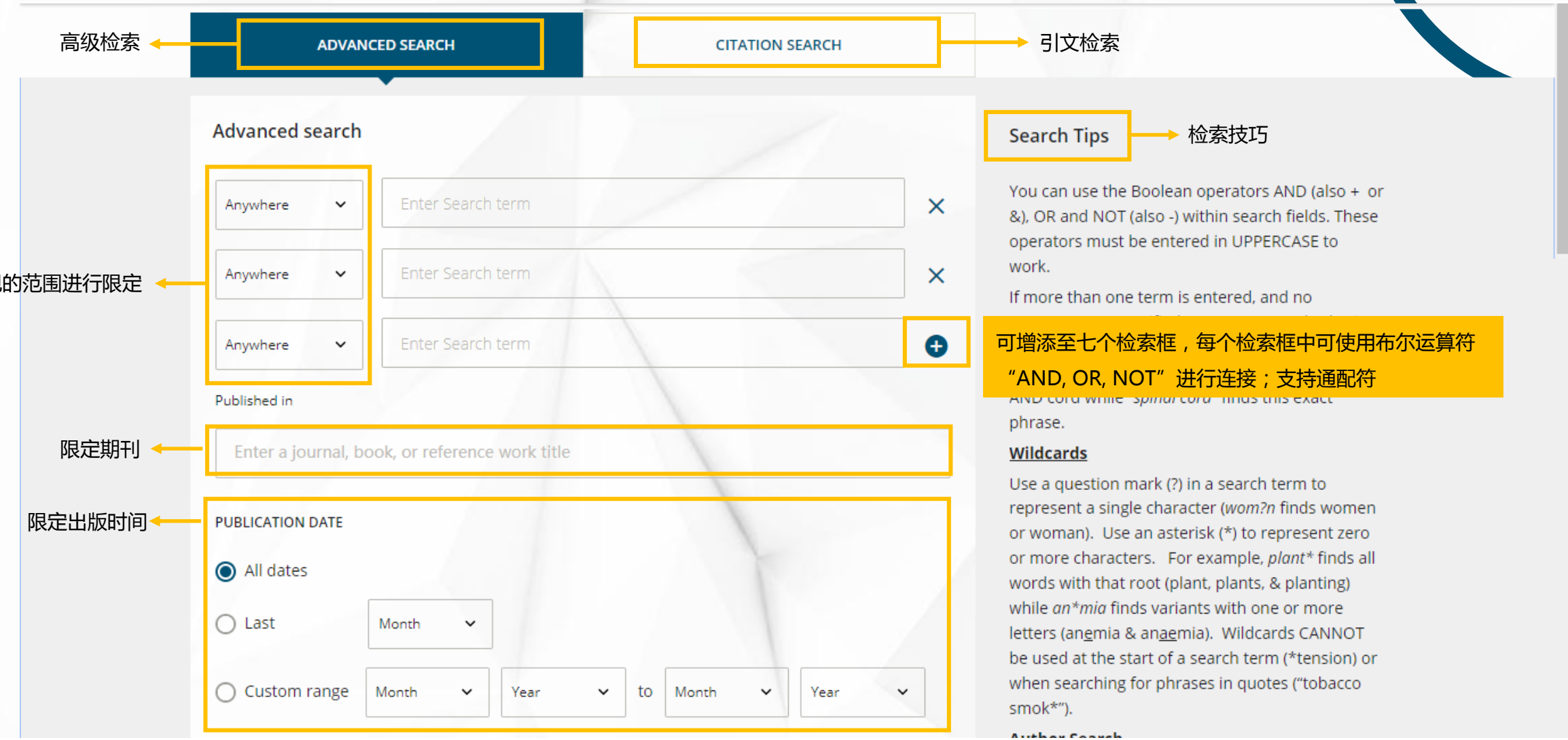

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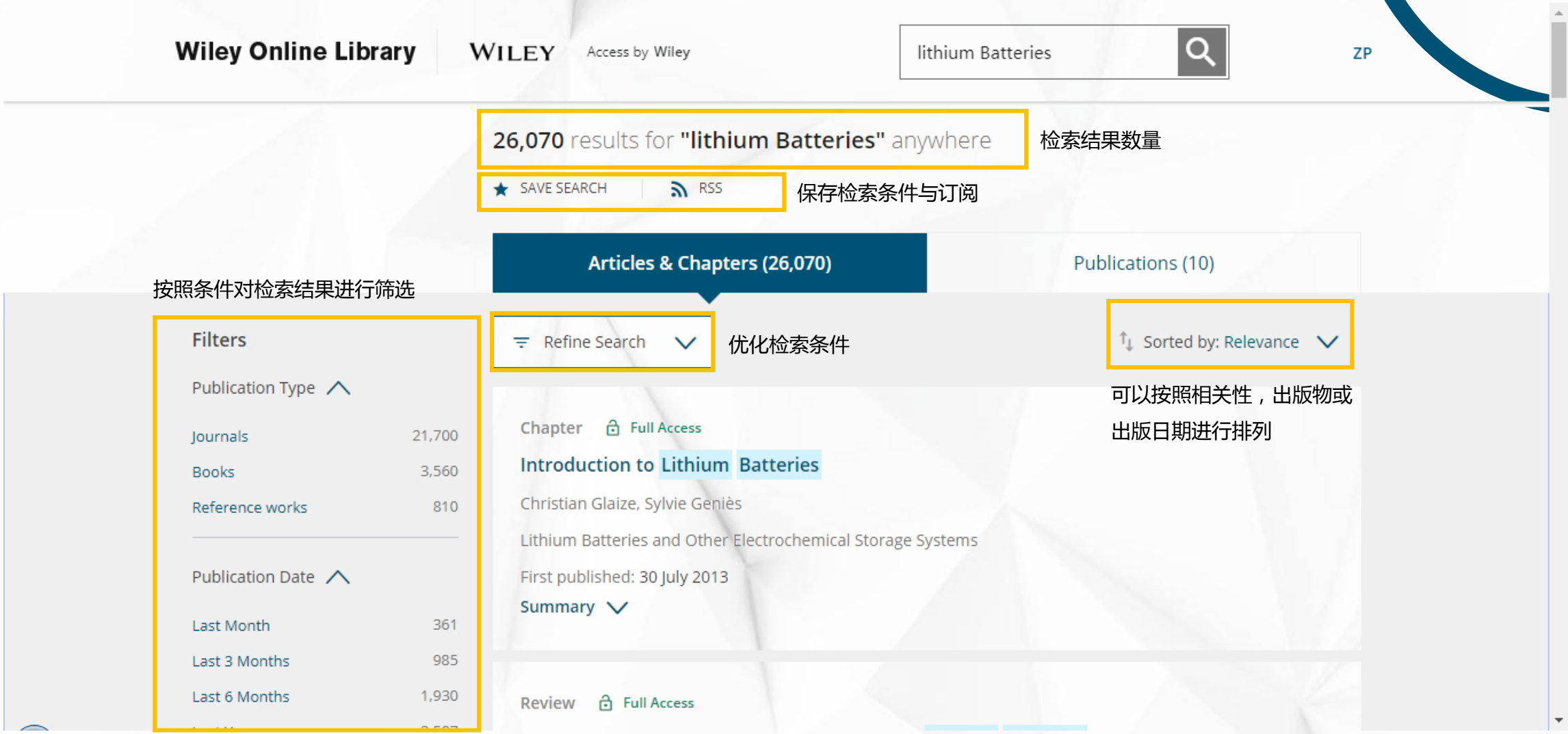

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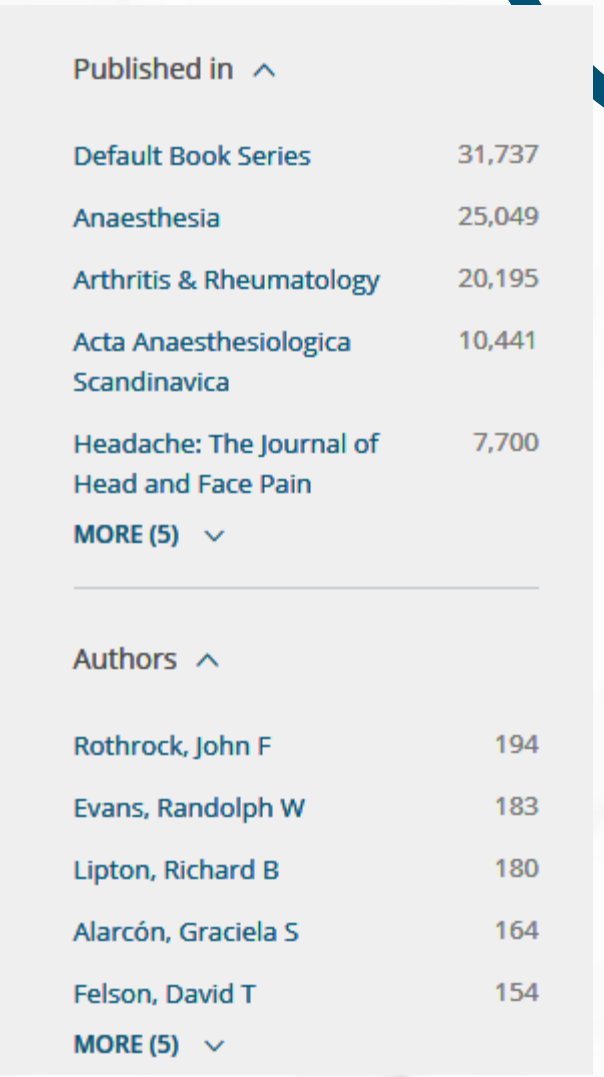

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Peter G. Bruce Prof., Bruno Scrosati Prof., Jean-Marie Tarascon Prof.

First published: 01 April 2008 | https://doi.org/10.1002/anie.200702505 | Cited by: 3898

† Thanks to Dr. Aurelie Debart for preparation of the frontispiece.

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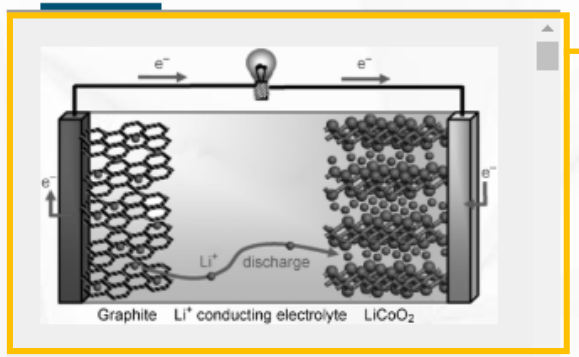

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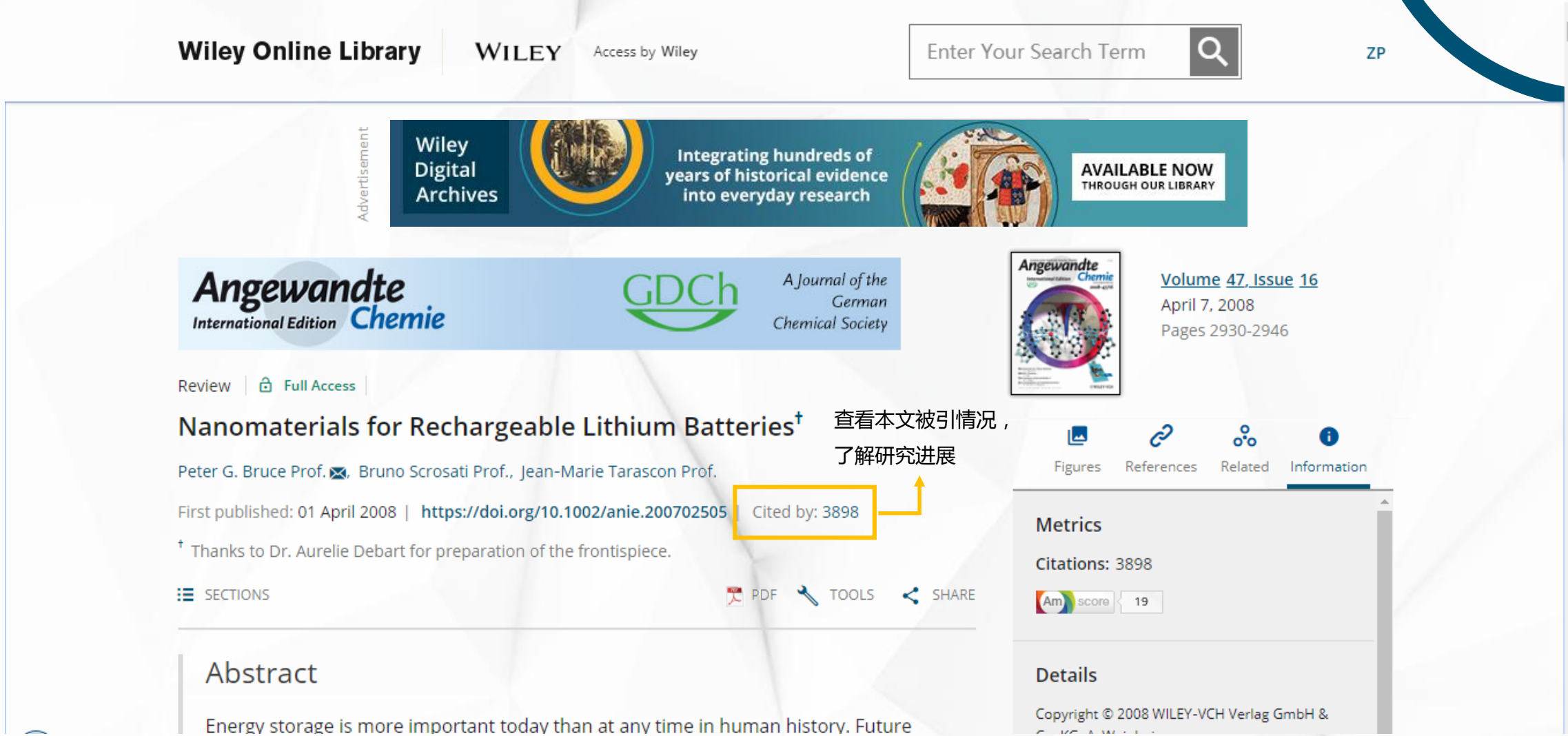

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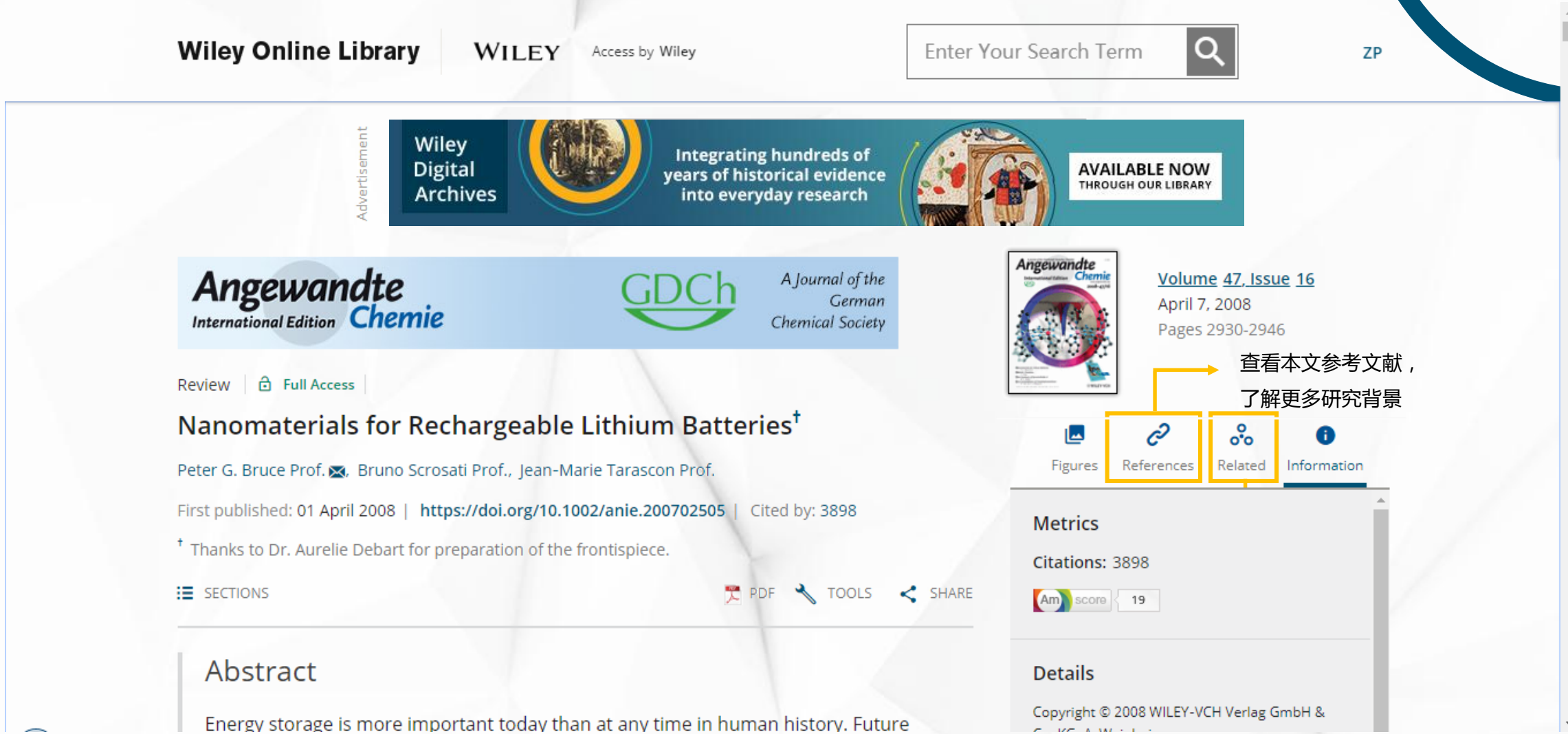

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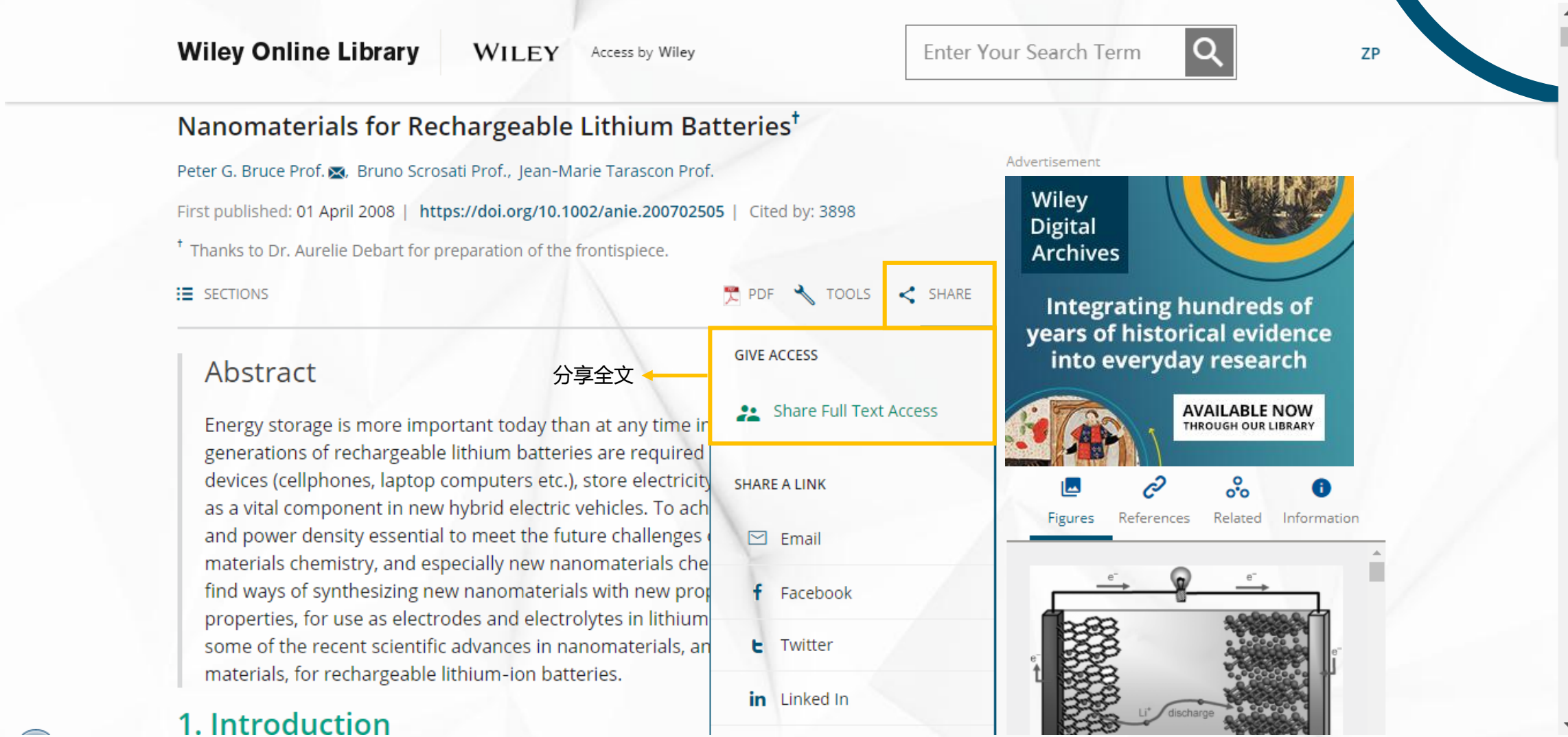

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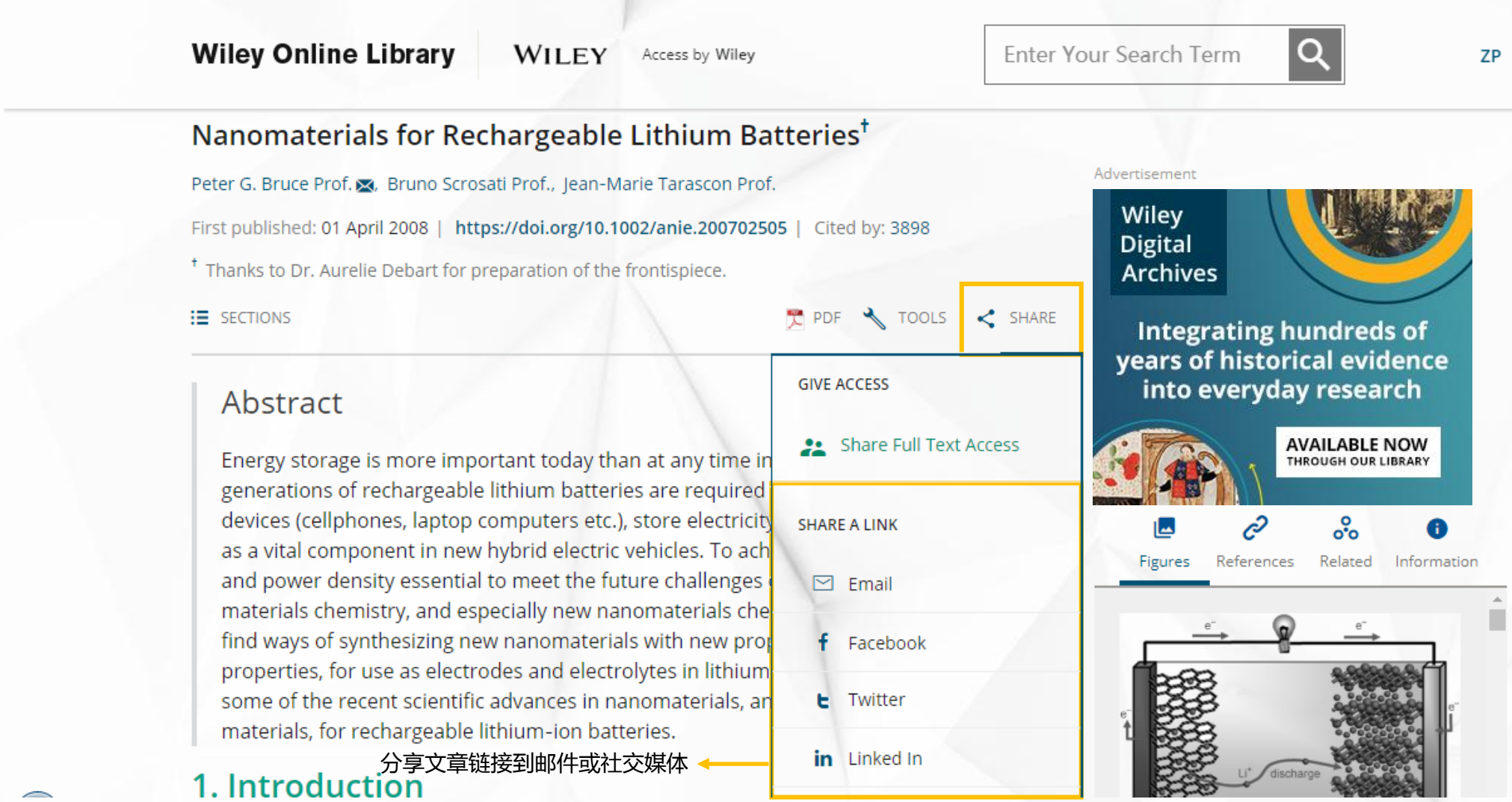

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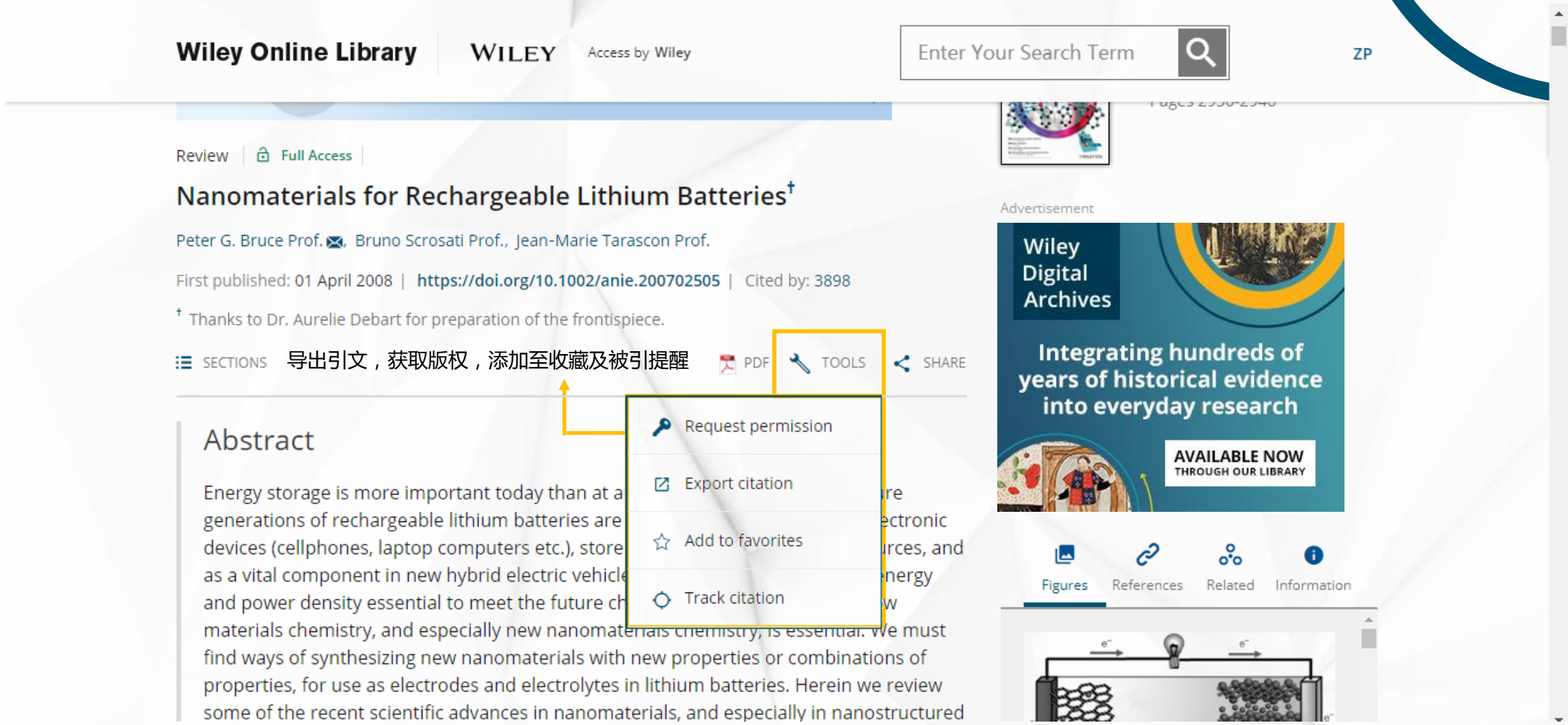

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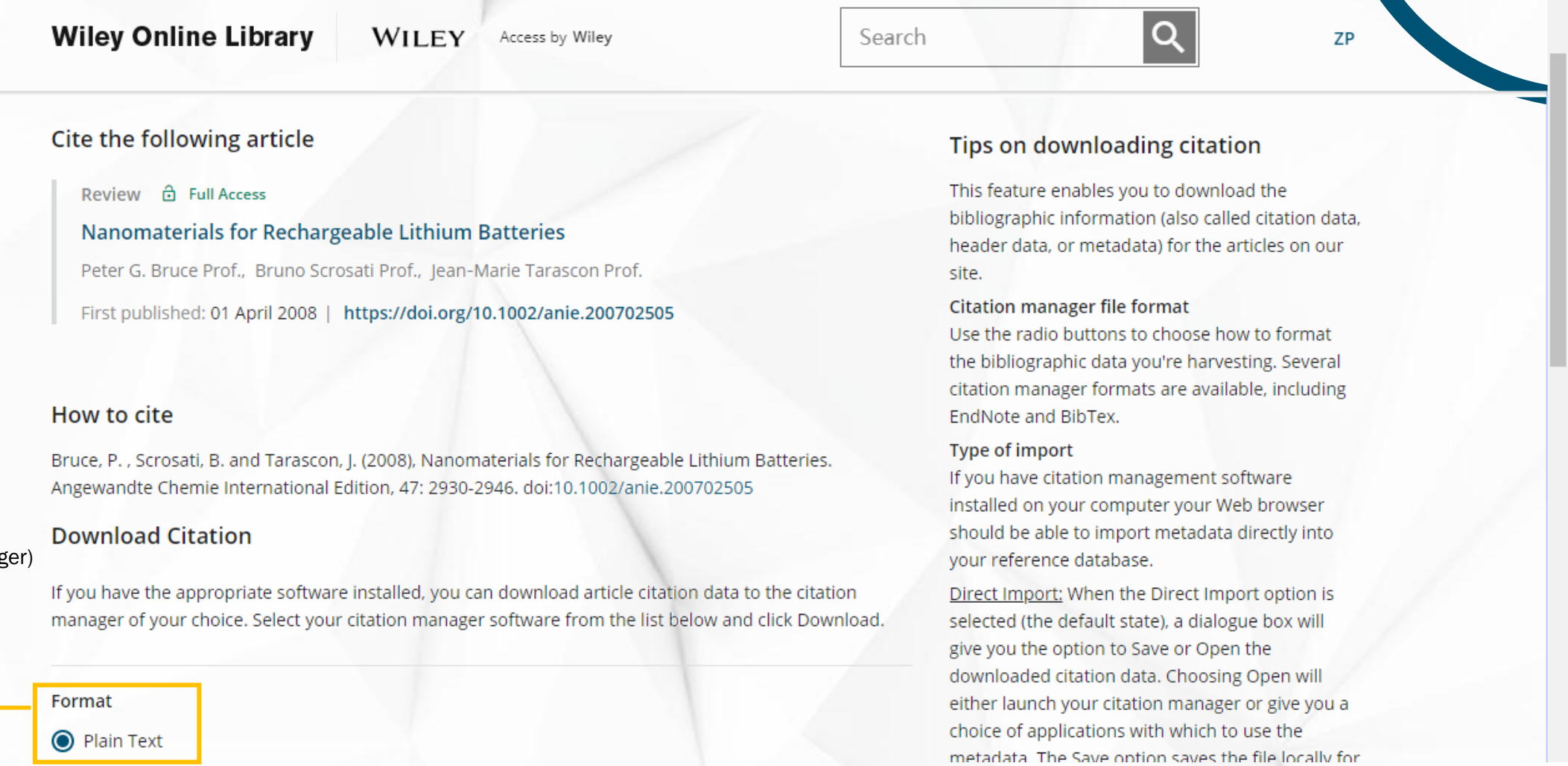

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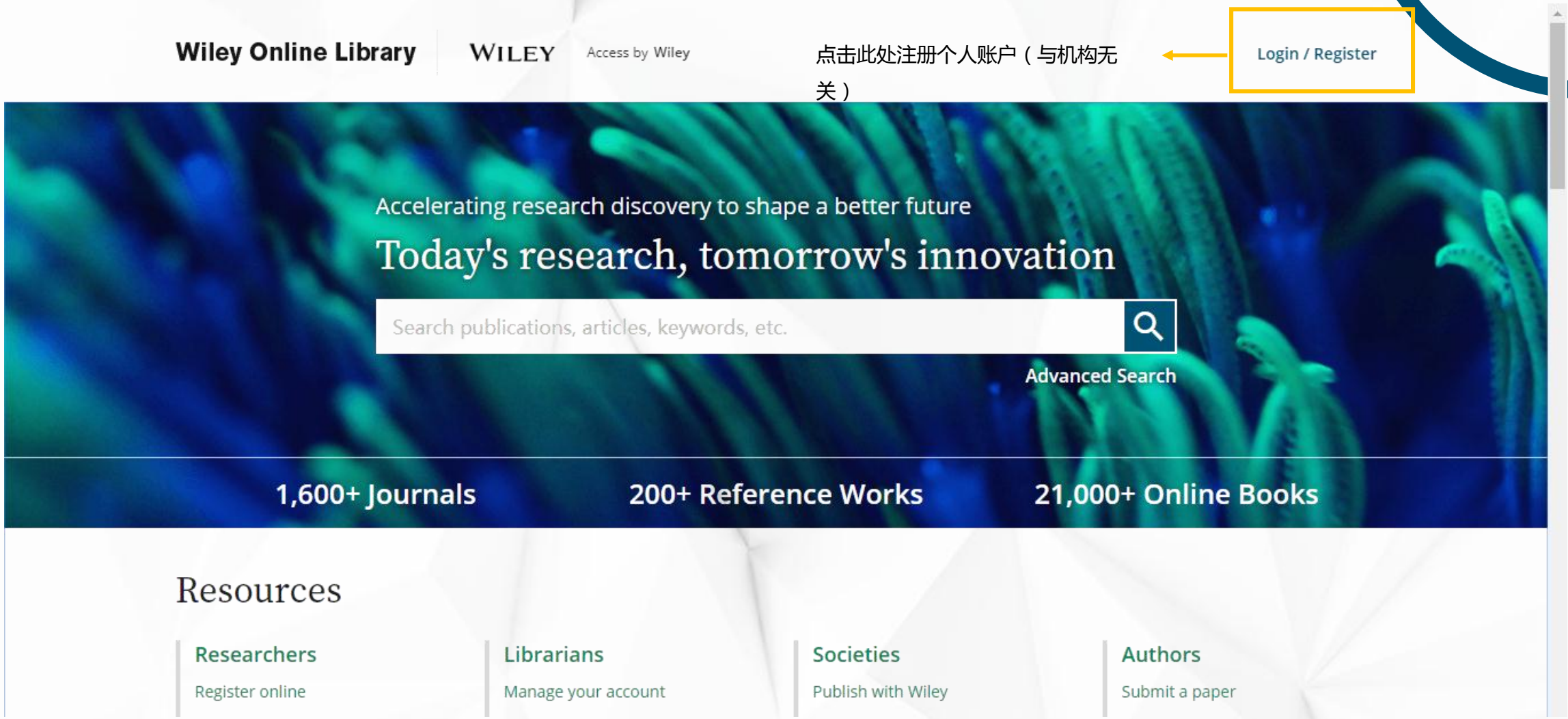

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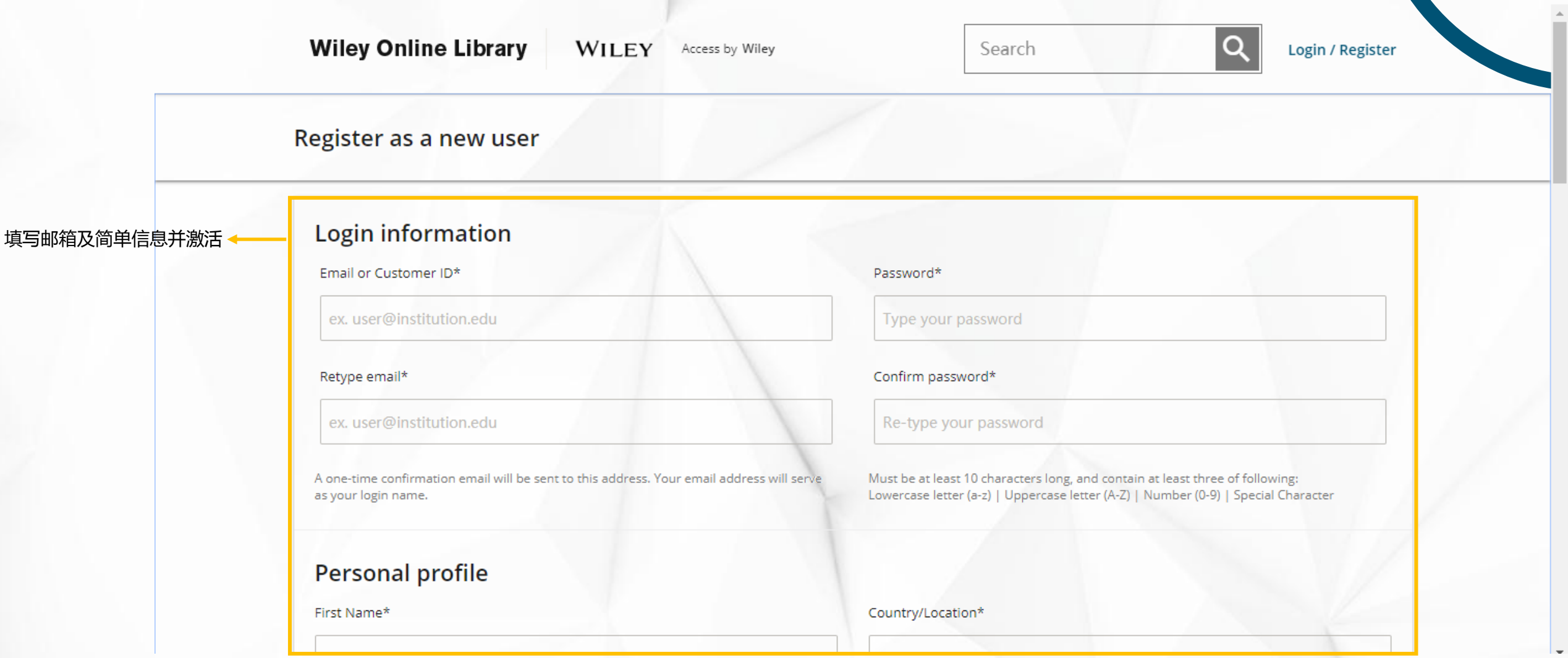

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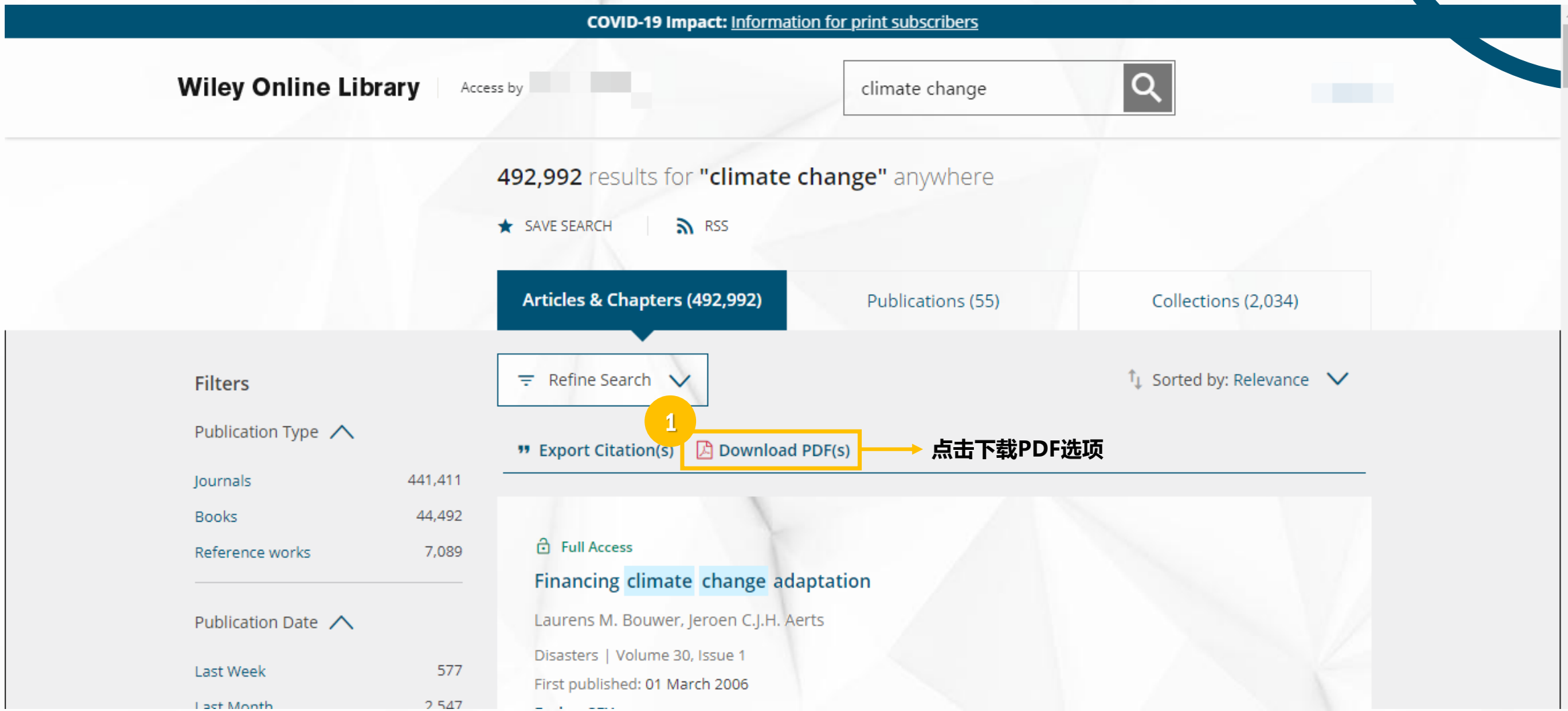
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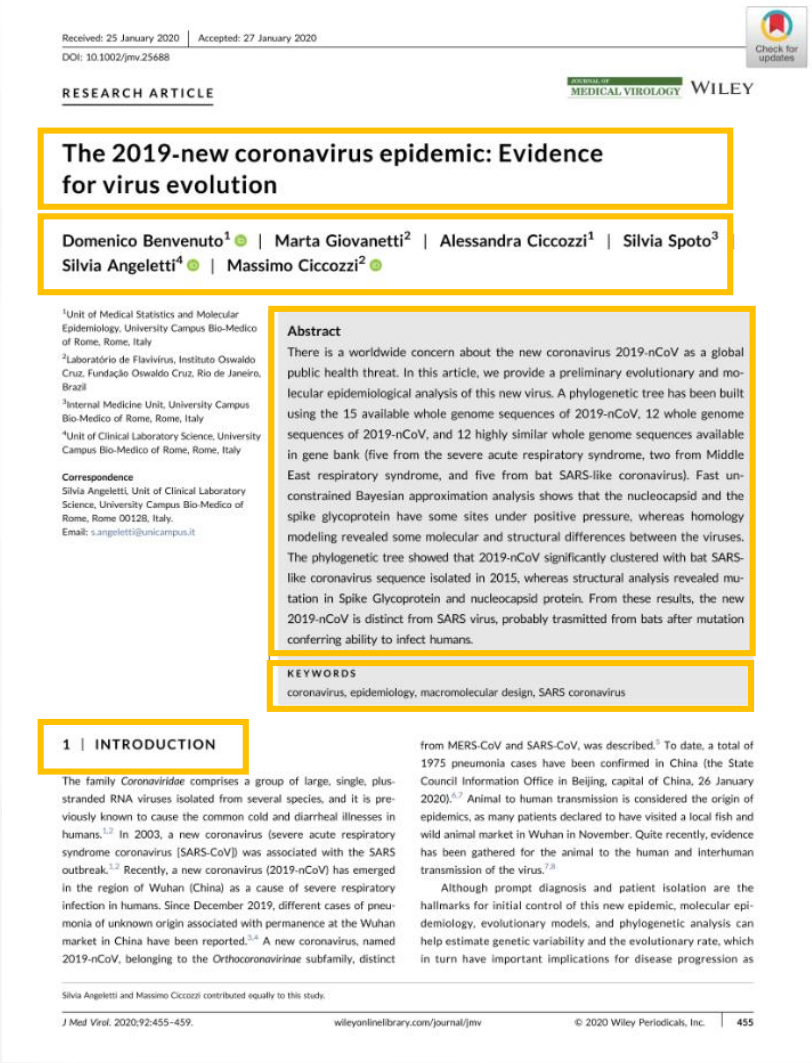

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DOI: 10.1002/jmv.25688

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

The 2019-new coronavirus epidemic: Evidence for virus evolution

Domenico Benvenuto[1] | Marta Giovanetti[2] | Alessandra Ciccozzi[1] | Silvia Spoto[3] | Silvia Angeletti[4] | Massimo Ciccozzi[2]

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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

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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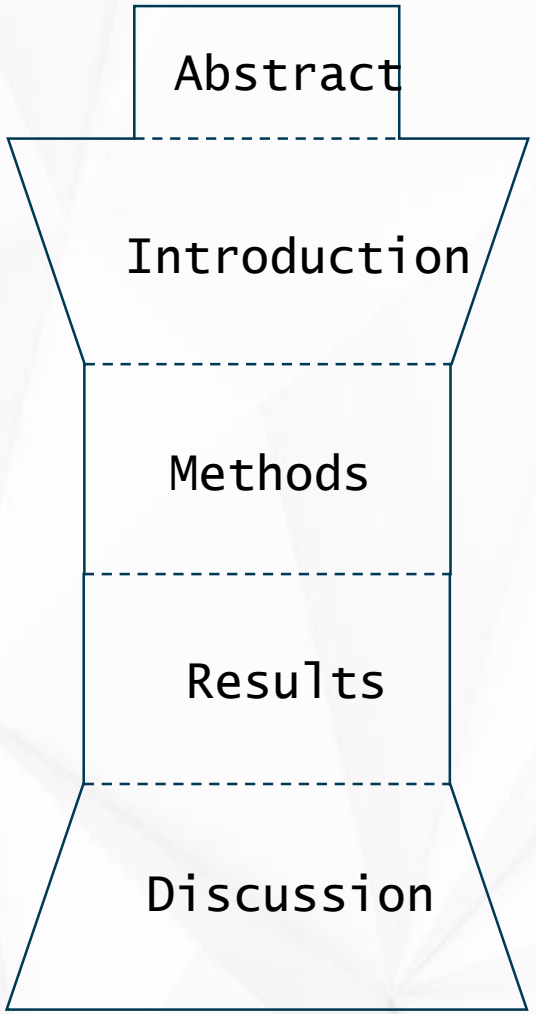

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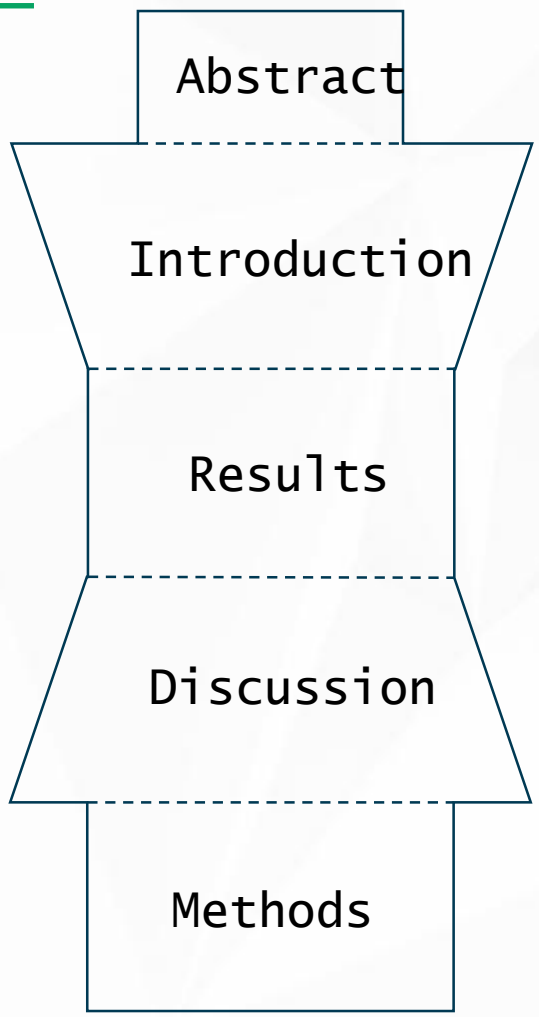

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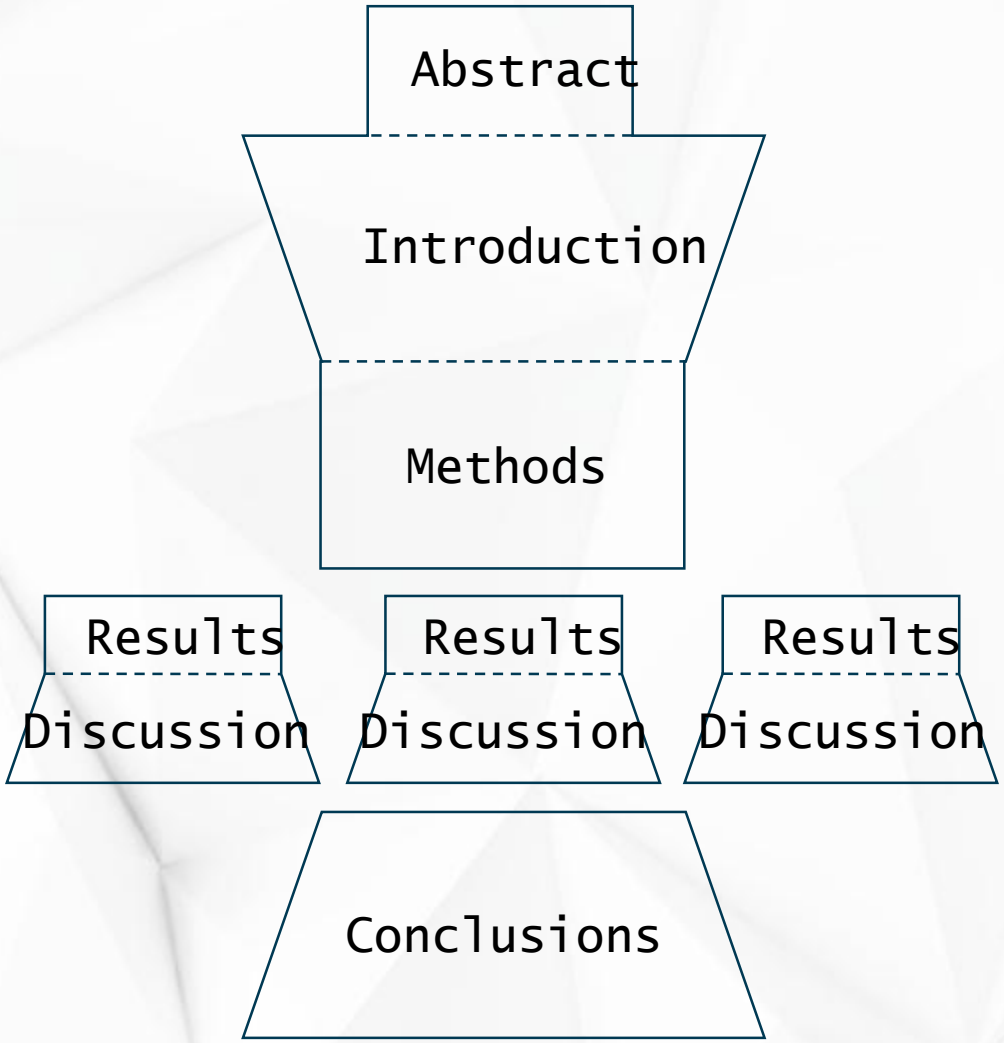

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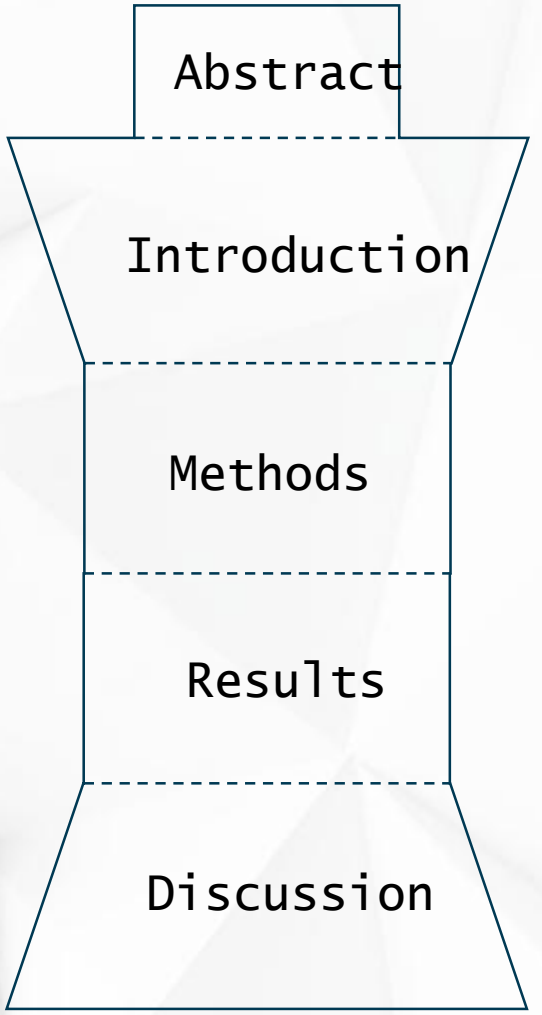

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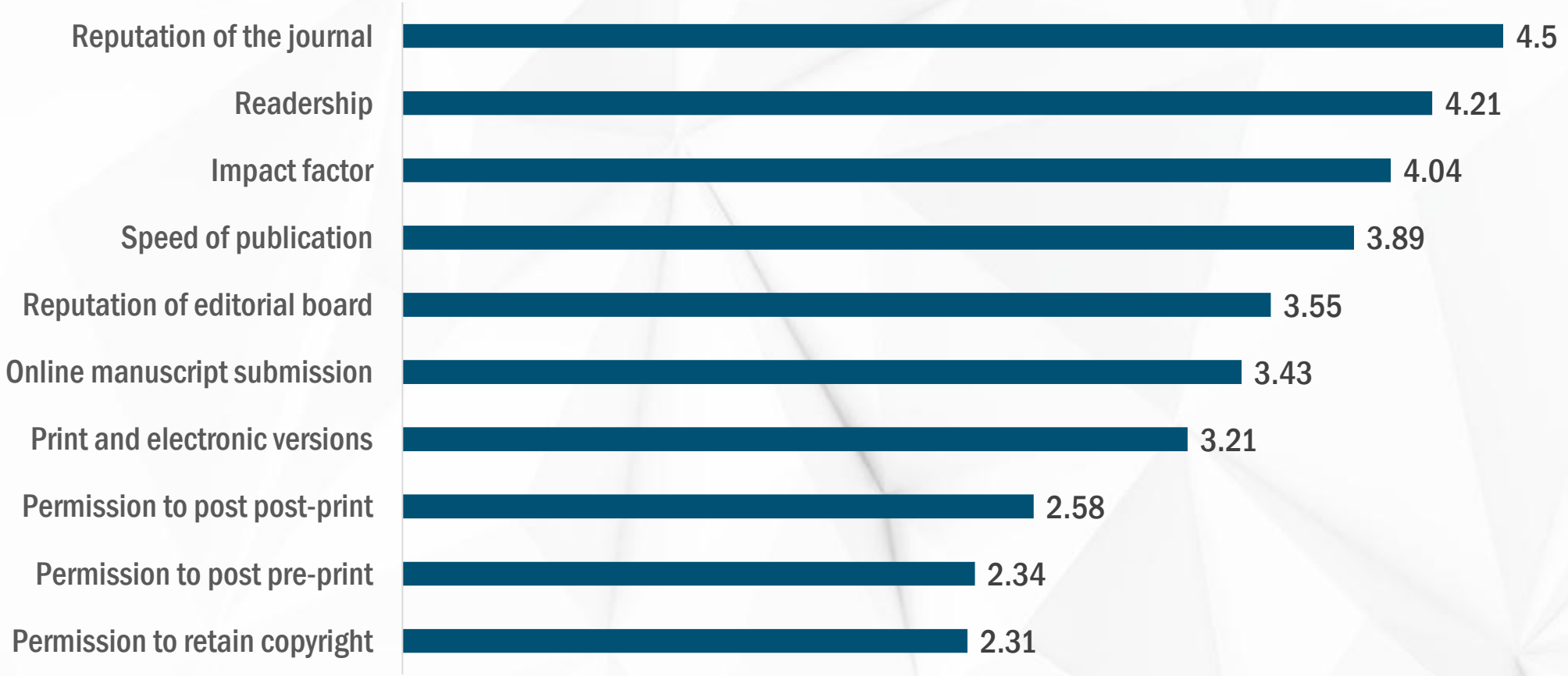

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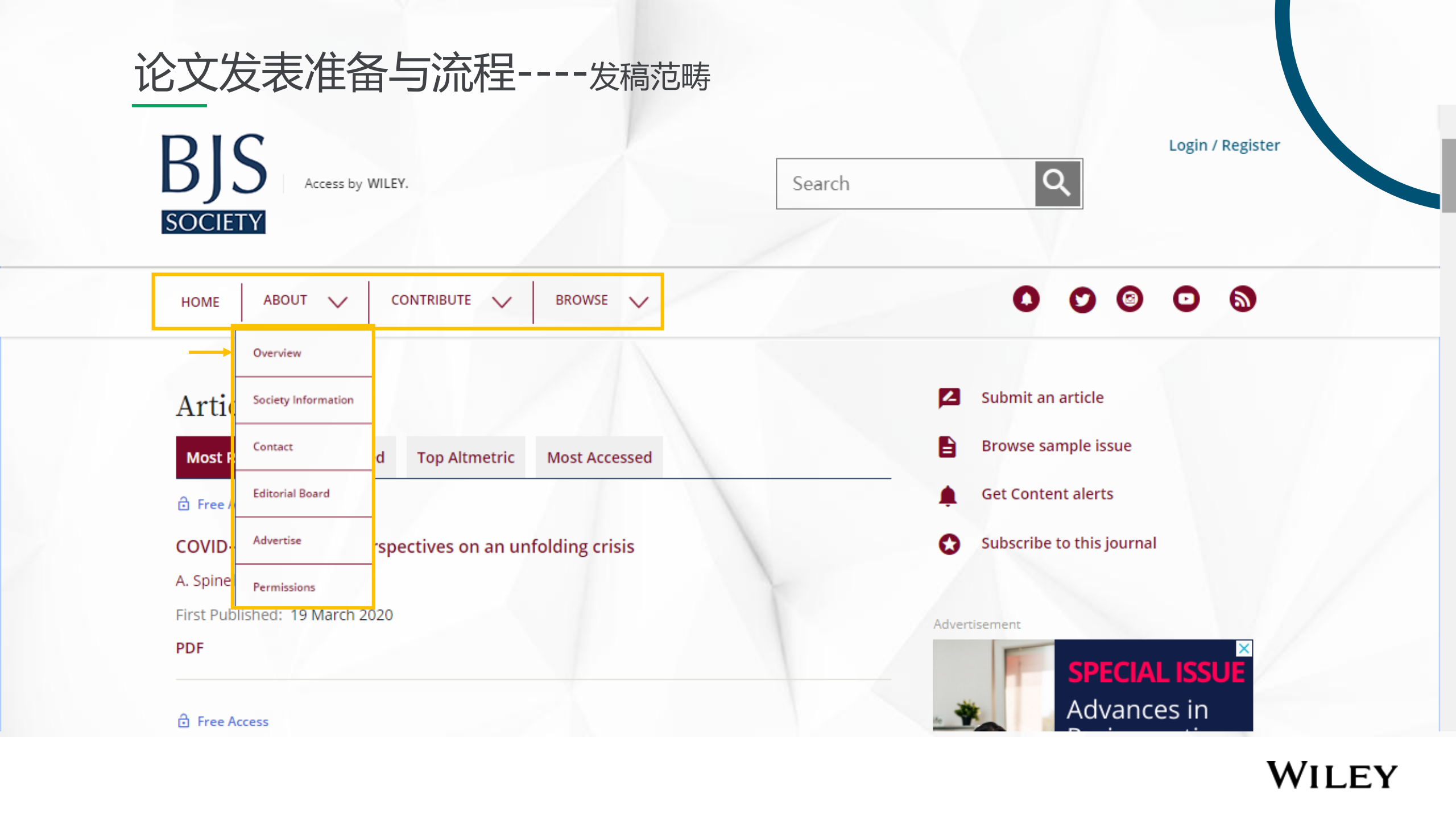

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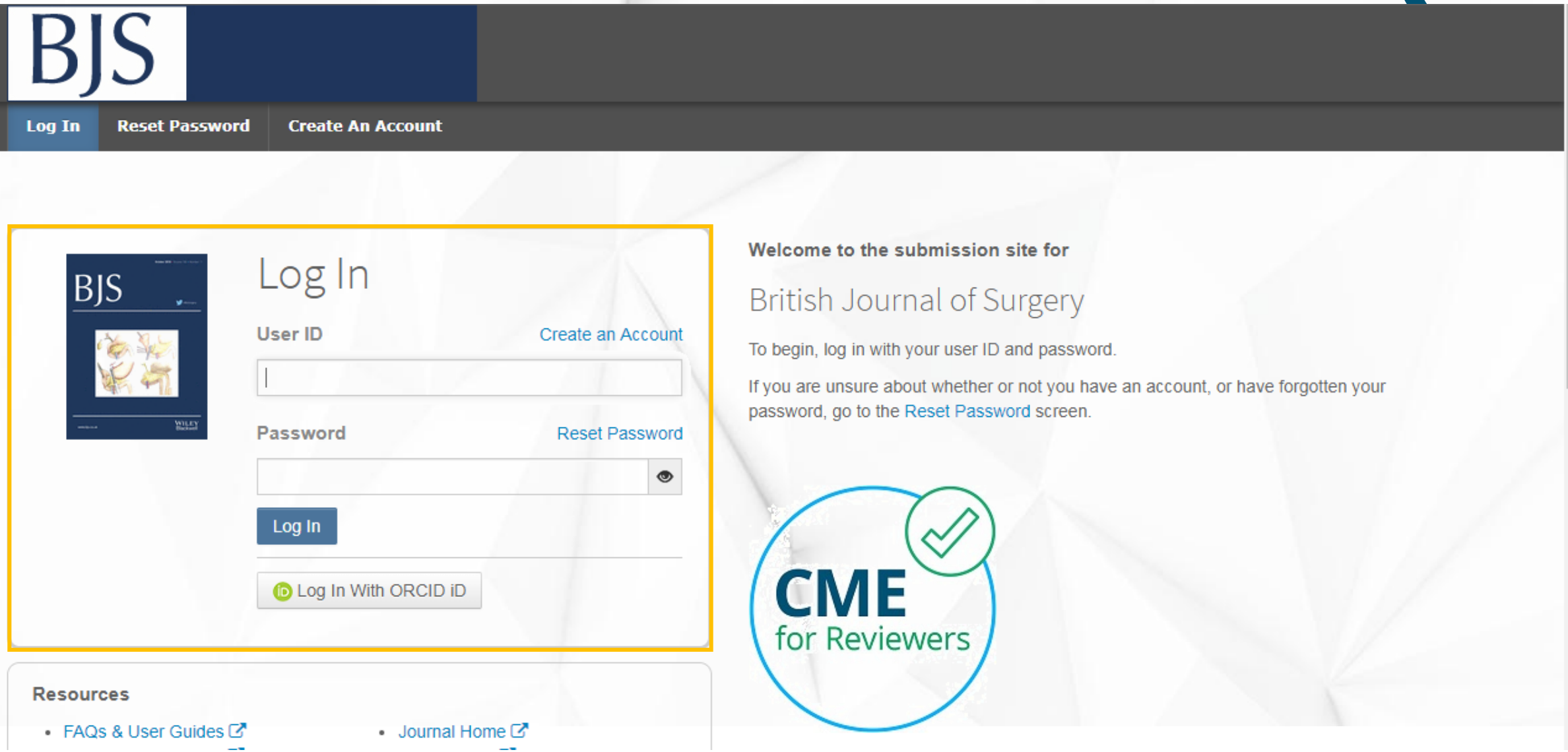

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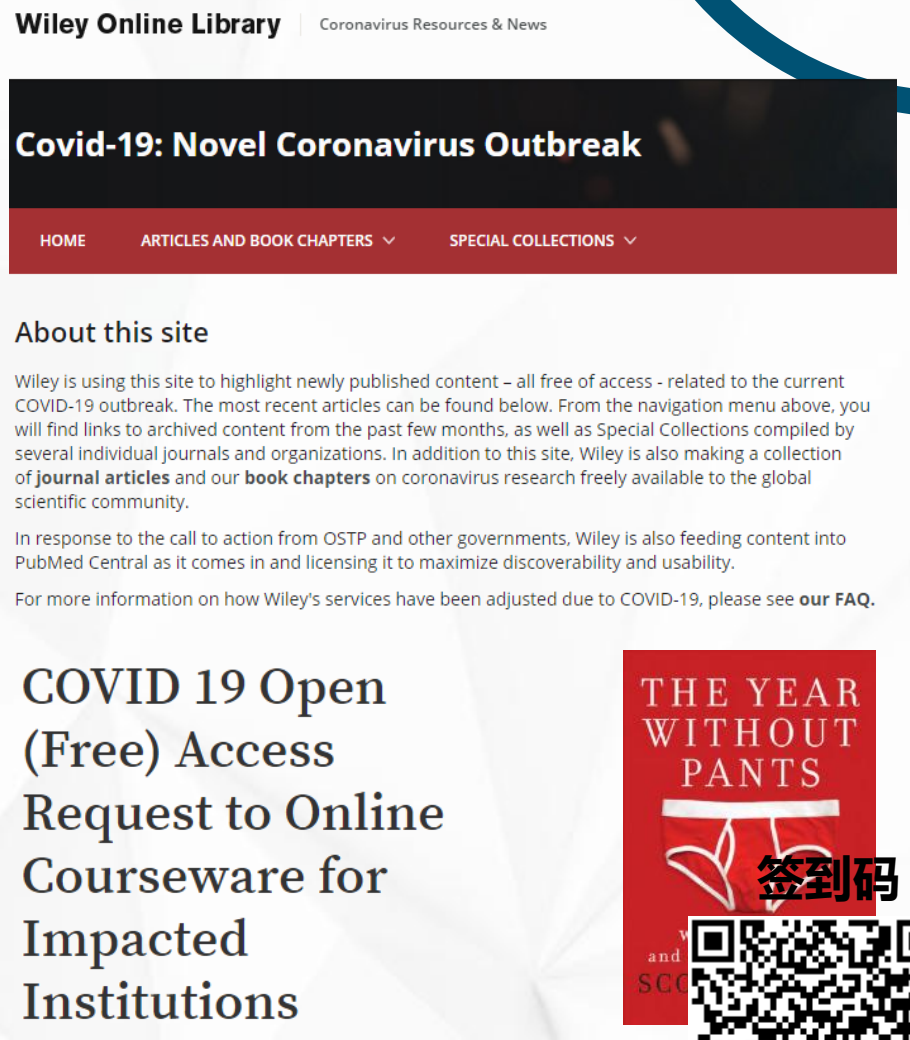

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