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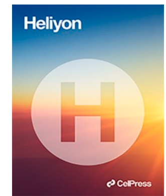

Genomic characterization and seed transmission of a novel unclassified partitivirus infecting *Polygonatum kingianum* Coll. et Hemsl

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ABSTRACT

This study identified a novel virus in the family *Partitiviridae* infecting *Polygonatum kingianum* Coll. et Hemsl, which is tentatively named polygonatum kingianum cryptic virus 1 (PKCV1). PKCV1 genome has two RNA segments: dsRNA1 (1926 bp) has an open reading frame (ORF) encoding an RNA-dependent RNA polymerase (RdRp) of 581 amino acids (aa), and dsRNA2 (1721 bp) has an ORF encoding a capsid protein (CP) of 495 aa. The RdRp of PKCV1 shares 20.70–82.50% aa identity with known partitiviruses, and the CP of PKCV1 shares 10.70–70.80% aa identity with known partitiviruses. Moreover, PKCV1 phylogenetically clustered with unclassified members of the *Partitiviridae* family. Additionally, PKCV1 is common in *P. kingianum* planting regions and has a high infection rate in *P. kingianum* seeds.

1. Introduction

Polygonatum kingianum Coll. et Hemsl, belonging to the family *Liliaceae*, is a medicinal plant with a planting area exceeding 11,000 hm^2 in Yunnan Province, China [1,2]. However, viral diseases have seriously affected *P. kingianum* in recent years. The viruses, include bean common mosaic virus (BCMV) [3], paris mosaic necrosis virus (PMNV) [4], and polygonatum kingianum virus 1 (PKgV1) [5], all of which belong to genus *Potyvirus* of the family *Potyviridae*. Virus-infected *P. kingianum* shows leaf mosaic, chlorosis, shrinkage, overall dwarfism as well as other symptoms that cause considerable yield and quality loss in *P. kingianum* [4].

Family *Partitiviridae* has 45 species covering five genera: *Alphapartitivirus*, *Betapartitivirus*, *Cryspovirus*, *Deltapartitivirus,* and *Gammapartitivirus*. An additional 15 species have been assigned to unclassified partitiviruses [6]. Members of the family *Partitiviridae* are isometric, 25–43 nm in diameter, and non-enveloped [6], and have two double-stranded RNA (dsRNA), separately encapsulated

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genomic segments [7]. DsRNA segment 1 is approximately 1.5–2.5 kbp and encodes RNA-dependent RNA polymerases (RdRp), while dsRNA segment 2 is approximately 1.2–2.4 kbp and encodes capsid protein (CP) [6]. Viruses in this family infect plants [8], fungi [9], or protozoa [10]. Plant-infecting partitiviruses (cryptic viruses) [11], are persistently and efficiently transmitted via seeds despite their lack of movement protein [8,11–14]. However, their transmission through vectors is unclear [6]. A single partitivirus -infection usually causes nonvisible symptoms in plants [15,16], but co-infection with other viruses commonly causes crop yield loss [17,18].

Therefore, this study identified a novel partitivirus, tentatively named polygonatum kingianum cryptic virus 1 (PKCV1). Through next-generation sequencing (NGS), RT-PCR and RACE, the complete genome sequence of PKCV1 was determined. Sequence analyses clarified PKCV1 taxonomic and phylogenetic status. Seed detection of PKCV1 confirmed the high infection rate of PKCV1 in *P. kingianum* seeds.

2. Materials and methods

2.1. Sample collection for next-generation sequencing (NGS)

During a field investigation in May 2021, a *P. kingianum* sample showed dwarfing, leaf chlorotic and curling, was collected in Shizong, Yunnan Province, China (Fig. S1). This sample was subsequently prepared for NGS sequencing.

2.2. Next-generation sequencing (NGS)

Total RNA was extracted using the MagMAX Plant RNA Isolation Kit (Thermo Fisher Scientific, WA, USA), and rRNA was removed using the Ribo-Zero Plant Kit (Illumina, CA, USA), cDNA was obtained through reverse transcription. Sequencing was performed using the X Ten HiSeq Illumina sequencing platform (Illumina, CA, USA) [19]. *De novo* sequence assembly was performed using SPAdes, and the assembled sequences were annotated using the BlastN and BlastX tools of NCBI.

2.3. RT-PCR, RACE and sequencing

Several RT-PCR primers were designed to amplify PKCV1 and verify the correctness of the *de novo* assembled viral sequence (Table S1). The Phanta Max Super-Fidelity DNA Polymerase (Vazyme Biotech Co.,Ltd., Nanjing, China) was used to amplify the target sequence. The PCR conditions involved: pre-denaturation at 95 °C for 3 min, denaturation at 95 °C for 15s, annealing at 54 °C for 15 s, extension at 72 °C for 5 s, and final extension at 72 °C for 5 min. The purified DNA fragment was attached to the pMD18-T vector and transferred to *Escherichia coli* DH5α competent cells by thermal excitation. The positive colonies were sent to Beijing Qingke Biotechnology Co., Ltd. (Kunming, China) for Sanger sequencing. Additionally, the SMARTer RACE 5'/3′ Kit (TaKaRa, Dalian, China) was used to amplify the 5′- and 3′- terminal sequences of the PKCV1 using 5′- and 3′- RACE specific primers (Table S1) following the manufacturer's instructions.

2.4. Sequence analysis

The DNAMAN (5.0) tool was used to analyze ORFs. Multi-sequence alignments were performed using MegAlign (Lasergene7.1). Phylogenetic trees were conducted by MEGA (7.0) based on the RdRp and CP amino acid sequences of partitiviruses. MEME (https://meme-suite.org/meme/tools/meme) [20] was used to analyze the amino acid motifs of partitiviruses.

2.5. PKCV1 detection from field samples

To investigate incidence rate of PKCV1 in this *P. kingianum* planting area, total 30 *P. kingianum* samples, including 16 symptomatic and 14 asymptomatic samples (Table S4), were collected from Shizong, Yunnan Province following the five-point sampling method [26]. Specific detection primers CP-F/R (Table S1) were used to amplify the partial CP gene of PKCV1 (1187bp), and all amplicons were verified by Sanger sequencing.

2.6. PKCV1 detection in *P. kingianum* seeds

A total of 16 *P. kingianum* seeds were randomly selected from the PKCV1 investigation area to detect the rate of PKCV1 infection in *P. kingianum* seeds. After manually removing the seed coat from each seed, the seed embryo was used for total RNA extraction and subsequent RT-PCR following the methods of PKCV1 detection. All amplicons were verified by Sanger sequencing.

3. Results

3.1. Viral sequence assembly

The NGS generated 24.72 M raw reads and 24.18 M clean reads with a mean length of 150 nucleotides (nt) after removing low-quality reads (Q30 > 95.45%, N50 = 369 bp). Finally, 55.114 contigs (>500bp) were obtained by *de novo* assembly. The BlastX using the NR databases identified two plant viruses. One of the viruses belongs to the family *Potyviridae*, which has the highest identity

with polygonatum kingianum mottle virus (PKgMV). Another virus belongs to the family *Partitiviridae*, which is a novel partitivirus tentatively named polygonatum kingianum cryptic virus 1 (PKCV1), was investigated further in this study.

The NGS analysis showed that a total of 1035 reads were mapped to the partitivirus dsRNA-1, and 330 reads were mapped to the partitivirus dsRNA-2. The average dsRNA-1 and dsRNA-2 sequencing depths were 160.74 and 57.26, with 95.12% and 98.65% 10 × coverage and 88.55% and 98.18% 30 × coverage, respectively. Besides, the contig NODE_3332 with 1911 nt was mapped to the complete sequence of partitivirus dsRNA1. Another contig, NODE_4759 with 1699 nt, was mapped to complete the sequence of partitivirus dsRNA2. RACE was conducted to amplify the 5′ and 3′ sequences of partitivirus and RT-PCR to amplify two target fragments corresponding to partitivirus dsRNA1 and dsRNA2 from the *P. kingianum* sample (Fig. S2A). Finally, the complete sequence of PKCV1 was assembled, and full-length dsRNA-1 and dsRNA-2 sequence were deposited in GenBank with the accession numbers OM807072 and OM807071.

3.2. Sequence analysis

The whole genome of PKCV1 is structurally similar to other partitiviruses, having two dsRNA segments, dsRNA-1 and dsRNA-2. DsRNA-1 has 1926 nt and encodes an RdRp with 67.9 kDa. The untranslated regions (UTRs) of dsRNA-1 contain 67 and 117 nt at the 5′- and 3′- terminal ends, respectively. Furthermore, dsRNA-2, has 1721 nt and encoded a CP protein of 54.6 kDa. The UTRs of dsRNA-2 were 88 nt and 145 nt at the 5′- and 3′- terminal regions, respectively (Fig. 1A). The 5′-UTR alignment showed that dsRNA-1 and dsRNA-2 of PKCV1 share highly conserved sequences (TAGTTTTCAAAAAA, TTAAAAACTT) in the 5′-UTR (Fig. 1B). The sequence conservation is typical in the *Partitivirus* genome structure, although the common 5′-UTR conserved sequences between dsRNA-1 and dsRNA-2 vary among species [21–23].

Besides, a multi-sequence alignment of PKCV1 with other partitiviruses showed that PKCV1 RdRp amino acid (aa) sequences share 20.70–82.50% identity with other partitiviruses, while PKCV1 CP aa sequences share 10.70–70.80% identity with other partitiviruses (Table S2). The RdRp and CP aa sequences of PKCV1 had the highest identities with unclassified partitivirus panax cryptic virus 3 (82.50 and 70.80%, respectively). Moreover, the RdRp and CP aa sequences of PKCV1 clustered with other unclassified partitiviruses (Fig. 2A and B).

A total of eight conserved motifs between PKCV1 and other selected partitiviruses were found in the RdRp aa sequence of partitiviruses (Fig. S3). One motif, RdRp-I, is highly conserved among unclassified partitiviruses (Fig. 3). Additionally, eight distinct conserved motifs were found in CP aa sequences of partitiviruses (Fig. S3). Seven motifs, including motif CP-I and CP-III ~ CP-VIII, are only present in the unclassified partitivirus. Another motif, CP-II, was conserved between unclassified partitiviruses and two alphapartitiviruses, white clover cryptic virus 1 (WCCV1) and beet cryptic virus 1 (BCV1) (Fig. 3).

3.3. PKCV1 detection in field and P. kingianum seeds

Briefly, 28 of the 30 *P. kingianum* field samples were positive for PKCV1 by RT-PCR, with a 93.3% incidence rate of PKCV1 on *P. kingianum* in investigation area (Fig. S2B). Moreover, 46.4% of the PKCV1-positive samples were asymptomatic, while 53.6% showed viral disease symptoms, including yellowing, mosaics, and mottling. In addition, the PKCV1 and PKgMV co-infection rate was 93.3% in this field (Table S4). The seed detection analysis showed that 15 of the 16 analyzed *P. kingianum* seeds had PKCV1, translating to 93.8% PKCV1 infection in *P. kingianum* seeds (Fig. S2C).

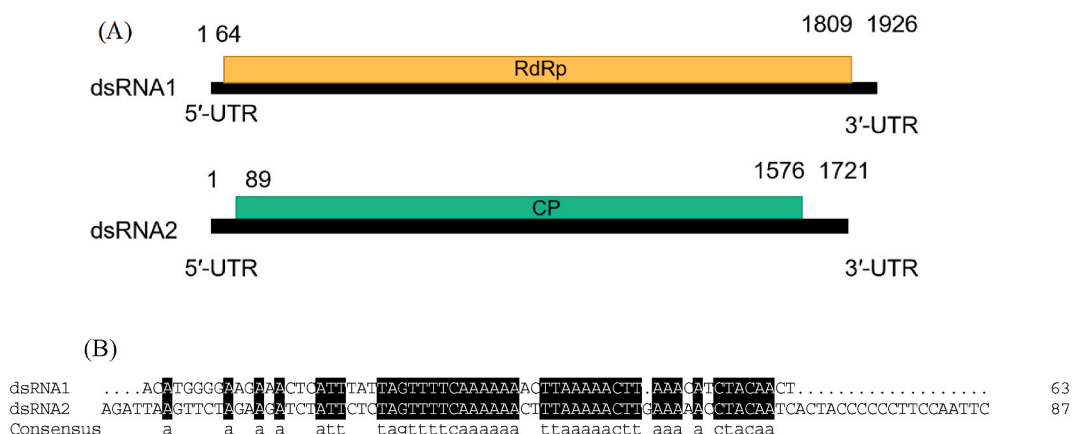

Fig. 1. Genomic characterization of polygonatum kingianum cryptic virus 1 (PKCV1). (A) Schematic diagram of the PKCV1 genome. The orange and green boxes indicate putative ORFs in dsRNA1 (RdRp) and dsRNA2 (CP). (B) 5′-UTRs sequence of the coding strands of the two PKCV1 genomic segments. Black colors indicate conserved sequences. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

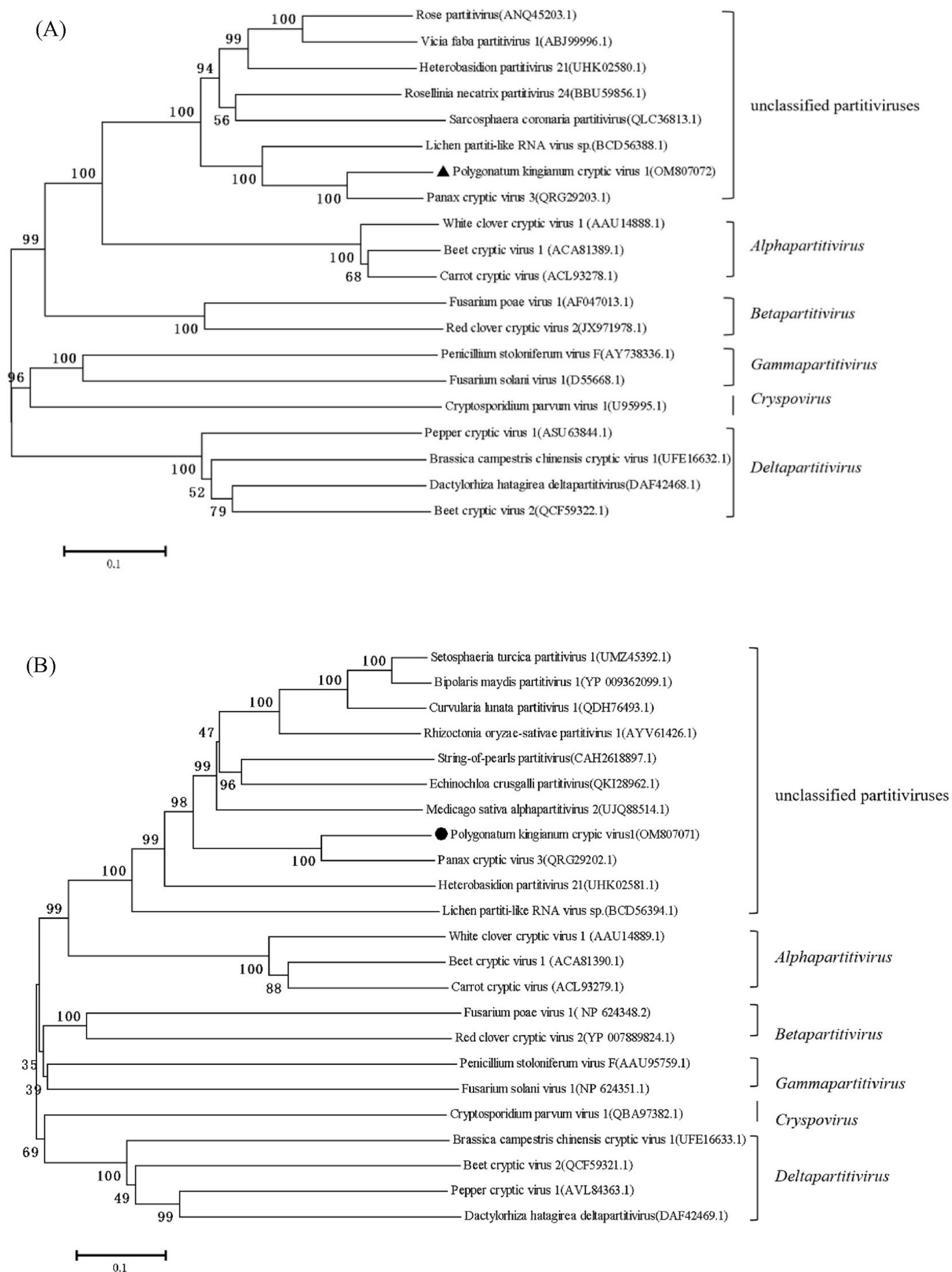

Fig. 2. Phylogenetic trees of partitiviruses based on RdRp and CP amino acid (aa) sequences. (A) Phylogenetic tree of partitiviruses based on RdRp aa sequences; (B) Phylogenetic tree of partitiviruses based on CP aa sequences. Neighbor-joining (NJ) tree were generated in MEGA 7 with 1000 bootstraps. The scale bar represents 0.1 substitutions per site.

4. Discussion

According to the ICTV classification criteria for the family *Partitiviridae*, the genome structure and protein lengths of the five genera have been clearly defined [6]. The phylogenetic results indicated that PKCV1 is most closely related to unclassified partitiviruses but does not belong to any genus in the family *Partitiviridae*. PKCV1 had the highest aa sequence identity (82.50% RdRp and 70.80% CP)

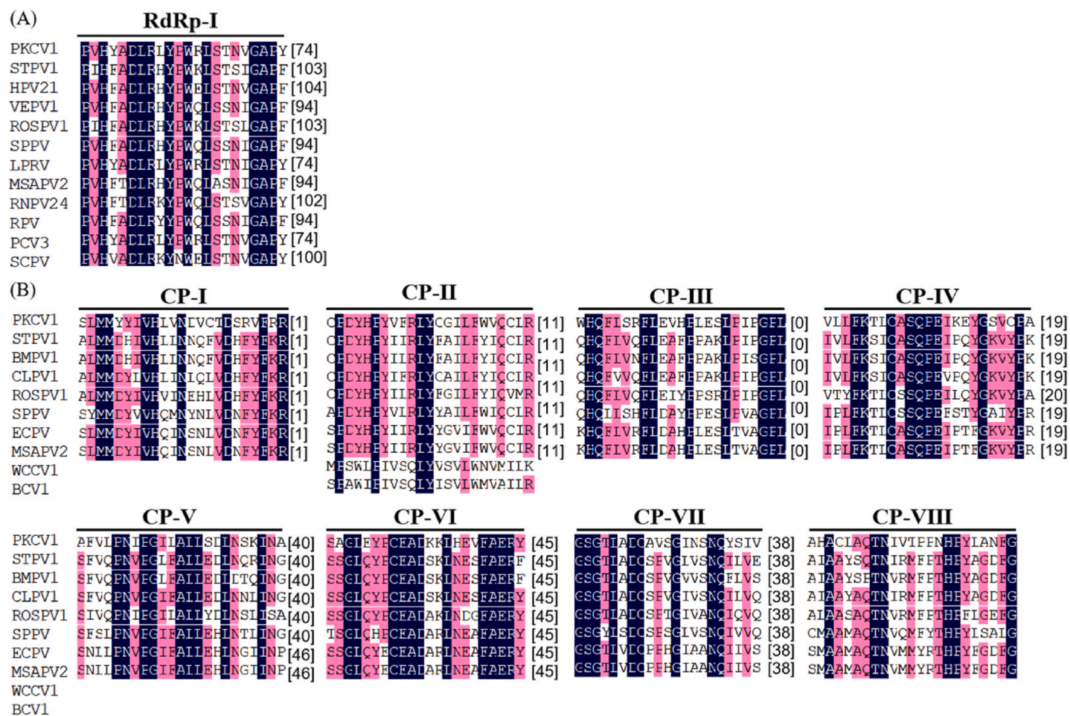

Fig. 3. Conserved motifs (RdRp and CP) between PKCV1 and selected partitiviruses. (A) Conserved motifs of RdRp in unclassified partitiviruses; (B) Conserved motifs of CP in unclassified partitiviruses and *Alphapartitivirus*. The shaded black and pink areas indicate conserved and semi-conserved amino acid residues, respectively. The numbers in square brackets represent to the number of aminc acid residues separating the motifs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

with panax cryptic virus 3. These values are well below the current demarcation criteria for a new species in the genus *Alphapartitivirus* (≤90 and ≤ 80% for aa identities for RdRp and CP, respectively) (https://ictv.global/report/chapter/partitiviridae/partitiviridae). Therefore, we suggest that the virus infecting *P. kingianum* is a new species and named polygonatum kingianum cryptic virus 1 (PKCV1).

Plant-infecting partitiviruses are vertically transmitted by seed [11,14]. The field investigation and seed detection results from this study showed high PKCV1 incidence in the field and a high infection rate in the *P. kingianum* seeds. At present, *P. kingianum* seedlings breeding mainly depends on seeds, indicating the risk of spreading PKCV1 by seeds. Moreover, the high rate of PKCV1 and PKgMV co-infection further underscores the need to determine the epidemic potential and pathological effects of co-infection on *P. kingianum*.

In general, the current research on partitiviruses in host plants is still rather limited. Some research has shown that partitiviruses usually co-infect hosts with other viruses, resulting in crop yield loss, but the interactions between viruses remain unclear [17,18]. Some reports show that partitiviruses has positive effects on the host. For example, the bed crypto viruses (BCV) of the family *Partitiviridae* increases the resistance of host plants to environmental stress [27]. The white clover cryptic partitivirus-1 (WCCV-1), a member of the family *Partitiviridae*, inhibits root nodule formation and improves host plant growth [28]. Understanding the biology of partitivirus infections is a clear scientific interest that will improve future breeding and management of cultivated crops [29].

5. Conclusions

High-throughput sequencing, RACE, and RT-PCR identified a novel partitivirus, tentatively named polygonatum kingianum cryptic virus 1 (PKCV1), infecting *P. kingianum*. A multi-sequence alignment showed that the RdRp of PKCV1 shares 20.7–82.50% aa identity with known partitiviruses and the CP of PKCV1 shares 10.7–70.80% aa identity with known partitiviruses. PKCV1 shares the highest identity with panax cryptic virus 3, an unclassified partitivirus, and PKCV1 is phylogenetically clustered to a clade of unclassified partitiviruses. We propose that the new taxon (PKCV1) is an unclassified partitivirus, following the classification criteria of ICTV [6].

Author contribution statement

Kuanyu Zheng: Conceived and designed the experiments; Wrote the paper.
Zhongkai Zhang: Conceived and designed the experiments; Wrote the paper.
Fan Zhang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Maosen Wang: Analyzed and interpreted the data.

Xiaoxia Su: Contributed reagents, materials, analysis tools or data.
Shaozhi Zhang: Contributed reagents, materials, analysis tools or data.
Tiantian Wang: Contributed reagents, materials, analysis tools or data.
Xue Zheng:Contributed reagents, materials, analysis tools or data.
Kuo Wu: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16719.

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