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Archaeogenetic Study of Prehistoric Rice Remains from Thailand and India: Evidence of Early Japonica in South and Southeast Asia

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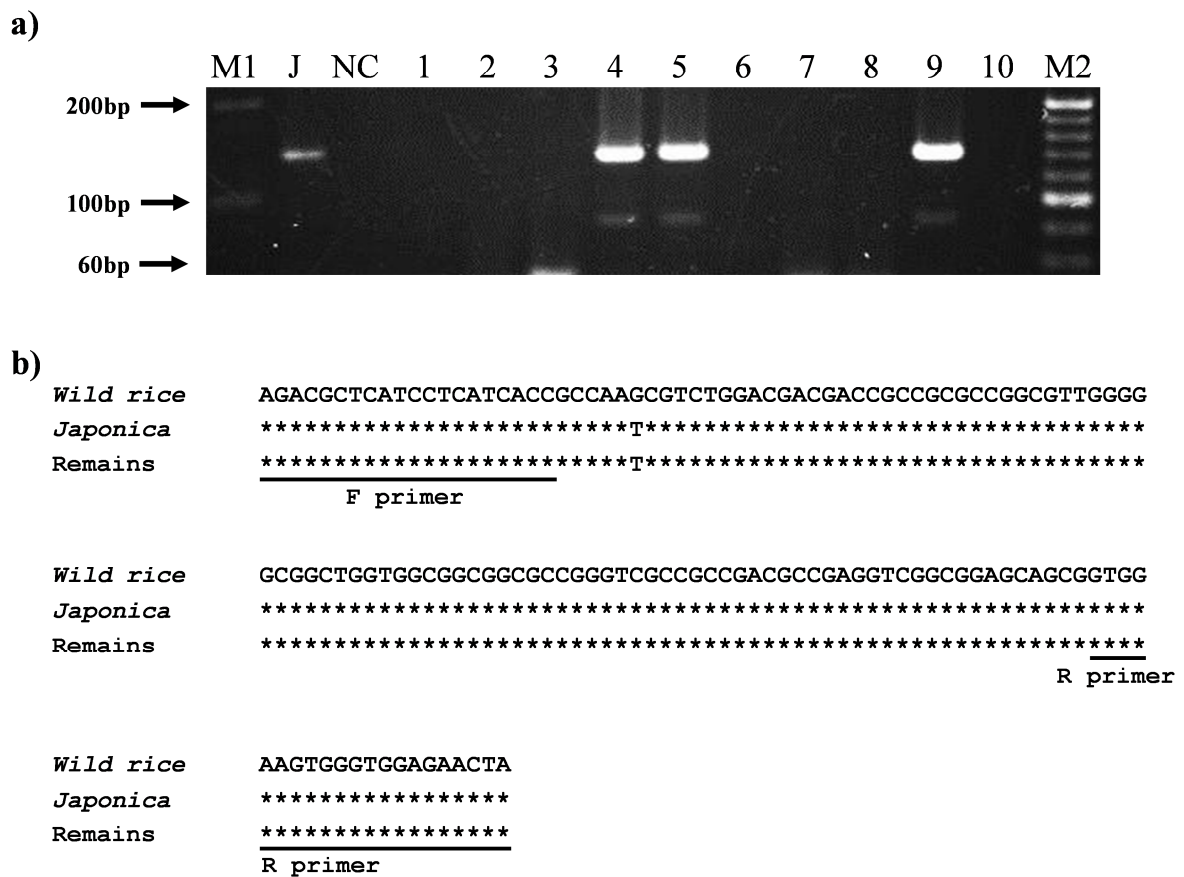
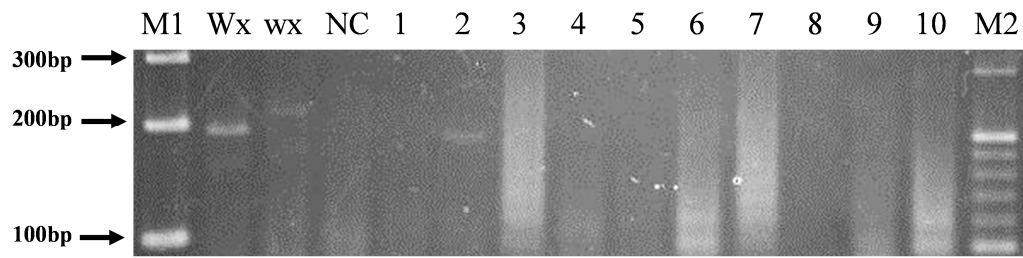


Figure S1: PCR amplification for the nuclear genome region *Sh4*; and the sequence analysis. **(a)** Electrophoresis of 10 PCR products amplified with specific primer sets, F2 and R2 primers. M1: 100bp DNA Ladder (TaKaRa, Japan), M2: 20bp DNA Ladder (TaKaRa, Japan), J: Modern *japonica* cv. 'Nipponbare', NC: negative control (dH₂O), 1-10: represent remains from Ban Non Wat V200 7:Σ3 Δ2 Samples 11-20. **(b)** Sequence detected in wild rice, *japonica* and archaeological remains from Ban Non Wat V200 7:Σ3 Δ2. *Wild rice*: Modern *O. rufipogon* (EU999926); *Japonica*: Modern *japonica* cv. 'Nipponbare' (NC_008397); Remains: from Ban Non Wat V200 7:Σ3 Δ2 represent 14, 15 and 19. "*" shows identical nucleotide sequencing to *Wild rice*.

a)

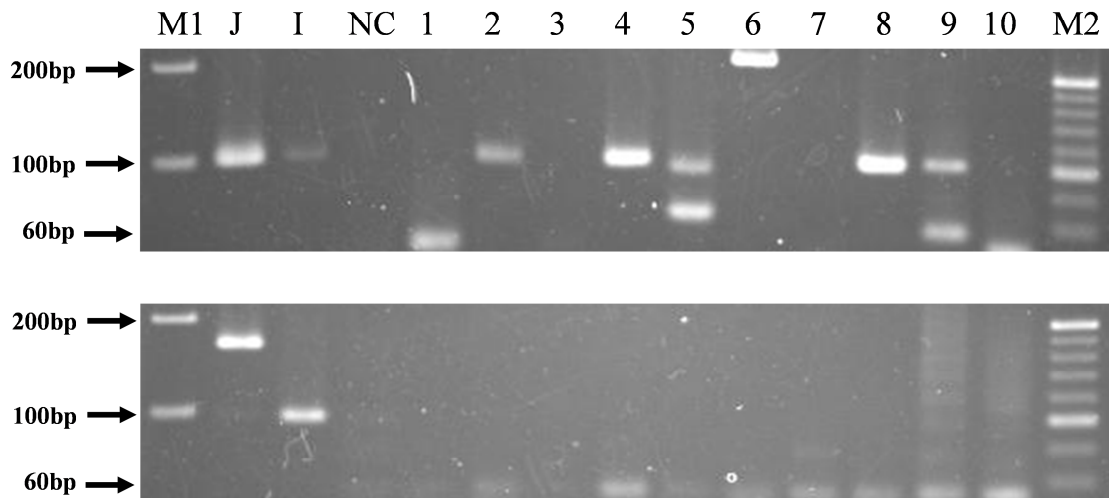


b)

Non-glutinous rice	TGCAGAGATCTTCCACAGCAACAGCTAGACAACCACCATG ~ (70 bp)~ CGTCGCTGCT
Glutinous rice	***** ~ (70 bp)~ *****
Remain	***** ~ (70 bp)~ *****
	<u>23F primer</u>
Non-glutinous rice	CGCCACGGGTTCAGGGCCTCAAGCCC-----CGCAGCCCC
Glutinous rice	*****ACGGGTTCAGGGCCTCAAGCCC*****
Remain	*****-----*****
Non-glutinous rice	GCAGCCCCGCCGGCGCGACGCGACGTCGCTCAGCGTGACGACCAGC
Glutinous rice	*****
Remain	*****
	<u>23R primer</u>

Figure S3: PCR amplification for the nuclear genome region *Waxy*; and the sequence analysis. **(a)** Electrophoresis of 10 PCR products amplified with specific primer sets, F and R primers. M1: 100bp DNA Ladder (TaKaRa, Japan), M2: 20bp DNA Ladder (TaKaRa, Japan), *Wx*: Modern *japonica* cv. 'Nipponbare', *wx*: Modern *japonica* glutinous cv. accession no. DQ280635, NC: negative control (dH₂O), 1-10: represent remains from Ban Non Wat K500 4:2 GEN Δ Samples 1-10. **(b)** Sequence detected in non-glutinous rice, glutinous rice and archaeological remains from Ban Non Wat K500 4:2 GEN Δ. *Non-glutinous rice*: Modern *japonica* cv. 'Nipponbare' (NC_008399); *Glutinous rice*: accession no. DQ280635; Remains: from Ban Non Wat K500 4:2 GEN Δ represent 12. "-" in the sequence symbolises a gap introduced to optimise alignment, whereas "*" shows identical nucleotide sequencing to *non-glutinous rice*.

a)



b)

```

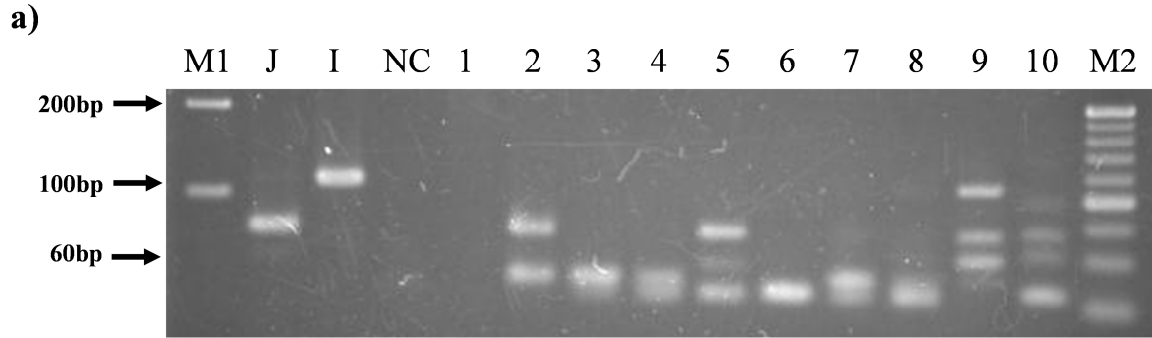
Indica      TGGATTTTCGAAAGTCAATTTTTCTTTCAATATCTTTACTTTTTTTCA-----
Japonica   *****GAATCCTATTTT
Remains    *****GAATCCTATTTT
           _____
           F1 primer

Indica      -----TTT
Japonica   TGTTC TTATACCCATGCAATAGAGAGCGAGTGGGAAAAGGGAGGTTACTTTTTTTCA***
Remains    TGTTC TTATACCCATGCAATAGAGAGCGAGTGGGAAAAGG
           _____
           R1 primer

Indica      TTCCCTTAAAAAATAGGCTTTCTTGCAAATAGGAATCATGGA
Japonica   *****
Remains    *****
           _____
           R2 primer

```

Figure S5: Examples of PCR amplification for the chloroplast genome region *Orf100* and the sequence analysis. **(a)** Electrophoresis of 10 PCR products amplified with F and R1 primer set (upper image) and F and R2 primer set (lower). M1: 100bp DNA Ladder (TaKaRa, Japan), M2: 20bp DNA Ladder (TaKaRa, Japan), J: Modern *japonica* cv. ‘Nipponbare’, I: Modern *indica* cv. ‘IR36’, NC: negative control (dH₂O), 1-10: represent remains from Noen U-Loke #105 Samples 1-10. **(b)** Sequence detected in *indica*, *japonica* and archaeological remains from Noen U-Loke. *Indica*: Modern *indica* (NC_008155); *Japonica*: Modern *japonica* cv. ‘Nipponbare’ (NC_001320); Remains: from Noen U-Loke represent samples 2,4,5,8 and 9. “-” in the sequence symbolises a gap introduced to optimise alignment, whereas “*” shows identical sequencing to *indica*.



b)

```

Indica          ATCAGTTCAAAGAATTTACTCTTAACAAATTCTTAGAGTATTTCTGGTAGAATTTAACAA
Japonica      *****-----
Remains          *****-----
                _____
                F2 primer

Indica          ATTCTTAGAGTATTTCTGGTAGAATTGGGGAGCATTAAGTATAAATA
Japonica      -----*****
Remains          -----*****
                _____
                R2 primer

```

Figure S6: Examples of PCR amplification for the chloroplast genome region *PetN-TrnC* and the sequence analysis, indicating presence of deletion characteristic of *japonica* rice in archaeological specimens. **(a)** Electrophoresis of 10 PCR products amplified with specific primer set, F and R primers. M1: 100bp DNA Ladder (TaKaRa, Japan), M2: 20bp DNA Ladder (TaKaRa, Japan), J: Modern *japonica* cv. 'Nipponbare', I: Modern *indica* cv. 'IR36', NC: negative control (dH₂O), 1-10: represent remains from Noen U-Loke #105 Samples 1-10. **(b)** Sequence detected in *indica*, *japonica* and archaeological remains from Noen U-Loke. *Indica*: Modern *indica* (NC_008155); *Japonica*: Modern *japonica* cv. 'Nipponbare' (NC_001320); Remains: from Noen U-Loke represent 2,5,9 and 10. "-" in the sequence symbolises a gap introduced to optimise alignment, whereas "*" shows identical sequencing to *indica*.

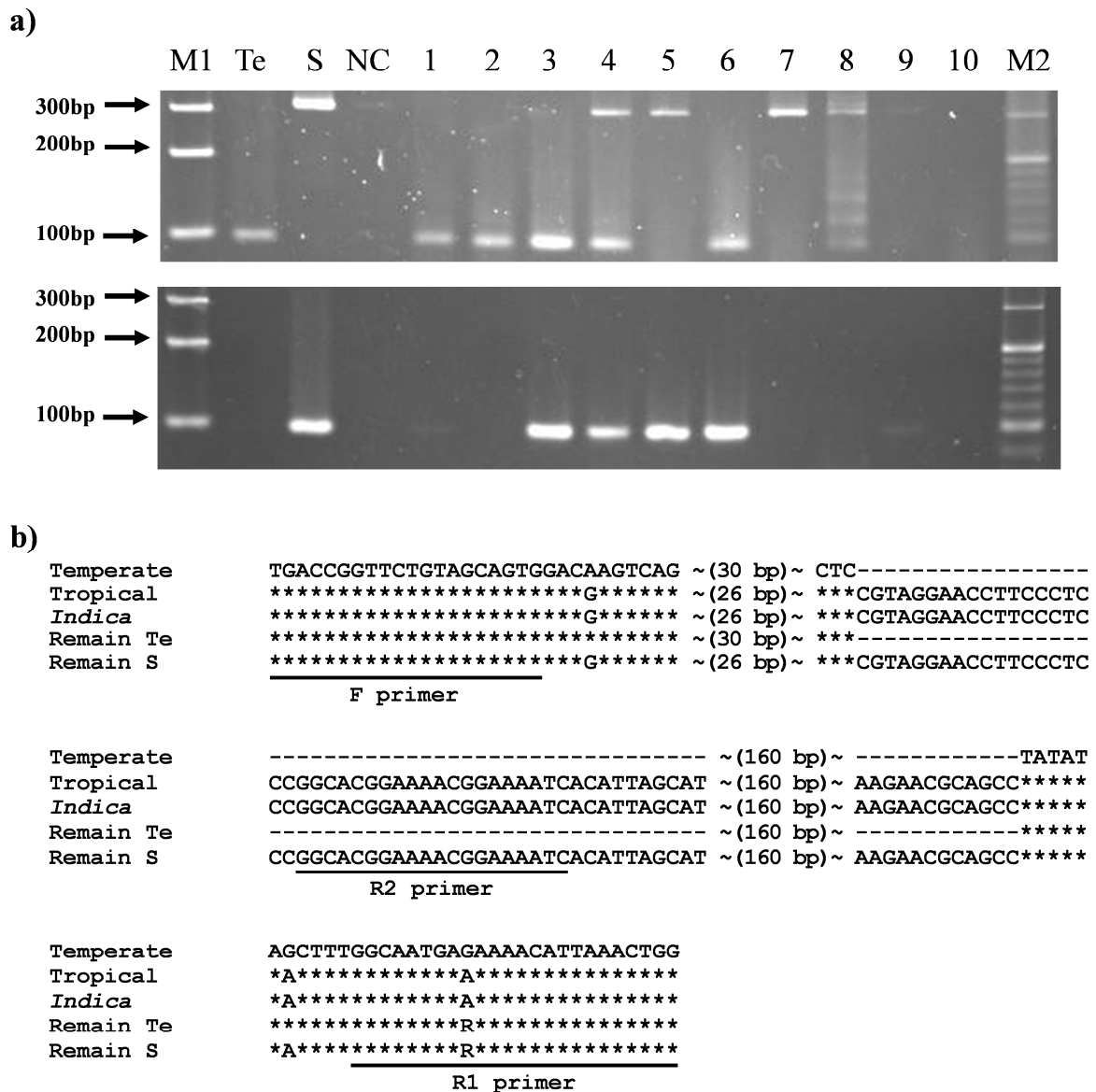


Figure S8: Polymorphism in a non-coding region in chromosome 6 (Ch6); and the sequence analysis. **(a)** Electrophoresis of PCR products amplified with specific primer sets, Ch6 F and R1 primers (upper) and Ch6 and R2 primers (lower). M1: 100bp DNA Ladder (TaKaRa, Japan), M2: 20bp DNA Ladder (TaKaRa, Japan), Te: Modern temperate *japonica* cv. 'Nipponbare', S: Modern tropical *japonica* cv. 'AC220', NC: negative control (dH₂O), 1-10: represent remains from Ban Non Wat K500 4:2 GEN Δ Sample 11-20. **(b)** Sequence detected in temperate *japonica*, tropical *japonica* and archaeological remains from Ban Non Wat K500 4:2 GEN Δ. *Temperate*: Modern *japonica* cv. 'Nipponbare' (NC_008399); *Tropical*: Modern *japonica* cv. 'AC220'; *Indica*: Modern *indica* cv. 'IR36'; *Remain Te*: from Ban Non Wat K500 4:2 GEN Δ represent samples 11, 12, 13, 14 and 16; *Remain S*: from Ban Non Wat K500 4:2 GEN Δ represent samples 14, 17, 18 and 19. "-" in the sequence symbolises a gap introduced to optimise alignment, whereas "*" shows identical nucleotide sequencing to *Temperate*.

