



Genetic Diversity and Natural Selection in 42 kDa Region of *Plasmodium vivax* Merozoite Surface Protein-1 from China-Myanmar Endemic Border

Xia Zhou^{1,2}, Ernest Tambo^{2,3}, Jing Su⁴, Qiang Fang⁵, Wei Ruan⁶, Jun-Hu Chen^{2,*}, Ming-Bo Yin^{4,*},
Xiao-Nong Zhou^{2,*}

¹Medical College of Soochow University, Suzhou 215123, P. R. China; ²National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, WHO Collaborating Centre for Tropical Diseases, National Center for International Research on Tropical Diseases, Key Laboratory of Parasite and Vector Biology, Ministry of Health, Shanghai 200025, P. R. China; ³Department of Biochemistry, Higher Institute of Health Sciences, Université des Montagnes, Bangangté BP208, Cameroon; ⁴School of Life Science, Fudan University, Shanghai 200438, P. R. China; ⁵Department of Microbiology and Parasitology, Bengbu Medical College, Bengbu 233030, P. R. China; ⁶Department of Parasitic Disease Control and Prevention, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou 310051, P. R. China

Abstract: *Plasmodium vivax* merozoite surface protein-1 (PvMSP1) gene codes for a major malaria vaccine candidate antigen. However, its polymorphic nature represents an obstacle to the design of a protective vaccine. In this study, we analyzed the genetic polymorphism and natural selection of the C-terminal 42 kDa fragment within PvMSP1 gene (PvMSP1₄₂) from 77 *P. vivax* isolates, collected from imported cases of China-Myanmar border (CMB) areas in Yunnan province and the inland cases from Anhui, Yunnan, and Zhejiang province in China during 2009-2012. Totally, 41 haplotypes were identified and 30 of them were new haplotypes. The differences between the rates of non-synonymous and synonymous mutations suggest that PvMSP1₄₂ has evolved under natural selection, and a high selective pressure preferentially acted on regions identified of PvMSP1₃₃. Our results also demonstrated that PvMSP1₄₂ of *P. vivax* isolates collected on China-Myanmar border areas display higher genetic polymorphisms than those collected from inland of China. Such results have significant implications for understanding the dynamic of the *P. vivax* population and may be useful information towards China malaria elimination campaign strategies.

Key words: *Plasmodium vivax*, merozoite surface protein-1, genetic polymorphism, natural selection, Myanmar, China

INTRODUCTION

Malaria is a major infectious disease in the Greater Mekong Subregion (GMS) in Asia. Although there has been a considerable decrease in the incidence of malaria in China [1], Yunnan Province still has the highest transmission area of vivax malaria in China, particularly in the southern border areas adjacent to Myanmar. *Plasmodium vivax* is also the most widely distributed species of all 5 human malaria parasites in Southeast Asia and accounts for 65% of malaria cases in Asia and South America [2]. More attention is being focused on malaria today than any time since the world's last efforts to achieve eradica-

tion over 40 years ago. The global community is now discussing strategies aimed at dramatically reducing malarial disease burden and the eventual eradication of all types of malaria everywhere. As a consequence, *P. vivax*, which has long been neglected and mistakenly considered benign, is now entering into the strategic debates taking place on malaria epidemiology and control, drug resistance, pathogenesis, and vaccines. Thus, contrary to the past, the malaria research community is becoming more aware and concerned about the widespread spectrum of illness and death caused by up to a couple of hundred million cases of vivax malaria each year [3].

Taking account of the facts above, availability of *P. vivax* malaria vaccine is highly desirable. Advanced studies on genetic diversity of the most variable domain of vaccine candidate *P. vivax* merozoite surface proteins (PvMSPs) in field isolates of different countries have been carried on and demonstrated that the diversity of MSPs in *P. vivax* is presumed be associated to parasite immune evasion and be important for the rationale

• Received 19 March 2017, revised 16 September 2017, accepted 18 September 2017.

* Corresponding authors

(junhuchen@hotmail.com; yinm@fudan.edu.cn; ipdzhouxn@sh163.net)

© 2017, Korean Society for Parasitology and Tropical Medicine

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

of malaria vaccine designs [4,5]. Since the 42 kDa fragment of *Plasmodium* merozoite surface protein-1 (PvMSP1) contains known B- and T-cell cell epitopes, a PvMSP1₄₂ vaccine antigen may be capable of conferring protection mediated by providing antigen-specific T-cell help for B-cells and antibody production [6]. Several previous studies have reported the presence of acquired antibodies against the C-terminus part of the protein called PvMSP1₁₉ or PvMSP1₄₂ antigens among individuals during natural *P. vivax* infections [7,8]. Immunological studies performed on animal models have also proved that the PvMSP1₁₉ or PvMSP1₄₂ is one of the promising vaccine candidates against asexual stages of the malaria [9]. Although genetic polymorphisms in the central repeat region of PvMSP1 has been investigated among other countries in Southeast Asia on *P. vivax* isolates [10], the data is not available for the C-terminus region of this antigen from southern border areas adjacent to Myanmar and the inland cases in China.

The present study aimed to identify the genetic diversity and haplotypes of the gene fragment coding PvMSP1₄₂ in *P. vivax* isolates of malaria cases in China-Myanmar border (CMB) areas and Yunnan, Zhejiang, and Anhui province of inland of China. Moreover, the natural selection of the gene fragment coding PvMSP1₄₂ was tested in 4 *P. vivax* populations from CMB areas and inland of China.

MATERIALS AND METHODS

Ethics statement

This study was conducted according to the principles expressed in the Declaration of Helsinki. Blood collections were made with full informed consent of the patients and following institutional ethical guidelines that were reviewed and approved by the ethics committee at National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention.

Blood samples, DNA extraction, and purification

Blood samples were obtained from 77 symptomatic and microscopically confirmed *P. vivax* malaria patients in China during 2009 to 2012. These examined samples included 59 imported cases of CMB areas based on their traveling history, including 14, 19, 17, and 9 cases each year from 2009 to 2012, respectively, and additional 18 patients from inland China. Among the 18 samples from inland China, 6 of them were from Anhui province collected in 2009, 6 from Zhejiang prov-



Fig. 1. The map of *P. vivax* samples collection. Sample collection areas in this study are indicated in black pentagrams (Anhui, Yunnan, and Zhejiang provinces from inland China) and red dot (China-Myanmar border area, CMB).

ince collected in 2009 and 6 from Yunnan province collected in 2010 (Fig. 1). Annual parasite incidence (API) for Anhui, Zhejiang and Yunnan province was 0.0210, 0.0352, and 0.2186 per 10,000 person-years in 2015 [11]. Because the transmission of malaria had been controlled in a very low level in local China, only limited sporadic cases of malaria inland were collected here. These samples collected from different villages in Yunnan province treated as the inland malaria cases from these febrile patients haven't been abroad within 1 month. All the patients' *P. vivax* infection were diagnosed by microscopic examination of thin and thick blood smears and further confirmed by nested PCR as described previously [12].

Genomic DNA was isolated from 200 μ l of venous blood which collected in a sterile heparinized tube from the patients who were found positive for *P. vivax*. Approximately 100 μ l of blood each patient was used and added 100 μ l PBS to get the final volume of 200 μ l. Then, DNA was extracted from the whole blood by using the QIAamp DNA mini kit (QIAGEN, Shanghai, China), according to the manufacturer's instructions. The purified DNA was dissolved in 150 μ l TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) and stored at -20°C until use.

PCR amplification and analysis of *P. vivax* field isolates

The *P. vivax* fragment (comprising PvMSP1₄₂ kDa amino ac-

ids) of PvMSP1₄₂ was amplified by polymerase chain reaction (PCR). In this study, the specific primer was designed according to the *P. vivax* MSP1 complete gene sequence of PlasmoDB (PVX_099980) [13]. The primers are: Pv1SF (5'-AGAAG AAAAC GTAGC AGCAA-3') and Pv1SR (5'-AAGCC CAGTT CAGTT CAGAA CTCA-3'). PCR reaction volumes were 50 μ l. The cycling parameters for PCR amplification was performed under the following conditions: initial denaturation 5 min at 94°C, 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min 30 sec, followed by a final extension at 72°C for 5 min. PCR mixture reagents contained 1 μ l of DNA, 0.5 units of ExTaq or LA Taq DNA polymerase (Takara, Shiga, Japan), 0.2 mM of each primer, 0.1 mM dNTPs in a 25 μ l of reaction mix with 1.5 mM MgCl₂. The PCR products were examined by electrophoresis in a 1% agarose gel, visualized with an ultraviolet trans-illuminator and purified with PCR purification kits (Qiagen). Then, the purified PCR products were sequenced using the forward primers on an ABI PRISM 3700 DNA capillary sequencer, by BGI Company (Shenzhen, Guangdong, China). All unique mutations were carefully checked, and ambiguous bases were confirmed by resequencing. We also carried out a BLAST search on PlasmoDB Genebank Database of *P. vivax* to compare these successfully sequenced isolates with those previously identified from China and the Asia-pacific subregion. Accurate alignment of the sequences was performed by in ClustalX version 2.0 [14]. The new sequences were deposited in GenBank with accession nos. JX490129-JX490156, JX993754, and JX993755.

Nucleotide diversity and natural selection test of *P. vivax* field isolates

Nucleotide and amino acid sequences were aligned using Clustal W in MEGA 5.0 [15]. First, nucleotide diversity (π) was computed in 100 bp sliding window and 25 bp step size using DnaSP v.5.0 [16]. Then, to detect natural selection acting on these coding sequences, the rates of non-synonymous (dN) to synonymous (dS) substitution (dN/dS) was calculated with DnaSP v.5.0. If the amino acid change is deleterious, purifying selection, then dN/dS < 1; only when the amino acid change offers a selective advantage, the dN/dS is > 1. In addition, Tajima's D was used to test neutrality of this gene fragment in DnaSP v.5.0 [16,17]. A remarkable negative value of Tajima's D reveals an excess of rare variants as expected under positive and negative selection or population size expansion. Whereas,

a significant positive value demonstrates an excess of high-frequency variant as expected under balancing selection or under population structure. Finally, to describe the genetic similarities among PvMSP1₄₂ haplotypes, we constructed networks by the median joining method from 41 unique haplotypes on the basis of PvMSP1₄₂ sequences in Network 4.5 [18].

Phylogenetic analysis of PvMSP1 from *P. vivax* field isolates

The phylogenetic relationships were derived from the PvMSP1₄₂ sequences. In case of individuals that carried an identical sequence (it is possible that individuals from different locations shared the same sequence), only 1 sequence was included for the tree reconstruction. The reference sequences were chosen from GenBank. Then, partitioned Neighbor-Joining method was performed in MEGA 5.0 [15,19] to construct the phylogenetic tree (with Kimura-2 parameter distance, branch support with 1000 bootstrap replicates, and complete deletion of gaps). MSP1₄₂ fragment gene of *P. cynomolgi* strain Berok was set as the out group.

RESULTS

Haplotype variations in *P. vivax* of different field isolates

We successfully amplified and sequenced the gene encoding PvMSP1₄₂ fragment (1,209 bp, corresponding to amino acid positions 1350-1752 in PvMSP1) shown in Supplementary Fig. S1. Of 77 isolates from 4 geographic locations, 41 haplotypes were detected on the entire PvMSP1₄₂ fragment (Table 1). From these 41 haplotypes, 30 were new haplotypes. Only 1 single haplotype was detected among 6 isolates from Anhui, all these samples were collected from Bengbu city and 2 haplotypes from 6 isolates in Zhejiang province. In contrast, other 6 isolates from Yunnan province distributed in different villages are detected in 6 different haplotypes and 35 haplotypes are detected from 59 imported cases of CMB areas.

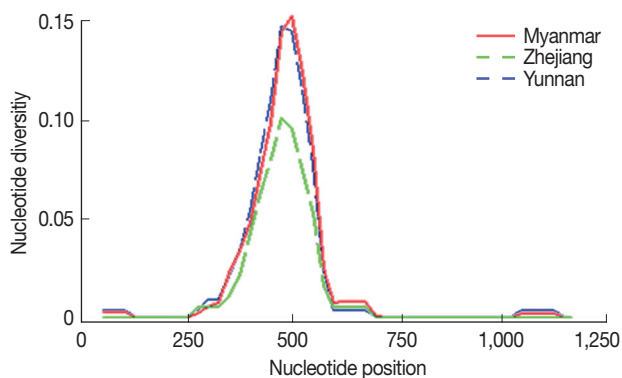
Only 3 haplotypes were detected for PvMSP1₁₉ fragment in comparison with 38 haplotypes for PvMSP1₃₃ fragment in the all sequenced isolates. The consistent pattern was observed in the *P. vivax* populations from CMB areas and inland China (Table 1).

Nucleotide diversity and natural selection of different PvMSP1₄₂ fragment from different *P. vivax* isolates

The overall nucleotide diversity (π) of PvMSP1₄₂ for all of 77

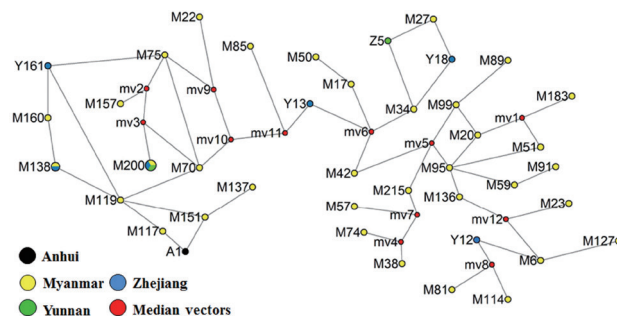
Table 1. Haplotype diversity, nucleotide diversity, and natural selection of *Plasmodium vivax* MSP1₄₂

Fragment	H ^a	π^b	dN ^c	dS ^d	dN/dS	Tajima's D	P-value
42 kDa							
All samples (n = 77)	41	0.01901	0.02188	0.01016	2.15354	2.44824	<0.05
Border areas ^f (n = 59)	35	0.01836	0.02085	0.01082	1.92699	2.01030	>0.05
Inland China (n = 18)	8	0.01317	0.01615	-0.00328	4.92378	0.48590	>0.05
Anhui (n = 6)	1	n.a. ^e	n.a.	n.a.	n.a.	n.a.	n.a.
Yunnan (n = 6)	6	0.01803	0.02234	0.00366	6.10383	0.23699	>0.05
Zhejiang (n = 6)	2	0.01235	0.01546	0.00209	7.39713	1.37681	>0.05
33 kDa							
All samples (n = 77)	38	0.02617	0.03026	0.01401	2.15989	2.57163	<0.05
Border areas (n = 59)	33	0.02523	0.02879	0.01489	1.93351	2.11738	<0.05
Inland China (n = 18)	8	0.01818	0.02237	0.00456	4.90570	0.54855	>0.05
Anhui (n = 6)	1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Yunnan (n = 6)	6	0.02467	0.03067	0.00509	6.02554	0.27447	>0.05
Zhejiang (n = 6)	2	0.01716	0.02159	0.00290	7.44483	1.37681	>0.05
19 kDa							
All samples (n = 77)	3	0.00063	0.00071	0.00037	1.91892	-0.76528	>0.05
Border areas (n = 59)	3	0.00073	0.00081	0.00048	1.68750	-0.73272	>0.05
Inland China (n = 18)	2	0.00033	0.00042	n.a.	n.a.	-1.16467	>0.05
Anhui (n = 6)	1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Yunnan (n = 6)	2	0.00098	0.00126	n.a.	n.a.	-0.93302	>0.05
Zhejiang (n = 6)	1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

^aH: the number of haplotypes.^b π : nucleotide diversity.^cdN: the rates of nonsynonymous substitutions.^ddS: the rates of synonymous substitutions.^en.a.: not applicable.^fBorder areas: China-Myanmar border areas.**Fig. 2.** Nucleotide diversity per site (π) at PvMSP1₄₂ from *P. vivax* isolates collected along the China-Myanmar border areas, local regions of Yunnan and Zhejiang provinces in inland China.

isolates was 0.01901, and π values were 0.01803 and 0.01235 for the isolates from Yunnan and Zhejiang province of inland China, respectively, as well as π value was 0.01836 for the isolates from the CMB areas, with the peak on nucleotide positions from 476 to 525 bp (Fig. 2), which located at the C-terminal 33 kDa fragment within PvMSP1 gene (PvMSP1₃₃) (Supplementary Figs. S2, S3).

The rates of non-synonymous (dN) to synonymous (dS) substitution (dN/dS) of PvMSP1₄₂ for all of 77 isolates was

**Fig. 3.** The network of PvMSP1₄₂ from *P. vivax* isolates collected along the China-Myanmar border areas, local regions of Anhui, Yunnan, and Zhejiang provinces in China.

2.15354, and the rates of dN/dS were 6.10383, 7.39713, and 1.92699 for the isolates from Yunnan, Zhejiang province of inland China and CMB areas, respectively, suggesting a positive selection for PvMSP1₄₂ of *P. vivax* populations from inland China and CMB areas. The overall Tajima's D value of PvMSP1₄₂ was 2.44824 ($P < 0.05$) for all of 77 isolates, and the Tajima's D values were 0.23699, 1.37681, and 2.01030 for the isolates from the Yunnan, Zhejiang province of inland China and CMB areas, respectively, which also indicated balancing selection for PvMSP1₄₂ of *P. vivax* populations from CMB areas.

or different villages in Yunnan province. The phylogenetic analysis revealed that newly identified haplotypes from China were clustered differently. The gene coding PvMSP1₄₂ from field isolates collected in Anhui province has only 1 haplotype and close to these isolates from Myanmar. Two different haplotypes of PvMSP1₄₂ were detected from Zhejiang isolates. One sequence coding PvMSP1₄₂ from Zhejiang province is close to these from Yunnan province and Myanmar, while another one also detected in Zhejiang isolates has been demonstrated to be close to isolates from Singapore and Thailand. All these genes coding PvMSP1₄₂ from Yunnan are from those patients who haven't been abroad within 1 month.

DISCUSSION

The *P. vivax* parasite exhibits higher genetic diversity than *P. falciparum*, especially for the gene families associated with merozoite invasion or immune response modulation (e.g., the *msp3*, *vir*, and *msp7* gene families) [20-22]. The high genetic diversity and natural selection of *P. vivax* vaccine targets is common existed in isolates world-wide [23,24]. The PvMSP1 locus codes for a major asexual blood-stage antigen currently proposed as a malaria vaccine candidate antigen. Reports of extensive polymorphism of this protein from field isolates and clones from different geographical areas remain a major challenge. Numerous studies on the genetic diversity of PvMSP1 in *P. vivax* field isolates have been carried out in many different geographic areas [25,26]. However, there is no available data for PvMSP1₄₂ from southern border areas adjacent to Myanmar and the inland cases in China.

In this study, we present several sets of genetic information for PvMSP1₄₂ of populations from inland China and CMB areas at first time. We found 35 and 8 haplotypes of PvMSP1₄₂ for the isolates from Myanmar and China during 2009-2012, respectively. We also documented varied types of haplotypes characteristic of high genetic diversity in the studied region compared to other endemic regions. This high genetic diversity of PvMSP1₄₂ fragments were consistent with that of *P. vivax* field isolates collected in Cambodia and Thailand [27].

Of the 41 haplotypes, 30 were new haplotypes including 28 of them from Myanmar, characterizing of multiple clonality. The same single haplotype was documented in each of the inland isolates from Anhui compared to those of Myanmar, 2 different haplotypes from isolates from Zhejiang and diverse multiple haplotypes found in Yunnan similar to Myanmar.

This finding indicated that geographical proximity between Myanmar and Yunnan China border which showed that vector dynamic and/or human motility might have been important contributing factors in malaria parasite transmission and degree of endemicity [28]. In recent years, malaria transmission has been controlled in a very low level in Anhui province, China and these cases collected here from Bengbu city were localized sporadic malaria cases [29]. These results are consistent with previous studies that genetic diversity of the malaria parasites has been shown to be associated with the levels of endemicity and transmission intensity.

Genetic diversity analysis revealed that the majority of polymorphic sites were in the 33 kDa portion and significant proportion of the identified polymorphisms occurred probably as result of reported positive selection pressure on this region while 19 kDa regions remained highly conserved. The similar results for the positive selection of PvMSP1₃₃ were reported in the *P. vivax* isolates from India and Sri Lanka several years ago [30,31]. The frequent occurrences of non-synonymous substitutions relative to synonymous ones and high value of Tajima's D indicate the polymorphism of antigen enable parasites to avoid host immune pressure and host immune responses likely play a role in maintaining the polymorphism of *P. vivax* MSP1 alleles.

The haplotype network demonstrated that parasite populations are highly heterogenetic and dynamics of the disease transmission in these endemic areas [32]. PvMSP1 gene codes for a major malaria vaccine candidate antigen. But its polymorphic nature represents an obstacle to the design of a protective vaccine. Present study will be helpful for the development of PvMSP1 based vaccine against *P. vivax* malaria and provide evidence driven knowledge towards development of effective control interventions in Myanmar and appropriate measures in achieving China malaria elimination goals. Noteworthy, Myanmar is one of the major malaria endemic countries in the South-East Asia region, the genetic diversity of the malaria parasite circulating in CMB areas provides additional supportive information. In total, we documented 11 synonymous and 112 non-synonymous haplotypes of which 71.11% and 36.66% previously reported. Of the 11 synonymous polymorphisms, 7 were previously identified. The change might be contributed to evolutionary and/or environmental changes characterized by different patterns compared to natural and geographical studies in the Great Mekong region.

Interestingly, the network analysis of identified haplotypes

of PvMSP1₄₂ showed that most prevalent haplotypes originated from Myanmar followed by Yunnan and Zhejiang provinces of China. This information is vital and indicates that understanding the genetic diversity and network provides insights into parasite strains dynamics in the region, and design of most appropriate programmes and interventions in reducing or blocking the transmission, curbing the spread of parasite as well as containment of increasing resistant strain in the Great Mekong Region.

Phylogenetic tree also showed a substantial degree of variability of the origin of the parasites. Although, all *P. vivax* clinical isolates, originated from the same species but analysis of these isolates showed distinct differences with the high prevalence of isolates from different countries of Myanmar, Thailand, Singapore, Bangladesh, South Korea, India, Vietnam, and Indonesia and different regions in China. Our findings are consistent with high malaria endemicity in Myanmar, where with the long borders proximity, haplotype diversity has been high comparable to the endemicity in vivax population from inland areas of China such as Anhui and Zhejiang province were lower. However, further studies on a larger population from these endemic geographic areas are required not only to determine the nationwide parasite genetic mapping and detailed malaria molecular epidemiology in CMB areas to provide evidence based decision and effective interventions [33,34].

ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Program of China (nos. 2016YFC1202000, 2016YFC1202001, 2016YFC1202003 and 2016YFC1200500), the National Natural Science Foundation of China (no. 81101266), the WHA-WHO Demonstration Project (no. UNOPS/ANDI/G/2016/01), the Foundation of National Science and Technology Major Program (no. 2012ZX10004-220), and the Fourth Round of Three-Year Public Health Action Plan (2015-2017) in Shanghai, P. R. China (no. GWIV-29).

CONFLICT OF INTEREST

We have no conflict of interest related to this work.

REFERENCES

1. Yin JH, Zhou SS, Xia ZG, Wang RB, Qian YJ, Yang WZ, Zhou XN. Historical patterns of malaria transmission in China. *Adv Parasitol* 2014; 86: 1-19.
2. Vogel G. The forgotten malaria. *Science* 2013; 342: 684-687.
3. Mueller I, Galinski MR, Baird JK, Carlton JM, Kochar DK, Alonso PL, del Portillo HA. Key gaps in the knowledge of *Plasmodium vivax*, a neglected human malaria parasite. *Lancet Infect Dis* 2009; 9: 555-566.
4. Wang Y, Ma A, Chen SB, Yang YC, Chen JH, Yin MB. Genetic diversity and natural selection of three blood-stage 6-Cys proteins in *Plasmodium vivax* populations from the China-Myanmar endemic border. *Infect Genet Evol* 2014; 28: 167-174.
5. Kassegne K, Abe EM, Chen JH, Zhou XN. Immunomic approaches for antigen discovery of human parasites. *Expert Rev Proteomics* 2016; 13: 1091-1101.
6. Longley RJ, Sattabongkot J, Mueller I. Insights into the naturally acquired immune response to *Plasmodium vivax* malaria. *Parasitology* 2016; 143: 154-170.
7. Chen JH, Chen SB, Wang Y, Ju C, Zhang T, Xu B, Shen HM, Mo XJ, Molina DM, Eng M, Liang X, Gardner MJ, Wang R, Hu W. An immunomics approach for the analysis of natural antibody responses to *Plasmodium vivax* infection. *Mol Biosyst* 2015; 11: 2354-2363.
8. Chen JH, Jung JW, Wang Y, Ha KS, Lu F, Lim CS, Takeo S, Tsuboi T, Han ET. Immunoproteomics profiling of blood stage *Plasmodium vivax* infection by high-throughput screening assays. *J Proteome Res* 2010; 9: 6479-6489.
9. Dutta S, Kaushal DC, Ware LA, Puri SK, Kaushal NA, Narula A, Upadhyaya DS, Lanar DE. Merozoite surface protein 1 of *Plasmodium vivax* induces a protective response against *Plasmodium cynomolgi* challenge in rhesus monkeys. *Infect Immun* 2005; 73: 5936-5944.
10. Putaporntip C, Jongwutiwes S, Sakihama N, Ferreira MU, Kho WC, Kaneko A, Kanbara H, Hattori T, Tanabe K. Mosaic organization and heterogeneity in frequency of allelic recombination of the *Plasmodium vivax* merozoite surface protein-1 locus. *Proc Natl Acad Sci U S A* 2002; 99: 16348-16353.
11. Zhang L, Zhou SS, Feng J, Fang W, Xia ZG. Malaria situation in the People's Republic of China in 2015. *Chin J Parasitol Parasit Dis* 2015; 34: 477-481 (in Chinese).
12. Zhou X, Huang JL, Njuabe MT, Li SG, Chen JH, Zhou XN. A molecular survey of febrile cases in malaria-endemic areas along China-Myanmar border in Yunnan province, People's Republic of China. *Parasite* 2014; 21: 27.
13. Aurrecochea C, Brestelli J, Brunk BP, Dommer J, Fischer S, Gajria B, Gao X, Gingle A, Grant G, Harb OS, Heiges M, Innamorato F, Iodice J, Kissinger JC, Kraemer E, Li W, Miller JA, Nayak V, Pennington C, Pinney DE, Roos DS, Ross C, Stoeckert CJ Jr, Treatman C, Wang H. PlasmoDB: a functional genomic database for malaria parasites. *Nucleic Acids Res* 2009; 37: 539-543.
14. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. Clustal W and Clustal X version 2.0. *Bioinformatics* 2007; 23: 2947-2948.

1. Yin JH, Zhou SS, Xia ZG, Wang RB, Qian YJ, Yang WZ, Zhou

15. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011; 28: 2731-2739.
16. Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 2003; 19: 2496-2497.
17. Thornton K. Recombination and the properties of Tajima's D in the context of approximate-likelihood calculation. *Genetics* 2005; 171: 2143-2148.
18. Bandelt HJ, Forster P, Röhl A. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 1999; 16: 37-48.
19. Zhang W, Sun Z. Random local neighbor joining: a new method for reconstructing phylogenetic trees. *Mol Phylogenet Evol* 2008; 47: 117-128.
20. Chen SB, Wang Y, Kassegne K, Xu B, Shen HM, Chen JH. Whole-genome sequencing of a *Plasmodium vivax* clinical isolate exhibits geographical characteristics and high genetic variation in China-Myanmar border area. *BMC Genomics* 2017; 18: 131.
21. Neafsey DE, Galinsky K, Jiang RH, Young L, Sykes SM, Saif S, Gujja S, Goldberg JM, Young S, Zeng Q, Chapman SB, Dash AP, Anvikar AR, Sutton PL, Birren BW, Escalante AA, Barnwell JW, Carlton JM. The malaria parasite *Plasmodium vivax* exhibits greater genetic diversity than *Plasmodium falciparum*. *Nat Genet* 2012; 44: 1046-1050.
22. Shen HM, Chen SB, Wang Y, Xu B, Abe EM, Chen JH. Genome-wide scans for the identification of *Plasmodium vivax* genes under positive selection. *Malar J* 2017; 16: 238.
23. Pearson RD, Amato R, Auburn S, Miotto O, Almagro-Garcia J, Amaratunga C, Suon S, Mao S, Noviyanti R, Trimarsanto H, Marfurt J, Anstey NM, William T, Boni ME, Dolecek C, Hien TT, White NJ, Michon P, Siba P, Tavul L, Harrison G, Barry A, Mueller I, Ferreira MU, Karunaweera N, Randrianarivelojosia M, Gao Q, Hubbart C, Hart L, Jeffery B, Drury E, Mead D, Kekre M, Campino S, Manske M, Cornelius VJ, MacInnis B, Rockett KA, Miles A, Rayner JC, Fairhurst RM, Nosten F, Price RN, Kwiatkowski DP. Genomic analysis of local variation and recent evolution in *Plasmodium vivax*. *Nat Genet* 2016; 48: 959-964.
24. Hupal DN, Luo Z, Melnikov A, Sutton PL, Rogov P, Escalante A, Vallejo AF, Herrera S, Arevalo-Herrera M, Fan Q, Wang Y, Cui L, Lucas CM, Durand S, Sanchez JE, Baldeviano GC, Lescano AG, Laman M, Barnadas C, Barry A, Mueller I, Kazura JW, Eapen A, Kanagaraj D, Valecha N, Ferreira MU, Roobsoong W, Nguitra-gool W, Sattabongkot J, Gamboa D, Kosek M, Vinetz JM, González-Cerón L, Birren BW, Neafsey DE, Carlton JM. Population genomics studies identify signatures of global dispersal and drug resistance in *Plasmodium vivax*. *Nat Genet* 2016; 48: 953-958.
25. Zhong D, Bonizzoni M, Zhou G, Wang G, Chen B, Vardo-Zalik A, Cui L, Yan G, Zheng B. Genetic diversity of *Plasmodium vivax* malaria in China and Myanmar. *Infect Genet Evol* 2011; 11: 1419-1425.
26. Bastos MS, da Silva-Nunes M, Malafronte RS, Hoffmann EH, Wunderlich G, Moraes SL, Ferreira MU. Antigenic polymorphism and naturally acquired antibodies to *Plasmodium vivax* merozoite surface protein 1 in rural Amazonians. *Clin Vaccine Immunol* 2007; 14: 1249-1259.
27. Parobek CM, Bailey JA, Hathaway NJ, Socheat D, Rogers WO, Juliano JJ. Differing patterns of selection and geospatial genetic diversity within two leading *Plasmodium vivax* candidate vaccine antigens. *PLoS Negl Trop Dis* 2014; 8: e2796.
28. Moore SJ, Min X, Hill N, Jones C, Zaixing Z, Cameron MM. Border malaria in China: knowledge and use of personal protection by minority populations and implications for malaria control: a questionnaire-based survey. *BMC Public Health* 2008; 8: 344.
29. Zhang HW, Liu Y, Zhang SS, Xu BL, Li WD, Tang JH, Zhou SS, Huang F. Preparation of malaria resurgence in China: case study of vivax malaria re-emergence and outbreak in Huang-Huai Plain in 2006. *Adv Parasitol* 2014; 86: 205-230.
30. Dias S, Longacre S, Escalante AA, Udagama-Randeniya PV. Genetic diversity and recombination at the C-terminal fragment of the merozoite surface protein-1 of *Plasmodium vivax* (PvMSP-1) in Sri Lanka. *Infect Genet Evol* 2011; 11: 145-156.
31. Thakur A, Alam MT, Sharma YD. Genetic diversity in the C-terminal 42 kDa region of merozoite surface protein-1 of *Plasmodium vivax* (PvMSP-1(42)) among Indian isolates. *Acta Trop* 2008; 108: 58-63.
32. Cui L, Yan G, Sattabongkot J, Cao Y, Chen B, Chen X, Fan Q, Fang Q, Jongwutiwes S, Parker D, Sirichaisinthop J, Kyaw MP, Su XZ, Yang H, Yang Z, Wang B, Xu J, Zheng B, Zhong D, Zhou G. Malaria in the Greater Mekong Subregion: heterogeneity and complexity. *Acta Trop* 2012; 121: 227-239.
33. Zhou XN, Bergquist R, Tanner M. Elimination of tropical disease through surveillance and response. *Infect Dis Poverty* 2013; 2: 1.
34. Chen SB, Ju C, Chen JH, Zheng B, Huang F, Xiao N, Zhou X, Ernest T, Zhou XN. Operational research needs toward malaria elimination in China. *Adv Parasitol* 2014; 86: 109-133.

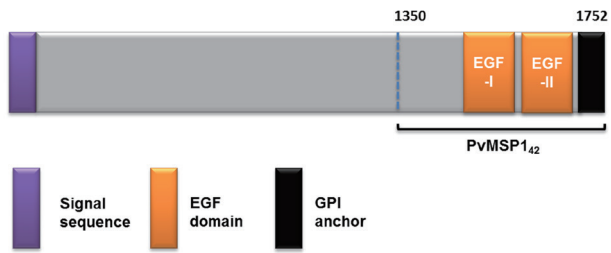


Fig. S1. Schematic diagram of *Plasmodium vivax* merozoite surface protein 1 (PvMSP1). The gene fragment encoding PvMSP1₄₂ (including 2 EGF domains and a glycosylphosphatidylinositol anchor) was amplified and sequenced.

Fig. S2. The nucleotide sequence alignment of PvMSP1₄₂ from *P. vivax* isolates collected along the China-Myanmar border areas, local regions of Anhui, Yunnan and Zhejiang provinces in inland China.

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)

Thursday, August 03, 2017 05:05 PM

	10	20	30	40	50	60	70	80
6.seq	80
17.seq	80
20.seq	80
22.seqT.....	80
23.seq	80
27.seq	80
38.seq	80
40.seq	80
42.seq	80
50.seqT.....	80
51.seqT.....	80
57.seq	80
59.seq	80
63.seq	80
66.seq	80
70.seq	80
74.seq	80
75.seqT.....	80
78.seq	80
81.seq	80
82.seq	80
85.seq	80
89.seq	80
91.seq	80
95.seq	80
98.seq	80
99.seq	80
114.seq	80
117.seq	80
118.seq	80
119.seq	80
120.seq	80
127.seqT.....	80
132.seq	80
133.seq	80
134.seq	80
136.seq	80
137.seq	80
138.seq	80
144.seq	80
145.seqT.....	80
150.seq	80
151.seq	80
153.seq	80
154.seq	80
156.seq	80
157.seqT.....	80
158.seq	80
215.seq	80
160.seqT.....	80
162.seqT.....	80
167.seq	80
172.seq	80
173.seq	80
183.seqT.....	80
191.seq	80
199.seq	80
200.seq	80
203.seq	80
Anhui_11.seq	80
Anhui_2.seq	80
Anhui_4.seq	80

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

Anhui_5.seq	80
Anhui_9.seq	80
Anhui_10.seq	80
Yunnan_T18.seq	80
Yunnan_161.seq	80
Yunnan_A66.seq	80
Yunnan_T7.seq	80
Yunnan_T12.seq	80
Yunnan_T13.seq	80
Zhejiang_8.seq	80
Zhejiang_2.seq	80
Zhejiang_3.seq	80
Zhejiang_5.seq	80
Zhejiang_6.seq	80
Zhejiang_7.seq	80

	90	100	110	120	130	140	150	160
6.seq								160
17.seq								160
20.seq								160
22.seq								160
23.seq								160
27.seq								160
38.seq								160
40.seq								160
42.seq								160
50.seq								160
51.seq								160
57.seq								160
59.seq								160
63.seq								160
66.seq								160
70.seq								160
74.seq								160
75.seq								160
78.seq								160
81.seq								160
82.seq								160
85.seq								160
89.seq								160
91.seq								160
95.seq								160
98.seq								160
99.seq								160
114.seq								160
117.seq								160
118.seq								160
119.seq								160
120.seq								160
127.seq								160
132.seq								160
133.seq								160
134.seq								160
136.seq								160
137.seq								160
138.seq								160
144.seq								160
145.seq								160
150.seq								160
151.seq								160
153.seq								160
154.seq								160
156.seq								160

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

157.seq	160
158.seq	160
215.seq	160
160.seq	160
162.seq	160
167.seq	160
172.seq	160
173.seq	160
183.seq	160
191.seq	160
199.seq	160
200.seq	160
203.seq	160
Anhui_11.seq	160
Anhui_2.seq	160
Anhui_4.seq	160
Anhui_5.seq	160
Anhui_9.seq	160
Anhui_10.seq	160
Yunnan_T18.seq	160
Yunnan_161.seq	160
Yunnan_A66.seq	160
Yunnan_T7.seq	160
Yunnan_T12.seq	160
Yunnan_T13.seq	160
Zhejiang_8.seq	160
Zhejiang_2.seq	160
Zhejiang_3.seq	160
Zhejiang_5.seq	160
Zhejiang_6.seq	160
Zhejiang_7.seq	160



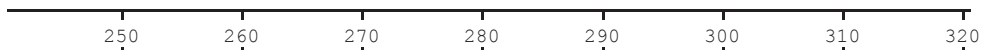
6.seq	240
17.seq	240
20.seq	240
22.seq	240
23.seq	240
27.seq	240
38.seq	240
40.seq	240
42.seq	240
50.seq	240
51.seq	240
57.seq	240
59.seq	240
63.seq	240
66.seq	240
70.seq	240
74.seq	240
75.seq	240
78.seq	240
81.seq	240
82.seq	240
85.seq	240
89.seq	240
91.seq	240
95.seq	240
98.seq	240
99.seq	240
114.seq	240
117.seq	240
118.seq	240

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

119.seq	240
120.seq	240
127.seq	240
132.seq	240
133.seq	240
134.seq	240
136.seq	240
137.seq	240
138.seq	240
144.seq	240
145.seq	240
150.seq	240
151.seq	240
153.seq	240
154.seq	240
156.seq	240
157.seq	240
158.seq	240
215.seq	240
160.seq	240
162.seq	240
167.seq	240
172.seq	240
173.seq	240
183.seq	240
191.seq	240
199.seq	240
200.seq	240
203.seq	240
Anhui_11.seq	240
Anhui_2.seq	240
Anhui_4.seq	240
Anhui_5.seq	240
Anhui_9.seq	240
Anhui_10.seq	240
Yunnan_T18.seq	240
Yunnan_161.seq	240
Yunnan_A66.seq	240
Yunnan_T7.seq	240
Yunnan_T12.seq	240
Yunnan_T13.seq	240
Zhejiang_8.seq	240
Zhejiang_2.seq	240
Zhejiang_3.seq	240
Zhejiang_5.seq	240
Zhejiang_6.seq	240
Zhejiang_7.seq	240



6.seq	320
17.seq	320
20.seq	320
22.seq	320
23.seq	320
27.seq	320
38.seq	320
40.seq	320
42.seq	320
50.seq	320
51.seq	320
57.seq	320
59.seq	320
63.seq	320

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

66.seq	320
70.seq	320
74.seq	320
75.seq	320
78.seq	320
81.seqC.....	320
82.seq	320
85.seq	320
89.seq	320
91.seq	320
95.seq	320
98.seq	320
99.seq	320
114.seqC.....	320
117.seq	320
118.seq	320
119.seq	320
120.seq	320
127.seq	320
132.seq	320
133.seq	320
134.seq	320
136.seq	320
137.seq	320
138.seq	320
144.seq	320
145.seq	320
150.seq	320
151.seq	320
153.seq	320
154.seq	320
156.seq	320
157.seq	320
158.seq	320
215.seq	320
160.seq	320
162.seq	320
167.seq	320
172.seq	320
173.seq	320
183.seq	320
191.seq	320
199.seq	320
200.seqC.....	320
203.seq	320
Anhui_11.seq	320
Anhui_2.seq	320
Anhui_4.seq	320
Anhui_5.seq	320
Anhui_9.seq	320
Anhui_10.seq	320
Yunnan_T18.seq	320
Yunnan_161.seq	320
Yunnan_A66.seq	320
Yunnan_T7.seqC.....	320
Yunnan_T12.seq	320
Yunnan_T13.seq	320
Zhejiang_8.seqC.....	320
Zhejiang_2.seqC.....	320
Zhejiang_3.seqC.....	320
Zhejiang_5.seq	320
Zhejiang_6.seqC.....	320
Zhejiang_7.seq	320

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

	330	340	350	360	370	380	390	400
6.seq						A.C		G.. 400
17.seq						A.C		G.. 400
20.seq			G.			A.C		G.. 400
22.seq						A.C		G.. 400
23.seq						A.C		G.. 400
27.seq			A.			A.C		G.. 400
38.seq			A.					G.. 400
40.seq				G.		A.C		G.. 400
42.seq								G.. 400
50.seq	C.							G.. 400
51.seq						A.C		G.. 400
57.seq			A.					G.. 400
59.seq						A.C		G.. 400
63.seq						A.C		G.. 400
66.seq						A.C		G.. 400
70.seq								G.. 400
74.seq			A.					G.. 400
75.seq								G.. 400
78.seq								G.. 400
81.seq			A.					G.. 400
82.seq						A.C		G.. 400
85.seq				G.				G.. 400
89.seq				G.				G.. 400
91.seq	C.					A.C		G.. 400
95.seq						A.C		G.. 400
98.seq						A.C		G.. 400
99.seq				G.				G.. 400
114.seq			A.					G.. 400
117.seq								G.. 400
118.seq								G.. 400
119.seq								G.. 400
120.seq						A.C		G.. 400
127.seq						A.C		G.. 400
132.seq						A.C		G.. 400
133.seq								G.. 400
134.seq			A.					G.. 400
136.seq						A.C		G.. 400
137.seq				G.				A.. 400
138.seq								G.. 400
144.seq						A.C		G.. 400
145.seq						A.C		G.. 400
150.seq								G.. 400
151.seq								G.. 400
153.seq						A.C		G.. 400
154.seq						A.C		G.. 400
156.seq						A.C		G.. 400
157.seq			A.					G.. 400
158.seq						A.C		G.. 400
215.seq								G.. 400
160.seq								G.. 400
162.seq			A.					G.. 400
167.seq						A.C		G.. 400
172.seq								G.. 400
173.seq								G.. 400
183.seq				G.		A.C		G.. 400
191.seq			A.			A.C		G.. 400
199.seq				G.				G.. 400
200.seq			A.					G.. 400
203.seq			A.					G.. 400
Anhui_11.seq								G.. 400
Anhui_2.seq								G.. 400
Anhui_4.seq								G.. 400

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
 Thursday, August 03, 2017 05:05 PM

Anhui_5.seq	400
Anhui_9.seq	400
Anhui_10.seq	400
Yunnan_T18.seqA.....A.C.....G..	400
Yunnan_161.seq	400
Yunnan_A66.seq	400
Yunnan_T7.seqA.....	400
Yunnan_T12.seq	400
Yunnan_T13.seqG..	400
Zhejiang_8.seqA.....	400
Zhejiang_2.seqA.....	400
Zhejiang_3.seqA.....	400
Zhejiang_5.seqA.....G..	400
Zhejiang_6.seqA.....G..	400
Zhejiang_7.seqA.....G..	400

	410	420	430	440	450	460	470	480		
6.seqC.....	A.....	G..	480	
17.seqT.....	480	
20.seqAG.....	G.A	480	
22.seq	AC.C.....	TG.C.....C.....	G..G.....	T..	480	
23.seqAG.....	G..	480	
27.seqT.....	480	
38.seqAG.....	G.A	480	
40.seqAG.....	G.A	480	
42.seqG..T.....	G.A	480	
50.seqT.....	480	
51.seqAG.....	G.A	480	
57.seq	AC.C.....	TG.C.....AG.....	G.A	480	
59.seqAG.....	G.A	480	
63.seqC.....	A.....	G..	480
66.seqAG.....	G..	480	
70.seq	AC.C.....	TGA...G.....C.....	G..G.....	T..	480	
74.seqAG.....	G.A	480	
75.seq	AC.C.....	TGA...G.....C.....	G..G.....	T..	480	
78.seq	AC.C.....	TGA...G.....C.....	G..G.....	T..	480	
81.seq	AC.C.....	TG.C.....C.....	A.....	G..	480
82.seqAG.....	G.A	480	
85.seqC.....	G..G.....	T..	480	
89.seqAG.....	G.A	480	
91.seqAG.....	G.A	480	
95.seqAG.....	G.A	480	
98.seqAG.....	G.A	480	
99.seqAG.....	G.A	480	
114.seq	AC.C.....	TG.C.....C.....	A.....	G..	480
117.seq	AC.C.....	TGA...G.....C.....	G..G.....	T..	480	
118.seq	AC.C.....	TGA...G.....C.....	G..G.....	T..	480	
119.seq	AC.C.....	TGA...G.....C.....	G..G.....	T..	480	
120.seqAG.....	G.A	480	
127.seqC.....	A.....	G..	480
132.seqAG.....	G.A	480	
133.seqT.....	480	
134.seqT.....	480	
136.seqAG.....	G.A	480	
137.seq	AC.C.....	TGA...G.....C.....	G..G.....	T..	480	
138.seq	AC.C.....	TGA...G.....C.....	G..G.....	T..	480	
144.seqAG.....	G.A	480	
145.seqC.....	A.....	G..	480
150.seqT.....	480	
151.seq	AC.C.....	TGA...G.....C.....	G..G.....	T..	480	
153.seqAG.....	G.A	480	
154.seqAG.....	G.A	480	
156.seqAG.....	G..	480	

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

157.seqAC..C.....TGA....G.....C.....G..G.....T...	480
158.seqC.....A.....G..	480
215.seqAG.....G.A	480
160.seqAC..C.....TGA....G.....C.....G..G.....T...	480
162.seqAC..C.....TGA....G.....C.....G..G.....T...	480
167.seqAG.....G.A	480
172.seqAC..C.....TGA....G.....C.....G..G.....T...	480
173.seqAC..C.....TGA....G.....C.....G..G.....T...	480
183.seqAG.....G.A	480
191.seqT.....	480
199.seqAG.....G.A	480
200.seqAC..C.....TGA....G.....C.....G..G.....T...	480
203.seqAG.....G.A	480
Anhui_11.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Anhui_2.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Anhui_4.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Anhui_5.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Anhui_9.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Anhui_10.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Yunnan_T18.seqT.....	480
Yunnan_161.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Yunnan_A66.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Yunnan_T7.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Yunnan_T12.seqAC..C.....TG..C.....C.....A.....G..	480
Yunnan_T13.seqT.....	480
Zhejiang_8.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Zhejiang_2.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Zhejiang_3.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Zhejiang_5.seqT.....	480
Zhejiang_6.seqAC..C.....TGA....G.....C.....G..G.....T...	480
Zhejiang_7.seqT.....	480

	490	500	510	520	530	540	550	560
6.seqA.....C.....A.....G.TA.....T.C.....	560						
17.seqC.....T.....G.....AGT.....C..AA..G.....G.....A.....	560						
20.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
22.seq	A.....C.....G.....C.....A.....C.....G.....	560						
23.seqA.....C.....A.....G.TA.....T.C.....	560						
27.seqC.....T.....G.....AGT.....C..AA..G.....G.....A.....	560						
38.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
40.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
42.seqT.....G.....AGT.....C..AA..G.....A.....	560						
50.seqC.....T.....G.....AGT.....C..AA..G.....G.....A.....	560						
51.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
57.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
59.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
63.seqA.....C.....A.....G.TA.....T.C.....	560						
66.seqA.....C.....A.....G.TA.....T.C.....	560						
70.seq	A.....G.....C.....A.....C.....G.....	560						
74.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
75.seq	A.....G.....C.....A.....C.....G.....	560						
78.seq	A.....G.....C.....A.....C.....G.....	560						
81.seqA.....AGC..A.....A..G.....G.TA.....T.C.....T.....	560						
82.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
85.seq	A.....C.....G.....C.....A.....C.....G.....	560						
89.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
91.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
95.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
98.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
99.seqT.....T.....A.....C.....A..G.A.....A.G.....T.....	560						
114.seqA.....AGC..A.....A..G.....G.TA.....T.C.....T.....	560						
117.seq	A.....A.....AGC..A.....G.....C.....G.....	560						
118.seq	A.....G.....C.....A.....C.....G.....	560						

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
 Thursday, August 03, 2017 05:05 PM

119.seq	A.....A.....AGC.A.....G.....C..G.....	560
120.seqT.....T.A.....C..A..G.A.....A.G.....T.....	560
127.seqA.....C.....A.....G.TA.....T.C.....	560
132.seqT.....T.A.....C..A..G.A.....A.G.....T.....	560
133.seq	..C.....T.....G..AGT...C..AA..G.....A.....	560
134.seq	..C.....T.....G..AGT...C..AA..G.....A.....	560
136.seqT.....A.....C..A..G.A.....A.G.....T.....	560
137.seq	A.....A.....AGC.A.....G.....C..G.....	560
138.seq	A.....A.....AGC.A.....G.....C..G.....	560
144.seqT.....T.A.....C..A..G.A.....A.G.....T.....	560
145.seqA.....C.....A.....G.TA.....T.C.....	560
150.seq	..C.....T.....G..AGT...C..AA..G.....A.....	560
151.seq	A.....A.....AGC.A.....G.....C..G.....	560
153.seqT.....T.A.....C..A..G.A.....A.G.....T.....	560
154.seqT.....T.A.....C..A..G.A.....A.G.....T.....	560
156.seqA.....C.....A.....G.TA.....T.C.....	560
157.seq	A.....A.....G.....C.....A.....C..G.....	560
158.seqA.....C.....A.....G.TA.....T.C.....	560
215.seqT.....T.A.....C..A..G.A.....A.G.....T.....	560
160.seq	A.....A.....AGC.A.....G.....C..G.....	560
162.seq	A.....A.....G.....C.....A.....C..G.....	560
167.seqT.....A.....C..A..G.A.....A.G.....T.....	560
172.seq	A.....A.....AGC.A.....G.....C..G.....	560
173.seq	A.....A.....AGC.A.....G.....C..G.....	560
183.seqT.....T.A.....C..A..G.A.....A.G.....T.....	560
191.seq	..C.....T.....G..AGT...C..AA..G.....A.....	560
199.seqT.....T.A.....C..A..G.A.....A.G.....T.....	560
200.seq	A.....T.....G.....C.....A.....C..G.....	560
203.seqT.....T.A.....C..A..G.A.....A.G.....T.....	560
Anhui_11.seq	A.....A.....AGC.A.....G.....C..G.....	560
Anhui_2.seq	A.....A.....AGC.A.....G.....C..G.....	560
Anhui_4.seq	A.....A.....AGC.A.....G.....C..G.....	560
Anhui_5.seq	A.....A.....AGC.A.....G.....C..G.....	560
Anhui_9.seq	A.....A.....AGC.A.....G.....C..G.....	560
Anhui_10.seq	A.....A.....AGC.A.....G.....C..G.....	560
Yunnan_T18.seq	..C.....T.....G..AGT...C..AA..G.....A.....	560
Yunnan_161.seq	A.....A.....AGC.A.....G.....C..G.....	560
Yunnan_A66.seq	A.....A.....AGC.A.....G.....C..G.....	560
Yunnan_T7.seq	A.....A.....G.....C.....A.....C..G.....	560
Yunnan_T12.seqA.....C.....A.....G.TA.....T.C.....	560
Yunnan_T13.seq	..C.....T.....G.....C.....A.....C..G.....	560
Zhejiang_8.seq	A.....A.....G.....C.....A.....C..G.....	560
Zhejiang_2.seq	A.....A.....G.....C.....A.....C..G.....	560
Zhejiang_3.seq	A.....A.....G.....C.....A.....C..G.....	560
Zhejiang_5.seq	..C.....T.....G..AGT...C..AA..G.....A.....	560
Zhejiang_6.seq	A.....A.....G.....C.....A.....C..G.....	560
Zhejiang_7.seq	..C.....T.....G..AGT...C..AA..G.....A.....	560

570 580 590 600 610 620 630 640

6.seq	640
17.seq	640
20.seq	640
22.seq	640
23.seq	640
27.seq	640
38.seq	640
40.seq	640
42.seq	640
50.seq	640
51.seq	640
57.seq	640
59.seq	640
63.seq	640

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

66.seq	640
70.seq	640
74.seq	640
75.seq	640
78.seq	640
81.seq	640
82.seq	640
85.seq	640
89.seq	640
91.seq	640
95.seq	640
98.seq	640
99.seq	640
114.seqA.....	640
117.seq	640
118.seq	640
119.seq	640
120.seq	640
127.seq	640
132.seq	640
133.seq	640
134.seq	640
136.seq	640
137.seq	640
138.seq	640
144.seq	640
145.seq	640
150.seq	640
151.seq	640
153.seq	640
154.seq	640
156.seq	640
157.seq	640
158.seq	640
215.seq	640
160.seq	640
162.seq	640
167.seq	640
172.seq	640
173.seq	640
183.seq	640
191.seq	640
199.seq	640
200.seq	640
203.seq	640
Anhui_11.seq	640
Anhui_2.seq	640
Anhui_4.seq	640
Anhui_5.seq	640
Anhui_9.seq	640
Anhui_10.seq	640
Yunnan_T18.seq	640
Yunnan_161.seq	640
Yunnan_A66.seq	640
Yunnan_T7.seq	640
Yunnan_T12.seq	640
Yunnan_T13.seq	640
Zhejiang_8.seq	640
Zhejiang_2.seq	640
Zhejiang_3.seq	640
Zhejiang_5.seq	640
Zhejiang_6.seq	640
Zhejiang_7.seq	640

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

	650	660	670	680	690	700	710	720
6.seq	720
17.seq	720
20.seq	720
22.seqT.	720
23.seq	...C	720
27.seq	...C	..A.	720
38.seq	...C	..A.	720
40.seq	720
42.seq	720
50.seq	720
51.seq	720
57.seq	720
59.seq	720
63.seq	720
66.seq	...C	720
70.seq	720
74.seq	...C	..A.	720
75.seq	720
78.seq	720
81.seq	...C	..A.	720
82.seq	720
85.seq	...C	720
89.seqT.	720
91.seq	720
95.seq	720
98.seq	720
99.seq	720
114.seq	...C	..A.	720
117.seq	...C	720
118.seq	720
119.seq	720
120.seq	720
127.seq	720
132.seq	720
133.seq	720
134.seq	720
136.seq	720
137.seqA.	720
138.seq	720
144.seqA.	720
145.seq	720
150.seq	720
151.seqA.	720
153.seqA.	720
154.seq	720
156.seq	...C	720
157.seq	...C	..A.	720
158.seq	720
215.seq	720
160.seq	720
162.seq	...C	..A.	720
167.seq	720
172.seq	...C	720
173.seq	720
183.seq	...C	720
191.seq	...C	..A.	720
199.seq	720
200.seqA.	720
203.seq	...C	..A.	720
Anhui_11.seq	...C	..A.	720
Anhui_2.seq	...C	..A.	720
Anhui_4.seq	...C	..A.	720

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

Anhui_5.seqC...A.....	720
Anhui_9.seqC...A.....	720
Anhui_10.seqC...A.....	720
Yunnan_T18.seq	720
Yunnan_161.seq	720
Yunnan_A66.seq	720
Yunnan_T7.seqA.....	720
Yunnan_T12.seq	720
Yunnan_T13.seq	720
Zhejiang_8.seqA.....	720
Zhejiang_2.seqA.....	720
Zhejiang_3.seqA.....	720
Zhejiang_5.seqC...A.....	720
Zhejiang_6.seqA.....	720
Zhejiang_7.seqC...A.....	720

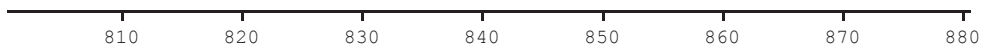
	730	740	750	760	770	780	790	800
6.seq	800
17.seq	800
20.seq	800
22.seq	800
23.seq	800
27.seq	800
38.seq	800
40.seq	800
42.seq	800
50.seq	800
51.seq	800
57.seq	800
59.seq	800
63.seq	800
66.seq	800
70.seq	800
74.seq	800
75.seq	800
78.seq	800
81.seq	800
82.seq	800
85.seq	800
89.seq	800
91.seq	800
95.seq	800
98.seq	800
99.seq	800
114.seq	800
117.seq	800
118.seq	800
119.seq	800
120.seq	800
127.seq	800
132.seq	800
133.seq	800
134.seq	800
136.seq	800
137.seq	800
138.seq	800
144.seq	800
145.seq	800
150.seq	800
151.seq	800
153.seq	800
154.seq	800
156.seq	800

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

157.seq	800
158.seq	800
215.seq	800
160.seq	800
162.seq	800
167.seq	800
172.seq	800
173.seq	800
183.seq	800
191.seq	800
199.seq	800
200.seq	800
203.seq	800
Anhui_11.seq	800
Anhui_2.seq	800
Anhui_4.seq	800
Anhui_5.seq	800
Anhui_9.seq	800
Anhui_10.seq	800
Yunnan_T18.seq	800
Yunnan_161.seq	800
Yunnan_A66.seq	800
Yunnan_T7.seq	800
Yunnan_T12.seq	800
Yunnan_T13.seq	800
Zhejiang_8.seq	800
Zhejiang_2.seq	800
Zhejiang_3.seq	800
Zhejiang_5.seq	800
Zhejiang_6.seq	800
Zhejiang_7.seq	800



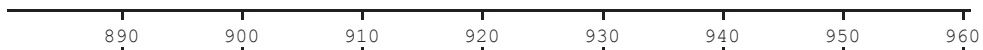
6.seq	880
17.seq	880
20.seq	880
22.seq	880
23.seq	880
27.seq	880
38.seq	880
40.seq	880
42.seq	880
50.seq	880
51.seq	880
57.seq	880
59.seq	880
63.seq	880
66.seq	880
70.seq	880
74.seq	880
75.seq	880
78.seq	880
81.seq	880
82.seq	880
85.seq	880
89.seq	880
91.seq	880
95.seq	880
98.seq	880
99.seq	880
114.seq	880
117.seq	880
118.seq	880

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

119.seq	880
120.seq	880
127.seq	880
132.seq	880
133.seq	880
134.seq	880
136.seq	880
137.seq	880
138.seq	880
144.seq	880
145.seq	880
150.seq	880
151.seq	880
153.seq	880
154.seq	880
156.seq	880
157.seq	880
158.seq	880
215.seq	880
160.seq	880
162.seq	880
167.seq	880
172.seq	880
173.seq	880
183.seq	880
191.seq	880
199.seq	880
200.seq	880
203.seq	880
Anhui_11.seq	880
Anhui_2.seq	880
Anhui_4.seq	880
Anhui_5.seq	880
Anhui_9.seq	880
Anhui_10.seq	880
Yunnan_T18.seq	880
Yunnan_161.seq	880
Yunnan_A66.seq	880
Yunnan_T7.seq	880
Yunnan_T12.seq	880
Yunnan_T13.seq	880
Zhejiang_8.seq	880
Zhejiang_2.seq	880
Zhejiang_3.seq	880
Zhejiang_5.seq	880
Zhejiang_6.seq	880
Zhejiang_7.seq	880



6.seq	960
17.seq	960
20.seq	960
22.seq	960
23.seq	960
27.seq	960
38.seq	960
40.seq	960
42.seq	960
50.seq	960
51.seq	960
57.seq	960
59.seq	960
63.seq	960

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

66.seq	960
70.seq	960
74.seq	960
75.seq	960
78.seq	960
81.seq	960
82.seq	960
85.seq	960
89.seq	960
91.seq	960
95.seq	960
98.seq	960
99.seq	960
114.seq	960
117.seq	960
118.seq	960
119.seq	960
120.seq	960
127.seq	960
132.seq	960
133.seq	960
134.seq	960
136.seq	960
137.seq	960
138.seq	960
144.seq	960
145.seq	960
150.seq	960
151.seq	960
153.seq	960
154.seq	960
156.seq	960
157.seq	960
158.seq	960
215.seq	960
160.seq	960
162.seq	960
167.seq	960
172.seq	960
173.seq	960
183.seq	960
191.seq	960
199.seq	960
200.seq	960
203.seq	960
Anhui_11.seq	960
Anhui_2.seq	960
Anhui_4.seq	960
Anhui_5.seq	960
Anhui_9.seq	960
Anhui_10.seq	960
Yunnan_T18.seq	960
Yunnan_161.seq	960
Yunnan_A66.seq	960
Yunnan_T7.seq	960
Yunnan_T12.seq	960
Yunnan_T13.seq	960
Zhejiang_8.seq	960
Zhejiang_2.seq	960
Zhejiang_3.seq	960
Zhejiang_5.seq	960
Zhejiang_6.seq	960
Zhejiang_7.seq	960

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

Anhui_5.seq	104
Anhui_9.seq	104
Anhui_10.seq	104
Yunnan_T18.seq	104
Yunnan_161.seq	104
Yunnan_A66.seq	104
Yunnan_T7.seq	104
Yunnan_T12.seq	104
Yunnan_T13.seq	104
Zhejiang_8.seq	104
Zhejiang_2.seq	104
Zhejiang_3.seq	104
Zhejiang_5.seq	104
Zhejiang_6.seq	104
Zhejiang_7.seq	104

	1050	1060	1070	1080	1090	1100	1110	1120
6.seq	112
17.seq	112
20.seq	112
22.seq	112
23.seq	112
27.seq	112
38.seq	112
40.seq	112
42.seq	112
50.seq	112
51.seq	112
57.seq	112
59.seq	G.....	112
63.seq	112
66.seq	112
70.seq	112
74.seq	112
75.seq	112
78.seq	112
81.seq	112
82.seq	G.....	112
85.seq	112
89.seq	112
91.seq	G.....	112
95.seq	112
98.seq	G.....	112
99.seq	112
114.seq	112
117.seq	112
118.seq	112
119.seq	112
120.seq	112
127.seq	112
132.seq	112
133.seq	112
134.seq	112
136.seq	112
137.seq	112
138.seq	G.....	112
144.seq	112
145.seq	112
150.seq	112
151.seq	112
153.seq	112
154.seq	112
156.seq	112

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

157.seq	112
158.seq	112
215.seq	112
160.seqG.....	112
162.seq	112
167.seq	112
172.seq	112
173.seqG.....	112
183.seq	112
191.seq	112
199.seq	112
200.seq	112
203.seq	112
Anhui_11.seq	112
Anhui_2.seq	112
Anhui_4.seq	112
Anhui_5.seq	112
Anhui_9.seq	112
Anhui_10.seq	112
Yunnan_T18.seq	112
Yunnan_161.seq	112
Yunnan_A66.seqG.....	112
Yunnan_T7.seq	112
Yunnan_T12.seq	112
Yunnan_T13.seq	112
Zhejiang_8.seq	112
Zhejiang_2.seq	112
Zhejiang_3.seq	112
Zhejiang_5.seq	112
Zhejiang_6.seq	112
Zhejiang_7.seq	112

	1130	1140	1150	1160	1170	1180	1190	1200	
6.seq	120
17.seq	120
20.seq	120
22.seq	120
23.seq	120
27.seq	120
38.seq	120
40.seq	120
42.seq	120
50.seq	120
51.seq	120
57.seq	120
59.seq	120
63.seq	120
66.seq	120
70.seq	120
74.seq	120
75.seq	120
78.seq	120
81.seq	120
82.seq	120
85.seq	120
89.seq	120
91.seq	120
95.seq	120
98.seq	120
99.seq	120
114.seq	120
117.seq	120
118.seq	120

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

119.seq	120
120.seq	120
127.seq	120
132.seq	120
133.seq	120
134.seq	120
136.seq	120
137.seq	120
138.seq	120
144.seq	120
145.seq	120
150.seq	120
151.seq	120
153.seq	120
154.seq	120
156.seq	120
157.seq	120
158.seq	120
215.seq	120
160.seq	120
162.seq	120
167.seq	120
172.seq	120
173.seq	120
183.seq	120
191.seq	120
199.seq	120
200.seq	120
203.seq	120
Anhui_11.seq	120
Anhui_2.seq	120
Anhui_4.seq	120
Anhui_5.seq	120
Anhui_9.seq	120
Anhui_10.seq	120
Yunnan_T18.seq	120
Yunnan_161.seq	120
Yunnan_A66.seq	120
Yunnan_T7.seq	120
Yunnan_T12.seq	120
Yunnan_T13.seq	120
Zhejiang_8.seq	120
Zhejiang_2.seq	120
Zhejiang_3.seq	120
Zhejiang_5.seq	120
Zhejiang_6.seq	120
Zhejiang_7.seq	120
<hr/>		
6.seq	120
17.seq	120
20.seq	120
22.seq	120
23.seq	120
27.seq	120
38.seq	120
40.seq	120
42.seq	120
50.seq	120
51.seq	120
57.seq	120
59.seq	120
63.seq	120

(Continued to the next page)

Fig. S2. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (Weighted)
Thursday, August 03, 2017 05:05 PM

66.seq	120
70.seq	120
74.seq	120
75.seq	120
78.seq	120
81.seq	120
82.seq	120
85.seq	120
89.seq	120
91.seq	120
95.seq	120
98.seq	120
99.seq	120
114.seq	120
117.seq	120
118.seq	120
119.seq	120
120.seq	120
127.seq	120
132.seq	120
133.seq	120
134.seq	120
136.seq	120
137.seq	120
138.seq	120
144.seq	120
145.seq	120
150.seq	120
151.seq	120
153.seq	120
154.seq	120
156.seq	120
157.seq	120
158.seq	120
215.seq	120
160.seq	120
162.seq	120
167.seq	120
172.seq	120
173.seq	120
183.seq	120
191.seq	120
199.seq	120
200.seq	120
203.seq	120
Anhui_11.seq	120
Anhui_2.seq	120
Anhui_4.seq	120
Anhui_5.seq	120
Anhui_9.seq	120
Anhui_10.seq	120
Yunnan_T18.seq	120
Yunnan_161.seq	120
Yunnan_A66.seq	120
Yunnan_T7.seq	120
Yunnan_T12.seq	120
Yunnan_T13.seq	120
Zhejiang_8.seq	120
Zhejiang_2.seq	120
Zhejiang_3.seq	120
Zhejiang_5.seq	120
Zhejiang_6.seq	120
Zhejiang_7.seq	120

Fig. S3. The amino acid sequence alignment of PvMSP1₄₂ from *P. vivax* isolates collected along the China-Myanmar border areas, local regions of Anhui, Yunnan, and Zhejiang provinces in inland China.

Alignment Report of Myanmar plus inland China.meg ClustalW (PAM250)

Thursday, August 03, 2017 05:06 PM

	10	20	30	40	50	60	70	80
6.seq
17.seq
20.seq
22.seqY.....
23.seq
27.seq
38.seq
40.seq
42.seq
50.seqY.....
51.seqY.....
57.seq
59.seq
63.seq
66.seq
70.seq
74.seq
75.seqY.....
78.seq
81.seq
82.seq
85.seq
89.seq
91.seq
95.seq
98.seq
99.seq
114.seq
117.seq
118.seq
119.seq
120.seq
127.seqY.....
132.seq
133.seq
134.seq
136.seq
137.seq
138.seq
144.seq
145.seqY.....
150.seq
151.seq
153.seq
154.seq
156.seq
157.seqY.....
158.seq
215.seq
160.seqY.....
162.seqY.....
167.seq
172.seq
173.seq
183.seqY.....
191.seq
199.seq
200.seq
203.seq
Anhui_11.seq
Anhui_2.seq
Anhui_4.seq

(Continued to the next page)

Fig. S3. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (PAM250)
 Thursday, August 03, 2017 05:06 PM

Anhui_5.seq	238
Anhui_9.seq	238
Anhui_10.seq	238
Yunnan_T18.seq	238
Yunnan_161.seqY.....	238
Yunnan_A66.seq	238
Yunnan_T7.seq	238
Yunnan_T12.seq	238
Yunnan_T13.seq	238
Zhejiang_8.seq	238
Zhejiang_2.seq	238
Zhejiang_3.seq	238
Zhejiang_5.seq	238
Zhejiang_6.seq	238
Zhejiang_7.seq	238

	90	100	110	120	130	140	150	160	
6.seq	T.....	NT.NE	478
17.seq	E.....	TH..	478
20.seq	V.....	T.....	K...E	478
22.seq	E.....	TA.NAQ	...A...E.D	478
23.seq	T.....	K...E	478
27.seq	K.....	T.....	TH..	478
38.seq	L.....	K.....	E.....	K...E	478
40.seq	V.....	T.....	K...E	478
42.seq	TH..E	478
50.seq	Q.....	E.....	TH..	478
51.seq	T.....	K...E	478
57.seq	K.....	E.....	TA.NAQ	...K...E	478
59.seq	T.....	K...E	478
63.seq	T.....	NT.NE	478
66.seq	T.....	K...E	478
70.seq	E.....	TA.NE.V	...A...E.D	478
74.seq	K.....	K...E	478
75.seq	E.....	TA.NE.V	...A...E.D	478
78.seq	E.....	TA.NE.V	...A...E.D	478
81.seq	L.....	K.....	E.....	TA.NAQ	...NT.NE	478
82.seq	T.....	K...E	478
85.seq	V.....	A...E.D	478
89.seq	V.....	K...E	478
91.seq	Q.....	T.....	K...E	478
95.seq	T.....	K...E	478
98.seq	T.....	K...E	478
99.seq	V.....	K...E	478
114.seq	L.....	K.....	E.....	TA.NAQ	...NT.NE	478
117.seq	E.....	TA.NE.V	...A...E.D	478
118.seq	E.....	TA.NE.V	...A...E.D	478
119.seq	E.....	TA.NE.V	...A...E.D	478
120.seq	T.....	K...E	478
127.seq	T.....	NT.NE	478
132.seq	T.....	K...E	478
133.seq	E.....	TH..	478
134.seq	K.....	TH..	478
136.seq	T.....	K...E	478
137.seq	V.....	K.....	TA.NE.V	...A...E.D	478
138.seq	E.....	TA.NE.V	...A...E.D	478
144.seq	T.....	K...E	478
145.seq	T.....	NT.NE	478
150.seq	E.....	TH..	478
151.seq	E.....	TA.NE.V	...A...E.D	478
153.seq	T.....	K...E	478
154.seq	T.....	K...E	478
156.seq	T.....	K...E	478

(Continued to the next page)

Fig. S3. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (PAM250)
 Thursday, August 03, 2017 05:06 PM

157.seqK.....E.....TA.NE.V...A...E.D.	478
158.seqT.....NT.NE	478
215.seqE.....K...E	478
160.seqE.....TA.NE.V...A...E.D.	478
162.seqK.....E.....TA.NE.V...A...E.D.	478
167.seqT.....K...E	478
172.seqE.....TA.NE.V...A...E.D.	478
173.seqE.....TA.NE.V...A...E.D.	478
183.seqV.....T.....K...E	478
191.seqK.....T.....TH...	478
199.seqV.....K...E	478
200.seqL.....K.....E.....TA.NE.V...A...E.D.	478
203.seqK.....K...E	478
Anhui_11.seqE.....TA.NE.V...A...E.D.	478
Anhui_2.seqE.....TA.NE.V...A...E.D.	478
Anhui_4.seqE.....TA.NE.V...A...E.D.	478
Anhui_5.seqE.....TA.NE.V...A...E.D.	478
Anhui_9.seqE.....TA.NE.V...A...E.D.	478
Anhui_10.seqE.....TA.NE.V...A...E.D.	478
Yunnan_T18.seqK.....T.....TH...	478
Yunnan_161.seqE.....TA.NE.V...A...E.D.	478
Yunnan_A66.seqE.....TA.NE.V...A...E.D.	478
Yunnan_T7.seqL.....K.....E.....TA.NE.V...A...E.D.	478
Yunnan_T12.seqE.....TA.NAQ.....NT.NE	478
Yunnan_T13.seqTH...	478
Zhejiang_8.seqL.....K.....E.....TA.NE.V...A...E.D.	478
Zhejiang_2.seqL.....K.....E.....TA.NE.V...A...E.D.	478
Zhejiang_3.seqL.....K.....E.....TA.NE.V...A...E.D.	478
Zhejiang_5.seqK.....TH...	478
Zhejiang_6.seqL.....K.....E.....TA.NE.V...A...E.D.	478
Zhejiang_7.seqK.....TH...	478

	170	180	190	200	210	220	230	240	
6.seq	...E.D.T..T..AK.T.....								718
17.seq	.Q.L.S.V.QN....LN.....								718
20.seq	...L..VN.QD.K.NE.I.....								718
22.seq	I.T..SD.T..T...S.....								718
23.seq	...E.D.T..T..AK.T.....								718
27.seq	.Q.L.S.V.QN....LN.....N.....								718
38.seq	...L..VN.QD.K.NE.I.....N.....								718
40.seq	...L..VN.QD.K.NE.I.....								718
42.seq	...L.S.V.QN....LN.....								718
50.seq	.Q.L.S.V.QN....LN.....								718
51.seq	...L..VN.QD.K.NE.I.....								718
57.seq	...L..VN.QD.K.NE.I.....								718
59.seq	...L..VN.QD.K.NE.I.....								718
63.seq	...E.D.T..T..AK.T.....								718
66.seq	...E.D.T..T..AK.T.....								718
70.seq	I...SD.T..T...S.....								718
74.seq	...L..VN.QD.K.NE.I.....N.....								718
75.seq	I...SD.T..T...S.....								718
78.seq	I...SD.T..T...S.....								718
81.seq	...E..AN..A..AK.T.....N.....								718
82.seq	...L..VN.QD.K.NE.I.....								718
85.seq	I.T..SD.T..T...S.....								718
89.seq	...L..VN.QD.K.NE.I.....								718
91.seq	...L..VN.QD.K.NE.I.....								718
95.seq	...L..VN.QD.K.NE.I.....								718
98.seq	...L..VN.QD.K.NE.I.....								718
99.seq	...L..VN.QD.K.NE.I.....								718
114.seq	...E..AN..A..AK.T.....N.....								718
117.seq	I...E..AN.AA...S.....								718
118.seq	I...SD.T..T...S.....								718

(Continued to the next page)

Fig. S3. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (PAM250)
 Thursday, August 03, 2017 05:06 PM

119.seq	I...E..AN.AA...S.....	718
120.seq	...L..VN.QD.K.NE.I.....	718
127.seq	...E.D.T..T..AK.T.....	718
132.seq	...L..VN.QD.K.NE.I.....	718
133.seq	.Q.L.S.V.QN.....LN.....	718
134.seq	.Q.L.S.V.QN.....LN.....	718
136.seq	...L..DN.QD.K.NE.I.....	718
137.seq	I...E..AN.AA...S.....N.....	718
138.seq	I...E..AN.AA...S.....	718
144.seq	...L..VN.QD.K.NE.I.....	718
145.seq	...E.D.T..T..AK.T.....	718
150.seq	.Q.L.S.V.QN.....LN.....	718
151.seq	I...E..AN.AA...S.....N.....	718
153.seq	...L..VN.QD.K.NE.I.....	718
154.seq	...L..VN.QD.K.NE.I.....	718
156.seq	...E.D.T..T..AK.T.....	718
157.seq	I...SD.T..T...S.....N.....	718
158.seq	...E.D.T..T..AK.T.....	718
215.seq	...L..VN.QD.K.NE.I.....	718
160.seq	I...E..AN.AA...S.....	718
162.seq	I...SD.T..T...S.....N.....	718
167.seq	...L..DN.QD.K.NE.I.....	718
172.seq	I...E..AN.AA...S.....	718
173.seq	I...E..AN.AA...S.....	718
183.seq	...L..VN.QD.K.NE.I.....	718
191.seq	.Q.L.S.V.QN.....LN.....N.....	718
199.seq	...L..VN.QD.K.NE.I.....	718
200.seq	I...SD.T..T...S.....N.....	718
203.seq	...L..VN.QD.K.NE.I.....N.....	718
Anhui_11.seq	I...E..AN.AA...S.....N.....	718
Anhui_2.seq	I...E..AN.AA...S.....N.....	718
Anhui_4.seq	I...E..AN.AA...S.....N.....	718
Anhui_5.seq	I...E..AN.AA...S.....N.....	718
Anhui_9.seq	I...E..AN.AA...S.....N.....	718
Anhui_10.seq	I...E..AN.AA...S.....N.....	718
Yunnan_T18.seq	.Q.L.S.V.QN.....LN.....	718
Yunnan_161.seq	I...E..AN.AA...S.....	718
Yunnan_A66.seq	I...E..AN.AA...S.....	718
Yunnan_T7.seq	I...SD.T..T...S.....N.....	718
Yunnan_T12.seq	...E.D.T..T..AK.T.....	718
Yunnan_T13.seq	.Q.L.SD.T..T...S.....	718
Zhejiang_8.seq	I...SD.T..T...S.....N.....	718
Zhejiang_2.seq	I...SD.T..T...S.....N.....	718
Zhejiang_3.seq	I...SD.T..T...S.....N.....	718
Zhejiang_5.seq	.Q.L.S.V.QN.....LN.....N.....	718
Zhejiang_6.seq	I...SD.T..T...S.....N.....	718
Zhejiang_7.seq	.Q.L.S.V.QN.....LN.....N.....	718

	250	260	270	280	290	300	310	320	
6.seq	958
17.seq	958
20.seq	958
22.seq	958
23.seq	958
27.seq	958
38.seq	958
40.seq	958
42.seq	958
50.seq	958
51.seq	958
57.seq	958
59.seq	958
63.seq	958

(Continued to the next page)

Fig. S3. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (PAM250)
Thursday, August 03, 2017 05:06 PM

66.seq	958
70.seq	958
74.seq	958
75.seq	958
78.seq	958
81.seq	958
82.seq	958
85.seq	958
89.seq	958
91.seq	958
95.seq	958
98.seq	958
99.seq	958
114.seq	958
117.seq	958
118.seq	958
119.seq	958
120.seq	958
127.seq	958
132.seq	958
133.seq	958
134.seq	958
136.seq	958
137.seq	958
138.seq	958
144.seq	958
145.seq	958
150.seq	958
151.seq	958
153.seq	958
154.seq	958
156.seq	958
157.seq	958
158.seq	958
215.seq	958
160.seq	958
162.seq	958
167.seq	958
172.seq	958
173.seq	958
183.seq	958
191.seq	958
199.seq	958
200.seq	958
203.seq	958
Anhui_11.seq	958
Anhui_2.seq	958
Anhui_4.seq	958
Anhui_5.seq	958
Anhui_9.seq	958
Anhui_10.seq	958
Yunnan_T18.seq	958
Yunnan_161.seq	958
Yunnan_A66.seq	958
Yunnan_T7.seq	958
Yunnan_T12.seq	958
Yunnan_T13.seq	958
Zhejiang_8.seq	958
Zhejiang_2.seq	958
Zhejiang_3.seq	958
Zhejiang_5.seq	958
Zhejiang_6.seq	958
Zhejiang_7.seq	958

(Continued to the next page)

Fig. S3. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (PAM250)
Thursday, August 03, 2017 05:06 PM

	330	340	350	360	370	380	390	400	
6.seq	119
17.seq	119
20.seq	119
22.seq	119
23.seq	119
27.seq	119
38.seq	119
40.seq	119
42.seq	119
50.seq	119
51.seq	119
57.seq	119
59.seqE.	119
63.seq	119
66.seq	119
70.seqE.	119
74.seq	119
75.seq	119
78.seq	119
81.seq	119
82.seqE.	119
85.seq	119
89.seq	119
91.seqE.	119
95.seq	119
98.seqE.	119
99.seq	119
114.seq	119
117.seq	119
118.seq	119
119.seq	119
120.seq	119
127.seq	119
132.seq	119
133.seq	119
134.seq	119
136.seq	119
137.seq	119
138.seqE.	119
144.seq	119
145.seq	119
150.seq	119
151.seq	119
153.seq	119
154.seq	119
156.seq	119
157.seq	119
158.seq	119
215.seq	119
160.seqE.	119
162.seq	119
167.seq	119
172.seq	119
173.seqE.	119
183.seq	119
191.seq	119
199.seq	119
200.seq	119
203.seq	119
Anhui_11.seq	119
Anhui_2.seq	119
Anhui_4.seq	119

(Continued to the next page)

Fig. S3. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (PAM250)
Thursday, August 03, 2017 05:06 PM

Anhui_5.seq	119
Anhui_9.seq	119
Anhui_10.seq	119
Yunnan_T18.seq	119
Yunnan_161.seq	119
Yunnan_A66.seqE.....	119
Yunnan_T7.seq	119
Yunnan_T12.seq	119
Yunnan_T13.seq	119
Zhejiang_8.seq	119
Zhejiang_2.seq	119
Zhejiang_3.seq	119
Zhejiang_5.seq	119
Zhejiang_6.seq	119
Zhejiang_7.seq	119

6.seq	...	120
17.seq	...	120
20.seq	...	120
22.seq	...	120
23.seq	...	120
27.seq	...	120
38.seq	...	120
40.seq	...	120
42.seq	...	120
50.seq	...	120
51.seq	...	120
57.seq	...	120
59.seq	...	120
63.seq	...	120
66.seq	...	120
70.seq	...	120
74.seq	...	120
75.seq	...	120
78.seq	...	120
81.seq	...	120
82.seq	...	120
85.seq	...	120
89.seq	...	120
91.seq	...	120
95.seq	...	120
98.seq	...	120
99.seq	...	120
114.seq	...	120
117.seq	...	120
118.seq	...	120
119.seq	...	120
120.seq	...	120
127.seq	...	120
132.seq	...	120
133.seq	...	120
134.seq	...	120
136.seq	...	120
137.seq	...	120
138.seq	...	120
144.seq	...	120
145.seq	...	120
150.seq	...	120
151.seq	...	120
153.seq	...	120
154.seq	...	120
156.seq	...	120

(Continued to the next page)

Fig. S3. Continued

Alignment Report of Myanmar plus inland China.meg ClustalW (PAM250)
Thursday, August 03, 2017 05:06 PM

157.seq	...	120
158.seq	...	120
215.seq	...	120
160.seq	...	120
162.seq	...	120
167.seq	...	120
172.seq	...	120
173.seq	...	120
183.seq	...	120
191.seq	...	120
199.seq	...	120
200.seq	...	120
203.seq	...	120
Anhui_11.seq	...	120
Anhui_2.seq	...	120
Anhui_4.seq	...	120
Anhui_5.seq	...	120
Anhui_9.seq	...	120
Anhui_10.seq	...	120
Yunnan_T18.seq	...	120
Yunnan_161.seq	...	120
Yunnan_A66.seq	...	120
Yunnan_T7.seq	...	120
Yunnan_T12.seq	...	120
Yunnan_T13.seq	...	120
Zhejiang_8.seq	...	120
Zhejiang_2.seq	...	120
Zhejiang_3.seq	...	120
Zhejiang_5.seq	...	120
Zhejiang_6.seq	...	120
Zhejiang_7.seq	...	120

Decoration 'Decoration #1': Hide (as '.') residues that match the Consensus exactly.