Mosquito Population Diversity and Abundance Patterns In Linzhi, Xizang, China

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Abstract

Background: In 2009, China CDC found the distribution of *Culex tritaeniorhynchus* and *Aedes albopictus* in Motuo county, Linzhi, Xizang and isolated the epidemic encephalitis V virus from *Culex tritaeniorhynchus*. However, with the road opened in Motuo County, the trade between people and mosquitoes transmission were more frequent. To illustrate patterns of mosquito population in Linzhi, Xizang, a mosquitoes surveillance was carried.

Methods: Adult mosquitoes were collected by light traps and human landing catches, larva by container survey in six counties of Linzhi during August and September 2019. The trapped adult mosquitoes were initially counted and identified according to morphological criteria, and a part of mosquitoes were further identified by PCR. The monitoring data were recorded by Excel 2007, analyzed by SPSS 20.0 software and R.

Results: Eight species of mosquitoes belonging to four genera were identified. *Culex pipiens pallens* (86.80%), *Armigeres subalbatus* (5.29%) and *Aedes vexans* (2.99%) were the top three species collected by 164 light traps on 12 nights with the average mosquito density was 21.20 (mosquitoes per trap per night); *Aedes albopictus* were only collected in Chayu County Xiachayu Town by human landing catches with high average mosquito density (26.33 mosquitoes per hour per person). The larva were collected from waste tires, cement tanks and drums in Bomi, Milin and Lang Counties. The results of molecular identification are consistent with morphological identification. *Aedes albopictus* and *Culex orientalis* were newly recorded species for the first time. It is preliminarily speculated that there is a high potential risk of dengue fever in Xiachayu Town and a certain epidemic risk of Japanese encephalitis, while the risk of malaria is low in Linzhi.

Conclusion: Mosquitoes had heterogeneous distribution throughout Linzhi, Xizang. The dominant mosquito species are *Culex pipiens pallens*. It is emphasized to carry out mosquito surveillance again during the peak period of mosquito activities so as to better understand the distribution and composition of local mosquitoes and assess the mosquito-borne disease risk in this area.

Background

Mosquito-borne diseases is a major public health problem worldwide^[1]. Mosquitoes spread many mosquito-borne viruses and parasites between people and animals, including viral infections such as West Nile disease, malaria, dengue fever and Chikungunya fever^[2–3]. Many infections cause high fevers often accompanied with pain or other flu-like symptoms^[4]. Mosquito-borne diseases are now being reported at high elevations^[5–7]. Field studies including mosquito surveillance enable efficient mosquito control and yields knowledge of when and where to take appropriate protective measures. In China, *Culex pipiens pallens*, *Culex tritaeniorhynchus*, *Anopheles sinensis* and *Aedes albopictus* are widely distributed. *Culex* is the vector of epidemic encephalitis virus, filariasis, West Nile virus and other pathogens. *Anopheles sinensis* carry Plasmodium, filariasis and other pathogens. The mosquitoes *Aedes aegypti* and *Aedes albopictus* are vectors of several globally important arboviruses including dengue virus, yellow fever virus and chikungunya virus (CHIKV). Due to the high risk of transmission caused by many pathogens, mosquito-borne diseases cause considerable incidence rate and mortality^[5–8], which is a serious public health problem^[9]. Changes in the distribution of population and density of mosquitoes are influenced by climatic conditions, physical geography and human activities^[10], which in turn affect the occurrence and prevalence risk of mosquito-borne diseases^[11].

During the investigation of Japanese encephalitis vector in Motuo County, Linzhi in 2009, China CDC found *Culex tritaeniorhynchus* and *Aedes albopictus*. The Japanese Encephalitis Virus Genotype V was isolated from *Culex tritaeniorhynchus*^[12]. *Culex tritaeniorhynchus* and *Aedes albopictus* are the vectors of epidemic encephalitis and dengue fever, respectively. Linzhi is located in the southeast of Xizang Autonomous Region of China, in the middle and lower reaches of the Yarlung Zangbo River. It is connected with Lhasa city in the west and shannan city in the southwest, Changduo city in the east and Naqu area in the north, and India and myanmar in the south. Linzhi is a sub-humid tropical climate with sufficient sunshine and abundant rainfall, with an annual precipitation of 400 ~ 2200mm. The average altitude of Linzhi is about 3000 m above sea level (Longitude 92°09′–98°47′, Latitude 26°52′–30°40′). Linzhi governs 6 counties and 1 district, which are Gongbujiangda County, Milin County, Motuo County, Bomi County, Chayu County, Lang County and the newly established Bayi District. Motuo County was the only county in China that did not have access to roads in the past. In 2010, it opened the garongla tunnel, and then successfully opened the roads in 2013.

With the opening of the road and the unique geographical location of Motuo County, people in other counties communicate with each other are more and more frequently, which makes the mosquitoes easily transported with the mode of transportation. When the mosquitoes arrive at the new suitable habitat, they propagate and breed by occupying more ecological niches, thus increasing the risk of mosquito distribution and the spread of mosquito-borne diseases. Once exist in high altitude areas, some mosquito species may threaten the health of humans and vertebrates due to their ability to transmit numerous diseases^[13–15]. In this study, we report the distribution and composition of mosquitoes in Linzhi, Xizang. We investigated the distribution and composition of mosquitoes to provide the first scientific assessment of mosquitoes and a foundation for development of measures to control mosquito-borne diseases in Linzhi in the future.

Methods

Study sites

Linzhi is located in the southeast of Xizang, in the middle and lower reaches of the Yarlung Zangbo River, with an area of 11,700 km² and a population of 231,000. The annual average temperature is 8.7°C, and annual cumulative precipitation is 650 mm.

The present study was undertaken in six sites of Linzhi during August and September 2019 (Fig. 1). Based on this geographic conditions and socio-economic characteristics to the Linzhi, and the actual field conditions, we chose six counties to fully represent the overall situation of the research. They included Bayi District (formerly Linzhi County), Chayu County, Xiachayu Town, Milin County, Bomi County and Lang County (Table 1)

Table 1
Description of study sites in Linzhi, Xizang

Study site	Location	Elevation (m)	Landscape	Climate zone	Annual mean temperature (°C)	Annual precipitation (mm)
Bayi district	N29°30.668',E94°37.808'	2985	Urbanized, mountain	Humid and semi humid monsoon climate	8.5	654
Chayu County	N28°40.176',E97°28.165'	2354	Mountain, forest	Subtropical mountain humid monsoon	15.1	794
Xiachayu Town	N28°29.377',E97°01.379'	1559	Animals, forest	Subtropical mountain humid monsoon	14.0	799
Milin County	N29°19.747',E94°21.497'	2962	Mountain, rivers	Temperate semi humid monsoon	8.2	641
Bomi County	N28°40.074',E97°27.791'	2352	Mountain, rivers	Subtropical mountain humid monsoon	8.5	977
Lang County	N29°04.312',E92°53.907'	3141	Mountain, rivers	Temperate semi humid monsoon	11.0	500

Mosquito collection and initial morphological identification

In this study, light traps and human landing catches were employed to collect adult mosquitoes and larva collected by container in August and September 2019. The surveillance method was the standard method released by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China and Standardization Administration of the People's Republic of China (surveillance methods for vector density-mosquito, GB/T 23797–2009).

Kung Fu Xiaoshuai miniature light traps (Photocatalytic Miewen Ying supply device; Wavelength: 2537Å; Power: 8W; Corporation: Wuhan Environmental Protection Technology Co., Ltd. Gemstar) were used to collect adult mosquitoes. Traps were hung away from interference by light sources, 1.5 m above the floor. They were turned on 1 hour before sunset (20:00) and turned off 1 hour after sunrise (08:00) in 2–3 consecutive nights.

Human landing catches were performed in the forest, Bush, residential area, garbage station, near village farmland, waste tires and other places when the time period of *Aedes albopictus* activity (15:00–18:00). Some members of staff at Linzhi CDC and China CDC expose their calves, sit still or walk slowly in the trap site to collect the mosquitoes. At the same time, an electric aspirator was employed for 30–60 minutes to collect mosquitoes.

Outdoor large and medium-sized water bodies (rivers, ponds, puddles, lakes, canals) and water storage containers in villages, streets and yards were collected for container investigation. Transfer larvae to a mineral water bottle containing half a bottle of raw water with a spoon or a straw and store them in a sampling tube containing 95% ethanol and 75% ethanol after being quickly killed in 80–90°C hot water.

Each morning, the trapped adult mosquitoes were initially counted and identified according to morphological criteria using the key developed by Lu BL^[16]. All collected mosquitoes were put into 1.5 mL centrifuge tubes individually and then transported to the laboratory of the Department of Vector Biology and Control in China CDC for further molecular identification.

For information on temperature (°C) and relative humidity (%), please visit <http://www.weather.com.cn>. During the collection process, GPS is used to obtain the longitude and latitude information.

Mosquito species molecular identification

Mosquitoes were preserved in 75–95% ethanol and stored dark at 4°C before molecular analyses. Head and thorax were selected for genomic DNA extraction using Micro Tissue Genomic DNA Extraction Kit (BioTeke, Wuxi, China) and and DNA/RNA Extractor–32 system (BioTeke). A 658 bp fragment of the COI region was PCR-amplified using the universal primers LCO1490 and HCO2198^[17]. DNA amplification was carried out in 25 µl reactions using 12.5 µl PCR Mix, 1 µl 10 µM of each primer, 1.5 µl template DNA and 9 µl ddH₂O. The amplification program consisted of one cycle at 94 °C for 5 minutes, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 minute, and 1 cycle at 72 °C for 5 minutes. PCR products were electrophoresed in 1.0% agarose gel, and then sent to sequence at TsingKe Co. Ltd (Beijing, China). Raw sequences were edited and assembled in SeqMan version 7.1.0 (in the Laser Gene package, DNASTAR, Madison, USA), and aligned using the Muscle algorithm (Edgar 2004) in MEGA X. The sequences of *Culex pipiens pallens* (MH463066.1), *Armigeres subalbalus* (KJ410334.1), *Aedes vexans* (AB738152.1) and *Culex orientalis* (KT358428.1), *Aedes albopictus* (KU738429.1) were downloaded from GenBank as outgroups. The neighbor joining (NJ) tree were constructed in MEGA X using Kimura 2-Parameter (K2P) substitution model, 1000 bootstrap replicates and the “pairwise deletion” option for missing data.

Statistical analysis

Information was recorded on the date of the collections, number of light traps, number of people, the composition of mosquitoes. Analysis including the mosquito composition ratio, mosquito density and other data was conducted by Excel 2007, SPSS 20.0 software and R, maps were generated by ArcGIS 10.6.

Mosquito density (mosquitoes per trap per night) = Number of collected mosquitoes/ (light traps* nights)

Mosquito density (mosquitoes per hour per person) = Number of collected mosquitoes/ (person* hour)

Ethics statement

Ethical approval for this study was obtained from the Ethical Committee of China CDC and permission was also obtained from Xizang Autonomous Region CDC and Linzhi CDC.

Results

Morphological and molecular identification

A total of 3648 mosquitoes were captured in the six sites in August and September 2019. Of these, 2359 (64.67%) mosquitoes were female 1289 (35.33%) mosquitoes were male (Table 2). Preliminary morphological identification demonstrated that these mosquitoes belonged to *Culex pipiens pallens*, *Armigeres subalbalus*, *Aedes vexans*, *Culex orientalis*, *Anopheles tessellatus*, *Anopheles gigas baileyi* and *Anopheles messeae*, *Aedes albopictus*. The neighbor joining tree (Fig. 2) based on COI DNA barcodes of five morphospecies revealed five distinct genetic clusters.

Table 2
The presence of the mosquitoes in different collections in Linzhi, Xizang

Study sites	Collection method	Date of the collections	No. of light traps/people	The number of mosquitoes	
				♀	♂
Bayi district	light trap	Aug.26th-27th	28	515	688
Bayi district	human landing catches	Aug.27th	8	5	0
Chayu County	light trap	Sep.2nd-3rd	36	737	439
Chayu County	human landing catches	Sep.2nd	4	0	0
Xiachayu Town	light trap	Aug.30th-Sep.1st	26	430	80
Xiachayu Town	human landing catches	Aug.30th	12	158	0
Milin County	light trap	Sep.9th-10th	26	288	10
Milin County	human landing catches	Sep.10th	7	8	0
Bomi County	light trap	Sep.4th-6th	20	188	3
Bomi County	human landing catches	Sep.4th	8	1	0
Lang County	light trap	Sep.7th-8th	28	69	29
Total	-	-	-	3648	

Mosquitoes collected by light traps

Using light traps (164light traps), mosquito surveillance was conducted from August 26 to September 10, 2019 (Fig. 3), 1203 mosquitoes (42.81% females, 57.19% males) were collected in Bayi District, 1177 mosquitoes (62.62% females, 37.38% males) were collected in Chayu County, 510 mosquitoes (84.31% females, 15.69% males) were collected in Xiachayu Town, 298 mosquitoes (95.67% females, 4.03% males) were collected in Milin County, 191 mosquitoes (98.43% females, 1.57% males) were collected in Bomi County, 98 mosquitoes (70.41% females, 29.59% males) were collected in Lang County (Table 3, Fig. 4).

Table 3
The density of mosquitoes in different sites by light traps in Linzhi, Xizang

The study sites	No. of light traps	<i>Culex pipiens pallens</i>		<i>Culex orientalis</i>		<i>Armigeres subalbatus</i>		<i>Aedes vexans</i>		<i>Anopheles</i>		Mean ¹
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
Bayi district	28	470	688	0	0	0	0	45	0	0	0	42.96
Chayu County	36	688	439	1	0	3	0	45	0	0	1	32.69
Xiachayu Town	26	207	25	83	15	134	40	1	0	5	0	19.62
Milin County	26	271	9	0	0	3	0	14	0	0	1	11.46
Bomi County	20	146	3	5	0	0	0	36	0	1	0	9.55
Lang County	28	44	28	0	0	4	0	20	0	1	1	3.50
Total	164	1826	1192	89	15	144	40	161	0	7	3	-
¹ Mean means mosquitoes per trap per night (light trap collection)												

The mean mosquito density was 42.96 (mosquitoes per trap per night), 32.69 (mosquitoes per trap per night), 19.62 (mosquitoes per trap per night), 11.46 (mosquitoes per trap per night), 9.55 (mosquitoes per trap per night), 3.50 (mosquitoes per trap per night) respectively (The sites were livestock sheds and houses were are many mosquitoes). Among them, 3064 (86.80%) belonged to *Culex pipiens pallens* are dominant species (Fig. 5), next 184 (5.29%) belonged to *Armigeres subalbatus*, 115 (2.99%) belonged to *Aedes vexans*, 104 (4.63%) belonged to *Culex orientalis*, a total of 10 (0.29%) *Anopheles tessellatus*, *Anopheles gigas baileyi* and *Anopheles messeae* with minimum proportion (Fig. 6). There was significant difference of the number of *Culex pipiens pallens* collected by light traps ($\chi^2=119.99$, $df=4$, $P<0.001$).

Mosquitoes by human landing catches

Aedes albopictus was only collected in Xiachayu Town, 158 mosquitoes were collected by human landing catches. The mean mosquito density was 26.33 (mosquitoes per hour per person). A 5 *Culex pipiens pallens* and 8 *Culex pipiens pallens* were collected in Bayi District and Milin County, respectively.

Mosquito larvae by container survey

The larvae were collected from waste tires, cement tanks and drums in Bomi, Milin and Lang counties. Through the preliminary morphological identification of the larvae, it clear that the larva species obtained by container survey methods were consistent with the adult species collected by light traps.

Discussion

Mosquito-borne diseases are spreading all over the world at an amazing speed. In recent years, there are more and more reports about mosquito-borne diseases^[18–20]. Based on morphological and molecular identification, this study revealed the composition and density of mosquito vectors and other relevant information in Linzhi, Xizang. Using light traps, it is found that the dominant mosquito species in Linzhi were *Culex pipiens pallens*, followed by *Armigeres subalbalus*, *Aedes vexans*, *Culex orientalis* and *Anopheles*. *Culex pipiens pallens* distributed in areas north of the Yangtse River, which is mainly distributed in the area below 3000 m above sea level^[21]. The highest elevation at which *Culex pipiens pallens* has been observed in China is 2,900 metres, in Mainling County, Nyingchi area, Tibet^[12], which is basically consistent with the findings of this study. The female mosquito of *Armigeres subalbalus* can stab and suck human blood fiercely. It is the mosquito family with greater harassment^[16]. Previous studies in Europe have demonstrated that *Aedes vexans* is not an effective vector of chikungunya virus but may play a secondary role in temperate regions where the mosquito is abundant^[22]. It is difficult to distinguish between *Culex orientalis* and *Culex mimulus* in morphology. The middle white ring of the beak is obvious which mainly distributed in Heilongjiang, Jilin and Liaoning in China, South Korea, Japan and other places abroad^[16]. In South Korea, it has been found that *Culex orientalis* can carry epidemic encephalitis V virus, which can be viewed as the second vector to transmit Japanese encephalitis except *Culex tritaeniorhynchus*^[23]. It was found that Bayi District had the highest mosquito density in the survey (42.96 mosquitoes per trap per night), Bayi District was located in the North Bank of the Yarlung Zangbo River in the south of Linzhi, Xizang, with sufficient water resources and wide wetland coverage area. Although the altitude was 2985 m, the vegetation coverage was good, and the population density of Bayi District is high. The distribution and density of mosquito species are closely related to the natural environment and human factors, and more mosquito breeding places will be formed due to frequent human activities. The distribution of *Culex orientalis* was found for the first time in Chayu and Bomi Counties. The density value of Chayu (3.77 mosquitoes per trap per night) is much higher than Bomi County (0.25 mosquitoes per trap per night). It is closely related to the climatic conditions of Chayu County. It has a low altitude area with high temperature, high precipitation and short sunshine time, which is suitable for mosquito breeding. *Anopheles* were found little in the survey, which were distributed in Chayu County, Xiachayu Town, Milin County, Bomi County and Lang County, it might be related to missing the local maximum temperature in the investigation season, and the other possible reason was that the cowshed preferred by *Anopheles* was in the vacant period. Compared with the collection of adult mosquitoes in 2009, the species of mosquito vectors are basically the same, but the diversity is lower than that in Motuo County. Due to the investigation of Japanese encephalitis vector in Linzhi, the selection of mosquito catching sites focused on the pig pen and outdoor environment suitable for the survival of *Culex tritaeniorhynchus*, resulting in the dominant species were *Culex pipiens pallens* in Linzhi different with Motuo (*Culex tritaeniorhynchus*).

Using human landing catches, *Aedes albopictus* was first found in Chayu County xiachayu town and only caught in this area with the high density (26.33 mosquitoes per hour per person). Xiachayu town has low altitude, mild climate and high rainfall, which is suitable for the breeding of *Aedes albopictus*^[24]. Combined with the discovery of *Aedes albopictus* in Motuo in 2009^[12], it is preliminarily speculated that there is a high potential epidemic risk of dengue fever in Chayu and Motuo County. Some research results show that seasonal weather change, vegetation height, population and land coverage will affect the population and distribution of *Aedes albopictus*, which are indicators to assess the risk of dengue transmission^[25, 26].

The container survey only collected larva in Bomi, Milin and Lang Counties. The results show that the preferred breeding place for mosquitoes is waste tires, cement cans and barrels, which are closely related to human beings.

Old tires are often found along the main roads or waste factories, where they are usually unattended and exposed to rain and sunshine for a long time. In communities with intermittent or inadequate water supply, cement tanks and buckets are the most common water storage containers. Although *Aedes albopictus* preferred water, no *Aedes albopictus* was found in this kind of habitat. It can be preliminarily inferred that *Aedes albopictus* did not occur population diffusion in Linzhi.

Aedes albopictus and *Culex orientalis* were found for the first time and were new records. In South Korea, Japan and other places, *Culex orientalis* is considered as an important vector for the transmission of Japanese encephalitis except *Culex tritaeniorhynchus*^[23]. It can not be considered that there is no epidemic risk of Japanese encephalitis in this area, so further virological testing is needed for certification. It has been reported to explore the correlation between malaria and meteorological factors in Motuo County, Xizang. The results show that temperature, relative humidity, rainfall and other meteorological variables are important environmental factors for malaria transmission^[27]. It can be preliminarily inferred that with the progress of malaria elimination, the local malaria epidemic risk has been at a very low risk level. Climate change, population flow and environmental change play an important role in mosquito distribution in Linzhi^[26]. In 2009, *Aedes albopictus* was first found in Motuo County, while in this study, *Aedes albopictus* was only found in Xiachayu. It is preliminarily speculated that there is mosquito diffusion barrier in Linzhi. Therefore, in order to better understand the local ecological characteristics of *Aedes albopictus*, it is urgent to further explore the possibility of population diffusion of *Aedes albopictus* in Linzhi. In the future, further warming is expected, and further economic development in Linzhi will lead to even greater movement of freight and people. These conditions raise the risk of outbreak of mosquito-borne diseases in a population with no prior exposure to such infections^[29, 30]. Therefore, it is urgent to strengthen the detection and monitoring of mosquito-borne diseases.

This study has limitations due to time and traffic restrictions, the research sites did not cover the whole area of Linzhi, but the six counties selected can fully represent the mosquito surveillance results of Linzhi. However, due to the maintenance of Motuo road traffic, the epidemic risk of mosquito diseases in Motuo County can only be preliminarily speculated. Secondly, the survey was carried out in August and September 2019 when the temperature is between 10°C-26°C slightly lower than the peak of mosquito activity. However, the results of this study can be used to preliminarily explore the species and distribution of mosquitoes in Linzhi, so as to speculate the epidemic risk of mosquito-borne diseases. The agricultural area along the border between China and Myanmar is rich in mosquitoes with obvious seasonality. The composition and density of mosquito vectors are greatly affected by the natural environment, and the risk of mosquito-borne disease transmission is relatively high^[31]. As a neighboring country of China, India has a wide range of mosquito species, and the prevalence of dengue fever, chikungunya fever, malaria and other mosquito borne diseases is serious^[32, 33]. Therefore, it is speculated that the mosquito density will increase in high temperature season, the diversity of mosquito species will increase, and the potential epidemic risk of various diseases will increase slightly. It is suggested that mosquito surveillance should be carried out again in Linzhi during the peak period of mosquito activity and the opening of Motuo road to obtain more accurate mosquito distribution and epidemic risk assessment of mosquito diseases. Mosquito monitoring is of great significance for the assessment of mosquito-borne diseases, which is consistent with the results of most studies^[34].

Mosquitoes had heterogeneous distribution throughout Linzhi, Xizang. With the increasingly developed traffic and global warming, it is necessary to further determine the composition and density of mosquitoes in this area, so as to assess the risk of disease transmission and establish an effective mosquito surveillance system. With the

continuous progress and development of Xizang's economy, people pay more attention to the pursuit of health. Mosquitoes can breed and breed in the high-altitude urban environment of Xizang, so that people have the risk of disease. Strengthened community health education and engagement should be conducted to better guarantee the health and life safety of local citizens^[35]. Mosquito-borne viruses pose a serious health threat to people. Mosquito surveillance is an essential part to control mosquito-borne diseases, and sometimes it is the only effective way to block or reduce the spread of these diseases (such as dengue fever)^[36, 37]. Using effective monitoring tools to characterize the species composition and density of mosquitoes is the most important step to control and assess the risk of mosquito-borne diseases outbreak^[28, 38]. It is suggested that *Aedes* vector monitoring should be carried out again in the high-risk counties in order to better grasp the species, distribution and growth and decline of mosquito vectors in the area, and increase the number and frequency of monitoring appropriately through monitoring data, so as to provide theoretical basis for scientific mosquito control and control.

Conclusion

In summary, our results revealed the mosquito density and population composition in Linzhi, which made up for the blank of mosquito surveillance in this area. The results showed that the dominant mosquito species in Linzhi were *Culex pipiens pallens*, and mosquitoes had heterogeneous distribution throughout Linzhi, Xizang. The mosquito density and species composition changed with the change of longitude, latitude and altitude. Among them, *Aedes albopictus* and *Culex orientalis* were found for the first time and were new records. It is speculated that it has a high potential epidemic risk of dengue fever in Chayu County Xiachayu Town, while the risk of malaria flow in Linzhi is low, and the epidemic risk of Japanese encephalitis can not be excluded. Due to the fact that there is no epidemic history of mosquito-borne diseases in the local, people's awareness of mosquito-borne diseases will be relatively weak. In view of the above situation, it is suggested that local citizens use physical barriers such as screens, nets and insecticides when the peak of mosquitoes. In the process of raising livestock, the distance between livestock and houses should be expanded as much as possible, and the awareness of mosquito prevention should be enhanced through the publicity of local communities or CDC to prevent the occurrence of mosquito-borne diseases. At the same time, local staff should timely clean up the waste tires, garbage stations and other mosquito breeding places to prevent the occurrence of mosquito breeding places, so as to minimize the risk of mosquito-borne diseases by controlling vector mosquitoes.

Abbreviations

China CDC: Chinese Center for Disease Control and Prevention; bp: base pair; COI: mitochondrial cytochrome c oxidase subunit 1 gene; LC01490 and HCO2198: abbreviation for universal primers; PCR: Polymerase Chain Reaction; XXZ18, XXZ20, XZ4, XZ19, XZ21, XZ22: the mosquitoes from Xiachayu town; XXZ19: the mosquito from Chayu County; XXZ22, XXZ12: the mosquitoes from Milin County; XXZ24: the mosquito from Bayi District; XXZ28: the mosquito from Bomi County; XZ3, XZ17: the mosquitoes from Lang County.

Declarations

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XRM conceived, designed the study. QYL, FXM, XDY, PCX, FTQ, YJY, BMCW and YHG participate in mosquito sampling work, XRM, PCX and YHG performed on mosquitoes identification, nucleic acid extraction, PCR amplification and sequencing.

XRM and DLM analyzed the data and interpreted the results. XRM drafted the article,

XRM, QYL, FXM, PCX, DLM and YHG jointly performed on revising the article. GLX and YHG gave suggestions and provided technical support throughout the experiments. All authors read and approved the final manuscript submitted.

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Figures

Figure 1

Map of the study sites in Linzhi, Xizang, China. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Neighbor joining tree for five morphospecies based on K2P distance in DNA barcodes. Numbers on branches represent bootstrap support (>70%) based on 1000 replicates; scale equals K2P genetic distance.

Figure 3

The number of mosquitoes in one day in Linzhi, Xizang, China

Figure 4

The percentage of mosquitoes in six sites by light traps.(a: Bayi District; b: Chayu County; c: Xiachayu Town; d:Milin County; e: Bomi County; f: Lang County;)

Figure 5

The percentage of *Culex pipiens pallens* in Linzhi, Xizang, China

Figure 6

The percentage of mosquitoes in Linzhi, Xizang, China