



## First report of a mosquito species “*Psorophora columbiae* (Dyar & Knab)” from Rajasthan, India

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### Abstract

Mosquitoes are important public health concern insects and many species are important vectors that transmit a wide variety of disease-causing pathogens i.e., protozoans, viruses, and nematodes. A mosquito species complex, *Psorophora confinnis* consists of three species as following; *Psorophora confinnis sensu stricto* (Lynch Arribalzaga), *Psorophora jamaicensis* (Theobald), and *Psorophora columbiae* (Dyar and Knab). *Psorophora columbiae* is an important vector for the Venezuelan Equine Encephalitis Virus that causes febrile illness and progressively severe encephalitis in both equines and humans. Mosquito samples were collected from Banswara district (Lat 23.558306° N & Long 74.37597° E) of Rajasthan, India during routine survey and sampling with the help of a mouth aspirator and transported to the laboratory. Standard identification keys were used to identify the mosquitoes at the species level. Based on morphological characteristics i.e., banding pattern of proboscis, palpi, wings, legs, and abdomen, it is confirmed as *Psorophora columbiae*. It was first time described by Dyar and Knab in 1906 from Grassymead, Virginia, and is commonly known as the dark rice field mosquito. This is the first report from the rice fields from Rajasthan in the Banswara district. Till now, there is no earlier report of this species from Rajasthan state and it will be a significant finding and contribution to understanding its distribution, behavior, and control of this species.

**Keywords:** *Psorophora columbiae*, Rajasthan, India, dark rice field mosquito, vector, venezuelan equine encephalitis

### Introduction

Mosquito species are an important vector for many diseases and are important public health concern insects. A wide range of disease-causing pathogens such as protozoans i.e., *Plasmodium* sps, viruses i.e., dengue, Zika, chikungunya, yellow fever, Japanese encephalitis and nematodes i.e., *Wuchereria bancrofti* are transmitted mainly by the species of *Anopheles*, *Aedes*, and *Culex* mosquitoes (WHO 2020)<sup>[19]</sup>. The mosquito species, *Psorophora columbiae* belongs to the *Psorophora confinnis* species complex which is composed of three species-*Psorophora confinnis sensu stricto* (Lynch Arribalzaga), *Psorophora jamaicensis* (Theobald), and *Psorophora columbiae* (Dyar and Knab) (Lanzaro *et al.* 2015; Bibbs *et al.* 2019)<sup>[7]</sup>. *Psorophora columbiae* was first time described by Dyar and Knab in 1906 from Grassymead, Virginia, along with three closely related species: *Psorophora floridense*, *Psorophora texanum*, and *Psorophora toltecum* (Lanzaro *et al.* 2015)<sup>[7]</sup>. Locally, it is known as Dark Rice Field Mosquito due to its high presence in rice growing regions and its dark coloration and also “glades mosquito” or “the Florida glades mosquito” due to its breeding association with grasslands (Bibbs *et al.* 2019)<sup>[2]</sup>.

It is widely distributed in Florida, across the United States, California, Mexico, Bahamas, Belize, Canada, Cayman Islands, Cuba, Honduras, Mexico, Panama, Central America, the Caribbean, and South America to Argentina (Rutgers 2022; Walter Reed Biosystematics Unit 2022)<sup>[15]</sup>.<sup>[8]</sup> *Psorophora columbiae* is an important vector for Venezuelan Equine Encephalitis (VEE), and anaplasmosis in cattle, and can also carry infective stages of canine heartworm, *Dirofilaria immitis* (Bibbs *et al.* 2019; Rutgers 2022)<sup>[2, 15]</sup>. It might be a vector for Rift Valley Fever Virus (RVFV) due to the tendency to feed on large mammals in areas with high populations (Turell *et al.* 2015)<sup>[16]</sup>. The

detection of West Nile Virus (WNV) in *Psorophora columbiae*, indicates it could be a vector for WNV (Pitzer *et al.* 2009)<sup>[11]</sup>.

An unexpected mosquito was collected during the routine survey and sampling in the Banswara district of Rajasthan, India. In preliminary observation, the collected mosquito is different and not belonging to the recorded and local mosquito fauna of Rajasthan. On behalf of morphological characters by comparing with the standard identification key it is identified and confirmed the species. Till now, there is no documentation on earlier reports of this species from the Rajasthan state of India, so it is the first documentation reporting of this species. The reporting has a high impact on understanding the distribution of this species and reporting of this species in new areas from where it was not previously reported.

### Material and Methods

#### Sampling site

Mosquito samples were collected during the month of October 2022 from Banswara district (Lat 23.558306° N & Long 74.37597° E) of Rajasthan state, India. Banswara is located in the southern part of Rajasthan, India between 23.11° to 23.56° N latitudes and 73.58° to 74.49° E longitudes. Due to the numerous islands on the Mahi River, it is also known as the city of hundred islands, and during the rainy season, many waterfalls become started to flow. From June 2022 to September 2022, approximately 1007.9 mm of rainfall was recorded and also recorded in starting of October 2022 (Central Flood Cell Rajasthan 2022)<sup>[4]</sup>. Rice cultivation is predominant near the sampling sites, making a suitable environment for breeding for mosquitoes. Figure 1 shows the geographical coordinates and location of the sample collection.

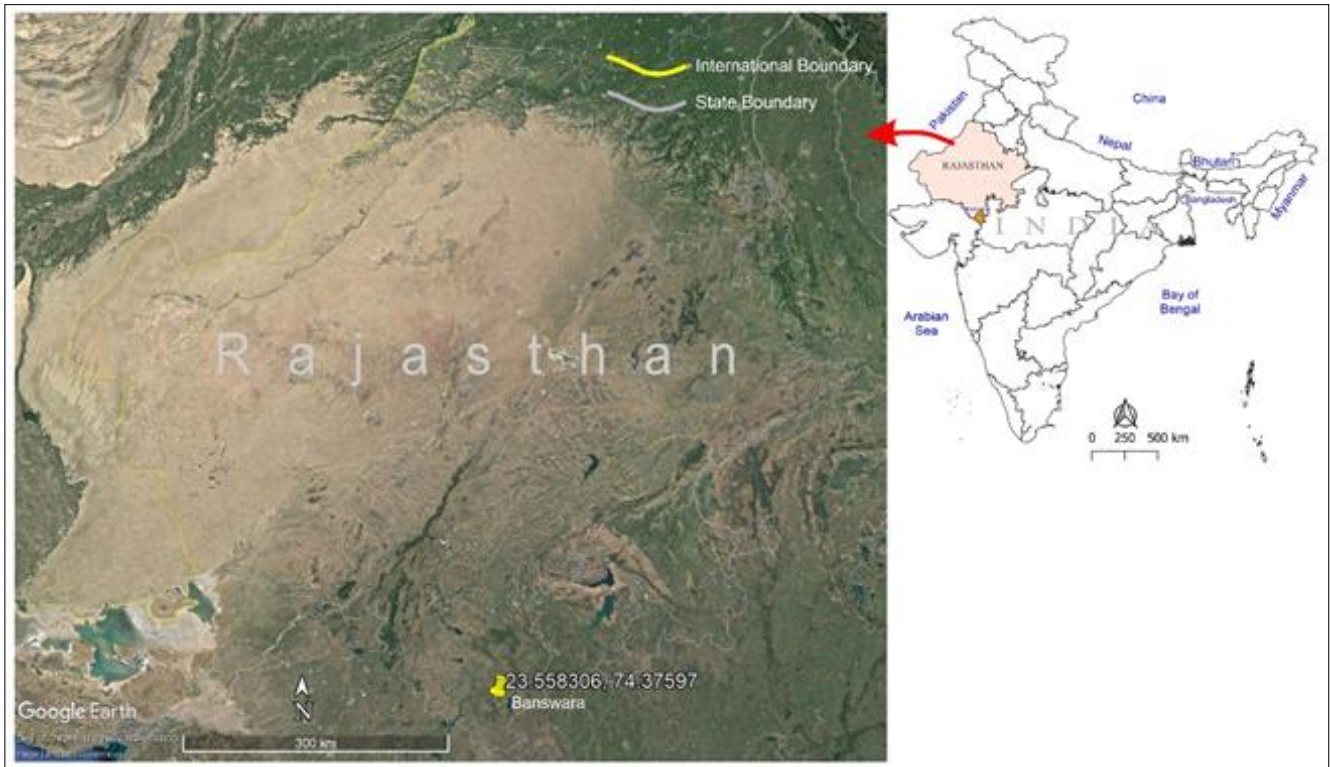


Fig 1

**Mosquito Collection, Transportation, and Identification**

Field collection of mosquitoes was carried out in nearby areas of the sampling sites. Adult mosquitoes were collected from indoor and outdoor houses and cattle sheds using a mouth aspirator.

After a successful collection, mosquitoes were carefully transferred in a caged cloth and transported to the laboratory for further processing and analysis. During the transportation, collected adult mosquitoes were allowed to feed on a 10% glucose solution soaked in a cotton plug.

After successful transportation to the laboratory, the mosquito was identified using different standard identification keys based on morphological characterization under stereo zoom microscopes (Bohart and Washino 1978; Varnado *et al.* 2012; Harrison *et al.* 2016; Rueda 2019; Becker *et al.* 2020) [3, 17, 6, 14, 1].

**Photography and Data Analysis**

Data were recorded and analyzed with the help of suitable software and instruments. Map to show the sampling site is constructed using QGIS 3.22.10, Google Earth Pro, and CorelDRAW 2020 software. Photographs and identification were taken placed under Leica S9i Stereomicroscope and Leica EZ4 W stereomicroscope while processed in CorelDRAW 2020 software.

**Results**

The collected mosquito species were identified as *Psorophora columbiae* based on their morphological characteristics. In adult females, distinguishing characteristics were observed and are used to identify the species as follows.

**General Appearance**

The collected mosquito sample is black in color with numerous white bands or patches on the legs and white and black scales on the wings (Figures 2a & 2b). Its abdomen

lies parallel to the resting surface, similar to the genus *Culex* and *Aedes*.



Fig 2a: Front view of Adult *Psorophora columbiae*

Fig 2b: Top view of Adult *Psorophora columbiae*

**Head**

The occiput is covered by white scales on the dorsal side with numerous erect scales (Figure 3). Palpi are dark and shorter than the proboscis with a pale tip and the clypeus is dark without white scales (Figure 4). The proboscis is scaled and dark in color with a broad pale band at the middle and a narrow pale tip (Figure 4).



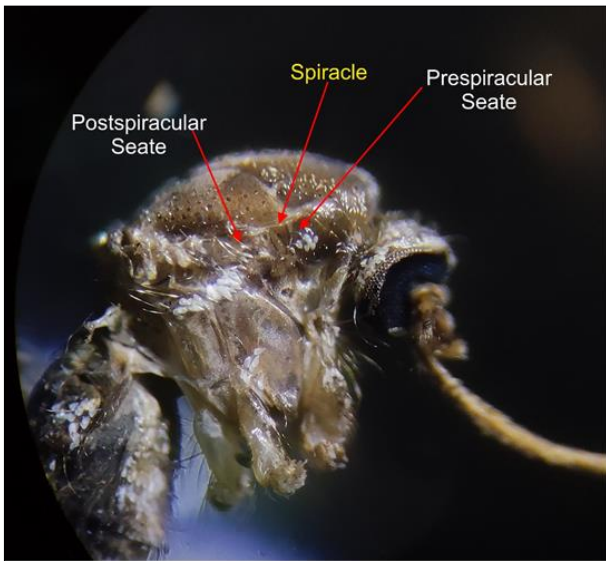
Fig 3: Occiput Shows covering of White scales and Erect scales



**Fig 4:** Band pattern and pale patches on Proboscis, Palpi, and Clypeus

**Thorax**

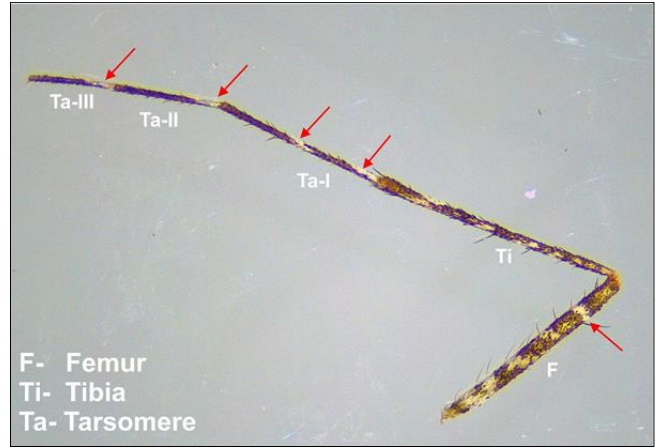
Pre- and post-spiracular setae are present on the thorax (Figure 5). Both wings are entirely covered with an undefined scattered pattern of black and white scales on veins (Figure 6 and Figure 2 top view). The hindleg's femur has numerous white patches of scales and a distinguishing subapical band of white scales (Figure 7). The tibia is dark with many white scaly spots throughout its length. (Figure 7). 1<sup>st</sup> tarsomere of the hind leg (Hind tarsomere 1) has a basal and median pale band of white scales while the remaining are with a white scaly ring (Figure 7). Tarsal claws are long, without basal teeth (Figure 8).



**Fig 5:** Prspiracular, Postspiracular setae, and Spiracle



**Fig 6:** Wing showing scattered pattern of black and white scales



**Fig 7:** Hind leg showing patches and banding pattern



**Fig 8:** Foreleg showing claws

**Abdomen**

1<sup>st</sup> Abdominal segment is with a pale scaly patch in the middle of the tergum, 2<sup>nd</sup> and 3<sup>rd</sup> tergum has a pale triangular patch on the apical side while the remaining 4<sup>th</sup> to 7<sup>th</sup> patches are in pairs and placed in the submedian position (Figure 9a). A mixed pattern of pale and dark scales covers the ventral side of the abdomen (Sterna) in which pale scales are predominant (Figure 9b).



**Fig 9a:** Dorsal view of abdomen showing Terga

**Fig 9b:** Ventral view of abdomen showing Sterna

## Discussion

Morphological characterization of adult females i.e., the pattern of dark and pale scales of wings, pattern of legs, pale band on the proboscis, and palpi confirm the species '*Psorophora columbiae*'. It is widely distributed in Florida, across the United States, California, Mexico, Bahamas, Belize, Canada, Cayman Islands, Cuba, Honduras, Mexico, Panama, Central America, the Caribbean, and South America to Argentina (Rutgers 2022; Walter Reed Biosystematics Unit 2022) <sup>[15, 18]</sup>. In the Banswara district of Rajasthan, this first occurrence is recorded during the routine survey and sampling of mosquitoes in October 2022. The high abundance and breeding of this species in rice growing fields and its dark coloration are referred to as the dark rice field mosquito in local areas (Olson and Huggins 1983) <sup>[10]</sup>.

Banswara is predominant in rice growing fields, heavy rainfall, the flow of the Mahi River, and the island collectively favor the breeding of *Psorophora columbiae* and make a suitable habitat for the establishment of this species. The high range of migration, and climatic and geographical conditions may also favor the invasion of this species in the rice fields of Banswara, and not much information is available about the previous reports of this species in this area. It is currently difficult to predict the timing of the arrival of this mosquito in Banswara and its abundance in this area as only a single specimen was found. Still, the biological and geographic ranges of this species are also unclear, and the reporting of this species to a new area is a point of concern as it is a vector for arboviruses.

*Psorophora columbiae* is considered a vector for Venezuelan equine encephalitis virus (VEEV) which affect both equines and humans that cause febrile illness and progressively severe encephalitis called Venezuelan Equine Encephalitis (VEE) disease, also anaplasmosis in cattles and canine heartworm (Lundberg *et al.* 2017; Bibbs *et al.* 2019; Rutgers 2022) <sup>[9, 2, 15]</sup>. However, studies suggest that it could be a vector for RVFV and WNV as pathogens were detected in *Psorophora columbiae* (Pitzer *et al.* 2009; Turell *et al.* 2015) <sup>[11, 16]</sup>. The establishment of this species could be an invitation to these diseases and a chance for the establishment in this area as well as in the nearby states of Rajasthan.

In recent years, many species i.e., *Aedes albopictus*, and *Anopheles stephensi* are being reported in new areas from where they were not previously reported (Roiz *et al.* 2011; World Health Organization 2022) <sup>[13, 20]</sup>. This may happen due to the movement, transportation, or exchange of resources, changes in lifestyle, land usage, and change in climate conditions (Leisnham and Juliano 2012) <sup>[8]</sup>. Studies also reported that *Psorophora columbiae* could migrate up to 14 km from their larval habitat, which also favors the invasion of this species in a new area (Robert *et al.* 1986) <sup>[12]</sup>. The climatic factor of Banswara favors the breeding that could lead to the establishment of this species as well as VEE disease.

In Rajasthan, it was not reported earlier and the first report of its occurrence could be a threat to be get infected with VEEV. However, it is widely distributed in the USA, but very few records of its occurrence are there from India. In India, its occurrence was reported in Madhya Pradesh state in 1998 and latest by July 2018 while in nearby countries of India, it is also reported from China (GBIF Backbone Taxonomy 2021) <sup>[5]</sup>. It can be a possibility of invasion from

Madhya Pradesh to Rajasthan and can be from China but the route of its invasion in Rajasthan, as well as India is still not clear.

## Conclusion

The collected mosquito sample was identified as *Psorophora columbiae* based on morphological characteristics. As it is a vector, the reporting of *Psorophora columbiae*, in a new area can be a threat to human health. This is the first reporting documentation in rice fields of Rajasthan, India, and will be a significant finding and contribution to the distribution, habitat, behavior as well as control of this species. Hence, it is important to undertake rigorous entomological surveillance for earlier detection of invasion for such important species. It is also important to prevent the establishment of this species in this area as well as in different areas of the country.

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## Authors' contributions

**Arti Prasad:** Mosquito identification; the concept of the manuscript; supervision.

**Pradeep Kumar Jangir:** Field collection of mosquitoes; mosquito identification, photography, and characterization; manuscript drafting & writing.

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## Availability of data and material

All supporting data to the study and findings are included in this article.

## Declarations

**Ethics approval:** The script does not require ethical approval.

**Consent to participate:** Not applicable.

**Consent for publication:** Not applicable.

**Conflicts of interest:** The author(s) have no conflict of interest.

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