

Integrated Morphological and Molecular Differentiation of *Culicoides oxystoma* and *Culicoides kingi* (Diptera: Ceratopogonidae) in India

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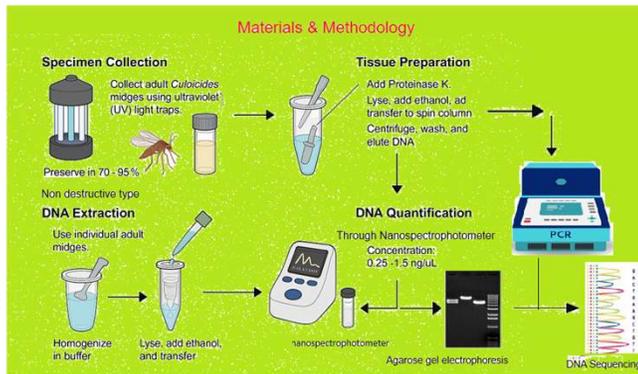
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INTRODUCTION & AIM

Culicoides biting midges transmit numerous livestock arboviruses (e.g. bluetongue [BTV], African horse sickness [AHSV], Epizootic hemorrhagic disease [EHDV], Schmallenberg virus) with major economic impact. For example, *C. oxystoma* is a known vector of Bluetongue virus in Asia and Africa and has been incriminated in Indian bluetongue transmission. Recent Indian studies identified *C. oxystoma* as dominant vectors of BTV around cattle. Worldwide, recognizing *C. oxystoma* and *C. kingi* is critical where they co-occur. However, both species are very small (1–3 mm) and exhibit overlapping characters, so vector surveillance must rely on robust identification. Classical keys note differences in wing pigmentation and body proportions, but these can be subtle. Female wings have similar shapes and spot patterns. Misidentifications are common unless detailed measures (e.g. ratios of antennal segments, palpal segments, spermathecae) are taken. Thus integrating molecular methods is increasingly used to confirm morphological determinations.

METHOD

Sample collection was performed at various target study areas by placing individual insect net, UV light traps near livestock premises at dawn & dusk and collected specimens preserved in 70% alcohol. Dissection performed using dissecting Stereoscopic Zoom Binocular in which head, thorax, wings, legs and genital part mounted on glass slides using Balsam Canada. After non-destructive type of DNA Extraction, PCR amplification was carried out with the primers used i.e. LCO1490 (5'-GGTCAACAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTCAGGGTGACCAAAAATCA-3'), which target a ~658 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene.



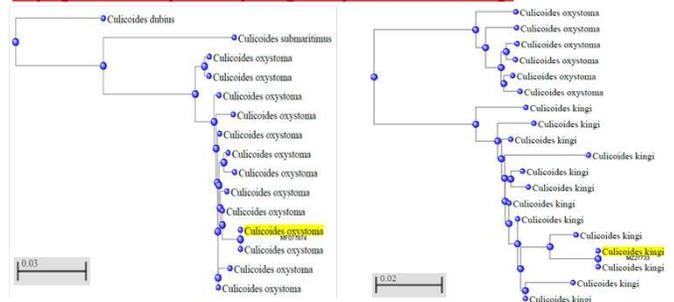
RESULTS & DISCUSSION

During the study period, a total of 143 biting midges were collected from the northern peninsular region of India. Of these, 78% were identified as females and 22% as males. Despite the limited sample size, *C. kingi* consistently displayed smaller measurements in comparison to *C. oxystoma*. These findings suggest that a combination of abdominal segment size and wing spot pattern provides an effective morphological criterion for species differentiation.

To the best of our knowledge, this is the first report that combines both morphological and molecular approaches to differentiate *C. oxystoma* from *C. kingi* in India. In this study, five out of thirteen evaluated morphometric variables in female specimens were found to be diagnostically valuable in supporting morphological identification. However, our findings show that the *C. oxystoma* specimens identified in this study through both morphological and molecular methods were closely related to populations from Tunisia, China, Israel, and Lebanon. Notably, they formed a distinct clade that was separate from *C. oxystoma* populations in Senegal, highlighting potential geographical variation within the species.

Feature	<i>Culicoides oxystoma</i>	<i>Culicoides kingi</i>
Wing	Distinctive pale spots and vein structures on the Radial sectors making wings as primary identifiers. Variations in these patterns can be observed among different species within the <i>Schultzei</i> group.	Wing patterns are less distinctive compared to <i>C. oxystoma</i> , making morphological identifiers more challenging.
Head	compound eyes barely separated with interfacetal hair	Similar head morphology with compound eyes and comparatively less interfacetal hair.
Antennae	Composed of 13 segments with sensilla cosioloconica on 2 nd , 3 rd , 4 th , 6 th , and 7 th flagellomeres, aiding in species differentiation.	Antennae structure is similar, but sensilla distributions may appear on 2 nd , 3 rd , 4 th , 5 th , 6 th , and 7 th flagellomeres
Maxillary Palp	Typically, five-segmented with a sensory pit on the third segment; length and shape of sensory pit deepens.	Similar segmentation; however, variations in length and shape of sensory pit is wide
Tibial Comb	Presence and arrangement of spines on the hind tibial comb are characteristic, usually 4 in number, used in species-level identification.	Tibial comb morphology is comparable, but spine count and arrangement may differ slightly.
Spermathecae	Females possess two spermathecae with one rudimentary spermatheca with specific shapes and sizes; these structures are critical for distinguishing closely related species.	Also having two spermathecae, but rudimentary spermatheca sometimes developed fully.

Phylogenetic analysis comparing *C. oxystoma* and *C. kingi*



CONCLUSION

In this study, we demonstrated that *C. oxystoma* can be effectively distinguished from *C. kingi* based on wing morphology and other morphological measurements. Ultimately, integrating both morphological and molecular approaches is essential for improving the systematics of *C. oxystoma*, a species of medical and veterinary importance due to its role as a disease vector.

FUTURE WORK / REFERENCES

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