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Review Article

Species Diversity, Ecology, and Pathogen Associations of *Culicoides* Biting Midges in Thailand: A Systematic Review and Meta-Analysis (2015–2025)

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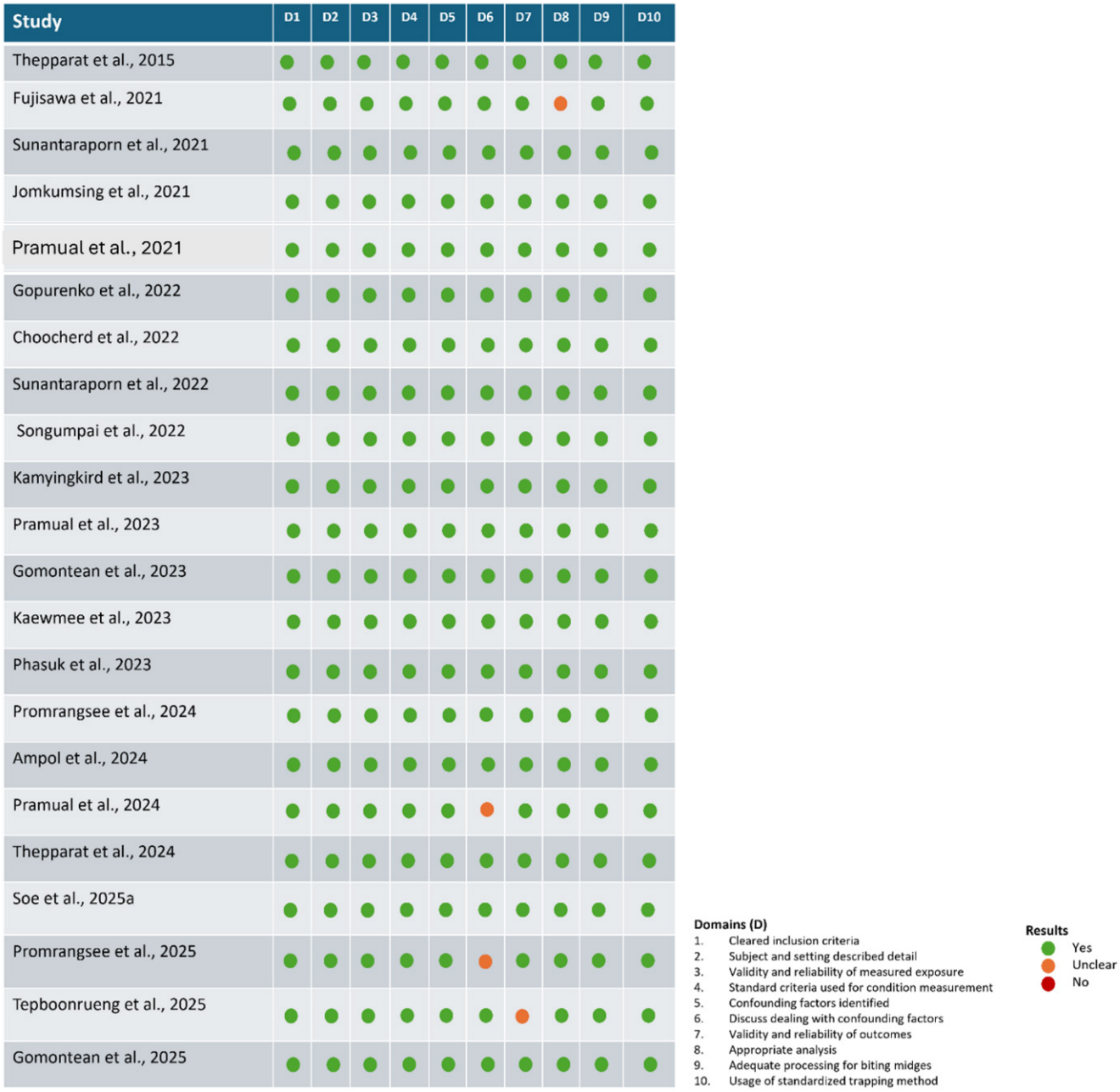
Supplementary File 1: PRISMA checklist.

Section and topic	Item #	Checklist item	Location where item is reported
Title			
Title	1	Identify the report as a systematic review.	Lines 1-2
Abstract			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Lines 26-29
Introduction			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Lines 45-113
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	
Methods			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Lines 137-144
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Lines 125-136
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Lines 125-144
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Lines 137-163 Suppl file 2
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Lines 137-163
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Lines 125-181 Table 1
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Table 1
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Suppl file 2
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Lines 164-196
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Lines 164-196
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Lines 164-196
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Lines 164-196
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Lines 164-196
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Lines 182-192
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	None
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Lines 145-163

Table continues on next page.....

Section and topic	Item #	Checklist item	Location where item is reported
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Lines 164-196
Results			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Lines 197-225
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Suppl file 2
Study characteristics	17	Cite each included study and present its characteristics.	Suppl file 3
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Suppl file 2
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Lines 197-300
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Lines 197-300
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Lines 197-353
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Figures 1-10
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Figure 10
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Suppl file 2
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Figures 2-10 Lines 197-353
Discussion			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Lines 354-539
	23b	Discuss any limitations of the evidence included in the review.	Lines 540-560
	23c	Discuss any limitations of the review processes used.	Lines 540-560
	23d	Discuss implications of the results for practice, policy, and future research.	Lines 561-580
Other information			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Lines 135-136
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Lines 126-136
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Lines 135-136
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Lines 598-600
Competing interests	26	Declare any competing interests of review authors.	Lines 598-600
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Lines 135-136

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al*. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71; For more information, visit: http://www.prisma-statement.org/



Supplementary File 2: Quality assessment of included studies. Analytical cross-sectional studies: 22

Supplementary File 3: Categories, variables and factor labels studied from the 22 articles.

Category	Variable	Factor label
Bibliometrics	**Title**	
	Publication year	2015-2025
	Indexed in JCR	Yes; No
	Journal quartile	Q1; Q2; Q3; Q4
Material and methods	Study the presence/abundance of *Culicoides*	Yes; No
	Study area	Southern; western; northern; northeastern; eastern
	State/District/Province	
	Sampling places	Mixed-livestock farms; peri-urban; horse stable; animal shelters; caves; plantations; ponds; streams; mangroves; beaches
	Publication period	2015; 2021; 2022; 2023; 2024; 2025

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Category	Variable	Factor label
	Study period	3 days – 7 year
	Sample size	
	Collection method	CDC light traps; net sweeping; Incandescent (IN-CND) light trap; White fluorescent (WHT-FLR) light trap; Ultraviolet fluorescent (UV-FLR) light trap; UV light-emitting diode (UV-LED); simple UV light traps; traps with white fine-mesh materials
	Diagnostic techniques	Taxonomic keys; COI gene specific PCR; COI barcoding
	Targeted gene for pathogen detection	ITS 1; 3'UTR-HSP70-I; ssu rRNA; gGAPDH; 12S rRNA; cyt *b*; BTV Partial S10
	Targeted gene for host blood meal analysis	cyt *b*; prepronociceptin (PNOC)
Results	*Culicoides* identification	Yes/No
	Culicoides genus	Culicoides
	Culicoides subgenera/group	*Trithecoides*; *Avaritia*; *Hoffmania*; *Haemophoructus*; *Meijerehelea*; *Beltranmyia*; *Remmia*; Clavipalpis; Ornatus; Shortti; Shermani
	Culicoides species	
	Number of identified genera	
	Number of identified subgenera and group	
	Most abundant species	
	Number of individuals of the most abundant species	
	Percentage of most abundant species of the total sampled	
	Temporal distribution	
	Pathogen detection in *Culicoides* Number of analyzed samples	Yes; No
	Number of positive samples	
	Pathogen genus	
	Pathogen species	
	Species of *Culicoides* in which the pathogen was detected	
	Host blood meal analysis	Human; Cattle; Water buffaloes; Goats; Chicken; Horses; Pigs; Dogs; Birds; Deer
	Other findings	

Supplementary File 4: The list of studies included in systematic review.

No.	Title	References
1	Ten species of *Culicoides* Latreille (Diptera: Ceratopogonidae) newly recorded from Thailand	Thepparat *et al.*, 2015
2	DNA barcode identification and molecular detection of bluetongue virus in *Culicoides* biting midges (Diptera: Ceratopogonidae) from western Thailand	Fujisawa *et al.*, 2021
3	Genetic variation, DNA barcoding and blood meal identification of *Culicoides* Latreille biting midges (Diptera: Ceratopogonidae) in Thailand	Jomkumsing *et al.*, 2021
4	*Culicoides* Latreille (Diptera: Ceratopogonidae) as potential vectors for *Leishmania martiniquensis* and *Trypanosoma* sp. in northern Thailand	Sunantaraporn *et al.*, 2021
5	Molecular detection of avian haemosporidian parasites in biting midges (Diptera: Ceratopogonidae) from Thailand	Pramual *et al.*, 2021
6	DNA barcoding of *Culicoides* Latreille (Diptera: Ceratopogonidae) From Thailand reveals taxonomic inconsistencies and novel diversity among reported sequences	Gopurenko *et al.*, 2022

Table continues on next page.....

No.	Title	References
7	Molecular identification of host blood meals and detection of blood parasites in *Culicoides* Latreille (Diptera: Ceratopogonidae) collected from Phatthalung Province, Southern Thailand	Sunantaraporn *et al*., 2022
8	Preliminary study on comparative efficacy of four light sources for trapping *Culicoides* spp. (Diptera: Ceratopogonidae) in Prachuap Khiri Khan Province, Thailand	Choocherd *et al*., 2022
9	First evidence of co-circulation of emerging *Leishmania martiniquensis*, *Leishmania orientalis*, and *Crithidia* sp. in *Culicoides* biting midges (Diptera: Ceratopogonidae), the putative vectors for autochthonous transmission in Southern Thailand	Songumpai *et al*., 2022
10	Molecular identification of *Culicoides* species and host preference blood meal in the African Horse Sickness outbreak-affected area in Hua Hin District, Prachuap Khiri Khan Province, Thailand	Kamyingkird *et al*., 2023
11	Molecular detection of filarial nematode from *Culicoides* biting midges (Diptera: Ceratopogonidae) in northeastern Thailand	Pramual *et al*., 2023
12	Diversity, abundance and host blood meal analysis of *Culicoides* Latreille (Diptera: Ceratopogonidae) from cattle pens in different land use types from Thailand	Gomontean *et al*., 2023
13	Natural infection with *Leishmania* (*Mundinia*) *martiniquensis* supports *Culicoides peregrinus* (Diptera: Ceratopogonidae) as a potential vector of leishmaniasis and characterization of a *Crithidia* sp. isolated from the midges	Kaewmee *et al*., 2023
14	Seasonal abundance of *Culicoides imicola* and *Culicoides oxystoma* in Prachuap Khiri Khan province, Thailand	Phasuk *et al*., 2023
15	Seasonal dynamics, *Leishmania* diversity, and nanopore-based metabarcoding of blood meal origins in *Culicoides* spp. in the newly emerging focus of leishmaniasis in Northern Thailand	Promrangsee *et al*., 2024
16	Seasonal abundance and diversity of *Culicoides* biting midges in livestock sheds in Kanchanaburi Province, Thailand	Thepparat *et al*., 2024
17	Nanopore-based surveillance of *Leishmania* parasites in *Culicoides* Latrielle (Diptera: Ceratopogonidae) caught from the affected community and Tham Phra Cave in Chiang Rai Province, the endemic area of leishmaniasis in Northern Thailand	Ampol *et al*., 2024
18	Population genetics of the biting midge *Culicoides oxystoma* Kieffer (Diptera: Ceratopogonidae) from Thailand and its genetic relationships with global populations	Pramual *et al*., 2024
19	Molecular detection of parasites and host preference in wildcaught *Culicoides* biting midges (Diptera: Ceratopogonidae) in Chiang Mai and Nakhon Si Thammarat Provinces, Thailand	Soe *et al*., 2025a
20	First record of mermithid parasitism in adult biting midges, *Culicoides huffi* (Diptera: Ceratopogonidae), collected from Southern Thailand, with ultrastructural and molecular characterization	Promrangsee *et al*., 2025
21	DNA barcoding of *Culicoides* biting midges (Diptera: Ceratopogonidae) and detection of *Leishmania* and other trypanosomatids in southern Thailand	Tepboonrueng *et al*., 2025
22	Immature habitats of *Culicoides* Latreille (Diptera: Ceratopogonidae) from Northeastern Thailand	Gomontean *et al*., 2025

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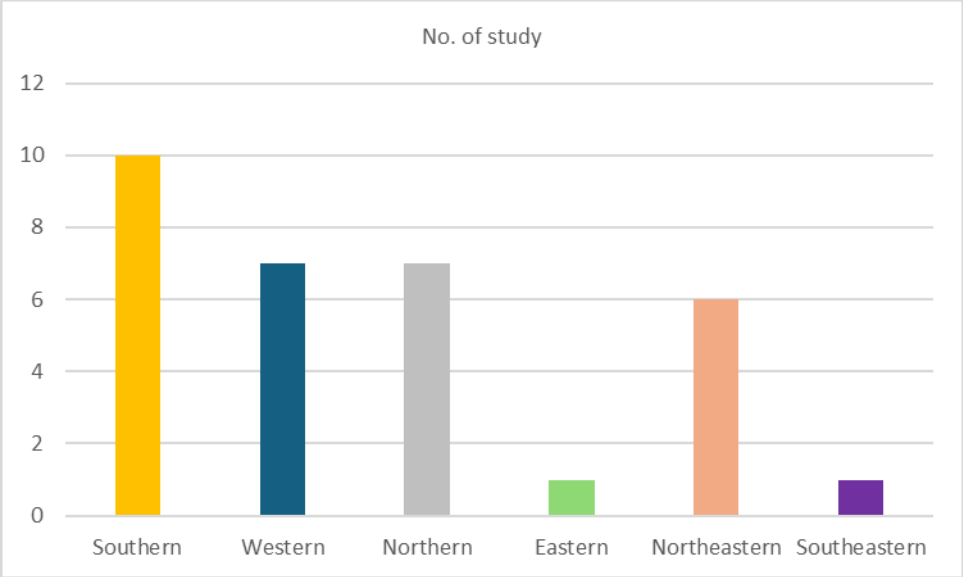
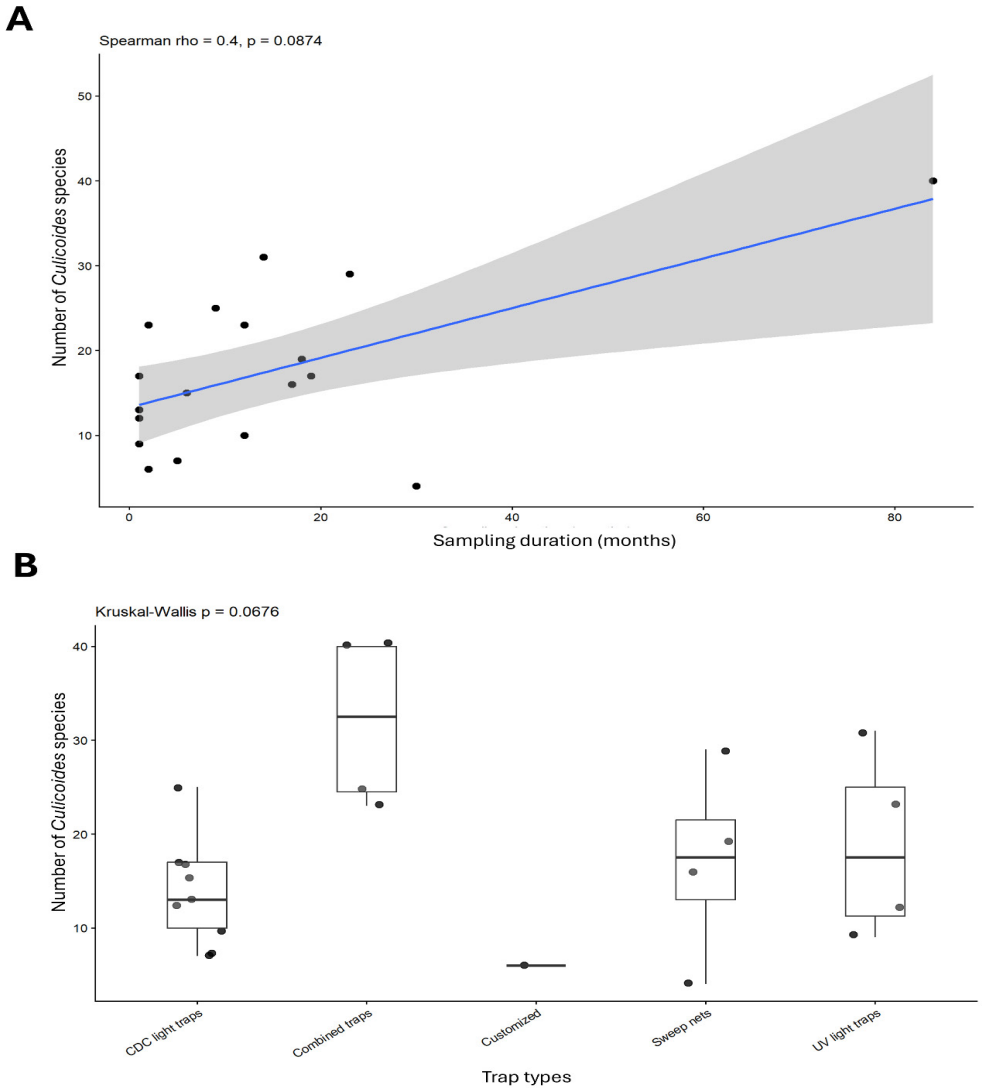


Supplementary File 5: Descriptive data according to geographical region of 22 included studies.



Supplementary File 6: Influence of sampling effort on *Culicoides* species richness in Thailand. (A) Relationship between sampling duration and the number of recorded *Culicoides* species. (B) Comparison of species richness among different trap types. No significant associations were observed for sampling duration (Spearman's rho = 0.4, p = 0.0874) or trap type (Kruskal–Wallis test, p = 0.0676).

Links Researchers

Supplementary File 7: Compiled information on *Culicoides* species and detected host blood meal.

No.	Host	Culicoides species	Targeted gene	References
1.	Human	*C. brevitarsis*	cyt *b* and prepronociceptin (PNOC)	Gomontean *et al*., 2023
		C. oxystoma		Kamyingkird *et al*., 2023
		C. imicola		Promrangsee *et al*., 2024
		Culicoides sp.		Soe *et al*., 2025a
		C. orientalis		Tepboonrueng *et al*., 2025
		C. sumatrae		
2.	Dogs	*C. brevitarsis*	cyt *b*	Sunantaraporn *et al*., 2022
		C. oxystoma		Kamyingkird *et al*., 2023
		C. imicola		
		C. peregrinus		
		C. guttifer		
3.	Birds	*C. brevitarsis*	cyt *b*	Gomontean *et al*., 2023
		C. flavicutatus		
4.	Cattle	*C. oxystoma*	cyt *b*	Jomkumsing *et al*., 2021
		C. peregrinus		Songumpai *et al*., 2022
		C. imicola		Sunantaraporn *et al*., 2022
		C. innoxius		Kaewmee *et al*., 2023
		C. fordae		Gomontean *et al*., 2023
		C. orientalis		Promrangsee *et al*., 2024
		C. (*Trithecoides*) spp.		Soe *et al*., 2025a
		C. mahasarakhamense		Tepboonrueng *et al*., 2025
		C. fulvus		
		C. elbeli		
		C. shortti		
		C. actoni		
		C. jacobsoni		
		C. insignipennis		
		C. gemmelus		
		C. sumatrae		
		C. huffi		
5.	Water buffaloes	*C. brevitarsis*	cyt *b*	Jomkumsing *et al*., 2021
		C. oxystoma		Gomontean *et al*., 2023
		C. peregrinus		
		C. immicola		
		C. actoni		
		C. brevipalpis		
		C. asiana		
		C. flavicutatus		
		C. fulvus		
		C. geminus		
		C. hegneri		
		C. innoxius		
		C. papifer		
		C. shortti		

Table continues on next page.....

No.	Host	Culicoides species	Targeted gene	References
		C. parahumeralis		
6.	Goats	*C. fordae*	cyt *b*	Songumpai *et al.*, 2022
		C. elbeli		Promrangsee *et al.*, 2024
		C. flavicutatus		
		C. shortti		
7.	Pigs	*C. oxystoma*	cyt *b*	Sunantaraporn *et al.*, 2022
		C. imicola		Kamyingkird *et al.*, 2023
		C. orientalis		Promrangsee *et al.*, 2024
		C. peregrinus		
		C. arakawae		
8.	Chickens	*C. oxystoma*	cyt *b*	Jomkumsing *et al.*, 2021
		C. arakawae		Sunantaraporn *et al.*, 2021
		C. guttifer		Sunantaraporn *et al.*, 2022
		C. actoni		Gomontean *et al.*, 2023
		C. (*Trithecoides*) spp.		Soe *et al.*, 2025a
		C. fulvus		Tepboonrueng *et al.*, 2025
		C. huffi		
		C. mahasarakhamense		
		C. shortti		
		C. peregrinus		
		C. jacobsoni		
		C. insignipennis		
		C. innoxius		
		C. gemmelus		
9.	Horses	*C. brevitarsis*	cyt *b*	Kamyingkird *et al.*, 2023
		C. oxystoma		
		C. imicola		
		C. peregrinus		
		C. actoni		
		C. flavipunctatus		
		C. innoxius		
		C.histrio		
		C. arakawae		
		C. geminus		
10.	Deer	*C. shortti*	cyt *b*	Promrangsee *et al.*, 2024
		C. actoni		

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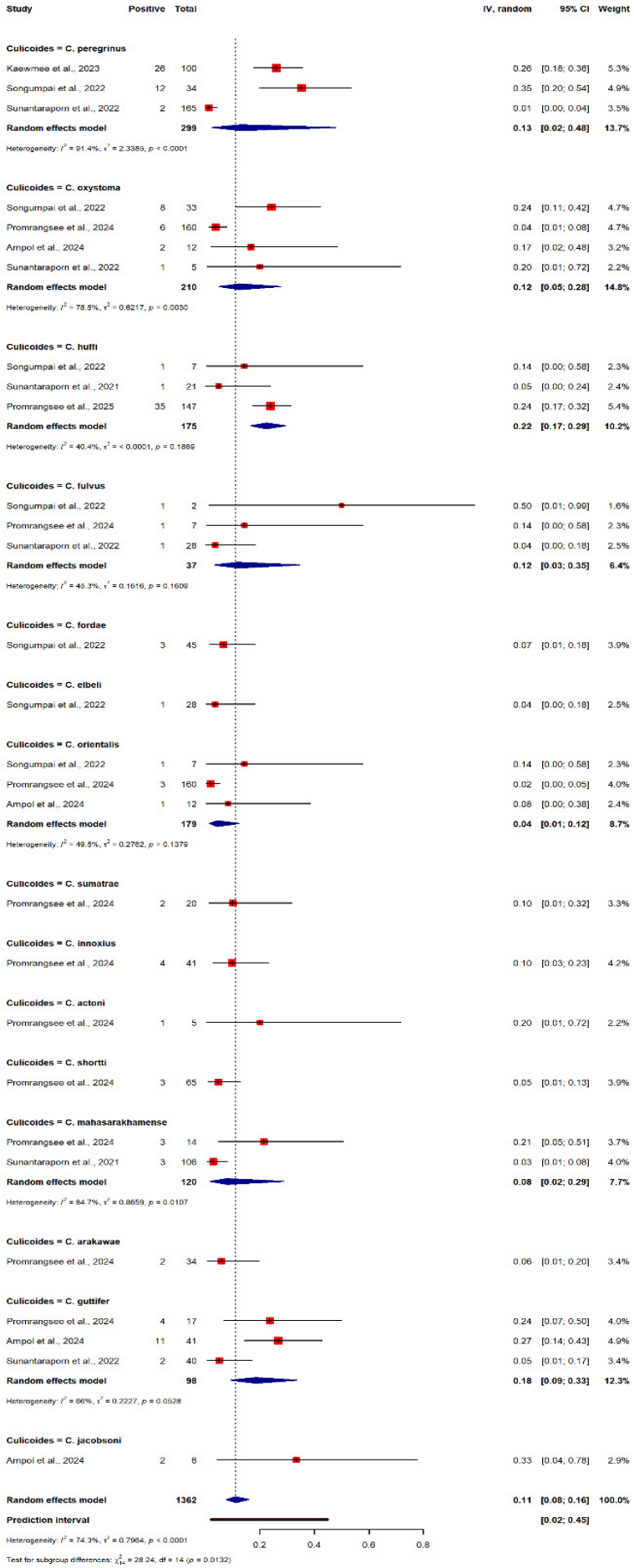
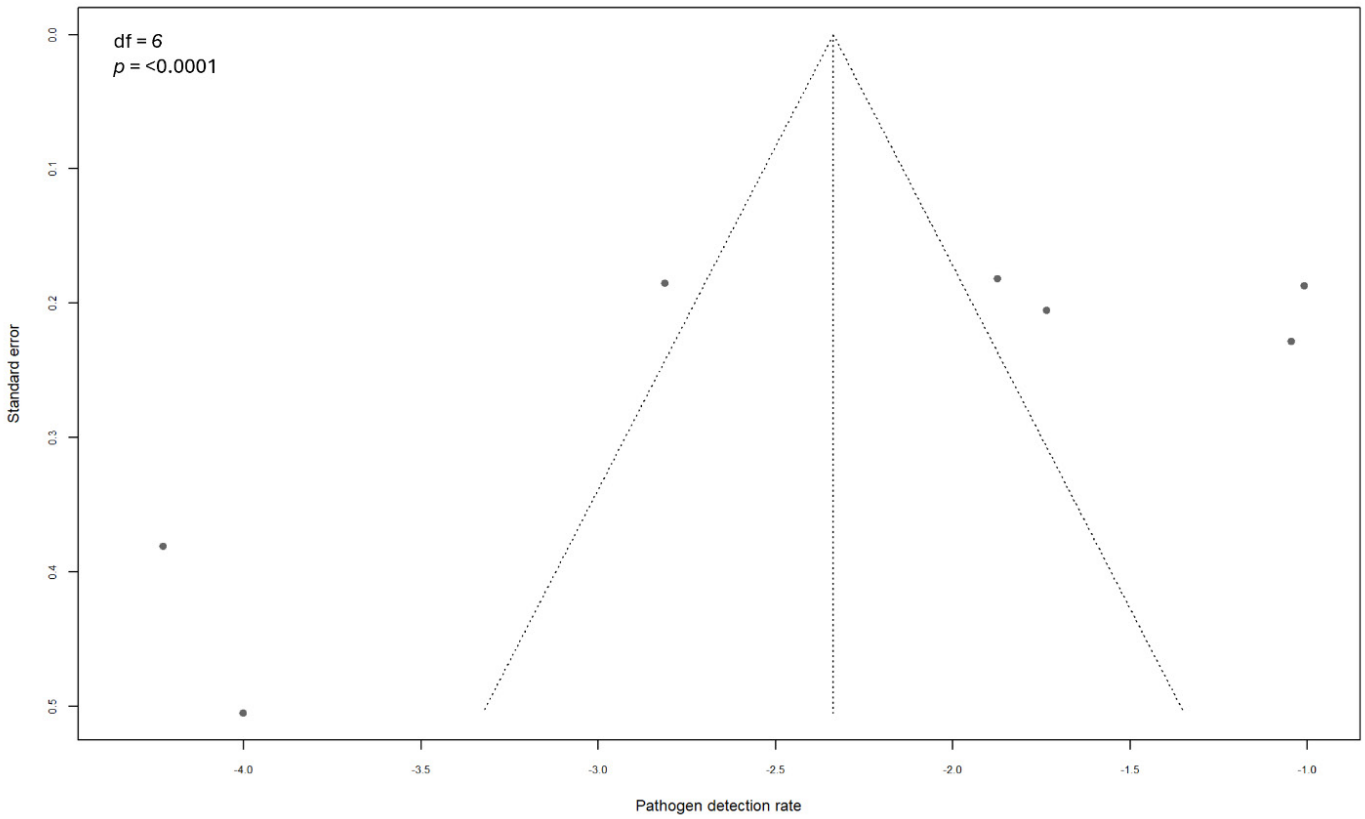


Supplementary File 8: Sub-group analysis according to *Culicoides* species.



Supplementary File 9: Funnel plot assessing publication bias for pathogen detection rates. Each point represents an individual study. Egger's regression test indicated no significant funnel plot asymmetry (p = <0.0001).

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