THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE MONTPELLIER SUPAGRO

En Biologie des populations et écologie

École doctorale GAIA – Biodiversité, Agriculture, Alimentation, Environnement, Terre, Eau
Portée par

Unité de recherche ASTRE, CIRAD-INRA

DETERMINANTS ENVIRONNEMENTAUX DE LA DISTRIBUTION DES *CULICOIDES* (DIPTERA : CERATOPOGONIDAE), MOUCHERONS VECTEURS DE VIRUS ANIMAUX D'INTERET ECONOMIQUE AU MAROC

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Le 24 octobre 2019

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Remerciements

Cette thèse est le résultat de trois ans de travail au sein de l'unité de parasitologie et maladies parasitaires de l'Institut Agronomique et Vétérinaire Hassan II, Rabat (Maroc) et de l'UMR ASTRE Cirad/INRA, Montpellier (France). Je ne serais jamais arrivée là, sans l'aide précieuse de beaucoup de personnes que je tiens à remercier.

Je tiens à remercier mes directeurs de thèse Khalid Khallayoune et Claire Garros, pour leur encadrement et leur soutien tout au long de cette période. Vous avez su m'apporter un soutien constant, une disponibilité, une écoute, une confiance et des conseils précieux et avisés à la hauteur de vos compétences et de vos qualités humaines.

Je tiens à remercier Thomas Balenghien mon encadrant, sans toi ce travail n'aurait jamais vu le jour, je te remercie infiniment pour ton écoute, ta disponibilité, ta patience, tes connaissances, tes critiques et tes conseils constructifs qui m'ont guidé tout au long de cette thèse. Tu as partagé avec moi sans compter ta passion pour les *Culicoides*. Ton expérience scientifique a été un grand apport pour moi.

Un grand merci à Intissar Boukhari, Ignace Rakotoarivony et Maxime Duhayon, pour leur précieuse aide sur le terrain, pour les identifications mais aussi pour votre soutien tout au long de ce travail.

Je tiens à adresser mes sincères remerciements à Karine Huber et Thierry Baldet, pour les conseils judicieux qu'ils ont su me donner.

Je remercie Laetitia Gardès et Antoine Mignotte pour leur initiation aux analyses moléculaires.

Je remercie également les Professeurs Abdelkbir Rhalem, Mohammed Bouslikhane et Ghita Chlyeh pour leurs encouragements.

Je tiens à remercier Dr Youssef Lhor, Dr Mounir Khayli, Dr Yassir Lezaar et l'ensemble des chefs de service vétérinaire, vétérinaires et techniciens de l'ONSSA pour l'appui logistique sur le terrain.

Merci à l'ensemble des personnes de l'unité de parasitologie et maladies parasitaires et de l'UMR Cirad/ASTRE, de m'avoir intégré en tant qu'entomologiste dans un monde pluridisciplinaire et pour les bons moments partagés.

Je remercie les membres du jury d'avoir accepté de se réunir pour évaluer ce travail. Merci pour le temps et l'attention que vous apporté à celui ci.

Un grand merci à mon fils, mes parents, ma grand-mère Ira, mon mari et mes amis qui ont toujours été là pour m'épauler, m'encourager et m'aider à surmonter de nombreuses épreuves.

Je dédie ce travail à Tiatia parti trop tôt.

Cette thèse a bénéficié d'un cofinancement de l'Institut Agronomique et Vétérinaire Hassan II et de l'UMR ASTRE Cirad/INRA dans le cadre d'un programme de bourse d'étude de doctorat. Je tiens à les remercier vivement. Cette étude a été soutenue par le Projet Hubert Curien (PHC) Toubkal (Toubkal/17/38 campus France 36867VH) et le Centre National de Recherche Scientifique et Technique (CNRST). Je les remercie. Je remecie également le projet Vectornet, financé par l'EFSA et l'ECDC d'avoir pris en charge la partie terrain.

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Liste des valorisations

Communication orales et écrites

1. **Bourquia M.,** Balenghien T., Rakotoarivony I., Gardès L., Chakrani M., Boukhari I., Huber K., Garros C., Khallaayoune K. **2017**. *Culicoides* composition in different host environments and updated checklist in Morocco **[poster].** 7th International Congress of the Society for Vector Ecology, Palma of Mallorca, Balearic Islands, Spain (1st-7th October 2017).
2. **Bourquia M.,** Balenghien T., Boukhari I., Rakotoarivony I., Duhayon M., Gardès L., Huber K., Garros C., Khallaayoune K. **2018**. How to obtain *Culicoides* abundance data at a national scale with a single collection season **[poster]**. Innovsur, Supagro Montpellier, France (16th-18th May 2018).
3. **Bourquia M.,** Balenghien T., Boukhari I., Rakotoarivony I., Duhayon M., Gardès L., Huber K., Garros C., Khallaayoune K. **2018**. How to obtain *Culicoides* abundance data at a national scale with a single collection season. XIth European Congress of Entomolgy, Napoli, Italy (2nd-6th July 2018).
4. **Bourquia M.,** Balenghien T., Rakotoarivony I., Boukhari I., Duhayon M., Baldet T., Wint W., Khayli M., Garros C., Khallaayoune K. **2018**. Towards a risk mapping of *Culicoides*-borne diseases in Morocco. 21st congress of the European Society for Vector Ecology, Palermo, Italy (22nd-26th October 2018).

Articles de rang A

1. **Bourquia M.**, Garros C., Rakotoarivony I., Gardès L., Huber K., Boukhari I., Delécolle J.C., Baldet T., Mignotte A., Lhor Y., Khallaayoune K., Balenghien T. 2019. Update of the species checklist of *Culicoides* Latreille biting midges (Diptera: Ceratopogonidae) of Morocco. 12:459. Parasites & Vectors.
2. **Bourquia M.**, Garros C., Rakotoarivony I., Boukhari I., Chakrani M., Huber K., Gardès L., Wint W., Thierry Baldet T., Khallaayoune K., Balenghien T. 2019. Composition and seasonality of *Culicoides* in different host environments in the Rabat region (Morocco). Revue d'Élevage et de Médecine Vétérinaire des Pays tropicaux. Accepted.
3. **Bourquia M.**, Garros C., Rakotoarivony I., Boukhari I., Duhayon M., Huber K., Mathieu B., Mignotte A., Baldet T., Wint W., Khayli M., Khallaayoune K., Balenghien T. 2019. Distribution of *Culicoides* species in Morocco, and environmental factors driving it. In prep

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Résumé

Les *Culicoides* sont de petits moucherons piqueurs hématophages, appartenant à la famille des Ceratopogonidae, le genre est présent sur toutes les surfaces terrestres, à l'exception de l'Antarctique. Une très faible proportion de la diversité du genre *Culicoides* a un rôle épidémiologique avec un impact significatif sur la santé humaine et animale. En effet, un certain nombre d'espèces est connu pour être impliqué dans la transmission biologique de virus, de protozoaires et d'helminthes. L'importance des *Culicoides* tient surtout à leur capacité à transmettre le virus de la fièvre catarrhale ovine et le virus de la peste équine, qui ont par le passé, provoqué des épizooties massives, à l'origine par exemple à la fin des années 1950 de la mort de 180 000 moutons dans le sud de la Péninsule ibérique et de 300 000 chevaux au Proche et Moyen-Orient. De plus, certaines espèces ont un comportement mammophile et sont des nuisances importantes pour les activités humaines de tourisme ou d'agroforesterie et harassent les animaux d'élevage (dermatite estivale récidivante chez les chevaux). Malgré cette importance vétérinaire les *Culicoides* restent un groupe de diptères peu étudié. Dans le but de mieux comprendre la distribution des *Culicoides* au Maroc, pays qui a connu des épizooties de fièvre catarrhale ovine et de peste équine, notre travail de thèse aborde à travers 3 volets la caractérisation des facteurs environnementaux déterminant l'abondance des espèces.

Le premier volet s'intéresse à la révision taxonomique de la diversité des espèces de *Culicoides* présente au Maroc avec une approche morphologique et moléculaire. Ce travail a permis la mise à jour de la liste d'espèces de *Culicoides* présentes au Maroc, de confirmer la présence d'au moins huit espèces vectrices -avérées ou potentielles- des virus de la fièvre catarrhale ovine (endémique depuis 2004), de la peste équine (éradiqué grâce à la vaccination, mais avec un risque de réintroduction) et de la maladie hémorragique épizootique (EHDV) (absent depuis 2006). Cette étude a aussi recensé pour la première fois 4 espèces nouvelles pour la faune marocaine, à savoir *Culicoides paolae, Culicoides subfagineus, Culicoides achrayi et Culicoides haranti.*

Le second volet s'attache à caractériser la diversité et la saisonnalité des espèces de *Culicoides* d'intérêt vétérinaire dans différents environnements d'élevage (bovin, ovin, équin). Nos résultats montrent que la diversité spécifique est la même quel que soit le type d'élevage, mais qu'elle varie significativement selon la saison. Des variations de l'abondance et de la dynamique sont observées entre les espèces, tout en étant caractéristique du régime

saisonnier du climat méditerranéen. L'impact des paramètres environnementaux, qui peuvent être à l'origine de l'abondance des *Culicoides,* a été étudié, en remettant en question le rôle potentiel de la pression atmosphérique.

Le dernier volet s'est intéressé à la détermination de la distribution spatiale des *Culicoides* à l'échelle nationale, ce qui nous a permit d'obtenir des cartes d'abondance pour les principales espèces recensées et de caractériser les facteurs environnementaux expliquant leur abondance à différentes échelles spatiales. Ce qui servira dans le futur à cartographier le risque de transmission des virus qui sont associés aux espèces vectrices de *Culicoides*. De telles cartes de risque sont importantes pour cibler les actions de surveillance et de lutte dans une perspective d'améliorer le rapport coût/bénéfice de ces programmes.

Mots clés : *Culicoides*, distribution, maladie vectorielle, écologie, saisonnalité, Maroc, facteurs écologiques, abondance

Summary

Culicoides are small blood-biting midges, belonging to the Ceratopogonidae family, the genus is present on all land surfaces except Antarctica. A very small proportion of the diversity of *Culicoides* has an epidemiological role with a significant impact on human and animal health. A few numbers of species are known to be involved in the biological transmission of viruses, protozoa and helminths. Some species with mammophilic behaviour are important nuisances of human tourism or agroforestry activities and harass farm animals -recurrent summer dermatitis in horses-. Despite this veterinary importance, *Culicoides* remain a poorly studied group of diptera. In order to better understand the distribution of *Culicoides* in Morocco, and to characterize the factors that determine their abundance, our study addressed 3 main components:

The first concerned the revision of the diversity of *Culicoides* species in Morocco based on morphological and molecular approaches, which made it possible to update the list of species of the country. This also enabled us to describe at least eight vector species - known or potential - of viruses of economic importance in animal health, namely bluetongue virus -endemic since 2004-, African horse sickness virus -eradicated by vaccination, with a risk of

reintroduction- and epizootic haemorrhagic disease virus (EHDV) -absent since 2006-. This study also allowed us to describe new species for Moroccan fauna, namely *Culicoides paolae, Culicoides subfagineus, Culicoides achrayi and Culicoides haranti.*

The second research component made it possible to characterize the diversity and seasonality of species of veterinary interest in different breeding (cattle, sheep and horse). It was concluded that species diversity remains the same regardless of the type of breeding, but that it varies greatly according to the season. Indeed, each species has its own seasonality with peaks of abundance at a specific period (s) of the year. It was concluded that the seasonal diet of *Culicoides* was typical of the Mediterranean climate. The impact of environmental parameters, which may be at the origin of *Culicoides* abundance, has been studied, questioning the potential role of atmospheric pressure.

The last component focused on determining the spatial distribution of *Culicoides* at the national level, which allowed us to obtain abundance maps for the main species and to characterize the environmental factors explaining their abundance at different spatial scales. This will be used in the future to map the risk of transmission of viruses by *Culicoides* vector species. Such risk maps are important to target monitoring and control actions with a view to improving the cost/benefit ratio of these programs.

Keywords: *Culicoides*, distribution, vector-borne disease, ecology, seasonality, Morocco, ecological factors, abundance

Résumé vulgarisé

Ces dernières décennies ont été marquées par l'apparition, l'émergence ou la réémergence de maladies humaines et animales. Parmi ces maladies, beaucoup sont des maladies à transmission vectorielle (transmises par un vecteur, c'est-à-dire un arthropode piqueur). Dans le domaine de la santé animale, deux maladies virales transmises par piqûre d'arthropode, la fièvre catarrhale ovine (FCO) et la peste équine, ont causés des pertes économiques considérables. En effet, la FCO est une maladie endémique au Maroc depuis 2004 (maladie présente habituellement dans une région ou une population déterminée), la peste équine quant à elle a été éradiqué en 2015, après que le pays ai connu plusieurs épizooties (maladie qui frappe simultanément un grand nombre d'animaux) par le passé. Ces maladies virales sont transmises par des moucherons hématophages, appelés *Culicoides*. Cette étude a été réalisée dans le but de mieux comprendre la distribution de ces moucherons au Maroc, mais aussi de caractériser les facteurs environnementaux qui déterminent leur abondance, ce qui servira dans le futur à cartographier le risque de transmission des virus qui sont associés aux espèces vectrices de *Culicoides*.

Summary for a broad audience

Recent decades have been affected by the emergence or re-emergence of human and animal diseases. Many of these diseases are vector-borne diseases (transmitted by a vector, i.e. a biting arthropod). In the field of animal health, two viral diseases transmitted by arthropod bites, bluetongue and African horse sickness have caused considerable economic losses. Indeed, bluetongue is an endemic disease in Morocco since 2004 (usually present in a specific region or population), while African horse sickness was eradicated in 2015, after the country had experienced several epizootic diseases (a disease that affects a large number of animals simultaneously) in the past. These viral diseases are transmitted by haematophagous midges, called *Culicoides*. This study was carried out in order to better understand the distribution of these midges in Morocco, but also to characterize the environmental factors that determine their abundance, which will be used in the future to map the risk of transmission of viruses associated with *Culicoides* vector species.

Préambule

Ce manuscrit de thèse est construit sur le modèle d'une thèse sur articles et se structure autour d'un premier chapitre de synthèse bibliographique (**Chapitre 1**), suivi des travaux de recherche formatés : un article publié (**Chapitre 2**), un article accepté en cours de publication (**Chapitre 3**), et enfin un article en préparation (**Chapitre 4**). Pour que la lecture soit facile et agréable, une brève synthèse au début de chaque chapitre a été rédigée, permettant de placer le contexte de l'étude et ses objectifs. Une discussion générale reprend les principaux résultats évoqués dans chaque chapitre, avec un recul sur leurs interprétations, et leurs comparaisons aux études passées. Finalement, la dernière partie comprend une conclusion générale ainsi que des perspectives à ce travail.

Introduction générale

Depuis plusieurs décennies, des maladies à transmission vectorielle animales et humaines ont connu des changements importants de leur épidémiologie (WHO, 2004). Le commerce international -intensification et mondialisation des échanges commerciaux-, la mobilité humaine -générant des cas importés pouvant amener à l'introduction de nouveaux pathogènes et leur transmission à la faveur de l'installation d'espèces exotiques dans de nouvelles zones, les facteurs socio-démographiques et les modifications de l'environnement par l'homme -changement des pratiques agricoles, déforestation, urbanisation désordonnée-, le changement climatique -modifications des précipitations, de la température, du vent- sont les principales causes de ces modifications épidémiologiques. Certaines maladies ont émergé dans de nouvelles régions du monde provoquant ainsi des pertes économiques monstrueuses mais aussi des graves problèmes de santé publique. D'autres quant à elles, ont ré-émergé réapparaissant dans des zones où elles avaient complètement disparu, ou ont connu une augmentation brutale de leur incidence alors qu'elle était faible auparavant (Gubler et al., 2001).

Dans le domaine de la santé animale, deux arboviroses transmises par des moucherons du genre *Culicoides*, la fièvre catarrhale ovine (FCO) et la peste équine, se sont démarquées ces 50 dernières années, avec des patrons épidémiologiques évoluant très vite engendrant des pertes économiques majeures (Mellor et al., 2000).

La peste équine, affecte les équidés et principalement le cheval chez qui le taux de létalité dépasse 95 % dans sa forme pulmonaire. La maladie a été éradiquée au Portugal et en Espagne suite à différentes épizooties après une introduction d'un hôte infecté grâce à la vaccination intensive des chevaux (Sellers et al., 1977, Zientara et al., 2016). Le Maroc a connu deux épizooties par le passé (Mellor, 1993), les derniers cas de peste équine ont été recensés en 1991, et le pays a été déclaré officiellement indemne en 2015. Cependant, la maladie continue toujours de sévir en Afrique subsaharienne, et le risque d'une potentielle réintroduction dans les pays indemnes n'est pas exclue (Purse et al., 2015).

La fièvre catarrhale ovine illustre le phénomène d'émergence d'une maladie dite « exotique » dans des pays tempérés. L'augmentation globale des températures et des abondances du vecteur afrotropical *Culicoides imicola* semblent avoir joué un rôle déterminant dans son émergence dans le bassin méditerranéen (Mellor et Wittman, 2002). La remontée vers le nord

des limites traditionnelles de transmission du virus de la FCO permet aujourd'hui de considérer le bassin méditerranéen comme une zone endémique (Purse et al., 2015). La FCO est considérée comme endémique au Maroc depuis son apparition en 2004 (OIE, 2004). Cette maladie a eu des conséquences dramatiques pour les filières, par l'impact sanitaire direct sur les ruminants domestiques (plus d'un million de moutons seraient morts dans le bassin méditerranéen), par les pertes de production, les problèmes de reproduction chez les bovins, les restrictions aux mouvements d'animaux en particulier dans le cadre du commerce international, et le coût des programmes de surveillance et de vaccination (Balenghien et Garros, 2017).

Le bassin méditerranéen, et plus particulièrement le Maroc de par sa situation de transition entre l'Afrique subsaharienne et l'Europe, est une zone géographique essentielle pour comprendre les phénomènes d'émergence, de diffusion et d'endémisation des maladies à transmission vectorielle. En effet, le pays présente une grande variété de paysages et comprend tous les étages bioclimatiques du climat méditerranéen dans leurs variantes, en passant du per–humide à l'aride.

Les différents épisodes de fièvre catarrhale ovine et de peste équine en Afrique du Nord ont motivé les chercheurs et les services vétérinaires à s'intéresser à la faune culicoïdienne en menant des enquêtes entomologiques ou en installant des systèmes de surveillance entomologiques. Ces études ont permis de mettre à jour la liste d'espèces -Algérie, Maroc et Tunisie- (Clastrier 1957, Callot et al., 1964, Callot et al., 1968, Callot et Kremer, 1969, Chaker, 1981, Szadziewski, 1984, Chaker et al., 2005, Hammami et al., 2008, Djerbal et Delécolle, 2009, , Slama et al., 2016 ; Sghaier et al., 2017), de définir leur distribution -Maroc et Tunisie- (Baylis et al., 1998, Bouayoune et al., 1998, Hammami et al., 2008, Lhor et al., 2015, Slama et al., 2016, Sghaier et al., 2017) et même de caractériser la saisonnalité de certaines espèces vectrices et les facteurs environnementaux déterminant cette dernière – Maroc- (Baylis et al., 1998, Lhor et al., 2015).

Dans la continuité de ces travaux de recherche, une revue des connaissances taxonomiques et bio-écologiques sur le genre *Culicoides* a été réalisée en région Nord-Africaine, en particulier au Maghreb, en synthétisant les travaux réalisés entre 1950 et 2017 (**Chapitre 1**).

Chapitre 1 : Les *Culicoides* et les agents pathogènes transmis de par le monde : focus sur la côte Nord-Africaine

Les *Culicoides* sont de petits moucherons piqueurs appartenant à la famille des Ceratopogonidae (la longueur du corps dépasse rarement trois millimètres) **(Encadré 1),** dont seule la femelle est hématophage. L'appareil de piqûre est court, de type solénophage (Mellor et al., 2000).

Encadré 1 : Identification morphologique et moléculaire des *Culicoides*

Les moucherons piqueurs du genre *Culicoides* se différencient des autres genres de la famille des Ceratopogonidae par certains critères notamment au niveau du patron alaire : la présence de deux cellules radiales de taille subégale, une nervure transverse r-m et une nervure médiane (M2) pédonculée (Figure 1). Au niveau des pattes postérieures, l'empodium, porté par le dernier segment du tarse, est rudimentaire. Au niveau de la tête, on retrouve chez les deux sexes, quinze articles antennaires qui se composent comme suit : scape + pédicelle + 8 articles courts + 5 articles longs chez la femelle et scape + pédicelle + 10 articles courts + 3 articles longs chez le mâle ; le quinzième article antennaire des *Culicoides* est dépourvu de micron ; Le palpe est composé de cinq articles dont les deux premiers sont soudés et le troisième présente une fossette sensorielle (Cornet, 1974 ; Kremer et al., 1987).

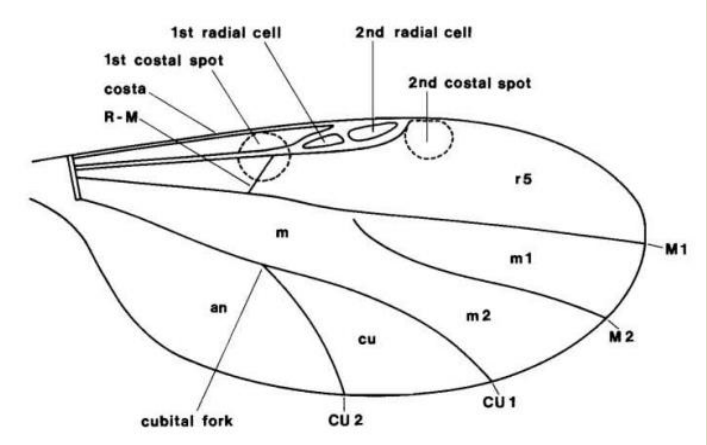

Figure 1 : Aile de *Culicoides*, montrant les principales cellules et nervures (Boorman, 1989).

L'identification à l'espèce repose en premier lieu sur la forme et la disposition des tâches alaires (Figure 2). Cependant ces tâches peuvent être absentes chez certaines espèces, et le recours à d'autres critères morphologiques devient dès lors indispensable. Par exemple, les yeux peuvent être soudés ou séparés, nus ou pubescents. D'autres critères peuvent être

observés et nécessitent une dissection et un montage des spécimens entre lame et lamelle. C'est le cas du rapport antennaire (11ème article/ 10ème article), de l'indice antennaire (les articles longs versus les articles courts), des sensilles coeloconiques (nombre et disposition) mais aussi d'autres sensilles antennaires (forme, taille, présence ou absence). Les palpes fournissent également des informations importantes notamment avec le rapport du troisième article sur les deux premiers, mais aussi la forme de la fossette sensorielle, sa taille et sa profondeur (Figure 3). Le thorax présente aussi des ornementations qui sont caractéristiques d'espèce. Les derniers segments abdominaux portent les structures génitales mâles et femelles. Chez les femelles, on examine le nombre, la forme et la taille des spermathèques, tandis que chez les mâles on observe l'aspect du genitalia (Figure 4) (Kremer, 1965 ; Cornet, 1974 ; Kremer, 1987 ; Glick, 1990 ; Rawlings, 1996).



Figure 2 : Exemples de motifs d'ailes de femelles *Culicoides*, de gauche à droite : *C. paolae* Boorman ; *C. saevus* Kieffer ; *C. parroti* Kieffer (clichés : M. Bourquia, 2019) ; *C. cataneii* Clastrier /*C. gejgelensis* Dzahafarov.

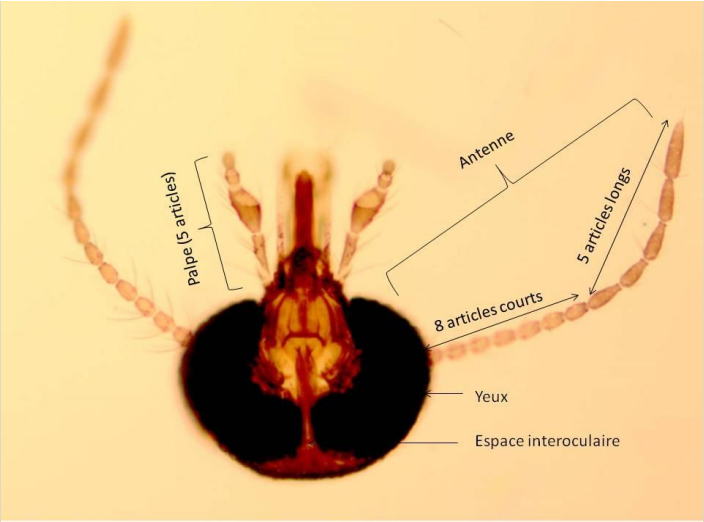

Figure 3 : Caractères morphologiques propres au genre Culicoides Latreille: tête d'une femelle de *Culicoides imicola* Kieffer, cliché : M. Bourquia, 2019).

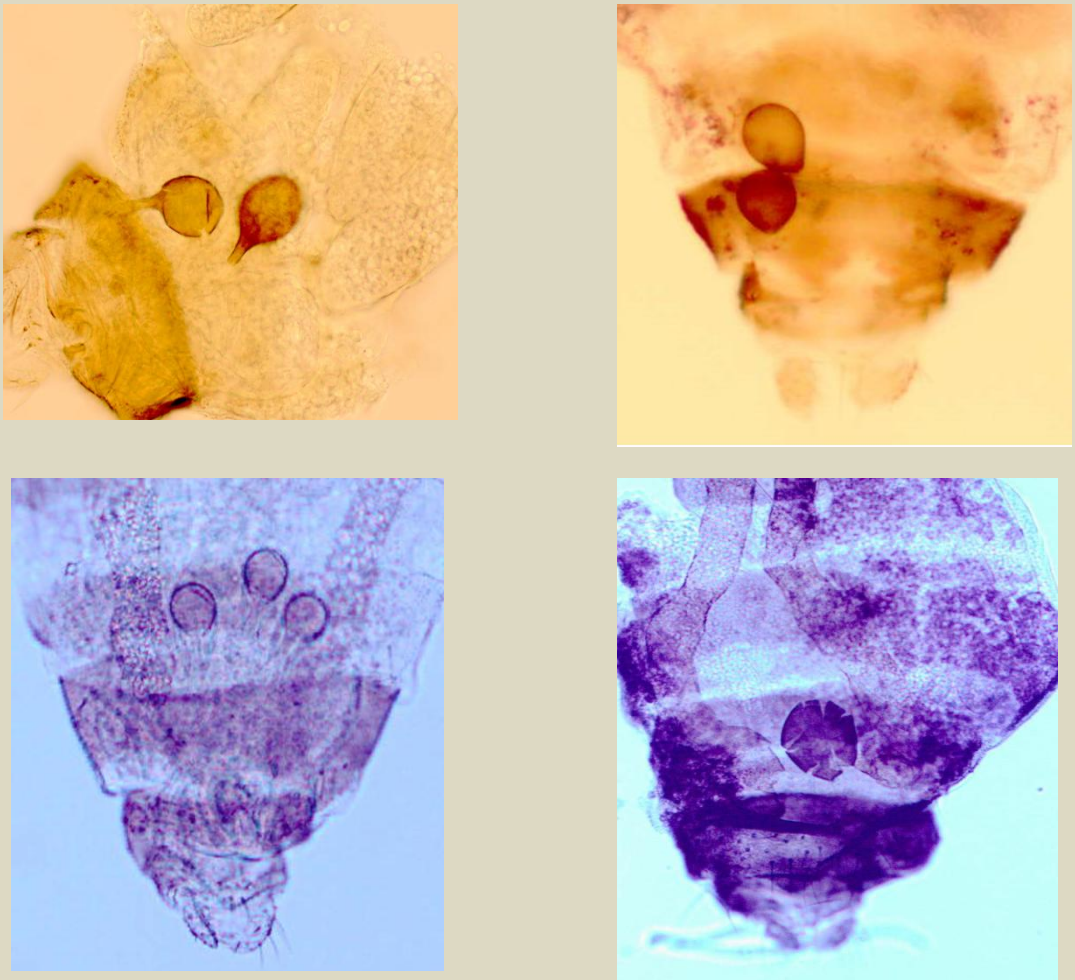

Figure 4 : Exemples de spermathèques servant à la diagnose des femelles de *Culicoides* (Clichés : M. Bourquia, 2019), de gauche à droite: *C. azerbajdzhanicus* Dzhafarov : deux spermathèques de forme particulière ; *C. imicola* Kieffer : deux spermathèques arrondies ; *C. saevus* Kieffer : trois spermathèques bien développées ; *C. circumscriptus* : une seule spermathèque de forme ovoïde.

L'identification moléculaire est privilégiée dans le cas de spécimens endommagés, d'espèces jumelles morphologiquement non différentiables et d'espèces difficilement différentiables sur la base de critères morphologiques. Ainsi, des tests utilisant l'amplification par PCR avec des sondes spécifiques d'espèces ont été développés pour la faune paléarctique.

Les outils moléculaires et leur développement ont eu de multiples applications dans le diagnostic d'espèce. Les principaux marqueurs utilisés chez les *Culicoides* sont le marqueur mitochondrial COI (Cytochrome oxydase subunit 1) qui a été utilisé pour les espèces proches de *C. imicola* (Linton et al., 2002), le complexe *Obsoletus* et *Pulicaris* (Pagès et Sarto i Monteys, 2005 ; Nolan et al., 2007 ; Pagès et al., 2009), et les marqueurs nucléaires ribosomiques ITS1 (Internal transcribed spacer) pour le complexe *Obsoletus* (Mathieu et al., 2007) et ITS2 pour le complexe *Pulicaris* (Gomulski et al., 2006). En outre, on a démontré aussi l'utilité du marqueur nucléaire carbamoyl phosphate synthétase 2, aspartate transcarbamylase, and dihydroorotase (CAD) dans la différenciation des espèces (Bellis 2013).

Une autre technique moléculaire (matrix-assisted laser desorption ionisation/spectrométrie de masse à temps de vol, MALDI-TOF MS) a démontré par ses multiples avantages (simplicité, rapidité et rentabilité) sa capacité de caractériser et identifier des insectes piqueurs (Kaufmann, 2011).

Le cycle de développement des *Culicoides* comprend 4 stades larvaires et un stade de nymphe qui se développent dans des milieux généralement décrits comme riches en matière organique d'origine animale ou végétale **(Encadré 2).** Cette exigence écologique est un déterminant clé qui limite la distribution, l'abondance et l'occurrence saisonnière des espèces de *Culicoides* (Mellor et al., 2000).

Encadré 2 : Généralités sur la bio-écologie des *Culicoides*

Les *Culicoides* adultes préfèrent vivre dans des endroits humides. Les mâles sont floricoles : ils se nourrissent de nectar, de sucre, de pollen, ainsi que de liquides provenant de la décomposition de matières organiques (Goetghebuer, 1952 ; Chaker, 1983) et fréquentent généralement le sommet des arbres (Birley et al., 1982 ; Rieb, 1982). Les femelles sont hématophages mais peuvent se nourrir de jus sucré pour assurer leur métabolisme de base. En effet, le repas de sang est nécessaire exclusivement à la maturation des œufs (Holmes et

Birley, 1987; Braverman, 1988). Certaines espèces comme *Culicoides circumscriptus* (Kieffer) et *Culicoides belkini* (Wirth et Arnaud) sont autogènes (ne nécessitent pas de repas sanguin pour la première ponte). La ponte survient quelques jours après le repas sanguin de la femelle. Les œufs sont déposés sur un substrat humide (Figure 5). Les œufs vont se transformer en larves qui vont subir 4 mues (L1, L2, L3, L4) (Chaker, 1983). Les larves sont vermiformes de couleurs claires, mobiles et dépourvues de pseudopodes. Elles se nourrissent de débris organiques ou sont prédatrices de nématodes, protozoaires, bactéries, champignons, algues, voire même de leurs propres congénères (cannibalisme) (Hill, 1947 ; Kettle, 1962 ; Chaker, 1983). Les larves se développent dans des gîtes larvaires de nature variées ou spécifique à l'espèce : bords des mares, étangs, trous d'arbres, fruits en décomposition, tourbières, excréments d'animaux ou creux de rocher riches en matière organique (Figure 5) (Rodhain et Perez, 1985; Balenghien et Delécolle, 2009). Arrivée à maturité, la larve de stade 4 se transforme en nymphe. Ce stade est peu mobile, ne se nourrit pas et vit grâce à ses réserves accumulées lors des différents stades larvaires. Elle possède un céphalothorax portant deux trompettes respiratoires qui assurent leur respiration aérienne. À l'émergence, les imagos sont d'abord clairs puis rapidement prennent couleur. Les *Culicoides* adultes peuvent vivre jusqu'à un mois et demi en condition de laboratoire (Nevill, 1971 ; Boorman, 1991). Les adultes s'accouplent quelques temps après l'éclosion, lors de vols nuptiaux ou au niveau du sol (Chaker, 1983). Les femelles stockent les spermatophores des mâles dans le ou les spermathèque (s) qu'elle a au niveau de la partie postérieure de l'abdomen.

Les *Culicoides* peuvent se disperser généralement par vol actif (jusqu'à 3 km du site d'émergence). Ce déplacement est justifié par sa recherche de repas sanguin (Lillie et al., 1985). Cependant, les *Culicoides* peuvent être dispersés par le vent sur des grandes distances (1 à 700 km) (dispersion passive) (Sellers et al., 1977 ; Sellers et al., 1979; Gibbs et Greiner, 1994).



Figure 5 : Quelques exemples d'habitats favorables au développement larvaire des *Culicoides* de haut en bas et de gauche à droite, tas de fumier; figuiers de barbarie, végétation émergée, terre boueuse (Clichés : M. Bourquia, 2017).

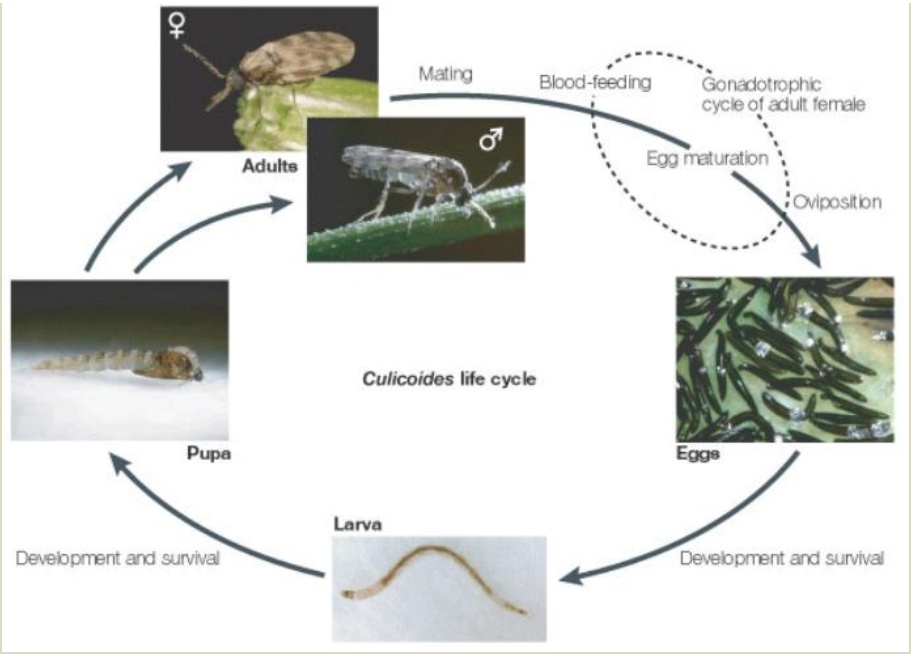

Figure 6 : Cycle biologique de *Culicoides* (Purse et al., 2015).

Plus de 1400 espèces sont décrites à travers le monde, et le genre est présent sur toutes les surfaces terrestres, à l'exception de l'Antarctique (Borkent, 2016). Une très faible proportion de la diversité du genre *Culicoides* joue un rôle épidémiologique avec un impact significatif sur la santé humaine et animale. Un certain nombre d'espèces est connu pour être des espèces impliquées dans la transmission biologique de virus, de protozoaires et d'helminthes **(Encadré 3)**. Certaines espèces avec un comportement mammophile sont des nuisances importantes des activités humaines de tourisme ou d'agroforesterie et harassent les animaux d'élevage (dermatite estivale récidivante chez les chevaux). Malgré cette importance vétérinaire les *Culicoides* restent un groupe de diptères peu étudié.

Encadré 3 : Importance médicale et vétérinaire des *Culicoides*

Les *Culicoides* provoquent des piqûres douloureuses et prurigineuses chez l'homme, et constitue ainsi un frein au tourisme dans certaines zones dans le monde. En raison de leur nuisance, certaines espèces ont été nommées *Culicoides diabolicus* ou *Culicoides irritans* (Mullen, 2009 ; Balenghien et Garros, 2017). Ils sont aussi responsables chez les chevaux par l'action de leur salive d'une réaction allergique appelée la dermatite estivale récidivante ou encore la dermatite saisonnière (Kleider et Lees, 1984).

Outre leur nuisance, les *Culicoides* transmettent un certain nombre de filaires. Chez l'homme,

en région afrotropicale, certaines espèces de *Culicoides* transmettent des filaires bénignes telles que *Mansonella perstans* et *M. streptocerca* (Golvan, 1983; Simonsen et al., 2010). Chez l'animal, les *Culicoides* sont vecteurs d'*Onchocerca cervicalis* chez les chevaux et *O. guttarosa* chez les bovins, provoquant des pathologies discrètes (Linley, 1985). Les *Culicoides* sont aussi responsables de la transmission des protozoaires notamment d'*Haemoproteus sp*. chez les oiseaux et d'*Hepatocystis kochi* chez les singes (Mathieu, 2011).

De loin le plus important au niveau sanitaire et économique, c'est bien le rôle des *Culicoides* dans la transmission des virus qui motivent leurs études. Plusieurs virus ont été isolés à partir de différentes espèces de *Culicoides* collectés sur le terrain, la plupart appartenant aux familles des Bunyaviridae, Reoviridae, et Rhabdoviridae (Mellor et al., 2000). Parmi les virus transmis par les *Culicoides*, deux *Orbivirus* (Reoviridae) sont particulièrement pathogènes engendrant des dégâts économiques non négligeables sur le cheptel : le virus de la fièvre catarrhale ovine (BTV) affectant les ruminants sauvages et domestiques, ayant une répartition mondiale et le virus de la peste équine (AHSV) affectant les équidés, ce virus est présent en Afrique subsaharienne, avec de rares incursions en Europe et en Asie (Mellor et al., 2000). Le virus de la maladie hémorragique des cervidés (EHDV) (*Orbivirus)* affecte une large gamme de vertébrés domestiques et sauvages, à l'instar du BTV, l'EHDV a une répartition mondiale (Gumm et al., 1984 ; Dulac et al., 1989), l'infection est asymptomatique chez les ruminants et peut des fois être confondue avec la fièvre catarrhale ovine chez certaines espèces de cerfs (Hoff et Trainer, 1978 ; Sohn et Yuill, 1991). Au Maroc, une épidémie de la maladie hémorragique des cervidés a eu lieu en 2006, depuis aucun cas n'a été déclaré. Le virus Oropouche (Bunyaviridae, genre *Orthobunyavirus*) est le seul virus ayant un impact sur la santé publique, provoquant une forte fièvre chez les personnes atteintes, pouvant même causer une méningite, ce virus est actuellement limité à l'Amérique du Sud (Figure 7).

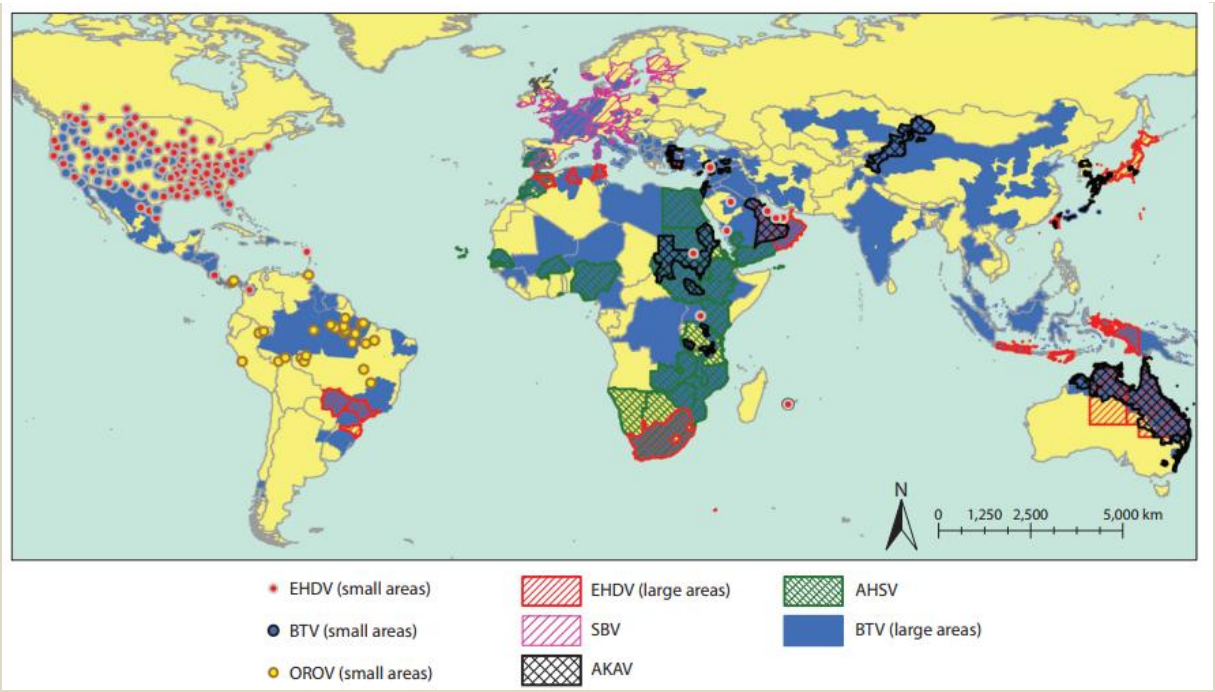

Figure 7 : Répartition connue de six arbovirus transmis par les *Culicoides* à partir de données sérologiques et de cas cliniques au cours des trois dernières décennies (Purse et al, 2015).

Les *Culicoides* transmettent également le virus de Schmallenberg (SBV) récemment découvert (*Bunyaviridae*) -sa distribution est pour l'instant limitée à l'Europe du Nord-, le virus de l'encéphalite équine (EEV), le virus de la fièvre bovine éphémère, le virus de Palyam et le virus Akabane (AKAV) qui est présent au Moyen orient, en Afrique, en Asie et en Australie (Figure 7) et qui provoque des anomalies congénitales chez les ruminants domestiques (Mellor, 2000).

La fièvre catarrhale ovine, la peste équine et la maladie hémorragique des cervidés figurent sur la liste des maladies à déclaration obligatoire de l'Organisation mondiale de la santé animale.

Au cours de ces dernières décennies, on a assisté à des foyers de maladies transmises par les *Culicoides* en dehors des zones historiques de distribution connues (Mellor et al., 2000). C'est le cas de la fièvre catarrhale ovine (FCO), maladie virale transmise par certaines espèces de *Culicoides*. En effet, cette maladie est devenue en quelques années un sujet de préoccupation majeure en santé animale en région tempérée et méditerranéenne. La FCO est décrite pour la première fois dans la province de Cap en Afrique du Sud à la fin du 19ème siècle, à la suite de

l'importation de moutons de race mérinos en provenance d'Europe. Elle est d'abord nommée "fièvre catarrhale paludique du mouton " en 1902 en raison de la croyance erronée que la maladie était causée par un parasite intracellulaire (Spreull, 1902). Sir Arnold Theiler montre que la maladie est causée par un agent filtrable -qui passe à travers un filtre de diatomite ou de porcelaine non émaillée avec le filtrat et qui reste virulent-, viral et elle est rebaptisée "fièvre catarrhale du mouton", en référence à la langue cyanosée observée chez certains moutons atteints (Spreull, 1905). La preuve d'une transmission biologique du virus de la FCO par les *Culicoides* est faite par Du Toit (1944) **(Encadré 4)**.

Encadré 4 : Mécanisme de transmission du virus

En 1943, Du Toit démontre avec succès que les *Culicoides* sont les vecteurs biologiques du virus de la fièvre catarrhale ovine et de la peste équine. En effet, il réussit à infecter des moutons sains avec le virus de la FCO en les exposant aux piqûres de moucherons nourris 10 jours plus tôt sur des moutons virémiques. Il a également réussi à infecter un cheval avec le virus de la peste équine selon le même principe.

Les *Culicoides* s'infectent uniquement lors de repas de sang provenant d'hôtes vertébrés virémiques. Il n'existe aucune preuve publiée suggérant une transmission verticale ou vénérienne chez les *Culicoides* contrairement à d'autres insectes vecteurs (moustiques, phlébotomes et tiques) (Walker et Davies, 1971). La période allant de l'ingestion d'un repas de sang infecté à la présence de particules virales dans les glandes salivaires permettant un repas de sang infectant s'appelle la période d'incubation extrinsèque. Pendant cette période, le virus pénètre dans les cellules épithéliales de l'intestin moyen et se réplique. Les nouvelles particules virales s'échappent à travers la membrane basale des cellules intestinales, se dispersent dans l'hémolymphe, se disséminent pour infecter les organes cibles secondaires y compris les glandes salivaires. Une fois que le virus se retrouve dans les glandes salivaires, il peut être transmis aux vertébrés pendant un repas sanguin. La durée de l'incubation extrinsèque dépend de la température (vecteur poikilotherme). Une température élevée raccourcit la période d'incubation extrinsèque (Megahed, 1956 ; Eaton et al., 1990).

Un certain nombre d'obstacles peuvent inhiber la dissémination du virus au sein du vecteur : échappement de l'infection mésentérique, à la dissémination, à la transmission transovarienne et à l'infection des glandes salivaires. Cependant, il est compliqué d'étudier la compétence vectorielle des *Culicoides* afin de statuer sur le rôle vecteur d'une espèce. En effet, rares sont les espèces qu'on peut facilement élever et entretenir au laboratoire vu la diversité d'habitats

larvaires de certaines espèces, qu'on ne peut pas reproduire au laboratoire (Boorman, 1974 ; Kremer et Lienhart, 1998).

En 1918, la FCO est décrite en Afrique de l'Ouest (Tomori et al., 1991). A partir de 1940, la FCO est décrite en Afrique Centrale puis dans le Bassin Méditerranéen (Brugère-Picoux et Rey, 2010). La première notification de la FCO dans le Bassin Méditerranéen (sérotype 10) a eu lieu en 1943 à Chypre, puis s'est étendue à la Turquie, Syrie, Oman et l'Arabie saoudite (Benkerroum, 2010). D'autres notifications ont suivies en Espagne en 1956, au Maroc à la même année dans les régions de Tanger-Tétouan-Al hoceima et Rabat-Salé-Kénitra et au Portugal en 1957 (Placidi, 1957, Gibbs et Greiner, 1994). En 1977, la maladie (sérotype 4) est observée en Turquie. Deux ans plus tard, c'est sur l'île grecque de Lesbos, en Mer Egée qu'elle est rapportée (Papadopoulos, 1996).

L'Europe continentale reste indemne de FCO jusqu'à la fin des années 1990. La maladie réapparaît dans les îles grecques du sud-est de la Mer Egée en 1998, et des cas sont enregistrés en 1999 en Grèce, en Bulgarie (Mellor et Wittman, 2002), en Tunisie (sérotype 2) (Hammami, 2004) et en Turquie. En 2000, des foyers de FCO sont notifiés pour la première fois en Algérie avec 28 foyers en 4 mois (Mellor and Wittman, 2002) en Italie (Sardaigne, Sicile et Calabre) en Espagne (îles Baléares) (Alba et al., 2004), en France (Corse) (Zientara et al., 2001) et à nouveau en Grèce et en Tunisie (sérotype 2 et 7), avec 72 foyers en 5 mois. A cette période, sachant les foyers dans les pays voisins (Algérie et Tunisie), les services vétérinaires marocains mettent en place un système de surveillance épidémiologique craignant que la maladie ne s'étende au Maroc. En effet, en 1999, environ 7040 sérums de bovins provenant de 32 provinces ont été analysés, deux pour cent des résultats se sont avérés positifs au BTV. En 2001, une deuxième enquête a été réalisée, cette fois ci, c'est 4 000 sérums de moutons qui ont été analysés. Près de 8,7 % ont été positifs. Cette augmentation du pourcentage a été la première alerte de la circulation virale dans le pays. Néanmoins aucune détermination des sérotypes du virus n'a été effectuée (Lhor, 2017). En 2001, la maladie s'étend en Europe du Sud-est, avec des foyers observés dans le Nord-Ouest de la Grèce, l'Ouest de la Bulgarie, la Turquie, le Kosovo, la Macédoine, le Monténégro et la Serbie (Baylis et Mellor, 2001). En 2004, la FCO apparaît au Maroc (sérotype 4) dans la région de Rabat-Salé-Kénitra (anciennement Gharb-Chrarda-Beni Hssen) (OIE, 2004). En 2006, le sérotype 1 est détecté en Algérie, et sans surprise progresse jusqu'au Maroc où les premiers cas sont reportés à l'est du pays (Cêtre-Sossah et al., 2011).

En 2008, les mesures sanitaires mises en œuvre par les services vétérinaires ont permis de contrôler les foyers déclarés au Maroc, mais, depuis 2009, le sérotype 1 et le sérotype 4 circulent conjointement pendant les périodes d'activité des *Culicoides* dans le nord et le sud du pays (Drif et al., 2013 ; Lhor et al., 2017). Le dernier recensement a fait état de 305 foyers de FCO dans tout le pays avec 1 456 cas entraînant 449 décès en 2017 (ONSSA, 2019).

Les *Culicoides* sont aussi des vecteurs d'une arbovirose létale touchant les équidés, la peste équine. La plus ancienne référence à la peste équine est décrite dans un manuscrit arabe «كتاب الأفي والكافية و الفصول الشافية» (Livre des dissertations suffisantes et des sections satisfaisantes) écrit vers l'an 728 de l'Hégire (1327-1328 de notre ère) (Mornet et Gilbert, 1968). Au Yémen, sévit une importante épizootie qui décime les populations d'équidés. La rapidité et la soudaineté de l'apparition de la maladie sont soulignées dans une anecdote : un acheteur débat le prix du cheval avec un vendeur. Pendant ce temps, l'animal tombe, un jetage s'écoule de ses naseaux puis meurt. La première description reconnue de la peste équine remonte au 18ème siècle, lors d'une épizootie qui provoqua la mort de 1700 chevaux dans la région du Cap en Afrique du Sud (Mornet et Gilbert, 1968). La zone historique de distribution de la maladie est le continent africain mais la maladie est sortie plusieurs fois de son berceau africain. La peste équine a provoquée trois épizooties majeures au cours du 20ème siècle, causant un impact économique considérable ; l'épizootie de 1943-1944 en Égypte et en Palestine, l'épizootie de 1959-1960 au Moyen-Orient (Iran, Irak et Liban), en Asie du Sud-Est (Pakistan et Inde) et en Europe (Turquie et Chypre) et celle de 1965-1966 dans le Maghreb (Howell, 1960 ; Rafyi, 1961 ; Gorhe et al., 1965 ; Mirchamsy et Hazrati, 1973).

En 1965, le sérotype 9 de la peste équine est diagnostiqué au Maroc pour la première fois dans les provinces de Nador, Al Hoceima, Tétouan et Tanger (Laaberki, 1969) puis s'étend à l'Algérie et à la Tunisie, traverse le détroit de Gibraltar en 1966 et atteint l'Espagne, où des cas sont enregistrés à Los Barrios et La Linea. L'hypothèse avancée serait la dispersion via les vents de *Culicoides* infectés (Sellers et al., 1977, Zientara et al., 2016). En 1988, la peste équine réapparaît en Espagne avec le sérotype 4 (Rodriguez et al., 1992 ; Sanchez-Vizcaino, 2004), suite à l'introduction de zèbres infectés (mais asymptomatiques) en provenance de Namibie et à destination du Safari Park de Madrid. L'Espagne connaitra plusieurs épizooties entre 1988 et 1990, qui s'étendront au Portugal et au Maroc (Mellor, 1993). Les mesures de prophylaxie sanitaire et de vaccination ont permis l'éradication de la peste équine en Espagne et au Portugal en 1990. Au Maroc, des cas sont déclarés entre 1989 et 1991 ; le dernier cas

officiel a été recensé le 10 octobre 1991 dans la province de Kénitra ; le pays bénéficie d'un statut indemne depuis 2015.

Les différents épisodes de FCO ou de peste équine en Afrique du Nord ont motivé les chercheurs et les services vétérinaires à s'intéresser à la faune culicoïdienne des pays concernés en menant des enquêtes entomologiques ou en installant des systèmes de surveillance entomologiques.

En Algérie, la première étude entomologique est réalisée par Clastrier (1957) après l'épizootie de peste équine démarrée en Egypte et en Palestine, il décrit des Ceratopogonidés dont quelques espèces de *Culicoides* à ailes tachetées. La deuxième étude (Callot et al., 1968) permet de décrire *Culicoides marcleti* et de préciser la distribution géographique des espèces. Enfin, Szadziewski, 1984 a fait état de 31 espèces pour le pays dont *C. imicola.* Des captures réalisées entre 2003 et 2006 (Delécolle, communication personnelle) et entre 2007 et 2009 (Djerbal et Delécolle, 2009) ont permis de rajouter une dizaine d'espèces à la liste des *Culicoides* recensés pour le pays.

En Tunisie, la première étude sur les *Culicoides* est réalisée par Callot et al. (1964), suite aux épizooties survenues au Moyen-Orient au début des années soixante. La deuxième (Callot et Kremer, 1969) a permis de décrire une nouvelle espèce pour la science *Culicoides jumineri*, la troisième (Chaker, 1982), a permis de décrire 22 espèces pour le pays. *Culicoides imicola* est signalé pour la première fois en 2005 (Chaker et al., 2005). Les études réalisées entre 2008 et 2016 ont ajouté une dizaine d'espèces supplémentaires (Hammami et al., 2008, Slama et al., 2016 ; Sghaier et al., 2017).

Au Maroc, plusieurs études ont été entreprises dans les années 1970, après la première épizootie de peste équine afin de décrire la faune culicoïdienne du pays (Callot et al. 1968 ; Bailly-Choumara et Kremer, 1970 ; Kremer et al., 1971 ; Kremer et al., 1975 ; Chaker et al., 1979 ; Kremer et al., 1979). Ces travaux ont permis de décrire une cinquantaine d'espèces dont *Culicoides imicola*, espèce afrotropicale vectrice confirmée des virus de la FCO et de la peste équine. Suite à la deuxième épizootie de peste équine, d'autres études ont été réalisées afin de mieux caractériser la diversité des *Culicoides* mais aussi de décrire l'impact des variables environnementales et climatiques sur la dynamique des populations de vecteurs avérés et suspects (principalement *C. imicola*) (Baylis et al., 1997 ; Baylis et al., 1998 ; Bouayoune et al.,1998). Une étude transversale est réalisée entre 2000 et 2003 par les services

vétérinaires marocains suite à l'émergence de la FCO en Algérie et Tunisie. Les échantillons collectionnés sont identifiés au niveau des groupes d'espèces à l'exception de *C. imicola*.

Le premier foyer de FCO au Maroc est signalé en septembre 2004 (OIE, 2004). Les enquêtes entomologiques réalisées au niveau des foyers décrivent la présence d'espèces déjà signalées comme *C. imicola* (Cêtre sossah et al., 2004). Entre 2009 et 2010, un nouveau réseau de surveillance entomologique est mis en place dans le cadre de la surveillance épidémiologique des maladies animales. Ce réseau est coordonné par les services vétérinaires nationaux (Lhor et al., 2015) et permet de mettre à jour la liste d'espèces présentes dans le pays.

A ce jour, malgré les mesures de lutte entreprises (lutte antivectorielle, vaccination), la FCO est endémique au Maroc et en Tunisie (pas de données disponibles pour l'Algérie), tandis que la peste équine a été éradiquée.

Problématique et objectifs

Contrairement à d'autres pays d'Afrique -Afrique du Sud, Sénégal- (Purse et al., 2015; Simonsen et al., 2011), il y a un manque considérable d'études du genre *Culicoides* en région Nord-Africaine.

Au Maroc, plusieurs études ont été entreprises dans les années 1970, après la première épizootie de peste équine afin de décrire la faune culicoïdienne du pays (Callot et al. 1968 ; Bailly-Choumara et Kremer, 1970 ; Kremer et al, 1971 ; Kremer et al, 1975 ; Chaker et al, 1979 ; Kremer et al, 1979). Ces travaux ont permis de décrire une cinquantaine d'espèces dont *Culicoides imicola*. Une étude transversale a été réalisée entre 2000 et 2003 par les services vétérinaires marocains suite à l'émergence de la FCO en Algérie et Tunisie. Les échantillons collectionnés ont été identifiés au niveau des groupes d'espèces à l'exception de *C. imicola*.

Le premier foyer de FCO au Maroc est signalé en septembre 2004 (OIE, 2004). Les enquêtes entomologiques réalisées à l'époque dans les foyers confirmés, ont permis de décrire la présence d'espèces déjà signalées comme *C. imicola* (Cêtre sossah et al., 2004). Entre 2009 et 2010, un nouveau réseau de surveillance entomologique est mis en place dans le cadre de la surveillance épidémiologique des maladies animales. Ce réseau est coordonné par les services vétérinaires nationaux, mais l'identification s'est faite selon le groupe d'espèces sauf pour *C. imicola* (Lhor et al., 2015).

D'autres études ont été réalisées afin de mieux caractériser la diversité des *Culicoides* mais aussi de décrire l'impact des variables environnementales et climatiques sur la dynamique des populations de vecteurs (Baylis et al., 1997 ; Baylis et al., 1998 ; Bouayoune et al., 1998). Ces études ont ciblé particulièrement *Culicoides imicola*, incriminée dans la transmission du virus de la peste équine dans ces régions. Les collectes n'ont été identifiées qu'au niveau du groupe d'espèces, et ne permettent donc pas d'utiliser ces données pour les espèces d'intérêt vétérinaire autres que *Culicoides imicola*, dont les abondances pourraient avoir été modifiées cette dernière décennie sous l'effet de l'augmentation globale des températures. En outre, de récentes infections expérimentales ont montré que d'autres espèces que *C. imicola* seraient compétentes pour le virus de la peste équine et celui de FCO (Carpenter et al., 2008; Foxi et al., 2016 ; Paslaru et al., 2018, Foxi et al., 2019).

Des captures récentes, réalisées par les services vétérinaires marocains (Lhor et al., 2015) ont permis de décrire précisément les dynamiques de populations de différentes espèces de

Culicoides au Maroc, mais dans un nombre de sites ne permettant pas une modélisation de leur distribution.

Ce travail de thèse vise à compléter ces précédents travaux, en réalisant l'inventaire faunistique des espèces d'intérêt vétérinaire à partir d'approche morphologique et moléculaire **(Chapitre 2),** en apportant de nouveaux éléments sur la saisonnalité et la diversité d'espèces d'intérêt dans différents environnements d'élevage **(Chapitre 3)** et en caractérisant la distribution spatiale des *Culicoides* à l'échelle du pays et les facteurs environnementaux qui la déterminent à différentes échelles spatiales **(Chapitre 4).** Cette étude est une étape préliminaire cruciale à la réalisation de cartes de risque de transmission des virus qui sont associés aux espèces vectrices, de telles cartes de risque sont extrêmement importantes pour cibler les actions de surveillance et de lutte dans une perspective d'améliorer le rapport coût/bénéfice de ces programmes.

L'ensemble de ces résultats sont discutés dans une dernière partie, suivie d'une conclusion.

Chapitre 2 : Révision de la diversité des espèces de *Culicoides* au Maroc et mise à jour de la liste d'espèces présentes dans le pays.

Dans le chapitre précédent, nous avons réalisé une revue des connaissances taxonomiques et bio-écologiques du genre *Culicoides* en région Nord-Africaine. Nous avons évoqué l'importance vétérinaire du genre *Culicoides*, et le mécanisme de transmission des arbovirus par ces moucherons. Environ 1400 espèces sont décrites dans le monde. En Afrique, l'inventaire des espèces de *Culicoides* le plus à jour est celui d'Afrique du Sud qui comptabilise environ 100 espèces (Labuschagne, 2016). En Afrique du Nord et plus précisément au Maghreb (Algérie, Maroc et Tunisie), les premiers inventaires d'espèces ont été réalisés suite aux épizooties de peste équine entre les années 1950 et 1970, ainsi qu'en 1990, et suite à l'émergence de la fièvre catarrhale ovine au début des années 2000, mais depuis aucune mise à jour d'espèce n'a été entreprise.

Dans la présente étude, nous avons cherché à réviser l'inventaire actuel des espèces de *Culicoides* présentes au Maroc, en se basant sur des connaissances taxonomiques et systématiques les plus récentes. Les études passées ont eu recours à des noms d'espèces qui sont maintenant considérés comme synonymes. L'identification morphologique des *Culicoides* n'étant pas facile, les variations des caractéristiques diagnostiques sont fréquentes et peuvent conduire à une fausse affiliation d'espèces. Par conséquent, nous avons voulu mettre à jour la liste d'espèces pour le Maroc, en se basant sur l'examen d'individus collectés dans la région de Rabat, avec des identifications morphologiques complétés par des identifications moléculaires.

L'établissement de cet inventaire est une étape préalable à l'élaboration d'un atlas des caractères diagnostiques. Mais permet aussi de planifier d'autres études écologiques, y compris des captures de *Culicoides* à grande échelle portant sur des espèces d'intérêt vétérinaire, afin d'être en mesure de cartographier les risques liés aux maladies transmises par ces moucherons.

Les résultats issus de cette étude font l'objet de l'article 1 publié dans le journal Parasites & Vectors. **Article 1 : Bourquia M.,** Garros C., Rakotoarivony I., Gardès L., Huber K., Boukhari I., Delécolle J.C., Baldet T., Mignotte A., Lhor Y., Khallaayoune K., Balenghien T. 2019. Update of the species checklist of *Culicoides* Latreille biting midges (Diptera: Ceratopogonidae) of Morocco. Parasites & Vectors. 12: 459.

Bourquia *et al. Parasites Vectors* (2019) 12:459
https://doi.org/10.1186/s13071-019-3720-4

Parasites & Vectors

RESEARCH Open Access

Update of the species checklist of *Culicoides* Latreille, 1809 biting midges (Diptera: Ceratopogonidae) of Morocco

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Abstract

Background: Investigations of *Culicoides* fauna, including inventories, were carried out in Morocco at different periods after the country had faced major bluetongue and African horse sickness outbreaks. However, no comprehensive reference publication has provided a clear overview of the *Culicoides* species diversity. This study reviewed available data on *Culicoides* biting midge species in Morocco from 1968 to 2015 (published and grey literature in French and English) in order to revise the current checklist, in light of state of the art taxonomic and systematic knowledge, and confirmed the checklist with morphological and molecular identifications of specimens collected from the region of Rabat.

Methods: Literature related to *Culicoides* collections in Morocco was collated. Authors were contacted to obtain raw data and additional information for the collections. Fresh *Culicoides* material was collected and examined from two sites around Rabat, the capital of Morocco. Each collected individual was examined and morphologically identified, if possible, to the species level. In addition, molecular identification was performed to separate closely related species, to confirm difficult morphological identifications and to confirm new species records.

Results: A total of 6121 individuals of *Culicoides* spp. were collected and analyzed and at least 17 species were identified: *C. cataneii/C. gejgelensis*, *C. circumscriptus*, *C. fagineus*, *C. festivipennis*, *C. imicola*, *C. jumineri*, *C. kingi*, *C. longipennis*, *C. montanus*, *C. newsteadi*, *C. obsoletus*, *C. paolae*, *C. parotti*, *C. puncticollis*, *C. sahariensis*, *C. scoticus* and *C. subfagineus*. Seven species were confirmed using phylogenetic analyses. Two new species records for Morocco are reported: *C. paolae* and *C. subfagineus*.

Conclusions: The Moroccan fauna of *Culicoides* now includes 54 valid species. Further work would certainly increase this total, as one of the clades we identified was not affiliated to any described and valid species.

Keywords: Species diversity, Inventory, *Culicoides*, Biting midges, Rabat region, Morocco

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Background

Biting midges of the genus *Culicoides* Latreille, 1809 (Diptera: Ceratopogonidae) are responsible for the transmission of economically important viruses in animal production, most notably African horse sickness virus (AHSV), bluetongue virus (BTV), epizootic hemorrhagic disease virus (EHDV) and the Schmallenberg virus which emerged in 2011 in Europe [1–4]. Over the past two decades, multiple incursions of BTV serotypes [5] into the Mediterranean basin and the rest of Europe have dramatically changed the epidemiology of *Culicoides*-borne diseases in this region and increased the need for up to date taxonomic knowledge. Indeed, although *Culicoides imicola* Kieffer, 1913 is known as a proven BTV vector species for a very long time [6, 7], recent BTV outbreaks, especially in regions where *C. imicola* is absent, have highlighted that other species are able to transmit BTV. Within species present in the Mediterranean region, some other species of the subgenus *Avaritia*, such as *Culicoides obsoletus* (Meigen, 1818) and *Culicoides scoticus* Downes & Kettle, 1952, are considered probable BTV vectors based on their ecological habits, on virus isolation or viral genome detections from field-collected individuals and on experimental infections [8–10]. Moreover, several species of the subgenus *Culicoides*, such as *Culicoides pulicaris* (Linnaeus, 1758), *Culicoides lupicaris* Downes & Kettle, 1952 *Culicoides punctatus* (Meigen, 1804) or *Culicoides newsteadi* Austen, 1921, are considered possible BTV vectors based on their ecological habits and on virus isolation or viral genome detections from field-collected individuals [11–14]. Finally, BTV genome has been found in the abdomen and thorax of *Culicoides paolae* Boorman, 1996 [15], questioning its role as possible vector.

Previous investigations of the *Culicoides* fauna of Morocco have been prompted by major epizootics of *Culicoides*-borne diseases. The first intensive faunistic inventory started after the large AHSV outbreaks affecting northern Africa and Spain in 1965–66 [16]. Various surveys conducted in 1968–69 and 1970–71 throughout the country reported the existence of many *Culicoides* species and described two new species: *Culicoides calloti* Kremer, Delécolle, Bailly-Choumara & Chaker, 1979 and *Culicoides landauae* Kremer, Rebholtz-Hirtzel & Bailly-Choumara, 1975 [17–21]. Two additional species, *Culicoides clastrieri* Callot, Kremer & Deduit, 1962 and *Culicoides duddingstoni* Kettle & Lawson, 1955, were collected in Fès-Meknès Region in 1972, but the information was not published (Delécolle, pers. comm.). Taking into account the current classification of the genus *Culicoides*, the checklist in the 1970s consisted of at least 54 species (Table 1).

A second AHS epizootic occurred in Spain in 1987, from where it spread to Morocco in 1989 and persisted until 1991 [22]. At that time, the Afrotropical species *C. imicola* was the only proven AHSV vector and was considered the only species involved in AHSV transmission in Morocco [23, 24]. As a result, surveys conducted in Morocco in 1994–95 were focused on characterizing the distribution and abundance of *C. imicola* [23, 25]. *Culicoides imicola* is very widely dispersed, found at altitudes of up to 1275 meters and under climatic conditions ranging from Mediterranean to Saharan, with the highest catches in the lower areas of the northwest (between Tangier and Rabat) and in Marrakech (Fig. 1). Other species reported were *Culicoides circumscriptus* Kieffer, 1918, *C. newsteadi*, *Culicoides puncticollis* (Becker, 1903) and specimens close to *Culicoides festivipennis* Kieffer, 1914 (mentioned under its synonym name, *Culicoides odibilis* Austen, 1921), but also *C. obsoletus* and *C. pulicaris*. The two latter referred more likely to 'group' than to single species. Indeed, authors confirmed that morphologically close species might have been grouped with *C. obsoletus* or *C. pulicaris*, as identification focused mainly on *C. imicola* [23].

The late 1990s were marked by the occurrence of massive and regular BTV outbreaks in the Mediterranean [5]. The scale of these successive epizootic diseases has led to the establishment of entomological surveillance networks dedicated to *Culicoides* in most Mediterranean countries. In Morocco, a cross-sectional study was therefore carried out in 2000–2003 by the veterinary services in 50 sites. This third study confirmed the wide distribution of *C. imicola*, which was the most frequent species in the collections [26], but did not provide additional inventory information, except for *C. imicola*; the samples collected by these authors were identified only at the level of species groups.

The first BTV outbreak in Morocco was reported in September 2004 [27]. Since then, BTV outbreaks have been reported every year, except for 2008, in the entire Moroccan territory, whereas the country faced EHDV outbreaks in 2006. Following snapshot entomological surveys carried out in BTV transmission sites [28], a new entomological surveillance network was set up in 2009–2010 as part of the epidemiological surveillance of animal diseases organized by the national veterinary services [29]. This fourth study resulted in the publication of an updated *Culicoides* checklist for Morocco, with the addition of a new species based on a single morphologically identified specimen: *Culicoides helveticus* Callot, Kremer & Deduit, 1962. The lack of molecular confirmation for this individual alone suggests caution in including this new species in the national checklist.

Table 1 *Culicoides* species list for Morocco

Species	Reference	Our data	Proposed checklist
C. azerbajdzhanicus Dzhafarov, 1962	[3, 4, 6]		✓
C. badooshensis Khalaf, 1961	[3, 4, 6]		✓
C. calloti Kremer, Delécolle, Bailly-Choumara & Chaker, 1979	[6, 7]		✓
C. cataneii Clastrier, 1957	[4, 6]	✓	✓
C. circumscriptus Kieffer, 1918	[1, 3, 4, 6, 9]	✓	✓
C. clastrieri Callot, 1962	[a]		✓
C. derisor Callot & Kremer, 1965	[4, 6]		✓
C. duddingstoni Kettle & Lawson, 1955	[a]		✓
C. dzhafarovi Remm, 1967	[4, 6]		✓
C. faghihi Navai, 1971	[5]		✓
C. fagineus Edwards, 1939	[4, 6]	✓	✓
C. festivipennis Kieffer, 1914	[1, 3, 4, 6, 9] (as synonym name *C. odibilis*)	✓	✓
C. helveticus Callot, Kremer & Deduit, 1962	[10]		
C. heteroclitus Kremer & Callot, 1965	[5]		✓
C. imicola Kieffer, 1913	[3, 4, 6, 8, 9] (as synonym name *C. pallidipennis*)	✓	✓
C. jumineri Callot & Kremer, 1969	[4, 6, 9] (as Jumineri group)	✓	✓
C. kibunensis Tokunaga, 1937	[5] (as synonym name *C. cubitalis*)		✓
C. kingi Austen, 1912	[2] (as Schultzei/Kingi group)	✓	✓
C. kurensis Dzhafarov, 1960	[9]		✓
C. landauae Kremer, 1975	[5]		✓
C. langeroni Kieffer, 1921	[3, 4, 6]		✓
C. longipennis Khalaf, 1957	[4]	✓	✓
C. marcleti Callot, Kremer & Basset, 1968	[4]		✓
C. maritimus Kieffer, 1924	[3, 4, 6]		✓
C. montanus Shakirzjanova, 1962	[4, 6]	✓	✓
C. newsteadi Austen, 1921	[1, 3, 4, 6, 9] (as synonym name *C. halophilus*)	✓	✓
C. obsoletus (Meigen, 1818)	[1, 3, 4, 6, 9]	✓	✓
C. odiatus Austen, 1921	[3, 4, 6–9] (as synonym names *C. lailae* and *C. indistinctus*)		✓
C. pallidicornis Kieffer, 1919	[4] (as Achrayi/pallidicornis group)		✓
C. pallidus Khalaf, 1957	[4] (as synonym name *C. stackelbergi*)		✓
C. paolae Boorman, 1996		✓	✓
C. parroti Kieffer, 1922	[3, 4, 6]	✓	✓
C. pictipennis (Staeger, 1839)	[3]		✓
C. picturatus Kremer & Deduit, 1961	[3, 4, 6]		✓
C. pseudopallidus Khalaf, 1961	[3, 4]		✓
C. pulicaris (Linnaeus, 1758)	[4, 6, 8, 9]		✓
C. pumilus (Winnertz, 1852)	[5]		✓
C. punctatus (Meigen, 1804)	[1, 3, 4, 6]		✓
C. puncticollis (Becker, 1903)	[1, 3, 4, 6, 9]	✓	✓
C. ravus de Meillon, 1936	[4, 6] (as synonym name *C. subravus*)		✓
C. saevus Kieffer, 1922	[1, 3, 4, 6]		✓
C. sahariensis Kieffer, 1923	[3, 4, 6–9] (as synonym name *C. coluzzi*)	✓	✓
C. santonicus Callot, Kremer, Rault & Bach, 1966	[3, 4, 6]		✓
C. schultzei (Enderlein, 1908)	[1]		✓
C. scoticus Downes & Kettle, 1952	[4, 6]	✓	✓
C. sejfadinei Dzhafarov, 1958	[4, 6]		✓
C. semimaculatus Clastrier, 1958	[5]		✓
C. sergenti Kieffer, 1921	[6] (as synonym name *C. mosulensis*)		✓
C. shaklawensis Khalaf, 1957	[5]		✓

Table 1 (continued)

Species	Reference	Our data	Proposed checklist
C. similis Carter, Ingram & Macfie, 1920	[4]		✓
C. simulator Edwards, 1939	[5]		✓
C. subfagineus Delécolle & Ortega, 1998		✓	✓
C. subfasciipennis Kieffer, 1919	[4, 6, 9]		✓
C. truncorum Edwards, 1939	[4] (as synonym name *C. sylvarum*)		✓
C. univittatus Vimmer, 1932	[3, 4, 6] (as synonym name *C. agathensis)*		✓
C. vidourlensis Callot, Kremer, Molet & Bach, 1968	[3]		✓

Notes: [1] Callot et al. (1968); [2] Kremer et al. (1970); [3] Kremer et al. (1971); [4] Kremer et al. (1975); [5] Chaker et al. (1979); [6] Kremer et al. (1979); [7] Baylis et al. (1997); [8] Bouayoune et al. (1998); [9] Cêtre-Sossah & Baldet (2004); [10] Lhor et al. (2015)

[a] Unpublished records of Delécolle

In the present study, we aimed to revise the current inventory of *Culicoides* species present in Morocco in light of the most up to date taxonomic and systematic knowledge. The studies in the 1970s used species names that are now considered as synonymous. Morphological identification of *Culicoides* is also difficult; variations in diagnostic characteristics are common and could lead to false affiliation of specimens [30, 31]. Therefore, we aimed to confirm the checklist for Morocco based on the examination of fresh material collected in the Rabat region, the Moroccan capital, by an integrative taxonomy approach using morphological identification supplemented by molecular identification to confirm species records.

Methods

Literature related to *Culicoides* collections in Morocco, including articles published in French, English and unpublished 'grey' literature (mainly reports or theses), was collated. Authors were contacted to obtain raw data on the collections and details on the level of identification (species or 'group').

In these previous studies, only morphological identification was used. We thus decided to collect and examine fresh *Culicoides* material from two sites around Rabat, a horse-riding center (33°56′N, 6°49′W) (site M1) and a goat farm (33°51′N, 6°51′W) (site M2) to make samples available for molecular identification. The objective was to confirm the record of some species for which morphological identification is difficult. Collection was made using an Onderstepoort Veterinary Institute black light suction trap operated from dusk until dawn, on two consecutive days every two weeks (from May to October 2016). When possible, collected individuals were morphologically identified to the species level using relevant morphological identification keys [32, 33].

Additionally, molecular identification was performed (i) to identify closely related species (such as *C. obsoletus*, *C. scoticus* or *Culicoides montanus* Shakirzjanova, 1962); (ii) to confirm difficult morphological identifications (damaged specimen or uncertain diagnostic features); and (iii) to confirm new species records. Genomic DNA was individually extracted using the NucleoSpin® Tissue DNA Kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions. For *C. obsoletus/C. scoticus*, the allele-specific PCR from Nolan et al. [34] was used to separate the two morphologically similar species. For other species, *cox*1 amplification and sequencing using primers were produced following procedure described by Bakhoum et al. [35], before final species assignment was done by phylogenetic reconstructions and BLAST search of GenBank entries and an Afrotropical BOLD database (AFCUL001-18 to AFCUL1131-18).

The newly generated *cox*1 sequences were edited in GENEIOUS R6 (Biomatters, http://www.geneious.com/) then aligned using the MUSCLE algorithm [36]. Maximum Likelihood (ML) phylogenetic trees were computed under a substitution model estimated using the JMODELTEST tool within MEGA X [37]. The Bayesian information criterion implemented within JMODELTEST was used to determine the most suitable evolutionary model; this was the general time reversible (GTR) model with gamma distributed (+G) rate variation among sites (5 categories; parameter = 0.6015) and proportion of invariable sites (+I) of 22.82%. Missing data, including indels, were excluded from the analysis. The tree was subjected to 1000 bootstrap replications to assess topological reliability. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value.

Specimens were stored in alcohol with one specimen per species slide-mounted and are available upon

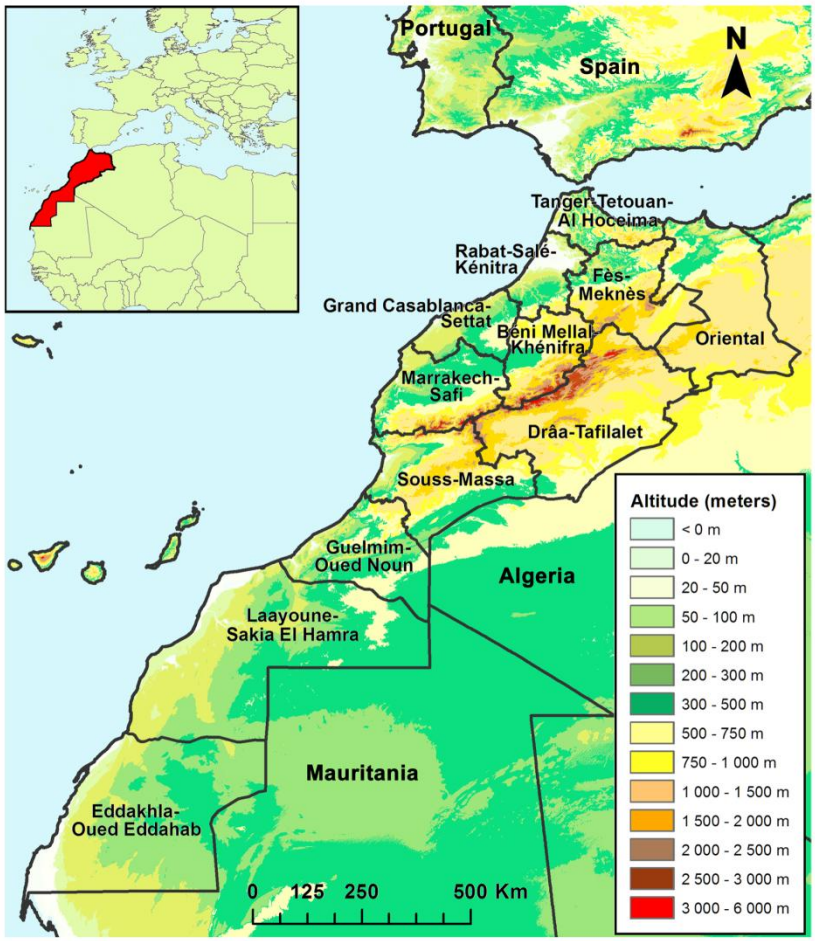

Fig. 1 Map of the regions in the Kingdom of Morocco

request to Maria Bourquia and Khalid Khallaayoune at Agronomic and Veterinary Institute Hassan II, Rabat, Morocco. Sequences were deposited in the GenBank database under the accession numbers MK732283–MK732313. Sequences from the GenBank and BOLD databases were used in the tree reconstruction. Sequences for *Culicoides halophilus* Kieffer, 1924 (GenBank: KX853271 and KX853272) were used although not considered as a valid species by the world catalogue of *Culicoides* [38].

Results

A total of 6121 *Culicoides* were examined, representing at least 17 species collected during a total of 12 collection sessions. Morphological identification confirmed the presence of 15 previously reported species and established two new records: *C. paolae* with 6 individuals and *Culicoides subfagineus* Delécolle & Ortega, 1998 with 3 individuals (Table 1). We dissected and mounted the wings of the different species including *C. paolae* and *C. subfagineus* (Fig. 2).

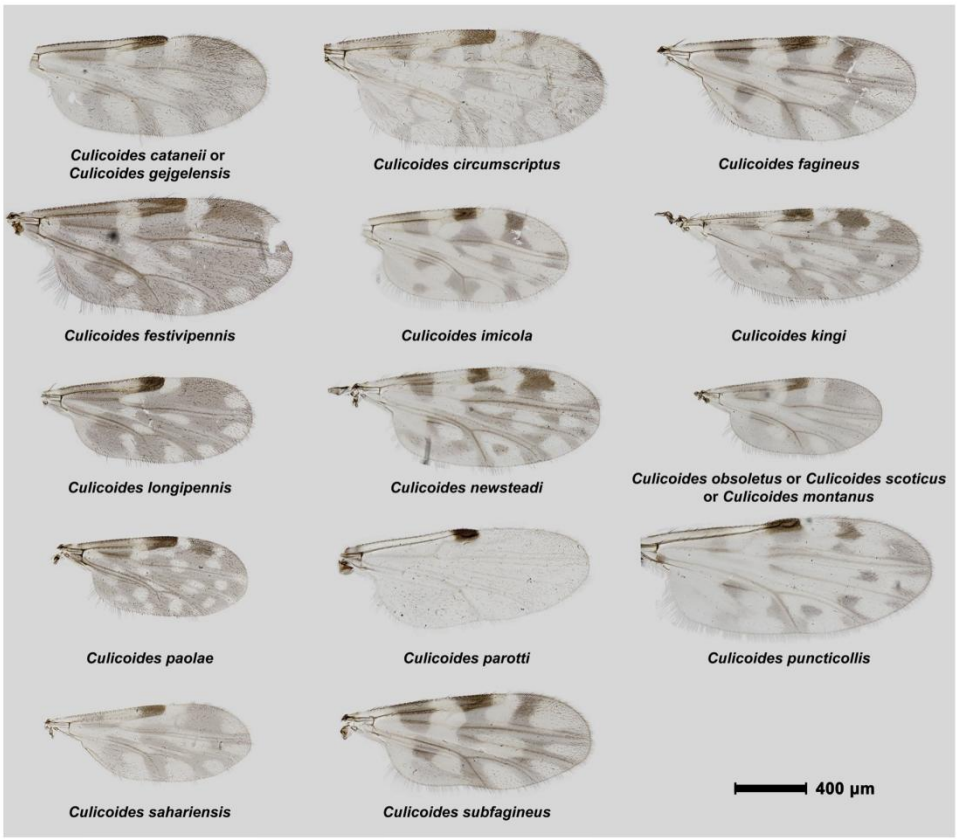

Fig. 2 Wing patterns of *Culicoides* spp. collected during the present survey

Molecular identification was performed on 105 individuals. A total of 71 females morphologically identified as *C. obsoletus*/*C. scoticus* were identified with the species-specific PCR [34] allowing the identification of 59 *C. obsoletus* and 7 *C. scoticus*. Five individuals could not be identified to the species level and were kept as *C. obsoletus*/*C. scoticus*. *cox*1 sequences were generated from 34 individuals of which 3 individuals did not produce exploitable sequences. Alignment was 390 bp long and included 31 sequences plus 135 references sequences retrieved from GenBank, BOLD or expert molecular library.

The resulting tree was used as a species affiliation tool and not to infer systematic relationships. Indeed, the node robustness was high (above 90%) only at the terminal nodes which allows affiliation at the species level. From the tree we infer the presence of *Culicoides cataneii* Clastrier, 1957 (2 sequences), *C. circumscriptus* (1 sequence), *C. imicola* (1 sequence), *Culicoides jumineri* Callot & Kremer, 1969 (5 sequences), *C. montanus* (1 sequence), *C. obsoletus* (1 sequence), *C. paolae* (6 sequences), *Culicoides sahariensis* Kieffer, 1923 (2 sequences), *Culicoides kingi* Austen, 1912 (3 sequences), *C. subfagineus* (3 sequences) and 6 sequences clustering in the same clade without voucher sequence (Fig. 3). Molecular affiliation of two sequences (MK732287 and MK732288) should be considered with caution. They clustered in proximity to *C. jumineri* sequences. However, these two individuals

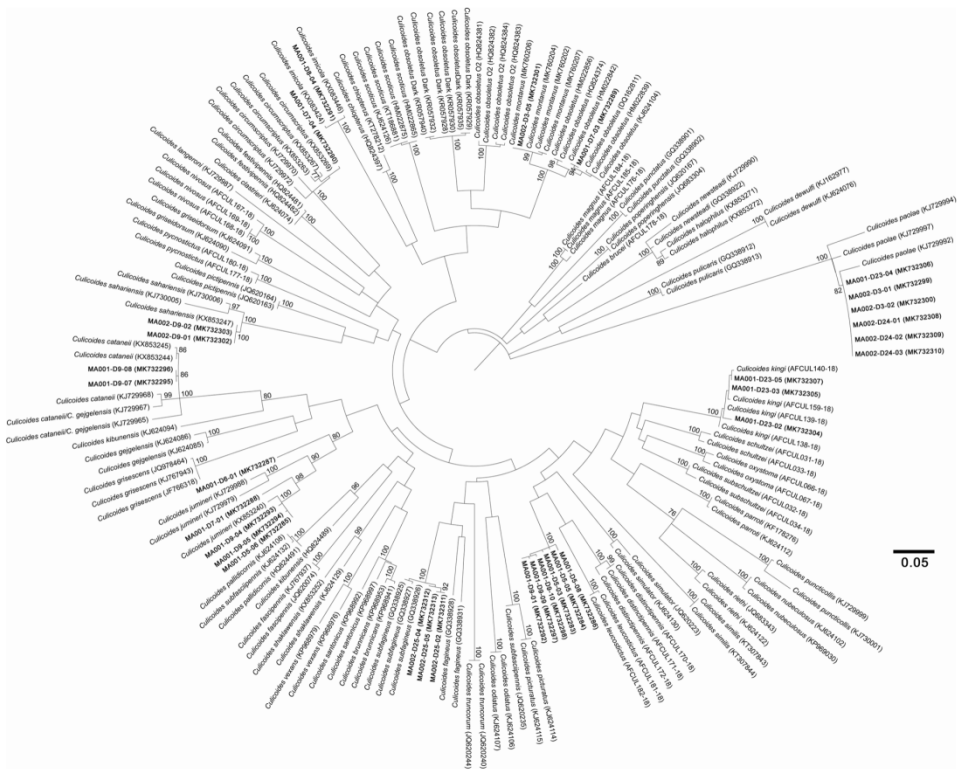

Fig. 3 Phylogenetic tree based on *cox*1 polymorphism. In bold, sequences produced in this study with GenBank reference numbers in brackets. The evolutionary history was inferred using the Maximum Likelihood method and GTR+G+I model. This analysis involved 166 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option); there were a total of 390 positions in the final dataset. The tree is drawn to scale, with branch lengths corresponding to the number of substitutions per site

could not be considered with certainty as *C. jumineri*, due to the bootstrap values being too low, highlighting genetic differences between these two sequences and the *C. jumineri* reference sequences.

Discussion

Species inventories allow completing and updating taxonomic and systematic revision to develop identification keys, establish national or regional species checklists and field guides, and make all the relevant information readily available through scientific publications and other dissemination tools. Regular updates of faunistic inventories of economically important biological groups are of great importance to maintain expertise and overcome taxonomic obstacles, train young scientists and ensure the stability of species community structure in an environment subject to the introduction and colonization of exotic species [39].

The present paper revised, updated and confirmed the checklist of *Culicoides* for Morocco using combined morphological and molecular identification and, to the authors' knowledge, reports for the first time *C. paolae* and *C. subfagineus* in the country. The revised checklist includes 54 species of *Culicoides* recorded from the territory of Morocco. However, it is noted that the southernmost Saharan region of Eddakhla-Oued Eddahab has, to our knowledge, never been prospected (Fig. 1). The neighboring region of Laayoune-Sakia El Hamra has only been prospected around Laayoune in 2009–2010 [29]. The few specimens collected belonged to *C. imicola*,

and to Newsteadi and Circumscriptus groups of species. Therefore, we have not included these two regions in Table 2. The small number of *Culicoides* specimens collected in Laayoune may be related to the arid climate prevailing in this region.

Our review of the literature highlighted the use of synonym names for 11 species (*C. sahariensis*, *C. imicola*, *Culicoides univittatus* Vimmer, 1932, *C. newsteadi*, *Culicoides odiatus* Austen, 1921, *C. festivipennis*, *Culicoides pallidus* Khalaf, 1957, *Culicoides ravus* de Meillon, 1936, *Culicoides kibunensis* Tokunaga, 1937, *Culicoides sergenti* Kieffer, 1921 and *Culicoides truncorum* Edwards, 1939). Some authors also re-evaluated their own collections. *Culicoides riethi* Kieffer, 1914 erroneously reported [17], was modified to *C. puncticollis* in 1971 [18]. Caution should therefore be exercised when using former publications. It is mandatory to refer to the world catalogue for *Culicoides* [38] to validate species names and update them if needed. Moreover, our literature review highlighted the lack of identification tools for *Culicoides* in Morocco, and more broadly in North Africa, as already published for the Western Palaearctic region [33].

The updated checklist for the country was established after a critical analysis of the literature and from our novel collections. *Culicoides begueti* Clastrier, 1957 is mentioned by Kremer et al. [18] from unpublished collections in the Marrakech region. As materials and references to these catches were not available, the species has been removed from the list pending further reports. The presence of individuals belonging to the 'Kingi-schultzei group' was reported by Bailly-Choumara & Kremer in Souss-Massa Region [17, 21]. According to our molecular and morphological analysis, this species could be *C. kingi*. *Culicoides helveticus* was collected as a unique individual by Lhor et al. [29]. This species can be confused with *Culicoides stigma* (Meigen, 1818) and *Culicoides parroti* Kieffer, 1922, which have the same wing pattern, but can be distinguished by the shape of the spermatheca [40]. In our collections, *C. parroti* was observed. Considering the known distribution of *C. helveticus* is the mountainous regions of the Palaearctic region such as in Russia [41–43], France [40], Sweden [44] and Ukraine [45], we considered it was appropriate to remove this species from Moroccan list of *Culicoides*.

Our molecular analysis revealed that the specimens morphologically identified as *C. obsoletus*/*C. scoticus* belong to *C. obsoletus*, *C. scoticus* and one specimen of *C. montanus*. Indeed, *C. montanus* is a rare species that is phylogenetically very close to *C. obsoletus* [46, 47] for which the taxonomic status is a little controversial [48, 49]. Recently, several authors have reported the existence of cryptic diversity within the *C. obsoletus* taxon [50, 51], namely *C. obsoletus* clade O2, *C. obsoletus* dark and one unnamed entity. Albeit from a limited number of individuals, we did not observe such cryptic diversity within the *C. obsoletus* taxon in Morocco.

A clade of 6 sequences (i.e. MK732297, MK732292, MK732298, MK732283, MK732284 and MK732286) received high bootstrap support. This clade is part of the same phylogroup as *C. santonicus* Callot, Kremer, Rault & Bach, 1966 which belongs to the Vexans group, grouping three species with *Culicoides brunnicans* Edwards, 1939 and *Culicoides vexans* (Staeger, 1839). We hypothesized that it is either a novel species or simply one without voucher sequences in molecular libraries. This could be investigated by increasing the number of voucher sequences to other Afrotropical or Palaearctic species. Moreover, the *C. jumineri* clade showed genetic variability that may indicate that cryptic species diversity is present within this clade.

To our knowledge, our study records *C. paolae* for the first time in Morocco, whereas it was previously known from Italy [52], Greece [53], Algeria [54] and Tunisia [55–57]. This species, largely distributed in the Mediterranean region, was not considered, until recently, to be associated with BTV transmission. Indeed, BTV genome was recovered from the thorax of field-collected individuals in Sardinia during BTV outbreaks [15], raising a possible role of this species in BTV transmission. Updating the distribution of this species is thus of primary interest. To our knowledge, we also describe *C. subfagineus* for the first time in the country. Our specimens were first morphologically identified as *Culicoides grisescens* Edwards, 1939 but tree reconstruction clearly assigned all three specimens to *C. subfagineus*. *Culicoides subfagineus* was recently reported for the first time in Portugal [58] and Germany [59]. Some authors confuse this species with *Culicoides fagineus* Edwards, 1939 and group it into *C. subfagineus/C. fagineus* as pairs of species [60].

Finally, Moroccan fauna is composed of species with a different geographical distribution and can be classified into three groups: (i) species with a wide distribution covering the Palaearctic region (from Scandinavia to the Mediterranean) such as *C. circumscriptus*, *C. clastrieri*, *C. duddingstoni*, *Culicoides dzhafarovi* Remm, 1967, *C. fagineus*, *C. festivipennis*, *C. jumineri*, *C. kibunensis*, *Culicoides kurensis* Dzhafarov, 1960, *Culicoides maritimus* Kieffer, 1924, *C. montanus*, *C. newsteadi*, *C. obsoletus*, *C. odiatus*, *C. parroti*, *Culicoides pictipennis* (Staeger, 1839), *Culicoides picturatus* Kremer & Deduit, 1961, *C. pulicaris*, *C. punctatus*, *C. puncticollis*, *Culicoides saevus* Kieffer, 1922, *C. scoticus*, *Culicoides shaklawensis* Khalaf, Ingram & Macfie, 1957, *Culicoides simulator* Edwards, 1939, *Culicoides subfasciipennis* Kieffer, 1919 and *Culicoides vidourlensis* Callot, Kremer, Molet & Bach, 1968; (ii) species distributed around the Mediterranean basin

Table 2 *Culicoides* species list for Morocco by region

Species	Tanger-Tetouan-Alhoceima	Oriental	Fès-Meknès	Rabat-Salé-Kénitra	Beni Mellal-Khénifra	Casablanca-Settat	Marrakesh-Safi	Drâa-Tafilalet	Souss-Massa	Guelmim-Oued Noun
C. azerbajdzhanicus			[4]		[4]		[3, 4]		[6]	[4, 6]
C. badooshensis	[3]		[4]	[4, 6]			[3, 6]			
C. calloti									[6, 7]	[6, 7]
C. catanei	[6]			[4, 6]						
C. circumscriptus	[3, 6]	[3]	[4, 6]	[1, 3, 4, 6, 9]		[4, 6]	[4, 6]		[6]	[4]
C. clastrieri			[a]							
C. derisor			[4]	[4, 6]						
C. duddingstoni			[a]							
C. dzhafarovi				[4]						[6]
C. faghihi									[5]	
C. fagineus				[4, 6]	[4]		[4]			
C. festivipennis	[1, 3, 6]			[4, 6, 9]		[6]	[3, 6]			
C. heteroclitus							[5]		[5]	
C. imicola	[8]	[8]	[4, 8]	[8, 9]	[8]		[3, 8]	[8]	[8]	[4, 6, 8]
C. jumineri		[6]	[4]	[4, 6, 9]			[6]		[6]	[4, 6]
C. kibunensis			[5]				[5]			
C. kingi									[2]	
C. kurensis				[9]						
C. landauae			[5]							
C. langeroni				[4]			[3]			[6]
C. longipennis			[4]				[4]			
C. marcleti			[4]							[4]
C. maritimus	[4]			[3, 4]			[6]			
C. montanus	[6]			[4]			[4, 6]			[4]
C. newsteadi	[1, 3, 6]		[4]	[3, 4, 6, 9]	[4, 6]	[4, 6]	[3, 6]	[3]	[3]	[4]
C. obsoletus	[6, 8]	[8]	[8]	[1, 3, 4, 6, 8]	[4, 8]	[6, 8]	[4, 6, 8]		[8]	[8]
C. odiatus			[4]	[4, 6, 9]			[3, 6]		[5]	[4, 6]
C. pallidicornis										[4]
C. pallidus										[4]
C. parroti				[4]			[3, 6]			
C. pictipennis				[3]						
C. picturatus	[3]			[4]	[6]	[6]				
C. pseudopallidus			[4]				[3, 4]			
C. pulicaris	[8]	[8]	[4, 8]	[4, 6, 8]	[4, 8]	[8]	[6, 8]	[8]	[8]	[4, 6]

Table 2 (continued)

Species	Tanger-Tetouan-Alhoceima	Oriental	Fès-Meknès	Rabat-Salé-Kénitra	Beni Mellal-Khénifra	Casablanca-Settat	Marrakesh-Safi	Drâa-Tafilalet	Souss-Massa	Guelmim-Oued Noun
C. pumilus			[5]				[5]			
C. punctatus	[1, 3]		[4]	[3, 4]		[6]				
C. puncticollis	[3]		[4, 6]	[1, 3, 4, 6, 9]			[3, 6]			
C. ravus							[4]		[6]	[4, 6]
C. saevus	[3]	[6]	[4, 6]	[1, 4, 9]			[1, 3, 6]	[6]	[6]	[4, 6]
C. sahariensis	[3]	[3]	[4]	[3, 4, 6, 9]			[3]	[3]		[4, 6]
C. santonicus	[3]			[4, 6]		[6]				
C. scoticus					[4]	[6]	[4, 6]			
C. sejfadinei		[6]						[6]		[4, 6]
C. semimaculatus	[5]					[5]				
C. sergenti		[6]								[6]
C. shaklawensis			[5]				[5]			
C. schultzei							[1]			
C. similis										[4]
C. simulator			[5]							
C. subfasciipennis	[6]		[4]	[4, 9]	[4]					
C. truncorum				[4]	[4]					
C. univittatus	[4, 6]		[6]	[3, 4, 6]			[6]			
C. vidourlensis	[3]			[3]			[3]			

Notes: [1] Callot et al. (1968); [2] Bailly-Choumara & Kremer (1970); [3] Kremer et al. (1970); [4] Kremer et al. (1971); [5] Kremer et al. (1975); [6] Chaker et al. (1979);[7] Kremer et al. (1979); [8] Bouayoune et al. (1998); [9] Cêtre-Sossah & Baldet (2004)

[a] Unpublished records of Delécolle

such as *Culicoides azerbajdzhanicus* Dzhafarov, 1962, *C. cataneii*, *Culicoides derisor* Callot & Kremer, 1965, *Culicoides faghihi* Navai, 1971, *Culicoides heteroclitus* Kremer & Callot, 1965, *C. jumineri*, *Culicoides langeroni* Kieffer, 1921, *Culicoides longipennis* Khalaf, 1957, *Culicoides marcleti* Callot, Kremer & Basset, 1968, *C. paolae*, *Culicoides pseudopallidus* Khalaf, 1961, *C. sahariensis*, *C. santonicus*, *Culicoides sejfadinei* Dzhafarov, 1958, *Culicoides semimaculatus* Clastrier, 1958, *Culicoides sergenti* Kieffer, 1921, *C. subfagineus*, *C. univittatus*; and (iii) Afrotropical species also present in the southern part of the Palaearctic zone, especially in the Mediterranean such as *C. imicola*, C. *kingi*, *C. ravus* and *Culicoides similis* Carter, 1920.

Some species have limited numbers of records in the literature making their known geographical distribution too sporadic to be assigned to the above groups: *Culicoides badooshensis* Khalaf, 1961 (reported in Irak, Iran, Morocco and Turkey), *C. pallidus* (Morocco), *C. landauae* (Morocco), *C. calloti* (Morocco) and *Culicoides pumilus* (Winnertz, 1852) (Ukraine, France and Morocco).

The two other countries of the Maghreb, Algeria and Tunisia, have many bio-climatic similarities such as a Mediterranean coastline in the north and Sahara desert in the south. The *Culicoides* fauna of these countries has been studied in two steps, as in Morocco. First, inventories were carried out in the 1950s and the 1980s in Algeria [61, 62], leading to the record of 31 species, and in the 1980s in Tunisia [63], leading to the record of 22 species. Then, inventories were completed during studies following the BTV emergence in the 2000s in the Maghreb and in the whole Mediterranean region. Today, the number of species recorded in Morocco (54 species) was slightly higher than in Algeria with 47 species [54] and in Tunisia with 35 species [55, 57, 64, 65]. The same increasing species richness gradient from Tunisia to Morocco is observed for mosquitoes [66], and may be related to the greater diversity of climates and landscapes found in Morocco, including the influence of the ocean climate prevailing on the northwest coast and not existing in the two neighboring countries.

Conclusions

The *Culicoides* fauna of Morocco includes 54 species, at least 8 of which are recognized vectors or potential vectors of *Culicoides*-borne viruses of economic importance in animal production. Among these, *C. imicola* is a proven BTV and AHSV vector [6, 7, 67] and was considered for a long time the only important vector. Most epidemiological studies have therefore focused on this species [23]. In this study, we confirmed by molecular assay the presence of both *C. obsoletus* and *C. scoticus* in Morocco, which are considered as probable BTV vectors in the western Palaearctic region [68, 69]. Moreover, we reported the presence of *C. kingi*, a potential EHDV vector [70, 71] and of *C. paolae*, which, together with *C. newsteadi*, *C. pulicaris* and *C. punctatus* [14, 15, 72], is regarded as a potential BTV vector. Establishing a comprehensive checklist of *Culicoides* for Morocco is a prerequisite for developing a barcode library and atlas of diagnostic characters, but also to plan further ecological studies, including large scale *Culicoides* collections focusing species of veterinary interest, to be able to carry out risk mapping for related *Culicoides*-borne diseases.

Abbreviations

AHSV: African horse sickness virus; BTV: bluetongue virus; EHDV: epizootic hemorrhagic disease virus.

Acknowledgments

The authors thank Matthew Baylis (University of Liverpool, UK) for sharing the raw data from 1994–1995 collections, François Thiaucourt for the help with the illustrations and finally the owner of the farms where 2016 collections were carried out.

Authors' contributions

MB, TB, CG and KK designed the study. MB and IB contributed to the collection of *Culicoides*. MB, IR, LG, KH, JCD and AM identified all *Culicoides* specimens. MB analysed the data. IR, LG, KH, IB, JCD, TB, AM and YL contributed to the manuscript after its first draft by MB, TB, CG and KK. All authors read and approved the final manuscript.

Funding

This study was supported by Vectornet, funded by European Foof Safety Agency and European Center for Disease Control. MB was supported by a Grant from PHC Toubkal (Toubkal/17/38 campus France 36867VH) and Centre National de Recherche Scientifique et Technique (CNRST).

Availability of data and materials

Specimens are stored in ethanol with one specimen per species slide-mounted and are available upon request to Maria Bourquia and Khalid Khallaayoune at Agronomic and Veterinary Institute Hassan II, Rabat, Morocco.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 27 May 2019 Accepted: 14 September 2019
Published online: 24 September 2019

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Chapitre 3 : Caractérisation de la diversité et la saisonnalité des espèces de *Culicoides* d'intérêt vétérinaire dans différents environnements d'élevages.

Les *Culicoides* sont très sensibles aux conditions météorologiques et environnementales, qui régulent leur dynamique de populations. Des changements de ces conditions auront généralement un impact important sur la dynamique de transmission des maladies transmises (Gubler et al, 2001).

Les conséquences d'une modification des éléments météorologiques ou climatiques, telles que les températures ou le régime des précipitations, peuvent affecter tous les étapes du cycle de vie des moucherons dépendant de facteurs biotiques.

Il est important d'identifier ces facteurs qui déterminent la dynamique saisonnière des *Culicoides* pour différentes raisons. Le caractère saisonnier des épizooties est souvent associé au pic d'abondance annuel des populations de *Culicoides*. Enfin, la présence de populations pendant les périodes non favorables peut entretenir une circulation virale toute l'année. Pour chaque espèce de *Culicoides*, la relation entre la saisonnalité et certaines variables climatiques -température et précipitations-semble varier d'un endroit à l'autre. Bien que la température et les précipitations déterminent probablement le caractère saisonnier de la majorité des espèces de *Culicoides*, certaines peuvent être influencées par d'autres facteurs (Mellor, 2000, Purse et al., 2015). De plus, le nombre de générations par an déterminera si les populations atteindront leur maximum plus tôt (dynamique univoltine) ou plus tard (dynamique multivoltine) au cours de la saison.
Les études traitant de l'influence des paramètres météorologiques et environnementaux sur les populations de *Culicoides* sont rares (Purse et al., 2012). Au Maroc, le profil saisonnier des populations de *C. imicola* a été décrit lors des prospections de 1994-1995 et 2009-2010 (Baylis et al., 1997 ; Lhor et al., 2015) mais n'a pas été analysé pour identifier les facteurs climatiques qui influent sur cette saisonnalité.

Pour apporter de nouvelles connaissances sur la composition d'espèces et la saisonnalité des *Culicoides* au Maroc, on a réalisé des captures dans trois élevages aux hôtes différents (centre équestre, fermes caprines et bovines) à Rabat. Les résultats issus de cette étude font l'objet de l'article 2 accepté dans le journal Revue d'Élevage et de Médecine Vétérinaire des Pays tropicaux. **Bourquia M.**, Garros C., Rakotoarivony I., Boukhari I., Chakrani M., Huber K., Gardès L., Wint W., Thierry Baldet T., Khallaayoune K., Balenghien T. 2019. Composition

and seasonality of *Culicoides* in different host environments in the Rabat region (Morocco). Revue d'Élevage et de Médecine Vétérinaire des Pays tropicaux. Accepted.

Composition and seasonality of *Culicoides* in three host environments in the Rabat region (Morocco)

Diversité et saisonnalité des *Culicoides* dans trois élevages aux hôtes différents dans la région de Rabat (Maroc)

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Abstract

Morocco has suffered several outbreaks of *Culicoides*-borne viruses in recent decades and most studies have focused on *Culicoides imicola*, considered for a long time as the only important vector. The change of bluetongue epidemiology in the Mediterranean Basin and Europe over the past two decades has highlighted the role of other *Culicoides* species in bluetongue virus (BTV) transmission. The objective of this study was to provide new insights on the *Culicoides* species composition and seasonality in three different host-environments (horse-riding center, goat and cattle farms) in Rabat, the capital of Morocco, where bluetongue has been endemic since 2004. Light/suction trap collections were carried out on two consecutive nights at fortnight intervals from May 2016 to May 2017. *Culicoides* were identified morphologically at the species level when possible. Multivariate analysis was used to compare the impact of the site/vertebrate species and the collection month on the species communities, as well statistical modelling was used to identify environmental drivers of the *Culicoides* seasonality. A total of 12,460 *Culicoides* individuals belonging to at least 15 different species was collected during the survey. *Culicoides imicola* was by far the most abundant species (71.4% of total catches). The site location, and thus the vertebrate species, did not influence the species composition, which was principally impacted by the month of collection. Surprisingly, the atmospheric pressure was the environmental parameter most frequently selected and a predictor in seasonal models. The potential impact of this meteorological parameter along with the other selected variables is discussed. Identifying the environmental parameters driving the *Culicoides* seasonal abundance is the first step to implementing robust *Culicoides* dynamic models that could later be used in transmission risk modelling.

Keywords: entomology, *Culicoides*, seasonality, bluetongue, Morocco

Résumé

Le Maroc a connu plusieurs épizooties liées à des virus transmis par les *Culicoides* au cours des dernières décennies, et la majorité des études associées se sont focalisées sur *Culicoides imicola*, considéré depuis longtemps comme le seul vecteur d'importance. Les changements apparus dans l'épidémiologie de la fièvre catarrhale du mouton (FCM) dans le bassin méditerranéen et en Europe au cours des 20 dernières années a souligné l'importance des autres espèces de *Culicoides* dans la transmission du virus de la FCM. L'objectif de cette étude était de fournir de nouvelles connaissances sur la composition d'espèces et la saisonnalité des *Culicoides* dans trois élevages aux hôtes différents (centre équestre, fermes caprines et bovins) à Rabat, capitale du Maroc, où la FCM est endémique depuis 2004. Des collectes ont été réalisées à l'aide de pièges lumineux pendant deux nuits consécutives tous les 15 jours de mai 2016 à mai 2017. Des analyses multivariées ont été utilisées pour comparer l'effet du site/espèce animale à l'effet du mois de collecte sur la composition d'espèces, et des méthodes de modélisation statistique ont été utilisées pour identifier les déterminants environnementaux de la saisonnalité des *Culicoides*. Un total de 12 460 *Culicoides*, appartenant à au moins 15 espèces différentes, a été capturé pendant l'étude. *Culicoides imicola* était de loin l'espèce la plus abondante (71,4 % du total des captures). Le site, et donc l'espèce hôte, influençait peu la composition d'espèce, qui était principalement influencée par le mois de collecte. De manière surprenante, la pression atmosphérique a été le paramètre environnemental le plus fréquemment sélectionné dans les modèles saisonniers. L'impact potentiel de ce paramètre météorologique et des autres variables sélectionnées est discuté. Identifier les paramètres environnementaux gouvernants l'abondance des *Culicoides* au cours de la saison est le premier pas pour construire des modèles robustes de dynamique des populations, qui pourront être utilisés ultérieurement dans des modèles estimant le risque de transmission.

Mots-clés : entomologie, *Culicoides*, saisonnalité, fièvre catarrhale du mouton, Maroc

1. INTRODUCTION

The *Culicoides* Latreille biting midges (Diptera: Ceratopogonidae) are small hematophagous insects, which are biological vectors of viruses responsible for major livestock diseases, such as bluetongue (BT), African horse sickness (AHS), epizootic haemorrhagic disease (EHD) or Schmallenberg disease (SB) (Purse et al., 2015). Several incursions of AHS and EHD have occurred in the recent decades in the Mediterranean region, though outbreaks were limited geographically and lasted only few years. In contrast, after sporadic incursions, the Mediterranean BT epidemiology changed radically in the last two decades. What happened in Morocco is an illustrative example of this epidemiological drift in BT situation compared to other *Culicoides*-borne viruses.

Morocco experienced AHS outbreaks in the 1960s (serotype 9) and in the late 1980s (serotype 4) (Mellor and Hamblin, 2004), and EHD outbreaks (serotype 6) in 2006 (Savini et al., 2011). During the 1980s AHS outbreak, observed circulation of AHSV was reported for 5 years before coming back to an absence of transmission (Mellor and Hamblin, 2004). No EHD clinical cases were reported after 2006. Bluetongue (serotype 10, BTV-10) was described for the first time in Morocco in 1956. The virus spread was controlled thanks to the implementation of a vaccination campaign and to autumnal temperature drop (Lhor et al., 2017). Meanwhile, other BTV serotypes were extensively transmitted in the Mediterranean Basin from 1998, but the disease had apparently remained absent in Morocco until BTV-4 emergence in 2004 (Lhor et al., 2017). In 2006, BTV-1 was reported in Algeria, and then spread to Morocco in 2006-2007 (Lhor et al., 2017). In 2008, sanitary policies implemented by veterinary services stopped these outbreaks, but the disease reappeared in 2009, with BTV-1 and BTV-4 co-circulating during *Culicoides* activity periods in both northern and southern parts of the country (Lhor et al., 2017**)**. The last census reported 305 BT outbreaks across the country with 1,456 cases leading to 449 livestock deaths in 2017. The BT endemic situation is weighing on the sheep industry, which has increased from 1.6 billion to 2.4 billion euros between 2008 and 2017. The national herd is currently estimated at 20.6 million head, including 2.5 million of the Sardi breed, which are reared for the Eid al-Adha sacrifice fest. The economic impact of BT includes direct losses due to mortality, weight loss, abortion, reduction of the fertility rate and the meat production efficiency; and indirect costs due to vaccination, monitoring and surveillance measures, also trade restrictions limiting the access to higher value markets **(**Balenghien and Garros, 2017**)**.

105 A total of 54 *Culicoides* species have been recorded in Morocco. This list was mainly
106 established in the 1970s after the 1965-1967 AHS outbreaks and has been comprehensively
107 reviewed and updated recently (Bourquia et al., in press).

108 *Culicoides* distributions were investigated in Morocco in 1994-1995 after the 1989-1991 AHS
109 outbreaks (Baylis et al., 1997) and in 2000-2003 just before the 2004 BT emergence (Lhor et
110 al., 2015). These studies focused mainly on *Culicoides imicola* Kieffer, as a proven AHSV
111 and BTV vector, which was, at that time, considered the only significant vector in the
112 Mediterranean region. The rest of the individuals were identified at the 'group' level, mainly
113 Obsoletus and Pulicaris 'groups' (see Bourquia et al., in press). The *C. imicola* abundance (as
114 the annually averaged mean daily trap catch) was found to be positively correlated with the
115 Normalized Difference Vegetation Index (NDVI) and negatively with the wind speed (Baylis
116 et al., 1997). NDVI was suggested to be related to soil moisture and thus to the suitability for
117 *C. imicola* breeding sites (Baylis et al., 1997). Wind speed was suggested to affect the
118 abundance of *C. imicola*, by increasing adult mortality rates through desiccation or more
119 likely, according to authors, through the loss of adults due to wind dispersal (Baylis et al.,
120 1997). The seasonal pattern of *C. imicola* populations was described in Morocco during the
121 1994-1995 and 2009-2010 surveys (Baylis et al., 1997; Lhor et al., 2015) but was not
122 analysed to identify climatic drivers. The *C. imicola* dynamic was described as mainly
123 unimodal, as in general, the catch of *C. imicola* peaked in late summer and autumn, with a
124 smaller peak in spring. In Senegal and South Africa, this species was associated with rainfall,
125 NDVI, temperature, wind speed and percentage cover of water bodies (Diarra et al., 2015).
126 Other studies including environmental parameters (related to land use, landscape and climate)
127 conducted in the Mediterranean Basin suggested that these parameters improve the prediction
128 of habitat suitability occupied by *C. imicola* as well as the accuracy of abundance estimates at
129 a local scale (Ippoliti et al., 2013).

130 The species composition and the seasonality of *Culicoides* in the Rabat region was thus
131 investigated to provide new insights on the role of *Culicoides* in BTV transmission by
132 highlighting the environmental drivers of the seasonality.

133 **2. MATERIAL AND METHODS**

134 **2.1. Study sites and entomological collections**

This study was conducted in the region of the Moroccan capital Rabat. The city is located on the Atlantic coast in north-western Morocco, with a Mediterranean climate (characterized by hot, dry summers and mild, wet winters.). The average annual precipitation reaches 492 mm and the average annual temperature is 17.1°C (1970-2000 seasonal averages from the WorldClim version 2, http://worldclim.org/version2). The presence of the Atlantic Ocean is conducive to a high relative humidity, which fluctuates depending on distance from the sea and time of the year.

Culicoides midges were collected around Rabat at two-week intervals from May to October 2016 in two sites, a cattle farm (33°57'44.1"N 6°48'11.7"W) and a goat farm (33°51'36.0"N 6°51'00.0"W), and from May 2016 to April 2017 in a horse-riding center (33°53'46.0"N 6°49'53.3"W), using a UV-light/suction trap (OVI type) manufactured by the Onderstepoort Veterinary Institute (Venter et al., 2009). The traps were placed near animals and were operated from dusk to dawn for two consecutive nights fortnightly. The *Culicoides* collected were transferred to 70% ethanol and then, for most individuals, identified morphologically to species-level under a stereomiscroscope using the key of Mathieu et al. (2012)**.** Additionally, molecular identification was performed (i) to differentiate morphologically close species (*Culicoides obsoletus* (Meigen), *Culicoides scoticus* Downes and Kettle or *Culicoides montanus* Shakirzjanova), (ii) to confirm morphological identifications if specimens were damaged and (iii) to confirm new species records (details are available in Bourquia et al., in press). Females of all species were age-graded into nulliparous, parous, gravid females or freshly blood-fed females after abdominal examination of pigmentation.

2.2. Environmental data

Daily rainfall estimates were obtained from 1983 to 2017 from the TAMSAT (Tropical Applications of Meteorology using SATellite data and ground-based observations, (https://www.tamsat.org.uk/data/archive") database (Maidment et al., 2014). Day and night land surface temperatures (LSTday and LSTnight), vegetation indices (Enhanced Vegetation Index EVI and Normalized Difference Vegetation Index NDVI) from 2000 to 2017 were extracted from version 6 MODIS MOD11A1 product (Wan et al., 2015) and from version 6 MODIS MOD13A1 (Didan, 2015) for each site location and sample date. Hourly climatic data, including 2m temperature, 2m dewpoint temperature, surface pressure, 10m u-component of wind and 10m v-component of wind, were extracted for 2016 and 2017 from the fifth generation ECMWF (European Centre for Medium-range Weather Forecasts)

atmospheric reanalysis (ERA5) of the global climate (Dee et al., 2011).The saturated vapour pressure and the environmental vapour pressure, and then the relative and the absolute humidity (see Shaman and Kohn (2009) for calculation details and definitions), were calculated from the 2m temperature and the 2m dewpoint temperature. Then, meteorological parameters from ECMWF-ERA5 were rescaled from hourly data to daily data (corresponding of the 24h which included the night collection) by computing the minimum, maximum and mean of each parameter.

2.3. Statistical analyses

Meteorological conditions during the collection years (2016 and 2017) were described by comparing the 2016 and 2017 monthly minimum and maximum temperatures (MODIS LSTday and LSTnight) and the 2016 and 2017 total rainfall (TAMSAT database) to the average of temperatures from 2000 to 2015 and rainfall from 1986 to 2015.

To broadly overview the diversity per site, species communities were described using a principal component analysis (PCA) on the log-transformed abundances. Then, a between-class analysis was carried out on this PCA to assess the importance of both site and collection month. Between-class analysis is a particular case of a PCA, where the variability between groups is optimized.

A correlation matrix was produced for all environmental variables to select non-correlated variables to be included in the modelling of *Culicoides* seasonal abundance. Then, cross correlation maps (CCM) were used to assess the correlation between the *Culicoides* abundance averaged between sites and the selected environmental variables at different time lags. The CCM were used to assess average or accumulated meteorological quantities over a period beginning at a first time lag and ending at a second time lag (see Brugger and Rubel (2013) for methodological details). Analysing the CCM determined which time lags of environmental variables may have had an impact on *Culicoides* abundance.

Finally, abundance was modelled using these environmental parameters and time lags (after centring and scaling) using a generalized linear model (GLM) or a negative-binomial log linear model to account for any over-dispersion of insect collection data. The model selection was based on the corrected Akaike's (cAIC) and on the Bayesian (BIC) information criteria. The validity of the selected GLM was assessed by: i) plotting the observed versus the fitted values and testing the Pearson's product moment correlation coefficient, ii) graphically checking the normality of the residuals, iii) graphically testing the linearity hypothesis

(random distribution of residuals around 0 after they have been plotted by fitted values) and iv) graphically confirming the homogeneity of residuals.

All statistical analyses and graphs were implemented using version 3.4.2 of the R software, using the *ade4* package for PCA and between class analysis, *fields* for CCM, *MuMIn* for calculating the corrected Akaike and Bayesian information criteria and *aod* for analysis of over-dispersed data.

3. RESULTS

3.1. *Culicoides* collections

The goat and cattle farms were sampled fortnightly over a period of 22 weeks (12 and 11 collections – last sampling was not considered due to electricity failure) during most of the *Culicoides* activity period (from mid-May 2016 to mid-October 2016). The horse-riding centre was sampled fortnightly for 48 weeks (22 collections) to measure the population seasonality over an entire year (from mid-May 2016 to the end of April 2017).

During sampling periods, the temperatures were quite similar to the normal seasonal conditions, with an average annual temperature of 20.5 °C in 2016 and 20.6 °C in 2017, compared to 20.1 °C for the 2000-2015 period (figure 1). The 2016 collections began with relatively dry period with a total of 125 mm from January to May 2016, compared to 193 mm on the same normal period (figure 1). In contrast, the winter of 2016/2017 was wetter than normal with 440 mm rainfall from September 2016 to May 2017 compared to 389 mm for the 2000-2015 period (figure 1).

Figure 1. 2016 and 2017 monthly average meteorological conditions (temperature and precipitations) compared to normal seasonal conditions at Rabat, Morocco.
Temperature data (day and night land surface temperatures) were extracted from the MOD11A1 version 6 product (Wan et al., 2015) using a 4 km² area from 2000 to 2017. Rainfall data were extracted from the TAMSAT database (Maidment et al., 2014) from 1986 to 2017.

3.2. *Culicoides* diversity and seasonality in the three collection sites

A total of 12,460 *Culicoides* individuals belonging to at least 15 different species were collected during the survey, including 12,053 (96.7%) females and 407 (3.3%) males (table I). From May to October 2016, during the 11 collections common to the three sites, the most

229 abundant species were *C. imicola* (71.4% of total catches), *Culicoides newsteadi* Austen
230 (6.4%), *Culicoides circumscriptus* Kieffer (6.4%), *Culicoides kingi* Austen (4.1%), *Culicoides*
231 *cataneii* Clastrier/*Culicoides gejgelensis* Dzhafarov (3.8%), *Culicoides puncticollis* (Becker)
232 (3.6%) *and C. obsoletus/C. scoticus* (2.2%). Altogether these species represented 97.9% of
233 the collected individuals (table I).

234 The structure of the PCA on log-transformed abundances was driven by the 7 most abundant
235 species (or pair of species), leading to a cumulative projected inertia of 70.4% on the first
236 3 axes. The site (or the dominant domestic vertebrate species) explained 17.1% of the PCA
237 inertia (p = 0.001, with a permutation test), whereas the collection month explained 26.7% of
238 the PCA inertia (p = 0.001). The 'site/host effect' was mainly due to a higher abundance of
239 *C. circumscriptus* in the cattle farm compared to both other sites (figure 2A), and also, but
240 less significant, to higher abundance of *C. imicola* in the horse-riding centre and of
241 *C. puncticollis* and *C. paolae* in the goat farm (see supplementary material I for abundance
242 per species and site). The between class analysis on the collection month highlighted the
243 higher abundances of *C. imicola* and *C. kingi* in August/September, of
244 *C. obsoletus/C. scoticus* and *C. newsteadi* in May/June and October, and secondary of
245 *C. puncticollis* in June/July (figure 2B).

246 **Figure 2.** Between-class analysis (particular case of a principal component analysis (PCA)
247 with optimisation of the variability between groups) with the site (A) and the collection month
248 (B) as group applied on the PCA carried out of the log-transformed 2016-2017 abundances.
249 Bart chart: percentage of inertia explained by the axes.
250 Culicoides *collections were carried out bimonthly using UV-light/suction trap (OVI type) in*
251 *three sites in Rabat (Morocco): a horse-riding centre (MA001), a goat farm (MA002) and a*
252 *cattle farm (MA003) from May (05) to October (10).*

253 *Culicoides imicola* populations increased progressively from spring to peak in September, see
254 supplementary material II, whereas *C. kingi* was abundant only in August/September.
255 Population densities of *C. circumscriptus*, *C. cataneii/C. gejgelensis* and *C. puncticollis* were
256 unimodal with a maximal abundance in August for the first and July for the latter two species
257 (suppl. mat. II). Finally, both *C. obsoletus/C. scoticus* and *C. newsteadi* populations showed
258 two peaks of abundance, the first in June and the second in October (suppl. mat. II).

259 **3.3. Influence of environmental parameters on *Culicoides* abundance**

The supplementary material III details the correlation matrices produced with all environmental parameters, including EVI and NDVI MODIS parameters, TAMSAT daily rainfall and ECMWF-ERA5 meteorological parameters. Comparison of the day and night land surface temperatures from MODIS products and the daily 2m temperature from the ECMWF-ERA5 showed high correlations (see supplementary material III for details). Therefore, only the temperature ECMWF-ERA5 data was used for the rest of the analysis. The temperature, which was highly correlated with the environmental vapour pressure, the relative and the absolute humidity, the atmospheric pressure, the wind speed, the rainfall and the EVI, which was highly correlated with the NDVI, were also retained.

Bivariate correlations between *C. imicola* abundance and the environmental variables highlighted a positive impact of temperature and absolute humidity for a large range of time lags (figure 3). The time lags that had the highest correlation coefficients with abundance were selected (Pearson's product moment correlation coefficient $\rho = 0.80$ for 25-27 days as time lags for temperature and $\rho = 0.75$ for 23-26 days for absolute humidity). In contrast, atmospheric pressure was negatively correlated with the *C. imicola* abundance (figure 3, $\rho = 0.83$ for 27-43 days). The CCM did not indicate any specific relation between the wind speed and the number of *C. imicola* collected. The best correlation was positive and obtained with the wind speed 36 days before the collection, which seemed incidental and without any biological sense (the loss of adults is generally due to wind dispersal the day of collection). Thus, only the wind speed on the day of collection was included as variable for modelling procedure. Finally, the EVI of the week of collection was negatively correlated with *C. imicola* abundances (figure 3).

Figure 3. Cross correlation maps (CCM) investigating bivariate correlations between the *Culicoides imicola* abundance averaged at the three collection sites around Rabat and the environmental variables at different time lags.
CCM at the coordinates j and k was $CCM_{j,k} = cor\ (\ Y_i\ ,\ X_{i-j,i-k}\ )$, Y_i the number of collected *Culicoides* (of a given species) at time i and $X_{i-j,i-k}$ an environmental quantity, averaged over a time period starting at time $i - j$ (time lag 1) and ending at $i - k$ (time lag 2), with $j \geq k$. Tested time lags were 12 weeks for EVI and 60 days for all other parameters. Only environmental variables correlated with the *Culicoides* abundances were included in the figure.
Each figure also showed the commonly used correlation coefficient r(0,0) (without time lags, $j = k = 0$) and the maximum correlation coefficient of the map r(k,j), highlighted by the black square symbol.

293 Table II contained the environmental parameters with the optimum time lags selected for the
294 modelling procedure after having analyzed the CCM for *C. newsteadi* (suppl. mat. IV),
295 *C. circumscriptus* (suppl. mat. V), *C. kingi* (suppl. mat. VI), *C. cataneii/C. gejgelensis* (suppl.
296 mat. VII), *C. puncticollis* (suppl. mat. VIII) and *C. obsoletus/C. scoticus* (suppl. mat. IX).

297 The selected GLM included atmospheric pressure (average the 27 to 43 days before
298 collections) and mean temperatures (averaged the 25 to 27 days before collections), and
299 correctly predicted the seasonal pattern of *C. imicola* populations (R^2 = 0.778, table III), *i.e.* a
300 slow increase of population from March to reach a maximum in September, and then a rapid
301 decrease from October to February, (figure 4).

302 **Figure 4.** Comparison of fitted and observed values of the *Culicoides imicola* abundance
303 averaged at the three collection sites around Rabat, at the collection days (A) and during the
304 entire season (B).

305 The selected models (table III), correctly predicted the seasonal pattern of *C. circumscriptus*
306 (R^2 = 0.752), *C. cataneii/C. gejgelensis* (R^2 = 0.754) and *C. puncticollis* (R^2 = 0.670), with a
307 lowest accuracy for the latter (see supplementary material X for details on model selection,
308 validation and predictions for all species). The selected models did not successfully predict
309 the bimodal seasonal patterns of *C. newsteadi* (R^2 = 0.463) and *C. obsoletus/C. scoticus* (R^2 =
310 0.700). The GLM was able to predict the abundance peaks of *C. newsteadi* populations in
311 2016 fall and 2017 spring, but not in 2016 spring (suppl. mat. X). Similarly, the GLM was
312 able to predict the abundance peaks of *C. obsoletus/C. scoticus* populations in 2016 and 2017
313 spring, but not fully in 2016 fall (suppl. mat. X). Finally, the selected model predicted the
314 general seasonal pattern of *C. kingi* populations, but failed to reproduce the November peak of
315 abundance (suppl. mat. X). The atmospheric pressure was selected as a significant predictor in
316 5 of 7 models, always with a negative correlation, rainfall in 4 models but only once with a
317 significant positive effect, the humidity in 2 models, the wind speed in 2 models with a
318 positive correlation, the EVI and the temperature only once each (table III).

319 **4. DISCUSSION**

320 *Culicoides* collections carried out in three different host environments in Rabat (Morocco)
321 confirmed the presence of at least 15 *Culicoides* species, among which were several proven or
322 probable vectors of arboviruses of veterinary interest. *C. imicola* is a proven BTV and AHSV
323 vector and a suspected EHDV vector (Purse et al., 2015). *C. obsoletus* and *C. scoticus* are

probable BTV vectors (Purse et al., 2015), whereas *C. newsteadi* and *C. paolae* are suspected BTV vectors (Foxi et al., 2016; Foxi et al., 2019).

Culicoides imicola was by far the dominant species in the three sites combined. It was reported as the most abundant and frequent species in Morocco (Baylis et al., 1997; Lhor et al., 2015). The species composition was quite similar between sites, the month of collection having more impact than the site/host species. Even if the site and host effects were intertwined, it is likely that species composition is driven first by site location due to the location of breeding sites. For instance, Larska et al. (2017) highlighted no difference in species composition between cattle and horse farms. The main difference in species composition was due to higher abundance of *C. circumscriptus*, known as a coastal species (Balenghien and Garros, 2017), in the cattle farm, which was the closest to the coast. Other abundant species were known to breed in specific types of sites. *Culicoides imicola* larvae develop in moist clay mud rich in nutrients exposed to sunlight or in moist or water saturated soils rich in organic matter (Braverman et al., 1974). Species such as *C. newsteadi* and *C. puncticollis* are found near substrates rich in water-saturated organic matter (Braverman et al., 1974). Larvae of *C. kingi* are more likely to be found in sunny and very salty mud (Cornet and Brunhes, 1994), whereas *C. cataneii* and *C. gejgelensis* larvae may be found in rivers or pond edges in wet meadows (Balenghien and Garros, 2017). *C. obsoletus* and *C. scoticus* may be considered ubiquitous as they develop in forest litter, tree holes, corn silage residues or composting manure (Balenghien and Garros, 2017). Finally, *C. paolae* larvae are considered specific to decaying prickly pear trees, which are common in the Mediterranean areas.

The seasonal pattern of *C. imicola* populations observed in Rabat is consistent with previous studies carried out in Morocco, with a peak in late summer/early autumn (Baylis et al., 1997), which was associated with BTV transmission in northwestern Morocco (Lhor et al., 2015). This seasonal pattern is usually observed in the Mediterranean Basin (Balenghien and Garros, 2017). The peak of abundance of *C. imicola* populations is observed at the same period (September/October) in Senegal with a tropical climate, where populations are highest during the rainy season (Diarra et al., 2014). Seasonal patterns of *C. obsoletus* and *C. scoticus* vary widely depending on the climate (Balenghien and Garros, 2017). In the Mediterranean Basin, as observed in Spain or Sardinia, they exhibit a first principal peak of abundance in May/June, and then a later secondary peak in October (Lucientes and Alarcón-Elbal, 2016; Foxi et al., 2016). These current observations are in accordance with these previous reports. The seasonal pattern of *C. newsteadi* populations highlighted the same bimodal distribution. This species is

abundant during spring in Morocco or Sardinia (Lhor et al., 2015; Foxi et al., 2016). Finally, *C. kingi* populations were mainly abundant in Rabat in November. On the contrary, in tropical regions of Sudan and Senegal, this species is most abundant in July/August during the rainy season, and also in April when temperatures are the highest (El Sinnary et al., 1985; Diarra et al., 2014).

Determining the environmental factors driving these seasonal patterns is useful not only to better understand the variations in abundance, but also to be able to predict abundance as a first step for transmission risk modelling. Other factors are, of course, involved. Adult population seasonality is also the consequence of long-term impact of environmental factors on the different steps of the life cycle, including the duration of the larval development, the longevity of both adult and immature stages, the size and frequency of egg laying, etc. (Purse et al., 2015). The same meteorological parameters, such as temperature, rainfall, or wind, may also have a short-term impact on the *Culicoides* flight activity leading to important daily variation of the proportion of the *Culicoides* population which is active and can be collected. Moreover, a single meteorological variable, such as temperature, may have a mainly positive impact on population dynamics at a given range of values, but mainly negative impacts at another range leading to non-linear effects on population abundances. These complexities may explain why many models have been developed to predict the presence and distribution of *Culicoides* species, in particular *C. imicola* in Europe, using climatic factors (Wittman et al., 2001), satellite imagery (Tatem et al., 2003) or a combination of both (Baylis and Rawlings, 1998), but only few have described the influence of meteorological and environmental parameters on *Culicoides* populations using statistical (Sanders et al., 2011; Rigot et al., 2012; Searle et al., 2013; Brugger and Rubel, 2013; Scolamacchia et al., 2014; Diarra et al., 2015) or mechanistic (White et al., 2017) modelling.

The influence of environmental parameters on the abundance was explored for species collected in 2016-2017 in Rabat. The atmospheric pressure was negatively correlated with the abundance of *C. imicola*, *C. newsteadi*, *C. circumscriptus*, *C. kingi* and *C. obsoletus/C. scoticus* at different time scales. Although never assessed in *Culicoides* seasonality modelling before, the atmospheric pressure has long been known to impact insect populations (Wellington, 1946), especially mating or phototaxis behavior (Pellegrino et al., 2013; Zagvazdina et al., 2015). At this stage, it is not possible to conclude if atmospheric pressure has a real impact on *Culicoides* population abundance or if this correlation is incidental (seasonal pattern similar to *Culicoides* seasonal pattern) or indirect (through

another meteorological parameter). This is the main limitation of statistical modelling. The rainfall is positively correlated to *C. newsteadi* and *C. cataneii/C. gejgelensis* abundances with time lags corresponding to 2 weeks before collections, but negatively to *C. circumscriptus* and *C. kingi* with longer time lags (about 40 days before collections). Rainfall may have a direct negative impact on *Culicoides* activity (Murray, 1991), and long-term effects on *Culicoides* abundance by increasing the availability of breeding sites or perhaps drowning nymphs (Nevill, 1967). Long-term positive effects were highlighted in areas with temperate climates (Brugger and Rubel, 2013) and short-term negative effects in both temperate (Sanders et al., 2011) and tropical climates (Diarra et al., 2015). The wind speed was positively correlated with the abundance of *C. cataneii/C. gejgelensis* and *C. obsoletus/C. scoticus* with a large time lag (up to 45 days before collections). Wind speed on the day of collection has often been reported as having a negative impact on abundance by reducing the flight activity (Sanders et al., 2011; Scolamacchia et al., 2014). Baylis et al. (1997) even reported that wind speed negatively affected the abundance of *C. imicola* in distribution modelling, through the loss of adults due to wind dispersal. The positive correlation shown in this study should thus be considered as incidental. It is worth noting that including the wind speed on the day of the collection within the model did not change the outcome (data not shown). Finally, the temperature, the absolute humidity and the EVI were rarely selected in models. This result contrasts with other studies where temperature was considered a main positive driver of *Culicoides* seasonal abundance (Sanders et al., 2011; Rigot et al., 2012; Brugger and Rubel, 2013; Scolamacchia et al., 2014; Diarra et al., 2015). The positive impact of humidity has been less rarely highlighted in other studies (Diarra et al., 2015) though it is known to impact adult survival (Purse et al., 2015). The NDVI, which is correlated to EVI, was associated with high abundance of *C. imicola* in distribution models (Baylis and Rawlings, 1998; Baylis et al., 1997; Tatem et al., 2003; Acevedo et al., 2010), but rarely to abundance in seasonality modelling (Diarra et al., 2015). In this latter study, NDVI was higher during the rainy season, and the association with *Culicoides* abundances may be co-incidental.

5. CONCLUSION

The primary objective of this study was to provide new insights on the *Culicoides* species composition and seasonality in Rabat, to better understand the role of *Culicoides* in BTV transmission in Morocco where BT is currently endemic. Neither the site location (except for the cattle farm where the coastal species *C. circumscriptus* was more abundant than the other

ones), nor the main vertebrate species, influenced the global species composition. The seasonal pattern of *Culicoides* described was typical of the Mediterranean climate. Finally, the impact of environmental parameters which may drive the *Culicoides* abundance was investigated, questioning the potential role of the atmospheric pressure.

ACKNOWLEDGMENTS

The authors would like to thank the owner of the farms where 2016-2017 collections were carried out. This study was supported by Vectornet, funded by EFSA and ECDC. Maria Bourquia was granted by PHC Toubkal (Toubkal/17/38 campus France 36867VH) and Centre National de Recherche Scientifique et Technique (CNRST).

AUTHOR CONTRIBUTIONS

MB, KK and TBale were involved in the conception and planned the study. MB, IR, IB, MC, LG and WW collected the data. MB, CG, KH and TBale analysed and interpreted the data. MB and TBale drafted the paper. CG, WW, TBald and KK revised and commented the manuscript.

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Table I. Total number of *Culicoides* per species collected, using every two weeks UV-light/suction trap (OIV type) collections, from May to October 2016 in a goat and a cattle farm (N = 12 and 11 collections) and from May 2016 to April 2017 in a horse-riding centre (N = 25) in the Rabat region (Morocco). The 'total' column was calculated using data from weeks when all sites were sampled, so as to compute a global abundance rank for each species.

	Riding centre				**Goat farm**				**Cattle farm**				**Total common collections**			
Species	No. Ind	No. F	No. M	*Rank*	No. Ind	No. F	No. M	*Rank*	No. Ind	No. F	No. M	*Rank*	No. Ind	No. F	No. M	*Rank*
Culicoides imicola	5,468	5,347	121	*1*	2,027	1,944	83	*1*	1,432	1,392	40	*1*	5,844	5,688	156	*1*
Culicoides newsteadi	276	274	2	*3*	131	130	1	*3*	270	237	33	*3*	524	489	35	*2*
Culicoides circumscriptus	68	60	8	*6*	79	75	4	*5*	434	399	35	*2*	522	480	20	*3*
Culicoides kingi	1,010	966	44	*2*	107	105	2	*4*	71	69	2	*6*	335	315	20	*4*
Culicoides cataneii/Culicoides gejgelensis	84	83	1	*5*	47	44	3	*6*	222	221	1	*4*	314	309	5	*5*
C. cataneii/C. gejgelensis	55	54	1		47	44	3		222	221	1					
Culicoides cataneii	2	2	0		0	0	0		0	0	0					
Culicoides gejgelensis	27	27	0		0	0	0		0	0	0					
Culicoides puncticollis	20	19	1	*9*	212	197	15	*2*	64	56	8	*7*	293	269	24	*6*
Obsoletus group	94	94	0	*4*	30	30	0	*8*	96	96	0	*5*	181	181	0	*7*
C. obsoletus/C. scoticus	40	40	0		4	4	0		8	8	0					
Culicoides obsoletus	41	41	0		23	23	0		55	55	0					
Culicoides scoticus	6	6	0		2	2	0		33	33	0					
Culicoides montanus	0	0	0		1	1	0		0	0	0					
Unknown species	7	7														
Culicoides paolae	11	11	0	*10*	35	33	2	*7*	9	9	0	*9*	46	44	2	*8*
Culicoides subfagineus	22	21	1	*8*	13	13	0	*9*	6	6	0	*10*	41	40	1	*9*
Culicoides longipennis	8	8	0	*12*	4	4	0	*10*	23	23	0	*8*	30	30	0	*10*
Culicoides fagineus	53	53	0	*7*	0	0	0		1	1	0	*14*	27	27	0	*11*
Culicoides jumineri	4	4	0	*14*	0	0	0		6	6	0	*10*	9	9	0	*12*
Culicoides univittatus	0	0	0		2	2	0	*12*	4	4	0	*12*	6	6	0	*13*

Culicoides parroti	9	9	0	*11*	0	0	0		0	0	0		4	4	0	*14*
Culicoides sahariensis	0	0	0		4	4	0	*11*	0	0	0		4	4	0	*14*
Culicoides festivipennis	0	0	0		0	0	0		2	2	0	*13*	2	2	0	*16*
Culicoides sp.	1	1	0		0	0	0		0	0	0					

Table II. Environmental variables and optimal time lags (in weeks for EVI and days for the rest of parameters) selected for modelling procedure after analysing of the cross correlation maps for each the most abundant *Culicoides* species collected in 2016-2017 in Rabat (Morocco).

Species	Temperature	Absolute humidity	Relative humidity	Atmospheric pressure	Wind speed	Rainfall	EVI
C. imicola	mean [25,27]	max [23,26]	-	mean [27,43]	mean [0,0]	-	[0,0]
C. newsteadi	-	-	max [7,11]	min [9,12]	-	[9, 17]	-
C. circumscriptus	min [23,25]	mean [6,25]	-	min [27,40]	-	[37,40]	[0,0]
C. kingi	mean [15,15]	mean [20,30]	-	max [29,39]	mean [27,45]	[37,40]	[0,2]
C. cataneii/ C. gejgelensis	min [14,14]	mean [20,30]	min [0,5]	max [33,35]	mean [27,45]	[9, 9]	[0,2]
C. puncticollis	max [2,4]	max [1,7]	min [23,25]	mean [15,15]	min [5,38]	[2,60]	[0,0]
C. obsoletus/C. scoticus	-	-	max [17,21]	max [6,8]	max [5,41]	[37,38]	-

Table III. Modelling *Culicoides* abundances assessed in 2016-2017 at Rabat (Morocco) by environmental parameters using generalized linear or binomial model: selected model, variables and estimates, and Pearson's correlation coefficient.

Species	Selected model	Selected variables and estimates	Pearson's correlation coefficient
C. imicola	GLM	Abun ~ 1.70^{***} - 0.77^{*} × Pres_Mean + 0.35° × Temp_Mean	ρ = 0.882 (p < 0.001), R^2 = 0.778
C. newteadi	GLM	Abun ~ 0.87^{***} - 0.23^{*} × Pres_Mean + 0.59^{*} × Rain	ρ = 0.680 (p < 0.001), R^2 = 0.463
C. circumscriptus	GLM.NB	Abun ~ 1.86^{***} - 1.49^{***} × Pres_Mean - 4.98^{*} × Rain	ρ = 0.867 (p < 0.001), R^2 = 0.752
C. kingi	GLM	Abun ~ 0.67^{***} - 0.53^{*} × Pres_Max - 0.35^{*} × Rain	ρ = 0.628 (p < 0.001), R^2 = 0.394
C. cataneii/C. gejgelensis	GLM	Abun ~ 0.51^{***} - 0.29^{***} × EVI + 0.14^{*} × Rain + 0.87^{***} × Wind_Mean	ρ = 0.868 (p < 0.001), R^2 = 0.754
C. puncticollis	GLM.NB	Abun ~ 1.56^{***} + 7.54^{***} × AH_Max - 5.06^{*} × RH_Min	ρ = 0.818 (p < 0.001), R^2 = 0.670
C. obsoletus/C. scoticus	GLM.NB	Abun ~ 1.42^{***} - 2.69^{*} × Pres_Max - 5.85^{**} × RH_Max + 1.07^{***} × Wind_Max	ρ = 0.837 (p < 0.001), R^2 = 0.700

Asterix denotes significance: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\circ}p < 0.1$

GLM: generalized linear model; GLM.NB: negative-binomial log linear model; Abun: log-transformed abundance for GLM or counts for NegBin; Pres: atmospheric pressure, Temp: temperature; Rain: total of rainfall; EVI: enhanced vegetation index; Wind: wind speed; AH: absolute humidity; RH: relative humidity

Figure 1 : 2016 and 2017 monthly average meteorological conditions (temperature and precipitations) compared to seasonal normal at Rabat, Morocco. Temperature data (day and night land surface temperatures) were extracted from the MOD11A1 version 6 product (Wan et al., 2015) using a 4 km² area from 2000 to 2017. Rainfall data were extracted from the TAMSAT database (Maidment et al., 2014 and Tarnavsky et al., 2014) from 1986 to 2017.

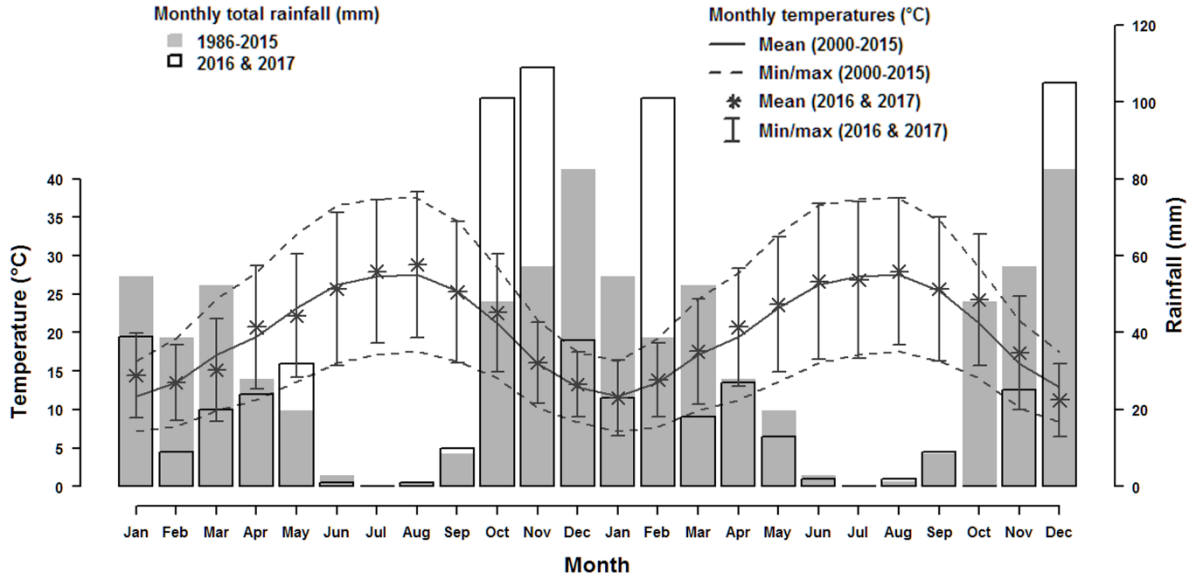


Figure 2 : Between-class analysis (particular case of a principal component analysis (PCA) with optimisation of the variability between groups) with the site (A) and the collection month (B) as group applied on the PCA carried out of the log-transformed 2016-2017 abundances. Bart chart: axes 1-4. *Culicoides* collections were carried out bimonthly using UV-light/suction trap (OVI type) in three sites in Rabat (Morocco): a horse riding centre (MA001), a goat farm (MA002) and a cattle farm (MA003).

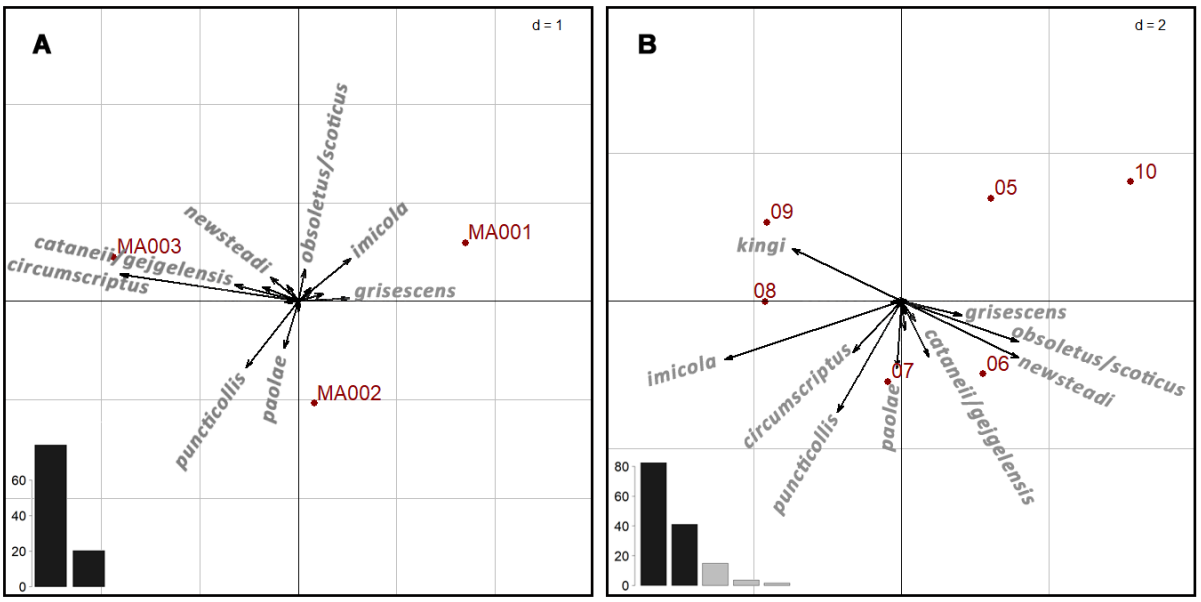


Figure 3 : Cross correlation maps (CCM) investigating bivariate correlations between the *Culicoides imicola* abundance averaged at the three collection sites around Rabat and the environmental variables at different time lags. CCM at the coordinates j and k was $CCM_{j,k} = cor\ (Y_i , X_{i-j,i-k})$, Y_i the number of collected *Culicoides* (of a given species) at time i and $X_{i-j,i-k}$ an environmental quantity, averaged over a time period starting at time $i - j$ (time lag 1) and ending at $i - k$ (time lag 2), with $j \geq k$. Tested time lags were 12 weeks for EVI and 60 days for all other parameters. Only environmental variables correlated with the *Culicoides* abundances were included in the figure. Each figure also shows the commonly used correlation coefficient r(0,0) (without time lags, j = k = 0) and the maximum correlation coefficient of the map r(k,j), highlighted by the black square symbol.

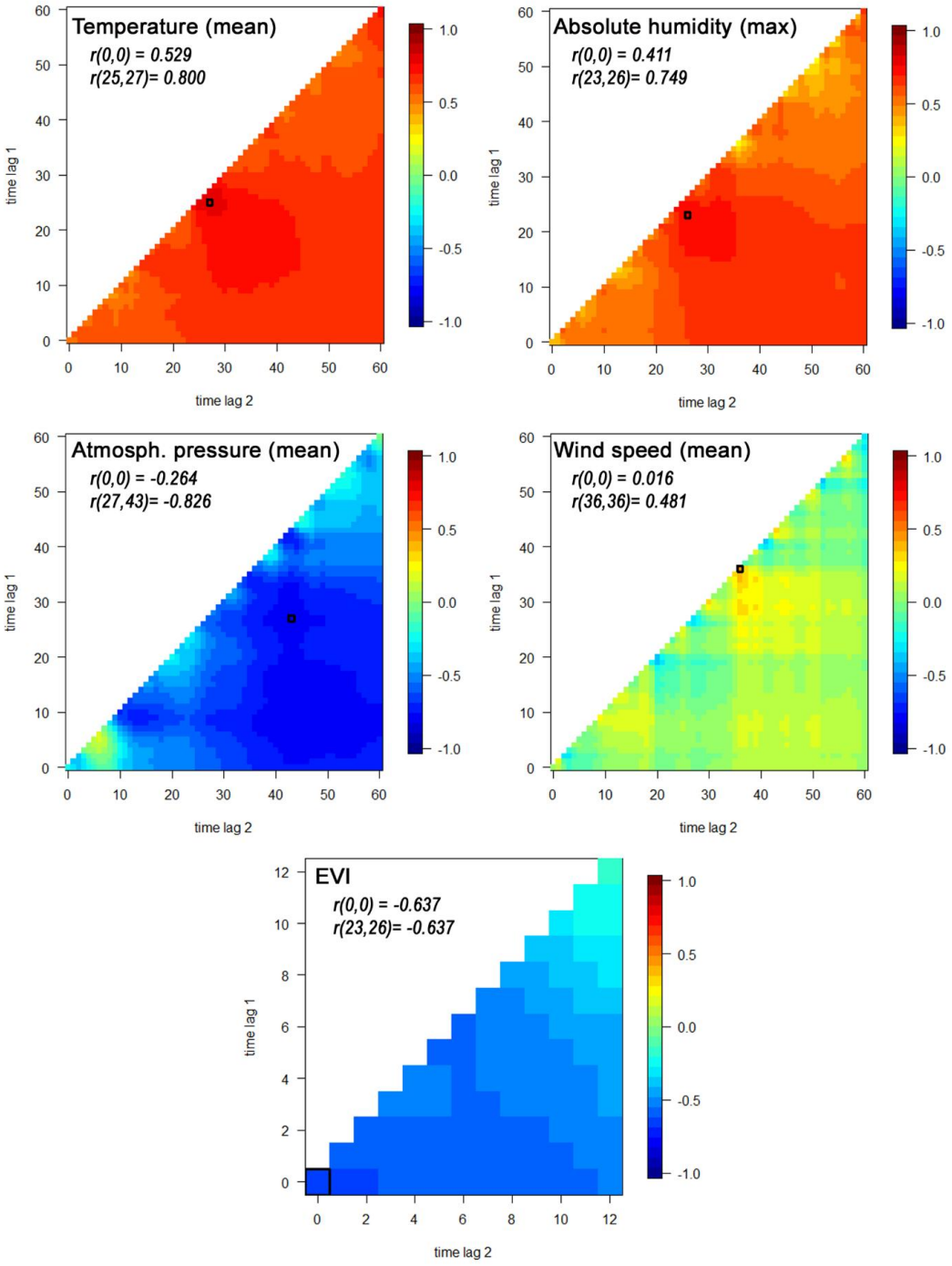
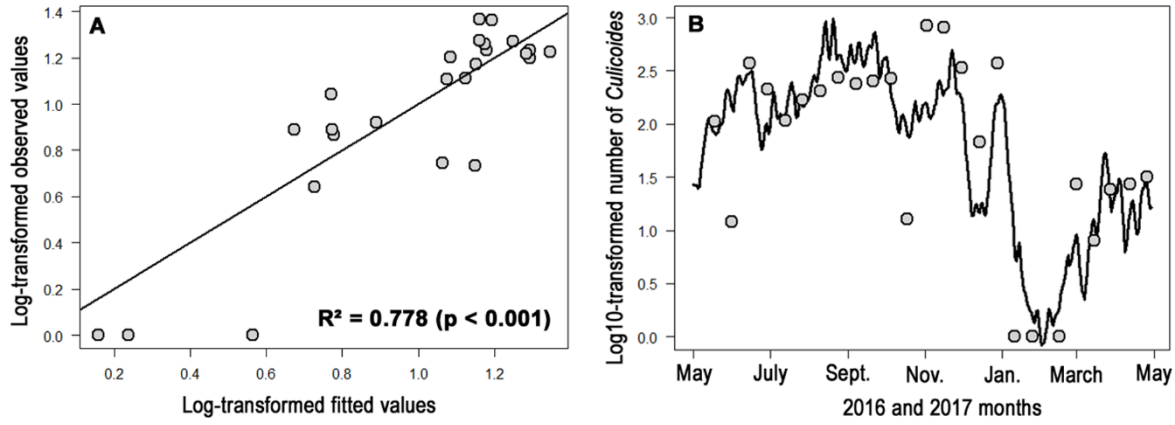
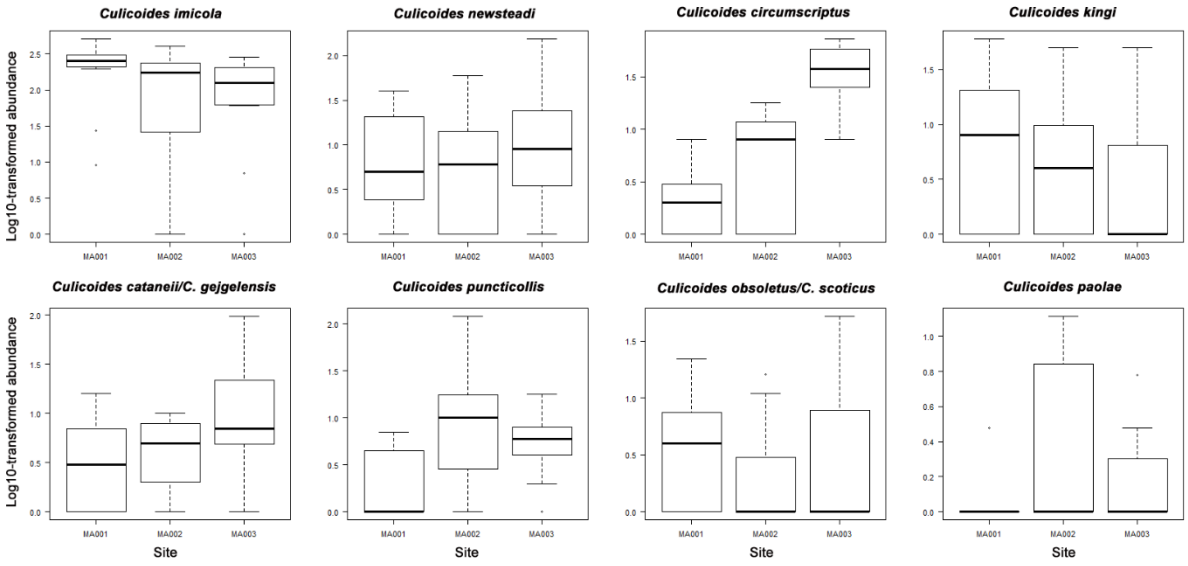


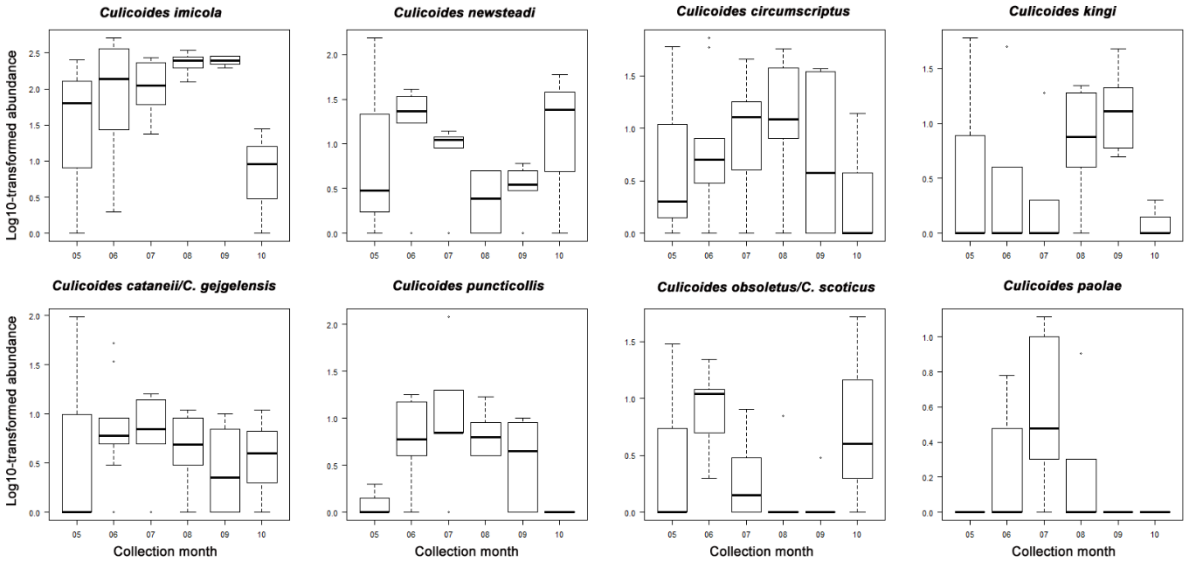
Figure 4 : Comparison of fitted and observed values of the *Culicoides imicola* abundance averaged at the three collection sites around Rabat, at the collection days (A) and during the entire season (B).



Supplementary data I : Log10-transformed number of *Culicoides* per species and per site. Bimonthly collections were carried, using UV-light/suction trap (OIV type), from May to October 2016 in a goat and a cattle farm (N = 12 and 11 collections) and from May 2016 to April 2017 in a horse riding centre (N = 25) in the Rabat region (Morocco).



Supplementary data II : Log10-transformed number of *Culicoides* per species and per collection month. Bimonthly collections were carried, using UV-light/suction trap (OIV type), from May to October 2016 in a goat and a cattle farm (N = 12 and 11 collections) and from May 2016 to April 2017 in a horse riding centre (N = 25) in the Rabat region (Morocco).



Supplementary file I : Description of environmental variables.

Environmental data had three different sources:

i) the TAMSAT (Tropical Applications of Meteorology using SATellite data and ground-based observations) database for daily rainfall estimates;

ii) ii) the MODIS products for day and night land surface temperatures (LSTday and LSTnight) and vegetation indices (Enhanced Vegetation Index EVI and Normalized Difference Vegetation Index NDVI);

iii) iii) the fifth generation ECMWF (European Centre for Medium-range Weather Forecasts) atmospheric reanalysis (ERA5) of the global climate hourly meteorological data, including 2m temperature, 2m dewpoint temperature, surface pressure, 10m u-component of wind and 10m v-component of wind.

To avoid collinearity between variables used to model the *Culicoides* abundances, we first compare temperature data from the MODIS source and from ECMWF-ERA5 source. Then, we compared all environmental parameters using correlation matrices.

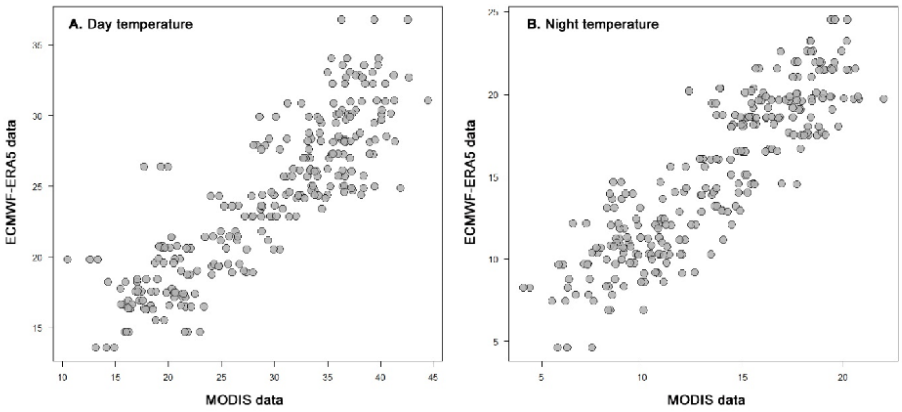


Figure 1. Comparison of 2016-2017 day and night temperatures extracted from MODIS products and from the ECMWF-ERA5 source at Rabat (Morocco)

Both day and night temperatures from MODIS and from ECMWF-ERA5 were correlated (Pearson's product-moment correlation $\rho = 0.864$ with $p < 0.001$ for day temperature, and $\rho =$

0.877 with $p < 0.001$ for day temperature) without any apparent bias. Thus, the ECMWF-ERA5 source will be preferred because of much better time resolution (hourly) than the MODIS products (weekly).

Environmental variables were divided into two subsets, and then 2-D scatter plots and correlation matrix plots were computed. The NDVI was strongly positively correlated to EVI ($\rho = 0.921$, Figure 2), and negatively with temperature [$\rho = -0.779$ for minimum (Figure 2) and $\rho = -0.749$ for maximum (Figure 3)]. Moreover, the environmental vapor pressure was strongly positively with temperature [$\rho = 0.880$ for minimums (Figure 2) and $\rho = 0.852$ for maximums (Figure 3)]. Thus, the NDVI and the environmental vapor pressure were not considered for the rest of the analysis.

Finally, we retained the EVI, the temperature, the atmospheric pressure, the relative humidity, the wind speed and the rainfall. The EVI was minimal in September, after the summer, and then increased progressively to reach a maximum March/April, and then decreased progressively (data not shown). The temperature highlighted a strong seasonal pattern, with highest temperatures during summer and lowest during winter, whereas the atmospheric pressure was the lowest during summer and highest during winter (Figure 4). The relative humidity seemed slightly impacted by seasons, but highlighted important intra-monthly variations. It was why absolute humidity was also computed as highlighting a clear seasonal pattern (Figure 4). The wind speed did not highlight any particular seasonal pattern, whereas the maximum wind speed seemed highest in spring and fall (Figure 4). Finally, the rainfall were observed from September to May, with highest precipitation during late fall/early winter (Figure 4).

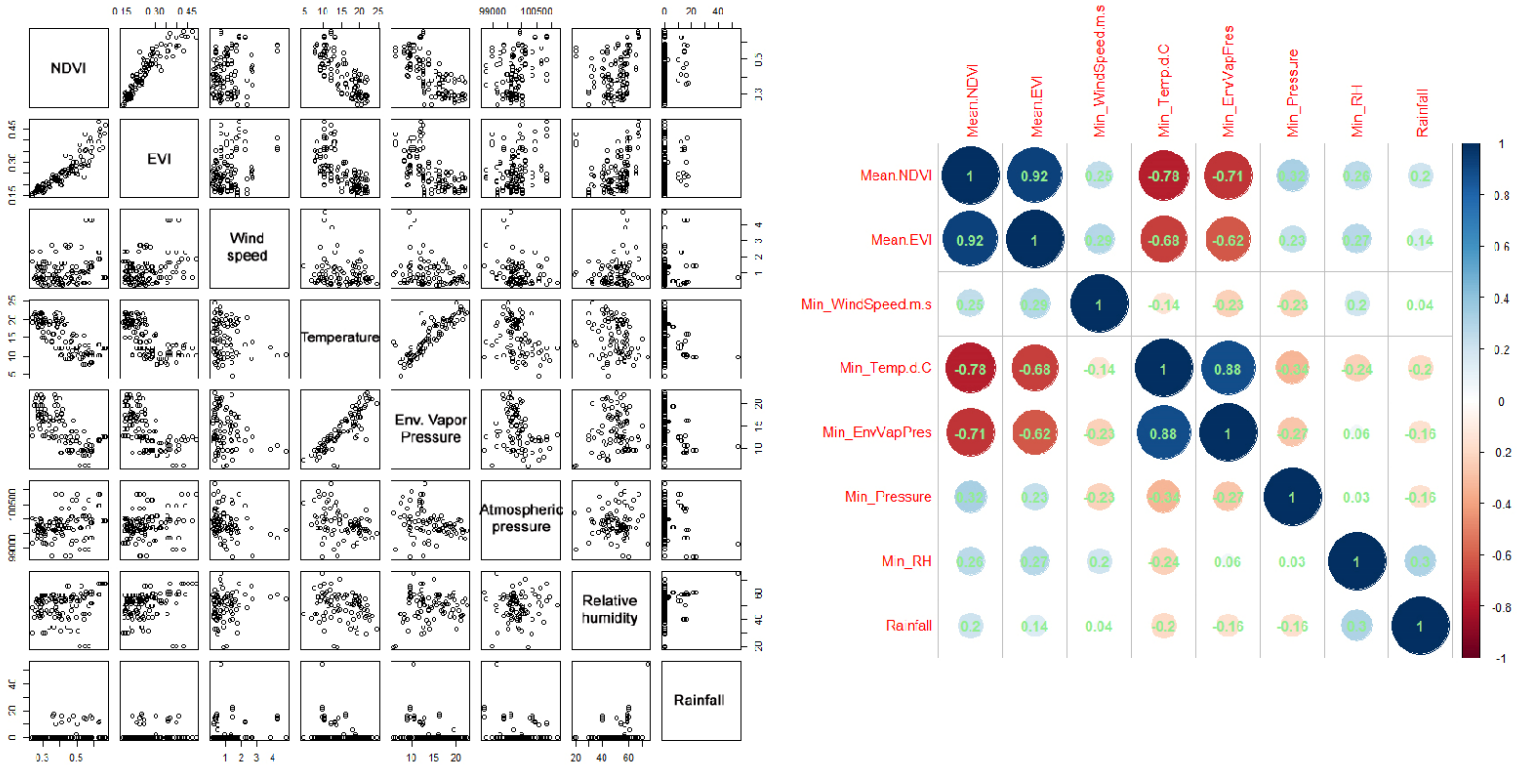


Figure 2. 2-D scatter plots and correlation matrix computed with the normalized difference vegetation index (Mean.NDVI), the enhanced vegetation index (Mean.EVI), and the minimums of wind speed (Min_WindSpeed.m.s), temperature (Min_Temp.d.C), environmental vapor pressure (Min_EnvVapPres), atmospheric pressure (Min_Pressure), relative humidity (Min_RH) and rainfall extracted from MODIS products and ECMWF-ERA5 source in 2016 and 2017 in Rabat (Morocco).

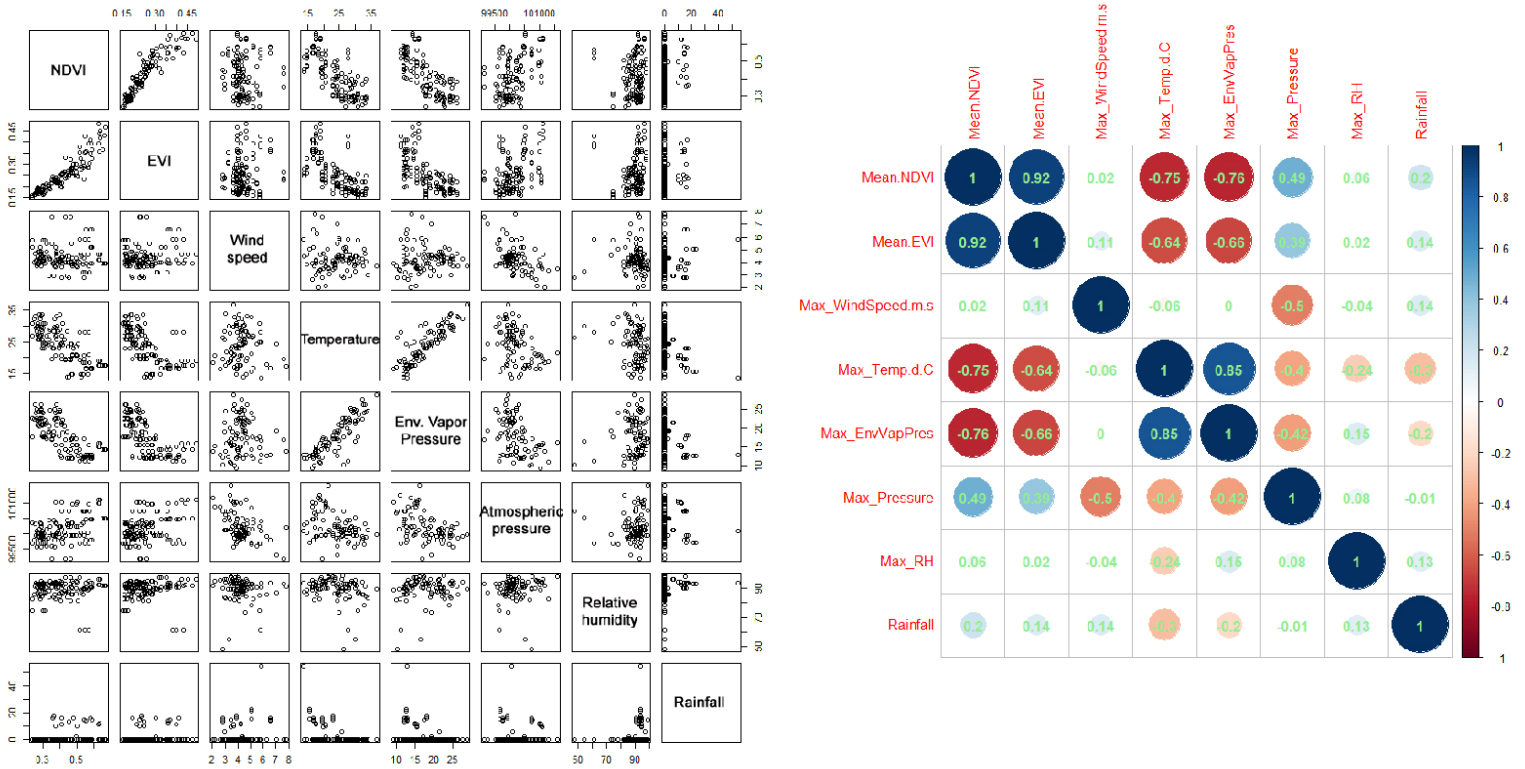


Figure 3. 2-D scatter plots and correlation matrix computed with the normalized difference vegetation index (Mean.NDVI), the enhanced vegetation index (Mean.EVI), and the maximums of wind speed (Max_WindSpeed.m.s), temperature (Max_Temp.d.C), environmental vapor pressure (Max_EnvVapPres), atmospheric pressure (Max_Pressure), relative humidity (Max_RH) and rainfall extracted from MODIS products and ECMWF-ERA5 source in 2016 and 2017 in Rabat (Morocco).

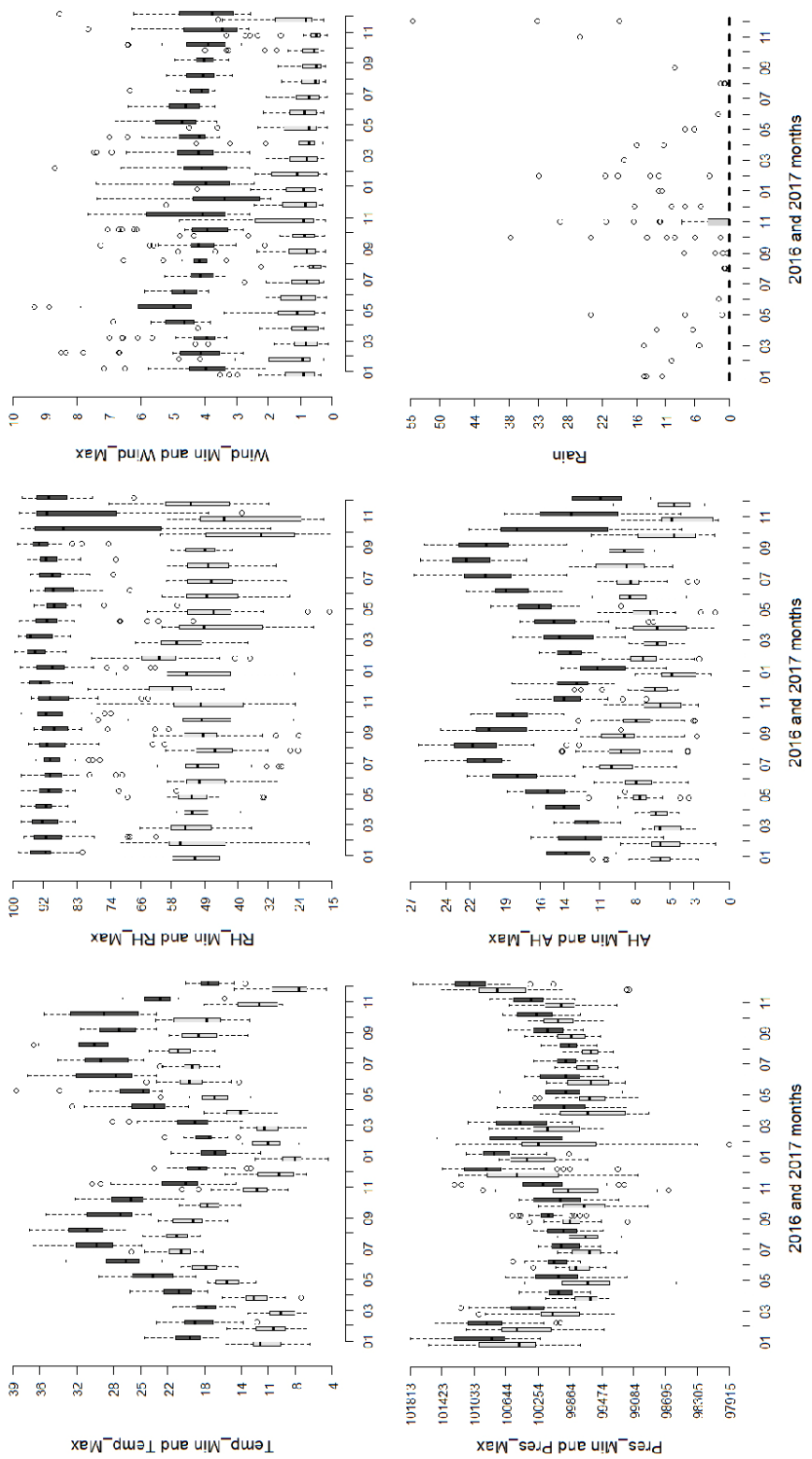
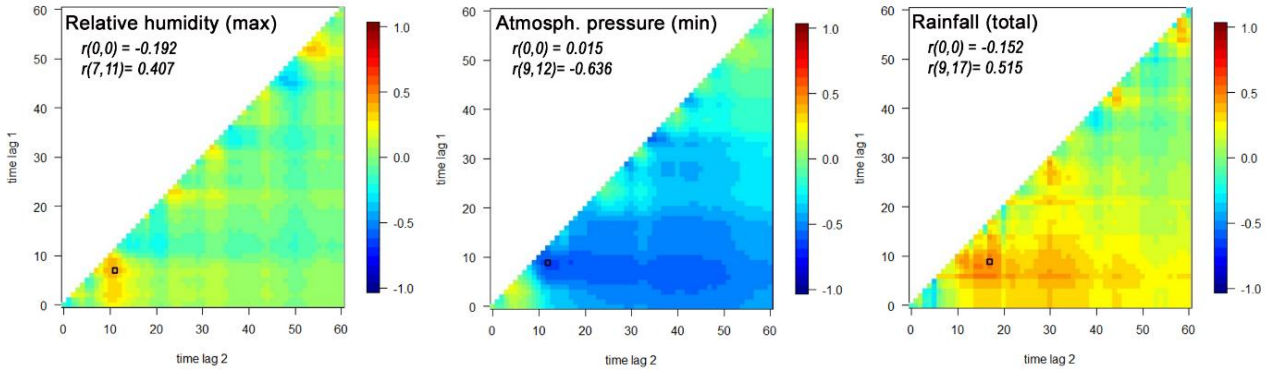
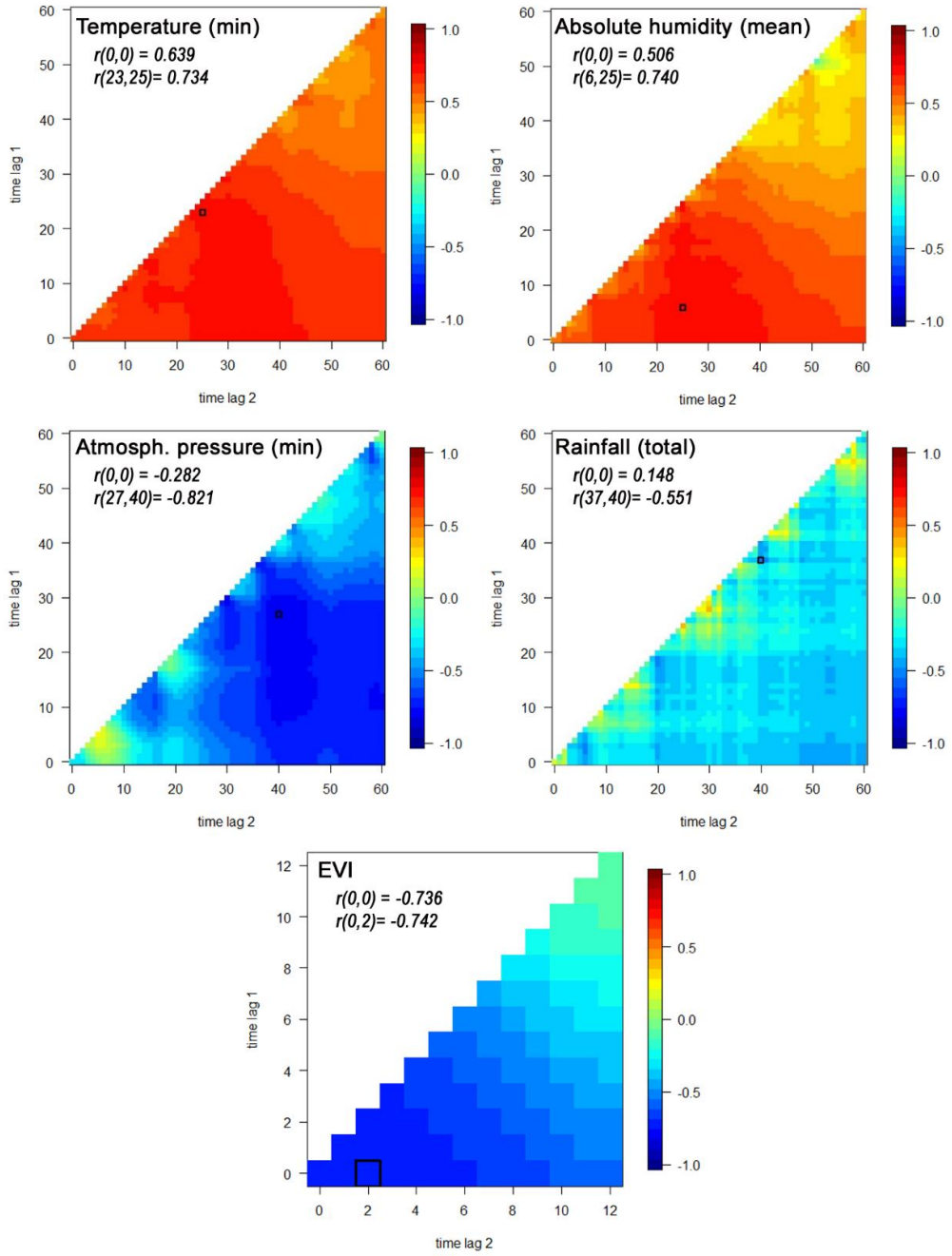


Figure 4. Boxplots highlighting the 2016-2017 season pattern of minimums and maximums of the environmental pattern, *i.e.* temperature, atmospheric pressure, relative humidity, absolute humidity, wind speed and rainfall (only daily amount) derived from ECMWF-ERA5 source at Rabat (Morocco).

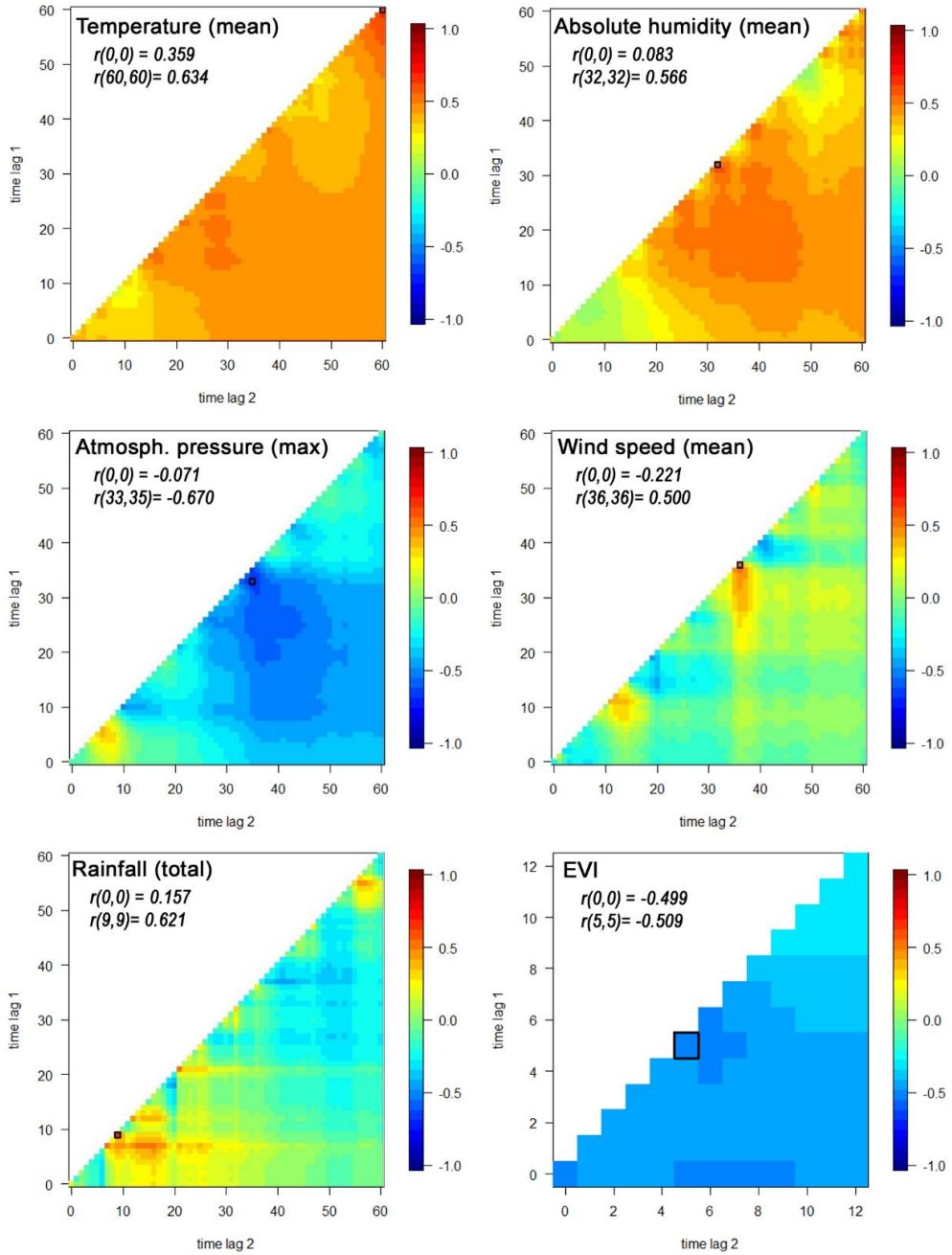
Supplementary data III : Cross correlation maps (CCM) investigating bivariate correlations between the *Culicoides newsteadi* abundance averaged at the three collection sites around Rabat and the environmental variables at different time lags. CCM at the coordinates *j* and *k* was $CCM_{j,k} = cor\ (\ Y_i\ ,\ X_{i-j,i-k}\ )$, Y_i the number of collected *Culicoides* (of a given species) at time *i* and $X_{i-j,i-k}$ an environmental quantity, averaged over a time period starting at time *i* - *j* (time lag 1) and ending at *i* - *k* (time lag 2), with $j \geq k$. Tested time lags were 12 weeks for EVI and 60 days for all other parameters. Only environmental variables correlated with the *Culicoides* abundances were included in the figure.



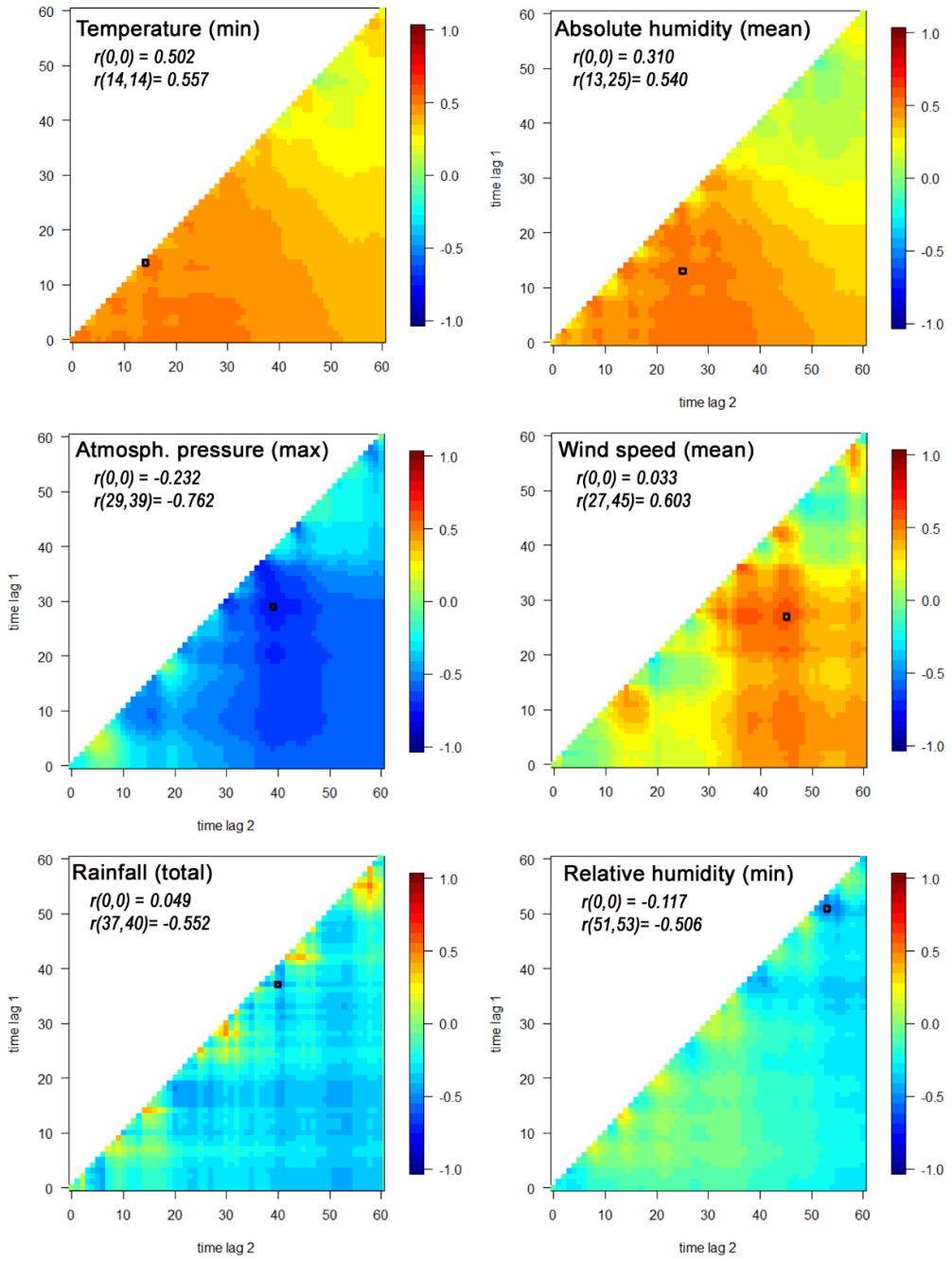
Supplementary data IV : Cross correlation maps (CCM) investigating bivariate correlations between the *Culicoides circumscriptus* abundance averaged at the three collection sites around Rabat and the environmental variables at different time lags. CCM at the coordinates j and k was $CCM_{j,k} = cor\ (\ Y_i\ ,\ X_{i-j,i-k}\ )$, Y_i the number of collected *Culicoides* (of a given species) at time i and $X_{i-j,i-k}$ an environmental quantity, averaged over a time period starting at time $i - j$ (time lag 1) and ending at $i - k$ (time lag 2), with $j \geq k$. Tested time lags were 12 weeks for EVI and 60 days for all other parameters. Only environmental variables correlated with the *Culicoides* abundances were included in the figure



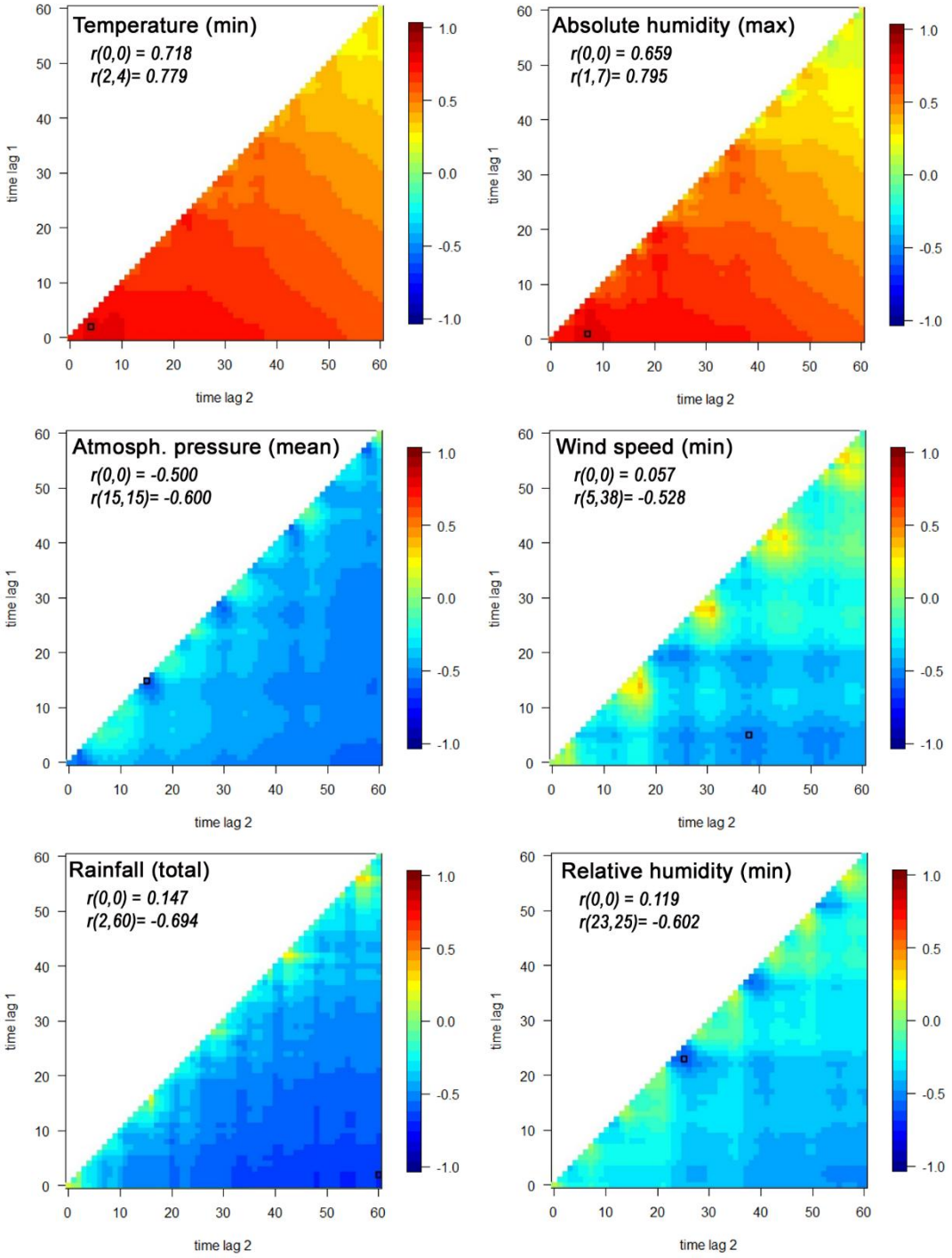
Supplementary data V : Cross correlation maps (CCM) investigating bivariate correlations between the *Culicoides kingi* abundance averaged at the three collection sites around Rabat and the environmental variables at different time lags. CCM at the coordinates j and k was $CCM_{j,k} = cor\ (\ Y_i\ ,\ X_{i-j,i-k}\ )$, Y_i the number of collected *Culicoides* (of a given species) at time i and $X_{i-j,i-k}$ an environmental quantity, averaged over a time period starting at time $i - j$ (time lag 1) and ending at $i - k$ (time lag 2), with $j \geq k$. Tested time lags were 12 weeks for EVI and 60 days for all other parameters. Only environmental variables correlated with the *Culicoides* abundances were included in the figure.



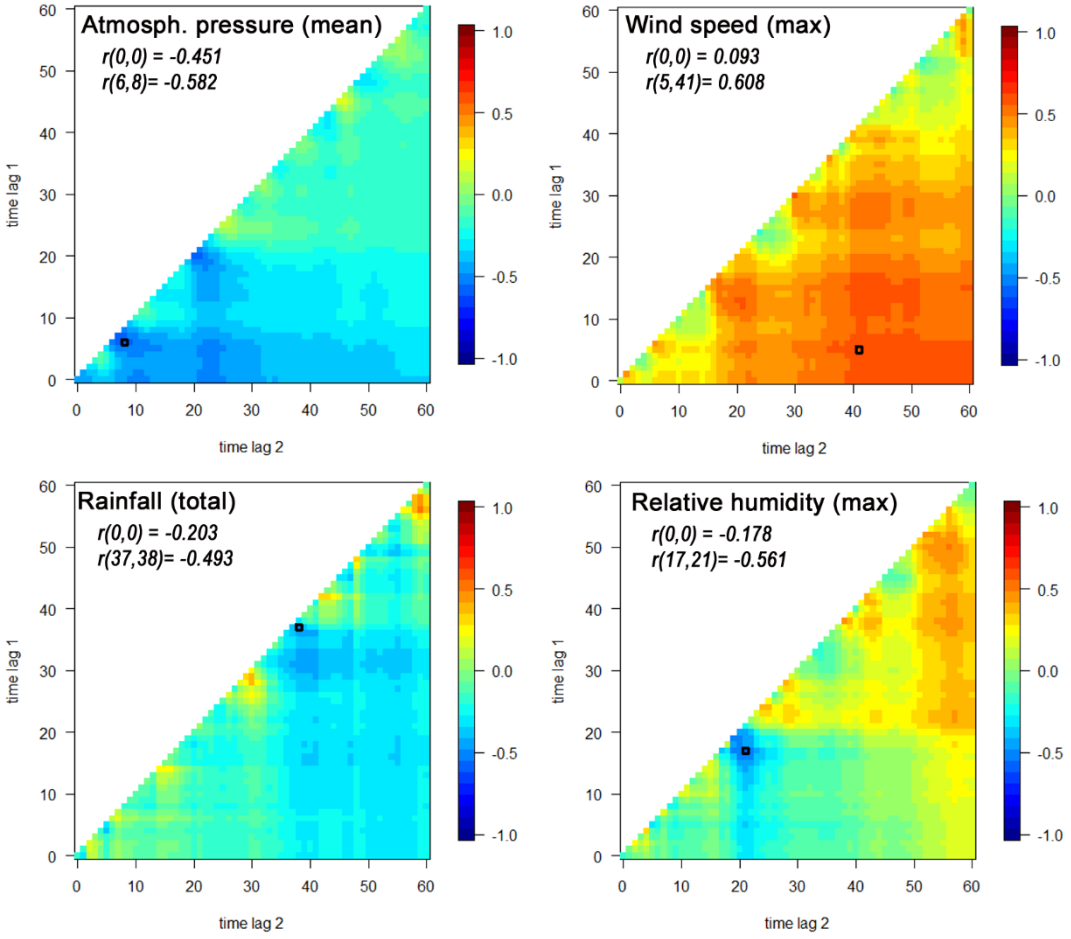
Supplementary data VI : Cross correlation maps (CCM) investigating bivariate correlations between the *Culicoides cataneii/Culicoides gejgelensis* abundance averaged at the three collection sites around Rabat and the environmental variables at different time lags. CCM at the coordinates j and k was $CCM_{j,k} = cor\ (\ Y_i\ ,\ X_{i-j,i-k}\ )$, Y_i the number of collected *Culicoides* (of a given species) at time i and $X_{i-j,i-k}$ an environmental quantity, averaged over a time period starting at time $i - j$ (time lag 1) and ending at $i - k$ (time lag 2), with $j \geq k$. Tested time lags were 12 weeks for EVI and 60 days for all other parameters. Only environmental variables correlated with the *Culicoides* abundances were included in the figure.



Supplementary data VII : Cross correlation maps (CCM) investigating bivariate correlations between the *Culicoides puncticollis* abundance averaged at the three collection sites around Rabat and the environmental variables at different time lags. CCM at the coordinates j and k was $CCM_{j,k} = cor\ (Y_i\ ,\ X_{i-j,i-k})$, Y_i the number of collected *Culicoides* (of a given species) at time i and $X_{i-j,i-k}$ an environmental quantity, averaged over a time period starting at time $i - j$ (time lag 1) and ending at $i - k$ (time lag 2), with $j \geq k$. Tested time lags were 12 weeks for EVI and 60 days for all other parameters.



Supplementary data VIII : Cross correlation maps (CCM) investigating bivariate correlations between the *Culicoides obsoletus/Culicoides scoticus* abundance averaged at the three collection sites around Rabat and the environmental variables at different time lags.
CCM at the coordinates j and k was $CCM_{j,k} = cor\ (\ Y_i\ ,\ X_{i-j,i-k}\ )$, Y_i the number of collected *Culicoides* (of a given species) at time i and $X_{i-j,i-k}$ an environmental quantity, averaged over a time period starting at time $i - j$ (time lag 1) and ending at $i - k$ (time lag 2), with $j \geq k$. Tested time lags were 12 weeks for EVI and 60 days for all other parameters. Only environmental variables correlated with the *Culicoides* abundances were included in the figure.



Supplementary file 2: Modeling the dynamics of *Culicoides* species

We modelled the abundance of the seven most abundant species collected at the three collection sites around Rabat in 2016-2017 using a generalized linear model (GLM) or a negative-binomial log linear model (GLM.NB) to account for the eventual over-dispersion of insect collection data. The environmental variables and the optimal time lags were selected using cross correlation maps (CCM). The same procedure was used for all species.

Firstly, all possible models were systematically produced. Different information criterions (including the uncorrected Akaike's information criterion AIC, the corrected Akaike's information criterion cAIC and the Bayesian information criterion BIC) were computed and were used to select the model (Tables 1 to 7).

The validity of the selected GLM was assessed by (Figures 1 to 14):

i) plotting the observed versus the fitted values and testing the Pearson's product moment correlation coefficient;
ii) checking graphically the normality of the residuals;
iii) testing graphically the linearity hypothesis (random distribution of residuals around 0 after have been plotted by fitted values);
iv) confirming graphically the homogeneity of residuals.

A simplified procedure (steps i and iii) was used for the GLM.NB.

Finally, the selected model was used to predict the daily *Culicoides* abundance from May 2016 to May 2017 to provide an overview of the seasonal pattern and to compare it with the observations (Figures 2B, 4B, 6B, 8B, 10B, 12B and 14B).

Table 1. Values of the information criterions for principal models computed to predict *Culicoides imicola* abundances assessed in 2016-2017 at Rabat (Morocco) by environmental parameters using a generalized linear model. The selected model is in bold.

Formula	AIC	cAIC	BIC
Abun ~ Pres_Mean + Temp_Mean	38.740	40.740	43.616
Abun ~ Pres_Mean	40.929	42.072	44.586
Abun ~ AH_Max + Pres_Mean	40.327	42.327	45.202
Abun ~ EVI + Pres_Mean + Temp_Mean	39.344	42.502	45.439
Abun ~ Pres_Mean + Temp_Mean + Wind_Mean	40.715	43.873	46.810
Abun ~ AH_Max + Pres_Mean + Temp_Mean	40.740	43.898	46.835
Abun ~ Pres_Mean + Wind_Mean	42.657	44.657	47.532
Abun ~ EVI + Pres_Mean	42.910	44.910	47.786
Abun ~ AH_Max + EVI + Pres_Mean	41.866	45.024	47.960
Abun ~ Temp_Mean	43.995	45.138	47.652
Abun ~ AH_Max + Pres_Mean + Wind_Mean	42.208	45.366	48.303
Abun ~ EVI + Pres_Mean + Temp_Mean + Wind_Mean	41.336	46.003	48.649
Abun ~ AH_Max + EVI + Pres_Mean + Temp_Mean	41.340	46.006	48.653
Abun ~ AH_Max + Pres_Mean + Temp_Mean + Wind_Mean	42.715	47.382	50.029
Abun ~ EVI + Pres_Mean + Wind_Mean	44.644	47.802	50.739
Abun ~ Temp_Mean + Wind_Mean	45.841	47.841	50.716
Abun ~ EVI + Temp_Mean	45.927	47.927	50.803
Abun ~ AH_Max + Temp_Mean	45.943	47.943	50.818
Abun ~ AH_Max + EVI + Pres_Mean + Wind_Mean	43.748	48.414	51.061
Abun ~ AH_Max + EVI + Pres_Mean + Temp_Mean + Wind_Mean	43.331	49.919	51.863

AIC: uncorrected Akaike's information criterion; cAIC: corrected Akaike's information criterion; BIC Bayesian information criterion; Abun: log-transformed abundance for GLM and counts for GLM.NB. Pres: atmospheric pressure; Temp: temperature; AH: absolute humidity; EVI: enhanced vegetation index; Wind: wind speed; Rain: total of rainfall; RH: relative humidity.

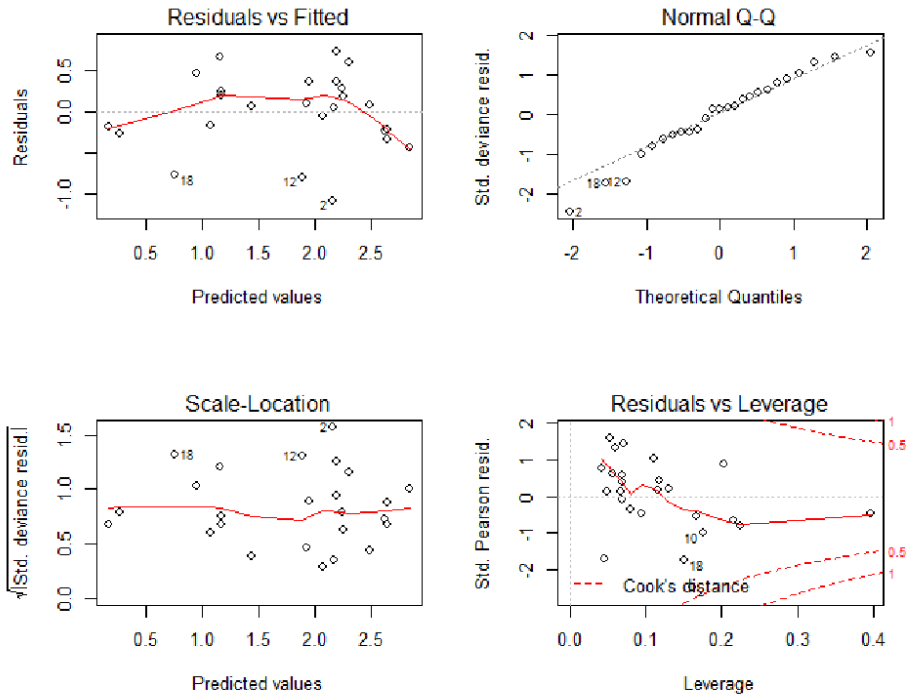


Figure 1. Assessment of the validity of the selected model (GLM, Abun ~ Pres_Mean + Temp_Mean) for *Culicoides imicola* abundance averaged at the three collection sites around Rabat using model residuals.
Residuals vs Fitted: evaluation of the linearity hypothesis (residuals must be generally uniformly distributed on both sides of the ground line). Normal Q-Q: evaluation of the homogeneity of residuals (residuals need to be distributed along the straight line); Scale-Location and Residuals vs Leverage: evaluation of the residuals homogeneity (residuals need to be distributed closed to the ground line).

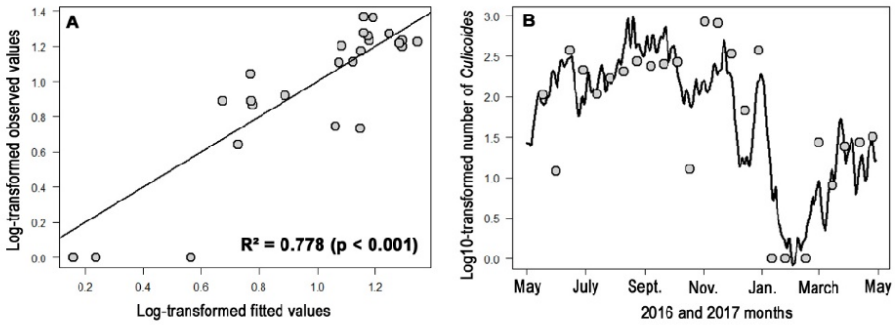


Figure 2. Comparison of fitted and observed values of the *Culicoides imicola* abundance averaged at the three collection sites around Rabat, at the collection days (A) and during the entire season (B).

Table 2. Values of the information criterions for principal models computed to predict *Culicoides newsteadi* abundances assessed in 2016-2017 at Rabat (Morocco) by environmental parameters using a generalized linear model. The selected model is in bold.

Formula	AIC	cAIC	BIC
Abun ~ Pres_Mean + Rain	30.952	32.952	35.827
Abun ~ Pres_Mean + Rain + RH_Max	31.846	35.003	37.940
Abun ~ Pres_Mean	34.112	35.255	37.769
Abun ~ Pres_Mean + RH_Max	34.355	36.355	39.230
Abun ~ Rain	35.347	36.489	39.003
Abun ~ Rain + RH_Max	35.409	37.409	40.285
Abun ~ RH_Max	39.757	40.900	43.414

AIC: uncorrected Akaike's information criterion; cAIC: corrected Akaike's information criterion; BIC Bayesian information criterion; Abun: log-transformed abundance for GLM and counts for GLM.NB. Pres: atmospheric pressure; Temp: temperature; AH: absolute humidity; EVI: enhanced vegetation index; Wind: wind speed; Rain: total of rainfall; RH: relative humidity.

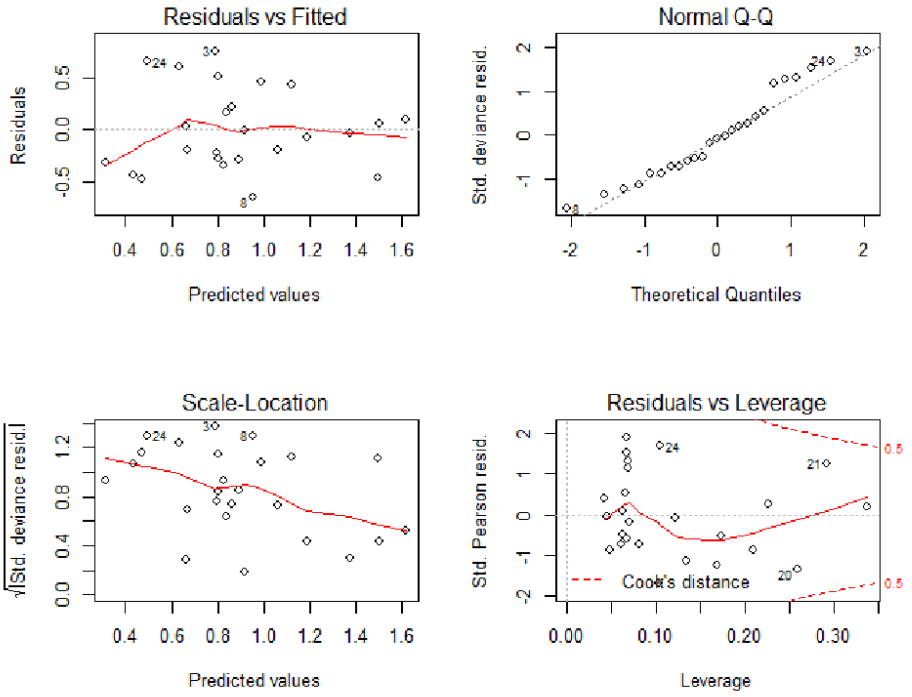


Figure 3. Assessment of the validity of the selected model (GLM, Abun ~ Pres_Mean + Rain) for *Culicoides newsteadi* abundance averaged at the three collection sites around Rabat using model residuals.
Residuals vs Fitted: evaluation of the linearity hypothesis (residuals must be generally uniformly distributed on both sides of the ground line). Normal Q-Q: evaluation of the homogeneity of residuals (residuals need to be distributed along the straight line); Scale-Location and Residuals vs Leverage: evaluation of the residuals homogeneity (residuals need to be distributed closed to the ground line).

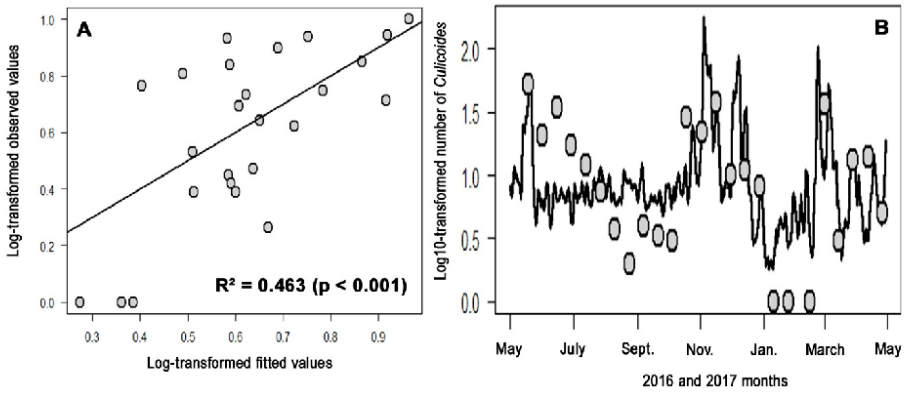


Figure 4. Comparison of fitted and observed values of the *Culicoides newsteadi* abundance averaged at the three collection sites around Rabat, at the collection days (A) and during the entire season (B).

Table 3. Values of the information criterions for principal models computed to predict *Culicoides circumscriptus* abundances assessed in 2016-2017 at Rabat (Morocco) by environmental parameters using a negative-binomial log linear model. The selected model is in bold.

Formula	**AIC**	**cAIC**	**BIC**
Abun ~ Pres_Mean	145.146	146.289	148.803
Abun ~ Pres_Mean + Rain	145.127	147.127	150.003
Abun ~ AH_Mean + Pres_Mean	145.598	147.598	150.473
Abun ~ AH_Mean + Pres_Mean + Temp_Mean	144.865	148.022	150.959
Abun ~ Pres_Mean + Temp_Mean	146.791	148.791	151.667
Abun ~ EVI + Pres_Mean	146.898	148.898	151.774
Abun ~ AH_Mean + Pres_Mean + Rain	146.395	149.553	152.489
Abun ~ Pres_Mean + Rain + Temp_Mean	146.931	150.089	153.025
Abun ~ AH_Mean + EVI + Pres_Mean	147.175	150.333	153.269
Abun ~ EVI + Pres_Mean + Rain	147.363	150.521	153.458
Abun ~ AH_Mean + Pres_Mean + Rain + Temp_Mean	146.313	150.980	153.626
Abun ~ AH_Mean + EVI + Pres_Mean + Temp_Mean	146.998	151.664	154.311
Abun ~ EVI + Pres_Mean + Temp_Mean	149.390	152.548	155.484
Abun ~ AH_Mean + EVI + Pres_Mean + Rain	148.079	152.746	155.393
Abun ~ EVI + Pres_Mean + Rain + Temp_Mean	149.377	154.043	156.690
Abun~ AH_Mean + EVI + Pres_Mean + Rain + Temp_Mean	148.401	154.989	156.933
Abun ~ EVI + Rain	162.758	164.758	167.633
Abun ~ Rain + Temp_Mean	165.273	167.273	170.149
Abun ~ AH_Mean + Rain	165.717	167.717	170.593

AIC: uncorrected Akaike's information criterion; cAIC: corrected Akaike's information criterion; BIC Bayesian information criterion; Abun: log-transformed abundance for GLM and counts for GLM.NB. Pres: atmospheric pressure; Temp: temperature; AH: absolute humidity; EVI: enhanced vegetation index; Wind: wind speed; Rain: total of rainfall; RH: relative humidity.

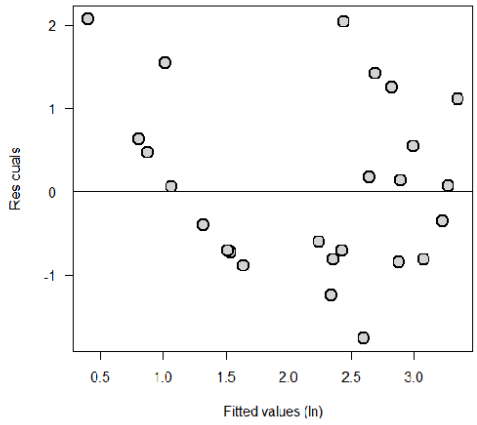


Figure 5. Assessment of the validity of the selected model (GLM.NB, Abun ~ Pres_Mean + Rain) for *Culicoides circumscriptus* abundance averaged at the three collection sites around Rabat using model residuals.

Evaluation of the residuals homogeneity (residuals need to be distributed closed to the ground line).

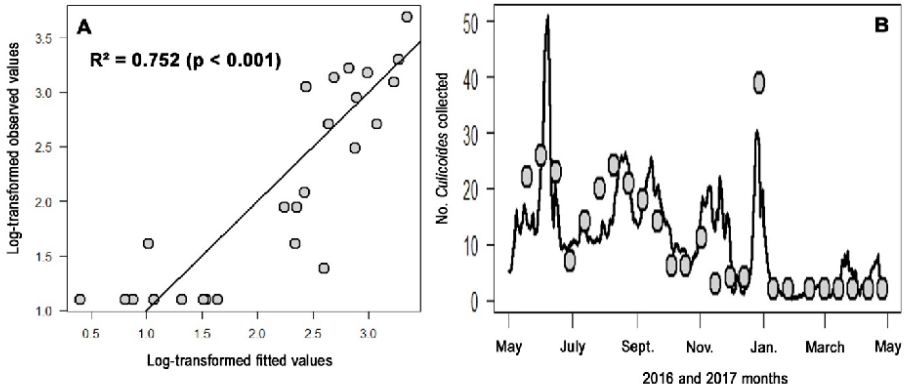


Figure 6. Comparison of fitted and observed values of the *Culicoides circumscriptus* abundance averaged at the three collection sites around Rabat, at the collection days (A) and during the entire season (B).

Table 4. Values of the information criterions for principal models computed to predict *Culicoides kingi* abundances assessed in 2016-2017 at Rabat (Morocco) by environmental parameters using a generalized linear model. The selected model is in bold.

Formula	**AIC**	**cAIC**	**BIC**
Abun ~ Pres_Max	54.400	55.543	58.057
Abun ~ Pres_Max + Rain	55.207	57.207	60.082
Abun ~ AH_Mean + Pres_Max	55.620	57.620	60.496
Abun ~ Pres_Max + Temp_Mean	55.691	57.691	60.566
Abun ~ AH_Mean	56.611	57.754	60.268
Abun ~ Pres_Max + Wind_Mean	56.002	58.002	60.878
Abun ~ EVI + Pres_Max	56.016	58.016	60.891
Abun ~ EVI + Rain	56.597	58.597	61.473
Abun ~ Temp_Mean	57.455	58.598	61.111
Abun ~ EVI	57.732	58.875	61.389
Abun ~ AH_Mean + Rain	57.550	59.550	62.426
Abun ~ Pres_Max + Rain + Wind_Mean	56.639	59.797	62.734
Abun ~ AH_Mean + EVI	57.862	59.862	62.738
Abun ~ Rain	58.726	59.869	62.383
Abun ~ Rain + Temp_Mean	57.875	59.875	62.750
Abun ~ EVI + Pres_Max + Rain	56.724	59.882	62.818
Abun ~ Pres_Max + Rain + Temp_Mean	56.904	60.062	62.998
Abun ~ AH_Mean + Pres_Max + Rain	56.930	60.088	63.025
Abun ~ AH_Mean + Temp_Mean	58.224	60.224	63.099
Abun ~ AH_Mean + Wind_Mean	58.313	60.313	63.188

AIC: uncorrected Akaike's information criterion; cAIC: corrected Akaike's information criterion; BIC Bayesian information criterion; Abun: log-transformed abundance for GLM and counts for GLM.NB. Pres: atmospheric pressure; Temp: temperature; AH: absolute humidity; EVI: enhanced vegetation index; Wind: wind speed; Rain: total of rainfall; RH: relative humidity.

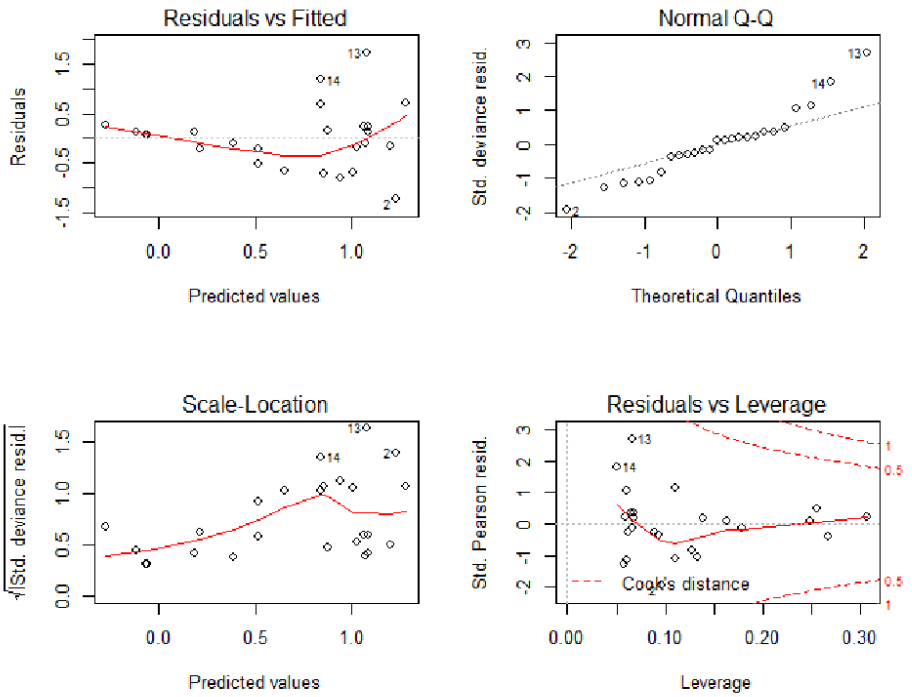


Figure 7. Assessment of the validity of the selected model (GLM, Abun ~ Pres_Max + Rain) for *Culicoides kingi* abundance averaged at the three collection sites around Rabat using model residuals.
Residuals vs Fitted: evaluation of the linearity hypothesis (residuals must be generally uniformly distributed on both sides of the ground line). Normal Q-Q: evaluation of the homogeneity of residuals (residuals need to be distributed along the straight line); Scale-Location and Residuals vs Leverage: evaluation of the residuals homogeneity (residuals need to be distributed closed to the ground line).

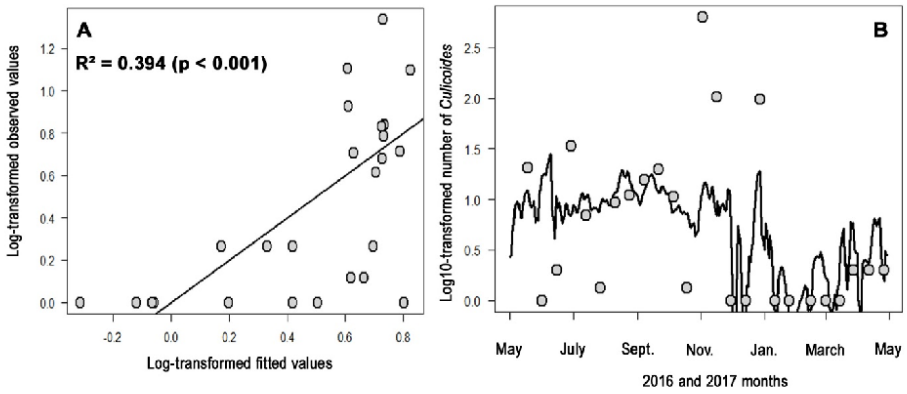


Figure 8. Comparison of fitted and observed values of the *Culicoides kingi* abundance averaged at the three collection sites around Rabat, at the collection days (A) and during the entire season (B).

Table 5. Values of the information criterions for principal models computed to predict *Culicoides cataneii/Culicoides gejgelensis* abundances assessed in 2016-2017 at Rabat (Morocco) by environmental parameters using a generalized linear model. The selected model is in bold.

Formula	**AIC**	**cAIC**	**BIC**
Abun ~ EVI + Rain + Wind_Mean	8.195	11.353	14.289
Abun ~ EVI + Wind_Mean	10.267	12.267	15.142
Abun ~ EVI + Rain + Temp_Min + Wind_Mean	8.789	13.455	16.102
Abun ~ Rain + Temp_Min + Wind_Mean	11.376	14.534	17.470
Abun ~ EVI + Pres_Max + Rain + Wind_Mean	10.013	14.680	17.326
Abun ~ EVI + Temp_Min + Wind_Mean	11.580	14.738	17.674
Abun ~ EVI + Pres_Max + Wind_Mean	11.591	14.749	17.686
Abun ~ AH_Mean + EVI + Rain + Wind_Mean	10.162	14.829	17.475
Abun ~ EVI + Rain + RH_Min + Wind_Mean	10.184	14.851	17.497
Abun ~ EVI + RH_Min + Wind_Mean	11.718	14.876	17.812
Abun ~ AH_Mean + EVI + Wind_Mean	12.254	15.412	18.348
Abun ~ Temp_Min + Wind_Mean	14.553	16.553	19.429
Abun ~ AH_Mean + EVI + Rain + Temp_Min + Wind_Mean	10.086	16.675	18.618
Abun ~ EVI + Rain + RH_Min + Temp_Min + Wind_Mean	10.569	17.158	19.101
Abun ~ Rain + RH_Min + Temp_Min + Wind_Mean	12.518	17.185	19.832
Abun ~ EVI + Pres_Max + Rain + Temp_Min + Wind_Mean	10.788	17.377	19.320
Abun ~ AH_Mean + Rain + Temp_Min + Wind_Mean	13.154	17.821	20.467
Abun ~ EVI + Pres_Max + Temp_Min + Wind_Mean	13.241	17.907	20.554
Abun ~ EVI + RH_Min + Temp_Min + Wind_Mean	13.355	18.022	20.668
Abun ~ Pres_Max + Rain + Temp_Min + Wind_Mean	13.361	18.028	20.674

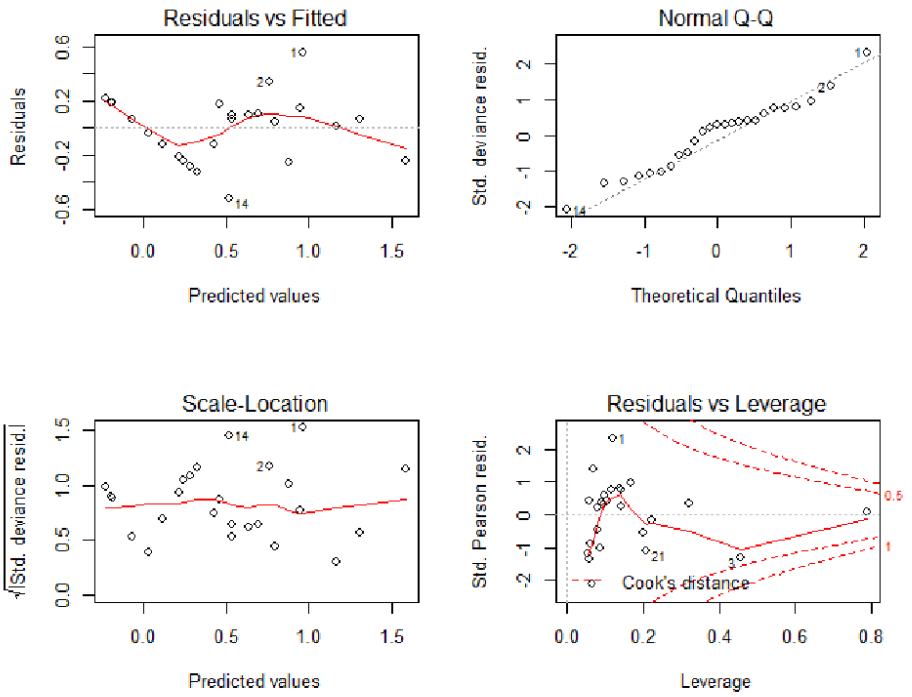


Figure 9. Assessment of the validity of the selected model (GLM, Abun ~ EVI + Rain + Wind_Mean) for *Culicoides cataneii/Culicoides gejgelensis* abundance averaged at the three collection sites around Rabat using model residuals.

Residuals vs Fitted: evaluation of the linearity hypothesis (residuals must be generally uniformly distributed on both sides of the ground line). Normal Q-Q: evaluation of the homogeneity of residuals (residuals need to be distributed along the straight line); Scale-Location and Residuals vs Leverage: evaluation of the residuals homogeneity (residuals need to be distributed closed to the ground line).

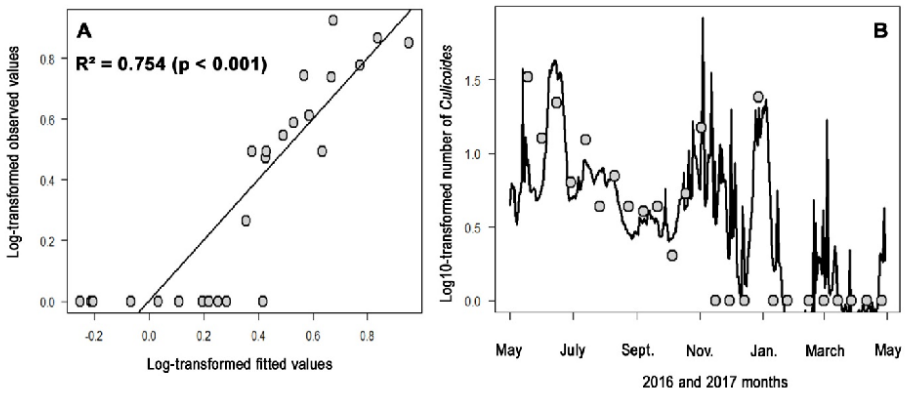


Figure 10. Comparison of fitted and observed values of the *Culicoides cataneii/Culicoides gejgelensis* abundance averaged at the three collection sites around Rabat, at the collection days (A) and during the entire season (B).

Table 6. Values of the information criterions for principal models computed to predict *Culicoides puncticollis* abundances assessed in 2016-2017 at Rabat (Morocco) by environmental parameters using a negative-binomial log linear model. The selected model is in bold.

Formula	AIC	cAIC	BIC
Abun ~ AH_Max + RH_Min	121.077	123.077	125.953
Abun ~ AH_Max	124.322	125.465	127.979
Abun ~ AH_Max + EVI + RH_Min	122.082	125.240	128.177
Abun ~ AH_Max + RH_Min + Wind_Min	122.114	125.272	128.209
Abun ~ AH_Max + RH_Min + Temp_Min	122.250	125.407	128.344
Abun ~ AH_Max + Rain + RH_Min	122.796	125.954	128.890
Abun ~ AH_Max + Pres_Mean + RH_Min	123.011	126.169	129.105
Abun ~ RH_Min + Temp_Min	125.288	127.288	130.163
Abun ~ AH_Max + Rain + RH_Min + Wind_Min	122.850	127.516	130.163
Abun ~ AH_Max + EVI + RH_Min + Wind_Min	122.927	127.594	130.240
Abun ~ AH_Max + Rain	125.420	127.420	130.295
Abun ~ Temp_Min	127.058	128.201	130.715
Abun ~ AH_Max + Rain + RH_Min + Temp_Min	123.407	128.074	130.721
Abun ~ AH_Max + EVI + RH_Min + Temp_Min	123.561	128.228	130.874
Abun ~ AH_Max + EVI + Rain + RH_Min	123.751	128.418	131.064
Abun ~ AH_Max + Temp_Min	126.277	128.277	131.153
Abun ~ AH_Max + EVI	126.299	128.299	131.174
Abun ~ AH_Max + Wind_Min	126.307	128.307	131.183
Abun ~ AH_Max + Pres_Mean	126.450	128.450	131.326
Abun ~ AH_Max + Pres_Mean + RH_Min + Temp_Min	124.026	128.692	131.339

AIC: uncorrected Akaike's information criterion; cAIC: corrected Akaike's information criterion; BIC Bayesian information criterion; Abun: log-transformed abundance for GLM and counts for GLM.NB. Pres: atmospheric pressure; Temp: temperature; AH: absolute humidity; EVI: enhanced vegetation index; Wind: wind speed; Rain: total of rainfall; RH: relative humidity.

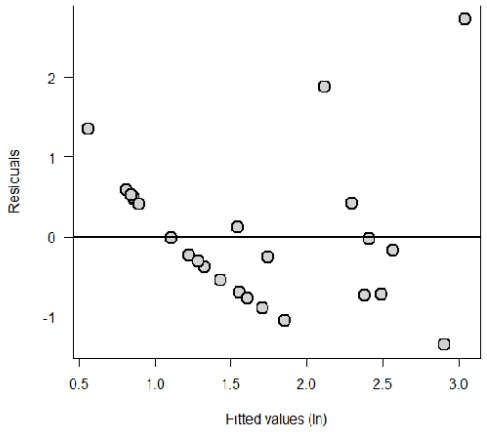


Figure 11. Assessment of the validity of the selected model (GLM.NB, Abun ~ AH_Max + RH_Min) for *Culicoides puncticollis* abundance averaged at the three collection sites around Rabat using model residuals. *Evaluation of the residuals homogeneity (residuals need to be distributed closed to the ground line).*

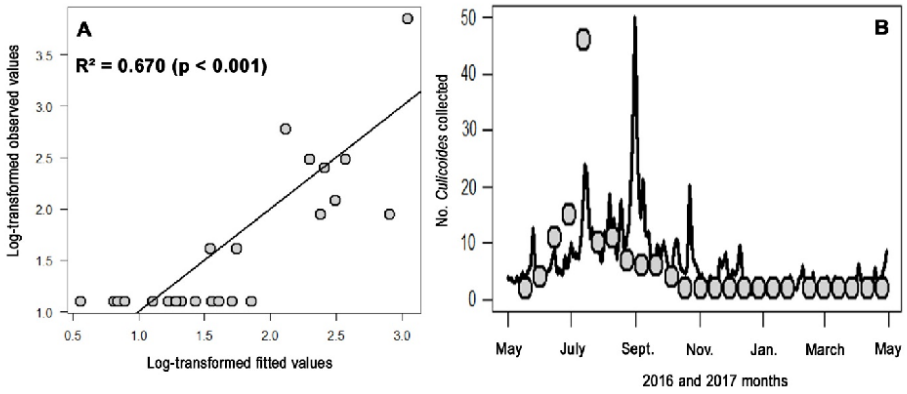


Figure 12. Comparison of fitted and observed values of the *Culicoides puncticollis* abundance averaged at the three collection sites around Rabat, at the collection days (A) and during the entire season (B).

Table 7. Values of the information criterions for principal models computed to predict *Culicoides obsoletus/Culicoides scoticus* abundances assessed in 2016-2017 at Rabat (Morocco) by environmental parameters using a negative-binomial log linear model. The selected model is in bold.

Formula	**AIC**	**cAIC**	**BIC**
Abun ~ Pres_Max + RH_Max + Wind_Max	121.746	124.904	127.841
Abun ~ RH_Max + Wind_Max	123.696	125.696	128.571
Abun~ Pres_Max + Rain + RH_Max + Wind_Max	121.924	126.591	129.237
Abun ~ Rain + RH_Max + Wind_Max	123.653	126.811	129.748
Abun ~ Pres_Max + Wind_Max	128.779	130.779	133.654
Abun ~ Pres_Max + Rain + Wind_Max	128.218	131.376	134.313
Abun ~ Pres_Max + Rain + RH_Max	128.850	132.008	134.944
Abun ~ Pres_Max + RH_Max	130.392	132.392	135.267
Abun ~ Pres_Max + Rain	130.798	132.798	135.673
Abun ~ Pres_Max	132.798	133.941	136.455
Abun ~ Rain + RH_Max	133.529	135.529	138.405
Abun ~ Rain + Wind_Max	134.945	136.945	139.821
Abun ~ RH_Max	136.574	137.717	140.231
Abun ~ Wind_Max	136.671	137.814	140.328
Abun ~ Rain	139.393	140.536	143.049

AIC: uncorrected Akaike's information criterion; cAIC: corrected Akaike's information criterion; BIC Bayesian information criterion; Abun: log-transformed abundance for GLM and counts for GLM.NB. Pres: atmospheric pressure; Temp: temperature; AH: absolute humidity; EVI: enhanced vegetation index; Wind: wind speed; Rain: total of rainfall; RH: relative humidity.

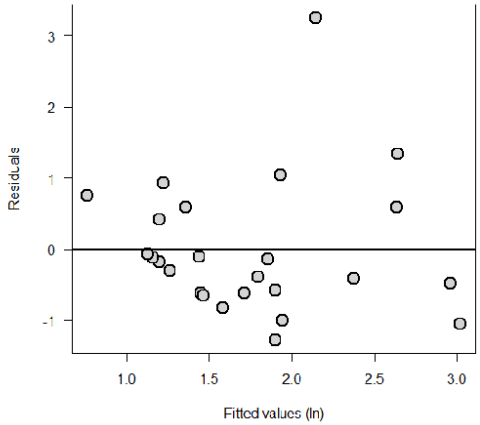


Figure 13. Assessment of the validity of the selected model (GLM.NB, Abun ~ Pres_Max + RH_Max + Wind_Max) for *Culicoides obsoletus/Culicoides scoticus* abundance averaged at the three collection sites around Rabat using model residuals.

Evaluation of the residuals homogeneity (residuals need to be distributed closed to the ground line).

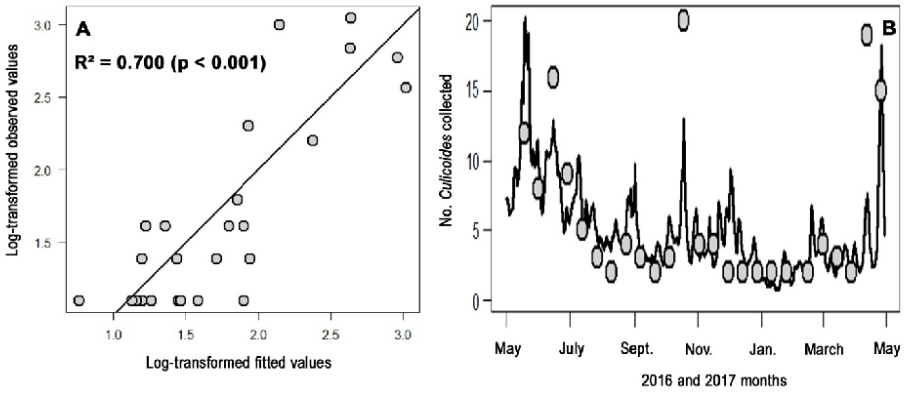


Figure 14. Comparison of fitted and observed values of the *Culicoides obsoletus/Culicoides scoticus* abundance averaged at the three collection sites around Rabat, at the collection days (A) and during the entire season (B).

Chapitre 4 : Détermination de la distribution spatiale des *Culicoides* à l'échelle nationale et caractérisation des facteurs environnementaux expliquant leur abondance.

Lorsqu'il s'agit de maladies à transmission vectorielle, l'analyse de la répartition spatiale des espèces vectrices de maladies peut apporter une contribution importante dans la compréhension de la dynamique des épizooties, la réalisation de cartes de risques aux gestionnaires de la santé et permettre l'élaboration de plans de riposte efficaces et ciblés dans l'espace et dans le temps.

Nous avons décidé d'étudier la distribution des espèces de *Culicoides* à l'échelle du pays. Ceci nous a permit de voir quelles espèces dominent et si elles présentent de fortes hétérogénéités en termes d'abondance. On a aussi caractérisé les facteurs environnementaux déterminant cette distribution. Cette étude est une étape préliminaire à la réalisation de cartes de risque de transmission des virus qui sont associés aux espèces vectrices, de telles cartes de risque sont extrêmement importantes pour cibler les actions de surveillance et de lutte dans une perspective d'améliorer le rapport coût/bénéfice de ces programmes.

Les résultats issus de cette étude font l'objet de l'article 3 en préparation. Article 3 : **Bourquia M.**, Garros C., Rakotoarivony I., Boukhari I., Duhayon M., Huber K., Mathieu B., Mignotte A., Baldet T., Wint W., Khayli M., Khallaayoune K., Balenghien T. 2019. Distribution of *Culicoides* species in Morocco, and environmental factors driving it. In prep

Environmental drivers of *Culicoides* (Diptera : Ceratopogonidae) species communities in Morocco

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Abstract

Culicoides biting midges (Diptera: Ceratopogonidae) are responsible for important nuisance and vectors of major arboviral pathogens for livestock. We aimed to investigate the influence of bioclimatic and environmental factors on *Culicoides* distribution in Morocco. We carried out two transversal snapshots from April to early July and from late August to October 2017 in 144 sites across the country using light/suction trap collections on two consecutive nights. *Culicoides* were identified when possible at the species level using morphological identification keys and complementary molecular assays. The impact of environmental and climatic factors on the species composition was assessed using a multivariate statistical approach. A total of 217,031 *Culicoides* individuals belonging to at least 30 different species were collected. *Culicoides imicola* (51.6% of total catches) was by far the most abundant species. Three groups of species were identified. The first group, including *Culicoides paolae, Culicoides circumscriptus, Culicoides puncticollis* and *C. imicola,* was dominant in regions characterized by humid to subhumid climate, by low to medium altitude (with low slope), by a soil rich in clay with a high salinity and hosting mainly poultry and goat farms. The second group shared the same climatic, topographic and host classes than cluster I, but differed in soil and land use. Indeed, it was in washed soils covered by mixed trees areas. The species that determine this group are: *C. sahariensis, C. pulicaris, C. obsoletus/C scoticus, C. kurensis*, heteroclitus/odiatus/pseudopallidus/picturatus group (HOPP), *C. lupicaris, C. fagineus, C. newteadi* and *C. catanneii/C gejgelensis.* The last group was dominant in arid, or even in per-arid for *C. kingi*, areas of plateaus of average to high altitude, characterized by shallow soil located on hard rocks and where host densities, except goat, were high. The species that determine this group are, *C. kingi, C. saevus/langeroni* and *C. parroti*.

Background

The transmission of vector-borne diseases (VBD) is driven by multiple ecological processes, which regulate the distribution and abundance of vectors. In the past, some successful programs of VBD control have relied upon the elimination of vector populations from the environment (Lemon et al., 2008). Characterizing the interactions between vectors and their abiotic and biotic environments is mandatory to understand the dynamics of VBD. Mathematical and statistical modelling can help to disentangle the complex relationships between the vector and the environment, including by identifying the ecological factors driving their distribution. Models allow representing in a simplified way a complex biological system and predicting its evolution under different scenarios (Valleron, 2000).

Culicoides biting midges (Diptera: Ceratopogonidae) are responsible for the biological transmission of major arboviruses, such as bluetongue and African horse sickness viruses (BTV and AHSV). As other insects, *Culicoides* do not highlight an uniform distribution. The boundaries of the area occupied by each species are subject to permanent fluctuations due to the continuous variations of one or more environmental factors. Undoubtedly, the climate is one of the fundamental drivers of the *Culicoides* distribution. The influence of climate is exerted and can be perceived at all scales of observation of the biosphere, this component itself results from a combination of elementary factors and variables (Purse et al., 2008). The influence of topography is primarily indirect, by altering other drivers. Its main variables, such as altitude and shape (relief form) as well as exposure and slope, interfere with all climatic factors by contributing very largely to the differentiation of microclimates, but also of local and even of regional climates. However, topography could also induce other ecological components. Topography largely conditions the modalities of pedogenesis, in particular through erosion and stagnation processes, and certain topographical situations are directly responsible for the development of a specific habitat: for instance, depressions leading to waterlogging (Lacoste and Salanon, 1999). Edaphic properties are considered to have important influences on *Culicoides* population sizes, highlighting the availability of breeding habitats (Braverman *et al.*, 1974; Conte *et al.*, 2007; Titeux *et al.*, 2009; Foxi & Delrio, 2010). Hosts may influence the distribution of species by the production of breeding habitats for dung-breeding species or as source of blood for these hematophagous species. The abundance of *Culicoides* species has been found either to be linked to land use (pastures) and host density (Purse *et al.*, 2012; Sanders *et al.*, 2011), or to be not affected by the number and type of farmed animals (De Liberato *et al.*, 2010). It is commonly assumed that variation

in *Culicoides* abundance can only be explained by the combination of several ecological factors because of their complex interactions with species-specific biological traits (Acevedo *et al.*, 2010).

In Morocco, extensive collections of *Culicoides* were carried out in 1994-1995, after the 1989-1991 AHSV outbreaks (Bouayoune et al., 1998), in 2000-2003, after BTV emergence in the Mediterranean basin (Fassi Fihri and Lhor, 2008), and in 2009, after the BTV emergence in Morocco (Lhor et al., 2015). These studies focused mainly on *C. imicola*, as a proven vector of AHSV and BTV, which was, at that time, considered the only significant vector in the Mediterranean region. After the BTV emergence in Europe, it has been confirmed that several other species could be implicated in the transmission of this arbovirus. The BTV has been isolated or its genome identified in field-collected individuals of several *Avaritia* species, such as *Culicoides obsoletus*, *Culicoides scoticus*, *Culicoides chiopterus or Culicoides dewulfi.* Moreover, the BTV genome was detected in field-collected individuals of *Culicoides pulicaris, Culicoides newsteadi*, *Culicoides punctatus*, *Culicoides lupicaris, Culicoides paolae* and of species belonging to Nubeculosus complex *(*Caracappa et al., 2005*,* Vanbinst et al., 2009, Romon et al., 2012; Goffredo et al., 2015, Foxi et al. 2019). This highlights the importance of bringing new insights on the *Culicoides* ecology in Morocco.

The present study aimed to describe the *Culicoides* species communities through Morocco and to characterize the environmental factors determining their composition. This is a crucial preliminary step to implement robust distribution model and then to develop maps assessing the risk of virus transmission. Such risk maps are extremely important to target surveillance and control actions and then to improve the cost/benefit ratio of these programs.

Material and methods

1. Study area

Morocco covers an area of 710,850 km^2 with a coastline of 3,500 km. It is located at the Northwest of the African continent, as part of the Maghreb. It is bordered to the north by the Mediterranean Sea, to the west by the Atlantic Ocean, to the south by Mauritania and to the east by Algeria. This position and the Moroccan relief lead to a great variety of climates, ecosystems and landscapes (plateaus, plains, mountains, oases, deserts...), flora and fauna. For instance, the annual average rainfall varies from less than 25 mm in the Sahara to nearly 2000

mm in the Rif Mountains. All bioclimatic stages based on the Emberger diagram exist in Morocco, from per-humid (limited to the crest of the Rif Mountains) to per-arid, including all thermal variants, from cold to warm winters.

2. Entomological investigations

a. Sampling design

The agro-ecological zones of Morocco were used to develop a stratified sampling strategy (Figure 1). We selected 24 veterinary services – which are the decentralized administrative structures of the Moroccan Animal Health Authority – distributed throughout the country and covering all agro-ecological zones, with the exception of the southern Saharan zones. At least 6 cattle farms per veterinary service were randomly selected from the ONSSA (National Office for the Health Security of Food Products) database to obtain, if possible, farms in each agro-ecological zone of the veterinary service (Figure 1). Only farms breeding more than 5 animals were considered to ensure the presence of several animals at the time of the visit.

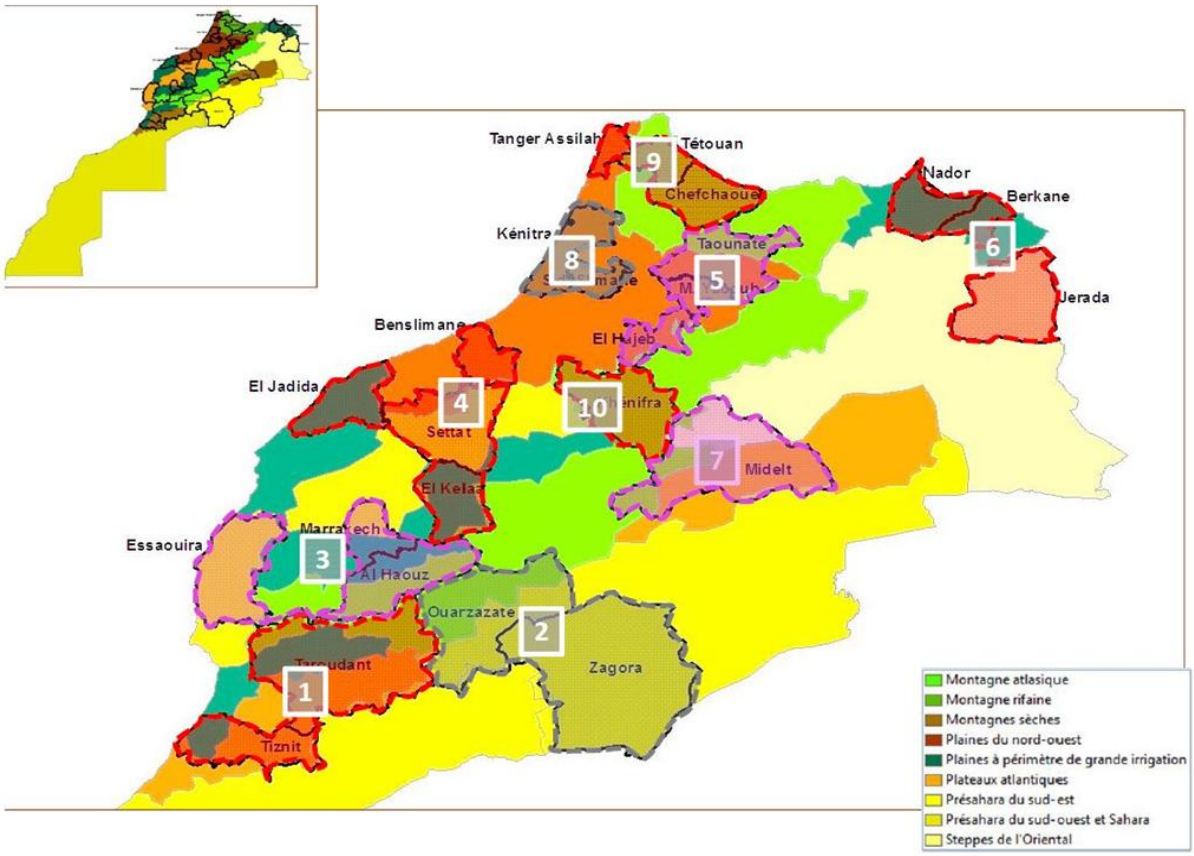


Figure 1. Localization of the 24 veterinary services selected to sample, using a stratified sampling strategy, all agro-ecological zones of Morocco. Numbers represent the order with which the sampling is temporally organized (see text for details).
1: Tiznit-Taroudant, 2: Ouarzazate-Zagora, 3: Marrakech-Essaouira-Al Haouz, 4: Benslimane-Settat-El Jadida-El Kelâa, 5: Elhajeb-MoulayYacoub-Taounate-, 6: Nador-Berkane-Jerada, 7: Midelt, 8: Kénitra-Sidi Slimane, 9: Tanger-Assilah-Tétouan-Chefchaouen

b. Culicoides *collections*

We gathered the raw data from the longitudinal monitoring carried out during the 1990s (Baylis et al., 1997) and during 2009-2010 (Lhor et al., 2005). These data were analyzed to determine the period of abundance peaks for the most common *Culicoides* species per climatic zone. This aimed to define the optimal period of the year for population sampling in each of the visited areas, i.e. the period when the abundance of the most common *Culicoides* species is expected to be at its highest, assuming that diversity and population dynamics are homogeneous within these spatial units. The timing of collection was planned according to this analysis.

We collected *Culicoides* midges using UV-light/suction traps manufactured by the Onderstepoort Veterinary Institute (Venter et al., 1998; Goffredo & Meiswinkel, 2004). The traps were placed near to animals and were operated from dusk to dawn for two consecutive nights in order to limit the impact of adverse weather conditions. Every site was sampled twice a year. The collected individuals were transferred to 70% ethanol.

c. *Identification of the collected individuals:*

When possible, collected individuals were morphologically identified at species level under a stereomiscroscope using the keys of Delécolle, (1985) and Mathieu et al. (2012). Additional molecular assays were carried out in case of morphologically close species (*C. obsoletus*, *C. scoticus* or *C. montanus*) or of difficult morphological identifications (damaged specimen or uncertain diagnostic features). Genomic DNA extraction was individually extracted using the NucleoSpin® Tissue DNA Kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instruction. COI amplification and sequencing using primers were produced following the procedure described by Bakhoum et al. (2018). The obtained sequences were BLASTED on Genebank.

3. Environmental data

To identify the environmental factors driving the *Culicoides* species communities, we gathered a wide range of variables related to climate, topography, soil, land use and vertebrate host. All of these categories are known to affect *Culicoides* abundance or distribution (Baylis et al., 1998, Baylis and Rawlings, 1998, Baylis et al. 1999, Tatem et al., 2003; Conte et al., 2007, Acevedo et al., 2010, Rigot et al., 2012, Ducheyne et al., 2013, Ippoliti et al. 2013).

Bioclimatic variables were obtained from the WorldClim database with a spatial resolution of from 30 seconds (~1 km^2) (http://www.worldclim.org/version2). These bioclimatic variables values were derived from monthly temperature and precipitation and represented annual trends (average of annual temperature, annual precipitation), seasonality (range of temperature and annual precipitation) and extreme climatic characteristics. Topography variables were obtained from the EarthEnv project (https://www.earthenv.org/topography). These included the altitude, the slope, and the roughness (aggregated by minimum, maximum, standard deviation, mean and median), variables assessing the topography heterogeneity (Terrain Ruggedness Index, the roughness, the Topographic Position Index and the Vector Ruggedness Measure), and finally categorical topographic variables (aggregated using Shannon and uniformity indices). Soil data were extracted from the Harmonized World Soil Database (http://webarchive.iiasa.ac.at/Research/LUC/External-World-soil-database/). We considered only data in the topsoil (0-30 cm), which include variables characterizing the texture, the structure, the chemical, the exchange and hydric capacities. Livestock density data were obtained from the global distribution of livestock maps produced by the FAO (http://www.fao.org/ag/againfo/resources/en/glw/GLW_dens.html). Finally, land use data were extracted from the EarthEnv project (https://www.earthenv.org/landcover). These data provide consensus information on the prevalence of 12 land-cover classes at 1-km resolution (Tuanmu and Jetz, 2014).

For datasets related to climate, topography and host densities, which included several variables, we first carried out correlation matrices to select variables and limiting collinearity. For the climatic dataset, a principal component analysis was carried out to decrease the number of variables. Then, a clustering procedure was applied using the CLARA (CLustering LARge Applications) algorithm aiming to obtain homogeneous classes of environmental factors. The number of classes was selected based on the PBM index (Bandyopadhyay et al., 2004). Moreover, we used the HWSD classification of soil and the dominant class of land use to aggregate soil and land use datasets.

All data layers were clipped on the Moroccan territory and projected in the same projection system with a spatial resolution of 1 km2 using R 3.6.0 statistical language environment (R coreteam, 2017) using of the R-package: raster (Hijmans, 2017), rgdal (Bivand et al, 2016), cluster (Maechler et al., 2019) and clusterCrit (Desgraupes, 2018).

4. Statistical analyses

Statistical analyses were performed using the R software (R. Core Team, 2017) and additional packages: *vegan* (Jari et al, 2007)*, ade4* (Dray and Dufour, 2007), *tdisplay* which includes the *vtest* function (Chemobay et al., 2005) and *ComplexHeatmap* (Gu, 2016).

a. Overview of species communities

The *Culicoides* species communities were first described plotting graphs of log abundance on rank and the species accumulation curves (*specaccum* function). Observed and extrapolated (*specpool* function using the Chao equation) species richness were calculated together with the evenness indices (*diversity* function).

We explored the structure of *Culicoides* species communities using a principal component analysis (PCA, dudi.pca function) performed on the centered and reduced abundance data. We considered only sites for which at least 900 individuals were collected, and species for which the total abundance was more than 100 individuals. A hierarchical clustering analysis (HCA, *hclust* function), using the Ward minimum variance method (Ward, 1963), on the Bray-Curtis distance (Bray & Curtis, 1957), was carried out on the PCA coordinates. This aimed to obtain groups of homogeneous diversity. Partition was described using heat maps (*Heatmap* function) and test values (vtest function). The test values are measurements of the distance between the within-class value and the overall value (Lebart et al., 2000). For continuous variables, test values compare the mean of X in the group k and the mean of X in the whole population, whereas for qualitative variable, test values compare the proportion of the population with the category j in a group k with the proportion of the population with the category j in the whole population.

b. Impact of environmental variables on the Culicoides *species community*

We assessed how classes of environmental factors (related to climate, topography, soil, host density and land use) affected the composition of *Culicoides* species community using a between-class analysis (*bca* function) on the species community PCA. Between-class analysis

is a particular case of PCA, where only variability between groups is optimized. This allows estimating the percentage of PCA inertia explained by the categorical variable. Then, the composition of *Culicoides* species in each class of these ecological drivers was described using test values on the log-transformed *Culicoides* abundances. Finally, a global overview of the results was given using a heat map (*Heatmap* function) carried out on the test values.

Results

1. Entomological collections

Among the 162 initially selected farms, 144 were sampled at least once, and 135 twice (Figure 2). The analysis of raw data from Baylis et al. (1997) and Lhor et al. (2015) highlighted bimodal *Culicoides* seasonality in most climatic zones, with highest abundance in spring and autumn – the month of the peak being related to the temperature. Therefore, collections were organized from late April to June with a south-northern gradient and from September to October with the opposite gradient (Figure 1). Farms located in high-altitude areas were visited in early July and late August- (Figure 1).

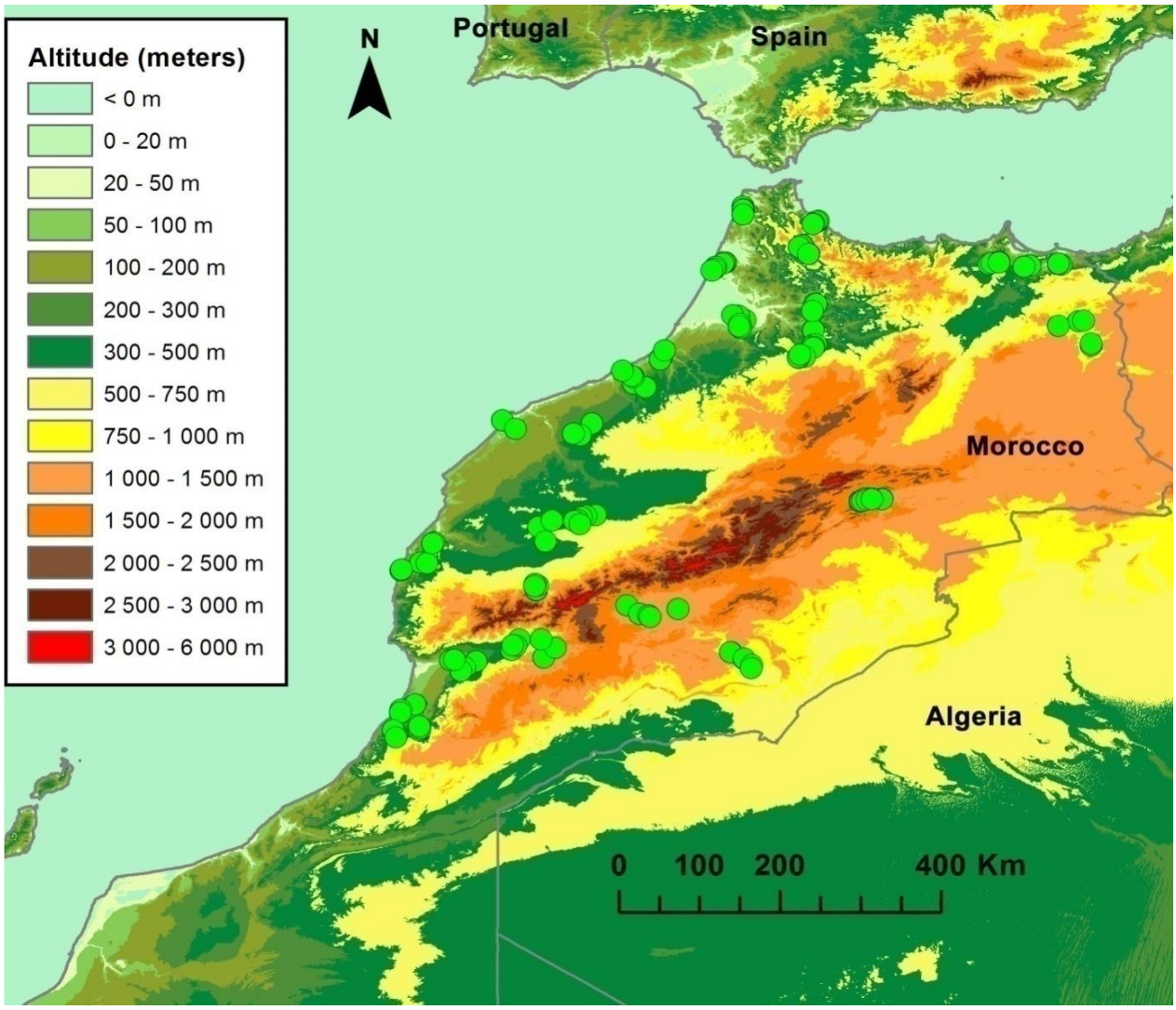


Figure 2. Localization of the 144 sites sampled for *Culicoides* abundance from April to October 2017 using UV-light/suction traps. The traps were placed near to animals and were operated from dusk to dawn for 2 consecutive nights.

2. Environmental classifications

The procedure leading to the environmental classifications was detailed in the supplementary material I. Briefly, the climate was described using 6 classes. These classes separated: (1) the Atlantic and Mediterranean coastal plains, (2) the high altitude plateaus, (3) the Atlas high altitude areas, (4) the arid areas influenced by the Atlas Mountains, and (5), (6) (7) the Saharan regions according to a gradient of ocean influence. The topography was described using 6 classes: classes 1 and 2 are the classes from low to medium altitude, and from low slope (class 2) to medium slope (class 1). Class 2 represents the Saharan zone, and the Atlantic coastal plains. Class 1 is mainly represented in the Rif, and in the margins of the Atlas Mountains. Classes 5 and 6 are plateaus (low slopes) of medium (class 5) or high (class

6) altitude. Class 5 includes the transition areas between the plains and landforms of the Atlas, and Class 6 includes the Oriental and the plateaus between the Anti and the High Atlas or between the High and the Middle Atlas. Finally, classes 3 and 4 are grouped into high (class 3) to very high (class 4) altitudes and slopes. They correspond to the High Atlas Mountains and the summits of the Anti-Atlas, the Middle Atlas and the Rif). Following the HWSD classification, we obtained 12 different types of topsoil in Morocco: 1. Leptosols (very shallow soil located on hard rocks or very calcareous materials), 2. Arenosols (unconsolidated sand deposits), 3. Calcisols (soil with a high accumulation of secondary calcium carbonate, widespread in arid and semi-arid environmental areas), 4. Fluvisols (genetically young soil in alluvial deposits), 5. Phaeozems (a dark soil with a high base status, but without secondary carbonates at one metre from the soil surface.), 6. Kastanozems (continental climate soil with accumulations of calcium carbonate or calcium sulphate at depth within 1.25 m of the surface), 7. Luvisols (leached soil, white and poor in clay on the surface and dark rich in clay at depth), 8. Regosols (very little evolved soil that constitutes the regs. It is a slightly altered loose bedrock.), 9. Vertisols (clay-rich soil containing a layer of aluminum oxide enclosed by two layers of silica tetrahedrons), 10. Planosols (soil with a light-coloured, coarse-textured, surface horizon that shows signs of periodic water stagnation and abruptly overlies a dense, slowly permeable subsoil with significantly more clay than the surface horizon.), 11. Cambisols (soil with a beginning of soil formation and weak differentiation evidenced from weak, mostly brownish discolouration and/or structure formation in the soil profile.), 12. Solonchaks (soil of high salinity, formed when evaporation is higher than rainfall inputs, under the influence of an outcropping groundwater table - permanent or seasonal.

Four different classes of host density were defined: (1) the coastal plains, which was the dominant class for all animals except goats, (2) the arc of the Atlas Mountains, where animal densities were average, and dominant for goats, and (3) two classes for pre-Saharan and Saharan regions, where animal density was low. Finally, the land use classification was obtained by computing the dominant class per pixel (evergreen/deciduous trees (class_01), evergreen broodleaf trees (class_02), deciduous broodleaf trees (class_03), mixed/other trees (class_04), shrubs (class_05), herbaceous vegetation (class_06), cultivated and managed vegetation (class_07), regularly flooded vegetation (class_08), urban/built up (class_09), snow/ice (class_10), Darren (class_11), open water (class_12). The dominant classes in Morocco were barren, cultivated vegetation and shrubs.

3. *Culicoides* diversity

A total of 217,031 *Culicoides* individuals belonging to at least 30 different species were collected during the survey, including 199,748 (92.0%) females and 17,283 (8.0%) males (Table 1). From April to October 2017, the most abundant species were *C. imicola* (51.6% of the collected individuals), *Culicoides saevus/Culicoides langeroni* (20.4%), *Culicoides kingi* (12.6%), *Culicoides newsteadi* (5.0%). Altogether, these species represented 89.6% of the collected individuals (Table 1). July was the month with the most individuals collected, while during this month traps were set in only one region (Khénifra-Beni Mellal). The number of *Culicoides* collected in period 1 and in period 2 were quite similar. Among the 10 most abundant species, the only difference was observed for *C. kingi*, which was more abundant during the second (autumn) than the first period (spring).

Molecular identification was performed on 282 individuals. A total of 201 females morphologically identified as *C. obsoletus*/*C. scoticus* were COI sequenced allowing the identification of 33 *C. obsoletus*, 38 *C. scoticus* and 80 *C. montanus*. 50 individuals were not identified at the species level and were kept as *C. obsoletus*/*C. scoticus*. We also were able to confirm the identification of *Culicoides langeroni, Culicoides shaklawensis, Culicoides subfagineus, Culicoides jumineri* and *Culicoides subfascipennis.*

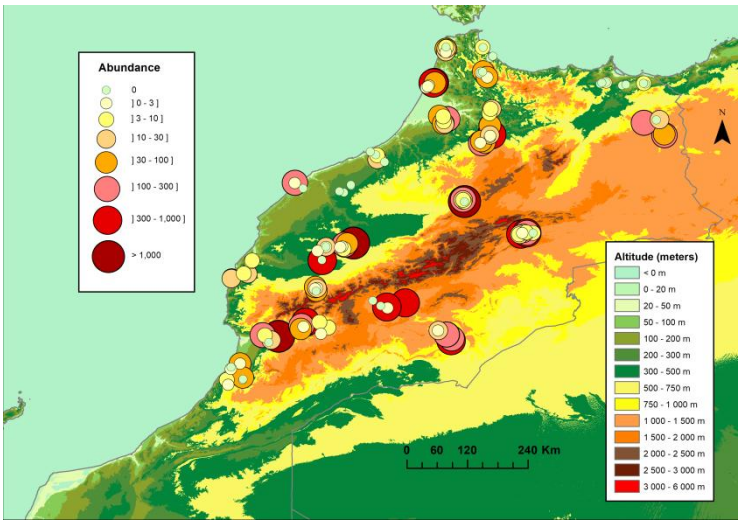
Table 1: Total number, global abundance, rank and occurrence of *Culicoides* collected, using UV-light/suction traps (OIV type), from April to October 2017, in 144 sites in Morocco.

Species	No. Individuals	No. Females	No. Males	Rank	Percentage	Cumulative percentage	Occurrence
Culicoides imicola	112,157	102,735	9,422	1	51.678	51.678	92.361
Culicoides saevus/Culicoides langeroni	44,442	42,032	2,410	2	20.477	72.155	79.167
Culicoides kingi	27,480	25,700	1,780	3	12.662	84.817	77.083
Culicoides newsteadi	10,884	10,649	235	4	5.015	89.832	55.556
Culicoides paolae	4,940	2,586	2,354	5	2.276	92.108	62.5
Culicoides obsoletus/scoticus	4,650	4,622	28	6	2.143	94.251	32.639
Culicoides circumscriptus	4,560	4,110	450	7	2.101	96.352	72.222
Culicoides kurensis	2,012	1,984	28	8	0.927	97.279	31.944
Culicoides puncticollis	1,896	1,654	242	9	0.874	98.152	45.139
Culicoides cataneii/gejgelensis	1,266	1,035	231	10	0.583	98.736	52.778
Culicoides sp.	729	728	1	11	0.336	99.072	22.917
Group heteroclitus/odiatus/picturatus/pseudopallidus	702	689	13	12	0.323	99.395	18.75
Culicoides parroti	384	361	23	13	0.177	99.572	14.583
Culicoides pulicaris	218	215	3	14	0.1	99.672	9.722
Culicoides sahariensis	154	111	43	15	0.071	99.743	14.583
Culicoides lupicaris	140	140	0	16	0.065	99.808	2.083
Culicoides fagineus	113	113	0	17	0.052	99.86	2.778
Culicoides punctatus	83	79	4	18	0.038	99.898	9.722
Culicoides subfagineus	60	58	2	19	0.028	99.926	4.861
Culicoides longipennis	53	49	4	20	0.024	99.95	6.25
Culicoides begueti	50	40	10	21	0.023	99.973	0.694
Culicoides achrayi	22	22	0	22	0.01	99.983	2.778
Culicoides festivipennis	20	20	0	23	0.009	99.993	1.389
Culicoides azerbajdzhanicus	6	6	0	24	0.003	99.995	0.694
Culicoides jumineri	5	5	0	25	0.002	99.998	2.083

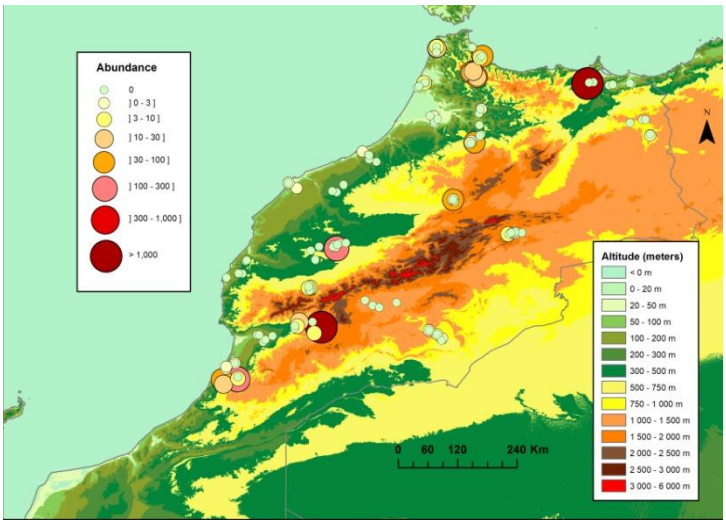
Culicoides subfasciipennis	3	3	0	26	0.001	99.999	1.389
Culicoides haranti	1	1	0	27	0	100	0.694
Culicoides shaklawensis	1	1	0	28	0	100	0.694

4. Distribution of the most abundant *Culicoides* species

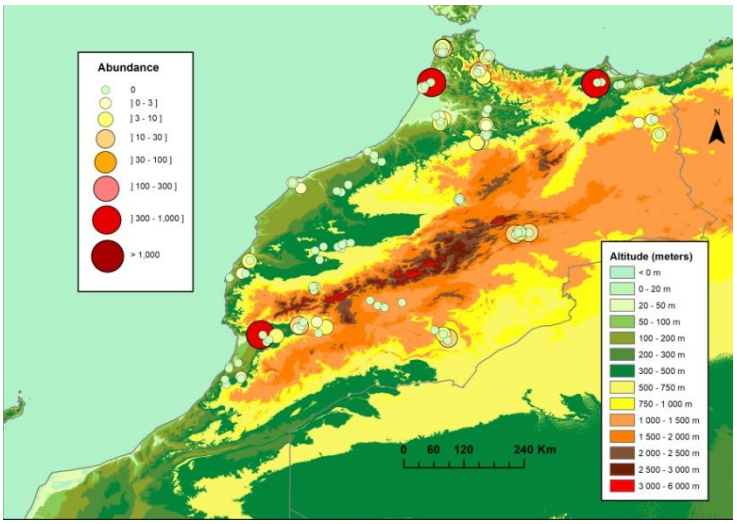
Culicoides imicola was widespread as found in most of the sites. This species was present in low density in the coastal plains, with the exception of El Jadida. It was abundant in the High Atlas, around the Marrakesh region, and found in the Souss valley (Taroudant), at the junction of the Middle Atlas and Eastern High Atlas ranges at an altitude of more than 1,500 m (Midelt), and in the Eastern Rif at an altitude of more than 1,500 m (Jerada-Oujda) (Figure 3). *Culicoides kingi* was rarer. It was limited to the eastern plains (Nador), to the Souss valley (Taroudant) and around the Marrakesh region (High Atlas) (Figure 3). *Culicoides saevus/ C. langeroni*, were found in the Atlantic coastal plains (Tangier), in the Souss valley (Taroudant) and along the eastern Mediterranean coast (Nador) (Figure 3). *Culicoides kurensis* was largely distributed, absent only around Settat (Casablanca region) (Figure 3). *Culicoides obsoletus/C. scoticus* were collected in the Atlantic coastal plains (Tangier) and in the High Atlas, around the Marrakech region (Figure 3). *Culicoides paolae* was collected at several locations, including the Oriental (Oujda and Berkane), the Rif and Atlas Mountains (at more than 1,000 m around Ouarzazate), the Draa Valley (Zagora) and in the Souss Plain (Taroudant) (Figure 3). *Culicoides newsteadi* was present in the southern High Atlas Mountains at more than 1,000m altitude (Ouarzazate), along the Mediterranean coast (Nador) and around Casablanca (Settat) (Figure 3). Finally, *Culicoides puncticollis* was limited to the Rabat region (Sidi Slimane) (Figure 3).



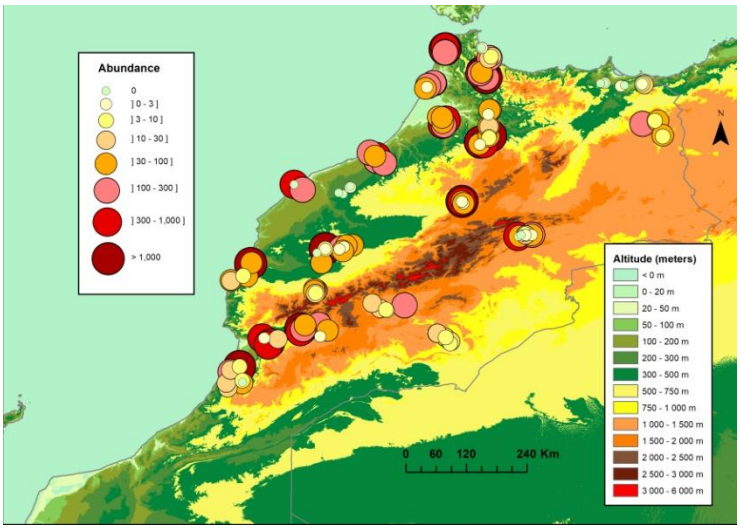
Culicoides imicola



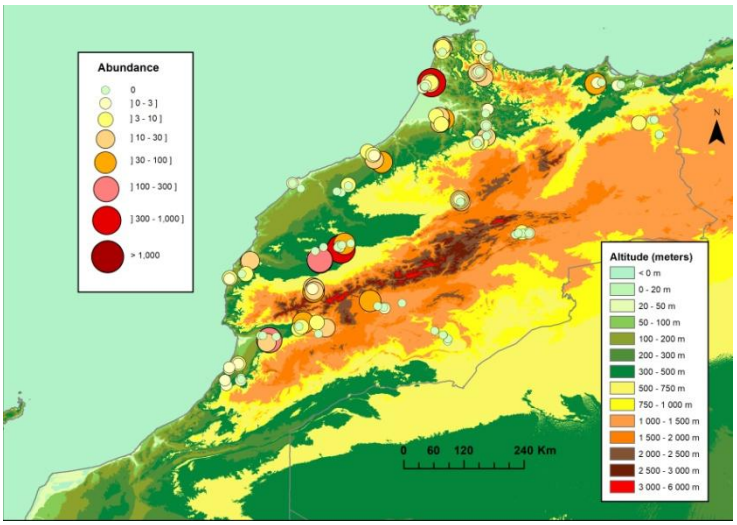
Culicoides kingi



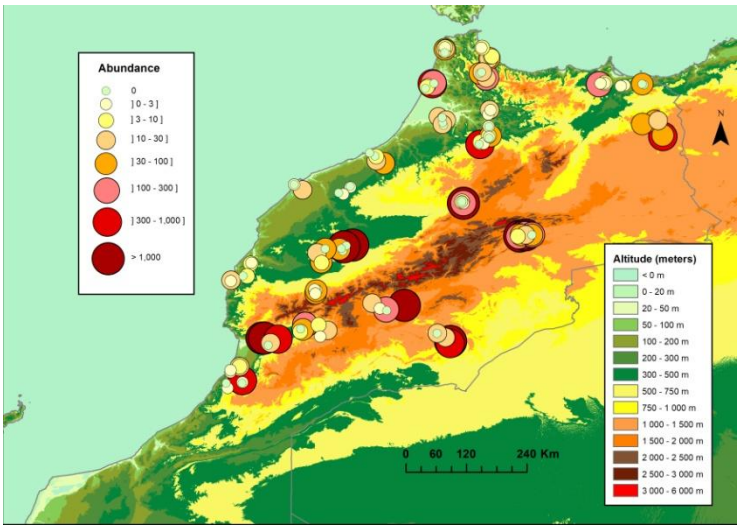
Culicoides saevus langeroni



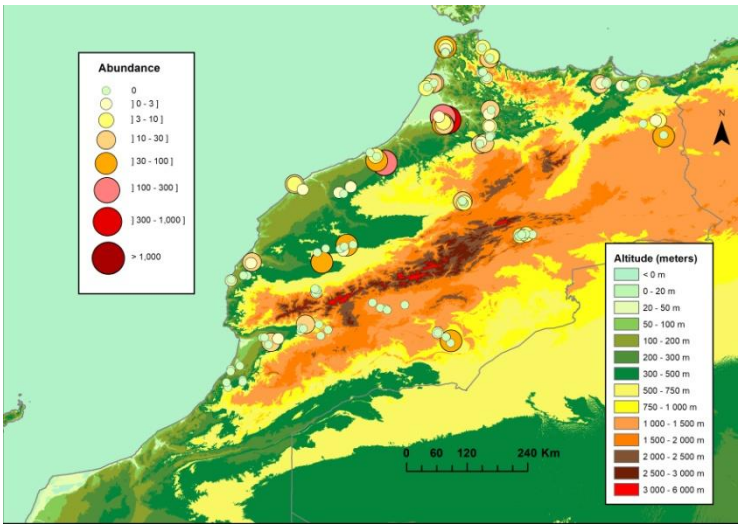
Culicoides kurensis



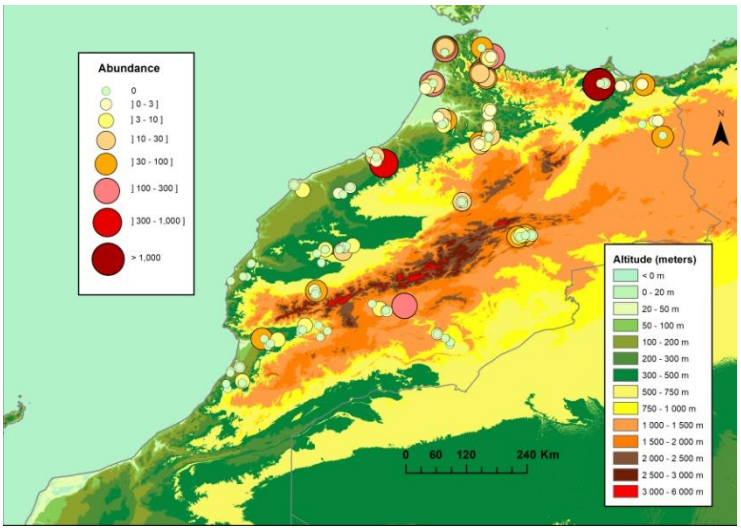
Culicoides obsoletus/Culicoides scoticus



Culicoides paolae



Culicoides puncticollis



Culicoides newsteadi

Figure 3. Spatial distribution of the 8 most abundant *Culicoides* species collected from April to October 2017, using OVI light traps.

5. *Culicoides* species communities and environmental drivers

The *Culicoides* species communities fitted the log-normal model (Figure 4A. The inventory seemed to be comprehensive (Figure 4B). The Shannon index (1.61) and the Evenness index (0.49) suggested a balanced species community with high specific diversity. However, the abundances of the three most abundant species were higher than predicted by the log-normal model (Figure 4A).

The predicted no. species ranged from 10 to 45 depending on the climate class, from 22 to 28 depending on the topography class, from 12 to 30 depending on the soil type, from 16 to 28 depending on the host class and from 18 to 38 depending on the land use dominant class (Table 2).

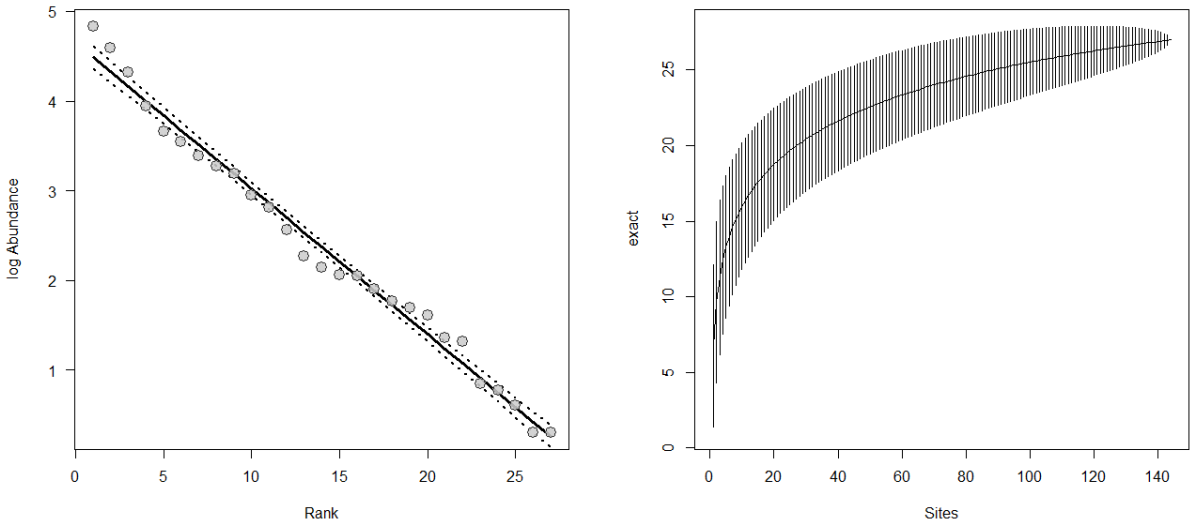


Figure 4A: graph of log abundance on rank. Figure 4B: accumulation curve of *Culicoides* species.

Table 2: observed and expected species richness in the different classes of environment. Collections were carried out in 144 sites in Morocco from April to October 2017 using UV-light suction traps (OVI model).

Environment	**Classes**	**No. observed species**	**No. predicted species (Chao)**
Climate	I	24	24.65
	II	18	19.11
	III	17	18.41
	IV	17	21.35
	V	17	47.00
	VI	10	10.41
Topography	I	18	21.55
	II	24	27.93
	V	21	24.91
	VI	18	21.82
Soil	I	15	20.64

	III	21	29.79
	IV	11	13.02
	VI	17	18.90
	VII	23	23.08
	VIII	10	12.25
	IX	13	18.33
	X	13	13.83
	XII	9	12.37
Host density	I	18	27.64
	II	23	23.65
	III	22	25.91
	IV	10	16.00
Land use	IV	15	18.37
	V	17	19.14
	VII	25	28.95
	XI	20	37.50

The figure 6 gave the outputs of the PCA carried out on the log-transformed abundance of the 10 most abundant species. The cumulative inertia was 51.8% on the first 2 axes and 75.1% on the first 4 axes. The first axis (31.8% of inertia) opposed *C. saevus/C. langeroni* and *C. kingi*, with the rest of the species. The second axis (20.0% of inertia) was marked by a size effect due to mainly *C. newsteadi* and secondary to *C. saevus/C. langeroni* and *C. imicola*. The third axis opposed mainly *C. kingi* and *C. circumscriptus* with *C. obsoletus/C. scoticus* and *C. newsteadi*, while the fourth axis opposed *C. imicola* with *C. puncticollis*.

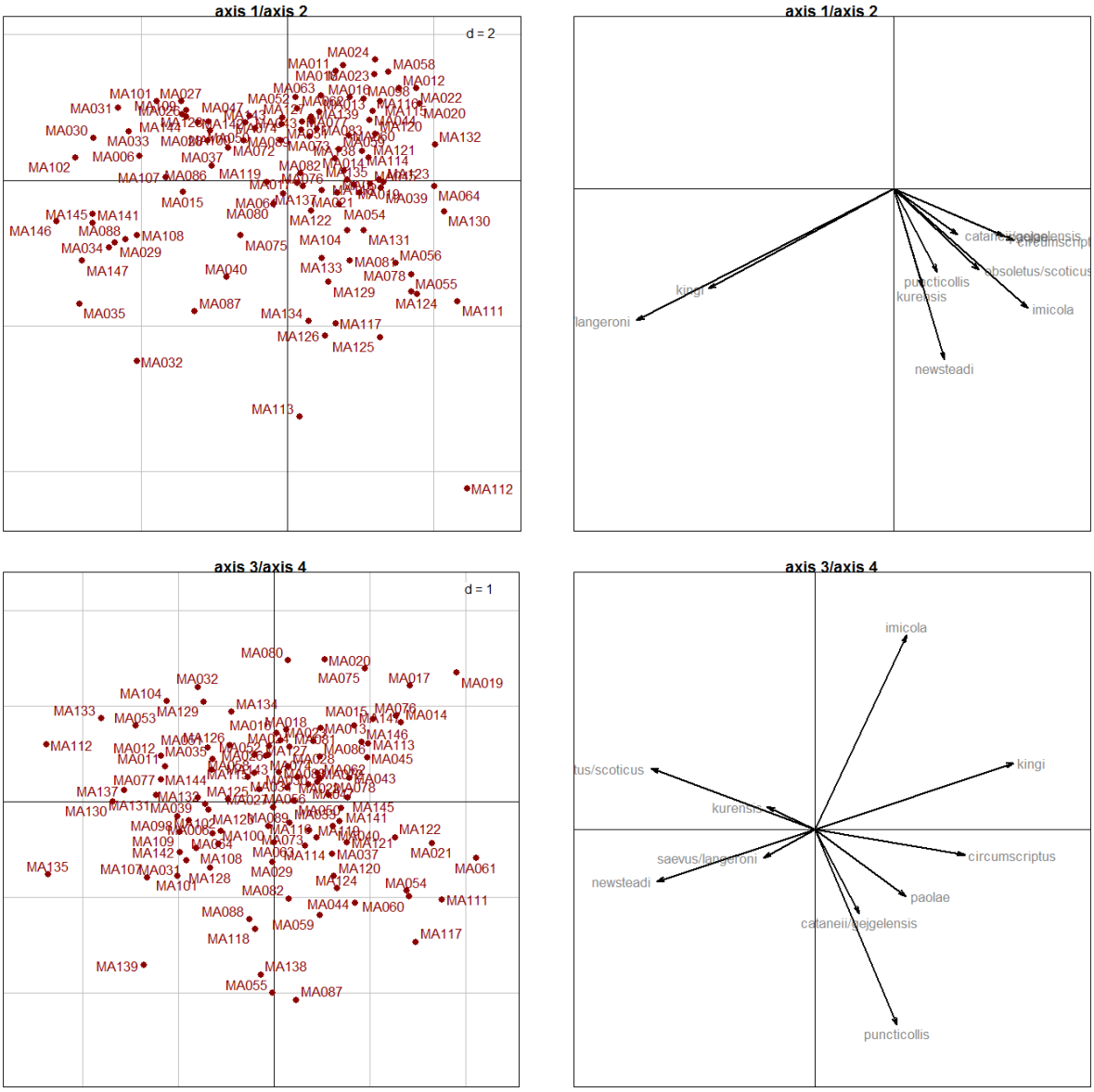


Figure 6: Principal component analysis carried out of the centered and reduced log-transformed data of abundance.

The clustering of the PCA coordinates highlighted 5 diversity clusters (Figure 7).

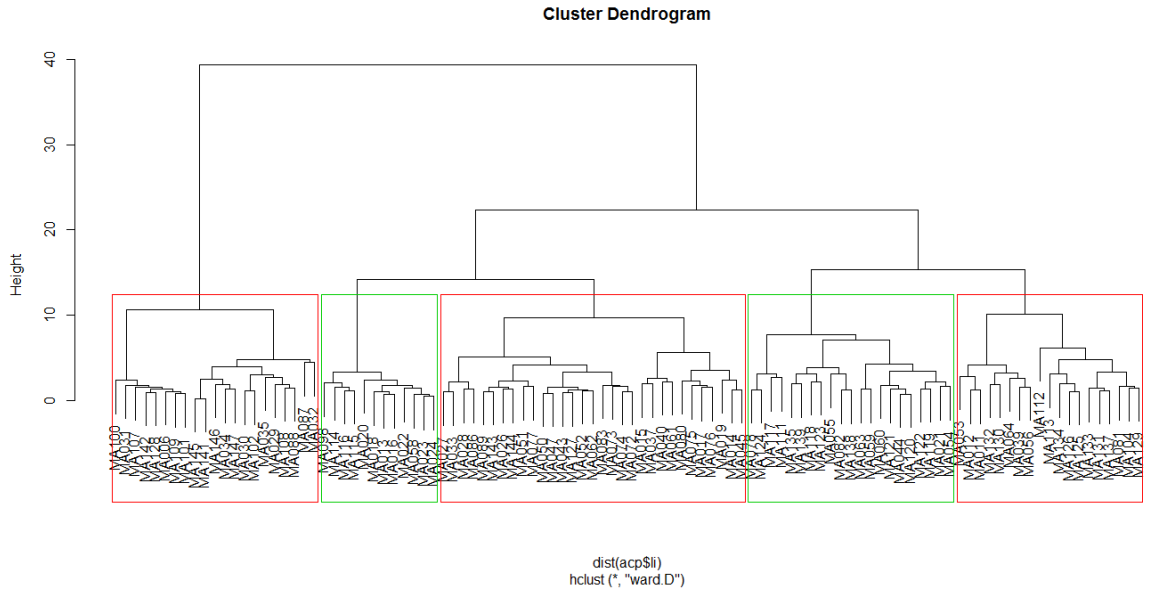


Figure 7: Hierarchical ascendant classification of the 144 sites, where *Culicoides* collections were carried out from April to October 2017. Ward method was applied on the Bray-Curtis distance between the PCA coordinates.

We used a test value to highlight the diversity characteristics of these clusters (Figure 8).

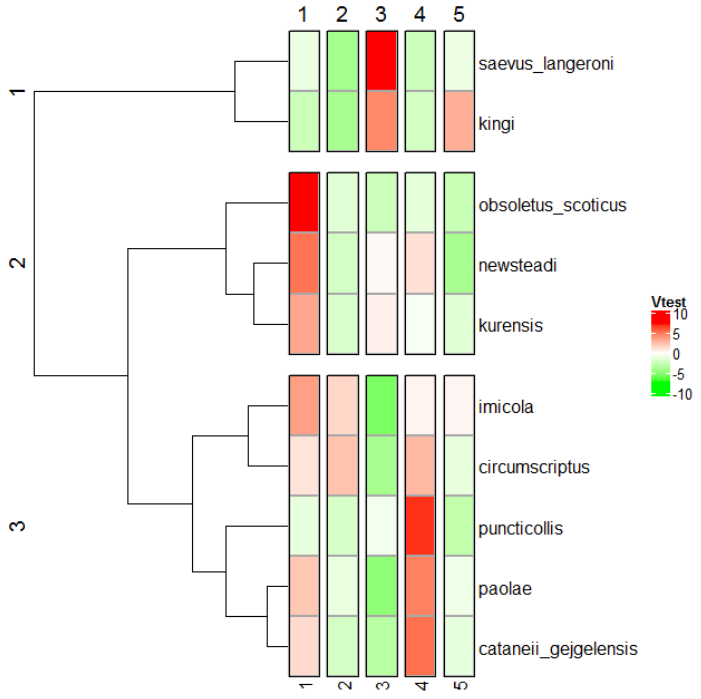


Figure 8: Typology of diversity groups described using a test value

The cluster I was characterized by high abundances of *C. obsoletus/C. scoticus*, *C. newsteadi*, *C. kurensis* and *C. imicola*, and the cluster II by presence of *C. imicola* and *C. circumscriptus*. The cluster III grouped the sites where *C. kingi* and *C. saevus/C. langeroni* were abundant,

whereas the cluster IV is characterized by high abundances of *C. puncticollis*, *C. cataenii/C. gejgelensis* and *C. paolae* and the cluster V by abundant collections of *C. kingi*.

The distribution of the diversity clusters (Figure 9) highlighted a consistent spatial structure. The cluster I is dominant in the coastal plains, the cluster II along the Mediterranean and Atlantic coasts, the cluster III in the eastern Rif and in the Atlas Mountains, the cluster IV in the coastal plains and in the High Atlas and the cluster V in the eastern Rif, in the Oriental, in the Draa valley, in the Souss plains, and in the costal Atlantic plains.

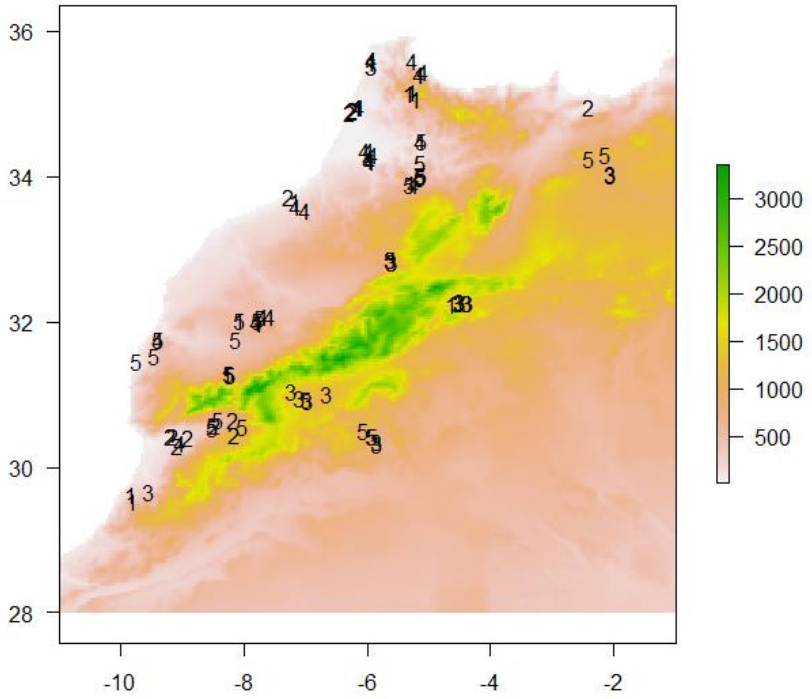


Figure 9: Mapping of the classification of the 144 sampled sites grouped into 5 diversity clusters

Crossing the *Culicoides* abundances with the ecological classes highlighted three clusters of *Culicoides* species (Figure 10). The cluster I included *C. paolae, C. circumscriptus, C. puncticollis and C. imicola*. It was dominant in humid to subhumid regions, in regions with low to medium altitude and low slope and with a soil rich in clay and high salinity, where goat, and secondary poultry, farming was dominant. The cluster II included several species, i.e. *C. sahariensis*, *C. pulicaris*, *C. obsoletus/C scoticus*, *C. kurensis*, the Hopp

group, *C. lupicaris*, *C. fagineus*, *C. newteadi*, and *C. catanneii/C gejgelensis*. It was dominant in humid to subhumid regions, in regions with low to medium altitude and medium slope, with washed soil, with mixed trees area, where goat, and secondary poultry, farming was dominant. The cluster III included *C. kingi*, *C. saevus/C. langeroni* and *C. parroti*. It was dominant in arid or even Saharan areas – *C. kingi* may be abundant in per-arid areas where almost all the months are dry, in regions of medium to high plateaus, with shallow soil located on hard rocks and where animal densities may be high.

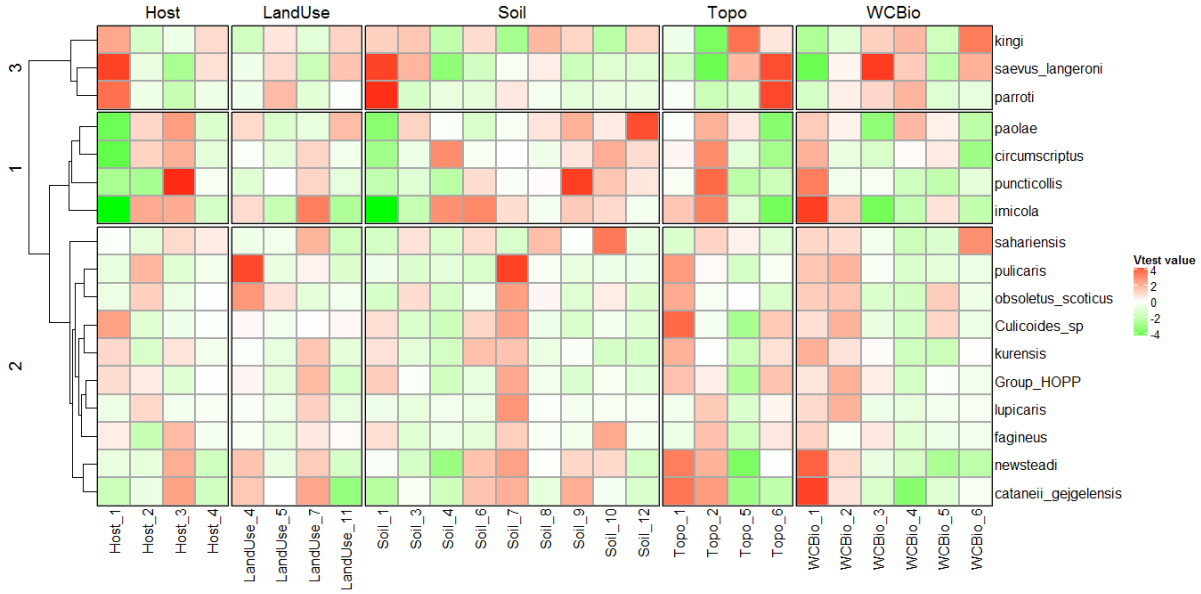


Figure 10. Description of the *Culicoides* species communities by ecological drivers, *i.e.* host, land use, soil, topography and climatic classes, using a heat map of the test values (measure of the distance between the within group and the overall values). The test values ranged from -4 to 4.

Discussion

Culicoides collections carried out at a national scale confirmed the presence of at least 30 *Culicoides* species, among which several were proven or probable vectors of arboviruses of veterinary interest: *C. imicola*, *C. obsoletus*, *C. scoticus, C. montanus*, *C. newsteadi* and *C. paolae*. *Culicoides montanus* was not properly described as a probable BTV or AHSV vector. However, we included in that list considering the difficulties to separate *C. montanus* from *C. obsoletus*. Two new species for the Moroccan fauna were identified: *Culicoides achrayi* and *Culicoides haranti*, which breeds in tree holes. The afrotropical species *Culicoides imicola* was by far the dominant species. This dominance matches previous reports in Morocco (Baylis et al., 1997; Lhor et al., 2015). This species was known to be dominant in countries under Mediterranean climate, such as South Africa, where *C. imicola* comprises more than 90% of the collections made near livestock (Meiswinkel et al., 2004) or Tunisia (Slama et al, 2015). *Culicoides imicola* remains abundant in other African countries, but in lower proportions (below 30%) in Namibia (Liebenberg et al, 2016).

The *Culicoides* collections highlighted a spatial pattern of species composition. We confirmed the classical differences in the *C. imicola* versus *C. obsoletus* distribution. Indeed, *C. imicola* seems perfectly adapted to Mediterranean climate, where *C. obsoletus* is present in its edges of distribution. We confirmed the known pattern, such as observed in Sardinia, where *C. imicola* is dominant in the coastal areas at lowers attitude, whereas *C. obsoletus* is restricted to more mountainous central areas (Slama et al., 2015). *Culicoides circumscriptus* was abundant in coastal plains. This species is known to breed mainly in brackish coastal marshes (Balenghien and Garros, 2017). *Culicoides kingi* was found in the arid zone of the Rif. This species has a large distribution covering the Sahel (up to northern Kenya) and the Maghreb (Mathieu et al., 2011; Bakhoum et al., 2013). The species *C. saevus* and *C. langeroni* were grouped together as they were difficult to separate due to the absence of wing spots. However, deep morphological investigations associated with molecular analyses confirmed the presence of both species in that group. *Culicoides saevus*, as a Palearctic species, has a wider distribution than *C. langeroni*, as a Mediterranean species (Mathieu et al., 2011). The distribution of this group of species was characterized by high abundances in the coastal plains and in the arid areas of the Rif.

Collection sites were grouped into five clusters of *Culicoides* diversity highlighting different ecological drivers. The cluster I was dominant in humid to subhumid regions, in regions with

low to medium altitude and low slope and with a soil rich in clay and high salinity. This cluster dominant in regions where extensive farming, especially of goats, is dominant, with frequent poultry farms. The cluster included 4 characteristic species, including *C. paolae*, *C. circumscriptus*, *C. puncticollis* and *C. imicola*. *Culicoides paolae* was described for the first time from southern Italy. It was suggested to feed mainly on horse (Boorman et al., 1996), although its antenal and palpal morphology have led other authors to speculate that it feeds preferentially in birds (Meiswinkel et al., 2004). This species is a potential vector of avian haemosporidians in arid ecosystems (Veiga et al, 2018). The *C. paolae* female lays her eggs on decaying prickly pear trees (Meiswinkel et al., 2004). The soil characteristics of this cluster, *i.e.* rich in clay with high salinity, are favorable to the development of this plant. *Culicoides circumscriptus* is also a potential vector of avian haemosporidians in arid ecosystems (Veiga et al, 2018). This species was known to breed in a wide range of habitats, including salt marshes (Zimmer et al., 2014). The abundance of *C. imicola* was described to be influenced by host density (especially horses) (Meiswinkel et al., 2004). Soil moisture can have an impact on the survival rate of the larval stages (Meiswinkel, 1998; Meiswinkel and Paweska, 2003). Indeed its larvae develop in moist clay mud rich in nutrients exposed to sunlight or in moist or water saturated soils rich in organic matter (Braverman et *al.*, 1974). In South Africa, *C. imicola* was observed to be absent from sandy coastal areas despite a mild prevailing climate (including adequate rainfall) and a large resident animal biomass (Meiswinkel, 1997). The absence of this species has been linked causally to soils with a sandy texture, which are known to have depleted moisture levels (especially in the surface layer) and to lack vital nutrients. Conte et al. (2007) evidenced that retentive soils was the most important factor for distinguishing between positive and negative *C. imicola* sites in the region of Calabria in Italy. The high abundances of *C. imicola* mirrored the situation in South Africa, where this species becomes highly abundant in areas with clay soils, especially following periods of continuous rain (Meiswinkel et 1998).

The cluster II shared the same climatic, topographic and host classes than cluster I, but differed in soil and land use. Indeed, it was in washed soils covered by mixed trees areas. This cluster was the most diverse and grouped: *C. sahariensis, C. pulicaris, C. obsoletus/C scoticus, C. kurensis*, a morphogroup including a mix of *C. heteroclitus*, *C. odiatus*, *C. pseudopallidus* and *C. picturatus* (Hopp group), *C. lupicaris, C. fagineus, C. newteadi* and *C. catanneii/C gejgelensis. Culicoides newsteadi* is known to be found near substrates rich in water-saturated organic matter (Braverman et al., 1974). Blood meal

analyses of *C. newsteadi* in a recent study (Slama et al., 2015), showed that this species is able to feed on poultry. *Culicoides cataneii* and *C. gejgelensis* larvae are regularly found in rivers or pond edges in wet meadows (Balenghien and Garros, 2017). *Culicoides sahariensis* Kieffer larvae are described in sludge sampled along the banks without vegetation. Wet litter in many boreal forests appears to be classical larval habitats for *C. pulicaris and Culicoides lupicaris* Downes & Kettle (Mirzaeva et al., 1976). It is interesting to mention that *C. circumscriptus* was described to share the same biotope with *C. puncticollis, C. cataneii and C. sahariensis* in other Mediterranean regions (Slama et al, 2015: Foxi, 2010). In this study, the environmental factors that determine the distribution of these species are not the same. *Culicoides obsoletus* and *C. scoticus* are considered ubiquitous as their larvae were found in various habitats, such as forest litter, tree holes, corn silage residues or composting manure (Balenghien and Garros, 2017). De Liberato et al. (2010) found that the abundance of the the Obsoletus 'group' was favored largely by partially or completely wooded land cover. The broader distribution of this group of species demonstrates its tolerance of a wider range of temperature, altitude and terrain slope. Contrary to *C. imicola*, the *C. obsoletus/C. scoticus* species can be found in both flat and steeply sloping terrain and at altitudes in excess of 2,000 m. The predominance of these species in areas with an elevated NDVI range indicates ecological preferences for habitats shielded from direct solar radiation. Our analysis suggested that mixed broadleaved/coniferous forests was associated with high abundance of the cluster II and of *C. obsoletus/C. scoticus*. This was coherent with previous observations evidencing that these closely related species breed in forest leaf litter (Amosova, 1956; Jamnback and Wirth, 1963; Dzhafarov, 1964). *Culicoides imicola* and *C. obsoletus/C. scoticus* failed into two different clusters. This pattern was observed in Italy (Conte et al., 2007), in continental Spain (Ortega et al., 1998), in Morocco (Bouayoune et al., 1998), in Portugal (Capela et al., 2003), in the Balearic Islands (Miranda et al., 2004) and in Corsica (Baldet et al., 2004). Both species are indeed commonly found in sympatry, but rarely co-dominated in the same localities.

The cluster III was dominant in arid, or even in per-arid for *C. kingi*, areas of plateaus of average to high altitude, characterized by shallow soil located on hard rocks and where host densities, except goat, were high. This cluster encompassed at least 3 species: *C. kingi, C. saevus/langeroni* and *C. parroti*. *Culicoides parroti* seems able to feed on sheep (Gerry et al. 2009; Mullens et al., 2010). Larvae are found in mud rich in organic matter, near water reservoirs, but also in shallow pools covered with grass (Zimmer et al, 2014). The *C. kingi*

larvae are more likely to be found in lake edges, but also in pond and puddle edges (Bakhoum et al., 2017). This species seems feeding mainly on cattle and secondarily on horses (Bakhoum et al., 2017).

Many distribution models, predicting *Culicoides* abundance, were developed using climatic predictors. It was evidenced that combining climatic variables with host, soil or landscape predictors improved greatly the performances of insect distribution models (Titeux et al., 2008). Calvete et al. (2008) found that incorporating variables related to host into bioclimatic models, predicting the patterns of occurrence of BTV-4 in Spain, vastly increased the variance explained. In Italy, both biotic (forest cover and vegetation activity) and abiotic (topography, temperature and aridity index) factors were found to drive occurrences of dominant populations of the Obsoletus 'group' (Conte et al., 2007). It was emphasized that landscape factors are key determinants to model abundance of species breeding in moist soil or dung with a highly temperature-dependent life cycle (Mellor et al., 2000). Our study aimed to explore the *Culicoides* species composition through Morocco, and to investigate environmental drivers among a large range of ecological factors. This is a first step to develop distribution models with highlighted environmental factors as predictors.

Conclusion

This study aimed to overview the *Culicoides* species communities through Morocco. Collecting insects at a large scale is a real methodological challenge. We used analyses of previously collected data to plan and optimize a national scale campaign able to assess *Culicoides* abundance during a single activity season. Then we investigated the relation between diversity clusters and a wide range of ecological factors to determine the environmental drivers of species composition and abundance. This is a first step to develop accurate distribution models that could be used to model the risk of BTV transmission. Such maps are required by agricultural and veterinary health authorities to target surveillance and control actions and to improve the cost/benefit ratio of these programs.

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Supplementary material: description of the procedure leading to the environmental classifications.

1. Bioclimatic variables

These variables were obtained from the WorldClim database with a spatial resolution of from 30 seconds (~1 km^2) (http://www.worldclim.org/version2). They were derived from monthly temperature and precipitation and represented annual trends (average of annual temperature, annual precipitation), seasonality (range of temperature and annual precipitation) and extreme climatic characteristics.

We compared the 19 bioclimatic variables using correlation matrices to avoid collinearity (Figure S1).

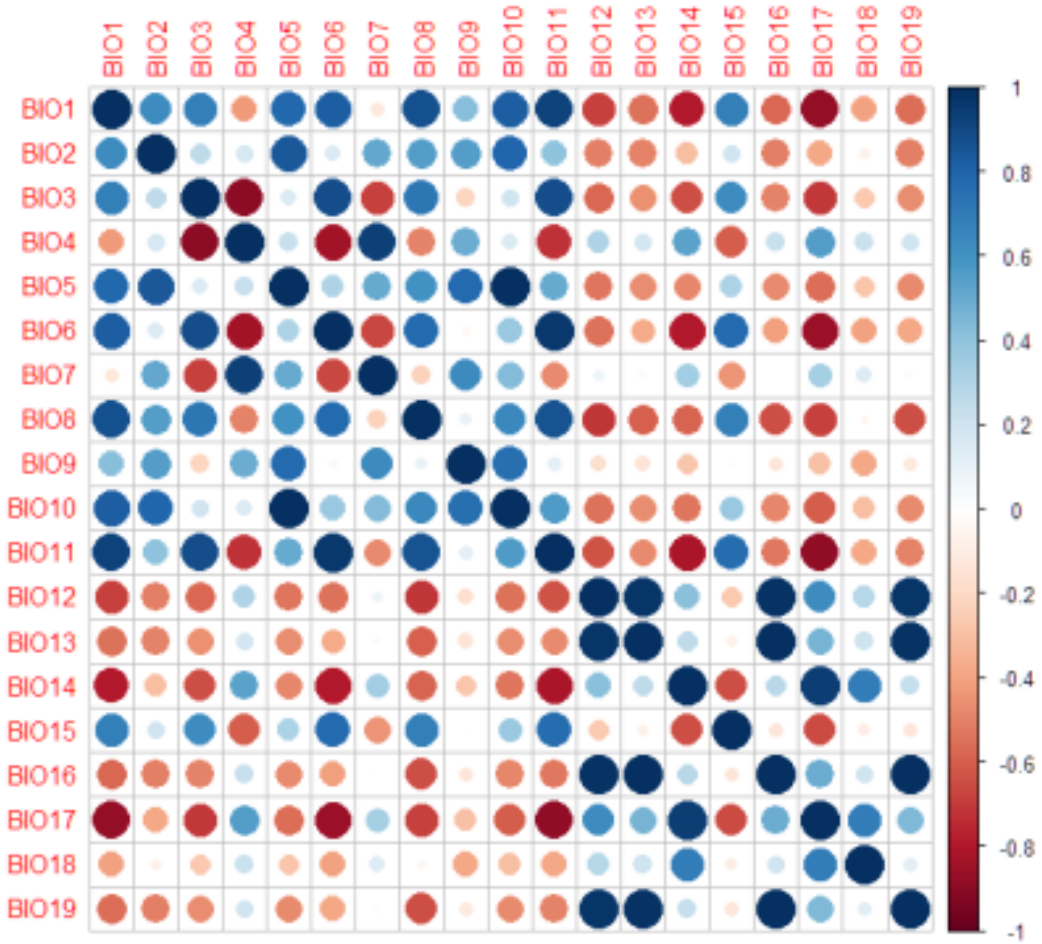

Figure S1: Correlation matrix computed with the 19 bioclimatic variables extracted from Wordclim version 2.

Finally, 10 bioclimatic variables were retained: Mean Diurnal Range (BIO2), Isothermality (BIO3), Temperature Annual Range (BIO7), Mean Temperature of Wettest Quarter (BIO8), Mean Temperature of Driest Quarter (BIO9), Mean Temperature of Warmest Quarter (BIO10), Mean Temperature of Coldest Quarter (BIO11), Annual Precipitation (BIO12), Precipitation Seasonality (BIO15), and Precipition of Driest Quarter (BIO17). A principal component analysis (PCA) was carried out to reduce the dimensions before applying the CLARA (CLustering LARge Applications) classification method. The final classification (Figure S2) encompassed 6 classes (selected using the PBM index).

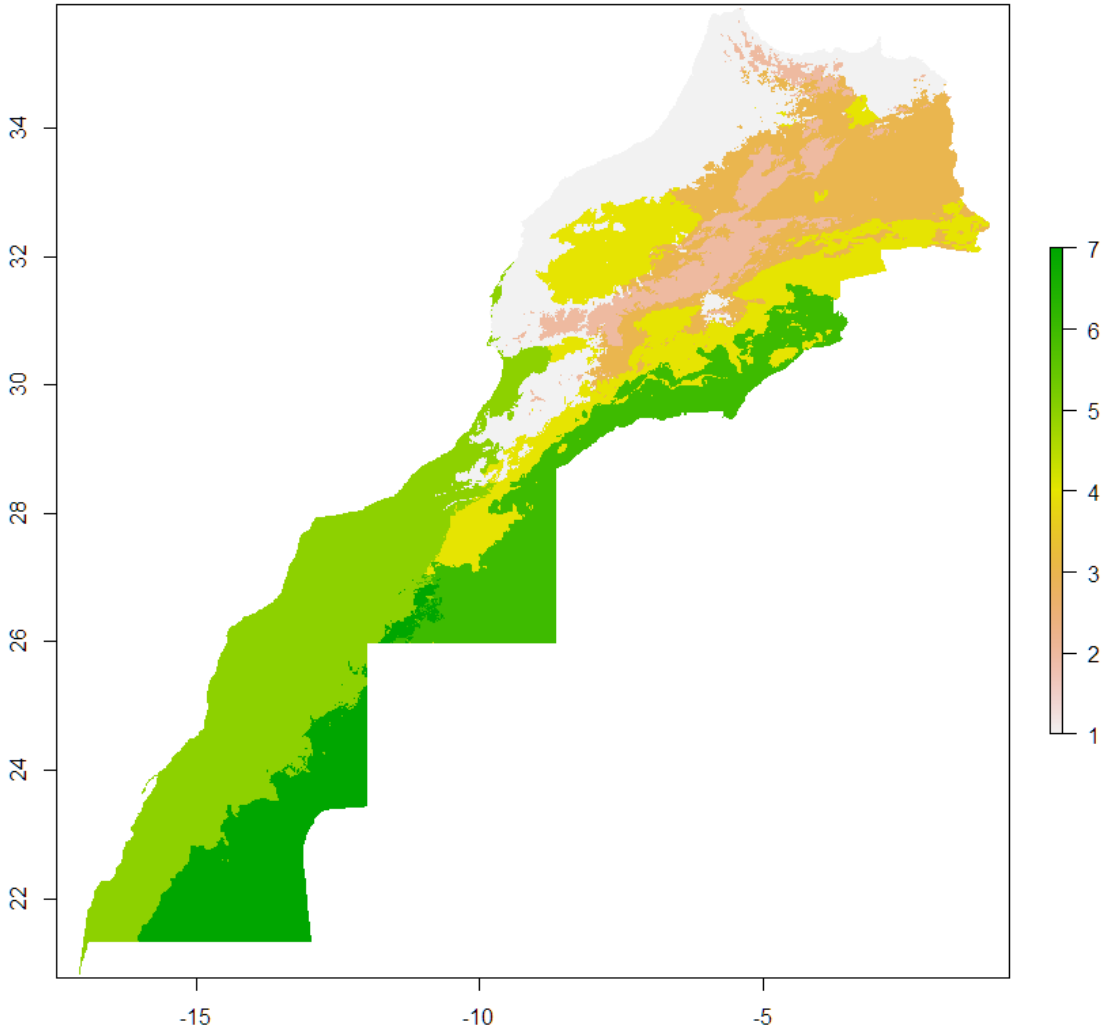

Figure S2: CLARA classification of climatic variables (6 classes)

2. Topography

Topography variables were obtained from the EarthEnv project (https://www.earthenv.org/topography). These included the altitude, the slope, and the

roughness (aggregated by minimum, maximum, standard deviation, mean and median), variables assessing the topography heterogeneity (Terrain Ruggedness Index, the roughness, the Topographic Position Index and the Vector Ruggedness Measure), and finally categorical topographic variables (aggregated using Shannon and uniformity indices).

Correlation matrix highlighted high degree of collinearity between variables (Figure S3).

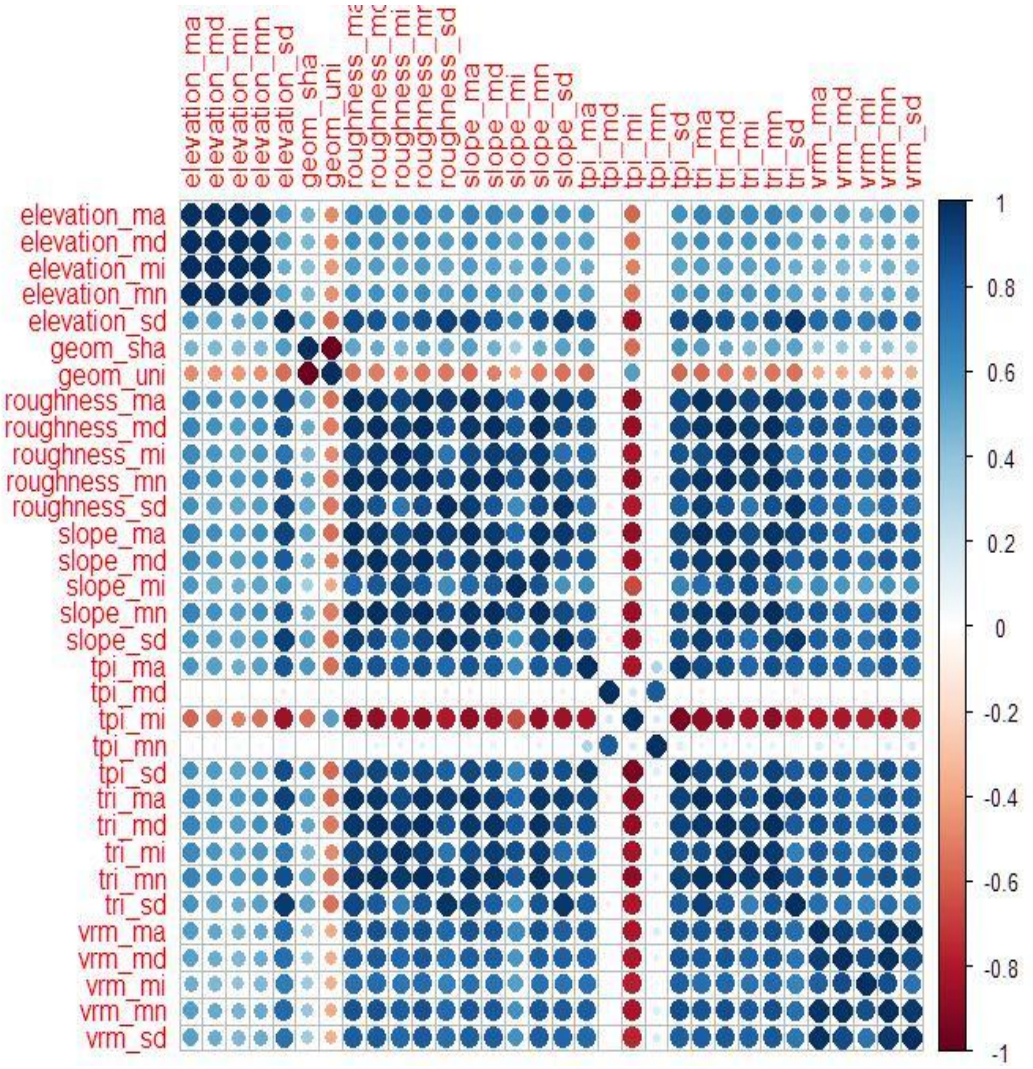

Figure S3: Correlation matrix computed with altitude, slope, and roughness; heterogeneity measurement variables : the Terrain Ruggedness Index (TRI), the Topographic Position Index (TPI) and the Vector Ruggedness Measure (VRM) - with the same aggregations, aggregated by minimum, maximum, standard deviation, mean and median, categorical topographic variables, with the majority geomorphological class (depression, valley, summit, etc.),

aggregation indices of categorical topographic variables: Shannon index and uniformity index.

CLARA classification method was applied on elevation_md and slope_md (Figure S4).

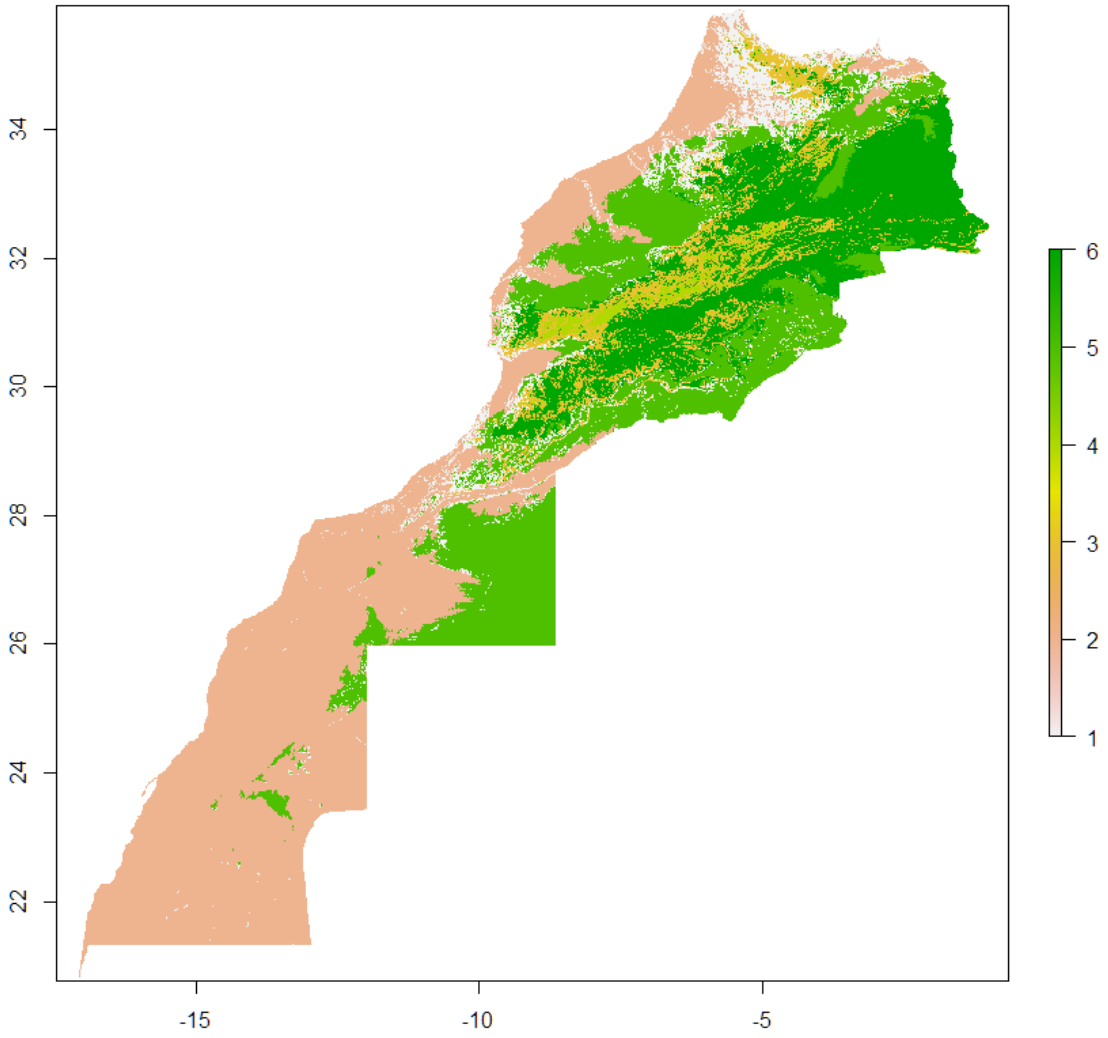

Figure S4: CLARA classification of topographic variables (6 classes)

Classes 1 (Rif region and edges of the Atlas Mountains) and 2 (Saharan regions and Atlantic coastal plains) are from low to medium altitude, and from low slope (class 2) to medium slope (class 1). Classes 3 and 4 are characterized by high (class 3) to very high (class 4) altitudes and slopes, and corresponded to the High Atlas Mountains and the peaks of the Anti-Atlas, the Medium Atlas and the Rif. Classes 5 and 6 are plateaus (low slopes) of medium (class 5)

or high (class 6) altitude. Class 5 included the transition areas between the plains and landforms of the Atlas, and Class 6 includes the Oriental and the plateaus between the Anti and the High Atlas or between the High and the Medium Atlas.

3. Soil

Soil data were extracted from the Harmonized World Soil Database (http://webarchive.iiasa.ac.at/Research/LUC/External-World-soil-database/). We considered only data in the topsoil (0-30 cm), which include variables characterizing the texture, the structure, the chemical, the exchange and hydric capacities.

We explored the collinearity between variables using correlation matrices (Figure S5).

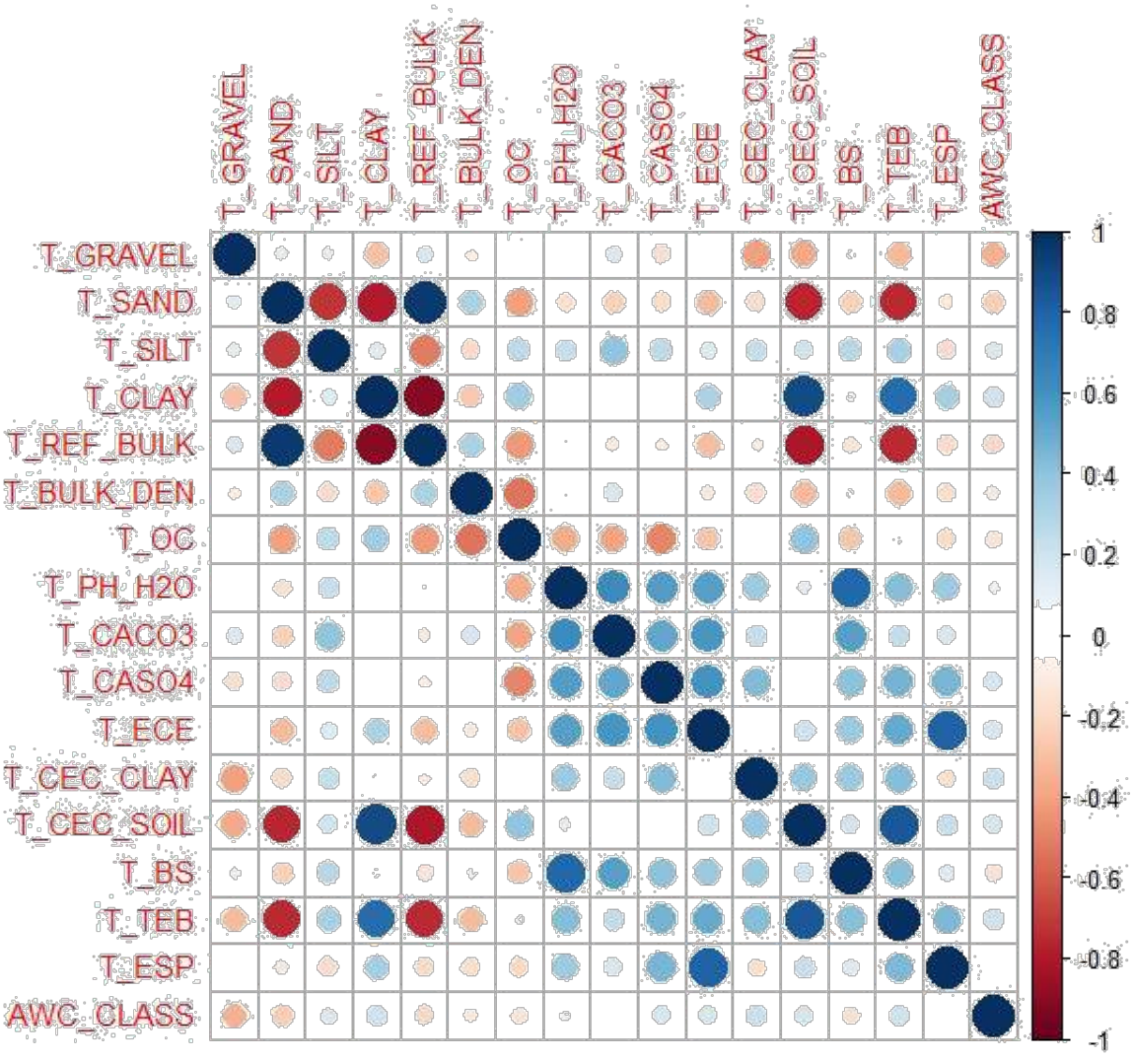

Figure S5: Correlation matrix computed with topsoil gravel content (t_gravel), topsoil silt

fraction (t_silt), topsoil clay fraction (t_clay), topsoil bulk density (t_bulk_den), topsoil pH (H_2O) (t_ph_h20), topsoil organic carbon (t_oc), topsoil Calcium Carbonate (t_caco3), topsoil Gypsum (t_caso4) and topsoil CEC clay.

Finally, we used the HWSD classification of soil (Figure S6).

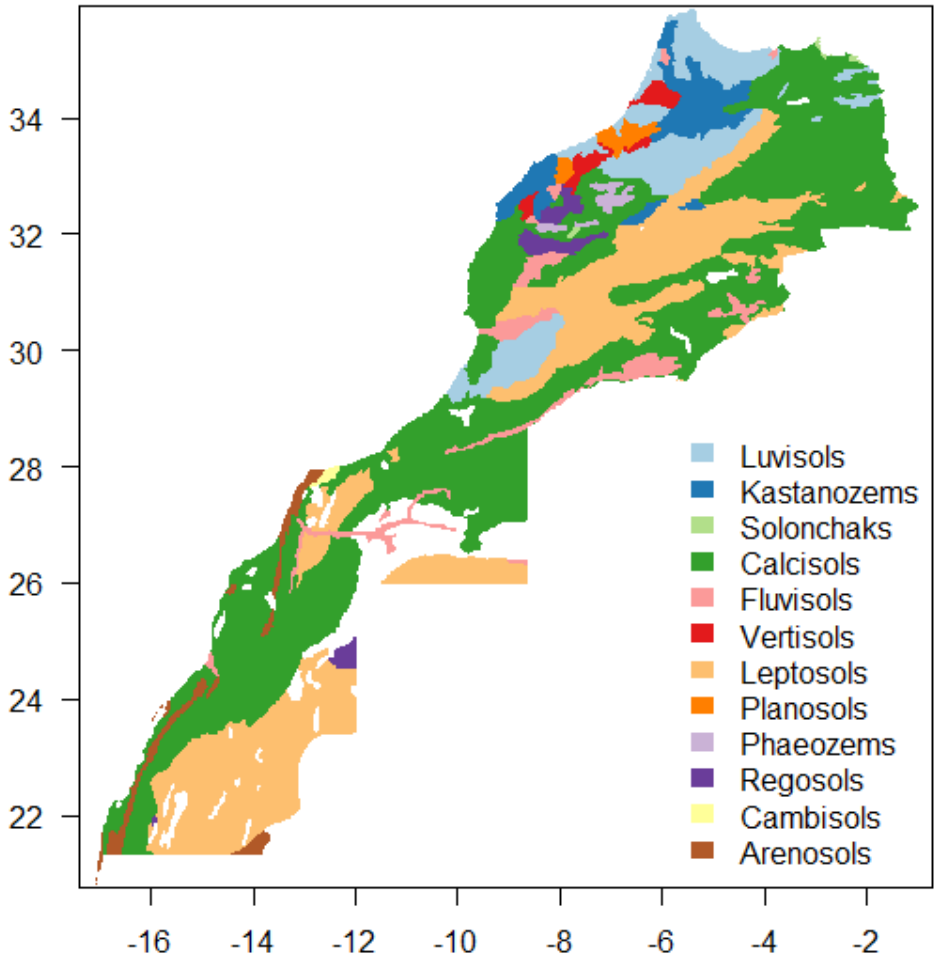

Figure S6: Classification of topsoil using the Harmonized World Soil Database

4. Host density

Livestock density data were obtained from the global distribution of livestock maps produced by the FAO (http://www.fao.org/ag/againfo/resources/en/glw/GLW_dens.html).

Few correlations were observed between host densities (Figure S7).

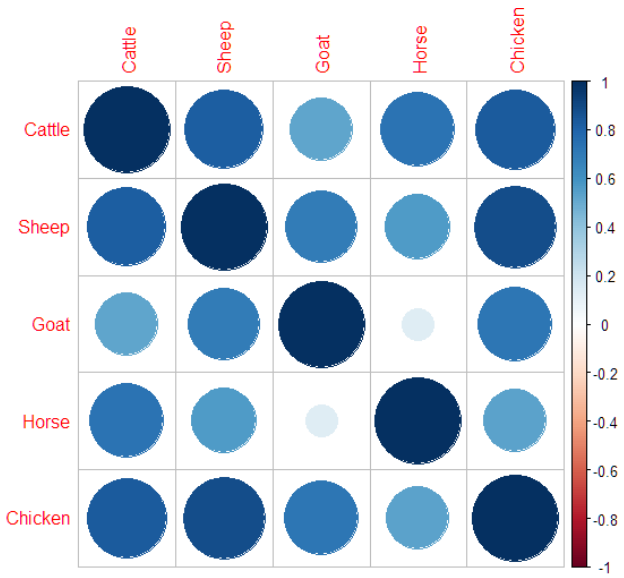

Figure S7: Correlation matrix computed with chicken, horse, cattle, sheep and goat density.

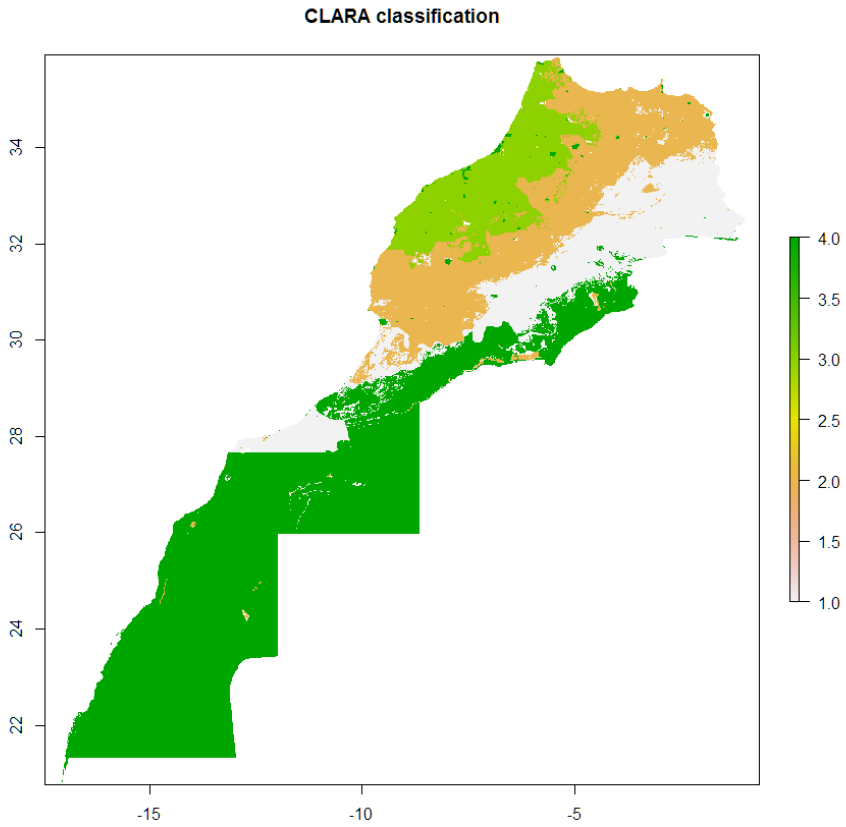

Figure S8: CLARA classification for host density classes

Four different classes of host density were defined (Figure S8): i) the coastal plains, which was the dominant class for all animals except goats, ii) the arc of the Atlas Mountains, where animal densities were average, and dominant for goats, and iii) two classes for pre-Saharan and Saharan regions, where animal density was low.

5. Land use

Land use data were extracted from the EarthEnv project (https://www.earthenv.org/landcover). These data provide consensus information on the prevalence of 12 land-cover classes at 1-km resolution.

Few classes were represented in Morocco (Figure S9).

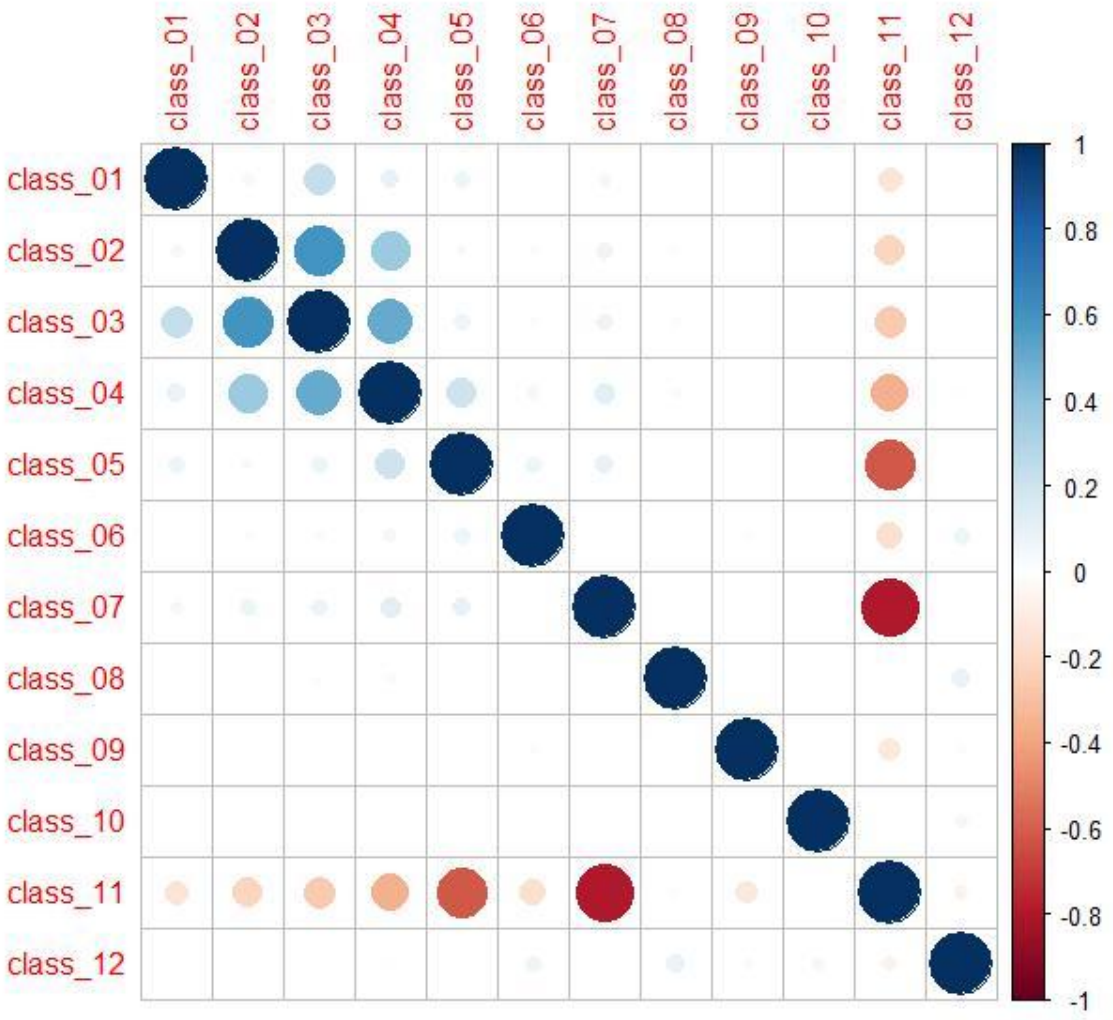

Figure S9: Correlation matrix computed with evergreen/deciduous trees (class_01), evergreen broodleaf trees (class_02), deciduous broodleaf trees (class_03), mixed/other trees (class_04), shrubs (class_05), herbaceous vegetation (class_06), cultivated and managed vegetation (class_07), regularly flooded vegetation (class_08), urban/built up (class_09), snow/ice (class_10), Darren (class_11), open water (class_12).

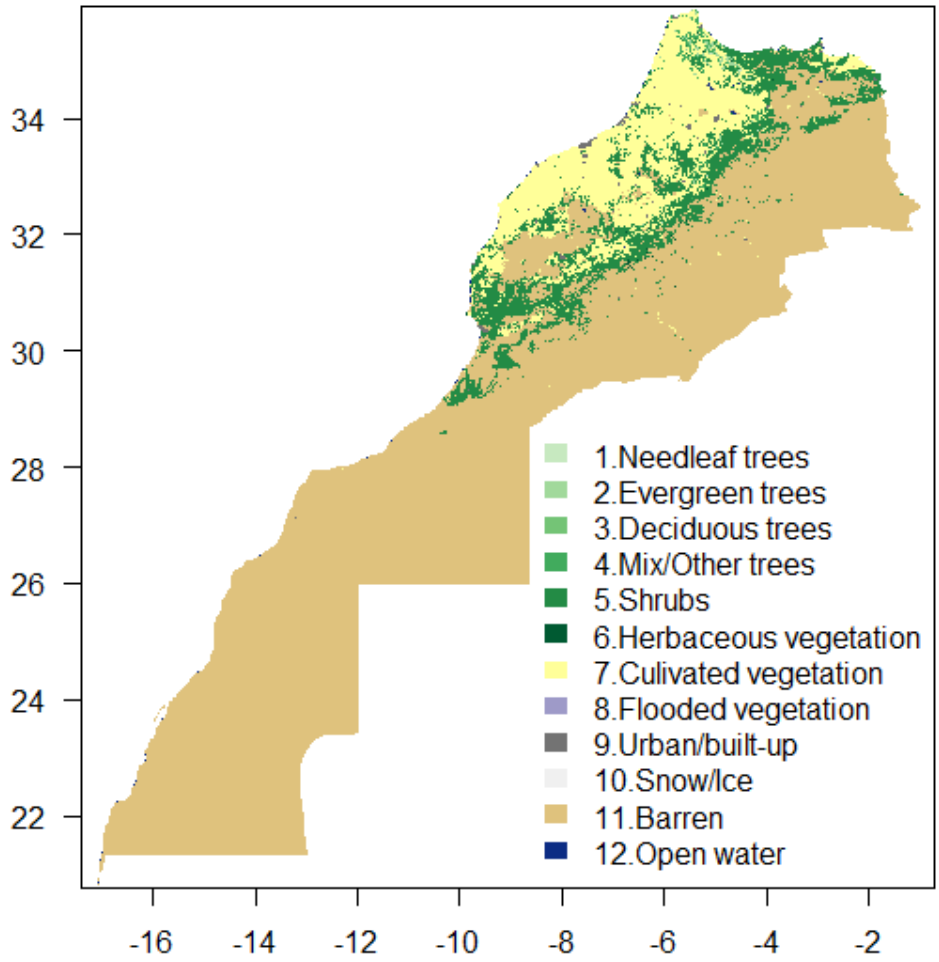

Figure S10: Land use classification using the dominant class per pixel.

Discussion

Les objectifs de cette thèse étaient (i) de réviser la diversité des espèces de *Culicoides* présentes au Maroc, afin de mettre à jour la liste d'espèces (ii) de caractériser la diversité et la saisonnalité des espèces de *Culicoides* d'intérêt vétérinaire dans différents environnements d'élevage (bovin, ovin/caprin, équin) et (iii) de déterminer la distribution spatiale des *Culicoides* à l'échelle nationale, et de caractériser les facteurs environnementaux expliquant cette distribution. L'originalité de ce travail est d'avoir combiner identification morphologique et moléculaire pour valider la liste d'espèces et assurer ainsi une qualité du jeu de données sur lequel repose la caractérisation des paramètres expliquant l'abondance et la saisonnalité. L'ampleur du travail réside dans la qualité et la quantité des identifications faites et la couverture géographique du matériel biologique qui donne aujourd'hui un jeu de données sans précédent pour le pays et la région pour construire des cartes de risque pour les virus transmis par les *Culicoides*.

Pour répondre à ces objectifs, nous avons réalisé une étude bibliographique approfondie des travaux et enquêtes réalisés sur le genre *Culicoides* au Maroc. Nous avons aussi contacté certains auteurs pour obtenir des données non publiées et des informations supplémentaires sur les collectes. En complément, nous avons collecté des spécimens aux alentours de la région de Rabat et nous les avons examinés à l'aide d'observations morphologiques et d'analyses moléculaires d'affiliation d'espèces. Cette étude nous a permis de mettre à jour la liste des espèces de *Culicoides* présentes au Maroc en y incluant deux nouvelles espèces pour la faune du pays **(Chapitre 2, article 1).** Une fois l'inventaire faunistique mis à jour, nous avons caractérisé la saisonnalité des populations de plusieurs espèces dans la région de Rabat, en comparant la diversité et la dynamique des populations dans trois types d'environnement (ovin, caprin, bovin). Ce travail a aussi permis d'identifier les facteurs météorologiques et environnementaux qui déterminent la dynamique saisonnière des *Culicoides* dans cette région (**Chapitre 3, article 2)**. La caractérisation de la saisonnalité est importante d'un point de vue épidémiologique. Enfin, nous nous sommes intéressés à la distribution spatiale des espèces d'intérêt à l'échelle du pays. Pour cela, deux campagnes de captures ont été réalisées à deux saisons, le printemps et l'automne, dans les différentes zones éco-climatiques du pays. En plus de poursuivre le travail de description de la diversité à l'échelle nationale, ce travail décrit et analyse le lien entre certains paramètres environnementaux et la distribution des *Culicoides* avec un intérêt particulier pour les communautés d'espèces **(Chapitre 4, article 3).**

Les principaux résultats de cette thèse mettent à jour, complètent et approfondissent le corpus de connaissances relatif à la diversité et la distribution des *Culicoides* présents au Maroc, travail indispensable pour l'élaboration de stratégies de contrôle et de gestion des populations dans le futur.

Avant le démarrage de ces travaux de thèse, la dernière mise à jour de la liste d'espèces au Maroc avait été réalisée en 2015 (Lhor et al., 2015), avec un dernier inventaire faunistique d'ampleur réalisé dans les années 1970 (Chaker et al., 1979). L'ensemble du matériel biologique collecté à l'époque a été identifié en utilisant des observations morphologiques. La lecture critique de la bibliographie a permis de confirmer et de souligner l'utilisation de 11 noms d'espèces aujourd'hui considérés comme invalides ou tombés en synonymie (Borkent, 2016). Aussi nous avons réalisé des ajustements dans la liste d'espèces publiée. Nous avons retiré de la liste *Culicoides begueti* mentionné par Kremer et al, 1970 et *Culicoides helveticus* reporté par Lhor et al., 2015 et nous avons inclus *C. kingi* dans la liste des espèces valides du Maroc.

Outre ce travail bibliographique, nous avons réalisé des captures à l'échelle régionale (Région de Rabat) et nationale couvrant toutes les zones éco-climatiques du Maroc afin de compléter la liste d'espèces. Cela nous a permis de mettre en évidence de nouvelles espèces pour la faune culicoïdienne marocaine, à savoir *C. subfagineus*, *C. paolae* (confirmés par des outils morphologiques et moléculaires), *C. haranti* et *C. achrayi* (confirmés morphologiquement par un expert). De plus, notre analyse phylogénétique a mis en évidence un clade de 6 séquences non affilié à des séquences de référence. Ce clade apparaît très proche de *Culicoides santonicus.* Deux hypothèses sont avancées pour justifier la non-affiliation de ce clade: (i) il s'agit d'une espèce nouvelle pour la science, (ii) il n'existe pas de séquence de référence disponible dans les bases de données moléculaires que nous avons consultées. Le clade *C. jumineri* montre une variabilité génétique importante, cela nous a conduit à nous interroger sur l'existence d'une diversité cryptique au sein de ce taxon. A ce stade, il est préliminaire d'avancer l'existence d'un nouveau taxon mais des études à venir pourraient s'y intéresser. Beaucoup d'individus, ayant un patron alaire proche, ont été groupés au sein d'un morphogroupe contenant 4 espèces : *C. heteroclitus*, *C. odiatus*, *C. pseudopallidus* et *C. picturatus* (groupe HOPP). Une analyse moléculaire de plusieurs individus appartenant à ce morphogroupe a été réalisée. Malheureusement la comparaison de nos séquences à celles disponibles dans les bases de données moléculaires n'a pas donné de résultats concluants. Nous avons alors gardé ce morphogroupe sans avoir pu déterminer exactement la composition

d’espèces au sein de celui-ci. Les espèces *C. saevus* et *C. langeroni* ont été regroupées car elles étaient difficiles à séparer à l’aide d’un examen rapide sous loupe binoculaire en raison de l'absence de tâches alaires. Cependant, des études morphologiques approfondies associées à des analyses moléculaires ont confirmé la présence des deux espèces au sein de ce morphogroupe.

L’espèce *C. paolae*, mise en évidence pour la première fois au Maroc, est largement répandue dans la région méditerranéenne, et n'était pas considérée jusqu'à récemment comme impliquée dans la transmission du virus de la FCO. Son rôle épidémiologique a cependant été suggéré suite à des travaux récents en Sardaigne (Foxi et al., 2019). En effet, le génome du virus a été détecté par des outils moléculaires dans le thorax d'individus collectés dans cette île. Sans que cela soit une preuve formelle de sa compétence vectorielle pour le virus de la FCO, ce résultat soulève la question d’un rôle possible de cette espèce qui mérite d’être exploré dans un futur proche.

Aujourd’hui, l’inventaire faunistique recense 56 espèces de *Culicoides* pour le territoire du Maroc. Pour des raisons d’éloignement géographique et de niveau faible de production animale, certaines régions ont été peu investiguées et la diversité y reste inconnue ou sous-estimée dans certaines régions. La région saharienne la plus méridionale d'Eddakhla-Oued Eddahab n'a jamais été prospectée à notre connaissance. La région voisine de Laayoune-Sakia El Hamra a, quant à elle, été prospectée, relativement récemment, autour de la seule ville de Laayoune (2009-2010). Le faible nombre d’espèces collectées dans cette région peut s’expliquer notamment par le climat aride (Lhor et al., 2015). Les prochaines campagnes entomologiques devraient cibler ces zones afin de compléter l’inventaire et de mettre à jour les cartes de distribution des espèces. Ceci semble d’autant plus important que ces zones sont très arides et singulières d’un point de vue de leur écosystème et pourraient ainsi présenter des spécificités au niveau de leur faune. A notre connaissance, il n’y a jamais eu d’inventaire de la faune du genre *Culicoides* de l’autre côté de la frontière administrative en Mauritanie.

Nos travaux montrent que la faune marocaine est composée d'espèces ayant une large répartition géographique en région paléarctique, méditerranéenne et afrotropicale, mais aussi d’autres ayant une répartition plus limitée.
La suite logique de ce travail d'inventaire serait de réaliser un outil d'aide à l'identification des *Culicoides* au Maroc, ou préférentiellement pour le Maghreb. Le nombre d’espèces recensées

au Maroc est supérieur à celui des autres pays du Maghreb (Algérie et Tunisie). La plus grande diversité des climats et des paysages peut expliquer cette différence. Sachant la difficulté d'identification du genre *Culicoides* et l'existence d'une faune issue d'au moins deux régions biogéographiques, il semble nécessaire de développer un outil d'aide à l'identification spécifique à destination des entomologistes et des opérateurs de la surveillance entomologique. Il éviterait de devoir consulter les clés existantes, mais pas nécessairement à jour, de chacune des deux régions géographiques. Cet outil pourrait prendre la forme d'une clé d'identification morphologique des stades adultes, d'un atlas de patrons alaires et des caractères diagnostiques ou mieux celui d'un identiciel en ligne comme déjà développé pour la faune de la région paléarctique ouest. Cet outil permettrait d'assurer les identifications en routine, de former plus facilement des étudiants ou des opérateurs et de pérenniser l'expertise du genre pour le pays et plus largement en Afrique du Nord.

Parmi les espèces recensées au cours de ce travail au moins huit d'entre elles sont impliquées dans la transmission d'arbovirus. En effet, *C. imicola* est un vecteur avéré de BTV et d'AHSV et a longtemps été considéré comme le seul vecteur d'importance. La plupart des études épidémiologiques se sont donc concentrées uniquement sur cette espèce. Dans notre travail, nous avons confirmé par analyses moléculaires la présence de *C. obsoletus* et de *C. scoticus* au Maroc, qui sont considérés comme des vecteurs probables de BTV dans la régions paléarctique occidentale. De plus, nous avons signalé la présence de *C. kingi*, vecteur potentiel d'EHDV et de *C. paolae*, qui, avec *C. newsteadi*, *C. pulicaris* et *C. punctatus*, est considéré comme un vecteur potentiel de BTV.

Pour définir le rôle vecteur d'une espèce, on se base généralement sur trois critères : la démonstration d'un contact entre l'hôte vertébré pertinent et l'espèce potentiellement vectrice dans les lieux et en période de transmission, la confirmation de la compétence vectorielle de l'espèce au laboratoire et les détections répétées d'agents infectieux chez des populations collectées sur le terrain, c'est-à-dire dans des conditions naturelles.
Le comportement mammophile de la plupart des espèces de *Culicoides* a été bien décrit ces dernières années dans le monde entier, même si ce caractère biologique peut varier selon la saison et la disponibilité de l'hôte (Viennet, 2011).

La compétence vectorielle, est la capacité intrinsèque et génétique d'une espèce à ingérer, multiplier et transmettre des agents pathogènes (Dye, 1992 ; Lord et al. 1996). Pour les

espèces d'arthropodes d'intérêt médical et vétérinaire, la compétence vectorielle d'une espèce est démontrée par l'expérimentation en laboratoire avec l'ingestion de sang infectieux puis un suivi régulier des titres viraux dans les différentes parties du corps de l'arthropode. Un nombre suffisant de femelles présentant du virus dans la salive est un bon indicateur d'une compétence vectorielle. Les études de compétence vectorielle sur les espèces de *Culicoides* sont rares contrairement à celles sur les Culicidés parce qu'elles sont difficiles à mettre en place (Balenghien et Garros, 2017).

Tout d'abord, le maintien des élevages de *Culicoides* en laboratoire est caractérisé par une forte mortalité lors de l'introduction des stades F0 collectés sur le terrain, par des accouplements inconstants, par des prises de repas sanguins limitées et/ou par un sex-ratio déséquilibré. Seuls deux élevages d'espèces de *Culicoides* ont pu être maintenus artificiellement avec succès dans le monde. Il s'agit de l'espèce néarctique *Culicoides sonorensis* et de l'espèce paléarctique *Culicoides nubeculosus* qui sont toutes les deux de grande taille (Kremer et Lienhart, 1998). Vu la difficulté de maintenir des élevages, certains auteurs ont utilisé des femelles prélevées sur le terrain pour mener des études sur la compétence vectorielle. Cela a été possible dans leur cas grâce à la forte abondance des populations de *Culicoides* autour des lieux d'expérimentation qui a permis de contrebalancer la mortalité dans l'échantillon prélevé (Carpenter et al., 2006 ; Ninio et al., 2010). Ainsi, la principale raison limitant la caractérisation du rôle vecteur des *Culicoides* s'appuie sur des contraintes et des limitations méthodologiques notamment celle d'évaluer le taux de transmission au laboratoire. La plupart des études ayant réussi à évaluer la compétence vectorielle des *Culicoides*, se sont retrouvées à chaque fois face à une compétence faible des espèces étudiées (Carpenter et al. 2006 ; Venter et al., 2006, Carpenter et al. 2008 ;Veronesi et al., 2008, Veronesi et al., 2013) On pourrait poser l'hypothèse que la compétence vectorielle n'est pas un facteur limitant. Les espèces pourraient alors compenser leur faible compétence par leur forte abondance qui est déterminée par différents facteurs écologiques.

Enfin, le niveau d'infection des populations dans des conditions naturelles peut être quantifié. Etant donné que les études sur la compétence des vecteurs sont difficiles à mener pour les *Culicoides*, le niveau naturel d'infection des populations, associé au contact hôte-vecteur, est habituellement utilisé comme une bonne approximation du rôle vecteur.

Les *Culicoides* sont des organismes poïkilothermes et sont par conséquent très sensibles aux conditions environnementales qui régulent la dynamique des populations pouvant entraîner une modification de la dynamique de la transmission des pathogènes (Gubler et al, 2001). La température et les précipitations déterminent probablement le caractère saisonnier de la majorité des espèces de *Culicoides*, certaines peuvent être influencées par d'autres facteurs (Mellor, 2000, Purse et al., 2015). Nous avons voulu caractériser les facteurs qui déterminent la saisonnalité des espèces de *Culicoides* au Maroc. En effet, la détermination des facteurs environnementaux à l'origine de cette saisonnalité est utile non seulement pour mieux comprendre les variations de l'abondance, mais aussi pour être en mesure de la prédire. Deux études ont défini le profil saisonnier des populations de *C. imicola* au Maroc (Baylis et al., 1997 ; Lhor et al., 2015) mais aucune analyse des facteurs climatiques qui influent sur cette saisonnalité n'a été entreprise.

Le profil saisonnier des populations de *C. imicola*, *C. obsoletus* et *C. scoticus* observé à Rabat est celui généralement observé dans le bassin méditerranéen (Venail et al., 2012), avec un pic à la fin de l'été/au début de l'automne pour C. imicola (Baylis et al., 1997 ; Lhor et al., 2015), et un pic principal d'abondance en mai/juin, puis un pic secondaire en octobre pour les espèces du complexe Obsoletus. La dynamique des populations de *C. newsteadi* est aussi bimodale, avec un pic d'abondance principal au printemps comme déjà démontré au Maroc (Lhor et al., 2015). Enfin, les populations de *C. kingi* sont principalement abondantes au mois de novembre dans la région d'étude. Ce profil est différent des dynamiques rapportées dans des régions tropicales. En effet, au Soudan et au Sénégal, cette espèce est plus abondante en juillet/août pendant la saison des pluies, et en avril lorsque les températures sont élevées (El Sinnary et al., 1985 ; Diarra et al., 2014).

La caractérisation des facteurs environnementaux a montré que les précipitations sont corrélées positivement ou négativement, avec des délais temporels variables, à l'abondance de certaines espèces, tandis que la température, l'humidité absolue et l'indice de végétation NDVI n'ont pas été sélectionnés pour nos modèles, contrairement aux précédentes études. Cependant nos résultats ont souligné l'importance de la pression atmosphérique, cette dernière est corrélée négativement à l'abondance de *C. imicola, C. newsteadi, C. circumscriptus, C. kingi et C. obsoletus/C. scoticus* à différentes échelles temporelles. A notre connaissance ce paramètre n'a jamais été utilisé dans des modèles de dynamique de populations de *Culicoides*. Pour autant, la pression atmosphérique est connue depuis longtemps pour avoir un impact sur

les populations d'insectes (Wellington, 1946), en particulier sur le comportement d'accouplement et de phototaxie (Pellegrino et al., 2013 ; Zagvazdina et al., 2015). A ce stade, nous ne pouvons pas dire explicitement que la pression atmosphérique a un réel impact sur l'abondance des populations de *Culicoides* car notre analyse mets en lumière un lien statistique et non un lien de causalité. L'approche la plus classique utilisée pour comprendre les tendances saisonnières des *Culicoides* reste les modèles statistiques corrélatifs (Sanders et al., 2011, Rigot et al., 2012, Brugger et Rubel, 2013, Searle et al., 2014), les modèles mécanistes mathématiques n'étant que très rarement déployés (White et al. 2017) en dehors des modèles de transmission (Gubbins et al. 2008, Græsbøll et al., 2012, O'Farrell et al. 2014). Bien que les modèles statistiques permettent de décrire le lien entre variable à expliquer et facteurs explicatifs, leurs prédictions ne sont souvent précises que dans les gammes de variations des facteurs explicatifs ayant permis la construction du modèle, et ces modèles sont difficilement extrapolables à de nouveaux territoires sans données supplémentaires. Ils donnent peu d'indications sur les mécanismes qui sous-tendent les réponses environnementales des systèmes. Par exemple, Searle et al. (2014) ont établi un lien entre la variabilité intraspécifique de la phénologie des *Culicoides* entre les sites et les années et un certain nombre de facteurs environnementaux, y compris la température, la densité des hôtes et l'habitat larvaire mais le fondement biologique de ces liens était incertain. En outre, il n'est généralement pas possible d'utiliser des modèles statistiques adaptés pour tester des scénarios, tels que l'évaluation des stratégies de contrôle (les modèles statistiques le faisant présupposent souvent une absence de rétroaction explicite entre les efforts de contrôle et la dynamique de la population vectorielle, c'est-à-dire un manque de mécanisme). En revanche, les modèles mécanistes offrent une plus grande souplesse, mais ils sont souvent difficiles à paramétrer et peuvent présenter des difficultés à représenter correctement les tendances saisonnières de l'abondance des vecteurs. Récemment, White et al. (2017) ont établi un nouveau modèle mecanistique adapté aux espèces du groupe Obsoletus. À l'aide de ce modèle, ils ont étudié les effets de la lutte chimique par les insecticides et montrer que les stratégies de lutte axées sur le pic automnal d'abondance chez les adultes ont le plus grand impact en termes de réduction de la population.

Par ailleurs, d'autres facteurs environnementaux pourraient être intéressants à incorporer comme la température ou l'humidité du sol, éléments importants pour la survie des larves de plusieurs espèces. Ce travail pourrait aussi se décliner à d'autres régions du pays pour mieux caractériser la dynamique des espèces réparties sur des climats différents et ainsi améliorer les

modèles de prédiction et renforcer l'hypothèse de causalité des liens entre variables environnementales et abondances.

Après avoir caractérisé les facteurs déterminant la saisonnalité des différentes espèces de *Culicoides* à l'échelle régionale, nous avons voulu caractériser ceux qui déterminent leur distribution spatiale à l'échelle du pays.

Nous avons pu décrire la distribution spatiale de plusieurs espèces. En effet nous avons confirmé les différences entre la distribution de *C. imicola* et celle de *C. obsoletus*. *C. imicola* semble parfaitement adapté au climat méditerranéen, tandis que *C. obsoletus* est présent dans des zones montagneuses. Ceci correspond à ce qui a été rapporté en Sardaigne, où *C. imicola* domine dans les zones côtières à faible altitude, alors que *C. obsoletus* est limité aux zones centrales plus montagneuses (Slama et al., 2015). *Culicoides circumscriptus* était abondant au niveau des plaines côtières. Cette espèce est connue pour présenter ses habitats larvaires principalement dans les marais côtiers saumâtres (Balenghien et Garros, 2017). *Culicoides kingi* a été trouvé dans la zone aride du Rif. Cette espèce a une large répartition couvrant le Sahel (jusqu'au nord du Kenya) et le Maghreb (Mathieu et al., 2011 ; Bakhoum et al., 2013). Les espèces *C. saevus* et *C. langeroni* sont caractérisées par des abondances élevées dans les plaines côtières et dans les zones arides du Rif.

L'analyse des facteurs environnementaux expliquant la distribution des espèces, nous a permis d'obtenir trois groupes ayant les mêmes caractéristiques écologiques. Au sein de chaque groupe, a été classé un certain nombre d'espèces répondant aux critères de celui-ci. Les variables environnementales utilisées étaient le climat, la topographie, le type de sol, la densité d'hôtes et l'utilisation du sol. Nous nous sommes essentiellement basés sur des études antérieures pour sélectionner ces variables qui influent sur la distribution et/ou l'abondance d'espèces (Baylis et al., 1998, Baylis et Rawlings, 1998, Baylis et al. 1999, Tatem et al., 2003; Conte et al., 2007, Acevedo et al., 2010, Rigot et al., 2012, Ducheyne et al., 2013, Ippoliti et al. 2013). Nous avons trouvé que toutes les variables à de degrés différents influent positivement ou négativement sur la distribution et l'abondance des espèces de *Culicoides* au Maroc. D'autres groupes de variables écologiques, comme celles liées à l'hydrologie, n'ont pas été explorés par manque de temps.

Cette étape doit être considérée comme une étape descriptive permettant d'explorer rapidement le lien entre communautés d'espèces et groupes de facteurs environnementaux.

Elle semble néanmoins nécessaire pour inclure, dans de futurs modèles de distribution d'espèces, l'ensemble des facteurs d'intérêt. En effet, de nombreux modèles de distribution, prédisant l'abondance des *Culicoides*, ont été élaborés à l'aide de prédicteurs climatiques. Il a cependant été démontré que la combinaison de variables climatiques avec des variables liées à l'hôte, au sol ou au paysage, améliorait grandement les performances des modèles de distribution des insectes (Titeux et al., 2008). Calvete et al. (2008) ont constaté que l'intégration de variables liées à l'hôte dans les modèles bioclimatiques prédisant les schémas d'occurrence du BTV-4 en Espagne, augmentait considérablement la variance expliquée. En Italie, une combinaison de facteurs biotiques (couvert forestier et activité de la végétation) et abiotiques (topographie, température et indice d'aridité) semblent déterminer la présence de populations dominantes du "groupe" Obsoletus (Conte et al., 2007). Il a été souligné que les facteurs écologiques sont des déterminants clés pour modéliser l'abondance des espèces, qui se reproduisent dans un sol humide ou dans les bouses de ruminants, et dont le cycle de vie dépend fortement de la température (Mellor et al., 2000).

L'ensemble du jeu de données obtenu va maintenant permettre de modéliser la distribution des principales espèces de *Culicoides*, afin de produire *in fine* des cartes de risque de transmission des virus de la FCO et de la peste équine, qui sont indispensables pour l'élaboration de stratégies de contrôle et de gestion des populations.

Conclusion et perspectives

L'ensemble de ce travail a permis de mettre à jour la liste d'espèces de *Culicoides* qui comprend aujourd'hui 56 espèces valides, dont huit d'entre elles sont des vecteurs reconnus ou potentiels de virus d'intérêt vétérinaire (*C. imicola, C. obsoletus, C. scoticus, C. montanus, C. pulicaris, C. newsteadi, C. paolae* et *C. kingi*), mais aussi d'approfondir les connaissances sur la distribution spatiale et temporelle de différentes espèces de Culicoides au Maroc. En effet de nouvelles connaissances sur la composition, la saisonnalité, la distribution spatiale des espèces de *Culicoides* et les facteurs explicatifs ont été apportées.

Caractériser la dynamique saisonnière des *Culicoides* peut permettre de comprendre et de prévoir la persistance et la propagation des virus transmis, afin d'optimiser les stratégies d'atténuation, notamment la vaccination des ruminants sensibles, la restriction aux mouvements et aux échanges de bétail, mais aussi la lutte antivectorielle (Carpenter et al. 2013). Bien que ces mesures réduisent la vitesse et l'étendue de la propagation des virus, elles imposent des coûts logistiques énormes aux régions touchées, et seront d'autant mieux préconisées qu'elles seront limitées aux périodes d'intérêt. Cette compréhension est particulièrement importante pour le Maroc où le virus de la fièvre catarrhale ovine est endémique depuis 2004.

Aussi dans la même perspective d'optimiser la lutte et la surveillance des maladies transmises par les *Culicoides* et notamment la fièvre catarrhale ovine, il est essentiel d'identifier les zones présentant un risque plus élevé de transmission à l'échelle nationale. Ceci nécessite de caractériser la répartition des espèces vectrices au Maroc, élément essentiel pour déterminer si des vecteurs sont présents sur l'ensemble du territoire national, si leur abondance est très variable ou non et ainsi identifier les zones de transmission à haut risque. Combiné à la répartition de la population des ruminants, cela permet de cibler la vaccination dans ces zones à haut risque. La première étape vers cet objectif implique l'amélioration des connaissances sur la distribution des vecteurs dans le pays et le lien avec les facteurs environnementaux pouvant influencer cette distribution.

Plusieurs études ont démontré la capacité de prédire la présence et/ou l'abondance des *Culicoides* à l'aide des variables météorologiques et environnementales principalement dérivées de l'imagerie satellitaire. Ces modèles ont été établis à l'aide de techniques statistiques telles que les modèles discriminants (Baylis et al., 2001), des modèles linéaires

généralisés (Wittman et al. 2001, Calistri et al., 2003 ; Conte et al. 2003, Guis, 2007, Cavete et al. 2008) et, plus récemment, l'exploration de données comme les forêts aléatoires (Peters et al. 2011, Ducheyne et al. 2013, Cianci et al., 2015).

En combinant les connaissances sur la saisonnalité et la modélisation de la distribution des espèces de *Culicoides*, nous serons dans la capacité de cibler dans le temps et l'espace les mesures préventives, notamment d'éventuelles méthodes de lutte antivectorielle.

La stratégie de lutte contre la fièvre catarrhale ovine mise en place par l'Office National de Sécurité Sanitaire des produits Alimentaires (ONSSA) repose sur quatre volets essentiels :

- la vaccination massive des ovins contre les sérotypes circulant dans le pays (1 et 4);
- la gestion sanitaire des foyers de fièvre catarrhale ovine déclarés ;
- la lutte contre le vecteur ;
- la surveillance des signes cliniques de la maladie.

En effet, suite à l'apparition de la FCO pour la première fois en 2004, des campagnes de vaccination des ovins ont été réalisées contre cette maladie (sérotype 4 en 2004 puis le séotype 1 et 4 depuis 2006) au niveau des zones à risque (limitées principalement à la partie nord du pays). En 2008, la campagne de vaccination a été généralisée à l'ensemble du Royaume. A ce jour, aucun plan de vaccination nationale n'est préétabli par les services compétents malgré une circulation virale endémique.

Les résultats de notre étude peuvent contribuer à planifier des campagnes de lutte réfléchies dans des périodes et endroits précis afin de limiter le coût de ces programmes.

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