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RESEARCH ARTICLE

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Molecular characterization of *Culex pipiens* (Diptera, Culicidae) in Reghaïa lake, Algeria

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ABSTRACT

Culex pipiens is the most common mosquito in Algeria. It is widely distributed and colonizes different habitats in urban and rural areas, thriving in both polluted and clean breeding water. In addition to being a biting nuisance, this mosquito is also the potential vector of West Nile virus (WNV) worldwide. This species includes two biological forms: Culex pipiens pipiens and Culex pipiens molestus, which are morphologically similar, but differ in their behavior and biology. For determining the molecular profile of Culex pipiens populations in the region of Reghaïa lake, a suburb of Algiers, 300 Culicinae larvae were collected in different habitats above and underground. Molecular typing using multiplex PCR, based on the microsatellite locus CQ11, of 130 adults randomly chosen was carried out. The results demonstrated the presence of the pipiens form (26.1%) and, for the first time in Algeria, the molestus form (43.1%) and a hybrid of the two forms (30.8%). The existence of these forms associated with the presence of migratory and sedentary wild birds known as potential carriers or reservoir hosts of WNV may represent a potential risk for the emergence of WNV in the region of Reghaïa lake.

Key words: *Culex, pipiens, molestus,* West Nile Virus, Algeria.

INTRODUCTION

Mosquitoes are among the most important vectors transmitting pathogens causing diseases such as malaria [1], Dengue fever [2] and West Nile virus (WNV) [3]. Members of the *Culex pipiens* species complex are among the most widely distributed mosquitoes in the world and are of both medical and veterinary importance [4]. The complex comprises several species, including *Cx. pipiens* and *Cx. quinquefasciatus*, which are the most abundant and ubiquitous Culicinae mosquitoes in temperate and tropical regions, respectively. *Culex pipiens* has two different biological forms: *pipiens* and *molestus* that are morphologically indistinguishable but differ in their eco-physiological and blood feeding behaviour [5, 6].*Culex pipiens* form *pipiens* is eurygamous (unable to mate in confined spaces), is anautogenous (only lays eggs after a blood-meal), diapauses during winter, occurs mainly aboveground and prefers blood feeding on avian hosts [7]. *Culex pipiens* form *molestus* is stenogamous (able to mate in confined spaces), autogenous (lays eggs without a blood-meal), occurs mainly in underground habitats and prefers biting mammals [7]. In Russia, these two forms occur in different habitats, while in southern Europe and USA, they were found to exist sympatrically allowing the generation of hybrids *pipiens*/*molestus* [8]. In North Africa, *Cx. p. molestus* was first described in Egypt [7]. More recently, the *molestus* form and hybrids

pipiens/*molestus* were reported in Morocco [9] which display good vectorial competence for the transmission of WNV [10]. Identifying members of the *Cx. pipiens* populations by morphological methods is difficult, time-consuming, and often limited to adult males. The rapid and accurate identification of these mosquitoes, which are responsible in the transmission of several vector borne diseases, is therefore one of the important critical to establish risk maps for outbreaks and to implement adapted control strategies. This study aims to determine the molecular profile of *Culex pipiens* populations collected in the region of Reghaïa lake, in Algeria. The sampled region is a suburb of Algiers which harbours several species of migratory birds (known as amplifiers of WNV) and where several patients have been identified carrying antibodies to WNV [11].

MATERIALS AND METHODS

Mosquito larvae were collected twice a month, between May and June 2013 using the "dipping" sampling method with fishy. Five locations in the Reghaïa lake were selected according to the habitat characteristics (n°1: 36°46'34.72"N, 3°20'3.51"E, n°2: 36°46'24.94"N, 3°20'10.04"E, n°3: 36°46'23.87"N, 3°20'12.98"E, n°4: 36°46'22.66"N, 3°20'13.00"E and n°5: 36°45'58.22"N, 3°20'23.18"E), either along the lakeshore (aboveground) or in manholes (underground) (Figure. 1).

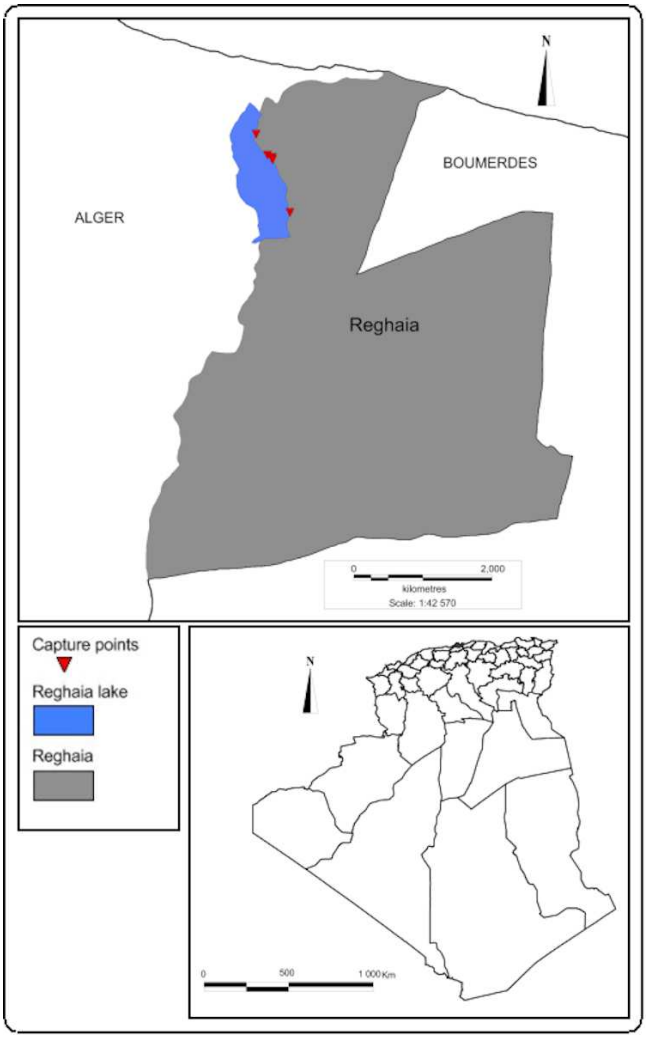

Fig 1. Collection sites of *Culex pipiens* larvae

A total of 300 larvae were collected and reared until the pupal stage under standardised conditions at 26°C and 12:12h photoperiod. Emerging adults were conserved at -20C° for subsequent molecular characterization.

One hundred thirty individuals selected randomly from the five locations were identified morphologically as belonging to the complex *Cx. pipiens* [12]. Then, they were further characterized as *molestus* or *pipiens* using a multiplex PCR assay [13]. One leg of each mosquito was used as DNA template in a 20µL reaction with a final concentration of 0.1 µmol/L of pipCQ11R and 0.15 µmol/L of molCQ11R and CQ11F (these primers amplify the flanking regions of the microsatellite locus CQ11), 2 mmol/L $MgCl_2$, 200 µmol/L of each dNTP mixture and 1 U/µL of Taq polymerase. The amplified DNA fragment size was 200bp for *pipiens* and 250bp for *molestus*, thus allowing detection of the two forms (and hybrids) in a single PCR reaction. Specimens of *Cx. pipiens molestus* from Turkey were used as control. Amplification products were visualised on a 2% agarose gel stained with ethidium bromide (Figure. 2).

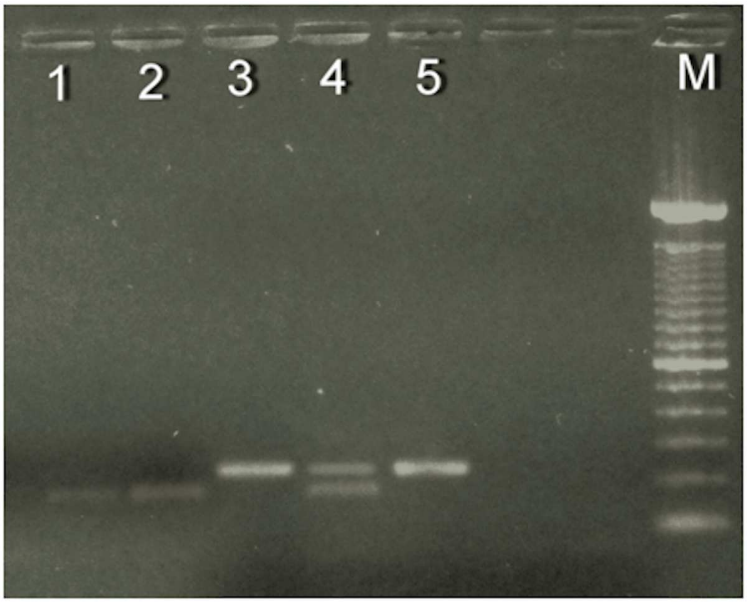

Fig 2. PCR amplification *of Cx. pipiens*. M: 100 pb size marker; Lanes 1and 2 *pipiens* form; Lane 3 *molestus* form; Lane 4 hybrid form; Lane 5 control *Cx. p. molestus* from Turkey

All PCR reactions were performed in triplicate to confirm the molecular profile of each mosquito.

RESULTS AND DISCUSSION

Overall, 130 *Cx. pipiens* were identified morphologically and subsequently, typed to the specific biological form (*pipiens*, *molestus*, hybrid) by PCR (Table. 1).

Table 1. *Culex pipiens* forms frequencies according to the breeding sites

	Site 1 (A) [40]	Site 2 (A) [60]	Site 3 (U) [100]	Site 4 (A) [45]	Site 5 (A) [55]	All sites [300]
pipiens	0	15 (42.8%)	6 (9.2%)	10 (100%)	3 (30%)	34 (26.1%)
molestus	3 (30%)	10 (28.6%)	36 (55.4%)	0	7 (70%)	56 (43.1%)
Hybrid	7 (70%)	10 (28.6%)	23 (35.4%)	0	0	40 (30.8%)
Total	10	35	65	10	10	130

A, aboveground; U, underground; [ ] Number of collected larvae by site.

Of the analysed specimens, 26.1% were identified as representing the *pipiens* form, 43.1% as *molestus* form and 30.8% as hybrid. The sympatric presence of *Cx. pipiens* biotypes were observed in 3 of the 5 locations studied. Females colonized similarly aboveground and underground habitats without any difference in the proportion of hybrids (Fisher exact test: $p>0.05$). To note, as expected, the *molestus* form was predominant in the underground site.

Our results provide, for the first time, the molecular evidence for the sympatric occurrence of *Cx. p. molestus*, and *Cx. p. pipiens*, and their hybrids in Algeria, thus allowing to bring out their existence in North Africa from Tunisia [14] to Morocco [9]. Despite the existence of a partial reproductive isolation barrier reported by *Urbanelli et al*. [15] between *Cx. pipiens* populations from aboveground and underground, a high rate of hybrids was noted in three of the five locations sampled. This may be due to the proximity of the manhole to the lakeshore, which can favour the meeting of the two forms and the subsequent generation of hybrids. The species of *Cx. pipiens* complex are known for their ability to adapt to different biotopes or habitats [16]. Spielman [17] noted that hybrids feed indiscriminately on avian or mammalian hosts. This opportunistic feeding behaviour could increase the risk of pathogen transfer such as WNV, from wild birds to mammals. Moreover, experimental infections of *Cx. pipiens* populations from different regions of Algeria confirmed their ability to transmit two arboviruses: WNV and Rift Valley Fever virus [10]. In addition, the region of Reghaïa lake which represents a suitable environment for mosquito breeding, harbours several migratory bird species considered to be a potential WNV amplifier such as the Common starling (*Sturnus vulgaris),* European robin (*Erithacus rubecula*),Common house martin*(Delichon urbica),* European turtle dove (*Streptopelia turtur*),Eurasian collared-dove (*Streptopelia decaocto*), Caspian gull (*Larus cachinnans*), Black-winged stilt (*Himantopus himantopus*),and Grey plover (*Pluvialis squatarola*) [18, 19]. The conjunction of these factors with the neighborhood of the habitations could favour the emergence of a WNV outbreak and thus, should be considered as a serious threat to public health. Further studies in this region should be emphasized on surveillance of WNV circulation for an early detection of outbreaks. This may be achieved through different strategies: i) frequent monitoring of chickens acting as sentinels (indicating the presence of WNV), ii) defining the prevalence of WNV-infected mosquitoes, iii) assessing the vector competence of these mosquito biotypes and iv) identifying areas at risk of WNV transmission established from the information gathered from the previous points.

In conclusion, molecular characterization of mosquitoes collected in the region of Reghaïa lake revealed the presence of *molestus* and hybrid forms of *Cx. pipiens* in addition to the previously reported *pipiens* form. This wetland area represents favorable conditions for the transmission of WNV, due to the presence of all three forms of *Culex pipiens* that may be involved in the transmission of WNV combined with the arrival in summertime of migratory birds.

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