



## Diversity, abundance and distribution of mosquitoes (Diptera, Culicidae) in Northeast Algeria

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**ABSTRACT.** Mosquitoes (Culicidae) are hematophagous insects that play a major role as vectors of diverse pathogens affecting human health. Understanding their distribution and diversity is essential for assessing pathogen risks and developing control strategies. This study investigated the Culicid fauna in the semi-humid region of northeastern Algeria (Annaba-El Tarf) over a period from October 2019 to July 2022. An entomological survey was conducted in natural and artificial larval habitats across rural and urban areas to account for factors influencing mosquito breeding capacity and distribution. Immature mosquitoes were collected and identified to assess species richness and abundance. A total of 5587 immature mosquitoes were collected from 111 positive larval habitats, representing four genera and nine species: *Culex pipiens*, *Cx. theileri*, *Cx. hortensis*, *Culiseta longiareolata*, *Cs. annulata*, *Aedes caspius*, *Ae. geniculatus*, *Ae. albopictus*, and *Anopheles labranchiae*. The genus *Culex* was the most prevalent, with *Culex pipiens* accounting for 63.25% of the total, followed by *Culiseta longiareolata* (23.41%). Species richness was lower in urban areas compared to rural areas, and natural larval habitats harboured more species than artificial ones. This study provides essential data on the distribution, abundance, and diversity of mosquito species in northeastern Algeria, contributing to understanding mosquito population dynamics and enabling better vector control and monitoring strategies, particularly in light of evolving human activities and climate change.

**Keywords:** *Aedes albopictus*, Annaba-El Tarf, co-occurrence, ecology, taxonomy

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## INTRODUCTION

Mosquitoes represent the largest group of biting Diptera (WHO, 1999). These hematophagous insects have existed on Earth for over 100 million years. They exhibit a wide range of species that can adapt their biology to various ecological conditions, allowing them to reproduce in diverse habitats (Becker et al., 2012). Globally, 3727 mosquito species have been recognized worldwide (Harbach, 2024); there are 53 species among others resident in Algeria, which are 15 species of *Aedes*, 15 species of *Anopheles*, 14

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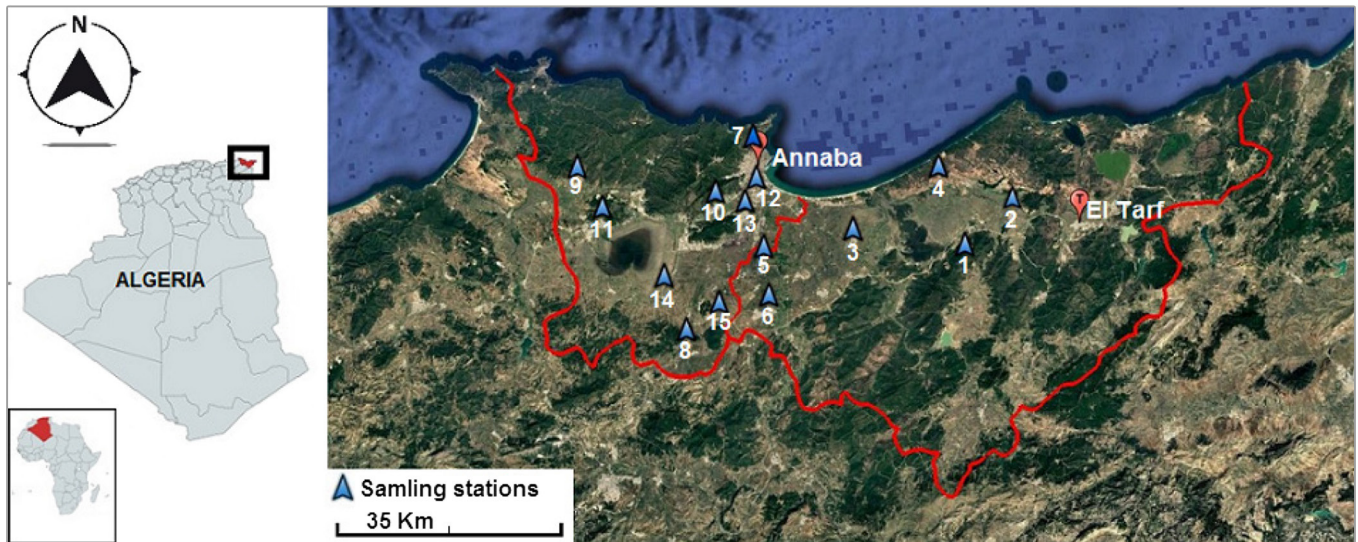
species of *Culex*, five species of *Culiseta*, two species of *Coquillettidia* and one species of *Orthopodomyia* (Merabti et al., 2021).

In recent years, the global and regional importance of vector-borne diseases (VBDs) has increased, particularly those caused by pathogens transmitted by mosquitoes, which cause significant human morbidity and mortality worldwide (Beerntsen et al., 2000; Valenzuela & Aksoy, 2018). The World Health Organization (WHO) reports that diseases caused by pathogens transmitted by mosquitoes contribute to approximately 17% of the total burden of infectious diseases (WHO, 2020). Mosquitoes (Culicidae) are significant carriers of pathogens, including metazoans (such as filarial nematodes), protozoa (such as malaria parasites), and various viruses (such as Dengue virus, Rift Valley virus, and West Nile virus) (Brenda et al., 2000; Tolle, 2009; Nebbak et al., 2022). The dispersal of these vectors has led to the transmission of numerous fatal diseases. Additionally, these small insects are a major source of nuisance (Ramirez et al., 2016). Indeed, anthropogenic activities (urbanization, agricultural and industrial development), including transcontinental exchanges and international trade (Khan et al., 2014; Saeed et al., 2019), along with climate change, have enabled the spread of mosquitoes and the emergence of the diseases they transmit (Gould & Higgs, 2009; Kearney et al., 2009; Kilpatrick & Randolph, 2012). In this context, effective vector control programs require detailed knowledge of the ecology and population dynamics of mosquito species, as well as a thorough understanding of the epidemiology of diseases caused by mosquito-transmitted pathogens (Amini et al., 2020). Mosquitoes have the ability to colonize a variety of natural and artificial aquatic habitats, including ponds, streams, ditches, swamps, marshes, temporary and permanent pools, tree holes, tyres, cans, flower vases, and bird feeders (Rueda, 2008). However, certain mosquito species show a preference for specific breeding sites, while others are more opportunistic, with habitats ranging from small and ephemeral to large and permanent (Bentley & Day, 1989; Juliano, 2009). They are highly sensitive and adapt quickly to habitat, climate change, and forest structure due to their high sensitivity and quick adaptation to changes in habitats, climate, and forest structure (Roiz et al., 2014; Caldwell et al., 2021; Young et al., 2021). Identifying larval habitats and understanding the factors influencing their development is essential for analyzing the spatial and temporal distribution of mosquito species (Novianto et al., 2021). This knowledge is crucial for implementing effective approaches to vector control and disease prevention (Ramasamy et al., 2011). Several studies on mosquito diversity, distribution, and ecology have been published in the same study region (Belayadi, 2010; Tahraoui, 2012; Amara Korba et al., 2016; Dahchar et al., 2017; Matoug, 2017; Arroussi et al., 2021). Therefore, the study of mosquito biodiversity should consider co-occurrence, density, and distribution patterns.

The objective of this study is to investigate mosquito populations in the semi-humid region (Annaba-El Tarf) in Northeast Algeria through a systematic survey using morphological identification based on dichotomous keys. The aim is to update the list of culicid fauna. Simultaneously, we will analyze the spatiotemporal distribution, structure, and composition of species. Additionally, we will provide ecological characteristics of culicid larvae, including habitat type and nature, associated species, and affinity indices.

## MATERIAL AND METHODS

**Study area.** Mosquito samples were collected from 15 stations in the North-East of Algeria, including six stations in El Tarf (36°45' N, 8°18' E) (Bouthelja, Sidi Kassi, Ben Mehidi, Sebaa, Chebaïta Mokhtar, and Drean) and nine stations in Annaba (36°54' N, 07°44' E) (Sidi Amar, El Bouni, Ain Sayed, Chorfa, Kheraza, Annaba, Berrahal, Ain Berda, and Tréat) (Fig. 1). Annaba and El-Tarf are classified as sub-humid cities. El-Tarf has an average temperature of 12 °C in winter and 28 °C in summer, respectively, and its average annual rainfall reaches 700 mm. The humidity level averages at 70%. March has the highest relative humidity (76%), while July has the lowest (60%). In contrast, Annaba has an average temperature of 18.4 °C throughout the year. The warmest month is August with a temperature of 30.3 °C, while the coldest month is February with an average temperature of 14.3 °C. The average humidity is 70.41%, and the precipitation averages 712 mm per year (650 and 1000 mm/year). Meteorological data (rainfall and temperature) were obtained from the local meteorological station.



**Figure 1.** Geographic map of Algeria (left) and its study area as well as sampling stations (right). **1.** Sidi Kassi; **2.** Bouteldja; **3.** Ben M'hidi; **4.** Sebaa; **5.** Chebaïta; **6.** Drean; **7.** Annaba; **8.** Ain berda; **9.** Tréat; **10.** Kherraza; **11.** Berrahal; **12.** El bouni; **13.** Sidi Amar; **14.** Chorfa; **15.** Ain Sayed.

**Sampling and identification.** Mosquito larvae were collected using the 'dipping' method (Papierok et al., 1975) during field trips conducted from October 2019 to July 2022. The method involved dipping a ladle into multiple locations (edges and center) of each site to sample the larvae. To collect fauna from larval habitats, two precise methods were employed. For sites with a water depth of less than 15 cm and rich in vegetation, a vacuum strainer with 0.1 mm mesh and a 10 cm diameter was used. For sites between 20 and 50 cm deep, we used a 500 ml container to collect the fauna. The collected fauna was then placed in plastic bottles filled with water from the surveyed site. The bottles were labelled with the date of sampling and station and were transported to the laboratory. The larvae brought to the laboratory are at different stages of development. Once they reach the fourth stage, they can be reliably identified. Species identification was conducted using a binocular magnifying glass and/or a light microscope after fixation between a slide and cover slip with a drop of glycerin. The software used for identifying species included the Mediterranean African Culicidae (Brunhes et al., 2000) and MosKeyTool *ver.2.3*, an interactive identification key for Euro-Mediterranean mosquitoes (Régine & Lebbe, 2013).

**Larval habitat characteristics.** This study considers the nature and type of larval habitats, including natural habitats such as swamps, peat bogs, puddles, ponds, and ditches, as well as artificial habitats resulting from poor maintenance of rainwater collection and drainage systems (e.g; sewers, Concrete ditch) and the accumulation of waste in gardens (e.g; water tanks, flower bunches, and tyres). The study also takes into account the habitat location, distinguishing between urban areas (city centers) and rural areas (away from human settlements).

**Affinity and association indices.** The analysis of co-occurring larval species was conducted using Fager and McGowan's affinity index and association index (Fager & McGowan, 1963). Assume that species found together in more than 50% of the collection sites have the same environmental needs. In other words, the presence of one co-occurring species suggests the presence of the other in the same habitat. A species is considered to have the same resource use if its affinity index value is greater than or equal to 0.5. The degree of association ranges from -1 (complete dissociation) to +1 (complete association). The calculation of affinity and association indices is done as follows:

$$\text{Affinity index} = \left[ \frac{J}{(nA + nB)^{\frac{1}{2}}} \right] - \left[ \frac{1}{2(2nB)^{\frac{1}{2}}} \right]$$

$$\text{Association index} = 2 \left[ \frac{J}{(A + B)} - 0,5 \right]$$

Where  $J$  on affinity index = number of joint occurrences,  $nA$  = total number of occurrences of species A,  $nB$  = total number of occurrences of species B. Nevertheless  $J$  value on association = the number of individuals of both species in samples where they occur together,  $A$  and  $B$  = the total number of individuals of both species in all samples.

**Diversity indices.** Simpson's Index of Diversity ( $1 - D$ ) was calculated using the formula:

$$1 - D = 1 - \frac{\sum n_i(n_i - 1)}{N(N - 1)}$$

Where  $n_i$  represents the number of individuals of the  $i^{\text{th}}$  species and  $N$  is the total number of individuals across all species in the studied habitat or country. Simpson's Index of Diversity reflects the probability that two individuals taken at random from the dataset are not the same species. Values for Simpson's Index of Diversity range between 0 and 1, with larger values representing greater diversity.

The Shannon-Weiner species diversity Index was also used as a measure of diversity and is calculated using the formula:

$$H' = - \sum_{i=1}^S P_i \cdot \log_2(P_i), \text{ where } P_i = \frac{n_i}{N}$$

This index reflects the uncertainty of correctly predicting the species of an individual randomly selected from the dataset. Higher values indicate greater uncertainty and, therefore, greater diversity. This method is sensitive to sample size, whereas the Simpson's Index puts more weight on dominant species and is hardly influenced by a few rare species. In addition, the Shannon-Weiner evenness index was calculated as:

$$E = \frac{H'}{\ln(S)}$$

Where  $S$  is the total number of species. Evenness values range from 0 to 1, with a value of 1 representing complete evenness, where all species are equally abundant.

**Data analysis.** Statistical analyses were calculated using SPSS V.23 statistical software (SPSS, 2015). A descriptive analysis was utilized to determine the abundance and frequency of mosquito larvae in various types and natures of larval habitats. The species richness of the different types and nature of larval habitats (urban and rural, natural and artificial) was comparatively analyzed using a Student's T-test. The relationship between the presence of mosquito larvae (negative/positive) and the type of larval habitat (natural/artificial, rural/urban) was determined using the Chi-square test. The relationship between cohabitation (isolated/cohabited) and species was also determined. To compare mosquito diversity across different sample sizes in each larval habitat type and assess sampling sufficiency, we generated rarefaction and extrapolation curves for species accumulation using iNEXT software (online version 2022). Comparisons were made between larval habitats in urban and rural areas, as well as between natural and artificial habitats. The analysis was conducted using 1000 randomizations without replacement and at a 95% confidence interval (Hsieh et al., 2016).

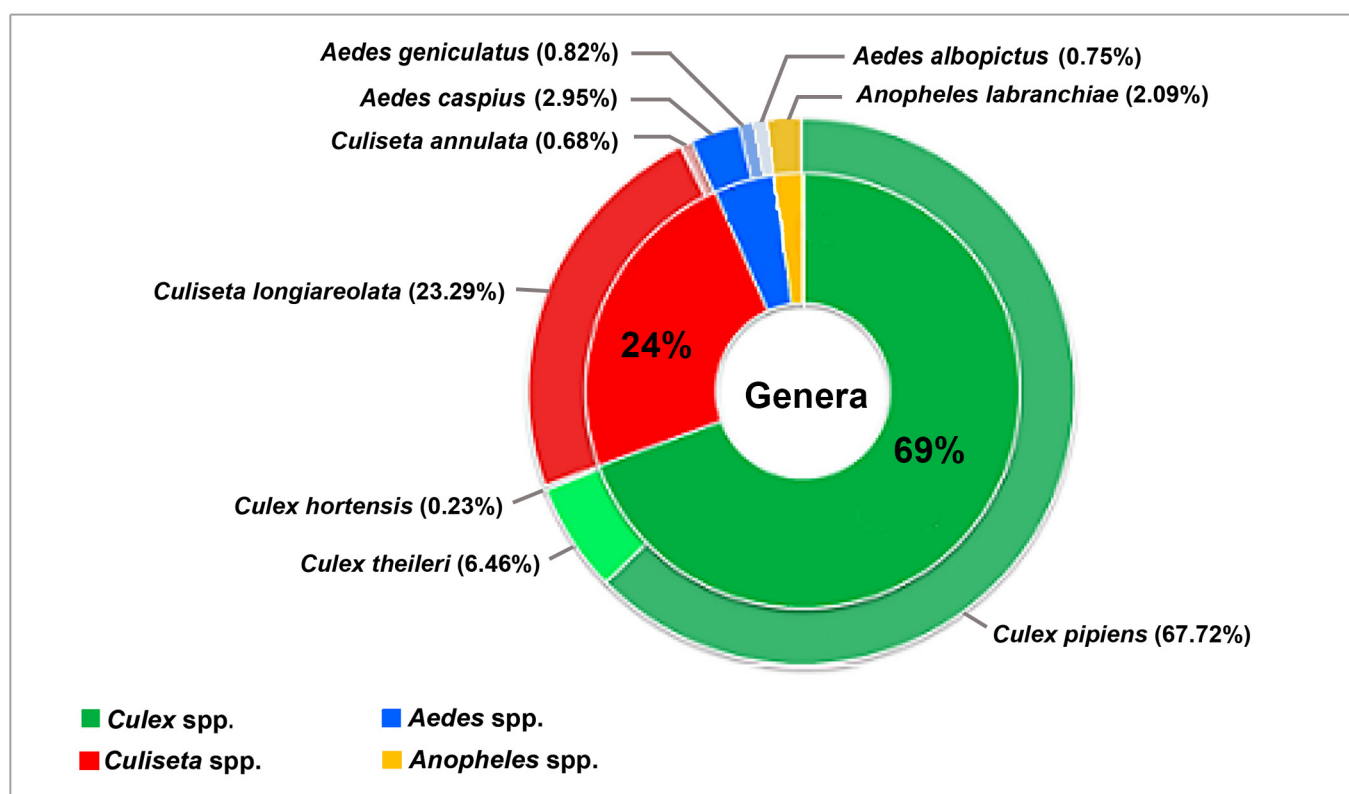
## RESULTS

**Species diversity.** A total of 5587 immature mosquitoes were collected and classified into 4 genera and 9 species. *Culex* was the most prevalent genus, accounting for 69.4% (95% CI: 68.2–70.6%), followed by *Culiseta* at 24% (95% CI: 22.8–25.1%), *Aedes* at 4.5% (95% CI: 4–5.1%), and *Anopheles* at 2.1% (95% CI: 1.7–2.5%). Out of the 9 species collected, *Culex pipiens* and *Culiseta longiareolata* were the most abundant, together representing 86.0% (95% CI: 85.1–86.9%,  $n = 4805$ ) of the identified specimens. *Culex pipiens* was

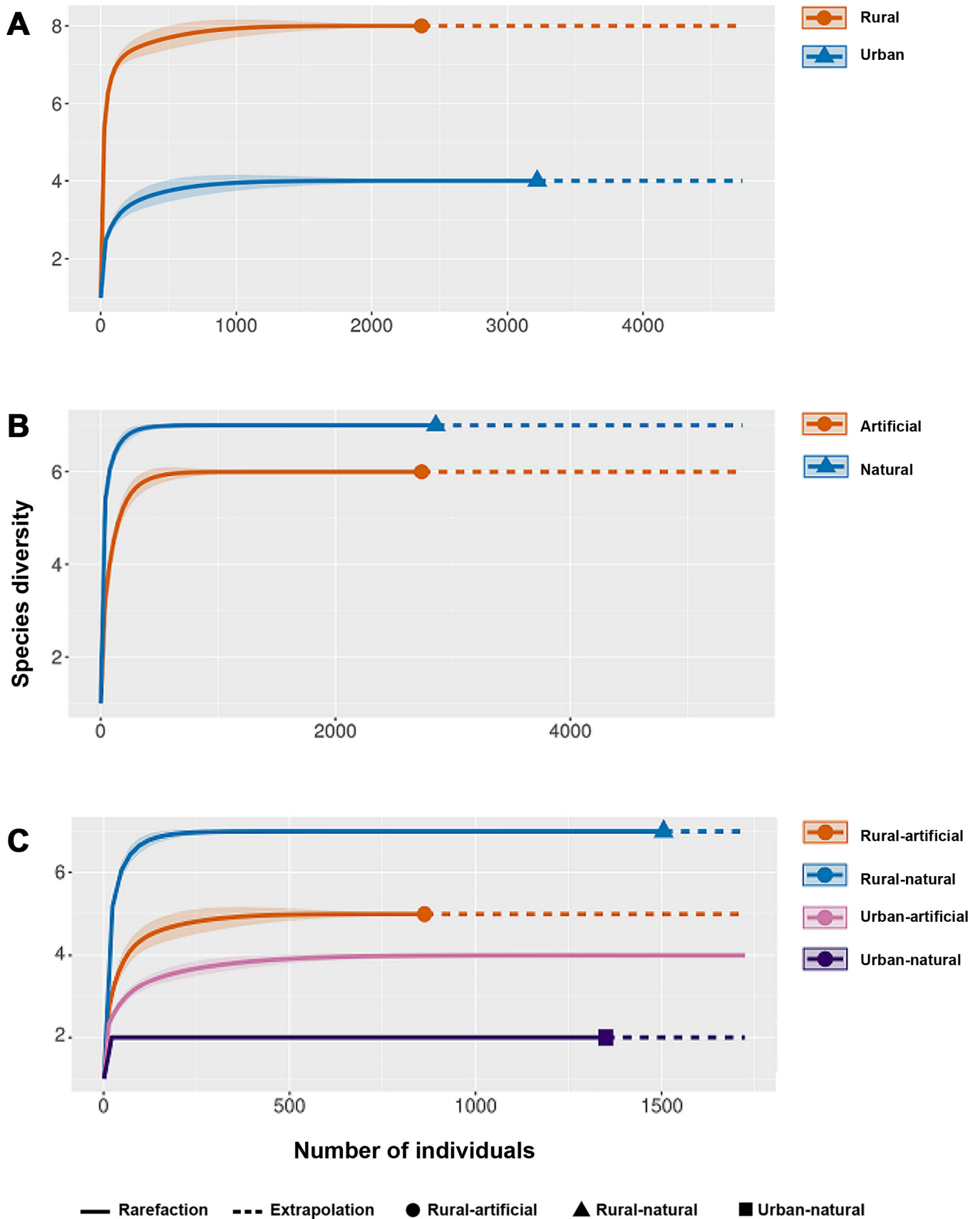
the most abundant, comprising 62.7% (95% CI: 61.4–64%,  $n = 3504$ ) of the mosquitoes sampled, followed by *Cx. longiareolata* with 23.3% (95% CI: 22.2–24.4%). *Culex theileri* and *Anopheles labranchiae* accounted for only 6.5% (95% CI: 5.8–7.1%) and 3.0% (95% CI: 2.5–3.4%), respectively, while the remaining species had rates below 1% (Fig. 2).

**Diversity analysis.** The diversity of mosquito fauna varied among the different aquatic habitats, as evidenced by the values of Simpson's diversity index ( $D$ ), Shannon-Wiener index ( $H'$ ), evenness ( $E$ ), and species richness ( $S$ ) among the study regions (Table 1). The Culicidae population sampled in the semi-humid region of Annaba-El Tarf exhibited varying levels of diversity ( $H = 1.61$ ,  $E = 51$ ). The rural area exhibited higher diversity ( $H = 1.89$ ,  $E = 0.63$ ) compared to the urban area ( $H = 1.03$ ,  $E = 0.52$ ). Natural aquatic habitats in the rural area were found to be the most diverse, with a more equal distribution of species abundance observed in the stand ( $H = 2.03$ ,  $E = 0.72$ ). In contrast, natural aquatic habitats in urban areas exhibit the lowest diversity ( $H = 0.96$ ) and a more even distribution of species abundances ( $E = 0.96$ ) (Table 1).

The Chi-square test showed that species richness was significantly dependent on the type and nature of the larval habitat ( $\chi^2 = 32.8$ ;  $df = 03$ ;  $P = 0.000^*$ ). Additionally, the species accumulation curve indicated that species richness was significantly higher in the rural area (8 species) than in the urban area (4 species) ( $T$ -test;  $t = 2.78$ ,  $df = 40.52$ ,  $P = 0.008$ ) (Fig. 3A). The contrast between the number of species in artificial and natural larval habitats was not surprising given the results of the  $t$ -test, which showed no significant difference ( $t = 0.78$ ,  $df = 11.49$ ,  $P = 0.45$ ) (Fig. 3B). Furthermore, the species accumulation curve demonstrates a significant difference in species richness between natural larval habitats in the rural (7 species), and urban areas (2 species) ( $T$ -test;  $t = -7.14$ ;  $df = 11.87$ ;  $P = 0.000^*$ ). However, there was no significant difference in species richness between artificial larval habitats in rural and urban areas ( $t = -1.83$ ,  $df = 29.68$ ,  $P = 0.07$ ) (Fig. 3C). We note that the curves have reached the asymptotic stage, indicating the effectiveness of sampling in this study.



**Figure 2.** Relative abundance of genera and species found in the different study area sites, Annaba-El Tarf, Algeria, 2019–2022.



**Figure 3.** Mosquito species accumulation curves; comparison of mosquito diversity. **A.** In rural and urban areas, Annaba-El Tarf, Algeria, 2019–2022; **B.** In natural and artificial habitats, Annaba-El Tarf, Algeria, 2019–2022; **C.** In different types and localities of larval habitats, Annaba-El Tarf, Algeria, 2019–2022.

**Table 1.** Ecological indices of structure in different localities and types of larval habitats in the semi-humid region, Annaba-El Tarf, Algeria, 2019–2022.

Habitats	No. sites	Species richness (S)	Shannon index (H)	Evenness (E)	Dominance (D)	Simpson_1-D	Nb of larvae
Localities	Rural	56	8	1.89	0.63	0.4	2367
	Urban	55	4	1.03	0.52	0.53	3220
Rural	Natural	43	7	2.03	0.72	0.33	1505
	Artificial	13	5	1.1	0.47	0.6	862
Urban	Natural	16	2	0.96	0.96	0.52	1349
	Artificial	39	4	1.06	0.53	0.55	1871
Type of larval habitat	Natural	59	7	1.87	0.66	0.37	2854
	Artificial	52	6	1.12	0.43	0.56	2733
TOTAL		9	1.61	0.51	0.45	0.55	5587

**Distribution and abundance.** (according to localities and type of larval habitats). In this study, a total of 5587 mosquito larvae were collected from 111 sites, with 2,367 larvae from 56 rural sites 42.4% (95% CI: 32.7–52%) and 3220 larvae from 55 urban sites 57.6% (95% CI: 48–67.3%) (Table 2). According to the Figure 4A, *Cx. pipiens* dominates larval habitats in rural areas 59.5% (95% CI: 57.5–61.5%), followed by *Cx. theileri* 5.3% (95% CI: 4.3–6.2%), whereas, in urban areas, larval habitats are mainly composed of *Cx. pipiens* 65.7% (95% CI: 64–67.3%) and *Cs. longiareolata* 33.4% (95% CI: 31.7–35%). A total of seven mosquito species were identified from 2,854 specimens collected from 59 natural habitats, with *Cx. pipiens* 55.7% (95% CI: 53.9–57.5%), *Cs. longiareolata* 20.5% (95% CI: 19–22%) and *Cx. theileri* 11.8% (95% CI: 10.6–13%) being the most dominant (Fig. 4B). Puddles were identified as a natural larval habitat for 23.3% (95% CI: 22.2–24.4%,  $n = 1303$ ) of the sampled larvae, followed by ditches 8.7% (95% CI: 7.9–9.4%,  $n = 388$ ), swamps 7.5% (95% CI: 6.8–8.1%,  $n = 200$ ) and peat bogs 6.8% (95% CI: 6.1–7.4%,  $n = 137$ ) (Table 2). while six species were identified among the 2733 mosquito larvae collected from 52 artificial larval habitats, with *Cx. pipiens* 70.0% (95% CI: 68.3–71.8%), and *Cs. Longiareolata* 26.2% (95% CI: 24.5–27.9%), being the most dominant (Fig. 4B). During this study, *Ae. albopictus* was only collected in pails and flower pots in the urban area. The remaining identified species were found in several habitats. Metal tanks 13.6% (95% CI: 12.7–14.5%,  $n = 762$ ) and concrete ditches 10.7% (95% CI: 9.9–11.6%,  $n = 600$ ) were the most productive artificial habitats. Diversity data for mosquitoes in both natural and artificial habitats are presented in Table 2. The Venn diagram presented in Figure 5A demonstrates that 33.33% (95% CI: 9–69.1%), of mosquito species (*Cx. pipiens*, *Cx. hortensis*, and *Cs. longiareolata*) were found in both rural and urban habitats, while 55.6% (95% CI: 22.6–84.7%) of the species (*Cx. theileri*, *Cs. annulata*, *Ae. caspius*, *Ae. geniculatus* and *An. labranchiae*) were collected only in the rural area. Further, Figure 5B showed that 44.4% (95% CI: 15.3–77.3%) of the mosquito species (*Cx. pipiens*, *Cx. theileri*, *Cs. longiareolata*, and *Ae. geniculatus*) were found in both natural and artificial larval habitats. In contrast, *Ae. albopictus* and *Cx. hortensis* were exclusively found in artificial habitats, representing only 22.2% (95% CI: 21.1–23.3%), of the inventoried species. Conversely, *Ae. caspius*, *Cs. annulata*, and *An. labranchiae* were only found in natural habitats.

**Co-occurrence, affinity and association indices.** According to (Table 3 and Table 4), Pearson's chi-squared test suggested that cohabitation was significantly dependent on species ( $\chi^2 = 190.01$ ;  $df = 08$ ;  $P = 0.000^*$ ) and aquatic habitat types ( $\chi^2 = 56.16$ ;  $df = 03$ ;  $P = 0.000^*$ ). Out of 111 positive larval habitats, 66.7% (95% CI: 57.5–75.9%,  $n = 74$ ) were colonized by the species *Cx. pipiens*, followed by *Cs. longiareolata* 39.6% (95% CI: 30.1–49.2%,  $n = 44$ ), *Cx. theileri* 22.5% (95% CI: 14.3–30.7%,  $n = 25$ ) and *An. labranchiae* 12.6% (95% CI: 6–19.2%,  $n = 14$ ). The remaining species (*Ae. geniculatus*, *Cx. hortensis*, *Cs. annulata*, *Ae. caspius*, and *Ae. albopictus*) occupied less than 10% (95% CI: 4–16%) of the sampled sites in varying proportions. Five species (*Cx. hortensis*, *Ae. geniculatus*, *Cs. annulata*, *Ae. caspius*, and *Ae. albopictus*) were found 100% co-occurred. However, *Cx. pipiens* was found isolated with a frequency of 40.5% (95% CI: 28.7–52.4%,  $n = 30$ ), followed by *Cs. longiareolata* at 31.8% (95% CI: 16.9–46.7%,  $n = 14$ ), *An. labranchiae* 28.6% (95% CI: 1.3–55.8%,  $n = 4$ ) and *Cx. theileri* 20.0% (95% CI: 4.3–35.7%,  $n = 5$ ).

**Table 2.** Culicidae population numbers and ecological indices of the structure by species among different aquatic habitat types, Annaba-El Tarf, Algeria, 2019–2022.

Sites		Species	Positive habitats					
Area	habitat types		Sites, n (%)	Larvae	Cohabitation		Isoled	
					Sites, n (%)	Larvae, n (%)	Sites, n (%)	Larvae, n (%)
RURAL (56)	Artificial (13)	<i>Cx. pipiens</i>	9 (69.23)	644	7 (77.78)	612 (95.03)	2 (22.22)	32 (4.97)
		<i>Cx. Theileri</i>	3 (23.08)	23	3 (100)	23 (100)	0 (0)	-
		<i>Cx. hortensis</i>	3 (23.08)	5	3 (100)	5 (100)	0 (0)	-
		<i>Cs. longiareolata</i>	6 (46.15)	165	5 (83.33)	143 (86.66)	1 (16.67)	22 (13.34)
		<i>Ae. geniculatus</i>	3 (23.08)	25	3 (100)	25 (100)	0 (0)	-
		<i>Cx. pipiens</i>	26 (60.47)	764	16 (61.54)	517 (67.67)	10 (38.46)	247 (32.33)
	Natural (43)	<i>Cx. Theileri</i>	22 (51.16)	338	17 (77.27)	228 (67.46)	5 (22.73)	110 (32.54)
		<i>Cs. longiareolata</i>	4 (9.30)	62	4 (100)	62 (100)	0 (0)	-
		<i>Cs. annulata</i>	4 (9.30)	38	4 (100)	38 (100)	0 (0)	-
		<i>Ae. caspius</i>	6 (13.95)	165	6 (100)	165 (100)	0 (0)	-
		<i>Ae. geniculatus</i>	6 (13.95)	21	6 (100)	21 (100)	0 (0)	-
		<i>An. labranchiae</i>	14 (32.56)	117	10 (71.43)	83 (70.94)	4 (28.57)	34 (29.06)
URBAN (55)	Artificial (39)	<i>Cx. pipiens</i>	30 (76.92)	1270	12 (40)	491 (38.66)	18 (60)	779 (61.34)
		<i>Cs. longiareolata</i>	18 (46.15)	551	12 (66.67)	337 (61.16)	6 (33.33)	214 (38.84)
		<i>Cx. hortensis</i>	2 (5.13)	8	2 (100)	8 (100)	0 (0)	-
		<i>Ae. albopictus</i>	2 (5.13)	42	2 (100)	42 (100)	0 (0)	-
	Natural (16)	<i>Cx. pipiens</i>	9 (56.25)	826	9 (100)	826 (100)	0 (0)	-
		<i>Cs. longiareolata</i>	16 (100)	523	9 (56.25)	350 (66.92)	7 (43.75)	173 (33.08)

**Table 3.** Species co-habitation frequencies by area and type of larval habitats in the semi-humid region, Annaba-El Tarf, Algeria, 2019–2022.

Species	Natural aquatic habitats							Artificial aquatic habitats					
	Peat bog	Puddle	Swamp	Pond	Ditch	wheel rut	Water hole	Sewer	Pail/flower pot	Metal tank	Road gully	Tyre	Concrete ditch
<i>Cx. pipiens</i>	137	910	200	0	388	0	103	327	255	604	56	96	428
<i>Cx. theileri</i>	17	0	96	151	74	0	0	0	0	0	0	2	21
<i>Cx. hortensis</i>	0	0	0	0	0	0	0	0	0	0	8	0	5
<i>Cs. longiareolata</i>	84	393	5	0	2	55	92	59	51	158	179	85	138
<i>Cs. annulata</i>	0	0	32	0	6	0	0	0	0	0	0	0	0
<i>Ae. caspius</i>	137	0	0	0	4	0	24	0	0	0	0	0	0
<i>Ae. geniculatus</i>	0	0	11	0	10	0	0	0	0	0	0	17	8
<i>Aedes albopictus</i>	0	0	0	0	0	0	0	0	42	0	0	0	0
<i>An. labranchiae</i>	3	0	72	42	0	0	0	0	0	0	0	0	0
<b>Total n (%)</b>	378 (6.4)	1303 (23.32)	416 (7.44)	193 (3.45)	484 (8.66)	55 (0.98)	219 (3.91)	386 (6.9)	348 (6.22)	762 (13.63)	243 (4.35)	200 (3.58)	600 (10.74)
<b>Shannon index (H)</b>	1.84	0.88	1.93	0.76	0.95	0	1.39	0.62	1.1	0.74	0.97	1.4	1.15
<b>Dominance (D)</b>	0.31	0.58	0.32	0.66	0.63	1	0.41	0.74	0.57	0.67	0.6	0.42	0.56
<b>Simpson index (1-D)</b>	0.69	0.42	0.68	0.34	0.33	0	0.59	0.26	0.43	0.33	0.4	0.58	0.44
<b>Species richness (S)</b>	5	2	6	2	6	1	3	2	3	2	2	4	5
<b>Evenness (E)</b>	0.79	0.88	0.75	0.76	0.37	-	0.88	0.39	0.69	0.74	0.97	0.7	0.5



**Table 4.** The occurrence and frequency of species coexistence in the semi-humid region, Annaba-El Tarf, Algeria, 2019–2022.

Species	Occasions of occurrence n (%)	Total number of larvae n (%)	Cohabitation		Isolated n(%)	
			Sites n (%)	Larvae n (%)	Sites n (%)	Larvae n (%)
<i>Cx. pipiens</i>	74 (66.66)	3504 (62.72)	44 (59.46)	2446 (43.78)	30 (40.54)	1058 (18.93)
<i>Cx. theileri</i>	25 (22.52)	361 (6.46)	20 (80)	251 (4.49)	5 (20)	110 (1.96)
<i>Cx. hortensis</i>	5 (4.50)	13 (0.23)	5 (100)	13 (0.23)	0	0.00
<i>Cs. longiareolata</i>	44 (39.64)	1301 (23.29)	30 (68.18)	892 (15.97)	14 (31.82)	409 (7.32)
<i>Cs. annulata</i>	4 (3.60)	38 (0.68)	4 (100)	38 (0.68)	0	0
<i>Ae. caspius</i>	6 (5.41)	165 (2.95)	6 (100)	165 (2.95)	0	0
<i>Ae. geniculatus</i>	9 (8.11)	46 (0.82)	9 (100)	46 (0.82)	0	0
<i>Ae. albopictus</i>	2 (1.80)	42 (0.75)	2 (100)	42 (0.75)	0	0
<i>An. labranchiae</i>	14 (12.61)	117 (2.09)	10 (71.42)	83 (1.48)	4 (28.58)	34 (0.60)
<b>TOTAL</b>		<b>5587 (100)</b>		<b>3976 (71.17)</b>		<b>1611 (28.83)</b>

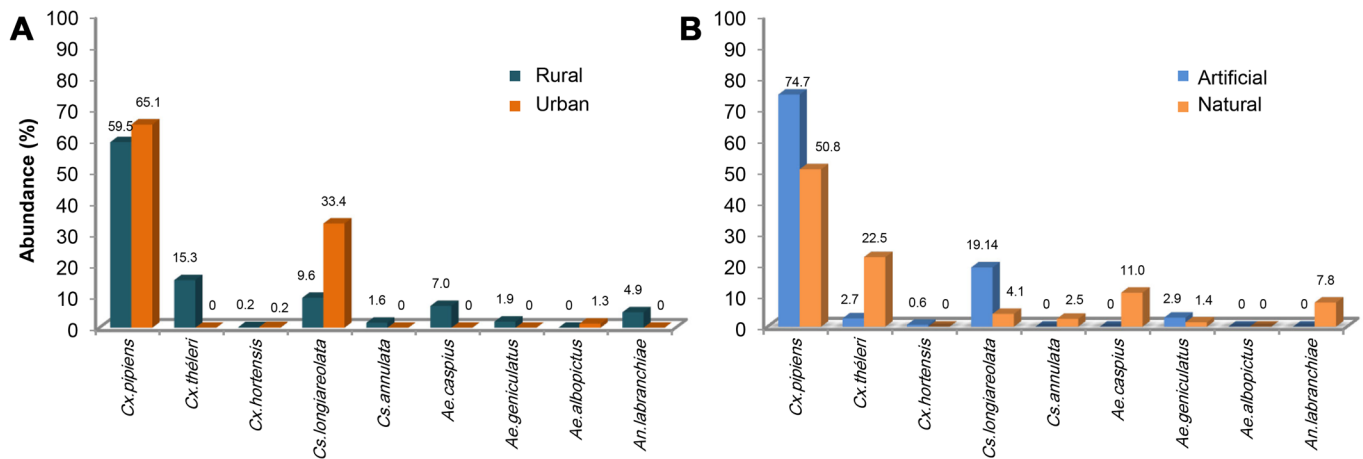
In the same context, *Cx. pipiens* showed the highest levels of intra- and inter-specific association compared to other species. The highest cohabitation was observed between *Cx. pipiens* and *Cs. longiareolata*, they were found in association on 24 occasions, corresponding to 54.6% (95% CI: 42.5–66.6%) and 32.4% (95% CI: 17.5–47.4%) of the total occasions, respectively. The association occurrences and percentage association occurrences of the remaining species are shown in Table 5. Affinity indices calculated between the nine most prevalent species pairs indicate that no significant affinity was found (at the 5% probability level). The affinity between *Cx. theileri* & *An. labranchiae* was 0.42, *Cs. longiareolata* & *Cx. pipiens* was 0.39, and *Cx. theileri* & *Cx. pipiens* was 0.21. Additionally, the affinity between the two pairs *Ae. geniculatus* & *Cx. pipiens* and *Ae. geniculatus* & *Cx. theileri* was 0.20. Also, the association index was negative, close to the value (-1) (Table 6).

**Table 5.** The association of mosquito larvae collected in the semi-humid region, Annaba-El Tarf, Algeria, 2019–2022.

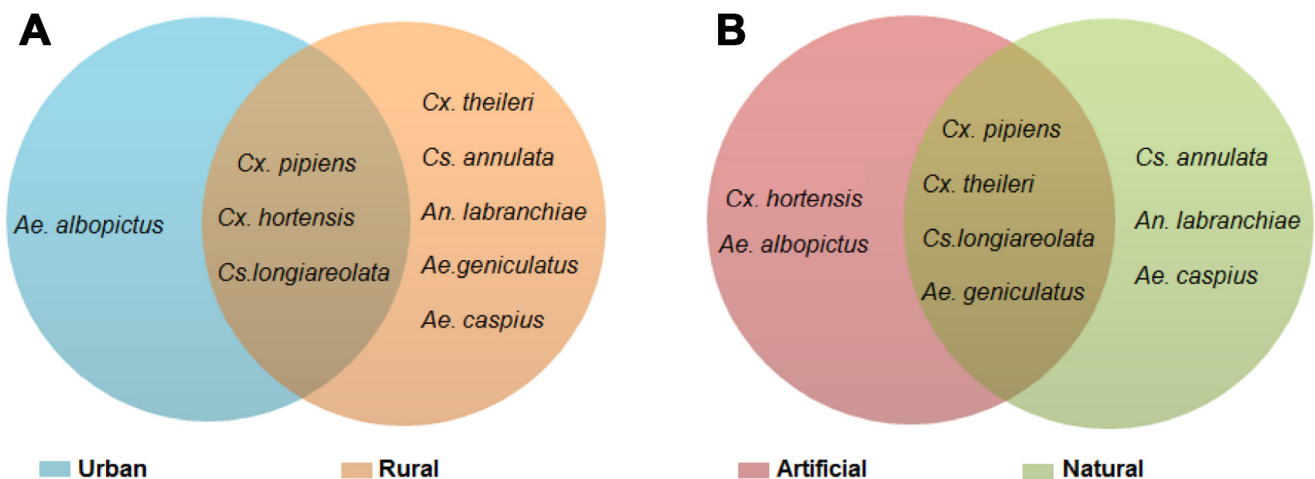
	<i>Cx. pipiens</i>	<i>Cx. theileri</i>	<i>Cx. hortensis</i>	<i>Cs. longiareolata</i>	<i>Cs. annulata</i>	<i>Ae. caspius</i>	<i>Ae. geniculatus</i>	<i>Ae. albopictus</i>	<i>An. labranchiae</i>
<b>Total occasions</b>	<b>74</b>	<b>25</b>	<b>5</b>	<b>44</b>	<b>4</b>	<b>6</b>	<b>9</b>	<b>2</b>	<b>14</b>
<i>Cx. pipiens</i>	*								
<i>Cx. theileri</i>	11	*							
<i>Cx. hortensis</i>	03	--	*						
<i>Cs. longiareolata</i>	24	05	04	*					
<i>Cs. annulata</i>	04	03	--	02	*				
<i>Ae. caspius</i>	06	02	--	02	--	*			
<i>Ae. geniculatus</i>	09	04	--	02	1	--	*		
<i>Ae. albopictus</i>	01	--	--	01	--	--	--	*	
<i>An. labranchiae</i>	04	09	--	01	1	1	1	--	*

**Table 6.** Matrix of association index (above the diagonal) and affinity index (below the diagonal) between pairs of larval species, Annaba-El Tarf, Algeria, 2019–2022.

	<i>Cx. pipiens</i>	<i>Cx. theileri</i>	<i>Cx. hortensis</i>	<i>Cs. longiareolata</i>	<i>Cs. annulata</i>	<i>Ae. caspius</i>	<i>Ae. geniculatus</i>	<i>Ae. albopictus</i>	<i>An. labranchiae</i>
<i>Cx. pipiens</i>	*	-0.77	-0.96	-0.36	-0.97	-0.83	-0.72	-0.98	-0.94
<i>Cx. Theileri</i>	0.21	*	--	-0.81	-0.77	-0.63	-0.30	--	-0.26
<i>Cx. hortensis</i>	0.06	--	*	-0.87	--	--	--	--	--
<i>Cs. longiareolata</i>	<b>0.39</b>	0.12	0.14	*	-0.99	-0.76	-0.99	-0.98	-0.99
<i>Cs. annulata</i>	0.09	0.17	--	0.06	*	--	-0.90	--	-0.51
<i>Ae. caspius</i>	0.14	0.09	--	0.06	..	*	--	--	-0.38
<i>Ae. geniculatus</i>	0.20	0.20	--	0.05	0.04	--	*	..	-0.91
<i>Ae. albopictus</i>	0.01	--	--	0.02	--	--	--	*	--
<i>An. labranchiae</i>	0.08	<b>0.42</b>	--	0.01	0.04	0.03	0.02	--	*



**Figure 4.** The relative abundance of the mosquito species. **A.** In rural areas compared to urban areas, Annaba-El Tarf, Algeria, 2019–2022; **B.** In natural habitats compared to artificial habitats, Annaba-El Tarf, Algeria, 2019–2022.



**Figure 5.** The Venn diagram shows the absolute occurrence of mosquito species. **A.** In rural and urban larval habitats; **B.** In artificial and natural larval habitats, Annaba-El Tarf, Algeria, 2019–2022.

## DISCUSSION

**Taxonomy and diversity comparisons.** In our study, we found nine species from four genera (*Culex*, *Culiseta*, *Aedes*, and *Anopheles*), representing 17% of the total mosquito species in Algeria, according to Merabti et al. (2021), who listed 53 species based on data from 1903 to 2021. It should be noted that the diversity of mosquitoes in our collections does not represent the entire semi-humid region of eastern Algeria, therefore, the results should be compared with previous studies in terms of consistency and study design. The genus *Culex* was mainly represented by *Cx. pipiens*, followed by *Cx. theileri* and *Cx. hortensis*. The *Culiseta* genus included two species: *Cs. longiareolata* and *Cs. annulata*. The genus *Aedes* was composed of three species: *Ae. caspius*, *Ae. geniculatus*, and *Ae. albopictus*. The genus *Anopheles* was represented by only one species, *An. labranchiae*. These species have also been reported in other studies within our study area, which overlaps with the results of our research As reported by Aouati (2009), six species were identified, including *Cx. pipiens*, *Cx. theileri* and *Cs. Annulata*. Tahraoui et al. (2012) reported the presence of 14 species in the humid ecosystem of El-Kala National Park (El Tarf), with *Cx. pipiens*, *Cx. theileri*, *Cx. hortensis*, *Cs. annulata* and *An. labranchiae*. In addition, Lafri et al. (2014) found two species in the Annaba state and El Tarf, namely *Cx. pipiens* and *Cs. longiareolata*. Similarly, Amara Korba et al. (2016)

reported the presence of thirteen species, including *Cx. pipiens*, *Cx. theileri*, *Cs. longiareolata*, *Cs. annulata* and *An. labranchiae*. Furthermore, Dahchar et al. (2017) identified twelve species, including *Cx. pipiens*, in the western region of Annaba. On the other hand, work conducted by Matoug (2017) in the Annaba and El-Tarf regions revealed the presence of eight species, including *Cx. pipiens*, *Cx. theileri*, *Cx. hortensis*, *Cs. longiareolata*, *Cs. annulata* and *Ae. caspius*. Further, Arroussi et al. (2021) reported the presence of eight species in urban areas of Annaba, including *Cx. pipiens*, *Cs. theileri*, *Cs. longiareolata*, *An. labranchiae* and *Ae. caspius*.

The absence of certain species may be mainly due to differences in the type and nature of larval habitats, their proximity to human activities, the presence or absence of vegetation cover, sampling methods, and the duration and period of the study (Amara Korba et al., 2016; Arroussi et al., 2021; Chahed et al., 2021). Furthermore, Okogun et al. (2003) suggested that the distribution of Culicidae could be influenced by various environmental factors such as the physicochemical properties of their aquatic habitats during the larval stage, as well as interspecific associations and climate. The composition of the mosquito fauna differed according to the location and type of sites, as shown by the calculated diversity indices. The highest diversity indices were found in natural sites in rural areas, followed by artificial sites in urban areas. This diversity can be explained by the high number and variety of rural larval habitats, especially natural ones (43 sites), compared to urban larval habitats, which are mostly artificial (39 sites). Furthermore, the larval habitats in rural are less influenced by human activities (especially chemical mosquito control), allowing the natural proliferation of different mosquito species. These results are consistent with previous studies in Algeria (Bouabida, 2014) and elsewhere (Versteirt et al., 2013; Johnston et al., 2014; Ferraguti et al., 2016; Farag et al., 2021; Perrin et al., 2022) where urban larval habitats generally have the lowest mosquito diversity.

According to Johnston et al. (2014), species richness is negatively correlated with factors such as human density and distance between urban areas and puddles. Further, the difference in the distribution of mosquito species can be observed globally based on their food preferences, anthropophilia (preferring human blood) or zoophilia (preferring animal blood). Furthermore, adult mosquitoes in urban areas have fewer and less diverse blood and sugar sources than those in natural habitats, especially in forested areas (Allison et al., 2014). Thus, natural larval habitats are characterized by a more developed and diversified plant cover, which ensures a favourable microclimate for mosquito larvae, which explains the increase in the specific richness of mosquitoes in natural sites. Additionally, Bouabida (2014) suggested that decomposing vegetation provides an environment rich in organic matter that would be favourable for the proliferation of several aquatic species, especially Culicidae. On the contrary, artificial larval habitats are temporary, small, and devoid of vegetation, making them an unfavourable environment for the establishment of diverse mosquito fauna. Our results are consistent with those (Sulesco et al., 2015; Almeida et al., 2020; Srisuka et al., 2022). The Venn diagram revealed that *Cx. pipiens* and *Cs. longiareolata* have good ecological plasticity and can tolerate different types and natures of larval habitats, whether natural, artificial, rural, or urban. This has been confirmed by previous work reporting the abundance and dominance of these two species (Messai et al., 2010; Merabti & Ouakid, 2011; Bouabida et al., 2012; Arroussi et al., 2021; Chahed et al., 2021).

**The vector potential.** Several mosquito species demonstrate remarkable adaptability, utilizing both urban and rural larval habitats. *Culex pipiens*, *Cs. longiareolata*, and *Cx. hortensis* are prime examples of such adaptability. *Culex pipiens* primarily occupy urban artificial sites such as metal tanks, sewers, and road gullies, thriving in stagnant, nutrient-rich water (Berchi, 2000; Schaffner et al., 2010). In rural areas, this species adapts to natural sites like peat bogs and swamps, exhibiting notable plasticity in both polluted and clear waters (Merabti & Ouakid, 2011; Chahed et al., 2021). This adaptability, coupled with its ability to transmit various pathogens, including the West Nile virus, makes *Cx. pipiens* a significant public health concern in both urban and rural settings (Amraoui et al., 2012; Nebbak et al., 2022; Ayhan et al., 2022). Similarly, *Cs. longiareolata* is common in urban artificial habitats, including sewers, pails, and flowerpots, but also inhabits rural natural sites such as valleys and ditches (Bouabida et al., 2012; Merabti et al., 2017). Despite being primarily ornithophilous, its vector potential in both settings

warrants attention. Comparatively, urban habitats offer more consistent breeding opportunities for these species due to the prevalence of stagnant water (González et al., 2020; Toma et al., 2020). *Culex hortensis* also displays remarkable adaptability by inhabiting both urban and rural environments. It thrives in urban sewers and concrete ditches while also utilizing rural wetlands as breeding sites, while its feeding preferences reduce its impact on humans (Brunhes et al., 2000).

Other species exhibit more specialized habitat preferences. *Aedes albopictus* demonstrates a contrasting pattern by exclusively adapting to urban habitats. Found in buckets, jars, and flowerpots in shaded gardens, its preference for clean, nutrient-rich water exemplifies its reliance on human-modified environments (Arroussi et al., 2021; Rouibi et al., 2023; Azari-Hamidian et al., 2024). Unlike other species, its absence from rural settings underscores its invasive nature and significant urban vector potential for diseases such as dengue and chikungunya (Reiter et al., 2006). This distinction highlights its unique ecological role, contrasting with species that traverse both urban and rural habitats, and emphasizes its public health relevance in cities. In contrast, some species are predominantly rural. *Culex theileri* inhabit both natural and artificial sites, with a predominance in natural sites such as ponds, swamps, ditches, and peat bogs. Meanwhile, *An. labranchiae* exclusively occupies rural natural sites like marshes and peat bogs with clear, shallow water (El Ouali Lalami et al., 2010; Merabti & Ouakid, 2011; Laboudi et al., 2012; Lounaci et al., 2016). *Aedes caspius* and *Cs. annulata* are predominantly associated with rural habitats, favouring natural sites such as ditches, swamps, and floodplains. *Aedes caspius* thrives in vegetation-rich floodplains, while *Cs. annulata* prefers organic-rich ditches exposed to sunlight (Merabti & Ouakid, 2011; Bouabida, 2014). *Aedes geniculatus* is exclusively associated with rural habitats. Its larvae were found in temporary natural habitats, such as swamps and ditches with clear water and abundant vegetation, as well as in artificial sites like tyres and concrete ditches rich in organic matter. However, the absence of tree holes (a primary habitat for this species) in this study may limit a comprehensive understanding of its habitat preferences (Brunhes et al., 2000). These species collectively highlight the ecological significance of rural areas for sustaining vector populations. Their roles as vectors for diseases such as West Nile virus, malaria, tularemia, and Usutu virus (Santa-Ana et al., 2006; Azari-Hamidian et al., 2009; Schaffner et al., 2010; Laboudi et al., 2012; Tabbabi et al., 2015; Mancini et al., 2017) necessitate focused surveillance in these habitats.

**Interspecies affinity and association indices.** The investigation of larval inter-species correlation and the measurement of affinity and association indices are important to disseminate key information for a better understanding of the biological role of mosquitoes in pathogen transmission and the co-occurrence of different mosquito species in their habitat (Novianto et al., 2021). The species sampled in our study area showed a tendency to coexist with other mosquito species, indicating a preference for colonizing positively cohabited larval habitats. These findings are consistent with those of Sultana et al. (2018) in Bangladesh. The study found that the most frequently collected species were often the sole occupants of their habitats. Specifically, *Cx. pipiens* accounted for 40.54% ( $n = 30$ ) of the collection, followed by *Cs. longiareolata* at 31.82% ( $n = 14$ ), *An. labranchiae* at 21.43% ( $n = 3$ ), and *Cx. theileri* at 20% ( $n = 5$ ). These findings suggest that these species have a competitive advantage in selecting and occupying oviposition sites that are not already occupied by other mosquito species. However, further research is needed to confirm this hypothesis. Indeed, *Cx. pipiens* exhibited the highest level of species distribution and association. However, it prefers to occupy its egg-laying site solitarily. These results are consistent with those of Azari-Hamidian (2007) and Nikookar et al. (2016) in Iran.

Fager and McGowan's association index and affinity index were calculated for all pairs of species that were collected together. *Anopheles labranchiae* and *Cx. theileri* were found to have an affinity as they appeared together nine (9) times, while they were collected a total of 14 and 25 times respectively. Although the affinity was significant ( $\geq 0.5$ ), it was not very large (0.42). These results are consistent with those of (Nabti & Bounechada, 2020), who found a positive correlation between *Cx. theileri* and *An. labranchiae* in the Setif region using Spearman's Rho test. This demonstrates that species have common requirements for the same type of habitat, which in turn affects larval development and biology through competition for food, exposure to predators, and sensitivity to pesticides (Nikookar et al., 2016).

However, there was no significant correlation between the other pairs, which may be attributed to the differing needs of these species. Additionally, the association index was negative, approaching the value of (-1), indicating inter-species dissociation despite being observed in the same habitat site. Currently, there is limited information available on species co-occurrence and association in Algeria. Additionally, no studies have been conducted on this topic in the East Algerian wetland. It is important to note that the lack of research in this area may hinder conservation efforts and further investigation is necessary. This study has several limitations that warrant attention. Although the sampling period was extensive, it coincided with the COVID-19 pandemic, which imposed significant restrictions on fieldwork and disrupted data collection. These challenges likely affected the dataset's representativeness, particularly in capturing seasonal variations in mosquito populations. Furthermore, the survey did not include all aquatic habitats, such as rocky outcrops, harbours, and tree holes, which may serve as niches for additional mosquito species. Moreover, reliance on a single sampling method may have limited the detection of certain species, potentially underestimating overall species richness and diversity. Additionally, the lack of detailed physicochemical analyses of larval habitats restricts a comprehensive understanding of the environmental factors driving species distribution and abundance.

To address these limitations, future research should integrate molecular identification techniques to enhance the accuracy of species identification, adopt diverse sampling methodologies to improve coverage, and conduct thorough characterizations of habitat parameters, including their physicochemical properties. These approaches will strengthen the ecological understanding of mosquito populations and inform more effective vector management strategies. This research is crucial for monitoring mosquito-borne pathogens and understanding the shifting distribution of vector species due to climate change. We identified nine species, including key vectors such as *Cx. pipiens*, *Cx. theileri*, *Cs. longiareolata*, *Cs. annulata*, *Ae. caspius*, *Ae. albopictus*, and *An. labranchiae*, which are highly adaptable to different habitats and climatic conditions. This study highlights the diversity of mosquitoes and provides valuable insights into the biological, ecological, and geographical factors that influence their distribution. Further research is needed to assess the impact of physicochemical parameters on mosquito habitats. Statistical analyses of these factors can improve the accuracy of surveillance programs, enhancing our understanding of mosquito behaviour and distribution. This will enable more effective vector control strategies and better public health interventions for diseases caused by mosquito-borne pathogens.

## AUTHOR'S CONTRIBUTION

The authors confirm their contribution to the paper as follows: M. Boumaza: collected data (sampling and identification), and performed analytical calculations. He also wrote and edited content; I. Kihel performed the analytic calculations and interpretation of the results; C. Zemouli: collected data and conducted bibliographic research; B. Merabti: contributed to the interpretation of the results, took the lead in writing the manuscript, and supervised the findings of this work; M.L. Ouakidi: supervised the study and proofread the manuscript for validation. The authors read and approved the final version of the manuscript.

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## AVAILABILITY OF DATA AND MATERIAL

The specimens listed in this study are deposited in the Ecology Laboratory of Marine and Coastal Environments (EMMAL), Badji Mokhtar University, Annaba and are available from the curator, upon request.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study only arthropod material, and all required ethical guidelines for the treatment and use of animals were strictly adhered to in accordance with international, national, and institutional regulations. No human participants were involved in any studies conducted by the authors for this article.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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## تنوع، فراوانی و انتشار پشه‌ها (Diptera, Culicidae) در شمال شرقی الجزایر

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**چکیده:** پشه‌ها (Culicidae) حشراتی خون‌خوار بوده و به عنوان ناقلین اصلی عوامل بیماری‌گر پاتوژن‌های مختلفی که بر سلامت انسان تأثیر می‌گذارند، شناخته می‌شوند. درک الگوهای انتشار و تنوع آن‌ها برای ارزیابی خطرات بیماری‌گرها و توسعه استراتژی‌های کنترلی ضروری است. در این مطالعه، فون پشه‌ها در منطقه نیمه‌مرطوب شمال شرقی الجزایر (عنابه-الطارف) در بازه زمانی از اکتبر ۲۰۱۹ تا ژوئیه ۲۰۲۲ بررسی شد. زیستگاه‌های طبیعی و مصنوعی لاروها در مناطق روستایی و شهری بررسی و ارزیابی شد تا عوامل مؤثر بر ظرفیت تکثیر و انتشار پشه‌ها مشخص شود. پشه‌های نابالغ، جمع‌آوری و شناسایی شدند تا تنوع گونه‌ها و فراوانی آن‌ها ارزیابی شود. در مجموع ۵۵۸۷ نمونه پشه نابالغ از ۱۱۱ زیستگاه جمع‌آوری شد که شامل ۹ گونه متعلق به چهار جنس شامل موارد ذیل بودند: *Aedes caspius*, *Cs. annulata*, *Culiseta longiareolata*, *Cx. hortensis*, *Cx. theileri*, *Culex pipiens*، *Anopheles labranchiae* و *Ae. albopictus*، *Ae. geniculatus* بیشترین فراوانی مربوط به گونه‌های جنس *Culex* بود و از بین آنها *Culex pipiens* ۶۳.۲۵٪ از کل فراوانی را تشکیل می‌دهد و پس از آن *Culiseta longiareolata* با ۲۳.۴۱٪ قرار دارد. تنوع گونه‌ها در مناطق شهری نسبت به مناطق روستایی کمتر بود و زیستگاه‌های طبیعی لاروها، دارای گونه‌های بیشتری نسبت به زیستگاه‌های مصنوعی بودند. این مطالعه داده‌های اساسی درباره انتشار، فراوانی و تنوع گونه‌های پشه در شمال شرقی الجزایر ارائه می‌دهد که به درک نوسانات جمعیت پشه‌ها کمک کرده و امکان توسعه بهتر استراتژی‌های کنترل و نظارت بر ناقلین را به ویژه با توجه به فعالیت‌های انسانی در حال تحول و تغییرات اقلیمی فراهم می‌آورد.

**واژگان کلیدی:** پشه آندس، عنابه، الطارف، هم‌زیستگاهی، اکولوژی، تاکسونومی