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Abstract: An extensive survey of aphid predatory coccinellid beetles (Coleoptera: Coccinellidae) was conducted in twentyseven sites in Tunisia over fourteen years from 2005 to 2019. In total, thirteen ladybird beetles (Adalia decempunctata, Chilocorus bipustilatus, Coccinella undecimpunctata, algerica, Coccinella Coccinella novemnotata, Harmonia axyridis, Hippodamia tredecimpunctata, Hippodamia variegata, Oenopia doublieri, Oenopia lyncea, Myrrha octodecimguttata Linnaeus, Scymnus fulvicollis, Scymnus nubilus) were identified as predators of aphid. Among them, C. algerica was the most abundant predator observed preving on twenty-two aphid species found in twenty-seven sites. The second most abundant predator was H. variegata observed feeding on ten aphid Four species (H. axyridis, O. species. doublieri, O. lyncea, S. nubilus) were the least abundant and were observed only in one site.

**Key words:** Coccinellid beetles, aphid, geographical distribution, Tunisia.

# Introduction

Coccinellidae (Lady beetles, ladybirds) comprise one of the largest and most abundant families of the super family Coccinelloidea with over 6.000 nominal species described throughout the world (Canepari, 2011, Robertson *et al.*, 2015). Most of them, including larva and adults, are entomophagous, predators of a wide range of hosts from hemipteran pests such as aphids, mealybugs and scale insects, to

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thrips (Thysanoptera) and mites (Acarina) all over the world (Raimundo et al., 2008). Predaceous groups have a great economic importance and are used today to control the populations of many arthropod pests, particularly aphid species and mealybugs (Agarwala and Dixon, 1992, Mdellel and Ben Halima Kamel, 2012). Some adult ladybirds visit flowers and feed on pollen and nectar, usually as supplement to their predatory diet (Biranvand et al., 2017). In addition to predation, some other species of ladybirds are phytophagous and feed on leaves of cultivated plant species (Cucurbitaceae, Fabaceae) and are considered pests (Koren et al., 2012). Some other species are mycophagous feeding mainly on powdery mildew fungi. Ladybirds are considered to be good indicator species because they are very sensitive to changes in the environment and can offer a better overall picture about the health of ecosystems. The degree of their adaptation as well as their efficiency in controlling aphid populations varies with the species and the environmental conditions (Dixon 2000). The ability of natural enemies to track their hosts will depend on their tolerance to environmental extremes relative to their herbivore hosts as well as their movement rates. Under climate change, the effectiveness of some biological control agents may change. Stireman et al. (2005) predicted that the frequency and intensity of pest outbreaks will increase as climate becomes more variable and disrupts the stability of existing biological control systems. Only few publications on the Coccinellidae in Tunisia exist providing little information about Coccinellidae fauna.

The majority of studies record the ladybird species as predators of pests especially aphids, but so far no comprehensive checklist exists (Mdellel *et al.*, 2015, Ben Halima Kamel *et al.*, 2018). The main goal of this paper is to contribute to our knowledge of diversity and geographical distribution of ladybirds in Tunisia and to present the first preliminary checklist of species recorded until now from Tunisia.

#### Materials and Methods

#### Study sites

The survey was carried out over the period from 2005 to 2019 in different agroecosystems in Tunisia. Localities, site/ zone-name, geographic coordinates and the date of sampling are given in Table 1.

#### Sampling methods

Ladybugs or the larvae of ladybugs and aphids were collected every two weeks from host plants. Ladybugs were collected weekly from a wide variety of habitats (agricultural land, gardens and parks, both on herbaceous and woody plants) using sweep nets, yellow traps and by hand picking. Captured adult insects were killed immediately with diethyl ether and were soon preserved in a ratio of 90% of ethyl alcohol (Eastop and Van Emden, 1972, Blackman and Eastop, 2000) for identification. The larvae of the ladybirds were taken to the laboratory, where they were reared on aphid colonies until adult emergence and were then preserved as described above.

### Aphid and ladybird identification

Alate and apterous adult aphids were removed from the samples and were preserved and mounted on microscope slides (Blackman and Eastop, 2000); they were identified using Leclant (1978) and Blackman and Eastop (1984) keys. The collected ladybirds were carefully studied under a binocular microscope to determine the scientific name of the species using Iablokoff- Khnzorian (1982) keys. They were then sent to Dr Sahraoui L. (Department of Agricultural and Forestry Zoology, ENSA El Harrach/ Algeria) for confirmation.

Climate characteristic and ladybird distribution relationship

The relationship between the climate characteristics of the survey sites (Figure 1) and the ladybird beetle diversity was studied. Several species from sites having the same climate were registered and compared.

#### Results

#### Ladybird species

The ladybird species collected over the period of fourteen years belong to thirteen species in three tribes. One species of the Chilicorini was collected, Chilochorus bipustalitus Linnaeus. The Coccinellini included ten species: Adalia decempunctata Linnaeus, Coccinella algerica Kovar, Coccinella novemnotata Herbst. Coccinella undecimpunctata Linnaeus, Hippodamia variegata Goeze, Hippodamia tredecimpunctata Linnaeus, Harmonia axyridis Pallas, *Oenopia* doublieri Mulsant, Oenopia lyncea Olivier, and Myrrha octodecimguttata Linnaeus. Two species of the Scymnini were collected, Scymnus fulvicollis Mulsant and Scymnus nubilus Mulsant (Figure 2).

#### Abundance of ladybird species

Thirteen ladybird species were identified. Among these, the most frequent species in time and sites was *C. algerica*, observed over the period between2005 and 2019 across twenty-seven sites in the north, middle, and south of Tunisia. *C. algerica* (eggs, larva, and adult) was observed feeding on twenty-two aphid species, found on the leaves and trunks of twenty host plants belonging to eight families (*Poaceae, Rosaceae, Rutaceae, Asteraceae, Solanaceae, Lythceae, Fabacea,* and *Pinaceae*). The second abundant species

Province	Site/Zone	Climate	Sampling period	Crops
Bizerte	Tinja	Sub-Humid	2017 - 2019 December to June	Cereal, Rape (Colza)
	Hammem-Sayala	Sub-Humid	2017-2018	Cereal, Vetch
Daia	Tarhouna		December to June	
Beja	Ghozzia			
	Tebaba			
	Eddir	Semiarid	2017-2018	Cereal, Vetch
Kef	Boulifa		December to June	
Xel	Sers			
	El-Houdth			
	Sidi-Khiar			
Siliana	Krib	Semi-arid	2017- 2019 December to June	Cereal, Faba
Ariana	Sidi-Thabet		2000 -2014 December to May	Peach, Potato
Ben-Arous	Mornag		2007- 2008 April to May	Peach, Apple
Zaghouane	Ras-El Gassaa		2007-2008	Alfah grass
	Takelsa		April to May 2007- 2008	Peach, Citrus
Nabeul	Korbous		April to May	Citrus
Sousse	Chott Mariem	S uperior arid	2007 – 2016 December to June	Peach, almond, artichoke Citrus, cucumber, melon, pomegranate, pepper.
	Sidi-Bouali		2000 – 2005 February to April	Citrus
	Hergla		2008 – 2014 December	Cereal
	Kalaa Kebira		2015 – 2016 May	Apple
	Akouda		2007 – 2016 December to May	Peaches, almond, apricot, Citrus
	Msaken		2007 – 2011 December to February	Potato
Monastir	Jammel	Superior arid	2008 – 2014 April to May	Peaches, almond, apricot, apple, plum
	El-Werdanine		2014 December	Potato
Mahdia	Sidi-Alouane	Superior arid	2008 – 2014 December to April	Cereal, almond, faba
Kairouan	Chebika	Superior arid	2015 – 2019 December to April	Citrus, cereal
Sfax	Manzel-Chaker	Inferior arid	2008 – 2015 April to May	Peaches, almond

Table 1. Sites, climates, and crops of the survey.

was *H. variegata*, recorded from six sites feeding on nine aphid species, found on seven infested host plants belonging to seven families (*Poaceae*, *Rosaceae*, *Rutaceae*, *Asteraceae*, *Solanaceae*, *Lythceae*, and *Pinaceae*) (Table 2). Five species (*C. indecimpunctata*, *C. novemnotata, H. tredecimpunctata, M. octodecimguttata, S. nubilus*) were observed, each at one site, feeding on one aphid species (Table 2). One species, *H. axyridis*, is an introduced species observed for the first time by Ben Halima Kamel et *al.*, (2018) (Table 3).

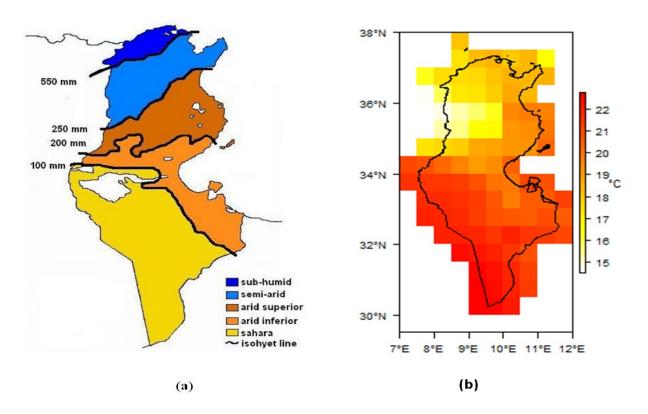


Figure 1. Tunisia climate map based to Koppen climate classification. (a): Average annual precipitation, (b) average annual temperature

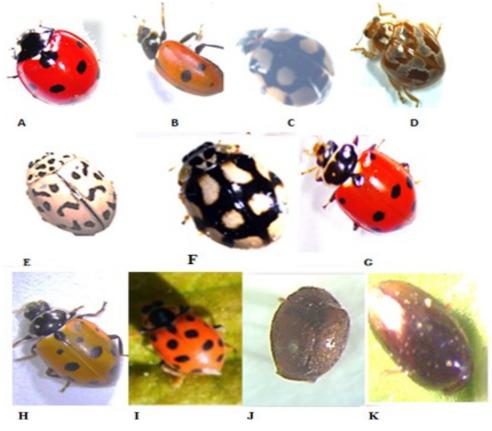


Figure 2. Ladybird species recorded in Tunisia. A: Coccinella algerica, B: Hippodamia variegata, C: Adalia decempunctata, D: Myrrha octodecimguttata, E: Oenopia doublieri, F: Oenopia lyncea, G: Coccinella novemnotata H: Coccinella undecimpunctata, I: Hippodamia tredecimpunctata, J: Scymnus fulvicollis, K: Scymnus nubilus.

Table 2. Ladybird species and aphid hosts.

Ladybird species	Aphid species prey
Chilochorus bipustalitus Linnaeus	Aphis spiraecola Patch
Adalia decempunctata L.	Cinara magrebica Mimeur, Eulachnus agillis Kaltenbach
<i>Coccinella algerica</i> Kovar <i>Coccinella novemnotata</i> Herbst	Aphis gossypii Glover, Aphis fabae Scopoli, Aphis punicae Passerini, Aphis pomi De Geer, A. spiraecola, Brachycaudus schwartzi Börner, Capitophorus eleagnus Olivier, C. magrebica, Diuraphis noxia Kurdjumov, Dysaphis plantaginea Passerini, E. agillis, Hyalopterus pruni Geoffroy, Hyalopterus amygdali Blanchard, Hyalopterus persikonus Miller, Myzus persicae Sulzer, Macrosiphum rosae L., Pterochloroides persicae Cholodkovsky, Rhopalosiphum padi L., Sitobion avenae Fabricius, Sitobion fragariae Wolke, Schizaphis graminum Rondani, Taxoptera aurantii Boyer de Fonscolombe. R. padi
Coccinella undecimpunctata Linnaeus	R. padi
Harmonia axyridis Pallas	C. magrebica, E. agillis.
Hippodamia variegata Goeze	A. gossypii, H. pruni, H. amygdali, M. persicae, P. persicae, A. punicae, Aphis neeri Fonscolombe, C. eleagnus, R. padi, S. avenae.
Hippodamia tredecimpunctata L	C. eleagnus
Oenopia doublieri Mulsant	H. pruni, H. amygdali, C. magrebica, E. agillis.
Myrrha octodecimguttata Linnaeus	E. agillis
Oenopia lyncea Olivier	C. magrebica, E. agillis.
Scymnus fulvicollis Mulsant	H. pruni, H. amygdali, M. persicae, A. punicae, Aphis pomi, A. spiraecola.

Table 3. Ladybird species and geographical distribution.

Ladybird species	Site/Zone
Chilochorus bipustalitus Linnaeus	Chott Mariem, Sidi Bouali, Akouda, Takelsa
Adalia decempunctata L.	Chott Mariem, Boulifa
<i>Coccinella algerica</i> Kovar	Tinja, Hammem-Sayala, Tarhouna, Ghozzia, Tebaba, Eddir, Boulifa, Sers, El-Houdth, Sidi-Khiar, Krib, Sidi-Thabet, Utique, Mornag, Ras-El Gassaa, Takelsa, Korbous, Chott Mariem, Sidi-Bouali, Hergla, Kalaa-Kebira, Akouda, Msaken, Jammel, El Ouerdanine, Sidi-Alouane, Chebika, Manzel-Chaker.
Coccinella novemnotata Herbst	Boulifa
Coccinella undecimpunctata Linnaeus	Boulifa
Harmonia axyridis Pallas	Chott Mariem
Hippodamia variegata Goeze	Sidi Thabet, Akouda, Chott Mariem, Jammel, El Ouerdanine, Chebika, Tinja, Hammem Sayala, Tarhouna, Ghozzia, Tebaba, Eddir, Boulifa, Sers, El-Houdth, Sidi Khiar, Krib
<i>Hippodamia tredecimpunctata</i> L.	Utique
Oenopia doublieri Mulsant	Chott Mariem
Oenopia lyncea Olivier	Chott Mariem
Myrrha octodecimguttata Linnaeus	Chott Mariem
Scymnus fulvicollis Mulsant	Chott Mariem, Jammel, Chebika.
Scymnus nubulis Mulsant	Jammel

Climate and ladybird distribution

The survey of the ladybird distribution demonstrated that certain species can live in sites with different climates (Table 4 and 5), while others were observed only at sites with a specific climate (Table 4).

*C. algerica* can be said to have the widest distribution and was found in all studied areas in which the annual average temperature ranges between 15 and 22 °C. *H. variegata* (second frequent species) and *A. decempunctata* were observed in sites belonging to two climates (semi-arid,

superior arid climates) with the average annual temperature ranging between 19 and 21°C. *O. doublieri, O. lyncea, S. fulvicollis* and *S. nubulis* were less distributed and were recorded only at sites belonging to superior arid climates where the average annual temperature ranges between 19 and 20°C. *H. tredecimpunctata* at Utique and *C. novemnotata* and *C. undecimpunctata* at Boulifa were found in semiarid climates in which the average annual temperature is 18°C. The subfamily Coccinellini is found in all climate areas of Tunisia (Table 5).

Species	Climate zone			
	Inferior arid	Superior arid	Semiarid	Subhumid
Chilochorus bipustalitus L.		+	+	
Adalia decempunctata		+	+	
Coccinella algerica	+	+	+	+
Coccinella novemnotata			+	
Coccinella undecimpuntata			+	
Harmonia axyridis		+		
Hippodamia variegata		+	+	
Hippodamia tredecimpunctata			+	
Oenopia doublieri		+		
Oenopia lyncea		+		
Myrrha octodecimguttata		+		
Scymnus fulvicollis		+		
Scymnus nubulis		+		

Table 5. Ladybird	l subfamily distribution	and function of climate.
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Subfamily		Climate zor	ie	
	Inferior arid	Superior arid	Semiarid	Subhumid
Chilicorini		+	+	
Coccinellini	+	+	+	+
Scymnini		+		

#### Discussion

Over the last decade, climate changes and agricultural practices (intensive agriculture and use of chemical pesticides) affected the insect's fauna and all living organisms. In this context, the current research aimed to highlight the species of *Coccinellidae* fauna present in different habitats and crops in Tunisia. Thirteen ladybird species were identified and *C. algerica* was found to be the most abundant species. This coccinellid is known as a generalist predator and was described based on small morphological differences with the North African populations. It was originally thought to be *Coccinella septumpunctata* L. (Sahraoui *et al.*, 2014; Sahraoui and Gourreau, 2000). Marin *et al.* (2010) demonstrated that these two species were genetically similar. Previous studies in Tunisia, described C. algerica, as larva and as adult, near a population of several aphid species on different host plants (Ben Halima Kamel and Ben Hammouda, 2005; Mdellel and Ben Halima Kamel, 2012; Ben Halima Kamel et al., 2013; Mdellel and Ben Halima Kamel; 2015). H. variegata, the second abundant species, recorded at six sites, was defined as an efficient predator of C. eleagnus on artichoke in Tunisia (Guesmi et al., 2011). It was also defined as an efficient predator of A. gossypii and other aphid species (Rondoni et al., 2014; Hodek and Honek 1996; Obrycki et al., 2009). In Australia, the lady beetle was recorded preying on twelve aphid species; one psyllid species was found to be feeding on various crops, weeds, and ornamental plants (Franzmann, 2002). The other identified species (A, A)decempunctata, C. undecimpunctata, C. novemnotata, H. axyridis, O. doublieri, O. lyncea and S. fulvicollis) were less abundant and have a small number of aphid species preys. Distribution and abundance of some species can be affected by the agroecosystem and the climate. All identified species were recorded in superior arid climates where temperature ranges between19 and 20°C. Mattias et al., (2014) showed that complex landscapes generally harbor higher abundance and richness of beneficial natural enemies than simple landscapes. In complex landscapes, the use of predator ladybirds or parasitoids is more efficient compared to simple landscapes and intensive agriculture (Landis et al., 2008). In addition, climate can impact the host plants, aphid population and their natural enemies. Indeed, aphids are sensitive to climatic conditions, with extreme rainfall and drought events or low winter temperatures directly causing mortality or reducing the population growth (Hulle et al., 2010). Narayandas and Alyokhin (2006) proved that rain can increase the predation risk by dislodging aphids from plants and forcing them to move between plants. However, temperature may facilitate predator effects when aphids are often more sensitive to extreme temperature

fluctuations than their predators (Bale et al., 2002). Extreme temperatures are stronger on aphids than predators when development rate and population density are affected (Bannerman and Roitberg, 2011). Thus, the small abundance of the ladybird species can be explained in relation to the cultural practices in some sites principally in the north of Tunisia (cereals) where a small number of aphid species was recorded, and major ladybird species were identified. Therefore, it is recommended that the impact of cultural practices, aphid diversity, and specificity of some ladybird species should be studied further in the future. Also, the mass rearing of some abundant species of ladybirds and their use in the biological control of aphids can be implemented.

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