Course 4

How to read and understand scientific writings

Types of Scientific Articles

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1 Introduction:

- The objective of scientific writing is to publish and communicate results with others; it is based on both description and analysis.
- So, the scientific writing includes surveys of previous research and descriptions of current methodologies and results. Consequently, science writers present facts and careful reasoning based on evidences.
- However, they must use techniques that ameliorate communication, and shape their presentations in an engaging and persuasive way.
- Therefore, most types of science writing follow a special format that frames the information presented in a defined template.
- The types of science writing outlined here are :
  1- The review article,
  2- The research proposal,
  3- The lab report/research paper

2 The review article (Literature Review):

Objective : a literature review is a summary and analysis of the relevant publications on a topic, we first have to understand what is meant by 'the literature'. In this case, 'the literature' is a collection of all of the relevant written sources on a topic. It will include both theoretical and empirical works. Both types provide scope and depth to a literature review.

Audience: large audience, because a review is more general in its focus than a research article.

Format:

2.1 Abstract

An abstract is a summary of the literature review. It is made up of the following parts :

- A contextual sentence about the motivation behind the research topic
- The thesis statement
- A descriptive statement about the types of literature used in the review
- Summarize of findings
- Conclusion(s) based upon the findings

2.2 Introduction

introduces the topic and its significance and provides a brief preview of the sub-topics or major trends to be covered in the paper :

- Define or identify the general topic, issue, or area of concern. This provides the reader with context for reviewing the literature.

- Identify related trends in what has already been published about the topic; or conflicts in theory, methodology, evidence, and conclusions; or gaps in research and scholarship; or a single problem or new perspective of immediate interest.
- Establish your reason (point of view) for reviewing the literature; explain the criteria to be used in analyzing and comparing literature and the organization of the review (sequence); and, when necessary, state why certain literature is or is not included (scope) -

2.3 Body

presents a survey of the stages or significant trends in the research

**Tips:* the body should contain both generalizations about the set of studies under review (written in the present tense) and citations of specific studies (past tense) to identify and verify observed trends.

Topic and concluding sentences of paragraphs and/or sections should synthesize research findings and may show differences and similarities or points of agreement/disagreement.

It can be organized in 3 ways :

- **Chronological**- by publication or by trend
- **Thematic**- organized around a topic or issue, rather than the progression of time
- **Methodical**- the focusing factor usually does not have to do with the content of the material. Instead, it focuses on the "methods" of the literature's researcher or writer that you are reviewing

2.4 Conclusion

Provides a final general overview of what is known and what is left to explore in the field. This section may discuss practical implications or suggest directions for future research.

2.5 Bibliography

Since a literature review is composed of pieces of research, it is very important to correctly cite the reviewed literature, both in the reviews body as well as in a bibliography/works cited.

Exemple :

VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 12, Number 6, 2012
© Mary Ann Liebert, Inc.
DOI: 10.1089/vbz.2011.0814

REVIEW ARTICLE

A Review of the Invasive Mosquitoes in Europe: Ecology, Public Health Risks, and Control Options

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Guy Hendrickx[2] Herve Zeller[5] and Wim Van Bortel[5]

Abstract

There has been growing interest in Europe in recent years in the establishment and spread of invasive mosquitoes, notably the incursion of *Aedes albopictus* through the international trade in used tires and lucky bamboo, with onward spread within Europe through ground transport. More recently, five other non-European aedine mosquito species have been found in Europe, and in some cases populations have established locally and are spreading. Concerns have been raised about the involvement of these mosquito species in transmission cycles of pathogens of public health importance, and these concerns were borne out following the outbreak of chikungunya fever in Italy in 2007, and subsequent autochthonous cases of dengue fever in France and Croatia in 2010. This article reviews current understanding of all exotic (five introduced invasive and one intercepted) aedine species in Europe, highlighting the known import pathways, biotic and abiotic constraints for establishment, control strategies, and public health significance, and encourages Europe-wide surveillance for invasive mosquitoes.

Key Words: *Aedes*—Europe—Invasive mosquitoes—Vector-borne disease.

Introduction

THE RECENT EMERGENCE OF BLUETONGUE VIRUS in northern Europe, large scale outbreaks of West Nile fever in North America, and an outbreak of chikungunya fever in Italy, reminds us of the very real risk of exotic pathogens being transported to Europe throughout the rapidly-changing, interconnected world. Such connectivity also increases the possibilities for movement of disease vectors, particularly mosquitoes and ticks, and this has created novel scenarios whereby established exotic vectors have facilitated transmission of previously tropical and subtropical pathogens in Europe.

Continued vector importation events, in combination with climatic and environmental changes, increase the likelihood of the establishment and adaptation of vectors to new environments. In the case of exotic invasive mosquitoes, new disease vector species are frequently being recorded in Europe, and the spread of these species within Europe is evidenced by the growing list of countries known to harbor certain species.

To address this problem, and to facilitate Europe-wide collaboration, the European Centre for Disease Prevention and Control (ECDC) established VBORNET, a network of European medical entomologists and public health experts, in order to assist ECDC activities involving arthropod vector surveillance within the European Union, and consequently to improve preparedness for vector-borne diseases (www.vbornet.eu).

This article focuses purely on the invasive mosquito species that have been imported into Europe over the past 40 years. The aim of this review is to provide an update on the current status of introduced invasive and intercepted aedine mosquito species, detail their geographic spread thus far, assess their pathways for importation and onward dissemination, to analyze their known vector status and potential public health risks for Europe, detail their biology, and to assess climatic thresholds for survival and strategies for control.

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Major generic changes within the tribe aedini were recently published (Reinert 2000; Reinert et al. 2004, 2006, 2008), leading to scientific debate and two or more names being simultaneously used for a single taxon. In this article we use the traditional names, with alternate names also shown: *Aedes aegypti*, also known as *Stegomyia aegypti* sensu (Reinert et al. 2004); *Aedes albopictus*, also known as *Stegomyia albopicta* sensu (Reinert et al. 2004); *Aedes atropalpus*, also known as *Ochlerotatus atropalpus* sensu (Reinert et al. 2004); *Georgecraigius atropalpus* sensu (Reinert et al. 2006); *Aedes japonicus*, also known as *Ochlerotatus japonicus* sensu (Reinert et al. 2004); *Hulecoeteomyia japonica* sensu (Reinert et al. 2006); *Aedes koreicus*, also known as *Ochlerotatus koreicus* sensu (Reinert et al. 2004); *Hulecoeteomyia koreica* sensu (Reinert et al. 2006); and *Aedes triseriatus*, also known as *Ochlerotatus triseriatus* sensu (Reinert et al. 2004).

Overview of Invasive Mosquito Species in Europe

Aedes albopictus

Of the invasive mosquito species discovered in Europe, the Asian Tiger mosquito, *Aedes* (*Stegomyia*) *albopictus*, probably presents the major threat to public health in Europe. It is listed as one of the top 100 invasive species by the Invasive Species Specialist Group (Invasive Species Specialist Group 2009), and is considered to be the most invasive mosquito species in the world. *Aedes albopictus* originated in Southeast Asia, but has spread during the last 30–40 years (Paupy et al. 2009) to North, Central and South America, parts of Africa, northern Australia, and several countries in Europe. Since its first appearance in Albania in 1979 and Italy in 1990, *A. albopictus* has been reported in 20 European countries (Fig. 1 and Table 1), including Albania, Belgium, Bosnia and Herzegovina, Croatia, France (including Corsica), Germany, Greece, Italy (including Sardinia and Sicily), Malta, Monaco, Montenegro, the Netherlands, San Marino, Serbia, Slovenia, Spain, Switzerland, and the Vatican City (European Centre for Disease Prevention and Control 2009; Gatt et al. 2009: Petric 2009), as well as Bulgaria, where it has been recently reported (O. Mikov, personal communication) and Turkey (O. Alten, K. Oter; personal communication). It is unclear whether populations in some countries of northwestern Europe will establish, particularly into the Netherlands, as the populations found in greenhouses are thought to be non-diapausing strains imported from southern China on lucky bamboo (Scholte et al. 2007). However, strains recently introduced to the

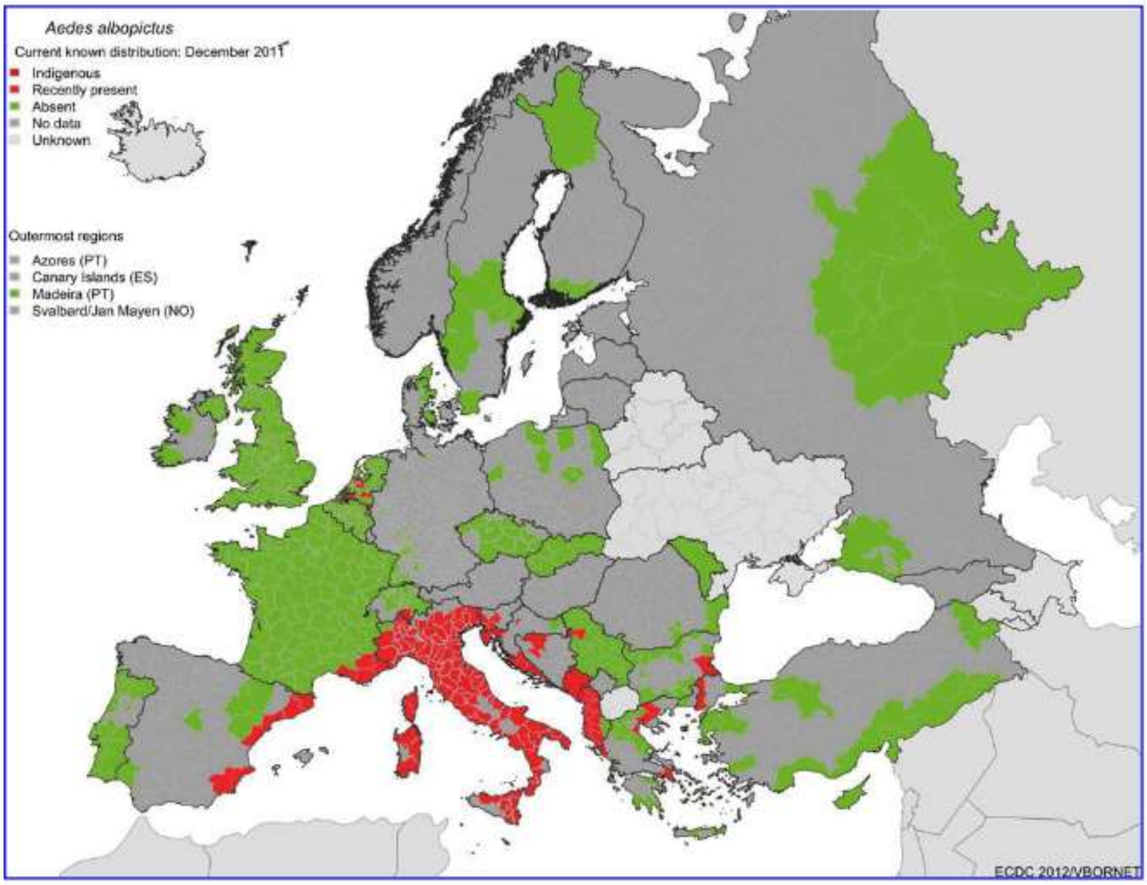


FIG. 1. The currently known distribution of *Aedes albopictus* in Europe in September 2011. The most recent updated map can be downloaded from www.vbornet.eu (ES, Spain; PS, Portugal; NO, Norway).

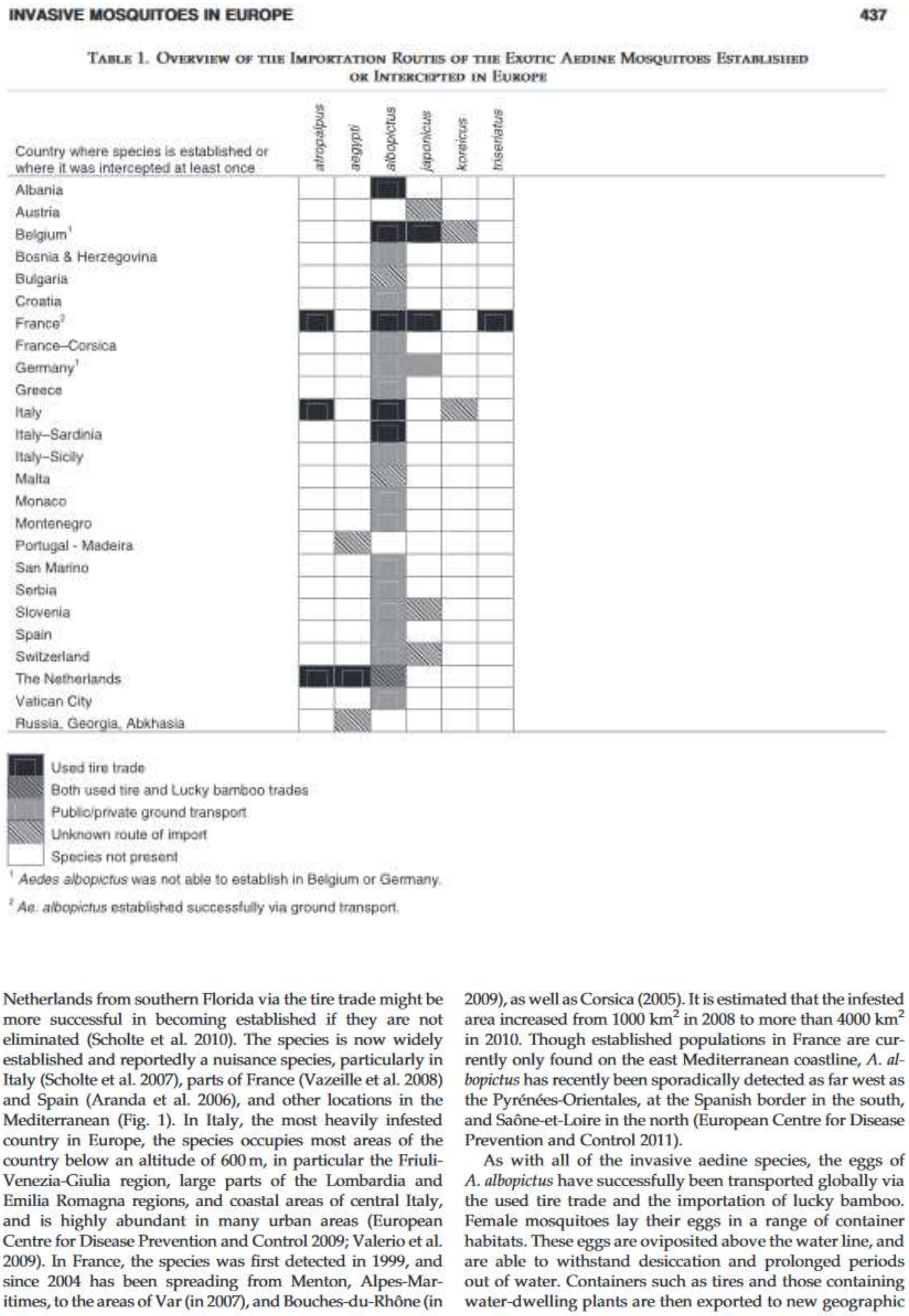


TABLE 1. OVERVIEW OF THE IMPORTATION ROUTES OF THE EXOTIC AEDINE MOSQUITOES ESTABLISHED OR INTERCEPTED IN EUROPE

Country where species is established or where it was intercepted at least once	*atropalpus*	*aegypti*	*albopictus*	*japonicus*	*koreicus*	*triseriatus*
Albania			■			
Austria				▨		
Belgium[1]			■	■	▨	
Bosnia & Herzegovina			▒			
Bulgaria			▨			
Croatia			▒			
France[2]	■		■	■		■
France–Corsica			▒			
Germany[1]			▒	▒		
Greece			▒			
Italy	■		■		▨	
Italy–Sardinia			■			
Italy–Sicily			▒			
Malta			▨			
Monaco			▒			
Montenegro			▒			
Portugal - Madeira		▨				
San Marino			▒			
Serbia			▒			
Slovenia			▒	▨		
Spain			▒			
Switzerland			▒	▨		
The Netherlands	■	■	▩			
Vatican City			▒			
Russia, Georgia, Abkhasia		▨				

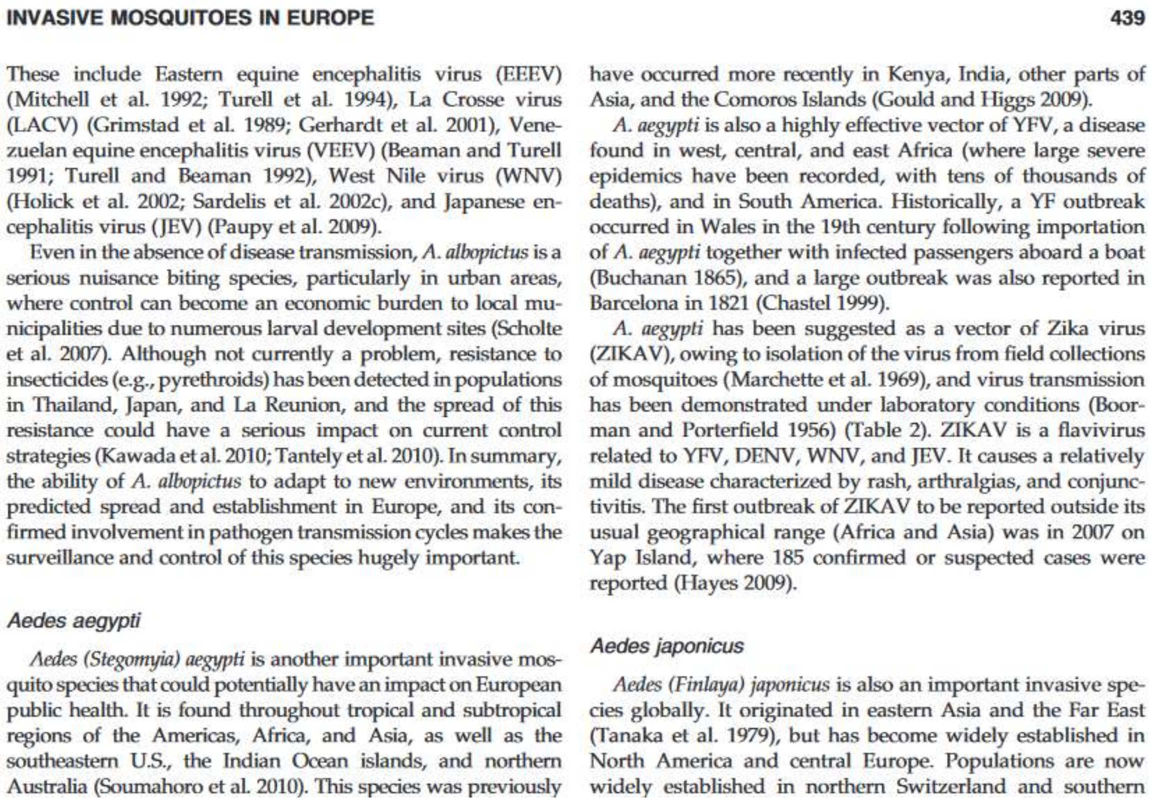
■ Used tire trade
▩ Both used tire and Lucky bamboo trades
▒ Public/private ground transport
▨ Unknown route of import
□ Species not present

[1] *Aedes albopictus* was not able to establish in Belgium or Germany.

[2] *Ae. albopictus* established successfully via ground transport.

Netherlands from southern Florida via the tire trade might be more successful in becoming established if they are not eliminated (Scholte et al. 2010). The species is now widely established and reportedly a nuisance species, particularly in Italy (Scholte et al. 2007), parts of France (Vazeille et al. 2008) and Spain (Aranda et al. 2006), and other locations in the Mediterranean (Fig. 1). In Italy, the most heavily infested country in Europe, the species occupies most areas of the country below an altitude of 600 m, in particular the Friuli-Venezia-Giulia region, large parts of the Lombardia and Emilia Romagna regions, and coastal areas of central Italy, and is highly abundant in many urban areas (European Centre for Disease Prevention and Control 2009; Valerio et al. 2009). In France, the species was first detected in 1999, and since 2004 has been spreading from Menton, Alpes-Maritimes, to the areas of Var (in 2007), and Bouches-du-Rhône (in 2009), as well as Corsica (2005). It is estimated that the infested area increased from 1000 km^2 in 2008 to more than 4000 km^2 in 2010. Though established populations in France are currently only found on the east Mediterranean coastline, *A. albopictus* has recently been sporadically detected as far west as the Pyrénées-Orientales, at the Spanish border in the south, and Saône-et-Loire in the north (European Centre for Disease Prevention and Control 2011).

As with all of the invasive aedine species, the eggs of *A. albopictus* have successfully been transported globally via the used tire trade and the importation of lucky bamboo. Female mosquitoes lay their eggs in a range of container habitats. These eggs are oviposited above the water line, and are able to withstand desiccation and prolonged periods out of water. Containers such as tires and those containing water-dwelling plants are then exported to new geographic



INVASIVE MOSQUITOES IN EUROPE **439**

These include Eastern equine encephalitis virus (EEEV) (Mitchell et al. 1992; Turell et al. 1994), La Crosse virus (LACV) (Grimstad et al. 1989; Gerhardt et al. 2001), Venezuelan equine encephalitis virus (VEEV) (Beaman and Turell 1991; Turell and Beaman 1992), West Nile virus (WNV) (Holick et al. 2002; Sardelis et al. 2002c), and Japanese encephalitis virus (JEV) (Paupy et al. 2009).

Even in the absence of disease transmission, *A. albopictus* is a serious nuisance biting species, particularly in urban areas, where control can become an economic burden to local municipalities due to numerous larval development sites (Scholte et al. 2007). Although not currently a problem, resistance to insecticides (e.g., pyrethroids) has been detected in populations in Thailand, Japan, and La Reunion, and the spread of this resistance could have a serious impact on current control strategies (Kawada et al. 2010; Tantely et al. 2010). In summary, the ability of *A. albopictus* to adapt to new environments, its predicted spread and establishment in Europe, and its confirmed involvement in pathogen transmission cycles makes the surveillance and control of this species hugely important.

Aedes aegypti

Aedes (Stegomyia) aegypti is another important invasive mosquito species that could potentially have an impact on European public health. It is found throughout tropical and subtropical regions of the Americas, Africa, and Asia, as well as the southeastern U.S., the Indian Ocean islands, and northern Australia (Soumahoro et al. 2010). This species was previously

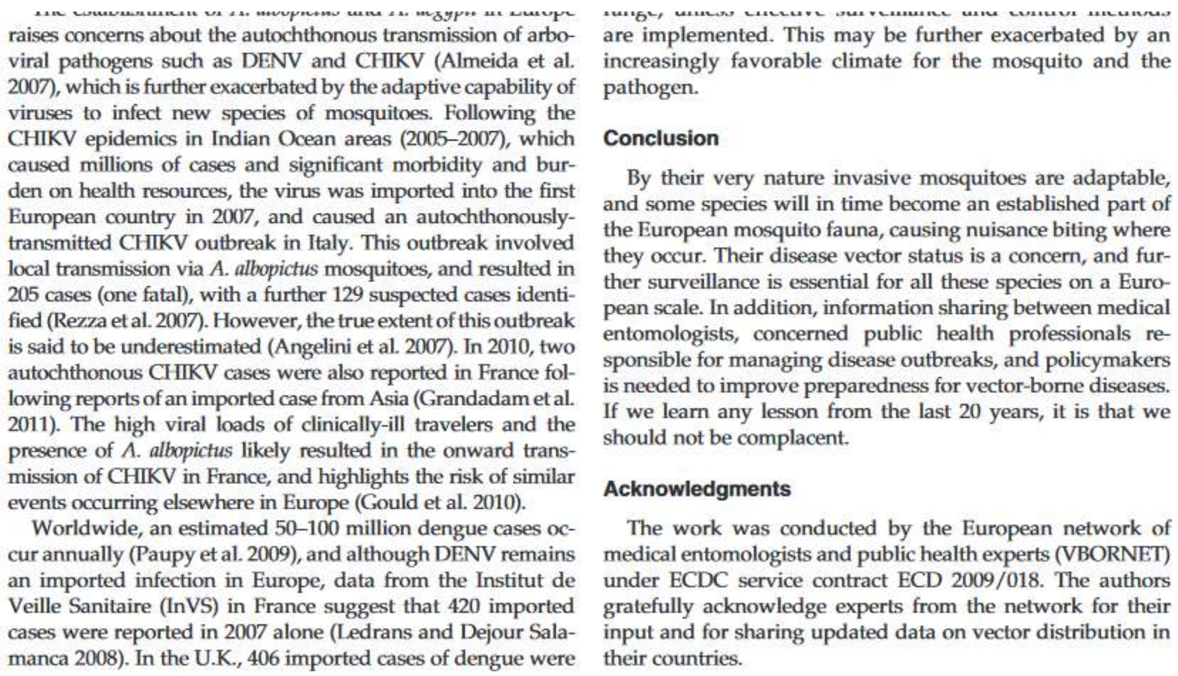
have occurred more recently in Kenya, India, other parts of Asia, and the Comoros Islands (Gould and Higgs 2009).

A. aegypti is also a highly effective vector of YFV, a disease found in west, central, and east Africa (where large severe epidemics have been recorded, with tens of thousands of deaths), and in South America. Historically, a YF outbreak occurred in Wales in the 19th century following importation of *A. aegypti* together with infected passengers aboard a boat (Buchanan 1865), and a large outbreak was also reported in Barcelona in 1821 (Chastel 1999).

A. aegypti has been suggested as a vector of Zika virus (ZIKAV), owing to isolation of the virus from field collections of mosquitoes (Marchette et al. 1969), and virus transmission has been demonstrated under laboratory conditions (Boorman and Porterfield 1956) (Table 2). ZIKAV is a flavivirus related to YFV, DENV, WNV, and JEV. It causes a relatively mild disease characterized by rash, arthralgias, and conjunctivitis. The first outbreak of ZIKAV to be reported outside its usual geographical range (Africa and Asia) was in 2007 on Yap Island, where 185 confirmed or suspected cases were reported (Hayes 2009).

Aedes japonicus

Aedes (Finlaya) japonicus is also an important invasive species globally. It originated in eastern Asia and the Far East (Tanaka et al. 1979), but has become widely established in North America and central Europe. Populations are now widely established in northern Switzerland and southern



raises concerns about the autochthonous transmission of arboviral pathogens such as DENV and CHIKV (Almeida et al. 2007), which is further exacerbated by the adaptive capability of viruses to infect new species of mosquitoes. Following the CHIKV epidemics in Indian Ocean areas (2005–2007), which caused millions of cases and significant morbidity and burden on health resources, the virus was imported into the first European country in 2007, and caused an autochthonously-transmitted CHIKV outbreak in Italy. This outbreak involved local transmission via *A. albopictus* mosquitoes, and resulted in 205 cases (one fatal), with a further 129 suspected cases identified (Rezza et al. 2007). However, the true extent of this outbreak is said to be underestimated (Angelini et al. 2007). In 2010, two autochthonous CHIKV cases were also reported in France following reports of an imported case from Asia (Grandadam et al. 2011). The high viral loads of clinically-ill travelers and the presence of *A. albopictus* likely resulted in the onward transmission of CHIKV in France, and highlights the risk of similar events occurring elsewhere in Europe (Gould et al. 2010).

Worldwide, an estimated 50–100 million dengue cases occur annually (Paupy et al. 2009), and although DENV remains an imported infection in Europe, data from the Institut de Veille Sanitaire (InVS) in France suggest that 420 imported cases were reported in 2007 alone (Ledrans and Dejour Salamanca 2008). In the U.K., 406 imported cases of dengue were

are implemented. This may be further exacerbated by an increasingly favorable climate for the mosquito and the pathogen.

Conclusion

By their very nature invasive mosquitoes are adaptable, and some species will in time become an established part of the European mosquito fauna, causing nuisance biting where they occur. Their disease vector status is a concern, and further surveillance is essential for all these species on a European scale. In addition, information sharing between medical entomologists, concerned public health professionals responsible for managing disease outbreaks, and policymakers is needed to improve preparedness for vector-borne diseases. If we learn any lesson from the last 20 years, it is that we should not be complacent.

Acknowledgments

The work was conducted by the European network of medical entomologists and public health experts (VBORNET) under ECDC service contract ECD 2009/018. The authors gratefully acknowledge experts from the network for their input and for sharing updated data on vector distribution in their countries.

3 The research proposal :

Objective: to convince a scientific audience that a proposed problem for investigation is worth exploring and that the proposed research approach will be effective. The proposal should present a specific, interesting research question and demonstrate the following:

- The question’s significance,
- The merit of the proposed research methods,
- The ways in which results will contribute to the solution of the problem,
- And the degree to which the research will advance the state of the science in this area.

A research proposal is simply a structured, formal document that explains

1- **what** the author planed to research (i.e. his research topic),
2- **why** it’s worth researching (i.e. his justification),
3- and **how** he planed to investigate it (i.e. his practical approach).

Audience: academic departments that grant approval for dissertation projects and funding agencies. Research proposals are often read by a broader range of readers than a journal article.

Format:

3.1 Introduction

Presents the specific objectives and scientific significance of the proposed research and also previews the rest of the paper. Because the audience may be more general than for the research article, the introduction provides a more comprehensive orientation to the topic and to the purpose of and need for the proposed research.

The introduction must achieve a few goals:

- Introduces the topic
- States the problem statement and the questions that the research aims to answer
- Provides context for the research

3.2 Background

In this part the author explains:

- *why* his research is necessary and

- *how* it relates to established research in his field.

The work might :

- complement existing research,

- strengthen it,

- or even challenge it

No matter how the work will “play with” other researchers’ work, the author needs to express it in detail in the research proposal.

This is also the section where the auther clearly define the existing problems that the research will address. By doing this, the reader will understand why the work is necessary.

In the background significance section, the auther also outline how he conductes his research. moreover, the reader can find in this section what questions and issues the auther will not cover in the research.

3.3 Literature review

In the literature review, the author introduces all the sources he planed to use in his research. This includes landmark studies and their data, books, and scholarly articles. A literature review isn’t merely a list of sources (that’s what the bibliography is for); a literature review delves into the collection of sources that the author chosed and explains how he used them in his research.

3.4 Research design, methods, and schedule

Following the research review, the author discuss his research plans. Therfore, the author in this section will cover these aspects:

- The type of research. Is he conducting qualitative or quantitative research? Is he collecting original data or working with data collected by other researchers?
- Whether he is doing experimental, correlational, or descriptive research
- The data he is working with. For example, if he is conducting research in the biological sciences, he will describe for example : the study area, the sample in question…etc. He will also cover how he selected the subjects and how he collected data.
- The tools useed to collect data. If the author runned experiments? Conducted surveys? Observed phenomena?
- All data collection methods were noted here along with *why there are effective methods for this specific research.*

Beyond a comprehensive look at the research itself, the reader will also find:

- The research timeline
- The research budget
- Any potential obstacles the author found and his plan for handling them

3.5 Suppositions and implications

In this part the author will explain how the work will contribute to his field. This section is perhaps the most critical to the research proposal’s argument because it expresses *exactly why* the research in question is necessary.

In this section, the author may note:

- Any ways his work can challenge existing theories and assumptions in his field
- How the work will create the foundation for future research
- The practical value that the findings will provide to practitioners, educators, and other academics in the same field
- The problems that the work can potentially help to fix
- Policies that could be impacted by the findings
- How the findings can be implemented and used in academia or other settings and how this will improve or otherwise transform these settings

In other words, this section isn't about stating the specific results the author has expected. Rather, it is where you state *how* the research findings will be valuable.

3.6 Conclusion

Just like any conclusion paragraph for an essay, the conclusion here will briefly summarize the research proposal and reinforce the research's stated purpose.

3.7 Bibliography

In addition to the literature review section, the bibliography section must be present. However, in this part the author will simply list the sources and their authors.

Sometimes, a full bibliography is not needed. The reader will find a scaled-down list of all the sources the authored cited in the work.

Example :

See Annex 1.

4 The lab report/research paper

Objective: to present either the results of primary research accomplished through study/experimentation or theoretical developments in a particular field. In both the lab report and research article, the writing is both descriptive/factual and analytical/persuasive. It accurately reports the details of the research but also attempts to convince readers of the importance of the research in terms of its contribution to the advancing knowledge in the field.

Audience: for the student writer, most often a professor or teaching assistant; for the report writer, a wider audience – a journal editor, peer reviewers, a community of specialists in a discipline, the general scientific community.

Format: follows the dominant format of articles in science journals – Introduction, Methods, Results, and Discussion (IMRAD).

4.1 Title

The title of the research article is one of the first indicators of the paper concepts. It will be concise, accurate, and informative. In a title, the reader will find the most relevant keywords of the article.

4.2 Keywords

Keywords are an essential part of producing a journal article; when reading a journal article, the reader will find that the keywords are ranking according to the level of their relation to the article subject.

Keywords help the readers to discover the article when conducting research using search engines.

4.3 Abstract

In the abstract, the author express the key points of his research, clearly and concisely. An abstract is always be well considered, as it is the primary element that readers come across.

An abstract is a short paragraph (around 300 words) that summarizes the findings of the journal article. Ordinarily an abstract will be comprised of:

- What the research is about
- What methods have been used
- What the main findings are

4.4 Introduction

An introduction not only introduces the topic, but it also demonstrate the argument in its broader academic field.

The purpose of the introduction is than:

- Introduces topic of investigation and its importance
- States central question or hypothesis
- Cites any relevant literature
- May identify gap in the research
- States objective or purpose

Finally the introduction will answer to these questios:

- Why is this topic significant or interesting?
- Why did the author undertake this investigation?

4.5 Main body

The main body is where the main arguments and evidence are located. Each paragraph will encapsulate a different notion and there will be clear linking between each paragraph.

Here we will find the material and methods, the results and the discussion.

4.5.1 Material and methods:

In this part, the author will Describe the research design and provide details of the experimental procedure step by step.

The author in this part will answer to these questions:

- How could someone else replicate the study or experiment?

4.5.2 Results:

In this part, the author reports in detail the findings of his investigation and provides figures and tables to support the text

The author in this part will answer to these questions:

- What actually happened?
- What are the findings in details?

4.5.3 Discussion:

Here, the author:

- summarizes results and draws tentative conclusions,
- add comments on results related to research question or hypothesis
- Examines other supporting or contradicting evidence
- Suggests refinements or applications of research
- Offers possibilities for further study

The author in this part will answer to these questions:

- Why did the author obtain these results?
- Did he obtain the expected results? Why or why not?
- How do the conclusions relate to other research on the topic?
- How might this study or experiment be improved or used?
- What next?

4.6 Conclusion

In the conclusion, the author interpret his results, where he summarises all of the concepts that he introduced in the main body of the text in order of most to least important. A new concepts can be introduced in this section.

4.7 References and citations

In this part, the author stats the list of references he used in his research, mostly they are the most strongly related papers to his research question.

4.8 Acknowledgements

Acknowledgements can appear to be a small aspect of the journal article, however it is still important. This is where the author acknowledge the individuals who do not qualify for co-authorship, but contributed to his article intellectually, financially, or in some other manner.

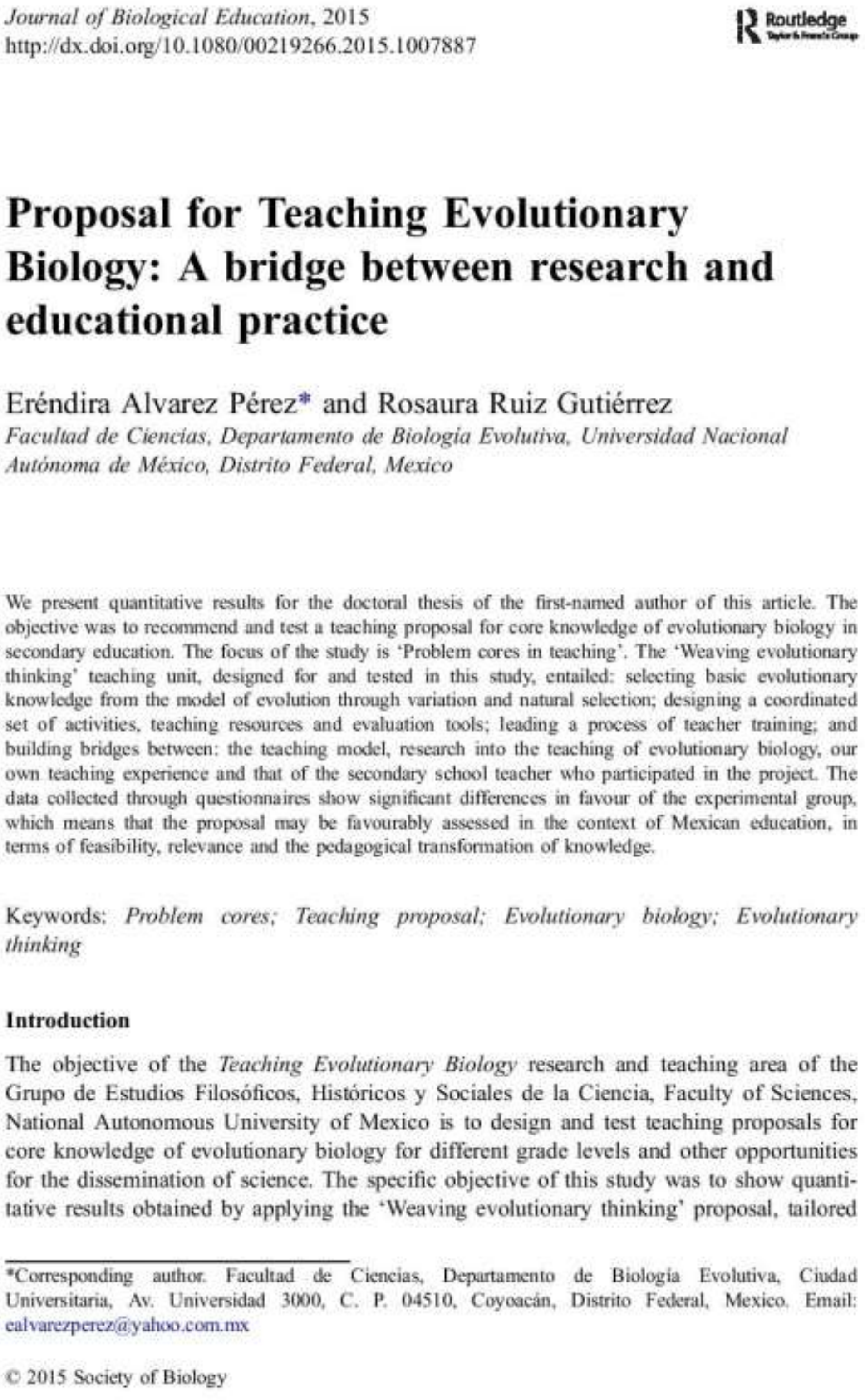
Example :

See Annex 2

Annex 1:

The document here is not complete, it is used merely as an example of research proposal. the entire document can be found in this address:

https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwjqv8OFh9f2AhUJiP0HHczWCqEQFnoECAIQAQ&url=https%3A%2F%2Fwww.tandfonline.com%2Fdoi%2Ffull%2F10.1080%2F00219266.2015.1007887&usg=AOvVaw34dWxTKSHAuAMv9reSigz1



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Proposal for Teaching Evolutionary Biology: A bridge between research and educational practice

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We present quantitative results for the doctoral thesis of the first-named author of this article. The objective was to recommend and test a teaching proposal for core knowledge of evolutionary biology in secondary education. The focus of the study is 'Problem cores in teaching'. The 'Weaving evolutionary thinking' teaching unit, designed for and tested in this study, entailed: selecting basic evolutionary knowledge from the model of evolution through variation and natural selection; designing a coordinated set of activities, teaching resources and evaluation tools; leading a process of teacher training; and building bridges between: the teaching model, research into the teaching of evolutionary biology, our own teaching experience and that of the secondary school teacher who participated in the project. The data collected through questionnaires show significant differences in favour of the experimental group, which means that the proposal may be favourably assessed in the context of Mexican education, in terms of feasibility, relevance and the pedagogical transformation of knowledge.

Keywords: *Problem cores; Teaching proposal; Evolutionary biology; Evolutionary thinking*

Introduction

The objective of the *Teaching Evolutionary Biology* research and teaching area of the Grupo de Estudios Filosóficos, Históricos y Sociales de la Ciencia, Faculty of Sciences, National Autonomous University of Mexico is to design and test teaching proposals for core knowledge of evolutionary biology for different grade levels and other opportunities for the dissemination of science. The specific objective of this study was to show quantitative results obtained by applying the 'Weaving evolutionary thinking' proposal, tailored

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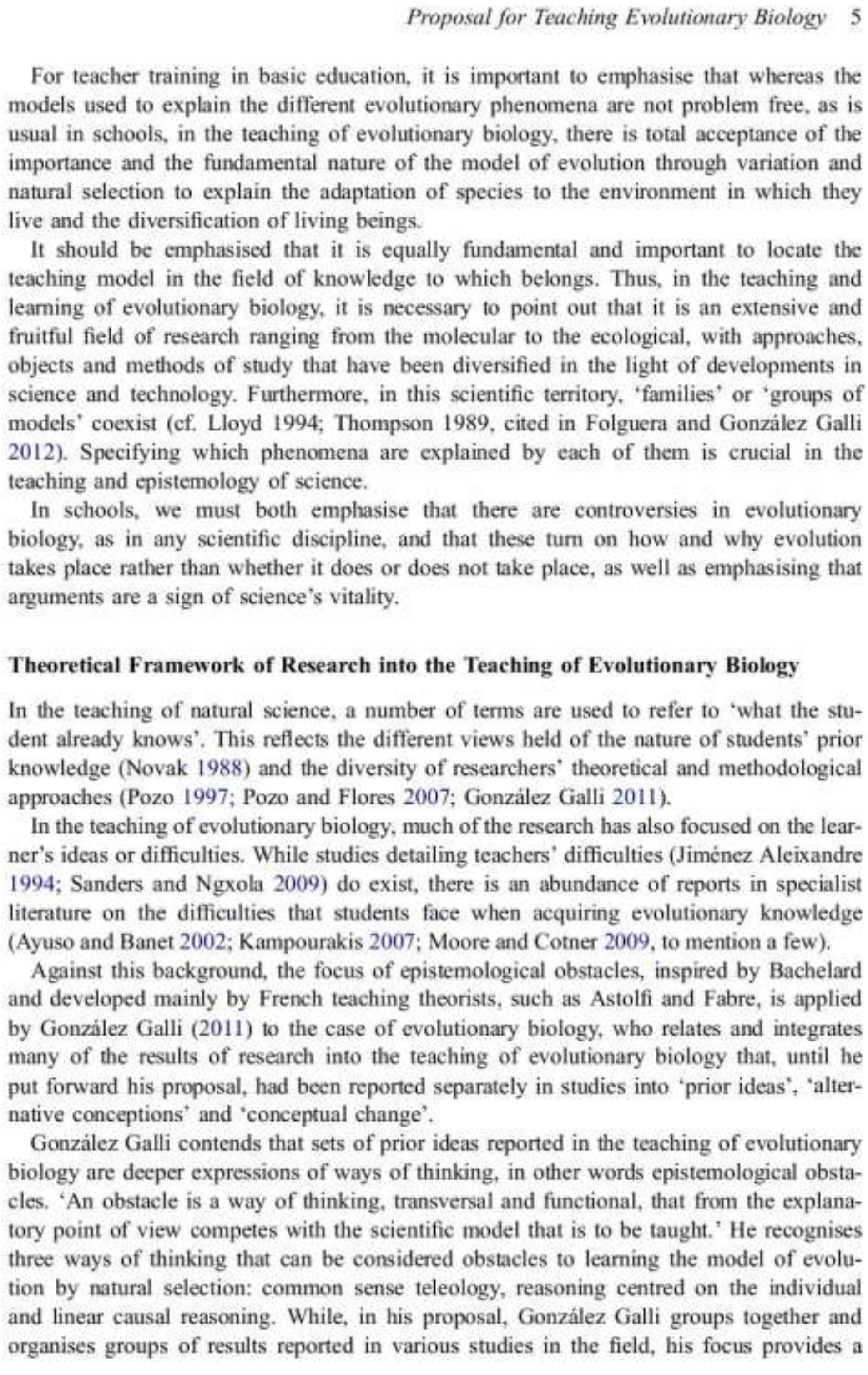

For teacher training in basic education, it is important to emphasise that whereas the models used to explain the different evolutionary phenomena are not problem free, as is usual in schools, in the teaching of evolutionary biology, there is total acceptance of the importance and the fundamental nature of the model of evolution through variation and natural selection to explain the adaptation of species to the environment in which they live and the diversification of living beings.

It should be emphasised that it is equally fundamental and important to locate the teaching model in the field of knowledge to which belongs. Thus, in the teaching and learning of evolutionary biology, it is necessary to point out that it is an extensive and fruitful field of research ranging from the molecular to the ecological, with approaches, objects and methods of study that have been diversified in the light of developments in science and technology. Furthermore, in this scientific territory, 'families' or 'groups of models' coexist (cf. Lloyd 1994; Thompson 1989, cited in Folguera and González Galli 2012). Specifying which phenomena are explained by each of them is crucial in the teaching and epistemology of science.

In schools, we must both emphasise that there are controversies in evolutionary biology, as in any scientific discipline, and that these turn on how and why evolution takes place rather than whether it does or does not take place, as well as emphasising that arguments are a sign of science's vitality.

Theoretical Framework of Research into the Teaching of Evolutionary Biology

In the teaching of natural science, a number of terms are used to refer to 'what the student already knows'. This reflects the different views held of the nature of students' prior knowledge (Novak 1988) and the diversity of researchers' theoretical and methodological approaches (Pozo 1997; Pozo and Flores 2007; González Galli 2011).

In the teaching of evolutionary biology, much of the research has also focused on the learner's ideas or difficulties. While studies detailing teachers' difficulties (Jiménez Aleixandre 1994; Sanders and Ngxola 2009) do exist, there is an abundance of reports in specialist literature on the difficulties that students face when acquiring evolutionary knowledge (Ayuso and Banet 2002; Kampourakis 2007; Moore and Cotner 2009, to mention a few).

Against this background, the focus of epistemological obstacles, inspired by Bachelard and developed mainly by French teaching theorists, such as Astolfi and Fabre, is applied by González Galli (2011) to the case of evolutionary biology, who relates and integrates many of the results of research into the teaching of evolutionary biology that, until he put forward his proposal, had been reported separately in studies into 'prior ideas', 'alternative conceptions' and 'conceptual change'.

González Galli contends that sets of prior ideas reported in the teaching of evolutionary biology are deeper expressions of ways of thinking, in other words epistemological obstacles. 'An obstacle is a way of thinking, transversal and functional, that from the explanatory point of view competes with the scientific model that is to be taught.' He recognises three ways of thinking that can be considered obstacles to learning the model of evolution by natural selection: common sense teleology, reasoning centred on the individual and linear causal reasoning. While, in his proposal, González Galli groups together and organises groups of results reported in various studies in the field, his focus provides a point of reference for locating, resizing and contrasting the approach of research into PCT.

The central idea of PCT is that teaching and learning processes link problems of different natures: students' and teachers' epistemological obstacles, students' and educators' training and careers, the nature of the teaching model, teaching strategies, the curricular space and format occupied by the topic, unfortunate images of evolution used as teaching resources, the treatment of the topic in text books and, most commonly, various combinations of these factors, which make the problems more difficult to deal with. These 'problem cores' may be related–and may even cause each other. Consequently, the results reported in the literature need to be organised and systematised. This means distinguishing between the sources of origin and differentiating their natures, problematising, establishing approaches to dialogue, organising results, designing and testing integrated teaching proposals, guided by field research. This is the case of the teaching unit that was designed and tested in this investigation.

The detail of the proposed approach is the reason underlying a publication in progress, in which it is stated that the main areas of incidence of teaching problems are as follows: evolutionary biology and the associated metadisciplines (epistemology and history) (cf. Adúriz-Bravo 2010, 2011); the implications and the philosophical and social conflicts associated with evolutionary knowledge: pedagogical transformation; educational philosophy and corresponding expressions of teaching; as well as the people who take part in the process (mainly students and teachers).

Proposal: Methodological framework

The selection of the basic evolutionary knowledge used to design the instruments for evaluating the learning, and the teaching proposal, was made from a standpoint of biological and teaching criteria.

From a range of authors (Darwin 1859; Dobzhansky et al. 1980; Eldredge 1985; Dawkins 2009; Mayr 2001, 2006; Gould 2002; Ruiz and Ayala 1999, 2002; Futuyma 2009), the following were identified, analysed, specified and related: the phenomena that explain the teaching model; the central concepts of the theory of evolution through variation and natural selection; and the facts, premises and principles that this theory is based on (cf. Olander 2013).

At the same time, problems identified in research into the teaching of evolutionary biology were systematised, organised and prioritised. It should be stressed that the traditional approach of conceptual change was not used (cf. Pozo 1997; Pozo and Flores 2007). Instead, it was decided to clarify the knowledge that, in the opinion of the researchers responsible for the project, helps to unravel the nodes of difficulty that arise during teaching interaction. Based on these considerations, the 'Weaving evolutionary thinking' teaching unit was designed.

The 'Weaving Evolutionary Thinking' Teaching Unit

The knowledge chosen was organised along two axes, as if the 'warp' and 'weft' in weaving, one set in parallel and the other interwoven with it (cf. García 2008) to create a

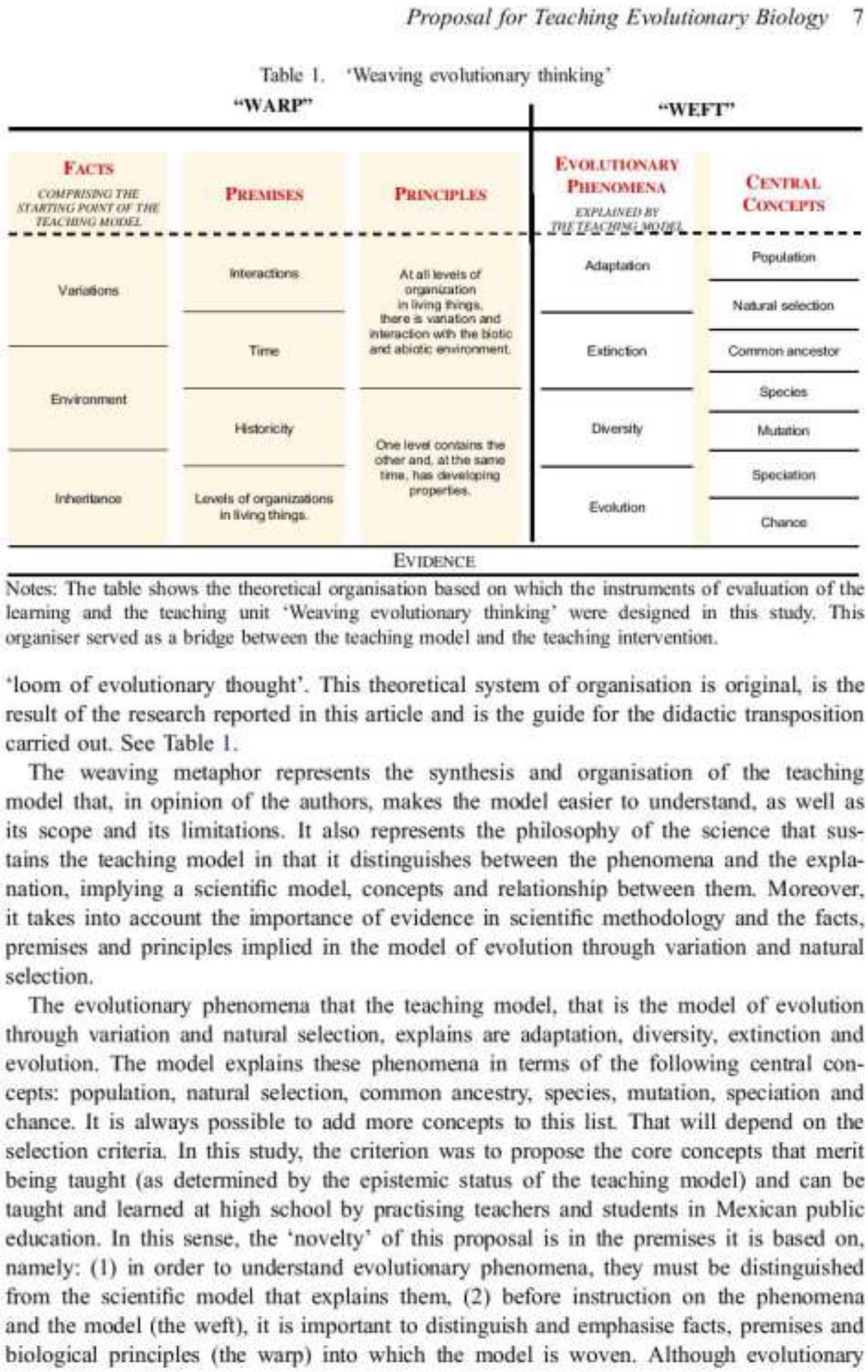

Table 1. 'Weaving evolutionary thinking'

"WARP"			"WEFT"	
FACTS *COMPRISING THE STARTING POINT OF THE TEACHING MODEL*	**PREMISES**	**PRINCIPLES**	**EVOLUTIONARY PHENOMENA** *EXPLAINED BY THE TEACHING MODEL*	**CENTRAL CONCEPTS**
Variations	Interactions	At all levels of organization in living things, there is variation and interaction with the biotic and abiotic environment.	Adaptation	Population
				Natural selection
	Time		Extinction	Common ancestor
Environment				Species
	Historicity	One level contains the other and, at the same time, has developing properties.	Diversity	Mutation
Inheritance				Speciation
	Levels of organizations in living things.		Evolution	Chance

EVIDENCE

Notes: The table shows the theoretical organisation based on which the instruments of evaluation of the learning and the teaching unit 'Weaving evolutionary thinking' were designed in this study. This organiser served as a bridge between the teaching model and the teaching intervention.

'loom of evolutionary thought'. This theoretical system of organisation is original, is the result of the research reported in this article and is the guide for the didactic transposition carried out. See Table 1.

The weaving metaphor represents the synthesis and organisation of the teaching model that, in opinion of the authors, makes the model easier to understand, as well as its scope and its limitations. It also represents the philosophy of the science that sustains the teaching model in that it distinguishes between the phenomena and the explanation, implying a scientific model, concepts and relationship between them. Moreover, it takes into account the importance of evidence in scientific methodology and the facts, premises and principles implied in the model of evolution through variation and natural selection.

The evolutionary phenomena that the teaching model, that is the model of evolution through variation and natural selection, explains are adaptation, diversity, extinction and evolution. The model explains these phenomena in terms of the following central concepts: population, natural selection, common ancestry, species, mutation, speciation and chance. It is always possible to add more concepts to this list. That will depend on the selection criteria. In this study, the criterion was to propose the core concepts that merit being taught (as determined by the epistemic status of the teaching model) and can be taught and learned at high school by practising teachers and students in Mexican public education. In this sense, the 'novelty' of this proposal is in the premises it is based on, namely: (1) in order to understand evolutionary phenomena, they must be distinguished from the scientific model that explains them, (2) before instruction on the phenomena and the model (the weft), it is important to distinguish and emphasise facts, premises and biological principles (the warp) into which the model is woven. Although evolutionary

teaching and learning this content with the aim of strengthening the knowledge covered in class.

Activity 5. 'The girl who slept in class' was a picture sequence through which it could be noted how, on a daily basis, causal chains of events that were originally unconnected intersect: the purpose here was to incorporate the concept of chance when explaining evolutionary phenomena, particularly the causal disconnect between the emergence of variation and selective pressures.

Methodological Design

Target Population, Selection of the Experimental Group and the Control Group

To evaluate and calibrate the proposal, two first-year secondary school groups from the same school and session were considered, as it is the only one of the three years of secondary education in which the Science 1 (Biology) course includes evolutionary biology content. The experimental and control groups, and the session, were chosen at random from the list of first-year groups that had been authorised by school management for participation in the project.

Both groups were given conventional instruction on the contents of evolutionary biology for approximately 3 months of the school year. In the experimental group, towards the end of the school year, 38 students received the teaching intervention for 3 h. Simultaneously, in the control group, 32 students received traditional instruction, emphasising knowledge of evolutionary biology, for the same number of hours, with the teacher deciding the content that, in his or her view, should be emphasised. In each case, the teachers had degrees in Biology from the same university (Universidad Nacional Autónoma de México).

Statistical methodology was designed with the aim of obtaining information that could be recorded and organised systematically to test, evaluate, calibrate and improve the proposal, and to refine an educational model that, with the appropriate adjustments, can be tested in the future in other school contexts. As with all experimental research, it is the model that is being tested.

It was hoped that the instruction imparted to the experimental group would have significant effects on learning compared with that imparted to the control group because, *inter alia*, the problems identified in the specialised literature on teaching and learning these topics were taken into account in the sequence of presentation, the activities and the specially designed teaching resources; awareness of earlier ideas was stressed and the epistemological obstacles underlying them were considered, among other aspects.

Design of Evaluation Tools

Specific questionnaires were designed to assess the educational proposal. The questions included were derived from categories and units of analysis and indicators, defined a priori, consistent with:

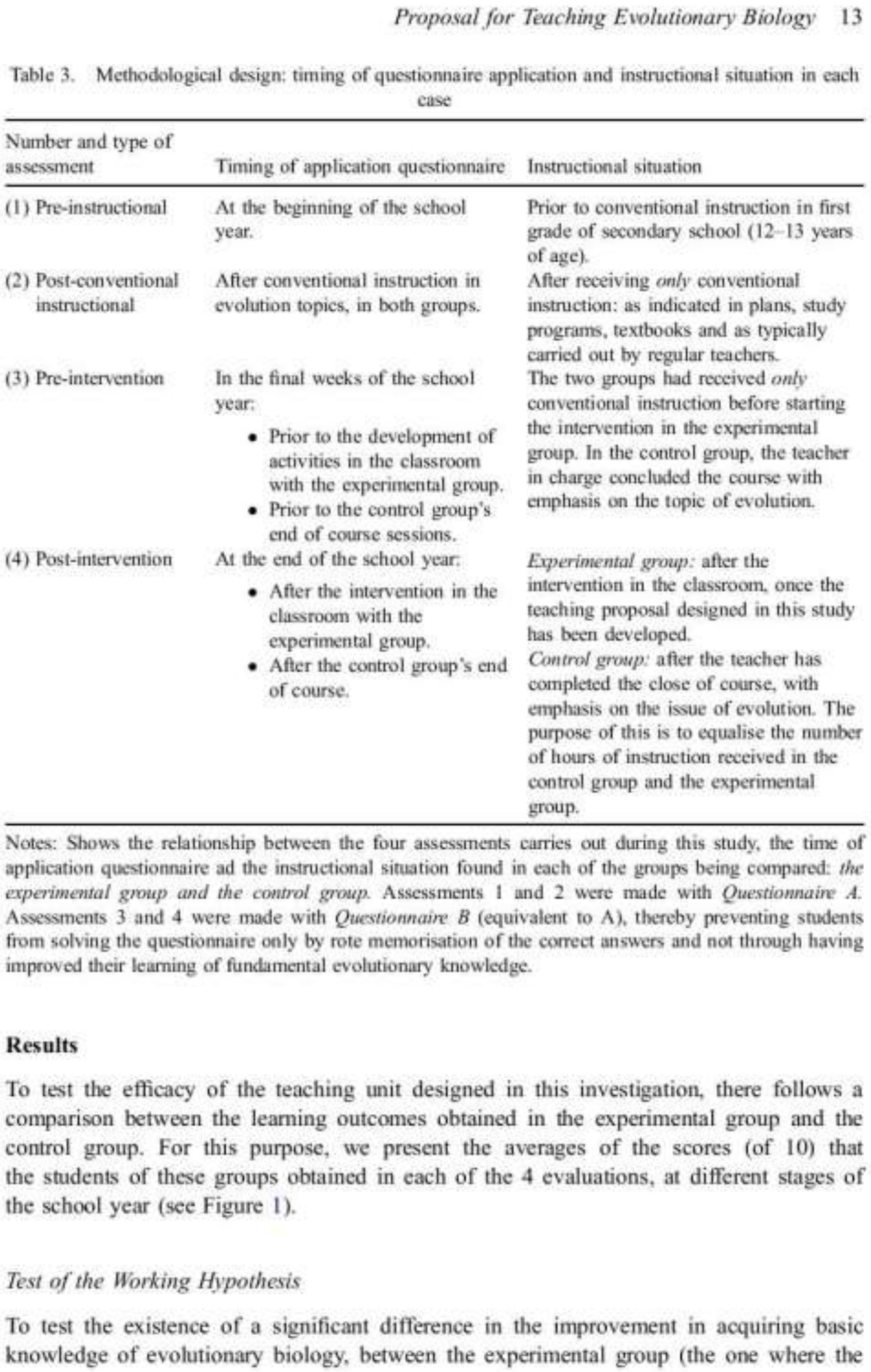

Table 3. Methodological design: timing of questionnaire application and instructional situation in each case

Number and type of assessment	Timing of application questionnaire	Instructional situation
(1) Pre-instructional	At the beginning of the school year.	Prior to conventional instruction in first grade of secondary school (12–13 years of age).
(2) Post-conventional instructional	After conventional instruction in evolution topics, in both groups.	After receiving *only* conventional instruction: as indicated in plans, study programs, textbooks and as typically carried out by regular teachers.
(3) Pre-intervention	In the final weeks of the school year: • Prior to the development of activities in the classroom with the experimental group. • Prior to the control group's end of course sessions.	The two groups had received *only* conventional instruction before starting the intervention in the experimental group. In the control group, the teacher in charge concluded the course with emphasis on the topic of evolution.
(4) Post-intervention	At the end of the school year: • After the intervention in the classroom with the experimental group. • After the control group's end of course.	*Experimental group:* after the intervention in the classroom, once the teaching proposal designed in this study has been developed. *Control group:* after the teacher has completed the close of course, with emphasis on the issue of evolution. The purpose of this is to equalise the number of hours of instruction received in the control group and the experimental group.

Notes: Shows the relationship between the four assessments carries out during this study, the time of application questionnaire ad the instructional situation found in each of the groups being compared: *the experimental group and the control group.* Assessments 1 and 2 were made with *Questionnaire A.* Assessments 3 and 4 were made with *Questionnaire B* (equivalent to A), thereby preventing students from solving the questionnaire only by rote memorisation of the correct answers and not through having improved their learning of fundamental evolutionary knowledge.

Results

To test the efficacy of the teaching unit designed in this investigation, there follows a comparison between the learning outcomes obtained in the experimental group and the control group. For this purpose, we present the averages of the scores (of 10) that the students of these groups obtained in each of the 4 evaluations, at different stages of the school year (see Figure 1).

Test of the Working Hypothesis

To test the existence of a significant difference in the improvement in acquiring basic knowledge of evolutionary biology, between the experimental group (the one where the

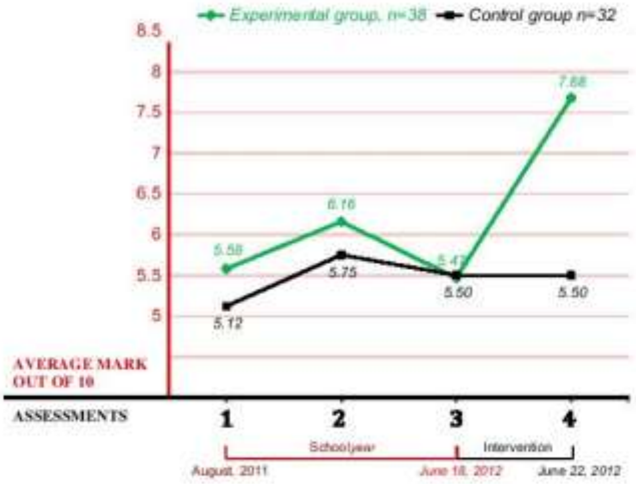

Figure 1. Comparison of average marks obtained by each group at each application of the questionnaires designed in this study

'Weaving evolutionary thinking' teaching proposal was applied) and the control group, hypothesis tests were performed.

The supposition of normality in the data distribution was confirmed by applying the D'Agostino skewness test, the D'Agostino kurtosis test and the D'Agostino omnibus test of normality.

The statistical analysis was performed using the scores obtained on each of the 4 evaluations applied to the control group and the experimental group (see Table 4). For this analysis, the *t*-test for Independent Samples was applied, with a level of significance of $\alpha = 0.05$.

A Paired Samples Test was subsequently performed using the scores obtained on the assessments applied before and after both groups received conventional instruction on evolutionary biology content (assessments 1 and 2). This was also performed on the test scores from evaluations 3 and 4, which in the control group were applied before and after

Table 4. Result of statistical analysis, *t*-test for Independent Samples

CG vs. XG	*n* CG	*n* GT	Mean CG	Mean GT	*t*	df	*P*
Ev1, CG–XG	32	38	5.12	5.58	−0.8316	68	0.4085
Ev2, CG–XG	32	38	5.75	6.16	−0.7000	68	0.4863
Ev3, CG–XG	32	38	5.50	5.47	0.0425	68	0.9662
Ev4, CG–XG	32	38	5.50	7.68	−4.2216	68	0.0001

Notes: Statistical analysis of the scores on each of the four reviews (Ev1, Ev2, Ev3 and Ev4), by students of the two groups analysed: CG, control group and XG, experimental group.

the course close emphasising evolution topics and in the experimental group before and after the classroom intervention using the teaching proposal. See Table 5.

Confidence intervals for the differences between the means and significance levels are reported in Table 5, where it can be seen that:

- In the control group, between the first and second evaluation, $p = 0.209$ (95% CI −0.368, 1.618); and between the third and fourth evaluation, $p = 1.000$ (95% CI −0.634, 0.634).
- In the experimental group, between evaluations 1 and 2 (before the intervention), $p = 0.208$ (95% CI −0.336, 1.494).
- In contrast to these results, in the experimental group, the confidence interval between the third and the fourth evaluations was $p = 0.000$ (95% CI 1.39, 3.03).

Analysis of Results

The working hypothesis was to sustain that the intervention in the experimental group would have a positive effect on student responses, that is there would be an advance in the learning of the evolutionary topics covered, without coming to the end of the spiral of successive approximations that these issues require and the intellectual challenge involved. The specific methodological objective was to seek out evidence of such progress.

The results show that:

There was no significant difference in the initial marks of the two groups (independent t-test, $p = 0.073$). This means that both groups started out at the same point with respect to the information reported on by the evaluation tool.

In neither of the two groups were there statistically significant differences between evaluations 1 and 2 (pre- and post-instruction), as may be corroborated in Table 5 (Paired

Table 5. Results of the statistical analysis, Paired Samples Test

		Paired differences							
					95% Confidence Interval of the Difference				
Group/pairs		Mean	Std. deviation	Std. error mean	Lower	Upper	t	df	Sig. (two-tailed)
CG	Ev2–Ev1	0.6250	2.7562	0.4872	−0.3687	1.6187	1.283	31	0.209
	Ev4–Ev3	0.0000	1.7598	0.3111	−0.6345	0.6345	0.000	31	1.000
XG	Ev2–Ev1	0.5789	2.7864	0.4520	−0.3369	1.4948	1.281	37	0.208
	Ev4–Ev3	2.2105	2.4949	0.4047	1.3905	3.0306	5.462	37	0.000

Notes: Statistical analysis of the scores on each of the four reviews (Ev1, Ev2, Ev3 and Ev4), by students of the two groups analysed: CG, control group and XG, experimental group.

that characterise evolutionary knowledge, the methodology used in this study suffers from the limited amount of time spent in the intervention and implementation of evaluations. Design of the control group was subject to restrictions because the school was involved. Nonetheless, in this study, the control group and the experimental group were as comparable as two groups of people in action may be. It is not reasonable to state that significant learning was achieved, to do so, long-term evaluations would have to be carried out and the logistics of the school did not permit them at this stage.

The core strength of the study is based on its having been applied in actual classroom conditions and having dealt with various sources of difficulty involved in teaching interactions, and which the literature usually reports on in an atomised way.

Conclusions

Based on the results of the statistical analysis, it can be said that the use of the 'Weaving evolutionary thinking' teaching unit with Mexican students at the public high school where the project was implemented had a significantly positive effect in the learning spiral, at high school level, of core evolutionary knowledge explaining evolution through variation and natural selection.

Given the above, the results of this project point in favour of the research approach being proposed (PCT) and its testing through this learning unit. This means that distinguishing the sources of the problems that are 'tied into' the teaching interaction, differentiating their nature, problematising, creating approaches for dialogue, organising results, designing and testing organic educational proposals, guided by research in the field–and, it must not be forgotten, research into teaching experience–can produce encouraging educational outcomes.

If it was possible to achieve the results shown with barely four hours' teacher training and 3 of classroom intervention, it is reasonable to conclude that the approach of the research and the teaching proposal, developed and tested in this project, is on the right track for the goal of teaching and learning core knowledge of evolutionary biology in basic education.

Although this study contributes to the discussion, it obviously has not exhausted it. The project is unfinished, still under construction, spiralling outwards, estimating, specifying and making adjustments to improve the proposals. While the results are encouraging, it is still necessary to analyse and rethink the subjects that caused the greatest learning difficulties: a case in point is the concept of population. Further, the concept of heredity will have to be emphasised. Quantitative results will have to be related to, be supplemented by, or if necessary, be confronted by the qualitative results generated in this project. This will be the basis for future publications.

Given that a research community does not only engage in dialogue, it above all states interests and activities; research through dialogue between approaches is provided to identify points in common, differences and to generate knowledge that is always subject to revision. Such is the purpose of this article.

Even given the methodological limitations of the project, which are explicitly recognised above, it is important to advance the articulation of theory and practice, which is

what this study focuses on. Teaching and research are highly responsible activities and require a regulating ideal that guides action. Making clarifications is also advancing. Building bridges between theory and practice involves risks and limitations, but the risks and limitations of doing nothing are greater. Education, educational research and teaching must be underpinned by the principle of hope, and on the combined efforts of those who, with care and determination, get involved in situations that can only be found in the direct experience of the classroom, in the challenges faced by practicing teachers, and the working conditions in which high school students in Mexico study.

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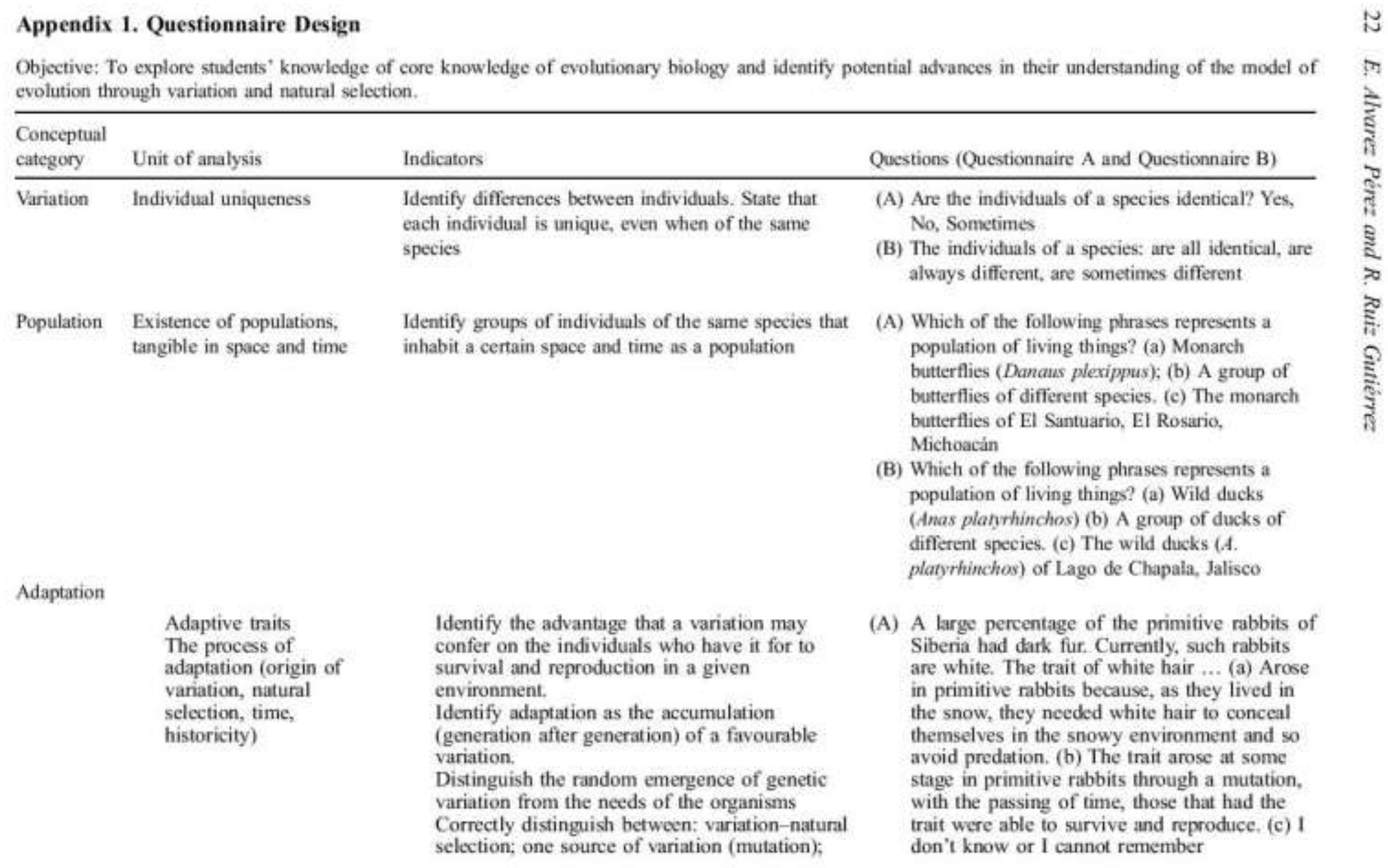

Appendix 1. Questionnaire Design

Objective: To explore students' knowledge of core knowledge of evolutionary biology and identify potential advances in their understanding of the model of evolution through variation and natural selection.

Conceptual category	Unit of analysis	Indicators	Questions (Questionnaire A and Questionnaire B)
Variation	Individual uniqueness	Identify differences between individuals. State that each individual is unique, even when of the same species	(A) Are the individuals of a species identical? Yes, No, Sometimes (B) The individuals of a species: are all identical, are always different, are sometimes different
Population	Existence of populations, tangible in space and time	Identify groups of individuals of the same species that inhabit a certain space and time as a population	(A) Which of the following phrases represents a population of living things? (a) Monarch butterflies (*Danaus plexippus*); (b) A group of butterflies of different species. (c) The monarch butterflies of El Santuario, El Rosario, Michoacán (B) Which of the following phrases represents a population of living things? (a) Wild ducks (*Anas platyrhinchos*) (b) A group of ducks of different species. (c) The wild ducks (*A. platyrhinchos*) of Lago de Chapala, Jalisco
Adaptation	Adaptive traits The process of adaptation (origin of variation, natural selection, time, historicity)	Identify the advantage that a variation may confer on the individuals who have it for to survival and reproduction in a given environment. Identify adaptation as the accumulation (generation after generation) of a favourable variation. Distinguish the random emergence of genetic variation from the needs of the organisms Correctly distinguish between: variation–natural selection; one source of variation (mutation);	(A) A large percentage of the primitive rabbits of Siberia had dark fur. Currently, such rabbits are white. The trait of white hair … (a) Arose in primitive rabbits because, as they lived in the snow, they needed white hair to conceal themselves in the snowy environment and so avoid predation. (b) The trait arose at some stage in primitive rabbits through a mutation, with the passing of time, those that had the trait were able to survive and reproduce. (c) I don't know or I cannot remember

Annex2:

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Mosquito biodiversity in Setif region (Algerian High Plains), density and species distribution across climate zones

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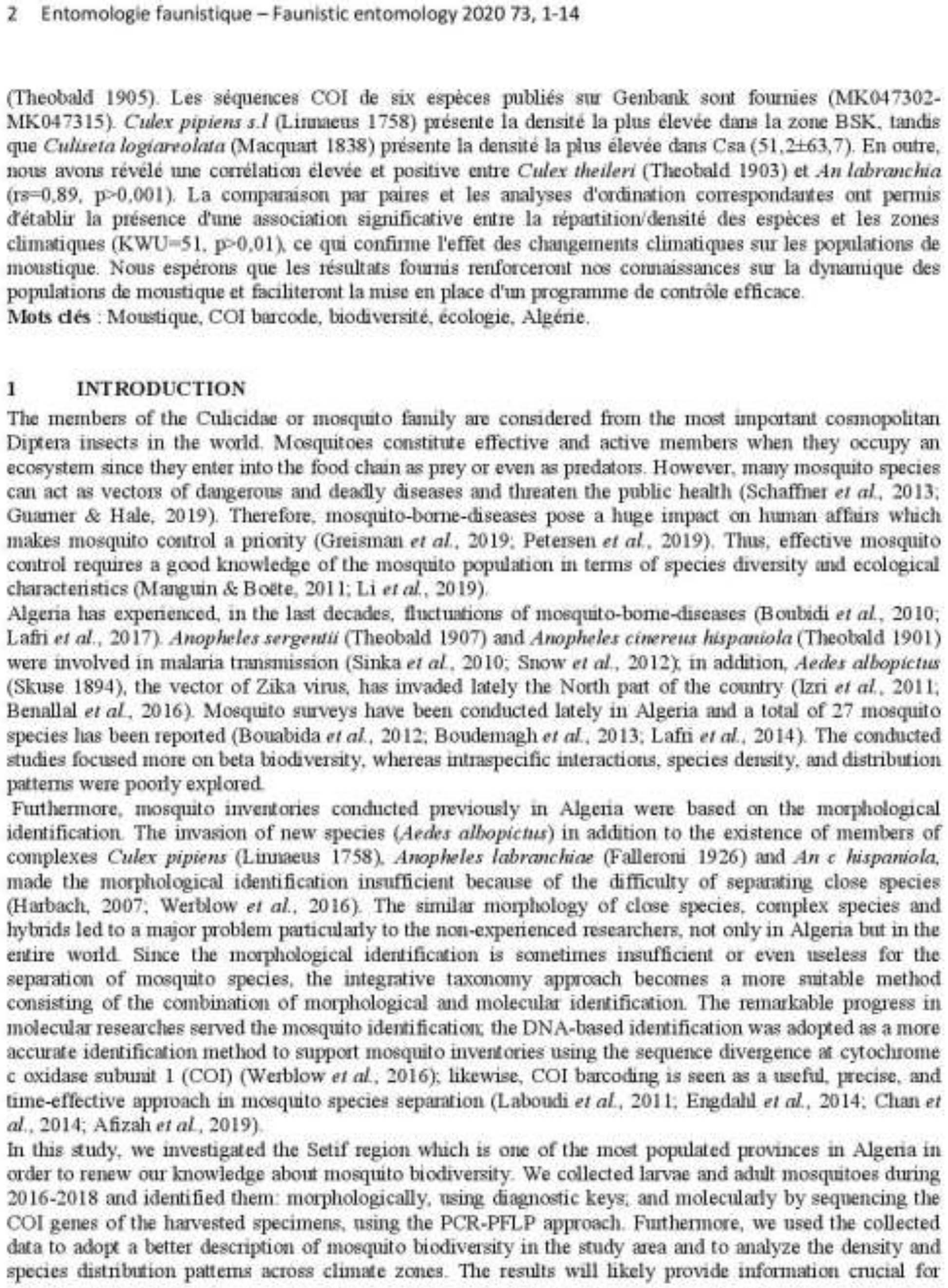
Algeria has experienced outbreaks related to mosquitoes; additionally, it is exposed at the present to the installation of the invasive species *Aedes albopictus* (Theobald 1907). In this context, we performed a mosquito inventory in the Algerian high plains (Setif region) from 2016 to 2018, in order to provide the list of mosquitoes in the study area and analyze their diversity, density and species distribution across two climate zones (Mediterranean Csa and steppe BSk Zones) using biostatistical tests. The identification of species was done using a combination of morphological and molecular approaches (COI barcoding). The sampling yielded the identification of nine mosquito species including the malaria vectors *Anopheles labranchiae* (Falleroni 1926) and *Anopheles cinereus hispaniola* (Theobald 1901). A new species *Culex simpsoni* (Theobald 1905) is also recorded. The COI sequences of six species are provided in Genbank (MK047302-MK047315). From the total sampled mosquitoes, *Culex pipiens s.l* (Linnaeus 1758) showed the highest density in BSk zone (34.7±8.9), while *Culiseta logiareolata* (Macquart 1838) showed the highest density (51.2±28.5) in Csa. Further, we have revealed a high and positive correlation between *Culex theileri* (Theobald 1903) and *An labranchiae* ($r_s=0.89$, $p<0.001$). Moreover, the pairwise comparison and Ordination Corresponding Analyses ascertained the presence of a significant association between species distribution/density and climate zones in the study area (K-W U=51, $p<0.01$), and confirm the effect of the climate changes on the mosquito population. The results provided will hopefully accentuate our knowledge about mosquito population dynamics and facilitate the installation of an effective control program.

Keywords: Mosquito; COI barcode; biodiversity; ecology Algeria.

L'Algérie a connu des épidémies liées aux moustiques. De plus, ce pays est actuellement exposé à l'installation de l'espèce invasive *Aedes albopictus* (Theobald 1907). Dans ce contexte, nous avons réalisé un inventaire des moustiques dans la région de Sétif de 2016 à 2018, afin de fournir une liste de moustiques dans la zone d'étude et d'analyser leur diversité, densité et répartition dans deux zones climatiques (méditerranéenne Csa et haute plaine BSK) en utilisant des tests biostatistiques. L'identification a été réalisée à l'aide d'une combinaison d'approches morphologiques et moléculaires (COI barcode). Nous avons identifié neuf espèces, dont les vecteurs du paludisme *Anopheles labranchiae* (Falleroni 1926) et *Anopheles cinereus hispaniola* (Theobald 1901). Nous signalons aussi la présence d'une nouvelle espèce *Culex simpsoni*



(Theobald 1905). Les séquences COI de six espèces publiés sur Genbank sont fournies (MK047302-MK047315). *Culex pipiens s.l* (Linnaeus 1758) présente la densité la plus élevée dans la zone BSK, tandis que *Culiseta logiareolata* (Macquart 1838) présente la densité la plus élevée dans Csa (51,2±63,7). En outre, nous avons révélé une corrélation élevée et positive entre *Culex theileri* (Theobald 1903) et *An labranchia* (rs=0,89, p>0,001). La comparaison par paires et les analyses d'ordination correspondantes ont permis d'établir la présence d'une association significative entre la répartition/densité des espèces et les zones climatiques (KWU=51, p>0,01), ce qui confirme l'effet des changements climatiques sur les populations de moustique. Nous espérons que les résultats fournis renforceront nos connaissances sur la dynamique des populations de moustique et faciliteront la mise en place d'un programme de contrôle efficace.

Mots clés : Moustique, COI barcode, biodiversité, écologie, Algérie.

1 INTRODUCTION

The members of the Culicidae or mosquito family are considered from the most important cosmopolitan Diptera insects in the world. Mosquitoes constitute effective and active members when they occupy an ecosystem since they enter into the food chain as prey or even as predators. However, many mosquito species can act as vectors of dangerous and deadly diseases and threaten the public health (Schaffner *et al.*, 2013; Guarner & Hale, 2019). Therefore, mosquito-borne-diseases pose a huge impact on human affairs which makes mosquito control a priority (Greisman *et al.*, 2019; Petersen *et al.*, 2019). Thus, effective mosquito control requires a good knowledge of the mosquito population in terms of species diversity and ecological characteristics (Manguin & Boëte, 2011; Li *et al.*, 2019).

Algeria has experienced, in the last decades, fluctuations of mosquito-borne-diseases (Boubidi *et al.*, 2010; Lafri *et al.*, 2017). *Anopheles sergentii* (Theobald 1907) and *Anopheles cinereus hispaniola* (Theobald 1901) were involved in malaria transmission (Sinka *et al.*, 2010; Snow *et al.*, 2012); in addition, *Aedes albopictus* (Skuse 1894), the vector of Zika virus, has invaded lately the North part of the country (Izri *et al.*, 2011; Benallal *et al.*, 2016). Mosquito surveys have been conducted lately in Algeria and a total of 27 mosquito species has been reported (Bouabida *et al.*, 2012; Boudemagh *et al.*, 2013; Lafri *et al.*, 2014). The conducted studies focused more on beta biodiversity, whereas intraspecific interactions, species density, and distribution patterns were poorly explored.

Furthermore, mosquito inventories conducted previously in Algeria were based on the morphological identification. The invasion of new species (*Aedes albopictus*) in addition to the existence of members of complexes *Culex pipiens* (Linnaeus 1758), *Anopheles labranchiae* (Falleroni 1926) and *An c hispaniola*, made the morphological identification insufficient because of the difficulty of separating close species (Harbach, 2007; Werblow *et al.*, 2016). The similar morphology of close species, complex species and hybrids led to a major problem particularly to the non-experienced researchers, not only in Algeria but in the entire world. Since the morphological identification is sometimes insufficient or even useless for the separation of mosquito species, the integrative taxonomy approach becomes a more suitable method consisting of the combination of morphological and molecular identification. The remarkable progress in molecular researches served the mosquito identification; the DNA-based identification was adopted as a more accurate identification method to support mosquito inventories using the sequence divergence at cytochrome c oxidase subunit 1 (COI) (Werblow *et al.*, 2016); likewise, COI barcoding is seen as a useful, precise, and time-effective approach in mosquito species separation (Laboudi *et al.*, 2011; Engdahl *et al.*, 2014; Chan *et al.*, 2014; Afizah *et al.*, 2019).

In this study, we investigated the Setif region which is one of the most populated provinces in Algeria in order to renew our knowledge about mosquito biodiversity. We collected larvae and adult mosquitoes during 2016-2018 and identified them: morphologically, using diagnostic keys; and molecularly by sequencing the COI genes of the harvested specimens, using the PCR-PFLP approach. Furthermore, we used the collected data to adopt a better description of mosquito biodiversity in the study area and to analyze the density and species distribution patterns across climate zones. The results will likely provide information crucial for mosquito control in the study area and highlight the effect of global climate change on mosquito populations. The ecological data was analyzed using bio-statistical analyses.

2 MATERIALS AND METHODS

2.1 Study area

Setif region of high plains Northeastern Algeria (36°03'N 5°31'E) stretches over a surface of 6 504km², the human population density is approximately 230 inhabitants/km². The human population is distributed in the different landscape structures according to the nature of their life activities. The agriculture constitutes an important sector in the study area due to the availability of farmlands and water surfaces (dams and rivers) (Rouabhi *et al.*, 2012; Rouabhi *et al.*, 2016). Setif region is characterized by heterogeneity of climate according to Köppen climate classification (Köppen *et al.*, 2011). We can differentiate two sectors: a north part of Csa climate, and a south part of BSk climate (semi-arid; cold and dry). Therefore, the sampling sites in the study area were regrouped within two groups:

- The first group: includes 16 sites within the region characterized by a Csa climate;
- The second group: regroups 4 sites within the region characterized by a BSk climate.

The distribution of the sampling sites in the study area and the limitation of the climate zones are illustrated in **Figure 1**.

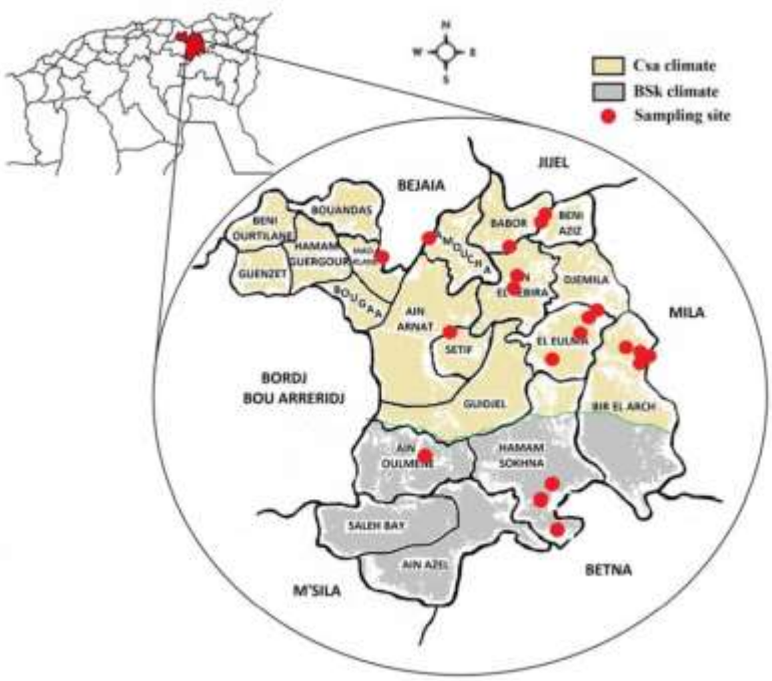


Figure 1: The geographical localization of Setif region (Algeria) and the distribution of the sampling sites (n=20) in two types of climate zones: Csa (Mediterranean climate) and BSk (steppe climate).

2.2 Sampling and morphological identification

The sampling was conducted during 2016-2018 and targeted larval and adult forms. The larvae sampling occurred using a standard dipper (1L) while adult sampling was done using simple CDC miniature light traps (Handmade: yellow light lamp and fan "12VDC").

The sampled specimens were first identified morphologically, 3rd and 4th instar larvae were identified alive (this operation preserve the setae that can be lost easily with the intense manipulation), or after being mounted for permanent preparations (Becker *et al.*, 2003). First and second instar larvae were reared in the breeding site water until they reached the fourth instar. The larvae identification was carried out using a microscope (Browser LCD MICRO 5MP microscope) with a camera built (5MP CNOS 1/2.5", 2560 x 1920 pixel array). Adults were analyzed using binocular microscope loupe. The morphological identification (larvae and adults) was done based on characters described by (Becker *et al.*, 2003) and the last version of interactive keys provided by the French National Research Institute for Sustainable Development (IRD) (Gunay *et al.*, 2018).

2.3 Molecular identification

DNA of the harvested and reared adults (n=24) was extracted from the legs (one of each specimen) using the DNeasy blood & tissue kit (Qiagen, Hilden, Germany) by following the handbook instructions. The PCR amplification of the COI barcode was performed in a total volume of 35 µl consisting of 10x reaction buffer, 2.5 mM MgCl 2; 200 µM of dNTPs; 28 pmol each primer LCO1490 and HCO2191 (Vrijenhoek, 1994); 2.5 U of TaqDNA polymerase. A volume of 3µl of genomic DNA was added to each PCR reaction and samples without DNA were included to exclude carryover contamination. The PCR procedure was as follows: initial denaturation stage and activation of the enzyme at 95°C for 2 minutes; 40 cycles at 94°C for 40 seconds, 50°C for 40 seconds and 72°C for 1 minute, followed by a final extension phase at 72 °C for 7 minutes. PCR products were examined on 1% agarose gel and the band's intensity was noted using a gel imaging system (ChemiDoc™ XRS+ System with Image Lab™ Software #1708265); both strands of the successful amplifications were sequenced at GATC Biotech (Konstanz, Germany). Sequencing results were analyzed using Geneious 10.2.3 software (https://www.geneious.com/) (Kearse *et al.*, 2012). The data of positive sequences were edited using BioEdit (Hall, 1999) and compared with sequences deposited in GenBank and Bold using BLASTn (Zhao & Chu, 2014). COI sequences were deposited in GenBank with the accession numbers from MK047302 to MK047315.

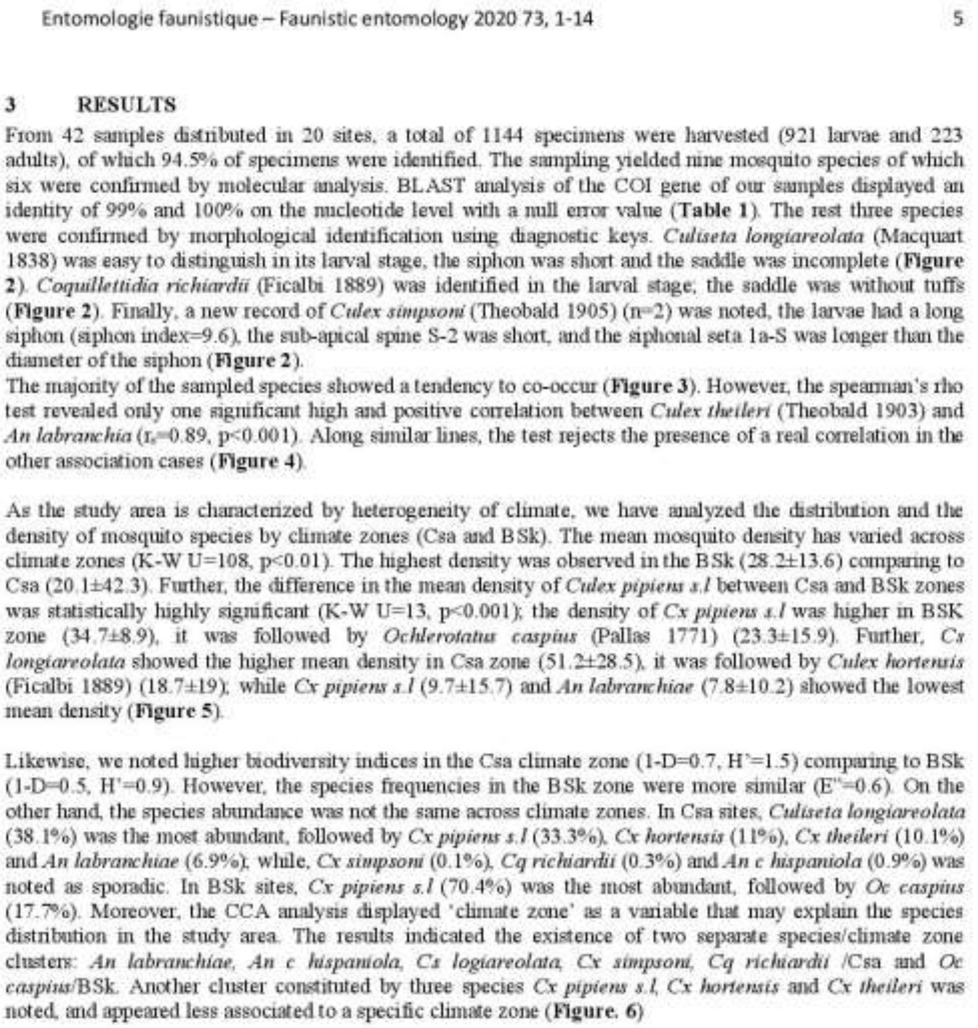
2.4 Data analysis

We analyzed the total mosquito population by calculating the abundance (the number of species specimens divided by the total number of samples), frequency in percentage (*f*) and mean density (Mean±Standard deviation). Only identified specimens were included.

The majority of the sampled larvae co-occurred; thus, we calculated the frequency of the species association and non-association, and the level of correlation between the co-occurred species using the *cor.test* (method=Spearman's" non-normality of data") and the *corrgram* package (Wright & Wright, 2018) in R studio Version 2.1.1335 (Team, 2018). The Spearman's correlation (r_s) was considered as weak if $0<r_s\leq0.4$, moderate if $0.4<r_s\leq0.7$ and as strong if $0.7<r_s<1$. Only species found more than one time was included in the analyses. Species found only one time were excluded for the correlation test.

Next, the difference in mosquito density between climate zones was analyzed using the non-parametric test Mann-Whitney U (non-normality and heteroscedasticity of data). Further, the mean density of the sampled mosquitoes was calculated by climate zone. The analysis performed using SPSS version 25.

Simultaneously, Alpha diversity within Csa and BSk climate zones was evaluated using species richness (S), Simpson index (1-D) (Simpson, 1949), Shannon index (H') (Shannon & Weaver, 1949) and Evenness (E") indices (Hill, 1973). Moreover, a canonical correspondence analysis (CCA) was performed to evaluate the effect of the variable 'climate zone' on mosquito distribution in the study area where sites and species constituted the axes 1 and 2. The diversity and multivariate analyses were conducted using PAST3 (Hammer *et al.*, 2001).



3 RESULTS

From 42 samples distributed in 20 sites, a total of 1144 specimens were harvested (921 larvae and 223 adults), of which 94.5% of specimens were identified. The sampling yielded nine mosquito species of which six were confirmed by molecular analysis. BLAST analysis of the COI gene of our samples displayed an identity of 99% and 100% on the nucleotide level with a null error value (**Table 1**). The rest three species were confirmed by morphological identification using diagnostic keys. *Culiseta longiareolata* (Macquart 1838) was easy to distinguish in its larval stage, the siphon was short and the saddle was incomplete (**Figure 2**). *Coquillettidia richiardii* (Ficalbi 1889) was identified in the larval stage; the saddle was without tuffs (**Figure 2**). Finally, a new record of *Culex simpsoni* (Theobald 1905) (n=2) was noted, the larvae had a long siphon (siphon index=9.6), the sub-apical spine S-2 was short, and the siphonal seta 1a-S was longer than the diameter of the siphon (**Figure 2**).

The majority of the sampled species showed a tendency to co-occur (**Figure 3**). However, the spearman's rho test revealed only one significant high and positive correlation between *Culex theileri* (Theobald 1903) and *An labranchia* (r_s=0.89, p<0.001). Along similar lines, the test rejects the presence of a real correlation in the other association cases (**Figure 4**).

As the study area is characterized by heterogeneity of climate, we have analyzed the distribution and the density of mosquito species by climate zones (Csa and BSk). The mean mosquito density has varied across climate zones (K-W U=108, p<0.01). The highest density was observed in the BSk (28.2±13.6) comparing to Csa (20.1±42.3). Further, the difference in the mean density of *Culex pipiens s.l* between Csa and BSk zones was statistically highly significant (K-W U=13, p<0.001); the density of *Cx pipiens s.l* was higher in BSK zone (34.7±8.9), it was followed by *Ochlerotatus caspius* (Pallas 1771) (23.3±15.9). Further, *Cs longiareolata* showed the higher mean density in Csa zone (51.2±28.5), it was followed by *Culex hortensis* (Ficalbi 1889) (18.7±19); while *Cx pipiens s.l* (9.7±15.7) and *An labranchiae* (7.8±10.2) showed the lowest mean density (**Figure 5**).

Likewise, we noted higher biodiversity indices in the Csa climate zone (1-D=0.7, H'=1.5) comparing to BSk (1-D=0.5, H'=0.9). However, the species frequencies in the BSk zone were more similar (E''=0.6). On the other hand, the species abundance was not the same across climate zones. In Csa sites, *Culiseta longiareolata* (38.1%) was the most abundant, followed by *Cx pipiens s.l* (33.3%), *Cx hortensis* (11%), *Cx theileri* (10.1%) and *An labranchiae* (6.9%); while, *Cx simpsoni* (0.1%), *Cq richiardii* (0.3%) and *An c hispaniola* (0.9%) was noted as sporadic. In BSk sites, *Cx pipiens s.l* (70.4%) was the most abundant, followed by *Oc caspius* (17.7%). Moreover, the CCA analysis displayed 'climate zone' as a variable that may explain the species distribution in the study area. The results indicated the existence of two separate species/climate zone clusters: *An labranchiae, An c hispaniola, Cs logiareolata, Cx simpsoni, Cq richiardii* /Csa and *Oc caspius*/BSk. Another cluster constituted by three species *Cx pipiens s.l, Cx hortensis* and *Cx theileri* was noted, and appeared less associated to a specific climate zone (**Figure. 6**)

Table 1: Comparaison of BLASTn results of the sampled species sequences with Genbank and BOLD accessions displayed an identity between 99% and 100% on the nucleotide level with a null error value.

Sampled species		Compared sequences			
Species	Accession	Species	Identy %	Accession	Country
Culex Pipiens s.l. (Linnaeus 1758)	MK047302 MK047304 MK047308 MK047309 MK047311 MK047314 MK047315	*Cx pipiens*	100	JQ958370	Iran
Culex theileri (Theobald 1903)	MK047307	*Cx theileri*	99.42	HE610459	Portugal
Culex hortensis (Ficalbi 1889)	MK047303 MK047305	*Cx hortensis*	99.10	MH807266	Austria
Anopheles labranchiae (Falleroni 1926)	MK047310	*An labranchiae*	99.54	HQ860410	UK
Anopheles cinereus hispaniola (Theobald 1901)	MK047312	*An cinereus*	99.93	Private sequences (BOLD system)	Morocco
Ochlerotatus caspius (Pallas 1771)	MK047306 MK047313	*Oc caspius*	99.85	KM258354	Belgium

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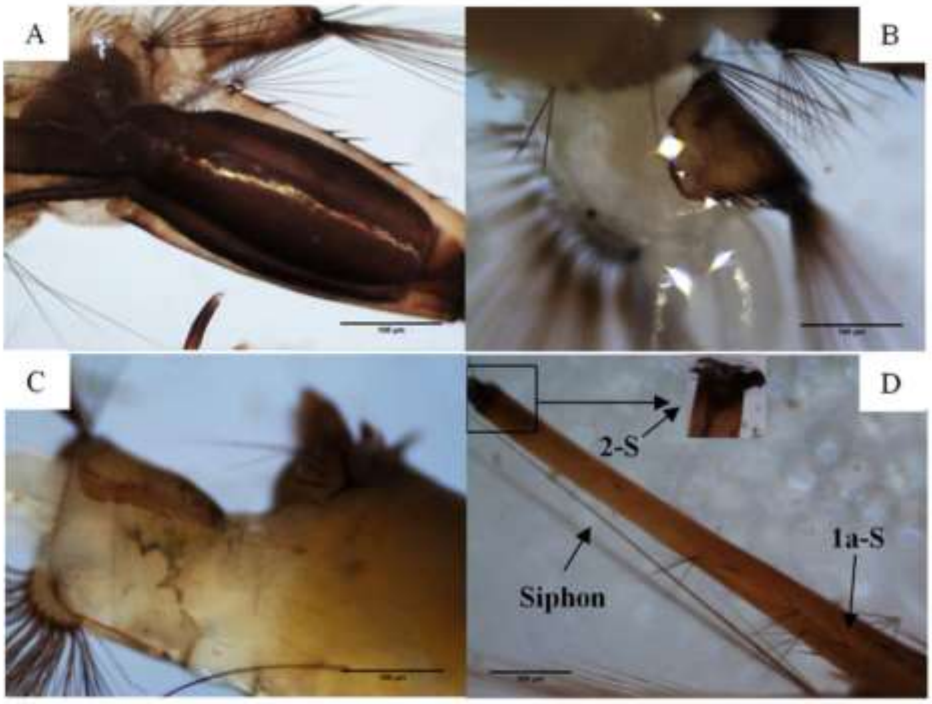


Figure 2: Morphological characters of three mosquito species: (A) siphon of *Culiseta longiareolata* (Macquart 1838) larvae. (B) Incomplete saddle in *Cs longiareolata* larvae. (C) Saddle without tuffs in *Coquillettidia richiardii* (Ficalbi 1889). (D) Long siphon in *Culex simpsoni* (Theobald 1905) with 1a-S longer than the siphon diameter and 2-S short.

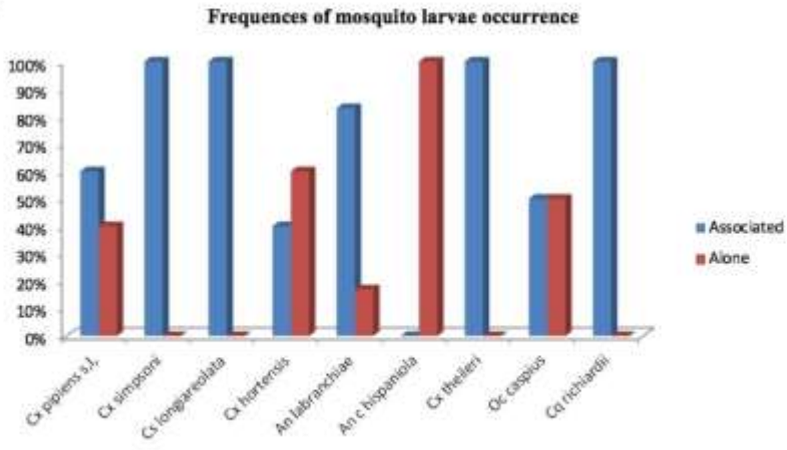


Figure 3: Frequencies of association and non-association in larvae occurrence for the sampled mosquito species (*Culex pipiens* (Linnaeus 1758), *Culex hortensis* (Ficalbi 1889), *Culex theileri* (Theobald 1903), *Culex simpsoni* (Theobald 1905), *Culiseta longiareolata* (Macquart 1838), *Ochlerotatus caspius* (Pallas 1771), *Coquillettidia richiardii* (Ficalbi 1889), *Anopheles labranchiae* (Falleroni 1926), *Anopheles cinereus hispaniola* (Theobald 1901)).

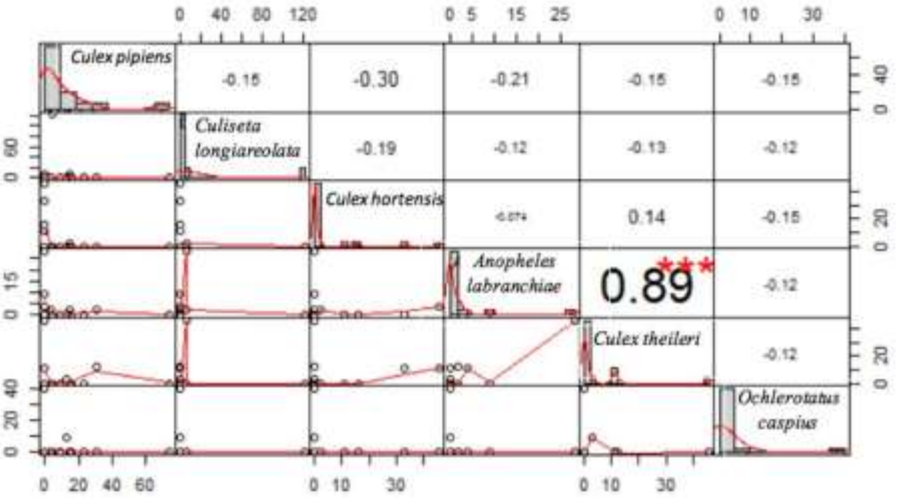


Figure 4: Correlogram of mosquito larvae co-occurrence within Setif region, Algeria (*Culex pipiens* (Linnaeus 1758), *Culex hortensis* (Ficalbi 1889), *Culex theileri* (Theobald 1903), *Culiseta*

longiareolata (Macquart 1838), *Ochlerotatus caspius* (Pallas 1771), *Anopheles labranchiae* (Falleroni 1926)). Upper are the correlation coefficients (rho) with the significance level (***=p-value<0.001). Lower are scatter plots with tendency curves. Values with no significant level refer to the absence of correlation.

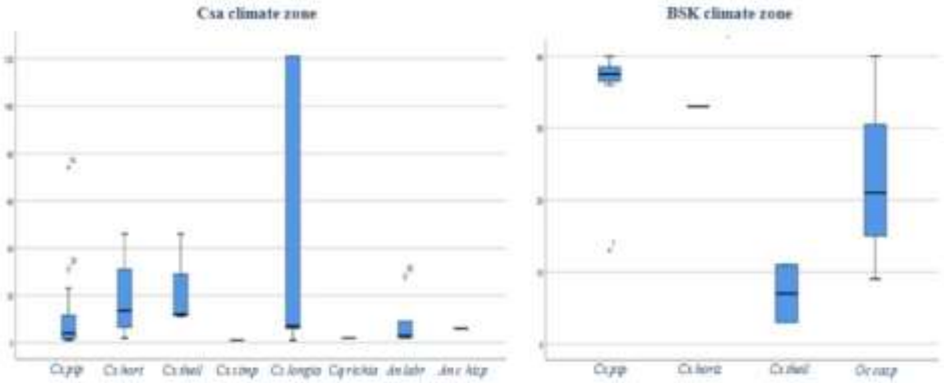
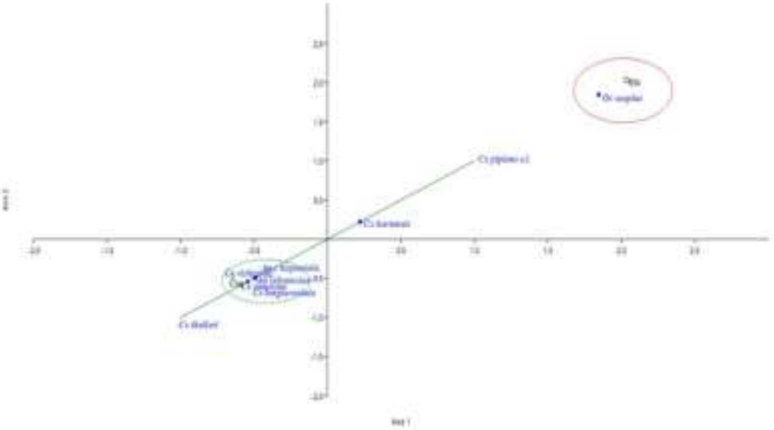


Figure 5: Box and whisker plot showing mean density of the mosquito species (*Culex pipiens* (Linnaeus 1758), *Culex hortensis* (Ficalbi 1889), *Culex theileri* (Theobald 1903), *Culex simpsoni* (Theobald 1905), *Culiseta longiareolata* (Macquart 1838), *Ochlerotatus caspius* (Pallas 1771), *Coquillettidia richiardii* (Ficalbi 1889), *Anopheles labranchiae* (Falleroni 1926), *Anopheles cinereus hispaniola* (Theobald 1901)) sampled from Setif province, from 2016 to 2018, in two different climate zones (BSk and Csa).



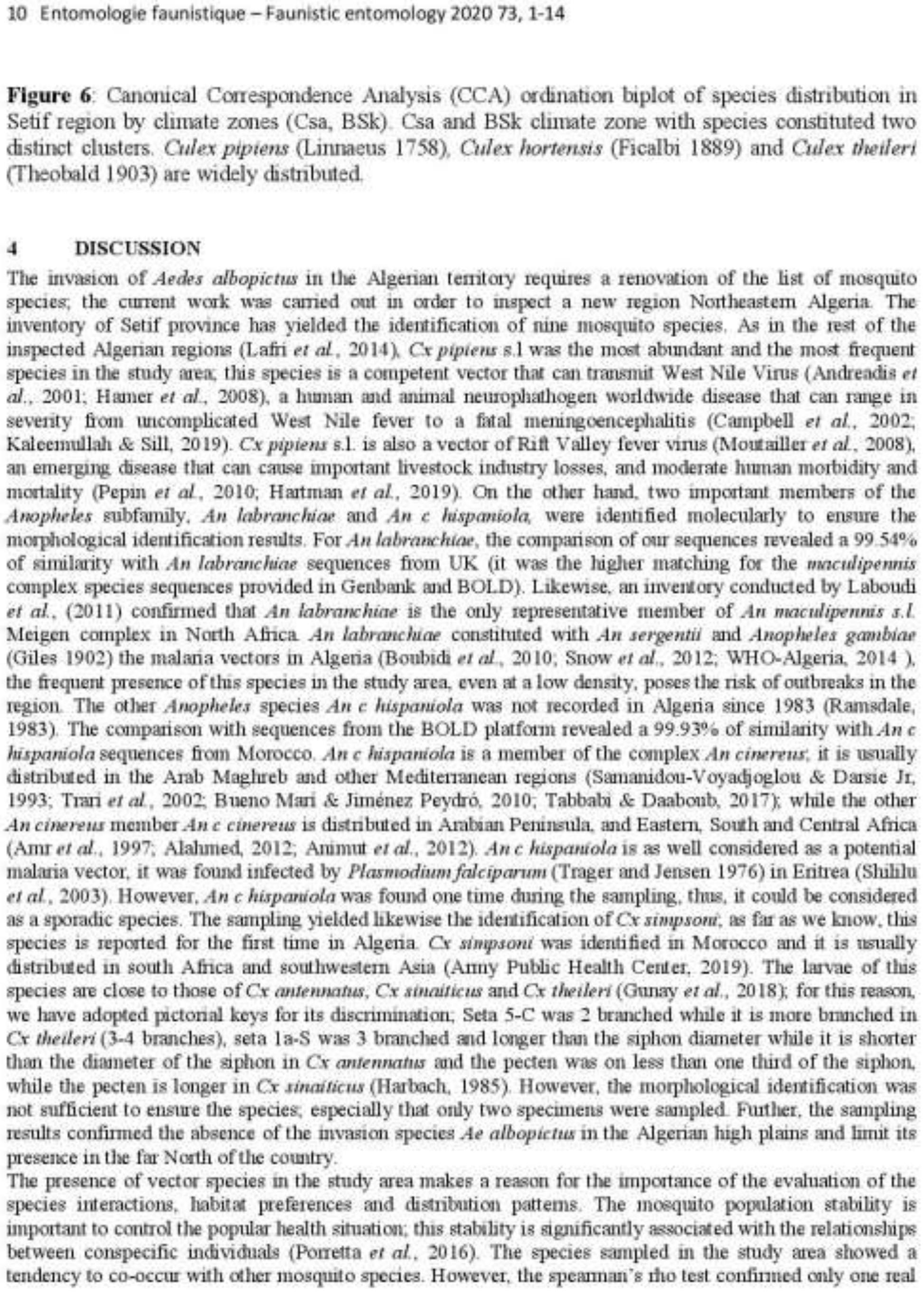
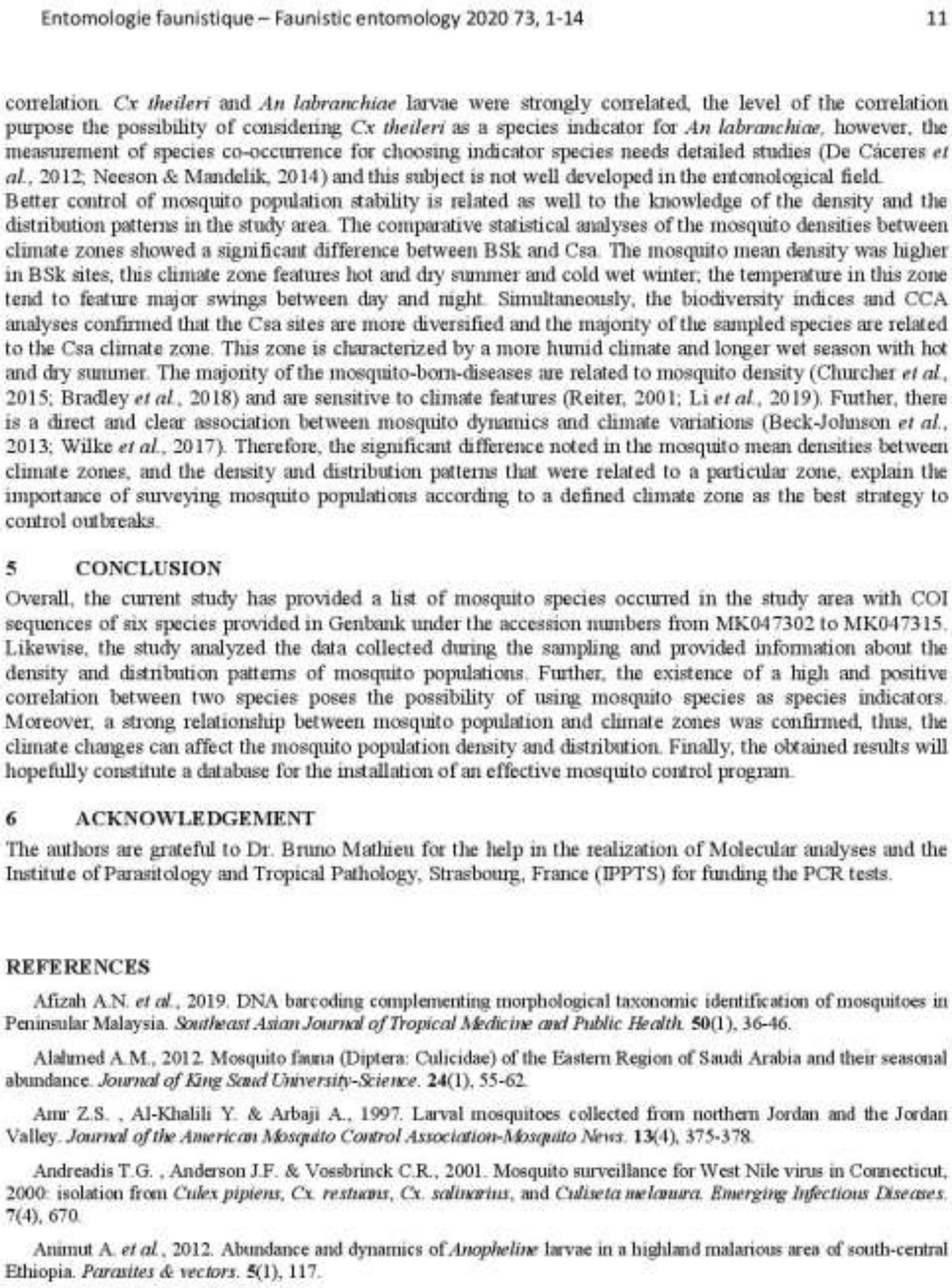


Figure 6: Canonical Correspondence Analysis (CCA) ordination biplot of species distribution in Setif region by climate zones (Csa, BSk). Csa and BSk climate zone with species constituted two distinct clusters. *Culex pipiens* (Linnaeus 1758), *Culex hortensis* (Ficalbi 1889) and *Culex theileri* (Theobald 1903) are widely distributed.

4 DISCUSSION

The invasion of *Aedes albopictus* in the Algerian territory requires a renovation of the list of mosquito species; the current work was carried out in order to inspect a new region Northeastern Algeria. The inventory of Setif province has yielded the identification of nine mosquito species. As in the rest of the inspected Algerian regions (Lafri *et al.*, 2014), *Cx pipiens* s.l was the most abundant and the most frequent species in the study area; this species is a competent vector that can transmit West Nile Virus (Andreadis *et al.*, 2001; Hamer *et al.*, 2008), a human and animal neurophathogen worldwide disease that can range in severity from uncomplicated West Nile fever to a fatal meningoencephalitis (Campbell *et al.*, 2002; Kaleemullah & Sill, 2019). *Cx pipiens* s.l. is also a vector of Rift Valley fever virus (Moutailler *et al.*, 2008), an emerging disease that can cause important livestock industry losses, and moderate human morbidity and mortality (Pepin *et al.*, 2010; Hartman *et al.*, 2019). On the other hand, two important members of the *Anopheles* subfamily, *An labranchiae* and *An c hispaniola*, were identified molecularly to ensure the morphological identification results. For *An labranchiae*, the comparison of our sequences revealed a 99.54% of similarity with *An labranchiae* sequences from UK (it was the higher matching for the *maculipennis* complex species sequences provided in Genbank and BOLD). Likewise, an inventory conducted by Laboudi *et al.*, (2011) confirmed that *An labranchiae* is the only representative member of *An maculipennis s.l.* Meigen complex in North Africa. *An labranchiae* constituted with *An sergentii* and *Anopheles gambiae* (Giles 1902) the malaria vectors in Algeria (Boubidi *et al.*, 2010; Snow *et al.*, 2012; WHO-Algeria, 2014), the frequent presence of this species in the study area, even at a low density, poses the risk of outbreaks in the region. The other *Anopheles* species *An c hispaniola* was not recorded in Algeria since 1983 (Ramsdale, 1983). The comparison with sequences from the BOLD platform revealed a 99.93% of similarity with *An c hispaniola* sequences from Morocco. *An c hispaniola* is a member of the complex *An cinereus*; it is usually distributed in the Arab Maghreb and other Mediterranean regions (Samanidou-Voyadjoglou & Darsie Jr, 1993; Trari *et al.*, 2002; Bueno Mari & Jiménez Peydró, 2010; Tabbabi & Daaboub, 2017); while the other *An cinereus* member *An c cinereus* is distributed in Arabian Peninsula, and Eastern, South and Central Africa (Amr *et al.*, 1997; Alahmed, 2012; Animut *et al.*, 2012). *An c hispaniola* is as well considered as a potential malaria vector, it was found infected by *Plasmodium falciparum* (Trager and Jensen 1976) in Eritrea (Shililu *et al.*, 2003). However, *An c hispaniola* was found one time during the sampling, thus, it could be considered as a sporadic species. The sampling yielded likewise the identification of *Cx simpsoni*; as far as we know, this species is reported for the first time in Algeria. *Cx simpsoni* was identified in Morocco and it is usually distributed in south Africa and southwestern Asia (Army Public Health Center, 2019). The larvae of this species are close to those of *Cx antennatus*, *Cx sinaiticus* and *Cx theileri* (Gunay *et al.*, 2018); for this reason, we have adopted pictorial keys for its discrimination; Seta 5-C was 2 branched while it is more branched in *Cx theileri* (3-4 branches), seta 1a-S was 3 branched and longer than the siphon diameter while it is shorter than the diameter of the siphon in *Cx antennatus* and the pecten was on less than one third of the siphon, while the pecten is longer in *Cx sinaiticus* (Harbach, 1985). However, the morphological identification was not sufficient to ensure the species; especially that only two specimens were sampled. Further, the sampling results confirmed the absence of the invasion species *Ae albopictus* in the Algerian high plains and limit its presence in the far North of the country.

The presence of vector species in the study area makes a reason for the importance of the evaluation of the species interactions, habitat preferences and distribution patterns. The mosquito population stability is important to control the popular health situation; this stability is significantly associated with the relationships between conspecific individuals (Porretta *et al.*, 2016). The species sampled in the study area showed a tendency to co-occur with other mosquito species. However, the spearman's rho test confirmed only one real



correlation. *Cx theileri* and *An labranchiae* larvae were strongly correlated, the level of the correlation purpose the possibility of considering *Cx theileri* as a species indicator for *An labranchiae*, however, the measurement of species co-occurrence for choosing indicator species needs detailed studies (De Cáceres *et al.*, 2012; Neeson & Mandelik, 2014) and this subject is not well developed in the entomological field.

Better control of mosquito population stability is related as well to the knowledge of the density and the distribution patterns in the study area. The comparative statistical analyses of the mosquito densities between climate zones showed a significant difference between BSk and Csa. The mosquito mean density was higher in BSk sites, this climate zone features hot and dry summer and cold wet winter; the temperature in this zone tend to feature major swings between day and night. Simultaneously, the biodiversity indices and CCA analyses confirmed that the Csa sites are more diversified and the majority of the sampled species are related to the Csa climate zone. This zone is characterized by a more humid climate and longer wet season with hot and dry summer. The majority of the mosquito-born-diseases are related to mosquito density (Churcher *et al.*, 2015; Bradley *et al.*, 2018) and are sensitive to climate features (Reiter, 2001; Li *et al.*, 2019). Further, there is a direct and clear association between mosquito dynamics and climate variations (Beck-Johnson *et al.*, 2013; Wilke *et al.*, 2017). Therefore, the significant difference noted in the mosquito mean densities between climate zones, and the density and distribution patterns that were related to a particular zone, explain the importance of surveying mosquito populations according to a defined climate zone as the best strategy to control outbreaks.

5 CONCLUSION

Overall, the current study has provided a list of mosquito species occurred in the study area with COI sequences of six species provided in Genbank under the accession numbers from MK047302 to MK047315. Likewise, the study analyzed the data collected during the sampling and provided information about the density and distribution patterns of mosquito populations. Further, the existence of a high and positive correlation between two species poses the possibility of using mosquito species as species indicators. Moreover, a strong relationship between mosquito population and climate zones was confirmed, thus, the climate changes can affect the mosquito population density and distribution. Finally, the obtained results will hopefully constitute a database for the installation of an effective mosquito control program.

6 ACKNOWLEDGEMENT

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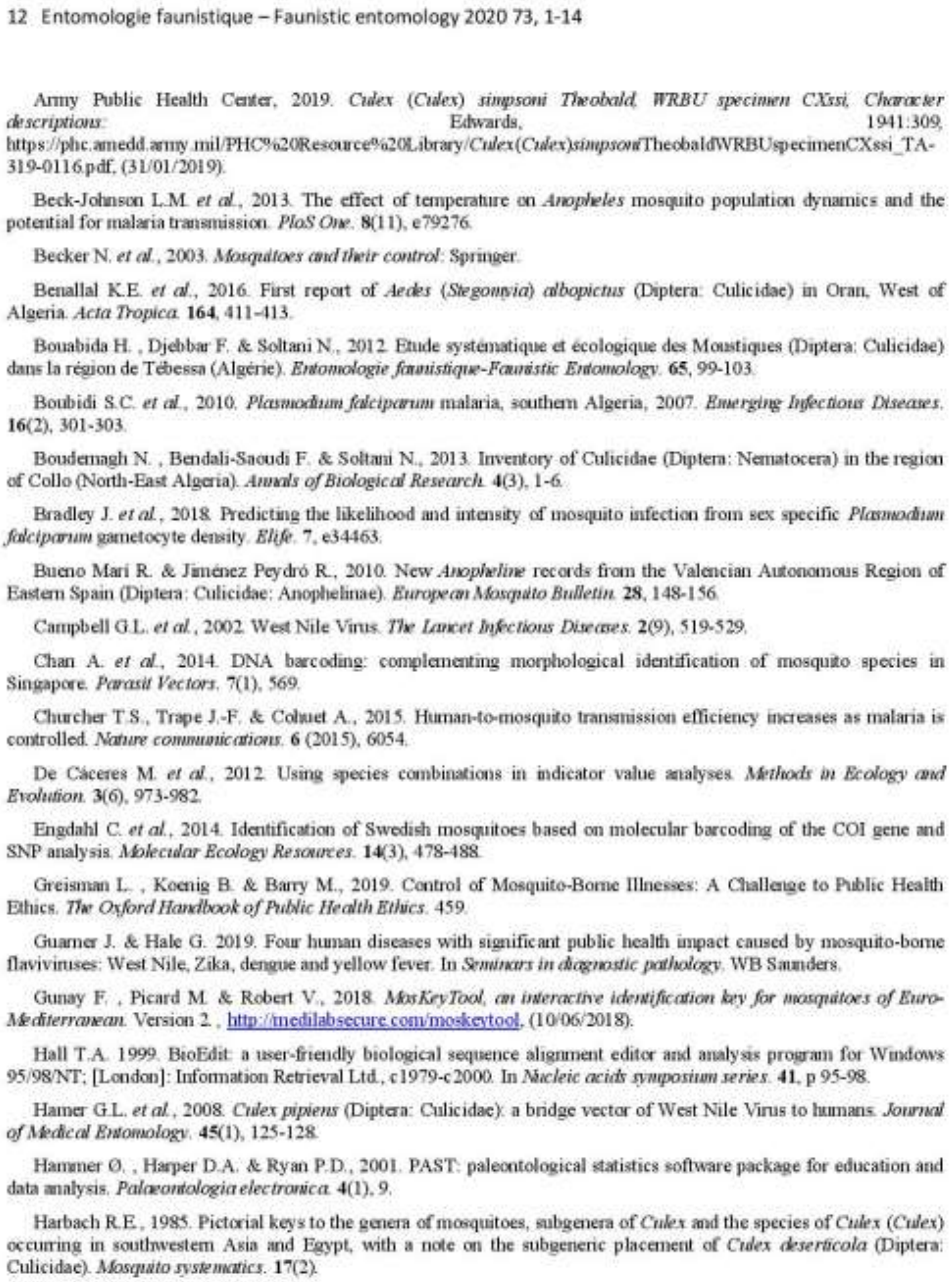

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