



**FACULTAD DE CIENCIAS**  
**DEPARTAMENTO DE BIOLOGÍA**

**Trabajo de Fin de Máster**

**¿INTERVIENE EL PARASITISMO EN LA  
DISTRIBUCIÓN BIOGEOGRÁFICA DE ÁCAROS  
ACUÁTICOS?**

**Presentado por:**

**Hiromi Isabel Yagui Briones**

**Director:**

**Antonio García-Valdecasas**

**Tutor:**

**María José Luciáñez**

**Madrid-España**

**2014**



**SCIENCE FACULTY**  
**BIOLOGY DEPARTMENT**

**Master Thesis Work**

**DOES PARASITISM MEDIATE WATER MITE**  
**BIOGEOGRAPHIC DISTRIBUTION?**

**Presented by:**

**Hiroimi Isabel Yagui Briones**

**Director:**

**Antonio García-Valdecasas**

**Advisor:**

**María José Luciáñez**

**Madrid-Spain**

**2014**

## GENERAL INDEX

I. INTRODUCTION .....	5
1.1. Basic introduction of the Clade Hydrachnidia.....	7
Taxonomy and origin .....	8
General characteristics and adult morphology. ....	10
Development a Development and Life History .....	11
Ecology and habitats.....	17
Loss of larval Parasitism.....	19
Global patterns in water mite distribution .....	20
Potential as indicators of environmental quality .....	21
Biogeography of microorganisms .....	22
1.2. Objectives.....	23
II. MATERIALS AND METHODS.....	24
2.1.Compilation of data set .....	24
2.2.Statistical analyses .....	26
III. RESULTS .....	29
3.1.Distribution types.....	29
3.2.Descriptive data.....	29
3.3.Resampling results .....	31
IV. DISCUSSION .....	35
V. CONCLUSIONS .....	38
VI. ACKNOWLEDGMENTS .....	39
VII. REFERENCES.....	40

## **ABSTRACT**

The biogeography of some organisms has been an intriguing issue to many scientists for several centuries. In the case of organisms with a parasite stage, complex variables may compound their dispersal abilities. The clade Hydrachnidia is a good example of the last statement. The predominant life styles of host-mediated dispersal in water mite's parasitic larvae lead us to expect a wider distribution pattern on those who possess it. But, does it actually occur in reality? We tested and compare the geographic distribution between those water mites that have parasitic larvae with those who have lost this stage. Our sample represents the total number of non-parasitic larvae described and 780 parasitic species. Species world distribution were obtained from specialized literature. The null hypothesis was that both means are not significantly different. We use the bootstrap statistic resampling method to compare the means. Contrary to what we expected, the result pointed out, not only that both particular life cycles have a different dispersion pattern, but also, that those with non-parasitic stage have more species with a wider geographic distribution.

### **Keywords:**

Water mites, non-parasitic larvae, parasitic larvae, geographic distribution

## I. INTRODUCTION

Scientific explanations for the pattern in the geographical distribution of organisms have played an important role in the emergence and argumentation in favor of Darwin's Natural Selection Theory of Evolution (Darwin, 1859). Traditionally, two different mechanisms has been used to develop explanation narratives for shared or sorted organism distribution: dispersal and vicariance (Zink et al., 2000). Basic assumptions under a dispersal scenario is the proposition that anything is possible even those considered highly improbable events (De Queiroz, 2014). Under this proposition, dispersal is a possible scenario for different sized organisms, living in different kinds or habitats and with a variety of life forms and cycles. Vicariance, in contrast, assumes that is the splitting of a previous continuous distribution and their derivatives what must give account of the differential distribution of plants and animals. Vicariance as an alternative to dispersal scenarios has been largely discussed in theory and in the case of particular biotas (Nelson & Platnick, 1980; Yoder & Nowak, 2006)

In either cases, vicariance or dispersal, the first fact to consider is the identity of the organisms under study and in relation to their distribution. A big team of naturalists, including botanists and zoologists have documented near 2 million species worldwide, since Linnaeus times (Chapman, 2009). This effort could be called "a preliminary inventory" not only for the presumed number of species waiting to be documented but for the real nature of those already found (May, 1988). It has been realized in the last decades that there are set of populations with a high morphological similarity by the traditional diagnostic characters used in that particular groups that may conceal what has been designated as cryptic species (Tzedakis et al., 2013).

The present work has originated in the confluence of these two organism axis: the singularity of species identity and the pattern of their distribution.

As stated Finlay (2002), microbial species do not have restricted geographical barriers and will not show biogeographic discreteness. Water mites as microscopic organisms, whose body size is within the range given by Finlay, have also been included into the presumption that “Everything is everywhere, but the environment selects” (Becking, 1934 in Fontanedo & Hortal, 2013, p.5053). Nevertheless, environmental and biological attributes influence the potential to disperse. According to Valdecasas et al. (2006) “abundance and distribution can lose its applicability in parasitic species,” (p. 134) being their distribution conditioned by the host.

Although a parasitic larval is a characteristic aspect of water mites, there are few species that appear to have lost this stage (Smith B. et al. 1998). The loss of parasitism in aquatic mites may affect its geographic distribution patterns; it could stop of being dependent of its size as they have a particular biology.

In the present study, we examine the effect of the loss of parasitism on geographical distribution patterns of Hydrachnidia. We expect to answer the question whether or not parasitism mediates water mite biogeographic distribution.

In consequence, we have organized our work in the following sections:

1.1. Basic introduction to the biology of the Clade Hydrachnidia.

1.2. Objectives

II. Material and methods

III. Results

IV. Discussion.

V. Acknowledgements

VI. References

VII. Appendices.

### **1.1. Basic introduction of the Clade Hydrachnidia.**

One heterogeneous subclass of modern arachnids that have become one of the most adaptable and ubiquitous clade of arthropods is mites (Acari). A specialize type of mites are usually found in freshwater habitats as abundant and diverse benthic arthropods with adaptations to survive in rivers, streams, lakes, ponds and other unusual habitats. Sometimes the area is so filled with water mites, as they are generally known, that, as “One square meter area of substratum from littoral weed beds in eutrophic lakes may contain as many as 2000 deutonymphs and adults representing up to 75 species in 25 or more genera” (Smith I. et al., 2010, p. 486).

There are nearly 6,000 species of water mites (Acari: Hydrachnidia) described (Di Sabatino et al. 2002 as cited in Więcek et al. 2013; Smith I. et al., 2010). They are grouped into seven superfamilies, 50 families and 300 genera (Di Sabatino et al., 2000). According to Smith I. et al. (2010), three of seven superfamilies, Hydrovolzioidea, Hydrachnoidea, and Eylaoidea, are probably representing natural groups; the last remaining, Hydryphantoidea, Lebertioidea, Hygrobatoidea, and Arrenuroidea are either paraphyletic or polyphyletic and require extensive revision.

Most aquatic mites have a very particular life cycle among Acari. It resembles the one of holometabolous insects, including a few dormant pupa-like stages, an active

larva, a deutonymph and an adult (Smith I. et al., 2010; Więcek et al., 2013). Water mites have a crucial coevolutionary relationship with some of the predominant insects in aquatic ecosystems: Diptera, Trichoptera, Coleoptera, Ephemeroptera, Plecoptera, Heteroptera and Odonata, interacting intimately with them at their various stages of their life. The larvae parasitize the insect, and in that way, the insect “help them” in a particular way to disperse and colonize other habitats (Martin, 2008 as cited in Więcek et al., 2013). The other stages of water mites, deutonymphs and adults, usually fed on mainly immature stages of insect. The complex life cycle of water mites could be the principal reason of the individual demands for abiotic and biotic components of their environments (Di Sabatino et al., 2000; Więcek et al., 2013). Most species need a particular habitat or microhabitat. As Di Sabatino et al. (2000) found, the species' composition in water mite communities is determined by “temperature, current speed, substratum type, physiographic and geomorphological factors.”

### **Taxonomy and origin**

Even though Linnaeus, Et. Geoffroy Saint-Hilaire and others had described some water mites, Otto Friedrich Müller is considered the initiator of the study of this group, with the publication in 1781 of the first monograph in which he described 49 species, all assigned to the same genus: Hydrachna (Valdecasas, 1981).

In 1928 K. Viets (as cited in Valdecasas, 1981) offers a classification of water mites including three superfamilies and 16 families and in 1936 published one of his most important works "Wassermilben oder Hydracarina," which produces a determinative key to all genera described to time. Viets can be considered the great hydrachnologist of the century; his works deal with wildlife from diverse places as Java, Australia, Europe, India, etc. In 1955-1956, he published his definitive work: "Die Wilben Süßwassers



und des Weeres," an essential working tool and reference for hydrachnologists ever since then, and where we can find the most complete synonymy to 1953 with the work of all authors on the theme until 1955 (Valdecasas, 1981).

Within the order Actinedida and suborder Parasitengona, Hydrachnidia, along with Stygothrombidoidea, Calyptostomatoidea, Trombidoidea, and Erythraeoidea, belong to an exceptionally natural group suggested by its morphological and behavioral data (Smith I. et al., 2010). The origin of the monophyletic clade dates back to the Jurassic - Triassic period (Smith & Cook, 1991 in Di Sabatino et al. 2000), following a radical reorganization of their life history traits and ontogenetic development, they were able to invade and diversify in the freshwater medium (Wiggins et al., 1980). The pupa-like dormant stages were adapted to resist unfavorable conditions of the unstable environments at that time, and the larval parasitism on flying insects, as said before, conferred advantages like dispersion and colonization of new habitats (Smith & Cook, 1991 as cited in Di Sabatino et al. 2000).

Davids, Belier and Mitchell (as cited in Smith I. et al., 2010, p. 486) stated in their studies, "water mites evolved from terrestrial stock, and hypotheses on their origin usually presume an ancestral terrestrial parasitengonine" as the ancestor that invade the aquatic habitat. An alternative hypothesis suggests that the ancestors may have been water mites resembling some actual Hydryphantoidea. As this is stated, water mites diverged from terrestrial ancestors with direct development. So the essential parasitengonine life history evolves as "a set of adaptations for exploiting spatially and temporally intermittent aquatic habitats" (Smith I. et al., 2010, p.486).

## General characteristics and adult morphology

The Hydrachnidia body has the same constitution of the Acari; it is divided into two regions named the gnathostome, at the front with mouth and feeding pieces, and the idiosoma, at the posterior end, bearing the legs and the genital region (Fig.1). Strongly divergent evolutionary tendencies accompanied the transition from aquatic to terrestrial life: “enlargement, multiplication, dislocation” (Di Sabatino et al., 2000).

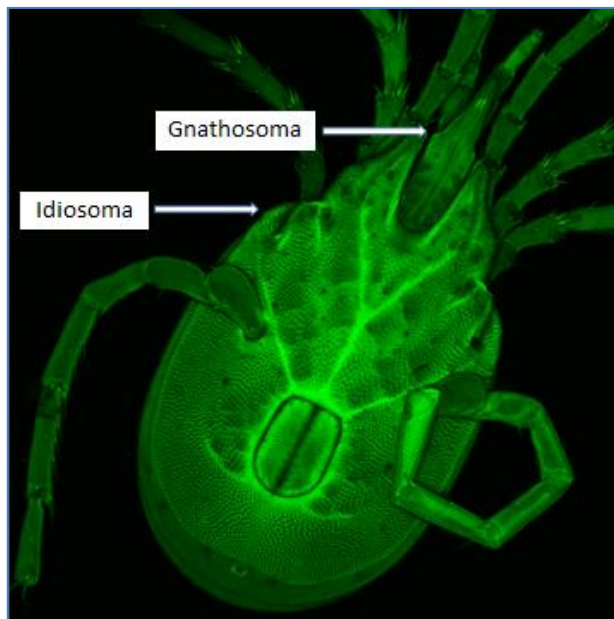


Fig.1 A maximum intensity projection image obtained from an image stack taken with a Leica Laser Scanning Confocal Microscope showing the ventral surface of *Torrenticola* sp.

Water mite's body sizes vary between 0.3-0.4 mm to 7-8 mm, although most species have a size between 0.5-1 mm. In contrast to other aquatic meiofauna, Hydrachnidia color is one superficial characteristic that make them very striking. In general, the color is obtained from a mix of substances the mite gain with food. A great number of species are red or green, but there are some blue ones, yellow ones and brown ones (Fig. 2). Other species with interstitial habits have lost the pigment, showing a whitish color

(Valdecasas, 1981). According to Meyer and Kabbe (as cited in Di Sabatino et al., 2000), the color patterns of some water mite species may be considered as “disruptive camouflage” and the bright color as a warning sign that is connected with the presence of defence glands in the idiosoma. Plesiotypically, water mites are globular in shape but there are also species flattened, elongated and rounded (Di Sabatino et al., 2000).

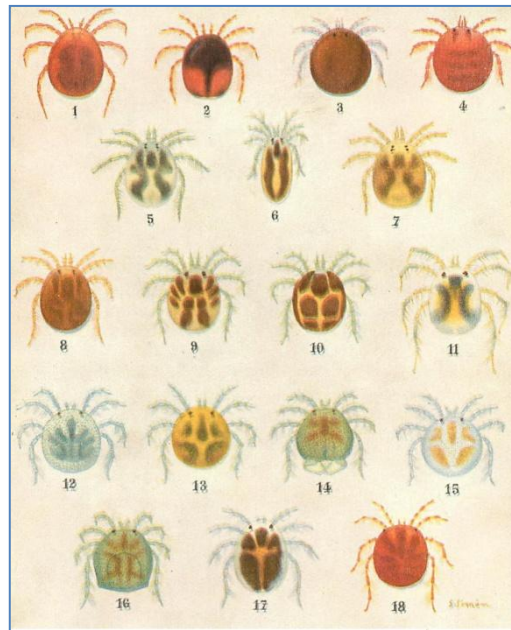


Fig.2 Different species of water mites found in Spain. 1, *Eylais*; 2, *Hydryphantus*; 3, 4, *Hidrachna*; 5, 7 and 8, *Limnesia*; 6, *Oxus*; 9, 10, *Hygrobatas*; 11, *Neumania*; 12-16 and 18, *Arrenurus*; 14, 17 *Arrenurus sinuator*. Arévalo (1929, p. 128)

### **Development and Life History**

All Hydrachnidia species have sexual reproduction, but cases of arrhenotoky (a particular type of parthenogenesis in which unfertilized eggs develop into males) and pseudoarrhenotoky (the paternal genome is eliminated) are considered in order to explain the different sex ratios found in nature and as an adaptation that restrains the population size (Baker & Proctor, as cited in Di Sabatino et al., 2000).

Most species of water mites are univoltine, having one generation per year, with females releasing multiple eggs (Smith I. et al., 2010). The number of eggs a female deposit varies between one or two in species like *Aturus scaber* to 14000 in species as *Eylais discreta* (Valdecasas, 1981).

After the eggs hatch, Hydrachnidia (and all Parasitengona) undergo a series of stages: prelarva, larva, protonymph, deutonymph, tritonymph and adult (Valdecasas, 1981; Smith I. et al., 2010) (Fig.3). The duration of each period is not stable among species and is influenced by the environment and the relationship with the insect host (Di Sabatino et al., 2000). In most species, the development from oviposition to adult takes 1-6 months (Stechmann, Hevers & Meyer in Di Sabatino et al., 2000) and the duration of adult life lasts from 6 weeks to 2 years (Di Sabatino et al., 2000).

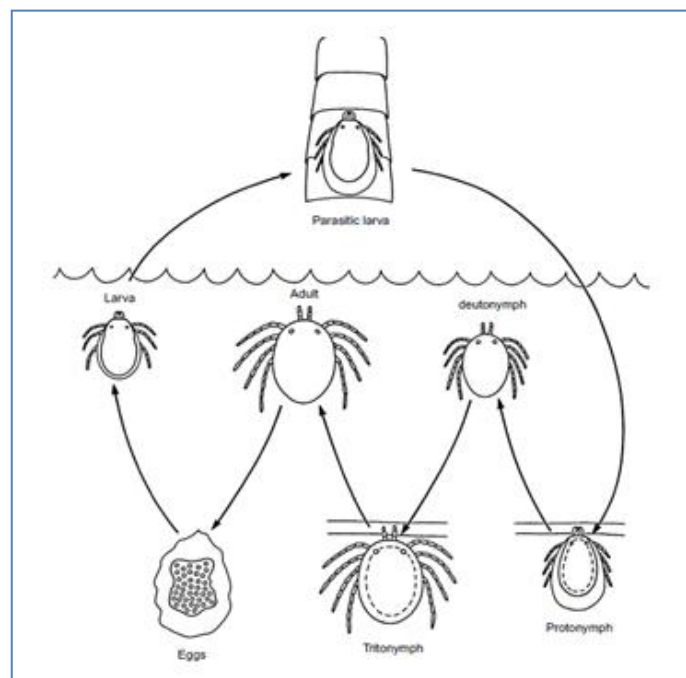


Fig. 3 Generalized water mite life cycle diagram (from I. Smith et al. 2010)

The various stages of water mite's postembryonic development are detailed next, emphasizing the larval phase given its implication in water mite's dispersal. Most of the information was taken from Smith I. et al. (2010).

### *Larva*

After hatching, the young hexapod larva seeks for a proper host and starts its ectoparasitic life being passively transported and fed by its host (Smith I. et al., 2010). Most water mites go through this kind of parasitic stage. It is precisely the parasitic phase the principal way of dispersal, the one that motivates big oscillations in the population present in a defined area, provoking local extinctions and a high dynamism in their temporal dimension (Valdecasas, 1981).

According to Smith B. (1988), there is a survival period for free living larvae before being parasitic that last from 4 days to 6 weeks, about 7 days for most species. A larva older than 7 days has difficulties to attach to a proper host; although those adapted to cold conditions, can survive longer periods remaining infective (Smith I. et al., 2010).

The effect of larval parasitism in their host may be quite significant. There are differences in the water mite growth when it is feeding on the host fluids; depending on the clade, it could be minor or substantial, and it could be related to the host mortality, survival rates, fecundity, and retarded growth (Di Sabatino et al., 2000). Davids (as cited in Di Sabatino et al., 2000) said that the whole structure of freshwater ecosystem may also be altered by aquatic larva parasitism.

Mitchell (as cited in Smith et al., 2010, p. 504 ), theorized, "the probability of success for larval water mites can be calculated as the product of the probabilities of discovering a host, attaching at an appropriate site on the host, completing engorgement on the host, and detaching from the host in a proper habitat."

#### a. Host Selection

Smith I. et al. (2010) stated that some species larvae search for a possible host on the surface film, showing "plesiotypical terrestrial behavior." Opportunities of these mites to contact hosts are occasionally events related with specific aspects of the host behavior (Smith I. et al., 2010). As Smith point out, the water mites that perform this type of strategy apparently repay the risk of failure in getting a host, by producing hundreds of eggs. Another type of host selection described by Mitchell (as cited in Smith I. et al., 2010) is presented by water mites that act like fully adapted aquatic organisms, swimming all over the water column or crawling on the substratum, actively seeking for hosts. Unlike the previously described mites, these are apparently more efficient as their females tend to lay a large number of smaller of eggs. This last type of host selection, as Smith I. (2010) explained, enhances the probability of accomplishment in finding a host and, in higher water mites, "facilitates preparasitic attendance of hosts during their final preadult instar."

Böttger (cited in Smith I. et al., 2009) stated that it seems that water mites find their host just by accidental contact, but there are some observations that confirm that the larvae present visible behavioral changes when the host is near, presenting evidence enough to show the importance of chemical and tactile cues.

#### b. Site Selection

After host selection, the larvae locate an appropriate attachment and start its feeding. They use their pedipalps to help stabilize the body, and pierce the host cuticle with their mouth parts (Smith I. et al., 2010). According to Åbro (as cited in Smith I. et al., 2010), some species of *Arrenurus* secure their attachment by the secretion of a substance that

strengthens when is in contact with the host, a particular case not observed in other groups (Redmond & Lanciani cited in Smith I., 2010).

Those species that show a preparasitic stage must pass through the penultimate instar of the host to the imago in the time of ecdysis (Smith I. et al., 2010). It is not that simple to transfer from one stage of the host to another, many parasitic larvae of fly hosts have some difficulty to locate the appropriate site on the pupal exuvia, and some others are unable to reach the adult as it emerges (Smith I. et al., 2010). As a result, “30–50% of preparasitic larvae fail to infect the adult host” (Smith & McIver cited in Smith I. et al., 2010).

#### c. Engorgement

The engorgement period is quite different among water mite taxa. Some larvae require 6–13 days to engorge (Lanciani, Böttger, Smith B., cited in Smith I. et al., 2010), but others may spend from 14 days to 10 months attached to the host (Davids, Wiggin, and Smith B., cited in Smith I. et al., 2010). It is not surprising that those species related to insects that have a short life, “especially nematoceros Diptera,” require short periods of engorgement, such as 24 hours in particular cases (Smith I. et al., 2010).

The size the larvae acquire during this period also varies substantially. As an example, those larvae that are parasitic of nematoceros flies increase some 3-4.3 times their original size (Smith I. et al., 2010) and those in the extreme case reported by Münchberg species from the subgenus *Brevicaudaturus* grew as much as 1300 times their original volume (as cited in Smith I. et al., 2010).

#### d. Detaching

The next step following engorgement is to detach from the host and return to an aquatic environment for a posterior development. There are many different cues such as host behavior, along with physical or chemical stimuli that mean the larvae the right moment to initiate the separation from the host (Böttger, as cited in Smith I. et al., 2010). Some species just need an environmental cue to induce detachment, but physiological cues related to the detection of hormones or other signs from the host, as oviposition is essential in others (Smith & Lughland, as cited in Smith I. et al., 2010).

#### *Protonymph (Nymphochrysalis)*

Those water mite larvae that successfully reenter into suitable aquatic habitats, usually seek a place to attach themselves, and become quiescent. That is when their protonymph stage starts. Inside the larval skin, huge structural reorganization occurs, the larval tissues are reabsorbed, and the active deutonymph starts its development (Smith I. et al., 2010).

#### *Deutonymph*

After a few days of being in the quiescent stage (Smith I. et al., 2010), the active and predaceous deutonymph emerge resembling the adult, but sexually immature (incomplete sclerotization, chaetotaxy and with a rudimentary or provisional genital field (Smith I. et al., 2010).

This stage varies in duration from a few days or weeks to several months depending on the group (Smith I. et al., 2010). Before entering the second quiescent stage, the individual feeds and grows in size. They embed their mouth parts in plant material or soft detritus and become the inactive tritonymph (Smith I. et al., 2010).



### *Tritonymph (Imagochrysalis)*

The tritonymph is the last quiescent instar, and it remains enclosed by the deutonymphal skin. During this rapid stage, that last as much as few days, the final metamorphosis and structural reorganization occurs to produce the adult (Smith I. et al., 2010).

### *Adult*

An unarmed adult emerges from the deutonymphal integument with general soft, docile and colorless body (Smith I. et al., 2010). However, almost immediately, it becomes active and completes its sclerotization, having distinctive color patterns that weren't evident before. The various groups of water mites show a wide range of shapes and arrangements of idiosomal sclerites, appearing as adaptations for living in aquatic environments with different physical or chemical characteristics (Smith I. et al., 2010).

As fast as the adult emerges, it become sexually mature and star to mate. As Smith I. et al. (2010) pointed out, in many taxas males emerge and develop a few days earlier than its females, and are ready to mate as soon as they become active in the habitat.

### **Ecology and habitats**

The water mite fauna is a very successful group that has invaded nearly all kinds of freshwater habitats (Fig.4, 5). According to Smith I. et al. (2010), the core of this success of water mite evolution, on exploiting and invading different new habitats, has depended on the development of adequate adaptative design for all instars. "Larval traits tend to promote parasitism and dispersal on hosts, during those of deutonymphs and adults favor feeding, growth, and reproduction in water" (Smith I. et al., 2010).



Fig.4 (a) Stony section of a mountain stream. (b) Amazonian river (río Napo, Ecuador) with typical gravel shore, habitat for interstitial fauna. (c) Temporary pond in the sierra de Guadarrama



Fig. 5 A fountain in Sierra de Guadarrama

Over time, the primary mechanism that promotes speciation and divergence on water mites have been the passive transport of their larvae on hosts giving them the opportunity to colonize new habitats. Nevertheless, as Smith I. et al. (2010) stated, in modern communities, most species or monophyletic groups are restricted to few analogous types of habitats. According to this, the correlation between individual clades and its habitats suggests “conservative factors such as adaptive requirements for locating hosts, prey, mates, and oviposition sites tend to constrain adaptive radiation” (Smith I. et al., 2010), also related to the evolution of new biological traits.

## **Loss of larval Parasitism**

The generalized basic life cycle strategies vary through all Hydrachnidia (Mitchell and Boëttger, as cited in Di Sabatino et al., 2000). As mentioned before, the larva that searches for hosts above the water surface have the highest risk of failure in locating a host and return to an adequate aquatic habitat. Therefore, one shift on the life cycle involves the elimination of the parasitic larval feeding, in that way, avoiding the risk of not found an appropriate host, but foregoing the dispersal mediated by them (Smith I. et al., 2010).

The lack of parasitism in water mites larva stage has taken place independently, at least in “29 species of water mites and one species of Trombidiidae” (Smith B., 1998). In all of them forgo larval feeding and any association with a host; those are independent cases within groups of species or genera where the rest have parasitic larvae (Smith B., 1998). There are also a small number of species that transform directly in protonymphs that develop inside the eggs from which only the deutonymph emerges (Di Sabatino et al., 2000). So, in some species, both the larvae and deutonymphs may appear to hatch from a single clutch (Smith B., 1998).

According to Di Sabatino et al., (2000), the lack of larval parasitism could be polyphyletic, having occurred at least 21 times. Lineages of water mites with non parasitic feeding larvae also exist in the same time with proximal populations or species that have parasitic larvae (Di Sabatino et al., 2000). Thus, as Smith B. (1998) stated, comparison of the two life history strategies has a tremendous potential for futures studies.

The life cycle that lack of a parasitic stage may confer some benefits through the accelerated development, but there are a drastically reduce in the dispersal capacity and

genetic variability (Di Sabatino et al., 2000). Comparison studies of closely related species with and without larval parasitism by Smith B. (1998) and Bohonak et al. (2004); shows that loss of larval parasitism correlates with an accelerated maturity and metamorphosis to adults of smaller size (Smith I. et al., 2010). In addition, studies verify that the female adults from with non-parasitic larvae produce smaller numbers of larger eggs; the extra nutrition that this means permits the larvae to forgo feeding (Smith B., 1998). There is also no apparent pattern in relation to habitat; both lineages occur in streams, ponds, lakes, and other regions (Smith B., 1998).

### **Global patterns in water mite distribution**

All superfamilies of water mites occur almost everywhere richly represented in all zoogeographic regions, except Antarctica (Cook, as cited in Di Sabatino et al., 2000). Smith and Cook (as cited in Di Sabatino et al., 2000), describe the primary distribution of geographical patterns as the result of vicariance due to plate tectonics and, in to a lesser degree, by its host dispersal. Dispersal between contiguous areas or across continents significantly modifies these geographical patterns as modern groups displaced the ancient ones progressively (Smith I. et al., 2010). According to Smith and Cook (as cited in Di Sabatino et al., 2000), for most taxa a more ancient Pangean, Gondwanan or Laurasian distribution is easily to recognize. Thus, there are several examples of an evident morphological similarity between unrelated taxa that have been apart over prolonged geohistorical times (Di Sabatino et al., 2000).

As stated in Di Sabatino et al. (2000), some families and subfamilies of water mites are limited to a certain geographic region: we can find species of Neocarinae, Bogatiidae, Chappuisidinae, Rutripalpidae or Huitfeldtiinae in the Holarctic region; Momonidinae distributed in the Palaearctic; Cowichaniinae, Laversiidae in the Nearctic;

Psammolimnesiinae and Ferradasiidae in the Neotropic; and Astacocrotonidae, Australiothyadinae and Gretacarinae in the Australian region. As pointed Gerecke (as cited in Di Sabatino et al., 2000), endemic species of water mites that live in a more restricted geographical area are rare, and most species are distributed over a larger geographical range. An interesting example is the one mentioned in Smith I. et al. (2010), showing that in many cases, "monotypic genera are known only from isolated, single or rare populations with particularly ecological requirements correlated with habitat specializations," as many interstitial genera that have relict distributions caused by the disappearance of their habitats requiring a long period of climatic stability to become reestablish there again. As Smith continues, the sister species of many Nearctic Tertiary relicts now inhabit similar areas in temperate Asia, resulting in strikingly discontinuous distributions. The species adapted to areas that have similar conditions and distributions of different genera were dramatically influenced by climatic cooling and glaciations during the late Pliocene and Pleistocene (Smith I. et al., 2010). As they said, "Genera, which show evidence of recent adaptive radiation, have reestablished pan-continental distributions while those with relatively few species often have remained within more limited areas" (Smith I. et al., 2010).

Because of its potential in the study events belonging to very distant geohistorical changes, water mites are also a very interesting group to investigate in terms of historical zoogeography (Di Sabatino et al., 2000).

### **Potential as indicators of environmental quality**

Water mites have been shown to be useful water quality bioindicators in most freshwater habitats, especially running water (Dohet et al., 2008) and rarely in lakes (Biesiadka & Kowalik, as cited in Smith I. et al., 2010).

The particular biological attributes of Hydrachnidia species makes them adapted and specialized to different limited ranges of physical and chemical traits (Di Sabatino et al. 2000, Smith I. et al., 2010). Because of their predacious lifestyle as immature or entirely developed adults, water mite species richness may reflect the diversity of prey and host species as well. In addition, variations in mite faunal diversity not only should reflect some changes in the aquatic ecosystem provoked by alterations in the physical and/or chemical variables of the area, but also indicates their own sensitivity to environmental conditions (Więcek et al., 2013). Various studies confirm that water mite species richness and abundance is seriously affected by chemical pollution or physical disturbance degradation (Di Sabatino & Cicolani, as cited in Di Sabatino et al. 2000).

### **Biogeography of microorganisms**

As many other ecological aspects, the biogeography of every living organism is also crucial regulated by its body size (Fontaneto & Hortal, 2013). For a long time, the biogeography of free-living microorganisms, whether or not they display cosmopolitan distributions and microbial dispersal limitations due to their small size, has been the subject of debate (Yang et al., 2010; Wilkinson et al., 2012; Fontaneto & Hortal, 2013).

During 1930 the main idea concerning microbial biogeography was that microbial species can easily disperse to anywhere on the planet (Wilkinson et al., 2012). Nevertheless, during the second half of the twentieth century, this concept was challenged (Yang et al., 2010). There are consistent exceptions that appear with the idea of cosmopolitan distributions, most of them related with habitat requirements regulate what could be found in any given place (Fierer & Jackson, as cited in Yang et al., 2010).

The transition region for cosmopolitan organism dispersion about the size of 1–10 mm established by general models for “size-mediated biogeographies,” tend to lack a

valid allegation when the particular biological traits of the specie comes into play (Valdecasas et al., 2006).

## **1.2.Objectives**

Given the limited information available about geographic distribution patterns of water mites and continuing with the previous research of Valdecasas et al. (2006) where the biogeography of small animals was assessed, this study aims to contribute to the knowledge of water mite biogeography and allow the development of further investigations on this amazing yet unexplored group.

As several authors stated, hosts play an important role on the dispersal of this clade. It is logical to think that the loss of parasitism limits their geographic distribution, and in that context, the main objective of this study is to evaluate distribution patterns according to the presence of larval parasitism on aquatic mites. We tested dispersal patterns related to the two types of larval biological development in order to solve the question given at the beginning of the work: “Does parasitism mediate water mite biogeographic distribution?”

## II. MATERIALS AND METHODS

### 2.1. Compilation of the data set

There are around 6,000 species of water mites documented in the world (Zhang, 2011). However, there are an additional number of subspecies that are not counted in that revision. As a compromise, we used as basic data, the list of water mites species compiled by Smit H. (2005) under the order Actinedida. It amounts to a list of 9,919 taxa.

Our test data are the 26 species mentioned by Smith B. (1998) as having loss their parasitic behavior in the larval stage. We sampled 30 groups of 30 species from H. Smit (2008) list generating random number between 1 and 9,919 from <http://www.random.org/> (last seen 2 June 2014), that is said that “the randomness comes from atmospheric noise, which for many purposes is better than the pseudo-random number algorithms typically used in computer programs.” The generator has been used by a certain number of scientific publications in refereed journals (<http://www.random.org/media/>). We selected groups of 30 numbers allowing for the possibility of having trouble to document particular species distribution, ending with 30 groups of 26 species distributions.

Worldwide distribution of the species selected was documented from Viets (1956) and Viets (1987) compilations, individual publications and the web page of Water Mites in Europe (<http://www.watermite.org/>; last seen 2 June 2014).

We obtain an average distance for each species taking the larger in cases they present more than two areas of geographic distribution. When the specie where just



registered in one location we assign the lowest value of the entire group, corresponding to 49.3 km found in *Knysnabates theroni* Cook, 2003. All distances were rounded to their entire number.

The distances were measured using the distance measurement tool from Google Maps Engine. First we create a map for each species and locate all the distribution data on it, and then obtain the distance between the two farthest points as shown in figures 6 to 8.

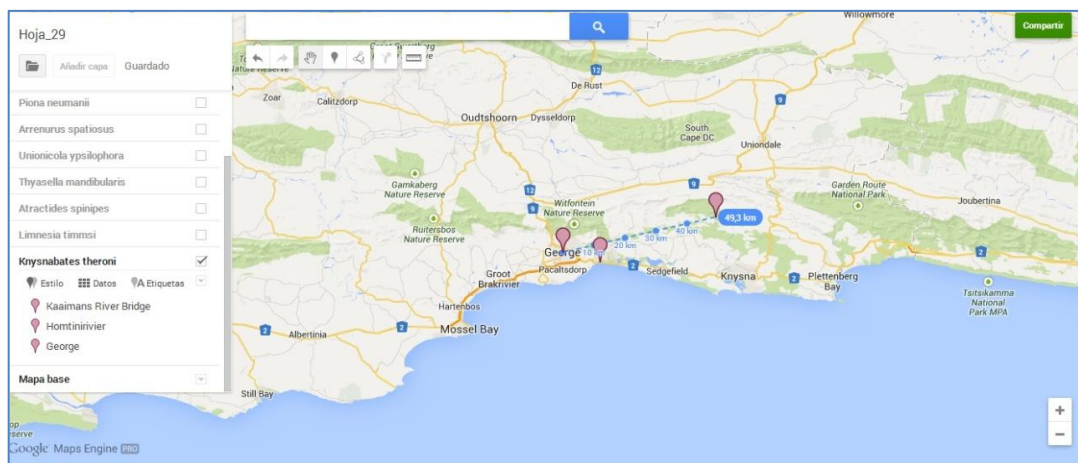


Fig. 6 Distribution Map of *Knysnabates theroni* Cook, 2003 generated in Google Maps Engine showing a local distribution.

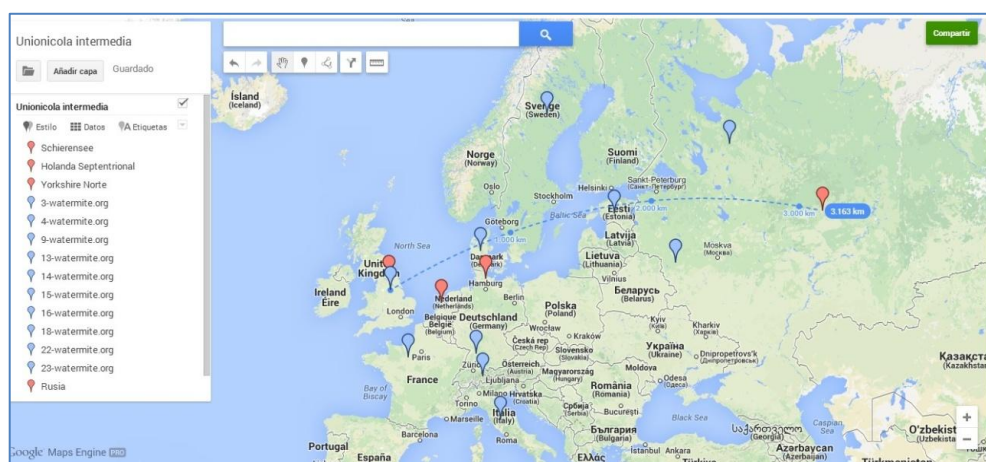


Fig. 7 Distribution Map of *Unionicola intermedia* (Koenike, 1909) generated in Google Maps Engine showing a continental distribution.

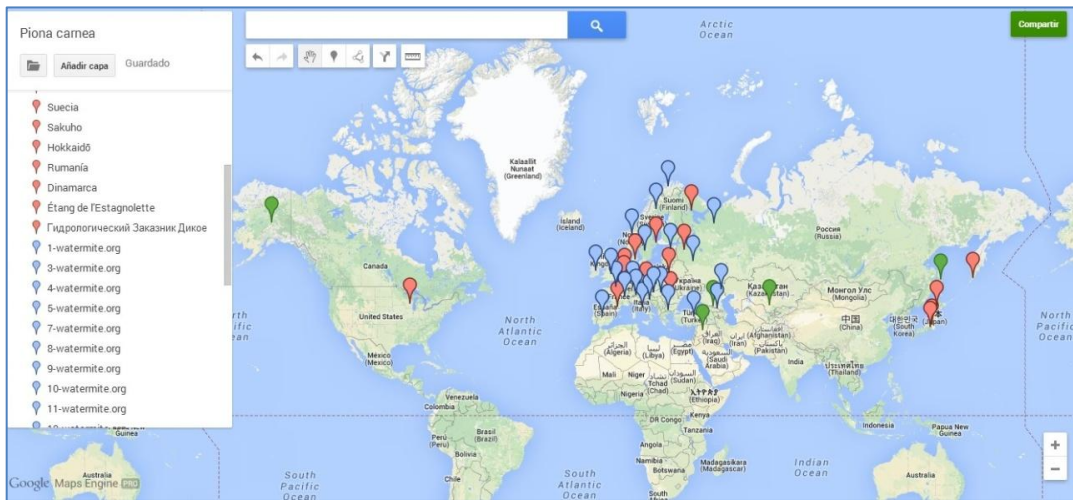


Fig. 8 Distribution Map of *Piona carnea* (Koch, 1836) generated in Google Maps Engine showing a circum worldwide distribution.

## 2.2. Statistical analyses

We have used several approaches to test the following null hypothesis:

$$\mu = \mu_0$$

Where  $\mu$  is the mean distribution distance of the parent population, that is, the water mite species that have a documented or presumed parasitic stage when larvae, and  $\mu_0$  is the mean distribution distance of the clique of water mite species that do not have a parasitic stage when larvae.

The basic idea is that if the parasitic stage affects the dispersal capacity of the water mite larvae, then both means will be different. And we add an additional criterion: we assume that the parasitic stage of insects is an aid to dispersal, so our hypothesis is that

the distribution of parasitic species will be greater than the distributions that have lost their parasitic stage.

Our selection of statistical methodology derives from the non-normal distribution of the two sets of data: the parasitic and the non-parasitic water mites (Fig. 9).

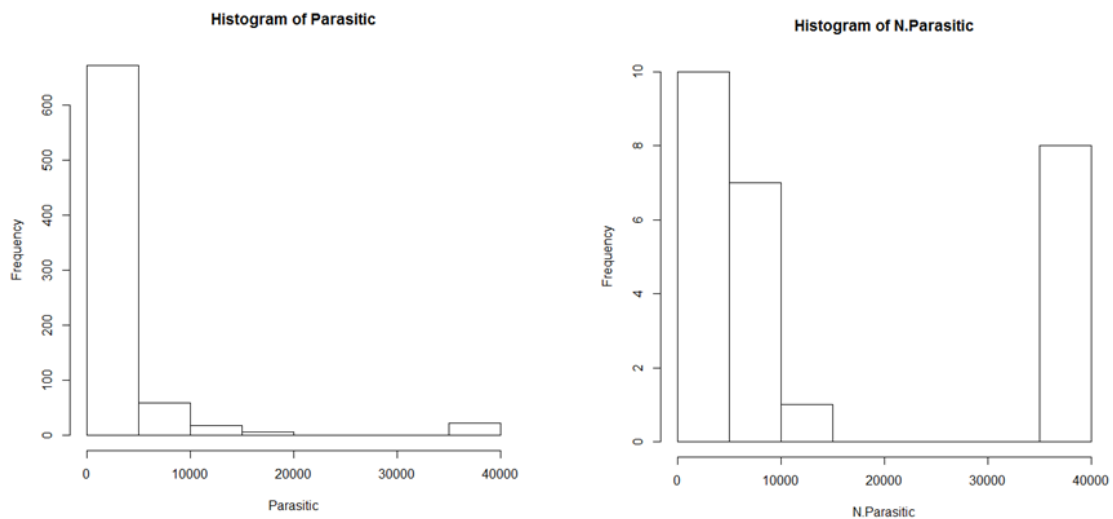


Fig. 9 Non-normal distribution of parasitic and non-parasitic water mites

### Resampling methods:

There is a large number of test available for non-parametric data (Siegel, 1957) but in the last 20 years, with the improving calculating capacities of computers a new realm of statistical methods have been developed and are available for the statistical user. These are called 'resampling methods' or computer intensive methods (Diaconis & Efron, 1983).

There are three main types of resampling techniques (Berger, n.d.): permutation test, bootstrap and Monte Carlo. In our case the bootstrap is the adequate technique to test between the range of distribution of parasitic and non-parasitic water mites, as it

allows estimate confidence intervals for our target parameter, the mean distribution of parasitic versus non parasitic water mite species. The bootstrap has been given a solid foundation by Efron (1994) although some others authors had proposed the same idea in a less formal detail (Simon, 1997). The basic idea of the bootstrap method is to sample with replacement the original sample to produce a distribution that will be used to know 'how singular' is our original sample.

We have done four resampling schemes, all of them based on a 'bootstrapping' procedure, using the software for statistical computing and graphics R with the 'boot' command available in the R library. Each case was carried out on 10.000 simulations.

- a) Bootstrap of the set of distance means of the 30 groups of 26 parasitic water mites. This would generate a distribution of bootstrapped means and their confidence limits. The value of the distance mean of the group on non-parasitic water mites is then compared against this distribution of values.
- b) Bootstrap of the total set of 31 distance means. We assume that the non-parasitic water mite group belongs to the 'universe' of water mites and obtain a distribution of means for the whole. Again, the value of the distance mean of the group on non-parasitic water mites is compared against this distribution of values.
- c) Bootstrap of the 780 distance values of parasitic water mites to obtain a distribution of bootstrapped to compare with the value of the distance mean of the non-parasitic water mites.
- d) Finally, we built a data set of 780 + 26 species and proceed as above.

### III. RESULTS

#### 3.1. Distribution types

As seen in the previous maps, water mites distribution can be classified in three different categories. According to the range of dispersal, water mites could have a local, continental or a worldwide distribution.

Table 1 shows that there is not a homogeneous distribution of species in the three categories, and it also shows a marked difference among the two types of water mite life cycle.

**Table 1** Distribution percentage of parasitic and non-parasitic water mite's larvae according to their type of distribution.

Distribution type	Parasitic larvae		Non parasitic larvae	
	N	%	N	%
Worldwide	22	2.8	8	30.8
Continental	265	34	17	65.4
Local	493	63.2	1	3.8

#### 3.2. Descriptive data

Table 2 includes a summary of the distribution data of the set of 780 parasitic water mites' species by subsets of 26 species. Table 3 gives the same statistics for the non parasitic group of 26 water mites.

**Table 2**

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
49	49	49	1291	1503	9363
49	49	49	2812	1320	39900
49	49	49	2659	1228	39900

49	49	49	1449	694	16260
49	49	49	1372	734.2	11090
49	49	670.5	3538	4002	39900
49	49	49	4526	2868	39900
49	49	49	1448	1675	9551
49	49	49	958.3	755.2	8762
49	49	49	3221	3451	39900
49	49	49	4762	4219	39900
49	49	479	4417	2872	39900
49	49	49	1710	1682	9861
49	49	49	1943	106.8	39900
49	49	49	3355	2192	39900
49	49	49	3551	2664	39900
49	49	49	1003	694	5491
49	49	49	1063	836.2	8431
49	49	49	2416	1224	39900
49	49	49	5970	3187	39900
49	49	49	2116	1354	16770
49	49	49	471.2	49	8022
49	49	49	2452	49	39900
49	49	191	1653	2322	8993
49	49	587.5	3738	3867	39900
49	49	49	1338	1948	8719
49	49	49	1505	2208	8108
49	49	479	4941	6164	39900
49	49	49	2903	2941	16260
49	49	49	6317	4822	39900

**Table 3**

<b>Min.</b>	<b>1st Qu.</b>	<b>Median</b>	<b>Mean</b>	<b>3rd Qu.</b>	<b>Max.</b>
49	3945	7420	16110	39900	39900

In the following figure (Fig. 10) we have superimposed the density distribution over the histogram of the means of parasitic group that clearly shows that their distributions positively skewed to the right).

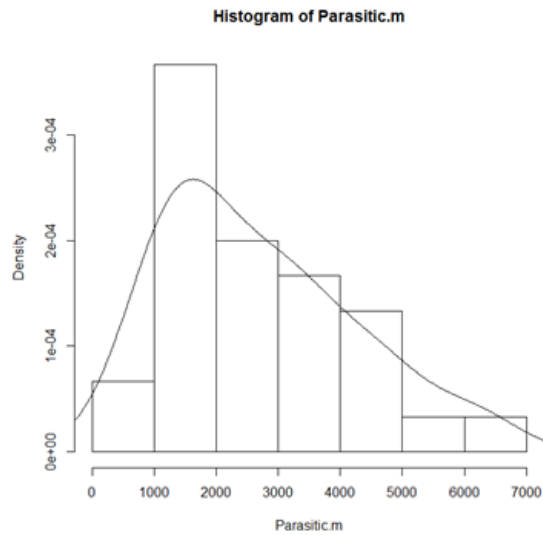


Fig. 10 Superposition of density distribution over the histogram of the means of parasitic group

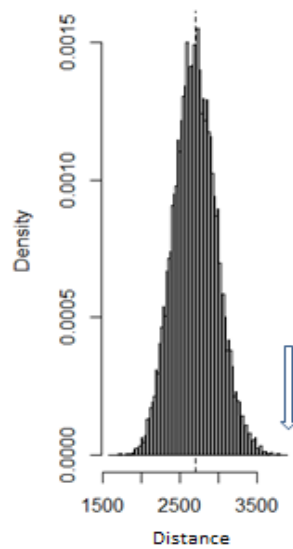
### 3.3.Resampling results:

a) Distribution of parasitic against non-parasitic means.

The bootstrapped statistics are:

Mean = 2.696, 3

Standard error = 277, 1



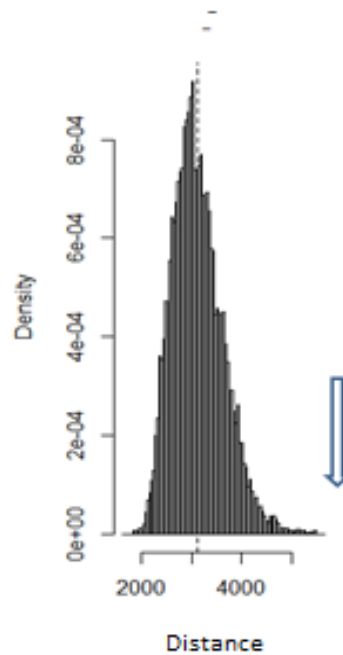
The dotted line indicates the mean of means of parasitic water mites and the arrow the value of the mean distance of non-parasitic water mites.

b) Resampling of the mean distances of parasitic and non parasitic water mites.

The bootstrapped statistics are:

Mean = 3129

Standard error = 504, 13



As before, the dotted line indicates the mean of means of parasitic and non parasitic water mites and the arrow the value of the mean distance of non-parasitic water mites.

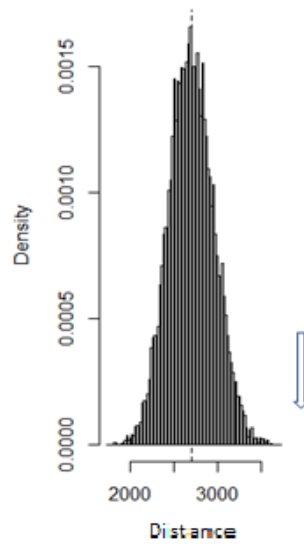
c) Bootstrap of the 780 distance values of parasitic water mites to obtain a distribution of bootstrapped means based on the whole sampled population.

The bootstrapped statistics are:

Mean = 2696, 7

Standard error = 253, 87



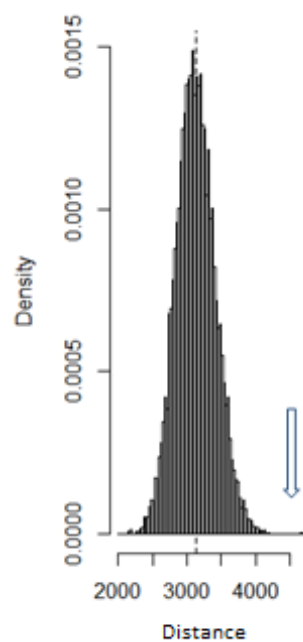


Same as before, the dotted line indicates the mean of original distance value of parasitic water mites and the arrow the value of the mean distance of non-parasitic water mites.

- d) Bootstrap of the 780 + 26 distance values of parasitic and non parasitic water mites to obtain a distribution of bootstrapped distance means to compare with the value of the distance mean of the non-parasitic water mites.

The bootstrapped statistics are:

Mean = 3129.4  
Standard error = 279.26



Finally, the dotted line indicates the mean of original distance value of parasitic and non parasitic water mites and the arrow the value of the mean distance of non-parasitic water mites.

#### IV. DISCUSSION

Distribution of organisms is not arbitrary, but they may depend from multiples causes (Darwin, 1859). Water mites have a complex life cycle, with a parasitic stage as larvae, two dormant phases and two predatory stages as nymphs and adults. They may only leave the water in the phoretic stage. It is assumed, that this parasitic stage makes dispersal easier (Davids et al., 2007)

In a previous work Valdecasas (Valdecasas et al., 2006) questioned Finlay (2002) statement that organisms below a certain size don't have a biogeography, meaning that below that size limit everything could be everywhere. Suggested explanations for the discordant results were particularities of life cycle like "resistance phase, parasitic stage or feeding habits" (Valdecasas et al., 2006).

In this work we have tried to advance into this question, improving first, on the empirical data used, and methodologically, with a different statistical strategy. Unlike Valdecasas et al. (2006) that counted distribution by areas we take linear distances from the two most extremes points of distribution of a species. This allows a hierarchical ordering of distributions without overlapping, a problem that appeared in Valdecasas et al. (2006).

The methodological strategy, besides the selections of distance as the variable to be explained, employ so called 'resampling methods', in this case 'the bootstrap' (Efron & Tibshirani, 1994), to use the sample to build the distribution of the population. The main critical aspect (but this is general to all the inferential statistics) is that the sample constitute a random sample.

Efron and Tibshirani (1994) have laid the bootstrap on a solid mathematical foundation. In our case, it may provide confidence limits for our target statistic. We have selected the mean distance per group because it is known that the mean behaves well with the bootstrap (Chernick & LaBudde, 2011).

Our first step was to provide a representative random sample of water mites species distribution. Our random data set constitutes around the 30% of all water mite species already known and considered well documented (Zhang, 2011). They were divided in groups of 26 species and their maximum distance distribution calculated. Mean distribution of each subset and mean of means was also calculated.

Our four resampling simulations were done on the premises that all of them should have a similar behavior as it resulted.

Our results, said in plain words, means that non-parasitic water mites have a wider distribution than parasitic species. This has been an unexpected result, and at first glance results counterintuitive. It may seem that being a parasite of a terrestrial insect one have a free ticket to disperse. However, it may not be so.

Dispersal of aquatic animals that may not stand desiccation requires continuity of the habitat. This may be so for stream dwelling species, at least for distributing along the same basin, but not necessarily between basins. A different situation occurs with ponds and lakes inhabitant. They have been considered continental island (Vuilleumier 1970) and dispersal from them is prone to the same vagaries as truly islands, desiccation being the main problem. To save discontinuity, water mites may require an external agent, a flying host, or be able to be physically transmitted by a biological or physical agent, either in their dormant stage or as deutonymph or adults. Valdecasas

(personal communication) found apparently viable *Arrenurus* individuals in the gut content of a duck in Bolivia. But this case and those findings of water mites in fishes are mainly anecdotic (Pešić et al. 2013). In consequence, dispersal for water mite's individuals may be risky and unpredictable.

What is true for water mites may be said as well for other aquatic animal. For aquatic insects with a terrestrial flying stage, finding a new aquatic habitat may be hazardous. Even more, if they have an additional 'cargo', as water mite larvae parasites, that may limit their range of dispersion, avoiding behavior to predators and the like (Di Sabatino et al., 2000), although it seems not to be always the case (Mlynarek et al., 2013)

In consequence, supposing that water mites have a mean probability to disperse ( $p$ ), the subset of parasitic water mites will have the compound of their probability and the probability of their host target to reach a new aquatic medium ( $q$ ). Assuming that both probabilities are independent (what is not necessarily true), we have:

$$p > (p * q)$$

That means that non-parasitic water mites have a higher probability of dispersal than their parasitic congeners, although that probability must be very low, as only a small set reaches the highest dispersal distance.

Other explanation for our result point to the possibility that the species with a very wide distribution are cryptic species, a phenomena found in other invertebrates groups but that has not been explored in water mites (Tzedakis et al., 2013). That is, what we are counting as one species may constitute a set of species minimally differentiable from a morphological point of view.

## V. CONCLUSIONS

We have done a review of the general biology of the clade Hydrachnidia. From their complex life cycle with parasitic and non-parasitic stages a complex pattern of geographical distributions arise. A very small subset of water mites species have overcome the parasitic stage providing for internal comparisons between both life cycles in relation to distribution.

There are substantial numbers of water mites' species with very broad distribution, even worldwide, others with medium sized distribution and a sizeable amount restricted or only known from one locality.

Due to the skewness of the distribution of geographical distances we have relied on resampling methods, more specifically, the bootstrap, for testing for differences between distances between parasitic and non-parasitic water mites species.

Contrary to expectations, the geographic dispersion in water mites' species without parasitic stage is wider than those found in parasitic species.

Our general conclusion is that the particular life cycle of parasitic and non parasitic water mites affects significantly their biogeography.

## **VI. ACKNOWLEDGMENTS**

In the first place I want to thank my director, Antonio García-Valdecasas, for encouraging me to start this project and for his invaluable support and constant lessons. The present study wouldn't have been possible without him. I will also want to give him the credits for the pictures shown in the present work. Thank you very much.

And finally to my tutor Maria José Luciañez, for always being available to me with the kindness that always characterizes her.

## VII. REFERENCES

- Arévalo, C. (1929). *La vida en las aguas dulces.* (Vol. 197). Editorial Labor. Available at: <http://www.bhl-europe.eu/bhle-read/bhle%3A10706a0ksn057#page/n139/mode/1up>. Last seen: 07 Jun 2014.
- Berger D. (n.d.). A gentle Introduction to resampling techniques. Web Interface for Statistics education. Claremont Graduate University. Available at: <http://wise.cgu.edu/downloads/Introduction%20to%20Resampling%20Techniques%20110901.pdf>. Last seen 4 June3 2014
- Bohonak, A. J., Smith, B. P., & Thornton, M. (2004). Distributional, morphological and genetic consequences of dispersal for temporary pond water mites. *Freshwater Biology*, 49(2), 170-180.
- Chapman, A. D. (2009). Numbers of living species in Australia and the world. Available at: [http://155.187.2.69/biodiversity/abrs/publications/other/species-numbers/2009/pubs/nl\\_saw-2nd-complete.pdf](http://155.187.2.69/biodiversity/abrs/publications/other/species-numbers/2009/pubs/nl_saw-2nd-complete.pdf). Last seen 27/05/2014
- Chernick, M. R., & LaBudde, R. A. (2011). *An introduction to bootstrap methods with applications to R.* Wiley Publishing.
- Darwin, C. (1859). *On the origin of species by means of natural selection.* Murray, London (1968). Chapters 11 and 12. Available at: <http://www.gutenberg.org/files/1228/1228-h/1228-h.htm>. Last seen 27/05/2014.



- Davids, C.; Di Sabatino, A.; Gerecke, R.; Gledhill, T.; Smit, H. & Van. (2007). Acari: Hydrachnidia in Gerecke, R. (ed.): Süßwasserfauna Mitteleuropas, 7/2-1, Chelicerata, Acari I: 241-376. Spektrum Elsevier, München.
- De Queiroz, A. (2014). *The Monkey's Voyage: How Improbable Journeys Shaped the History of Life*, Perseus Books Group.
- Di Sabatino, A. D., Gerecke, R., & Martin, P. (2000). The biology and ecology of lotic water mites (Hydrachnidia). *Freshwater Biology*, 44(1), 47-62.
- Diaconis, P., & Efron, B. (1983). Computer-intensive methods in statistics. *Scientific American*, 248(5), 16-130. Available at: <https://statistics.stanford.edu/sites/default/files/BIO%2083.pdf>. Last seen: 05 Jun 2014.
- Dohet, A., Ector, L., Cauchie, H. M., & Hoffmann, L. (2008). Identification of benthic invertebrate and diatom indicator taxa that distinguish different stream types as well as degraded from reference conditions in Luxembourg. *Animal Biology*, 58(4), 419-472.
- Efron, B., & Tibshirani, R. J. (1994). *An introduction to the bootstrap* (Vol. 57). CRC press.
- Finlay, B. J. (2002). Global dispersal of free-living microbial eukaryote species. *Science*, 296 (5570), 1061-1063.
- Fontaneto, D., & Hortal, J. (2013). At least some protist species are not ubiquitous. *Molecular ecology*, 22(20), 5053-5055.

- May, R. M. (1988). How many species are there on earth? *Science* (Washington), 241(4872), 1441-1449.
- Meyer, E., & Kabbe, K. (1991). Pigmentation in water mites of the general *Limnochares* Latr. and *Hydrodoma* Koch (Hydrachnidia). In *The Acari* (pp. 379-391). Springer Netherlands.
- Mlynarek, J. J., Knee, W., & Forbes, M. R. (2013). Relative geographic range of sibling species of host damselflies does not reliably predict differential parasitism by water mites. *BMC ecology*, 13(1), 50.
- Nelson, G., & Platnick, N. I. (1980). A vicariance approach to historical biogeography. *Bioscience*, 30(5), 339-343
- Pešić, V., Chatterjee, T., Das, M. K., & Bordoloi, S. (2013). A new species of water mite (Acari, Hydrachnidia) from Assam, India, found in the gut contents of the fish *Botia dario* (Botiidae). *Zootaxa*, 3746(3), 454-462.
- Siegel, S. (1957). Nonparametric statistics for the behavioral sciences. *The American Statistician*, Vol. 11, No. 3 (Jun., 1957), pp. 13-19. Available at: <http://www.mun.ca/biology/quant/siegel.pdf>. Last seen 07/06/2014. Last seen: 05 Jun 2014.
- Simon, J. L. (1997). *Resampling: The new statistics*. Duxbury Press. Available at: <http://www.resample.com/intro-text-online/> Last seen 4 June3 2014
- Smit, H. (2005). Synopsis of the described Actinedida of the world. In: Hallan, J. (coord.) *Index of the described animalia of the world*. Available at: <https://insects.tamu.edu/research/collection/hallan/Acari/Family/Actinedida1.htm>; Last seen: 22/05/2014).

- Smith, B. P. (1988). Host-parasite interaction and impact of larval water mites on insects. *Annual review of entomology*, 33(1), 487-507.
- Smith, B. P. (1998). Loss of larval parasitism in parasitengonine mites. *Experimental & applied acarology*, 22(4), 187-199.
- Smith, I. M., Cook, D. R. & Smith, B. P. (2010) Water Mites (Hydrachnidiae) and Other Arachnids. In: Thorp, J. & Covich, A. (eds) Chapter 15: Ecology and Classification of North American Freshwater Invertebrates. 3rd ed. Academic Press, San Diego (Elsevier), pp 485-586.
- Tzedakis, P. C., Emerson, B. C., & Hewitt, G. M. (2013). Cryptic or mystic? Glacial tree refugia in northern Europe. *Trends in ecology & evolution*, 28(12), 696-704.
- Valdecasas A.G. (1981). Las hidracnelas de la Sierra del Guadarrama: taxonomía, distribución y ecología, Doctoral Thesis, Universidad Complutense, Madrid, Spain.
- Valdecasas, A. G., Camacho, A. I., & Peláez, M. L. (2006). Do small animals have a biogeography?. *Experimental & applied acarology*, 40(2), 133-144.
- Viets K. O. (1987) Die Milben des Susswassers (Hydrachnellae und Halacaridae (part.), Acari). 2: Katalog. Sonderbaende des Naturwissenschaftlichen Vereins in Hamburg 8:1-1012.
- Viets, K. O. (1956). Die Milben des Susswassers und des Meeres. Zewwiter und Dritter Teil, Gustav Fischer, Sttugart, Jena, 870 pp.
- Vuilleumier, F. (1970). Insular biogeography in continental regions. I. The northern Andes of South America. *American Naturalist*, 373-388.

- Więcek, M., Martin, P., & Gąbka, M. (2013). Distribution patterns and environmental correlates of water mites (Hydrachnidia, Acari) in peatland microhabitats. *Experimental and Applied Acarology*, 61(2), 147-160.
- Wiggins, G. B., Mackay, R. J., & Smith, I. M. (1980). Evolutionary and ecological strategies of animals in annual temporary pools. *Archiv für Hydrobiologie supplement*, 58(97), 206.
- Wilkinson, D. M., Koumoutsaris, S., Mitchell, E. A., & Bey, I. (2012). Modelling the effect of size on the aerial dispersal of microorganisms. *Journal of Biogeography*, 39(1), 89-97.
- Yang, J., Smith, H. G., Sherratt, T. N., & Wilkinson, D. M. (2010). Is there a size limit for cosmopolitan distribution in free-living microorganisms? A biogeographical analysis of testate amoebae from polar areas. *Microbial ecology*, 59(4), 635-645.
- Yoder, A. D., & Nowak, M. D. (2006). Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. *Annu. Rev. Ecol. Evol. Syst.*, 37, 405-431.
- Zhang, Z. Q. (2011). Order Trombidiformes Reuter, 1909. Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness. *Z. Q. Zhang, Zootaxa*. 3148: 129-138
- Zink, R. M., Blackwell-Rago, R. C., & Ronquist, F. (2000). The shifting roles of dispersal and vicariance in biogeography. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267(1442), 497-503.