

Evolutionary ecology, biogeography and conservation of water beetles in Mediterranean saline ecosystems

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ABSTRACT

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Among the variety of Mediterranean aquatic habitats, inland saline ecosystems are considered particularly rare and have been much less studied than other freshwater systems. Previous research has emphasised the ecological and evolutionary singularity of these environments and their great conservation value, as they are extremely endangered and also include a highly specialised biota with a high degree of endemism and genetic diversity. However, until recently, our knowledge about the biogeographical patterns and evolutionary processes of the lineages inhabiting these particular systems has been very sparse. This paper is a compilation and summary of the principal results obtained from various recent studies that were developed in the framework of the PhD thesis of the first author and focused on the water beetle diversity of the Mediterranean inland saline waters. The evolution of salinity tolerance, the main factors driving diversification on saline lineages and the application of all this information to provide relevant data for the conservation of aquatic saline biodiversity are addressed. Finally, further objectives are identified to progress in our understanding and the conservation of Mediterranean saline biodiversity.

Key words: Inland saline waters, aquatic insects, salinity tolerance evolution, dispersal capacity, cryptic diversification, lotic and lentic habitats, species vulnerability, climate change.

RESUMEN

Ecología evolutiva, biogeografía y conservación de los coleópteros acuáticos en ecosistemas salinos del Mediterráneo

Entre la variedad de hábitats acuáticos de la cuenca Mediterránea, los ecosistemas salinos continentales se consideran particularmente raros y han sido mucho menos estudiados que otros sistemas de agua dulce. Investigaciones previas han destacado la alta singularidad ecológica y evolutiva de estos ambientes y su alto valor en términos de conservación, puesto que están extremadamente amenazados e incluyen una biota muy especializada con altos grados de endemidad y diversidad genética. Sin embargo, hasta ahora nuestro conocimiento sobre los patrones biogeográficos y los procesos evolutivos de los linajes de estos sistemas ha sido muy escaso. Este artículo es una compilación y resumen de los resultados principales obtenidos en varios estudios recientes desarrollados en el marco de la Tesis Doctoral de la primera autora y centrados en la diversidad de coleópteros acuáticos de las aguas continentales salinas del Mediterráneo. Se presentan distintos tópicos como la evolución de la tolerancia a la salinidad, los principales factores que afectan a la diversificación de los linajes salinos y el uso de toda esta información para proporcionar datos relevantes para la conservación de la biodiversidad acuática salina. Finalmente, se identifican nuevos objetivos para futuros trabajos y poder así seguir avanzando en nuestro conocimiento y conservación de la biodiversidad salina del Mediterráneo.

Palabras clave: Aguas salinas continentales, insectos acuáticos, evolución de la tolerancia a la salinidad, capacidad de dispersión, diversificación críptica, hábitat lótico y lenítico, vulnerabilidad de las especies, cambio climático.

INLAND SALINE WATERS IN THE MEDITERRANEAN REGION

The Mediterranean basin region is one of the world's biodiversity hotspots. It constitutes one of the regions of highest biogeographical interest and displays a complex geological history that includes glaciations, tectonics and contact between African and Euroasiatic biotas (Médail & Quézel, 1999; Rosembaum *et al.*, 2002). These complex processes have left their fingerprints on Mediterranean biodiversity, particularly in the case of aquatic lineages where the isolated and fragmented nature of their habitats and their broad heterogeneity could have exacerbated the effects of such a knotty history (e.g., Bonada *et al.*, 2009, Múrria *et al.*, 2012).

Among the wide variety of Mediterranean aquatic habitats, inland saline ecosystems have gone relatively unnoticed until recently and have been much less studied than the more common freshwater systems. However, different studies focused on saline waters in recent years have described their high singularity and interest at multiple levels (Millán *et al.*, 2011). These naturally saline waters have salinities ranging from 3 g/L to more than 200 g/L (i.e., six times the mean salinity of the sea) due to the presence of specific lithologies in their watersheds or influxes of seawater from the coastline. Indeed, sedimentary rocks such as calcareous (limestones and marls) and evaporitic outcrops (consisting mainly of anhydrite, gypsum, halite and sylvine), which originated from the recurrent marine incursions and subsequent regressions of the continent that have occurred since the Triassic in the Mediterranean region (Duggen *et al.*, 2003), are usually linked to inland saline waters. These aquatic habitats are scarce in the western Palearctic region and are particularly rare in Europe, where they are mostly located around the Mediterranean Basin (in the Iberian Peninsula, mainly in the Guadalquivir, Segura, Júcar and Ebro watersheds) and display a strongly patchy and restricted distribution (Millán *et al.*, 2011). This scattered disposition, together with their natural stressful conditions mainly associated with the osmotic stress imposed by the high salini-

ties, makes the Mediterranean saline waters an ideal model for exploring the mechanisms involved in the generation and distribution of biodiversity in space and time.

However, until now, most research on inland waters in Europe and the Mediterranean basin has mainly focused on freshwater systems, with much less attention given to inland saline waters and their biodiversity (Millán *et al.*, 2011). This is particularly dramatic because these ecosystems have been recognised as among the most endangered European aquatic ecosystems (Velasco *et al.*, 2006; Sánchez-Fernández *et al.*, 2008). They suffer severe dilution and eutrophication processes as a consequence of the extreme changes in land use (mainly intensive agriculture) that have occurred in recent decades (Millán *et al.*, 2011; Gutiérrez-Cánovas *et al.*, 2013). In addition, climate change is expected to synergistically affect these and other Mediterranean aquatic ecosystems (Moss *et al.*, 2009). Rising global temperatures and the increased frequency and intensity of droughts and extreme flow events could drastically affect aquatic biodiversity, especially in the most arid areas (Sala *et al.*, 2000; Heino *et al.*, 2009).

SALINE BIODIVERSITY: WATER BEETLE LINEAGES

Inland saline waters are very productive ecosystems (Velasco *et al.*, 2003, 2006) but are inaccessible for most organisms due to their toxic salinity levels. Water salinity constitutes a strong evolutionary pressure and is one of the main environmental factors constraining the ecological niches of aquatic species. As a result, an important decrease in taxon richness occurs along the salinity gradient (Williams, 1998; Pinder *et al.*, 2005; Arribas *et al.*, 2009), which is characterised by a high community turnover (Gutiérrez-Cánovas *et al.*, 2013).

Among the simplified communities of macro-invertebrates in saline waters, Coleoptera and Diptera are the most diverse and include taxa specialised to the full range of habitats across the salinity gradient (Millán *et al.*, 2011). The suc-

cess and potential of diversification of the beetles over all habitats is broadly recognised; they represent almost 25 % of all known animal life-forms and occur in all of the world's ecosystems (Hunt *et al.*, 2007). In inland waters, water beetles constitute one of the richest and most abundant groups of aquatic macroinvertebrates, with many species also occurring in the Mediterranean region (approximately 1000 species, Ribera, 2000) and particularly in the Iberian Peninsula (486 species, Millán *et al.*, 2014). Aquatic Coleoptera

are present across the entire spectrum of aquatic habitats, including the three main types of saline waters: hyposaline, mesosaline and hypersaline systems (Arribas *et al.*, 2009; Millán *et al.*, 2011). The studies focusing on water beetles (or more generally on macroinvertebrates) in Mediterranean saline waters show convergent results about the large number of endemic species displaying high habitat specificity (Abellán *et al.*, 2009; Sánchez-Fernández *et al.*, 2011), high physiological uniqueness (Grueber & Bradley, 1994;

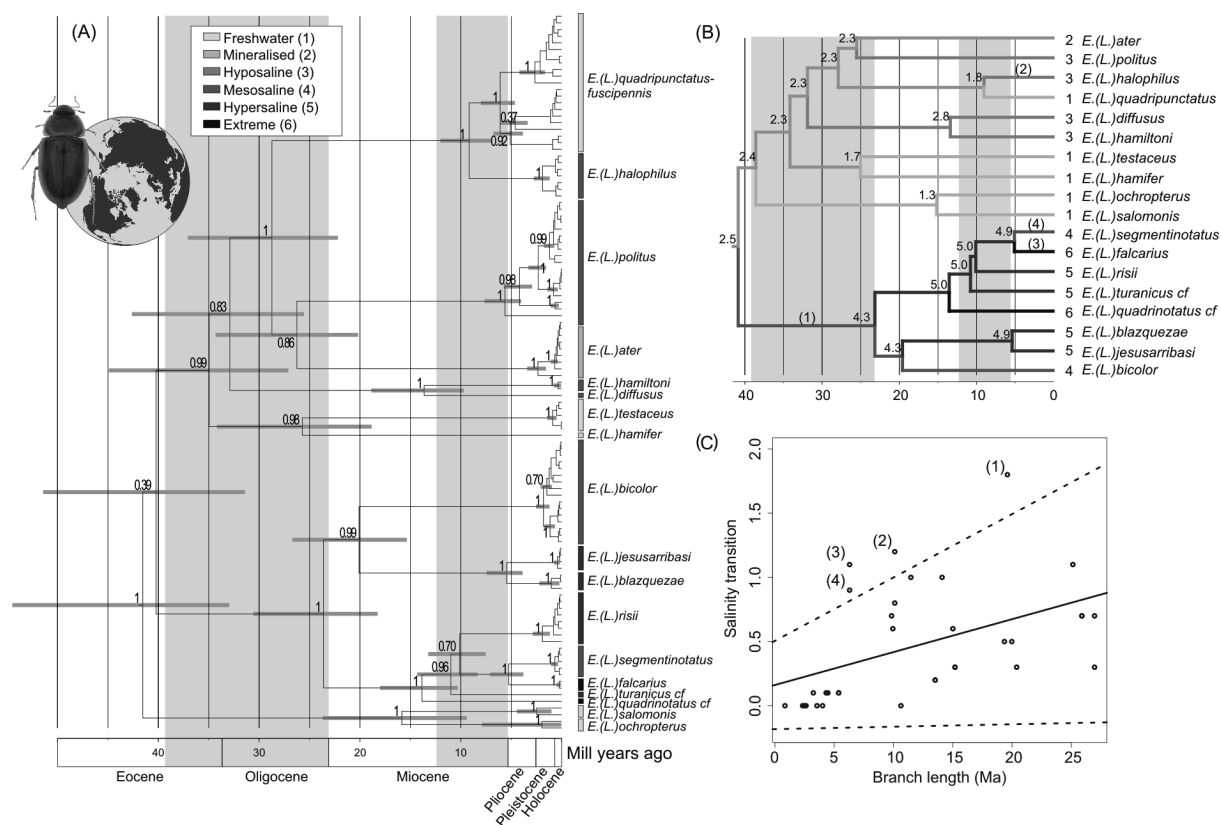


Figure 1. Tempo and mode of the multiple origins of salinity tolerance in the water beetle subgenus *Lumetus* (family Hydrophilidae) (A) Ultrametric time-calibrated tree for the subgenus using five mitochondrial and two nuclear genes. Numbers above nodes: posterior probabilities. Grey bars on nodes: 95 % confidence intervals for node ages (Ma). Coloured vertical bars: salinity tolerance of each species. Shaded areas: events of global aridification in the northern hemisphere. (B) Reconstruction of ancestral salinity tolerance. (C) Plot of branch lengths with absolute ranges of the salinity transitions for the ancestral reconstruction. Discontinuous lines: 99 % confidence intervals of the significant regression line (solid line). Numbers: branches indicated in (B). Modified from Arribas *et al.*, 2014. *Marco temporal y origen múltiple de la tolerancia a la salinidad en un subgénero de coleópteros acuáticos Lumetus (familia Hydrophilidae)* (A) *Árbol ultramétrico calibrado del subgénero construido en base a cinco genes mitocondriales y dos nucleares. Números sobre los nodos: probabilidades posteriores. Barras grises: Intervalos de confianza al 95 % para la edad de los nodos (Ma). Barras verticales coloreadas: tolerancia a la salinidad de cada especie. Áreas sombreadas: eventos globales de aridificación en el hemisferio norte.* (B) *Reconstrucción ancestral de la tolerancia a la salinidad.* (C) *Longitudes de rama frente al rango de salinidad de las transiciones para la reconstrucción ancestral. Líneas discontinuas: Intervalos de confianza al 99 % para la regresión significativa (línea continua). Números: ramas indicadas en (B). Modificado de Arribas et al., 2014.*

Sánchez-Fernández *et al.*, 2010) and extraordinary levels of genetic diversity within their lineages (Abellán *et al.*, 2007; Muñoz *et al.*, 2008). However, further studies of the biogeographical patterns and evolutionary processes of such lineages are still essential to estimate, understand and preserve the biodiversity of inland saline waters.

EVOLUTION, BIOGEOGRAPHY AND CONSERVATION IN MEDITERRANEAN SALINE WATERS

As shown above, the high singularity of Mediterranean saline waters at multiple levels (from their spatial distribution at global and regional scales to their naturally stressful habitat conditions) makes these systems an interesting model for further exploration of the evolution and biogeography of their lineages. Here we summarise and discuss some of the main results obtained in various recent studies, which were developed in the framework of the PhD thesis of the first author (Arribas, 2013) and focused on the water beetle diversity of the Mediterranean inland saline waters. Such studies combined multiple methodologies, such as phylogenetics, morphometrics, thermal physiology and ecological modelling, to contribute to a better understanding of the determinants and mechanisms of diversification and persistence of species in these stressful systems and to provide relevant data for the conservation of their biodiversity. Despite it is increasingly recognised that a comprehensive study of ecological and evolutionary processes on lineages requires integration across a vast array of disciplines (Whittaker *et al.*, 2005), such transversal research is still scarce in the case of the continental waters, and it constitutes a minor part of the limnological research. In regards to the Iberian Peninsula, some broad approaches to the biogeographical and evolutionary patterns of freshwater macroinvertebrates have been performed (e.g., Ribera & Vogler, 2004; Ribera *et al.* 2001, 2011; Bonada *et al.*, 2009; Múrria *et al.*, 2012), but studies are still scant when considering the great promise of these systems

for macroecological and evolutionary research (Hortal *et al.*, 2014; Pauls *et al.*, 2014). The need for integrated knowledge about these processes is particularly pressing in biodiversity hotspots such as the Mediterranean region and in the most endangered ecosystems such as the inland saline waters, where both the challenges and payoffs of conservation measures are expected to be the greatest (Myers *et al.*, 2000).

Evolution of the tolerance to salinity

The study of saline tolerance evolution has been proposed as particularly important for clarifying the general processes that allow organisms to address extreme conditions; however, until now, it has been mainly centred on terrestrial or marine systems (e.g., Flowers *et al.*, 2010; Whitehead *et al.*, 2013), while inland waters have remained largely unexplored. This lack of study is despite the fact that continental waters comprise a much broader salinity gradient and patchy mosaic that could potentially result in intricate evolutionary histories mediated by salinity tolerance. In insects, it has been proposed that, in general, adaptation to saline water is a highly derived characteristic, most likely arising in species already adapted to freshwater (Bradley *et al.*, 2009). However, the few studies on mosquito larvae that have dealt with this topic revealed much more complex evolutionary patterns of salinity tolerance (Grueber & Bradley, 1994; Albers & Bradley, 2013) and significant gaps in our knowledge gaps still exist concerning the adaptation trajectory to saline waters.

In a recent study, Arribas *et al.* (2014) used the potential offered by the subgenus *Enochrus* (*Lumetus*), an inland aquatic lineage of water beetles that includes species adapted across the full salinity gradient, to explore the process of adaptation to salinity and its temporal framework. The time-calibrated phylogeny of this beetle group showed that salinity tolerance appeared multiple times with evidence of some accelerated transitions from freshwater to high salinities associated with periods of global aridification (see Fig. 1). On the basis of these results and the strong positive correlation found between salinity tolerance

and the aridity of the habitats in which these beetle species are found, Arribas *et al.* (2014) suggested that tolerance to salinity may be based on a co-opted mechanism originally developed for drought resistance.

The emergence of a parallel function or capability associated with a regulatory mechanism developed for different purposes has been proposed as being relatively frequent in the evolution of characters (Gould & Vrba, 1982). Because of the similar physiological basis of tolerances to desiccation and osmotic stress, i.e., the physiological mechanism for avoiding internal water loss (Cloudsley-Thompson, 2001; Bradley, 2008), it seems reasonable to hypothesise that in some insect groups the selection for reducing water loss (i.e., water absorption and ion excretion by the rectum) could secondarily lead to the possible toleration of saline waters and subsequent diversification in these habitats. Global aridification events have already been identified as a driver of diversification in multiple lineages (e.g., Pepper *et al.*, 2011; Pinceel *et al.*, 2013); and because of the complex climatic and geographical history of Europe and the Mediterranean Basin, this could be particularly relevant in the area (Postigo Mijarra *et al.*, 2009). The evolution of saline tolerance as a result of a process of exaptation linked to desiccation tolerance could potentially have acted on other aquatic lineages adapted to saline waters and driven the strong diversification found across the saline gradient of Mediterranean inland waters.

Additionally, the cost of ionic and water regulation will likely be detrimental to other metabolic functions. Therefore, a trade-off between competitive ability and adaptation to osmotic stress has been suggested for different aquatic groups, with tolerant species displaying a selective disadvantage in more competitive habitats, such as those with less stressful conditions (Herbst, 2001; Latta *et al.*, 2012). In accord with such trade-offs, several studies covering a broad salinity gradient of inland waters have reported a strong inverse relationship between species richness and salinity in a variety of aquatic systems (e.g., Williams, 1998; Pinder *et al.*, 2005; Gutiérrez-Cánovas *et al.*, 2013). Similarly, some recent physiological

studies on insects inhabiting saline waters (i.e., Carbonell *et al.*, 2012; Céspedes *et al.*, 2013; Pallarés *et al.*, 2015) have reported an important mismatch between their fundamental and realised salinity niches, because specimens tolerated freshwater in the laboratory but were absent in such low-mineralised conditions in the field. Such results are also concordant with a trade-off between the competitive ability of species and their increase in salinity tolerance. Similarly, Arribas *et al.* (2014) did not find any evidence of a reversal from saline to freshwater in the *Enochrus* (*Lumetus*) phylogeny (see Fig. 1), again pointing to salinity specialisation as a single-direction habitat transition among aquatic insects.

Diversification in saline waters

The macroinvertebrate communities of saline waters, particularly those of saline streams, usually include endemic species displaying high habitat specificity, restricted geographical ranges and often occurring as highly isolated populations (Millán *et al.*, 2011). In addition, different studies have reported high and spatially structured genetic diversity within aquatic saline lineages, which together point to an important role of dispersal limitation driving diversification in Mediterranean saline systems (Muñoz *et al.*, 2008; Abellán *et al.*, 2009). As examples, Abellán *et al.* (2007) and subsequently Sánchez-Fernández *et al.* (2011) reported an extraordinarily high genetic diversity, phylogeographic structure and climatic niche divergence among the Iberian populations from the Segura, Jucar and Guadalquivir watersheds for the hypersaline water beetle *Ochthebius glaber*. Similarly, Arribas *et al.* (2013) found that poor habitat connectivity in the distribution of saline streams in the western Mediterranean (e.g., resulting from the sea or mountain ranges) constitute effective barriers to gene flow between populations of another highly specialised saline water beetle (i.e., the *Enochrus falcarius* complex, Fig. 2). Indeed, the subsequent study by Arribas *et al.* (2014) reported the two splits between southern European and northern African saline lineages of this complex as being coherent with a post-Messinian vicariant

speciation (the *E. jesuarribasi*-*E. blazquezae* and *E. falcarius*-*E. risii* lineages shown in Fig. 1). Despite the fact that most of these species are

winged and able to fly, different studies point to a low dispersal ability for these aquatic beetles, likely driven by the long-term stability and patchy

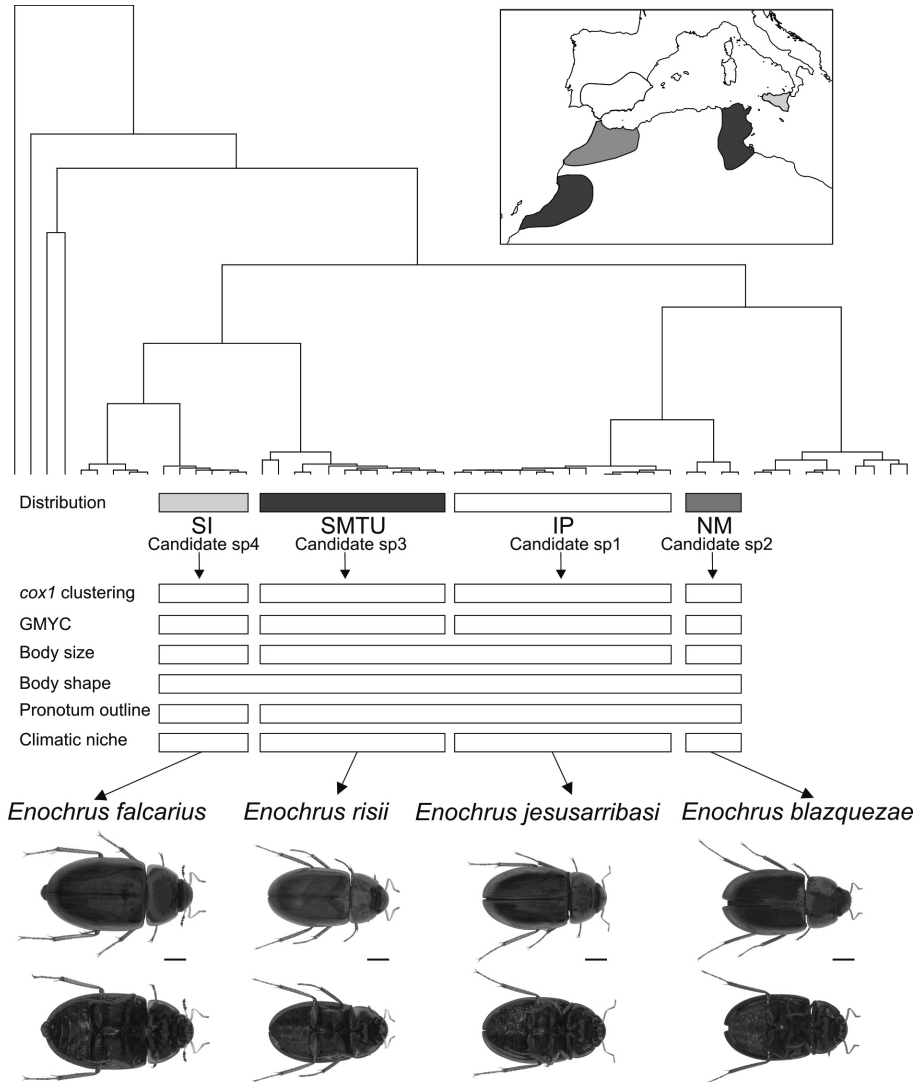


Figure 2. Synthetic representation of the integrative taxonomy approach combining geographical, molecular, morphometric and ecological information on the *Enochrus falcarius* species complex and the four species delimited within this complex. Hypotheses about the candidate species based on the phylogenetic analyses (four mitochondrial genes and one nuclear gene) and biogeographical information (SI Sicily, SMTU Southern Morocco and Tunisia, IP Iberian Peninsula, NM Northern Morocco). Evaluation of lineage divergence between candidate species using (i) molecular cluster delimitation (*cox1* clustering and GMYC analyses), (ii) morphometry (both linear body morphometrics and pronotum outlines) and (iii) ecological niche similarity estimates. Scale bars correspond to 1 mm. Modified from Arribas *et al.*, 2013. *Representación esquemática de la aproximación de taxonomía integrativa combinando información geográfica, molecular, morfométrica y ecológica para el complejo de especies Enochrus falcarius, así como las cuatro especies delimitadas dentro de éste. Hipótesis sobre especies candidatas basadas en los análisis filogenéticos (cuatro genes mitocondriales y un nuclear) e información biogeográfica (SI Sicilia, SMTU Sur de Marruecos y Túnez, IP Península Ibérica, NM Norte de Marruecos). Evaluación de la divergencia de linajes entre las especies candidatas basada en (i) delimitación por agrupamiento molecular (agrupamiento basado en *cox1* y análisis GMYC), (ii) morfometría (lineal y basada en perfiles del pronoto) y (iii) estimas de similaridad de nicho ecológico. Líneas de escala de 1 mm. Modificado de Arribas *et al.*, 2013.*

distribution of Mediterranean saline streams (e.g., Arribas *et al.*, 2012b; Abellán *et al.*, 2012). Taken together, the long-term extrinsic barriers defining allopatric lineages within the Mediterranean region seem to result in a profound limitation of gene exchange among isolated populations of saline invertebrate taxa. Thus, allopatric speciation across the patchily distributed Mediterranean saline streams seems to be an important process in the diversification of these lineages.

Another convergent result of the various studies on the diversity of saline waters is the high morphological similarity found among the lineages despite their high levels of molecular and even ecological divergence (e.g., Gómez *et al.*, 2002; Abellán *et al.*, 2007; Arribas *et al.*, 2013, see Fig. 2). Arribas *et al.* (2013) hypothesised that the extreme conditions of saline waters could promote morphological stasis between divergent lineages because they undergo a strong selection for behavioural, physiological or morphological characters, similar to what has been found in other extreme habitats such as the Arctic tundra, underwater karst or deep-sea environments (Bickford *et al.*, 2007). Thus, the high degree of habitat specialisation observed among saline species, mainly physiological tolerance to the extreme thermal and osmotic conditions of saline waters (e.g., see Sánchez-Fernández *et al.*, 2010; Arribas *et al.*, 2012b), could be producing cryptic diversification within these groups.

Regarding the influence of the habitat in the diversification of aquatic lineages, one of the most important habitat constraints is the division between standing (lentic) and running (lotic) waters (Hutchinson, 1957). Lentic and lotic waters fundamentally differ in their stability and persistence (i.e., lentic systems tend to be geologically shorter-lived and more ecologically unstable than lotic systems), which promotes differential selection in the colonisation capacity of species living in them. This leads to differences in population genetic structure, geographical range size and speciation rates in lentic versus lotic lineages (Ribera, 2008). A few studies have highlighted that this pattern is very marked in the case of the lineages inhabiting Mediterranean saline waters and is present even between closely related

species, where the lotic species are mainly restricted to southern localities, while the lentic taxa tend to occupy broader areas (e.g., Abellán *et al.*, 2009). Such congeneric dichotomies in habitat occupation also make saline water beetles an ideal model for further investigating the association between habitat type and geographical range size. Therefore, Arribas *et al.* (2012b) used both lotic and lentic saline species of the water beetle species complex *Enochrus bicolor* to decipher the suite of traits comprising the species' colonisation capacity (mainly its dispersal and establishment potential) to determine differences in the range size of these species. These results note that the clear differences in range size between the lotic and lentic taxa of this species complex are mainly driven by a higher dispersal ability rather than a broader fundamental niche breadth. However, physiological studies on other lineages of saline water beetles (i.e., Pallarés *et al.*, 2012; Céspedes *et al.*, 2013) have also identified thermal and osmotic niche differences as potentially driving different range sizes between related lotic and lentic species. Despite the fact that different colonisation-related traits such as wing morphology or thermal and osmotic tolerances seem to have emerged in the different saline insect lineages studied, overall, these results highlight the habitat stability mediated by the division between lotic/lentic as a fundamental constraint driving diversification in saline waters.

In addition to the lotic/lentic character, other geomorphological, hydrological and physico-chemical characteristics of the aquatic habitats (most of them highly dependent on substrate type) have been shown to have a fundamental influence on aquatic communities (e.g., Bonada *et al.*, 2005; Velasco *et al.*, 2006). In the case of some saline endemic species of water beetles, Abellán *et al.* (2012) showed that the lithologic substrate, rather than present and past climatic conditions, is a key factor determining the distribution and genetic structure of such saline species. Some recent results concerning multiple lineages of saline beetles across Europe and northern Africa (Arribas, 2013) are consistent with this lithology-mediated distribution of saline aquatic diversity and highlight that in the case of the saline lotic

species, the low habitat availability mediated by the patchy distribution of saline substrates could restrict their present and future potential to track a changing climate.

Conservation of saline water biodiversity

Evaluating the impact of climate change on biodiversity presents major challenges to conservation biology, forcing us to revisit previous tools

and principles based on how species are able to respond to this impact (Heller & Zavaleta, 2009). Species may be affected by climatic warming in very different ways, despite having broadly similar ecological and biogeographical traits. Elucidating the drivers of the vulnerability of species to global warming (i.e., the persistence of a species and its potential for shifting range; Williams *et al.*, 2008) is very relevant for understanding particular threats and designing specific

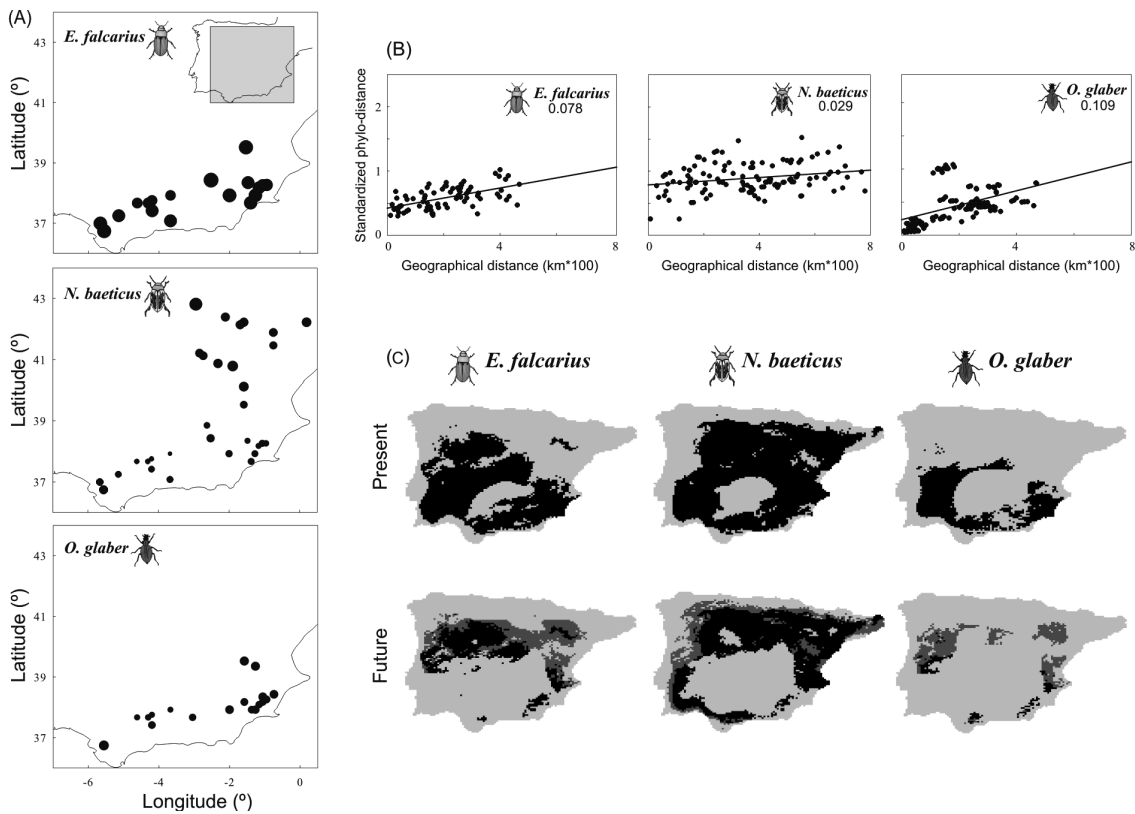


Figure 3. Comparative view of drivers of species vulnerability under climate change (CCM3 scenario for the year 2100) in three saline water beetle species (*Enochrus falcarius*, *Nebrioporus baeticus* and *Ochthebius glaber*). (A) Geographic location and future mean thermal safety margins (TSM Future) of each species locality. Circle diameter is proportional to TSM Future. (B) Dispersal capacity of each species as estimated by the increase of standardised phylogenetic distance (based on *cox1* phylogenetic trees), with geographical distance among localities. The slope of the regression line (in all cases significant at p -value < 0.05) is indicated in the upper-right corner of each graph. (C) Climatically suitable areas for each species as estimated by the multidimensional-envelope procedure for the present and future. Grid cells representing turnover are shown in dark grey. Modified from Arribas *et al.*, 2012a. *Marco comparativo de los determinantes de vulnerabilidad ante el cambio climático (escenario CCM3 para el año 2100) en tres especies de coleópteros acuáticos salinos (Enochrus falcarius, Nebrioporus baeticus y Ochthebius glaber). (A) Márgenes térmicos de seguridad en el futuro (TSMFuture) para cada localidad y especie. Diámetro proporcional a TSMFuture. (B) Capacidad de dispersión de cada especie estimada en base al incremento de la distancia filogenética estandarizada (basada en árboles filogenéticos para *cox1*) frente a la distancia geográfica entre localidades. La pendiente de la regresión lineal (en todos los casos significativa p -value < 0.05) se indica en cada gráfico. (C) Áreas con condiciones climáticas adecuadas para cada especie, estimadas para el presente y futuro con un procedimiento de envoltura multidimensional. Las celdas que se estimaron adecuadas en el futuro pero no en el presente se muestran en gris oscuro. Modificado de Arribas *et al.*, 2012a.*

management strategies. However, such evaluations are very scarce, particularly with regard to insects (but see Thomas *et al.*, 2011). In a recent study focused on threatened water beetles that inhabit Iberian saline streams, Arribas *et al.* (2012a) showed that despite having a broadly similar ecology and biogeography, these species are likely to respond to climate change in very different ways due to differences in species-specific traits (Fig. 3). These species thus represent an ideal model to show how an exploration of the different drivers of the vulnerability of species to climatic warming (i.e., their capacity to persist and potential to shift their ranges in response to global warming) could provide interesting information to guide conservation strategy decisions to help species cope with this impact. Overall, these results highlighted that species occupying Iberian saline streams could undergo major habitat reductions in the southern basins as a consequence of global warming (i.e., the Segura and Guadalquivir basins) because in these localities, the climatic conditions are predicted to drastically change and even rise to levels near the upper thermal limits of these species. Because of the low dispersal potential reported in some of these saline stream biotas, not only habitat conservation and population management but also *in situ* mitigation measures could be required for the future maintenance of southern populations of these species. At the same time, conservation of a network of saline streams across the Ebro basin could be critical if we are to preserve Iberian saline diversity, because this area is expected to serve as a future reservoir for different endangered saline species (Arribas *et al.*, 2012a, Fig. 3).

Moreover, discovering diversity is a potentially important factor influencing future conservation decisions (Witt *et al.*, 2006; Condon *et al.*, 2008), especially for groups such as insects that suffer an enormous taxonomic deficit (i.e., the ratio of expected taxa to named taxa). The detection and evaluation of cryptic diversity is highly dependent upon the application of integrative taxonomic approaches (Schlick-Steiner *et al.*, 2010). For instance, identifying geographical and habitat-related patterns in the distribution of cryptic

species could not only promote conservation of such covert endangered taxa but also discover unknown pockets of endemism and diversity that might warrant reconsideration for particular habitats or conservation sites (Bickford *et al.*, 2007; Nair *et al.*, 2012). This seems to be the case for saline environments in the Mediterranean region, which are increasingly recognised as having cryptic diversity usually associated with morphological stasis (e.g., Gómez *et al.*, 2002; Abellán *et al.*, 2007).

Furthermore, considering the dramatic decline and loss of saline habitats as a consequence of the rapid changes in land use that is taking place in some Mediterranean areas (Millán *et al.*, 2011), recognising and evaluating such cryptic diversity and applying appropriate conservation measures are of immediate importance. Recent studies have achieved such goals with water beetle lineages (Sánchez-Fernández *et al.*, 2011; Arribas *et al.*, 2013). As an example, Arribas *et al.* (2013) developed an integrative taxonomic study on the *Enochrus falcarius* complex (until then considered a single entity without conservation status), which resulted in the delimitation of four new species within this species complex (*E. jesus-arribasi* sp. n., *E. blazquezae* sp. n., *E. risii* sp. n. and *E. falcarius*). All of these species were evaluated as vulnerable and should be included in national and international lists of endangered species. Together, all of this data emphasises that applying integrative taxonomic approaches to lineages from Mediterranean saline streams may be fundamental for the proper evaluation and conservation of their biodiversity and may drive the discovery, naming and evaluation of cryptic species, as well as the modification of previously inadequately assessed vulnerability categorisations.

FURTHER OBJECTIVES

Further efforts are required to advance our understanding, future management and conservation of Mediterranean saline biodiversity. It is first necessary to achieve a proper inventory of the diversity of saline systems and the identification of common ecological and biogeographical patterns

across multiple saline lineages. Saline lineages hold great promise to delve deeply into some of the main and even partially unknown topics in ecology, evolution and biogeography, such as the extension of island biogeographical theory to continental substrate-mediated environments or the identification of the specific processes driving the generation of diversity in extreme conditions. Additionally, a broader exploration of this diversity is also fundamental for a better management of Iberian, and even Mediterranean, inland saline waters. In this sense, the long-term biomonitoring of specific saline populations could provide relevant information for calibrating and validating species vulnerability categorisations and conservation strategies under climate change.

A second goal is the determination of the origin and mechanisms of salinity tolerance in different aquatic groups and its possible interactions with tolerance to desiccation and extreme temperatures. Studies on the physiological basis of desiccation and salinity tolerances are needed to identify the mechanisms underlying such correlations and their generalisations to other aquatic groups. Advances in this research area could provide a fundamental basis for further predictions about the adaptation of aquatic biotas to the salinisation and the aridification processes expected under global change. Additionally, further exploration of the metabolic cost of osmoregulation and its possible trade-offs with other biological traits, such as fecundity, dispersal or thermal tolerance, could be fundamental for understanding the evolutionary process and ecological consequences of salinity tolerance.

Finally, further efforts should be made concerning the communication and translation of all the knowledge generated to the policy makers, environmental managers and society in general, a still-pending but critical challenge if we are to preserve the biological legacy of the Mediterranean saline ecosystems.

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