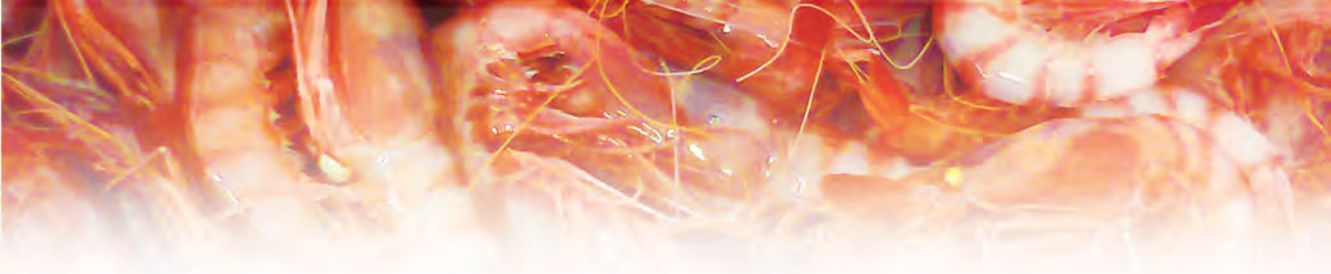
PHYLOGEOGRAPHICAL ANALYSIS OF TWO ARISTED SHRIMPS, ARISTEUS ANTENNATUS AND ARISTAEMORPHA FOLIACEA (CRUSTACEA: ARISTEIDAE), WITH IMPLICATIONS FOR RESOURCE CONSERVATION

Maria Victoria FERNÁNDEZ HERNÁNDEZ

Dipòsit legal: GI. 160-2013
http://hdl.handle.net/10803/98477



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Doctoral thesis

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2012

Programa de Doctorat de Ciències Experimentals i Sostenibilitat

Directed by:

Dra. Maria Inés Roldán Borassi

In candidacy for the degree of Doctor by *Universitat de Girona*

Universitat de Girona

La Dra. Maria Inés Roldán Borassi, Professora Titular de Genètica del Departament de Biologia de la Universitat de Girona,

CERTIFICA

que aquest treball, titulat Phylogeographical Analysis of Two Aristeid Shrimps, *Aristeus antennatus* and *Aristaeomorpha foliacea* (Crustacea, Aristeidae), with implications for resource conservation, que presenta Maria Victoria Fernández Hernández per a l'obtenció del títol de Doctora, ha estat realitzat sota la meva direcció i que compleix els requeriments per poder optar a Menció Internacional.

Girona, 23 de Juliol de 2012

Maria Inés Roldán Borassi

A Manuel Hernández,

mi abuelo

Acknowledgements

The development of this thesis has been possible thanks to:

- *Ministerio de Educación y Ciencia (*Spain) for a predoctoral fellowship (FPI, BES-2007-15865).

- *Ministerio de Educación y Ciencia (*Spain) for financial support to the project CTM2006-00785 and *Ministerio de Ciencia e Innovación* (Spain) for financial support to the project AGL2009-09228.

- Prof. Dr. Alberto Castelli and Dr. Ferruccio Maltagliati from *Dipartamento di Biologia*, *Universitá di Pisa* (Italy) for hosting a short visit for training in statistical analysis.

- Dra. Federica Pannacciulli for hosting 5 months stage in *Laboratorio di ecologia molecolare* at *Centro Richerche Ambiente Marino*, St Teresa-ENEA, La Spezia (Italy).

- Organizing committee of the IX Colloquium Crustacean Decapoda Mediterranean held in September 2008 in Torino (Italy) for student grant which covered accommodation costs to assist to the conference.

- *Universitat de Girona* and *Vicens Vives* Universities Network for DRAC *Formació Avançada* mobility grant to assist the course "Molecular Evolution, Phylogenetics and Phylogenomics" held in 12-16 May 2008 by the Bioinformatics Department of *Centro de Investigación Príncipe Felipe*, Valencia (Spain).

- Organizing committee of the XXXVIII meeting of *Sociedad Española de Genética* held in September 2011 in Murcia (Spain) for student grant which covered registration costs.

Agraïments personals

Com tots sabem aquest apartat és fàcil i difícil de redactar alhora. Un pot simplement dir que agraeix a tothom i que cadascú que llegeixi aquest apartat sap on entra la seva part d'agraïment. No obstant, crec que és lícit dedicar unes línees a cadascun d'aquests, encara que el llistat pugui ser molt llarg. Al cap i a la fi han sigut 5 anys des que vaig iniciar aquesta etapa a la UdG, un trajecte en el que moltes persones m'ha aportat molt, tant en lo professional com en lo personal; persones ja conegudes i d'altres noves, cap d'elles quedarà en l'oblit després d'aquest viatge, encara que mai més ens tornem a veure. I si potser algú creu que també mereix aquestes línees després de tot el llistat, si us plau, contacteu-me que faré una fe d'errates per tal d'incloure'l ;).

Començant per l'apartat professional, primer de tot, vull agrair a la meva directora de tesi, la Marina, per confiar en mi i escollir-me com a doctoranda. Que aunque hablemos el mismo idioma, en muchas ocasiones parecía lo contrario, pero aún y así hemos conseguido finalizar esta tesis. Por enseñarme lo necesario que es releer toda la literatura, ir a los principios y desempolvar viejos textos "*to get the whole picture*". Por estar siempre dispuesta a ejercer como directora y transmitir todo el conocimiento de que dispone. No puedo dejar de agradecerle el darme la oportunidad de asistir a congresos, nacionales e internacionales, así como las varias estancias en Italia. Por todo esto, así como por tantas otras charlas no profesionales delante de un café, gracias.

En segon ordre haig de mencionar en Ferruccio, que encara que ja ha sigut citat als agraïments formals, mereix un espai més personal. His contribution to this work has been immense, being a great source of knowledge, both in the analytical part as well as in the discussion of results. He's been always willing to dedicate some minutes to solve any doubt, even the most basic ones. Finally, I am grateful to his patience, comprehension and support, especially during these lasts months. *Per tutto questo, grazie mille!!*

Similarly I am grateful to Federica for allowing me to stay 5 months in her lab, where aside from teaching me a new technique, she welcomed me in her team and her house making me feel at home. Here I have to include Piero as well, for guidance when arriving to San Terenzo and showing me the best place where to have aperitivos. But also his advice on ISSRs amplification and data analysis whenever I have needed it; thanks for being a fantastic colleague. *Grazie anche a voi!!.*

Així mateix, estic molt agraïda a en Siscu per tots els mails i xerrades que m'han permès entendre una mica més a aquesta interessant espècie que és la gamba vermella.

Continuant amb l'equip de gambes, haig d'agrair a la Sandra el introduir-me en el món del ADN mitocondrial, i per detectar tots aquells errors que a la resta se'ns passen desapercebuts. I com a últim membre de l'equip de gambes, el "jefe", en Jordi, perquè encara que va esvalotat como jo, troba aquells cinc minuts per parar-se, escoltar-te i donar-te la seva opinió; a tots dos, gràcies.

Ja fora de l'equip de gambes, haig d'agrair a l'Oriol tots els seus consells tècnics, sense els quals moltes seqüències no haurien sortit, així com totes les hores de laboratori que hem compartit; i a la Cesca amb qui la xerrameca de primera hora del matí feia molt més fàcil començar els matins foscos d'hivern. De la mateixa manera, agraeixo la companyia i paciència de tots aquells que han treballat amb mi, colze amb colze en el laboratori, Aldo, Josep, Laura, Carolina, que m'han patit com a instructora i que m'han ensenyat lo difícil que és ensenyar a algú. Finalment, estic contenta d'haver pogut compartir moments amb la resta de membres del LIG (Carles, Jose Luis, Núria, Rosa, Raquel, Manel, Alexandra, David i Luis), ja fos en dinars o congressos. I no puc oblidar-me de la Mercedes, que durant el poc temps que vàrem coincidir i un cop hagués marxat, sempre ha mostrat el seu suport.

Altres membres de la UdG que mereixen estar en aquest apartat són la Roser, magnifica secretària, i en Narcís, fantàstic conserge, que fan molt més fàcils qualssevol tràmit a la UdG.

Ja passant a l'apartat personal, són molts els becaris que han passat per la UdG des de que jo vaig començar, alguns ja no hi són, i d'altres tot just comencen, però amb tots s'ha compartit alguna cosa, ni que fos un dinar a les taules de fusta. Tots ells et fan sentir que formes part d'un gran equip, i és amb aquest gran equip que he compartit xerrades (existencials o no), dinars, sopars, excursions i viatges que no s'oblidaran.

Particularment, estic contenta d'haver pogut pertànyer a l'exclusiu grup d'EECC, on lloc de treball, locutori, centre d'operacions alhora que rebost, hi tenia cabuda. Allí he compartit espai amb surers (Marçal, Pau, Roger, Olga), micros (Anna, Ariadna, Arantxa, Mireia Fillol, Mireia López, Núria, Olga), bioquímics (Jess, Montse, Pere, Sònia) i genètics (Alexandra, David). Aquest són els residents però molts altres han passat per allí, ja fos per participar en les esbojarrades activitats (regals de tesina, tesi i competicions nadalenques), per fer ús del telèfon, o demanar paper i bolígraf: Anna (BQ), Ariadna (BQ), Clara, Cristina, David (BQ), Dolors, Gela, Marc Yeste, Marc Llirós, Maria, Mariona, Marta, Meritxell, Olaya, Roger (BQ), Sara. No obstant, estic especialment agraïda a les BQ105 per adoptar-me (Montse, Clara, Dolors, Sònia Cristina, Marta) i a en David, per esdevenir tant bon company de taula, laboratori i professió.

I també vull manifestar la meva gratitud als membres de UdG.doc, Benito, Lorenzo, Josep, Miquel, Sergi, David (BQ i Gen), Mirèia (Politècnica), Clara, Dolors, Montse; per compartir l'esperit de lluita, dedicar el vostre

temps i voler aconseguir les millors condicions per a tots nosaltres. Tots ells han fet que els mals moments no semblessin tan dolents i que els bons fossin ben recordats.

Però no tot el treball s'ha realitzat a Girona, gran part de la feina es va realitzar a Itàlia, on haig d'agrair a tota la gent que allà vaig conèixer. First, Michele, for hosting me once in his flat while barely knowing me, for taking me around with his friends making sure I didn't get bored, for those deep discussions about life and science; and for being such a great colleague, thank you. Second, I am grateful to Marco Valente, Sara Fiorenzani and Lisa Lupi, for all the unforgettable time at ENEA, in the lab, during coffee walks; and outside the office; for all the dinners, parties and diving trips. Y no puedo olvidar a Patricia por resultar una perfecta compañera de piso, de baile y de viajes, así como por resolver todas mis dudas con su profundo conocimiento de la oceanografía física del Mediterráneo, gracias amiga!

També, està tota aquella gent, que fora de l'ambient professional et donen energies per continuar endavant. El Dr. Chapela, qui em recorda que un té que "take it easy", per tants bons moments entre bambolines, i per proveir copies de bibliografia. Dr. Tysklind, who introduced me to the field of molecular ecology, who is a good advisor, colleague and most of all friend. La Dra. Jiménez, quien des de el día en que nos conocimos en clase de Citología con la Dr. Durfort ha sido compañera de estudio, penas y alegrías, y como no de ésta también ha formado parte. Dr. Pumpkin, who is always there. La Núria, amb qui tantes tardes de quintos he compartit intentant arreglar el món, i qui és el meu punt de connexió amb la realitat fora d'aquesta bombolla en que els científics ens trobem. To all of you, a hearted thanks!!!

Finalmente, todo este trabajo no habría sido posible sin el apoyo de mi familia; mi madre referente de superación personal, quien empieza a comprender lo "complicado e interesante" que es esto de la genética; a mi padre, por su continuo interés y consejos que me devuelven a la tierra y me hacen mirar el mundo con perspectiva; my sister, que me visita allá donde el trabajo me lleva; i Edu, per aguantar el meu humor variable, especialment en aquest últim any. Per què a la seva manera també m'aconsella i em recorda el que és prioritari en cada moment. Per recordar-me que s'ha de sortir a passejar.

A tots vosaltres

gràcies, gracias, thank you, grazie

List of publications derived from the thesis

This thesis is presented as a compendium of four scientific publications, whose impact factor and position within subject category are detailed according to the last update (2011) of the Journal Citation Report:

Fernández MV, Heras S, Maltagliati F, Turco A, Roldán MI (2011) Genetic structure in the blue and red shrimp, *Aristeus antennatus* and the role played by hydrographical and oceanographical barriers. Marine Ecology-Progress Series 421:163-171. doi:10.3354/meps08881

Impact Factor of the journal Marine Ecology-Progress Series in 2011: 2.711. 5 year Journal Impact Factor = 3.086. This journal was found in the position 12/97 (Q1) of the subject category Marine and Freshwater Biology.

Fernández MV, Maltagliati F, Pannacciulli F, Roldán MI (2011) Analysis of genetic variability in *Aristaeomorpha foliacea* (Crustacea, Aristeidae) using DNA-ISSR (Inter Simple Sequence Repeat) markers. Comptes Rendus Biologies, 334:705-712. doi: 10.1016/j.crvi.2011.07.005

Impact Factor of the journal Comptes Rendus Biologies in 2011: 1.533. 5 year Journal Impact Factor = 1.826. This journal was found in the position 44/84 (Q3) of the subject category Biology.

Fernández MV, Heras S, Maltagliati F, Roldán MI (2012) Deep genetic divergence in giant red shrimp *Aristaeomorpha foliacea* (Risso, 1827) across a wide distributional range. Journal of Sea Research, xxx:xxx-xxx. doi: 10.1016/j.seares.2012.08.004

Impact Factor of the journal Journal of Sea Research in 2011: 2.598. 5 year Journal Impact Factor = 2.683. This journal was found in the position 13/97 (Q1) of the subject category Marine and Freshwater Biology.

Fernández MV, Heras S, Viñas J, Maltagliati F, Roldán MI. Comparative phylogeography of two Aristeid shrimps of high commercial interest (*Aristeus antennatus* and *Aristaeomorpha foliacea*) using nuclear and mitochondrial markers. Accepted by PLoS One with minor revisions the 6th September 2012

Impact Factor of the journal PLoS One in 2011: 4.092. 5 year Journal Impact Factor = 4.537. This journal was found in the position 12/84 (Q1) of the subject category Biology.

CONTENTS

RESUM

La conservació d'espècies, especialment aquelles explotades i d'alt interès econòmic, depèn d'un bon coneixement de la seva biologia, així com del desenvolupament de plans de gestió adequats. Per aquest motiu, la informació genètica resulta d'utilitat a la gestió de pesqueries mitjançant la identificació d'unitats reproductivament aïllades i genèticament diferenciades (estocs genètics). Les gambes vermelles, *Aristeus antennatus* i *Aristaeomorpha foliacea* són decàpodes marins amb un alt valor econòmic. Viuen en els fons tous dels canons submarins i són particularment abundants entre 600-800 m de profunditat. Ambdues espècies presenten un ampli rang de distribució en el Mar Mediterrani (MED), Oceà Atlàntic (AO) i Canal de Moçambic (MOZ) on són parcialment simpàtriques. En aquesta tesi, s'han analitzat genèticament els caladers més importants d'*A. antennatus* i *A. foliacea* mitjançant una sèrie de marcadors moleculars (seqüenciació de gens mitocondrials i nuclears, i ISSRs) per abordar estudis de microevolució (genètica de poblacions) i macroevolució (filogeografia comparada). *A. antennatus* va presentar els valors més alts de diversitat genètica a MOZ, seguit d'AO, Mediterrani Oriental (EM) i Mediterrani Occidental (WM). Per *A. foliacea* els valors més alts de diversitat genètica es varen detectar al nord-oest d'Austràlia (AUS) mentre que a WM, EM i MOZ es varen trobar valors similars. En ambdues espècies s'ha observat una divergència genètica significativa i una restricció del flux gènic associada a barreres geogràfiques i hidrogràfiques. L'Estret de Sicília i el gir del Peloponès s'identificaren com principals barreres al flux gènic entre WM i EM, per *A. antennatus* i *A. foliacea* respectivament. L'Estret de Gibraltar també va resultar ser una barrera entre WM i AO per *A. antennatus*. En conjunt, per a cada espècie s'han identificat quatre estocs genètics, associats a quatre regions geogràfiques. A escala macroevolutiva, els resultats revelen el monofiletisme d'*A. antennatus* i confirmen la seva relació congenèrica amb *Aristeus virilis*. Per *A. foliacea* s'han detectat tres llinatges mitocondrials geogràficament restringits a MED, MOZ i AUS però les anàlisis nuclears tan sols han identificat l'existència de dos llinatges, MED-MOZ i AUS. L'anàlisi multilocus confirma el monofiletisme del llinatge AUS aportant evidències moleculars de l'existència d'una espècie genètica diferent. Per concloure, els resultats obtinguts fonamenten les bases per a la conservació d'*A. antennatus* i *A. foliacea* a diferents escales evolutives i demostren que la informació genètica pot ser de gran ajuda en l'estudi de la biodiversitat marina.

RESUMEN

La conservación de especies, especialmente aquellas explotadas y de elevado interés económico, depende tanto de conocer su biología como de desarrollar precisos planes de gestión. Así, la información genética resulta ser útil en la gestión de las pesquerías mediante la identificación de unidades reproductivamente aisladas y genéticamente diferenciadas (estocs genéticos). Las gambas rojas *Aristeus antennatus* y *Aristaeomorpha foliacea* son decápodos marinos de alto valor económico, habitan los fondos blandos de los cañones submarinos y son muy abundantes entre 600-800 m de profundidad. Ambas especies poseen un amplio rango de distribución en el Mar Mediterráneo (MED), Océano Atlántico (AO) y Canal de Mozambique (MOZ) donde son parcialmente simpátricas. En esta tesis, se han analizado genéticamente los caladeros más importantes de *A. antennatus* y *A. foliacea* mediante una batería de marcadores moleculares (secuenciación directa de genes mitocondriales y nucleares, e ISSRs) con el objetivo de realizar estudios microevolutivos (genética de poblaciones) y macroevolutivos (filogeografía comparada). En *A. antennatus* los valores más elevados de diversidad genética se detectaron en MOZ, seguido de AO, Mediterráneo Oriental (EM) y Mediterráneo Occidental (WM). En *A. foliacea* los mayores valores de diversidad genética se detectaron en el Noroeste de Australia (AUS) mientras que WM, EM y MOZ presentaron valores similares. En ambas especies se ha observado una significativa diferenciación genética y una restricción al flujo génico asociadas a barreras geográficas e hidrográficas. Entre WM y EM las barreras son el Estrecho de Sicilia y el giro del Peloponeso para *A. antennatus* y *A. foliacea* respectivamente. Además para *A. antennatus* el Estrecho de Gibraltar es una barrera al flujo génico entre WM y AO. Así, para cada especie se han identificado cuatro estocs genéticos, asociados a cuatro regiones geográficas. A escala macroevolutiva, los resultados revelan el monofiletismo de *A. antennatus* y confirman la relación congenérica con *Aristeus virilis*. En *A. foliacea* se han detectado tres linajes mitocondriales geográficamente asociados a MED, MOZ y AUS aunque solo dos MED-MOZ y AUS, han sido detectados mediante genes nucleares. El monofiletismo del linaje AUS se corrobora con el análisis multilocus evidenciando la existencia de una nueva especie genética. En suma, los resultados obtenidos sientan las bases para la conservación de *A. antennatus* y *A. foliacea* a diferentes escalas evolutivas y demuestran que la información genética es una herramienta importante en el estudio de la biodiversidad marina.

SUMMARY

The conservation of species, especially those with high levels of exploitation and economic interest, relies on a deep knowledge of biology and ecology of the species concerned, as well as on the definition of accurate management plans. Genetic information can greatly contribute to fisheries management by identifying reproductively isolated units, which are genetically different from one other (genetic stocks). *Aristeus antennatus* and *Aristaeomorpha foliacea* are commercially important decapods that inhabit on muddy bottoms, with major abundances found between 600 - 800 meters depth. Both species present a large distributional range and can be found living in partial sympatry in the Mediterranean Sea (MED), Atlantic Ocean (AO) and Mozambique Channel (MOZ). In this thesis, the genetic analysis of harvesting grounds of *A. antennatus* and *A. foliacea* was approached using a battery of molecular markers (sequencing of mitochondrial and nuclear genes, and ISSRs) to address microevolutionary (population genetics) and macroevolutionary (comparative phylogeography) studies. In *A. antennatus* the highest genetic diversity values were detected in MOZ, followed by AO, Eastern Mediterranean (EM) and Western Mediterranean (WM). In *A. foliacea* the highest levels of genetic diversity were detected in North-Western Australia (AUS) whilst WM, EM and MOZ presented similar levels of genetic variability. Significant levels of genetic divergence were detected for both species which were associated with geographical and hydrographical barriers to gene flow. The Strait of Sicily and the Peloponnesian gyre were identified as major barriers to gene flow between WM and EM, for *A. antennatus* and *A. foliacea* respectively. For *A. antennatus* the Strait of Gibraltar was also identified as barrier to gene flow between WM and AO. Overall, four genetic sotcks associated to four geographical regions were identified in each species. At macroevolutionary scale, *A. antennatus* was identified as a single monophyletic species and genetically close to *Aristeus virilis,* consistent to congeneric species relationship. In *A. foliacea* a deep split among three mitochondrial lineages geographically restricted to the MED, MOZ and AUS was detected but nuclear analyses only identified the existence of two lineages: MED-MOZ and AUS. The multilocus comparison supports the monophyletic status of the AUS lineage providing molecular evidence of the existence of a different genetic species. These results set the basis for the conservation of *A. antennatus* and *A. foliacea* at different evolutionary scales and demonstrate that genetic information can greatly contribute to the re-evaluation of marine biological diversity.

"If knowledge can create problems,
it is through ignorance that we can solve them"

Isaac Asimov (1920 - 1992)

GENERAL INTRODUCTION

The purpose of this introduction is to provide the reader with the basic knowledge on aristeid shrimps, population genetics and the application of the latest in fisheries management, so the contents of this work can be understood. "All man by nature desire to know" (Aristotle, 384 BC-322 BC, Metaphysics) and an extension of this desire is mankind's will to control and exploit resources at its hands. *Aristeus antennatus* and *Aristaeomorpha foliacea* shrimps are highly appreciated culinary resources that constitute the basis of a commercially important deep-sea bottom fishery (Bensch et al. 2008). Therefore, the maintenance of these resources is important for a large part of the fisheries sector of many countries. But in order to ensure the continuity of a resource, a complete understanding of a species biology, ecology and evolutionary history is imperative for the implementation of precise management strategies (Everhart & Youngs 1981).

With the advent of molecular techniques, the study of the marine environment, where the organisms under study are relatively inaccessible for direct field observations, gained a whole different approach (Avise 1998a). Genetic data allowed the accurate definition of population boundaries, the identification of species and filling in the knowledge gaps concerning organism behaviour, natural history, and current and past population demographic factors, which in turn can be highly relevant to conservation efforts (Avise 1998a). Among commercially important species, genetics has particular applications in forensic studies, management decisions and conservation plans (Avise 2004).

Milestones in population genetics

Back in 1850s, an Austrian monk named Gregor Johann Mendel realized that "characters" were being transferred from parents to offspring. By hybridizing varieties of peas Mendel realized that "characters" (color, shape and texture) were being transferred in a regular way which allowed him to come up with two generalizations: the law of segregation and the law of independent assortment (Bateson 1901). His results and laws of inheritance were eventually published in the Natural History Society of Brünn in 1865, but passed almost unnoticed and remained in oblivion for at least 30 years (Bateson 1909). Parallel in time, two British naturalists, Charles Darwin and Alfred Russel Wallace, arrived independently to the conclusions that environmental pressures were most likely the evolutionary cause for species to diverge; these ideas were presented as a joint essay to the Linnean Society of London in 1858. A year later Darwin's book "The Origin of Species" (1859) came out and constituted the fundational stone of the field of evolutionary biology (Seward 1909).

Mendel's work was rediscovered in the 1900s stimulating much theoretical and experimental work. In 1908 Godfrey Harold Hardy in England and Wilheim Weinberg in Germany, independently, demonstrated that in a population of randomly mating individuals, gene frequencies remained essentially unchanged from one generation to the next; or what is known as the Hardy-Weinberg (HW) equilibrium (Box 1). Because the HW equilibrium informs on population structure, it became a central concept to many genetic diversity and differentiation models; it also permits to predict the proportion of diallelic genotypes in next generations following random mating given initial allele frequencies (Hartl & Clark 1988).

Box 1. Hardy-Weinberg (HW) equilibrium (after Beebee & Rowe 2008)

HW assumptions:
- random mating (panmixia) within the population
- negligible effects of mutation or migration (closed system)
- infinitely large population size
- Mendelian inheritance
- no selection acts

HW expectation: in a situation of equilibrium, the proportion of homozygotes (AA, aa) and heterozygotes (Aa) for a single locus, with two alleles, should follow the relationship:

$$p^2 + 2pq + q^2 = 1$$

Knowing the absolute number of individuals presenting each of the three possible genotypes [AA (p^2), Aa ($2pq$), aa (q^2)] in a population of diploid organism, the relative frequencies of the two alleles (p, q) in a situation of HW equilibrium should be:

$$p = \frac{2(\text{n°AA}) + (\text{n°Aa})}{2[(\text{n°AA}) + (\text{n°Aa}) + (\text{n°aa})]};\ q = \frac{2(\text{n°aa}) + (\text{n°Aa})}{2[(\text{n°AA}) + (\text{n°Aa}) + (\text{n°aa})]}$$

The values obtained for p and q can then be used to calculate the expected proportion of the three genotypes (AA, Aa, aa) under a situation of HW equilibrium; a x^2 test can determine whether the expected genotype proportions differ significantly from the observed ones:

$$x^2 = \Sigma \frac{(Observed - Expected)^2}{Expected}$$

In the 1920s and early 30s, Ronald Fisher, John Burdon Sanderson Haldane and Sewall Wright, published mathematical works linking Mendelian genetics with evolutionary theory based on natural selection, what became to be known as neo-Darwinism or “the modern synthesis” (Mayr 1993).

In the 1960s, the application of molecular methods to population genetics revealed the existence of extensive genetic variation within most natural populations. These findings came as a surprise given that if natural selection was acting on populations it would be expected that all but most fit alleles were removed from a population (Hartl & Clark 1988). The large amount of variability detected would mean that the majority of such variation should be selectively neutral and that this variation could be maintained or lost over time essentially by chance (i.e. genetic drift) (Kimura 1968). Thus genetic drift and neutrality arose in contrast to the neo-Darwinism notion that natural selection was

the all-sufficient agent of evolution, also setting the basements for the later formulation of the neutral theory of molecular evolution (Kimura 1968).

Describing populations

Many detailed definitions of population have been described based on the field of study or the underlying objectives of the researcher. Yet, two major types of biological definitions (Box 2) can be identified (Crawford 1984; Waples & Gaggiotti 2006): those reflecting an ecological paradigm and those reflecting an evolutionary paradigm. The ecological paradigm has been traditionally used in the management context because this was the natural way to approach the problem of defining populations. It primarily considers population dynamic processes (*m*, number of migrants) to identify whether two demographic units should be treated jointly or independently. However it is being replaced by the evolutionary paradigm because not only it considers the number of migrants but also those that actually reproduce and contribute with genes to the new generation (Waples & Gaggiotti 2006).

Box 2. Population definitions (after Waples & Gaggiotti 2006)

Ecological paradigm
- group of individuals of the same species occupying a particular space at a particular time (Krebs 1994)
- group of individuals of the same species that live together in an area of sufficient size that all requirements for reproduction, survival and migration can be met (Huffaker et al. 1984)
- group of organisms occupying a specific geographical area or biome (Lapedes 1978)

Evolutionary paradigm
- community of individuals of a sexually reproducing species within which mating takes place (Dobzhansky 1970)
- group of interbreeding individuals that exist together in space and time (Hedrick 2000)
- group of individuals of the same species living in close enough proximity that any member of the group can potentially mate with any other member (Hartl & Clark 1988)

Variations of population definition:
- Stock, demographic unit, deme, interaction group, natural population, local population

According to the evolutionary paradigm, the reproductive cohesiveness of a population (F_{ST}) is determined by the effective population size (N_e), the migration rate (m) and the local recruitment

(births and deaths of resident individuals) (Wright 1931). In order to estimate the level of genetic cohesion of a population two models of gene flow between subpopulations were formulated, the island model (Wright 1931) and the stepping stone model (Slatkin 1993) (Figure 1).

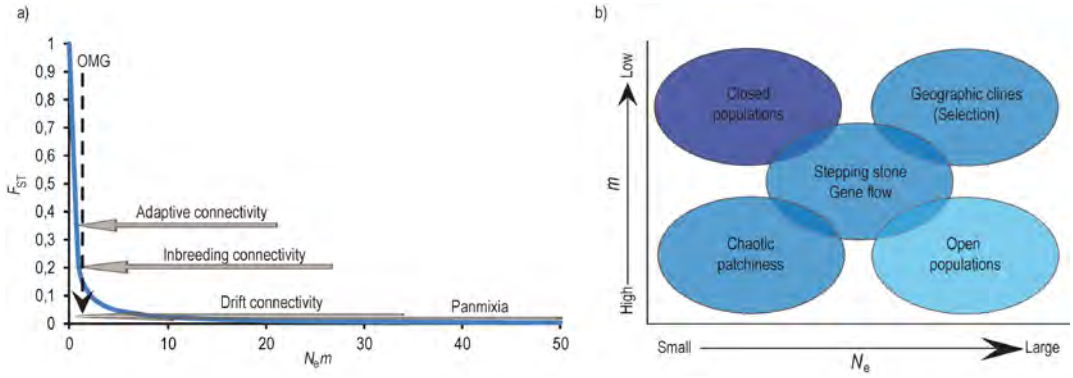


Figure 1. Levels of genetic connectivity according to a) the island model (after Lowe & Allendorf 2010), b) the stepping stone model (after Hellberg et al. 2002).

In the island model, all subpopulations are linked by equals levels of gene flow irrespective of intervening geographical distances, and Wright (1931) showed that genetic differentiation among subpopulations of diploid organisms was inversely related to the effective migration rate (N_em) [$F_{ST}\approx1/(1+4\ N_em)$]. According to the island model, two groups of individuals might constitute a unique population (panmixia) when $N_em = N_e\,(n\text{-}1)/n$. Even values as low as of N_em =1 are enough to keep a balance between genetic drift and inbreeding (Wright 1951) which has been later coded as the One Migrant per Generation rule (OMG - Mills & Allendorf 1996). The maintenance in each of these two extremes of reproductive cohesion is rather difficult because of departures from random mating and the influence of genetic drift; hence, natural populations usually present intermediate levels of genetic connectivity (Figure 1a). Drift connectivity is suggested for subpopulations with nearly equal allele frequencies; adaptive connectivity for subpopulations with the potential to spread advantageous alleles; and interbreeding connectivity when gene flow is enough to reduce the harmful effects of interbreeding (Lowe & Allendrof 2010) (Figure 1a). The island model is mostly adequate for two-population cases or multiple equally-spaced populations. However when only adjacent populations exchange migrants and dispersal is related to geographic distance, the stepping stone model (Slatkin 1993) seems to reflect better the organization of populations (Hellberg et al. 2002). Following this model, populations can be completely closed (all recruits from

within) when N_em is small, or completely open (all recruits from other populations) when N_em is large. Between these two extremes of dispersal, populations may show gradually reduced genetic similarity with increasing geographical isolation owing to restricted dispersal (Figure 1b).

Box 3. Population diversity estimators (after Beebee & Rowe 2008)

Heterozygosity: the observed or expected (under HW equilibrium) proportion of heterozygotes in a population.
H_I: mean observed heterozygosity per individual (within a subsample)
H_S: mean expected heterozygosity within random mating subsamples = $2p_iq_i$
H_T: expected heterozygosity in random mating total samples = 2pq

Wright's (1951) *F*-statistics
F_{IT} – overall fixation index - correlation of alleles within an individual relative to the entire population; equivalently, the departure of genotype frequencies from HW expectations relative to the entire population. Range of values: (-1, +1) = no observed heterozygotes - excess of heterozygotes

$$F_{IT} = (H_T\text{-}H_I)/H_T$$

F_{IS} – inbreeding coefficient - correlation of alleles within an individual relative to the subpopulation in which it occurs; equivalently, the average departure of genotype frequencies from HW expectations within populations. Range of values: (-1, +1) = no observed heterozygotes - excess of heterozygotes

$$F_{IS} = (H_S\text{-}H_I)/H_S$$

F_{ST} – fixation index - correlation of randomly chosen alleles within the same subpopulation relative to the entire population; equivalently, the proportion of genetic diversity due to allele frequency differences among populations. Range of values: (0, +1) = same populations – different populations

$$F_{ST} = (H_T\text{-}H_S)/H_T$$

Ultimately, conclusions about levels of connectedness between populations are based on the genetic similarity between these populations. Wright (1951, 1969) developed *F*-statistics as a tool for describing the levels of genetic connectivity within and among populations based on the variance of allele frequencies and expected heterozigosity levels (Box 3). Now, F_{ST} was one of three interrelated parameters [$(1\text{-}F_{IT}=(1\text{-}F_{IS})/(1\text{-}F_{ST})$] that served as a quantitative guide for many population and evolutionary genetic assessments, as it could be statistically tested by permutation test, and compared across works (Holsinger & Weir 2009). Wright (1943) indicated that genetic

differentiation is by no means negligible when F_{ST} is as small as 0.05; even if F_{ST} was ≈ 0.001 but statistically significant, panmixia cannot be considered. There is no actual agreement on the level of genetic divergence that identifies two subpopulations apart, so genetic analyses can only be used to assess whether the observed estimate of genetic divergence is significantly higher or lower than a predefined threshold value (Palsboll et al. 2006). With the advent of molecular techniques and the development of different types of molecular markers (gene sequencing and microsatellites), variants of *F*-statistics have been developed adapting its formulation to the properties of each marker (Holsinger & Weir 2009). For haplotypic data Φ-statistics are the analogous to classical *F*-statistics and are calculated using the analysis of molecular variance (AMOVA, Excoffier et al. 1992). AMOVA is based on an analysis of variance framework that allows for incorporation of the appropriate evolutionary model, and permits to conduct analysis on different hierarchical levels.

A breaking point comes when the levels of genetic divergence are very high (F_{ST} close to 1), that as previously seen characterize inbreeding or even closed populations. This is usually the genetic signal of an interruption of gene flow for an extended period of time and if interruption is persisted for long enough, differences in morphology, behaviour, ecological preferences and mechanisms of reproductive isolation (RIMs) may ultimately appear which in other terms means that these populations may diverge into new species (de Queiroz 2007). But as there was discussion on what constitutes a population, the problem of defining species is no different (see next section). Some authors have tried to establish genetic thresholds to identify new species based on genetic distances of specific molecular markers among recognised different species (Johns & Avise 1998; Lavery et al. 2004). However, no arbitrary magnitude of molecular genetic divergence can provide an infallible metric to establish specific status. Furthermore, because species differ from each other not by a single Mendelian difference, but by a number of small differences, information from multiple datasets is necessary to finally tell two species apart (Avise 2004).

Systematics

Systematics is the field of science that studies the biological diversity and its origins. It has three main activities: (1) recognition of basic entities of biological diversity (species), (2) classification of species in a hierarchical scheme according to their evolutionary relationships, and (3) provide keys

for species identification and data on their distribution (Schuh & Brower 2009). Yet the key question is "what constitutes a species?" which has been focus of discussion early since Darwin's evolutionary theory (Mallet 2001); hence, through history many definitions of species have been given for sexually reproducing organisms (Mayden 1997) which sometimes responded to user-specific needs (Box 4).

Systematics, as currently practiced, has its origins in the mid-eighteenth century in the work of the Swedish botanist and naturalist Carl Nilsson Linæus (Schuh & Brower 2009) who established the binomial system of biological classification. To Linæus, species were the lowest particular kinds of organism which must present three specific characteristics: 1) distinct and monotypic, 2) immutable and created as such and, 3) breeding true (Claridge et al. 1997). However, since its establishment its theoretical content has experienced several renaissances. Particular concern has been given among scientist to what a species really is, how these are formed and where to draw the lines between them. After Linæus' work two ways of species identification were followed; on the one hand, taxonomists working with dead museum specimens, heavily relied on morphological differences to define species; on the other hand, naturalists emphasized breeding criteria and reproductive communities as species (Claridge et al. 1997). The publication of "The origin of species" (Darwin 1859) supposed a shift on the perception of species which were not further seen as static forms; rather species were considered arbitrary stages of the divergence process from a common ancestor from whom specific characters were inherited. However, the issue remained on how species are formed. To naturalists, like Darwin, natural selection was the most important force in speciation; instead, mutationists believed that speciation involved non-adaptive and macromutational steps (Coyne & Orr 2004).

The development of "the modern synthesis" added importance to the weight of evolutionary processes on the origin and formation of species. Dobzhansky (1937) stated in his work "Genetics and the origin of species" that a continuous evolutionary process could produce genetically and morphologically discrete groups living in one habitat, and stressed the importance RIMs in the formation of new species. Mayr (1942) complemented the genetic work of Dobzhansky with studies on the natural history and biogeography of species, synthesized in "Systematics and the origin of species". He further coined the Biological Species Concept (BSC) which defines species as "groups

of actually or potentially interbreeding natural populations that are reproductively isolated from other groups, thus representing independent units of evolution" (Mayr 1942). Also, he recognized that the identification of species based solely on morphological traits lead to some difficulties owing to (1) conspicuous morphological differences among individuals and populations (intraspecific variation) and (2) the virtual absence of morphological differences among certain sympatric populations (sibling species) that otherwise have all the characteristics of good species (genetic difference and reproductive isolation) (Mayr 1970).

Box 4. Representative species concepts and definitions (after Avise 2004)

Biological Species Concept (BSC) – "systems of populations: the gene exchange between these systems is limited or prevented by a reproductive isolating mechanisms or perhaps by a combination of several mechanisms" (Dobzhansky 1937; later modified by Mayr 1942)

Evolutionary Species Concept (ESC) – "a lineage (ancestral-descendant sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies" (Simpson 1951)

Phylogenetic Species Concept (PSC) – "a monophyletic group composed of "the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent" (Cracraft 1983)

Recognition Species Concept (RSC) – "the most inclusive population of biparental organisms which share a common fertilizations system" (Paterson 1985)

Cohesion Species Concept (CSC) – "the most inclusive population of individuals having the potential for cohesion through intrinsic cohesion mechanisms" (Templeton 1989)

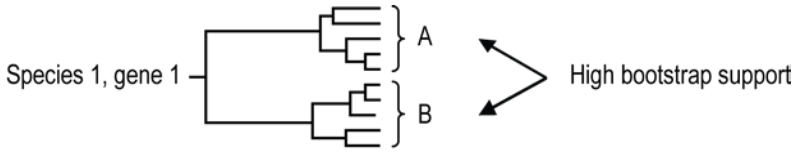
Concordance Principles (CP) – "a suggested means of recognizing species by the evidence of concordant phylogenetic partitions at multiple genetic attributes" (Avise & Ball 1990)

The BSC preponderates nowadays, and it is now widely and popularly accepted that species are defined by "a group of living organisms consisting of similar individuals capable of exchanging genes or interbreeding" (www.oxforddictionary.com, visited 02-05-2012). However, some difficulties were found when trying to apply the BSC (Avise 2004): (1) the discretionary judgements that are often required about the specific status of closely related forms living in allopatry, and (2) how much genetic exchange disqualifies populations from status as separate biological species (Avise 2004). These critics and the advent of genetic tools, and hence molecular systematics, revolutionized again the way in which species were defined and seen. Particular acceptance

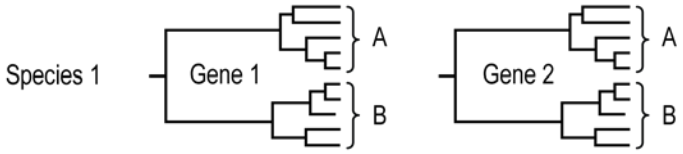
Box 5. Genealogical concordance (after Avise 2000)

Schematic description of the four aspects of genealogical concordance in phylogeographic inference. A and B are distinctive phylogroups in a gene tree.

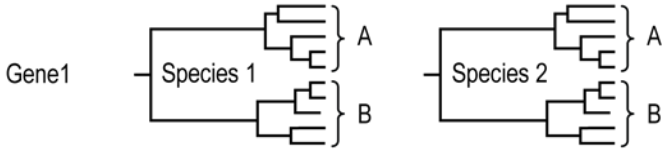
I Concordance across sequence characters within a gene.
Relevance: yields statistical significance for putative gene-tree clades.



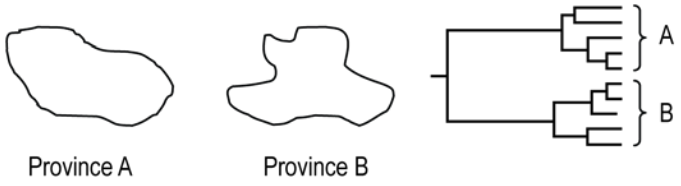
II Concordance in significant genealogical partitions across multiple genes within a species.
Relevance: establishes that gene-tree partitions register phylogenetic partitions at the population or species level.



III Concordance in the geography of gene-tree partitions across multiple codistributed species.
Relevance: implicates shared historical biogeographic factors in shaping intraspecific phylogenies.



IV Concordance of gene-tree partitions with spatial boundaries between traditionally recognized biogeographic provinces.
Relevance: implies shared historical biogeographic factors in shaping intraspecific phylogenies and organismal distributions.



received the Phylogenetic Species Concept (PSC) proposed by Cracraft (1983) who suggested that units of biodiversity should be identified as monophyletic groups composed of "the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and

descent". Advocates of the PSC argued that BSC lacked a sufficient phylogenetic perspective (de Queiroz & Donoghe 1988) and that "reproductive isolation should not be part of species concepts" because it could be operationally misleading (Frost & Hillis 1990). However, the PSC implied that even single synapomorphies were sufficient to identify a monophyletic aggregate of individuals worthy of recognition as a phylogenetic species but with the fine-scale resolving power of molecular techniques that would mean that even individuals could be raised to phylogenetic species, with associated warnings of taxonomic inflation (Isaac et al. 2004). Thus, the major problem of the PSC was the utilitarian rationale for defining each such diagnosable biological unit as a distinct species (Avise & Wollenberg 1997).

Avise & Ball (1990) proposed that the desirable elements of the BSC and the PSC could be reconciled under principles of multilocus genealogical concordance (Box 5). Accordingly, genealogical distinction must come in principle from empirical evidence of concordant genetic partitions across multiple, independent, genetically based molecular traits. They further suggested that the biological and taxonomic category "species" should continue to refer to groups of actually or potentially interbreeding populations isolated by intrinsic RIMs from other such groups. Within such units, the term "subspecies" should refer to groups of actually or potentially interbreeding populations (normally allopatric) that are genealogically highly distinctive from other such groups (Avise 2004).

Phylogeography

During the 1970s and 1980s mtDNA was adopted as tool of preference for genetic studies due to its lack of recombination and its maternal inheritance (for more details see next section). Its lack of recombination became especially practical because it meant that information could be ordered phylogenetically within a species, yielding an intraspecific genealogy (gene genealogy) interpretable as the matriarchal component of an organism pedigree, like surnames in a family (Avise 2000). These realizations together with the neutral theory contributed to the development of mathematical and statistical models for gene genealogies (Kingman 1982; Hudson 1983; Tajima 1983) later coded as the coalescent theory (Figure 2). Accordingly, individuals in any extant population invariably trace back, or "coalesce", to a common ancestor and so it tries to predict gene

genealogies and the time passed between the introduction of a mutation in a population to its distribution across the population (Kingman 1982).

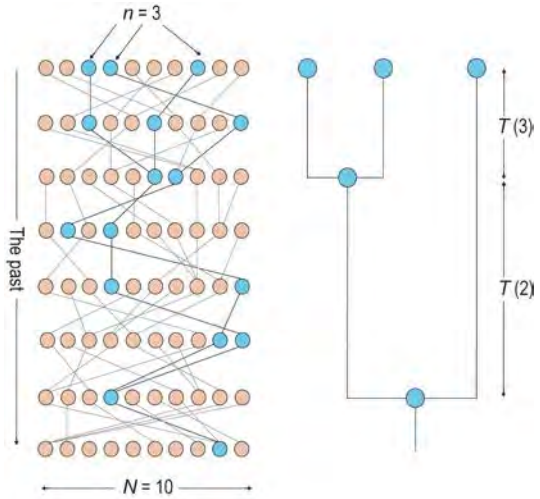


Figure 2. The coalescent theory (after Rosenberg 2002). Genealogy for a population of ten haploid individuals. *N*, sample size; *n*, number of allelic copies in the population. The black lines trace the ancestries of three sampled lineages back to a single common ancestor, whose subgenealogy with coalescence events [*T*(3) and *T*(2)] is given.

As the amount of studies looking at mtDNA genetic variation in natural populations piled up, Avise et al. (1987) suggested that the joint examination of (1) phylogenetic relationships among mtDNA molecules and (2) geographic distributions of the phylogenetic groupings, constituted the basements of a discipline that might be termed intraspecific phylogeography. They also felt that phylogeography should assume a place in evolutionary studies at least commensurate with ecogeography. As subdiscipline of biogeography, phylogeography balances traditional ecogeographic views by emphasizing historical aspects of the contemporary spatial distributions of gene lineages (Avise et al. 1987). Thus, phylogeography is a field of study concerned with the principles and processes governing the geographical distribution of genealogical lineages, expecially those at the intraspecific level (Avise 1998a).

The demographic history of a species is prompted to leave a footprint on its levels of genetic diversity and in the pattern of DNA substitutions among individuals, that will depend on the direction (growth or decline) and tempo (ancient or recent) of this change (Grant & Bowen 1998). The neutral theory of evolution says that the amount of genetic variation in a population will be positively related

to the size of the population which also determines the probability that any two sequences drawn from a population will coalesce to a point in time (Kingman 1982). Based on these priors, several tests have been developed to determine whether demographic changes occurred in the past or not. The ratio of haplotypes vs nucleotide mutations, the frequency of haplotypes within a population (Mismatch Distribution - MMD; Rogers & Harpending 1992) and the phylogenetic relationships among these haplotypes (represented by median-joining network; Bandelt et al. 1999), can give inferences on past demographic events.

Furthermore, following the neutral theory assumption that DNA sequences might evolve at a roughly constant rate, paleographical information in combination with fossil records can help date the evolutionary rate of molecular markers employed in genetic studies, what is also known as calibration of mtDNA clocks (Avise 2000). With this information inferences can be made on the approximate date when these demographic events took place. However, several studies have confirmed that mtDNA variation might not be completely neutral and its evolution can be influenced by selective pressures or extreme genetic drifts (Meiklejohn et al. 2007). This can inflate estimates of divergence times and misleading estimates of time since common ancestry; consequently, phylogeography expanded its focus to nuclear markers, with subsequent modification of coalescence calculation (Avise 2000).

Molecular markers

Molecular markers are polymorphic proteins or DNA fragments that present sufficient variability to conduct studies of intraspecific or interespecific variability (Hillis et al. 1996). Also, for their implementation in molecular statistical analyses, molecular markers should only be subjected to neutral variation (Mortiz & Hillis 1996). The first markers were based on organic molecules that were thought to be taxon specific, such as turacin which is exclusive of musophagidae birds (Church 1869). However, very few of these markers proved useful in animal taxonomy. Instead, Smithies (1955) discovered variants in serum blood proteins through electrophoretic separation in a starch gel support, and Hunter & Markert (1957) identified that different variants of the same enzyme having identical function could be present in an individual, the so-called isozymes. Many studies used allozymes to address ecological and evolutionary questions (May 1992) but researchers

realized that large part of genetic variation was probably unnoticed due to redundancy of genetic code. Thus, further improvements in the resolution of genetic differences required developing new methods of DNA analysis. The first of those were RFLP (1970s) and minisatellite DNA fingerprinting (1980s), followed by direct sequencing of DNA (Sanger 1975). With the development of polymerase chain reaction (PCR) method (Saiki et al. 1988), not only further techniques were developed but also opportunities for micro and macro evolutionary studies were widened (Hillis et al. 1996b; Avise 2004).

With the wide assortment of molecular markers available nowadays (Table 1), it is important to know the characteristics of each of these markers (Sunnucks 2000) and choose according to the objectives of the study and the economic resources affordable by researchers, as there is no single best marker suitable for all purposes (Féral 2002; Avise 2004). Favourable attributes to consider in the choice of final marker are: (1) assayable by PCR, so even with small quantities of DNA amplification will be possible; (2) rapid development and screening; (3) single-locus as opposed to multilocus markers, which despite are technically convenient present dominant inheritance hampering analytical analysis; (4) present high genetic variability and sensitivity for the question to answer; (5) allele frequencies and gene genealogy, which can yield information on demographic trends and help to reconstruct current and historical processes (Sunnucks 2000).

Nowadays, evolutionary studies mostly rely on direct analysis of DNA. DNA sequencing and microsatellites are the most widespread molecular markers because of their capacity to yield gene genealogies (Hillis et al. 1996; Avise 2000; Avise 2004). Yet a major drawback of these methods is the necessity to design marker-specific primers (Sunnucks 2000). Within DNA sequencing, and particularly mtDNA, the existence of conserved primers suitable for a wide range of species (Palumbi et al. 1991), makes population studies more straight-forward and economically accessible; whereas microsatellite primers have to be created de novo for almost each species, hampering the results of a study if all resources and time have to be devoted to the design of specific primers. On the other hand, multilocus PCR-based techniques (ISSR, AFLP, RAPD) present analytical drawbacks, such as dominance or not possibility to infer gene genealogy. However, these are technically convenient because they allow conducting molecular analyses without any prior DNA

sequence information, present large variability and their total cost may be significantly reduced (Hillis et al. 1996).

Table 1. Main attributes of molecular markers used in evolutionary studies (after Sunnucks 2000).

	PCR assay	Rapid transfer	Single locus	Codominant	Overall variability	Gene genealogy
Mitochondrial and chloroplast DNA						
Sequence	Yes	Yes	Yes	Yes	Low-high	Yes
RFLP	No, large	Yes	No	Yes	Low-medium	Yes
Multilocus nuclear						
DNA fingerprints	No, large	Yes	No	No	High	No
ISSR	Yes	Yes	No	No	High	No
RAPD	Yes	Yes	No	No	High	No
AFLP	Yes	Yes	No	No	Medium-High	No
Single locus nuclear (single copy nuclear, scn)						
Allozymes	No	Yes	Yes	Yes	Low-medium	Rarely
Minisatellites	Yes	Few	Yes	Yes	High	Rarely
Microsatellites	Yes	Some	Yes	Yes	High	Yes
Sequence	Yes	Yes	Yes	Yes	Low-High	Yes
Anonymous scn	Yes	No	Yes	Yes	Medium	Yes
Specific scn	Yes	Some	Yes	Yes	Medium	Yes
rDNA	Yes	Yes	No	No	Low-medium	Yes

DNA sequencing: mitochondrial vs nuclear genes

Direct gene sequencing is the highest possible level of DNA resolution in genetic analyses as it permits to know the information of a section of the genome base per base (Hillis et al. 1996b). It also presents most of the properties that make a molecular marker desirable and both nuclear and organelle genes can be analyzed this way. In the early 1970s several sequencing methods were developed [e.g. chemical sequencing, by Maxam & Gilbert (1977)] but current automated sequencers (Smith et al. 1986) are based on the dideoxy method (Sanger et al. 1977). Sanger's method consists in the addition of radioactive labelled dideoxy nucleotide triphosphates (ddNTPs), to an amplification reaction mix. ddNTPs are nucleotides lacking the 3'-OH group necessary for the formation of the phosphodiester bond between two nucleotides. Modified nucleotides will terminate DNA strand elongation generating fragments of different length that can be separated by electrophoresis and visualized by autoradiography. In the original method (Sanger et al. 1977), four

sequencing reactions had to be run, one for each radiolabelled ddNTP. Current automated sequencers use fluorescently labelled nucleotides. Each ddTNP is labelled with fluorescent dyes, each with different wavelength of fluorescence and emission, which will be detected with a fluorescence detector after migrating through a capillary electrophoresis. The sequence information is directly transmitted to a computer (Smith et al. 1986; Hillis et al. 1996b).

The mtDNA (Box 6) is a circular double-stranded molecule (in animals) of which a large number of copies can be found in each somatic cell (100-1000 copies) in contrast to the single nuclear copy. This makes mtDNA an easier molecule to amplify than nuclear DNA which becomes especially practical when the biological tissue may be small or degraded. Furthermore, mtDNA presents a series of attributes, namely maternal inheritance, lack of recombination, neutral evolution, and smaller effective population size and higher evolutionary rate than nuclear DNA, which make it a useful tool for phylogeographycal analyses and phylogenetic inferences among closely related species (Hillis et al. 1996a; Avise 2000; Avise 2004).

Its smaller effective size is a consequence of its haploid nature and the fact that it is inherited through the oocyte cytoplasm (maternal inheritance). This means that for every copy of mtDNA inherited there are four copies of nuclear DNA passed on (i.e. mtDNA presents ¼ smaller effective population size than nuclear DNA). The maternal inheritance of mtDNA further implies that this molecule does not suffer recombination therefore the whole genome behaves as a single locus with all copies within an organism being equal (homoplasmy). Therefore, heterozygote positions are rarely found in mtDNA analyses, which makes sequence analyses straighter forward than for nuclear DNA (although methodological and analytical solutions exist to solve nuclear heterozygote positions, see Hare 2001). The lack of recombination also implies that the mitochondrial gene content and order is strongly conserved across taxa (with very few duplications, no introns, and very short intergenic regions, Gissi et al. 2008), thus PCR primers can be designed which will work across a wide range of taxonomic units, (universal primers, Palumbi et al. 1991). Yet, because mtDNA does not have protective histones or DNA reparation system during replication (Wilson et al. 1985), it is more exposed to the oxidative phosporylation metabolism of the inner membrane (Ritcher et al. 1988) and the accumulation of errors is larger (Bogenhagen 1999). It is estimated that mtDNA evolves about 5-10 times faster than typical single-copy nuclear DNA (Brown et al. 1979).

The final result is a molecule with variable regions (e.g. the non-coding region) flanked by highly conserved ones (e.g. ribosomal DNA).

Box 6. mtDNA structure in *Penaeus monodon* (after Wilson et al. 2000)

Structural properties:

- Circular closed molecule consisting of a heavy (H) and a light (L) chain
  - 15984 base pairs (bp)
  - 37 conserved genes (22 tRNAs, 2 rRNA and 13 mRNA)
  - non-coding region (991 bp) which holds initiation of transcription
- Gene order and genetic code as in *Drosophila yakuba*
  - UAG codifies for aminoacid rather than STOP codon

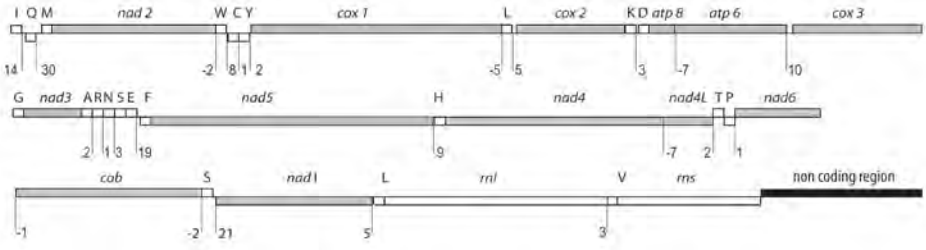


Figure 3. Gene order in *Penaeus monodon* mitochondrial genome. tRNA genes are represented by the single-letter code for the amino acid they codify for. Numbers indicate bp between genes, with negative numbers representing probable overlaps between genes (e.g. arp8 and arp6 are separated by -7bp. ATGTTAA). GenBank Accession number AF217843.

Finally, mtDNA is supposed to evolve in a nearly neutral fashion because the genes it codifies for are mostly involved in basic metabolic functions (respiration) and because adaptive mutations are very rare (Galtier et al. 2009). So polymorphism will be mostly influenced by demographic events in population history than by selection. Furthermore, as no recombination occurs within the molecule, differences are only due to accumulated mutational events since the divergence from the ancestor; also, assuming constant evolutionary rate, divergence levels between two mitochondrial sequences should roughly reflect divergence times (Avise 2000; Hillis et al. 1996). Therefore, mtDNA genes have often been used for molecular clock calibrations (Avise 2000). However, within single taxa different mitochondrial genes present different evolutionary rates because of gene-specific evolutionary constraints (Moritz et al. 1987), which has led to use different mitochondrial regions for different analytical purposes. Within crustaceans the genes codifying for the 16S rRNA and for the

subunit I of the Cytochrome *c* Oxidase (COI) (Box 7) have been the primary tool for studies of phylogenetic relationships (e.g. Schubart et al. 2000; Lavery et al. 2004; Voloch et al. 2005), intraspecific phylogeographical analyses (e.g. Roman & Palumbi 2004; Reuschel et al. 2010) and species identification (i.e. barcoding, Costa et al. 2007).

However, because of the fast evolutionary rate, mtDNA may present saturation when trying to resolve deep evolutionary nodes across distant taxa (Anderson et al. 2004). In contrast, a set of nuclear genes can be selected from distinct chromosomes, each with its different evolutionary rate, such that each gene tree provides an independent estimate of the species tree (Moore 1995). The protein-coding nuclear genes phosphoenolpyruvate carboxykinase (PEPCK) and Sodium-potassium ATPase α-subunit (NaK) (Box 7) have been previously used to resolve phylogenetic relationships within insecta (Friedlander et al. 1996), bilaterian metazoans (Anderson et al. 2004) and decapoda crustacea (Tsang et al. 2008; Ma et al. 2009).

Box 7. Molecular markers employed in this study (GenBank)

Mitochondrial genes

- 16S rRNA is a structural, non-coding gene, whose transcript is the small subunit ribosomal RNA that in a conserved secondary structure, and in association with proteins, forms the large subunit of mitochondrial ribosomes.
- COX 1 codifies for the subunit 1 of the Cytochrome *c* Oxidase (COI), the last enzyme in the respiratory electron transport chain of mitochondria that catalizes the reduction of oxygen to water. It is a transmembrane protein of the mitochondrial membrane in eukaryotes.

Nuclear genes

- PEPCK codifies for the enzyme with the same name (phosphoenolpyruvate carboxykinase). Enzyme PEPCK catalyzes the first step of gluconeogenesis, interconverting oxaloacetate and phosphoenolpiruvate in organisms ranging from bacteria to human.
- NaK codifies for the sodium-potassium ATPase α-subunit that when binded with the β-subunits forms an heterodimer responsible for maintaining electrochemical potential differences across cell membranes which is essential for cell signalling and secondary transport. Although in vertebrates the α- and β-subunits have evolved into multiple copies, sodium-potassium ATPase remains a single copy gene in invertebrates.

Inter Simple Sequence Repeat (ISSR)

Inter Simple Sequence Repeat (ISSR) consists in the amplification of DNA fragments between two closely and inversely oriented microsatellites (Figure 4, Zietkiewicz et al. 1994). Since microsatellites are known to be arbitrarily spread along the genome, amplifications of ISSR are

multilocus and polymorphic (Zietkiewicz et al. 1994). A repeat motif is used as priming site, thus primers have a simple sequence repeat, usually 15-30 nucleotide in length with degenerate/redundant anchoring at either the 5' (e.g. BDB$[CA]_7$) or at the 3' (e.g. $[TCC]_5$RY) end (Bornet & Branchard 2001). Where the primer successfully locates microsatellite regions within amplifiable distance, a band of particular size for that locus is generated, which represents the intervening strand of DNA between the microsatellites (Figure 4). For all these attributes ISSRs result appropriate markers, inexpensive and fast, especially when no previous knowledge of species' genome exists (Wolfe & Liston 1998; Abbot 2001). Results are anonymous, typically dominant, diallelic mendelian markers (Wolfe & Liston 1998) where each band is considered a separate locus (Abbot 2001).

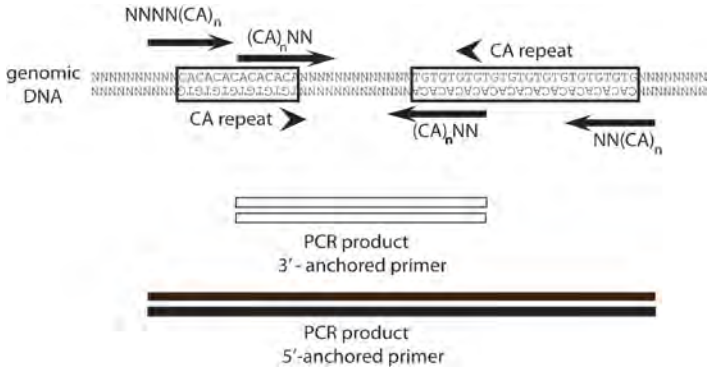


Figure 4. ISSR functioning (after Zietkiewicz et al. 1994)

These markers were initially used by plant biologists (Bornet & Branchard 2001) for studies of hybridization in cultivated plants (Wolfe 2005). But since Abbot (2001) evaluated their utility for population-level studies in invertebrate species, their use extended to the whole animal kingdom, from marine invertebrates (Pannacciulli et al. 2009 and references therein) to vertebrates (Machkour-M'Rabet et al. 2009 and references therein) with extensive application in population genetic studies and species identification (Casu et al. 2009).

Aristeid shrimps

Caridea, Procarididea, Stenopodidea and Dendrobranchiata, are the main shrimp-like lineages within decapoda (de Grave & Fransen 2011). Caridea, Procarididea and Stenopodidea are

infraorders of the suborder Pleocyemata (Burkenroad 1963) whilst Dendrobranchiata (Bate 1888) is itself a single suborder (Box 8) characterized by the morphology of the gills, and by the extension of the pleura of the second somite. In Caridea species the pleura of the second somite overlaps those of the first and third somite, but in Dendrobranchiata the pleura of the second somite only overlaps that of the third somite. Accordingly, several authors suggested that the common term shrimp should refer to Caridea species and prawn should be restricted to Dendrobranchiata species (Poore et al. 2008 and cites therein). British English uses more frequently the term prawn whilst American English uses more frequently the term shrimp (Poore et al. 2008). But shrimp and prawn are interchangeable names in literature. *Aristeus antennatus* is commonly known as “blue and red shrimp”, and *Aristaeomorpha foliacea* is recognized as “giant red shrimp”; despite Aristeidae species should be referred to as prawns, to avoid confusion with the large bulk of published literature, they will be referred to as shrimps throughout this work.

Aristeus antennatus (Risso 1816) (Box 9) and *Aristaeomorpha foliacea* (Risso 1827) (Box 10) are the two most important commercial species of the Aristeidae family in the Mediterranean Sea. Giuseppe Antonio Risso (1777-1845), called Antoine Risso, was a French naturalist that first identified *A. antennatus* (Figure 5) and *A. foliacea* (Figure 7) in the Ligurian Sea (Mediterranean Sea) in the 19th century. Because of the large geographical distribution of both *A. antennatus* and *A. foliacea*, these same species were later described by other authors in the Atlantic, Indian and Pacific Oceans giving place to a large range of synonyms (de Grave & Fransen 2011). However, after agreement on synonyms the full distribution of each species was given by the carcinologist Lipke Bijdeley Holthuis (1921-2008). Accordingly, *A. antennatus* is present across the Mediterranean Sea, in the Eastern Atlantic Ocean, and in the Western Indian Ocean; the distribution of *A. foliacea* partly overlaps that of *A. antennatus* but it is further distributed in the Western Atlantic, Eastern Indian Ocean and New Zealand (Holthius 1980) (see Figure 6 and Figure 8 for details).

A. antennatus and *A. foliacea* present the typical diagnosable characters of Dendrobranchiata, namely: (1) dendrobranchaita gills, (2) chelae on the first three pairs of pereiopods, (3) second pleomere with pleura does not overlap the first, (4) prominent hinges between pleomeres, (5) eggs released directly into the water which hatch as lecithotrophic nauplius, (6) presence of petasma in males, (7) pleopods without appendix interna.

Box 8. Biological classification of *Aristeus antennatus* and *Aristaeomorpha foliacea* (highlighted in blue) (after de Grave & Fransen 2011)

The family Aristeidae currently contains 25 species in 9 genera, plus one fossil genus, *Archaeosolenocera* Carriol & Riou 1991 (Martin & Davis 2001; Tavares & Martin 2010).

Phylum arthropoda
SubPhylum Crustacea
Class Malacostraca
Sclass Eumalacostraca
Superorder Eucarida Calman 1904
Order Decapoda Latreille 1802 (Deca = ten, poda = legs)
Suborder Pleocyemata Burkenroad 1963
Infraorder Caridea Dana 1852
Infraorder Procaridídea Felgenhauer & Abele 1983
Infraorder Stenopodidea Bate 1888
Suborder Dendrobranchiata Bate 1888 (Dendro = tree, branching; chiata = gills)
Superfamily Sergestoidea Dana 1852
Superfamily Penaeoidea Rafinesque 1815
Family Benthesicymidae Wood-Mason & Alcock 1891
Family Penaeidae Rafinesque 1815
Family Sicyoniidae Ortmann 1898
Family Solenoceridae Wood-Mason & Alcock 1891
Family Aristeidae Wood-Mason 1891
Genus *Aristaeomorpha* Wood-Mason & Alcock 1891
Aristaeomorpha foliacea Risso 1827
Aristaeomorpha woodmasoni Calman 1925
Genus *Aristaeopsis* Wood-Mason & Alcock 1891
Genus *Aristeus* Duvernoy 1840
Aristeus alcocki Ramadan 1938
Aristeus antennatus Risso 1816
Aristeus antillensis Milne-Edwards & Bouvier 1909
Aristeus pallidicauda Komai 1993
Aristeus semidentatus Bate 1881
Aristeus varidens Holthius 1952
Aristeus virilis Bate 1881
Genus *Austropenaeus* Pérez Farfante & Kensley 1997
Genus *Hemipenaeus* Bate 1881
Genus *Hepomadus* Bate 1881
Genus *Parahepomadus* Crosnier 1978
Genus *Plesiopenaeus* Bate 1881
Genus *Pseudoaristeus* Crosnier 1978

Box 9. *Aristeus antennatus* (Risso 1816) identification sheet

FAO names: blue and red shrimp (En), crevette rouge (Fr), gamba rosada (Es)
Color: nacreous pink profusely interspersed with violet on the dorsal regions of carapace and around the joints of abdominal segments
Carapace length (CL): ♀♂ 10 to 18 cm, max 22 cm
Depth range: 80 to 3300m depth (Campillo 1994; Sardà et al. 2004a)

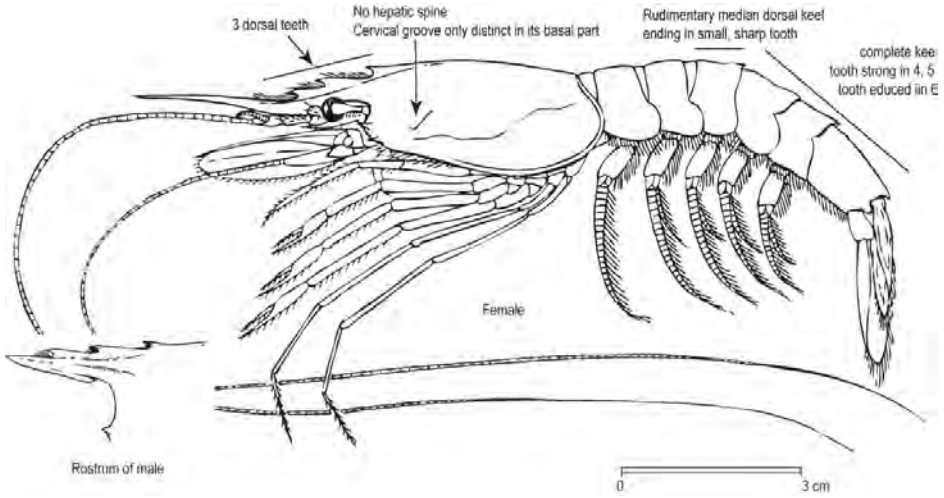


Figure 5. *A. antennatus* schematic description (after Fischer et al. 1981)

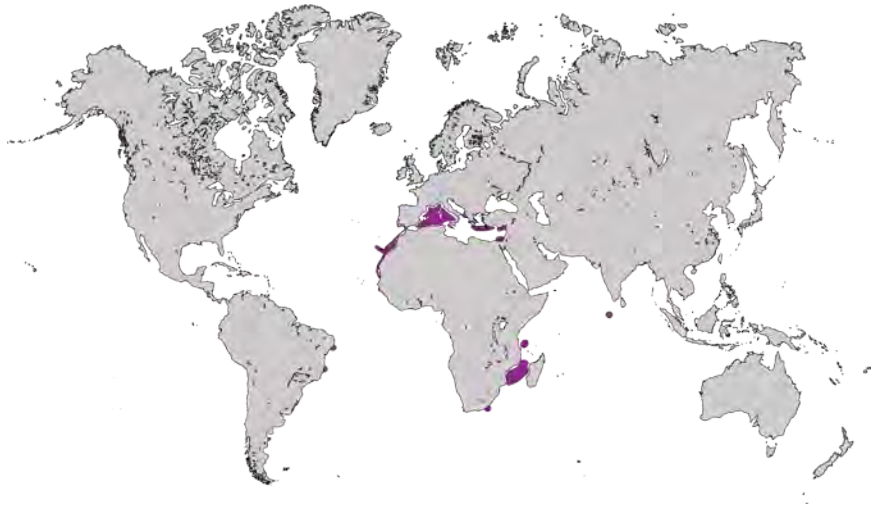


Figure 6. *A. antennatus* geographical distribution: Western Atlantic: Bahia and Espirito Santo (Brazil, Serejo et al. 2007); Eastern Atlantic: from south Portugal to Cape Verde Islands and Mediterranean Sea (Holthuis 1980); Indo-West Pacific: Zanzibar, Maldive Islands, Mozambique and South Africa (de Freitas 1985).

Box 10. *Aristaeomorpha foliacea* (Risso 1827) identification sheet

FAO names: giant red shrimp (En), gambon rouge (Fr), gamba española (Es)
Color: wine red with darker violet reflections on the upper side of carapace
Carapace length (CL): ♂ 13 to 14 cm, max 17 cm; ♀ 17 to 20 cm, max 22.5 cm
Depth range: 123 to 1100 m depth (Politou et al. 2004)

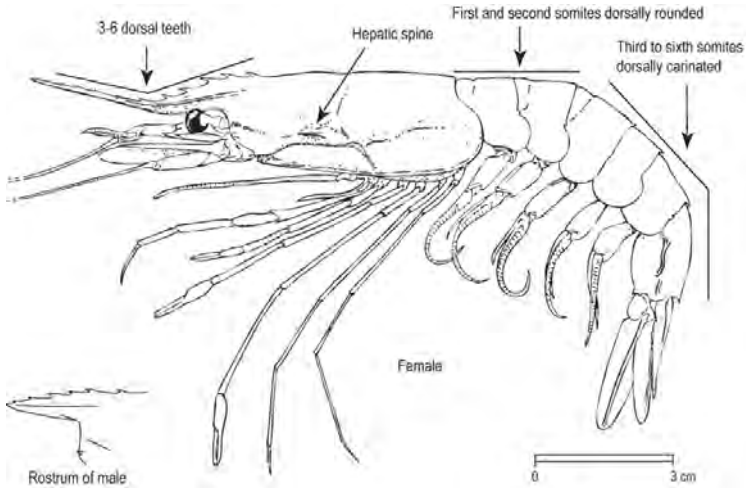


Figure 7. *A. foliacea* schematic description (after Fischer et al. 1981)

Figure 8. *A. foliacea* geographical distribution. Western Atlantic: South of Massachusetts to the Straits of Florida, Gulf of Mexico, Colombia, Venezuela; Eastern Atlantic: Bay of Biscay to South Western Sahara and Mediterranean Sea (Holthuis 1980); Indo-West Pacific: Eastern South Africa, Mozambique, Madagascar, Reunion, Maldive Islands, Sri Lanka, Indonesia, Philippines, China Sea, Japan, Australia, New Zealand, New Caledonia, Wallis and Futuna Islands, Fiji (Dall 2001).

In addition, as members of the family Aristeidae (Wood-Mason 1891) they are characterized by inhabiting in deep waters (mostly found between 200 and 2000 m depth), by their reddish coloration, sexual dimorphism and needle-like rostrum (Holthius 1980; Tavares & Martin 2010). Aristeid females are larger than males. The rostrum presents 3 to 6 dorsal rostral/postrostral teeth and no ventral teeth which is usually elongated in females and young juveniles but short in adult males. Eyestalks present a tubercle on its inner border. Upper antennular flagellum is short. Carapace lacks postorbital and pterygostomian spines but it sometimes presents cervical and postcervical grooves which most often reach dorsal midline. The athrobranchs on the penultimate thoracic segment are well developed and telson presents movable spines (Fisher et al. 1981).

Compared biology

A large bulk of literature exists concerning *Aristeus antennatus* and *Aristaeomorpha foliacea* distribution (Cau et al. 2002; Company et al. 2004; Politou et al. 2004), ecology (Sardà & Cartes 1993, 1997; D'Onghia et al. 2005; Company et al. 2008; Maynou 2008), reproductive biology (Demestre 1995; Orsi Relini & Relini 1998a; Kapiris & Thessalou-Legaki 2006, 2009) and fisheries viability (Carbonell et al. 1999; Politou et al. 2001; Ragonese et al. 2001; Carbonell & Azevedo 2003; Maynou et al. 2003). However, most of this information refers to the Western Mediterranean, Strait of Sicily and Ionian Sea, where both species constitute the main target for demersal deep-water fisheries (Scopus search for "Aristeus" gave 165 publications between 1950-2011 and "Aristeus Mediterranean" gave 103 publications between 1988-2011; "Aristaeomorpha" gave 61 results between 1971-2012 and "Aristaeomorpha Mediterranean" gave 40 results between 1994-2012). Other studies concerning their distribution and fisheries viability have been done in other Mediterranean regions (Aegean Sea, Mytilineou et al. 2006; Algerian waters, Mouffok et al. 2008a, 2008b; Turkish waters, Ozcan et al. 2009), as well as in Atlantic waters (Cascalho & dos Santos 1994; dos Santos & Cascalho 1994; Figuereido et al. 2001), Mozambique Channel (Sobrino et al. 2010), North-Western Australia (Wadley 1994), Brazil (Pezzuto et al. 2006; Serejo et al. 2007; Dallagnolo et al. 2009), Mexico (Gracia et al. 2010) and Colombia (Paramo & Saint-Paul 2011).

A. antennatus and *A. foliacea* are nektobenthonic species that inhabit on muddy bottoms of the upper and middle slope, usually associated to submarine canyons. The highest abundances of both

species occur between 600-800 m depth, where they often co-exist; however, *A. antennatus* presents a wider bathymetric range (80 to 3300 m depth; Campillo 1994; Sardà et al. 2004a) than *A. foliacea* (123 to 1100 m depth; Politou et al. 2004). Both species are active predators of epifauna and infauna (bivalves, polychaetes, amphipods, ophiuroids, Cartes & Sardà 1989; Chartosia et al. 2005) and perform nocturnal upward migrations along the slope in search for food to which their shallower presence has been associated (Cartes et al. 1993; Bello & Pipetone 2002).

Aristeidae engage in external fertilization and an open thelycum stores and transfers the spermatophore (Tavares & Martin 2010). Mature males can be found throughout the year although spermatogenesis is discontinuous and seasonal changes occur in testicular activity (DeSantis et al. 1998). Mating activities occur from late winter to late spring (January-May), and copula would seem to stimulate ovary development and spawning, which takes place in summer. Thus the cycle is as follows: initial ovary development, molting, mating, vitellogenesis and spawning (Kapiris & Thessalou-Legaki 2009). These features are common to both red shrimps, only that reproductive activity of *A. foliacea* starts earlier than that of *A. antennatus*; main mating activity precedes the appearance of gonad maturation by four months in *A. foliacea* (January-April) and by only two months in *A. antennatus* (March-April) (Kapiris & Thessalou-Legaki 2009).

The eurybathic condition of *A. antennatus* permits the species to adapt its population density and structure to the energy available at different depth ranges (Cartes & Demestre 2003; Sardà et al. 2003; Company et al. 2008). Three different stratums (<1000 m, 1000-1500 m, >1500 m depth) have been identified, with density, sex and size segregation (Sardà et al. 2004a) (Figure 9). In the upper stratum (<1000 m depth) the largest abundances can be found (1000 ind/km^2), the population is mainly composed of large females and the species performs seasonal movements along submarine canyons in relation to reproductive activities (Demestre & Martín 1993); it is within this stratum that fishing activity also takes place, so they are called fishing grounds (Sardà et al. 2002). From late winter to early summer, the large part of the population forms elongated shoals at around 600-800 m on the open slope outside the canyons (so-called *baranes*) and is mainly composed of mature females (Tobar & Sardà 1987). From mid-summer to mid-winter, individuals move upwards in the canyon, and fishing takes place along the canyon walls from the

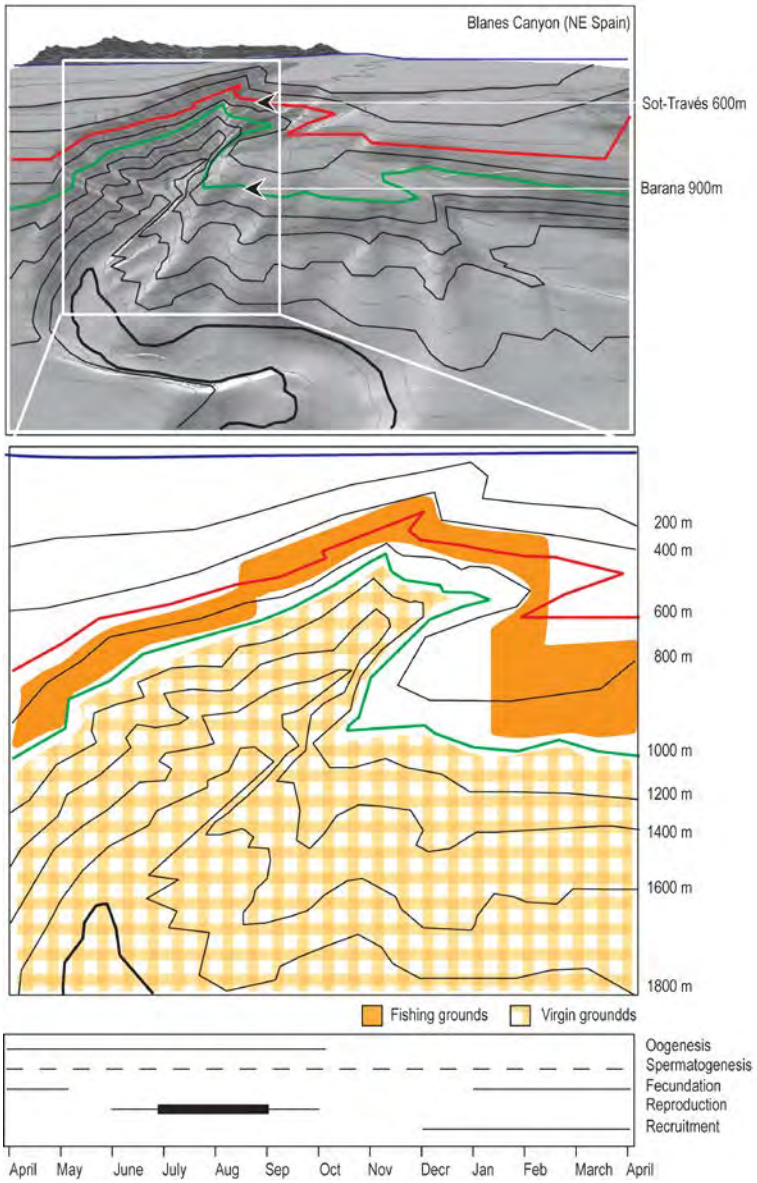


Figure 9. Bathymetry of the Blanes Canyon (Spain), after Sardà et al. (2009), and schematic distribution of *A. antennatus* along the canyon according to reproductive period and season.

mid-canyon to the canyon head, known locally as *sot-través* (400-600 m) fishing ground (Sardà & Cartes 1993, 1997; Tudela et al. 2003). In winter and early spring, males are relatively abundant over slope areas, which would make possible physical contact with females (Sardà et al. 1997). After mating, males would return to deeper grounds. Although many studies looking at the spatial

mobility of the individuals have been focused on the Catalan submarine canyons, Tursi et al. (1996) suggested this seasonality behaviour could be extrapolated to other localities of the Western Mediterranean where canyons are an important geological feature (Tudela et al. 2003). In the middle (1000-1500 m, 300 ind/km^2) and lower stratums (>1500 m, <50 ind/km^2), also called the virgin grounds because there is no fishing activity, density and size of individuals tends to decrease (Sardà et al. 2002; Sardà et al. 2004a), whilst the number of males and juveniles increases until reaching sex proportions not significantly different from 1:1 (Sardà & Cartes 1993). This distribution of individuals, together with recruitment taking place below 1200 m depth, suggests a response to food competition or predation. The smallest individual so far collected (6.4 mm CL) was found in December at approximately 1250 m depth (Sardà & Cartes 1997).

There is less biological information regarding *A. foliacea* (see previous records in Scopus), and it mostly refers to Italian fishing grounds (Belcari et al. 2003). However, *A. foliacea* is also distributed on muddy bottoms, associated to submarine canyons, and presents spatio-temporal distribution correlated to bio-ecological aspects (D'Onghia et al. 1998). In winter, maturity of males begins and so their proportion increases on the fishing bottoms (D'Onghia et al. 1998). During winter-spring season both, females and males, migrate to the upper slope, where main mating activity occurs during late-spring and summer. Then, *A. foliacea* individuals displace again to deeper grounds (D'Onghia et al. 1998). Spawning takes place in summer (Papaconstantinou & Kapiris 2003; Kapiris & Thessalou-Legaki 2009) and recruitment occurs in winter-early spring at around 750-800 m depth (D'Onghia et al. 1998; Papaconstantinou & Kapiris 2001).

Little is known about the larval stages of red shrimps after eggs have been released, but a model of reproductive dynamics has been proposed for *A. antennatus* based on the discovery of protozoeas II and III, and zoea I larval stages near red shrimp fishing grounds off the Balearic Islands (Carbonell et al. 2010). The model suggests that newly hatched larvae would perform an ontogenetic migration up through the water column to surface waters, where food availability is higher and posterior larval stages can successfully develop (Carbonell et al. 2010). When the dicapodid stage had been reached, a second ontogenetic migration would take place to adult's habitat in deep waters (Sardà et al. 2004a). Between the two ontogenetic migrations, larvae could

be transported by oceanic currents, as Carbonell et al. (2010) also found larval stages far from the adult fishing grounds.

As previously stated, both species coexist in large part of their distribution range, but in the Mediterranean Sea, where the large part of studies have been undertaken, their distribution is patchy and presents an antagonistic longitudinal gradient (Cau et al. 2002; Politou et al. 2004) being *A. antennatus* more abundant in the Western Mediterranean basin, with numbers decreasing towards the Eastern Mediterranean basin, and vice versa for *A. foliacea* (Cau et al. 2002; Politou et al. 2004) (Figure 11). Two main hypotheses have been considered by several authors to try to explain this difference in distribution, as next detailed.

On the one hand, Ghidalia & Bourgois (1961) suggested that *A. antennatus* would be more associated to waters with a temperature of 12.8 ºC and a salinity level of 38.4 psu, values typically observed in the Western Mediterranean Deep Waters (WMDW, below 1000 m depth, 12.73 ºC, 38.43 psu) which forms in winter at the Gulf of Lion (Millot 1999). Contrary, *A. foliacea* would be associated to waters with slightly higher temperature and salinity (13.5 ºC, 38.5 psu) typical of the Levantine Intermediate Water (LIW, between 200-1000 m depth, 14.00–13.28 ºC, 38.7–38.5 psu), which dominates in Eastern Mediterranean and flows above the WMDW (Ghidalia & Bourgois 1961). These conditions would be considered the optimal ones for these species to reach their maximum yields, but temperature alone is not the delimiting factor, as both species have been detected in other regions with different temperatures, e.g. *A. antennatus* occurs in the Eastern basin at 13.9 ºC (Politou et al. 2004) and between 11-12 ºC in the Atlantic Ocean (Ribeiro-Cascalho 1988). In a recent multidisciplinary study carried out in the Blanes canyon (Spain, Figure 9) Sardà et al. (2009) concluded that presence and abundance of *A. antennatus* is conditioned by environmental but also hydrodynamic conditions. Accordingly, *A. antennatus* would prefer relatively cold (13.1-13.2 ºC) and salty (>38.5 psu) waters and low currents with moderate variability (Sardà et al. 2009). The case of *A. foliacea* is less clear; changes in water salinity rather than temperature have been suggested to explain the disappearance of *A. foliacea* from certain regions of the Mediterranean, as in the Ligurian Sea (Murenu et al. 1994); Capezzuto et al. (2010) detected a significant correlation between an increase in temperature and salinity in the western Ionian Sea with an increase in giant red shrimp biomass over a period of 5 years; instead, Cartes et al. (2011) detected a decrease in

A. foliacea in the Balearic Basin that was associated to a reduction of the O_2 content as a consequence of an increase in temperature and salinity of the LIW.

On the other hand, some authors have considered the effect of fisheries pressure for the actual different distribution. *A. foliacea's* recruitment and distribution occurs almost exclusively at depths where fishing pressure is exerted, whilst the existence of virgin grounds for *A. antennatus* represents that a section of the population would be safe from fishing activity, contributing at the same time to the replenishment of the fished population (Sardà et al. 2002, 2010). Orsi Relini & Relini (1998b) and Relini & Orsi Relini (1987) detected a decline in the number of *A. foliacea* catches in the Gulf of Genova (Ligurian Sea). Campillo (1994) detected by 1984 a total absence of *A. foliacea* in the Gulf of Lion were previously it was captured in the same quantities as *A. antennatus*. Similarly, D'Onghia et al. (2005) detected lower abundances of *A. foliacea* in the western part of the Ionian Sea than in the eastern side, where directed exploitation is almost absent (see next section).

Aristeid fisheries

Red shrimps, *A. antennatus* and *A. foliacea*, became the target of artisanal deep-water bottom trawl fisheries in 1930s in the Ligurian Sea due to their large size and high commercial value; around 1940s its exploitation had extended to other regions of North-Western Mediterranean (Gulf of Lion, Catalan Sea, Balearic Sea) (Sardà et al. 2004b). As technology improved and fleets were developed in Western Mediterranean bordering countries, the number of captures also increased, until 1970s when the first collapse of red shrimps fisheries was detected (Figure 10) (Bensch et al. 2008). By 1985, *A. antennatus* fishing stocks showed signs of recovery but not those of *A. foliacea* (Fiorentino et al. 1998) which disappeared from Ligurian and Gulf of Lion grounds, remaining restricted to Sardinian and Strait of Sicily area (Campillo 1994). During following decades, fishing activity extended in terms of time, space and effort and newer areas were explored to evaluate the potential of the resource (e.g. Algeria, Tunisia, Albania, Greece; Bianchini & Ragonese 1994). As the number of catches reached a steady state (Figure 10), the tendency of trawling vessels was to increase the power and to improve the technological equipment which has provoked

fluctuations in the number of landings and situations of fully exploitation to overexploitation (Fiorentino 2000).

In the necessity to manage fisheries resources, the Food and Agricultural Organization (FAO) created the General Fisheries Commission for the Mediterranean (GFCM) which entered into force in 1952. The GFCM is the regional body to promote the development, conservation, rational management and best utilization of living marine resources, as well as the sustainable development of aquaculture in the Mediterranean, Black Sea and connecting waters (FAO Statistical Area 37). Matters concerning deep-sea fisheries are studied through Subcommittees (COPEMed, Adriamed, EastMed, and MedSudMed) and Working Groups (e.g Working Groups on Demersal Species, Stock Assessment Methodologies). The Mediterranean Sea is divided into Geographical Sub-Areas (GSAs, Res. GFCM/31/2007/2; Figure 11) for specific evaluation of the resources. *A. antennatus* and *A. foliacea* were included in the priority species list in 2006 for assessment and management purposes in the Mediterranean (GFCM SAC 2006).

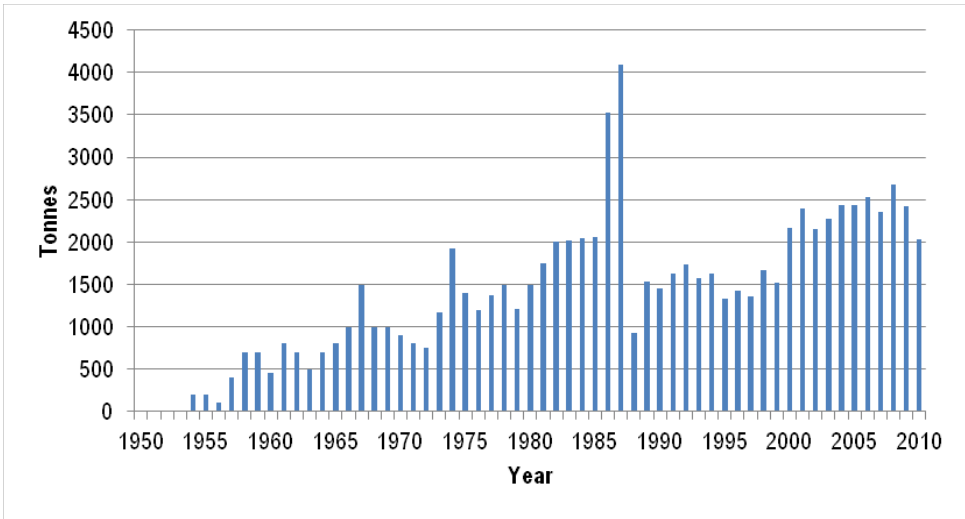


Figure 10. *Aristeus antennatus* total fishing captures between 1954 and 2009, according to FAO. Some years the number of captures was estimated and in certain regions of the Strait of Sicily, often *A. antennatus* and *A. foliacea* figures are reported together (FAO, species fact sheet – http://www.fao.org/fishery/species/3422/en).

Within the Mediterranean, *A. antennatus* is mostly captured in the Western Mediterranean (GSA 1, 5, 6, 9, 10, 11) and secondly in the Strait of Sicily (GSA 15, 16) and Ionian Sea (GSA 19, 20) whilst *A. foliacea* is mostly harvested in the Strait of Sicily and Western Sardinia (GSA 11, 15, 16) with

small appearance along the Spanish coasts (GSA 5, 6) (Bensch et al. 2008). The main countries that go after these resources are Spain and Italy, and secondly, Algeria, Tunisia and Albania (Bensch et al. 2008). At present fishing activity is almost exclusively artisanal in structure, The exploitation of red shrimps is usually carried out by specialized trawlers that operate near the shore and perform daily trips to the shelf or upper slope (600-800 m depth) (Sardà et al. 2004b) except for some areas (e.g. the Sicilian Channel) where large trawlers operate on a more industrial scale (Sardà et al. 2004b).

The average trawling fleet in the Mediterranean Sea for blue and red shrimp is 21 m long which has an engine power of 243 kW and a Gross Register Tonnage (GRT) of 66 tons (Sardà 2000). Exceptions occur in GSA 16 where trawlers from Mazara del Vallo go to Aegean Sea and Levant Sea due to the reduction in catches in the Strait of Sicily since 2004 (GFCM 2010) which implies fleets have to be larger with refrigerator chambers. The exploitation of red shrimps in Greek waters of the Ionian Sea (GSA 20) by greek fleet has not yet been developed as the commercial trawl fishing is traditionally exercised no deeper than 400-500 m, partly due to the lack of engines and experience of fishermen (Mytilineou et al. 2006) and partly as a consequence of the narrow and steep grounds (Politou et al. 2004); however, these authors also say that potential for exploitation exists and some occasional exploitation has begun. Whenever possible, the fleet specializes in one or other species, but in those areas where they co-occur they are reported together in catch reports (Sardà et al. 2004b; Bensch et al. 2008).

Assessment of stocks has been conducted in most of its exploitation areas, although data is not updated for all GSAs, especially those that have been characterized as overexploited long ago. In 2002 stock assessments for *A. antennatus* in Northern Alborán Sea (GSA 1), Northern Spain (GSA 6) and Balearic Islands (GSA 5) pointed at stocks being fully exploited (GFCM 2002). By 2004, GSA 1 was described as slightly overexploited (GFCM 2004) whilst GSA 5 and GSA 6 were determined overexploited by 2006 (GFCM 2006) and have remained so ever since (GFCM 2008, 2011). GSA 1 and GSA 6 landings fluctuated from 300 to 400 tonnes (tn) per year between 1996 and 1999, and until 2006 landings were around 300 tn per year, for what scientific advise recommended not to increase the fishing effort but to implement a reduction of 10%. By 2011, a

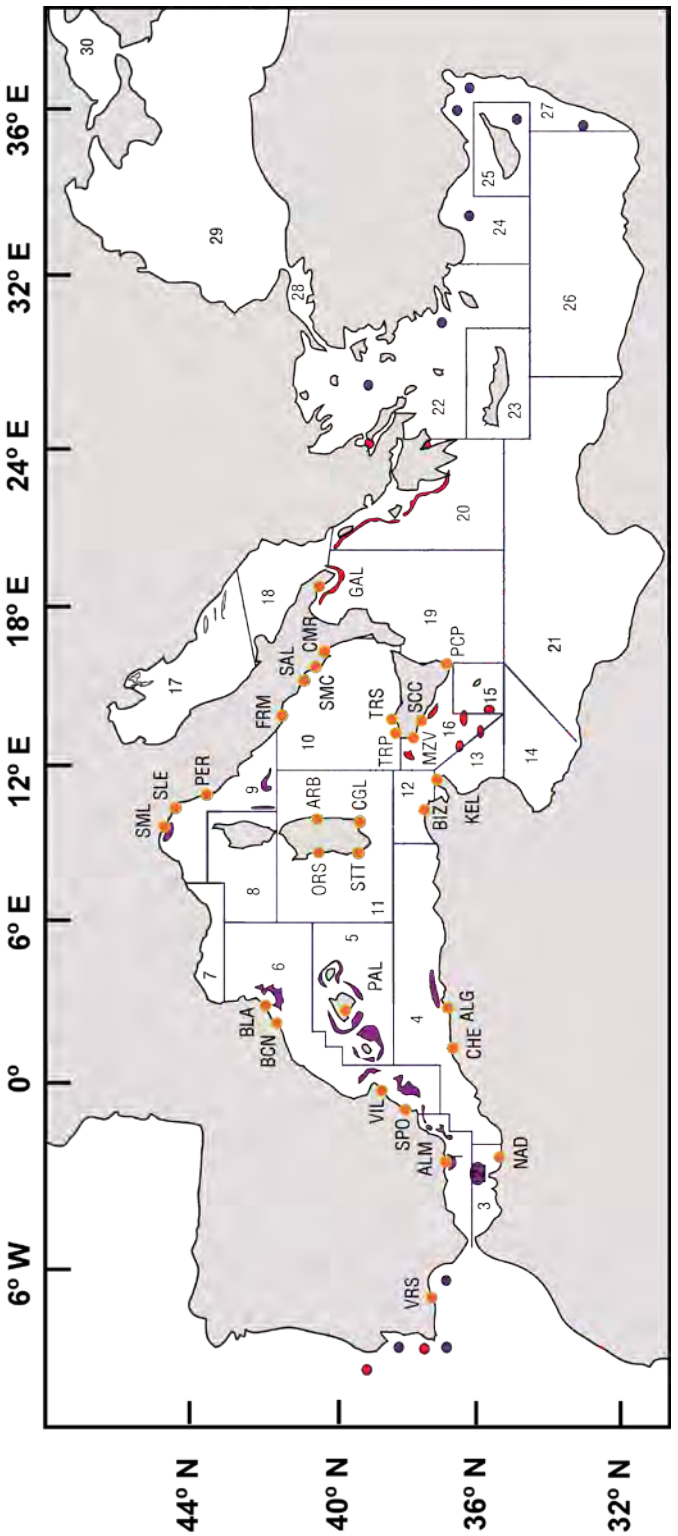


Figure 11. Map of Mediterranean Sea with indication of the main red shrimps harvesting areas (in red giant red shrimp, in purple blue and red shrimp), as well as Geographical Sub Areas (1-29) according to GFCM and main fishing ports (orange dots) targeting red shrimps (Sardà 2000). Ports abbreviations: ALG - Algeria, CHE - Cherchell, NAD - Nador, VRS – Vila Real de Santo Antonia, ALM – Almeria, SPO – Santa Pola, VIL – Vila Joiosa, PAL – Palma, BCN – Barcelona, BLA - Blanes, SML – Santa Margherita Ligure, SLE – Sestri Levante, PER – Porto Ercole, FRM - Formia, SAL - Salerno, SMC – S. Maria di Castellabate, CMR – Marina di Camerota, TRS - Terrasini, TRP - Trapani, ORS – Orisiano, STT – Sant'Antionico, CGL – Cagliari, ARB – Arbatax, MZV – Mazara del Vallo, SCC - Sciaca, PCP – Portopalo di Capopassero, GAL – Gallipoli, BIZ - Bizerte, KEL - Kelibia.

reduction of 30-50% of fishing effort was recommended for GSA 5, where annual landings are around 150 tn since 2000 (GFCM 2011). *A. antennatus* in Ligurian Sea (GSA 9) was considered overexploited in 2010 (GFCM 2010). The waters around Sardinia (GSA 11) hold both red shrimps, *A. antennatus* and *A. foliacea*, but exploitation is not even all around for what only certain areas were considered overexploited in 2004 (GFCM 2004). Stock assessments specifically referring to *A. foliacea* only report on the strait of Sicily (GSA 15, 16) and the species was considered to be overexploited in 2009 (GFCM 2009) and in 2010 a 30% reduction on fishing effort was advised (GFCM 2010).

Outside the Mediterranean Sea, *A. antennatus* was registered for first time in Portuguese coasts in 1968 (dos Santos & Cascalho 1994) and became part of Portuguese trawl fishery. However, in 1994 catches had reduced by one third in four years (Cascalho & dos Santos 1994), reaching overexploitation status (Figuereido et al. 2001). In North-Western Australia, *A. foliacea* was found on commercial quantities in 1985 with a maximum of 420 tn in 1987 (Wadley 1994). However, a substantial decline in catch rates was detected between 1987 and 1992, until a situation of compete stock depletion in 1993; it now appears as by-catch (Samplakis et al. 2010). In the Mozambique Channel both red shrimps are harvested by industrial fisheries that consist of a joint venture between the government of Mozambique and foreign companies from Japan, Portugal and Spain, which make up for more than 70% of total allowable catch (TAC) (FCP 2007). Recently, new fishing grounds for *A. foliacea* have been detected off Brazil (Dallagnolo et al. 2009), Mexico (Gracia et al. 2010) and Colombia (Paramo & Saint-Paul 2011), but no directed fisheries have yet been established.

Current state of Aristeidae genetics' knowledge

Scarce studies have look at the population genetics of *A. antennatus*, but there is no published literature about population genetics of *A. foliacea*. The first genetic work to address the population structure of *A. antennatus* consisted in an allozymic and morphometric analysis of samples collected across the Mediterranean Sea and adjacent Atlantic waters (Sardà et al. 1998) (Figure 12). No genetic divergence was detected among localities, not even among Western Mediterranean, Eastern Mediterranean and Atlantic Ocean, but significant morphological differences were observed

(Figure 12). Thus, the morphological differences were attributed to phenotypic plasticity of the species (Sardà et al. 1998) to the hydrological and ecological characteristics of the three regions studied (Sarà 1985).

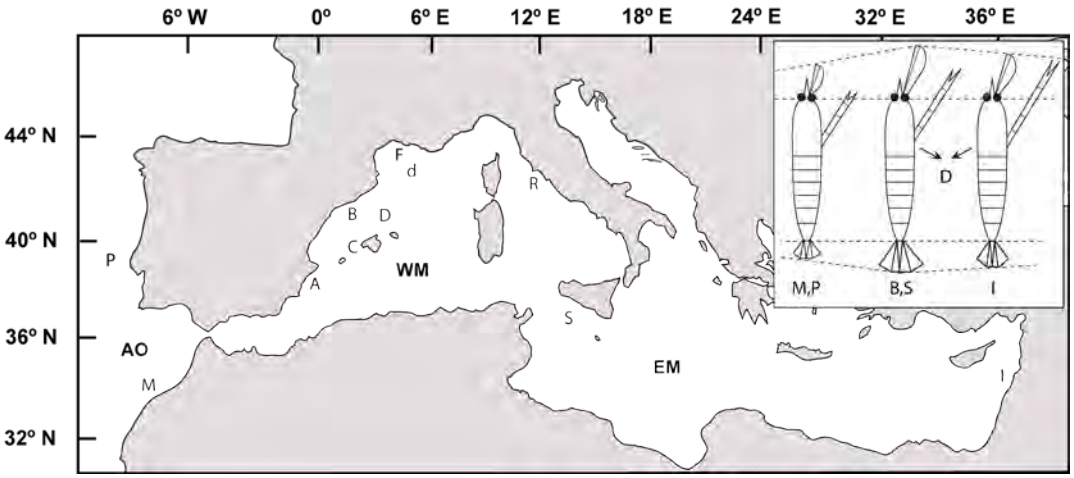


Figure 12. Map of the Mediterranean Sea with sampling locations of Sardà et al. (1998) for *Aristeus antennatus* with conceptual diagram of morphometric differences between sampled locations (proportions not drawn to scale). P - Portugal; M - Morocco; A – Alicante, B – Barcelona, D – Barcelona deep sample (> 1000 m), C – Palma, F – Marseille, d – Marseille deep sample (> 1000 m), R – Rome, S – Mazara, I – Israel. AO - Atlantic Ocean, WM - Western Mediterranean, EM - Eastern Mediterranean.

A decade after Sardà et al. (1998)'s work, several authors have addressed the population genetics of *A. antennatus* in the Western Mediterranean Sea, by means of different molecular techniques (Figure 13). First, Roldán et al. (2009) analyzed the genes codifying for 16S rRNA and COI, then Maggio et al. (2009) studied the variability of the mitochondrial control region (CR), and finally microsatellites were developed and applied by Cannas et al. (2008, 2011). All these studies detected an absence of genetic divergence within the Western Mediterranean basin. Roldán et al. (2009) indicated that both pelagic larvae and adult migration may account for the low levels of genetic divergence observed between localities distant more than 500 kms apart. Maggio et al. (2009) suggested that the absence of deep-sea barriers also contributed to the large genetic homogeneity. Cannas et al. (2011) further hypothesized that adult gene flow was mostly sustained by female dispersal whose migration is facilitated by the Levantine Intermediate Waters.

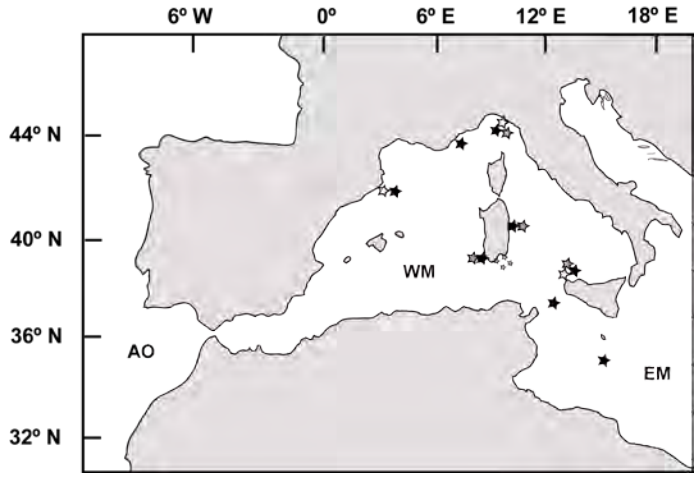


Figure 13. Map of the Mediterranean Sea with sampling locations by Roldán et al. (2009) in white, Maggio et al. (2009) in black and by Cannas et al. (2011) in grey. AO - Atlantic Ocean, WM - Western Mediterranean, EM - Eastern Mediterranean.

A different approach was attained by Sardà et al. (2010) who tried to see whether the fishing and virgin grounds presented genetic differences, using 16S rRNA mitochondrial marker. The analysis detected high levels of genetic homogeneity among four bathymetrical samples (350, 700, 1100, 1500 m depth) of *A. antennatus* from off Barcelona (Spain), giving reason to believe that deeper-dwelling stocks are not isolated from exploited stocks, in agreement with previous hypothesis by Sardà et al. (2003). It was suggested that *A. antennatus* in the Catalan Sea was more likely to be organized in a metapopulation-like structure (Levins 1969), i.e. as a network of local populations connected by different degrees of gene flow, a portion of which faces substantial likelihood of extinction (sinks) only that it is maintained by organisms coming from neighbouring areas (sources). Accordingly, the shallow-dwelling harvested stocks can be considered sinks while deeper-dwelling stocks are sources (Sardà et al. 2010) that would be exerting a "rescue effect" (Pulliam 1988) on the shallower groups threatened by fisheries pressure and environmental disturbances like major cascading events (Company et al. 2008).

From a macroevolutionary point of view, the phylogenetic position of *A. antennatus* and *A. foliacea* within the Aristeidae family has never been specifically addressed. Only once, *A. foliacea* was included as part of a major study looking at the phylogeny of Penaeoidea (Ma et al. 2009) (Figure 14). Ma et al. (2009) found the divergence of penaeid-like and aristeid-like species in the

late Permian (~ 250 Mya). Since fossil records indicate that Paleozoic crustaceans predominantly inhabited shallow marine environments in the tropical Laurentia region, it is likely that penaeoid species had a shallow origin in Laurentia from which the aristeid-like shrimps evolved to offshore environments and the Aristeidae family started radiation during the late Cretaceous (~ 73 Mya) (Ma et al. 2009).

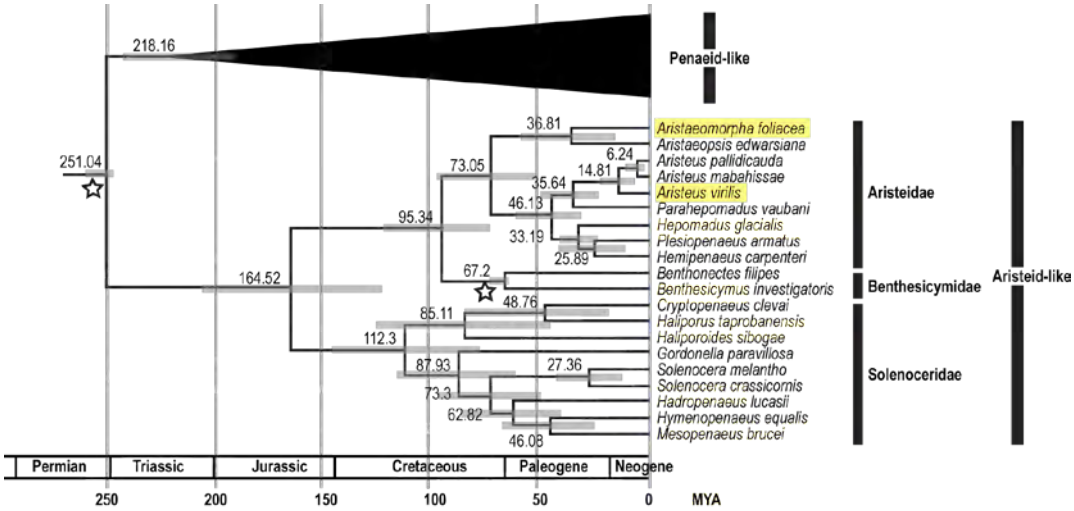


Figure 14. Phylogenetic tree of aristeid-like species (*Aristaeomorpha foliacea* and *Aristeus virilis* highlighted) showing molecular divergence estimates in million years based on a relaxed phylogenetic analysis of combined PEPCK and NaK sequence data. Grey bars show 95% credibility intervals and posterior mean age adjacent to each node. Stars near nodes indicate fossil calibration points (extracted and modified from Ma et al. 2009).

Objectives

Previous knowledge on the genetics of *Aristeus antennatus* and *Aristaeomorpha foliacea* is scarce or inexistent, despite their high economic importance. However, genetic data can bring important information to management measures and conservation strategies of species with commercial interest. Therefore, the aim of this work is to expand the genetic knowledge of these species whilst trying to understand the biological and ecological rationale behind the patterns found. To do so, several molecular markers have been developed to accomplish the following specific objectives:

1. To estimate genetic variability and genetic divergence in *A. antennatus* across a wide distributional range, with special emphasis in the Mediterranean Sea in order to
    a. infer intraspecific genealogy
    b. identify barriers to gene flow
    c. contribute to the definition of stocks based on genetic criteria
2. To estimate genetic variability and genetic divergence in *A. foliacea* across a wide distributional range, with special emphasis in the Mediterranean Sea in order to
    a. infer intraspecific genealogy
    b. identify barriers to gene flow
    c. contribute to the definition of stocks based on genetic criteria
3. To compare the phylogeographycal patterns of *A. antennatus* and *A. foliacea* in order to
    a. infer the evolutionary history of these species and evaluate the role played by present and past factors
    b. contribute to the phylogeny of Aristeidae by inferring the phylogenetic relationship between *A. antennatus*, *A. foliacea* and *Aristeus virilis*

"The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them"

Sir William Bragg (1862 - 1942)

RESULTS

The main results obtained from this work are presented in the form of four scientific papers, according to the objectives previously stated. Scientific papers (articles) are presented from microevolution (population genetics) scale of each of both species to macroevolution scale (comparative phylogeography), which coincide with chronological order of publication.

In the first article, the population genetics of the most economically important red shrimp in the Mediterranean Sea, *Aristeus antennatus*, were examined extending the work by Roldán et al. (2009). Mitochondrial molecular markers (16S rRNA and COI) were used to analyze eleven main fishing grounds along Mediterranean Sea, adjacent Atlantic waters and Indian Ocean. This work was the first one to reveal signals of genetic structure in *A. antennatus* and to identify the hydrographical

processes and geographical barriers which cause restriction to gene flow, providing useful data for its management.

The second article is the result of a five months research placement at the *Centro Richerche Ambiente Marino S. Teresa* - ENEA (La Spezia, Italy) where the population genetics genetic *Aristaeomorpha foliacea* were addressed for first time. Because no previous genetic studies had ever been conducted in this species, the analysis was conducted using ISSR markers. The analysis of six Mediterranean localities, from Western and Eastern basins, and one sample from the Mozambique Channel revealed the existence of high levels of gene flow and no evidence of genetic structure, which was attributed to the to hypervariability of the marker employed together with the high potential for larval and adult dispersal of the species.

Given the informative results obtained for *A. antennatus* using mitochondrial markers (Article I) and the lack of genetic structure detected in *A. foliacea* with ISSRs over a similar geographical scale (Article II) it was decided to readdress the population structure of *A. foliacea* using COI as molecular marker. Then, in the third article, specific primers for *A. foliacea* were designed and genetic analysis was conducted on eighth localities, seven previously analyzed with ISSRs and one from North-Western Australia. Our results indicated a significant level of genetic differentiation and the existence of three mitochondrial lineages geographically restricted to the Mediterranean Sea, the Mozambique Channel and North-Western Australia. Also restriction to gene flow was detected within Mediterranean Sea.

Under the sight of genetic results obtained within species level for *A. antennatus* (Article I) and *A. foliacea* (Article III) it was opportune to focus on the evolutionary change that occurs at or above level of species. In the last article (Article IV) a novel approach was conducted using multilocus (mitochondrial: COI; nuclear: PEPCK and NaK) analysis in order to seek the comparative phylogeography. Only one lineage of *A. antennatus* without geographical pattern was revealed, but a deep evolutionary split at species level was detected in *A. foliacea*, with consequent implications on taxonomy and conservation.

Article I - Genetic structure in the blue and red shrimp, *Aristeus antennatus*, and the role played by hydrographical and oceanographical barriers

Fernández MV, Heras S, Maltagliati F, Turco A, Roldán MI
Marine Ecology-Progress Series (2011) 421:163-171
doi:10.3354/meps08881

Fernández M.V., Heras S., Maltagliati F., Turco A., Roldán M.I. “Genetic structure in the blue and red shrimp *Aristeus antennatus* and the role played by hydrographical and oceanographical barriers”. *Marine ecology progress series*. Vol. 421 : p. 163-171

Online publication date: January 17, 2011

http://dx.doi.org/10.3354/meps08881

http://www.int-res.com/abstracts/meps/v421/p163-171/

ABSTRACT:

The blue and red shrimp *Aristeus antenantus* supports an important commercial fishery in the Western Mediterranean, adjacent Atlantic waters and Mozambique Channel (western Indian Ocean). This study investigates its genetic structure by examining a total of 506 individuals from Mediterranean, Atlantic and Indian Ocean locations. In order to identify putative genetic stocks, sequences from 16S rDNA (546 bp) and COI (514 bp) genes were used. Genetic diversity, estimated by haplotypic and nucleotidic diversity, was lower in the Western Mediterranean than in samples from other locations. The high haplotypic diversity of the Eastern Mediterranean, Atlantic and Indian Ocean samples reflects the occurrence of a number of private haplotypes, which are also responsible for significant genetic divergence between these samples and the Western Mediterranean ones. The analysis of mismatch distributions, neutrality tests, and star-like patterns present in the network of haplotypes provided consistent inference of past population expansion in the Western Mediterranean, Atlantic and Mozambique Channel regions. Our study provides the first evidence of genetic structuring in *A. antennatus* across its distributional range.

KEY WORDS:

Aristeus antennatus · COI · 16S rDNA · Genetic structure · Atlantic Ocean · Mediterranean Sea · Western Indian Ocean

Article II - Analysis of genetic variability in *Aristaeomorpha foliacea* (Crustacea, Aristeidae) using DNA-ISSR (Inter Simple Sequence Repeat) markers

Fernández MV, Maltagliati F, Pannacciulli F, Roldán MI

Comptes Rendus Biologies (2011) 334:705-712
doi: 10.1016/j.crvi.2011.07.005

Maria Victoria Fernández, Ferruccio Maltagliati, Federica G. Pannacciulli, Maria Inés Roldán. “Analysis of genetic variability in *Aristaeomorpha foliacea* (Crustacea, Aristeidae) using DNA-ISSR (Inter Simple Sequence Repeats) markers”. *Comptes rendus biologies.*Vol. 334, October 2011 : p. 705-712

http://dx.doi.org/10.1016/j.crvi.2011.07.005,

http://www.sciencedirect.com/science/article/pii/S1631069111001983

Abstract

This work reports the first genetic data of *Aristaeomorpha foliacea*, a marine decapod of high commercial value, from six Mediterranean localities and one new fishing ground in the Mozambique Channel. The use of five Inter Simple Sequence Repeat (ISSR) primers provided 150 polymorphic loci. Average estimates of genetic diversity did not significantly differ among sampled localities, with a mean value of heterozygosity $H = 0.105 \pm 0.015$. Analysis of molecular variance (AMOVA) allocated > 98% of genetic variability to the within-sample component, displaying values higher than those previously reported in ISSR studies on marine invertebrates. Cluster analyses did not detect geographically or genetically distinct groups. The observed lack of large-scale genetic differentiation is discussed in relation to the high potential for larval dispersal of the species and to features of the marker employed.

Keywords

- Marine decapod;
- *Aristaeomorpha foliacea*;
- Genetic variability;
- Gene flow;
- DNA-ISSRs

Article III - Deep genetic divergence in giant red shrimp *Aristaeomorpha foliacea* (Risso, 1827) across a wide distributional range

Fernández MV, Heras S, Maltagliati F, Roldán MI
Journal of Sea Research (2012) xxx:xxx-xxx
doi: 10.1016/j.seares.2012.08.004

M.V. Fernández, S. Heras, F. Maltagliati, M.I. Roldán.” Deep genetic divergence in giant red shrimp *Aristaeomorpha foliacea* (Risso, 1827) across a wide distributional range”. *Journal of sea research.* Available online 14 August 2012

In Press, Corrected Proof — Note to users

http://dx.doi.org/10.1016/j.seares.2012.08.004,

http://www.sciencedirect.com/science/article/pii/S138511011200127X

Abstract

The giant red shrimp, *Aristaeomorpha foliacea*, is a commercially important species in the Mediterranean Sea (MED), Mozambique Channel (MOZ), and north western Australia (AUS). 685 bp of the mitochondrial COI gene was sequenced in 317 individuals from six Mediterranean and two Indian Ocean localities. Genetic diversity estimates of Indian Ocean samples were higher than those of MED counterparts. AMOVA, phylogenetic tree, haplotype network and Bayesian assignment analyses detected three haplogroups, corresponding to MED, MOZ and AUS, separated by three and 38 mutational steps, respectively. Within MED shallow genetic divergence between populations was dependent on local oceanographical characteristics. Mismatch distribution analysis and neutrality tests provided a consistent indication of past population expansion in each region considered. Our results provide the first evidence of genetic structure in *A. foliacea* and suggest a scenario of allopatric speciation within the Indian Ocean that, however needs deeper examination.

Highlights

► The giant red shrimp is an economically important marine resource. ► High levels of genetic diversity were detected in all regions considered. ► Three highly divergent mtDNA lineages were detected, suggesting allopatric speciation. ► Divergence is consistent with current hydrographical and oceanographical barriers.

Keywords

- Mitochondrial Genetic Diversity;
- Allopatric Divergence;
- Fisheries;
- Mediterranean Sea;
- Indian Ocean

Article IV - Comparative phylogeography of two Aristeid shrimps of high commercial interest (*Aristeus antennatus* and *Aristaeomorpha foliacea*) using nuclear and mitochondrial markers

Fernández MV, Heras S, Viñas J, Maltagliati F, Roldán MI

Accepted by PLoS One with minor revisions the 6th September 2012

Comparative phylogeography of two Aristeid shrimps of high commercial interest (*Aristeus antennatus* and *Aristaeomorpha foliacea*) using nuclear and mitochondrial markers

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Funding: This work was supported by a grant from Ministerio de Educación y Ciencia, Spain (CTM2006-00785) and a grant from Ministerio de Ciencia e Innovación, Spain (AGL2009-09228) to MIR. MVF benefited from a predoctoral fellowship from Ministerio de Educación y Ciencia, Spain (BES-2007-15865).

Competing interests: The authors have declared that no competing interests exist.

Abstract

Phylogeographical studies can reveal hidden patterns of the evolutionary history of species. Comparative analyses of closely related species can further help to disentangle the relative contributions of processes responsible for such patterns. In this work, the phylogeography of two aristeid species, *Aristeus antennatus* and *Aristaeomorpha foliacea*, was approached by multiple genetic markers. *Aristeus antennatus* and *Aristaeomorpha foliacea* are two marine shrimp species of high commercial importance that are exploited in the Mediterranean Sea (MED) and in Mozambique Channel (MOZ), where they occur in partial sympatry. 50 individuals of *Aristeus antennatus* from Western and Eastern Mediterranean (WM and EM, respectively), Atlantic Ocean (AO) and MOZ, and 40 individuals of *Aristaeomorpha foliacea* from WM, EM, MOZ North-Western Australia (AUS) were analyzed with two nuclear (PEPCK and NaK) and one mitochondrial (COI) genes. Within the study area, where the two species co-occur, differences were found between their phylogeographical patterns, suggesting that intrinsic response to environmental changes played different roles in the two species. Only one major *Aristeus antennatus* lineage was found across its distributional range. Instead, a deep evolutionary split within *Aristaeomorpha foliacea* was observed in which genetic diversity followed a geographical pattern associated to MED-MOZ and AUS. AUS lineage of *A. foliacea* deserves to be considered as a distinct species, with consequent implications in taxonomy and resource management.

Keywords: *Aristeus antennatus*, *Aristaeomorpha foliacea*, *Aristeus virilis*, compared phylogeography, PEPCK, NaK, COI, sequencing, Mediterranean Sea, Indian Ocean, new species.

Introduction

Aristeid shrimps (Aristeidae, Wood-Mason 1891) are a group of commercially important species within the superfamily Penaeoidea (Rafinesque 1815), which are also known as red shrimps because of their body coloration [1]. *Aristeus antennatus* (Risso, 1816) and *Aristaeomorpha foliacea* (Risso, 1827) are the two most economically valuable species of the Aristeidae family. They occur in sympatry in large part of their geographical and bathymetrical distribution. Bathymetrically, *Aristeus antennatus* can be found between 80 [2] and 3300 m depth [3], whilst *Aristaeomorpha foliacea* is distributed between 120 and 1000 m depth [4] with a maximum abundance for both species between 400 and 800 m depth [3, 4]. *Aristeus antennatus* is distributed in the Mediterranean Sea and adjacent Atlantic Ocean, from Portugal to Cape Verde Islands [5]. In the Indian Ocean, its distribution is restricted to the Maldives Islands, Zanzibar Island, Mozambique and South Africa (Freitas 1985). *Aristaeomorpha foliacea* is found in the same geographical range of *Aristeus antennatus*, but in the Indian Ocean its distribution is much wider, reaching North-Western Australia coastal waters [6]. Since 1930s both shrimp species have been harvested in the Mediterranean Sea, constituting an important directed fishery that nowadays represents the 30% income of many local Mediterranean ports [7]. Moreover, a second major area of exploitation for red shrimps has been recently established in the Mozambique Channel [8]. Other minor harvesting areas exist off the coast of Portugal for *Aristeus antennatus* [9] and off North-Western Australia for *Aristaeomorpha foliacea* [6].

Despite the high economic value of both species, little is known about their biology, ecology and population genetics outside the Mediterranean Sea. In addition, information on phylogenetic relationships within Aristeidae and with other Penaeoidea families is very scarce. Only two surveys included aristeid species as a part of reviews on the phylogeny of Decapoda (*Aristeus virilis*), [10] and the phylogeny of Penaeoidea (*Aristaeomorpha foliacea*) [11]. From a population genetic perspective, a number of studies on *Aristeus antennatus* and *Aristaeomorpha foliacea* were consistent in showing a general picture of relative genetic homogeneity within the Mediterranean Sea [12-16]. Instead, mitochondrial genetic analysis including also extra-Mediterranean samples detected moderate to high levels of genetic differentiation in both species [17, 18]. For example, genetic analysis of *Aristeus antennatus* based on mtDNA markers detected significant genetic differentiation among Western Mediterranean, Eastern Mediterranean, Atlantic Ocean and Mozambique Channel [17]. Similarly, genetic analysis of *Aristaeomorpha foliacea* detected three mtDNA monophyletic lineages corresponding to Mediterranean Sea, Mozambique Channel and North-Western Australia [18].

Given that these two shrimp species are taxonomically closely related and partially sympatric, with large similarities in adult ecology and reproductive biology [19], they are ideal candidates for a study of comparative phylogeography. This kind of studies can provide sound information on the origin and evolutionary history of species and help in the identification of evolutionary isolated areas, altogether providing information on conservation strategies [20]. Phylogeographical analyses should be based on multiple loci of different nature, because different genes may be responding idiosyncratically to the evolutionary forces operating upon populations [21]. One of the major drawbacks of these studies [17, 18] is that they were conducted using mtDNA which partially reflects the evolutionary history of the species. Given the low number of genetic studies conducted

on these two species, in this work we have used available molecular markers from literature which have proven satisfactory within the studies these were employed. Consequently, COI was chosen as mitochondrial marker and, phosphoenolpyruvate carboxykinase (PEPCK) and sodium–potassium ATPase α -subunit (NaK) were chosen as nuclear markers. PEPCK and NaK participate in fundamental cellular functions in the animal kingdom and are well-conserved throughout evolution [10]. PEPCK codifies for the enzyme that catalyzes the first step of gluconeogenesis, interconverting oxaloacetate and phosphoenolpiruvate in organisms ranging from bacteria to human [10, and references therein]. NaK codifies for the sodium-potassium ATPase α-subunit that when binded with the β-subunits forms an heterodimer responsible for maintaining electrochemical potential differences across cell membranes which is essential for cell signalling and secondary transport [10 and references therein]. The objective of this study was to compare the phylogeographical patterns of *Aristeus antennatus* and *Aristaeomorpha foliacea*, to determine how these two species have been influenced by present or historical factors. In addition, this work gives an insight into the phylogenetic relationships between *A. antennatus, A. foliacea* and *Aristeus virilis*.

Methods

Sampling

A sub-sample of 50 *Aristeus antennatus* and 40 *Aristaeomorpha foliacea* individuals were selected from previous works based on mtDNA [17, 18]. Selection of individuals was performed according to two criteria: i) each different putative biogeographical region had to be represented by 10 individuals, ii) maintenance of previous levels of within locality mitochondrial genetic variability (Table S1 and Table S2). Biogeographical regions selected for *Aristeus antennatus* [17] were Alborán Sea (ALB), Western Mediterranean (WM), Eastern Mediterranean (EM), Atlantic Ocean (AO) and Mozambique Channel (MOZ) (Table 1); Gulf of Lion was selected as representative of WM and Ionian Sea as representative of EM. The biogeographical regions selected for *Aristaeomorpha foliacea* [18] were WM, EM, MOZ and North-Western Australia (AUS); Cabrera was selected as representative of WM and Aegean Sea as representative of EM (Table 1). Finally, three individuals of *Aristeus virilis* were also included in the analysis (collected in Mozambique Channel, MOZ0308 survey by the *Instituto Español de Oceanografia*). Available GenBank sequences for the three studied genes were found only for *Penaeus monodon* (COI: PRJNA11894, PEPCK: EU427213, NaK: EU427144) and *Solenocera crassicornis* (COI: AY264902, PEPCK: FJ441211, NaK: FJ441166), which belongs to the same superfamily (Penaeoidea), and were used as outgroup species in phylogenetic analyses.

DNA extraction, PCR amplification and sequencing

DNA extraction of ethanol-preserved *Aristeus virilis* samples, polymerase chain reaction (PCR) and sequencing of COI followed the procedures outlined in Fernández et al. [18]. Amplification of PEPCK was performed with primers described in Tsang et al. [10]. New primers for NaK amplification were designed based on 48 Penaeoidea sequences available in GenBank [10, 11]. Final primers were NaK-fAr (5'-TGGCTGCCAGTATGSCAAGA-3', for Aristeidae), NaK-rAa (5'-CGGAGGATCAATCATCGACA-3', for *Aristeus* spp.), NaK-rAf (5'-CGGAGGATCAATCATGGACA-3', for *Aristaeomorpha foliacea*). Amplifications for PEPCK and NaK were carried out in a reaction mix containing 1-3 µl of template DNA (25 ng μl^{-1}), 1X PCR reaction buffer, 3mM $MgCl_2$, 200 µM dNTPs,

200 nM of each primer, and 0.03 U of DNA polymerase (Ecotaq, Ecogen) in a 30 µl final volume. The PCR profile for both nuclear genes was as follows: 3 min at 94 ºC for initial denaturation, followed by 35 cycles of 30 s at 94 ºC, 30 s at 57 ºC and 55 ºC annealing temperatures for PEPCK and for NaK respectively, 90 s at 72 ºC with a final extension for 10 min at 72 ºC. Non-template controls were run in all PCRs to ascertain that no cross contamination took place. PCR products were verified on 1% agarose gel with ethidium bromide (0.5 mg/ml). Sequences were cleaned for sequencing by treating with exonuclease I and shrimp alkaline phosphatase [22]. DNA sequencing reactions were carried using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions and read in an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) at the *Laboratori d'Ictiologia Genètica*, *Universitat de Girona*, Spain.

Sequence data analysis

Nucleotide sequences were aligned and edited in SeqScape v2.5 (Applied Biosystems) employing as reference the partial regions of COI, PEPCK and NaK genes from *Aristaeomorpha foliacea* (GenBank accession numbers: BJN676306, FJ441125 and FJ441170, respectively). Final edition and concatenation of the three genes were performed with BioEdit v7.0.4.1 [23]. Ambiguous nuclear positions (i.e. double peaks in the chromatogram, corresponding to putative heterozygote sites) were left unresolved for all subsequent analyses, except for heterozygosity calculations. PHASE algorithm [24, 25], as implemented in DnaSP v5 [26], was used to reconstruct putative alleles of each nuclear gene (coded as Allele 1 and Allele 2 in Table S2).

Genetic variability estimates were calculated on each gene, separately. Haplotype and nucleotide diversity were calculated for COI gene with DnaSP v5, and expected heterozigosity was obtained for PEPCK and NaK genes with the online version of Genepop v4.0.10 [27] after haplotype reconstruction. A partition homogeneity test [28] was carried out with PAUP* v4.0 b10 [29], in order to assess the correctness of using the concatenated dataset for phylogenetic inference. The program jModelTest v0.1 [30] was used to run a hierarchical series of tests based on the Akaike Information Criterion (AIC) to identify the best-fit model of nucleotide substitution for the concatenated dataset of all the species studied considered among 88 models tested. The best model was TrN [31] with $\alpha = 0.566$, $i = 0.563$, and base frequency A = 0.261, C = 0.255, G = 0.215, T = 0.269.

Maximum likelihood (ML) [32] and Neighbor-Joining (NJ) [33] analyses were conducted in PAUP. NJ was based on a maximum likelihood distance matrix; ML was performed with an heuristic search and tree bisection-reconnection branch-swapping algorithm, 100 replicates and as-sis was chosen for sequence addition. Robustness of trees was tested using bootstrap analyses [34] with 1000 replicates. Furthermore, MrModeltest v2.3 [35] was used to estimate best evolutionary model under Bayesian Inference (BI) analysis. The model selected was SYM [36] with $\alpha = 0.691$, $i = 0.576$, and equal base frequencies; successively, a Bayesian phylogenetic tree was constructed with MrBayes v3.1.2 [37] with metropolis-coupled Markov Chain Monte Carlo algorithm. Four replicate runs were carried out with the value of four Markov chains per run for 2×10^6 generations. The chain was sampled every 100 generations to obtain 20 000 sampled trees. The first 5 000 trees (25%) were discarded as the burn-in phase. A final consensus tree with branch length and clade credibility (posterior probability) was generated with the 75% remaining samples.

Analysis of molecular variance (AMOVA) [38] and Φ-statistics for concatenated dataset was conducted with Arlequin v3.5 [39]. Significance of Φ-statistics was estimated by a permutation test with 10 000 pseudoreplicates. This analysis was used to partition genetic variance in the among- and within-sample components for *Aristeus antennatus* and *Aristaeomorpha foliacea* and datasets. Tamura-Nei genetic distances [31], with a gamma distribution (shape parameter = 0.566) and considering the composition bias among sequences of concatenated dataset, were calculated between lineages detected by phylogenetic trees (NJ, ML, BI). Genetic distances of mitochondrial COI and nuclear molecular markers (NaK and PEPCK) were calculated following Tamura & Nei [31] and Kimura 2 parameters [40] models, respectively. Standard errors were obtained after 10 000 replicates. Correction between groups for all genes was calculated (D_A) [41]. All genetic distances were calculated with MEGA v5 [42]. Evolutionary relationships among phylogenetic groups from concatenated and mitochondrial dataset was inferred by constructing a NJ tree after 10 000 replicates. A median-joining network of COI haplotypes was constructed using NETWORK v4.600 [43].

Results

From a total of 50 *Aristeus antennatus*, 42 individuals were successfully sequenced for PEPCK (536 bp) and 27 for NaK (498 bp) gene, providing 5 and 26 different genotypes, respectively (Table S2). From a total of 40 *Aristaeomorpha foliacea*, 39 amplified for PEPCK and 30 for NaK gene, providing 20 and 6 different genotypes, respectively. The analysis of three *Aristeus virilis* provided three different COI (514 bp) haplotypes plus one PEPCK and two NaK different genotypes. Sequences were deposited in GenBank (Table S2). The partition homogeneity test did not reveal incongruence between molecular markers (p = 0.194) allowing their combination for successive analyses. The final concatenated dataset consisted of 55 sequences (23 *Aristeus antennatus,* 30 *Aristaeomorpha foliacea* and 2 *Aristeus virilis*) and the two outgroup sequences that presented 236 parsimony informative sites from a total of 1 548 bp.

Genetic diversity

The comparative analysis of mitochondrial COI gene indicated that *Aristaeomorpha foliacea* presented a total degree of mitochondrial diversity (h = 0.927 ± 0.022) higher than that detected for *Aristeus antennatus* (h = 0.777 ± 0.059). Instead, *Aristeus antennatus* exhibited levels of haplotypic and nucleotidic diversity in MOZ and EM higher than those obtained for *Aristaeomorpha foliacea*. On the contrary, in the WM *Aristaeomorpha foliacea* exhibited haplotype diversity higher than that of *Aristeus antennatus*, although nucleotide diversity values were similar (Table 1). Private haplotypes in all geographical regions were detected for both species (Table S2).

Genetic variability of PEPCK was higher in *Aristaeomorpha foliacea*, which showed 12 different alleles, whilst *Aristeus antennatus* exhibited only four (Table 1 and Table S2). This outcome is also reflected by the heterozigosity values, which are higher in *Aristaeomorpha foliacea* (H_O = 0.62) than in *Aristeus antennatus* (H_O = 0.43) (Table 1). Each species was clearly distinguished by their genotypes. *Aristeus antennatus* did not exhibit region specific genotypes, but *Aristaeomorpha foliacea* presented private genotypes in MED (Af-ph1, Af-ph2, Af-ph3, Af-ph7, Af-ph8, Af-ph12, Af-

ph13, Af-ph16) and AUS (Af-ph20), and some alleles of those genotypes (Allele 11, 12, 13, 16) were private of regions as well (Table S2).

NaK genetic variability was higher in *Aristeus antennatus* than in *Aristaeomorpha foliacea*, as revealed by the higher number of alleles detected in the former species (38 *vs*. 6) (Table 1 and Table S2). However, observed heterozigosity values were higher in *Aristaeomorpha foliacea*, in which all individuals were heterozygotes (H_O = 1), than in *Aristeus antennatus* (H_O > 0.75) (Table 1). Almost each individual of *Aristeus antennatus* exhibited a different genotype, with the exception of genotype Aa-nh3, which occurred in WM and EM; hence, no geographical association could be drawn (Table S2). Instead, relationships between NaK genotypes and geographical origin of samples were detected in *Aristaeomorpha foliacea*; AUS presented a private genotype (genotype Af-nh6, Table S2) due to the presence of a private allele (allele 44, Table S2) and MOZ showed two private genotypes (genotype Af-nh1 and Af-nh5, Table S2), and a private allele (allele 40, Table S2).

Phylogeographical analysis

NJ, ML and BI analyses for concatenated dataset generated identical tree topologies (Figure 1). Two major lineages corresponding to *Aristeus* and *Aristaeomorpha* genera were identified. Within the *Aristeus* lineage, *Aristeus virilis* and *Aristeus antennatus* clustered into exclusively monophyletic lineages. Within *A. antennatus* no clear associations between geographical distribution and sequences obtained was detected. Within the *Aristaeomorpha* clade, two major phylogroups and geographical association of genetic diversity were detected. One corresponding to AUS and the second including MED and MOZ, where MED appears monophyletic (Figure 1).

The lack of a clear geographical pattern in the distribution of genetic diversity in *Aristeus antennatus* was corroborated by the low and non-significant "among samples" component of molecular variance (9.6 %, Φ_{ST} = 0.096, p = 0.079) (Table 2). Conversely, the high levels of genetic divergence detected in *Aristaeomorpha foliacea* (87.4 % of variance among samples, Φ_{ST} = 0.874, p < 0.001) supported the existence of genetic differentiation at regional level (Table 2). The average within-species genetic distance [31] from concatenated dataset was lower for *Aristeus antennatus* (D = 0.0033 ± 0.0008) than that found in *Aristaeomorpha foliacea* (D = 0.0094 ± 0.0015) (Table 3); net genetic distances between *A. foliacea* lineages, MED-MOZ and AUS, (D_A = 0.0226 ± 0.0038) was about the half of genetic distance between *Aristeus* species (D_A = 0.0492 ± 0.0062) (Table 3). Deep divergence of *Aristaeomorpha foliacea* lineages associated to three biogeographical regions was also detected in NJ tree based on genetic distances within and between species of Table 3 (Figure 2a).

Genetic distances from nuclear data (Table S3) among Aristeidae species of this study were higher for NaK (D_A = 0.0304 ± 0.0048 to D_A = 0.1083 ± 0.0105) than for PEPCK (D_A = 0.0056 ± 0.0022 to D_A = 0.0238 ± 0.0044). These values fall within the range previously reported by Ma et al. [11] for the family Aristeidae (NaK D = 0.01-0.099, PEPCK D = 0.002-0.036). Very low genetic distance values were obtained between MED and MOZ regions of *Aristaeomorpha foliacea*, which were then pooled together to estimate the genetic distance between MED-MOZ and AUS regions (NaK D_A = 0.0029 ± 0.0013, PEPCK D_A = 0.0002 ± 0.0001). The values of mitochondrial genetic distance between Aristeidae species (D_A = 0.1143 – 0.1949) were of the same order of magnitude of those values with outgroup species (Table S4). The values of genetic distance between *Aristaeomorpha foliacea* regions MED-AUS (D_A = 0.0690 ± 0.0117) and MOZ-AUS (D_A = 0.0698 ± 0.0117) are

equivalent to the 61% genetic distance between true congeneric species (*Aristeus antennatus* and *Aristeus virilis*, $D_A = 0.1143 \pm 0.0153$); instead, the genetic distance between MED and MOZ *Aristaeomorpha foliacea* geographical regions is equivalent to the 8% ($D_A = 0.0090 \pm 0.0040$) (Table S4).

The median-joining network of haplotypes clearly separated *Aristeus antennatus, Aristeus virilis* and *Aristaeomorpha foliacea* by a large number of mutational steps and connected the three species in a circle (Figure 3). *Aristeus antennatus* consisted of a single network connected to *Aristeus virilis* subnetwork by 52 mutational steps (MSs), whilst *Aristaeomorpha foliacea* presented three subnetworks, each corresponding to one of the regions considered. The subnetwork corresponding to MOZ connected with i) MED subnetwork through four MSs, ii) AUS subnetwork through 32 MSs, and iii) *Aristeus antennatus* through 89 MSs. The subnetwork corresponding to AUS connects with *Aristeus virilis* through 89 MSs.

Discussion

Genealogical concordance is expected to be found among closely related taxa, even more if they share the same habitat and are co-distributed [21]. However, instances of discordance in phylogenetic patterns among co-distributed closely related marine species have been described [44, 45]. This study provides another example of different evolutionary histories between two partially sympatric species.

Genealogical concordance within species

Phylogeographical analysis of *Aristeus antennatus* showed concordant patterns across genes. Previous mitochondrial genetic analysis detected significant genetic differences between the Mediterranean Sea (MED), the Atlantic Ocean (AO) and the Mozambique Channel (MOZ) [17]. However, the presence of common haplotypes among these regions (Figure 3) indicates that mitochondrial lineage sorting has not been completed with is corroborated by the combined analyses of mitochondrial and nuclear genes carried out in the present ($\Phi_{ST} = 0.096$, $p = 0.079$), highlighting the monophyletic status of *Aristeus antennatus* (Figure 1 and Figure 2). Conversely, phylogeographical analysis of *Aristaeomorpha foliacea* showed discordant genetic partitions across multiple and independent (mitochondrial and nuclear) loci. Previous mtDNA genetic analysis detected three highly differentiated lineages that were geographically characterised: MED, MOZ and North-Western Australia (AUS) [18]. The combined mitochondrial and nuclear markers employed in the present study corroborated that mitochondrial signature of genetic divergence ($\Phi_{ST} = 0.874$, $p < 0.001$), confirming the existence of a clearly differentiated AUS lineage (Figure 1). However, the mitochondrial reciprocal monophyly detected between MED and MOZ (Figure 3) was not fully supported by nuclear markers (Table S3), which placed the individuals of these two regions within the same clade (Figure 1). Incomplete lineage sorting of nuclear markers may account for such results. If a matrilinear tree for two isolated populations has just barely achieved a status of reciprocal monophyly, then about 3x more time is required for a typical nuclear gene to achieve the same status through lineage sorting [46]. Consequently, not enough time would have passed for the mitochondrial monophylies of MED and MOZ to be reflected in the nuclear intraspecific phylogeny.

Genealogical discordance between species

Discordant patterns have been detected within the regions in which *Aristeus antennatus* and *Aristaeomorpha foliacea* co-occur (MED and MOZ). The following hypothesis is advanced: after a vicariant event separated the populations of MED and MOZ of *A. antennatus* and *A. foliacea*, evolutionary forces and/or ecological processes would have had a minor effect on *A. antennatus* than on *A. foliacea*, shaping intraspecific phylogenies differently, as discussed below.

Based on COI genetic distances and using 0.83-1.2% evolutionary rate for COI gene (as reviewed in Ketmaier et al. [47], the divergence between MED and MOZ regions of *Aristaeomorpha foliacea* has been estimated at ca. 500 kyr (Table S4). The Benguela upwelling system is now considered a major barrier for many marine organisms between eastern and western South African coasts [48]. The final closure of the Isthmus of Panama provoked changes in ocean circulation and marked the transition to a period of cold climate worldwide. As a result, the Benguela Current (BC) responded with pronounced upwelling system at 2.1-1.9 Mya with further intensifications during Pleistocene glacial cycles at ~ 0.6 Mya. Consequently, average surface temperature lowered from the 26 ºC in the mid-Pliocene (3.5 Mya) to approximately 18 ºC in modern times [49]. The intensification of the BC upwelling system could have acted as vicariant event causing the disappearance of *Aristeus antennatus* and *Aristaeomorpha foliacea* within its area of influence (between Cape Verde and South Africa) where currently there is no knowledge of their presence [5]. However, because of their relatively thin cuticula, shrimps tend to be underrepresented in the fossil record [1], not allowing to test this vicariant hypothesis.

The reasons why divergence occurred faster in *Aristaeomorpha foliacea* than in *Aristeus antennatus* could be related to differential life-history traits. For example, it has been suggested that *Aristaeomorpha foliacea* would be more sensitive to changes in environmental conditions, due to its higher susceptibility to low levels of dissolved oxygen in the water [50], implying that it might have been more susceptible to Pleistocene climatic changes than *Aristeus antennatu*s. In contrast, because the water column acts as a natural buffer against climatic oscillations, deep water masses remain more stable than superficial waters [51]. Since *Aristeus antennatus* occurs at greater depths than *Aristaeomorpha foliacea*, the former species could have found refugia in deeper waters during glacial cycles, being its populations less affected by environmental changes. Instead, *Aristaeomorpha foliacea* populations would have suffered cycles of reduction and expansion of its populations accelerating the divergent process observed.

Interspecific phylogeny and speciation

This study showed the close relationship between *Aristeus virilis* and *Aristeus antennatus* based on multilocus analyses which is consistent with congeneric species level (Figure 1, Table S3). In contrast, a unique lineage defined *A. antennatus* throughout the study area, which covers most of its spatial distributional range. Instead, two clearly distinguished monophyletic lineages of *Aristaeomorpha foliacea* were detected, whose genetic distance (D_A = 0.0226 ± 0.0038) was almost the half of that detected between true congeneric species (Table 3).

Through the speciation process, divergent lineages suffer changes in different genotypic and phenotypic properties that lead to morphological differentiation, reproductive isolation and ecological differentiation; yet these changes do not all occur at the same time, and they do not even necessarily occur in a regular order [52]. Therefore, when gene flow is restricted between lineages for a long time, reproductive isolating mechanisms (RIMs) and morphological differences will

eventually appear [52]. The fact that the Biological Species Concept (BSC) [53] in this deep-sea marine species cannot be experimentally tested does not imply that both lineages have not developed RIMs. Also, it is well known that decapods and particularly penaeids species present large genetic differences with apparently no morphological variability [54-57]. Palumbi & Benzie [56] proposed a combination of two factors to explain the differences in molecular and morphological evolution in penaeids species: i) an accelerated rate of mitochondrial evolution, ii) a slow rate of morphological divergence due to stabilizing selection on morphological or ecological characters.

Before 1920's there were two recognized species in the genus *Aristaeomorpha*, *A. foliacea* (Risso 1827) from the Mediterranean Sea and Eastern Atlantic, and *A. rostridentata* (Spence Bate 1888) from the Indo-Pacific [58]. Calman [60] compared Spence Bate's holotype of *A. rostridentata* from Fiji Islands with, first, individuals *A foliacea* from the Mediterranean Sea and Atlantic coast of Morocco and, second, individuals of *A. rostridentata* from the Indian Seas (Arabian Sea, Bay of Bengal and Adaman Sea). Calman [58] did not find any single constant morphological difference between *A. rostridentata* holotype and the individuals of *A. foliacea* from the Mediterranean Sea, but Calman [58] did find distinctive morphological differences between *A. rostridentata* holotype and the individuals from the Indian Seas. This led him to consider, first, *A. rostridentata* from Fiji Islands as a synonym of *A. foliacea* and, second, the individuals from the Indian Seas as a distinct species for which he proposed the name *Aristaeomorpha wood-masoni*, after Wood-Mason, Alcock and Kemp, who had already pointed out some of these morphological differences.

The results of Calman [58]'s work in conjunction with the levels of genetic divergence detected in this study between *A. foliacea* from MED-MOZ and AUS (Figure 2) suggests that cryptic species may have been further overlooked in *A. foliacea* and that allopatric speciation is taking place. Calculations of time since divergence based on COI genetic distance indicate that the AUS lineage would have split about 2.88-4.20 Mya (Figure 2b and Table S4). Given that the AUS lineage of *A. foliacea* showed strong support from multilocus genealogical concordance, inhabits in a recognized distinct biogeographical province (North-Western Australia, [59]) and presents advanced levels of multilocus divergence since the split from a parental lineage, we suggest that *A. foliacea* in North-Western Australia should be considered a distinct species. Thus, following the rules of the International Commission on Zoological Nomenclature, we propose to retake *Aristaeomorpha rostridentata* for the Australian lineage. Furthermore, within MED-MOZ lineage *Aristaeomorpha foliacea* presented monophyletic mitochondrial clades that would have split about 0.5-0.3 Mya (Figure 1, Figure 2b and Table S4). These two mitochondrial monophyletic lineages (MED and MOZ) can be considered as evolutionary significant units (ESU) according to the definition given by Moritz [60]: "populations that are reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci". Therefore each of these species and ESUs should be re-evaluated independently in terms of its potential risk of depletion; and management agencies, e.g. FAO, should develop ESU-specific management plans and conservation measures.

Concluding remarks

Conservation of biodiversity mostly relies on the taxonomic unit of species as working tool. Therefore, the correct delimitation of species boundaries is essential; yet it is a difficult task that has been focus of discussion early since Darwin's proposal of the morphological species criterion [61]. In some occasions, new species have been named without morphological or reproductive evidence

claiming to the existence of allopatric monophyletic clades and cripticity. Based on genetic evidence, *Aristaeomorpha foliacea* in North-Western Australia would be regarded as a separate and distinct species. We believe that an in-depth morphological comparison of the distinct lineages is necessary in order to find diagnosable morphological differences, as occurred with other penaeids [57]. Finally, at this moment *A. foliacea* could be considered a cosmopolitan species as punctual records have been recorded in the Western Pacific and Western Atlantic coast [5]. Given the results of this study, we encourage performing genetic and morphological analyses throughout the whole distribution range of the species as similar situations of cripticity may arise.

Acknowledgements

Authors wish to thank A.M. Carbonell, M. Castro, L. Hidalgo, K. Kapiris, C.Y. Politou, I. Sobrino and A. Souplet for their help in sample collection and A. McCallum and C. Rowley, from the Museum Victoria (Melbourne Australia), for providing Australian individuals (Museum Victoria Catalogue numbers J58332, J58318, J58321, J58347, J58334).

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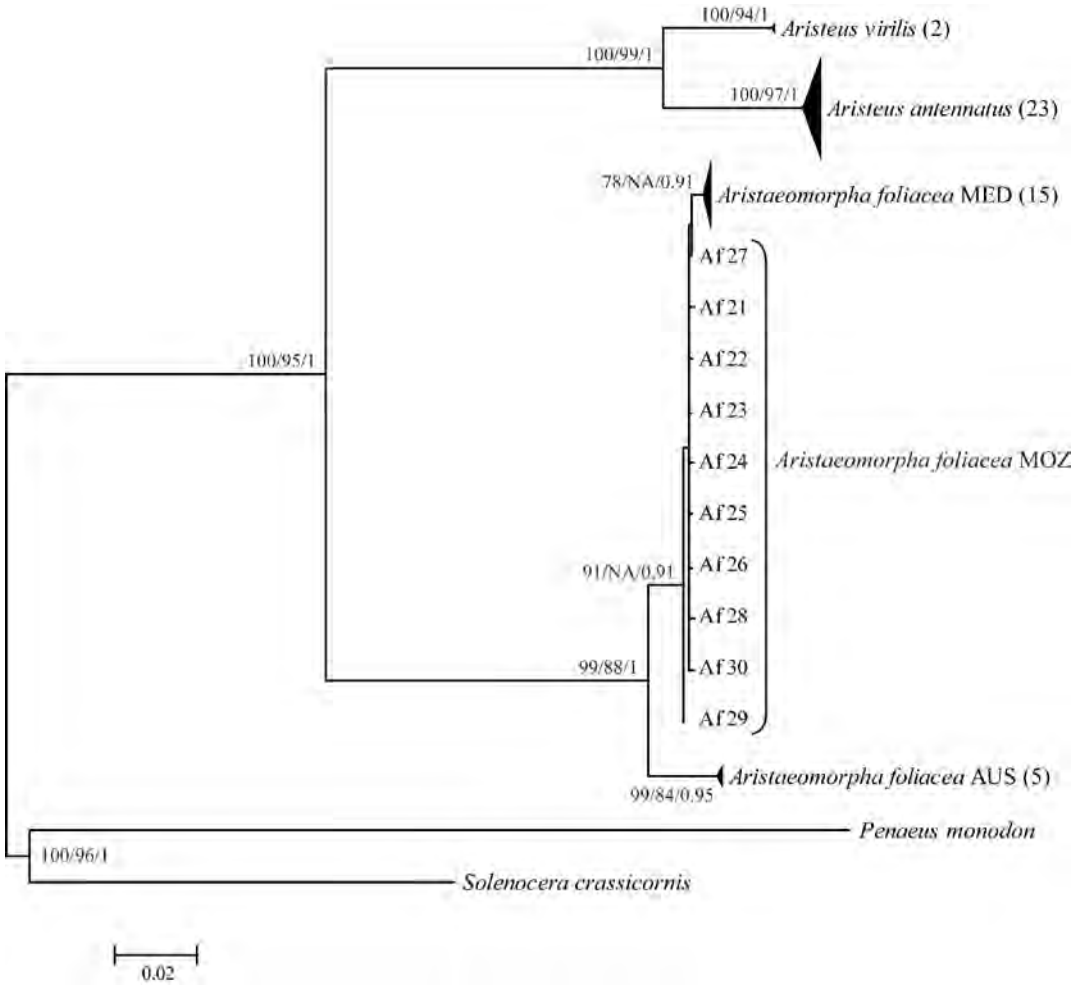

Figure 1. Maximum Likelihood condensed tree based on concatenated dataset. Tree was inferred from 57 sequences of concatenated COI, PEPCK and NaK fragments (1 548 bp), based on 236 parsimony informative sites. *Solenocera crassicornis* and *Penaeus monodon* were used as outgroup. The numbers on nodes indicate bootstrap values for Neighbor-Joining, and Maximum Likelihood trees, and posterior probability values for Bayesian tree, respectively. Triangle sizes are proportional to the number of sequences present in the cluster (number in brackets). Location codes are as in Table 1. NA: not available.

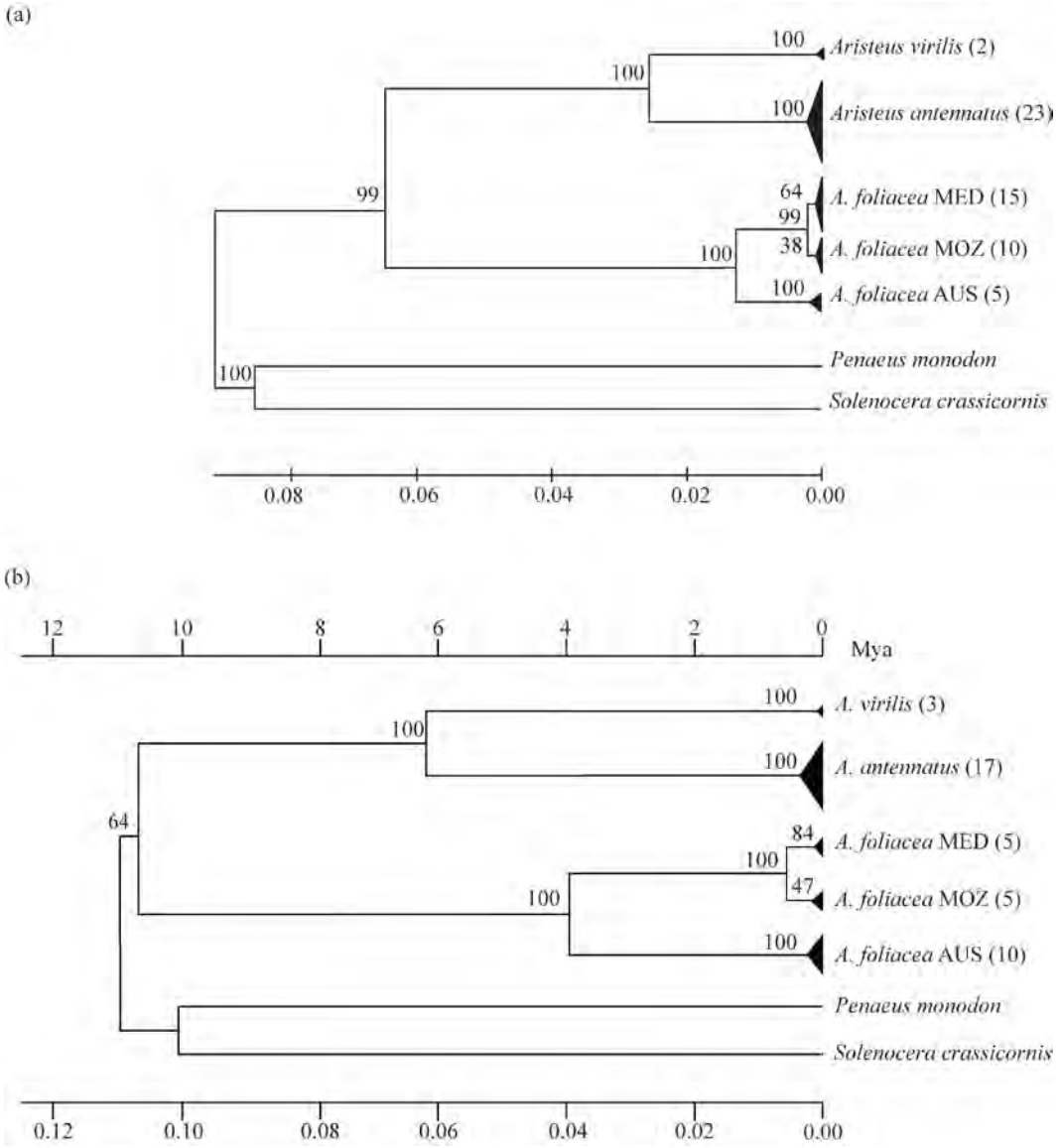

Figure 2. Neighbor-Joining tree based on Tamura-Nei genetic distances. (a) Condensed tree of genetic distances from concatenated loci of Table 3; (b) condensed tree of genetic distances from COI data of Table S4 with bar on top showing estimated time since divergence (Mya) using 1.015% as mean of 0.83-1.2% evolutionary rate. *Solenocera crassicornis* and *Penaeus monodon* were used as outgroups. The numbers on nodes indicate bootstrap values (≥75) after 10 000 replicates. Triangle sizes are proportional to the number of sequences present in the cluster (number in brackets).

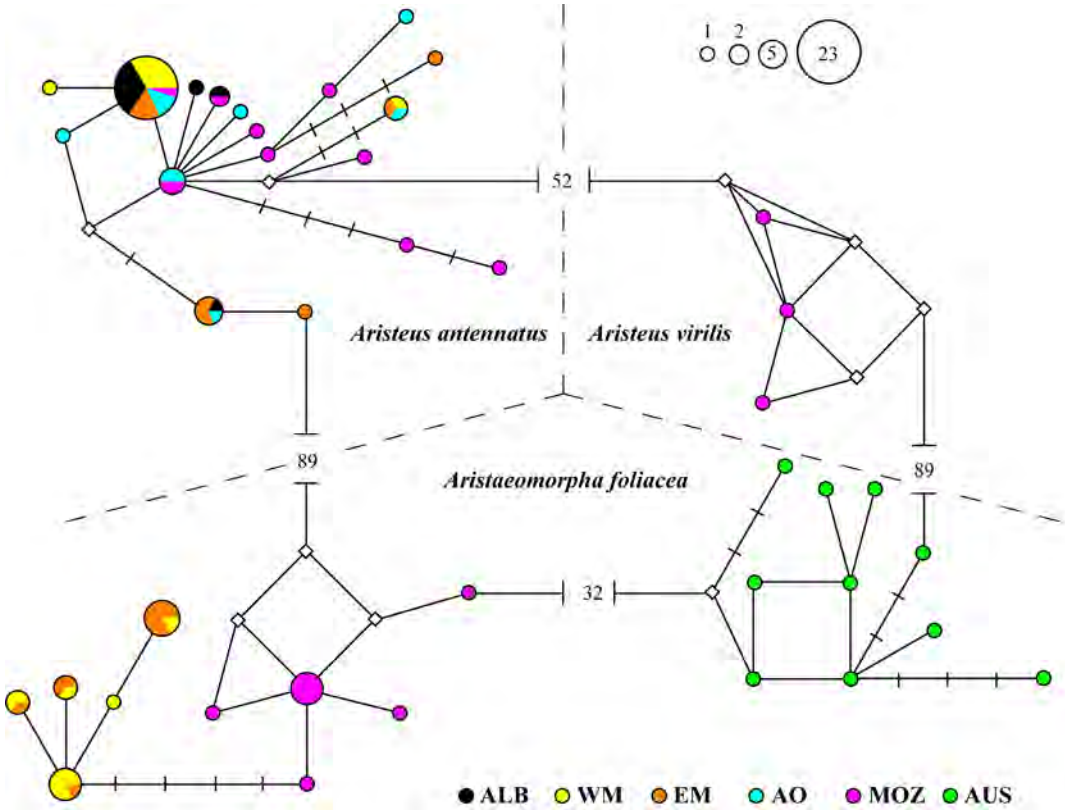

Figure 3. Median-joining network of COI haplotypes detected for the three species studied. The area of each circle is proportional to the number of individuals exhibiting that haplotype. Each line in the network represents one mutational step, vertical bars and white rhombi represent mutational steps and median vectors, respectively, both interpreted as missing or undetected haplotypes. Location codes are as in Table 1.

Table 1. Estimates of genetic diversity obtained for each molecular marker employed*.

Species	CODE	Geographical coordinates	COI (514 bp)					PEPCK (536 bp)			NaK (498 bp)		
Biogeographical region			n	nh	*h* ± S.D.	np	π ± S.D.	n	F	*Ho*	n	F	*Ho*
Aristeus virilis	Av												
Mozambique Channel	MOZ	17º 36' S, 38º 26' E	3	3	1.000 ± 0.074	2	0.0026 ± 0.002	3	2	1	2	2	0.5
Aristeus antennatus	Aa												
Alborán Sea	ALB	35º 59' N, 03º 05' W	10	4	0.533 ± 0.180	6	0.0028 ± 0.002	10	4	0.40	5	9	0.80
Western Mediterranean	WM	42º 35' N, 04º 13' E	10	3	0.378 ± 0.181	5	0.0019 ± 0.002	6	3	0.16	5	9	0.75
Eastern Mediterranean	EM	37º 37' N, 21º 03' E	10	5	0.800 ± 0.100	12	0.0072 ± 0.004	4	4	0.57	7	12	0.86
Atlantic Ocean	ATL		10	7	0.911 ± 0.077	11	0.0052 ± 0.004	9	4	0.44	2	4	1
Mozambique Channel	MOZ	17º 32' S, 38º 29' E	10	9	0.978 ± 0.054	12	0.0062 ± 0.004	10	4	0.50	8	16	1
Total *A. antennatus*			50	17	0.777 ± 0.059	24	0.0051 ± 0.001	42	4	0.43	27	38	0.88
Aristaeomorpha foliacea	Af												
Western Mediterranean	WM	39º 02' N, 02º 39' E	10	5	0.756 ± 0.130	4	0.0022 ± 0.002	10	10	0.70	7	5	1
Eastern Mediterranean	EM	37º 17' N, 22º 53' E	10	4	0.644 ± 0.152	4	0.0032 ± 0.002	10	10	0.60	8	2	1
Mozambique Channel	MOZ	25º 57' S, 34º 38' E	10	5	0.667 ± 0.163	5	0.0019 ± 0.002	10	6	0.60	10	4	1
North-Western Australia	AUS	14º 51' S, 121º 26' E	10	10	1.000 ± 0.045	12	0.0614 ± 0.004	9	5	0.50	5	2	1
Total *A. foliacea*			40	20	0.927 ± 0.022	47	0.0311 ± 0.004	39	12	0.60	30	6	1

*Number of individuals (n), number of haplotypes (nh), number of polymorphic sites (np), haplotype (*h*) and nucleotide (π) diversity with standard deviation (S.D.) of mtDNA COI; number of alleles (F), observed heterozigosity (*Ho)* of nuclear loci PEPCK and NaK.

Table 2. Analysis of molecular variance (AMOVA) for concatenated data (1548 bp).

Species	Source of variations	df	Variance components	%	Φ-statistics	*P*
A. antennatus	Among samples	4	0.23459	9.57	Φ_{ST} = 0.096	0.079
	Within samples	18	2.21667	90.43		
A. foliacea	Among samples	3	7.70177	87.36	Φ_{ST} = 0.874	< 0.001
	Within samples	26	1.11429	12.64		

Table 3. Matrix of Tamura-Nei genetic distance measures for concatenated data (1548 bp) for species and lineages detected in Figure 1*.

	A. virilis	*A. antennatus*	*A. foliacea*	*P. monodon*	Af MED	Af MOZ	Af AUS
A. virilis	0.0013 ± 0.00086						
A. antennatus	0.0492 ± 0.00616	0.0033 ± 0.00076					
A. foliacea	0.1203 ± 0.01242	0.1232 ± 0.01339	0.0094 ± 0.00147				
P. monodon	0.2175 ± 0.02067	0.2108 ± 0.01854	0.1880 ± 0.01709				
S. crassicornis	0.1550 ± 0.01475	0.1535 ± 0.01427	0.1546 ± 0.01506	0.1683 ± 0.01609			
Af MED	0.1248 ± 0.01270	0.1276 ± 0.01353			0.0016 ± 0.00066		
Af MOZ	0.1248 ± 0.01271	0.1254 ± 0.01346			0.0028 ± 0.00132	0.0008 ± 0.00035	
Af AUS	0.1206 ± 0.01228	0.1284 ±0.01326			0.0227 ± 0.00406	0.0242 ± 0.00434	0.0025 ± 0.00082

Standard error estimates after 10000 replicates. In bold are reported within species and within lineage mean values. Location codes are as in Table 1.

"No policy is sustainable without a public that broadly understands why it is necessary and sees the world the way you do"

Thomas L. Friedman, 2000
The Lexus and the Olive Tree: Understanding Globalization

GENERAL DISCUSSION

Five massive extinctions have been documented through the history of the earth, one each in the final of the Ordovician, Devonian, Permian, Triassic and Cretaceous periods. These major global biotic turnovers were tightly associated with physical events that lay outside the normal climatic and other physical disturbances which species, and entire ecosystems, experience and survive (Eldredge 1998). It has been estimated that Earth is currently losing something on the order of 27 000 species per year, which is between 1 000 and 10 000 times higher than the "background" or expected natural extinction rate (Wilson 1993). This rate of extinction has been considered so high that it has been labeled as the biodiversity crisis, and if present trend continues it will soon

constitute the sixth mass extinction (Leakey & Lewin 1995). By 1970s, scientists became very aware that the main cause for this rapid decline was largely due to the influence of human activities: transformation of the landscape, environmental pollution, overexploitation of species, and the introduction of alien species. Even the oceans, with their vast size and composition that provides this environment some buffer against anthropogenic modifications, have suffered profound impacts on its marine biodiversity (Avise 1998). Not only populations of marine mammals and large vertebrates have seen significantly reduced their population sizes, but also fishes and invertebrates have been depleted severely or forced to extinction by human harvesting (Malakoff 1997).

Around 1976 Michael Soulé adopted the term conservation biology "drawing on established disciplines (e.g. ecology, fisheries) and integrating them in pursuit of a coherent goal: the protection and perpetuation of the Earth's biological diversity" (Meine et al. 2006). Human related factors reduce population sizes therefore inbreeding and loss of genetic diversity are unavoidable. Since inbreeding reduces reproduction and survival rates, and loss of genetic diversity reduces the ability of populations to cope with environmental change, it was early clear that genetics played an important part in the conservation of species (Soulé 1985) and that any attempt to conserve biological diversity passes by maintaining the genetic variability of organisms (species, sub-species or populations) (Frankham 2003). Conservation genetics emerged as subdiscipline of conservation biology (Meffe & Carroll 1997) and encompasses genetic management of populations, resolution of taxonomic uncertainties, and the use of molecular genetic analyses in forensics and to understanding species' biology (Frankham 2003).

A large number of productive commercial fisheries have collapsed in the present century (Allendorf et al. 1987). Fish and secondly shellfish are the most important source of protein for many cultures, and collapse of fisheries not only threats the main component of their diet, but posses at risk their economical and social stability (Everhart & Youngs 1981). As detailed in the General Introduction section (pp 33-35), *Aristeus antennatus* and *Aristaeomorpha foliacea* are the basis of a large socio-economic sector in the Mediterranean Sea. Therefore, the conservation of these resources is not only important for the maintenance of the species itself but for the continuity of a large economic and social sector of coastal localities (Lleonart & Maynou 2003).

Fisheries management was defined as the application of scientific knowledge to the problems of providing optimum yield of commercial fisheries products (Everhart & Youngs 1981) so it ranges from individual fishermen's concern to problems of international magnitude (Everhart & Youngs 1981). Fisheries managers have to consider a wide range of biological, ecological, economical, political and social issues in the final definition of a stock. This has resulted in a multiple number of stock definitions (Box 11), largely depending on which issue (biological, ecological, economical, political or social) presented major repercussions (Nelson & Soulé 1987; Carvalho & Hauser 1994; Coyle 1998). Because fisheries management has largely been concerned with the immediate resource of interest, that is, the abundance and size of the organisms available for harvesting, definition of fishing units traditionally has relied on the biological and ecological characteristics (Allendorf et al. 1987). Little attention has been directed towards understanding the genetics of these populations, which informs on groups of organisms largely demographically independent from such other groups (Allendorf et al. 1987). The development of the fields of conservation biology and conservation genetics prompted the increase of genetic studies in species of commercial interest for its application in management plans because the exploitation of a resource based on genetic parameters (Box 11) guarantees the persistence of the stock (Allendorf et al. 1987). Yet, integration of genetic information into actual management has been slow, and explicit and quantitative inclusion of genetic data into management models is rare (Waples et al. 2008); especially if genetic information contradicted practical, political or economical priorities (Carvalho & Hauser 1994). Such a narrow perspective may be economically advantageous in the short-term, but it is doomed to fail the test of time (Allendorf et al. 1987). There are several examples in which the no consideration or application of genetic information in the delimitation of a particular stock has resulted in management failure, interpreted as overexploitation or extirpation of local autonomous populations (Graves 1998; Waples et al. 2008; Reiss et al. 2009). Once this occurs, these populations are unlikely to recover via natural recruitment of foreign individuals over ecological timescales relevant to immediate management interests (Nelson & Soulé 1987; Carvalho & Hauser 1994). Therefore, holistic approaches (incorporating interespecific interactions and physical environmental influences) are desirable to maximize sustainability and reduce uncertainty in predictions which would eventually lead to overfished stocks (Botsford et al. 1997; Coyle 1998).

Box 11. Most commonly used definitions of stock (extracted from Coyle 1998)

Biological Stock: intraspecific group of randomly mating individuals with temporal and spatial integrity (Ihssen et al. 1981)

Genetic Stock: reproductively isolated units which are genetically different from each other (Ovenden 1990)

Environmental or Phenotypic Stock: two groups of fish that may not be considered genetically distinct but that may have adapted separately to their respective environments (Coyle 1998)

Harvest Stock: locally accessible fishing resources in which fishing pressure on one resource has no effect on the abundance of fish in another contiguous resource (Gauldie 1988)

Fishery Stock: group of fish exploited in a specific area or by a specific method (Smith et al. 1990)

Moritz (1994) described Management Units (MU) as populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles, indicating that they represent populations connected by such low levels of gene flow that they are functionally independent (Moritz 1994). In the literature of commercial fisheries, MUs are equivalent to genetic stocks (Ryman & Utter 1987; Ovenden 1990; Moritz 1994) and a synonymy has been established between these two terms in current scientific literature (Avise 2004). Accordingly, 4 MU have been detected for *A. antennatus* (Article I) with geographical delimitation corresponding to WM, EM, AO and MOZ; similarly, 4 MUs with geographic delimitation have been detected for *A. foliacea* (Article III) corresponding to WM, EM, MOZ and AUS (Table 2). The correct delineation of MUs and their geographical extent is necessary to accurately design management plans (Pella & Milner 1997).

In that sense, the more accurately MU defined in this work is that of the WM, for *Aristeus antennatus* (Article I), due to the large number of samples available in the region which permitted to get precision on the hydrographical and geographical points acting as barriers to gene flow between this MU and adjacent ones. Accordingly, restriction of gene flow between *A. antennatus* from WM with EM and AO MUs seems to be restricted by the Straits of Sicily and Strait of Gibraltar respectively (Article I). No further genetic structure was detected within the WM despite the fact that the Almería-Orán Front or the Balearic Front have been questioned as putative barriers to gene flow for coastal fishes (Galarza et al. 2009) and coastal crustaceans (García-Merchán et al. 2011). However, the lack of no further subdivision within the WM does necessarily mean that smaller-scale

substructure might exist. Because mtDNA is clonal and maternally inherited it cannot provide information about male migration or male gene flow. Also, being only a single marker, with a relatively slow molecular evolutionary rate, it has less power for small geographical scale population genetic analyses than a full suite of nuclear markers like microsatellites (Waples et al. 2008). Surprisingly, a lack of genetic structure was detected on a microsatellite (8 loci) survey on samples from the Ligurian Sea, Tyrrhenian Sea and Algerian Sea (Cannas et al. 2011) (Figure 13).

The MUs of the EM, AO and MOZ have been defined based on genetic analyses from just one location; the extension of *A. antennatus* is known to be larger in each of these geographical areas, and it would have been desirable to analyze samples from the extremes of its distribution, i.e. Atlantic Moroccan, Argelian, Tunisian, Ionian Italian and Lebanon waters, as well as other settlements adjacent to the Mozambique Channel. However, despite intensive effort it was impossible to obtain samples from these fishing grounds (Roldán pers. comm.). These samples would have allowed defining the geographical extent of each of these MUs with major precision. Until that is possible, with the genetic information available it is better to consider the existence of four MUs and to extend the consideration of such to the whole geographical area of influence.

For *A. foliacea,* the anticyclonic gyre at the south of the Peloponnesian peninsula was identified as the major barrier to gene flow between the two MUs detected in the Mediterranean Sea (Article III). However, as in the case of *A. antennatus*, it would have also been desirable to analyze fishing grounds from the Levantine Sea and adjacent Atlantic Ocean, where the species is recorded to exist, but its quantities are not enough to sustain directed fisheries (Gönülal et al. 2010; PescProf). Likewise, in order to delimitate the extension of MOZ and AUS MUs, sampling should be extended to neighboring fishing grounds (e.g. South-Africa, SWIOFP 2009).

After observing the genetic structure detected for the two red shrimps in the Mediterranean Sea, it is possible that a combination of both, Strait of Sicily and Pelopponesian gyre may be difficulting the migration of individuals (larvae and adults) and gene flow between the two basins. Nevertheless, from the genetic results obtained in this work, it can be concluded that 4 MUs exists for each Aristeidae species studied in this work and that fisheries management should be carried out in a coordinated manner among the countries harvesting each specific MU.

Each of these MUs presents relatively high levels of genetic variability, indicating that these genetic stocks would be still genetically healthy and distant from inbreeding problems. However, the levels of genetic variability are not equal across regions or MUs. Particularly, the MU of *A. antennatus* in the WM is the one presenting the lowest levels of genetic variability. Theoretical and empirical observations indicate that loss of genetic diversity might occur over a span of decades (e.g. Ruzante et al. 2001) and in some cases a significant decline in genetic diversity has been associated to a continuous exploitation history (e.g. Smith et al. 1990; Hauser et al. 2002). In the WM *A. antennatus* has been the object of commercial exploitation since 1930s (Sardà et al. 2004b) in a continuous manner with increase in fishing effort with time, and as seen in the General Introduction (pp. 33, 34), reductions in population size due to fishing pressure and physical disturbance have been documented (Relini & Orsi Relini 1987; Company et al. 2008; respectively). This has eventually resulted in a significant decrease in the number of catches over time, leading to the recognition of overexploitation of the resource in some GSAs and the temporal closure of local ports (e.g. Palamós fishermen association). Although there is no temporal information on the evolution of the genetic variability within the WM MU of *A. antennatus*, the low levels of genetic variability, in combination with the high fishing pressure, should be taken as a precautionary measure and a genetic sign of overexploitation. The reasons why this resource has not yet become depleted, as happened with *A. foliacea*, is its metapopulation structure, in which virgin grounds would be the sources that perform rescue effects on the sink-fishing grounds (Sardà et al. 2010) (Figure 15).

On the other hand, the WM MU of *A. antennatus* presents clear evidence of a past population expansion as indicated by the star-like networks, mismatch distributions and neutrality tests (Article I). Genetic signal of a past population expansions are usually associated to bottleneck or founder events (Rogers & Harpending 1982). However, the same genetic signature of a past population expansion event can be created when only a reduced part of the population contributes to the next generation of reproducing adults, usually by external agents, the so-called Sweepstake Reproductive Success (SRS, Hedgecock 1994). Invertebrate species are particularly prone to suffer SRS events, because of their high fecundity and mortality rates at early life stages (Hedgecock 1994). Also, fishing activity is intensive during summer time because of the easier access to the

resource, which has migrated to shallower grounds (400-600 m) for mating activities. This combination means that fishing activity in summer mostly removes large females, leaving reproduction only to small females. Small females undertake a larger number of molts than large females, and because *A. antennatus* presents an open thelycum (Demestre & Fortuño 1992), with external mating and fertilization, smaller females are more prone to lose the spermatophore (Sardà 2004a). Therefore, it is hypothesized that the genetic signal of a past population expansion in the WM is caused by a series of sweepstake reproductive success aggravated by fishing pressure.

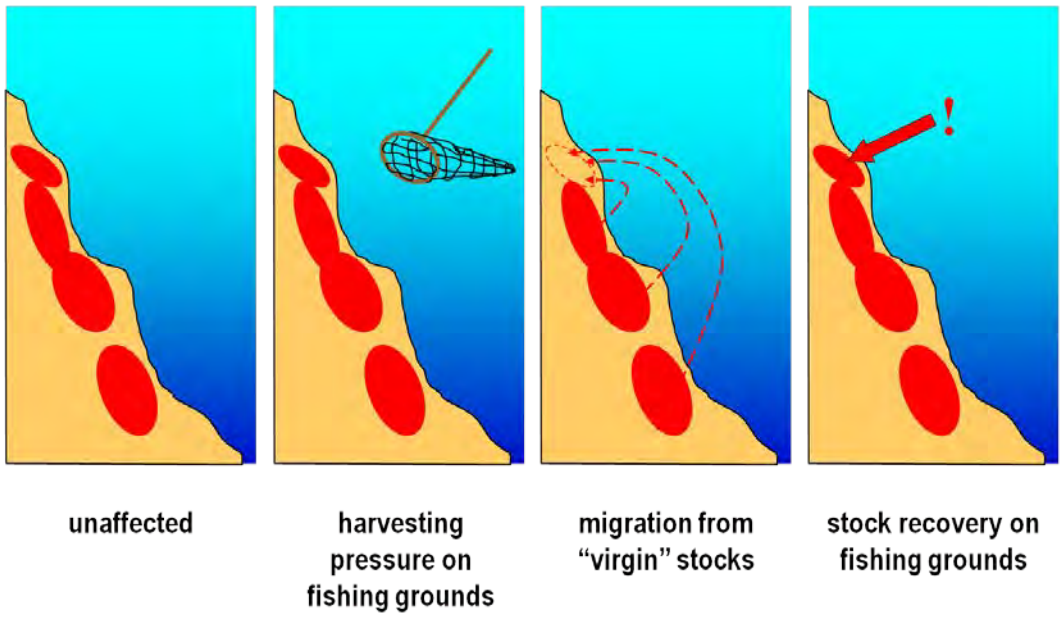

Figure 15. Metapopulation-like structure of *Aristeus antennatus* populations. Extracted from Sardà et al. (2008).

At present, no TACs (Total Allowable Catch) or other types of adaptive management exists for *A. antennatus* and *A. foliacea* in the Mediterranean Sea (Lleonart & Maynou 2003) and only recommendations on harvesting quotas and effort control are given, as already seen for the different GSAs, in the Aristeid fisheries section (pp 35-37). Likewise, there is no coordinated exploitation of the resource because the narrowness of the continental shelf implies that in few areas demersal stocks are shared, by any of the 21 bordering countries of the Mediterranean Sea (Caddy 1998). Extra measures regarding technological equipment have been applied in an equal manner to all GFCM Members. In 2005 all Members of the GFCM were recommended to prohibit the use of

towed dredges and trawl nets fisheries at depths beyond 1000m depth (Rec. GFCM/29/2005/1) which particularly protects virgin grounds of *A. antennatus* from fishing pressure with consequent benefit for the fishing grounds (Figure 15). In 2007, all GFCM Members agreed on a voluntary implementation of at least a 40 mm square mesh codend in bottom trawling (Res. GFCM/31/2007/3) which should have become extensively implemented by 31 May 2010 in all trawlers exploiting demersal resources (Rec. GFCM/31/2007/1).

From the genetic results (Article I, Article III) it is recommended to further implement in the WM MU two management measures. First, temporal genetic monitoring could help to promptly identify whether fisheries are causing negative genetic effects (i.e. a decrease of genetic variability over time) (Ferris & Berg 1987). Second, a coordinated closure of the fishing activity during reproductive season would allow a larger part of the population to contribute to next generations (Nelson & Soulé 1987).

Recommendations for *A. antennatus* in WM MU are transferable to the EM MU as well, as it is foreseeable that exploitation of these until now barely exploited stocks will increase in the short future (Mytilineou et al. 2006; Garofalo et al. 2007; GFCM SAC 2010). Likewise, similar precautionary measures should be applied to *A. foliacea*, especially for its higher susceptibility to fisheries pressure. In fact, Politou et al. (2004) already pointed out that in case the fisheries for *A. foliacea* developed in eastern Ionian waters, fisheries closure should be implemented during summer.

Fisheries in the Mozambique Channel started in 1968 and at present it consist of joint ventures between the Government of Mozambique and foreign companies from Japan, Spain and Portugal that have 70% of the TAC (Sobrino et al. 2009). Vessels are freezer trawlers that undertake 20 to 40 days trips in offshore waters (EEZ) and process the product at sea (SWIOFP 2012). The fisheries is directed towards deep crustaceans and *A. antennatus* and *A. foliacea* are the main catches together with *Haliporoides triarthus*. The last stock assessment (2011) indicated that *A. foliacea* was underexploited and recommendation for an increase in catches was given (SWIOFP 2012). However, for *A. antennatus* apparently there is no stock assessment and no species-specific management strategies are defined because the species is considered as part of the deep-water prawn target group of the Mozambican fishery (SWIOFP 2012). In the Proceedings of the Regional

Workshop for Component 2 (SWIOFP 2009), it was considered that aside from abundance, distribution, size composition and general biological information, genetic data was necessary on *A. antennatus* and *A. foliacea* to assess the regionality of exploited stocks, as basis for developing appropriate management plans (SWIOFP 2009). The only available genetic data for this species is that reported in this work. The high levels of genetic variability recorded for both species, guarantee safe room for further exploitation, from the genetic point of view. However, in both species, signs of past population expansion were also detected that although not as intense as those of *A. antennatus* in the WM, the sign should nevertheless be taken as precautionary given the experience from WM stocks. Therefore, same safeguard measures, closure of fishery during reproductive period and implementation of temporal genetic controls, are advised for this region.

Table 2. Number of conservation units of *Aristeus antennatus* and *Aristaeomorpha foliacea* found in this study according to level of genetic divergence: Management Unit (MU), Evolutionary Significant Unit (ESU), or species.

	MUs	ESUs	Species
Aristeus antennatus	4 – WM, EM, AO, MOZ	1	1
Aristaeomorpha foliacea	4 – WM, EM, MOZ, AUS	3 – MED, MOZ, AUS	2

Until now it has been addressed the role of conservation genetics in the management of populations, but genetic information can have important ramifications in legal aspects of species conservation. Since its establishment in 1948, the International Union for Conservation of Nature (IUCN, www.iucn.org) has assessed the conservation status of species and subspecies on a global scale. However, in a conference held in July 1985 at the Zoological Society of Philadelphia (USA) it was realized that "if conservation seeks to preserve genetic variability, then conservation plans should be based on conservation of genetic pools" (Ryder 1986). The outcome was that what should be preserved is "a subset of the more inclusive entity species, which possess genetic attributes significant for the present and future generations", or what is also known as Evolutionary Significant Unit (ESU) (Ryder 1986). One of the first implementations of the ESU concept was made by Waples (1991) for the conservation of the Chinook salmon under the USA Endangered Species Act (ESA). Waples (1991) proposed that a population should be considered distinct for purposes of the ESA if that satisfied two criteria - reproductive isolation and genetic differentiation form conspecific populations – i.e. if that population represented an ESU of the biological species. Ryder (1986) and

Waples (1991) agreed in that an ESUs should be geographically discrete, but it remained unclear the genetic criteria to implement. The principles of genealogical concordance proposed by Avise & Ball (1990) (Box 5) offered a good solution for critically evaluating evolutionary depths of population separation using molecular data. However, Moritz (1994) found that reciprocal monophyly for both mitochondrial and nuclear data was overly restrictive, and proposed instead that ESUs should be "reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci". ESUs now have both important legal and biological ramifications under the ESA, the Australian Endangered Species Protection Act and parallel legislation in other countries (Fraser & Bernatchez 2001).

Within this work (Article IV) *A. antennatus* has been identified as a monophyletic species and a single ESU throughout its distributional range. Instead, in *A. foliacea* (Article IV) up to three ESUs have been recognized geographically restricted to Mediterranean Sea, Mozambique Channel and North-Western Australia (Table 2). This information has double repercussions. First, the definition of ESUs permits to detect the regional origin (Mediterranean Sea, Mozambique Channel or North-Western Australia) of *A. foliacea* which is especially useful within the field of forensics to perform product traceability, consumer protection and regulatory enforcement, in particular with respect to illegal, unreported or unregulated fishing (Odgen 2008). Second, both *A. antennatus* and *A. foliacea* have already been included in the priority species list drafted by SAC of GFCM on its ninth session (Rome, 24-27 October 2006) (AdriaMed 2007). Other species in this list have been recently included in the IUCN red list, e.g. *Homarus gammarus* appears as least concerned species (Butler et al. 2001) or *Thunnus thynnus* is considered endangered (Collette et al. 2011). If ever *A. antennatus* or *A. foliacea* reached very low abundance levels in any of its current fishing grounds as to merit legal protection, this should be performed following ESU designations which would ensure the perpetuity of the genetic pool from which re-stocking programs could act. Currently, the biological condition of the species which lives at great depths and the lack of aquaculture technological facilities do not allow maintaining red shrimps in captivity; therefore, conservation measures for these species must rely on maintaining a minimum viable population size.

A. foliacea has been traditionally recognized as a single, broadly distributed (Figure 8), species; however genetic data (Article IV) suggests this is not the case, and that there are at least two

genetic species (Table 2), one in the Mediterranean Sea and Mozambique Channel (*Aristaeomorpha foliacea*) and another in North-Western Australia (*Aristaeomorpha rostridentata*) (Box 12). There is now the opportunity to evaluate each of these species in terms of its management and conservation measures. The genetic status of *A. foliacea* in Western Atlantic, and rest of Indo-Pacific localities is unknown but it would not be surprising that similar deep genetic divergences would appear among individuals morphologically undistinguishable (cryptic species). Finally, our results clearly demonstrate that cryptic diversity has been often underestimated in decapod crustaceans (Knowlton 1986), highlighting the important contribution that genetic studies make to the estimation of overall biodiversity levels.

Box 12. Biological classification of the genus *Aristaeomorpha* and geographical distribution of its species, according to the results of this study

Family Aristeidae Wood-Mason 1891
Genus *Aristaeomorpha* Wood-Mason & Alcock 1891
Aristaeomorpha foliacea Risso 1827
Aristaeomorpha rostridentata Spence Bate 1881
Aristaeomorpha woodmasoni Calman 1925

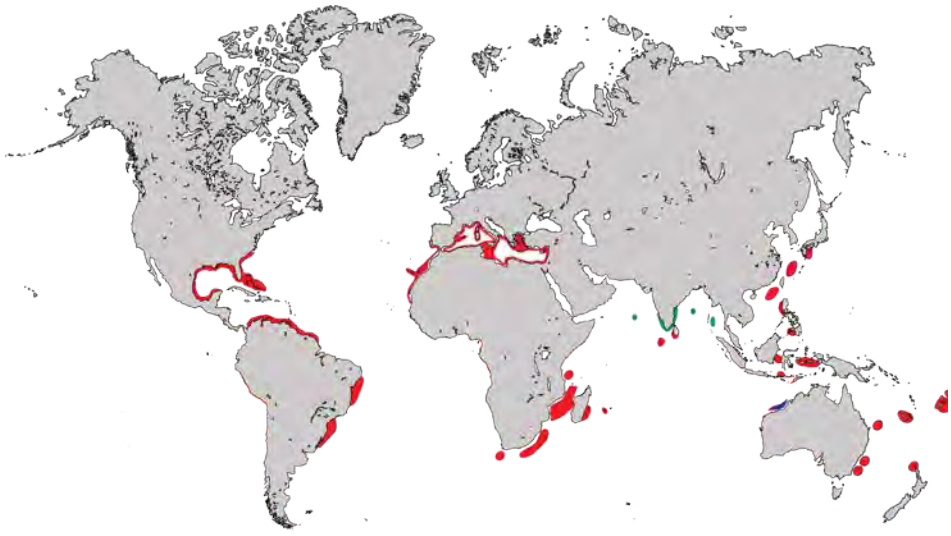

Figure 16. Distribution of *Aristaeomorpha* spp. *A. foliacea* (in red): Mediterranean Sea, Atlantic and Indo-West Pacific Oceans. *A. rostridentata* (in blue): North-Western Australia. *A. woodmasoni* (in green): Arabian Sea, Bay of Bengal and Adaman Sea.

"Imagination is more important than knowledge"

Albert Einstein (1879 - 1955)

CONCLUSIONS

In this work, genetic analyses were performed to study the population genetics and compared phylogeography of *Aristeus antennatus* and *Aristaeomorpha foliacea*. From the results obtained, the following conclusion can be extracted:

1- *A. antennatus* presents high levels of genetic diversity but not evenly distributed. Higher genetic diversity values were detected in Mozambique Channel, followed by the Atlantic Ocean, Eastern Mediterranean and Western Mediterranean. Probably, the lower genetic variability of the Western Mediterranean is the consequence of a continuous intensive exploitation.

2- Two barriers to gene flow were indentified: the Strait of Gibraltar between Western Mediterranean and Atlantic Ocean and the Strait of Sicily between Eastern and Western Mediterranean. The Almería-Orán front did not significantly restrict gene flow. The genetic divergence among the four regions studied (Western Mediterranean, Eastern Mediterranean, Atlantic Ocean and Mozambique Channel) indicate each of these regions should be considered a distinct Management Unit for fishery purposes.

3- For *A. foliacea* high levels of genetic diversity were detected. Western Mediterranean, Eastern Mediterranean and Mozambique Channel presented similar level of genetic variability but North-Western Australia showed the highest values where almost each individual presented a different haplotype.

4- The Peloponnese gyre was detected as the most likely restrictor to gene flow between Western and Eastern Mediterranean. The genetic divergence among the four geographical regions studied (Western Mediterranean, Eastern Mediterranean, Mozambique Channel and North-Western Australia) indicates each of these regions should be considered a distinct Management Unit for fishery purposes.

5- ISSR markers did not result suitable to study the population genetics of *A. foliacea* given their hypervariability and possible saturation would be shadowing possible real genetic divergence.

6- This is the first work in which mitochondrial genetic analyses have proved useful to detect genetic differentiation of *A. antennatus* and *A. foliacea* at microevolutionary scale.

7- *A. antennatus* and *A. foliacea* presented discordant phylogeographic patterns across geographical regions where these species lives in partial sympatry. The surgence of the Benguela current (2 Mya) was a major vicariant event between the Mediterranean and Mozambique populations of both species. Posterior Pleistocene climatic fluctuations caused greater instabilities on *A. foliacea* than in *A. antennatus* populations, accelerating the process of divergence between *A. foliacea* lineages.

8- Mitochondrial and nuclear markers identified *A. antennatus* as a single genetic species throughout a wide distributional range. The close genetic relationship detected between *Aristeus antennatus* and *Aristeus virilis* are consistent with congeneric species.

9- In *A. foliacea* three monophyletic lineages were identified, corresponding to Mediterranean Sea, Mozambique Channel and North-Western Australia. However, nuclear markers only detected two monophyletic groups: Mediterranean - Mozambique Channel and North-Western Australia.

10- The amount of genetic divergence detected between the North-Western Australian lineage of *A. foliacea* and the Mediterranean-Mozambique Channel lineage were comparable to levels of genetic divergence recorded for species level. It is suggested to recognize North-Western Australian lineage as a genetic different species, despite the apparent lack of morphological differences, and it is proposed the name *Aristaeomorpha rostridentata* (previously assigned by Bate in 1881).

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GLOSSARY

Admixture: the result of interbreeding between two or more previously isolated populations within a species.

AFLP: a method for identifying polymorphism in DNA sequences using restriction enzymes, DNA linkers, PCR amplification and gel electrophoresis.

Allopatric (speciation): generation of new species where populations are physically separated from each other.

Allozymes: electrophoretic expression of allelic proteins at a particular locus.

Antennula: the first paired cephalic appendage.

Background selection: describes the selective removal of rare deleterious mutations from a population. In general, background selection results in a reduction in levels of neutral polymorphism.

Barcoding: the use of diagnostic gene sequences as identifiers of particular species.

Coalescent theory: describes the genealogical relationships among individuals in a Wright-Fisher population.

Dicapodid (=postlarva): final larval stage that resembles a miniature adult but is not sexually mature.

Divergence: describes variable sites within species.

Effective population size (N_e): is the size of an idealized population, such as described by the Wright-Fisher model, that has the same magnitude of genetic drift, the effective population size is always less than the actual population size due to: unequal number of males and females; overlapping generations; non-poisson distribution of fecundity; non-random mating, e.g. population structure.

Eurybathic: capable of living on the bottom in both depth and shallow waters, can tolerate a wide range of depths; contrary to stenobathic.

Eyestalk: Peduncle or unfaceted part of the eye supporting the cornea.

Genetic distance: extent to which populations differ from one another with respect to allele frequences or DNA sequences at a particular locus.

Genetic drift: changes in allele frequency that occur by chance, essentially as a random sampling of available gametes each generation.

Homoplasmy: a cell or organism having all copies of the mitochondrial DNA identical.

Isozymes: distinguishable molecules found in the same organism which catalyze the same reaction.

Kinetic energy: energy of motion. The word "kinetic" is derived from the Greek word meaning to move, and the word "energy" is the ability to move.

Locus (pl. loci): the location in the DNA occupied by a particular gene.

Mutation rate: refers to the rate at which changes are incorporated in a nucleotide sequence during the process of evolution.

Mysis (Penaeoidea): second stage of the zoeal development in which the larvae undergo major changes in appearance, and the body becomes shrimplike.

Natural selection: reproductive differential rate of individuals in a population as a consequence of their differential physiologic, genetics and behaviour characteristics. Natural selection can drive mutations to

fixation, due to positive selection for an adaptive mutation, or to loss, due to negative selection against a deleterious mutation, or to intermediate frequency in a population, due to balancing selection.

Nauplius (pl. nauplii): free-swimming, microscopal larval stage, after hatching from egg, characterized by bearing median simple eye and 3 pairs of setose (functional appendages destined to become the antennules, antennae and mandibles).

Nektobenthic: organisms typically associated with the benthos that swim actively in the water column at certain periods.

Panmixia: random mating of individuals throughout a population.

Pereiopods: one of five pairs of posterior appendages or legs attached to the cephalothorax.

Pleomere: one of six segments (somites) of abdomen (pleon); each bears pair of appendages (pleopods). Last pleomere bears uropods and is followed by telson.

Pleopods: one of the biramous paired appendages typically arising ventrally from each of the anterior five abdominal somites. In the shrimps, they are primarily swimming organs.

Pleura (pleurite): lateral part of integument of somite, most clearly visible in abdomen of shrimp-like decapods, where they may form prominent lateral extensions.

Polymorphism: the existence of different multiple forms, for example alleles or nucleotide positions.

Postorbital spine: spine situated near the orbital margin posterior to the antennal spine.

Postorbital groove: on each side of carapace, groove close to and parallel with margin of orbit.

Petasma: a complex genital structure attached to the mesial margins of the protopodites of the first pair of pleopods in male penaeidean shrimps.

Prawn: common term referring to members of the Dendrobranchiata order.

Priority species: fish and wildlife species requiring protective measures and/or management guidelines to ensure their perpetuation, according to the Washington Department of Fish and Wildlife, USA.

Protozoea (Penaeoidea): first stage within the zoeal development characterized by the presence of natatory exopods on some or all of the thoracic appendages and by pleopods being absent or rudimentary.

RAPD: a method for detecting polymorphism in DNA sequences using random primers in a PCR followed by gel electrophoresis.

Regional Fisheries Bodies (RFB): a mechanism through which states or organizations that are parties to an international fishery agreement or arrangement work together towards the conservation, management and/or development of fisheries.

RFLP: a method for identifying polymorphism in DNA sequences using restriction enzymes and gel electrophoresis.

Shrimp: common term applied to members of the infraorder Caridea.

Somite: a body segment, especially of the abdomen.

Stenobathic: living within narrow limits of depth, opposed to eurybathic.

Substitution rate: the rate at which the replacement of one nucleotidic base by another occurs.

Sympatric (speciation): generation of new species among individuals living in the same area.

Telson: terminal unit of the abdomen bearing the anus.

Thelycum (pl. thelca): the female genitalia consisting of modifications of the posterior two, or sometimes three thoracic sternites (XII-XIV) serving for the storage or transfer of the sperm, usually in spermatophores, and often shielding seminal receptacles.

Zoea (Penaeoidea): larval stage after nauplii. The zoeal development is divided into two stages, protozoea and mysis.

Glossary based on:

Beebee T, Rowe G (2008) An introduction to Molecular Ecology. 2nd Ed. Oxford University Press, New York, USA

Brusca RC, Brusca GJ (2002) Invertebrates. Sinauer Associates Sunderland, Massachusetts, USA

Hartl DL, Clark AG (1988) Principles of Population Genetics. Sinauer Associates, Sunderland, Massachusetts, USA

Perez Farfante I, B Kensley B (1997) Penaeoid and Sergestoid Shrimps and Prawns of the World: Keys and Diagnoses for the Families and Genera. Memoires du Museum National d'Histoire Naturelle, 175, 233 pp

Assembling the Tree of Life: Decapoda – http://decapoda.nhm.org, visited 06-16-2012

Merriam Webster dictionary, an encyclopaedia Britannica company - www.merriam-webster.com, visited 06-16-2012

ABBREVIATIONS

AFLP - Amplified Fragment Length Polymorphism

bp - Base pair

BSC – Biological Spcecies Concept

COI - Subunit I of the Cytochrome C Oxidase

CR – Control Region

16s rRNA - small subunit of ribosome 16s ribonucleid acid

ddDNA - dideoxy nucleotides

DNA - Deoxyribonucleic acid

GFCM – General Fisheries Commission for the Mediterranean

H – Heterozygosity

ISSRs - Intern Simple Sequence Repeats

ESU - Evolutionary Significant Unit

F_{ST} - Fixation index

mtDNA - mitochondrial DNA

MMD - Mismatch Distribution

NaK - Sodium-potassium ATPase α-subunit

MRCA - Most Recent Common Ancestor

MU - Management Unit

Mya – Million years ago

OMG – One Migrant per Generation

PCR - Polymerase Chain Reaction

PEPCK - Phosphoenolpyruvate carboxykinase

PSC – Phylogenetic Species Concept

rDNA – ribosomal DNA

RAPD - Randomly Amplified Polymorphic DNA

RFLP – Restriction Fragment Length Polymorphism

RIMs - Reproductive Isolating Mechanisms

SAC – Scientific Advisory Committee

tn - tonnes

SUPPLEMENTARY MATERIAL

Supplementary material Article I

The following supplement accompanies the article

Genetic structure in the blue and red shrimp *Aristeus antennatus* and the role played by hydrographical and oceanographical barriers

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Marine Ecology-Progress Series 421:163–171 (2011)

Supplement. These additional data include a complete list of haplotypes for single (16S rDNA and COI) and concatenated genes. In addition, data analysis of single genes and mismatch distribution of concatenated genes are provided.

Table S1. *Aristeus antennatus*. List of 16S rDNA haplotypes detected in the eleven sampling sites with respective GenBank accession numbers. Accession numbers in bold refer to previously detected haplotypes by Roldán et al. (2009) and Sardà et al. (2010).

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	TOTAL	GenBank
h1	0.002						1						1	EU908298
h2	0.642	11	34	37	39	28	48	36	23	27	18	2	303	EU977139
h3	0.193	17	4	3	2	1	3	2	10	2	18	29	91	EU977140
h4	0.002						1						1	EU908312
h5	0.006				1		1		1				3	EU908381
h6	0.002						1						1	EU977163
h7	0.002						1						1	EU908332
h8	0.002						1						1	EU908351
h9	0.002						1						1	EU908353
h10	0.002						1						1	EU908355
h11	0.002								1				1	GU972605
h12	0.002								1				1	GU972606
h13	0.002								1				1	EU908391
h14	0.002									1			1	EU908402
h15	0.006							1		2			3	EU908405
h16	0.002									1			1	EU908414
h17	0.002									1			1	EU977162
h18	0.002									1			1	EU908430
h19	0.017	4	1		1			1				1	8	EU977149
h20	0.002	1											1	GU972607
h21	0.002	1											1	GU972608
h22	0.002	1											1	GU972609
h23	0.002			1									1	GU972610
h24	0.002			1									1	GU972611

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	TOTAL	GenBank
h25	0.002			1									1	GU972612
h26	0.004											2	2	GU972613
h27	0.002											1	1	GU972614
h28	0.002											1	1	GU972615
h29	0.004		1									1	2	GU972616
h30	0.002											1	1	GU972617
h31	0.002											1	1	GU972618
h32	0.002											1	1	GU972619
h33	0.002											1	1	GU972620
h34	0.002											1	1	GU972621
h35	0.002											1	1	GU972622
h36	0.002											1	1	GU972623
h37	0.004		1									1	2	GU972624
h38	0.002											1	1	GU972625
h39	0.004							2					2	GU972626
h40	0.002							1					1	GU972627
h41	0.002							1					1	GU972628
h42	0.002							1					1	GU972629
h43	0.002							1					1	GU972630
h44	0.002		1										1	GU972631
h45	0.002		1										1	GU972632
h46	0.002		1										1	GU972633
h47	0.002		1										1	EU977173
h48	0.002		1										1	GU972634
h49	0.002		1										1	GU972635

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	TOTAL	GenBank
h50	0.002		1										1	GU972636
h51	0.002		1										1	GU972637
h52	0.002		1										1	GU972638
h53	0.002		1										1	GU972639
h54	0.002		1										1	GU972640
h55	0.002		1										1	GU972641
h56	0.002										1		1	GU972642
h57	0.002										1		1	GU972643
h58	0.002										1		1	GU972644
h59	0.002					1							1	GU972645
h60	0.002					1							1	GU972646
h61	0.002					1							1	GU972647
h62	0.002					1							1	GU972648
h63	0.002				1								1	GU972649
h64	0.002				1								1	GU972650
h65	0.002				1								1	GU972651
		35	53	43	46	33	59	46	37	35	39	46	472	

Table S2. *Aristeus antennatus*. List of COI haplotypes detected in the eleven sampling sites with respective GenBank accession numbers. Accession numbers in bold refer to previously detected haplotypes by Roldán et al. (2009).

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	TOTAL	GenBank
h1	0.004						2						2	EU908436
h2	0.656	12	39	34	40	34	46	44	28	30	16	3	326	EU908437
h3	0.002						1						1	EU908439
h4	0.002						1						1	EU908440
h5	0.002						1						1	EU908442
h6	0.004		1				1						2	EU908446
h7	0.002						1						1	EU908447
h8	0.002						1						1	EU908463
h9	0.002						1						1	EU908469
h10	0.002						1						1	EU908481
h11	0.004				1		1						2	EU908485
h12	0.006	1					1		1				3	EU908486
h13	0.002						1						1	EU908490
h14	0.054	4	4	1					5	1	12		27	EU908497
h15	0.024	3		2		1		1	2		3		12	EU908498
h16	0.002								1				1	EU908501
h17	0.002								1				1	GU972652
h18	0.002								1				1	EU908512
h19	0.002								1				1	EU908514
h20	0.002								1				1	EU908528
h21	0.002								1				1	EU908529
h22	0.004			1					1				2	EU908537
h23	0.002								1				1	EU908538

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	TOTAL	GenBank
h24	0.010	1								4			5	EU908539
h25	0.002									1			1	EU908561
h26	0.002									1			1	EU908563
h27	0.002	1											1	GU972653
h28	0.006	1	1									1	3	GU972654
h29	0.002	1											1	GU972655
h30	0.002	1											1	GU972656
h31	0.006	2									1		3	GU972657
h32	0.028	6		1								7	14	GU972658
h33	0.002	1											1	GU972659
h34	0.002	1											1	GU972660
h35	0.006	1		1							1		3	GU972661
h36	0.002	1											1	GU972662
h37	0.002			1									1	GU972663
h38	0.004		1	1									2	GU972664
h39	0.002			1									1	GU972665
h40	0.002											1	1	GU972666
h41	0.002											1	1	GU972667
h42	0.006											3	3	GU972668
h43	0.002											1	1	GU972669
h44	0.002											1	1	GU972670
h45	0.002											1	1	GU972671
h46	0.008											4	4	GU972672
h47	0.002											1	1	GU972673
h48	0.002											1	1	GU972674

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	TOTAL	GenBank
h49	0.002											1	1	GU972675
h50	0.002											1	1	GU972676
h51	0.002											1	1	GU972677
h52	0.002											1	1	GU972678
h53	0.002											1	1	GU972679
h54	0.002											1	1	GU972680
h55	0.002											1	1	GU972681
h56	0.004											2	2	GU972682
h57	0.008											4	4	GU972683
h58	0.002											1	1	GU972684
h59	0.002											1	1	GU972685
h60	0.002											1	1	GU972686
h61	0.004											2	2	GU972687
h62	0.004											2	2	GU972688
h63	0.002											1	1	GU972689
h64	0.002											1	1	GU972690
h65	0.004					1		1					2	GU972691
h66	0.004							1			1		2	GU972692
h67	0.002							1					1	GU972693
h68	0.002							1					1	GU972694
h69	0.002							1					1	GU972695
h70	0.002							1					1	GU972696
h71	0.002		1										1	GU972697
h72	0.002		1										1	GU972698
h73	0.002		1										1	GU972699

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	TOTAL	GenBank
h74	0.002		1										1	GU972700
h75	0.002		1										1	GU972701
h76	0.002		1										1	GU972702
h77	0.002		1										1	GU972703
h78	0.002										1		1	GU972704
h79	0.002										1		1	GU972705
h80	0.002										1		1	GU972706
h81	0.002										1		1	GU972707
h82	0.002										1		1	GU972708
h83	0.002										1		1	GU972709
h84	0.002					1							1	GU972710
h85	0.002					1							1	GU972711
h86	0.002					1							1	GU972712
h87	0.002				1								1	GU972713
h88	0.002				1								1	GU972714
h89	0.002				1								1	GU972715
h90	0.002				1								1	GU972716
h91	0.002				1								1	GU972717
h92	0.002				1								1	GU972718
		37	53	43	47	39	59	51	44	37	40	47	497	

Table S3. *Aristeus antennatus*. Estimates of genetic diversity for 16S rDNA and COI gene. Sample size (N), number of sequence obtained (n), number of haplotypes (Nh), number of polymorphic sites (Np), haplotype (h) and nucleotide (π) diversity for each locality and the total.

		16S rDNA						COI				
Region	Locality	N	n	Nh	Np	*h* ± S.E.	π ± S.E.	n	Nh	Np	*h* ± S.E.	π ± S.E.
Atlantic Ocean (AO)	Faro, Portugal	38	35	6	6	0.669 ± 0.056	0.0017 ± 0.0003	37	15	23	0.863 ± 0.042	0.0062 ± 0.0008
Alborán Sea (AS)	Alborán Sea, Spain	53	53	17	17	0.589 ± 0.081	0.0016 ± 0.0003	53	12	13	0.458 ± 0.085	0.0020 ± 0.0005
Western Mediterranean (WM)	Almería, Spain	45	43	5	4	0.259 ± 0.086	0.0005 ± 0.0002	43	9	14	0.378 ± 0.095	0.0019 ± 0.0007
	Sóller, Spain	48	46	7	7	0.283 ± 0.087	0.0007 ± 0.0003	47	8	11	0.278 ± 0.086	0.0012 ± 0.0005
	Cabrera, Spain	40	33	6	6	0.284 ± 0.102	0.0007 ± 0.0003	39	6	8	0.243 ± 0.091	0.0009 ± 0.0004
	Palamós, Spain	59	59	10	10	0.339 ± 0.080	0.0007 ± 0.0002	59	13	18	0.395 ± 0.082	0.0016 ± 0.0004
	Gulf of Lion, France	51	46	9	8	0.389 ± 0.092	0.0009 ± 0.0002	51	8	13	0.258 ± 0.081	0.0014 ± 0.0005
	Genoa, Italy	44	37	6	5	0.553 ± 0.072	0.0011 ± 0.0002	44	12	16	0.589 ± 0.085	0.0039 ± 0.0008
	Palermo, Italy	40	35	7	7	0.407 ± 0.104	0.0010 ± 0.0003	37	5	8	0.338 ± 0.096	0.0016 ± 0.0006
Eastern Mediterranean (EM)	Ionian Sea, Greece	40	39	5	4	0.587 ± 0.041	0.0012 ± 0.0002	40	12	18	0.758 ± 0.050	0.0058 ± 0.0006
Indian Ocean (IO)	Mozambique Channel, Mozambique	48	46	16	13	0.606 ± 0.086	0.0015 ± 0.0003	47	28	39	0.961 ± 0.015	0.0070 ± 0.0007
	Total	506	472	65	59	0.551 ± 0.024	0.0013 ± 0.0001	497	92	75	0.566 ± 0.027	0.0034 ± 0.0002

Table S4. *Aristeus antennatus*. Hierarchical analysis of molecular variance (AMOVA) for 16S rDNA and COI gene. Regions code as in Appendix 3.

		16S rDNA					COI				
Hypothesis	Source of variation	df	Components	%	Φ-statistics	*P*	df	Components	%	Φ-statistics	*P*
Unstructured	Among samples	10	0.08513	22.88	Φ_{ST} = 0.229	< 0.001	10	0.11542	13.21	Φ_{ST} = 0.132	< 0.001
	Within samples	461	0.28698	77.12			486	0.75818	86.79		
Five regions	Among regions	4	0.13258	31.41	Φ_{CT} = 0.314	0.018	4	0.18502	19.56	Φ_{CT} = 0.196	0.003
(AO, AS, WM, EM, IO)	Among samples within regions	6	0.00257	0.61	Φ_{SC} = 0.008	0.004	6	0.00261	0.28	Φ_{SC} = 0.003	0.001
	Within samples	461	0.28698	67.98	Φ_{ST} = 0.320	< 0.001	486	0.75818	80.16	Φ_{ST} = 0.198	< 0.001
Four regions	Among regions	3	0.10765	25.96	Φ_{CT} = 0.259	0.044	3	0.15482	16.53	Φ_{CT} = 0.165	0.009
(AS+AO, WM, EM, IO)	Among samples within regions	7	0.02007	4.84	Φ_{SC} = 0.065	<0.001	7	0.02373	2.53	Φ_{SC} = 0.030	<0.001
	Within samples	461	0.28698	69.20	Φ_{ST} = 0.307	<0.001	486	0.75818	80.94	Φ_{ST} = 0.191	<0.001
Four regions	Among regions	3	0.17745	38.01	Φ_{CT} = 0.380	0.005	3	0.24831	24.63	Φ_{CT} = 0.246	0.006
(AO, AS+WM, EM, IO)	Among samples within regions	7	0.00248	0.53	Φ_{SC} = 0.009	< 0.001	7	0.00161	0.16	Φ_{SC} = 0.002	< 0.001
	Within samples	461	0.28698	61.46	Φ_{ST} = 0.385	< 0.001	486	0.75818	75.21	Φ_{ST} = 0.248	< 0.001
Three regions	Among regions	2	0.09743	26.02	Φ_{CT} = 0.260	0.022	2	0.18058	21.63	Φ_{CT} = 0.216	0.023
(AO, AS+WM, EM)	Among samples within regions	7	0.00277	0.74	Φ_{SC} = 0.010	0.009	7	0.00392	0.47	Φ_{SC} = 0.006	0.002
	Within samples	416	0.27428	73.24	Φ_{ST} = 0.268	< 0.001	440	0.65050	77.90	Φ_{ST} = 0.221	< 0.001

Table S5. *Aristeus antennatus*. Fu's (1997) F_S and Ramos-Onsins & Rozas' (2002) R_2 neutrality tests for 16S rDNA and COI genes for samples pooled within regions and total. ^{ns} non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

	16S rDNA		COI	
Region	Fu's F_S	R_2	Fu's F_S	R_2
Atlantic Ocean	-1.709^{ns}	0.088^{ns}	-5.165*	0.063*
Western Mediterranean	-104.782***	0.008*	-95.080***	0.011***
Eastern Mediterranean	-1.450^{ns}	0.092^{ns}	-2.292^{ns}	0.077^{ns}
Indian Ocean	-18.841***	0.035**	-22.081***	0.038***
Total	-138.196***	0.007*	-162.021***	0.011**

Table S6. *Aristeus antennatus*. List of concatenated 16S rDNA and COI haplotypes detected in the eleven sampling sites.

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	Total	Haplogroup
h1	0.002						1						1	HG1
h2	0.536	7	29	31	34	23	38	32	19	21	14		248	HG1
h3	0.002						1						1	HG1
h4	0.002						1						1	HG2
h5	0.002						1						1	HG1
h6	0.002						1						1	HG2
h7	0.002						1						1	HG1
h8	0.002						1						1	HG1
h9	0.006				1		1		1				3	HG1
h10	0.002						1						1	HG1
h11	0.002						1						1	HG1
h12	0.002						1						1	HG2
h13	0.002						1						1	HG1
h14	0.002						1						1	HG1
h15	0.002						1						1	HG1
h16	0.004				1		1						2	HG1
h17	0.004						1		1				2	HG2
h18	0.002						1						1	HG1
h19	0.002						1						1	HG1
h20	0.002						1						1	HG1
h21	0.002						1						1	HG1
h22	0.045	3	2	1					5		10		21	HG2
h23	0.019	2		1		1		1	1		3		9	HG2

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	Total	Haplogroup
h24	0.002								1				1	HG2
h25	0.002								1				1	HG2
h26	0.002								1				1	HG1
h27	0.002								1				1	HG1
h28	0.002								1				1	HG1
h29	0.002								1				1	HG1
h30	0.002								1				1	HG1
h31	0.002								1				1	HG2
h32	0.002								1				1	HG1
h33	0.004			1					1				2	HG1
h34	0.009									4			4	HG1
h35	0.004							1		1			2	HG1
h36	0.002									1			1	HG2
h37	0.002									1			1	HG1
h38	0.002									1			1	HG2
h39	0.002									1			1	HG2
h40	0.002									1			1	HG1
h41	0.009	2								1		1	4	HG1
h42	0.002	1											1	HG2
h43	0.006	1	1									1	3	HG2
h44	0.002	1											1	HG2
h45	0.002	1											1	HG2
h46	0.002	1											1	HG2
h47	0.002	1											1	HG2
h48	0.011	4										1	5	HG2

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	Total	Haplogroup
h49	0.004	1		1									2	HG2
h50	0.011	2										3	5	HG2
h51	0.002	1											1	HG1
h52	0.002	1											1	HG2
h53	0.002	1											1	HG2
h54	0.006	1		1							1		3	HG2
h55	0.002	1											1	HG2
h56	0.002	1											1	HG1
h57	0.002	1											1	HG1
h58	0.002			1									1	HG1
h59	0.002			1									1	HG1
h60	0.002			1									1	HG1
h61	0.002			1									1	HG1
h62	0.004		1	1									2	HG1
h63	0.002											1	1	HG2
h64	0.002											1	1	HG2
h65	0.002											1	1	HG2
h66	0.002											1	1	HG2
h67	0.002											1	1	HG2
h68	0.004											2	2	HG2
h69	0.002											1	1	HG1
h70	0.002											1	1	HG2
h71	0.006											3	3	HG2
h72	0.002											1	1	HG2
h73	0.002											1	1	HG2

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	Total	Haplogroup
h74	0.002											1	1	HG2
h75	0.002											1	1	HG2
h76	0.002											1	1	HG2
h77	0.002											1	1	HG2
h78	0.002											1	1	HG2
h79	0.002											1	1	HG2
h80	0.002											1	1	HG2
h81	0.002											1	1	HG2
h82	0.002											1	1	HG2
h83	0.002											1	1	HG2
h84	0.002											1	1	HG2
h85	0.002											1	1	HG2
h86	0.002											1	1	HG2
h87	0.002											1	1	HG2
h88	0.006											3	3	HG2
h89	0.002											1	1	HG2
h90	0.004			1								1	2	HG1
h91	0.002											1	1	HG2
h92	0.004											2	2	HG2
h93	0.002											1	1	HG2
h94	0.002											1	1	HG1
h95	0.002											1	1	HG2
h96	0.002											1	1	HG2
h97	0.004							2					2	HG1
h98	0.002							1					1	HG1

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	Total	Haplogroup
h99	0.004					1		1					2	HG1
h100	0.004							1			1		2	HG1
h101	0.002							1					1	HG1
h102	0.002							1					1	HG2
h103	0.002							1					1	HG2
h104	0.002							1					1	HG1
h105	0.002							1					1	HG1
h106	0.002							1					1	HG1
h107	0.002							1					1	HG1
h108	0.002		1										1	HG1
h109	0.002		1										1	HG1
h110	0.002		1										1	HG2
h111	0.002		1										1	HG2
h112	0.002		1										1	HG1
h113	0.002		1										1	HG1
h114	0.002		1										1	HG1
h115	0.002		1										1	HG1
h116	0.002		1										1	HG2
h117	0.002		1										1	HG1
h118	0.002		1										1	HG1
h119	0.002		1										1	HG2
h120	0.002		1										1	HG1
h121	0.002		1										1	HG1
h122	0.002		1										1	HG1
h123	0.002		1										1	HG1

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	Total	Haplogroup
h124	0.002		1										1	HG1
h125	0.002		1										1	HG1
h126	0.002		1										1	HG1
h127	0.002										1		1	HG1
h128	0.002										1		1	HG2
h129	0.002										1		1	HG2
h130	0.002										1		1	HG2
h131	0.002										1		1	HG1
h132	0.002										1		1	HG2
h133	0.002										1		1	HG1
h134	0.002										1		1	HG2
h135	0.002										1		1	HG1
h136	0.002										1		1	HG2
h137	0.002					1							1	HG1
h138	0.002					1							1	HG1
h139	0.002					1							1	HG1
h140	0.002					1							1	HG1
h141	0.002					1							1	HG1
h142	0.002					1							1	HG1
h143	0.002					1							1	HG2
h144	0.002				1								1	HG2
h145	0.002				1								1	HG1
h146	0.002				1								1	HG1
h147	0.002				1								1	HG2
h148	0.002				1								1	HG1

Haplotype	Freq	Faro	Alborán Sea	Almería	Sóller	Cabrera	Palamós	Gulf of Lion	Genoa	Palermo	Ionian Sea	Mozambique	Total	Haplogroup
h149	0.002				1								1	HG2
h150	0.002				1								1	HG1
h151	0.002				1								1	HG1
h152	0.002				1								1	HG1
		34	53	41	45	32	58	46	37	32	39	46	463	

Figure S1. *Aristeus antennatus*. Median-joining nework of haplotypes detected for 16S rDNA (A) and COI (B) genes from the sampling locations of the Atlantic Ocean (black), Western Mediterranean Sea (yellow), Eastern Mediterranean Sea (red) and Indian Ocean (blue). The area of each circle is proportional to the number of individuals exhibiting that haplotype. Each line in the network represents one mutational step, and vertices represent missing or undetected haplotypes.

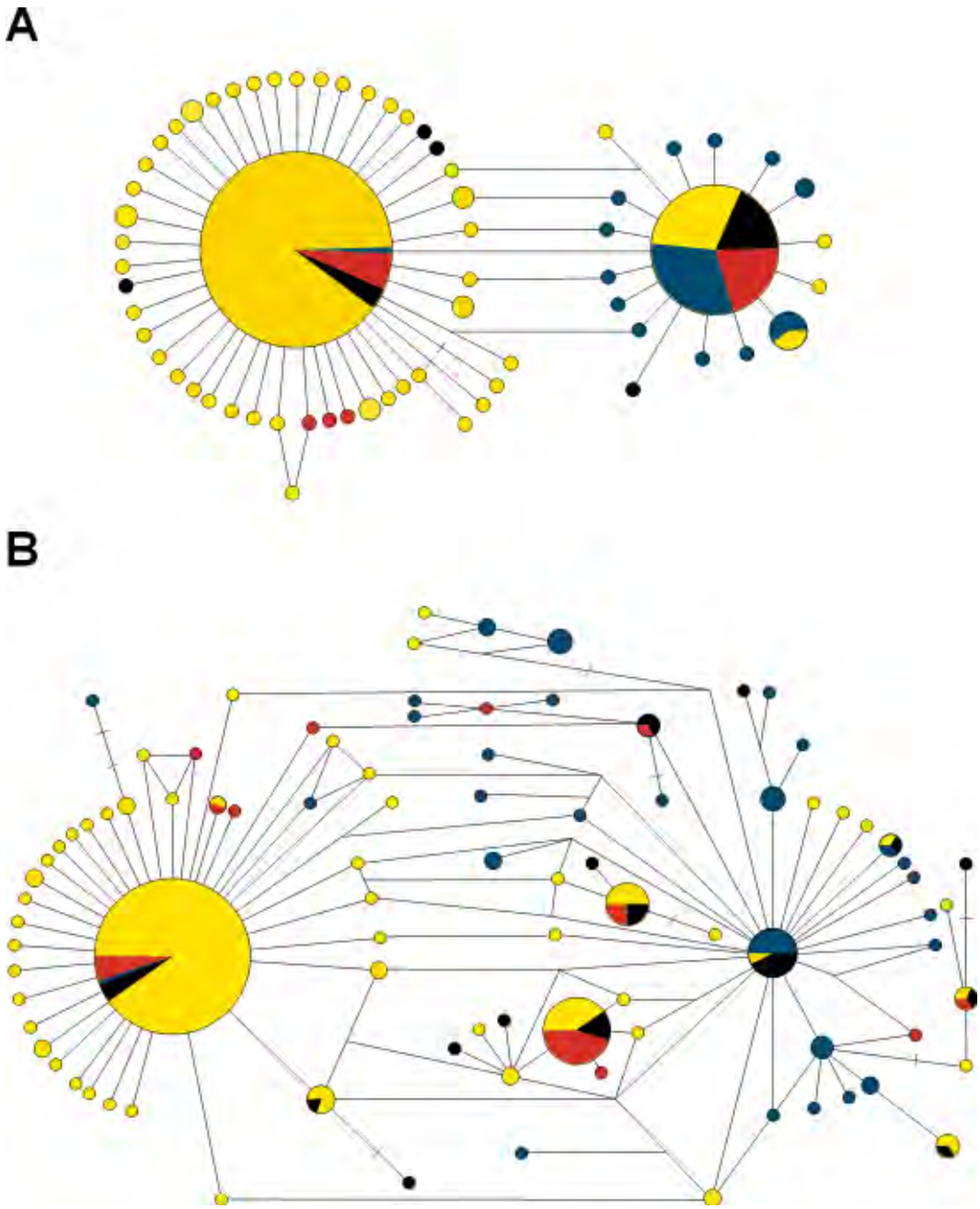
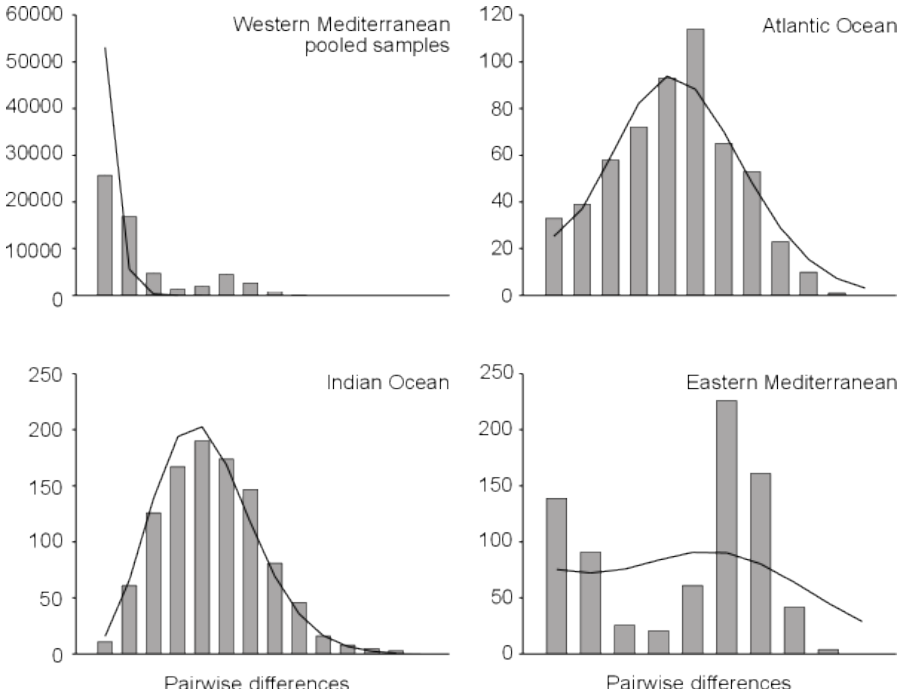


Figure S2. *Aristeus antennatus*. Frequency distributions of the number of pairwise nucleotide differences (mismatch) between merged haplotypes for the four regions considered. Solid line is the theoretical distribution under the assumption of population expansion.



Supplementary material Article II

The following supplement accompanies the article

Analysis of genetic variability in *Aristaeomorpha foliacea* (Crustacea, Aristeidae) using DNA-ISSR (Inter Simple Sequence Repeat) markers

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Comptes Rendus Biologies 334:705-712 (2011)

Supplement. These additional data include a complete list of all ISSRs loci detected and their frequency in each of the localities sampled.

Appendix 1. Presence (1) or absence (0) of ISSR fragments in the seven local samples of *Aristaeomorpha foliacea*. Loci present in all samples are in bold and loci identified as under selection by Bayescan (see text) are underlined. The locus 121 is an autapomorphy.

Primer	Locus	IBI		TYR		MAZ		PPA		ION		AEG		MOZ	
			freq		freq		freq		freq		freq		freq		freq
IT1	1	1	0,0204	1	0,0208	1	0,0769	0	0	1	0,0540	0	0	1	0,0238
	2	1	0,1428	1	0,0416	1	0,1794	1	0,1666	1	0,0540	1	0,1590	1	0,0476
	3	0	0	1	0,0208	0	0	1	0,0555	0	0	1	0,0227	0	0
	4	1	0,1020	1	0,0833	1	0,1025	1	0,0555	1	0,0810	1	0,1363	1	0,1190
	5	1	0,2449	1	0,2083	1	0,1538	1	0,1666	1	0,0810	1	0,3181	1	0,1190
	6	1	0,7142	1	0,7083	1	0,8974	1	0,4722	1	0,5945	1	0,7954	1	0,8333
	7	1	0,0816	1	0,0416	1	0,0256	1	0,0277	1	0,0270	1	0,0454	1	0,0238
	8	1	0,0612	1	0,1041	1	0,0769	1	0,0277	0	0	1	0,1136	1	0,0714
	9	1	0,4285	1	0,3333	1	0,2307	1	0,1944	1	0,2973	1	0,5	1	0,1666
	10	1	0,2653	1	0,2083	0	0	1	0,1111	1	0,0810	1	0,0454	0	0
	11	1	0,1836	1	0,2708	1	0,4615	1	0,2777	1	0,1621	1	0,2954	1	0,1428
	12	1	0,0204	1	0,0416	1	0,0512	1	0,0277	1	0,0270	0	0	1	0,0714
	13	1	0,0612	1	0,0208	1	0,0256	1	0,0277	1	0,0270	1	0,0681	1	0,0476
	14	1	0,0408	0	0	0	0	1	0,0277	0	0	1	0,0454	1	0,0238
	15	1	0,0612	0	0	1	0,0512	1	0,0277	1	0,0540	1	0,1136	1	0,0238
	16	1	0,1632	1	0,125	1	0,3076	1	0,0833	1	0,1891	1	0,3636	1	0,1904
	17	1	0,3469	1	0,25	1	0,0769	1	0,0833	1	0,1891	1	0,1590	1	0,2381
	18	1	0,1428	1	0,2083	1	0,1025	1	0,0277	1	0,0540	1	0,1818	1	0,1428
	19	1	0,0612	1	0,1041	0	0	1	0,0555	1	0,0540	1	0,0909	1	0,1190
	20	1	0,1836	1	0,1458	1	0,0769	1	0,0555	0	0	1	0,2727	1	0,0476
	21	1	0,0408	1	0,1041	0	0	0	0	1	0,1351	1	0,0681	0	0
	22	1	0,1428	1	0,1875	1	0,0512	1	0,0277	1	0,1621	1	0,1818	1	0,0714
	23	1	0,1224	1	0,125	1	0,3076	1	0,0277	1	0,1621	1	0,2727	1	0,0476
	24	1	0,0204	1	0,1041	1	0,0256	1	0,0555	1	0,0270	1	0,0454	0	0
	25	1	0,1428	1	0,0625	1	0,1794	1	0,1111	1	0,0540	1	0,25	1	0,0476
	26	1	0,2857	1	0,1666	1	0,4615	1	0,1111	1	0,1891	1	0,3636	1	0,0952
	27	1	0,0612	1	0,125	0	0	0	0	1	0,0270	1	0,0681	0	0
	28	1	0,2040	1	0,1041	1	0,1794	0	0	1	0,2162	1	0,2727	1	0,0476
	29	1	0,1632	1	0,0208	1	0,0256	0	0	1	0,0270	1	0,0227	0	0
	30	1	0,2244	1	0,0416	1	0,0769	0	0	1	0,0810	1	0,1136	1	0,1428
	31	1	0,0612	1	0,0625	1	0,1282	1	0,0555	1	0,0540	1	0,2272	1	0,0714
	32	1	0,1020	0	0	1	0,0512	0	0	1	0,0810	1	0,1363	0	0
	33	1	0,0816	1	0,0208	0	0	0	0	1	0,0270	1	0,1136	1	0,0714
	34	1	0,4081	1	0,1875	1	0,2307	1	0,0833	1	0,1081	1	0,4318	1	0,1428
IT2	35	1	0,6326	1	0,7708	1	0,5897	1	0,5	1	0,6486	1	0,6363	1	0,7381
	36	1	0,0612	1	0,1041	1	0,1538	1	0,0277	1	0,0540	1	0,1136	1	0,1428
	37	1	0,0204	1	0,0625	1	0,0512	0	0	0	0	1	0,0227	0	0
	38	1	0,0816	1	0,0833	0	0	1	0,1388	1	0,0270	1	0,0909	0	0
	39	1	0,0408	1	0,0416	1	0,0256	1	0,0277	0	0	0	0	1	0,0238
	1	1	0,0408	1	0,0416	0	0	0	0	1	0,0810	0	0	0	0
	41	1	0,4081	1	0,1458	1	0,3076	1	0,5833	1	0,4594	1	0,4318	1	0,0714
	42	1	0,1224	1	0,2916	1	0,1538	1	0,1388	1	0,0540	1	0,0681	1	0,0238
	43	1	0,1836	1	0,1458	1	0,3076	0	0	1	0,1621	1	0,2272	1	0,0952
	44	1	0,1632	1	0,0416	1	0,0769	1	0,0833	1	0,1081	1	0,1136	1	0,0714

Primer	Locus	IBI		TYR		MAZ		PPA		ION		AEG		MOZ	
	45	0	0	1	0,0416	1	0,1025	1	0,0277	0	0	1	0,0909	1	0,0238
	46	1	0,0612	1	0,0416	1	0,0512	1	0,0277	1	0,0540	1	0,0227	1	0,0476
	47	1	0,0204	1	0,0208	0	0	0	0	1	0,0270	0	0	0	0
	48	0	0	0	0	1	0,0769	0	0	1	0,0810	1	0,0454	1	0,0238
	49	0	0	0	0	0	0	0	0	0	0	1	0,0454	1	0,0238
	50	1	0,0204	1	0,0208	0	0	0	0	0	0	0	0	1	0,0238
	51	0	0	0	0	0	0	1	0,0277	0	0	0	0	1	0,0238
	52	1	0,0204	1	0,0208	0	0	0	0	0	0	1	0,0227	0	0
	53	1	0,0204	1	0,0208	0	0	0	0	0	0	0	0	1	0,0238
	54	1	0,0204	1	0,0208	0	0	1	0,0277	1	0,0270	1	0,0227	0	0
	55	0	0	1	0,0416	0	0	0	0	1	0,0270	0	0	0	0
	56	1	0,0204	0	0	0	0	0	0	0	0	1	0,0227	0	0
	57	1	0,0816	1	0,0625	1	0,0256	0	0	0	0	1	0,0454	0	0
	58	1	0,4489	1	0,3541	1	0,3589	1	0,3055	1	0,2432	1	0,5681	1	0,1190
	59	1	0,1020	0	0	0	0	0	0	0	0	1	0,0681	0	0
	60	1	0,2244	1	0,5	1	0,5897	1	0,1944	1	0,4054	1	0,25	1	0,4523
	61	1	0,1428	1	0,2083	1	0,1282	1	0,1388	1	0,0270	1	0,1363	0	0
	62	1	0,0204	0	0	1	0,0769	1	0,0277	1	0,0270	0	0	1	0,1666
	63	1	0,0204	1	0,0208	1	0,0256	1	0,0277	0	0	1	0,0454	1	0,0238
IT3	64	0	0	1	0,0208	1	0,0256	0	0	1	0,0270	0	0	0	0
	65	1	0,1020	1	0,0833	1	0,1025	1	0,0833	1	0,0540	1	0,0681	1	0,0238
	66	1	0,1020	1	0,0833	1	0,0256	0	0	0	0	1	0,1136	0	0
	67	1	0,0408	1	0,0416	1	0,1282	0	0	0	0	0	0	0	0
	68	0	0	0	0	0	0	1	0,0555	0	0	1	0,0227	1	0,0238
	69	1	0,0816	1	0,0416	1	0,0256	1	0,0833	1	0,0270	0	0	0	0
	70	1	0,3061	1	0,2083	1	0,2051	1	0,3888	1	0,2162	1	0,1363	1	0,1904
	71	1	0,4081	1	0,4166	1	0,6410	1	0,1666	1	0,5405	1	0,3181	1	0,7142
	72	1	0,2857	1	0,2291	1	0,0769	1	0,25	1	0,1351	1	0,2954	1	0,2381
	73	1	0,1020	1	0,1041	1	0,1538	1	0,1111	1	0,0810	1	0,2045	1	0,0238
	74	1	0,1836	1	0,1041	1	0,0512	1	0,0277	1	0,0270	1	0,1363	1	0,0238
	75	1	0,1836	1	0,2291	1	0,3076	1	0,2222	1	0,2702	1	0,1818	1	0,4047
	76	1	0,0204	1	0,0416	1	0,2051	1	0,0555	1	0,0540	1	0,0454	1	0,0952
	77	1	0,1428	1	0,1041	1	0,0256	1	0,0277	1	0,0270	1	0,1363	1	0,0238
	78	1	0,0204	1	0,0416	0	0	1	0,0555	1	0,0540	0	0	0	0
	79	0	0	1	0,0416	0	0	0	0	0	0	1	0,0227	1	0,0952
	80	1	0,0816	1	0,0208	1	0,0256	0	0	1	0,0270	1	0,0454	1	0,0238
	81	1	0,0408	0	0	0	0	0	0	1	0,0270	0	0	0	0
	82	1	0,0204	0	0	1	0,0256	0	0	0	0	1	0,0227	0	0
	83	1	0,1020	0	0	1	0,0512	1	0,0277	0	0	1	0,0909	1	0,0238
	84	1	0,2244	1	0,1666	1	0,2307	1	0,1666	1	0,0810	1	0,0681	1	0,0238
	85	1	0,0816	1	0,0833	1	0,1025	0	0	1	0,0540	1	0,0681	1	0,0714
	86	1	0,2244	1	0,1875	1	0,1282	1	0,0555	1	0,1081	1	0,1818	1	0,0476
	87	1	0,1428	1	0,0625	1	0,0256	1	0,0277	1	0,0270	1	0,1136	0	0
	88	1	0,0204	1	0,0625	1	0,0512	0	0	0	0	1	0,0227	0	0
	89	1	0,0612	1	0,0416	1	0,0256	1	0,0833	0	0	1	0,0454	1	0,0238
SAS2	90	1	0,1020	1	0,0833	1	0,1025	1	0,1111	1	0,1351	1	0,1590	1	0,0952
	91	1	0,3673	1	0,3958	1	0,2307	1	0,3611	1	0,1351	1	0,2954	1	0,0476
	92	1	0,1224	1	0,0416	1	0,1794	1	0,1944	1	0,1621	1	0,1363	1	0,0952

Primer	Locus	IBI		TYR		MAZ		PPA		ION		AEG		MOZ	
	93	1	0,0204	1	0,0416	1	0,2051	1	0,0277	1	0,1081	1	0,0681	1	0,2142
	94	0	0	1	0,0416	0	0	0	0	1	0,0810	0	0	1	0,0476
	95	0	0	1	0,0208	1	0,0256	0	0	1	0,0270	1	0,0227	0	0
	96	1	0,0408	1	0,1041	1	0,0256	1	0,0833	0	0	1	0,0454	1	0,0476
	97	1	0,1836	1	0,0833	1	0,2051	1	0,0555	1	0,2162	1	0,2727	1	0,2142
	98	1	0,0408	1	0,1666	1	0,0512	1	0,0277	1	0,0270	1	0,0227	1	0,0476
	99	1	0,0204	0	0	1	0,0512	0	0	0	0	1	0,0227	1	0,0238
	100	1	0,0204	0	0	1	0,0256	0	0	0	0	1	0,0454	1	0,0714
	101	1	0,0408	1	0,0208	0	0	0	0	1	0,0270	1	0,0227	1	0,1428
	102	1	0,1428	1	0,1666	1	0,2051	1	0,0833	1	0,1081	1	0,1363	1	0,3809
	103	1	0,3061	1	0,4583	1	0,4871	1	0,1944	1	0,3783	1	0,4545	1	0,3095
	104	1	0,1224	1	0,1666	0	0	1	0,0277	1	0,0270	1	0,2954	1	0,0476
	105	1	0,3061	1	0,25	1	0,1538	1	0,25	1	0,1891	1	0,2954	1	0,3095
	106	1	0,0408	1	0,0208	0	0	1	0,0277	0	0	1	0,0454	1	0,0238
	107	1	0,1428	1	0,0833	1	0,0256	1	0,0833	1	0,1081	1	0,0909	1	0,1190
	108	1	0,1020	1	0,0833	1	0,1025	1	0,0277	1	0,0810	1	0,1363	1	0,1428
	109	1	0,0612	1	0,0416	1	0,0256	1	0,0555	1	0,0810	0	0	1	0,1190
	110	1	0,0612	1	0,0416	1	0,1025	1	0,0555	1	0,0540	1	0,0681	1	0,1190
	111	1	0,0204	1	0,125	1	0,2307	1	0,0833	1	0,0540	1	0,0227	1	0,0714
	112	1	0,1836	1	0,2083	1	0,2051	1	0,1388	1	0,1621	1	0,2272	1	0,1666
	113	1	0,0612	1	0,1875	0	0	0	0	0	0	1	0,1818	0	0
	114	1	0,3877	1	0,2083	1	0,2307	1	0,1388	1	0,2162	1	0,4090	1	0,2619
	115	1	0,0612	1	0,0833	1	0,0512	1	0,0277	1	0,0270	1	0,0681	0	0
	116	1	0,1020	1	0,2291	1	0,1025	1	0,0277	1	0,1351	1	0,1590	1	0,0952
	117	0	0	1	0,0416	1	0,0256	0	0	1	0,0270	1	0,0227	1	0,0476
	118	1	0,1020	1	0,1875	1	0,1025	1	0,0555	1	0,1891	1	0,2045	1	0,0952
	119	1	0,1632	1	0,0833	1	0,2307	1	0,0555	1	0,1351	1	0,1818	1	0,1666
	120	1	0,1224	1	0,1458	1	0,1282	1	0,0277	1	0,1081	1	0,0227	1	0,1428
SAS3	121	0	0	0	0	0	0	0	0	0	0	0	0	1	0,0714
	122	0	0	0	0	1	0,0256	0	0	0	0	1	0,0227	1	0,0476
	123	1	0,0408	1	0,0208	1	0,0256	1	0,0277	0	0	1	0,0227	1	0,0952
	124	1	0,7346	1	0,7708	1	0,2307	1	0,3888	1	0,4324	1	0,3863	1	0,3333
	125	1	0,0204	1	0,0416	1	0,0769	1	0,0555	0	0	0	0	1	0,0714
	126	0	0	1	0,0416	1	0,0256	0	0	1	0,0540	0	0	1	0,0476
	127	1	0,0612	1	0,0416	1	0,1025	1	0,0277	1	0,0810	1	0,1136	1	0,1428
	128	1	0,1836	1	0,0625	1	0,1025	1	0,0555	1	0,1081	1	0,0909	1	0,1190
	129	1	0,2040	1	0,0833	1	0,1794	1	0,1944	1	0,3243	1	0,2045	1	0,2857
	130	1	0,1020	1	0,1666	1	0,1538	1	0,0555	1	0,0270	1	0,1136	1	0,0238
	131	1	0,0408	1	0,0833	1	0,1025	1	0,0277	0	0	1	0,0454	1	0,0476
	132	1	0,0816	1	0,0833	0	0	1	0,1666	1	0,1351	1	0,0909	1	0,0714
	133	1	0,0408	1	0,0208	1	0,0256	1	0,0555	0	0	1	0,0454	1	0,0476
	134	0	0	1	0,0416	1	0,0512	1	0,0555	1	0,0270	0	0	1	0,0476
	135	1	0,0612	1	0,0625	0	0	1	0,0277	1	0,1351	1	0,0454	1	0,2142
	136	1	0,1428	1	0,1458	1	0,1538	1	0,0555	1	0,1081	1	0,1363	1	0,1190
	137	1	0,2449	1	0,1666	1	0,0256	1	0,1111	1	0,0540	1	0,2272	1	0,0238
	138	1	0,5306	1	0,5833	1	0,6153	1	0,2222	1	0,5135	1	0,6136	1	0,6428
	139	1	0,0612	1	0,0833	0	0	1	0,0555	1	0,0270	1	0,0227	0	0
	140	1	0,2244	1	0,1875	1	0,2051	1	0,0833	1	0,1351	1	0,1136	1	0,1666

Primer	Locus	IBI		TYR		MAZ		PPA		ION		AEG		MOZ	
	141	1	0,0612	1	0,0416	1	0,0256	0	0	0	0	0	0	0	0
	142	1	0,3061	1	0,1666	1	0,1794	1	0,0833	1	0,2432	1	0,1818	1	0,2857
	143	1	0,5102	1	0,5	1	0,4871	1	0,2777	1	0,2432	1	0,6136	1	0,3571
	144	1	0,9795	1	0,8958	1	0,9743	1	0,7222	1	0,9729	1	0,9318	1	0,9761
	145	1	0,1224	1	0,25	1	0,1538	1	0,1388	0	0	1	0,1136	1	0,1190
	146	1	0,0204	1	0,0416	1	0,0256	0	0	1	0,0270	0	0	1	0,0238
	147	1	0,0816	1	0,0416	1	0,0512	0	0	0	0	1	0,0227	1	0,0238
	148	1	0,0204	1	0,0208	1	0,0512	1	0,0555	1	0,0270	0	0	1	0,0238
	149	1	0,1020	1	0,0416	1	0,1025	0	0	1	0,0270	1	0,0909	1	0,0476
	150	0	0	1	0,0208	0	0	0	0	1	0,0810	0	0	1	0,0238

Supplementary material Article III

The following supplement accompanies the article

Deep genetic divergence in giant red shrimp *Aristaeomorpha foliacea* (Risso, 1827) across a wide distributional range

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Journal of Sea Research (2012) xxx:xxx-xxx

Supplement. These additional data include a complete list of haplotypes for COI gene and a list of the variable nucleotide positions that define each haplotype.

Table S1. *Aristaeomorpha foliacea*. List of COI haplotypes detected in the eight sampling sites with respective GenBank accession number. N = number of sequences per haplotype in each locality and total. Freq: frequency of each haplotype per locality and total. Sample codes are as in Table 1.

Haplotype	GenBank Acc. Number	IBI		TYR		MAZ		PPA		ION		AEG		MOZ		AUS		TOTAL	
		N	Freq	N	Freq	N	Freq	N	Freq	N	Freq	N	Freq	N	Freq	N	Freq	N	Freq
h1	JN676306	29	0.57	23	0.51	12	0.3	16	0.43	4	0.11	4	0.09					88	0.278
h2	JN676307	4	0.08	6	0.13	17	0.43	7	0.19	23	0.62	30	0.68					87	0.274
h3	JN676308	9	0.18	8	0.18	5	0.13	4	0.11	3	0.08	2	0.05					31	0.098
h4	JN676309	1	0.02	2	0.04	1	0.03	1	0.03									5	0.016
h5	JN676310	1	0.02							1	0.03							2	0.006
h6	JN676311	1	0.02															1	0.003
h7	JN676312	1	0.02			1	0.03	1	0.03									3	0.009
h8	JN676313	1	0.02															1	0.003
h9	JN676314	1	0.02															1	0.003
h10	JN676315	1	0.02					1	0.03									2	0.006
h11	JN676316	1	0.02															1	0.003
h12	JN676317	1	0.02	3	0.07	2	0.05	3	0.08	3	0.08	7	0.16					19	0.060
h13	JN676318			1	0.02													1	0.003
h14	JN676319			1	0.02													1	0.003
h15	JN676320			1	0.02													1	0.003
h16	JN676321					1	0.03											1	0.003
h17	JN676322					1	0.03											1	0.003
h18	JN676323							1	0.03									1	0.003
h19	JN676324							1	0.03									1	0.003
h20	JN676325							1	0.03									1	0.003
h21	JN676326							1	0.03									1	0.003
h22	JN676327									1	0.03							1	0.003
h23	JN676328									1	0.03							1	0.003
h24	JN676329									1	0.03							1	0.003
h25	JN676330											1	0.02					1	0.003
h26	JN676331													28	0.67			28	0.088
h27	JN676332													3	0.07			3	0.009
h28	JN676333													1	0.02			1	0.003

Haplotype	GenBank Acc. Number	IBI		TYR		MAZ		PPA		ION		AEG		MOZ		AUS		TOTAL	
		N	Freq	N	Freq	N	Freq	N	Freq	N	Freq	N	Freq	N	Freq	N	Freq	N	Freq
h29	JN676334													1	0.02			1	0.003
h30	JN676335													1	0.02			1	0.003
h31	JN676336													1	0.02			1	0.003
h32	JN676337													1	0.02			1	0.003
h33	JN676338													1	0.02			1	0.003
h34	JN676339													1	0.02			1	0.003
h35	JN676340													1	0.02			1	0.003
h36	JN676341													1	0.02			1	0.003
h37	JN676342													1	0.02			1	0.003
h38	JN676343													1	0.02			1	0.003
h39	JN676344															1	0.05	1	0.003
h40	JN676345															2	0.1	2	0.006
h41	JN676346															1	0.05	1	0.003
h42	JN676347															1	0.05	1	0.003
h43	JN676348															1	0.05	1	0.003
h44	JN676349															2	0.1	2	0.006
h45	JN676350															1	0.05	1	0.003
h46	JN676351															1	0.05	1	0.003
h47	JN676352															1	0.05	1	0.003
h48	JN676353															1	0.05	1	0.003
h49	JN676354															1	0.05	1	0.003
h50	JN676355															1	0.05	1	0.003
h51	JN676359															1	0.05	1	0.003
h52	JN676357															1	0.05	1	0.003
h53	JN676358															1	0.05	1	0.003
h54	JN676359															1	0.05	1	0.003
h55	JN676360															1	0.05	1	0.003
h56	JN676361															1	0.05	1	0.003
h57	JN676362															1	0.05	1	0.003

Table S2. *Aristaeomorpha foliacea.* Variable nucleotide positions defining 57 haplotypes in the 657 bp of COI region. The fixed nucleotide position for the Mozambique Channel haplotypes is highlighted in grey and the fixed nucleotide positions for Australian haplotypes are in bold.

Haplotype Code number	Variable nucleotide positions
	1111111111222222222222223333333333344444444444555555556666666666
	2233567889011237899900345667779999002344557780111244467801223567002223555 67
	2365802736870324081476735703258016958081469173404953694083847970236037536954
h1	GTAAAAAAATTAATCTAGATCCACGTATTTCGTCTATCACTTCACATCTTGAAACGTGAGGTATTCAATGCGTTCG
h2	....G.....A.....
h3	..G.....
h4	.....T.....
h5	.....T.....
h6	.....G.....T.....
h7	.....A.....
h8	.....T.....
h9	..G.....C.....
h10	.....G.....
h11	.....T.....A.....
h12	.....T.....
h13	.....G.....
h14	..G.....A.....
h15	..G.....G.....
h16	.....T.....A.....
h17	.....C.....
h18	..G.....C.....
h19	.....G.....
h20	..G.....G.....
h21	.....G.....
h22	.....G.....
h23	....G.....A.....G.....
h24	.....A.....
h25	.....G.....
h26	A.....C.....G.....G.....G.....C.....

Haplotype Code number	Variable nucleotide positions
	1111111111222222222222223333333333344444444444555555556666666666
	2233567889011237899900345667779999002344557780111244467801223567002223555 67
	2365802736870324081476735703258016958081469173404953694083847970236037536954
h28	A.....C.....G.....G.....G.....C...C...
h29	A.....C....A.....GC.....G.....G.....C.....
h30	A.....A.C.....G.....G....G...G.....C.....
h31	A.....C.....T.....G.....G.....G.....C.....
h32	A.....C.....C....G.....G.....G.....C.....
h33	A.....G.....C.....G.....G.....G.....C.....
h34	A.....GC.....G.....G.....G.....C.....
h35	A.....C.....G.....G.....G.....C.....A
h36	A.....C.....T.....G.....G.....G.....C.....
h37	A.....C.....G.....G.....G.....
h38	A.....C.....G.....G..C.....G.....C.....
h39	ACG**G**..**GG**.**CC**GGC**T**CGA..T..TA**CG**CACAA.T...TG.C**C**...GC.**CCA**..**G**...**A**G..C**C**.C**T**....T**A**.**C**..
h40	.CG**G**..**GG**.**CC**GGC**T**C.A..TT.TA**CG**CACAA.T...T..C**C**...GCT**CCA**..**G**...**A**G..C**C**.C**T**.....**A**.**C**.A
h41	ACG**G**..**GG**.**CC**GGC**T**CGA..T..TA**CG**CACAA.T...T..C**C**...GC.**CCA**..**G**...**A**G..C**C**.C**T**....T**A**.**C**..
h42	.CG**G**..**GG**.**CC**GGC**T**C.A..TT.TA**CG**CACAA.T...T..C**C**...GCT**CCA**..**G**...**A**G..C**C**..**T**.....**A**.**C**.A
h43	ACG**G**..**GG**.**CC**GGC**T**C.A..T..TA**CG**CACAA.T...T..C**C**...GCT**CCA**..**G**...**A**G...**C**.C**T**.....**A**.**C**.A
h44	.CG**G**..**GG**.**CC**GGC**T**C.A..T..TA**CG**CACAA.T...T..C**C**...GCT**CCA**..**G**...**A**G.AC**C**.C**T**.....**A**.**C**.A
h45	.CG**G**..**GG**.**CC**G.C**T**C.A..TT.TA**CG**CACAA.T...T..C**C**...GCT**CCA**..**G**...**A**G..C**C**.C**T**.....**A**.**C**.A
h46	.CG**G**..**GG**.**CC**GGC**T**C.A..T..TA**CG**CACAACT..CT..C**C**...GCT**CCA**..**G**...**A**G..C**C**.C**T**.....**A**.**C**.A
h47	.CG**G**..**GG**.**CC**GGC**T**C.A..T..TA**CG**CACAA.T...T..C**C**...GCT**CCA**..**G**...**A**GA.C**C**.C**T**.....**A**.**C**.A
h48	.CG**G**.G**GG**.**CC**GGC**T**..A..T..TA**CG**CACAA.T...T..C**C**...GCT**CCA**..**G**.A.**A**G..C**C**.C**T**.....**A**.**C**.A
h49	.CG**G**..**GG**.**CC**GGC**T**C.A..T..TA**CG**CACAA.TC..T..C**C**...GCT**CCA**..**G**...**A**GA.C**C**.C**T**.....**A**.**C**.A
h50	.CG**G**..**GG**.**CC**GGC**T**C.A..T..TA**CG**CACAA.T...T..C**C**T..GCT**CCA**..**G**...**A**G.AC**C**.C**T**.....**A**.**C**.A
h51	..G**G**..**GG**.**CC**GGC**T**C.A..T..TA**CG**CACAA.T...TG.C**C**.T.GCT**CCA**..**G**...**A**G..C**C**.C**T**.....**A**.**C**.A
h52	.CG**G**..**GG**.**CC**GGC**T**C.A..T..TA**CG**CACAA.T.G.T..C**C**...GCT**CCA**..**G**...**A**G..C**C**.C**T**.....**A**.**C**.A
h53	ACG**G**..**GG**.**CC**GGC**T**C.A..TT.TA**CG**CACAA.T...T..C**C**...GCT**CCA**..**G**...**A**G..C**C**.C**T**.....**A**.**C**.A
h54	.CG**G**..**GG**.**CC**GGC**T**C.A..T..TA**CG**CACAA.T...T..C**C**..AGCT**CCA**..**G**...**A**GA.C**C**.C**T**.....**A**.**C**.A
h55	.CG**G**.G**GG**.**CC**GGC**T**..A..T..TA**CG**CACAA.T...T..C**C**...GCT**CCA**..**G**...**A**G..C**C**.C**T**.....**A**.**C**.A
h56	.CG**G**..**GG**.**CC**GGC**T**C.A..T..TA**CG**CACAA.T...T..C**C**...GCT**CCA**..**G**...**A**GA.C**C**..**T**.....**A**.**C**.A
h57	.CG**G**..**GG**.**CC**GGC**T**C.A..TT.TA**CG**CACAA.T...T..C**C**...GC.**CCA**..**G**...**A**G..C**C**.C**T**.....**A**.**C**GA

Supplementary material Article IV

The following supplement accompanies the article

Comparative phylogeography of two Aristeid shrimps of high commercial interest (*Aristeus antennatus* and *Aristaeomorpha foliacea*) using nuclear and mitochondrial markers

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Accepted by PLoS One with minor revisions the 6th September 2012

Supplement. These additional data includes a complete list of haplotypes (COI) and genotypes (PEPCK, NaK). In addition comparison of mitochondrial genetic diversity values and genetic distances tables for nuclear and mitochondrial genes is provided.

Table S1. Comparison of COI genetic diversity estimates detected in the present and previous works. Haplotype diversity (*h*), nucleotide diversity (π), standard deviation (S.D.). ALB: Alborán Sea, WM: Western Mediterranean, EM: Eastern Mediterranean, AO: Atlantic Ocean, MOZ: Mozambique Channel, AUS: North-Western Australia.

A. antennatus	Present work		Fernández et al. [17]	
Location	*h* ± S.D.	π ± S.D.	*h* ± S.D.	π ± S.D.
ALB	0.533 ± 0.1801	0.0029 ± 0.0022	0.458 ± 0.085	0.0020 ± 0.0005
WM	0.378 ± 0.1813	0.0020 ± 0.0016	0.258 ± 0.081	0.0014 ± 0.0005
EM	0.800 ± 0.1001	0.0073 ± 0.0045	0.758 ± 0.050	0.0058 ± 0.0006
AO	0.911 ± 0.0773	0.0052 ± 0.0034	0.863 ± 0.042	0.0062 ± 0.0008
MOZ	0.978 ± 0.0540	0.0062 ± 0.0040	0.961 ± 0.015	0.0070 ± 0.0007
A. foliacea	Present work		Fernández et al. [18]	
Location	*h* ± S.D.	π ± S.D.	*h* ± S.D.	π ± S.D.
WM	0.756 ± 0.1295	0.0022 ± 0.0017	0.649 ± 0.068	0.0015 ± 0.0002
EM	0.644 ± 0.1518	0.0032 ± 0.0024	0.511 ± 0.081	0.0019 ± 0.0003
MOZ	0.667 ± 0.1633	0.0020 ± 0.0016	0.557 ± 0.093	0.0011 ± 0.0002
AUS	1.000 ± 0.0447	0.0062 ± 0.0034	0.990 ± 0.018	0.0058 ± 0.0007

Table S2. GenBank accession numbers for each individual analyzed. COI haplotype number (ch), PEPCK (ph) and NaK (nh) genotype number and number of alleles. Empty rows correspond to individuals with unsuccessful amplification. The individuals used in phylogenetic analyses are in bold. Location codes as in Table 1.

Code		COI (514 bp)		PEPCK (536 bp)				NaK (498 bp)			
Individual	Location	GenBank	Haplotype	GenBank	Genotype	Allele 1	Allele 2	GenBank	Genotype	Allele 1	Allele 2
Av1	MOZ	JQ928293	Av-ch1	JQ928267	Av-ph1	17	18	JQ928233	Av-nh1	45	45
Av2	MOZ	JQ928294	Av-ch2	JQ928267	Av-ph1	17	18	JQ928234	Av-nh2	45	46
Av3	MOZ	JQ928295	Av-ch3	JQ928267	Av-ph1	17	18				
Aa1	ALB	EU908437	Aa-ch2	JQ928270	Aa-ph3	1	1	JQ928240	Aa-nh6	3	13
Aa2	ALB	EU908437	Aa-ch2	JQ928268	Aa-ph1	3	3	JQ928241	Aa-nh7	21	21
Aa3	ALB	EU908437	Aa-ch2					JQ928242	Aa-nh8	6	27
Aa4	ALB	EU908437	Aa-ch2	JQ928268	Aa-ph1	3	3				
Aa5	ALB	EU908437	Aa-ch2	JQ928268	Aa-ph1	3	3	JQ928243	Aa-nh9	28	29
Aa6	ALB	EU908437	Aa-ch2	JQ928268	Aa-ph1	3	3				
Aa7	ALB	EU908437	Aa-ch2								
Aa8	ALB	EU908446	Aa-ch6								
Aa9	ALB	EU908497	Aa-ch14								
Aa10	ALB	GU972654	Aa-ch28	JQ928269	Aa-ph2	3	4	JQ928244	Aa-nh10	11	30
Aa11	WM	EU908437	Aa-ch2	JQ928268	Aa-ph1	3	3	JQ928235	Aa-nh1	21	22
Aa12	WM	EU908437	Aa-ch2	JQ928269	Aa-ph2	3	4	JQ928236	Aa-nh2	19	19
Aa13	WM	EU908437	Aa-ch2	JQ928270	Aa-ph3	1	1				
Aa14	WM	EU908437	Aa-ch2	JQ928271	Aa-ph4	1	2				
Aa15	WM	EU908437	Aa-ch2	JQ928268	Aa-ph1	3	3	JQ928237	Aa-nh3	4	13
Aa16	WM	EU908437	Aa-ch2	JQ928268	Aa-ph1	3	3	JQ928238	Aa-nh4	25	26
Aa17	WM	EU908437	Aa-ch2	JQ928270	Aa-ph3	1	1				
Aa18	WM	EU908437	Aa-ch2	JQ928269	Aa-ph2	3	4	JQ928239	Aa-nh5	23	24
Aa19	WM	EU908498	Aa-ch15	JQ928270	Aa-ph3	1	1				
Aa20	WM	GU972691	Aa-ch65	JQ928269	Aa-ph2	3	4				
Aa21	EM	EU908437	Aa-ch2	JQ928269	Aa-ph2	3	4	JQ928237	Aa-nh3	4	13

Code		COI (514 bp)		PEPCK (536 bp)				NaK (498 bp)			
Individual	Location	GenBank	Haplotype	GenBank	Genotype	Allele 1	Allele 2	GenBank	Genotype	Allele 1	Allele 2
Aa22	EM	EU908437	Aa-ch2	JQ928272	Aa-ph5	4	4	JQ928245	Aa-nh11	1	37
Aa23	EM	EU908437	Aa-ch2	JQ928270	Aa-ph3	1	1	JQ928246	Aa-nh12	35	36
Aa24	EM	EU908437	Aa-ch2					JQ928247	Aa-nh13	29	31
Aa25	EM	EU908497	Aa-ch14	JQ928269	Aa-ph2	3	4				
Aa26	EM	EU908497	Aa-ch14					JQ928248	Aa-nh14	1	38
Aa27	EM	EU908497	Aa-ch14	JQ928271	Aa-ph4	1	2	JQ928249	Aa-nh15	32	32
Aa28	EM	EU908498	Aa-ch15								
Aa29	EM	GU972657	Aa-ch31	JQ928269	Aa-ph2	3	4	JQ928250	Aa-nh16	33	34
Aa30	EM	GU972661	Aa-ch35	JQ928270	Aa-ph3	1	1				
Aa31	AO	EU908437	Aa-ch2	JQ928271	Aa-ph4	1	2				
Aa32	AO	EU908437	Aa-ch2	JQ928269	Aa-ph2	3	4				
Aa33	AO	EU908437	Aa-ch2	JQ928269	Aa-ph2	3	4				
Aa34	AO	GU972658	Aa-ch32	JQ928270	Aa-ph3	1	1				
Aa35	AO	GU972658	Aa-ch32	JQ928270	Aa-ph3	1	1				
Aa36	AO	EU908497	Aa-ch14	JQ928271	Aa-ph4	1	2	JQ928251	Aa-nh17	3	4
Aa37	AO	EU908498	Aa-ch15	JQ928270	Aa-ph3	1	1				
Aa38	AO	GU972657	Aa-ch31	JQ928270	Aa-ph3	1	1				
Aa39	AO	EU908486	Aa-ch12					JQ928252	Aa-nh18	1	2
Aa40	AO	EU908539	Aa-ch24	JQ928270	Aa-ph3	1	1				
Aa41	MOZ	GU972658	Aa-ch32	JQ928271	Aa-ph4	1	2	JQ928253	Aa-nh19	5	6
Aa42	MOZ	GU972658	Aa-ch32	JQ928269	Aa-ph2	3	4	JQ928254	Aa-nh20	11	12
Aa43	MOZ	GU972672	Aa-ch46	JQ928269	Aa-ph2	3	4				
Aa44	MOZ	GU972668	Aa-ch42	JQ928271	Aa-ph4	1	2	JQ928255	Aa-nh21	7	8
Aa45	MOZ	GU972682	Aa-ch56	JQ928272	Aa-ph5	4	4	JQ928256	Aa-nh22	13	14
Aa46	MOZ	GU972683	Aa-ch57	JQ928272	Aa-ph5	4	4	JQ928257	Aa-nh23	15	16
Aa47	MOZ	GU972687	Aa-ch61	JQ928270	Aa-ph3	1	1				
Aa48	MOZ	GU972688	Aa-ch62	JQ928269	Aa-ph2	3	4	JQ928258	Aa-nh24	17	18
Aa49	MOZ	EU908437	Aa-ch2	JQ928268	Aa-ph1	3	3	JQ928259	Aa-nh25	19	20

Code		COI (514 bp)		PEPCK (536 bp)				NaK (498 bp)			
Individual	Location	GenBank	Haplotype	GenBank	Genotype	Allele 1	Allele 2	GenBank	Genotype	Allele 1	Allele 2
Aa50	MOZ	GU972654	Aa-ch28	JQ928268	Aa-ph1	3	3	JQ928260	Aa-nh26	9	10
Af1	WM	JN676306	Af-ch1	JQ928273	Af-ph1	5	5	JQ928262	Af-nh2	39	41
Af2	WM	JN676306	Af-ch1	JQ928274	Af-ph2	11	12				
Af3	WM	JN676306	Af-ch1	JQ928275	Af-ph3	9	13				
Af4	WM	JN676306	Af-ch1	JQ928276	Af-ph15	13	14				
Af5	WM	JN676306	Af-ch1	JQ928273	Af-ph1	5	5	JQ928262	Af-nh2	39	41
Af6	WM	JN676307	Af-ch2	JQ928277	Af-ph5	9	10	JQ928263	Af-nh3	41	43
Af7	WM	JN676308	Af-ch3	JQ928278	Af-ph6	8	9	JQ928264	Af-nh4	41	42
Af8	WM	JN676308	Af-ch3	JQ928279	Af-ph7	13	15	JQ928264	Af-nh4	41	42
Af9	WM	JN676312	Af-ch7	JQ928280	Af-ph8	7	13	JQ928262	Af-nh2	39	41
Af10	WM	JN676317	Af-ch12	JQ928274	Af-ph2	11	12	JQ928262	Af-nh2	39	41
Af11	EM	JN676306	Af-ch1	JQ928281	Af-ph8	7	13	JQ928264	Af-nh4	41	42
Af12	EM	JN676307	Af-ch2	JQ928282	Af-ph10	15	15	JQ928264	Af-nh4	41	42
Af13	EM	JN676307	Af-ch2	JQ928283	Af-ph11	5	6				
Af14	EM	JN676307	Af-ch2	JQ928284	Af-ph12	5	8	JQ928264	Af-nh4	41	42
Af15	EM	JN676307	Af-ch2	JQ928285	Af-ph13	13	13	JQ928264	Af-nh4	41	42
Af16	EM	JN676307	Af-ch2	JQ928286	Af-ph14	8	8	JQ928264	Af-nh4	41	42
Af17	EM	JN676307	Af-ch2	JQ928287	Af-ph15	13	14				
Af18	EM	JN676308	Af-ch3	JQ928288	Af-ph16	11	16	JQ928264	Af-nh4	41	42
Af19	EM	JN676317	Af-ch12	JQ928286	Af-ph14	7	15	JQ928264	Af-nh4	41	42
Af20	EM	JN676317	Af-ch12	JQ928290	Af-ph18	7	7	JQ928264	Af-nh4	41	42
Af21	MOZ	JN676331	Af-ch26	JQ928290	Af-ph18	7	7	JQ928261	Af-nh1	39	40
Af22	MOZ	JN676331	Af-ch26	JQ928286	Af-ph14	8	8	JQ928261	Af-nh1	39	40
Af23	MOZ	JN676331	Af-ch26	JQ928278	Af-ph6	8	9	JQ928261	Af-nh1	39	40
Af24	MOZ	JN676331	Af-ch26	JQ928283	Af-ph11	5	6	JQ928265	Af-nh5	40	42
Af25	MOZ	JN676331	Af-ch26	JQ928277	Af-ph5	9	10	JQ928265	Af-nh5	40	42
Af26	MOZ	JN676331	Af-ch26	JQ928286	Af-ph14	8	8	JQ928261	Af-nh1	39	40
Af27	MOZ	JN676332	Af-ch27	JQ928286	Af-ph14	8	8	JQ928262	Af-nh2	39	41

Code		COI (514 bp)		PEPCK (536 bp)				NaK (498 bp)			
Individual	Location	GenBank	Haplotype	GenBank	Genotype	Allele 1	Allele 2	GenBank	Genotype	Allele 1	Allele 2
Af28	MOZ	JN676336	Af-ch31	JQ928277	Af-ph5	9	10	JQ928264	Af-nh4	41	42
Af29	MOZ	JN676334	Af-ch29	JQ928286	Af-ph14	8	8	JQ928265	Af-nh5	40	42
Af30	MOZ	JN676335	Af-ch30	JQ928291	Af-ph19	8	10	JQ928264	Af-nh4	41	42
Af31	AUS	JN676352	Af-ch47								
Af32	AUS	JN676346	Af-ch41	JQ928282	Af-ph10	15	15				
Af33	AUS	JN676356	Af-ch51	JQ928286	Af-ph14	8	8	JQ928266	Af-nh6	41	44
Af34	AUS	JN676348	Af-ch43	JQ928278	Af-ph6	8	9				
Af35	AUS	JN676349	Af-ch44	JQ928289	Af-ph17	7	15	JQ928266	Af-nh6	41	44
Af36	AUS	JN676350	Af-ch45	JQ928291	Af-ph19	8	10	JQ928266	Af-nh6	41	44
Af37	AUS	JN676357	Af-ch52	JQ928277	Af-ph5	9	10				
Af38	AUS	JN676351	Af-ch46	JQ928291	Af-ph19	8	10	JQ928266	Af-nh6	41	44
Af39	AUS	JN676358	Af-ch53	JQ928292	Af-ph20	10	10	JQ928266	Af-nh6	41	44
Af40	AUS	JN676362	Af-ch57	JQ928291	Af-ph19	8	10				

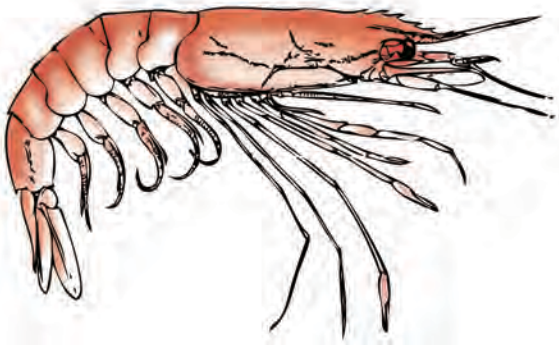
Table S3. Matrix of K2P genetic distances between all lineages for nuclear genes. NaK genetic distances are given below diagonal and PEPCK genetic distances above diagonal.

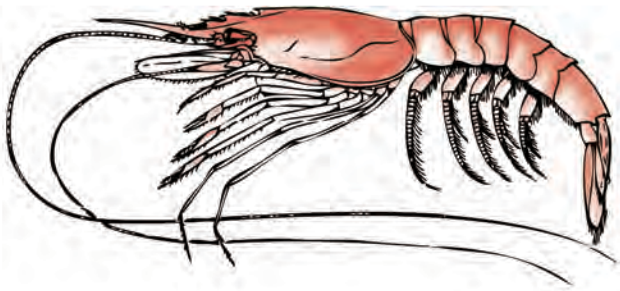
	A. virilis	*A. antennatus*	*A. foliacea*	Af MED	Af MOZ	Af AUS
A. virilis		0.0056 ± 0.0022	0.0238 ± 0.0044	0.0240 ± 0.0044	0.0236 ± 0.0045	0.0243 ± 0.0046
A. antennatus	0.0304 ± 0.0048		0.0214 ± 0.0041	0.0217 ± 0.0041	0.0213 ± 0.0042	0.0219 ± 0.0043
A. foliacea	0.0966 ± 0.0104	0.1083 ± 0.0105				
Af MED	0.0973 ± 0.0104	0.1089 ± 0.0104			0.0005 ± 0.0004	0.0001 ± 0.0003
Af MOZ	0.0970 ± 0.0104	0.1088 ± 0.0103		0.0025 ± 0.0009		0.0005 ± 0.0001
Af AUS	0.0995 ± 0.0106	0.1113 ± 0.0029		0.0036 ± 0.0017	0.0035 ± 0.0015	

Table S4. Matrix of Tamura-Nei genetic distance calculated for COI dataset (514 bp) between all lineages and species. Genetic distances are given (below diagonal) and estimated times (Mya) since divergence (above diagonal), using 0.83-1.2% evolutionary rate (reviewed in Ketmaier et al. [48]). Location codes as in Table 1.

	A. virilis	*A. antennatus*	*A. foliacea*	*P. monodon*	*S. crassicornis*	Af MED	Af MOZ	Af AUS
A. virilis		7.07-4.87	13.40-9.27	15.81-10.94	12.28-8.49	13.40-9.27	13.36-9.24	12.87-8.90
A. antennatus	0.1143 ± 0.0153		12.29-8.50	15.00-10.37	11.71-8.10	13.40-9.27	13.04-9.02	13.63-9.43
A. foliacea	0.1929 ± 0.0194	0.1949 ± 0.0186		13.13-9.08	11.29-7.81			
P. monodon	0.2625 ± 0.0284	0.2489 ± 0.0263	0.2137 ± 0.0214		12.03-8.32	14.05-9.72	13.62-9.42	14.70-10.17
S. crassicornis	0.2038 ± 0.0240	0.1944 ± 0.0225	0.1874 ± 0.0207	0.1997 ± 0.0238		12.58-8.55	12.36-8.55	12.39-8.57
Af MED	0.2224 ± 0.0246	0.2225 ± 0.0249		0.2333 ± 0.0260	0.2088 ± 0.0241		0.54-0.38	4.16-2.88
Af MOZ	0.2217 ± 0.0245	0.2165 ± 0.0246		0.2261 ± 0.0256	0.2052 ± 0.0237	0.0090 ± 0.0040		4.20-2.91
Af AUS	0.2136 ± 0.0236	0.2262 ± 0.0252		0.2441 ± 0.0257	0.2056 ± 0.0235	0.0690 ± 0.0117	0.0698 ± 0.0117	

Cover illustration (front): *Aristeus antennatus* from Blanes canyon collected at Blanes fishing market © MI Roldán Borassi; (back): *Aristeus antennatus* (right) and *Aristaeomorpha foliacea* (left) schematic drawings modified from Fisher et al. (1981) by Ana Fernández Hernández.





"I liked very much the sections General Introduction and General discussion, which are the right prologue and epilogue for inserting in a general framework the four papers proposed as the core of the thesis activity...a colleague who works on other fields not so related with the arguments of this thesis. will appreciate, like me, all the content of this work"
(Expert 1)

"This thesis is a strong and comprehensive document that probes the population genetic structures of two aristeid shrimp species, the bases of their distinct structures in sympatry, their congeneric relationships, and the overall management and implications of these data and their synthesis."
(Expert 2)