MEGALOPA "*big eyes*" Leach 1793

APLICACIÓN DE TÉCNICAS MORFOLÓGICAS Y MOLECULARES EN LA IDENTIFICACIÓN DE LA MEGALOPA

de Decápodos Braquiuros de la Península Ibérica

Elena Marco-Herrero

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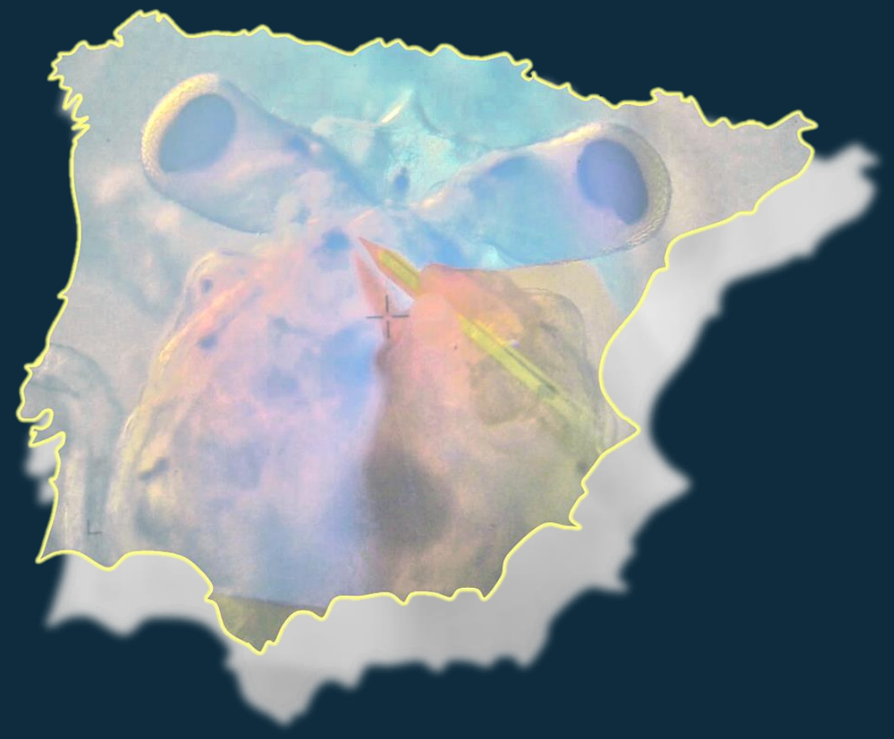

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Tesis Doctoral, Valencia 2015

CSIC
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Vniversitat de València

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TESIS DOCTORAL

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Valencia, septiembre 2015

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CSIC

Consejo Superior de Investigaciones Científicas

Als naninets

AGRADECIMIENTOS-AGRAÏMENTS

Colaboración y ayuda prestada por diferentes instituciones:

- Ministerio de Ciencia e Innovación (actual Ministerio de Economía y Competitividad) por la concesión de una Beca de Formación de Personal Investigador FPI (BES-2010-033297) en el marco del proyecto: *Aplicación de técnicas morfológicas y moleculares en la identificación de estados larvarios planctónicos de decápodos braquiuros ibéricos* (CGL2009-11225)
- Departamento de Ecología y Gestión Costera del Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC)
- Club Náutico del Puerto de Santa María
- Centro Andaluz de Ciencias y Tecnologías Marinas (CACYTMAR)
- Instituto Español de Oceanografía (IEO), Centros de Mallorca y Cádiz
- Institut de Ciències del Mar (ICM-CSIC) de Barcelona
- Institut de Recerca i Tecnología Agroalimentàries (IRTA) de Tarragona
- Centre d'Estudis Avançats de Blanes (CEAB) de Girona
- Universidad de Málaga
- Natural History Museum of London
- Stazione Zoologica Anton Dohrn di Napoli (SZN)
- Universitat de Barcelona

AGRAÏSC – AGRADEZCO

En primer lugar quisiera agradecer a mis directores, el Dr. Jose A. Cuesta y el Dr. Ferran Palero, por darme la oportunidad de desarrollar este trabajo y la posibilidad de conocer el mundo científico. A Jose A. por el apoyo y por creer en mí para hacer esta tesis, además, gracias por transferirme conocimientos, técnicas pero sobretodo la motivación e ilusión por trabajar con las ya famosas "megalopas". A Ferran por esos tiempos de su tiempo libre, e ingeniando enseñándome en el mundo filogenético, también agradecerle sus conocimientos y sobretodo sus buenos consejos de este mundo de la investigación.

Al Instituto de Ciencias Marinas de Andalucía, incluida la gran administración, y en especial a Mª Ángeles y Fran. Al departamento de Ecología y Gestión Costera, a los más zoólogos, Dr. Pilar Drake, compartiendo experiencias con *Afro*; a Dr. Alberto Arias, al que admiro por su arte y por ser una leyenda viva, y al recién jubilado Dr. Antonio Rodríguez, el responsable de iniciar la saga de los larvarios y dejar tan gran descendencia; a Mariana, que cuida de todos, a Carlos por su compañía en el despacho, por su trabajo y ayuda; A Dulce e Irene, mucho aprendí de ellas. Y para acabar con el departamento, a la parte oceanógrafos, gracias por incluirme en los desayunos, comidas, cenas,... como una más. Ya no estás aquí, una medusera ecuatoriana, pero gracias por los primeros años de Cádiz juntitas.

Agradecida de formar parte del grupo Megalopadn: A Dr. Nacho González-Gordillo, gracias y mil gracias por el kit-kat, y por guiarme en mis inicios de las disecciones e ilustraciones de las larvas. A Dr. Pere Abelló y Dr. Guillermo Guerao, por enseñarme tanto de estas peculiares larvitas y transmitir la emoción de trabajar e investigar bajo lupa. A Dr. Enrique García-Raso y Dr. Enrique Macpherson por tener la posibilidad de aprender de sus conocimientos y brindarme su ayuda.

A la Dr Mª Grazia Mazzocchi y la Dr. Marta Pascual, por haberme permitido realizar las estancias del doctorado con ellas y aprender en otros grupos de investigación.

Esto me lleva a Napoli, a todos los de Stazione Zoologica Anton Dohrn que estuvieron ahí para ayudarme y sacarme de paseo!! Davide, Laura, Lucia, Iole, Giaunluca e Ferdinando meraviglioso campionamento!!! Vistas al Vesuvio con pizza en mano, mi piace! Ahí obtuve conocimiento y experiencia en el trabajo, pero una familia Napolitana y un hogar!!! Grazie mille a Piera, Giorgio, Yesil y Yolanda, mi manchi!! Y en Barcelona y el maravilloso curso de filogenia!! Y que buenos compañeros conocí allí, eixos Mallorquins! uno de adopción jajajaj Sergio no cuelas como lugareño de la ISLA. Gracias Sergio por estar ahí, y oír mis estreses, en calidad de... ya

sabes de que!!! Tú apunta mi recomendación para cuando te toque a ti pasar este final ;). Ací aprofite com a Mallorquina, per agraïr a Asvin, recent Doctora!!! I companya de congrés i altra friki de les larves.

El mundillo de los braquiuros también me llevo a estar en el Natural History Museum, que guaiii!!! Sumergida en la colección de los cangrejos. Y ver ejemplares de siglos atrás, gracias a Paul F. Clark por darme esa oportunidad y permitirme aprende de él, otro apasionado de las larvas de los crangrejos a la inglesa. Gracias a Felipe (canario-inglés) el curator de las plantas de museo y que me enseñó la parte desconocida del NHM.

Pasando a los muestreos, a las benditas salidas de campo!! En primer lugar de estos agradecimientos es a Antonio y David, por facilitarme en todo lo posible esos muestreos en luna llena con marea entrante. Agradecer a Josep Mª i Guiomar (IRTA, Tarragona) y a Ferran (CEAB, Girona) que nos ayudaron en las campañas del Mediterráneo. I gràcies a Eustaqui Palero per la disponibilitat dels mostrejos a Cullera (València).

Mi inicio investigador, aquí agradecer enormemente a mi directora de tesis de Master, Dr. Mercedes Fernández, de la unidad de zoología marina, del Isntituto Cavanilles, no hay palabras exactas que reflejen el trato que recibí, y todo lo que aprendí de ella, gracias Merche! Bendita asignatura la de “rastros de aves y mamíferos”, con Dr. Javier Aznar y Merche de profes, ellos fueron los culpables de que empezase a investigar, a preguntar y querer respuestas de otra manera, sí, sin lugar a dudas los mejores profesores que he tenido!! Aún recuerdo un brindis de Javi en Cuenca, *por un futuro brillante!!!* Que buenos brindis y recuerdos. Agradecer a la gente que forma la Unidad de Zoología Marina, queda lejos en el tiempo, pero el recuerdo fue tan bueno que se mantiene, gràcies: Neus, Patri, Isa, Raúl, Juanma, Paula, esa salidas en barco a ver cetáceos no se puede olvidar y las necropsias tampoco jajaja

Agradecer a las ”Single Ladies” Isa (la más veterana para mí, gracias, tú fuiste mi primera Lady in Cái), fugada Marga, Sori, Marina, Cori- Kyro, Emma y Vicky por lo buenos momentos que se pasan junto a ellas. Y a la gente que va y viene, y en su momento estuvo cerquita compartiendo algún momento perruno, campero, bichero, playero, cachondeo, relax,…y hacen que estar aquí sea más llevadero!!

Mil Mil Gracias, Gràcies, Thanks!!! a los que me habéis ayudado en la parte perruna, en estar ahí, para mí pero también para mi sombra, a Anabel y Lennon (un punk2) gracias,!! Y como no a Javi, un indispensable de mi vida, gracias por estar ahí en la pseudo-distancia, para Duna y para mí, llevártela de vacaciones y sacarnos al campo, en medio de la nada y hacernos

desconectar, disfrutando del campo como los viejos tiempos, no solo nuestros, sino también como los lugareños cabreros de hace 50 años jajajaj asilados en Mordor.

Al portento, mi pilar en Cádiz, gracias Parce por acompañarme aquí.

A les meues perletes del Túria, gràcies per fer-me sentir com si no me n'haguera anat de la Terreta, sempre esteu ahí Raquel i Vera, Mari, Neus i el hippies, Sara, Imma, Horten, i Felipe, la gent que mai desapareix. I a Paco, eixe afèrrim de València i al Micalet, ajjajaja peça important de la meua vida, allí i ací. Gràcies per no perdres en la distància.

A un escritorl Norteño!! Quien dice que en la distancia no pueden echarte un cable para pasar una tabla excel, Mila esker Andoni! y por los kit kats literarios ;)

El meu coret, els meus *naninets i Duna!!* Sense ells açó no sería possible, són els que están darrere, facilitant-me que puga seguir i arribar lluny, i naninets aconseguit!!! I que dir dels xics de la meua vida: el *tete*, *Miquel* i *Joan*, *Silvia* tenim que compartir-los jajajaj gràcies, simple i ras "per estar" els millors.

Gràcies fins i tot als que no estan :)

ÍNDICE

Índice

ABREVIACIONES

Aest	aestetascos
An	antena
Au	anténula
C	carpo
Ca	región cardíaca
Ch	quela
CL	longitud cefalotórax
Co	coxa
Cs	espina cardíaca
CW	anchura cefalotórax
Car	carina
D	dáctilo
En	endopodo
Epb	región epibranquial
Ex	exópodo
Fr	región frontal
F1-7	flagelo 1-7
He	región hepática
I	isquio
In	región intestinal
ISp	espina isquial
M	mero
Me	región mesobranquial
Meg	región metagástrica
Mes	región mesogástrica
Met	región metabranquial
O	región orbital
P	propodio
Per1-5	pereiópodo 1-5
Pl1-5	pleonitos 1-5
Pr	región protogástrica
Pro	protópodo
Prot	protuberancia
Prs	proceso
Pu	segmento peduncular
PrsSp	proceso espinoso
R	rostro
Sba	esternito basal
Sp	espina
Sst1-5	esternito1-5
Subs	seta subterminal
T	telson
U	urópodo
Ur	región urogástrica
Z	zoea

RESUMEN

INTRODUCCIÓN

Entre los crustáceos decápodos, el Infraorden Brachyura Linnaeus, 1758 es el grupo más diverso y de mayor éxito evolutivo, con aproximadamente 7.000 especies pertenecientes a 98 familias (Tsang et al. 2014). Los braquiuros, comúnmente llamados cangrejos, han conquistado casi todos los hábitats y numerosos nichos ecológicos (De Grave et al. 2009; Ahyong et al. 2011). La mayoría de las especies son marinas, aunque también existen especies de agua dulce o incluso especies terrestres.

El desarrollo larvario de los braquiuros suele constar de dos fases de vida libre y planctónicas (con las escasas excepciones de aquellos con desarrollo directo, principalmente de agua dulce): zoea (con varios estadios) y megalopa (Anger 2006). La megalopa es una fase de transición entre la zoea planctónica y la fase juvenil y adulta, típicamente bentónicas (Rice 1981). La notable variación de morfología, comportamiento y hábitat entre larvas y adultos representa un gran problema a la hora de identificar las larvas del zooplancton. La morfología de las formas larvarias es difícil de relacionar con la de los adultos y, aunque a veces las larvas se pueden distinguir morfológicamente, no resulta sencillo atribuirlas a la forma adulta correcta (Bucklin 2010). La falta de datos *a priori* que relacionen los estadios larvarios con la especie a la que pertenecen, ha ralentizado el avance en el conocimiento de la fase megalopa.

De las 140 especies de braquiuros conocidas en la Península Ibérica, solo se dispone de descripciones fiables de la megalopa de 67 especies (< 48%). En la última década se han empezado a aplicar nuevas técnicas que minimizan estas limitaciones y/o restricciones, y que permiten avanzar a un mayor ritmo en el conocimiento de la morfología larval de los braquiuros y sus aplicaciones, como la filogenia y sistemática moleculares (Ampuero et al. 2010; Spiridonov et al. 2014).

Una de estas nuevas técnicas fue presentada en 2003 por el doctor Paul Hebert y colaboradores quienes propusieron la utilización de una región pequeña del genoma como DNA barcode (código de barras genético), al gen citocromo oxidasa 1 (Cox1) (Hebert et al. 2003). El código de barras de ADN ha demostrado ser muy útil tanto para diferenciar especies (Costa et al. 2007) como para la diferenciación entre poblaciones de una misma especie (Palero et al. 2008; García-Merchán et al. 2012). Además del Cox1, el gen mitocondrial de la subunidad ribosomal 16S también ha demostrado ser una herramienta eficiente en estudios sistemáticos de crustáceos decápodos (Schubart et al. 2000; Ahyong et al. 2007). La aplicación de técnicas moleculares para la identificación de megalopas en muestras del plancton, nos ha permitido

incrementar el número de especies para las que se conoce este estadio larval, y que a partir de ahora pueden ser identificadas directamente del plancton en base a su morfología (Weeb 2006).

Se podría concluir que una clasificación sistemática adecuada, que refleje las relaciones filogenéticas entre los diferentes taxa, debería representar un compendio de todas las fuentes de información disponibles, considerando siempre que existan los datos larvales.

Objetivos

El principal objetivo de esta tesis es optimizar la aplicación de técnicas morfológicas y moleculares que faciliten la identificación de las megalopas de los braquiuros colectadas del plancton en la Península Ibérica.

Una vez identificadas, las megalopas serán descritas cuando no se disponga de información previa o re-descritas si las descripciones originales son incompletas. Estas nuevas descripciones y re-descripciones permitirán, junto con las descripciones ya existentes, elaborar una clave ilustrada.

Los *objetivos específicos* de cada Sección/Capítulo son:

SECCIÓN I Capítulo 1

- Generar una base de datos completa con las secuencias de ADN de dos marcadores moleculares (16S y Cox1) para todas las especies de braquiuros de la Península Ibérica.
- Actualizar el listado de braquiuros de la Península Ibérica.
- Revisar la validez taxonómica y posición sistemática de las especies de braquiuros de la Península Ibérica.

SECCIÓN II-III Capítulos 2-7

- Nuevas aportaciones en el conocimiento de la morfología de la megalopa de diferentes especies:
  - Descripciones y re-descripciones de desarrollos larvarios completos a partir de hembras ovígeras.
  - Descripciones y re-descripciones de desarrollos larvarios y del estadio megalopa, obtenidas del plancton e identificados con técnicas moleculares (16S y Cox1).

SECCIÓN IV Capítulo 8

- Elaborar una clave ilustrada, que facilite la correcta identificación de las megalopas de los braquiuros ibéricos.

MATERIAL Y MÉTODOS

En este resumen, los materiales y métodos se describen por separado para cada uno de los capítulos.

RESULTADOS

Se han obtenido un total de 3.445 ejemplares, de los cuales 331 corresponden a adultos y 3.114 a megalopas. En los Apéndices I-II, se resume toda la información/resultados obtenida para cada especie de braquiuro de la Península Ibérica.

Referente a los adultos, se han conseguido ejemplares de 132 especies de las 140 especies de braquiuros de la Península Ibérica. Se han obtenido secuencias "Código de barras" para el marcador Cox1 de 118 especies y para el marcador 16S de 115 especies. De aquellas especies para las que no se han conseguido, en Genbank se dispone de secuencias de 11 especies para el marcador 16S y de 9 especies para Cox1. Englobando todas las secuencias se pueden identificar el 90% de las especies conocidas de braquiuros ibéricos.

En cuanto a las megalopas, de los 3.114 ejemplares colectados del plancton, se han identificado mediante técnicas morfológicas y moleculares un total de 57 especies. De estas, 12 especies corresponden a braquiuros para los que no se conocía la morfología de la megalopa, y se describen por primera vez, 4 especies descritas previamente, que consideramos la descripción morfológica insuficiente, y 41 especies ya descritas, en las que el desarrollo puede estar completo, o carecer de la descripción de algún/os caracteres y la consideramos aquí incompleta. Estas últimas han sido analizadas y comparadas con las descripciones originales. Además de los ejemplares obtenidos del plancton e identificados morfológica y molecularmente, se han conseguido 8 especies provenientes de depósitos en Museos (NHM e ICM), que han sido analizadas y comparadas, al igual que las identificadas con técnicas moleculares. Sumando las 27 especies para las que están descritas sus megalopas, pero de las que no obtuvimos ejemplares, se consigue un total de 92 especies de las que disponemos de información morfológica. Esto supone un 65.7% de las especies, aportando la presente tesis un 26.5% de información morfológica de las megalopas de los braquiuros ibéricos a una clave de identificación.

A continuación se detallan los resultados de cada capítulo:

SECCIÓN I Actualización de la fauna de braquiuros de la Península Ibérica

Capítulo 1 Annotated checklist of brachyuran crabs (Crustacea: Decapoda) of the Iberian Peninsula (SW Europe)

Introducción

Han pasado casi 50 años desde que un grupo de reputados carcinólogos (viz. Lipke B. Holthuis, Isabella Gordon y Jacques Forest) finalizaran la obra póstuma de Ricardo Zariquiey Álvarez (1968), "Crustáceos decápodos de la Península Ibérica". Desde entonces no se ha publicado una lista de la fauna de decápodos que cubra específicamente este área, y era necesaria una actualización. Hay un esfuerzo concertado por parte de todos los carcinólogos para verificar la validez de los taxones, utilizando múltiples herramientas como la caracterización ecológica, morfología larvaria y técnicas moleculares (Schubart et al. 2001; Spivak y Schubart 2003; Marco-Herrero et al. 2013). El presente trabajo resume todos los cambios en la carcinofauna ibérica desde Zariquiey Álvarez (1968), y proporciona a los científicos una lista de clasificación actualizada. Además, incluye una revisión a fondo del estado actual de la presencia en esta región de especies exóticas de braquiuros.

Material y Métodos

Para la elaboración de esta lista se han revisado todas las publicaciones sobre braquiuros de la Península Ibérica aparecidas desde 1968, además se han utilizado datos no publicados o en preparación. También se han revisado varios especímenes del Museo de Historia Natural (Londres), del Museo Nacional de Historia Natural (París) y de las Colecciones biológicas de referencia del Instituto de Ciencias del Mar (Barcelona), usando morfología y/o técnicas moleculares. Esta lista cubre todas las especies de braquiuros presentes en la Península Ibérica e Islas Baleares, incluyendo especies de agua dulce, marina (de aguas profundas hasta intermareal), y salobres (estuarios, lagunas costeras, pantanos, estanques). La actualización sistemática sigue la clasificación de Ng et al. (2008), pero también se tienen en cuenta los últimos cambios en determinados taxones (por ejemplo, Spiridonov et al. 2014).

Resultados

La lista actual de braquiuros de la Península Ibérica consta de un total de 140 especies, 35 especies más de las 105 especies válidas enumeradas en Zariquiey Álvarez (1968).

Observaciones

Los cambios en la sistemática han afectado a la clasificación original, se actualiza a 20 superfamilias, 36 familias y 77 géneros. Este incremento en el número de especies se debe que algunas especies han sido citadas en aguas ibéricas debido a la expansión natural de su rango

de distribución desde áreas cercanas (Mediterráneo y Atlántico), otras especies son introducciones mediadas por las actividades antropogénicas y algunas por descripciones de nuevas especies. Además, se han sinonimizado dos especies. Algunos de estos cambios, basados en evidencias de la morfología de las larvas y/o datos moleculares, se detallan en esta revisión. Aunque no se espera que las descripciones de nuevas especies de cangrejos se produzcan a un ritmo significativo, sí es esperable un incremento en el número de especies en la Península Ibérica como resultado de la introducción de especies exóticas.

SECCIÓN II Descripciones morfológicas de las larvas de braquiuros ibéricos a partir de hembras ovígeras cultivadas en laboratorio

Capítulo 2 Morphology of the larval stages of *Macropodia czernjawskii* (Brandt, 1880) (Decapoda, Brachyura, Inachidae) reared in the laboratory

Introducción

El género *Macropodia* Leach, 1814 está representado en el Atlántico Noreste y en aguas del Mediterráneo por 9 especies. Actualmente el desarrollo larvario completo se conoce sólo para 4 especies del género: *M. tenuirostris* (Ingle 1982; Salman 1981), *M. rostrata* (Ingle 1982), *M. longipes* (Guerao y Abelló 1997) y *M. parva* (González-Gordillo y Rodríguez 2001). En el presente estudio se describe e ilustra en detalle el desarrollo larvario completo (dos estadios zoea y la megalopa) de *Macropodia czernjawskii* y se compara con otras especies del género.

Material y Métodos

Una hembra ovígera de *Macropodia czernjawskii* fue colectada en el intermareal de la playa El Chato (Cádiz, SO España), el 10 de septiembre de 1999. El cultivo se realizó de 417 zoeas que eclosionaron el 17 de septiembre. Para mejorar la observación de las estructuras en el microscopio de la larvas se siguió un protocolo de tinción (Landeira et al. 2009). La descripción y figuras están dispuestas de acuerdo con las normas propuestas por Clark et al. (1998).

Resultados

El desarrollo de las larvas de *M. czernjawskii* consta de dos zoeas y una megalopa. El desarrollo larvario se completa en un mínimo de 8 días (aparición del primer cangrejo). La duración y la supervivencia de cada estadio larval se muestran en la Fig. 1 del Capítulo 2. La descripción detallada e ilustraciones se pueden observar en las Figs. 2-7 del Capítulo 2.

Discusión

La superfamilia Majoidea Samouelle, 1819 cuenta con más de 900 especies (De Grave et al. 2009). Aunque ocupan diferentes hábitats marinos, comparten un conjunto de caracteres larvarios que los distinguen del resto de braquiuros. Varios trabajos han utilizado las características larvarias para estudiar las relaciones filogenéticas de los Majoidea (Rice 1980, 1988; Marques y Pohle 1998, 2003). La familia Inachidae consta de 204 especies en 37 géneros (Ng et al. 2008; De Grave et. al 2009), pero tan solo para 26 especies (12 géneros) se tienen datos larvarios, por lo que actualmente es demasiado pronto para definir caracteres que distingan a esta familia. Por otro lado, algunas publicaciones indican que existen fuertes diferencias intragenéricas (ver Oh y Ko 2011). Dentro del género *Macropodia* se encuentran 17 especies válidas, de las cuales 9 habitan en aguas de la Península Ibérica. El análisis de los diferentes caracteres larvarios conocidos del género, y la nueva descripción de las zoeas y megalopa de *M. czerjawskii*, permite separar las especies en 3 grupos combinando varios caracteres, dejando a *M. czerjawskii* como única especie en un grupo distinto. El carácter que la separa del resto de especies del género es la ausencia de espinas laterales en la furca del telson en las zoeas, separándola también de la mayoría de majoideos.

Capítulo 3 Morphology of the larval stages of a Mediterranean population of the allochthonous Say's mud crab, *Dyspanopeus sayi* (Decapoda: Brachyura: Panopeidae)

Introducción

La distribución natural de *Dyspanopeus sayi* (Smith 1869), abarca la costa atlántica de América del Norte desde Florida a Canadá (Nizinski 2003). *Dyspanopeus sayi* es una especie eurihalina y euritérmica, que habita en estuarios y aguas marinas costeras someras (Schubart et al. 2012). Se considera una especie invasora en otras partes del mundo como resultado de las actividades humanas (Davidson y Simkanin 2012). La cita más reciente se da en el Mediterráneo occidental, lo que constituyó el primer registro para la costa de la Península Ibérica (Schubart et al. 2012).

En el presente estudio se describe e ilustra en detalle el desarrollo larvario completo (cuatro zoeas y una megalopa) de *Dyspanopeus sayi* a partir de cultivo en el laboratorio. Además se comparan con estadios larvarios de *D. sayi* colectados en el plancton y con las descripciones de los otros dos Panopeidae que habitan en la Península Ibérica.

Material y Métodos

Tres hembras ovígeras de *Dyspanopeus sayi* fueron colectadas en la Bahía de los Alfacs (NO Mediterráneo) en agosto de 2010, y el cultivo larvario se realizó a partir de 100 zoeas I. Además, se llevó a cabo un muestreo cualitativo del plancton durante el 24 y 25 de septiembre de 2012 en la Bahía de los Alfacs, Delta del Ebro.

Para mejorar la observación en el microscopio de las larvas se realizó un protocolo de digestión y tinción (Marco-Herrero et al. 2012). Las descripción y figuras están dispuestas de acuerdo a los estándares propuestos por Clark et al. (1998).

Resultados

En las muestras de plancton se identificaron un total de 9 zoeas I, 2 zoeas II y 1 zoea IV de *Dyspanopeus sayi* que se utilizaron para la comparación merística y morfométrica con las larvas obtenidas en el laboratorio. El desarrollo larvario de *Dyspanopeus sayi* consta de 4 zoeas y una megalopa, y se completa en un mínimo de 15 días (aparición de la primera megalopa). La descripción detallada y las ilustraciones del desarrollo se puede observar en las Figs. 3-9 del Capítulo 3.

Discusión

Hay una población bien establecida de *Dyspanopeus sayi* en el área de la Bahía de Alfacs, Delta del Ebro. La colecta de ejemplares adultos y hembras ovígeras desde 2005 (Schubart et al. 2012; Guerao obs. pers.) y la aparición en el plancton de las fases larvarias en septiembre de 2012 confirma que la especie está establecida.

La familia Panopeidae es un complejo con gran heterogeneidad entre las formas larvarias (Martin 1988). Los estadios larvarios descritos han sido comparados con larvas del plancton capturadas en la misma zona, con descripciones previas de esta especie y con descripciones de los estadios larvarios de las otras dos especies de Panopeidae que habitan en la Península Ibérica: *Panopeus africanus* y *Rhithropanopeus harrisii*. Se han encontrado diferencias destacables en algunos caracteres de los estadios, zoea y la megalopa, lo cual podría poner en duda la posición de estas especies dentro de la misma familia. Los estudios futuros utilizando técnicas moleculares en combinación con la morfología de las larvas podrían arrojar luz sobre las relaciones filogenéticas reales en esta familia de braquiuros.

Capítulo 4 Larval development of the pea crab *Afropinnotheres monodi* Manning, 1993 (Decapoda, Pinnotheridae) using plankton-collected and laboratory-reared specimens: Effects of temperature

Introducción

La familia Pinnotheridae (De Haan, 1833) está compuesta de pequeños cangrejos simbióticos. Debido a su pequeño tamaño y estilo de vida simbiótica, se sabe poco sobre su ciclo de vida, rasgos reproductivos, desarrollo larvario y sistemática (Becker y Türkay 2010; Palacios-Theil et al. 2009). *Afropinnotheres monodi* Manning, 1993 es un cangrejo africano que recientemente ha llegado a las costas del SO de Europea (Subida et al. 2011). Se ha encontrado habitando en 7 especies diferentes de bivalvos con distintos grados de prevalencia (Drake et al. 2014). Además, como en muchas especies africanas se reproduce durante todo el año (Drake et al. 1998, 2014). Este período reproductivo largo, junto con su amplia gama de especies huésped, le proporciona una clara ventaja para una expansión a nuevas áreas y establecerse de forma exitosa.

El objetivo de este estudio fue evaluar el efecto de la temperatura en la supervivencia y duración del desarrollo larvario, así como completar la descripción de las zoeas y la megalopa.

Material y Métodos

En el estuario del río Guadalete, en la Bahía de Cádiz (SO España), se colectaron 62 zoeas (Zoea I- Zoea IV) y 13 megalopas identificadas previamente como Pinnotheridae, entre finales de primavera de 2006 y verano de 2012. Además, 2 hembras ovígeras fueron colectadas en el interior de la almeja *Scrobicularia plana* en el Río San Pedro (Bahía de Cádiz) el 2 de diciembre de 2011 y el 8 de mayo de 2012. La identificación de las larvas del plancton se basa en secuencias parciales del gen 16S del ADNmt (Marco-Herrero et al. 2013, 2014). Las secuencias 16S ADNmt obtenidas se compararon con las especies de pinnoteridos ibéricas depositadas en Genbank. Las disecciones, dibujos y mediciones siguen la metodología de trabajos anteriores realizados según Marco-Herrero et al. (2012, 2014). Las descripciones y figuras se organizan de acuerdo a los estándares propuestos por Clark et al. (1998). Para comprobar si la temperatura afecta al desarrollo de cada estadio larvario se realizaron pruebas estadísticas.

Resultados

El desarrollo larvario de *A. monodi* consta de 4 zoeas y una megalopa. En las larvas cultivadas, la duración de cada estadio zoea, y su patrón temporal de mortalidad, variaban dependiendo de la temperatura (Figura 1 del Capítulo 4). El tiempo transcurrido desde la eclosión de las larvas hasta la megalopa fue de alrededor de 25 días a 25°C, y más de 40 días a 19ºC. La descripción

detallada y las ilustraciones de cada estadio larval se pueden observar en las Figs. 3-6 del Capítulo 4.

Discusión

El desarrollo de la fase dispersiva de *Afropinnotheres monodi* esta modulado por la temperatura. Debido a esto, se esperaría un mayor reclutamiento en las poblaciones parentales durante el verano, mientras que el resto del año se daría en nuevas ubicaciones. Este patrón estacional del desarrollo de la fase dispersiva podría contribuir a la expansión de esta especie invasora en aguas europeas. La información que aporta este estudio podría ayudar a establecer una alerta temprana para la detección de esta especie africana.

SECCIÓN III "Código de barras" de ADN como herramienta para la identificación de larvas colectadas en el plancton y estudios de sistemática molecular

Capítulo 5 Morphology of the megalopa of the mud crab, *Rhithropanopeus harrisii* (Gould, 1841) (Decapoda, Brachyura, Panopeidae), identified by DNA barcode

Introducción

El primer registro de una población de *R. harrisii* en la Península Ibérica fue realizado por Cuesta et al. (1991) en el estuario del Guadalquivir. *Rhithropanopeus harrisii* es un cangrejo eurihalino típicamente asociado con los hábitats de estuarios. En 1925, Connolly describió las cuatro etapas zoea y la megalopa de esta especie, basado en cultivos de laboratorio. Más tarde, también fue descrito por Hood (1962) y Chamberlain (1962), pero todas las descripciones son incompletas según la propuesta de normalización de las descripciones larvarias de braquiuros hecha por Clark et al. (1998).

El uso de marcadores moleculares ha demostrado ser una poderosa herramienta para proporcionar una identificación precisa de muestras de plancton (Pan et al. 2008, Pardo et al. 2009, Ampuero et al. 2010, Marco-Herrero et al. 2013). La identificación de la megalopa tradicionalmente se ha basado en características morfológicas a partir de cultivos en laboratorio, pero a veces puede ser simplemente imposible conseguir una identificación precisa de muestras del plancton. En este estudio se utilizó 16S como código de barras de ADN. El gen 16S ha demostrado ser una herramienta eficaz en los estudios de los crustáceos decápodos (Schubart et al. 2000, Porter et al. 2005; Ahyong et al. 2007).

En el presente estudio, en contraste con las descripciones tradicionales, las megalopas se obtuvieron del plancton y fueron identificadas con el código de barras de ADN.

Material y Métodos

En Julio de 2007 se colectaron 28 megalopas de *R. harrisii* y 4 de zoeas I en abril de 2011, en el estuario del rio Guadalete (Cádiz-SW España). Las 4 zoea I, se cultivaron en el laboratorio hasta que mudaron a megalopa. La identificación de las larvas del plancton se basa en secuencias parciales del gen 16S del ADNmt (Marco-Herrero et al. 2013). Los dibujos y mediciones se siguieron según la metodología detallada en el Capítulo 6.

Discusión

La re-descripción de la morfología larvaria de los braquiuros es inusual, aunque es necesario cuando las descripciones anteriores son erróneas o incompletas. Se necesita una descripción correcta de las fases larvarias para ser utilizada posteriormente en estudios filogenéticos y para la identificación precisa de muestras de plancton. La morfología de la megalopas de *R. harrisii* descritas en el presente trabajo no se ajustan por completo a las típicas de las megalopas de Panopeidae, aunque Martin (1984) incluyó *R. harrisii* en el Grupo I, basándose en los caracteres de las zoeas y megalopas. Algunos caracteres morfológicos varían ampliamente de los de otras especies de panopeidos, lo que podría emitir algunas dudas sobre la posición de la especie en la misma familia. Además, se obtuvieron megalopas anómalas de *R. harrisii* en los cultivos de laboratorio. Esta morfología anómala se relaciona con los problemas asociados a las condiciones de los cultivos.

Capítulo 6 Larval morphology of the family Parthenopidae, with the description of the megalopa stage of *Derilambrus angulifrons* (Latreille, 1825) (Decapoda: Brachyura), identified by DNA barcode

Introducción

La familia Parthenopidae MacLeay de 1838 se divide actualmente en dos subfamilias: Parthenopinae MacLeay, 1838 con 123 especies y Daldorfiinae Ng & Rodriguez, 1986, con 17 especies (Ng et al. 2008). La morfología de los adultos partenópidos se ha examinado recientemente y se han propuesto varios cambios en su sistemática (Tan y Ng 2004; Tan y Low 2014). Sin embargo, hay muy poca información sobre su morfología larvaria y la mayoría de las descripciones sólo consiguen los primeros estadios de la fase zoea. Solo se conocen dos desarrollos larvarios completos realizados a partir de cultivos en el laboratorio: *Platylambrus serratus* (H. Milne Edwards, 1834) y *Enoplolambrus validus* (De Haan, 1837). Para las especies

restantes, las descripciones de su desarrollo son parciales o inexistentes. En el presente trabajo, se comparan y analizan todos los datos larvarios disponibles relacionados con los partenópidos.

Derilambrus angulifrons se conoce en el Atlántico oriental, como el suroeste de España (Cuesta Mariscal y González-Gordillo, 1992) y el Mar Mediterráneo (d'Udekem d'Acoz, 1999). Otro partenópido que se encuentra en la Península y fue descrito a partir de muestras de plancton por Thiriot en 1973 es *Parthenopoides massena* se, este cangrejo se distribuye en el NE del Atlántico de Europa a Guinea y las costas del Mediterráneo (d'Udekem d'Acoz, 1999). En el presente estudio la megalopa de *Derilambrus angulifrons* se identifica con técnicas moleculares y se describe e ilustra en detalle por primera vez. Además, la megalopa de *Parthenopoides massena* se compara con la descripción anterior de Thiriot (1973) y se separa morfológicamente *de Derilambrus angulifrons*.

Material y Métodos

Tres megalopas de *Derilambrus angulifrons* fueron capturadas en julio de 2007 en el plancton del estuario del Guadalete (Cádiz-SW España) y dos megalopas de *Parthenopoides massena* se colectaron en dos estaciones diferentes en el mar Mediterráneo, una en el Golfo de Nápoles en septiembre de 2009 y otra en las Islas Baleares en julio de 2010. La identificación de las larvas del plancton se basa en secuencias parciales del gen 16S del ADNmt y el gen Cox1 (Marco-Herrero et al. 2013).

Resultados

Las secuencias de las megalopas obtenidas en el estuario del rio Guadalete encajan perfectamente con las de *Derilambrus angulifrons* y las de las megalopas de las Islas Baleares y de Nápoles encajan con las secuencias *de Parthenopoides massena.* Ambas secuencias obtenidas en este trabajo han sido depositadas en Genbank. Las larvas se describen de acuerdo a los estándares propuestos por Clark et al. (1998). Las ilustraciones de la descripción se pueden observar en las figs. 1-4 del Capítulo 7.

Discusión

Las relaciones sistemáticas de los Parthenopidae ha sido motivo de controversia durante mucho tiempo. Desde 1862 hasta la actualidad, su posición sistemática ha cambiado de Calappidae (Strahl 1862) a Brachyryncha (Yang 1971), pasando por Cancridae (Lebour 1928; Aikawa 1935) y Oxyryncha (Bouvier 1940; Balss 1957). Guinot (1977; 1978) elevó Parthenopidae a una superfamilia en la sección Heterotremata, que más tarde fue corroborado con la morfología de las larvas (Rice 1980). Tan (2004) y Tan y Ng (2007) han llevado a cabo la revisión más reciente y completa de Parthenopoidea. A pesar de todos estos estudios, sus relaciones filogenéticas

están aún sin resolver, y sólo queda claro que la familia no está relacionada con Majoidea (Yang 1971; Ahyong et al. 2007). Sin embargo, en base a la morfología adulta se ha sugerido que existen relaciones con Aethroidea, Calappoidea, Trapezoidea y Plagusiidae, entre otros (Tan y Ng 2007), y que en base a la morfología de las larvas se relacionan con Cancroidea (Lebour 1928; Aikawa 1937) y Cyclometopa en general (Rice 1980).

Los estudios larvarios han contribuido a la resolución de problemas en la clasificación sistemática de cangrejos braquiuros (Marques y Pohle 1998; Clark y Guerao 2008; Clark 2009; Marco-Herrero et al. 2013). Sin embargo, todavía hay pocos datos sobre el desarrollo larvario para los partenópidos y la mayoría de las descripciones larvarias se ocupan sólo de los primeros estadios zoea de muestras del plancton.

Las larvas de partenópidos no poseen ningún carácter único que las distinga del resto de las superfamilias de braquiuros (Yang 1971; Rice 1980), pero hay un conjunto de características que se pueden usar para identificarlas, detalladas en el Capítulo 7. En el presente estudio se describe por primera vez la megalopa de *Derilambrus angulifrons* a partir de muestras colectadas del plancton e identificados por código de barras de ADN. Rice (1981) examinó el significado filogenético de las megalopas de braquiuro y comentó que esta etapa era la única fase del ciclo de vida braquiuro que no habían sido examinados previamente para las clasificaciones. Más tarde Martin (1988) estudió el significado filogenético de la megalopa en el caso de los Xanthidae. Es difícil aplicar la morfología de la megalopa para inferir relaciones filogenéticas para Parthenopoidea, teniendo en cuenta que en la actualidad solo se conocen descripciones para cinco especies. Se necesitan nuevos datos sobre la morfología larval de más géneros de Parthenopinae y representantes de la subfamilia Daldorfiinae, así como nuevas filogenias moleculares que comprenden miembros de todas las superfamilias Heterotremata, con una representación más amplia de especies Parthenopidae, Cancridae, Aethridae y Calappidae para determinar la posición filogenética de este taxón.

Capítulo 7 The systematic position of *Ergasticus* (Decapoda, Brachyura) and allied genera, a molecular and morphological approach

Introducción

La superfamilia Majoidea Samouelle, 1819 está representada por aproximadamente 950 especies que se encuentran distribuidas por todo el planeta ocupando múltiples hábitats, desde zonas intermareales hasta profundidades de más de 1.000 metros (D'Udekem d'Acoz 1999; De Grave et al 2009; Richer de Forges y Poore 2008). Las clasificaciones actuales de la superfamilia

Majoidea se basan principalmente en la morfología de los adultos (Garth 1958; Griffin y Tranter 1986). Sin embargo, recientes revisiones taxonómicas parecen sugerir que los rasgos morfológicos de los adultos pueden ser incongruentes con los caracteres larvarios (Clark y Webber 1991; Marques y Pohle 2003). Los análisis basados en marcadores moleculares parecen corroborar las relaciones filogenéticas basadas en la morfología larval (Hultgren et al. 2009).

Ergasticus clouei A. Milne-Edwards, 1882 es un cangrejo majoideo raro y la única especie conocida del género (Ng et al. 2008). No se sabe mucho sobre la biología y del ciclo de vida de esta especie. Sobre la base de ejemplares adultos, *Ergasticus* ha sido tradicionalmente asignado a la familia Inachidae MacLeay, 1838 (Balss 1957; Manning y Holthuis 1981; Ng et al., 2008), aunque algunos autores la han situado en Pisinae Dana, 1851 (Bouvier 1940; Zariquiey Álvarez 1968). Aunque el adulto de *Ergasticus* entra dentro de la definición actual de los Inachidae, un estudio reciente basado en la morfología de la primera zoea cuestionó su posición sistemática (Guerao y Abelló 2007). Sin embargo, dadas las dificultades encontradas para cultivar la zoea II y megalopa, los resultados obtenidos en ese estudio fueron limitados e impidieron la evaluación de la sistemática de *Ergasticus.*

El presente estudio tiene como objetivo resolver las incertidumbres de la asignación de *Ergasticus* dentro de la familia Inachidae, describiendo la morfología completa de su desarrollo larvario a partir de muestras obtenidas del plancton identificadas mediante análisis de ADN. Además, se realizó un análisis filogenético completo, incluyendo secuencias de ADN de representantes de varias familias de majoideos.

Material y Métodos

Dos campañas de investigación multidisciplinarias se llevaron a cabo durante el otoño de 2009 y el verano de 2010. Se recogieron un total de 218 muestras de meso-zooplancton y 66 muestras de macro-zooplancton. De estas, 2 zoeas I, 4 zoeas II y 2 megalopas se asignaron provisionalmente a una especie de májido no identificada. Además, de forma independiente se colectaron dos individuos de *E. clouei* en Almería, cerca de Cabo de Gata, y otro ejemplar adulto de *E. clouei* en Mallorca. Las larvas se describieron siguiendo las normas estandarizadas propuestas por Clark et al. (1998).

La metodología molecular utilizada en este estudio se detalla en el Capítulo 5 de esta tesis. Con el fin de llevar a cabo un análisis filogenético completo, las alineaciones de cada conjunto de datos de genes se realizaron utilizando v3.6 MUSCLE (Edgar 2004). Para evitar ambigüedades, se utilizó Gblocks v0.91b software (Castresana 2000) para la alineación del gen

16S rDNA. La selección combinada del esquema de partición de mejor ajuste para la alineación y el modelo de sustitución de nucleótidos para cada partición se llevó a cabo utilizando el nuevo método objetivo implementado en PartitionFinder (Lanfear et al. 2012). El software BEAST (Drummond y Rambaut 2007) se utilizó para inferir las relaciones filogenéticas entre las muestras, y para generar datos de consenso de los árboles posteriores. El enfoque del factor de Bayes se utilizó para comparar los diferentes modelos (Nylander et al. 2004).

Resultados

La serie completa de las larvas (zoea I, zoea II y megalopa) del cangrejo *Ergasticus clouei* se describe e ilustra, basándose en muestras de plancton del Mediterráneo. La zoea II y la megalopa, previamente desconocidos, se describen aquí por primera vez. Las secuencias parciales de los genes 16S rDNA y Cox1 confirmó la asignación de estas larvas a *Ergasticus clouei*.

Se aplicaron métodos de análisis hasta obtener el árbol filogenético consenso (Fig. 1 del Capítulo 5) que mostró una agrupación altamente significativa de la zoea y megalopa con el adulto *Ergasticus clouei,* revelando la identidad de las larvas. Con el fin de probar con apoyo estadístico las hipótesis previas establecidas (es decir *Ergasticus* pertenece a Inachidae), se calcularon factores de Bayes comparando con la topología del árbol obtenido bajo un modelo sin restricciones contra topologías limitadas. Los valores de probabilidad logarítmica obtenidos del árbol sin restricciones (-8258,55 ± 0,78) fueron significativamente mayores que las obtenidos a partir del árbol restringido (-8262,9 ± 1,07). Según el factor de Bayes obtenido (BF = 8.70), se puede concluir que existe un fuerte apoyo para la eliminación de *Ergasticus* de la familia Inachidae, y su agrupación con los Oregoniidae Garth, 1958.

Discusión

El presente estudio describe por primera vez el desarrollo larvario completo de *Ergasticus clouei* gracias a la utilización del método de código de barras de ADN a partir de larvas recogidas en el plancton. Los estados larvarios identificados genéticamente de *E. clouei,* muestran las características generales indicadas por Rice (1980) para las larvas de Majoidea, sin embargo, no encajaban en la típica definición actual de la familia Inachidae (Marco-Herrero et al. 2012; Marques y Pohle 2003; Rice 1980). En relación con esto, la descripción detallada nos permitió observar un carácter notable en las zoeas de *E. clouei*: la presencia de una espina en la parte ventral de la furca del telson. Después de una revisión exhaustiva de la literatura, lo más probable es que esta espina sea homóloga a la espina presente en algunos Majoidea no inachidos (Rice 1980; Ingle 1992). Además, presentó una serie de caracteres, tales como la

morfología de las antenas y el patrón de setación de las piezas bucales que les coloca más cerca de la familia Oregoniidae. Como tal, *E. clouei* debe ser posicionado en Oregoniidae porque comparte con ellos más caracteres que con cualquier otra familia de Majoidea.

De acuerdo con la presente revisión de la morfología de las larvas y contrario a nuestras expectativas dada la actual clasificación de los Majoidea, el análisis molecular filogenético no mostró que *E. clouei* se agrupara con los géneros de los inachidos probados (*Macropodia*, *Podochela*, *Inachus* y *Metoporhaphis*). En cambio, tanto las secuencias de las larvas como de los adultos de *E. clouei* se agruparon con los géneros de Oregoniidae como *Chionoecetes* Krøyer, 1838 y *Hyas* Leach, 1814. En este estudio, tanto la información morfológica de todos los estadios larvarios, como los análisis de las secuencias de ADN (16S y Cox1), proporcionan pruebas concluyentes para apoyar la eliminación de *Ergasticus* de la familia Inachidae, y situarlo junto con los miembros de la familia Oregoniidae. Por lo tanto, nuestros resultados también evidencian que las fases del desarrollo larvario de los braquiuros proporcionan características morfológicas fiables para ayudar a resolver las relaciones filogenéticas entre géneros.

SECCIÓN IV. Clave ilustrada de las megalopas de braquiuros de la Península Ibérica

Capítulo 8 Illustrated key for the identification of brachyuran megalopae (Crustacea, Decapoda) of Iberian Peninsula (SW Europe)

Introducción

La mayoría de los invertebrados marinos presentan ciclos de vida complejos, con varias fases de desarrollo cuya morfología difiere mucho de la que finalmente adopta el adulto (Anger 2006). Este es el caso de los crustáceos decápodos braquiuros, conocidos comúnmente como cangrejos. Los braquiuros, con las escasas excepciones de aquellos con desarrollo directo, pasan por una etapa larvaria planctónica con dos fases, zoea y megalopa, ambas muy diferentes morfológicamente entre si y con respecto al adulto (Rice 1981). Este hecho supone un inconveniente a la hora de identificarlas cuando son colectas en el plancton (Bucklin 2010).

La identificación de las megalopas se ha basado tradicionalmente en características morfológicas, pero en ocasiones, es imposible conseguir una identificación precisa.

Existen claves para la identificación de larvas braquiuros para diferentes regiones (por ejemplo; Ingle 1992; Paula 1996; Báez 1997; Bullard 2003; dos Santos y González-Gordillo 2004; Pessani et al. 1998, 2004; Rice & Tsukimura 2007; González et al. 2009; Korn y Kornienko 2010; Koetter et al. 2012), pero no existen estudios específicos para la Península Ibérica.

Actualmente con la combinación de las claves de Ingle (1992) y Pessani et al. (2004) sólo se pueden identificar 55 de las 140 especies de la Península Ibérica (Marco-Herrero et al. 2015).

El uso de marcadores moleculares es una herramienta poderosa para identificar con precisión las muestras de plancton (Pan et al. 2008; Pardo et al. 2009; Ampuero et al. 2010; Marco-Herrero et al. 2013). En este estudio, se han identificado las megalopas colectadas del plancton mediante el uso de secuencias parciales de los genes mitocondriales 16S y Cox1 como códigos de barras de ADN. El principal objetivo del presente estudio es la optimización de la aplicación conjunta de técnicas moleculares, en particular el análisis de secuencias mitocondriales de ADN (16S y Cox1), y el análisis morfológico, en la identificación precisa de las megalopas de braquiuros ibéricos obtenido directamente de muestras planctónicas. Las nuevas descripciones, junto con los anteriormente existentes, han permitido la creación de una clave de identificación ilustrada, destinada a ayudar a la correcta identificación de este importante grupo de organismos planctónicos.

Métodos

La clave Ilustrada proporciona información morfológica de la megalopa de 92 especies de braquiuros de la Península Ibérica, pero todas las especies no han podido ser separadas en la clave debido a las dificultades encontradas relacionadas con la poca variabilidad morfológica de especies estrechamente relacionadas, especialmente algunos géneros (por ejemplo *Liocarcinus*). La clave se basa en megalopas obtenidos a partir de muestras de plancton e identificadas por los genes mitocondriales 16S y Cox1, a partir de cultivo de laboratorio, colecciones de los museos y de la literatura de las larvas. Siempre que ha sido posible, las muestras obtenidas del plancton se compararon con la descripción original y se volvieron a dibujar a partir de los especímenes capturados. El trabajo realizado con las megalopas obtenidas se detalla en el Apéndice I. En la clave de identificación se han utilizado solamente los caracteres morfológicos externos de las larvas que son fáciles de observar utilizando un microscopio estereoscópico, sin disección del espécimen (Plate I). Esta clave no refleja ninguna disposición sistemática de las familias de los braquiuros.

Resultados

La clave ilustrada se puede observar en el Capítulo 8 de la presente tesis, además de las figuras realizadas hasta el momento.

CONCLUSIONES

1. Se ha generado por primera vez una gran base de datos moleculares para los braquiuros ibéricos. Se han conseguido secuencias para el marcador 16S de 115 especies, y para el "código de barras" (Cox1) de 118 especies. De las 233 secuencias obtenidas, 118 son nuevas aportaciones: 57 nuevas secuencias para el gen Cox1 y 61 nuevas secuencias para el marcador 16S. El 90% de las especies ibéricas podrán ser identificadas a nivel molecular, con los marcadores genéticos utilizados.

2. Se completó un inventario de la fauna de braquiuros presentes en la Península Ibérica, actualizando el trabajo de Ricardo Zariquiey Álvarez (1968) para esta región y de Cédric d'Udekem d'Acoz (1999) para Europa. Los cambios en la sistemática realizados en los últimos años han afectado la clasificación original, por lo que ahora se cuenta con 20 superfamilias, 36 familias y 77 géneros. Además de nuevas aportaciones para la carcinofauna ibérica.

3. Hay 10 nuevas especies para la ciencia presentes en agua ibéricas:

Calappa tuerkayana, *Chaceon inglei*, *Chaceon mediterraneus*, *Homologenus boucheti*, *Maja brachydactyla*, *Macropodia deflexa, Macropodia parva, Monodeus guinotae Pilumnus* sp. y *Pisa* sp.

4. Se han encontrado 17 especies ya conocidas, pero citadas en la Península Ibérica después de 1968: *Afropinnotheres monodi*, *Brachynotus atlanticus, Brachynothus gemmellaroi*, *Calappa pelii*, *Chaceon affinis*, *Cryptosoma cristatum*, *Cymonomus normani*, *Ethusina talismani*, *Geryon trispinosus*, *Liocarcinus maculatus*, *Liocarcinus mcleayi*, *Microcassiope minor*, *Paractaea monodi*, *Paragalene longicrura*, *Pisa carinimana*, *Velolambrus expansus* y *Xantho sexdentatus*.

5. Hay un total de 10 especies exóticas introducidas en los últimos 30 años en aguas ibéricas: *Callinectes exasperatus*, *Callinectes sapidus, Charybdis feriata*, *Dyspanopeus sayi*, *Eriocheir sinensis, Hemigrapsus takanoi*, *Pachygrapsus gracilis, Percnon gibbesi*, *Pilumnopeus africanus* y *Rhithropanopeus harrisii.*

6. Diferentes estudios morfológicos y moleculares sobre las especies: *Brachynotus gemmellaroi*, *Calappa tuerkayana*, *Geryon trispinosus*, *Macropodia parva* y *Monodaeus guinotae* apuntan a que se trata de posibles sinonimias de *B. sexdentatus*, *C. granulata*, *G. longipes*, *M. rostrata* y *M. couchii*, respectivamente, pero se precisa aun confirmación.

7. Se describen las megalopas de 12 especies para las cuales no se tenía conocimiento de su morfología: *Afropinnotheres monodi, Derilambrus angulifrons, Ergasticus clouei, Macropodia czerjawskii*, *Liocarcinus maculatus*, *L. vernalis*, *L. zariquieyi*, *Planes minutus, Pilumnus* sp., *Pisa* sp., *Portunus hastatus* y *Sirpus zariquieyi.*

8. Se re-describen las megalopas de 4 especies: *Atelecyclus undecimdentatus Dyspanopeus sayi*, *Percnon gibbesi* y *Rhithropanopeus harrisii*.

9. Los análisis de las secuencias de dos genes (16S rDNA y Cox1) revelaron grandes diferencias entre *Ergasticus* y otros miembros de la familia Inachidae, incluyendo el género tipo *Inachus*. El análisis filogenético realizado y la comparación de caracteres larvales sugieren la eliminación de *Ergasticus* y géneros relacionados (*Bothromaia, Pleisticanthoides, Parapleisticantha* y *Pleistacantha*) de la familia Inachidae y situarlos dentro de la familia Oregoniidae como una subfamilia separada, Pleistacanthinae Števčić, 2005.

10. La base de datos molecular obtenida para los marcadores (16S y Cox1), supone una contribución importante a futuro, para la realización de diferentes estudios y/o aplicaciones:

a. Estudios filogenéticos que servirán como base para resolver la filogenia de varios grupos complicados y difíciles de estudiar en la Península Ibérica. En particular, se encuentran ya en preparación los estudios de la familia Inachidae y del género *Ebalia*.

b. Estudiar la dinámica de poblaciones de cangrejos, sobre todo aquellas especies de interés comercial, ya que se podrán determinar sus periodos de reclutamiento y su área de dispersión, épocas de reproducción, zonas de dispersión y concentración larval, así como áreas de asentamiento y reclutamiento, imprescindible para una gestión sostenible de sus pesquerías.

c. Proporciona una correcta identificación de ejemplares juveniles (muy difíciles de reconocer incluso por expertos), ejemplares incompletos, o incluso realizar estudios sobre relaciones tróficas entre especies (e.g. braquiuros en contenidos estomacales de *Thunnus* sp.).

d. Permite la detección temprana de especies tanto de aquellas que están ampliando su rango de distribución como de especies invasoras, como por ejemplo el nuevo *Pinnotheres* sp. Este cangrejo africano es simbionte, por lo que el adulto es más difícil de encontrar. En este estudio en preparación, se detectó el desarrollo completo en el plancton mediante la morfología larval y se confirmó mediante técnicas moleculares que no se trataba de ninguna de las especies de pinnotheridos ibéricos conocidos.

11. Las nuevas megalopas descritas y re-descritas han ampliado la información morfológica de esta fase larval de los cangrejos ibéricos con respecto a los trabajos de Ingle (1992) para especies Atlánticas y de Pessani et al. (2004) para las Mediterráneas, que permitían identificar las megalopas de hasta un total de 55 especies de braquiuros ibéricos. Esta tesis proporciona información morfológica nueva para 37 especies, lo que supone un incremento del 26.5% y permite incluir información morfológica de 92 especies en la clave que se ha desarrollado.

12. El estudio combinado de la morfología y marcadores moleculares nos permite concluir que no todas las 92 especies pueden discriminarse a nivel de morfología de la megalopa. Se ha encontrado poca o ninguna variabilidad fenotípica entre especies de algunos géneros, como por ejemplo el caso de *Liocarcinus* y *Brachynotus* que necesitan un mayor esfuerzo y revisión detallada de otros caracteres para obtener unos mejores resultados (en preparación).

13. Del estudio morfológico minucioso de las megalopas, se detectan caracteres importantes, que se consideraban constantes, que muestran variabilidad intraespecífica, tanto en ejemplares colectados del plancton como obtenidos en laboratorio. Por ejemplo: setación de los urópodos y segmentación de la antena.

14. Se añade la descripción de la placa esternal de todos los ejemplares estudiados. Un carácter apenas descrito hasta ahora. La nueva comparación de este carácter, nos permite confirmar que es constante dentro de una misma especie, llegando a ser en algunos grupos constante a nivel de género. Por lo tanto, en combinación con otros caracteres, se debería de considerar un nuevo carácter morfológico de utilidad en la identificación de las megalopas.

15. El área de estudio comprende todas las aguas de la Península Ibérica, tanto las marinas (desde la zona intermareal a profundidades aproximadas de 1.200 metros) como las continentales. El gran tamaño de la zona a estudiar y los costos asociados a los muestreos de plancton, impidieron que los muestreos fueran uniformes y periódicos en toda el área. Por tanto, especies menos frecuentes y de distribución local pueden no estar incluidas en las muestras analizadas. En cualquier caso, las secuencias disponibles permitirán identificar a nivel molecular el 90% de las especies ibéricas.

INTRODUCCIÓN GENERAL

Entre los crustáceos decápodos, el Infraorden Brachyura Linnaeus, 1758 es el grupo más diverso y de mayor éxito evolutivo, con aproximadamente 7.000 especies pertenecientes a 98 familias (Tsang et al. 2014). El número de especies sigue incrementándose con gran rapidez en los últimos años ya que aún existen hábitats poco estudiados, como por ejemplo los manglares tropicales y las profundidades oceánicas (Ng et al. 2008). En la **Sección I** / **Capítulo 1** de esta tesis se presenta una actualización de las especies de la Península Ibérica**:**

Elena Marco-Herrero, Pere Abelló, Pilar Drake, J Enrique García-Raso, J Ignacio González-Gordillo, Guillermo Guerao, Ferran Palero, José A Cuesta (2015) **Annotated checklist of brachyuran crabs (Crustacea: Decapoda) of the Iberian Peninsula (SW Europe)** Scientia Marina 79(2): 243-256

Los braquiuros, comúnmente llamados cangrejos, han conquistado casi todos los hábitats y numerosos nichos ecológicos (De Grave et al. 2009; Ahyong et al. 2011). La mayoría de las especies son marinas, aunque también existen especies de agua dulce o incluso especies terrestres. Esta riqueza de especies y diversidad ecológica es aún más sorprendente cuando se considera la edad de este grupo. Los primeros representantes del Infraorden Brachyura aparecen tarde en el registro fósil en comparación con otros grupos de crustáceos decápodos, en el Jurásico inferior (sobre 180 Mya para ser exactos) (Haug et al. 2015). A pesar de este origen tardío, los cangrejos se diversificaron muy rápidamente tanto morfológica como ecológicamente entre el Cretácico medio (alrededor de 100 Mya) y el Eoceno (unos 50 Mya) (Brösing 2008; Luque 2015).

El ciclo de vida de los braquiuros, consiste de una etapa larvaria, generalmente de vida libre y planctónica, con un número variable de estadios, seguida de una etapa juvenil que puede ser libre o asociada a un hospedador (en el caso de especies simbiontes), también con un número variable de estadios y finalmente una etapa adulta que se desarrolla en el hábitat propio de cada especie, que es muy variable, como se ha señalado con anterioridad (Anger 2006).

El término “larva” hace referencia a cualquier forma inmadura post-embrionaria que difiere morfológicamente del adulto y que, bien de forma gradual (anamorfosis) o por medio de cambios más abruptos (metamorfosis), se desarrolla hasta alcanzar la forma del adulto (Martin et al. 2014). En el caso de los braquiuros, con las escasas excepciones de aquellos con desarrollo directo (principalmente especies de agua dulce), el desarrollo larvario es metamórfico, y consta de dos fases: zoea y megalopa (Figura 1). La fase zoea se caracteriza porque la locomoción de la larva se realiza mediante los apéndices cefalotorácicos bucales, concretamente los

maxilípedos. Esta fase puede constar de un número variable de estadios, desde sólo una o dos zoeas (en los Majoidea Samouelle, 1819) hasta las 8 o 12 zoeas que pueden presentar algunos Grapsoidea MacLeay, 1838, y Portunoidea Rafinesque, 1815.

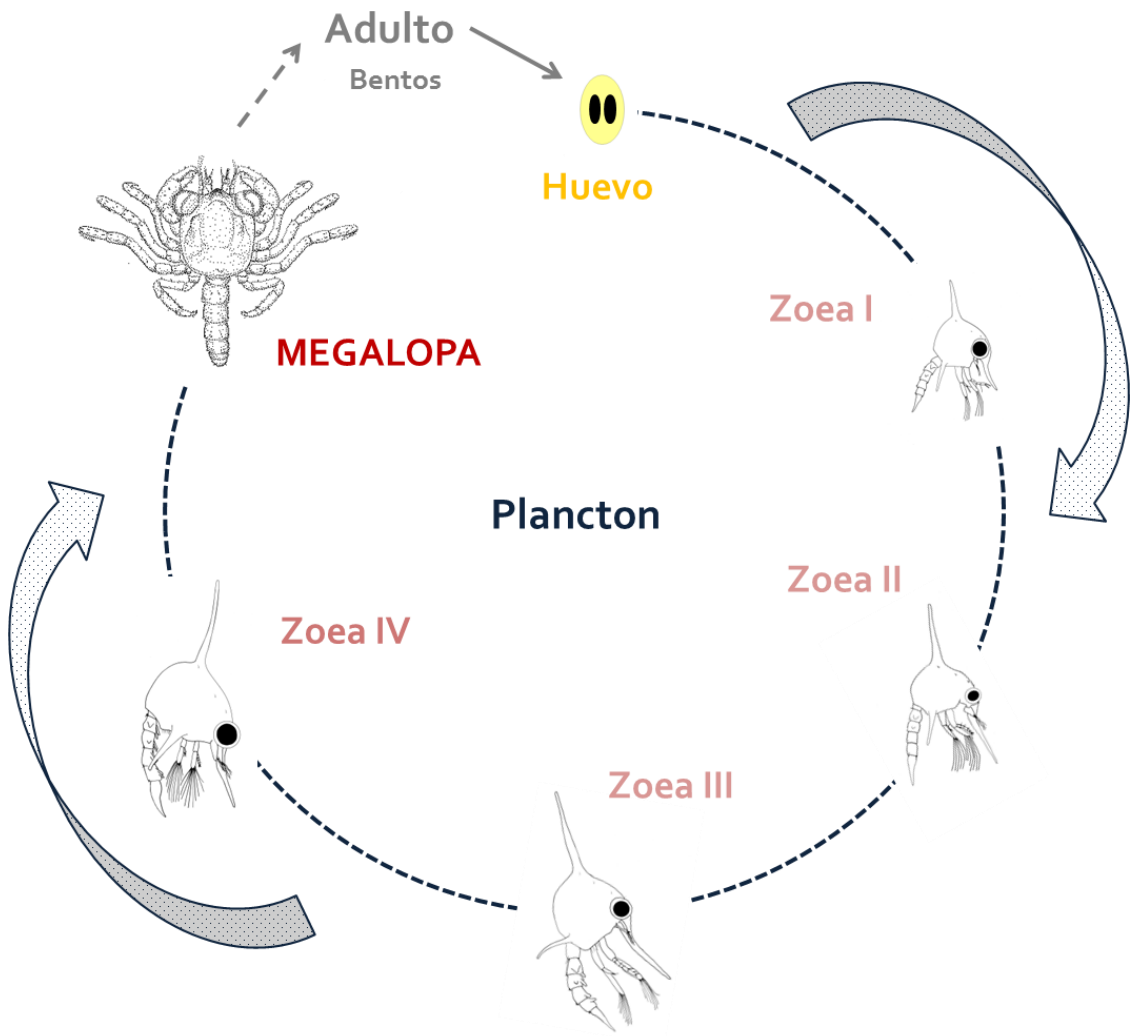

Figura 1. Desarrollo larvario de braquiuros (cangrejos), dos fases planctónicas: zoea y megalopa. Ejemplo esquemático de *Afropinnotheres monodi*.

La fase megalopa, un nombre que proviene del griego y significa “ojos grandes”, es en la que se centra la presente tesis. Consta de un solo estadio, aunque en alguna familia, como los Hymenosomatidae MacLeay, 1838 o alguna especie como *Metopaulias depressus* (González-Gordillo et al. 2010) no se presenta, y el último estadio zoea muda directamente al primer juvenil. La megalopa es una fase de transición entre la zoea planctónica y la fase juvenil y adulta bentónica. Esta fase aún mantiene la capacidad natatoria (pero impulsada en este caso por los pleópodos del pleon), y su morfología general recuerda más a la de un cangrejo adulto, presentando estructuras que le permiten fijarse al sustrato y mudar al primer estadio juvenil.

Dado que es la fase intermedia entre el plancton y el bentos, difiere tanto morfológica como ecológicamente respecto de las fases zoea y juvenil y adulta (Rice 1981).

Los caracteres larvarios propios de las fases zoea y megalopa incluyen tanto características morfológicas como de comportamiento asociado a la alimentación y locomoción, presentando adaptaciones a un estilo de vida planctónica y permitiendo la explotación de recursos distintos de aquellos utilizados en la fase adulta (Anger 2006).

Esta notable variación de morfología, comportamiento y hábitat entre larvas y adultos, representa un gran problema a la hora de identificar las larvas del zooplancton. La morfología de las formas larvarias no guarda ninguna similitud con la de los adultos, por lo que son difíciles de relacionar, y por tanto identificar a nivel de especie, incluso a veces las larvas se distinguen morfológicamente, pero no resulta fácil atribuirla a la forma adulta correcta (Bucklin 2010). Este problema supone una falta de información que impide estudios detallados sobre la dinámica de poblaciones de los cangrejos, la determinación de sus periodos de reclutamiento y su área de dispersión, en general, cualquier tipo de investigación sobre relaciones tróficas planctónicas, o inventarios de biodiversidad de taxones o áreas concretas. Además, en el caso de las especies de interés comercial, el poder identificar estos estados larvarios en el plancton es necesario para una gestión sostenible de sus pesquerías (Eaton et al. 2003; Freire et al. 2002).

La existencia de un proceso de metamorfosis en los crustáceos decápodos fue reconocida hace menos de 200 años por Thomson (1828), que observó que especies descritas del plancton, como *Zoea pelagica* Bosc, 1802, eran realmente formas larvarias de braquiuros y no formas adultas (Ingle 1992; Anger 2001). Siendo así, que el término “megalopa” (asignado a una especie, *Megalopa armata*, por Leach en 1814), que define la última fase larvaria de los braquiuros, se propuso por Williamson en 1962, reemplazando el término post-larva de Gurney utilizado hasta el momento (Gurney 1939; Rice 1981, 1993).

A lo largo del S. XIX y la primera mitad del XX, la mayoría de las descripciones morfológicas de los estados larvarios se basaron en ejemplares recogidos directamente del campo. Esto dejó considerables dudas sobre la correcta identidad taxonómica o la integridad de la serie del desarrollo que había sido reconstruido a partir de muestras de plancton, y que a veces eran combinados con observaciones de cultivos en laboratorio, que en su gran mayoría solo conseguían una o dos mudas larvarias. Además, las hembras ovígeras a veces eran erróneamente identificadas o mal etiquetadas, y no se aportaba información de si fueron depositadas en algún museo, cosa que en la actualidad se exige ya que permite confirmar y revisar el material depositado en caso necesario (Ingle 1992).

Una de las mayores aportaciones al avance del conocimiento de las larvas de los braquiuros en la primera mitad del S. XX, fue la de Marie Victorie Lebour (1927, 1928), que perfeccionó y describió la técnica del cultivo en laboratorio de estas larvas. En sus años de investigación, dedicada a los estudios de los braquiuros, aportó información de la morfología larval de 40 especies (Lebour 1931, 1934, 1944). A partir del trabajo de Lebour y con aportaciones nuevas como Bocquet (1954), Bourdillon-Casanova (1960), Heegaard (1963), Goldstein (1971) y Christiansen (1973), las descripciones se vuelven más fiables. En los últimos 50 años se han realizado cultivos larvales en laboratorio a partir de hembras ovígeras para describir los diferentes estadios larvarios y conocer la morfología larval de una determinada especie con un 100% de certeza (Rice 1993; Anger 2001). Con esta metodología, se han descrito en la tesis dos desarrollos larvarios completos, formando parte de los **Capítulos 2-3** de la **Sección II**:

- Elena Marco-Herrero, Antonio Rodríguez, José A Cuesta (2012) **Morphology of the larval stages of *Macropodia czernjawskii* (Brandt, 1880) (Decapoda, Brachyura, Inachidae) reared in the laboratory** Zootaxa 3338: 33-48

- Elena Marco-Herrero, Guillermo Guerao, José A Cuesta (2013) **Morphology of the larval stages of a Mediterranean population of the allochthonous Say's mud crab, *Dyspanopeus sayi* (Decapoda: Brachyura: Panopeidae)** Scientia Marina 77(2): 341-352

Además, un mejor manejo de los cultivos de larvas bajo condiciones controladas en el laboratorio ha permitido realizar otro tipo de estudios relacionados con la ecofisiología, comportamiento, nutrición, etc. Un ejemplo, donde se estudia el impacto de la temperatura en el desarrollo larvario de *Afropinnotheres monodi*, se presenta en la tesis dentro de la **Sección III,** el **Capítulo 4.**

- Elena Marco-Herrero, Pilar Drake, JIgnacio González-Gordillo, José A Cuesta (2015) **Larval development of the pea crab *Afropinnotheres monodi* Manning, 1993 (Decapoda, Pinnotheridae) using plankton-collected and laboratory-reared specimens: Effects of temperature** Marine Biology Research (Aceptado)

Las metodologías empleadas en los estudios de la biología y taxonomía larval, llevadas a cabo hasta el momento por la mayoría de los investigadores, tienen una serie de restricciones y/o limitaciones para el avance de esta ciencia (Anger 2006):

1. El tamaño del material de estudio. Las larvas son muy pequeñas con respecto los adultos, por lo tanto el esfuerzo para obtenerlas es mayor.

2. En estudios de laboratorio, la producción de larvas en cantidades suficientes requiere de unos métodos tediosos y consume mucho tiempo. Por otra parte, las condiciones de cría artificiales pueden comportar artefactos morfológicos, difíciles de identificar, y como consecuencia pueden dar descripciones larvales erróneas.

3. Las muestras tomadas del campo no aportan información relativa a la duración de incubación o muda, o sobre la alimentación o condiciones ambientales.

Además las larvas en fase megalopa, aún las identificadas a nivel de especie, son a menudo morfológicamente bastante similares, y hay muy pocas claves para su identificación (Haug et al. 2015).

La falta de datos *a priori* que relacionen los estados larvarios con la especie a la que pertenecen, han causado un avance moderado en el conocimiento de la fase megalopa. De las 140 especies de braquiuros conocidas en la Península Ibérica, solo se dispone de descripciones fiables de sus estados larvarios para 67 especies, de las cuales 10 son incompletas. Estas descripciones incompletas, hacen que se tengan que actualizar los datos realizando re-descripciones que aporten toda la información necesaria para su correcta caracterización morfológica. En el **Capítulo 5 de la Sección III** se presenta 1 re-descripción:

- Elena Marco-Herrero, J Ignacio González-Gordillo, José A Cuesta (2014) **Morphology of the megalopa of the mud crab, *Rhithropanopeus harrisii* (Gould, 1841) (Decapoda, Brachyura, Panopeidae), identified by DNA barcode** Helgoland Marine Research 68: 201-208

Como se ha comentado anteriormente, la megalopa (Figura 2) difiere mucho en morfología respecto a las otras fases del ciclo de vida de los braquiuros por ser una fase de transición, por lo cual es muy útil conocer todos los rasgos posibles, y así poder validar caracteres que nos permitan identificarlas pero también observar y comprender cuales pueden ser característicos a nivel de género o familia. Dado que en el pasado se realizaron descripciones que no han podido ser validas a nivel taxonómico por la carencia de rigor científico, Clark et al. (1998) propusieron un estándar para las descripciones larvarias de este taxón para homogenizar las descripciones y no perder información valiosa y que facilite futuros estudios comparados. En la actualidad, se han realizado avances, y se han empezado a aplicar las nuevas técnicas moleculares que minimizan estas limitaciones y/o restricciones antes citadas, y que están permitiendo avanzar a un mayor ritmo en el conocimiento de la morfología larval de los braquiuros y sus aplicaciones, como la filogenia y sistemática.

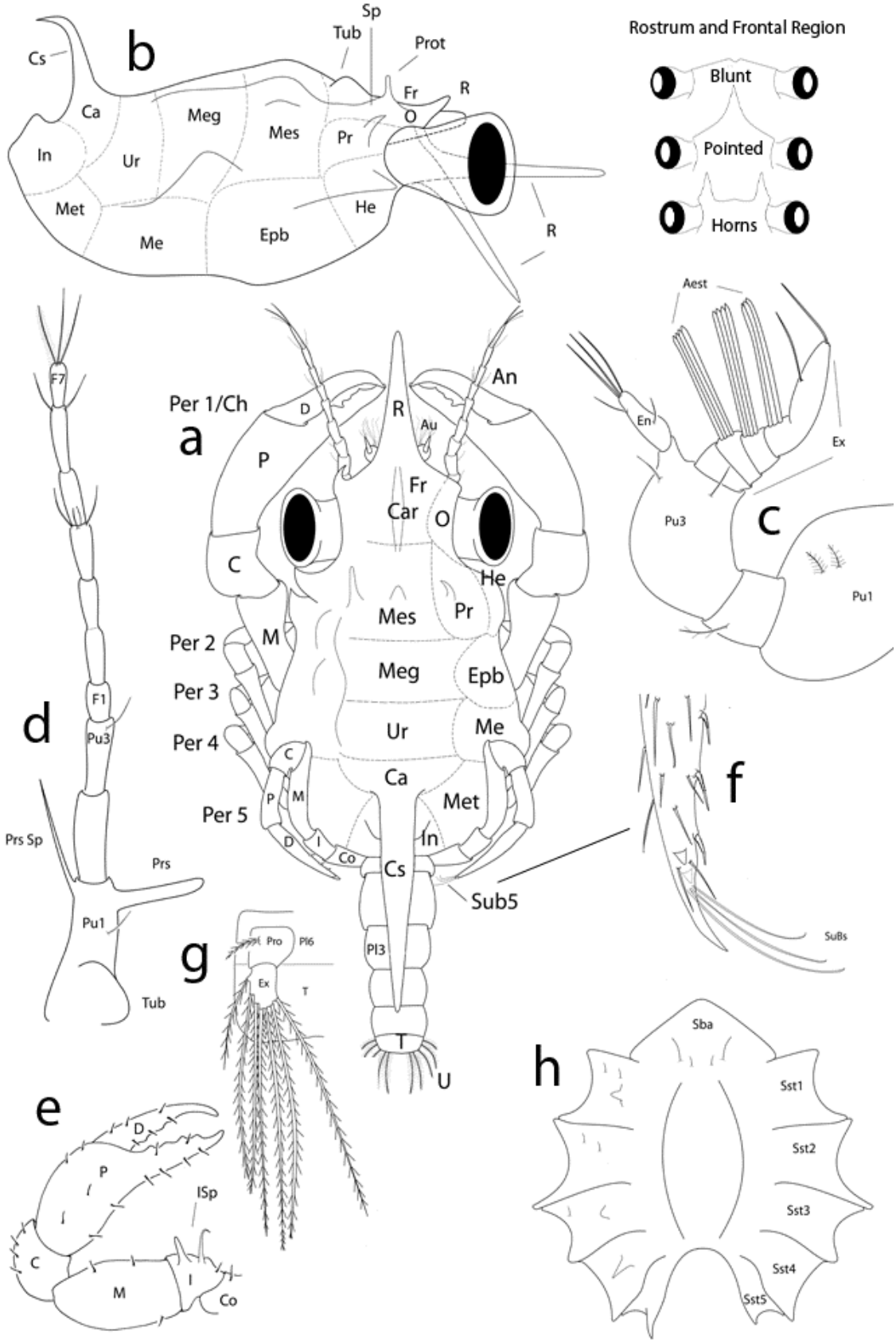

Figura 2. Esquema de los caracteres externos más relevantes de una megalopa.

Una de estas nuevas técnicas fue presentada en 2003 por el doctor Paul Hebert de la Universidad de Guelph, Ontario (Canadá), quien propuso la utilización de una región pequeña del genoma mitocondrial como DNA barcode (código de barras genético), ofreciéndonos un novedoso mecanismo para la identificación de especies (Herbt et al. 2003). El código de barras de ADN, basado en la secuenciación de un fragmento del gen codificante para el citocromo oxidasa, subunidad I, Cox1, resuelve las grandes dificultades que presenta la cría de las larvas en cautividad. Por otro lado, el uso del código de barras de ADN no significa que la taxonomía tradicional haya perdido importancia, sino que ahora cuenta con una nueva herramienta, un complemento valiosísimo, que facilita en gran parte el trabajo de identificación de especies, particularmente cuando se trata de grupos de organismos muy similares (con pocas diferencias morfológicas), fases iniciales del ciclo de vida que presentan una morfología diferente a la del adulto, o cuando los caracteres taxonómicos aún no están diferenciados (o incluso cuando se trata de ejemplares incompletos).

Desde la propuesta inicial del doctor Hebert hasta la actualidad, han surgido numerosos proyectos muy ambiciosos, así como la aplicación de esta técnica en el estudio e identificación de estadios larvales, aunque no siempre utilizando Cox1 como marcador genético (Sewell et al. 2006; Wong et al. 2014). Pardo et al. (2009) y Ampuero et al. (2010) fueron los primeros que describieron megalopas colectadas del plancton e identificadas mediante esta técnica molecular, utilizando en ambos casos el marcador 16S. En la presente tesis se incluyen 3 artículos pertenecientes a la **Sección III (Capítulos 5-7)** en las que se describe la megalopa de tres especies identificadas directamente del plancton utilizando la metodología del código de barras de ADN (uno de estos trabajos ha sido citado anteriormente):

- Elena Marco-Herrero, J Ignacio González-Gordillo, José A Cuesta (2014) **Morphology of the megalopa of the mud crab, *Rhithropanopeus harrisii* (Gould, 1841) (Decapoda, Brachyura, Panopeidae), identified by DNA barcode** Helgoland Marine Research 68(2): 201-208

- Elena Marco-Herrero, J Ignacio González-Gordillo, Jose A. Cuesta (2015) **Larval morphology of the family Parthenopidae, with the description of the megalopa stage of *Derilambrus angulifrons* (Latreille, 1825) (Decapoda: Brachyura), identified by DNA barcode** Journal of the Marine Biological Association of the United Kingdom 95(3): 513-521

- Elena Marco-Herrero, Asvin P Torres, José A Cuesta, Guillermo Guerao, Ferran Palero, Pere Abelló (2013) **The systematic position of *Ergasticus* (Decapoda, Brachyura) and allied genera, a molecular and morphological approach** Zoologica Scripta 42(4): 427-439

El código de barras de ADN ha demostrado ser muy útil tanto para diferenciar especies (Harrison 2004; Lefébure et al. 2006; Costa et al. 2007) como para la diferenciación entre poblaciones de una misma especie (Palero et al. 2008; García-Merchán et al. 2012). Además del Cox1, el gen mitocondrial de la subunidad ribosomal 16S, también ha demostrado ser una herramienta eficiente en estudios sistemáticos de crustáceos decápodos (Schubart et al. 2000; Porter et al. 2005; Ahyong et al. 2007), tanto en el establecimiento de especies nuevas, como para dilucidar la validez taxonómica cuestionable de especies muy cercanas (Schubart et al. 1998, 2001a, b; Spivak y Schubart 2003). Del mismo modo, ha permitido mostrar cómo diferentes poblaciones de una misma especie, deberían ser realmente consideradas especies diferentes (Cuesta y Schubart 1998; Schubart et al. 2001). Parece, por tanto, una herramienta eficaz para la correcta y fiable asignación de una muestra a una especie en el caso de los decápodos braquiuros.

Esta técnica molecular de identificación de braquiuros con secuencias genéticas (genes 16S y Cox1), es también un mecanismo que permite confirmar y/o detectar la presencia de especies introducidas o desconocidas en aguas de la Península Ibérica, no solo de adultos, sino de sus larvas, lo cual puede ser indicador de que una especie está bien establecida ya que completa su ciclo en aguas de la Península. Un ejemplo de este avance en el conocimiento temprano de la presencia de una nueva especie de braquiuro en aguas europeas es el artículo: "*Larval morphology and DNA barcode as valuable tools in early detection of biological invasions. A new pea crab invading European waters as an example"* (en preparación). En este caso tanto la morfología larval, como las secuencias de 16S y Cox1, señalan que se trata de una especie de Pinnotheridae no presente hasta ahora en aguas europeas, pero de la que aún no se han encontrado ejemplares adultos y por tanto no ha sido identificada a nivel de especie.

El aplicar las técnicas moleculares en la identificación de megalopas de muestras del plancton, nos ha llevado a un incremento del número de especies para las que se conoce este estadio larval (Weeb 2006). Y que a partir de ahora podrán ser identificadas directamente del plancton en base a su morfología. Hasta el momento no existía una clave de megalopas específica para la Península Ibérica, así que para poder identificar las megalopas, se contaba con la clave de Ingle (1992) para las especies del Océano Atlántico, y la clave de Pessani et al. (2004) para las del Mar Mediterráneo. Entre ambas se pueden identificar en la Península 55 de las 140 especies. En la tesis actual se aporta una nueva clave más completa para la Península Ibérica y que ha sido realizada con la combinación de las metodologías morfológica y molecular **(Sección IV / Capitulo 8)**:

- Elena Marco-Herrero, Ferran Palero, José A Cuesta (2015) **Illustrated key for the identification of brachyuran (Crustacea, Decapoda) megalopae of Iberian Peninsula (SW Europe)** ***(en preparación)***

Como en la mayoría de los grupos de crustáceos, la sistemática de los braquiuros ha ido variando a lo largo de los últimos años. Hay pocos estudios que hayan abordado la filogenia global del Infraorden Brachyura, pero entre ellos se pueden destacar algunos que han usado técnicas moleculares como el de Spears et al. (1992) basado en el 18S rRNA, o Ahyong et al. (2010), Tsang et al. (2014) realizada con 6 genes nucleares y dos genes mitocondriales. Los de Rice (1980, 1981, 1983) basados en la morfología de las larvas, y también hay varios estudios que utilizaron la morfología espermática (Jamieson 1991, 1994; Guinot et al. 1994; Jamieson et al. 1995) y análisis de la morfología del intestino anterior (Brösing et al. 2002, 2007). Las clasificaciones más recientes y aceptadas se basan en la combinación de estudios moleculares con la morfología de los adultos (Ng et al. 2008; Lai et al. 2011; Karasawa et al. 2011).

Como se ha visto hasta ahora, y más allá de estudios descriptivos a nivel de especies, los caracteres larvales son reconocidos como una fuente importante de información para los análisis filogenéticos y sistemáticos. Haeckel (1866) ya propuso la importancia de estudiar la evolución con un nuevo enfoque, creando una combinación entre la biología del desarrollo y la evolutiva que hoy se conoce como "Biología Evolutiva del Desarrollo" o "Evo-Devo" (Gilbert 2003; Hossfeld y Olsson 2003). Desde los tiempos de Haeckel, la morfología larval y las secuencias sucesivas de las fases del desarrollo, se han utilizado con frecuencia como criterios para desentrañar relaciones filogenéticas entre los taxones de crustáceos (Williamson 1982; Scholtz 2003).

La zoea, por ejemplo, ha sido objeto de especial atención en varios niveles taxonómicos dentro del Infraorden Brachyura, en su mayoría dentro de familias (Rice 1980, 1981, 1983; Marques y Pohle 1995, 1998, 2003; Pohle y Marques 1998, 2000; Clark 2000; Ng y Clark 2000a, b; Santana et al. 2004). En algunos casos, la información de la morfología larval ha demostrado que los caracteres de los adultos pueden ocultar convergencias y por tanto no reflejar correctamente las verdaderas relaciones filogenéticas (Ng y Clark 2000a, b). Del mismo modo, la morfología de la fase megalopa puede indicar relaciones filogenéticas dentro o entre familias de los braquiuros (Martin 1988; Rice 1988). Entre los diferentes caracteres larvales estudiados para los análisis filogenéticos se encuentran: número y posición de setas y espinas, presencia/ausencia de determinados segmentos o apéndices, la aparición de setas en una

secuencia con el desarrollo, su tasa de desarrollo, y la expresión de articulaciones en determinados apéndices (Clark 2000, 2005).

Se podría concluir que una clasificación sistemática adecuada, que buscase reflejar las relaciones filogenéticas entre los diferentes taxa, debería representar un compendio de todas las fuentes de información de las que disponemos, considerando los datos larvales. Sin embargo, a pesar de la información que proporcionan los estadios larvarios, el hecho de no poseer una extensa documentación de los caracteres clave hace que la aplicación en las clasificaciones de esta información se realice a niveles taxonómicos inferiores como familia o género, como ejemplo el artículo del **Capítulo 7** *(citado anteriormente)*. Estudios recientes como en el caso de la revisión de la superfamilia Portunoidea (Spiridonov et al. 2014), todavía se realizan básicamente con la combinación de datos moleculares y la morfología de los adultos, y aunque señalan el alto valor que aporta la morfología larvaria en los estudios filogenéticos, y la utilizan en aquellos grupos para los que hay descripciones conocidas, también indican que hay un gran escasez de datos en esta superfamilia, y que es algo a priorizar en futuros estudios.

OBJETIVOS

El principal **objetivo** de esta tesis es optimizar la aplicación de técnicas morfológicas y moleculares que faciliten la identificación de las megalopas de los braquiuros colectadas del plancton en la Península Ibérica.

Las megalopas, una vez identificadas, serán descritas cuando no se disponga de información previa, o re-descritas si las descripciones originales son incompletas. Estas nuevas descripciones y re-descripciones junto con las existentes, permitirán elaborar una clave ilustrada, dirigida a investigadores y técnicos, que facilite la correcta identificación de estos organismos tan importantes en el zooplancton.

Los *objetivos específicos* de cada Sección/capítulo son:

SECCIÓN I

Capítulo 1

- Generar una base de datos completa con las secuencias de ADN de dos marcadores moleculares (16S y Cox1) para todas las especies de braquiuros de la Península Ibérica.
- Actualizar el listado de braquiuros de la Península Ibérica.
- Revisar la validez taxonómica y posición sistemática de las especies de braquiuros de la Península Ibérica.

SECCIÓN II-III

Capítulos 2-7

- Nuevas aportaciones en el conocimiento de la morfología de la megalopa de diferentes especies.
    - Descripciones y re-descripciones de desarrollos larvarios completos a partir de hembras ovígeras.
    - Descripciones y re-descripciones de desarrollos larvarios y del estadio megalopa, obtenidas del plancton e identificados con técnicas moleculares (16S y Cox1).

SECCIÓN IV

Capítulo 8

- Elaborar una clave ilustrada, que facilite la correcta identificación de las megalopas de los braquiuros ibéricos.

SECCIÓN I

ACTUALIZACIÓN DE LA FAUNA DE BRAQUIUROS DE LA PENÍNSULA IBÉRICA

CAPÍTULO 1

Annotated checklist of brachyuran crabs (Crustacea: Decapoda) of the Iberian Peninsula (SW Europe)

Elena Marco-Herrero, Pere Abelló, Pilar Drake, J Enrique García-Raso, J Ignacio González-Gordillo, Guillermo Guerao, Ferran Palero, José A Cuesta (2015) Annotated checklist of brachyuran crabs (Crustacea: Decapoda) of the Iberian Peninsula (SW Europe) Scientia Marina 79(2): 243-256

Scientia Marina 79(2)
June 2015, 243-256, Barcelona (Spain)
ISSN-L: 0214-8358
doi: http://dx.doi.org/10.3989/scimar.04161.27A

Annotated checklist of brachyuran crabs (Crustacea: Decapoda) of the Iberian Peninsula (SW Europe)

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Summary: Almost 50 years have passed since a group of reputed carcinologists (*viz*. Lipke B. Holthuis, Isabella Gordon and Jacques Forest) finished the posthumous work of Ricardo Zariquiey Álvarez (1968) on decapod crustaceans of the Iberian Peninsula. No lists of decapod fauna specifically covering this area have been published since then, and an update is needed. The current list of brachyuran crabs of the Iberian Peninsula comprises 140 species, which is 35 species more than the 105 valid species listed in Zariquiey Álvarez (1968). Systematic changes have affected the original classification, so now there are 20 superfamilies, 36 families and 77 genera. Additional species have been recorded in Iberian waters due to natural range expansions from nearby areas (Mediterranean and Atlantic), introductions by anthropogenic activities, and description of new taxa. Also, two species were synonymized. Several of these changes, based on evidence from larval morphology and/or molecular data, are detailed in this review. Although descriptions of crab species new to science are not expected to occur at a significant rate, an increase in the number of species in the Iberian Peninsula is expected to result from the introduction of alien species.

Keywords: checklist; Brachyura; Crustacea; Decapoda; crab; Iberian Peninsula.

Lista comentada de los cangrejos braquiuros (Crustacea: Decapoda) de la península Ibérica (SO Europa)

Resumen: Han pasado casi 50 años desde que un grupo de reputados carcinólogos (viz. Lipke B. Holthuis, Isabella Gordon y Jacques Forest) finalizaran la obra póstuma de Ricardo Zariquiey Álvarez (1968), "Crustáceos decápodos de la Península Ibérica". Desde entonces no se ha publicado una lista de la fauna de decápodos que cubra específicamente este área, y era necesaria una actualización. La lista actual de braquiuros de la Península Ibérica consta de un total de 140 especies, 35 especies más de las 105 especies válidas enumeradas en Zariquiey Álvarez (1968). Los cambios en la sistemática han afectado la clasificación original, por lo que ahora hay 20 superfamilias, 36 familias y 77 géneros. Otras especies han sido citadas en aguas ibéricas debido a expansiones naturales de su rango de distribución desde áreas cercanas (Mediterráneo y Atlántico), a las introducciones mediadas por las actividades antropogénicas y a la descripción de nuevas especies. Además, se han sinonimizado dos especies. Varios de estos cambios, basados en evidencias de la morfología de las larvas y/o datos moleculares, se detallan en esta revisión. Aunque no se espera que las descripciones de nuevas especies de cangrejos para la ciencia se produzcan a un ritmo significativo, si es esperable un incremento en el número de especies en la Península Ibérica como resultado de la introducción de especies exóticas.

Palabras clave: lista; Brachyura; Crustacea; Decapoda; cangrejo; península Ibérica.

Citation/Como citar este artículo: Marco-Herrero E., Abelló P., Drake P., García-Raso J.E., González-Gordillo J.I., Guerao G., Palero F., Cuesta J.A. 2015. Annotated checklist of brachyuran crabs (Crustacea: Decapoda) of the Iberian Peninsula (SW Europe). Sci. Mar. 79(2): 243-256. doi: http://dx.doi.org/10.3989/scimar.04161.27A

Editor: C. Froglia.

Received: October 17, 2014. **Accepted**: February 23, 2015. **Published**: March 31, 2015.

INTRODUCTION

Almost 50 years have passed since a group of reputed carcinologists (*viz*. Lipke B. Holthuis, Isabella Gordon and Jacques Forest) finished the posthumous work of Ricardo Zariquiey Álvarez (1968) on decapod crustaceans of the Iberian Peninsula. This geographic area has over 6000 km of coastline and is washed by the warm and oligotrophic Mediterranean Sea in the east and by the colder Atlantic Ocean in the west, which converge at the Strait of Gibraltar (Fig. 1). The high environmental heterogeneity and the proximity between the European and African continents provide suitable conditions for a particularly diverse marine fauna. The extensive information compiled by Zariquiey Álvarez regarding habitat, spawning season and distribution of Iberian decapods made L.B. Holthuis (Zariquiey Álvarez 1968) state that "this peninsula is, in the present moment, one of the best known areas of South Europe concerning decapod fauna". After the work of Zariquiey Álvarez (1968), several authors have published updated lists of decapod fauna at different geographical scales, from European species by d'Udekem d'Acoz (1999) and Türkay (2001) to worldwide brachyuran decapods by Ng et al. (2008). However, none of these has specifically covered the diversity found around the Iberian Peninsula; and an update is needed for this area.

A great number of changes concerning the crustacean species found around the Iberian Peninsula have taken place in the last decades. These changes can be due to systematic modifications such as synonymizations (qualitative) or due to non-corroborated presence or newly reported species for the area (quantitative). The systematic research landscape on decapod crustaceans has changed dramatically in the last few decades as well. A general tendency during the last few years has been to increase the number of families, in most cases simply by raising the rank of extant subfamilies. Today's most widely used classifications have all appeared after the work of Zariquiey Álvarez (1968), including those by Guinot (1977), Bowman and Abele (1982), Martin and Davis (2001) or Ng et al. (2008). De Grave et al. (2009) have also listed all known suprageneric taxa of decapod crustaceans, with estimates on the number of valid species within each group. There is a concerted effort by carcinologists worldwide to check the validity of taxa using multiple tools such as ecological characterization, larval morphology and molecular techniques (Schubart et al. 2001, Reuschel and Schubart 2006, Marco-Herrero et al. 2013a).

The infraorder Brachyura Linnaeus, 1758 may be claimed to contain the highest degree of diversity among decapod crustaceans and includes both crab species with an important role in trophic webs as well as others of commercial interest. The main species of commercial interest found in Iberian waters are *Maja brachydactyla, Maja squinado, Cancer pagurus* and *Necora puber*; but *Calappa granulata, Carcinus maenas, Carcinus aestuarii, Liocarcinus depurator, Geryon longipes* and *Uca tangeri* are also important.

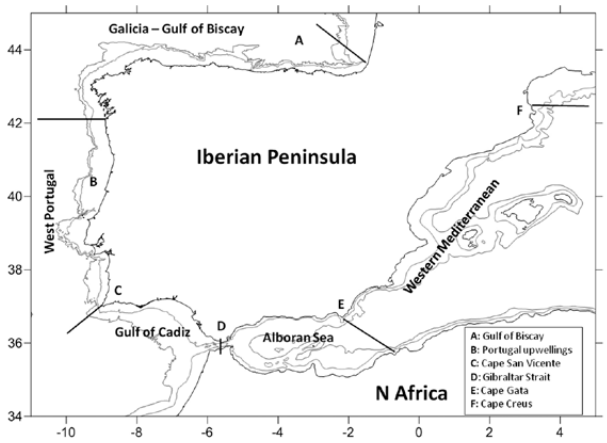

Fig. 1. Map of the Iberian Peninsula and nearby waters showing the different areas considered here to characterize the spatial distribution of brachyuran species. The 200- and 1000-metre isobaths are shown. Abbreviations: A, Gulf of Biscay; B, Portugal upwellings; C, Cape San Vicente; D, Gibraltar Strait; E, Cape Gata; F, Cape Creus.

Occasionally, other species may be seen in markets, such as *Macropipus tuberculatus, Paromola cuvieri* and *Cancer bellianus*. Several allochthonous species have been recorded in recent years and some may even show well-established populations. The present work summarizes all changes in Iberian carcinofauna since Zariquiey Álvarez (1968), and provides scientists with an updated classification list. Furthermore, the current status of brachyuran alien species throughout this region is thoroughly reviewed.

MATERIALS AND METHODS

The updated list of Iberian brachyuran crabs was drawn up in the context of the MEGALOPADN research project, which is focused on the use of morphological and molecular techniques for identifying planktonic larval stages. For the compilation of this list, all publications since 1968 about the distribution of brachyuran crabs were checked, including previous lists for Iberian or European regions, data from Internet databases such as WoRMS (http://www.marinespecies.org/), GBIF (http://www.gbif.org/species) and Observadores del Mar (http://www.observadoresdelmar.es/), systematic data, new records, and unpublished or in preparation data. Several contributions need to be highlighted here, mainly the works on European decapods carried out by d'Udekem d'Acoz (1999) and Türkay (2001), but also several specific works on Iberian carcinofauna (García Raso 1984, 1985, 1989, 1993, 1996, García Raso et al. 1987, García and Corbera 2007). In order to clarify the taxonomic status of controversial species and genera, the authors have checked multiple vouchers from the Natural History Museum (London), Muséum National d'Histoire Naturelle (Paris) and the Biological Reference Collections of the Institute of Marine Sciences (Barcelona) using morphology or molecular techniques.

This checklist covers all brachyuran species present in the Iberian Peninsula and Balearic Islands (see Fig. 1), including marine (from deep water to intertidal),

brackish (estuaries, costal lagoons, marshes, ponds) and freshwater species (note that *Eriocheir sinensis* is considered a freshwater species here, although it depends on seawater for reproduction). The updated systematic classification follows Ng et al. (2008), but also considers the latest changes in particular taxa (e.g. new results by Spiridonov et al. (2014) on the Portunoidea). Superfamilies are listed by systematic order following the Sections and Subsections as currently accepted, and by alphabetical order within them. Families, genera and species are also listed by alphabetical order within their respective superfamilies and families. The tribe level has not been considered and the use of subgenera is left at a minimum.

All changes with respect to the work by Zariquiey Álvarez (1968) are explained, including new species, introduced alien species, synonyms, systematic modifications, species that reach Iberian waters by increasing their distribution range, and species no longer found in the Iberian Peninsula.

RESULTS

A total of 140 crab species are reported around the Iberian Peninsula, and their distribution is indicated in Table 1. This represents about half of the 284 brachyuran species known in European waters, of which 40 are freshwater crabs (d'Udekem d'Acoz 1999). It is also noteworthy that about two thirds of the currently accepted brachyuran superfamilies (Ng et al. 2008, Spiridonov et al. 2014) are represented in the Iberian carcinofauna.

REMARKS

Systematic changes have affected the original classification of Brachyura, so instead of the 5 superfamilies, 20 families and 58 genera considered in Zariquiey Álvarez (1968), a total of 20 superfamilies, 36 families and 77 genera are presented here.

The current account of brachyuran crabs of the Iberian Peninsula adds another 35 to the 105 valid species in Zariquiey Álvarez (1968). Though a total of 113 brachyuran species were listed in his seminal work, five of these (*Parthenope miersii, Portunus sayi, Euchirograpsus americanus, Grapsus grapsus* and *Percnon planissimum*) should not be considered here because they are synonyms or misidentifications, or their presence in Iberian waters has not been confirmed. The *Xantho incisus* subspecies (*X. incisus incisus* and *X. incisus granulicarpus*) mentioned in Zariquiey Álvarez (1968), and considered as proper species by some authors (e.g. Mavidis et al. 2008), are not valid anymore. Although the morphology may be questionable (see García Raso et al. 1987, Mavidis et al. 2008), a recent genetic study did not allow their differentiation and *X. incisus* is considered here a synonym of *X. hydrophilus* (Reuschel and Schubart 2006).

Some additional species are now present in Iberian waters due to natural range expansions from nearby areas (Mediterranean and Atlantic), accidental introductions by anthropogenic activities, and species new to science. For example, two species (*Pisa carinimana* and *Paractaea monodi*) had not been recorded along the Iberian coasts before Zariquiey Álvarez (1968). Several of these modifications are detailed below, most of them based on evidence from larval morphology and/or molecular data.

Species no longer found in the Iberian Peninsula

As noted above, five species included in Zariquiey Álvarez (1968) should not be considered as present in Iberian waters:

1. *Parthenope miersii* (A. Milne-Edwards and Bouvier, 1898)

This species has been collected only twice, the first time corresponding to a male collected at 112 m depth in the Gulf of Cádiz and used as holotype by A. Milne-Edwards and Bouvier (1898, 1900) and the second time corresponding to another male at 135-150 m depth in the Cape San Vicente (Nunes-Ruivo 1961). D'Udekem d'Acoz (1999) questioned the validity of this species based on two male specimens only, and Türkay (2001) considered that *P. miersii* is a synonym of *Spinolambrus macrochelos*.

2. *Portunus sayi* (Gibbes, 1850)

The records of this species in Cabo Espartel (NW Africa close to Gibraltar Strait) and in the Balearic Islands (Zariquiey Álvarez 1968) should be considered juveniles of *Portunus hastatus* according to Türkay (1987).

3. *Euchirograpsus americanus* A. Milne-Edwards, 1880

This species is only distributed in the western Atlantic, and all reports in Mediterranean and eastern Atlantic must be referred to *E. liguricus* (see Türkay 1975).

4. *Grapsus grapsus* (Linnaeus, 1758)

This species was reported along the coast of Portugal, once in Setubal (Osório 1905) and later on in Sesimbra (Vilela 1936). *G. grapsus* is mainly distributed in the western Atlantic, while *Grapsus adscensionis* is the main eastern Atlantic species of this genus (see Manning and Chace 1990). It is hard to imagine that *G. grapsus* could occur along the Iberian coast and pass unnoticed, taking into account the habitat (intertidal rocky shores) and typical size of this species. Given that there are no other reports for these species along the Iberian coast, *G. grapsus* was not included in this checklist.

5. *Percnon planissimum* (Herbst, 1804)

Zariquiey Álvarez (1968) reports this species as rare in the coastal and sub-coastal waters of Portugal. No reports have been published confirming its presence in Iberian waters. In the last few years though, *Percnon gibbesi* has been collected throughout the Mediterranean, and specifically from Mediterranean localities on the Iberian coast.

Table 1. – List of brachyuran species present in the Iberian Peninsula. Abbreviations: ALB, Alboran Sea; GC, Gulf of Cadiz; G-GB, Galicia-Gulf of Biscay; MED, Mediterranean Sea; WP, West Portugal. (+) present; ([1]) García Raso (unpublished data); ([2]) Rufino (unpublished data); ([3]) Cuesta et al. (unpublished data); (?) not confirmed. Species marked with an asterisk were questioned in recent and ongoing studies as possible synonyms, and could be removed from the Iberian carcinofauna in the near future.

Taxa/Species	Distribution				
	G-GB	WP	GC	ALB	MED
BRACHYURA Linnaeus, 1758					
PODOTREMATA Guinot, 1977					
CYCLODORIPPOIDEA Ortmann, 1892					
Cymonomidae Bouvier, 1898					
Cymonomus granulatus (Thomson, 1873)	(+)	(+)	(+)	(+)	(+)
Cymonomus normani Lankester, 1903	(+)	(+)			
DROMIOIDEA de Haan, 1833					
Dromiidae de Haan, 1833					
Dromia personata (Linnaeus, 1758)	(+)	(+)	(+)	(+)	(+)
HOMOLODROMOIDEA Alcock, 1899					
Homolodromiidae Alcock, 1899					
Dicranodromia mahieuxii A. Milne-Edwards, 1883	(+)				
HOMOLOIDEA de Haan, 1839					
Homolidae de Haan, 1839					
Homola barbata (Fabricius, 1793)	(+)	(+)	(+)	(+)	(+)
Homologenus boucheti Guinot and Richer de Forges, 1995			(+)		
Paromola cuvieri (Risso, 1816)	(+)	(+)	(+)	(+)	(+)
Latreilliidae Stimpson, 1858					
Latreillia elegans Roux, 1830		(+)	(+)	(+)[1]	(+)
EUBRACHYURA de Saint Laurent, 1980					
HETEROTREMATA Guinot, 1977					
CALAPPOIDEA de Haan, 1833					
Calappidae de Haan, 1833					
Calappa granulata (Linnaeus, 1758)	(+)	(+)	(+)	(+)	(+)
Calappa pelii Herklots, 1851				(+)	
Calappa tuerkayana Pastore, 1995 *					(+)
Cryptosoma cristatum Brullé, 1837			(+)[2]	(+)	
CANCROIDEA Latreille, 1802					
Atelecyclidae Ortmann, 1893					
Atelecyclus rotundatus (Olivi, 1792)	(+)	(+)	(+)	(+)	(+)
Atelecyclus undecimdentatus (Herbst, 1783)	(+)	(+)	(+)	(+)	(+)
Cancridae Latreille, 1802					
Cancer bellianus Jonhson, 1861	(+)	(+)			
Cancer pagurus Linnaeus, 1758	(+)	(+)	(+)		(+)
CORYSTOIDEA Samouelle, 1819					
Corystidae Samouelle, 1819					
Corystes cassivelaunus (Pennant, 1777)	(+)	(+)	(+)	(+)	(+)
DORIPPOIDEA MacLeay, 1838					
Dorippidae MacLeay, 1838					
Medorippe lanata (Linnaeus, 1767)		(+)	(+)	(+)	(+)
Ethusidae Guinot, 1977					
Ethusa mascarone (Herbst, 1785)		(+)	(+)	(+)	(+)
Ethusina talismani A. Milne-Edwards and Bouvier, 1897		(+)			
ERIPHIOIDEA MacLeay, 1838					
Eriphiidae MacLeay, 1838					
Eriphia verrucosa (Forskål, 1775)	(+)	(+)	(+)	(+)	(+)
GONEPLACOIDEA MacLeay, 1838					
Goneplacidae MacLeay, 1838					
Goneplax rhomboides (Linnaeus, 1758)	(+)	(+)	(+)	(+)	(+)
Progeryonidae Števčić, 2005					
Paragalene longicrura (Nardo, 1869)					(+)
LEUCOSIOIDEA Samouelle, 1819					
Leucosiidae Samouelle, 1819					
Ebalia cranchii Leach, 1817	(+)	(+)	(+)	(+)	(+)
Ebalia deshayesi Lucas, 1846	(+)	(+)		(+)	(+)
Ebalia edwardsii Costa, 1838			(+)	(+)	(+)
Ebalia granulosa H. Milne-Edwards, 1837	(+)		(+)	(+)	(+)
Ebalia nux A. Milne-Edwards, 1883	(+)	(+)	(+)	(+)	(+)
Ebalia tuberosa (Pennant, 1777)	(+)	(+)	(+)	(+)	(+)
Ebalia tumefacta (Montagu, 1808)	(+)	(+)	(+)	(+)	(+)
Ilia nucleus (Linnaeus, 1758)			(+)	(+)	(+)
Merocryptus boletifer A. Milne-Edwards and Bouvier, 1894					(+)
MAJOIDEA Samouelle, 1819					
Epialtidae MacLeay, 1838					
Acanthonyx lunulatus (Risso, 1816)			(+)	(+)	(+)
Anamathia rissoana (Roux, 1828)		(+)		(+)	(+)
Herbstia condyliata (Fabricius, 1787)	(+)		(+)	(+)	(+)
Lissa chiragra (Fabricius, 1775)		(+)	(+)	(+)	(+)
Pisa armata (Latreille, 1803)	(+)	(+)	(+)	(+)	(+)
Pisa carinimana Miers, 1879			(+)	(+)	
Pisa hirticornis (Herbst, 1804)				?	(+)
Pisa muscosa (Linnaeus, 1758)				(+)	(+)
Pisa nodipes (Leach, 1815)	(+)	(+)		(+)	(+)

Taxa/Species	Distribution				
	G-GB	WP	GC	ALB	MED
Pisa tetraodon (Pennant, 1777)	(+)	(+)	(+)	(+)	(+)
Pisa sp. Marco-Herrero et al. (in prep.)				(+)	(+)
Rochinia carpenteri (Thomson, 1873)	(+)	(+)	(+)	(+)	(+)
Inachidae MacLeay, 1838					
Achaeus cranchii Leach, 1817	(+)		(+)	(+)	(+)
Achaeus gracilis (Costa, 1839)	(+)	(+)	(+)	(+)	(+)
Dorhynchus thomsoni Thomson, 1873	(+)	(+)	(+)	(+)	(+)
Inachus aguiarii Brito Capello, 1876	(+)[3]	(+)	(+)	(+)	
Inachus communissimus Rizza, 1839			(+)	(+)	(+)
Inachus dorsettensis (Pennant, 1777)	(+)	(+)	(+)	(+)	(+)
Inachus leptochirus Leach, 1817	(+)	(+)	(+)	(+)	(+)
Inachus phalangium (Fabricius, 1775)	(+)	(+)		(+)	(+)
Inachus thoracicus Roux, 1830		(+)	(+)	(+)	(+)
Macropodia czernjawskii (Brandt, 1880)			(+)	(+)	(+)
Macropodia deflexa Forest, 1978	(+)	(+)			
Macropodia linaresi Forest and Zariquiey Álvarez, 1964	(+)		(+)	(+)	(+)
Macropodia longipes (A. Milne-Edwards and Bouvier, 1899) *	(+)	(+)	(+)	(+)	(+)
Macropodia longirostris (Fabricius, 1775)				(+)	(+)
Macropodia parva Van Noort and Adema, 1985 *		(+)	(+)		
Macropodia rostrata (Linnaeus, 1761)	(+)	(+)	(+)	(+)	(+)
Macropodia tenuirostris (Leach, 1814)	(+)	(+)	(+)	(+)	(+)
Majidae Samouelle, 1819					
Eurynome aspera (Pennant, 1777)	(+)	(+)	(+)	(+)	(+)
Eurynome spinosa Hailstone, 1835	(+)		(+)	(+)	(+)
Maja brachydactyla Balss, 1922	(+)	(+)	(+)	(+)	
Maja crispata Risso, 1827	(+)	(+)	(+)	(+)	(+)
Maja goltziana d'Oliveira, 1888	(+)	(+)			(+)
Maja squinado (Herbst, 1788)				(+)	(+)
Oregonidae Garth, 1958					
Ergasticus clouei A. Milne-Edwards, 1882	(+)	(+)	(+)	(+)	(+)
PALICOIDEA Bouvier, 1898					
Palicidae Bouvier, 1898					
Palicus caronii (Roux, 1828)				(+)	(+)
PARTHENOPOIDEA MacLeay, 1838					
Parthenopidae MacLeay, 1838					
Derilambrus angulifrons (Latreille, 1825)			(+)	(+)	(+)
Distolambrus maltzami (Miers, 1881)	(+)			(+)	(+)
Parthenopoides massena (Roux, 1830)	(+)	(+)	(+)	(+)	(+)
Spinolambrus macrochelos (Herbst, 1790)		(+)	(+)[1]	(+)	(+)
Velolambrus expansus (Miers, 1879)				(+)	
PILUMNOIDEA Samouelle, 1819					
Pilumnidae Samouelle, 1819					
Pilumnopeus africanus (De Man, 1902)	(+)				
Pilumnus aestuarii Nardo, 1869					(+)
Pilumnus hirtellus (Linnaeus, 1761)	(+)	(+)	(+)	(+)	(+)
Pilumnus inermis A. Milne-Edwards and Bouvier, 1894		(+)	(+)	(+)	(+)
Pilumnus spinifer H. Milne-Edwards, 1834	(+)	(+)	(+)	(+)	(+)
Pilumnus villosissimus (Rafinesque, 1814)			(+)	(+)	(+)
Pilumnus sp. d'Udekem d'Acoz and Schubart (in prep.)			(+)		(+)
PORTUNOIDEA Rafinesque, 1815					
Carcinidae MacLeay, 1838					
Carcinus aestuarii Nardo, 1847				(+)	(+)
Carcinus maenas (Linnaeus, 1758)	(+)	(+)	(+)	(+)	(+)
Portumnus latipes (Pennant, 1777)	(+)	(+)	(+)	(+)	(+)
Xaiva biguttata (Risso, 1816)	(+)			(+)	(+)
Geryonidae Colosi, 1923					
Chaceon affinis (A. Milne-Edwards and Bouvier, 1894)	(+)				
Chaceon mediterraneus Manning and Holthuis, 1989					(+)
Chaceon inglei Manning and Holthuis, 1989	(+)				
Geryon longipes A. Milne-Edwards, 1881	(+)		(+)	(+)	(+)
Geryon trispinosus (Herbst, 1803) *	(+)	(+)			
Pirimelidae Alcock, 1899					
Pirimela denticulata (Montagu, 1808)	(+)	(+)	(+)	(+)	(+)
Sirpus zariquieyi Gordon, 1953		(+)	(+)	(+)	(+)
Polybiidae Ortmann, 1893					
Bathynectes longipes (Risso, 1816)		(+)		(+)	(+)
Bathynectes maravigna (Prestandrea, 1839)	(+)	(+)	(+)	(+)	(+)
Liocarcinus bolivari (Zariquiey Álvarez, 1948)			(+)		(+)
Liocarcinus corrugatus (Pennant, 1777)	(+)	(+)	(+)	(+)	(+)
Liocarcinus depurator (Linnaeus, 1758)	(+)	(+)	(+)	(+)	(+)
Liocarcinus holsatus (Fabricius, 1798)	(+)	(+)			
Liocarcinus maculatus (Risso, 1827)	(+)		?	(+)	(+)
Liocarcinus marmoreus (Leach, 1814)	(+)	(+)		(+)	(+)
Liocarcinus mcleayi (Barnard, 1947)		(+)	(+)		
Liocarcinus navigator (Herbst, 1794)	(+)	(+)	(+)	(+)	(+)
Liocarcinus pusillus (Leach, 1815)	(+)	(+)	(+)		
Liocarcinus vernalis (Risso, 1816)	(+)	(+)	(+)	(+)	(+)

Taxa/Species	Distribution				
	G-GB	WP	GC	ALB	MED
Liocarcinus zariquieyi Gordon, 1968				(+)	(+)
Macropipus tuberculatus (Roux, 1830)	(+)	(+)	(+)	(+)	(+)
Necora puber (Linnaeus, 1767)	(+)	(+)	(+)	(+)	(+)
Polybius henslowii Leach, 1820	(+)	(+)	(+)	(+)	(+)
Portunidae Rafinesque, 1815					
Callinectes exasperatus (Gerstaecker, 1856)			(+)		
Callinectes sapidus Rathbun, 1896	(+)	(+)	(+)		(+)
Charybdis (*Charybdis*) *feriata* (Linnaeus, 1758)					(+)
Portunus (*Portunus*) *hastatus* (Linnaeus, 1767)				(+)	(+)
Thiidae Dana, 1852					
Thia scutellata (Fabricius, 1793)	(+)	(+)	(+)	(+)	(+)
XANTHOIDEA MacLeay, 1838					
Panopeidae Ortmann, 1893					
Dyspanopeus sayi (Smith, 1869)					(+)
Panopeus africanus A. Milne-Edwards, 1867	(+)	(+)	(+)		
Rhithropanopeus harrisii (Gould, 1841)		(+)	(+)		
Xanthidae MacLeay, 1838					
Microcassiope minor (Dana, 1852)				(+)	?
Monodaeus couchii (Couch, 1851)	(+)		(+)	(+)	(+)
Paractaea monodi Guinot, 1969				(+)	(+)
Xantho hydrophilus (Leach, 1814)	(+)	(+)	(+)	(+)	(+)
Xantho pilipes A. Milne-Edwards, 1867	(+)	(+)	(+)	(+)	(+)
Xantho poressa (Olivi, 1792)		(+)	(+)	(+)	(+)
Xantho sexdentatus (Miers, 1881)			(+)		
THORACOTREMATA Guinot, 1977					
GRAPSOIDEA MacLeay, 1838					
Grapsidae MacLeay, 1838					
Pachygrapsus gracilis (Saussure, 1858)	(+)				
Pachygrapsus marmoratus (Fabricius, 1787)	(+)	(+)	(+)	(+)	(+)
Pachygrapsus maurus (Lucas, 1846)				(+)	(+)
Pachygrapsus transversus (Gibbes, 1850)		(+)	(+)	(+)	(+)
Planes minutus (Linnaeus, 1758)	(+)	(+)	(+)	(+)	(+)
Plagusiidae Dana, 1851					
Euchirograpsus liguricus H. Milne-Edwards, 1853		(+)	(+)	(+)	(+)
Percnidae Števčić, 2005					
Percnon gibbesi (H. Milne-Edwards, 1853)		(+)		(+)	(+)
Varunidae H. Milne-Edwards, 1853					
Asthenognathus atlanticus Monod, 1933	(+)		(+)	(+)	
Brachynotus atlanticus Forest, 1957			(+)	(+)	
Brachynotus foresti Zariquiey Álvarez, 1968					(+)
Brachynotus sexdentatus (Risso, 1827)			(+)	(+)	(+)
Eriocheir sinensis H. Milne-Edwards, 1853	(+)	(+)	(+)		
Hemigrapsus takanoi Asakura and Watanabe, 2005	(+)				
OCYPODOIDEA Rafinesque, 1815					
Ocypodidae Rafinesque, 1815					
Uca (*Afruca*) *tangeri* (Eydoux, 1835)		(+)	(+)		
PINNOTHEROIDEA de Haan, 1833					
Pinnotheridae de Haan, 1833					
Afropinnotheres monodi Manning, 1993			(+)		
Nepinnotheres pinnotheres (Linnaeus, 1758)	(+)	(+)	(+)	(+)	(+)
Pinnotheres pisum (Linnaeus, 1767)	(+)	(+)	(+)	(+)	(+)

New species present in Iberian waters (since 1968)

In this first group we have included 10 species new to science and reported in Iberian waters after the work of Zariquiey Álvarez (1968). Note that *Monodaeus guinotae* is considered as an invalid species and is not included in the checklist.

1. *Homologenus boucheti* Guinot and Richer de Forges, 1995

This deep-sea species was described to comprise the eastern Atlantic populations of *Homologenus rostratus* (A. Milne-Edwards 1880). In the Iberian Peninsula, it had only been reported (as *H. rostratus*) in the south of Portugal (García Raso 1996).

2. *Calappa tuerkayana* Pastore, 1995

This new species was described by Pastore (1995) from the Ionian Sea, and has been reported in the Balearic Islands (García 2002, García and Corbera 2007) and Atlantic waters (d'Udekem d'Acoz 2001). The validity of *C. tuerkayana* was already questioned by Holthuis (2001), and new molecular evidence indicates that this species represents juvenile stages of *Calappa granulata* (Abelló and Palero in prep.). Therefore, *C. tuerkayana* should be excluded from the Iberian checklist in the near future.

3. *Pisa* sp. Marco-Herrero et al. (in prep.)

A megalopa stage collected in Balearic waters has been assigned to the genus *Pisa* using molecular data. However, the DNA sequence obtained does not correspond to any of the species of this genus found in Iberian waters (Marco-Herrero et al. in prep.). In addition, a small specimen of this genus, morphologically different to other known Iberian species, has been found in the Alboran Sea (García Raso et al. unpublished).

4. *Macropodia deflexa* Forest, 1978

Forest described this *Macropodia* species as being closely related to *M. czernjawskii*, and its taxonomic validity has been questioned by d'Udekem d'Acoz (1999). The species has been reported in the Gulf of Biscay, Galicia, Portugal and the Gulf of Cádiz (Forest 1978, Fernández Cordeiro et al. 2006).

5. *Macropodia parva* Van Noort and Adema, 1985

D'Udekem d'Acoz (1999) suggested that this species could be attributed to juvenile stages of *M. rostrata.* Molecular evidence obtained by the authors support this assumption (Marco-Herrero et al. in prep.), so this species should also be excluded from the Iberian checklist in the near future.

6. *Maja brachydactyla* Balss, 1922

This species was established by the recognition of two distinct taxa within *M. squinado sensu lato*: *M. squinado sensu stricto* in Mediterranean waters and *M. brachydactyla* in Atlantic waters. The first hints given by Neumann (1998) were recently confirmed by DNA analyses (Sotelo et al. 2008). It should be pointed out that *M. brachydactyla* is also known to occur in the western Alboran Sea (Abelló et al. 2014).

7. *Pilumnus* sp. d'Udekem d'Acoz and Schubart (in prep.)

The taxonomy of *Pilumnus* is controversial, with no clear distinction between several species (d'Udekem d'Acoz 1999, Mavidis et al. 2009). Recent molecular studies on the European representatives of this genus have established six different operative taxonomic units, one of them corresponding to a yet undescribed species present in the Gulf of Cádiz (Oliveira-Biener et al. 2010, Schubart and Aichinger 2013).

8. *Chaceon mediterraneus* Manning and Holthuis, 1989

This deep-sea species is known so far from Mediterranean waters only. It has been reported in both the western (Cartes 1993) and eastern (Kitsos et al. 2005) Mediterranean basins.

9. *Chaceon inglei* Manning and Holthuis, 1989

This species was described based on a female specimen obtained during the Challenger expedition. It has been reported from Iceland, Scotland, southwest England, the Bay of Biscay, Madeira, and the Canary and Azores Islands (Manning and Holthuis 1989). In Iberian waters, it has been reported (as *Geryon affinis*) off Vigo (northwest Spain) (d'Udekem d'Acoz 1999, Araújo et al. 2009).

Invalid species:

1. *Monodaeus guinotae* Forest, 1976

This species has been recorded in southwest Portugal (d'Udekem d'Acoz 1999), southwest Spain, the Alboran Sea (García Raso 1996) and the Balearic Islands (García and Gracia 1996). The differences between this species and *Monodaeus couchii* are very small, and Mavidis et al. (2008) consider this species identical to *M. couchii*. Indeed, the recent molecular phylogeny study by Reuschel and Schubart (2006) indicates that *M. guinotae* should be considered a synonym of *M. couchii.*

Species reported after 1968

This second group includes 17 species that were known by 1968 but had not been reported in Iberian waters. It also includes one invalid species:

1. *Cymonomus normani* Lankaster, 1903

This eastern Atlantic species was reported in Portuguese waters by Türkay (1976).

2. *Calappa pelii* Herklots, 1851

This is a West African species collected once in the Chafarinas Islands (Silvestre, identification confirmed by Galil et al. 2002)

3. *Cryptosoma cristatum* Brullé, 1837

This species has been reported from the Macaronesian archipelagos (Madeira, Azores, Canarias, Cape Verde), and also in Málaga and Alboran Sea by García Raso (1993). There is an unpublished photography of a specimen collected in Algarve (Portugal) in 2008 (M.M. Rufino pers. comm.).

4. *Ethusina talismani* A. Milne-Edwards and Bouvier, 1897

This is a West African species, reported from South Portugal by García Raso (1996).

5. *Paragalene longicrura* (Nardo, 1869)

This is a rare species with a wide distribution, from the Aegean Sea (eastern Mediterranean) to Madeira (eastern Atlantic), reported in the Balearic Islands by García Socias (1985) and Gili and Macpherson (1987).

6. *Pisa carinimana* Miers, 1879

This species is known to occur from the Canary Islands (topotypic locality) to Angola, and was recently observed for the first time in Madeira (Ramalhosa et al. 2014). It was collected in Melilla (Mediterranean North Africa) by Zariquiey Álvarez (1968) and for the first time in the Alboran Sea by García Raso (1981, 1984), and in the Gulf of Cádiz by González-Gordillo et al. (1990).

7. *Velolambrus expansus* (Miers, 1879)

Also reported as *Parthenope expansa*, this species is distributed from the eastern Atlantic to the eastern Mediterranean. It was collected from the Alboran Sea by García Raso (1989, 1996).

8. *Chaceon affinis* (A. Milne-Edwards and Bouvier, 1894)

This eastern Atlantic species was found off Galicia (Northwestern Spain) by González Gurriarán and Méndez (1986).

9. *Geryon trispinosus* (Herbst, 1803)

This species, very similar to the northeastern Atlantic *G. longipes,* has been captured off Galicia (Urgorri et al. 1990) and Portugal (Vilela 1936, Türkay 1976).

10. *Liocarcinus maculatus* (Risso, 1827)

This species has been confounded with *L. pusillus* and *L. zariquieyi*, but Froglia and Manning (1982) summarized their distinctive morphological traits. It is mainly present in the Mediterranean Sea, with some occurrences in the eastern Atlantic. It has been collected in the Alboran Sea (García Raso 1984, 1996) and along the Catalan coast (Abelló et al. 1988, 2002).

11. *Liocarcinus mcleayi* (Barnard, 1947)

A synonym of *Xaiva mcleayi* and *Polybius mcleayi*. It was recorded in Portugal (as *Macropipus zariquieyi*) by Neves (1978), and in the South of Spain (Barbate, Cádiz) by García Raso and Manjón Cabeza (1996).

12. *Paractaea monodi* Guinot, 1969

This species was reported from North Africa (Melilla) as *Actaea rufopunctata* (Zariquiey, 1968). Specimens (as *Paractaea rufopunctata*) were caught in the Alboran Sea (García Raso and Barrajón 1983, García Raso 1990). It is also known from the Balearic Islands (Corbera et al. 1993).

13. *Microcassiope minor* (Dana, 1852)

An Atlantic species that has also been reported from Almeria and the Alboran Sea (García Raso and López de la Rosa 1992).

14. *Xantho sexdentatus* (Miers, 1881)

This tropical and subtropical Atlantic species is distributed from Senegal to the western Sahara (d'Udekem d'Acoz 1999), and the closest records to the Iberian Peninsula so far correspond to the Azores and Canary Islands (Fransen 1991). A specimen, identified by DNA barcoding in the context of the MEGALOPADN project as *X. sexdentatus*, has been collected in Rota (Cádiz). This constitutes a new report for the Iberian fauna.

15. *Brachynotus atlanticus* Forest, 1957

This western Atlantic species has been reported for Iberian waters in the Gulf of Cádiz (García Raso 1985, González-Gordillo et al. 1990) and the Alboran Sea (García Raso 1984, 1985).

16. *Afropinnotheres monodi* Manning, 1993

This African pinnotherid was recently reported for the first time in the Gulf of Cádiz by Subida et al. (2011), which also constituted the first record in European waters. This was the third report for this species worldwide. Although it is probably a case of natural range expansion, an introduction by fouling should not be discarded, given that the mussel *Mytilus galloprovincialis* is one of its main hosts (Drake et al. 2014).

Invalid species:

1. *Brachynothus gemmellaroi* (Rizza, 1839)

The westernmost record for this Mediterranean endemic species was reported as *B. gemmellari* in the Ebro Delta (Guerao et al. 1995). Although it is still considered a valid species (e.g. WoRMS), initial DNA evidence suggests that *B. gemmellari* should be considered an ecophenotype of *Brachynotus sexdentatus* (Schubart et al. 2001).

Newly introduced alien species

Human introduction of alien/allochthonous species has become an important biodiversity concern (Zenetos et al. 2010). Crab species are not an exception and up to ten alien species have recently been found in Iberian waters, namely: *Hemigrapsus takanoi, Eriocheir sinensis, Percnon gibbesi, Dyspanopeus sayi, Rhithropanopeus harrisii, Callinectes sapidus, Charybdis feriata, Callinectes exasperatus, Pachygrapsus gracilis* and *Pilumnopeus africanus* (Cuesta Mariscal et al. 1991, Abelló and Hispano 2006, García-de-Lomas et al. 2010, Castejón and Guerao 2013, Marco-Herrero et al. 2013b, Almon et al. 2014, Cuesta et al. 2015). The first six species from this list show established populations in Iberian waters:

1. *Hemigrapsus takanoi* Asakura and Watanabe, 2005

The first European records of this varunid crab from Asia were identified as *Hemigrapsus penicillatus* (de Haan, 1835) since *H. takanoi* was not described at that time. Asakura and Watanabe (2005) described *H. takanoi* as a new species and differentiated it from *H. penicillatus*. In the Iberian coast, *H. takanoi* was first reported from Laredo (Gulf of Biscay) (Noël et al. 1997), and it is now well established in several localities of this region (Dauvin et al. 2009).

2. *Eriocheir sinensis* H. Milne-Edwards, 1853

The Chinese mitten crab is native to the east coast of China, from Hong Kong to North Korea. In the Iberian Peninsula, *E. sinensis* has been established in the Guadalquivir Estuary, SW Iberian Peninsula (Garcia-de-Lomas et al. 2010). It has been reported from the Tagus estuary (Cabral and Costa 1999) and Zumaia (Gulf of Biscay) (Martínez and Adarraga 2006), but there are no data about stable populations in these localities.

3. *Rhithropanopeus harrisii* (Gould, 1841)

The Harris mud crab is native to the Atlantic coast of North America, from the southern Gulf of Saint Lawrence (Canada) to the Gulf of Mexico. *R. harrisii* has been established in the Guadalquivir and Guadalete estuaries, SW Iberian Peninsula (Cuesta Mariscal et al. 1991, Rodríguez et al. unpublished data) and it was also reported in the Mondego estuary (Portugal) (Gonçalves et al. 1995).

4. *Dyspanopeus sayi* (Smith, 1869)

Say's mud crab is native to the northwestern Atlantic Ocean from Canada to Florida. This species has been established in the Ebro Delta, NE Iberian Peninsula (Schubart et al. 2012, Marco-Herrero et al. 2013b).

5. *Percnon gibbesi* (H. Milne-Edwards, 1853)

Zariquiey Álvarez (1968) reported *Percnon planissimum* (Herbst, 1804) as being a species very rarely present in Portuguese waters, but these records have not been confirmed. Instead, the Atlantic species *Perc-*

non gibbesi has been recently recorded in different localities throughout the Mediterranean (see Katsanevakis et al. 2011). In the Iberian Peninsula, the species has been reported in the Balearic Islands (García and Reviriego 2000, Müller 2001), Alicante (Acosta 2003), the Columbretes Islands and Barcelona (Abelló et al. 2003), Murcia (Félix-Hackradt et al. 2010), Almeria (Junta de Andalucía, GEOBIO 2010), Valencia (Palero unpublished data) and Granada (de la Roza, personal comm.). Megalopa stages and early juvenile specimens have recently been collected from Cullera (Valencia). Citizen science reports, mediated through the website "Observadores del Mar", show that the species is now widely reported along the Mediterranean coasts from Cape Palos to Catalonia, as well as in the Balearic Islands. It is not yet clear whether this Mediterranean expansion from the Atlantic is a natural process or was mediated by human activities (accidental transport in ballast water or specimens released from pet trade).

6. *Callinectes sapidus* Rathbun, 1896

Some adult specimens of the American blue crab were recently captured in the Ebro Delta, but more data are needed to determine whether this species is definitely established. The species can be considered rare in other areas of the Iberian Peninsula (Castejón and Guerao 2013), although a recent report of one ovigerous female from the Sado estuary might indicate the establishment of a small population (Ribeiro and Verissimo 2014).

Some casual reports are known for the remaining alien species of this checklist, including a single adult female of the Indo-Pacific portunid *Charybdis feriata* caught in Barcelona (Abelló and Hispano 2006), one male specimen of *Callinectes exasperatus* collected in the Bay of Cádiz (Cuesta et al. 2015), and four specimens of *Pilumnopeus africanus* and two specimens of *Pachygrapsus gracilis* collected in Galicia (NW Spain) by Almon et al. (2014).

When species native from distant localities are reported within Iberian waters, there is little doubt that they were introduced through human activities (intentional or accidentally). However, this is not necessarily the case for species native from nearby areas in West and North Africa which have been recently found in the Alboran Sea and Gulf of Cádiz (*Calappa pelii, Cryptosoma cristatum,* and *Afropinnotheres monodi*). These were not considered as introduced species in the present account, but this hypothesis cannot be discarded.

Systematic remarks

The scientific names of some species considered by Zariquiey Álvarez (1968) have changed due to new systematic studies or synonymizations, and these are listed in Table 2. Other systematic changes refer to higher taxonomic levels, and these will be addressed here.

The first main change in the systematics of brachyuran crabs after 1968 was the proposal of new sections and subsections by Guinot (1977, 1978, 1979), de Saint Laurent (1979, 1980), and Guinot and Bouchard (1998). Based on the male and female genital apertures, these authors separated brachyuran crabs into Dromiacea and Eubrachyura, or the subsections Podotremata, Heterotremata and Thoracotremata. Morphological and molecular analyses do not reveal monophyly within Podotremata, so the most recent classifications divide it into three sections: Dromiacea, Cyclodorippoidea and Raninoida (De Grave et al. 2009). According to these changes, the old term Reptantia (present in Zariquiey Álvarez 1968) was removed from the classification. Considering just those superfamilies present in Iberian waters, most changes correspond to splits of old taxa into several new superfamilies. For example, the superfamily Corystoidea (which comprised the families Atelecyclidae, Cancridae, Corystidae, Pirimelidae and Thiidae) now comprises Corystidae only, while Atelecyclidae and Cancridae have been placed in the new superfamily Cancroidea, and Pirimelidae and Thiidae have been relocated within the Portunoidea (Spiridonov et al. 2014). All changes at the superfamily level are listed in Table 3, including new family composition.

Some of the most important changes affect the assignment of genera to new families, which cannot be appreciated in Table 3. For example, the former Majidae family suffered important changes due to its elevation into a superfamily (Majoidea) and the elevation to family level of previous subfamilies: Majidae, Epialtidae, and Inachidae. Some authors have raised Pisidae as well (Hendrickx 1995), but we followed here the more conservative classification of Ng et al. (2008) considering Pisiinae as a subfamily of Epialtidae. A recent study based on larval morphology and DNA did not support a clear separation between epialtid and pisid crabs (Hultgren and Stachowitz 2008). Neverthe-

Table 2. – Previous and current names of brachyuran species present in the Iberian Peninsula renamed since Zariquiey Álvarez (1968), listed by alphabetical order.

Previous names (as in Zariquiey Álvarez 1968)	Current names (as in Ng *et al*. 2008)
Achaeus gordonae	*Achaeus gracilis*
Actaea rufopunctata	*Paractaea monodi*
Bathynectes superbus	*Bathynectes maravigna*
Carcinus mediterraneus	*Carcinus aestuarii*
Dicranodromia mayheuxi	*Dicranodromia mahieuxii*
Ebalia cranchi	*Ebalia cranchii*
Ebalia edwardsi	*Ebalia edwardsii*
Heterocrypta maltzani	*Distolambrus maltzami*
Macropipus arcuatus	*Liocarcinus navigator*
Macropipus bolivari	*Liocarcinus bolivari*
Macropipus corrugatus	*Liocarcinus corrugatus*
Macropipus depurator	*Liocarcinus depurator*
Macropipus holsatus	*Liocarcinus holsatus*
Macropipus marmoreus	*Liocarcinus marmoreus*
Macropipus puber	*Necora puber*
Macropipus pusillus	*Liocarcinus pusillus*
Macropipus vernalis	*Liocarcinus vernalis*
Macropipus zariquieyi	*Liocarcinus zariquieyi*
Maja verrucosa	*Maja crispata*
Medaeus couchi	*Monodaeus couchii*
Parthenope angulifrons	*Derilambrus angulifrons*
Parthenope macrochelos	*Spinolambrus macrochelos*
Parthenope massena	*Parthenopoides massena*
Pinnotheres pinnotheres	*Nepinnotheres pinnotheres*
Pisa corallina	*Pisa hirticornis*
Xantho incisus granulicarpus	*Xantho hydrophilus*
Xantho incisus incisus	*Xantho hydrophilus*

Table 3. – Previous and current names (and family composition) of the superfamilies of brachyuran crabs present in the Iberian Peninsula, listed by systematic order.

Previous names (as in Zariquiey Álvarez 1968)		Current names (as in Ng et al. 2008 and Spiridonov et al. 2014)	
Superfamily	Family	Superfamily	Family
Dromiacea	Dromiidae	Homolodromioidea	Homolodromiidae
		Dromioidea	Dromiidae
	Homolidae	Homoloidea	Homolidae
	Latreillidae		Latreillidae
Oxystomata	Dorippidae	Dorippoidea	Dorippidae
			Ethusidae
		Cyclodorippoidea	Cymonomidae
	Calappidae	Calappoidea	Calappidae
	Leucosiidae	Leucosioidea	Leucosiidae
Corystoidea	Corystidae	Corystoidea	Corystidae
	Atelecyclidae	Cancroidea	Atelecyclidae
	Canceridae		Cancridae
	Pirimelidae*		
	Thiidae*		
Brachyryncha	Goneplacidae	Goneplacoidea	Goneplacidae
			Progeryonidae
	Grapsidae	Grapsoidea	Grapsidae
			Percnidae
			Plagusiidae
			Varunidae
	Ocypodidae	Ocypodoidea	Ocypodidae
	Palicidae	Palicoidea	Palicidae
	Pinnotheridae	Pinnotheroidea	Pinnotheridae
	Portunidae	Portunoidea	Carcinidae
			Portunidae
			Pirimelidae*
			Polybiidae
			Thiidae*
			Geryonidae**
	Xanthidae	Pilumnoidea	Pilumnidae
		Xanthoidea	Xanthidae
			Panopeidae
		Eriphioidea	Eriphiidae
Oxyrhyncha	Majidae	Majoidea	Majidae
			Epialtiidae
			Inachidae
			Oregoniidae
	Parthenopidae	Parthenopoidea	Parthenopidae

* Pirimelidae and Thiidae have been relocated in Portunoidea according to Spiridonov et al. (2014)
**Geryonids were considered to belong to Xanthidae in Zariquiey Álvarez (1968)

less, larval morphology and DNA data suggest that *Ergasticus clouei* should be moved from Inachidae to Oregoniidae, a family that was not present in Iberian waters (Marco-Herrero et al. 2013a).

Within the Grapsoidea superfamily, the former Grapsidae is now restricted to the previous subfamily Grapsinae, while other subfamilies that acquired familial level (e.g. Varunidae, Plagusiidae and Percnidae) are also present in Iberian waters (see Schubart et al. 2002, Schubart and Cuesta 2010). Some genera and species have also changed their placement, such as *Euchirograpsus* that was considered a Varuninae and is now within the Plagusiidae.

The superfamily Portunoidea is still under discussion. Geryonid crabs, which were previously considered as part of Xanthidae, now belong to Geryonidae. Schubart and Reuschel (2009) proposed a new taxonomy based on molecular evidence for the Pirimelidae (traditionally placed in Cancroidea), Polybiidae, Carcinidae and Thiidae (according to Ng et al. 2008, but currently in its own superfamily Thioidea), and these changes obtained further support from Spiridonov et al. (2014). Several portunoid genera are currently under study and new modifications are expected.

The Parthenopidae family has also experienced strong changes after the work of Tan and Ng (2007). In the case of Iberian species, all generic names have been modified (new combinations) and the three species of *Parthenope* are now considered as one species of *Spinolambrus* and the monotypic genera *Derilambrus* and *Parthenopoides*. The former parthenopid genus *Heterocrypta*, a monotypic genus also found in Iberian waters, is now named *Distolambrus*.

Another important change affects the systematic position of *Asthenognathus atlanticus*, a former pinnotherid crab that is now considered a member of the subfamily Asthenognathinae (Grapsoidea: Varunidae). This modification, based on larval and molecular evidences provided by Cuesta et al. (2005), was already included in Ng et al. (2008).

Within the Ocypodidae, Spivak and Cuesta (2009) proposed to elevate the subgenus *Afruca* to genus level, based on larval morphology and supported by previous phylogenies of the genus *Uca*, and then named the species inhabiting the southwest of the Iberian Peninsula as *Afruca tangeri*. However, this proposal has not been followed in later studies and *Afruca tangeri* is maintained in this checklist as *Uca* (*Afruca*) *tangeri*.

Finally, morphological studies by Forest (1978) and García Raso et al. (1987) have questioned the validity of *Macropodia longipes* and considered its possible synonymy with *Macropodia tenuirostris*. Studying the larval development of both species, Guerao and Abelló (1997) also mentioned that "… the two species are closely related phylogenetically". Both species are maintained as reported in Zariquiey Álvarez (1968), but their taxonomic status will be clarified with ongoing molecular phylogenetics research.

The Iberian carcinofauna: future changes

Further modifications are still ongoing in brachyuran systematics, so changes in the Iberian carcinofauna are expected to come in the near future. The improvements on the application of molecular tools and phylogenetic inference methods and the use of larval morphology are expected to bring further changes in the systematics of brachyuran crabs. These will have an impact at several taxonomic levels, from species to superfamilies. For example, preliminary studies carried out by our team on the molecular phylogeny of the Inachidae family, or some genera like *Ebalia, Liocarcinus* and *Pisa*, point to the presence of synonymy, the necessity to split some taxa into new species, and the erection of new genera. The validity of some species is currently questioned, such as the case of *Calappa tuerkayana* (possible synonym of *Calappa granulata*), *Geryon trispinosus* (possible synonym of *Geryon longipes*), *Macropodia parva* (as synonym of *Macropodia rostrata*) and *Macropodia longipes* (as synonym of *Macropodia tenuirostris*).

Although descriptions of crab species new to science are not expected to occur at a significant rate, an increase on the number of taxa in the Iberian Peninsula will surely result from the human introduction of alien species, as well as the natural expansion of species from West and North Africa and the eastern Mediterranean. Likely candidates to expand the checklist include *Pinnotheres pectunculi* (presumably present in the Iberian Peninsula as a native species; d'Udekem d'Acoz pers. comm.), *Hemigrapsus sanguineus, Portunus sayi* and maybe *Potamon ibericum* (introduced to the Cagne river in southern France between 1975 and 1983 [Noël and Guinot, 1983]). Species of warmer waters will expand their geographic range through "tropicalization" and climate change will favour the expansion of thermophilic species (Verges et al. 2014). Although not all of their predictions have been fulfilled, the likely arrival of alien species was already mentioned by Almaça (1985) and García Raso et al. (1987), and new species are expected to arrive in the near future.

ACKNOWLEDGEMENTS

Thanks are due to Félix Pérez (ICM), Enrique Macpherson (CEAB), Paul Clark (NHM), Laure Corbari (MNHN), Paula Martin-Lefevre (MNHN) and to J.-C. Dauvin (Univ. Caen Basse-Normandie) for facilitating access to key specimens to complete the present study. Thanks are also due to Cedric d'Udekem d'Acoz, Pierre Nöel and Carlo Froglia for criticism and suggestions that clearly improved the manuscript. This research was carried out within the framework of the MEGALOPADN project (CGL2009-11225) funded by the "Ministerio de Economía y Competividad (MINECO)" Spanish Plan R+D+I and FEDER. Marco-Herrero acknowledges the pre-doctoral FPI Fellowship (BES-2010-033297), and the present study was carried out as part of her PhD Thesis.

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SECCIÓN II

DESCRIPCIONES LARVARIAS DE BRAQUIUROS IBÉRICOS A PARTIR DE HEMBRAS OVÍGERAS Y CULTIVOS EN LABORATORIO

CAPÍTULO 2

Morphology of the larval stages of *Macropodia czernjawskii* (Brandt, 1880) (Decapoda, Brachyura, Inachidae) reared in the laboratory

E Marco-Herrero, A Rodríguez, JA Cuesta (2012) Morphology of the larval stages of *Macropodia czernjawskii* (Brandt, 1880) (Decapoda, Brachyura, Inachidae) reared in the laboratory Zootaxa 3338: 33-48

Zootaxa 3338: 33–48 (2012)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Morphology of the larval stages of *Macropodia czernjawskii* (Brandt, 1880) (Decapoda, Brachyura, Inachidae) reared in the laboratory

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Abstract

The complete larval development of *Macropodia czernjawskii* (Brandt, 1880), is described and illustrated for the first time. Larvae were reared in the laboratory and development consisted of two zoeal stages and a megalopa. The main difference in the zoeal stages is the absence of lateral spines on the telson furcae, which allow it to be distinguished from the remaining species of *Macropodia* as well as from the zoeae of most majoids.

Key words: Brachyura, Majoidea, Inachidae, zoea, megalopa, *Macropodia czernjawskii*

Introduction

The spider crab genus *Macropodia* Leach, 1814, is represented in the northeastern Atlantic and Mediterranean waters by nine species: *M. czernjawskii* (Brandt, 1880), *M. deflexa* Forest, 1978, *M. intermedia* Bouvier, 1940, *M. linaresi* Forest & Zariquiey-Álvarez, 1964, *M. longipes* (Milne-Edwards & Bouvier, 1899), *M. longirostris* (Fabricius, 1775), *M. parva* Noort & Adema, 1985, *M. rostrata* (Linnaeus, 1761), and *M. tenuirostris* (Leach, 1814). *Macropodia czernjawskii* is found in the Eastern Atlantic and Mediterranean Sea (D'Udekem d'Acoz 1999), where it inhabits rocky intertidal pools and bottoms with algae at depths of 0.3–80 m (García Raso 1984; Zariquiey-Álvarez 1968).

The complete larval development reared in the laboratory is known for only four species of *Macropodia*: *M. tenuirostris* (Salman 1981), *M. rostrata* (Ingle 1982, 1992), *M. longipes* (Guerao & Abelló 1997) and *M. parva* (González-Gordillo & Rodríguez 2001). Lebour (1927, 1928) had previously described the larval development of *M. deflexa* (as *M. egyptia*), *M. tenuirostris* (as *M. longirostris*), and *M. rostrata*, but descriptions and illustrations were brief and incomplete. The first zoea of *M. linaresi* was described by Guerao *et al*. (1998).

The complete larval development (two zoeal stages and the megalopa) of *M. czernjawskii* is herein described and illustrated in detail and compared with the known development of other species of the genus.

Material and methods

One ovigerous individual of *Macropodia czernjawskii* was collected by hand from intertidal pools off El Chato beach (Cadiz, southwestern Spain) (36º 28' 30'' N 06º 15' 40'' W), on 10 September 1999. The ovigerous crab was placed in an aquarium containing filtered and well-aerated sea water at a salinity of 32 ± 1 ‰ and keep at 26 ± 1?C. A total of 417 zoeae hatched on 17 September, the 300 most actively swimming zoeae were transferred to 2 L glass bottles (150 ind. L^{-1}) with aeration, and constant temperature (25 ± 1°C) for mass culture. Zoea I larvae were fed with a mix of rotifer *Brachionus plicatilis* (Müller, 1786) (fed with *Nannochloropsis gaditana* Lubián, 1982) and nauplii of *Artemia* sp., and from ZII to first crab with only fresh nauplii of *Artemia* sp. All reared larvae were maintained under the same constant conditions of temperature and salinity mentioned above. Seawater was changed daily, and culture was checked daily for exuviae and dead larvae and it was finished when all megalopae moulted to the first crab instar. Exuviae and specimens of all stages were fixed in 4% neutral formalin for later examination.

Accepted by P. Castro: 1 May 2012; published: 7 Jun. 2012

For an easier microscopic observation of larval structures and setation a digestion-stain procedure (adjustment of that described by Landeira *et al*. 2009) was carried out. Entire specimens were first placed for 10 minutes in a watch glass with 2 ml of heated lactic acid. Immediately after, 3 drops of Clorazol Black stain (0.4 g Clorazol Black powder dissolved in 75 ml 70% Ethanol) were added to the heated solution. The specimen was removed from the solution after 5–10 minutes and placed on a slide with lactic acid before proceeding with the dissection of the mouthparts.

Drawings and measurements were made using a Wild MZ6 and Zeiss Axioskop compound microscope with Nomarski interference, both equipped with a *camera lucida*. All measurements were made by using an ocular micrometer. Descriptions and measurements of different larval stages were based on at least 10 specimens of each stage, but due to the exceptional feature found in the telson (absence of lateral spines on the furcae), 30 additional zoea I, and 25 zoea II, were also checked for only this character. Description and figures are arranged according to the standards proposed by Clark *et al*. (1998).

Measurements taken in zoeal stages were: rostro-dorsal length (RDL) measured from frontal margin to tip of dorsal spine; cephalothorax length (CL) measured from frontal margin (between the eyes) to posterolateral cephalothoracic margin; cephalothoracic dorsal spine length (DSL) distance from base to tip of dorsal spine; antenna length (AL) from base of the antennal peduncle to tip of the spinous process. For the megalopa, cephalothorax length (CL) measured from the frontal to posterior margin of cephalothorax; cephalothorax width (CW) as the cephalothorax maximum width.

The parental female and complete larval series have been deposited in the Museo Nacional de Ciencias Naturales (MNCN) under accession number MNCN 20.04/867 (parental female), MNCN 20.04/867 (Zoeae I), MNCN 20.04/8678 (Zoeae II) and MNCN 20.04/8679 (Megalopae).

Results

The larval development of *M. czernjawskii* consists of two zoeal stages and a megalopa. At 25 ± 1°C and 32 ± 1 ‰ salinity the larval development is completed in a minimum of 8 days (appearance of the first crab). The duration and survival of each larval stage is show in Fig. 1. The first zoeal stage is described in detail, and only the main differences in subsequent stages are noted.

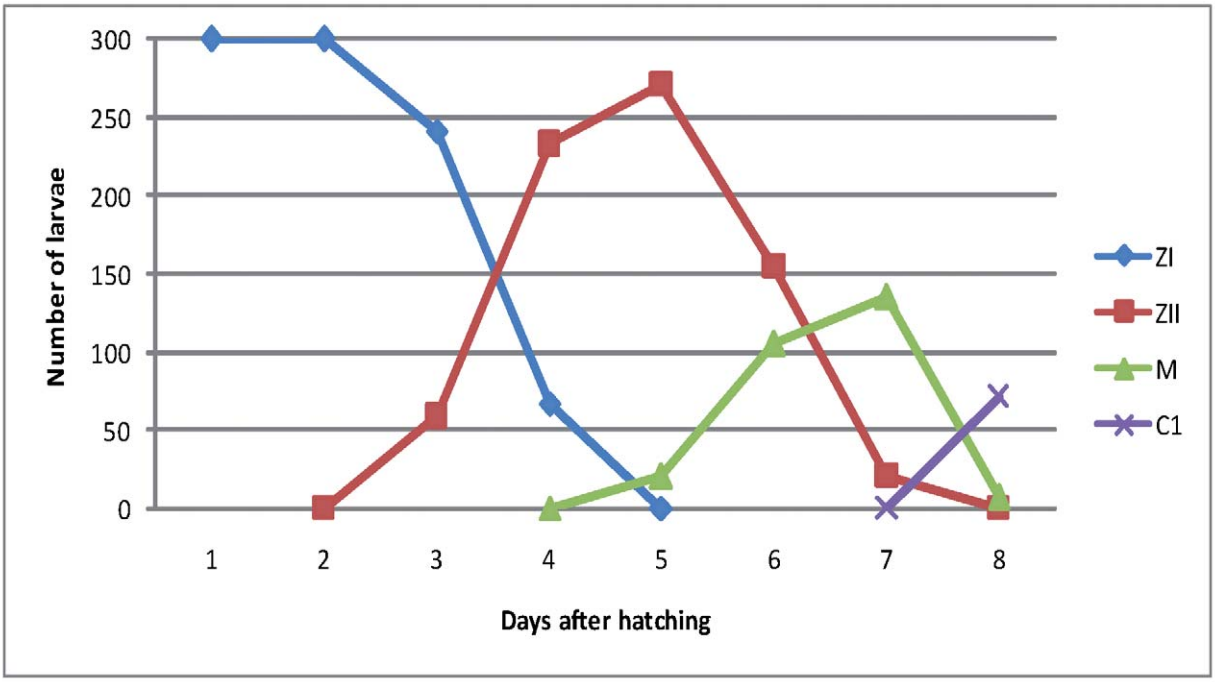

FIGURE 1. Rearing records of *Macropodia czernjawskii* (Brandt, 1880) reared at 25 ± 1°C and 32 ± 1 ‰ salinity. ZI, zoea I; ZII, zoea II; M, megalopa; C1, first crab.

Description of larvae

First zoea

(Figs. 2A, B, H; 3A, D; 4A, D; 5A, D; 7A, D)

Size: RDL = 1.152 ± 0.03; CL = 0.569 ± 0.05 mm; DSL = 0.688 ± 0.09 mm; AL = 0.607 ± 0.05 mm, N= 10.

Cephalothorax (Figs. 2A, B, H): Globose and smooth with well-developed dorsal spine, slightly curved backward; rostral and lateral spines absent; anteromedian ridge present; dorsomedian tubercle absent; pair of anterodorsal and posterodorsal simple setae; posterolateral margin with densely plumose "anterior seta", two sparsely plumose setae and minute denticles; eyes sessile.

Antennule (Fig. 3A): Uniramous, unsegmented and conical, endopod absent; exopod with 4 terminal aesthetascs (two long and two shorter) and 1 simple seta.

Antenna (Fig. 3D): Biramous, spinuous process of protopod very long with two rows of distal spinules; unsegmented and short endopod; exopod slightly shorter than protopod, with 2 medial simple setae and distal spinules.

Mandible: Incisor and molar process developed, irregularly dentate; palp absent.

Maxillule (Fig. 4A): Coxal endite with 7 plumodenticulate setae; basial endite with 4 terminal setae (3 cuspidate, 1 plumodenticulate), 2 subterminal plumodenticulate setae and 1 proximal plumose seta; endopod 2-segmented with 0, 3 sparsely plumose setae; epipodal and exopodal seta absent.

Maxilla (Fig. 4D): Coxal endite not bilobed with 7 plumodenticulate setae; basial endite bilobed with 5 + 4 plumodenticulate setae; unsegmented endopod not bilobed, with 4 setae (3 sparsely plumose, 1 distal simple shorter); exopod (scaphognathite) with 9 marginal plumose setae plus one stout plumose process.

First maxilliped (Fig. 5A): Epipod present without setae. Coxa without setae; basis with 9 medial sparsely plumodenticulate setae arranged as 2+2+2+3; endopod 5-segmented, longer than exopod, with 3, 2, 1, 2, 5 (4 terminal + 1 subterminal) sparcely plumodenticulate setae; exopod 2-segmented with 4 terminal plumose natatory setae.

Second maxilliped (Fig. 5D): Coxa without setae; basis with one sparcely plumodenticulate seta; endopod 3-segmented, with 0, 0, 4 setae, (2 subterminal + 2 terminal); exopod 2-segmented with 4 terminal plumose natatory setae.

Third maxilliped: Present as biramous buds.

Pereiopods: Present as incipient buds, cheliped bilobed.

Pleon (Fig. 7A, D): Five somites; somite I without setae; somite II–V with pair of minute simple setae on posterodorsal margin; somite II with pair of forwardly directed dorsolateral processes, somites III–V with long and terminally acute posterolateral processes.

Pleopods: Incipient pleopods bud on somites II–V.

Telson (Fig. 7A): Bifurcated, with deep median cleft; 2 pairs of 3 serrulate setae on posterior margin, medial setae longest; telson furcae without spines, and distally spinulate.

Second zoea

(Figs. 2C, D; 3B, E; 4B, E; 5B, E; 7B, E)

Size: RDL = 1.030 ± 0.07; CL = 0.606 ± 0.01 mm; DSL = 0.576 ± 0.08 mm; AL = 0.684 ± 0.05 mm, N=10.

Cephalothorax (Figs. 2C, D): Anteromedian ridge more pronounced than zoea I; 3 pairs of anterodorsal simple setae; well developed supraocular process; eyes stalked and movable.

Antennule (Fig. 3B): Exopod terminally with 6 terminal aesthetascs (3 long, 3 shorter) and 1 simple seta.

Antenna (Fig. 3E): Endopod more elongated.

Mandible: Palp bud present.

Maxillule (Fig. 4B): Basial endite with 5 terminal setae (3 cuspidate, 2 plumodenticulate), 2 subterminal plumodenticulate setae and 1 proximal plumose seta; exopodal seta present.

Maxilla (Fig. 4E): Basial endite with 5 + 5 sparsely plumodenticulate setae; endopod now with fourth seta sparsely plumose and of the same length of the rest; scaphognathite (exopod) with 18 plumose marginal setae.

First maxilliped (Fig. 5B): Exopod with 6 terminal plumose natatory setae.

Second maxilliped (Fig. 5E): Basis without setae; exopod with 6 terminal plumose natatory setae.

Pleon (Figs. 7B, E): Posterolateral spines more elongated.

Pleopods (Figs. 7B, E): Biramous more elongated, endopod buds present.

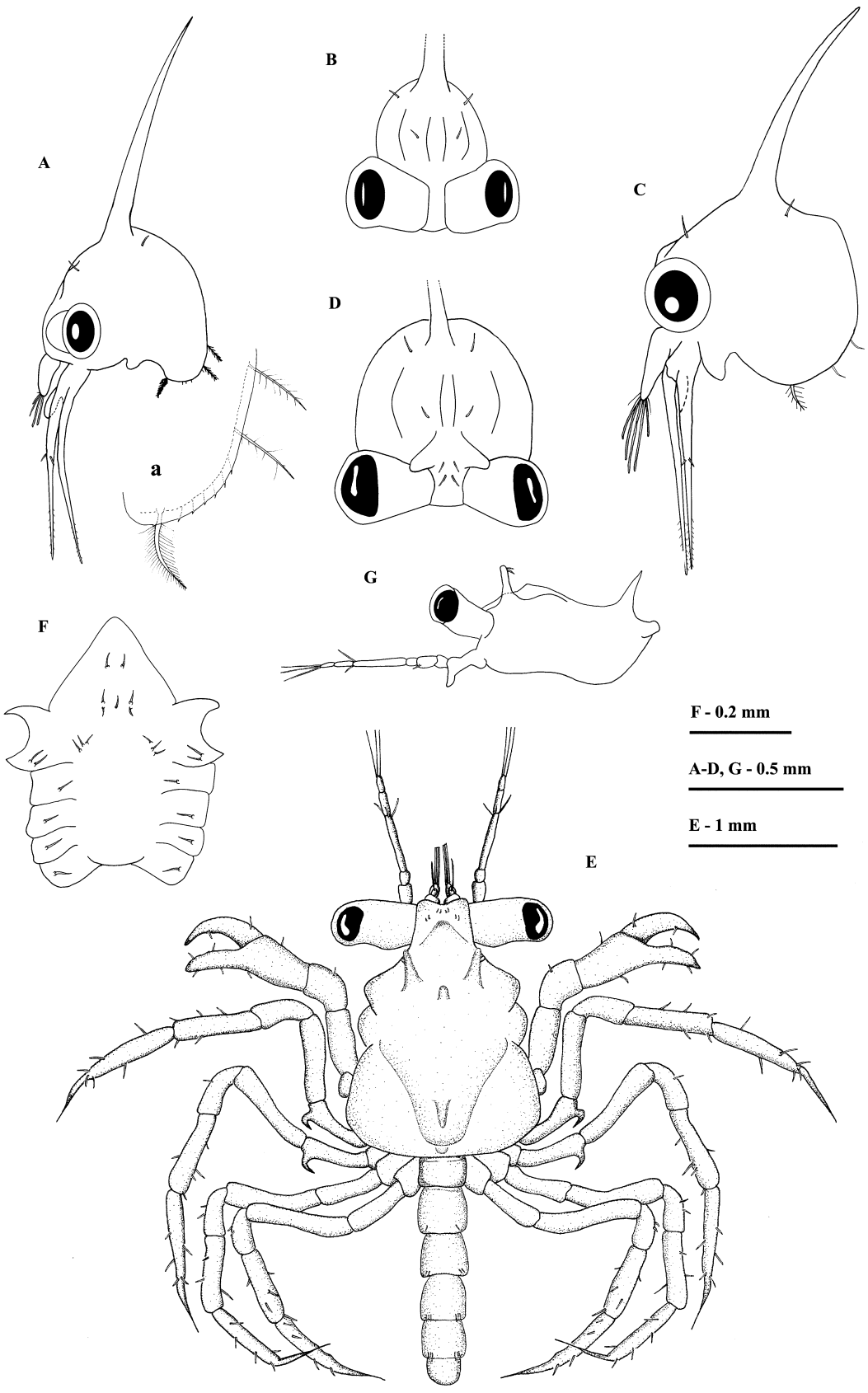

FIGURE 2. *Macropodia czernjawskii* (Brandt, 1880). Zoea I, cephalothorax, A: lateral view; a: posterolateral margin detail; B: frontal view. Zoea II, cephalothorax, C: lateral view; D: frontal view. Megalopa, E: dorsal view; F: sternum; G: lateral view of cephalothorax.

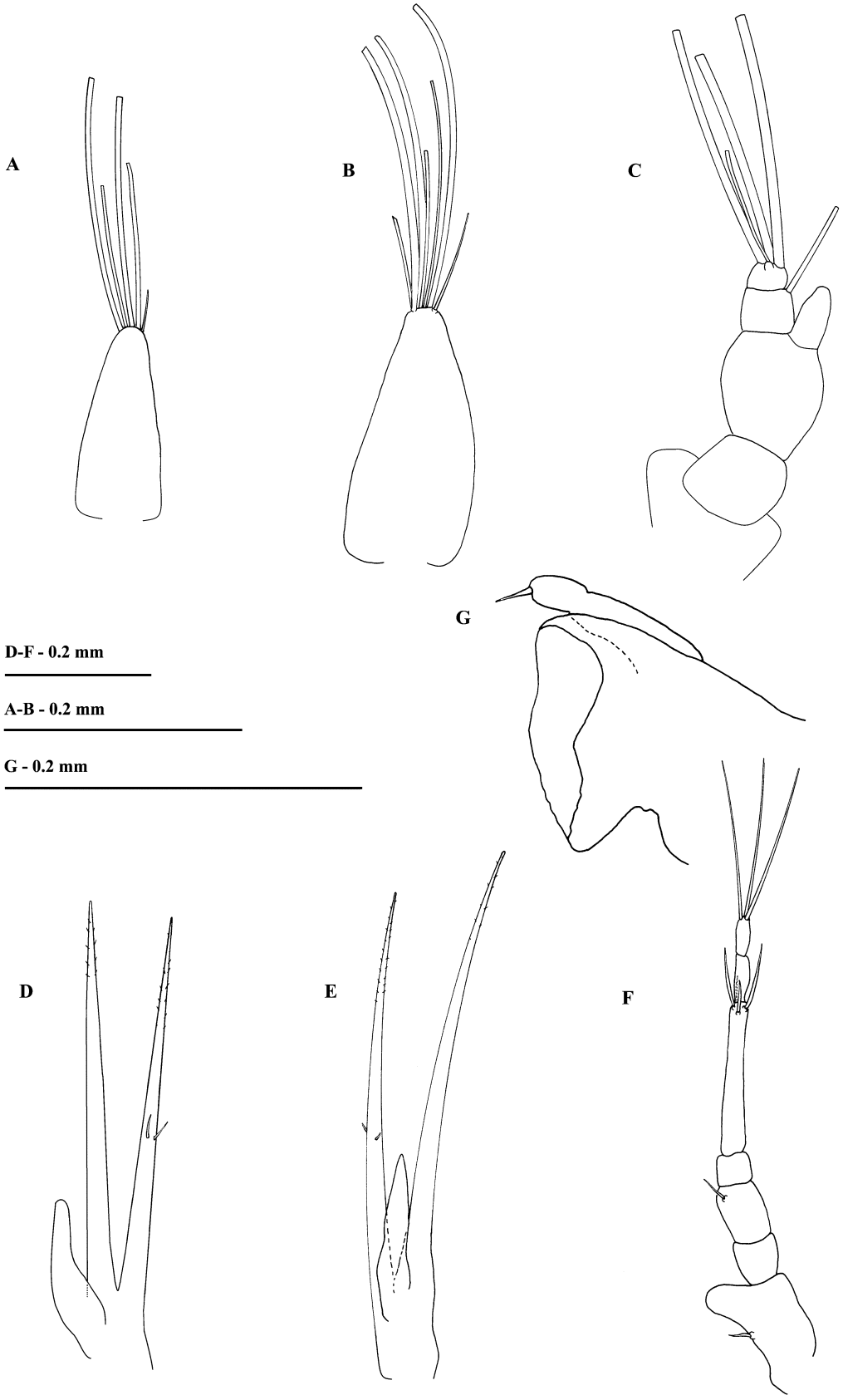

FIGURE 3. *Macropodia czernjawskii* (Brandt, 1880). Antennule, A: zoea I; B: zoea II; C: megalopa. Antenna, D: zoea I; E: zoea II; F: megalopa. Mandible, G: megalopa.

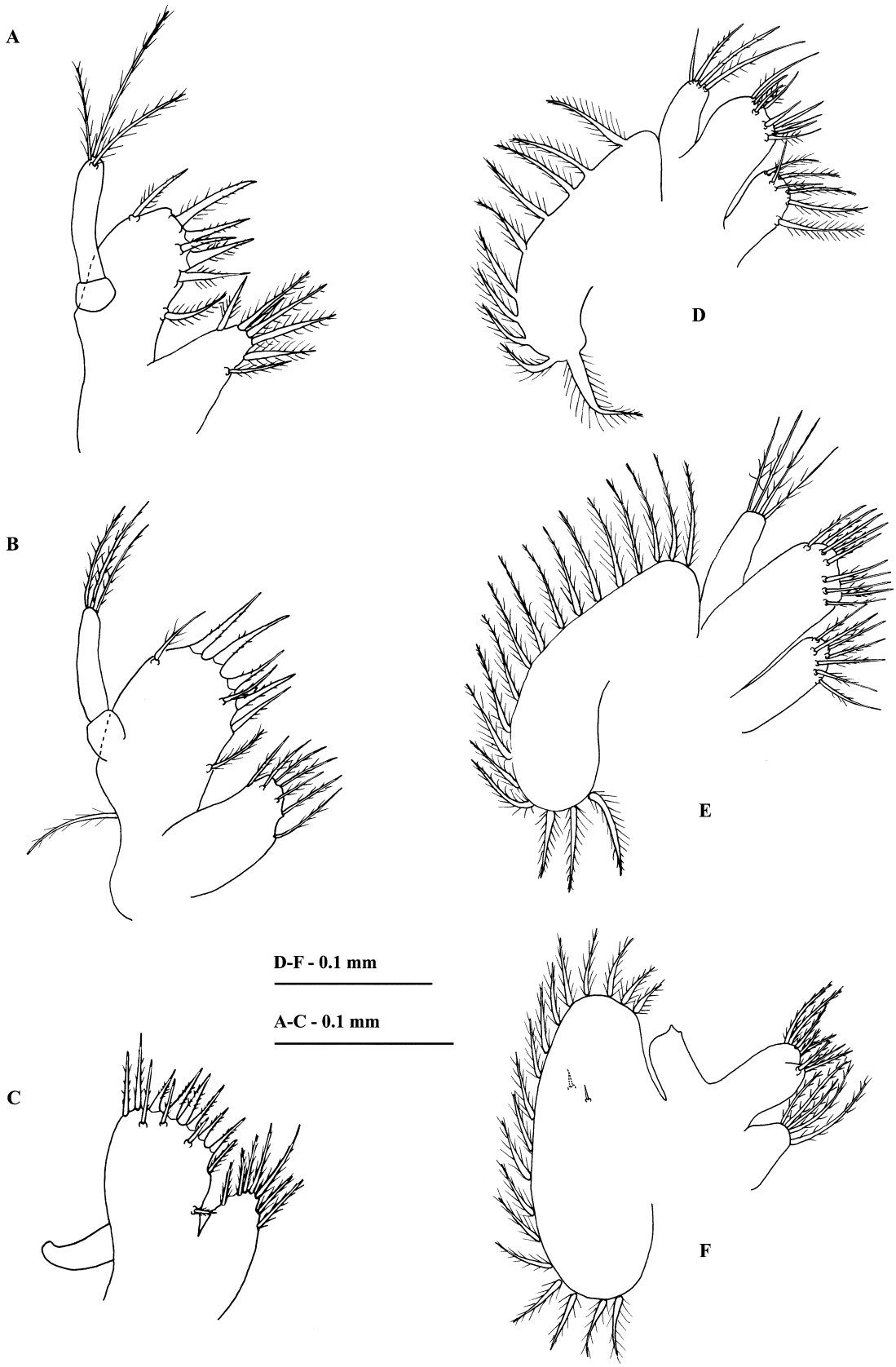

FIGURE 4. *Macropodia czernjawskii* (Brandt, 1880). Maxillule, A: zoea I; B: zoea I; C: megalopa. Maxilla, D: zoea I; E: zoea II; F: megalopa.

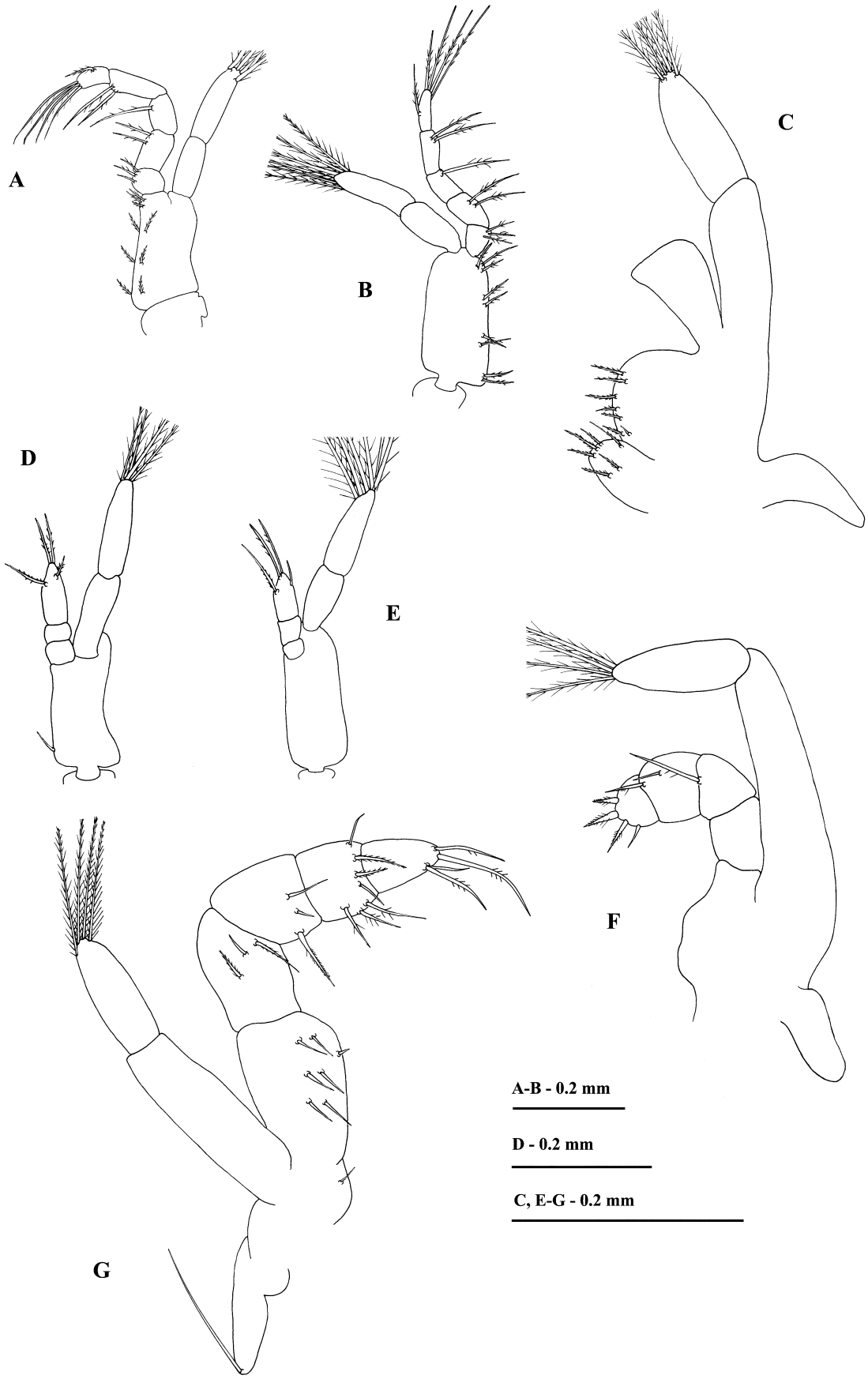

FIGURE 5. *Macropodia czernjawskii* (Brandt, 1880). First maxilliped, A: zoea I; B: zoea II; C: megalopa. Second maxilliped, D: zoea I; E: zoea II; F: megalopa. Third maxilliped, G: megalopa.

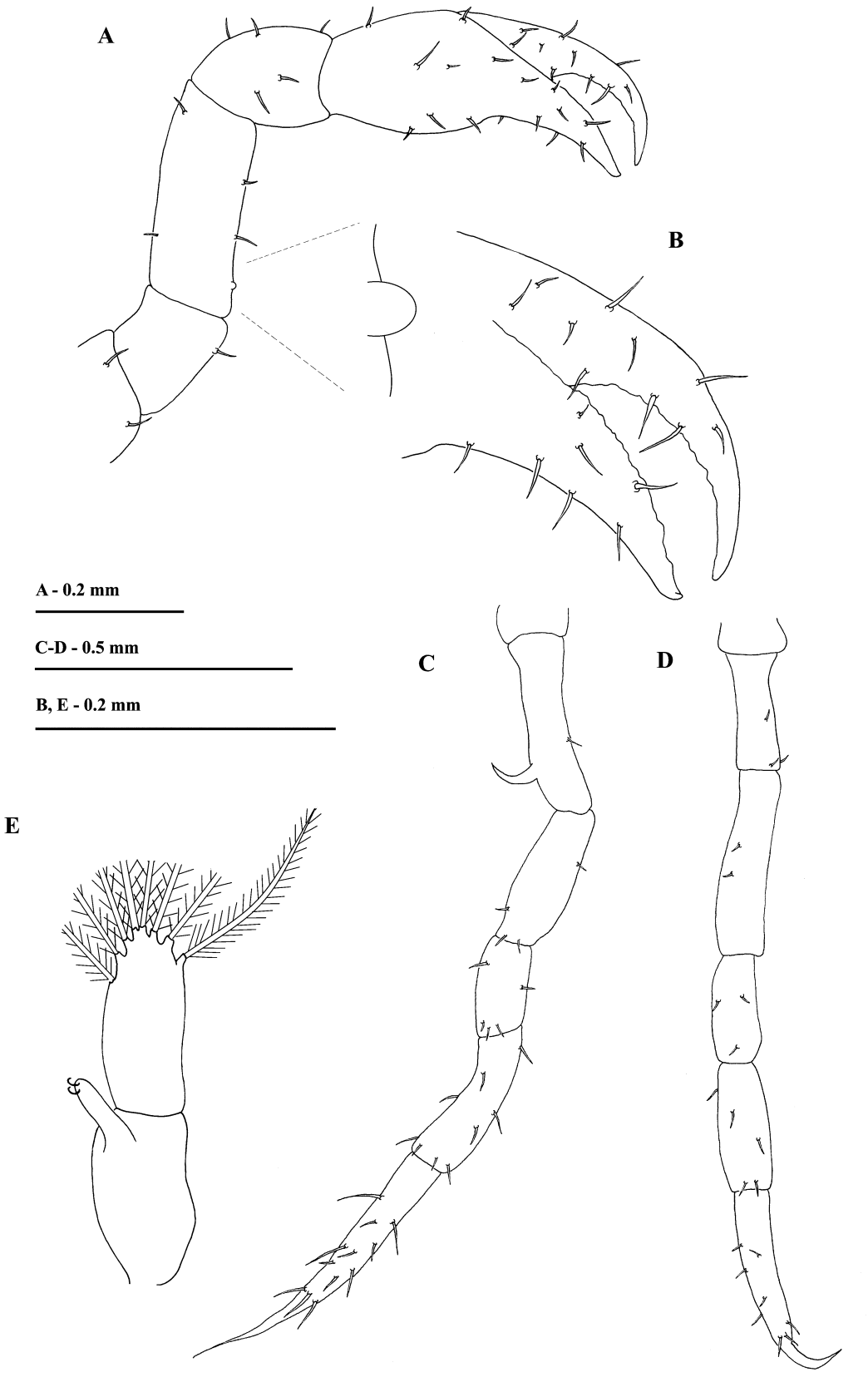

FIGURE 6. *Macropodia czernjawskii* (Brandt, 1880). Megalopa, A: Cheliped, with detail of tubercle on merus; B: detail of distal part of propodus and dactylus; C: second pereiopod; D: fourth pereiopod; E: Pleopod.

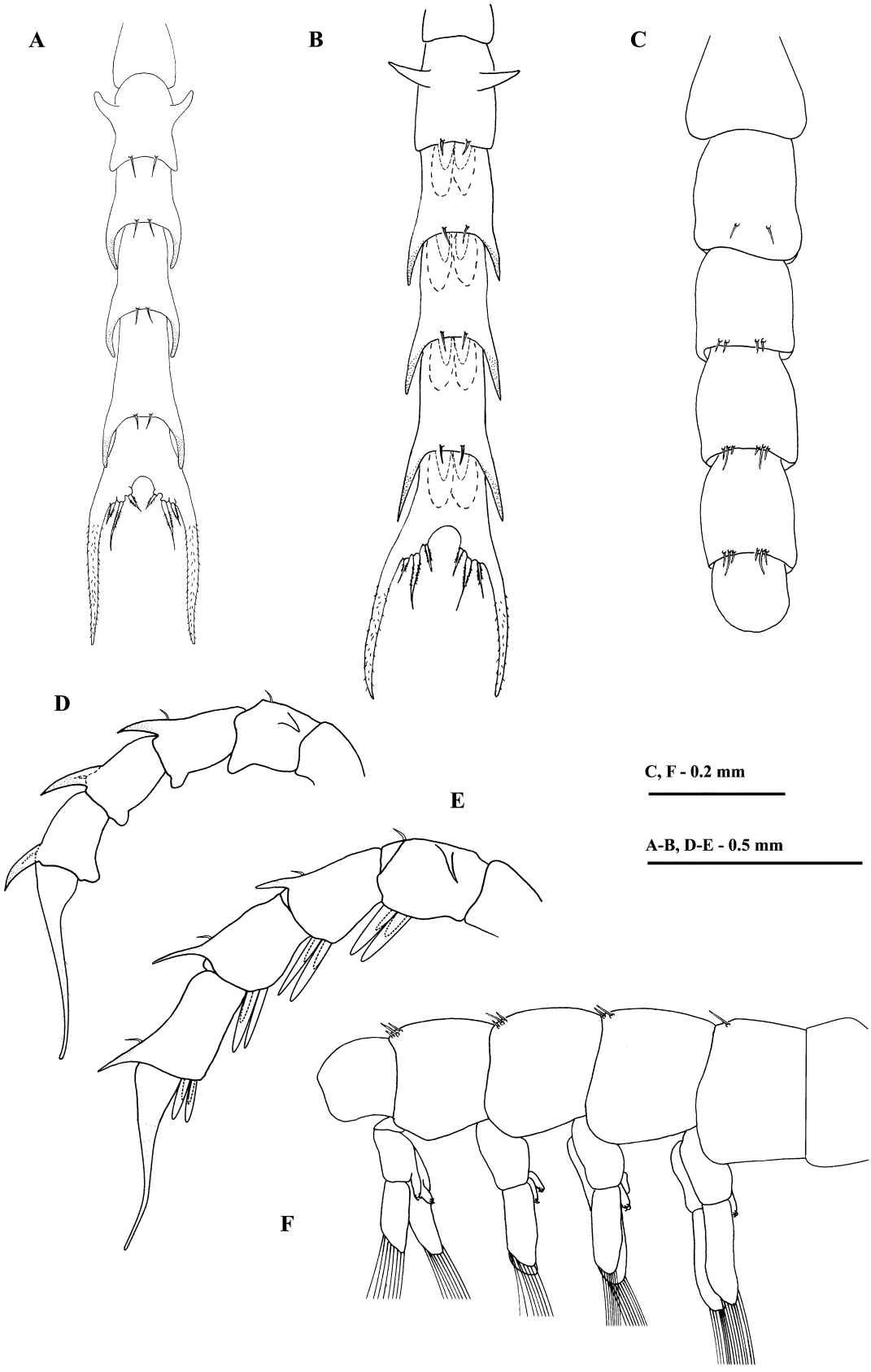

FIGURE 7. *Macropodia czernjawskii*(Brandt, 1880). Abdomen, dorsal view, A: zoea I; B: zoea II; C: megalopa. Abdomen, lateral view, D: zoea I; E: zoea II; F: megalopa.

Megalopa
(Figs. 2E–G; 3C, F, G; 4C, F; 5C, F, G; 6A–D; 7C, F)

Size: CL = 0.833 ± 0.045 mm; CW = 0.631 ± 0.035 mm; N= 10

Cephalothorax (Figs. 2E, G): Longer than broad, with small rostrum, directed ventrally; each protogastric region with dorsally directed blunt process with pair of plumose setae; one tubercle on mesogastric region and on posterodorsal margin; prominent long spine present on cardiac region; four pairs of simple setae on frontal region as drawn.

Antennule (Fig. 3C): Peduncle 3-segmented, without setae; unsegmented endopod without setae; exopod 2-segmented, proximal segment with 1 and distal segment with 4 aesthetascs.

Antenna (Fig. 3F): Peduncle 3-segmented with 1, 0, 1 setae respectively, proximal segment with stout ventrally directed process; flagellum 4-segmented with 0, 4, 0, 3 setae respectively.

Mandible (Fig. 3G): Palp unsegmented with one terminal simple seta.

Maxillule (Fig. 4C): Basial endite with 7 terminal setae (4 cuspidate, 3 plumodenticulate), 5 subterminal sparsely plumodenticulate setae and 1 proximal plumose seta; endopod reduced, unsegmented and without setae.

Maxilla (Fig. 4F): Coxal endite with 5 terminal plumose setae; basial endite bilobed with 3 + 5 sparsely plumodenticulate setae; endopod unsegmented and without setae; exopod with 18–20 marginal plumose setae plus 1 small simple seta on each lateral surface.

First maxilliped (Fig. 5C): Epipod without setae; coxal endite with 6 plumose setae; basial endite with 7 sparsely plumodenticulate setae; endopod reduced, unsegmented and without setae; exopod 2-segmented, with 4 plumose setae on distal segment.

Second maxilliped (Fig. 5F): Epipod present, without setae; protopod without setae. Endopod 4-segmented with 0, 1 (plumose), 2 (1 simple and 1 plumodenticulate), and 4 (plumodenticulate) setae; exopod 2-segmented, with 4 terminal plumose setae on distal segment.

Third maxilliped (Fig. 5G): Epipod with 1 terminal long seta; protopod with 1 simple seta; endopod 5-segmented, with 7, 3, 3, 7, 4 setae respectively; exopod 2-segmented with 4 plumose setae on distal segment.

Pereiopods (Figs. 6 A–D): Cheliped with a small proximal ventral tubercle on merus; pereiopods II–V slender and setose, with dactyl terminally acute; ischium of pereiopods II–III with prominent curved hook-shape spines. Setation as illustrated.

Sternum (Fig. 2 F): Setation as shown in the illustration.

Pleon (Figs. 7C, F): Five somites, somite VI absent; somite I without setae; somite II with one pair of posterodorsal simple setae; somite III with two pairs of posterodorsal simple setae; somite IV–V with one pair posterodorsal of 3 simple setae.

Pleopods (Fig. 6E): Present on somites II–V; endopods with 2 cincinuli; exopods with 8 long plumose natatory setae.

Telson (Figs. 7C, F): Longer than broad without setae.

Discussion

The Majoidea is one of the most species-rich groups of Brachyura and has more than 900 species (Ng *et al*. 2008; De Grave *et al*. 2009). Although these many species inhabit different marine habitats and have a diversity of adaptations as well as a wide variety of zoeal and megalopal forms, they share a set of larval characters that distinguish them from the rest of the brachyuran superfamilies: only two zoeal stages, the scaphognathite of the zoea I has at least nine marginal plumose setae and the apical stout process is greatly reduced, zoea II with developed pleopods (Rice 1980; Van Dover *et al*. 1982), megalopa lacking sensory setae on the dactylus of the fifth pereiopods, uropods may be absent, and when present, they have no more than eight setae and the antennal flagellum never more than five segments (Rice 1988).

Several works have used larval features to study the phylogeny and familial relationships of Majoidea (Rice 1980, 1988; Clark & Webber 1991; Marques & Pohle 1998, 2003; Pohle & Marques 2000). With respect to Inachidae, these studies agree in considering the family to be monophyletic when *Macrocheira kaempferi* (Temminck, 1836) and *Stenorhynchus* Lamarck, 1818 are removed. Some of these authors even consider Inachidea as the most

derived majoid family. Recent studies (Hultgren *et al*. 2009; Hultgren & Stachowicz 2008) combined larval morphology data and molecular evidence to also support the monophyly of Inachidae, but as inachid species are poorly represented in these analyses it is not possible to reach definitive conclusions at present. In general, the molecular results are congruent with those derived from larval morphology in the rest of the majoid families.

Inachidae consists of 204 species in 37 genera (Ng et al. 2008; De Grave *et al*. 2009), but larval data are only available for 26 species (12 genera). Therefore, it is currently too early to define larval features that characterize this family, which is made even more difficult since the known data indicate that there are strong intergeneric differences (see Oh & Ko 2011). Some of this larval morphological evidence has led authors to suggest removing species like *Platymaia wyvillethomsoni* Miers, 1886 and *Ergasticus clouei* A. Milne-Edwards, 1882, from Inachidae (Oh & Ko 2011; Guerao & Abelló 2007). Therefore, new larval descriptions of genera without larval data, and new molecular analyses that represent a wider number of inachid genera are needed to shed light on the real familial composition and phylogenetic relationships.

Macropodia comprises 17 valid species, of which nine inhabit northeast Atlantic and Mediterranean waters. There is currently larval data for only six species, all of them belonging to this Atlanto-Mediterranean group. As it has been previously pointed out (Guerao & Abelló 1997; González-Gordillo & Rodríguez 2001) the morphology of the larval stages of the genus *Macropodia* is very similar among the different species. It is therefore not easy to find consistent characters that can be used to distinguish them. First, the morphometry can be compared (data shown in Table 1). Although differences are obvious, especially between the largest (*M. tenuirostris*) and the smaller larvae (*M. czernjaswkii*), these kinds of differences have to be considered with care due to the latitudinal (temperature) effect on size, as previously demonstrated in other species (*Metacarcinus magister* (Dana, 1852), as *Cancer magister*, Shirley *et al*. (1987)). In the present study this is clear for *M. rostrata*, which shows differences between the larvae from the U.K. and those from SW Spain (see Table 1). Even in larvae collected from the same area, these measurements need to be carefully analyzed since there are also data that correlate larval intraspecific differences in size with parental female size (Sato & Suzuki 2010), and differences that depend on the season of the year (Pardo *et al*. 2009). More interesting are differences in ratios, for example between the DSL and CL, which makes it possible to see which larvae have a long dorsal cephalothoracic spine, and not just obtain an absolute measure. In this case, while the zoea I of *M. rostrata* from the U.K. has the longest DS (1.3–1.4 mm), it is the zoea I of *M. linaresi* that has the highest DSL/CL ratio (1.98–2.0) (see Table 1).

Table 2 summarizes the main morphological and meristic features that differ among *Macropodia* larval stages. Other minor differences (especially in the number of setae) are not listed because they may be more related to the size of the larvae rather than being a remarkable difference. According to these data, three main groups can be distinguished: The first comprises *M. rostrata*, *M. parva* and *Macropodia* S13, and is characterized by the antennal morphology of the zoeae, which have a rounded tip of the protopod, and exopod and protopod without spinules, the megalopae without a cheliped isquial spine and only one small tubercle in the merus of the cheliped. *Macropodia tenuirostris* and *M. longipes* form a second group that is characterized by the antenna of zoeae having protopod and exopod spinulated with acute tips, and megalopae with one spine on the isquium and two well-developed spines on the merus of the cheliped. *Macropodia linaresi* can be included in this group but only based on features of the zoea I, because there are currently no data on the zoea II and megalopa. The third group is represented solely by *M. czernjawskii*. It shows intermediate characters, sharing the antennal morphology of the zoeae of *M. tenuirostris*, *M. longipes* and *M. linaresi*, and the spinulation of the cheliped of the megalopa of *M. rostrata* and *M. parva*. However, it does have a particular trait that distinguishes it from other *Macropodia* zoeae: the absence of a lateral spine on the telson furcae. This character is really exceptional in Majoidea, as all known zoeae in this superfamily have at least one pair of well-developed lateral spines on the telson furcae, with only a few exceptions: four species of *Doclea* Leach, 1815 (see Krishnan & Kannuandi 1988) that do not have spines on the telson furcae, and *Pyromaia tuberculata* (Lockington, 1877) that only has a pair of small dorsal spines (see Fransozo & Negreiros-Fransozo 1997; Luppi & Spivak 2003). *Macropodia* has been defined, within inachids, as the most derived genus due to reduction in number of segments, appendages and setation, for example, the setation of the endopods of maxillule (0, 3), maxilla (2+2) and the second maxilliped (0, 0, 4), among others. This absence of spines on the telson furcae could be in line with this characteristic, and more larval descriptions of the remaining species of this genus are necessary in order to confirm this.

TABLE 1. Morphometric differences between larval stages of the *Macropodia* species with larval data known. Abbreviations: CL, cephalothorax length; DSL, dorsal spine of the cephalothorax length; AL, antenna length; ZI, zoea I, ZII, zoea II; nd, no data;[(1)] *M. rostrata* larvae from San Pedro River (SW Spain) were obtained from an ovigerous female collected in August 1999;[(2)] larvae from plankton samples attributed to *M. linaresi*, *M. tenuirostris* or *M. longipes*. All data in mm.

		Zoeal stages								**Megalopa**		
		CL		**DSL**		**DSL/ CL**		**AL**				
Species (reference)	**Origin**	**ZI**	**ZII**	**ZI**	**ZII**	**ZI**	**ZII**	**ZI**	**ZII**	**CL**	**CW**	**CL/CW**
M. tenuirostris (Salman 1981)	Isle of Man (U.K.)	0.98	1.19	1.14	0.89	1.16	0.74	0.9-1.0	1.05	1.14	0.98	1.16
M. tenuirostris (Ingle 1982)	Isle of Man (U.K.)	0.9-1.0	1.1-1.2	1.0-1.1	nd	1.11	nd	0.9-1.0	1.1-1.2	1.5	nd	nd
M. rostrata (Ingle 1982)	Isle of Man (U.K.)	0.7-0.8	0.8-0.9	1.3-1.4	1.1-1.2	1.85-1.75	1.9-1.3	1.2	1.3-1.4	1.2-1.3	nd	nd
M. rostrata[(1)] (present study)	San Pedro River (SW Spain)	0.66	0.78	1.03	0.9	1.56	1.15	0.87	0.95	0.98	0.76	1.3
M. longipes (Guerao&Abelló 1997)	Delta EbroRiver (W Mediterranean)	0.75-0.77	0.9	0.90-0.94	0.80-0.82	1.2	0.88-1.1	0.80-0.83	0.87-0.89	1.1-1.15	0.78-0.82	1.40-1.41
M. linaresi (Guerao *et al.* 1997)	Cape La Nao (W Mediterranean)	0.60-0.63	-	1.20-1.25	-	2-1.98	-	0.64-0.66	-	-	-	-
M. parva (González-Gordillo & Rodríguez 2000)	El Chato beach (SW Spain)	0.63	0.85	0.60	0.92	0.95	1.08	0.61	0.81	1.0	0.78	1.28
M. czernjawskii (present study)	El Chato beach (SW Spain)	0.57	0.61	0.69	0.58	1.21	0.95	0.60	0.68	0.83	0.63	1.31
Macropodia S13[(2)] (Paula 1987)	S. Torpes Bay (SW Portugal)	0.74	0.79	1.15	1.19	1.55	1.51	nd	nd	1.09	nd	nd

TABLE 2. Main morphological and meristic differences between larval stages of the *Macropodia* species with larval data known. Abbreviations: Pp, Posterodorsal protuberance; Sl, slightly; Pt, protopod tip; s, setation; ss, simple setae; es, exopodal seta; sp., spine; [(1)] 0,1+2 in SW Spain zoea I; [(2)] absent in SW Spain and Tunisia zoeae II; [(3)]1, 0, 1 setae in SW Spain and Ingle´s 1992 description of megalopae; [(4)]according to Paula (1987) no difference respect to Ingle´s (1982) description of *M. rostrata*; [(5)] data from SW Spain larvae; other abbreviations and references as in Table 1.

Species	***M. tenuirostris***	***M. longipes***	***M. linaresi***	***M. rostrata***	***M. parva***	***Macropodia* S13**	***M. czernjawskii***
Zoea I							
Cephalothorax Pp	present	present	present	absent	absent	absent	absent
Dorsal spine	Sl curved distally	Sl curved distally	Straight	Straight	Sl curved distally	Sl curved distally	Sl curved distally
Antennule s	4a, 1ss	3a, 2ss	3a, 2ss	2a, 2ss	4a, 1ss	nd	4a, 1ss
Antenna Pt	acute	acute	acute	rounded	rounded	rounded	acute
Antennal protopod	spinulated	spinulated	spinulated	not spinulated	not spinulated	not spinulated	spinulated 2 rows
Maxillule endopod s	0, 1+2	0, 1+2	0, 1+2	0, 3[(1)]	0, 3	0, 1+2	0, 3
Telson furcae	spinulated	spinulated (minute spinules)	spinulated (minute spinules)	spinulated (very minute spinules)	spinulated (very minute spinules)	nd	spinulated (minute spinules)
Telson lateral spines	present	present	present	present	present	present	absent
Zoea II							
Cephalothorax Pp	present	present	-	absent	absent	absent	absent
Dorsal spine	straight	Sl curved distally	-	curved	curved	straight	curved
Maxillule es	present	present	-	present [(2)]	absent	present	present
Pleonal somite 1 s	2	0	-	0	0	0	0
Megalopa							
Antennal peduncle s	1, 0, 1	0, 0, 1	-	0, 0, 1 [(3)]	1, 0, 1	0, 0, 1[(4)]	1, 0, 1
Cheliped isquium sp.	1	1		0	0	0[(4)]	0
Cheliped merus sp.	2	2	-	1 (minute)	1 (minute)	1 (minute)[(4)]	1 (minute)
Sternal plate s	nd	nd	-	6 [(5)]	6	nd	28
Pleonal somite 5 s	2+2	2+2	-	1+2+2+1	3+3	2+2	3+3

Within each group mentioned above, separations can be made as follows: the zoea I of *M. linaresi* has a long, straight dorsal spine of the carapace, which is shorter and slightly curved distally in *M. tenuirostris* and *M. longipes*. Differences between these two last species are more difficult to find. In the zoeal stages the only differences are the antennules formula (see Table 2), the setation of the scaphognathite of the maxilla of zoea II (which could be related to size), and two dorsal setae on abdominal somite 1 that are present in *M. tenuirostris* and absent in *M. longipes*. There are evident differences in the overall morphology of the megalopa, as the cardiac spine of the cephalothorax is more than twice as long as the protograstic spines, and the first segment of the antennal peduncle does not have setae in *M. longipes*, while in *M. tenuirostris* the megalopa cardiac and protograstic spines have a similar length, and one setae is present in the first segment of the antennal peduncle. Likewise, differences between the larval stages of *M. rostrata* and *M. parva* are not easy to observe. In this case there are additional difficulties due to differences reported in *M. rostrata* larvae from different geographical origins. Starting with this intraspecific variability in *M. rostrata*, two main groups can be separated based on the presence or absence of exopodal seta on the maxillule of zoea II: larvae from the Isle of Man and Plymouth (Ingle 1982) as well as from S. Torpes Bay (SW Portugal) (described by Paula (1987) as *Macropodia* S13) have exopodal seta; and larvae from Carthage-Salammbo (Tunisia) (Ingle 1982) and San Pedro River (SW Spain) (unpublished material from larvae reared by AR) do not have exopodal seta. Other features for making comparisons are confusing due to inaccuracies between the original description by Ingle (1982) and a posterior re-description (Ingle 1992). For example, in his first work Ingle (1982) only described one medial seta on the exopod of the antenna of zoea I, and that the megalopa had setation of the antennal peduncle 0, 0, 1; however, later in his 1992 description he described the antennal exopod of the zoea I as having two medial setae, and 1, 0, 1 setae on the antennal peduncle of the megalopa. Although Paula (1987) initially attributed the larvae described as *Macropodia* S13 to *M. linaresi*, *M. tenuirostris* or *M. longipes*, it should actually be identified as *M. rostrata* based on the zoeal antennal morphology. Alternatively, these larvae could belong to *M. longirostris* or *M. intermedia*, which are two other species present in the area and for which there are currently no larval data. The minor differences with respect to *M. rostrata* from the U.K. are the same as those of the larvae from SW Spain (see Table 2), and are therefore presumably related to intraspecific variability due to geographical origin.

There are no significant differences when the larval stages of *M. rostrata* and *M. parva* are compared (see Table 2), because re-examination of the larval stages of several specimens of *M. parva* deposited at the Instituto de Ciencias Marinas (accession number MJ/2000-3) showed that some differential characters, such as the presence of one seta at the basis of the second maxilliped of zoea II and two dorsal setae on the telson of the megalopa, described by González-Gordillo & Rodríguez (2001) were absent in the re-examined larvae. Therefore, this could be a mistake in the original description or a less frequent feature. These characters are in any case not useful for characterizing these larvae.

González-Gordillo & Rodríguez (2001) suggested that *M. rostrata* and *M. parva* could be subspecies due to strong homogeneity in the larval morphology, and the similar morphological characteristics of adults, which even coexist in the same habitat in the Gulf of Cádiz. In addition, D´Udekem d´Acoz (1999) questioned the validity of *M. parva* due to the inaccurate description of the species, which is mainly based on small specimens. Initial data from an ongoing work on the molecular phylogeny of *Macropodia* suggest that *M. rostrata* and *M. parva* should be considered as the same species due to similar sequences of the mitochondrial genes 16S and Cox1 (Marco-Herrero *et al*. unpublished data). Therefore, the slight differences observed in larval stages (see Tables 1, 2) should be attributed to intraspecific variability.

Acknowledgments

We are grateful to Peter Castro and one anonymous referee for useful suggestions and corrections that clearly improved the manuscript. This study was funded by the Spanish “Ministerio de Ciencia e Innovación, Plan Nacional I+D” and the European FEDER funds through project MEGALOPADN (CGL2009–11225). The “Ministerio de Ciencia e Innovación” also supported a FPI PhD grant to the first author; results here presented are part of her PhD thesis project.

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CAPÍTULO 3

Morphology of the larval stages of a Mediterranean population of the allochthonous Say's mud crab, *Dyspanopeus sayi* (Decapoda: Brachyura: Panopeidae)

E Marco-Herrero, G Guerao, JA Cuesta (2013) Morphology of the larval stages of a Mediterranean population of the allochthonous Say's mud crab, *Dyspanopeus sayi* (Decapoda: Brachyura: Panopeidae) Scientia Marina 77(2): 341-352

Scientia Marina 77(2)
June 2013, 341-352, Barcelona (Spain)
ISSN: 0214-8358
doi: 10.3989/scimar.03815.15A

Morphology of the larval stages of a Mediterranean population of the allochthonous Say's mud crab, *Dyspanopeus sayi* (Decapoda: Brachyura: Panopeidae)

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SUMMARY: In this study the complete larval development (including four zoeal stages and a megalopa stage) of *Dyspanopeus sayi* is described and illustrated in detail based on larvae reared in the laboratory. Ovigerous females were collected in Alfacs Bay, Ebro Delta (NW Mediterranean) where the species was recently introduced. These larval stages were compared with others collected in the plankton from the same area, the larval stages described previously for this species and the larval stages of the two other Panopeidae that inhabit the Iberian Peninsula: *Panopeus africanus* and *Rhithropanopeus harrisii*. Differences were found in both zoeal and megalopal larval characters between *D. sayi* and the other two species, which could raise doubt about their placement in the same family.

Keywords: Panopeidae, *Dyspanopeus sayi*, allochthonous species, larval stages, zoea, megalopa.

RESUMEN: Morfología de los estadios larvarios de una población mediterránea del cangrejo alóctono, *Dyspanopeus sayi* (Decapoda: Brachyura: Panopeidae). – En este estudio se describe e ilustra en detalle el desarrollo larvario (4 estadios zoea y un estadio megalopa) de *Dyspanopeus sayi*, a partir de larvas cultivadas en el laboratorio. Las hembras ovígeras fueron capturadas en la Bahía de Alfacs (NO Mediterráneo), donde la especie ha sido recientemente introducida. Los estadios larvarios descritos han sido comparados con larvas del plancton capturadas en la misma zona, con descripciones previas de esta especie y con descripciones de los estadios larvarios de las otras dos especies de Panopeidae que habitan en la Península Ibérica: *Panopeus africanus* y *Rhithropanopeus harrisii*. Se han encontrado diferencias destacables en algunos caracteres de los estadios, zoea y megalopa, lo cual podría poner en duda la posición de estas especies dentro de la misma familia.

Palabras clave: Panopeidae, *Dyspanopeus sayi*, especie alóctona, estadio larvario, zoea, megalopa.

INTRODUCTION

The native distribution of Say's mud crab, *Dyspanopeus sayi* (Smith 1869), encompasses the Atlantic coast of North America from Florida to Canada (Nizinski 2003). It is considered an invasive species in other parts of the world as a result of human activities and has probably been accidentally transported in ballast water like many other marine invertebrates (Davidson and Simkanin 2012). Currently, outside of its native range, *D. sayi* has been recorded from southwest England, Queens Dock, Swansea (Wales) (Ingle 1980, Clark 1986), on the French and Dutch coasts of the North Sea (Vaz *et al.* 2007), and more recently in the Black Sea (Micu *et al*. 2010). It is also present in the Mediterranean Sea, where it has been collected in Venice, the Marano and Varano lagoons, and in the Po River Delta (western Adriatic Sea) (Froglia and Speranza 1993, Mizzan 1995, Florio *et al.* 2008). The most recent record is from the western Mediterranean, which constituted the first record for the coast of the Iberian Peninsula (Schubart *et al*. 2012). Figure 1 shows the current distribution of *Dyspanopeus sayi* worldwide.

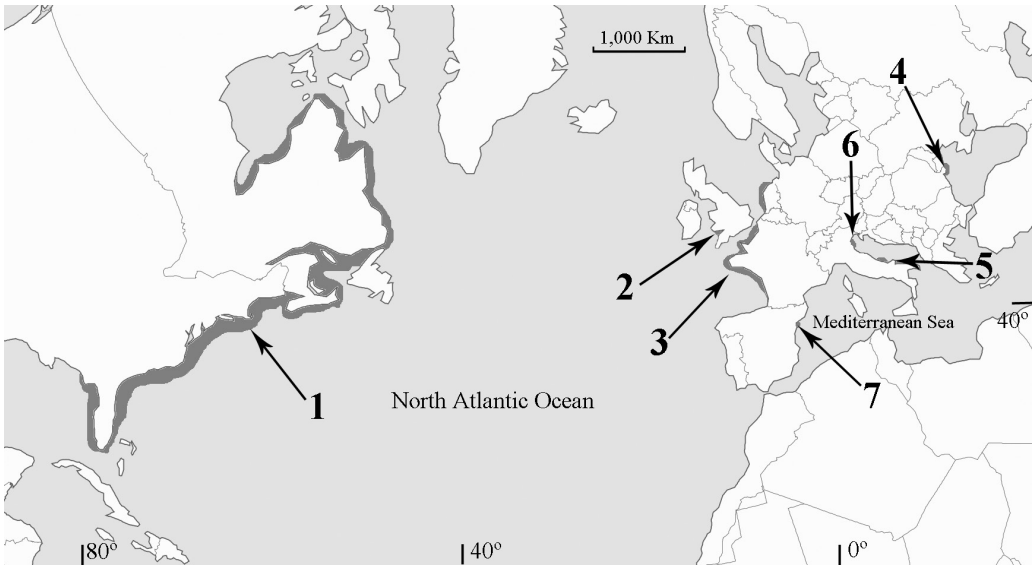

FIG 1. – Worldwide distribution of Say's mud crab *Dyspanopeus sayi* (Smith, 1869). References: 1, Nizinski (2003); 2, Ingle (1980), Clark (1986); 3, Vaz *et al.* (2007); 4, Micu *et al*. (2010); 5, Florio *et al*. (2008); 6, Froglia and Speranza (1993), ICES (2005); 7, Schubart *et al*. (2012).

Dyspanopeus sayi is a euryhaline and eurythermic species that inhabits estuaries and shallow coastal marine waters (see Schubart *et al*. 2012). This species is the second Panopeidae that has established large populations in estuarine habitats of the Iberian Peninsula. The first species was *Rhithropanopeus harrisii* (Gould 1841), reported for the first time in the Guadalquivir River (south Atlantic coast of Spain) (Cuesta *et al.* 1991). Another panopeid inhabiting the Iberian Peninsula is *Panopeus africanus* A. Milne-Edwards, 1867, which is an endemic crab with a wide distribution from Angola to Portugal (Rodríguez and Paula 1993).

A number of authors have partially or completely studied the larval development of the three species: Rodríguez and Paula (1993) described *Panopeus africanus*; Connolly (1925), Hood (1962), Chamberlain (1962), and Kurata (1970) studied *Rhithropanopeus harrisii* and Marco-Herrero *et al.* (2012) recently redescribed the megalopa stage; and Birge (1883), Hyman (1925), Chamberlain (1957, 1961), Kurata (1970), Clark (2007) and Schubart *et al.* (2012) studied *Dyspanopeus sayi*. With the exception of the zoea I, the descriptions of the remaining three zoeal stages and the megalopa of *Dyspanopeus sayi* are incomplete, not detailed and poorly illustrated according to the modern standardization of brachyuran larval descriptions (Clark *et al.* 1998).

In the present study the complete larval development (four zoeal stages and a megalopa) of *Dyspanopeus sayi* is described and illustrated in detail based on larvae reared in the laboratory. We compared these larval stages with those collected in the plankton, and the larval stages described previously for the other two Panopeidae that inhabit the Iberian Peninsula, namely *P. africanus* and *R. harrisii.*

MATERIALS AND METHODS

Three ovigerous females of *Dyspanopeus sayi* of 16 to 18 mm cephalothorax length were collected by beam trawls from sandy-muddy bottoms of Alfacs Bay, Ebro Delta (40°40'N, 0°40'E) covered by the seagrass *Cymodocea nodosa* and the alga *Caulerpa prolifera* (see Pérez and Camp 1986, Fusté 1988), in August 2011. Specimens were transported to the laboratories of the IRTA (Institut de Recerca i Tecnologia Agroalimentàries) in Sant Carles de la Ràpita. The females were kept in 40-L aquariums. After hatching, actively swimming zoea I were transferred to 500-mL beakers (n=5). A total of 20 larvae were placed in each beaker. The subsequent rearing cultures were conducted at a constant salinity of 34, a temperature of 18±1°C and a natural photoperiod of *ca*. 12 h light per day (early spring condition). After the water had been changed, the Chlorophyceae *Tetraselmis chuii* and *Artemia* sp. naupii were provided daily as feed.

A qualitative plankton survey was carried out on 24 and 25 September 2012 in the Alfacs Bay, Ebro Delta using a plankton net with a mouth opening of 0.25 m^2 and mesh size of 250 μm. Samples were taken during daytime, fixed in ethanol (96%), and later sorted in the laboratory under a Wild MZ6 compound microscope.

For easier observation of larvae structures and setation under microscope, a digestion-stain procedure (Marco-Herrero *et al*. 2012) was carried out. Initially, entire specimens were placed for 10 minutes in a watch glass with 2 ml of heated lactic acid. Immediately after, 3 drops of Clorazol Black stain (0.4 g Clorazol Black powder dissolved in 75 ml 70% ethanol) were added to the heated solution. After 5-10 minutes, the specimen was removed from the solution and placed on a slide

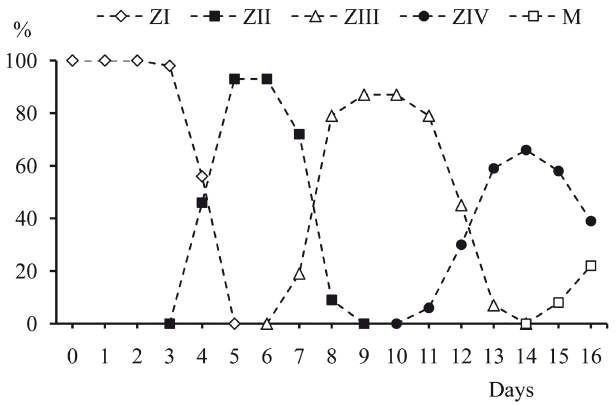

FIG 2. – Percentage survival and duration of larval stages of *Dyspanopeus sayi* reared under laboratory conditions (18±1°C and 34±1 salinity). ZI-IV, zoea I-IV; M, megalopa.

with lactic acid, in order to proceed with the dissection of the appendages.

Drawings and measurements were made using a Wild MZ6 and Zeiss compound microscope with Nomarski interference, both equipped with a *camera lucida*. All measurements were made by an ocular micrometer. Descriptions and measurements of different larval stages were based on at least 10 specimens of each stage from culture larvae. In Figure 7 first and second maxilliped of zoea I are drawn without exopod, and plumose natatory setae of the maxillipeds exopods of zoeae II-IV are drawn truncated. Description and figures are arranged according to the standards proposed by Clark *et al.* (1998).

Measurements taken for the zoeal stages were: rostrodorsal length (RDL), distance from the tip of the rostral spine to the tip of the dorsal spine; cephalothorax length (CL) between eyes (base of the rostrum) to the posterolateral carapace margin; cephalothorax width (CW) as the distance between the tips of lateral spines; rostral spine length (RL) from base of eye to tip of rostral spine; and antennal length (AL) from base of eye to tip of the protopod. Measurements for the megalopa included: CL measured from the frontal to posterior margin of carapace; CW as the carapace maximum width.

A larval series has been deposited at the Biological Collections of Reference of the Institut de Ciències del Mar (ICM-CSIC) in Barcelona under accession numbers ICMD13022501-3.

RESULTS

In the plankton samples a total of 9 zoeae I, 2 zoeae II and 1 zoea IV of *Dyspanopeus sayi* were identified and used for morphological and meristical comparison with those reared in the laboratory.

The larval development of *Dyspanopeus sayi* consists of four zoeal stages and a megalopa. At 18±1°C and 34 salinity the zoeal development was completed in a minimum of 15 days (appearance of the megalopa). The duration and survival of each larval stage is shown in Figure 2. The first zoeal stage is redescribed (see Schubart *et al*. 2012) and only the main differences in subsequent stages are described.

Larval description

Dyspanopeus sayi (Smith, 1869)

Zoea I

(Figs 3A, G; 5 A, E; 6 A, E; 7 A, E; 8 A, D)

Size: RDL=1863.5±12.4 μm; CL=514.4 ± 31.9 μm; CW=612.5±31.5 μm; RL=786.0±12.2 μm; AL=832.3±50.0 μm; N=10.

Cephalothorax (Fig. 3A, G): dorsal spine straight and well developed with small tubercles over the surface; rostral spine straight and slightly longer than dorsal spine; ventral caparace margin without setae, 1 pair of posterodorsal simple setae; eyes sessile.

Antennule (Fig. 5A): uniramous, unsegmented and conical; endopod absent; exopod with 4 terminal aesthetascs (two long and two shorter) and 1 simple seta.

Antenna (Fig. 5E): protopod long, equal in length to rostral spine, with rounded tip and without spines; exopod reduced to a minute bud with 1 small terminal simple seta; endopod absent.

Mandible: incisor and molar process developed; palp absent.

Maxillule (Fig. 6A): coxal endite with 7 plumodenticulate setae; basial endite with 5 terminal setae (3 cuspidate and 2 plumodenticulate); endopod 2-segmented with 1,2 subterminal + 4 terminal sparsely plumose setae respectively; epipod and exopod setae absent.

Maxilla (Fig. 6E): coxal endite bilobed with 4+4 plumodenticulate setae; basial endite bilobed with 5+4 plumodenticulate setae; unsegmented endopod bilobed, with 3 and 2 subterminal + 3 terminal sparsely plumose setae, respectively; exopod (scaphognathite) with 4 marginal plumose setae plus one stout plumose process.

First maxilliped (Fig. 7A): coxa with 1 sparcely plumose seta; basis with 10 medial sparsely plumodenticulate setae arranged as 2+2+3+3; endopod 5-segmented, longer than exopod, with 3,2,1,2,5 (1 subterminal + 4 terminal) sparcely plumodenticulate setae; exopod 2-segmented with 4 terminal plumose natatory setae.

Second maxilliped (Fig. 7E): coxa without setae; basis with 4 sparsely plumodenticulate setae arranged 1+1+1+1; endopod 3-segmented, with 1, 1, 5 (2 subterminal + 3 terminal) setae; exopod 2-segmented with 4 terminal plumose natatory setae.

Third maxilliped: absent.

Pleon (Fig. 8A, D): five pleonites. Pleonite 1 without setae; pleonites 2-5 with a pair of minute simple setae on posterodorsal margin; pleonite 2 with pair of forwardly directed dorsolateral processes and pleonite 3 with smaller dorsolateral processes backward directed.

Pleopods: absent.

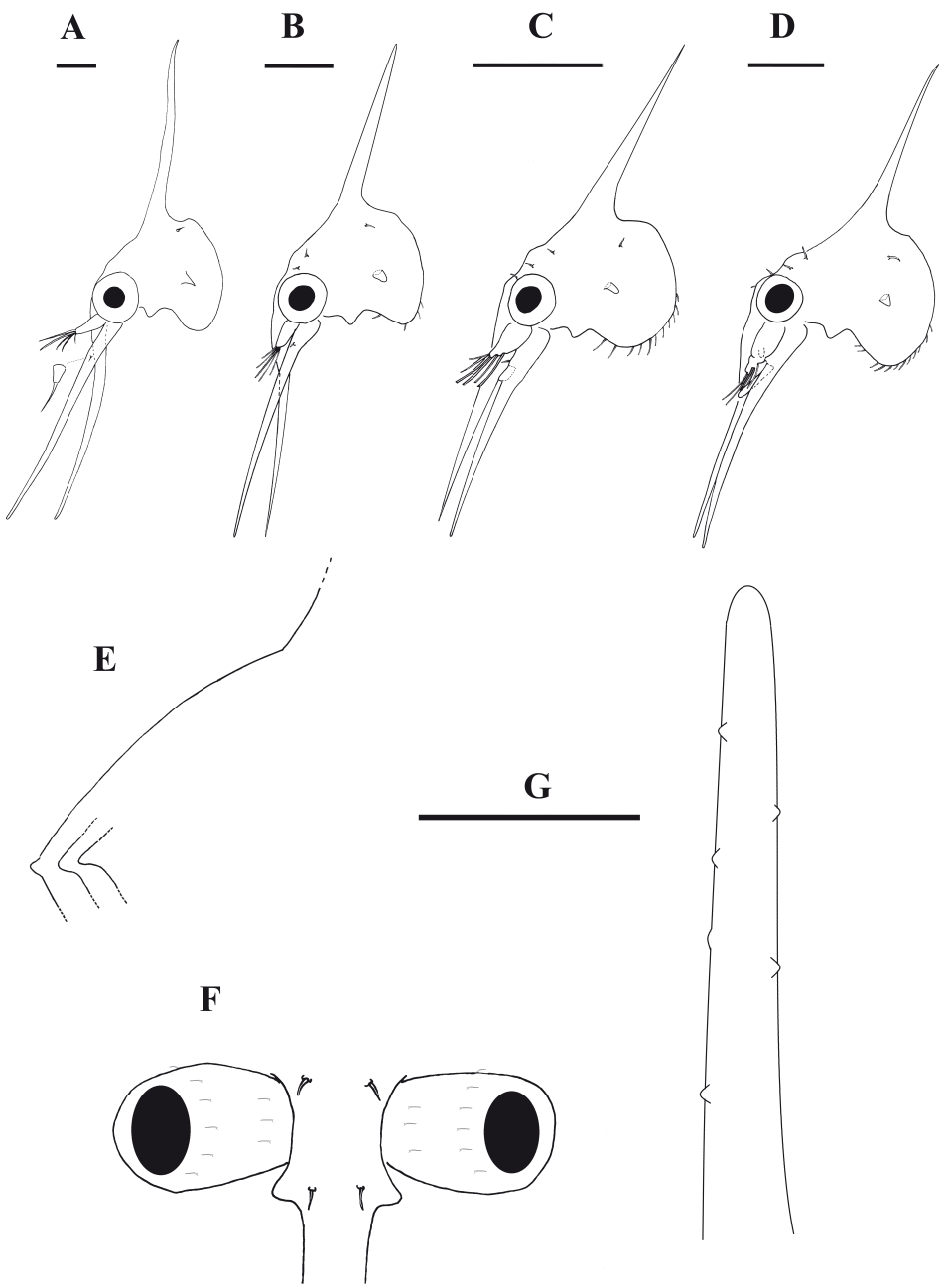

FIG 3. – *Dyspanopeus sayi*. Cephalothorax, lateral view. A, zoea I; B, zoea II; C, zoea III; D, zoea IV; E, cephalothorax, lateral spine, zoea II-IV; F, detail of the frontal view of rostrum of zoea IV; G, detail of distal part of dorsal spine of zoea I. Scale bars = 0.5 mm.

Telson (Fig. 8A, D): bifurcated, with deep median cleft; 2 pairs of 3 serrulate setae on posterior margin, medial setae longest; furcae with dorsal spine on mid part, and not spinulated.

Zoea II
(Figs 3B, E; 6B; 8E)

Size: RDL=2033±28.8 μm; CL=582.6±20.6 μm; CW=593±41.6 μm; RL=830±60.3 μm; AL=889.3±39 μm, N=10.

Cephalothorax (Fig. 3B, E): anteromedian ridge more pronounced than zoea I; 2 pairs of anterodorsal simple setae; 4 setae on ventral margin including 1 plumose anterior seta and 3 sparsely setose posterior setae; eyes stalked and movable.

Antennule: exopod with 5 terminal aesthetascs (2 long and 3 shorter) plus one small seta.

Antenna: Endopod present as a small bud.

Maxillule (Fig. 6B): basial endite with 8 setae (4 terminals cuspidate, 3 subterminal plumodenticulate and 1 proximal plumose seta); exopodal seta present.

Maxilla: scaphognathite with 11 plumose marginal setae.

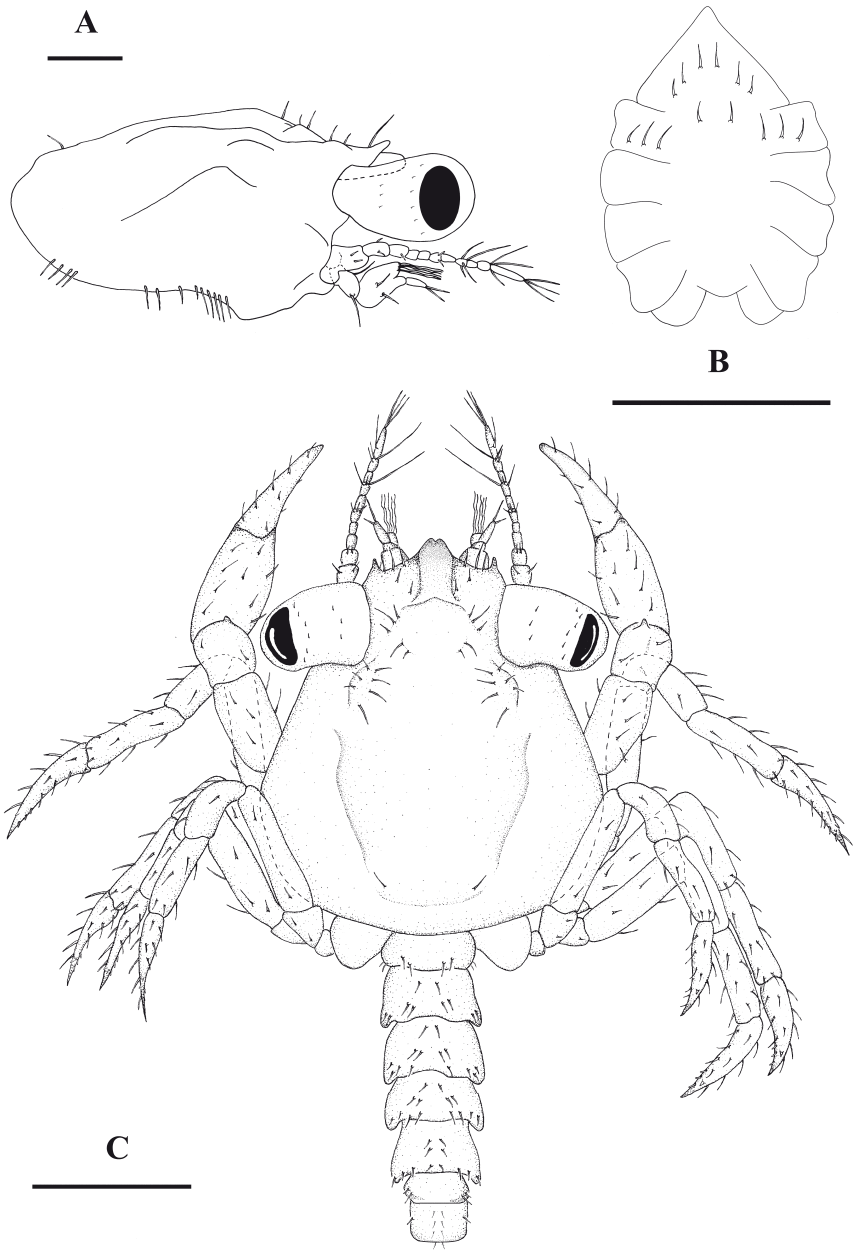

FIG 4. – *Dyspanopeus sayi*. Megalopa. A, cephalothorax, lateral view; B, sternum; C, whole dorsal view. Scale bars = 0.5 mm.

First maxilliped: exopod with 6 terminal plumose natatory setae.

Second maxilliped: exopod with 6 terminal plumose natatory setae.

Third maxilliped: present as undifferentiated bud.

Pleon (Fig. 8E): pleonite 1 with 1 mid-dorsal seta.

Pleopods: all present as undifferentiated buds.

Zoea III

(Figs 3C, E; 5F; 6F; 7B; 8F)

Size: RDL=2644±57.9 μm; CL=862.5±17.6 μm; CW=756.5±35 μm; RL=988.3±72.3 μm; AL=1051.3±74.1 μm, N=10.

Cephalothorax (Fig. 3C, E): anteromedian ridge more pronounced than zoea II; 3 pairs of anterodorsal simple setae; 11 setae on ventral margin including 1 plumose anterior seta and 10 sparsely setosed posterior setae.

Antennule: exopod with 6 aesthetascs (4 terminal and 2 subterminal).

Antenna (Fig. 5F): endopod more elongated.

Maxillule: coxal endite with 8 terminal plumodenticulate setae; basial endite with 9 setae (3 subterminal plumodenticulate setae, 5 terminal cuspidate setae and 1 proximal plumose seta).

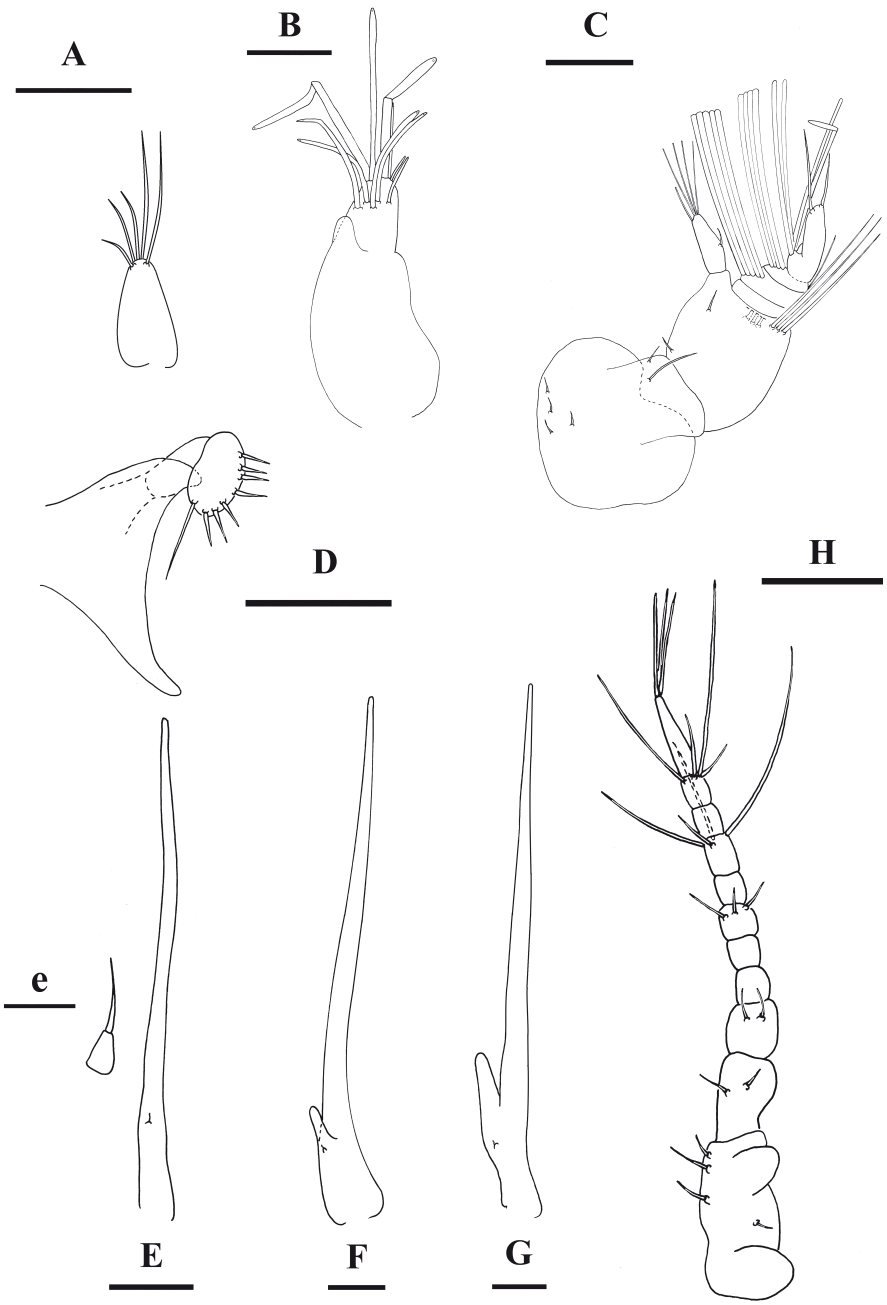

FIG 5. – *Dyspanopeus sayi*. Antennule. A, zoea I; B, zoea IV; C, megalopa. Mandible, D, megalopa. Antenna, E, zoea I; e, detail of exopod; F, zoea III; G, zoea IV; H, megalopa. Scale bars = 0.1 mm.

Maxilla (Fig. 6F): basial endite with 5+5 plumodenticulate setae; scaphognatite with 18-19 plumose marginal setae.

First maxilliped (Fig. 7B): endopod segment 5 with 6 (2 subterminal +4 terminal) sparsely plumodenticulate setae; exopod with 8 terminal plumose natatory setae.

Second maxilliped: exopod with 9 terminal plumose natatory setae.

Third maxilliped: unsegmented and without setae, differentiated in endopod and exopod buds.

Pereiopods: unsegmented and without setae. First pair bilobed (cheliform), pereiopod 2-5 as elongated buds.

Pleon (Fig. 8F): six pleonites plus telson.

Pleopods (Fig. 8F): present on pleonites 2-6 as small buds, endopods absent.

Zoea IV

(Figs 3D-F; 5B, G; 6C, G; 7C, F; 8B, G)

Size: RDL=3080.5±51.6 μm; CL=1059.0±14.1 μm; CW=927.5±24.7 μm; RL=1312.0±35.3 μm;

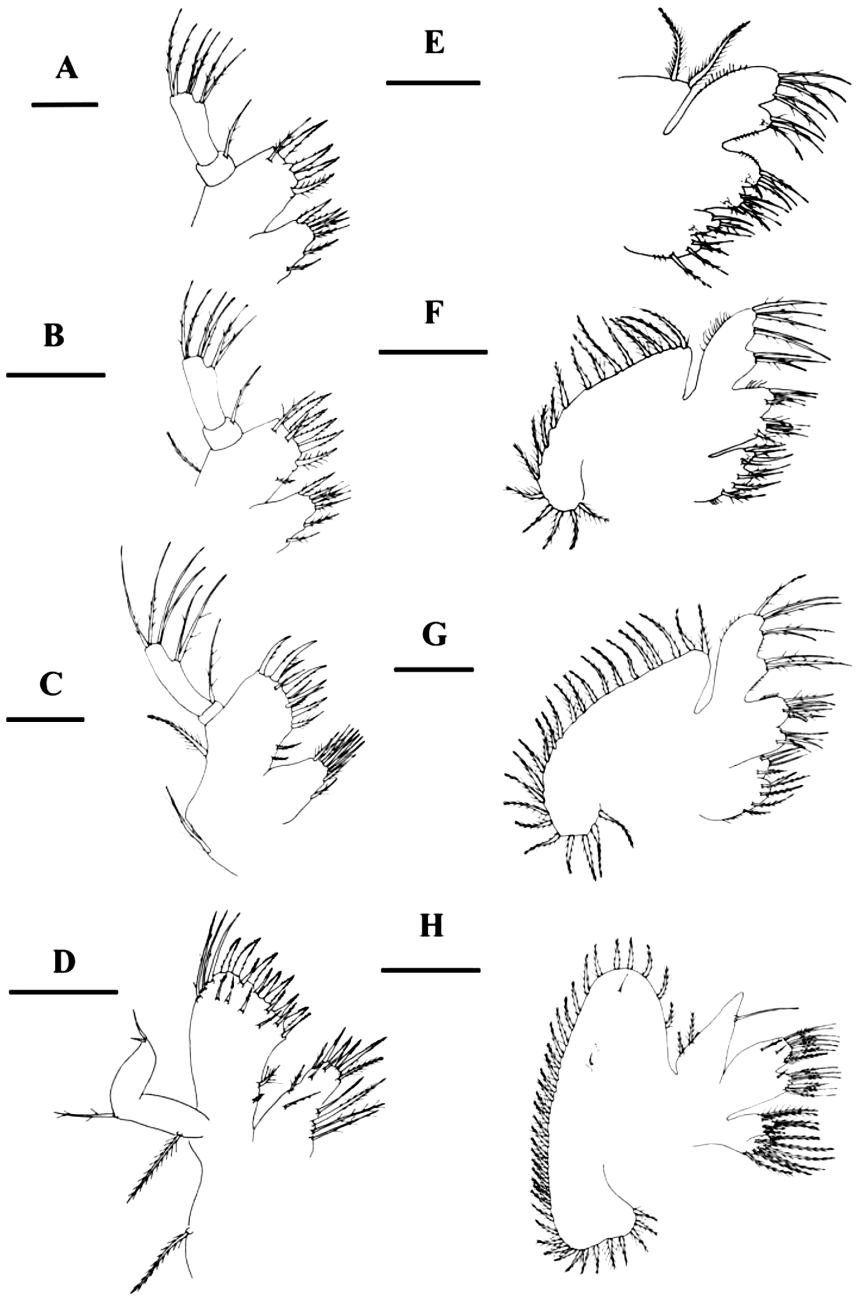

FIG 6. – *Dyspanopeus sayi*. Maxillule. A, zoea I; B, zoea II; C, zoea IV; D, megalopa. Maxilla, E, zoea I; F, zoea III; G, zoea IV; H, megalopa. Scale bars = 0.1 mm.

AL=1378.0±31.7 μm, N=10.

Cephalothorax (Fig. 3D-F): anteromedian ridge more pronounced than zoea III; 4 pairs of anterodorsal simple setae; 16 setae on ventral margin including 1 plumose anterior seta and 15 sparsely setosed posterior setae.

Antennule (Fig. 5B): endopod present; exopod with 10-11 aesthetascs (5 subterminal and 5-6 terminal).

Antenna (Fig. 5G): endopod more elongated.

Maxillule (Fig. 6C): coxal endite with 9-10 plumodenticulate setae; basial endite with 11-12 setae (4-5 subterminal plumodenticulate, 5 terminal cuspidate, 2 proximal plumose seta); epipodal seta present.

Maxilla (Fig. 6G): coxal endite with 5+4 plumodenticulate setae; basial endite with 5-6+6-7 plumodenticulate setae; scaphognatite with 24-27 plumose marginal setae.

First maxilliped (Fig. 7C): coxa with 2 sparsely plumose setae; exopod with 9 terminal plumose natatory setae. Epipodite present.

Second maxilliped (Fig. 7F): exopod with 11 terminal plumose natatory setae.

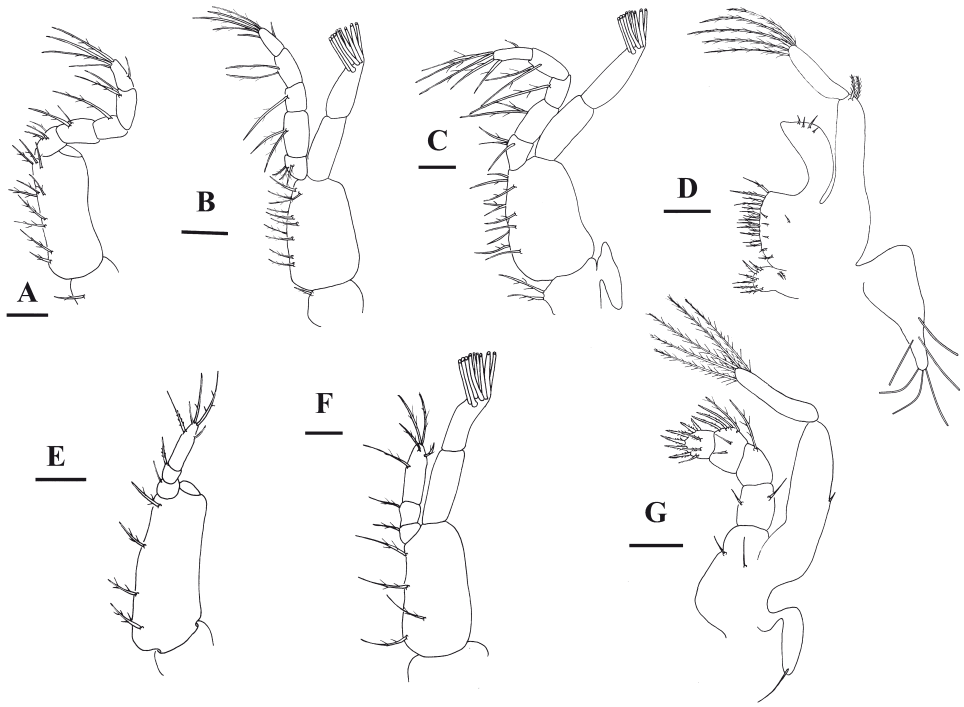

FIG 7. – *Dyspanopeus sayi*. First maxilliped: A, zoea I; B, zoea III; C, zoea IV; D, megalopa. Second maxilliped: E, zoea I; F, zoea IV; G, megalopa. Scale bars = 0.1 mm.

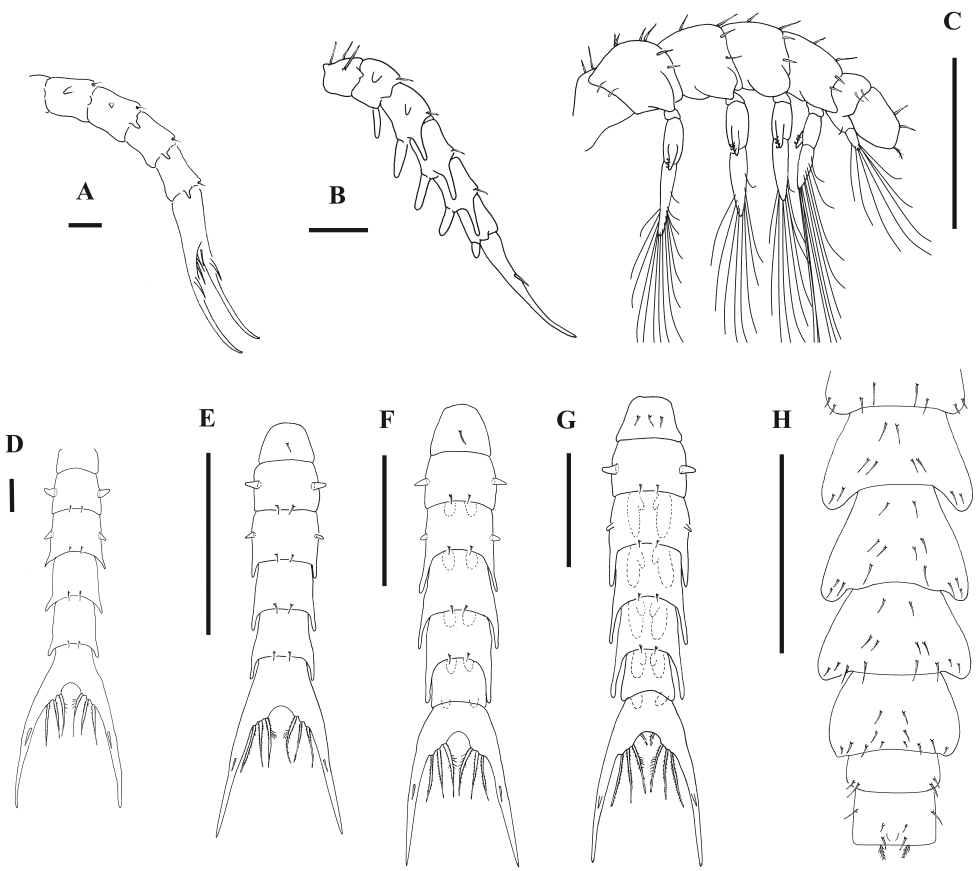

FIG 8. – *Dyspanopeus sayi*. Abdomen, lateral view: A, zoea I; B, zoea IV; C, megalopa. Abdomen, dorsal view: D, zoea I; E, zoea II; F, zoea III; G, zoea IV; H, megalopa. Scale bars = 0.5 mm.

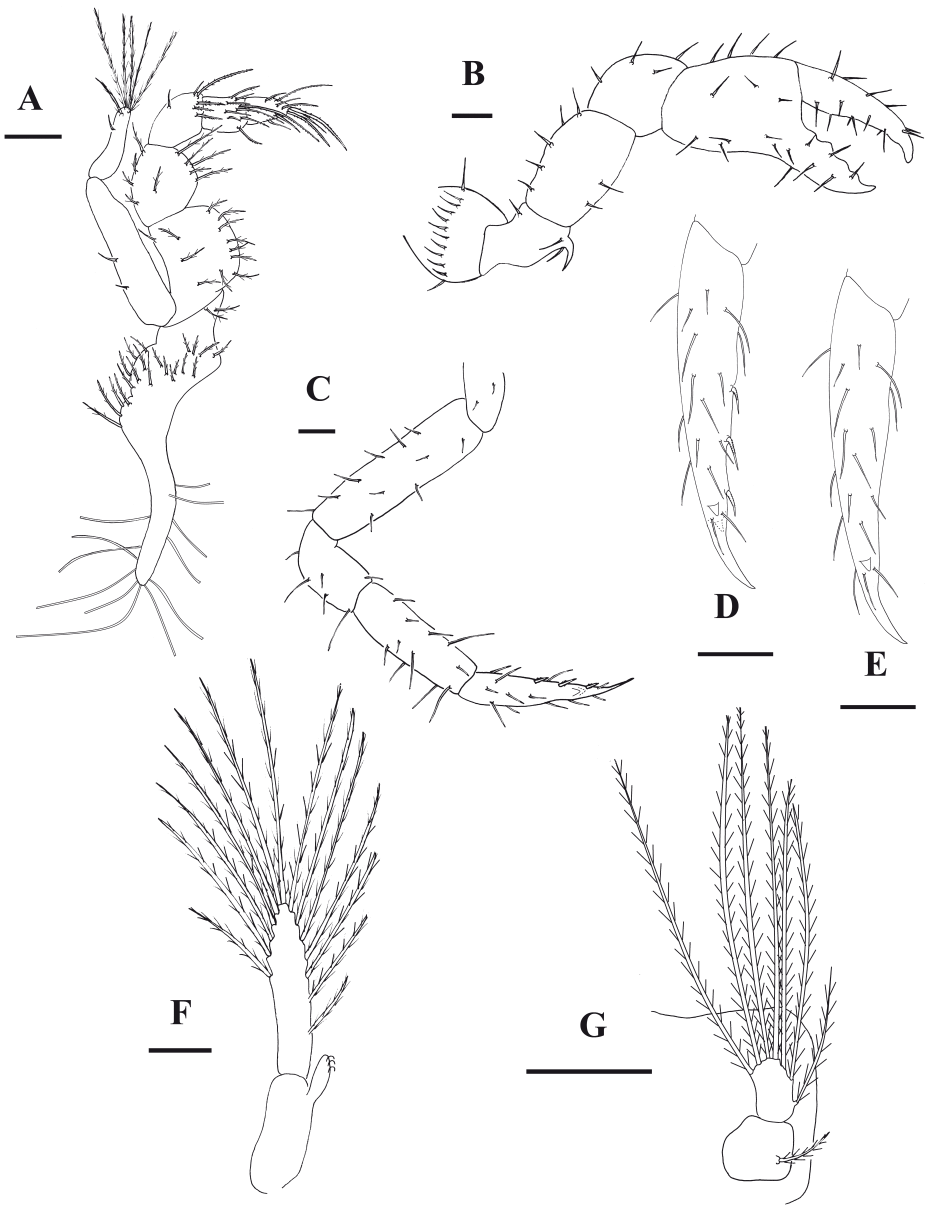

FIG 9. – *Dyspanopeus sayi*. Megalopa. A, third maxilliped; B, cheliped; C, pereiopod 3; D, dactylus pereiopod 4; E, dactylus pereiopod 5; F, pleopod 2; G, uropod. Scale bars = 0.1 mm.

Third maxilliped: rudimentary, slightly segmented, without setae.

Pereiopods: all slightly segmented, without setae.

Pleon (Fig. 8B, G): Pleonite I with 3 mid-dorsal setae.

Pleopods (Fig. 8B, G): biramous buds now with endopod present more elongated than previous stage.

Telson (Fig. 8B, G): one extra pair of short setae on inner posterior margin.

Megalopa

(Figs 4 A-C; 5C, D H; 6D, H; 7D, G; 8C, H; 9)

Size: CL=1130±100 μm; CW=1060±100 μm; N=15

Cephalothorax (Fig. 4A-C): frontal margin is obliquely downward with 2 lateral spines, rostrum ending in a median triangular notch with bifid tip; the peduncle of eyes with 9 small setae on dorsal part; 1 pair of protogastric and mesobranchial protuberance present; setation as shown.

Antennule (Fig. 5C): peduncle 3-segmented, with 4,2,2 plus 2 pairs of 3 long plumodenticulate setae, respectively; endopod unsegmented with 1 medial, 1 subterminal and 4 terminal setae; exopod 4-segmented, with 0,5,4,4 aesthetascs and 0,0,1,2 setae.

Antenna (Fig. 5H): peduncle 3-segmented with 4,2,2 setae; flagellum 8-segmented with 0,0,3,0,4,0,4,3 setae, respectively.

Mandible (Fig. 5D): palp 2-segmented, with 8 terminal setae on distal segment.

Maxillule (Fig. 6D): coxal endite with 14 plumose setae; basial endite with 2 setae on lower margin, 10 subterminal sparsely plumodenticulate setae and 8 terminal cuspidate setae; endopod unsegmented with 1 proximal and 2 terminal setae.

Maxilla (Fig. 6H): coxal endite with 7+6 terminal plumose setae; basial endite with 7+8 sparsely plumodenticulate setae; endopod unsegmented with 1 long simple seta; scaphognathite with 38-40 marginal plumose setae plus 2 small simple setae on each lateral surface.

First maxilliped (Fig. 7D): coxal endite with 2 proximal and 6 terminal plumodenticulate setae; basial endite with 13 plumodenticulate terminal setae plus 7 proximal simple setae; endopod unsegmented with 4 setae on distal part; exopod 2-segmented, with 2 terminal plumodenticulate setae on proximal segment, and 5 plumose setae on distal segment; epipod well developed with 6 setae.

Second maxilliped (Fig. 7G): protopod without setae; endopod 5-segmented with 2 simple, 2 simple, 1 plumodenticule, 2 simple + 4 plumodenticulate, and 3 proximal simple + 6 terminal plumodenticulate setae, respectively; exopod 2-segmented, with 1 medial simple seta on proximal segment and 5 terminal plumose setae on distal segment; epipodite reduced with 1 terminal seta.

Third maxilliped (Fig. 9A): protopod with 19 plumodenticulate setae; endopod 5-segmented, with 12,10,6,9,9 plumodenticulate setae respectively; exopod 2-segmented with 2 medial simple setae on proximal segment and 5 terminal plumose setae on distal segment plus 1 subterminal simple seta; epipodite well development with 4 proximal plumodenticulate setae and 12 long setae on distal part.

Pereiopods (Fig. 9B-E): cheliped sparsely setose as shown, prominent curved spine on ischium; pereiopods 2-5 thin and setose, inner margin of dactyli with 3 stout spines and 2 shorter lateral spines, except dactylus of pereiopod 5 with no spines on inner margin and only one shorter lateral spine.

Sternum (Fig. 4B): maxilliped sternites completely fused with 8 setae, cheliped sternites with 3 setae each, sternal sutures are interrupted medially.

Pleon (Fig. 8C, H): six pleonites plus telson; setation as shown.

Pleopods (Figs 8C, 9F, G): biramous, present on pleonites 2-5; endopod with 3 cincinuli in all four pairs; exopod with 12-14 long plumose natatory setae; uropods uniramous with 1, 7 long plumose natatory setae on proximal and distal segment respectively.

Telson (Fig. 8C, H): rectangular, truncated with a pair of lateral setae, and posterior margin with 2 sparsely plumose setae, 2 pairs of dorsal setae, and 1 ventral pair.

DISCUSSION

There is a well-established population of *Dyspanopeus sayi* in the area of Alfacs Bay, Ebro Delta, NW Mediterranean, as evidenced by the collection of mature males and ovigerous females since 2005 (see Schubart *et al*. 2012; Guerao pers. obs.) and by the occurrence of larval stages in the plankton of the bay in September 2012. The presence of zoea I, zoea II and zoea IV in these samples from the inner part of the bay suggests that this species carries out its complete larval development in the bay, independently of the possibility of larval dispersal offshore. The scattered populations of this species in the Mediterranean compared with the continuous distribution long the French and Dutch coastline (see Fig. 1) is due to the low number and location of appropriate habitats in the Mediterranean, as there are no estuaries with a gradient of mixed salinities. The most similar habitats are lagoons, deltas, and closer bays, which are ecosystems with brackish waters; however, these habitats are not abundant in the Mediterranean basin. This scarcity of available habitats also explains the low number of populations of the other panopeid *Rhithropanopeus harrisii* introduced into the Mediterranean, in contrast with its wider distribution on the European Atlantic coasts (Projecto-García *et al*. 2010).

The zoeal stages collected in the Alfacs Bay plankton were measured and dissected. No significant differences were found in size, morphology or setation with respect to the cultured material. This is important information for examining morphological descriptions of larvae because meristic data obtained from specimens reared in the laboratory can be considered as valid, regardless of possible anomalies due to culture conditions (González-Gordillo and Rodríguez 2000, Wehrtmann and Albornoz 2003, Marco-Herrero *et al*. 2012).

According to previously published data, the duration of zoeal development of *D. sayi* ranges from 14 days at 21°C to 27 days at 14°C (Chamberlain 1957). Kurata (1970) reported complete zoeal development in a minimum of 15 days (April culture) and 14 days (May culture) but did not give data on temperature. Data obtained in the present study, 15 days at 18°C, fall within the range mentioned above and corroborate the well-known relationship between temperature and duration of decapod larval development (Anger 2001).

Previous descriptions of larval stages of *D. sayi,* except the zoea I described by Clark (2007) and Schubart *et al.* (2012), are incomplete, brief or inaccurate. Illustrations are also incomplete and in some cases are of low quality. The present study provides, for the first time, data on cephalothorax setation for zoea II-IV, in addition to information on the right setation pattern of mouthparts. Setation is described for the megalopa stage and illustrations of the sternum are provided for the first time, in addition to illustrations of the ischial spine of the cheliped, spinulation and the dactyli of the pereiopods. The following are some of the noteworthy differences: a fourth pair of inner serrulated setae was observed on the telson margin in zoea IV in the present study rather than in zoea III as reported by Chamberlain (1961); and the short "feeler" on the dactylus of pereio-

TABLE 1. – Main morphological and meristic differences between larval stages of *Dyspanopeus sayi*, *Panopeus africanus* and *Rhithropanopeus harrisii*. Abbreviations: dsl, cephalothorax dorsal spine length; rsl, cephalothorax rostral spine length; sp., spines; plp, posterolateral process; seg., number of segment; s., setation; sbls, subterminal long setae.

	Dyspanopeus sayi	*Panopeus africanus*	*Rhithropanopeus harrisii*
Reference	Present study	Rodríguez and Paula, 1993	Kurata (1970), Marco-Herrero *et al.* (submitted)
Zoeal stages			
dsl/rsl ratio	dsl < rsl	dsl ≥ rsl	2 dsl < rsl
Antennal protopod sp.	absent	present (ZI-III)	present (minute)
Antennal protopod tip	rounded	acute	acute
Pleonite 3 dorsolateral process	present	present	absent
Pleonite 5 plp / pleonite length	plp ≤ pleonite length	plp ≤ pleonite length	plp > 2 pleonite length
Telson lateral sp.	absent	2	absent
Megalopa stage			
Spines on frontal margin	present	present	absent
Antennal flagellum seg. (s.)	8(0,0,3,0,4,0,4,3)	8(0,0,2-4,0,3-4,0,4,3-4)	6(0,0,0,0,0,0)
Mandibular palp seg. (s.)	2 (0,8)	3 (0,0,8-9)	2 (0,5)
Cheliped ischial spine	present (curved)	present (curved)	sometimes (never curved)
Maxilliped sternite s.	8	6*	6
Cheliped sternite s.	3	3*	4-6
Pereiopods 2-5 sternites s.	0	0*	1-4
Pereiopod 5, dactylus sp/sbls	1/0	0/3	0/0
Uropods s.	1, 7	1, 7-8	0, 3-4

*Marco-Herrero *et al.* (unpublished)

pod 5 described by Kurata (1970) was not observed in the present study. Other differences in the setation pattern (fewer setae) could be due to miscounts.

Panopeidae in its present composition (see Ng *et al.* 2008) is a complex family with high heterogeneity between larval forms (Martin 1988). In some cases, this variability in larval morphology has been used as evidence to support taxonomic changes in the family, such as the recent establishment of the genus *Acantholobulus* Felder and Martin 2003, for some species of *Panopeus* H. Milne-Edwards, 1843. The larval stages of *D. sayi* fit in Group I of the classification of the Xanthid larvae by Martin (1984), which also includes *R. harrisii* and *Panopeus africanus;* however, it should be pointed out that other panopeid species have been placed in other groups of this classification based on larval morphology. This may suggest that this family needs to be studied further, taking into account other evidence based on adult morphology, and using techniques such as DNA analysis combined with larval morphology. Although they are all in Martin's Group I, the zoeal stages of the three Iberian panopeids show clear differences that allow easy identification. They also show differences in the megalopa stage, a larval phase which is not considered in the classification by Martin (1984). The main differences observed in the zoeal stages are observed in the ratio between the dorsal and rostral spine lengths, as well as in the antennal and pleonal morphology (see Table 1). Zoeal stages of *Panopeus africanus* have well-developed dorsal and rostral spines that are similar in size in zoea I; however, the dorsal spine is longer than the rostral spine in the other three zoeal stages. In *R. harrisii* and *D. sayi*, both cephalothoracic spines are well developed, but the rostral spine is longer than the dorsal spine, and in *R. harrisii* it is more than twice as long as the dorsal spine. The antenna of the zoeae of the three species are characterized by a reduced exopod, although it is less reduced in *Panopeus africanus*, which even has a fourth zoeal stage that shows two terminal setae instead of one, as in the rest of the zoeal stages and in the other two species. In all cases the protopod is as long as the rostral spine, but the tip is acute in *P. africanus* and *R. harrisii* and rounded in *D. sayi* with no spinulation. *Panopeus africanus*, however, has strong spines increasing in size towards the tip in zoea I and with fewer spinules in subsequent stages; in *R. harrisii* there are only minute spinules in the distal part. Differences were also observed in the pleon morphology, such as the number of dorsolateral processes, which were only present in pleonite 2 in *R. harrisii* and in pleonites 2 and 3 in *P. africanus* and *D. sayi*. This is one of the features characterizing Group I; therefore, *R. harrisii* must be considered an exception with respect to this character. Other differences in pleonal features are shown in Table 1.

The megalopa stage also shows clear differences between the three species (see Table 1). The rostrum is similar in *P. africanus* and *D. sayi*, although the spines at the basal angles, called "horns" in some papers, are more developed and acute in *P. africanus*. The megalopa of *R. harrisii* does not have these spines on the rostrum. The chelipeds are also similar in *P. africanus* and *D. sayi* but the strongly curved ischial spine is not present in *R. harrisii* (in some cases there is a small spine but it is never curved). The main difference in the megalopa stage is the number of segments of the antennal flagellum: there are eight in *P. africanus* and *D. sayi*, and six in *R. harrisii*. Other differences are seen in the mandibular palp, sternum, the dactyli of pereiopods, and uropods, and are shown in Table 1.

These larval differences allow larvae of the three panopeids inhabiting Iberian Peninsula waters to be accurately identified; however, the fact that species be-

longing to the same family may show large differences could raise doubts about whether they really belong to the same family. Future studies using molecular techniques in combination with larval morphology could shed light on the real phylogenetic relationships in this complex brachyuran family.

ACKNOWLEDGEMENTS

This study was funded by the Spanish "Ministerio de Ciencia e Innovación, Plan Nacional I+D" and the European FEDER funds through project MEGALOPADN (CGL2009-11225). The "Ministerio de Ciencia e Innovación" also supported a FPI PhD grant to EMH (BES-2010-033297); results here presented are part of her PhD thesis project. Financial support to GG (postdoctoral fellowship; INIA) was provided by the Ministry of Science and Research. The authors would like to thank Agustí Benito, a fisherman of Sant Carles de la Ràpita, for his collaboration in catching crabs. Paul Clark kindly shared his own unpublished description of the first zoeal stage of *Dyspanopeus sayi*. We are grateful to two anonymous referees for useful suggestions and corrections that clearly improved the manuscript.

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Scient. ed.: E. Macpherson.
Received January 27, 2013. Accepted February 28, 2013.
Published online April 15, 2013.

CAPÍTULO 4

Larval development of the pea crab *Afropinnotheres monodi* Manning, 1993 (Decapoda, Pinnotheridae) using plankton-collected and laboratory-reared specimens: Effects of temperature

E Marco-Herrero, P Drake, JI González-Gordillo, JA Cuesta (2015) Larval development of the pea crab *Afropinnotheres monodi* Manning, 1993 (Decapoda, Pinnotheridae) using plankton-collected and laboratory-reared specimens: Effects of temperature Marine Biology Research (Aceptado)

Larval development of the pea crab *Afropinnotheres monodi* Manning, 1993 (Decapoda, Pinnotheridae) using plankton-collected and laboratory-reared specimens: Effects of temperature.

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Running head: Larval development of *Afropinnotheres monodi*

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Abstract

The aim of this study was to assess the effect of temperature on the survival and duration of larval development in the African pea crab *Afropinnotheres monodi*, as well as to describe its larval stages. We studied larvae reared in the laboratory and also specimens collected from plankton of the Gulf of Cadiz at two different temperatures. According with results of this study, larval development of *A. monodi* involves four zoea stages and one megalopa, and lasts around 25 days at 25°C, and longer than 40 days at 19°C. Such temperature-related duration of this dispersive phase may be causing a higher recruitment of new individuals to parental populations during summer, but a higher dispersal to new locations during the rest of the year: a seasonal pattern of dispersion which could favour the successful expansion of this non-native species into European waters. The identification of the larval stages in plankton samples, and of adult specimens, was carried out using morphological characters and molecular techniques. Both the 16S mtDNA sequence of this species now available in GenBank and the larval descriptions provided by this study could help to establish an early alert for the detection of this African species on its northward expansion.

Keywords: Afropinnotheres monodi, *megalopa, morphology, Pinnotheridae, temperature effects, zoea, 16Smt DNA.*

Introduction

Pinnotheridae De Haan, 1833 is a brachyuran family comprised of small symbiotic crabs. Due to their small size and symbiotic life style little is known about their life history, reproductive traits, larval development and systematics (Becker & Türkay 2010; Palacios-Theil et al. 2009). Recent molecular studies have reduced the genera so far attributed to the subfamily Pinnotherinae *sensu stricto* De Haan, 1833 to only twenty-five and 152 species (Palacios-Theil et al. 2009). Known species of this subfamily are characterised by a typical trilobated telson in the zoea stages. There are only larval data for 8 of its genera *viz*: *Afropinnotheres* Manning, 1993, *Buergeres* Ng and Manning, 2003, *Gemotheres* Campos, 1996, *Nepinnotheres* Manning, 1993, *Orthotheres* Sakai, 1969, *Ostracotheres* H. Milne Edwards, 1853, *Pinnotheres* Bosc, 1802, and *Zaops* Rathbun, 1900.

Afropinnotheres monodi Manning, 1993 is an African pea crab that recently arrived onto southwestern European coasts, having been reported from several localities in the Gulf of Cadiz and south of Portugal (Subida et al. 2011). Until then, this small pinnotherid crab had a recorded distribution restricted to four localities, two in Morocco and two in Mauritania, but with no data on their hosts (Manning 1993). The populations inhabiting in the Gulf of Cádiz have now been studied, and data are available on their hosts as well as on the period of reproduction (Drake et al. 2014). *A monodi* species has a high number of bivalve hosts, and has been collected with varying degrees of prevalence, from *Scrobicularia plana* (da Costa, 1778), *Cerastoderma glaucum* (Bruguière, 1789), *Chamelea gallina* (Linnaeus, 1758), *Donax trunculus* Linnaeus, 1758, *Mactra stultorum* (Linnaeus, 1758), *Spisula solida* (Linnaeus, 1758), *Ruditapes decussates* (Linnaeus, 1758), and the mussel *Mytilus galloprovincialis* Lamarck, 1819, in which the ovigerous females reach a larger size and therefore with a higher number of eggs (Drake et al. 2014). In a similar way to other species originated from African, *A. monodi* reproduce throughout the year, although the lowest number of ovigerous female was observed in autumn and of zoea I in winter (Drake et al. 1998). This long reproductive period, together with its wide range of host species, offers a clear advantage for a successful establishment and expansion to new areas. However, the maximum distance that larvae can disperse tends to be temperature-dependent, in temperate areas, with a remarkable temperature seasonal pattern (Lindley 1990; Dickey-Collas et al. 2000; Pfeiffer-Hoyt & McManus 2005).

The aim of this study was to assess the effect of temperature on the survival, and on the duration of larval development for the species, as well as to describe the larval stages. This could help to determine how the number of larval stages and duration of larval development could contribute to the successful expansion of the species in

European waters. Knowledge of the larval morphology could allow for the identification of the various stages found in plankton samples, and to help differentiate them from larvae of other European pea crab species. Taking into account the difficulties in pinnotherid taxonomy, especially for non-specialists, a molecular marker could also help in the identification of the larval stages of *A. monodi*.

Material and Method

Plankton collected larvae

The estuary of the Guadalete River in the Bay of Cadiz (SW Spain) was sampled in various periods between the late spring of 2006 and the summer of 2012. Plankton net with a mesh size of 500 µm was deployed at a fixed point on the docks at the Marina of the Puerto Santa María, Cadiz, Spain (for more sampling details see Olaguer-Feliú et al. 2010). Samples were taken at intervals of 24 hours and transported to the laboratory where decapod larval stages were sorted and fixed in ethanol (90%), for posterior morphological and molecular studies.

Larval cultures

Two ovigerous females were recovered from inside the clam *Scrobicularia plana* in the Rio San Pedro inlet (36º31' N, 6º12' W), Bay of Cadiz (SW Spain) on 2 December 2011 and 8 May 2012. They were then placed in aquaria containing filtered and well-aerated sea water with a salinity of 32 ± 1‰ and kept at 19 ± 2ºC (December) and 25 ± 2ºC (May). A total of 311 and 417 zoeae hatched on 4 December 2011 and 17 May 2012, respectively. The 100 most actively swimming zoeae of each hatch were transferred individually to plates with 6 containers of 10 mL each, and the rest were placed in 2 L glass bottles with aeration for mass culture. Due to the small size of all larval stages, from zoea I to megalopa, they were fed ad libitum with the rotifer *Brachionus plicatilis* Müller, 1787 (fed with *Nannochlorosis gaditana* Lubián, 1982). All reared larvae were maintained under the same constant conditions of temperature and salinity mentioned above for the ovigerous females. Cultures were checked for exuviae and dead larvae. Exuviae and specimens of all stages were fixed in ethanol (90%) for later examination.

Material studied from plankton samples

A total of 62 unidentified zoeae (various stages between zoea I-IV) and 13 unidentified megalopae attributed to Pinnotheridae were collected during the period of study from the plankton of Guadalete River. Although several of these larvae were identified initially using the 16S mtDNA marker, measurements and dissections were also carried out for comparison with the larvae of *Afropinnotheres monodi* reared at the laboratory from ovigerous females.

Larval morphology and taxonomic account

Dissections, drawings and measurements were made using the same methodology as described in previous works by the present authors (for details see Marco-Herrero et al. 2012, 2014). The long setae on the distal exopod segments of the first and second maxillipeds were drawn truncated. Descriptions and figures were arranged according to the standards proposed by Clark et al. (1998). Parental females and series of all larval stages reared in the laboratory and plankton samples of *Afropinnotheres monodi*, were deposited at the ICMAN Decapod collection under numbers of accession ######## (pending).

In the taxonomic account the first zoeal stage is described in detail, and only the main differences in subsequent stages are also noted.

Molecular identification

The identification of plankton larval stages was based on partial sequences of the 16S mtDNA gene. DNA extraction from larvae, PCR and sequencing followed the same protocols used in previous works by the present authors (for more details see Marco-Herrero et al. 2013, 2014). The 16S mtDNA sequences obtained were compared with those from Iberian pinnotherid species deposited in Genbank: *Pinnotheres pisum* (Linnaeus, 1767), AM180694 and *Nepinnotheres pinnotheres* (Linnaeus, 1758), EU935001, and a new as yet unpublished sequence of *Afropinnotheres monodi* obtained in the present study. These new sequences obtained from larvae and adults of *A. monodi* were deposited in Genbank under accession numbers ######### (pending).

Data analysis

The effect of water temperature on the duration of each larval stage was ascertained by carrying out one-way ANOVA tests and Student-Newman-Keuls a posteriori tests. Prior to the statistical analyses data were logged transformed to homogenized variances. When data did not met ANOVA assumptions, statistical differences were assessed using non-parametrical Kruskall-Wallis ANOVA tests and box and whisker plots. Differences in mortality rates related to development stage and temperature were tested by using chi-squared test.

Results

Taxonomy

Class Malacostraca Latreille, 1802

Order Decapoda Latreille, 1802

Family Pinnotheridae De Haan, 1833

Genus *Afropinnotheres* Manning, 1993

Afropinnotheres Manning, 1993: 130. Type species:

Afropinnotheres monodi Manning, 1993

Afropinnotheres monodi Manning, 1993

Zoea I (Figures 3A, a, 4A-C, 5A-D, 6A, B), Zoea II (Figures 3B; 6C), Zoea III (Figures 3C; 6D),

Megalopa (Figures 3E-G; 4G-I; 5H-L; 6F-J)

Material examined

Zoea I Size: RDL =1.265 ± 0.03 mm; CL = 0.567 ± 0.017 mm; CW = 0.786 ± 0.019 mm; RL = 0.411 ± 0.026 mm; DL = 0.397± 0.029 mm; N=10.

Zoea II Size: RDL = 1.733 ± 0.17 mm; CL = 0.630 ± 0.013 mm; CW = 1.069 ± 0.015 mm; RL = 0.592 ± 0.079 mm; DL = 0.613 ± 0.056 mm, N=10.

Zoea III Size: RDL = 2.122 ± 0.175 mm; CL = 0.831 ± 0.052 mm; CW = 756.5 ± 35 mm; RL = 0.709 ± 0.063 mm ; DL = 0.757 ± 0.090 mm, N=10.

Zoea IV Size: RDL = 2.437 ± 0.149 mm; CL = 0.917 ± 0.037 mm; CW = 1.380 ± 0.103 mm; RL = 0.797 ± 0.092 mm; DL = 0.862 ± 0.034 mm, N=10.

Megalopa Size: CL = 0.6489 ± 0.022 mm; CW = 0.553 ± 0.025 mm; N= 10.

Description

Zoea I

Cephalothorax (Figure 3A, a): Dorsal and rostral spines straigth and well developed. Lateral spines long and down directed in the typical position in Pinnotheridae, close to posterior angle of ventral margin. One pair of posterodorsal and 3 pairs of anteromedian simple setae. Posterior and ventral margin without setae. Eyes sessile.

Antennule (Figure 4A): Biramous, unsegmented and conical. Endopod absent. Exopod with 3 terminal aesthetascs (2 long, 1 short), without setae.

Antenna (Figure 4B): Protopod process present as a minute simple seta. Endopod present as small bud. Exopod absent.

Mandible (Figure 4C): Well developed, incisor and molar process developed. Palp absent.

Maxillule (Figure 5A): Coxal endite with 5 plumodenticulate setae. Basial endite with 7 terminal setae (5 terminals cuspidate, 2 subterminal plumodenticulate). Endopod 2-segmented, proximal segment without setae, and with 4 terminal (2+2) sparsely plumose setae on distal segment. Epipod and exopod setae absent.

Maxilla (Figure 5B): Coxal endite single lobed, with 5-6plumodenticulate setae. Basial endite bilobed, with 4+5 plumodenticulate setae. Unsegmented endopod bilobed, 1 long plumodenticulate seta on proximal lobe, and 2 long plumodenticulate setae on distal lobe. Exopod (scaphognathite) with 4 plumose marginal setae plus one stout plumose process.

First maxilliped (Figure 5C): Coxa with 1 sparsely plumose seta. Basis with 10 medial sparsely plumodenticulate setae arranged as 2+2+3+3. Endopod 5-segmented with 2,2,1,2,5 (1 subterminal + 4 terminal) sparsely plumodenticulate setae. Exopod unsegmented, with 4 terminal plumose natatory setae.

Second maxilliped (Figure 5D): Coxa without setae. Basis with 4 sparsely plumodenticulate setae arranged 1+1+1+1. Endopod 2-segmented with 0, 1 subterminal serrulate + 4 terminal (2 long plumodenticulate, 2 short, 1 plumodenticulate and 1 simple) setae. Exopod unsegmented with 4 terminal plumose natatory setae.

Third maxilliped: Absent.

Pleon (Figures 3A; 6A): 5 pleonites. Pleonite 1 without setae. Pleonites 2-5 with a pair of minute simple setae on posterodorsal margin. Pleonite 2 with pair of forwardly directed dorsolateral processes and pleonite 3 with smaller dorsolateral processes laterally directed.

Pleopods: Absent.

Telson (Figures 6A,B): Trilobed with 2 pairs of 3 serrulate setae on posterior margin, inner setae longest; each lateral lobes covered with spinules distally.

Zoea II

Cephalothorax (Figure 3B): Eyes stalked and movable.

Antennule: Exopod with 4 terminal aesthetascs plus one small seta.

Antenna: Protopod process (seta) reduced in size.

Maxillule: Exopodal seta present.

Maxilla: Scaphognathite with 8 plumose marginal setae.

First maxilliped: Exopod with 6 terminal plumose natatory setae.

Second maxilliped: Exopod with 6 terminal plumose natatory setae.

Pleon (Figure 6C): Pleonite 1 with one mid-dorsal seta.

Zoea III

Cephalothorax (Figure 3C): Ventral margin with 1 highly plumose and 3 sparsely setose setae.

Antennule: Exopod with 6 aesthetascs (4 terminal and 2 subterminal).

Antenna: Protopod process absent. Endopod enlarged.

Maxillule: Coxal endite with 8 terminal plumodenticulate setae. Basial endite with 10 setae (3 subterminal plumodenticulate, 6 terminal cuspidate and 1 proximal plumose seta).

Maxilla: Basial endite with 6+5 plumodenticulate setae. Scaphognathite with 14-15 plumose marginal setae.

First maxilliped: Exopod with 8 terminal plumose natatory setae.

Second maxilliped: Exopod with 7-8 terminal plumose natatory setae.

Third maxilliped: Present as undifferentiated buds.

Pereiopods: All present as buds, slightly segmented, first pair chelate.

Pleon (Figure 6D): Pleonite 1 with 3 mid-dorsal setae.

Pleopods: Present on pleonites 2-5 as small buds, endopods absent.

Zoea IV

Cephalothorax (Figure 3D): Ventral margin with 1 highly plumose and 4 sparsely setose setae.

Antennule (Figure 4D): Endopod bud present. Exopod with 7 aesthetascs (2+2 subterminal and 3 terminal).

Antenna (Figure 4E): Endopod more elongated.

Mandible (Figure 4F): Palp present as unsegmented bud without setae.

Maxillule (Figure 5E): Coxal endite with 6-7plumodenticulate setae. Basial endite with 10-11 setae (4 subterminal plumodenticulate, 6 terminals cuspidate, 1 proximal plumose seta). Exopod and epipod setae present.

Maxilla (Figure 5F): Coxal endite with 9plumodenticulate setae. Basial endite with 6+6 plumodenticulate setae. Scaphognathite with 18-19 plumose marginal setae.

First maxilliped: Coxa with 2 sparsely plumose setae. Exopod with 9 terminal plumose natatory setae.

Second maxilliped: Exopod with 9 terminal plumose natatory setae.

Third maxilliped (Figure 5G): Biramous. Endopod and exopod present as slightly segmented buds, without setae. Epipod bud present.

Pereiopods: Cheliped and pereiopods slightly segmented without setae.

Pleon (Figure 6E): Pleonite I with 4 mid-dorsal setae.

Pleopods (Figure 6E): Biramous buds more elongated with endopod present.

Megalopa

Cephalothorax (Figures 3E, F): Slightly longer than broad. Rostrum small, ventrally deflected (approximately 70º), with median longitudinal depression. Protogastric, cardiac and mid-posterior region with tubercles. Eyes stalked.

Antennule (Figure 4G): Peduncle 3-segmented, with 6 (5 plumodenticulate, 1 simple), 2 simple, 1 simple setae, respectively. Endopod unsegmented, proximal with 1subterminal and 3 terminal simple setae. Exopod 4-segmented, with 0,0,5,5-6 aesthetascs and 0,0,1,0 setae respectively.

Antenna (Figure 4H): Peduncle 3-segmented without setae and flagellum 3-segmented with 0, 3 (2 long sparsely setose, 1 long simple) and 2 (1 long sparsely setose, 1 simple) setae, respectively.

Mandible (Figure 4I): Palp 2-segmented with 2 terminal simple setae on distal segment.

Maxillule (Figure 5H): Coxal endite with 13 plumose setae. Basial endite with 2 simple setae on lower margin, 6 subterminal plumodenticulate setae and 6 terminal cuspidate setae. Endopod unsegmented with 1 terminal seta. Exopod seta reduced to simple setae.

Maxilla (Figure 5I): Coxal endite bilobed with 9+5 terminal plumose setae. Basial endite bilobed with 5+8plumodenticulate setae. Endopod unsegmented without setae. Scaphognathite with 29-33 marginal plumose setae plus 2 small simple setae, one on each lateral surface.

First maxilliped (Figure 5J): Coxal endite with 5 plumodenticulate setae. Basial endite with 3-4 plumodenticulate terminal setae. Endopod unsegmented with 3 simple setae. Exopod 2-segmented with 2 terminal plumodenticulate setae on proximal segment, and 4 plumose setae on distal segment.

Second maxilliped (Figure 6K): Protopod without setae. Epipodite of triangular shape with 2 terminal long seta. Endopod 4-segmented with 0, 1 long sparsely setose, 5 (4 plumodenticulate, 1 simple), 3 plumodenticulate setae, respectively, dactylus inserted subterminally on propodus. Exopod 2-segmented with 1 medial and 1 subterminal simple setae on proximal segment and 4 terminal plumose setae on distal segment.

Third maxilliped (Figure 5L): Protopod with 9 plumodenticulate setae. Epipodite well development with 19 proximal plumodenticulate and 10 long terminal setae. Endopod 4-segmented, ischium and merus fused with 1 simple basal setae and 4 marginal plumodenticulate and 2 medial simple and 2 terminal simple setae, carpus with 4 (3 plumodenticulate, 1 simple) terminal and 1 medial simple setae, propodus with 4 (3 terminal, 1 subterminal) plumodenticulate setae, dactylus with 3 (2 terminal, 1 subterminal) plumodenticulate setae. Exopod 2-segmented, proximal segment without setae and 3 terminal plumose setae on distal segment.

Pereiopods (Figures 6F, G): All segments well differentiated. Cheliped sparsely setose as shown. Pereiopods 2-5 thin and setose.

Sternum (Figure 3G): Maxillipeds and cheliped sternites fused with 6 simple setae. Sternites of pereiopods 2-5 with 2,1,1,1 simple setae respectively.

Pleon (Figure 6H): Six pleonites, setation as shown.

Pleopods (Figures 6I, J): Biramous, present on pleonites 2-5. Endopod of pleonites 2-4 with 3 cincinuli and exopod with 8 long terminal plumose natatory setae. Endopod of pleonite 5 with 2 cincinuli and exopod with 6 long terminal plumose natatory setae. Uropods absent.

Telson (Figure 6H): Rounded with 2 pairs of simple setae on terminal margin.

Molecular study

The 16S mtDNA sequences obtained from adult and larvae of *Afropinnotheres monodi* consist of 552 bp (excluding the primers). All sequences fit 100%, and only one haplotype is shared for all specimens analyzed. Pairwise genetic distances between *A. monodi* and the other Iberian pinnotherids species, *Pinnotheres pisum*, (Genbank AM180694, 553 bp) and *Nepinnotheres pinnotheres* (Genbank: EU935001, 550 bp), indicate stronger differences with *P. pisum* (0.991) than with *N. pinnotheres* (0.056). Furthermore, studying mutation rate of these sequences respect to that of *Afropinnotheres monodi*, 50 mutations (9.05% divergence rate) are observed in *P. pisum* and 30 mutations (5.45% divergence rate) in *N. pinnotheres*. These clear differences allow accurate identification of the three species based on this molecular marker.

Effects of temperature on the larval development

As observed both in specimens recovered from the field (natural plankton) and those in laboratory cultures, the larval development of *A. monodi* consists of four zoea stages and one megalopa.

In reared larvae, the duration of each zoea development stage, and its temporal pattern of mortality, varied depending on the temperature (Figure 1). The time from larval hatching to the megalopa stage was around 25 days at 25°C, and longer than 40 days at 19°C; concurrently, 25% of zoeae reared at 25°C and 19°C was still alive after 18 and 33 days post-hatching, respectively.

Mean (±SE) duration of each zoea stage fluctuated between: 5.68 ± 0.16 days (d) at 25ºC and 8.20 ± 0.09 d at 19ºC for Zoea I; 4.83 ± 0.16 d at 25ºC and 9.46 ± 0.42 d at 19ºC for Zoea II; 6.14 ± 0.55 d at 25ºC and 16.88 ± 0.35 d at 19ºC for Zoea III; and 8.50 ± 0.70 d at 25ºC for Zoea IV (Figure 2). For each zoea stage, the development duration was significantly shorter for larvae reared at 25°C than for those reared at 19°C ($P < 0.05$). No significant differences in development duration were observed between Zoea I, II and III reared at 25°C, whereas larvae spent a significantly longer period as Zoea IV at this temperature; similarly, there was no significant difference between the development duration of Zoea I and II stages of larvae reared at 19°C, whereas they spent a longer period as Zoea III ($P < 0.05$).

The mean mortality rate of each zoea stage was always lower for larvae reared at 25°C than at 19°C (Table I), although such differences were not statistically significant ($P > 0.05$). An increased pattern of mortality rates was observed from early to older zoea stages at both tested temperatures. Namely, differences in mortality among different zoea stages at each temperature were statistically significant ($P < 0.01$), except between Zoea I and II and between Zoea III and IV reared at 19and 25°C, respectively ($P > 0.05$). In addition, there was a higher mortality ($P < 0.05$) between slower growing specimens (development duration above the mean) for Zoea I at both temperatures, and for Zoea II and III at 25°C (Table I).

Morphological comparison of plankton and reared larvae

A total 25 zoeae of various stages, and 13 megalopae recovered from the plankton were identified by comparison of their 16S mtDNA sequences, and once morphological characters were established the rest of zoeae (37 specimens) were identified by morphology. All sequences obtained fit 100% with the 16S mtDNA sequence of adult specimens of Afropinnotheres monodi, and any others corresponded to other native pinnotherids (*Pinnotheres pisum* and *Nepinnotheres pinnotheres*). All these specimens were used for size measurements and for morphological

comparison. No differences in size or morphology (including setation patterns) were found between plankton and laboratory reared larvae, for this reason larvae from both origins were used in the descriptions.

Discussion

Dispersal capability of *A. monodi*

Among the few complete larval development records known for Pinnotherinae *s.s.* the number of larval stages varied from between 2 to 4, although 3 was the most frequent (Palacios-Theil et al. 2009). *Afropinnotheres monodi* with 4 zoea stages may be considered to have an extended development, which represents an advantage for dispersal success. In fact, although all zoea stages of *A. monodi* have been collected in estuarine waters, pointing out to a possible retention mechanism, the species has been also found in bivalves that inhabit breakwaters and intertidal rock shores (Drake et al. 2014), suggesting a partial larval exportation out of the most sheltered estuarine habitats.

The global duration of the planktonic phase also plays a relevant role in species dispersal. The duration of the larval development of decapod crustaceans is greatly dependent on temperature (Dawirs 1979; Lárez et al. 2000; Anger et al. 2003; Barría et al. 2005). Indeed, a 6°C decrease in the average temperature of larval culture of the African pea crab *A. monodi* seems to double the time spent by individuals in its most dispersive zoea phase (Figure 1). In the studied area, coastal seawater temperatures follow a clear seasonal pattern, with monthly mean temperatures between ≈ 15 ± 1°C in January-February and ≈ 25 ± 1°C in July-August (Navarro & Ruiz 2006; García-Lafuente et al. 2012); similarly, the lowest chlorophyll a concentrations are observed in winter, whereas the chlorophyll a maximum appears in spring followed by a second bloom either in summer or fall (Establier et al. 1990; Navarro & Ruiz 2006). Accordingly, although larval stages and ovigerous females of *A. monodi* were found all the year round in the Bay of Cadiz, an autumnal decrease in the reproductive activity has been observed (Drake et al. 1998, 2014). The temperatures used in the larval cultures in this study corresponded to both extremes of the temperature range during the period of maximal larval density of *A. monodi* in the field. Thus, as suggested for other decapod crustaceans from temperate areas (Dawirs 1979; Lindley 1990), we hypothesise that the duration of the planktonic phase of *A. monodi* in the studied area is modulated by temperature and, consequently, its dispersal capacity follows a seasonal pattern. Thus a higher recruitment of new individuals to parental populations would be expected during the warmest summer, and a higher dispersal to new locations during the rest of the year.

Food availability and suitability are other factors affecting the duration of larval development (Anger 2001; D'Urban Jackson et al. 2014). *Artemia* nauplii have been successfully used as larval food for some brachyurans (Dawirs 1979; Anger 1983, 1991; Gonçalves et al. 1995; Barría et al. 2005). Since the small size of *A. monodi* larvae did not permit the use of *Artemia* nauplii as food, they were fed instead with rotifers pre-fed with algae. At the tested temperatures, the high global larval mortality and the higher mortality rate observed for larvae with slower developing growth (unhealthy larvae) may indicate that rotifers and algae were not the most suitable food for rearing pea crab larvae. However, algae and concentrated plankton were successfully used to feed larvae of other pinnotherid species (Sandoz & Hopkins 1947; Atkins 1955). Furthermore, zoea mortality rates shown in this study were lower than those derived from available information on *A. monodi* zoea abundance in the studied area: zoea IV represented 0.2% and 1.1% of *A. monodi* zoea collected when the mean water temperature was 24.9ºC (July) and 19.3ºC (October), respectively (derived from data published in Drake et al. 1998). Mortality could be selectively removing the less fit individuals of the population in the field, and as a result larval duration of survivors could be slightly shorter than that estimated under culture conditions (Dickey-Collas et al. 2000).

Pinnotherids parasitize commercially exploited bivalves (Silas & Alagarswami1967; Sun et al. 2006; Mena et al. 2014), with a significant loss of production detected in some shellfish farms (Trottier et al. 2012). As males and females of *Afropinnotheres monodi* display an asymmetrical use of different bivalve hosts, the strongest infestation by this pea crab was expected to be found in shellfish exploitations located where their various hosts used coexist; that is, in sheltered waters as bays, inlets, rías and harbours (Drake et al. 2014). Furthermore, as this is an African species that seems to be in a clear northward expansion, under the current scenario of increasing temperature, it could represent a threat to European bivalve aquaculture in the near future.

Larval morphology

The larval morphology of *A. monodi* is, in general terms, similar to that of other Pinnotherinae *s.s.* However, the setation pattern of the sternum of the megalopa is a feature never described before for the Pinnotherinae *s.s.*; therefore, comparison with other species was not possible. Since this is a useful character for intrageneric comparison (Marco-Herrero et al. 2012) it has been described herein. Another interesting feature is the setation of the ventral margin of the cephalothorax, and *A. monodi* has 4 and 5 setae in Zoea III and IV, respectively. This setation has only been described in zoeae of three other Pinnotherinae (*Pinnotheres pisum*, *Viridotheres gracilis* (Bürger, 1895) and *Nepinnotheres pinnotheres*), and in all these cases marginal setae appear in Zoea II and the subsequent

stages. In *A. monodi* the first seta is highly plumose, similar to the "anterior" seta previously described like that typical of majids zoeae (Clark et al. 1998).

A first zoea stage from plankton samples of Selvagens Islands was tentatively attributed to *A. monodi* by Lindley et al. (2002). The brief description, the position of lateral spines of the cephalothorax, the bifurcated telson, and the general illustration indicates clearly that this larva does not belong to *Afropinnotheres monodi*, and not even to any other Pinnotheridae.

The description of the first zoea stage of *Afropinnotheres larissae* Machkevsky, 1992 by Machkevsky (1999) is brief and incomplete. Thus, differences in setation pattern with respect to the first zoea of *A. monodi* should be attributed to mistakes or overlooked setae, rather than to real intrageneric differences. The general morphology clearly resembles that of *A. monodi*, although the dorsal, rostral and lateral spines are shorter and the ratio between dorsal and rostral spine lengths is smaller.

Larval development of the two native species of Iberian waters, *Nepinnotheres pinnotheres* and *Pinnotheres pisum*, was described by Atkins (1955). The zoea stages of *N. pinnotheres* (as *Pinnotheres veterum*), and *P. pisum* were also previously briefly described by Lebour (1928), and the first zoea of *P. pisum* latter by Rice (1975). The 4 zoea stages of *A. monodi* are easily distinguished from those of *P. pisum* by the presence of a well-developed dorsal spine on the cephalothorax of the former species (see Table II). Although *N. pinnotheres* has well-developed dorsal, rostral and lateral spines on the cephalothorax, as is in *A. monodi*, *N. pinnotheres* has only 2 zoea stages. Therefore *N. pinnotheres* zoeae I and II show characters of more advanced stages like periopods, and pleopods buds on the Zoea I, and mandibular palp in the Zoea II.

Megalopae of *N. pinnotheres* can be easily differentiated from those of *A. monodi* and *P. pisum* by having an antennal flagellum with only 2 segments instead of 3 in the other two species (see Table II). Megalopae of *P. pisum* can be distinguished from those of *A. monodi* by: the second segment of antennal flagellum of *P. pisum* without setae, whereas those of *A. monodi* present 3 long setae; the antennular flagellum is unsegmented in *P. pisum* and 4-segmented in *A. monodi*; pleon of *P. pisum* presents only 5 pleonites and that of *A. monodi* 6 pleonites.

Conclusion

As the dispersal capability of *A. monodi* seems to be modulated by temperature, we hypothesise that higher recruitment of new individuals to parental populations should occur during the warmest summer, while higher dispersal to new locations should take place during the rest of the year. This temperature-related feature could

facilitate a faster northward expansion of *A. monodi*, and consequently, the infestation of European bivalve aquaculture installations by this pea crab in the near future. Thus, the information provided on the morphology of larval stages and on the genetic marker may be of use in establishing an early alert for detection of this African species in more northern European locations. Nevertheless, a long-term monitoring of seasonal larval abundance pattern in the area is needed to confirm the proposed hypothesis.

Acknowledgements

We want to thank the "Real Club Nautico" of the Puerto Santa María (Cádiz, Spain) for kindly allowing us to use their facilities. We are especially grateful to Reyes Sánchez García and Ainhoa Olaguer-Feliú who helped in collecting the larvae and Carlos Sánchez Nieto for his assistance in the laboratory work. Thanks are due to Translations group and especially to Alicia Moguilevsky by revision and correction of English. Three anonymous reviewers, the subject editor Matz Berggren and the editor Franz Uiblein provided useful comments and corrections that clearly improved the manuscript.

Funding

The research was carried out within the framework of the projects MEGALOPADN (CGL2009-11225), "Transporte y reclutamiento larvario de crustáceos bentónicos litorales: importancia de los agentes forzadores costeros y regimen mareal" (CTM2005-00024/MAR) and AFROBIV (CGL2014-53557-P), co-financed by the "Ministerio de Economía y Competividad (MINECO)" Spanish Plan R+D+I and ERDF, European Union. Elena Marco-Herrero acknowledges the pre-doctoral FPI Fellowship supported by MINECO (BES-2010-033297).

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Tables and figures

Table I. Mean mortality (%) of different larval stages at 19 and 25°C, as well as mortality for larvae with rapid (RD, below mean stage duration) and slow (SD, above mean stage duration) development. Remarked in bold: zoeal mean mortalities that were significantly higher (Chi-squared test, $P < 0.05$) at 19 °C than at 25 °C; and SD mortality that were significantly higher than the corresponding SD.

	19 °C			25 °C		
	Mean	RD	SD	Mean	RD	SD
Z I	**33.00**	20.97	**52.63**	21.00	0.00	**51.22**
Z II	**55.00**	50.00	58.82	41.8	33.33	**51.35**
Z III	**78.4**	86.36	66.67	69.6	43.75	**83.33**
Z IV	md	md	md	85.7	88.89	80.0

Table II. Main meristic and morphological differences between zoeal and megalopa stages of *Afropinnotheres monodi*, *Nepinnotheres pinnotheres* and *Pinnotheres pisum*. Abbreviations: DS, dorsal spine; (-), absent; (+), present; F, flagellum; seg., number of segments.

	Afropinnotheres monodi	***Nepinnotheres pinnotheres***	***Pinnotheres pisum***
Reference	Present study	Atkins (1955)	Atkins (1955)
No. Zoeal stages	4	2	4
Zoea			
Cephalothoracic DS	(+)	(+)	(-)
Z I pereiopods	(-)	buds	(-)
Z I pleopods	(-)	buds	(-)
Z II pereiopods	(-)	elongated buds	(-)
Z II pleopods	(-)	elongated buds	(-)
Megalopa			
Antennular F seg.	4	4	1
Antennal F seg.	3	2	3
No. pleonites	6	6	5

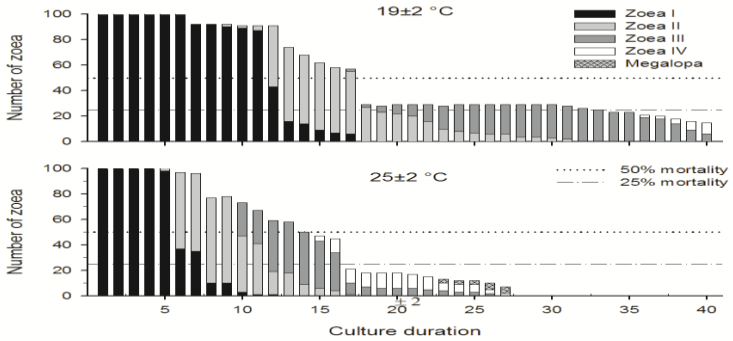

Figure 1. Temporal pattern of larval development stages of *Afropinnotheres monodi* Manning, 1993 reared at 19 and 25 °C.

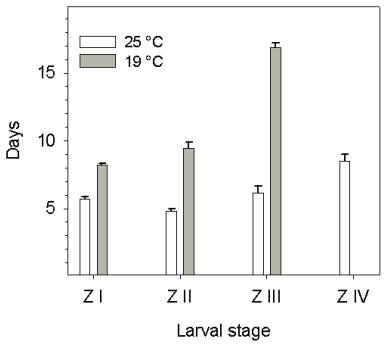

Figure 2. Mean duration of different zoeal stages of *Afropinnotheres monodi* Manning, 1993 reared at 19 and 25 °C.

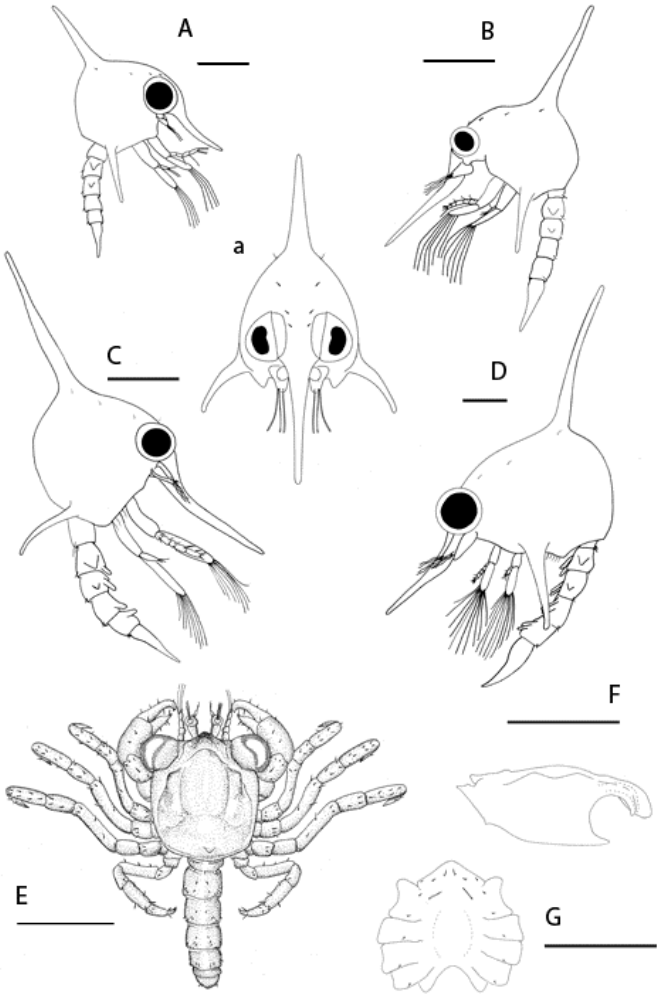

Figure 3. *Afropinnotheres monodi* Manning, 1993. Lateral view, A: Zoea I, B: Zoea II, C: Zoea III, D: Zoea IV, a: Detail of the frontal view of rostrum of zoea I. Megalopa, E: Dorsal view, F: Lateral view, G: Sternum. Scale bars = 0.5 mm.

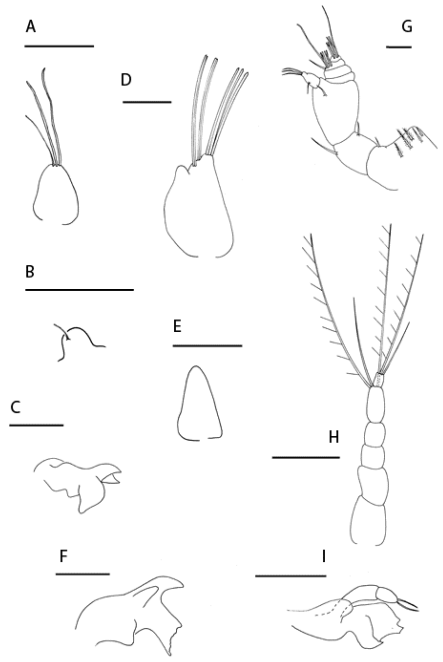

Figure 4. *Afropinnotheres monodi* Manning, 1993. Antennule, A: Zoea I, D: Zoea IV, G: Megalopa. Mandible, C: Zoea I, F: Zoea IV, I: Megalopa. Antenna, B: Zoea I, E: Zoea IV, H: Megalopa. Scale bars = 0.1 mm.

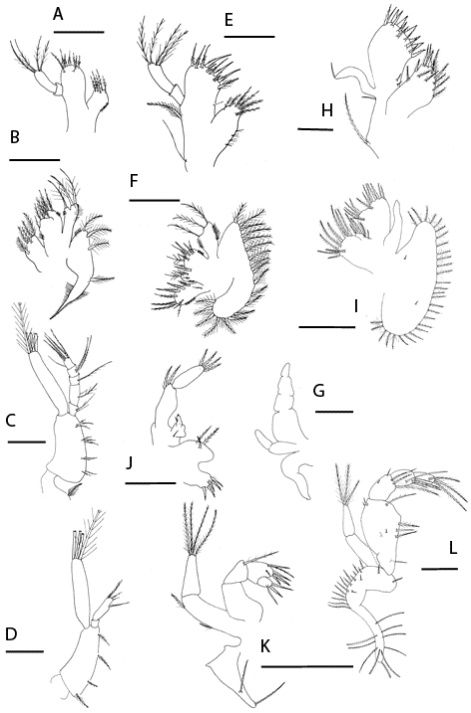

Figure 5. *Afropinnotheres monodi* Manning, 1993. Maxillule, A: Zoea I, E: Zoea II, H: Megalopa. Maxilla, B: Zoea I, F: Zoea IV, I: Megalopa. First maxilliped, C: Zoea I, J: Megalopa. Second maxilliped, D: Zoea I, K: Megalopa. Third maxilliped; G: Zoea IV, L: Megalopa. Scale bars = 0.1 mm.

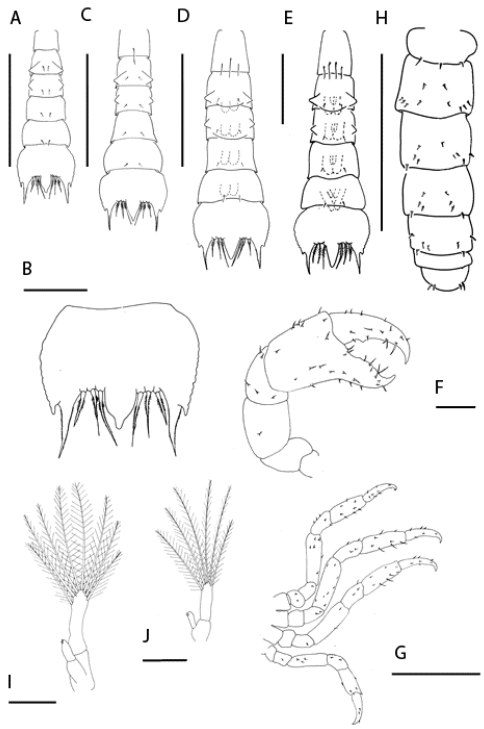

Figure 6. *Afropinnotheres monodi* Manning, 1993. Abdomen, dorsal view, A: Zoea I, C: Zoea II, D: Zoea III, E: Zoea IV, H: megalopa. Telson, B: Zoea I. Megalopa, F: Cheliped, G: Pereiopods, I: Pleopods I-III, J: Last pleopod. Scale bars = 0.5 mm (A, C-E, G, H), Scale bars = 0.1 mm (B, F, I, J).

SECCIÓN III

“CÓDIGO DE BARRAS” DE ADN COMO HERRAMIENTA PARA LA IDENTIFICACIÓN DE LARVAS COLECTADAS EN EL PLANCTON Y ESTUDIOS DE SISTEMÁTICA MOLECULAR

CAPÍTULO 5

Morphology of the megalopa of the mud crab, *Rhithropanopeus harrisii* (Gould, 1841) (Decapoda, Brachyura, Panopeidae), identified by DNA barcode

E Marco-Herrero, JI González-Gordillo, JA Cuesta (2014) Morphology of the megalopa of the mud crab, *Rhithropanopeus harrisii* (Gould, 1841) (Decapoda, Brachyura, Panopeidae), identified by DNA barcode Helgoland Marine Research 68(2): 201-208

Helgol Mar Res (2014) 68:201–208
DOI 10.1007/s10152-014-0381-8

ORIGINAL ARTICLE

Morphology of the megalopa of the mud crab, *Rhithropanopeus harrisii* (Gould, 1841) (Decapoda, Brachyura, Panopeidae), identified by DNA barcode

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Received: 13 June 2013 / Revised: 10 January 2014 / Accepted: 16 January 2014 / Published online: 28 January 2014

Abstract The morphology of the megalopa stage of the panopeid *Rhithropanopeus harrisii* is redescribed and illustrated in detail from plankton specimens identified by DNA barcode (16S mtDNA) as previous descriptions do not meet the current standard of brachyuran larval description. Several morphological characters vary widely from those of other panopeid species which could cast some doubt on the species' placement in the same family. Besides, some anomalous megalopae of *R. harrisii* were found among specimens reared at the laboratory from zoeae collected in the plankton. These anomalous morphological features are discussed in terms of problems associated with laboratory rearing conditions.

Keywords *Rhithropanopeus harrisii* · Panopeidae · Megalopa · Barcode · 16S · Morphology · Anomalies

Introduction

Currently, three species of Panopeidae are known for the Iberian Peninsula, *Panopeus africanus* (A. Milne Edwards, 1867), *Dyspanopeus sayi* (Smith, 1869) and *Rhithropanopeus harrisii* (Gould, 1841). While *P. africanus* is an Iberian native species distributed from the Gulf of Cadiz (SW Spain) to the Mondego estuary (NW Portugal), the other two panopeids are introduced species. These are among the most widespread introduced brachyuran species in the world. *D. sayi* is native to the Atlantic coast of North America from Florida to Canada (Nizinski 2003) and has been introduced to coastal areas of southwest England, Queens Dock, Swansea (Wales) (Ingle 1980; Clark 1986), to the French and Dutch coasts of the North Sea (Vaz et al. 2007), the Black Sea (Micu et al. 2010), and more recently to the Mediterranean Sea, Venice, the Marano and Varano lagoons, the Po River Delta (western Adriatic Sea) (Froglia and Speranza 1993; Mizzan 1995; Florio et al. 2008) and to the east coast of the Iberian Peninsula (Schubart et al. 2012).The first report of a population of *R. harrisii* for the Iberian Peninsula was made by Cuesta et al. (1991) for the Guadalquivir estuary, but populations are present in many European Atlantic estuaries, as well as in some Mediterranean locations. The species has been extensively studied from several perspectives such as ecology, phylogeography and larval biology (Gonçalves et al. 1995; Forward 2009; Projecto-Garcia et al. 2010).

Rhithropanopeus harrisii is a euryhaline crab typically associated with sheltered estuarine habitats. Connolly (1925) described its four zoeal stages and the megalopa, based on larvae reared from eggs in the laboratory. Further descriptions were provided by Hood (1962) and Chamberlain (1962), but the best illustrations of the larval stages are shown in Costlow and Bookhout (1971) (as underlined by Forward 2009). Nevertheless, all descriptions are incomplete compared to the current standard of brachyuran larval descriptions proposed by Clark et al. (1998).

Communicated by H.-D. Franke.

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Traditionally, descriptions of larvae have been accomplished from specimens cultivated in the laboratory under controlled conditions (temperature, salinity, density and absence of predators), and the specimens commonly originated from a single or sometimes from two ovigerous females. These circumstances may contribute to conceal the morphological variability of larvae that can be found in the field, a phenomenon already discussed in the literature for brachyuran larvae (Cuesta et al. 2002).

The use of molecular markers has demonstrated to be a powerful tool in providing accurate identifications for plankton specimens (Pan et al. 2008; Pardo et al. 2009; Ampuero et al. 2010; Marco-Herrero et al. 2013a). The identification of megalopae has traditionally been based on morphological characteristics, but sometimes, it is impossible to get an accurate identification with this approach. In the present study, we used partial sequences of the mitochondrial gene 16S as DNA barcode to identify the megalopae collected in the plankton. The 16S marker has proven to be an effective tool in studies of decapod crustaceans (Schubart et al. 2000; Porter et al. 2005; Ahyong et al. 2007), not only for the establishment of new species, but also to elucidate the taxonomic validity of closely related species (Schubart et al. 1998, 2001; Spivak and Schubart 2003).

In contrast to traditional descriptions, the megalopae of the present study were obtained from the plankton and identified by DNA barcode. Furthermore, in order to provide a definite morphological description of the megalopa stage of *R. harrisii*, comparisons were made not only with previous descriptions, but also with another set of megalopae which were reared in the laboratory from four zoeae I collected in the plankton.

Materials and methods

Collection of the megalopae

Twenty-eight megalopae of *R. harrisii* were collected in July 2007 and four zoeae I in April 2011, all from the plankton of the Guadalete estuary (Cádiz-SW Spain) (36°35'24.09″N 6°13′46.19″W).

Rearing and description of the megalopae

All megalopae collected were preserved directly in 80 % ethanol. The four zoeae I were placed in beakers containing filtered and well-aerated sea water at a salinity of 32 ± 1 ‰ and a temperature of 26 ± 1 °C. The larvae were fed with the rotifer *Brachionus plicatilis* (fed with *Nannochloropsis gaditana*). Rearing was finished when all zoeae had molted to the megalopa instar. Megalopa descriptions were based on 10 specimens identified by DNA barcode.

To facilitate the microscopical observation of larvae structures, a digestion-stain procedure was carried out. Firstly, entire specimens were placed for about 10 min in a watch glass with 2 ml of heated lactic acid. Immediately afterward, three drops of Clorazol Black stain (0.4 g Clorazol Black powder dissolved in 75 ml 70 % EtOH) were added to the heated solution. After 5–10 min, the specimen was removed from the solution and placed on a slide with lactic acid in order to proceed with the dissection of the appendages (Landeira et al. 2009).

Drawings and measurements were made using a Leica MZ6 and Zeiss Axioskop compound microscope with Nomarski interference, both equipped with a *camera lucida*. All measurements were made by an ocular micrometer. The measurements taken were cephalothorax length (CL) as the distance from the tip of the rostrum to the posterior margin of the cephalothorax and cephalothorax width (CW) as the maximum width of the cephalothorax. Two megalopae identified by DNA barcode were deposited at the Biological Collections of Reference of the Institut de Ciències del Mar (ICM-CSIC) in Barcelona, under accession numbers ICMD13121701 and ICMD13121702.

DNA extraction, amplification and sequencing

The identification of the megalopae was based on partial sequences of the 16S rDNA gene. Total genomic DNA was extracted from muscle tissue from 1 to 2 pereiopods of each megalopa and incubated for 1–24 h in 300 µl lysis buffer at 65 °C. Protein was precipitated by addition of 100 µl of 7.5 M ammonium acetate and subsequent centrifugation, and DNA precipitation was obtained by addition of 300 µl isopropanol and posterior centrifugation. The resulting pellet was washed with ethanol (70 %), dried, and finally resuspended in Milli-Q distilled water.

Target mitochondrial DNA from the large subunit rRNA (16S) gene was amplified with polymerase chain reaction (PCR) and the following cycling conditions for reactions: 2 min at 95 °C, 40 cycles of 20 s at 95 °C, 20 s at 45–48 °C, 45 s at 72 °C, and 5 min at 72 °C. Primers 1472 (5′-AGA TAG AAA CCA ACC TGG-3′) (Crandall and Fitzpatrick 1996) and 16L2 (5′-TGC CTG TTT ATC AAA AAC AT-3′) (Schubart et al. 2002) were used to amplify 540 bp of 16S. PCR products were sent to NewBiotechnic and Biomedal companies to be purified and then two-directional sequencing.

Sequences were edited using the software Chromas version 2.0. The final sequences were blasted on GenBank database to get the best BLAST matches for an accurate identification. Sequences are accessible in GenBank under the accession numbers KJ125076-KJ125077.

Results

Barcode identification

Using the BLAST utility (http://blast.ncbi.nlm.nih.gov/Blast.cgi), the sequences obtained from the megalopae were compared with those deposited in GenBank. The sequences perfectly fit those of *R. harrisii*, more specifically, no difference (100 % match) was found between the 16S sequence for 546 bp and sequences of *R. harrisii* from Woodland Beach, Delaware, USA (ULLZ 3836), GenBank accession number AJ274697.

Nevertheless, three out of four megalopae reared in the laboratory from specimens collected as zoeae I in the plankton did not show the general morphology and all setation patterns of those megalopal stage of *R. harrisii* which had been directly collected in the plankton. According to the DNA barcode, however, these specimens clearly belong to the same species. We have considered these specimens as "anomalous megalopa" and have provided an additional description of this type of larva.

Description of the megalopa

(Figs. 1a–e; 2a, b, d, e, g; 3a, c, d; 4a–e; 5a, d, e)
Size: CL = 1.18 ± 0.05 mm; CW = 1.02 ± 0.05 mm; $N = 5$

Cephalothorax (Fig. 1a, b) Rostrum is short and obliquely downward with 2 lateral simple setae at base, anterior end with a median triangular notch; the pedunculated eyes with 8 small simple setae each; hepatic region swollen; one pair each of protogastric, mesobranchial and cardiac protuberances present; and broader posterior part, margins setose.

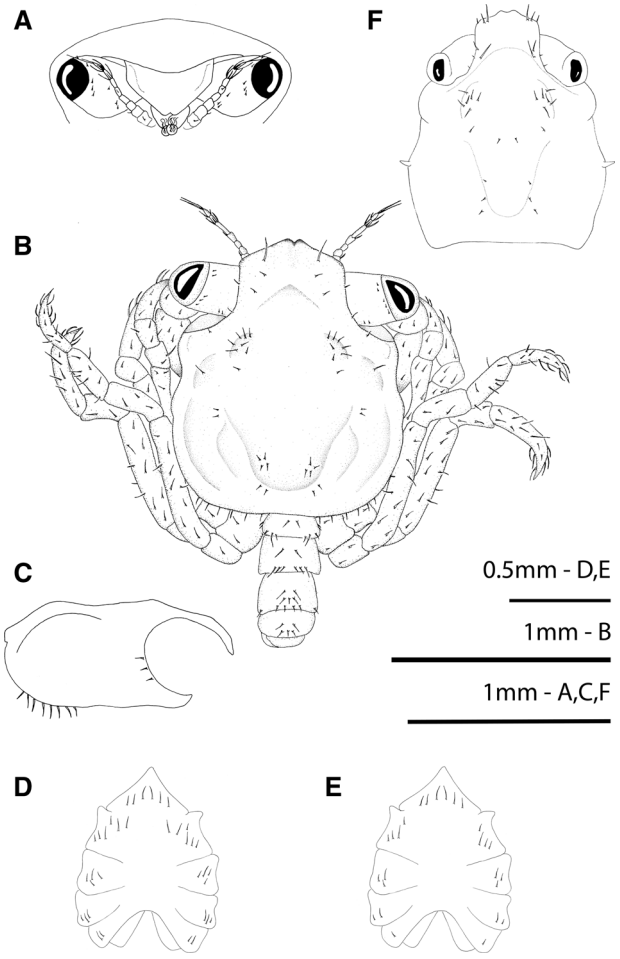

Fig. 1 *Rhithropanopeus harrisii* (Gould, 1841). Megalopa, **a** frontal view; **b** dorsal view; **c** lateral view of the cephalothorax; **d**, **e** sternum; **f** anomalous megalopa, dorsal view

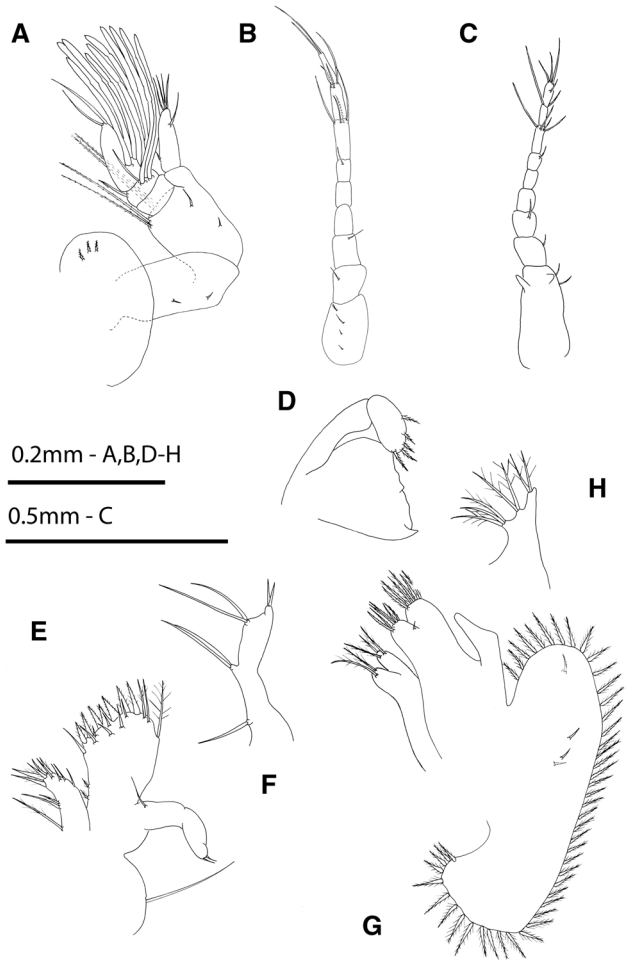

Fig. 2 *Rhithropanopeus harrisii* (Gould, 1841). Megalopa, **a** antennule; **b** antenna; **c** anomalous antenna; **d** mandible; **e** maxillule; **f** endopod of maxillule of the anomalous specimen; **g** maxilla; **h** endopod of maxilla of the anomalous specimen

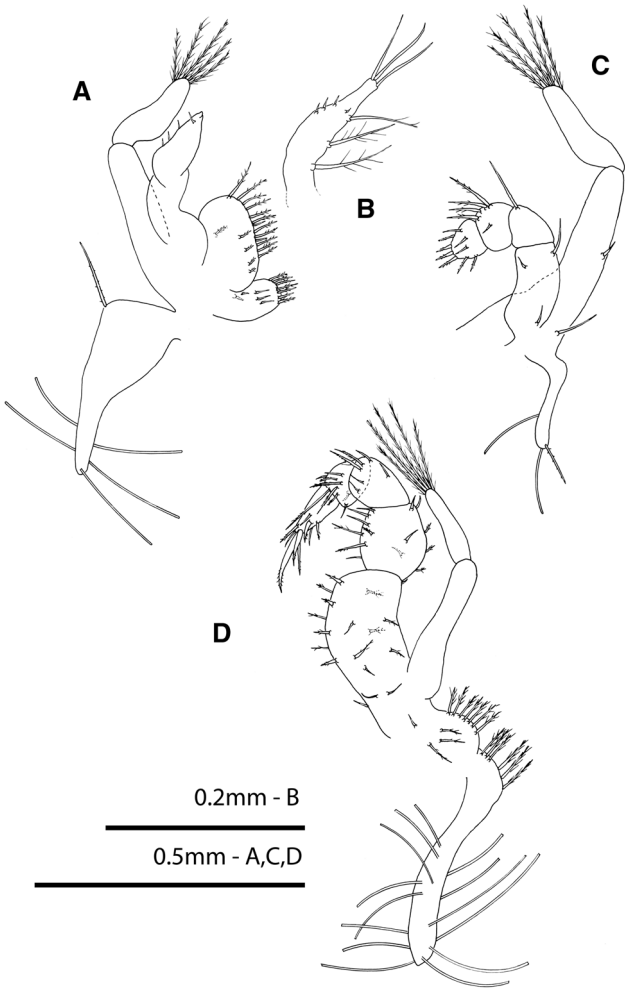

Fig. 3 *Rhithropanopeus harrisii* (Gould, 1841). Megalopa, **a** first maxilliped; **b** endopod of first maxilliped of the anomalous specimen; **c** second maxilliped; **d** third maxilliped

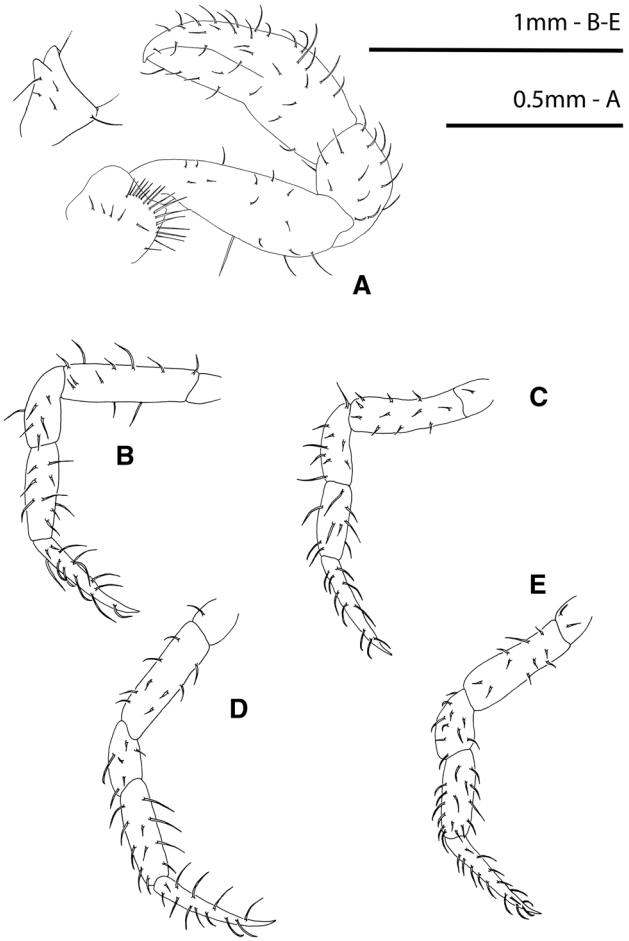

Fig. 4 *Rhithropanopeus harrisii* (Gould, 1841). Megalopa, **a** cheliped, with detail of the ischium spine; **b** second pereiopod; **c** third pereiopod; **d** fourth pereiopod; **e** fifth pereiopod

Antennule (Fig. 2a) Peduncle three-segmented, with 3 short simple setae on first segment, 2 short simple setae on median segment and 2 short simple setae plus 2 pairs of long plumodenticulate setae on distal segment; endopod unsegmented with 1 basal simple seta, 1 subterminal simple seta and 3 terminal simple setae; exopod three-segmented, with 10 aesthetascs (arranged 0, 4, 6) and 4 setae (arranged 0, 2, 2 setae).

Antenna (Fig. 2b) Peduncle three-segmented with 6 setae (arranged 4, 1, 1); flagellum six-segmented with 10 simple setae (arranged 0, 0, 1, 4, 3, 2).

Mandible (Fig. 2d) Palp two-segmented, with 5 terminal short plumodenticulate setae on distal segment.

Maxillule (Fig. 2e) Coxal endite with 12 plumose setae; basial endite with 16 setae (3 terminal plumodenticulate, 1 terminal sparsely plumose, 7 terminal cuspidate, 3 subterminal plumodenticulate, and 2 proximal plumodenticulate); endopod unsegmented with 1 proximal and 2 terminal simple setae; and long epipodal seta present.

Maxilla (Fig. 2g) Coxal endite bilobed with 2 + 3 terminal plumose setae; basial endite bilobed with 6 + 6 sparsely plumodenticulate setae; endopod unsegmented and without setae; scaphognathite with 45–47 marginal plumose setae plus 2 small simple setae on each lateral surface.

First maxilliped (Fig. 3a) Epipod well developed, triangular shaped, with 5 long simple setae and 1 proximal plumodenticulate seta; coxal endite with 5 inner simple setae and 7 terminal plumose setae; basial endite with 1 inner + 4 subterminal + 11 terminal sparsely plumodenticulate setae plus 2 terminal short simple setae; endopod unsegmented with 4 short terminal simple setae; exopod two-segmented, with 5 long terminal plumose setae on distal segment.

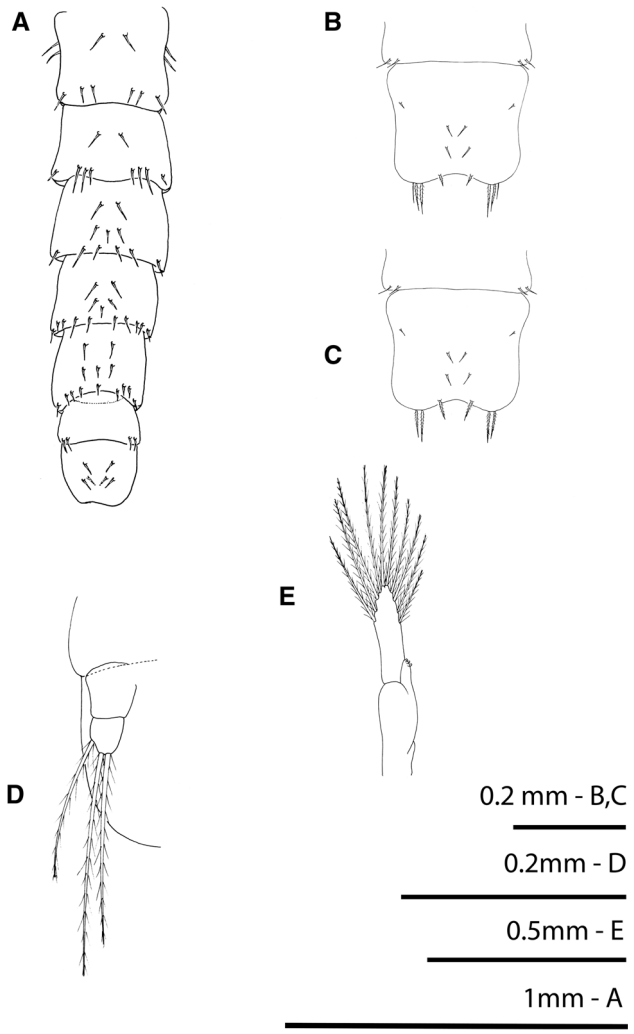

Fig. 5 *Rhithropanopeus harrisii* (Gould, 1841). Megalopa, **a**: pleon, dorsal view; (**b–c**) telson of an anomalous megalopa; **d** uropod; **e** third pleopod

Second maxilliped (Fig. 3c) Reduced epipod with 2 simple setae and 1 plumodenticulate seta; endopod five-segmented, with 1 simple, 2 simple, 1 simple, 4 plumodenticulate + 1 short simple, and 3 proximal simple + 6 terminal plumodenticulate setae, respectively; exopod two-segmented, with 2 simple setae on proximal segment and 5 long terminal plumose setae on distal one.

Third maxilliped (Fig. 3d) Epipod well developed with a proximal marginal row of 6 plumose setae and 14 long simple setae; protopod with a marginal row of 7 plumose setae and 1 simple + 3 plumose inner setae; endopod five-segmented, with 19, 14, 6, 9 and 9 setae, respectively; exopod two-segmented with 5 long plumose setae on distal segment.

Pereiopods (Fig. 4a–e) Pereiopods 2–5 thin and setose, with long subterminal setae on dactyli. Cheliped robust and setose without remarkable recurved spines, only sometimes a small spine, never recurved.

Sternum (Fig. 1d, e) Maxilliped sternites completely fused with 6 simple setae, cheliped sternites with 4 or 6 simple setae each, pereiopod sternites 2–5 with 3 or 4, 2 or 3, 1 or 2, and 0 simple setae, respectively; sternal sutures are interrupted medially. There are two forms according to setation; the most common is illustrated in Fig. 1.

Pleon (Fig. 5a) Six somites plus telson; setation as shown.

Pleopods (Figs 5d, e) Biramous except uropods present on somites 2–5; endopod with 3 cincinuli; exopod with 10 long plumose natatory setae; uropod with 3 or 4 natatory setae on distal segment.

Description of anomalous megalopae

(Figs. 1f, 2c, f, h, 3b, 5b, c)
Size: CL = 1.12–1.14 mm; CW = 0.92–0.98 mm; $N = 2$

All three specimens exhibited the following deviations from the typical form: cephalothorax with different shape, bearing vestiges of zoeal lateral spines, and a reduced number of setae (Fig. 1f); antennular peduncle with remains of exopodal and protopodal processes as spines (Fig. 2c); endopod of maxillule with a setation pattern of 1, 2, 2, 2 as in the zoeal endopod of the maxillule (Fig. 2f); endopod of maxilla with setation 3, 2, 2 as in the zoeal maxillar endopod (Fig. 2h); endopod of first maxilliped with 3 terminal long setae plus 1 + 1 + 1 long inner plumose setae (Fig. 3b); telson with 2–3 terminal setae in the place of furcal arms and 1 pair of marginal setae as zoeal stage (Figs 5b, c).

Discussion

Redescriptions of brachyuran larval stages are unusual, although they are necessary when previous descriptions are brief, incomplete, inaccurate or deficient, making them useless for reliable identifications. There are some cases of redescriptions in the recent literature. For instance, *Aratus pisonii* (H. Milne Edwards, 1837) was redescribed by Cuesta et al. (2006) considering that the previous description by Warner (1968) referred to a clearly anomalous megalopa. The most recent redescription of *D. sayi* by Marco-Herrero et al. (2013b) was necessary because the several previous descriptions were brief and inaccurate and thus inappropriate for comparative taxonomic studies. Correct descriptions of larval stages are needed for phylogenetic studies and accurate identifications of plankton-

collected specimens. In the case of *R. harrisii*, the several previous descriptions of the megalopa from both laboratory-reared larval stages and from plankton-collected specimens are all incomplete and inaccurate and do not meet the standard proposed by Clark et al. (1998), currently followed by the majority of decapod larval morphologists.

Since the previous descriptions do not allow for an accurate identification of plankton-collected specimens, the DNA barcode was used instead. Current molecular tools ensure a correct identification of specimens collected in the field, which present clear advantages over specimens which have been reared in the laboratory. In particular, field-collected larvae allow for obtaining a better representation of natural morphological variability compared with larvae originated from only one or two ovigerous females cultured in the laboratory. In the present study, the 16S sequences of the 10 studied megalopae, collected in the Guadalete estuary for morphological description, fit at 100 % the 16S sequence of *R. harrisii* from Delawere (USA) deposited in GenBank.

The morphology of the megalopae of *R. harrisii* described in the present work do not completely match the typical characters of the megalopa stages of panopeids, although Martin et al. (1984), based on zoeal morphology, included *R. harrisii* in the Group I together with the majority of panopeids. Even when the classification was based on megalopal features, the species was attributed to Group I (Martin 1988). The main differences relate to rostrum morphology, the number of segments of the antennular flagellum and the spinulation of the ischium of the cheliped.

The typical panopeid megalopa rostrum presents a remarkable spine at each basal angle, called "horns" in some papers, but these are missing in *R. harrisii*. The antennular flagellum of *R. harrisii* shows six segments while eight segments are present in other panopeids such as *D. sayi* (see Marco-Herrero et al. 2013b) and *P. africanus* (see Rodríguez and Paula 1993). The number of segments of the antennular flagellum is considered to be a conservative character at family level in other taxa (Cuesta 1999). Finally, the absence of a remarkable recurved spine on the cheliped ischium is another marked contrast to the majority of panopeids. Together with the above-mentioned differences, this feature could challenge the phylogenetic position of this species. Future molecular phylogenetic studies will help to resolve this question raised by the larval morphology.

The setation patterns of maxillule, maxilla, first, second and third maxillipeds, and sternum are described in the present work for the first time. As to the setation pattern of the sternal plates, some variability was observed, although the proportions between sternites were always similar.

In the identification key to the megalopa stages of the Mediterranean Brachyura by Pessani et al. (2004), *R. harrisii* is differentiated by bearing three long plumose terminal setae on the distal segment of the uropod in contrast to "uropod exopod with more than 3 setae." Megalopae in the present study showed either three or four setae, and in one case, this variability occurred in the same specimen. The same variability in the setation on the exopods of the uropods has already been described by Kurata (1970).

In the present work, we also studied megalopae grown from zoeae, which I had been collected in the plankton and raised in the laboratory. There is some evidence that the culture conditions (temperature and/or feeding) were suboptimal. The megalopae which developed under these conditions showed an anomalous morphology. This kind of anomalies has already been reported in other species and not only for larvae raised in the laboratory (Willems 1982; Cuesta and Anger 2001), but also for larvae collected in the field (Cuesta et al. 2002). In all these cases, the anomalies referred to morphological character of the zoeal phase, such as the presence of short lateral spines in the cephalothorax and the setation patterns of maxillule and maxilla endopods. The available data suggest that morphological anomalies in the megalopa stage are the result of suboptimal environmental conditions (temperature, salinity, food), and that such deficiencies can occur not only during laboratory rearing but also in the natural environment.

Acknowledgments We want to thank the "Real Club Náutico" of the Puerto Santa María (Cádiz, Spain) for kindly allowing us to use their facilities. We are especially grateful to Reyes Sánchez García and Ainhoa Olaguer-Feliú who helped in collecting the larvae, Carlos Sánchez Nieto for his assistance in the laboratory work, and Dulce Subida for her careful language corrections. Thanks are also due to anonymous referees and Prof. Heinz-Dieter Franke for his editorial work. The research was carried out within the framework of the projects MEGALOPADN (CGL2009-11225) and "Transporte y reclutamiento larvario de crustáceos bentónicos litorales: importancia de los agentes forzadores costeros y regimen mareal" (CTM2005-00024/MAR), both co-financed by the "Ministerio de Economía y Competividad (MINECO)" Spanish Plan R + D + I and European Union FEDER funds. Elena Marco-Herrero acknowledges the predoctoral FPI Fellowship supported by MINECO (BES-2010-033297).

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CAPÍTULO 6

Larval morphology of the family Parthenopidae, with the description of the megalopa stage of *Derilambrus angulifrons* (Latreille, 1825) (Decapoda: Brachyura), identified by DNA barcode

E Marco-Herrero, JI González-Gordillo, JA Cuesta (2015) Larval morphology of the family Parthenopidae, with the description of the megalopa stage of *Derilambrus angulifrons* (Latreille, 1825) (Decapoda: Brachyura), identified by DNA barcode Journal of the Marine Biological Association of the United Kingdom 95(3): 513-521

Journal of the Marine Biological Association of the United Kingdom, 2015, 95(3), 513–521.
doi:10.1017/S0025315414001908

Larval morphology of the family Parthenopidae, with the description of the megalopa stage of *Derilambrus angulifrons* (Latreille, 1825) (Decapoda: Brachyura), identified by DNA barcode

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Although Parthenopidae is a brachyuran decapod family comprising almost 140 species, there is little knowledge about its larval morphology. There are only two complete larval developments reared in the laboratory and some larval stages described for seven species. In the present work these data are compared and analysed. A summary is made of the larval features that characterize parthenopids that can be used to distinguish them from other brachyuran larvae. In addition, the megalopa stage of Derilambrus angulifrons *and* Parthenopoides massena *was collected from plankton and identified by DNA barcodes. The morphology of the megalopa of* D. angulifrons *is described for the first time, and that of* P. massena *is compared with a previous description.*

Keywords: larval morphology, megalopa, DNA barcode, Parthenopidae, *Derilambrus angulifrons*, *Parthenopoides massena*, brachyura, Decapoda, plankton, crabs

Submitted 16 December 2013; accepted 7 August 2014; first published online 15 December 2014

INTRODUCTION

The family Parthenopidae MacLeay, 1838 is currently divided into two subfamilies: Parthenopinae MacLeay, 1838 and Daldorfiinae Ng & Rodriguez, 1986. Daldorfiinae comprises four genera with 17 species and Parthenopinae 32 genera and 123 species (Ng *et al.*, 2008).

The adult morphology of the parthenopids has been examined recently and several changes in its systematics were proposed (Tan & Ng, 2007; Tan & Low, 2014). However, there is very little information about their larval morphology and most larval descriptions deal only with the first zoeal stages (ZI). Complete larval development is only known for two species, *Platylambrus serratus* (H. Milne Edwards, 1834) by Yang (1971) and *Enoplolambrus validus* (De Haan, 1837) by Kurata and Matsuda (1980) and Terada (1985). For the remaining species, the larval development descriptions are partial or unavailable. The first known description, assigned to *Lambrus massena* (Roux, 1830), was published by Gourret (1884), and later Cano (1893) described three zoeal stages as *Lambrus* sp. Already in the 19th century, Aikawa (1937) described the first zoea of *Enoplolambrus validus* (as *Lambrus validus*) and Lebour (1944) identified and illustrated one megalopa from plankton attributed to Parthenopidae. Bourdillon-Casanova (1960) and Heegaard (1963) reported the first zoeal stage of *Parthenopoides massena* (as *Lambrus massena*). Thiriot (1973) also reported the ZI of *Distolambrus maltzami* (Miers, 1881) (as *Heterocrypta maltzami*) reared in the laboratory and five zoeal stages and one megalopa from plankton of *P. massena*. Heegaard (1963) described the first zoeal stage of *Derilambrus angulifrons* (Latreille, 1825) (as *Lambrus angulifrons*) and Kurata (1970) illustrated and described the first zoea of *Heterocrypta granulata* (Gibbes, 1850). More recently, Guerao and Abelló (1999) described the first zoeal stage of *Spinolambrus macrochelos* (Herbst, 1790) (as *Parthenope macrochelos*), and Ng and Clark (2000) described the first zoeal stage of *Rhinolambrus pelagicus* (Rüppell, 1830), both from larvae hatched in the laboratory. Rice and Williamson (1977) and Paula (1987) attributed larvae described from plankton samples to parthenopids but did not identify genus or species.

In the present work, we compare and analyse all these data, revise the larvae from plankton attributed to this family, and make a summary of the larval features that characterize parthenopids and which can be used to distinguish them from other brachyuran larvae.

Many brachyurans are clearly distinguishable in adult form but have larval and juvenile forms that are difficult to identify to species level. In some instances, the larvae are distinguishable but not easily matched with the correct adult form. A classic tool for helping to identify larvae collected in the field is to use complete descriptions of larvae obtained in laboratory cultures from clearly identified parental females.

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Current molecular tools such as DNA barcoding ensure that specimens collected in the field are identified correctly. These specimens collected in the field have clear advantages over specimens which have been reared in the laboratory; for example, González-Gordillo & Rodríguez (2000) reported morphological differences between larvae collected in the plankton and those reared in the laboratory from ovigerous females, although both inhabit the same locality.

The use of molecular markers has demonstrated to be a powerful tool for accurately identifying plankton specimens (Pan *et al.*, 2008; Pardo *et al.*, 2009; Ampuero *et al.*, 2010; Marco-Herrero *et al.*, 2013). In the present study, we identified the megalopa stages of *Derilambrus angulifrons* and *Parthenopoides massena*, collected in the plankton, using partial sequences of the mitochondrial genes 16S and Cox1 as DNA barcodes.

Derilambrus angulifrons is known from the eastern Atlantic: south-western Spain (Cuesta Mariscal & González-Gordillo, 1992) and the Mediterranean Sea (d'Udekem d'Acoz, 1999) at depths from 2 m (Števčić, 1990) to 40 m (Zariquiey Álvarez, 1968). In this area this species lives on sandy mud, muddy detritus and coralligenous bottoms (d'Udekem d'Acoz, 1999). *Parthenopoides massena* is distributed in the east Atlantic from northern Europe to Guinea and Mediterranean coasts (d'Udekem d'Acoz, 1999) where they inhabit mainly sandy and calcareous algae bottoms at 3–141 m depth (Zariquiey Álvarez, 1968; Števčić, 1990).

In the present study the megalopa of *Derilambrus angulifrons* is described and illustrated in detail for the first time and the megalopa of *Parthenopoides massena* is compared with the previous description by Thiriot (1973).

MATERIALS AND METHODS

Collection of the megalopae

Megalopae were collected in the course of three different projects. Three megalopae of *Derilambrus angulifrons* were captured in July 2007 from the plankton of the Guadalete estuary (Cádiz-SW Spain) (36°35′24.09″N 6°13′46.19″W) in a campaign of plankton sampling in this estuary in the context of the project 'Transporte y reclutamiento larvario de crustáceos bentónicos litorales: importancia de los agentes forzadores costeros y regimen mareal' (CTM2005-00024/MAR). Two megalopae of *Parthenopoides massena* were collected in two different stations in the Mediterranean Sea, one in the Gulf of Naples (40°49′10.51″N 14°14′05.09″E) in September 2009 and another one off the Balearic Islands (39°43.27′N 02°13.07′E) in July 2010.

Morphological descriptions

Drawings and measurements were made using a Wild MZ6 and Zeiss Axioskop compound microscope with Nomarski interference, both equipped with a *camera lucida*. All measurements were made using an ocular micrometer. Descriptions were based on all collected megalopae. The following measurements were taken for the megalopa: cephalothorax length (CL), measured from the tip of rostrum to posterior margin of cephalothorax; and cephalothorax width (CW), measured as the cephalothorax maximum width (mesobranchial regions). In Figures 3B, C and 4B the plumose setae are drawn truncated.

The larvae are described using the basic malacostracan somite plan from anterior to posterior and appendage segments are described from proximal to distal, endopod then exopod (Clark *et al.*, 1998).

DNA extraction, amplification and sequencing

The identification of larval stages was based on partial sequences of the 16S rDNA and Cox1 genes. Total genomic DNA was extracted from muscle tissue from pereiopods of the megalopae, and incubated for 1–24 h in 300 µl lysis buffer at 65°C. Protein was precipitated by addition of 100 µl of 7.5 M ammonium acetate and subsequent centrifugation, and DNA precipitation was obtained by addition of 300 µl of isopropanol and posterior centrifugation. The resulting pellet was washed with ethanol (70%), dried, and finally resuspended in Milli-Q distilled water.

Target mitochondrial DNA from the 16S rRNA and Cox1 genes was amplified with polymerase chain reaction (PCR) using the following cycling conditions: 2 min at 95°C, 40 cycles of 20 s at 95°C, 20 s at 45–48°C, 45 s (16S) or 47 s (Cox1) at 72°C, and 5 min 72°C. Primers 1472 (5′- AGA TAG AAA CCA ACC TGG -3′) (Crandall & Fitzpatrick, 1996) and 16L2 (5′-TGC CTG TTT ATC AAA AAC AT-3′) (Schubart *et al.*, 2002) were used to amplify 540 bp of 16S, while primers COH6 (5′- TAD ACT TCD GGR TGD CCA AAR AAY CA -3′) and COL6b (5′- ACA AAT CAT AAA GAT ATY GG -3′) (Schubart & Huber, 2006) allowed amplification of 670 bp of Cox1. PCR products were sent to New Biotechnic and CISA-INIA companies to be purified and then bidirectionally sequenced.

Sequences were edited using the software Chromas version 2.0. The obtained final DNA sequences were compared with those from adult specimens of several Iberian brachyuran crabs obtained in the context of the MEGALOPADN project. Adult and larval sequences for both genes are deposited in GenBank under accession numbers (KP057806-KP057819).

RESULTS

Barcode identification

In the context of the MEGALOPADN project we have obtained the DNA mitochondrial sequences of 16S and Cox1 genes for almost all the Iberian brachyuran crabs. Therefore we can compare the sequences obtained from the megalopae with those in our alignments and database. For Parthenopidae we have got the sequences of the Iberian representatives of *Derilambrus angulifrons*, *Distolambrus maltzani*, *Parthenopoides massena* and *Spinolambrus macrochelos*. The sequences of the megalopae from Guadalete estuary perfectly fit those of *Derilambrus angulifrons* and those of the megalopae from the Balearic Islands and Naples with the sequences of *Parthenopoides massena*. No differences (100% match) were found between the 16S (546 bp) and Cox1 (667 bp) sequences of *D. angulifrons* and the Guadalete estuary megalopae. Also the Mediterranean megalopae sequences math 100% with 16S sequence of *P. massena*. In the case of Cox1, while the Naples megalopa sequence (613 bp) also matches

100% with those of *P. massena*, the Balearic Island megalopa sequence differs in 4 mutations out of 667 bp from the Cox1 sequence of *P. massena*.

MEGALOPA DESCRIPTION

Family Parthenopidae MacLeay, 1838
Genus *Derilambrus* Tan & Ng, 2007
Derilambrus angulifrons (Latreille, 1825)
(Figures 1 & 2)

Size: CL = 1.78 ± 0.08 mm; CW = 0.91 ± 0.06 mm; N = 3
Cephalothorax (Figure 1A, B) Longer than broad, with long, thin and straight rostrum with 3 pairs of minute setae; a pair of lobes on the mesobranchial regions with hepatic regions moderately inflated; 2 tubercles, 1 on metagastric region and 1 on urogastric region; prominent long spine present on cardiac region backwards with few minute unpaired setae; setation as drawn; dorsal organ present; eyes stalked.
Antennule (Figure 2A) Peduncle 3-segmented with 7, 2, 2 simple setae; unsegmented endopod with 1 medial, 1 subterminal and 3 terminal simple setae; exopod 4-segmented with 0, 0, 1, 2 simple setae; segments 2–4 with 4, 4 and 3 aesthetascs respectively.
Antenna (Figure 2B, C) Crenulated peduncle 3-segmented with 2, 1, 1 simple setae respectively, proximal segment with stout and ventrally directed process; flagellum 7-segmented with 0, 0, 0, 4, 0, 3, 5 simple setae respectively.
Mandible (Figure 2D) Palp 2-segmented with 8 plumodenticulate terminal setae on distal segment.
Maxillule (Figure 2E) Coxal endite with 8 plumose setae plus 4 plumodenticulate setae on margin; basial endite with 14 marginal cuspidate, 10 subterminal plumodenticulate, and 2 proximal plumose setae; endopod unsegmented with 1 terminal simple setae; long exopodal simple seta present.
Maxilla (Figure 2F) Coxal endite bilobed with 9 + 5 terminal plumose setae; basial endite bilobed with 5 + 5 sparsely plumodenticulate setae; endopod unsegmented with 3 short plumodenticulate setae on base; exopod (scaphognathite) with 47–48 marginal plumose setae plus 3 small simple setae, 2 dorsal and 1 ventral, on lateral surface.
First maxilliped (Figure 3A) Epipod triangular shaped with 8 setae, 2 proximal plumodenticulate and 6 distal long setae; coxal endite with 13 plumose setae; basial endite with 17 sparsely plumodenticulate setae; endopod reduced, unsegmented and with 2 simple setae; exopod 2-segmented with 1 plumodenticulate distal seta on proximal segment and 5 terminal plumose setae on distal segment.

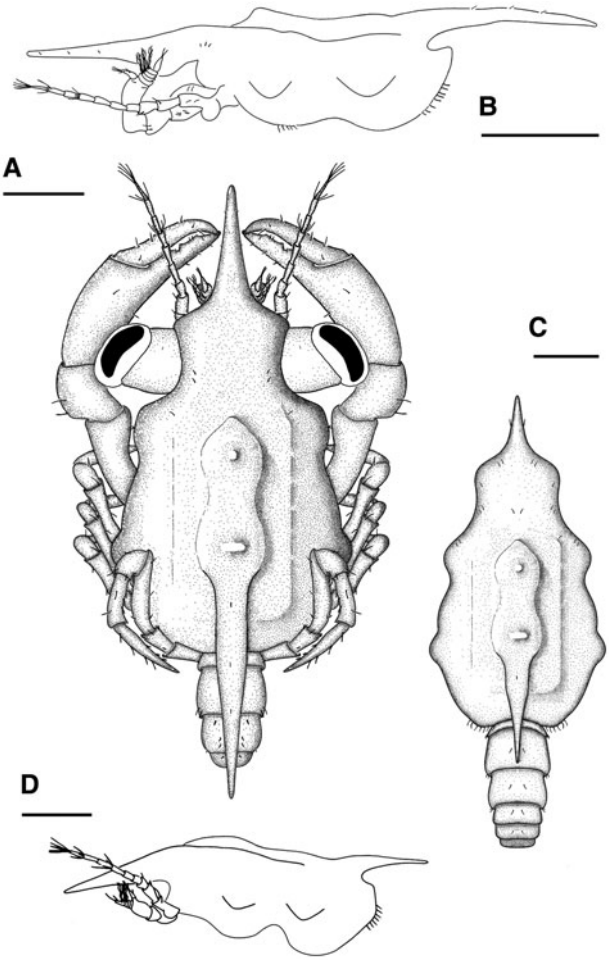

Fig. 1. *Derilambrus angulifrons* (Latreille, 1825) Megalopa, (A) general dorsal view; (B) lateral view of the cephalothorax. *Parthenopoides massena* (Roux, 1830) Megalopa, (C) dorsal view; (D) lateral view of the cephalothorax. Scale bars= 0.5 mm.

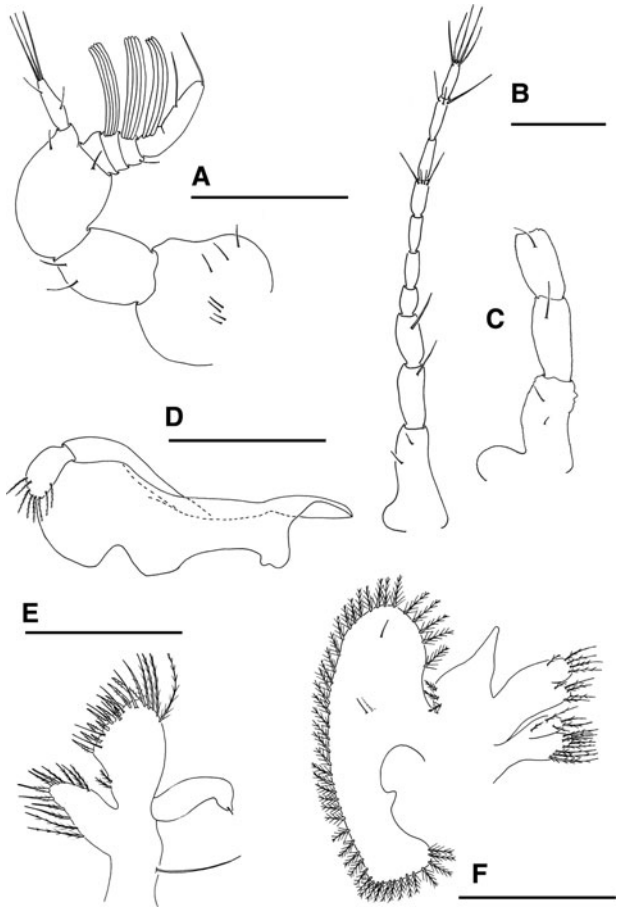

Fig. 2. *Derilambrus angulifrons* (Latreille, 1825) Megalopa, (A) antennule; (B) antenna, (C) detail of the peduncle of antenna; (D) mandible; (E) maxillule; (F) maxilla. Scale bars = 0.2 mm.

Second maxilliped (Figure 3B) Epipod reduced without setae; protopod with 1 simple seta; endopod 5-segmented with 1 (simple), 2 (simple), 1 (long simple), 7 (plumodenticulate) and 9 (3 cuspidate, 6 plumodenticulate) setae, respectively; exopod 2-segmented with 1 medial simple seta on proximal segment and 5 terminal plumose setae on distal segment.
Third maxilliped (Figure 3C) Epipod with 6 subterminal and 1 terminal long setae; protopod with 12 plumodenticulate setae; endopod 5-segmented, margin of the proximal segment denticulate, and 19, 10, 6, 8, 7 sparsely plumose setae respectively; exopod 2-segmented with 1 distal simple seta on proximal segment and 7 terminal plumose setae on distal segment.
Pereiopods (Figure 3D–G) Cheliped setation as drawn, fixed finger lower margin with 2 prominent teeth; pereiopods II–V thin and setose, inner margin of dactyl with 3 stout ventral spines and 1 pair subterminal shorter spines; setation as illustrated. Long setae (feelers) on dactylus of pereiopod V absent.
Sternum (Figure 4C) Maxilliped sternites completely fused with 2 simple setae, cheliped sternites with 3 simple setae each, pereiopod sternites 2–5 without setae; sternal sutures are interrupted medially.
Pleon (Figure 4A, B) Six pleonites; pleonite I without setae; setation of pleonites II–VI as shown; pleonite VI reduced.
Pleopods (Figure 4B, D & E) Present on pleonites II-VI; endopods unsegmented with 3 cincinuli; exopod unsegmented with 11–14 long plumose natatory setae; uropod 2-segmented, proximal segment without setae, distal segment with 4 terminal plumose natatory setae.
Telson (Figure 4A) Reduced, subquadrate, with 1 pair of dorsal setae.

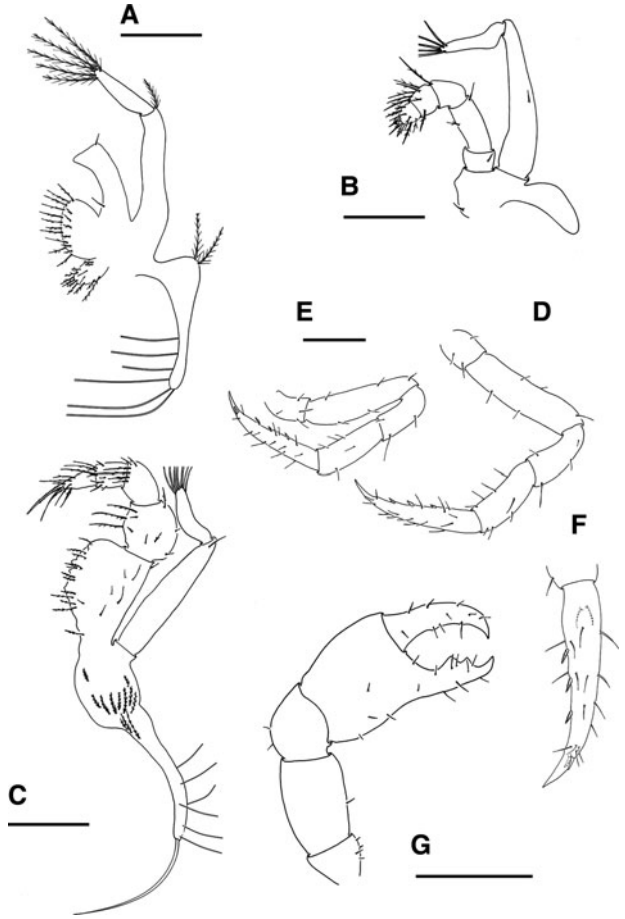

Fig. 3. *Derilambrus angulifrons* (Latreille, 1825) Megalopa, (A) first maxilliped; (B) second maxilliped; (C) third maxilliped; (D) second pereiopod; (E) fifth pereiopod; (F) detail of the dactylus of pereiopods II–V (G) cheliped. Scale bars = (A–E) 0.2 mm and (F) 0.5 mm.

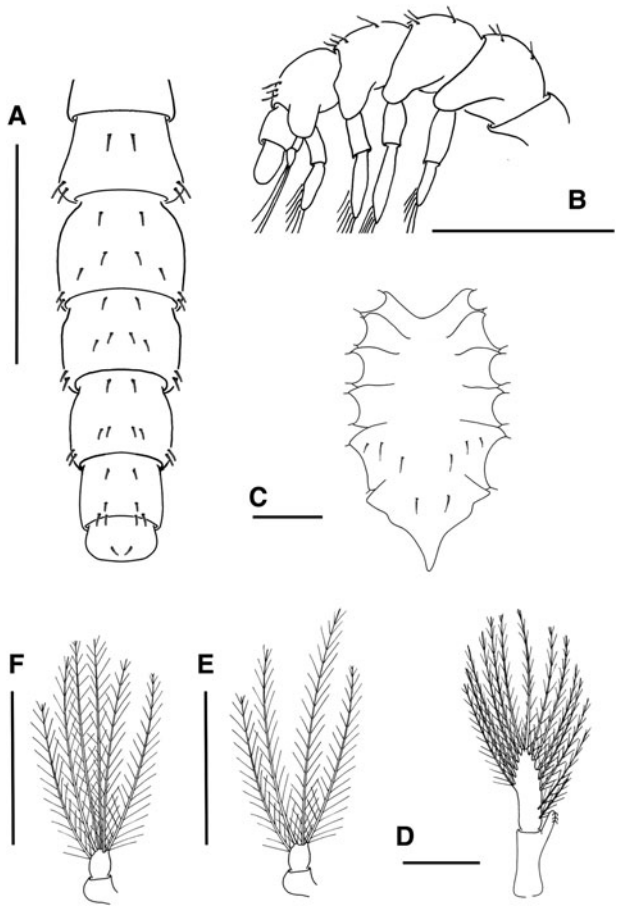

Fig. 4. *Derilambrus angulifrons* (Latreille, 1825) Megalopa, (A) pleon, dorsal view; (B) pleon, lateral view; (C) sternum; (D) third pleopod; (E) uropod. *Parthenopoides massena* (Roux, 1830) Megalopa, (F) uropod. Scale bars = (A, B) 0.5 mm and (C, D) 0.2 mm.

DISCUSSION

The systematic relationships of Parthenopidae have been controversial for a long time. In several works since 1862 to the present, its systematic position has changed from Calappidae (Strahl, 1862) to Brachyryncha (Yang, 1971), passing through Cancridae (Lebour, 1928; Aikawa, 1935) and Oxyryncha (Bouvier, 1940; Balss, 1957). Guinot (1977, 1978) elevated the Parthenopidae to a superfamily in the section Heterotramata, which was later corroborated with larval morphology (according to Rice, 1980), and currently this is the most widely accepted status. Tan (2004) and Tan & Ng (2007) have carried out the most recent and comprehensive revision of Parthenopoidea, which Ng *et al.* (2008) follows. According to these authors, Parthenopoidea contains only one family, Parthenopidae, divided into two subfamilies, Daldorfiinae (4 genera and 17 species) and Parthenopinae (32 genera and 123 species). In spite of all these studies, its phylogenetic relationships are still unresolved, and it is only clear

that it is not related to Majoidea (Yang, 1971; Ahyong *et al.*, 2007). However, it has been suggested that based on adult morphology there are relationships with Aethroidea, Calappoidea, Trapezoidea and Plagusiidae, among others (see Tan & Ng, 2007), and based on larval morphology there are relationships with Cancroidea (Lebour, 1928; Aikawa, 1937) and Cyclometopa in general (Rice, 1980).

Larval studies have contributed to the resolution of problems in the systematic classification of brachyuran crabs (Rice, 1980; Marques & Pohle, 1998; Clark & Guerao, 2008; Clark, 2009; Marco-Herrero *et al.*, 2013) because the morphology of larval stages gives an insight into the relationships between brachyuran taxa. Larval characters may reflect relationships even better than adult morphology (Rice, 1980). Nevertheless, there are still few data on larval development for parthenopids and most larval descriptions deal only with the first zoeal stages and partial descriptions of intermediate zoeae from plankton samples. In the present study we compare all known descriptions of the larval stages of parthenopids (see Tables 1 & 2).

In parthenopid larvae there is no single character that distinguishes them from the rest of the brachyuran superfamilies (see Yang, 1971; Rice, 1980) but there is a set of features that can be used to identify them. Summarizing the set of characters proposed by Yang (1971) and Rice (1980), including some modifications and new features, the 9 diagnostic characteristics of the parthenopid zoeal stages are: (i) the cephalothorax has well developed and smooth dorsal, lateral, and rostral spines and the dorsal and rostral spines are longer than cephalothorax length; (ii) the antenna shows a long protopodal process (but never reaching the tip of the rostral spine) with 2 rows of spinules, an exopod about 2/3 of the protopod length with 2 unequal length terminal setae (the longer seta can reach the tip of protopod, and in some cases is described as setulose); (iii) endopod of maxillule and maxilla with 1,2 + 2 + 2 and 2 + 2 + 3 setae respectively; (iv) basis of maxillipeds 1 and 2 with 2 + 2 + 2 + 2 and 1 + 1 + 1 + 1 setae respectively; (v) endopod of maxilliped 2 with 1,1,4 setae; (vi) dorsolateral processes are present on pleonal somites II and III; (vii) usually long acute posterolateral processes on somites III-V; (viii) telson forks bear one pair of well-developed dorsomedial spines and sometimes there are 1 or 2 lateral setae present; (ix) three pairs of posterior processes on telson through development. Moreover, Yang (1971) described another character: a well-developed forehead and posterodorsal protuberances on the cephalothorax that appears in the majority of parthenopids (absent in *Rhinolambrus pelagicus* by Ng & Clark, 2000), although this is also very common in larvae of other brachyurans.

According to the few previous studies describing the complete larval development of parthenopids the number of zoeal stages is variable. Four were described for *Enoplolambrus validus* (see Terada, 1985) and five for *Parthenopoides massena* (see Thiriot, 1973) and *Platylambrus serrata* (see Yang, 1971), although in this last case an extra sixth zoeal stage was also recorded. The common characters related to changes through development are, besides the general increase in the number of setae, the appearance of the sixth somite of the pleon from zoea III on, and the addition of one plumodenticulate seta on the distal segment of the endopod of the first maxilliped also from zoea III on.

The megalopa stage has only been described for three species of parthenopids, *P. serrata*, *P. massena* and *E.*

Table 1. Morphological comparison of the known zoea I of Parthenopidae

	DEAN Heegaard (1963)	DIMA Thiriot (1973)	ENVA Terada (1985)	HEGR Kurata (1970)	PAS14 Paula (1987)	PAMA Thiriot (1973)	PLSE Yang (1971)	RHPE Ng and Clark (2000)	SPMA Guerao and Abelló (1999)
No zoeal stages	nd	nd	4	nd	nd	5	5–6	nd	nd
CDRLsp	Present	Present	Present	Present	Present	Present	Present	Present	Present
Exp/Antenna (length ratio)	1/4	1/3	1/3	1/4	1/3	1/3	1/3	1/3	1/4
Maxillule End (s)	0,0,2 + 2 + 2[1]	0,2 + 2 + 2[2]	1, 2 + 2 + 2	0,2 + 2 + 2[2]	0, 2 + 2 + 2[2]	0,0,2 + 2 + 2[1,2]	1,2 + 2 + 2	0, 2 +2 + 2[2]	1, 2 + 2 + 2
Maxillule Coxal/Basial (s)	8/5	6/5	7/5	nd	nd	6/5	6/5	7/5	7/5
Maxilla End (s)	2 + 2 + 2	2 + 2 + 2	2 + 2 + 3	2 + 2 + 2	2 + 2 + 3	2 + 2 + 3	2 + 2 + 3	2 + 2 +3	2 + 2 + 3
Maxilla Coxal/Basial (s)	11/7	8/8	8/8	nd	nd	8/8	8/8	8/8	8/8
Mxp 1 End (s)	2,2,2,2,4[2]	2,2,0,2,5[2]	2,2,1,2,5	nd	nd	2,2,1,2,4[2]	2,2,1,2,5	2, 2, 1, 2, 5	2,2,1,2,5
Mxp 1 Basis (s)	2 + 2 + 2 + 2	2 + 2 + 2 + 2	2 + 2 + 2 + 2	nd	nd	2 + 2 + 2 + 2	2 + 2 + 2 + 2	2 + 2 + 2 + 2	2 + 2 + 2 + 2
Mxp 2 End (s)	1,3[2]	1,1,4	1,1,4	0,1,4[2]	1,1,4	1,1,4	1,1,4	1, 1, 4	1,1,4
Mxp 2 Basis (s)	1[2]	1 + 1 + 1[2]	1 + 1 + 1 + 1	nd	nd	1 + 1 + 1 + 1	1 + 1 + 1 + 1	1 + 1 + 1 + 1	1 + 1 + 1 + 1
Telson furca (sp)	1D/1L	1D	1D	1D	1D/2L	1D	1D	1D/1L	1D/1L

CDRLsp, cephalothorax dorsal, rotral and lateral spines; D, dorsal; DEAN, *Derilambrus angulifrons*; DIMA, *Distolambrus maltzani*; End, endopod; ENVA, *Enoplolambrus validus*; Exp, exopod; HEGR, *Heterocrypta granulate*; L, lateral; Mxp, maxilliped; nd, no data; PAMA, *Parthenopoides massena*; PAS14, unidentified parthenopid larvae collected in the plankton; PLSE, *Platylambrus serrata*; RHPE, *Rhinolambrus pelagicus*; s, setation; sp, spines; SPMA, *Spinolambrus macrochelos*.

[1]Probably a mistake, it is 2-segmented.

[2]Probably authors' mistakes, some seta overlooked.

Table 2. Morphological comparison of the known megalopa of Parthenopidae

	DEAN Present study	**ENVA Terada (1985)**	**PAMA Thiriot (1973)**	**PAMAa Present study**	**PAMAb Present study**	**PLSE Yang (1971)**
Rostral + Cardiac (sp)	Present	Present	Present	Present	Present	Present
Cardiac (sp) length	Until 4th Pls	Until 5th Pls[1]	Until 3rd Pls	Until 3rd Pls	Until 3rd Pls	Until 2nd Pls
MR + HR	Prominent	No prominent[2]	Prominent	Prominent	Prominent	Prominent + sp
Antenna Pe (s)	2,1,1	3,1,1[3]	2,1,1	2,1,1	2,1,1	1,1,1
Antenna Fl (s)	0,0,0,4,0,3,5	0,4,3,4[4]	0,4,3,3-5[5]	0,0,0,4,0,3,5	0,0,0,4,0,3,5	0,0,0,3,0,3,4[6]
Maxilla Ssc (s)	2D + 1V	nd	3D	2D + 1V	2D + 1-2V	3D/V
5th P Feelers	Absent	1 subterminal	Absent	Absent	Absent	1 subterminal
Uropod (s)	0,4	1,5	0,4–7	0,6	1,6	0,4

Fl, flagellum; HR, hepatic region; MR, mesobranchial region; P, pereiopod; PAMAa, *Parthenopoides massena* megalopa from Balearic Island plankton; PAMAb, *P. massena* megalopa from Gulf of Naples plankton; Pe, peduncle; Pls, pleon somite; Ssc, scaphognathite surface; V, ventral; rest of abbreviations as in Table 1.

[1]Until 3rd Pls according to Kurata & Matsuda (1980).

[2]Prominent and with spines, according to Kurata & Matsuda (1980).

[3]0,1,0, according to Kurata & Matsuda (1980).

[4]0,0,0,2,0,3,4, according to Kurata & Matsuda (1980).

[5]Thiriot (1973) overlooked the segmentation of the first three segments.

[6]Based on the drawing of Fig 8a by Yang (1971).

validus, and now in the present study it is also described for *D. angulifrons*. Although it is early to draw conclusions about the typical morphological characters for megalopa of parthenopids, all known megalopae share the features listed in Table 2. The main distinctive characters are: (i) the presence of well-developed rostral and cardiac spines horizontally directed, (ii) antennal flagellum with seven segments, (iii) 3 simple setae on the scaphognathite surface and (iv) dactylus of fifth pereiopod without feelers (only 1 long seta described in *P. serrata*) and with 3 ventral spines and 1 pair of subterminal spines.

In the present study the megalopa of *Derilambrus angulifrons* is described for the first time based on three specimens collected in the plankton and identified by DNA barcode. These megalopae show all common characters described above as typical of parthenopid megalopae. The main distinctive feature that separates them from the only other known megalopae of the family with an overlapping distribution, *Parthenopoides massena*, is the length of the cardiac spine. In *D. angulifrons* the cardiac spine is longer, exceeding the third somite of the pleon, while that of *P. massena* is shorter and never reaches the third pleonal somite. In the present study, two megalopae of *P. massena* collected in the plankton have also been identified by DNA barcode techniques. Comparing them with the megalopa described by Thiriot (1973) from plankton samples confirmed that the assignment of these megalopae to *P. massena* was correct. Nevertheless, we found one difference between the two megalopae studied: the antennal flagellum is 7-segmented, while Thiriot (1973) described only 4 segments. This fact affects the key for the identification of Mediterranean brachyuran megalopae by Pessani *et al.* (2004) who based the identification of *Parthenopoides massena* (according to Thiriot, 1973) on the number of antennal segments. This dichotomy separates *P. massena* (8–9-segmented) from *Cancer pagurus* Linnaeus, 1758 and two species of *Atelecyclus* Leach, 1814 (11-segmented), the numbers for *P. massena* should be corrected to 10-segmented, which will still make a valid separation possible. A feature not described by Thiriot (1973) is the sternal plate, which in the two specimens studied here has the same setation as *D. angulifrons* (see Figure 4C). In addition, the number of setae of the uropods described by Thiriot (1973) was 0, 4–7, but in the two specimens studied here it was 0, 6 and 1, 6.

With respect to the other larval stages collected in the plankton samples and attributed to Parthenopidae, not all the zoeae described by Rice and Williamson (1977) as ASM16-ASM19 fit exactly with the features mentioned above for parthenopid zoeae. While ASM16 and ASM17 are clearly zoeae II-V of unidentified parthenopids, ASM18 and ASM19 show remarkable differences, for example they have different types of antennae (exopod very reduced), and the spines of the cephalothorax have spinules. ASM18 also differs in the setation of the endopod of the maxillule and second maxilliped, and in the case of ASM19 (zoeae II–III) the telson has a fourth pair of the distal process. Although Rice & Williamson (1977) state that these differences correspond to intergeneric variability and that the specimens definitely belong to the parthenopids we believe that some of the differences, especially those in the mouthpart setation pattern, are not acceptable as intrafamilial variability. Unfortunately there are still a lot of brachyuran families without larval data. Therefore, at this point it is not possible to attribute ASM18 and ASM19 to another family with certainty, although in some aspects they are close to Xanthoidea and Cancroidea.

Paula (1987) described zoea I of unidentified parthenopids as *Parthenope* S14 and Parthenopidae S15. *Parthenope* S14 clearly corresponds to a zoea of Parthenopidae, with a setation of the endopod of the maxillule 0, 2 + 2 + 2. The absence of this seta in the proximal segment was also described in the zoeal stages of *P. massena*, according to Heegaard (1963) and Thiriot (1973), and *D. angulifrons* (see Heegaard, 1963) and *Rhinolambrus pelagicus* (see Ng & Clark, 2000), although it is present in other species (see Table 1). Normally this is not a setation pattern that shows variability at intrafamilial level; therefore, the significance of this variability is not currently easy to evaluate due to the low number of species studied. Kurata and Matsuda (1980) describe '1 rudimentary seta on proximal segment which may be very difficult to see in early stages'; therefore that this seta was overlooked by some authors cannot be discarded. Paula (1987) states that

Parthenopidae S15 resembles ASM19 (Rice & Williamson, 1977); therefore, according to the issues mentioned above, these larvae must not be attributed to this family.

There is also a megalopa collected in the plankton attributed to Parthenopidae by Lebour (1944). She gave a brief description and illustration, and based on the elongated cheliped, long rostral and cardiac spines, and lack of feelers on the dactyl of the fifth pereiopods, it was attributed to Parthenopidae. All these characters support this identification, except the general shape of the cephalothorax and the long chelipeds, as they are very different with respect to the rest of the known megalopae of parthenopids. Especially the chelae that clearly resemble those of the adult forms. It is possible that this stage could be an intermediate anomalous specimen between megalopa and first crab.

Cano (1891) described a megalopa that he assigned to *Goneplax rhomboides* Linnaeus, 1758, but later Ingle & Clark (1983) when they described the complete larval development of *G. rhomboides* showed that Canós megalopa does not belong to this species. However, according to the description, although brief and incomplete, in the figures it is clear that it corresponds to a parthenopid larva because it shares the characters described above for parthenopid megalopa.

Rice (1981) examined the phylogenetic significance of the brachyuran megalopae and commented that this stage was the only phase of the brachyuran life cycle that had not been previously examined for classificatory evidence. Later Martin (1988) studied the phylogenetic significance of the brachyuran megalopa in the case of Xanthidae. It is difficult to apply the megalopa morphology to infer phylogenetic relationships for Parthenopoidea considering that currently there are only known descriptions for five species. The most conspicuous features are the characteristic cephalothorax with long rostral and cardiac spines, and a pair of lobes on the mesobranchial region with hepatic regions moderately inflated. The long rostral and cardiac spines are features shared with Cancridae (see for example the megalopae of *Atelecyclus rotundatus* by Hong & Ingle (1987) and *Cancer pagurus* by Ingle (1981)), but it can be distinguished from them by the number of segments of the antennal flagellum and setae of the uropods, as well as by the absence of feelers on the dactylus of the fifth pereiopod.

Relationships between Parthenopidae and Cancridae have been proposed in the past (Lebour, 1928; Aikawa, 1935) but there have been no new studies on this matter since then. The first molecular phylogeny including data of parthenopids was made in the context of their systematic position with respect to Majoidea (Hultgren & Stachowicz, 2008), where it is clear that there are no relationships with majoids, and in a global phylogeny of Podotremata (Ahyong *et al.*, 2007) where its systematic relationships was not resolved. In both cases, representatives of Cancridae were not included in the molecular phylogenies. However, in a recent exhaustive phylogeny of brachyuran crabs (Tsang *et al.*, 2014) an important number of taxa have been analysed and on this occasion representatives of Crancridae have been included. The results place Parthenopidae in the same clade as Aethridae, Cancridae and Calappidae, with a closer relationship with *Calappa philargius* (Linnaeus, 1758), the only representative of Calappidae. While relationships with Cancridae are as expected those with Calappidae are not supported by larval data.

New data on the larval morphology of more genera of Parthenopinae and representatives of the subfamily Daldorfiinae, as well as new molecular phylogenies comprising members of all Heterotramata superfamilies, with a wider representation of Parthenopidae, Cancridae, Aethridae and Calappidae species are needed to determine the phylogenetic position of this taxon.

ACKNOWLEDGEMENTS

We are especially grateful to Carlos Sánchez Nieto for his assistance in the laboratory work, Catherine Stonehouse for her careful language corrections and Asvin P. Torres (Instituto Español de Oceanografía) and. Maria Grazia Mazzocchi (Stazione Zoologica Anton Dohrn) who helped in collecting the megalopae of *P. massena*.

FINANCIAL SUPPORT

The research was carried out within the framework of the projects MEGALOPADN (CGL2009-11225) and 'Transporte y reclutamiento larvario de crustáceos bentónicos litorales: importancia de los agentes forzadores costeros y regimen mareal' (CTM2005- 00024/MAR), both co-financed by the 'Ministerio de Economía y Competividad (MINECO)' Spanish Plan R + D + I and FEDER. Elena Marco-Herrero acknowledges the pre-doctoral FPI Fellowships supported by MINECO (BES-2010-033297 and EEBB-I-13-07501).

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CAPÍTULO 7

The systematic position of *Ergasticus* (Decapoda, Brachyura) and allied genera, a molecular and morphological approach

E Marco-Herrero, AP Torres, JA Cuesta, G Guerao, F Palero, Pere Abelló (2013) The systematic position of *Ergasticus* (Decapoda, Brachyura) and allied genera, a molecular and morphological approach Zoologica Scripta 42(4): 427-439

Zoologica Scripta

The systematic position of *Ergasticus* (Decapoda, Brachyura) and allied genera, a molecular and morphological approach

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Submitted: 5 December 2012
Accepted: 23 March 2013
doi:10.1111/zsc.12012

Marco-Herrero, E., Torres, A.P., Cuesta, J.A., Guerao, G., Palero, F. & Abelló, P. (2013). The systematic position of *Ergasticus* (Decapoda, Brachyura) and allied genera, a molecular and morphological approach. —Zoologica Scripta, *42*, 427–439.

The complete larval series (zoea I, zoea II and megalopa) of the crab *Ergasticus clouei* is described and illustrated based on plankton samples from Mediterranean waters. The zoea II and megalopal stages, previously unknown, are described here for the first time. Nucleotide sequence analysis of two gene regions (16S rDNA and Cox1 genes) confirmed the assignment of these larvae to *Ergasticus clouei*. The molecular analyses and the morphology of the larval stages revealed large differences between *Ergasticus* and *Inachus*, the type genus of the family Inachidae. In fact, *E. clouei* larvae presented a series of morphological characters, such as antennal shape and mouthparts setation pattern that placed them closer to the family Oregoniidae. The phylogenetic analyses also showed significant support for the monophyly of the Oregoniidae + *Ergasticus* group. The data argue for removal of *Ergasticus* and the related genera (*Bothromaia*, *Pleisticanthoides*, *Parapleisticantha* and *Pleistacantha*) from the Inachidae and their placement within the Oregoniidae as a separate subfamily, Pleistacanthinae Števčić, 2005. Our results demonstrate that larval stages provide reliable morphological traits, independent from those of adults, to help resolving relationships among Majoidea genera.

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Introduction

Members of the crab superfamily Majoidea Samouelle, 1819 comprise one of the most diversified groups within Brachyura (Ng *et al.* 2008). The superfamily is represented by approximately 950 species distributed all around the planet and occupying multiple habitats, from intertidal zones to depths over 1000 m (D'Udekem d'Acoz 1999; Richer de Forges & Poore 2008; De Grave *et al.* 2009). Probably due to this large morphological diversity, members of the Majoidea have had a confusing taxonomic history (Miers 1879; Garth 1958; Griffin & Tranter 1986; Martin & Davis 2001; Ng *et al.* 2008). Current familial and subfamilial classifications in the Majoidea are mostly based on adult morphology, especially on eyestalk or antennal shape and spination patterns (Garth 1958; Griffin & Tranter 1986). However, recent taxonomic revisions seem to suggest that these adult morphological traits may in some cases be incongruent with larval characters (e.g. see Clark &

Webber 1991; Marques & Pohle 2003). In the largest phylogenetic study published to date, including sequences of both mitochondrial (16S, Cox1) and nuclear (28S) markers for 37 majoid species, Hultgren & Stachowicz (2009) found that phylogenetic relationships inferred from genetic data are in some cases incongruent with adult morphology. Most interestingly, the molecular-based analyses corroborated phylogenetic relationships based on larval morphology (Hultgren *et al.* 2009).

Despite larval morphology providing a valuable set of characters to resolve majoid systematics, the larval forms of many species are still undescribed. Most plankton-captured larval stages are not identified to species level due to the scarceness of full larval descriptions and due to the specialized and time-consuming identification work needed for visualizing the precise morphological features. The low variability observed may not allow specific identifications based on morphological criteria even if larval descriptions are available (Ingle 1992). However, with the rapid development of new tools based on molecular analysis, more accurate species identification has been made available in recent years (see Hebert *et al.* 2003). One obvious advantage of DNA barcoding comes from the fact that genetic markers do not change during the ontogeny of the organism. Therefore, molecular-based identification is most useful when there are no obvious means to match adults with larval stages or when larval rearing cannot be completed (Palero *et al.* 2008; Ampuero *et al.* 2010).

Ergasticus clouei A. Milne-Edwards, 1882 is a rare majoid crab and the only known species of the genus (Ng *et al.* 2008). Specimens have been reported along the western coasts of Africa and Europe, from Cape Verde Islands to the Bay of Biscay, including the Açores, Madeira and the Canary Islands as well as throughout the Mediterranean (Zariquiey Álvarez 1968; Manning & Holthuis 1981; Guerao & Abelló 2007). *Ergasticus* has been recorded within a large bathymetric range, from 70 to 1000 m (D'Udekem d'Acoz 1999), but it is mostly found between 250 and 800 m, that is, from the continental shelf break to the upper and middle slope (Manning & Holthuis 1981; Abelló *et al.* 2002). The precise biogeographic range of the species is not yet fully understood due to the sparse captures in benthic or epibenthic samples. Hardly anything is known about the biology and life history of the species. As in most Majoidea, *E. clouei* shows a strong sexual dimorphism in claw length, being much longer and stronger in adult males than in females (Zariquiey Álvarez 1968). Even though no information is available on the reproductive biology of the species, ovigerous females have been recorded in May, June and July.

On the basis of adult characters, *Ergasticus* has been traditionally assigned to the Inachidae MacLeay, 1838 (Balss 1957; Manning & Holthuis 1981; Ng *et al.* 2008), although some authors have placed it within the Pisinae Dana, 1851 (Bouvier 1940; Zariquiey Álvarez 1968). Inachid crabs are grouped together mostly for showing eyes without orbits and generally long eyestalks, either non-retractile or retractile against the sides of carapace or against an acute postocular spine affording no concealment (Garth 1958; Manning & Holthuis 1981; Griffin & Tranter 1986). Although adult *Ergasticus* fall within the current adult definition of Inachidae, given that their eyes are retractile against an acute postocular spine, a recent study based on the morphology of the first zoeal stage questioned its systematic position (Guerao & Abelló 2007). However, given the difficulties found in reaching further zoea and megalopa stages, the results obtained in that study were limited and prevented the assessment of *Ergasticus* systematics.

The present study aims at resolving the uncertainties on the assignment of *Ergasticus* to the Inachidae by describing the complete morphology of all its larval stages, identified through DNA analyses of plankton samples and comparing them to previous descriptions of the larval stages of other majoid genera. Furthermore, a complete phylogenetic analysis was performed, including DNA sequences from representatives of several majoid families.

Material and methods

Sampling methods

Two multidisciplinary research surveys were conducted on board R/V 'Sarmiento de Gamboa' off the Balearic Archipelago (western Mediterranean) during late autumn (29 November to 18 December 2009) and summer (11 to 30 July 2010). These surveys aimed, among other objectives, at studying the meroplankton communities found at two stations over 200 and 900 m depth (shelf break and middle slope, respectively). These stations were located west and south of Mallorca Island (Balearic and Algerian sub-basins, respectively) and belong to areas with distinct water masses and different environmental conditions (Pinot *et al.* 2002; López-Jurado *et al.* 2008).

A total of 218 depth-stratified mesozooplankton samples were collected using a multinet HYDRO-BIOS in 2009 and a Multiple Opening–Closing Net and Environmental Sensing System (MOCNESS) in 2010 (Olivar *et al.* 2012). The mouth opening of these nets was 0.25 and 1 m^2, respectively, and their mesh size was 333 μm. Both devices were towed at ~2 knots, performing oblique-stratified hauls from near bottom to the surface. A total of 66 macrozooplankton samples were collected using an Isaac-Kidd midwater trawl (IKMT) of 3 m^2 with a codend mesh size of 3 mm. The fishing speed was three knots and the effective tow duration was 30 min. Immediately after collection, IKMT samples were preserved in ethanol 96%, while the remaining samples were stored and fixed in buffered 5%

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formalin, because they were mainly aimed for ichthyoplankton studies. Once in the laboratory, decapod crustacean larvae were sorted and identified to species level and developmental stage whenever possible, using available descriptions and keys (Dos Santos & Lindley 2001; Dos Santos & Gonzalez-Gordillo 2004; Pessani *et al.* 2004). In total, two zoeae I, four zoeae II and two megalopae were recorded and tentatively assigned to an unidentified majoid species.

Samples from several adult majoid species were collected independently by demersal trawling during a fishery research survey (MEDITS-ES-2003) carried out in May 2003 along the western Mediterranean on board R/V 'Cornide de Saavedra'. In particular, two *E. clouei* individuals were collected off Almeria near Cape Gata at depths between 500 and 600 m and kept in ethanol 96%. Another adult specimen of *E. clouei* was also collected by an epibenthic beam trawl off Mallorca during the IDEADOS-2010 research survey in July 2010 on board F/V 'Punta des Vent'. The *E. clouei* adult specimens included in this study have been deposited at the Biological Collections of Reference of the Institut de Ciències del Mar (CSIC) in Barcelona under accession numbers ICMD290/1994 and ICMD13032201.

Morphological descriptions

Drawings and measurements were made using a Wild MZ6 and Zeiss Axioskop (Carl Zeiss Microscopy, Jena, Germany) compound microscope with Nomarski interference, both equipped with a *camera lucida*. All measurements were made using an ocular micrometer. Descriptions are based on two zoeae I, three zoeae II and two megalopae, and measurements of different larval stages are based on all specimens obtained. The following measurements were taken for the zoeal stages: cephalothoracic dorsal spine length (DL) distance measured from base to tip of the dorsal spine; cephalothoracic rostral spine length (RL) distance measured from base to tip of the rostral spine; rostrodorsal length (RDL) distance measured from the tip of the rostral spine to the tip of the dorsal spine; cephalothorax length (CL) measured from between eyes (base of the rostrum) to the postero-lateral cephalothorax margin; cephalothorax width (CW) measured from the tip of one lateral spine to the tip of the other lateral spine. For the megalopa: cephalothorax length (CL) measured from the base of rostrum to posterior margin of cephalothorax; cephalothorax total length (CTL) measured from the tip of the rostrum to posterior margin of cephalothorax and cephalothorax width (CW) as the cephalothorax maximum width (excluding the hepatic protuberances).

The larvae are described using the basic malacostracan somite plan from anterior to posterior, and appendage segments are described from proximal to distal, endopod then exopod (Clark *et al.* 1998). All larval specimens have been dissected and used for descriptions, with the exception of one zoea II that has been deposited at the Centre Oceanogràfic de les Balears in Palma de Mallorca (Spain), with catalogue number ID2-0710-E9N2-ZII.

DNA extraction, amplification and sequencing

The identification of larval stages was based on partial sequences of the 16S rDNA and Cox1 genes. Total genomic DNA was extracted from muscle tissue from two pereiopods of one megalopa, from the pleon of one zoea II, and from one pereiopod of each of the three adult specimens of *Ergasticus clouei*, and incubated for 1–24 h in 300 μL lysis buffer at 65 °C. Protein was precipitated by addition of 100 μL of 7.5 M ammonium acetate and subsequent centrifugation, and DNA precipitation was obtained by addition of 300 μL of isopropanol and posterior centrifugation. The resulting pellet was washed with ethanol (70%), dried and finally resuspended in Milli-Q distilled water (Merck Millipore, Darmstadt, Germany).

Target mitochondrial DNA from the 16S rRNA and Cox1 genes was amplified with polymerase chain reaction (PCR) using the following cycling conditions: 2 min at 95 °C, 40 cycles of 20 s at 95 °C, 20 s at 45–48 °C, 45 s (16S) or 47 s (Cox1) at 72 °C and 5 min 72 °C. Primers 1472 (5′- AGA TAG AAA CCA ACC TGG -3′) (Crandall & Fitzpatrick 1996) and 16L2 (5′-TGC CTG TTT ATC AAA AAC AT-3′) (Schubart *et al.* 2002) were used to amplify 540 bp of 16S, while primers COH6 (5′- TAD ACT TCD GGR TGD CCA AAR AAY CA -3′) and COL6b (5′- ACA AAT CAT AAA GAT ATY GG -3′) (Schubart & Huber 2006) allowed amplification of 670 bp of Cox1. PCR products were sent to New Biotechnic and Biomedal companies to be purified and then bidirectionally sequenced.

Sequences were edited using the software CHROMAS version 2.0 (Technelysium Pty Ltd, Brisbane, Australia). Adult and larval sequences for both genes are deposited in Genbank under accession numbers KC866326-KC866329 and KC866335-KC866338. The obtained DNA sequences were compared with sequences from adult specimens of several Iberian brachyuran crabs obtained in the context of the MEGALOPADN project or from public databases (Table S1).

Phylogenetic analyses and hypothesis testing

In order to carry out a complete phylogenetic analysis, alignments of each gene data set were conducted using MUSCLE v3.6 (Edgar 2004). To avoid alignment ambiguity for the 16S rDNA gene, gaps and hypervariable regions were excluded from further analysis using GBLOCKS software

v0.91b (Castresana 2000). The combined selection of the best-fit partitioning scheme for the alignment and the nucleotide substitution model for each partition was carried out using the new objective method implemented in PARTITIONFINDER (Lanfear *et al.* 2012). The BEAST software (Drummond & Rambaut 2007) was used to infer phylogenetic relationships among samples (two independent runs starting from a random tree; estimated base frequencies; Yule tree prior; 50 000 000 generations, sampling every 1000th tree with a 10% burn-in) and to generate consensus data from the posterior trees.

It has been shown that the 'uncorrelated relaxed-clock' models, in which the mutation rates in each branch are allowed to vary within particular constraints, perform better than strict molecular clock or the correlated models (Drummond *et al.* 2006). Therefore, the Bayesian relaxed-clock uncorrelated lognormal approach was used here as implemented in BEAST v1.7.4 (Drummond & Rambaut 2007) with the corresponding model of sequence evolution previously inferred for each gene partition.

Other than the unconstrained search, BEAST runs were carried out using the same conditions, but including a constrained search in order to test the hypothesis of the genus *Ergasticus* belonging to the same monophyletic clade as the other inachid genera analysed (i.e. *Macropodia* Leach, 1814; *Podochela* Stimpson, 1860; *Inachus* Weber, 1795 and *Metoporhaphis* Stimpson, 1860). The Bayes factor approach was used to compare the different models (Nylander *et al.* 2004), evaluating the hypothesis (H0) that our constrained and unconstrained topologies explain the data equally well, vs. the alternative hypothesis (H1) that constrained BI searches provide a poorer explanation of the data. The Bayes factor is calculated as twice the difference in the harmonic mean 2 lnL scores (2 ln B01) between alternative hypotheses (Brandley *et al.* 2005) and these values are compared to the framework provided by Kass & Raftery (1995) where <0 is evidence against H1, 0–2 provides no evidence for H1, 2–6 is positive support for H1, 6–10 is strong support for H1, and >10 is very strong support for H1 (see Nylander *et al.* 2004; Brandley *et al.* 2005).

Results

Among the decapod crustacean larvae found in the samples, those referred to *Ergasticus clouei* were captured during July 2010 at shelf break station located south of Mallorca. The zoeal stages were captures with MOCNESS, between 250 and 100 m, and the megalopa stage was captured with IKMT from 272 m to surface.

Larval description

The zoea I is completely redescribed and in subsequent stages only differences are highlighted.

Ergasticus clouei A. Milne-Edwards 1882 (Figs 1–6).

Zoea I. Size: RDL = 2.69–2.90 mm; CL = 1.05–1.06 mm; CW = 1.40 mm; RL = 0.61–0.66 mm; DL = 1.33–1.27 mm; $N = 2$.

Cephalothorax (Fig. 1A). With long dorsal spine, strongly curved distally backwards without setae; rostral spine slightly longer than antenna; lateral spines present; each latero-ventral margin with one densely plumose 'anterior seta', followed by two additional sparsely plumose setae; one pair of antero-dorsal setae, one pair of postero-dorsal setae present; eyes sessile.

Antennule (Fig. 2A). Uniramous, smooth, conical; endopod absent; exopod unsegmented with four terminal aesthetascs of different diameter/width and 1 minute seta.

Antenna (Fig. 2D). Biramous, protopod very long with two rows of spinules (one with 13–15 spinules of different sizes, second with only 4 minute spinules); endopod bud present; one-segmented exopod shorter than the spinous process, with two unequal subterminal setae.

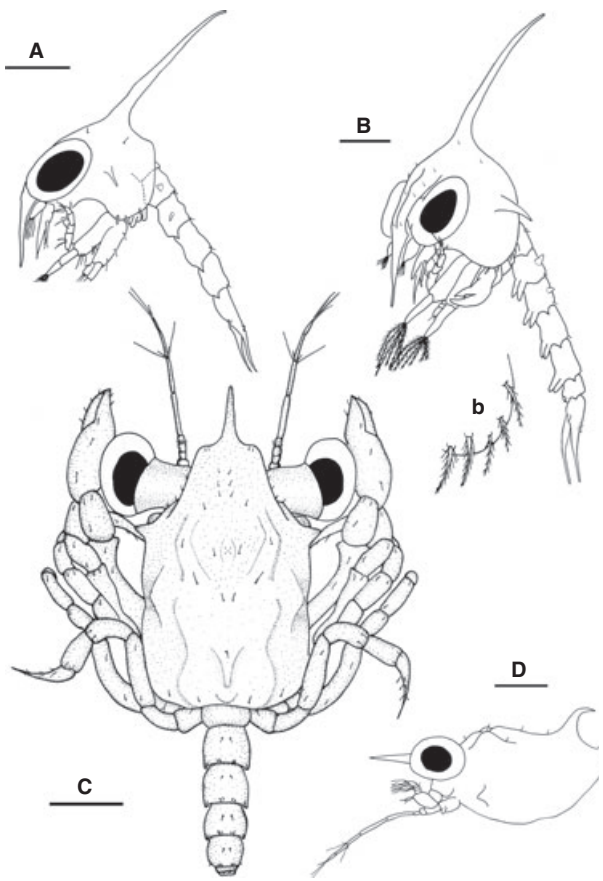

Fig. 1 *Ergasticus clouei*. General lateral view, —A. zoea I; —B. zoea II. Ventral margin of cephalothorax detail, b: zoea II. Megalopa, —C. dorsal view; —D. lateral view. Scale bars = 0.5 mm.

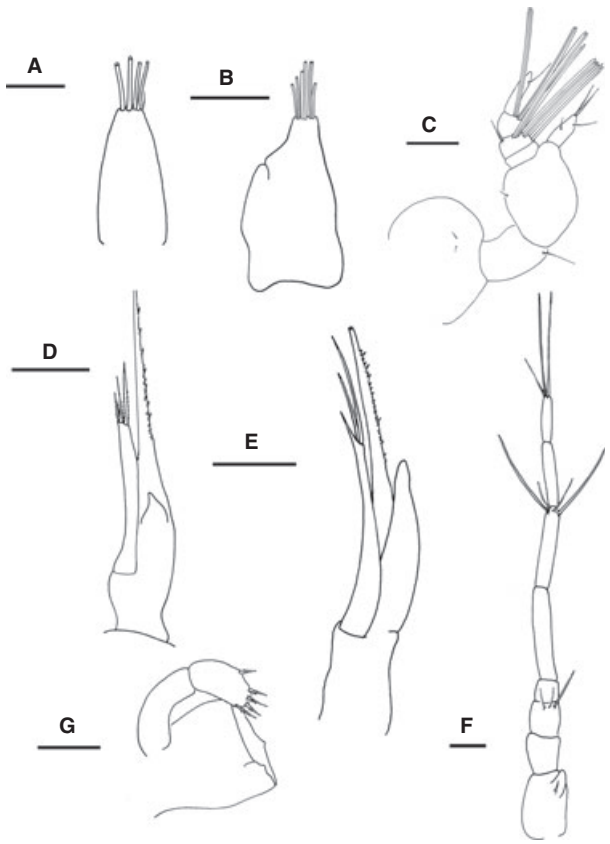

Fig. 2 *Ergasticus clouei*. Antennule, —A. zoea I; —B. zoea II; —C. megalopa. Antenna, —D. zoea I; —E. Zoea II; —F. megalopa. Mandible, —G. megalopa. Scale bars = 0.1 mm.

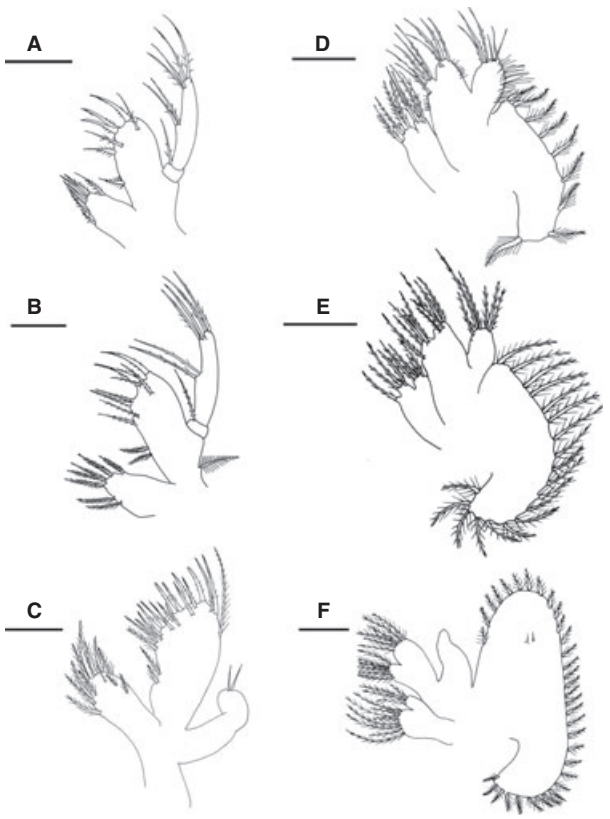

Fig. 3 *Ergasticus clouei*. Maxillule, —A. zoea I; —B. zoea II; —C. megalopa. Maxilla, —D. zoea I; —E. zoea II; —F. megalopa. Scale bars = 0.1 mm.

Mandible. Incisor and molar processes differentiated; mandibular palp (endopod) absent.

Maxillule (Fig. 3A). Coxal endite with seven setae; basial endites with seven setae (four cuspidate); endopod two-segmented, proximal segment with one seta, distal segment with two medial, two subterminal and two terminal setae; exopodal seta absent.

Maxilla (Fig. 3D). Coxal endite bilobed with 4 + 4 setae; basial endite bilobed with 5 + 4 setae; unsegmented endopod not bilobed, with six terminal setae and microtrichia on lateral margin; exopod (scaphognathite) margin with nine plumose setae, including distal process.

First maxilliped (Fig. 4A). Coxa with one seta; basis with 10 setae arranged 2 + 2 + 3 + 3; endopod five-segmented with 3, 2, 1, 2, 5 (one subterminal, four terminal) setae, respectively; exopod incipiently two-segmented, distal segment with four terminal long natatory plumose setae.

Second maxilliped (Fig. 4B). Coxa without setae; basis with three setae arranged 1 + 1 + 1; endopod three-segmented with 0, 1, 6 setae, respectively; exopod incipiently two-segmented, distal segment with four terminal long natatory plumose setae.

Third maxilliped. Present as small bud.

Pereiopods. Present as small buds.

Pleon (Figs 1A and 5A). With five pleomeres; pleomeres 2 and 3 with one pair of dorso-lateral processes; pleomeres 3–5 with one pair of short postero-lateral processes; pleomere 1 without setae, pleomeres 2–5 with one pair of postero-dorsal setae; pleopods absent.

Telson (Fig. 5A, a). Telson furcae with one pair of ventral and two pairs of dorsal spines; inner margin with three pairs of serrulate setae.

Zoea II. Size: RDL = 3.34–3.97 mm; CL = 1.08–1.51 mm; CW = 1.71 mm; RL = 0.84–0.98 mm; DL = 1.61–2.02 mm; $N = 3$.

Cephalothorax (Fig. 1B, b). Antero-median region with five pairs of setae, one pair of setae near the base of dorsal spine; each latero-ventral margin with two additional setae (one plumose + one sparsely plumose). Eyes stalked.

Antennule (Fig. 2B). Exopod with seven aesthetascs; endopod bud present.

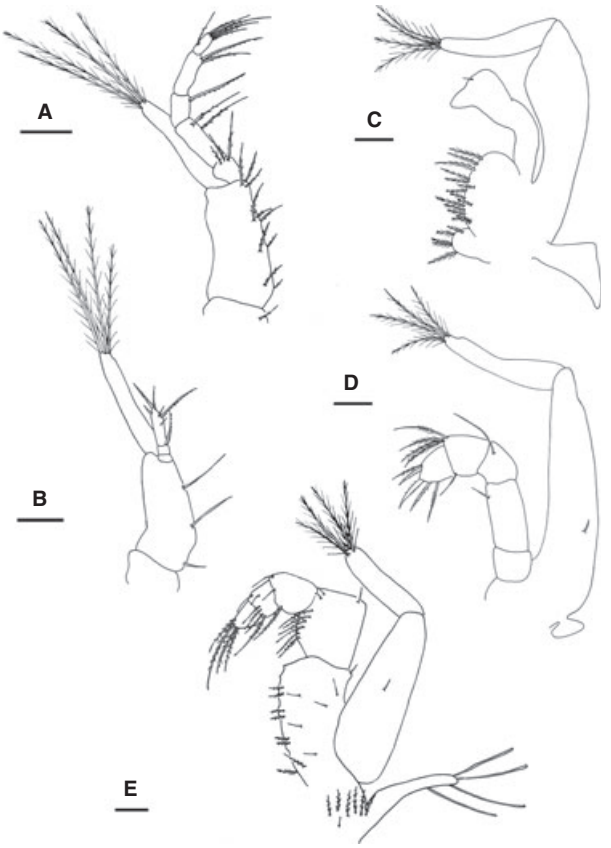

Fig. 4 *Ergasticus clouei*. First maxilliped, —A. zoea I; —C. megalopa. Second maxilliped, —B. zoea I; —D. megalopa. Third maxilliped, —E. megalopa. Scale bars = 0.1 mm.

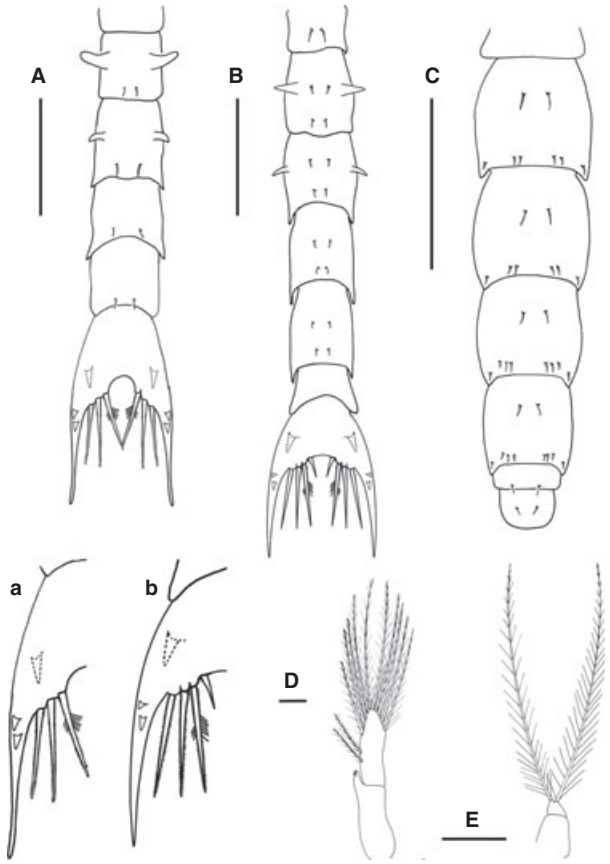

Fig. 5 *Ergasticus clouei*. Pleon, dorsal view, —A. zoea I; —B. zoea II; —C. megalopa. Detail telson, a: zoea I; b: zoea II. Megalopa, —D. 3rd pleopod; —E. uropod. Scale bars A–C = 0.5 mm, D–E = 0.1 mm.

Antenna (Fig. 2E). Endopod longer, almost reaching half length of protopod. Protopod with two rows (one with 20–22 spinules of different sizes, and another one with only 4–5 minute spinules).

Mandible. Palp bud present.

Maxillule (Fig. 3B). Basial endite with 10 setae (five cuspidate); one long plumose exopodal seta on outer margin.

Maxilla (Fig. 3E). Basial endite with 5 + 5 setae; scaphognathite with 19–20 marginal plumose setae.

First and second maxillipeds. Exopod distal segment with six long plumose natatory setae.

Third maxilliped and pereiopods. More prominent buds than in first stage; cheliped bilobed.

Pleon (Figs 1B and 5B). With six pleomeres, first pleomere with two long mid-dorsal setae, pleomeres 2–5 with one pair of mid-dorsal simple setae; pleomeres 2–5 with long pleopod buds, endopod buds present.

Telson (Fig. 5B, b). Inner margin with one pair of additional setae.

Megalopa

Size: CL = 1.90–1.85 mm; CTL = 2.31–2.30 mm; CW = 1.21–1.24 mm; $N = 2$.

Cephalothorax (Fig. 1C, D). Longer than broad, with long, thin and straight rostrum; hepatic regions with one anterior subacute tubercle; each protogastric region with dorsally directed blunt process with two setae; one tubercle on mesogastric region and posterodorsal margin; prominent long curved spine present on cardiac region; setation as drawn. Dorsal organ present. Eyes stalked.

Antennule (Fig. 2C). Peduncle three-segmented with 2, 1, 1 simple setae; unsegmented endopod with one medial, one subterminal and two terminal simple setae; exopod four-segmented with 0, 1, 0, 1 simple setae, second segment with 11 and third segment with four aesthetascs.

Antenna (Fig. 2F). Peduncle three-segmented with 1, 0, 3 simple setae, respectively, proximal segment with stout and ventrally directed process; flagellum five-segmented with 0, 0, 4, 0, 3 simple setae, respectively.

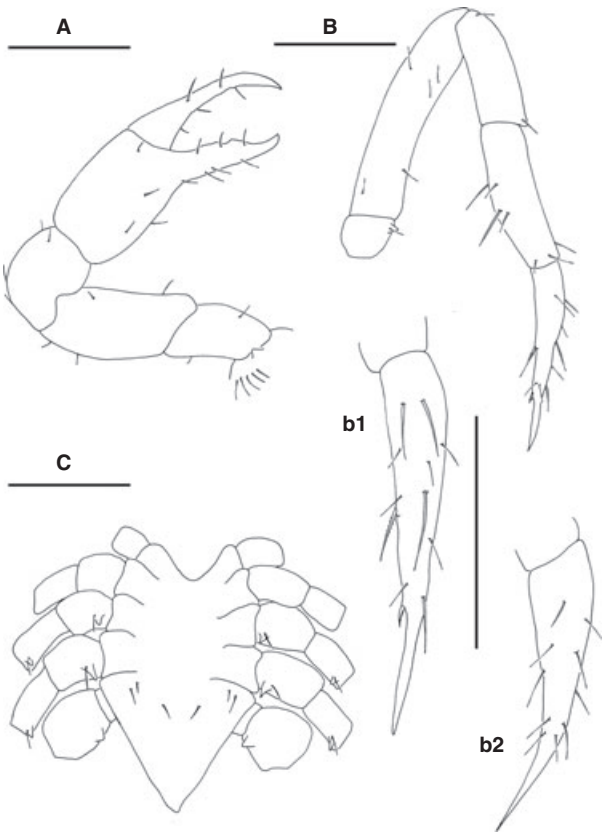

Fig. 6 *Ergasticus clouei*. Megalopa, —A. cheliped; —B. 3rd pereiopod, b1: dactylus II; b2: dactylus V; —C. sternum. Scale bars = 0.5 mm.

Mandible (Fig. 2G). Palp two-segmented with five terminal plumo-denticulate setae on distal segment.

Maxillule (Fig. 3C). Coxal endite with five subterminal plumose setae and seven plumose setae on margin; basial endite with 20 setae: six marginal cuspidate, nine subterminal plumo-denticulate and five proximal plumose setae; endopod unsegmented with two terminal setae.

Maxilla (Fig. 3F). Coxal endite bilobed with 8 + 4 terminal plumose setae; basial endite bilobed with 6 + 6 sparsely plumodenticulate setae; endopod unsegmented and without setae; exopod (scaphognathite) with 34 marginal plumose setae and one small simple seta on each lateral surface.

First maxilliped (Fig. 4C). Epipod triangular shaped without setae; coxal endite with five plumose setae; basial endite with 14 sparsely plumodenticulate setae; endopod reduced, unsegmented with one subterminal seta; exopod two- segmented, with four terminal plumose setae on distal segment.

Second maxilliped (Fig. 4D). Epipod reduced without setae; protopod without setae; endopod five-segmented with 0, 1 (simple), 1 (long simple), 5 (plumo-denticulate) and 5 (two cuspidate, three plumo-denticulate) setae; exopod two-segmented, with one submedial simple seta on proximal segment and four terminal plumose setae on distal segment.

Third maxilliped (Fig. 4E). Epipod relatively small with one subterminal and three terminal long setae; protopod with seven plumo-denticulate setae; endopod five-segmented, with 14, 9, 3, 6, 4 setae, respectively, ischium with denticulate margin; exopod two-segmented with one medial simple seta on proximal segment and four terminal plumose setae on distal segment.

Pereiopods (Figs 1C and 6A, B, b1, b2). Cheliped with a small proximal ventral tubercle on coxa, setation as drawn; pereiopods 2–5 slender and setose, with dactyli terminally acute; each ischium of pereiopods 2–3 with small spine; pereiopods 2–4 each with one plumo-denticulate seta and one stout serrulate spine on inner margin of dactylus. Setation as illustrated.

Sternum (Fig. 6C). Setation as illustrated.

Pleon (Figs 1C and 5C). Six pleomeres plus telson; pleomere 1 without setae; setation of pleomeres 2–6 as shown; sixth pleomere reduced.

Pleopods (Figs 5D,E). Present on pleomeres 2–5; endopods unsegmented with three cincinnuli; exopod unsegmented with 12 long plumose natatory setae. Uropods two-segmented, proximal segment without setae, distal segment with two terminal plumose natatory setae.

Telson (Fig. 5C). Small with one pair of dorsal setae.

DNA analysis

The initial length of the aligned dataset for the 16S rRNA and Cox1 genes was 446 and 611 bp, respectively. After running GBlocks, a total of 1027 positions were kept for further analyses (97% of the original 1057 positions). The best-fit partitioning scheme for the alignment included four partitions (one per codon position within Cox1 and another partition including the 16S gene region) and the nucleotide substitution models selected for each partition were TrNef+G (COI_1st), HKY+I (COI 2nd), HKY+G (COI 3rd) and GTR+I+G (16S rRNA). The performance of the BEAST runs was assessed using TRACER v1.5 (http://tree.bio.ed.ac.uk/software/tracer/), a freeware graphical tool for visualization and diagnostics of MCMC output. The effective sample size was >200 in all BEAST runs, indicating convergence of the MCMC chains. The consensus phylogenetic tree (Fig. 7) showed a highly significant clustering of the zoea and megalopa with the adult *Ergasticus clouei*, pointing out the actual identity of the larvae.

In order to test the statistical support for previously established hypotheses (i.e. *Ergasticus* belongs to Inachidae), Bayes factors were computed comparing the tree topology obtained under the unconstrained model against the constrained topologies. The log-likelihood values obtained from

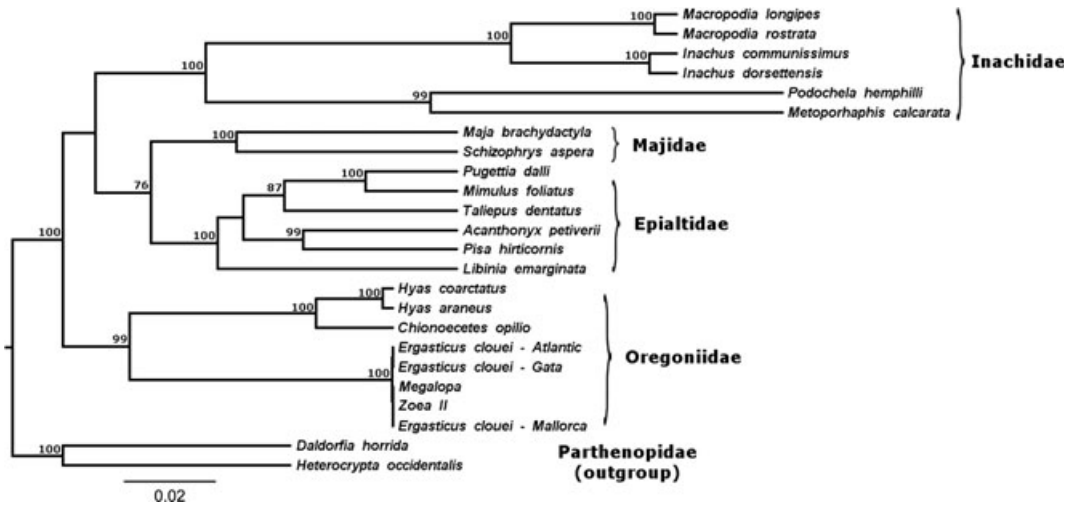

Fig. 7 Phylogenetic tree based on 16S rRNA and Cox1 genes sequence data set, showing the position of the larval specimens genetically analysed.

the unconstrained tree (-8258.55 ± 0.78) were significantly larger than those obtained from the constrained tree (-8262.9 ± 1.07). According to the large Bayes factor obtained (BF = 8.70), it can be concluded that there is strong support for the removal of *Ergasticus* from the Inachidae and its clustering with the Oregoniidae Garth, 1958.

Discussion

The present study describes for the first time the complete larval development of *Ergasticus clouei* thanks to the use of DNA barcoding methods on larvae collected from the plankton. The genetically identified larval stages of *E. clouei* show the general characteristics listed by Rice (1980) for Majoidea larvae: presence of two zoeal stages, with at least nine marginal setae on the scaphognathite of the first zoea and with developed pleopods in the second zoea. However, the morphology of the larval stages of *E. clouei* did not fit into the typical Inachidae as defined at present (see Marco-Herrero *et al.* 2012; Marques & Pohle 2003; Rice 1980). Clear differences were found, such as the presence of two subterminal setae in the exopod of the antenna, rostral and lateral carapace spines, additional spines on the telson furcae, subterminal setae on the distal endopod segment of the maxillule or the basis of first maxilliped with 2 + 2 + 3 + 3 setae (see Table 1).

In relation with this, the detailed description carried out in this study allowed us to notice a remarkable character of *E. clouei* zoeal stages unnoticed by Guerao & Abelló (2007): the presence of furcal spines in the ventral side of the telson. After a thorough review of the literature, this spine was found most likely to be homologous to the large lateral spine present in some non-inachid Majoidea (Rice 1980; Ingle 1992). If this is the case, the number of spines of the telson would be a character shared by *E. clouei* with the Majidae Samouelle, 1819, members of which also possess three spines (one large and two small) on each telson furca, but all in a lateral position (Rodriguez 2002; Guerao *et al.* 2008).

The fact that rostral and lateral spines are not found in Inachidae, but are present in Oregoniidae (Table 2), Majidae and some Pisinae (see Santana *et al.* 2004), that the setation pattern of the basis of the first maxilliped (2 + 2 + 3 + 3) is present in Oregoniidae and some Pisinae but not in Inachidae and that the presence of three spines on the telson furcae is also observed in Majidae (but not in Inachidae) further supports the necessity to remove *Ergasticus* from the Inachidae. The morphology of the larval antenna seems to be particularly important in this regard. As described by Clark & Webber (1991) for the Japanese giant spider crab *Macrocheira kaempferi* (Temminck, 1836), the antennal type observed in *Ergasticus* (with two subterminal setae on the exopod) is found in Oregoniidae and Majidae genera, but not in Inachidae (see Pohle 1991; Rodriguez 2002). Finally, the fact that larvae of Oregoniidae and *E. clouei* are the only majoids that possess two mid-dorsal setae on the fifth pleomere of the second zoea and a five-segmented antennal flagellum in the megalopa indicates that they are closely related. As such, *E. clouei* should be placed in Oregoniidae because it shares with them more characters than with any other majoid families.

In agreement with the present review of larval morphology and contrary to our expectations given the current classification of the Majoidea, the molecular phylogenetic analyses also did not show *E. clouei* grouping with the tested inachid genera (*Macropodia*, *Podochela*, *Inachus* and *Metoporhaphis*). Instead, both the larvae and adult *E. clouei* sequences clustered with Oregoniidae genera such as

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Table 1 Morphological comparison between larval stages of selected Inachidae genera (according to Ng *et al.* 2008)

								Inachidae *s.s.*			
Taxa	*Cyrtomaia owstoni*	*Ergasticus clouei*	*Eurypodius latreille*	*Macrocheira kaempferi*	*Platymaia wyvillethomsoni*	*Pleistacantha sanctijohannis*	*Stenorhynchus lanceolatus S. seticornis**	*Achaeus cranchii** *A. tuberculatus*	*Inachus thoracicus*	*Macropodia czernjwaskii*	*Podochela riisei*
Zoea I											
Cephalothorax rsp	Present	Present	Present	Present	Present	Present	Absent	Absent	Absent	Absent	Absent
Cephalothorax lsp	Present	Present	Present	Present	Absent	Present	Present (3) Absent*	Absent	Absent	Absent	Absent
Antenna exopod (s)	2 subt	2 subt	2 subt	2 subt	2 subt	2 subt	2 medial	2 medial	2 medial	2 medial	2 medial
Maxillule endopod (s)	1, 2 + 4	1, 2 + 4	1, 2 + 4	1, 2 + 4	1, 2 + 4	1, 2 + 4	0, 4	0, 4*	0, 4	0, 3	0, 4
Maxilla endopod (s)	4(?)	6	6	6	5	6	5	3*	4	4	4
Maxilla coxal end (s)	5 + 4	4 + 4	4 + 4	4 + 4	5 + 4	nd	4 + 4	nd	3 + 4	3 + 4	3 + 4
Maxilliped 1 basis (s)	0 + 1 + 2 + 2(?)	2 + 2 + 3 + 3	2 + 2 + 3 + 3	2 + 2 + 3 + 3	2 + 2 +2 + 3	nd	2 + 2 + 2 + 3	2 + 2 + 3 + 3*	2 + 2 + 2 + 3	2 + 2 + 2 + 3	2 + 2 + 2 + 3
Maxilliped 2 basis (s)	2 (?)	3	2 (3 in ZII)	3	3	nd	3	0*	1	1	2
Pleomere 3 dlp	Present	Present	Present	Present	Present	Present	Present	Absent	Absent	Absent	Absent
Zoea II											
Pleomere 4 (mds)	nd	Present	Present	Absent	Present	nd	Absent	nd	Absent	Absent	nd
Pleomere 5 (mds)	nd	Present	Present	Absent	Present	nd	Absent	nd	Absent	Absent	nd
Pleomere 6	nd	Present	Present	Present	Present	nd	Present	Absent	Absent	Absent	nd
Telson inner margin (ss)	nd	4 + 4	4 + 4	3 + 3	4 + 4	nd	3 + 3	nd	3 + 3	3 + 3	nd
Megalopa											
Antennule endopod (s)	nd	4	0, 6	nd	nd	nd	3	nd	0	0	nd
Antenna flagellum (se)	nd	5	5	nd	nd	nd	4	3	3–4	3–4	nd
Pleomere 6	nd	Present	Present	nd	nd	nd	Present	Absent	Absent	Absent	nd

An asterisk is used to indicate the different character states observed when studying several species of the same genus; dlp, dorsolateral processes; lsp, lateral spine; mds, mid-dorsal setae; nd, no data; rsp, rostral spine; s, setae; se, segments; subt, subterminal; ss, serrulate setae.

References: *Achaeus tuberculatus* (see Kurata 1969), *Achaeus cranchii* (see Ingle 1992), *Cyrtomaia owstoni* (see Iwata *et al.* 1991), *Ergasticus clouei* (see Guerao & Abelló 2007 and present study), *Eurypodius latreille* (see Campodonico & Guzman 1972), *Inachus thoracicus* (see Guerao *et al.* 2002), *Macrocheira kaempferi* (see Clark & Webber 1991), *Macropodia czernjwaskii* (see Marco-Herrero *et al.* 2012), *Platymaia wyvillethomsoni* (see Oh & Ko 2011), *Pleistacantha sanctijohannis* (see Kurata 1969), *Podochela riisei*, (see Yang 1967), *Stenorhynchus lanceolatus* (see Paula & Cartaxana 1991), and *Stenorhynchus seticornis* (see Yang 1976).

Table 2 Morphological comparison between larval stages of *Ergasticus clouei*, Oregoniidae (represented by the genera *Chionocetes* and *Hyas*) and Inachidae (represented by the genera *Inachus* and *Macropodia*) showing only those characters shared with oregoniids and that differ in inachids (there were no characters shared with inachids that would differ in oregoniids)

Taxa	*Ergasticus clouei*	Oregoniidae *Chionocetes Hyas*	Inachidae *Inachus Macropodia*
Zoea I			
Cephalothorax rsp	Present	Present	Absent
Cephalothorax lsp	Present	Present	Absent
Antenna exopod (s)	2 subterminal	2 subterminal	2 medial
Maxillule endopod (s)	1, 2 + 4	1, 2 + 4	0, 4
Maxilla endopod (s)	6	6	4, 5
Maxilla coxal end (s)	4 + 4	4 + 4	3 + 4
Maxilliped 1 basis (s)	2 + 2+3 + 3	2 + 2+3 + 3	2 + 2+2 + 3
Maxilliped 2 basis (s)	3	>1	$\leq$1
Pleomere 3 dlp	Present	Present	Absent
Zoea II			
Pleomere 4 (mds)	Present	Present	Absent
Pleomere 5 (mds)	Present	Present	Absent
Pleomere 6	Present	Present	Absent
Telson inner margin (ss)	4 + 4	4 + 4	3 + 3
Megalopa			
Antennule endopod (s)	4	4	0
Antenna flagellum (se)	5	5	3–4
Pleomere 6	Present	Present	Absent

dlp, dorsolateral processes; lsp, lateral spine; mds, mid-dorsal setae; rsp, rostral spine; s, setae; se, segments, ss, serrulate setae.

References: *Ergasticus clouei* (see Guerao & Abelló 2007 and present paper); *Chionocetes, C. opilio* (see Motoh 1973), *C. japonicus* (see Motoh 1976); *Hyas, H. araneus* (see Christiansen 1973; Pohle 1991), *H. ursinus* (see Kornienko & Korn 2010); *Inachus, I. dorsettensis* (see Ingle 1977), *I. thoracicus* (see Guerao *et al.* 2002); *Macropodia, M. parva* (see González-Gordillo & Rodríguez 2001), *M. czernjwaskii* (see Marco-Herrero *et al.* 2012).

Chionoecetes Krøyer, 1838 and *Hyas* Leach, 1814. Our results using the Bayes Factor approach on alternative phylogenetic hypotheses showed strong support for the removal of *Ergasticus* from the Inachidae and the clustering of *Ergasticus* with the Oregoniidae.

As pointed out by Griffin & Tranter (1986), the limitations on the definition of the Inachidae based on few adult traits have caused the family to become cluttered over the years by various species with long eyestalks, but not necessarily resembling other inachids in other characteristics. Based on larval morphology, Oh & Ko (2011) have recently suggested that *Platymaia wyvillethomsoni* Miers, 1886 as well as *Pleistacantha sanctijohannis* Miers, 1879 (larvae described by Kurata 1969) are closer to *Macrocheira kaempferi* than to any other Inachidae (see Table 1). They proposed that *P. wyvillethomsoni* should not be placed within the Inachidae, although they did not suggest a new placement, stating that 'future investigations should check their taxonomic status'. In a review of the Inachoididae Dana, 1851, Guinot (2012) has also proposed changes in the generic composition of the Inachidae. She advocated the transfer of *Stenorhynchus* Lamarck, 1818, from Inachidae to Inachoididae (resurrecting Stenorhynchinae Dana, 1851) and also suggested a reappraisal of Inachidae to reinstate the subfamilies Inachinae Macleay, 1838, Podochelinae Neumann, 1878 and Anomalopodinae Stimpson, 1871.

During a recent visit to the Natural History Museum (NHM) in London, one of the authors (FP) was able to review the adult morphology of several Inachidae genera available in the NHM collections. The shape of the male first gonopod, which is commonly used as a key character in majoid systematics, had never been described in *Ergasticus*. This ongoing revision of adult morphology clearly showed that the three Inachidae genera, *Ergasticus*, *Bothromaia* Williams & Moffitt, 1991 and *Pleistacantha* Miers, 1879 present a distinct type of gonopod, bearing a subdistal papilla (see also Ahyong *et al.* 2005). Note that the genus *Pleistacantha* has been recently regarded as polyphyletic and *Pleisticanthoides* Yokoya, 1933 and *Parapleisticantha* Yokoya, 1933 resurrected (see Ng & Richer de Forges 2012; Richer de Forges *et al.* 2013). All these genera can be distinguished from other Inachidae by their long 'rostral' horns, markedly divergent and the strong spines at the base of the pseudorostrum and the supraorbital margin. Given our results from larval morphology, genetic markers and the observations from adult morphology, it is proposed to remove the five genera *Ergasticus*, *Bothromaia*, *Pleisticanthoides*, *Parapleisticantha* and *Pleistacantha* from the Inachidae and place them within the Oregoniidae as a new subfamily. Even though Števčić (2005) treated the Pleistacanthini as a tribe within the Inachinae MacLeay, 1838, his establishment of the group has nomenclatural priority and therefore the authors propose the name Pleistacanthinae Števčić, 2005.

The clear similarities in larval form between *Ergasticus* and *Cyrtomaia* Miers, 1886, *Eurypodius* Guérin, 1825 or *Platymaia* Miers, 1886 indicate that these genera should also be removed from Inachidae and placed within Oregoniidae. These observations would support Griffin & Tranter (1986), whom already mentioned 'at least superficial resemblances to *Chionoecetes* of the Oregoniinae' with regard to adult morphology of *Cyrtomaia* and *Platymaia* species. Nevertheless, extending the assessment of the taxonomic position of all these genera (particularly all above mentioned such as *Macrocheira*, *Platymaia* and *Stenorhynchus*) would demand a more comprehensive review of the whole family that goes beyond this work.

In this study, the results obtained from both morphological information of all larval stages as well as the analyses of DNA sequences (16S rDNA and Cox1 genes) provide conclusive evidence to support the removal of *Ergasticus* from

the family Inachidae and its placement together with members of the family Oregoniidae. Therefore, our results also evidence that developmental stages of brachyurans provide reliable morphological characteristics to help resolving the phylogenetic relationships among majoid genera.

Acknowledgements

The research was carried out within the framework of the projects MEGALOPADN (CGL2009-11225) and IDEADOS (CTM2008-04489-C03), funded by the 'Ministerio de Economía y Competividad (MINECO)' Spanish Plan R + D + I and FEDER. The authors are very grateful to all colleagues who participated in the IDEADOS surveys and to the crew of the R/V 'Sarmiento de Gamboa' and of the F/V 'Punta des Vent'. We also wish to thank all participants in the MEDITS-ES-2003 cruise on board the R/V 'Cornide de Saavedra' from which adult *Ergasticus clouei* were collected. We are especially grateful to Antonina dos Santos who helped in the larvae identification and to Carlos Sánchez Nieto for his assistance in the laboratory work. F. Palero acknowledges funding from the Spanish Government, Ministry of Economy and Competitiveness (BFU2011-24112). E. Marco-Herrero and A.P. Torres acknowledge the predoctoral FPI Fellowships supported by MINECO (BES-2010-033297) and Regional Government of the Balearic Islands, Conselleria d'Educació, Cultura i Universitats, select as part of an operational programme co-financed by the Fondo Social Europeo, respectively. Thanks are also due to Peter Ng and an anonymous referee for their comments and corrections that clearly improved the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Species used in the present study and gene sequences included in the phylogenetic analysis. Abbreviations; ND, no data.

SECCIÓN IV

CLAVE ILUSTRADA DE LAS MEGALOPAS DE BRAQUIUROS DE LA PENÍNSULA IBÉRICA

CAPÍTULO 8

Illustrated key for the identification of brachyuran (Crustacea, Decapoda) megalopae of Iberian Peninsula (SW Europe)

E Marco-Herrero, F Palero, JA Cuesta (2015) Illustrated key for the identification of brachyuran (Crustacea, Decapoda) megalopae of Iberian Peninsula (SW Europe) *(in prep.)*

ILLUSTRATED KEY FOR THE IDENTIFICATION OF BRACHYURAN (CRUSTACEA, DECAPODA) MEGALOPAE OF IBERIAN PENINSULA (SW EUROPE)

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Summary

An identification key has been constructed for the megalopae of 92 species of Iberian Brachyuran crabs. The key is based on examination of megalopae obtained from plankton samples and identified by mitochondrial genes 16S and Cox1 as DNA barcodes, and larvae previously described in the literature. This key is based mainly on external morphological features visible without dissection of larvae.

Keywords: Brachyura, crab, Iberian Peninsula, key, larva, megalopa, DNA barcode.

Introduction

Many marine invertebrates present complex life cycles comprising several developmental stages which clearly differ morphologically from those finally reached by the adult (Anger 2006). This is the case of the brachyuran crustacean decapods, commonly called crabs. Most brachyuran crabs, excepting a few species that show direct development, pass through a planktonic larval period with two phases, zoea and megalopa, which are very different from each other and from the adult form (Rice 1981; Martin et al. 2014). This represents an important drawback in the identification of the brachyuran larvae collected from the plankton (Bucklin 2010), which is further complicated by the fact that reliable larval descriptions are only available for a small amount of known brachyuran species. In comparison with zoeal stages, megalopae are not as well studied (Rice 1981). Megalopae identification has been traditionally based on morphological characteristics, but occasionally, it is impossible to get an accurate identification using this approach only.

While keys for the identification of brachyuran larvae are available for different regions (e.g. Wear & Fielder 1985; Ingle 1992; Paula 1996; Báez 1997; Anosov 2000; Bullard 2003; dos Santos & González-Gordillo 2004; Pessani et al. 1998, 2004; Rice & Tsukimura 2007; Gonzalez et al. 2009; Kornienko & Korn 2009; Korn & Kornienko 2010; Koetter et al. 2012), no specific studies exist for the Iberian Peninsula. The keys by Ingle (1992) and Pessani et al. (2004) only allow us to identify 55 of the 140 species that have been reported in the Iberian Peninsula (Marco-Herrero et al. 2015).

This gap in the knowledge of brachyuran larval taxonomy is in turn responsible by an important amount of problems encountered by researchers when studying population dynamics, recruitment events, larval dispersal and colonization, functioning of planktonic trophic webs (inter-specific interactions) and, overall, any kind of biodiversity research concerning this taxon. Besides its ecological importance, the gathering of high-quality data for the accurate identification of brachyurans is also essential for the sustainable management of the fisheries of commercial species, since it is the starting point to determine reproductive periods and larval dispersal and aggregation channels, as well as to recognize settlement and recruitment areas (Eaton et al. 2003; Freire et al. 2002). Nowadays, molecular tools such as DNA barcoding facilitate the identification of larval specimens collected in the field. This approach has

clear advantages over using morphology of larvae reared in the laboratory from ovigerous females; for example, González-Gordillo & Rodríguez (2000) reported morphological differences between plankton-collected larvae and those reared in the laboratory.

The use of molecular markers is a powerful tool for accurately identifying plankton specimens (Pan et al. 2008; Pardo et al. 2009; Ampuero et al. 2010; Marco-Herrero et al. 2013). In the present study, we identified megalopa stages from plankton samples by using partial sequences of the mitochondrial genes 16S and Cox1 as DNA barcodes. The main objective of the present study is the optimization of the joint applicability of molecular techniques, particularly the analysis of mitochondrial DNA sequences (16S and Cox1), and morphological analysis, in the accurate identification of Iberian brachyuran megalopae obtained directly from planktonic samples. The new descriptions, together with the formerly existing ones, have allowed the creation of an illustrated identification key, which is intended to assist in the correct identification of this important group of planktonic organisms.

Methods

Illustration key provided morphological information for 92 species of brachyuran megalopae of Iberian Peninsula (40°18′0″ N, 3°43′0″ W), but not all species can be identified because of difficulties in the morphological differentiation of closely related species especially some genera (e.g. *Liocarcinus* and *Brachynotus*).

The key is based on examination of megalopae obtained from plankton samples and identified by mitochondrial genes 16S and Cox1 as DNA barcodes, from laboratory culture, museum collections and larval literature. Whenever possible, samples obtained from the plankton were compared with the original description and redrawn from captured specimens. The work done with the megalopae obtained is detailed in Appendix I.

This key is based mainly on external morphological characteristics visible. All sternum of megalopae analysed were described. The following measurements were taken for the megalopa: cephalothorax length (CL), measured from the tip of rostrum to posterior margin of cephalothorax; and cephalothorax width (CW), measured as the cephalothorax maximum width.

For the identification key we tried to use only the external morphological characters of larvae that are easy visible using a stereomicroscope, without specimen

dissection (Plate 1). This key does not reflect any systematic arrangement of the Brachyuran families.

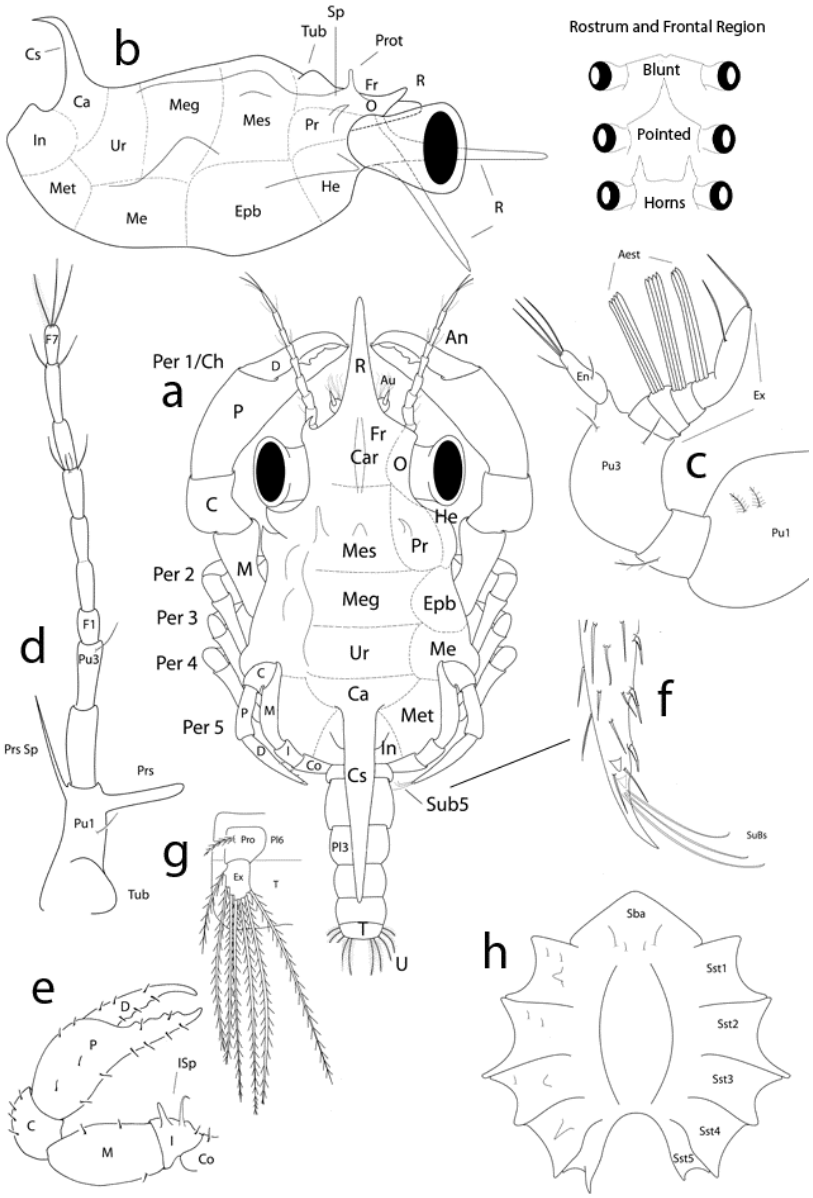

Plate I

Schematic megalopa with selected appendages used in the key: **a**, general dorsal view; **b**, lateral view; **c**, antennule; **d**, antenna; **e**, cheliped; **f**, dactylus of pereiopod 5; **g**, uropod; **h**, sternum.

Aest aesthetacs, **An** antenna**, Au** antennule, **C** carpus, **Ca** cardiac region, **Ch** chela, **Co** coxa, **Cs** cardiac spine, **Car** carinae**, D** dactylus, **Distr** distribution, **En** endopod, **Epb** epibranchial region, **Ex** exopod**, Fr** frontal region, **F1-7** flagellum 1-7, **He** hepatic region, **I** ischium, **In** intestinal region, **ISp** ischial spine, **M** merus, **Me** mesobranchial region, **Meg** metagastric region, **Mes** mesogastric region, **Met** metabranchial region, **O** orbital region, **P** propodus, **Per1-5** pereiopods 1-5, **Pl1-5** pleonites 1-5, **Pr** protogastric region, **Pro** protopod, **Prot** protuberance, **Pu** peduncular segment, **Prs** process, **R** rostrum, **Sba** sternite basal, **Sp** spine, **SpPrs** spinous process, **Sst1-5** sternite 1-5, **Subs** subterminal seta, **T** telson, **Tub** Tubercle, **U** uropod, **Ur** urogastric region.

Illustrated key to brachyuran megalopae of Iberian Peninsula

1 **a)** With remarkable spines or pronounced process on the cephalothorax (excluding the frontal region and rostrum) ……..……...…….……..…**(2)**
Plate II-III.

b) Without remarkable spines or process on the cephalothorax, but in some cases with small tubercles or protuberances…… **(17)**
Plate IV-

2 **a)** Dorsal surface of the cephalothorax with numerous conical spines and short plumose setae. Antennal flagellum with 12 segments. Uropod biramous, the inner rami carry <20 setae and the outer rami carry <30 setae…… ***Dromia personata***
Plate II; Figs. 1a-c.

CL: 2.30 – 2.5 mm

Distr.: G-GB, WP, GC, ALB, MED

b) Dorsal surface of the cephalothorax without spines but with numerous setae. Frontal region with acute rostrum and a pair of lateral spines. Antennal flagellum with 31-36 segments. Uropod, biramous the inner rami carry 43-49 setae and the outer rami carry 57-63 setae…..……..…….…..……. ***Homola barbata***
Plate II; Figs. 2a, b.

CL: 7.25-7.50 mm

Distr.: G-GB, WP, GC, ALB, MED

c) Dorsal surface of the cephalothorax without numerous spines or setae, but some remarkable ones on some regions…… **(3)**
Figs. 3-10

3 **a)** Two pairs of long spines on frontal region, one pair of long spines on protogastric region, two medially placed mesogastric spines, and prominent cardiac spine present. Rostrum long and thin. Basal segment of antennal peduncle with a spiniform process still longer than rostrum. Antennal flagellum 4-segmented. Cheliped and pereiopods 2-5 with long and prominent spines…….……..……***Dorhynchus thomsoni***
Plate II; Figs. 3a-c

CL: 2.9 mm

Distr.: G-GB, WP, GC, ALB, MED

b) One pair of very long and diverging processes on the orbital region, a prominent spine with a small anterior spine on the mesogastric region, and one pair of hook-shaped spines on the epibranchial region. Antennal flagellum 7-segmented……..… ***Latreillia elegans***
Plate II; Figs. 4a, b.

CL: 4.1 mm

Distr.: WP, GC, ALB, MED

c) Not as above, these characters not combined.…….…….. **(4)**

4 a) Cephalothorax without cardiac spine ……..…….……..…….**..... (5)**
Plate III; Figs. 3a- 4a.

b) Cephalothorax with cardiac spine present and a pair of remarkable processes on the protogastric region. Antennal flagellum 4-segmented with 0,4,0,3 setae. Pereiopod 2-3 with ischial hook. Dactylus of pereiopod 5 without feelers. Pleon 5-segmented. Uropods absent……..…….. **(6)** ***Macropodia* spp.**
Plate II; Figs. 5-7

c) Cephalothorax with cardiac spine well-developed…...……..……..... **(8)**
Plate III

5. a) Cephalothorax with only one stout spine on each outer protogastric region. Pereiopods 4-5 smaller. Dactylus of pereiopod 5 without feelers.... ***Medorippe lanata***
Plate III; Figs. 3a, b

Distr.: WP, GC, ALB, MED

b) Cephalothorax with a pair of curved spines on medial protogastric region. Cheliped with well-developed ischial spine. Pereiopod 2-4 with coxal and ischial spines. Dactylus of 5 pereiopod with 3 long feelers..……***Goneplax rhomboides***
Plate III; Figs. 4a, b

CL: 1.8-1.9 mm

Distr.: G-GB, WP, GC, ALB, MED

6 a) Cheliped with 1 tubercle on ischium and 1 tubercle and 1 spine on merus…...… **(7)**
Plate II; Fig. 7b

b) Cheliped with only 1 minute tubercle on merus. Antennal peduncle process on proximal segment shorter than segment length……..…. ***Macropodia czernjawskii***
Plate II; Figs. 5a-e.

CL: 0.83 mm

Distr.: GC, ALB, MED

7 a) Spine on cardiac region slightly recurved and twice longer than protuberances on protogastric region. Antennal peduncle process on proximal segment shorter than segment length ……..***Macropodia longipes/ M. tenuirostris***
Plate II; Figs. 7a, b.

CL: 1.10-1.15 mm

Distr.: G-GB, WP, GC, ALB, MED

b) Spine on cardiac region posterior-dorsally directed and longer than protuberances on protogastric region. Antennal peduncle process on proximal segment longer than segment length…….……..…….…… ***Macropodia rostrata***
Plate II; Figs. 6a-c.

CL: 1.2-1.3 mm

Distr.: G-GB, WP, GC, ALB, MED

8 **a)** Cardiac spine well developed and dorsally directed……..…….…… **(9)**

b) Cardiac spine well developed and posteriorly directed……..…..…… **(11)**

9 **a)** Cardiac spine long and stout (90º). One mesobranchial tubercle on each side. Rostrum long. Antennal flagellum 4-segmented. Pereiopod 2-4 with coxal and ischial spines. Dactylus of pereiopod 5 without feelers. Uropod exopod with 5-6 long terminal natatory setae…….……..... ***Rochinia carpenteri***
Plate II; Figs. 8a-c.

CL: 2.3-2.5 mm

Distr.: G-GB, WP, GC, ALB, MED

b) Cardiac spine short and dorsally directed…….…..……..… **(10)**
Plate III; Figs. 1b, 2b.

11 **a)** Cardiac spine short and acute. Antennal flagellum 7-segmented. Cheliped with well-developed ischial spine. Dactylus of pereiopod 5 lanceolate with 3 long feelers. Uropod exopod with 7 long terminal natatory setae……..……..***Portumnus latipes***
Plate III; Figs. 1a-d.

CL: 1.69-2.59 mm

Distr.: G-GB, WP, GC, ALB, MED

b) Cardiac spine short and recurved. Antennal flagellum 5-segmented. Cheliped without spines. Dactylus of pereiopod 5 without feelers. Uropod exopod with 2 long terminal natatory setae. …..……..……..…… ***Ergasticus clouei***
Plate III; Figs. 2a-d.

CL: 1.90 mm

Distr.: G-GB, WP, GC, ALB, MED

12 a) Cardiac spine short. Rostrum trifid. Prominent orbital and two anterolateral spines. Cephalothorax with a pair of curved spines on medial protogastric and hepatic region. Antennal flagellum 17- segmented…….....…. ***Corystes cassivelaunus***
Plate III; Figs. 5a-d.

CL: 2.3-2.5 mm

Distr.: G-GB, WP, GC, ALB, MED

b) Cardiac spine long……...……..……..… **(12)**
Plate III; Figs. 7-10 a, b.

13 a) Cheliped without ischial or coxal spine or tubercle……..……. **(13)**
b) Cheliped with ischial or coxal spine or tubercle…….…… **(16)**
Fig. 8d.

14 a) Dactyl of 5 pereiopod with 3 feelers…….……..…….**(14)**
b) Dactyl of 5 pereiopod without feelers…….…….**.....**……..**(15)**

15 a) Uropod exopod with 13 long terminal natatory setae. Antennal flagellum 8-segmented (0,2,4,1,5,0,4,6)…….....***Cancer bellianus***

CL: exceeding 3.5 mm

Distr.: G-GB, WP

b) Uropod exopod with 10-11 long terminal natatory setae. Antennal flagellum 8-segmented (0,1-2,4,0,4-5,0-1,4,5) ……...……...……..... ***Atelecyclus rotundatus***
Plate III; Fig. 6a.

CL: 2.60-2.82 mm

Distr.: G-GB, WP, GC, ALB, MED

16 a) Uropod exopod with 6 long terminal natatory setae. Cardiac spine overpass pleonite 2……... ***Parthenopoides massena***
Plate III; Figs. 10a-c.

CL: 1.6-2.43 mm

Distr.: G-GB, WP, GC, ALB, MED

b) Uropod exopod with 4 long terminal natatory setae. Cardiac spine longer, overpass pleonite 4……..…….. ***Derilambrus angulifrons***
Plate III; Figs. 9a-c.

CL: 1.78 mm

Distr.: GC, ALB, MED

17 a) Cheliped with ischial spine. Pereiopod 2 with a well-developed coxal spine. Uropod exopod with 8-9 long terminal natatory setae. Antennal flagellum 8-segmented (0,0,4,0,4,0,4,5)…..…….…..……. ***Cancer pagurus***
Plate III; Figs. 8a-d.

CL: 2.4 mm

Distr.: G-GB, WP, GC, MED

b) Cheliped with coxal tubercle. Pereiopod 2 with coxal and ischial spines. Uropod exopod with 10 long terminal natatory setae. Antennal flagellum 8-segmented (0,0,4,0,5,0,4,5)…..... ***Atelecyclus undecimdentatus***
Plate III; Figs. 7a, b.

CL: 2.39- 2.59mm

Distr.: G-GB, WP, GC, ALB, MED

New description in appendix

18 a) Cheliped well developed, long or large .....……..…..……..…… **(18)**
Plate ; Figs.

b) Cheliped proportional to the cephalothorax…….…..……. **(19)**

19 a) Cheliped large, dorso-ventrally compressed. Heterochely with right-handedness. Teeth of the right propodus more developed. Frontal region and rostrum gradually deflected ventrally. Antennal flagellum 8-segmented (0,0,4,2,2,4,2,3,4). Uropod exopod with 20-21 long terminal natatory setae……..…..... ***Calappa granulata***

CL: 4.6 mm

Distr.: G-GB, WP, GC, ALB, MED

b) Cheliped longer, with dactylus longer than the palm. Dactylus pereiopod 5 without feelers. Rostrum directed obliquely downward, very small. Antennal flagellum 4-

segmented (0,0,0,3). Uropod exopod with 11-12 long terminal natatory setae ……..…….....…***Ilia nucleus***

CL: 1.7-1.8 mm

Distr.: GC, ALB, MED

19 a) Frontal margin with a pair of submedian "horns" present……...……... **(20)**

b) Frontal margin with a pair of frontal submedian horns with a long rostrum seems trident. Antennal flagellum 9-segmented. Uropod protopod with 5 plumose setae and uropod exopod with 25 long terminal natatory setae ……..……...….***Percnon gibbesi***

CL: 5.2-5.9 mm

Distr.: WP, ALB, MED

c) Frontal margin without "horns"……..……...……...…….**(29)**

20 a) Rostrum blunt or flat ……..…..…….…. **(21)**

b) Rostrum pointed or with rostral spine directed obliquely downwards .....…. **(24)**

21 a) Pleon 5-segmented, posterodorsal margin of pleonits 2-4 with a pair of spinous process. A pair of protogastric small spines present. Antennal flagellum 3-segmented (0,4,3). Dactylus of pereiopod 5 without feelers. Uropod absents…..... **(22)** ***Inachus* spp.**

b) Pleon 5-segmented, pleonits 2-4 with 1,2,1,2 pairs of posterio-dorsal spinous process. A pair of protogastric small spines present……...***Achaeus cranchii***

CL: 0.90 mm

Distr.: G-GB, GC, ALB, MED

22 a) Cheliped with 4 spines on merus. Propodus of pereiopod 5 with 16 setae ……..***Inachus leptochirus***

Distr.: G-GB, WP, GC, ALB, MED

b) Cheliped with 3 spines on merus…….……..…….…… **(23)**

23 a) Propodus of pereiopods 2-4 with 16,16,17 setae respectively. Antennule peduncle 1,0,1……..……...……...…….....***Inachus dorsettensis***

CL: 1,3 mm

Distr.: G-GB, WP, GC, ALB, MED

b) Propodus of pereiopods 2-4 with 17,17,16 setae respectively. Antennule peduncle 0,0,1……..……..…...***Inachus phalangium***

Distr.: G-GB, WP, ALB, MED

c) Propodus of pereiopods 2-4 with 17,17,15 setae respectively. Antennule peduncle 0.0.1…….……..**.....*Inachus thoracicus***

Distr.: WP, GC, ALB, MED

24 a) Cheliped without ischial spine but with meral stout spine. Pereiopods 2-3 with ischial spine. Pereiopods 2-4 with coxal spine. Posterior margin of telson with 4 setae. Uropod protopod with 1 plumose setae and exopod with 9-10 long terminal natatory setae. Antennal flagellum 7 or 8-segmented (0,(0),4,0,5,0,4,4-5)…….……..***Monodaeus couchii***

CL: 1mm

Distr.: G-GB, GC, ALB, MED

b) Cheliped with ischial spine. …….……..……..…… **(25)**

25 a) Pereiopods 2-4 without coxal spines……..……..…..……**(26)**

b) Pereiopods 2-4 with coxal spines…….**.....**……..……..…. **(27)**

26 a) Dactylus of pereiopod 5 with 3 feelers. Antennal flagellum setation 0,0,3,0,4,0,4,3. Uropod protopod with 1 plumose setae and exopod with 7-8 long terminal natatory setae. ……..……..***Panopeus africanus***

CL: 1mm

Distr.: G-GB, WP, GC

b) Dactylus of pereiopod 5 without feelers. Antennal flagellum setation 0,0,3,0,4,0,4,3. Uropod protopod with 1 plumose setae and exopod with 7 long terminal natatory setae …..……..……..……..…...***Dyspanopeus sayi***

CL: 1.30 mm

Distr.: MED

27 a) Antennal flagellum setation 0,0,3,0,5,0,4,5. Pereiopods 2-4 with ischial spine. Dactylus of pereiopod 5 with 3 feelers Uropod protopod with 1 plumose setae and exopod with 9 long terminal natatory setae …..……...……..…….. ***Xantho pilipes***

CL: 1,22-1,52 mm

Distr.: G-GB, WP, ALB, MED

b) Antennal flagellum setation 0,0,3,0,4,0,4,5……..…...……..…. **(28)**

28 a) Pereiopod 2 without ischial spine. Dactylus of pereiopod 5 with 3 feelers Uropod protopod with 1 plumose setae and exopod with 10 long terminal natatory setae……..……...….***Xantho hydrophilus***

CL: 1,6 mm

Distr.: G-GB, WP, ALB, MED

b) Pereiopod 2 with small ischial spine. Dactylus of pereiopod 5 with 3 feelers. Uropod protopod with 1 plumose setae and exopod with 9 long terminal natatory setae. ……...…….…***Xantho poressa***

CL: 1,45 mm

Distr.: WP, GC, ALB, MED

29 a) Without rostrum…….....……. **(30)** ***Ebalia* spp**.

b) With rostrum……..…… **(31)**

30 a) Antennal flagellum setation 0,0,0-1,3,4. Dactylus of pereiopod 5 without feelers Uropod exopod with 5 (4-6) long terminal natatory setae……..***Ebalia tuberosa***

CL: 1,22 mm

Distr.: G-GB, WP, GC, ALB, MED

b) Antennal flagellum setation 0,0,0-1,3,4. Uropod exopod with 4 long terminal natatory setae……..…….....… ***Ebalia cranchii // Ebalia tumefacta***

CL: *E. cranchii* ¿?? CL: 1,21 mm *E. tumefacta*

Distr.: G-GB, WP, GC, ALB, MED *E. cranchii* and *E. tumefacta*

31 a) Antennal flagellum < 7-segmented ……..……..……...… **(32)**

b) Antennal flagellum ≥ 7-segmented.…….…..... **(35)**

32 a) Antennal flagellum 4-segmented (0,0,4,4). Pereiopod 2 with ischial spine. Uropod exopod with 5 long terminal natatory setae. Dactylus of pereiopod 5 without feelers. Telson posterior margin with 2 setae……***...Acanthonyx lunulatus***

CL: 1,18-1,26 mm

Distr.: GC, ALB, MED

b) Antennal flagellum 6-segmented ……..……..…..…….. **(33)**

33 a) Cheliped with ischial spine. …….……...…….……...…… **(34)**

b) Cheliped without ischial spine but with small tubercle on coxa. Antennal flagellum setation 0,0,4,0,5,1+4. Uropod exopod with 6-7 long terminal natatory setae. Pereiopods 2-4 with propodial setae ……..……..…..……***Sirpus zariquieyi***

CL: 1.39-1.52 mm

Distr.: WP, GC, ALB, MED

34 a) Uropod protopod with 0-1 plumose setae and exopod with 8 long terminal natatory setae. Antennal flagellum setation 0,4,5,4,4,4…..*****Liocarcinus marmoreus***

CL: 2 mm

Distr.: G-GB, WP, ALB, MED

b) Uropod protopod with 2 plumose setae and exopod with 11 long terminal natatory setae. Antennal flagellum setation 0,4,5,1,4,5…..**.....**…***Polybius henslowii***

CL: 5.50 mm

Distr.: G-GB, WP, GC, ALB, MED

35 a) Antennal flagellum 7-segmented ……..…..……..…… **(36)**

b) Antennal flagellum 8-segmented ……..…...…..…… **(37)**

36 a) Antennal flagellum setation 0,2,0,4,0,3,4. Cheliped without coxal or ischial spines. Pereiopods 2-4 with 5,5,5,4 setae stout. Dactylus of pereiopod 5 lanceolate with 2 feelers……...…….…… ***Thia scutellata***

CL: 2.20 mm

Distr.: G-GB, WP, GC, ALB, MED

b) Antennal flagellum setation 0,0,4,0,5,1,4. Cheliped with ischial spine. Pereiopods 2-4 with propodial setae. Dactylus of pereiopod 5 not lanceolate with 3 feelers…….**....**……..….***Pirimela denticulata***

CL: 1.80 mm

Acknowledgements

The research was carried out within the framework of the project MEGALOPADN (CGL2009-11225) funded by the 'Ministerio de Economía y Competividad (MINECO)' Spanish Plan R + D + I and FEDER. The authors are very grateful to all colleagues who participated in the MEGALOPADN. We are especially grateful to Asvin P. Torres, Enrique Macpherson for their assistance provided in obtaining samples, to Paul F. Clark for help in the Natural History Museum of London, to Mº Grazzia Mazzocchi for help in the Stazione Zoologica Anton Dorhn of Naples, to Marta Pascual in the University of Barcelona and Carlos Sánchez Nieto for his assistance in the laboratory work. E. Marco-Herrero acknowledge the predoctoral FPI Fellowships supported by MINECO (BES-2010-033297)

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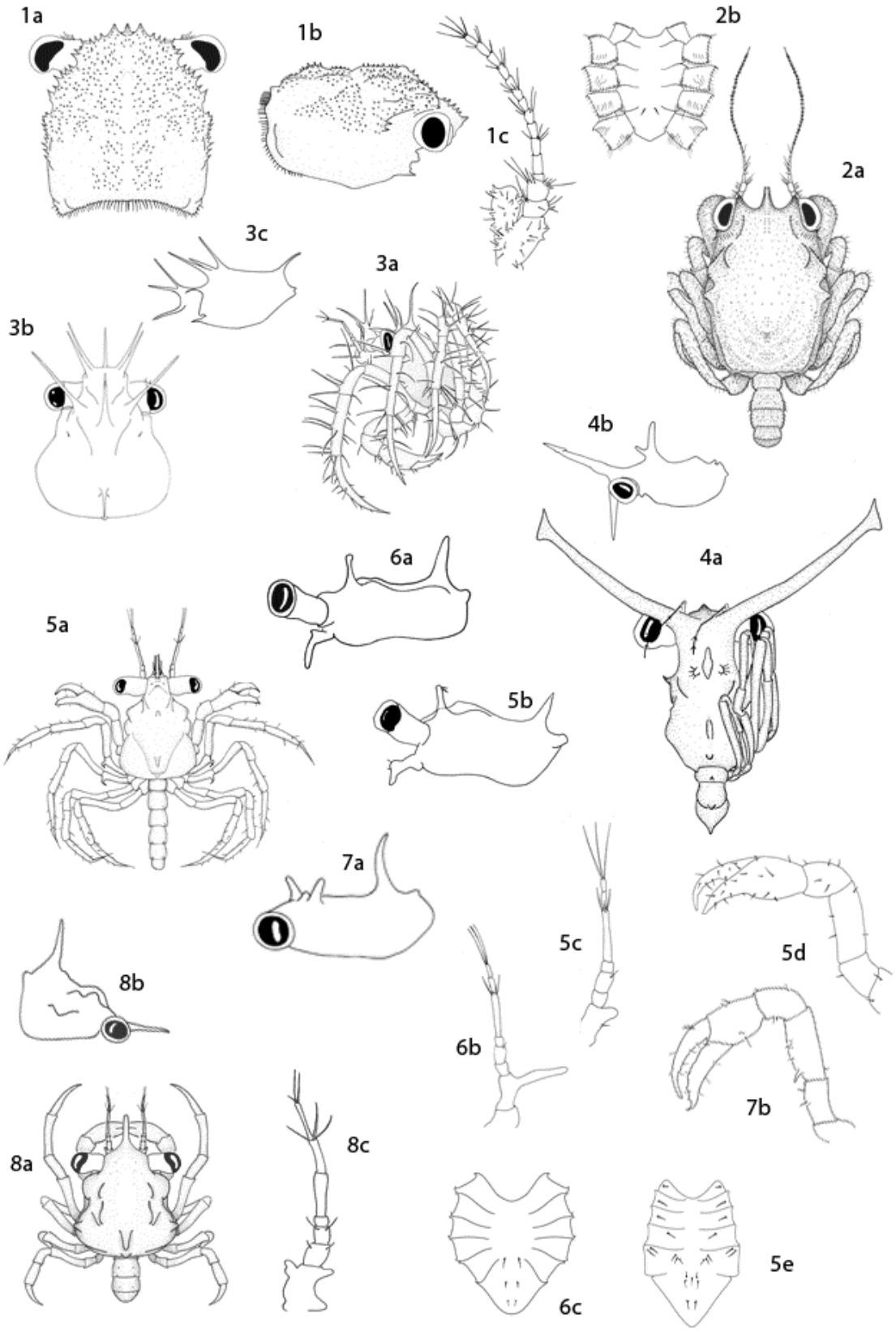
Figures Caption

Plate II

Fig. **1** *Dromia personata* (after Rice et al. 1970): **a**, cephalothorax; **b**, lateral view; **c**, antenna. Fig. **2** *Homola barbata*: **a**, dorsal view; **b**, sternum. Fig. **3** *Dorhynchus thomsoni* (after Williamson 1982): **a**, general view; **b**, cephalothorax; **c**, lateral view. Fig. **4**; *Latreillia elegans* (after Rice 1982): **a**, general view; **b**, lateral view. Fig. **5** *Macropodia czernjawskii*: **a**, general view; **b**, lateral view; **c**, cheliped; **d**, sternum. Fig. **6** *M. rostrata*: **a**, lateral view; **b**, antenna; **c**, sternum. Fig. **7** *M. longipes* (after Guerao & Abelló 1997): **a**, lateral view; **b**, cheliped. Fig. **8** *Rochinia carpenteri* (after Ingle 1979): **a**, general view; **b**, lateral view; **c**, antenna.

Plate III

Fig. **1** *Portunus latipes***: a**, general view; **b**, lateral view; **c**, sternum; **d**, antenna. Fig. **2** *Ergasticus clouei*: **a**, general view; **b**, lateral view; **c**, antenna; **d**, uropod. Fig. **3** *Medorippe lanata* (after Gilet 1952): **a**, general view; **b**, antenna. Fig. **4** *Goneplax rhomboides*: **a**, general view; **b**, lateral view. Fig. **5** *Corystes cassivelaunus*: **a**, general view; **b**, lateral view; **c**, antenna; **d**, sternum. Fig. **6** *Atelecyclus rotundatus*: **a**, sternum. Fig. **7** *Atelecyclus undecimdentatus*: **a**, general view; **b**, lateral view. Fig. **8** *Cancer pagurus*: **a**, general view; **b**, lateral view; **c**, sternum; **d**, cheliped. Fig. **9** *Derilambrus angulifrons*: **a**, general view; **b**, lateral view; **c**, uropod. Fig. **10** *Parthenopoides massena*: **a**, general view; **b**, lateral view; **c**, uropod**.**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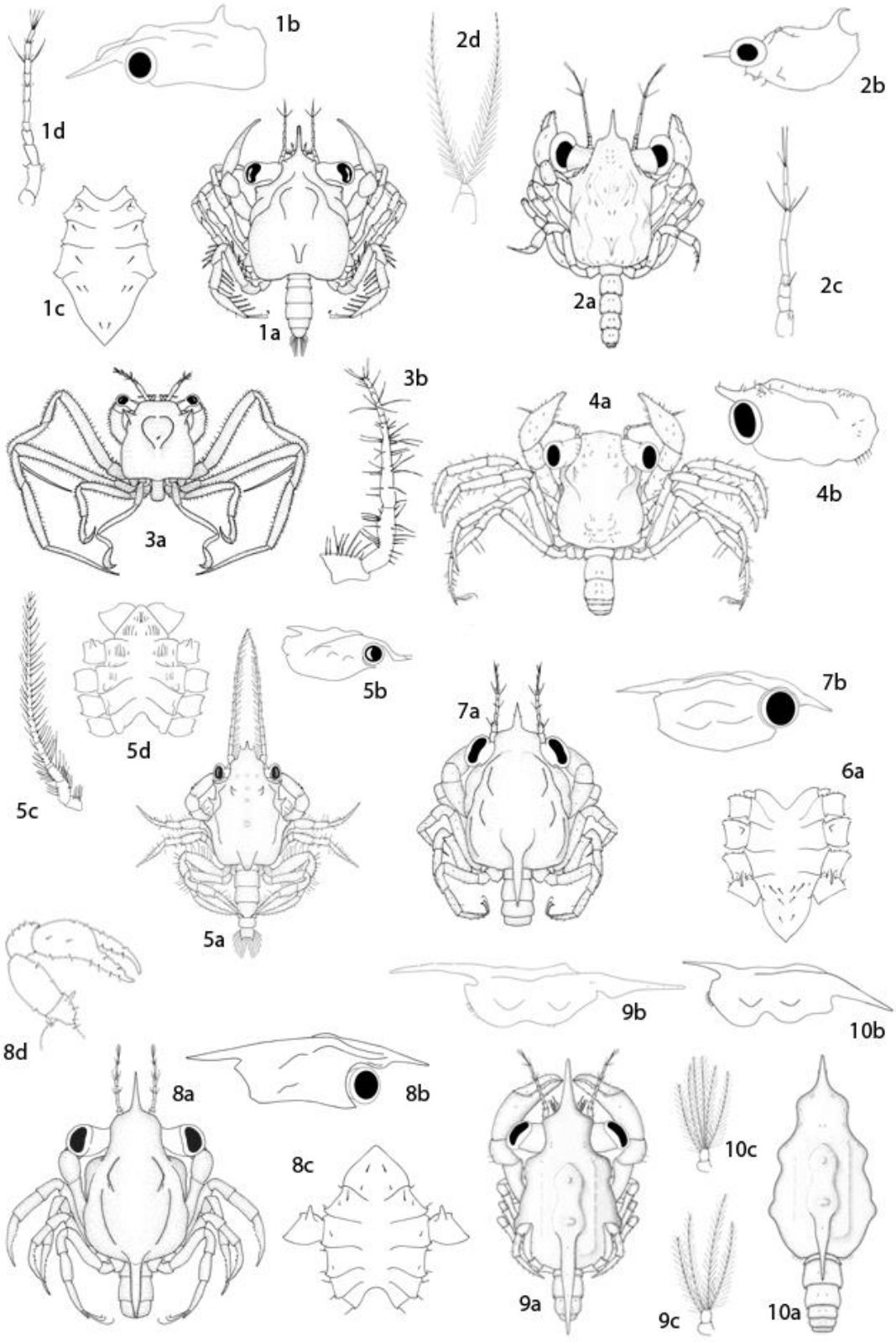
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Appendix I. List of Brachyuran crab species from the Iberian Peninsula indicating state of megalopa description (**C,** complete, **I,** incomplete or **U,** undescribed), original references, samples being analyzed in this work (**rd**, re-drawn from the original description, **RD**, re-described; **X**, checked and re-drawn, **Nw**, new description), and species included for the first time in an identification key (**FK**). Other abbreviations: (-) No data; **Sin**, synonym.

Taxa/Species	Status	Reference	Analysis	Key
BRACHYURA Linnaeus, 1758				
PODOTREMATA Guinot, 1977				
Cymonomidae Bouvier, 1898				
Cymonomus granulatus (Thomson, 1873)	U	-	-	
Cymonomus normani Lankester, 1903	U	-	-	
Dromiidae de Haan, 1833				
Dromia personata (Linnaeus, 1758)	I	Rice et al. 1970	rd	
Homolodromiidae Alcock, 1899				
Dicranodromia mahieuxii A. Milne-Edwards, 1883	U	-	-	
Homolidae de Haan, 1839				
Homola barbata (Fabricius, 1793)	C	Rice 1974	X	
Homologenus boucheti Guinot & Richer de Forges, 1995	U	-	-	
Paromola cuvieri (Risso, 1816)	U	-	-	
Latreilliidae Stimpson, 1858				
Latreillia elegans Roux, 1830	C	Rice 1982	rd	
EUBRACHYURA de Saint Laurent, 1980				

HETEROTREMATA Guinot, 1977				
Calappidae de Haan, 1833				
Calappa granulata (Linnaeus, 1758)	C	Guerao et al. 1998	rd	
Calappa pelii Herklots, 1851	U	-	-	
Calappa tuerkayana Pastore, 1995	U	-	Sin	
Cryptosoma cristatum Brullé, 1837	U	-	-	
Atelecyclidae Ortmann, 1893				
Atelecyclus rotundatus (Olivi, 1792)	C	Hong & Ingle 1987	X	
Atelecyclus undecimdentatus (Herbst, 1783)	C*	***in prep.***	**RD**	**FK**
Cancridae Latreille, 1802				
Cancer bellianus Jonhson, 1861	I	Ingle 1998	-	
Cancer pagurus Linnaeus, 1758	C	Ingle 1998	X	
Corystidae Samouelle, 1819				
Corystes cassivelaunus (Pennant, 1777)	I	Ingle & Rice 1971	X	
Dorippidae MacLeay, 1838				
Medorippe lanata (Linnaeus, 1767)	C	Gilet 1952	rd	**FK**
Ethusidae Guinot, 1977				
Ethusa mascarone (Herbst, 1785)	I	Cano 1892	rd	**FK**
Ethusina talismani A. Milne-Edwards & Bouvier, 1897	U	-	-	
Eriphiidae MacLeay, 1838				
Eriphia verrucosa (Forskål, 1775)	I	Lumare & Gozzo 1972	X	
Goneplacidae MacLeay, 1838				

Goneplax rhomboides (Linnaeus, 1758)	C	Ingle & Clark 1983	X	
Progeryonidae Števčić, 2005				
Paragalene longicrura (Nardo, 1869)	U	-	-	
Leucosiidae Samouelle, 1819				
Ebalia cranchii Leach, 1817	I	Kurian 1956	X	**FK**
Ebalia deshayesi Lucas, 1846	U	-	-	
Ebalia edwardsii Costa, 1838	U	-	-	
Ebalia granulosa H. Milne Edwards, 1837	U	-	Sin	
Ebalia nux A. Milne-Edwards, 1883			-	
Ebalia tuberosa (Pennant, 1777)	C	Salman 1982	rd	
Ebalia tumefacta (Montagu, 1808)	C	Salman 1982	rd	**FK**
Ilia nucleus (Linnaeus, 1758)	C	Bartilotti et al. 2009	X	**FK**
Merocryptus boletifer A. Milne-Edwards & Bouvier, 1894	U	-	-	
Epialtidae MacLeay, 1838				
Acanthonyx lunulatus (Risso, 1816)	C	Guerao & Abelló 1996	X	
Anamathia rissoana (Roux, 1828)	U	-	-	
Herbstia condyliata (Fabricius, 1787)	I	Cano 1893	X	**FK**
Lissa chiragra (Fabricius, 1775)	C	Guerao et al. 2003	rd	**FK**
Pisa armata (Latreille, 1803)	C	Ingle & Clark 1980	rd	
Pisa carinimana Miers, 1879	U	-	-	
Pisa hirticornis (Herbst, 1804)	U	-	-	
Pisa muscosa (Linnaeus, 1758)	U	-	-	

Pisa nodipes (Leach, 1815)	U	-	-	
Pisa tetraodon (Pennant, 1777)	C	Rodríguez 1997	rd	
Pisa sp. Marco-Herrero et al. (in prep.)	U	***in prep.***	**Nw**	**FK**
Rochinia carpenteri (Thomson, 1873)	C	Ingle 1979	rd	
Inachidae MacLeay, 1838				
Achaeus cranchii Leach, 1817	I	Bocquet 1954	rd	
Achaeus gracilis (Costa, 1839)	U	-	-	
Dorhynchus thomsoni Thomson, 1873	I	Williamson 1982	rd	
Inachus aguiarii Brito Capello, 1876	U	-	-	
Inachus communissimus Rizza, 1839	U	-	-	
Inachus dorsettensis (Pennant, 1777)	C	Ingle 1977	X	
Inachus leptochirus Leach, 1817	I	Clark 1983	-	
Inachus phalangium (Fabricius, 1775)	I	Clark 1983	-	
Inachus thoracicus Roux, 1830	C	Guerao et al. 2002	rd	**FK**
Macropodia czernjawskii (Brandt, 1880)	C	Marco-Herrero et al. 2012	**Nw**	**FK**
Macropodia deflexa Forest, 1978	U	-	-	
Macropodia linaresi Forest and Zariquiey Álvarez, 1964	U	-	-	
Macropodia longipes (A. Milne-Edwards and Bouvier, 1899)	C	Guerao & Abelló 1997	rd	**FK**
Macropodia longirostris (Fabricius, 1775)	U	-	-	
Macropodia parva Van Noort and Adema, 1985	C	González-Gordillo & Rodríguez 2001	Sin	
Macropodia rostrata (Linnaeus, 1761)	C	Ingle 1982	X	
Macropodia tenuirostris (Leach, 1814)	C	Salman 1981	Sin	

Majidae Samouelle, 1819				
Eurynome aspera (Pennant, 1777)	C	Salman 1982	X	
Eurynome spinosa Hailstone, 1835	I	Ingle 1981	X	**FK**
Maja brachydactyla Balss, 1922	C	Guerao et al. 2008	X	**FK**
Maja crispata Risso, 1827	C	Rodríguez 2002	X	**FK**
Maja goltziana d'Oliveira, 1888	C	Paula 1988	rd	
Maja squinado (Herbst, 1788)	C	Guerao et al. 2008	X	
Oregonidae Garth, 1958				
Ergasticus clouei A. Milne-Edwards, 1882	U	Marco-Herrero et al. 2013	**Nw**	**FK**
Palicidae Bouvier, 1898				
Palicus caronii (Roux, 1828)	U	-	-	
Parthenopidae MacLeay, 1838				
Derilambrus angulifrons (Latreille, 1825)	C	Marco-Herrero et al. 2015	**Nw**	**FK**
Distolambrus maltzami (Miers, 1881)	U	-	-	
Parthenopoides massena (Roux, 1830)	C	Thiriot 1973	X	
Spinolambrus macrochelos (Herbst, 1790)	U	-	-	
Velolambrus expansus (Miers, 1879)	U	-	-	
Pilumnidae Samouelle, 1819				
Pilumnopeus africanus (De Man, 1902)	U	-	-	
Pilumnus aestuarii Nardo, 1869	U	-	-	
Pilumnus hirtellus (Linnaeus, 1761)	I	Ingle 1983	X	
Pilumnus inermis A. Milne-Edwards & Bouvier, 1894	U	-	-	

Pilumnus spinifer H. Milne Edwards, 1834	I	Guerao et al. 2005	X	**FK**
Pilumnus villosissimus (Rafinesque, 1814)	U	-	X?	
Pilumnus sp. d´Udekem d´Acoz and Schubart (in prep.)	U	***in prep***	**Nw**	**FK**
Carcinidae MacLeay, 1838				
Carcinus aestuarii Nardo, 1847	C	Rice & Ingle 1975	X	
Carcinus maenas (Linnaeus, 1758)	C	Rice & Ingle 1975	X	
Portumnus latipes (Pennant, 1777)	C	Paula 1988	X	
Xaiva biguttata (Risso, 1816)	I	Lebour 1944	rd	**FK**
Geryonidae Colosi, 1923				
Chaceon affinis (A. Milne-Edwards and Bouvier, 1894)	U	-	-	
Chaceon mediterraneus Manning and Holthuis, 1989	U	-	-	
Chaceon inglei Manning and Holthuis, 1989	U	-	-	
Geryon longipes A. Milne-Edwards, 1881	C	Guerao et al. 1996	X	
Geryon trispinosus (Herbst, 1803)	I	Ingle 1979, 1992	X	
Pirimelidae Alcock, 1899				
Pirimela denticulata (Montagu, 1808)	C	Flores & Paula 2000	X	
Sirpus zariquieyi Gordon, 1953	U	***in prep.***	**Nw**	**FK**
Polybiidae Ortmann, 1893				
Bathynectes longipes (Risso, 1816)	C	Ingle 1985	X	
Bathynectes maravigna (Prestandrea, 1839)	U	-	-	
Liocarcinus bolivari (Zariquiey Álvarez, 1948)	U	-	-	
Liocarcinus corrugatus (Pennant, 1777)	C	Kim & Hong 1999	X	

Liocarcinus depurator (Linnaeus, 1758)	C	Guerao et al. 2006	X	
Liocarcinus holsatus (Fabricius, 1798)	C	Rice & Ingle1975	X	**FK**
Liocarcinus maculatus (Risso, 1827)	U	***in prep.***	**Nw**	**FK**
Liocarcinus marmoreus (Leach, 1814)	C	Rice & Ingle 1975	X	
Liocarcinus mcleayi (Barnard, 1947)	U	-	-	
Liocarcinus navigator (Herbst, 1794)	I	Ingle 1992	X	
Liocarcinus pusillus (Leach, 1815)			X	
Liocarcinus vernalis (Risso, 1816)	U	***in prep.***	**Nw**	**FK**
Liocarcinus zariquieyi Gordon, 1968	U	***in prep.***	**Nw**	**FK**
Macropipus tuberculatus (Roux, 1830)	I	Rice & Ingle 1978	X	**FK**
Necora puber (Linnaeus, 1767)	C	Rice & Ingle 1975	X	
Polybius henslowii Leach, 1820	I	Ingle 1992	X	
Portunidae Rafinesque, 1815				
Callinectes exasperatus (Gerstaecker, 1856)	U	-	-	
Callinectes sapidus Rathbun, 1896	I	Costlow & Bookhout 1959	rd	
Charybdis (*Charybdis*) *feriata* (Linnaeus, 1758)	I	Fielder 1984/ Hu 1983	rd	**FK**
Portunus (*Portunus*) *hastatus* (Linnaeus, 1767)	U	***in prep.***	**Nw**	**FK**
Thiidae Dana, 1852				
Thia scutellata (Fabricius, 1793)	C	Ingle 1984	rd	
Panopeidae Ortmann, 1893				
Dyspanopeus sayi (Smith, 1869)	C	Marco-Herrero et al. 2013	**RD**	**FK**
Panopeus africanus A. Milne-Edwards, 1867	C	Rodríguez & Paula 1993		**FK**

Rhithropanopeus harrisii (Gould, 1841)	C	Marco-Herrero et al. 2014	**RD**	
Xanthidae MacLeay, 1838				
Monodaeus couchii (Couch, 1851)	C	Ingle 1983	X	
Microcassiope minor (Dana, 1852)	U	-	-	
Paractaea monodi Guinot, 1969	U	-	-	
Xantho hydrophilus (Leach, 1814)	I	Ingle 1983	X	
Xantho pilipes A. Milne-Edwards, 1867	C	Paula & Santos 2000	X	**FK**
Xantho poressa (Olivi, 1792)	C	Rodríguez & Martin 1997	X	
Xantho sexdentatus (Miers, 1881)	U	-	-	
THORACOTREMATA Guinot, 1977				
Grapsidae MacLeay, 1838				
Pachygrapsus gracilis (Saussure, 1858)	C	Cházaro-Olvera & Rocha-Ramírez 2007	rd	**FK**
Pachygrapsus marmoratus (Fabricius, 1787)	I	Guerao et al. 1997	X	
Pachygrapsus maurus (Lucas, 1846)	U	-	-	
Pachygrapsus transversus (Gibbes, 1850)	C	Flores et al. 1998	X	**FK**
Planes minutus (Linnaeus, 1758)	U	***in prep.***	**Nw**	**FK**
Plagusiidae Dana, 1851				
Euchirograpsus liguricus H. Milne Edwards, 1853	U	-	-	
Percnidae Števčić, 2005				
Percnon gibbesi (H. Milne Edwards, 1853)	I	***in prep.***	**RD**	**FK**
Varunidae H. Milne Edwards, 1853				
Asthenognathus atlanticus Monod, 1933	C	Bocquet 1965	rd	

Brachynotus atlanticus Forest, 1957	C	Rodríguez et al. 1993	X	
Brachynotus foresti Zariquiey Álvarez, 1968	U	-	-	
Brachynotus sexdentatus (Risso, 1827)	C	Cuesta et al. 2000	X	
Eriocheir sinensis H. Milne Edwards, 1853	C	Montú et al. 1996	rd	
Hemigrapsus takanoi Asakura and Watanabe, 2005	U	-	-	
Ocypodidae Rafinesque, 1815				
Uca (*Afruca*) *tangeri* (Eydoux, 1835)	C	Rodríguez & Jones 1993	X	**FK**
Pinnotheridae de Haan, 1833				
Afropinnotheres monodi Manning, 1993	C	Marco-Herrero et al. 2015	**Nw**	**FK**
Nepinnotheres pinnotheres (Linnaeus, 1758)	C	Atkins 1955	rd	
Pinnotheres pisum (Linnaeus, 1767)	C	Atkins 1955	rd	

CONCLUSIONES

1. Se ha generado por primera vez una gran base de datos moleculares para los braquiuros ibéricos. Se han conseguido secuencias para el marcador 16S de 115 especies, y para el "código de barras" (Cox1) de 118 especies. De las 233 secuencias obtenidas, 118 son nuevas aportaciones: 57 nuevas secuencias para el gen Cox1 y 61 nuevas secuencias para el marcador 16S. El 90% de las especies ibéricas podrán ser identificadas a nivel molecular, con los marcadores genéticos utilizados.

2. Se completó un inventario de la fauna de braquiuros presentes en la Península Ibérica, actualizando el trabajo de Ricardo Zariquiey Álvarez (1968) para esta región y de Cédric d'Udekem d'Acoz (1999) para Europa. Los cambios en la sistemática realizados en los últimos años han afectado la clasificación original, por lo que ahora se cuenta con 20 superfamilias, 36 familias y 77 géneros. Además de nuevas aportaciones para la carcinofauna ibérica.

3. Hay 10 nuevas especies para la ciencia presentes en agua ibéricas:

Calappa tuerkayana, *Chaceon inglei*, *Chaceon mediterraneus*, *Homologenus boucheti*, *Maja brachydactyla*, *Macropodia deflexa, Macropodia parva, Monodeus guinotae Pilumnus* sp. y *Pisa* sp.

4. Se han encontrado 17 especies ya conocidas, pero citadas en la Península Ibérica después de 1968: *Afropinnotheres monodi*, *Brachynotus atlanticus, Brachynothus gemmellaroi*, *Calappa pelii*, *Chaceon affinis*, *Cryptosoma cristatum*, *Cymonomus normani*, *Ethusina talismani*, *Geryon trispinosus*, *Liocarcinus maculatus*, *Liocarcinus mcleayi*, *Microcassiope minor*, *Paractaea monodi*, *Paragalene longicrura*, *Pisa carinimana*, *Velolambrus expansus* y *Xantho sexdentatus*.

5. Hay un total de 10 especies exóticas introducidas en los últimos 30 años en aguas ibéricas: *Callinectes exasperatus*, *Callinectes sapidus, Charybdis feriata*, *Dyspanopeus sayi*, *Eriocheir sinensis, Hemigrapsus takanoi*, *Pachygrapsus gracilis, Percnon gibbesi*, *Pilumnopeus africanus* y *Rhithropanopeus harrisii.*

6. Diferentes estudios morfológicos y moleculares sobre las especies: *Brachynotus gemmellaroi*, *Calappa tuerkayana*, *Geryon trispinosus*, *Macropodia parva* y *Monodaeus guinotae* apuntan a que se trata de posibles sinonimias de *B. sexdentatus*, *C. granulata*, *G. longipes*, *M. rostrata* y *M. couchii*, respectivamente, pero se precisa aun confirmación.

7. Se describen las megalopas de 12 especies para las cuales no se tenía conocimiento de su morfología: *Afropinnotheres monodi, Derilambrus angulifrons, Ergasticus clouei, Macropodia czerjawskii*, *Liocarcinus maculatus*, *L. vernalis*, *L. zariquieyi*, *Planes minutus, Pilumnus* sp., *Pisa* sp., *Portunus hastatus* y *Sirpus zariquieyi.*

8. Se re-describen las megalopas de 4 especies: *Atelecyclus undecimdentatus Dyspanopeus sayi*, *Percnon gibbesi* y *Rhithropanopeus harrisii*.

9. Los análisis de las secuencias de dos genes (16S rDNA y Cox1) revelaron grandes diferencias entre *Ergasticus* y otros miembros de la familia Inachidae, incluyendo el género tipo *Inachus*. El análisis filogenético realizado y la comparación de caracteres larvales sugieren la eliminación de *Ergasticus* y géneros relacionados (*Bothromaia, Pleisticanthoides, Parapleisticantha* y *Pleistacantha*) de la familia Inachidae y situarlos dentro de la familia Oregoniidae como una subfamilia separada, Pleistacanthinae Števčić, 2005.

10. La base de datos molecular obtenida para los marcadores (16S y Cox1), supone una contribución importante a futuro, para la realización de diferentes estudios y/o aplicaciones:

a. Estudios filogenéticos que servirán como base para resolver la filogenia de varios grupos complicados y difíciles de estudiar en la Península Ibérica. En particular, se encuentran ya en preparación los estudios de la familia Inachidae y del género *Ebalia*.

b. Estudiar la dinámica de poblaciones de cangrejos, sobre todo aquellas especies de interés comercial, ya que se podrán determinar sus periodos de reclutamiento y su área de dispersión, épocas de reproducción, zonas de dispersión y concentración larval, así como áreas de asentamiento y reclutamiento, imprescindible para una gestión sostenible de sus pesquerías.

c. Proporciona una correcta identificación de ejemplares juveniles (muy difíciles de reconocer incluso por expertos), ejemplares incompletos, o incluso realizar estudios sobre relaciones tróficas entre especies (e.g. braquiuros en contenidos estomacales de *Thunnus* sp.).

d. Permite la detección temprana de especies tanto de aquellas que están ampliando su rango de distribución como de especies invasoras, como por ejemplo el nuevo *Pinnotheres* sp. Este cangrejo africano es simbionte, por lo que el adulto es más difícil de encontrar. En este estudio en preparación, se detectó el desarrollo completo en el plancton mediante la morfología larval y se confirmó mediante técnicas moleculares que no se trataba de ninguna de las especies de pinnotheridos ibéricos conocidos.

11. Las nuevas megalopas descritas y re-descritas han ampliado la información morfológica de esta fase larval de los cangrejos ibéricos con respecto a los trabajos de Ingle (1992) para especies Atlánticas y de Pessani et al. (2004) para las Mediterráneas, que permitían identificar las megalopas de hasta un total de 55 especies de braquiuros ibéricos. Esta tesis proporciona información morfológica nueva para 37 especies, lo que supone un incremento del 26.5% y permite incluir información morfológica de 92 especies en la clave que se ha desarrollado.

12. El estudio combinado de la morfología y marcadores moleculares nos permite concluir que no todas las 92 especies pueden discriminarse a nivel de morfología de la megalopa. Se ha encontrado poca o ninguna variabilidad fenotípica entre especies de algunos géneros, como por ejemplo el caso de *Liocarcinus* y *Brachynotus* que necesitan un mayor esfuerzo y revisión detallada de otros caracteres para obtener unos mejores resultados (en preparación).

13. Del estudio morfológico minucioso de las megalopas, se detectan caracteres importantes, que se consideraban constantes, que muestran variabilidad intraespecífica, tanto en ejemplares colectados del plancton como obtenidos en laboratorio. Por ejemplo: setación de los urópodos y segmentación de la antena.

14. Se añade la descripción de la placa esternal de todos los ejemplares estudiados. Un carácter apenas descrito hasta ahora. La nueva comparación de este carácter, nos permite confirmar que es constante dentro de una misma especie, llegando a ser en algunos grupos constante a nivel de género. Por lo tanto, en combinación con otros caracteres, se debería de considerar un nuevo carácter morfológico de utilidad en la identificación de las megalopas.

15. El área de estudio comprende todas las aguas de la Península Ibérica, tanto las marinas (desde la zona intermareal a profundidades aproximadas de 1.200 metros) como las continentales. El gran tamaño de la zona a estudiar y los costos asociados a los muestreos de plancton, impidieron que los muestreos fueran uniformes y periódicos en toda el área. Por tanto, especies menos frecuentes y de distribución local pueden no estar incluidas en las muestras analizadas. En cualquier caso, las secuencias disponibles permitirán identificar a nivel molecular el 90% de las especies ibéricas.

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APÉNDICE I

Apéndice I. Tabla resumen con la información disponible referente a las descripciones originales y trabajo realizado en esta tesis para cada megalopa/especie de braquiuro de la Península ibérica. **C** Descripción completa, **I** Descripción incompleta, **N** Nueva descripción, **NC** Nueva contribución en una clave de identificación, **D** Larva desconocida, **R** Redescripción, **Rd** Redibujada a partir de la descripción original, **X** Redibujados a partir de ejemplares estudiados. Otras observaciones: (**-**) Sin datos disponibles, **Sin** Sinonimia ***** Se confirma la asignación de una megalopa colectada del plancton por Paula (1987) a la especie *Atelecyclus undecimdentatus*.

Taxa/Species	Estado de la descripción	Referencia	Análisis	Clave
BRACHYURA Linnaeus, 1758				
PODOTREMATA Guinot, 1977				
Cymonomidae Bouvier, 1898				
Cymonomus granulatus (Thomson, 1873)	D	-	-	
Cymonomus normani Lankester, 1903	D	-	-	
Dromiidae de Haan, 1833				
Dromia personata (Linnaeus, 1758)	I	Rice et al. 1970	Rd	
Homolodromiidae Alcock, 1899				
Dicranodromia mahieuxii A. Milne-Edwards, 1883	D	-	-	
Homolidae de Haan, 1839				
Homola barbata (Fabricius, 1793)	C	Rice 1974	X	
Homologenus boucheti Guinot and Richer de Forges, 1995	D	-	-	
Paromola cuvieri (Risso, 1816)	D	-	-	
Latreilliidae Stimpson, 1858				
Latreillia elegans Roux, 1830	C	Rice 1982	Rd	
EUBRACHYURA de Saint Laurent, 1980				

HETEROTREMATA Guinot, 1977				
Calappidae de Haan, 1833				
Calappa granulata (Linnaeus, 1758)	C	Guerao et al. 1998	Rd	
Calappa pelii Herklots, 1851	D	-	-	
Calappa tuerkayana Pastore, 1995	D	-	Sin	
Cryptosoma cristatum Brullé, 1837	D	-	-	
Atelecyclidae Ortmann, 1893				
Atelecyclus rotundatus (Olivi, 1792)	C	Hong & Ingle 1987	X	
Atelecyclus undecimdentatus (Herbst, 1783)	C*	***in prep.***	**R**	**NC**
Cancridae Latreille, 1802				
Cancer bellianus Jonhson, 1861	I	Ingle 1998	-	
Cancer pagurus Linnaeus, 1758	C	Ingle 1998	X	
Corystidae Samouelle, 1819				
Corystes cassivelaunus (Pennant, 1777)	I	Ingle & Rice 1971	X	
Dorippidae MacLeay, 1838				
Medorippe lanata (Linnaeus, 1767)	C	Gilet 1952	Rd	**NC**
Ethusidae Guinot, 1977				
Ethusa mascarone (Herbst, 1785)	I	Cano 1892	Rd	**NC**
Ethusina talismani A. Milne-Edwards and Bouvier, 1897	D	-	-	
Eriphiidae MacLeay, 1838				
Eriphia verrucosa (Forskål, 1775)	I	Lumare & Gozzo 1972	X	
Goneplacidae MacLeay, 1838				
Goneplax rhomboides (Linnaeus, 1758)	C	Ingle & Clark 1983	X	
Progeryonidae Števčić, 2005				
Paragalene longicrura (Nardo, 1869)	D	-	-	
Leucosiidae Samouelle, 1819				

Ebalia cranchii Leach, 1817	I	Kurian 1956	X	**NC**
Ebalia deshayesi Lucas, 1846	D	-	-	
Ebalia edwardsii Costa, 1838	D	-	-	
Ebalia granulosa H. Milne Edwards, 1837	U	-	Sin	
Ebalia nux A. Milne-Edwards, 1883	U	-	-	
Ebalia tuberosa (Pennant, 1777)	C	Salman 1982a	Rd	
Ebalia tumefacta (Montagu, 1808)	C	Salman 1982/ Paula 1987	Rd	**NC**
Ilia nucleus (Linnaeus, 1758)	C	Bartilotti et al. 2009	X	**NC**
Merocryptus boletifer A. Milne-Edwards and Bouvier, 1894	D	-	-	
Epialtidae MacLeay, 1838				
Acanthonyx lunulatus (Risso, 1816)	C	Guerao & Abelló 1996	X	
Anamathia rissoana (Roux, 1828)	D	-	-	
Herbstia condyliata (Fabricius, 1787)	I	Cano 1893	X	**NC**
Lissa chiragra (Fabricius, 1775)	C	Guerao et al. 2003	Rd	**NC**
Pisa armata (Latreille, 1803)	C	Ingle & Clark 1980	Rd	
Pisa carinimana Miers, 1879	D	-	-	
Pisa hirticornis (Herbst, 1804)	D	-	-	
Pisa muscosa (Linnaeus, 1758)	D	-	-	
Pisa nodipes (Leach, 1815)	D	-	-	
Pisa tetraodon (Pennant, 1777)	C	Rodríguez 1997	Rd	
Pisa sp. Marco-Herrero et al. (in prep.)	D	***in prep.***	**N**	**NC**
Rochinia carpenteri (Thomson, 1873)	C	Ingle 1979	Rd	
Inachidae MacLeay, 1838				
Achaeus cranchii Leach, 1817	I	Bocquet 1954	Rd	
Achaeus gracilis (Costa, 1839)	D	-	-	
Dorhynchus thomsoni Thomson, 1873	I	Williamson 1982	Rd	

Inachus aguiarii Brito Capello, 1876	D	-	-	
Inachus communissimus Rizza, 1839	D	-	-	
Inachus dorsettensis (Pennant, 1777)	C	Ingle 1977	X	
Inachus leptochirus Leach, 1817	I	Clark 1983	-	
Inachus phalangium (Fabricius, 1775)	I	Clark 1983	-	
Inachus thoracicus Roux, 1830	C	Guerao et al. 2002	Rd	**NC**
Macropodia czernjawskii (Brandt, 1880)	D	Marco-Herrero et al. 2012	**N**	**NC**
Macropodia deflexa Forest, 1978	D	-	-	
Macropodia linaresi Forest and Zariquiey Álvarez, 1964	D	-	-	
Macropodia longipes (A. Milne-Edwards and Bouvier, 1899)	C	Guerao & Abelló 1997	Rd	**NC**
Macropodia longirostris (Fabricius, 1775)	D	-	-	
Macropodia parva Van Noort and Adema, 1985	C	González-Gordillo & Rodríguez 2001	Sin	
Macropodia rostrata (Linnaeus, 1761)	C	Ingle 1982	X	
Macropodia tenuirostris (Leach, 1814)	C	Salman 1981	Sin	
Majidae Samouelle, 1819				
Eurynome aspera (Pennant, 1777)	C	Salman 1982	X	
Eurynome spinosa Hailstone, 1835	I	Ingle 1981	X	**NC**
Maja brachydactyla Balss, 1922	C	Guerao et al. 2008	X	**NC**
Maja crispata Risso, 1827	C	Rodríguez 2002	X	**NC**
Maja goltziana d'Oliveira, 1888	C	Paula 1988	Rd	
Maja squinado (Herbst, 1788)	C	Guerao et al. 2008	X	
Oregonidae Garth, 1958				
Ergasticus clouei A. Milne-Edwards, 1882	D	Marco-Herrero et al. 2013	**N**	**NC**
Palicidae Bouvier, 1898				
Palicus caronii (Roux, 1828)	D	-	-	
Parthenopidae MacLeay, 1838				

Derilambrus angulifrons (Latreille, 1825)	D	Marco-Herrero et al. 2015	N	NC
Distolambrus maltzami (Miers, 1881)	D	-	-	
Parthenopoides massena (Roux, 1830)	C	Thiriot 1973	X	
Spinolambrus macrochelos (Herbst, 1790)	D	-	-	
Velolambrus expansus (Miers, 1879)	D	-	-	
Pilumnidae Samouelle, 1819				
Pilumnopeus africanus (De Man, 1902)	D	-	-	
Pilumnus aestuarii Nardo, 1869	D	-	-	
Pilumnus hirtellus (Linnaeus, 1761)	I	Ingle 1983	X	
Pilumnus inermis A. Milne-Edwards and Bouvier, 1894	D	-	-	
Pilumnus spinifer H. Milne Edwards, 1834	I	Guerao et al. 2005	X	NC
Pilumnus villosissimus (Rafinesque, 1814)	D	-	X	
Pilumnus sp. d´Udekem d´Acoz and Schubart (in prep.)	D	***in prep***	N	NC
Carcinidae MacLeay, 1838				
Carcinus aestuarii Nardo, 1847	C	Rice & Ingle 1975	X	
Carcinus maenas (Linnaeus, 1758)	C	Rice & Ingle 1975	X	
Portumnus latipes (Pennant, 1777)	C	Paula 1988	X	
Xaiva biguttata (Risso, 1816)	I	Lebour 1944	Rd	NC
Geryonidae Colosi, 1923				
Chaceon affinis (A. Milne-Edwards and Bouvier, 1894)	D	-	-	
Chaceon mediterraneus Manning and Holthuis, 1989	D	-	-	
Chaceon inglei Manning and Holthuis, 1989	D	-	-	
Geryon longipes A. Milne-Edwards, 1881	C	Guerao et al. 1996	X	
Geryon trispinosus (Herbst, 1803)	I	Ingle 1979b, 1992	X	
Pirimelidae Alcock, 1899				
Pirimela denticulata (Montagu, 1808)	C	Flores & Paula 2000	X	

Sirpus zariquieyi Gordon, 1953	D	***in prep.***	**N**	**NC**
Polybiidae Ortmann, 1893				
Bathynectes longipes (Risso, 1816)	C	Ingle 1985	X	
Bathynectes maravigna (Prestandrea, 1839)	D	-	-	
Liocarcinus bolivari (Zariquiey Álvarez, 1948)	D	-	-	
Liocarcinus corrugatus (Pennant, 1777)	C	Kim & Hong 1999	X	
Liocarcinus depurator (Linnaeus, 1758)	C	Guerao et al. 2006	X	
Liocarcinus holsatus (Fabricius, 1798)	C	Rice & Ingle1975	X	**NC**
Liocarcinus maculatus (Risso, 1827)	D	***in prep.***	**N**	**NC**
Liocarcinus marmoreus (Leach, 1814)	C	Rice & Ingle 1975	X	
Liocarcinus mcleayi (Barnard, 1947)	D	-	-	
Liocarcinus navigator (Herbst, 1794)	I	Ingle 1992	X	
Liocarcinus pusillus (Leach, 1815)			X	
Liocarcinus vernalis (Risso, 1816)	D	***in prep.***	**N**	**NC**
Liocarcinus zariquieyi Gordon, 1968	D	***in prep.***	**N**	**NC**
Macropipus tuberculatus (Roux, 1830)	I	Rice & Ingle 1978	X	**NC**
Necora puber (Linnaeus, 1767)	C	Rice & Ingle 1975	X	
Polybius henslowii Leach, 1820	I	Ingle 1992	X	
Portunidae Rafinesque, 1815				
Callinectes exasperatus (Gerstaecker, 1856)	D	-	-	
Callinectes sapidus Rathbun, 1896	I	Costlow & Bookhout 1959	Rd	
Charybdis (*Charybdis*) *feriata* (Linnaeus, 1758)	I	Fielder 1984/ Hu 1983	Rd	**NC**
Portunus (*Portunus*) *hastatus* (Linnaeus, 1767)	D	***in prep.***	**N**	**NC**
Thiidae Dana, 1852				
Thia scutellata (Fabricius, 1793)	C	Ingle 1984	Rd	
Panopeidae Ortmann, 1893				

Dyspanopeus sayi (Smith, 1869)	I	Marco-Herrero et al. 2013	R	NC
Panopeus africanus A. Milne-Edwards, 1867	C	Rodríguez & Paula 1993		NC
Rhithropanopeus harrisii (Gould, 1841)	I	Marco-Herrero et al. 2014	R	
Xanthidae MacLeay, 1838				
Monodaeus couchii (Couch, 1851)	C	Ingle 1983	X	
Microcassiope minor (Dana, 1852)	D	-	-	
Paractaea monodi Guinot, 1969	D	-	-	
Xantho hydrophilus (Leach, 1814)	I	Ingle 1983	X	
Xantho pilipes A. Milne-Edwards, 1867	C	Paula & Santos 2000	X	NC
Xantho poressa (Olivi, 1792)	C	Rodríguez & Martin 1997	X	
Xantho sexdentatus (Miers, 1881)	D	-	-	
THORACOTREMATA Guinot, 1977				
Grapsidae MacLeay, 1838				
Pachygrapsus gracilis (Saussure, 1858)	D	-	-	
Pachygrapsus marmoratus (Fabricius, 1787)	D	Guerao et al. 1997	X	
Pachygrapsus maurus (Lucas, 1846)	D	-	-	
Pachygrapsus transversus (Gibbes, 1850)	C	Flores et al. 1998	X	NC
Planes minutus (Linnaeus, 1758)	D	***in prep.***	N	NC
Plagusiidae Dana, 1851				
Euchirograpsus liguricus H. Milne Edwards, 1853	D	-	-	
Percnidae Števčić, 2005				
Percnon gibbesi (H. Milne Edwards, 1853)	I	***in prep.***	R	NC
Varunidae H. Milne Edwards, 1853				
Asthenognathus atlanticus Monod, 1933	C	Bocquet 1965	Rd	
Brachynotus atlanticus Forest, 1957	C	Rodríguez et al. 1993	X	
Brachynotus foresti Zariquiey Álvarez, 1968	D	-	-	

Brachynotus sexdentatus (Risso, 1827)	C	Cuesta et al. 2000	X	
Eriocheir sinensis H. Milne Edwards, 1853				
Hemigrapsus takanoi Asakura and Watanabe, 2005	D	-	-	
Ocypodidae Rafinesque, 1815				
Uca (*Afruca*) *tangeri* (Eydoux, 1835)	C	Rodríguez & Jones 1993	X	**NC**
Pinnotheridae de Haan, 1833				
Afropinnotheres monodi Manning, 1993	p	Marco-Herrero et al. 2015	**N**	**NC**
Nepinnotheres pinnotheres (Linnaeus, 1758)	C	Atkins 1955	Rd	
Pinnotheres pisum (Linnaeus, 1767)	C	Atkins 1955	Rd	

APÉNDICE II

Apéndice II. Especímenes obtenidos en el presente estudio para cada una de las especies de braquiuros de la Península Ibérica. Se detalla el número de ejemplares adultos estudiados junto con la institución que los cede y las secuencias logradas a partir de estos. Además se indican, para cada especie, las secuencias disponibles en Genbank para los genes 16S y Cox1.

Taxa/Especies	Número Ejemplares	Institución	16S	Cox1	Genbank
BRACHYURA Linnaeus, 1758					
PODOTREMATA Guinot, 1977					
Cymonomidae Bouvier, 1898					
Cymonomus granulatus (Thomson, 1873)	1	ICM	x-N	x	Cox1
Cymonomus normani Lankester, 1903	1 (Formol)	NHM	-	-	-
Dromiidae de Haan, 1833					
Sternodromia spinirostris (Miers, 1881)	1 (Formol)	NHM	-	-	-
Dromia personata (Linnaeus, 1758)	1	ICM	x-N	x	Cox1
Homolodromiidae Alcock, 1899					
Dicranodromia mahieuxii A. Milne-Edwards, 1883	3	ICM	x-N	x-N	-
Homolidae de Haan, 1839					
Homola barbata (Fabricius, 1793)	1	ICM	x-N	x	Cox1
Homologenus boucheti Guinot & Richer de Forges, 1995	1	MNHN	x-N	x-N	-
Paromola cuvieri (Risso, 1816)	1	ICM	x-N	x-N	-
Latreilliidae Stimpson, 1858					
Latreillia elegans Roux, 1830	1	ICM	x-N	x	Cox1
EUBRACHYURA de Saint Laurent, 1980					
HETEROTREMATA Guinot, 1977					
Calappidae de Haan, 1833					
Calappa granulata (Linnaeus, 1758)	1	ICM	x-N	x	Cox1
Calappa pelii Herklots, 1851	1	ICM	x-N	x-N	-

Calappa tuerkayana Pastore, 1995					
Cryptosoma cristatum Brullé, 1837	1	NHM	x-N	x-N	-
Atelecyclidae Ortmann, 1893					
Atelecyclus rotundatus (Olivi, 1792)	3	ICMAN	x	x	16S/Cox1
Atelecyclus undecimdentatus (Herbst, 1783)	1	ICM	x	x-N	16S
Cancridae Latreille, 1802					
Cancer bellianus Jonhson, 1861	2	NHM	x-N	x	Cox1
Cancer pagurus Linnaeus, 1758	1	ICMAN	x	x	16S/Cox1
Corystidae Samouelle, 1819					
Corystes cassivelaunus (Pennant, 1777)	1	ICM	x	x	16S/Cox1
Dorippidae MacLeay, 1838					
Medorippe lanata (Linnaeus, 1767)	1	ICM	x	x	16S/Cox1
Ethusidae Guinot, 1977					
Ethusa mascarone (Herbst, 1785)	1	ICMAN	x-N	x-N	-
Ethusina talismani A. Milne-Edwards & Bouvier, 1897	1 (Formol)	NHM	-	-	-
Eriphiidae MacLeay, 1838					
Eriphia verrucosa (Forskål, 1775)	1	ICMAN	x	x	16S/Cox1
Goneplacidae MacLeay, 1838					
Goneplax rhomboides (Linnaeus, 1758)	1	ICMAN	x	x	16S/Cox1
Progeryonidae Števčić, 2005					
Paragalene longicrura (Nardo, 1869)	1	ICM	x-N	x-N	-
Leucosiidae Samouelle, 1819					
Ebalia cranchii Leach, 1817	5	NHM	x-N	x-N	-
Ebalia deshayesi Lucas, 1846	12	UMA	x-N	x-N	-
Ebalia edwardsii Costa, 1838	3	UMA	x-N	x-N	-
Ebalia granulosa H. Milne Edwards, 1837	6	ICM	x-N	x-N	-

Ebalia nux A. Milne-Edwards, 1883	5	ICM	x-N	x	Cox1
Ebalia tuberosa (Pennant, 1777)	16	ICM	x-N	x-N	-
Ebalia tumefacta (Montagu, 1808)	1	ICMAN	x-N	x-N	-
Ilia nucleus (Linnaeus, 1758)	1	ICMAN	x-N	x-N	-
Merocryptus boletifer A. Milne-Edwards & Bouvier, 1894	1 (Formol)	ICM	-	-	-
Epialtidae MacLeay, 1838					
Acanthonyx lunulatus (Risso, 1816)	3	ICMAN	x	x	16S/Cox1
Anamathia rissoana (Roux, 1828)	2 (Formol)	ICM	-	-	-
Herbstia condyliata (Fabricius, 1787)	2	ICM/UMA	x	x	16S/Cox1
Lissa chiragra (Fabricius, 1775)	2	ICM	x-N	x-N	-
Pisa armata (Latreille, 1803)	3	UMA	x-N	x-N	-
Pisa carinimana Miers, 1879	1	UMA	x-N	x-N	-
Pisa hirticornis (Herbst, 1804)	1	ICM	x-N	x-N	-
Pisa muscosa (Linnaeus, 1758)	3	UMA	x-N	x-N	-
Pisa nodipes (Leach, 1815)	4	ICM	x-N	x-N	-
Pisa tetraodon (Pennant, 1777)	5	ICM/ICMAN	x	x	16S/Cox1
Pisa sp. Marco-Herrero et al. (in prep.)	1	IEO	x-N	x-N	-
Rochinia carpenteri (Thomson, 1873)	1	ICM	x-N	x-N	-
Inachidae MacLeay, 1838					
Achaeus cranchii Leach, 1817	6	ICMAN	x-N	x-N	-
Achaeus gracilis (Costa, 1839)	2	ICM/ICMAN	x-N	x-N	-
Dorhynchus thomsoni Thomson, 1873	6	ICM	x-N	x-N	-
Inachus aguiarii Brito Capello, 1876	5	NHM	x-N	x-N	-
Inachus communissimus Rizza, 1839	2	ICM	x-N	x-N	-
Inachus dorsettensis (Pennant, 1777)	5	ICM	x	x	Cox1
Inachus leptochirus Leach, 1817	7	UMA	x	x	Cox1

Inachus phalangium (Fabricius, 1775)	3	ICMAN	x-N	x-N	-
Inachus thoracicus Roux, 1830	4	ICM	x-N	x-N	-
Macropodia czernjawskii (Br&t, 1880)	2	ICMAN	x-N	x-N	-
Macropodia deflexa Forest, 1978	4	NHM	x	-	-
Macropodia linaresi Forest & Zariquiey Álvarez, 1964	6	ICM	x-N	x-N	-
Macropodia longirostris (Fabricius, 1775)	2	ICM	x-N	x-N	-
Macropodia parva Van Noort & Adema, 1985	5	ICMAN	x	x	16S/Cox1
Macropodia rostrata (Linnaeus, 1761)	9	ICMAN	x-N	x	Cox1
Macropodia tenuirostris (Leach, 1814)	3	ICMAN	x	x	16S/Cox1
Majidae Samouelle, 1819					
Eurynome aspera (Pennant, 1777)	2	ICM	x-N	x-N	-
Eurynome spinosa Hailstone, 1835	2	UMA	x-N	x-N	-
Maja brachydactyla Balss, 1922	1	ICM	x	x	16S/Cox1
Maja crispata Risso, 1827	2	ICM	x	x	16S/Cox1
Maja goltziana d'Oliveira, 1888	1	ICM	-	-	16S/Cox1
Maja squinado (Herbst, 1788)	1	ICM	-	-	16S/Cox1
Oregonidae Garth, 1958					
Ergasticus clouei A. Milne-Edwards, 1882	1	ICM	x-N	x-N	-
Palicidae Bouvier, 1898					
Palicus caronii (Roux, 1828)	1	ICM	x	x-N	16S
Parthenopidae MacLeay, 1838					
Derilambrus angulifrons (Latreille, 1825)	1	ICM/ICMAN	x-N	x-N	-
Distolambrus maltzami (Miers, 1881)	1	ICM	x-N	x-N	-
Parthenopoides massena (Roux, 1830)	2	ICM	x-N	x-N	-
Spinolambrus macrochelos (Herbst, 1790)	1	ICM	x-N	x-N	-
Velolambrus expansus (Miers, 1879)	2 (Formol)	NHM	-	-	-

Pilumnidae Samouelle, 1819					
Pilumnopeus africanus (De Man, 1902)	1	ICMAN	x-N	x-N	-
Pilumnus aestuarii Nardo, 1869	0		-	-	-
Pilumnus hirtellus (Linnaeus, 1761)	3	ICMAN	x	x	16S/Cox1
Pilumnus inermis A. Milne-Edwards & Bouvier, 1894	1	UMA	-	-	Cox1
Pilumnus spinifer H. Milne Edwards, 1834	5	ICM/UMA	x-N	x	Cox1
Pilumnus villosissimus (Rafinesque, 1814)	1	ICMAN/UMA	x-N	x	Cox1
Pilumnus sp. d´Udekem d´Acoz & Schubart (in prep.)	1	ICMAN/UMA	x-N	x	Cox1
Carcinidae MacLeay, 1838					
Carcinus aestuarii Nardo, 1847	1	ICMAN	x	x	16S/Cox1
Carcinus maenas (Linnaeus, 1758)	1	ICMAN	x	x	16S/Cox1
Portumnus latipes (Pennant, 1777)	6	ICMAN	x	x-N	16S
Xaiva biguttata (Risso, 1816)	2	ICM/NHM	-	-	-
Geryonidae Colosi, 1923					
Chaceon affinis (A. Milne-Edwards & Bouvier, 1894)	1 (Formol)	MNHN	-	-	16S/Cox1
Chaceon mediterraneus Manning & Holthuis, 1989	3	ICM	x-N	x-N	-
Chaceon inglei Manning & Holthuis, 1989	1 (Formol)	NHM	-	-	-
Geryon longipes A. Milne-Edwards, 1881	2	ICM	-	x	16S/Cox1
Geryon trispinosus (Herbst, 1803)	0		-	-	16S
Pirimelidae Alcock, 1899					
Pirimela denticulata (Montagu, 1808)	3	ICMAN	x	x-N	16S
Sirpus zariquieyi Gordon, 1953	0	ICMAN	x*	x*-N	16S
Polybiidae Ortmann, 1893					
Bathynectes longipes (Risso, 1816)	2	ICM	x-N	x-N	-
Bathynectes maravigna (Prest&rea, 1839)	1	ICM	x-N	x-N	-
Liocarcinus bolivari (Zariquiey Álvarez, 1948)	3	ICM/ICMAN	x-N	x-N	-

Liocarcinus corrugatus (Pennant, 1777)	1	ICMAN	x		16S/Cox1
Liocarcinus depurator (Linnaeus, 1758)	3	ICM	x	x	16S/Cox1
Liocarcinus holsatus (Fabricius, 1798)	1	ICM	-	x	16S/Cox1
Liocarcinus maculatus (Risso, 1827)	5	ICM	-	x	16S/Cox1
Liocarcinus marmoreus (Leach, 1814)	0		-	-	16S/Cox1
Liocarcinus mcleayi (Barnard, 1947)	1	UMA	-	-	-
Liocarcinus navigator (Herbst, 1794)	2	ICMAN	x	-	16S/Cox1
Liocarcinus pusillus (Leach, 1815)	1	ICM	-	x	16S/Cox1
Liocarcinus vernalis (Risso, 1816)	3	ICMAN	x	-	16S/Cox1
Liocarcinus zariquieyi Gordon, 1968	4	ICMAN	x-N	x-N	-
Macropipus tuberculatus (Roux, 1830)	1	ICM	x	x	16S/Cox1
Necora puber (Linnaeus, 1767)	1	ICMAN	x	x	16S/Cox1
Polybius henslowii Leach, 1820	2	ICM	x	x	16S/Cox1
Portunidae Rafinesque, 1815					
Callinectes exasperatus (Gerstaecker, 1856)	1	ICMAN	x	-	16S
Callinectes sapidus Rathbun, 1896	2	ICM	x	x	16S/Cox1
Charybdis (*Charybdis*) *feriata* (Linnaeus, 1758)	1	ICM	x	x	16S/Cox1
Portunus (*Portunus*) *hastatus* (Linnaeus, 1767)	1	ICMAN	x	x-N	16S
Thiidae Dana, 1852					
Thia scutellata (Fabricius, 1793)	5	ICM/UMA/NHM	-	-	16S
Panopeidae Ortmann, 1893					
Dyspanopeus sayi (Smith, 1869)	2	ICM	x	x	16S/Cox1
Panopeus africanus A. Milne-Edwards, 1867	7	ICMAN	x	x	16S/Cox1
Rhithropanopeus harrisii (Gould, 1841)	2	ICMAN	x	x	16S/Cox1
Xanthidae MacLeay, 1838					
Monodaeus couchii (Couch, 1851)	1	ICM	x	x	16S/Cox1

Microcassiope minor (Dana, 1852)	2	UMA	-	-	-
Paractaea monodi Guinot, 1969	1	UMA	-	-	16S/Cox1
Xantho hydrophilus (Leach, 1814)	8	ICMAN	x-N	x	Cox1
Xantho pilipes A. Milne-Edwards, 1867	2	ICMAN	x	x	16S/Cox1
Xantho poressa (Olivi, 1792)	3	ICMAN	x	x	16S/Cox1
Xantho sexdentatus (Miers, 1881)	1	ICMAN	x	x	16S/Cox1
THORACOTREMATA Guinot, 1977					
Grapsidae MacLeay, 1838					
Pachygrapsus gracilis (Saussure, 1858)	2	ICMAN	x	x	16S/Cox1
Pachygrapsus marmoratus (Fabricius, 1787)	1	ICMAN	x	x	16S/Cox1
Pachygrapsus maurus (Lucas, 1846)	1	ICM	x	x	16S/Cox1
Pachygrapsus transversus (Gibbes, 1850)	3	ICMAN	x	x	16S/Cox1
Planes minutus (Linnaeus, 1758)	3	Malaspina	x	x-N	16S
Plagusiidae Dana, 1851					
Euchirograpsus liguricus H. Milne Edwards, 1853	1	ICM	x-N	x-N	-
Percnidae Števčić, 2005					
Percnon gibbesi (H. Milne Edwards, 1853)	1	ICMAN	x	x	16S/Cox1
Varunidae H. Milne Edwards, 1853					
Asthenognathus atlanticus Monod, 1933	2	IEO	x	-	16S
Brachynotus atlanticus Forest, 1957	2	ICMAN	x	x-N	16S
Brachynotus foresti Zariquiey Álvarez, 1968	0		-	-	16S
Brachynotus sexdentatus (Risso, 1827)	1	ICMAN	x	x-N	16S
Eriocheir sinensis H. Milne Edwards, 1853	1	ICMAN	x	x	16S/Cox1
Hemigrapsus takanoi Asakura & Watanabe, 2005	0		-	-	16S/Cox1
Ocypodidae Rafinesque, 1815					
Uca (*Afruca*) *tangeri* (Eydoux, 1835)	1	ICMAN	x	x	16S/Cox1

Pinnotheridae de Haan, 1833					
Afropinnotheres monodi Manning, 1993	13	ICMAN	x-N	x-N	-
Nepinnotheres pinnotheres (Linnaeus, 1758)	3	ICMAN	x	-	16S
Pinnotheres pisum (Linnaeus, 1767)	3	ICM/ICMAN	x	x	16S/Cox1

ICM Institut de Ciències de Mar; **ICMAN** Instituto de Ciencias Marinas de Andalucía; **IEO** Instituto Oceanográfico Español: Campañas MEDITS-INDEMARES-ARSA; **NHM** Natural History Museum (London); **Malaspina** Proyecto Malaspina; **MNHN** Muséum National d'Histoire Naturelle (París); **UMA** Universidad de Málaga; X Secuencia obtenida; **x-N** Secuencia nueva; (-) Sin datos.

* Secuencias de *Sirpus zariquieyi* obtenidas a partir de larvas del plancton.