Possible amphi-Atlantic dispersal of Scyllarus lobsters (Crustacea: Scyllaridae): molecular and larval evidence

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Abstract

DNA methods may contribute to better understand larval dispersal of marine lobsters. The molecular analysis of phyllosoma specimens from the East Atlantic facilitated for the first time here the description of Scyllarus subarctus Crosnier, 1970 larvae. The identification of S. subarctus phyllosome from Cabo Verde confirmed that this species has a much wider geographic distribution than previously thought. Moreover, the phylogenetic analyses placed S. depressus from the Western Atlantic together with the African species S. subarctus, instead of other American Scyllarus. In fact, S. depressus and S. subarctus formed a strongly supported clade with comparatively low genetic differentiation, suggesting the possibility that they might be recently-diverged sister taxa with an amphi-Atlantic distribution. Support for this is provided by the examination of S. subarctus larvae and the lack of any qualitative character that would allow for differentiation between the adults of S. subarctus and S. depressus. The results obtained highlight the challenges of current Scyllarus systematics and the need for further research on Atlantic slipper lobsters.

Key words: Slipper lobster, phylogenetics, amphi-Atlantic distribution, planktonic larval duration, DNA barcoding

Introduction

Slipper lobsters, Scyllaridae Latreille, 1825, constitute a monophyletic group of crustaceans characterized by possessing a completely flattened last antennal segment (Spanier 1991; Haug et al. 2015). Together with palinurid lobsters, scyllarid lobsters have a unique larval form particularly adapted to planktonic life and long-distance dispersal, the phyllosoma (Palero & Abello 2007). This planktonic phase contains multiple stages that finally transform into a benthic decapod, taking up to 2 years to fully develop and metamorphose depending on species (Booth et al. 2005; Palero et al. 2014a). Scyllaridae includes more than 90 species consigned to 20 genera (Chan 2010) and comprises four subfamilies: Arctidinae Holthuis, 1985 (including Scyllarides Gill, 1898 and Arctides Holthuis, 1960), Ibacinae Holthuis, 1985 (Ibacus Leach, 1815, Evibacus Smith, 1869 and Parribacus Dana, 1852), Theninae Holthuis, 1985 (Thenus Leach, 1816) and Scyllarinae Latreille, 1825 (Scyllarus Fabricius, 1775 and 13
additional new genera proposed by Holthuis (1960, 1985, 2002). The latest molecular and phyllosoma morphology results support the monophyly of Arctidinae, Theninae and Scyllarinae, but Ibacininae appears to be a paraphyletic group (Yang et al. 2012; Bracken-Grissom et al. 2014; Palero et al. 2014b). The study of phyllosoma larvae is a difficult task though, mainly due to the great difficulty in rearing them in the laboratory and establishing the identification of plankton-caught material.

Subsequent to the revision of Scyllarus by Holthuis (2002), only 9 species were retained within the original genus. Four species are distributed in Western Atlantic waters: S. americanus (Smith, 1869), S. chacei Holthuis, 1960, S. depressus (Smith, 1881) and S. planorbis Holthuis, 1969; and 5 in European and African waters: S. arctus (Linnaeus, 1758), S. caparti Holthuis, 1952, S. paradoxus Miers, 1881, S. pygmaeus Bate, 1888 and S. subarctus Crosnier, 1970. Scyllarus americanus, S. depressus, and S. chacei have been recorded from North Carolina to Brazil (Holthuis 1960; Robertson 1968b; Lyons 1970; Tavares 1997), and S. planorbis has a restricted distribution ranging from Honduras to Suriname (Dall’Occo 2010). With regard to the Eastern Atlantic species, S. arctus and S. pygmaeus are commonly reported from the Mediterranean Sea and North-East Atlantic (Pessani & Mura 2007; Palero et al. 2011), while S. paradoxus is limited to the Mediterranean Sea and North-East Atlantic (Pessani & Mura 2007; Palero et al. 2011), while S. paradoxa has been assigned to S. subarctus (Muñoz et al. 2012; Garcia-Isarch et al. 2015), suggesting that this species may have an intertropical distribution.

Complete larval descriptions for American Scyllarus were provided by Robertson (1968a, 1971, 1979) from laboratory breed material, with some series complemented using planktonic specimens. In comparison, for Eastern Atlantic species, only the final phyllosoma and nisto stages of S. arctus and S. pygmaeus have been confirmed by DNA barcoding (Palero et al. 2008, 2009a, 2011). The identification of lobster larvae from Africa has been limited by difficulties in obtaining ethanol-preserved planktonic material, so that species assignments from previous records remain uncertain (Lindley et al. 2004). Recently, a large collection of phyllosomae from Eastern Atlantic waters was obtained during the Migrants and Active Flux in the Atlantic Ocean (MAFIA) project expedition. DNA methods allow for the first time the identification and description of S. subarctus phyllosoma stages VII, IX, and X. The present study confirms that S. subarctus has a much wider geographic distribution than previously thought and suggests the possibility of amphi-Atlantic dispersal of Scyllarus larvae.

Materials and methods

The phyllosomae used for molecular analysis and descriptions were obtained during the MAFIA cruise between 3rd and 29th April 2015. A total of 13 stations were sampled on board of the RV Hesperides, which crossed the Atlantic from Salvador de Bahia, Brazil, to Las Palmas, Canary Islands. Micronekton samples were collected with a mid-water trawl (Mesopelagos net) with a mean mouth opening of 5 × 7 m and a final cod-end of 4 mm. This system allowed discriminating samples from different levels into the water column to depths around 1000 m. Phyllosoma specimens from the MAFIA cruise were obtained near Cabo Verde, 900–1000 km away from continental Africa. Sampling co-ordinates, date and depth are shown in Table I. Samples were preserved in absolute ethanol and registered in the invertebrate collections of the Universidad de Cádiz.

Phyllosoma specimens showing identical morphological traits to our DNA-identified material from Cabo Verde were found among the Institutt de Ciències del Mar collections (Table I). These specimens were sampled during the SNEC-II cruise from North of Lüderitz (18°S 10°30'E), at about 217 km off the Namibian coast. A Rectangular Mid-water Trawl plankton net with 1 m opening and 200 µm mesh was used to sample a single station between 0 and 200 m depth. SNEC-II collected specimens were, however, preserved in formalin and their specific identification was based on morphology only. Finally, reference DNA sequences were obtained from type specimens of Scyllarus subarctus deposited in the Muséum National d’Histoire Naturelle, France, and GenBank (see Table I for accession codes).

DNA analyses. Total genomic DNA extraction was performed using the Chelex-protK method (Palero et al. 2010). The standard universal primers for the 16S rDNA gene (Marco-Herrero et al. 2013) were used for DNA barcoding, since this marker shows a higher amplification rate than COI primers in Achelata (Palero et al. 2009b;
Amplifications were carried out with ~30 ng of genomic DNA in a reaction containing 1 U of Taq polymerase (Amersham), 1 × buffer (Amersham), 0.2 mM of each primer and 0.12 mM dNTPs. The polymerase chain reaction (PCR) thermal profile used was 94°C for 4 min for initial denaturation, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 30 s and a final extension at 72°C for 4 min. Amplified PCR products were purified with QIAquick PCR Purification Kit (QIAGEN Inc.) before direct sequencing of the product. The sequences were obtained using the kit BigDye v3.1 (Applied Biosystems) on an ABI Prism 3770. The chromatograms for each DNA sequence were checked using the software BioEdit ver. 7.2.5 (Hall 1999). Sequence alignment was conducted using the program Muscle ver. 3.6 (Edgar 2004) with default parameters. Selection of the nucleotide substitution model was performed according to the BIC criterion as implemented in MEGA v7 (Kumar et al. 2016). The aligned sequences and selected evolutionary model were used to estimate genetic distances and the corresponding Maximum Likelihood phylogenetic tree in MEGA.

**Larval description.** The larval accounts were based on the malacostracan somite plan, described from anterior to posterior and proximal to distal (Clark et al. 1998; Palero et al. 2016). Morphological illustrations of the larvae were drawn using a camera lucida attached to a Leica high-performance stereo microscope (M165C, Leica Microsystems) and the maxillae and mandibles were dissected before drawing. The stage assignment of *Scyllarus* phyllosoma larvae is based on Robertson (1968a, 1971) and Webber & Booth (2001). The following measures were taken for each individual analysed: total length (TL) from the anterior margin of the cephalic shield between the eyes to the posterior margin of the telson; cephalic length (CL) from the anterior to the posterior margin of the cephalic shield; cephalic width (CW) measured at the widest part of the cephalic shield; pleon length (PDL) from the anterior margin of the pleon to the posterior margin of the telson.

**Results**

**Molecular identification of phyllosoma larvae.** DNA sequences obtained from the MAFIA phyllosomae and adult specimens included 428 bp positions after alignment. The DNA substitution model selected according to the BIC method was the Hasegawa-Kishino-Yano model (HKY) with invariant positions. The rate variation model allowed for 61% of the sites to be evolutionarily invariable. The phylogenetic tree obtained by Maximum Likelihood (Ln = -1652.65) strongly supported the species-level assignment of the Cabo Verde larvae, clustering with adult *S. subarctus* and genetic distances below 0.01 (ranging between 0.005 and 0.007). *S. depressus* and *S. subarctus* formed a monophyletic clade with high bootstrap support (97%) (Fig. 1). Genetic distances between *S. subarctus* and *S. depressus* (between 1.9 and 2.4%) were 3 times lower than those observed between *S. subarctus* and other African species (5.8 to 7.9%) and 8 times lower than genetic distances with species from America (between 14.2 and 17.3%).

**Morphological description.** A total of 18 specimens, 11 from the MAFIA cruise and 7 from SNEC-II, were used for morphological characterization of *S. subarctus* phyllosomae. The larvae could be assigned to 3 different stages based on morphology namely, stage VII, IX (subfinal) and X (final), and which also correspond with separate groups based on total length. Correlation between TL and both CL and CW values was linear, with CL (CL=0.67 TL + 1.67; $R^2=0.981$) increasing much faster than CW (CW=0.51 TL + 2.02; $R^2=0.985$) during these late stages.

**Scyllarus subarctus** Crosnier, 1970

**Phyllosoma, stage VII (PHMF 13, PHMF 51)**

**Dimensions.** N = 7; TL = 9.1–10.9 mm; CL = 6.4–7.7 mm; CW = 7.4–8.7 mm; PDL = 1.1–1.4 mm.

**Cephalic shield** (Fig. 2A). Sub-rectangular; 1.2 × wider than long.

**Antennule** (Fig. 5A). Peduncle 3-segmented, last segment shorter and carrying two flagella (primary and accessory); accessory flagellum longer than primary, unsegmented with 2 setae in external side and 1–3 longer setae in the apical region; primary flagellum unsegmented with 8–9 rows of sensory setae (aesthetasc). **Antenna** (Fig. 5A). Biramous and unsegmented; longer than antennule.
Mandibles (Fig. 5D, G). Asymmetrical dentition. Left mandible (Fig. 5D) larger and with more teeth on incisor process than right (Fig. 5G). Right mandible teeth are curved towards molar process while teeth of left mandible are elongated. Both mandibles with abundant small teeth distributed over surface and molar process crowned with many denticles.

Maxillule (Fig. 5J). Uniramous. Coxal and basal endites with 7 setae (2 and 3 strong setae, respectively). Palp (endopod) absent.

Maxilla (Fig. 6A). Endites, endopod and exopod (scaphognathite) not differentiated.

First maxilliped (Fig. 6A). Unsegmented and cone-shaped; rudimentary bud.

Second maxilliped (Fig. 6D). Five-segmented, with 0, 1, 2, 10, 3 setae respectively.

Third maxilliped (Fig. 4, 6H, G). Five-segmented, with ventral coxal spine; distal part of propodus and dactyl densely setose. Two serrated and curved setae in distal end of propodus.

Pereiopods (Fig. 2A–E; 6L). P1–4 biramous with ventral coxal spine and 5-segmented endopod; basis-ischio-merus (fused) with abundant spines scattered over the surface. Two large distal spines on ischio-merus and carpus; with long and strong spines on distal end of propodus, increasing in length from P1 to P4. Exopods with 22–26, 21–24, 18–22, 14–18 annulations respectively, each annulation carrying two long setae. Dorsal side of P1–3 covered with many spines, fewer on P4. P5 rudimentary and 2-segmented; exopod absent.

Pleon (Fig. 6L). Undeveloped and unsegmented; with 4 pairs of rudimentary pleopods. Biramous uropods undeveloped. Telson with 2 long processes and 4 setae on posterior margin (one pair on dorsal and one pair on ventral sides).

FIGURE 1. Molecular Phylogenetic tree obtained by Maximum Likelihood. Only bootstrap support values above 80 are shown. Larval images adapted from Robertson (1968a, 1968b, 1971) and Palero et al. (2008, 2011).
**Figure 2.** *Scyllarus subarctus*, Phyllosoma stage VII (PHMF 13, PHMF 51). (A) ventral view, (B) dactylus of first pereiopod; (C) dactylus of second pereiopod, (D) dactylus of third pereiopod, (E) dactylus of fourth pereiopod. Scale bars: A = 1 mm; B-E = 500 µm.

**Figure 3.** *Scyllarus subarctus*, Phyllosoma subfinal stage (PHMF 56, PHMF 48, SNECII-E89_02). (A) ventral view, (B) dactylus of first pereiopod, (C) dactylus of second pereiopod, (D) dactylus of third pereiopod, (E) dactylus of fourth pereiopod. Scale bars: A = 42 mm; B-E = 500 µm.
Phyllosoma, subfinal stage (PHMF 56, PHMF 48, SNECHI-E89_02)

Dimensions. N = 3; TL = 19.4–20.3 mm; CL = 12.6–13.1 mm; CW = 15.3–16.2 mm; PDL = 4.1–5.4 mm.

Cephalic shield (Fig. 3A). Subrectangular, 1.2× wider than long.

Antennule (Fig. 5B). Accessory flagellum slightly longer than primary. Primary flagellum with 13–14 rows of aesthetascs.

Antenna (Fig. 5B). Widening inner ramus. Same length as antennule.

Mandibles (Fig. 5E, H). Similar to stage VII but with more teeth on both mandibles.

Maxillule (Fig. 5K). Uniramous. Coxal endite with 12 setae (2 long and strong, and 10 small setae) and basial endite with 13 setae (3 long and strong, and 10 small setae).

Maxilla (Fig. 6B). Endite and endopod poorly differentiated. Scaphognathite (exopod) rectangular shaped and with small anterior and posterior expansions. Lateral process of endite with trapezoidal shaped.

First maxilliped (Fig. 6B). Rudimentary and slightly bilobed.

Second maxilliped (Fig. 6B). 5-segmented with 0,1,3,13,3 setae respectively. Spines of fourth segment form a crown around the base of dactyl.

Third maxilliped (Fig. 6I, J). More spines than previous stage.

Pereiopods (Fig. 3A–E; 6M). P1–4 with more spines than stage VII; exopods with 32–34, 27–34, 30–32, and 23–30 annulations respectively. P5 without exopod, 3-segmented and reaching base of uropods; coxa with ventral spine and 2 spines on ischio-merus.

Pleon (Fig. 6M). Four pairs of bilobed pleopods longer and narrower than stage VII; bilobed uropods; margin

FIGURE 4. Scyllarus subarctus, Phyllosoma final stage (PHMF 92). (A) ventral view, (B) dactylus of first pereiopod, (C) dactylus of second pereiopod, (D) dactylus of third pereiopod, (E) dactylus of fourth pereiopod, (F) left side of thorax, dorsal view, (G) detailed view of distal part of proximal exopod segment. Scale bars: A = 2 mm; B-F = 1 mm; G = 0.1 mm.
of telson is concave; elongated processes of telson shorter with respect to telson length. Two rows of 14–15 setae on ventral and dorsal side of telson.

**FIGURE 5.** *Scyllarus subarctus*, (A)–(C) antennule and antenna, (D)–(F) left mandible, (G)–(I) right mandible, (J)–(L) maxillule of stage VII, subfinal and final stage respectively. Scale bars: A and B = 1 mm; C = 2 mm; D, E, G and J = 100 µm; F, H and I = 200 µm; K and L = 500 µm.
FIGURE 6. *Scyllarus subarctus*, (A)–(C) maxilla and first maxilliped, (D)–(F) second maxilliped, (H)–(K) third maxilliped, (L)–(N) pleon and fifth pereiopod, ventral view, (O) pleopods of stage VII, subfinal and final stage respectively. Scale bars: A, B, D, H and I = 500 µm; C, E, F, G, L, M and O = 1 mm; N = 2 mm.
### TABLE 1. List of specimens used in the present study. Sampling information includes date, coordinates and depth. Morphological measurements (in millimetres) of phyllosomae include total length (TL), cephalic length (CL), cephalic width (CW) and pleon length (PDL).

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Phyllosoma, final stage (PHMF 92)

Dimensions. N = 8; TL = 27.0–35.1 mm; CL = 15.5–20.7 mm; CW = 19.6–25.9 mm; PDL = 7.0–10.2 mm.

Cephalic shield (Fig. 4A). Rectangular, 1.3 × wider than long.

Antennule (Fig. 5C). Accessory flagellum unsegmented; primary flagellum shorter than accessory, unsegmented, with 16–17 rows of sensory setae.

Antenna (Fig. 5C). Longer than antennule.

Mandibles (Fig. 5F, I). Similar to stage VII, but internal row of teeth approaches the external row so that both rows meet.

Maxillule (Fig. 5L). Coxal and basal endite with 11 and 10 setae respectively. Palp (endopod) absent.

Maxilla (Fig. 6C). Endite and endopod poorly differentiated with 3 setae on superior margin of lateral process of endite. Scaphognathite (exopod) without setae, flattened and anterior and posterior parts considerably expanded.

First maxilliped (Fig. 6C). Unsegmented and bilobed; outer lobe flattened and round; inner lobe conic-shaped and shorter.

Second maxilliped (Fig. 6F). 5-segmented with 0, 1, 3, 15, 4 setae respectively; exopod bud present.

Third maxilliped (Fig. 6K). Densely setose.

Pereiopods (Fig. 4A–G; 6N). Exopods of P1–4 with 32–38, 27–38, 32–35 and 29–33 annulations respectively. One spine-like seta present at the distal end of the proximal segment of exopod. P5 reaching uropods, 5-segmented with ventral coxal spine, 2 distal spines on ischio-merus, carpus and propodus.

Gills (Fig. 4F). Gill buds present: mxp3 and P1 with 1 pleurobranch, 1 arthrobranch and 2 podobranchs; P2–4 with 2 pleurobranches, 1 arthrobranch, 2 podobranchs; P5 with 1 pleurobranch.

Pleon (Fig. 6N, O). Pleopods biramous. Posterior margin of telson rounded with two postero-lateral processes. Two rows of 17–22 setae on dorsal and ventral sides of telson.

Discussion

The ethanol-preserved phyllosoma material collected by MAFIA facilitated the identification of Scyllarus subarctus larvae using molecular techniques and the description of its late developmental stages. Phyllosomae of S. subarctus are consistently larger than those from closely-related species such as S. arctus (Palero et al. 2011) or S. pygmaeus (Palero et al. 2008), reaching over 3 cm in total length in the final stage. The most distinctive morphological characteristic of S. subarctus phyllosomae is that pereiopods are covered with abundant spines. All pereiopods show 2 strong spines on the carpus (occasionally 3, one smaller) and one spine-like seta on the distal end of the proximal segment of the exopod, although it can be easily broken and it is not always visible. Such spine-like setae have never been described in a phyllosoma before, so it could either be a species-specific trait of S. subarctus or it may be a previously overlooked character. Even though morphological traits are seldom shared between phyllosoma and adult stages, the third finger-like lobe of the antennal flagellum is pointed and protruding in the final phyllosoma stage, a characteristic also present on S. subarctus adults. The final stage described here also presents the greatest number of sensory setae on the antennule and annulations on P1 exopod typically found in Scyllarus larvae (Robertson 1968a, 1971; Webber & Booth 2001; Palero et al. 2008, 2011). Scyllarus subarctus late stage phyllosomae share a rectangular cephalic shape with other congeneric species such as S. arctus and S. pygmaeus, but the TL/CW ratio is lower in S. subarctus than in S. pygmaeus or S. arctus.

Phylogenetic analyses separate Western Atlantic Scyllarus (excluding S. depressus) from East Atlantic taxa. Scyllarus depressus formed a strongly supported clade together with S. subarctus, an African species, which suggests that they could be a single species with an amphitropical distribution (see also Yang et al. 2012; Bracken-Grissom et al. 2014). Crosnier (1970) did not provide any qualitative character that would allow for differentiation between the adults of these two species, and distinguished them based in the more slender appearance of S. subarctus, the anterior part of its median carina directed upwards or a sternum widening much less towards the back. Further support for the sister relationship of S. subarctus and S. depressus was provided by larval morphology, with previous descriptions of S. depressus phyllosomae being remarkably similar to the MAFIA specimens (Robertson 1968b, 1971). Scyllarus depressus and S. subarctus larvae both possess many spines scattered over the pereiopods, identical first maxilliped and a comparatively long P5. Almost identical larvae and comparatively low genetic differentiation levels imply recent divergence between both S. depressus and S.
subarctus, suggesting the possibility that they might be a single species with an amphi-Atlantic distribution. The synonymy of species from American and African waters has been proposed in other marine taxa based on larval evidence (i.e. Sebastidae fish; Sabates & Olivar 1990), and amphi-Atlantic patterns have been observed in Grapsidae crabs (Schubart 2011) with long planktonic larval duration (>2 months; see Cuesta et al. 2011).

Long planktonic larval duration could explain the amphi-Atlantic pattern observed here, since some Scyllarus species have a comparatively long larval phase which would allow for transoceanic dispersal (e.g. 75 days for S. depressus; Robertson 1971). Previous simulation studies based on Atlantic Ocean dynamics suggest that phyllosomae could disperse between continents following a stepping-stone path through offshore islands (Rudorff et al. 2009). The distribution of S. subarctus is still poorly known, but it might be present in islands along the mid-Atlantic ridge, such as Azores or Ascension Island. In a recent study, several adult specimens from Guinea Bissau and Mauritania waters, in the Northern hemisphere, have been tentatively assigned to this species, expanding its range from 17ºS to 20 N (García-Isarch & Muñoz 2015; García-Isarch et al. in press). DNA sequences obtained from phyllosoma larvae collected near Cabo Verde and type specimens of S. subarctus from Angola are shown here to be identical. Marine currents might contribute to phyllosoma dispersal over long distances and could explain this wide distribution (Lass & Mohrholz 2008). Little is known about phyllosoma dispersal however, and passive movements could be restricted by eddies (Chiswell & Booth 1999) or modified by behavioural interaction with jellyfish (Booth et al. 2005; O’Rorke et al. 2015).

Larval morphology and molecular phylogeny results obtained in the present study highlight the need for a revision of Scyllarus systematics, with S. depressus being much closer to African species, in particular to S. subarctus, than to another American species. These results highlight unexpected evolutionary relationships within Scyllarus, and suggest that more fundamental research is required on African slipper lobsters. Future investigations should focus on revising morphological characters in both adults and larvae and obtaining supplementary molecular data.

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