



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* phyllosomae 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 decapodid, 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 Ibacinae 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 Senegal, Sierra Leone, Guinea and St. Tomé (Forest 1963), and *S. caparti* extends mostly along the East Atlantic coast in tropical and subtropical latitudes (Holthuis 1952), with an isolated specimen reported by Frogli (1974) from the Adriatic Sea. Although originally described from South-East Atlantic waters (Crosnier 1970; Macpherson 1991), some specimens from Guinea Bissau and Mauritania have been tentatively 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 East 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 Institut 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 m² 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;

Bracken-Grissom *et al.* 2014). 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 ($L_n = -1652.65$) 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 (aesthetascs).

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).

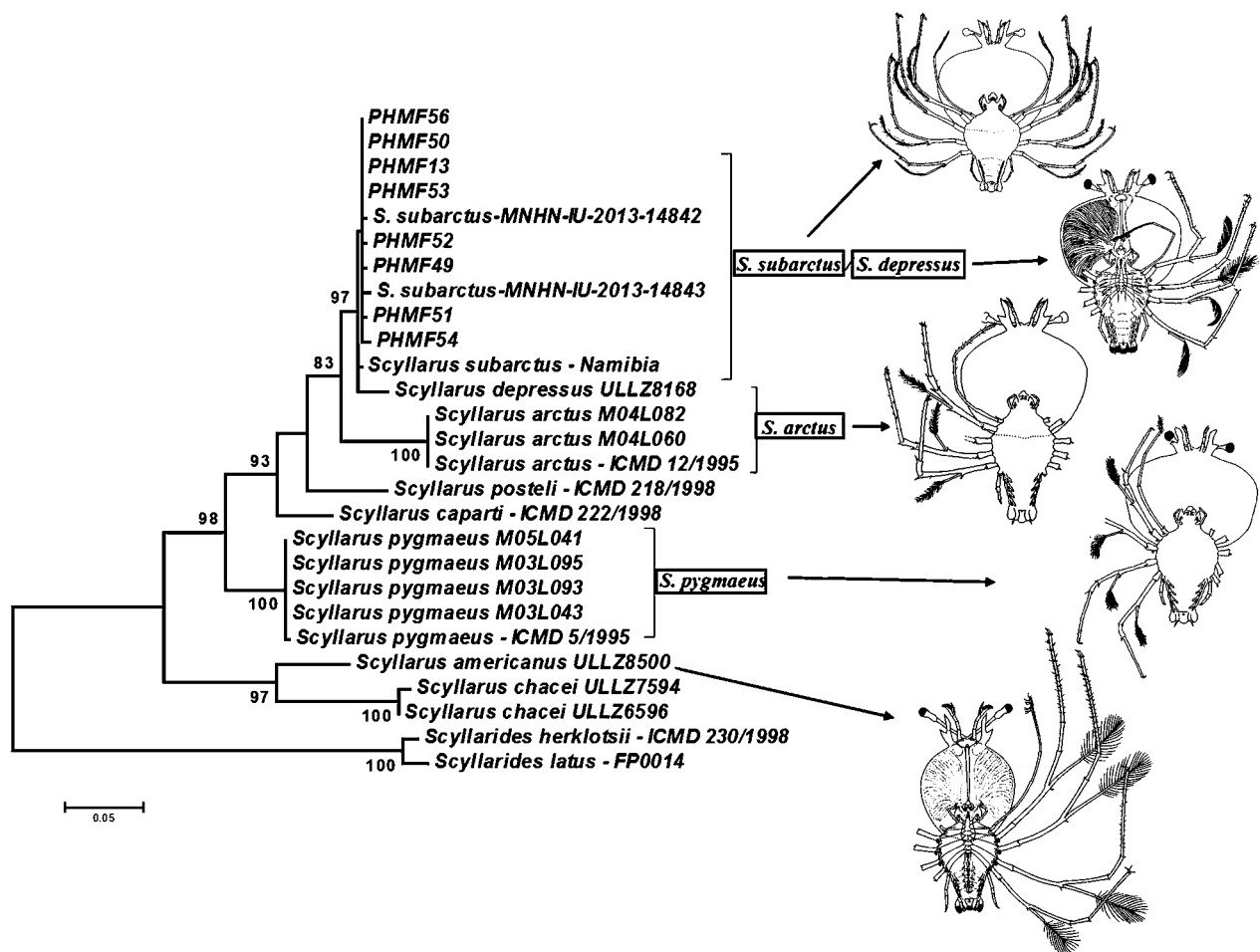


FIGURE 1. Molecular Phylogenetic tree obtained by Maximum Likelihood. Only bootstrap support values above 80 are shown. Larval images adapted from Robertson (1968a, 1968b, 1971) and Palero *et al.* (2008, 2011).

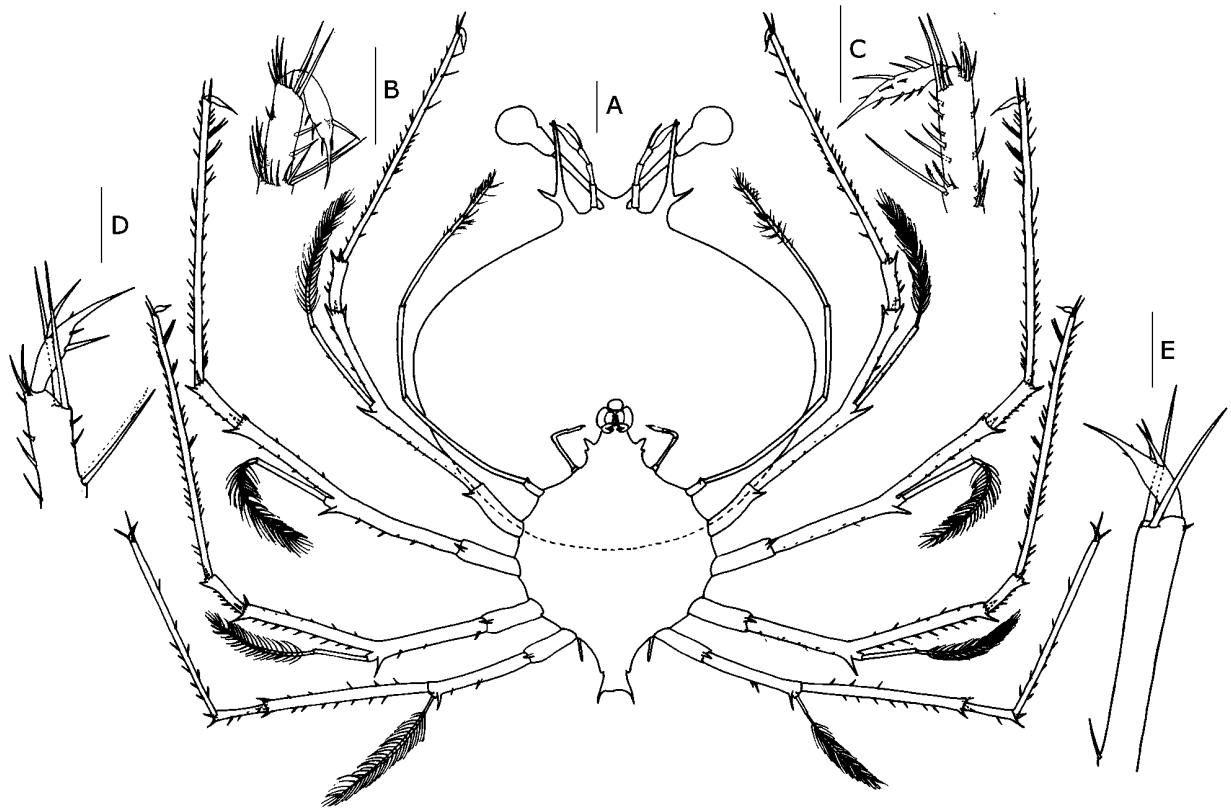


FIGURE 2. *Scyllarus subarctus*, Phyllosoma stage VII (PHMF 13, PHMF 51). (A) ventral view, (B) dactylus of first pereiopod; (C) dactylus of second pereiopod, (D) dactylus of third pereiopod, (E) dactylus of fourth pereiopod. Scale bars: A = 1 mm; B-E = 500 μ m.

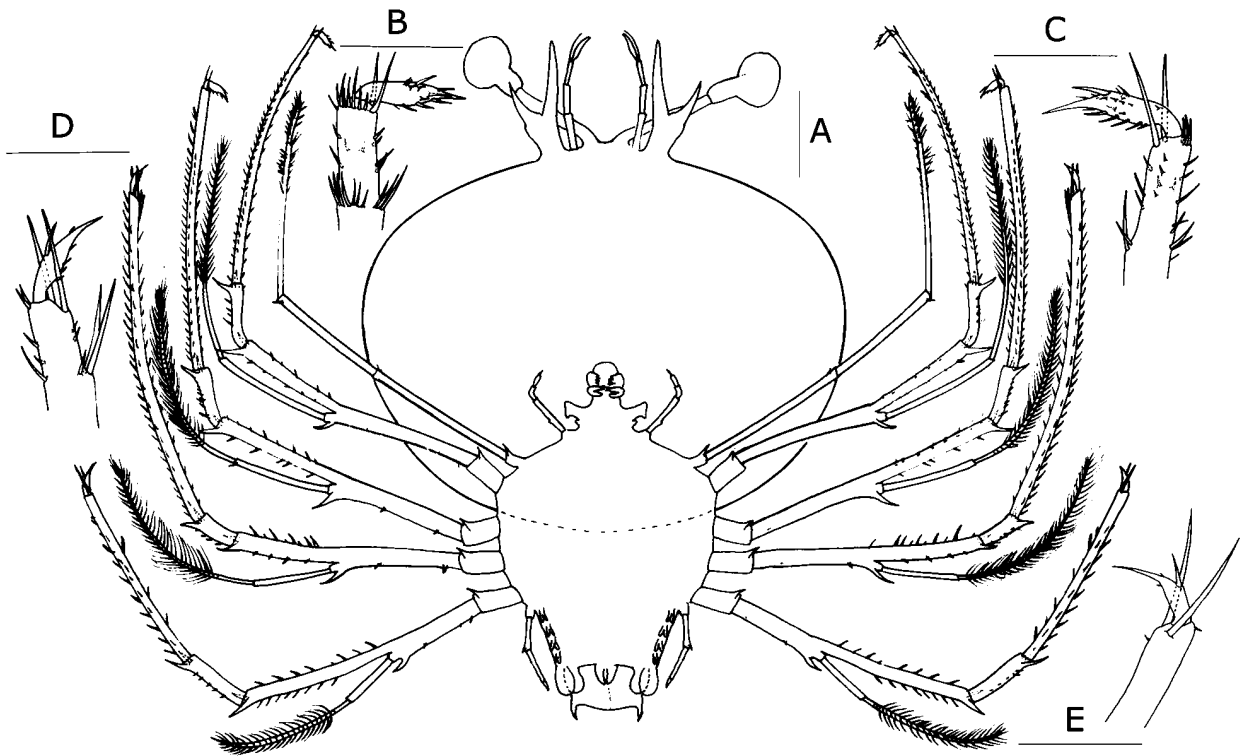


FIGURE 3. *Scyllarus subarctus*, Phyllosoma subfinal stage (PHMF 56, PHMF 48, SNECII-E89_02). (A) ventral view, (B) dactylus of first pereiopod, (C) dactylus of second pereiopod, (D) dactylus of third pereiopod, (E) dactylus of fourth pereiopod. Scale bars: A = 42 mm; B-E = 500 μ m.

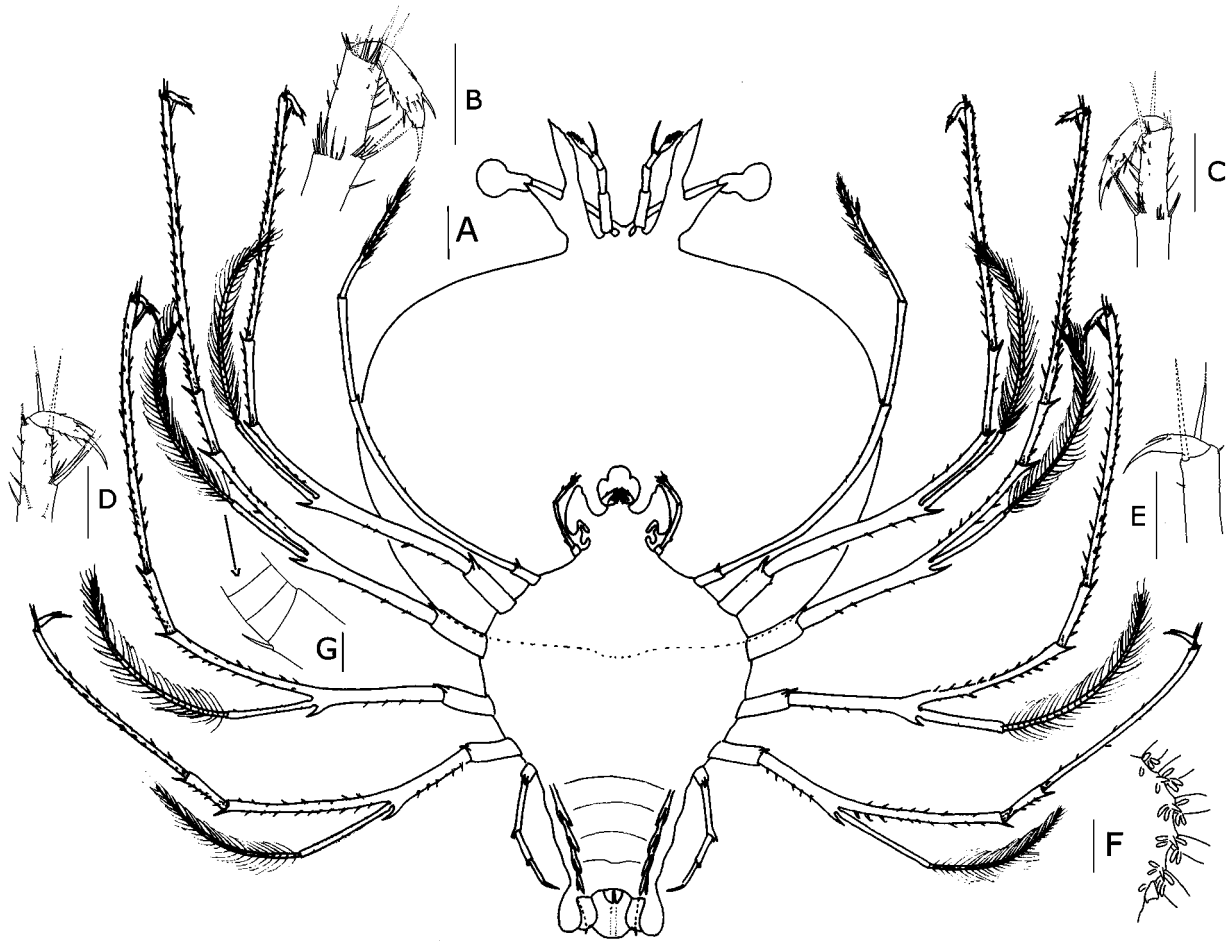


FIGURE 4. *Scyllarus subarctus*, Phyllosoma final stage (PHMF 92). (A) ventral view, (B) dactylus of first pereiopod, (C) dactylus of second pereiopod, (D) dactylus of third pereiopod, (E) dactylus of fourth pereiopod, (F) left side of thorax, dorsal view, (G) detailed view of distal part of proximal exopod segment. Scale bars: A = 2 mm; B-F = 1 mm; G = 0.1 mm.

Phyllosoma, subfinal stage (PHMF 56, PHMF 48, SNECII-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 basal 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

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.

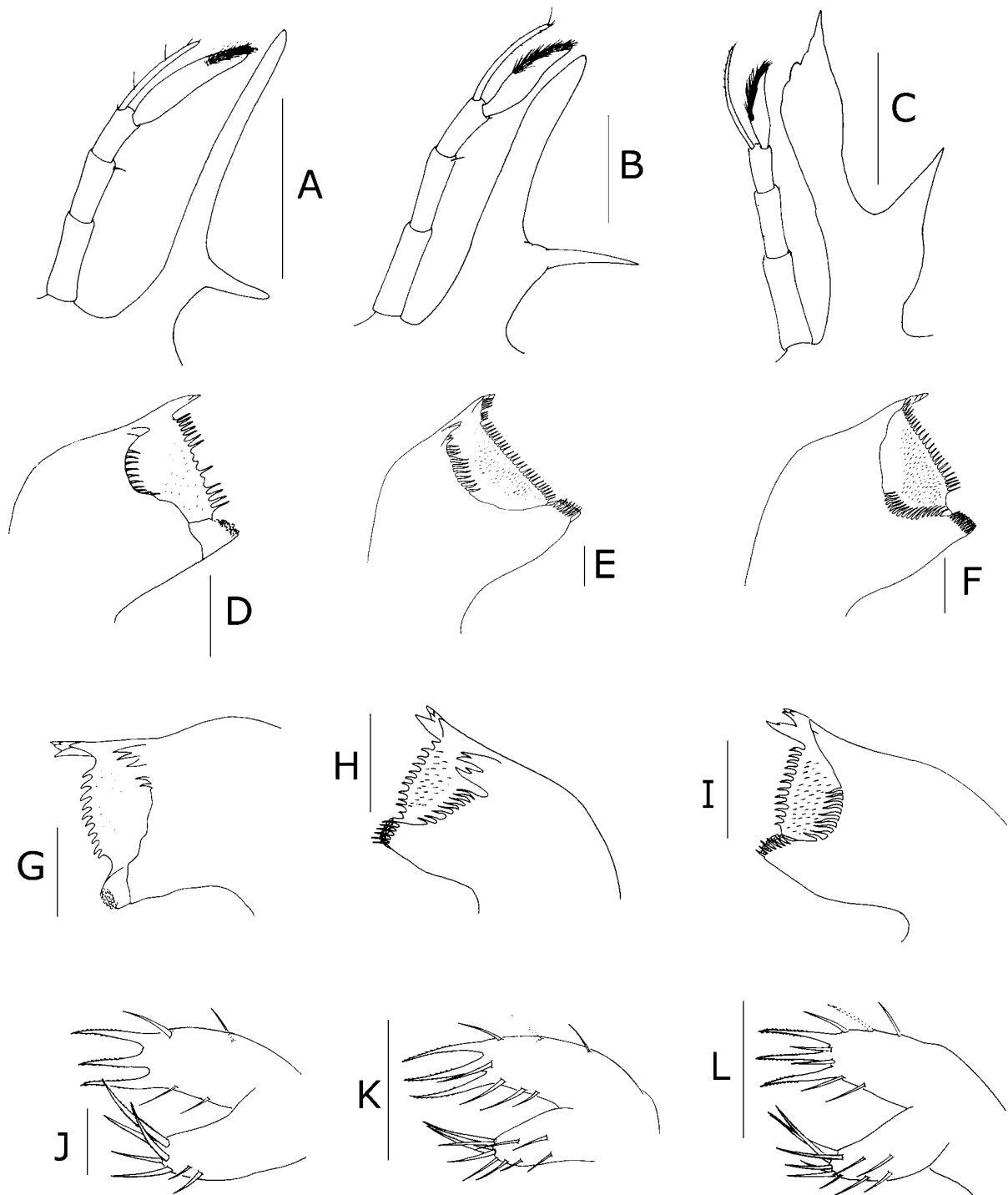


FIGURE 5. *Scyllarus subarctus*, (A)–(C) antennule and antenna, (D)–(F) left mandible, (G)–(I) right mandible, (J)–(L) maxillule of stage VII, subfinal and final stage respectively. Scale bars: A and B = 1 mm; C = 2 mm; D, E, G and J = 100 μ m; F, H and I = 200 μ m; K and L = 500 μ m.

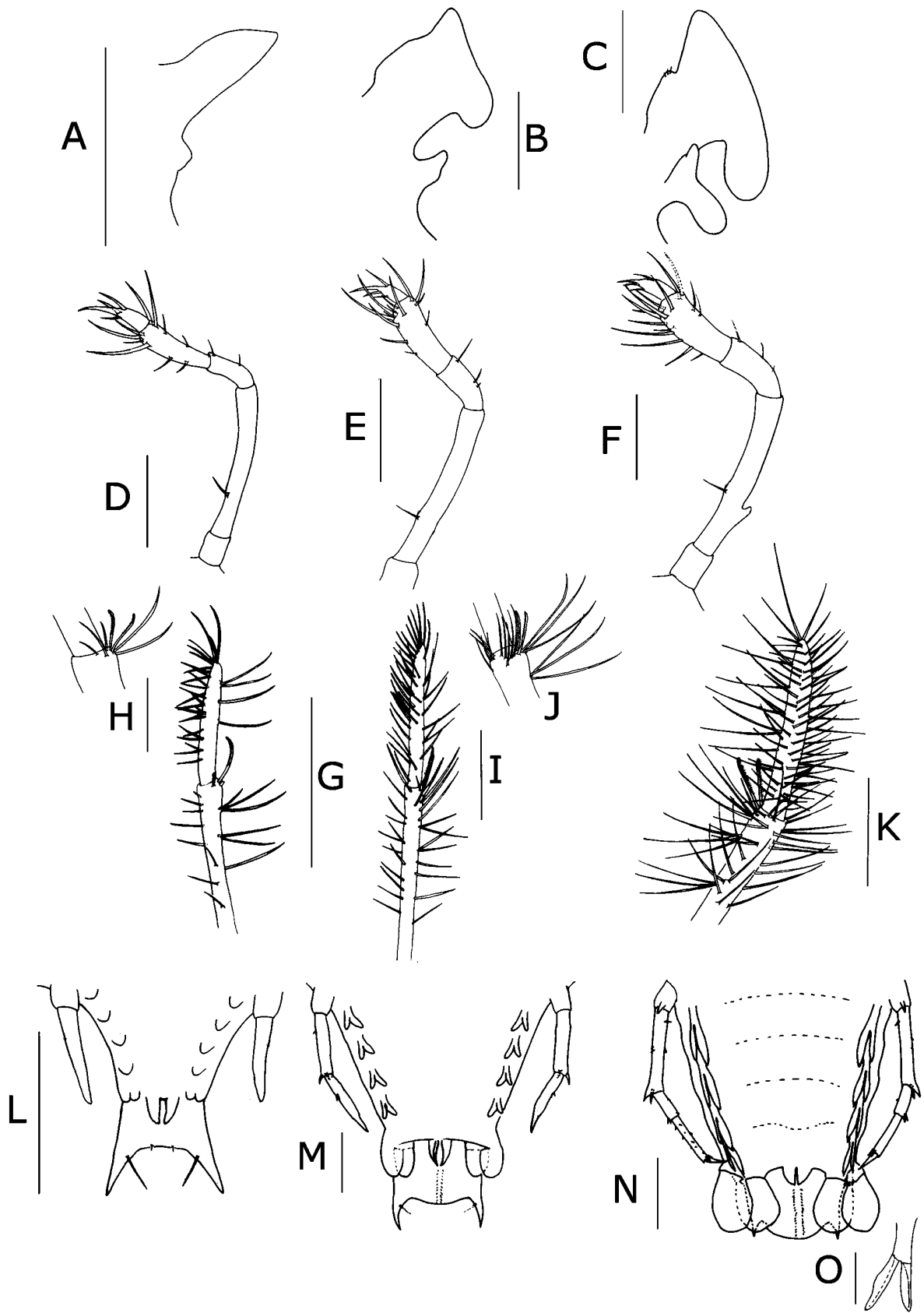


FIGURE 6. *Scyllarus subarctus*, (A)–(C) maxilla and first maxilliped, (D)–(F) second maxilliped, (H)–(K) third maxilliped, (L)–(N) pleon and fifth pereiopod, ventral view, (O) pleopods of stage VII, subfinal and final stage respectively. Scale bars: A, B, D, H and I = 500 μ m; C, E, F, G, L, M and O = 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).

Stage	Specimen ID	Museum code	Cruise	Date	Latitude	Longitude	Depth	TL	CL	CW	PDL	GenBank Acc.
Adult	<i>Scyllarides herklotsii</i>	ICMD 230/1998	---	20/01/1985	11.6500	-17.3333	217	---	---	---	---	FJ174906
Adult	<i>Scyllarides latus</i>	FP0014	---	---	---	---	---	---	---	---	---	FJ174907
Adult	<i>Scyllarus americanus</i>	ULLZ8500	---	07/27/1998	27.4520	-80.2655	---	---	---	---	---	JN701732
Adult	<i>S. arctus</i>	ICMD 12/1995	---	13/01/1993	40.6128	0.5937	25-23	---	---	---	---	DQ079732
Adult	<i>S. caparti</i>	ICMD 222/1998	---	30/12/1984	11.3000	-17.0833	33	---	---	---	---	FJ174909
Adult	<i>S. chacei</i>	ULLZ7594	---	07/04/2006	28.6210	-84.4730	---	---	---	---	---	JN701734
Adult	<i>S. chacei</i>	ULLZ6596	---	06/01/2004	24.5968	-83.4748	---	---	---	---	---	JN701733
Adult	<i>S. depressus</i>	ULLZ8168	---	06/06/2005	22.2678	-90.7143	---	---	---	---	---	JN701735
Adult	<i>S. posteli</i>	ICMD 218/1998	---	07/02/1985	11.5833	-17.2500	58-55	---	---	---	---	FJ174910
Adult	<i>S. pygmaeus</i>	ICMD 5/1995	---	30/10/1991	41.6785	2.8124	40-35	---	---	---	---	FJ174908
Adult	<i>S. subarctus</i>	MNHN-IU-2013-14842	---	18/08/1968	16.6166	11.3666	126	---	---	---	---	MF460387
Adult	<i>S. subarctus</i>	MNHN-IU-2013-14843	---	18/08/1968	16.6166	11.3666	126	---	---	---	---	MF460388
Adult	<i>S. subarctus</i>	ICMD 299/1991	---	25/03/1981	-17.4833	11.3833	300-293	---	---	---	---	FJ174912
VII	PHMF 13	MA017008PN07DPH01	MAFIA	19/04/2015	20.26	-24.25	100-0	9.6	6.6	7.7	1.3	MF460389
Sub-Final	PHMF 48	MA017008PED05PH01	MAFIA	19/04/2015	20.26	-24.25	100-0	19.4	12.8	15.3	4.1	---
Final	PHMF 49	MA017008PED05PH02	MAFIA	19/04/2015	20.26	-24.25	100-0	35.1	20.7	25.9	10.2	MF460391
VII	PHMF 50	MA017008PED05PH03	MAFIA	19/04/2015	20.26	-24.25	100-0	10.9	7.7	8.7	1.1	MF460392
VII	PHMF 51	MA017008PED05PH04	MAFIA	19/04/2015	20.26	-24.25	100-0	10.0	7.0	8.1	1.2	MF460393
VII	PHMF 52	MA017008PED05PH05	MAFIA	19/04/2015	20.26	-24.25	100-0	9.3	6.5	7.5	1.2	MF460394
VII	PHMF 53	MA017008PED05PH06	MAFIA	19/04/2015	20.26	-24.25	100-0	10.8	7.6	9.4	1.7	MF460395
VII	PHMF 54	MA017008PED05PH07	MAFIA	19/04/2015	20.26	-24.25	100-0	9.1	6.4	7.4	1.4	MF460396
Sub-Final	PHMF 56	MA019009PEN05PH01	MAFIA	21/04/2015	16.16	-26.03	100-0	20.3	13.1	16.2	5.4	MF460390

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TABLE 1. (Continued)

Stage	Specimen ID	Museum code	Cruise	Date	Latitude	Longitude	Depth	TL	CL	CW	PDL	GenBank Acc.
Final	PHMF_91	MA025012PEN00PH19	MAFIA	27/04/2015	9.56	-25.99	250-0	27.5	15.9	20.5	7.9	---
Final	PHMF_92	MA025012PEN00PH20	MAFIA	27/04/2015	9.56	-25.99	250-0	28.6	16.7	21	7	---
VII	E89_01	ICMD001081	SNEC-II	25/04/1986	-18	10.55	200-0	9.7	6.8	8.2	1.1	---
Sub-Final	E89_02	ICMD001082	SNEC-II	25/04/1986	-18	10.55	200-0	19.4	12.6	15.4	4.1	---
Final	E89_03	ICMD001083	SNEC-II	25/04/1986	-18	10.55	200-0	27.0	15.5	19.6	8.2	---
Final	E89_04	ICMD001084	SNEC-II	25/04/1986	-18	10.55	200-0	27.2	15.8	19.5	7.8	---
Final	E89_05	ICMD001085	SNEC-II	25/04/1986	-18	10.55	200-0	28.6	16.9	21.7	8.4	---
Final	E89_06	ICMD001086	SNEC-II	25/04/1986	-18	10.55	200-0	28.7	16.9	21.5	8.4	---
Final	E89_07	ICMD001087	SNEC-II	25/04/1986	-18	10.55	200-0	31.6	16.4	20.0	8.2	---
Final	<i>S. arctus</i>	ICMD-69/2007	MEDITITS	16/05/2003	41.9058	3.5156	401-450	---	---	---	---	GQ922071
Final	<i>S. arctus</i>	ICMD-68/2007	MEDITITS	16/05/2003	42.1091	3.5930	401-450	---	---	---	---	GQ922070
Final	<i>S. pygmaeus</i>	---	MEDITITS	19/05/2005	38.0803	0.0001	601-650	---	---	---	---	GQ922075
Final	<i>S. pygmaeus</i>	ICMD-64/2007	MEDITITS	19/05/2004	38.9838	0.4998	701-750	---	---	---	---	GQ922074
Sub-Final	<i>S. pygmaeus</i>	---	MEDITITS	23/05/2004	41.0235	1.3763	101-150	---	---	---	---	GQ922073
Final	<i>S. pygmaeus</i>	ICMD-63/2007	MEDITITS	05/05/2003	38.1211	-0.0626	251-300	---	---	---	---	GQ922072

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). Subrectangular, 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 pleurobranchs, 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 amphi-Atlantic 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 17°S 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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