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Chromosomal mapping of ribosomal clusters and telomeric sequences $(TTAGG)_n$ in nine species of lobsters (Crustacea, Decapoda)

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Abstract
Lobsters are ubiquitous, economically important decapod crustaceans with apparently conflicting evolutionary relationships. Here, we describe the chromosomal location of the major (45S rDNA) and minor (5S rDNA) ribosomal gene families in four species of Astacidea and five of Achelata, using two-color FISH. The major ribosomal family is located in 4–16 sites per diploid chromosome set, with *Nephrops norvegicus* (Nephropidae) showing the highest number described so far in Decapoda. The 5S rDNA is located in two sites in eight species; only in the crayfish *Procambarus clarkii* the 5S FISH signals were detected in four sites together with additional weaker signals. Furthermore, in *N. norvegicus* the minor ribosomal genes are syntenic with one major ribosomal cluster. Moreover, we located by two-color FISH the pentanucleotide $(TTAGG)_n$ telomeric repeat in the Nephropidae studied, showing the occurrence of a colocalization with 45S ribosomal sequences in *Homarus gammarus*. The comparison of chromosomal locations of repetitive sequences in Mediterranean, Atlantic, and South African lobster species as well as in marine and freshwater ones provides information on chromosomal evolution and cytotaxonomy of Decapoda.

Keywords: *Decapoda*, *FISH*, *$(TTAGG)_n$*, *interstitial telomeric sequences (ITSs)*, *28S rDNA*, *5S rDNA*

Introduction

Decapoda is a highly diversified group with a complex, often debated systematics and a vast geographical distribution. They appeared approximately 455 million years ago (Mya, late Ordovician) and include around 17500 living and 3000 extinct species (Schram & Dixon 2004; Wolfe et al. 2019). Among them, lobsters (Achelata, Astacidea, Glypheidea, and Polycheida) have a rich fossil record dating back 360 Mya and are a very important fishing and economic resource (Patek et al. 2006; Bracken-Grissom et al. 2014). Cytogenetic information on lobsters is still scarce and mostly reflecting only chromosome number and morphology, mainly because the karyotype consists of numerous small-sized chromosomes, >100 in most species studied to date. This causes possible counting errors, aggravated by the presence, in some species, of B chromosomes namely supernumerary chromosomes found in many eukaryotic species, mostly heterochromatic, with irregular inheritance and interindividual variation (Jones & Rees 1982; Camacho 2005; Jones et al. 2008). As regard basic karyotype parameters (Table I), Astacidea species (clawded lobsters and freshwater crayfish) have more than 100 chromosomes and among them, crayfish show the highest diploid numbers, mostly >170 chromosomes (Mlinarec et al. 2011, 2016); among crayfish, *Procambarus clarkii* (Cambaridae) shows 2 n = 188 (Salvadori et al. 2014). In the family Nephropidae, *Homarus* and *Nephrops* are closely related genera (Bracken-Grissom et al. 2014), with *N. norvegicus* showing the highest 2 n and the presence of large, mainly heterochromatic, supernumerary chromosomes, which are punctual chromosomal markers of the species (Table I; Deiana et al. 1996). The two North Atlantic *Homarus* species are very similar, distinguishable from each other by

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Table I. FISH data on Astacidea-Achelata decapods.

Taxa/species	2n/ n/ Bs (mode)	FISH	45S sites/loci: chr location	5S sites/loci: chr location	ITS presence	References
Suborder Pleocyemata Infraorder Astacidea Family Nephropidae						
Homarus gammarus	2n 120–130/ n 62–63/	45S-, 5S-rDNA, (TTAGG) repeat	12/6: short arms	2/1: short arm	ITS colocalized with 45S loci	2); this study
H. americanus	2n (135–136)/ n 64–68/ 2–4 Bs	45S-, 5S-rDNA, (TTAGG) repeat	12/6: 10 short arm, 2 long arm distal region	2/1: short arm		3); this study
Nephrops norvegicus	2n 131–140/ n 64/ 7–9 Bs	45S-, 5S-rDNA, (TTAGG) repeat	16/8: 8 short arm, 8 pericentromeric region. One syntenic with 5S site.	2/1: long arm interstitial region. Sintenic with a 45S locus located in the short arm		1); this study
Family Cambaridae						
Procambarus clarkii	2n (188)/ n (94)	45S-, 5S-rDNA, (TTAGG) repeat	8/4: 4 subtelo, 4 acro	4/2: 1 distal region, 1 short arm + additional smaller signals	ITS	4); this study
Family Astacidae						
Astacus astacus	2n 176/ n (88)	45S rDNA	4/2: 1 acro long arm, 1 meta short arm			5)
Astacus leptodactilus	2n 180/ n (90)	45S rDNA	4/2 + 1 additional smaller signal			5)
Austropotamobius pallipes	2n 176 (possible XX-XY)	45S rDNA	4/2: 1 submeta long arm, 1 acro-meta long arm (possible XY)			6)
A. torrentium	2n 176 (possible XX-XY)	45S rDNA	4/2: 1 submeta long arm, 1 acro-meta long arm (possible XY)			6)
Infraorder Achelata Family Palinuridae						
Palinurus elephas	2n 138–150/4-13 Bs	45S-, 5S-rDNA, (TTAGG) repeat	10/5 + Bs	2/1: short arm		7); 8); this study
P. mauritanicus	2n 113–130/2-8 Bs	45S-, 5S-rDNA, (TTAGG) repeat	4/2: small chrs + 3–5 Bs	2/1: pericentromeric region		9); 8); this study
P. gilchristi	2n 120–132/ n 60–72/3-7 Bs	45S-, 5S-rDNA, (TTAGG) repeat	4/2: small chrs + 3–6 Bs	2/1: distal region		10); 8); this study

(*Continued*)

Table I. (Continued).

Taxa/species	2n/ n/ Bs (mode)	FISH	45S sites/loci: chr location	5S sites/loci: chr location	ITS presence	References
Jasus lalandii	2n (148)/ n (74)	45S rDNA, (TTAGG) tel repeat, 5S rDNA	6/3: 4 large and 2 medium chrs. All colocalized with telomeric sequences	2/1: long arm distal region	ITS; 3 colocalized with 45S loci	8); this study
Panulirus regius	2n 158/ n 79	45S rDNA, (TTAGG) tel repeat, 5S rDNA	4/2: medium sized chrs			11); 8)
Family Scyllaridae						
Scyllarus arctus	2n 70–72/ n 35–36	45S-, 5S-rDNA, (TTAGG) repeat	4/2: interstitial region	2/1: long arm interstitial region		12); 8; this study

ITS: interstitial telomeric sequence; Bs: B (supernumerary) chromosomes; chr: chromosome; meta: metacentric chr, submeta: submetacentric chr, subtelo: subtelocentric chr, acro: acrocentric chr; tel: telomere(s).

References: 1) Deiana et al. (1996). 2) Salvadori et al. (2002). 3) Coluccia et al. (2001). 4) Salvadori et al. (2014). 5) Mlinarec et al. (2011). 6) Mlinarec et al. (2016). 7) Coluccia et al. (2006). 8) Salvadori et al. (2012). 9) Coluccia et al. (2003). 10) Coluccia et al. (2005). 11) Cannas et al. (2004). 12) Deiana et al. (2007).

slight morphological and genetic differences (Jørstad et al. 2007; Wahle et al. 2013; Ellis et al. 2017); cytogenetically the karyotype of *H. americanus* has higher chromosome number than *H. gammarus* and presents small and heterochromatic supernumerary (B) chromosomes (Coluccia et al. 2001; Salvadori et al. 2002).

Among Achelata (true lobsters), in the Scyllaridae family, *Scyllarides latus* (subfamily Arctidinae) shows about three times genome size (GS) values and double chromosome number compared to *Scyllarus arctus* and *S. pigmaeus* (subfamily Scyllarinae), (Table I; Deiana et al. 1999, 2007). These data show a sharp cytogenetic separation among these species accompanied by a marked difference in body size (Holthuis 1991) and based on the fossil record (Webber & Booth 2007), suggesting a trend toward a reduction of genome size and chromosome numbers. The species of the Palinuridae family show chromosome numbers >100 and medium to high haploid GS values (Deiana et al. 1999) with *Jasus lalandii* having both the highest GS and chromosome number (Deiana et al. 1999, 2007; Salvadori et al. 2012).

Moreover, all studied species of the *Palinurus* genus *(P. gilchristi*, *P. mauritanicus*, and *P. elephas)* present a variable number of small, heterochromatic B chromosomes which were not found in the other studied species of Palinuridae: *Panulirus regius* and *Jasus lalandii* (Coluccia et al. 2003, 2005, 2006; Cannas et al. 2004; Salvadori et al. 2012).

As regards the heterochromatin distribution in lobsters, large blocks of AT-rich DNA located mainly in the centromeric/pericentromeric regions, have been reported for many Decapoda species (Mlinarec et al. 2011, 2016; Salvadori et al. 2012; González-Tizón et al. 2013).

In lobsters, fluorescence *in situ* hybridization (FISH) has been applied to only 10 species for chromosome mapping of highly conserved sequences: the major ribosomal gene family (45S rDNA, located in the Nucleolar Organizer Region and containing genes encoding for 18S, 5.8S, and 28S rRNAs) and/or the arthropod pentameric telomeric $(TTAGG)_n$ repeat (Table I). In two species, the presence of interstitial telomeric sequences (ITSs) has been reported: in the long arm of the largest chromosome of the complement in *P. clarkii* (Salvadori et al. 2014) and colocalized with 45S rDNA in *J. lalandii* (Salvadori et al. 2012).

With the aim to deepen our knowledge on the karyology of Decapoda, mapping of ribosomal and telomeric sequences using two-color FISH was conducted on four species of Astacidea and five species of Achelata.

In Astacidea, we located for the first time 45S and 5S ribosomal genes and telomeric $(TTAGG)_n$ repeat on the chromosomes of three species of Nephropidae: the eastern Atlantic and Mediterranean *Homarus gammarus* and *Nephrops norvegicus* and the western Atlantic *H. americanus*. Furthermore, the 5S rDNA was also localized in the worldwide invasive crayfish from North American *Procambarus clarkii* (Cambaridae), in which we had previously located 45S rDNA and telomeric $(TTAGG)_n$ repeat (Salvadori et al. 2014). In Achelata, we located both 45S rDNA and 5S rDNA in the eastern Atlantic and Mediterranean *Scyllarus arctus* (Scyllaridae) and 5S rDNA in four species of the Palinuridae family: the eastern Atlantic and Mediterranean *Palinurus elephas* and *P. mauritanicus*, and the South African *Palinurus gilchristi* and *Jasus lalandii*, in which we had already located 45S rDNA and telomeric $(TTAGG)_n$ repeat (Salvadori et al. 2012).

We performed two-color FISH experiments to study the possible synteny between the two ribosomal families and to ascertain the presence of interstitial telomeric sequences and the eventual colocalization of telomeric/ribosomal sequences already highlighted in one Achelata species (Salvadori et al. 2012).

The comparison of chromosomal mapping of repetitive sequences in Mediterranean, Atlantic, and South African species as well as in marine and freshwater lobster species could provide information on chromosomal evolution and cytotaxonomy of Decapoda

Materials and methods

This research followed all applicable international, national, and/or institutional guidelines for the care and use of animals.

In this study, five Mediterranean, two South African, and one Atlantic marine species, as well as a freshwater species, were analyzed. The specimens of Mediterranean species were captured in the southern Sardinia (Mediterranean Sea) and/or collected from the local fish market, in particular 10 males of *Homarus gammarus* (Linnaeus, 1758), 10 males of *Nephrops norvegicus* (Linnaeus, 1758) (Nephropidae), 10 males of *Scyllarus arctus* (Linnaeus, 1758) (Scyllaridae), 20 males of *Palinurus elephas* (Fabricius, 1787) and 15 males of *P. mauritanicus* Gruvel, 1911 (Palinuridae). The specimens of the Atlantic species were purchased at the fish market, in particular ten males from three different commercial stocks of the Nephropidae

H. americanus H. Milne Edwards, 1837, six males of *Palinurus gilchristi* Stebbing, 1900, and ten males of *Jasus lalandii* (H. Milne-Edwards, 1837) (Palinuridae) from commercial stocks by South Africa. Thirty males of the freshwater Cambaridae species *Procambarus clarkii* (Girard, 1852), invasive in Europe, were captured with lobster pots along the South Sardinian rivers and ponds. The specimens were determined following Holthuis (1991).

Chromosome preparations were obtained by direct methods from testis, according to Salvadori et al. (2012). Direct methods are the only techniques available for obtaining chromosome preparations from crustaceans. In particular, the air-drying technique is based on tissue dissociation, followed by hypotonization and fixation of cells in suspension (Doussou De Bazignan & Ozouf-Costaz 1985). The most used tissue is testis since a good number of metaphases may be obtained, both mitotic and meiotic metaphase I and II plates were analyzed, useful to study chromosome number and the presence of B chromosomes.

The major ribosomal gene unit (45S rDNA that contains the genes encoding 18S, 5.8S, and 28S rRNAs), has been localized using 28S rDNA probes obtained by PCR amplification of the DNA of *Anguilla anguilla, Nephrops norvegicus*, and *Homarus gammarus* using the universal primers D1F and D1R (Zardoya & Meyer 1996). The 5S rDNA probes have been obtained by PCR amplification of the DNA of *Homarus gammarus* using the primers listed by Pelliccia et al. (1998). For *P. clarkii*, 5S rDNA probes were obtained by PCR amplification of the *P. clarkii* DNA using two different primer sets: the primers of *H. gammarus* listed by Pelliccia et al. (1998), and the degenerated primers based on the 5S rDNA sequence of several crustaceans, listed by Huan et al. (2010); furthermore, both high and low stringency conditions have been applied in post-hybridization washings according to Schwarzacher and Heslop-Harrison (2000).

The pentameric telomeric probe $(TTAGG)_n$ was amplified by non-template PCR following Ijdo et al. (1991) using the primers F: $(TTAGG)_5$ and R: $(CCTAA)_5$.

All probes were labeled with biotin-16-dUTP or digoxigenin-11-dUTP, using a nick translation kit (Roche Diagnostics), following manufacturer's instructions. Two-color FISH with 28S and 5S rDNA or 28S rDNA and telomeric $(TTAGG)_n$ probes was performed according to Schwarzacher and Heslop-Harrison (2000). Biotin-labeled probes were detected with avidin conjugated to FITC or extra-avidin conjugated to TRITC (Sigma-Aldrich); digoxigenin labeled probes were detected with anti-digoxigenin conjugated to FITC (Sigma-Aldrich).

Chromosomes were counterstained using DAPI (Sigma-Aldrich) or propidium iodide (Sigma-Aldrich). The slides were subsequently Wright's stained for the study of size, shape, and number of mitotic and meiotic chromosomes. Over 20 metaphase plates were analyzed per treatment. Metaphase plates were observed under a Zeiss Imager M1 fluorescence microscope, and images were captured with a Hamamatsu digital camera C8484 and processed using a karyotyping- and FISH-dedicated image analysis system (Cromowin Plus, TESI Imaging). When allowed by chromosome feature, we defined chromosome location of probes as interstitial, pericentromeric, and terminal. Since the chromosomes were often too small to define the exact signal location on the chromosomes, we indicated as "short arm" the cases in which the signal completely covered the short arm of small chromosomes. For the same reason, the shape of chromosomes has not been reported in many cases.

Results

Astacidea-Nephropidae, 28S and 5S rDNA two-color FISH

Homarus gammarus. The 28S rDNA signals were localized in the short arm of six medium-sized chromosome pairs, visible in six tetrads in meiotic metaphase I (Figure 1a) and in six chromosomes of a meiotic metaphase II (inset in Figure 1a). The 5S rDNA signals were localized in the short arm of one medium-sized chromosome pair, other than the 45S-bearing pairs, visible in one tetrad in meiotic metaphase I (Figure 1a) and in one chromosome in meiotic metaphase II (inset in Figure 1a). After inverted DAPI staining, the meiotic bivalents are clearly identifiable (Figure 1b).

H. americanus. The 28S rDNA signals were localized in six chromosome pairs: in the short arm of five medium-small pairs and terminally in the long arm of one medium-sized pair, visible in mitotic metaphase (Figure 1c) and in six chromosomes of a meiotic metaphase II (inset in Figure 1c). In meiotic metaphase I, the 28S rDNA signals were localized in six tetrads, and no signals were detected on B chromosomes. The 5S rDNA signals were localized in two sites in the short arm of one medium-sized chromosome pair, other than the 45S-bearing chromosome pairs (Figure 1c). After Wright's staining, the mitotic chromosomes are clearly identifiable (Figure 1d).

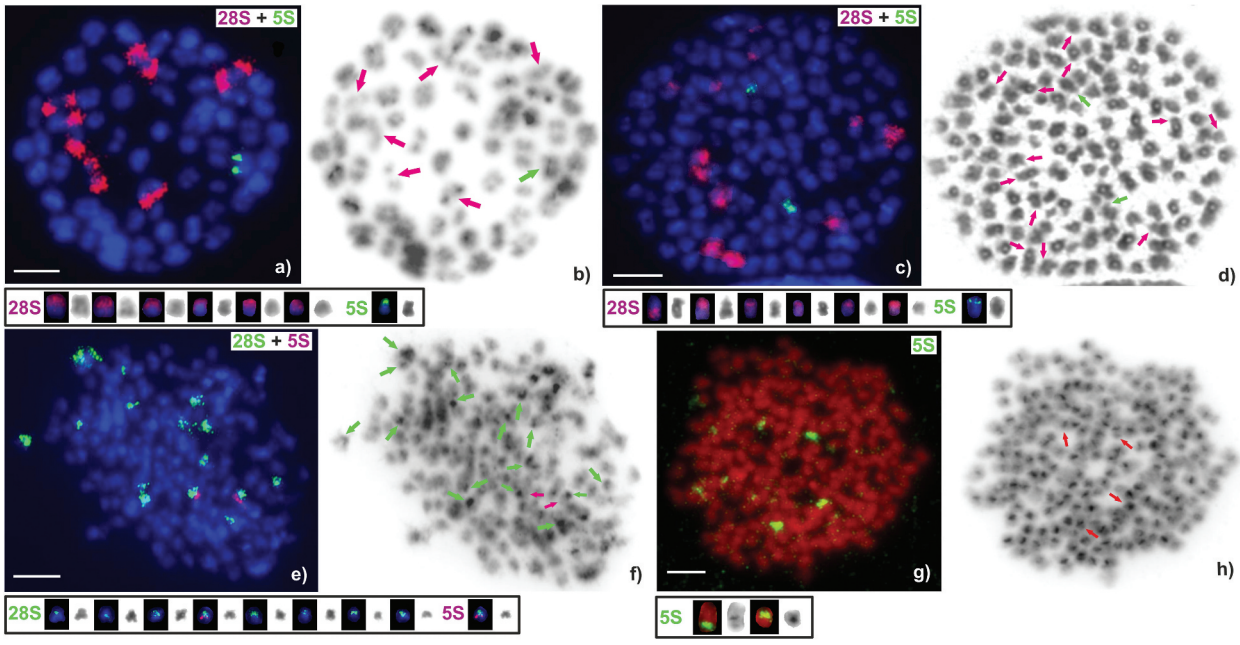

Figure 1. (a-f) Chromosomes of Nephropidae lobsters after 28S rDNA and 5S rDNA two-color FISH. (a) *H. gammarus* meiotic metaphase I: 28S rDNA is red labeled in 6 tetrads and 5S rDNA is green labeled in 1 tetrad, not syntenic; (b) in the same metaphase plate after inverted DAPI staining the arrows indicate the labeled meiotic bivalents; the inset shows the labeled chromosomes from a meiotic metaphase II after FISH and inverted DAPI staining; (c) *H. americanus* mitotic metaphase: 28S rDNA is red labeled in 12 chromosomes and 5S rDNA is green labeled in 2 chromosomes, not syntenic; (d) in the same metaphase after Wright's staining the arrows indicate the labeled chromosomes; the inset shows the marked chromosomes from a meiotic metaphase II after FISH and inverted DAPI staining; (e) *N. norvegicus* mitotic metaphase: 28S rDNA is green labeled in 16 chromosomes and 5S rDNA is red labeled in 2 chromosomes; the 5S rDNA signals are located in a NOR-bearing pair close to –but not colocalized with- one 28S rDNA cluster; the inset shows the labeled chromosomes from a meiotic metaphase II; (f) in the same metaphase after inverted DAPI staining the arrows indicate the labeled chromosomes; (g) 5S rDNA FISH in *P. clarkii* mitotic metaphase: 5S rDNA probe sharply labeled 4 chromosomes: the signals were located in the distal region of a medium sized metacentric pair and in the short arm of an acrocentric pair, showed in the inset. Moreover, numerous additional chromosomes presented weaker signals; in (h) the same metaphase plate after inverted DAPI staining is shown, arrows indicate the four sharply labeled chromosomes. Scale bar, 5 μm.

N. norvegicus. The 28S rDNA signals were localized in eight chromosome pairs: in the short arm of four pairs and in the centromeric-pericentromeric region of four pairs, observable in mitotic metaphase (Figure 1e) and in eight chromosomes of a meiotic metaphase II (inset in Figure 1e). The 5S rDNA signals were localized in one chromosome pair coincident with a medium-sized NOR-bearing pair, close to – but not colocalized with – one 28S rDNA cluster; the 5S rDNA was at an interstitial position in the long arm, while the 28S rDNA signals were spread on the short arm (Figure 1e and inset). After inverted DAPI staining, the mitotic chromosomes are clearly identifiable (Figure 1f).

Astacidea-Cambaridae, 5S rDNA FISH

Procambarus clarkii. 5S rDNA signals were localized in two chromosome pairs: distally in the long arm of a medium-sized pair and in the short arm of a small pair, visible in mitotic metaphase (Figure 1g) and showed in the inset and by arrows in Figure 1h. Furthermore, numerous additional weaker signals were detected using both different primer sets and different stringency conditions in FISH experiments (Figure 1g). After inverted DAPI staining, the mitotic chromosomes are clearly identifiable (Figure 1h).

Astacidea-Nephropidae, 28S and (TTAGG)$_n$ telomeric repeat two-color FISH

The pentameric telomeric (TTAGG)$_n$ probe produced, in some chromosomes, extended fluorescent signals in the three clawed lobsters. In *H. gammarus*, the two-color FISH revealed that the 28S rDNA (Figure 2a), and the telomeric (Figure 2b) sequences were colocalized in four out of six NOR-bearing pairs (Figure 2c). The colocalization of telomeric repeat and 28S rDNA did not occur in *H. americanus* and *N. norvegicus* (data not shown).

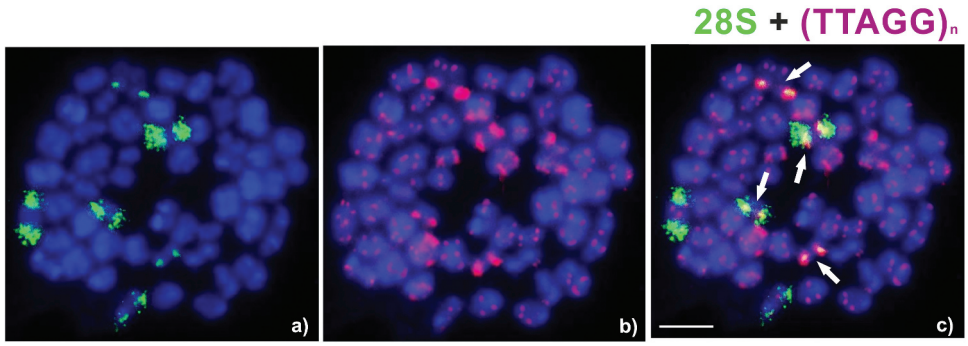

Figure 2. 28S and $(TTAGG)_n$ telomeric repeat two-color FISH. Meiotic metaphase I of *H. gammarus*: (a) 28S rDNA (green fluorescence) and (b) $(TTAGG)_n$ telomeric repeat (red fluorescence). In (c) the combination of the two images is shown; arrows indicate the four tetrads where the two probes are colocalized. Scale bar, 5 μm.

Achelata-Palinuridae, 28S and 5S rDNA two-color FISH

Palinurus elephas. The minor ribosomal gene family was localized in the short arm of one small-sized chromosome pair other than the NOR-bearing pairs, observable in a tetrad in meiotic metaphase I (Figure 3a) and in one chromosome of a meiotic metaphase II (inset in Figure 3a). After Wright's staining, the mitotic chromosomes are clearly identifiable (Figure 3b). The NOR-bearing chromosomes were variable in number because 28S rDNA clusters were also localized on some B chromosomes (Coluccia et al. 2006).

P. mauritanicus. The minor ribosomal gene family was localized in the centromeric-pericentromeric region of one pair, other than the NOR-bearing pairs, observable in mitotic metaphase (Figure 3c) and in one chromosome of a meiotic metaphase II (inset in Figure 3d). After Wright's staining, the mitotic chromosomes are clearly identifiable (Figure 3d). The NOR-bearing chromosomes were variable in number as some 28S sites were localized on B chromosomes (Coluccia et al. 2003).

P. gilchristii. The minor ribosomal gene family was localized distally in the long arm of one chromosome pair other than the NOR-bearing pairs, visible in mitotic metaphase (Figure 3e) and in one chromosome of a meiotic metaphase II (inset in Figure 3e). After Wright's staining, the mitotic chromosomes are clearly identifiable (Figure 3f). The NOR-bearing chromosomes were variable in number as some sites were also localized on B chromosomes (Coluccia et al. 2005).

Achelata-Palinuridae, 5S rDNA and $(TTAGG)_n$ telomeric repeat two-color FISH

Jasus lalandii. The minor ribosomal gene family was localized distally in the long arm of a medium-sized chromosome pair, observable in a mitotic metaphase (Figure 3g) and in one chromosome of a meiotic metaphase II (inset in Figure 3h). Telomeric signals labeled 10 expanded terminal regions; six of these were found colocalized with 28S rDNA sequences (Salvadori et al. 2012); as a consequence, the 5S rDNA was not syntenic to the 45S rDNA. In the same metaphase plate after Wright's staining the chromosomes are identifiable (Figure 3h).

Achelata-Scyllaridae, 28S and 5S rDNA FISH

Scyllarus arctus. After 28S rDNA FISH fluorescence signals were detected interstitially in two meta-submetacentric large chromosome pairs, visible in two tetrads in meiotic metaphase I (Figure 3i) and in two chromosome pairs in mitotic metaphases. After 5S rDNA FISH, the signals were interstitially located in the long arm of one medium-sized submetacentric chromosome pair, observable in one tetrad in meiotic metaphase I (Figure 3m) and in one chromosome of a meiotic metaphase II (inset in Figure 3m). In the same metaphase plate after Wright's staining the chromosomes are identifiable (Figure 3n).

Discussion

Within decapod crustaceans, only 50 species (10 families) over a total of 17.500 (233 families) have been studied with a karyological approach. Herein, we focused on the FISH localization of highly conserved sequences, namely the major (45S rDNA) and minor (5S rDNA) ribosomal gene families as well as the pentameric telomeric $(TTAGG)_n$ arthropod repeat, on lobsters of Astacidea and Achelata taxa and compared the results with classic cytogenetic data.

FISH data on the Astacidea and Achelata species, together with their basic karyotype parameters are summarized in Table I. As regards the

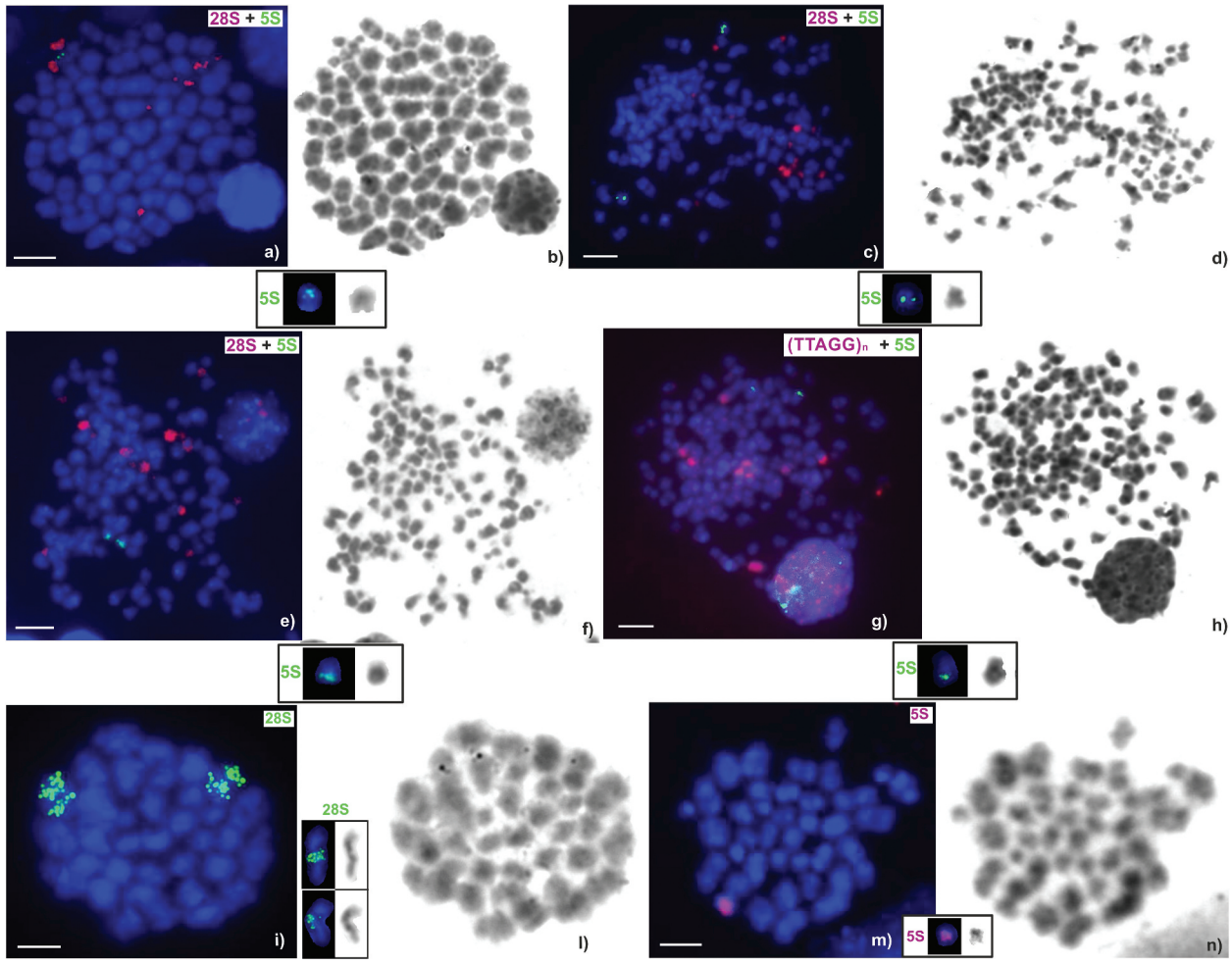

Figure 3. (a-f) 28S rDNA and 5S rDNA two-color FISH. (a, b) *P. elephas* meiotic metaphase I, (c, d) *P. mauritanicus* mitosis, and (e, f) *P. gilchristi* mitosis. In all species, the 5S rDNA (green fluorescence) is located in one chromosome pair, not syntenic with 28S rDNA (red fluorescence); the insets show the 5S rDNA labeled chromosomes from a meiotic metaphase II plate after FISH and inverted DAPI staining. (g) 5S rDNA (green fluorescence) and $(TTAGG)_n$ telomeric repeat (red fluorescence) two-color FISH in *J. lalandii* mitotic metaphase: the 5S signals are located in two sites, distally in the long arm of a medium sized chromosome pair, the telomeric probe marked ten expanded telomeric regions; the inset shows the 5S rDNA marked chromosomes from a meiotic metaphase II plate after FISH and inverted DAPI staining. (i, l, m, n) *S. arctus* chromosomes. (i) after 28S rDNA FISH the signals (green fluorescence) are located in two tetrads in meiotic metaphase I. The inset shows the labeled chromosomes from a mitotic plate (one for each pair). (m) after 5S rDNA FISH the signals (red fluorescence) are located in one tetrad in meiotic metaphase I; the inset shows the signals located interstitially in the long arm of one medium sized chromosome in a meiotic metaphase II. In b, d, f, h, l, n the Wright's staining of the same metaphase plates after FISH allowed the identification of chromosomes/meiotic bivalents. Scale bar, 5 μm.

heterochromatin distribution in lobsters, large blocks of AT-rich DNA located mainly in the centromeric/pericentromeric regions, clearly shown by DAPI staining, have been reported for many Decapoda species (Mlinarec et al. 2011, 2016; Salvadori et al. 2012; González-Tizón et al. 2013).

Ribosomal genes

In lobsters, the major ribosomal family (45S rDNA, NOR) is located in 4–16 chromosome sites (2–8 loci). The present data show a sharp differentiation between Achelata and Astacidea with the three Nephropidae species presenting the highest number of NORs among the Decapoda species studied (Table I). In particular, the highest number (16 sites) of 45S rDNA clusters was found in *N. norvegicus;* furthermore, in this species the 45S and 5S ribosomal families have a syntenic position in one of the eight NOR-bearing pairs, in a close but not overlapping location. These data support the peculiar cytogenetic features of *N. norvegicus*.

A high number of 45S rDNA sites does not necessarily go together with a high number of

chromosomes, as shown by *Scyllarus arctus*, which has one of the lowest diploid number in lobsters (70–72) (Deiana et al. 2007) and harbors four 45S rDNA sites, the same number as some species of the *Austropotamobius* and *Astacus* genera which have a high diploid number (2 n = 176–180) (Mlinarec et al. 2011, 2016).

Furthermore, in the three investigated species of *Palinurus*, some B chromosomes were entirely labeled by the 28S rDNA probe (Salvadori et al. 2012; present data). The overall presence in *Palinurus* species of B chromosomes with similar features and often carrying 45S rDNA, together with the very small size of NOR-bearing chromosomes and the lower chromosome number compared to other Palinuridae, could indicate an important karyotype reshaping and be related to the very rapid speciation process hypothesized for this genus (Palero et al. 2009; Tsang et al. 2009).

To date, this is the second report of 5S rDNA location in Decapoda. It is noteworthy that in lobsters, in contrast to the high number of 45S rDNA clusters, 5S rDNA was found in only one chromosome pair in eight out of nine studied species. Similarly, the only additional decapod species investigated (*Fenneropenaeus chinensis*) presents only one 5S-bearing pair (Huan et al. 2010).

Conversely to the other studied species, in the crayfish *P. clarkii*, we localized the 5S rDNA in four sites and even in numerous additional chromosomes. This extensive dispersion of 5S rDNA sequences could be due to the presence of small 5S rDNA clusters in the genome (Cabral-de-Mello et al. 2011a) or to the presence of satellite DNA derived from 5S rDNA, as it has been reported in several species (Martins et al. 2006; Vittorazzi et al. 2011).

Sochorová et al. (2018) set up an rDNA database containing FISH information on 45S and 5S rDNA location in 1343 animal species. They highlighted a general trend of low numbers of both 5S and 45S ribosomal clusters in animal genomes; the median number was close to two sites (single locus/1C) per ribosomal family. However, in each group, they found karyotypes which largely deviate from the average, and different studies indicate likely independent amplification events of 45S and 5S rDNA. In arthropods, a higher number of sites for the 5S rDNA compared to the 45S rDNA is reported, with most of the data referring to insects. Among them, the family Acrididae has been deeply investigated (Cabral-de-Mello et al. 2011a) revealing an extensive variation in the number of cluster for both rDNA types; on the contrary, in the subfamily Scarabaeinae (Coleoptera, Scarabaeidae) it has been reported a large variability of the 45S rDNA clusters when compared to the consistency of the 5S clusters (Cabral-de-Mello et al. 2011b), as we have found in lobsters. It is noteworthy that 5S rDNA clusters was found in only one chromosome pair in all Decapoda studied, even distantly related, except for the crayfish *Procambarus clarkii*.

Taking into account the difficulty of determining the precise location of FISH signals on small chromosomes, the 45S rDNA signals are terminally located, often spread all over the short arm in most of the studied lobsters and in pericentromeric positions in few species. Similarly, also regarding the 5S rDNA chromosomal location, we found it at a terminal location in seven out of nine species and in an interstitial position in *N. norvegicus* and in *Scyllarus arctus*.

Telomeric repeats

In the three Nephropidae investigated species, we pointed out the occurrence of some extended telomeric regions. Furthermore, in eight sites of *H. gammarus,* the telomeric sequences were found colocalized to 45S rDNA; and following the classification made by Ocalewicz (2013), they could be considered as interstitial telomeric sequences (ITSs). This same colocalization has been found in many plants and animals. Among arthropods, the pentameric telomeric sequence was reported colocated with ribosomal genes in all species of stick insects (order Phasmatodea), even those belonging to distantly related genera, thus appearing to be an ancestral character (Scali et al. 2016; Liehr et al. 2017, 2020). On the other hand, among decapods, the telomeric sequences interspersed with rDNA appear to be an accidental event, found in one out of four Achelata species, the spiny lobster *Jasus lalandii* (Salvadori et al. 2012) and in one out of four Astacidea species (present data).

The origin of this colocalization, present in plant and animal genomes is still debated; however, whatever its origin, this interspersion turns out to be a hot spot of genomic recombination, enhancing differentiation and karyotype plasticity (Meyne et al. 1990; Salvadori et al. 1995; Ocalewicz 2013; Dvorackova et al. 2015; Scali et al. 2016, 2020; Liehr et al. 2017; Aksenova & Mirkin 2019).

This research provided information from both classic cytogenetic techniques and from FISH of highly conserved sequences: major (45S rDNA) and minor (5S) ribosomal gene families as well as

telomeric repeats in nine species of lobsters belonging to Astacidea and Achelata. Our data, even if referring to a low number of species, seem to suggest a clear trend of decapods toward two sites (single locus) of 5S rDNA, and a variable number of loci of the 45S rDNA per genome. These data could contribute to a better understanding of the Decapoda karyotype evolution and of the relationships between the two ribosomal gene families in favor of their likely independent amplification. The molecular cytogenetic approach also provided useful clues for tracing phylogenetic relationships among species difficult to be analyzed through classical cytogenetic approach.

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No potential conflict of interest was reported by the author(s).

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References

Aksenova AY, Mirkin SM. 2019. At the beginning of the end and in the middle of the beginning: Structure and maintenance of telomeric DNA repeats and interstitial telomeric sequences. Genes (Basel) 10(2):118. DOI:10.3390/genes10020118.

Bracken-Grissom HD, Ahyong ST, Wilkinson RD, Feldmann RM, Schweitzer CE, Breinholt JW, Bendall M, Palero F, Chan T-Y, Ferlder DL, Robles R, Chu K-H, Tsang L-M, Kim D, Martin JW, Crandall KA. 2014. The emergence of lobsters: Phylogenetic relationships, morphological evolution and divergence time comparison of an ancient group (Decapoda: Achelata, Astacidea, Glypheidea, Polychelida). Systematic Biology 63(4):457–479. DOI:10.1093/sysbio/syu008.

Cabral-de-Mello DC, Cabrera J, López-León MD, Camacho JPM. 2011a. Evolutionary dynamics of 5S rDNA location in acridid grasshoppers and its relationship with H3 histone gene and 45S rDNA location. Genetica 139:921–993. DOI:10.1007/s10709-011-9596-7.

Cabral-de-Mello DC, Oliveira SG, de Moura RC, Martins C. 2011b. Chromosomal organization of the 18S and 5S rRNAs and histone H3 genes in Scarabaeinae coleopterans: Insights into the evolutionary dynamics of multigene families and heterochromatin. BMC Genet 12:88. DOI:10.1186/1471-2156-12-88.

Camacho JPM. 2005. B chromosomes. In: Gregory TR, editor. The evolution of the genome. Amsterdam: Elsevier Academic Press. pp. 224–273.

Cannas R, Deiana AM, Salvadori S, Coluccia E. 2004. Preliminary data on the karyology of *Palinurus regius* De Brito Capello, 1864 (Crustacea, Decapoda). Biologia Marina Mediterranea 11(2):721–723.

Coluccia E, Cau A, Cannas R, Milia A, Salvadori S, Deiana AM. 2001. Mitotic and meiotic chromosomes of the American lobster *Homarus americanus* (Nephropidae, Decapoda). Hydrobiologia 449:49–152. DOI:10.1023/A:1017557523022.

Coluccia E, Deiana AM, Cannas R, Salvadori S. 2006. Study of the nucleolar organizer regions in *Palinurus elephas* (Crustacea: Decapoda). Hydrobiologia 557:5–8. DOI:10.1007/s10750-005-1302-2.

Coluccia E, Salvadori S, Cannas R, Deiana AM. 2003. Study of mitotic and meiotic chromosomes of *Palinurus mauritanicus* (Crustacea, Decapoda). Biologia Marina Mediterranea 10 (2):1069–1071.

Coluccia E, Salvadori S, Cannas R, Milia A, Deiana AM. 2005. First data on the karyology of *Palinurus gilchristi* (Crustacea, Decapoda). Biologia Marina Mediterranea 12(1):661–663.

Deiana AM, Cau A, Coluccia E, Cannas R, Milia A, Salvadori S, Libertini A. 1999. Genome size and AT-DNA content in thirteen species of Decapoda. In: Schram FR, von Vaupel Klein JC, editors. Crustacean and the biodiversity crisis. Leiden: Brill Academic Publishers. pp. 981–985.

Deiana AM, Coluccia E, Milia A, Salvadori S. 1996. Supernumerary chromosomes in *Nephrops norvegicus* L. (Crustacea, Decapoda). Heredity 76:92–99. DOI:10.1038/hdy.1996.12.

Deiana AM, Libertini A, Cau A, Cannas R, Coluccia E, Salvadori S. 2007. Genetics of slipper lobters. In: Lavalli KL, Spanier E, editors. The biology and fishery of slipper lobsters. Boca Raton (FL): CRC Press Taylor & Francis Group. pp. 53–67.

Doussou De Bazignan M, Ozouf-Costaz C. 1985. Une tecnique rapid d'analyse chromosomique appliquée a sept especies de poissons antarticques. Cybium 9:57–74.

Dvorackova M, Foitova M, Fajkus J. 2015. Chromatin dynamics of plant telomeres and ribosomal genes. The Plant Journal 83:18–37. DOI:10.1111/tpj.12822.

Ellis CD, Hodgson DJ, Daniels CL, Collins M, Griffiths AGF. 2017. Population genetic structure in European lobsters: Implications for connectivity, diversity and hatchery stocking. Marine Ecology Progress Series 563:123–137. DOI:10.3354/meps11957.

González-Tizón AM, Rojo V, Menini E, Torrecilla Z, Martínez-Lage A. 2013. Karyological Analysis of the Shrimp *Palaemon serratus* (Decapoda: Palaemonidae). Journal of Crustacean Biology 33(6):843–848. DOI:10.1163/1937240X-00002185.

Holthuis LB. 1991. FAO specie catalogue, vol 13: Marine Lobster of the world. An annotated and illustrated catalogue of species of interest to fisheries known to date. FAO fisheries Synopsis No 125. Rome: FAO. pp.171–226.

Huan P, Zhang X, Li F, Zao C, Zhang C, Xiang J. 2010. Chromosomal localization of 5S rDNA in Chinese shrimp (*Fenneropenaeus chinensis*): A chromosome-specific marker for chromosome identification. Chinese Journal of Oceanology and Limnology 28:233–238. DOI:10.1007/s00343-010-9278-9.

Ijdo JW, Wells RA, Baldini A, Reeders ST. 1991. Improved telomere detection using a telomere repeat probe (TTAGGG)n generated by PCR. Nucleic Acid Research 19 (17):4780. DOI:10.1093/nar/19.17.4780.

Jones RN, Gonzales-Sanchez M, Gonzales-Garcia M, Vega JM, Puertas MJ. 2008. Chromosomes with a life of their own. Cytogenetic and Genome Research 120:265–280. DOI:10.1159/000121076.

Jones RN, Rees H. 1982. B chromosomes. London: Academic Press.

Jørstad KE, Prodohl PA, Agnalt AL, Hughes M, Farestveit E, Ferguson AF. 2007. Comparison of genetic and morphological methods to detect the presence of American lobsters, *Homarus americanus* H. Milne Edwards, 1837 (Astacidea: Nephropidae) in Norwegian waters. Hydrobiologia 590:103–114. DOI:10.1007/s10750-007-0762-y.

Liehr T, Buleu O, Karamysheva T, Bugrov A, Rubtsov N. 2017. New insights into Phasmatodea Chromosomes. Genes 8 (327):1–15. DOI:10.3390/genes8110327.

Martins C, Ferreira IA, Oliveira C, Foresti F, Galetti PM. 2006. A tandemly repetitive centromeric DNA sequence of the fish *Hoplias malabaricus* (Characiformes, Erythrinidae) is derived from 5S rDNA. Genetica 127:133–141. DOI:10.1007/s10709-005-2674-y.

Meyne J, Baker RJ, Hobart HH, Hsu TC, Ryder OA, Ward OG, Wiley JE, Wurster-Hill DH, Yates TL, Moyzis RK. 1990. Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes. Chromosoma 99 (1):3–10. DOI:10.1007/BF01737283.

Mlinarec J, Muži M, Pavlica M, Šrut M, Klobučar G, Maguire I. 2011. Comparative karyotype investigations in the European crayfish *Astacus astacus* and *A. leptodactylus*. Crustaceana 84 (12–13):1497–1510. DOI:10.1163/156854011X607015.

Mlinarec J, Porupski I, Maguire I, Klobučar G. 2016. Comparative karyotype investigations in the white-clawed crayfish *Austropotamobius pallipes* (lereboullet, 1858) species complex and stone crayfish *A. torrentium* (Schrank, 1803) (Decapoda: Astacidae). Journal of Crustacean Biology 36 (1):87–93.

Ocalewicz K. 2013. Telomeres in Fishes. Cytogenetic and Genome Research 141:114–125. DOI:10.1159/000354278.

Palero F, Lopes J, Abello P, Macpherson E, Pascual M, Beaumont MA. 2009. Rapid radiation in spiny lobsters (*Palinurus* spp) as revealed by classic and ABC methods using mtDNA and microsatellite data. BMC Evolutionary Biology 9:263. DOI:10.1186/1471-2148-9-263.

Patek SN, Feldmann RM, Porter M, Tshudy D. 2006. Phylogeny and Evolution. In: Phillips B, editor. Lobsters, biology, management, aquaculture and fisheries. Oxford, UK: Blackwell Publishing Ltd. pp. 113–138.

Pelliccia F, Barzotti R, Volpi EV, Bucciarelli E, Rocchi A. 1998. Nucleotide sequence and chromosomal mapping of the 5S rDNA repeat of the crustacean *Proasellus coxalis*. Genome 41 (1):129–133. DOI:10.1139/g97-110.

Salvadori S, Coluccia E, Deidda F, Cau A, Cannas R, Deiana AM. 2012. Comparative cytogenetics in four species of Palinuridae: B chromosomes, ribosomal genes and telomeric sequences. Genetica 140:429–437. DOI:10.1007/s10709-012-9691-4.

Salvadori S, Coluccia E, Deidda F, Cau A, Cannas R, Lobina C, Sabatini A, Deiana AM. 2014. Karyotype, ribosomal genes, and telomeric sequences in the crayfish *Procambarus clarkii* (Decapoda: Cambaridae). Journal of Crustacean Biology 34 (4):525–531. DOI:10.1163/1937240X-00002247.

Salvadori S, Coluccia E, Milia A, Cannas R, Deiana AM. 2002. Studio dei cromosomi mitotici e meiotici di *Homarus gammarus* (Crustacea, Decapoda). Biologia Marina Mediterranea 9(1):889–891.

Salvadori S, Deiana AM, Coluccia E, Floridia G, Rossi E, Zuffardi O. 1995. Colocalization of (TTAGGG)n telomeric sequences and ribosomal genes in Atlantic eels. Chromosome Research 3:54–58. DOI:10.1007/BF00711162.

Scali V, Coluccia E, Deidda F, Lobina C, Deiana AM, Salvadori S. 2016. Co-localization of ribosomal and telomeric sequences in *Leptynia* (Insecta: Phasmatodea). Italian Journal of Zoology 83:285–290. DOI:10.1080/11250003.2016.1219403.

Scali V, Deidda F, Coluccia E, Cannas R, Melis R, Salvadori S. 2020. Parental species and hybrid descendants of Bacillus (Insecta Phasmatodea) show different patterns of highly amplified, colocalized ribosomal and telomeric sequences. The European Zoological Journal 87(1):272–281. DOI:10.1080/24750263.2020.1764640.

Schram FR, Dixon CJ. 2004. Decapod phylogeny: Addition of fossil evidence to a robust morphological cladistic data set. Bull. Mitsunami Fossil Museum 31:1–19.

Schwarzacher T, Heslop-Harrison JS. 2000. Practical *in situ* Hybridization. Oxford: BIOS Scientific Publishers. pp. 203.

Sochorová J, Garcia S, Gálvez F, Symonová R, Kovařík A. 2018. Evolutionary trends in animal ribosomal DNA loci: Introduction to a new online database. Chromosoma 127:141–150. DOI:10.1007/s00412-017-0651-8.

Tsang LM, Chan TY, Cheung MK, Chu KH. 2009. Molecular evidence for the Southern Hemisphere origin and deep-sea diversification of spiny lobsters (Crustacea: Decapoda: Palinuridae). Molecular Phylogenetics and Evolution 51 (2):304–311. DOI:10.1016/j.ympev.2009.01.015.

Vittorazzi SE, Lorenço LB, Del-Grande ML, Recco-Pimentel SM. 2011. Satellite DNA derived from 5S rDNA in *Physalaemus cuvieri* (Anura, Leiuperidae). Cytogenetic and Genome Research 134(2):101–107. DOI:10.1159/000325540.

Wahle RA, Castro KM, Tully O, Cobb S. 2013. *Homarus* in Phillips BF. Lobsters biology management aquaculture and fisheries. 2nd ed. Oxford, UK. pp. 237–274.

Webber WR, Booth JD. 2007. Genetics of slipper lobters. In: Lavalli KL, Spanier E, editors. The biology and fishery of slipper lobsters. Boca Raton (FL): CRC Press Taylor & Francis Group. pp. 25–53.

Wolfe JM, Breinholt JW, Crandall KA, Lemmon AR, Lemmon EM, Timm LE, Siddall ME, Bracken-Grissom HD. 2019. A phylogenomic framework, evolutionary timeline and genomic resources for comparative studies of decapod crustaceans. Proceedings of the Royal Society B: Biological Sciences 286(1901):20190079. DOI:10.1098/rspb.2019.0079.

Zardoya R, Meyer A. 1996. Evolutionary relationships of the coelacanth, lungfishes, and tetrapods based on the 28S ribosomal RNA gene. Proceedings of the National Academy of Sciences of the USA 93(11):5449–5454. DOI:10.1073/pnas.93.11.5449.