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TTAGG telomeric repeats in chromosomes of some insects and other arthropods

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We studied the occurrence of the TTAGG telomere repeats by fluorescence *in situ* hybridization (FISH) and Southern hybridization in ten insect species and two other arthropods. $(TTAGG)_n$-containing telomeres were found in three Lepidoptera species, the silkworm *Bombyx mori* (in which the telomeric sequence was recently discovered), the flour moth *Ephestia kuehniella*, and the wax moth *Galleria mellonella*, in one species of Hymenoptera, the honey bee *Apis mellifera*, in one species of Coleoptera, the bark beetle *Ips typographus*, in one species of Orthoptera, the locust *Locusta migratoria*, and in a crustacean, the amphipod *Gammarus pulex*. They were absent in another species of Coleoptera, the mealworm *Tenebrio molitor*, in two representatives of Diptera, *Drosophila melanogaster* and *Megaselia scalaris*, in a species of Heteroptera, the bug *Pyrrhocoris apterus* and in a spider, *Tegenaria ferruginea*. Our results, which confirm and extend earlier observations, suggest that $(TTAGG)_n$ was a phylogenetically ancestral telomere motif in the insect lineage but was lost independently in different groups, being replaced probably by other telomere motifs. In the Coleoptera this must have happened rather recently as even members of the same family, Curculionidae, differ with respect to the telomeric DNA.

Key words: Telomere, fluorescence *in situ* hybridization, FISH, Southern hybridization, insect phylogeny, *Apis*, *Bombyx*, *Ephestia*, *Galleria*, *Drosophila*, *Megaselia*, *Ips*, *Tenebrio*, *Pyrrhocoris*, *Locusta*, *Gammarus*, *Tegenaria*

Introduction

Telomeres, the physical ends of chromosomes in eukaryotes, are essential structural components of the chromosomes. They are specialized DNA-protein structures that prevent the chromosomes from end-to-end fusions and protect chromosomal ends from gradual erosion during successive rounds of semi-conservative DNA replication (Review: Blackburn 1991). The mode of securing the telomere functions is remarkably conserved between lower and higher eukaryotes. In most organisms, telomeric DNA consists of tandem arrays of simple sequence; one strand is G-rich and its 3' end points to the chromosome end. The repetitive sequences are synthesized and maintained at the DNA ends by a specialized reverse transcriptase called telomerase (Blackburn 1990; Greider 1996).

Although there is diversity in the composition of telomeric repetitive sequences among different organisms (Review: Zakian 1995), some sequences are characteristic for whole taxonomic groups. The human telomeric sequence $(TTAGGG)_n$ (Moyzis *et al.* 1988), *e.g.*, has been found in all vertebrates examined (Meyne *et al.* 1989) and the $(TTTAGGG)_n$ sequence appears to be conserved in the plant kingdom (Cox *et al.* 1993; Fuchs *et al.* 1995) with few exceptions (*e.g.*, Pich *et al.* 1996).

Insects represent the most numerous group of organisms in the animal kingdom, with a large variety of cytogenetic mechanisms. Three different types of DNA telomere organization have been described in this group: a pentanucleotide sequence repeat, $(TTAGG)_n$, from the silkworm *Bombyx mori* (Okazaki *et al*. 1993), HeT-A and TART transposable elements in *Drosophila melanogaster* (Biessmann *et al.* 1990, Levis *et al.* 1993), and complex tandem repeats in the midge genus *Chironomus* (Nielsen & Edstr m 1993, Zhang *et al*. 1994). The $(TTAGG)_n$ repeat was also found in telomeres of the ant *Myrmecia* (Meyne *et al*. 1995) by fluorescence *in situ* hybridization (FISH) and appears to be a widespread telomere DNA motif among insects according to the very suggestive Southern hybridization data of Okazaki *et al*. (1993).

In this paper, we present Southern hybridization data and - since crosshybridizing sequences may not be telomeric - complementary FISH data on the occurrence of the $(TTAGG)_n$ motif among insects with emphasis on model species and economically important insect species. *Bombyx mori* and *Drosophila melanogaster* serve as a positive and, respectively, negative control. The inclusion of a spider and a crustacean species adds helpful information for the discussion of the phylogenetic implications.

Materials and methods

Specimens

We used three species of Lepidoptera. The wild-type strain WT-C of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Pyralidae), is kept in the laboratory of F.M. České Budějovice, Czech Republic (for its origin see Marec 1990). A laboratory wild-type strain of the wax moth, *Galleria mellonella* L. (Pyralidae), was provided by Tom Tonka (České Budějovice). The silkworm, *Bombyx mori* L. (Bombycidae), originated from the collection of mutants kept in the laboratory of K.S. (Sapporo, Japan), and we used hybrid larvae from crosses between C124 females and N124 males (Hiratsuka 1969). Larvae of workers and adult drones of the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), were obtained from colonies kept at the "Haustier-Schutzpark Warder" (Germany) by Jürgen Güntherschulze. Two representatives of Diptera, *Drosophila melanogaster* Meigen (the white-eyed strain *w*/*w*) and the phorid fly *Megaselia scalaris* Loew (wild-type strain 'Wien', for its origin see Johnson *et al.* 1988), were from laboratory cultures kept in Lübeck (Germany). A sample from a laboratory population of the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), was obtained from Jaroslav Pavelka (České Budějovice). Adults of the spruce bark beetle, *Ips typographus* L. (Coleoptera: Curculionidae), were collected by Jiři Zelen in umava mountains (Czech Republic) and sent to us by Martina Žurovcová (both České Budějovice). The laboratory wild-type strain of the red firebug, *Pyrrhocoris apterus* (L.) (Heteroptera: Pyrrhocoridae), was a gift from Radom r Socha (České Budějovice). The African migratory locust, *Locusta migratoria* L. (Orthoptera: Acrididae), was from a laboratory colony of Hans-G nter Mertl (L beck). Subadult and adult stages of the spider *Tegenaria ferruginea* (Panzer) (Arachnida, Araneae: Agelenidae) were collected by Ji Kr l (Prague, Czech Republic) in Prague. Specimens of the amphipod crustacean, *Gammarus pulex* (L.) (Crustacea, Amphipoda: Gammaridae) were collected near L beck, Germany, by Garnet Suck (Lübeck).

Chromosome preparations

Mitotic and meiotic chromosomes were prepared from gonads of lepidopteran larvae of both sexes, male pupae of flies, adult males of beetles, an adult male of the locust, and male larvae of the bug. In the honey bee, we prepared mitotic chromosomes from freshly hatched larvae of workers and meiotic chromosomes from testes of adult drones. In *G. pulex*, chromosome preparations were made from embryos and testes. In most cases, specimens were dissected in a saline solution (Glaser 1917) and fixed for 15-30 min in freshly prepared Carnoy fixative (ethanol, chloroform, acetic acid, 6:3:1). Cells were dissociated in 60% acetic acid and spread on the slide using a heating plate at 50 C (Traut 1976). In some cases, dissected tissues were swollen for 10(-20) min in a hypotonic solution (83 mM

KCl and 17 mM NaCl, Marec & Traut 1993) and then fixed. In the spider, we used preparations from testes of subadult males; the preparations were made by a modified dropping technique (Kr l 1994). Briefly, testes were dissected out in the saline solution, pretreated in a hypotonic solution (0.075 M KCl) for 25 min and fixed for 6-9 h in freshly prepared methanol- acetic acid (3:1) at 5 C. Then the testes were transferred into fresh fixative, dispersed with a fine Pasteur pipette, and centrifuged for 5 min at 2000 x g. The sedimented cells were resuspended in fresh fixative to an optimal concentration and dropped onto slides.

Shortly after drying, all preparations were passed through an ethanol series (70%, 80% and 96%; 30 second each) and stored at -20 C.

$(TTAGG)_n$ telomere probe

We devised two oligomers, 5'-TAGGTTAGGTTAGGTTAGGT-3' and 5'-CTAACCTAACCTAACCTAAC-3' (custom-made by MWG-Biotech GmbH, Ebersberg, Germany, with phosphorylated 5'-ends) which when mixed, annealed to two stable double-stranded DNA forms with 2-base 3' and 3-base 5' protruding ends, respectively. Telomere probes were produced by ligating annealed oligomers using T4 DNA ligase (Boehringer Mannheim GmbH, Germany) according to the standard procedure. This approach resulted in DNA molecules with a mean length of 0.2 kb (Fig. 1, Ligation). The probe, when hybridized to chromosome spreads, detected the telomeres but exhibited uneven hybridization signals and a high background. We obtained better signal-to-noise ratios with $(TTAGG)_n$ probes generated according to Ijdo *et al.* (1991) with few modifications. For this non-template PCR method, reactions were carried out in 20 l reaction volumes containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 M of each dNTP, 0.5 M of each of the two primers and 2 units of *Taq* DNA polymerase (GibcoBRL, Life Technologies Inc., Karlsruhe, Germany). An initial period of 90 sec at 94 C was followed by 30 cycles of 45 sec at 94 C, 30 sec at 52 C and 60 sec at 72 C, and concluded by a final extension step of 10 min at 72 C. The amplified product consisted of a heterogeneous population of DNA molecules with a mean length of about 1 kb (Fig. 1, PCR). Samples of the molecules were cloned into a plasmid (pMOS*blue* T-vector Kit, Amersham Int. plc, Buckinghamshire, England). Sequencing of a 550 bp insert confirmed its composition of tandem TTAGG repeats with a total of 3 mis-incorporations (not shown). The PCR-generated probe was used for both, Southern hybridization and fluorescence *in situ* hybridization.

Southern hybridization

Genomic DNAs were isolated according to Blin and Stafford (1976). The telomere probe was labeled by nick translation with [γ-^{32}P]dCTP using the BioNick Labeling System (GibcoBRL, Life Technologies Inc., Karlsruhe, Germany). From each species, 1.5 g of genomic DNA was digested

with *Hin*dIII, separated on 1% agarose gel, and blotted onto a nylon membrane (NY 13N NYTRAN, Schleicher & Schuell, Dassel, Germany). Hybridization of DNAs with 1 g of telomeric probe was done overnight at 68 C. Posthybridization washes were carried out at 68 C twice with each 0.4 M and 0.2 M phosphate buffer (pH 6.4) containing 0.1% SDS, followed with three high-stringency washes in 0.04 M phosphate buffer/0.1% SDS at 50 C. Detection of ^{32}P was performed with an Kodak autoradiography film X-OMAT AR (exposed for two days at -80 C).

Fluorescence *in situ* hybridization (FISH)

Chromosome preparations were removed from the freezer, passed through an ethanol series and air-dried. To reduce background, they were incubated in 5x Denhardt's solution (50x Denhardt is 1% Ficoll, 1% polyvinylpyrrolidone, 1% bovine serum albumin) for 30 min at 37 C. Subsequently, chromosomes were denatured in 70% deionized formamide, 2xSSC (1xSSC is 0.15 M sodium citrate, 0.015 M NaCl, pH7.0) for 4 min at 72-75 C, immediately dehydrated in a cold ethanol series and air-dried.

Telomere probes were labeled by nick translation with biotinylated dUTP (Bio-16-dUTP) using a BioNick Labeling System (GibcoBRL, Life Technologies Inc., Karlsruhe, Germany). For each slide, we prepared 10 l hybridization mix containing 50 ng biotin-labeled telomere probe, 25 g salmon sperm DNA (Sigma), 50% deionized formamide, 10% dextran sulphate and 2xSSC.

The hybridization mixture was denatured for 5 min at 90 C. Hybridization was for 18-22 h at 37 C. Posthybridization washes were three times for 5 min at 46 C in 50% formamide, 2xSSC, five times for 2 min at 46 C in 2xSSC, three times for 5 min at 62 C in 0.1xSSC, and 30-40 min at room temperature in 4xSSC, 0.1% Tween 20.

After preincubation in blocking buffer (2.5% BSA in 4xSSC) for 20 min at room temperature, hybridization signals were detected with Cy3-conjugated streptavidin (Jackson ImmunoRes. Labs., Inc., West Grove, Pennsylvania) and one round of enhancement with biotinylated antistreptavidin (Vector Labs., Inc., Burlingame, California) and Cy3-conjugated streptavidin. The preparations were counterstained with 0.5 g/ml DAPI (4'6-diamidino-2-phenylindole; Sigma) in PBS (0.15 M NaCl, 0.05 M NaH_2PO_4, pH 7.4), 1% Triton X-100, and mounted in antifade (0.233 g 1,4-diazobicyclo(2.2.2)-octane, 1 ml 0.2 M HCl, pH 8.0, 9 ml glycerol).

Black and white images of the chromosomes were recorded separately for each fluorescent dye with a cooled CCD camera using the Pinkel filter set for the Zeiss Axioskop microscope. Images were pseudocoloured (blue for DAPI and red for Cy3) and superimposed with the aid of an image processing program, Adobe Photoshop Version 4.01.

Results

Southern hybridization

The $(TTAGG)_n$ probe was hybridized to *Hin*dIII-digested genomic DNAs of ten insect species, a spider and a crustacean (Fig. 2). DNAs of three lepidopteran species, *Bombyx mori*, *Ephestia kuehniella*, and *Galleria mellonella*, the coleopteran species *Ips typographus* (bark beetle), the hymenopteran *Apis mellifera,* the orthopteran *Locusta migratoria,* and the amphipod crustacean *Gammarus pulex* crossreacted with the probe. They displayed a diffuse smear of hybridization signal in the high molecular weight range with superimposed weaker (*B. mori*, *E. kuehniella*, *G. mellonella*, *A. mellifera*) or stronger (*I. typographus, L. migratoria*) bands. A second coleopteran species, *Tenebrio molitor,* produced two very faint bands. By contrast, DNAs of two flies, *Drosophila melanogaster* and *Megaselia scalaris*, the heteropteran *Pyrrhocoris apterus,* and the spider *Tegenaria ferruginea* did not hybridize with the probe. With respect to *B. mori* and *D. melanogaster* our results confirm those of a similar experiment by Okazaki *et al*. (1993).

Fluorescence *in situ* hybridization (FISH)

Lepidoptera

The location of the signals can best be evaluated in the long pachytene chromosomes that represent the preferred stage for chromosome research in Lepidoptera. The pachytene complements of both, *G. mellonella* males and females, were composed of 30 bivalents, consistent with the published haploid chromosome number (Ostrjakowa-Warschawer 1937). In pachytene complements of females, the sex-chromosome bivalent, WZ, can be recognized as the longest bivalent with a partially heterochromatinized W chromosome (Traut *et al.* 1999, Wang *et al.* 1993). Twin hybridization signals of the $(TTAGG)_n$ probe were typically located at both ends of autosomal and sex chromosomal bivalents, each signal representing the end of one of the synapsed homologous chromosomes (Figs. 3 and 4).

In *B. mori*, pachytene nuclei of both sexes displayed 28 bivalents in accordance with the published haploid chromosome number (see Tazima 1964). $(TTAGG)_n$ hybridization signals were restricted to the chromosomal ends (Fig. 6). Terminal location of the signals was also found in mitotic prometaphase complements of both sexes as well as in prometaphase complements of male meiosis II (not shown) when chromosomes look like thick rods. In the more or less spherical to dumbbell-shaped metaphase I chromosomes of male meiosis, however, the signals were regularly observed inside the highly condensed chromatin mass (Fig. 5). It is not clear yet whether this was

due to terminalization of chiasmata or a particular arrangement of chromosomes at this stage of bivalent development.

Pachytene nuclei of *E. kuehniella* displayed 30 bivalents (Schulz & Traut 1979). The telomere probe hybridized to the ends of the pachytene bivalents in female and male meiosis (not shown). In postpachytene nuclei of female meiosis, clusters of hybridization signals were observed at bivalent ends (Fig. 7) instead of the twin signals typical for the pachytene stage *sensu stricto*. In some bivalents, signals were stretched out from the chromosomal ends, indicating that the $(TTAGG)_n$ probe labeled the very ends of the chromosomes. This phenomenon was also observed in females of *G. mellonella* and *B. mori* but not in males of the three species. The sex difference may be related to the different course of meiosis. In Lepidoptera, female meiosis is achiasmatic whereas male meiosis is chiasmatic and follows a normal sequence of meiotic events (Rasmussen 1977; Traut 1977).

Hymenoptera

The diploid chromosome number in *A. mellifera* is 2n = 32 (Petrunkewitsch 1901, Beye & Moritz 1995). We found a limited number of mitotic prometaphase and metaphase chromosome plates in larval tissues of honeybee workers. Hybridization signals were observed at both ends of acrocentric and metacentric chromosomes (Fig. 8). Interphase nuclei of worker bees as well as those from drones, nuclei of spermatids and spermatozoa displayed strong signals of the telomere probe (not shown).

Diptera

As expected from Southern hybridization, no hybridization signal of the telomere probe was observed after FISH on chromosomes of the two fly species, either in the four chromosome pairs of *D. melanogaster* (Fig. 9) or the three chromosome pairs of *M. scalaris* (Fig. 10).

Coleoptera

Virkki (1960) described a haploid chromosome number of n=15 for the bark beetle, *I. typographus*. We found n=16 instead. Chromosome complements in mitotic metaphase consisted of 32 elements, and 16 bivalents were regularly observed in meiotic metaphase I of males. The telomere probe hybridized to chromosome ends at the male pachytene stage (Fig. 11), at other stages of meiosis and at mitosis (not shown).

Ten bivalents were found in meiosis of *T. molitor* males in accordance with Stevens (1905). After FISH, no $(TTAGG)_n$ signal appeared on pachytene bivalents (Fig. 12) or on chromosomes at other stages of male meiosis (not shown). Thus the two faint bands seen in Southern hybridization do not represent telomeric DNA.

Heteroptera

In accordance with published accounts (see Socha 1993, and references therein), 23 chromosomes of various length were observed in mitoses of *P. apterus* males. No hybridization signal of the telomere probe was found, either on the chromosomes (Fig. 13) or on interphase nuclei (not shown). This confirms the results from Southern hybridization.

Orthoptera

The male karyotype of *L. migratoria* consists of 2n=23 telocentric chromosomes (see White 1973). Spermatogonial mitoses displayed $(TTAGG)_n$ signals at both chromosome ends (Fig. 14). In diplotene and diakinesis of males, positions of $(TTAGG)_n$ signals depended on the configuration of chiasmata while those in the X-chromosome univalent were always terminal (Fig. 15).

Arachnida

Chromosome preparations from testes of subadult males of the spider *T. ferruginea* showed 40 mitotic chromosomes, 39 acrocentrics and one metacentric (Fig.16; *cf.* Kr l 1994). In accordance with the results from Southern hybridization, the telomere probe did not hybridize to the chromosomes.

Crustacea

In preparations of *G. pulex* testes, we found 52 chromosomes in spermatogonial mitoses and 26 bivalents in metaphase I, confirming the chromosome number reported by Orian & Callan (1957). The chromosomes were rather similar in size and form, metacentric or submetacentric according to the prominent DAPI-positive block of presumably pericentric heterochromatin. After FISH, mitotic prometaphase chromosomes displayed either single or double $(TTAGG)_n$ hybridization signals at the ends (Fig. 17). When sister chromatids were separated, they showed one hybridization signal at each end (not shown). Particularly strong signals were observed in meiotic chromosomes: pachytene bivalents and metaphase I bivalents (not shown).

Discussion

In this study, ten representatives from six insect orders and two non-insect arthropods were screened by Southern hybridization and FISH for the presence of the pentanucleotide telomere repeat TTAGG, discovered by Okazaki *et al.* (1993) in the silkworm, *B. mori*. The sequence was shown to be a component of the telomeres in six insect species and a crustacean whereas it was absent in four other

insect species and a spider. Thus it is a widespread though not the only telomere motif in insects and it occurs in other arthropods.

Although insects as a group are heterogeneous with respect to the presence or absence of the $(TTAGG)_n$ telomere motif, some phylogenetic branches of the insects appear to be homogeneous (Table 1). The three species of Lepidoptera investigated by FISH have this telomere motif, and crosshybridization of a $(TTAGG)_5$ probe with genomic DNA in Southern blots (Okazaki *et al.* 1993) suggests its presence in four more lepidopteran species. On the other hand, the genomes of the seven dipterans probed so far, lack this telomere motif, and a simple sequence telomere motif is probably also absent in another dipteran species, the mosquito *Anopheles gambiae* (Roth *et al*. 1997). Telomere elongation in the dipterans *D. melanogaster* and *A. gambiae* is telomerase-independent. In *Drosophila* it is achieved by insertions of telomere-associated HeT-A and TART transposons (Biessmann *et al.* 1990, Levis *et al.* 1993), in *Anopheles* probably by unequal recombination (Roth *et al.* 1997).

The insect order Coleoptera, in contrast, is heterogeneous. While *I. typographus* possesses the $(TTAGG)_n$ motif, it is evidently absent in *T. molitor*. The Southern hybridization data of Okazaki *et al.* (1993) included, five species of Coleoptera presumably possess the $(TTAGG)_n$ telomere motif while it is clearly absent in four other species. Presence or absence is irrespective of phylogenetic relationships (for phylogeny of Coleoptera see Kukalov -Peck & Lawrence 1993). More specifically, *I. typographus* undoubtedly possesses $(TTAGG)_n$-containing telomeres while this motif is absent in *Sipalinus gigas* (Okazaki *et al.* 1993), a species that belongs to the same family, Curculionidae. These results suggest phylogenetically recent changes of the telomere composition in Coleoptera.

The basic phylogenetic question then is whether the $(TTAGG)_n$ telomere motif evolved independently several times in the insect lineage or whether it was an ancestral character and was lost several times in different branches of the insect phylogeny. The cladogram in Fig. 18 combines present knowledge of the insect phylogeny and of the occurrence of the $(TTAGG)_n$ telomere motif. Since we assume that maintenance of the TTAGG telomere repeats depends on the presence of a functional telomerase, we consider the appearance of a new simple sequence telomere repeat a rare evolutionary event. Loss of the simple sequence telomere motif may be non-destructive if an alternative pathway of telomere elongation exists, a scenario suggested to explain the evolution of the *Drosophila* telomeres (Biessmann & Mason 1997). Another scenario, proposed for *Chironomus*, is the gradual evolution of complex repeats from simple repeats, thereby retaining some functional features (Nielsen & Edstr m 1993). The presence of the $(TTAGG)_n$ telomere motif in several branches of the insect phylogenetic tree and in another athropod, the crustacean amphipod *G. pulex*, most probably also in a second crustacean species, the decapod *Penaeus semisulcatus*

(Okazaki *et al.* 1993), argue in favour of the conservation and occasional loss of an ancestral $(TTAGG)_n$ telomere motif during the evolution of insects.

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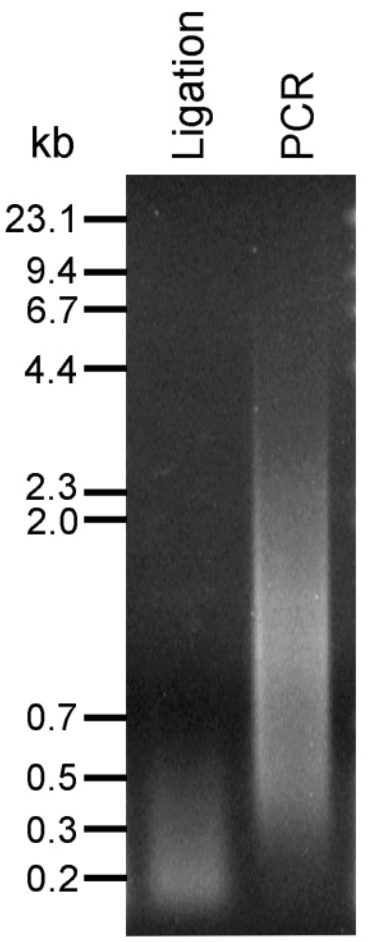
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Drosophila
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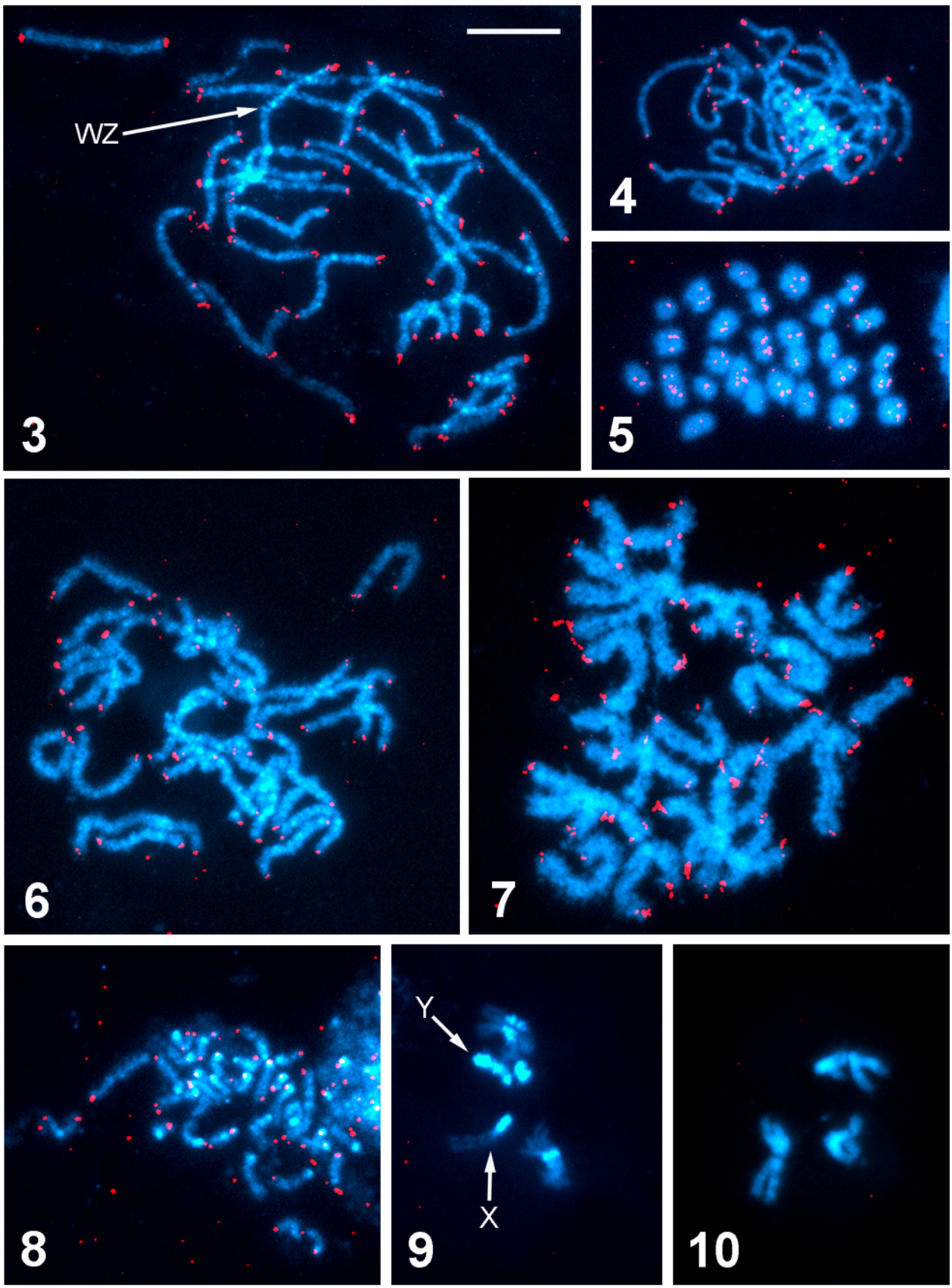
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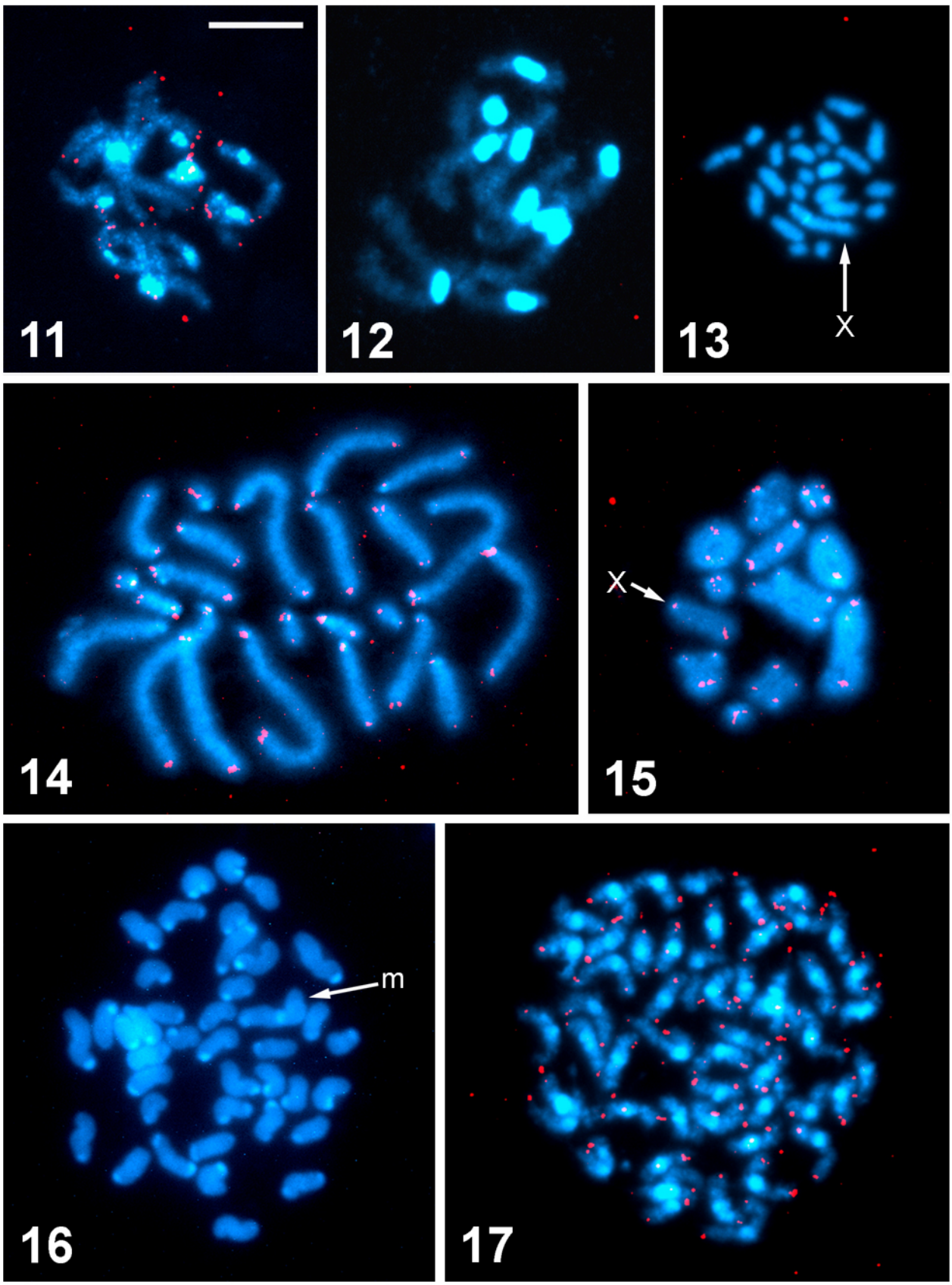
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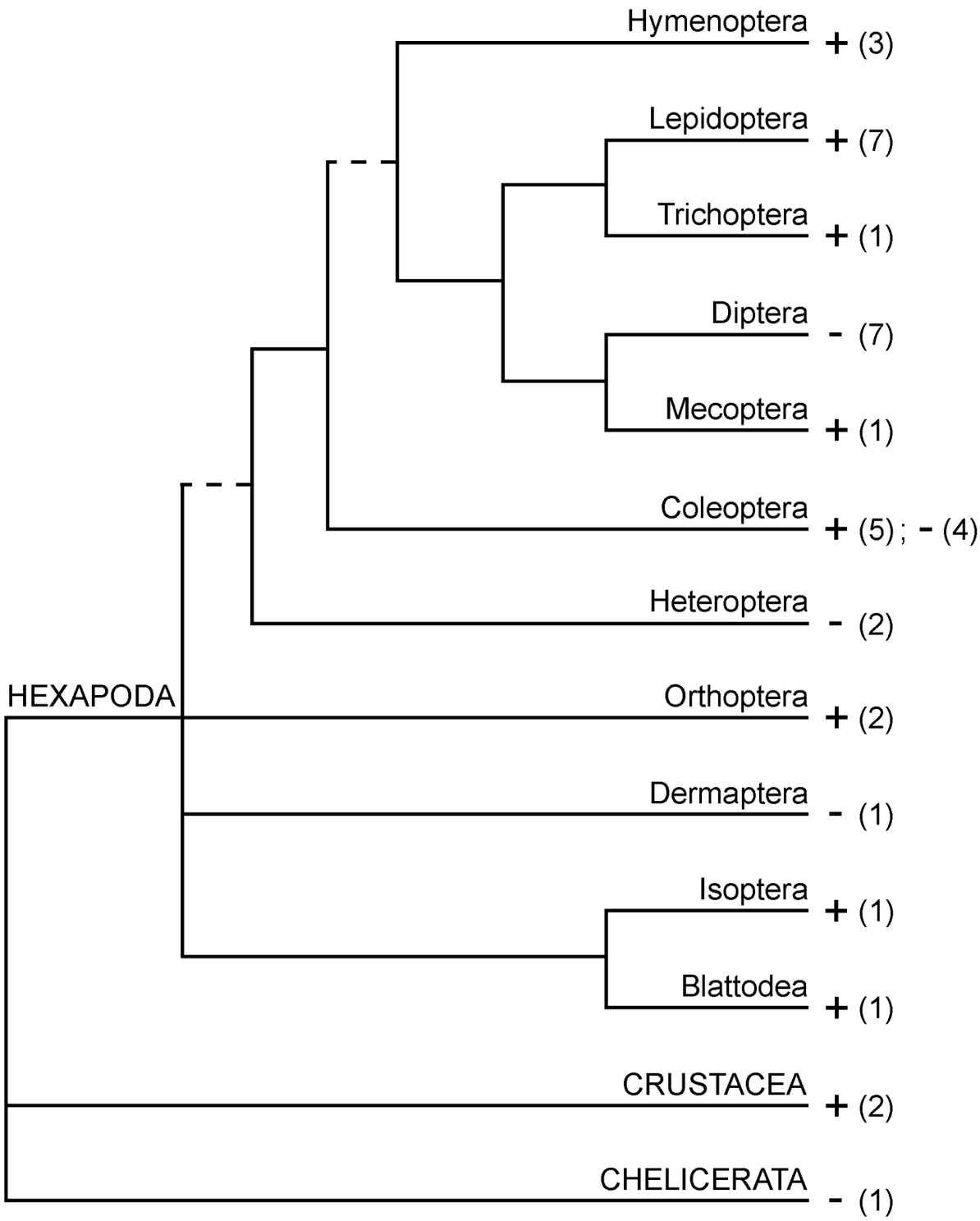
Hymenoptera
+ (3)
Lepidoptera
+ (7)
Trichoptera
+ (1)
Diptera
- (7)
Mecoptera
+ (1)
Coleoptera
+ (5) ; - (4)
Heteroptera
- (2)
HEXAPODA
Orthoptera
+ (2)
Dermaptera
- (1)
Isoptera
+ (1)
Blattodea
+ (1)
CRUSTACEA
+ (2)
CHELICERATA
- (1)

FIGURE LEGENDS

Figure 1. Electrophoresis of $(TTAGG)_n$ telomere probe samples in a 1.5% agarose gel stained with ethidium bromide. The left lane shows a sample produced by ligation of annealed oligomers, the right lane one produced by non-template PCR.

Figure 2. Southern hybridization of *Hin*dIII-digested genomic DNAs from ten insect species, a spider (*Tegenaria*), and an amphipod (*Gammarus*) with the $(TTAGG)_n$ probe. Full species names are given in Materials and methods.

Figures 3-10. FISH of the $(TTAGG)_n$ telomere probe (red signals) to chromosome spreads counterstained with DAPI. **Figure 3.** *Galleria mellonella* female, pachytene complement with a sex-chromosome bivalent, WZ. **Figure 4.** *Galleria mellonella* male, pachytene bivalents. **Figure 5.** *Bombyx mori* male, meiotic metaphase I, note internal location of hybridization signals in most bivalents. **Figure 6.** *Bombyx mori* female, pachytene bivalents. **Figure 7.** *Ephestia kuehniella* female, postpachytene bivalents, some with stretched-out signal chains (arrowheads). **Figure 8.** *Apis mellifera*, mitotic prometaphase chromosomes of a worker bee larva. **Figure 9.** *Drosophila melanogaster* male, mitotic chromosomes, note somatic pairing of the three autosome pairs. **Figure 10.** *Megaselia scalaris* male, mitotic prometaphase with somatic pairing of homologues. Bar = 10 m.

Figures 11-17. FISH of the $(TTAGG)_n$ telomere probe (red signals) to chromosome spreads counterstained with DAPI. **Figure 11.** *Ips typographus* male, pachytene bivalents with $(TTAGG)_n$ signals and DAPI-positive blocks of heterochromatin. **Figure 12.** *Tenebrio molitor* male, pachytene bivalents with large blocks of DAPI-positive heterochromatin. **Figure 13.** *Pyrrhocoris apterus* male, mitotic metaphase. **Figure 14.** *Locusta migratoria* male, mitosis. **Figure 15.** *Locusta migratoria* male, diakinesis, note positions of hybridization signals in autosomal bivalents as opposed to those in the univalent X chromosome. **Figure 16.** *Tegenaria ferruginea* male, mitotic metaphase, note DAPI-positive centromere regions and a long metacentric chromosome (m). **Figure 17.** *Gammarus pulex* male, mitotic prometaphase, note DAPI-positive pericentric heterochromatin. Bar = 10 m.

Figure 18. Phylogeny of insect orders and two other arthropod lineages examined for the presence (+) or absence (-) of the $(TTAGG)_n$ motif by FISH and/or Southern hybridization (for details see Table 1). Numbers of species examined are given in brackets. The cladogram is based on Kristensen (1991) and Štys & Zrzavy (1994). Interrupted lines indicate groupings that are considered doubtfully monophyletic.

Table 1. Arthropod species tested for the presence of $(TTAGG)_n$ telomere sequences

Group	Species	FISH	Southern hybridization	Other telomere structure
Hymenoptera	*Apis mellifera*	+ [7]	+ [7]	
	Myrmecia ssp.	+ [3]		additionally $(TTAGGG)_n$ [3]
	Manica yessensis		+ [4]	
Lepidoptera	*Antheraea pernyi*		+ [4]	
	Antheraea yamamai		+ [4]	
	Bombyx mandarina		+ [4]	
	Bombyx mori	+ [4] [7]	+ [4] [7] [8]	
	Ephestia kuehniella	+ [7]	+ [7]	
	Galleria mellonella	+ [7]	+ [7]	
	Samia cynthia ricini		+ [4]	
Trichoptera	*Stenopsyche japonica*		+ [4]	
Diptera	*Chironomus ssp.*		- [6]	340 / 350 bp repeats [6]
	Drosophila melanogaster	- [7]	- [4] [7]	HeT-A [1], TART [2] elements
	Eristalomya tenax		- [4]	
	Megaselia scalaris	- [7]	- [7]	
	Neoitamus angusticornis		- [4]	
	Sphyximorphoides pleuralis		- [4]	
	Tabanus trigonus		- [4]	
Mecoptera	*Protidricerus japonicus*		- [4]	
Coleoptera	*Anomala cuprea*		- [4]	
	Arhopalus coreanus		+ [4]	
	Diacanthous undosus		+ [4]	
	Gonocephalum sexuale		- [4]	
	Hydrochara affinis		±[4]	
	Ips typographus	+ [7]	+ [7]	
	Melanotus legatus		+ [4]	
	Sipalinus gigas		- [4]	

	Spondylis buprestoides		+ 4)	
	Tenebrio molitor	- 7)	± 7)	
Hemiptera (Heteroptera)	*Halyomorpha mista*		- 4)	
	Pyrrhocoris apterus	- 7)	- 7)	
Hemiptera (other)	*Acyrthosiphon pisum*		± 4)	
	Terpnosia nigricosta		± 4)	
Orthoptera	*Diestrammena japonica*		+ 4)	
	Locusta migratoria	+ 7)	+ 4) 7)	
Dermaptera	*Forficula scudderi*		- 4)	
Isoptera	*Hodotermopsis japonicus*		+ 4)	
Blattodea	*Periplaneta fuliginosa*		+ 4)	
Crustacea	*Asellus aquaticus*			$(TTAGGG)_n$ 5)
	Gammarus pulex	+ 7)	+ 7)	
	Penaeus semisulcatus		+ 4)	
Chelicerata	*Tegenaria ferruginea*	- 7)	- 7)	

+: $(TTAGG)_n$ signal present; -: $(TTAGG)_n$ signal absent.; : ambiguous weak signal.

References: 1) Biessmann *et al.* (1990); 2) Levis *et al.* (1993); 3) Meyne *et al.* (1995); 4) Okazaki *et al.* (1993); 5) Pelliccia *et al.* (1994); 6) Nielsen & Edstr m (1993), Zhang *et al.* (1994), L pez *et al.* (1996); 7) this paper.