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Authors: Passamonti, Marco, Mantovani, Barbara, and Scali, Valerio

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Characterization of a Highly Repeated DNA Family in Tapetinae Species (Mollusca Bivalvia: Veneridae)

Marco Passamonti*, Barbara Mantovani and Valerio Scali

Dipartimento di Biologia Evoluzionistica Sperimentale, Università di Bologna, via Selmi 3; I-40126 Bologna, Italy

ABSTRACT—A repetitive DNA family (*phBgl*II400) was characterized in the clam species *Tapes philippinarum* (Veneridae Tapetinae). The tandemly repeated sequences are AT-rich and show a mainly pericentromeric localization, as most satellite DNAs do. Sequence analysis of *phBgl*II400 DNA family showed a high level of intraspecific homogeneity. Furthermore a 200 bp subunit motif within the 400 bp monomer was apparent as well as the existence of two main "open reading frames" along the 400 bp sequence.

In order to investigate the possible distribution of this DNA family among Veneridae, Southern blot analyses were performed on genomic DNAs of *Tapes decussatus*, *Venerupis aurea* and *Paphia undulata* (Tapetinae), *Callista chione* (Pitarinae), *Chamelea gallina* (Chioninae) and *Venus verrucosa* (Venerinae). The *phBgl*II400 family has been found in two additional Tapetinae, namely *V. aurea* and *P. undulata*, but not in *T. decussatus* or other analyzed species. This pattern of sat-DNA distribution supports the high level of differentiation of *T. decussatus* observed in the previous gene-allozyme analysis. All of these suggest a better allocation of *T. decussatus* to a genus different from that of *T. philippinarum*.

INTRODUCTION

Tapes philippinarum, *Tapes decussatus*, *Venerupis aurea* and *Paphia undulata* (subfamily Tapetinae, Fischer, 1887) represent some of the economically most important bivalves. Their taxonomic position and phyletic relationships are questioned. On the basis of shell morphology, "*decussatus*" and "*philippinarum*" taxa have been invariably considered congeneric and ascribed to *Tapes* Von Muehlfeldt, 1811; "*aurea*" has been most often ascribed to *Venerupis* Lamarck, 1818 (Fischer-Piette and Metivier, 1971), but, recently, also to *Paphia* Roding, 1798 (Sabelli *et al*., 1990). Finally, "*undulata*" has been always ascribed to *Paphia*. On the other hand, morphological data sharply contrast allozyme and mitochondrial DNA analyses (Borsa and Thiriot-Quiévreux, 1990; Canapa *et al*., 1996; Passamonti *et al*., 1997). In particular, biochemical and mtDNA findings do not support the assignment of *T. decussatus* and *T. philippinarum* to the same genus.

A useful tool to a further understanding of the phyletic relationships within the genus *Tapes* and the Tapetinae subfamily, could be a molecular analysis of tandemly repeated DNA sequences in their genomes. Eukaryotic genomes embody large amounts of tandemly repeated, non-coding DNA sequences, so-called satellite DNA (John and Miklos, 1979). Sat-DNA is highly variable in complexity and copy number; the repeated units can range from 3, 4 to thousand base pairs and may represent from a few units to more than 50% of the genomic complement and can be arranged into either short interspersed or long tandemly repeated units (Singer, 1982; Miklos, 1985).

The general significance of satellite DNA is rather controversial and several hypotheses have been developed to assign this fraction of the eukaryote genome a role. It has been involved in heterochromatin constitution (Gershenson, 1933, 1940; Pardue, 1975; Brutlag, 1980), chromosome pairing (Salser *et al*., 1976; Fry and Salser, 1977), rearrangements (Hatch *et al*., 1976), tridimensional organization of the interphasic nucleus (Hilliker *et al*., 1980; Manuelidis, 1982), gene amplification events (Bostock and Clark, 1980) and chromosome - mitotic spindle interactions through a peculiar class of centromeric sat-DNA-binding proteins (CENP), that preferably interact with DNA curvatures caused by $d(A \cdot T)_{n \geq 5}$ stretches (Masumoto *et al*., 1989). Alternative hypotheses suggest that sat-DNA lacks any function except its own survival (Doolittle and Sapienza, 1980; Orgel and Crick, 1980). Since it often corresponds to a heterochromatic noncoding genomic fraction, some models have been produced to explain its maintenance and evolutionary processes, such as "molecular drive" and "concerted evolution" (Dover, 1982, 1986, 1989; Charlesworth *et al*., 1994).

Although a peculiar sat-DNA family is usually restricted to a single or a few closely related species, sometimes sat-DNAs show remarkable similarities in taxonomically distant taxa; highly conserved satellite DNAs can be spread over a whole group of phylogenetically related organisms (Arnason

* Corresponding author: Tel. +39-51-354168; FAX. +39-51-251208.

et al., 1984; Cremisi *et al.*, 1988) or even be widely distributed among members of evolutionarily distant groups (Abad *et al.*, 1992). It should be noted, however, that a higher level of homogeneity has been commonly found in intraspecific sequence analyses than in inter-specific comparisons. Therefore this approach could help when traditional investigations of taxonomy appear to be inadequate or even give controversial results at the specific level (Bachmann *et al.*, 1993).

Therefore we started a genomic DNA screening of *T. philippinarum*, *T. decussatus*, *V. aurea* and *P. undulata*, all belonging to the Tapetinae subfamily. A sat-DNA family was first detected in *T. philippinarum* and its distribution in the remaining species was analyzed. Comparisons were afterwards made also with genomic DNAs of *Callista chione* (subfamily Pitarinae), *Chamelea gallina* (subfamily Chioninae) and *Venus verrucosa* (subfamily Venerinae).

MATERIALS AND METHODS

Nine individuals of seven Veneridae species from different localities were analyzed: two *T. philippinarum* (*ph*Sc - Scardovari Lagoon, Po river estuary; *ph*Ga - Ganzirri Lagoon, Sicily) and *T. decussatus* (*de*Sf - Sfax, Tunisia; *de*Ch - Chioggia, Venice Lagoon); one each of *V. aurea* (*au*Ci - Civitanova Marche, Middle Adriatic Sea), *P. undulata* (*un*Th - Thailand), *C. chione* (*ch*Ch - Chioggia, Venice Lagoon), *C. gallina* (*ga*Ch - Chioggia, Venice Lagoon) and *V. verrucosa* (*ve*Ch - Chioggia, Venice Lagoon).

Genomic DNAs were isolated by homogenizing a single foot-muscle in 160 mM sucrose, 80 mM EDTA and 100 mM Tris-HCl, pH 8 buffer and incubated at 65°C for 1 hr after addition of 0.5% SDS and 1 μg/ml of Proteinase K (Boehringer). Homogenates were then extracted for 3-5 times with phenol and/or chloroform. SDS separation was performed using potassium acetate at the final concentration of 1.2 M for 30' on ice and centrifuging for 15' at 15,000 rpm. The DNA was precipitated by the addition of 2 volumes of ethanol, centrifuging for 15' at 12,000 rpm. The DNA was recovered in TE 1 × buffer (10 mM Tris-HCl pH 8 and 1 mM EDTA) and treated with 50 μg/ml of RNAse A (Boehringer) for 10' at room temperature. The DNA was finally precipitated with 0.2 M sodium acetate and 2 volumes of ethanol. From 50 to 200 μg of genomic DNA were obtained through this procedure.

Genomic DNAs were digested with 5 units of specific endonucleases/μg of DNA for 7-8 hr, according to the manufacturer's instructions. The following endonucleases were used: *Acc*, *Alu*I, *Apa*I, *Ava*I, *Bam*HI, *Bcl*I, *Bgl*II, *Dde*, *Dra*I, *Eco*RI, *Hae*III, *Hin*dII, *Hin*dIII, *Hpa*, *Msp*I, *Nde*, *Nsi*I, *Pst*I, *Rsa*I, *Sac*I, *Sal*I, *Sma*I, *Stu*I, *Taq*I. All restricted DNAs were separated on 1.2% agarose gels in 1 × TBE buffer (90 mM Tris-borate; 2 mM EDTA) and then transferred onto a Nylon Membrane (Boehringer) following the protocol of Southern (1975).

From the *Bgl*II digested genomic DNAs of *T. philippinarum* (Scardovari and Ganzirri), a band, at about 400 bp, was cut and eluted from a 1.2% agarose gel using the Genomed JETsorb Gel Extraction Kit (cat. n. 110150). Eluted DNAs were cloned into the *Bam*HI sites of pGEM -7Zf(+) (Promega) or pUC19 (+) (Pharmacia) plasmid vectors.

V. aurea and *P. undulata* monomers, only observed in Southern blots, could not be cloned directly, owing to their extremely low quantity. Therefore, a PCR amplification was performed with 5'-AGATTTCCGCATGGC-3' and 5'-GACGTGTTCCTTGGG-3' primers, designed on the already obtained *T. philippinarum* sequence; these primers amplified the second part of the 400 bp sequence, since no good couple of primers could be designed to amplify the whole monomer. Primers were designed using PCR PLAN software in the PC/GENE 6.6 software package (© 1991, IntelliGenetics, 700 East El Camino Real, Mountain View, California). The obtained fragments were therefore cloned into a pCR-Script plasmid vector with the Stratagene pCR-Script™ Amp SK(+) Cloning Kit (Cat. n. 211189).

Labeling and Southern blot hybridizations were performed according the DIG-DNA kit instructions by Boehringer.

The nucleotide sequences of the recombinant clones were determined using the AutoRead™ Sequencing Kit (Pharmacia) with T7 and SP6 universal primers and a ALF™9000 automated sequencer (Pharmacia). Acrylamide gels were casted using ReadyMix Gels, ALF™ grade (Pharmacia).

Sequence alignments, consensus sequence, mutation distribution and codon analysis were performed using the Clustal algorithm and the Sequence Navigator software (Applied Biosystems). Genetic distances and cluster analyses were performed using the MEGA program (ver. 1.01, © S. Kumar, K. Tamura and M. Nei, 1993); in particular was utilized the "Kimura's two parameters" mutational model (Kimura, 1980) in computing distances.

Giemsa and NOR karyotypes and *in situ* hybridizations were performed on chromosome preparations from the gill tissue of *T. philippinarum* from Scardovari. Living clams were treated overnight with 0.005% colchicine in marine water. The dissected gills were hypotonically shocked with 1% w/v sodium citrate solution and then fixed with Carnoy's liquid (3:1, ethanol: acetic acid). Slides were prepared using a slightly modified air-drying technique by Crozier (1968).

Some slides were directly Giemsa stained, whereas others were treated with a silver impregnation technique, according to Marescalchi and Scali (1990), to evidence the active nucleolar organizing regions (NOR).

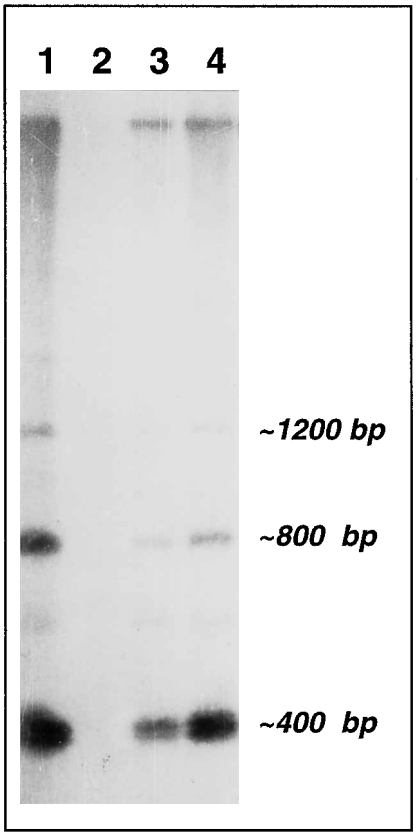

Fig. 1. Southern blot hybridization (at 1% stringency) of the *Bgl*II restricted DNAs of Tapetinae *T. philippinarum* from Scardovari (1), *T. decussatus* from Sfax (2), *P. undulata* from Thailand (3) and *V. aurea* from Civitanova (4). Approximate base-pair lengths are indicated.

Table 1. Alignment of the *phBgl*II400 DNA family cloned monomers of *T. philippinarum* from Scardovari (p*ph*Sc) and Ganzirri (p*ph*Ga). A consensus sequence, with *Bgl*II-like site (underlined) is shown. Asterisks (∗) indicate gaps introduced to increase sequence similarity. Last digits of numerals are aligned with the corresponding nucleotide.

	10	20	30	40	50	60	70	80	90	100
cons	AGATCTCCGC	ATGGCTAGGT	cACTTACCAG	CGATTTGCTT	TTTCTGTATA	TAAATTTCGG	gAAcAAACAT	CAAtTTTACG	AGCTTGCCgT	TCAAAAGCCA
p*ph*Sc4	----------	----------	A----G----	----------	----------	----------	---A------	----------	-----A----	----------
p*ph*Sc10	----------	----------	-----G----	-------A--	----------	----------	---A------	----------	-----A----	----------
p*ph*Sc11	----------	----------	----------	----------	----------	----------	A---------	--G-------	--------A-	----------
p*ph*Ga3	----------	----------	A----G----	--------*-	----------	----------	-*--------	---*--C---	C----A----	---------G
p*ph*Ga4	----------	----------	-----G----	----------	------C---	----------	A---------	---C------	-----A----	----------

	110	120	130	140	150	160	170	180	190	200
cons	TTTGACCAAT	TCCAATGCAA	ATTCGATTTT	GTACAACTAG	AGAGGGTCTT	CTTTGCACAT	GAAAAGGCCT	aAAGCCCTAA	CCCAAGTAAT	ATGTCCAGGA
p*ph*Sc4	----------	----------	----------	----------	T-T------A	A---------	----------	----------	----------	----------
p*ph*Sc10	----------	----------	----------	----------	----------	----------	-----A----	-----G----	-T--------	----------
p*ph*Sc11	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------
p*ph*Ga3	----------	----------	----------	----------	----------	------G---	----------	G---------	----------	-----T----
p*ph*Ga4	----------	----------	----------	----------	--------C-	----------	--------A-	T---------	----------	----------

	210	220	230	240	250	260	270	280	290	300
cons	GATTTCCGCA	TGGCTTGGTC	ATTTTTTGCA	CCGGCGATTT	GITTCTTTCT	CTATGTAAAT	TTCGGGAAGG	AACATCATAT	TCACGAGCCT	ACCATTCAAA
p*ph*Sc4	----------	------C-----	----------	----------	-*--------	G---------	---------A	----------	----------	----------
p*ph*Sc10	----------	----------	----------	----------	-*--------	----------	---------A	----------	----------	----------
p*ph*Sc11	----------	----------	----------	----------	-G--------	----------	----------	----------	----------	----------
p*ph*Ga3	----------	----------	----------	----------	-*--------	----------	---------A	---C------	----------	----------
p*ph*Ga4	A---------	--T-------	----------	---------C	-*--------	----------	---------A	----------	----------	----------

	310	320	330	340	350	360	370	380	390	400	
cons	AACCATTTGA	TCAATTCCGT	TGCAAATTTG	ATTTTGTACA	ACTTGAGGGG	GTCTkCTTTG	CACATgaiTa	AaGGCTTAAA	GCCCTAACCC	AAGGAACACG	TCCATG
p*ph*Sc4	----------	----------	----------	----------	----------	----AA----	-----*****	----------	---T------	----------	------
p*ph*Sc10	----------	----------	----------	----------	----------	---AG-----	-----*****	---TT-----	----------	----------	------
p*ph*Sc11	----------	----------	----------	----------	----------	----T---*-	-------A--	----------	----------	----------	------
p*ph*Ga3	----------	----------	---------GA	----------	----------	----G-----	-------*-C	-*--------	----------	----------	------
p*ph*Ga4	----------	----------	----------	----------	TT--------	----T-----	-------*--	-*--------	----------	----------	---G--

In situ hybridization was performed following Maluszynska and Heslop-Harrison (1993) with a DIG-labeled monomer obtained from the p*ph*Sc11 recombinant clone. Slides were examined under a Zeiss Axioskop fluorescence microscope with a fluorescein-specific filter set. Photomicrographs were taken on an Ektachrome Panther P1600 color-reversal film. The chromosomes were then printed at a final magnification of 1250x.

RESULTS

A restriction analysis of the genomic DNAs of *T. philippinarum*, *T. decussatus*, *V. aurea* and *P. undulata* was carried out. A ladder-like pattern was observed on ethidium bromide stained gels of the *Bgl*II restricted DNAs of *T. philippinarum*, its monomer band being localized at about 400 bp position. The cloning of the 400 bp monomer led to 8 recombinant clones (p*ph*Sc1, p*ph*Sc4, p*ph*Sc7, p*ph*Sc10-14) from Scardovari and 9 (p*ph*Ga1-5, p*ph*Ga7-10) from Ganzirri.

Southern blot hybridizations of restricted DNAs of all analyzed Veneridae species were also performed with the DIG-labeled p*ph*Sc11 clone. In *T. philippinarum*, *V. aurea* and *P. undulata*, they evidenced a main multimeric ladder with a 400 bp monomer and an intervening fainter ladder with a 200 bp monomer in the *Bgl*II restricted DNAs (Fig. 1). On the other hand, through this technique no related sequences were detected in *T. decussatus* (Fig. 1) as well as in the remaining Veneridae (*C. chione*, *C. gallina* and *V. verrucosa* - not shown). The multimeric pattern showed that the units were tandemly repeated and the family of related sequences was named *phBgl*II400.

Sequences of 5 *T. philippinarum* randomly cloned monomers from Scardovari and Ganzirri were obtained (Table 1). The sequence was AT rich (about 60%) and embodied a few $d(A \cdot T)_{n\geq 5}$ stretches. Alignment analysis evidenced clear homology between all analyzed sequences and indicated that the monomer length of the family ranged from 400 to 405 bp, with small variation caused by either single insertion/deletions (bases 39, 62, 74, 242, 359, 368 and 372) or multiple ones (only observed between positions 365-370 in clones p*ph*Sc10 and p*ph*Sc4). The highest concentration of indels was localized in the 359-372 stretch of the sequence. As a consequence, very high sequence homology, ranging from 97.5 to 99.3%, resulted.

The number of base-pair differences ascribable to point mutation was not evenly distributed throughout the sequence, ranging from 0 (positions 1-20, 51-60, 101-140, 221-230, 241-250, 281-320, 231-240, 401-410) to 4 (positions 141-150) every 10 bp.

Between bases 200 and 205 a *Bgl*II-like site (AGATTT, underlined in Table 1), was observed. Such a sequence might explain the secondary fainter ladder with a 200 bp monomer, observed in Southern blots, because a point mutation of the *Bgl*I site (AGATCT) into a *Bgl*II-like restriction site (AGATTT) in a subset of the sequences could result in both 400 bp (more numerous) and 200 bp (fewer) monomers. The mutated site

Table 2. Alignment between the 200 bp subunits of the p*ph*Sc11 monomer. #1 = first part (bp 1-199) of the p*ph*Sc11 sequence; #2 = second part (bp 200-405) of the p*ph*Sc11 sequence. Bars (|) indicate homologous sites. Asterisks (*) indicate gaps introduced to increase sequence similarity.

```
phSc11  #1  AGATCTCCGCATGGCTAGGTCACTT*****ACCAGCGATTTGCTTTTT*CTGTATATAAAT
            |||| |||||||||||| ||||| ||      ||| |||||||| || || || ||| |||||
phSc11  #2  AGATTTCCGCATGGCTTGGTCATTTTTTGCACCGGCGATTTGGTTCTTTCTCTATGTAAAT

phSc11  #1  TTCGGAAACAAACATCAGTTTTACGAGCTTGCCATTCAAAAGCCATTTGACCAATTCCAAT
            ||||| ||  |||||||  || |||||| | |||||||||| |||||||| |||||||  |
phSc11  #2  TTCGGGAAGGAACATCATATTCACGAGCCTACCATTCAAAAACCATTTGATCAATTCCGTT

phSc11  #1  GCAAATTCGATTTTGTACAACTAGAGAGGGTCTTCTTTGCACATGA**AAAGGCCTAAAGC
            ||||||| |||||||||||||| ||| |||||||||| |||||||| ||||||  ||||||
phSc11  #2  GCAAATTTGATTTTGTACAACTTGAGGGGGTCTTCTT*GCACATGAATAAAGGCTTAAAGC

phSc11  #1  CCCCAA*****GTAATATGTCCAGG
            |  |  ||     |  ||  | |||||  |
phSc11  #2  C*CTAACCCAAGGAACACGTCCATG
```

Table 3. "Kimura two parameters" genetic distances between randomly cloned *T. philippinarum* monomers

	p*ph*Sc11	p*ph*Sc10	p*ph*Sc4	p*ph*Ga3	p*ph*Ga4
p*ph*Sc11	–				
p*ph*Sc10	0.039	–			
p*ph*Sc4	0.042	0.047	–		
p*ph*Ga3	0.045	0.050	0.053	–	
p*ph*Ga4	0.037	0.056	0.058	0.059	–

has been used as a landmark to align the first part (bp 1-199) on the second one (bp 200-405) of the sequence of the cloned p*ph*Sc11 monomer. When aligned in this way, the two subunits (~200 bp) showed a high level of similarity, although differed in length for deletions/insertions and in nucleotide sequence for point mutations (Table 2).

Codon usage analysis of the whole 405 bp consensus sequence evidenced only three non-sense codons and two main open reading frames (ORF): the first one from base 2 to base 169 and the second one from 173 to 367 (56 and 65 codons in length, respectively).

"Kimura's two parameters" genetic distances were low, ranging from 0.037 to 0.059 (Table 3); furthermore no geographic differences between clones were found. None of neighbor-joining or maximum parsimony dendrograms (not shown) supported geographic differentiation.

PCR amplification and cloning allowed to obtain recombinant clones for both *V. aurea* (p*au*CiPCR5 and p*au*CiPCR26) and for *P. undulata* (p*un*ThPCR1 and p*un*ThPCR17) with a 200 bp monomer. Two PCR recombinant clones of *T. philippinarum* were also obtained for comparison (p*ph*ScPCR10 and p*ph*ScPCR11). Southern blot hybridization with the DIG-labeled p*ph*Sc11 clone (not shown) demonstrated the homology of the obtained sequences to the *phBgl*II400 family.

Alignment of sequenced 200 bp stretches evidenced a high level of similarity among clones (0.990-0.995%): only 13 point mutations were observed (Table 4) and "Kimura's two parameters" distances ranged from 0.000 to 0.042 (Table 5). Chromosome analysis of *T. philippinarum* specimens from Scardovari constantly showed a 2n = 38 metaphase complement, with mainly meta- and submetacentric chromosomes. Moreover, silver staining technique invariably revealed a homozygous condition of active nucleolar organizing regions (NORs) on long arms of pair 17. *In situ* hybridization of *T. philippinarum* metaphases evidenced a mainly pericentromeric localization of the *phBgl*II400 family on most chromosomes (Fig. 2). However, the rather homogeneous morphology of *T. philippinarum* chromosomes and their strong contraction in C-metaphases did not allow a very clear karyotype definition from FISH labeled sets. Giemsa and NOR-stained metaphases are available upon request.

DISCUSSION

Previous allozyme analyses have evidenced high levels of genetic divergence between *T. philippinarum* and *T. decussatus*; *T. philippinarum* is genetically more similar to *P. undulata* and *V. aurea* than to *T. decussatus* (Passamonti *et al.*, 1997). Analyses of 16S rRNA sequences in mtDNA (Canapa *et al.*, 1996) appear to be in line with gene-enzyme findings.

Some features of the *phBgl*II400 family are informative. Southern blot fits within the above reported phyletic frame, since the *phBgl*II400 family appears to be shared by all the Tapetinae species presently analyzed with the only exception of *T. decussatus*. This reinforces the high level of differentia-

Table 4. Alignment of the PCR-obtained 200 bp sequences from the *phBgl*II400 DNA family of *T. philippinarum* from Scardovari (*ph*Sc), *V. aurea* from Civitanova Marche (*au*Ci) and *P. undulata* from Thailand (*un*Th). A consensus sequence, with underlined primers' annealing sites, is shown.

```
                    10        20        30        40        50        60        70        80        90       100
cons                AGATTCCGC ATGGCTTGGC CATTTTTTGC ACCGGCGATT TGTTCTTTCT CTATGTAAAT TTCGGGAAGA AAICATCATA TTCACGAGCC TACCATTCAA
pphScPCR1           ---------- ---------- ---------- ---------- ---------- ---------- ---------- --*------- ---------- ----------
pphScPCR12          ---------- ---------- ---------- ---------- ---------- ---------- ---------- --A------- ---------- ----------
pauCiPCR5           ---------- ---------- ---------- ---------- ---------- ---------- ---------- --*------- -C-------- ----------
pauCiPCR26          ---------- ---------- ---------- C--------- ---------- ---------- G--------- G-*------- ---------- ----------
punThPCR1           ---------- ---------- ---------- ---------- ---------- ---------- ---------- --*------- ---------- ----------
punThPCR17          ---------- ---------- ---------- ---------- ---------- ---------- ---------- --*------- ---------- ----------

                   110       120       130       140       150       160       170       180       190       200
cons                AAACCATTTG ATCAATTCCG TTGCAAATTT GATTTTGTAC AACTTGAGGG GGTCTTCTTT GAACATGAAA AGTCTTAAAG CCCTAACCCA AGGAACAGTC
pphScPCR1           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pphScPCR12          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pauCiPCR5           ---------- ---------- ---------- ---------- *--------- ---------- C--------- ---------- ---------- ----------
pauCiPCR26          ---------- ---------- ---------- ---------- TT-------- ---------- C--------- T--------- C--------- ----------
punThPCR1           ---------- ---------- G--------- ---------- ---------- C--------- C--------- TC-------- G--------- ----------
punThPCR17          ---------- ---------- ---------- ---------- ---------- AG-------- C--------- T--------- ---------- ----------
```

Table 5. "Kimura two parameters" genetic distances between PCR-obtained 200 bp sequences of the *phBgl*II400 family in three Tapetinae species (*T. philippinarum*, *ph*; *V. aurea*, *au*; *P. undulata*, *un*).

	p*ph*Sc PCR1	p*ph*Sc PCR12	p*au*Ci PCR5	p*au*Ci PCR26	p*un*Th PCR1	p*un*Th PCR17
p*ph*ScPCR1	–					
p*ph*ScPCR12	0.000	–				
p*au*CiPCR5	0.010	0.010	–			
p*au*CiPCR26	0.036	0.036	0.036	–		
p*un*ThPCR1	0.031	0.031	0.030	0.042	–	
p*un*ThPCR17	0.026	0.026	0.025	0.042	0.031	–

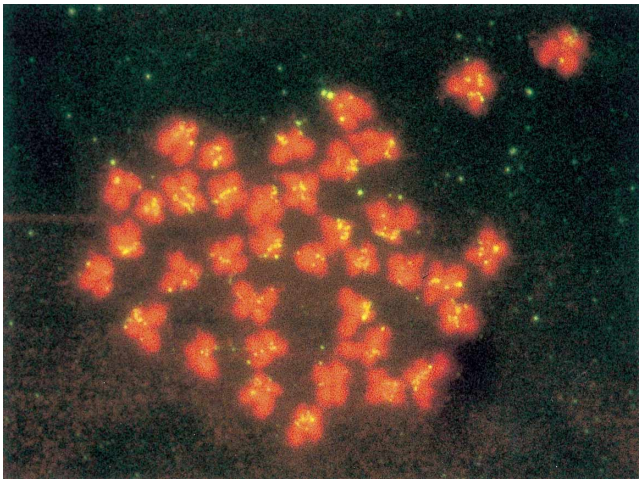

Fig. 2. FISH labeled *in situ* hybridization of a *T. philippinarum* metaphase with a digoxigenin-labeled monomer obtained from the p*ph*Sc11 recombinant clone.

tion of *T. decussatus* from the other Tapetinae taxa, which form a sound cluster. As a consequence, these data sets suggest that the two *Tapes* species could be separated to different genera.

While *V. aurea* is found only in the Mediterranean basin, *T. philippinarum* and *P. undulata* are Indo-Pacific clams in origin, their evolutionary divergence dating back, at least, to the closure of Tethys Ocean. Even if at present *T. philippinarum* and *V. aurea* have become artificially sympatric, the hypothesis of a recent "horizontal" transfer of these sequences appears very unlike, since neither natural hybridization has been observed, nor artificial hybrids have been obtained. The assumption seems to be inconsistent with the observation that intra-specific sequence divergence in the 405 bp monomers of *T. philippinarum* is somewhat higher than the inter-specific one, as obtained from the PCR sequences. It is to be noted, however, that this comparison is heavily biased by the non-random working of the PCR-cloning procedure: likely, the observed distances are an outcome of the primer design performed on *T. philippinarum*. As a consequence, PCR amplified sequences of *V. aurea* and *P. undulata* would have artificially increased similarity to those of *T. philippinarum*.

Very little is known about satellite DNA in the Mollusca; in the Bivalvia, only available for *Mytilus edulis* (Ruiz-Lara *et al.*, 1992). No definite hypotheses on the function of these sequences can be put forward. The high inter- and intra-specific sequence similarity of the *phBgl*II400 family may be attributed to the homogenization phenomena of the "concerted evolution"; these proceed through genomic turnover mechanisms (such as unequal crossing over, slippage replication and rolling circle amplification) and bisexual reproduction (Dover, 1982, 1986, 1989; Charlesworth *et al.*, 1994). Even the existence of some sort of selective constraints, such as satellite DNA-centromeric protein (CENP) interactions or hypothetical ORF transcription, could play a role in the maintenance of a high similarity of the *phBgl*II400 monomeres.

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