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Molecular cloning and sequence analysis of cDNAs encoding for *Boophilus microplus, Haemaphysalis longicornis* and *Rhipicephalus appendiculatus* actins

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Abstract

The nucleotide and deduced amino acid sequences of the actins from ticks, *Boophilus microplus, Haemaphysalis longicornis* and *Rhipicephalus appendiculatus,* have been determined. Nucleotide sequence analysis showed open reading frames of 1128-nucleotide-long encoding proteins of 376 amino acids with a predicted molecular weight of 41.82 kDa each. Comparison between the nucleic acid and deduced amino acid sequences as well as structural and phylogenetic analyses of these genes confirmed the high similarity among actins from ticks in comparison to other species.

Keywords: Actin; Tick; *Boophilus microplus*; *Haemaphysalis longicornis*; *Rhipicephalus appendiculatus*

1. Introduction

Actin genes have been widely used as an important source of information for evolution and population studies in many different species. But, in spite of the evolutionary and economical importance of ticks and their extreme diversity, to date, very little is known

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about the structure and diversity of the actin genes in ticks. The GenBank database has only one full-length actin sequence of the soft tick *Ornithodoros moubata* (AY547732), so we focused our interest on the cloning and characterization of the actin cDNAs of the three species of economically important ticks.

In Asian and Oceania countries, *Haemaphysalis longicornis*, a tick commonly infesting cattle and dogs, is an important vector of *Theileria* and *Babesia*, which are responsible for economically important diseases (Minami et al., 1980). *Boophilus microplus* constitutes one of the major problems in cattle husbandry in America due to the direct effects tick parasitism have on weight gain of cattle and the transmission of *Babesia* spp. and *Anaplasma marginale* (Mendiola et al., 1996). *Rhipicephalus appendiculatus* is the most important vector of *Theileria parva,* the causative agent of East Coast fever, in Eastern, Central and Southern Africa (Estrada-Peña, 2001).

Actin and microtubules together make up a part of the cytoskeleton of cells. Actin filaments are mainly structural elements and provide the cell with its overall shape and allow it to form contacts with its substrate. Moreover, actin is involved in a variety of processes including cellular motility, intracellular transport, cytoplasmatic streaming and endocytosis (Van Troys et al., 1999). The actin cytoskeleton, along with myosin, also generates the forces used by cells to crawl through tissues in an intact animal.

Actin is highly conserved among species and is a member of a protein superfamily, which is believed to have evolved from a common ancestor (Flaherty et al., 1991; Bork et al., 1992). There are three main actin isotypes (alpha, beta and gamma) which show >90% amino-acid homology among isotypes and >98% homology within members of a particular isotypic group. The majority of the isotype heterogeneity is located in the amino-terminal. The four mammalian muscle actin genes are expressed in different types of muscle (skeletal, cardiac, smooth and enteric) and two cytoskeletal isoforms (β and γ) are found in all cells. In insects and other invertebrates, muscle and cytoplasmic actins are more similar to mammalian cytoplasmic actins than to mammalian muscle actins (Vandekerckhove and Weber, 1978). Arthropod muscle actin genes are believed to have emerged from an ancestral cytoplasmic actin gene within the arthropod phylum, whereas vertebrate muscle actin genes evolved within the chordate lineage (Mounier et al., 1992).

The amino-terminus of each actin monomer is localized on the periphery of the double-helix in F-actin (Holmes et al., 1990), and this site is also thought to interact with myosin (Rayment et al., 1993). Actin is a highly conserved protein found in probably all eukaryotic cells where it typically makes up 10% of the total cell protein content in muscles (Santos et al., 1997) and 1–5% of the total weight of non-muscle cells (Lodish et al., 2000). Characterization of the protein products of these genes revealed that they encode a family of 35–45 kDa proteins, and contain two putative nuclear export signals (NES) at residues 170–181 and 211–222 (Wada et al., 1998).

The nucleic acid sequence and molecular structure of actins have been determined in a large number of species and the studies about actins from protozoa, plants and vertebrates have shown that they were highly conserved along evolution due to severe functional constraints (Van Troys et al., 1999).

It is possible to infer molecular phylogenies, which are important to establish the relationships among different organisms, using the sequence of an actin gene or protein structure. In this case, regions of sequence variation may identify either sequences at which the constraints are smaller, or sequences that are important for the specific functions of the different isoforms.

In the present study, we cloned and compared actin cDNAs from *B. microplus*, *H. longicornis* and *R. appendiculatus*. We also inferred properties of the deduced protein domains involved in protein transport regulation.

2. Materials and methods

2.1. Isolation of the B. microplus actin

2.1.1. Animals

B. microplus ticks (Porto Alegre strain) were reared in bovines, which were brought from a tick-free area and housed in individual tick-proof pens on slatted floors. Eggs were obtained 1 and 20 days after the beginning of the oviposition, and 1–5, 5–10 and 15–20-day-old larvae were collected after egg hatching. Nineteen-day-old partially-engorged females and

males were collected directly from the cattle and fully-engorged females were collected after dropping from the cattle (Da Silva Vaz et al., 1994).

2.1.2. Tissue dissections

Dissections of ticks were performed under a light microscope. Ticks were submerged in phosphate buffered saline (PBS) and held down with a pair of soft tissue forceps. The salivary gland, ovary and midgut from *B. microplus* were separated from the rest of the tick. Tissues used in the preparation of RNA were rapidly removed, frozen in liquid N_2, and stored at −80 °C upon use. (Ferreira et al., 2002).

2.2. Cloning of actin cDNA of B. microplus

A 905-bp fragment from a conserved region of the *Echinococcus granulosus* actin (EgactI) (Da Silva et al., 1993), corresponding to the nucleotides 935–1840 (obtained by digestion with the restriction enzymes *Nco*I and *Bam*HI) was labeled with [α-^{32}P] dATP using random oligonucleotide primers.

The *E. granulosus* actin probe was used to screen 5×10^4 individual clones from *B. microplus* cDNA library (Renard et al., 2000) by filter hybridization on nitrocellulose. Hybridization was carried out overnight at 37 °C in hybridization buffer (6X SSC) containing 5% (w/v) cow non-fat dry milk, 200 μg/ml denatured salmon sperm DNA and DNA probe. Filters were then washed for 30 min each in 6X SSC at room temperature, followed by 2X SSC, 1X SSC, 0.5X SSC and 0.2X SSC at 65 °C.

Two clones out of five positive clones were isolated and excised into plasmid, and the inserts analyzed by digestion with restriction enzymes. Both clones were sequenced on an ABI-PRISM 377 automated DNA sequencer (Perkin-Elmer) at Molecular Genetics Facility, University of Georgia, Athens–Georgia, USA. One clone, named ActBm1, was then further characterized.

2.3. Isolation of actin cDNAs from H. longicornis and R. appendiculatus

2.3.1. Animals

H. longicornis and *R. appendiculatus* ticks were fed on Japanese White rabbits in an established laboratory colony. Partially-engorged females were collected after dropping from the rabbit. All unfed ticks were maintained at 27–28 °C and 90% relative humidity.

2.3.2. Tissue dissections

Dissections of ticks were performed as described above. The dorsal cuticle was cut out, and the salivary gland and midgut from *H. longicornis* were separated from the rest of the tick.

2.4. Cloning of actin cDNAs from H. longicornis and R. appendiculatus

The *H. longicornis* actin coding sequence was amplified from the cDNA library (Mulenga et al., 1999) and the *R. appendiculatus* actin coding sequence was amplified from cDNA synthesized using total RNA isolated from larvae tissues. Two primers were used based on the *B. microplus* actin gene: FOR-A (5′- ATGTGTGACGACGAGGTTGCCG-3′) which corresponds to the 5′ end, and REV-A (5′-TTAGAAGCACTTGCGGTGGACAATG-3′), which corresponds to the 3′ end. The amplified cDNA products were separated by agarose gel electrophoresis. Fragments around 800 bp, which are within the expected size for the actins molecular weights, were excised and purified by Glassmilk DNA purification kit (BIO 101 Systems). The actin cDNA was directly ligated into the pGEM-T vector, and transformed into DH5α strain of *Escherichia coli* (GIBCO-BRL Life Technologies). The recombinant plasmids containing the full-length cDNA were identified by inserted segment size and by PCR using the actin primers. The plasmids were purified and the nucleotide sequences of the inserts were determined on an 8-capillary Beckman CEQ2000 automated sequencer.

2.5. RNA isolation and RT-PCR of B. microplus actin

RNA was isolated from ticks at different developmental stages (1–7-day and 20-day old-eggs, 1–5-day and 20-day-old larvae, partially-engorged females, fully-engorged females and males) and from different tissues (ovary and salivary glands of fully- and partially-engorged females, respectively, and midgut of fully-engorged females). Total RNA and mRNA were isolated using the TRIzol reagent (Invitrogen) and Micro mRNA Purification Kit (Amersham-Pharmacia) respectively, as described by the manufacturers.

Five micrograms of total RNA or 500 ng of mRNA were submitted to reverse transcription (RT) before PCR. The RT reaction was performed at 42 °C for 60 min in the presence of oligo-dT (Amersham-Pharmacia) and SuperScript II (Gibco-BRL Life Technologies) according to the manufacturer's instructions.

Two oligonucleotides, the 5′ primer (5′-GCATC-CACGAGACCACG-3′), named primer 3, and the 3′ primer (5′-GGGGTGTAGAAGGAAGG-3′), named primer 1, were used to amplify a 339-bp region of Bmact1. The PCR was performed using 2 μl of the RT reaction, 10 pmol of each primer (3 and 1) and 2.5 U Taq polymerase, in a final volume of 50 μl. Samples were denatured for 5 min at 94 °C and amplification was achieved through 30 cycles of 30 s at 94 °C, 30 s at 54 °C and 30 s at 72 °C. Following the last cycle a final extension (72 °C, 10 min) was carried out. The actin PCR products were fractionated on 2% agarose gel electrophoresis and stained with ethidium bromide.

2.6. RNA isolation and RT-PCR of H. longicornis actin

RNA was isolated from ticks at different developmental stages (4 day-feeding larvae, 4 day-feeding nymph and 4 day-feeding female) and from different tissues (midgut, salivary glands and carcasses obtained by direct dissection of females). Total RNA was isolated using the TRIzol reagent (Invitrogen), as described by the manufacturer.

The first-strand cDNA was synthesized from 5 μg of total RNA. The reactions were performed at 42 °C for 60 min and at 72 °C for 15 min in the presence of oligo-dT (Hokkaido System Science, Japan) and RAV2 (Takara, Japan) according to the manufacturer's instructions. For PCR amplification two oligonucleotide primers were synthesized based on cDNA sequences of actin genes: 5′-TGTGACGACGAGG-TTGCCG-3′, which corresponds to nucleotides 48–67; and 5′-GAAGCACTTGCGGTGGACAATG-3′ complementary to the 3′-end. The PCR conditions were: 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 90 s, and then a final extension at 72 °C for 7 min. The actin PCR products were fractionated on 1.5% agarose gel electrophoresis and stained with ethidium bromide.

2.6.1. Genomic sequence

Genomic DNA from *H. longicornis* and *B. microplus* larvae were isolated with Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's protocol and stored at −20 °C.

The cloning of *H. longicornis* and *B. microplus* genomic actins was performed after PCR amplification using the primers FOR-A and REV-A (described above). The cycling reaction was carried out with an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 62 °C and 2 min at 72 °C. The final extension was performed at 72 °C for 7 min. The amplified DNA products were separated by agarose gel electrophoresis. Fragments were excised and purified by Glassmilk DNA purification kit (BIO 101 Systems) and cloned into pGEM-T vector as described above. The recombinant plasmids containing the DNA were identified by PCR and the nucleotide sequences of the inserts were determined as described above.

2.6.2. Sequence analyses

Analyses of nucleotide and deduced amino acid sequences were carried out using BioEdit version 5.0.6 software program (Hall, 1999). Alignment of the sequences was performed by the Clustal W program, and phylogenetic analyses using the Neighbor Joining Method were carried out by the Phylogeny Inference Package (PHYLIP) version 3.5c (Felsenstein, 1989) provided by the Biology WorkBench Web-based Tool (Subramaniam, 1998) and NJplot program (Perrière and Gouy, 1996). Bootstrap support was assessed using 1000 replicates.

The GenBank accession numbers are: *Bombyx mori* X05185 (actin 1), *Drosophila melanogaster* NM079076, *Drosophila virilis* AF358264 (cytoskeletal protein), *Litopenaeus vannamei* AF300705 (β-actin), *Manduca sexta* L13764, *Marsupenaeus japonicus* AB055975 and *Ornithodoros moubata* AY547732.

3. Results

The complete nucleotide sequences of actin coding sequences of *B. microplus*, *H. longicornis* and *R. appendiculatus* were determined and the sequence data reported herein have been submitted to GenBank under accession no. AY255624 for *B. microplus*

(ActBm1), AY254898 for *H. longicornis* (ActHl1) and AY254899 for *R. appendiculatus* (ActRa1). The actin ORFs identified have 1128 nucleotides and encode 376 amino acids (Fig. 1). Within the coding regions the identity is 98% between *B. microplus* and *R. appendiculatus*, 96% between *B. microplus* and *H. longicornis*, 96% between *H. longicornis* and *R. appendiculatus*. The deduced amino acid sequences of

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ActBm1   1   MCDDEVAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQ 50
ActHl1   1   .................................................. 50
ActRa1   1   .................................................. 50

ActBm1   51  KDSYVGDEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPE 100
ActHl1   51  .................................................. 100
ActRa1   51  .................................................. 100

ActBm1   101 EHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTT 150
ActHl1   101 .................................................. 150
ActRa1   101 .................................................. 150

ActBm1   151 GIVLDSGDGVSHTVPIYEGYALPHAILRLDLAGRDLTDYLMKILTERGYS 200
ActHl1   151 .................................................. 200
ActRa1   151 .................................................. 200

ActBm1   201 FTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVIT 250
ActHl1   201 .................................................. 250
ActRa1   201 .................................................. 250

ActBm1   251 IGNERFRCPEALFQPSFLGMESCGIHETTYNSIMKCDVDIRKDLYANTVL 300
ActHl1   251 N................................................. 300
ActRa1   251 ................P................................. 300

ActBm1   301 SGGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASL 350
ActHl1   301 .................................................. 350
ActRa1   301 .................................................. 350

ActBm1   351 STFQQMWISKQEYDESGPSIVHRKCF 376
ActHl1   351 ........................... 376
ActRa1   351 ........................... 376
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Fig. 1. The putative amino acid sequences of *Boophilus microplus* (ActBm1), *Haemaphysalis longicornis* (ActHl1), and *Rhipicephalus appendiculatus* (ActRa1) actins showing two nuclear export signals (NES-1, residues 170–181; NES-2, residues 211–222) (underlined), which contain four hydrophobic residues (in bold) (leucine, isoleucine or valine) with the characteristic spacing present in established NES sequences. Putative propeptide of two amino acids (Met1-Cys2) are shown double underline.

the three actins show only one difference between one another. Furthermore, the three actins have a predicted molecular mass of 41.82 kDa and a theoretical pI of 5.3 calculated by Compute pI/Mw (Bjellqvist et al., 1993).

When compared to the rabbit skeletal muscle actin, the three tick actins were shown to possess identical residues in positions that affect the binding to ATP (residues Lys214,Glu215, Gly303, Tyr307 and Lys337) and Ca^{+2} (residues Asp12, Lys19, Gln138 and Asp155). Also, all residues participating in the DNase I binding region (residues Arg40-Gly47, Lys62-Ile65, Thr204 and Glu208) can also be found in these tick actins. Furthermore, all residues required to form actin filaments (322–325, 286–289, 166–169, 375 and 110–112) are present in the tick actins (Fig. 1) (Kabsch and Vandekerckhove, 1992).

We have also found two nuclear export signals (NES) (NES-1, residues 170–181; NES-2, residues 211–222) (Fig. 1), which are leucine-rich domains necessary to the shuttle between nucleus and cytoplasm. It is noteworthy that these sites and several flanking residues are conserved in the predicted amino acid sequence of these tick actins, suggesting that these proteins are cytoplasmic. In addition, a probable propeptide of two amino acids (Met1-Cys2) (Gunning et al., 1983) is present at the N-terminus of the three tick actins (Fig. 1).

A search made against the nucleotide databases with BLAST (Altschul et al., 1997) and MView (Brown et al., 1998) revealed that the nucleotide acid sequence of tick actins have similarities ranging from 77 to 90% with actins of 46 other species and from 87 to 91% with actins of 7 other species of arthropods (data not shown). Subsequently, we investigated the evolutionary relationships between the actins isolated from ticks and actins from other arthropod species. We constructed a phylogenetic tree using the complete coding sequences of actins from these ticks and seven other arthropod species. The phylogenetic tree demonstrates that the *B. microplus* actin coding sequence is more closely related to the *R. appendiculatus* sequence than to the *H. longicornis* sequence. In addition, the tree illustrates that hard ticks can be classified into a separate cluster away from other arthropod species (100% bootstrap value). The *O. moubata* (a soft tick) actin sequence is away from the hard tick and insect clusters (Fig. 2).

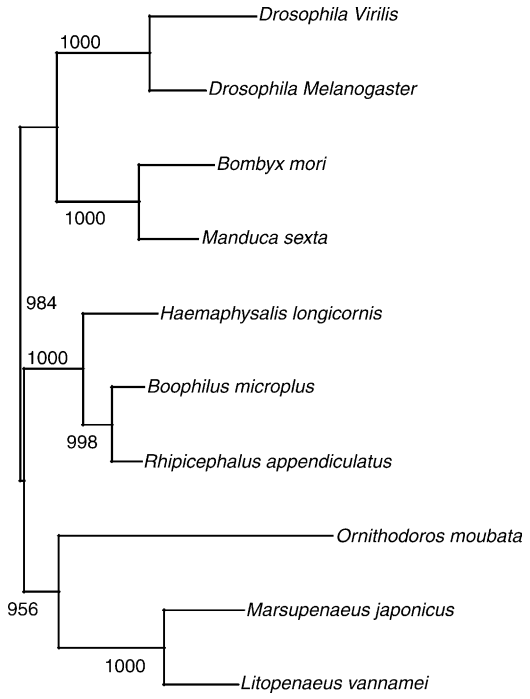

Fig. 2. Dendrogram based on actin coding sequences of *Boophilus microplus*, *Haemaphysalis longicornis*, *Rhipicephalus appendiculatus* and other arthropod species. GenBank accession numbers for the actin sequences are shown in the material and methods. Bootstrap values of 1000 simulations are shown at the branches.

In addition, we describe the exon–intron organization of the genes of ActBm1 and ActHl1 by comparative analysis of the amplification products generated by PCR, using either genomic DNA or cDNA clones as template. The *H. longicornis* actin gene is divided in three exons with 126, 487 and 518 pb and two introns with 1174 and 324 pb, and the *B. microplus* actin gene is divided in two exons with 126 and 1005 pb and one intron with 916 pb (Fig. 3).

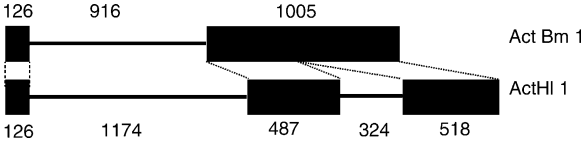

Fig. 3. Schematic diagram of the *Boophilus microplus* (ActBm1) and *Haemaphysalis longicornis* (ActHl1) actin genes. The filled boxes and lines represent coding sequences and introns, respectively. Numbers indicate the lengths of sequences.

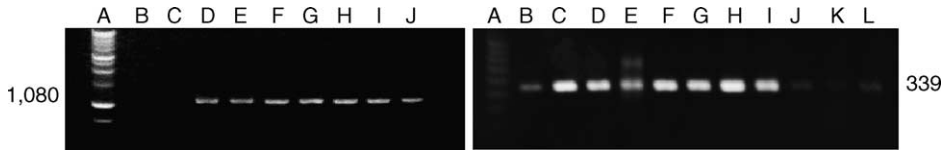

Fig. 4. RT-PCR amplification of tick actin cDNA fragments from RNA poli-A$^+$ of different tissues/developmental stages. (1) Amplicons of *Haemaphysalis longicornis* (a) 1-kb ladder; (b) negative PCR control; (c) negative RT control; (d) plasmid containing *H. longicornis* actin cDNA; (e) 4-day feeding larva; (f) 4-day feeding nymph; (g) 4-day feeding female; (h) 4-day feeding female salivary gland; (i) 4-day feeding female gut; (j) 4-day feeding female carcasses. (2) Amplicons of *Boophilus microplus* (a) 100-bp ladder; (b) egg (1–7-day-old); (c) egg (20-day-old); (d) larva (1–5-day-old); (e) larva (20-day-old); (f) partially-engorged female; (g) male; (h) fully-engorged female; (i) fully-engorged females ovary; (j) partially-engorged females salivary gland; (k) partially-engorged females gut; (l) fully-engorged females gut. Samples were submitted to electrophoresis on agarose gel electrophoresis and stained with ethidium bromide.

All identified intron–exon boundaries are consistent with the GT–AG-rule (Breathnach et al., 1978).

In order to determine the temporal and tissue expression patterns of the tick actin genes, RT-PCR was performed using *B. microplus* and *H. longicornis* mRNAs from different tissues and developmental stages. The results showed a single band in all tissues and developmental stages of both ticks, (Fig. 4), indicating that these genes are ubiquitously transcribed.

4. Discussion

This study reports on the identification and characterization of the *B. microplus*, *H. longicornis* and *R. appendiculatus* actin cDNAs, and provides the first putative full actin sequences of hard ticks. At the present time, no actin sequences of ticks have been made available to infer the actin evolution in this order or the relationships of the tick actins with those from other arthropods.

Analysis of nucleotide sequences and the deduced amino acid-sequences of the cloned actin cDNAs of *B. microplus*, *H. longicornis* and *R. appendiculatus* showed that they are closely related. Each of the tick actins differ only on one amino acid replacement in the mature protein, and in the three proteins this substitution results in conservative amino acid replacement (Fig. 1).

Two leucine-rich nuclear export signal-like sequences (NES-1 and NES-2) were identified in the middle part of the molecule (Fig. 1), both of which were shown to be functional and conserved in all actin isoforms (Wada et al., 1998). By analyzing the inferred tertiary structure of these actins it is possible to notice that the two sequences are in the external portion of the molecules and have hydrophobic characteristics that suggest a transporting function for the molecule (Wada et al., 1998).

In mammals, two main types of actins have been identified based on the primary structure and tissular localization: muscle actins with four isoforms, two in striated muscle (skeletal and cardiac actins), and cytoplasmic actins with two isoforms, beta and gamma actins, in non-muscle cells. The arthropod muscle actins, however, differ from the vertebrate muscle isoforms to such an extent that it seems likely that muscle actin genes have arisen independently in both chordate and arthropod phyla. This suggests that the muscle actins arose from cytoplasmic isoforms at least twice during animal evolution (Mounier et al., 1992). The urochordate muscle actins possibly represent a transition from a non-muscle-like sequence to a vertebrate muscle-like sequence. The invertebrate muscular actins are more related to vertebrate β-actins than to vertebrate α-actins. As shown by Mounier et al. (1992), insect muscle actins form a distinct family of closely related proteins characterized by the presence of about 10 muscle-specific residues. In insect actins, the muscle isoform-specific residues are Asp (3), Ile (76), Ala (232), Thr (234), Val (278), Ile (325) and Gly (368). In addition, Ala (5) is an adult muscle-specific residue, and Met (299) a larval-and-adult muscle-specific residue. Although the consensus positions for insect actins may not be the same in acari, it is indicative that *B. microplus*, *H. longicornis* and *R. appendiculatus* actins are cytoplasmic actins, as positions 278, 325 and 368 do not match the consensus residues. Indeed, the actin genes were shown by RT-PCR to be

ubiquitously expressed in all tissues and developmental stages examined including 1-day-old eggs devoid of any kind of musculature, which is also consistent with a cytoplasmic localization (Fig. 4).

When alignments were made between the *B. microplus*, *H. longicornis* and *R. appendiculatus* coding sequences, identities in the range of 96–98% were found between one another. The deduced nucleotide sequences were also compared with known actin sequences from 47 species, and a high degree (89.6%) of identity was observed (data not shown).

Actin contains a small and a large domain, ATP and Ca^{+2} reside in the cleft between these two domains (Kabsch and Vandekerckhove, 1992). In agreement with the high level of sequence conservation, tick actins present identical residues in positions that affect the binding of ATP and Ca^{+2} (Fig. 1). Also, all residues participating in the DNase I binding region, which can be found in almost all actins (Kabsch and Vandekerckhove, 1992), can also be found in these tick actins (Fig. 1), which suggests that these actins may also interact with DNase I. Furthermore, the comparison of a theoretical 3D-structure of tick actins with a 3D-structure of rabbit skeletal muscle actin (Schwede et al., 2003) reveals the conservation of the cleft between the small and large domains, indicating, that the Ca^{+2} and ATP-binding region is present in tick actins (data not shown).

The actin coding sequences of these ticks and seven other species of arthropods were used to perform a phylogenetic analysis. The amino acid sequences were not used because of their extremely low divergence. The phylogenetic analysis of actins indicated that hard tick actins form a distinct cluster from other arthropod actins (Fig. 2). When the partial sequence of *Ixodes scapularis* actin (GenBank accession number AY547732) was analyzed together with the sequences studied, it clustered within the same subgroup of tick actins (data not shown), which is in accordance with the classic taxonomy system. Interestingly, the tick actins characterized in this work (putative cytoplasmic isoforms) clustered together, while the *O. moubata* actin sequence clustered with muscular isoforms of arthropods, indicating that this sequence may represent a different tick actin isoform.

The phylogenetic analysis attempts to demonstrate the evolutionary relationships between hard tick actins. During the last years, molecular phylogenetics has significantly redesigned our views on species definition and evolution, and actin genes are useful tools for phylogenetic analyses since actins are highly conserved among different organisms. However, there are difficulties in constructing phylogeny relationships in many large groups of organisms due to insufficient data available from many orders and families. Under these circumstances our analysis fills a gap, providing the first report on the description of structure and sequence characterization of hard tick actins.

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