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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