Soil and freshwater rhabditid nematodes (Nematoda, Rhabditida) from Iran: A compendium

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Ebrahim Shokoohi and Joaquín Abolafia

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Contents

Summary

Iran is a vast country located in the Middle East with different climates, which make the country rich in terms of animal biodiversity. Nematological research inside the country has a high importance. This study presents valuable things of research on free-living bacterivorous nematodes that are present in terrestrial and aquatic ecosystems in Iran which belong to the order Rhabditida. A total of 88 free-living bacterivorous species belonging to 39 genera and 15 families are included. To date, the richest genera with regards to species numbers are *Panagrolaimus* (9 species), *Chiloplacus* (7 species) and *Acrobeles* (7 species). The most distributed genus is *Panagrolaimus*, isolated from different habitats. The current taxonomic status, description, distribution and illustrations are given for all species.

Keywords: Iran, monograph, nematode, Rhabditida.

Resumen

Irán es un gran país situado en Oriente Medio, el cual presenta diferentes climas que hacen que el país sea rico en términos de biodiversidad animal. La investigación en Nematología dentro del país tiene gran importancia. El presente trabajo es el resultado del estudio de los nematodos de vida libre bacteriófagos en Irán, lo cuales pertenecen al orden Rhabditida. Un total de 88 especies, pertenecientes a 39 géneros y 15 familias son incluidas. De todos ellos, los géneros más abundantes en número de especies son Panagrolaimus (9 especies), Chiloplacus (7 especies) y Acrobeles (7 especies), siendo Panagrolaimus el género más ampliamente distribuido, apareciendo en un mayor número de hábitats diferentes. Descripción, distribución geográfica, biología e ilustraciones se aportan para todas las especies.

Palabras clave: *Irán, monografía, nematodos, rabdítidos.*

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The first author: first and totemost, I praise God, the almighty for providing me this opportunity and granting me the capability to proceed in compiling this book successfully. This book owes its existence to the help, support and inspiration of several people.

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This book is dedicated to my lovely parents:
Mr. Mohammadrasoul Shokoohi and Mrs. Sarvar Ourang
for their endless love, support and encouragement.

Preface

The primary purpose of this book is to release data concerning the free-living bacterivorous nematodes from Iran. In addition, this book aims to improve the existing knowledge on rhabditid nematodes, especially for nematologists interested in this field inside and outside the country. We believe this compilation will help to unify research efforts on this major group of free-living nematodes. Obviously this book is not an in-depth coverage of the subject; we have only tried to give the taxonomic aspects, based on the literature and our own research experience, and have presented all species of the order Rhabditida described from Iran to date. Considerable emphasis has been placed on the description of the species. Numerous illustrations from the original descriptions have been reproduced and redrawn, while characters considered useful for species identification are emphasized in the text. In addition, we have accentuated the importance of using all available information to enable accurate identification. This includes morphological data and illustrations from original descriptions. Furthermore, keys for Iranian representatives of the included nematode group are presented. We believe the book can be helpful to scientists who are interested in members of the Rhabditida, occurring all over the world. Thanks are due to the nematologists and other scientists who have contributed directly and indirectly to the information presented.

E. Shokoohi
J. Abolafia

I. Introduction

What is a rhabditid nematode?

Members of the order Rhabditida are free living and microbivorous and mainly feed on bacteria. Some rhabditids are also parasites of animals. The majority of rhabditid nematodes exist in the soil; however, they have been found as parasites in the human body, compost, animal dung, etc. (Andrássy, 2005). The main characters used to distinguish rhabditid nematodes from other nematodes are their tubular stoma, usually having a tripartite pharynx with a distinct valvular apparatus present in the basal bulb (rarely not tripartite pharynx or lacking valves at the basal bulb) and a tail lacking a spinneret.

Importance of free-living rhabditid nematodes

The effect on the mineralization of soil resulting from the utilization of ingested food is performed directly by free-living nematodes. Free-living nematodes can mineralize up to 10% of the soil compared to Protozoa, which is capable of mineralizing 40% (Gaugler and Bilgrami, 2004). Free-living nematodes indirectly alter the soil microbial population by injecting new substrates containing microorganisms (e.g. bacteria and fungi), which are deposited into the rhizosphere (Gaugler and Bilgrami, 2004). Ingham *et al.* (1985) showed that environments with high densities of *Pelodera* and *Acrobeloides* were also associated with high bacterial density. As a result, more nitrogen is available in the soil for plant uptake due to high mineralization rates. Furthermore, ammonia (NH4+) is excreted by free-living nematodes, which can increase soil fertility and thus promote plant growth. Findings also suggest that *Caenorhabditis elegans*, a well-studied bacterivorous species, is associated with higher numbers of activated bacteria in soil environments than fungivores (Gaugler and Bilgrami, 2004). Free-living nematodes also play an important role in the soil food web (Majdi and Traunspurger, 2015).

Caenorhabditis elegans was the first multicellular eukaryote to have its genome completely sequenced; totaling 99.75 Mb of sequence data and approximately 20512 predicted protein-coding genes (Spieth *et al.*, 2014). Fierst *et al.*, (2015) revealed the total gene number in *Caenorhabditis* between 20964 for *C. elegans* and 34696 for *C. sinica*. A number of physical and database resources have been developed by the *C. elegans* community to facilitate large-scale studies of gene expression and function. These tools are also invaluable for gaining insight into the function of gene homologues from plant-parasitic nematodes (Costa *et al.*, 2007).

Caenorhabditis elegans has been isolated in mushroom substrate in Bulgaria, Hungry, France, United Kingdom and USA. Some bacteria such as *Bacillus cereus* and *Enterobacter amnigenus*, which are symbiotic of *C. elegans*, prevent *Agaricus bisporus* mycelia (Nagesh and Parvatha Reddy, 2000). *Diploscapter* spp. can transmit the plant-bacterial pathogens during the harvest of vegetables and fruits (Gibs *et al.*, 2005). *Pelodera strongyloides* is very important in terms of health and cause skin diseases in dogs, sheep, cows, guinea pigs and humans (Yeruham and Perl, 2005). *Halicephalobus gingivalis* can cause brain, heart, kidney, mouth, nose, spinal cord, adrenal glands, stomach, liver and bone disease in horses. Some cases have also been reported in humans (Kinde *et al.*, 2000).

Two genera of the rhabditid group, viz. *Steinernema* and *Heterorhabditis*, have a global distribution and are used as a biological agent for pest management. Worldwide, over 16 species of *Heterorhabditis* and 60 species of *Steinernema* have been reported (Nguyen and Hunt, 2007). The different species of entomopathogenic nematodes (EPNs) vary in the range of insects they attack, environmental needs, and stability in commercial products (Gaugler, 1999). Some species of EPNs may also control a particular pest more effectively than others. Therefore, these nematodes are important and appropriately used for biological control.

II. Historical outline

The first genus of rhabditid nematodes was described as *Rhabditis* by Dujardin (1845). The afore mentioned author described four species and introduced *R. terricola* as a type species. Subsequently, Schultze (1857) described *Diplogaster micans* and Bastian (1865) four new *Rhabditis* species, as well as new *Diplogaster* and *Cephalobus* species. De Man (1876, 1880, 1884) described 37 *Rhabditis* species and the genus *Teratocephalus*.

Örley (1880) included the genera *Anguillula*, *Cephalobus*, *Rhabditis* and *Teratocephalus* in the family Rhabditidae. Maupas (1900, 1915, 1916, 1919) added new species to this family and studied its biology and reproductive behavior. In 1922, Micoletzky subdivided Rhabditidae into four subfamilies viz. Cylindrolaiminae, Plectinae, Rhabditinae and Bunonematinae (Micoletzky, 1922). However, at that time the subfamily Rhabditinae included the genera *Rhabditis*, *Diploscapter*, *Cephalobus*, *Chambersiella*, *Teratocephalus,* and *Rhodolaimus* without any proven relationship. Some years later, Chitwood (1933) proposed the order Rhabditida, while Filipjev (1934) and Chitwood and Chitwood (1934) created the family Cephalobidae with two subfamilies, viz. Cephalobinae and Dubayliinae.

Based on the rhabdia structure (associated with the stoma), Thorne (1937) proposed three Cephalobidae subfamilies, viz. Chambersiellinae, Panagrolaiminae and Acrobelinae. Subsequently, Goodey (1951) subdivided the family Rhabditidae into three subfamilies including Rhabditinae, Diploscapterinae and Bunonematinae. Osche (1952) took into account the phylogeny of the order Rhabditida and divided *Rhabditis* into seven genera, viz. *Rhabditis*, *Choriorhabditis*, *Telorhabditis*, *Caenorhabditis*, *Mesorhabditis*, *Teratorhabditis* and *Protorhabditis*. In 1958, Andrássy placed Teratocephalidae under the suborder Cephalobina (Andrássy, 1958).

Years later, Thorne (1961) subdivided Rhabditidae into five subfamilies (Rhabditinae, Protorhabditinae, Poikilolaiminae, Diploscapterinae and Bunonematinae) and Cephalobidae into two subfamilies (Chambersiellinae and Turbatricinae). Paramonov (1962, 1964) proposed two superfamilies (Rhabditoidea and Cephaloboidea) and placed Cephalobidae, Panagrolaimidae and

Teratocephalidae under the superfamily Cephaloboidea, while the superfamily Rhabditoidea included Carabonematidae, Neoaplectanidae, Rhabdiasidae, Rhabditidae, Bunonematidae and Strongylidae. Later, Goodey (1963) elevated Brevibuccinae to family level (Brevibuccidae) and added Protorhabditinae and Allionematinae to the family Rhabditidae, and Pterigorhabditinae to the family Bunonematidae. He also elevated the family Teratocephalidae to order level.

Sudhaus performed extensive that has been comprehensive to date work (Sudhaus, 1974a; 1974b; 1974c; 1976a; 1976b; 1977; 1978; 1980) on the family Rhabditidae and specifically studied its biology, ecology, morphology, and phylogeny (2001, 2011). Although Andrássy (1978) placed Teratocephalina and Diplogasterina in the order Rhabditida, Maggenti (1981) re-classified Diplogasterina as an order. Andrássy (1984) studied the order Rhabditida and proposed four suborders, viz. Diplogasterina, Rhabditina, Cephalobina and Teratocephalina. However, Sudhaus and Fürst von Lieven (2003) placed all diplogasterid nematodes under the family Diplogasteridae. Malakhov (1994) divided this order into three suborders including Rhabditina, Cephalobina and Drilonematina. Lorenzen (1994) studied the order Rhabditida and proposed the superfamilies Rhabditoidea (Cephalobidae, Myolaimidae, Panagrolaimidae, Bunonenatidae and Rhabditidae) and Diplogasteroidea (Pseudodiplogasteroididae, Diplogasteridae). Furthermore, Andrássy (2005) presented valuable information on Rhabditida.

De Ley and Blaxter (2002) used molecular data (based on 18S rDNA) to substantiate a new (and current) taxonomic scheme of the order Rhabditida. The latter authors placed Teratocephalidae as *incertae sedis* in the order Rhabditida. This order now comprises three suborders, viz. Rhabditina, Myolaimina, Tylenchina and Spirurina. Finally, Andrássy (2005) and Abolafia *et al.* (2011) retained Teratocephalina as a valid suborder.

III. General morphology

Body size and shape.- Nematodes of the order Rhabditida are generally about 1-mm long, however, from 0.18 mm in *Protorhabditis hortulana* (Abolafia and Peña-Santiago, 2016) up to 12 mm in some representatives of the family Steinernematidae (De Ley, 1995). In general, in a transversal section their body shape is round or cylindrical. After relaxation, the body shape of females and males represent that of a "C" and "J", respectively.

Cuticle.- The cuticle of rhabditid nematodes comprises an epicuticle, cortical layer, median layer and basal layer (Fig. 1). However, considerable variation between different Rhabditida taxa also occurs. While *Zeldia punctata* has a punctate cuticle, *Acrobeles complexus* has a "double" cuticle. Similarly, the genera *Cuticularia*, *Cuticonema* and *Myolaimus* have double cuticles, but the outer cuticle layer is unstable. Furthermore, the cuticle of all taxa that belong to this order is annulated. In Heteropleuronematidae and Bunonematidae, warts are present in the side of the body that allow the nematode to escape a predator (De Ley, 1995). The lateral fields, which consist of lateral winds or alae separated by longitudinal incisures running along the side of the body, are easily observable

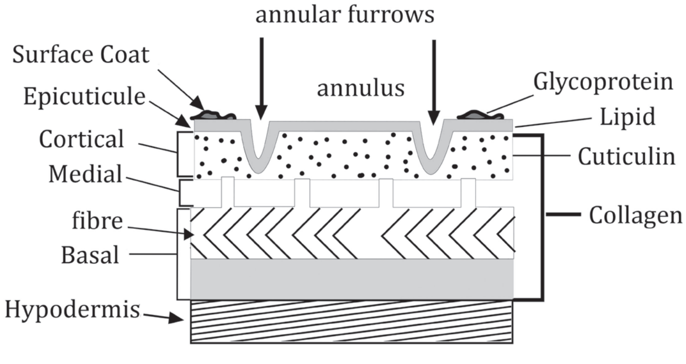

Figure 1. Structure of the cuticle of *Caenorhabditis elegans* (based on Page and Johnstone, 2007).

on the members of the Cephalobomorpha under a light microscope. However, this characteristic is hardly visible in other taxa, and inconspicuous within the members of Diplogasteromorpha (De Ley, 1995).

Anterior end.- The lips of most members of the order Rhabditida are six rounded and conspicuous (Fig. 2); however, some variations occur: expanded (Elaphonematidae), lobed or denticulate (Cephalobidae), amalgamated in pairs (Panagrolaimidae), offset from the adjacent part of body, or totally fused (Osstellidae).

Also, further adaptations of the lip region are associated with some members of the order Rhabditida. The genus *Ablechroiulus*, for example, has six brush-like structures surrounding the lip region, each of which comprises 2-6 hair-like structures (bristle). While the genus *Matthesonema* has eight similar structures, *Bicirronema* has a unique feather-like structure also surrounding the lip region. Although these structures can be used to identify and describe species, especially those classified under Bunonematomorpha, they remain poorly studied. Another example of variation is found in the genus *Diploscapter* (Figs. 7E & 62A) where modified membrane-like, fan-shaped lateral lips are present. *Diploscapter* is also characterized by the presence of two subdorsal and two subventral modified lip pairs forming cuticularized hook-like labial appendages (Andrássy, 1983).

Labial probolae.- The labial probolae (Fig. 3), always three in number, only exist in Cephalobomorpha (families Cephalobidae and Elaphonematidae).

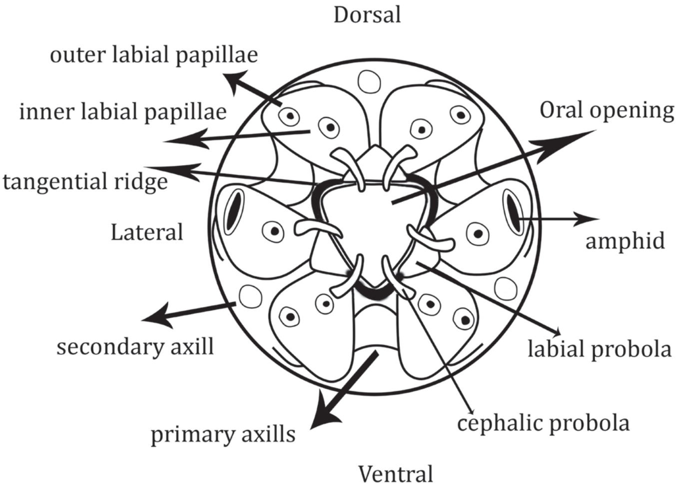

Figure 2. Structure of the lip region (based on Shokoohi and Abolafia, 2011).

Thorne (1925) was the first to report on this structure, while Rashid *et al.* (1989) studied it in more detail.

This structure serves the function of collecting bacteria (Xiao *et al.*, 2014) that represent the only food source of these families. Although the presence of labial probolae is a very important characteristic used for species identification, substantial differences also occur between genera: *Pseudacrobeles* and *Cephalobus* have reduced rounded labial probolae while *Acrobeles* has long bifurcated and dentate labial probolae (Fig. 4).

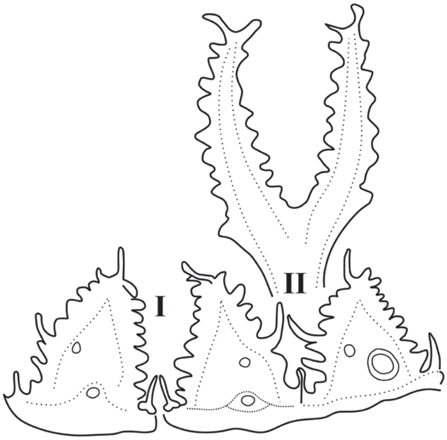

Figure 3. Lip region of *Acrobeles andalusicus*. I : Primary axils. II : Secondary axils (after Shokoohi *et al.*, 2007).

Stoma.- The stoma was studied for the first time by Steiner (1933), followed by Andrássy (1962), Goodey (1963) and Paramonov (1968). However, a more recent study by De Ley *et al.* (1995) provided the current standards for characterizing the different parts of the stoma (Fig. 5). De Ley *et al.* (1995) considered that the stoma comprises three parts, viz. cheilostom, gymnostom and stegostom. The cheilostom comprises the labial region of the cuticle, the gymnostom is naked and consists of epidermal tissue, and the stegostom is enveloped by the pharyngeal musculature. The latter structure comprises four sections, viz. prostegostom, mesostegostom, metastegostom and telostegostom (De Ley *et al.*, 1995).

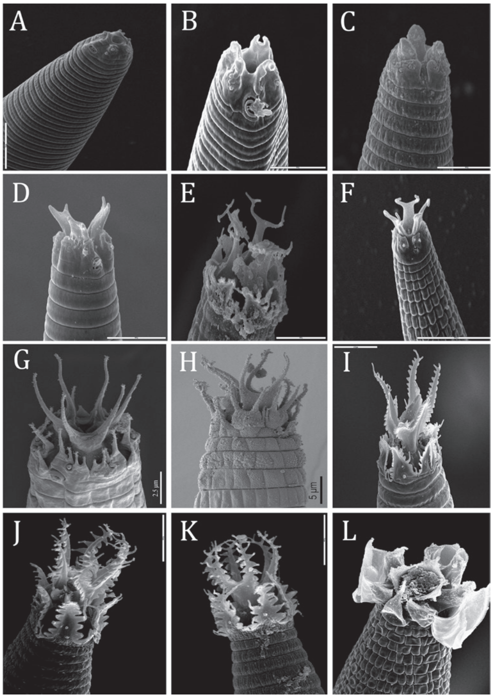

Figure 4. Scanning electron microscopy photographs of the lip region of Cephalobomorpha. A: *Pseudacrobeles iranicus*. B: *Eucephalobus mucronatus*. C: *Acrobeloides nanus*. D: *Chiloplacus bisexualis*. E: *Cervidellus hamatus*. F: *Stegelleta ophioglossa*. G: *Nothacrobeles abolafiai*. H: *Paracrobeles hebetocaudatus*. I: *Acrobeles andalusicus*. J: *Acrobeles iranicus*. K: *Acrobeles singulus*. L: *Acromoldavicus skrjabini*.

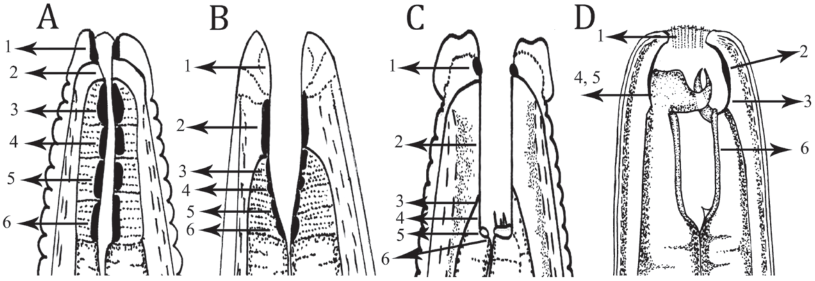

Figure 5. Morphology of the stoma. A: *Pseudacrobeles iranicus* (Cephalobomorpha). B: *Panagrolaimus rigidus* (Panagrolaimomorpha). C: *Cruznema tripartitum* (Rhabditomorpha). D: *Mononchoides adjunctus* (Diplogasteromorpha). 1: Cheilostom. 2: Gymnostom. 3-6: Stegostom (3: Prostegostom. 4: Mesostegostom. 5: Metastegostom. 6: Telostegostom).

Teratocephalina: Stoma is funnel shaped with visible rhabdia. No tooth available in the stoma. Cheilostom and gymnostom wide, stegostom narrow. Stegostom involved uniformly by the pharyngeal muscle (Andrássy, 2005; Hernández and Jordana, 1988).

Myolaimina: Stoma is roomy, tooth bearing and divided into two parts. The anterior part includes large cheilostom. The posterior part includes gymnostom and stegostom. Stegostom is involved uniformly by the pharyngeal muscle. A large tooth exists between the cheilostom and gymnostom. Two subventral teeth exist in the stegostom part. Stoma shifted slightly ventrally and anisotopic (Andrássy, 2005; Bärmann *et al.*, 2009).

Cephalobomorpha: Stoma tubular with short cheilostom with rounded, bar-shaped or comma-shaped strongly refringent rhabdia, very short gymnostom and long stegostom with small refringent rhabdia. The stegostom is involved uniformly by the pharyngeal muscle (Andrássy, 2005) (Fig. 6).

Panagrolaimomorpha: Stoma funnel shaped, with short cheilostom with poorly refringent rhabdia, long gymnostom with extremely developed and refringent rhabdia and short "V"-shaped stegostom with poorly refringent rhabdia and involved uniformly by the pharyngeal muscle. First two parts with wide lumen and stegostom narrow lumen. Metastegostom occasionally with denticle (Andrássy, 2005; Abolafia and Peña-Santiago, 2006).