

***Rhinoceronema unicornensis* gen. n., sp. n. (Cosmocercoidea: Atractidae) from Indian one horned Rhinoceros (*Rhinoceros unicornis* Linnaeus, 1758) captivated at the Alipore Zoological Garden, Kolkata, India**

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Abstract During survey of nematodes from the faecal samples of *Rhinoceros unicornis* Linnaeus, 1758 remaining captivated in the Alipore Zoological Garden, Kolkata, India 2,500 specimens were recovered in 3 years from March, 2007 to February, 2010. After processing the specimens were identified as a member of the family Atractidae (Railliet 1917) Travassos 1919 which have 22 valid genera. The present specimens differs from all other valid attractid genera in the features of cephalic region, cup-shaped buccal cavity with a pair of teeth, structure of the oesophagus and coarse transversely striated body. A new genus *Rhinoceronema* has been created to accommodate the present newly erected type species *R. unicornensis*. A Key to the genera of family Atractidae is added.

Keywords Nematode · Atractidae · *Rhinoceronema unicornensis* gen. n., sp. n. · Rhinoceros · Alipore Zoological Garden · West Bengal · India

Introduction

During the survey of gastrointestinal parasites of the Indian one horned rhinoceros (*Rhinoceros unicornis* Linnaeus, 1758), remaining captivated at the Alipore Zoological Garden, Kolkata, India by faecal examination, 2,500 nematode specimens were recovered within 3 years period

from March, 2007 to February, 2010. After careful processing and observation the specimens were identified and placed under the family Atractidae (Railliet, 1917) Travassos, 1919. The family Atractidae was previously placed in the superfamily Oxyuroidea but Chabaud and Petter (1960) placed it under the superfamily Cosmocercoidea Travassos, 1925 and their classification is now widely accepted. In 1978, Chabaud accepted 20 genera in the family Atractidae while, Adamson and Baccam (1988) listed 14 genera only and characterized the family having two types of ovary, monodelphic and didelphic. *Fitzsimmonsia* Petter, 1966, and *Probstmayria* Ransom, 1907 have didelphic ovary while, *Atractis* Dujardin, 1845; *Cobboldina* Leiper, 1911; *Crossocephalus* Railliet, 1909; *Cyrtosomum* Geddoelst, 1919; *Grassenema* Petter, 1959; *Labiduris* Schneider, 1866; *Leiperenia* Khalil, 1922; *Monhysterides* Baylis and Daubney, 1922; *Orientattractis* Petter, 1966; *Parattractis* Sarmiento, 1959; *Proattractis* Caballero, 1971; and *Rondonia* Travassos, 1919 have monodelphic ovary. Bursey et al., 2009 included the genus *Nouvelnema* Petter, 1959, in the didelphic attractid genera. Subsequently, *Pseudocyrtosomum* Gupta and Johri, 1987; *Buckleyattractis* Khalil and Gibbons, 1988; *Diceronema* Gibbons et al., 1996; *Klossinemella* Costa, 1961; *Podocnemattractis* Gibbons et al., 1995; *Paraorientattractis* Gibbons et al., 1997; *Rhinoclemmysnema* Gibbons and Platt, 2006 and *Pneumoatractis* Bursey et al., 2009 have been added to monodelphic group. Moravec and Thatcher (1997) has synonymized *Proattractis* Caballero, 1971 with *Klossinemella* Costa, 1961. The present specimens do not fit well with any of these valid monodelphic genera but fit well with the family Atractidae (Railliet, 1917) Travassos, 1919. Thus at present there are 22 valid genera and a new genus *Rhinoceronema* has been erected to accommodate the present new type species *R. unicornensis*.

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Materials and methods

Total 2,500 nematode specimens collected during the study period from the faeces of (*Rhinoceros unicornis* Linnaeus, 1758) were fixed in 4 % FA (formalin: glacial acetic acid, 4:1) and subsequently stored in 70 % alcohol. Most of the specimens after fixation were dehydrated slowly (Seinhorst, 1959, 1966; Mondal and Manna, 2010), and were mounted in anhydrous glycerin and sealed with paraffin wax. Specimens were observed in different magnification under Zeiss trinocular research microscope. Figures were drawn with the aid of Camera Lucida. Mature larvated female specimens and juvenile females with little ovarian development were recovered from the stool samples of rhinoceros for 3 consecutive years. Measurements of the mature females are only provided. All measurements are in micrometer (μm), unless otherwise stated. The

average measurements of 15 female specimens were taken and calculated \pm standard deviation. Range is mentioned in parenthesis.

Results

Rhinoceronema gen. n. (Figs. 1, 2)

Diagnosis: Nematoda, Cosmocercoidea, Atractidae. Worms are small, stout (Figs. 1A, 2A). Cuticle with coarse transverse striations; mouth with 6 prominent lips—one dorsal, one ventral, two subdorsal and two subventral in position, each lip is provided with a distinct papilla. Cephalic region with a pair of lateral amphids; buccal cavity small cup-shaped with a pair of teeth, varies from 2.31–7.5 in length and 2.7–7.5 in width (Figs. 1B, F, 2B, C, H). Oesophagus is

Fig. 1 Camera Lucida drawings of *Rhinoceronema unicornensis* gen. n., sp. n. **A** Whole body, **B**. Head, **C**. Gonad and vulval and anal region, **D**. Anterior region. **E**. Tail region, **F**. Cephalic region, *en face* view, **G**. Position of the excretory pore at the oesophageal-intestinal junction, **H**. Vulva and anal region

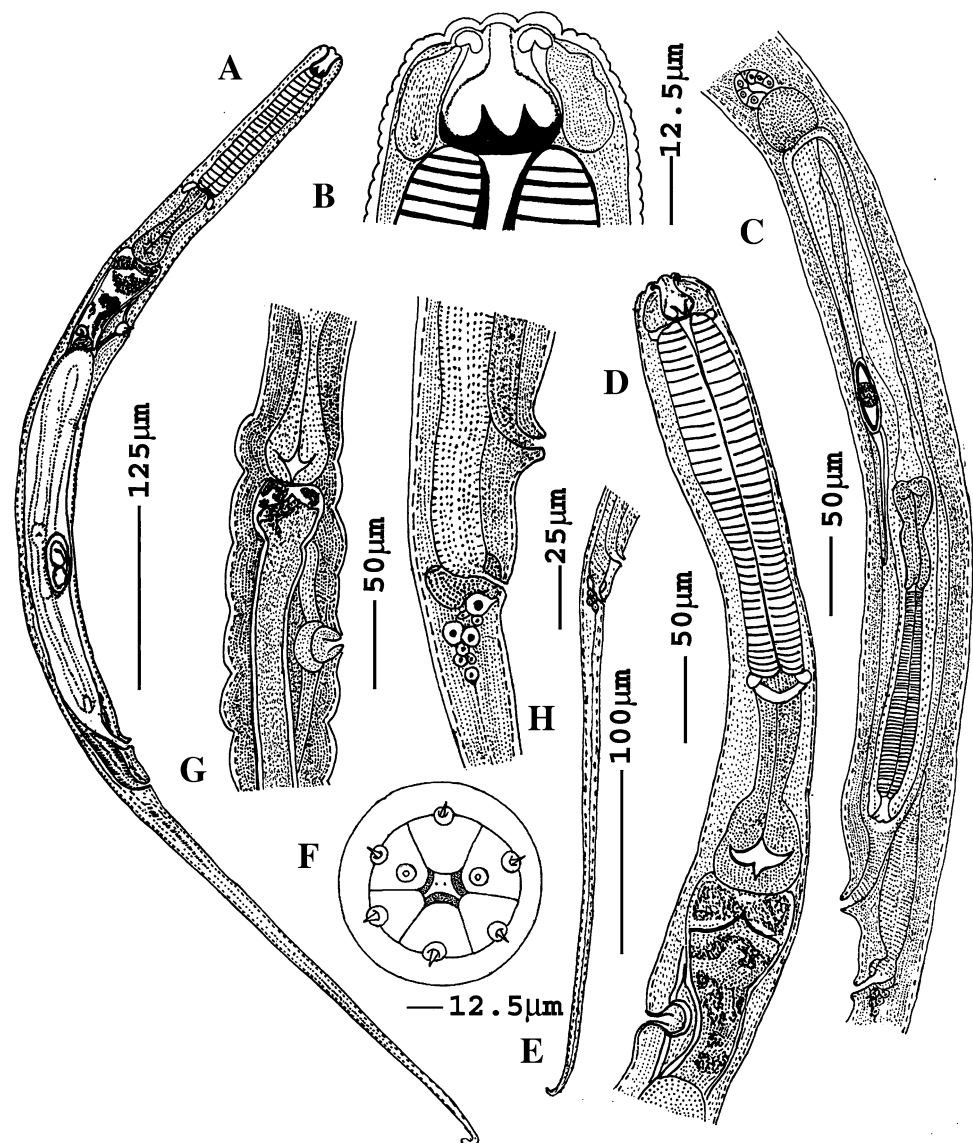
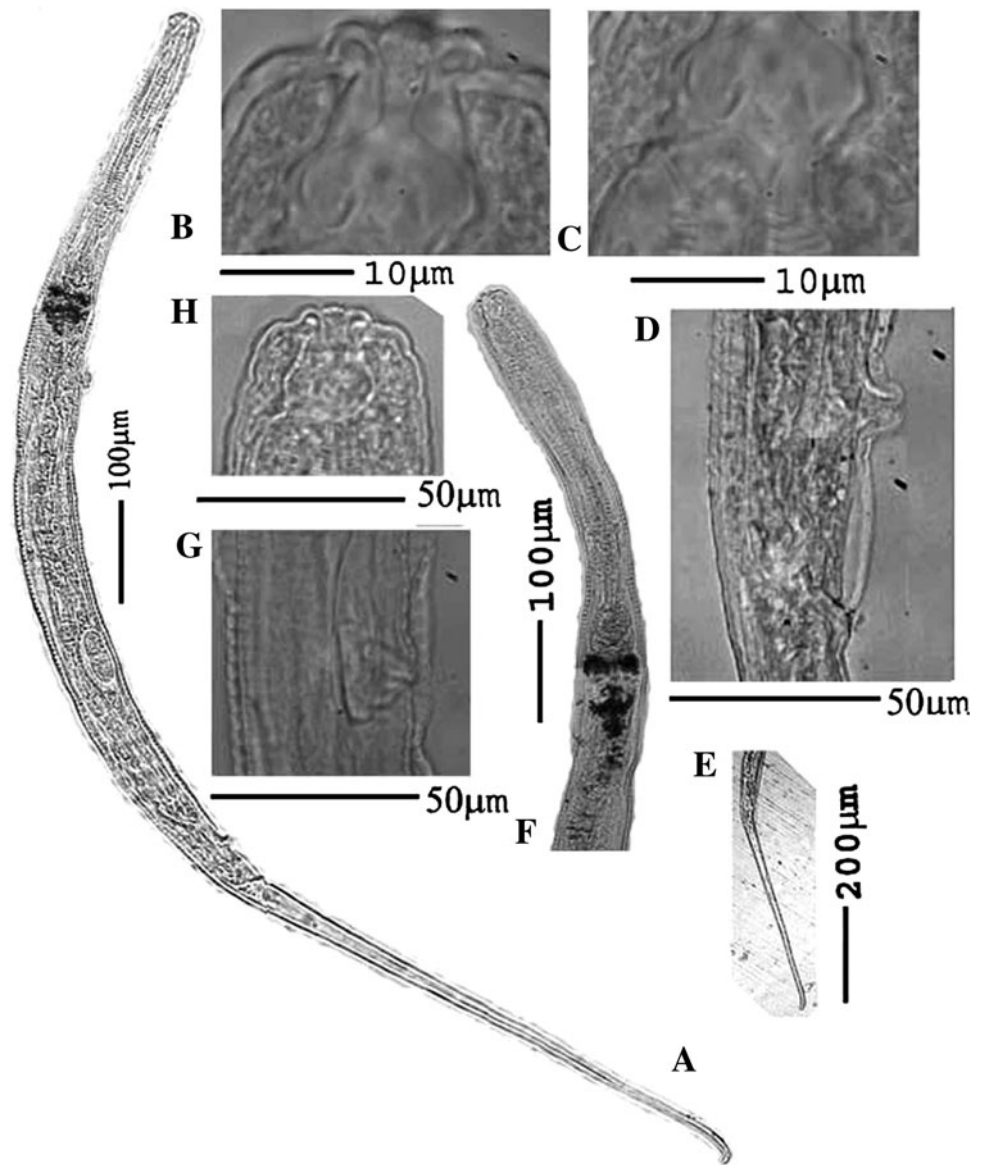


Fig. 2 Photomicrographs of *Rhinoceronema unicornensis* gen. n., sp. n. **A.** Whole body, **B.** Head region showing lips and buccal cavity, **C.** Buccal cavity showing a pair of teeth, **D.** Position of vulva and anus, **E.** Tail region, **F.** Anterior region, **G.** Excretory pore, **H.** Head region



clearly divided into corpus, isthmus and bulb, where the corpus is wider than isthmus, and the bulb is furnished with a vulvular apparatus (Figs. 1D, 2F). Excretory pore situated a little posterior to the oesophageal–intestinal junction (Figs. 1D, G, 2G). Females are monodelphic, viviparous and vulva remains close to the anus (Figs. 1C, E, H, 2D). Female tail long, filamentous that ends into a blunt tip (Figs. 1E, 2E). Male not known.

Rhinoceronema unicornensis gen. n., sp. n. (Figs. 1, 2)

Description: type species

General: Worms are whitish, stout delicate hair-like, within 2 mm in length (Figs. 1A, 2A). Female tail

filamentous, very long, $\sim >50\%$ of the entire body length and ends into a blunt tip (Figs. 1E, 2E).

Female (Holotype and 14 paratypes)

Body $1.54 \text{ mm} \pm 0.09$ (1.33–1.72) mm long and 68.75 ± 9.1 (50–87.5) wide; Head diameter 42.24 ± 3.3 (39.6–49.5); Length of the lips 6.01 ± 1.2 (4.25–7.5); Buccal cavity measuring 23.98 ± 3.8 (16.5–29.7) long and 17.42 ± 3.6 (13.2–23.1) thick; anterior oesophagus 228 ± 16.7 (188.1–247.5) long and 30.8 ± 5.2 (23.1–39.6) wide, posterior oesophagus 68.16 ± 5.2 (56.1–75.9) long and 17.82 ± 4 (13.2–23.1) wide, bulb 53.02 ± 4.7 (42.9–59.4) long and 43.34 ± 3.2 (36.3–46.2) wide. Nerve ring at 234.96 ± 16 (198–260.7) and excretory pore at 416.66 ± 28.5 (375–475),

both from anterior end; vulva post equatorial at 919.16 ± 56.8 (775–1037.5) and anus at 975.8 ± 58.5 (825–1,100) both from anterior end. Distance from vulva to anus 56.66 ± 6.45 (50–62.5). Gonad 500.83 ± 76.8 (325–587.5) long, monodelphic, uterus filled with more than two well developed larvae (1,000–1,300 long and 25–37.5 wide) and one or two eggs (66.0–100.0 long and 23.1–49.5 wide).; Tail filamentous, 569.23 ± 40.9 (512.5–612.5) long.

| | |
|-------------------------|---|
| Male | Not found |
| Deposition of Specimens | Holotype (♀) with two Paratypes in one slide; and 3 Paratypes (♀♀) in three separate slides, deposited at present to the nematode collection of Parasitology research unit, Department of Zoology, University of Calcutta, India Accession Nos. 000081 N/10; 000082 N/10; 000083 N/10; 000084 N/10; |
| Type Host | Indian one horned rhinoceros (<i>Rhinoceros unicornis</i> Linnaeus 1758) |
| Location | Unknown, recovered from faecal matter of the host |
| Type Locality | Alipore Zoological Garden, Kolkata, West Bengal, India |
| Duration of Collection | March, 2007 to February, 2010 |
| Type species | <i>Rhinoceronema unicornensis</i> gen. n., sp. n. |

Discussion

The oesophagus is clearly divided into, anterior and posterior part in the present specimen; the posterior part is with a distal bulb furnished with a vulvulus apparatus. Females viviparous, vulva situated in the posterior part of the body. So the present specimen is placed in the family Atractidae (Railliet, 1917) Travassos, 1919.

Due to the presence of noncosmocercid oesophagus, anterior cuticular structures, monodelphic reproductive system and absence of specialized anterior cuticular structures, the genera *Atractis*, *Cyrtosomum*, *Leiperenia*, *Monhysterides*, *Paratractis*, *Rondonia*, and *Pneumoatractis* come closer to the present specimen. The present genus differs from all other genera of the family Atractidae in

having a cup-shaped buccal capsule with a pair of sub-ventral teeth, non cosmocercid oesophagus, body with coarsely transverse striation restricted before the beginning of the tail. The present specimen has a smooth dorsal surface, while *Paratractis*, *Buckleyatractis*, and *Podocnematractis* have scale-like cuticular projections along the dorsal surface of the body (Khalil and Gibbons, 1988; Gibbons et al., 1995), and *Paraorientatractis* has semi-annules on the dorsal surface of the body (Gibbons et al., 1997). The present specimen has no cuticular formations in the cephalic region but *Cobboldina*, *Crossocephalus*, *Grassenema*, *Klossinemella*, and *Orientatractis* have specialized cuticular formations around the mouth (Chabaud, 1978). *Pseudocyrtosomum* is separated from the present specimen due to the position of the vulva and the structure of the oesophagus. Again, *Labiduris* is separated from the present specimen due to the presence of ventrally directed mouth (Chabaud, 1978). In *Leiperenia*, the anterior part of the oesophagus is one-fourth of total length (Chabaud, 1978), while in the present specimen, the anterior part is more than half of total length. *Pneumoatractis* have 3 prominent lips and cuticle with fine transverse striations while, the present specimen have 6 prominent lips and cuticle with coarse transverse striations. The present specimens have very prominent lips whereas *Monhysterides* lacks lips (Chabaud 1978). In the present specimen, the excretory pore is situated posterior to the esophageal bulb, whereas in *Rhinoclemmysnema* and *Cyrtosomum* the excretory pore is situated anterior to the esophageal bulb. In *Diceronema*, spines are restricted to the ventral surface of the female tail (Gibbons et al., 1996), whereas, the present specimen shows smooth ventral surface of the female tail. The present specimen have a cup-shaped buccal capsule with a pair of subventral teeth, while it is absent in the genus *Atractis*. The females of the present specimen have separate openings for vulva and anus, whereas the females of *Rondonia* possess a cloaca (Chabaud, 1978).

Lastly, the present specimen can further be separated from *Fitzsimmonsma* Petter, 1966 and *Probstmayria* Ransom, 1907 by the nature of reproductive system (monodelphic reproductive system vs. didelphic reproductive system in the last two).

Etymology: Considering all these differences the present genus is new to science and the authors suggest the name of the new genus as *Rhinoceronema* and the type species as *Rhinoceronema unicornensis* gen. n., sp. n. after the generic and species name of its host respectively, captivated at the Alipore Zoological Garden in Kolkata, West Bengal, India.

Key to the Genera of the Family Atractidae (Railliet, 1917) Travassos, 1919 following Chabaud (1978) (23 genera are included here including the present new genus) :

1. Didelphic 2
 Monodelphic 4
2. Oral opening elongated in lateral axis. Pharyngeal part of oesophagus forming small buccal cavity. Parasites of intestine of Hyracoidea.
 *Nouvelnema* Petter, 1959
 Oral opening not elongated . Pharyngeal part of oesophagus not forming buccal cavity. 3
3. Tail of male rather short. Parasites of reptiles..... *Fitzsimmonsma* Petter, 1966
 Tail of male terminated by long filament. Parasites of intestine of Equidae and Primates. *Probstmayria* Ransom, 1907
4. Parasites of mammals5
 Parasites of reptiles , fishes and amphibians. 9
5. Mouth simple. 6
 Mouth with cuticular formations 7
6. Mouth with two rudimentary lips, with cup- shaped buccal capsule. Parasites of Rhinoceros *Diceronema* Gibbons, *et al.*, 1996
 Mouth with 6 lips , cup- shaped buccal cavity with a pair of sub ventral teeth.
 *Rhinoceronema* gen. n.
 Mouth with more than 6 lips , without buccal capsule. Parasites of elephant.
 *Leiperenia* Khalil, 1922.
7. Pharynx without pectinated laminae. Parasites of intestine of Hyracoidea.
 *Grassinema*, Petter, 1959.
 Pharynx with pectinated laminae 8
8. Mouth with 3 pairs of pectinated laminae. Parasites of equides and Rhinoceros.
 *Crossocephalus* Railliet, 1909.
 Mouth with two large lateral pointed elevations and no lamina. Parasites of intestine of Hippopotamus*Cobboldina* Leiper, 1911.
9. Cuticle without spines. 10
 Cuticle with spines. 19

10. Vulva opening into rectum forming cloaca. *Rondonia* Travassos,, 1919.
 Vulva separate from rectum and anus. 11

11. Mouth without lips. Parasites of fish and reptiles.
 *Monhysterides* Baylis and Daubney, 1922.
 Mouth with 3 to 6 lips. 12

12. Oral opening apical in position. 13
 Oral opening ventrally displaced. Subventral lip with a fringe . Parasites of
 intestine of tortoise. *Labiduris* Schneider, 1866.

13. Mouth with cuticular formations 14
 Mouth without cuticular formations 15

14. Lips with small tongue-like cuticular expansions surrounding oral opening.
 Parasites of intestine of fish. *Klossinemella* Costa, 1961.
 Lips without small tongue-like cuticular expansions surrounding oral opening.
 Parasites of intestine of tortoise. *Orientatractis* Petter, 1966.

15. Mouth with three lips 16
 Mouth with four lips *Paraorientatractis* Gibbons *et al.*, 1997.
 Mouth with six lips 17

16. Vulva close to anus . Lung parasites of turtles
 *Pneumotractis* Bursey *et al.*, 2009
 Vulva pre or post-equatorial. Lung parasites of turtles.
 *Pseudocyrtosomum* Gupta & Johri, 1987.

17. Tail short. Parasites of turtle. *Cyrtosomum* Gedeolst, 1919.
 Tail long. 18

18. Buccal capsule present. Excretory-pore anterior to oesophageal bulb. Parasites
 of turtle *Rhinoclemmysnema* Gibbons and Platt, 2006.
 Buccal capsule absent. Excretory-pore posterior to oesophageal bulb. Parasites of
 Reptiles , amphibians and fishes. *Atractis* Dujardin, 1845.

19. Cuticle covered with many spines alternating in diagonal rows. 20
Only posterior dorsal surface of the body with or without spines. Parasites of
intestine of reptiles..... *Podocnematractis* Gibbons *et al.*, 1995.
20. Spines extend along entire length of body. in diagonal rows. Parasites of intestine
of turtle. *Paratractis* Sarmiento, 1959
Spines vary in arrangement and differing in shape and size. Parasites of intestine
of Reptiles *Buckleyatractis* Khalil and Gibbons, 1988.

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References

- Adamson ML, Baccam D (1988) Systematic revision of the Atractidae sensu Chabaud (1978) (Nematoda: Cosmocercoidea); *Maracaya belemensis* n.sp. and *Apletana albae* n. sp. from *Amphisbaena alba* in Brazil. Can J Zool 66:1857–1864
- Baylis HA, Daubney R (1922) Report on the parasitic nematodes in the collection of the Zoological Survey of India. Memoirs Indian Mus 7:263–347
- Bursey CR, Reavill D, Greiner E (2009) *Pneumotractis podocnemis* n. gen., n.sp. (Nematoda: Atractidae) from the yellow-spotted Amazon River Turtle, *Podocnemis unifilis* (Testudines: Pelomedusidae). Comp Parasitol 76:149–153
- Caballero GR (1971) Contribucion al conocimiento de lo nematodes que parasitan a los reptiles de Mexico. I. Descripcion de *Proatractis parvicapitronata* n.g., n. sp., parasite de Testudines. Rev Biol Trop 18:149–154
- Chabaud AG (1978) CIH Keys to the Nematode parasites of vertebrates. No. 6. Key to genera of the superfamilies Cosmocercoidea, Seuratoidea, *Heterakoidea* and *Subuluroidea* Commonwealth Agricultural Bureaux, Farnham Royal, Buckinghamshire, UK p 71
- Chabaud AG, Petter AJ (1960) *Sur les nematodes Atractides*. Libro Homenaje, E (ed) Caballero, pp 465–470
- Costa SCG (1961) Sobre a sistemática da familia cobboldinae Skrzabin, 1948 (Nematoda). Atas Sociedade de Biologia do Rio de Janeiro. 5:30–32
- Dujardin MF (1845) Histoire naturelle des helminthes or vers intestinaux. Paris, p 654
- Geddoelst L (1919) Un oxyuridé nouveau parasite d'un reptile. Comptes Rendus Society Biology Paris 82:910–913
- Gibbons LM, Platt TR (2006) *Rhinoclemmysnema* n. g. and three new species of nematodes of the family Atractidae (Cosmocercoidea), with notes on the helminth fauna of *Rhinoclemmys pulcherrima* (Testudines: Bataguridae) in Costa Rica. J Helminthol 80:333–340
- Gibbons LM, Khalil LF, Marinkelle CJ (1995) A new nematode genus, *Podocnematractis*, for *Atractis ortleppi* Thapar, 1925 (Cosmocercoidea, Atractidae) and the description of another new species, *P. colombianaensis* from turtles *Podocnemis* spp. in Colombia. Syst Parasitol 30:47–56
- Gibbons LM, Knapp SE, Krecsek RC (1996) *Diceronema versteriae* gen. n., sp. n. (Atractidae: Cosmocercoidea) from the black rhinoceros, *Diceros bicornis* in South Africa. J Helminthol Soc Wash 63:98–104
- Gibbons LM, Khalil LF, Marinkelle CJ (1997) *Paraorientatractis semiannulata* n. g., n. sp. (Cosmocercoidea: Atractidae) from the large intestine of the side-necked turtle, *Podocnemis unifilis* Troschel, 1848 (Testudines: Pelomedusidae) in Brazil. Mem Inst Oswaldo Cruz 92:359–364
- Gupta V, Johri S (1987) On a new genus *Pseudocyrtosomum* (Atractidae: Nematoda) from *Kachuga dhongoka* Gray from Lucknow, U.P. Indian J Helminthol xxxix: 83–87
- Khalil M (1922) A preliminary note on some new nematode parasites from the elephant. Ann Mag Nat History 9:212
- Khalil LF, Gibbons LM (1988) Two nematodes, *Paratractis hystrix* (Diesing, 1851) and *Buckleyatractis marinkelli* n. g., n. sp. (Atractidae: Cosmocercoidea) from *Podocnemis* spp. in Colombia. Syst Parasitol 12:187–198
- Leiper T (1911) Some new parasitic nematodes from tropical Africa. Proc Zool Soc Lond. 12:549–555
- Linnaeus C (1758) Systema Naturae per Regna Tria Naturae, secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis. Tomus I. *Laurentii Salvii, Holmiae (Stockholm)*, 1–823
- Mondal S, Manna B (2010) *Probstmayria bengalensis* n.sp. (Cosmocercoidea: Atractidae) in captive Indian one horned rhinoceros (*Rhinoceros unicornis*) from Alipore Zoological Garden, Calcutta, West Bengal, India. Proc Zool Soc Calcutta 63(2):129–134
- Moravec F, Thatcher VE (1997) New data on the morphology and systematic status of *Klossinella iheringi* (Nematoda: Atractidae) from an Amazonian serrasalmid fish. Folia Parasitol 44:48–54
- Petter AJ (1959) Deux nouveaux genres de nematodes Atractides, parasites du daman des rochers [*Procavia rufigera* (Ehrenberg)]. Bulletin de la Société Zoologique de France 84:195–204
- Petter AJ (1966) Équilibre des espèces dans les populations de nematodes parasites du colon des tortues terrestres. Mémoires du Muséum national d'Histoire naturelle 39:3–252
- Railliet A (1909) *Probstmayria vivipara*, nematode du cheval. Recueil de Médecine Vétérinaire. Paris, 86:336
- Railliet A (1917) L'oxyurose des équidés. Recueil de Médecine Vétérinaire 93:517–541
- Ransom BH (1907) *Probstmayria vivipara* (Probstmayr, 1865) Ransom, 1907, a nematode of horses heretofore unreported from the United States. Trans Am Microsc Soc 27:33–41

- Sarmiento L (1959) Description of *Paratractis hystrix* (Diesing, 1851) gen. nov. (Nematoda: Atractidae) from *Podocnemis dumeriliana*. J Parasitol 45:65–68
- Schneider A (1866) Monographie Der Nematoden. G. Reimer. Berlin, Germany, p 57
- Seinhorst JW (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4:67–69
- Seinhorst JW (1966) Killing nematodes for taxonomic study with hot f. a. 4:1. Nematologica. 12:175
- Travassos L (1919) Contributions á l'étude de la fauna helminthologique du Brasil. No. VIII. Les espèces brésiliennes du genera *Tetrameres* Crippling, 1846. Memorias do institute Oswaldo Cruz. 11, Portuguese text 71–79, French Text 63–71
- Travassos L (1925) Contribuição ao conhecimento da fauna helminthologica dos batráchios do Brasil. Nematódeos intestinais. Sciencia Medica 3:673–687