UNIVERSITI PUTRA MALAYSIA

DESCRIPTIVE MORPHOLOGY, MORPHOMETRY, COMMUNITY STRUCTURE AND SPATIAL DISTRIBUTION OF HELMINTH PARASITES IN THE MALAYAN BOX TURTLE, *CUORA AMBOINENSIS* (CHELONIA: BATAGURIDAE) FROM PENINSULAR MALAYSIA

REUBEN SUNIL KUMAR SHARMA

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By

REUBEN SUNIL KUMAR SHARMA

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Veterinary Science at the Faculty of Veterinary Medicine Universiti Putra Malaysia

May 2001
Dedicated to future chelonian parasitologists, who, unless science stagnates, will find themselves compelled to review my opinions.

“"We are as dwarfs mounted on the shoulders of giants, so we can see more and farther than they; yet not by virtue of the keenness of our eyesight, not through the tallness of our stature, but because we are raised and borne aloft upon that giant mass”

— John of Salisbury —
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Veterinary Science

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May 2001

Chairman: Associate Professor Dr. Rehana Abdullah Sani, DVM, Ph.D
Faculty: Veterinary Medicine

Thirty-six wild-caught C. amboinensis from Peninsular Malaysia, comprising adults and juveniles of both sexes, were examined for helminth endoparasites. A total of 11 species (5 nematodes and 6 trematodes) were recovered from various organs, namely, Serpinema octorugatus (small intestine), Falcaustra duyagi (large intestine), Falcaustra sp. 1 (large intestine), Oxyurid larvae sp. 1 (liver), Oxyurid larvae sp. 2 (large intestine), Atractidae sp. (large intestine), Parorientodiscus magnus (large intestine), Digenea sp. 1 (liver), Digenea sp. 2 (large intestine), Polystomoides malayi (urinary bladder), and Polystomoides asiaticus (oral cavity). Seven of these helminths were not previously recorded in C. amboinensis, while 4 species, namely Falcaustra sp. 1, Digenea spp. 1 and 2, and the Atractidae sp. may be new species and geographical records, and require further taxonomic workout.

The morphology and morphometry of 4 helminth species, namely, S. octorugatus, F. duyagi, P. magnus and P. malayi were studied in detail. A rigorous redescription of S. octorugatus was done with the aid of SEM and Pepsin-HCL
digestion. New information was obtained including the number and arrangement of
the buccal ridges, the absence of the lateral papillae, the addition of a fifth post anal
papillae and the complex structure of the spicule tip. This study also demonstrated
the usefulness of the relative positions of the caudal papillae and derived ratios, in
the characterisation of the genus *Serpinema*.

The helminth community structure of *C. amboinensis* in relation to age, gender
and lifestage categories was investigated for the first time. This chelonian had a rich
assemblage of helminths (observed richness = 11; expected richness, Chao2 = 9.17),
with high diversity (Shannon’s index = 0.78) and low evenness (0.36). This supports
previous claims that *C. amboinensis* is agile in food habits and habitat selection.
Two helminths, namely *S. octorugatus* and *F. duyagi* exhibited high intensity and
prevalence, and were designated as core and dominant species, while *Falcaustra* sp.
1 and Oxyurid sp. 1 were regarded as unsuccessful pioneers. The helminth
community structure between the various hosts age, gender and lifestage categories
were different, and may be attributed to disparate feeding habits and habitat
utilisation.

Results on the spatial distribution of the helminths found in the alimentary canal
of *C. amboinensis* indicate that the worms are site specific, with a relatively narrow
niche width. Further, interspecific competition may not be a prominent factor in
determining the habitat restriction of helminths in this turtle host. Significant and
positive correlation (Spearman’s rank coefficient = 0.45, P < 0.05) was found
between the intensity and prevalence of *S. octorugatus* and the packing density of the
mucosal folds in the cranial duodenum. These helminths may actively select this niche, as the close proximity of the mucosal folds arranged in a complex labyrinth provide structural support and added traction, which function to stabilise the attachment interface. The large intestinal nematode (*F. duyagi*), on the contrary, appeared to select its habitat based on physiological rather than morphological cues. It is likely that this luminal feeder is attracted to the prolonged retention of food material in the large intestine, and the presence of particulate digesta and abundant micro-organisms.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

DESCRIPTIVE MORPHOLOGY, MORPHOMETRY, COMMUNITY STRUCTURE, AND SPATIAL DISTRIBUTION OF HELMINTH PARASITES IN THE MALAYAN BOX TURTLE, \textit{CUORA AMBOINENSIS} (CHELONIA: BATAGURIDAE) FROM PENINSULAR MALAYSIA

Oleh

REUBEN SUNIL KUMAR SHARMA

Mei 2001

Pengerusi: Profesor Madya Dr. Rehana Abdullah Sani, DVM, Ph.D

Fakulti: Perubatan Veterinar

Tiga puluh enam ekor \textit{Cuora amboinensis} liar dari Semenanjung Malaysia, yang terdiri daripada dewasa dan juvana dari kedua-dua jantina, telah diperiksa untuk parasit helmin. Sejumlah 11 spesis (5 nematoda dan 6 trematoda) telah dijumpai dari pelbagai organ iaitu, \textit{Serpinema octorugatus} (usus kecil), \textit{Falcaustra duyagi} (usus besar), \textit{Falcaustra} sp. 1 (usus besar), \textit{Oxyurid larvae} sp. 1 (hati), \textit{Oxyurid larvae} sp. 2 (usus besar), \textit{Atractidae} sp. (usus besar), \textit{Parorientodiscus magnus} (usus besar), \textit{Digenea} sp. 1 (hati), \textit{Digenea} sp. 2 (usus besar), \textit{Polystomoides malayi} (pundi kencing), dan \textit{Polystomoides asiaticus} (mulut). Peringkat juvana \textit{P malayi} dan \textit{P. asiaticus}, masing-masing dijumpai didalam ginjal dan paru-paru. Tujuh daripada helmin yang jumpai, pada \textit{C. amboinensis} adalah rekod baru. Empat spesis lain, terutamanya, \textit{Falcaustra} sp. 1, \textit{Digenea} spp. 1 dan 2, serta spesis \textit{Atractidae} mungkin merupakan rekod lokasi dan spesis yang baru. Walau bagaimanapun, pemeriksaan taxonomi selanjutnya diperlukan sebelum helmin ini dapat diberi gelaran spesis baru.

Struktur komuniti helmin pada *C. amboinensis* menurut umur, jantina dan peringkat hidup telah dikaji buat pertama kalinya. Spesis kura-kura ini dijangkiti dengan *richness* helmin yang agak tinggi (Index Shannon’s = 0.78) dan *evenness* yang rendah (0.36). Dua spesis helmin (*S. octorugatus* dan *F. duyagi*) menunjuk intensiti dan prevalens yang tinggi, lalu dikenalpasti sebagai *core species*. Menurut *dominance ranking*, *S. octorugatus*, *F. duyagi* dan spesis Atractidae diberi designasi *dominant species*, manakala *Falcaustra* sp. 1 dan *Oxyurid* sp. 1 dikenalpasti sebagai *unsuccessful pomeer*. Perbezaan struktur komuniti helmin diantara jantina dan umur mungkin disebabkan oleh perbezaan makanan, keluasan kawasan merayau dan penggunaan habitat. Walau bagaimanapun, maklumat ekologi *C. amboinensis* diperlukan sebelum sebarang inferens konkrit dapat dikemukakan.
Keputusan penyebaran helmin di dalam salur pemakanan menunjukkan bahawa cacing ini menepat di kawasan-kawasan tertentu dan terhad dari segi keluasan habitat. Pertandingan diantara spesis mungkin tidak mustahak dalam menentukan habitat masing-masing. Intensiti dan peratus jangkitan didapati signifikan dan berkadar positif (Spearman’s rank coefficient = 0.45, P < 0.05) dengan kemampatan lipatan pada lapisan mukosa. Dicadangkan bahawa spesis helmin ini memilih kawasan duodenum secara aktif, kerana struktur labyrinth mukosa memberi sokongan untuk mencengkam Helmin usus besar (F. duryagi) tidak menunjukkan afiniti kepada perbezaan morfologi usus, malahan mungkin tertarik kepada makanan dan mikro-organisma yang terdapat di dalam segmen usus ini.
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I certify that an Examination Committee met on 18\textsuperscript{th} May 2001 to conduct the final examination of Reuben Sunil Kumar Sharma on his master of Veterinary Science thesis entitled "Descriptive Morphology, Morphometry, Community Structure, and Spatial Distribution of Helminth Parasites in \textit{Cuora amboinensis} (Chelonia: Bataguridae) from Peninsular Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and the Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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Date: 14 JUN 2001
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

Reuben Sunil Kumar Sharma

Date: 29-5-2001
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<td><em>Serpinema octorugatus</em> from the intestines of <em>Cuora amboinensis</em>. A) ventral and B) lateral view of male tail end</td>
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<td>3</td>
<td>Light micrographs of <em>Serpinema octorugatus</em> from the small intestine of <em>Cuora amboinensis</em>. A) lateral cephalic end of female B) dorsal cephalic end of female C) vulva region of female D) tail end of female ending in a tri pronged tip (mucrons) E) lateral view of male tail end F) ventral view of male tail end</td>
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<td>Scanning electron micrographs of <em>Serpinema octorugatus</em> from the small intestine of <em>Cuora amboinensis</em>. A) apical view showing the lateral pairs of peribuccal shields (bs); B) dorsal view of buccal opening C) dorsal view of cephalic region D) lateral view of cephalic region E) body with fine transverse striations F) vulva region of female showing the prominent anterior vulva flap (avf) and vulva opening (vu)</td>
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<td>Scanning electron micrographs of <em>Serpinema octorugatus</em> from the small intestine of <em>Cuora amboinensis</em>. A) dorso-ventral view of the inner surface of the buccal capsule showing the buccal ridges (br) and connecting cylinder (cc) B) close-up of the connecting cylinder showing the separation (arrow) from the buccal capsule proper. C, D, E, F) lateral views of the inner surface of the buccal capsule illustrating the variation in the arrangement and numbers of buccal ridges</td>
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<td>Scanning electron micrographs of <em>Serpinema octorugatus</em> from the small intestine of <em>Cuora amboinensis</em>. A) male tail end B) cloacal region of male C) tail tip of female D) male spicule lateral view E) close-up of the lateral aspect of the spicule F) dorsal view of the spicule</td>
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<td>7</td>
<td>Scanning electron micrographs of <em>Serpinema octorugatus</em> from the small intestine of <em>Cuora amboinensis</em>. A) anterior view of the buccal capsule B) dorsal view of the buccal capsule C) posterior view of the buccal capsule showing the basal ring (br) and basal opening of the buccal capsule D,E,F) lateral views of the buccal capsule illustrating the variation in architecture which may be attributed to age-related chitinization, basal ring (br), connecting cylinder (cc)</td>
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Falcaustra duyagi from the large intestine of Cuora amboinensis. A) anterior end B) lateral view of male tail end C) ventral view of male tail end

Light micrographs of Falcaustra duyagi from the large intestine of Cuora amboinensis A) cephalic end B) anterior end showing the different segments of the oesophagus C) mid-body of gravid D) distal end of female showing the anal opening (an) E) tail end of typical Falcaustra morulated (1) and undeveloped (2) eggs found in the faeces of Cuora amboinensis.

Scanning electron micrographs of Falcaustra duyagi from the large intestine of Cuora amboinensis. A) lateral cephalic end B) apical view showing the arrangement of the cephalic papillae (cp) and lips (lp) C) tail end of female D) tail end of male with protruding spicules (sp) and caudal pseudosuckers (cps) E) tail tip of male showing the caudal papillae F) excretory pore. Scale bars and primary magnification as indicated on the respective plates.

Ventral view of Parorientodiscus magnus from the large intestine of Cuora amboinensis.

Light micrographs of Parorientodiscus magnus from the large intestine of Cuora amboinensis. A) anterior portion B) ovary (ov), Mehli's gland (mg) and caeca (ce) C) the anterior (at) and posterior (pt) testes are lobed and tandem in orientation D) developed egg in the uterus E) scanning electron micrograph showing the ventral sucker (vs) and ridges (vrs). F) light micrograph of Polystomoides malayi showing the arrangement and structure of the genital spines.

Ventral view of Polystomoides malayi from the urinary bladder of Cuora amboinensis.

Scanning electron and light micrographs of Polystomoides malayi from the urinary bladder of Cuora amboinensis. A, B) anterior portion showing the prominent oral sucker (os) and pharyngeal bulb (ph) C, D) opisthaptor with six large suckers (hs) and one pair of large opisthaptoral hooks (hk) E) undeveloped egg with the characteristic trematode operculum (arrow); F) genital pore (gp) surrounded by numerous small genital spines (gs).
16 Unidentified species of Atractidae from the large intestine of *Cuora amboinensis*. A) anterior end B) cephalic end C) male tail end showing the characteristic curved tip D) close-up of male anal region

17 Light micrographs of an unidentified species of Atractidae from the large intestine of *Cuora amboinensis*. A) anterior end of female showing the anterior (at) and posterior (pt) portions of the oesophagus B) typical form of the male with a curled posterior end and pointed tail C) the tail end of the female is straight, slender and pointed D) mid-body of female showing numerous larvae (lav) in the uterus E) posterior end of the female showing the close proximity of the vulva (vu) and anus (an) F) unidentified Oxyurid larvae encapsulated in the liver of *Cuora amboinensis* ...

18 Scanning electron micrographs of an unidentified species of Atractidae from the large intestine of *Cuora amboinensis*. A, B and C) apical view showing the different position of the lips and cephalic papillae D) caudal end of female illustrating the close proximity of the vulva (vu) and anus (an) E) caudal end of male F) male with extruded spicule (sp).

19 Light micrographs of *Falcaustra* sp. 1 from the large intestine of *Cuora amboinensis*. A) cephalic end showing the lips (lp) and pharynx (ph) B) anterior end of female showing the different segments of the C) mid-body region of female D) tail end of female showing the anus (an) and simple pointed tip E) tail end of male F) a single pseudosucker (ps) was present on the tail.

20 Ventral view of *Polystomoides asiaticus* from the oral cavity of *Cuora amboinensis*.

21 Light micrographs of *Polystomoides asiaticus* found in the oral cavity of *Cuora amboinensis*. A) anterior portion B) mid-body C) close-up of the genital bulb showing the structure and arrangement of the genital hooks (ghk) D) posterior end showing the opisthaptor (ops), hooks and suckers; E and F) immature *P. asiaticus* found in the lungs showing the undeveloped pharynx and opisthaptor.

22 Light micrographs of unidentified digenetic trematodes found in *Cuora amboinensis*. A) Digenea sp. 1 from the liver – elongated form with eggs in the uterus; B) the same species - contracted form C) Digenea sp. 2 from the large intestine D) the same species – anterior end showing the prominent oral pouches (op) and oesophageal bulb (eb).
23 A) Species accumulation curve (SAC) of helminth parasites in 36 *Cuora amboinensis* from Peninsular Malaysia, irrespective of age and gender, B) SAC for juvenile males (n=10), C) SAC for juvenile females (n=6), D) SAC for adult males (n=10), E) SAC for adult females (n=10)

24 Species accumulation curve (SAC) of helminth parasites in 36 *Cuora amboinensis* from Peninsular Malaysia, with regards to age and gender A) SAC for adults (n=20), B) SAC for juveniles (n=16), C) SAC for males (n=20), D) SAC for females (n=16)

25 Collective frequency distribution of the helminth species richness in *Cuora amboinensis* from Peninsular Malaysia, irrespective of age and gender categories

26 Frequency distribution of the helminth species richness in *Cuora amboinensis* from Peninsular Malaysia, irrespective of age and gender categories

27 Scattergram of prevalence versus log mean intensity of helminths in *Cuora amboinensis* from Peninsular Malaysia, irrespective of age and gender categories. Core species appear in the upper-right quadrant

28 Comparative scattergrams of prevalence versus log mean intensity of helminths in *Cuora amboinensis* from Peninsular Malaysia, with regards to the various lifestages, age and gender categories. Core species appear in the upper-right quadrant

29 Helminth species richness (number of species), general spatial distribution, and presence of empty niches (blocks with diagonal lines) in the various sections of the alimentary tract of *Cuora amboinensis* from Peninsular Malaysia

30 Percentage of infection in the various segments of the alimentary tract in the overall sample population of *Cuora amboinensis* from Peninsular Malaysia

31 Linear distribution (represented by boxes) of the various helminth species within the alimentary tract of *Cuora amboinensis* from Peninsular Malaysia

32 Mean intensity of the dominant (D) and codominant (CD) helminth species in the various intestinal segments of 4 lifestage categories of *Cuora amboinensis* from Peninsular Malaysia

33 Mean abundance of the dominant (D) and codominant (CD) helminth species in the various intestinal segments of 4 lifestage categories of *Cuora amboinensis* from Peninsular Malaysia
Scanning electron and light micrographs of the intestinal mucosal relief of *Cuora amboinensis* showing the arrangement and extent of the mucosal folds and mucosal epithelial cells. Small intestine segment 1 (Si 1) – A, C, E; Small intestine segment 2 (Si 2) – B, D, F.

Scanning electron and light micrographs of the intestinal mucosal relief of *Cuora amboinensis* showing the arrangement and extent of the mucosal folds and mucosal epithelial cells. Small intestine segment 3 (Si 3) – A, C, E; Small intestine segment 4 (Si 4) – B, D, F.

Scanning electron and light micrographs of the intestinal mucosal relief of *Cuora amboinensis* showing the arrangement and extent of the mucosal folds and mucosal epithelial cells. Small intestine segment 5 (Si 5) – A, C, E; Small intestine segment 6 (Si 6) – B, D, F.

Scanning electron and light micrographs of the intestinal mucosal relief of *Cuora amboinensis* showing the arrangement and extent of the mucosal folds and mucosal epithelial cells. Small intestine segment 7 (Si 7) – A, C, E; Large intestine segment 1 (Li 8) – B, D, F.

Scanning electron and light micrographs of the intestinal mucosal relief of *Cuora amboinensis* showing the arrangement and extent of the mucosal folds and mucosal epithelial cells. Large intestine segment 2 (Li 9) – A, C, E; Large intestine segment 3 (Li 10) – B, D, F.

Linear distribution of *Serpinema octorugatus* in the various intestinal segments of adult *Cuora amboinensis*, in relation to intestinal morphology and stereology.

Linear distribution of *Falcaustra duyagi* in the various intestinal segments of adult *Cuora amboinensis*, in relation to intestinal morphology and stereology.

Gross appearance and scanning electron micrographs showing the distribution of *Serpinema octorugatus* in the oesophagus (A, C, D) and anterior duodenum (B, E) and *Falcaustra duyagi* in the large intestine (F) of *Cuora amboinensis*. Scanning electron micrograph (D) illustrates the attachment interface in the oesophagus.