

**Ultrastructural and Other Studies on
Cestodes of the Genus *Moniezia*
Infecting Egyptian Ruminants**

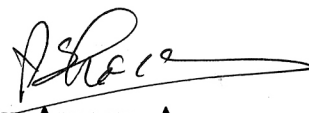
**A thesis submitted for the award
of Ph.D. degree in
Zoology (Parasitology)**

**By
Hanan Mohamed Abdel Wahab Elbaz
(M.Sc.)**

Supervised by



**Prof. Dr. Abdel Hafez H.
Mohammed**
Emeritus Professor of
Protozoology
Department of Zoology-
Faculty of Science – Ain
Shams University



**Prof. Dr. Ameen A.
Ashour**
Professor of Parasitology
Department of Zoology
Faculty of Science – Ain
Shams University



Dr. Hoda Abdel Halim Taha
Assistant Professor of Parasitology
Department of Zoology
Faculty of Science – Ain Shams University

**Department of Zoology
Faculty of Science
Ain Shams University
2008**

بسم الله الرحمن الرحيم

وقالوا لولا نزل عليه آية من ربه قل إني الله فأور
عني آية ينزل آية ولكن أنزلهم لا يعلموا. وما من
دولة في الأرض ولا طائر يطير بجناحيه إلا أمتنا لكم
ما فرطنا في الكتاب من شيء، نعم إلى ربهم يحشروا.

سورة الأنعام/ ٣٧-٣٨

Ultrastructural and Other Studies on Cestodes of the Genus *Moniezia* Infecting Egyptian Ruminants

Supervisors:

Prof. Dr. Abdel Hafez H. Mohammed

Emeritus Professor of Protozoology

Faculty of Science – Ain Shams University

Prof. Dr. Ameen A. Ashour

Professor of Parasitology

Faculty of Science – Ain Shams University

Dr. Hoda Abdel Halim Taha

Assistant Professor of Parasitology

Faculty of Science – Ain Shams University

Biography

Name: Hanan Mohamed Abdel Wahab Elbaz.

Date and place of birth: 2/ 5/ 1970.

Dekernes / Dakahlia Governorate.

Date of graduation: May, 1991.

Degree awarded: M.Sc. Zoology- Mansura
university.

Date of registration: 12 /11 / 2001.

FROM AND TO

ALLAH

Acknowledgement

I am deeply grateful and indebted to Professor Dr. Abdel Hafez H. Mohammed, Emeritus Professor of Protozoology, Zoology Department, Faculty of Science, Ain Shams University for his kind supervision, valuable criticism and critical reading of the manuscript.

I would like also to express my sincere gratitude and deep thanks to Professor. Dr. Ameen A. Ashour, professor of Parasitology, Department of zoology, Faculty of Science , Ain Shams University , for planning of the study , his valuable suggestions , kind supervision and reading of the manuscript.

Special thanks are due to Dr. Hoda Abdel Halium Taha, Assistant Professor of Parasitology, Department of Zoology, Faculty of Science, Ain Shams University, for her help, encouragement, and her constant aid in practical and technical work during this thesis.

Abstract

The present work deals with an electron microscopical investigation of the egg, vitelline cells, tegument, spermatogenesis and sperm in three cyclophyllidean cestodes that infect sheep and cows in Egypt. These were *Moniezia expansa*, *M. benedeni*, and *Avitellina centripunctata*. The three species were also compared using the RAPD-PCR technique.

Finally, a chromosomal study was also performed to differentiate between the two species of Genus *Moniezia* (*Moniezia expansa* & *M. benedeni*).

Keywords: Cestoda, Cyclophyllidae, Anoplocephalidae, ultrastructure, Egg, vitelline cells, tegument, spermatogenesis, sperm, *Moniezia expansa*, *M. benedeni*, *Avitellina centripunctata*, RAPD-PCR, chromosomes.

Contents

Acknowledgement.....	i
Abstract	ii
Contents	iii
List of Abbreviations.....	vi
List of Figures.....	viii
List of Tables.....	xx
General introduction and Aim of the work	1
Bases of taxonomy.....	5
Classification of family Anoplocephalidae.....	8
Genus <i>Moniezia</i>	11
Genus <i>Avitellina</i>	12
Aim of the work.....	12
Chapter I: Materials and methods	
1-Materials.....	14
1.1- <i>Moniezia</i> species.....	14
Systematic position.....	15
Diagnostic features of the two <i>Moniezia</i> species according to Khalil <i>et al.</i> ,1994.....	15
1.2- <i>Avitellina centripunctata</i>	16
Systematic position.....	16
Diagnostic features of <i>Avitellina centripunctata</i> according to Khalil <i>et al.</i> ,1994.....	17
2- Methods.....	18
I- Light Microscope Examination.....	18
A- Whole mount preparation.....	18
B- Semi-thin sections.....	19
1- Tegument, Egg and Vitelline cells.....	20
2- Spermatogenesis.....	20
II-Transmission Electron Microscope (TEM)Examination.....	20
1- Tegument, Egg and Vitelline cells.....	20

2- Spermatogenesis.....	21
III- Scanning Electron Microscope (SEM)	
Examination.....	22
IV-Chromosome Preparation	22
V- Molecular Techniques.....	24
Protocol 1: Isolation of DNA by CTAB	
precipitation.....	24
Procedure applied.....	26
Protocol 2: PCR Amplification.....	27
Primer selection.....	28
PCR preparation and conditions.....	29
Agarose gel electrophoresis.....	30
Data analysis.....	31
Buffers and Reagents.....	32
Chapter II: Egg and Vitelline cells	
Introduction.....	34
Results.....	37
1- <i>Moniezia expansa</i>	37
Shell formation.....	38
2- <i>Avitellina centripunctata</i>	39
Shell formation.....	40
Discussion.....	41
Chapter III: Tegument	
Introduction.....	66
Results.....	70
Discussion.....	74
Chapter IV: Spermatogenesis	
Introduction.....	98

Results.....	106
Genus <i>Moniezia</i>	
1- Spermatogenesis.....	106
2- Spermatozoon.....	108
Genus <i>Avitellina</i>	
Spermatozoon of <i>Avitellina centripunctata</i>	110
Discussion.....	115
Chapter V: Molecular characterization for the three species	
Introduction.....	161
Results.....	167
Discussion.....	195
Chapter VI: Chromosomal study	
Introduction.....	205
Results.....	209
A-mitosis.....	209
B-meiosis.....	209
Discussion.....	211
Summary and Conclusion	224
References	230
Arabic Summary.....	

List of Abbreviation

ac: Apical cone
ax: Axoneme
c: Centrioles
cb: Crested bodies
cp: Cytophore
ct: Cortical microtubules
cy: Cytoplasm
d: Doublets
dp: Electron-dense peripheral material
dz: Differentiation zone
EB: Embryonic block
EL: Embryophoric lacunae
g: Electron-dense granules
h: Heterochromatin
IE: Inner envelope
L: Lipids
M: Plasma membrane
m: Mitochondria
ML: Superficial muscle layer
Mo: Mature oocyte
Mv: Mature vitellocyte
n: Nucleus

nu: Nucleolus
Oc: Oocytes
OE: Outer envelope
ONC: Oncosphere
OV: Ovary
Po: Primary oocyte
ps: Primary spermatocytes
Pv: Paravitelline cells
rer: Rough endoplasmic reticulum
s: Singlets
Sd: Sheath of electron-dense periaxoneal material
sp: Spermatid
St: Transverse striation
Sv: Spindle-shaped vitellocytes
Ts: Tegumental syncytium
Tsc: Tegument-secreting cells
U: Uterus
Vg: Vitelline granules
Vs: Vitelline cells
W: Wall of intracytoplasmic electron-dense material

List of Figures

Fig. 1: Whole mount of <i>Moniezia expansa</i> showing the genital pore in the mature proglottid with the cirrus (penis) partly protruded	45
Fig. 2: Whole mount of <i>Moniezia expansa</i> showing the vitelline gland surrounded by the ovary in the proglottid.....	46
Fig. 3: Transmission electron micrograph of the mature proglottid of <i>M. expansa</i> showing spherical primary oocytes.....	47
Fig.4: Transmission electron micrograph of the mature proglottid of <i>Moniezia expansa</i> showing spherical immature vitellocytes.....	48
Fig.5: Transmission electron micrograph of the mature proglottid of <i>Moniezia expansa</i> showing some mature spindle-shaped vitellocytes.....	49
Fig.6: Transmission electron micrograph of the mature proglottid of <i>Moniezia expansa</i> showing a mature vitellocyte.....	50
Fig.7: Transmission electron micrograph of the mature proglottid of <i>M. expansa</i> showing the mature oocyte.....	51

Fig.8: Transmission electron micrograph of the mature proglottid of <i>M. expansa</i> showing the mature oocyte and the vitelline cells.....	52
Fig.9: Transmission electron micrograph of the mature proglottid showing <i>Moniezia expansa</i> (Dipylidium type) few vitelline cells associate with the zygote.....	53
Fig.10: <i>Moniezia expansa</i> : semithin section of the gravid proglottid showing fully developed eggs (oncospheres) packed into the uterus.....	54
Fig.11: <i>Moniezia expansa</i> : Enlarged from the previous Figure showing one oncosphere.....	55
Fig.12: <i>Moniezia expansa</i> : Transmission electron micrograph of the gravid proglottid showing one oncosphere.....	56
Fig.13: <i>Moniezia expansa</i> : Transmission electron micrograph of the oncosphere showing the inner envelope IE, which is responsible for the formation of the embryophore and oncospherical membranes.....	57
Fig. 14: Transmission electron micrograph of the gravid proglottid of <i>M. expansa</i> taken through the embryophore near its internal margin.....	58

Fig. 15: Whole mount of <i>Avitellina centripunctata</i> showing that each pregravid proglottid has one set of genitalia and genital pores alternate irregularly.....	59
Fig. 16: <i>Avitellina centripunctata</i> : enlarged from the previous figure showing the uterus lies transversely in the middle portion of the proglottid, and there is one ovary attached to it.....	60
Fig. 17: Whole mount of <i>Avitellina centripunctata</i> showing that each gravid proglottid has a posterior median opaque line formed by the uterus and paruterine organ filled with eggs.....	61
Fig. 18: <i>Avitellina centripunctata</i> : Transmission electron micrograph of the mature proglottid showing primary oocytes beside them the paravitelline cells.....	62
Fig. 19: <i>Avitellina centripunctata</i> :Transmission electron micrograph of the mature proglottid showing the characteristic paravitelline cells.....	63
Fig. 20: <i>Avitellina centripunctata</i> :Transmission electron micrograph of the mature proglottid showing the smooth ovary which containing the oocytes.....	64
Fig. 21: <i>Avitellina centripunctata</i> : enlarged from the previous figure showing ovary with many oocytes inside.....	65