Seasonal Variations in Germ Cell Production and Reproductive Activity of the Great Bandicoot Rat, *Bandicota indica* (Bechstein, 1800) in Southern Thailand

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ชื่อวิทยานิพนธ์ ความแปรผันในฤดูกาลการสร้างเซลล์สืบพันธุ์และการสืบพันธุ์ของหนูพุกใหญ่ (*Bandicota indica*) ในภาคใต้ของประเทศไทย
ผู้เขียน นายณัตฐาวุฒิ ฐิติปราโมทย์
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บทคัดย่อ

ศึกษาความแปรผันในฤดูกาลการสร้างเซลล์สืบพันธุ์และการสืบพันธุ์ของหนูพุกใหญ่ (*Bandicota indica*) ในนาข้าวจังหวัดพัทลุง ภาคใต้ของประเทศไทย โดยเก็บตัวอย่างทุกสองเดือนเป็นระยะเวลา 2 ปีตั้งแต่เดือนกันยายน 2547 ถึงเดือนกันยายน 2549 และประเมินภาวะการสืบพันธุ์ของหนูจากน้ำหนักตัว น้ำหนักและโครงสร้างเนื้อเยื่อของอวัยวะสืบพันธุ์ จำนวนหนูตั้งท้อง คุณภาพของอสุจิ และโครงสร้างโดยละเอียดของเซลล์หลอดเก็บตัวอสุจิด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องผ่าน (TEM) ผลการศึกษาพบว่าการสืบพันธุ์ของหนูพุกใหญ่พบมากในช่วงฤดูฝน (เดือนกันยายน ถึงเดือนมกราคม) โดยพบหนูตั้งท้อง 79.3% ของจำนวนหนูตั้งท้องทั้งหมด และรังไข่และอัณฑะของหนูโตเต็มวัยมีน้ำหนักมากกว่าอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับช่วงฤดูแล้ง (เดือนกุมภาพันธ์ ถึงเดือนสิงหาคม) (p<0.05) จากการศึกษาโครงสร้างเนื้อเยื่อของรังไข่แสดงให้เห็นว่า การเจริญของฟอลลิเคิลสามารถพบได้ในหนูเพศเมียโตเต็มวัยทุกตัว แต่จะพบมากในช่วงฤดูฝนโดยมีจำนวนฟอลลิเคิลที่เจริญเต็มที่มากกว่าช่วงฤดูแล้งอย่างมีนัยสำคัญทางสถิติ (p<0.05) นอกจากนี้โครงสร้างเนื้อเยื่อของอัณฑะแสดงให้เห็นว่าแม้หนูเพศผู้มีน้ำหนักอัณฑะมากในช่วงฤดูฝนแต่สามารถพบกระบวนการสร้างอสุจิได้ในหนูทุกตัวรวมทั้งในช่วงฤดูแล้ง จากการศึกษาความสัมพันธ์ระหว่างการสืบพันธุ์ของหนูพุกใหญ่กับสิ่งแวดล้อมพบว่า การสืบพันธุ์ของหนูมีความสัมพันธ์กับระยะเพาะปลูกของข้าว (p<0.05) โดยหนูพุกใหญ่สามารถสืบพันธุ์ได้ทุกระยะของการปลูกข้าว แต่พบการสืบพันธุ์สูงสุดในระยะการเจริญพันธุ์ของข้าว

การศึกษารูปร่างและคุณภาพของอสุจิแสดงให้เห็นว่า หนูชนิดนี้มีรูปร่างอสุจิหลายแบบทั้งลักษณะของส่วนหัวและหางอสุจิ โดยพบรูปร่างหัวอสุจิอย่างน้อย 9 แบบ และรูปร่างหางอสุจิอย่างน้อย 14 แบบในหนูเพศผู้ทุกตัว และอสุจิแบบที่พบมากและจัดว่าปกติคืออสุจิที่มีหัวลักษณะทรงกลมรี หรือทรงกรวย และหางอสุจิแบบตรง จากการศึกษาความแปรผันคุณภาพของอสุจิซึ่งได้ประเมินจากเปอร์เซ็นต์ของอสุจิที่มีรูปร่างปกติในประชากรอสุจิทั้งหมด พบว่า คุณภาพของอสุจิแปรผันตามฤดูกาล โดยในช่วงฤดูฝนหนูเพศผู้มีอสุจิที่มีหัวปกติ (67.4%) และหางปกติ (36.1%) มากกว่าในช่วงฤดูแล้งอย่างมีนัยสำคัญทางสถิติ (p<0.05) และ

เปอร์เซ็นต์ของอสุจิที่มีหัวและหางปกติมีแนวโน้มสูงที่สุดในระยะการเจริญพันธุ์ของข้าว (68.6% และ 33.4% ตามลำดับ) และน้อยที่สุดในระยะเก็บเกี่ยว (47.7% และ 17.0% ตามลำดับ) ซึ่งสอดคล้องกับความแปรผันของน้ำหนักอัณฑะ จากโครงสร้างโดยละเอียดของอสุจิด้วยกล้อง TEM พบว่า แม้ว่าหนูพุกใหญ่มีอสุจิที่มีรูปร่างหลากหลาย แต่อสุจิเหล่านี้มีลักษณะร่วมกันบางประการคือ (1) หัวอสุจิมีลักษณะไม่แบนทั้งสองด้าน (2) หัวอสุจิไม่พบส่วนเพอร์ฟอราทอเรียมและส่วนอีควอทอเรียล (3) นิวเคลียสมีแวคิวโอลจำนวนมากอยู่ภายใน และ (4) หางอสุจิส่วนกลางและส่วนหลักมีเอาท์เทอร์ เดนซ์ ไฟเบอร์ที่มีลักษณะเป็นรูปตัวซี ซึ่งลักษณะที่ร่วมกันเหล่านี้ของอสุจิหนูพุกใหญ่แตกต่างจากอสุจิของหนูชนิดอื่น

จากการศึกษาชนิดของเซลล์บุผนังหลอดเก็บตัวอสุจิพบว่าเยื่อบุหลอดเก็บตัวอสุจิส่วนปลายของหนูในช่วงฤดูฝนมีจำนวน clear cell มากกว่าในฤดูแล้งอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) และการศึกษาโครงสร้างโดยละเอียดของเซลล์ในหลอดเก็บตัวอสุจิแสดงให้เห็นว่าในช่วงฤดูฝน clear cell มีลักษณะเป็นเซลล์ที่มีการดูดซึมโดยที่ผิวเซลล์ด้านบนพบกระบวนการเอนโดไซโตซิส และภายในไซโทพลาสซึมประกอบด้วยมัลติเวซิคูลาร์ บอดี้ และเวซิเคิลที่มีเยื่อหุ้มจำนวนมาก นอกจากนี้ในช่วงฤดูฝนยังพบ principal cell มีลักษณะเป็นเซลล์ที่มีการคัดหลั่งโดยพบเอนโดพลาสมิกเรติคูลัมที่เด่นชัดในไซโทพลาสซึม และมีเวซิเคิลที่มีเยื่อหุ้มอยู่ภายในไซโทพลาสซึมด้านบนของเซลล์และระหว่างสเตอริโอซีเลีย

การศึกษาครั้งนี้สรุปว่า หนูพุกใหญ่ในภาคใต้ของประเทศไทยสามารถสืบพันธุ์ได้ตลอดทั้งปี แต่สืบพันธุ์มากที่สุดในช่วงฤดูฝนซึ่งมีอาหารอุดมสมบูรณ์จากที่ข้าวเติบโตในระยะการเจริญพันธุ์ โดยช่วงเวลาดังกล่าวหนูเพศเมียมีการเจริญของฟอลลิเคิลภายในรังไข่มากที่สุดและหนูเพศผู้มีคุณภาพอสุจิสูงที่สุด และเซลล์เยื่อบุหลอดเก็บตัวอสุจิส่วนปลายแสดงลักษณะการดูดซึมและการคัดหลั่งซึ่งอาจมีส่วนช่วยทำให้อสุจิเจริญเต็มที่พร้อมปฏิสนธิ นอกจากนี้หนูพุกใหญ่เพศผู้ทุกตัวมีอัณฑะขนาดเล็ก (ประมาณ 0.42% ของน้ำหนักตัว) และมีอสุจิหลายรูปแบบมากเมื่อเทียบกับหนูชนิดอื่น นอกจากนี้หนูพุกใหญ่ทั้งเพศเมียและเพศผู้ทุกตัวมีต่อมพรีพูเตียวขนาดใหญ่กว่าหนูชนิดอื่นซึ่งอาจเกี่ยวข้องกับการหลั่งฟีโรโมนสำหรับการจัดลำดับทางสังคม ผลการศึกษาเหล่านี้ชี้ให้เห็นว่า ช่วงเวลาการสืบพันธุ์ของหนูพุกใหญ่คล้ายกับหนูนาท้องขาว (*Rattus argentiventer*) แต่อาจแตกต่างกันในชีววิทยาการสืบพันธุ์และการจัดลำดับทางสังคม

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ABSTRACT

This study investigated the seasonal variation in germ cell production and reproductive activity of the Great Bandicoot rat, *Bandicota indica*, in a ricefield at Phatthalung province, southern Thailand. Animals were collected bimonthly over a period of 24 months between September 2004 and September 2006. Body mass, the weights of reproductive organs, the gonadal histology, the incidence of pregnancy, and the sperm quality, as well as the ultrastructure of the epididymal cells were used to assess the reproductive condition of this species. It was shown that the highest incidence of reproductive activity occurred in the wet season (September-January) when ~79.3% of all pregnancies were detected and the ovary and testis of the adults were significantly heavier than those in the dry season (February-August) (p<0.05). Ovarian histology studies showed that ovarian follicular development (or folliculogenesis) was present in all ovaries sampled at any time however it occurred more frequently in the wet season when the number of the mature (Graafian) follicles was significantly higher than in the dry seasons (p<0.05). The testicular histology revealed that, regardless of the testes weight, spermatogenesis was occurring in all animals sampled even those obtained from the dry season. The reproductive activity of *B. indica* was strongly related with the rice cultivation stage (p<0.05). Although *B. indica* reproduction could occur at any of the rice cultivation stages but the main breeding occurred during the generative stage of the rice plant in the wet season.

The investigation of the morphology and quality of *B. indica* sperm showed that this species had an unusually high pleiomorphic sperm morphology both in their head and tail characteristics. At least 9 different sperm head and 14 sperm tail morphotypes were found in all individuals with the morphology of the normal

spermatozoa being a globular or conical head and a straight tail. In relation to the seasonal variation in sperm quality which was assessed based on the percentage of sperm with normal morphology it was found that the sperm collected in the wet season showed a significantly higher percentage of normal sperm heads (67.4%) and normal sperm tails (36.1%) than those obtained in the dry season (57.9% and 20.2%, respectively) ($p<0.05$). In addition, the percentage of normal sperm heads and tails tended to be highest during the generative stage of the rice plant (68.6% and 33.4%, respectively) and were lowest during the harvesting period (47.7% and 17.0%, respectively) and this was paralleled by their testis weights. TEM investigations also revealed that although *B. indica* spermatozoa had various sperm head and tail morphotypes, but some characteristics were common such as (1) their heads were not bilaterally flattened, (2) a perforatorium and equatorial segment were not found in any of the spermatozoa, (3) their nuclei had many prominent nuclear vacuoles (4) and the midpiece and principal piece contained C-shaped outer dense fibres. These characteristics of *B. indica* spermatozoa differed from those of other rats.

The study of the cell types along the epididymal epithelium showed that the number of clear cells from the wet season bandicoots was significantly higher than those of the dry seasons ($p<0.05$) but the percentages of other cell types did not vary to the same extent. Ultrastructural observations of the caudal epididymis indicated that the clear cells had an absorptive function during the wet season occurring endocytosis at their apical surface and had many multivesicular bodies (MVBs) and membrane-bounded vesicles within their cytoplasm. On the other hand, the principal cells in the wet season exhibited secretory activity as they had a distinct endoplasmic reticulum in their cytoplasm and membrane-bounded vesicles in either their apical cytoplasm or among their stereocilia.

In conclusion, the Great Bandicoot rats in southern Thailand breed throughout the year with their maximum germ cell production and reproductive activities occurring in the wet season when the rice is in the generative stage (good food availability). In this period, female rats have a maximum ovarian follicular development and in males the sperm quality is highest and the epididymal epithelium exhibits both absorptive and secretory activities that probably related to the sperm maturation. Regardless of the time of the year, male *B. indica* has small testis

(~0.42% of body weight) and a highly pleiomorphic sperm morphology comparing to other murid rodents. Furthermore, the preputial glands, that probably secrete pheromones for social interactions are surprisingly large in both male and female bandicoot rats, unlike other rats. These findings indicate that *B. indica* reproduces at a similar time to the sympatric ricefield rat, *Rattus argentiventer* but has a different breeding biology and perhaps social organization.

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LIST OF ABBREVIATIONS AND SYMBOLS

A	=	Antrum
AbH	=	Abnormal sperm head
AbT	=	Abnormal sperm tail
Ac	=	Acrosome
AH	=	Apical hook
B	=	Basal cell
B. bengalesis	=	*Bandicota bengalensis*
B. indica	=	*Bandicota indica*
BF	=	Bent flagellum
BF.PCD	=	Bent flagellum with proximal cytoplasmic droplet
BF.DCD	=	Bent flagellum with distal cytoplasmic droplet
BM	=	Bent midpiece
BM.CD	=	Bent midpiece with cytoplasmic droplet
BN	=	Bent neck
BN.CD	=	Bent neck with cytoplasmic droplet
Bp	=	Basal plate
B. savilei	=	*Bandicota savilei*
BW	=	Body weight
C	=	Clear cell
CE	=	Cytoplasmic extension
Ce	=	Centriole
CF	=	Coarse fibres in sperm tail
CO	=	Cumulus oophorus
CR	=	Corona radiate
Ct	=	Captitulum
DAPI	=	4’-6-diamidino-2-phenylindole dihydrochloride
db	=	Electron-dense bodies
D.CD	=	Distal cytoplasmic droplet
E	=	Epididymides, epididymis

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

EM	=	Electron microscope
EpW	=	Epididymides weight
ER	=	Endoplasmic reticulum
et al.	=	Et. Ali (Latin), and others
F	=	Flagella, flagellum
FD	=	Flagellar deformity
FS	=	Fibrous sheath in sperm tail
FSH	=	Follicle stimulating hormone
G	=	Growing stage of rice plant
GA	=	Golgi apparatus
Gf	=	Mature or Graafian follicle
GH	=	Gonadotrophin hormone
GnRH	=	Gonadotrophin releasing hormone
Gr	=	Generative stage of rice plant
H	=	Halo cell
Hv	=	Harvesting stage of rice plant
If	=	Implantation fossa
L	=	Lumen
Ldb	=	Lysosome like dense body
LH	=	Luteinizing hormone
LM	=	Light microscope
M	=	Mitochondria
M.	=	*Mus* spp.
MD	=	Midpiece deformity
MP	=	Midpiece of sperm tail
MVBs	=	Multivesicular bodies
N	=	Nucleus
NBF	=	Neutral buffered formalin
ne	=	Nucleus extension

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

NH	=	Normal sperm head
No.GF	=	Number of mature or Graafian follicles
No.PlS	=	Number of placental scars
NT	=	Normal sperm tail
nv	=	Nucleus vacuoles
Oc	=	Oocyte
Od	=	Oviduct
Ov	=	Ovary or ovaries
OvW	=	Ovary weight
P	=	Principal cell
PADL	=	Postacrosomal dense lamina
PBF	=	Phosphate buffered saline
P.CD	=	Proximal cytoplasmic droplet
Pf	=	Primary follicle
Pn	=	Penis
Pp	=	Preputial glands
PpW	=	Preputial glands weight
Ps	=	Prostate glands
PsW	=	Prostate glands weight
R.	=	*Rattus* spp.
Rsd	=	Round spermatids
Rt	=	Resting stage of rice plant
S	=	Stereocilia
SAM	=	Subacrosomal space with electron dense material
Sc	=	Primary spermatocytes
SCs	=	Segmented columns
Sd	=	Spermatids
S.E.	=	Standard error
SE Asia	=	Southeast Asia

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

SEM	=	Scanning electron microscope
SG	=	Stratum granulosum
Sg	=	Spermatogonia
ST	=	Scrotum
St	=	Sperm tail
SV	=	Secretion vesicle
Sv	=	Seminal vesicles
SvW	=	Seminal vesicles weights
Sz	=	Spermatozoa
T	=	Testis or testes
TEM	=	Transmission electron microscope
TI	=	Theca interna
TSH	=	Thyroid-stimulating hormone
TW	=	Testis weight
U	=	Uterus
UW	=	Uterus weight
V	=	Membrane-bounded vesicles
Vg	=	Vagina
ZP	=	Zona pellucida

CHAPTER 1
INTRODUCTION

Several species of murine rodents occur in the ricefields of Southeast (SE) Asia with species in the genus *Rattus* being the most common and including the ricefield rat, *Rattus argentiventer*, the lesser ricefield rat, *R. losea*, and the black or the roof rat, *R. rattus* (Marshall, 1977; Boonsong *et al.,* 1999). In Thailand, apart from these *Rattus* species there are two species of *Mus, M. caroli* and *M. cervicolor*, and two species of bandicoot rats in the genus *Bandicota* found in the ricefields (Boonsong *et al.,* 1999). These murine species cause considerable damage to the ricefield crops and are thus of considerable economic importance to the country (Boonsong *et al.,* 1999). The relationships among these murine species are not well known but there is some evidence that species of *Bandicota* are closely related to *Rattus* (Gadi and Sharma, 1983; Carleton and Musser, 2005). Although there is some controversy as to the number of species of *Bandicota* (Boonsong and Felten, 1989; Musser and Brothers, 1994), at least three species, *Bandicota indica*, *B. bengalensis*, and *B. savilei* are currently recognized (Musser and Brothers, 1994; Aplin *et al.,* 2003b). The Great Bandicoot rat, *B. indica*, is so far the largest murine rodent found in the ricefields of southern Thailand. This species has a wide distribution ranging from India and Sri Lanka to southern China, Taiwan, Malaysia and Indonesia (Marshall, 1977; Aplin *et al.,* 2003a).

Studies on the timing of the reproductive activity of murine rodents in the ricefields of SE Asia have largely focused on the ricefield rat, *R. argentiventer*, a major economic pest in parts of SE Asia. This species can reproduce throughout the year (Brown *et al.*, 2005) with the peak of breeding activity corresponding closely to the generative or reproductive stage of rice crop when rice is the best food source (Lam, 1987; Tristiani and Murakami, 1998; 2003; Brown *et al.*, 2005). Similarity, the lesser ricefield rat, *R. losea*, in Vietnam also reproduces at the time after the early generative stage of rice plants (Brown *et al.*, 2005). These findings indicate that nutritional factors, particularly the presence of rice in the generative stage, may

trigger reproduction of these *Rattus* in the ricefields, but whether that is also the case for the Great Bandicoot rat is unknown.

In relation to the study of the seasonal variation in reproduction, the reproductive status can be determined in several ways e.g. based on the external characteristics of gonads and the levels of reproductive hormone. However, perhaps one of the accurate indicator of the degree of gonadal and reproductive activity is by histological observation of the reproductive organs and investigation of the sperm quality (Wildt, 1999; Couto and Talamoni, 2005). During a breeding period, the gonads and accessory sex organs of both males and females become active, but in addition the sperm quality tends to be high and this of course increases the potential for fertilization (Couto and Talamoni, 2005; de Haas van Dorsser and Strick, 2005; Muteka *et al.,* 2006a). Sperm quality is associated with spermatogenesis in the testes and sperm maturation in the epididymis (Hamilton, 1975; Hermo *et al.,* 1988). Sperm morphology has been reported to be the best parameter for predicting sperm fertilizing capacity (Wild, 1999; Kubo-Irie *et al.,* 2005), and thus is used to evaluate sperm quality. Sperm morphology of mammalian species including most species of murine rodents includes the shape and size of the head, length of midpiece, and total length of sperm tail (Bedford and Hoskins, 1990; Gage, 1998). However, recently it has become evident that a few species have variable sperm morphology within and between individuals in some mammals such as the red velt rat, *Aethomys ineptus* in Africa (Breed, 1995), the spinifex hopping mouse, *Notomys alexis* in Australia (Bauer and Breed, 2006), and *Bandicota* in SE Asia (Breed, 1993; 2004). Within the *Bandicota* genus, *B. bengalensis* has a homogeneous sperm population that is generally consistent in morphology and similar in form to that of closely related genera of murine rodents. However, the other two species within this genus, *B. savilei* and *B. indica*, have highly divergent and pleiomorphic sperm populations (Breed, 1993; 1998). Therefore, it is interesting to focus on the reproduction of *B. indica* which is a one of the most serious pests in the ricefields of Thailand (Boonsong *et al.,* 1999).

Current investigations on the reproduction of the Great Bandicoot rat are far less extensive than reproductive studies of the ricefield rat. The oestrous cycle of the Great Bandicoot rat is around 8 days and the gestation length is about 26 days,

which is several days longer than that of *Rattus* species (Boonsong, 1984). Apart from this observation, subsequent studies on a few wild caught individuals have found that *B. indica* possesses rather small testes (Breed and Taylor, 2000) and produced pleiomorphic sperm (Breed, 1993; 1998) as well as having an unusual germ cell organization in the testes (Worawittayawong *et al.,* 2005). Despite *B. indica* having these unusual phenomena, unlike *Rattus* and other rats, little is known about the seasonal variation in germ cell production and reproductive activity of this species in Thailand. In addition, the relationship between its reproduction and a diet of rice plant in various stages of growth is not clear. Therefore, the present study is carried out to investigate the seasonal changes in germ cell production and the reproductive activities of *B. indica* in the ricefield. The *B. indica* sperm quality as well as the histological changes and cell activity of epididymal cells in various reproductive stages have also been studied by transmission electron microscope (TEM). The environmental conditions, particularly the relation of the rice cultivation stage to the reproductive activities of *B. indica* are discussed.

Literature reviews

1. Biology of the Great Bandicoot rat

1.1 Taxonomy of the Great Bandicoot rat (Marshall, 1977)

Phylum Chordata

Class Mammalia

Order Rodentia

Family Muridae

Subfamily Murinae

Genus *Bandicota*

Bandicota indica Bechstein, 1800

The *Bandicota* genus contains three well recognized species *Bandicota indica* (the Great or Greater Bandicoot rat), *B. bengalensis* (the Lesser or Bengal Bandicoot rat), and *B. savilei* (the Savile's Bandicoot rat also referred to as the Lesser Bandicoot rat) (Corbet and Hill, 1992; Musser and Brothers, 1994; Aplin *et al.,* 2003b; Carleton and Musser, 2005). They are terrestrial rats which are distinguished by their blunt-nose, the straight, cross-wise lamellae of the molars and 44-46 chromosomes including 8 to 9 pairs of metacentrics (Marshall, 1977). Most studies have indicated that these *Bandicota* rats are closely related to *Rattus* (Gadi and Sharma, 1983; Musser and Brothers, 1994; Watts and Baverstock, 1994). The name *Bandicota indica* is synonymous with *B. mordax*, Thomas, 1916 and *B. siamensis*, Kloss, 1919 (Marshall, 1977).

1.2 General morphology of the Great Bandicoot rat

Bandicota indica is the largest terrestrial rat and has a ferocious nature. It has a distinctly shaggy and blackish-brown dorsal fur with numerous black guard hairs that project through the dorsal fur, especially along the middle of the back and on the rump (Figs. 1A-B), and its incisor is broader than that of *Rattus* (Fig. 1C). The tail is usually shorter than the head-body length and is uniformly dark. The manus and feet are clothed in black hairs and bear strong claws adapted for digging (Marshall, 1977; Aplin *et al.,* 2003a). The foot of *B. indica* is broader and thicker than in the other *Bandicota* species (Fig. 1D) (Aplin *et al.,* 2003a; 2003b). Adult bandicoot rats

in Thailand have the body weight of up to 545 g, a head-body length of up to 276 mm and the maximum tail length of 244 mm (Marshall, 1977). The mammary gland arrangement of this species is 3+3 (Marshall, 1977; Aplin *et al.,* 2003a).

1.3 Distribution and habitat of the Great Bandicoot rat

The Great Bandicoot rat, *B. indica*, is widely distributed across South and Southeast Asia including Thailand, while *B. savilei* is also found around Thailand except in the Thai peninsular, whereas *B. bengalensis* is not found in Thailand (Marshall, 1977; Musser and Brothers, 1994). *B. indica* is found in many rural areas including varied cropping systems but is especially common in rice fields and in rural village and urban environments. It has not been found in any purely natural habitat in Thailand (Marshall, 1977; Boonsong *et al.,* 1999; Aplin *et al.,* 2003a). The bandicoot rat is a good digger and constructs elaborate burrows at the edges of fields, in dikes and stream banks, and even along city streets (Marshall, 1977). *B. indica* exploits a wide range of both aquatic and terrestrial foods, including mollusks, crustaceans, water lily fruit, water hyacinths, insects, earthworms, and field crops such as rice, vegetables (including tubers), fruits, and nuts (Aplin *et al.,* 2003a). This species has also been reported to be one of the most serious pests in field crops, especially in rice fields and oil palm plantations (Boonsong *et al.,* 1999).

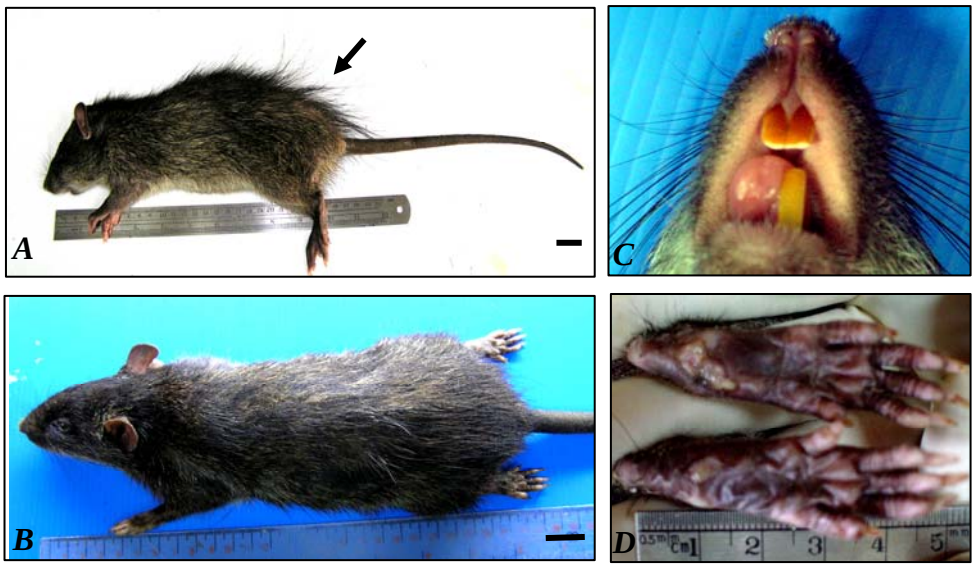


Figure 1. External characteristics of mature *Bandicota indica*. Note the black guard hairs on dorsal fur of adult rat (arrow). Scale bars= 2 cm.

1.4 Reproductive biology of the Great Bandicoot rat

The captive male Great Bandicoot rat in central Thailand reaches maturity as early as 120 days and up to 180 days of age. The perforation of the vagina of the female appears at the age of 120 days to 150 days. Adult females have an oestrous cycle of 8.0±1.2 days and a gestation period of 26.6±1.6 days. The litter size is 6.6±2.9 young with 1-2 litters per year. Postpartum-estrus occurs in this species. There is a sexual dimorphism with the male being bigger than the female *B. indica*. The longevity of the female varies from 1.5-2.7 years, while that of male is 2.5-3.3 years (Boonsong, 1984). On the other hand, the wild-caught *B. indica* in India has an oestrous cycle of 4-8 days and a shorter gestation period (23±1.2 days). The vaginal perforation occurs at 190-210 days after birth (Aplin *et al.,* 2003a). Subsequent studies on a few wild caught *B. indica* in Thailand found a rather small testis of about 0.33 % of body mass (Breed and Taylor, 2000) and sperm of variable morphology (Breed, 1993; 1998), as well as having an unusual germ cell organization in the seminiferous epithelium as the multi-cellular stage, like in humans and some other primates (Worawittayawong *et al.,* 2005). In relation to the reproductive seasonality of *B. indica* it has been little investigated around South and Southeast Asia. In Thailand, its reproduction has been observed in only two populations in the ricefields at Suphanburi and Chinat provinces, central Thailand, where the rice is grown twice per year (Sudto, 1987). The results revealed that the breeding seasons of this species occur twice per year during the dry season (December-February) and the wet season (May-June) which corresponds closely to the periods of the generative to harvesting stages of both rice crops (Sudto, 1987). On the other hand, *B. indica* in the marshland habitat in Sagor Island of India can reproduce throughout the year with a peak of pregnant animals occurring in the post-monsoon to mid-summer seasons (Aplin *et al*., 2003a).

2. Sperm production and breeding season in mammals

Spermatogenesis is the process of sperm production by which spermatogonia develop into spermatozoa within the seminiferous tubules of the testis. In most adult mammals, testes do not remain uniformly active throughout the year and maximum activity usually corresponds to the breeding season when the spermatogenic and androgenic functions of the testes are maximally developed (Lincoln, 1981; Muteka *et al.,* 2006a; Couto and Talamoni, 2005). The maximal spermatogenic activity of the testes results in a high spermatogenic output (e.g. high sperm concentration and highly motile spermatozoa) and in the case of animals that have a high pleiomorphic sperm morphology, there is a high proportion of morphologically normal spermatozoa (Goeritz *et al.,* 2003; de Haas van Dorsser and Strick, 2005). On the other hand, the regression of testes in the non-breeding season results in the spermatogenesis proceeding only as far as the very early preleptotene stage of meiosis (Johnson and Everitt, 2004), with no spermatozoa being produced for several months of the year and during this period the epididymis becomes devoid of spermatozoa (Lincoln, 1981) or has a very low sperm concentration as well as a greater number of abnormal spermatozoa in the population (Goeritz *et al.,* 2003; de Haas van Dorsser and Strick, 2005). However, in some seasonal breeder mammals e.g. Hairy-tailed bolo mouse (*Bolomys lasiurus*) and Nembian cheetah (*Acinonyx jubatus*), males exhibit no apparent manifestation of seasonal testicular activity with spermatogenesis and sperm quality being similar in the reproductively active and inactive periods (Couto and Talamoni, 2005; Crosier *et al.,* 2007).

3. Polymorphic sperm morphology in the rat

The spermatozoon is the end product of the process of spermatogenesis and has a very precise and limited function to carry genetic material from the male to the oocyte. The sperm needs to pass through the vagina, uterus, and oviduct and then penetrate the zona pellucida of the oocyte. The cellular organelles of the spermatozoon are highly modified for motility (Bedford and Hoskins, 1990; Eddy and O'Brian, 1994). The spermatozoon has two main components, the head and the flagellum or tail, which are joined at the neck. The sperm head consists of the acrosome, the nucleus, and small amounts of cytoskeletal material. The sperm tail is a specialized motile apparatus containing an axoneme surrounded by outer dense fibres, an elaborate fibrous sheath and, in the anterior midpiece region, mitochondria (Fawcett, 1970; Bedford and Hoskins, 1990; Eddy and O'Brian, 1994).

Eutherian mammals generally produce spermatozoa that are highly characteristic and consistant in morphology for a particular species with most orders of eutherians having a sperm head that is oval or paddle-shaped, bilaterally flattened, a symmetrical nucleus and a basally attached tail (Fawcett, 1970; Bedford and Hoskins, 1990; Eddy and O'Brian, 1994). However, in the rodent Superfamily Muroidea, with includes the common laboratory rats and mice in the Family Muridae, most species have a sperm head that is hook-shaped with a highly complex and asymmetrical acrosome and an elaborate cytoskeletal rostral projection, a perforatorium, and a long sperm tail attached to the lower concave surface of the sperm head (Bishop and Austin, 1957; Lalli and Clermont, 1981; Breed, 2004). Nevertheless, there are a few species within this superfamiliy that have a highly pleiomorphic sperm population that lacks an apical hook and in which the sperm tail is generally much shorter (Breed, 2004; Breed *et al.,* 2007), like those of the leopard (de Haas van Dorsser and Strick, 2005), cheetah (Crosier *et al.,* 2007) and human (Kubo-Irie *et al.,* 2005). The divergent sperm morphology generally occurs in species with relative small testes (Breed and Taylor, 2000). For instance in Africa, the red velt rat, *Aethomys ineptus*, has a sperm morphology that is highly divergent from other species in the same genus, and produces sperm of high variability (Breed, 1995). In Australia another species of murine rodent, *Notomys alexis*, has also been found to

have a highly variable sperm population in both wild caught as well as laboratory bred individuals, regardless of their age (Bauer and Breed, 2006).

In SE Asia, many species of murine rodents are found and by far the majority of them appear to have homogenous sperm populations, however, in the genus, *Bandicota*, this is not the case (Breed, 1993; 2004). In this genus, *B. bengalensis* has been found to have a sperm head structure that is generally consistent in morphology and similar to that of other genera of murines including the laboratory rat (Breed, 1998). Their sperm heads are falciform in shape, have a bilaterally flattened nucleus and the sperm tail is attached to the head on the lower concave surface (Fig. 2A). However the other two species within this genus, *B. savilei* and *B. indica,* have a totally different sperm morphology from that of *B. bengalensis* and all other rats and mice (Breed, 1993; 1998). They have spermatozoa with heads that are pleiomorphic but are generally either bulbous or globular. The nuclei of these spermatozoa are more or less circular in cross-section for most of their length and capped by a huge acrosome (Figs. 2B,C). Furthermore, in some of the individuals investigated, a high frequency of sperm abnormalities is evident (Breed, 1993; 1998) and these individuals have also small testes (Breed and Taylor, 2000). These interspecies differences in sperm morphology within this genus indicate that both the mode of control of the spermatozoon shape within the testis, and also perhaps some aspects of the sperm-egg interaction at the time of fertilization, may have diverged markedly between the species of *Bandicota* with that of *B. bengalensis* probably representing the ancestral condition and *B. indica* as well as *B. savilei*, exhibiting a more recently derived status (Breed, 1998).

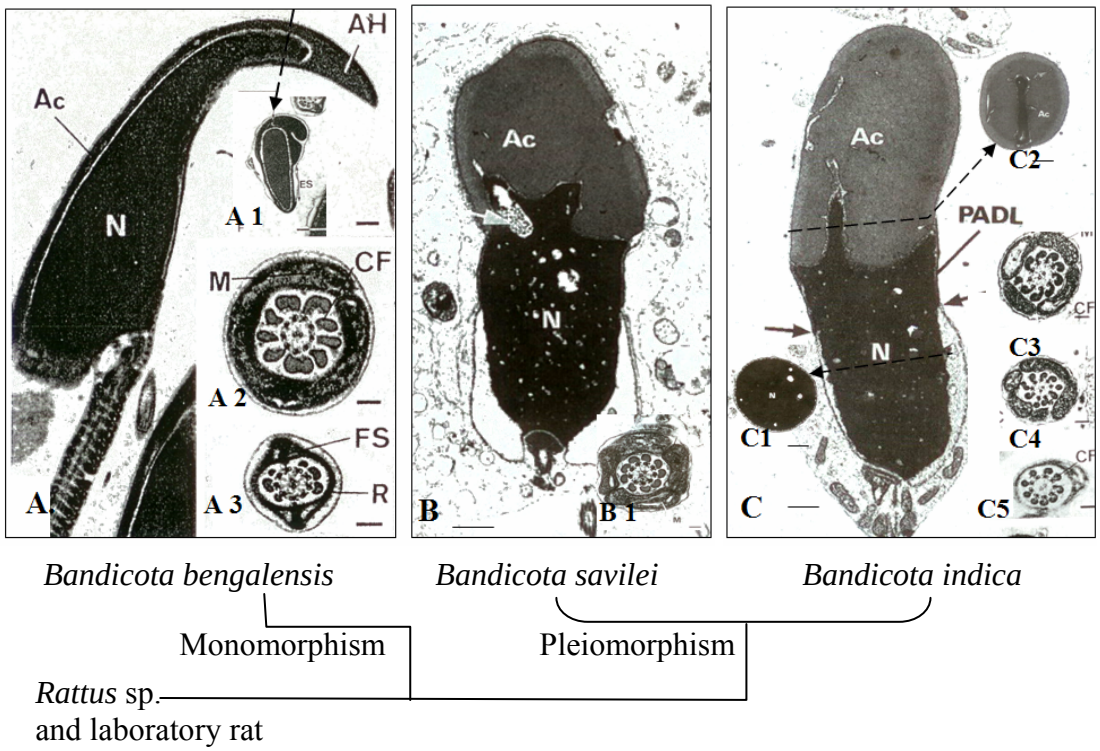


Figure 2. The sperm morphology of *Bandicota* genus: *B. bengalensis* (*A*), *B. savilei* (*B*) and *B. indica* (*C*). Sagittal longitudinal section (*left*) and cross section (*C1*) of sperm head as well as transverse sections of tail sperm (*right bottom; A2,3, B1, C3,4,5*). Note, nuclear vacuoles in which a group of chromatin fibres is present (white arrow). Ac=acrosome, AH=apical hook, CF= coarse fibres, FS= fibrous sheath, M=mitochondria, PADL=postacrosomal dense lamina, R=ribs, black arrow= posterial ring. Scale bars: *A*=600 nm, *A1*=870 nm, *A2,3*=120 nm, *B*=440 nm, *B1*=100 nm, *C*= 400 nm, *C1*= 230nm, *C2*=200 nm, *C3,4,5*=150 nm (Breed, 1993; 1998).

4. Structure and function of the epididymal epithelium

The epididymal duct is a single highly convoluted duct, closely applied to the surface of the testis, extends from the anterior to the posterior pole of that organ and is held more or less firmly, depending on the species to the tunica albuginea by connective tissue. The duct is coiled into segments demarcated by connective tissue septula and the organ is contained within a fibrous tissue capsule. The segment into which the ductuli efferentes empty is usually referred to as the initial segment and the remainder of the epididymis is loosely divided into three parts termed the caput, corpus, and cauda epididymis (Hamilton, 1975; Setchell *et al.,* 1994). The epididymal duct is lined with pseudostratified columnar epithelium (Ross *et al.,* 2003) that consists of five cellular types e.g. principal, basal, holo, apical, and clear cells (Reid & Cleland, 1957). *Principal cells* are a dominant epididymal cell whereas *basal cells* occur sporadically along the duct but appear to be more common in the cauda region. *Halo cells* are found at all levels along the duct and exhibit an empty cytoplasmic area surrounding the nuclei (Reid & Cleland, 1957; Hamilton, 1975). *Apical cells* have many characteristics of their adjacent principal cells, and are found only in the initial segment. *Clear cells* are characterized by dense accumulations of glycolipid or lipid droplets in the cell base, contain many lysosome like dense bodies, and multivesicular bodies as well as lipofuscin material in the apical cytoplasm. Short microvilli can be seen on the luminal surface. These clear cells are only found nearer to the tail region of the rat epididymis (Hamilton, 1975; Kumar *et al.,* 1980; Hermo *et al.,* 1988).

The epididymis, an androgen-dependent organ, plays a key role in the maturation and storage of spermatozoa (Bedford, 1975). The functions of the epididymal epithelium are absorption and secretion that are responsible for creating the ideal conditions for making spermatozoa ready for fertilizing and motility (Hamilton, 1975; Fornes and De Rosas, 1991). In its absorptive activity, the epididymis can absorb both the particulate material and the luminal fluid into the vesicles and vacuoles in their cytoplasm (Hamilton, 1975). Among epididymal cells, the principal and clear cells have been implicated in the absorptive function that are capable of absorbing portions of spermatozoa and have evidence of epithelial spermiophagy (Hamilton, 1975; Flickinger *et al.,* 1978; Hermo *et al.,* 1988). In the rat

and hamster, principal cells exhibit absorptive activity in the proximal epididymis (caput and corpus segments), but the clear cells may have an absorptive function in the distal epididymis where they are numerous (Flickinger *et al.,* 1978; Hermo *et al.,* 1988). In its secretory function, the epididymis can synthesize certain compounds, and there is evidence to indicate that some of them are secreted into the epididymal lumen. In this activity, the rough endoplasmic reticulum in the principal cells plays a role in the synthesis of secretory protein, while the smooth endoplasmic reticulum correlates with the synthesis of steroids (Hamilton, 1975; Flickinger *et al.,* 1978). The presence of membrane-bounded vesicles in the apical cytoplasm and among stereocilia of epididymal cell is a possible form of secretion of the epididymal epithelium and contributes to the intraluminal environment (Fornes and De Rossas, 1991).

5. Climate and rice plants in southern Thailand.

Southern Thailand is located in the Peninsular Thailand where there are several sizeable coastal plains and a mountain chain running along its western coast. The climate in this region is strongly influenced by the tropical monsoon system; the northeast monsoon (October to February) and the southwest monsoon (May to October). In addition, two principal seasons characterize the climatic periods: the dry season from February to May, and the wet season from May to January with heavy rain expected during September to January (The Thai Meteorological Department; http://www.tmd.go.th). Rice is generally grown either once or twice a year, however, in southern Thailand including Phatthalung province, rice is mostly cultivated only once a year. Rice cultivation can be divided into 4 main stages; growing, generative or reproductive, harvesting, and resting stages (modified from Tristiani and Murakami 1998; 2003) (Fig. 3).

1. The growing stage is characterized by 1.1) preparation of the land by ploughing; 1.2) the germination period during which the seeds are sown and plants grow into seedlings; and 1.3) the maximum tillering step.
2. The generative stage is characterized by 2.1) the initiation of the primordial panicle when the bulb of the rice plant initially develops; 2.2) the booting step

when maximum bulb and stalk growth take place; 2.3) the flowering stage when the flowers appear; 2.4) the milky step when seeds form a milky liquid; 2.5) the ripening stage when seeds ripen for harvest.

3. The harvesting stage is when rice is harvested.
4. The resting stage is when there is no planting in the ricefield.

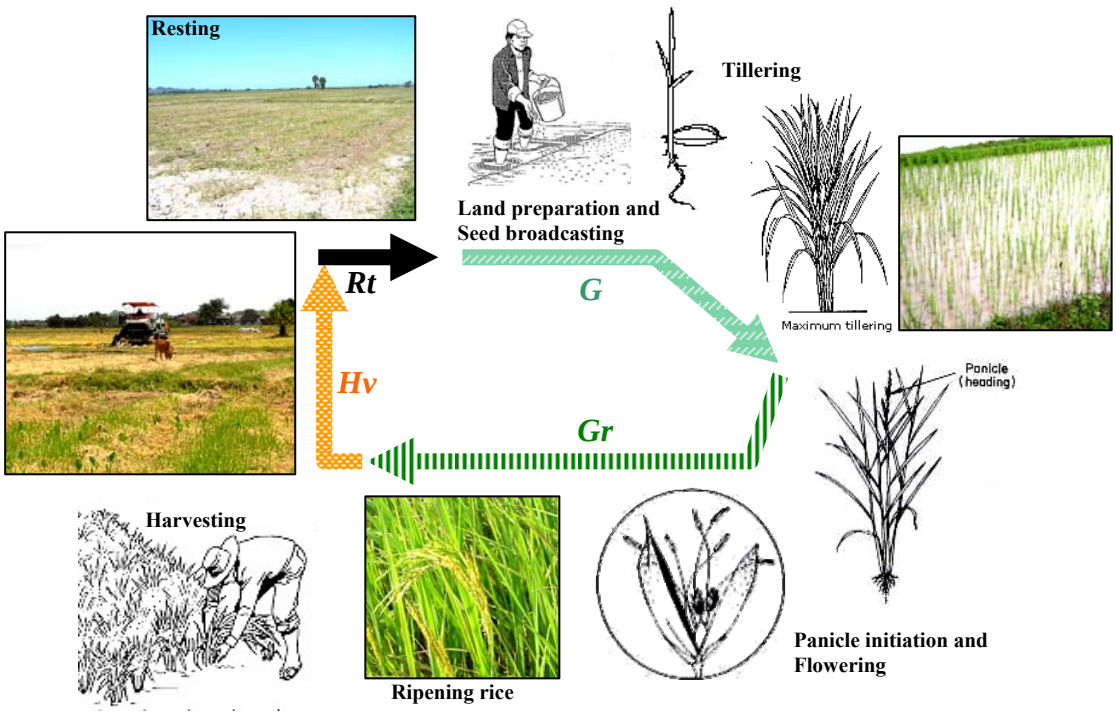


Figure 3. Phenology of the rice plant at the study site. *G*= Growing stage, *Gr*= Generative stage, *Hv*= Harvesting stage, and *Rt*= Resting stage.

Research questions

1. Does germ cell production and reproductive activity including sperm quality of *B. indica* in southern Thailand vary between seasons?
2. Does the seasonal variation in the gonadal and reproductive activities of *B. indica* correlate with the ambient environmental conditions?

Objectives of this study

The objectives of this study are as follows:

1. To determine the seasonal changes in germ cell production and reproductive activities including the sperm quality of *B. indica*.
2. To investigate the epididymal cell activities of *B. indica* in relation to the variable sperm quality and its reproductive condition.
3. To investigate the relationship between *B. indica* reproductive activities and the environmental conditions, especially the stage of rice cultivation.

CHAPTER 2
MATERIALS AND METHODS

1. Study site and sample collection

The Great Bandicoot rats, *B. indica*, obtained for the present study came from rice fields in Donpradu subdistrict, Pakphayun district, Phatthalung province, southern Thailand (7^{o} 16' N, 100^{o} 18' E) (Fig. 4). The region of Pakphayun district has two principal seasons, the wet and the dry seasons. In this study, a wet season period is categorized when the heavy rain and the high water level in the ricefield occur. The data of the monthly total rainfall at Phatthalung province from the Thai Meterological Department for a period of 12 years (1995-2006) showed that the heavy rain occurs between September to December, and includes January in some years, with having the highest rainfall in either November or December (Table 3 in Appendix A). January is a transition period from the wet to the dry seasons when rainfall gradually decreases, but in this month the water level in paddy field is still high. Thus, in this study, the study site has a wet season from September to January and a dry season from February to August (The Pakphayun Public Health; http://www.pyhd .moph.go.th/data/gen.pdf). Samples were collected bimonthly over a period of 24 months between September 2004 and September 2006. Animals* were randomly trapped using 50 metal live-traps (18 x 35 x 18 cm^3) per time placed on the ground of the paddy field at the study site (approximately 2 km^2). They were then transported to the laboratory at the Department of Biology, Prince of Songkla University, Hat Yai, Songkhla province.

Environmental parameters in the study site (phenology of rice, temperature, rainfall, humidity and photoperiod) were recorded monthly for the two year period of study when these environmental factors were available from the nearest station of the Thai Meteorological Department, at Pakphayun district. The phenology of rice was recorded directly from the study site.

* The animal collection of this work was under the Ethics Committee approval (No. MOE.0521.11/244 Ref. 12/51).

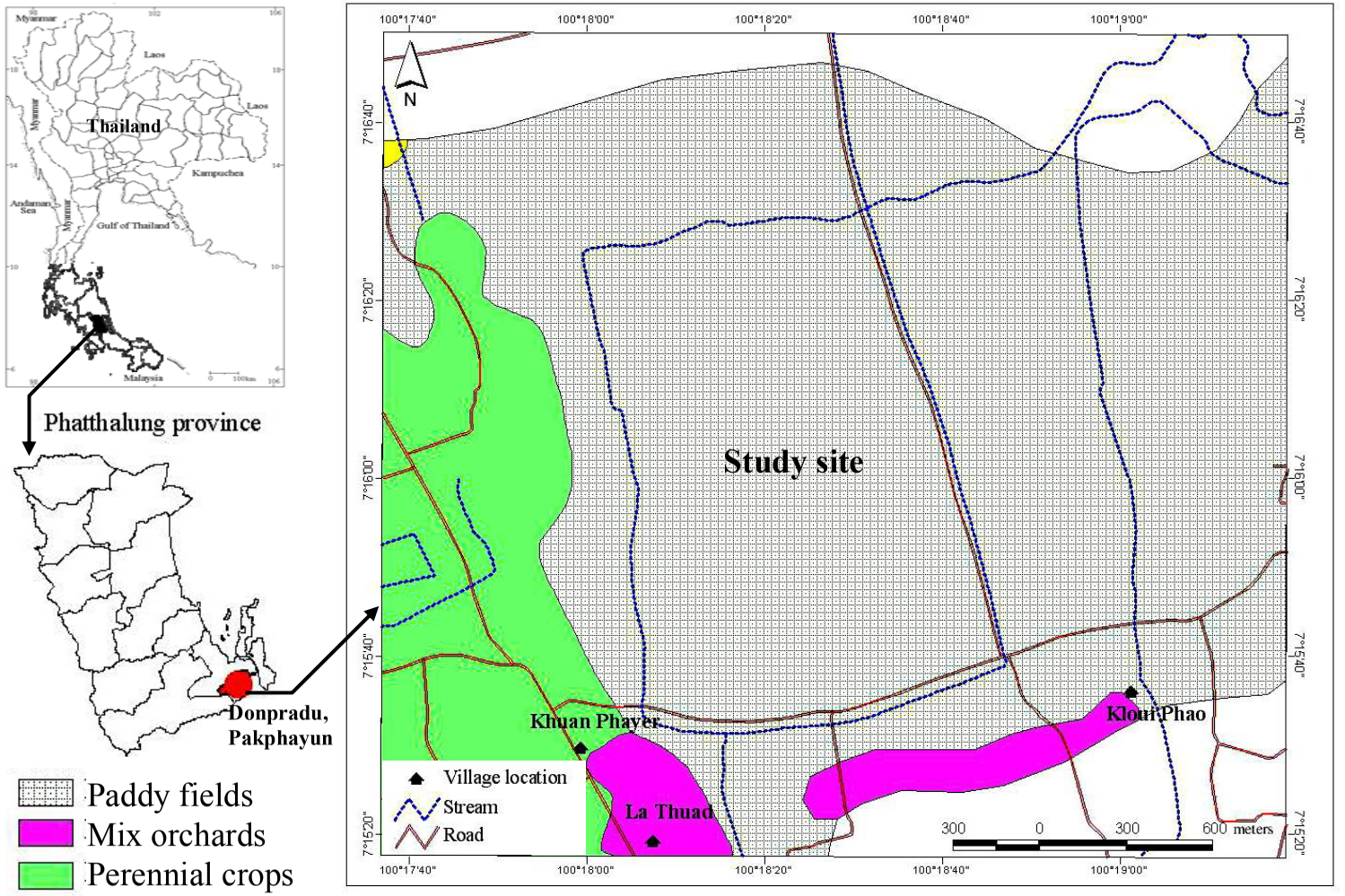


Figure 4. Map of study site and surrounding area. (printed by GEO-Informatics Research Center for Natural Resource and Environment, Prince of Songkla Univerisity)

2. Assessment of reproductive status

Animals were anaesthetized using intraperitoneal injection of pentobarbitone sodium (50 mg kg^{-1} bodyweight; Nembutal, Libourne, France). The body mass of each specimen was then recorded and the animals were classified as either juveniles or adults according to their fur characteristics, body weights, and head-body lengths (Fig. 5). Males were considered to be mature if the head-body length exceeded 265 mm and females were considered as sexual mature if the head-body length was at least 245 mm and bodymass exceeded 395 g (Boonsong, 1984). Adult females also generally had prominent teats and perforate vaginae. The testes, epididymides, seminal vesicles, prostates and preputial glands from the males, and the ovaries, uteri and preputial glands from the females were removed, cleaned, and weighed to the nearest 1 mg. In females the number of embryos, fetuses, and placental scars, if present, was recorded. Pregnancies were only detected when uterine swellings were present thus animals with preimplantation embryos would have been undetected.

3. Light microscopic (LM) investigations

3.1 Female rats

Ovaries from non-pregnant adult *B. indica* females with the greatest ovary weight in each sample were prepared for histology. Ovaries were fixed in Bouin's fluid for about 18-24 h, processed through the paraffin embedding technique (Bancroft and Gamble, 2002), and serially sectioned at 6 μm thicknesses. All ovarian sections were stained with Harris's haematoxylin and eosin, and observed with a light microscope for determining the ovarian follicles. Ovarian follicular development was assessed according to the guidelines of Ross *et al.* (2003). The number of mature (or Graafian) follicles from each ovary of all females was recorded when the serial sections of the mature follicles showed the presence of an antrum, the cumulus oophorus and the corona radiata as well as the visible oocyte.

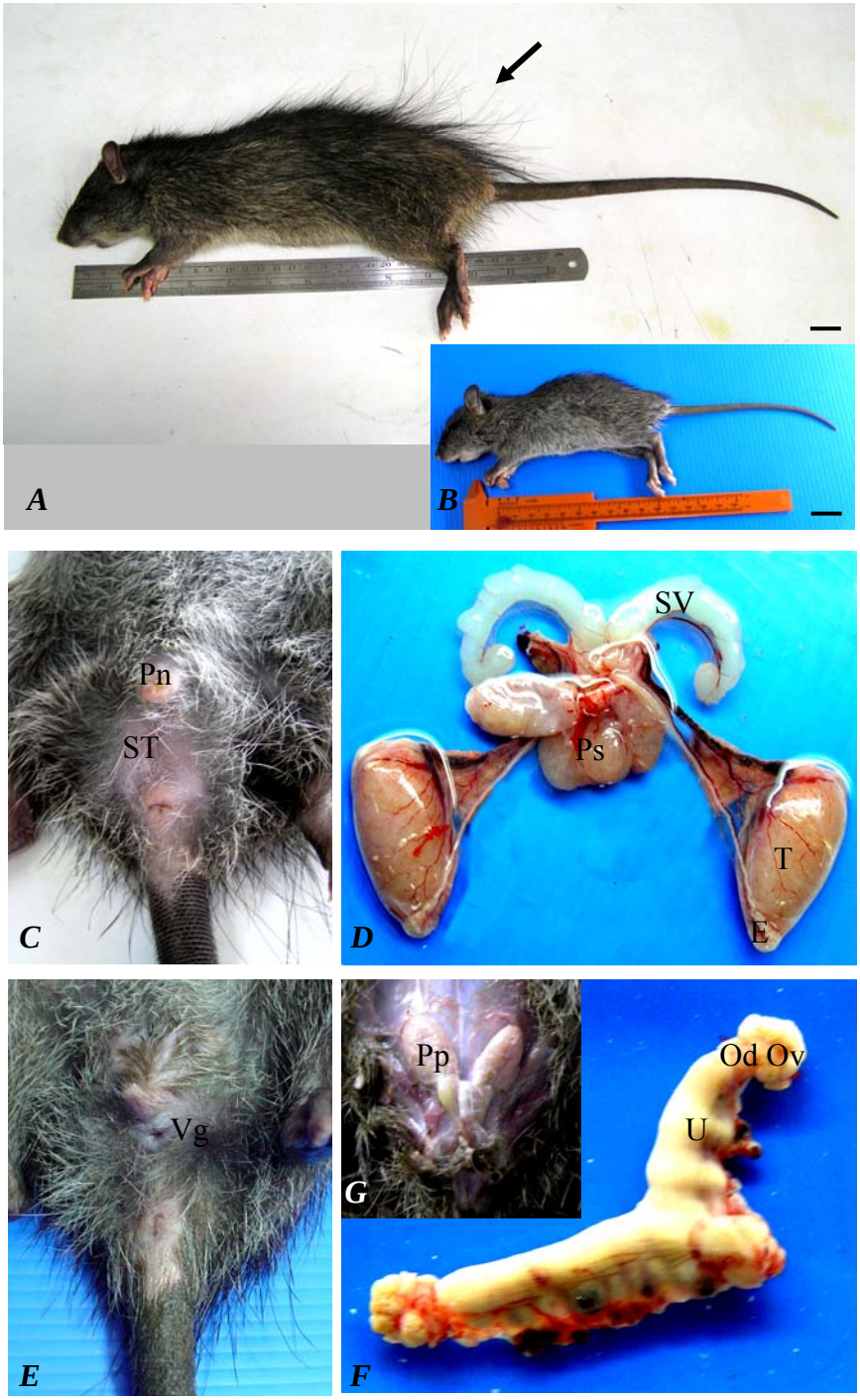


Figure 5. External characteristic of mature (*A*) and immature (*B*) *Bandicota indica* and morphology of reproductive organs in male (*C,D*) and female (*E,F*) as well as the preputial glands (*G*) of mature *B. indica*. Note the black guard hair on dorsal fur of adult rats (arrow). E= epididymis, Ov= ovary, Od= oviduct, Pn= penis, Pp= preputial glands, Ps= prostate glands, ST= scrotum, SV= seminal vesicle, T= testes, U= uterus, and Vg= vagina. Scale bars= 2 cm.

3.2 Male rats

Testes from adult *B. indica* males with the greatest testes weight in each sample were removed and fixed in either Bouin's fluid or 10% neutral buffered formalin, and processed for paraffin sectioning (Bancroft and Gamble, 2002). The 6 μm thickness sections were stained with Harris's haematoxylin and eosin, and 50 cross-sections of seminiferous tubule per testis were examined for the spermatogenic activity with a light microscope at 400x and 1000x magnifications.

Epididymides from immature and mature male *B. indica* were fixed with 10% neutral buffered formalin for LM investigation or 3% glutaraldehyde and 3% paraformaldehyde for the electron microscopic (EM) study. This study mainly focused on the cauda epididymis where is involved in the sperm maturation and storage. For the epididymal histological study, small pieces of 10% neutral buffered formalin fixed cauda epididymis were processed following standard histology techniques (Bancroft and Gamble, 2002). Epididymides were sectioned at 6 μm thicknesses, stained with Harris's haematoxylin and eosin, and observed with a light microscope. The diameters of the epididymal lumen and tubules as well as the epithelium height were measured by using the ocular micrometer at 400x and 1000x magnifications. The different cell types in the epididymal epithelium were examined.

3.3 Evaluation of sperm morphology and quality

The cauda segment of the right epididymis from each individual that had been fixed with 10% neutral buffered formalin, was removed and placed in 0.1 M phosphate buffered saline (PBS). Spermatozoa were stripped from the tubules and a small drop (~0.05 ml) of sperm suspension was placed on a glass slide. The sperm morphology was observed using the phase contrast and/or Nomarski differential interference optics microscope. Sperm head and tail morphologies were determined and recorded from 200 heads and 100 tails of randomly selected spermatozoa per animal. The sperm quality was assessed based on the percentage of normal sperm morphology in the population. The criteria used for classification of the sperm morphology were as follows:

For assessing the normality of the sperm head structure, it is assumed that common head shapes within the epididymal sperm population of males collected

at a time when females were also pregnant in the population represented sperm that have the potential to fertilize eggs and can thus be classified as ‘normal’. The shapes of these sperm heads ranged from globular to conical with the latter narrowing towards the site of the tail attachment. Where the sperm head differed from this in either (1) shape, (2) size or (3) more than one head attached to one tail, they were categorized as ‘an abnormal sperm head’.

For categorizing the normal shape of the sperm tail, it is assumed that sperm with tails that were either straight or had a gentle bend were ‘normal’ with the latter being fixed at the time that sperm motility was occurring. Where the sperm tail differed from this in either (1) having a sharp bend, a twisted bend, or a coiled in the neck or along the sperm tail length, (2) presence of cytoplasmic droplets in the midpiece, (3) or more than one flagellum attached to one head, they were assumed to be ‘an abnormal sperm tail’.

4. Electron microscopic (EM) investigations

This study investigated the ultrastructure of the sperm morphology and the epididymal epithelium from the cauda region of mature *B. indica* using the transmission electron microscope (TEM). Comparisons of the epithelial ultrastructure between the cauda epididymis of the adult *B. indica* and either the caput segment of the adult *B. indica*, the cauda epididymis of immature *B. indica*, or the cauda epididymis of the closely related species, *B. bengalensis* were also investigated. The epididymides of *B. indica* were obtained from males with the greatest testes weight in each of the seasons of this study, whereas the cauda epididymides of *B. bengalensis* were obtained from 4 sexually mature males collected by Assoc. Prof. Dr. William G. Breed in Penang, Malaysia ($5^{o}25'$N, $100^{o}19'$E), during the dry season of April 1994. The small pieces of these epididymides of *B. indica* and *B. bengalensis* were rapidly fixed in 3% glutaraldehyde and 3% paraformaldehyde, made up in 0.1 M phosphate buffer, pH 7.4. Specimens were post fixed in 1% osmium tetroxide, dehydrated through a graded series of acetones and subsequently embedded in Epoxy resin. Subsequently, 0.5 to 1 μm thick sections were stained with toluidine blue, and the ultrathin sections were stained with uranyl acetate and lead citrate, and examined with

a Philips CM 100 TEM in Adelaide Microscopy, the University of Adelaide, Australia and a JEOL JEM-2010 TEM in the Scientific Equipment Center, PSU, Thailand.

For the scanning electron microscopy (SEM), fixed spermatozoa extruded from the cauda epididymides of adult *B. indica* were placed on a polylysine coated coverslips. Spermatozoa were dehydrated by passing the coverslips through a graded series of acetones and then critical point-dried, and sputter-coated with gold and carbon. The external characteristics of the sperm morphologies were investigated with a Philips XL20 SEM in Adelaide Microscopy, the University of Adelaide, Australia and a JEOL JSM-5800LV SEM in the Scientific Equipment Center, PSU, Thailand.

5. Data analysis

The data were statistically analyzed using the SPSS program version 11.5 for Windows and the differences were considered significant when $p<0.05$. The Chi-square test was used to compare the number of pregnancies between the wet and the dry seasons. The differences of body mass, the weights of reproductive organs, the number of fetuses, placental scars and mature follicles, and the sperm quality as well as the epididymal parameters among those samples taken throughout the study period were statistically tested with either the One-way Analysis of Variance (ANOVA) when the equal variances assumed or the Brown-Forsythe of Robust test for non-equal variances (see Vanichbuncha, 2006). The relationships between the cultivation stage of the rice plant and the male and female reproductive activities were statistically analyzed by either the One-way ANOVA when the equal variances assumed or the Brown-Forsythe of Robust test for non-equal variances. The Unpaired Student's *t* test was used to compare the body mass, the weights of reproductive organs and preputial glands, the number of fetuses, placental scars and mature follicles in the uterus, the sperm quality, the epididymal parameters between those from the wet and the dry seasons. The Multiple Linear Regression and Pearson's Correlation tests were used to investigate the relationship between environmental factors (temperature, rainfall, humidity and photoperiod) and the reproductive activities of males and females.

CHAPTER 3
RESULTS

1. Ambient environmental conditions at the study site

The region of Pakphayun at Phatthalung province, where this study was performed, has a wet season from September to January and a dry season from February to August (see in the materials and methods for detail). During the time of the present investigation the rainfall in the two wet seasons differed markedly with around three times as much rain falling in the wet season of the year 2005 than that of 2004 (Fig. 6). In this region, the average ambient temperature was 28 ^{o}C with a drop of 1-4 ^{o}C in the wet season and the average relative humidity was 80% being highest in December during the end of the wet season. The photoperiod was 12.1 h/day in average and slightly varied throughout the year (Fig. 6).

In the study area, rice was grown once per year with the beginning of cultivation in all paddy fields being synchronous. The cultivation period was divided into four main stages: the growing, generative, harvesting and resting stages. The growing stage was approximately four months long from the time of the land ploughing in the late dry season (June or July) to the maximum tillering of rice in the wet season (September or October). The generative stage of the rice plant took place in the wet season from October to December, and the harvesting occurred in a period of the late wet season to the early dry season (January-March). The resting stage included the rest of the dry season (March-June) (Fig. 6).

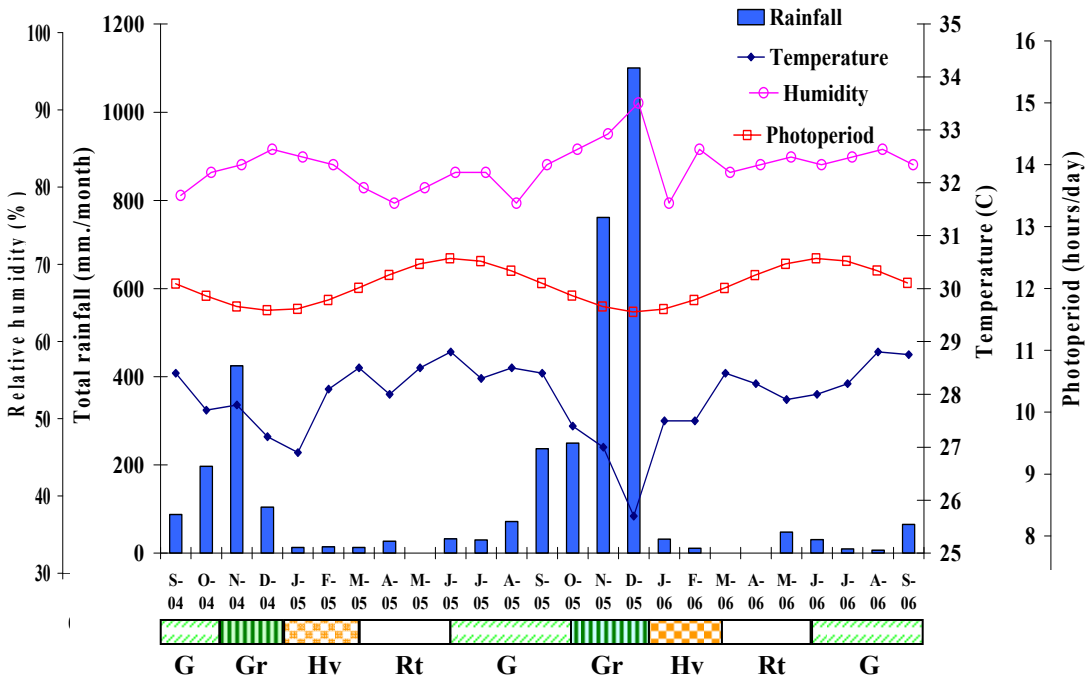


Figure 6. Monthly total rainfall and mean temperature, photoperiod, and relative humidity between September 2004 and September 2006 at the study site (source: The Thai Meteorological Department). The bars represent the cultivation stage of the rice plant: growing (G), generative (Gr), harvesting (Hv), resting (Rt) stages.

Table 1. The occurrence of pregnant and immature rats and the average number (± S.E.) of fetuses in *B. indica* during September 2004 to September 2006.

Seasons	Months	Number of total adult females	Pregnant animals		Number of immature male and female rats
			Number of pregnancies	Average number of fetuses	
Wet I	Sep-04	13	2	6.5±0.5	0
	Oct-04	17	3	5.3±0.3	3
	Dec-04	13	5	8.8±0.8	2
Dry I	Feb-05	0	0	-	3
	May-05	6	1	3	9
	July-05	4	0	-	19
Wet II	Sep-05	4	0	-	5
	Nov-05	13	5	6.2±0.7	0
	Jan-06	19	5	8.8±1.0	4
Dry II	Mar-06	14	2	4.5±0.5	19
	May-06	7	2	7.0±2.0	2
	Jul-06	9	1	8	0
Wet III	Sep-06	10	3	8.7±2.6	2

Seasons: Wet (September-January); Dry (February-August).

2. Variations in female reproductive activities

Data on the reproductive biology of the female bandicoot rats showed that many females (23 out of 29 pregnancies) were visibly pregnant in the wet season (Table 2). In the first year of the study all 10 out of 11 pregnancies occurred from a total of 43 individual investigations in the wet season with just one animal out of 10 being pregnant in the subsequent dry season (Tables 1,2; Fig. 7). In the second year of the study period, apart from there being no pregnancies (out of 4 individuals) at the beginning of wet season in September 2005, 5/13 of the females were found to be pregnant with another 5/19 being pregnant in January during the end of the wet season (Table 1; Fig. 7). For the four remaining sampling periods (March-September 2006), one or more pregnant females were found with a total of 8 out of 40 females examined in this period being pregnant (Fig. 7).

During the first wet season, the average number of fetuses in the first three samples ranged from 5.3 to 8.8, whereas the one pregnant animal in the subsequent dry season had only three fetuses (Table 1). In the second wet season, the average number of fetuses was 6.2 and 8.8 in November 2005 and January 2006 samples, respectively. Five pregnant females collected later in the dry season had 4.5 fetuses in March, 7.0 in May and 8 in July 2006 on average (Table 1). In the last sample (September 2006), the number of fetuses averaged 8.7 for 3 pregnant animals (Table 1). Even though only one animal out of the 10 collected in the first dry season was visibly pregnant, four out of nine adult females collected at this time had a significantly higher number of placental scars (Table 2; Table 1 in Appendix A) indicating that they had been pregnant sometime previously. Almost all individuals obtained in the second dry season also had placental scars (Table 2; Table 1 in Appendix A). The greatest number of immature rats (19 rats) occurred in samples collected in the dry season, mainly in July 2005 and March 2006 (Table 1), indicating that a cohort of individuals had been borne and entered the population in the recent past.

The body mass of non-pregnant adult females was 512 ± 6.7 g (n= 100) with no statistical significant differences among the samples collected (p>0.05) (Table 2). The average ovary weights was significantly greater in the wet season

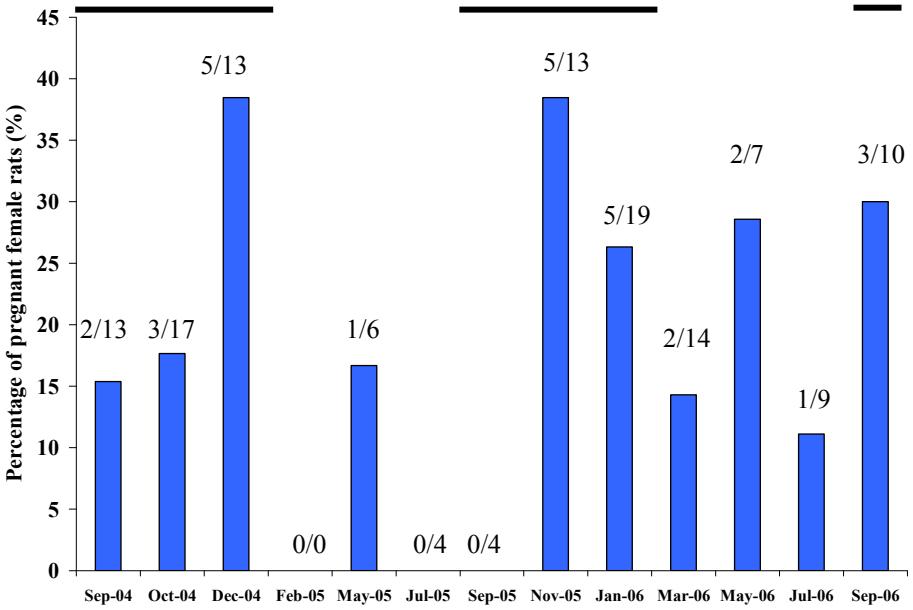


Figure 7. Percentage of pregnant female *B. indica* during September 2004 to September 2006. The number above the histogram is the number of pregnant versus the total number of adult females. The black lines represents the period of the wet season.

Table 2. Mean (± S.E.) body mass, the weights of female reproductive organs and preputial gland, and the number of pregnant animals, mature follicles, and placental scars of adult female *B. indica* during the wet and the dry seasons.

Seasons	Non-pregnant females (Adult)							Number of pregnant females (% of total pregnancies)
	n	Body mass (g)	Ovary weight (mg)	Uterus weight (mg)	Preputial gland weight (mg)	No. of mature follicles/ rat	No. of placental scars/ rat	
All								
Wet	66	506±8	90±7[a]	541±50	325±26[*]	4.9±0.3[a]	10.5±0.8	23 (79.3%)
Dry	34	525±12	64±5[b]	471±49	319±44	3.8±0.3[b]	12.2±1.0	6 (20.7%)
Year I								
Wet	33	503±10	92±9[a]	558±87[a]	-	5.2±0.6[a]	8.7±0.9[a]	10 (34.5%)
Dry	9	496±24	43±5[b]	204±31[b]	198±27	2.8±0.4[b]	16.5±2.3[b]	1 (3.5%)
Year II								
Wet	26	514±14	100±12[a]	583±59	346±31	4.4±0.3	13.0±1.3	10 (34.5%)
Dry	25	536±13	71±6[b]	567±54	363±57	4.5±0.5	11.3±1.0	5 (17.2%)
Year III								
Wet	7	485±23	39±6	304±36	248±39	5.3±0.6	8.6±1.5	3 (10.3%)

Values within a column followed by a different superscript differ statistically (Unpaired student's *t* test, $p<0.05$). Seasons: Wet (September-January); Dry (February-August). Incomplete data from fewer samples ($n=33$) than the total sample numbers ($n=66$) are shown with an asterisk (*).

(90 mg) than in the dry season samples (64 mg) (p<0.05) (Table 2). The ovary weights ranged from 83.1 to 106.6 mg for the 33 non-pregnant animals obtained in the first wet season (92 mg) that was significantly higher than that of the following dry season (43 mg) (p<0.05) (Table 2). In the first dry season, the ovary weight averaged 50 mg in May 2005 and was significantly lowest in July 2006 (35.3 mg) (Fig. 8). During the second year of study, significantly heavier ovaries were obtained in the wet season (100 mg) than those in the dry season (71 mg) (p<0.05) (Table 2). In the second wet season, ovary weight was 36.5 mg in September 2005 and began to increase in November (58.9 mg) and was heaviest in January 2006 (142.6 mg) (Fig. 8). The ovary weights of the last four samples were low and ranged from 38.5 to 75 mg (Fig. 8).

The uterus weights of individuals were significantly greater in the first wet season samples (558 mg) than in those collected in the dry season (204 mg) (p<0.05) (Table 2; Fig. 9), whereas in the second year of study, uterus weights were not significantly different between the wet (538 mg) and dry seasons (567 mg) (p>0.05) (Table 2; Fig. 9). Preputial glands, that are a conspicuous feature in both the female and male bandicoot rats, were not available for *B. indica* in the first wet season. However, their weights during the first dry season (198 mg) were less than those in the second wet season (346 mg) (Table 2; Fig. 9).

Ovarian histology revealed that the ovarian follicular development or folliculogenesis occurred in ovaries of all adult non-pregnant *B. indica* throughout the year with evidence of the presence of mature follicles (Graafian follicles) (Figs. 9, 10). The number of mature follicles varied between seasons but was significantly greater in the wet season samples (4.9) than those of the dry season samples (3.8) (p<0.05) (Table 2). Likewise in the first year of study, a significantly higher number of mature follicles were found in the 11 animals collected in the wet (5.2) than in the dry seasons (2.8) (p<0.05) (Table 2) with a peak of the follicles (7.9) occurring in September 2004 in the wet season (Fig. 9). Two samples in the first dry season (May and July 2005) had a low number of mature follicles and were lowest in July 2005 (Fig. 9). In the second year of study, the number of mature follicles was not significantly different between the wet (4.4) and dry (4.5) seasons (p>0.05) (Table 2).

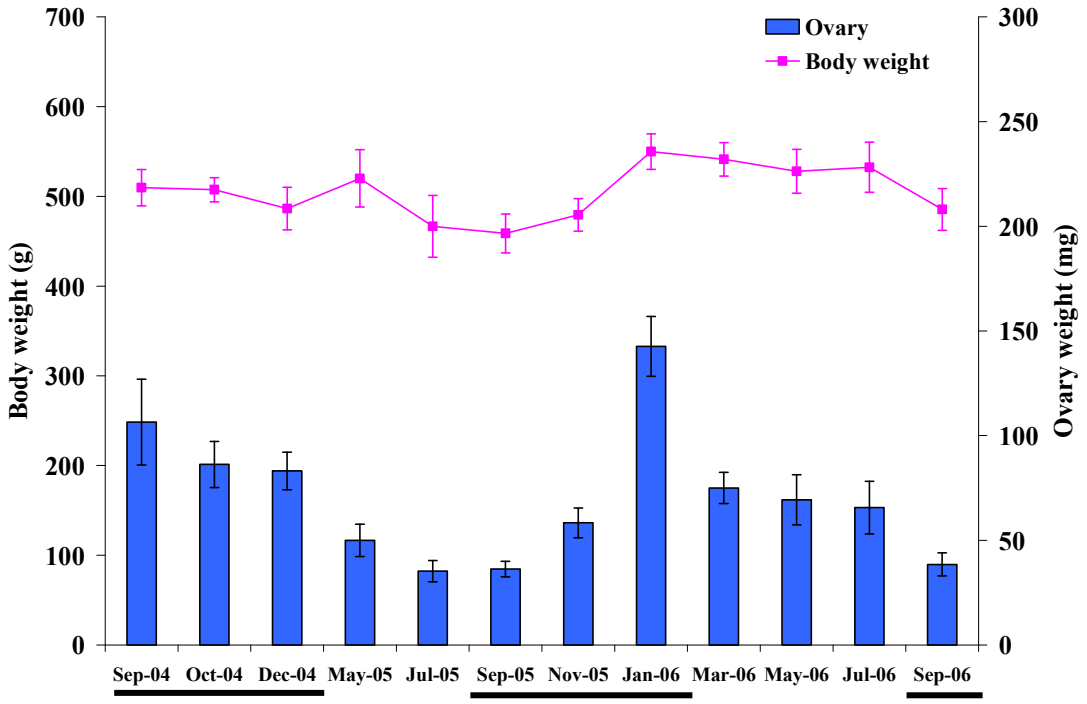


Figure 8. Means (±S.E.) body weight and ovary weight in adult female *B. indica* during September 2004 to September 2006. The black line represents the periods of the wet season.

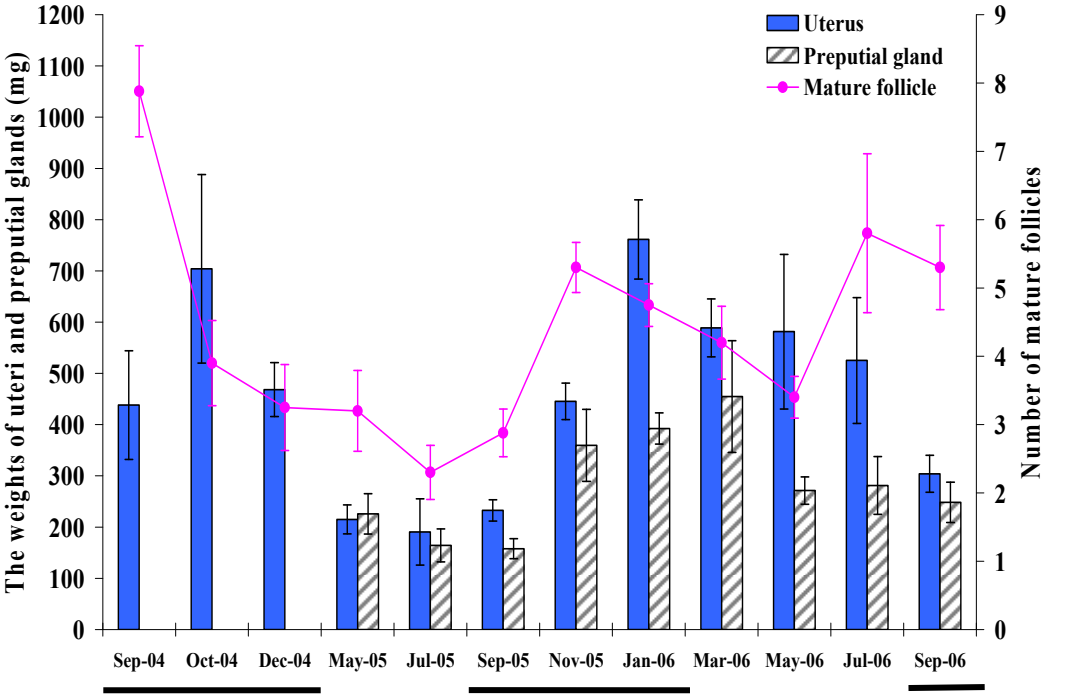


Figure 9. Seasonal variations in the uterus weight, preputial gland weight and the number of mature follicles in adult female *B. indica* (Mean±S.E.). The black line represents the period of the wet seasons. Preputial glands were not available from September to December 2004.

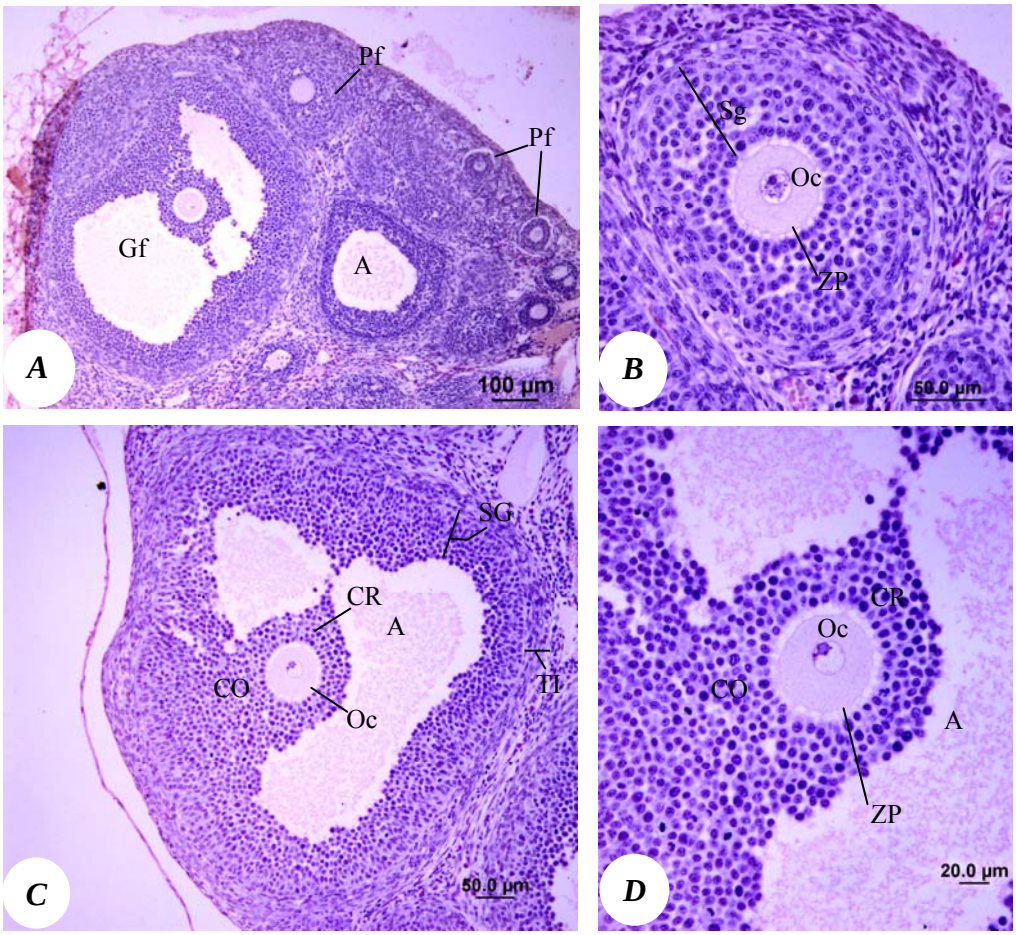


Figure 10. LM photomicrographs of the ovary in *B. indica* (H&E). *A:* The cortex of an ovary containing various ovarian follicles; the primary (Pf) and the mature or Graafian follicles (Gf). *B:* The primary follicle shows stratum granulosum (SG) surrounding the oocyte (Oc). *C-D:* The mature follicles with a large antrum (A) containing an oocyte (Oc) embedded within the cumulus oophorus (CO). CR= corona radiata, TI= theca interna, ZP=zona pellucida.

In addition to the relationship between the female reproductive activities of *B. indica* and the environmental conditions, a multiple linear regression analysis showed that their reproduction were not significantly correlated to the environmental factors; ambient temperature, rainfall, photoperiod and humidity ($p>0.05$) (Table 9 in Appendix E). However, these reproductive activities coincided with the cultivation stage of the rice plant (growing, generative, harvesting and resting stages) ($p<0.05$; ANOVA). Their body weight, ovary weight, and preputial gland weight were highest during the harvesting period (546 g, 111.4 mg, and 421 mg, respectively) (Table 3). The number of placental scars was not significantly different between the cultivation stages of the rice plant ($p>0.05$). Ovarian histology revealed that mature follicles were found in all samples throughout the cultivation stages of the rice plant, but the number of follicles was not significantly different among the stages of the rice plant ($p>0.05$) (Table 3). The occurrence of pregnant females was not significantly different between the stages of the rice plant ($p>0.05$; Chi-square), but the number of pregnancies tended to be high during the growing to the generative stages of the rice plant (9 and 10 animals, respectively) and decreased in the harvesting period (7 animals) as well as being lowest during the resting period (3 animals) (Table 3). The average number of fetuses was also not significantly different between the cultivation stages of the rice plant ($p>0.05$), but it was higher in a period between the growing and harvesting stage (6.9, 7.5 and 7.6, respectively) than those of the animals collected in the resting period (5.7) (Table 3). However, the occurrence of immature rats was significantly different among the cultivation stages of the rice plant ($p<0.05$; Chi-square). The greatest number of immature rats occurred in the growing and harvesting stages of the rice plant (29 and 26 animals, respectively), whereas in the generative period it was lowest (3 animals) (Table 3).

Table 3. Comparison of mean (±S.E.) body mass, the weights of female reproductive organs and preputial gland, and the number of pregnant females, fetuses, placental scars, mature follicles and immature *B. indica* among the cultivation stage of the rice plant.

Rice plant cycle	Adult pregnant females		Adult non-pregnant females							Number of Immature male and female rats (% of total immature rats)
	n (% of total pregnancies)	Number of fetuses	*n*	Body weight (g)	Ovary weight (mg)	Uterus weight (mg)	Preputial gland weight* (mg)	Number of placental scars/ rat	Number of mature follicles/ rat	
Growing stage	9 (31.0%)	6.9±1.0	48	502±9^{ab}	72.1±7.1^{b}	472.9±66.2	229±25^{b}	10.3±1.1	4.6±0.4 (*n*=28)	29 (42.7%)†
Generative stage	10 (34.5%)	7.5±0.7	16	483±15^{b}	70.8±6.4^{b}	456.9±30.9	360±70^{ab}	10.4±0.8	4.7±0.4 (*n*=7)	2 (2.9%)†
Harvesting stage	7 (24.1%)	7.6±1.0	26	546±14^{a}	111.4±10.7^{a}	681.7±51.2	421±52^{a}	11.6±0.8	4.4±0.3 (*n*=9)	26 (38.2%)†
Resting stage	3 (10.4%)	5.7±1.8	10	524±19^{ab}	59.7±7.5^{b}	398.6±94.6	249±24^{b}	14.6±2.7	3.3±0.3 (*n*=10)	11 (16.2%)†

Values within a column followed by a different differ statistically (ANOVA or Brown-Forsythe of Robust test, $p<0.05$).

†A statistically significant difference between values within a column (Chi-squares test, $p<0.05$).

Incomplete data from fewer samples ($n=67$) than the total sample numbers ($n=100$) are shown with an asterisk (*).

Growing stage = June-October; Generative stage = October-December; Harvesting stage = January-March; and Resting stage = March-June.

3. Variations in male reproductive activities

3.1 Variation in body mass and reproductive organs of the male *B. indica*

The body weight of 15 adult males in the first wet season ranged from 529 g to 580 g (Fig. 11) and the average body weight (557 g) was statistically significantly greater than that of 11 males in the dry season (482 g) ($p<0.05$) (Table 4). In the first dry season, the body weight averaged 549 g for the 4 animals obtained in May 2005, and was lowest in July 2005 (443 g) (Fig. 11). During the second year of study, significant differences of body weight between seasons were not found ($p>0.05$) (Table 4), but the lowest body weights (400 g) were found in the 2 animals obtained in March 2006 during the dry season. In the last three samples (May to September 2006), the body weights gradually increased and ranged from 460 g to 536 g (Fig. 11).

The average testis weight of adult males was 0.42±0.02 % of body weight and in the wet season it was significantly higher (2.41 g) than in the dry season (1.83 g) ($p<0.05$) (Table 4). Testis weights from individuals during the first wet season were around 0.5% of body weight but this dropped to 0.3% of body weight late in the dry season (1.73 g) and was statistically significantly lower than those for the animals collected in the preceding wet season (2.74 g) ($p<0.05$) (Table 4; Fig. 13). In the second wet season, testis weights were similar to that of the first wet season. Average testis weights dropped to around 0.2% body weight at the beginning of the second dry season (March 2006) but in the last two samples of this season (May-July 2006) they became similar to those of the second wet season samples (Fig. 11). The results, showed there was no statistically significant differences between the two seasons of the second year study ($p>0.05$) (Table 4). In the last sample (September 2006), the testis weights were 0.4% of the body weight (Fig. 11).

Testicular histology revealed that spermatogenic activity was taking place in all adult *B. indica* males from every studied month in the wet and the dry seasons with evidence for the presence of spermatozoa in the seminiferous tubules (Table 5; Fig. 12). Nevertheless, a few of the seminiferous tubules in 8 of the 44 males had degenerating germ cells with only spermatogonia, and occasional primary spermatocytes (Table 5; Fig. 12B-D). The diameter of these seminiferous tubules was

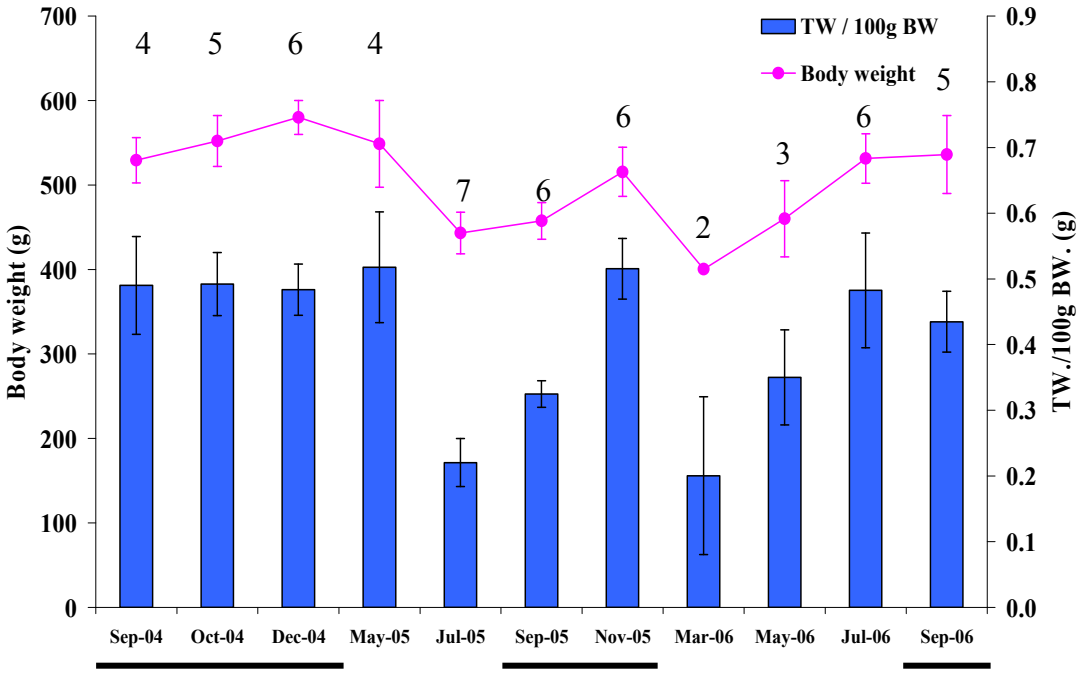


Figure 11. Seasonal variations of the body weight and the testis weight/100g body weight (±S.E.) in the adult male *B. indica* between September 2004 and September 2005. The black lines represents the period of the wet season. Numbers above the graph indicates the number of animals.

Table 4. Comparison of the mean (± S.E.) body mass and the weights of testes, seminal vesicles, prostate glands, epididymides and preputial glands of adult *B. indica* between the wet and the dry seasons.

Seasons	*n*	Body weight (g)	Testis weight (g)	TW./100g BW. (g)	Seminal vesicle weight (g)	Prostate gland weight (g)	Epididy-mis weight (g)	Preputial gland weight (g)
All								
Wet	32	527±13	2.41±0.13^{a}	0.45±0.02^{a}	0.32±0.03^{a}	0.19±0.02	0.28±0.06*	0.57±0.09*
Dry	22	485±18	1.83±0.25^{b}	0.36±0.04^{b}	0.19±0.04^{b}	0.20±0.03	0.25±0.03	0.47±0.09
Year I								
Wet	15	557±15^{a}	2.74±0.18^{a}	0.50±0.03^{a}	0.36±0.05^{a}	0.13±0.02	-	-
Dry	11	482±28^{b}	1.73±0.39^{b}	0.33±0.06^{b}	0.13±0.05^{b}	0.14±0.03	0.24±0.05	0.24±0.06
Year II								
Wet	12	487±19	2.04±0.19	0.42±0.04	0.28±0.03	0.22±0.02	0.21±0.01	0.59±0.13
Dry	11	488±25	1.93±0.32	0.40±0.06	0.25±0.06	0.26±0.04	0.26±0.03	0.70±0.15
Year III								
Wet	5	536±46	2.32±0.27	0.43±0.05	0.33±0.05	0.24±0.07	0.45±0.18	0.51±0.09

Values within a column followed by a different superscript differ statistically (student's *t* test, $p<0.05$).
Seasons: Wet (September- January); Dry (February-August)
Incomplete data from fewer samples ($n=17$) than the total sample numbers ($n=32$) are shown with an asterisk (*).
Epididymides, and preputial glands were not available from Sep-Dec 2004 (Wet season I)

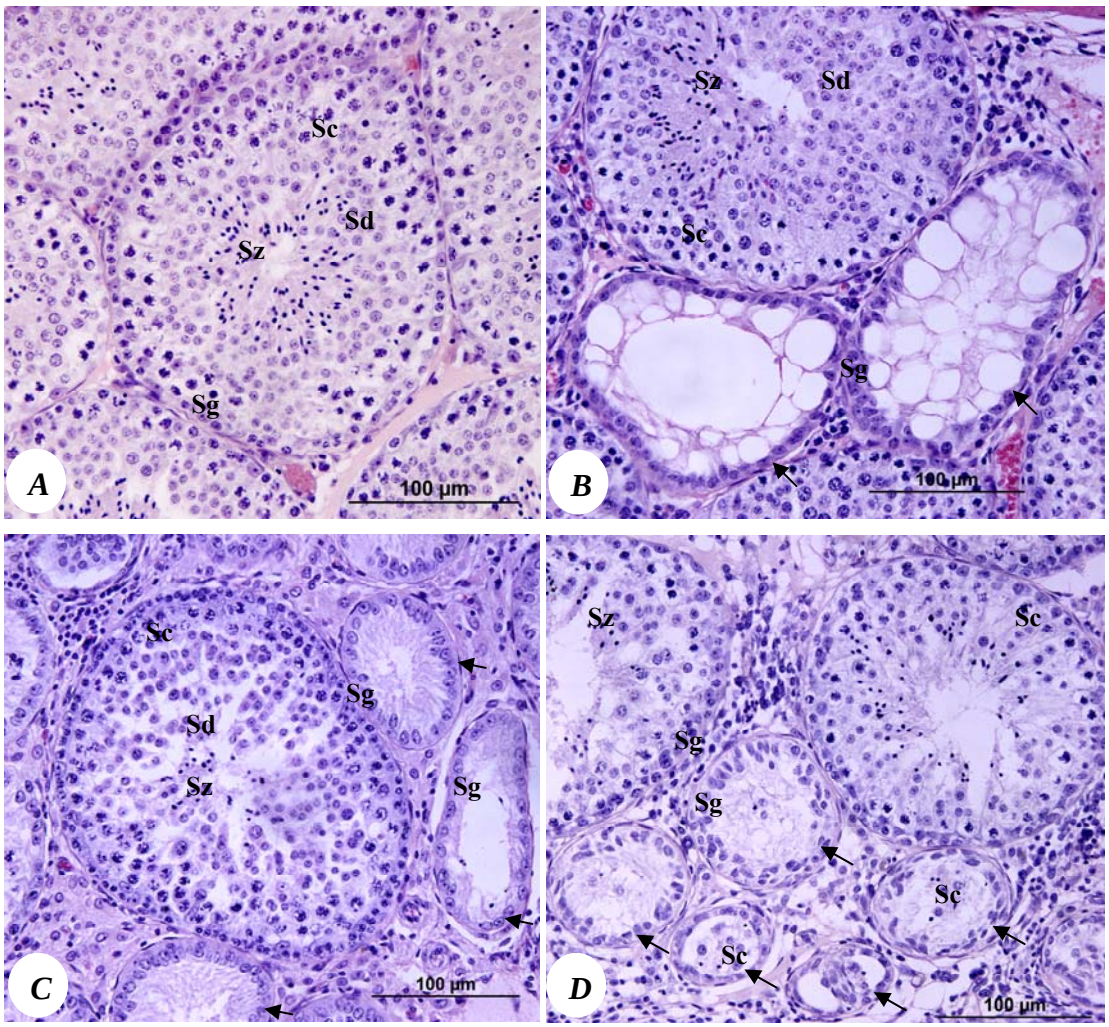


Figure 12. LM photomicrographs of mature *B. indica* testes (H&E). *A:* Active spermatogenesis occurred in seminiferous tubules in all seasons. *B-D:* Absence of spermatogenesis in some seminiferous tubules (arrows) of males in the dry season. Sg= Spermatogonia, Sc= Primary spermatocytes, Sd= Spermatids, Sz= Spermatozoa.

Table 5. The number of individuals and their reproductive condition based on the testicular histology of mature male *B. indica* during the wet and the dry seasons.

Number of mature males	Seasons				
	Year 1		Year 2		Year 3
	Wet	Dry	Wet	Dry	Wet
-Histological examinations	11	7	11	10	5
-Presence of spermatogenesis within all seminiferous tubules (%)	9(82%)	5(71%)	10(91%)	7(70%)	5(100%)
-Absence of spermatogenesis within some seminiferous tubules (%)	2(18%)	2(29%)	1(9%)	3(30%)	0(0%)

Seasons: Wet (September- January); Dry (February-August).

considerately less than those in which sperm were being produced (Fig. 12B-D). Of the males in which this was found, 5 out of 8 were individuals collected in the dry season (Table 5) and these males had smaller testis weights, but in all cases there were other seminiferous tubules in the same testes in which full spermatogenesis was occurring (Fig. 12B-D).

Comparing the male accessory sex glands, the weight of the seminal vesicles was statistically significantly lighter in the dry season (0.19 g) compared to the wet season (0.32 g) (p<0.05) (Table 4). In the second year of study a similar trend was evident but the differences were not statistically significant (p>0.05). The epididymides and preputial glands were not available in the first 3 samples collected between September and December 2004 (Fig. 13). However the weights of the epididymis, prostate gland, and preputial glands were not significantly different between the wet and the dry seasons during the rest of the study period (Table 4).

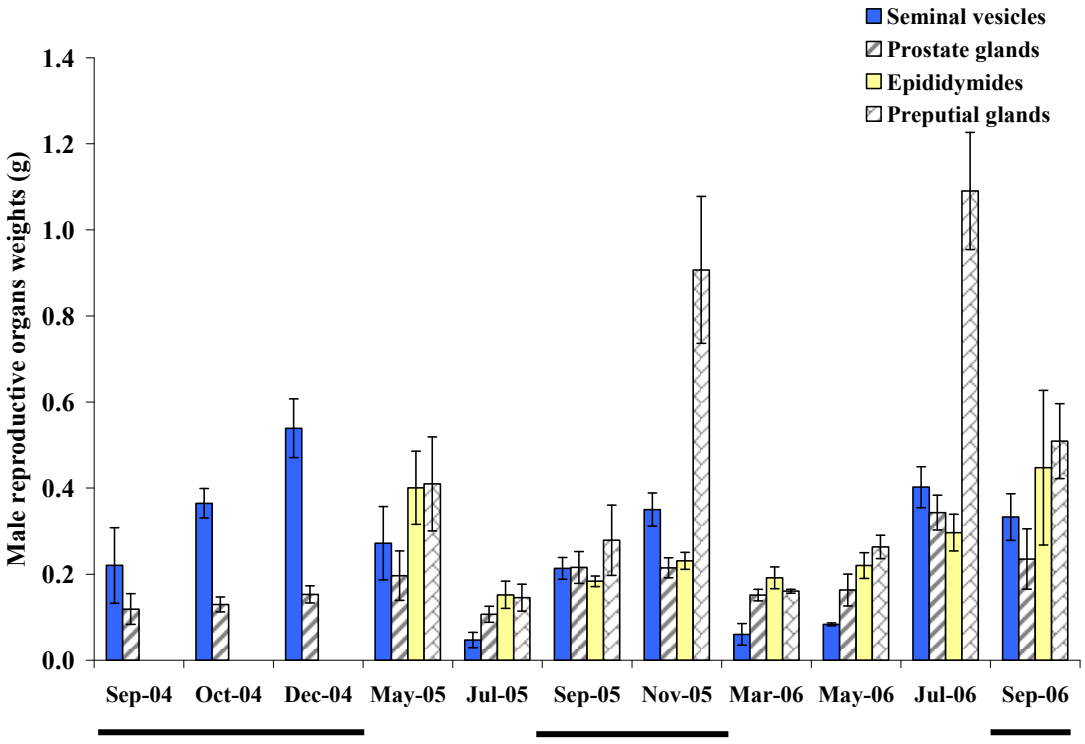


Figure 13. Comparison of the weights of the accessory sex glands and epididymides in adult male *B. indica* between September 2004 and September 2006. Values are the mean ± S.E. The black lines represents the period of the wet season. Epididymides and preputial glands were not available in September to December 2004.

When assessing any relationship between the male reproductive activity and the environmental conditions, it was found that the male reproductive characteristics were related to the phenology of the rice plant ($p<0.05$), but not to any other of the environmental factors measured (temperature, total rainfall, photoperiod and humidity) ($p>0.05$) (Table 9 in Appendix E). The testes weight and seminal vesicle weight as well as preputial gland weight of mature male *B. indica* were significantly different between the stages of the rice plant ($p<0.05$) but not that of the body weight, the testis weight/100g body weight, the weights of the prostate gland and epididymis ($p>0.05$). However, the tendencies for differences of these male reproductive parameters, excepting the epididymis weight, were similar and were highest in the generative stage of the rice plant but were lowest in the harvesting period (Table 6).

Table 6 Comparison of body weights and the weights of male reproductive organs and preputial gland of the adult male *B. indica* between the cultivation stages of the rice plant.

Rice plant cycle	*n*	Body weight (g)	Testes weight (g)	TW./100g BW. (g)	Seminal vesicle weight (g)	Prostate gland weight (g)	Epididymis weight* (g)	Preputial gland weight* (g)
Growing stage	33	503±14	2.03±0.17^{ab}	0.39±0.03	0.25±0.03^{ab}	0.20±0.02	0.26±0.04	0.49±0.09^{ab}
Generative stage	12	548±19	2.70±0.13^{a}	0.50±0.03	0.41±0.05^{a}	0.20±0.02	0.23±0.02	0.91±0.17^{a}
Harvesting stage	2	400	0.80±0.47^{b}	0.20±0.12	0.06±0.03^{b}	0.15±0.01	0.19±0.03	0.16±0.01^{b}
Resting stage	7	511±37	2.36±0.45^{a}	0.45±0.06	0.19±0.06^{ab}	0.18±0.03	0.32±0.06	0.35±0.07^{ab}

Values within a column followed by a different superscript differ statistically (ANOVA or Brown-Forsythe of Robust test, $p<0.05$).
Incomplete data from fewer samples ($n=39$) than the total samples number ($n=54$) are shown with an asterisk (*).
Growing stage = June-October; Generative stage = October-December; Harvesting stage = January-March; and Resting stage = March-June.

3.2 Variation in the sperm quality of *B. indica*

3.2.1 Morphology and variation of 'normal' sperm

Normal sperm head: Light (LM) and electron microscopy (TEM and SEM) investigations demonstrated considerable pleiomorphism in the cauda epididymal spermatozoa of *B. indica* in all samples. At least nine different sperm head morphotypes were found in all individuals (Table 7) with the morphologically normal spermatozoa ranging from globular to conical heads (Fig. 14). In the epididymides, only ~63.8% of all spermatozoa were considered to be those of normal sperm heads and consisted of ~29.9% with globular heads and ~33.9% with conical heads (Table 7). The globular sperm heads were globular or oval shaped and rounded anteriorly and the width of the apical region is similar to that of the basal region (Figs. 14A,C). The head length varied from 5 to 6 μm with a maximal width of 2.5 μm. The conical sperm heads were characterized by a cone shape and being wider and rounded at the apical end and narrowed to a small point at the basal end that was connected to the tail (Figs. 14B,D). The sperm heads had a mean length of 5.5 μm (range of 5 to 6 μm).

Normal sperm tail: The sperm tail that possessed straight, slightly wavy, or had only a gentle bend in the tail with no shape, was defined as being 'normal' (Fig. 15). ~30% of the sperm population had this structure (Table 7), and the normal sperm tails occurred with any of the sperm head shapes. The average total tail length was about 54.01 μm and had a 11.38 μm long midpiece section and a 42.63 μm principal-end piece length (Table 8).

In relation to the seasonal variations of the sperm quality *B. indica* the proportion of normal sperm heads was significantly higher in the wet season samples (67.4%) than those of the dry season samples (57.9%) ($p<0.05$) (Table 9). In the first year of study, the percentage of normal sperm heads ranged from 74.1 to 78.7 % for the first three samples obtained in the wet season and this was significantly greater (76.6%) than those collected in the first dry season (61.4%) ($p<0.05$) (Table 9; Fig. 16). In the first dry season, the proportion of normal sperm heads remained high in the early season (May 2005), but it decreased to be lowest in July 2005 (48.9%) (Fig. 16). During the second year of study, the number of normal sperm heads did not significantly differ between the wet (58.5%) and the dry seasons (55.3%) ($p>0.05$)

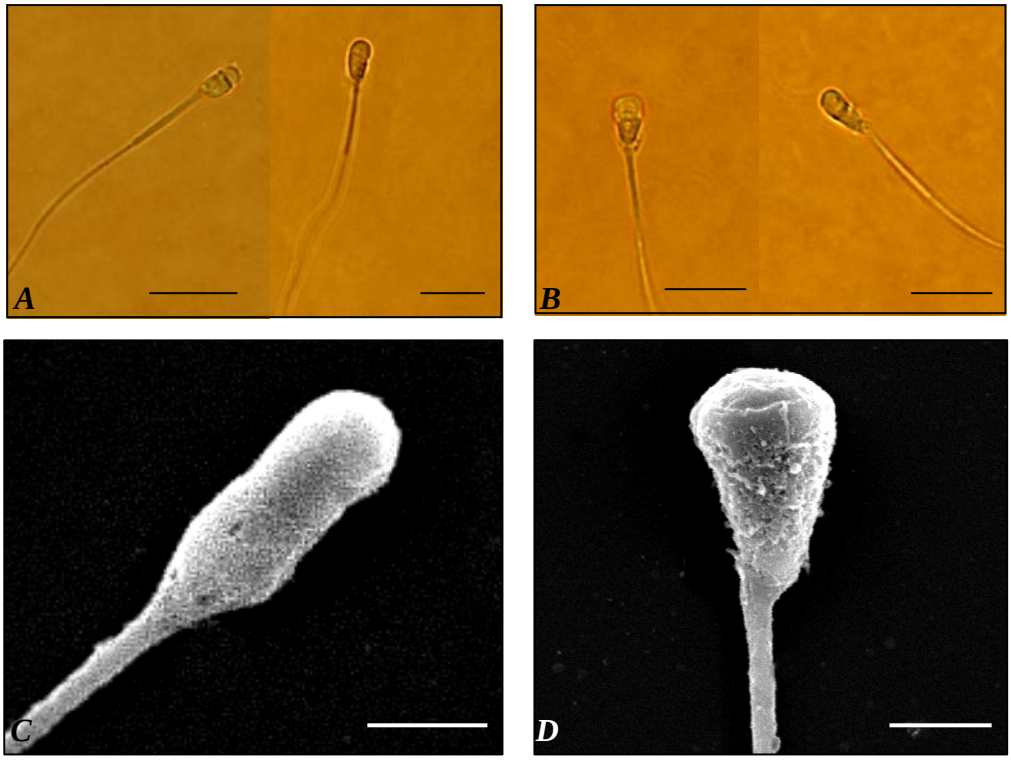


Figure 14. The normal sperm head of *B. indica*; the globular (*A,C*) and conical heads (*B,D*). *A-B*: Phase contrast and *C-D*: SEM photomicrographs of spermatozoa. Scale bars= 2.5 µm (*C-D*), and 10 µm (*A-B*).

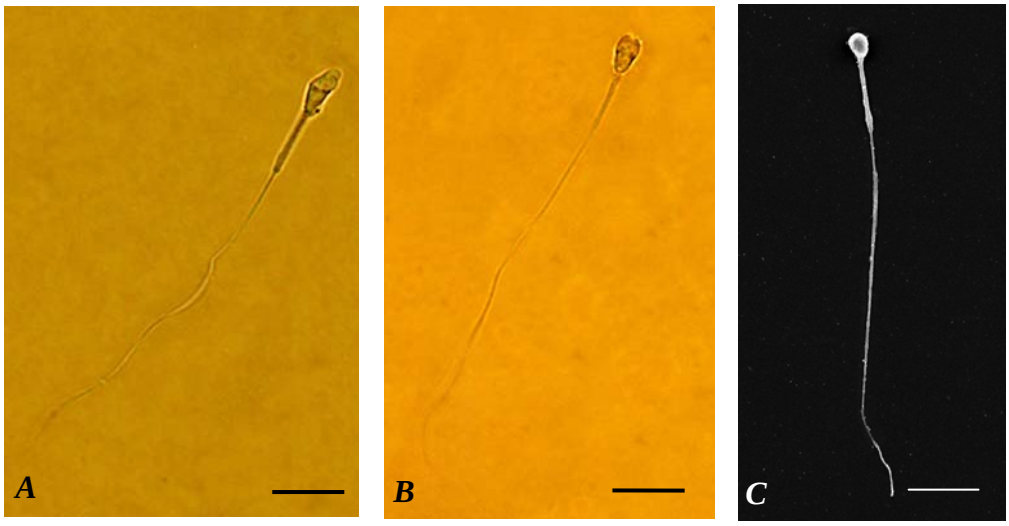


Figure 15. Phase contrast (*A-B*) and SEM (*C*) photomicrographs showing the 'normal' sperm tail of *B. indica*. Scale bars= 10µm.

Table 7. Incidence of structurally normal and abnormal spermatozoa in the epididymis of *B. indica* (*n*=51 males)

Trait	Mean±S.E.
Head sperm morphology (%)	
➢ **Normal sperm head**	***63.8 ± 1.6***
- Globular or Bulbous head	29.9 ± 1.1
- Conical head	33.9 ± 1.4
➢ **Abnormal sperm head**	***36.2 ± 1.6***
1. Round head	5.4 ± 0.4
2. Spear head	4.1 ± 0.4
3. Triangular head	3.1 ± 0.2
4. Two head attached one tail	3.8 ± 0.3
5. Three head attached one tail	0.0 ± 0.0
6. Macrocephalic head	14.5 ± 1.0
7. Irregular head	5.3 ± 0.5
Tail sperm morphology (%)	
➢ **Normal sperm tail (straight)**	***30.1 ± 1.8***
➢ **Abnormal sperm tail**	***69.9 ± 1.8***
❖ *Midpiece deformity*	*55.8 ± 1.7*
1. Proximal cytoplasmic droplet (P.CD)	12.7 ± 0.8
2. Distal cytoplasmic droplet (D.CD)	13.5 ± 0.9
3. Bent neck (BN)	9.4 ± 0.6
4. Bent neck with cytoplasmic droplet (BN.CD)	4.3 ± 0.4
5. Bent midpiece (BM)	2.9 ± 0.4
6. Bent midpiece with cytoplasmic droplet (BM.CD)	0.3 ± 0.1
7. Thickness or rugged midpiece	12.7 ± 0.9
❖ *Flagellar deformity*	*14.1 ± 0.9*
8. Bent flagellum (BF)	6.8 ± 0.5
9. Bent flagellum with proximal cytoplasmic droplet (BF.PCD)	0.8 ± 0.2
10. Bent flagellum with distal cytoplasmic droplet (BF.DCD)	0.7 ± 0.1
11. Biflagella	3.2 ± 0.4
12. Triflagella	0.3 ± 0.1
13. Coiled tail	2.4 ± 0.4

Table 8. Intra-individual variation in sperm tail length (± S.E.) of *B. indica* (*n*=51 males)

Sperm tail length (µm)	n^a	Mean	Range of average sperm tail lengths (±S.E.)
Midpiece	2336	11.38±0.06	9.1±0.3 to 15.7±0.4
Principal and end pieces	2336	42.63±0.20	31.6±1.0 to 60.7±1.0
Total sperm tail	2336	54.01±0.23	42.3±1.2 to 75.6±1.1

[a] Total number of spermatozoa

(Table 9). The average number of normal sperm heads was 58.4% in September 2005 and 58.6% in November 2005 of the second wet season, whereas it was lowest in March 2006 (47.8%), and was 51%, 60.1% and 60.8% in the May, July and September 2006 samples, respectively (Fig. 16). For the normal sperm tails, the percentages were significantly higher in the wet season (36.1% in all years, 44.4% in the 1st year and 28.8% in the 2nd year) than those in the dry seasons (20.2%, 25.2%, and 16.5%, respectively) ($p<0.05$) (Table 9). During the first year of study, the normal sperm tails ranged from 39.4% to 48.4% of the sperm population in the 3 samples collected in the first wet season, whereas in the first dry season, the percentage was 31.6% in May 2005 and this decreased to be lowest in July 2005 (18.8%) (Fig. 16). In the second wet season, the sperm with normal tails were 30.2% and 27.4% in the September and November 2005 samples, respectively (Fig. 16). In 2006, normal sperm tails decreased to their lowest percentage from the March-July samples (a range of 16.3 to 17.5%) and was 28.4% in September 2006 (Fig. 16).

Table 9. Comparison of the percentages of normal sperm heads and tails of male *B. indica* between the wet and the dry seasons during September 2004-September 2006.

Seasons	*n*	Percentage of normal spermatozoa (Mean±S.E.)	
		Normal sperm heads	Normal sperm tails
All			
Wet	32	67.4±2.0[a]	36.1±2.0[a]
Dry	19	57.9±2.4[b]	20.2±1.7[b]
Year 1			
Wet	15	76.6±1.4[a]	44.4±2.7[a]
Dry	8	61.4±4.9[b]	25.2±3.2[b]
Year 2			
Wet	12	58.5±1.7	28.8±1.8[a]
Dry	11	55.3±1.9	16.5±0.8[b]
Year 3			
Wet	5	60.8±5.6	28.4±1.1

Values within a column followed by a different superscript differ statistically (student's *t* test, $p<0.05$).
Seasons: Wet (September- January); Dry (February-August).

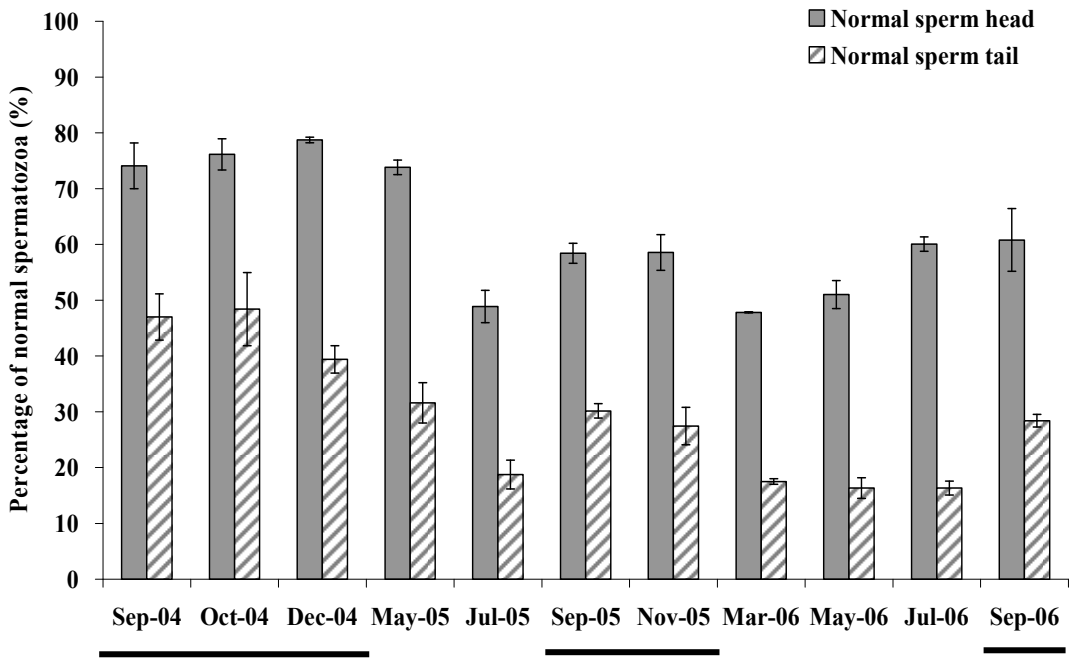


Figure 16. Seasonal variations in the percentage (±S.E.) of normal sperm heads and tails in male *B. indica* during September 2004-September 2006. The black lines represents the period of the wet season.

Table 10. The percentage of normal sperm heads and tails in the cultivation stages of rice plants during September 2004 to September 2006.

Rice plant cycle	*n*	Percentage of normal spermatozoa (Mean±S.E.)	
		Normal sperm head	Normal sperm tail
Growing stage	30	62.9±2.0	30.9±2.6
Generative stage	12	68.6±3.4	33.4±2.7
Harvesting stage	2	47.7±0.1	17.0±0.0
Resting stage	7	64.0±4.8	25.1±3.7

Growing stage = June-October; Generative stage = October-December; Harvesting stage = January-March; and Resting stage = March-June.

In addition to the relationship between the sperm quality of *B. indica* and the environmental conditions, the results showed that the sperm quality was not significantly correlated with any of the environmental conditions including the cultivation stages of rice plant (p>0.05). However, during the cultivation stages of the rice plants the percentages of normal sperm head and tail tended to be highest in the generative stage (68.6% and 33.4%, respectively) and were lowest in the harvesting period (47.7% and 17.0%, respectively) (Table 10) and this was correlated with the male reproductive organ weights.

3.2.2 Morphology and variation of 'abnormal' sperm

Abnormal sperm heads: Apart from the 'normal' sperm heads of globular and conical heads, the spermatozoa that differed from this in either their shape, size of sperm, or having more than one head attached to one tail, were categorized as 'abnormal'. About 36.2% of all of the *B. indica* spermatozoa samples investigated had bizarre forms of heads (Table 7). The spermatozoa with abnormal heads were categorized into at least seven different sperm head morphotypes (Fig. 17). Round sperm heads with a distinctly round head shape (Figs. 17A, 18A). Sperm heads that tapered to a sharp point apically were called spear heads (Figs. 17B, 18B). Triangular heads looked like the conical shaped heads but the apical end was squared off not rounded (Figs. 17C, 18C). Differences occurred in the number of sperm heads attached to one tail, with some sperm having two (bicephalic) (Figs. 17D, 18D) or three heads (tricephalic) (Fig. 17E). Macrocephalic sperm had obviously enlarged heads that were approximately twice as large as the other sperm heads and the head length was more than 7 μm with a maximal width of 4.2 μm (Figs. 17F, 18E). Spermatozoa that were not categorized into the above sperm head morphologies or had rough sperm head surfaces, had irregularly shaped sperm heads (Fig. 17G).

Abnormal sperm tail: Apart from 'normal' sperm with straight tails, the others that differed from this in having either a sharp angle, and being tightly coiled between head and tail along its length, a cytoplasmic droplet on the midpiece of the sperm tail or more than one flagellum attached to the sperm head, were assumed to have an 'abnormal' sperm tail. Many defective forms of sperm tail were present in all of the *B. indica* samples investigated. They were divided into 13 morphotypes and any of the different tail morphologies occurred with any of the sperm head morphologies (Figs. 19, 20). The characteristics of the 13 deformed forms of the sperm tails were as follows:

1. *Proximal cytoplasmic droplet (P.CD)*: Straight tail sperm with a cytoplasmic droplet at the proximal region of the midpiece (Figs. 19A, 20A).

2. *Distal cytoplasmic droplet (D.CD):* Straight tail sperm retained a cytoplasmic droplet at the distal region of the midpiece (Fig. 19B).

3. *Bent neck (BN):* Sperm tail bent at the connecting or neck region, with the bent angle being $\leq 90^{o}$ (Figs. 19C, 20B).

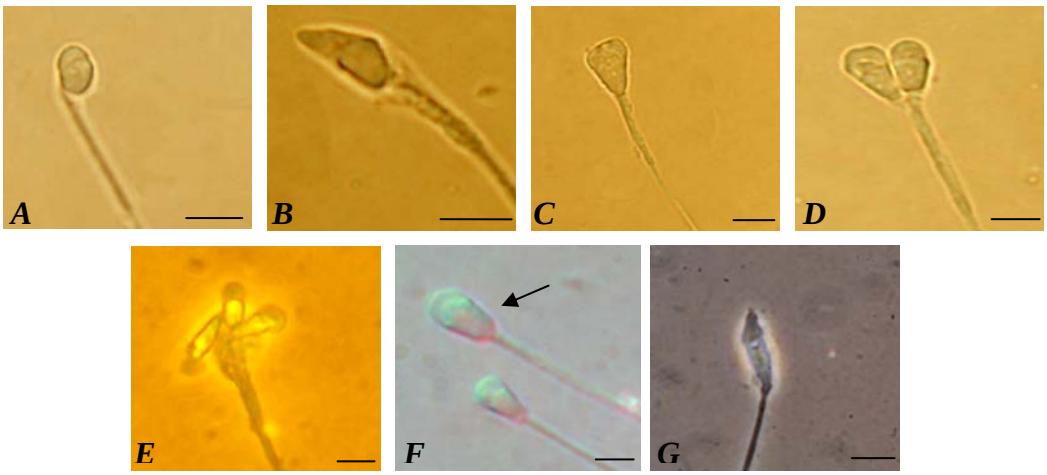


Figure 17. Phase contrast and Nomarski photomicrographs showing the deformities of the sperm head in adult male *B. indica*. *A*: round head, *B:* spear head, *C*: triangular head, *D:* two/ *E*: three heads attached to one tail, *F*: macrocephalic head (arrow), and *G*: irregular head. Scale bars = 5µm.

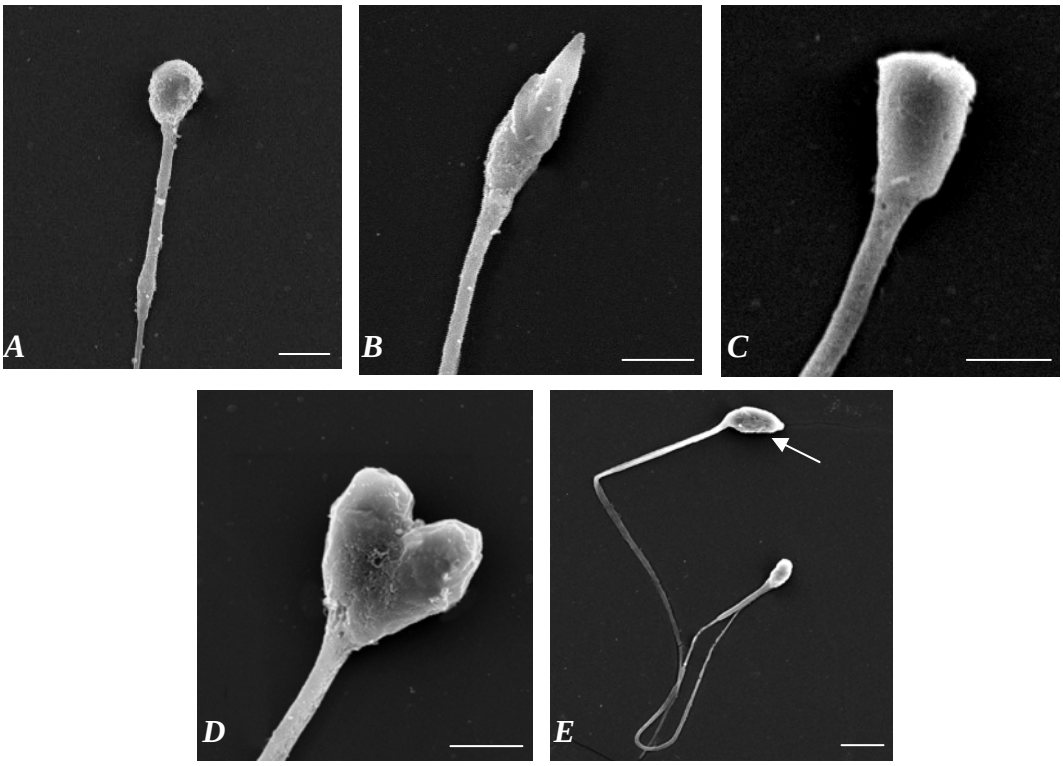


Figure 18. SEM photomicrographs showing the deformities of the sperm head morphotypes in the adult male *B. indica*. *A*: round head, *B:* spear head, *C*: triangular head, *D*: two heads attached to one tail, and *E*: macrocephalic head (arrow). Scale bars = 2.5 µm (*A*-*D*), 5 µm (*E*).

4. *Bent neck with cytoplasmic droplet (BN.CD):* Bent neck sperm retained a cytoplasmic droplet at either the proximal or distal half of the midpiece (Figs. 19D, 20C).

5. *Bent midpiece (BM):* Sperm tail bent at the midpiece and might be either sharp or rounded but must be bent by 90^{o} or less (Figs. 19E, 20D).

6. *Bent midpiece with cytoplasmic droplet (BM.CD):* Bent midpiece sperm with a residual cytoplasmic droplet in either the proximal or distal half of the midpiece (Fig. 19F).

7. *Thickness or rugged midpiece:* The midpiece of the sperm tail was either thicker than usual or with a rugged surface (Fig. 19G).

8. *Bent flagellum (BF):* The principal and end piece of the sperm tail was bent by less than 90^{o} and the bent end was either sharp, round or u-shaped (Figs. 19H, 20E).

9. *Bent flagellum with proximal cytoplasmic droplet (BF.P.CD):* Bent flagellum with a residual cytoplasmic droplet in the proximal midpiece (Figs. 19I, 20F).

10. *Bent flagellum with distal cytoplasmic droplet (BF.D.CD):* Bent flagellum with a retained cytoplasmic droplet in the distal midpiece (Fig. 19J).

11. *Biflagella:* Sperm with two tails attached to one sperm head (Figs. 19K, 20G).

12. *Triflagella:* Sperm with three tails attached to the one sperm head (Figs. 19L, 20H).

13. *Coiled tail:* Sperm tail curled one or more times at any point along the tail length (Figs. 19M, N, 20I).

In the epididymis, about 70% of the sperm population had deformed sperm tails. Malformations of the sperm midpiece (Figs. 19A-G) was the most common having the highest frequency of ~55.8% of all spermatozoa and the majority of these spermatozoa had a cytoplasmic droplet on the proximal (12.7%) and distal (13.5%) regions of the midpiece as well as having a thicker or rugged midpiece (12.7%) (Table 7). Spermatozoa with flagellar deformity (Figs. 19H-N) comprised ~14.1% of all the spermatozoa observed (Table 7).

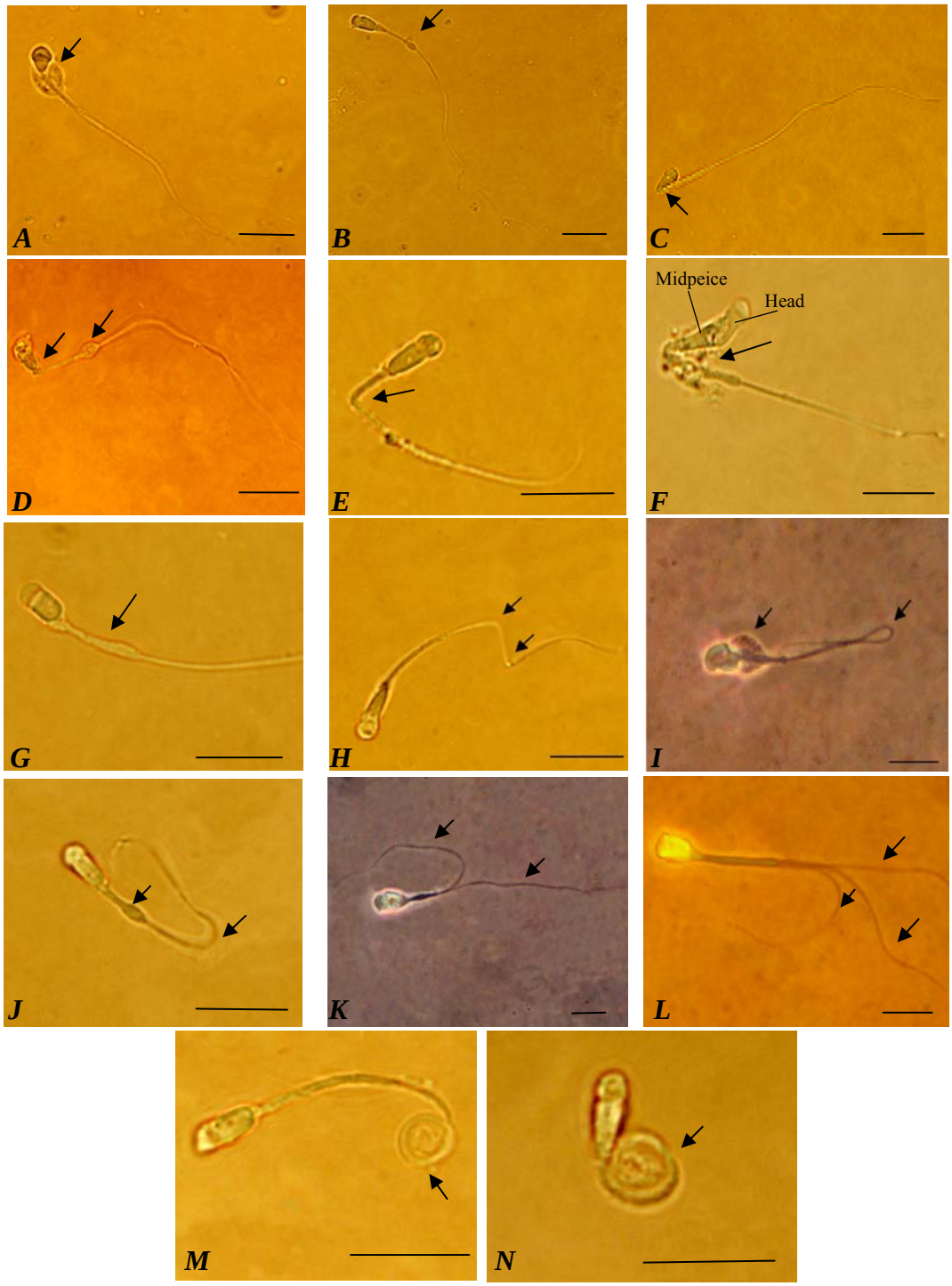


Figure 19. Phase contrast photomicrographs of abnormal sperm tails in *B. indica*. *A-B:* Straight sperm tail with a proximal cytoplasmic droplet (*A*) and distal (*B*) midpieces. *C-D:* Bent neck (*C*) and having a cytoplasmic droplet (*D*). *E-F:* Bent midpiece (*F*) and having a cytoplamic droplet (*F*). *G*: Thicker or rugged midpiece. *H-J:* Bent flagellum (*H*) and having a proximal (*I*) and distal (*J*) cytoplasmic droplet. *K*: Biflagella. *L*: Triflagella. *M-N*: Coiled tail. Arrows show the marked point of each abnormal sperm tail. Scale bars= 10 µm.

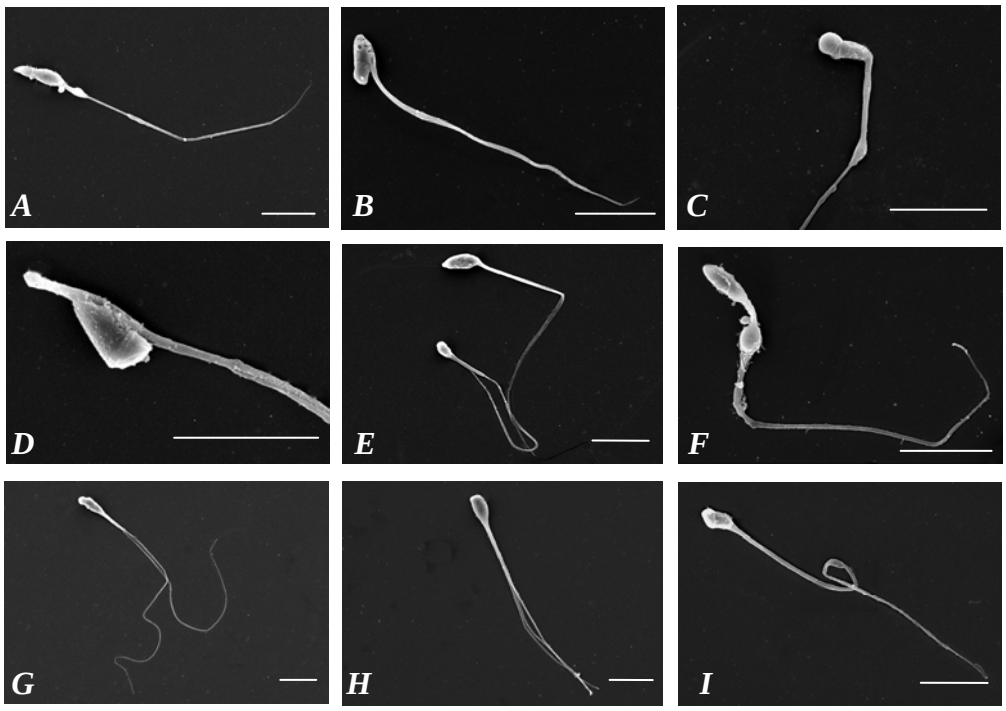


Figure 20. SEM photomicrographs of abnormal sperm tails in *B. indica*. *A*: Straight tail sperm having a proximal cytoplasmic droplet. *B-C:* Bent neck (*B*) and having a cytoplasmic droplet (*C*). *D:* Bent midpiece. *E-F*: Bent flagellum (*E*) and having a proximal cytoplasmic droplet (*F*). *G:* Biflagella. *H*: Triflagella. *I*: Coiled sperm tail. Scale bars = 10μm.

Table 11. Comparison of the percentage of abnormal sperm heads and tails of male *B. indica* between the wet and the dry seasons during September 2004-September 2006

Seasons	*n*	Percentage of abnormal sperm (Mean±S.E.)			
		Abnormal sperm head	Abnormal sperm tail		
			Total	Midpiece deformity	Flagellar deformity
All					
Wet	32	32.7±2.0[a]	63.9±2.0[a]	50.9±2.0[a]	13.0±1.2
Dry	19	42.1±2.4[b]	79.8±1.7[b]	64.0±1.9[b]	15.8±1.1
Year 1					
Wet	15	23.4±1.4[a]	55.6±2.7[a]	44.3±2.9[a]	11.2±2.0
Dry	8	38.6±4.9[b]	74.8±3.2[b]	61.4±3.7[b]	13.5±1.6
Year 2					
Wet	12	41.5±1.7	71.2±1.8[a]	55.9±2.5[a]	15.3±1.9
Dry	11	44.7±1.9	83.5±0.8[b]	65.9±1.7[b]	17.5±1.4
Year 3					
Wet	5	39.2±5.6	71.6±1.1	58.6±1.4	13.0±0.3

Values within a column followed by a different superscript differ statistically (Student's *t* test, $p<0.05$).
Seasons: Wet (September-January); Dry (February-August).

In addition to the seasonal variation of the proportion of abnormal spermatozoa, the sperm head abnormalities were significantly higher in the dry season (42.1%) than those of the wet season (32.7%) ($p<0.05$) (Table 11). Likewise, in the first year of study, the percentage of abnormal sperm heads was significantly greater in the dry season (38.6%) than the wet season (23.4%) ($p<0.05$) with the abnormal sperm heads being highest (51.1%) in July 2005 when the most prominent sperm defect was a macrocephalic head (37.1% of total head abnormalities) (Table 12). During the second year of study, the percentage of abnormal sperm heads was not different between seasons ($p>0.05$) (Table 11), however the highest proportion of defective sperm heads were found at the beginning of the dry season (March 2006) (52.3%) when 44.4% of these bizarre sperm heads were macrocephalic (Table 12). For the abnormal sperm tails, the percentage of these sperm were significantly higher in samples collected in the dry seasons (79.8% in all years, 74.8% in the 1st year and 83.5% in the 2nd year) compared to those of the wet seasons in both years of study (63.9%, 55.6% and 71.2%, respectively) ($p<0.05$) with the midpiece deformity being the highest in each season of both years (Table 11). In the first year of study, the percentage of abnormal sperm tails ranged from 51.6% to 60.6% in the wet season, whereas in the subsequent dry season, it was 68.4% in May 2005 and increased to be highest (81.3%) in July 2006 with 22.6% of them having the proximal cytoplasmic droplets (Table 13). During the second dry season, the abnormal sperm tails were high in March 2006 (82.5%), tended to be highest in May-July 2006 (83.7%) when the most prominent sperm defect was the thicker/more rugged midpiece (21.2%) and with distal cytoplasmic droplets (22%) (Table 13).

Table 12. Seasonal variation of the percentage (±S.E.) of abnormal sperm head morphotype in *B. indica* between September 2004 and September 2006.

Abnormal sperm	2004			2005				2006			
head morphotypes	Sep	Oct	Dec	May	Jul	Sep	Nov	Mar	May	Jul	Sep
***n*[a]**	4	5	6	4	4	6	6	2	3	6	5
Normal head	74.1±4.1	76.1±2.8	78.7±0.5	73.8±1.3	48.9±2.9	58.4±1.8	58.6±3.2	47.7±0.1	51.0±2.5	60.1±1.3	60.8±5.6
Abnormal head	25.9±4.0	23.9±2.8	21.3±0.5	26.2±1.3	51.1±2.9	41.6±1.8	41.5±3.2	52.3±0.1	49.0±2.5	40.0±1.3	39.2±5.6
-Round head	32.4±12.1	27.6±6.6	25.8±3.5	7.5±2.8	17.4±4.1	13.2±2.0	11.7±1.4	10.2±0.9	13.3±3.7	10.3±0.5	11.5±2.9
-Spear head	13.7±5.1	9.9±4.3	7.5±3.8	11.6±4.5	8.6±3.5	10.8±1.5	13.1±4.4	6.5±0.9	10.6±4.6	16.6±3.1	8.9±2.3
-Triangular head	12.5±3.5	8.3±2.1	5.7±1.2	6.7±2.8	10.2±1.3	8.3±1.0	6.9±2.1	7.4±0.0	10.1±0.7	8.6±1.4	8.7±1.3
-Bicephalic head	8.1±2.7	17.3±2.8	22.9±3.6	16.0±5.3	11.3±2.2	10.4±1.5	10.8±2.0	5.5±0.0	7.2±4.1	4.1±0.5	8.4±1.4
-Tricephalic head	0	0	0.4±0.4	0	0.2±0.2	0	0	0	0	0	0
-Macrocephalic head	26.1±8.8	25.4±6.1	29.8±4.1	40.5±3.2	37.1±4.5	42.5±2.2	43.9±6.3	44.4±0.0	44.6±3.8	42.3±3.4	49.5±3.7
-Irregular head	7.2±2.9	11.5±4.2	7.8±2.6	17.7±3.5	15.1±3.2	14.7±2.8	13.5±1.4	25.9±0.0	14.2±1.8	18.0±1.7	13.0±3.3

[a] Total number of individual Great Bandicoot rats.
Seasons: Wet (September-January); Dry (February-August)

Table 13. Seasonal variation of the percentage (±S.E.) of 'abnormal' sperm tail morphotypes in *B. indica* between September 2004 and September 2006.

Abnormal sperm tail morphotypes	2004			2005				2006			
	Sep	Oct	Dec	May	Jul	Sep	Nov	Mar	May	Jul	Sep
***n*[a]**	4	5	6	4	4	6	6	2	3	6	5
Normal	47.0±4.1	48.4±6.6	39.4±2.5	31.6±3.6	18.8±2.6	30.2±1.3	27.5±3.4	17.5±0.5	16.3±1.9	16.3±1.2	28.4±1.1
Abnormal	53.0±4.1	51.6±6.6	60.6±2.5	68.4±3.6	81.3±2.6	69.8±1.3	72.6±3.4	82.5±0.5	83.7±1.9	83.7±1.2	71.6±1.1
1.Midpiece abnormality	79.2±10.2	84.8±3.2	76.2±6.1	81.0±1.1	82.5±4.4	78.0±3.6	79.0±4.5	80.0±0.7	77.0±4.2	79.5±2.6	81.8±0.7
-Proximal CD	23.7±1.5	18.8±3.3	10.6±2.0	15.6±5.4	22.6±4.0	19.3±3.4	17.3±2.9	26.1±0.4	16.3±0.1	18.4±2.6	18.3±2.1
-Distal CD	19.8±3.9	23.1±1.7	13.2±3.5	22.7±6.0	17.3±4.5	16.6±2.8	17.0±3.3	12.1±0.1	16.1±4.4	22.0±2.0	29.1±3.6
-Bent neck	17.8±2.9	22.5±4.7	25.2±2.5	11.1±1.5	13.4±2.0	11.2±1.3	10.5±3.0	4.8±0.0	11.5±1.5	10.5±1.4	10.4±1.5
-Bent neck with CD	5.5±1.9	5.9±1.5	6.8±2.0	5.6±2.2	10.2±2.3	7.1±1.8	6.3±1.8	4.8±0.0	5.9±1.7	3.9±1.0	5.0±1.3
-Bent midpiece	2.3±1.2	4.5±1.4	3.7±0.7	7.0±2.9	2.7±1.5	2.9±1.0	7.3±3.2	2.4±0.0	4.4±1.4	4.1±1.6	3.4±1.5
-Bent midpiece with CD	0	0.4±0.4	0.6±0.4	1.0±0.7	0.3±0.3	0.7±0.3	0	0	1.6±0.4	0.4±0.2	0
-Thickness or rugged midpiece	10.0±3.4	9.6±4.4	16.4±3.4	17.7±2.5	16.0±3.9	20.2±2.7	20.2±4.6	29.7±0.4	21.2±1.3	20.2±1.4	15.6±1.3
2 Flagellar abnormalities	20.8±10.2	15.2±3.2	23.8±6.1	19.0±1.1	17.5±4.4	22.0±3.6	21.0±4.5	20.0±0.7	23.0±4.2	20.5±2.6	13.2±0.7
-Bent flagellum	9.7±4.2	4.5±1.2	9.0±2.0	10.8±1.8	5.0±2.2	9.1±1.4	11.2±2.4	15.8±0.1	14.7±2.1	10.2±1.2	9.2±1.2
-Bent flagellum with proximal CD	1.1±1.1	0.4±0.4	1.1±0.5	0.6±0.4	1.6±1.0	0.7±0.7	1.3±0.7	1.2±0.0	0.8±0.4	2.2±1.1	0.6±0.3
-Bent flagellum with distal CD	1.6±1.6	0.4±0.4	2.0±0.5	1.1±0.4	0.6±0.4	0.7±0.3	0	0	0.4±0.4	0.6±0.3	3.1±0.3
-Biflagella	3.8±1.4	8.5±3.0	4.8±2.7	2.2±1.1	5.3±0.8	6.7±1.9	3.5±2.2	1.8±0.6	5.5±1.7	4.3±2.1	2.8±0.4
-Triflagella	0	0.9±0.6	0	0	0.6±0.3	0.5±0.3	0.8±0.5	0	0	1.0±0.5	0
-Coiled tail	4.7±2.8	0.4±0.4	7.2±4.0	3.9±0.7	4.4±1.3	4.3±1.2	3.8±1.9	1.2±0.0	1.6±0.8	2.2±0.6	2.5±1.1

[a] Total number of individual Great Bandicoot rats. Seasons: Wet (September-January); Dry (February-August). CD= cytoplasmic droplets

3.3 Ultrastructure of *B. indica* spermatozoa

3.3.1 Ultrastructure of normal spermatozoa

TEM sections of the cauda epididymides showed the luminal epididymis containing many spermatozoa and some round spermatids. Spermatozoa of *B. indica* had various sperm morphotypes in both their head and tail shapes (previously described in the above section). However some of the characteristics of these spermatozoa were similar in that (1) the heads were not bilaterally flattened and the nucleus was more or less circular in cross section for most of its length (Fig. 21E), (2) there was no recognizable perforatorium or equatorial segment in any of the spermatozoa (Figs. 21A-B,D) and (3) the nucleus had many prominent nuclear vacuoles (Figs. 21A-B,D-G) as well as (4) the midpiece and principal piece of the sperm tail contained C-shaped outer dense fibres (Figs. 21H,I).

Normal head spermatozoa: longitudinal sections of the globular heads showed that the 2.2-4.5 μm–long nuclei were generally paddle-shaped, being broader basally but the apical region had extensions of varying shapes and size (Figs. 21A,D-F). The nucleus had electron-dense chromatin and in spite of the occurrence of many nuclear vacuoles, some nuclei had a few large vacuoles containing globular fibres (Figs. 21A,D). The acrosome occurred as a massive cap over the nucleus and was also variable in morphology but was generally a bulbous structure, that was rounded at its apex (Figs. 21A,F). In the conical sperm head, the nucleus was generally cone-shaped tapering basally but the anterior region was variable in shape having nuclear extensions (Fig. 21B). The nuclear length varied from 2.9 to 4.2 μm with a maximum width of ~1.5 μm. The acrosome was large and occurred as a bulbous structure over the anterior region of the nucleus (Fig. 21B). In addition, these normal sperm heads which were both globular and conical, exhibited a narrow subacrosomal space (SAM) with a modest amount of electron-dense material occurring between the inner acrosomal membrane and the nucleus (Figs. 21A-B,D). The postacrosomal dense lamina (PADL) had typical projections that passed towards the overlying plasmalemma, but was short (Figs. 21B,D) and the posterior ring was situated in the anterior third of the nucleus (Figs. 21B,F). Caudal to the posterior ring of the nucleus, some granular cytoplasmic material was present between the nuclear envelope and the plasma membrane (Figs. 21B,F). The caudal surface of the nucleus had a concavity,

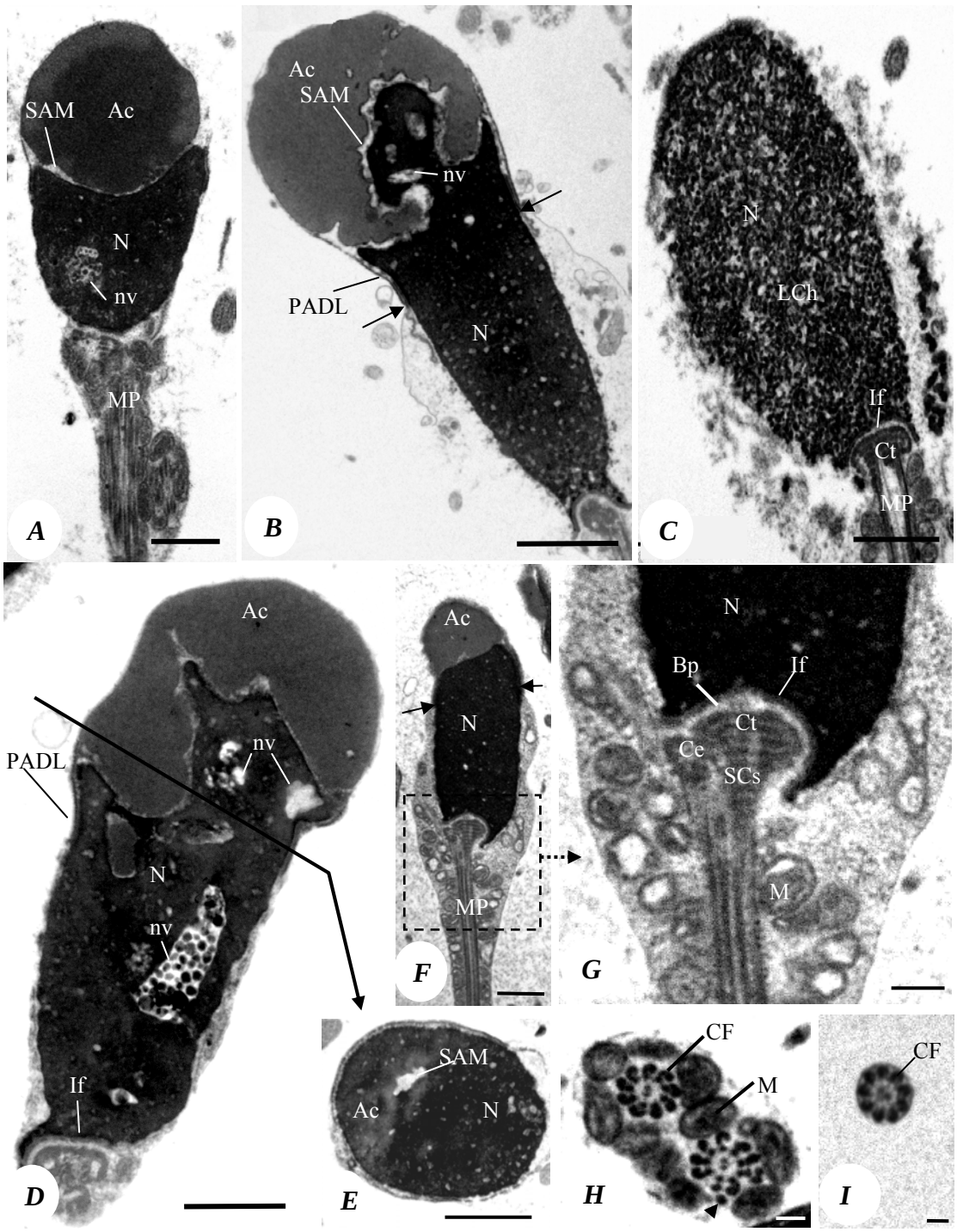


Figure 21. TEM photomicrographs of the normal sperm heads; globular (*A*,*D*) and conical heads (*B*), and the normal sperm tail (*F-I*). *C*: A dry season spermatozoon showing loose chromatin condensation (Lch) of the nucleus (N). *E:* Transverse section of a normal sperm head. *F-G:* Sperm flagellum attached to the nucleus by the implantation fossa (If). *H-I:* Cross-sections of the midpiece (MP) (*H*) and principal piece (*I*) with C-shape of coarse fibres (CF). Ac= acrosome, Bp= basal plate, Ce= proximal centriole, Ct= capitulum, M= mitochondria, nv= nuclear vacuoles, PADL= postacrosomal dense lamina, SAM= subacrosomal space with electron dense material, SCs= segmented columns, Arrows= posterior ring, Arrowhead= accessory dense fibres. Scale bars= 0.2 µm (*H-I*), 0.3 µm (*G*) and 1 µm (*A-F*).

the implantation fossa, for attachment to the conforming capitulum of the connecting piece (Figs. 21B-G). In relation to the comparison of sperm ultrastructure between seasons, some spermatozoa from animals obtained in the dry season exhibited different characteristics from those in the wet season. The dry season spermatozoon had incomplete chromatin condensation showing heterogeneity or loose chromatin in the nucleus (Fig. 21C).

Normal sperm tail: the connecting piece was attached mid-basally to the spermatozoon head at the implantation fossa (Figs. 21B-F), and was composed of a basal plate, capitulum and segmental columns (Fig. 21G). The basal plate was generally a thin electron lucid zone and constituted an anchor for the fibres that were joined to the capitulum of the tail's connecting columns. The capitulum was T-shaped and at its apex was slightly round (Figs. 21C,G). In the interior of the neck region immediately beneath the capitulum the proximal centriole occurred (Fig. 21G). Peripheral to the connecting pieces, scattered mitochondria were present (Figs. 21F-G). The axoneme of the sperm tail contained a central pair of microtubules and had nine outer doublet microtubules arranged in a circle around the central pair that extended throughout the length of the sperm tail. In the midpiece, mitochondria were similar to those of the sperm of other murids wrapped in helix around the outer dense fibres and the mitochondrial matrix was dense and homogeneous (Fig. 21H). The structures of the nine outer dense fibres differed from those of other murine sperm. They were C-shaped in transverse section and all fibres were similar in morphology with a more electron-dense outer cortical region; sometimes one or more accessory dense fibres could be seen (Fig. 21H). A transverse section of the principal piece revealed that the longitudinal column of the fibrous sheath was very small and thus the ribs were not distinguishable, but the axonemal filament and the dense fibres were similar those of the midpiece (Fig. 21I).

3.3.2 Ultrastructure of abnormal spermatozoa

Abnormal sperm head: The TEM study showed that, in spite of the similarity of some sperm characteristics in the pleiomorphic spermatozoa, the sperm heads differed in either their shapes, size of nuclei and acrosomes or the whole sperm heads. The fine structure of four out of seven abnormal sperm head morphotypes were examined; spear head, triangular head, bicephalic head and macrocephalic head (Fig. 22). The spear head sperm had a cone-shaped nucleus that tapered basally, was concave apically, and had many nuclear vacuoles (Fig. 22A). The acrosome was cone-shaped in the opposite direction, long and tapered at the spear tip and a blunt end next to the nucleus. The SAM and PADL were also found next to the concave end of the nucleus (Fig. 22A). In the triangular head (Fig. 22B), the nucleus was concave at the apical end and was electron dense with many vacuoles. The acrosome was squared off, not rounded, at the anterior end with extensions into the nuclei. The posterior ring was situated in the lower half of the head length. The posterior region of the nuclei was attached with two tails at the implantation fossa (Fig. 22B). The bicephalic heads exhibited two nuclei with one huge acrosome (Figs. 22C-D) that extended over the nucleus and each nucleus was attached to a sperm tail at its implantation fossa (Fig. 22C). The nuclei had many small plus a few large vacuoles with or without some materials inside (Figs. 22C,D) and some nuclei had extensions into the acrosome (Fig. 22D). Some bicephalic heads had one nucleus capped by two acrosomes one at each pole. The nucleus was electron-dense with many nuclear vacuoles (Fig. 22E). In the macrocephalic head, the nucleus was about twice as large as that in normal spermatozoa and it occupied two-thirds of the sperm head as well as having many nuclear vacuoles (Fig. 22F). The acrosome occurred as a huge cap over the nucleus and its size was similar to that of the normal spermatozoa. The SAM was similar to the normal spermatozoa but the PADL differed, in being longer than the usual sperm PADL. The posterior ring was located in the lower half of the nucleus (Fig. 22F).

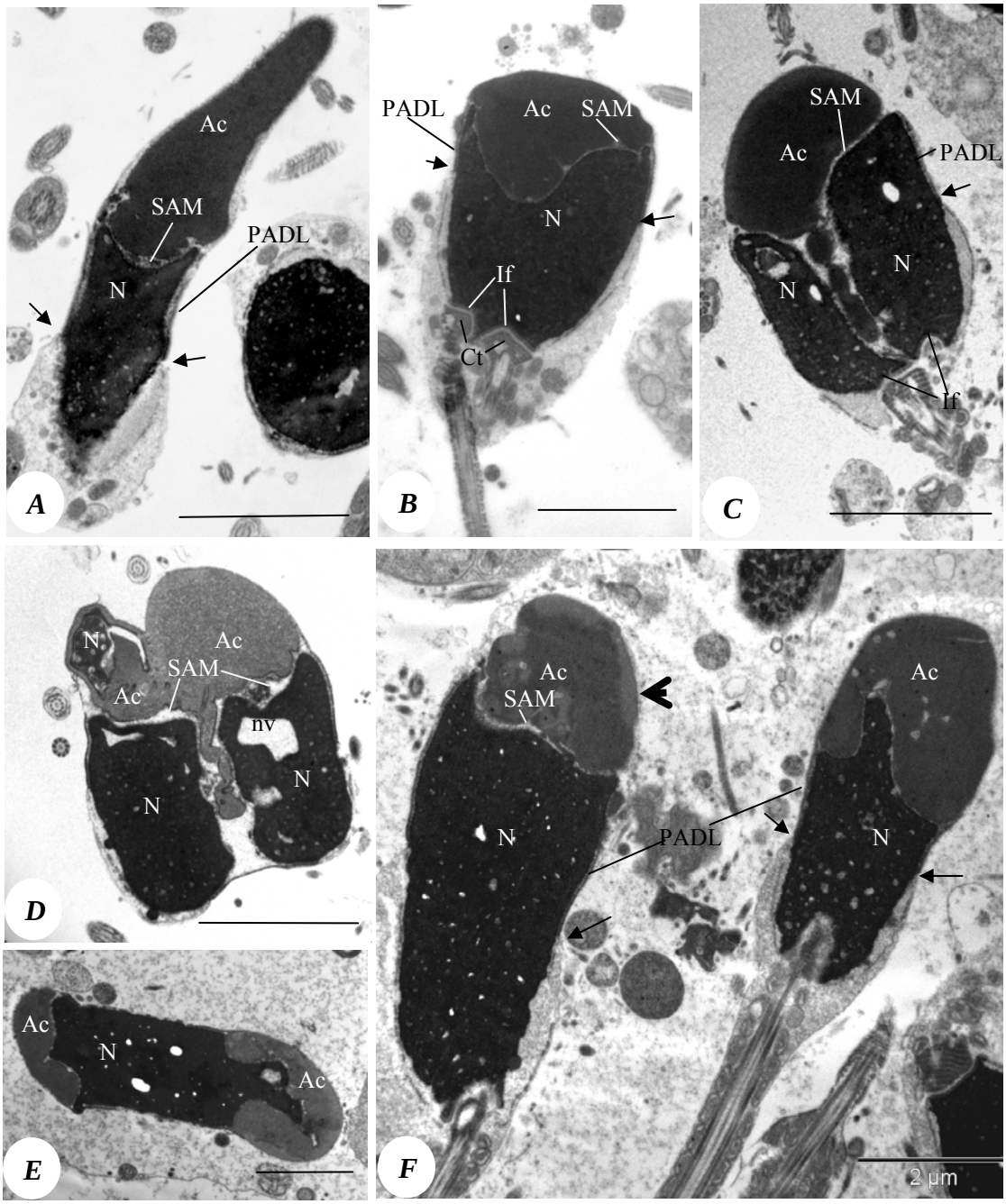


Figure 22. Sagittal sections (TEM) of abnormal sperm head morphotypes in the male *B. indica*. *A*: spear head, *B*: triangular head, *C-E*: bicephalic head, and *F:* macrocephalic head (arrowhead). Ac= acrosome, Ct= capitulum, If= implantation fossa, N= nucleus, nv= nuclear vacuoles, PADL= postacrosomal dense lamina, SAM= subacrosomal space with electron dense material, Arrows= posterior ring. Scale bars = 2 µm.

Abnormal sperm tail: the ultrastructure of the various types, but not all, of abnormal sperm tail were identified as follows: presence of a cytoplasmic droplet, bent neck, and biflagellate (Fig. 23). The straight tail sperm with a cytoplasmic droplet at the distal region of the midpiece showed that the cytoplasmic droplet contained various membrane-bounded vesicles of different sizes and also the C-shaped lamellae (Fig. 23A). A bent neck abnormality had a dislocated implantation fossa, basal plate and a capitulum that was not situated at the mid-basal region of the nucleus. The axoneme at the neck region was bent at an angle of 90^{o} (Fig. 23B). The biflagellate spermatozoa had two axonemes attached to one sperm head by a double symmetrical implantation fossa with a sheath of mitochondria surrounding the midpieces (Figs. 23C-D).

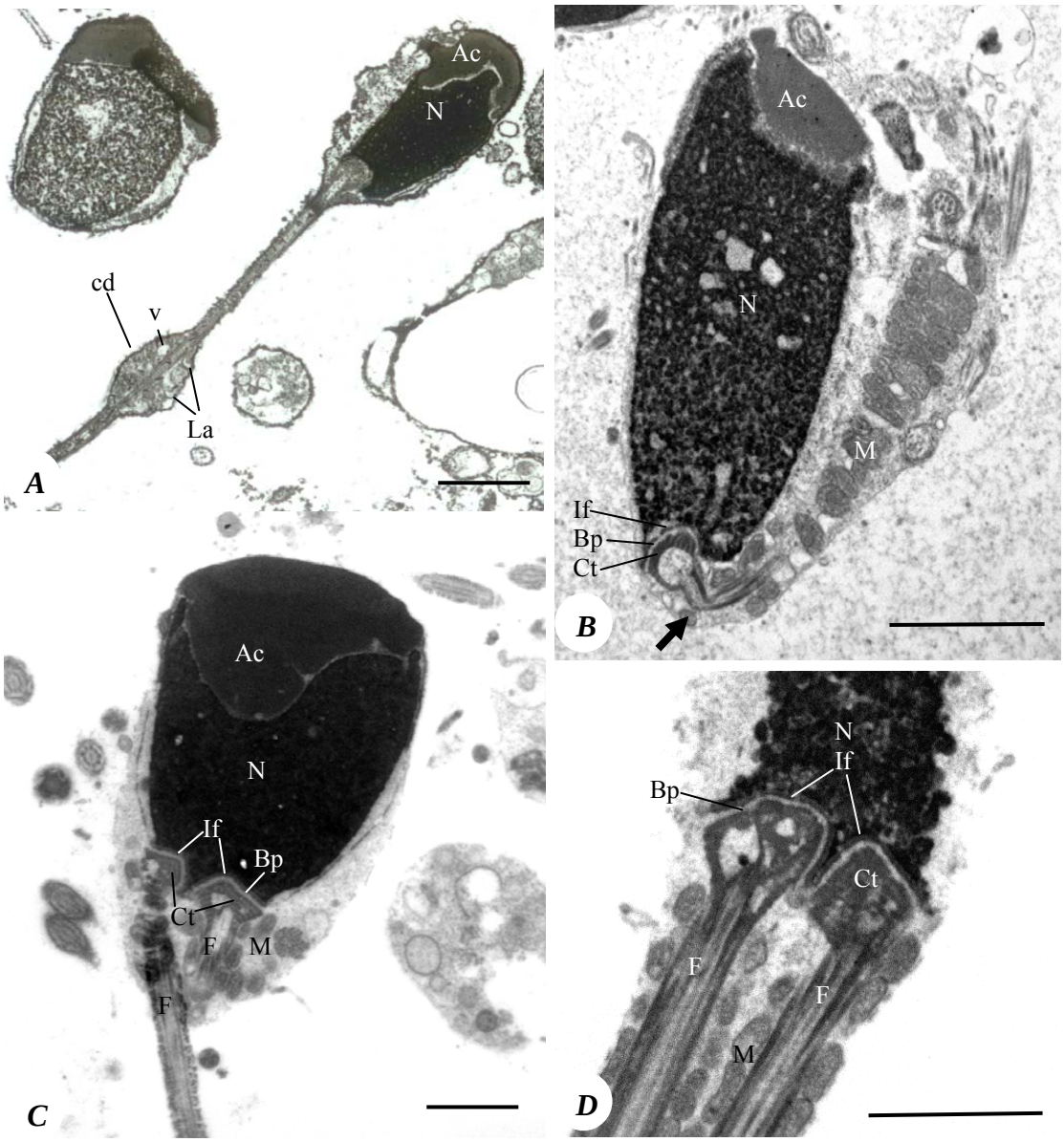


Figure 23. TEMs of abnormal sperm tails in *B. indica*. *A*: Straight tail sperm with a distal cytoplasmic droplet (cd). *B*: Bent neck sperm showing the axoneme bent at the connecting region (arrow). *C-D*: Biflagellar sperm with two flagella (F). Ac= acrosome, Bp= basal plate, Ct= capitulum, If= implantation fossa, La= lamellae, M= mitochondria, N= nucleus. Scale bars=1µm (*C-D*), 2µm (*A-B*).

3.4 Variation in epididymal histology and cell activities of *B. indica* and *B. bengalensis*

The *B. indica* epididymis was a single elongated and coiled tubule that had been divided into three regions; caput, corpus and cauda segments. In this study, the caput and cauda epididymides, not the corpus region, were selected to investigate. The structure and cell activities of these epididymides were shown as follows:

LM study of sectioned B. indica cauda epididymis: The cauda epididymis, where the sperm maturation and storage are occurred, is composed of pseudostratified columnar epithelium. The epithelial height of the caudal epididymis in the wet season samples was significantly higher (18.7 μm) than in those animals collected in the dry season (10.1 μm) ($p<0.05$) (Table 14) and the average height was greatest in November 2005 (22.8 μm) (Table 2 in Appendix A). The diameter of the tubules in the caudal epididymis were not significantly different between the seasons ($p>0.05$) (Table 14), but the diameters were greater in the wet than in the dry seasons. The maximum diameter occurred in November 2005 (390.8 μm) (Table 2 in Appendix A). The cauda epididymal cells have been classified into four different cell types as principal, basal, clear, and halo cell. During the wet season, the caudal segment was contained spermatozoa and a few round spermatids in the lumen (Figs. 24A-C). Clear cells had an abundance of vesicles near their apex and many electron-dense bodies in their basal region (Figs. 24A,B). Some clear cells had cytoplasmic extensions into the lumen (Fig. 24B). Compared to the dry period, the halo and basal cells did not show significant morphological changes at the LM level. During the dry period (Figs. 24D-F), the principal cells showed morphological modifications with some cells having cytoplasmic extensions into the lumen, especially in the July 2005 samples (Figs. 24D,E). Compared to the wet period, the clear cells had no cytoplasmic extensions and low numbers of cytoplasmic vesicles near their apex as well as having an electron-dense body in the basal region (Figs. 24D-E). The luminal epididymis contained more round spermatids and fewer spermatozoa in the dry season than in the wet season (Fig. 24F). Furthermore, the different cell types in the caudal epididymis were quantified during the wet and dry seasons. The results showed that the principal cells constituted the predominant epithelial population in the

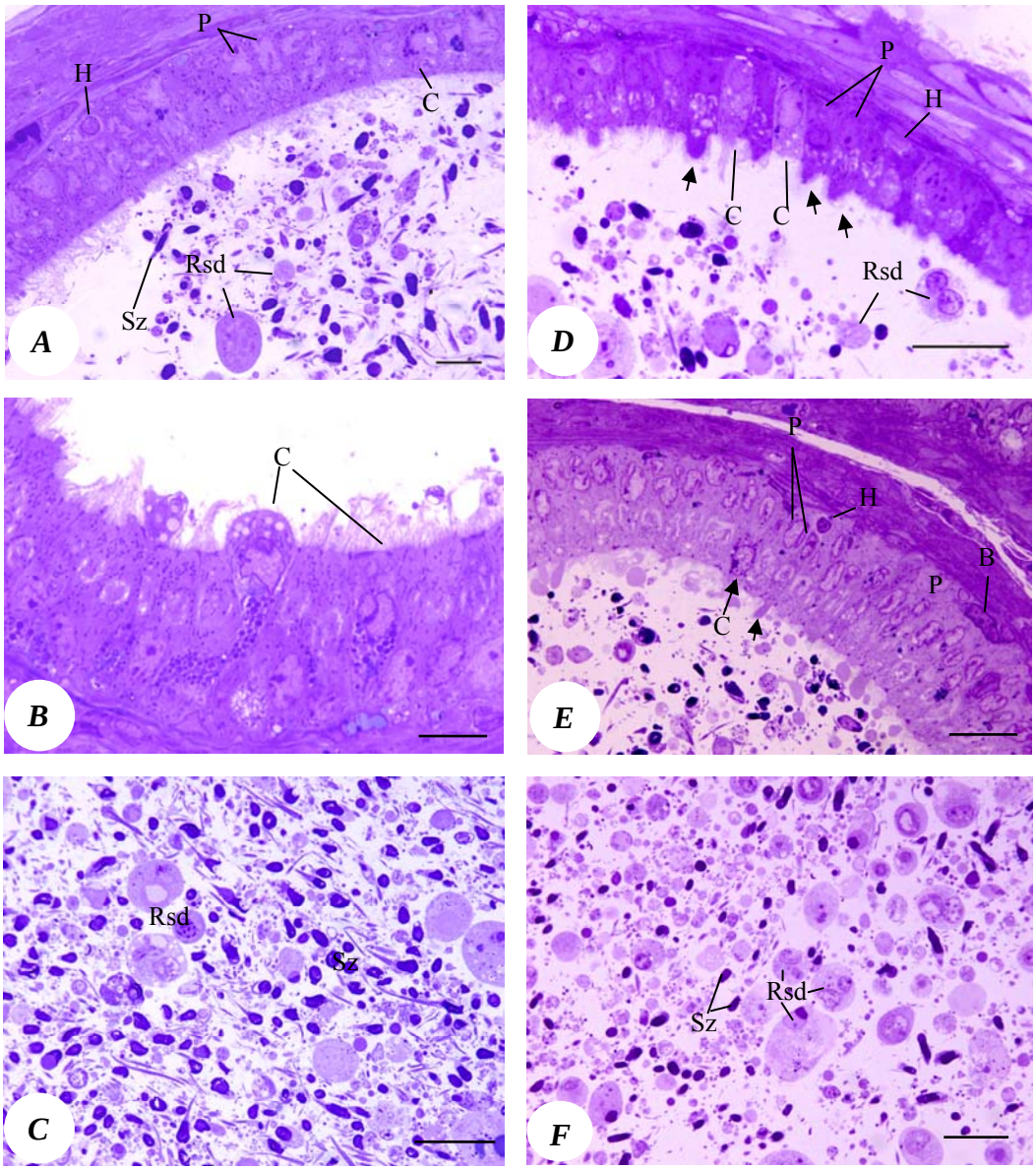


Figure 24. Transverse sections of the caudal region of the *B. indica* epididymis during the wet (*A-C*) and the dry (*D-F*) seasons. *C,F*: Various cells in the epididymal lumen; spermatozoa (Sz) and round spermatids (Rsd) (Toluidine blue stained). B= basal cell, C= clear cell, H= halo cell, P= principal cell, arrows= cytoplasmic extension. Scale bars= 10 µm (*A,B*), 20 µm (*C-F*).

Table 14. Comparison of the epithelium height, the epididymal diameter, and the distribution of epididymal cell types and round spermatids in the cauda segment of the epididymis of selected adult male *B. indica* between the wet and the dry seasons (Mean±S.E.).

Seasons	Epididymal parameters (µm)			Distribution of epididymal cell types (% of total epididymal cells)					Round spermatids in lumen (%)
	n	Epithelium height	Epididymal diameter	*n*	Principal cell	Clear cell	Halo cell	Basal cell	
Wet	4	18.7±0.8[a]	337.1±21.4	5	68.8±1.3	11.0±0.8[a]	4.9±0.4	15.3±0.9	36.3±3.6 (*n*=8)
Dry	3	10.1±0.5[b]	326.1±13.0	3	71.5±1.1	7.3±0.8[b]	6.3±0.6	14.9±0.8	56.7±7.6 (*n*=2)

n = Total number of individual Great Bandicoot rats.
Values within a column followed by a different superscript differ statistically (student's *t* test, $p < 0.05$).
Seasons: Wet (September-January); Dry (February-August)

epididymal duct of all samples, and the percentage of these cells was not significantly different between seasons ($p>0.05$) (Table 14). The basal cells, located at the base of the epithelium, did not exhibit significant quantitative variations between the two periods ($p>0.05$) (Table 14). For the clear and halo cells, the frequency of these cells was low when compared to the other cellular types. The percentage of clear cells was greatest in November 2005 (12.4%) (Table 2 in Appendix A) and was significantly higher in the wet season (11.0%) than in the dry season sample (7.3%) ($p<0.05$) (Table 14). Halo cells did not exhibit significant quantitative variation between the two periods ($p>0.05$) (Table 14) but the lowest percentage of these cells occurred in March 2006 (3.9%) which is part of the dry season (Table 2 in Appendix A). In the lumen of the cauda epididymis, the spermatozoa and the round spermatids were found in all samples investigated. Round spermatids did not exhibit significant quantitative variations between seasons ($p>0.05$) but had higher numbers in the dry season (Table 14).

TEM study of B. indica cauda epididymis: the ultrastructure of the caudal epididymal cells in the adult *B. indica* are shown in Figures 25 and 26. During the wet season, the principal cells had their highest incidence of membrane-bounded vesicles in the apical cytoplasm and among their stereocilia. The smaller vesicles were located on the apical surface, whereas the larger vesicles presumably involved in secretion were located in the lumen (Figs. 25A,B). The micropinocytotic infoldings found in the apical cytoplasm did not extend into the lumen (Fig. 25B). A variety of cytoplasmic vesicles; multivescular bodies (MVBs), electron-dense bodies, and small vesicles were evident in their cytoplasm (Figs. 25B,C). Compared to the dry season, the number of MVBs and electron-dense bodies were lower in the wet season samples (Fig. 25C). The endoplasmic reticulum and Golgi apparatus were clearly visible in their cytoplasm (Figs. 25D,E). Mitochondria were loosely scattered at the apex of these cells with lower numbers detected in the dry season samples (Fig. 25A). The halo cells showed significant morphological changes between seasons with a darker nucleus in the wet than the dry seasons (Fig. 25F) but this was not the case for the basal cells (Figs. 25A,F). The clear cells showed a higher number of MVBs, membrane-bounded vesicles, and lysosome like dense bodies in the supranuclear cytoplasm in the wet samples than those from animals obtained in the dry season

(Figs. 25G-K). Electron-dense bodies were located in both the supra- and especially subnuclear cytoplasm of these cells with a higher number in the wet season samples than those of the dry season samples (Fig. 25G). In some clear cells, their apical surfaces were either round, swollen or extended into the lumen (Figs. 25G,J). Endocytosis was found at the base of the microvilli of these cells (Figs. 25I,K). Although rare, images were seen of microvilli appearing to bend over and approach the adjacent area of the cell surface as if to incorporate the contents of the luminal fluid (Fig. 25K). Most mitochondria were distributed around the apical cytoplasm with higher numbers in the wet season rather than the dry season (Fig. 25J). Endoplasmic reticulum and Golgi apparatus were rarely found in these cells.

During the dry season (Fig. 26), the principal and clear cells in the cauda epididymis showed conspicuous morphological modifications. Some principal cells had cytoplasmic extensions into the lumen (Figs. 26A,B) and micropinocytotic infoldings at their apical region (Figs. 26A,F). The spermatozoa were found among their stereocilia (Figs. 26B,E). The endoplasmic reticulum and Golgi apparatus were not distinct in the cytoplasm (Fig. 26C), however, the supranuclear cytoplasm contained abundant mitochondria, MVBs and electron-dense bodies with these organelles being present in higher numbers in the dry season samples than in the wet season samples (Figs. 26A,D-F). Clear cells showed a lower number of MVBs, lysosome like dense bodies, membrane-bounded vesicles, electron-dense bodies and mitochondria in the cytoplasm during the dry season than the wet season samples (Figs. 26G-I). The apical surface of these cells was not extended or swollen. Endocytosis and bent microvilli were rarely found (Figs. 26G-I). The endoplasmic reticulum and Golgi apparatus of these cells were not different between seasons. The halo cells in the dry season were significantly different from those of the wet season and had a paler nucleus and many nuclear extensions in some cells (Fig. 26J).

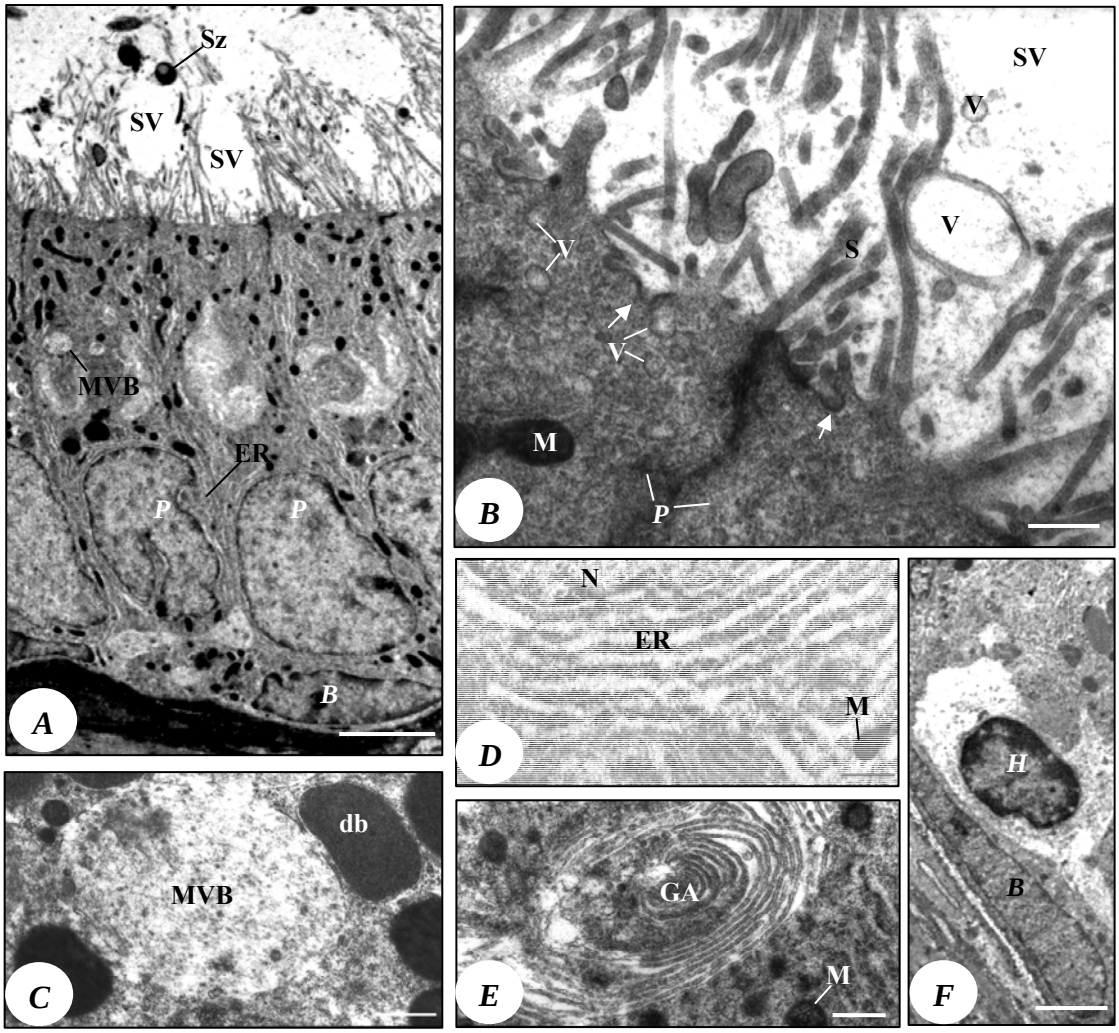
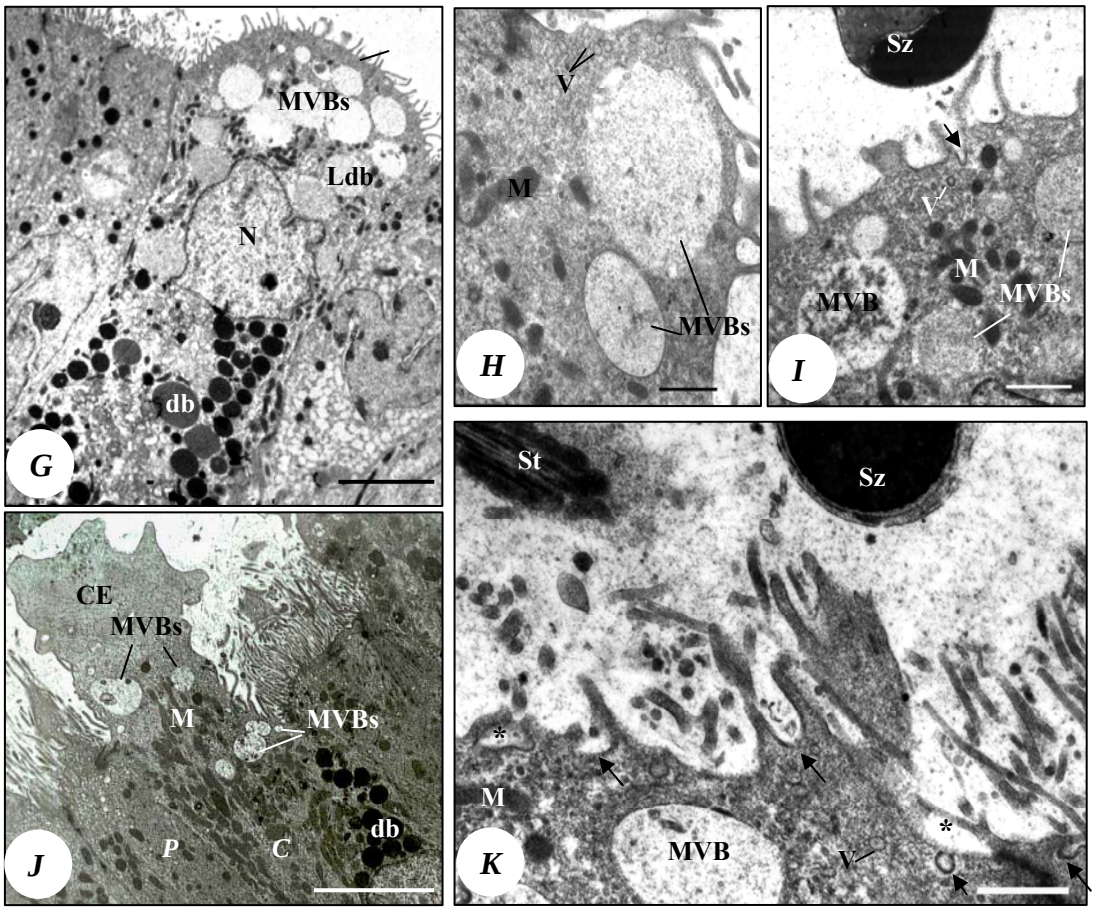


Figure 25. TEMs of the caudal region of the *B. indica* epididymis during the wet season. *A-E*: Principal cells (P) showing the incidence of membrane-bounded vesicles (V) and micropinocytotic inflolding (white arrows) at the apex, and having a distinct endoplasmic reticulum (ER) and Golgi apparatus (GA) as well as a few multivesicular bodies (MVBs) and electron-dense bodies (db) in their cytoplasm. *F:* Halo cell (H) with dark nucleus. *G-K*: Clear cells (C) showing many MVBs, membrane-bounded vesicles (V), lysosome dense bodies (Ldb) and electron-dense bodies (db) in their cytoplasm and having endocytosis (black arrows) and microvilli bent over (asterisk) at the apical region. The incidence of a swollen cell surface or cytoplasmic extension (CE) in some clear cells. B= basal cell, M= mitochondria, N= nucleus, St= sperm tail, Sv= secretion vesicle, Sz= spermatozoa. Scale bars= 1µm (*B-E*), 2µm (*H,J*), 2.5µm (*F,I*), and 5µm (*A,G,K*).



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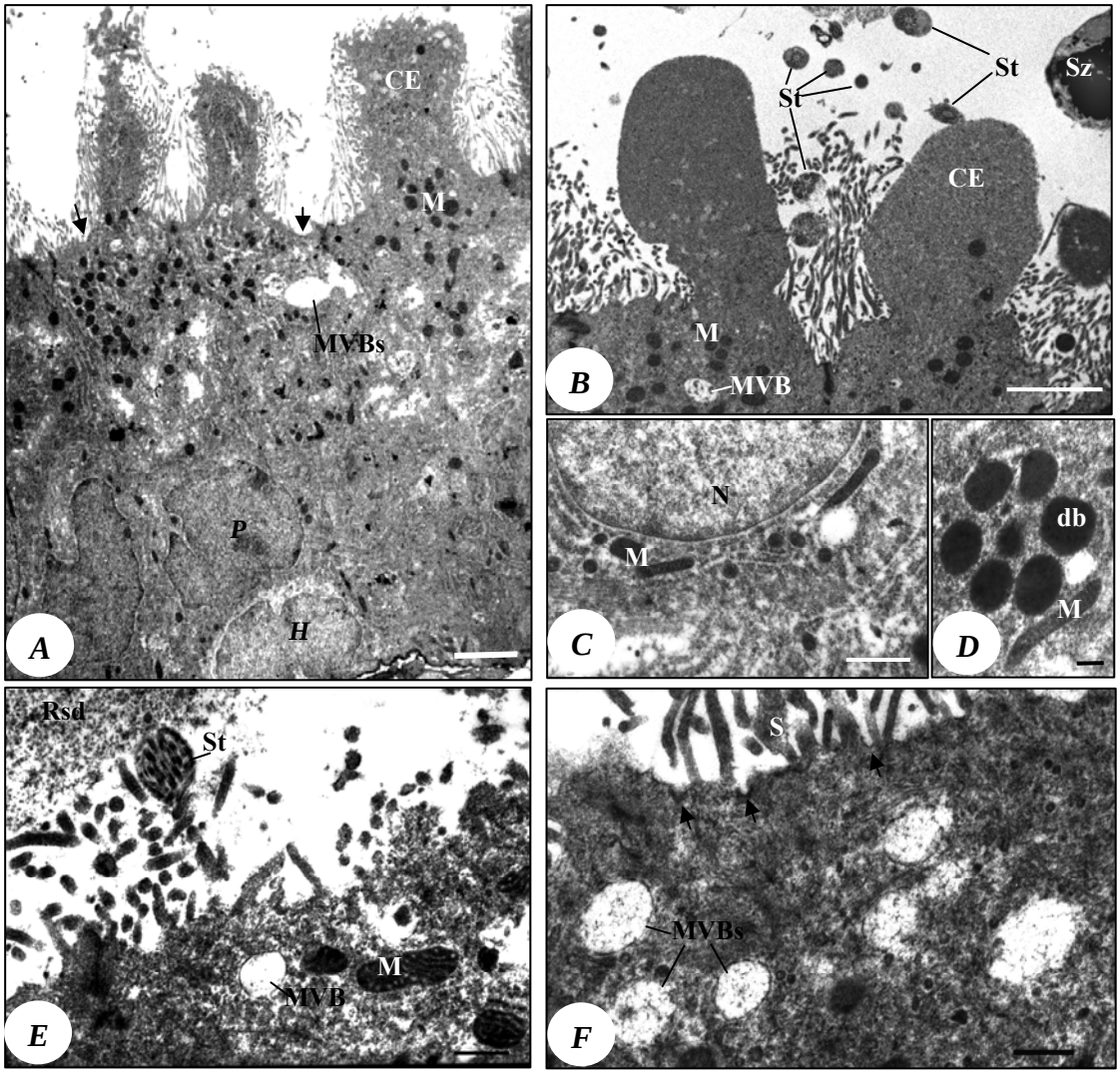

Figure 26. TEMs of the caudal region of the *B. indica* epididymis during the dry season. *A-F:* Principal cells (P) showing the incidence of cytoplasmic extensions (CE) and pinocytosis (black arrows) at the apical region and many multivescular bodies (MVBs) and electron-dense bodies (db) in the cytoplasm. *G-I*: Clear cells (C) showing the microvilli bent over (asterisk) and a few MVBs, lysosome like dense bodies (Ldb) and electron-dense bodies (db) in the cytoplasm. *J*: Halo cell (H) with a pale nucleus. B= basal cell, M= mitochondria, N= nucleus, Rsd= round spermatids, S= stereocilia, St= sperm tail, Sz= spermatozoa. Scale bars= 1µm (*D-F,I*), 2µm (*C,H*), 2.5µm (*B,G*), 3µm (*A*), 5µm (*J*).

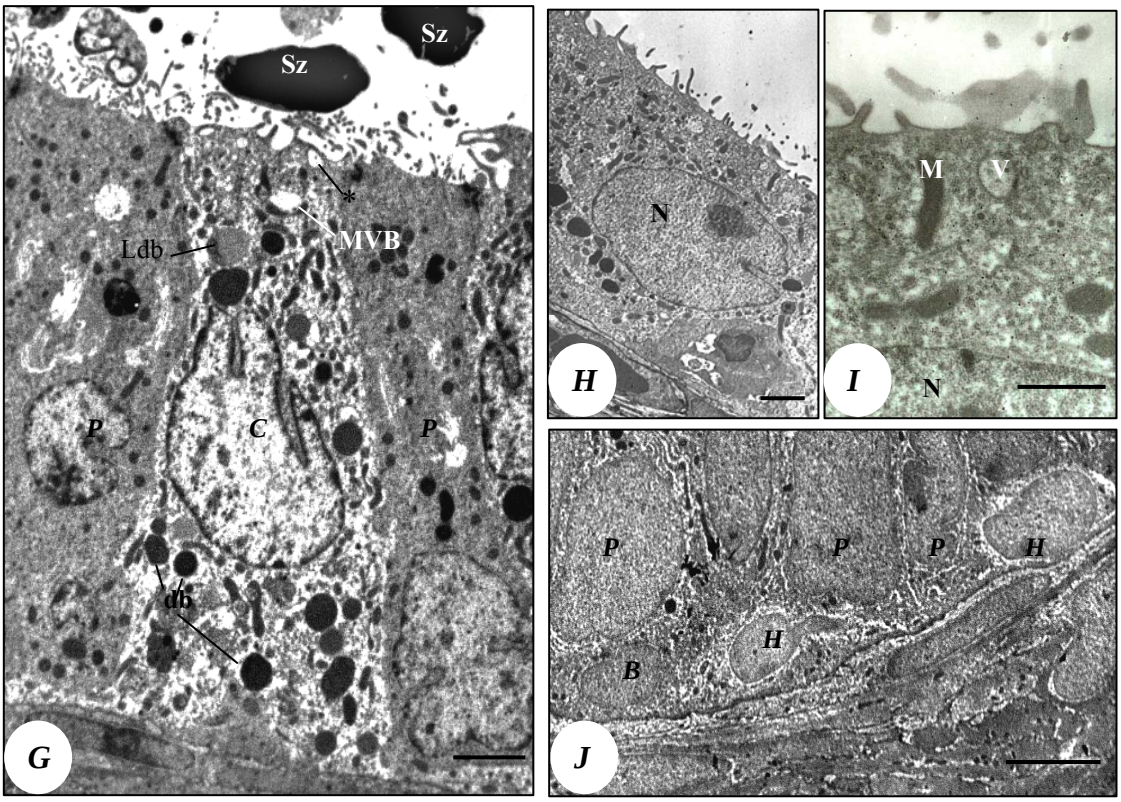

Figure 26 (Continued).

LM study of B. indica caput epididymis: a comparative study was made of the epididymal histology between the caput and cauda segments of the same animal obtained from animals with maximum testes weight (on November 2005 of the wet season). The epithelium height of the epididymides was not significantly different between these segments, however the diameters of the epididymal tubule of the cauda epididymis were wider than those of the caput segment ($p>0.05$) (Table 15). In the caput region, the epithelium was surprisingly composed of four epididymal cells (principal, halo, basal, and clear cells) as normally clear cells are not generally found in this region (Hamilton, 1975). The percentage of principal cells in the cauda region was significantly higher (64.9%) than in the caput segment (54.74%) ($p<0.05$) (Table 15). The abundance of clear cells also differed markedly with about double the percentage in the caput region (35.9%) compared to those in the cauda segment (13.7%) ($p<0.05$) (Table 15). In contrast, basal cells in the caput segment were present in significantly lower number than in the terminal region of the epididymis ($p<0.05$) (Table 15). There were no significant quantitative variations in the halo cell percentages between the epididymal regions ($p>0.05$) (Table 15). The caput epididymal lumen contained round spermatids and a few spermatozoa (Figs. 27A,B). In this segment, the principal cells are characterized by being columnar with elongated microvilli (Fig. 27A). Clear cells were surprisingly found in the caput segment of the epididymis but these clear cells were mostly type II clear cells that are identified with basally situated nuclei (see Kumar *et al.,* 1980). They differed from those type I clear cells in the cauda segment that their nuclei are situated centrally (Figs. 27A,B). Halo cells and basal cells were rarely found.

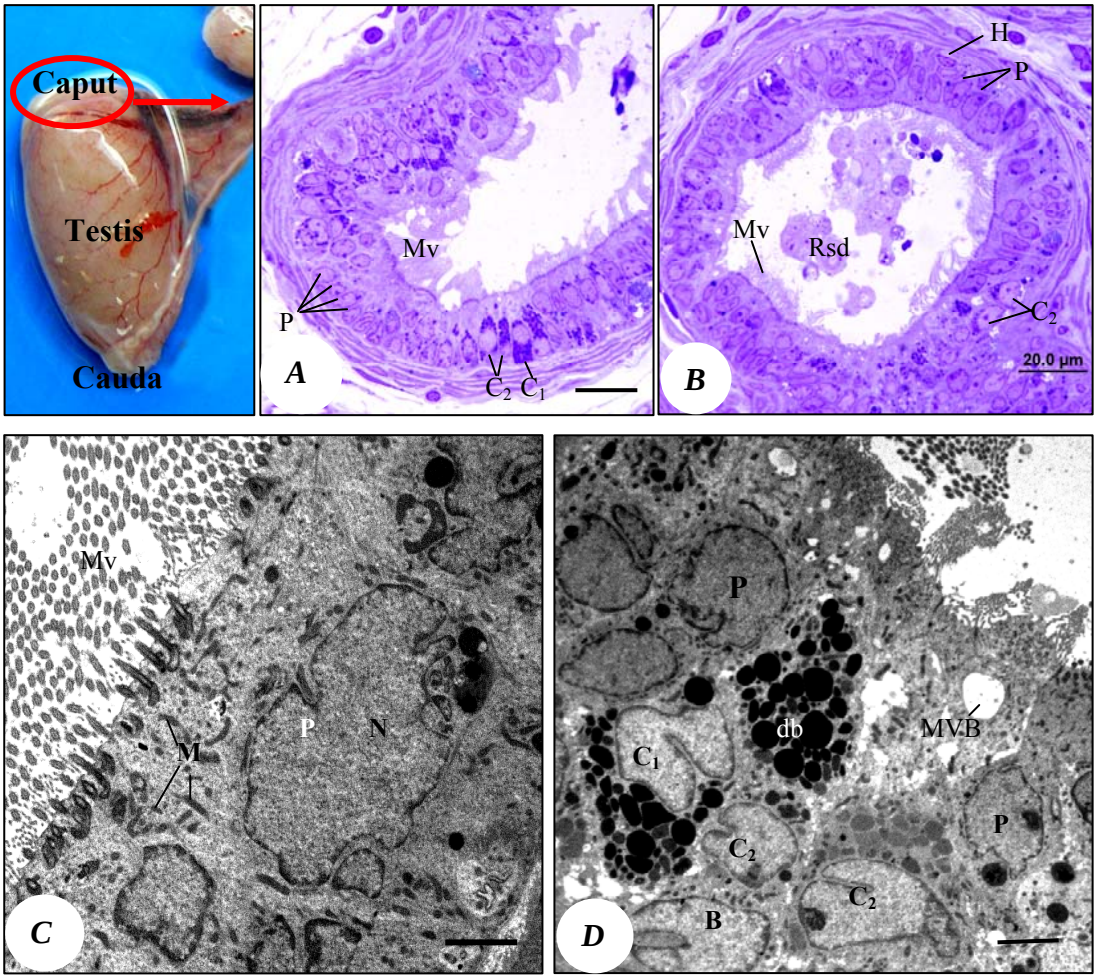

Figure 27. The caput epididymis segment of the adult *B. indica* during a period of maximum testis weight (November 2005). *A-B*: LM photomicrographs showing numerous principal (P) and clear cells (C) (Toluidine blue). *C-D*: TEM photomicrographs showing the principal cells with elongated microvilli (Mv) and clear cells having many electron-dense bodies (db) and a few multivesicular bodies (MVBs). B= basal cell, H= halo cell, N= nucleus. Scale bars= 2µm (*C-D*) and 20 µm (*A-B*).

Table 15. Comparison of the epithelium height, the diameters of the lumen and tubules, and the distribution of epithelial cells between the caput and cauda regions of the epididymides in the same adult *B. indica* during a period of maximum testis weight (November 2005) (Mean±S.E.).

Epididymal segment	N^a	Epididymal parameters (µm)		N^a	Distribution of epididymal cell types (% of total epididymal cells)			
		Epithelium height	Epididymal diameter		Principal cells	Clear cells	Halo cells	Basal cells
Caput	16	27.2±1.3	93.8±8.0^a	5	54.7±1.8^a	35.9±2.4^a	4.5±0.7	4.9±1.3^a
Cauda	16	24.5±1.0	143.8±9.4^b	15	64.9±1.6^b	13.7±1.0^b	4.7±0.6	16.7±1.5^b

N^a= Total number of tubular cross section investigated.
Values within a column followed by a different superscript differ statistically (student's *t* test)

TEM study of the B. indica caput epididymis: the fine structure of the caput epididymal cells of *B. indica* in the wet season showed that the principal cells had distinctly elongated microvilli and a lower number of cytoplasmic vesicles (MVBs, electron-dense bodies and small vesicles), mitochondria, endoplasmic reticulum, and Golgi apparatus than these cells in the cauda segment (Figs. 27C,D). The clear cells had a high number of electron-dense bodies in the sub- and supranuclear cytoplasm (Fig. 27D). These clear cells had a lower number of multivesicular bodies in the caput than in the cauda segment of the epididymis (Fig. 27D). The basal and halo cells showed no significant morphological differences between the two epididymal segments.

***LM and TEM studies of the cauda segment of the epididymis of immature B. indica during the dry season*:** the caudal epididymal epithelium had a high number of principal cells, while other epididymal cells; clear, basal and halo cells, were rare (Fig. 28A). The principal cells had scattered mitochondria and MVBs and a low number of Golgi apparatuses in their cytoplasm (Fig. 28B). Clear cells had a lower number of MVBs and no lipid droplets or electron-dense bodies in the cytoplasm. The nuclei had a concavity and were more electron-dense in their outer cortical region. Many mitochondria were dispersed throughout the cytoplasm (Fig. 28B).

TEM study of the cauda segment of the epididymis of B. bengalensis during the dry season: the epididymal lumen contained spermatozoa that had monomorphic morphology (such as a falciform head) (Fig. 29A, inset). Compared to the *B. indica* cauda epididymis in the dry season, the principal cells of *B. bengalensis* had a lower number of MVBs and cytoplasmic vesicles. The endoplasmic reticulum and Golgi apparatus were abundant in these cells (Fig. 29B). In comparison to *B. indica*, the clear cells had a higher number of MVBs (Figs. 29A,B). However, the clear cells of both bandicoot species in the dry season exhibited similar characteristics with a low number of electron-dense bodies and no cytoplasmic extensions (Fig. 29B).

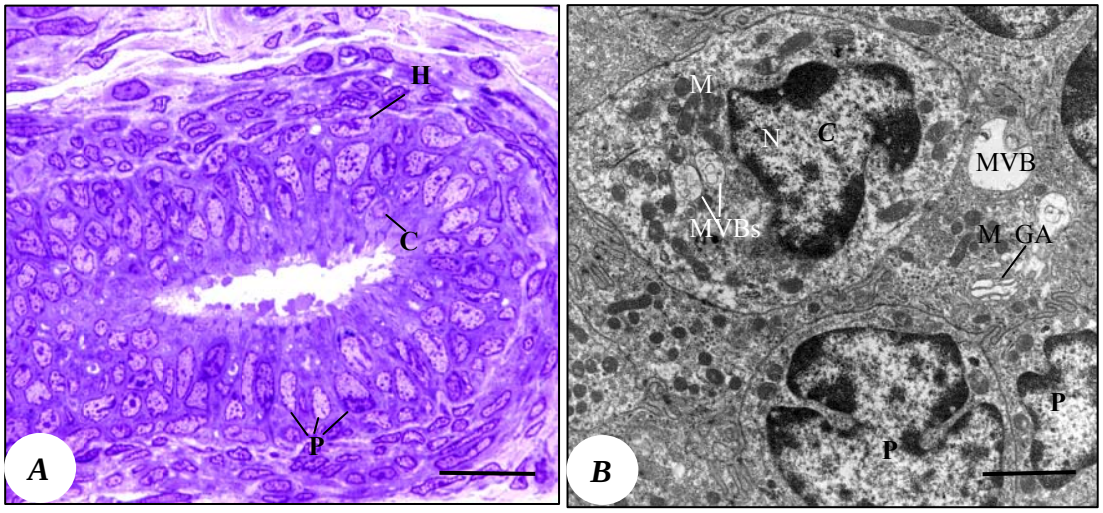

Figure 28. Transverse sections of the caudal segment of the epididymis in immature *B. indica* during the dry season. *A*: LM photomicrographs showing the epithelium with many principal cells (P) (Toluidine blue stain). *B*: TEM section showing the clear cell (C) with a few multivescular bodies (MVBs). H= halo cell, GA= Golgi apparatus, M= mitochondria, N= nucleus. Scale bars= 2µm (*B*), 20 µm (*A*).

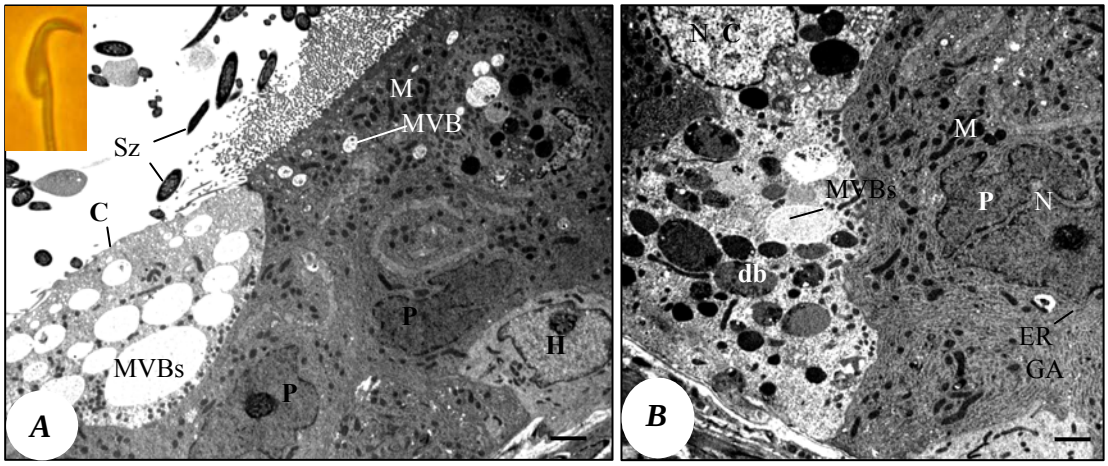

Figure 29. TEMs of the caudal segment of the epididymis in mature *B. bangalensis* during the dry season. *A:* Clear cell (C) showing many multivesicular bodies (MVBs) in the supranuclear cytoplasm. *B:* Principal cell (P) with distinct endoplasmic reticulum (ER) and Golgi apparatus (GA). Phase contrast of falciform sperm head (*A*, inset). H= halo cell, db= electron-dense bodies, M= mitochondria, N= nucleus, Sz= spermatozoa. Scale bars= 1.5 µm (*B*), 2 µm (*A*).

CHAPTER 4
DISCUSSION

Maximum reproductive activity of the *B. indica* population in the ricefield at Pakphayun district, Phatthalung province coincided with the occurrence of the wet season with many females visibly pregnant and having heavy ovaries and testes. However, the number of pregnant females and reproductive activity differed between the two dry seasons. In the first dry season, only one individual out of 10 was pregnant and this individual had only three fetuses present which is about half the usual fetal number for this species (Boonsong, 1984; this study). In the second dry season, about 17 % of the total adults obtained at that time were pregnant females. Furthermore, in the second dry season the reproductive organs weights of the non-pregnant females and mature males were generally similar to those of animals sampled in the previous wet season, whereas in the first dry season these weights tended to be lower. However, regardless of their weights, the follicular development in ovaries and the spermatogenesis in testes were still occurring, all females had mature follicles and sperm were being produced in all males even during the first dry season. Nevertheless, in some cases, a few of the individual seminiferous tubules of some males obtained in the dry season were aspermatogenic. Thus it would appear that, unlike in *B. bengalensis* either at Ludhiana in India or Islamabad in Pakistan, where a clear breeding season occurs (Kaur and Guraya, 1983; Hussain *et al.,* 1994), there is not a discrete breeding season in *B. indica* and that reproduction continues into the dry season in at least some years when it followed a period of very high rainfall in the preceding wet season as happened in the second year of this study. A similar finding has been reported by many previous studies that heavy precipitation causes delay or interruptions of the onset of breeding e.g. the multimammate rat *Mastomys natalensis* in Tanzania, the reproductive activity of females but not males responded to heavy rainfall with a 2-3 months delay (Leirs *et al.,* 1994) and the dusky rat, *Rattus colletti*, in Darwin cannot breed under very wet conditions (when heavy rain and flooding occurred) or under very dry conditions (when insufficient food is available) (Medsen and Shine, 1999).

The question thus arises, is the occurrence of maximum reproduction of this species in the wet season due to the environmental conditions during this period? Previous studies have indicated that the reproductive performance of small mammals is affected by either the quality or the abundance of available food, and also by temperature and social factors (Sadleir, 1969). Thus, in this study the relationship between *B. indica* reproduction and the environmental factors (phenology of rice plants, temperature, total rainfall, photoperiod and humidity) were observed. The results showed that their reproductive activity was significantly related with the rice cultivation stage ($p<0.05$) but not with the four remaining environmental factors examined. This result was similar to the previous observations on rats living in ricefields where the nutritional quality of the rice plant has an important influence on their reproduction and population (Lam, 1987; Hussain *et al.,* 1994; Tristiani and Murakami, 1998; 2003; Brown *et al.,* 2005). All the individuals in this study were obtained within a small defined area of rice fields where harvesting of rice takes place once per year. *B. indica* in southern Thailand breeds during the generative stage of the rice plant when there is a high percentage of pregnancies and the male reproductive organs are heaviest, but they also breed at other times of the year. These results are similar to those found for the ricefield rat (*Rattus argentiventer*) and the lesser ricefield rat (*R. losea*) in Vietnam (Brown *et al.,* 2005). However, in central Thailand where the rice is grown twice a year *B. indica* and *B. savilei* exhibit two clear breeding periods during the flowering (as generative stage) to harvesting stages of the rice plant (Sudto, 1987) and in Malaysia and Indonesia breeding by *R. argentiventer* is confined to the generative stages of rice crops with little breeding occurring outside this period (Lam, 1983; Tristiani *et al.,* 1998; Leung *et al.,* 1999). As with the ricefield rats in Vietnam and the Great Bandicoot rats in southern Thailand the fact that breeding can occur throughout the year, is probably due to the varied diets available for these animals (Marshall, 1977; Leung *et al.,* 1999; Aplin *et al.,* 2003a). However, previous studies have indicated that the high quality milky and ripening grain in the generative stage of the rice plant provides the optimal food for the reproduction and survival of rats (Tristiani and Murakami, 1998; 2003; Leung *et al.,* 1999; Brown *et al*., 2005). Bomford (1987) has reported that milk-ripe seeds of rice plants were full-sized or nearly so, and their endosperms contained starch granules,

even though they were still green and if squeezed exuded a milky juice. Chemicals in milk-ripe seeds may contain naturally occurring oestrogen or chemicals (e.g. 6-methoxybenzoxazolinone; 6-MBOA) that stimulate reproductive activity (Berger *et al.,* 1981; Tristiani and Murakami, 1998). While the rats including *B. indica* and *R. argentiventer* feed on rice plants in all stages of growth, the palatability and presumably the nutritive value differs between the stages (Tristiani and Murakami, 1998). Thus, the main breeding stages of *B. indica* in southern Thailand coincided with the generative stage of the rice plant as they responded to the availability of energy-rich foods, in the same way as did *B. bengalensis* (Hussain *et al.,* 1994) and *R. argentiventer* (Tristiani and Murakami, 1998; Leung *et al.,* 1999; Brown *et al*., 2005). This hypothesis is supported by the occurrence of the greatest intensity of rat damage during the generative stage of the rice plant and many immature rats were found during the following period (harvesting) with the gestation period being about 27 days (Boonsong, 1987). Furthermore, during the harvesting stage, the female *B. indica* had the heaviest reproductive organs, but the lowest number of pregnancies and the male reproductive organs were at their lightest. This may be caused by the rapidly decline of high quality food when the ricefields are disturbed by harvesters and other harvesting activities at this period (Leung *et al.,* 1999; Tristiani and Murakami, 2003). As a result, the reproductive activity of *B. indica* decreases during the harvesting stage of the rice plant, as for *R. argentiventer* (Tristiani *et al.,* 1998). In addition, the photoperiod may be a major regulating factor influencing the timing of reproduction of many mammals in the temperate, sub-tropical and tropical zone, especially above 10^{o} latitude (Bronson and Heideman, 1994). Many rodents in these regions cease breeding as the day length becomes shorter following the end of summer and the onset of autumn such as in *Aethomys ineptus* and *A. namaquensis* from southern Africa (25^{o} 34'N) (Muteka *et al.,* 2006b). By contrast, mammals and rodents living within 10^{o} of equator appear to rely on cues other than photoperiod to signal changes in reproductive physiology (Sadleir, 1969; Bronson and Heideman, 1994). Similarity, this study revealed that the reproductive activity of *B. indica* in southern Thailand where is at 7^{o}16'N latitude, was not significantly correlated to the photoperiod (p>0.05), like the reproduction of the Nile grass rat, *Arvicanthis niloticus*, in Kenya (2^{o}S) (Nunes *et al.,* 2002). Furthermore, several studies of animals living in tropical

and arid region have revealed that their reproduction is necessarily linked to rainfall and nutritional improvement or a combination of these factors (Sadleir, 1969). The seasonal variations of weather, particularly rainfall, influences food availability that in turn affects the life strategies and reproduction of rodents (Bronson and Heideman, 1994; Leirs *et al.,* 1994) such as *Arvicanthis niloticus, Mastomy natalensis* (Taylor and Green, 1976), *Lophuromys flavopuncatus*, *Grammomys dolichurus* and *Praomys delectorum* (Mukundi *et al.,* 2006). In the present study, although the rainfall had little significant influence on *B. indica* reproduction ($p>0.05$), the breeding of this species was related to the season and the cultivation stage of the rice plant with high reproductive activity being found during the generative stage in the wet season. It indicates that the heavy rain in the wet season may result in the rice to growing well in its generative stage and provide the optimal high quality food for promoting rat reproduction. Therefore, the nutritional factors appear to be essential for *B. indica* reproduction, just as they are for *R. argentiventer* (Tristiani and Murakami, 1998), when food intake may play a pivotal role in determining the onset of ovulation and other aspects of female reproduction as well as influencing spermatogenesis of the male mammals. However the reproductive responses to food may differ among species (Blank and Desjardrins, 1984). It is well known that a reduction in food availability inhibits reproduction by influencing the pulsatile release of GnRH which depresses the secretion of LH, and growth hormone, whereas FSH is affected by food restriction only if the restriction is severe and greatly prolonged (Bronson and Heideman, 1994). Thus, steroidogenesis is more sensitive to food restriction than gametogenesis (Bronson and Heideman, 1994; Tinney *et al.,* 2001). It may be one possible cause of the occurrence of the ovarian follicular development and the spermatogeneis in *B. indica* being active in any stage of rice plant cultivation including even during the resting stage (as food restriction) when the weights of reproductive organs decreased, as with *Mus musculus* (Blank and Desjardrins, 1984) and *Saccostomus campestris* (Tinney *et al.,* 2001).

In addition to the sperm morphology of murid rodents, several studies have revealed that most species within the subfamily Murinae produce sperm with consistent morphology and falciform sperm heads (Breed, 2002; 2004). Nevertheless a few species of murine rodents have a highly pleiomorphic sperm population e.g. the

red veld rat, *Aethomys ineptus* and three species of *Lophuromys* from Africa (Breed, 1995), the spinifex hopping mouse, *Notomys alexis* from Australia (Bauer and Breed, 2006) and a few others from southeast Asia (Breed and Young, 1986; Breed, 1993). Previous observations on the Great Bandicoot rat of central Thailand have shown that, in the few individuals investigated, there is not only sperm with highly divergent morphologies but they are also normally highly pleiomorphic (Breed, 1993; 1998). However, the question arises is the sperm pleiomorphism previously seen in the sperm populations of the cauda segment due to inadvertent selection of males that were not optimally reproductively active at the time they were sampled for their spermatozoa? The present study tried to address this question. The results showed that the high variations in sperm morphology were found in all *B. indica* samples including even males with larger testes that implied they had fully active spermatogenesis. *B. indica* spermatozoa could be categorized into at least nine different sperm head and fourteen different sperm tail morphotypes but the morphologically normal spermatozoa ranged from globular to conical heads and a straight tail. The spermatozoa of this species are totally different in nearly all of their morphological characteristics from those of *B. bengalensis* and *Rattus* whose sperm heads are consistently falciform (Breed, 1998; 2004), in spite of the genus *Bandicota* being considered a close relative of *Rattus* (Gadi and Sharma, 1983; Musser and Brother, 1994; Watts and Baverstock, 1994). One possibility is that the environment when fertilization occurs and the surrounding barriers of the egg have an indirect influence on sperm head shape (Bedford, 1991; Breed, 1998). Thus in *Bandicota*, a causal relationship may possibly exist between the zona thickness and the structural organization of the sperm head. The incidence of abnormal spermatozoa has been reported to be high in a few wild mammals e.g. Arabian leopard (41-72% abnormality) (de Haas van Dorsser and Strick, 2005), Namibian cheetah (~81% abnormality) (Crosier *et al.,* 2007). Similarity, in this study, *B. indica* had highly abnormal spermatozoa with about 36.2% having abnormal sperm heads and 70% abnormal sperm tails. The most prominent sperm defect was a macrocephalic head and distal cytoplasmic droplets. The reasons for the high incidence of morphologically abnormal spermatozoa in *B. indica* is not known but pleiomorphic spermatozoa have often been assumed to be due to either the absence of genetic

heterozygosity within the population as happens with inbreeding e.g. cheetahs, florida panthers, snow leopards (Wildt *et al*., 1987), or old age e.g. some strains of laboratory mice (Krzanowska, 1981), hamster (Calvo *et al.,* 1999) and black-footed ferret (Wolf *et al.,* 2000). However, in the case of a few murine rodents including *N. alexis* and *B. indica*, they invariably have highly variable sperm morphology regardless of their age and inbreeding (Bauer and Breed, 2006; Breed, 1998; my observation). TEM observations of *B. indica* spermatozoa revealed that although their spermatozoa had various sperm head morphotypes with differences in either shape or size of the nucleus, acrosome, and tail, but some characteristics were common such as (1) these sperm heads were not bilaterally flattened, (2) the nucleus had many prominent vacuoles and extended into acrosome with being various shapes and sizes, (3) sperm heads had no perforatorium and equatorial segment, and (4) sperm tails were short in length with having C-shaped outer dense fibres. These structures of *B. indica* spermatozoa are most unusual in a eutherian mammal that normally has a bilaterally flattened nucleus, a resilient inner acrosomal membrane, an apical accumulation of subacrosomal material, and an acrosomal equatorial segment (Bedford, 1991). These features of eutherian sperm have been suggested as being adaptations of the spermatozoon that allow them to penetrate the zona pellucida and the equatorial sperm segment initially makes contact with the oolemma at the time of the sperm-egg fusion (Bedford, 1991; Bedford and Hoskins, 1990). The absence of some of these morphological characteristics in *B. indica* spermatozoa thus indicates a possible different mechanism of zona penetration (Breed, 1993; 1998). Previous observations on these *Bandicota* species have indicated that *B. indica* has an unusually thin zona pellucida (~ 3.5 μm of thickness) compared to that of *B. bengalensis* (Breed, 1998). Therefore, the Great Bandicoot rats have been confirmed as having a highly developed and pleiomorphic epididymal sperm population regardless the time of year. The unusual sperm characteristics of *B. indica* indicate that testicular function in the control of spermatozoon shape, and also some aspects of the sperm-egg interaction during fertilization may have diverged markedly between the different species of *Bandicota* with *B. bengalensis* probably representing the ancestral condition and *B. indica* and *B. savilei* exhibiting a more recently derived state (Breed, 1993; 1998).

In relation to the variation in sperm quality of ricefield *B. indica* in southern Thailand, the percentage of sperm with morphologically normal heads and tails was determined for most of the individuals collected, both in the dry and the wet seasons. The proportion of normal morphologically spermatozoa showed a seasonal variation that parallelled the testis weight and the frequency of normal heads and tails was significantly higher in the wet season than those collected in the dry season, especially during the first year of study. Thus clearly the season in which the animals were collected did indeed affect the quality of the sperm produced although it appears that spermatogenesis did not ever cease completely. This finding is similar to previous studies of some other mammalian species e.g. Friesland bulls (Vilakazi and Webb, 2004), Arabian leopards (de Haas vas Dorsser and Stick, 2005) that also showed the testes produce the highest quality of normal spermatozoa during the breeding time but that spermatogenesis still occurs at other times of year. During fertilization, the mechanisms that reduce the numbers of sperm passing into and through the oviduct would allow only the more vigorous spermatozoa to reach the site of fertilization. However previous studies on deformed spermatozoa indicate that the abnormal spermatozoa do have fertilizing ability (Smith *et al.,* 1970), but the potential for fertilization and the ability to bind to and penetrate the zona pellucida by abnormal sperm are lower than normal sperm (Howard *et al.,* 1993; Vos *et al.,* 2003). In this study, the proportion of abnormal spermatozoa from *B. indica* was high during the dry season and therefore had a low potential for fertilization. It coincided with the occurrence of a low number of pregnant females during this season. Moreover, TEM sections of *B. indica* spermatozoa revealed that several sperm from some animals obtained in the dry season had a heterogenous nucleus with uncompleted chromatin condensation. The results were similar to a previous study of the ram (*Ovis aries*) and brown bear (*Ursus arctos*) as their spermatozoa are more decondensed outside the breeding period (Garcia-Macias *et al.,* 2006). Although the seasonal variation in sperm quality has not been investigated well in murine rodents, there have been several studies of sperm quality in large mammals that indicate that low sperm numbers and a high incidence of sperm defects are assumed to associate with a reduction in scrotal circumference during high ambient temperatures (Vilakazi and Webb, 2004) and/or poor nutrition or food restriction (Morais *et al.,* 2002). In the

present study, the sperm quality of *B. indica* were not significantly correlated with environmental factors including the cultivation stage of the rice plant ($p>0.05$), however the normal sperm heads and tails of this species tended to be high during the generative stage of the rice plant when it is assumed that high quality food is available (see above). *B. indica* normally has a small testis within a small scrotum (Breed and Taylor, 2000; my observation) and this implies its scrotal circumference changes little with the ambient temperature. Therefore, the seasonal variation in sperm quality of *B. indica* in southern Thailand may be more associated with changes in food availability than changes in the ambient temperature, like the ocelot (*Leopardus pardalis*), margay (*L. wiedii*) and tigrina (*L. tigrinus*) (Morais *et al.,* 2002).

Sperm abnormalities have been reported to originate in either the testes during spermatogenesis (as primary abnormalities e.g. abnormal head shape, bicephalic, biflagellate, a coiled tail) or the excurrent duct system during sperm transport (as secondary abnormalities e.g. cytoplasmic droplets) (Wildt, 1999). The sperm quality thus relates to the spermatogenesis in the testes and the activities in epididymis. It is assumed that the epididymal epithelium produces a favorable environment for sperm maturation by secretion and absorption processes (Bedford, 1975; Hemilton, 1975). In this study, seasonal variations in the epididymal structure and activity of *B. indica* showed that the cauda epididymides during the dry season exhibited a narrow tubular diameter with low columnar epithelium and having numerous round spermatids in the lumen. The epithelium also had a higher number of principal cells compared to that of the wet season. The principal cells in the cauda segment of the wet season had many cytoplasmic extensions into the lumen, micropinocytotic infolding on their apical surface, and a high number of mitochondria, multivesicular bodies (MVBs), electron-dense bodies, and small vesicles. These structures seem to offer the most likely morphological manifestation of the principal cells absorptive processes (Hamilton, 1975; Flickinger *et al.,* 1978). The MVBs, which are numerous in the principal cells of the cauda segment of the epididymis of the rat, function as digestive vacuoles or phagocytososomes (absorptive activity) (Friend, 1969). Thus, the principal cells of the *B. indica* cauda epididymis may exhibit a high potential for absorptive processes during the dry season. The absorptive processes in these principal cells of *B. indica* differ from that found in the

laboratory rats whose absorption is generally found in the proximal region of the epididymis (Hamilton, 1975; Flickinger *et al.,* 1978; Moore and Bedford, 1975; Robaire and Hermo, 1988; Hermo *et al.,* 1988). In addition, the apical principal cells of the wet season contained the membrane-bounded vesicles among their stereocilia, and they also had a distinct endoplasmic reticulum and Golgi apparatus. The membrane-bounded vesicles have been reported to be a possible form of a secretory system of the epididymal epithelium and this contributes to the intraluminal environment for sperm maturation (Fornes and De Rosas, 1991). Thus, in this study these principal cells in the wet season samples may play a role in the secretory activity. Furthermore, during the wet season, the clear cells also exhibited a greater number of MVBs, membrane-bounded vesicles, and lysosome like dense bodies (Lbd) in the supranuclear cytoplasm, and with a large electron-dense body on the base of this cell that these structures seem to be the morphological manifestation of absorptive activity (Hamilton, 1975; Flickinger *et al.,* 1978; Hermo *et al.,* 1988). Consistently, the previous studies of epididymal activity indicate that, in the rat, the clear cells are involved chiefly in absorptive activities on the distal epididymis (Hamilton, 1975; Flickinger *et al.,* 1978; Moore and Bedford, 1979; Robaire and Hermo, 1988; Hermo *et al.,* 1988). As one of their functions clear cells have an active role in the uptake and disposal of the contents of the cytoplasmic droplets that originate from the spermatozoa (Hermo *et al.,* 1988). The cytoplasmic droplets are released from the spermatozoa, they then break up in the lumen, and their released contents, appear to be endocytosed selectively by the clear cells (Hermo *et al.,* 1988). However, the disposal of cytoplasmic droplets in the rat appears to be markedly different from that described for the brushtailed possum by Temple-Smith (1984) in which intact cytoplasmic droplets detached from spermatozoa are phagocytosed by specialized principal cells in a distinct region of the epididymis. The morphological characteristics of these specialized principal cells in the brushtailed possum seem to be similar to the type II clear cells of the rat as described by Kumer *et al.* (1980). In this study, the clear cells in the cauda epididymis of *B. indica* thus exhibited a high absorptive activity in the wet season that their activity may relate to the uptake and disposal of the contents of the cytoplasmic droplets by endocytosis in the clear cell as found for the Sherman rat (Hermo *et al.,* 1988). The occurrence of this activity

coincided with a decreasing proportion of the abnormal sperm tails that had a cytoplasmic droplet on the midpiece (both distal and proximal regions) of *B. indica* during the wet season.

In the caput epididymis of *B. indica*, the presence of clear cells in the epithelium was surprising as this was different from other rats as their clear cells are generally a significant part of the epithelium of the tail of the epididymis (Hamiliton, 1975). The percentage of clear cells in the caput segment was about twice that of the cauda segment and most of these clear cells were of the type II variety (as described by Kumer *et al.*, 1980). TEM of these type II clear cells showed a high number of electron-dense bodies and a low number of MVBs in the cytoplasm. It indicates that the clear cells in the caput segment may also have absorptive activity but not so distinctly as in the cauda segment.

The presence of these clear cells with absorptive activity in the caput and cauda segments (in the wet season) and in the principal cells of the cauda segment (in the dry season) implies that absorption by the epididymal epithelium in *B. indica* occurred throughout the length of the epididymal tubules, unlike the situation with laboratory rats. This difference may be one reason why the testis and epididymis of *B. indica* are smaller than those of the common rat and this will allow a faster transit time for their spermatozoa along the epididymides, as is the case for *Notomys alexis* (Perice and Breed, 2001). This finding suggests that the maturation of spermatozoa in this species occurs rapidly in the upper regions of the epididymis, a possibility that needs to be investigated.

The results of the seasonal variations in epididymal activity of *B. indica* indicated the high absorptive activity of principal cells during the dry season, whereas in the wet season (when the maximum reproductive activity occurring), the high secretory activity of the principal cells and the high absorptive activity in the clear cells were established by virtue of their structures. Observations of the epididymal activity of the cauda segment of the epididymides of *B. bengalensis* that had been collected in Penang, Malaysia in April 1994 (the dry season), exhibited a similar ability for secretion in the principal cells as they also had a distinct endoplasmic reticulum and Golgi apparatus, and also their clear cells had a high number of MVBs again indicating a secretory function. In this study *B. bengalensis*

were collected from a different location than in a previous study that had shown the existence of a distinct breeding period for this species (February-October with peaks at April to August-September) (Kaur and Guraya, 1993) In this study animals were collected in April and have been assumed that they occurred in their main breeding season. The cauda epididymides of *B. bengalensis* thus exhibits active secretory functions of their principal cells and absorptive functions in their clear cells during a period of maximum reproductive activity, just as is the case in *B. indica*.

Unfortunately, in this study, adult *B. indica* were rarely collected in February 2005 and January 2006 that coincided with rice being harvested and thus the study area was being disturbed by harvesting activity. A previous study has indicated that rats probably moved away from either the unavailability of food or the harvesting disturbances to an area that had food of a higher quality (as during the generative stage of rice crop) (Boonsong *et al.,* 1984). The distance of *B. indica* migration has been reported to range from 90 metres to 1 kilometre (Boonsong *et al.,* 1984; Sudto, 1987). Moreover, several studies of the demography and movement of rats have indicated that rainfall inundates the rat habitat, and the rats are forced to migrate to higher ground, and thus rats have probably moved out of the ricefield during flooding (Boongsong *et al.,* 1999; Madsen and Shine, 1999). In the case of this study, the study site was greatly flooded in December 2005 of the second year study with the water levels being about 90 cm height. It may be a cause of the decreasing *B. indica* population during this time. Thus, the reduction of the *B. indica* population during February 2005 and January 2006 may due to the inundation in study site and the disturbance of the rat habitat by harvesting activity so rats moved from the study site to nearby crops where were not flooded and the rice was in its milky or ripening stages.

The present study has clearly shown that *B. indica* males invariably have small testes with a maximum weight of around 0.5% of their body weight occurring at the height of the breeding period in the wet season. A highly pleiomorphic sperm morphology and sperm abnormalities of this speices appear to be a characteristic feature of their sperm populations regardless of the time of year. This contrasts with most species of murine rodents, including that of another species within the same genus *B. bengalensis* and the closely related species *R. argentiventer* both of

which have a much more consistent sperm morphology (Breed, 1998; 2004) and larger testes (Breed and Taylor, 2000). In various groups of mammals it has been shown that the relative testes size is associated with the type of breeding system. In the potentially intense intermale sperm competition within species, the efficiency of sperm production, and sperm size for animals that mate promiscuously, or have a multimale breeding system, males have relatively larger testes and higher sperm production as well as larger sperm size (longer sperm tail), than do those that mate monogamously or exhibit a single male mating system (Harcourt *et al.,* 1981; 1995; Harvey and Harcourt, 1984; Kenagy and Trombulak, 1986; Peirce and Breed, 2001). In multi-mating animals, one of the possible reasons why a longer sperm may be selected, is that a longer sperm may be able to swim faster and reach the ova sooner, thus outcompeting rival smaller sperm (Gomendio and Roldan, 1991). The Great Bandicoot rat showed relatively small testis size and small sperm size (short sperm tail) (Breed *et al.,* 2007; this study) and thus indicates that, unlike the ricefield rats, there may be low levels, or an absence of, intermale sperm competition and hence perhaps a monogamous mating. Therefore, it appears from the reproductive anatomy that the ricefield rats and the Great Bandicoot rats exhibit very different breeding systems. Furthermore, they may also differ in their social organization as their preputial glands, which probably secrete pheromones for social interactions and territorial defense, are surprisingly large in both sexes of the Great Bandicoot rat but in the ricefield rat only the males have large preputial glands. The findings from this study will apply and help to refine the management and control of the rat population by focusing management prior to the onset of the main breeding periods.

CHAPTER 5
CONCLUSIONS

The conclusions of these findings are as follows:

1. The Great Bandicoot rat, *Bandicota indica*, in ricefield of southern Thailand can breed throughout the year with the ovarian follicular development and the spermatogenesis being found in all individuals. However, the maximum reproductive activity of this species occurred in the wet season (September to January) that showed most females being visibly pregnant and the high number of mature follicle as well as the heavy male and female reproductive organs.

2. The reproduction of male and female *B. indica* was related to the cultivation stage of the rice plant ($p<0.05$). The maximum reproductive activity coincided with the generative stage of the rice plant when the number of pregnancies and mature follicles were highest as well as the male reproductive organs being heaviest.

3. *B. indica* has a highly pleiomorphic sperm morphology and sperm abnormalities appear to be a characteristic feature of their sperm populations regardless of the time of year. The spermatozoa could be divided into at least 9 different sperm head and 14 different sperm tail morphotypes, and the morphologically normal spermatozoa ranged from globular to conical heads and a straight tail. The spermatozoa of this species are totally different from those of *B. bengalensis* and *Rattus* whose spermatozoa have a consistent falciform sperm head.

4. TEM of *B. indica* spermatozoa revealed that all sperm morphotypes possessed the common characteristics as: (1) the heads were not bilaterally flattened, (2) the nucleus had many prominent vacuoles, (3) the heads had no recognizable perforatorium and equatorial segment, and (4) the midpiece and principal piece contained C-shaped outer dense fibres. These features of *B. indica* spermatozoa are most unusual compared to spermatozoa of other eutherian mammals.

5. The sperm quality of *B. indica* varied between seasons that the proportion of normal spermatozoa was significantly high in the wet season. Although the sperm quality was not significantly correlated with the environmental conditions

including the rice cultivation stage (p>0.05), but the percentage of normal sperm heads and tails tended to be highest in the generative stage and lowest in the harvesting stage of the rice plant.

6. The principal cells of the cauda epididymis in the adult male *B. indica* showed a remarkably role in absorption during the dry season and secretion in the wet season. In addition, the clear cells had a high absorptive activity during the wet season.

7. The relatively small testes and highly pleiomorphic sperm morphology of male *B. indica* indicates that this species differs from the ricefield rat in its breeding system, by being monogamous whereas the large preputial glands in the female bandicoot rats suggest a divergent social organization.

Suggestions:

This research raises some interesting points that should be given attention in future as follows:

1. *B. indica* that was collected bimonthly over a period of 24 months had the differences in reproductive activities between the two study years. The intervals of animal collection may be insufficient for clarify its reproductive activity. So, further study should investigate the variation in its reproductive activity with monthly intervals.

2. The study site is too limited that may not be a good representation of *B. indica* population in southern Thailand. Thus, it needs further investigations in other areas of ricefield in southern Thailand.

3. The spermatozoa of *B. indica*, unlike other eutherian mammals, have no perforatorium and equatorial segment in their sperm heads that may cause a markedly different mechanism of the zona penetration and the sperm-egg fusion from that of the typical eutherian mammalian pattern. Therefore, further study on the mechanism of fertilization in this species is suggested.

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APPENDICES

Appendix A

Table 1. Seasonal variations in the number of placental scars in adult non-pregnant female *B. indica* during September 2004 to September 2006.

Seasons	Months	Total number of adult females	No. of animals with placental scars	No. of placental scars	
				Mean±S.E.	Range
Wet I	Sep-04	11	7	8.4±1.3	4-12
	Oct-04	14	13	7.6±1.5	1-18
	Dec-04	8	7	11.1±1.4	5-16
Dry I	Feb-05	0	0	0	0
	May-05	5	2	20.5±0.5	20-21
	Jul-05	4	2	12.5±0.5	12-13
Wet II	Sep-05	4	3	28.0±2.1	24-31
	Nov-05	8	7	9.6±0.7	7-12
	Jan-06	14	14	11.4±0.9	5-18
Dry II	Mar-06	12	11	11.8±1.6	6-23
	May-06	5	3	10.7±2.3	7-15
	Jul-06	8	7	10.9±1.8	4-18
Wet III	Sep-06	7	7	8.6±1.4	5-16

Table 2. Mean (± S.E.) epithelium height, the epididymal diameter, and the distribution of epithelial cell types in the cauda region of the epididymis of selected adult male *B. indica* during September 2004-September 2006.

Seasons	Months	*N* (*n*[a])	Epididymal parameters (µm)		Distribution of epididymal cell types (% of total epididymal cells)				
			Epithelium height	Epididymal diameter	*N* (*n*[a])	Principal cell	Clear cell	Halo cell	Basal cell
Dry 1	Jul-2005	1(16)	8.2±0.3[e]	296.3±19.0[ab]	1(5)	69.3±2.5	10.0±1.7[ab]	7.2±1.1[ab]	13.6±1.1
Wet 2	Sep-2005	1(16)	13.3±1.0[bc]	328.1±20.1[ab]	1(5)	70.2±3.6	11.4±1.3[a]	4.1±0.6[c]	14.4±2.4
	Nov-2005	2(32)	22.8±1.1[a]	390.8±35.6[a]	3(15)	67.2±1.6	12.4±0.9[a]	5.2±0.6[abc]	15.2±1.2
Dry 2	Mar-2006	1(16)	12.0±0.8[cd]	341.9±14.7[ab]	1(5)	73.8±1.4	7.4±0.4[bc]	3.9±0.3[c]	14.9±1.3
	May-2006	1(12)	10.1±0.9[de]	345.0±33.9[ab]	1(5)	71.4±1.6	4.8±0.7[c]	7.7±0.7[a]	16.3±1.7
Wet 3	Sep-2006	1(16)	15.6±0.6[b]	258.8±17.5[b]	1(5)	72.2±1.8	6.6±0.5[bc]	4.9±1.1[bc]	16.3±1.4

N = Number of animal investigated
n^a = Total number of tubular cross section investigated.
Values within a column followed by a different superscript differ statistically (ANOVA or Brown-Forsythe of Robust test, $p<0.05$).
Seasons: Wet (September-January); Dry (February-August)

Table 3. Total rainfall of Phatthalung province for the 12-year period (1995-2006).

Months	Monthly total rainfall of each year (mm./month)											
	1995	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
January	53.0	85.7	11.1	9.6	321.9	164.6	385.9	10.2	111.1	8.2	67.4	2.6
February	21.0	63.6	214.0	0.6	617.0	149.7	73.8	0.0	7.8	66.3	0.0	97.3
March	133.6	5.7	68.3	44.2	30.6	410.9	246.0	13.6	98.4	10.0	70.3	140.4
April	55.2	108.9	84.3	9.6	131.3	176.0	59.1	98.5	38.0	55.8	8.3	191.8
May	24.0	73.3	73.9	133.7	208.8	65.5	129.4	173.7	27.8	121.3	70.3	133.5
June	6.7	85.8	63.1	66.5	91.4	139.8	82.6	122.5	47.5	78.9	25.3	118.4
July	132.1	54.5	162.3	200.4	40.3	10.0	79.0	30.9	85.3	52.6	59.4	18.1
August	261.8	95.1	184.5	159.0	57.1	141.2	27.9	141.2	27.9	6.6	96.8	44.6
September	73.6	102.2	184.5	211.7	104.7	112.3	80.9	50.3	62.3	327.4	56.6	161.3
October	172.4	141.8	242.0	316.7	247.4	106.2	237.9	144.5	303.6	264.2	342.1	269.4
November	324.7	291.6	477.9	401.3	327.9	922.4	411.2	834.1	389.5	600.4	702.2	310.0
December	173.2	935.5	474.5	556.4	383.0	436.4	441.5	437.3	400.2	226.8	1505.5	232.9

The data were available from the Thai Meterological Department at Phatthalung province.

Appendix B

Tissue processing for paraffin sections (Bancroft and Gamble, 2002)

1. **Formulae for fixative solution**

1.1 Bouin's fluid

Saturated aqueous picric acid solution	75 ml
40% formaldehyde	25 ml
Glacial acetic acid	5 ml

1.2 10% Neutral buffered formalin (NBF)

40% formaldehyde	100 ml
Distilled water	900 ml
Sodium dihydrogen phosphate monohydrate	4 g
Disodium hydrogen phosphate anhydrate	6.5 g

2. **Tissue processing**

Fixation

Bouin's fluid	18-24 hours
(Transfer to 70% alcohol for washing out picric acid) or	
10% Neutral buffered formalin	12 hours or overnight
(Wash in running water 15- 30 minutes or up to 1 hour)	

Dehydration

70% ethanol	2 hours-overnight
95% ethanol	2 hours (2 times)
Absolute ethanol	2 hours
Absolute ethanol	overnight

Clearing

Xylene	1 hour

Infiltration

Xylene: wax (1:1)	1 hour
Wax1	1 hour
Wax2	1 hour

Embedding Tissue is embedded in paraffin

Sectioning Cut at 5-6 μm using microtome

Appendix C

Staining methods for paraffin sections (Bancroft and Gamble, 2002)

1. Preparation of solution

1.1 Harris's hematoxyline (Harris, 1900 cited by Bancroft and Gamble, 2002)

Hematoxyline	2.5 g
Absolute alcohol	25 g
Potassium alum	50 g
Distilled water	500 ml
Mercuric oxide	1.25 g
Glacial acetic acid	20 ml

The heamotoxylin is dissolved in the absolute alcohol, and is then added to the alum, which has previously been dissolved in the warm distilled water in a 2-litre flask. The mixture is rapidly brought to the boil and the mercuric oxide is then slowly and carefully added. Plunging the flask into cold water or into a sink containing chopped ice rapidly cools the stain. When the solution is cold, the acetic acid is added, and the stain is ready for immediate use.

1.2 Eosin

Stock solution (1% Alcoholic Eosin)

Eosin Y	10.0 g
Distilled water	50.0 ml
95% ethanol	940.0 ml

Working solution

Stock (1% Alcoholic Eosin)	1 part
95% ethanol	1 part

2. Staining processing

Deparaffinization section

Xylene	2 minutes (2 times)
Absolute ethanol	2 minutes (2 times)

Hydration

95% ethanol	2 minutes (2 times)
Running tap water	5 minutes
Staining	
Hematoxylin	6 minutes
1% acid alcohol (Differentiate)	5 seconds
Tap water	2 minutes
Saturated lithium carbonate (Blueing or neutrlize)	30 seconds
Distilled water	1-2 minutes
Eosin	30 seconds-1 minute
95% ethanol	5-10 dips (2 times)
Dehydration	
Absolute ethanol	2 minutes (2 times)
Clearing	
Xylene	2 minutes (2-3 times)
Mounting with Permount	

Appendix D

Tissue processing for electron microscopy (EM) (Bancroft and Gamble, 2002)

1. Preparation of solution

1.1 *Phosphate buffer* (0.1 mol/l, pH 7.4)

Stock reagents

Solution A

Disodium hydrogen orthophosphate (Na_2HPO_4 anhydrous)	14.2 g
Distilled water	1.0 l

Solution B

Sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$)	15.6 g
Distilled water	1.0 l

Mix 40.5 ml of solution A with 9.5 ml of solution B. The pH should be checked and adjusted if necessary, using 0.1 mol/l hydrochloric acid or 0.1 mol/l sodium hydroxide.

1.2 *Paraformaldehyde (3%)- glutaraldehyde (3%) fixative* (base on Glauert, 1972; Karnovsky, 1965 cited by Bancroft and Gamble, 2002)

0.2 mol/L phosphate buffer, pH 7.4	50 ml
Paraformaldehyde	2.0 g
25% aqueous glutaraldehyde	12 ml
Distilled/deionized water	to 100 ml

Completely dissolve paraformaldehyde in buffer using heat and with continuous stirring. It may be necessary to add a few drops of 1.0 mol/L sodium hydroxide to clarify the solution. Cool the solution rapidly under running water. Add aqueous glutaraldehyde. Check the pH of the mixture and adjust if necessary to pH 7.4 and then add distilled water to make 100 ml.

2. Tissue processing for transmission electron microscopy (TEM)

Fixation	
3% paraformaldehyde 3% glutaraldehyde	2-4 hours
Wash in 0.2 M. phosphate buffer	15 minutes (2 times)
Post-fix in 1% OsO_4 in phosphate buffer	1 hours
Wash in 0.2 M. phosphate buffer	15 minutes
Dehydrate	
30% ethanol	30 minutes
50% ethanol	15 minutes
70% ethanol	15 minutes
75% ethanol	15 minutes
80% ethanol	15 minutes
85% ethanol	15 minutes
90% ethanol	15 minutes
95% ethanol	15 minutes
100% ethanol	15 minutes
100% ethanol in $CuSO_4$	15 minutes (4 times)
Propylene oxide	30 minutes (2 times)
Infiltration	
Propylene oxide: resin (2:1)	overnight
Propylene oxide: resin (1:2)	7 hours
Pure resin	overnight
Embedding	
Polymerize at 60 ^{o}C for 24 hours	

3. Preparation of spermatozoa for scanning electron microscope (SEM)

Coverslip coating	
Drop Poly-L-lysine solution on coverslip	5 minutes
(1:10 dilution with MiliQ reverse osmosis water)	
Sperm preparation	
Centrifuge sperm solution	
Drop sperm suspension from sediment	5-10 minutes

Fix with EM fixative	15 minutes
(3% paraformaldehyde 3% glutaraldehyde)	
Wash with 0.1 M phosphate buffer	
Dehydration	
30% acetone	10 minutes
50% acetone	10 minutes
70% acetone	10 minutes
80% acetone	10 minutes
90% acetone	10 minutes
100% acetone	overnight
Critical drying (at 31 ^{o}C; pressure= 73.8 bar)	
Coat specimen with gold	

4. Staining grids for transmission electron microscopy (TEM)

4.1 Place lead citrate in the centrifuge for 10 minutes at 3000/ min

4.2 Place up to 2 grids in Uranyl acetate stain, allow sink and have sections facing up for 9 minutes

4.3 Take out of Uranyl acetate using forceps and wash grids one at a time in 70% alcohol and three distilled waters about 20 daps each

4.4 Dry the back of the grid and inside of the forcep on the filter paper

4.5 Drop lead citrate on parafilm and place sodium hydroxide pellets nearly

4.6 Place the grid on lead citrate for 9 minutes cover with glass lid

4.7 Wash grid in four distilled water about 20 daps each, and then dry the back of the grid and inside of the forceps, place in a dry labeled petri dish.

Appendix E

Table 4. Chi-square test for the number of pregnant female *B. indica* during the wet and dry season of both years of study.

No. of pregnancies	df (c-1)	χ^2 calculated	χ^2 table	Sig. (p=0.05)
Year 1	1	0.248	3.841	No significant
Year 2	1	0.183	3.841	No significant
Both years	1	0.557	3.841	No significant

Table 5. Analysis of Variance (ANOVA) for the weights of body, ovaries, uteri, and preputial glands, and the number of placental scars as well as the number of mature follicles of nonpregnant adult females, and also the number of fetuses of pregnant *B. indica* throughout the study period.

Test of Homogeneity of Variance

Females reproductive parameters	Levene Statistic	df1	df2	Sig.
Body weight	0.9738124	11	88	0.476
Ovary weight	5.0755164	11	88	0.000
Uterus weight	3.7982892	11	88	0.000
Preputial glands weight	1.2323696	8	58	0.297
Number of placental scars	0.9058473	11	71	0.539
Number of mature follicles	3.6601893	11	96	0.000
Number of fetuses	1.7450414	7	19	0.015

ANOVA

Females reproductive parameters			Sum of Squares	df	Mean Square	F	Sig.
Non pregnant adult females	OvW	Between Groups	101709.913	11	9246.356	6.040	.000
		Within Groups	134714.052	88	1530.841		
		Total	236423.964	99			
	UW	Between Groups	3057017.443	11	277910.677	2.317	.015
		Within Groups	10555578.227	88	119949.753		
		Total	13612595.670	99			
	No of Mature follicles	Between Groups	228.517	11	20.774	6.148	.000
		Within Groups	324.400	96	3.379		
		Total	552.917	107			
Pregnancies	No of Fetuses	Between Groups	80.695	8	10.087	2.067	.093
		Within Groups	92.733	19	4.881		
		Total	173.429	27			

BW= Body weight; OvW= Ovaries weight; UW= Uteri weight; PpW= Preputial glands weight.

Brown-Forsythe of Robust test of Equality of Mean

Females reproductive parameters	Statistic[a]	df1	df2	Sig
Body weight	1.6675005	11	64.089787	0.101
Preputial glands weight	2.9058557	8	28.764443	0.017
Number of placental scars	8.7733598	11	47.151867	0.000

[a] Asymptotically F distributed

Table 6. Unpaired student's *t* test for the weights of body, ovaries, uteri, and preputial glands, and the number of placental scars of nonpregnant adult female *B. indica* between wet and dry seasons during both years of study.

Independent Samples Test

Year or study	Parameters	Equal variances	Levene's Test for Equality of Variances		t-test for Equality of Means						
					t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
			F	Sig.						Lower	Upper
All	BW	Y	.139	.711	-1.411	98	.161	-19.7175	13.97252	-47.44548	8.01055
		N			-1.390	64.076	.169	-19.7175	14.18464	-48.05390	8.61896
	Ov W	Y	9.092	.003	2.607	98	.011	26.1383	10.02681	6.24045	46.03620
		N			3.148	97.988	.002	26.1383	8.30359	9.66010	42.61655
	UW	Y	.465	.497	.892	98	.374	62.9234	78.35897	-85.57748	225.4242
		N			.998	89.106	.321	62.9234	70.06944	-69.30085	209.1476
	Pp W	Y	.410	.524	.120	65	.905	.0062	.05184	-.0973	.10975
		N			.121	53.827	.904	.0062	.05146	-.09696	.10941
	No PlS	Y	.116	.734	-1.1272	81	.207	-1.69	1.332	-4.344	.955
		N			-1.345	51.971	.185	-1.69	1.266	-4.223	.834
	No GF	Y	.000	.998	2.528	106	.013	1.08	.428	.233	1.931
		N			2.507	99.209	.014	1.08	.432	.226	1.939
Year 1	BW	Y	1.416	.241	.295	40	.770	6.8687	23.30197	-40.22635	53.96373
		N			.264	11.181	.796	6.8687	25.99358	-50.22970	63.96708
	Ov W	Y	4.003	.052	2.938	40	.005	48.8283	16.62114	15.23570	82.42086
		N			4.893	38.619	.000	48.8283	9.97872	28.63804	69.01853
	UW	Y	3.783	.059	2.086	40	.043	353.7677	169.57773	11.03830	696.49706
		N			3.820	38.051	.000	353.7677	92.61584	166.28499	541.25037
	No PlS	Y	.009	.925	-3.102	29	.004	-7.76	2.501	-12.875	-2.643
		N			-3.110	3.952	.036	-7.76	2.495	-14.720	-.799
	No GF	Y	10.341	.003	3.554	40	.001	2.48	.697	1.068	3.886
		N			3.637	34.810	.001	2.48	.681	1.094	3.860
Year 2	BW	Y	.183	.671	-1.105	49	.274	-21.5692	19.51292	-60.78191	17.64345
		N			-1.108	48.691	.273	-21.5692	19.46637	-60.69463	17.55617
	Ov W	Y	15.136	.000	2.170	49	.035	29.4702	13.58216	2.17578	56.76453
		N			2.198	35.455	.035	29.4702	13.40675	2.26550	56.67481
	UW	Y	.000	.993	.197	49	.844	15.7812	79.99560	-144.97595	176.53841
		N			.198	48.766	.844	15.7812	79.82120	-144.64495	176.20741
	Pp W	Y	.581	.449	-.256	49	.799	-.0165	.06424	-.14556	.11263
		N			-.254	37.093	.801	-.0165	.06493	-.14801	.11508
	No PlS	Y	.544	.465	.938	43	.354	1.63	1.733	-1.869	5.119
		N			.958	41.631	.344	1.63	1.696	-1.799	5.049
	No GF	Y	3.629	.062	-.146	54	.884	-.08	.562	-1.209	1.045
		N			-.151	47.185	.880	-.08	.542	-1.172	1.008

Y= Equal variances assumed; N= Equal variances not assumed

BW= Body weight; OvW= Ovaries weight; UW= Uteri weight; PpW= Preputial glands weight; No PlS= number of placental scars, No GF= Number of mature follicles.

Table 7. Chi-square test for the number of pregnant females and immature *B. indica* on the cultivation stage of the rice plant (growing, generative, harvesting, and resting stages).

Parameters	df (c-1)	χ^2 calculated	χ^2 table	Sig. (p=0.05)
Number of pregnancies	3	6.837	7.815	No significant
Number of immatures	3	18.886	7.815	Significant

Table 8. Analysis of Variance (ANOVA) for the weights of body, ovaries, uteri, and preputial glands, and the number of placental scars as well as the number of mature follicles of nonpregnant adult females and also the number of fetuses of pregnant *B. indica* on the cultivation stage of the rice plant (growing, generative, harvesting, and resting stages).

Test of Homogeneity of Variance

Female reproductive parameters	Levene Statistic	df1	df2	Sig.
Body weight	0.6566325	3	96	0.581
Ovary weight	2.915959	3	96	0.038
Uterus weight	2.7639677	3	96	0.046
Preputial glands weight	0.8036937	3	63	0.496
Number of placental scars	2.8234411	3	79	0.044
Number of mature follicles	7.4397603	3	104	0.000
Number of fetuses	0.304046	3	24	0.822

ANOVA

	Female reproductive parameters		Sum of Squares	df	Mean Square	F	Sig.
Non-pregnant adult females	Ovaries weight	Between Groups	34086.399	3	11362.133	5.391	.002
		Within Groups	202337.565	96	2107.683		
		Total	236423.964	99			
	Uteri weight	Between Groups	996780.442	3	332260.147	2.528	.062
		Within Groups	12615815.228	96	131414.742		
		Total	13612595.670	99			
	Number of placental scars	Between Groups	96.871	3	32.290	1.036	.381
		Within Groups	2463.081	79	31.178		
		Total	2559.952	82			
	Number of mature follicles	Between Groups	28.290	3	9.430	1.869	.139
		Within Groups	524.627	104	5.044		
		Total	552.917	107			

Brown-Forsythe of Robust test of Equality of Mean

Female reproductive parameters	Statistic[a]	df1	df2	Sig
Body weight	4.3219701	3	59.251453	0.008
Preputial glands weight	5.6173371	3	33.585464	0.003
Number of fetuses	0.4214992	3	11.386249	0.741

[a] Asymptotically F distributed

Table 9. Multiple Linear Regression for the relationship between the reproductive activity of *B. indica* and environmental conditions (temperature, humidity, photoperiod and total rainfall)

Reproductive parameters		df	F	Sig.	R square	Adjust R^2
Male	Body weight	4,6	0.359	0.830	0.193	-0.345
	Testes weight	4,6	0.428	0.785	0.222	-0.297
	TW/ 100g BW	4,6	0.793	0.793	0.217	-0.305
	Seminal vesicles weight	4,6	0.845	0.545	0.360	-0.066
	Prostate glands weight	4,6	1.363	0.349	0.476	0.127
	Epididymides weight	4,3	0.197	0.925	0.208	-0.847
	Preputial glands weight	4,3	0.571	0.705	0.432	-0.325
	% of normal sperm head	4,6	1.539	0.303	0.506	0.177
	% of normal sperm tail	4,6	4.541	0.055	0.752	0.586
Female	Body weight	4,7	1.143	0.410	0.395	0.049
	Ovary weight	4,7	1.290	0.512	0.406	0.296
	Uterus weight	4,7	1.587	0.278	0.476	0.176
	Preputial gland weight	4,4	2.132	0.241	0.681	0.361
	No. of pregnant females	4,7	1.441	0.120	0.410	0.201
	No. of placental scars	4,7	0.276	0.885	0.136	-0.358

Table 10. Analysis of variance (ANOVA) for body weight, the weights of testes, epididymides, seminal vesicles, prostate glands, and preputial glands, and the testes weight/100g body weight of adult male *B. indica* throughout the study period.

Test of Homogeneity of Variance

Male reproductive parameters	Levene Statistic	df1	df2	Sig.
Body weight	1.492599	10	43	0.175
Testes weight	1.191072	10	43	0.323
Testes weight/ 100g body weight	1.008205	10	43	0.452
Epididymides weight	3.572142	7	31	0.006
Seminal vesicles weight	2.22635	10	39	0.036
Prostate glands weight	3.05662	10	37	0.006
Preputial glands weight	3.737825	7	31	0.005

ANOVA

Male reproductive parameters		Sum of Squares	df	Mean Square	F	Sig.
Epididymides weight	Between Groups	.399	7	.057	2.080	.076
	Within Groups	.849	31	.027		
	Total	1.248	38			
Seminal vesicles weight	Between Groups	.985	10	.099	9.019	.000
	Within Groups	.426	39	.011		
	Total	1.412	49			
Prostate glands weight	Between Groups	.245	10	.024	3.178	.005
	Within Groups	.285	37	.008		
	Total	.530	47			
Preputial glands weight	Between Groups	4.686	7	.669	10.527	.000
	Within Groups	1.971	31	.064		
	Total	6.658	38			

Brown-Forsythe of Robust test of Equality of Mean

Male reproductive parameters	Statistic[a]	df1	df2	Sig
Body weight	2.66438	10	26.83661	0.021
Testes weight	4.647277	10	20.40804	0.002
Testes weight/ 100g body weight	3.505189	10	19.26989	0.009

[a] Asymptotically F distributed

Table 11. Unpaired student's *t* test for body weight and male reproductive organ weights of adult male *B. indica* between wet and dry seasons during both years of study.

Year or study	Parameters	Equal variances	Levene's Test for Equality of Variances		t-test for Equality of Means						
					t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
			F	Sig.						Lower	Upper
All	BW	Y	1.125	.294	1.948	52	.057	42.5483	21.84282	-1.28254	86.37913
		N			1.898	41.032	.065	42.5483	22.41933	-2.72739	87.82398
	TW	Y	12.290	.001	2.257	52	.028	.5818	.25778	.06455	1.09910
		N			2.081	32.453	.045	.5818	.27963	.01256	1.15109
	TW/ BW	Y	13.545	.001	2.167	52	.035	.0922	.04257	.00682	.17767
		N			1.978	31.119	.057	.0922	.04663	-.00285	.18734
	Ep W	Y	.069	.794	.481	37	.633	.0284	.05913	-.09135	.14825
		N			.450	23.875	.657	.0284	.06325	-.010213	.15903
	Sv W	Y	3.935	.053	2.848	48	.006	.1306	.04507	.04001	.22126
		N			2.811	38.784	.008	.1306	04647	.03662	.22465
	PsW	Y	1.093	.301	-.312	46	.7567	-.0097	.03107	-.07223	.05284
		N			-.306	39.700	.761	-.0097	.03169	-.07375	.05436
	Pp W	Y	.559	.459	.732	37	.469	.0996	.13600	-.17598	.37514
		N			.745	36.308	.461	.0996	.13375	-.17160	.37076
Year 1	BW	Y	7.738	.010	2.581	24	.016	75.5879	29.28138	15.15409	136.02167
		N			2.401	15.464	.029	75.5879	31.48834	8.64717	142.52858
	TW	Y	10.434	.004	2.557	24	.017	1.0137	.39644	.19545	1.83189
		N			2.341	14.291	.034	1.0137	.43296	.08682	1.94051
	TW/ BW	Y	8.450	.008	2.733	24	.012	.1605	.05872	.03930	.28167
		N			2.510	14.521	.024	.1605	.06393	.02384	.29713
	Sv W	Y	.265	.612	3.302	20	.004	.2311	.06999	.08509	.37709
		N			3.302	19.561	.004	.2311	.06999	.08488	.37730
	PsW	Y	3.310	.086	-.289	18	.776	-.0094	.03245	-.07754	.05881
		N			-.305	16.265	.765	-.0094	.03074	-.07445	.05572
Year 2	BW	Y	.782	.387	-.050	21	.961	-1.5455	31.08391	-66.18798	63.09707
		N			-.049	19.398	.961	-1.5455	31.36560	-67.10326	64.01235
	TW	Y	3.395	.080	.286	21	.778	.1044	.36474	-.65412	.86293
		N			.281	16.639	.782	.1044	.37195	-.68163	.89044
	TW/ BW	Y	2.562	.124	.336	21	.740	.0237	.07061	-.12314	.17056
		N			.329	16.605	.746	.0237	.07202	-.12851	.17593
	Ep W	Y	1.666	.211	-1.660	21	.112	-.0493	.02967	-.11097	.01245
		N			-1.615	14.389	.128	-.0493	.03050	-.11451	.01600
	Sv W	Y	6.997	.015	.454	21	.654	.0288	.06335	-.10297	.16049
		N			.443	15.189	.664	.0288	.06492	-.10947	.16699
	PsW	Y	2.351	.140	-1.059	21	.302	-.0439	.04150	-.13023	.04237
		N			-1.036	15.908	.316	-.0439	.04243	-.13391	.04605
	Pp W	Y	.369	.550	-.511	21	.615	-.1029	.20142	-.52175	.31599
		N			-.508	20.087	.617	-.1029	.20254	-.52527	.31950

Y= Equal variances assumed; N= Equal variances not assumed. BW=Body weight; TW=Testes weight; TW/BW=Testes weight/100g body weight; EpW= Epididymides weight; SvW=Seminial vesicles weight; PtW= Prostate glands weight; PsW=Preputial glands weight.

Table 12. Analysis of Variance (ANOVA) for the percentage of the normal and abnormal morphologies of sperm heads and tails including midpiece deformity and flagellar deformity of the adult male *B. indica* throughout the study period.

Test of Homogeneity of Variance

Percentage of sperm morphotypes	Levene Statistic	df1	df2	Sig.
Normal sperm head	3.465639	10	40	0.002
Abnormal sperm head	3.465639	10	40	0.002
Normal sperm tail	3.65146	10	40	0.002
Abnormal sperm tail	3.652769	10	40	0.002
Midpiece deformity	1.433712	10	40	0.201
Flagellar deformity	1.95293	10	40	0.066

ANOVA

Percentage of sperm morphotypes		Sum of Squares	df	Mean Square	F	Sig.
Normal sperm head	Between Groups	5292.360	10	529.236	13.285	.000
	Within Groups	1593.514	40	39.838		
	Total	6885.874	50			
Abnormal sperm head	Between Groups	5292.360	10	529.236	13.285	.000
	Within Groups	1593.514	40	39.838		
	Total	6885.874	50			
Normal sperm tail	Between Groups	5941.681	10	594.168	12.077	.000
	Within Groups	1967.887	40	49.197		
	Total	7909.567	50			
Abnormal sperm tail	Between Groups	5941.628	10	594.163	12.086	.000
	Within Groups	1966.507	40	49.163		
	Total	7908.134	50			

Brown-Forsythe of Robust test of Equality of Mean

Percentage of sperm morphotypes	Statistic[a]	df1	df2	Sig
Midpiece deformity	4.711617	10	22.02143	0.001
Flagellar deformity	1.077649	10	24.676	0.415

[a] Asymptotically F distributed

Table 13. Unpaired student's *t* test for the percentage of normal and abnormal morphologies of sperm heads and tails including midpiece deformity and flagellar deformity of adult male *B. indica* between the wet and dry seasons during both years of study

Year or study	Percentage of sperm morphotypes	Equal variances	Levene's Test for Equality of Variances		t-test for Equality of Means						
			F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
										Lower	Upper
All	% NH	Y	.757	.389	3.005	49	.004	9.4816	3.15484	3.14169	15.82147
		N			3.069	40.441	.004	9.4816	3.08964	3.23930	15.72386
	% AbH	Y	.757	.389	-3.005	49	.004	-9.4816	3.15484	-15.82147	-3.14169
		N			-3.069	40.441	.004	-9.4816	3.08964	-15.72386	-3.23930
	% NT	Y	2.706	.106	5.487	49	.000	15.8898	2.89610	10.06988	21.70973
		N			6.083	44.394	.000	15.8898	2.61195	10.63922	21.14038
	% AbT	Y	2.706	.106	-5.487	49	.000	-15.8898	2.89610	-21.70570	-10.06571
		N			-6.083	44.394	.000	-15.8898	2.61195	-21.13684	-10.63458
	% MD	Y	2.184	.146	-4.446	49	.000	-13.0920	2.94501	-19.0102	-7.17384
		N			-4.826	44.394	.000	-13.0920	2.71289	-18.54972	-7.63438
	% FD	Y	1.112	.297	-1.547	49	.128	-2.7937	1.80615	-6.42325	.83593
		N			-1.681	44.394	.099	-2.7937	1.66204	-6.13712	.54980
Year 1	% NH	Y	25.811	.000	3.789	21	.001	15.2767	4.03187	6.89193	23.66140
		N			2.977	8.162	.017	15.2767	5.13226	3.48238	27.07095
	% AbH	Y	25.811	.000	-3.789	21	.001	-15.2767	4.03187	-23.66140	-6.89193
		N			-2.977	8.162	.017	-15.2767	5.13226	-27.07095	-3.48238
	% NT	Y	.575	.457	4.428	21	.000	19.2583	4.34948	10.21310	28.30356
		N			4.627	16.291	.000	19.2583	4.16240	10.44723	28.06943
	% AbT	Y	.572	.458	-4.425	21	.000	-19.2519	4.35029	-28.29878	-10.20495
		N			-4.624	16.290	.000	-19.2519	4.16325	-28.06481	-10.43892
	% MD	Y	.158	.695	-3.527	21	.002	-17.0176	4.82465	-27.05105	-6.98423
		N			-3.621	15.513	.002	-17.0176	4.69926	-27.00512	-7.03017
	% FD	Y	4.171	.054	-.739	21	.468	-2.2342	3.02198	-8.51877	4.05033
		N			-.867	20.751	.396	-2.2342	2.57662	-7.59652	3.12808
Year 2	% NH	Y	.137	.715	1.225	21	.234	3.1470	2.56988	-2.19740	8.49134
		N			1.223	20.683	.235	3.1470	2.57372	-2.21037	8.50431
	% AbH	Y	.137	.715	-1.225	21	.234	-3.1470	2.56988	-8.49134	2.19740
		N			-1.223	20.683	.235	-3.1470	2.57372	-8.50431	2.21037
	% NT	Y	6.343	.020	6.141	21	.000	12.2629	1.99686	8.11017	16.41559
		N			6.337	15.177	.000	12.2629	1.93516	8.14236	16.38340
	% AbT	Y	6.369	.020	-6.148	21	.000	-12.2594	1.99415	-16.40646	-8.11234
		N			-6.343	15.189	.000	-12.2594	1.93261	-16.37419	-8.14461
	% MD	Y	1.373	.254	-3.259	21	.004	-10.0160	3.07332	-16.40734	-3.62470
		N			-3.318	19.134	.004	-10.0160	3.01831	-16.33042	-3.70162
	% FD	Y	1.118	.302	-.921	21	.367	-2.2434	2.43557	-7.30842	2.82166
		N			-.936	19.663	.361	-2.2434	2.39741	-7.24979	2.76303

Y= Equal variances assumed; N= Equal variances not assumed.
NH=Normal sperm head; AbH=Abnormal sperm head; NT=Normal sperm tail; AbT=Abnormal sperm tail; MD=Midpiece deformity; FD=Flagellar deformity.

Table 14. Analysis of Variance (ANOVA) for epithelium height, tubular diameter and the percentage of epididymal cells of cauda segments of the epididymides in selected adult males *B. indica* throughout the study period.

Test of Homogeneity of Variance

Epididymal parameters	Levene Statistic	df1	df2	Sig.
Epididymal height	6.965674	5	102	0.000
Epididymal diameter	1.921171	5	45	0.110
% of principal cell	1.29809	5	34	0.288
% of clear cell	1.7021092	5	34	0.161
% of halo cell	1.4114189	5	34	0.245
% of basal cell	1.7112025	5	34	0.159

ANOVA

Epididymal parameters		Sum of Squares	df	Mean Square	F	Sig.
Epididymal height	Between Groups	3174.931	5	634.9863	37.88229	0.000
	Within Groups	1709.733	102	16.76209		
	Total	4884.664	107			
	Total	651643.220	58			

Brown-Forsythe of Robust test of Equality of Mean

Epididymal parameters	Statistic[a]	df1	df2	Sig
Epididymal diameter	3.326807	5	30.39225	0.016
% of principal cell	1.5802064	5	18.216499	0.215
% of clear cell	10.417451	5	16.921389	0.000
% of halo cell	3.5197022	5	19.838453	0.019
% of basal cell	0.3829985	5	21.099722	0.855

[a] Asymptotically F distributed

Table 15. Unpaired student's *t* test for epithelium height, tubular diameter and the percentage of epididymal cells and round cells in the lumen of the cauda segment of the epididymides in selected adult males *B. indica* during the wet and dry seasons.

Independent Samples Test

Epididymal parameters	Equal variances	Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Epididymal height	Y	32.394	.000	9.486	122	.000	9.7429	1.02711	7.70963	11.77617
	N			11.457	119.710	.000	9.7429	.85042	8.05909	11.42671
Epididymal diameter	Y	3.756	.058	-.919	57	.362	-26.2715	28.57490	-83.49176	30.94876
	N			-1.079	55.357	.285	-26.2715	24.34095	-75.04479	22.50180
% Principal cell	Y	3.853	.057	-1.436	38	.159	-2.6776	1.86414	-6.45135	1.09615
	N			-1.576	37.215	.123	-2.6776	1.69874	-6.11890	.76370
% Clear cell	Y	.666	.420	3.197	38	.003	3.6963	1.15635	1.35537	6.03717
	N			3.330	33.406	.002	3.6963	1.11009	1.43881	5.95372
% Halo cell	Y	1.230	.274	-1.866	38	.070	-1.3513	.72424	-2.81749	.11482
	N			-1.826	27.623	.079	-1.3513	.74025	-2.86861	.16594
% Basal cell	Y	.161	.691	-.273	38	.786	-.6933	2.53973	-5.83475	4.44808
	N			-.267	27.541	.792	-.6933	2.59825	-6.01961	4.63294
% Round cell in lumen	Y	.005	.946	-2.524	8	.036	-20.4077	8.08590	-39.05379	-1.76156
	N			-2.424	1.483	.178	-20.4077	8.41754	-71.87850	31.06315

Y= Equal variances assumed; N= Equal variances not assumed

Table 16. Unpaired student's *t* test for epithelium height, tubular diameter and the percentage of epididymal cells in the caput and cauda segments of the epididymides of adult *B. indica* during a period of maximal testes weight (November 2005 sample in the wet season)

Independent Samples Test

Epididymal parameters	Equal variances	Levene's Test for Equality of Variances		t-test for Equality of Means						
				t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		F	Sig.						Lower	Upper
Epididymal height	Y	1.180	.286	-1.661	30	.107	-2.6563	1.59905	-5.92195	.60945
	N			-1.661	28.650	.108	-2.6563	1.59905	-5.92841	.61591
Epididymal diameter	Y	.058	.814	3.532	14	.003	51.2500	14.50831	20.13277	82.36723
	N			3.532	13.902	.003	51.2500	14.50831	20.11227	82.38773
% Principal cell	Y	1.569	.226	-4.153	18	.001	-12.4653	3.00172	-18.77172	-6.15895
	N			-5.216	11.234	.000	-12.4653	2.39003	-17.71243	-7.21823
% Clear cell	Y	.354	.559	11.508	18	.000	23.5327	2.04497	19.23634	27.82899
	N			9.281	5.203	.000	23.5327	2.53569	17.09032	29.97501
% Halo cell	Y	.885	.359	-.618	18	.544	-.6947	1.12318	-3.05437	1.66504
	N			-.775	11.150	.455	-.6947	.89687	-2.66544	1.27611
% Basal cell	Y	2.843	.109	-4.592	18	.000	-10.3693	2.25810	-15.11343	-5.62524
	N			-5.729	11.041	.000	-10.3693	1.80990	-14.35108	-6.38759

Y= Equal variances assumed; N= Equal variances not assumed.

Table 17. Analysis of Variance (ANOVA) for body weight, the weights of testes, epididymides, seminal vesicles, prostate glands, and preputial glands, the testes weight/ 100g body weight, and the percentage of normal sperm heads and tails as well as the sperm tail length of adult male *B. indica* on the cultivation stage of the rice plant (growing, generative, harvesting, and resting stages).

Test of Homogeneity of Variance

Male reproductive parameters	Levene Statistic	df1	df2	Sig.
Body weight	3.881464	3	50	0.014
Testes weight	8.079389	3	50	0.000
Testes weight/ 100g body weight	2.632983	3	50	0.060
Epididymides weight	0.832784	3	35	0.485
Seminal vesicles weight	1.529606	3	46	0.219
Prostate glands weight	3.52506	3	44	0.022
Preputial glands weight	3.004473	3	35	0.043
Percentage of normal sperm head	2.1225251	3	47	0.110
Percentage of normal sperm tail	1.4120548	3	47	0.251

ANOVA

Male reproductive parameters		Sum of Squares	df	Mean Square	F	Sig.
Body weight	Between Groups	42910.927	3	14303.642	2.352	.083
	Within Groups	304135.319	50	6082.706		
	Total	347046.245	53			
Testes weight	Between Groups	7.970	3	2.657	3.201	.031
	Within Groups	41.492	50	.830		
	Total	49.462	53			
Prostate glands weight	Between Groups	.005	3	.002	.148	.930
	Within Groups	.525	44	.012		
	Total	.530	47			
Preputial glands weight	Between Groups	1.385	3	.462	3.065	.041
	Within Groups	5.272	35	.151		
	Total	6.658	38			

Brown-Forsythe of Robust test of Equality of Mean

Male reproductive parameters	Statistic[a]	df1	df2	Sig
Testes weight/ 100g body weight	2.830496	3	5.566731	0.135
Epididymides weight	1.03748	3	19.98158	0.397
Seminal vesicles weight	7.041518	3	20.21459	0.002
Percentage of normal sperm head	2.7956306	3	20.425494	0.066
Percentage of normal sperm tail	2.796141	3	30.035	0.057

[a] Asymptotically F distributed

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Lists of Publications and Proceedings

Breed, W.G., Bauer, M., Wade, R., **Thitipramote, N.,** Suwanjarat, J. and Yelland, L. 2007. Intra-individual variation in sperm tail length in murine rodents. Journal of Zoology. 272: 299-304.

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