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A review of endoparasitic acarines of Malaysia with special reference to novel endoparasitism of mites in amphibious sea snakes and supplementary notes on ecology of chiggers

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Abstract. Some 2,000 species of mites of the family Trombiculidae are known in the world. The 6-legged larvae are mostly ectoparasites of reptiles, birds, mammals and invertebrates. Their 8-legged active nymphs and adults are free-living predators. In the Asia-Pacific region, a few species in various genera are vectors of scrub typhus and scrub-itch. In this a paper, a very bizarre trombiculid species, *Vatacarus ipoides* Southcott 1957, endoparasitic in the trachea of the amphibious sea snake, *Laticauda colubrina* (Schenider) is re-described based mostly on new-born larvae reared in the laboratory. Life history study of the mite produced very novel and interesting results. A brief account of the life-cycle was presented at the first laboratory demonstration of the Malaysian Society of Parasitology and Tropical Medicine Meeting by Nadchatram and Audy (1965). The life history is illustrated and described here in greater detail. The active nymphal, and the akinetic teleiophane stages are bypassed, which is unusual in the life-cycle of the family Trombiculidae. Also, the larva is the only stage in the life-cycle that feeds. The sexes are predetermined in the larval neosomatic stage and give rise to small males and bigger females. Having obtained adults of the species, by rearing, it is deemed unnecessary for the original proposal by Southcott to erect a new family, Vatacaridae, because the adults share all the attributes of the family Trombiculidae. The male and female obtained through laboratory rearing are illustrated for the first time. Relationship of *V. ipoides* with *Laticauda* snakes show close host-specificity, in a group of acarines that are generally habitat-specific. Possible explanations for their association are discussed. The unusual morphology and the formation of new structures during an instar is of ontogenetic and evolutionary importance. The hypertrophic larvae are superficially vermiform, rather than typically acarine in shape. This, and other biological features, necessitated the proposal of new morphological terms, and they are discussed here.

INTRODUCTION

An essential component of investigations of tropical or arthropod-borne endemic infections is the systematic collection of the incriminating agents to investigate their biomedical and scientific importance on a broad basis. International cooperation between scientists at the IMR and scientists with common interest from various universities and institutions in America, Australia, Europe and elsewhere, and the free exchange of material, made it possible to better understand the Malaysian organisms of diverse forms and life-styles, and their medical or potential medical importance.

For example, in scrub typhus research, the study of parasites on a broad basis also contributed to our knowledge of ecology and host-parasite-habitat relationships, which in turn, helped in the use of animals and their ectoparasites as ecological "labels" or markers to monitor the effects of deforestation on the natural environment of the Malaysian countryside (Audy, 1948; 1954; 1956a; 1956b; 1958; 1960).

As I look back over some 35 years of my happy and fulfilling association with the Institute for Medical Research through biomedical studies of ticks and mites, I realize how much I owed it to others and how dependant I was on the ideas and achievements of other learned peers and colleagues, nationally and internationally. Working in a research laboratory in a specialized field in a somewhat isolated environment for many years, the realization that we must always be reminded that the vigour of a research organization must finally depend on the range and degree of its contacts with the outside world for fruitful endeavours in the best interest of science and the nation, especially in a field of study which is unique to the country. Our vigorous activities and collaborative participation gained international popularity, and many overseas scientists interested in ticks and mites for their scientific and public health importance spent days, weeks, months and, in some cases more than a year through grants they applied for and received from various sources.

Participation by international researchers proved to be of mutual benefit. Some 500 parasitic and free-living mites, and ticks were discovered and described in collaboration with overseas specialists, and their bionomics clarified through field investigations and life history studies in our laboratory. These Malaysian investigations also contributed to the discovery of other parasites, both ecto- and endoparasites, published elsewhere by various specialists. There are many new emerging and re-emerging diseases lurking on the door steps of the rainforest ecosystem rich in the diversity of biosystems. Understanding the natural history of infectious and vector-transmitted diseases must be an on-going programme in our country and in any other country like ours in the tropical rainforest ecosystem, rich in fauna and flora. Early in my years I was made to learn to be broad based in investigations of parasites, without being rigidly directed by knowledge or suspicion of their ability to transmit disease. Understanding the parasites in relationship with the animal hosts has proven equally important in science.

Known Malaysian endoparasitic mites of terrestrial birds and animals

Instances of the broad based investigations of parasites in Malaysia were the discoveries of the numerous endoparasitic mites that were found living naturally in the lungs of monkeys, stomachs of fruit bats, as well as the nasal and respiratory passages of birds and ground mammals. Though these studies were incidental to the investigation of the vectors and potential vectors of scrub typhus, the discovery broadened our knowledge of acarine parasitism. Therefore, the opportunity is taken to briefly summarise endoparasitism of terrestrial reptiles, birds, mammals and invertebrates recorded in Malaysia. With the first discovery of the intranasal biotope of chiggers in Malaysia in 1952, a total of 86 species in 16 genera of chigger mites infesting intranasal passages of vertebrates were recorded in a world review up to 1970, and of these 14 were Malaysian species (Nadchatram, 1970a). Over a 40-year period many more species representing many other families of mites were found in various biotopes, especially those parasitic in birds by non-trombiculid families of mites, e.g. Rhinonyssidae, Ereynetidae, Cytoditidae and Turbinoptidae (Nadchatram *et al*, 1964). Subsequently, additional species of Rhinonyssidae were found on migratory birds in Malaysia (IMR Annual Report, 1977: p. 23). The monkey lung mite, *Pneu-monyssus simicola* Banks was found naturally infecting the lungs of the Silver Leaf Monkey, *Presbytis cristatus* (Stiller *et al*., 1974 and IMR Annual Report, 1974: p. 85). It is noteworthy that the nymphal stage of the mite was not found from among the thousands of specimens collected. Many other species of monkeys and tree shrews were also examined for lung mites, with negative results. Fain (1969) discusses the adaptation of parasitism of mites on various animal hosts. The lungs of the colubrid land snake, *Natrix chrysarga* was found infected with *Entonyssus asiaticus* (Fain, 1961; IMR Annual Report 1975: pp.34-35; Stiller *et al.*, 1977). More than a hundred specimens were collected from 4 of 14 snakes of *N. chrysarga*. Bats are also not spared by endoparasitic mites. The stomaches of fruit bats were infected with *Gastronyssus bakeri* Fain (Fain, 1955; Stiller 1977b; IMR Annual Report 1975: p. 35). Of 52 fruit bats collected, 36.5% of two species of fruit bats were found harbouring the mites in their stomach.

Another group of mites that are very specialized and restricted in their biotope are demodicid mites of the species *Demodex intermedius* Lukoschus *et al.* that were found in the Meibomian glands of the eye lids of the tree-shrew, *Tupaia glis* in Bukit Fraser, Malaysia (Lukoschus *et al.*, 1984). All stages of the vermiform mites were found and described, followed by a discussion of its taxonomy and pathology. Meibomian glands associated with the eyes are multi-alveolar glands with holocrine secretion of a fatty paste-like sebum. Large amounts of this sebum, essential for the normal function of the cornea, are spread on the cornea by the movements of the eye-lids. All stages of the life-cycle were found in the glands of the upper and lower eye-lids of both eyes. The mites were found in 4 of 17 *T. glis* examined and *D. sabani* in several species of rodents (Desch *et al.*, 1984). The genus *Demodex* is found in humans throughout the world living in all stages of the cycle in facial pores and hair follicles of the eye-brows as observed by the author on himself. Two species of human *Demodex* are known – *D. folliculorum* (Simon) and *D. brevis* (Akbulatova). It is also worthy of note that many different groups of arthropods are hosts to endoparasitic mites (Fain and Lukoschus, 1983).

Endoparasitism in amphibious sea snake

The main object of this paper is the description of an extremely classical example of endoparasitism of sea snakes. The biology of the mites of the family Trombiculidae found in the trachea of the Amphibious Sea Snake, *Laticauda colubrina* (Schenider) (plate I) is described in more detail from the earlier synoptic description by Nadchatram and Audy (1965) which was presented as a laboratory demonstration, organized by the then Malaysian Society of Parasitologists, as the Malaysian Society of Parasitology and Tropical Medicine was known then.

The family Trombiculidae is one of the largest families in the order Acari, with some 2,000 known species in the world.

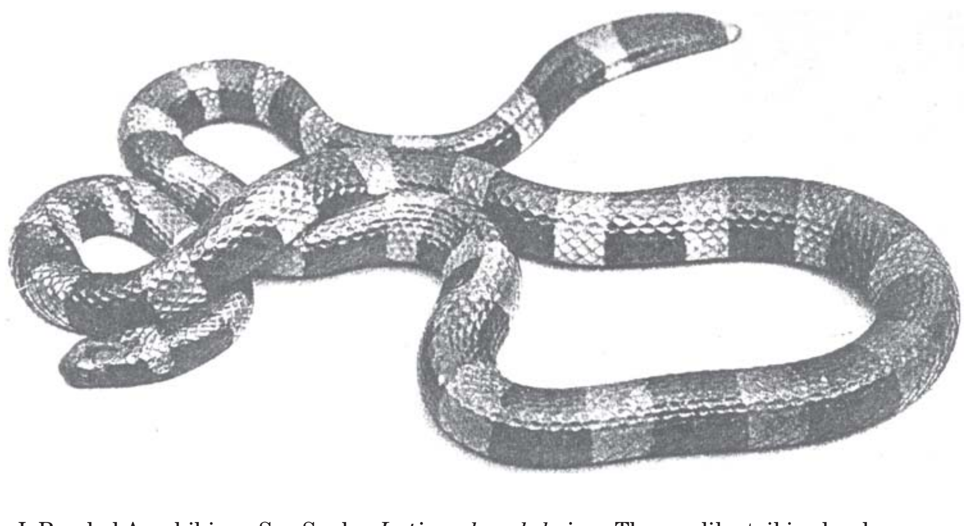

Plate I. Banded Amphibious Sea Snake, *Laticauda colubrina.* The oar-like tail is clearly seen, upper right. (After Ditmars, 1962).

The 6-legged larvae are mostly ectoparasites of reptiles, birds, mammals and invertebrates. Members of this family are also the important vectors of scrub typhus. In the Asia- Pacific region, including Malaysia, at least a dozen species of larval mites in various genera of this family are either vectors of scrub typhus or the causative agents of scrub-itch. The 8-legged active nymphs and adults are free-living predators. The Institute for Medical Research, Kuala Lumpur, Malaysia has a colourful background in investigations on various aspects of scrub typhus research, since 1925.

In the study of the life history of *Vatacarus ipoides*, carried out by the author at the Institute for Medical Research, Kuala Lumpur, it was found that two stages in the life-cycle were bypassed, the larvae developed from a single moulting stage into adults without feeding, a very unusual phenomenon. The bypassing of the active nymphal stage and the quiescent teleiophane stage (an example of tachygenesis) is unique among mites of the family Trombiculidae. The revelation of this unusual phenomenon in 1960 prompted much excitement. The unique morphology and the formation of new structures during an instar was sufficiently novel and of sufficient ontogenetic and evolutionary importance to deserve new descriptive terms as stated by Audy. Consequently, Audy *et al* (1963) proposed new terms to define the new structures in *V. ipoides* and a number of other species of trombiculid mites. This was followed by a review of examples of external transformations in a variety of insects, acarines and crustaceans (Audy *et al* 1972). However, in this paper only the morphological structures and biological features that apply to *Vatacarus ipoides* are discussed. The terms and definitions as applied to this species and presented here are from that of Audy et al. 1963 and 1972, as defined below:

Neosomy (new term, n. Greek neo – new, soma – body), meaning the process of new formation of structures or tissues in a single active instar of an arthropod, at present recorded only for certain groups of trombiculid larvae and represented by development of new integument material;

Neosome is a new derivative term: the developed product of neosomy to be distinguished from earlier stages of the instar, as in plate II.

Neosomule is a new external structure resulting from neosomy

Neosomatic structures or organs: new descriptive term. Distinct structures of adaptive value and usually of taxonomic importance.

The bizarre mites found in the respiratory system of amphibious sea snakes of the genus *Laticauda*, are capable of increasing greatly in size to a worm-like or maggot-like creature with neosomatic structures. The term **ipomorphy** was proposed by Southcott (1957) to denote such a modification, in groups of animals not normally so.

In this paper, the larva of *V. ipoides* is re-described and illustrated based for the most part on unengorged or newly hatched larvae obtained from rearing the mites in the laboratory, for the first time. Description of neosomatic or engorged larvae follows the description, with slight modification, by Southcott, 1957. The adult male and female are also illustrated for the first time, obtained from the mites reared in the laboratory, to exemplify the features of an adult trombiculid mite of normal contour that emerged from the hypertrophic larvae bearing corniculate papillae, defined as neosomules and are vermiform rather than typically of the oval or round shape of larval mites of the family Trombiculidae. This led Southcott to propose a new family, Vatacaridae to accommodate the genus and species. However, laboratory rearing of this mite has shown that the adult mites have all the characteristic morphological features of the family Trombiculidae, and of normal trombiculid form, hence there is no valid reason to erect a new family, Vatacaridae. However, consideration may be given for

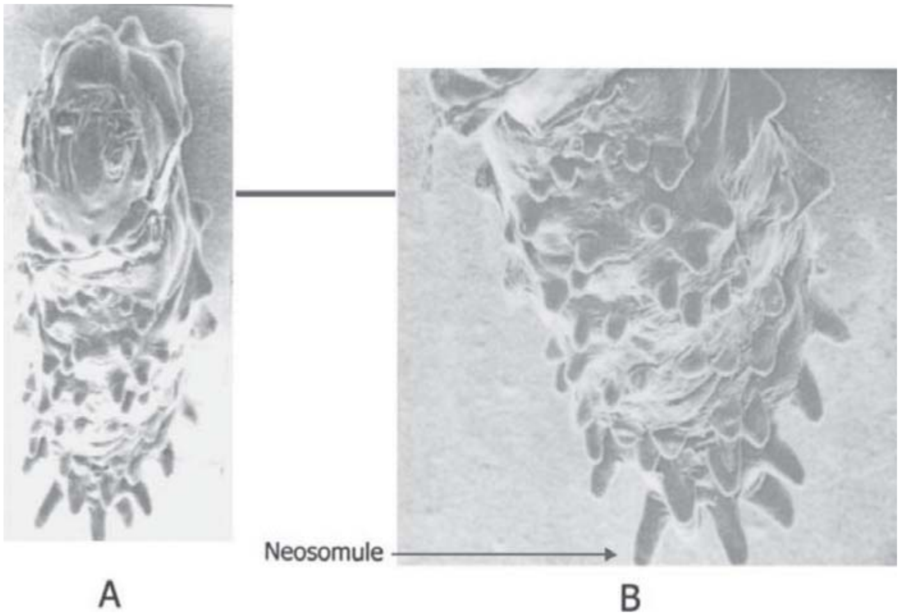

Plate II. A, Scanning electron micrograph of ventral view of entire engorged larva or neosome of *V. ipoides*. B, posterior ¾ of the neosome or larva of *V. ipoides* showing ambulacral papillae (neosomules).

subfamily ranking of this bizarre creature in the larval stage. However, the taxonomic treatment is best left to the actively interested acarologists.

TAXONOMY

Revision of the genus *Vatacarus* Southcott, 1957
(based on larval characters)

A mite of the family Trombiculidae, found in the respiratory system of the genus *Laticauda*, maggot-like in appearance in the engorged state (neosome), capable of increasing greatly in size, with conicular projections or neosomules developing only in the engorged specimens. Urstigmata present. Trachea, though not at all seen in whole mounted specimens, are apparent in larval pelts of *V. ipoides*. Cheliceral fangs recurved, hinged. Palpal tibial claw 2-pronged. Palpal tarsal formula (PTF) 7BS. Eyes 2 + 2, visible only in unengorged larva, not discernable in engorged specimens. Scutum trapezoidal, shoulders present. 2 anterolateral setae (AL) placed below anterior margin, 1 anteromedian seta (AM) and 2 posterior lateral setae (PL) situated well above line of posterior margin. A pair of filiform sensillae. Genu of leg III multisetose. Empodia of tarsus I to III setiform.

Re-description of *Vatacarus ipoides* Southcott
(mostly based on new-born larva, figs. 1-8)

(Terminology in description follows that of Nadchatram and Dohany (1974). The re-description is based on unengorged (UL) larvae reared in the laboratory for the first time, and from neosomatic or fully engorged larvae collected from the host. (The identity of the mite species was confirmed by Dr. Southcott).

Unfed larva 0.45 x 0.21 mm, partially fed larva (PEL) elongated, length 2.94 mm; width across idiosoma 1.2 mm, width across hysterosoma, or posterior idiosome 0.97 mm; engorged larva or neosome 4.0 - 4.8 mm x 2.0 - 2.3 mm. Colour in life of UL and PEL yellow to light orange, EL bright orange.

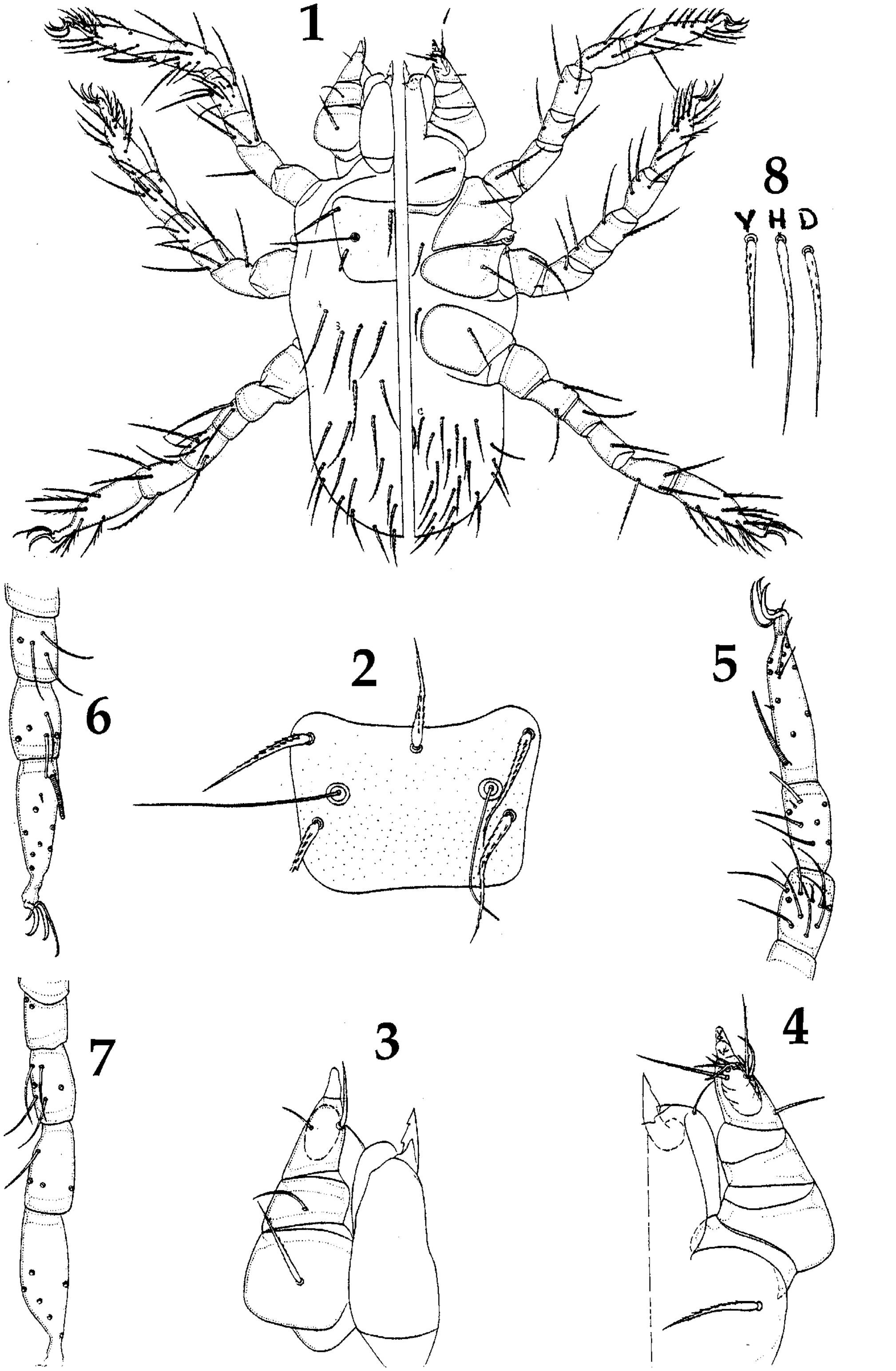

Figure 1–8. Unengorged Larva of *Vatacarus ipoides* Southcott:– 1, dorsal and ventral aspects of idiosome; 2, scutum; 3,4, dorsal and ventral aspects of gnathosome; 5,6,7, distal segments of leg I, leg II and leg III; 8, humeral, dorsal and ventral setae.

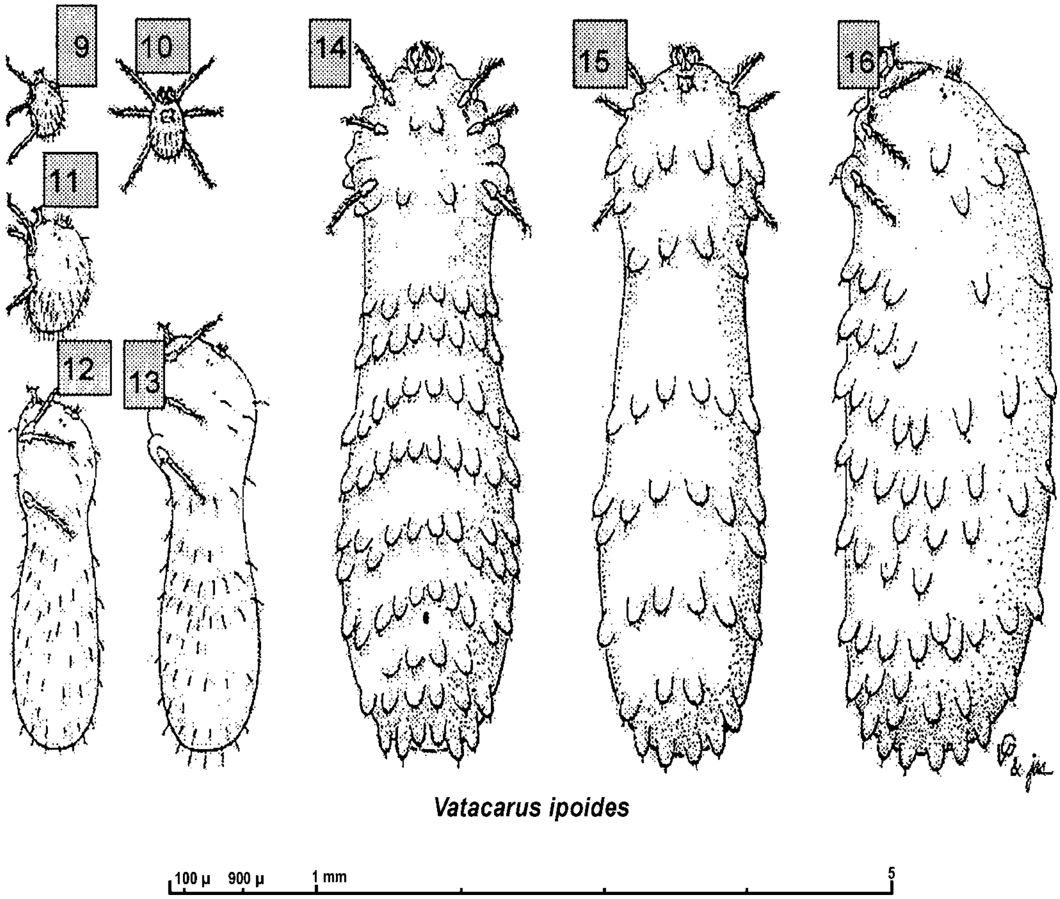

Figure 9–16. Neosomy in *Vatacarus ipoides* Southcott, larva (9–10) new born, lateral and dorsal view; (11–13), progressive intermediate degrees of enlargement, lateral view; (14–16), neosome, ventral, dorsal and lateral view (after Audy *et al.*, 1963).

UL and PEL free of conical projections or neosomes of idiosome. UL broadly oval, PEL idiosome swollen, hysterosoma greatly elongate. EL elongate, conicular projections or neosomes more prominent dorsolaterally and posteriorly. ***Eyes*** 2 + 2, placed on ocular plate; anterior eye round and oculate, posterior eye ovoid and indistinct; ocular plate situated laterally in the posterior half of scutum, the midline of plate being in line with PL base. (Eyes not apparent in engorged specimens, but visible in UL and PEL, less so in the latter, and not easily discernable in old mounts.) Eyes, in live UL and PEL visible as red spots under low magnification. ***Gnathosome:*** Fairly heavily chitinised, chelicera (37 um), flexed with pointed apex, a small distal denticle and a big hook-like ventral denticle. Galeal seta (15 um) nude, fine and tapering. A pair of coxal setae with adpressed ciliations. ***Palpal formula*** b/b/bNN + 7BS (PTF); palpal setae weak, femoral and dorsotibial setae more developed than others and have swollen bases; dorso-tibial seta stoutest; lateral seta and ventral seta of tibia normally ciliated, the latter two nude and tapering. Palpal tarsus with a long, blunt subterminala, a subapical sensory seta, and 3 fairly long, and 4 short setae with short indistinct barbules and appearing almost nude. ***Claw*** 20 um with 2 strong prongs, ventral strongly recurved with pointed apex, dorsal claw less recurved and blunt. ***Scutum***: (also see scanning electron micrograph, plate III)

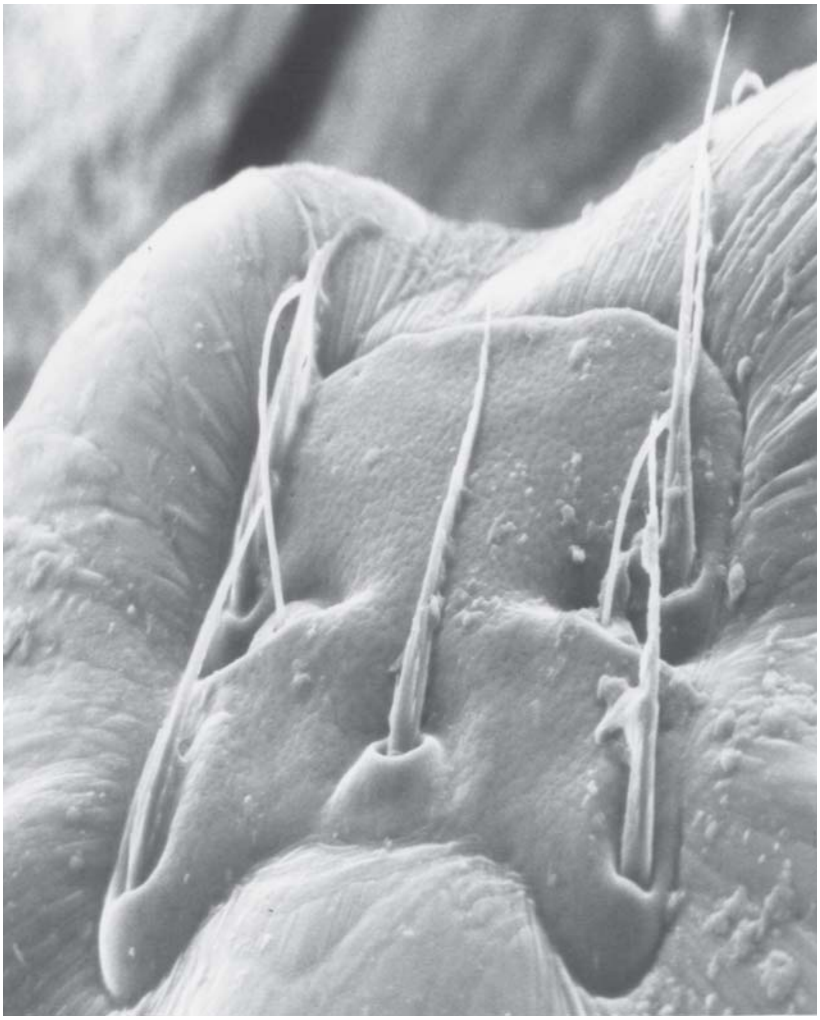

Plate III. Electron micrograph of scutum of engorged larva.

trapezoidal, anterior width greater than posterior, anterior shoulders not evenly rounded, lateral margins sinuous, posterior margin slightly concave medially; posterior margin more distinct than the other scutal margins, but not chitinised. Punctations close-set and evenly distributed. Scutal setae with slightly expanded bases, but tapering distally, with minute barbs. PL>AM>AL, the difference in measurements being gradual. Sensillae long and filiform (85 um).

Standard measurements (in um) of scutum, mean of 4 unfed larvae:

AW	A-AL	PW	P-PL	SB	ASB	PSB	AP	AM	AL	PL	SENS
96	14	82	36	59	49	69	32	52	48	65	85

Dorsal setae (56 um), slightly swollen at base, and tapering distally. DS arranged in somewhat transverse rows of 2. 6. 4. 6. .6. 4. 2. 2 (0) in unfed larva. Ventral setae (VS) 2 pairs ciliated sternal setae, plus 40- 44 true ventral setae 32 um long. Both DS and VS are inserted on neosomules or conical projections in engorged larvae.

Legs: 7-segmented and fairly sclerotized. Tarsal claws I-III stout; empodia long, thin and delicate, and easily broken in engorged specimens. Coxae I and II well separated in engorged neosomes and partially engorged larvae, but less so in the latter. Coxae I and II almost fused in ULs. Ordinary setae have very short adpressed ciliations and therefore appear nude. Coxae I to III unisetose, the setae being slightly swollen at base. Sensory and barbed setae as follows: ***Leg I***: tarsus + pretarsus 95 x 41, tarsala elongate and blunt (44), microtarsala situated at tip of tarsala; a pretarsala, a long subterminala, a small tapering parasubterminala placed close behind subterminala, and 20-22 barbed setae. 2 tibialae, distal one stout, blunt and striated, proximal slender and pointed; a fairly long microtibiala placed below and in line with distal tibiala, 6 barbed setae; 6 tapering genualae (varies from 5-7 in some specimens), l microgenuala and 4 ciliated setae; remaining 3 basal segments with 5, 1,1 ciliated setae. The seta on basifemur nude and pointed. Coxa I 76 x 56. ***Leg II :*** **t**arsus + pretarsus 75 x 40 um, a short cigar-shaped striated tarsala (26 um), a microtarsala above tarsala plus 15-16 ordinary setae; 2 tibialae, the distal striated and blunt, proximal tapering plus 5 ordinary setae; 3 subequal, tapering genualae and 3 ordinary setae; other 3 basal segments with 4, 1, 1 ordinary setae. Coxa II: 80 x 84 um. ***Leg III :*** Tarsus + pretarsus 82 x 40 um. One indistinct mastitarala and 13 ordinary seate; a pointed tibiae + 3 ordinary setae; other 3 basal segments with 3, 2, 1 ordinary setae. Coxa III 86 x 52 um.

Remarks on taxonomy: It is interesting to note that ordinary or non-sensory setae are of two kinds - setae without basal expansion and those with swollen bases as in the genus *Babiangia* -complex. Radiating lines around scutum and coxae I and II were observed in some engorged larvae, but not so in UL or PEL. Evidence of trachea was found in larval pelts, but not on larval specimens. It is also noteworthy that variations in numerous taxonomic characters were observed which is either related to sexual differences or other intraspecific variations.

Southcott in his description of *V. ipoides* stated that the empodia tended to be retroflexed, and had figured it so. He had, since, examined some of the specimens we sent him, and has noted (pers. commun.) that the empodia are setiform.

Material examined: The mites were found in the air-sacs (tracheae) of the Amphibious Sea Snake, *Laticauda colubrina* (Schneider), found in low tide in the mangrove tree holes and in rock holes in a tidal reef, near Pulau Sudong, about 5 miles off Singapore by M. Nadchatram in October, 1959.

General remarks on slide preparation
Making slide preparations of the neosomes of *Vatacarus* caused considerable technical problems at first due to their large size and cylindrical shape. The cuticle of the species in the engorged state was fatty, leathery, and thick-walled. Potassium hydroxide was an unsatisfactory clearing agent, because specimens cleared in it disintegrated when mounted. At first it was possible to mount neosome *Vatacarus* only after dissecting it into several parts, a method that is still useful for detailed study of certain taxonomical characters. Subsequently, a somewhat satisfactory method for making whole mounts was developed. After a few tiny holes made in the posterior end, the specimen was placed between 2 slides, and gradual pressure was applied and the glass slides were tied together with a string. Some of the liquid fatty content oozed out through the artificial pores on pressure. The specimen was then placed in a petri dish while it was still pressed between the slides, flooded in lactic-glycerine. After a week the specimen was transferred to the PVA mounting medium. The method was not ideal, but it was quite satisfactory. (These notes were made in the early 1960s, when the choice mounting medium was PVA or Polyvinal Alcohol. The medium of choice nowadays is Hoyer's medium. This medium is found to be satisfactory because age-old mounts are easily recoverable and remounted.)

The adult male and female *V. ipoides*
Synopsis of the descriptions of the adult male and female *Vatacarus ipoides* Southcott (Fig. 17-28, male; Fig. 29- 40, female).

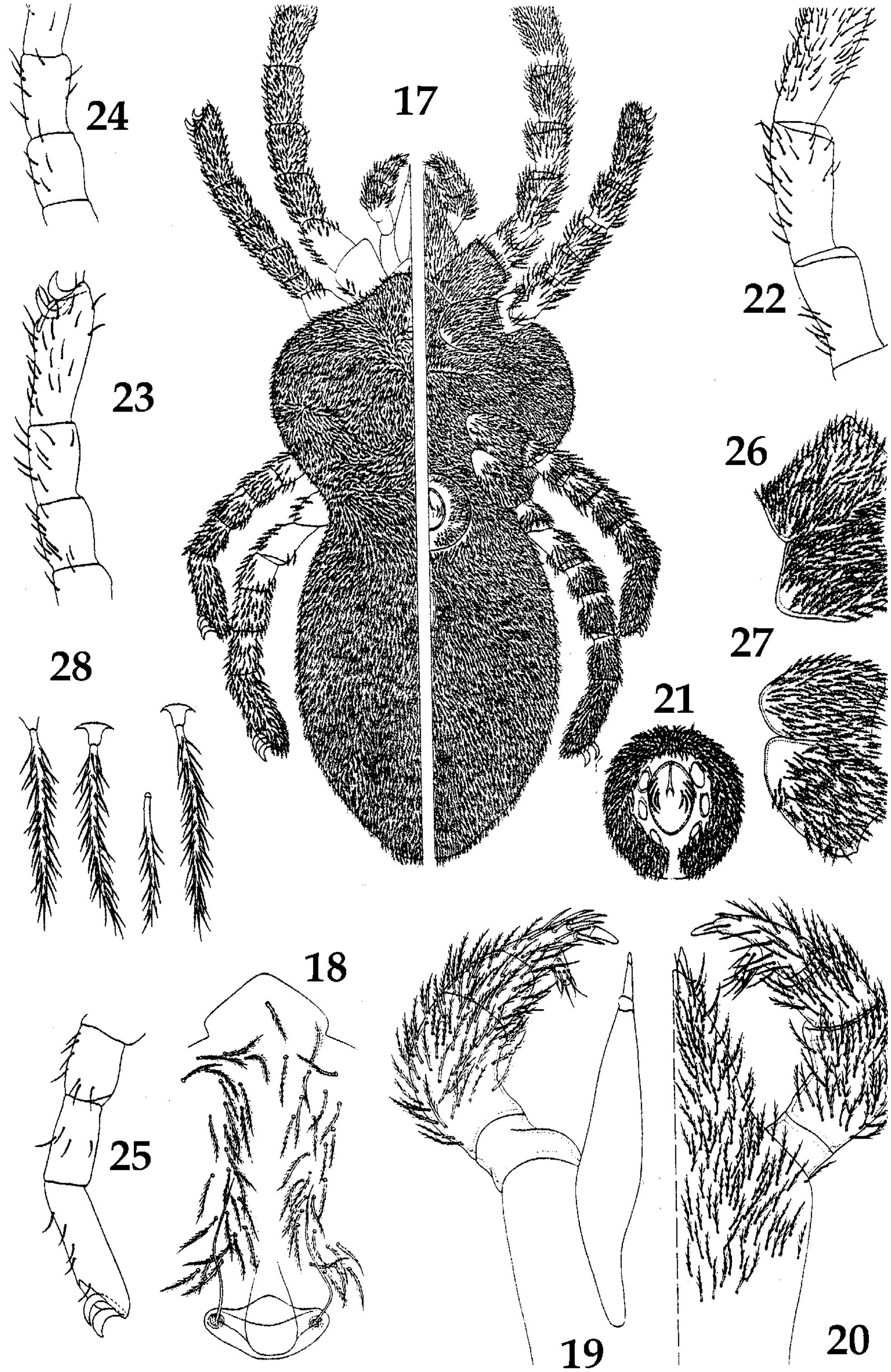

Figure 17–28. Male *Vatacarus ipoides* Southcott:– 17, dorsal and ventral aspects of idiosome; 18, crista and epistome; 19,20, dorsal and ventral aspects of gnathosome; 21, male genitalia; 22,23,24,25, distal segments of legs I, II, III, IV; 26, coxae I and II; 27, coxae III and IV; 28, dorsal and ventral idiosomal setae.

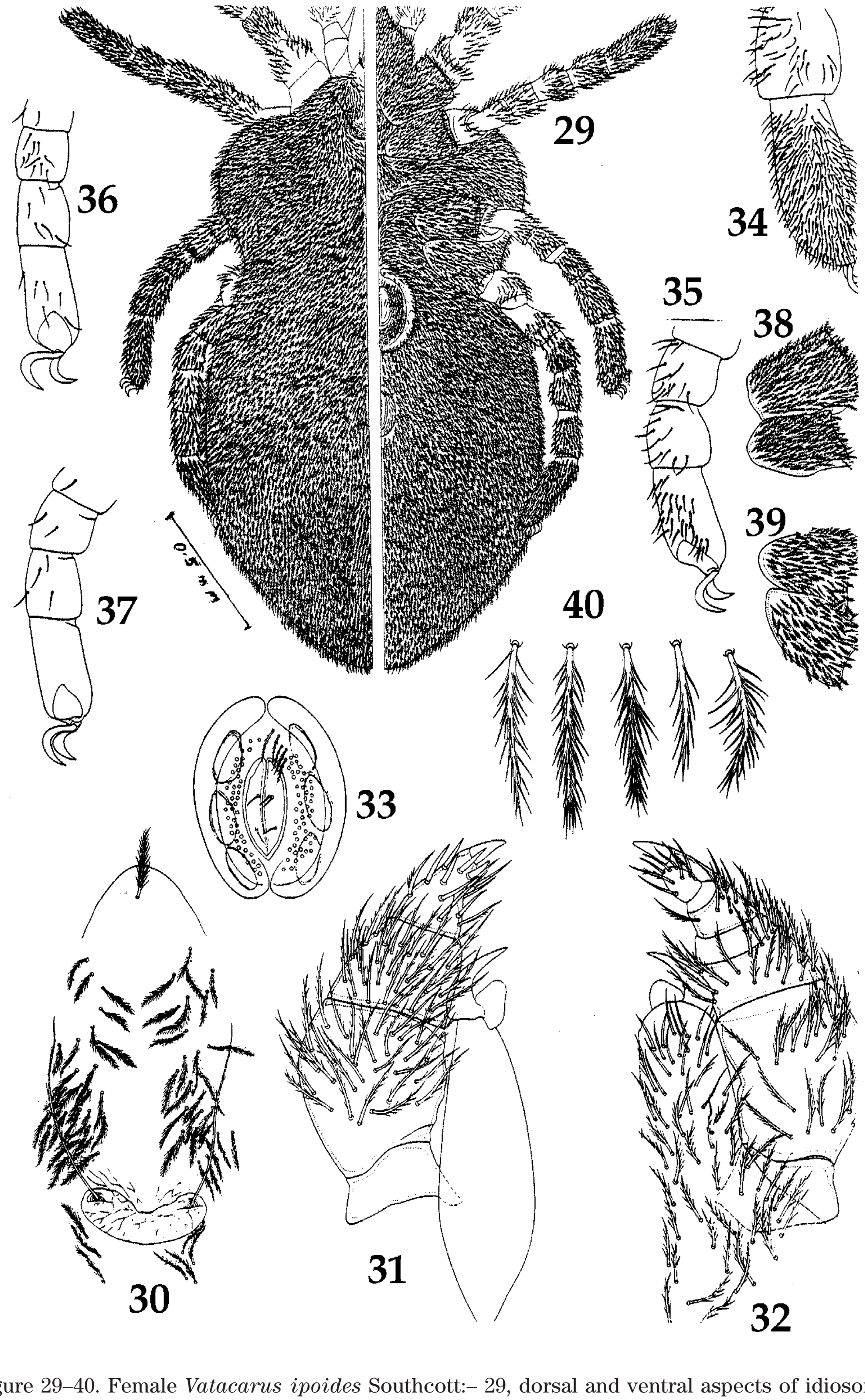

Figure 29–40. Female *Vatacarus ipoides* Southcott:– 29, dorsal and ventral aspects of idiosome; 20, crista and epistome; 31,32, dorsal and ventral aspects of gnathosome; 33, female genitalia; 34,35,36,37, distal segments of legs I, II, III, IV; 38, coxae I and II; 39, coxae III and IV; 40, dorsal and ventral idosomal setae.

(Some drastic changes and movements had taken place in the acarology laboratory a few years ago after my retirement, when the slide collections of these and other material were either lost or misplaced. Therefore, the synopsis presented here is based on the illustrations only. However, it is felt justified to publish the illustrations to show the typical contour of the slide preparation of the adult mites, which were directly reared in the laboratory by the author. The publication of the illustrations would also be of some help to future workers. Also, the illustrations were prepared in Japan, at the U.S. Army 406 laboratory, at considerable expense and labour and would prove it to be wasteful if they are not published).

The adult mites emerged directly from the neosomatic larvae. They were orange coloured in life. Live males were slightly smaller and slender.

The adults are of the general facies of trombiculid mites, with the usual scutum or crista and epistome, the genitalia of male and female show 3 pairs of genital discs (nymphs only have two pairs). The gnathosome and legs are also of normal contour. However, the dorsal aspect of tibia of the gnathosome bears 3 pairs of spur-like setae just proximal of the claw, as in other trombiculid mites. The male was more elongate and smaller than the female, the idiosome of male more slender. Male 2.0 to 2.5 mm, with propodosoma 1.2 mm, hysterosoma 1.7 mm. The constriction of the idiosome is characteristic for mites of the family Trombiculidae, the constriction of both male and female is between third and fourth pair of legs. Idiosome of female 3.0 to 3.5 mm with hysterosoma wider than propodosoma. As in other trombiculid mites it has more setae of the ordinary barbed type to give the adult a velvety appearance.

BIOLOGY

The life history of *Vatacarus ipoides*

The amphibious sea snakes, *Laticauda colubrina* located near Pulau Sudong, Singapore were seen in most of the tree holes by J.R. Audy, and rock holes in the tidal reef at low tide by M.N. As many as 5 or 6 in each of the many holes or crevices in the coral reef were found. A total of 18 snakes were collected and transported in metal tanks to the Institute for Medical Research, Kuala Lumpur, where the live snakes were kept in tanks filled with sea water. The snakes were dissected from time to time, to recover the mites alive. The trachea or air-sac was slit open to expose the entire windpipe. All of the 18 snakes examined were infested with mites, which were subsequently identified as *V. ipoides*, and confirmed by Dr Southcott. The older and larger snakes harboured the most number of mites, 60 being the largest number found in a single big snake. The bigger, maggot-like mites or neosomes were confined to the upper region of the trachea, a few were found dispersed in the posterior portion of the trachea, but none were found in the lung-sac. Almost all mites found were fully fed. Lungs of amphibious sea snakes are much larger than their land based relatives. Most chigger mites increase their volume some 20 x with engorgement, *Vatacarus* larvae increase 1,500 x or more. The legs, functional in the unfed stage, are useless, relatively minute appendages of the neosome. The cornicular projections or neosomules (plate II) have an obvious function enabling the larval mites to move in the mucus secretions in the manner of maggots or worms (plate IV A). The progressive degrees of enlargement from new-born to neosomatic larvae is as illustrated in figures (Fig. 9–16).

The engorged or maggot-like mites in batches of 15 x 4, were reared in porous pots made of unglazed clay, about 1½” in height and 1¾” wide (Nadchatram,1968). The pots were boiled several times to remove soluble minerals before use. A pinch of sterile soil was introduced to simulate natural conditions. A glass cover was placed over the pot to contain the mites within the pot. The ambient temperature of the area housing the breeding unit was min. 25.5ºC, max. 30ºC, with RH

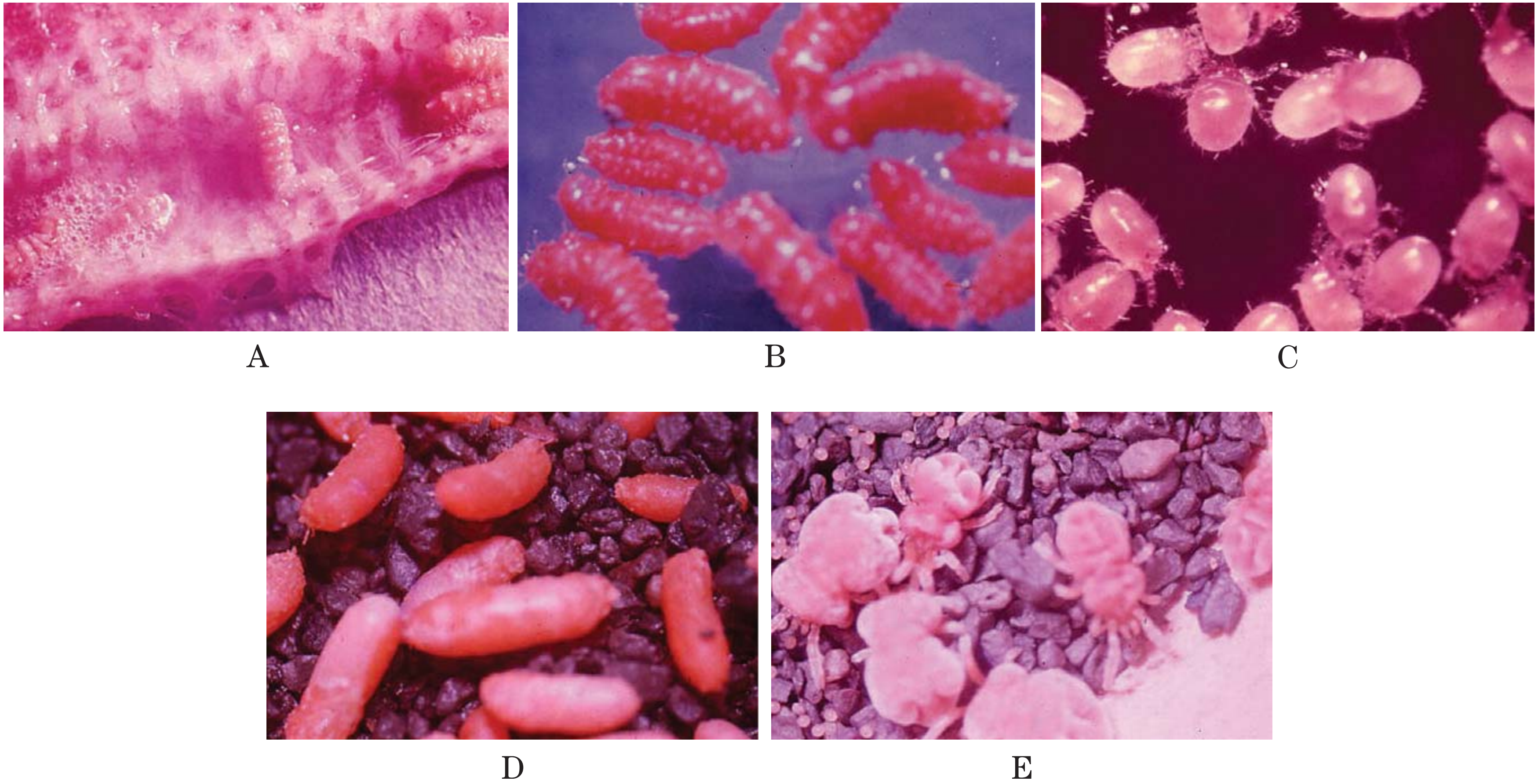

Plate IV. Pictures taken under low power magnification (by author) showing some aspects of the life cycle of *Vatacarus ipoides*. A, mites seen in the mucus of trachea of amphibious sea snake. B, a cluster of *V. ipoides* – neosomes. C, fully engorged larvae of *Leptotrombidium deliense* to compare with neosome (engorged larvae) of *V. ipoides*. D, Cigar shaped "pupae" showing the absence of neosomules. E, adult male and females (females bigger than male).

63 - 87 %. The larvae were reared to adults, and F1 eggs and larvae were obtained.

The neosomatic larvae were sluggish and moved very slowly or not at all. Two sizes of neosomatic larvae, some smaller than the others, were seen. In the former case it was possible to determine if the mite was alive by the contraction and expansion of the body. On the third day after being placed in the breeding pot the larvae became quiescent, without visible movement. From this stage onwards the cornicular projections or neosomules (plate II) gradually disappeared until the body was smooth-surfaced, and resembled an inflated, elongate balloon, or shaped like a Havana cigar (plate IV D). This change was completed by the end of the fifth day, and 9 days later, the adults of two sizes emerged. The adults were orange coloured, the contour was typically of trombiculid form, with medial body constriction. It was at first thought that the post-larval stages were nymphs, but was subsequently established (more from their behaviour than anything else) that they were, in actual fact, sexually separable adults. Only half of the larvae emerged as adults. Of the others, many were attacked by fungi, and a few failed to metamorphose for unknown reasons.

The development of adults directly from larvae is an extreme example of tachygenesis. Both sexes of adults were obtained, and it is highly probable that the smaller, neosomatic larvae gave rise to the males, and the bigger ones to females, suggesting that sex differentiation is predetermined, hence the sexes were easily separable, (plate IV E) unlike in other species of trombiculid adults, which needed to be turned-over for a ventral view, to examine the genitalia to determine the sex. The males in this species were about 2.5 to 3 mm, which are approximately the size of *Blankaartia acuscutellaris*, a scrub-itch chigger, and bright orange in colour. The females were 1.5 times bigger than the males, and lighter orange. The ratio of males to females was 3 to 2.

Both males and females were normally active. One day after the first batch of adults emerged, a few tiny mould-like structures were seen here and there in a pot containing both sexes. The head of this mould-like structure (sperm-sac) was small and round and was supported on a very thin stalk. (Some of the stalks were without the head, suggesting that the sperm-sac were taken by the female) The head of the sperm-sac capsule was pallid and translucent. On careful examination it was established that the structures were, indeed, spermatophores with the sperm packets. Adults, both male and female, that emerged subsequently were segregated by keeping them in separate pots for two days, but spermatophores were not seen in the pot containing the males. However, within about an hour after the males and females were brought together, three stalks of spermatophore with sperm packets or sacs were seen. This showed that the males were stimulated to deposit the sperm only in the presence of the "fairer sex". Though it was not observed by us, it is most likely that the females take up the sperm through the genitalia, leaving only the empty packet and stalk as demonstrated by the female *Blankaartia acuscutellaris*. This unique method of insemination and fertilisation of the female trombiculid mites was described by Lipovsky *et al.* (1957). Whether or not a ritualistic dance precedes spermatophore deposition, as in some other groups of acarines, is not known. However, the mode of transfer of the male sperm packet to the female further supports the fact that *Vatacarus* is a member of the family Trombiculidae. Two virgin females were isolated immediately upon emergence, but they did not produce eggs till the day they died 30 and 36 days later. From these observations it is perceived that parthenogenesis does not occur in this species. Also, the adult mites seem to have a much shorter life span (6 to 8 weeks) compared with adults of *Leptotrombidium deliense* or *Blankaartia acuscutellaris* whose life span under laboratory conditions is about a year, or longer.

Eggs were seen 12 days following the deposition of spermatophores, and one female continued to oviposit for 24 days.

Eggs were laid singly at long intervals, the number per day ranging from 1 to 7. The number of eggs laid by a single female was 52, the average number of eggs laid varied. To observe the number of eggs laid, and the duration of egg laying, 3 fertile females were placed in 3 separate pots. In pot # 1: 46 eggs were laid in 17 days; the female died on the 27th day; highest number of eggs laid was 7, on the 9th day. In pot #2: 52 eggs were laid in 22 days, the highest number laid was 6, on the 15th day, the female died on 43rd day. In pot #3: 42 eggs were laid in 24 days; the female died on 30th day, highest number of eggs laid was 6, on 7th day. The eggs were orange, round and a little bigger than those of *Leptotrombidium deliense*, the vector of scrub typhus. Deutovums were seen 7 days after ovipositon, and larvae hatched 16 days later.

The newly emerged larvae were approximately 2 x the size of newly hatched larvae of *L. deliense*, and the idiosome under low-power magnification was clearly divided into a broad anterior half and a narrow posterior half. On hatching, the unfed larvae crawled up the pot and rested in small clusters underneath the glass cover, as new born larvae of *L. deliense* and *B. acuscutellaris* did. The larvae in the unfed state were yellowish-orange and quite active, and lived for a maximum of 18 days. Adult females lived for 8 weeks, and the males for 5- 6 weeks. During the period under observation cannibalism among the adults did not occur.

Four of the host snakes were kept alive in sea water. One of them died 3 weeks after capture. Six engorged larval mites were recovered from the tank that held the snake, and 20 more from the dead snake. The last 3 snakes died 64 days after their capture. A total of 57 engorged larvae were found in the tracheae. In all the 18 snakes examined, almost all the larvae were engorged; only about 10 partially engorged larvae were found among the several hundreds that were recovered from the snakes. Not a single unengorged larva was found. The feeding time of the larval mite is unknown. It is suspected that the feeding time is short, because almost all the mites found in the snakes were mostly fully engorged. The life-cycles of *Blankaartia acuscutellaris*, a species with a typically normal trombiculid life cycle, and *V. ipoides* are presented in tabular form, for comparison

The normal life-cycle of mites of the family Trombiculidae consists of the egg, deutovum, active parasitic larva, akinetic nymphophane, active free-living nymph, akinetic teleiophane, and finally the free-living adult. In *Vacacarus*, the active nymphal and the akinetic teleiophane

Table 1. Life cycle of *V. ipoides* compared with *B. acuscutellaris*

Stages of life-cycle	*Blankaartia acuscutellaris* No. of days	*Vatacarus ipoides* No. of Days
Engorged larvae placed in breeding pot	0	0
Larvae quiescent in	2-3	"Pupal stage" 8
Nypmphs emerged (fed on culicine eggs)	6-7	lacking
Nymphs quiescent in	8-9	lacking
Adults emerged in	8-9	9-10
Spermatophore deposited in	1-2	1
Eggs laid after	12-13	10-12
Deutovum developed in	5	7
Eggs hatched in	10-12	14
Development period of F1 from EL to UL	48-56	48-52

stages are absent. However, the total number of days required to complete the life-cycle is not significantly different.

The breeding experiment has established a few interesting biological facts, as listed below:

(1) The protonymphal and active nymphal stages are bypassed, the neosomatic larvae giving rise to adults of different sexes, after a single moulting stage. This is an example of tachygenesis and is unique, and unusual for the family Trombiculidae.

(2) The larva is the only stage in the life-cycle that fed, the free-living adults did not feed, but produced eggs. (Normally, free-living adults feed on other arthropods or their eggs before producing eggs.)

(3) The sexes of the adults were easily distinguished, the male being smaller than the female, the sex difference is probably predetermined in the embryonic stage, but quite evident in the neosomatic larval stage. (Sexes of normal adults are of similar size.)

(4) The male deposited sperm packets only in the presence of the opposite sex.

(5) Cannibalism was not seen to occur among adults.

(6) Newly emerged larvae, male and female adults had short life span. For example, ectoparasitic unfed larvae lived for up to 18 weeks, not 18 days, free-living adults lived for over a year, not for 6 to 8 weeks.

(7) Normal chiggers increased their volume with engorgement some 20 x, but *Vatacarus* larvae increased 1,500 x or more.

(8) When the new born larva enter the respiratory track and begins to feed, the mite is a completely new creature compared with other ectoparasitic trombiculid mites larvae.

The following freshwater snakes were examined for tracheal mites but were found uninfected: 12 Dog-Faced Water snakes, *Cerberus honchos*, a common species in fresh and tidal waters, especially in muddy creeks near the coast. 20 Puff-Faced Water Snake, *Homalopsis buccata*, common in freshwater ponds and swamps, both near the coast and far inland; and 5 Elephant's Trunk Snakes, *Acrochordus javanicus*, common in tidal streams.

In addition, 39 specimens of true sea snakes of 6 different species held in captivity by the Penang Hospital for research on sea snake anti venom, were examined for mites in the trachea, with negative results.

RESULTS AND DISCUSSION

Relationship of *V. ipoides* with the host, *L. colubrina* – a possible explanation

The habitat and behaviour of the Amphibious Sea Snake is based on Sharma (1973), Tweedie (1983), Ditmars (1962), and from my observations and inferences.

The Amphibious Sea Snake, *Laticauda colubrina*, is the only local species found in the tidal reefs and mangrove tree holes of Singapore, and possibly the west coast of Peninsular Malaysia. It has broad abdominal plates like terrestrial snakes, although the tail is wide, flat and paddle-like. It is said to be encountered on the beach where it seeks shelter under boulders or in rocky niches. It also lays its eggs on land, unlike other sea-snakes which are viviparous and entirely aquatic. This species also frequents mangroves, and off the near-by island of Pulau Sudong, where the author collected as many as 5-6 snakes from a single rock hole in the tidal reef. Although considered potentially dangerous, this species has never been known to attack anyone in the sea in this area. It appears to be quite nonaggressive and makes no attempt to bite even when molested. According to Tweedie (1983) the sea snakes are common and found in numbers on the shore on small islands, hiding in crevices or under stones, off Singapore. The land area near Raffles Lighthouse had been reported as a nesting site. Species of

Laticauda are known for their wide distribution across the Pacific and Indian Oceans. They are associated with marine waters and rocky areas or coral along the seacoast. Nadchatram and Radovsky (1971) described a second species of *Vatacarus* from *L. colubrina* from Lan Yu, a small island of unknown name, 70 km SE of Taiwan. The endoparasitic mites are supposedly common wherever the snakes are found. In 1980, a collection was received from Professor A. Fain of Belgium. The collection of mites were from the lung sacs of *L. colubrina* and *L. laticaudata* found in New Guinea and Caledonia and preserved since 1878. The species was close to *Vatacarus*, and described as *Iguanacarus alexfaini* Nadchatram 1980.

The sea snakes have a cylindrical body, with a laterally compressed, paddle-like tail. A salt gland under the tongue gives amphibious snakes the ability to expel excess salt absorbed from the marine environment. Whether or not this biological feature would be a factor which is likely to facilitate endoparasitism of the amphibious sea snakes is not known. The genus *Laticauda* appears to have evolved from an elapid stem much later than the other sea-snakes. In their natural habitat, the snakes feed primarily on eels, but will occasionally prey on small fishes. When feeding they have been seen to trap small fish in rock crevices with coils of the body and then seize them. *Laticauda* species. (there are 3 known species, *colubrina*, *semifasciata*, and *laticaudata*) are the only sea snakes known to lay eggs on land, either on the sand or under it. Courtship and mating occur on land. The breeding habitat must be protected sufficiently to provide shade and shelter. Some form of biological mechanism must trigger the fed larvae to leave the hosts when the snakes come ashore for the mites to continue their free-living phase. Snakes of *Laticauda* species spend much time on land and, therefore, are the only ones which could have any kind of "den" relationship that could be utilised by the mite parasite for spreading from snake to snake. Also, because of the gregarious behaviour of these snakes, they must have recognised sites to which they come for egg laying and other reasons.

There has to be a very close relationship between the snake and the parasites. Large populations of the snakes are said to be found on relatively small islands during the breeding season. The rock holes and the holes in mangrove trees are only the resting sites during low tide. These holes were examined, but did not yield the free-living adults of the mite. Also all the larvae found in the tracheae of the snakes were mostly fully engorged, but did not detach, only waiting for the snakes to go ashore. The sea snakes leave the ocean for land at about 10 day intervals, usually at night, to digest food, engage in courtship, lay eggs, and slough skin. This is when the engorged or replete larvae leave the host to continue with the progression of their life cycle. In the breeding season, the movement of the snakes from sea to land is said to be more frequent.

Tick Infestation

It is ecologically noteworthy that a species of tick, *Amblyomma nitidum* Hirst and Hirst, 1910 was found on at least three separate occasions by Audy *et al.* (1960). A female adult, nymphs and a few larvae were collected, which supports the "den" behaviour of the amphibious sea snake on land Audy *et al.* Warburton (1933) described *A. laticaudae* from the shed skin of *L. colubrina* held at the Raffles Museum, Singapore. Some doubt has been cast on the status of the *Amblyomma* species infesting this species of sea snake (Petney & Keirans, 1995). They concluded that both *nitidum* and *laticaudae* are valid species based on hypostomal dentition of male specimens. *A. laticaudae* possess 3/3 dentition, whereas *A. nitidum* has 4/4 dentition in the male. For a species of sea snake that lives and breeds in complete ecological isolation from other species of snakes, and where the ticks are identical in other taxonomic ways, it is very unlikely that on the bases of hypostomal dentition the two forms can be regarded as separate species. I support, as Audy *et al* did, Kohls' contention that the ticks represent a single species. Kohls, in Audy *et al* (1960) had compared the Singapore specimens with the descriptions of *A. nitidum* and *A. laticaudae* and had observed the difference in hypostomal dentition in the males, and had suggested that this could be due to variation. Kohls (1957) concluded that *laticaudae* is a junior synonym of *A. nitidum*. From the literature reviewed, *A. nitidum* is host-specific to amphibious sea snakes.

Habitat and Ecological Niche of Chiggers

With reference to the behaviour of the larval trombiculids mites, Audy (1956a) and other observers Nadchatram & Dohany, (1974) and Traub & Wisseman (1974) have noted that trombiculid mites generally show little or no host-specificity, but rather a varying degree of habitat-specificity, with a few notable exceptions, for example, in birds, a few genera of chiggers are primary parasites showing host preference. Like-wise with reptiles and bats in Malaysia. Years of studies in Malaysia have borne it out. Though habitat-specificity may give an appearance of host-specificity in many genera of trombiculid mites, it is, however, a well established fact that all the mite vectors of scrub typhus and the mites causing scrub-itch, which are red or orange in colour, are habitat-specific (Audy, 1968). It is now clear, based on Malaysian studies of host-parasite relationship of the thousands of reptiles, birds, bats and other mammals, that certain groups of mites pertain particularly to reptiles (e.g. the genera *Eltonella*, *Babiangia* and *Fon-secia*), and others almost exclusively to ground mammals (e.g. the genera *Gahrlie-pia*, *Walchia*, and *Helenicula)*. Certain groups, especially closely related to their hosts in one way or another, show signs of developing true host-specificity on birds of some genera (e.g. *Neoschoengatia*, *Tori-trombicula* and *Odontacarus*, and on bats (e.g. *Chiroptella*, *Trombigastia*, and *Riedlinia)*.

Based on approximately 35 years of field and laboratory investigations carried out at the Institute for Medical Research, Kuala Lumpur, Malaysia, 148 species of trombiculid mites or chiggers were recorded. Taxonomical, biological and ecological investigations were conducted on most of the species, with reference to understanding their epidemiological importance. On the basis of these studies an ecological classification was conceived and published by Nadchatram (1970b). The classification divided the Malaysian chigger mites into seven different ecological categories. Alland (1967) stated that all scientific theories have three things in common. They are simple; they explain a great deal; they can be tested through either observation or experimentation, or both. According to him, Darwin's theory of evolution is one such elegant theory. With a few basic assumptions, Darwin's theory attempts to explain the development and diversification of life. Nadchatram (1970) following Darwin's concept endeavoured to illustrate the diversification of the trombiculid mites or chiggers to better understand their epidemiological importance or significance. The following criteria were used to arrive at the ecological classification.

A. Colour of chiggers; all chiggers are soft bodied, the exoskeleton sclerotized only in certain regions, their colour range from white or pallid, to yellow, orange to red. The colour is mostly influenced by the habitat; **B**. natural host preference of chiggers based on the examination of thousands of reptiles, birds and mammals; **C.** natural habitat preference of new born chiggers; **D**. breeding experiments using a standard rearing technique; **E**. morphological and taxonomical characteristics of the chigger species.

A major attribute of this classification is its provision of an ecological framework within which the present wealth of chigger-related information may be considered. It was shown that all the orange and red chiggers living on the ground-surface were relatively highly adaptable to fluctuating environmental conditions, and parasitic on all animals, including man. The sensillae were either filiform or filamentous. The tarsus of the palp always has a basal tarsala and a number of barbed setae, varying from 3 to 7, and with or without nude subterminala(s). This is expressed as a palpal tarsus formula (PTF). For detailed ex-planation see Nadchatram & Dohany, 1974. For the purpose of this discussion the PTF determines the ecological affinity of the chiggers, so that 7B or 7BS is associated with orange or red chiggers that infest a variety of species of host animals and are tolerant to changing environmental conditions, whereas species of chiggers with 3B and 4B are white or pale coloured, restricted in their host and habitat and sensitive to changing environmental conditions. The orange or red ones were chiggers of Eco-logical Group I, the group that included all the species of chigger mites of epidemio-logical importance. Conversely, those species of chiggers of Ecological Group II were pallid or white, or pale yellow, and were sensitive to changing environmental conditions and the free-living post-larval stages were cryptozoic and lived in ground burrows of rodents. Sensillae were club-shaped or globose and PFT 3B, 4B, 4BS or 5B. Of the other groups i.e. Ecological Group III to VII, the determining factors were based on habitat and host. Group VI is discussed here because it involves the two species found hypodermal in frogs, and endo-parasitic in sea snakes, respectively. Both species, *Vercammenia hendricksoni* Audy and Nadchatram and *V. ipoides* were orange in colour, suspected to be living in close association in the same habitat as the hosts. Whether or not they are adaptable to fluctuating environmental conditions will not be known until the nest sites are investigated to determine the behaviour of the adult mites. The PTF is 7BS in larval *V. ipoides* and 7B in *Ve. hendricksoni* as in other surface dwelling chiggers. As mentioned earlier the larvae and adults of both species were orange coloured. However, based on the know-ledge gained from our laboratory studies of *V. ipoides* and the morphological modifica-tions seen in the larval stage, and based on the abbreviated life-cycle and short life span, it is sufficiently evident to postulate that *V. ipoides* is a host-specific parasite, and that the adults are surface dwelling within the confines of the snake's breeding sites. There is an extremely close relation-ship between the parasite and host, because of the clustering behaviour of the larval mites and the "den" relationship of the sea snakes.

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