

## RESEARCH NOTES

### RECORDS OF *CYTAMOEBA* LABBÉ, 1894, AND *TODDIA* FRANCA, 1910, IN SOME ANURANS FROM THE MALAYAN PENINSULA†

Examination of Giemsa-stained blood films showed that 63 of 344 anurans surveyed for blood protozoa in Peninsular Malaysia and Singapore (Sullivan and Sullivan, 1976. *Southeast Asian J. Trop. Med. Pub. Hlth.*, 7: 493) were also infected with *Cytamoeba* Labbé, 1894, and *Toddia* Franca, 1910. Although readily identifiable in blood films, the taxonomic status of these two parasites is uncertain since their alleged protozoan or viral affinities have not been confirmed (John-

ston, 1975. *J. Protozool.*, 22:529). Numerous records for these cell inclusions exist for the Americas, but published records for *Cytamoeba* and *Toddia* in Asia are scant (Johnston, 1975. *Ibid.*).

Johnston (1975. *Ibid.*) lists *Cytamoeba* from *Rana cancrivora* in the Philippines and from *R. japonica* and *R. rugosa* in Japan. Manwell (1964. *J. Protozool.*, 11:526) found "vacuoles filled with rod-shaped bodies" or bacteria in blood cells of *R. guentheri* in Taiwan, a description consistent with that of *Cytamoeba*. In the present survey, 63 of 344 anurans (18%) examined were infected with *Cytamoeba* (Table 1). *Rana erythraea* showed

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Table 1

Hosts and localities of *Cytamoeba* in the Malayan peninsula.  
[No. positive (No. examined)]

| Localities       | <i>Bufo melano-</i><br><i>sticticus</i> | <i>Rana cancri-</i><br><i>vora</i> | <i>Rana ery-</i><br><i>thraea</i> | <i>Rana lin-</i><br><i>limo-</i><br><i>charis</i> | <i>Rana mac-</i><br><i>ro-</i><br><i>don</i> | <i>Rhaco-</i><br><i>phorus</i><br><i>leuco-</i><br><i>mystax</i> | Other   | Total    |
|------------------|---|------------------------------------|-----------------------------------|---|--|--|---------|----------|
| Johore:          |   |                                    |                                   |   |  |  |         |          |
| Johore Bahru     | 1 (5)                                   |                                    |                                   |   | 0 (1)  |  |         | 1 (6)    |
| Kota Tinggi      | 0 (11)                                  | 0 (1)                              |                                   |   | 1 (1)  |  | 2 (13)* | 3 (26)   |
| Negeri Sembilan: |   |                                    |                                   |   |  |  |         |          |
| Ulu Jempol       | 0 (2)                                   | 1 (15)                             | 15 (23)                           | 3 (9)   | 7 (47)                                       | 0 (10)   | 1 (9)** | 27 (115) |
| Pahang:          |   |                                    |                                   |   |  |  |         |          |
| Kuala Tahan      |   |                                    |                                   |   |  | 0 (9)  |         | 0 (9)    |
| Tasek Bera       | 0 (6)                                   |                                    | 21 (43)                           |   | 0 (1)  | 0 (5)  |         | 21 (55)  |
| Tioman Island    |   |                                    | 1 (1)                             |   |  | 0 (9)  |         | 1 (10)   |
| Perak:           |   |                                    |                                   |   |  |  |         |          |
| Hilir District   | 0 (11)                                  |                                    | 5 (6)                             |   | 0 (1)  |  |         | 5 (18)   |
| Perlis:          |   |                                    |                                   |   |  |  |         |          |
| Kangar           | 0 (1)                                   |                                    |                                   | 0 (25)  |  |  |         | 0 (26)   |
| Selangor:        |   |                                    |                                   |   |  |  |         |          |
| Kuala Lumpur     | 0 (23)                                  |                                    |                                   |   | 0 (36)                                       |  |         | 0 (59)   |
| Singapore:       |   | 1 (4)                              | 3 (4)                             | 0 (1)   |  |  |         | 4 (9)    |
| Other:           | 0 (3) <sup>+</sup>                      |                                    | 0 (1) <sup>++</sup>               |   | 1 (5) <sup>+++</sup>                         | 0 (2) <sup>++++</sup>  |         | 1 (11)   |
| Total            | 1 (62)                                  | 2 (20)                             | 45 (78)                           | 3 (35)  | 9 (92)                                       | 0 (35)   | 3 (22)  | 63 (344) |

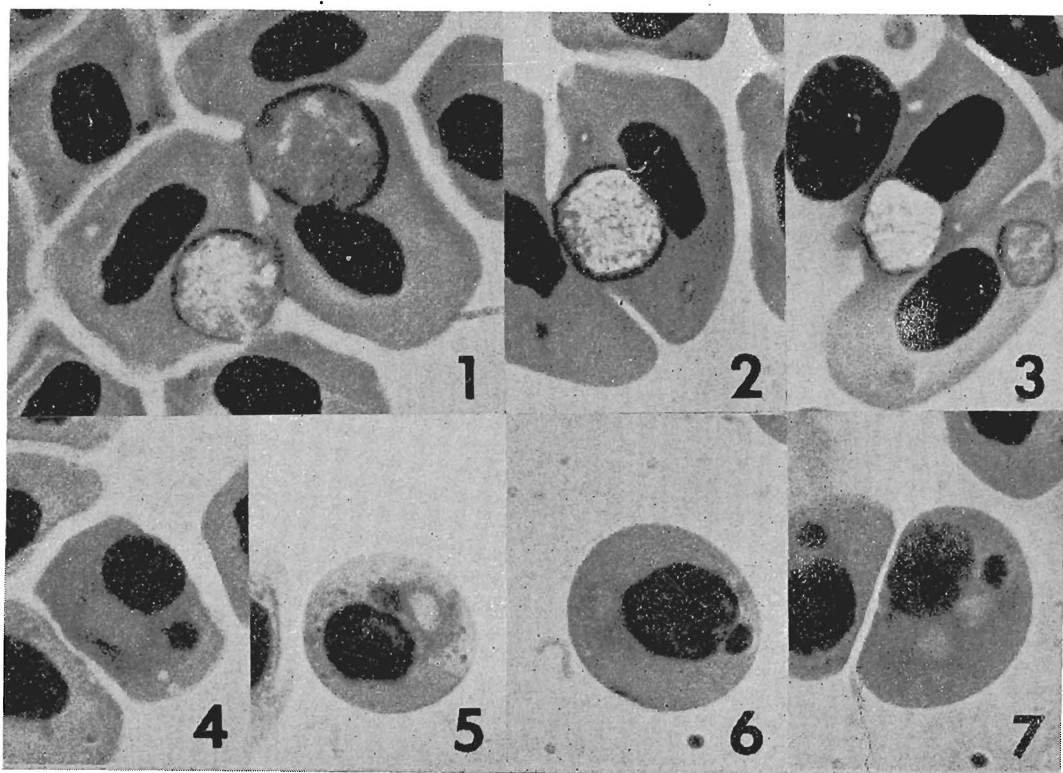
\**Rana hosei*. \*\**Leptobranchium hasselti*. +Kuala Pilah, Negeri Sembilan. ++Fraser's Hill, Pahang. +++Gombak and ++++Bukit Lanjan, Selangor.

the highest rate of infection (58%) with six other species showing lower infection rates: *R. hosei* 15%, *Leptobrachium hasselti* 11%, *R. cancrivora* 10%, *R. macrodon* 10%, *R. limnocharis* 9%, and *Bufo melanostictus* 2%. All 35 *Rhacophorus leucomystax* examined for *Cytamoeba* were negative.

The *Cytamoeba* observed in these studies generally exhibited one of two morphological forms. One form was a uniformly pink-staining, dense, circular mass occasionally containing one or two irregular clear areas (Fig. 1); the second form was a much less dense pink-staining circular mass, containing pink or red rods of various lengths (Figs. 2, 3). The largest parasite seen of either form measured 20  $\mu\text{m}$  in diameter. Some specimens of both parasites appeared to have an

incomplete membrane (Fig. 3), while some parasites of both forms exhibited a heavily staining dark violet membrane (Fig. 2). Less frequently, small, oval-shaped parasites were seen and rarely were there amoeboid forms. In red blood cells containing large parasites, there was a displacement of the host cell nucleus.

Mathis and Leger (1912. Cited by Wenyon, 1926. *Protozoology*. Vol. 2, pp. 779-1563, Baillière, Tindall and Cox, London) recorded *Toddia* in *Bufo melanostictus* in Tonkin. In Malaysia *Toddia* was found only in the blood of two *Leptobrachium hasselti* (< 1% of all anurans examined) from Ulu Jempol, Negeri Sembilan. The infections consisted of the parasite, a violet-staining mass up to 3  $\mu\text{m}$  in diameter with no apparent nucleus, and a



Figs. 1-7—(X 1500). 1-3: *Cytamoeba* sp. from *Rana erythraea*; 4-7: *Toddia* sp. from *Leptobrachium hasselti*. See text for details.

blue crystal (Fig. 4) which appeared to increase in size as the parasite increased in size. Some parasitized erythrocytes showed degenerative changes and displacement of the nucleus. The parasite and crystal generally occurred on the same side of the host cell nucleus (Figs. 4, 5), but this configuration was not constant (Fig. 6). Rarely a cell containing a single parasite with two crystals was observed (Fig. 7).

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## ENTONYSSUS ASIATICUS FAIN, 1960 (ACARI : ENTONYSSIDAE), A LUNG PARASITE OF MALAYSIAN SNAKES WITH NOTES ON THE IMMATURE STAGES†

Lung mites were first discovered in Malaysian snakes in 1962 when one of us (M.N.) recovered 20 mites from the respiratory tract of a colubrid snake, *Natrix chrysarga* (Boie), collected from the Ulu Gombak Forest Reserve, State of Selangor, West Malaysia. The mites were identified as *Entonyssus asiaticus* Fain, 1960; this identification was later confirmed by Fain.

In 1975, 74 colubrid snakes, representing 6 species of the genus *Natrix* Laurenti and preserved in 70% ethanol in the collection of the Division of Medical Ecology, Institute for Medical Research, were examined to learn more about the prevalence and distribution of *E. asiaticus* in Malaysia. The snakes had been collected from 1958 to 1975 from Sabah, East Malaysia (North Borneo) and five states in West Malaysia. Mites from these snakes were cleared briefly in lactophenol, mounted in Hoyer's medium, and studied under phase contrast, as had been the mites recovered in 1962.

Three of 13 (23.1%) specimens of *N. chrysarga* had lungs and tracheae infested with *E. asiaticus*, whereas the other 5 species, comprising 61 specimens, were negative for lung mites. The infested snakes were from two areas of Selangor: Bukit Lagong Forest Reserve, Kepong and Bukit Mandol, Klang. Prevalence of mites recovered from these snakes and from the snake examined in 1962 is given in Table 1.

Comparison of female mites with descriptions by Fain (1960. *Rev. Zool. Bot. Afr.*, 62: 269; 1961. *Bull. Inst. Roy. Sci. Nat. Belg.*,

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37: 1) and with the specimens previously collected by M.N., confirmed that these acarines were *E. asiaticus*, otherwise known only from Java and Sumatra in the snakes, *Natrix subminiata* (Schlegel) and *N. chrysarga* (Fain, 1961. *loc. cit.*). This is the first published record of this mite in Malaysia.

Of 105 mites recovered from the four specimens of *N. chrysarga*, 17 were males and 85 females, providing a sex ratio of 1:5 ( $p < 0.001$ , chi squared), which suggests that *E. asiaticus* may be parthenogenetic. Two fully developed larvae were expelled from mounted females by coverslip compression, and a nymph was recovered from the lung parenchyma. This is the first record of immature instars of *E. asiaticus*. The apparent absence of a membrane surrounding the larvae indicated that this species may be larviparous. Although the precise instar of the nymphal specimen could not be determined, the length of legs I and IV (350 and 365  $\mu$ ) confirmed it to be a nymph. That this mite was firmly attached to the lung suggested that the nymphal stage may feed. The poor condition of the immature specimens, probably the result of prolonged storage in alcohol, precluded their description and illustration. Finally, the preponderance of adult mites in the snakes is difficult to interpret, but it is of interest that a similar observation was made for the halacarid mite, *Pneumonyssus simicola* Banks, which infested the lungs of a captive macaque (Furman *et al.*, 1974. *Lab. Anim. Sci.*, 24: 622).

Other members of this genus of mites from Asia include: *Entonyssus philippinensis* Fain, 1961 from *Fordonia leucobalia* (Schlegel) (Philippines) and *Natrix piscator* (Schneider) (India), and *Entonyssus javanicus* Fain, 1961

Table 1

Prevalence of *Entonyssus asiaticus* in the lungs and trachea of colubrid snakes from West Malaysia and Sabah.

| Host                                 | Locality       | No. snakes positive/examined | No. mites recovered from individual hosts |
|--------------------------------------|----------------|------------------------------|---|
| <i>Natrix chrysarga</i> (Boie)       | Negri Sembilan | 0/1                          | 8, 20, 21, 56                             |
|                                      | Pahang         | 0/2                          |   |
|                                      | Perak          | 0/1                          |   |
|                                      | Selangor       | 4/10                         |   |
|                                      |                | 4/14 (28.6%)                 |   |
| <i>Natrix maculata</i> (Edeling)     | Selangor       | 0/20                         |   |
| <i>Natrix petersi</i> (Boulenger)    | Selangor       | 0/20                         |   |
| <i>Natrix piscator</i> (Schneider)   | Kedah          | 0/1                          |   |
| <i>Natrix sarawacensis</i> (Gunther) | Sabah          | 0/1                          |   |
| <i>Natrix trianguligera</i> (Boie)   | Negri Sembilan | 0/4                          |   |
|                                      | Selangor       | 0/15                         |   |
|                                      |                | 0/19                         |   |

from *Natrix vittata* Linnaeus (Java) (Fain, 1961. *loc. cit.*). Although *N. piscator* and *N. subminiata*, a host of *E. asiaticus* in Java, occur in Malaysia, Malaysian populations of these snakes have not been examined for lung mites, except for one specimen (*N. piscator*), which was negative (Table 1).

Acarine associations with other species of Malaysian land snakes are poorly known, but the respiratory tract of the amphibious sea snake, *Laticauda colubrina* (Schneider) was found by one of us (M.N.) to be infested by the trombiculid mite, *Vatacarus ipoides* Southcott; the host relationship and unusual biology of this mite were reported by Nadchatram and Audy (1965. *Med. J. Malaya*, 20: 80).

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