THE HÆMATOZOA OF AUSTRALIAN BATRACHIANS No. 1.

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IN carrying out a systematic census of the hæmatozoa of Australian vertebrates, it is our intention to record from time to time the various species met with. In the present paper we propose to deal with *trypanosomes* in Queensland frogs and a *haemogregarine* in *Hyla caerulea*, White, from the Sydney district.

The following is a list of the various species of frogs searched by us but in which haematozoa were not found. The dates, localities and number examined are also given:—

Species.	Date.	Locality.	No
Hyla lesueurii, D.&B.	April 1910	Kilroy, Queensland	(1)
Hyla aurea, Lesson	Nov. 1910	Sydney	(1)
	Nov. 1910	Richmond	(7)
	Mar. 1910	Sydney	(8)
Hyla nasuta, Gray	Mar. 1910	Harrisville, Queensl.	(2)
Hyla affinis ?	Mar. 1910	Harrisville, Queensl.	(3)
Crinia sp. ?	Mar. 1910	Harrisville, Queensl.	(2)
Limnodynastes dorsalis, Gray	Dec. 1909	Sydney	(1)
—— *ornatus*, Gray	July 1910	Queensland	(2)
—— *peronii*, Dum. and Bibr.	Mar. 1910	Sydney	(4)
Pseudophryne bibronii, Günth.	July 1910	Queensland	(1)
Unidentified frogs	Mar. 1910	Harrisville, Queensl.	(2)

List of species in which haematozoa have been found by us:

Hyla caerulea, White—October 1909, Sydney, haemogregarines in two out of two specimens examined; Dec. 1909, Sydney, one examined (nil); January 1910, Sydney in one out of two examined.

Limnodynastes tasmaniensis, Günther—December 1893, Myrtletown, Queensland; trypanosomes present.

Limnodynastes ornatus, Gray? March 1910, Harrisville; trypanosomes present.

With the exception of the trypanosome and the haemogregarine to be described further on, we are not aware of any other haematozoa having been recorded from Australian batrachians. We are indebted to the kindness of Dr. T. L. Bancroft for all the films from Queensland frogs.

Trypanosomes of Frogs (Figs. 2 – 10).

We have received from Dr. T. L. Bancroft, a number of specimens of blood from Queensland frogs, and amongst them were two instances in which trypanosomes were present. One, a frog from Harrisville, in which well preserved trypanosomes were found with ease, was probably *Limnodynastes ornatus*, Gray; in the other, a slide dated "Myrtletown, December 3rd, 1893," in which the species of frog had been indentified as *Limnodynastes tasmaniensis*, Günther, only one or two specimens of trypanosomes were seen and the stain had faded, whilst re-staining was not very successful.

Description of the trypanosome from the Harrisville frog:—All the specimens examined were very similar in appearance, differing from each other, mainly in breadth. The posterior ends were gradually attenuated. The prominent kinetonucleus were far removed from this end, and close in front of each was the reddish nucleus. The protoplasm of the body stained a deep blue; occasion-

ally assuming a streaked appearance but no definite ribbing could be discerned. Sometimes small vacuolar-like spaces were noticed in front of the nucleus. The undulating membrane was very distinct and deeply folded; owing to the situation of the kinetonucleus it naturally did not occupy the posterior part of the parasite. The flagellum was strikingly visible, running along the edge of the membrane and usually crossed the body in its anterior portion; it ended in a well-marked free flagellum.

In view of the marked pleomorphism of *Trypanosoma rotatorium* (Mayer), as described by Laveran and Mesnil,[1] we hesitate to separate this trypanosome from that species. Nevertheless the wide geographical separation of the frogs of Australia from those of Europe, coupled with the probably enormous lapse of years since these species of trypanosomes or their hosts, now respectively occupying these localities, were associated, gives reason to suppose that future research may reveal specific differences between the parasites. This is supported by the fact that our specimens seem decidedly shorter than *T. rotatorium*, Mayer, *T. mega*, Dutton and Tod, and *T. karyozeukton*, Dutton and Todd, and to have the nucleus situated distinctly nearer the posterior end.[2] In these respects it seems to closely resemble *T. nelspruitense*, Laveran, from a Transvaal frog. It is readily distinguishable from *T. inopinatum*, Sergent.

The trypanosome of *Limnodynastes tasmaniensis* resembles in appearance the remarkable *T. mega* of Dutton and Todd, as figured by Laveran and Mesnil; as these authors consider that this species is merely a form of *T. rotatorium* and as our specimen stained so poorly as to make detailed examination impossible, it must also be provisionally placed

[1] Laveran and Mesnil, "Trypanosomes and Trypanosomiases," Engl. transl. by Nabarro, 1907, p. 465 etc.

[2] Lühe in Mense's "Handbuch der Tropenkrankheiten," III, 1906, p. 89 - 91; Laveran and Mesnil, *l.c.*, p. 465 – 476.

under the name of *T. rotatorium*. Its posterior end was very long and finely pointed; the nucleus appeared as a prominent faintly-stained area, and a little behind it the undulating membrane could be seen to take origin, indicating the proximity of the centrosome to the nucleus. The protoplasm stained a fairly deep blue and seemed somewhat granular; the undulating membrane, which was deeply folded, was only just discernible.

Measurements of the Trypanosomes in micromillimetres.

(Nos. 1, 2, broad form, from Harrisville frog; Nos. 3–8, narrow forms, from same frog; No. 9 trypanosome of *Limnodynastes tasmaniensis*.) Harrisville frog's red blood corpuscles, 17·8 μ × 8·9 μ; nucleus, 5·34 μ × 3·56 μ.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Posterior end to kinetonucleus	8·0	8·9	5·3	8·9	4·0	5·3	4·0	8·9	9·0?
Kineto. to edge of nucleus	1·8	2·2	3·5	1·8	2·6	3·5	2·2	1·8	3·0?
Nucleus length	3·5	3·5	2·2	1·8	1·8	1·8	1·3	1·8	2·5
Nucleus to anterior end	19·6	23·1	21·4	19·6	14·2	16·0	16·0	19·6	24·0
Free flagellum	26·7	19·6	7·0	19·6	9·3	9·0	15·0	17·8	4·5?
Total length	59·6	57·3	39·4	51·7	31·9	35·6	38·5	49·0	43·0
Total length without flagellum	32·9	37·7	32·0	32·1	22·6	26·0	23·5	31·2	38·5
Greatest width including undulating membrane	8·9	8·5	4·8	4·4	4·0	3·4	4·0	5·2	7·0
Breadth of undulating membrane	3·5	3·5	1·8	1·8	2·6	1·8	2·2	2·6	3·4

Dr. Bancroft recently informed us that he had found trypanosomes in the following frogs in Queensland:—*Hyla nasuta*, Gray, and *Hyla lesueurii*, Dum. and Bibr.

HÆMOGREGARINA (LANKESTERELLA) HYLÆ, n.sp.

(Figs. 11 - 22.)

The red blood corpuscles of the common "Green Tree-frog" of Australia, *Hyla caerulea*, White, appear to be very frequently parasitised by a haemoprotozoon belonging to the Haemogregarinidæ. This sporozoon has been detected in three out of five specimens of this species examined by us. Although we have sought for haematozoa in the allied "Golden Frog" *Hyla aurea*, Lesson (sixteen specimens) we have not yet succeeded in finding any. The infected frogs came from the Sydney district, but the species ranges over the whole of the warmer portions of Australia.

The parasites showed the typical features of a batrachian *Haemogregarina* (*Lankesterella*). They did not vary much in size, the length being about 0·009 to 0·011 mm., with a maximum breadth of 0·003 mm., though on one occasion a form having a breadth of 0·005 mm. was noticed. The parasites were generally much thinner varying from 0·0016 to 0·0025 mm. Many of the narrower forms were immature. The usual shape of the parasite in the red cell was that of an open crescent, its concavity most often facing the host-nucleus, but occasionally turned from it. In parasites which had escaped from their hosts, the two sides were however, almost alike. In the centre of the concavity there was often present a more or less extensive bulging. In no case did the parasite possess a tail.

The protoplasm of the parasite proper stained a pale bluish colour. It was usually studded with fine reddish granules. The nuclear band was often nearer one end than the other, and sometimes in these cases the part of the parasite beyond the nucleus was non-granular, while the rest of the parasite was distinctly granular. In a number of cases the characteristic reticulate nucleus was not dis-

cernible as such, but instead, opposite the centre of the concavity of the parasite, was grouped a number of purplish granules which at times extended beyond this area as scattered grains. A vacuole was often associated with the mass. In the heavily parasitised frog, unusual appearances were noticed which suggested either that these granules were not of the same nature as the nucleus proper, (our specimens were all Giemsa stained), or else that portion of the nuclear chromatin separated itself from the nucleus proper. The appearances were as follows :— The group of granules referred to seemed to emigrate into the bulge lying first of all close to the parasite proper, sometimes in a non-staining vacuolar-like space, and free in the bulge. In many of these cases, the true band-like nucleus itself could be distinctly recognised quite apart from the mass, and situated more towards one end of the parasite, the bulge being then a little to the other end. Further, in the same specimen, a number of rounded, comparatively large, purplish chromatic-like bodies were seen (one in each cell—once two in a cell) free in the protoplasm of the red cell, occasionally close to the bulge, but more often far removed from this and even to be seen opposite the convex border of the parasite. It was noted that in these cases, the bulge was slightly developed or absent. The protoplasm of the parasite proper seemed often sharply differentiated from this bulged area, and in some cases the outline of the body proper could be traced trasversing the bulge, distinctly following the regular curve of this side. There were, further, sometimes differences in staining reactions by Giemsa's method: the parasite itself staining a definite bluish tint, with or without granules, and the bulge being a paler blue or colourless and vacuolar like, or with a colourless centre, but a deeper blue periphery. In some free forms of the parasite which were seen, it is note-

worthy that the bulge was not noticeable. In the heavily infected frog, some mitosing red cells were met with.

It is difficult to know what the bulge and chromatic-like mass represent. It must be borne in mind that the specimens obtained were dry blood-films stained by Giemsa's method and that, at the time, we were unable to adopt the more exact methods for fixation or staining advocated by Minchin. The question of distortion from drying and the accentuation of size of chromatic structures by the stain must be taken into account. Allowing for these imperfections, however, it is obvious that the bulge exists, and that masses are often present in the parasite which resemble chromatin by Giemsa staining, and yet are sometimes distinct from the nucleus. We have seen these chromatin-like masses collecting opposite the bulge, passing into it, and finally grouping in it, and we have further seen free bodies suggesting that these masses finally escape from the bulge and wander into the host's protoplasm. Is the chromatin-like mass excreted through the bulged area? Is it really chromatic in nature and a device for the reduction in the amount of chromatin of the nucleus proper? These are points which we hope in the future to be able to resolve. It should be noted that these remarkable appearances associated with the bulging were noted in only one of the infected frogs, and in this specimen the infection of red cells by the parasites was very heavy.

The animal occupied various positions in the host cell, the more common situation being diagonal. Occasionally it lay across the host cell between the nucleus, which might or might not be displaced. Sometimes its position was between the nucleus and the side of the cell. In one instance it was located longitudinally between the nucleus and one end of the erythrocyte. Not infrequently the host nucleus was more or less displaced, but in no case was it

ejected from the red cell. The red cell itself was neither enlarged nor distorted as often happens in the case of reptilian erythrocytes when infested by haemogregarines.

Double infection was common in one of the films examined and even triple infection was seen. In all observed cases of double infection the two parasites were lying side by side either parallel to each other or forming a narrow open V. No schizogonic stages were met with. The films were taken from the heart blood and were stained by Giemsa. The type slide of this parasite for which the name *Haemogregarina (Lankesterella) hylae* is proposed, has been presented to the Trustees of the Australian Museum, Sydney, co-types being retained in the Bureau of Microbiology, Sydney.

The finding of the haemogregarine so commonly in *Hyla caerulea* but not in *H. aurea* calls for remark. The two frogs are commonly found together in swamps, but, while *H. aurea* is essentially a swamp inhabiting species, *H. caerulea* is very frequently arboreal in its habits. The occurrence of the haemogregarine in one of these often associated species and not in the other, suggests either that the infection by the protozoon is specific in character for the one frog or that its alternative host has access to *H. caerulea* and not to *H. aurea*, which latter view seems to us unlikely. The haemogregarines of certain frogs, *Rana trigrina* and *R. hexydactyla*, are stated by Patton[1] to be transmitted by a leech. The same probably holds good for the above described parasite.

In addition to haematozoa, we have examined frogs for intestinal and other protozoa. Though not in any sense blood parasites, yet we consider it worth while to record the results here :—

[1] W. S. Patton, Parasitology, 1, p. 319, 1908.

Myxobolus sp.

This sporozoon is met with at times in *Hyla aurea* in the Sydney district. It occurs in enormous numbers in the genital organs, the whole organ becoming converted into a swollen tube filled with a whitish fluid containing myriads of these parasites. Attention has already been called to the presence of these *Myxosporidia* in this host.[2] They have not been found in any of the other species examined.

Opalina sp.

This degenerate ciliate infusorian is comparatively common in the intestine of *Hyla aurea*, *H. caerulea*, *Limnodynastes peronii*, and *L. dorsalis*, in Sydney district. It has not been looked for in other frogs.

Nyctotherus sp.

This actively swimming ciliate infusorian is very common in the above mentioned four species locally. There is also a very large form present in *Hyla aurea* measuring 0·47 mm. by 0·324 mm. Whether this is a distinct species from the smaller and commoner form we cannot yet say.

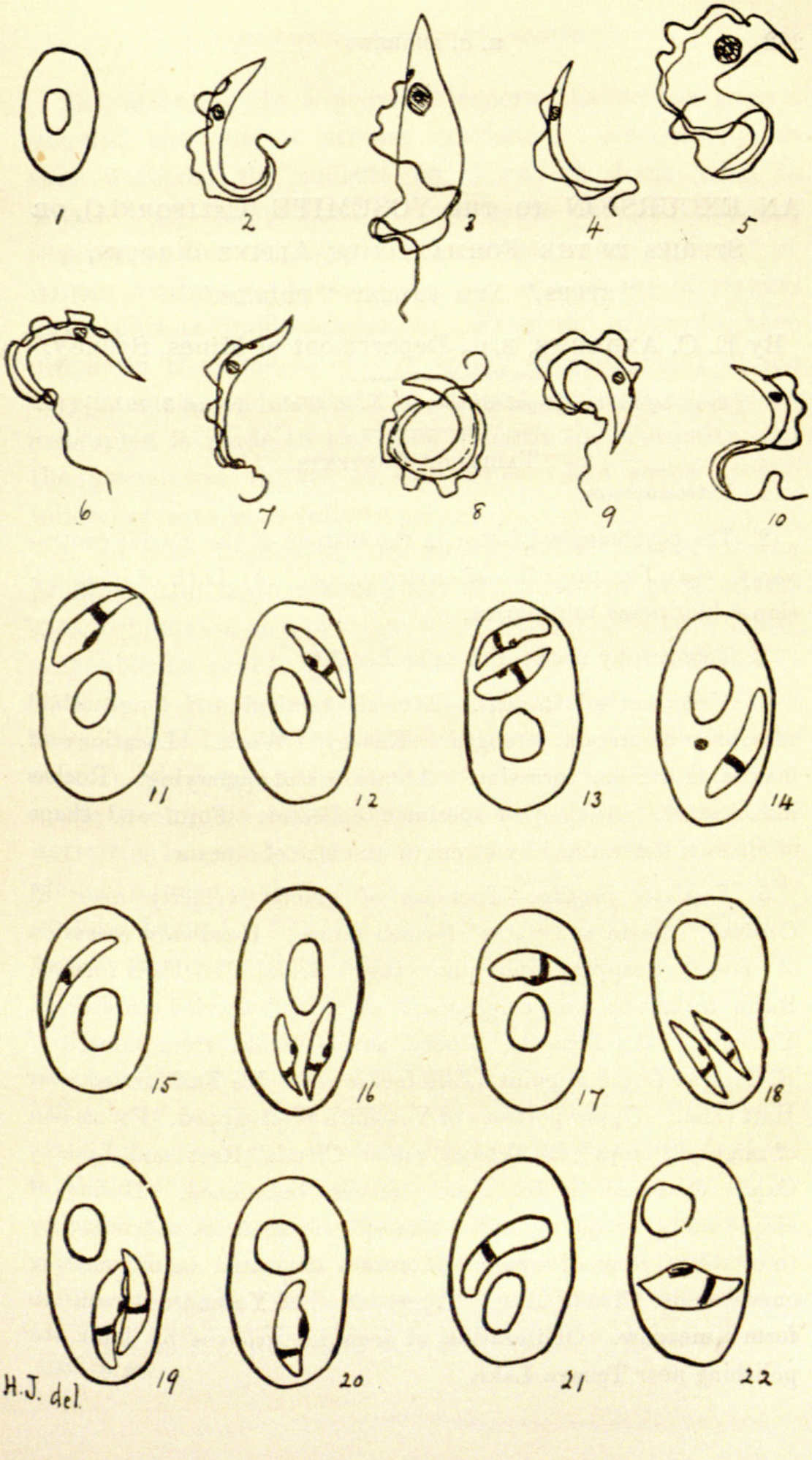
Explanation of Diagram.

Fig. 1. Normal corpuscle of Harrisville Frog, for comparison with the Trypanosome.

Figs. 2 – 10. *Trypanosoma rotatorium?* showing pleomorphism.

Figs. 11 – 22. Erythrocytes of *Hyla caerulea* parasitised by *Haemogregarina hylae*.

[2] A. W. Fletcher, *Proc. Austr. Assoc. Adv. Science*, I, 1887, 337. W. A. Haswell, *Proc. Linn. Soc. N. S. Wales*, v, 1890, p. 661. T. H. Johnston, This Journal, Vol. XLIII, 1909, p. xxviii.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