

Toddia Detected from *Rana (Babina) holsti* Boulenger, 1892, in Okinawa Island

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Abstract: A new type of *Toddia* was detected in the erythrocyte of *Rana holsti* Boulenger, 1892, which was captured in a northern part of Okinawa Island. *Toddia*-body is pale red in colour and round in shape. The diameter of the body is 1-3 microns. The body has no visible cytoplasm or no other structure under microscopic examination. In the infected cell with *Toddia*-body, small triangular crystal was seen at the edge of the host cell nucleus. The crystal is pale blue, and the length of a side of the triangle is 2-5 microns. In this infected case, *Trypanosoma* sp. was also found on the same blood smear. The present report appeared to be the first record of *Toddia* from Asia.

According to Wenyon (1926), the genus *Toddia* was originally described as a number of the Protozoa, but he treated it as one of the parasites of doubtful nature. He introduced *Toddia bufonis* França, 1911, which was detected in the red blood cells of *Bufo regularis* in Portuguese Guinea. Before his description was published, a similar parasite had already been reported from Amphibia in West Africa by Dutton, Todd, and Tobey (1907). According to Marquardt and Yaeger (1967), Scorza and Boyer (1956) described *Toddia prima* from *Rana galamensis*, *Toddia francai* from *Bufo melanosticus* and *Toddia carbonelli* from *Bufo marinus*.

Toddia is seen in the erythrocyte of the cold-blooded animals as a red staining spherical structure (*Toddia*-body in the present paper) after Giemsa staining. Rods of a crystalline nature associate with *Toddia*-body. Recently Marquardt and Yaeger (1967) pointed out that *Toddia* might belong not to Protozoa but to DNA virus. This opinion was supported by

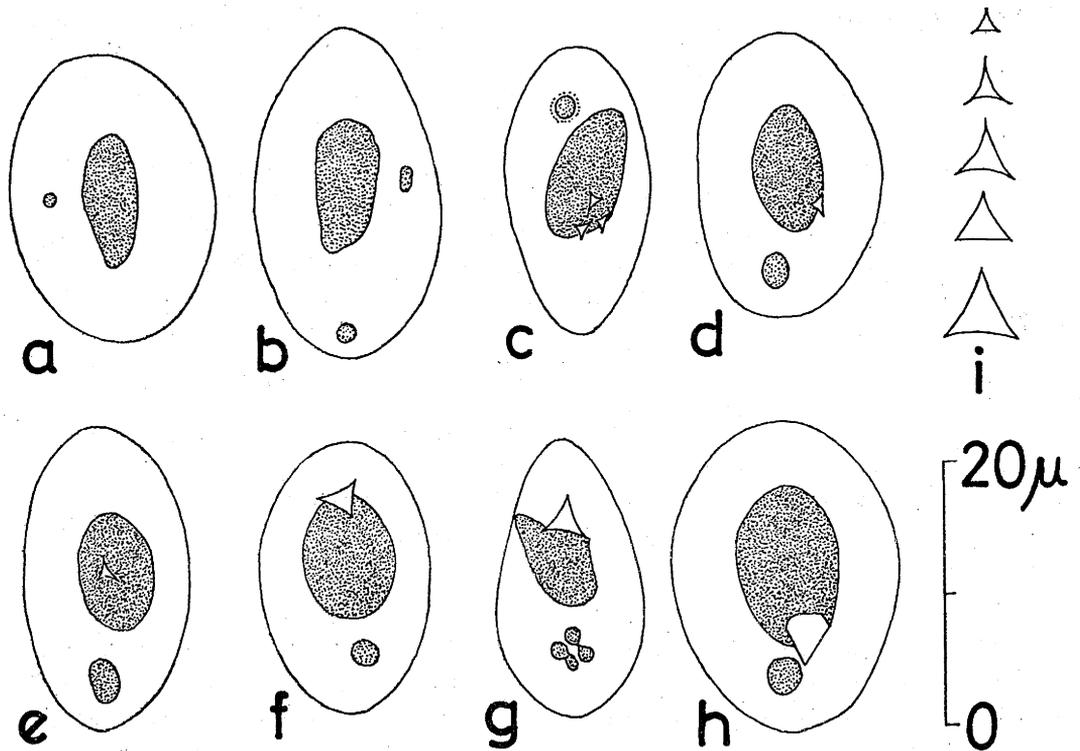


Fig. 1. *Toddia*-body and accompanied triangular crystal in the erythrocyte of *Rana holsti*.
 a-b. *Toddia*-body without crystal.
 c. three crystals associated with a *Toddia*-body.
 d-h. *Toddia*-bodies and crystals.
 i. exoerythrocytic crystals.

Johnston (1975) too.

Toddia has not been reported from Asia, but in July, 1976, the present authors observed a new type of *Toddia* in the red blood cell of *Rana holsti* in Okinawa Island. Therefore, this is described below in more detail.

TODDIA DETECTED FROM RANA HOLSTI

The morphological features of *Toddia* were examined after staining the blood smears with Giemsa. *Toddia* was detected from one of *Rana holsti* (frog.no. 76-7-1-13). The distribution of *Rana holsti* is limited only in Okinawa Island. The authors examined a number of frogs collected in the survey area, but no *Toddia* was detected from other frogs. Since *Toddia* was seen in almost all of erythrocytes in this particular case, it was impossible to compare the size of the infected erythrocyte and the normal erythrocyte, but no hypertrophy of the infected cells was recognizable. *Toddia*-body is pale red in colour and round in shape.

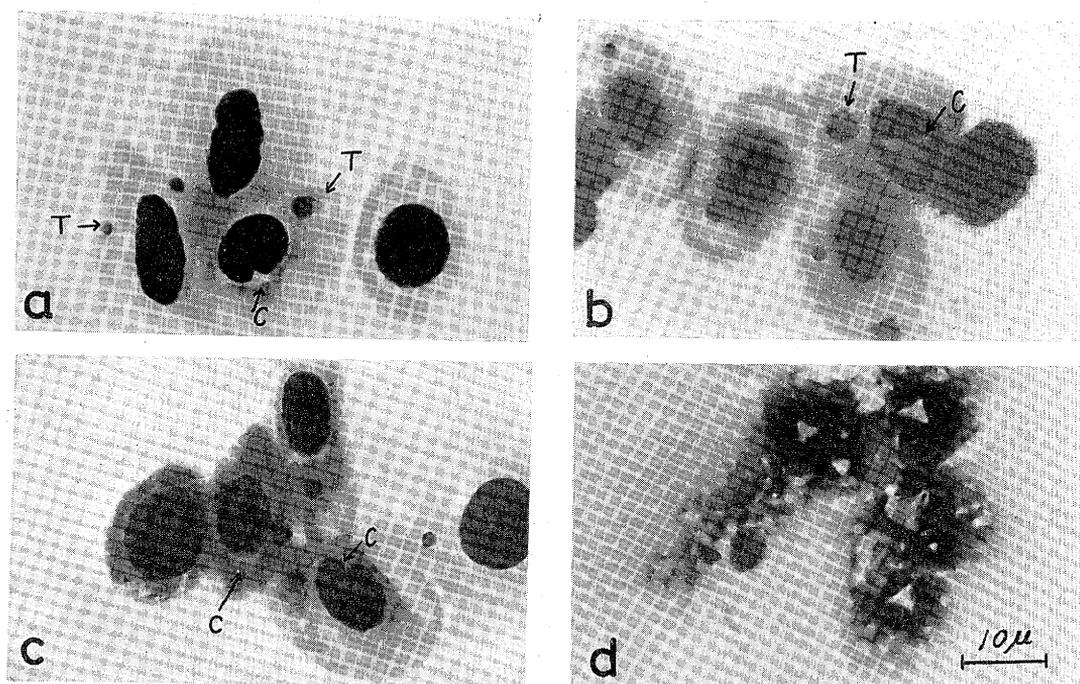


Fig. 2. Photographs of *Toddia*-body and accompanied triangular crystals in the erythrocyte of *Rana holsti*.
 a-c. *Toddia*-bodies (T) and crystals (C).
 d. exoerythrocytic crystals.

The body seems to be either homogeneous or granular. The diameter of the body is 1-3 microns. *Toddia*-body has no visible cytoplasm nor any recognizable structure as shown in Figs. 1-2. In the infected cells, small triangular crystal associated with *Toddia*-body is seen at the edge of the host cell nucleus. The crystal is pale blue and usually there is some distance between *Toddia*-body and the crystal. The length of a side of the crystal is about 2-5 microns, but when *Toddia*-body is still too small as shown in Fig. 1, a and b, no crystal has been detected. Larger *Toddia*-body usually has a single crystal, but in some cases, 2-3 crystals accompanied with one *Toddia*-body (Fig. 1, c). The crystal usually locates in the cytoplasm of the host cell and one angle of the crystal attaches into the host cell nucleus like a wedge. *Toddia*-body has never been observed outside of the erythrocyte, but the crystal is often seen in the serum. Those exoerythrocytic crystals are comparatively larger than the intraerythrocytic crystals (Fig. 1, i, and Fig. 2, d).

This *Toddia* seems apparently to be a new type morphologically, but the authors do not believe that this parasite belongs to Protozoa, because no cytoplasm nor any other structure was observed under light microscopic examination. This is the reason why the authors did not give any new specific name.

Host: *Rana (Babina) holsti* Boulenger, 1892, (Amphibia: Ranidae). The frog was captured on July 1, 1976, by the authors.

Locality: Yona, Kunigami-son, northern part of Okinawa Island, Japan.

Blood smear: Smears taken from the frog (frog no. 76-7-1-13) are in the collection of the one of the authors (Miyata) at the Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University. Two other smears will be deposited in the Collection of the Wellcome Museum of Medical Science, London.

In addition to *Toddia*, a kind of *Trypanosoma* was also detected from those blood smears, and this will be described elsewhere.

DISCUSSION

Toddia was originally described from the blood of a toad, *Bufo regularis*, in Africa (França, 1911). Scorza and Boyer (1956) described three new species from Anura in Venezuela. More recently, Arcay de Peraza and McLure (1971) found *Toddia* from the electric eel, *Electrophorus electricus*. From various reptiles, *Toddia* or *Toddia*-like bodies were reported by Bremer (1895), Laveran (1903), Marquardt and Yaeger (1967), and Arcay de Peraza *et al.* (1971): *Terrpene carolina* (= *Testudo carolina*), *Chelydra serpentina*, *Emys lutaria*, *Damonia revesii*, *Agkistrodon piscivorus leucostoma*, and *Iguana iguana*. Those reptiles belong to Celonia (first four species), Ophidia (fifth species) or Sauria (last species), and all the *Toddia* detected from reptiles were recorded from North and South Americas. Until now, *Toddia* was not reported from Asia, and the present paper might be the first record on *Toddia* from Asia. The biological nature of *Toddia* is not determined until now. The general appearance of *Toddia* is somewhat similar to that of the genus *Pirhaemocyton* and *Immanoplasma*. From Japan, *Pirhaemocyton* has been reported in the blood of snakes collected in Nagasaki by Miyata (1974). According to the ultrastructure reported by Stehbens and Johnston (1966), the *Pirhaemocyton*-body had the structure of cytoplasmic assembly pool or factory for the production of icosahedral viruses and the albuminoid body was a vacuole. The genus *Immanoplasma*, which was found from fishes, is related parasite with *Pirhaemocyton* and *Toddia*. Johnston and Davies (1973) pointed out that *Immanoplasma* resembled *Pirhaemocyton* in its ultrastructure. Stoltz (1971) included *Toddia* and *Pirhaemocyton* in one of suspected icosahedral cytoplasmic deoxyriboviruses. Marquardt and Yaeger (1967) pointed out that *Toddia* might be a DNA virus as shown in *Pirhaemocyton*. Johnston (1975) also repeated his opinion in a useful review of *Pirhaemocyton* and other, possibly related infections of poikilotherms, including *Toddia* as one of the related genera.

The present authors prefer to follow their opinion, and believe that *Toddia* is actually not belong to the phylum Protozoa, because of the absence of nucleus or cytoplasm and other structures. For these reasons, the authors did not give any specific name for this *Toddia* detected from *Rana holsti* in Okinawa. Much more studies on *Toddia*-body and associated crystal will be necessary.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to Dr. Masuhisa Tsukamoto, the Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University, for his advices and encouragements in this study.

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沖縄本島産ホルストガエルから発見された *Toddia* について

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Toddia は、古くは、原生動物と考えられていたが、最近の研究者の多くは、DNAウイルスが盛んに増殖している部分ではないかと述べている。従来アジアからは、*Toddia* は、全く報告されていなかった。ところが、たまたま昨年6月から7月にかけて、沖縄本島北部 国頭村与那で、捕獲したホルストガエルのうち1匹の末梢血の赤血球内から、今まで報告されたことのない新しい型の *Toddia* を発見したので報告した。今回発見された *Toddia* は、宿主赤血球の細胞質内のみ寄生し、ギムザ染色では、直径1~3ミクロンの赤染する球形の小体である。この小体に付属して、1辺2~5ミクロンの淡青色に染まる三角形の結晶が、宿主細胞の核に接して見出される。この結晶は、核の上あるいは側面にくさびのようにくこんでおり、そのため宿主細胞の核は、三角形の凹みが生じている。結晶は、しばしば血清中にもみられ、一般に血清中の結晶は、赤血球の中の結晶より大きい。これらの小体および結晶以外の構造物は、光学顕微鏡ではみとめられない。*Toddia* と類似する *Pirhaemocyton* や *Immanoplasma* は、いずれも冷血動物の赤血球内に寄生するもので、かつて原生動物と考えられていた。しかし電顕により、あとの2つは、ウイルスらしいことが知られてきた。*Toddia* については、まだ微細構造の報告はないが、*Toddia* は、*Pirhaemocyton* と似ており、おそらく微細構造も類似しているものと考えられる。今までアフリカと新大陸からだけ知られていた *Toddia* が、沖縄から発見されたことは、この謎の寄生体の地理的分布を考える上で誠に興味深い。*Toddia* およびそれに類似する寄生体については、宮田著「寄生原生動物—その分類・生態・進化」(B5判1600頁、文部省研究成果刊行費申請中)の中で、詳しく解説しておいた。

熱帯医学 第19巻 第2号 123-128頁, 1977年6月