

Re-description of *Trypanosoma corvi* Stephens and Christophers, 1908 Emend. Baker, 1976 and Remarks on the Trypanosomes of the Avian Family Corvidae

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The trypomastigote, epimastigote and amastigote stages of Trypanosoma corvi Stephens and Christophers, 1908 emend. Baker, 1976 from the peripheral blood, heart and bone marrow are described herein. Other trypanosomes described from the Corvidae are compared to T. corvi and their status is discussed.

Keywords: *Trypanosoma corvi* - redescription - trypanomastigote - amastigote - epimastigote

In 1885, Danilewsky described *Trypanosoma avium* from the European owl *Athene noctua*. Since that time, some 98 species and subspecies of avian trypanosomes have been described (Bishop & Bennett 1992). Most of these species are based on the presumption that avian trypanosomes show marked host specificity, a presumption shown to be false, at least in part, through the experimental studies of Bennett (1961, 1970a, b), Baker (1956a, b, c), and Woo and Bartlett (1982), who have all transmitted trypanosomes from one host to other avian hosts of different families. Recent studies on trypanosomes (Woo & Bartlett 1982, Kirkpatrick & Terway-Thompson 1986, Kirkpatrick et al. 1986) have stressed the value of using culture techniques and, in some cases, the identification of trypanosomes is approaching an analogous technology to that used by bacteriologists with differential growth on various culture media. However, these techniques have not as yet been universally applied and morphological description of the parasites in natural preparations is still of vital importance.

In 1908, Stephens and Christophers described *T. corvi* as follows: "*T. corvi* (S and C), in *Corvus splendens*, 40-56 by 3-5 μm ". This description, unaccompanied by illustrations, was a *nomen nudum* according to Article 12 of the International Code of Zoological Nomenclature. Therefore, the name is not an available name but may be made available later for the same or different

concept. However, in 1976, Baker recognized that this was a *nomen nudum* and re-described the species from material he obtained from *C. frugilegus* in the United Kingdom. He designated the type host as *C. splendens* from India (the same host as specified by Stephens & Christophers) without having seen the material from this species, with additional material from the United Kingdom specimens of *C. frugilegus*. His rationale for this action was that this term would be used to apply to the large "*Megatrypanum*-like" trypanosomes found in corvids. One can question the wisdom and validity of this move as the only indication that Stephens and Christophers were looking at a "*Megatrypanum*" lies in the length measurements that they provided. However, this action has been taken and the species name is currently in the literature and it seems rather pointless at this time to change this designation.

In 1982, Woo and Bartlett described *T. ontarioensis* from the North American corvid, *C. brachyrhynchus*. This trypanosome was about half the size of *T. corvi*, resembling the small avian trypanosomes described by a number of authors from South American birds, and lacked the typical striated appearance of the "*avium*" group of trypanosomes. The species also differed from *T. corvi* in a number of cultural characters and was a clearly distinct species. Woo and Bartlett (1982) also found what they presumed to be *T. paddae* in the same corvid host, and this parasite was again considerably larger than *T. ontarioensis*. Chatterjee and Ray (1971) described *T. avium bakeri* from the bulbul, *Pycnonotus jocosus* in India. Baker (1976) emended the name of this striated trypanosome to *T. brimonti bakeri* nov. comb. and removed it from consideration as a trypanosome of the corvids. Bennett (1961,

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1970b) studied the life cycle, morphology and transmission of a striated trypanosome originally isolated from a saw-whet owl (*Aegolius acadicus*) which he termed *T. avium*. He subsequently transmitted this trypanosome to a large number of species of North American birds of several families, including blue jays (*Cyanocitta cristata*) of the family Corvidae. He also described trypanosomes from naturally infected blue jays which had the same morphological appearance and developmental characteristics in invertebrate hosts as *T. avium*. Baker (1976) felt that this designation was in error, and in an extensive discussion, following Laveran's (1903) initial restriction of the term *T. avium* to strigids only, further restricted the term *T. avium* to those trypanosomes that were derived from Old World Strigiformes only, and restricted trypanosomes of similar morphology found in North American (New World) birds to the species *T. confusum* Luhe, 1906. Baker acknowledged that his own use of the name *T. avium* (1956a) for striated trypanosomes from corvids was in error and validated the Stephens and Christophers (1908) name *T. corvi* by presenting a valid description, although not one based on material from the type host which occurred several thousand kilometres away.

In 1976, 17 tree pies (*Dendrocitta vagabunda*, Corvidae) were examined for blood parasites over a period of one month; two of the birds were found to harbour trypanosomes in the blood. The birds were sacrificed and various stages of the parasite were found in the blood, liver and bone marrow. This trypanosome from the tree pie is presented herein as a re-description of *T. corvi* Stephens and Christophers, 1908 emend. Baker, 1976, as it comes from a corvid host from the same geographical area as the original region, in contrast to the description by Baker (1956a) based on material from corvids in the United Kingdom. The description also presents additional material obtained from bone marrow preparations and thus extends Baker's (1956a) description, which was based only on circulating blood forms as well as some forms seen in NNN culture.

MATERIALS AND METHODS

Seventeen tree pies, *D. vagabunda* (Latham), were captured around Barasat and Basirhat, 24-Parganas District of West Bengal, and maintained in the laboratory for about one month. Two of the birds had trypanosomes in the peripheral blood. The birds were sacrificed and preparations of peripheral blood, heart blood and bone marrow mixed with citrated saline were made. Some of the citrated preparations were examined under cover glass preparations to study the living trypanosomes, while blood smears and tissue imprints were stained with Leishman's, Wright's or

Giemsa's stain for more critical morphological study.

Cross-infection studies were also carried out by intra-peritoneal inoculations of citrated heart blood and bone marrow into two pigeons (*Columba livia intermedia*), two white-throated munias (*Lonchura malabarica*), two white-breasted water hens (*Amaurornis phoenicurus*), four house crows (*Corvus splendens*) and two tree pies (*D. vagabunda*). One bird of each species was retained as an untreated control and the inoculated birds were examined at irregular intervals for the presence of trypanosomes. The birds were sacrificed after 7-15 days and heart blood and bone marrow (tibia-tarsus) examined for the presence of the parasites.

Re-description of *Trypanosoma corvi* Stephens and Christophers, 1908 emend. Baker, 1976.

Type host: the house crow, *Corvus splendens* Vieillot.

Type locality: India.

Trypomastigote (Fig. 1; A-D; Table) - Trypomastigotes were rare in the peripheral blood (0-3 parasites per slide), slightly more numerous in the heart blood and numerous in citrated preparations of bone marrow and occurred as slender, intermediate and broad morphs which varied in width, length and the shape of the caudal end. The cytoplasm was increasingly coarser in the broad form and the number of cytoplasmic vacuoles increased. Longitudinal striations (myonemes) were most pronounced in the intermediate forms. An oval vacuole (2-3 μm in diameter) adjacent to the kinetoplast, and larger than the other cytoplasmic vacuoles, was a constant feature. The kinetoplast was round to oval, stained deep red and was 0.9-1.8 μm in diameter. The nucleus was granulated, staining pink with the granules arranged around the periphery on some occasions. A large vacuole, 3.0-6.0 μm was occasionally lodged within the nucleus. The anterior end gradually tapered to a point in all three morphs. The slender and intermediate forms were most frequently encountered and may be considered as most typical of this species of trypanosome.

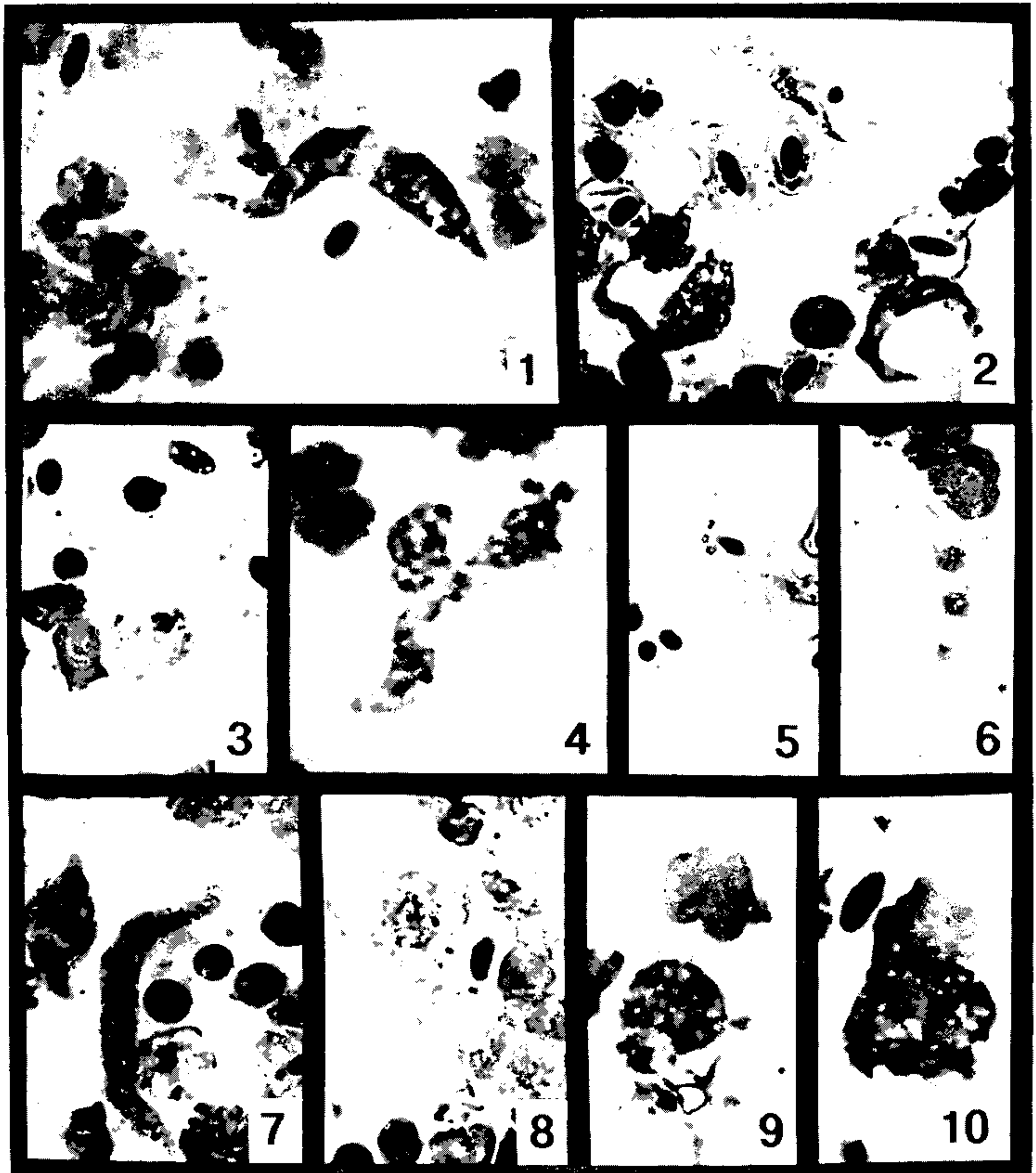
Epimastigote (Figs 3; E, F) - Epimastigotes were spear-shaped with both ends tapering. They measured 15.0-21.0 μm in length (excluding the free flagellum), 2.5-3.8 μm in width at the level of the nucleus; nucleus round, 2.4-3.0 μm in diameter, staining pink and located centrally, 5.5-11.5 μm from the posterior end. Cytoplasm somewhat coarse and staining blue. Undulating membrane narrow but distinct, free flagellum 7.0-9.0 μm in length. Material from bone marrow.

Amastigote (Figs 3, 4; G-I) - Amastigote typically round, 5.0-7.5 μm in diameter, with central-

ly or eccentrically placed spherical nuclei. Cytoplasm somewhat coarse and vacuolated. All specimens from bone marrow preparation.

Basis of description - All material from the tree pie, *Dendrocitta vagabunda* (Latham), col-

lected by NC Nandi at Barasat, North 24-Parganas district, West Bengal, India, 15 June 1976. All material deposited in the National Zoological Collections at the Zoological Survey of India, Calcutta.



35 micrometers

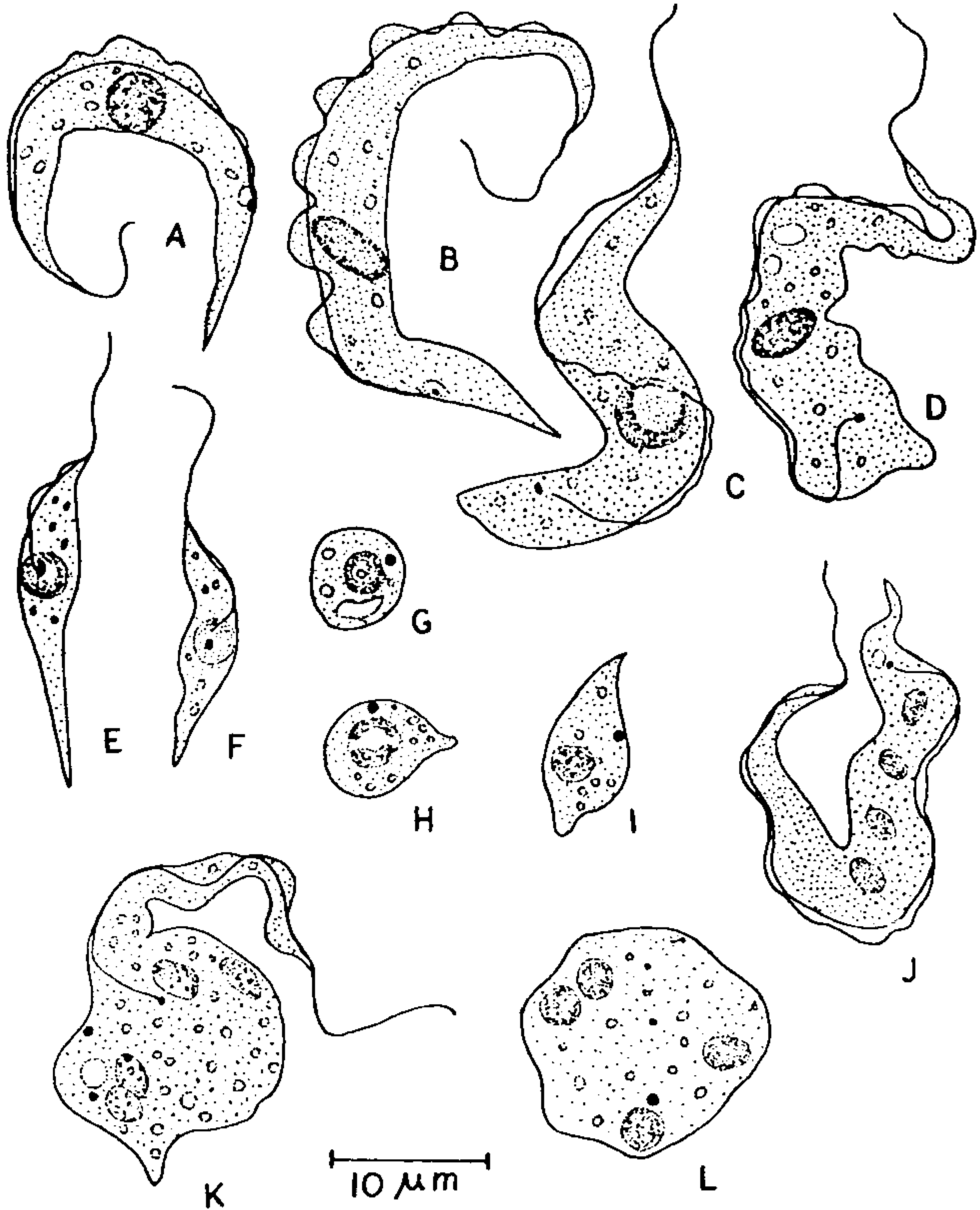
Fig. 1: trypomastigote morph (from blood smears). Figs 2-10 from bone marrow preparations. Fig. 2: epimastigote form. Figs 3, 4: amastigote forms: round (Fig. 3) and elliptical (Fig. 4). Fig. 5: trypomastigote with three nuclei and single kinetoplast. Fig. 6: trypomastigote with two nuclei and single kinetoplast. Fig. 7: trypomastigote with four nuclei and single kinetoplast. Fig. 8: amastigote with two nuclei and two kinetoplasts. Fig. 9: amastigote with four nuclei and three kinetoplasts. Fig. 10: irregularly-shaped trypomastigote with four nuclei and four kinetoplasts.

TABLE

Morphological parameters (in μm) of three forms of *Trypanosoma corvi* from *Dendrocitta vagabunda* compared with measurements of the same species by Baker (1956a), Subramanian and Singh (1962) and of *Trypanosoma ontarioensis* by Woo and Bartlett (1982)

Distance	From tree pie (this paper) ^a			Baker (1956a) ^b	Subramanian and Singh (1962) ^c	<i>T. ontarioensis</i> ^d
	slender	intermediate	broad			
Posterior end to kinetoplast (PK)	6.5-10.0	7.0-8.5	3.5-6.0	10.9 (4.7-20.0)	7.5 (5.5-13.2)	1.4 (0.8-2.0)
Kinetoplast to centre of nucleus (KN)	8.0-10.0	11.5-13.5	7.5-9.0	-	-	6.8 (5.9-9.2)
Posterior end to centre of nucleus (PN)	15.0-19.5	17.5-21.5	12.5-14.5	22.3 (15.7-27.7)	-	8.3 (7.0-11.6)
Centre of nucleus to anterior end (NA)	18.0-19.5	30.5-32.5	22.0-23.5	-	16.2 (12.5-19.5)	9.6 (7.5-16.7)
Length of body without free flagellum (PA)	34.8-38.0	48.5-54.0	35.5-37.5	49.2 (35.0-57.5)	34.7 (29.0-44.0)	18.0 (15.3-24.5)
Free flagellum (FF)	5.0-7.0	7.5-9.0	9.0-11.5	7.4 (4.5-10.2)	-	8.4 (5.7-11.6)
Width excluding undulating membrane (BW)	3.5-4.5	5.0-6.0	7.0-8.0	4.6 (3.5-6.0)	5.5 (3.5-7.0)	2.6 (2.0-3.9)
PK/PA	0.21	0.15	0.14	0.22	-	0.17
PN/PA	0.48	0.36	0.38	0.45	-	0.46
Nuclear index (PN/NA)	0.94	0.64	0.58	-	-	0.86
Kinetoplast index (PN/KN)	2.00	1.66	1.64	-	-	1.22

^a = from *Dendrocitta vagabunda*. ^b = from *Corvus frugillegus*. ^c = from *Garrulus lanceolatus*. ^d = from *Corvus brachyrhynchos*.



Camera lucida drawings of *Trypanosoma corvi* from the tree pie, *Dendrocitta vagabunda*. A-D: trypomastigote morphs (all from blood smears) - A: slender morph. B-C: intermediate morph. D: broad morph. Figs E-L: from bone marrow. E, F: epimastigote forms. G, H, I: amastigote forms. J-L: possible divisional forms. J: trypomastigote with four nuclei and single kinetoplast. K: irregularly-shaped trypomastigote with four kinetoplasts and four nuclei. L: amastigote with four nuclei and single kinetoplast.

COMMENTS

The frequency of occurrence of the different forms in the bone marrow was as follows: amastigotes (9%), epimastigotes (15%), trypomastigotes (76%). Only 18% of the forms were dividing, 82% were non-dividing parasites.

Dividing forms of the trypanosome (Figs 5-10) were encountered only in the bone marrow of the tree pies. In addition to the typical lon-

gitudinal binary fission, the trypomastigotes were also found to divide by a form of plasmotomy in which elongated tri- or tetra-nucleated trypomastigotes probably gave rise to epimastigotes (Figs J-L). Most of the multinucleated trypomastigotes possessed a single kinetoplast, indicating that the kinetoplast did not divide synchronously with the nucleus. Normally, the multiplication in the trypomastigote occurs in a definite sequence, in-

volving successively division of the basal body, flagellum, kinetoplast and nucleus, culminating in the cleavage of the cytoplasm (Hoare 1972). However, Hoare's work was conducted on mammalian trypanosomes and the same sequence may not always be followed by avian species of the group. However, Bennett (1961, 1970b) demonstrated this sequence in the division of *T. avium* reproducing in the mid-gut of *Aedes aegypti*, so this sequence is not totally foreign to the avian species. A few dividing amastigotes (Fig. L) with not more than four nuclei were seen in the smears but a dividing epimastigote was not detected. Baker (1956a) also described multi-nucleated crithidial forms in culture that were similar to those seen in the bone marrow preparations described here.

The dimensions of the trypanosomes from the tree pie presented here are similar to those given by Baker for *T. corvi* (Table). Although the measurements are slightly smaller, the range of the measurements embraces those presented by Baker (1956a) and they are clearly the same species. The sample size here is considerably larger than that presented by Baker, who worked with a total of only four specimens (three from the rook *C. frugilegus* and one from the jackdaw *C. monedula*). Thus the mean values presented for *T. corvi* by Baker are not really statistically of importance. Although three morphs could be distinguished within the tree pies (including bone marrow), the range of measurements for each morph broadly overlap the next morph and the measurements really present a continuum of what is a highly pleomorphic species.

Subramanian and Singh (1962) described *T. garruli* from the black-throated jay, *Garrulus lanceolatus* from northern India. The description of the species, including both measurements and illustrations, are clearly those of *T. corvi* (Table). The two authors were unaware of Baker's (1956a, b, c) work as they failed to cite any of his publications and compare their material with his. They also describe their trypanosome as lacking a free flagellum, a condition (see below) that is frequently associated with moribund trypanosomes, either through faulty preparation or because they were obtained from dead birds. We consider *T. garruli* Subramanian and Singh, 1962 to be a synonym of *T. corvi*. Zeiniev (1975) named *T. dschunkowskii* from the Eurasian jay *G. glandarius*. Unfortunately, as with all Zeiniev's names presented in this paper (Peirce & Bennett 1979), they were listed only in a table without illustration or description and are all *nomina nuda*. *T. dschunkowskii* is hereby declared a *nomen nudum*.

The measurements of *T. ontarioensis* (Table) given by Woo and Bartlett (1982) indicate a trypanosome which is considerably smaller in all

dimensions and is clearly a distinct species that is readily separable from *T. corvi*. Thus the two trypanosome species described from the Corvidae are easily separated. Baker (1976, p. 160) concludes that there are probably no more than 12 valid species of avian trypanosomes. On the basis of descriptions presented by Baker (1976) and Bennett (1961) these can be roughly grouped morphologically into the "*avium*-like" species with marked striations (myonemes), the "*paddae*-like" group in which the kinetoplast is close to the nucleus, the "*calmettei*-like" series in which the kinetoplast is nearly at the posterior end, and *T. everetti* Molyneux, 1973 "which does not resemble any previously described avian trypanosome" (Molyneux 1973a).

Baker considers that *T. johnstoni* Dutton and Todd, 1903 and *T. delhiense* Grewal, 1963 may be distinct on the basis that they lack a free flagellum. However, this is difficult to accept as most trypanosomes have a free flagellum, even though such flagella may not be long or very distinct as in the stumpy forms of *T. brucei*. It is more likely that blood smears containing trypanosomes lacking free flagella were made from dead or moribund birds and that the trypanosomes involved were either dead or moribund. Such trypanosomes, in our experience, rapidly change their morphology, including the loss of the free flagellum. *T. ontarioensis* is typical of New World (especially South American) avian trypanosomes which are extremely small, usually less than half the length of those seen in Old World birds. In the over 130,000 records of the International Reference Centre for Avian Haematozoa, there is only a single record of such "*ontarioensis*-like" trypanosomes outside of the New World (in the collared flycatcher *Ficedula albicollis* from Gotland, Sweden). Baker (1976) also considers that *T. macfieii* Cotton, 1970 is a distinct species on the basis of its life cycle and vector. However, morphologically it falls within the "*everetti*" group and, as the life cycles and natural vectors of most avian trypanosomes are unknown, this becomes a difficult character to use for speciation of avian trypanosomes at this time. As virtually all of the named avian trypanosomes fall within the above species complexes, it is difficult and probably of little value to make specific comparisons of the morphological forms seen in a variety of species of birds. It is probably better to assume at least a familial specificity at this time, and then synonymize as opportunity and life cycle and cross-transmission experiments confirm or disprove the species status of any given form. It is on this basis that we are assuming that *T. corvi* is a distinct species, as both culture and cross-transmission experiments using natural vectors have been carried out. The re-description of the morphological

forms is given to further substantiate the specific status of this trypanosome and to provide a broader base for interpretation of the morphometric measurements than was previously available.

While a degree of plasmotomy and asynchrony was demonstrated in bone marrow preparations, the significance of these observations is as yet unclear. This type of observation has been so rarely made on avian trypanosomes it is probably unwise to generalize further until many more such observations have been made on other species of avian trypanosomes. However, the appearance of amastigote and epimastigote forms in the avian host was also reported by both Molyneux (1973b) for epimastigotes of *T. bouffardi* and by Chatterjee and Ray (1970) for both amastigote and epimastigote forms of *T. avium bakeri* (= *T. brimonti bakeri*).

The results of the cross-transmission studies in which macerated infected bone marrow or heart blood was inoculated into a variety of bird species proved to be negative. The fact that neither the type host house crow nor the tree pie became infected suggested that the transfer technique left something to be desired and little can be concluded from these trials.

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