THE SPECIES OF *HAEMOPROTEUS, LEUCOCYTOZOON* AND *TRYPANOSOMA* OF THE AUSTRALIAN HONEYEATER FAMILY MELIPHAGIDAE (AVES: PASSERIFORMES)

GORDON F. BENNETT, DEBORAH SQUIRES-PARSONS AND TARMO POLDMAA

Bennett, G. F., Squires-Parsons, D. & Poldmaa, T. 1994 12 01: The species of *Haemoproteus, Leucocytozoon* and *Trypanosoma* of the Australian honeyeater family Meliphagidae (Aves: Passeriformes). *Memoirs of the Queensland Museum* 37(1): 13-18. Brisbane. ISSN 0079-8835.

The avian haematozoan species of *Haemoproteus, Leucocytozoon* and *Trypanosoma* occurring in the Australian Honeyeater family Meliphagidae, first described in 1909 and 1910, are re-described using modern criteria. The confusion surrounding the systematic position of these parasites when they were first described is resolved. □ *Meliphagidae, Haemoproteus, Leucocytozoon, Trypanosoma, Queensland, avian haematozoa.*

G.F. Bennett & D.Squires-Parsons, International Reference Centre for Avian Haematozoa and Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3X9; T. Poldmaa, Department of Biology, Queen's University, Kingston, Ontario, Canada, K7L 3N6; 28 July 1994.

Cleland & Johnston (1909) described *Haemoproteus ptilotis, H. philemon* and *H. meliornis* from the meliphagids *Ptilotis* (= *Meliphaga*) *chrysops, Philemon corniculatus* and *Meliornis* (= *Phylidonyris*) *novaehollandiae*, which they collected by shooting at Milson Island in the Hawkesbury River and in Sydney. In 1910 they described *Trypanosoma anellobiae* from *Anellobia* (= *Anthochaera*) *chrysoptera* which was collected near Brisbane, Queensland. They obtained further samples of a number of species of meliphagids from the environs of Eidsvold, Queensland and in 1911 they identified *Trypanosoma anellobiae* from *Myzomela sanguiniolenta, Ptilotis* (= *Meliphaga*) *fusca, Entomyzon cyanotis* and *Myzantha garrula* (now *Manorina melanocephala*). In 1911, they described and illustrated what they called the "intracorpuscular" or "*Leucocytozoon*" stage of *Trypanosoma anellobiae* and presented a discussion of how this stage was part of the life cycle of the trypanosome. However, the illustrations clearly indicate a species of *Leucocytozoon*. Johnston (1912) referred to *Leucocytozoon anellobiae* in a table and inadvertently established a specific designation.

Clearly, there is considerable confusion surrounding the identity of the avian haematozoa described by Cleland & Johnston from the Meliphagidae. In 1990-93, a large sample of Noisy Miners (*Manorina melanocephala*) from Laidley, Wivenhoe Dam and the Toohey Forest of Queensland were examined for blood parasites during the course of a study of their mating and social systems. These birds were infected with *Haemoproteus, Leucocytozoon* and *Trypanosoma* and thus gave the opportunity to re-describe these parasites using modern criteria and evaluate their systematic position.

MATERIALS AND METHODS

Blood samples from 173 Noisy Miners (*Manorina melanocephala*) in three locations in Queensland (Laidley, Wivenhoe Dam and Toohey forest) were collected via jugular venipuncture during the period 1990-1993. A blood smear from each sample was prepared in the field, air-dried and fixed in ethanol within 12 hours. Smears were then sent to the International Reference Centre for Avian Haematozoa (IRCAH) where they were re-fixed in 100% methanol and stained with Giemsa's stain at a pH 7.2 and examined for parasites. Forty-five birds were infected with species of *Haemoproteus, Leucocytozoon* and *Trypanosoma* and an additional seven birds were infected with *Plasmodium vaughani*, which constitutes the first Australian record of this parasite.

The blood parasites were drawn with the aid of a camera lucida and the morphometric parameters were determined with the aid of a Zeiss MOP-3 Digital Analyser. The parameters for the haemoproteids were measured by the protocols established by Bennett & Campbell (1972) as modified by Forrester et al. (1977). The parameters used for the leucocytozoids were established by Bennett et al. (1991). The morphometric parameters and derived indices for the trypanosomes follow the generally accepted measurements for this group (Woo & Bartlett, 1982). All measurements are presented as the

mean with the standard deviation in parentheses. All photomicrographs were taken with a Zeiss Photoscope III. In the re-descriptions of the haemoproteids and leucocytozoids, the measurements for the males are not presented in the interests of brevity. However, if the dimensions are markedly different from those of the macrogametocyte, these are mentioned in text.

Through the courtesy of Dr Penny Berents of the Australian Museum in Sydney, the original material used by Cleland & Johnston was made available for study.

TAXONOMIC REVIEW

Haemoproteus ptilotis (Cleland & Johnston, 1909) emend. Coatney, 1936

TYPE HOST

Ptilotis chrysops (Latham), now *Meliphaga chrysops* (Latham).

TYPE LOCALITY

Milson Island, Hawkesbury River, New South Wales.

Uninfected erythrocytes. N = 25. Erythrocyte 12.1 (0.8)µm in length, 6.1 (0.6) µm in width and 58.1 (7.4)µm^2 in area; erythrocyte nucleus 5.2 (0.5)µm in length, 1.9 (0.2)µm in width and 7.7 (1.2)µm^2 in area.

Immature gametocytes. Youngest forms seen were usually lateral to the erythrocyte nucleus, but sometimes in a polar position; margin entire.

Macrogametocyte (Fig. 1A). N = 35. Infected erythrocyte 12.8 (1.0)µm in length (6% hypertrophy), 7.1 (0.6)µm in width (16% hypertrophy) and 72.0 (8.6)µm^2 in area (24% hypertrophy); infected erythrocyte nucleus 5.1 (0.6)µm in length (2% atrophy), 2.1 (0.3)µm in width (10% hypertrophy) and 8.5 (1.5)µm^2 in area (10% hypertrophy). Parasite halteridial, entire in outline and occupying 58% of the area of the host cell-parasite complex. Parasite somewhat sausage-shaped, 12.1 (1.2)µm in length, 3.5 (0.6)µm in width at the middle of the parasite and 41.8 (6.4)µm^2 in area; parasite nucleus ovoid to round in outline, median in position, 2.2 (0.1)µm in length, 1.4 (0.1)µm in width and 2.4 (0.7)µm^2 in area; pigment granules average 10 (1.7) granules per parasite, small and scattered randomly throughout parasite cytoplasm; erythrocyte nucleus only slightly displaced laterally, NDR (Nuclear Displacement Ratio) = 0.6 (0.2) where the NDR represents the degree of lateral displacement of the cell through the action of the parasite (Bennett et al., 1990:194), vacuoles not prominent and volutin granules not seen.

Microgametocyte (Fig. 1B). N = 10. Microgametocytes closely similar to macrogametocytes in all dimensions except for the larger parasite nucleus typical of the microgametocytes of all the apicomplexan parasites; parasite nucleus central with volutin granules concentrated at the poles and not occupying the area occupied by the large parasite nucleus.

BASIS OF REDESCRIPTION

Blood films 115027 and 124757 from Noisy Miners *Manorina melanocephala* collected by Poldmaa in Queensland, Australia from Toohey Forest on 7 October 1990 and Wivenhoe Dam on 6 September 1992 respectively.

COMMENTS

Haemoproteus ptilotis is a small, halteridial haemoproteid that occupies less than 60% of the host cell-parasite complex. It has few and rather small pigment granules. It is considered to be a distinct species on the basis of its occurrence in the family *Meliphagidae*, following the assumption that haemoproteids are host family or subfamily specific (Bennett & Peirce, 1988). *Haemoproteus ptilotis* fits the description presented by Cleland & Johnston (1909) as far as can be followed. The illustrations presented by these authors are essentially the same as those figured including the few, small pigment granules per parasite. The measurements presented by Cleland & Johnston lie well within the range of those presented above. Unfortunately, the hapantotype (cotype of Cleland & Johnston, 1909) slide of *H. ptilotis* had degraded beyond use. Not only had the stain faded but the erythrocytes themselves were disintegrating and no parasites could be distinguished at any place on the blood film. In essence, the existing hapantotype slide is worthless and only the original description and illustrations of the species remains to define it.

Cleland & Johnston (1909), using the generic designation of *Halteridium*, subsequently emended by Coatney in 1936 to *Haemoproteus*, also described *Haemoproteus philemon* from *Philemon corniculatus* and their illustrations and dimensions of the parasite in this bird are essentially the same as the description for *H. ptilotis*, including the small parasite with few pigment granules. They mention that although a few of the parasites of *H. philemon* were larger than those of *H. ptilotis*, ". . little difference could be detected and the identity or otherwise of the two

must await the investigation of other stages in their life histories" (Cleland & Johnston, 1909:84). They also described *Haemoproteus meliornis* from *Meliornis* (= *Phylidonyris*) *novaehollandiae*. This bird had an intense infection with many erythrocytes having multiple invasion of parasites. Their illustrations of this parasite indicate many of the parasites were immature, but the mature forms are unquestionably the same as *H. ptilotis* and the dimensions of the cells are within the range of those cited in the redescription above. Regrettably, the hapantotype slide of *H. meliornis* is in equally poor shape as that of *H. ptilotis* and nothing could be seen on the smear except disintegrating cells that had lost their stain and the slide is essentially worthless. The blood film of *H. philemon* was fortunate to have been cover-slipped with Canada balsam and the cells on the smear were intact although badly faded. As described originally, this smear contained cells with up to four immature parasites. However, only two mature parasites were seen and these, as far as could be determined given the lack of staining, appeared to be similar to their illustrations in 1909 and to those used in the re-description above. Although the hapantotype slides of these parasites are useless for taxonomic purposes, on the basis of the original descriptions and line drawings presented by Cleland & Johnston (1909), all three species described from the meliphagids by Cleland & Johnston are the same species. By page priority (ICZN, Section 69B (11)), therefore, *Haemoproteus ptilotis* is the name of the haemoproteid in the Meliphagidae and *Haemoproteus philemon* and *Haemoproteus meliornis* fall as synonyms.

Leucocytozoon anellobiae (Cleland & Johnston, 1911) emend. Johnston, 1912

TYPE HOST

The little wattle bird, *Anthochaera chrysoptera* (Latham).

TYPE LOCALITY

Brisbane, Queensland, Australia.

Macrogametocyte (Fig. 1C). N = 51. Parasite with round morphs only. Parasite broadly ovoid to round, with a maximum diameter of 11.3 (1.6)µm, minimum diameter of 9.5 (0.9)µm, a periphery of 33.1 (3.5)µm and an area of 85.4 (15.6)µm^2, occupying 80% of the area of the host cell-parasite complex; parasite nucleus round to broadly ovoid and sometimes elliptical, 4.0 (0.5)µm in length, 2.8 (0.5)µm in width and 8.2 (1.9)µm^2 in area, without a marked karyosome, occupying 9.7% of the area of the parasite; vacuoles small and not prominent; volutin granules not seen; nucleus of host cell-parasite complex usually as a ribbon but sometimes as a cap, 23.1 (8.7)µm^2 in area and covering 14.9 (4.8)µm of the periphery of the parasite (44%) and occupying 21% of the area of the host cell-parasite complex; host cell-parasite complex 108.5 (22.6)µm^2 in area.

Microgametocyte (Fig. 1D). N = 14. Microgametocyte similar to the macrogametocyte in most respects except for the usual larger nucleus and pale staining that occurs in the apicomplexan parasites. The microgametocyte on average is 5-10% larger in most dimensions than the macrogametocyte and the host cell-parasite nucleus is larger and covers a greater amount of the periphery of the parasite (66% compared to 44% for the macrogametocyte).

BASIS OF DESCRIPTION

HAPANTOTYPE: Blood film no. 115021 from *Manorina melanocephala* collected by Poldmaa at Toohey Forest, Queensland on 7 October 1990.

PARAHAPANTOTYPES: Blood film no. 8872 from the Noisy Miner *Manorina melanocephala* collected by Bennett at Kenmore, Queensland on 7 September 1968; blood film no. 124710 from the same species collected by Poldmaa at Wivenhoe Dam, Queensland on 15 July 1992.

COMMENTS

This is a small round leucocytozoid, one of the smallest of the species described. It is considered to be a distinct species on the basis of the presumed familial/subfamilial specificity demonstrated for a number of species of *Leucocytozoon* (Bennett et al., 1991). Bennett & de Swardt (1989) believed that *Leucocytozoon anellobiae* also occurred in the South African Gurney's sugarbird (*Promerops gurneyi*) which was at that time classified as a meliphagid. However, this genus is now believed to have little or no relationship with the Australian Meliphagidae and has been placed in its own family, the Promeropidae (although some authorities consider them to be in the subfamily Promeropinae of the Meliphagidae). On comparison of the sugar bird material with that from the Australian noisy miner, it was evident that the South African species was much larger and that Bennett and de Swardt (1989) were in error. Therefore, the South African leucocytozoid was described as

Leucocytozoon deswardti by Bennett et al. (1992).

Cleland & Johnston (1910) described *Trypanosoma anellobiae* from *Anellobia* (= *Anthochaera*) *chrysoptera* and in 1911, elaborated on this parasite with remarks based on finding this trypanosome in several other species of the Meliphagidae. They were also convinced that the "*Leucocytozoon*" stage was the intracorpuscular stage of the trypanosome life cycle, a commonly held view at the time. This view may have been prompted by observation of the highly fusiform (almost trypanosome-like) appearance of *Leucocytozoon ziemanni* of owls, birds which are frequently concurrently infected with both parasites. Johnston (1912) referred in a table to *Leucocytozoon anellobiae*. The footnote to this specific name reads "The name *Leucocytozoon anellobiae* is here given to a blood parasite found by Dr. Cleland and myself in several species of birds. We believe it to be a phase in the life history of *Trypanosoma anellobiae* (Cleland & Johnston). I have used the above name as possessing specific value. Should our opinion as to the specific identity of the two forms be correct, then the name *L. anellobiae* becomes a synonym, or, to be more exact, it refers to a particular phase of *T. anellobiae*." Thus Johnston inadvertently created *Leucocytozoon anellobiae* as a valid species. Whether inadvertent or not, the name stands as the valid designation of the leucocytozoid of the Australian meliphagids and is herein so recognised. Cleland & Johnston (1910) did not indicate the disposition of the material used to define the "*Leucocytozoon*" stage of *Trypanosoma anellobiae*. However, when Johnston (1912) created *Leucocytozoon anellobiae*, he was in the Department of Biology of the University of Queensland. There is no trace of this material at the Queensland Museum or on record at other Australian institutions as far as is known (Lester Cannon, pers. comm.). In as much as that no "type" material was designated for *L. anellobiae*, we are designating hapantotype and parahapantotype slides from *Manorina melanocephala*, one of the hosts from which the "*Leucocytozoon*" stage was described and are birds from the type locality.

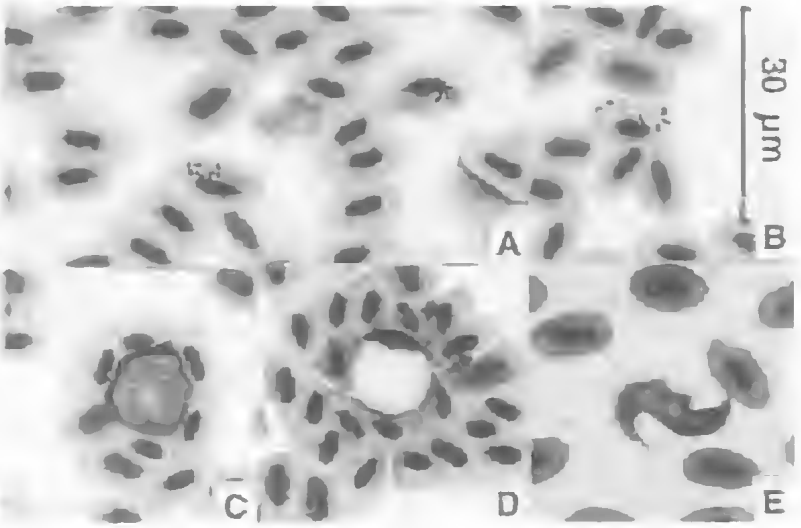

FIG. 1. A, *Haemoproteus ptilotis*, two macrogametocytes; B, *Haemoproteus ptilotis*, microgametocyte; C, *Leucocytozoon anellobiae*, macrogametocyte; D, *Leucocytozoon anellobiae*, microgametocyte; E, *Trypanosoma anellobiae*.

Trypanosoma anellobiae
Cleland & Johnston, 1910.

TYPE HOST

The little wattle bird, *Anthochaera chrysoptera* (Latham).

TYPE LOCALITY

Brisbane, Queensland, Australia.

Trypomastigote (Fig. 1E). N = 6. Trypomastigote small and slender, averaging 25.6 (2.3)µm in length and 5.7 (0.9)µm in width at the position of the nucleus. Kinetoplast 2.7 (0.8)µm from posterior end and 9.7 (0.6)µm from the nucleus. Nucleus 12.3 (0.8)µm from the anterior end which has a long free flagellum averaging 10.8µm (only two free flagellae measured). Trypomastigote 82.0 (12.7)µm^2 in area, nucleus 15.6 (3.5)µm^2 in area, the nucleus representing 19% of the area of the parasite. The distance from the posterior end to the kinetoplast represents 10% of the length of the trypanosome, while the distance of the centre of the nucleus from the posterior end is 48% of the length of the parasite, the nucleus approximately at the mid-point of the trypomastigote. The width of the trypanosome at the centre of the nucleus is 22% of the length of the organism.

BASIS OF DESCRIPTION

Blood film No. 115082 from *Manorina melanocephala* collected by Poldmaa at Toohey Forest, Queensland on 18 October 1990.

COMMENTS

Cleland & Johnston (1910) described *Trypanosoma anellobiae* from *Anellobia chrysoptera* (now *Anthochaera chrysoptera*) from a bird shot at Brisbane, Queensland. The infection was light and their description indicates that the trypomastigote was about 0.035mm in length with a maximum breadth of 0.002mm. The "kinetonucleus" was situated 0.003mm from the posterior end. They could not see the nucleus of the organism and they did not detect a free flagellum. They concluded that the undulating membrane was very narrow and believed it to be short. Their illustrations (Plate xxxiv, figs 6, 11) are clearly those of the trypanosome illustrated in Fig. 1E of this study. The measurements they presented are closely similar to those presented herein, especially with respect to the position of the kinetoplast. It is clear that the trypanosome in the Noisy Miner is the same as that described by Cleland & Johnston. The hapantotype slide of *Trypanosoma anellobiae* was examined and found to be in the same condition as described for the hapantotype slides of *Haemoproteus ptilotis* and *H. meliornis*. Only a few of the erythrocytes were intact and the stain had faded to the extent that the blood smear was a monocolour. The blood smear was reported to also contain two species of microfilariae. However, no trace of these parasites could be found. The blood smear is unacceptable as the basis for definition of a taxon.

On the basis of the original description, *Trypanosoma anellobiae* is a small trypanosome that lacks the striated appearance and larger size of the *T. avium* group; it is also easily separated from *T. paddae*, *T. corvi* and *T. hannai* on the basis of its much smaller size and distinctive derived ratios. It is smaller than *T. bouffardi* but shares the same slender appearance of this trypanosome and also differs in that the kinetoplast is much closer to the posterior end. The length of *T. anellobiae* is within the same range as *T. everetti*, and the placement of the kinetoplast is similar in both of these species. However, *T. everetti* is a broader trypanosome (which gives the organism a "stumpy" appearance) with a nucleus that occupies about 26% of the area of the trypomastigote and cannot be confused with *T. anellobiae*. On the other hand, *T. anellobiae* is remarkably similar to *T. ontarioensis* Woo and Bartlett, 1982. Both trypanosomes are small, slender and with the kinetoplast located close to the posterior end; measurements cited herein lie within the ranges quoted by Woo & Bartlett for their species. Both species have a long free flagellum that is about one-half the body length and the derived ratios are essentially the same. It would be essentially impossible to separate the two species on the basis of their morphometrics and appearance. *Trypanosoma ontarioensis* was originally described from a corvid in Ontario, Canada but its appearance is similar to the numerous small trypanosomes that have been inadequately described from South American birds and many of these South American trypanosomes can undoubtedly be assigned to this species. In addition, *T. ontarioensis* has been recorded from Sweden and appears to have a broad distribution. *Trypanosoma ontarioensis* is a readily cultured trypanosome, doing particularly well on diphasic blood-agar medium, producing infective cultured forms in two weeks. While the isolation of Australia would suggest that *T. anellobiae* is a distinct species and no attempt will be made to synonymize the two species at this time, the close similarity of the two species to each other requires experimental confirmation of their identity.

This study has also highlighted what will become a major problem for museums and repositories of hapantotype material of the Protozoa. The hapantotype material of the Cleland & Johnston species were 84-85 years of age and had deteriorated to the extent they were of no value as the basis for the definition of the taxons they were supposed to represent. It is almost 90 years since the first edition of the International Code for Zoological Nomenclature was published and the practice of establishing type material became mandatory although type material had frequently been designated decades before. Stained protozoal and similar material does age with time and as indicated in this study, in 85 years had deteriorated beyond use. While the use of coverslips and mounting media does aid in the preservation of the cells, the problem of stain fading over a long period still occurs. The preservation of hapantotype material will become a serious problem that will have to be addressed by curators of this type of material and should be addressed as a priority by the International Commission for Zoological Nomenclature.

ACKNOWLEDGEMENTS

The financial support of the Natural Sciences and Engineering Research Council of Canada to both the first and last authors is gratefully acknowledged.

LITERATURE CITED

BENNETT, G. F. & CAMPBELL, A. G. 1972. Avian Haemoproteidae. I. Description of *Haemoproteus fallisi* n. sp. and a review of the haemoproteids of the family Turdidae. Canadian Journal of Zoology 50: 1269-1275.

BENNETT, G. F., EARLÉ, R. A. & PEIRCE, M. A. 1992. The leucocytozoidae of South African birds: the Passeriforms. Onderstepoort Journal of Veterinary Research 59: 235-247.

BENNETT, G. F., EARLÉ, R. A., PEIRCE, M. A., HUCHZERMEYER, F. W. & SQUIRES-PARSONS, D. 1991. Avian Leucocytozoidae: the leucocytozoids of the Phasianidae sensu lato. Journal of Natural History 25: 1407-1428.

BENNETT, G.F. & PEIRCE, M. A. 1988. Morphological form in the avian Haemoproteidae and an annotated checklist of the genus *Haemoproteus* Kruse, 1890. Journal of Natural History 22: 1683-1696.

BENNETT, G.F., PEIRCE, M. A. & EARLÉ, R. A. 1990. The haemoproteids of the shrikes of the the avian family Laniidae (Passeriformes). South African Journal of Zoology 25: 194-198.

BENNETT, G.F. & DE SWARDT, D. H. 1989. First African record of *Leucocytozoon anellobiae* (Apicomplexa: Leucocytozoidae) in Gurney's sugarbird, *Promerops gurneyi*. Ostrich 60: 171.

CLELAND, J. B. & JOHNSTON, T. H. 1909. Descriptions of new haemoprotozoa from birds in New South Wales, with a note on the resemblance between the spermatazoa of certain honeyeaters (Fam. Meliphagidae) and spirochaetetrypanosomes. Journal of the Proceedings of the Royal Society of New South Wales 43: 75-96.

CLELAND, J. B. & JOHNSTON, T. H. 1910. The haematozoa of Australian birds. - No. I. Transactions of the Royal Society of Australia 34: 100-114.

CLELAND, J. B & JOHNSTON, T. H. 1911. The haematozoa of Australian birds, No. II. Journal of the Proceedings of the Royal Society of New South Wales 45: 415-444.

COATNEY, G. R. 1936. A check-list and host-index of the genus *Haemoproteus*. Journal of Parasitology 22: 88-105.

FORRESTER, D. J., GREINER, E. C., BENNETT, G. F & KIGAYE, M. M. 1977. Avian Haemoproteidae. 7. A review of the haemoproteids of the family Ciconiidae (storks) and descriptions of *Haemoproteus brodkorbi* sp. nov. and *H. peircei* sp. nov. Canadian Journal of Zoology 55: 1268-1274.

INTERNATIONAL CODE OF ZOOLOGICAL NOMENCLATURE. 1985. (International Trust for Zoological Nomenclature in association with British Museum (Natural History) London).

JOHNSTON, T. H. 1912. Internal parasites recorded from Australian birds. Emu 12: 105-112.

WOO, P. T. K. & BARTLETT, C. M. 1982. *Trypanosoma ontarioensis* n. sp. and *T. paddae* from Corvus brachyrhynchos in Ontario, Canada with notes on the biology of *T. ontarioensis* n. sp. Canadian Journal of Zoology 60: 2107-2115.