



Opalinidae (Slopalinida) in Madagascan Anura: *Zelleriella* Metcalf, 1920 and *Protoopalina* Metcalf, 1918

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Abstract

Between January and April, 1995, 142 Madagascan anurans collected at nine sites and comprising 61 species in three families were checked for opalinids in the cloaca. *Zelleriella madagascariensis* n. sp. was found in two of three *Dyscophus insularis* (Microhylidae) from Morondava; *Protoopalina devinckae* n. sp. in one *Ptychadena mascareniensis* (Ranidae) from Morondava; *P. primordialis* (Awerinzew, 1913) in two *Ptychadena mascareniensis* from Antananarivo; *P. drachi* Tuzet & Knoepfler, 1968 in three of seven *Ptychadena mascareniensis* from Antananarivo and Andasibe; *P. legeardi* n. sp. in one *Mantidactylus lugubris* (Ranidae) and one of two *M. grandidieri* both from Andasibe; *P. grolleau* n. sp. in one *Scaphiophryne marmorata* (Microhylidae) from Andasibe; *P. charvisi* n. sp. in two *S. calcarata* from Morondava; and *P. perantonii* n. sp. in three *Aglyptodactylus madagascariensis* (Ranidae) from Andasibe. The biogeographical significance of these findings is discussed.

Introduction

This paper is the first of two dealing with opalinids occurring in Madagascan Anura and reviews the binucleate genera *Zelleriella* Metcalf, 1920 and *Protoopalina* Metcalf, 1918. Both genera have never been reported in Madagascan anurans. Metcalf (1923, 1940) is the only author to have described opalinid species (three) from Madagascan anurans. All three species were multinucleate opalinids. Madagascar has three families of Anura. We have also included the families Mantellidae (genus *Mantella*) and Rhacophoridae (genus *Boophis*) as part of the Ranidae, which now represent a very large family in Madagascar. There are no Apoda or Caudata in Madagascar.

Materials and methods

The methods used are described in Delvinquier et al. (1991a). Collections were made between January and April, 1995 at nine sites (six areas) in Madagascar. Two areas (Andasibe and Andapa) are East Coast rain forest habitats at above 1,000 m elevation. The area in Antananarivo (Central High plateau) is a garden in a semi-urban environment. Two areas (Miandrivazo

and Ankazobe) are open fields in the Central plateau. The last area (Morondava) on the West Coast is a dry sclerophyll forest. A total of 142 specimens, representing 61 species in three families of Anura were dissected and checked for the presence of opalinids. Details concerning the number of Anura collected at each site, with latitude, longitude and map numbers, are given in Table I. The following anurans were examined (the number of specimens dissected is given in parentheses): Hyperoliidae (Hyperoliinae): *Heterixalus betsileo* (3); *H. luteostriatus* (3); *H. punctatus* (3); Microhylidae (Cophylinae): *Anodonthyla boulengeri* (2); *Platypelis grandis* (1); *P. occultans* (1); *P. pollicaris* (3); *Plethodontohyla* sp. a (1) [see Glaw & Vences, 1994]; (Dyscophinae): *Dyscophus insularis* (3); (Scaphiophryninae): *Paradoxophyla palmata* (2); *Scaphiophryne brevis* (2); *S. calcarata* (2); *S. marmorata* (1); Ranidae (Mantellinae): *Mantella aurantiaca* (1); *M. baroni* (1); *M. betsileo* (3); *M. crocea* (2); *M. laevigata* (2); *M. madagascariensis* (2); *Mantidactylus aglavei* (juvenile) (1); *M. albofrenatus* (4); *M. alutus* (2); *M. betsileanus* (5); *Mantidactylus* n. sp. (cf. *betsileanus*) (1); *M. bicalcaratus* (2); *M. biporus* (5); *M. blommersae* (5); *M. cornutus* (2); *M. femoralis* (4); *M. fimbriatus* (2); *M. grandidieri* (2); *M. gran-*

disonae (2); *M. guttulatus* (1); *M. leucomaculatus* (1); *M. liber* (5); *M. lugubris* (1); *M. cf. lugubris* (1); *M. malagasius* (2); *M. mocquardi* (1); *M. opiparis* (2); *M. redimitus* (2); *M. rivicola* (2); *M. tornieri* (1); (Raninae): *Ptychadena mascareniensis* (7); *Tomopterna labrosa* (7); (Rhacophorinae): *Aglyptodactylus laticeps* (3); *A. madagascariensis* (3); *Boophis boehmei* (4); *B. cf. burgeri* (1); *B. elenae* (1); *B. goudoti* (1); *B. guibei* (3); *B. idae* (1); *B. luteus* (2); *B. cf. madagascariensis* (1); *B. pauliani* (4); *B. rappiodes* (2); *B. sibilans* (2); *B. tephraeomystax* (2); *B. viridis* (2); *B. xerophilus* (2).

All measurements are listed in Table II. The nomenclature for the Anura is based on Glaw & Vences (1994). Geographical coordinates are based on topographical maps from the Institut National de Géodésie et Cartographie in Antananarivo. Specimens of opalinids have been deposited with The Natural History Museum, London (BM), the United States National Museum (USNM), the American Museum of Natural History (AMNH) and the Museum of Comparative Zoology (MCZ) at Harvard.

***Zelleriella madagascariensis* n. sp.** (Figures 1,2,19; Table II)

Type-specimens: BM 1997:9:18:1; USNM 50967; AMNH 810; MCZ 92B.

Etymology: This species is named after Madagascar.

Hosts and locality: Observed in 2 of 3 *Dyscophus insularis* (Microhylidae) from Camp forestier of CFPF forest, Kirindy, near Morondava (20°03' S, 44°39' E, map 7).

Morphology

Ovoid to pyriform in body shape with 2 nuclei situated in the anterior region of the cell. One nucleus is placed in a slightly more anterior position than the other.

Comments

This is the first *Zelleriella* species described from Madagascar and we propose to name it *Z. madagascariensis* n. sp. It is a rather small species occurring solely in a microhylid species of the subfamily Dyscophinae from the West coast of Madagascar. Other Afrotropical *Zelleriella* spp. are quite different in body shape and also larger in size (see Delvinquier et al., 1991b).

***Protoopalina devinckae* n. sp.** (Figures 3,4,22; Table II)

Type-specimens: BM 1997:9:18:2; USNM 50966.

Etymology: This species is named for Mrs J. Devinck in recognition of her help with the literature search.

Host and locality: Observed in one *Ptychadena mascareniensis* from Camp forestier, CFPF forest, Kirindy, near Morondava (20°03' S, 44°39' E, map 7).

Morphology

Body stocky, with a rounded anterior extremity that may be narrower than the rest of the body (Figure 3). The posterior extremity possesses a sharp pointed tail which becomes more prominent in older trophozoites. Two pear-shaped nuclei are placed, one above the other, along the antero-posterior axis of the cell.

Comments

This species is quite unlike any other *Protoopalina* we found because of the thickness of its body and the presence of a conspicuous posterior extremity. It approaches in shape *P. tronchini* Delvinquier, 1987 and *P. pattersoni* Delvinquier et al., 1995. The latter species also occurs in *Ptychadena mascareniensis* from South Africa. But the presence of a sharp-pointed tail in our specimens makes it quite different from *P. pattersoni* which was described with a posterior pad in older trophozoites (Delvinquier et al., 1995). We therefore propose to erect *P. devinckae* n. sp.

***Protoopalina primordialis* (Awerinzew, 1913) Metcalf, 1918** (Figures 5,6,21; Table II)

Type-specimens: BM 1997:9:18:3; USNM 50965; AMNH 811.

Hosts and locality: Observed in 2 *Ptychadena mascareniensis* from the garden of the Institut Pasteur in Antananarivo (18°55' S, 47°32' E, map 8).

Morphology

Resembling *P. intestinalis* (Ehrenberg, 1838) in shape. Smaller trophozoites have a stocky body, which becomes elongate and slender in larger forms. Nuclei close to each other and situated in the anterior region of the body.

Comments

This species has also been observed in a large number of African Anura including juveniles of *Ptychadena*

Table I. Number of Madagascan anurans collected at each site.

Localities Species	Number examined	Dates collected
Area 1. Antananarivo		
Site 1. Garden in the Institut Pasteur (18°55' S, 47°32' E, map 8)		01/95
<i>Mantidactylus alutus</i>	2	
<i>Ptychadena mascareniensis</i>	4	
Area 2. Andasibe		
Site 1. Near Réserve Spéciale d'Analamazaotra (18°56' S, 48°25' E, map 8)		01/95
<i>Aglyptodactylus madagascariensis</i>	3	
<i>Anodonthyla boulengeri</i>	2	
<i>Boophis boehmei</i>	3	
<i>Boophis elenae</i>	1	
<i>Boophis goudoti</i>	1	
<i>Boophis guibei</i>	3	
<i>Boophis idae</i>	1	
<i>Boophis luteus</i>	2	
<i>Boophis pauliani</i>	4	
<i>Boophis rappiodes</i>	2	
<i>Boophis sibilans</i>	2	
<i>Boophis viridis</i>	2	
<i>Heterixalus betsileo</i>	3	
<i>Heterixalus punctatus</i>	2	
<i>Mantella aurantiaca</i>	1	
<i>Mantella baroni</i>	1	
<i>Mantella crocea</i>	2	
<i>Mantella madagascariensis</i>	2	
<i>Mantidactylus albofrenatus</i>	1	
<i>Mantidactylus betsileanus</i>	4	
<i>Mantidactylus cf. betsileanus</i>	1	
<i>Mantidactylus bicalcaratus</i>	1	
<i>Mantidactylus blommersae</i>	5	
<i>Mantidactylus cornutus</i>	2	
<i>Mantidactylus grandidieri</i>	2	
<i>Mantidactylus grandisonae</i>	2	
<i>Mantidactylus guttulatus</i>	1	
<i>Mantidactylus liber</i>	3	
<i>Mantidactylus lugubris</i>	1	
<i>Mantidactylus malagasius</i>	2	
<i>Mantidactylus mocquardi</i>	1	
<i>Mantidactylus opiparis</i>	1	
<i>Mantidactylus redimitus</i>	1	
<i>Mantidactylus tormieri</i>	1	
<i>Paradoxophyla palmata</i>	2	
<i>Platypelis pollicaris</i>	1	
<i>Plethodontohyla sp. a</i>	1	
<i>Ptychadena mascareniensis</i>	2	
<i>Scaphiophryne marmorata</i>	1	
Site 2. An'Ala (18°56' S, 48°28' E, map 8)		01/95
<i>Mantidactylus opiparis</i>	1	
<i>Mantidactylus redimitus</i>	1	

Table I. Continued.

Localities Species	Number examined	Dates collected
Area 3. Miandrivazo		
Site 1. Ambalapaka, 60 km south of Miandrivazo, on N34 (20°00' S, 45°32' E, map 7)		01/95
<i>Tomopterna labrosa</i>	3	
Area 4. Morondava		
Site 1. Camp forestier, CFPF forest in Kirindy (20°03' S, 44°39' E, map 7)		01/95
<i>Aglyptodactylus laticeps</i>	3	
<i>Boophis tephraeomystax</i>	2	
<i>Boophis xerophilus</i>	2	
<i>Dyscophus insularis</i>	3	
<i>Heterixalus luteostriatus</i>	3	
<i>Mantella betsileo</i>	3	
<i>Ptychadena mascareniensis</i>	1	
<i>Scaphiophryne brevis</i>	2	
<i>Scaphiophryne calcarata</i>	2	
<i>Tomopterna labrosa</i>	4	
Area 5. Andapa		
Site 1. Réserve Naturelle Intégrale Marojezy, Camp 1 (14°26' S, 49°46' E, map 4)		02/95
<i>Mantella laevigata</i>	2	
<i>Mantidactylus aglavei</i> (juvenile)	1	
<i>Mantidactylus cf lugubris</i>	1	
<i>Mantidactylus femoralis</i>	2	
<i>Mantidactylus leucomaculatus</i>	1	
<i>Mantidactylus rivicola</i>	2	
<i>Platypelis grandis</i>	1	
<i>Platypelis occultans</i>	1	
Site 2. Réserve Naturelle Intégrale Marojezy, Camp 3 (14°26' S, 49°46' E, map 4)		03/95
<i>Boophis cf. burgeri</i>	1	
<i>Boophis cf. madagascariensis</i>	1	
<i>Mantidactylus albofrenatus</i>	2	
<i>Mantidactylus bicalcaratus</i>	1	
<i>Mantidactylus biporus</i>	2	
<i>Mantidactylus fimbriatus</i>	2	
<i>Mantidactylus redimitus</i>	1	
Site 3. Réserve Spéciale Anjanaharibe-Sud (14°48' S, 49°41' E, map 4)		02/95
<i>Heterixalus punctatus</i>	1	
Area 6. Ankazobe		
Site 1. Réserve Naturelle Ambohitantely (18°10' S, 47°17' E, map 8)		04/95
<i>Boophis boehmei</i>	1	
<i>Mantidactylus betsileanus</i>	1	
<i>Mantidactylus albofrenatus</i>	1	
<i>Mantidactylus biporus</i>	3	
<i>Mantidactylus femoralis</i>	2	
<i>Mantidactylus liber</i>	2	
<i>Platypelis pollicaris</i>	2	

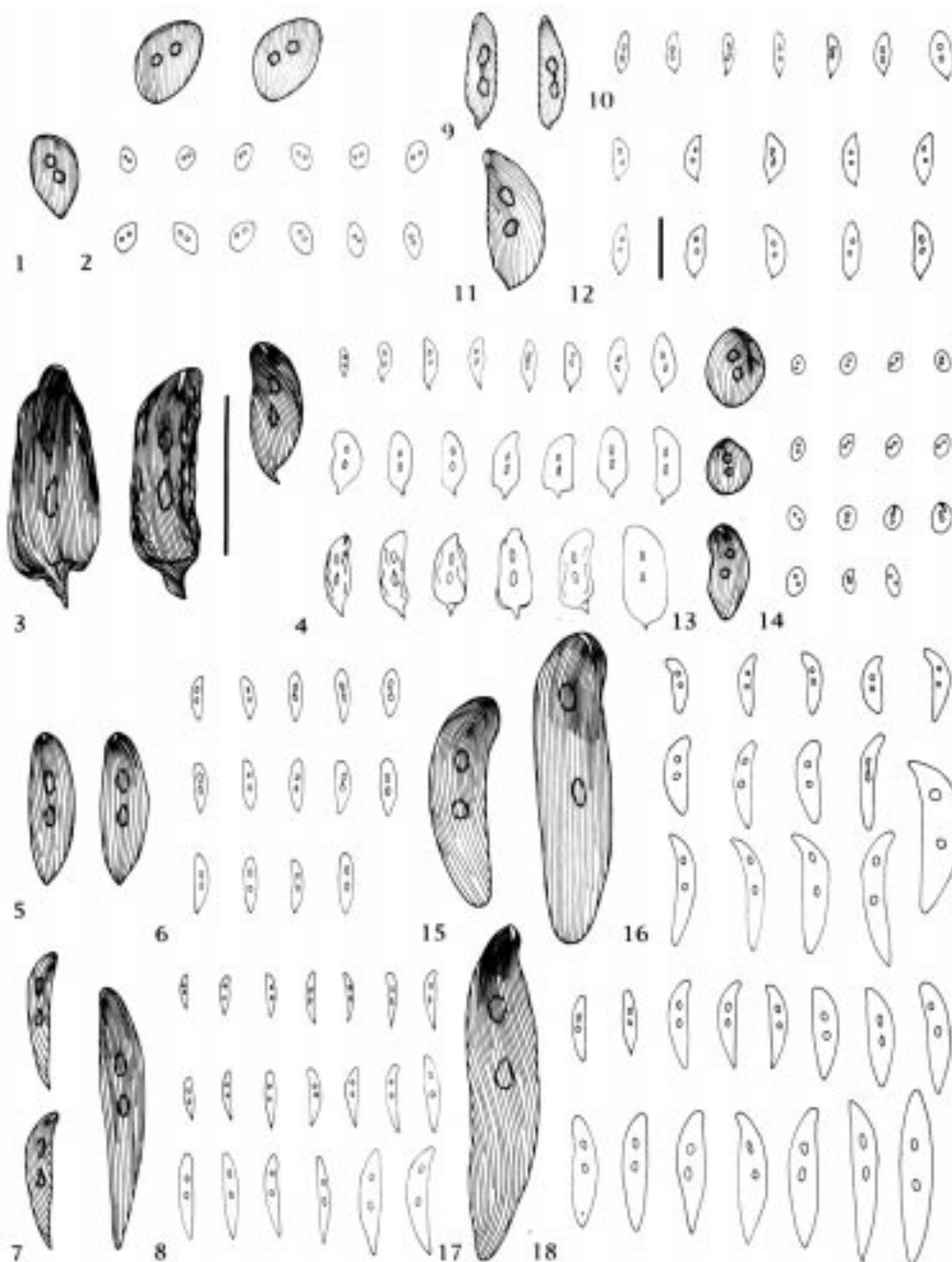


Figure 1-18. 1-2. *Zelleriella madagascariensis* n. sp. 1. Arrangement of the kinetids on one or both faces, ex *Dyscophus insularis* from Camp forestier of CFPF forest, Kirindy, near Morondava. 2. Specimens from the same host and locality. 3-4. *Protoopalina devinckae* n. sp. 3. Showing the arrangement of the kinetids on one face, ex *Ptychadena mascareniensis* from Camp forestier, CFPF forest, Kirindy, near Morondava. 4. Specimens from the same host and locality. 5-6. *P. primordialis*. 5. Arrangement of the kinetids on both faces, ex *Ptychadena mascareniensis* from the garden of the Institut Pasteur in Antananarivo. 6. Specimens from the same host and locality. 7-8. *P. drachi*. 7. Arrangement of the kinetids on one face, ex *Ptychadena mascareniensis* from the garden of the Institut Pasteur in Antananarivo. 8. Specimens from the same host and locality. 9-12. *P. legeardi* n. sp. 9. Arrangement of the kinetids on one face, ex *Mantidactylus lugubris* from near the Réserve Spéciale d'Analamazaotra, Andasibe. 10. Specimens from the same host and locality. 11. Arrangement of the kinetids on one face, ex *Mantidactylus grandidieri* from the same locality. 12. Specimens from the same host and locality. 13-14. *P. charvisi* n. sp. 13. Arrangement of the kinetids on one face, ex *Scaphiophryne calcarata* from Camp forestier, CFPF forest, Kirindy, near Morondava. 14. Specimens from the same host and locality. 15-16. *P. grolleaui* n. sp. 15. Arrangement of the kinetids on one face, ex *S. marmorata* from near the Réserve Spéciale d'Analamazaotra, Andasibe. 16. Specimens from the same host and locality. 17-18. *P. perantonii* n. sp. 17. Arrangement of the kinetids on one face, ex *Aglyptodactylus madagascariensis* from near the Réserve Spéciale d'Analamazaotra, Andasibe. 18. Specimens from the same host and locality. Scale-bars: 1, 3, 5, 7, 9, 11, 13, 15, 17, 100 μm ; 2, 4, 6, 8, 10, 12, 14, 16, 18, 100 μm .

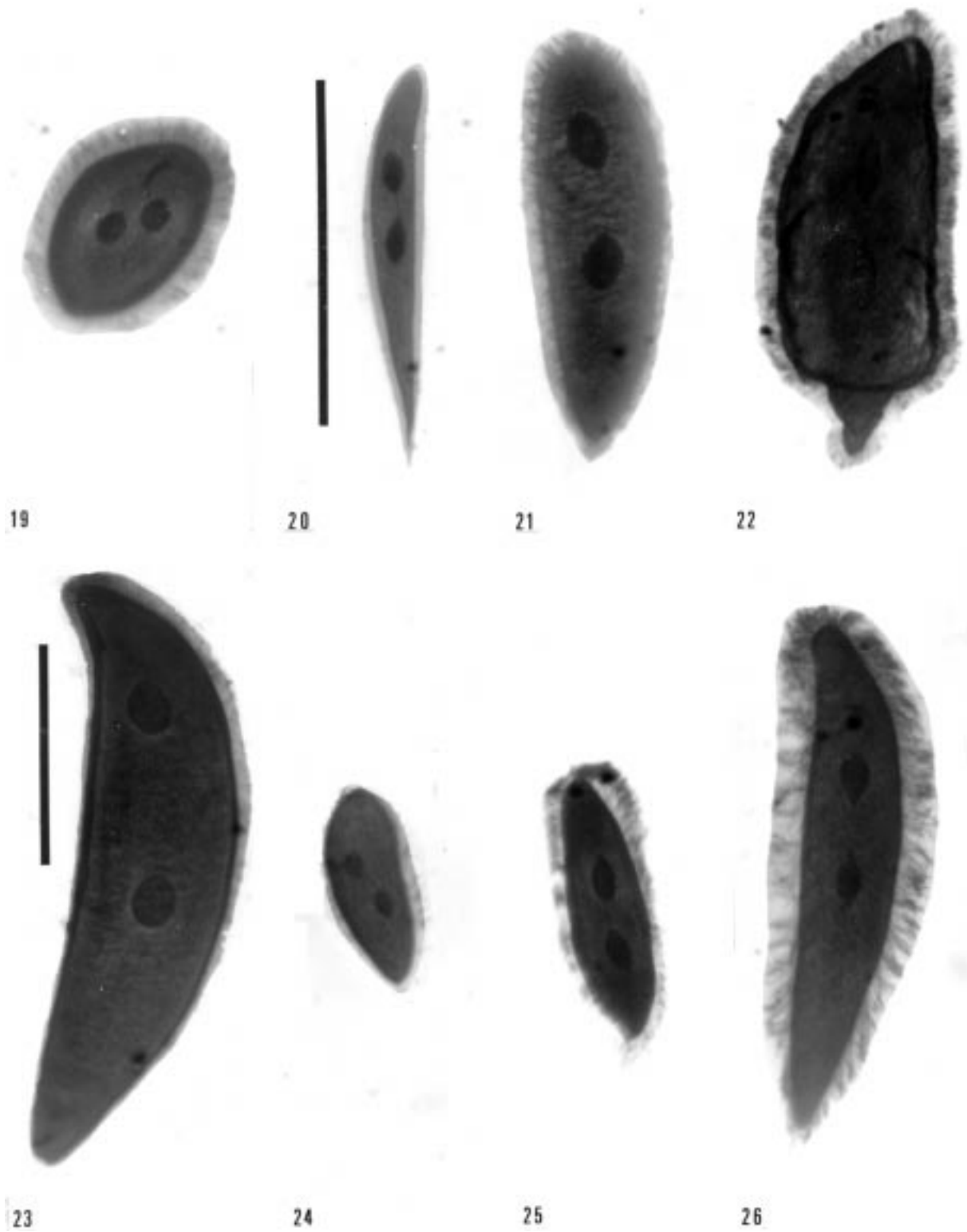


Figure 19-26. 19. *Zelleriella madagascariensis* n. sp. from *Dyscophus insularis*. 20. *Protoopalina drachi* from *Ptychadena mascareniensis*. 21. *P. primordialis* from *Ptychadena mascareniensis*. 22. *P. devinckae* n. sp. from *Ptychadena mascareniensis*. 23. *P. grolleaui* n. sp. from *Scaphiophryne marmorata*. 24. *P. charvisi* n. sp. from *S. calcarata*. 25. *P. legeardi* n. sp. from *Mantidactylus lugubris*. 26. *P. perantonii* n. sp. from *Aglyptodactylus madagascariensis*. Scale-bars: 19–22, 24, 25, 100 μm ; 23, 26, 100 μm .

Table II. Measurements (in micrometres and rounded to the nearest whole number) of *Zelleriella* and *Protoopalina* spp. in Madagascan anurans.

Host family Host species	N	Length \pm SD (range)	Width \pm SD (range)	Comments
<i>Zelleriella madagascariensis</i> n.sp.				
Microhylidae: Dyscophinae				
<i>D. insularis</i>	25	50 \pm 11 (31–67)	28 \pm 7 (20–44)	Present data
<i>Protoopalina devinckae</i> n. sp.				
Ranidae: Raninae				
<i>P. mascareniensis</i>	25	123 \pm 31 (58–184)	43 \pm 14 (15–68)	Present data
<i>Protoopalina primordialis</i> (Awerinzew, 1913) Metcalf, 1918				
Ranidae: Raninae				
<i>P. mascareniensis</i>	25	89 \pm 15 (52–107)	21 \pm 5 (14–35)	Present data
<i>Protoopalina drachi</i> Tuzet & Knoepffler, 1968				
Ranidae: Raninae				
<i>P. mascareniensis</i>	50	103 \pm 20 (73–168)	19 \pm 7 (12–43)	Present data
<i>Protoopalina legeardi</i> n. sp.				
Ranidae: Mantellinae				
<i>M. grandidieri</i>	25	80 \pm 11 (55–105)	28 \pm 5 (20–44)	Present data
<i>Protoopalina grolleau</i> n. sp.				
Microhylidae: Scaphiophryinae				
<i>S. marmorata</i>	25	148 \pm 42 (88–218)	33 \pm 6 (25–46)	Present data
<i>Protoopalina charvisi</i> n. sp.				
Microhylidae: Scaphiophryinae				
<i>S. calcarata</i>	25	44 \pm 13 (38–64)	24 \pm 4 (18–33)	Present data
<i>Protoopalina perantonii</i> n. sp.				
Ranidae: Rhacophorinae				
<i>A. madagascariensis</i>	25	194 \pm 78 (84–353)	31 \pm 13 (13–50)	Present data

Abbreviations: N, Number of specimens measured; SD, Standard deviation.

Host generic abbreviations: A., *Aglyptodactylus*; D., *Dyscophus*; M., *Mantidactylus*; P., *Ptychadena*; S., *Scaphiophryne*.

mossambica (see Delvinquier et al. 1995, for review). We have now found it in two specimens of *P. mascareniensis* from Madagascar.

***Protoopalina drachi* Tuzet & Knoepffler, 1968**
(Figures 7,8,20; Table II)

Type-specimens: BM 1997:9:18:4; USNM 50964; AMNH 812.

Hosts and localities: Observed in 3 of 7 *Ptychadena mascareniensis* (Ranidae) 2 from the garden of the Institut Pasteur in Antananarivo (18°55' S, 47°32' E,

map 8) and one from near Réserve Spéciale d'Analamazaotra, Andasibe (18°56' S, 48°25' E, map 8).

Morphology

Slender, elongate body with a gradually tapering posterior extremity. Nuclei become far apart from each other in larger trophozoites.

Comments

This species has been reported on a number of occasions in various species of the African genus *Ptychadena* (see Delvinquier et al., 1995, for review). We have now observed it in *P. mascareniensis* from Madagascar.

***Protoopalina legeardi* n. sp.** (Figures 9–12,25; Table II)

Type-specimens: BM 1997:9:18:5, 1997:9:18:6; USNM 50963.

Etymology: This species is named for Mr Luc Legeard. *Hosts and locality:* Observed in one *Mantidactylus lugubris* (Ranidae) and one of 2 *M. grandidieri* both from near the Réserve Spéciale d'Analamazaotra, Andasibe (18°56' S, 48°25' E, map 8).

Morphology

Stocky body with a sharp pointed extremity. Nuclei close to each other and placed in the middle portion of the body.

Comments

This *Protoopalina* was only found in three specimens of *Mantidactylus* from Andasibe. Infections were never abundant. It resembles in shape small trophozoites of *P. primordialis*, but larger trophozoites of our specimens do not show the slender body observed in Awerinzew's species. Also, the nuclei are placed in the middle region of the body whereas they are situated anteriorly in the case of *P. primordialis*. No other Afrotropical *Protoopalina* species exhibits a similar body shape (see Delvinquier et al., 1995). We propose to name this opalinid *P. legeardi* n. sp.

***Protoopalina grolleau* n. sp.** (Figures 15,16,23; Table II)

Type-specimens: BM 1997:9:18:7; USNM 50962; AMNH 813; MCZ 46B.

Etymology: This species is named for Mr Bernard Grolleau in recognition for his help in translating Latin texts relating to the history of opalines.

Host and locality: Observed in one *Scaphiophryne marmorata* (Microhylidae) from near the Réserve Spéciale d'Analamazaotra, Andasibe (18°56' S, 48°25' E, map 8).

Morphology

Cucumber-shaped body with a pointed anterior extremity in larger trophozoites. Two rounded nuclei are placed in the anterior region of the body and quite distant from each other in larger trophozoites.

Comments

The smaller trophozoites of this species resemble, in both the shape and in position of the nuclei, those of *P. hylarum* (Raff, 1911) (see Delvinquier, 1987) and of *P. octomixa* Fantham, 1930 (see Delvinquier et al., 1995). Our specimens are however more slender than the trophozoites of these two species. Other Afrotropical *Protoopalina* have quite different body shapes (see Delvinquier et al., 1995). We, therefore, propose to erect *P. grolleau* n. sp.

***Protoopalina charvisi* n. sp.** (Figures 13,14,24; Table II)

Type-specimens: BM 1997:9:18:8; USNM 50961; AMNH 814.

Etymology: This species is named for Mr Christophe Charvis in recognition of his help with photographs.

Hosts and locality: Observed in 2 *Scaphiophryne calcarata* from Camp forestier, CFPF forest, Kirindy, near Morondava (20°03' S, 44°39' E, map 7).

Morphology

Small trophozoites typically with a rounded body which becomes wedge-shaped in older forms (Figure 13). Two small rounded nuclei occur close to each other in the middle region of the cell.

Comments

This is a very small species of *Protoopalina* occurring in a species of microhylid. The two infections were not abundant. The presence of small rounded trophozoites makes it unlike any other Afrotropical *Protoopalina* (see Delvinquier et al., 1995). We propose to name it *P. charvisi* n. sp.

***Protoopalina perantonii* n. sp.** (Figures 17,18,26; Table II)

Type-specimens: BM 1997:9:18:9; USNM 50960; AMNH 815; MCZ 14A.

Etymology: This species is named for Mr Jacques Pérantoni who kindly provided accommodation in Antananarivo.

Hosts and locality: Observed in 3 *Aglyptodactylus madagascariensis* (Ranidae: Rhacophorinae) from near the Réserve Spéciale d'Analamazaotra, Andasibe (18°56' S, 48°25' E, map 8).

Morphology

Slender body with a narrow anterior end and a tapering posterior extremity. Two pear-shaped nuclei are situated in the anterior half of the body.

Comments

This species was only observed in *Aglyptodactylus*. It approaches in body shape the larger trophozoites of the Afrotropical species *P. primordialis*, but this species has a rounded anterior extremity. Also, the distance between the two nuclei, for specimens of similar size, is different: they are much closer to each other in the case of *P. primordialis*. Based on these differences, and because no other Afrotropical *Protoopalina* species has this combination of characters, we propose to name this species *P. perantonii* n. sp.

Discussion

Binucleate opalinid genera are not commonly encountered in Madagascan anurans. The genus *Zelleriella* is especially rare. We only observed it in the microhylid *Dyscophus insularis* from the west coast of Madagascar. The genus *Protoopalina* is most often found in *Ptychadena mascareniensis*, a ranid species also occurring on the African continent and known to harbour species of that genus (see Delvinquier et al., 1995). We found three different species of *Protoopalina* in the seven specimens we dissected of which two of the species are also found in African ranids. There is an apparently relictual occurrence of other species of *Protoopalina* in frogs, such as the ranid *Aglyptodactylus madagascariensis* and the microhylids *Scaphiophryne calcarata* and *S. marmorata*. Blanc & Paulian (1996) supported the view that the archaic group Scaphiophryninae were probably in Madagascar during the early stages of formation of the Mozambique

Channel, in the early Cretaceous (136 mya) and before the arrival of bufonids on the east coast of Africa. It is interesting to note that most of the binucleate opalines we found are rather small in size and were never abundant in any individual, except for *Ptychadena*. This ranid occurs abundantly in the country and its arrival is now regarded as recent and possibly through human introduction. The case of *Protoopalina legeari* is interesting, considering the large sample of *Mantidactylus* species we looked at. Again, specimens were never abundant and the infection could be seen as accidental rather than natural.

The possibility of a relictual occurrence of binucleate opalines in Madagascan anurans calls for comments. Brookes' (1965) statement on the discontinuous distribution of *Protoopalina* and *Zelleriella*, and the apparent dominance of *Opalina* suggesting that older opalinid groups have possibly been replaced or displaced in their hosts by more recent descendants, appears to be verified once again. Delvinquier & Marinkelle (1997) suggested that the isolation of *Zelleriella* and the limited invasion of multinucleated opalinid genera, in particular *Opalina*, in South America was until recently responsible for the great variety of species of *Zelleriella* occurring in Neotropical frogs. It is interesting to note that, outside South America and Australia, the genus *Zelleriella*, when present, has a tendency to occur in ancient groups of Anura, such as the North American Pelobatidae (*Scaphiopus*) and in many microhylids.

In terms of the evolution and distribution of the binucleate opalines in Madagascan anurans, we must look at Madagascar from a Gondwanan perspective. The initial position of Madagascar was located on the side of Kenya and flanked by India on its east coast and the Antarctic continent at its southern tip. Madagascar started to separate from Eastern Africa during the late Jurassic-early Cretaceous (136 mya) and this was completed by c. 110 mya. The separation of Madagascar, India and the Australian-Antarctic continental bloc occurred at the beginning of late Cretaceous (c. 83 mya) (Fröhlich, 1996). Madagascar became an island and the opalinids were able to evolve there in isolation. According to Duellman & Trueb (1986), only ranids (including rhacophorinids), hyperoliids and microhylids were present on this plate. Delvinquier & Patterson (1993) have placed the origin of all opalinid genera before the initial break-up of Gondwana in the late Jurassic. For the Neotropical Amphibia, Delvinquier & Marinkelle (1997) have agreed with Brookes (1965) that the relative isolation

of the genus *Zelleriella* and its strong speciation on the South America was correlated with the absence of multinucleate opalinids until a more recent period, the Pliocene (7–3 mya). We believe that competition, whether physiological or ecological, is directly related to the rare occurrence of the binucleate opalinid genera in Madagascar. Our findings show the overwhelming presence of the genus *Opalina* in Madagascan Anura (genera *Boophis* and *Mantidactylus*). The genus *Cepedea* appears to be restricted to the hyperoliid genus *Heterixalus*, which is closely related to the African genus *Hyperolius* (Delvinquier et al., unpublished results).

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